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A Monoclonal Antibody Defines a Geographically Conserved Surface Protein Epitope of *Babesia equi* Merozoites

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Babesiosis is a tick-borne hemoparasitic disease affecting horses worldwide. To investigate mechanisms of immunity to this parasite, the antibody response of infected horses to *Babesia equi* merozoite proteins was evaluated. Immunoprecipitation of *B. equi* merozoite antigens with sera from infected horses revealed 11 major proteins of 210, 144, 108, 88, 70, 56, 44, 36, 34, 28, and 25 kDa. Monoclonal antibody (MAb) 36/133.97, which binds to live merozoites, immunoprecipitated proteins of 44, 36, 34, and 28 kDa. When immunoprecipitations were performed with in vitro translation products of merozoite mRNA, MAb 36/133.97 immunoprecipitated proteins of 38, 28, 26, and 23 kDa which comigrated with proteins immunoprecipitated by sera from infected horses at 10^{-3} to 10^{-4} dilutions. In Western blot analysis, MAb 36/133.97 recognized proteins of 44, 36, 34, and 28 kDa, and a 28-kDa protein was identified by sera from infected horses at a dilution of 10^{-4}. MAb 36/133.97 bound to *B. equi* isolates from Florida and Europe. Furthermore, the binding of MAb 36/133.97 to merozoite proteins was inhibited by sera of infected horses from 19 countries. Collectively, these data indicate MAb 36/133.97 binds to a geographically conserved peptide epitope on multiple *B. equi* merozoite proteins, including a merozoite surface protein, and MAb 36/133.97 reacts with a *B. equi* protein immunodominant in infected horses.

Equine babesiosis, caused by *Babesia equi* or *Babesia caballi*, is a tick-borne hemoprotozoan disease of horses (10, 16, 26). Clinical disease is characterized by fever, anemia, and icterus (10, 26), most likely arising from hemolysis caused by merozoites, the intraerythrocytic stage of equine *Babesia* infection. Mortality rate is high during initial infection of horses introduced into enzootic regions (26), and horses which survive initial infection are protected from clinical disease upon subsequent challenge (26). It is hypothesized that this immunity acquired by horses in enzootic areas is the result of persistent infection (26).

Merozoite surface proteins are important in the pathogenesis of hemoprotozoan diseases because of their role in parasite recognition of, attachment to, and penetration of host erythrocytes (12). Antigens recognized by antibody from hosts demonstrating immunity to clinical disease during *Plasmodium* spp., *B. rhodhaini*, *B. bovis*, and *B. bigemina* infection include surface proteins of merozoites (3, 8, 11, 19, 30), the only blood stage of the parasite that is extracellular and directly accessible to serum antibody (11). It was demonstrated that cattle immune to infection with *B. bovis* had high-titered antibody preferentially directed against four immunodominant merozoite surface proteins (8). Invasion of erythrocytes by merozoites of *Plasmodium knowlesi* was inhibited by immune sera (3), and inhibition of *P. falciparum* merozoite invasion of erythrocytes in vitro required high concentrations of specific antibodies (25). These observations suggest that antibody to merozoite surface proteins may block erythrocyte invasion in vivo and that these proteins should be tested as potential immunogens.

Detection of antibodies has been the method of choice for diagnosis of infection with equine *Babesia* spp. (4–6, 17, 27–29); however, the specificity or role of antibodies in the acquired protective immunity against clinical disease following equine *Babesia* infection has not been determined.

To investigate mechanisms of the acquired protective immunity, the antibody response to *B. equi* merozoite proteins expressed during parasitemia was evaluated. The results presented in this report identify *B. equi* merozoite proteins recognized by sera from infected horses. Also described is monoclonal antibody (MAb) 36/133.97, which binds to a geographically conserved peptide epitope on multiple *B. equi* merozoite proteins, including a merozoite surface protein, and reacts with a *B. equi* protein immunodominant in infected horses.

