INFECTION AND IMMUNITY, Mar. 1991, p. 990–995
0019-9567/91/030990-06$02.00/0

Vol. 59, No. 3

Characterization of Bovine Cellular and Serum Antibody Responses during Infection by *Cryptosporidium parvum*

WILLIAM M. WHITMIRE AND JAMES A. HARP*

Metabolic Diseases and Immunology Research Unit, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010

Received 10 September 1990/Accepted 14 December 1990

Cellular and serum antibody responses of calves were monitored for 23 days after oral inoculation of the calves with oocysts of *Cryptosporidium parvum*. In vitro blastogenic responses of peripheral blood lymphocytes were assessed after stimulation with a *C. parvum* preparation. Specific lymphocyte blastogenic responses to the parasite were detected 2 days after inoculation. Parasite-specific antibody titers were demonstrable 7 days after inoculation with oocysts and achieved peak levels 9 days after inoculation, coinciding with oocyst shedding at 5 to 10 days after inoculation. Both lymphocyte and antibody responses remained elevated until the termination of the experiment. Immunoblotting the *C. parvum* preparation with serum from an infected calf revealed six major parasite antigens. Five of these antigens reacted on immunoblots from 7 to 14 days after inoculation with oocysts. A parasite antigen of approximately 11,000 molecular weight demonstrated intense reactivity on immunoblots from 7 to 23 days after inoculation. The 11,000-molecular-weight antigen also reacted on immunoblots with parenterally raised antioocyst and antisporozoite rabbit sera. These results indicate that cell-mediated as well as humoral immune responses are initiated by cryptosporidial infection in calves and that the 11,000-molecular-weight parasite antigen is immunodominant.

Cryptosporidium parvum is an obligate intracellular protozoan parasite that can cause enteric disease in various vertebrate species, including cattle and humans (5, 19). Once considered rare, cryptosporidiosis is now recognized as being cosmopolitan in nature (2, 4).

Disease caused by the parasite in immunocompetent hosts is self-limiting, and it appears that the status of the immune system determines the severity of and resistance to cryptosporidiosis (5, 19). For example, cryptosporidiosis in immunocompromised individuals can develop into a life-threatening situation, yet the removal of immunosuppression in people with reversible immunodeficiencies results in recovery from the disease (5). The exact immune mechanisms responsible for this resistance have not been defined. However, Ungar et al. (23) reported that chronic infection with *C. parvum* could be established in neonatally infected mice that had been treated in vivo with anti-CD4 alone or in conjunction with anti-CD8 monoclonal antibodies. Furthermore, chronically infected nude (T-cell-deficient) mice ceased oocyst shedding following adoptive transfer of lymphoid cells from immune mice (23). These data indicate that T-cell-mediated immune responses are necessary for both resistance to and recovery from cryptosporidiosis.

Recently, we described in vitro blastogenic responses to *C. parvum* by spleen lymphocytes from *C. parvum*-exposed mice (25). We have now characterized lymphocyte blastogenic and serum antibody responses during *C. parvum* infection in calves.

MATERIALS AND METHODS

Parasite and antigen preparations. *C. parvum* oocysts were separated from calf feces (17) and purified further by discontinuous sucrose gradients (1). Purified oocysts were either used to infect experimental animals or processed with slight modifications into a preparation that was described in a previous report (25). Briefly, purified oocysts were sterilized by exposure for 30 min at room temperature to 2.5% peracetic acid in 0.15 M phosphate-buffered saline (PBS), washed three times in PBS, and adjusted to a concentration of 5.6×10^7 oocysts ml^{-1} in 9 ml of sterile water. Aliquots of the oocyst suspension were homogenized by mixing with zirconium beads on a minibead beater (Biospec Products, Bartlesville, Okla.) as previously described (25). Following three cycles of freezing and thawing, the pooled homogenate was centrifuged at 1,200 × *g* for 15 min, and 1 ml of sterile 10× PBS was added to the resulting supernatant (*C. parvum* preparation). The *C. parvum* preparation was stored at −80°C and used in the enzyme-linked immunosorbent assay (ELISA) and in lymphocyte blastogenesis and immunoblotting assays.

