Cytokines and Inflammatory Mediators Do Not Indicate Acute Infection in Cystic Fibrosis

JOANNE M. WOLTER,1* ROBYN L. RODWELL,2 SIMON D. BOWLER,3 AND JOSEPH G. MCCORMACK1

Department of Infectious Disease,1 Haematology Department,2 and Department of Respiratory Medicine,3 Mater Adult Hospital, South Brisbane 4101, Queensland, Australia

Received 24 June 1998/Returned for modification 22 September 1998/Accepted 4 December 1998

Various treatment regimens and difficulties with research design are encountered with cystic fibrosis (CF) because no standard diagnostic criteria exist for defining acute respiratory exacerbations. This study evaluated the role of serial monitoring of concentrations of selected cytokines and inflammatory mediators in serum and sputum as predictors of respiratory exacerbation, as useful outcome measures for CF, and to guide therapy. Interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), neutrophil elastase-α-1-protease inhibitor complex (NE complex), protein, and α-1-protease inhibitor (α-1-PI) were measured in serum and sputum collected from CF patients during respiratory exacerbations and periods of well-being. Levels of NE complex, protein, and α-1-PI in sputum rose during respiratory exacerbations and fell after institution of antibiotic therapy (P = 0.078, 0.001, and 0.002, respectively). Mean (± standard error of the mean) levels of IL-8 and TNF-α were extremely high in sputum (13,780 ± 916 and 249.4 ± 23.5 ng/liter, respectively) but did not change significantly with clinical deterioration of the patient (P > 0.23). IL-8 and TNF-α were generally undetectable in serum, and therefore these measures were unhelpful. Drop in forced expiratory volume in 1 s was the only clinical or laboratory parameter that was close to being a determinant of respiratory exacerbation (P = 0.055). This study provides evidence of intense immunological activity occurring continually within the lungs of adult CF patients. Measurement of cytokines and inflammatory mediators in CF sputum is not helpful for identifying acute respiratory exacerbations.

Cystic fibrosis (CF) is the most common inherited fatal disease affecting Caucasians. Lung disease is the primary cause of morbidity and mortality due to chronic infection with Pseudomonas aeruginosa. The diagnosis of acute respiratory infection in individuals with CF is difficult because conventional parameters of acute infection such as fever, raised leukocyte count, deterioration in lung function, and positive sputum culture are not always helpful (17). Consequently, identification of exacerbations is necessarily based upon subjective feelings of deterioration from the patient.

The problem of diagnosing acute infection in CF has implications for therapy. Repeated antibiotic therapy usually requires hospitalization and has been implicated in the development of multiresistant strains of P. aeruginosa (3, 14). Consequently, most centers treat patients with antibiotics on an as required basis. This is despite increasing evidence that significant inflammatory processes continue in the lung during periods of wellness (2, 6, 10, 13).

The chronicity of lung disease in CF, plus the tendency for acute respiratory infections to present in an atypical fashion, poses problems for research design. It would be useful to have some other indicator of infection and inflammation as a diagnostic tool and as a way to monitor disease and guide therapy. Earlier studies suggested a relationship between concentrations in sputum and serum of certain cytokines and clinical state (4, 5, 13, 15, 18).

The aims of this study were to measure the concentrations of selected inflammatory mediators in serum and sputum by commercially available assays, during changing clinical circumstances of patients, to assess their predictive value in anticipating exacerbations and their role as outcome measures in CF-related research. The cytokines and inflammatory mediators selected for study included interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), neutrophil elastase-α-1-protease inhibitor complex (NE complex), protein, and α-1-protease inhibitor (α-1-PI). These play an important role in the pathogenesis of the CF lung lesion (4, 5, 7, 15), are readily detectable by commercial assays, and may prove to be useful in monitoring disease activity in CF.

MATERIALS AND METHODS

Population. Adults with CF attending the Mater Adult Hospital, Brisbane, Australia, were assessed consecutively over a 12-month period during routine outpatient clinic visits and during acute respiratory exacerbations. Informed consent was obtained. This project was approved by the Mater Hospital Research Ethics Committee. Classification of respiratory exacerbations and decision to treat were made by the treating physician. Exacerbations were defined functionally as a deterioration in symptoms perceived by the patient and included an increase in dyspnea, increased sputum production, decline in forced expiratory volume in 1 s (FEV1) compared with previous best, and fever. A researcher (J.W.) assessed each patient at the clinic and during exacerbations independently of the treating physician.

