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Differentiation of *Naegleria fowleri* from *Acanthamoeba* Species by Using Monoclonal Antibodies and Flow Cytometry

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Monoclonal antibodies to *Naegleria fowleri* and *Acanthamoeba polyphaga* were analyzed by enzyme-linked immunosorbent assay, indirect immunofluorescence microscopy, and fluorescence flow cytometry to assess specificity and cross-reactivity with axenically cultured *N. fowleri* and *Acanthamoeba* spp. Four monoclonal antibodies to *N. fowleri* were specific for *N. fowleri* and had no reactivity to *A. polyphaga*. Similarly, four monoclonal antibodies to *A. polyphaga* did not react with *N. fowleri*. Two of the four monoclonal antibodies to *A. polyphaga* did not react with other *Acanthamoeba* spp. tested, while two of the antibodies demonstrated a high degree of cross-reactivity with a putative *Acanthamoeba castellanii* strain by immunofluorescence microscopy; this was confirmed by fluorescence flow cytometry for one of the antibodies. These monoclonal antibodies were used to identify *Acanthamoeba* trophozoites in infected brain sections of a patient who died of suspected *Acanthamoeba*-caused granulomatous amoebic encephalitis, demonstrating potential utility in the direct identification of *N. fowleri* and *Acanthamoeba* spp. in clinical specimens.

Naegleria fowleri and *Acanthamoeba* species are the causative agents of primary amoebic meningoencephalitis and granulomatous amoebic encephalitis, respectively (4, 7, 13, 16, 22, 29). In addition, keratitis due to *Acanthamoeba* species in contact lens wearers has become increasingly common (1, 10, 17). These ubiquitous organisms are found in freshwater lakes, streams, and ponds; thermally polluted water; warm springs; soil; and chlorinated pools (7, 8, 18, 20, 22, 38). *N. fowleri* infects healthy individuals after swimming or other types of exposure to contaminated waters. In contrast, *Acanthamoeba* infection of the central nervous system occurs in debilitated or immunocompromised individuals (including those with acquired immunodeficiency syndrome) and is usually not associated with exposure to contaminated water (2–4, 9, 11, 15, 21, 25, 27, 29, 39). The neurologic diseases produced by *N. fowleri* and *Acanthamoeba* species, as well as *Acanthamoeba* keratitis, are difficult to diagnose and are refractory to treatment (1, 4, 10, 22). Although the outcome of both central nervous system diseases is usually fatal, the clinical courses are dramatically different. The clinical course of *Naegleria* neurologic infection has a sudden onset and a fulminant course due to diffuse hemorrhagic necrotizing meningoencephalitis, while *Acanthamoeba* neurologic infection is usually manifested with a subacute-to-chronic illness with signs of increased intracranial pressure and focal neurologic deficit due to granulomatous brain lesions (4, 16, 22, 25). However, there are several reports of primary amoebic meningoencephalitis due to *Acanthamoeba* spp. (2, 11, 39).

Only two cases of survival because of successful drug therapy in patients with primary amoebic meningoencephalitis have been reported, both in instances in which therapy was initiated early (12, 27). Thus, early diagnosis may be critical to ensuring a favorable prognosis. In this report, we demonstrate the potential clinical utility of monoclonal antibodies to *N. fowleri* and *Acanthamoeba polyphaga* as diagnostic tools. In addition, the monoclonal antibodies specific for *A. polyphaga* may be useful in the classification of *Acanthamoeba* spp., which is occasionally based on morphologic criteria alone (14, 35). We used indirect immunofluorescence microscopy with axenic organisms and infected brain sections and enzyme-linked immunosorbent assay (ELISA) to assess the specificity of the monoclonal antibodies. In addition, fluorescence flow cytometry was used to corroborate the data obtained.

MATERIALS AND METHODS

Cell culture and production of polyclonal antisera. *N. fowleri* LEE (ATCC 30894) (9a) harvested in log phase and fixed in 1% Formalin was the generous gift of Francine Marciano-Cabral. *N. fowleri* ATCC 30896 (5) Formalin-fixed or pelleted frozen organisms harvested in log phase were the kind gift of Thomas Fritche. *A. polyphaga* ATCC 30461 (37) and *Acanthamoeba culbertsoni* OC-3A, an isolate from a river (ATCC 30866), were obtained from the American Type Culture Collection (Rockville, Md.). A putative *Acanthamoeba castellanii* isolate was obtained from the Veterans Administration Hospital, Seattle, Wash., and was identified morphologically. *Acanthamoeba* spp. were grown in medium containing peptone, yeast extract, and glucose (34) and cultured at room temperature. Organisms in log phase were harvested by decanting the medium, chilling the flasks for 10 min on ice, and washing the organisms in ice-cold phosphate-buffered saline (PBS) (pH 7.6, 0.01 M). Organisms used for indirect immunofluorescence assay (IFA), flow cytometry, and immunization were fixed in 1% Formalin in PBS overnight at 4°C. The organisms were washed three times in cold PBS to remove excess Formalin before use. Organisms used for ELISA experiments were harvested as described above, washed three times in PBS at 4°C, and frozen at −70°C until use. Rabbit polyclonal antisera to *N. fowleri* or *A. polyphaga* were produced essentially as previously described (32).

