IgG antibodies in early *Pseudomonas aeruginosa* infection in cystic fibrosis

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Abstract

The relationship between IgG antibodies to *Pseudomonas aeruginosa* and its isolation from sputum was determined in 100 patients with cystic fibrosis observed at intervals of two months for a median period of one year. Only one patient had a raised antibody titre (≥22.9 ELISA units) before isolation of *P. aeruginosa*. Initially 65 patients were antibody negative, of whom 48 were also culture negative. Of 24 patients with positive sputum culture and negative antibodies, seven became antibody positive at a median (range) 15 (6–25) months later. The remaining 17 patients continued antibody negative until the end of the study at a median range 15 (1–123) months after becoming culture positive. This latter group were younger and had more intermittently positive sputum cultures. In general positive IgG antibody titres do not predate isolation of *P. aeruginosa*, but in some patients are present soon after acquisition of infection. A positive titre indicates significant exposure to *P. aeruginosa* and could be used to detect infection in patients unable to produce sputum and possibly indicate the effect of early antipseudomonal treatment.

Serum IgG antibody titres to *Pseudomonas aeruginosa* may be a sensitive and early indicator of the colonisation of the lungs with this organism in patients with cystic fibrosis. It has been reported that antibody titres are raised before isolation of *P. aeruginosa* from sputum and that antibody titres may fall after antibiotic treatment at this early stage of infection. *P. aeruginosa* infection of the chest in cystic fibrosis is the major determinant of survival in older children and adults. Once established in the airways, as indicated by regular isolation from sputum, *P. aeruginosa* is extremely difficult to eradicate with antibiotic treatment. The detection of serum antibodies might be a means of early diagnosis of the presence of the organism in the respiratory tract, which would allow early antibiotic intervention with partial or complete eradication of the organism at this time, although such recommendations are based on limited data.

We examined the relationship between the serum anti-*P. aeruginosa* IgG antibody titres and the presence of organisms in the sputum cultures of patients with cystic fibrosis.

Patients and methods

**PATIENTS**

Patients were recruited from the adult and paediatric cystic fibrosis clinics at Nottingham City Hospital based on their willingness to give regular blood and sputum samples. One hundred patients from a total of 130 agreed to take part and were studied prospectively. Samples for *P. aeruginosa* antibody measurement were either serum samples from venepuncture blood samples or whole blood spots, collected directly onto Guthrie cards from a finger puncture. Such samples are interchangeable in our assay.

Blood and sputum samples were collected at approximately two monthly intervals from a range of patients with cystic fibrosis, who were categorised into chronic, intermittent, or negative for *P. aeruginosa* infection on the basis of sputum cultures. Patients were considered to have chronic infection if *P. aeruginosa* was isolated on three or more occasions during a 12 month period, and intermittent infection was defined as any isolation in the year before the study or one or two isolations during any 12 month period during the study. The study had ethics committee approval.

**ASSAYS**

Specific IgG antibody to *P. aeruginosa* was determined in an antibody capture enzyme linked immunosorbent assay (ELISA). Soluble antigens, prepared from *P. aeruginosa* serotypes 11–17 of the International Antigenic Typing Scheme, were used to coat microtiter plates.

Gelatin was used to block areas of the plate where the antigen had not adhered. After incubation with diluted serum or blood spot eluates, biotinylated antihuman IgG was used to probe for the captured antipseudomonal antibodies. This was followed by avidin peroxidase and peroxidase substrate to give a colour signal. This reaction was stopped with sodium hydroxide and absorbance read at 455 nm.

**CALCULATIONS**

On each plate, four wells were left without coating antigen and, after blocking, were exposed to a pooled control, low *P. aeruginosa* antibody titre serum to quantify non-specific binding to IgG. A high titre *P. aeruginosa* antibody serum was used as reference, each unknown sample was expressed in ELISA units (EU), which was calculated as a percentage of this value after subtracting the non-specific binding optical density (OD, the mean of the low control wells) from both.