Assessment points and outcome measures. Assessments of lung function and quality of life (QOL) were carried out during routine clinic visits and on admission to hospital (day 0) and on days 1, 3, and 10 of therapy. QOL was measured by using the Chronic Respiratory Disease Questionnaire (CRQD) as described by Guyatt et al. (8). The CRQD is a measure of change in dyspnea, fatigue, emotion, and mastery and is a widely used measure of QOL for patients with chronic lung disease. Scores recorded on a seven-point Likert scale were compared before and after therapy, and the differences were calculated. The range of possible scores for each domain were as follows: dyspnea, 5 to 35; fatigue, 7 to 49; emotion and mastery, 4 to 28; total score, 20 to 140. Lower scores represent higher morbidity.

Oral temperature was taken during acute respiratory exacerbations, and fever was defined as temperature equal to or greater than 38°C. Sputum and serum samples were collected at each assessment for measurement of inflammatory mediators. Use of oral, nebulized, or intravenous (IV) antibiotics, oral and inhaled steroids, and DNase was noted.

Sample collection and storage. Sputum. Sputum samples were collected at each assessment by expectoration, and excess saliva was removed by blotting. Samples were immediately vortexed with equal volumes of phosphate-buffered

* Corresponding author. Mailing address: University Department of Medicine, Mater Adult Hospital, Raymond Terrace, South Brisbane, Queensland 4101, Australia. Phone: 61 7 3840 8916/8518. Fax: 61 7 3840 1548. E-mail: j.wolter@mailbox.uq.edu.au.
saline (PBS) and then centrifuged for 5 min at 7,000 × g. The supernatant was immediately removed and stored in aliquots at −70°C prior to testing.

(ii) Serum. Five to ten milliliters of venous blood was obtained by venipuncture at each assessment by aseptic technique. Samples were allowed to clot for 15 min. Specimens were centrifuged for 15 min at 720 × g, and the serum was stored in aliquots at −70°C prior to testing.

(iii) Controls. As sputum samples for use as controls are not available from normal subjects, we elected to use bronchoalveolar lavage (BAL) specimens as controls. These samples were obtained from two patients undergoing bronchoscopy and BAL as a planned diagnostic procedure. Patient A was an 82-year-old man with squamous cell carcinoma of the lung, and patient B was a 68-year-old woman with atypical mycobacterium infection. Aliquots of these samples were run with each assay to monitor interassay variation.

Serum samples from two individuals were used as internal controls. Patient C was a 67-year-old male with sepsis and patient D was a healthy 23-year-old female. Aliquots of these samples were also run with each assay.

Determination of cytokines and inflammatory mediators. (i) IL-8. IL-8 levels were determined for duplicate sputum supernatant and serum samples by using a commercially available coated-well, sandwich enzyme immunoassay (sensitivity, <1 pg/ml; Titerzyme IL-8 EIA kit; Perspective Diagnostics). Briefly, IL-8 present in samples was bound to IL-8 monoclonal antibody precoated onto a 96-well plate. Excess sample was removed by washing. Rabbit antibody to IL-8 bound to captured IL-8, and excess antibody was removed by washing. Enzyme-conjugated goat anti-rabbit immunoglobulin G (IgG) bound to the sandwich, tetramethylbenzidine (TMB) substrate was added after the unreacted conjugate was washed, and the colored product formed was proportional to the amount of IL-8 in the sample. Stop solution (1 N HCl) ended the reaction, and the absorbance was measured at 450 nm on an enzyme-linked immunosorbent assay (ELISA) reader (Beckman Array instrument). The normal range of serum protein is 57 to 85 g/liter.

Determination of cytokines and inflammatory mediators. (ii) TNF-α. TNF-α levels were also determined by coated-well, sandwich enzyme immunoassay (sensitivity 16.9 pg/ml in buffer diluted and 8.34 pg/ml in serum diluted; Titerzyme TNF-α EIA kit). Briefly, TNF-α present in samples was bound to TNF-α monoclonal antibody precoated onto a 96-well plate. Excess sample was removed by washing. Polyclonal rabbit antibody to TNF-α bound to captured TNF-α and excess antibody was removed by washing. Enzyme-conjugated goat anti-rabbit IgG bound to the sandwich, TMB substrate was added after the unreacted conjugate was washed, and the colored product formed was proportional to the amount of TNF-α in the sample. Stop solution (1 N HCl) ended the reaction, and the absorbance was measured at 450 nm on an ELISA reader (Beckman Array instrument).

