

Humoral Response in a Patient with Cutaneous Nocardiosis

Mathias W. Horne^a Arnd Gandorfer^b Jürgen Heesemann^a Andreas Roggenkamp^a

^aMax von Pettenkofer Institute for Hygiene and Medical Microbiology and ^bDepartment of Ophthalmology, Ludwig Maximilian University, Munich, Germany

Key Words

Nocardiosis • Immunoblot • Indirect immunofluorescence • Cutaneous abscess • *Nocardia brasiliensis*

Abstract

The clinical appearance of infection due to *Nocardia* spp. varies widely. The low sensitivity of direct microscopy and the slow growth of the organism challenge the laboratory diagnosis. We present the case of a skin abscess in an immunocompetent man caused by *Nocardia brasiliensis*. Diagnosis was made by cultivation and 16S rRNA sequencing. Using indirect immunofluorescence and Western blot, a strong antibody response to the *N. brasiliensis* isolate could be demonstrated. Serological tests might therefore be useful for the diagnosis and management of nocardial infections.

Copyright © 2000 S. Karger AG, Basel

The spectrum of disease caused by nocardial infections is broad and varies from self-limited local or inapparent infection to an aggressive and possibly fatal disease [1, 2]. The most commonly recognized species *Nocardia asteroides* (sensu lato) is mainly recovered from immunocompromised patients and causes pulmonary or disseminated disease. In contrast, infections due to *Nocardia brasiliensis* are predominantly seen in immunocompetent hosts and typically extend from local infection of the skin but may even-



Fig. 1. Clinical appearance of an abscess due to *N. brasiliensis* in a 58-year-old immunocompetent patient.

usually spread and cause fatal disseminated infection [3].

Currently, the diagnosis of *Nocardia* infection is still mainly based upon direct microscopy and culture of the organism [1]. The low sensitivity of a single microbiological evaluation and the delayed growth of the organism therefore challenge the clinical suspicion for correct diagnosis and optimal treatment. The presented case of a cutaneous nocardiosis caused by *N. brasiliensis* could

be confirmed using biochemical tests and 16S rRNA sequencing [4, 5].

A 54-year-old man without any known underlying disease presented to his physician with a local tender swelling above his right upper eyelid. Two weeks earlier, he had been in Tenerife, Canary Islands, where he had spent 1 week on holiday bathing and walking in the mountainous area of this island. The patient denied any local injury to the site of infection. Few days after his arrival at

home he noticed increasing local redness and swelling. Purulent material was evacuated repeatedly during the following days. He was finally admitted to the ophthalmology department for surgical drainage of this abscess. Physical examination at admission was normal except for local swelling and induration; no fever or lymphadenopathy was detected (fig. 1). Swab samples were taken intraoperatively and grew irregularly stained gram-positive branching filamentous bacteria forming large mycelium-like aggregates. Staining of older cultures showed breaking up of mycelium into coccobacillus-shaped elements. Partial acid-fastness was demonstrated by modified Kinyoun staining (fig. 2). Biochemical differentiation was delayed but identified the organism as being *N. brasiliensis*, which was confirmed by the national reference laboratory. At that time the patient had received parenteral imipenem for 10 days followed after discharge by oral erythromycin for 1 month and reported resolution of all signs and symptoms of infection. This antimicrobial regimen was based on susceptibility testing using the disk diffusion method. No recurrent disease or complication was reported over an observation period of 1 year.

Sulfonamides have been the mainstay of antimicrobial therapy for human nocardiosis. In fact, a resistance rate of 70% was reported for imipenem in one study examining 23 strains of *N. brasiliensis* with the broth microdilution technique, but all isolates showed susceptibility to erythromycin [6]. However, surgical drainage probably contributed predominantly to clinical amelioration.