Experimental animals. Four colostrum-deprived Holstein-Friesian bull calves were obtained from a local dairy and kept in strict isolation at the National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture (Ames, Iowa) large-animal facilities. Within 4 h of birth, a commercial colostrum replacer (Colostrx; Protein Technologies, Inc., Petaluma, Calif.) was administered to the calves per the manufacturer's instructions. Thereafter, calves were fed nonmedicated milk replacer twice daily for the duration of the experiment.

Beginning at 1 week of age, fecal (daily) and blood (two times a week for 5 weeks, for a total of 10 bleeds) samples were collected from each calf. Three of the calves were orally inoculated with 10^7 purified *C. parvum* oocysts at 2 weeks of age, and one calf was kept free of *C. parvum* infection. Subsequently, all calves were monitored for fecal shedding of oocysts (8), serum antibody levels, and in vitro lymphocyte responsiveness to *C. parvum*.

Lymphocyte blastogenesis assay. The lymphocyte blastogenesis assay was performed as previously described, with minor changes (25). Venous blood collected aseptically from each calf was mixed with anticoagulant (9 parts whole blood:1 part 2× acid citrate dextrose) and centrifuged at

* Corresponding author.

1,000 × *g* for 20 min. Five milliliters of the top layer of cells was aspirated and diluted 1:6 in PBS. Peripheral blood mononuclear cells (PBMC) were separated from erythrocytes by density gradient centrifugation (450 × *g* for 40 min) over Histopaque (specific gravity, 1.084; Sigma Chemical Co., St. Louis, Mo.). Following collection of PBMC by aspiration, residual erythrocytes were lysed, and after two washes in PBS, PBMC were suspended at a concentration of 2×10^6 cells ml^{-1} in RPMI 1640 culture medium (GIBCO, Grand Island, N.Y.) supplemented with 20 mM L-glutamine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 100 U of penicillin ml^{-1} and 100 μg of streptomycin (Sigma) ml^{-1}. Assays were set up in triplicate in 96-well flat-bottomed microtiter plates (Becton Dickinson, Lincoln Park, N.J.) by adding 0.1 ml of PBMC suspensions (2×10^5 cells $well^{-1}$) to wells containing 0.1 ml of RPMI with 20% fetal bovine serum (RPMI-FBS; Hyclone, Inc., Logan, Utah), mitogen (8 μg of concanavalin A [ConA] ml^{-1} in RPMI-FBS), or dilutions (1:100, 1:500, or 1:5,000) of the *C. parvum* preparation in RPMI-FBS. After 72 h (ConA) or 120, 168, or 216 h (*C. parvum* preparation) of incubation at 39°C in a 95% air–5% CO_2 humidified atmosphere, 0.5 μCi of [*methyl*-^{3}H]thymidine (specific activity, 25 Ci mM^{-1}; Amersham Corp., Arlington Heights, Ill.) in 50 μl of RPMI was added to each well. PBMC cultures were further incubated for 18 h and then harvested onto glass fiber filters. Incorporation of [^{3}H]thymidine was detected by liquid scintillation counting. Stimulation indices were calculated by dividing mean counts per minute for stimulated PBMC cultures by mean counts per minute for unstimulated (control) PBMC cultures. Results are shown as the stimulation index or mean stimulation index ± standard error of the mean of PBMC cultures from each group of animals (noninfected and infected, respectively). Results were analyzed by Student's *t* test or single-factor analysis of variance (18).

Detection of bovine parasite-specific antibody. The ELISA used in the present study was the same as that previously described (6), except that a 1:20 dilution of the *C. parvum* preparation in 50 μl of PBS was used to coat individual wells of microtiter plates. Serum samples corresponding to the 10 sequential bleeds of each calf were assessed for parasite-specific antibody. Parasite-specific antibody titers are reported herein as the reciprocal or mean reciprocal ± standard error of the mean of the highest dilution at which positive ELISA results were obtained from noninfected and infected animals, respectively.

