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Production of Monoclonal Antibodies to *Naegleria fowleri*, Agent of Primary Amebic Meningoencephalitis

G. S. VISVESVARA,[1]* M. J. PERALTA,[1]† F. H. BRANDT,[1] M. WILSON,[1] C. ALOISIO,[2] AND E. FRANKO[1,3]‡

Divisions of Parasitic Diseases[1] and Host Factors,[2] Center for Infectious Diseases, Centers for Diseases Control, Atlanta, Georgia 30333, and School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27514[3]

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Monoclonal antibodies (MAbs) to *Naegleria fowleri*, the etiologic agent of primary amebic meningoencephalitis (PAM), have been produced and used as probes to identify *N. fowleri* amebae in brain sections of patients who died of that disease. These MAbs were characterized for their specificity by the indirect immunofluorescence assay (IIF), dot immunobinding assay (DIBA), and enzyme-linked immunotransfer blot technique (EITB). The MAbs reacted intensely with all strains of *N. fowleri* tested originating from different geographic areas in the IIF and DIBA tests, but showed no reactivity with four other species of *Naegleria*, *N. gruberi*, *N. jadini*, *N. lovaniensis*, and *N. australiensis*, or a strain of *Acanthamoeba castellanii*. In the EITB assay the MAbs reacted with the antigens of *N. fowleri* and produced intensely staining bands at the 160-, 104-, 93-, and 66-kilodalton (kDa) regions and several minor bands at the 30- and 50-kDa regions. The MAbs also reacted with the antigens of *N. lovaniensis* and produced a darkly staining band at 160 kDa and a diffusely staining band at 116 kDa, indicating that these antigens were shared by the two species. The MAbs, however, showed no reactivity with *N. jadini* and *N. gruberi* in the EITB assay.

Primary amebic meningoencephalitis (PAM) is a fulminating disease of children and young adults that generally leads to death within 5 to 10 days. It is caused by a small free-living ameboflagellate, *Naegleria fowleri*. Of the more than 100 cases of PAM that have been reported worldwide, over 50 have occurred in the United States. Analysis of these cases has revealed that almost all of the infected individuals had a history of swimming in freshwater lakes, pools, or ponds (5, 7). Pathogenic *N. fowleri* has been isolated from various bodies of water (5, 7), particularly those with normally or artificially elevated temperatures (5, 7, 9, 10, 18). The disease is acquired by aspiration of water containing the amebas during swimming or other water sports.

Pathogenic *N. fowleri* trophozoites are indistinguishable from nonpathogenic *Naegleria* species such as *N. gruberi*, *N. jadini*, and *N. lovaniensis* and the mildly pathogenic *N. australiensis* by light microscope. *N. fowleri*, however, differs from *N. gruberi* and *N. jadini* in its ability to grow at higher temperatures (4, 5, 7) and its antigenic structure (4, 5, 7, 15). *N. fowleri* cannot be easily distinguished from *N. lovaniensis* because both species grow at elevated temperatures, cause cytopathic effects on cell culture, and are antigenically very similar (9).

Kohler and Milstein (6) developed a method to produce large quantities of monoclonal antibodies (MAbs) of defined specificity. MAbs developed by this method have been successfully used to detect *Toxoplasma gondii* antigens in human sera, *Trypanosoma cruzi* antigenemia in mice, and circumsporozoite proteins in *Plasmodium*-infected mosquitos (3). The development of MAbs to *N. fowleri* would potentially be a useful diagnostic tool for identifying *N. fowleri* amebae in human tissue and the environment and for differentiating species of *Naegleria* isolates. In this report, we describe the development of 14 cell lines that secrete MAbs to *N. fowleri* made by fusing myeloma cells with spleen cells derived from BALB/c mice immunized with *N. fowleri* trophozoites. High-titered ascitic fluids were produced in BALB/c mice by intraperitoneal (i.p.) injection of five of these cell lines. Finally, we used these MAbs as probes to identify *N. fowleri* amebae in brain tissues of PAM cases and to distinguish *N. fowleri* from other *Naegleria* species by the indirect immunofluorescence technique (IIF), dotimmunobindingassay(DIBA),andenzyme-linkedimmunoelectrotransfer blot (EITB) assay.

