

## Molecular Size Variations in an Immunoprotective Protein Complex among Isolates of *Anaplasma marginale*

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A major surface protein complex from the Florida isolate of *Anaplasma marginale* has been previously shown to induce protection in immunized cattle and has been proposed as the basis of a subunit vaccine against anaplasmosis. This complex in the Florida isolate is composed of two noncovalently associated polypeptides with molecular masses of 105 and 100 kilodaltons (kDa). The analogous protein complex from four geographically different isolates of *A. marginale* was immunoprecipitated and compared with the protein complex of the Florida isolate. The polypeptides of the complex varied in apparent molecular mass among the isolates. By using antibodies recognizing epitopes on each polypeptide of the Florida isolate, the antigenic identity of the polypeptides in the analogous complexes was determined. The polypeptides recognized by the neutralizing monoclonal antibody 22B<sub>1</sub>, which recognizes a 105-kDa polypeptide in the Florida isolate, ranged from 70 to 100 kDa in the other isolates. Those polypeptides recognized by rabbit antiserum R911, which recognizes a 100-kDa polypeptide in the Florida isolate, ranged from 97 to 100 kDa. The surface-exposed peptides in the complexes were compared by limited enzymatic digestion to assess structural homology among isolates. Despite the marked variations in molecular weight, there were conserved peptides between the 22B<sub>1</sub>-reactive polypeptides and between the R911-reactive peptides. Determination of the role of the conserved peptides in inducing immunity will be critical in the application of these polypeptides as the basis of a subunit vaccine for bovine anaplasmosis.

Bovine hemoparasite infections are a significant economic hindrance to the improvement of meat, milk, and fiber production in lesser-developed nations. The most prevalent of these diseases, anaplasmosis, is enzootic to nearly half the world's livestock production regions (18). Responsible for an estimated 100 million dollars per year in economic losses in the United States alone (16), anaplasmosis generates severe losses through mortality, reduced weight gains, and decreased milk production. Control of the causative rickettsia, *Anaplasma marginale*, is presently attempted by chemotherapy, vector control, and vaccination. However, current forms of vaccination, including live, attenuated strains (24) or killed, whole-organism preparations, have proven inadequate for widespread use, and the U.S. Agency for International Development has placed a high priority on the development of more effective immunization (18).

A promising approach towards immunoprophylaxis is a subunit vaccine. Monoclonal antibodies against an erythrocyte-stage surface protein of the Florida isolate of *A. marginale* neutralize infectivity (20). This major surface protein, designated MSP-1, is recognized by postinfection antisera from cattle immune to *A. marginale*, regardless of the isolate used to infect the cattle (21). MSP-1 has the ability to induce protection in immunized cattle against both a homologous (20) and a heterologous (G. H. Palmer, T. C. McGuire, and A. F. Barbet, unpublished data) *A. marginale* challenge and has been proposed as the basis of a subunit vaccine.

When purified from the Florida isolate of *A. marginale*, MSP-1 consists of a complex of two separate gene products (3), with apparent molecular masses of 105 and 100 kilodal-

tons (kDa), designated AmF105 and AmF100, respectively (by genus, species, isolate, and molecular mass in kilodaltons). The exact nature of the noncovalent association between AmF105 and AmF100 is unknown, but the polypeptides may be resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis under reducing conditions. AmF105 bears a neutralization-sensitive epitope common to all isolates of *A. marginale* examined in the United States, Israel, and Kenya (21a).

Demonstrated differences in morphology (10), antigenicity (17), virulence (11), tick transmissibility (27), protein structure (2), and ability to induce cross-protection (12) exist among the various isolates of *A. marginale*. Because an MSP-1-based subunit vaccine must protect against multiple isolates of *A. marginale* within a region, it is essential to determine whether differences exist between the polypeptides of the MSP-1 complex among various isolates. In this study, we examined these polypeptides from four antigenically distinct isolates and compared them with AmF105 and AmF100. The antigenic identity and apparent molecular mass of each polypeptide were determined, and the surface-exposed peptides were compared by partial proteolysis to assess structural homology among isolates.

