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Mycobacterial isolations in young adults with cystic fibrosis

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ABSTRACT In 223 patients admitted to hospital with cystic fibrosis mycobacteria were found in the sputa of seven. All of these cases were identified over a six year period after the introduction of routine examination and culture of sputum for acid fast bacilli in patients with cystic fibrosis. The organisms isolated were *Mycobacterium tuberculosis* in three patients, *M chelonei* in one, *M fortuitum* in one, and unidentified mycobacteria in two. The diagnosis was not suspected on clinical grounds in any of the cases; in one patient, however, night sweats were a prominent feature before diagnosis. In four of the patients direct sputum smear examination did not reveal the organism, which was grown subsequently in culture. An unusual phenomenon of liquefaction of the Lowenstein-Jensen culture medium was encountered in five of the seven patients described, which in one case made identification and sensitivity testing of the organism impossible. This phenomenon has been observed in sputum cultures from other patients with cystic fibrosis but not in other pulmonary diseases. Immunological studies performed in three of the patients showed normal numbers of peripheral blood T and B lymphocyte in all three; in vitro lymphocyte transformation to tuberculin PPD was, however, reduced in the patient with extensive *M fortuitum* infection, which proved fatal. Mycobacteria may be present in the sputa of patients with cystic fibrosis more often than previously recognised and therefore sputum examination and culture for mycobacteria should be performed periodically in these patients.

Mycobacterial infections are apparently rare in cystic fibrosis.[1] In a series of over 700 patients with cystic fibrosis Wood *et al*[1] found only two cases of active pulmonary tuberculosis during 18 years. Three more of their patients had *Mycobacterium fortuitum* isolated from their sputa and one of these subsequently died. In none of them, however, was a conclusive diagnosis of *M fortuitum* infection established. Boxerbaum[2] reported one case of fatal infection with *M chelonei* in a patient with cystic fibrosis, the diagnosis being confirmed at necropsy. He found seven other patients with cystic fibrosis in a series of 460 from whom rapidly growing mycobacteria (Runyon group IV) were isolated; these, however, were thought to be casual isolations.

A clinical diagnosis of mycobacterial infection is extremely difficult in these patients in view of their longstanding respiratory symptoms, frequent infective exacerbations, and pre-existing, often extensive radiographic abnormalities. The diagnosis is usually not suspected until mycobacteria are seen in or cultured from sputum. In these circumstances the distinction between infection, casual isolation, and (in the case of non-tuberculous mycobacteria) saprophytic colonisation is difficult. We have reviewed our experience of patients with cystic fibrosis who have had mycobacteria isolated from their sputa.

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Methods

Over a period of 16 years 286 patients with cystic fibrosis have attended our hospital for treatment and follow up. Most of this population are adults with a mean age of 22 years (range 11–50 years). During the first 10 years there were no mycobacterial isolations. In the last six years, however, our policy has been to examine sputum periodically for acid fast bacilli from all patients with cystic fibrosis admitted to hospital (223 patients). During these years

mycobacteria have been isolated from the sputum in seven of these patients. In three of them the organism was *M tuberculosis*, in three non-tuberculous mycobacteria were isolated, and in one patient the organism remained unidentified. Identification of the organisms and in vitro sensitivity testing was performed in the hospital tuberculosis laboratory with standard techniques.[3] Three of the patients also underwent immunological investigation. Total numbers of circulating T lymphocytes were determined on the basis of spontaneous rosette formation with sheep erythrocytes at 4°C[4] and total numbers of circulating B lymphocytes by the demonstration of surface immunoglobulin.[4] Lymphocyte function was also assessed in these patients by assaying in vitro lymphocyte transformation in response to phytohaemagglutinin (PHA) and tuberculin purified protein derivative (PPD) by the method described by Barnes *et al*.[5] In two patients with isolations of non-tuberculous mycobacteria differential skin tests were performed with PPD derived from *M chelonei* and *M fortuitum*. We reviewed the clinical, bacteriological, and immunological data on these patients.

