RESISTANCE TO NOCARDIA BRASILIENSIS INFECTION IN MICE IMMUNIZED WITH EITHER NOCARDIA OR BCG

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Abstract
Different vaccination procedures to increase the mechanisms of host resistance to Nocardia brasiliensis were studied in mice. When mice were challenged in the footpad, 2 × 10⁸ N. brasiliensis 20 days after footpad inoculation with either viable or killed N. brasiliensis, the mice demonstrated significant resistance to infection when compared with noninfected and nonimmunized mice. The degree of resistance seems to be correlated with the delayed-type hypersensitivity response in the vaccinated animals. Vaccination with another acid-fast bacilli, BCG, afforded both a mild protection and low DTH reactivity. Antibody levels to Nocardia were similar in either Nocardia- or BCG-treated groups indicating that they do not play an important role in resistance to infection by N. brasiliensis.

Introduction
Nocardia brasiliensis is one of the etiological agents of mycetoma; the disease is a chronic granulomatous infection which usually remains localized, with spread due to direct extension through the tissues (6). This mycosis appears as fistulous tumors in which the bacteria take the form of aggregations of mycelia (microcolonies) known as actinomycotic granules (8). Nocardia is considered a facultative intracellular parasite. Once phagocytized it may be killed and digested; it may remain viable and possibly replicate or persist within the cell in an altered form; or the antigens of the bacteria may initiate a cellular and humoral immune response (1). Nevertheless, immunity to infection with those bacteria which can survive and multiply in host macrophages is effected mainly by cell-mediated mechanisms: the development of this type of immunity is invariably accompanied by a state of specific delayed-type hypersensitivity (DTH) to antigens of the infecting organisms and depends on the acquisition by the host of macrophages with increased antibacterial mechanisms (12). Mice infected with N. brasiliensis display DTH reactions to Nocardia antigens which are associated with an increase in mononuclear phagocytic activity (11). The knowledge that animals with DTH are capable of showing an accelerated immune response to reinfection with the homologous organism, even when the delayed sensitivity was induced by injecting dead organisms in oil (4), led us to study the effect of immunization with viable and dead Nocardia on resistance to infection with the homologous micro-organism. In addition, attempts were made to increase cell-mediated immunity (CMI) by BCG vaccination. In this report we demonstrate that stimulation of CMI by means of immunization with the homologous organisms provides a way to importantly diminish the lesions caused by N. brasiliensis infection in the mouse.

Materials and methods

Animals
Carworth Farm strain (CF1) female mice from a colony maintained at the Facultad de Medicina, Universidad
Nacional Autónoma de México, México City, were used at 6–8 weeks of age. The animals were housed in plastic cages and fed Purina Lab Chow and water ad libitum.

Organisms

Strain UPHG-24 of *N. brasiliensis* obtained from the culture collection at the Instituto de Investigaciones Biomédicas, UNAM, México, has been previously described (14). It was grown in Proskauer and Beck medium as modified by Youmans and Karlson (19).

*Mycobacterium bovis* BCG strain was obtained from the Instituto de Higiene, Secretaría de Salubridad y Asistencia, México, D. F.

Purified cytoplasmic extract (PCE) from *N. brasiliensis*.

PCE from *N. brasiliensis* was obtained as described previously (14). In brief, *Nocardia* was ruptured in a Ribi cell fractionator at 30,000 psi and differentially centrifuged. The last centrifugation step was performed at 144,000 × *g* for 3 h. The supernatant fraction was then dialyzed against water, lyophilized, and stored at −20 °C until used.

Infection and immunization

To determine whether *Nocardia* infection conferred resistance to challenge with the homologous microorganism, mice were inoculated in the footpad with 0.1 ml of a suspension containing 2 × 10⁸ viable *N. brasiliensis* incorporated in incomplete Freund’s adjuvant (IFA). For immunization, mice were inoculated in the footpad with 0.1 ml of a colloidal suspension containing 2 × 10⁸ *N. brasiliensis*, which had been autoclaved at 22 psi for 20 min and an equal volume of IFA.

BCG immunization was performed by inoculating 0.1 ml of a colloidal suspension containing 4 × 10⁶ viable microorganisms incorporated into IFA, into the footpad. This dose of BCG has been previously shown to be, in our hands, the optimal dose to generate nonspecific protection against other intracellular organisms (15).

In cases where two immunizations or infective doses were used, the second inoculation was injected subcutaneously into the nuchal area, a week later.

Challenge with *N. brasiliensis*

To determine whether infection or immunization of mice conferred resistance to challenge with the homologous organism, the induction of mycetoma after footpad challenge was investigated. For this purpose, 20 days after the infecting or immunizing dose(s), mice were challenged with *N. brasiliensis* by footpad inoculation of 2 × 10⁸ viable *N. brasiliensis* in 0.1 ml of 0.15 M NaCl. The challenge dose was always given in the limb that have not been previously inoculated. The presence of *Nocardia* was determined by means of direct examination, and by bacteriological and histological studies performed on the challenged footpad of each experimental mouse as described elsewhere (11).

Determination of antibodies to *N. brasiliensis*

All animals were bled from the retroorbital sinus on the day of harvest. Antibodies in sera from each panel of bleedings were determined by indirect hemagglutination (IHA). Sheep erythrocytes were sensitized with PCE from *Nocardia* by the glutaraldehyde method (18). In brief, 1 mg of PCE in 0.5 ml phosphate-buffered saline (PBS) (0.15 M NaCl and 0.001 M sodium phosphate buffer, pH 7.2) was added to 0.1 ml packed, glutaraldehyde-fixed (17) sheep erythrocytes in 5 ml of 0.1 M sodium acetate buffer, pH 5.0, and the mixture was agitated overnight at room temperature. The cells were then washed six times with PBS and stored at 4 °C in PBS with 0.1% sodium azide until use. One-tenth ml of a 2.5% suspension of sensitized erythrocytes were added to 0.1 ml of serial twofold dilutions of the sera in round-bottom microtiter plates (Cooke Engineering Co., Alexandria, Va.); hemagglutination readings were performed after 24 h incubation at 4 °C.

DTH test in the footpad

PCE 50 μg obtained from *N. brasiliensis* in 50 μl of 0.15 M NaCl, was injected into the right hind footpad of the treated or normal mice. In the treated group, the PCE was inoculated in the leg not immunized, infected, or challenged with *Nocardia*. After 24 h the thickness of each hind foot was measured to 0.05 mm with dial-gauge calipers (Starret Co., Athol, Mass.). Histological studies were performed in the skin at test sites in infected and noninfected control mice as previously described (11).

Statistical analyses

The significance of the difference between means was estimated as previously described in detail (11).