### MATERIALS AND METHODS

**Source of *A. marginale* organisms.** Five field isolates of *A. marginale* were used in this study. The isolates are designated by the original location of isolation (17): Florida (F), southern Idaho (I), northern Texas (T), Virginia (V), and Clarkston, Wash. (W). The isolates were collected (11) and stored in liquid nitrogen as an infected-blood stabilate (15) before being used to initiate infections. Thawed stabilate (20 ml) from each isolate was inoculated intramuscularly into splenectomized 4-month-old, male Holstein calves. Calves

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were monitored daily by determination of percent parasitemia and hematocrit.

**<sup>35</sup>S labeling of organisms.** *A. marginale* initial bodies were radiolabeled during short-term erythrocyte culture, as described previously (2). Barbet et al. (2) demonstrated that the radiolabel is incorporated exclusively into the initial bodies during this procedure. Briefly, approximately 10 ml of blood was drawn from each calf when its parasitemia reached between 15 and 30%. The blood was washed three times with Hanks balanced salt solution without calcium or magnesium and then was washed once more with Eagle minimal essential medium containing Earle salts, 10% fetal bovine serum, 2 mM L-glutamine, 100 µg of streptomycin per ml, and 100 U of penicillin per ml. After each sterile wash, the buffy coat was removed. Washed erythrocytes were diluted 1:8 in minimal essential medium, and [<sup>35</sup>S]methionine (125 µCi/ml) was added. The suspension was incubated at 37°C with 5% CO<sub>2</sub> in air for 48 h. Cultures were collected and washed four times in Hanks balanced salt solution. Organisms were lysed by dilution in a 50 mM Tris (pH 8.0) buffer containing 5 mM EDTA, 5 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM *N*-alpha-*p*-tosyl-L-lysyl-chloromethyl ketone (TLCK), 1% Nonidet P-40 (NP-40), and 0.1% SDS, and frozen at -70°C until used.

**<sup>125</sup>I surface labeling of organisms.** *A. marginale* initial bodies were purified from infected-blood stabulates as previously described (22). The isolated organisms were suspended in 250 µl of phosphate-buffered saline (10 mM sodium phosphate, 14 mM NaCl [pH 7.4]). Approximately 5 × 10<sup>8</sup> isolated *A. marginale* initial bodies were labeled with <sup>125</sup>I (1 mCi) by the lactoperoxidase method (26). Free iodine was removed by G-50 column chromatography, and incorporation of the label was determined by trichloroacetic acid precipitation.

**Antibodies.** All antibodies were prepared and screened as previously described (7). 22B<sub>1</sub> is a neutralizing monoclonal antibody recognizing an epitope of AmF105 common to multiple isolates of *A. marginale* (17, 20). T1E1 is a monoclonal antibody specific for the variable surface glycoprotein of *Trypanosoma brucei* and serves as a negative control. R911 is a polyclonal, monospecific rabbit antiserum recognizing the complete recombinant polypeptide, AmF100, as expressed in *Escherichia coli* (3). R907 is a rabbit serum against *E. coli* without the inserted AmF100 gene and serves as a negative control for R911. R767 and R865 are rabbit antisera recognizing all mouse antibody subclasses.

**Immunoprecipitation and electrophoresis of surface proteins.** Labeled *A. marginale* initial bodies were disrupted by detergent and sonication, centrifuged, and filtered, as previously described (22). Approximately 10<sup>6</sup> trichloroacetic acid-precipitable cpm of <sup>35</sup>S-labeled proteins or 10<sup>7</sup> trichloroacetic acid-precipitable cpm of <sup>125</sup>I-labeled proteins were diluted with TEN buffer (20 mM Tris, 5 mM EDTA, 10 mM NaCl [pH 7.6]) with 1% NP-40 and 0.1% SDS. Either 5 µg of 22B<sub>1</sub> (or T1E1) or 10 µl of R911 (or R907) was added, and the solution was incubated at 4°C for 40 min. A 10-µl quantity of R767 was added to the 22B<sub>1</sub> (T1E1) precipitations, followed by another 40-min incubation at 4°C. A 100-µl quantity of a 10% (wt/vol) suspension of protein A-bearing *Staphylococcus aureus* was added, and the solution was incubated for 30 min at 4°C. Pellets were washed six times with TEN with NP-40 and SDS; the final four washes were done with an additional 2 M NaCl. The pellets were suspended and washed two more times in TEN without detergent. The precipitates were suspended in 50 µl of sample buffer (2% SDS, 2.5% 2-mercaptoethanol, 25 mM Tris [pH 6.8], 15%

