SHORT ARTICLE

CASES OF *MYCOBACTERIUM BORSTELENSE* AND *M. ABSCESSUS* INFECTION OBSERVED IN BELGIUM

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CASE HISTORIES

Case no. 1. Miss M. P., a nurse born in 1948, had been treated for diabetes mellitus since 1961. In February 1967 she was admitted to hospital with acetonæmia. About 3 wk later, red macules appeared at the site of insulin injections received during her stay in hospital. These became swollen and painful after a further 2 wk and yellow pus was seen when they were incised. Microscopic examination of the pus showed numerous acid-fast bacilli, and rapidly growing mycobacteria appeared in the cultures.

All the lesions were incised and treated locally with a quaternary ammonium antiseptic. By May, all the sites had healed leaving reddish-purple macules 5–7 mm in diameter. The tuberculin reaction remained negative.

The patient had observed that only the site of injections received during her stay in the hospital became inflamed. The syringes used in hospital were kept in alcohol, but at home the patient boiled her syringes.

Case no. 2. Mrs W. D., born in 1943, married with one child, had resided in Rwanda since 1962. There was nothing else significant in her personal or family history. On 22 Aug. 1968, she fell through a ceiling in Rwanda and sustained two cuts, one 20 cm long on her left thigh and the other 10 cm long on her left leg. Both wounds were stitched in Bujumbura after excision of the margins; 10 days later, necrosis developed in the wounds which opened widely. She returned to Belgium on 25 Sept. 1968 and entered hospital at the Institute for Tropical Medicine, Antwerp.

The extensive necrotic wounds were excised and treated with local applications of tetracycline and proteolytic enzymes. Improvement was considerable and the patient left the hospital on 26 Oct. 1968 to be treated as an out-patient. However, during the following days, hard subcutaneous plaques developed in the vicinity of the old scars. The overlying epidermis gradually became tender and some of the lesions ulcerated, producing a gelatinous fluid. No bacteria were seen in a Gram-stained film of this material, but a Ziehl-Neelsen stain showed numerous acid-fast bacilli. A strain of rapidly growing mycobacteria was isolated on Löwenstein-Jensen (LJ) medium and on blood agar within 3 days. A biopsy specimen showed a non-specific granulomatous lesion.

BACTERIOLOGICAL OBSERVATIONS

Both of the strains (M.P. and W.D.) were rapidly growing mycobacteria that formed colonies within 2–3 days on egg media and blood agar and somewhat more slowly on nutrient or glycerol agar. Their cultural, biochemical and serological characters were examined in detail, along with those of the following reference strains: *Mycobacterium abscessus* no. ATCC19977, *M. borstelense* no. ATCC 19235 and 19237, and *M. fortuitum* no. ATCC6841.

Methods

Colonial morphology was studied on modified corn-meal agar (Jones and Kubica, 1965). The cultures were examined for growth at 20°, 37°, 40°, 42° and 45°C, acid production from

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carbohydrates and utilisation of organic acids as carbon source (Gordon and Mihm, 1959), growth on MacConkey agar (Gordon and Mihm; Desmijter, 1962), niacin production

TABLE
Cultural characters of strains *M.P.* and *W.D.*, and of reference strains of *Mycobacterium borstelense*, *M. abscessus* and *M. fortuitum*

Test	Result of tests with					
	<i>M. borstelense</i>				<i>M. abscessus</i>	<i>M. fortuitum</i>
	strain M.P.	strain W.D.	no. ATCC19235	no. ATCC19237	no. ATCC19977	no. ATCC6841
Growth at:						
20°C and 37°C	+	+	+	+	+	+
40°C	—	+	—	—	+	+
42°C and 45°C	—	—	—	—	—	—
Niacin production	—	—	—	+	—	—
Nitrate reduction	—	—	—	—	—	+
Tween 80 hydrolysis in 5 days	—	—	—	—	—	+
Arylsulphatase production	+	+	+	+	+	+
Reduction of iron ammonium citrate	—	—	—	—	—	+
Catalase production:						
at room temperature*	50	80	30	100	100	30
after 30 min. at 68°C	+	+	+	+	+	+
Growth on MacConkey agar (6 days)	—	—	—	—	—	+
Acid formed from:						
glucose, mannose and trehalose	+	+	+	+	+	+
fructose	—	—	—	—	—	+
sorbitol, inositol, arabinose and rhamnose	—	—	—	—	—	—
mannitol	—	...	—	—	—	—
lactose	...	—	—	—	—	—
raffinose	—	...	—	—	—	—
dulcitol	...	—	—	—	—	—
xylose	...	...	—	—	—	—
Utilisation of:						
benzoate and oxalate	...	...	—	—	—	—
citrate	+	—	+	+	—	+
lactate	+	+	+	+	+	+
malate, succinate and fumarate	...	...	+	+	+	+
Growth in presence of:						
1 µg isoniazid per ml	+	+	+	+	+	+
25 and 50 µg ethambutol per ml	—	+	+	+	+	—
Mouse pathogenicity test	—	—	—	—	—	+
Growth on LJ+5 per cent NaCl	—	+	—	—	+	...

+ = Positive result; — = negative result; ... = test not done.

* Semi-quantitative method of Kubica *et al.* (1966); height of column of gas bubbles to nearest 10 mm.