MATERIALS AND METHODS

Amebae. Eight strains of *N. fowleri* (CA-66, HB-1, HB-3, HBWS-1, HB-5, 01230, PA90, and MD), two strains of *N. lovaniensis* (Aq/9/1/45D and 76/15/250,) two strains of *N. gruberi* (EG and NB-1), and one strain of *N. jadini* were grown in modified Nelson medium as described previously (15), except the concentration of fetal bovine serum was reduced from 10 to 5%. *N. australiensis* was grown in TYPH medium (0.5% Trypticase [BBL Microbiology Systems], 1% yeast extract, 1% Panmede, 1% glucose, 0.0025% hemin, 0.00132 M potassium phosphate [monobasic], 0.014 M sodium phosphate [dibasic], and 10% fetal bovine serum). Hemin was first dissolved in 2.5 ml of triethanolamine and then added to the medium, and the pH was adjusted to 7.2. The medium was filtered through Whatman no. 1 filter paper and autoclaved at 121°C for 15 min; when cooled, 10% fetal bovine serum was added aseptically. *Acanthamoeba castellanii* ATCC 30001 was grown in proteose-peptone-yeast extract-glucose (PYG) medium (14). *N. fowleri* CA-66, HB-1, and HB-3 and *N. gruberi* EG were obtained from the late professor W. Balamuth; *N. fowleri* 01230 and PA90 and *N. jadini* were obtained from Professor Jadin; and the two *N. lovaniensis* strains were obtained from J. DeJonckheere. *N. fowleri* MD was isolated in 1980 from the cerebrospinal fluid (CSF) of a 14-year-old girl at the University of California, Los Angeles, and strains HBWS-1 and HB-5 were

* Corresponding author.
† Present address: Instituto de Microbiologia da UFRS, Rio de Janeiro 21941, Brazil.
‡ Present address: Regional Laboratories, Georgia Department of Human Resources, Atlanta, GA 30303.

TABLE 1. Origins and pathogenicities of selected strains of *Naegleria* spp. used in this study

Species	Strain	Place of origin	Host or source	Mouse pathogenicity
N. fowleri	CA-66	Australia	Human	4+
	HB-1	Florida	Human	4+
	HB-3	Czechoslovakia	Human	4+
	HBWS-1	Georgia	Human	4+
	HB-5	Texas	Human	4+
	01230	Belgium	Human	4+
	PA90	Australia	Domestic water supply	4+
	MD	California	Human	4+
	NY	New York	Human	
N. australiensis	ATCC 30958	Australia	Flood drainage water	2+
N. lovaniensis	Aq/9/1/45D	Belgium	Thermally polluted water	−
	76/15/250	Belgium	Thermally polluted water	−
N. jadini	0400	Belgium	Private swimming pool	−
N. gruberi	Eg	California	Soil	−
	NB-1 (= 1518/1A)	United Kingdom	Soil	−

isolated at the Centers for Disease Control in 1978 from a 14-year-old girl from Georgia (8) and from the CSF of a 17-year-old girl from Edinburgh, Tex. All strains were grown at 37°C except *N. gruberi*, *N. jadini*, and *A. castellanii*, which were grown at room temperature. The origins and pathogenicities of the various strains are described in Table 1.

Immunization of mice. Initial attempts to produce hybridomas by fusing myeloma cells with spleen cells obtained from lethally infected mice failed. Since the infected mice died with typical signs of PAM 4 to 6 days after intranasal instillation of 10,000 *N. fowleri* HBWS-1 trophozoites, they probably did not have time to produce antibodies. Therefore, four 8-week-old female BALB/c mice were injected i.p. with a mixture of 10^5 washed *N. fowleri* HBWS-1 trophozoites and Freund complete adjuvant in a volume of 0.1 ml.

Fifteen days later, the mice were given a second i.p. injection of 0.1 ml of sonicated amebae (without Freund adjuvant) containing about 40 to 50 μg of protein. The four mice were bled from the orbital sinus 4 days after the booster shot, and the serum was separated and tested for antibody activity by the IIF test.