MATERIALS AND METHODS

***B. equi* isolates.** A *B. equi* isolate was obtained in 1976 from a horse in Florida and cryopreserved as a blood stabilate containing 10% dimethyl sulfoxide in liquid nitrogen. A nonsplenectomized horse (H5) was infected with 30 ml of the Florida *B. equi* first-passage stabilate containing 5.6×10^6 viable organisms per ml. Viability was determined by incubating merozoites with fluorescein diacetate (FDA) as previously described (24). This horse was monitored for clinical disease and parasitemia. During ascending parasitemia, 200 ml of whole blood was passaged to a splenectomized horse. At peak parasitemia (49%), infected erythrocytes were collected and stored in liquid nitrogen as a blood stabilate containing packed erythrocytes 1:1 with a cryopreservant of 20% (wt/vol) polyvinylpyrrolidone and 2% (wt/vol) glucose in Puck's saline G (GIBCO Laboratories, Chagrin Falls, Ohio) (21). Aliquots (25 ml) of washed packed infected erythrocytes were frozen at −70°C.

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The Europe isolate of *B. equi* was obtained from a mare from Georgia, USSR (15). A splenectomized pony was infected with the Europe isolate, and blood smears for indirect immunofluorescence assay (IFA) were prepared.

In vitro translation of *B. equi* mRNA. *B. equi* merozoite mRNA was isolated from infected erythrocytes by modification of previously described methods (18). A 25-ml aliquot of washed packed infected erythrocytes was thawed in the presence of equal volumes of guanidinium isothiocyanate (4.0 M guanidinium isothiocyanate [Bethesda Research Laboratories, Gaithersburg, Md.], 0.1 M Tris-HCl [pH 7.5], 1% 2-mercaptoethanol, 2% Sarkosyl, 0.01 M EDTA [pH 7.6]). Lysates were sequentially extracted with buffered phenol, phenol-chloroform-isoamyl alcohol, and ether before nucleic acids were ethanol precipitated. Polyadenylated mRNA was isolated by poly(U)-Sephadex (Bethesda Research Laboratories) chromatography. In parallel, mRNA was isolated from 25 ml of washed packed uninfected erythrocytes. Stained smears of washed infected erythrocytes revealed less than 1 leukocyte per 10^4 erythrocytes. Integrity of mRNA was evaluated by the migration of rRNA species in 1% agarose gel. Merozoite mRNA was translated in vitro (Promega, Madison, Wis.), using 2 μg of polyadenylated mRNA per reaction and a nuclease-treated rabbit reticulocyte lysate (13, 22). The rabbit reticulocyte lysate was chosen because it lacks microsomal membranes necessary for processing events such as signal peptide cleavage and core glycosylation (33).

Radiolabeling of *B. equi* proteins. Defibrinated blood from a splenectomized horse infected with the Florida isolate of *B. equi* was collected when ascending parasitemia reached 5%. Erythrocytes were washed twice in Puck's saline G to remove the majority of buffy coat cells. A final wash was made in serum- and amino acid-free medium 199 (Hazleton Laboratories, Lenexa, Kans.). Short-term cultures were established in 25-cm^2 flasks at a 10% erythrocyte suspension in amino acid-free medium 199 containing 40% autologous, preinoculation horse serum, 1% penicillin G, streptomycin, amphotericin B, 25 μCi (500 μCi total) each of tritiated isoleucine, lysine, tyrosine, valine, and arginine per ml (respective specific activities, 110.8, 97.4, 46.7, 64.6, and 53.3 Ci/mmol; Dupont-New England Nuclear, Boston, Mass.) and buffered with 10 mM 3-[*N*-tris-(hydroxymethyl) methylamino]-2-hydroxy propanesulfonic acid, pH 7.35. Metabolic labeling proceeded during an 18-h incubation period at 37°C in 5% CO_2 and ambient air. The labeled cells were then washed and solubilized as previously described (19). In vitro translation products were labeled with [^{35}S]methionine at 0.8 mCi/ml per reaction.

Production of MAb. Eight-week-old BALB/c mice were immunized subcutaneously with 10^7 viable merozoites in 0.1 ml of phosphate-buffered saline (PBS) emulsified in an equal volume of Freund's complete adjuvant. Merozoites for MAb production were prepared from stabilates containing a 49% parasitemia. The stabilates were diluted with 2 volumes of PBS and centrifuged at 2,500 × *g* for 5 min. Pellets were lysed for 30 s with an equal volume of distilled water, diluted with 3 ml of PBS, vortexed gently, and centrifuged at 400 × *g* for 5 min. The supernatant was centrifuged at 2,500 × *g* to pellet the merozoites. Two additional immunizations consisting of the same number of parasites in incomplete Freund's adjuvant were given subcutaneously at 10-day intervals. The mice were then immunized intravenously with 10^7 viable merozoites in 0.1 ml of PBS 72 h prior to fusion. Cell fusions and cloning by limiting dilution were performed as described previously (23). Heavy-chain isotypes were identified by enzyme-linked immunosorbent assay (ELISA), and concentrations of antibodies were determined by immunodiffusion (14). Supernatants from the initial fusion and from limiting-dilution clones were screened by IFA with acetone-fixed *B. equi* organisms.

