INFECTION AND IMMUNITY, Dec. 1985, p. 881–886
0019-9567/85/120881-06$02.00/0

Vol. 50, No. 3

Presence of Common Antigens, Including Major Surface Protein Epitopes, between the Cattle (Intraerythrocytic) and Tick Stages of *Anaplasma marginale*

GUY H. PALMER,[1]†* KATHERINE M. KOCAN,[2] SELWYN J. BARRON,[3] JAKIE A. HAIR,[4] ANTHONY F. BARBET,[1] WILLIAM C. DAVIS,[1] AND TRAVIS C. McGUIRE[1]

Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040,[1] and Departments of Veterinary Pathology,[2] Medicine and Surgery,[3] and Entomology,[4] Oklahoma State University, Stillwater, Oklahoma 74078

Received 1 July 1985/Accepted 11 September 1985

Epitopes of major surface proteins of the intraerythrocytic cattle stage of *Anaplasma marginale* were demonstrated in the midgut stage of the organism within the infective tick host *Dermacentor andersoni*. These proteins were common to all *A. marginale* isolates tested and at all stages of parasitemia. Sera from cattle immunized with the tick midgut stage of *A. marginale* immunoprecipitated multiple-erythrocyte-stage proteins, as demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The major proteins recognized (primarily >14 and <200 kilodaltons [kDa]) included two major-erythrocyte-stage surface proteins of 36 and 105 kDa molecular size. To confirm the presence of common tick and erythrocyte *A. marginale* antigens with the immunized cattle sera, we purified the 36-kDa erythrocyte-stage protein by monoclonal immunoaffinity chromatography and developed an enzyme-linked immunosorbent assay based on the purified protein. All sera from cattle immunized with tick-stage *A. marginale* and cattle infected with various isolates of *A. marginale* developed antibodies to the 36-kDa protein. The potential immunoprophylactic, diagnostic, and epidemiologic value of the major epitopes common to both the invertebrate and mammalian stages of *A. marginale*, especially the 36-kDa protein, is discussed.

Anaplasmosis is an arthropod-borne hemoparasitic disease of cattle and other ruminants caused by the rickettsia *Anaplasma marginale*. Infection is endemic in one-third to one-half of the worldwide livestock production regions, and the disease remains, along with African trypanosomiasis, babesiosis, theileriosis, and heartwater, the greatest obstacle to meat, milk, and fiber production in third-world nations (17). Infection results from inoculation of cattle with either a tick stage (via transtadial replication in the midgut epithelium of a variety of ixodid ticks) or the intraerythrocytic stage (after mechanical transmission on the mouthparts of biting flies or by blood-contaminated fomites, i.e., syringes or dehorning instruments) (22). The clinical disease is similar regardless of the source of infection and is characterized by intraerythrocytic parasitism and severe hemolytic anemia (28). Dramatic weight loss, abortion, and death may occur during the acute phase of the disease (1). Cattle recovered from acute infection remain persistently infected with a low-level parasitemia and serve as a reservoir of the organism for infection of ticks or mechanical transmission to cattle (26).

The severe constraints posed by anaplasmosis to livestock production both in the United States and developing nations and the inadequacy of current immunoprophylaxis led to the recent National Research Council recommendation that high priority be given to development of an effective vaccine (17). An effective vaccine would need to provide protection against all of the many isolates of *A. marginale* (with demonstrated antigenic, cross-protective, structural, and virulence differences) as well as against both infected blood and tick challenge (2, 13–15). Five major surface proteins with apparent molecular sizes of 105, 86, 61, 36, and 31 kilodaltons (kDa) have been identified so far with a neutralizing antibody (19) and are recognized by immune bovine anti-*A. marginale* sera regardless of the strain. The 105- and the 36-kDa proteins (Am105 and Am36) were shown to bear determinants common to all *A. marginale* isolates tested thus far (15).

In the present communication we report the results of experiments designed to investigate the existence of similar epitopes in the tick stages of *A. marginale*.

MATERIALS AND METHODS

Cattle sera. (i) Cattle infected with erythrocyte-stage *A. marginale*. Seronegative (by the complement fixation test) Holstein calves were inoculated intramuscularly with parasitized erythrocytes (10^{10} initial bodies), and infection was monitored daily by examination of Wright stained blood smears for parasites. Isolates of *A. marginale* used included the Florida isolate (five calves), the North Texas isolate (three calves), and the Virginia isolate (four calves). The antigenic, cross-protective, and structural differences between these isolates have been previously characterized (2, 13–15).