Cell fusion. Spleens from two mice that were IIF positive were removed and teased, and the cells were extracted. The spleen cells were suspended in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2 mM glutamine, 2×10^{-5} M 2-mercaptoethanol, and 10% fetal bovine serum (complete RPMI), counted, and centrifuged at $150 \times g$. The sedimented spleen cells were fused with SP-2/OAG14 myeloma cells as previously described (6). After 2 to 3 weeks of hybrid cell growth, the culture supernatants were screened for antibody activity by the IIF technique. Hybrid cultures that were positive for antibody activity were cloned and recloned by limiting dilution in 96-well plates with 10^5 normal spleen cells per ml as the feeder layer.

Ascitic fluid. Antibody-producing clones were injected i.p. into BALB/c mice (0.5 ml containing 10^6 cells per mouse) to produce ascites. The mice had been injected i.p. with 0.5 ml of pristane (2,6,10,14-tetramethyl pentadecane; Aldrich, Milwaukee, Wis.) 7 to 10 days earlier. Ascitic fluids derived from one clonal culture were pooled and centrifuged at $600 \times g$ for 30 min to sediment cells. The supernatant was passed through membrane filters (0.20-μm pore size), the antibody titer was determined, and the supernatants were stored frozen in small quantities under the vapor phase of liquid nitrogen. The antibody-producing clones were also frozen as described above in complete RPMI medium containing 10% dimethyl sulfoxide.

IIF test. Antibody activity in the sera of immunized mice, culture supernatants of hybrid cells, and ascitic fluids was analyzed by the IIF test. The IIF test was performed by using 1% Formalin-killed amebae affixed to each well of 12-well Teflon-coated slides. Sera from mice were diluted serially twofold beginning at 1:2. The hybrid cell culture supernatants were used undiluted and at a 1:10 dilution. The ascites were diluted serially fourfold, beginning at 1:16, with phosphate-buffered saline (PBS), pH 7.6, as the diluent. One drop of each dilution of the test sample was transferred to individual wells of the antigen slides, and the slides were incubated for 30 min at 37°C. The IIF test was performed as described previously (17). Fluorescein-conjugated immunoglobulin G (IgG) fraction of goat anti-mouse immunoglobulins (IgA, IgG, and IgM) (lot 17,543; Cappel Laboratories, West Chester, Pa.) was used at a dilution of 1:50. Evans blue diluted at 1:25,000 was used as the counterstain.

The IIF test was also performed on 6-μm tissue sections cut from Formalin-fixed paraffin-embedded brain tissue from humans as well as from mice that had died of PAM after they were inoculated with *N. fowleri*. The sections were deparaffinized, hydrated, and washed once in PBS before they were covered with 0.5-ml amounts of the appropriate ascitic fluids diluted 1:1,000. The slides were incubated at 37°C for 30 min, washed three times with PBS, and allowed to dry. The sections were covered with the fluorescein-conjugated anti-mouse immunoglobulin diluted 1:50 in PBS containing 1:25,000 Evans blue, incubated for 30 min, washed three times in PBS as before, and mounted in phosphate-buffered glycerol, pH 8.2. The slides were examined and photographed with a Leitz Ortholux fluorescence microscope equipped with Ploempak and mercury vapor lamp.

Immunodiffusion. For determining the antibody class and subclass of the MAbs, the supernatant from each clone was allowed to react with specific antisera to IgA, IgM, IgG1, IgG2a, IgG2b, and IgG3 in the Ouchterlony double-diffusion system.