Polyacrylamide gel electrophoresis. The *C. parvum* preparation was diluted 1:2 in 2× sodium dodecyl sulfate (SDS) solubilizing solution (4% SDS, 0.5 M Tris [pH 6.8], 20% glycerol, with or without 10% 2-mercaptoethanol) and solubilized at 100°C for 5 min. Solubilized samples as well as prestained molecular weight standards (Bio-Rad Laboratories, Richmond, Calif.) were subjected to SDS-polyacrylamide gel electrophoresis in 12 or 16.5% polyacrylamide slab gels with a Mini Protean II gel apparatus (Bio-Rad) and the discontinuous buffer system described by Laemmli (12). Following electrophoresis at 200 V for approximately 50 min, gels were subjected to immunoblotting techniques.

Immunodetection of parasite antigens. Reduced or nonreduced (with or without 2-mercaptoethanol, respectively) *C. parvum* preparation proteins were electrophoretically transferred to nitrocellulose (0.1-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) in a Mini Trans-Blot Cell (Bio-Rad) (21) for 2 h at 100 V. Following transfer, nitrocellulose sheets were incubated in 0.3% fish skin gelatin (Norland Products, Inc., New Brunswick, N.J.) and 0.05% Tween 20 in Tris-buffered saline (20 mM Tris base–0.5 M NaCl [pH 7.5] in distilled water) overnight at 4°C to block nonspecific binding sites. Nitrocellulose sheets were then cut into 4-mm-wide strips and probed with bovine antisera from sequential blood samples (corresponding to the ELISA samples; diluted 1:10 or 1:20 in blocking buffer) for 2 h at room temperature under constant agitation. After exposure to a 1:400 dilution of horseradish peroxidase-conjugated rabbit anti-bovine immunoglobulin G (IgG; heavy and light chain specific; Cappel Laboratories, Cochranville, Pa.) in blocking buffer for 1 h at room temperature, bound peroxidase activity was developed with peroxidase substrate solution (7).

Nitrocellulose strips containing *C. parvum* preparation proteins transferred from a 12% SDS-polyacrylamide gel, as described above, were also probed with three types of rabbit antisera (diluted 1:100 in blocking buffer). One type of rabbit antiserum was produced by several parenteral inoculations of purified intact oocysts, and a second type was produced by a similar immunization regimen with purified sporozoites only. A third type was normal preimmunization serum obtained prior to inoculations with the parasite. These rabbit antisera were generous gifts from Tom Casey (National Animal Disease Center). Specific binding of rabbit IgG to nitrocellulose-bound parasite antigens was subsequentially detected by using biotin-conjugated goat anti-rabbit IgG (Sigma) with horseradish peroxidase-conjugated streptavidin (Jackson Immunoresearch Laboratories, Inc., West Grove, Pa.) and peroxidase substrate solution.

Relative molecular weights (M_r) of parasite antigens were estimated by comparing their relative mobilities with the relative mobilities of prestained molecular weight standards which had been transferred to the same nitrocellulose sheets.

RESULTS

The three infected calves shed oocysts in their feces, with the average onset beginning at 5 days after initial oral inoculation (DAI) with *C. parvum* oocysts. Mean duration of oocyst shedding was 5 days, with individual fecal samples exhibiting one to five oocysts per high-power (500×) field. Oocyst shedding by the infected calves was accompanied by moderate nonhemorrhagic diarrhea, whereas the noninfected calf did not shed oocysts or have diarrhea.

PBMC from all calves demonstrated a significant response ($P \leq 0.05$) to ConA in the lymphocyte blastogenesis assay (data not shown). There were no significant differences ($P > 0.05$) between the ConA responses of PBMC from any of the four calves. The level of ConA responsiveness did not change significantly ($P > 0.05$) at any time during the study.

Mean responses to the *C. parvum* preparation by PBMC from the infected calves from 7 days before inoculation with oocysts to the day of inoculation were not significantly different ($P > 0.05$) from the responses of the noninfected calf (Fig. 1). Following oocyst inoculation, the responses to the *C. parvum* preparation by PBMC from the infected calves increased, while responses by cells from the noninfected calf remained the same (Fig. 1). Between 16 and 23 DAI, the *C. parvum* preparation-specific responses of PBMC from the infected calves were significantly greater ($P \leq 0.05$) than the mean of their responses prior to oocyst inoculation and the response of the noninfected calf throughout the study (Fig. 1).

