

全身紅斑性狼瘡病人之皮膚 *Mycobacterium kansasii* 感染：藉由基因鑑定和型態鑑定診斷

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Cutaneous *Mycobacterium kansasii* Infection in a Patient with Systemic Lupus Erythematosus : Diagnosed by Genotyping and Phenotyping

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Mycobacterium kansasii infections of the skin are rarely seen and present variable clinical and histologic features. Most patients with *M. kansasii* infection have altered immunity, caused either by immunosuppressants or underlying diseases. Laboratory identification of the organism is essential for proper diagnosis and selection of appropriate antimycobacterial medication. We report cutaneous *M. kansasii* infection in a patient with systemic lupus erythematosus. DNA was extracted from skin biopsy specimen and a portion of *HSP65* gene was amplified by polymerase chain reaction (PCR). The DNA sequence of the amplified product was compatible with that of *M. kansasii*. Culture of the skin biopsy specimen also yielded *M. kansasii* in 8 weeks. Treatment including isoniazid, ethambutol and rifampin was instituted right after the diagnosis was established. No new lesions have been noted for three months after initiation of the three combined therapy. (Dermatol Sinica 20 : 160-164, 2003)

Key words: *Mycobacterium kansasii*, Heat shock protein 65 (hsp65)

Mycobacterium kansasii 引起的皮膚感染相當少見而且在臨床和組織學上的表現相當多樣。大多數感染 *M. kansasii* 的病人免疫系統多有異常：可能來自使用免疫抑制劑或有其他內在疾病。實驗室鑑別致病菌對於確立診斷和使用正確的抗分枝桿菌藥物相當重要。我們報告一例全身紅斑性狼瘡之病人皮膚感染 *M. kansasii*。DNA 由皮膚切片的檢體抽取出來，*HSP65* 基因的一部份經由聚合酶鏈鎖反應 (PCR) 放大。放大產物經由直接定序的結果和 *M. kansasii* 的序列資料相符合。皮膚切片去做組織培養在八個禮拜後也長出 *M. kansasii*。診斷確立後病

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人接受 isoniazid, ethambutol 和 rifampin 之治療。在開始併用三種藥物治療之後的三個月內，沒有新的皮膚病灶再出現。(中華皮誌 21 : 160-164, 2003)

INTRODUCTION

Mycobacterium kansasii (*M. kansasii*) is a common and virulent pathogen. However, primary cutaneous *M. kansasii* infection has rarely been reported, totally to only 34 cases since 1965.¹ The identification of *Mycobacterium* species by traditional phenotyping only is sometimes difficult.² Mycobacterial genotyping is based on detecting differences in the genomic nucleotide sequences, and is a very powerful diagnostic tool with high accuracy.³ We report a case of cutaneous *M. kansasii* infection, diagnosed by mycobacterial genotyping and phenotyping, in a patient with systemic lupus erythematosus (SLE).

CASE REPORT

A 34-year-old Taiwanese woman with a 9-year history of SLE and being treated with prednisolone (15 mg/day) and cyclosporine (100 mg/day) presented with a two-week history of indurated erythematous nodules over bilateral thighs and buttocks. Examination revealed four warm indurated nodules on the

posterior aspect of her bilateral thighs (Fig. 1). The nodules measured 2 cm in diameter. She did not have lymphadenopathy. No other household member had experienced pulmonary mycobacterial infection. She denied keeping fish tanks or exposure to untreated well water. Lupus profundus was suspected and a lesion was biopsied. Two weeks later, the skin lesions progressed to 5 indurated nodules on the bilateral thighs and buttocks. Some nodules became fluctuant with pus discharge. One month later, the previous biopsy became ulcerated at the right posterior thigh.

Laboratory examination showed a white



Fig. 1
There are erythematous indurated nodules over bilateral posterior thigh .