(ii) Cattle immunized with tick-midgut-stage *A. marginale*. The laboratory rearing of *Dermacentor andersoni* ticks, infection of nymphs on *A. marginale* parasitemic calves, and incubation of subsequently molted infected adults have been previously described (7, 20). The morphology and infectivity of the isolated tick stages in gut cells of adults have also been described in detail (8, 9). Briefly, 2,000 to 3,000 *D. andersoni*

* Corresponding author.
† Present address: Department of Comparative and Experimental Pathology, University of Florida, Gainesville, FL 32610.

nymphs were placed in orthopedic stockinettes attached to each of three *A. marginale*-infected calves with an approximate parasitemia of 2%. The rapid phase of tick feeding therefore coincided with the acute phase of anaplasmosis, during which parasitemia increases sharply (7). Engorged nymphs were placed in 90 to 98% humidity at 25°C with a 14-h photophase and allowed to molt. The resultant adult ticks were maintained under these conditions for 1 month to ensure that any erythrocyte-stage antigens (not detectable in the tick midgut by indirect fluorescent antibody method after 2 days postattachment) remaining from the blood meal were catabolized (3). During the molting period, tick stages progress through a morphologically defined development cycle of colony types (8). Groups of 200 adult ticks that were infected as nymphs were incubated at 37°C for 48 h to stimulate formation of higher colony densities (7, 10). Midguts were dissected and homogenized in RPMI 1640 medium with ground-glass grinders and centrifuged at 500 × *g*, and the supernatant containing *A. marginale* was lyophilized and stored at 4°C until used to immunize cattle. Ticks exposed to uninfected calves were treated identically, and midguts were dissected, homogenized, and centrifuged as described for the infected ticks. Seronegative Holstein calves were splenectomized and then immunized intramuscularly and intravenously twice at 6-week intervals with tick-stage *A. marginale* emulsified in Freund incomplete adjuvant. Each immunization consisted of organisms isolated from 1,200 homogenized midguts. The immunized animals were monitored twice weekly by examination of Wright stained blood smears for parasites and by the complement fixation tests. Splenectomized calves were used to eliminate the possibility of a subclinical parasitemia resulting from the tick-stage organism in the immunogen—splenectomized calves will develop acute anaplasmosis from one infective particle, as previously demonstrated by limiting dilution (6). Furthermore, 50 ml of blood from one of the four immunized calves was directly inoculated into a susceptible, splenectomized calf, which did not become infected (later demonstrated to be susceptible to acute anaplasmosis after infective blood challenge).

[^{35}S]methionine radiolabeling of *A. marginale*. *A. marginale* initial bodies (Florida isolate) were radiolabeled with [^{35}S] methionine during short-term in vitro erythrocyte culture as described previously (2). Barbet et al. (2) demonstrated that the radiolabel incorporates exclusively into the *A. marginale* initial body by this procedure. Briefly, blood was drawn from splenectomized calves during ascending parasitemia of acute infection, washed in Hanks balanced salt solution with removal of the buffy coat after centrifugation, and diluted into Eagle minimal essential medium without methionine (10% fetal bovine serum, 2 mM L-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml). The cultures were incubated for 48 h with 125 μCi of [^{35}S]methionine per 5 × 10^8 parasitized erythrocytes at 37°C and 5% CO_2 in air. The cultures were washed four times in Hanks balanced salt solution, and the pelleted erythrocytes were disrupted in a 50 mM Tris (pH 8.0) buffer containing 5 mM EDTA, 5 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, 0.1 M *N*-α-*p*-tosyl-L-lysyl-chloromethyl ketone, 1.0% Nonidet P-40, and 0.1% sodium dodecyl sulfate (SDS). The pellets were frozen at −70°C until used.

^{125}I-surface radiolabeling of *A. marginale*. *A. marginale* initial bodies were purified from infected erythrocytes by a procedure previously described (19). Approximately 5 × 10^8 purified initial bodies or uninfected bovine erythrocyte membranes were surface radiolabeled with 1.0 mCi of ^{125}I in a lactoperoxidase-mediated reaction (23). Free iodine was removed by column chromatography (G-50 media; Bio-Rad Laboratories, Richmond, Calif.) and dialysis against phosphate-buffered saline (PBS).

Immunoprecipitation. The immunoprecipitation of [^{35}S] methionine or ^{125}I-radiolabeled *A. marginale* proteins was performed by a modification of a described technique (24). The disrupted, radiolabeled *A. marginale* were centrifuged at 135,000 × *g* for 60 min, passed through a 0.45-μm filter, and sonicated at 100 W for 15 s, and 10^6 trichloroacetic acid-precipitable cpm was added to 2 μl of bovine serum and incubated at 4°C for 30 min. Rabbit anti-bovine immunoglobulin M (IgM), IgG1, and IgG2 (100 μl) were added and incubated at 4°C for 30 min followed by a similar incubation with 100 μl of 10% (vol/vol) protein A-bearing *Staphylococcus aureus*. The precipitates were washed six times with TEN buffer (20 mM Tris-hydrochloride, 5 mM EDTA, 0.1 M NaCl, 15 mM NaN_3 [pH 7.6]) containing 1.0% Nonidet P-40 and, for the first four washes, 2 M NaCl. The precipitated radiolabel was eluted by boiling the staphylococci-bound complexes for 3 min in 50 μl of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) buffer followed by centrifuging at 1,000 × *g*. The eluted radiolabeled antigens were either frozen at −30°C until use or applied directly to polyacrylamide gels.

