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Comparison of *Cryptosporidium parvum* and *Cryptosporidium wrairi* by Reactivity with Monoclonal Antibodies and Ability To Infect Severe Combined Immunodeficient Mice

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Twenty-three monoclonal antibodies raised to *Cryptosporidium parvum* and 12 raised to *C. wrairi* reacted with equal intensity with the heterologous species. Despite demonstration of a close immunologic relationship between these two species, *C. wrairi* did not induce persistent infection in severe combined immunodeficient mice as did *C. parvum*.

Cryptosporidium parvum, a protozoan reported to infect the gastrointestinal tracts of numerous mammals, is a particularly important cause of infection in newborn calves and in humans (11, 12). *C. parvum* infects infant mice and guinea pigs but not adult animals (9, 12). *C. wrairi* was first described in guinea pigs (23). The differences between the two species have been described previously (8, 9, 21, 22).

One purpose of this study was to determine if species-specific monoclonal antibodies (MAbs) could be raised to either *C. parvum* or *C. wrairi*. Such reagents would be useful for distinguishing the two species from each other. In addition, because *C. parvum* infection is important in persons with AIDS (15), *C. wrairi* infection may also be a threat to these patients. Persistent infection by *C. parvum* has been established in nude mice (4) and in mice with severe combined immunodeficiency disease (SCID) (17, 18). Therefore, another purpose of this study was to determine if SCID mice could be persistently infected with *C. wrairi*.

The *C. parvum* isolate was originally obtained from H. Moon (National Animal Disease Center, Ames, Iowa). Oocysts were isolated by using an ether extraction method (20). *C. wrairi* was isolated during a spontaneous outbreak of cryptosporidiosis in guinea pigs (8) and has been passaged in guinea pigs. Feces were collected and passed through sieves (6). Oocysts were purified from sieved material by using sucrose gradients (3, 10).

Titers of polyclonal mouse sera were determined, or hybridoma supernatants were screened with an indirect fluorescent-antibody method using freshly excysted *C. parvum* or *C. wrairi* sporozoites (20). Some hybridoma supernatants were screened with an indirect fluorescent-antibody slide test using fixed antigen to reduce the amount of *C. wrairi* antigen needed (2). Hybridoma supernatants positive by the slide test were confirmed with the indirect fluorescent-antibody test by using freshly excysted sporozoites.

MAbs to *C. parvum* and *C. wrairi* were prepared as described previously (1, 19). Twenty-three MAbs raised to *C. parvum* and 12 raised to *C. wrairi* reacted with equal intensity and had the same surface pattern with both species of cryptosporidia (Table 1).

Western blotting (immunoblotting) was done with 14 MAbs to determine if more than one antigen would be recognized by different MAbs. For this study, *C. parvum* sporozoite antigen was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (19). Immunoglobulin concentrations were determined (The Binding Site, San Diego, Calif.) and adjusted to 2 μg/ml. A nonspecific MAb of the same isotype was used as a negative control. Western blotting was done with ECL Western Blotting Reagent (Amersham, Amersham, United Kingdom) in accordance with the vendor's instructions. Results are shown in Table 1.

Including the 9 MAbs previously studied (9, 22) and the 35 MAbs in the present study, a total of 44 cross-react with both species. Apparently, major epitopes have been highly conserved, despite differences in biologic behavior (9, 22) and chemical composition of surface structures (21) between the two species. A spectrum of antigens was detected with Western blots, although at least four of the MAbs tested recognized the same antigens. Three of the MAbs tested for cross-reactivity in this study, MAbs 16.29, 17.41, and 18.44, were shown previously to neutralize infectivity of both sporozoites and merozoites of *C. parvum* for infant mice (5, 19). In addition, persistent infection of nude mice and SCID mice was significantly decreased after treatment with MAb 17.41 (4, 18). Another neutralizing MAb to *C. parvum* recognizes a 15-kDa glycoprotein on the surface of sporozoites of both *C. parvum* and *C. wrairi* (22). Lastly, hyperimmune bovine colostrum raised to *C. parvum*, mixed with sporozoites of *C. wrairi* and injected intraintestinally into guinea pigs, markedly reduced the severity of infection by *C. wrairi* compared with non-hyperimmune colostrum (14). These studies showing cross-reactive neutralizing activity of antibodies raised to the two species suggest that guinea pigs infected with *C. wrairi* could be useful for studying the immune response to putative protective antigens of *C. parvum*. This is particularly true since guinea pigs with a mature immune response are infected with *C. wrairi* (8), whereas only immature or immunosuppressed animals are infected with *C. parvum* (12, 18).

