Role of alginate in infection with mucoid *Pseudomonas aeruginosa* in cystic fibrosis

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Abstract

Background Chronic bronchopulmonary infection with mucoid, alginate producing *Pseudomonas aeruginosa* occurs characteristically in patients with cystic fibrosis. Alginate may be a virulence factor for *P aeruginosa* infection in such patients.

Methods Forced vital capacity (FVC), nutritional state and the antibody response to *P aeruginosa* were determined at regular intervals from three years before chronic *P aeruginosa* infection to 10 years afterwards in 73 patients with cystic fibrosis. All patients were treated intensively with antipseudomonal chemotherapy during the study period.

Results FVC was reduced in all patients who subsequently developed *P aeruginosa* infection before they acquired the infection, indicating significant pre-existing lung damage when compared with patients who remained free of *P aeruginosa*. Lung function and nutritional state remained unchanged after 10 years of infection, except in the patients who died of *P aeruginosa* lung infection. The FVC and height and weight of patients infected with non-mucoid strains of *P aeruginosa* were similar to those of uninfected patients. Patients infected with mucoid strains had poorer lung function and nutritional state for the first five years after infection compared with patients with non-mucoid strains. Such infection was also associated with greater IgG and IgA antibody responses to *P aeruginosa* standard antigen compared with non-mucoid infection. Concentrations of antibody to alginate were similar in patients with non-mucoid and mucoid infection. Noticeably increased concentrations of IgA antibodies to *P aeruginosa* standard antigen were observed early after the onset of infection in patients who subsequently died.

Conclusion Alginate producing *P aeruginosa* infection is associated with a hyperimmune response and poor clinical condition, suggesting that alginate production is a virulence factor in such infections in patients with cystic fibrosis.

cause of death in 95% of patients with cystic fibrosis,^{1,2} and mucoid *P aeruginosa* was cultured from 94% of Danish patients with cystic fibrosis at death.³ Typically the airways are colonised initially with non-mucoid strains of *P aeruginosa*^{4,5} and after a variable period of colonisation, usually one to two years, mucoid strains emerge that produce large amounts of the extracellular polysaccharide alginate. Compared with non-mucoid strains, infection with mucoid *P aeruginosa* is associated with a poorer clinical state,⁶ lower pulmonary function,^{7,8} and greater risk of death.⁷

Infection with mucoid strains was also associated with an increased humoral immune response to *P aeruginosa*.^{9,10} An increased antibody response is associated with poorer pulmonary function^{7,11} and the lung damage in cystic fibrosis is mediated by immune complexes.^{12,13} This suggests that alginate is a virulence factor in chronic *P aeruginosa* pulmonary infection in cystic fibrosis.¹⁴

Our objective was to test the hypothesis that alginate producing, mucoid *P aeruginosa* is more virulent than non-mucoid strains. We studied 73 patients with cystic fibrosis classified according to their sputum bacteriology longitudinally and determined their antibody responses to *P aeruginosa* alginate and somatic antigens, lung function, and nutritional state for three years before and for up to 10 years after the onset of chronic *P aeruginosa* lung infection.

Patients and methods

The diagnosis of cystic fibrosis was based on repeatedly raised electrolyte concentrations in sweat and characteristic clinical features. The criteria for entry into the study were that the onset of chronic *P aeruginosa* infection was after 1975, that the patient had been followed up monthly at the Danish Cystic Fibrosis Centre at Rigshospitalet before the onset of infection, and that sputum bacteriology had been recorded for at least eight months of each year. Data were recorded prospectively and included forced vital capacity (FVC), height, weight, and sputum bacteriology. Since 1976 all patients with chronic *P aeruginosa* infection have been admitted to the centre every three months for intravenous courses of antipseudomonas chemotherapy.¹⁵

BACTERIOLOGY

The onset of chronic *P aeruginosa* infection was defined as the time when *P aeruginosa* had been grown in consecutive monthly sputum cultures over six months. Sputum was obtained by

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Mucoid *Pseudomonas aeruginosa* is isolated from most patients with cystic fibrosis, in whom it causes a chronic, incurable lung infection. Bronchopulmonary disease is the

expectoration or by endolaryngeal aspiration in patients unable to expectorate. The samples were cultured on standard laboratory media and *P. aeruginosa* identified by standard tests.¹⁶ The mucoid phenotype was identified visually by the appearance of colonies having a typical morphology with an abundance of watery and viscous slime. Strains not showing these characteristics were termed non-mucoid. Patients having both mucoid and non-mucoid colonies in the same sample (a common finding) were classified as harbouring mucoid strains. Strains were defined as virulent when they were associated with a raised antibody response and poor lung function.