glycerol, 0.002% bromophenol blue) and boiled, and the supernatants were either frozen at -20°C or used directly on 5% polyacrylamide gels containing 4 M urea under reducing conditions. <sup>14</sup>C-labeled proteins for molecular mass comparisons consisted of the following: myosin, 200 kDa; phosphorylase *b*, 92.5 kDa; bovine serum albumin, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor (soybean), 21.5 kDa; lysozyme, 14.3 kDa; cytochrome *c*, 12.5 kDa; aprotinin, 6.5 kDa; B chain of bovine insulin, 3.4 kDa; and A chain of bovine insulin, 2.3 kDa. The gels were fixed in 40% methanol for 30 min, and <sup>35</sup>S-labeled gels were processed for fluorography by immersion in En<sup>3</sup>Hance (New England Nuclear Corp.) and then in deionized water. Vacuum-dried gels were exposed to Kodak XAR-5 X-ray film at -70°C. Gels containing iodine-labeled proteins were exposed to X-ray film with an intensifying screen.

**Western blots (immunoblots).** *A. marginale* initial bodies were isolated, suspended in electrophoresis sample buffer, and separated on a 5% polyacrylamide gel with 4 M urea. The polypeptides were transferred onto nitrocellulose (pore size, 0.2 µm) blocked with 1% bovine hemoglobin in a pH 7.6 buffer containing 17 mM NaCl, 10 mM Tris, and 0.1 mM phenylmethylsulfonyl fluoride. The nitrocellulose was incubated for 30 min with the above buffer plus 0.1% SDS, 1% Triton X-100, 1 mM EDTA, and either a 1:100 dilution of R911 or R907 or 2 µg of 22B<sub>1</sub> or T1E1 per ml. The nitrocellulose was washed in the same buffer, and the 22B<sub>1</sub> or T1E1 filters were incubated for an additional 30 min with a 1:2,000 dilution of R865, followed by four more washes. All nitrocellulose filters were then incubated for 30 min with 10<sup>7</sup> cpm of <sup>125</sup>I-labeled protein A and washed 10 times, the final 5 without hemoglobin. The nitrocellulose was dried, covered with Saran Wrap, and exposed to X-ray film, as above.

**Antigen identity of immunoprecipitated polypeptides.** Autoradiographs were aligned with dried gels, and the <sup>125</sup>I-labeled bands immunoprecipitated by 22B<sub>1</sub> or R911 were excised. The gel was placed back on X-ray film to ascertain that the protein bands had been properly removed. The gel fragments were rehydrated in TEN buffer with NP-40 and SDS and electroeluted into dialysis tubing, with a molecular weight cutoff of 10,000, at 150 V and 25 mA for 3 h. The current was reversed for 10 min, and the gel fragments were pelleted by centrifugation. The supernatant was collected, and the eluted polypeptides were individually reimmunoprecipitated with 22B<sub>1</sub> and R911, as described above.

**Structural relationship of immunoprecipitated polypeptides.** Autoradiographs were aligned with dried gels, and the <sup>125</sup>I-labeled polypeptides were cut out as described above. The gel fragments were then placed into the wells of the stacking gel and allowed to rehydrate in running buffer. Polypeptides were digested in the gel for 45 min with 1.5 µg of *S. aureus* V8 protease, as previously described (4). Polyacrylamide gels (10% polyacrylamide) were fixed, vacuum dried, and exposed to X-ray film, as described above.

## RESULTS

**Immunoprecipitation of *A. marginale* proteins.** The major polypeptides precipitated by 22B<sub>1</sub> have apparent molecular masses of approximately 105 kDa in the Florida, 97 kDa in the south Idaho, 89 kDa in the north Texas, 70 kDa in the Virginia, and 86 kDa in the Washington isolates (Fig. 1). Other bands were present with apparent molecular masses of 100 kDa in the Florida, Virginia, and Washington isolates, 97 kDa in the Idaho isolate, and 98 kDa in the Texas isolate.

