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Monoclonal Antibodies to *Nocardia asteroides* and *Nocardia brasiliensis* Antigens

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Nocardia asteroides **and** ***Nocardia brasiliensis*** **whole-cell extracts were used as antigens to generate monoclonal antibodies (MAbs). Six stable hybrid cell lines secreting anti-*Nocardia* spp. MAbs were obtained. These were characterized by enzyme-linked immunosorbent assay, Western blot (immunoblot), and immunofluorescence assay. Although all the MAbs exhibited different degrees of cross-reactivity with *N. asteroides* and *N. brasiliensis* antigens as well as with culture-filtrate antigens from *Mycobacteria* spp., they have the potential for use as reagents in the purification of *Nocardia* antigens.**

Nocardia asteroides, *Nocardia brasiliensis*, and *Nocardia otitidiscaviarum* can act as primary pathogens in cases of actinomycetoma or appear as causative agents of secondary opportunistic infections (i.e., nocardiosis) in patients suffering from diseases or conditions that suppress their normal immune responses (6). In either case, the diagnosis of *Nocardia* infections relies on obtaining an appropriate clinical sample, which may require the use of invasive procedures, especially in cases of nocardiosis, and the subsequent isolation of the agent in culture followed by its biochemical identification. Growth in primary culture and identification often requires several days to several weeks, as nocardiae are slow growers. Therefore, serological tests for the diagnosis of *Nocardia* infections have been investigated as alternatives to current microbiological laboratory procedures. However, most of the serological tests that have been developed have failed to yield the desired sensitivity or specificity (or both). Cross-reactions of *Nocardia* culture-filtrate or whole-cell-extract antigens with sera from cases of tuberculosis or leprosy (or both) have been reported by several investigators (3, 13), as has a lack of reactivity in sera from documented cases of *Nocardia* infections (3). These findings have led to the search of specific *Nocardia* antigens of diagnostic value.

In 1985, Sugar et al. succeeded in identifying two immunodominant *N. asteroides* antigens by the use of the Western blotting (immunoblot) technique (14). One of them, a 55-kilodalton protein, has been found to be present also in *N. brasiliensis* and *N. otitidiscaviarum* and has been tested in serological assays yielding promising results (1, 2). However, the nature or localization (or both) of those antigens remains unknown.

In 1986, El-Zaatari and co-workers separated *Nocardia* antigens by electrofocusing and used these antigens to generate several monoclonal antibodies (MAbs) (5). Only two of these were found to lack cross-reactivity with mycobacterial antigens. Therefore, the investigators envisioned that they could be used as probes for *N. asteroides* antigens of clinical significance.

In the present work, we report the generation of a series of MAbs to antigens of *Nocardia* spp. These were characterized by enzyme-linked immunosorbent assay (ELISA), Western blot (immunoblot), and indirect immunofluorescence assay. The MAbs were developed for use in the eventual localization, purification, and characterization of *Nocardia* antigens and to possibly serve as diagnostic reagents.

MATERIALS AND METHODS

Nocardia **strains.** *N. brasiliensis* 27-78 and 267-78 were obtained from P. Lavalle (Instituto Dermatológico Pascua, Mexico City, Mexico), and *N. asteroides* N-71 and N-63 were obtained from the Centers for Disease Control (Atlanta, Ga.).

Growth of ***Nocardia*** **strains.** The organisms were grown at 37°C in Sabouraud glucose broth with shaking (150 rpm) in a controlled-environment incubator-shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) for 15 days. At the end of the incubation period, the organisms were removed from the medium by filtration through AE-20 membranes (pore size, 0.8 μm; Schleicher & Schuell, Inc., Boston, Mass.), washed with saline, and used for the preparation of the *Nocardia* antigens.