SDS-PAGE of immunoprecipitates and autoradiography. The immunoprecipitates were electrophoresed on 7.5 to 17.5% continuous gradient polyacrylamide gels under reducing conditions (27). The position of the ^{35}S-radiolabeled protein bands was revealed by fixation with a solution of 10% (wt/vol) tricholoracetic acid, 10% (vol/vol) acetic acid, and 30% (vol/vol) methanol, followed by processing for fluorography with En^{3}Hance (New England Nuclear Corp., Boston, Mass.), vacuum drying, and exposure to Kodak XAR-2 X-ray film at −70°C. The ^{125}I-radiolabeled proteins were fixed in polyacrylamide gels with 30% (vol/vol) methanol and 10% (vol/vol) acetic acid, followed by vacuum drying and exposure to X-ray film with Cronex Quanta III intensifying screens (Du Pont Co., Wilmington, Del.) at −70°C.

Identification of monoclonal antibodies to Am36. The production and initial screening by indirect immunofluorescence assay of monoclonal antibodies (MAbs) to *A. marginale* initial bodies have been previously described (4). MAbs positive by indirect immunofluorescence assay on initial bodies in acetone-fixed parasitized erythrocytes but unreactive with uninfected bovine erythrocytes or erythrocyte membranes of infected erythrocytes were used to immunoprecipitate ^{35}S-radiolabeled *A. marginale* proteins to identify MAbs reactive with Am36. The immunoprecipitation was done as described for the bovine sera; however, rabbit anti-mouse immunoglobulin (IgA, IgM, IgG1, IgG2a, IgG2b, and IgG3) was used as the second antibody. All MAbs were from double-cloned cell lines and had been concentrated to 0.1 mg/ml and the isotype determined prior to immunoprecipitation (15). Identification of precipitates by SDS-PAGE and fluorography was done as described for the bovine sera immunoprecipitates.

Purification of Am36. Am36 was purified from *A. marginale* initial bodies by monoclonal immunoaffinity chromatography. Pristane (Aldrich Chemical Co., Inc., Milwaukee, Wis.)-treated BALB/c mice were injected intraperitoneally with 3 × 10^6 hybridoma cells (ANA058A_2, twice-cloned cell lines producing anti-Am36 antibody of the IgG1 subclass), and ascitic fluid was withdrawn 10 to 14 days later and used as an antibody source. Immunoglobulin was

precipitated from the ascitic fluid with 50% (vol/vol) saturated ammonium sulfate and after dialysis was chromatographed on a DE-52 ion-exchange column (Whatman Chemicals Ltd., Kent, England) with elution with a 0 to 0.2 M NaCl continuous gradient. The purity of the isolated fractions was confirmed by SDS-PAGE with Coomassie blue staining to detect heavy and light chains. The purified immunoglobulin was coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) with 10 mg of protein per ml of settled beads as described previously (5).

Approximately 10^{12} *A. marginale* initial bodies were purified from parasitized erythrocytes by ultrasonic disruption and differential centrifugation as previously described (19). The isolated initial bodies were disrupted in 50 mM Tris (pH 8.0) buffer containing 1.0% Nonidet P-40, 0.1% SDS, and proteolytic inhibitors and applied to the immunoaffinity column at 25 to 30 ml/h. Unbound proteins were removed by washing with 100 column volumes of TEN buffer and Am36 was eluted with 50 mM Tris with 0.5% deoxycholate and 2 M KSCN; after extensive dialysis against PBS, the purity of the eluted Am36 was confirmed by SDS-PAGE with silver staining to detect proteins (16). The concentration of the purified Am36 was determined by a modification of the Lowry assay (21). Detergent extracts of 10^{12} uninfected erythrocytes were prepared and applied to the affinity column with elution under identical conditions.

Am36-based ELISA. For AM36-based enzyme-linked immunosorbent assay (ELISA), AM36 was passively adsorbed to Immulon I polystyrene microtiter plates (Dynatech Laboratories, Alexandria, Va.) overnight at 4°C with 20 ng of protein in 50 μl of 0.1 M sodium bicarbonate buffer (pH 9.6) per well. Optimal antigen concentration, conjugate dilution, and enzyme substrate concentration were determined by a checkerboard assay (29). Unbound sites on the wells were blocked by incubating with PBS containing 0.1% gelatin at 37°C for 2 h, followed by extensive rinsing with PBS containing 0.2% Tween 20 (Sigma Chemical Co., St. Louis, Mo.) and then addition of 50 μl of bovine sera with incubation at 37°C for 1 h. The plates were again rinsed with PBS-Tween followed by incubation for 1 h at 37°C with 50 μl of rabbit anti-bovine IgM, IgG1, and IgG2 antibody conjugated to horseradish peroxidase. The plates were again rinsed, and 50 μl of 5-aminosalicylate containing 0.005% H_2O_2 was added and allowed to react for 45 min at room temperature. The color reaction was quantitated with optical density 450 nm/630 nm on a Dynatech MR 600 microtiter ELISA reader.