The mean serum antibody response by the three infected calves showed an appreciable rise in titer compared with that

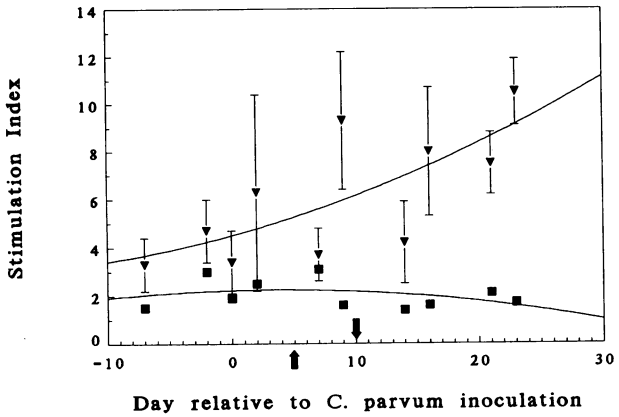

FIG. 1. Blastogenic responses of bovine PBMC from noninfected (■) or infected (▼) calves to the *C. parvum* preparation over the course of infection by the parasite. Arrows mark mean onset (day 5) and cessation (day 10) of oocyst shedding by infected calves. Curve fit lines were generated from second-order polynomial functions. Error bars indicate standard errors of the mean.

of the noninfected calf by 7 DAI (Fig. 2). Mean antibody titers for the infected calves peaked at 9 DAI and remained elevated for the duration of the experiment (Fig. 2). Preinoculation levels of parasite-specific antibody were low for all of the calves, and the noninfected calf did not ever produce titers of specific antibody above baseline level during the experiment (Fig. 2).

Immunoblots of the *C. parvum* preparation probed with serum from one of the infected calves are shown in Fig. 3. At 7 to 9 DAI, several antigens of >47,000 M_r were reactive with parasite-specific antibody. However, this reactivity was absent by 14 DAI. There was faint reactivity with a 20,000-M_r antigen from 9 to 14 DAI as well. A small antigen of approximately 11,000 M_r reacted intensely from 7 DAI to the termination of the experiment at 23 DAI. The 11,000-M_r antigen appeared to be the sole reactive parasite antigen after 14 DAI. Sera from other infected calves gave similar results, with slight variability in reactivities with the higher-M_r parasite antigens. However, sera from all of the

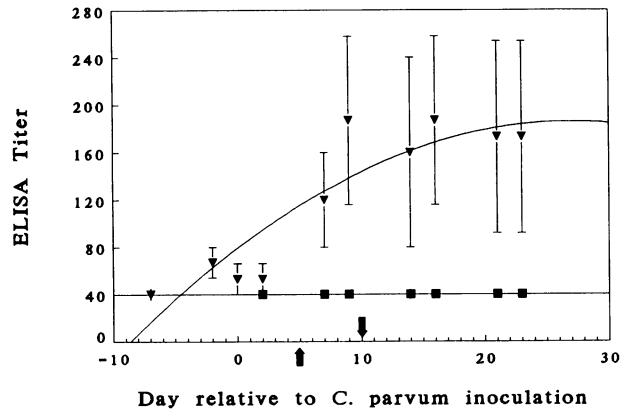

FIG. 2. Serum antibody responses of noninfected (■) or infected (▼) calves to the *C. parvum* preparation over the course of infection by the parasite as described in the legend to Fig. 1. Curve fit lines were generated from second-order polynomial functions. Error bars indicate standard errors of the mean.

