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Simple Diagnosis of *Encephalitozoon* sp. Microsporidial Infections by Using a Panspecific Antiexospore Monoclonal Antibody

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Microsporidia (phylum Microspora) have recently become recognized as common opportunistic protozoans in the United States and worldwide, particularly affecting immunodeficient patients. Microsporidian organisms within the genus *Encephalitozoon* are the cause of nephrologic, ophthalmic, pneumologic, gastroenteric, and systemic infections. However, diagnosis of the small spores by light microscopy is difficult, even with newly developed and improved staining techniques. We have developed an anti-*Encephalitozoon* species monoclonal antibody-based immunoassay for easy diagnosis. A hybridoma was produced and selected following one main criterion: recognition by immunofluorescence of all known *Encephalitozoon* spores affecting humans. The selected monoclonal antibody-secreting hybridomas were characterized by enzyme-linked immunosorbent assay, immunofluorescence, Western blot, and immunoelectron microscopy using *Encephalitozoon* species from fresh and fixed samples from patients and from in vitro cultures. In the immunofluorescence assay, one monoclonal antibody, termed 3B6, strongly recognized *Encephalitozoon cuniculi*, *E. hellem*, and *E. intestinalis*. Monoclonal antibody 3B6 bound to other microsporidia (*Nosema* and *Vairimorpha* spp.) without cross-reacting with any other parasite, including *Enterocytozoon bieneusi*, fungus, or bacterium tested. In immunoelectron microscopy assays, monoclonal antibody 3B6 bound to the exospore of *Encephalitozoon* species, while in Western blot assays, it recognized three to seven antigens with molecular masses ranging from 34 to 117 kDa. We have developed a sensitive and specific monoclonal antibody-based immunoassay to diagnose common microsporidian infections, particularly with *Encephalitozoon* species. This is a new tool for identifying spores in bodily fluids and biopsy samples and is an efficient diagnostic test. Additionally, monoclonal antibody 3B6 can serve to assess the prevalence of microsporidial infections in immunodeficient and immunocompetent patients.

Since the onset of the AIDS epidemic, several parasites belonging to the phylum Microspora have emerged as important opportunistic pathogens, affecting particularly the gastrointestinal tracts, respiratory tracts, urinary tracts, and conjunctivae of patients with AIDS and other immunocompromised patients (24). Microsporidia are small obligate intracellular protozoan parasites characterized by a proliferative merogonic stage followed by a sporogonic stage which results in highly resistant spores. Mature spores contain a coiled tubular extrusion apparatus which, when stimulated by the appropriate environment, is rapidly propelled for injecting sporoplasm contents into new host cells (14, 25).

The most common microsporidial opportunistic organisms in patients with AIDS have been identified recently as *Enterocytozoon bieneusi* in 1985 (6), *Encephalitozoon hellem* in 1991 (7), and *Encephalitozoon intestinalis* in 1993 (called *Septata intestinalis* until 1995 [4, 12]). Although both *E. bieneusi* and *E. intestinalis* affect mainly the gastrointestinal tract, *E. bieneusi* has also been reported as the cause of cholecystitis, cholangitis, bronchitis, pneumonia, sinusitis, and rhinitis, and *E. intestinalis* has been reported as the cause of disseminated infections. *E. hellem* has been described as the cause of keratoconjunctivitis and disseminated infections (24). The prevalence of microsporidiosis is poorly known and almost certainly underdiagnosed due to the difficulties in detecting and identifying these organisms, even with recently improved diagnostic staining methods. Here we describe the development, characterization, and use of an antimicrosporidia monoclonal antibody, namely, 3B6. A 3B6-based immunofluorescence assay could be a reliable diagnostic test to identify microsporidian parasites within the genus *Encephalitozoon.*

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MATERIALS AND METHODS

Animals. Adult 6-week-old female and male BALB/c mice for hybridoma development and ascites production, respectively, were purchased from Jackson Laboratories (Bar Harbor, Maine) and allowed to acclimate for a minimum of 5 days before any experimental procedure was initiated. All mice were housed in filter-topped plastic cages with wood chip bedding and maintained at 12-h photoperiod cycles. Food (Agway Prolab Animal Diet Rat, Mouse, Hamster 3000; Agway Inc., Syracuse, N.Y.) and water were provided ad libitum.

