

Monoclonal Antibodies to a Specific 54-Kilodalton Antigen of *Nocardia* spp.

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Two monoclonal antibodies (MAbs) of the immunoglobulin G2A isotype, reacting with a *Nocardia*-specific 54-kDa antigen, were generated. As determined by Western blot (immunoblot), both MAbs reacted only with the 54-kDa band. As determined by indirect immunofluorescence or enzyme immunoassay with whole microorganisms, the MAbs did not react with *Nocardia* cells. One of the MAbs showed weak cross-reactivity with mycobacterial antigens, while the other showed no cross-reactivity.

Nocardia are gram-positive, acid-fast-variable, branching, filamentous bacteria. Opportunistic nocardial disease, usually caused by *Nocardia asteroides*, has been associated with patients with an impaired host defense mechanism (17). Infection in normal hosts (16) and nosocomially acquired infections (2) have also been reported.

Most of the serodiagnosis tests that have been developed have met with limited success because of cross-reactions of nocardial antigens with sera from cases of tuberculosis and leprosy (10, 18) or lack of sensitivity (5). In a previous work (7), we partially purified a 54-kDa *Nocardia*-specific antigen which was first detected by Sugar et al. (1, 20). We report here the production and characterization of two monoclonal antibodies (MAbs) to the 54-kDa *Nocardia* antigen as a step toward the standardization of reagents for immunodiagnosis of nocardiosis.

The 54-kDa antigen was partially purified by a previously published method (7) and was used for the immunization of a 3-month-old female LOU/C rat. On day 0, 15 µg of antigen in 350 µl of physiological saline and 550 µl of Freund's complete adjuvant was injected intraperitoneally. Intraperitoneal booster injections of 30 µg of antigen in 600 µl of physiological saline were given on days 14 and 16. On day 16, the spleen cells were fused with rat myeloma cells (IR983F) by using polyethylene glycol 4000 (3) (spleen cells/IR983F ratio, 5:1). Maintenance of hybridoma cultures and expansion of antibody-positive wells were carried out as described by Bazin (3). The selected hybridoma cell lines were cloned by limiting dilution on peritoneal feeder cells.

The production of MAbs in ascites was performed as described previously (12, 19), with minor modifications. A subcutaneous tumor was induced in a 2-month-old LOU/C rat by the injection of 2×10^6 hybridoma cells. Cells of this solid tumor were obtained by homogenization of peripheral tumor tissue in a Potter-Elvehjem homogenizer in phosphate-buffered saline (0.15 M, pH 7.2) (PBS). Two-milliliter aliquots of hybridoma suspensions were injected intraperitoneally into 3-month-old LOU/C.Igk-1b rats additioned with 2 ml of a 1:1 mixture of Freund's incomplete adjuvant and pristane. Clotting was induced by Topostasin (Roche Diagnostics, Division of Hoffmann-La Roche, Inc., Nutley, N.J.). The fluid phase was separated from the clot by centrifugation (10 min, $20,000 \times g$), and lipids were ex-

tracted with trichlorotrifluoroethane. Merthiolate (0.01%) and phenylmethylsulfonyl fluoride (0.8 mg/ml) were added before storage at -30°C .

The MAbs were purified from the ascitic fluid by allotype-specific affinity chromatography on an affinity matrix consisting of MAb MARK3 (Biosys, Compiègne, France) coupled to Sepharose-4B beads (Pharmacia, Uppsala, Sweden) (4, 19).

Screening of MAbs was carried out according to standard enzyme immunoassay (EIA) procedures (8), using partially purified antigen and cell culture supernatants or purified MAbs as primary antibodies. By cloning by limiting dilution and ascites production, two clones were selected for further characterization. These clones were called EB-NA1 and EB-NA2. Both MAbs were of the immunoglobulin G2A isotype, as determined by an indirect double sandwich EIA (13).

The avidities of the selected MAbs were compared by using an indirect EIA procedure (21). Twofold serial dilutions of purified MAbs, starting with a maximal concentration of 80 µg/ml in PBS-Tween 20, were added to the wells.

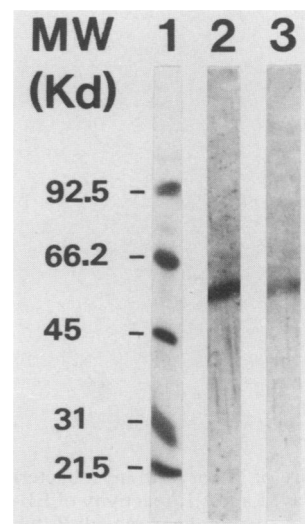


FIG. 1. Reactivity of MAbs with 54-kDa antigen by Western blot assay. Lanes: 1, molecular weight markers; 2, reactivity of EB-NA1; 3, reactivity of EB-NA2.