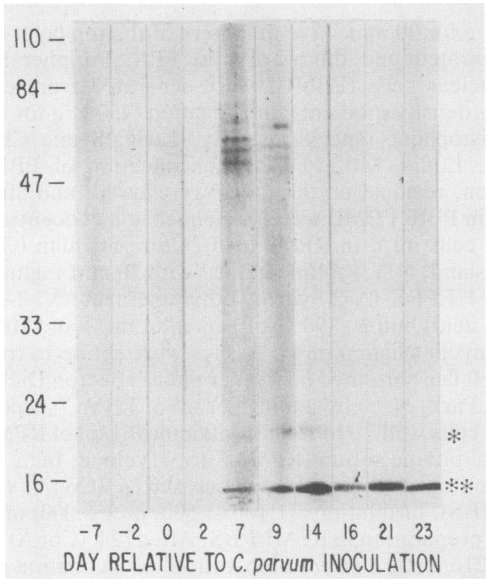

FIG. 3. Immunoblots of reduced *C. parvum* preparation (transferred from a 12% SDS-polyacrylamide gel) probed with serum samples obtained from a calf on the days indicated. The calf was orally inoculated with 10^7 *C. parvum* oocysts on day 0. *, 20,000-M_r antigen that is probably analogous to the 23,000-M_r parasite antigen (Fig. 5); **, immunodominant 11,000-M_r parasite antigen. Molecular weight standards (in thousands) are indicated on the left.

infected calves reacted with the 11,000-M_r antigen beginning at 7 DAI and continuing until termination of the experiment (data not shown).

Further characterization of the 11,000-M_r parasite antigen was achieved by probing an immunoblot from a 16.5% SDS-polyacrylamide gel containing both reduced and nonreduced *C. parvum* preparations with serum collected from an infected calf at 23 DAI. A single reactive antigen was easily recognizable at 11,000 and 13,000 M_rs on the reduced and nonreduced immunoblots, respectively (Fig. 4).

Numerous parasite antigens ranging from >110,000 to approximately 11,000 M_r reacted with rabbit antioocyst or antisporozoite antisera on immunoblots of the reduced *C. parvum* preparation (Fig. 5). For the most part, the two types of antisera reacted with the separated parasite antigens in a similar fashion but with differing intensities. One notable difference was the intense reaction of antioocyst serum with a 23,000-M_r antigen that was not detected with antisporozoite serum (Fig. 5). The two types of antisera reacted with the 11,000-M_r antigen with similar intensities (Fig. 5). Normal preimmunization rabbit serum did not react with immunoblots of the reduced *C. parvum* preparation (Fig. 5).

DISCUSSION

Cell-mediated immunity is generally considered necessary for resistance to intracellular parasites (13). Since mice and humans with defective T-cell functions or depleted T-cell populations can become chronically infected with *C. parvum*, it appears that cell-mediated immunity is involved in recovery from as well as resistance to the disease (4, 8, 23). In a recent report, we described the ability of the *C. parvum* preparation to specifically stimulate spleen lymphocytes from mice that had received multiple oral exposures to the parasite (25). The present study extends this finding to include lymphocytes from the peripheral circulation of

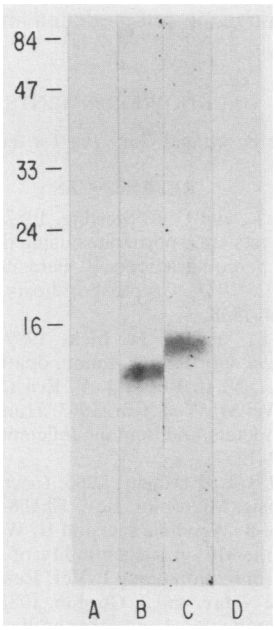

FIG. 4. Immunoblots of reduced (lanes A and B) and nonreduced (lanes C and D) *C. parvum* preparations transferred from a 16.5% SDS-polyacrylamide gel. Lanes A and D were probed with preinoculation calf serum, whereas lanes B and C were probed with serum taken from a calf 23 DAI as described in the legend to Fig. 3. Molecular weight standard (in thousands) are indicated on the left.

calves that received a single oral inoculum of oocysts. Specific blastogenic responses to *C. parvum* by bovine lymphocytes were detectable soon after oocyst inoculation; however, a substantial variation in lymphocyte responsiveness by infected animals at 2 to 14 DAI was evident. Less variation in increased lymphocyte responsiveness was present by 16 to 23 DAI. In a similar study, peripheral blood lymphocytes from calves infected with *Eimeria bovis* exhibited variable in vitro blastogenic responses to an *E. bovis* antigen preparation until 18 DAI (11). By 21 DAI, the increased lymphocyte response to *E. bovis* antigen was essentially the same between animals (11). The variable in vitro bovine lymphocyte responses to antigen in previous and present studies may be related to the fact that the animals were from an outbred population.