(iii) NE complex. NE complex was measured by using a commercially immunoassay kit (Merck PMN Elastase 12589). Briefly, NE complex with α-1-PI in the samples was added to antibodies attached to the well of the tube. Antibodies bound to alkaline phosphatase were added and bound to the protease inhibitor end of the complex. Excess antibody was washed away, and the enzymatic activity of the complexed alkaline phosphatase was measured photometrically at 405 nm. The quantity of dye formed was directly proportional to the concentration of NE complex present in the sample. A standard curve was prepared from six standards, and unknown values were calculated from the standard curve.

(iv) α-1-PI. α-1-PI was measured by a fully automated commercial assay on a Beckman Array instrument. α-1-PI antibody complexed with α-1-PI in the sample, causing an increase in light scatter proportional to the α-1-PI concentration. The signal was automatically converted to concentration units after calibration. The normal range of α-1-PI in serum, according to the manufacturer, is 0.83 to 1.99 g/liter.

(v) Protein. Sputum protein levels were determined by the Lowry method (12) and have been reported for CF as ranging from 39.9 to 79.5 mg/g (17). Total serum protein was determined with Kodak Ektachem Clinical Chemistry Slides based at each assessment. Albumin was determined as protein reacting with cupric ion in an alkaline medium. Absorbance was read at 540 nm. The normal range of serum protein is 57 to 85 g/liter.

Statistical methods. By using Kolmogorov-Smirnov tests, all continuous variables were determined to be approximately normally distributed. Exceptions were examined further for skewness, and none had coefficients outside the acceptable range of −2 to +2. Consequently, parametric t tests, analysis of variance, and regression models were fitted to describe the variations in lung function, QOL, and cytokine levels by exacerbation or clinic visit status and to determine the QOL and cytokine data. Logistic regression was used to consider the determinants of exacerbation.

The observation unit was the clinic or exacerbation assessment. Where numbers permitted, patients were included in the models as a blocking term; however, the available sample size rarely allowed this. Statistical significance for all tests was defined at the conventional 95% level, and all t tests used were two-tailed. To examine for improvement with therapy, analysis of exacerbations was divided into days 0 and 1 and day 2 onward.

RESULTS

Subjects. All patients approached agreed to participate in the study. There were 10 patients (5 male) aged between 15 and 44 (mean, 23.8) years. During exacerbations, patients were treated variably with oral, IV, and nebulized antibiotics. Use of oral or nebulized antibiotics as maintenance therapy was noted. There were 61 assessments; 26 were clinic visits and 35 assessments were during infective respiratory exacerbations. The median total assessments per subject was 4 (range, 1 to 17), the median number of clinic visits was 2 (range, 1 to 7), and the median assessments during exacerbations was 3 (0 to 10). There were 17 exacerbations. On 14 occasions individuals reported shortness of breath on the first day of an exacerbation, 11 reported sputum changes, and a fever was recorded on only four occasions. Although not always reaching statistical significance, we report associations below that are worthy of note and difficult to completely discount.

Lung function. FEV1 (percent predicted) was significantly higher during clinic visits than during day 0 to 1 exacerbation assessments (Table 1), but these values were similar after day 2 (P = 0.008). There were no other significant differences between clinic or exacerbation assessments in any of the other lung function variables measured. There was no association between lung function measures and antibiotic, steroid, or DNase use (P > 0.30 in all cases), with the exception of percent forced vital capacity (FVC) predicted, being significantly higher in the presence of steroid use (P = 0.001), and percent mean expiratory flow (MEF) rate predicted, being higher when DNase was used (P = 0.002).

TABLE 1. Mean ± SEM lung function comparing clinic and exacerbation visits

| Lung function | % Predicted |
|---------------|-------------|
| Clinic assessment | Exacerbation day 0 to 1 | Exacerbation day 2 to 10 | Significance<sup>a</sup> |
| FEV1 | 50.9 ± 2.7 | 44.6 ± 3.5 | 51.7 ± 6.0 | 0.008 |
| FVC | 62.0 ± 3.1 | 61.1 ± 4.4 | 61.7 ± 7.1 | 0.47 |
| MEF | 27.4 ± 3.1 | 21.9 ± 4.2 | 30.7 ± 6.9 | 0.94 |

<sup>a</sup> Overall significance of differences in means across clinic and exacerbation days 0 to 1 and day 2 to 10 assessments. Paired significance not tabulated.