TABLE 1. CI ELISA for assessment of antibodies to *B. equi* merozoite proteins recognized by MAb 36/133.97

Country of origin	OD at serum dilution of[a]:				CI titer[b]
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
Argentina	0.252	0.483	1.130	1.027	10^{-2}
Austria	0.563	0.703	0.826	0.948	10^{-2}
Brazil	0.126	0.236	0.641	0.824	10^{-3}
Chile	0.650	0.866	1.241	1.315	10^{-1}
Colombia	0.180	0.713	1.259	1.191	10^{-2}
Ecuador	0.247	0.543	1.055	1.263	10^{-2}
England	0.292	0.816	1.233	1.237	10^{-1}
France	0.238	0.608	1.110	1.229	10^{-2}
Italy	0.378	0.804	1.181	1.292	10^{-1}
Netherlands	0.148	0.266	0.740	1.093	10^{-2}
North Yemen	0.663	0.851	1.166	1.193	10^{-1}
Panama	0.240	0.484	1.066	1.139	10^{-2}
Peru	0.185	0.540	1.012	1.077	10^{-2}
Poland	0.601	1.000	1.247	1.185	10^{-1}
Saudi Arabia	0.420	0.771	1.218	1.266	10^{-1}
Spain	0.295	0.607	0.687	0.733	10^{-3}
Trinidad	0.269	0.594	1.143	1.227	10^{-2}
United States	0.202	0.377	1.012	1.264	10^{-2}
Venezuela	0.325	0.771	1.244	1.324	10^{-1}

[a] OD of MAb 36/133.97 reaction with *B. equi* merozoites with equine serum at the specified dilution. OD for isotype control MAb with *B. equi* merozoites = 0.153 ± 0.05 (n = 8).

[b] Dilution of serum reducing OD values to less than 3 standard deviations below the mean for control horses (<0.73) in CI ELISA with MAb 36/133.97. OD for control horses at a 1/2 dilution = 0.97 ± 0.08 (n = 68). Controls included preinoculation sera of H5 and SN76N8401 (control serum from the National Veterinary Services Laboratory, Ames, Iowa).

Immune sera from horses experimentally and naturally infected with *B. equi*. Serum was obtained from an adult horse (H5) infected intravenously twice at a 2-month interval with a Florida isolate of *B. equi*. After 50 ml of serum was obtained, the initial inoculation of H5 was with 30 ml of a first-passage stabilate of a Florida isolate of *B. equi*. This stabilate in 10% dimethyl sulfoxide contained 5.6 × 10^6 viable merozoites per ml. The second inoculation was with a 2.0-ml stabilate containing a 49% parasitemia prepared as described for *B. equi* isolates. Equine sera that tested positive for antibodies to *B. equi* by the complement fixation test (9) were obtained from the National Veterinary Services Laboratory, U.S. Department of Agriculture, Ames, Iowa. These sera were obtained from horses in 19 countries (Table 1).

Immunoprecipitation and SDS-PAGE. Immunoprecipitation of radiolabeled antigen was performed as previously described (19). A total of 1 × 10^6 to 2 × 10^6 trichloroacetic acid-precipitable counts of antigen and 10 μg of MAb or 10 μl of equine immune serum were used in each precipitation. Immune complexes were precipitated with protein A (Pansorbin; Calbiochem, San Diego, Calif.) or protein G (ImmuBind; Genex, Gaithersburg, Md.). Metabolically radiolabeled antigen, in vitro-translated proteins, or immunoprecipitates were boiled for 3 min in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer (final concentrations of 25 mM Tris [pH 6.8], 2% [wt/vol] SDS, 15% [vol/vol] glycerol, 2.5% 2-mercaptoethanol, and a few crystals of bromophenol blue) and were electrophoresed in a 7.5 to 17.5% SDS-polyacrylamide gradient slab gel with

a 5% stacking gel (31). SDS-polyacrylamide gels were processed for autoradiography as described previously (2). ^{14}C-labeled standards used for molecular weight comparisons (Amersham, Arlington Heights, Ill.) consisted of myosin (200,000), phosphorylase *b* (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lysozyme (14,300).