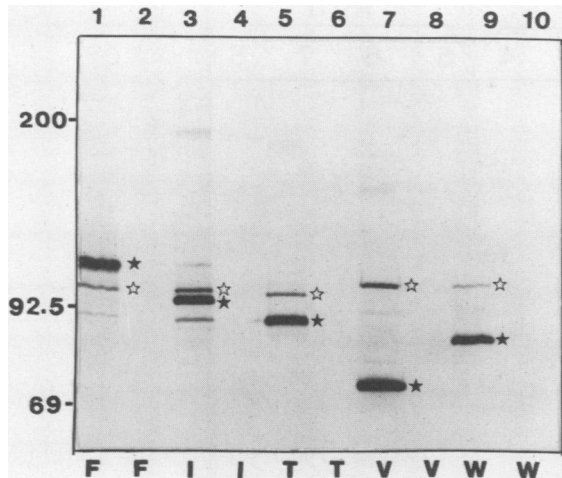


FIG. 1. Polyacrylamide electrophoresis of *A. marginale* polypeptides immunoprecipitated by monoclonal antibodies 22B<sub>1</sub> (lanes 1, 3, 5, 7, and 9) or T1E1 (lanes 2, 4, 6, 8, and 10), as described in Materials and Methods. F, I, T, V, and W represent the Florida, south Idaho, north Texas, Virginia, and Clarkston, Wash., isolates of *A. marginale*, respectively. The polypeptides later demonstrated to be recognized by 22B<sub>1</sub> (★) and polypeptides later demonstrated to be recognized by rabbit antiserum R911 (☆) (see text and Table 1) are indicated. The numbers to the left of the gel represent the positions of the molecular mass markers (in kilodaltons).

Polypeptides ranging from 82 to 92 kDa occasionally appeared in most isolates but were inconsistent. The major polypeptides precipitated by R911 have apparent molecular sizes of 100 kDa in the Florida, Virginia, and Washington isolates, 97 kDa in the Idaho isolate, and 98 kDa in the Texas isolate (Fig. 2). Similarly, fainter bands appeared with mo-

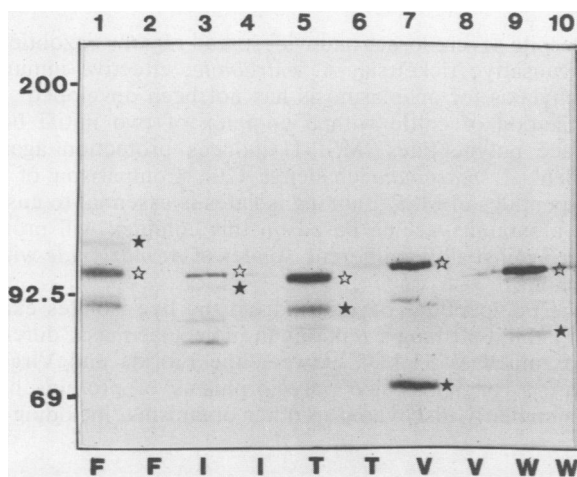


FIG. 2. Polyacrylamide electrophoresis of *A. marginale* polypeptides immunoprecipitated by rabbit antisera R911 (lanes 1, 3, 5, 7, and 9) or R907 (lanes 2, 4, 6, 8, and 10), as described in Materials and Methods. F, I, T, V, and W represent the Florida, south Idaho, north Texas, Virginia, and Clarkston, Wash., isolates of *A. marginale*, respectively. The polypeptides later demonstrated to be recognized by R911 (☆) and polypeptides later demonstrated to be recognized by monoclonal antibody 22B<sub>1</sub> (★) (see text and Table 1) are indicated. The numbers to the left of the gel represent the positions of the molecular mass markers (in kilodaltons).

lecular masses correlating with the molecular masses of the major bands immunoprecipitated by 22B<sub>1</sub> (i.e., 70 to 105 kDa). No proteins were precipitated by either T1E1 or R907.

**Western blots.** The apparent molecular masses of the major polypeptides recognized by 22B<sub>1</sub> in the Western blot were the same as those precipitated by 22B<sub>1</sub> above, i.e., 105, 97, 89, 70, and 86 kDa for the Florida, Idaho, Texas, Virginia, and Washington isolates, respectively (Fig. 3A). Similarly, the polypeptides recognized by R911 in the Western blot had the same molecular weights as the primary polypeptides immunoprecipitated by R911 (Fig. 3B). Upon increased exposure of the autoradiographs, lower-molecular-weight polypeptides infrequently appeared in blots with both antibodies, ranging in molecular mass from 85 to 92 kDa. No proteins were recognized by T1E1 or R907.