TABLE 2. Mean ± SEM QOL scores comparing clinic and exacerbation assessments

| QOL indicator | Clinic assessment | Exacerbation day 0 to 1 | Exacerbation day 2 to 10 | Significance<sup>a</sup> |
|---------------|------------------|-------------------------|-------------------------|-------------------------|
| Total score | 86.6 ± 3.9 | 79.6 ± 6.5 | 84.6 ± 10.9 | 0.060 |
| Emotion | 33.4 ± 1.6 | 30.5 ± 2.0 | 34.4 ± 2.7 | 0.039 |
| Fatigue | 15.8 ± 1.0 | 14.6 ± 1.9 | 15.0 ± 2.3 | 0.117 |
| Mastery | 20.3 ± 1.2 | 19.3 ± 1.6 | 21.2 ± 2.9 | 0.047 |
| Dyspnea | 16.8 ± 1.0 | 14.2 ± 1.7 | 16.1 ± 2.7 | 0.035 |

<sup>a</sup> Overall significance of differences in means across clinic and exacerbation days 0 to 1, and day 2 to 10 assessments. Paired significances were not tabulated.
QOL. With the exception of fatigue, mean QOL scores were significantly higher during clinical assessments than during day 0 to 1 of an exacerbation and were similar after day 2 ($P < 0.047$) (Table 2). Mastery and total QOL scores were significantly associated with percent FEV1 predicted ($P < 0.017$) (Fig. 1). Dyspnea scores correlated with percent FVC predicted ($P = 0.025$).

With the exception of dyspnea scores, mean QOL scores were all significantly higher in the absence of antibiotic use (oral, IV, or nebulized) ($P < 0.052$ in all cases; $P = 0.101$ for dyspnea). This was similarly true for steroid use ($P < 0.053$ in all cases; $P = 0.44$ for dyspnea). There was no association between QOL and DNase use ($P > 0.135$).

Correlation between serum and sputum samples. The numbers of paired serum-sputum samples for each cytokine tested at each assessment varied according to available sample volume. Paired samples were available for protein for all 61 assessments and were available for 53 assessments for NE complex and 44 assessments for α-1-PI. There was a significant correlation between serum and sputum values of protein ($r = 0.33; P = 0.010$). There was no correlation between sputum and serum values of NE complex or α-1-PI ($P > 0.440$ in all cases).

IL-8. Mean (± standard error of the mean [SEM]) sputum level of IL-8 was 13,780 ± 916 ng/liter. The coefficient of variation (CV) values for within run and between run precision, determined by replicate testing of control samples, was <8.4%. Figure 2 illustrates the individual variation in IL-8 levels measured from an individual during well periods and hospitalizations for respiratory exacerbations.

Assays for IL-8 were performed on 33 serum samples during exacerbations and clinic visits from 10 patients. IL-8 was undetectable in all serum samples, and testing of serum samples did not proceed further.

TNF-α. Mean (± SEM) sputum level of TNF-α was 249.4 ± 23.5 ng/liter. CV was 16% for between run precision and 22% for within run precision, determined by replicate testing of control samples.

Assays for TNF-α were performed on serum samples from patients both during exacerbations and clinic visits. The detection limit of this test is reported as 8.34 pg/ml. A total of 60% of clinic and exacerbation samples tested were below 10 pg/ml and therefore beneath the detectable limits of the test. Testing of serum samples did not proceed further.

NE complex, α-1-PI, and protein. Replicate testing of control plasma and serum indicated that plasma values were consistently 57 to 59% of serum values. The mean (± SEM) sputum level of NE complex was 106.4 ± 12.8 µg/liter. CV values of <13.3% were obtained for between run precision. The mean (± SEM) total serum level of NE complex was 369.3 ± 28.8 µg/liter. The mean (± SEM) sputum level of α-1-PI was 0.0724 ± 0.007 g/liter. The mean (± SEM) serum level of α-1-PI was 2.25 ± 0.08 g/liter. The mean (± SEM) sputum protein level was 1.20 ± 0.05 g/liter. Sputum protein CV values of <5.6% for within run precision and <25% for between run precision were obtained by replicate testing of control samples. The mean (± SEM) serum protein level was 78.6 ± 0.89 g/liter.

