Molecular Epidemiology of *Mycobacterium abscessus*, with Focus on Cystic Fibrosis

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*Mycobacterium abscessus* has been isolated increasingly often from the respiratory tracts of cystic fibrosis (CF) patients. It is not known whether these organisms are transmitted from person to person or acquired from environmental sources. Here, colony morphology and pulsed-field gel electrophoresis (PFGE) pattern were examined for 71 isolates of *M. abscessus* derived from 14 CF patients, three non-CF patients with chronic respiratory *M. abscessus* infection or colonization, one patient with mastoiditis, and four patients with infected wounds, as well as for six isolates identified as environmental contaminants in various clinical specimens. Contaminants and wound isolates mainly exhibited smooth colony morphology, while a rough colony phenotype was significantly associated with chronic airway colonization (*P* = 0.014). Rough strains may exhibit increased airway-colonizing capacity, the cause of which remains to be determined. Examination by PFGE of consecutive isolates from the same patient showed that they all represented a single strain, even in cases where both smooth and rough isolates were present. When PFGE patterns were compared, it was shown that 24 patients had unique strains, while four patients harbored strains indistinguishable by PFGE. Two of these were siblings with CF. The other two patients, one of whom had CF, had not had contact with each other or with the siblings. Our results show that most patients colonized by *M. abscessus* in the airways have unique strains, indicating that these strains derive from the environment and that patient-to-patient transmission rarely occurs.

*Mycobacterium abscessus* is an environmental mycobacterial species ubiquitous in soil and water (4, 43). It can grow in distilled water and has been isolated from water treatment plants, domestic water supply systems, and aquaria, where it may participate in biofilm formation (34).

*M. abscessus* may cause cutaneous infections after contact with contaminated water in public baths. Such infections usually resolve without complications, but medical or surgical treatment is sometimes needed (7, 20). *M. abscessus* is also capable of colonizing the respiratory tract and causes disease in certain groups of individuals. One such group is Caucasian females above 60 years of age. While most of them have no apparent underlying abnormality, 40% have some predisposing condition such as lung disease, lung transplantation, achalasia, or recurrent vomiting (5). Another group at risk for *M. abscessus* colonization is patients with cystic fibrosis (CF). In some CF patients, acquisition of *M. abscessus* is associated with poor clinical outcome, while in others colonization may persist for several years without evident disease or deterioration of pulmonary function (9, 27, 28, 30). *M. abscessus* is inherently multi-resistant and difficult to treat, and consequently its isolation in CF is of great concern.

*M. abscessus* has been reported to be isolated increasingly often from CF patients in several countries (8, 14, 30, 35). This appears to be true also in western Sweden, where the first case in the CF population was discovered in 1997. After that year, an increase in *M. abscessus*-colonized patients gave rise to the suspicion that person-to-person transmission had occurred between individuals at the West Swedish CF Centre at the Queen Silvia Children’s Hospital in Göteborg. In the present study, pulsed-field gel electrophoresis (PFGE) was used to identify respiratory *M. abscessus* isolates from CF patients treated in western Sweden and at other Swedish CF centers, as well as respiratory isolates from other patient groups. We also examined wound isolates acquired from environmental sources during the tsunami catastrophe in Thailand in 2004, as well as *M. abscessus* isolates from various patient samples, where the bacterium was identified as an environmental contaminant. The aim was to determine whether CF patients cared for at a certain center shared strains, suggesting patient-to-patient spread. A second aim was to determine whether *M. abscessus* strains colonizing the respiratory tract were more related to each other than to wound isolates or contaminants.

**MATERIALS AND METHODS**

**Patient groups and *M. abscessus* isolates.** In all, 71 isolates of *M. abscessus*, isolated from 28 patients, were included in the study (Table 1). Fourteen patients had CF; nine of them were seen at the West Swedish CF Centre at the Queen Silvia Children’s Hospital in Göteborg. Three were cared for at the Stockholm CF Centre. Two were cared for at the Uppsala CF Centre and referred to the Sahlgrenska University Hospital in Göteborg for lung transplantation. Three non-CF patients with *M. abscessus* lung infection/colonization (two women, 56 and 84 years of age, and one 54-year-old male) contributed a total of
nine isolates (Table 1). An isolate from a patient with M. abscessus mastoiditis was also included. Four victims of the tsunami catastrophe in Asia on 26 December 2004 contributed one wound isolate each.

Six additional patients contributed one isolate each, derived from various clinical samples. Follow-up samples from these patients were negative for M. abscessus, whose presence was regarded as the result of transient colonization without clinical significance.