Results

CLINICAL FEATURES

The mean age of the patients at the time of isolation of the mycobacteria was 22·6 years (range 18–36 years). All of the patients had well documented cystic fibrosis, with a sweat sodium concentration of over 70 mmol (mEq)/l. Four of them were diagnosed in infancy, one at 5 years, one at 12 years, and one at 35 years of age. All had a long history of cough and sputum production and all but one had suffered from recurrent chest infections from childhood.

The presenting symptoms and radiographic abnormalities at the time of mycobacterial isolation are shown in table 1. Six of the seven patients experienced an increase in cough and sputum production before the isolation of mycobacteria. In five of them the duration of change in symptoms was relatively short, ranging from two weeks to four months. One patient, however, had a two year history of increase in cough and sputum production with weight loss; night sweats were a prominent feature before the isolation of mycobacteria. Two patients had a history of recurrent pneumothoraces. In the first patient pneumothorax occurred simultaneously with the isolation of mycobacteria from his sputum, and in the second recurrent pneumothoraces antedated the isolation of mycobacteria by 12 months. Both patients required surgical pleurodesis.

CHEST RADIOGRAPHS

Before the isolation of mycobacteria all of the patients had abnormalities of varying extent on their chest radiographs consistent with cystic fibrosis (table 1). These included ring shadows, parallel line shadows, nodular shadowing, and atelectasis. In five of the patients definite new radiological abnormalities were present at the time of isolation of mycobacteria.

Patient 1 had recently developed evidence of allergic bronchopulmonary aspergillosis presenting with asthma, considerable blood eosinophilia, and positive immediate and late skin reactions to aspergillus protein. One month after starting oral cor-

Table 1 *Clinical date on the seven patients*

Patient No	*Sex*	*Age**	*Presenting symptoms and duration (m)*	*Previous radiographic abnormality*	*Change in radiograph**
1	M	17	Cough, sputum; dyspnoea (1)	Linear shadows both upper zones, few small ring shadows, irregular shadowing both lung fields	Cavity and infiltration left upper zone, left pneumothorax
2	F	20	No change	Widespread ill defined nodular shadowing, some bronchial wall thickening	No change
3	M	19	Cough, sputum (2)	†	Shadowing right upper zone, contracted right upper lobe, parallel line shadows and ring shadows bilaterally
4	M	18	Cough, sputum (4)	Generalised bronchial wall thickening, some parallel line and ring shadows	Widespread infiltration right upper lobe
5	F	36	Pleurisy, sputum (½)	Bilateral upper lobe contraction and some ill defined shadowing left upper zone	Consolidation right base with some proximal infiltration
6	F	19	Cough, sputum (½)	Widespread poorly defined nodular shadowing	Considerable generalised increase in nodular shadowing
7	F	29	Cough, sputum, weight loss (24); night sweats (12)	Ill defined shadowing both upper zones with bronchial wall thickening and some ring shadows	Cavity left apex with progressive widespread large ring shadow formation

*At time of isolation of mycobacteria.
†No previous recent film for comparison.

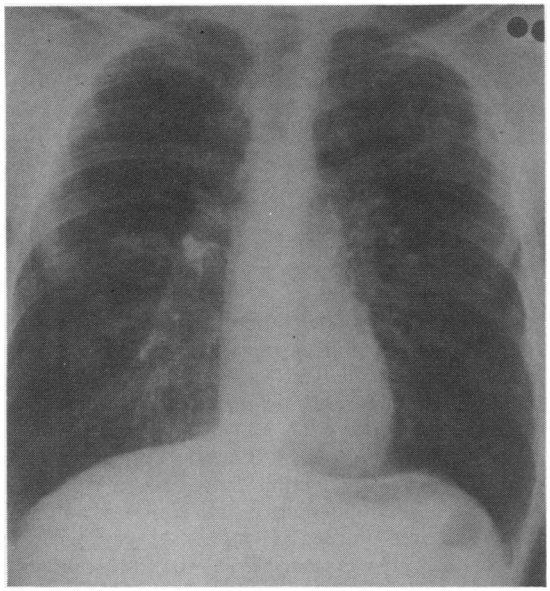

Fig 1 *Chest radiograph of patient 1 showing a new cavitated shadow in the left upper zone.*