CLASSIFICATION OF PATIENTS

The bacteriological records of all patients with chronic *P. aeruginosa* infection attending the centre at the beginning of 1988 were examined and patients to be included in the study were identified by the above criteria. Some patients did not satisfy these criteria because their onset of infection was before 1976, they had been transferred to the centre after the onset of *P. aeruginosa* infection, or they had not attended regularly. Patients were categorised according to their bacteriological results.

(1) Patients with classic bacteriology had non-mucoid strains initially and intermittently for about six months followed by a period of one to two years of continuous isolation of non-mucoid strains. The mucoid strains started to appear at this point and were cultured continuously thereafter. The median age at onset of infection was 10 years (range 1–16 years) for the 23 patients in this group and the median duration of infection at the time of the study was 10 years (range 6–13 years). Twenty one patients were available for analysis at the final observation (see below).

(2) Patients with non-mucoid bacteriology were chronically infected with non-mucoid *P. aeruginosa* with no mucoid strains isolated from any sputum sample ($n = 14$). The median age at onset of infection was 9 years (range 1–13 years) and the median duration of infection 10 years (range 5–12 years). Nine patients were assessed at the last observation.

(3) Patients with mucoid bacteriology had mucoid colonies at the first isolation of *P. aeruginosa* and remained chronically infected with mucoid strains ($n = 9$). Their median age at onset of infection was 12 years (range 0–31 years) and the median duration of infection nine years (range 4–13 years). Six patients were assessed at the final observation.

(4) Non-colonised patients with cystic fibrosis from whom *P. aeruginosa* had never been isolated were used as a control group ($n = 15$). They were matched for age with the infected patients, and at the end of the study their median age was 19 years (range 12–32 years). At the last observation 13 patients were assessed.

(5) Patients with fatal *P. aeruginosa* infection were included ($n = 12$). The onset of infection was after 1976 and the median age at onset was 12.5 years (range 0–22 years). The duration of infection from onset to death was 7.2 years

(2.1–11.5 years). All patients were infected with mucoid strains at the time of death and in four the initial strain was mucoid. The rest showed the same pattern as patients with classic bacteriology. Six patients remained at the final observation for assessment.

CLINICAL MEASUREMENTS

Clinical assessments were made and serum obtained from each patient every year for three years before the onset of infection; every six months for the first two years after onset; every year from two to five years after onset; and every two years from five years after the onset of infection. FVC was measured by spirometry as the best of three measurements at each clinic visit. A mean value of FVC at each time was expressed as a percentage of predicted values for sex and height.¹⁷ Because the onset of infection usually takes place in childhood around the age of 10 years paediatric predictions were used. For the sake of comparison the same predictions were used for patients aged 18 years or older.

Height and weight were measured on standard clinic scales at each visit; the mean of the recordings during an interval were expressed as the percentage deviation from the 50th centile of predicted values for Danish children.¹⁸ For patients older than 18 years the predicted value at age 18 years was used.

TESTS ON SERUM

Serum samples were stored at -20°C until analysis. All samples were analysed for precipitating antibodies against *P. aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae* by crossed immunoelectrophoresis.¹⁹ To determine specific antipseudomonal antibody activity the serum sample with the highest number of precipitating bands in a given study interval was selected.

ANTIGENS

P. aeruginosa alginate was isolated and purified from mucoid *P. aeruginosa*.²⁰ The alginate did not cross react with antibodies against lipopolysaccharide or protein antigens of *P. aeruginosa*. A mixture of three serologically cross reacting alginates was used.²⁰

P. aeruginosa standard antigen Water soluble antigens were produced from each of 17 serotypes of the international antigenic typing scheme of *P. aeruginosa*;¹⁹ equal volumes of each were mixed and designated standard antigen.

