

## Immunization of Cattle with the MSP-1 Surface Protein Complex Induces Protection against a Structurally Variant *Anaplasma marginale* Isolate

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**The *Anaplasma marginale* surface protein complex MSP-1 of the Florida isolate is composed of a 105-kilodalton (kDa) polypeptide, which bears a neutralization-sensitive epitope, and a 100-kDa polypeptide. Antigenically similar polypeptides in the Okanogan, Wash. (Washington-O), isolate MSP-1 are 86 and 100 kDa, respectively. Immunization of cattle with Florida isolate MSP-1 induced antibody titers to both MSP-1 polypeptides and protected cattle against homologous and heterologous challenge.**

Rickettsial pathogens vary antigenically and structurally among strains and consequently may vary in their ability to induce cross-protection against heterologous strains (3, 5, 7, 8, 19). In bovine anaplasmosis, caused by the intraerythrocytic rickettsia *Anaplasma marginale*, cattle that have recovered from acute infection are protected against homologous challenge but are usually susceptible to infection with heterologous isolates (7, 8). Significantly, the Florida isolate of *A. marginale* appears to induce postinfection immunity against heterologous isolates (8, 17, 20). Consequently, we have used the Florida isolate to identify surface proteins for vaccine development (14). Immunization of cattle with a 100- to 105-kilodalton (kDa) surface protein complex, identified in the Florida isolate with neutralizing antibodies, induces protection against challenge with a homologous isolate of *A. marginale* (12). The ability of the 100- to 105-kDa surface complex, designated major surface protein-1 (MSP-1), to induce heterologous protection has not been tested.

The Florida isolate MSP-1 is composed of two noncovalently linked polypeptides of 105 and 100 kDa (2). The 105-kDa polypeptide (referred to in reference 2 as 105U) bears surface-exposed epitopes, including a neutralization-sensitive epitope conserved among *A. marginale* isolates (12, 13, 16). The 100-kDa polypeptide, previously referred to as 105L, also has surface-exposed epitopes (2). Although MSP-1 is conserved as a bimolecular complex in different *A. marginale* isolates, the molecular size of the polypeptide components varies markedly among isolates (11). The polypeptide bearing the conserved neutralization-sensitive epitope is 105 kDa in the Florida isolate MSP-1 complex and is 70 to 100 kDa in the complexes of the five isolates characterized to date (11). The second component of the MSP-1, 100 kDa in the Florida isolate, varies by approximately 3 kDa among the five isolates (11).

The Okanogan, Wash. (Washington-O), isolate differs antigenically, morphologically, and in protein composition from the Florida isolate (1, 6, 7, 9). Despite the antigenic differences, the neutralization-sensitive epitope on the MSP-1 is conserved (9, 12). Comparison of MSP-1 between the Florida and Washington-O isolates was done by using immunoprecipitation and immunoblotting with antibodies previously defined against each of the Florida MSP-1 polypep-

tides (2). Following radiolabeling of *A. marginale* proteins with [<sup>35</sup>S]methionine during short-term in vitro cultivation of each isolate (1), 10<sup>5</sup> cpm (acid precipitable) were reacted with either monoclonal antibody ANA 22B1 (specific for the 105-kDa polypeptide) or rabbit antibody R911 (specific for the 100 kDa polypeptide). Bound complexes were precipitated with protein A-bearing *Staphylococcus aureus*, and the specifically bound polypeptides were identified by polyacrylamide gel electrophoresis and fluorography. As previously demonstrated (11), immunoprecipitation of Florida isolate proteins with either monoclonal antibody ANA 22B1 (Fig. 1) or rabbit antibody R911 (data not shown) precipitated both the 105-kDa polypeptide and the 100-kDa polypeptide. The Washington-O isolate polypeptides precipitated by either antibody were 100-kDa and 86-kDa components (Fig. 1). Therefore, as expected, the bimolecular nature of MSP-1 is conserved in the Washington-O isolate.