Unknown sample EU = (Unknown OD – low control OD)/ (High control OD – low control OD)
Intra-assay and interassay variation was determined from eight replicates per plate of two blood spot eluate samples.

STATISTICAL METHODS
Data from the control subjects was non-normally distributed and was log transformed to calculate the mean and SD. The mean + 1·96 SD was taken as the upper limit of antibody concentration in controls. Other non-normally distributed data is expressed as median and non-parametric confidence interval or range if stated. For paired comparisons differences were normally distributed and were analysed by Student’s t test; p < 0·05 was accepted as indicating a significant difference.

Results
(1) CONTROLS
A normal range was determined from 32 healthy adults and children of a similar age range to the patients with a mean (+1·96 SD) of 5·3 (22·9) EU. Mean interassay variation was 8·6% and intra-assay variation 7·1% (coefficient of variation).

(2) PATIENTS
Over a study period of three years 100 patients were monitored for a median period of 1·0 year. Median age at entry was 11·3 years (range 2 months–28 years) and the median number of samples per patient was four (range 3–5).

At entry to the study
Initially 34 patients had P aeruginosa cultured from their sputum and a positive antibody titre (group I, fig 1). Seventeen patients were culture positive but antibody titre negative (group II) and one was culture negative and antibody positive (group III). Forty eight patients were both culture and antibody negative (group IV, table).

Changes occurring during the study
During the study group I patients showed no significant change in antibody titres (70·7 EU v 79·2 EU, mean difference 2·4 EU, p = 0·5). The effect of acquisition of P aeruginosa and the subsequent antibody response was sought in the group IV patients who were initially culture and antibody negative. Of these 48 patients 39 remained in group IV and showed no change in antibody titres (4·5 EU v 3·4 EU, mean difference 0·6 EU, p = 0·37). The remaining nine patients changed their status during the study: seven cultured P aeruginosa from their sputum but remained antibody negative; one patient became culture and antibody positive simultaneously and one became antibody positive before becoming culture positive. This gave a total of 24 patients exhibiting a group II pattern (seven from group IV and 17 initially in group II) during the study. Of these 24 patients seven became group I patients when positive antibody titres developed, however, there was a median lag period of 15 months (range 6–25 months) from first culture to a positive titre. Of these
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seven patients, five were chronically culturing *P. aeruginosa* from the sputum and two were only intermittently isolating this organism. The remaining 17 patients with positive sputum culture (group II) have not yet shown an antibody response to median time from first culture of *P. aeruginosa* to the end of the study was 15 months (range 1–123 months). These 17 patients tended to be younger than the other seven patients making up the 24 with group II features (median range of 8·5 (1·0–24·8) years v 14·6 (5·2–19·3) years). Six of this subgroup of patients were chronically isolating *P. aeruginosa* and 11 were intermittently isolating this organism from their sputum. Initially only one of the 100 patients studied had positive antibody titres with negative sputum cultures (group III), although a positive sputum culture was documented 13·2 months after the first positive serum result. During the study one other patient met group III criteria for six months before becoming both antibody titre and culture positive. In patients infected with *P. aeruginosa* and later developing antibodies there was some variability of the antibody titre during the rising phase, but such changes were unrelated to anti-pseudomonal antibiotic treatment. Examples of the pattern of antibody development in two patients are given in fig 2.

Discussion

In our study 24 patients developed positive sputum cultures for *P. aeruginosa* before the development of a positive antibody titre, whereas only two patients showed a positive IgG titre preceding isolation of *P. aeruginosa* from their sputum. In a single patient antibodies became positive at the same time as sputum culture. One third of the patients with a delayed response became antibody positive between six and 25 months after the first isolation of *P. aeruginosa* from their sputum by which time most were chronically infected. The remainder had not developed an antibody response by the end of the study, although one to 123 months had elapsed from the first isolation of *P. aeruginosa*. The median follow up period of this group was only 15 months and more patients may have become antibody positive with a longer period of observation. Those without positive antibodies by the end of the study tended to be younger and had more intermittent infections, which could explain their longer lag period. They may have been more effective at clearing bacterial products from the respiratory tract than older patients who may have more accumulated lung injury and poorer airways clearance.