**Antigenic identity of immunoprecipitated polypeptides.** We decided to analyze which polypeptides in the complex contained epitopes that were recognized by each antibody, 22B<sub>1</sub> or R911. <sup>125</sup>I-labeled initial bodies were immunoprecip-

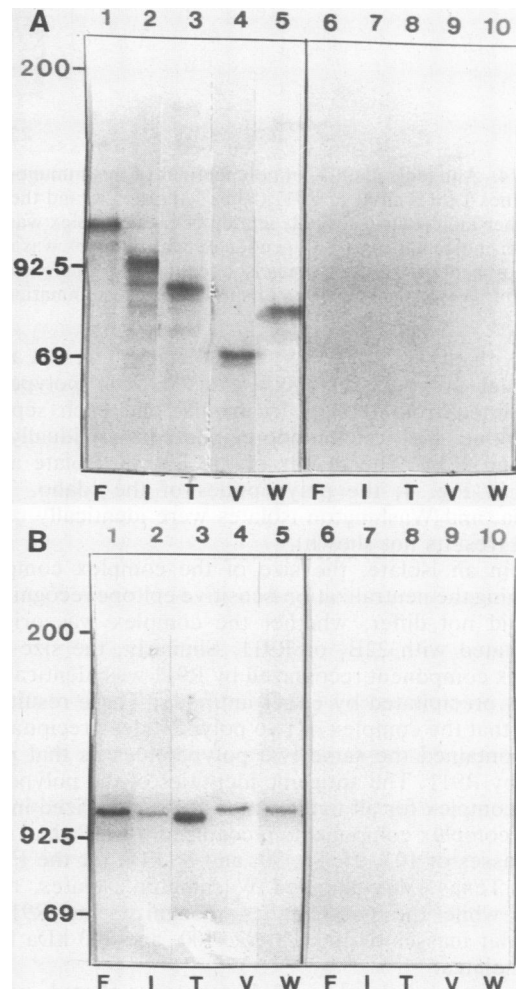


FIG. 3. Western blot analyses of *A. marginale* initial body proteins separated by SDS-polyacrylamide gel electrophoresis and probed with (A) monoclonal antibodies 22B<sub>1</sub> (lanes 1 through 5) or T1E1 (lanes 6 through 10), or (B) rabbit antisera R911 (lanes 1 through 5) or R907 (lanes 6 through 10), as described in Materials and Methods. F, I, T, V, and W represent the Florida, south Idaho, north Texas, Virginia, and Clarkston, Wash., isolates of *A. marginale*, respectively. The numbers to the left of the gel represent the positions of the molecular mass markers (in kilodaltons).