The type strain of M. abscessus ATCC 19977 was obtained from the Culture Collection University of Göteborg (CCUG 20993).

Isolation and identification of M. abscessus from patient samples. Direct microscopy for mycobacteria and culture were done according to routine procedures at the laboratory (18). M. abscessus was isolated on Lowenstein-Jensen solid medium. Mycobacteria were identified by the Hallberg staining technique (16). Briefly, bacterial slides were heated to 80°C in a solution containing NaCl, cooled to room temperature, rinsed in distilled water, decolorized in 8% HNO3 and 70% ethanol, and counterstained with fuchsine.

Species were identified by using a biochemical test panel (tolerance to 5% NaCl, iron uptake, Tween hydrolysis, urease, nitrate reduction) (24), by examination of the mycoid acid pattern by high-pressure liquid chromatographic analysis (1, 2), and by sequencing of the 16S RNA gene (19). For the most recent isolates (2005 onward), these methods were complemented with the GenoType Mycobacterium CM test (HAIN Lifescience, Nehren, Germany) (21).

All isolates were stored at room temperature in vials with Lowenstein-Jensen medium until analysis.

**Table 1. Description of Mycobacterium abscessus isolates used in this study**

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>No. of patients</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>14</td>
<td>51</td>
</tr>
<tr>
<td>Non-CF lung infection/chronic colonization</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mastoiditis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Wounds (tsunami victims)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Transient colonization/contaminationa</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Total 28 71 52 14 5

a Includes 33 sputum, 3 bronchiolaseal lavage, and 1 pleural fluid sample.

b Includes four blood cultures and one sample from a central venous catheter.
c Includes eight sputum and one bronchial lavage sample.
d Includes two sputum, two bronchial lavage, and two pleural fluid samples.
e Includes one wound sample appearing on a single culture occasion in a sample from a non-CF patient, without associated clinical symptoms, and followed by negative sample.

Results

Occurrence of M. abscessus in CF patients and clinical correlates. Of 140 patients with CF included in the regular sputum screening programs at the Queen Silvia Children’s Hospital, nine (6%) had been positive for M. abscessus on at least one occasion by the end of 2005. Details for these patients are shown in Table 2. The first case appeared in 1997. Among the nine CF patients positive for M. abscessus, five (patients 1, 2, 3, 4, and 5) (Table 2) were considered to have clinical disease caused by this bacterium and were treated for this for at least some period. For patient 6, the contribution of M. abscessus to the clinical disease was difficult to assess, but he received antimycobacterial treatment for at least 3 months. Since 2005,
this patient has had five negative cultures. One CF patient (no. 9) was persistently colonized by *M. abscessus* without clear clinical impact. This patient was treated for *Mycobacterium avium* complex (MAC) infection in 1996. This organism was eradicated, but in 1997 the patient was infected with *M. abscessus* and has been colonized since. Two CF patients (no. 10 and 11) were thought to have *M. abscessus* as a transient colonizer and did not receive any antimycobacterial treatment.

Two CF patients (a female born in 1976 and a male born in 1992) from the Stockholm/Uppsala region were referred to Göteborg for lung transplantation. They had been colonized by *M. abscessus* since 2000 and 2002, respectively. Both developed disseminated *M. abscessus* disease posttransplantation, for which they received treatment. Three CF patients, cared for at the Stockholm CF Centre, were interpreted as having persistent airway colonization with *M. abscessus*, probably of clinical significance. One of these patients (no. 14; see the Fig. 2 dendrogram) was treated for a MAC infection 10 years previously with rifabutin, ethambutol, and amikacin. Ethambutol was switched to clarithromycin and clofazimine because of side effects. The MAC strain was eradicated, but *M. abscessus* was isolated from sputum from this patient during MAC treatment.

The *M. abscessus* strain developed in vitro resistance to all tolerated drugs, but the patient was given gamma interferon with clinical effect. The *M. abscessus* strain was never eradicated, but this patient has been without mycobacterial treatment for the past 5 years.

**Colon** *M. abscessus* **in relation to clinical picture.** Two types of colony appearances were noted after culture of *M. abscessus* on either horse blood or Middlebrook 7H10 agar (Fig. 1). One phenotype was smooth and shiny and resembled that of the type strain, ATCC 19977. The other form had larger, rough, and markedly waxier colonies. The two forms were stable during subculture.

