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Cloning and Expression of a cDNA Encoding Epitopes Shared by 15- and 60-Kilodalton Proteins of *Cryptosporidium parvum* Sporozoites

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A cDNA (CP15/60) encoding epitopes of *Cryptosporidium parvum* 15- and 60-kDa sporozoite proteins was isolated and expressed in *Escherichia coli* toward the goal of developing an immunogen for producing high-titer anticryptosporidial colostrum. Antisera prepared in rats to native *C. parvum* 15-kDa protein and used to identify the CP15/60 bacteriophage clone recognized both 15- and 60-kDa in vitro translation products derived from sporozoite RNA. Antisera specific for recombinant CP15/60 antigen recognized native 15- and 60-kDa *C. parvum* sporozoite proteins by immunoblotting and identified both surface and internal antigens on *C. parvum* sporozoites by immunofluorescence staining. Northern (RNA) and Southern blot hybridization experiments using sporozoite RNA and DNA indicated that CP15/60 DNA is transcribed as a single 1.4-kb RNA species from a single-copy gene. Recombinant CP15/60 antigen was recognized by hyperimmune colostrum from cows immunized with *C. parvum* oocyst-sporozoite protein and by convalescent-phase sera from *C. parvum*-infected calves.

The protozoan *Cryptosporidium parvum* infects the intestinal epithelium and, to a lesser extent, extraintestinal epithelia of a wide range of mammalian species causing severe diarrhea that can be life threatening. The parasite is spread by a fecal-oral route. The young and immunosuppressed are at high risk. Numerous reports of cryptosporidiosis in children attending day care have been reported (7). Although in most individuals the disease is self-limiting and protective immunity develops after a primary infection, cryptosporidiosis is a major cause of death in immunodeficient hosts, such as persons afflicted with AIDS. As in humans, the young of animals are most susceptible to infection. Cryptosporidiosis is a major disease of dairy and beef calves in the United States. At present, no prophylactic therapy is available to prevent this parasitic disease in humans or animals (3, 7). However, several researchers have shown that in mice and humans administration of hyperimmune bovine colostrum (HBC), prepared by immunizing cows with extracts of *C. parvum* oocysts, can confer passive immunity against cryptosporidiosis (4–6, 15, 25, 26, 28). Also, monoclonal antibodies and immune serum specific for *C. parvum* sporozoites can neutralize the parasite and either prevent or lessen the severity of infection in animals (1, 2, 17, 18, 23). Although protection against *C. parvum* may be achieved by this type of immunotherapy, the development of resistance to cryptosporidiosis is dependent on T lymphocytes and secreted lymphokines, in particular, gamma interferon (8, 13, 14, 27). The humoral response leading to production of protective antibodies specific for *C. parvum* may be dependent on T-cell signaling, but in persons with severe immunodeficiency T-cell-mediated immunity is dysfunctional. Passive administration of hyperimmune serum or colostrum that is inhibitory for cryptosporidial parasites may be the only viable alternative for preventing or treating infection in such individuals. The purpose of the present study was to clone and express the gene encoding an epitope on the immunodominant *C. parvum* 15-kDa sporozoite protein (23). The recombinant CP15 antigen could then be used to immunize cows for production of HBC that may confer passive immunity to humans and calves against cryptosporidiosis.

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MATERIALS AND METHODS

Preparation of parasite nucleic acid and protein. *C. parvum* oocysts (2×10^8 per preparation) were recovered from feces of cows during days 4 to 14 of a severe cryptosporidial infection. Oocysts were concentrated by sucrose flotation followed by purification over a cesium chloride gradient by described procedures (23). After centrifugation, oocysts were resuspended in 5.0 ml of 10 mM Tris-HCl (pH 8.0)–1 mM EDTA (TE) and immersed dropwise into a mortar containing liquid nitrogen. The frozen parasites were ground in liquid nitrogen to a fine powder which was transferred to a tube containing the appropriate extraction buffer. Total *C. parvum* RNA was isolated by immersing the parasite extract in a mixture of guanidinium thiocyanate, sodium citrate, phenol, and sodium acetate followed by centrifugation and ethanol precipitation by standard procedures (31). DNA of *C. parvum* was prepared by treating the parasite extract with 1% sodium dodecyl sulfate (SDS) and 50 μg of proteinase K (Gibco/BRL, Gaithersburg, Md.) per ml in TE at 50°C for 2 h, followed by phenol-chloroform extraction (16). Oocyst protein was prepared by resuspending the parasite extract in 10 mM Tris-HCl (pH 7.3)–1 mM $MgCl_2$ in the presence of phenylmethylsulfonyl fluoride, sonicating for 20 s on a microson at setting 10 (Heat Systems Inc., Farmingdale, N.Y.), and treating with 5 μg each of DNase and RNase per ml for 15 min on ice (9).