Production of monoclonal antibodies. Monoclonal antibodies to *N. fowleri* and *A. polyphaga* were produced by the

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method of Kohler and Milstein (19). For immunization, BALB/c mice were intraperitoneally injected with 10^7 Formalin-fixed organisms in PBS with no adjuvant, rested 14 days, reimmunized intraperitoneally, rested 14 days, and sacrificed 3 days after a final intravenous boost with 10^4 organisms. Fusions were performed by using spleen cells of immunized mice and the NS-0 myeloma cell line (19). Screening of wells was performed by ELISA as described previously (32, 33). The feeding, minicloning, cloning by limiting dilution, and production of ascitic fluid were performed as described previously for production of monoclonal antibodies to *Trichomonas vaginalis* and *Entamoeba histolytica* (32, 33). Purification of immunoglobulin G (IgG) class monoclonal antibodies from ascitic fluid was performed by protein A column chromatography, and antibodies were isotyped by ELISA using a commercial kit (Hyclone Laboratories, Logan, Utah).

Clinical specimens. Brain sections were obtained at necropsy, Formalin fixed, and embedded in paraffin. Slides of brain tissue from a child who died of suspected *Acanthamoeba* neurologic infection were prepared with freshly cut sections from a paraffin block. "Uninfected" brain sections obtained from an individual who died of non-amoeba-related causes were used as a control. Prior to performance of indirect immunofluorescence, the slides were deparaffinized by treatments for 3 min each in three changes of xylene, 3 min in absolute ethanol, 3 min in 95% ethanol, and 3 min in distilled water and dried. Slides were stained immediately by indirect immunofluorescence or stored at −70°C for up to 1 week.

ELISA. ELISA was performed essentially as previously described for *T. vaginalis* and *E. histolytica* (32, 33). Briefly, 50 μl of a freeze-thawed suspension of *Acanthamoeba* or *N. fowleri* trophozoites containing 1 mg of protein per ml was seeded into each well of a 96-well microtiter plate (Immulon II; Dynatech Laboratories, Inc., Chantilly, Va.), and the antigens were allowed to adsorb overnight at 4°C. Excess antigen was removed by one wash with PBS, and the plates were blocked with 5% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) in PBS for 1 h at 37°C and then washed once in PBS. To each well was added 50 μl of a dilution of monoclonal antibody-bearing ascitic fluid serially diluted (1:2,000 to 1:64,000) in PBS containing 0.05% Tween 20 (PBS-Tween). The plates were incubated for 1 h at 37°C and washed four times in PBS-Tween, and 50 μl of a 1:1,000 dilution of peroxidase-conjugated affinity-purified rabbit anti-mouse immunoglobulins (Zymed, San Francisco, Calif.) was added to each well. The plates were incubated for 1 h at 37°C, washed four times in PBS-Tween, washed two times in PBS, and developed for 30 min in a phosphate-citrate buffer (pH 5.0) with *o*-phenylenediamine and peroxidase. Positive controls included rabbit polyclonal anti-*Naegleria* serum and rabbit polyclonal anti-*A. polyphaga* serum with peroxidase-conjugated goat anti-rabbit immunoglobulins (Zymed). Negative controls included monoclonal antibody specific for *T. vaginalis* and monoclonal antibody specific for *E. histolytica* with peroxidase-conjugated rabbit anti-mouse immunoglobulins (Zymed).