Parasites. Isolates of *Encephalitozoon cuniculi* and *E. hellem* were obtained from Govinda Visvesvara at the Centers for Disease Control and Prevention. An alveolar isolate of *E. intestinalis* (previously *Septata intestinalis*) (4, 12) was purchased from the American Type Culture Collection (ATCC) (catalog number 50506; ATCC Rockville, Md.). *Vairimorpha plodiae*, *Vairimorpha ephestiae*, *Nosema apis*, *Nosema algerae*, *Glugea atherinae*, and *Pleistophora hyphessbryconis* were originally obtained from Tomas Tonka at the Institute of Entomology of the Czech Academy of Sciences, České Budějovice, Czech Republic. *Enterocytozoon bieneusi* samples in stool specimens were kindly provided by Shew Chan from Meridian Diagnostics (Columbus, Ohio). With the exception of *E. bieneusi* and insect microsporidia, parasites were maintained in vitro in Vero cells (ATCC) with RPMI-1640 medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 1% L-glutamine (Gibco Laboratories), 1% sodium bicarbonate, and 10% fetal bovine serum (Gemini Bioproducts, Inc., Calabasas, Calif.). Spores were isolated and purified from cells by size exclusion chromatography (Bio-Rad Laboratories, Hercules, Calif., and Sigma, St. Louis, Mo.). Spores from each species were used to coat 10-well Hendley diagnostic slides previously treated with poly-L-lysine (0.01% [wt/vol]; Sigma) and stored at −80°C until

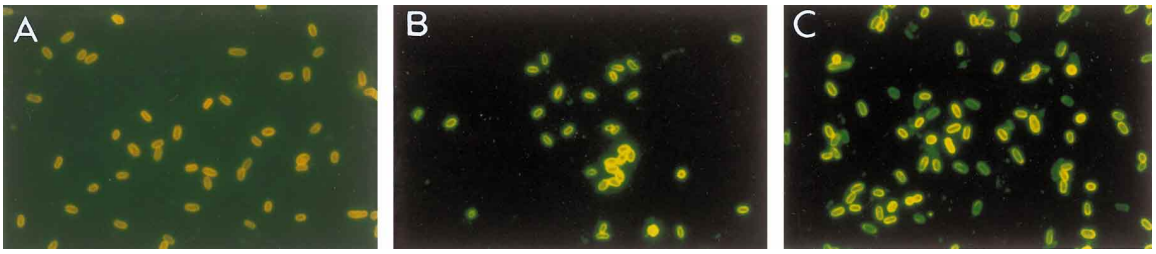

FIG. 1. Spores of *E. intestinalis* (A), *E. cuniculi* (B), and *E. hellem* (C) stained by indirect immunofluorescence using monoclonal antibody 3B6. Magnification, ×1,000. Bar = 1 μm.

used. Purified spores were also utilized for antigen preparation (enzyme-linked immunosorbent assay [ELISA] and Western blot). Briefly, they were disrupted by six freeze-thaw cycles and centrifuged (10,000 × *g*, 30 min), and protein concentration in the supernatant was determined with bicinchoninic acid (Pierce Chemical Company, Rockford, Ill.). Spore antigen was stored at −80°C prior to use.

Production and screening of anti-*Encephalitozoon* sp. monoclonal antibody-producing hybridomas. Female BALB/c mice were immunized intraperitoneally three times at 3-week intervals with 10^6 *E. cuniculi* spores mixed 1:1 in Ribis adjuvant (Ribis Immunochem Research Inc., Hamilton, Mont.). Seven days after each immunization, sera were screened by ELISA and immunofluorescence as described below to determine parasite-specific antibody titers. Sera were stored at −80°C and used as positive controls during all immunoassays. Fusion of spleen cells with SP2/O myelomas as well as maintenance and selection of hybridomas was performed as previously described (9). Hybridomas were selected based on positive ELISA and immunofluorescence reactivity to spores of all *Encephalitozoon* spp. tested. Only one monoclonal antibody, namely, 3B6, was selected, frozen, and cloned by standard procedures (9).