The antigenic identity of each Washington-O MSP-1 polypeptide was determined by immunoblotting. Approximately 100 µg of solubilized whole-organism antigen (Washington-O isolate) per lane was electrophoresed in 5% polyacrylamide gels containing 4 M urea to separate the MSP-1 polypeptides. The antigens were electrophoretically transferred to 0.45-µm-pore-size nitrocellulose and reacted with either monoclonal antibody ANA 22B1 or rabbit antibody R911, and antibody binding was detected with <sup>125</sup>I-protein A. Monoclonal antibody 22B1, which binds the Florida isolate 105-kDa polypeptide, bound the 86-kDa component but not the 100-kDa component of the Washington-O MSP-1 (Fig. 2). Rabbit antibody R911, which recognizes the Florida isolate 100-kDa polypeptide, bound only to the 100-kDa component in the Washington-O MSP-1 (Fig. 2). Therefore, the polypeptide bearing the neutralization-sensitive epitope is approximately 19 kDa smaller in the Washington-O isolate than in the Florida isolate. In contrast, the size of the second polypeptide, shown to have minor size variation among other characterized isolates, is similar in both the Washington-O and Florida isolates.

The ability of MSP-1 to induce antibody to each polypeptide component and to induce cross-protective immunity was assessed by immunization of cattle with Florida isolate MSP-1. MSP-1 was purified from the Florida isolate *A. marginale* by using monoclonal immunoaffinity chromatography as previously described (12). Seronegative cattle were

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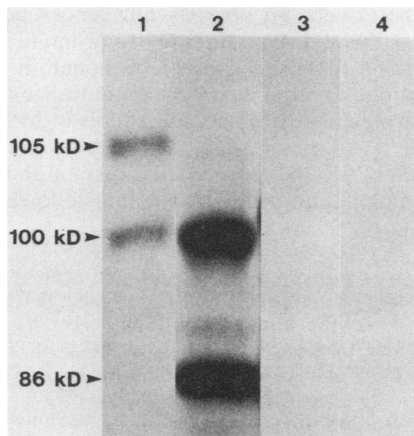


FIG. 1. Comparison of MSP-1 complexes between the Florida and Washington-O isolates of *A. marginale*. Organisms from each isolate were radiolabeled with [<sup>35</sup>S]methionine, detergent disrupted, and immunoprecipitated with monoclonal antibody ANA 22B1, which recognizes a conserved MSP-1 epitope. Immunoprecipitates were identified by polyacrylamide gel electrophoresis with fluorography. Florida isolate proteins were immunoprecipitated with monoclonal antibody ANA 22B1 (lane 1) or control monoclonal antibody TRYP 1E1 (lane 3). Washington-O isolate proteins were immunoprecipitated with ANA 22B1 (lane 2) or TRYP 1E1 (lane 4). Arrows in the left margin designate the apparent molecular masses, in kilodaltons, of the polypeptides.

immunized with 50 µg of MSP-1 emulsified in complete Freund adjuvant for the initial immunization and in incomplete adjuvant for three subsequent immunizations at 3-week intervals. Control seronegative cattle were immunized with 50 µg of ovalbumin emulsified in identical adjuvants and boosted on an identical schedule. Following the last immunization, antibody titers to each MSP-1 component were determined by endpoint titration by using serial dilutions of sera in immunoblots (4, 15). All cattle immunized with