Am36-based inhibition radioimmunoassay. Am36, and Am105 (isolated by affinity chromatography; G. H. Palmer, A. F. Barbet, W. C. Davis, T. C. McGuire, Proceedings of the Sixth Annual Conference on Food Animal Veterinary Medicine, in press), purified intact *A. marginale* initial bodies, uninfected bovine erythrocyte membranes (19), or tick midgut homogenates from uninfected *D. andersoni* were incubated overnight at 4°C with a 1:5,000 dilution of rabbit anti-Am36 serum (produced by four injections of 25 μg of Am36 emulsified in complete Freund adjuvant at 2-week intervals), and 50 μl of the mixture was incubated for 1 h at room temperature on polyvinyl chloride microtiter plates with 5 ng of Am36 coated per well. The plates were washed five times with TEN buffer. The plates were then incubated for 1 h at room temperature with 50,000 cpm ^{125}I-labeled protein A, washed five times with TEN, and the counts per minute per well were determined. The percent inhibition was determined relative to background counts per minute obtained with normal rabbit serum preincubated with 2 μg of Am36.

RESULTS

Immunoprecipitation of ^{35}S-radiolabeled *A. marginale*. Serum from cattle infected with parasitized erythrocytes specifically recognized primarily numerous [^{35}S]methionine-radiolabeled *A. marginale* initial body proteins of apparent molecular size >14 and <200 kDa (Fig. 1, lane 2). This same pattern has previously been shown regardless of the isolate used to infect the cattle, although isolate differences are apparent in two-dimensional gel electrophoresis (2). The sera from cattle immunized with tick-midgut-stage *A. marginale* recognized multiple [^{35}S]methionine-radiolabeled erythrocyte-stage proteins in the 14- to 200-kDa range (Fig. 1, lanes 4, 6, 8, and 10), although fewer proteins were precipitated and the precipitated bands were less intense than with the serum from infected cattle (Fig. 1, lane 2). Proteins of molecular weights corresponding to two of the initial body major surface proteins are recognized by the invertebrate-stage immunized cattle sera (Am105 most prominently in Fig. 1, lane 4 but faintly in lanes 6, 8, and 10, position marked by an arrowhead; Am36 faintly in Fig. 1, lanes 4, 6, 8, and 10, position marked by an arrowhead) indicating the possible presence of common epitopes between those initial body proteins and the tick stage of the organism. Preimmunization sera (Fig. 1, lanes 3, 5, 7, and 9) or sera from cattle immunized with midgut homogenates from uninfected ticks did not precipitate any [^{35}S] methionine-labeled *A. marginale* proteins.

Immunoprecipitation of ^{125}I-surface radiolabeled *A. marginale*. The significance of shared epitopes between the erythrocytic and tick stages of *A. marginale* in protection depends on whether these epitopes are present on the surface of the organism. To determine whether the erythrocyte-stage surface proteins were among the shared epitopes as is suggested by the similar molecular weights, purified *A.*

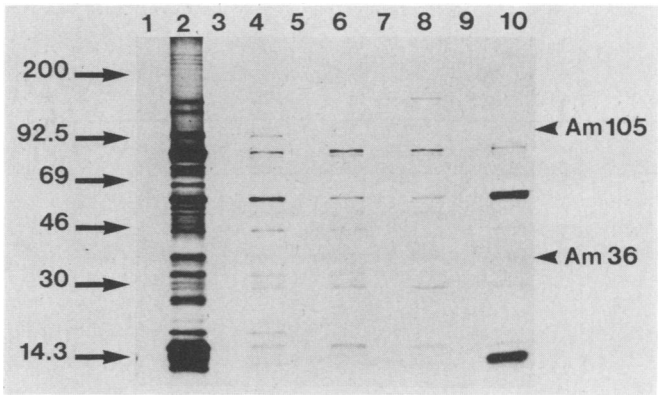

FIG. 1. Identification of erythrocyte-stage *A. marginale* proteins recognized by sera from cattle immunized with tick-midgut-stage *A. marginale*. *A. marginale*-infected erythrocytes were metabolically radiolabeled with [^{35}S]methionine during in vitro culture, detergent disrupted, and immunoprecipitated with sera from cattle infected with parasitized erythrocytes (preinfection sera, lane 1; postinfection sera, lane 2) or sera from cattle immunized with tick-stage *A. marginale* (preimmunization sera, lanes 3, 5, 7, and 9; postimmunization sera, lanes 4, 6, 8, and 10). Arrowheads on the right margin indicate the position of erythrocyte-stage major surface proteins Am36 and Am105. ^{14}C molecular weight standards are in thousands (arrows at the left margin).