The clinical origin of smooth and rough isolates is shown in Table 3. Sixteen patients, most of whom were CF and non-CF patients with chronic colonization of the airways, yielded only rough isolates. A few isolates were isolates causing transient colonization of the respiratory tract.

Among the rough isolates, 81% (13/16) were isolated from patients with chronic colonization/disease in the airways, while only 10% (1/10) of the smooth ones were from this patient group. This difference was significant ($P = 0.0014$, Fisher’s exact test).
**PFGE analysis of M. abscessus isolates.** Seventy-one M. abscessus isolates from 28 patients and the M. abscessus type strain were examined by PFGE after digestion of bacterial DNA by AseI to determine their strain identity (Fig. 2).

From each of 10 patients, several isolates of M. abscessus were obtained from successive specimens. The longest interval between the first and last isolates analyzed was almost 4 years (CF patient 2) (Table 2). All isolates analyzed from a single patient yielded the same PFGE pattern. Smooth and rough isolates from the same patient also had identical PFGE patterns.

Figure 2 shows the relatedness of the 28 patient strains after digestion with AseI. Twenty-two of the patients had isolates with unique PFGE patterns. Six patients harbored strains that appeared to be identical or nearly identical. We designated these strains cluster A. In five patients (CF patients 1, 2, 10, and 11 and non-CF patient 20), the strain had identical PFGE patterns within cluster A (named A1), while the strain colonizing the sixth patient (CF patient 6) differed by a single band and was termed A2. Except for the cluster, the isolates from CF patients living in the Göteborg area were no more related to one another than to isolates from CF patients living in the Stockholm/Uppsala area or to isolates acquired in Thailand.

Two of the four patients (no. 1 and 11) harboring cluster A1 were siblings, both suffering from CF. The older sibling (no. 1) has been permanently colonized by M. abscessus since 2000 and has clinical disease caused by this bacterium (Table 2). The younger sibling (no. 11) was positive for M. abscessus in sputum on one occasion only, in 2001. Thereafter, screening cultures have been negative. She never received antituberculosis treatment. The third patient with a strain belonging to cluster A1 (no. 10) is a CF patient, living in western Sweden but no longer cared for at the CF center in Göteborg. She has had only one M. abscessus-positive culture. To our knowledge, she has had no social contacts with the CF siblings. The fourth patient (no. 20) having an A1 cluster strain is an elderly woman from another town. She was seen at the local hospital for unspecified pleuritis, and M. abscessus was isolated from the pleural fluid on a single occasion in 2002 but was not interpreted as the etiology of her pleuritis. We received no other patient samples harboring the cluster strain from this hospital during this year. Patient no. 2, whose strain belonged to cluster A and was termed A1b, is a CF patient also colonized by Burkholderia cepacia. Due to this colonization, she has been kept segregated from other CF patients.

**TABLE 3. Colony morphology among M. abscessus strains**

<table>
<thead>
<tr>
<th>Sample type or patient group</th>
<th>No. of patients yielding:</th>
<th>Smooth isolates</th>
<th>Rough isolates</th>
<th>Rough and smooth isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical disease, CF</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chronic colonization, CF</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic colonization, non-CF</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastoiditis, non-CF</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient colonization</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient colonization</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound infection samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 28)</td>
<td>16</td>
<td>10</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

* Colony appearance (rough or smooth) when cultured on Middlebrook 7H10 agar or horse blood agar.

**DISCUSSION**

During the last decade we have noted an increasing prevalence of M. abscessus in sputum samples from CF patients cared for at the West Swedish CF Centre. Since 1997, when the first case of M. abscessus colonization was discovered in the CF population of 140 patients, one new case per year has occurred on average. The staff has changed little in our laboratory over the past 15 years, and samples have been prepared and cultured almost in the same manner since 1993. Furthermore,
because *M. abscessus* grows more rapidly than most other environmental mycobacteria, it is less easily overlooked than other mycobacterial species in sputum samples. Taking these observations together, we consider it likely that there has been a true increase in the prevalence of respiratory colonization by *M. abscessus* in the last decade. A similar increase has also been reported from several CF centers in Europe and the United States (9, 15, 26, 28, 30, 35).

In patients without CF from whose airway samples environ-
Mental mycobacteria have been isolated (n = 62 during 2005), the MAC is far more common than M. abscessus (33 versus 4 patients). Thus, M. abscessus appears to display a predilection for colonization of the airways in patients with CF.