ENZYME LINKED IMMUNOSORBENT ASSAYS (ELISAS)

The antibody response was measured by four ELISAs that detected IgG and IgA antibodies specific for the two different *P. aeruginosa* antigens. All samples from a single patient were run in triplicate on the same assay plate, which also included an internal standard. Details of the assays have been published.^{11,21} All reagent volumes were 100 μl .

Alginate ELISA Plates (MicroWell, Nunc, Tåstrup, Denmark) (96 wells) were coated with 10 $\mu\text{g/ml}$ purified *P. aeruginosa* alginate in phosphate buffered saline (PBS) pH 7.4 for one hour

at 35°C and residual binding sites were blocked with 0.1% Tween 20 (Polysorbate, Sigma, St Louis, Missouri) in PBS for one hour. IgG antibodies were detected in serum samples diluted 1:4000 in PBS-Tween that were incubated in the wells for one hour at 35°C. After extensive washings peroxidase conjugated goat-antihuman IgG (γ chain, Tago, Burlingame, California) diluted 1:10 000 in PBS-Tween was added for one hour at 35°C. IgA antibodies to alginate were detected by incubating serum diluted 1:125 for one hour followed by reaction with peroxidase conjugated rabbit-antihuman IgA diluted 1:1000 (α chain, Dako, Glostrup, Denmark).

Standard antigen ELISA Standard antigen (2.2 μ g/well) was coated on MicroWell plates for IgG antibodies or Maxisorb plates (Nunc, Denmark) for IgA antibodies for one hour at 35°C. Blocking, washing, and dilution was carried out with PBS-Tween. Serum was diluted 1:4000, the peroxidase conjugated anti-IgG (Tago) 1:7500, and anti-IgA (Dako) 1:1000. Visualisation of the antigen-antibody reaction was similar for all four ELISAs by adding sodium citrate (pH 5.0, 0.1 M) containing 1,2 phenylenediamide-dihydrochloride (3.7 mM, Dako) and hydrogen peroxide (6.5 mM) for one hour at room temperature before the reaction was stopped by the addition of 1M sulphuric acid. Optical density was measured by an automatic ELISA reader (BioRad, Model 3550, Richmond, California) programmed to calculate the antibody response in ELISA units by dividing the mean optical density of the sample by the mean optical density of the internal standard.

Although the assays were optimised to yield an optical density less than 3 (the upper limit of the automatic reader) the optical density of some samples exceeded this and these were further diluted 1:40 000 and the assays repeated. The results were multiplied by a dilution factor (6.8 for IgG against standard antigen, 4.0 for IgA against standard antigen and 4.0 for IgA against alginate) determined by the linear regression slope for 10 samples analysed in six doubling dilutions. Measurement of IgA antibodies against alginate never exceeded the range so further dilutions were unnecessary.

The performance characteristics of the assays have been described previously.²¹ The within plate variation ranged from 9% to 19%, the plate to plate variation from 11% to 16%, and the day to day variation from 13% to 19%.

ANALYSIS

For description of distributions and comparison between groups non-parametric analyses were used. For unpaired data the Mann-Whitney U test was used and for paired data the Wilcoxon signed rank statistic. The level of significance was 5% for a two tailed comparison.

Results

There was no difference in the median age at onset of *P aeruginosa* infection among the four

categories of infected patients, or between the infected patients and those from whom colonies were not isolated.

PATIENTS WITHOUT COLONISATION

Patients with no growth of *P aeruginosa* had either no or a very low antibody response to *P aeruginosa* antigens throughout the observation period (figure 1, A–D) when compared with the infected patients ($p < 0.0001$). The FVC was about 90% of predicted values and did not change during the observation period of 13 years (figure 1, E). Height and weight ranged from 92% to 100% and from 98% to 101% of the median for healthy Danish children (data not shown). The weight for height median value fluctuated around 100% of predicted values (figure 1, F).