ticosteroid treatment a new cavitated shadow appeared in the left upper zone (fig 1). The patient developed a left pneumothorax two weeks later. Patient 4 developed diffuse infiltration confined to the right upper zone, which persisted for five months until anti tuberculosis treatment was started. Patient 5 developed symptoms of right sided pleurisy, which was accompanied by new shadowing and a pleural reaction at the right base. In patient 6 existing widespread nodular shadowing became much more prominent, particularly in the left mid zone, at the time of mycobacterial isolation. Another patient (case 7) had existing bilateral apical shadowing and diffuse ring shadows but showed dramatic increase in these changes during the two years before mycobacteria were isolated (fig 2). This corresponded to the history of increase in cough and sputum, weight loss, and night sweats, which were initially thought to be compatible with cystic fibrosis. In one patient no previous radiographs were available for comparison as he had been recently referred to the unit, and in one patient (case 2) no significant change in the chest radiograph was apparent.

Apical disease was a prominent feature in five of the seven patients, although generalised changes were also present in all seven. In four of these five patients the upper lobes were contracted with hilar elevation, which was bilateral in three and right sided in one. There was cavitation of the upper lobes in two cases.

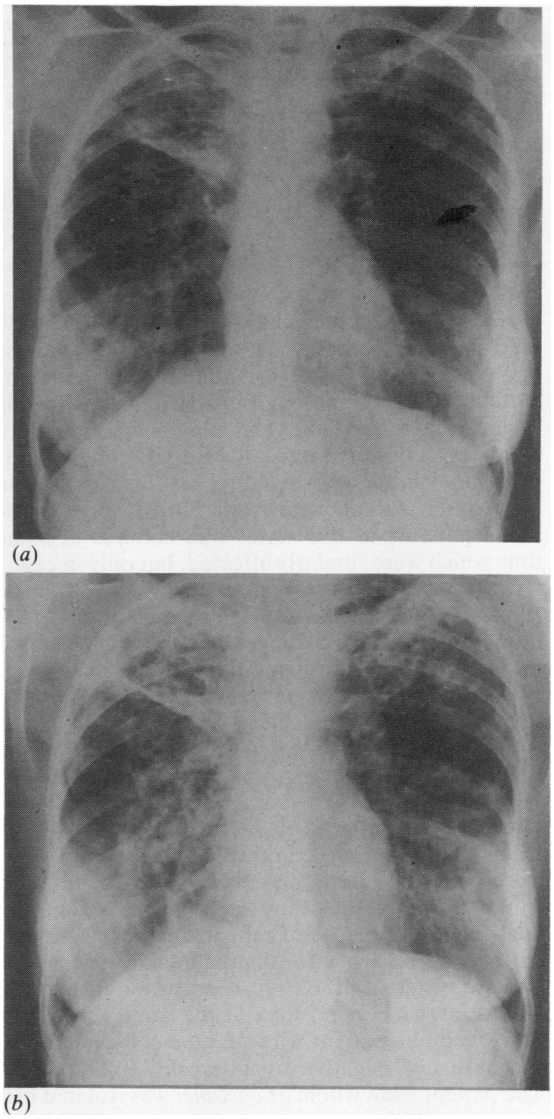

Fig 2 *Chest radiographs of patient 7 (a) two years before isolation of Mycobacterium fortuitum and (b) at the time of isolation.*

BACTERIOLOGY

M tuberculosis was isolated from three of the seven patients. Non-tuberculous mycobacteria were isolated from three more—namely, *M fortuitum* in one, *M chelonei* in one, and an unclassified mycobacterium in the third patient. In the final patient it was not possible to identify the organism in culture despite the finding of large numbers of acid fast bacilli on direct smear examination on many occasions. The cultures from this patient were repeatedly

Table 2 *Isolations of mycobacteria and culture contamination in patients with cystic fibrosis*

