The incidence of anaerobes in the sputum of patients with cystic fibrosis

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Summary. The number of anaerobes in selected sputum samples from patients with cystic fibrosis (CF) was investigated. When cultured by a semi-quantitative method, 26 (23.85%) of 109 sputum specimens from 21 CF patients contained $>10^5$ cfu of anaerobic organisms/ml. Nine (42.7%) of the 21 patients produced sputum containing such concentrations of anaerobes on at least one occasion. Anaerobes were isolated from repeated sputum specimens from five patients. The anaerobes most often isolated were *Bacteroides disiens*, pigmented *Bacteroides* spp. and anaerobic gram-positive cocci. Anaerobes were isolated more often from sputum liquefied by sonication than from unliquefied sputum, suggesting that they were unlikely to be oropharyngeal contaminants.

Introduction

Cystic fibrosis (CF), the commonest genetically inherited disease among Caucasian children, is characterised by abnormal bronchial mucus secretion leading to chronic bacterial pulmonary infection and progressive respiratory dysfunction which is nearly always fatal. Progression of the pulmonary disease may be slowed by intensive physiotherapy and treatment of acute exacerbations of infection with antimicrobial agents directed against the pathogens most frequently isolated from CF patients, i.e., in the early years *Staphylococcus aureus* and *Haemophilus influenzae* with *Pseudomonas aeruginosa* as a later acquisition (Smith, 1986). However, other organisms have been associated with infection in CF, including *Streptococcus pneumoniae*, enterobacteria, viruses, fungi, mycoplasmas and chlamydiae.

In recent years, several workers have assessed the role of anaerobes in pulmonary infection in CF. Brook and Fink (1983) and Thomassen *et al.* (1984) reported the isolation of anaerobes from transtracheal and thoracotomy specimens (lung biopsies and parenchymal aspirates) from CF patients. The importance of anaerobes in other types of pulmonary infection in children is well recognised, especially in the USA (Bartlett and Finegold, 1974; Brook and Finegold, 1980). In the present study, the number of anaerobes in the sputum of CF patients was measured by use of a semi-quantitative method, similar to that described by Thomassen *et al.* (1984).

Materials and methods

Specimen collection

During 7 months, 109 good quality sputum specimens were obtained from 21 CF patients, aged 5–17 years (mean 5.2 specimens/patient, range 1–17 specimens/patient). All specimens were obtained under the supervision of a physiotherapist at routine outpatient attendances or during admission for treatment of acute exacerbations of infection. Specimens from inpatients were obtained on admission and twice weekly thereafter.

Bacteriological examination

Macroscopically non-purulent specimens were discarded. The remainder were examined as soon as possible, usually between 30 min and 3 h after collection. By examining a gram-stained film, the number of pus cells (polymorphonuclear leucocytes) and epithelial cells in each specimen was determined. Cultures were made from specimens with >10 pus cells and less than five epithelial cells per high power field ($\times 400$) only. This resulted in a total of 109 specimens being cultured.

Cultures were made initially from untreated, undiluted samples, selecting the most purulent part of the specimen. The specimens were then liquefied by sonication, with an MSE sonicator (Scientific Instruments, Sussex Manor Park, Crawley, West Sussex) for 10 s at an amplitude of 12 μ m and diluted 1 in 2, 1 in 20 and 1 in 100 in normal saline. Cultures were then made from each of these dilutions by seeding by standard streaking one loopful (0.01 ml) on to a plate of Fastidious Anaerobe Agar (Lab M, Topley House, PO Box 19, Bury, Lancs) supplemented with lysed horse blood 5%. A metronidazole 5- μ g disk was placed in the inoculum well and the plates were incubated anaerobically for 7 days at 37°C in an

atmosphere of N₂ 80%, CO₂ 10%, and H₂ 10% in an anaerobic jar.

Anaerobes were recognised by a zone of inhibition around the metronidazole disk. Only anaerobes growing from the 1 in 100 dilution (indicating concentrations of > 10⁵ cfu/ml of sputum) were considered to be of possible significance. Anaerobes growing only from the lower dilutions were disregarded as probable oral contaminants.

Gram-negative anaerobic bacilli were identified by tests of dye and bile tolerance, pigment production, antibiotic resistance, sugar fermentation, indole production, gelatin digestion and aesculin hydrolysis (Duerden *et al.*, 1980). Anaerobic gram-positive cocci were not identified further and anaerobic gram-negative cocci were assumed to be veillonellae. Anaerobic gram-positive bacilli were not isolated.

Results

Anaerobes were isolated in numbers greater than 10⁵ cfu/ml from 26 (23.85%) of the 109 specimens. They were isolated from nine (42.7%) of the 21 patients on at least one occasion.

The anaerobic species isolated are shown in table I. Those most commonly isolated were *Bacteroides disiens* and anaerobic gram-positive cocci (eight isolates each). Pigmented *Bacteroides* spp. were found in seven samples (*B. intermedius*, two; *B. asaccharolyticus*, three; unidentified, two). One specimen contained two types of anaerobe (*B. asaccharolyticus* and an anaerobic coccus) and one contained three (*B. disiens*, *B. ureolyticus* and a veillonella).

Of the 26 specimens containing anaerobes in numbers greater than 10⁵ cfu/ml, 16 were taken during acute exacerbations and 10 during routine outpatient attendances. Four or more specimens were taken from only 10 of the 21 patients.

