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EXPERT'S CORNER: A PERSONAL APPROACH

Actinomycetoma by *Nocardia brasiliensis*: A neglected disease and a surprising laboratory for experimental medicine

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Introduction

Mycetoma was first described in 1842 in Madura, India, and was known for a long time as Madura foot. This chronic infection may be produced by *Madurella mycetomatis* or *Madurella grisae* (Eumycetoma) fungi. The *Nocardia brasiliensis*, *Actinomadure madurae*, and *Streptomyces somaliensis* bacteria are responsible for the infection known as actinomycetoma. Mycetoma is a chronic infectious disease that produces severe deformation of the infected area, with ulcers, abscesses, and swelling that drains a serous material. The drained liquid may contain micro colonies of the offending microbe that are named granules; the color and morphology of these granules aid in their identification. The painless lesion affects the lower extremities of young males more frequently than females.[1,2]

The diagnosis of mycetoma is made by clinical findings and confirmed by microbiological cultures to isolate the etiological agent. The microbe responsible for the infection can be difficult to identify in part because of the slow growing characteristics of the bacteria or fungi as well as a lack of sensitive molecular tests in clinical use. Serological tests using immunological techniques were used extensively during the second part of past century, without success. This can be now explained by the low sensitivity of the older laboratory test used and by the cross reactivity of crude extracts made of different Actinomycetes. Recently, we developed an ELISA test to detect specific anti-*N. brasiliensis* immunodominant antigens in serum. This ELISA test has been successfully used to confirm the presence of *N. brasiliensis* in infected patients and assess their response to treatment. The anti-*N. brasiliensis* antibody concentration correlates with disease progression and cure, and the ELISA test is now recommended by the American Microbiological Laboratory Techniques Manual.[3,4] Before the ELISA test was created, a large group of patients attended the University Hospital's Dermatology Service in our institution and provided their sera before, during and after their successful treatment. These sera were of great help in identifying the immunodominant antigens of a protein nature from a *N. brasiliensis* batch culture; P61, P24 and P38 is their molecular weights.[5] These three antigens were most frequently recognized by the sera of patients with an active disease, according to the extensive Western blot analysis.[6]

Treatment of an actinomycetoma includes antibiotics, but resistance may appear in some cases, and the need for surgical removal of the affected extremity is frequent. Even though actinomycetoma is a chronic infection produced by bacteria, there is no vaccine for prevention currently available. Also, the taxonomy of Actinomycetes is complex, and the associated morbidity is high. Recently, The World Health

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Organization (WHO) listed Mycetoma as a Neglected Tropical Disease that deserves further attention for rapid diagnosis, to study and develop new treatments and hopefully for prevention through vaccination.

Reproducing the actinomycetoma infection in experimental mice has been of great help in understanding the humoral and cellular immune response to *Nocardia brasiliensis*. We succeed in creating the most useful experimental actinomycetoma model in different strains of mice using the *N. brasiliensis* strain isolated from a local patient by Dr. Oliverio Welsh. This strain, identified at the Center for Disease Control (CDC) by June Brown, is now registered by our group under number ATCC 700358. Immune response to *N. brasiliensis* antigens during active infection have demonstrated a solid systemic antibody and inflammatory cytokine production coexisting simultaneously with local immunosuppression.[7,8] High pro-inflammatory cytokines both in serum and in local infected tissue characterize this experimental model. Granuloma formation around the microcolonies of the *N. brasiliensis* is similar to the ones induced by *M. tuberculosis and M. leprae*, with abundant macrophages, foamy cells, collagen formation, lymphocytes and plasma cells. The local findings of active acute infection with intense neutrophil response coexisting with chronic infection deserves further investigation to clarify this complex interaction. Another interesting finding is a large amount of new vessel formation during the course of the infection, providing an extraordinary model to study angiogenesis in this model. We made possible the isolation and purification of *N. brasiliensis* protein antigens, and also generated monoclonal antibodies. We created a green fluorescent plasmid in the *N. brasiliensis* ATCC700358 as a necessary tool to study and understand the complex host–parasite interaction of facultative intracellular bacteria such as *Mycobacterium tuberculosis*, *Nocardia brasiliensis*, and *Salmonella Typhi* in the host. The actinomycetoma experimental model in mice thus represents a living laboratory to study complex immunological mechanisms of cytokine production, regulation and interaction with other cytokines and with local cells. Also, angiogenesis, granuloma formation, and the mechanisms of foamy cells in host protection can now be addressed using this model.

Information obtained in our experimental mice model can be useful in human patients since the model mimics most of the clinical and immunological findings exactly. Such information is necessary before developing a vaccine to prevent this bacterial disease that is endemic in countries as Sudan, Senegal, Tunisia, Venezuela, and Mexico.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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