Patient No	Organism	No of positive		Contaminated cultures (No)
		smears	cultures	
1	Unidentified	13	0	25
2	*M tuberculosis*	0	1	1
3	Non-tuberculous mycobacterium (unclassified)	0	1	3
4	*M tuberculosis*	0	2	0
5	*M tuberculosis*	0	1	0
6	*M chelonei*	6	3	3
7	*M fortuitum*	15	18	4

reported as "contaminated" and did not yield mycobacterial colonies. This did not appear to be due to the usual type of contamination with, for instance, fungi but was characterised by liquefaction of the culture medium. This phenomenon has not been encountered in our laboratory in other conditions, but has been observed frequently with sputum from other patients with cystic fibrosis. Five of the seven patients in this series had cultures at some time which were similarly affected, but only in case 1 was there a complete failure to culture the organism (table 2). Acid fast bacilli were seen on direct smear in only three of the patients, two of whom also had multiple positive cultures. Of the four smear negative patients, three had only single positive cultures; two of these, however, were *M tuberculosis* and in the other there was repeated "contamination" of the cultures.

SKIN TESTS AND IMMUNOLOGICAL STUDIES

Delayed intradermal skin tests with tuberculin PPD were performed in four of the seven patients (table 3). In patient 1, in whom the mycobacterium was not identified because of repeated culture contamination, the response to tuberculin skin testing was initially negative at 1/1000 concentration, but one month later it was positive at the same concentration. Another patient with *M tuberculosis* (case 5) was skin test negative to tuberculin PPD 1/1000. The patient from whom *M chelonei* was isolated had a negative skin test reaction to tuberculin PPD but a strongly positive reaction to purified extract derived from *M chelonei*. The reactions to skin tests with tuberculin PPD and extracts derived from *M fortuitum* and *M chelonei* were all negative in the patient from whom *M fortuitum* was repeatedly isolated.

Numbers of peripheral blood T and B lymphocytes and in vitro lymphocyte transformation in response to phytohaemagglutinin were normal in all three patients studied. Lymphocyte responses to tuberculin PPD were normal in two of these patients (cases 1 and 5) but reduced in the patient with *M fortuitum* infection (case 7).

CLINICAL SIGNIFICANCE AND RESPONSE TO TREATMENT

Details of in vitro drug sensitivities, treatment, and response are shown in table 4.

Two of the patients from whom *M tuberculosis* was isolated (cases 4 and 5) had changes in both symptoms and chest radiographs before the isolation of the mycobacteria. They have both been treated with rifampicin, isoniazid, and ethambutol. The first patient completed 24 months' treatment of rifampicin and isoniazid (with ethambutol for the first two months), with clinical and radiographic improvement, and has remained sputum negative. The second has been receiving treatment for three months and is progressing satisfactorily. The other patient with a single culture positive for *M tuberculosis* (case 2) had no change in symptoms or chest radiograph. This patient is still under assessment and has received no treatment. There has been no change in

Table 3 *Results of delayed skin tests and lymphocyte studies in four patients*

Patient No	Organism	Skin test		Peripheral blood T and B cell counts	In vitro lymphocyte transformation	
		Tuberculin PPD	PPD-C* PPD-F		PHA	PPD
1	Unidentified	−, +	ND	Normal	Normal	Normal
5	*M tuberculosis*	−	ND	Normal	Normal	Normal
6	*M chelonei*	−	PPD-C +	ND	ND	ND
7	*M fortuitum*	−	PPD-C − PPD-F −	Normal	Normal	Reduced

ND—test not done; + = positive, − = negative.
*PPD-C—PPD derived from *M chelonei*; PPD-F—PPD derived from *M fortuitum*.

Table 4 *Drug sensitivities, treatment, and response*

Patient No	*Organism*	*In vitro* drug resistance (R) and sensitivity (S)*	*Drugs given and duration*	*Radiographic response*	*Clinical response*
1	Unidentified		Ri, I (30 m), S (6 w)	Cavity closure	Sputum negative after 5 m. Died during treatment
2	*M tuberculosis*	Fully sensitive	Nil	No change	Stable
3	Non-tuberculous (unclassified)	R to all but cycloserine	Nil	Progression of multiple cavities right upper zone	Some deterioration
4	*M tuberculosis*	Fully sensitive	Ri, I, E (in progress 3 m)	Some resolution	Stable with treatment
5	*M tuberculosis*	S to all but pyrazinamide	Ri, I (24 m), E (2 m)	Pleurisy resolved, apical shadowing improved	Stable with treatment
6	*M chelonei*	R to all drugs	Ri, I plus amikacin, erythromycin (in progress 4 m)	Some resolution	Stable with treatment
7	*M fortuitum*	R to all drugs	Ri, I, E, pyrazinamide, cycloserine (5 m) plus amikacin, erythromycin (3 m)	Rapid progression	Sputum positive throughout. Died during treatment