Indirect immunofluorescence microscopy. Briefly, for indirect immunofluorescence microscopy, 5×10^4 trophozoites fixed in 1% Formalin were placed onto glass slides with a pipette and allowed to dry at 37°C for 1 h. The organisms were fixed to the glass slides by being fixed in acetone for 10 min and then covered with monoclonal antibody-bearing ascitic fluid, protein A-purified monoclonal antibody, or rabbit polyclonal antiserum, which were diluted in PBS containing 1% bovine serum albumin. The slides were incubated in a moist chamber for 1 h, washed three times in PBS, and covered with an appropriate dilution of fluorescein-conjugated rabbit anti-mouse immunoglobulins (Zymed) or fluorescein-conjugated goat anti-rabbit immunoglobulins (Zymed). The optimal concentrations of antigen, primary monoclonal antibody, and fluorescein conjugate were determined by block titration. After incubation for 30 min in a moist chamber at 37°C, the slides were washed three times in PBS and allowed to air dry (in the dark), and cover slips were mounted with a glycerol mounting fluid (Difco Laboratories, Detroit, Mich.). The slides were subsequently examined for fluorescence by using a Leitz fluorescence microscope and scored as 0 to 4+ according to fluorescence intensity; 4+ indicated extremely bright apple green fluorescence; negative samples were scored as 0. Controls consisted of fluorescence conjugate only or monoclonal antibodies specific for *T. vaginalis* or *E. histolytica* plus fluorescein conjugate. These monoclonal antibodies were demonstrated to have no reactivity to *Acanthamoeba* spp. or *N. fowleri* by ELISA (data not shown).

The slides of brain sections were treated essentially as described above. Four monoclonal antibody-bearing ascitic fluids specific for *Acanthamoeba* spp. (AC2B, AC3B, AC7B, and AC9B) or four monoclonal antibody-bearing ascitic fluids specific for *N. fowleri* (N3B, N4A, N5A, and N6A) were pooled and used at a 1:100 dilution. Negative controls included fluorescein conjugate only and uninfected brain sections stained with each pool of monoclonal antibodies followed by staining with fluorescein conjugate. Positive controls included rabbit anti-*A. polyphaga* serum and rabbit anti-*N. fowleri* serum, which were tested at a 1:500 dilution.

Flow cytometry. For flow cytometry, 10^6 Formalin-fixed *A. polyphaga*, "*A. castellanii*," or *N. fowleri* cells were suspended in 1 ml of PBS to which was added 0.5 ml of monoclonal antibody-bearing ascitic fluid or protein A-purified monoclonal antibody (1 mg/ml), and the suspensions were incubated for 1 h at 37°C with occasional gentle agitation. The cells were washed four times in ice-cold PBS, suspended in 0.5 ml of a solution of fluorescein-conjugated rabbit anti-mouse immunoglobulins (diluted 1:20), and incubated at 37°C for 1 h with occasional gentle agitation. The cells were washed four times in ice-cold PBS, suspended in 1 ml of PBS, and analyzed immediately by flow cytometry. Fluorescence activity was measured on a Coulter Epics 751 fluorescence-activated cell sorter (Coulter Electronics, Inc., Hialeah, Fla.) using a single 488-nm-wavelength argon laser operated at 200 mW. Analysis of antibody specificity and cross-reactivity was performed by using the computer program IMMUNO from Coulter.

RESULTS

Monoclonal antibodies and isotypes. Eight monoclonal antibodies produced to either *A. polyphaga* (AC2B, AC3B, AC7B, and AC9B) or *N. fowleri* (N3B, N4A, N5A, and N6A) were used in this study. The isotypes of the monoclonal antibodies and organisms used to generate the antibodies are listed in Table 1. Monoclonal antibodies used as controls have been described elsewhere (32, 33).

ELISA. The immunological specificities of the eight monoclonal antibodies to *A. polyphaga* or *N. fowleri* were initially determined by ELISA. The four anti-*A. polyphaga* monoclonal antibodies did not react with *N. fowleri*. Similarly, the four anti-*N. fowleri* monoclonal antibodies did not react with *A. polyphaga* (Fig. 1; data shown for N3B and AC2B only).

TABLE 1. Murine monoclonal antibodies used for ELISA, IFA, and flow cytometry

Immunizing organism and antibody	Isotype
A. polyphaga ATCC 30461	
AC2B	IgG3
AC3B	IgG3
AC7B	IgG3
AC9B	IgM
N. fowleri ATCC 30896	
N3B	IgG3
N4A	IgM
N5A	IgM
N6A	IgM

In addition, monoclonal antibodies did not react with medium components demonstrating specificity to the organisms (data not shown).