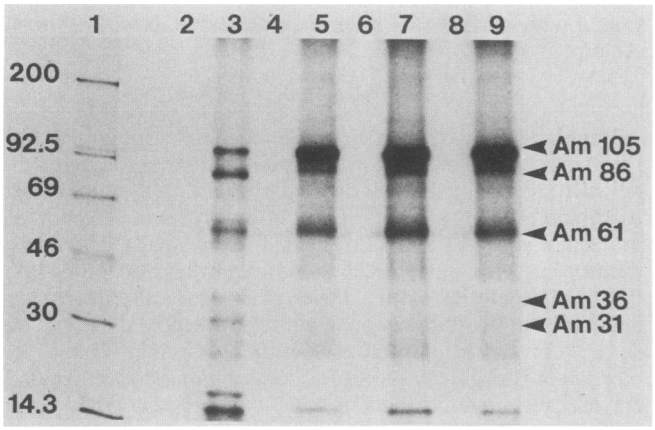

FIG. 2. Identification of erythrocyte-stage *A. marginale* surface proteins recognized by sera from cattle immunized with tick-stage *A. marginale*. Initial bodies purified from *A. marginale*-parasitized erythrocytes were surface radiolabeled with ^{125}I with lactoperoxidase, disrupted, and immunoprecipitated with sera from cattle immunized with tick-stage *A. marginale* (preimmunization sera, lanes 2, 4, 6, and 8; postimmunization sera, lanes 3, 5, 7, and 9). ^{14}C molecular weight standards are in thousands (lane 1).

marginale initial bodies were surface radiolabeled with ^{125}I, disrupted with detergent, and immunoprecipitated with sera from cattle immunized with the tick stage. Postimmunization sera recognized four to five major surface proteins of the erythrocyte-stage *A. marginale*, including Am105, Am86, Am61, and Am36 (Fig. 2, lanes 3, 5, 7, and 9). There was no reactivity of these postimmunization sera with identically

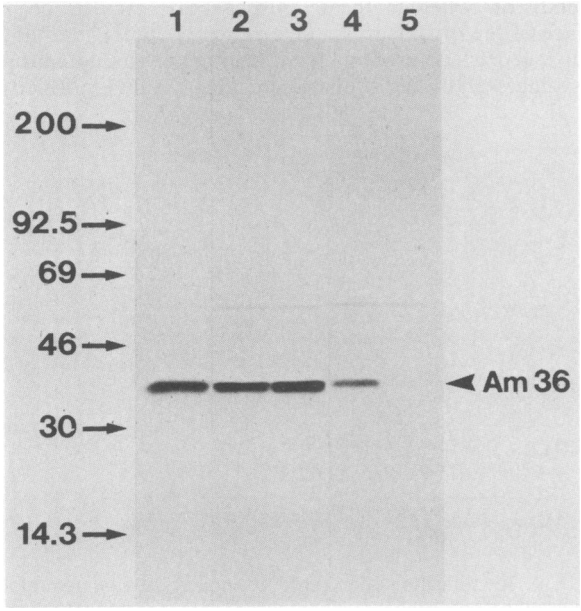

FIG. 3. Identification of MAbs to Am36. Erythrocyte-stage *A. marginale* proteins were metabolically radiolabeled with [^{35}S] methionine during in vitro culture and, after detergent disruption, immunoprecipitated with supernatant from twice-cloned hybridoma cell lines with rabbit anti-mouse immunoglobulin and *S. aureus* bearing protein A. Identified cell lines producing anti-Am36 antibody are $ANA050A_2$ (lane 1), $ANA058A_2$ (lane 2), $ANA066A_2$ (lane 3), and $ANA070A_2$ (lane 4). TRYP 1E1 (an anti-*Trypanosoma brucei* MAb) is included as a negative control (lane 5). ^{14}C molecular weight standards are in thousands (arrows left margin).

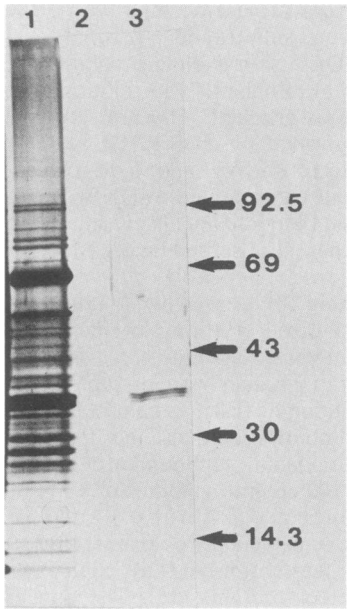

FIG. 4. Purification of Am36 by monoclonal immunoaffinity chromatography. Approximately 10^{12} detergent-disrupted erythrocyte-stage *A. marginale* were applied to a Sepharose 4B-$ANA050A_2$ chromatography column, and after extensive washing, Am36 was eluted with 50 mM Tris (pH 8.0) containing 0.5% deoxycholate and 2 M KSCN. The extract applied to the column (lane 1) and the purified Am36, after dialysis against PBS (lane 3), were electrophoresed on SDS-PAGE with silver-staining detection of protein. Lane 2 is unloaded as a control for silver-staining artifacts. ^{14}C molecular-weight standards are in thousands (right margin). Extracts of 10^{12} uninfected erythrocytes did not bind to the Sepharose 4B-$ANA050A_2$.

radiolabeled uninfected erythrocytes. In addition, preimmunization sera and sera from cattle immunized with tick midgut homogenates from uninfected *D. andersoni* were unreactive with ^{125}I-labeled *A. marginale*.

