Association of Mucoid Encapsulated *Moraxella duplex* var. *nonliquefaciens* with Chronic Bronchitis

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The recovery of two strains of highly mucoid encapsulated *Moraxella duplex* var. *nonliquefaciens* from the sputum of two patients suffering from chronic bronchitis is described. The biochemical, morphological, and pathogenic characteristics of this microorganism are discussed.

Members of the genus *Moraxella*, notably *M. lacunata* and *M. liquefaciens*, have long been recognized primarily for their eye pathogenicity (7). *M. duplex* var. *nonliquefaciens* may also produce a mild conjunctivitis. In diseases other than those affecting the eye, *M. duplex* var. *nonliquefaciens* has been isolated particularly when it occurs in the mucoid form.

Henriksen (1) described a mucoid strain of *M. duplex* var. *nonliquefaciens* that he recovered from the sputum of a 55-year-old laborer suffering from bronchitis and bronchopneumonia. Murray and Truant (5) reported the isolation of a similar mucoid strain from a throat culture that they identified according to Henriksen's criteria. Kaffka (4) also reported the isolation of two mucoid strains from healthy carriers and one nonmucoid strain from a 73-year-old patient suffering from bronchopneumonia. Peel (6) isolated a mucoid strain from the sputum of a 76-year-old male patient suffering from chronic bronchitis.

This report concerns the predominant isolation of two highly mucoid encapsulated strains of *M. duplex* var. *nonliquefaciens* from sputum and the evaluation of these mucoid variants as potential pathogens.

**CLINICAL STUDIES**

Case no. 1. An 84-year-old white male was admitted to the City Hospital Center at Elmhurst on 23 February 1967 because of severe dyspnea and cyanosis. The patient presented with a 50-year history of chronic bronchitis and a 10-year history of emphysema. He had a chronic cough with copious, purulent sputum accompanied by frequent attacks of dyspnea. His admission diagnosis was chronic bronchitis with recent superimposed acute infection. Temperature at admission was 99 F (37.22 C).

Laboratory tests showed that hemoglobin level was 11.1 g/100 ml and leukocyte count was 6,900. X-ray examination of the lungs showed old fibrotic calcifications from tuberculosis treated 10 years previously. Bacteriological examination of the sputum failed to reveal the presence of tubercle bacilli. However, routine cultures showed the predominant growth of mucoid *M. duplex* var. *nonliquefaciens*, accompanied by a few colonies of *Staphylococcus aureus*, *pneumococci*, *Haemophilus influenzae*, nonhemolytic streptococci, and *Escherichia coli*. The mucoid organism proved susceptible in vitro, by the disc-agar diffusion method, to many antibiotics (Table 1).

The patient was placed on tetracycline twice a day. After 24 hr, sputum cultures still showed the persistence of the same microorganism, but cultures became negative at 48 and 72 hr after admission. Sputum production diminished, probably as a result of the antimicrobial therapy, and the patient was discharged on 6 March 1967, 11 days after admission. Tetracycline treatment was continued, and the patient was referred to the pulmonary clinic for followup.

Case no. 2. A 61-year-old white male was admitted to City Hospital Center at Elmhurst on 5 July 1967 with a chief complaint of anorexia, chronic cough, and weight loss. The admission findings were chronic bronchitis and emphysema, cirrhosis of the liver due to chronic alcoholism, abdominal distress, and anemia.

Laboratory tests showed that hemoglobin level was 11.1 g/100 ml and leukocyte count was 6,900. X-ray examination of the lungs showed old fibrotic calcifications from tuberculosis treated 10 years previously. Bacteriological examination of the sputum failed to reveal the presence of tubercle bacilli. However, routine cultures showed the predominant growth of mucoid *M. duplex* var. *nonliquefaciens*, accompanied by a few colonies of *Staphylococcus aureus*, *pneumococci*, *Haemophilus influenzae*, nonhemolytic streptococci, and *Escherichia coli*. The mucoid organism proved susceptible in vitro, by the disc-agar diffusion method, to a variety of antimicrobial agents (Table 1).

The patient was placed on ampicillin, 250 mg four
Injection of 1 ml of the sputum from case 1 into the peritoneal cavity of two white mice proved nonlethal after 48 hr. However, when 1 ml of pure culture isolates from the above cases con-

**FIG. 1.** Blood-agar plate showing growth of mucoid colonies of Moraxella duplex var. nonliquefaciens.

**TABLE 1.** Antimicrobial susceptibility of the isolated mucoid Moraxella duplex var. nonliquefaciens

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Tetracycline</th>
<th>Chloramphenicol</th>
<th>Erythromycin</th>
<th>Streptomycin</th>
<th>Penicillin</th>
<th>Neomycin</th>
<th>Ampicillin</th>
<th>Lincomycin (2 μg)</th>
<th>Oxacillin (1 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5²</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

² Disc concentrations of antibiotic giving distinct zone of inhibition.

b R = resistant.