(i) ELISA. ELISA plates were coated with 5 μg of the respective microsporidian spore homogenate per ml, blocked with 1% nonfat milk solution, and incubated with the appropriate hybridoma supernatants and controls. After incubation, the plates were incubated with peroxidase-labelled goat anti-mouse immunoglobulin M (IgM)-IgG antiserum and developed with 2,2′-azino-di(3-ethylbenzthiazoline sulfonate) (ABTS) (Kirkegaard and Perry, Gaithersburg, Md.) (6). Those hybridomas secreting *Encephalitozoon* sp.-specific antibodies were further assayed by immunofluorescence.

(ii) Immunofluorescence assay. Supernatants of selected hybridomas, along with immune and negative preimmune mouse sera, were incubated for 40 min in separate spore-coated wells inside a humidified chamber. Following washing, each well was incubated for 40 min with fluorescein-conjugated goat anti-mouse affinity-purified antisera (Kirkegaard and Perry) with Evans blue (Sigma). Slides were mounted with DABCO-glycerol solution (Sigma), and spores were visualized with an Olympus epifluorescence microscope (magnification, ×400).

(iii) Ascites production. Groups of male BALB/c mice were primed with 750 μl of pristane, and 11 days later the mice in each group were injected intraperitoneally with 10^7 3B6 hybridoma cells. The generated ascites was screened by indirect immunofluorescence using purified spores. The monoclonal antibody 3B6 protein concentration was determined with bicinchoninic acid and by antibody capture ELISA standardized with known Ig concentrations run in each ELISA plate. The titer of the ascites was determined, and the ascites was stored at −80°C.

Characterization of anti-*Encephalitozoon* sp. monoclonal antibody 3B6. (i) Isotyping. The selected monoclonal antibody 3B6 was identified with a dipstick mouse monoclonal antibody isotyping kit (Life Technologies, Gaithersburg, Md.).

(ii) Western blots. *Encephalitozoon* sp. spores grown in vitro were separated from Vero cells by size exclusion chromatography. Purified spore homogenates of *E. intestinalis*, *E. cuniculi*, and *E. hellem* were separately boiled for 2.5 min in sample buffer, and proteins were separated on a 4 to 15% polyacrylamide-sodium dodecyl sulfate gradient and transferred to nitrocellulose membranes (1.25 h at 100 V). Membranes were blocked with 1.5% nonfat milk solution and placed in a multiscreen apparatus which divided the membrane into individual strips to be probed independently. Each strip was incubated for 1.5 h with either supernatant or ascites containing monoclonal antibody 3B6, murine immune sera, preimmune murine sera, or an irrelevant monoclonal antibody IgG2b (11) as isotype control. Following washing, membranes were incubated for 2 h with affinity-purified peroxidase-labelled goat anti-mouse IgM-IgG anti-serum and developed with 4-chloronaphthol (Kirkegaard and Perry).

(iii) Monoclonal antibody 3B6-based immunogold electron microscopy. Cultures of *E. intestinalis*, *E. cuniculi*, and *E. hellem* as well as stool samples from patients with suspected cases of microsporidiosis were fixed in cacodylate buffer containing 3% formaldehyde and 1% glutaraldehyde. Samples were mixed with Bactoagar, fixed, washed, and dehydrated as described below. Samples were then embedded in LR White Hard resin and cut. Grids with sections were blocked for 10 min by using 0.1% bovine serum albumin and 0.1% Tween and incubated overnight at 4°C with either monoclonal antibody 3B6 or an isotype control. After washing, samples were incubated with gold-conjugated goat anti-mouse affinity-purified IgG (Sigma) and washed and fixed with 0.5% glutaraldehyde. Samples were then washed, stained with uranyl acetate, and examined with a Phillips 420 electron microscope.

(iv) Cross-reactivity studies. The reactivity of monoclonal antibody 3B6 with other microsporidia, bacteria, parasites, and fungi was assessed by indirect immunofluorescence assay.

Use of monoclonal antibody 3B6 in diagnosis using stool and biopsy samples and comparison with other methods. Stool and biopsy samples were evaluated for the presence of microsporidia by immunofluorescence assay, the Calcofluor method and electron microscopy. Stool samples were concentrated by using ethyl acetate prior to the immunoassay. Livers from severe-combined-immunodeficient (SCID) mice previously infected with *E. cuniculi* were embedded in paraffin blocks. The samples were cut, rehydrated with decreasing percentages of ethanol solutions, and processed for indirect immunofluorescence.