Western immunoblotting. Western blotting was performed on a miniblotter 25 (Immunetics, Cambridge, Mass.) by modification of published techniques (32). Merozoite antigen was prepared from stabilates containing a 49% parasitemia as described for MAb production. Control erythrocyte antigen was prepared identically to merozoite antigen and was obtained from stabilates prepared from an uninfected horse. Pelleted merozoites were added to equal volumes of SDS-PAGE sample buffer and boiled for 10 min. Merozoite proteins separated in SDS-PAGE (as described above) were electrophoretically transferred overnight to nitrocellulose filters in 25 mM Tris–190 mM glycine buffer containing 20% (vol/vol) methanol. Filters were blocked for 2 h in 0.17 M NaCl–0.01 M Tris–0.1 mM phenylmethylsulfonyl fluoride–1.0% (wt/vol) bovine hemoglobin (buffer A). Serum (50 μl) or MAb (10 μg) was diluted in buffer A with the addition of 0.1% (wt/vol) SDS–0.1% (vol/vol) Triton X-100–1.0 mM EDTA (buffer B). Bound antibodies were detected by incubation for 1 h each in second antibody (rabbit anti-horse or rabbit anti-murine immunoglobulin) and ^{125}I-protein A in buffer B. Filters were washed three times in buffer B after incubation with equine serum or MAb, second antibody, and ^{125}I-protein A, followed by three washes in buffer B without hemoglobin before drying and autoradiography. ^{14}C-labeled molecular weight standards were the same as for SDS-PAGE (see above).

IFA. (i) Fixed *B. equi*. IFA of acetone-fixed *B. equi* was performed as described previously (20). Bound murine or equine antibodies were detected with fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin or goat anti-horse immunoglobulin.

(ii) Live *B. equi*. Merozoites for live IFA were prepared from stabilates containing a 49% parasitemia as described for MAb production. Live IFA was performed by minor modification of previously described methodology (7). Merozoite pellets resuspended in 100 μl of PBS were incubated with 25 μg of MAb 36/133.97. After a 30-min incubation at room temperature, the cells were washed three times with 10% normal goat serum in PBS, diluted to 975 μl with normal goat serum-PBS, and added to 12.5 μg of goat anti-mouse antibody conjugated with tetramethylrhodamine isothiocyanate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). Samples were incubated for 30 min, washed three times with PBS, and mixed with 2.0 μl of a 5-mg/ml solution of FDA. Samples were incubated for 15 min, washed once with PBS, resuspended in 100 μl of PBS, and examined in a wet mount by phase and fluorescence microscopy. A total of 757 FDA-positive merozoites were examined for reactivity to MAb 36/133.97.

CI ELISA. A competitive inhibition (CI) ELISA was established to test for a direct relationship between proteins recognized by immune equine sera and MAb 36/133.97. Merozoites were prepared as described for MAb production. Merozoite preparations were diluted to 40 μg/μl in PBS containing 20 mM $MgCl_2$ and treated with an equal volume of lysis buffer (50 mM Tris [pH 8.0], 5 mM EDTA, 5 mM iodoacetamide, 0.1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, and 1.0 mM phenylmethylsulfonyl fluoride in 1.0% Nonidet P-40). Lysates were placed on ice for 15 min and then centrifuged at 1,500 × *g* for 15 min, and the supernatant was collected. Four microliters of supernatant adjusted to 0.20 μg of protein per μl was added to individual wells of Immulon 2 flat-bottom plates (Dynatech Laboratories, Chantilly, Va.) and incubated overnight at room temperature. Each well was blocked for 2 h with 350 μl of 20% milk in PBS containing 0.2% Tween 20 (buffer A). Equine sera were diluted in buffer A to a final volume of 290 μl and added to the wells. Samples were incubated for 30 min, 0.125 μg of MAb 36/133.97 in 10 μl of buffer A was added, and the reaction mixture was incubated for 1 h at room temperature. Wells were washed three times with PBS containing 0.2% Tween 20 (buffer B). Biotinylated equine anti-murine immunoglobulin G (IgG; Vector Laboratories, Burlingame, Calif.) in buffer A was added, incubation was continued for 30 min, and the wells were washed three times with buffer B. Addition of avidin-conjugated alkaline phosphatase (Vector Laboratories) in buffer B was followed by a 30-min incubation. Wells were washed three times with buffer B, and 100 μl of a 1.0-g/μl solution of *p*-nitrophenyl phosphate in 100 mM $NaHCO_3$ (pH 9.5) with 10 mM $MgCl_2$ (Sigma Laboratories, St. Louis, Mo.) was added to each well. Following a 30-min incubation, reactions were stopped with 50 μl of 0.2 M EDTA and the optical density (OD) was read at 405 nm on a Dynatech MR-5000 ELISA plate reader.