We hypothesized that colonization by M. abscessus in CF patients could have occurred because one or a few clones had spread via social contacts or through contaminated equipment used for clinical examination at the CF clinic. PFGE has previously been used to retrospectively analyze M. abscessus outbreaks, all of which were found to involve a single strain usually originating from the water supply system, with no spread between patients (38, 41, 42). However, in our study most CF patients carried unique strains of M. abscessus. The only exception was a cluster of two siblings and two other patients who harbored a strain indistinguishable by PFGE (cluster A1a).

The samples from the patients harboring the cluster strain were never processed in the same month in the laboratory, and samples from the siblings were not obtained on the same occasion, making contamination via the laboratory highly unlikely. The siblings both had CF. One of them had been permanently colonized since 2000, while the other was positive on a single occasion only, in 2001. It is reasonable to believe that the colonized sibling may have passed the strain to her sister. Transfer of P. aeruginosa strains between siblings with CF has been observed (36), which supports our statement.

The third patient with a cluster A1a strain, a CF patient not cared for in Göteborg, had no known contacts with the siblings. The fourth patient with a cluster A1a strain was an elderly woman cared for at the local hospital in another town for other causes. She was positive for M. abscessus on a single occasion.

FIG. 3. PFGE analyses of cluster A after AseI and XbaI digestion of DNA. (A) AseI PFGE gel corresponding to the dendrogram in panel B. Lanes 1, 6, and 11, reference strain S. aureus NCTC 8325 (not shown in dendrogram). Lane 2, patient 3, isolate 05*1230; lane 3, patient 6, isolate 05*2299; lane 4, patient 10, isolate 05*2316; lane 5, patient 1, isolate 01*5153; lane 7, patient 1, isolate 03*4569; lane 8, patient 2, isolate 04*2226; lane 9, patient 20, isolate 02*1284; lane 10, patient 11, isolate 01*1526. (B and C) AseI (B) and XbaI (C) PFGE patterns presented as similarity dendrograms and schematic views of bands analyzed. *, isolate 01*5153; **, isolate 03*4569.
only, without associated symptoms. Thus, except for the possible transmission of clone A1a between the two siblings, we found no evidence of spread of M. abscessus between CF patients cared for at the West Swedish CF Centre. Furthermore, isolates from western Sweden were no more similar to one another than isolates from other geographical locations.

As M. abscessus is ubiquitous in nature, our results suggest that this bacterium is acquired from the environment by individual patients and that spread between patients is uncommon.

Such “spontaneous” colonization by M. abscessus might have increased in CF patients. One reason may be that better control of “classical” respiratory pathogens such as S. aureus and P. aeruginosa might have paved the way for opportunists with lower inherent pathogenicity, including M. abscessus. One example of this was the two CF siblings; one of them was permanently colonized by M. abscessus, harboring no other pathogen besides Aspergillus fumigatus, whereas the other sibling, positive only once for M. abscessus, had all classical CF pathogens in sputum, i.e., S. aureus, Haemophilus influenzae, P. aeruginosa, and Stenotrophomonas maltophilia. The patients colonized with M. abscessus might also have subtle deficiencies in their defense systems. Two CF patients who were both colonized/infected with M. abscessus underwent lung transplantation. Both developed disseminated disease postoperatively but survived. Both fatal and nonfatal post-lung-transplant infections with M. abscessus have been described (3,11,22,33). Clearly, immunosuppression is involved in aggravation of the disease, but whether any abnormalities underlie respiratory colonization per se remains to be shown.

The three non-CF patients with chronic pulmonary M. abscessus colonization/infection studied had no known immunological deficiencies, besides old age in one case. One patient had previously suffered from MAC disease and had developed bronchiectatic changes and A. fumigatus colonization. Allergic bronchopulmonary aspergillosis and systemic steroids have been identified as risk factors for mycobacterial disease (26). The second patient was a smoker with chronic obstructive pulmonary disease (COPD), while the third was a healthy nonsmoking middle-aged woman. This fits with the previously identified risk groups for pulmonary colonization with M. abscessus (middle-aged female nonsmokers, COPD patients, and patients previously treated for mycobacterial disease) (5).

We observed no apparent relatedness between strains causing a certain type of disease manifestation. Four isolates were from patients who sustained injuries which became contaminated with dirty water in the tsunami catastrophe in Thailand in 2004. These infections manifested as skin abscesses. These isolates interpreted as contaminants or transient colonizers from patients who sustained injuries which became contaminated with dirty water in the tsunami catastrophe in Thailand in 2004. These infections manifested as skin abscesses. These isolates interpreted as contaminants or transient colonizers...