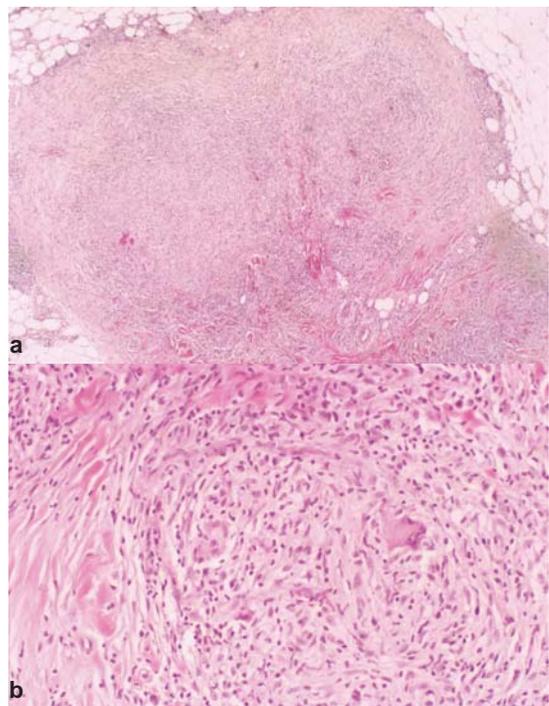


Fig. 2
Histological examination showed granulomatous inflammation in the reticular dermis and subcutis. (a) H & E, x40 (b) H & E, x200

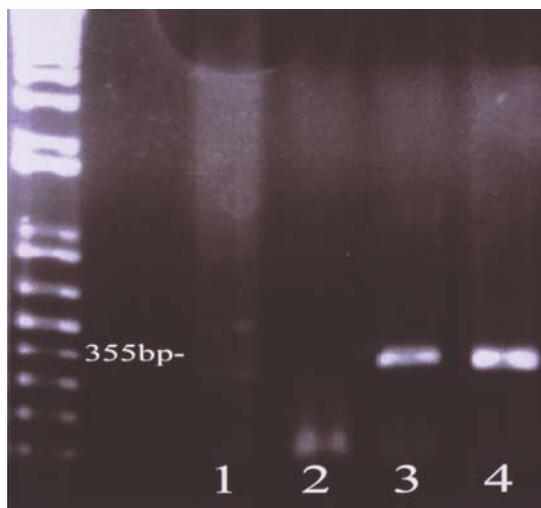


Fig. 3

PCR result of mycobacterium HSP65. Lane 1: buffer control without template DNA. Lane 2: negative control with normal skin DNA. Lane 3: positive control with sequencing proved *M. kansasii* DNA. Lane 4: After a round of 40 cycles, the amplicon in our case is visible as a 355-bp band.

blood cell count of 6350/ μ l (normal 4000-10000/ μ l), anti-nuclear antibody >1:2560 homogenous (normal <1:320 homogeneous), anti-dsDNA 465.16 IU/ml (normal <12.0), C3 72.3 mg/dl (normal 81.61-118.41 mg/dl), and C4 17.5 mg/dl (normal 16.7-38.2 mg/dl). Histopathologic examination of the biopsy specimen revealed granulomatous inflammation in the reticular dermis and subcutis (Fig. 2). Tissue cultures failed to grow any common aerobic or anaerobic bacteria or fungi. Chest radiographs showed no evidence of pulmonary infection. Sputum cultures gave a negative result. The mycobacteria culture of tissue specimen obtained from skin biopsy grew *M. kansasii* in 8 weeks, which was sensitive to rifampin 1.0 μ g/ml, ethambutol 10 μ g/ml, and streptomycin 10.0 μ g/ml. A PCR, using DNA extracted from the biopsy specimen with the method and primers described by Telenti *et al.*⁸, was also performed to amplify a fragment of the gene encoding for mycobacterium heat shock

protein 65 (hsp 65). The demonstration of *HSP65* gene in the biopsy specimen confirmed the mycobacterium infection (Fig. 3). Direct DNA sequencing of the PCR amplicons revealed data compatible with the DNA sequence of *M. kansasii*.^{4,5} A diagnosis of cutaneous *M. kansasii* was thus established and the patient was treated with rifampin (600 mg per day), isoniazid (300 mg per day), and ethambutol (800 mg per day). The nodules and ulcers improved after three weeks of treatment and no new lesions has been found for 3 months after initiation of the three-combined therapy.