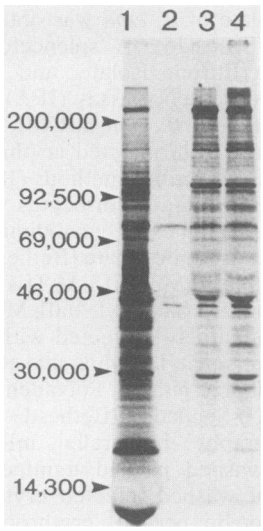

FIG. 1. Immunoprecipitation of ^{3}H-amino acid-labeled merozoite-associated proteins of *B. equi* with serum from experimentally infected horse H5. Shown are labeled protein profile (lane 1), preinoculation serum (lane 2), postinoculation serum (2 months after primary infection) (lane 3), and post-second inoculation serum (1 month after second infection; 3 months after primary infection) (lane 4).

RESULTS

Immunoprecipitation of *B. equi* merozoite proteins with equine serum. Figure 1 shows immunoprecipitation of *B. equi* merozoite proteins with pre- and postinoculation serum from horse H5 infected with a Florida isolate of *B. equi*. The major *B. equi* merozoite proteins recognized by antibodies from this horse have apparent molecular masses of 210, 144, 108, 88, 70, 56, 44, 36, 34, 28, and 25 kDa. Immunoprecipitations with sera from 10 additional experimentally infected

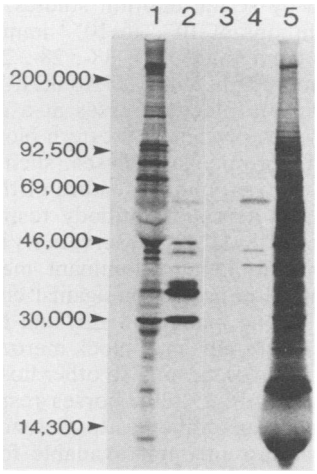

FIG. 2. Immunoprecipitation of ^{3}H-amino acid-labeled merozoite-associated proteins of *B. equi* with MAb 36/133.97. Shown are postinfection serum, horse H5 (lane 1), MAb 36/133.97 (lane 2), MAb isotype control (lane 3), protein A control (lane 4), and labeled protein profile (lane 5).

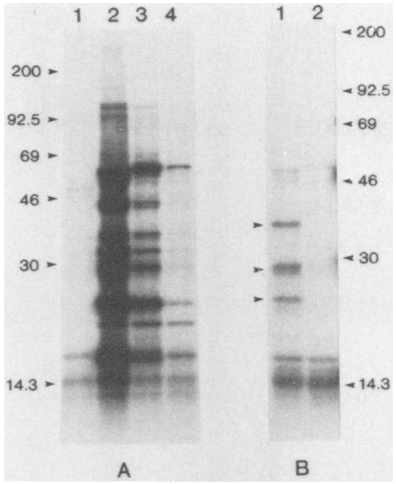

FIG. 3. Comparisons of immunoprecipitations of [^{35}S]methionine-labeled in vitro translation products with dilutions of sera from experimentally infected horse H5 (A) and MAb 36/133.97 (B). (A) 10^{-2} dilution of H5 preinoculation serum (lane 1), 10^{-2} dilution of H5 postinoculation serum (lane 2), 10^{-3} dilution of H5 postinoculation serum (lane 3), 10^{-4} dilution of H5 postinoculation serum (lane 4). (B) MAb 36/133.97 (lane 1) and MAb isotype control (lane 2). Arrowheads indicate locations of 38-, 28- to 26-, and 23-kDa proteins.

and 2 naturally infected horses provided similar results (data not shown).

