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Kinetics of *Cryptosporidium parvum* Sporozoite Neutralization by Monoclonal Antibodies, Immune Bovine Serum, and Immune Bovine Colostrum

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Monoclonal antibodies, immune bovine serum, and immune bovine colostral whey neutralized infectivity of *Cryptosporidium parvum* sporozoites for mice in a time-dependent manner. Immune colostral whey neutralized sporozoites more rapidly and completely than immune serum, monoclonal antibody (MAb) 18.44, or a combination of MAb 18.44 and MAb 17.41. Mice were partially protected against oral challenge with *C. parvum* oocysts when treated with immune colostral whey, MAb 17.41, or a combination of MAb 17.41 and MAb 18.44.

Cryptosporidiosis, caused by the protozoan parasite *Cryptosporidium parvum*, is a common cause of diarrhea in humans, calves, and other mammals (2, 6, 9, 10, 12, 14, 17, 19). The disease is self-limiting in animals with competent immune systems but persistent and life threatening in immunodeficient hosts (2, 3, 6, 7, 8, 13, 18). Infection is initiated by sporozoites released from oocysts in the intestinal tract. Disease in the immunodeficient host is maintained by cyclical infection of intestinal epithelial cells both by type I merozoites and by sporozoites released from thin-walled oocysts. The widespread distribution of the organism, its transmissibility among mammalian species, and the absence of effective therapeutic agents have made *C. parvum* a significant cause of disease in patients with genetically based or acquired immunodeficiency disorders (2, 3, 6–8, 10, 12–14).

Our goal is to develop immunologic regimens for the prevention and treatment of cryptosporidiosis. We hypothesize that infection may be prevented by neutralizing sporozoites in the intestinal lumen before they penetrate epithelial cells. In this study, we tested the rate and magnitude of sporozoite neutralization by four immune reagents and compared those results with the abilities of the reagents to protect mice against oral challenge with *C. parvum* oocysts.

Two monoclonal antibodies (MAbs) reactive with surface antigens on viable *C. parvum* sporozoites were obtained from hybridomas that were previously described (15). Antibody 18.44 (immunoglobulin G3 [IgG3] isotype) reacted with a nonprotein sporozoite surface antigen which eluted in the void volume of a Bio-Gel A column (exclusion limit, 500,000 daltons). The antibody bound to 100% of viable sporozoites and neutralized their infectivity when incubated with them for 30 min. The concentration of MAb 18.44 in cell culture supernatants was 640 μg/ml. Ascitic fluid collected from mice injected with 18.44 hybridoma cells contained 18 mg of IgG3 per ml. Antibody 17.41 (IgM isotype) reacted with sporozoite antigens of 98, 55, and 28 kilodaltons and neutralized sporozoites when incubated with them for 30 min. It was present in cell culture supernatants at a concentration of 100 μg/ml. Ascitic fluid collected from mice injected with 17.41 hybridoma cells contained 1.9 mg of IgM per ml. EqT6 and EqT12 MAbs were used as IgG3 and IgM MAb isotype controls, respectively. The production of EqT6 and EqT12 and their reactivity with equine thymocytes have been described (20, 21). Immune bovine serum was obtained by immunizing cattle with excysted oocysts and sporozoites. The production, sporozoite antigen specificity, and neutralizing activity of this immune serum were previously described (15, 16). The immune bovine colostrum was produced by intramuscular and intramammary immunization of cattle with *C. parvum* oocysts (4). Whey from this colostrum neutralizes sporozoite infectivity and partially protects mice against challenge with *C. parvum* oocysts (5). This same immune colostrum partially protected oocyst-challenged calves (4). Similar colostrum was successfully used to treat an acquired immunodeficiency syndrome patient with *Cryptosporidium* infection (B. L. P. Ungar, D. J. Ward, R. Fayer, and C. A. Quinn, J. Am. Med. Assoc., in press).