Preparation of ***Nocardia*** **antigens.** All of the following steps were done at 4°C unless otherwise stated. The organisms obtained were disrupted in a Bead Beater (Biospec Products, Bartlesville, Okla.). An 8-min breakage time yielded approximately 90 to 95% broken cells as determined microscopically by Gram-stained smears. Large cellular debris, intact cells, and glass beads were removed by low-speed centrifugation. The resulting supernatant fluid was further centrifuged at 40,000 × *g* for 15 min. Again, the resulting supernatant fluid was removed and saved, constituting the *Nocardia* whole-cell-extract antigen. Protein determination was carried out on the resulting supernatant fluid by the method of Lowry et al. (11). The supernatant fluids were then dispensed in small portions and kept frozen at −70°C until use.

Mycobacterial antigens. Lyophilized culture-filtrate antigens from *Mycobacterium tuberculosis* Hv37, *Mycobacterium intracellulare*, *Mycobacterium fortuitum*, *Mycobacterium kansasii*, *Mycobacterium scrofulaceum*, and *Mycobacterium avium* were given to us by L. F. Affronti (George Washington University, Washington, D.C.).

Immunization protocol. Four female BALB/c mice (Jackson Laboratories, Bar Harbor, Maine) were injected intra-

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peritoneally with 50 μg of *N. brasiliensis* 27-78, and two mice were injected intraperitoneally with 50 μg of *N. asteroides* 71 antigens in complete Freund adjuvant. At 28 days after the first injection, the mice received a second dose of 50 μg of antigen in saline. Three days after this booster, mice were bled from the retroorbital plexus and sacrificed by cervical dislocation.

Production of hybridomas and isotyping of clones. The technique of Kohler and Milstein was used (8). Briefly, spleens were removed aseptically from the immunized mice and minced. The mincings were washed with RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.), and spleen cells were centrifuged at 1,200 rpm for 8 min. The supernatant fluid was decanted, and cells were suspended in RPMI 1640 and underlaid with Ficoll-Hypaque solution. After centrifugation at 1,200 rpm for 20 min, the lymphocytes and mononuclear cells were removed from the gradient interface. These were washed twice with serum-free RPMI 1640 and were adjusted to a concentration of 2.5×10^6 cells per ml. Murine myeloma cells (SP2/0-Ag 14) were mixed in a ratio of 3:10 with the splenic lymphocytes. The mixed cells were centrifuged at 1,400 rpm for 5 min, the supernatant was decanted, and 1 ml of a 50% solution of sterile polyethylene glycol 1500 (pH 7.8) (Hana Biologics, Inc., Berkeley, Calif.) was added. The pellet was gently shaken and left undisturbed for 2 min, and 1 ml of RPMI 1640 was added. Fifteen milliliters more of RPMI 1640 was then added at a rate of 3 ml/min. Cells were spun down at 1,200 rpm for 4 min, suspended in complete Dulbecco modified Eagle medium (containing 10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, 1.6×10^{-5} M thymidine [HAT medium], and 10% fetal calf serum), and plated into 96-well plates (Costar, Cambridge, Mass.). The cells were fed HT medium (50% volume) on day 5 after the fusion. When the growth of hybrids was detected microscopically (usually 10 to 14 days after the fusion), the supernatant culture fluid was removed and assayed with an ELISA (see below). Cells from antibody-positive wells were transferred to 24-well plates (Costar) in HT medium and then were cloned twice by limiting dilution. Positive subclones were expanded to large cultures or injected into mice to produce ascites and were frozen in liquid nitrogen. Isotyping of the MAbs was done with the Ouchterlony double diffusion test as previously described (12). Monoclonal immunoglobulins from either hybridoma supernatant fluid or ascites were placed in the outside wells of an Ouchterlony plate. The center wells contained goat anti-mouse antibody to either immunoglobulin G (IgG) or IgM (Sigma Chemical Co., St. Louis, Mo.).

ELISA for detection of *Nocardia*-specific MAbs. Indirect ELISAs were performed with disposable polystyrene 96-well microdilution plates (Costar) for the solid phase. The *Nocardia* antigens (and later mycobacterial antigens) were appropriately diluted in phosphate-buffered saline (PBS), pH 7.4, to a concentration of 30 μg/ml. Two hundred microliters of antigen (6 μg) was added per well, and the plates were stored at 4°C overnight. When the plates were used, the wells were emptied by shaking and each well was washed three times with PBS-0.05% Tween 20 buffer.