Indirect immunofluorescence. We tested the reactivities of the eight monoclonal antibodies to three species of *Acanthamoeba* (*A. polyphaga*, "*A. castellanii*," and *A. culbertsoni*) and one strain of *N. fowleri*. Anti-*N. fowleri* monoclonal antibodies were specific for *N. fowleri* (Fig. 2A and 3A; data shown for N3B only) and did not cross-react with the three *Acanthamoeba* species tested (Fig. 3; data shown for *A. polyphaga*). The four monoclonal antibodies generated to *A. polyphaga* demonstrated 4+ fluorescence with *A. polyphaga* (Fig. 2B; data shown for AC2B) and did not react with *A. culbertsoni* (Table 2). In addition, two of the anti-*A. polyphaga* monoclonal antibodies (AC7B and AC9B) reacted with the "*A. castellanii*" strain (Table 2).

Brain sections from a patient diagnosed as having *Acanthamoeba*-caused granulomatous amoebic encephalitis by histopathologic and clinical criteria were tested by indirect immunofluorescence with a pool of *A. polyphaga*-specific monoclonal antibodies (1:100), a pool of *N. fowleri*-specific monoclonal antibodies (1:100), rabbit polyclonal anti-*A. polyphaga* serum (1:500), and rabbit polyclonal anti-*N. fowleri* serum (1:500). The pool of anti-*Acanthamoeba* monoclonal antibodies reacted with trophozoites but not

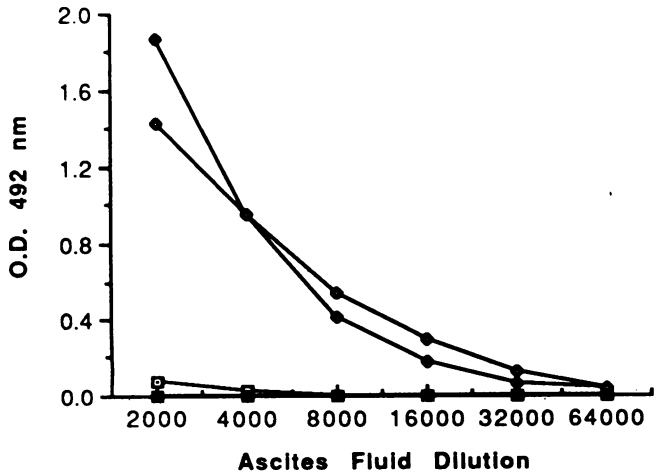

FIG. 1. ELISA reactivities of monoclonal antibody generated to *A. polyphaga* (AC2B) and monoclonal antibody generated to *N. fowleri* (N3B) to *A. polyphaga* and *N. fowleri*. AC2B reacted with *A. polyphaga* (◆) but not *N. fowleri* (□). N3B reacted with *N. fowleri* (◇) but not *A. polyphaga* (■). O.D., Optical density.

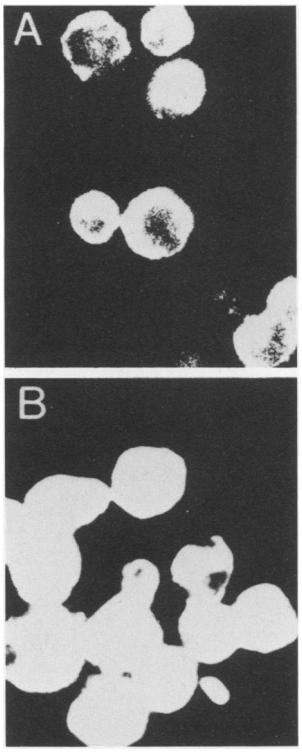

FIG. 2. Indirect immunofluorescence of monoclonal antibodies. (A) Reactivity of monoclonal antibody N3B with *N. fowleri*, showing 4+ immunofluorescence. (B) Reactivity of monoclonal antibody AC2B with *A. polyphaga*, showing 4+ immunofluorescence.

with cysts in the infected brain sections and failed to react with uninfected brain tissue (Fig. 4). Approximately five trophozoites were seen per brain section; however, numerous unstained cysts were present. In contrast, the pool of *N. fowleri* monoclonal antibody-bearing ascitic fluids did not react with the cysts or trophozoites in the infected brain sections or with uninfected brain sections (data not shown). When infected brain sections were stained with rabbit polyclonal antiserum, anti-*A. polyphaga* serum demonstrated 4+ fluorescence with trophozoites while anti-*N. fowleri* serum detected trophozoites which were weakly fluorescent (1+) (data not shown). Rabbit polyclonal antisera did not react with cysts present in the infected brain sections or with uninfected brain sections.