Identification of MAbs to Am36. We screened 119 hybridomas producing MAbs indirect immunofluorescence assay positive on initial bodies in *A. marginale*-infected erythrocytes (negative on uninfected erythrocytes and erythrocyte membranes of infected erythrocytes) for anti-Am36 antibody by immunoprecipitation. We identified four twice-cloned cell lines producing anti-Am36 MAbs: $ANA050A_2$ (IgG1), $ANA058A_2$ (IgG2a), $ANA066A_2$ (IgG1), and $ANA070A_2$ (IgG1) (Fig. 3).

Am36-based ELISA. Approximately 1.0 mg of Am36 was purified from 10^{12} isolated *A. marginale* initial bodies by immunoaffinity chromatography on a 1.0-ml Sepharose 4B-$ANA050A_2$ column. The purity of the Am36 was confirmed by SDS-PAGE with detection of proteins by silver staining (Fig. 4). The purified Am36 was used as the antigen base for a microtiter ELISA for anti-Am36 bovine antibody. All sera from cattle immunized with tick-stage *A. marginale* and sera from cattle infected with parasitized erythrocytes (Florida, Virginia, and North Texas isolates) contained antibodies to Am36 (Table 1). Preinfection and preimmunization sera were unreactive with Am36.

Am36-based inhibition radioimmunoassay. The Am36-rabbit anti-Am36 binding was inhibited by 2 ng of Am36 or 10^5 purified intact initial bodies (>90% inhibition with 200 ng of Am36 or 10^7 purified initial bodies) but not by purified Am105 (Palmer et al., in press) or uninfected bovine eryth-

TABLE 1. Reactivity of cattle sera with Am36

Inoculum source	No. of sera	Am36 titer
Florida isolate-infected erythrocytes	6	>1:1,000
North Texas isolate-infected erythrocytes	3	>1:1,000
Virginia isolate-infected erythrocytes	4	>1:1,000
Tick-stage immunized cattle	4	1:100

rocyte membranes (Fig. 5). In addition, tick midgut homogenates (2 μg to 2 mg) from *D. andersoni* fed on uninfected cattle did not inhibit Am36 binding.

DISCUSSION

Our recent identification of five major erythrocyte-stage surface proteins recognized by neutralizing antibody (molecular sizes, 105, 86, 61, 36, and 31 kDa) and the presence of epitopes of two of these proteins (Am36 and Am105) on all six *A. marginale* isolates examined led to this investigation of the occurrence of these proteins in the invertebrate stage of *A. marginale*. Previous work has demonstrated that at least some antigens were common to both stages by using polyspecific bovine postinfection serum to bind invertebrate-stage organisms in situ as detected by indirect immunofluorescence assay, immunoferritin labeling, and peroxidase-anti-peroxidase labeling (11, 12, 18, 25). Our objective was to determine whether either of the two erythrocyte-stage surface proteins bearing isolate-common epitopes shared antigens with the morphologically dissimilar tick stage.

The immunoprecipitation of ^{35}S-radiolabeled erythrocyte-stage proteins by the anti-tick stage sera clearly demonstrates that there are antigens common to both stages. Both Am36 and Am105 were recognized by all anti-tick-stage sera; however, the number and the intensity of the precipitated radiolabeled bands are less than those with sera from erythrocyte-stage infection. Undoubtedly, the use of splenectomized calves for tick-stage immunization diminished the humoral response to the immunogen; however, we felt this was necessary to eliminate the possibility of a subclinical erythrocyte-stage parasitemia. The precipitated antigens therefore likely represent a minimal number of common antigens between the two stages. To confirm the recognition of Am36 and Am105 by the tick-stage immunized sera, we immunoprecipitated surface-radiolabeled *A. marginale* initial bodies and identified Am105, Am86, Am61, and Am36 as sharing common epitopes with tick-stage proteins. Whether these epitopes are present on the surface of the tick stage of *A. marginale* and, therefore, are susceptible to neutralizing antibody raised against purified erythrocyte-stage proteins is not currently known. The use of monospecific antibody or MAb (presently available for Am36 and Am105) in colloidal gold labeling studies on sections of tick-stage organisms in tick midgut epithelium is presently being examined to determine the location of these epitopes. Additionally, whether these stage-common epitopes include those Am105 epitopes previously shown to be capable of inducing neutralizing MAb will be determined by these techniques (G. H. Palmer et al., in press). Identification of antigens capable of inducing neutralizing antibody common among antigenically diverse isolates and common among the two stages of the organism would provide specific antigens for testing as protective immunogens in cattle.