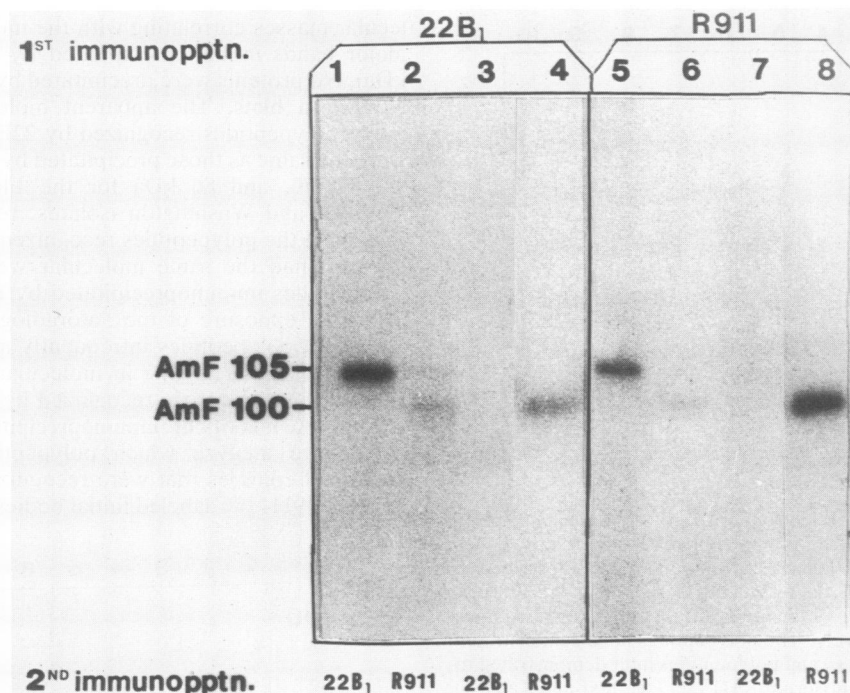


FIG. 4. Antigenic identity of polypeptides of the immunoprotective complex of *A. marginale*. The complex was immunoprecipitated with 22B<sub>1</sub> (lanes 1 through 4) or R911 (lanes 5 through 8) and the individual polypeptides were excised, as described in Materials and Methods. The higher-molecular-mass polypeptide of each complex was reprecipitated by either 22B<sub>1</sub> (lanes 1 and 5) or R911 (lanes 2 and 6). Similarly, the lower-molecular-mass polypeptide of each complex was reprecipitated by either 22B<sub>1</sub> (lanes 3 and 7) or R911 (lanes 4 and 8). The identity of the polypeptides of the Florida isolate of *A. marginale* is demonstrated above; the results for the south Idaho, north Texas, Virginia, and Clarkston, Wash., isolates are not shown but are summarized in Table 1. immunopptn., Immunoprecipitation.

itated with either 22B<sub>1</sub> or R911 and electrophoresed, and the individual polypeptides were excised. The polypeptides were eluted from the gel fragments, and each separated polypeptide was reimmunoprecipitated individually with R911 and 22B<sub>1</sub>. The results of the Florida isolate are depicted in Fig. 4; the polypeptides of the Idaho, Texas, Virginia, and Washington isolates were identically reprecipitated (results not shown).

Within an isolate, the size of the complex component containing the neutralization-sensitive epitope recognized by 22B<sub>1</sub> did not differ, whether the complex was originally precipitated with 22B<sub>1</sub> or R911. Similarly, the size of the complex component recognized by R911 was identical when initially precipitated by either antibody. These results confirmed that the complex of two polypeptides precipitated by 22B<sub>1</sub> contained the same two polypeptides as that precipitated by R911. The antigenic identities of the polypeptides in the complex for all five isolates are summarized in Table 1. The complex components recognized by 22B<sub>1</sub> had molecular masses of 105, 95, 89, 70, and 86 kDa for the Florida, Idaho, Texas, Virginia, and Washington isolates, respectively, while the polypeptides recognized by R911 had molecular masses of 100, 98, 97, 100, and 100 kDa for the same isolates.

**Structural relationship of immunoprecipitated polypeptides.** The polypeptides of the complex were individually examined by partial proteolysis to analyze structural polymorphisms between isolates (Fig. 5A and B). The 22B<sub>1</sub>-reactive polypeptides all exhibited cleavage peptides of apparent sizes of 58, 38.5, 27.5, 16.5, and 11.8 kDa. Isolate-specific peptide bands could be identified between 15 and 20 kDa in most isolates. The polypeptides recognized by R911 were cleaved into major fragments with approximate molec-

ular masses of 29, 23, and 10.8 kDa in all five isolates. In addition, several fragments between 16 and 19 kDa were present in every isolate, and a few isolate-specific peptides were visible between 32 and 45 kDa in most isolates.

## DISCUSSION

Despite severe losses and widespread regions enzootic for the causative rickettsia, *A. marginale*, effective immunoprophylaxis for anaplasmosis has not been developed. Immunization of cattle with a complex of two initial body surface polypeptides (MSP-1) induces protection against virulent *A. marginale* challenge (20). Comparison of the polypeptides of MSP-1 among isolates is essential to ensure that a subunit vaccine based on this complex will protect cattle against all the different isolates of *A. marginale* within a region.