(i) Calcofluor. Calcofluor staining was performed as previously described (19). Briefly, ethyl acetate-purified samples were placed in poly-L-lysine-coated slides, fixed with methanol, and incubated for 10 min in a 1% solution of Calcofluor white M2R (fluorescent brightener 28; Sigma) with 0.01% Evans blue. Slides were examined with an Olympus epifluorescence microscope with 425-nm and 460-nm exciting and block filters, respectively.

(ii) Electron microscopy. Stool samples were concentrated by ethyl acetate sedimentation. Sediments of each sample were mixed with 2% Bactoagar solution (Difco) and were allowed to solidify during centrifugation (10 min at 500× *g*). Portions of spore-containing agar were carefully removed, fixed, and washed in sucrose solution (5 g of sucrose in 100 ml of 0.15% cacodylate buffer). Following ethanol dehydration, samples were embedded in Epon-Araldite, cut in ultrathin slices (MT-2B, MT6000, Ultracut microtome), and stained with uranyl acetate and lead citrate. The stained sections were examined with a Phillips 420 electron microscope.

Statistical analysis. ELISA results were analyzed by one-way analysis of variance and by Student's *t* test. Experimental data were compared to controls. *P* values of 0.05 or less were considered significant.

RESULTS

Generation of an anti-*Encephalitozoon* sp. monoclonal antibody. Following multiple fusions of *E. cuniculi*-immunized murine spleen cells with SP2/O myeloma cells, the derived hybridomas were screened by immunofluorescence and ELISA using in vitro-derived spores from *E. intestinalis*, *E. cuniculi*, and *E. hellem* and their homogenates, respectively. One antibody-secreting hybridoma was selected from over 1,000 hybridomas, as it was the only one that met the criterion of recognition of all *Encephalitozoon* species which cause human disease ($P < 0.0001$). The hybridoma selected, named 3B6, strongly stained the perimeter of the spores, which were then easily recognized with either a ×400 or a ×1,000 magnification (Fig. 1). Hybridoma 3B6 was cloned three times by limiting dilution, isotyped as IgG2b(κ), expanded in both ascites and culture, and subjected to thorough screening with *Encephalitozoon* and other microsporidian species (Table 1). The antibody 3B6 appears to be genus specific, with low cross-reactivity to other microsporidia of the genera *Vairimorpha* and *Nosema.*

Characterization of monoclonal antibody 3B6. (i) Western blots of *Encephalitozoon* species using monoclonal antibody 3B6. Purified and electroseparated *E. intestinalis*, *E. cuniculi*, and *E. hellem* spore antigens transferred into nitrocellulose membranes exhibited up to seven bands with different molec-

TABLE 1. Reactivity of monoclonal antibody 3B6 to human and animal microsporidial by indirect immunofluorescence

Species	Origin	Source	Result	Titer
Encephalitozoon cuniculi	Human	Cell culture	Positive	1:16,000
Encephalitozoon cuniculi	Human	SCID mouse liver (fixed)	Positive	1:16,000
Encephalitozoon hellem	Human	Cell culture	Positive	1:32,000
Encephalitozoon intestinalis	Human	Cell culture	Positive	1:32,000
Encephalitozoon sp.	Lizard	Lizard tissue (fixed)	Positive	1:16,000
Enterocytozoon bieneusi	Human	Stool sample	Negative	
Vittaforma corneae	Human	Cell culture	Negative	
Vairimorpha ephestiae	*Ephestia kuehniella*[a]	Infected caterpillar	Positive	1:500
Vairimorpha plodiae	*Plodia interpunctella*[a]	*Galeria melonella* tissue	Positive	1:10,000
Nosema apis	*Apis melifera*	Midgut of infected bee	Positive	1:4,000
Nosema algerae	*Anopheles stephensi*	Infected mosquito tissue	Positive	1:500
Pleistophora hyphessbryconis	*Paracheiron inesi*	Fish muscle	Negative	
Glugea atherinae	*Atherina boyeri*	Fish muscle	Negative	