Sera, culture fluids, or ascites to be tested were appropriately diluted in PBS, and the solution (100 μl per well) was added to the plates. Culture fluids from the original fused hybrid cells were screened undiluted for antibody activity; ascites were diluted 1:4. Plates were incubated at room temperature for 1 h; then they were shaken dry and washed four times with the PBS-Tween 20 buffer. Goat anti-mouse immunoglobulins conjugated with peroxidase (Sigma) were added (100 μl per well; 1:1,000 dilution in PBS with 1% bovine serum albumin), and the plates were incubated for 45 min at room temperature. The conjugate was discarded, plates were washed five times with PBS-Tween 20, and freshly prepared *ortho*-phenylenediamine (40 mg in 100 ml of citric acid-phosphate buffer [24.3 ml of 0.1 M citric acid, 25.7 ml of 0.2 M phosphate, and 50 ml of distilled water] containing 40 μl of 30% hydrogen peroxide) was added (100 μl per well). After 20 min of incubation at room temperature, the reaction was stopped by the addition of 25 μl of 8 N sulfuric acid and the wells were read with an ELISA Minireader II (Dynatech Laboratories, Inc., Alexandria, Va.). Positive, negative, and conjugate controls were included. Readings of more than 0.2 were considered positive.

Immunoblotting procedure. Sodium dodecyl sulfate (10%) gels were prepared by the method of Laemmli (10). Antigen samples containing 60 μg of protein were solubilized in sample buffer and heated for 2 min at 100°C. Electrophoresis was carried out until the dye front reached the bottom of the gel. Molecular weight standards were included in each run. Immunoblotting was performed by a method similar to that of Towbin et al. (16). Briefly, the proteins were electrophoretically transferred to nitrocellulose paper at 100 mA for 19 h. A portion of the membrane containing the molecular weight standards and one lane of each of the antigens was stained with amido black. The other portion of the membrane was incubated with 5% nonfat dry milk in Tris-buffered saline (0.05 M Tris, pH 7.4, containing 0.5 M NaCl; TBS) for 30 min. The sheets were subsequently incubated with antiserum, undiluted hybridoma supernatant fluids, or a 1:100 dilution of ascites and then were washed four times with TBS-05% Tween 20 buffer for 30 min. After this, the membranes were incubated for 1 h with peroxidase-conjugated goat anti-mouse IgG (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) that had been diluted 1:300 in TBS-1% gelatin. This was followed by two washes in TBS-Tween 20 and two washes in TBS. Finally, the antigenic bands were developed by the addition of 4-chloro-1-naphthol and hydrogen peroxide.

Indirect immunofluorescence. Drops (100 μl) of 5-day-old *Nocardia* cultures grown in Sabouraud glucose broth with shaking were placed on clean slides and mixed with 50 μl of 0.01% bovine serum albumin. Smears were carefully prepared, allowed to air dry, and fixed with ethanol, chloroform, and Formalin (60:30:10) for 3 min. The smears were then washed with 95% ethanol for 4 min and twice in distilled water and were allowed to air dry. Fixed smears of *M. tuberculosis* (gift of the Center for Control of Tuberculosis from the San Juan Department of Health, San Juan, P.R.) and *Corynebacterium xerosis* were also treated in the same manner.

Undiluted hybridoma supernatant fluids, a 1:20 dilution of ascites, or a 1:4,000 dilution of mouse polyclonal antiserum in PBS was added to the smears. The samples were incubated for 30 min at 37°C in a humidified chamber. Samples were then washed three times in PBS and later in distilled water. A 200-μl sample of fluorescein isothiocyanate-conjugated goat anti-mouse IgG or IgM (Boehringer Mannheim) diluted 1:40 in PBS and mixed 1 to 1 with a 1:1,000 Evans blue solution (final dilution, 1:80) was then applied to each smear and incubated for an additional 30 min in a humidified chamber. Finally, the slides were washed three times in PBS for 5 min, once with distilled water, and allowed to air dry. Cover slips were mounted with 1 drop of 90% glycerol in PBS. The preparations were examined with a fluorescence microscope (Olympus Corp. of America, New Hyde Park,