In vitro translation of *C. parvum* sporozoite RNA. *C. parvum* sporozoite RNA was translated into protein to determine whether the transcript encoding CP15 was present and could be incorporated into cDNA for cloning into the bacteriophage expression vector. Heat-denatured and quick-

chilled total RNA (20 μg per reaction) and poly(A)$^+$ RNA (2 μg per reaction) from sporozoites were combined with rabbit reticulocyte lysate, 10 μCi of ^{35}S-methionine (ICN Radiochemicals, Costa Mesa, Calif.), and standard-reaction in vitro translation (IVT) reagents following directions provided by the manufacturer (Gibco/BRL). Negative control reactions containing either no RNA or RNA from the coccidian *Eimeria acervulina*, which does not express CP15, were included in each assay. The reaction mixtures were incubated at 30°C for 30 min, the reactions were stopped by placing on ice, and the mixtures were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting (immunoblotting) (see below).

Preparation and screening of *C. parvum* sporozoite cDNA bacteriophage expression libraries. Poly(A)$^+$ RNA was isolated by passing 210 μg of total *C. parvum* RNA through an oligo(dT) spun column and used to prepare cDNA following procedures supplied by the manufacturer (Stratagene, La Jolla, Calif.). Sporozoite cDNA was fractionated by gel filtration through a Sepharose CL4B column (Pharmacia/LKB, Piscataway, N.J.) into two size categories—greater than 350 bp (CP1) and between 200 and 350 bp (CP2)—and then introduced into the UNIZAP XR bacteriophage expression vector by techniques provided by the manufacturer (Stratagene). Unamplified CP1 was composed of 2.4×10^6 phage particles, and CP2 was composed of 2.1×10^6 phage particles; both contained greater than 95% recombinant clones. The libraries were amplified on *Escherichia coli* XL1 Blue and immunoscreened for expression of recombinant CP15 antigen by using polyclonal rat sera prepared against native *C. parvum* 15-kDa protein (below).

Generation of polyclonal rat antisera. Oocysts of *C. parvum* were purified on CsCl gradients (21), washed four times in phosphate-buffered saline (PBS), and freeze-thawed three times to release antigen. Proteins from 1.4×10^9 oocysts were separated by SDS–12% PAGE. A small vertical strip of the gel was excised and stained with Coomassie blue for visualization purposes. The strip was realigned with the rest of the gel, and a horizontal strip corresponding to the area of CP15 was excised. CP15 was electroeluted from the gel by using a BioRad model 422 electroeluter, dialyzed against distilled water overnight, and divided into three aliquots. One aliquot was emulsified in Freund's complete adjuvant and injected subcutaneously (s.c.) into a male Sprague-Dawley outbred rat (Harlan Sprague-Dawley). The second aliquot was mixed with Freund's incomplete adjuvant and injected s.c. 2 weeks later. The third aliquot was injected s.c. without adjuvant 2 weeks later. The rat was bled by heart puncture 4 days after the last injection. In accordance with approved animal procedures, the rat was anesthetized during each immunization and the heart puncture.

In vivo excision and DNA sequencing of *C. parvum* cDNA. Immunoreactive UNIZAP XR bacteriophage expressing CP15 epitopes were made clonal by repeated rounds of screening with rat anti-CP15 sera at bacteriophage densities that allowed single-plaque isolation. Recombinant pBluescript containing *C. parvum* cDNA was prepared by an in vivo excision protocol supplied by the manufacturer (Stratagene). Plasmid DNA was prepared (30) and used to determine the DNA sequence of the insert cDNA with Sequenase and reagents for dideoxy sequencing following techniques provided by the manufacturer (U.S. Biochemical, Cleveland, Ohio). The predicted amino acid sequence of the encoded recombinant protein was based on the reading frame of UNIZAP XR and the DNA sequence of the cDNA. Putative N-glycosylation sites (Asn-X-Ser or Asn-X-Thr) were identified by analysis with the Intelligenetics program (Intelligenetics, Inc., Mountain View, Calif.). Transmembrane regions and signal sequences were identified by standard techniques (29).