Flow cytometry. The visual interpretations of immunofluorescence reactivity of monoclonal antibodies with axenic organisms were analyzed and quantitated by fluorescence flow cytometry. Ninety-degree-light-scatter-versus-forward-angle-light-scatter profiles were used to assess the homogeneity of cell populations by cell complexity and size (Fig. 5). Flow cytometry detected fluorescence in 99.6% of *N. fowleri* cells when they were reacted with monoclonal antibody N3B (Fig. 6A) and 94% of *A. polyphaga* cells when they were reacted with AC2B (Fig. 6B). In contrast, there was no significant degree of fluorescence measured by N3B reacted against *A. polyphaga* organisms (Fig. 6B, inset 2). This demonstrated the specificity of N3B for *N. fowleri* and corroborated the data obtained from slide IFA. Conversely, when antibody specific for *A. polyphaga* was tested against *N. fowleri*, a low degree of fluorescence was detected, demonstrating that the monoclonal antibody did not cross-react with *N. fowleri* (Fig. 6A, inset 2). Similarly, when three

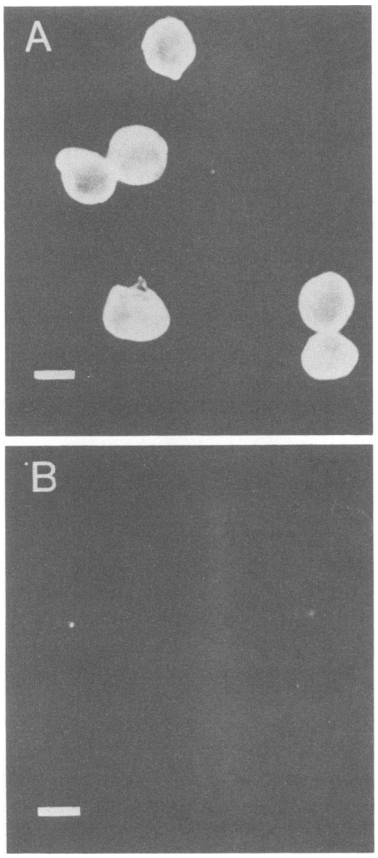

FIG. 3. Indirect immunofluorescence reactivities of monoclonal antibodies. Bar = 12 μm. (A) Reactivity of monoclonal antibody N3B with *N. fowleri*, showing 4+ immunofluorescence. (B) Reactivity of monoclonal antibody N3B with *A. polyphaga*, showing no immunofluorescence.

monoclonal antibodies (AC2B, AC3B, and AC7B) generated to *A. polyphaga* were analyzed by flow cytometry, each demonstrated a high degree of fluorescence with *A. polyphaga*. Monoclonal antibodies AC2B and AC3B showed little or no fluorescence with "*A. castellanii*" (Fig. 7A and B, insets 2). However, the degrees of fluorescence of monoclonal antibody AC7B with *A. polyphaga* and with "*A. castellanii*" were similar (Fig. 7C, insets 1 and 2, respectively). The histograms (Fig. 7A to C) demonstrate the percent difference in fluorescence between *A. polyphaga* and "*A. castellanii*" after subtraction of fluorescence data of inset 2 from inset 1. The percent difference in staining of monoclonal antibody AC7B to *A. polyphaga* and "*A. castellanii*" was 42, indicating a high degree of cross-reactivity, while the percent difference in staining of monoclonal antibody AC2B or AC3B was 91, indicating little or no cross-reactivity.

TABLE 2. Reactivities of monoclonal antibodies by IFA

Organism and monoclonal antibody	Reactivity[a]			
	N. fowleri	*A. polyphaga*	"*A. castellanii*"	*A. culbertsoni*
N. fowleri				
N3B	4+	−	−	−
N4A	4+	−	−	−
N5A	4+	−	−	−
N6A	4+	−	−	−
A. polyphaga				
AC2B	−	4+	−	−
A3B	−	4+	−	−
AC7B	−	4+	3+	−
AC9B	−	4+	3+	−

[a] Fluorescence was scored from 0 to 4+; 4+ indicates extremely bright apple green fluorescence; − indicates no fluorescence.

