Case Report

Interstitial pulmonary inflammation due to Microbacterium sp. after heart transplantation

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Case report

A 44-year-old man underwent heart transplantation on 8 August 2000 as a consequence of ischaemic cardiomyopathy (previous extended anterior myocardial infarction). Transplantation and the post-operative course were uncomplicated and the patient was discharged on 31 August 2000 and prescribed a cyclosporin A, azathioprine and corticosteroid therapy (cyclosporin blood levels were expected to be 400 ng dl⁻¹). Ten days later, the patient showed an acute organ rejection documented by endomyocardial biopsy (Grade 2, Dallas Classification, with diffuse and aggressive infiltrate) and was treated with high doses of intravenous corticosteroid (500 mg methylprednisolone per day for 3 days). Following this therapy, the rejection successfully reversed (Grade 1A at 1 month after treatment).

Twelve days after corticosteroid treatment, the patient was readmitted with fever, neck pain, oesophageal discomfort during deglutition and a cough. At that time, the white blood cell count was 0.56 cells ml⁻¹ with 80 % neutrophils, 15 % lymphocytes and a CD4/CD8 ratio of 0.4, thus showing intense immunosuppression. Fever quickly rose to 38.3°C. Several blood and sputum cultures were negative for aerobic and anaerobic bacteria and for fungal cells. Before and after the transplant, the patient had been monitored for cytomegalovirus by the pp65 antigenaemia test, whose results were constantly negative. At the time of readmission, a further test for cytomegalovirus pp65 antigenaemia was performed and the result again was negative. A chest radiograph showed interstitial inflammation of the inferior left pulmonary lobe. After a few days, pleural effusion developed, as evidenced by physical examination. Computerized axial tomography examination confirmed pulmonary inflammation and pleural effusion, but excluded mediastine involvement. Initially, the patient was treated empirically with meropenem (500 mg three times per day), clarithromycin (500 mg per day), ciprofloxacin (200 mg twice a day) and fluconazole (150 mg per day), all intravenously, and oral trimethoprim/sulfamethoxazole (800 mg per day). This therapy did not lower the fever, nor did it attenuate the other symptoms. After 5 days, a selective bronchoalveolar washing was performed and the aspirate was examined. Direct Gram staining showed the presence of Gram-positive bacilli, whilst observation for Pneumocystis jiroveci (Pneumocystis carinii) after Giemsa and toluidine blue O staining yielded negative results. The aspirate was not checked for the presence of cytomegalovirus, but was cultured for aerobic and anaerobic bacteria, as well as for fungi. Culture on blood agar and MacConkey agar under aerobic conditions yielded growth in pure culture of a Gram-positive coryneform bacterium that was found to be sensitive to vancomycin. Previous antibiotics were replaced by vancomycin (500 mg four times per day) and this therapy was prolonged for 20 days until complete resolution of radiographic and clinical symptoms. The patient was discharged after 31 days in a good condition.

A coryneform bacterium was isolated from the bronchoalveolar aspirate of a patient with interstitial pulmonary inflammation. Commercial systems identified the isolate as Corynebacterium sp. or Aureobacterium sp./Corynebacterium aquaticum, but 16S rRNA gene analysis unequivocally attributed it to the genus Microbacterium. This represents the first documented case of Microbacterium pulmonary infection.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of the OVE isolate reported in this paper is AY960683.
Microbiological studies