The isolation of Am36 and development of an ELISA based on purified Am36 allowed confirmation of the immunological cross-reactivity between the two stages of *A. marginale*. In addition, this assay was capable of detecting cattle infected with any of the three antigenically diverse isolates tested, indicating that Am36 epitopes are common among different isolates. Isolate-common Am36 epitopes have also been demonstrated by the reactivity of anti-Am36 MAbs with eight antigenically distinct *A. marginale* isolates (15). We are currently determining the sensitivity and spec-

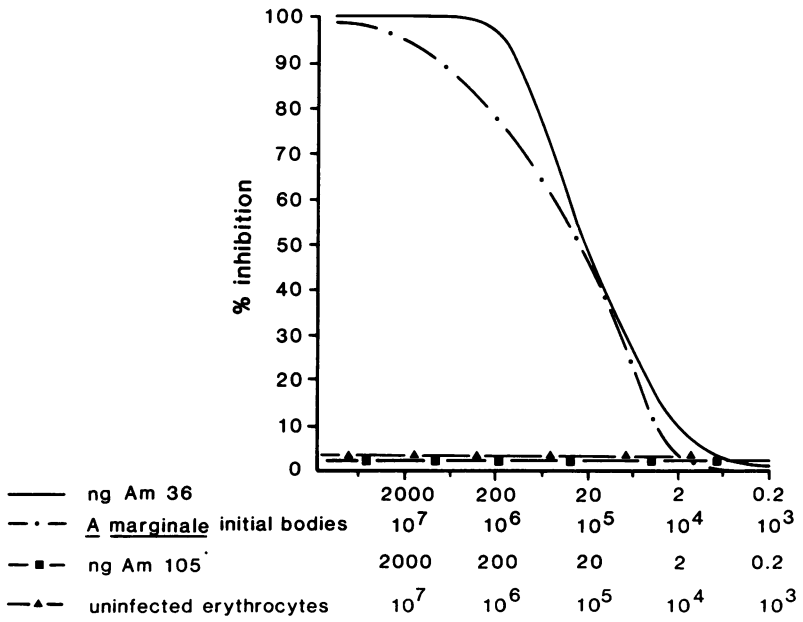

FIG. 5. Inhibition radioimmunoassay based upon Am36 and rabbit anti-Am36. Inhibition was achieved by purified Am36 (——) and intact isolated initial bodies (–·–) but not by purified Am105 (–■–) or uninfected bovine erythrocyte membranes (–▲–). Tick midgut homogenates from uninfected *D. andersoni* also did not inhibit Am36 binding.

ificity of this assay for use in diagnosis of persistently infected cattle.

The development of an inhibition radioimmunoassay based on the common-stage antigen Am36 capable of detecting 10^5 initial bodies (or 2 to 20 ng of Am36) provides a sensitive assay for detection of antigen in cell culture studies, immunologic detection of recombinant expressed proteins, and detection of individual or pooled infected ticks. The sensitive inhibition by intact initial bodies is consistent with surface localization of Am36 previously reported (19). Presently, the only assay for demonstrating infected ticks is by inoculation into or feeding on susceptible cattle, an expensive and cumbersome method that limits investigation of vector-host interaction. The use of a sensitive quantitative assay should allow progress in characterization of vertebrate and invertebrate infection.

ACKNOWLEDGMENTS

This work was supported by U.S. Department of Agriculture, Agricultural Research Service, Hemoparasitic Diseases Research Unit cooperative agreement 58-9-AHZ-2-663, United States-Israel Binational Agricultural Research and Development Fund grant US-344-80, U.S. Department of Agriculture special research grant 83-CRSR-2-2194, and Public Health Service pathology training grant GM 07943 from the National Institutes of Health.

We thank Teresa Gel Harkins and Jan Carlson for technical assistance and David Stiller for valuable discussions.