DIBA. DIBA was performed by the method of Bennet and Yeoman (1) with certain modifications as described by Franko (E. A. Franko, Ph.D. thesis, University of North Carolina, Chapel Hill, 1984). Briefly, nitrocellulose paper (BA85; Schleicher & Schuell, Inc., Keene, N.H.) was cut into strips measuring 116 by 78 mm so they would fit a microtiter plate (Costar, Cambridge, Mass.). The nitrocellulose strip was placed on the lid of a microtiter plate that was

illuminated from beneath, and the strip was marked on the edges with ink to aid in alignment. Either the desired antigen sample(s) or urea buffer (2 μl) was placed as a dot in the center of each of the wells that appeared as reliefs on the nitrocellulose strip. Amebic antigens were prepared by sonicating the washed amebae in 8.0 M urea buffered with 0.05 M Tris, 0.3 M KCl, and 2 mM EDTA, pH 8.0, and the protein content was assayed by the Bradford method (2). The antigen sample and the diluent (urea buffer) alternated in the wells. The strips were dried at room temperature and then soaked for 2 h at 37°C in TBS buffer (20 mM Tris hydrochloride, 150 mM NaCl), pH 7.4, containing 10% horse serum (blocking solution [11]). The strips were dried at room temperature to minimize nonspecific binding of serum protein.

The microtiter plates, before they were used, were also filled with the blocking solution and allowed to stand at 37°C for 2 h. After the solution was aspirated from the wells, the plates were stored at 4°C until used, usually within 2 days. The ascites were diluted with blocking solution, and 100 μl of each dilution was added to alternate wells of the plate. The nitrocellulose strip was moistened in the blocking solution, blotted to remove excess fluid, placed face downward on the plate, and sealed with Parafilm (American Can Co., Greenwich, Conn.). Several layers of bibulous paper followed by the plate lid were then placed on the Parafilm, and the whole package was held tightly with two large clamps. The package was inverted, tapped firmly to eliminate air bubbles, and shaken for 4 h at room temperature. The package was then disassembled, and the nitrocellulose strip was washed first with TBS containing 0.05% Nonidet P-40 (Sigma Chemical Co., St. Louis, Mo.) for 1 min with sharp agitation and subsequently three times with washing buffer for 15 min per wash.

After the final wash, the nitrocellulose strips were incubated for 2 h in a 1:1,000 dilution of peroxidase-conjugated goat anti-mouse IgG in blocking buffer. The strips were then washed three times in TBS for 10 min per wash. Color was developed by reacting the strip with TBS solution containing 3 mg of 4-chloro-1-naphthol (Aldrich Chemical Co.) per ml and a 0.01% (vol/vol) of 30% H_2O_2 for 10 min. A positive reaction appeared as a purple-blue dot at the site of antigen dotting. No colored spot was seen when negative serum or buffer was used. The strips were finally washed in several changes of distilled water, air dried, and stored in the dark.

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Tsang et al. (13) on vertical slab gels containig a gradient of 5 to 20% acrylamide and with a discontinuous buffer system. The sonicated *Naegleria* antigens were centrifuged at 20,000 × *g* for 30 min at 4°C, and the supernatants were collected and stored in small portions at −70°C. Antigen samples were treated with a solution containing 10% SDS, 9 M urea, and 0.05 M Tris hydrochloride, pH 8.0, to obtain a final concentration of 1% SDS and 1 μg of protein per μl. Ten microliters of bromphenol blue solution (0.5% bromphenol blue in 80% glycerol buffered with 0.05 M Tris hydrochloride, pH 8.0) was included in each 100 μl of sample before heating for 10 min at 65°C in a water bath. Gels prepared for visualization of components were loaded with 0.25 μg of protein per mm of gel and silver stained (12) after electrophoresis. Gels prepared for EITB were loaded with 1 μg of protein per mm of gel and electrophoresed.

EITB. Separated proteins were electrophoretically transferred to nitrocellulose membrane (Schleicher & Schuell; BA83, 0.2-μm pore size) at a constant voltage of 100 V for 1 h. The membranes were washed four times for 5 min each in PBS-T (PBS, pH 7.2, containing 0.3% Tween 20) and cut into 3-mm strips that were incubated overnight at room temperature while being rocked in the appropriate ascitic fluid diluted 1:100 in PBS-T. The strips were then washed four times, 5 min per wash, in PBS-T and incubated at room temperature in a 1:2,000 dilution of affinity-purified goat anti-mouse IgG conjugated with horseradish peroxidase. After three changes in PBS-T and one wash in PBS without Tween, the strips were developed for 10 min in a solution containing 5 mg of 3,3'-diaminobenzidine tetrahydrochloride and 10 μl of 30% H_2O_2 in 100 ml of PBS, pH 7.2. They were then washed with distilled water and dried at room temperature. Five MAbs were tested against antigens of five *N. fowleri* strains, two *N. gruberi* strains, and one strain each of *N. lovaniensis* and *N. jadini*.