[a] Lepidoptera.

ular masses, according to the species, when probed with monoclonal antibody 3B6. As seen in Fig. 2, monoclonal antibody 3B6 recognized three bands in *E. intestinalis* antigen (53, 102, and 117 kDa), four bands in *E. hellem* antigen (53, 63, 102, and 117 kDa), and seven bands in *E. cuniculi* antigen (34, 40, 46, 53, 63, 102, and 117 kDa). Each test included SP2/O hybridoma supernatant and preimmune mouse sera as negative controls and yielded no visible reactivity. In addition, Vero cell homogenates probed with monoclonal antibody 3B6 yielded no visible bands. Treatment with periodate did not change the reactivity of any of the *Encephalitozoon* sp. antigenic determinants recognized.

(ii) Immunoelectron microscopy. In order to identify the precise binding of monoclonal antibody 3B6 on microsporidian stages, in vitro-maintained *Encephalitozoon* spp. were tested by immunoelectron microscopy. We observed that monoclonal antibody 3B6 bound exclusively to the exospore. This exospore-specific reactivity was observed both in mature spores (Fig. 3A) and in developing sporonts (Fig. 3B). This reactivity was also seen with *E. intestinalis* spores present in stool samples but not with *E. bieneusi* spores.

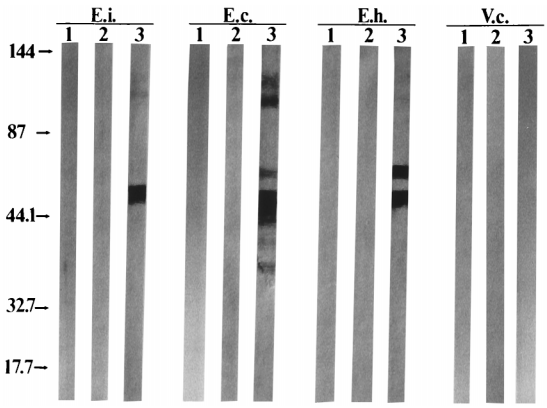

FIG. 2. Western blot of sodium dodecyl sulfate-separated components of *E. intestinalis* (E.i.), *E. cuniculi* (E.c.), *E. hellem* (E.h.), and control Vero cells (V.c.). Columns 1 were blotted with SP2/O hybridoma supernatant; columns 2 were blotted with preimmune mouse sera. Both served as negative controls. Columns 3 were blotted with monoclonal antibody 3B6. These Western blots of electroseparated microsporidian spore antigens show three bands in *E. intestinalis* antigen (53, 102, and 117 kDa), four bands in *E. hellem* antigen (53, 63, 102, and 117 kDa), and seven bands in *E. cuniculi* antigen (34, 40, 46, 53, 63, 102, and 117 kDa).

(iii) Cross-reactivity studies. By immunofluorescence, monoclonal antibodies 3B6 in supernatant and ascites were assessed for cross-reactivity to enteropathogenic bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Proteus vulgaris*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Yersinia enterocolitica*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Enterococcus faecalis*), other intestinal parasites (*Cryptosporidium parvum*, *Giardia intestinalis*, *Cyclospora cayetanensis*), and yeasts from stool samples. There was no cross-reactivity to any of the organisms evaluated.