The polypeptides of MSP-1 from the five isolates examined exhibit striking variations in molecular mass, differing by as much as 35 kDa between the Florida and Virginia isolates. Extensive size polymorphisms of proteins have been similarly discovered in other organisms, including the

TABLE 1. Antigenic identity of the polypeptides in the immunoprotective MSP-1 complex of *A. marginale*

Major complex component	Apparent molecular mass (kDa) in isolate <sup>a</sup> :				
	F	I	T	V	W
Recognized by 22B <sub>1</sub>	105	95	89	70	86
Recognized by R911	100	98	97	100	100

<sup>a</sup> Isolates: F, Florida; I, south Idaho; T, north Texas; V, Virginia; W, Clarkston, Washington.

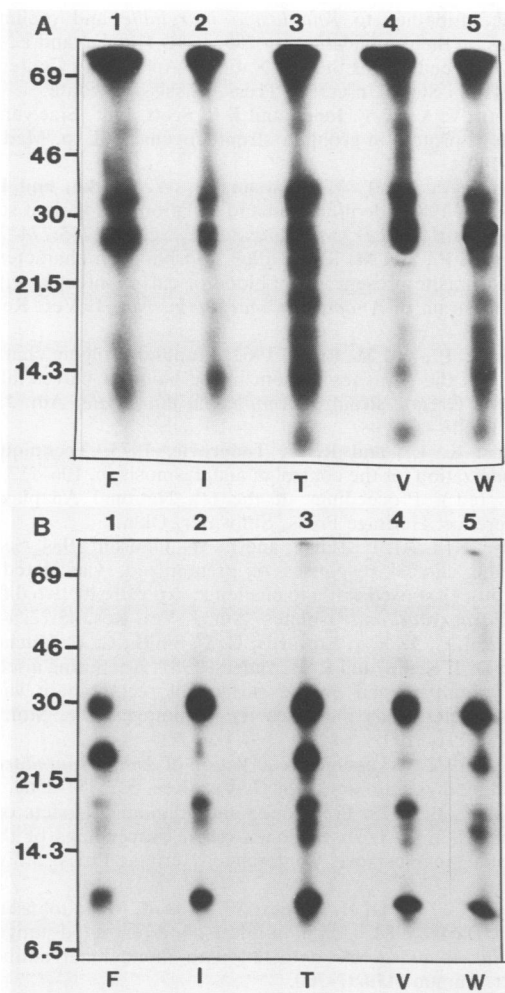


FIG. 5. Structural identity of polypeptides of the immunoprotective complex of *A. marginale*. Polypeptides were excised and partially digested as described in Materials and Methods. F, I, T, V, and W represent the Florida, south Idaho, north Texas, Virginia, and Clarkston, Wash., isolates of *A. marginale*, respectively. (A) 22B<sub>1</sub>-reactive polypeptides. Lanes: 1, AmF105; 2, AmI95; 3, AmT89; 4, AmV70; 5, AmW86. (B) R911-reactive polypeptides. Lanes: 1, AmF100; 2, AmI98; 3, AmT97; 4, AmV100; 5, AmW100. The numbers to the left represent the positions of the molecular mass markers (in kilodaltons).

M protein of group A streptococci (8), the variant-specific surface glycoprotein of *Trypanosoma vivax* (9), and the circumsporozoite (6, 30) and S-antigens (5) of *Plasmodium falciparum*. Considerable protein size differences among rickettsial species have been reported less frequently; only slight protein size variations were found between strains of *Rickettsia rickettsii* (1), although the 110-kDa protein of the Karp isolate of *Rickettsia tsutsugamushi* appears to vary by as much as 20 kDa in the Kato and Gilliam strains (19, 28).

Wide divergence among the isolate proteins could be critical to the application of the polypeptides of the Florida isolate MSP-1 as the foundation of a subunit vaccine. However, the Florida MSP-1 polypeptides have been demonstrated as capable of inducing protection not only against the Florida isolate of *A. marginale*, but also against a heterologous Washington (Okanogan) isolate (20). All of the isolates used in this study, as well as others from the United States, Israel, and Kenya, contain the neutralization-sensitive epi-

tope recognized by the monoclonal antibody 22B<sub>1</sub> (21a), supporting the possibility of an AmF105-based vaccine. The Florida polypeptides are the largest of all the isolates examined, suggesting potential determinants essential for protection have not been lost or deleted, as is possible with a vaccine based on the lower-molecular-weight polypeptides of the other isolates. Besides being one of the more virulent isolates (12, 23–25, 29), the Florida isolate is also the most widely cross-protective of all the isolates tested so far (13, 24, 29). This may imply that there may be more conserved determinants on the polypeptides of the Florida isolate.