TABLE 2. Reactivities of MAbs with selected strains of *N. fowleri*

MAb	Isotype	Reactivity[a]							
		CA-66	HB-1	HB-3	HBWS-1	HB-5	01230	PA90	MD
IV-DI-20	IgG1	1,024	512	2,048	1,024	1,024	1,024	1,024	512
IV-DI-23	IgG1	1,024	1,024	1,024	1,024	1,024	1,024	1,024	1,024
IV-DI-27	IgG1	1,024	512	1,024	1,024	1,024	1,024	1,024	1,024
IV-DI-30	IgG1	1,024	512	1,024	1,024	1,024	1,024	1,024	1,024
IV-DI-35	IgG1	1,024	512	1,024	1,024	1,024	1,024	1,024	1,024

[a] Reciprocals of highest dilution of the MAbs that gave a 1+ reaction in the IIF test.

RESULTS

Antibody response of immunized mice. Sera obtained from immunized mice were assayed for antibody activity with the HBWS-1 strain of *N. fowleri* as the antigen in the IIF test. Pooled sera from unimmunized mice served as negative controls. The fluorescence responses of the sera were graded on a scale of 1+ to 4+, with 4+ representing the brightest fluorescence. The reciprocal of the highest dilution of the test serum that reacted at 1+ was defined as the titer of that serum. The normal pooled mouse sera did not show any fluorescent activity even at a 1:2 dilution. Sera from all four immunized mice exhibited a 2+ reaction at the 1:16 through 1:64 dilutions. Thereafter the fluorescence intensity decreased with the dilution of the serum. Sera from two mice gave titers of 512, whereas the other two sera gave titers of 64 and 128, respectively.

Isolation of antibody-producing hybrids. Macroscopic and microscopic examination of the 96 wells revealed growth of hybrid cells in 21 wells. IIF analysis of the supernatants from these wells revealed 4+ antibody activity in one well (IV-D1) and 1+ or ± activity in four other wells (III-A3, IV-D1B, II-A1, and II-B4). Hybrid IV-D1 was selected for further study and cloned by limiting dilution. Of the 400 wells examined, growth was observed in 40 wells, and supernatant from 16 wells was positive for anti-*N. fowleri* antibody. Five of these clones (IV-D1-20, -23, -27, -30, and -35) were selected for further study.

When reacted with antisera specific for various mouse immunoglobulin classes or subclasses, the supernatant fluid from these five clones produced precipitin lines only against anti-IgG1. All five MAbs reacted 4+ with the eight strains of *N. fowleri* tested but not with *N. australiensis*, *N. gruberi*, *N. jadini*, *N. lovaniensis*, or *A. castellanii*. The extent of

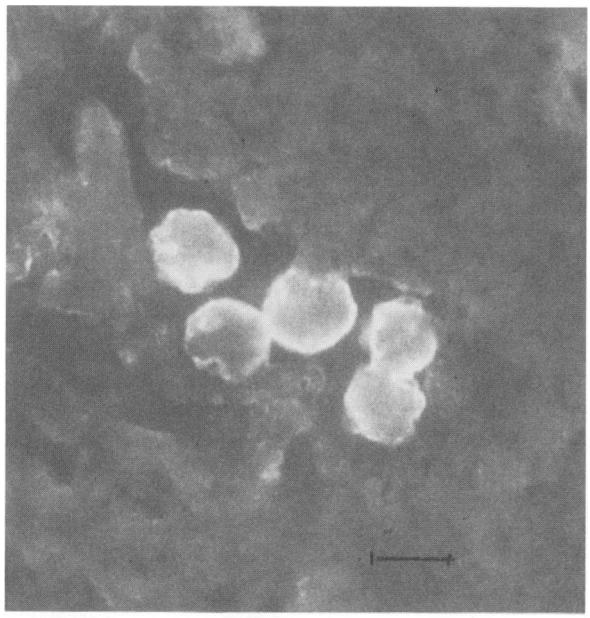

FIG. 1. IIF staining of *N. fowleri* amebae in brain section from a patient with PAM. The section was first treated with MAb IV-DI-30 and then with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulins. Bar, 10 μm.

reactivity of the MAbs with the various strains of *N. fowleri* is shown in Table 2.