Table 3 demonstrates changes in sputum levels of cytokines and inflammatory mediators during periods of well-being and during exacerbations. Table 4 shows associations with the use of DNase, oral or inhaled steroids, and antibiotic use plus associations between the measured levels of cytokines and inflammatory mediators in sputum and serum and clinical outcomes.

Time to next exacerbation and determinants of an exacerbation. This analysis was restricted to clinic visits and considered the number of days until the next exacerbation. There was no significant association between QOL, lung function, or any of the serum or sputum levels of inflammatory mediators and days to next admission ($P > 0.12$ in all cases).

Percent drop in FEV1, total QOL score, amount of sputum protein, and sputum (NE complex, IL-8 and TNF-α) per gram of protein were considered in a logistic regression model. Percent drop in FEV1 was identified as a likely determinant of a respiratory exacerbation ($P = 0.055; P > 0.21$ for all other variables). Figure 3 illustrates the relationships between percent drop in FEV1, total QOL score, amount of sputum protein, and amount of sputum NE complex in well patients versus patients with respiratory exacerbations. Conventionally, a 15% reduction in FEV1 compared with normal is considered to represent a significant drop in lung function in subjects with asthma. No similar standard exists for CF. If 15% is chosen as a significant reduction in FEV1, the sensitivity of this test as a
diagnosis for respiratory exacerbations in this population is 86.7%. The specificity of the test is 57.9%, and the positive and negative predictive values are 61.9 and 84.6%, respectively. Performance of other parameters as tests of an exacerbation of CF were not examined as all were less significant than drop in FEV1.

DISCUSSION

This study examined sputum and serum levels of a panel of cytokines and inflammatory mediators shown previously to be of potential significance for CF. The objectives were to assess pathogenic changes during infections, to determine the role of cytokines as predictors of disease status, and to guide therapy. Adult patients with long-standing CF formed the study population. Disease status was determined by lung function, as measured by spirometry, and QOL utilizing the CRDQ.

We found that sputum levels of protease inhibitors and protein rose during respiratory exacerbations and fell after initiation of therapy. Levels of IL-8 and TNF were extremely high in sputum but did not change significantly with clinical disease. In contrast, serum levels of proinflammatory cytokines were undetectable. The discordance observed between sputum and serum levels supports the concept of a florid inflammatory response that is compartmentalized to the local environment. This response cannot be detected systemically in the serum with the assays employed. We were unable to demonstrate a statistically reliable or clinically useful relationship between levels of these markers and patient status. Although not reaching statistical significance, the percent drop in FEV1 proved to be the best determinant of an exacerbation.

In this study we elected to use sputum rather than BAL specimens, which have been used in past literature. Variations in sputum quality, timing of collection with physiotherapy, effect of inhaled therapies such as DNase and antibiotics on sputum after collection, and the possibility for sputum to represent regional activity only are all potential problems. These issues though are equally problematic for BAL samples. Sputum is readily available in CF and does not require the patient to undergo a moderately invasive procedure. Furthermore, BAL specimens are unlikely to ever become part of routine clinical monitoring.

The very high levels of IL-8 found in the present study are consistent with findings by others (1, 4, 9, 11, 16). The levels detected in an adult CF population with long-standing colonization by *P. aeruginosa*, however, were higher than those reported previously in pediatric populations (1, 4, 9), where levels have been shown to increase with age (9). In our study, mean age was 23.9 years and mean IL-8 level was 13,050 ng/liter, compared with 1,036 ng/liter from children with a mean age of 29 months (1) and 1,298 ng/liter with a mean age of 0.48 years (9). Our data imply that IL-8 production is higher in the adult CF lung, although whether this represents a true age-related effect or reflects disease severity in older patients is uncertain.