Northern (RNA) and Southern blot hybridization experiments. Total *C. parvum* sporozoite RNA (20 μg per lane) was subjected to formaldehyde gel electrophoresis (19) and transferred to Nytran paper (Schleicher and Schuell, Keene, N.H.). Sporozoite DNA of *C. parvum* (2 μg per lane) was digested with various restriction enzymes, fractionated by agarose electrophoresis, and transferred to Nytran paper (20). An equimolar amount of insert cDNA relative to the estimated size of the *C. parvum* genome (about 6×10^6 bp [11]) was also electrophoresed and transferred to determine the copy number of the cloned gene fragment. Thus, 200 pg of insert DNA or 1.2 ng of restriction enzyme-digested plasmid DNA {[6×10^2 (size of insert in base pairs)/6×10^6 (size of *C. parvum* genome in base pairs)] × 2 (amount of genomic DNA electrophoresed in adjacent lanes in micrograms)} was analyzed. Insert cDNA was isolated by double digestion of recombinant pBluescript plasmid DNA with *Eco*RI and *Xho*I (Gibco/BRL) and subsequent agarose electrophoresis. The insert cDNA was excised from the gel, isolated by using the Prep-A-Gene kit following procedures supplied by the manufacturer (BioRad, Richmond, Calif.), and radiolabeled with ^{32}P-dCTP with random oligomers and Klenow DNA polymerase by the manufacturer's instructions (Gibco/BRL). The cDNA probes were denatured by heating in a boiling water bath for 5 min, quick-chilled in an ice bath, and applied to both Southern and Northern blots that had been prehybridized as described previously (10). After 24 h of hybridization with radiolabeled cDNA, blots were washed once with 2× SSC–0.1% SDS (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature (RT) followed by three washes in 0.2× SSC–0.1% SDS at 50°C. After being dried, blots were exposed to Kodak XAR film and stored at −70°C until development.

High-level expression of *C. parvum* cDNA in *E. coli* and analysis by Western blotting. Insert cDNA was isolated by digesting recombinant pBluescript plasmid DNA with both *Bam*HI and *Xho*I, subsequent agarose electrophoresis, excision from the gel, and treatment with the Prep-A-Gene reagents (BioRad). Purified cDNA was subcloned into *Bam*HI-*Xho*I-digested pTrcHis (Invitrogen, San Diego, Calif.) by using T4 DNA ligase (Gibco/BRL) and expressed in *E. coli* JM101 by isopropylthiogalactopyranoside (IPTG) induction by methods supplied by the manufacturer. After 4 h of IPTG induction, *E. coli* harboring recombinant or nonrecombinant pTrcHis plasmid was harvested by centrifugation and resuspended in sonication buffer containing protease inhibitors (see above). The *E. coli* was subjected to three freeze-thaw cycles and sonication on ice for 20 s, followed by DNase and RNase treatment. Expression of *C. parvum* cDNA was evaluated by SDS-PAGE followed by electrophoretic transfer to Immobilon paper (24) (Millipore, Bedford, Mass.). Western blots containing recombinant and nonrecombinant protein from uninduced and IPTG-induced *E. coli* were treated with PBS containing 1% nonfat dry milk (PBS-NFDM) for 1 h at RT to block nonspecific binding in later steps. The protein-impregnated paper was then probed for 4 to 24 h at RT with either anti-CP15 immune serum or monoclonal antibody 5C3 (23), followed by a 2-h incubation with biotin-labeled anti-mouse immunoglobulin G (IgG) (0.5 mg/ml; Vector Laboratories, Burlingame, Calif.) and then a 1-h incubation with avidin-peroxidase (0.25 mg/ml; Vector Laboratories). The Western blots were washed three times