All five MAbs identified amebae in the brain sections from PAM patients as well as those of mice experimentally infected with *N. fowleri* at a dilution of 1:1,000. The trophozoites in the sections fluoresced bright apple green (Fig. 1), whereas the surrounding tissues showed no fluorescence. The MAbs did not react with the amebae in the brain sections of patients who had died of granulomatous amebic encephalitis due to *A. castellanii*.

DIBA. A checkerboard titration was performed to determine the optimal concentrations of the antigens and conjugate. A range of concentrations of the antigens from 5.0 to 0.125 μg per spot and conjugate dilutions from 1:500 to 1:15,000 was evaluated. On the basis of the checkerboard titration, the optimum concentration of antigen was 0.5 μg per spot and the optimum concentration of the conjugate was 1:5,000. Positive reactions were visualized as a purple dot. The intensity of the color varied from dark purple when the MAb was used at a 1:16 dilution to pale blue when the dilution of the MAb was 1:2,048. Total disappearance of color occurred at a dilution of 1:4,096. Neither of the negative controls (irrelevant ascitic fluid and normal mouse serum) reacted with the dotted antigens at a dilution of 1:16. Reactivities of the five MAbs with *N. fowleri* antigens are shown in Fig. 2. The titers obtained with DIBA were comparable to those obtained with IIF. The antigenic extracts from the other four species did not shown any reactivity, even at a dilution of 1:16.

SDS-PAGE. Figure 3 shows the silver-stained protein bands of nine *Naegleria* isolates resolved on an SDS-PAGE gel. All five strains of *N. fowleri* originating from different geographic areas appear to be very similar except for some minor differences, indicating the molecular homogeneity of these strains. In contrast, the protein profiles of the other *Naegleria* strains appear to be distinctly different not only from those of *N. fowleri* but also from one another. Even the two strains of *N. gruberi* appear to be distinct from each other in their protein profile. All five strains of *N. fowleri* had multiple major protein bands with relative molecular weights

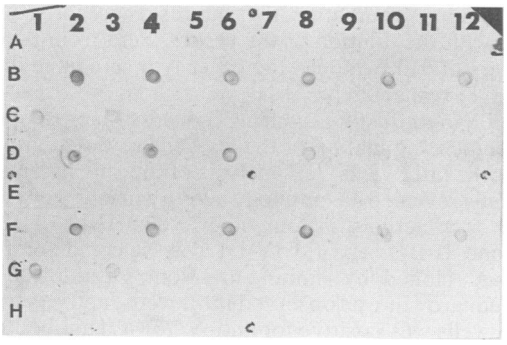

FIG. 2. DIBA patterns of MAbs IV-DI-20, -27, and -28 in reaction with *N. fowleri* antigens. Odd-numbered wells in rows A, C, E, and G and even-numbered wells in rows B, D, F, and H were spotted with *N. fowleri* HBWS-1 antigen (0.5 μg per spot). Row A: Wells 1, 3, and 5 received normal mouse serum at 1:16 dilution, and wells 7, 9, and 11 received irrelevant ascites at 1:16 dilution. Rows B and C: Wells received MAb IV-DI-20. Rows D and E: Wells received MAb IV-DI-27. Rows F and G: Wells received MAb IV-DI-28. All test wells in row H received buffer.