Studies to date have concentrated on correlating levels of

| Cytokine or inflammatory mediator in sputum (amt) | Level of cytokine or inflammatory mediator | Significance* |
|-----------------------------------------------|------------------------------------------|---------------|
|                                               | Clinic | Exacerbation day 0 to 1 | Exacerbation day 2 to 10+ |               |
| IL-8 (ng/liter)                               | 14,732 ± 1,297 | 13,617 ± 1,695 | 12,072 ± 1,968 | 0.324 |
| TNF (ng/liter)                                | 210 ± 28.4 | 306 ± 40.7 | 235 ± 68.7 | 0.230 |
| NE complex (μg/liter)                         | 105.2 ± 11.6 | 113.1 ± 11.9 | 99.4 ± 13.7 | 0.078 |
| α-1-PI (g/liter)                              | 0.05 ± 0.002 | 0.08 ± 0.01 | 0.10 ± 0.02 | 0.002 |
| Protein (g/liter)                             | 1.07 ± 0.06 | 1.29 ± 0.09 | 1.33 ± 0.12 | 0.001 |
| NE complex/g of protein                       | 116 ± 19.19 | 101 ± 12.7 | 80 ± 10.3 | 0.340 |
| α-1-PI/g of protein                           | 0.05 ± 0.005 | 0.06 ± 0.007 | 0.06 ± 0.01 | 0.039 |

* Overall significance of differences in means across clinic and exacerbation day 0 to 1 and day 2 to 10 assessments. Paired significances were not tabulated.
cytokines and inflammatory mediators to clinical factors such as lung function or clinical score. Given that these parameters are not always useful indicators of well-being for CF, it is not surprising that relationships between the two have not been consistently reproduced in the literature. We have attempted to find a relationship between levels of cytokines and inflammatory mediators and QOL. QOL measurements are a broad measure of well-being that encompass more symptomatology than isolated clinical measurements. The CRDQ assesses dyspnea, fatigue, emotional well-being, and mastery (feeling of control of the illness and therapy). QOL did not correlate with any serum parameters but was significantly associated with sputum α-1-PI and TNF-α levels and with FEV1 (percent predicted). The correlation with sputum TNF-α levels did not change significantly with exacerbations (P = 0.230). QOL appears to fluctuate with lung function and with levels of some inflammatory parameters present in sputum and as such may be a useful tool in evaluating the effects of therapy for CF. However, its use as a diagnostic tool of CF exacerbations is limited.

We have been unable to statistically correlate measurements of inflammatory mediators with exacerbations of disease. However, this study was not designed to pick up differences of any particular magnitude, since there was no a priori expectation about the size of any of the clinical versus day 0 to 1 differences. There was wide variation in the values for NE complex, TNF-α, and IL-8, which made small group differences difficult to detect statistically. Retrospective calculations suggested that power to detect the observed differences was moderate to low (≤63%). Indeed, with the observed standard deviations and small sample size per group, it was only possible to detect differences larger than 74 U of NE complex, 241 U of TNF-α, and 8,241 U of IL-8. Notwithstanding the relatively low power, the observed clinic versus day 0 to 1 differences were not considered large enough to be clinically important. Thus, it is unlikely that measurement of these parameters will be a useful modality to the clinician or for research outcome for CF.

We have measured a range of factors important in the pathology of the CF lung lesion. We have been unable to find a correlation between levels of inflammatory mediators in sputum and serum and clinical parameters measured in patients during periods of well-being and during acute respiratory exacerbations. Our data indicate that these markers would be unreliable as predictors of disease status.

Our results indicate continual intense inflammation and immunological activity occurring within the lungs of adult CF patients with long-standing colonization by P. aeruginosa. Antibiotic therapy in isolation addresses only the microbiological component of bacteria-host-immune response interaction in CF and would seem to be hopelessly inadequate in controlling...
inflammation in patients with severe disease. Immunomodulatory therapies rather than new antipseudomonal agents may prove to be the next advance in CF therapy.

ACKNOWLEDGMENTS

This study was supported by the Cystic Fibrosis Association of Queensland, The John P. Kelly Mater Research Foundation, and the EMA and MC Henker Postgraduate Medical Research Scholarship. We give grateful thanks to D. Battistutta, Medical Biostatistics.