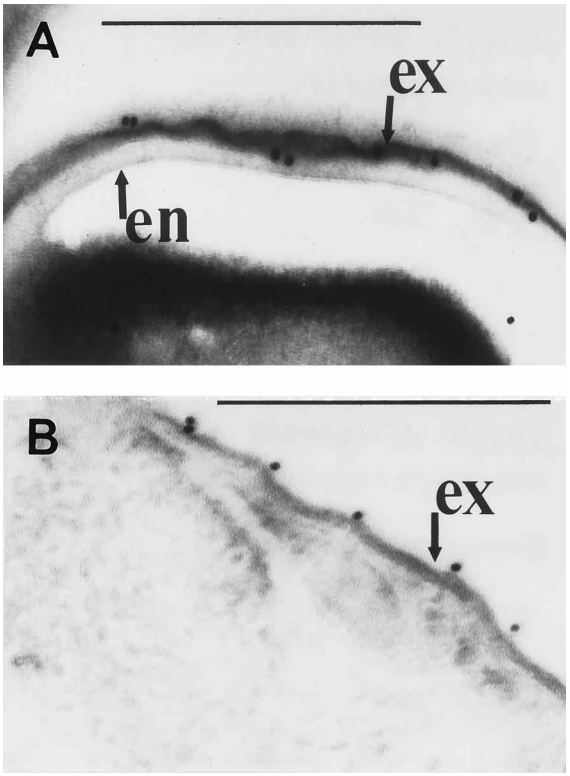

FIG. 3. Immunogold transmission electron micrographs of *E. cuniculi* treated with 3B6 monoclonal antibody. (A) Detail of mature spore showing the exospore (ex) and endospore (ed). Gold beads are bound to the exospore. (B) Sporont within the parasitophorous vacuole in which endospore is not yet well defined. Gold beads are seen in the developing exospore. Bar = 0.1 μm.

(iv) Reactivity in fixed samples. Next, fresh *E. intestinalis*, *E. cuniculi*, and *E. hellem* spores derived from in vitro culture were fixed with different fixatives. Fixation with either 3 and 4% glutaraldehyde, 10% formaldehyde, 4% OsO_4-periodate, or a combination of 4% formaldehyde with 1% glutaraldehyde did not affect fluorescence reactivity compared to fresh spores of all species examined.

Use of monoclonal antibody 3B6 in diagnosis of microsporidia in stool samples and comparison with other methods. (i) Electron microscopy. By the agar-centrifugation method, stool particles of similar weight and size migrated to delimited areas in the solidified agar. Microsporidian spores were found in areas containing numerous yeast and yeast-like organisms. *E. bieneusi* (Fig. 4A) was easily differentiated from *E. intestinalis* (Fig. 4B) based on the characteristic arrangement of the coiled polar tubes of each species.

(ii) Comparisons of diagnostic methods. Fixed and fresh stool samples from patients suspected of having intestinal microsporidiosis were compared by immunofluorescence, the Calcofluor method, and electron microscopy. As seen in Table 2, monoclonal antibody 3B6 recognized samples diagnosed as being microsporidia by Calcofluor. The microsporidian species of each positive sample was confirmed by electron microscopy, and *E. intestinalis* (Fig. 5A) but not *E. bieneusi* spores were recognized by monoclonal antibody 3B6.

Immunofluorescence was also performed on liver sections obtained from *E. cuniculi*-infected SCID mice, showing brightly stained foci of infected liver cells (Fig. 5B).

DISCUSSION

Though the pathogenic importance of microsporidia in the population at large remains to be determined, it is apparent that these protozoans are an important cause of infection among the immunodeficient, particularly patients with AIDS. Within the phylum Microspora, at least 10 species in up to six genera are known to cause human disease, and more are likely to be identified and reclassified as the human immunodeficiency virus-positive population increases (2, 4–7, 12, 22, 24). Recent awareness of the significance of these organisms has resulted in improved methods for detection, but microsporidiosis is still perhaps underdiagnosed. Part of the problem is that diagnostic tools for identification of microsporidia, such as chemofluorescent optical brightening agents like Calcofluor (18), chromotrope stains (23), or modified stains (10, 13, 15, 17), have not been used routinely by clinical laboratories. In addition, staining by the above techniques is not microsporidian specific.

Immunofluorescence assay with monoclonal antibody 3B6 is highly sensitive and specific, and we found it superior to the Calcofluor method since there was no cross-reactivity with other bacteria, fungi, or parasites evaluated. In using the immunofluorescence protocol described herein, there is low background in the fecal specimens examined and spores appear brightly stained. Because the monoclonal antibody 3B6 targets the exospores of *Encephalitozoon* spp., visualization of spores at a relatively low amplification is easy, even for the inexperienced eye. Polyclonal and polyclonal-absorbed antibodies for immunoassay may have lot-to-lot variation for wide diagnostic purposes, and the use of monoclonal antibodies that are species specific (1, 3, 16, 20, 21, 26) may not be helpful for identification of more than one microsporidian species. Monoclonal antibody 3B6 appears to be genus specific and recognizes *Encephalitozoon* spp. and related microsporidian species with less sensitivity. Thus, the application of monoclonal antibody 3B6 in immunofluorescence identification of *Encephalitozoon* infections offers a new diagnostic tool for clinical laboratories. In fact, it could serve, along with other techniques, to differentiate *Encephalitozoon* spp. from *E. bieneusi* infections as the former can be treated with albendazole while the latter cannot (8, 24).