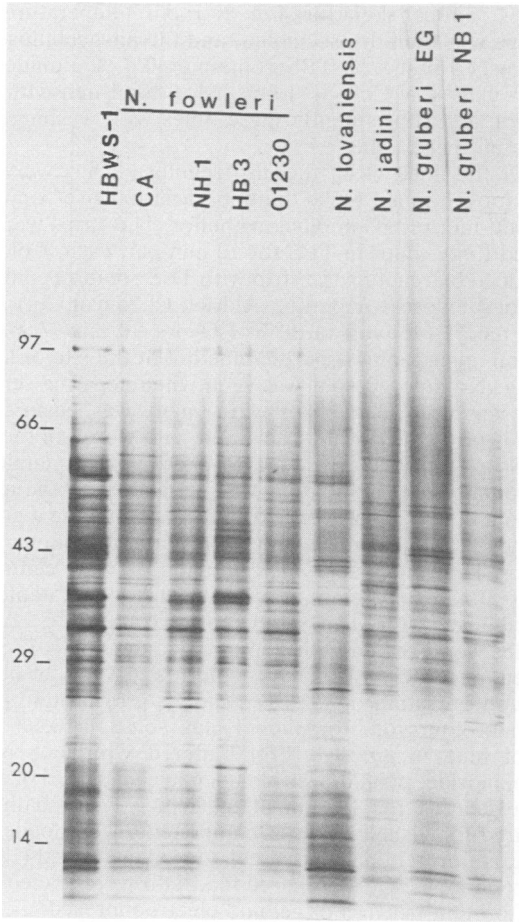

FIG. 3. Patterns of *Naegleria* spp. antigens resolved by SDS-PAGE. Positions of size markers (in kilodaltons) are indicated. CA, CA-66.

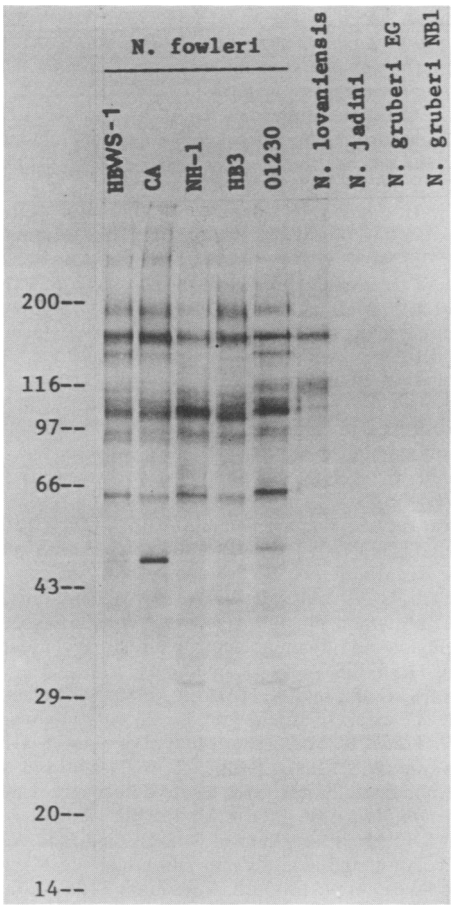

FIG. 4. EITB patterns depicting the reactivity of MAb IV-DI-30 with nine *Naegleria* isolates belonging to four species. Positions of size markers (in kilodaltons) are indicated. CA, CA-66.

(MWs) of 97,000, 36,000, 34,000, 30,600, and 10,000. *N. lovaniensis* shared with *N. fowleri* certain proteins with MWs of 52,000 and 34,000, but had unique bands at 39,000, 31,400, and 25,800 MW. The two strains of *N. gruberi* and *N. jadini* shared with *N. fowleri* and *N. lovaniensis* certain proteins in the low-MW range (20,000 MW) but differed considerably in those proteins with higher MWs.

EITB. Figure 4 shows the reactivities of MAb 30 with the antigens of various *Naegleria* spp. and strains. This MAb, representative of the five MAbs tested by immunoblot, reacted intensely with antigens at 160,000, 104,000, 93,000, and 66,000 daltons and moderately with an antigen at 190,000 daltons for all five *N. fowleri* strains. This MAb also reacted with two antigens of *N. lovaniensis* at 160,000 and 116,000 daltons. It did not react with antigens of either *N. jadini* or *N. gruberi*.

DISCUSSION

We describe the successful production of MAbs to *N. fowleri*. These MAbs were characterized by several different techniques, including IIF, DIBA, and EITB assays. The results of these analyses indicate that the five MAbs are identical in their specificities and the clones secreting these MAbs were derived from one clone.