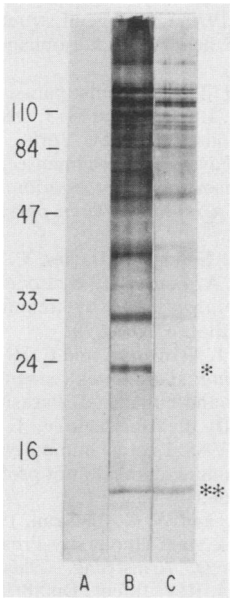

FIG. 5. Immunoblots of reduced *C. parvum* preparation (transferred from a 12% SDS-polyacrylamide gel) probed with normal rabbit serum (lane A), antioocyst rabbit serum (lane B), or antisporozoite rabbit serum (lane C). Relative positions of the 23,000-M_r and (*) 11,000-M_r (**) parasite antigens are indicated. Molecular weight standards (in thousands) are indicated on the left.

In the present study, ConA responses of peripheral blood lymphocytes from infected calves during acute infection were the same as preinoculation responses or responses by lymphocytes from the noninfected calf. Therefore, low preinoculation lymphocyte responses to the *C. parvum* preparation were not due to functional immaturity of PBMC. This also indicates that infection by *C. parvum* did not suppress or enhance bovine lymphocyte responsiveness. Similar findings have been reported for coccidiosis caused by *E. bovis* (11); however, other parasitic diseases such as toxoplasmosis and leishmaniasis may cause immunosuppressive episodes during infection (13).

Infection by *Cryptosporidium* spp. usually results in the production of parasite-specific antibody (3, 22). In the present study, bovine serum antibody titers against *C. parvum* were detectable by 7 DAI and were still elevated at 23 DAI. These results substantiate previous work by others; for example, parasite-specific serum antibodies were detected at 6 and 7 DAI in experimentally infected gnotobiotic calves and colostrum-deprived lambs, respectively (9, 26). Antibody levels in infected gnotobiotic calves were still elevated at 18 DAI, and lambs demonstrated elevated titers for up to 48 DAI (9, 26).

No reactivity was seen on immunoblots with infected calf serum until 7 DAI, which is the first day that parasite-specific antibody was detected by ELISA. Moreover, 7 DAI was roughly equivalent to the midpoint of the oocyst-shedding period. Bovine serum antibody recognized at least six major antigens in the *C. parvum* preparation. However, reactivity to five of these antigens was short term, with loss of reactivity occurring at 14 to 16 DAI. Conversely, Mead et al. (16), using immunoblots of a membrane preparation of *C. parvum* sporozoites, demonstrated reactivity of bovine serum antibodies to several high-M_r antigens for up to 28 weeks after infection. A 20,000-M_r antigen that became evident at 3 weeks, reacted strongly for up to 16 weeks, and lost reactivity by 20 weeks after infection was also reported (16). In a similar study, Hill et al. (9) reported the presence of at least 25 *C. parvum* antigens of >48,500 M_r that reacted with serum and fecal antibodies from infected lambs. Those investigators were able to detect these antigens as well as a 23,000- and a 15,000-M_r antigen for up to 6 weeks after infection (9). Differences in *C. parvum* preparations and sample processing for SDS-polyacrylamide gel electrophoresis may have accounted for the differences in results between previous investigations and the present study. In the present study, the faint 20,000-M_r antigen detected on immunoblots with calf serum at 7 to 16 DAI is probably the same as the 23,000-M_r antigen that reacted with antioocyst rabbit serum.

An 11,000-M_r parasite antigen was clearly visible from 7 to 23 DAI on bovine immunoblots. It appears that the 11,000-M_r antigen was responsible for the entire ELISA reactivity following 14 DAI. Additional characterization by immunoblotting indicated that the 11,000-M_r antigen may

contain intrachain disulfide bonds, since this antigen did not migrate as far under nonreducing conditions as under reducing conditions. Using serum from mice that had been orally inoculated with *C. parvum* oocysts, Luft et al. (14) detected a parasite antigen of <14,000 M_r under nonreducing conditions. This finding is particularly interesting because these investigators obtained their isolate of *C. parvum* from our laboratory. It is possible that the 11,000-M_r antigen described here, the <14,000-M_r antigen (14), and the 15,000-M_r antigen described by Hill et al. (9) are one and the same. A 9,000- to 10,000-M_r antigen of *C. parvum* has also been shown to react intensely with IgA in hyperimmune bovine colostrum (20).