Our findings contrast with an earlier report that IgG antibodies to *P. aeruginosa* may predate isolation of the organism from sputum. This may be due to differences in the frequency of sampling, the assay methods used, or the microbiological facilities available in the two centres. A major difference from the methods used in our study was the use of whole bacterial cell antigens derived from seven local strains which represented 85% of isolates of *P. aeruginosa* in their patients over a six month period. Each antigen was used independently to coat assay wells and the results from the seven determinations per sample were accumulated to derive an antibody titre. The normal range for their assay was derived from 17 patients. No intra-assay and interassay variation or other performance characteristics of the assay were quoted. In our assay a soluble extract of 17 standard serotypes containing 64 antigens was used. We used 32 healthy subjects to define a control range for antibody values. This gave a wide allowance for variation of antibody titres within this population, although the choice of such a cut off is fairly arbitrary and not directly comparable with other assay systems and healthy populations. It is possible that increases in antibody titre occurring before isolation of *P. aeruginosa* from the sputum, that have been reported by Brett et al, may be due to variation within the true normal range. An increased frequency of sampling would have helped to determine whether this was so and would have given a better defined profile for their patients. In addition, we were also unable to demonstrate a clear reduction in antibody titres after antibiotic treatment in the period after acquisition of infection. This might be expected, because a fall in antibody titre is only likely to occur after the long term eradication of *P. aeruginosa*, which seldom happens with traditional antibiotic regimens.

It was hoped that detection of antibodies before sputum culture became positive would allow the detection of infection, particularly in children too young to produce sputum samples, and could be used as an indicator of early infection at a time when aggressive anti-pseudomonal treatment might be beneficial. IgA antibodies may be a better indicator of early infection. A negative antibody titre in the presence of either chronic or intermittent isolation of the organism from sputum is difficult to interpret and is of no management value in patients unable to provide sputum. Absence of an antibody response in patients isolating the organism from their sputum may represent a period of less active injury, possibly because of a less intense host immune response or partly be due to better airway clearance mechanisms.

A positive titre of IgG antibody indicates a significant degree of exposure to *P. aeruginosa*, however, even if sputum culture is negative, intermittently positive, or unavailable. In this situation, specific antibiotic treatment could sensibly be instituted, particularly if there was a deterioration in respiratory function. This approach may be particularly applicable to children where early treatment of *P. aeruginosa* may reduce the rate of lung destruction, although more studies are needed to demonstrate any long term benefits. Combination of our assay system and bloodspot sampling provides a means of monitoring *P. aeruginosa* acquisition in children from an early age.

JSE and RJR were Cystic Fibrosis Research Trust Clinical Fellows and SMC was supported by the Cystic Fibrosis Research Trust.

1 Brett MM, Ghoneim ATM, Littlewood JM, Losowsky MS.
Food and stimulation are good for children

It is my purpose to draw your attention to an article published in the Lancet on 6 July 1991 (Grantham-McGregor et al, Lancet 1991;338:1–5). This paper describes research into the nutrition of stunted children in Jamaica. A house to house survey in Kingston identified 127 poor, undernourished, stunted children aged between 9 and 24 months all of whom were more than 2 SDs below the mean for height. These poor, deprived, undernourished children were allocated to four groups.

The first group were given no extra food or stimulation but they were visited once a week. The research workers considered giving this group a placebo but it was considered unethical to give them a low energy supplement, so they were given nothing. The second group were given extra psychosocial stimulation. That is, the mothers were taught how to play with their children and given toys for the purpose. The third group were given extra food and the fourth, both food and stimulation.

After two years those given nothing had deteriorated as regards developmental quotient. The groups given supplementation or stimulation alone showed modest benefit, and the group given both supplementation and stimulation showed clear benefit. Twelve months into the study the measurements of developmental quotient showed the control group to have deteriorated considerably but the study was continued for another year.

So feeding poor, deprived, undernourished children is good for them and giving them a more stimulating environment is also good for them. I thought you’d like to know that.

ARCHIVIST