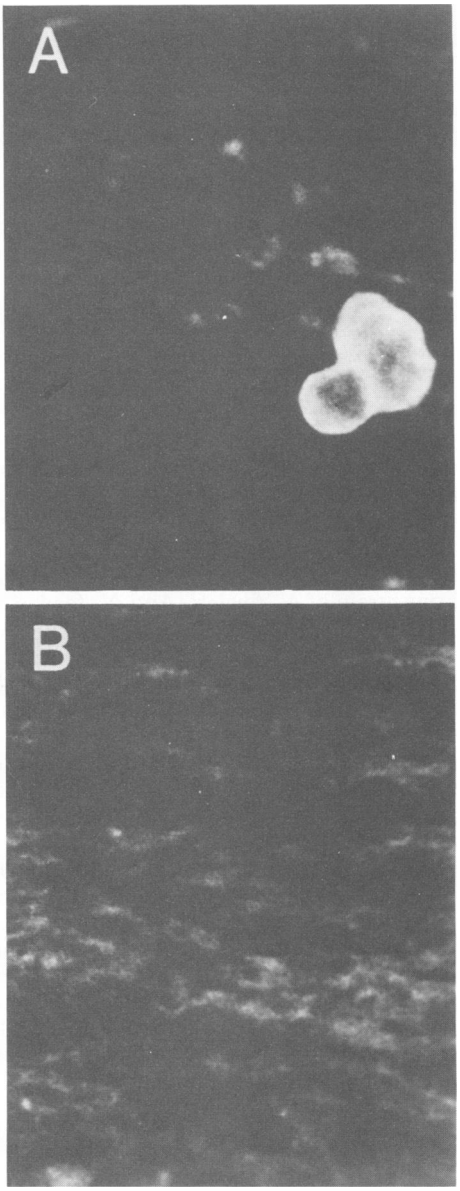

FIG. 4. Indirect immunofluorescence reactivities of monoclonal antibodies against infected and uninfected brain sections. (A) Reactivity of a pool of monoclonal antibodies specific for *Acanthamoeba* species against infected brain section of a child obtained at necropsy, showing fluorescent trophozoites and no fluorescence of cysts. (B) Reactivity of *Acanthamoeba*-specific monoclonal antibody pool against uninfected brain section, showing no fluorescence.

DISCUSSION

Although *N. fowleri* and *Acanthamoeba* species cause a variety of diseases, are highly pathogenic under certain

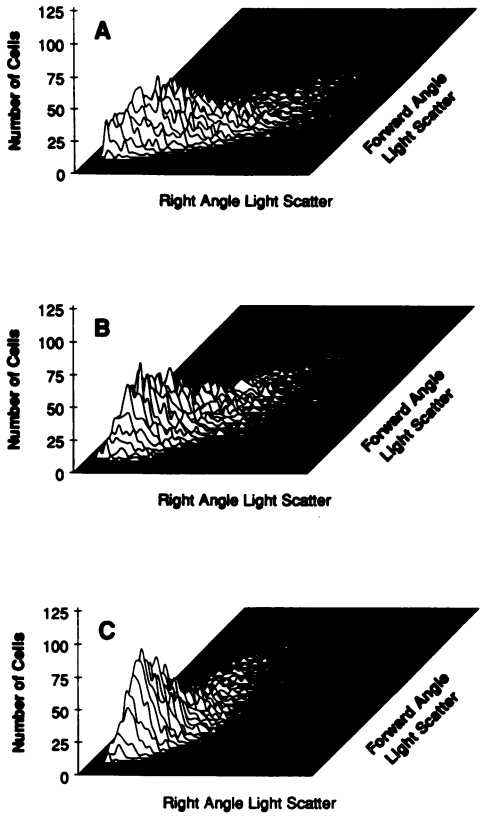

FIG. 5. Three-dimensional diagrams (90° light scatter versus forward angle light scatter) of the cell populations used for fluorescence flow cytometry, showing homogeneous populations with respect to cell complexity and size. (A) *N. fowleri* cell profile; (B) *A. polyphaga* cell profile; (C) "*A. castellanii*" cell profile.