Colonies of the isolate (OVE isolate) were non-haemolytic on 5% sheep blood agar and showed a bright yellow pigmentation at room temperature. The isolate was lactose-non-fermenting, catalase-positive and oxidase-negative. The Vitek 2 identification system (bioMérieux) identified the strain as Corynebacterium sp., whilst the API Coryne system (bioMérieux) identified it as Aueobacterium sp./Corynebacterium aquaticum with a 98.9% level of confidence. However, in contrast to the reported reactions for C. aquaticum (Funke et al., 1994; Grove et al., 1999), the isolate did not reduce nitrate and was non-motile. Using the API Coryne system, the isolate gave positive results for pyrazinamidase, alkaline phosphatase, hydrolysis of urea, and fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose and glycerol. Hydrolysis of gelatin was negative using API Coryne, but positive by inoculation on nutrient gelatin medium (Difco) after 3 days of incubation at 22°C. The numerical profile of the isolate on the API Coryne system was 2570004, similar to the 2550004 profile obtained with the type strain (CIP 102402T) of Microbacterium liquefaciens (reclassified as Microbacterium liquefaciens) and to the 5570004 profile obtained with the type strain (CIP 64.13T) of C. aquaticum (reclassified as Leifsonia aquatica). The main biochemical characteristics of the OVE clinical isolate are shown in Table 1, compared with those reported in the literature for the two above-mentioned species. Manual analysis of the overall reactions produced by the two identification systems used allowed us tentatively to identify our isolate as A. liquefaciens, which is not included in the identification database of the identification systems considered above. According to the Vitek 2 system, Gram-positive Susceptibility Test Card AST-GP61 and Gram-negative Susceptibility Test Cards AST-GN08 and AST-GN11, the isolate was susceptible to cefepime, ceftriaxone, cefuroxime, cephalothin, erythromycin, imipenem, piperacillin, rifampicin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin, but was resistant to cefazidine, gentamicin, meropenem and oxacillin. The MICs for imipenem and meropenem were also determined by the Etest (AB BIODISK) and were 6 and 16 mg l⁻¹, respectively.

In order to determine the exact phylogenetic position of the OVE isolate, the 16S rRNA gene was amplified by PCR and sequenced by GenomeExpress (Montreuil, France). The 16S rRNA gene sequence of our isolate was used for a BLAST search in the EMBL/GenBank database and showed high similarity with members of the genus Microbacterium. The partial 16S rRNA gene sequence (1446 bp) of our isolate was further compared and aligned to published 16S rRNA gene sequences searched with the taxonomy browser of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and retrieved from GenBank. The retrieved 16S rRNA gene sequences belonged to the type strains of 27 different species in the genus Microbacterium and to the L. aquatica type strain (Fig. 1). The EMBL/GenBank accession numbers for the 16S rRNA sequences are given in Fig. 1.

Sequence alignment was performed using CLUSTAL_W, and phylogenetic analysis, including calculation of percentage sequence similarity, construction of a phylogenetic tree and an assessment of the tree topology by bootstrap analysis, was

Table 1. Phenotypic characteristics of the study isolate and available information for closely related Microbacterium spp. and L. aquatica (Behrendt et al., 2001; Funke et al., 1994; Grove et al., 1999; Laffineur et al., 2003; Lau et al., 2002; Nolte et al., 1996; Schumann et al., 1999)

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<td>M. foliorum</td>
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<td>M. keratanolyticum</td>
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<td>M. superdae*</td>
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*Characters determined by the Vitek and API Coryne systems, with the exception of colour of colony and motility.
†Gelatin hydrolysis was negative in the API Coryne system and was observed only by inoculation on nutrient gelatin medium.
carried out using MEGA software version 3.0 (Kumar et al., 2004), using the Kimura two-parameter model as a method of substitution and the neighbour-joining method to construct the phylogenetic tree. In the phylogenetic tree (Fig. 1), the OVE clinical isolate clustered together with Microbacterium oxydans (98.9% sequence similarity), Microbacterium luteolium (98.8%), Microbacterium foliorum (98.7%), Microbacterium phyllosphaerae (98.6%), Microbacterium saerdae (98.6%), Microbacterium paraoxydans (98.6%) and Microbacterium keratanolyticum (98.6%) whilst it showed only 93.8% similarity to the L. aquatica sequence. Although low bootstrap values were obtained with 1000 pseudoreplicate datasets, the clustering was confirmed by the unweighted pair group method with arithmetic averages and the minimum evolution method (data not shown).