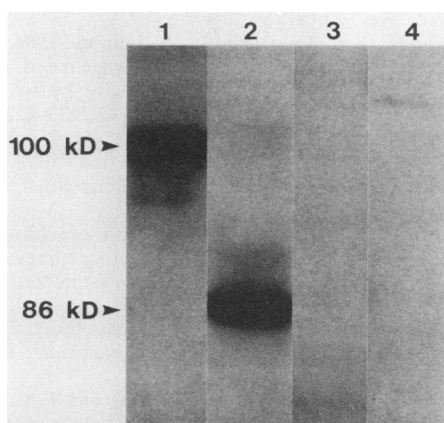


FIG. 2. Antigenic identity of MSP-1 polypeptides in the Washington-O isolate of *A. marginale*. Washington-O isolate antigens were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Antibody binding was detected by reaction with <sup>125</sup>I-protein A, followed by autoradiography. Lanes: 1, rabbit antibody R911 (defined against the Florida isolate 100-kDa polypeptide); 2, monoclonal antibody ANA 22B1 (defined against the Florida isolate 105-kDa polypeptide); 3, control rabbit antibody (against *E. coli*); 4, control monoclonal antibody TRYP 1E1.

TABLE 1. Protection of cattle immunized with Florida isolate MSP-1 against challenge with the *A. marginale* Florida isolate

Immunogen and animal no.	Titer of antibody to:		DPC to 1% rickettsemia <sup>a</sup>	Peak rickettsemia (%)	Low PCV (%) <sup>b</sup>
	105-kDa polypeptide	100-kDa polypeptide			
<b>MSP-1</b>					
B209	256,000	128,000	23	4.5	22.5
B211	256,000	128,000	Uninfected	0.0	29
B218	256,000	256,000	26	2.2	25.5
B226	128,000	64,000	Uninfected	0.0	29
B227	128,000	128,000	26	4.0	24.5
<b>Ovalbumin</b>					
B219	— <sup>c</sup>	—	16	25.6	13
B220	—	—	18	8.1	23
B222	—	—	25	9.7	22
B224	—	—	16	8.9	23.5
B225	—	—	15	10.1	26

<sup>a</sup> Rickettsemia was determined by daily microscopic examination of Wright-stained blood smears for 75 DPC.

<sup>b</sup> PCV, Packed-cell volume.

<sup>c</sup> —, Sera from all ovalbumin-immunized cattle were unreactive with *A. marginale* polypeptides, including MSP-1, at the lowest dilution tested, 1:500.

MSP-1 developed antibody to both polypeptide components (Tables 1 and 2). On the basis of responses of all MSP-1-immunized cattle, there was no significant difference ( $P = 0.68$ ) in the titers of the MSP-1 components, as evaluated with the paired *t*-test (18). The range of titers was from 1:64,000 to 1:256,000 against each polypeptide. Sera from all ovalbumin-immunized cattle were unreactive against *A. marginale* antigens, including both MSP-1 polypeptides (Tables 1 and 2).

Five MSP-1-immunized cattle and five ovalbumin-immunized cattle were challenged by intramuscular inoculation of 10<sup>10</sup> Florida isolate-infected erythrocytes derived from cryopreserved stabilate (homologous challenge). All five ovalbumin-immunized cattle developed microscopically detectable rickettsemia and had 1.0% infected erythrocytes in a mean of 18 days postchallenge (DPC) (Table 1). Cattle in this

TABLE 2. Protection of cattle immunized with Florida isolate MSP-1 against challenge with the *A. marginale* Washington-O isolate

Immunogen and animal no.	Titer of antibody to:		DPC to 1% rickettsemia <sup>a</sup>	Peak rickettsemia (%)	Low PCV (%) <sup>b</sup>
	105-kDa polypeptide	100-kDa polypeptide			
<b>MSP-1</b>					
B187	64,000	128,000	Uninfected	0.0	36
B194	64,000	128,000	Uninfected	0.0	35
B196	128,000	128,000	Uninfected	0.0	33
B203	64,000	64,000	Uninfected	0.0	35
B208	128,000	128,000	Uninfected	0.0	33
<b>Ovalbumin</b>					
B189	— <sup>c</sup>	—	25	4.8	27.5
B195	—	—	25	3.6	25
B197	—	—	29	3.0	27.5
B207	—	—	22	5.5	26.5
B217	—	—	25	4.6	26

<sup>a</sup> Rickettsemia was determined by daily microscopic examination of Wright-stained blood smears for 75 DPC.