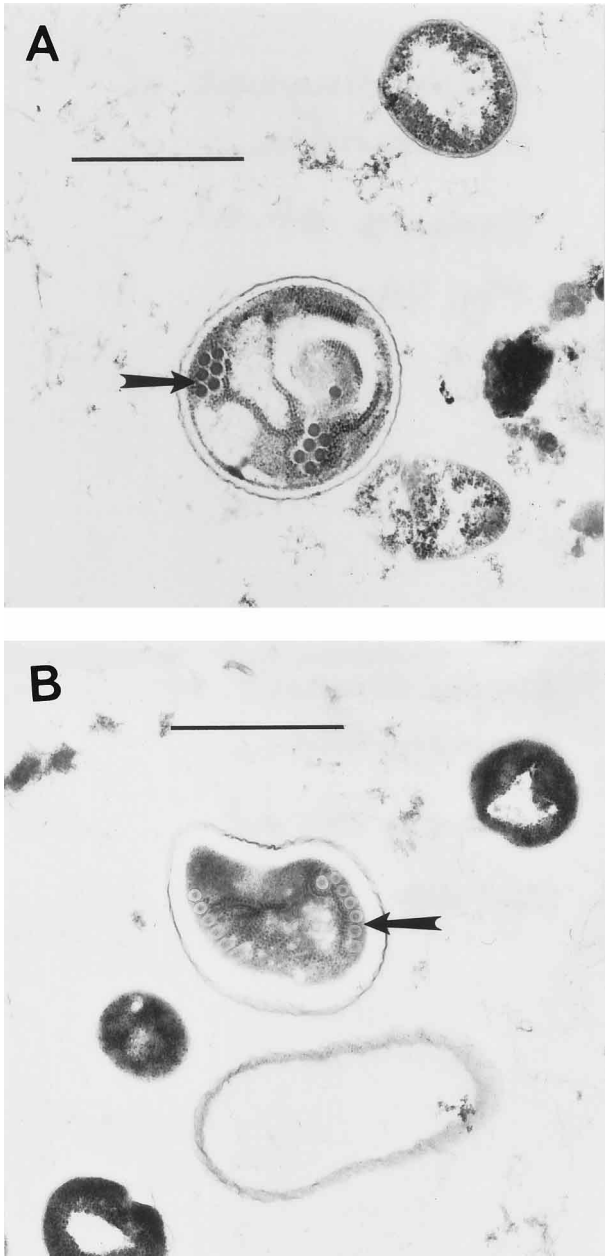

FIG. 4. Transmission electron micrographs of microsporidian spores from stool samples of human immunodeficiency virus-infected patients. (A) *Enterocytozoon bieneusi* from patient 13; (B) *E. intestinalis* from patient 10. The arrangement of the polar coils (arrows) is the main diagnostic feature for species identification. Bar = 1 μm.

We found that both fresh and fixed stool samples can be used for identification and that other secretory or excretory fluids as well as biopsy samples can be suitable for diagnosis. This could be useful for retrospective studies using stored tissue samples. In addition, the monoclonal antibody 3B6 offers a new vehicle for epidemiological studies geared to determining the prevalence of microsporidia, including features of

TABLE 2. Comparison of three diagnostic methods for microsporidia performed with diarrheal samples[a]