**Discussion**

In recent years, a limited number of infections caused by Gram-positive coryneform bacteria identified as C. aquaticum, Aureobacterium spp. (particularly A. liquefaciens), Microbacterium spp. or CDC coryneform group A-4 and A-5 have been described (Funke et al., 1997a; Grove et al., 1999; Lau et al., 2002). In particular, only three detailed clinical descriptions of Aureobacterium spp. infections have been published, whilst a larger number of clinical isolates of this micro-organism have been described (Funke et al., 1994, 1997b, 1998; Grove et al., 1999; Nolte et al., 1996; Saweljew et al., 1996). The first report of the isolation of Aureobacterium spp. from clinical specimens was published by Funke et al. (1994), who were able to identify seven strains of this unusual pathogen among 11 clinical isolates of yellow-pigmented Gram-positive coryneform bacteria, tentatively identified as C. aquaticum. The isolates were obtained from a variety of clinical samples: drainage of a polytraumatized patient, blood culture, cerebrospinal fluid, peritoneal fluid from a patient submitted to chronic ambulatory dialysis, abdominal deep wound of a kidney/pancreas-transplanted patient, epidural abscess and a soft-tissue infection. Unfortunately, no other clinical details were reported. However, only three reports have been published in which clinical details of Aureobacterium infections are described (Grove et al., 1999; Nolte et al., 1996; Saweljew et al., 1996). In
addition, isolates belonging to the genus Microbacterium have rarely been encountered in clinical specimens; of six cases reviewed by Funke et al. (1997a), three were endophthalmitis, two were septicaemic infections and one was mitral valve endocarditis. A nosocomial outbreak of Microbacterium bacteraemia involving six oncological patients has been described by Alonso-Echanove et al. (2001). The first documented case of catheter-related Microbacterium bacteraemia was reported by Lau et al. (2002). Formal identification at the species level and description of Microbacterium paraoxydans was achieved by Laffineur et al. (2003) for a yellow-pigmented coryneform rod isolated from the blood of a patient with leukaemia. In almost all described Aureobacterium or Microbacterium infections, patients were immunocompromised and/or suffering debilitating diseases. In our case, the patient was also immunodeficient because of corticosteroid treatment following heart transplantation. A Gram-positive coryneform bacterium was isolated from his bronchoalveolar aspirate in pure culture, suggesting that the isolate was responsible for the interstitial pulmonary inflammation. The patient recovered after treatment with vancomycin, which had been selected according to the results of in vitro susceptibility testing of the isolate. The case of Aureobacterium infection described by Grove et al. (1999) was treated empirically with cefazidime, gentamicin and imipenem, but the patient recovered only after vancomycin was added.

The isolate from our patient was identified as Corynebacterium sp. and Aureobacterium sp./C. aquaticum by two different commercial identification systems. At present, Aureobacterium species have been proposed for inclusion in the genus Microbacterium (Takeuchi & Hatano, 1998), whilst C. aquaticum is classified in the genus Leifsonia (Evtushenko et al., 2000). C. aquaticum has been defined as strictly motile (Funke et al., 1997b; Leifson, 1962), whilst our isolate was non-motile. Furthermore, the numerical profile obtained by the API Coryne system was very similar to that shown by the type strain of A. liquefaciens (reclassified as M. liquefaciens), whereas it clearly differed from that of the type strain of C. aquaticum (reclassified as L. aquatica). Finally, our clinical isolate proved genotypically distant from the registered sequence of this latter species. In contrast, BLAST and phylogenetic analyses showed high 16S rRNA gene similarities with some species of the genus Microbacterium, namely M. oxydans, M. luteolum, M. foliorum, M. phyllosphaerae, M. liquefaciens (formerly A. liquefaciens), M. saperdae, M. paraoxydans and M. keratanolyticum, the closest phylogenetic neighbours with 98·9–98·1% similarity. On the basis of these results, the isolate can be classified in the genus Microbacterium. With the exception of M. liquefaciens, at least two phenotypic characteristics differentiated our isolate from the most closely related Microbacterium species (Table 1 and references therein), but, unfortunately, the biochemical data available from the literature are incomplete. Since genetic distances among species seem to be very low in the genus Microbacterium (Funke et al., 1998; Schumann et al., 1999), it is possible that our isolate represents a novel species.

Although Laffineur et al. (2003) reported a Microbacterium isolate from a bronchial aspirate, to our knowledge, this is the first detailed study regarding the isolation of a Microbacterium sp. from interstitial pulmonary inflammation. The isolation in pure culture in the absence of other pathogens suggests that the isolate was responsible for the inflammation. Laboratories should always be alert to the possibility of a novel organism when different commercial or conventional systems do not agree on the identification of an isolate. Our results confirm that 16S rRNA gene sequencing provides a powerful tool for the definitive identification of clinical coryneform bacterial isolates (Funke et al., 1994; Grove et al., 1999; Laffineur et al., 2003; Lau et al., 2002; Tang et al., 2000).

References