Infection due to *Nocardia* spp. causes an intense immunological stimulation [7]. To demonstrate this immunological response, immunoblotting was performed using bacterial cell lysate and patient and control sera. The *N. brasiliensis* strain was grown in 100 ml brain-heart infusion broth at 27°C for 48 h. After centrifugation, the pellet was resuspended in 500 µl phosphate-buffered saline, and 50 µl sterile glass beads (Sigma, St. Louis, Mo., USA) were added. Bacterial cells were lysed by three cycles of vigorous shaking with a MM2000 shaker (Retsch, Haan, Germany) for 30 min, interrupted by heating at 100°C for 30 min and freezing at -20°C for 1 h. Unlysed whole cells and large debris were removed by centrifugation at 10,000 g for 10 min. The supernatant was mixed with sample buffer, boiled and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Separated proteins were electrotransferred to a nitrocellulose membrane and an immunoblot was performed using patient



Fig. 2. Modified Kinyoun stain showing partial acid-fastness of *N. brasiliensis*. Magnification ×250.

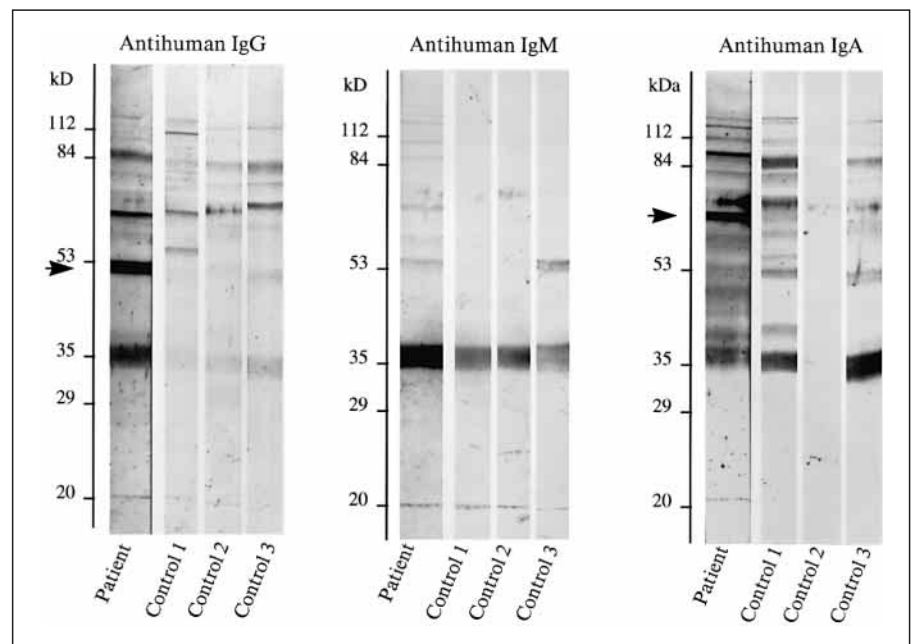


Fig. 3. Immunoblot for IgG, IgM and IgA with bacterial cell lysate and patient as well as control serum from 3 healthy blood donors. A 50-kD (IgG) and a 70-kD (IgA) protein were found to react specifically with the patient's serum.

and control serum and consequent antihuman IgG, IgM and IgA staining. A significant humoral response was found for IgG (a 50-kD protein) and IgA (a 60-kD protein); 3 control samples remained negative (fig. 3). Further-

more an indirect immunofluorescence test was performed and showed a strong humoral response to surface antigens of the isolated *N. brasiliensis* strain in the patient's serum sample whereas the control sera were nega-