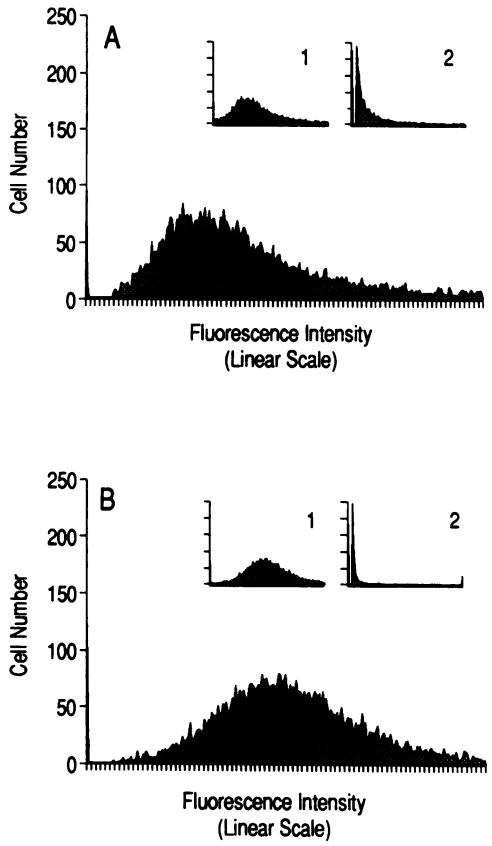

FIG. 6. Fluorescence flow cytometry analysis of specificities of monoclonal antibodies N3B and AC2B against *N. fowleri* and *A. polyphaga*. (A) Reactivities of *N. fowleri* with monoclonal antibodies N3B and AC2B. Histogram is derived from data in inset 2 (*N. fowleri* reacted with unrelated antibody AC2B) subtracted from data in inset 1 (*N. fowleri* reacted with N3B), demonstrating specificity of monoclonal antibody for *N. fowleri* organisms and 99.6% positivity of *N. fowleri* by fluorescence. (B) Reactivities of *A. polyphaga* with monoclonal antibodies AC2B and N3B. Histogram is derived from data from inset 2 (*A. polyphaga* reacted with unrelated antibody N3B) subtracted from data in inset 1 (*A. polyphaga* reacted with AC2B), demonstrating specificity of monoclonal antibody for *A. polyphaga* organisms and 94% positivity of *A. polyphaga* by fluorescence.

circumstances, and require different drug treatments, there are few tests available for their rapid identification and differentiation. Current methods for rapid detection of amoebae in tissue or corneal scrapings include Giemsa, Gomori, and iron-hematoxylin-eosin stains (1, 22). However, these techniques require highly trained personnel and a strong clinical suspicion of amoebic infection to identify organisms in stained specimens. Novel fluorescent stains such as Calcofluor white and fluorescein-conjugated lectins have also been used to stain corneal scrapings for *Acanthamoeba* spp. (26, 28). Indirect immunofluorescence with rabbit polyclonal antiserum has been used for staining tissue in suspected cases of amoebic meningoencephalitis or keratitis (10, 11, 36, 39), but considerable cross-reactivity has been reported (36) and the sensitivity of these antibodies is suspect. Clearly, reagents which specifically detect these organisms in clinical specimens and can identify *Acanthamoeba* spp. to the species level are needed.

In a previous report, Visvesvara et al. (37) generated monoclonal antibodies against *N. fowleri* which did not react with other *Naegleria* spp. or with *A. castellanii* and were useful in the diagnosis of primary amoebic meningoencephalitis postmortem. Our results also support the diagnostic potential of *N. fowleri*-specific monoclonal antibodies and demonstrate the ability of monoclonal antibodies to differentiate between *Acanthamoeba* and *Naegleria* species in one case of granulomatous amoebic encephalitis (postmortem) by indirect immunofluorescence reactivity with infected brain sections. Using the monoclonal antibodies to *Acanthamoeba* spp., we detected *Acanthamoeba* spp. in the brain tissue of a 22-month-old male who died 6 weeks after the onset of a progressive neurologic disease. The disease was manifested by right-sided focal seizures followed several days later by incoordination, tremor, and irritability. The neurologic examination revealed early papilledema, right-sided hemiparesis, ataxia, and bilateral Babinski signs. A computerized tomography scan of the brain showed multiple bilateral hemispheric and cerebellar lucent lesions with ring enhancement after intravenous contrast injection. The child's neurologic status deteriorated, he became unresponsive and quadriparetic, and he died of respiratory failure. The autopsy revealed multiple round necrotic and hemorrhagic lesions in both frontal lobes, basal ganglia, the brain stem, and the cerebellum. The histologic changes in the brain consisted of granulomatous necrotic areas that contained abundant trophozoites and cysts of amoebic organisms. Using the monoclonal antibodies to *A. polyphaga* and *N. fowleri*, we have confirmed the clinical diagnosis of *Acanthamoeba* infection. Although rabbit polyclonal anti-*A.*

