Fecal Isolation of *Pseudomonas aeruginosa* from Patients with Cystic Fibrosis

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Fecal isolation of *Pseudomonas aeruginosa* was observed in 8 of 10 patients with cystic fibrosis who at the time of sampling also exhibited colonization of the respiratory tract. In contrast, *P. aeruginosa* cells were isolated at low frequency (9.1%) from the stools of 44 patients with cystic fibrosis with no previous history of chronic colonization. The results of this study suggest that the gastrointestinal tract is not a significant chronic reservoir of *P. aeruginosa* prior to pulmonary colonization.

A major factor responsible for morbidity and mortality in patients with cystic fibrosis (CF) is the effect of the disease on the lungs, in particular the sequelae of chronic colonization with *Pseudomonas aeruginosa* (9, 14). Initial asymptomatic colonization of the respiratory tract with typical nonmucoid *P. aeruginosa* is followed by phenotypic changes in the organism and subsequent intermittent episodes of exacerbation and progressive pulmonary deterioration (7, 13, 15). Early detection of *P. aeruginosa* is of considerable importance in determining the prognosis for patients with CF, planning therapeutic strategies, and understanding the etiology of pulmonary colonization. In addition, since the major virulence determinant associated with *P. aeruginosa* pathogenesis (derepressed alginate biosynthesis resulting in a mucoid colonial appearance) has been shown to be regulated by the chromosomal mutations *muc* and *alg* (2–4, 7, 12), it seems reasonable to suggest that primary colonization by typical nonmucoid *P. aeruginosa* provides an essential microbial reservoir from which the virulence-associated phenotype arises.

At present, the nature of the site(s) of early asymptomatic colonization remains obscure. Reports of upper respiratory tract colonization sites include maxillary sinuses (18), tongue, buccal mucosa, and saliva (11). Lindemann et al. (11) stressed the difficulty of identifying oral colonization and distinguishing it from sputum contamination because of the high concentration of bacteria in sputum. A number of studies in non-CF patients who are compromised by underlying disease or immune-suppressive therapy have indicated that fecal carriage of *P. aeruginosa* increases the risk of subsequent opportunistic infection (16, 20). There have been few reported studies (10, 17) of the incidence of fecal carriage of *P. aeruginosa* in patients with CF, perhaps reflecting the relative difficulty in obtaining compliance, and these reports provide few details of whether the isolates were mucoid or nonmucoid and no indication of the presence or absence of pulmonary colonization. We considered that a study of fecal carriage of *P. aeruginosa* might provide valuable information, particularly if the study included patients who at the time of investigation did not harbor *P. aeruginosa* in their respiratory tracts.

MATERIALS AND METHODS

**Study group.** The study group comprised 54 patients with CF who regularly attend the Edinburgh CF clinics. The investigation was carried out during the period from February to September 1987. All patients had received antibiotic therapy on previous occasions, but no patient was receiving antibiotic treatment at the time of sampling. The age range of the patients was 2 months to 33 years. At the time of sampling, 10 patients were known to be chronically colonized in the upper respiratory tract by mucoid *P. aeruginosa*.

Informed consent was obtained from the parent or guardian of each participant and, when appropriate, from the subjects themselves.

**Bacteriology.** Stools were mixed with an equal volume of sterile physiological saline and emulsified by vortexing. Fecal emulsion (0.1 ml) was spread onto the surface of *Pseudomonas* isolation agar (PIA; Difco Laboratories, Detroit, Mich.) and incubated at 37°C for 48 h; a further 0.1 ml was inoculated into acetamide enrichment broth (10), and after 24 h of incubation at 37°C, a loopful was removed from the enrichment broth, plated onto PIA, and incubated as described before. Expectorated sputum was obtained after chest physiotherapy, and after liquefaction with sputolysin (Behring Diagnostics, La Jolla, Calif.), quantitative culture for *P. aeruginosa* was carried out by plating 0.1-ml volumes of diluted homogenized sputum onto PIA. *P. aeruginosa* was identified by characteristic features, including production of pyocyanin; when necessary, identification of nonpigmented isolates growing on PIA was by a positive oxidase reaction and identity as *P. aeruginosa* was confirmed by the API 20NE system (API-bioMérieux, Vercieu, France). Ten representative colonies from each *P. aeruginosa*-positive specimen were further characterized by pyocin typing by using a technique suitable for mucoid variants (5).