Patient no.	Determination by:		
	Calcofluor method	Transmission electron microscopy	Monoclonal antibody 3B6 immunofluorescence
1	Microsporidia	*E. intestinalis*	Positive
2	Negative	No spores found[b]	Positive
3	Negative	Yeast	Negative
4	Negative	*E. intestinalis*	Positive
5	Negative	Yeast	Negative
6	Negative	Negative	Negative
7	Negative	*Enterocytozoon bieneusi*	Negative
8	Negative	*E. bieneusi*	Negative
9	Microsporidia	*E. intestinalis*	Positive
10	Microsporidia	*E. intestinalis*	Positive
11	Microsporidia	*E. bieneusi*	Negative
12	Negative	Yeast and bacteria	Negative
13	Microsporidia	*E. bieneusi*	Positive
14	Negative	Negative	Negative
15	Negative	Negative	Negative
16	Microsporidia	*E. bieneusi*	Negative
17	Microsporidia	*E. bieneusi*	Negative
18	Microsporidia	*E. bieneusi*	Negative
19	Microsporidia	*E. bieneusi*	Negative
20	Microsporidia	*E. bieneusi*	Negative

[a] Results recorded and expressed according to the technique used.
[b] Limited amount of sample did not allow further evaluation by transmission electron microscopy.

transmission such as potential intermediate hosts and reservoirs of infection, e.g., water and food sources. However, results could be biased due to the recognition of non-human-infecting microsporidian species (e.g., *Nosema* spp. and *Vairimorpha* spp. of insects). This fact could allow monoclonal antibody 3B6 to prove useful in the study of animal and insect microsporidia. In addition, it could offer new alternatives, such as affinity chromatography or immunomagnetic separation using the antibody as a ligand, for isolation and purification of microsporidian spores.

In our results, only one sample positive by immunofluorescence was found to be *E. bieneusi* by electron microscopy. It could be that the patient had a dual microsporidian infection with a few *Encephalitozoon* spores, explaining why a predominance of *E. bieneusi* spores was detected by electron microscopy. Additionally, no microsporidia were found in the fecal sample from patient 2 by either the Calcofluor method or electron microscopy but microsporidian spores were observed by immunofluorescence. One explanation could be that no spores were present in the small amount of material used for electron microscopy evaluation. The sensitivity of our antibody 3B6 in immunofluorescence could enhance detection of spores which can go undetected by other methodologies.

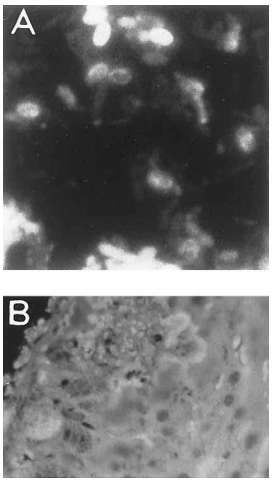

FIG. 5. Reactivity of monoclonal antibody 3B6 with fixed microsporidian samples by indirect immunofluorescence. (A) Electron microscopy-confirmed *E. intestinalis* fecal spores; (B) foci of parasitized cells in a liver section derived from a systemic *E. cuniculi* infection in a SCID mouse.

The molecular weights of antigens recognized by antibody 3B6 in *E. intestinalis*, *E. cuniculi*, and *E. hellem* were similar to and within the range of the molecular weights previously described for these species (21, 26). One difference was the number of bands recognized. Thus, the exospore antigenic determinants in the three *Encephalitozoon* species recognized by monoclonal antibody 3B6 appear to be highly conserved. The targeted epitopes appeared to be proteins, since periodate treatment did not decrease the reactivity of any of the bands by Western blot. It has been shown that the endospore is composed of chitin-like substances and the exospore is made of protein (24). Because monoclonal antibody 3B6 recognized similar antigens among *Encephalitozoon* sp. spores, we suggest that exospores in all three species could contain similar proteins which may differ in expression. This occurrence could have further implications. For example, we have observed that monoclonal antibody 3B6 reduced infectivity of *E. cuniculi*, *E. intestinalis*, and *E. hellem* in vitro (unpublished observations). It could be that there are neutralizing-sensitive epitopes among the exospore antigens recognized by antibody 3B6. The characterization of these and other epitopes could reveal potential targets for vaccine development, chemotherapy, and immunotherapy. These studies are warranted in view of the recurrence of microsporidian infections in patients with AIDS following treatment with chemotherapeutic agents.

In conclusion, we have developed a monoclonal antibody targeted to *Encephalitozoon* sp. exospore antigens which can be used in an easy and reliable as well as sensitive and specific immunofluorescence assay to detect microsporidian spores. This assay may increase efficiency in microsporidian diagnosis using excretory or secretory fluids and biopsy specimens.

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