Antioocyst and antisporozoite rabbit sera displayed similar reactive patterns with the *C. parvum* preparation, and both identified several parasite antigens, including the 11,000-M_r antigen. The similar reactivities of the two types of rabbit sera are probably due to the fact that oocysts of *C. parvum* contain sporozoites. Therefore, antigens from both parasite stages were presented to rabbits parenterally immunized with oocysts. The 11,000-M_r parasite antigen reacted with both types of rabbit antisera, which indicates that this antigen is of sporozoite origin. However, investigations with sporozoite antiserum exhaustively adsorbed with intact oocysts, or with specific monoclonal antibodies, will be necessary to confirm this hypothesis.

The most notable difference in reactivity patterns between the two types of rabbit antisera on immunoblots is displayed by the 23,000-M_r antigen. This antigen reacted only with rabbit serum raised against oocysts and not with that raised against sporozoites. The 23,000-M_r antigen has been described and shown by others to be present on the surface of sporozoites (15, 16, 24). Why this discrepancy exists in the present study is unclear. It is possible that the rabbit used for the production of antisporozoite serum was not responsive to this particular parasite antigen. In one study, only one of four people who contracted cryptosporidiosis from exposure to infected calves reacted to the 23,000-M_r antigen in immunoblots (3).

The intensity and duration of reactivity of antisera from calves orally infected with *C. parvum* to the 11,000-M_r antigen demonstrate the natural immunodominancy of this parasite antigen. Because of this immunodominancy, it is possible that the 11,000-M_r antigen is partially responsible for the responsiveness to the *C. parvum* preparation by peripheral blood lymphocytes from infected calves. The circumsporozoite protein of *Plasmodium falciparum*, which is involved with the generation of protective antibody responses, also contains T-cell epitopes (10). In any event, because the 11,000-M_r *C. parvum* antigen displays immunodominancy and is a component of a preparation that can stimulate lymphocytes of infected hosts, it may play an important role in the generation of protective immune responses toward the parasite.

In summary, calves experimentally infected with *C. parvum* demonstrated a mean oocyst-shedding period occurring at 5 to 10 DAI. At 2 DAI, specific lymphocyte responses to *C. parvum* were detected, whereas parasite-specific serum antibodies were not detected until 7 DAI, or midway through the oocyst-shedding period. Both lymphocyte responsiveness and levels of serum antibodies against *C. parvum* remained elevated until the termination of the experiment at 23 DAI. Immunoblotting with infected calf serum identified an immunodominant 11,000-M_r parasite antigen in the *C. parvum* preparation. Future investigations will determine the role of this antigen in cell-mediated immune responses to *C. parvum*.

ACKNOWLEDGMENTS

We thank Bruce Pesch and Gary Fry for technical support.