Times a day, which resulted in a marked decrease in the number of *Moraxella* colonies that developed from a 24-hr post-therapy sputum culture. At 48 hr after the initiation of antimicrobial therapy, the sputum culture became negative for this microorganism.

The patient improved, gained weight, and was subsequently discharged with referral to the gastrointestinal clinic for the followup of a bilateral diverticulosis discovered by X-ray examination.

**RESULTS**

In the two cases studied, the direct Gram smears of the sputum revealed the presence of numerous gram-negative, markedly encapsulated coccobacilli with squared ends (Fig. 2 and 3). As the microscopic morphology resembled that of *Klebsiella*, direct quellung reactions were performed with specific antisera, with negative results.

**FIG. 2.** Direct smear of sputum. Many encapsulated diplobacilli can be seen.

**FIG. 3.** India ink preparation from 24-hr blood-agar culture, demonstrating large capsules.
MORAXELLA FROM CHRONIC BRONCHITIS

Fig. 4. Smear of 24-hr blood-agar culture showing the marked pleomorphism. Irregular stained, vacuolated, and filament forms are seen.

taining approximately 600 million organisms (MacFarland no. 2) were injected into the peritoneal cavity of white mice, lethal results were obtained in two out of four mice in case 1, and in all three mice in case 2. The organisms were recovered from the heart blood of all mice that expired.

The organisms grew well on blood- and chocolate-agar, particularly under 10% CO₂ tension, but, as contrasted to *Klebsiella*, they failed to grow on MacConkey or Endo agar. They were nitrate and oxidase positive, penicillin sensitive, and lacked either oxidative or fermentative powers when grown in Cystine Trypticase Agar (BBL) or phenol red agar base containing 1% dextrose, sucrose, lactose, maltose, mannitol, and xylose. After 24 hr, Gram-stained smears of cultures grown on blood-agar plates showed marked pleomorphism ranging from typical gram-negative coccobacilli with square ends to large irregular stained filaments. Vacuolation and involution forms were common (Fig. 4). According to Henriksen (2), these morphological and biochemical characteristics are typical of this microorganism. Table 2 shows the biochemical characteristics observed by various investigators with mucoid *M. duplex* var. *nonliquefaciens*. The differences between this microorganism and *Klebsiella* are obvious.

**DISCUSSION**

The mucoid strains isolated from the sputum were pathogenic for white mice when massive doses of the organism were injected into the peritoneal cavity. The observation of Henriksen and Kaffka (1, 4), that only the mucoid strains are pathogenic for mice, has been confirmed by our studies. However, in our cases, as in the cases of Henriksen and Peel, the mucoid strains recovered were from symptomatic patients, whereas Kaffka recovered his strains from healthy carriers. Henriksen (3) recovered 99 isolates of *M. duplex* var. *nonliquefaciens* from 875 nose cultures, all nonmucoid and nonpathogenic for white mice. In our laboratory, 50 isolates of the same nonmucoid organism were recovered from a variety of clinical sources (eye, nose, throat, sputum, blood, etc.); none of these strains proved pathogenic to white mice, even in massive doses. It seems that the presence of increased amounts of capsular material is paramount for the elicitation of mouse pathogenicity.
<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Associated disease</th>
<th>Source</th>
<th>Biochemical reaction</th>
<th>Mouse pathogenicity</th>
<th>Penicillin susceptibility</th>
<th>Mucoid colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Loeffler's liquefaction</td>
<td>Oxidase</td>
<td>Glucose</td>
<td>Lactose</td>
</tr>
<tr>
<td>Henrikson</td>
<td>M</td>
<td>55</td>
<td>Bronchitis and bronchopneumonia</td>
<td>Sputum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Murray and Truant Kaffka</td>
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<td>Not given</td>
<td>Bronchopneumonia and chronic bronchitis</td>
<td>Throat</td>
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<td>+</td>
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<tr>
<td></td>
<td>F</td>
<td>55</td>
<td>Meningitis survey</td>
<td>Throat</td>
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<td>-</td>
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<tr>
<td></td>
<td>F</td>
<td>32</td>
<td>Meningitis survey</td>
<td>Sputum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peel</td>
<td>M</td>
<td>76</td>
<td>Chronic bronchitis</td>
<td>Sputum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bottone and Allerhand</td>
<td>M</td>
<td>84</td>
<td>Chronic bronchitis</td>
<td>Sputum</td>
<td>-</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>61</td>
<td>Chronic bronchitis</td>
<td>Sputum</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Not reported.
As pointed out by Henriksen, rare recovery in the laboratory of this microorganism may be attributed to (i) its CO₂ and moisture requirements for growth, and (ii) its morphological similarity to Klebsiella and occasionally to Neisseria, which may confuse identification. However, pleomorphism, when observed, is a particular characteristic of this microorganism, which may aid in identification.

The possible etiological role of this organism in chronic bronchitis is problematical. Its predominance in the sputum culture and the concomitant therapeutic response upon elimination of these organisms by appropriate antibiotic therapy does suggest a possible etiological significance.

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Literature Cited