COMPARISON OF PATIENTS WITH MUCOID AND NON-MUCOID INFECTION

Before infection antibody concentrations for *P aeruginosa* did not differ between patients who subsequently developed mucoid infection and those who developed non-mucoid infection (figure 1, A–D). Before sputum culture became positive these patients had significantly higher IgA antibody concentrations for *P aeruginosa* than did those without colonisation. This difference was seen for IgA antibodies to alginate for a period of one year (figure 1, A) and for standard antigen for three years before infection (figure 1, B). Infection with non-mucoid *P aeruginosa* was associated with an antibody response to alginate that did not differ from that of infection with mucoid strains (figure 1, A and C). Patients with mucoid infection had a greater IgA response to standard antigen from 1 to 7 years after infection and a greater IgG antibody response from 0 to 5 years than did the patients with non-mucoid infection (figure 1, B and D). Similar differences occurred with precipitating antibodies (data not shown).

Before infection patients who subsequently acquired infection with mucoid strains had a significantly lower FVC than patients with non-mucoid infection (figure 1E). Both groups had significantly poorer FVC than the non-colonised controls (figure 1E). Patients infected with a mucoid organism showed a gradual decline in FVC during the preinfection period, reaching a nadir of 50% of predicted values a year after the onset of infection. The FVC then improved slowly and five years after the onset of infection had reached preinfection values, where it remained for the rest of the observation period. There was no difference in FVC in this group at the beginning and end of the observation period. The FVC in patients infected with non-mucoid strains had started to improve before the infection became established. Over the 10 years of infection with non-mucoid strains there was no difference in FVC in this group compared with non-colonised patients (figure 1E) whereas the FVC in patients with mucoid infection was significantly worse than that of patients with non-mucoid infection during the five years after the onset of infection (figure 1, E).

The height and weight of patients with non-mucoid infection was similar to predicted values (data not shown) and the corresponding weight for height was around 100% of predicted (figure 1F). The nutritional state of patients with non-mucoid infection did not differ from that of non-colonised patients whereas mucoid infection was associated with a significantly lower weight (approximately 85% of predicted values) for the first five years of infection when compared with patients with non-mucoid infection. The height of patients with mucoid infection (range 95–98%) did not differ from that of the other groups of patients (data not shown); the weight for height was significantly lower for the first five years of mucoid infection (figure 1F).

COMPARISON OF PATIENTS WITH CLASSIC BACTERIOLOGY AND MUCOID INFECTION

The course of the antibody response and clinical measurements in 23 patients with the classic pattern of bacteriology are shown in figure 2 (A–F). The IgA antibody response to alginate did not differ significantly, except that it was lower from two years before the onset of infection to

three years after (figure 2, A). The IgA antibodies to standard antigen were lower in this group of patients than in those with mucoid infection throughout the whole observation period, though this was only significant during the early stages of infection (0–3 years) (figure 2, B). The IgG antibodies to standard antigen or alginate were significantly lower only during the first year after the onset of infection and overlapped in the later stages of chronic infection (figure 2, C and D). Precipitating antibodies followed the same trend (data not shown), being significantly lower in patients with classic bacteriology for the first 18 months of infection.

The opposite pattern was observed when the antibody responses in patients with classic bacteriology were compared with those in patients with non-mucoid infection. In the early stages of infection (0–3 years) no difference was noted, after which the response was significantly greater in patients with classic bacteriology. No differences in clinical measures were observed.

Before and at the onset of chronic *P. aeruginosa* infection the patients with classic bac-

Figure 1 Comparison of changes over 14 years in patients with cystic fibrosis with mucoid and non-mucoid infection and non-colonised patients. The antibody responses to *P. aeruginosa* alginate (A and C) and to *P. aeruginosa* standard antigen (B and D), forced vital capacity (E), and weight for height (F) are shown. All values shown are medians. Closed circles with solid lines represent patients infected with mucoid *P. aeruginosa* ($n = 9$), open squares and dashed lines patients infected with non-mucoid *P. aeruginosa* ($n = 14$), and closed squares patients not colonised with *P. aeruginosa* ($n = 15$).

teriology had a median FVC of about 70% of predicted values (figure 2, E), which was significantly less than that of patients without colonisation but similar to that in patients with mucoid or non-mucoid infection. In the patients with classic bacteriology lung function was unchanged over the 10 years of infection. For one year before and up to three years after acquisition of *P aeruginosa* lung function was significantly better than in those with mucoid infection (figure 2, E). The lung function in patients with mucoid infection then improved and for the rest of the observation period no difference was found. The weight for height did not differ between patients with mucoid and classic infection (figure 2, F).