TABLE 1. Immunoglobulin isotype and interspecies reactivity of MAbs

MAb[a]	Source of antigen	Source of MAb	Immunoglobulin isotype[b]	A_{490} in ELISA for indicated organism			
				N. asteroides		*N. brasiliensis*	
				71	63	27	267
3.13.10	*N. asteroides*	Ascites	IgM	1.26	1.25	1.20	1.10
3.14.12	*N. asteroides*	Ascites	IgM	1.06	1.12	1.10	1.09
36.6.5	*N. brasiliensis*	Ascites	IgG	1.32	1.20	1.60	1.65
47.2.4	*N. brasiliensis*	Supernatant	IgG	0.49	0.55	0.66	0.72
54.7.2	*N. brasiliensis*	Supernatant	IgM	1.15	1.16	1.45	1.54
54.7.3	*N. brasiliensis*	Supernatant	IgM	1.61	1.50	1.52	1.87

[a] Secreted by subclones selected after the second limiting dilution.
[b] Determined by double immunodiffusion test.

N.Y.), and photomicrographs of stained microorganisms were taken with an Olympus camera.

Cross-reactivity of MAbs with mycobacterial antigens. Lyophilized mycobacterial culture-filtrate antigens, provided by L. F. Affronti, were reconstituted in PBS, and their protein concentrations were determined by the method of Lowry et al. (11). To check the specificity of the anti-*Nocardia* spp. MAbs, the mycobacterial antigens were used in the ELISAs in a concentration of 4 μg per well.

RESULTS

Isolation and characterization of MAbs. Six hybrid cell lines, designated 3.13.10, 3.14.12, 36.6.5, 47.2.4, 54.7.2, and 54.7.3, were recovered from fusions involving the SP2/0 myeloma cells and spleen cells from BALB/c mice immunized as previously described. Hybrid cell lines 36.6.5, 47.2.4, 54.7.2, and 54.7.3 were produced from mice immunized against *N. brasiliensis* whole-cell-extract antigens. The other two cell lines, 3.13.10 and 3.14.12, were produced against *N. asteroides* antigens. The isotypes of the six MAbs secreted by the parental cell lines were determined by double immunodiffusion. MAbs 3.13.10, 3.14.12, 54.7.2, and 54.7.3 were of the IgM isotype, whereas the other two were of the IgG class (Table 1). All of the MAbs reacted with both *N. asteroides* and *N. brasiliensis* antigens when tested by ELISA (Table 1).

Identification of *Nocardia* antigens recognized by the MAbs. The Western blotting technique was used to identify *Nocardia* cellular components which had specific antigenic reactivity for the MAbs. Samples of *N. asteroides* and *N. brasiliensis* whole-cell extracts containing 60 μg of protein were run in 10% sodium dodecyl sulfate-polyacrylamide gels (Fig. 1). The separated polypeptides were then transferred to nitrocellulose sheets for immunological analysis. Table 2 shows the results obtained. MAbs 3.13.10, 3.14.12, 54.7.2, and 54.7.3 recognized more than one polypeptide band in both *N. asteroides* and *N. brasiliensis* antigens (Table 2). In contrast, MAb 47.2.4 only recognized one single polypeptide band in the *N. brasiliensis* preparation. MAb 36.6.5 did not react in the blots.

Indirect immunofluorescence assays. In indirect immunofluorescence assays, the six MAbs tested reacted with both *N. asteroides* and *N. brasiliensis* filaments (Fig. 2). Moreover, the MAbs recognized the bacterial surface (Fig. 2b). When tested with *M. tuberculosis* cells, a high degree of nonspecific fluorescence was observed, making it difficult to assess the degree of reactivity of the MAbs. However, with *C. xerosis*, no fluorescence was observed (Table 2).