*Drugs tested against rifampicin (Ri), isoniazid (I), ethambutol (E), streptomycin (S), *p*-aminosalicylic acid, ethionamide, pyrazinamide, cycloserine, TBI, capreomycin, kanamycin.

the radiograph and no recurrence of acid fast bacilli in the sputum.

The mycobacterium in case 1 remained unidentified, but in view of the recurrent isolations, change in symptoms, and cavitated new shadow in the left upper zone the patient was treated with rifampicin and isoniazid for 30 months and streptomycin for the first six weeks. Treatment was followed by initial clinical improvement. The sputum became smear negative after five months and there was eventual closure of the cavity. This, together with conversion of the tuberculin skin test after one month, suggested that the organism may well have been *M tuberculosis*. Unfortunately, the patient subsequently developed recurrent, severe infective exacerbations with *Pseudomonas aeruginosa* and died while still receiving antimycobacterial treatment.

In the two patients from whom *M chelonei* and *M fortuitum* respectively were isolated, the organisms were found on many occasions on direct sputum examination and multiple positive cultures were obtained. Both organisms were resistant in vitro to all antituberculous drugs tested. In retrospect, it is likely that patient 7 had had active *M fortuitum* infection for over two years before the organism was isolated, but the symptoms and radiographic changes during this time were quite compatible with the natural history of lung disease in cystic fibrosis alone. This patient failed to respond to multiple chemotherapy including amikacin and erythromycin (table 4) and died after five months while still receiving treatment. The diagnosis of mycobacterial infection was confirmed at necropsy. The patient with *M chelonei* isolations presented to us recently and after three months of chemotherapy with a combination of rifampicin, isoniazid, amikacin, and erythromycin there has been definite clearing of the widespread nodular shadowing on the chest radiograph accompanied by clinical improvement.

The final patient (case 3) had a single positive culture containing a non-tuberculous mycobacterium (unclassified). This patient presented with increase in cough and sputum at the time of isolation but no previous chest radiographs were available for comparison. He has received no antimycobacterial treatment and there has been no recurrence of mycobacteria in his sputum during two years. Follow up chest radiographs, however, show an appreciable increase in the right upper zone shadowing with further contraction and ring shadow formation in the right upper lobe.

Discussion

Pulmonary mycobacterial infection is a rare but potentially serious complication of cystic fibrosis.[1 2] The chronic pulmonary disease associated with this condition may in itself predispose to mycobacterial infection and other factors present in many of these patients, such as poor nutritional state, diabetes mellitus, and steroid treatment, may add to this risk. The diagnosis may easily be missed clinically and is usually discovered first in the microbiology laboratory. The first major problem after the isolation of mycobacteria from the sputum of a patient with cystic fibrosis is determining the clinical significance. In normal circumstances the diagnosis of significant infection would be based on a combination of clinical, radiographic, and bacteriological findings: for example, development of new respiratory symptoms or increase in existing ones, repeated isolations of the organism, radiological appearances consistent with mycobacterial infection, and a positive

response to chemotherapy. All of these developments, however, may be confounded in an adult patient with cystic fibrosis. Chronic cough and sputum production are almost universal and infective exacerbations are common. Systemic symptoms of fatigue, malaise, weight loss, and fever are also very common in cystic fibrosis. Patients rarely complain of night sweats, however, and this was a dominant symptom in one of our patients with mycobacterial infection. Night sweats may therefore be an important discriminating symptom in patients with cystic fibrosis who develop mycobacterial infection.