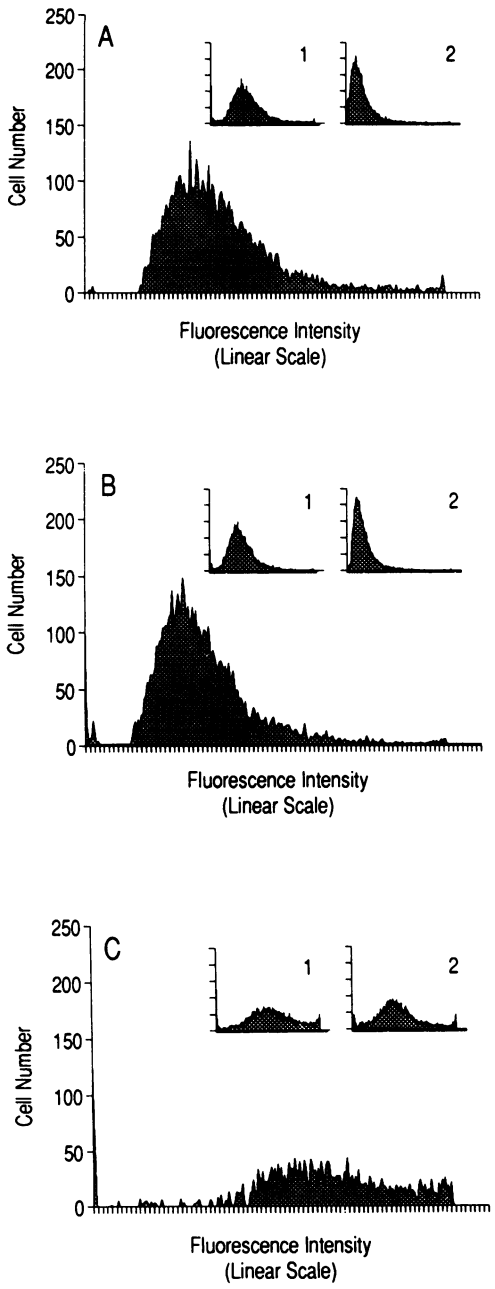

FIG. 7. Fluorescence flow cytometric analysis of cross-reactivities of monoclonal antibodies specific for *A. polyphaga* AC2B (A), AC3B (B), and AC7B (C) against *A. polyphaga* and "*A. castellanii*." Inset 1 of each panel shows the degree of reactivity of monoclonal antibody against *A. polyphaga*. Inset 2 of each panel shows the degree of reactivity of monoclonal antibody against "*A. castellanii*." Histograms in panels A, B, and C demonstrate the differences in degree of fluorescence reactivity after subtracting the data from control histogram (heterologous organism, inset 2) from data from the test histogram (homologous organism, inset 1).

polyphaga serum also detected fluorescent trophozoites in the infected brain sections, weak fluorescence of trophozoites was seen with rabbit polyclonal anti-*N. fowleri* serum. This cross-reactivity of anti-*Acanthamoeba* serum with *N. fowleri* renders the antiserum less desirable for use in clinical diagnosis than the highly specific monoclonal antibodies without careful titration of the antiserum and adequate controls.

Muldrow et al. (23) used rabbit polyclonal antisera prepared against various *Naegleria* and *Acanthamoeba* species and flow cytometry to demonstrate the increased sensitivity of the flow cytometric technique in measuring fluorescence compared with immunofluorescence microscopy. We used flow cytometry to obtain a quantitative analysis of the specificities of N3B for *N. fowleri* and AC2B for *A. polyphaga*, as well as a quantitative measurement of the cross-reactivities of three monoclonal antibodies generated to *A. polyphaga* with "*A. castellanii*." These data confirm the immunological specificities of the monoclonal antibodies made by visual qualitative observations in IFA studies and quantitative ELISAs. In addition, flow cytometry detected a low level of fluorescence reactivity of monoclonal antibodies AC2B and AC3B with "*A. castellanii*" cells which was not detected by immunofluorescence microscopy, demonstrating the sensitivity of the test. Although flow cytometry cannot be used to analyze organisms in tissue specimens or corneal scrapings, this technique may be useful for distinguishing amoebae cultured from clinical or biological specimens containing multiple species. In four cases of single-eye infections, multiple species of *Acanthamoeba* were recovered (6).

These monoclonal antibodies are also potentially of benefit in the clinical diagnosis of *Acanthamoeba* keratitis. Keratitis due to *Acanthamoeba* spp. has been frequently confused with herpes simplex virus stromal keratitis, resulting in delays in proper treatment (1, 10). Drug therapy alone is usually ineffective and susceptibilities to antimicrobial agents vary among different strains and species of *Acanthamoeba* (1, 10, 24, 30, 31). Since penetrating keratoplasty is usually most effective when performed prior to extensive corneal involvement (1, 10), early identification of *Acanthamoeba* infection is crucial.

Studies are under way to characterize these antibodies with respect to antigenic recognition by immunoprecipitation of metabolically labeled organisms (the monoclonal antibodies do not immunoblot) and to determine their reactivities with larger numbers of both *Naegleria* and *Acanthamoeba* strains and species, as well as to generate monoclonal antibodies to other species of *Acanthamoeba*.

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