C. wrairi and *C. parvum* oocysts were treated with 3% peracetic acid for 10 min before inoculation into 8-week-old female C.B-17/Icr/Imd-*scid* mice (Imdyne Corporation, San Diego, Calif.). Forty mice were randomly assigned to two

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TABLE 1. Characteristics of MAbs reactive with *C. parvum* and *C. wrairi*

MAb	Antigen source[a]	Parasite stage[b]	Immunofluorescence pattern	Western blot reactivity[c]
18.280	Cp	O		ND
60/62.62	Cw	O		ND
16.111	Cp	S	Diffuse	ND
18.247				
18.433				
18.44				
15.15	Cp	S	Multifocal	ND
15.20				
16.12				3
16.29				3
16.160				2
16.175.2				ND
16.239				ND
16.312B				ND
16.4				3
16.412				3
16.502				ND
17.41				ND
18.199				2
18.313				1
18.326.81				2
18.398				4
18.417B				1
18.97				ND
60/11.3	Cw	S	Multifocal	ND
60/12.21				ND
60/37.5				ND
60/40.28				ND
60/49.37				ND
60/85.1				ND
60/85.8				ND
62/96.3				5
62/148.2				1
62/251.3				1
60/89.3	Cw	S	Apical ends	ND

[a] Cp, *C. parvum*; Cw, *C. wrairi*.
[b] O, Oocyst; S, sporozoite.
[c] Western blot reactivities: 1, negative; 2, band migrated with dye front; 3, three bands ranging in size from <50 to 250 kDa; 4, two bands, one migrating with the dye front and the other at <49 kDa; 5, several bands (<50 to 250 kDa). ND, not done.

treatment groups of 15 mice and a control group of 10 uninoculated mice. Each mouse in a treatment group was inoculated by gavage via a blunted 22-gauge needle with 10^6 *C. wrairi* or *C. parvum* oocysts in 250 μl of phosphate-buffered saline. Mice from each experimental or control group were housed separately in groups of five in filter-topped cages. Experimental groups were handled with separate forceps to prevent cross-contamination. Eight weeks after inoculation, the mice were euthanatized with ether, weighed, and necropsied. The intestinal tracts were collected immediately after euthanasia, cut into 2.5-cm sections, and fixed longitudinally in buffered Formalin. Giemsa-stained longitudinal sections representing the entire length of the intestinal tract of each mouse were prepared. In the *C. wrairi*-treated group, the entire length of the gastrointestinal tract of each mouse was examined microscopically for the presence of cryptosporidia. Experience had shown that the distal 3 to 4 mm of the stomach and the cecum were specifically infected in SCID mice with persistent *C. parvum* infections (unpublished data). Tissue infectivity scores were assigned to these sites (18). In addition, bile duct, liver, pancreatic duct, and lung sections were taken for histopathologic analysis. In a second experiment, seven SCID mice were inoculated with 10^7 *C. wrairi* oocysts and examined as detailed previously. The same two inocula of *C. wrairi* oocysts (10^5) used to inoculate SCID mice were inoculated by stomach tube into each of five and two 3-week-old, female, specific-pathogen-free Hartley guinea pigs (Charles River Laboratories, Portage, Mich.), respectively. These animals were euthanized 1 week after inoculation, and a Giemsa-stained section of the distal ileum was examined microscopically for cryptosporidia. None of the SCID mice inoculated with either 10^6 or 10^7 *C. wrairi* oocysts were infected with cryptosporidia. All seven guinea pigs were infected with cryptosporidia. In contrast, all 15 SCID mice inoculated with *C. parvum* oocysts were infected with cryptosporidia. The average cumulative intestinal score for mice in this group was 4.4. The biliary tracts of 4 of 15 mice were infected.

Infant mice infected with *C. wrairi* shed far fewer oocysts than did those infected with *C. parvum* (9, 21). Also, oocysts isolated from mice infected with *C. wrairi* do not reinfect guinea pigs (9). These findings, along with the failure of *C. wrairi* to establish persistent infection in SCID mice, indicate that the mouse is an unnatural host for *C. wrairi*.

Germ-free SCID mice were much less resistant to infection with *C. parvum* than were SCID mice with a microflora (13). Gamma interferon production in SCID mice by a non-T-cell pathway requires tumor necrosis factor and a soluble product of bacterial origin (24). Furthermore, SCID mice given anti-gamma interferon antibody develop early overwhelming infections after inoculation with *C. parvum* (7, 16). Therefore, *C. wrairi* may be even more affected by these nonspecific immune mechanisms than *C. parvum*. Because adult humans are not resistant to *C. parvum* infection, as are adult mice, the resistance of SCID mice to establishment of a persistent infection by *C. wrairi* does not rule out the possibility that *C. wrairi* can infect immunodeficient humans.

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