LONGITUDINAL STUDY OF PATIENTS WHO DIED FROM *P AERUGINOSA* INFECTION

Patients who died from *P aeruginosa* infection had a similar IgA and IgG antibody response to *P aeruginosa* to that of those infected with mucoid strains (figure 2, A–D). When compared with patients with classic bacteriology the patients who died had significantly greater

IgA antibody responses to standard antigen and alginate during the whole period of observation (figure 2, A and B). The IgG antibody responses were not different (figure 2, C and D) although precipitating antibodies were significantly greater for the first five years of infection (data not shown).

Before infection the patients who died had similar FVC values to patients with mucoid, non-mucoid, and classic bacteriology but significantly lower values than patients without colonisation. After one year of infection patients with classic or non-mucoid bacteriology had a significantly higher FVC; after four years after the onset of infection the mucoid group had a higher FVC than those who died (figure 2, E).

From the start of infection those who subsequently died had a significantly lower body weight than patients with classic bacteriology. No difference in height was observed (data not shown). They did not differ from patients with mucoid infection until the infection had persisted for five years, after which the weight for height was significantly lower (figure 2, F).

Figure 2 Comparison of changes in patients with cystic fibrosis with classic bacteriology with changes in patients who died and in those who had mucoid *P aeruginosa* infection. The antibody responses to *P aeruginosa* alginate (A and C) and to *P aeruginosa* standard antigen (B and D), forced vital capacity (E), and weight for height (F) are shown. All values are medians. Closed circles with solid lines represent patients infected with mucoid *P aeruginosa* ($n = 9$), open circles are patients with classic bacteriology ($n = 23$), and crosses patients with cystic fibrosis who died of chronic *P aeruginosa* infection ($n = 12$). The scales of the ordinates are different from those in figure 1. Data from patients with mucoid infection appear in both figures for comparison.

ANTIBODY RESPONSE TO *S. AUREUS* AND *H. INFLUENZAE*

S. aureus and *H. influenzae* are common respiratory pathogens and may influence the course of disease. Before the onset of infection those who became infected with mucoid strains had significantly more precipitating antibodies to *S. aureus* than did any of the other groups (data not shown). After onset there were no significant differences in the antibody responses to *S. aureus* and *H. influenzae* between any of the groups. In particular, those who died had a similar antibody response to that of the survivors. The responses remained constant throughout the period of infection and ranged from two to four precipitins (data not shown).

Discussion

The presence of alginate producing mucoid *P. aeruginosa* is characteristically associated with chronic pulmonary infection in patients with cystic fibrosis. In some cystic fibrosis centres 80% or more of the patients harbour mucoid strains.³ Acquisition of mucoid strains usually leads to a deteriorating clinical state that coincides with systemic humoral hyperimmunisation.^{14,22} Evidence has accumulated suggesting that *P. aeruginosa* antibodies contribute to the pathogenicity of progressive lung damage in cystic fibrosis. Tissue damage is thought to be due to inflammation mediated by immune complexes, which cause release of proteases from polymorphonuclear leucocytes.^{12,13} Any bacterial product that induces a noticeable antibody response must therefore be regarded as a virulence factor in cystic fibrosis lung disease.