The existing chest radiographic abnormalities in adult patients with cystic fibrosis are often quite extensive, making it difficult to assess any new shadowing related to mycobacterial infection. For the same reason the chest radiograph cannot readily be used as an indication of response to treatment. Useful information can still be obtained from the chest radiograph, however, and definite radiographic changes occurring in relation to mycobacterial isolation were present in three of our patients. The predominance of apical disease in five of the patients is of interest and is a common feature of mycobacterial infection in other circumstances. Several reports of the radiographic findings in young adults with cystic fibrosis, however, have noted that the disease affected predominantly upper lobes.[6] The reason for this pattern of distribution remains obscure. The association between cystic fibrosis and allergic bronchopulmonary aspergillosis, another disease which may predominantly affect the upper lobes, is well recognised[7 8] and one of our patients (case 1) fulfilled the criteria for this diagnosis.[9]

Perhaps the most important criterion for the diagnosis of mycobacterial infection is the repeated isolation of the organism from the sputum. The three patients in this series from whom *M tuberculosis* was isolated were all smear negative and only single positive cultures were obtained in two of them; but, as *M tuberculosis* is the only culturable mycobacterial species that has no known free living saprophytic forms, all clinical isolates of this organism should probably be regarded as significant.[10] This is not the case with the other mycobacterial species and prolonged saprophytic colonisation of the respiratory tract with the rapidly growing *M fortuitum* and *chelonei* organisms has been described.[11] On the other hand, in a series of nine patients with histologically proved pulmonary lesions due to *M chelonei,* organisms were scanty and often absent from the sputum.[12] The observation that sputum from many patients with cystic fibrosis appears to have a deleterious effect on Lowenstein-Jensen medium, often destroying the culture, is of considerable importance and prevents both identification of the organism and sensitivity testing. In view of this the actual incidence of mycobacterial isolates from these patients is unknown and may be higher than recorded. It is tempting to speculate that this phenomenon may indicate the presence of substances in the sputum of some patients with cystic fibrosis—for example, proteases—which could directly damage lung tissue. We are currently investigating this phenomenon further to establish its cause and possibly develop a better method of culturing specimens from patients with cystic fibrosis for mycobacteria.

Assessment of the response to treatment is again complicated in these patients in view of the natural history of the condition. In the two cases where there was definite evidence of infection with fully sensitive organisms (*M tuberculosis*) satisfactory bacteriological and radiographic responses were obtained with standard first line chemotherapy. The optimum duration of chemotherapy is not known in such patients. It has been shown that in some bacterial infections in patients with cystic fibrosis alveolar macrophages may contain live bacilli which are able to replicate when short term antimicrobial therapy is stopped,[13] and possibly this could occur in mycobacterial infection. There is therefore some experimental evidence to support prolonged chemotherapy. The two cases of infection due to highly resistant organisms (*M chelonei* and *M fortuitum*) represent considerable management problems. Although there are no clinical studies to support the combination of amikacin and erythromycin, recent in vitro studies have shown mycobacteria of Runyon group IV to be susceptible to these drugs.[14 15] Our patient with *M fortuitum* infection showed no response to these agents, but she had very extensive disease at the time of diagnosis. The patient with *M chelonei* infection is still undergoing treatment and the initial radiographic improvement has been encouraging. The third patient with a resistant non-tuberculous mycobacterium (unclassified) had only a single positive culture with no recurrence in subsequent specimens and has received no antimycobacterial treatment. There has, however, been considerable radiographic progression of disease in the right upper lobe and the question remains whether these changes may be related to cryptic mycobacterial infection.

These cases illustrate the difficulties associated with isolation of mycobacteria in patients with cystic fibrosis. The currently accepted criteria for the diagnosis of significant mycobacterial infection cannot be applied satisfactorily in the presence of cystic fibrosis. Similar problems of diagnosis arise in other groups of patients with chronic pulmonary disease where non-tuberculous mycobacteria are isolated,[11]

emphasising that more specific methods of diagnosis in these patients are urgently required. It may be helpful to establish skin reactivity to tuberculin and other mycobacterial purified protein derivatives, if available, at presentation. This could provide a useful indication of infection if conversion occurs at a later date, although delayed hypersensitivity skin reactions may be suppressed in the severely debilitated patients. In view of the possibility that mycobacteria may be present in the sputum of patients with cystic fibrosis more often than previously recognised, it is important that regular sputum examinations for acid fast bacilli are carried out and that improved methods of culture for these patients are sought.

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