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TABLE 1. Characterization of MABs to *N. asteroides*

MAB	Immunoglobulin isotype	Reactivity (A_{490}) with 54-kDa antigen ^{a,b}	Avidity constant (1/M)	Reactivity by indirect immunofluorescence	Reactivity (A_{490}) with whole cells ^b
EB-NA1	IgG2A ^c	1.50	10^8	None	0.15
EB-NA2	IgG2A	1.30	4×10^7	None	0.18

^a A_{490} absorbance at 490 nm. Concentration of antigen, 100 ng/ml.

^b Determined by EIA.

^c IgG2A, immunoglobulin G2A.

Each experiment was carried out in triplicate. The avidity constant (K) was calculated as the inverse of the average MAB molarity (1/M) at half maximum binding as determined by the three assays. The avidity constants of EB-NA1 and EB-NA2 were 10^8 /M and 4×10^7 /M, respectively (Table 1).

In addition to the EIA, three methods were used to assess anti-54-kDa-antigen MAB activity. The Western blot (immunoblot) assay was performed as previously described (7). By this technique, only the 54-kDa band was revealed by both MABs (Fig. 1). An indirect immunofluorescence test was made by following the procedure of Jiménez et al. (11). The procedure using the EIA with whole *Nocardia* cells adsorbed on microtitration plates was previously described (6). The serum of a patient with well-defined nocardiosis was used as a positive control for indirect immunofluorescence and EIA with whole *Nocardia* cells. Neither MAB reacted with *Nocardia* cells by either indirect immunofluorescence assay or EIA with whole *Nocardia* cells.

By following the procedure of Matthews and Burnie (14), mycobacterial antigens (kindly provided by G. Marchal, Institut Pasteur) or partially purified nocardial antigen, both at concentrations of 1 and 10 mg/ml, were used in a dot immunobinding assay to check the specificity of the anti-*Nocardia* MABs. No cross-reactivity with mycobacterial antigens, either culture-filtrate antigens or tuberculin, was

exhibited for EB-NA1. For EB-NA2, weak cross-reactivity was observed only with tuberculin (Fig. 2).

The 54-kDa antigen was apparently not exposed to the microbial surface, as shown by indirect immunofluorescence experiments that were negative with all MABs tested. This was confirmed by the lack of reaction of MABs with whole *Nocardia* cells as determined by EIA.

Antigenic factors displaying a specificity for *N. asteroides* were characterized by El-Zaatar et al. (9) by using the enzyme-linked immunoelectrotransfer blot technique adapted to isofocused polyacrylamide gels. The relationship, if any, between the antigens detected by these MABs and the 54-kDa antigen is unknown.

MABs produced by Jiménez et al. (11) showed cross-reactivity with *Mycobacterium tuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, and *M. fortuitum*; this cross-reactivity may be viewed as undesirable for diagnostic purposes. MABs which reacted strongly with *Nocardia* spp. were those that showed the highest degree of cross-reactivity with *Mycobacterium* spp. This was not totally unexpected, because both genera are antigenically related (15) and the antigens used were unpurified whole-cell extracts. However, they recognized surface antigenic components of the *Nocardia* spp., as demonstrated by indirect immunofluorescence.

The 54-kDa antigen was previously characterized as a glycoprotein (18a). When serum specimens were heated for 10 min at 100°C, reaction of MABs with the 54-kDa antigen was abolished (data not shown). This observation suggests that our MABs reacted with the protein part of the antigen and that the 54-kDa epitope was not located on the carbohydrate portion of the molecule.

The MABs selected in this study are candidates for use in the development of a sensitive and specific diagnostic test for nocardiosis.

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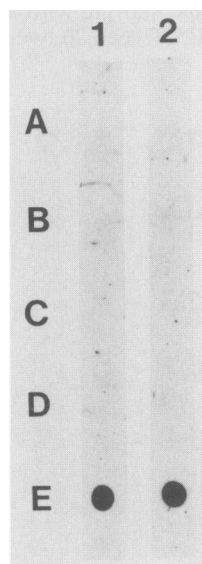


FIG. 2. Reactivity of MABs with mycobacterial antigens by dot immunobinding assay. Lanes: 1, reactivity of EB-NA1; 2, reactivity of EB-NA2. Lines: A, tuberculin, 1 mg/ml; B, tuberculin, 10 mg/ml; C, culture-filtrate antigens, 1 mg/ml; D, culture-filtrate antigens, 10 mg/ml; E, positive control (partially purified 54-kDa antigen, 1 mg/ml).

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