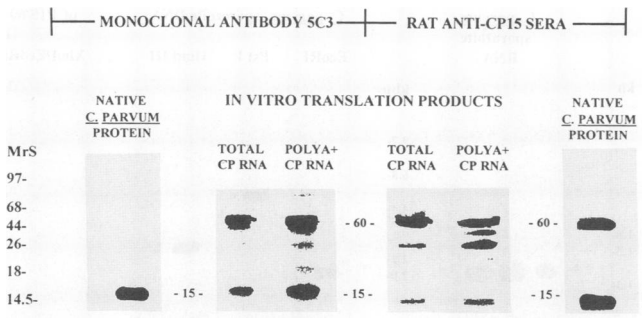

FIG. 1. Immunostaining of Western blots containing *C. parvum* sporozoite protein or in vitro translation products derived from *C. parvum* total RNA or poly(A)$^+$ RNA. The primary immunoreagent was either monoclonal antibody 5C3 or rat anti-native CP15 sera. MrS, 10^3 molecular weight standards.

with PBS containing 0.05% Tween 20 for 5 min per wash between each incubation step and developed by the addition of 0.5 mg of 4-chloro-1-naphthol per ml and 0.015% H_2O_2 substrate in PBS (Sigma Chemical Co., St. Louis, Mo.). HBC obtained from a cow that had received intramammary immunizations of *C. parvum* oocyst extract, as well as convalescent-phase serum from a *C. parvum*-infected calf, was used to probe Western blots containing recombinant CP15/60 protein. Colostrum from a nonimmunized cow and fetal calf serum were used as controls. Biotinylated anti-bovine IgG (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) followed by avidin-peroxidase was used to detect bovine antibody binding.

Purification of recombinant CP15 protein. Protein extracts from IPTG-induced *E. coli* containing either recombinant or nonrecombinant plasmid were separated by preparative SDS-PAGE and transblotted to nitrocellulose membrane. Western blots were treated with Ponceau S stain (Sigma) to visualize the recombinant protein, which was then excised as a horizontal strip from the paper. A horizontal strip from an adjoining region of the Western blot containing nonrecombinant protein was also excised. Both strips were cut into 0.5-cm pieces and placed in separate tubes containing 2.0% SDS and 1.0% Triton X-100 to elute the proteins from the paper (12). The eluted proteins were analyzed by SDS-PAGE

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GCA CGA GTA TTG ATA AAA GAA AAA CAA AAC ATG GGT AAC TTG AAA TCC TGT TGT
Ala Arg Val Leu Ile Lys Glu Lys Gln Asn MET Gly Asn Leu Lys Ser Cys Cys   18
|------------------------ SIGNAL SEQUENCE ------------------------|

TCT TTT GCC GAT GAA CAC TCC CTA ACC TCT ACT CAA CTA GTA GTT GGA AAT GGT
Ser Phe Ala Asp Glu His Ser Leu Thr Ser Thr Gln Leu Val Val Gly Asn Gly   36
---------- SIGNAL SEQUENCE ----------|                          |- NGLY-
                                                                 |••

TCA GGA GCT TCA GAA ACT GCT TCC AAC CAC CCC CAA GAA GAA GTT AAT GAT ATC
Ser Gly Ala Ser Glu Thr Ala Ser Asn His Pro Gln Glu Glu Val Asn Asp Ile   54
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•••••••••••••••••••••••• TRANSMEMBRANE REGION ••••••••••••••••••••••••
AAT ACT TTT AAT GTA AAG TTA ATA ATG CAA GAT AGA AGT AAG CTT GAC TGC GAG
Asn Thr Phe Asn Val Lys Leu Ile MET Gln Asp Arg Ser Lys Leu Asp Cys Glu   72
•••••••••|

GTA GTA TTT GAT AGC ACA AGT ATT TCG CTT TCT GGA GAT GGA AAT GTC AGA AAT
Val Val Phe Asp Ser Thr Ser Ile Ser Leu Ser Gly Asp Gly Asn Val Arg Asn   90

ATT GCT TTG GAT GAA ATC ACC AAT TAT TAT ATT CAA AGG AAG AGC TTT CTA GAG
Ile Ala Leu Asp Glu Ile Thr Asn Tyr Tyr Ile Gln Arg Lys Ser Phe Leu Glu  108