Neutralization kinetics studies were performed with hybridoma cell culture supernatants, serum, and colostral whey as follows. *C. parvum* sporozoites were isolated by ion-exchange chromatography as previously described (16). Sporozoites (2×10^5 in 10 μl of Hanks balanced salt solution or RPMI 1640 medium) were incubated with 25 μl of immune reactant or nonimmune control solution in 96-well round-bottom microdilution plates for 0, 10, 20, or 30 min at 37°C. After incubation, the contents of a well were drawn into a microdilution syringe and infused per anus into the intestinal tracts of BALB/c mice, 3 to 5 days old, as described previously (16). Removable rubber patches were applied to the perineum of each mouse to prevent defecation. Patches were removed after 4 h, and the mice were returned to their dams. Four days after injection, intestinal tracts were removed from each mouse and scored for intensity of infection by histologic examination (16). Infectivity scores for individual mice ranged from 0 (absence of infection) to a maximum of 9 (organisms present in >67% of mucosal epithelial cells in ileum, cecum, and colon).

Experiments to assess protection against oocyst challenge were performed with hybridoma ascitic fluids, serum, and

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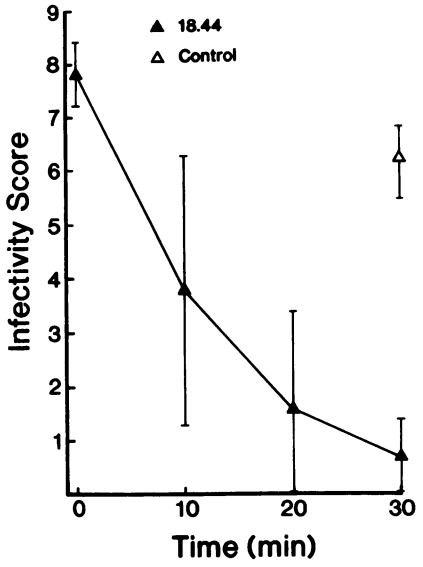

FIG. 1. Kinetics of neutralization of *C. parvum* sporozoites by MAb 18.44. Data are means ± standard deviations of three experiments comparing infectivity scores of mice injected with 2×10^5 sporozoites previously incubated for the indicated times with neutralizing MAb 18.44 or isotype control MAb EqT6.

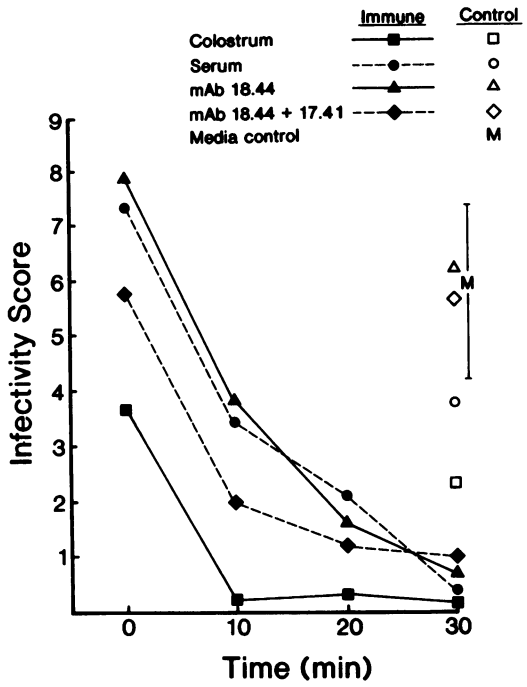

FIG. 2. Kinetics of neutralization of *C. parvum* sporozoites by immune bovine colostral whey, immune bovine serum, MAb 18.44, and the combination of MAbs 17.41 and 18.44. M and error bars, Mean ± standard deviation of infectivity scores from 12 experiments with mice injected with sporozoites incubated for 30 min in Hanks balanced salt solution or RPMI 1640 medium.

colostral whey as follows. BALB/c mice, 3 to 5 days old, were separated from dams for 1 h to minimize gastric content. They were then treated by gastric intubation with 65 μl of immune or nonimmune control solution. Individual mice were placed in paper cups and kept at 37°C for 2 h. Oocysts were isolated and treated with peracetic acid, as described previously (9, 16). Oocysts (2×10^4) in 10 μl of medium were mixed with 55 μl of the appropriate immune or control solution and administered by gastric intubation to individual mice. Mice were then returned to their dams. Each mouse was treated by gastric intubation with 65 μl of relevant immune or control solution at 20 h postoocyst injection. Mice were necropsied 96 h postchallenge, and intestinal tracts were scored for intensity of *C. parvum* infection as described above.

