Monoclonal Antibody Binding to a Surface-Exposed Epitope on Cowdria ruminantium That Is Conserved among Eight Strains

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Monoclonal antibodies (MAB) binding to Cowdria ruminantium elementary bodies (EB) were identified by enzyme-linked immunosorbent assay, and surface binding of one MAB (446.15) to intact EB was determined by immunofluorescence, immunogold labeling, and transmission electron microscopy. MAB 446.15 bound an antigen of approximately 43 kDa in immunoblots of eight geographically distinct strains. The MAb did not react with Ehrlichia canis antigens or uninfected bovine endothelial cell lysate and may be useful in diagnostic assays and vaccine development.

Heartwater is an often fatal tick-borne disease of domestic and wild ruminants caused by Cowdria ruminantium, and it remains a major constraint to efficient livestock production in sub-Saharan Africa (22, 30). In some sub-Saharan African countries, Amblyomma variegatum is the most important vector (30), while in Zimbabwe, Amblyomma hebraeum is most important. Heartwater has been detected in the Caribbean islands and is a threat to the North and South America, which have potential tick vectors (1, 2, 31). Detection of C. ruminantium depends on diagnostic tests; however, the current tests lack specificity, as evidenced by the detection of antibodies in sera from regions where heartwater does not occur (15). Some of the lack of specificity is caused by cross-reacting antigens in Ehrlichia species (7). In addition, the development of effective vaccines is constrained by a lack of knowledge of both the required target antigens and immunologic effector mechanisms (25–27, 29). The observation that protective immunity can be induced in some goats and sheep with culture-attenuated or inactivated organisms (12, 17, 19) indicates that induction of protective immune responses in ruminants by using subunits is possible. Toward this end, a protein that can induce a protective response against C. ruminantium challenge in some mice was identified using a DNA vaccine vector (21). This report describes a monoclonal antibody (MAB) that reacts with a surface-exposed epitope on C. ruminantium elementary bodies (EB) that is conserved among eight disease-causing strains of the organism. The use of MAB 446.15 for surface protein identification may be useful in the development of more specific diagnostic reagents for heartwater, and the protein could be evaluated as a component of a subunit vaccine.

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and reacted with 100 μl of fluorescein-conjugated rabbit antibody to mouse immunoglobulins (Organon Teknika, Durham, N.C.) diluted 1:100 in PBS. The mixture was incubated for 30 min at room temperature, washed three times with PBS, and examined by fluorescence microscopy. The specificity of the immunofluorescence was controlled by reacting EB with an isotype control (immunoglobulin M [IgM]), MAb WM25. Seven IgM MAbs (320.1.8, 442.3.21, 443.3.2, 446.15.22, 447.3.26, 447.3.15, and 447.3.24) binding to *C. ruminantium* EB were identified by IFA. All the MAbs caused clumping of the EB, indicating that they were reacting with epitopes on the EB surface (Fig. 1A). The isotype control MAb WM25 did not bind to EB (Fig. 1B). Evan’s blue (1%) was used in IFAs as the quenching agent. MAb 446.15.22 (designated 446.15) was selected for more detailed evaluation.

**Demonstration of MAb 446.15 binding to the surface of EB by immunogold electron microscopy.** To verify that MAb 446.15 was binding to the EB surface, intact EB were reacted with MAb followed by antibodies to mouse IgM that were conjugated to gold particles. These EB were then embedded, and ultrathin sections were examined by transmission electron microscopy. Intact EB prepared as described above were incubated for 30 min in 1:10 and 1:100 dilutions of MAb 446.15, isotype control MAb WM25, or PBS. The suspension was washed three times with PBS containing 1% bovine serum albumin before incubation in gold conjugated to goat anti-mouse IgM antibodies (Biocell, Cardiff, United Kingdom) for 30 min. EB were then washed twice in PBS and fixed at room temperature by adding equal volumes of 4% glutaraldehyde and 0.4% picric acid in 0.2 M sodium cacodylate buffer (pH 984).
Identification of the antigen bound by MAb 446.15 and conservation of the recognized epitope in *C. ruminantium* strains. To identify the antigen recognized by MAb 446.15 and to determine if the epitope is conserved on molecules of similar size, immunoblotting was done. Antigens from culture-strains were Crystal Spring (lane 1), Welgevonden (lane 2), Ball (lane 3), Umbanein (lane 4), Nigeria (lane 5), Highway (lane 6), Zwimba (lane 7), and Plum Tree (lane 8). MAb 446.15 did not react with *E. canis* antigens (lane 9) or uninfected bovine endothelial cell lysates (lane 10). Values on the left are molecular masses (in kilodaltons).

7.3) for 3 min before washing three more times and postfixing in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h. Following another three washes, EB were block stained with aqueous 2% uranyl acetate for 4 h and processed with graded acetone into an Epon-araldite resin mix. Ultrathin sections (50 to 70 nm thick) were cut, mounted on copper grids, counterstained with Reynold’s lead citrate, and viewed in a Zeiss (Oberkochen, Germany) EMICA. The presence of gold particles on the outer surface of EB further demonstrated that MAb 446.15 bound a surface-exposed epitope on these organisms (Fig. 2A). Similar results were obtained with MAbs 442.3.21 and 443.3.2 (data not shown). There were no significant amounts of gold particles in the control preparations incubated first with isotype control MAb WM25 or PBS followed by the gold-labeled second antibody (Fig. 2B). Immunogold staining for localizing antibody binding to surface antigens has been used previously with *Chlamydia trachomatis*, *C. ruminantium*, and *Babesia bigemina* (10, 13, 24).
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