TTG AAA GTA GTG CTG GAA TCA GCG ATT CCG ACA ATT GTG TTG CAA TTC ATC TCA
Leu Lys Val Val Leu Glu Ser Ala Ile Pro Thr Ile Val Leu Gln Phe Ile Ser  126

AAG AAT CAG GAA ACT GTA TTC CCC TTT TCT TTA ATA ATT CGC AAG ACA AAG AAA
Lys Asn Gln Glu Thr Val Phe Pro Phe Ser Leu Ile Ile Arg Lys Thr Lys Lys  144

GAT TTG TTG CAA CAG CAA ACA AAT TCA AAC CAA ACT TTA ACT AAA ACA GGA ATT
Asp Leu Leu Gln Gln Gln Thr Asn Ser Asn Gln Thr Leu Thr Lys Thr Gly Ile  162
                                  |-NGLY -|

TCA TTA ATT CTG GAT TTG TTT TTT CCA AAC CCT ATA AAT AAA ATC CAA ATA GCT
Ser Leu Ile Leu Asp Leu Phe Phe Pro Asn Pro Ile Asn Lys Ile Gln Ile Ala  180

TTT CTT CAA TAA CAGGGAGAGAATTAATAAAATATTTTTGATTAAAAAAAAAAAAAAAAAA
Phe Leu Gln  .
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FIG. 2. DNA sequence and predicted amino acid sequence of recombinant CP15/60 cDNA. (Coding sequence is shown 5′ to 3′; numerals to right of sequence reflect amino acid residue numbers.)

followed by Coomassie blue staining or transblotting to nitrocellulose membrane and immunostaining with specific sera.

Immunofluorescence staining of parasites. Sporozoites were obtained by excystation of purified *C. parvum* oocysts by standard methods (18). Excysted sporozoites were washed several times in PBS and then either stored live on ice, air-dried onto glass slides, or treated for 5 min with 1% paraformaldehyde (PF). PF-treated parasites were washed several times with PBS and then air dried onto glass slides. Untreated live sporozoites and PF-treated and air-dried parasites were incubated in PBS containing 1% bovine serum albumin (BSA) for 1 h at RT. After preincubation in PBS-BSA, the parasites were exposed for 1 h at 4°C to a 1:100 dilution of mouse antiserum specific for purified recombinant CP15/60 protein in PBS–BSA–0.05% sodium azide. To prepare immune sera, three mice received two intraperitoneal injections of purified recombinant CP protein in incomplete Freund's adjuvant (see above). Sera from mice immunized with nonrecombinant *E. coli* protein were used as negative controls. After incubation with mouse antisera, the *C. parvum* sporozoites were washed three times in PBS and treated for 1 h with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Sigma), followed by three additional PBS washes. The slides were then examined under epifluorescence to visualize the antibody binding pattern.

Nucleotide sequence accession number. The GenBank accession number of the reported sequence is L08612.

RESULTS

Recognition of parasite in vitro translation products by immune serum. Rat anti-CP15 sera recognized 15- and 60-kDa proteins on Western blots of native *C. parvum* protein and IVT products of similar molecular sizes from *C. parvum* RNA (Fig. 1). Monoclonal antibody 5C3 recognized similar-sized IVT products and the 15-kDa native protein, but not the 60-kDa native protein (Fig. 1). The binding of rat antisera to the 60-kDa IVT product appeared much greater than that to the 15-kDa IVT product. There was no apparent difference in the level of binding by monoclonal antibody 5C3 to either IVT product (Fig. 1). There was negligible recognition of negative control reactions by all of the immunoreagents (data not shown).

Identification and DNA sequencing of recombinant CP15/60 cDNA. An immunoreactive UNIZAP XR bacteriophage clone, designated CP15/60, was identified by screening about 10^6 CP1 bacteriophage clones with rat anti-CP15 sera. Recombinant pBluescript plasmid DNA was generated by in vivo excision and subjected to dideoxy sequencing. The insert cDNA was about 600 bp and contained a long poly(A) tail (Fig. 2). The predicted amino acid sequence appeared to contain two N-linked glycosylation sites (residues 35 to 37 and 154 to 156), a signal sequence (residues 1 to 27), and a transmembrane region (residues 36 to 57).