Eight strains of *N. fowleri* originating from different geographic areas were found to be antigenically homogeneous, as demonstrated by the reactivities of these strains with all five MAbs in all of the tests.

In the IIF test, all five MAbs reacted with all eight *N. fowleri* strains and produced titers ranging from 512 to 2,048. Interestingly, the entire surface of the trophozoite antigens fluoresced uniformly at lower dilutions of the MAbs, but at the higher dilutions fluorescence was confined to the surface membranes. None of these MAbs reacted even at the 1:16 dilution with any of the other *Naegleria* species or *A. castellanii*, indicating species specificity.

In the DIBA, all five MAbs also reacted with *N. fowleri* HBWS-1 and MD and produced titers ranging from 1,024 to 2,048. The intensity of the color developed when NF-27 was interacted with the antigens was slightly less than that developed by the other MAbs. None of the five MAbs reacted with the other *Naegleria* species, indicating once again the species specificity of these MAbs.

We have also shown that these MAbs can be used successfully to identify trophozoites of *N. fowleri* in the brain sections of patients who died of PAM and also in the brain sections of mice that were infected experimentally with the HBWS-1 strain of *N. fowleri*. These MAbs, however, did not cross-react with amebae in brain sections from patients who died of granulomatous amebic encephalitis due to *A. castellanii*. This demonstrates the specificity of these MAbs, and therefore these MAbs can be successfully used to differentially diagnose PAM infections retrospectively.

The EITB assay with the five MAbs to *N. fowleri* HBWS-1 revealed that the five strains of *N. fowleri* shared antigens at 160, 104, 93, and 66 kilodaltons (kDa). Minor differences were observed within the strains, particularly in the region of 30 to 50 kDa. Such intraspecific differences have been reported before, especially for esterase enzymes of *N. fowleri* (16). None of the MAbs reacted with other species of *Naegleria* (one strain each of *N. australiensis*, *N. lovaniensis*, and *N. jadini* and two strains of *N. gruberi*) in the IIF test and DIBA. However, in the EITB assay the five MAbs tested reacted intensely with two antigens from *N. lovaniensis* of 160 and 116 kDa, as well as several minor antigens that were also observed in *N. fowleri* strains. The reasons for the cross-reactivity observed between these two species in EITB but not in DIBA or IIF tests are not clear.

Currently the diagnosis of PAM is dependent on recognition of the amebae in the CSF and an astute microscopist with adequate training and experience to distinguish amebae from other host cells. In addition, it is possible that in the early stages of the disease process, amebic antigen rather than intact amebae may be present in the CSF. In such cases, a test that can detect amebic antigen in the CSF would greatly facilitate early detection of the disease so that appropriate therapy could be instituted. The DIBA may prove to be an ideal test in such a situation. For example, in the DIBA the checkerboard titration assay revealed that 0.5 μg per spot was the optimum concentration of the antigens. However, a careful analysis of the test revealed that at lower dilutions, for example, 1:16, the MAbs were able to detect antigens with concentrations of ≤0.125 μg per spot. This indicates that a test with these MAbs may be successfully developed to detect even nanogram quantities of *N. fowleri* antigens in the CSF of patients with PAM. Such a test will greatly facilitate early detection of the disease so that intervention with appropriate therapy may be instituted. PAM due to *N. fowleri* is now almost always fatal (4, 5, 7, 8).

In the environment, especially in thermally polluted lakes, power plant effluents, etc., both pathogenic and nonpathogenic *Naegleria* spp. are found (4, 5, 7, 9, 10, 18). In the

absence of any clear-cut morphologic differences between *N. fowleri* and other *Naegleria* spp., isoenzyme analysis or animal pathogenicity tests should be done to differentiate pathogenic *N. fowleri* from other *Naegleria* spp. Both of these procedures require growing large numbers of organisms, which is not only time-consuming but expensive. With the help of species-specific MAbs that react only with *N. fowleri* in the IIF test, however, even very small numbers of *N. fowleri* amebae concentrated directly from the contaminated water can be identified quickly.

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