<sup>b</sup> PCV, Packed-cell volume.

<sup>c</sup> —, Sera from all ovalbumin-immunized cattle were unreactive with *A. marginale* polypeptides, including MSP-1, at the lowest dilution tested, 1:500.

control immunized group developed a mean peak of 12.5% infected erythrocytes. The mean low packed-cell volume, a measure of the anemia characteristic of acute anaplasmosis, was 21.5%. In contrast, two of the five MSP-1-immunized cattle did not develop detectable rickettsemia (Table 1). The three MSP-1 vaccinates that were infected upon challenge were partially protected as determined by the significant prolongation of the prepatent period (DPC to 1% infected erythrocytes) as compared with the ovalbumin-immunized cattle ( $P < 0.025$ , pooled  $t$ -test). As a group, the MSP-1-immunized cattle had significantly lower peak rickettsemia (mean of 2.1%) ( $P < 0.025$ ) and were significantly less anemic (mean packed cell volume of 26%) ( $P = 0.055$ ) than ovalbumin-immunized cattle.

Cross-protective immunity induced by MSP-1 immunization was tested by heterologous challenge with intramuscular inoculation of  $10^{10}$  Washington-O isolate-infected erythrocytes derived from cryopreserved stabilate. The Washington-O isolate was less virulent than the Florida isolate, as judged by challenge infections in the control cattle (Table 2). The Washington-O isolate caused significantly less severe disease on the basis of all three parameters: DPC to 1.0% infection, peak rickettsemia, and minimum packed-cell volume ( $P < 0.03$  for all parameters by the pooled  $t$ -test) (18). All five ovalbumin-immunized cattle challenged with Washington-O isolate *A. marginale* developed microscopically detectable rickettsemia in a mean of 25 DPC (Table 2). The ovalbumin-immunized cattle reached a mean peak of 4.3% infected erythrocytes and a mean low packed-cell volume of 26.5%. In contrast to the challenge infections in the ovalbumin-immunized cattle, none of the five MSP-1-immunized cattle challenged with the Washington-O isolate developed microscopically detectable infection (Table 2). The packed cell volumes were unchanged from prechallenge levels (data not shown).

Protection against antigenically and structurally variant isolates of *A. marginale* is a primary requirement for development of an improved vaccine (10). The identification of isolates structurally variant in the MSP-1 raised the possibility that, despite conservation of a neutralization-sensitive epitope, the MSP-1 may not induce significant protection against challenge with a heterologous isolate (11). The demonstration that immunization of cattle with the Florida isolate MSP-1 induced complete protection against challenge with the Washington-O isolate indicates that MSP-1 epitopes relevant to cross-protection are conserved. Determination of the extent of conservation among isolates and the ability of MSP-1 immunization to induce widely cross-protective immunity requires challenge with additional isolates. The contribution of each MSP-1 polypeptide to the protective immunity is unknown. Both polypeptides have surface-exposed epitopes and are immunogenic when presented in the MSP-1 complex. The requirement for each polypeptide in a vaccine will be determined by using individual purified recombinant-derived polypeptides for immunization.

The complete protection afforded the five calves challenged with the Washington-O isolate versus the complete protection in only two Florida isolate-challenged calves is probably the result of the Washington-O being significantly less virulent. Complete protection of only a proportion of the MSP-1-immunized cattle following Florida isolate challenge was similar to the results of immunization experiments previously reported (12). The basis for the differences in protection is not clear; there were no significant differences in antibody responses to either MSP-1 component between completely protected cattle and partially protected cattle.

Similarly, there were no obvious differences between the two groups in the ability of antibody to promote *A. marginale* opsonization (data not shown). Although in vitro incubation of antibody with *A. marginale* neutralizes infectivity (12, 14), the mechanism of neutralization in MSP-1-immunized cattle is unknown. Identification of the basis for neutralization in vivo is needed to understand the difference between complete and partial protection following virulent challenge.

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