Cross-reactivity of anti-*Nocardia* spp. MAbs with mycobacterial antigens. Culture-filtrate antigens from *M. tuberculosis*, *M. intracellulare*, *M. scrofulaceum*, *M. kansasii*, and *M. fortuitum* were used in an ELISA to determine the possible cross-reactivity of the anti-*Nocardia* spp. MAbs with antigens from *Mycobacteria* spp. We determined that all MAbs exhibited different degrees of cross-reactivity with the mycobacterial antigens (Table 3). MAbs 54.7.2 and 54.7.3, which reacted strongly with *Nocardia* spp., were the ones that showed the highest degree of cross-reactivity with *Mycobacteria* spp.

DISCUSSION

The primary purpose of this study was to develop and characterize a panel of MAbs against *N. asteroides* and *N. brasiliensis* that could be used for the analysis of *Nocardia* antigens and, ultimately, for the development of diagnostic tests and in the study of the immune response to *Nocardia* spp.

A total of three fusions were performed between SP2/0-Ag14 myeloma cells and lymphocytes from BALB/c mice immunized with *N. asteroides* or *N. brasiliensis* whole-cell extracts. We obtained six stable hybrid cell lines secreting MAbs which were further characterized by ELISA, Western blot, and the indirect immunofluorescence assay. In ELISA, all the MAbs were found to react with homologous and heterologous *Nocardia* antigens. In addition, they reacted with antigens from various *Mycobacterium* spp. in the

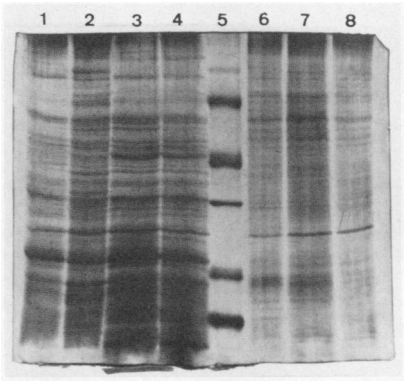

FIG. 1. Silver stain of 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis separated *Nocardia* whole-cell-extract protein antigens. Lanes: 1 and 2, two different batches of *N. brasiliensis* 267-78 antigens; 3 and 4, two different batches of *N. brasiliensis* 27-78 antigens; 5, molecular weight standards (from top to bottom: phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysosyme); 6, *N. asteroides* N-63 antigens; 7 and 8, *N. asteroides* N-71 antigens.

TABLE 2. Differential reactivities of MAbs in ELISA, Western blot, and the indirect immunofluorescence assay

Reagent	A_{490} in ELISA for:		No. of bands in Western blot for:		Presence (+) or absence (−) of fluorescence in indirect immunofluorescence assay for:			
	N. asteroides 71	*N. brasiliensis* 27	*N. asteroides* 71	*N. brasiliensis* 27	*N. asteroides* 71	*N. brasiliensis* 27	*M. tuberculosis*	*C. xerosis*
3.13.10	1.26	1.20	3	11	+	+	±[a]	−
3.14.12	1.06	1.10	3	6	+	+	±[a]	−
36.6.5	1.32	1.60	None	None	+	+	±[a]	ND[b]
47.2.4	0.49	0.66	None	1	+	++	ND	ND
54.7.2	1.15	1.45	10	10	+	++	±[a]	ND
54.7.3	1.61	1.52	1	10	+	++	±[a]	−
Anti-*N. brasiliensis* 27 mouse serum	1.00	1.33	3	4	+	++	ND	ND
N. asteroides 63 mouse serum	1.15	0.68	1	3	−	−	ND	ND
Normal mouse serum	ND	ND	None	None	−	−	ND	ND
PBS	0.00	0.00	ND	ND	−	−	−	−
cDMEM[c]	0.00	0.00	ND	ND	ND	ND	ND	ND

[a] Fluorescence difficult to assess because of background.
[b] ND, Not done.
[c] Complete Dulbecco modified Eagle medium.

ELISA. This was not totally unexpected because both genera are antigenically related (4, 9, 17) and because the antigen was given in Freund complete adjuvant. Since the latter includes killed *Mycobacterium butyricum* cells as part of its formulation, it is possible that shared antigens between *Nocardia* and *Mycobacterium* spp. acted as immunodominant epitopes in the antigen mixture. Although this cross-reactivity may be viewed as undesirable, the fact that the MAbs recognize surface antigenic components of the bacteria, as demonstrated by immunofluorescence, could be of significance in the purification of *Nocardia* spp.-specific antigens, such as the ones described by Sugar et al. (14).