REFERENCES

1. **Arrowood, M. J., and C. R. Sterling.** 1987. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic percoll gradients. J. Parasitol. **73**:314–319.
2. **Current, W. L.** 1985. Cryptosporidiosis. J. Am. Vet. Med. Assoc. **12**:1334–1338.
3. **Current, W. L., and P. H. Bick.** 1989. Immunobiology of *Cryptosporidium* spp. Pathol. Immunopathol. Res. **8**:141–160.
4. **Current, W. L., N. C. Reese, J. V. Ernst, W. S. Bailey, M. B. Heyman, and W. M. Weinstein.** 1983. Human cryptosporidiosis in immunocompetent and immunodeficient persons. N. Engl. J. Med. **308**:1252–1257.
5. **Fayer, R., and B. L. P. Ungar.** 1986. *Cryptosporidium* spp. and cryptosporidiosis. Microbiol. Rev. **50**:458–483.
6. **Harp, J. A., D. B. Woodmansee, and H. W. Moon.** 1989. Effects of colostral antibody on susceptibility of calves to *Cryptosporidium parvum* infection. Am. J. Vet. Res. **50**:2117–2119.
7. **Hawkes, R., E. Niday, and J. Gordon.** 1982. A dot-immunoblotting assay for monoclonal and other antibodies. Anal. Biochem. **119**:142–147.
8. **Heine, J., H. W. Moon, and D. B. Woodmansee.** 1984. Persistent *Cryptosporidium* infection in congenitally athymic (nude) mice. Infect. Immun. **43**:856–859.
9. **Hill, B. D., D. A. Blewett, A. M. Dawson, and S. Wright.** 1990. Analysis of the kinetics, isotype and specificity of serum and coproantibody in lambs infected with *Cryptosporidium parvum*. Res. Vet. Sci. **48**:76–81.
10. **Hoffman, S. L., L. T. Cannon, Sr., J. A. Berzofsky, W. R. Majarian, J. F. Young, W. Lee Maloy, and W. T. Hockmeyer.** 1987. *Plasmodium falciparum*: sporozoite boosting of immunity due to a T-cell epitope on a sporozoite vaccine. Exp. Parasitol. **64**:64–70.
11. **Hughes, H. P. A., W. M. Whitmire, and C. A. Speer.** 1989. Immunity patterns during acute infection by *Eimeria bovis*. J. Parasitol. **75**:86–91.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Liew, F. Y.** 1989. T cells against parasitic diseases, p. 329–346. *In* M. Feldmann, J. Lamb, and M. J. Owen (ed.), T cells, 1st ed. John Wiley & Sons, Inc., New York.
14. **Luft, B. J., D. Payne, D. Woodmansee, and C. W. Kim.** 1987. Characterization of the *Cryptosporidium* antigens from sporulated oocysts of *Cryptosporidium parvum*. Infect. Immun. **55**: 2436–2441.
15. **Lumb, R., P. S. Smith, R. Davies, P. J. O'Donoghue, H. M. Atkinson, and J. A. Lanser.** 1989. Localization of a 23 000 MW antigen of *Cryptosporidium* by immunoelectron microscopy. Immunol. Cell. Biol. **67**:267–270.
16. **Mead, J. R., M. J. Arrowood, and C. R. Sterling.** 1988. Antigens of *Cryptosporidium* sporozoites recognized by immune sera of infected animals and humans. J. Parasitol. **74**:135–143.
17. **Moon, H. W., D. B. Woodmansee, J. A. Harp, S. Abel, and B. L. P. Ungar.** 1988. Lacteal immunity to enteric cryptosporidiosis in mice: immune dams do not protect their suckling pups. Infect. Immun. **56**:649–653.
18. **Snedecor, G. W., and W. G. Cochran.** 1980. Statistical methods, 7th ed. The Iowa State University Press, Ames.
19. **Soave, R., and D. Armstrong.** 1986. *Cryptosporidium* and cryptosporidiosis. Rev. Infect. Dis. **8**:1012–1023.
20. **Tilley, M., R. Fayer, A. Guidry, S. J. Upton, and B. L. Blagburn.** 1990. *Cryptosporidium parvum* (Apicomplexa: Cryptosporidiidae) oocyst and sporozoite antigens recognized by bovine colostral antibodies. Infect. Immun. **58**:2966–2971.
21. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose

sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.

22. **Tzipori, S., and I. Campbell.** 1981. Prevalence of *Cryptosporidium* antibodies in 10 animal species. J. Clin. Microbiol. **14**:455–456.
23. **Ungar, B. L. P., J. A. Burris, C. A. Quinn, and F. D. Finkelman.** 1990. New mouse models for chronic *Cryptosporidium* infection in immunodeficient hosts. Infect. Immun. **58**:961–969.
24. **Ungar, B. L. P., and T. E. Nash.** 1986. Quantification of specific antibody response to *Cryptosporidium* antigens by laser densitometry. Infect. Immun. **53**:124–128.
25. **Whitmire, W. M., and J. A. Harp.** 1990. In vitro murine lymphocyte blastogenic responses to *Cryptosporidium parvum*. J. Parasitol. **76**:450–452.
26. **Williams, R. O.** 1987. Measurement of class specific antibody against cryptosporidium in serum and faeces from experimentally infected calves. Res. Vet. Sci. **43**:264–265.