REFERENCES

1. Balough, K., M. Weinberger, W. Smits, R. Ahrens, and R. Fick. 1995. The relationship between lung infection and inflammation in the early stages of lung disease from cystic fibrosis. Pediatr. Pulmonol. 20:63–70.
2. Bonfield, T. L., J. R. Panuska, M. W. Konstan, K. A. Hilliard, J. B. Hilliard, H. Ghanim, and M. Berger. 1995. Inflammatory cytokines in cystic fibrosis lungs. Am. J. Respir. Crit. Care Med. 152:2111–2118.
3. Ciolfi, O., B. Giwerman, S. Pedersen, and N. Hoiby. 1994. Development of antibiotic resistance to Pseudomonas aeruginosa during two decades of antipseudomonal treatment at the Danish CF Center. APMIS 102:674–680.
4. Dean, T. P., Y. Dai, J. K. Shute, M. K. Church, and J. O. Warner. 1993. Interleukin-8 concentrations are elevated in bronchoalveolar lavage and sera of children with cystic fibrosis. Pediatr. Res. 34:159–161.
5. Elborn, J. S., S. M. Cordon, D. Parker, F. M. Delamere, and D. J. Shale. 1993. The host inflammatory response prior to death in patients with cystic fibrosis and chronic Pseudomonas aeruginosa infection. Respir. Med. 87:603–607.
6. Elborn, J. S., S. M. Cordon, and D. J. Shale. 1993. Host inflammatory responses to first isolation of Pseudomonas aeruginosa from sputum in cystic fibrosis. Pediatr. Pulmonol. 15:287–291.
7. Greally, P., M. J. Hussein, A. J. Cook, A. P. Sampson, P. J. Piper, and J. F. Price. 1993. Sputum tumor necrosis factor-alpha and leukotriene concentrations in cystic fibrosis. Arch. Dis. Child. 68:389–392.
8. Guyatt, G. H., L. R. Berman, M. Townsend, S. O. Pugsley, and L. W. Chambers. 1987. A measure of quality of life for clinical trials in chronic lung disease. Thorax 42:773–778.
9. Khan, T. Z., J. S. Wagener, T. Bost, J. Martinez, F. J. Accurso, and D. W. H. Riches. 1995. Early pulmonary inflammation in infants with cystic fibrosis. Am. J. Respir. Crit. Care Med. 151:1075–1082.
10. Konstan, M. W., K. A. Hilliard, T. A. Norvell, and M. Berger. 1994. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild disease suggest ongoing infection and inflammation. Am. J. Respir. Crit. Care Med. 150:448–454.
11. Kronborg, G., M. B. Hansen, M. Svensson, A. Fomsgaard, N. Hoiby, and K. Bandtzen. 1993. Cytokines in sputum and serum from patients with cystic fibrosis and chronic Pseudomonas aeruginosa infection as markers of destructive inflammation in the lungs. Pediatr. Pulmonol. 15:292–297.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
13. Meyer, K. C., J. R. Lewandowski, J. J. Zimmerman, D. Nunley, W. J. Calhoun, and G. A. Doppio. 1991. Human neutrophil elastase and elastase/alpha-1-antiprotease complex in cystic fibrosis. Am. Rev. Respir. Dis. 144:580–585.
14. Mouton, J. W., J. G. den Hollander, and A. M. Horrevorts. 1993. Emergence of resistance amongst Pseudomonas aeruginosa isolates from patients with cystic fibrosis. J. Antimicrob. Chemother. 31:919–926.
15. O’Connor, C. M., K. Gaffney, J. Keane, A. Southey, N. Byrne, S. O’Mahoney, and M. X. Fitzgerald. 1993. Alpha-1 proteinase inhibitor, elastase activity, and lung disease severity in cystic fibrosis. Am. Rev. Respir. Dis. 148:1665–1670.
16. Richman-Eisenstat, J. V., P. G. Jores, C. A. Hebert, J. Ueki, and J. A. Nadel. 1993. Interleukin-8 is an important chemoattractant in sputum of patients with chronic airways disease. Am. J. Physiol. 264:L413–L418.
17. Smith, A. L., G. Redding, C. Doershuk, D. Goldmann, E. Gore, B. Hilman, M. Marks, R. Moss, B. Ramsey, T. Rubio, et al. 1988. Sputum changes associated with therapy for endobronchial exacerbations in cystic fibrosis. J. Pediatr. 112:547–554.
18. Strieter, R. M., A. E. Kock, V. B. Antony, R. B. Fick, T. J. Sandiford, and L. L. Kunkel. 1994. The immunopathology of chemoattractant cytokines: the role of interleukin-8 and monocyte chemoattractant protein-1. J. Lab. Clin. Med. 123:183–197.