(Konno, 1956), nitrate reduction (Virtanen, 1960), arylsulphatase activity (Pattyn and Hermans-Boveroulle, 1965), Tween 80 hydrolysis (Wayne, Doubek and Russell, 1964), reduction of ammonium citrate (Szabó and Vandra, 1963) and catalase production (Kubica *et al.*, 1966). Isoniazid and ethambutol sensitivity were determined by the proportion method

(Buttiaux, Beerens and Tacquet, 1966). Agglutination tests were done as described by Pattyn (1970a). 0.2 mg bacteria was inoculated intravenously into mice, which were observed for 6 wk before being killed. Macroscopic and microscopic examinations were made of lungs, liver, spleen and kidneys.

Results

The cultures from our two cases resembled *M. abscessus* and *M. borstelense* rather than *M. fortuitum*. On modified corn-meal agar they did not form the filamentous types of colony we designate as Sm(f) and R(f), which are seen only in *M. fortuitum* and *M. peregrinum* among the rapidly growing mycobacteria (Pattyn, 1970b). The rest of their cultural and biochemical characters are summarised in the table, which shows that, like the reference strains of *M. abscessus* and *M. borstelense*, they did not acidify fructose, grow readily on MacConkey agar, reduce nitrate, hydrolyse Tween 80 within 5 days or reduce iron ammonium citrate. The reference strain of *M. fortuitum* gave positive results in all these tests.

M. fortuitum and *M. peregrinum* are pathogenic for the mouse, and on intravenous injection produce a "spinning" or "rolling" disease and renal abscesses (authors' unpublished observations). Strains M.P. and W.D., like the reference strains of *M. abscessus* and *M. borstelense*, were not pathogenic for mice.

Antigenic differences between rapidly growing mycobacteria can be detected by means of the agglutination reaction; Pattyn (1970a) found that *M. fortuitum* and *M. peregrinum* differ from each other, and that both differ from *M. abscessus* and *M. borstelense*, which are themselves indistinguishable antigenically. Stanford and Gunthorpe (1969) made similar distinctions by means of immunoprecipitation reactions.

Strains M.P. and W.D. were agglutinated by a serum prepared against *M. abscessus* (no. ATCC19977) but not by a *M. fortuitum* or a *M. peregrinum* antiserum. Strain M.P. was agglutinated by a serum prepared against strain W.D., which also agglutinated *M. borstelense* strains no. ATCC 19235 and 19237. Our strains were also examined in immunoprecipitation tests by Dr J. L. Stanford, who confirmed their identity as *M. abscessus* or *M. borstelense*.

As shown previously (Portaels and Pattyn, 1970), *M. abscessus* and *M. borstelense* are in general more resistant to ethambutol than are *M. fortuitum* and *M. peregrinum*. Strain W.D. grew in the presence of 50 µg ethambutol per ml; strain M.P., on the other hand, grew in the presence of 5 µg per ml but not in the presence of 25 µg per ml of the antibiotic, and is in this respect atypical.

Differentiation between *M. abscessus* and *M. borstelense* is made on the results of three tests; the former grows on LJ medium with 5 per cent. added NaCl, does not use citrate as sole carbon source and grows at 40°C. Strain M.P. is therefore identified as *M. borstelense* and strain W.D. as *M. abscessus*.

DISCUSSION

Gordon and Smith (1953, 1955) distinguished three species among rapidly growing mycobacteria: *M. smegmatis*, *M. phlei* and *M. fortuitum*. Many other species have since been described. Recently, however, numerical analysis of results obtained in an International Cooperative Study on rapidly growing mycobacteria (report in preparation) showed the existence of eight species: *M. smegmatis*, *M. phlei*, *M. fortuitum*, *M. peregrinum*, *M. abscessus*, *M. borstelense* (Bönicke, 1965), *M. vaccae* and *M. diernhoferi*.

M. fortuitum and *M. peregrinum* on the one hand and *M. abscessus* and *M. borstelense* on the other are closely related species. Our present results, and those of Stanford and Beck (1969) show that both *M. abscessus* and *M. borstelense* can be readily distinguished from *M. fortuitum*, though our results for lactate utilisation are at variance with those of Stanford and Beck, since we found that all members of the two former species utilised lactate.

In the International Cooperative Study it was possible to differentiate between *M. abscessus* and *M. borstelense* by means of a small number of tests, so that the two species were distinguishable at the 85 per cent. but not at the 80 per cent. similarity level. Stanford

and Beck considered that the type strains of *M. abscessus*, *M. borstelense*, *M. runyuncii* and *M. friedmanii* belonged to a single species. In a note added to this paper they state that the correct name for this species is *M. chelonae*.

M. fortuitum, *M. peregrinum*, *M. abscessus* and *M. borstelense* are the only species among the rapidly growing mycobacteria at present known to be potentially pathogenic for man (Wells, Agius and Smith, 1955; Limbos *et al.*, 1961; Vandepitte *et al.*, 1962; Beck, 1965; Moore and Frerichs, 1953; Corpe, Smith and Stergus, 1961; Inman *et al.*, 1969). *M. abscessus* and *M. borstelense* are saprophytes that cause human disease rarely, and only when accidentally introduced through the skin. This may occur as a result of faulty injection technique (Moore and Frerichs; Inman *et al.*; and our own first case). Our second case is unique in that infection occurred in Central Africa. We cannot say whether the organism was introduced at the time of wounding or during the subsequent surgical treatment.

SUMMARY

Cases of multiple cutaneous abscesses due to *Mycobacterium borstelense* and *M. abscessus* are described. One occurred in Belgium after insulin injections and the other as a result of a wound infection sustained in Central Africa.

The differentiation of *M. borstelense* from *M. abscessus*, *M. fortuitum* and *M. peregrinum* is discussed.

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