Immunoprecipitation of *B. equi* antigens with MAb 36/133.97. An autoradiograph comparing immunoprecipitation of merozoite proteins with MAb 36/133.97 and equine immune serum is shown in Fig. 2. MAb 36/133.97, isotyped as IgG1, immunoprecipitated proteins with approximate molecular masses of 44, 36, 34, and 28 kDa which comigrated with proteins immunoprecipitated by serum from infected horse H5.

IFA of fixed and live merozoites with MAb 36/133.97. The epitope recognized by MAb 36/133.97 is conserved on at least two isolates of *B. equi*, as determined by reactivity in IFA. MAb 36/133.97 reacted with both the Florida and Europe (15) isolates of *B. equi* at a final concentration of 0.66 μg/ml. Up to 100% of merozoites from the Florida and Europe isolates of *B. equi* reacted with MAb 36/133.97 in fixed IFA. MAb 36/133.97 did not react with uninfected erythrocytes or *B. caballi* in IFA. At the same concentrations, IgG1 isotype control MAb and rabbit anti-mouse second antibody did not react with *B. equi*-infected erythrocytes.

The surface reactivity of MAb 36/133.97 was demonstrated by its binding to viable (FDA-positive) merozoites. Approximately 80% of isolated merozoites stained with FDA and 64% (482 of 757) of FDA-positive merozoites reacted diffusely with MAb 36/133.97.

Protein character of the epitope and immunodominance of the protein recognized by MAb 36/133.97. Equal volumes of washed packed erythrocytes from infected and uninfected horses yielded 5.7 and 0.22 μg of polyadenylated RNA. The small amounts of polyadenylated RNA isolated from uninfected erythrocytes provided insufficient incorporation of [^{35}S]methionine from in vitro translation for use in immunoprecipitations. Immunoprecipitation of in vitro-translated *B. equi* mRNA with serum from infected horse H5 and with MAb 36/133.97 is shown in Fig. 3. MAb 36/133.97 immunoprecipitated proteins at 38, 28, 26, and 23 kDa (Fig. 3B, arrowheads) which comigrated with proteins immunoprecipitated by serum from horse H5 at 10^{-3} to 10^{-4} dilutions (Fig. 3A). In vitro translation products derived from rabbit reticulocyte lysate are not glycosylated (33). Therefore, immunoprecipitation of these products by MAb 36/133.97 indicates that the binding site recognized by this antibody is a protein epitope. Immunoprecipitation of in vitro-translated *B. equi* mRNA with sera from four naturally infected horses provided similar results (data not shown).

In Western blot analysis, MAb 36/133.97 did not react with antigen from uninfected erythrocytes (data not shown); however, it recognized proteins of 44, 36, 34, and 28 kDa prepared from stabilates of infected erythrocytes (Fig. 4, arrowheads). Evaluation of diluted horse sera demonstrated reactivity with a 28-kDa protein at a dilution of 10^{-4} (Fig. 4).

Relatedness of proteins recognized by sera from *B. equi*-infected horses and MAb 36/133.97. Relatedness of proteins recognized by MAb 36/133.97 and sera from *B. equi*-infected horses was investigated by a CI ELISA. Sera from 34 noninfected horses allowed MAb 36/133.97 to bind in the CI ELISA with OD values of 0.97 ± 0.08. Thus, inhibition of MAb binding to *B. equi* merozoites was considered significant at OD values of <0.73, corresponding to mean OD minus 3 standard deviations. Sera from infected horses from 19 countries significantly inhibited the binding of MAb 36/133.97 to isolated merozoites (Table 1). At a 10^{-1} dilution, sera from all infected horses uniformly inhibited binding in the CI ELISA. Some of these sera also inhibited the binding of MAb 36/133.97 at dilutions of 10^{-2} and 10^{-3} (Table 1).

DISCUSSION

This study demonstrates that horses infected with *B. equi* produce antibodies reactive with at least 11 merozoite proteins ranging from 210 to 25 kDa. The described murine MAb 36/133.97 reacts with a protein epitope on 44-, 36-, 34-, and 28-kDa merozoite antigens. Through a competitive binding assay, we showed that horses infected with *B. equi* through-