DISCUSSION

M. kansasii is a slow-growing, photochromogenic mycobacterium (Runyon group I), first isolated in 1953 by Buhler and Pollak.⁶ *M. kansasii* grows best on Lowenstein-Jensen culture medium held at 37°C. This photochromogen characteristically produces yellow pigment within 24 hours of bright light exposure and has positive catalase and negative niacin reactions. The natural habitat of *M. kansasii* includes water supplies, swimming pools, and sewage.⁷ *M. kansasii* accounts for 3% to 4% of all pathogenic mycobacterial isolates in the United States.⁸

M. kansasii infections are sporadic, with no evidence of person-to-person transmission.⁶ Cutaneous *M. kansasii* infections are rare. The first case was described by Mayberry *et al.* in 1965.^{9,10}

The clinical appearance of cutaneous *M. kansasii* infection is heterogeneous and includes sporotrichoid nodules, pustules, crusted ulcers, verrucous or erythematous plaques, abscess, and seromas.^{3,11} Infections occurred predominantly in middle-aged men^{3,12} as reviewed by Breathnach *et al.*¹² and Czelusta *et al.*³ In majority of the patients, either the upper or lower limbs were affected.¹² In most cases, the infection was confined to one anatomic region. Most cases with infection at multiple skin sites were caused by means of autoinoculation rather than hematogenous spread.¹² The majority of the patients had evidences of altered immunity,

either an iatrogenic condition due to the use of corticosteroid or other immunosuppressants or some underlying diseases. The underlying conditions included human immunodeficiency virus infection, hematologic malignancies, autoimmune diseases, and renal transplantation.^{3,12}

The skin lesions in our patient were initially diagnosed as lupus profundus. Lupus profundus occurs in about 2% of patients with SLE.¹³ Lupus profundus usually manifests as deep nodules or plaques that occasionally ulcerate and are similar to those of cutaneous *M. kansasii* infection. Differentiation in this case relies heavily on histologic examination and microbial studies.³ The histologic pattern of *M. kansasii* infection depends on the stage of the infection and the immune status of the host.¹⁴ In immunocompromised patients, it shows a neutrophilic inflammatory infiltrate with occasional abscess formation. In immunocompetent hosts, a granulomatous infiltrate, with or without necrosis, is seen in more than 90% of cases.¹⁷

Mycobacterial phenotyping is based on evaluating the characteristics expressed during in vitro cultivation. Phenotyping may sometimes be ambiguous to interpret, even by experienced personnel.² Moreover, a discrepancy between the results of phenotyping and genotyping has been reported.^{1,5,15} Genotypic methods for the identification of mycobacteria have been developed in recent years. The *HSP65* gene, which is present in all mycobacteria, is more variable than the 16S rRNA gene in sequence and is therefore useful for the identification of genetically related species. Sequence variations in the *HSP65* gene can be used to identify both slowly growing and rapid growing mycobacteria to species level. It provides a rapid and accurate method for identification of *Mycobacterium* species.¹⁵⁻¹⁸ Both phenotyping and genotyping gave a yield of *M. kansasii* in our case, with a high confidence level.

The most recent treatment guideline described by the American Thoracic Society¹⁹ suggests that cutaneous infection can be treated by the same regimen outlined for pulmonary

disease in adults, i.e., triple therapy with isoniazid (300 mg), rifampin (600 mg), and ethambutol (25 mg/kg for the first 2 months then 15 mg/kg) given daily for 18 months or at least 12 months after clearance of the lesions.¹⁹ The addition of a fourth drug such as clarithromycin or azithromycin has been proposed.²⁰ Some reports demonstrated successful treatment with minocycline²¹ or erythromycin.²² Reduction of the immunosuppressant or steroid dosage may sometimes be required to achieve the therapeutic effect of the antibiotics.¹²

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