Northern and Southern blot hybridization studies. Hybridization of CP15/60 cDNA to Northern blots containing *C. parvum* total RNA revealed a 1.4-kb transcript (Fig. 3). The 1.4-kb RNA species was present in about equal concentrations from three separate oocyst extractions. Southern blot hybridization of CP15/60 to genomic DNA of *C. parvum* sporozoites showed single hybridizing bands in DNA digested with *Eco*RI and *Pst*I and two bands in DNA digested with *Hin*dIII (Fig. 3). The latter result was not unexpected since the cDNA contains a single *Hin*dIII site. CP15/60 appears to be a single or low-copy-number gene, since

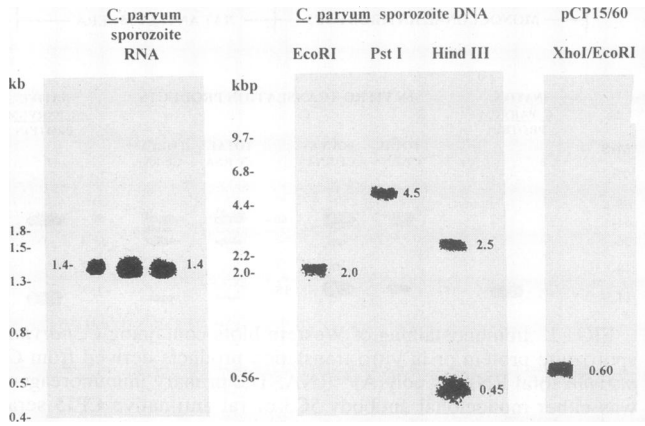

FIG. 3. Northern and Southern blot hybridization of CP15/60 cDNA to formaldehyde gel-separated *Cryptosporidium parvum* total RNA and 0.8% agarose gel-separated *C. parvum* genomic DNA. RNA samples derived from three separate nucleic acid extractions were compared. An equimolar amount of insert cDNA (relative to the amount of genomic DNA in adjacent lanes) was electrophoresed as *Xho*I-*Eco*RI digestion products of CP15/60 plasmid DNA. kb, RNA size standards (BRL); kbp, DNA size standards (*Hin*dIII-digested lambda DNA; BRL).

hybridization of the insert cDNA to equimolar amounts of CP15/60 and genomic DNA showed hybridizing bands of similar intensity.

Recognition of native and recombinant *C. parvum* proteins by immune serum, monoclonal antibody, and hyperimmune bovine colostrum. In immunoblotting experiments, rCP15/60 was recognized as a 40-kDa fusion protein by both monoclonal antibody 5C3 and rat anti-native CP15 sera (Fig. 4). This fusion protein was about 13 kDa larger than expected on the basis of size of the open reading frame of CP15/60 (22 kDa) and the peptide encoded by the parental plasmid (5 kDa). Antisera prepared in mice by immunization with purified rCP15/60 antigen bound epitopes of native *C. parvum* 15- and 60-kDa proteins (Fig. 4). Sera from mice immunized with nonrecombinant *E. coli* protein bound neither antigen. HBC from a cow immunized with *C. parvum* oocyst-sporozoite protein and hyperimmune bovine serum

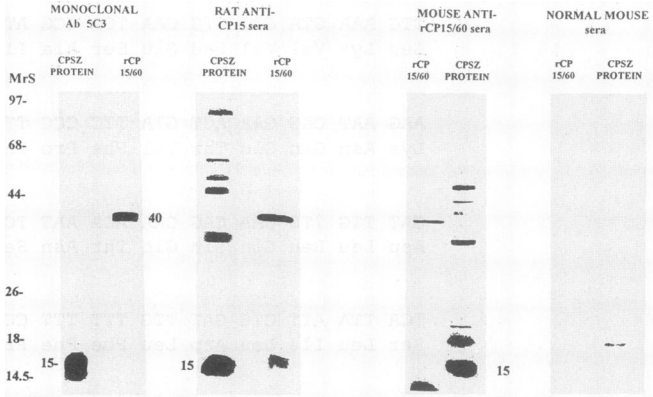

FIG. 4. Immunostaining of Western blots containing native *C. parvum* sporozoite protein and purified recombinant CP15/60 protein. The primary immunoreagent was either monoclonal antibody 5C3, rat anti-native CP15 sera, or mouse anti-recombinant CP15/60 sera. MrS, 10^3 molecular weight standards.