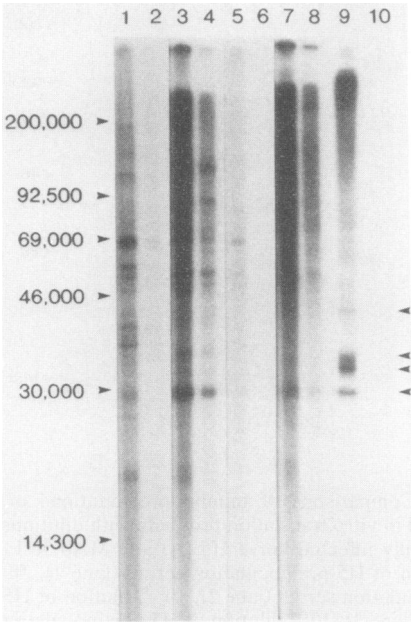

FIG. 4. Comparisons of dilutions of sera from infected horses and MAb 36/133.97 in Western blots: H5 preinoculation serum, 10^{-3} (lane 1) and 10^{-4} (lane 2); H5 postinoculation serum, 10^{-3} (lane 3) and 10^{-4} (lane 4); SN76N8401 (control serum from the National Veterinary Services Laboratory, Ames, Iowa), 10^{-3} (lane 5) and 10^{-4} (lane 6); naturally infected horse serum, 10^{-3} (lane 7) and 10^{-4} (lane 8); MAb 36/133.97 (lane 9); and MAb isotype control (lane 10). Arrowheads indicate locations of 44-, 36-, 34-, and 28-kDa proteins.

out the world consistently produce antibodies to the antigens associated with this epitope. The 28-kDa antigen is of particular interest because it is immunodominant in infected horses, as evidenced by its ability to induce high-titered antibody responses in both naturally and experimentally infected horses.

The protein nature of the epitope recognized by MAb 36/133.97 was demonstrated by immunoprecipitation of 38-, 28-, 26-, and 23-kDa in vitro translation products of merozoite mRNA by MAb 36/133.97. The rabbit reticulocyte lysate used lacks microsomal membranes necessary for processing events such as core glycosylation (33). Therefore, the lower apparent molecular weights of the proteins immunoprecipitated by MAb 36/133.97 from in vitro translation products of merozoite mRNA than of native *B. equi* merozoite proteins are most likely due to the lack of a secondary processing event such as glycosylation in the in vitro translation system. The reactivity of MAb 36/133.97 in Western blots with 44-, 36-, 34-, and 28-kDa proteins indicates that the epitope bound by MAb 36/133.97 is present on all four proteins. The organizational relationship of the four proteins recognized by MAb 36/133.97 was not defined by this study. However, the results of this study are compatible with two explanations. The epitope recognized by MAb 36/133.97 may be encoded by multiple mRNA. Alternatively, or in concert with expression by multiple mRNA, the four proteins may represent a precursor-product relationship.

The surface location of antigen associated with the epitope recognized by MAb 36/133.97 was demonstrated by binding of MAb 36/133.97 to viable merozoites. The immunodominance of the 28-kDa antigen recognized by MAb 36/133.97 was determined by serum dilution studies. Sera of horses examined at dilutions of 10^{-3} to 10^{-4} immunoprecipitated proteins which comigrated with 38-, 28-, 26-, and 23-kDa proteins immunoprecipitated by MAb 36/133.97. Like MAb 36/133.97, sera from infected horses at a dilution of 10^{-4} recognized a 28-kDa protein in Western blots.

In related hemoprotozoan diseases, such as malaria and bovine babesiosis, a prominent feature of the host reaction to infection is a preferential antibody response to protein antigens of merozoites (1, 8). Observations indicate that the immune response to immunodominant merozoite surface proteins is involved in protection against clinical disease in bovine babesiosis and malaria (8, 25) and that antibody to merozoite surface proteins may block merozoite invasion of erythrocytes in vivo (3). Similar to other hosts infected with hemoparasites (1, 3, 8, 19, 30), horses respond to *B. equi* infection by producing antibodies to merozoite proteins.

Vaccination is currently not available for *B. equi*. Our strategy for development of immunoprophylaxis is to identify and characterize surface antigens of the merozoite, because this stage is infective for erythrocytes and is accessible to the host immune system (11, 12). The data of this report identify means for the further characterization and isolation of a surface merozoite protein of *B. equi* which contains a geographically conserved protein epitope. The isolation of proteins recognized by MAb 36/133.97 and their use as immunogens will provide insights into their role in the acquired protective immunity against clinical disease of *B. equi*-infected horses. Additionally, the immunodominance of the 28-kDa protein bound by MAb 36/133.97 and the geographic conservation of the protein epitope recognized by MAb 36/133.97 indicate potential for use in diagnosis.

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