One unusual feature of MSP-1 is that the polypeptides are integrally associated, despite the large size variations between isolates. The reimmunoprecipitations clearly demonstrate that the polypeptides precipitate as a complex, regardless of which polypeptide is initially recognized by the antibody. The polypeptides tend to dissociate during immunoprecipitation, probably due to the stringent washing conditions used (3). As a result, more of the polypeptide containing the epitope initially recognized is precipitated, as demonstrated by the greater intensity of this polypeptide band.

The MSP-1 appears to consist of just the two major polypeptides, although lower-molecular-weight polypeptides were inconsistently precipitated as well. Excision and reimmunoprecipitation of some of these fainter polypeptides confirmed these polypeptides as being either recognized by the rabbit antiserum, R911, or not precipitated at all (results not shown). These polypeptides may be breakdown products of the higher-molecular-weight proteins, which either occur naturally or result from handling during the procedures described above. Alternately, they may be unrelated proteins which are also complexed but dissociated to a greater degree by the washing steps of the immunoprecipitation protocol. Another possibility is that the smaller, R911-reactive polypeptides are the products of one of the other two to four copies of the gene for this protein (3); this seems less likely, as the polypeptides varied in size, even among identically treated immunoprecipitations of the same isolate, and there is presently no evidence that the other gene copies are expressed. Only one copy of the gene for the 22B<sub>1</sub>-reactive polypeptide has been detected by hybridization experiments (D. R. Allred, A. F. Barbet, G. H. Palmer, and T. C. McGuire, unpublished data).

Upon extremely long exposures of the autoradiographs of the 22B<sub>1</sub> immunoprecipitations or the Western blots using 22B<sub>1</sub>, some proteins of very high molecular mass became visible at approximately 150 to 225 kDa. The different sizes of these high-molecular-mass proteins correspond to the sizes of the major 22B<sub>1</sub>-reactive complex polypeptides; that is, the largest of these high-molecular-weight proteins is found in the Florida isolate, the next largest is found in Idaho, the smallest is found in Virginia, etc. These high-molecular-weight proteins may be precursors or dimers of the 22B<sub>1</sub>-reactive polypeptides, or, like the *N*-acetyl-D-glucosamine-containing bands seen in the precipitations of the M protein of *Streptococcus pyogenes* (8), may contain noncovalently attached glycolipids, other complexed proteins, or cell wall components.

The polypeptides in the MSP-1 complex appear to be generally homologous in structure. Despite size variations as large as 35 kDa, there are several conserved peptides between the 22B<sub>1</sub>-reactive polypeptides. Similarly, the pattern of fragments resulting from a digestion of the R911-reactive polypeptides is very similar among isolates, suggesting regions of structural homology. Identification of any role for



these conserved peptides in inducing protection may be important for determining the mechanisms of immunity and of size variation.

Proteins containing multiple repeats may migrate anomalously in SDS-polyacrylamide gels (14); therefore, apparent molecular mass differences observed here may not be exact. The best way to precisely determine structural differences in MSP-1 between isolates is to define the primary structure via the gene sequence. Currently, research is in progress to complete the DNA sequence for the AmF105 gene; data suggest that the AmF105 gene contains a region of 87 bases tandemly repeated seven times or more (A. F. Barbet, G. H. Palmer, T. M. Harkins, D. A. Allred, and T. C. McGuire, unpublished data), and like the circumsporozoite genes of *P. falciparum* (6, 30), the 22B<sub>1</sub>-reactive polypeptides in other isolates may differ in the length, sequence, or number of repeats.

Repeated sequences may be important in determining the rate and processes of variation. While the polypeptides have not been observed to alter in size during several passages in cattle, the exact rate of the recombination, deletion, or duplication events which may alter these proteins is not known. Similarly, the relationship of these repeated sequences and the protein heterogeneities described to the induction of protective immunity remains to be established. Defining conserved immunoprotective determinants and the mechanism of structural variation will be critical to the application of MSP-1 in a subunit vaccine for bovine anaplasmosis.

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