**RESULTS**

Ten patients were known to exhibit chronic pulmonary colonization by mucoid and nonmucoid *P. aeruginosa* prior to this investigation. In the study, colonization in these patients was confirmed and ranged from $10^6$ to $10^8$ CFU/ml of homogenized sputum. Concurrently, *P. aeruginosa*-positive fecal cultures were obtained from eight of these patients and of these eight *P. aeruginosa*-positive stools, six samples harbored mucoid variants. In each case, the *P. aeruginosa*
isolated from the stool was found to belong to the same pyocin type as that present in the sputum. Of the four patients who were known to exhibit intermittent pulmonary colonization by nonmucoid P. aeruginosa, but whose sputum did not contain P. aeruginosa at the time of stool collection, three harbored nonmucoid P. aeruginosa in their stools. Forty patients had shown no previous history of respiratory colonization by P. aeruginosa, and sputum from these patients was confirmed as P. aeruginosa-free at the time of stool culture. From this collection of 40 stool samples, P. aeruginosa (nonmucoid) was isolated from only one specimen. In stools and sputum found to be P. aeruginosa positive, pyocin typing of 10 colonies from each primary plate did not produce evidence of multiple colonization with more than one strain of P. aeruginosa.

DISCUSSION

Rates of fecal carriage of P. aeruginosa differ widely in published reports and illustrate different carriage rates in different populations (16). In most studies, however, there is general agreement that carriage rates are less than 10% in healthy subjects but may rise to greater than 40% in hospitalized patients (1, 16). In a typical study, Yoshioka et al. (19) found the carriage rate of P. aeruginosa in infants delivered at home to be 6% compared with 57% in hospital-born infants. Fecal carriage rates also showed a seasonal variation, ranging in healthy adults from 4.7% in April to 43% in July.

To our knowledge, there has been no attempt to focus on the possibility of gastrointestinal carriage of nonmucoid P. aeruginosa as a reservoir for subsequent pulmonary colonization in patients with CF. Kelly et al. (10) reported 9 isolations of P. aeruginosa from 34 fecal samples but did not indicate whether isolates were mucoid or whether the patients had concurrent pulmonary colonization. Roy et al. (17) investigated the fecal flora of 23 CF patients and found that 7 patients had P. aeruginosa in their stools, 6 of whom were on antibiotics at the time; with one exception, the P. aeruginosa isolates were mucoid. In our study, the demonstration of simultaneous fecal carriage of mucoid P. aeruginosa belonging to the same pyocin type in 8 of the 10 patients with concurrent pulmonary colonization enhances the evidence of Roy et al. (17) and suggests a considerable degree of intestinal contamination with mucoid P. aeruginosa from the respiratory tract. This hypothesis is supported by the fact that mucoid forms of P. aeruginosa are rarely found in P. aeruginosa-positive stools in non-CF patients nor, in our experience, from the stools of patients with CF in the absence of pulmonary colonization. If the gastrointestinal tract is an important reservoir preceding pulmonary colonization, it would be reasonable to argue that fecal carriage in CF patients would be relatively high compared with that found in healthy individuals. This was not observed in the present study, which showed a fecal isolation rate (9.1%) within the normal range in those patients not exhibiting chronic pulmonary colonization. The relatively low isolation rate could not be attributed to culture techniques in view of the high incidence achieved concurrently in those patients with pulmonary colonization. Similarly, since stool cultures were investigated over a 6-month period from February to September, it seems unlikely that a seasonal variation, as reported by Yoshioka et al. (19), would exert a significant effect. Studies of fecal isolation rates in individual CF centers may be influenced by antibiotic usage as well as by the incidence of pulmonary P. aeruginosa colonization which is relatively low in the Edinburgh clinics (8). We deliberately excluded patients on current antibiotic therapy from this study to avoid any suppression of isolation rate by residual antibiotic present in the stools; all patients, however, had received antibiotic therapy on previous occasions. In CF and non-CF patients, colonization of the individual sputum and stools by more than one strain of P. aeruginosa has been reported (6, 7) and ranges from 3 to 15%; multiple colonization is more common in hospitalized patients than in patients treated at home. We took this factor into account in the present study but found no evidence of multiple colonization in the P. aeruginosa-positive specimens investigated in this study.

The four patients from whom non-mucoid P. aeruginosa had been isolated intermittently in the previous 3 years provided an interesting and unexpected result. At the time of sampling, three of these patients harbored nonmucoid P. aeruginosa in their stools, although P. aeruginosa was not cultured from their sputum. It is arguable that these patients reflect natural carriage or perhaps represent a small subpopulation of CF patients in whom intermittent stool carriage of nonmucoid P. aeruginosa precedes pulmonary carriage or vice versa. The future isolation of P. aeruginosa from such patients will be followed with interest. From the present study, we conclude that fecal contamination by P. aeruginosa resulting from pulmonary colonization occurs in the majority of patients with CF and that in the CF population investigated in this study the gastrointestinal tract does not form an important chronic reservoir of nonmucoid P. aeruginosa prior to colonization of the respiratory tract.

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LITERATURE CITED