The aim of our study was to test the hypothesis that alginate is a virulence factor in *P. aeruginosa* infection in patients with cystic fibrosis. In most patients the initial infecting strain is non-mucoid, but only a small proportion of strains remain so. In this study it was possible to select a group of patients with stable mucoid or non-mucoid infection throughout 10 years of observation, which allowed the virulence properties of alginate to be assessed. We found that infection with alginate producing mucoid *P. aeruginosa* was associated with a poorer clinical condition, although this does not constitute proof of a causal relation. Evidence in support of alginate being a virulence factor is as follows. The antibody response was greater and the FVC and nutritional state were poorer in patients with mucoid infection compared with patients infected with non-mucoid strains, while non-mucoid strains were associated with a pattern of disease that was similar to that in patients not infected with *P. aeruginosa*. Non-mucoid strains could therefore be considered harmless in cystic fibrosis.⁶ All the patients who died were infected with mucoid strains. The only known bacterial product mucoid strains produce in excess compared with non-mucoid strains is alginate. Mucoid strains must be considered to be deficient organisms because they are serum sensitive and polyagglutinable owing to loss of the lipopolysaccharide side chain and because

they produce less toxins than non-mucoid strains.^{23,24} It may be argued that alginate is not a virulence factor because the patients infected with these organisms—that is, those with mucoid and classic bacteriology—were able to maintain lung function and nutritional state virtually unchanged during a long period of infection. The patients in this study, however, do not represent the natural course of infection because they were treated intensively with antibiotics. Furthermore, these patients had survived the infection for about 10 years and constitute a selected group. In addition, they were unable to improve their FVC above preinfection values in contrast to those with non-mucoid infection. A contribution by other bacterial products to the observed differences cannot be ruled out, but we believe that alginate is the most important bacterial factor to determine the course of *P. aeruginosa* lung infection in cystic fibrosis.

Patients who later acquired either mucoid or non-mucoid infection had a greater IgA antibody response than non-colonised patients before infection was apparent. This indicates that *P. aeruginosa* was present, possibly intermittently, and was immunogenic in the lungs up to two years before it was isolated by sputum culture. The absence of *P. aeruginosa* on culture may not necessarily therefore represent absence of *P. aeruginosa* in the lungs; it may be present intermittently or in quantities that are too small to be detected (lower limit 5×10^2 organisms/ml sputum). Brett *et al* showed that specific IgG antibodies became detectable before isolation of the organism,²⁵ but we were unable to confirm this. Determination of IgA antibodies, however, may be useful for early detection of infection. Frequent bacteriological examinations, eventually using broncho-alveolar lavage fluid or bigger culture inocula, may be advisable when there is clinical or serological suspicion of *P. aeruginosa* infection.

The data presented confirm our earlier findings that patients with non-mucoid strains produce antibodies to alginate.¹¹ The term non-mucoid is therefore misleading if it is assumed to mean that the organism does not produce alginate. The mucoid nature of *P. aeruginosa* is unstable on subsequent culture,²⁶ and the bacteria in vivo may exist in the mucoid phenotype.²⁷ The genes for alginate biosynthesis are present in non-mucoid strains^{22,28} and a low level of alginate production has been reported for non-mucoid *P. aeruginosa*.^{29,30} A greater antibody response is associated with the presence of mucoid strains than of non-mucoid strains (figure 1, A–D). This increased antibody response could be because alginate is an adjuvant or a polyclonal B cell activator, though we have found that alginate is not a polyclonal B cell activator (unpublished observations). This is in accordance with our finding that the antibody response to *P. aeruginosa* alone is increased in patients with mucoid infection.

The antibody response in patients with classic bacteriology showed a similar response initially to that seen for non-mucoid strains, changing after three years to the course seen in

patients with mucoid strains (figure 2). The serological response of patients who died from *P aeruginosa* infection did not differ from that in patients with mucoid infection. Interestingly, however, the IgA antibody response to standard antigen was significantly greater during the early stage of infection in those who subsequently died than in survivors with the classic pattern of colonisation. The concentration of IgG antibodies did not discriminate between the subsequent progress of disease. Circulating immune complexes are associated with increased IgA concentrations³¹ and high total IgA concentrations occurred in patients who subsequently developed severe pulmonary complications.³² Patients who died had higher IgA concentrations than those who survived.³³ Determination of IgA antibodies may be useful to monitor the progression of lung disease. It is not possible from this study to determine whether increased IgA concentrations are an epiphenomenon of more aggressive infection or have a causal relation to the disease process. IgA coats *P aeruginosa* in the lungs.³⁴ Specific IgA antibodies may promote the persistence of bacteria in the lungs by blocking the attachment of opsonising IgG antibodies,³⁵ by inhibiting intracellular bacterial lysis,³⁶ or by steric interference with complement fixation.³⁷ IgA antibodies may also be more directly involved: acute lung injury has been induced experimentally in rats by IgA immune complexes.^{38, 39}