MAb 18.44 neutralized sporozoites in a time-dependent manner (Fig. 1). Neutralization was significant after 20 min but greatest following 30 min of incubation. Immune bovine serum and a combination of MAb 17.41 and MAb 18.44 neutralized sporozoites at rates and magnitudes comparable to those observed with MAb 18.44 (Fig. 2). However, neutralization of sporozoites with immune colostral whey was virtually complete following 10 min of incubation. To determine the reason for this more rapid neutralization by colostral whey, sporozoites were examined for evidence of agglutination following incubation with immune colostral whey, control colostral whey, and RPMI 1640 medium. Sporozoites remained individualized in all three reagents, indicating that agglutination of organisms was not the major reason for neutralization. However, the viability of sporozoites, as assessed by the fluorescein diacetate reaction (11), was reduced to 40% following incubation with immune colostral whey, compared with 80% in control colostral whey and >95% in RPMI 1640 medium.

The ability of immune bovine serum, immune bovine colostral whey, and ascitic fluids containing MAb 17.41 and MAb 18.44 to protect mice against oral challenge with oocysts was determined (Table 1). Neither immune bovine serum nor MAb 18.44 ascites provided significant protection compared with their respective controls. Immune colostral whey significantly reduced infectivity scores in mice challenged with 2×10^4 oocysts. Infectivity scores were also significantly reduced in mice treated with ascitic fluid containing MAb 17.41 or pooled ascitic fluids containing MAb 17.41 and MAb 18.44. Reduced parasite loads have also been observed by Arrowood et al., who treated mice with combinations of MAbs reactive with *C. parvum* sporozoite antigens (1).

The rapid neutralization of sporozoites by immune colostrum observed in the current study may explain protection against an oocyst challenge observed here and in calves (4), as well as the positive treatment effects reported by others who used immune bovine colostrum to treat a child with

TABLE 1. Protection of mice against oral challenge with 2×10^4 *C. parvum* oocysts

Reagent	Infectivity score		P^a
	Immune	Control	
Bovine serum	4.9 ± 1.3	6.0 ± 2.1	NS
MAb 18.44 ascites	7.3 ± 1.9	8.3 ± 0.9	NS
MAb 17.41 ascites	4.3 ± 2.0	8.3 ± 0.9	<0.0005
MAb 18.44 + MAb 17.41 ascites	1.9 ± 2.1	8.3 ± 0.9	<0.0005
Bovine colostral whey	2.0 ± 1.9	7.3 ± 1.1	<0.0005

[a] Differences between immune and respective control groups were evaluated by Student's one-tail *t* test. Groups contained 8 to 12 mice each. Significance conclusions were verified by repeat experiments. NS, Not significant.

persistent cryptosporidiosis (18). The ability to quickly neutralize sporozoites within the gut lumen prior to infection of epithelial cells may be important in reducing initial infection following ingestion of oocysts and in diminishing autoinfection caused by sporozoites released from thin-walled oocysts produced in the infected host (2).

Ascitic fluids containing a combination of MAb 17.41 and MAb 18.44 partially protected mice against oral oocyst challenge, as evidenced by reduced infectivity scores. In contrast to rapid sporozoite neutralization by colostral whey, sporozoite neutralization mediated by the combination of MAbs 17.41 and 18.44 was neither more rapid nor profound than that observed with immune reagents that lacked the ability to protect mice against oocyst challenge (MAb 18.44 alone and immune bovine serum). The fact that antibody concentrations in cell culture supernatants used for sporozoite neutralization studies were lower than concentrations of ascitic fluids used for oocyst challenge experiments may account for this disparity. Cell culture supernatants were used in the sporozoite neutralization studies to simplify interpretation of the role of the MAb in mediating neutralization. Ascitic fluids were used in the oocyst challenge protection experiments to maximize the amount of neutralizing antibody delivered to the intestinal lumen. Therefore, the results of the sporozoite neutralization and oocyst challenge studies involving MAbs are not directly comparable. It is clear, however, that MAb 17.41 can reduce the intensity of infection in mice challenged with *C. parvum* oocysts. Sporozoite antigens recognized by MAbs 17.41 and 18.44 are potentially useful for induction of protective immune responses against infection by *C. parvum*.

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