LITERATURE CITED

1. **Alderink, F. J., and R. Dietrich.** 1981. Anaplasmosis in Texas: epidemiologic and economic data from a questionnaire survey, p. 27–44. *In* R. J. Hidalgo and E. W. Jones (ed.), Proceedings of the Seventh National Anaplasmosis Conference. Mississippi State University Press, Mississippi State.
2. **Barbet, A. F., L. W. Anderson, G. H. Palmer, and T. C. McGuire.** 1983. Comparison of proteins synthesized by two different isolates of *Anaplasma marginale*. Infect. Immun. **40**:1068–1074.
3. **Bram, R. A., and R. D. Romanowsky.** 1970. Recognition of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles (*Dermacentor venustus* marx) by the fluorescent antibody method. I. Smears of nymphal organs. J. Parasitol. **56**:32–38.
4. **Davis, W. C., T. C. McGuire, L. W. Anderson, K. L. Banks, S. D. Seifert, and M. I. Johnson.** 1981. Development of monoclonal antibodies to *Anaplasma marginale* and preliminary studies on their application, p. 285–305. *In* R. J. Hidalgo and E. W. Jones (ed.), Proceedings of the Seventh National Anaplasmosis Conference. Mississippi State University Press, Mississippi State.
5. **Kasper, L. H., J. H. Crabb, and E. R. Pfefferkorn.** 1983. Purification of a major membrane protein of *Toxoplasma gondii* by immunoabsorption with a monoclonal antibody. J. Immunol. **130**:2407–2412.
6. **Kliewer, I. O., E. J. Richey, E. W. Jones, and W. E. Brock.** 1973. The preservation and minimum infective dosage of *Anaplasma marginale*, p. 39–41. *In* E. W. Jones (ed.), Proceedings of the 6th National Anaplasmosis Conference. Heritage Press, Stillwater, Okla.
7. **Kocan, K. M., S. J. Barron, D. Holbert, S. A. Ewing, and J. A. Hair.** 1982. Influence of increased temperature on *Anaplasma marginale* Theiler in the gut of *Dermacentor andersoni* Stiles. Am. J. Vet. Res. **43**:32–35.
8. **Kocan, K. M., S. A. Ewing, D. Holbert, and J. A. Hair.** 1982. Morphologic characteristics of *Anaplasma marginale* Theiler in midgut epithelial cells of *Dermacentor andersoni* Stiles. Am. J. Vet. Res. **43**:586–593.
9. **Kocan, K. M., J. A. Hair, and S. A. Ewing.** 1980. Ultrastructure of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles and *Dermacentor variablis* (Say). Am. J. Vet. Res. **41**:1966–1976.
10. **Kocan, K. M., D. Holbert, S. A. Ewing, J. A. Hair, and S. J. Barron.** 1983. Development of colonies of *Anaplasma marginale* in the gut of incubated *Dermacentor andersoni*. Am. J. Vet. Res. **44**:1612–1620.
11. **Kocan, K. M., K. C. Hsu, and J. A. Hair.** 1980. Demonstration of *Anaplasma marginale* Theiler in *Dermacentor variablis* (Say) by ferritin-conjugated antibody technique. Am. J. Vet. Res. **41**:1977–1981.
12. **Kocan, K. M., K. D. Teel, and J. A. Hair.** 1980. Demonstration of *Anaplasma marginale* Theiler in ticks by tick transmission, animal inoculation, and fluorescent antibody studies. Am. J. Vet. Res. **41**:183–186.
13. **Kreier, J. P., and M. Ristic.** 1963. Anaplasmosis. X. Morphologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of *Anaplasma marginale*. Am. J. Vet. Res. **24**:676–687.
14. **Kreier, J. P., and M. Ristic.** 1963. Anaplasmosis. XI. Immunoserologic characteristics of the parasites present in the blood of calves infected with the Oregon strain of *Anaplasma marginale*. Am. J. Vet. Res. **24**:688–696.
15. **McGuire, T. C., G. H. Palmer, W. L. Goff, M. I. Johnson, and W. C. Davis.** 1984. Common and isolate-restricted antigens of *Anaplasma marginale* defected with monoclonal antibodies. Infect. Immun. **45**:697–700.
16. **Morrissey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117**:307–310.
17. **National Research Council (ed.).** 1982. Priorities in biotechnology research for international development—proceedings of a workshop, p. 1–9. National Academy Press, Washington, D.C.
18. **Oberst, R. D., K. M. Kocan, J. A. Hair, and S. A. Ewing.** 1981. Staining characteristics of colonies of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles. Am. J. Vet. Res. **42**:2006–2009.
19. **Palmer, G. H., and T. C. McGuire.** 1984. Immune serum against *Anaplasma marginale* initial bodies neutralizes infectivity for cattle. J. Immunol. **133**:1010–1015.
20. **Patrick, C. D., and J. A. Hair.** 1975. Laboratory rearing procedures and equipment for multi-host ticks. J. Med. Entomol. **12**:389–390.
21. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable to membrane proteins. Anal. Biochem. **83**:346–356.
22. **Richey, E. J.** 1981. Bovine anaplasmosis, p. 767–772. *In* R. J. Howard (ed.), Current veterinary therapy—food animal practice. The W. B. Saunders Co., Philadelphia.
23. **Rovis, L., A. F. Barbet, and R. O. Williams.** 1978. Characterization of the surface coat of *Trypanosoma congolense*. Nature (London) **271**:654–656.
24. **Shapiro, S. Z., and J. T. August.** 1976. The use of immunoprecipitation to study the synthesis and cleavage processing of viral proteins. J. Immunol. Methods **13**:153–158.
25. **Staats, J. J., K. M. Kocan, J. A. Hair, and S. A. Ewing.** 1982. Immunocytochemical labeling of *Anaplasma marginale* Theiler in *Dermacentor andersoni* Stiles with peroxidase-antiperoxidase technique. Am. J. Vet. Res. **43**:979–983.
26. **Swift, B. L., and G. M. Thomas.** 1983. Bovine anaplasmosis: elimination of the carrier state with injectable long-acting oxytetracycline. J. Am. Vet. Med. Assoc. **183**:63–65.
27. **Takacs, B.** 1979. Electrophoresis of proteins in polyacrylamide slab gels, p. 81–105. *In* T. Lefkovitz and B. Persin (ed.), Immunological methods. Academic Press, Inc., New York.
28. **Theiler, A.** 1910. *Anaplasma marginale*. The marginale points in the blood of cattle suffering from a specific disease, p. 7–64. *In* A. Theiler (ed.), Report of the government veterinary bacteriologist (1908–1909). Transvaal Department of Agriculture, Republic of South Africa.
29. **Voller, A., A. Bartlett, and D. E. Bidwell.** 1976. Enzyme immunoassays for parasitic diseases. Trans. R. Soc. Trop. Med. Hyg. **70**:98–106.