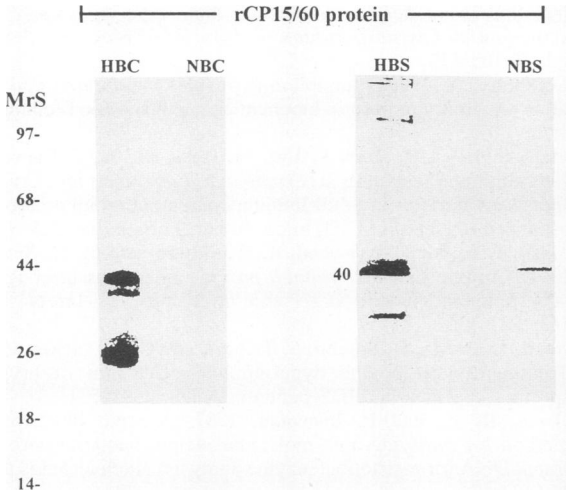

FIG. 5. Immunostaining of Western blots containing purified recombinant CP15/60 protein with either hyperimmune colostrum from a cow immunized with *C. parvum* oocyst extract or immune serum from a calf infected with *C. parvum*. MrS, 10^3 molecular weight standards.

obtained from a *C. parvum*-infected calf recognized purified rCP15/60 protein, while recognition of rCP15/60 by normal bovine colostrum was negligible (Fig. 5). There was lower but measurable binding of fetal calf serum to the recombinant protein. Both HBC and hyperimmune bovine serum appeared to recognize a lower-molecular-size protein in this preparation which we believe is a degradation product. It is interesting that the lower-molecular-size protein is similar in size (26 kDa) to that predicted by the open reading frame of CP15/60 (Fig. 5).

Immunofluorescence staining of *C. parvum* sporozoites. Antisera prepared against rCP15/60 bound an antigen on the surface of live *C. parvum* sporozoites (Fig. 6A). This antigen appeared to be distributed unevenly on the sporozoite surface. Immunofluorescent-antibody staining of PF-treated or dried sporozoites showed that the antigen was also a component of internal structures at the apical end of the parasite (Fig. 6B and C).

DISCUSSION

The present study describes cloning and expression of a cDNA that encodes epitopes shared by *C. parvum* sporozoite 15- and 60-kDa proteins. The evidence for this is that mouse antiserum specific for rCP15/60 recognizes native 15- and 60-kDa proteins. More critical to the goal of producing a "protective" immunogen is that rCP15/60 was recognized by monoclonal antibody 5C3, which has been used to confer passive immunity against cryptosporidiosis. This epitope was not apparent on native CP60 since the monoclonal antibody did not react with the 60-kDa protein by immunoblotting. The absence of recognition may be related to the amount of native CP60 present since overdevelopment of the immunoblotting reaction has shown a faint 60-kDa band (unpublished observations). The protein may be present in higher concentrations in *C. parvum* merozoites since a 60-kDa antigen is detectable on immunoblots of protein from this developmental stage of the parasite (22). It may also be that native CP60 undergoes in situ posttranslational modification that affects the monoclonal antibody-specific epitope. Such processing would not occur under in vitro translation conditions.