The propensity for *P aeruginosa* to infect patients with cystic fibrosis is not understood, but despite uniform treatment principles patients who developed chronic *P aeruginosa* infection had poorer lung function before the onset of infection than those who did not become infected for the next 10–15 years (figures 1E and 2E). This confirms data indicating that pulmonary disease tends to be more advanced in those who later acquire *P aeruginosa*,³³ but contradicts the findings of Kerem *et al*, who found no difference in lung function one year before colonisation, although 64% of their patients acquired *P aeruginosa* infection before 7 years of age, when cooperation with pulmonary function testing is less reliable.⁴⁰ Our data suggest that significant lung damage had occurred before the onset of *P aeruginosa* infection, which is in keeping with the observation that *P aeruginosa* is not usually the first pathogen in the lung in cystic fibrosis. The patients with mucoid infection had a higher antibody response to *S aureus* in the years preceding the onset of *P aeruginosa* infection, possibly indicating a predilection of mucoid strains for severely damaged airways. The implication of this finding is that more vigorous treatment of bacterial pathogens—for example, *S aureus* and *H influenzae*—and perhaps also viral episodes, which may precede *P aeruginosa* infection,⁴¹ may be helpful.

In patients given intensive treatment a large proportion will maintain similar lung function to that before infection, indicating that infection in these patients does not necessarily lead to a progressive decline in lung function as described previously⁴² and supporting earlier

reports from our own¹⁵ and other centres.⁴⁰ Obviously there is a subset of patients whose condition deteriorates and in whom the infection is fatal; further studies are needed to identify the factors responsible for this unfavourable clinical course.

The influence of malabsorption on lung function and the influence of respiratory infection on nutritional state are matters for debate. In our study weight and height were similar in patients with classic bacteriology or non-mucoid infection and in non-colonised patients. There was no difference in treatment other than antipseudomonal chemotherapy between the groups, nutritional advice and pancreatic enzyme supplementation being the same. These findings support the view that failure to thrive in cystic fibrosis relates more closely to chronic pulmonary infection than to pancreatic insufficiency, which can be corrected by enzyme substitution.⁴³ Patients in a centre recommending a high energy diet had a better nutritional state and increased survival rates in one study.⁴⁴ We rarely use hyperalimentation and have only recommended a high energy diet for the past five years. Low body weight in cystic fibrosis may be due to increased concentrations of tumour necrosis factor alpha induced by *P aeruginosa* infection.⁴⁵ Our data show that pulmonary function can be maintained despite chronic *P aeruginosa* infection and that nutritional states similar to those of children without cystic fibrosis can be established. This is in keeping with our previous results showing that aggressive anti-pseudomonal chemotherapy is associated with improved survival^{15, 46} and accords with the strong correlation seen between survival and the use of antibiotics.⁴⁷ We believe that aggressive antibiotic treatment of lung infections is mandatory in cystic fibrosis.

Our study has shown that the clinical condition of patients with mucoid infection is impaired compared with that of patients with non-mucoid infection and that the condition did not improve with chemotherapy. The importance of alginate in infection is likely to be related to the amount produced as small amounts are produced by non-mucoid strains. The role of alginate in the persistence of chronic infection is well described. We suggest that in addition to its being an antigen its role in the pathogenesis of lung disease is as an immunological adjuvant contributing broadly to increased formation of immune complexes. The genetics of alginate biosynthesis is an area of active research interest and the possibility of engineering a switchback to the non-mucoid phenotype could be immensely important for patients with cystic fibrosis infected with mucoid strains.

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