The Western blot experiments with the anti-*Nocardia* spp. MAbs revealed some interesting findings. Four of the six MAbs (3.13.10, 3.14.12, 54.7.2, 54.7.3) detected multiple bands on the blots containing *N. asteroides* and *N. brasiliensis* antigens separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Since those antibodies also reacted with the mycobacterial antigens in ELISA, it is likely that they recognize shared lipid, carbohydrate, or peptide epitopes (7, 17). Moreover, one could infer that the epitopes were located in the surface of the *Nocardia* cells, since the cell walls bound the MAbs as observed in the indirect immunofluorescence experiments.

Of the other two MAbs tested on the blots, one (47.2.4) detected a single protein band in the *N. brasiliensis*-separated antigens, while the other (36.6.5) did not exhibit any reactivity. This discordant behavior of MAb 36.6.5, which gave positive ELISA and indirect immunofluorescence assay reactions, is possibly due to its recognition of conformational epitopes that are lost when the antigens are subjected to the sodium dodecyl sulfate-polyacrylamide gel electrophoresis procedure (15).

While the absolute specificity of MAbs may be an obvious

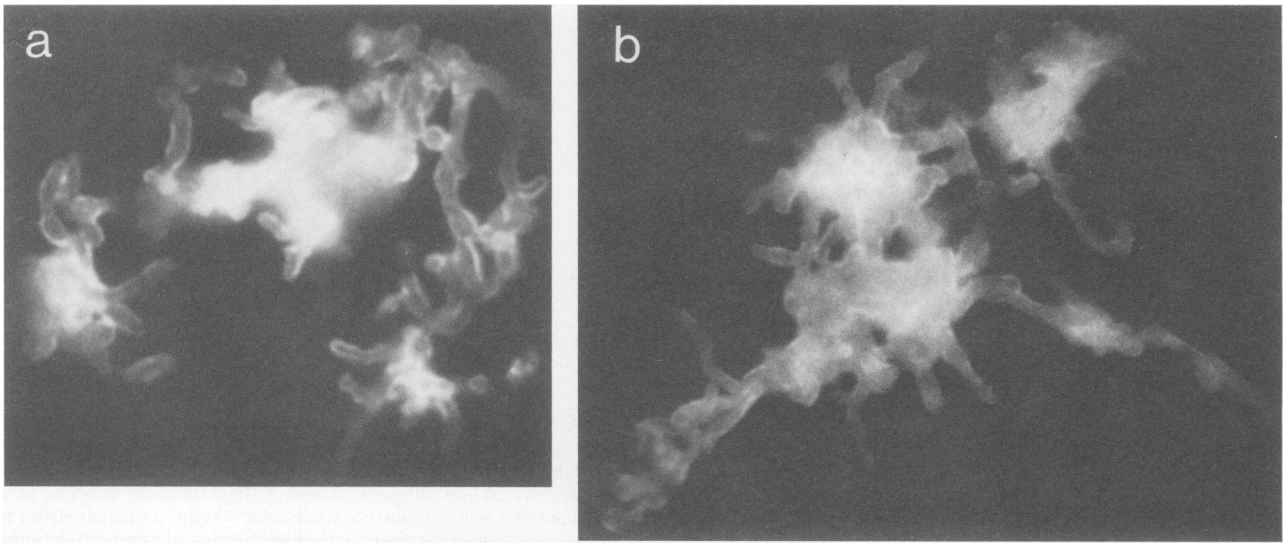

FIG. 2. Indirect immunofluorescence photographs of *N. brasiliensis* 27-78 incubated with heterologous monoclonal antibody 3.14.12 (a) and *N. asteroides* 71 incubated with heterologous monoclonal antibody 3.13.10 (b) (magnification, ×1,200).