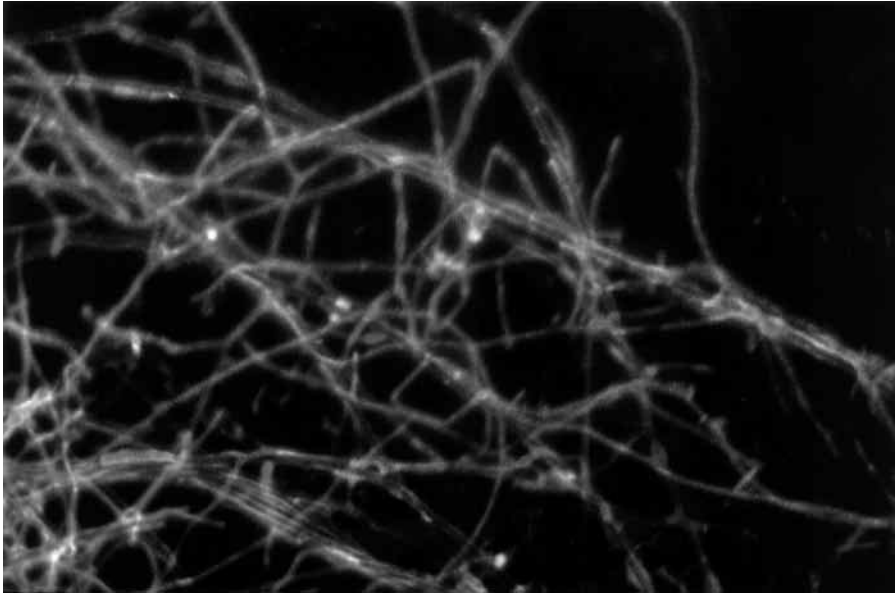


Fig. 4. Indirect immunofluorescence test with *N. brasiliensis*. The first antibody incubation was performed with patient serum (1:50 dilution) followed by FITC-conjugated antihuman IgG. Control sera showed negative staining. Magnification $\times 1,000$.

tive (fig. 4). Several nocardial proteins are under investigation for use in routine diagnostic serology [8, 9]. Isolation and purification of a 61-kD protein (P61) have recently been described for the diagnosis of *Nocardia brasiliensis*. However, serological reactivity against another described immunogenic protein with a molecular mass of 24 kD (P24) could not be demonstrated in the presented case [8].

This report demonstrates the strong humoral response against nocardial antigens even in localized infection. This suggests that serological tests such as indirect immunofluorescence and immunoblot may be of value for diagnostic evaluation and the clinical monitoring of nocardiosis. However, the strong humoral response even to localized infection might also hamper the value of serology due to the high seroprevalence in endemic geo-

graphic regions. Since antigenic cross-reactivity to related bacteria such as mycobacteria and actinomycetes was described, the development of sensitive and specific serological tests against isolated nocardial proteins is needed.

References

- 1 Lerner PI: Nocardiosis. *Clin Infect Dis* 1996; 22:891–905.
- 2 McNeil MM, Brown JM: The medically important aerobic actinomycetes: Epidemiology and microbiology. *Clin Microbiol Rev* 1994;7:357–417.
- 3 Smego RA, Gallis HA: The clinical spectrum of *Nocardia brasiliensis* infection in the United States. *Rev Infect Dis* 1984;6:164–180.
- 4 Kjelstrom JA, Beaman BL: Development of a serological panel for the recognition of nocardial infections in a murine model. *Diagn Microbiol Infect Dis* 1993;16:291–301.
- 5 Boiron P, Stynen D: Immunodiagnosis of nocardiosis. *Gene* 1992;115:219–222.
- 6 McNeil MM, Brown LM, Jarvis WR, Ajello L: Comparison of species distribution and antimicrobial susceptibility of aerobic actinomycetes from clinical specimens. *Rev Infect Dis* 1990; 12:778–783.
- 7 Beaman BL, Beaman L: *Nocardia* species: Host-parasite relationships. *Clin Microbiol Rev* 1994;7:213–216.
- 8 Vera-Cabrera L, Salinas-Carmona MC, Welsh O, Rodriguez MA: Isolation and purification of two immunodominant antigens from *Nocardia brasiliensis*. *J Clin Microbiol* 1992;30:1183–1188.
- 9 Salinas-Carmona MC, Welsh O, Casillas SM: Enzyme-linked immunosorbent assay for serological diagnosis of *Nocardia brasiliensis* and clinical correlation with mycetoma infections. *J Clin Microbiol* 1993;31:2901–2906.