Table I. Anaerobes isolated from the sputum of CF patients

Organism	Number of isolates	Number of patients
<i>B. disiens</i>	8	3
<i>B. intermedius</i>	2	1
<i>B. ureolyticus</i>	2	2
<i>B. oralis</i>	2	2
<i>B. asaccharolyticus</i>	3	2
Unidentified pigmented <i>Bacteroides</i> spp.	2	1
Anaerobic gram-positive cocci	8	5
Veillonellae	2	2
Total	29*	

*One specimen yielded three different isolates and another, two.

Anaerobes in numbers greater than 10⁵ cfu/ml were isolated from repeated specimens from five of the 10, and the same *Bacteroides* spp. was present in more than one specimen from three of the five (table II).

It was particularly noteworthy that anaerobes were not isolated readily from undiluted, unliquefied sputum. Of the 29 isolates of anaerobes from 26 sputa, only four (13.8%) grew heavily from undiluted sputum, as well as from sputum diluted up to 1 in 100. Thirteen (44.8%) of the isolates were not isolated from undiluted specimens; they grew on plates inoculated with the sonicated and diluted specimens only. A further 12 isolates (41.3%) grew scantily in cultures of undiluted sputum, but more profusely when the specimens had been liquefied and diluted.

Discussion

Most studies of anaerobic pulmonary infection have been made in the USA, where examination of sputum collected by transtracheal puncture or at bronchoscopy have shown rates of such infection ranging from 21 to 35% for both community and hospital acquired pneumonia (Ries *et al.*, 1974; Pollack *et al.*, 1983; Bartlett *et al.*, 1986). In the UK, anaerobes are considered to be of significance in certain pulmonary conditions only, such as bronchiectasis, lung abscess and aspiration pneumonia. Because bronchiectasis occurs in CF, the presence of anaerobes in the lungs of such patients is to be expected. Thus, Brook and Fink (1983) isolated anaerobes from four of six transtracheal aspirates from patients with CF. Thomassen *et al.* (1984) found anaerobes in numbers greater than 10⁵ cfu/ml in two of 10 such patients from whom both sputum and thoracotomy specimens were cultured anaerobically. Using a semi-quantitative technique, they found excellent correlation between the numbers of anaerobes isolated from both types of specimen. Based on the principle that bacterial infections of the lower respiratory tract are almost invariably associated with bacterial concentrations greater than 10⁵ cfu/ml of secretions, other workers also have found that quantitative culture minimises false positive results from oral contamination in the diagnosis of anaerobic lung infection (Bartlett and Finegold, 1978; Winterbauer *et al.*, 1983; Winterbauer, 1985).

In the present study, the likelihood that the anaerobes cultured were from the mouth was reduced by culturing only specimens minimally contaminated with saliva, as assessed by examination of a gram-stained film, and by quantitative

Table II. Patients from whom anaerobes were isolated more than once

Patient no.	Age	Number of specimens		Date of collection	Anaerobes isolated
		taken	positive for anaerobes		
1	17	17	6	15/3	<i>B. intermedius</i>
				10/3	<i>B. asaccharolyticus</i>
				30/3	Pigmented bacteroides*
				18/5	<i>B. intermedius</i>
				23/7	<i>B. asaccharolyticus</i> plus anaerobic coccus
				14/8	Pigmented bacteroides*
2	10	6	5	12/6	<i>B. disiens</i>
				15/6	<i>B. disiens</i>
				17/6	<i>B. disiens</i>
				18/6	<i>B. disiens</i>
				13/7	<i>B. disiens</i>
3	7	5	2	9/4	Anaerobic coccus
				13/4	Anaerobic coccus
4	11	14	5	27/4	Anaerobic coccus
				18/5	<i>B. disiens</i>
				19/5	Veillonella
				3/8	Anaerobic coccus
5	14	8	3	24/8	<i>B. disiens</i>
				14/7	Anaerobic coccus
				27/7	Anaerobic coccus
				7/9	<i>B. ureolyticus</i>

*Did not survive subculture.

culture to detect bacterial concentrations greater than 10^5 cfu/ml of sputum. It is noteworthy that organisms growing in such concentrations were often detected after sonication and dilution only, suggesting either that they were sequestered deep within the viscid mucus produced by patients with CF, or within the pus cells, and supporting the view that they were derived from the lungs rather than from the mouth.

Anaerobes were cultured from 23.85% of all processed specimens and at least once from nine of the 21 patients. The significance of this is difficult to determine, especially as only occasional specimens were taken from most patients. Anaerobes were isolated more than once from the sputum of five of the 10 patients from whom multiple specimens were taken. Different anaerobic species were isolated from three of the five on different occasions; it is, therefore, difficult to attribute much importance to these isolates. On the other hand, the same single *Bacteroides* spp. was isolated on five consecutive occasions from one patient, suggesting that this may have been clinically important.

The anaerobes isolated were, as expected, those

likely to be found colonising the oropharynx and were broadly similar to those found in other studies of anaerobic pulmonary infection (Bartlett, 1987), in which pigmented *Bacteroides* spp. and anaerobic cocci are commonest. However, fusobacteria were common in other studies but none was found here. Similarly, *B. fragilis* was not isolated in this study, whereas in other studies, small but significant numbers have been found.

The pathogenic potential of anaerobes cannot be assessed from this study. Most of the antimicrobial drugs being taken by nearly all the patients when the specimens were collected would have had some activity against at least some of the anaerobes isolated, but whether this activity increases the patients' response to such chemotherapy is impossible to assess. However, the observation that anaerobes were isolated as often from patients attending clinic in a quiescent phase of the disease as from those admitted with acute exacerbations suggests a colonising rather than a pathogenic role. This, and the value of specific anti-anaerobe antibiotic treatment in CF require further investigation.

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