TABLE 3. Reactivity of MAbs against mycobacterial antigens as determined by ELISA

Mycobacterial antigen	A_{490} for indicated MAb					
	3.13.10[a]	3.14.12[a]	36.6.5[a]	47.2.4[b]	54.7.2[b]	54.7.3[b]
M. tuberculosis	0.37	0.32	0.26	0.25	1.29	1.58
M. intracellulare	0.33	0.28	0.23	0.22	1.2	1.26
M. scrofulaceum	0.38	0.58	0.31	0.29	0.69	0.77
M. kansasii	0.46	0.57	0.40	0.25	0.95	1.07
M. fortuitum	0.40	0.52	0.31	0.22	0.50	0.60

[a] Analyzed with ascites diluted 1:4.
[b] Analyzed with undiluted culture supernatant fluid.

requirement for a diagnostic test, cross-reactive antibodies such as the ones described here have the potential for application as reagents that recognize shared mycobacterial and nocardial antigens. The use of such cross-reactive reagents could result in the purification of *Nocardia* spp.-specific antigens by affinity chromatography which, in turn, may prove to be of value in the development of a non-cross-reactive, highly specific diagnostic reagent or in the definition of the epitopes responsible for stimulating host cellular reactions during invasive infection.

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LITERATURE CITED

1. **Angeles, A. M., and A. M. Sugar.** 1987. Identification of a common immunodominant protein in culture filtrates of three *Nocardia* species and use in etiologic diagnosis of mycetoma. J. Clin. Microbiol. **25**:2278–2280.
2. **Angeles, A. M., and A. M. Sugar.** 1987. Rapid diagnosis of nocardiosis with enzyme immunoassay. J. Infect. Dis. **155**: 292–296.
3. **Blumer, S. O., and L. Kaufman.** 1979. Microimmunodiffusion test for nocardiosis. J. Clin. Microbiol. **10**:308–312.
4. **Cummins, C. S.** 1962. Chemical composition and antigenic structure of cell walls of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Actinomyces* and *Arthrobacter*. J. Gen. Microbiol. **2**:35–50.
5. **El-Zaatari, F. A., E. Reiss, A. M. Yakrus, S. L. Bragg, and L. Kaufman.** 1986. Monoclonal antibodies against isoelectrically focused *Nocardia asteroides* proteins characterized by the enzyme-linked immunoelectrotransfer blot method. Method. Diagn. Immunol. **4**:97–106.
6. **Harris, G. K.** 1980. Nocardiosis: a literature review and a case report of *Nocardia asteroides* infection. Am. J. Med. Technol. **46**:44–48.
7. **Hunter, S. W., H. Gaylord, and P. J. Brennan.** 1986. Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. J. Biol. Chem. **261**:12345–12351.
8. **Kohler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
9. **Kwapinski, J. B. G., E. H. Kwapinski, J. Dowler, and G. Horsman.** 1973. The phyloantigenic position of *Nocardiae* revealed by examination of cytoplasmic antigens. Can. J. Microbiol. **19**:955–964.
10. **Laemmli, U. K.** 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
12. **Ouchterlony, O.** 1949. Antigen-antibody reaction in gels. Acta Pathol. Microbiol. Scand. **26**:507–515.
13. **Shainhouse, J. Z., A. C. Pier, and D. A. Stevens.** 1978. Complement fixation antibody test for human nocardiosis. J. Clin. Microbiol. **8**:516–519.
14. **Sugar, A. M., G. K. Schoolnik, and D. A. Stevens.** 1985. Antibody response in human nocardiosis: identification of two immunodominant culture-filtrate antigens derived from *Nocardia asteroides*. J. Infect. Dis. **151**:895–901.
15. **Thorpe, R., C. R. Bird, and M. Spitz.** 1984. Immunoblotting with monoclonal antibodies: loss of immunoreactivity with human immunoglobulins arises from polypeptide chain separation. J. Immunol. Methods **73**:259–265.
16. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedures and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
17. **Voiland, A., and G. Michel.** 1985. Structural studies of the cell wall polysaccharide of *Nocardia asteroides* R 399. Can. J. Microbiol. **31**:1011–1018.