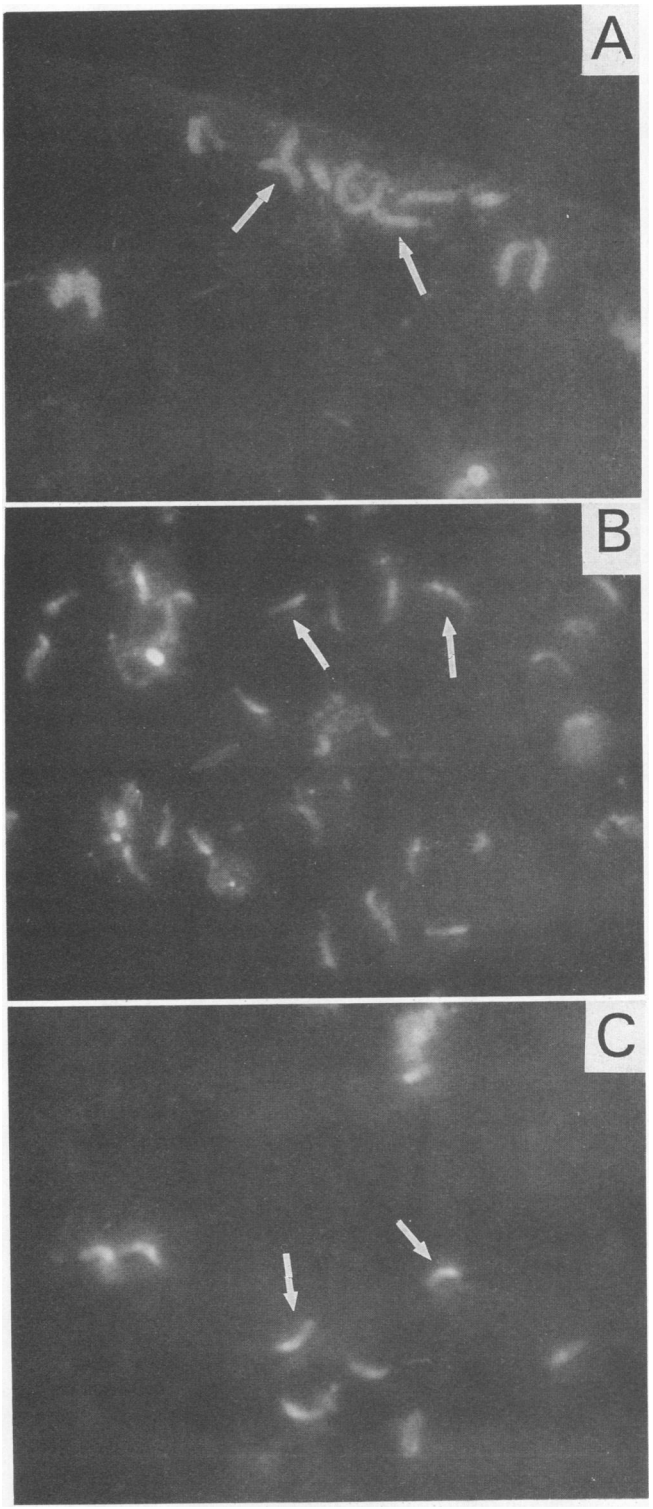

FIG. 6. Indirect immunofluorescence staining of live (A), PF-fixed (B), or dried (C) *C. parvum* sporozoites with mouse anti-recombinant CP15/60 sera followed by fluorescein isothiocyanate-labeled rabbit anti-mouse IgG (Sigma).

The relationship between native CP15 and CP60 proteins is unclear. Both proteins electrophoresed under either reducing or nonreducing conditions show similar levels of immunoreactivity after Western blotting and exposure to immune serum (data not shown). Therefore, CP60 does not appear to be composed of subunits of CP15 joined by disulfide cysteine residues. However, we have observed that native CP15 eluted from Immobilon paper in acetonitrile will, upon drying, form a 60-kDa protein that does not react after SDS-PAGE and immunoblotting with CP15-specific monoclonal antibody 5C3. Whether this manipulation with organic solvent and drying reproduces a natural process in the parasite remains to be determined.

As indicated by Northern blot hybridization, CP15/60 DNA binds to a 1.4-kb RNA transcript of *C. parvum* sporozoites, a size similar to that required for a hypothetical 60-kDa protein. Although CP15/60 DNA is about 0.6 kbp in length, representing 40% of the total transcript, it contains sequences sufficient to encode the monoclonal antibody 5C3-reactive CP15 epitope. As discussed above, *E. coli* harboring recombinant plasmid is capable of generating this epitope. Furthermore, both HBC prepared against *C. parvum* sporozoite protein and convalescent-phase bovine serum from calves infected with *C. parvum* recognize rCP15/60. Thus, rCP15/60 should be useful in generating high-titer anti-CP15 colostrum that may be utilized in immunotherapy of cryptosporidiosis.

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