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# Immunogenic B-Cell Epitopes of *Babesia bovis* Rhoptry-Associated Protein 1 Are Distinct from Sequences Conserved between Species

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***Babesia bovis*** **merozoite apical membrane polypeptide Bv60 was found to be rhoptry associated by immunoelectron microscopy and was redesignated rhoptry-associated protein 1 (RAP-1). The N-terminal 300 amino acids of RAP-1 have a high level of sequence similarity to the same N-terminal region of p58, its homolog from *Babesia bigemina*. However, the interspecies conserved sequences did not include RAP-1 surface-exposed B-cell epitopes as defined by monoclonal antibodies. Furthermore, neither heterologous *B. bigemina* immune nor monospecific anti-p58 bovine serum binds to whole RAP-1, indicating that the major B-cell epitopes recognized by these sera are also not encoded by the conserved sequences. Truncated RAP-1, expressed by a subclone encoding the N-terminal 235 amino acids, is weakly bound by antibodies in heterologous sera. A peptide representing the longest conserved amino acid sequence (amino acids 121 to 134) in this region is also weakly bound by antibodies in immune bovine sera, and rabbit antibodies induced by and strongly reactive with the peptide alone fail to bind native or denatured RAP-1. Thus, although the conserved region may contain one or more poorly immunogenic B-cell epitopes, these epitopes are inaccessible to antibody in whole RAP-1. The results indicate that the major immunogenic B-cell epitopes of RAP-1, including surface-accessible epitopes bound by monoclonal antibodies, are distinct from the conserved sequences representing putative functional domains.**

Cattle that have recovered from acute infection with the hemoprotozoan *Babesia bovis* or *Babesia bigemina* are immune (7). This immunity is mediated, in part, by antibody directed against the merozoite surface (8). Thus, our approach for babesial vaccine development includes the identification of conserved B-cell epitopes on proteins that are exposed on the merozoite surface. In *Babesia* spp. and related hemoprotozoa, both merozoite outer membrane and apical complex polypeptides have surface-exposed epitopes and are involved in attachment to and invasion of host erythrocytes (18). The accessible regions of these polypeptides that mediate the functions necessary for attachment and invasion are targets for antibody-based neutralization.

*B. bovis* Bv60 is an apical membrane protein with surface-exposed epitopes on the extracellular merozoite (4, 19). The first 300 amino acids (aa) of Bv60 have a high level of sequence identity and similarity to p58, its homolog from *B. bigemina* (13, 22, 23). In addition, both gene products have significant sequence similarity to other apical complex polypeptides of related genera (13, 22, 23). The strict conservation of numerous short oligopeptide motifs between species, including a stretch of 14 aa, suggests that the conserved regions perform a critical function (22). We hypothesize that antibody directed against B-cell epitopes encoded by sequences in the conserved domains will contribute significantly to merozoite neutralization.

In this study, we localized Bv60 (redesignated RAP-1, rhoptry-associated protein 1) to the *B. bovis* rhoptry by immunoelectron microscopy. We then addressed whether the interspecies conserved oligopeptide motifs in RAP-1 included B-cell epitopes and whether they were accessible to antibody in native RAP-1. To investigate the relationship between the conserved oligopeptides and B-cell epitopes, sequences encoding surface-exposed RAP-1 B-cell epitopes were mapped by using monoclonal antibodies (MAbs) and compared with conserved sequences. In addition, the relationship of B-cell epitopes recognized by antibodies in immune bovine sera to conserved and divergent regions was established.

## MATERIALS AND METHODS

**Parasites.** The biologically cloned strains of *B. bovis* (Mo7) and *B. bigemina* (JG-29) were obtained by limiting dilution of continuously cultured Mexico isolates as previously described (4, 5, 13). All strains were maintained as cryopreserved stabilates in liquid nitrogen (15). The organisms were grown in long-term microaerophilous stationary-phase culture by previously described techniques (6).

**Antibodies.** MAbs BABB75A4, MBOC79B2, 23/53.156, and 23/38.120, reactive with merozoite surface-exposed and strain-common epitopes on *B. bovis* RAP-1, and MAb 14/16.1.7, reactive with a *B. bigemina* merozoite surface-exposed and strain-common epitope on p58, were developed against the Mexico strains of *B. bovis* and *B. bigemina*, respectively, and have been previously described (18, 19). MAb ANA22B1, reactive with surface polypeptide MSP-1a of *Anaplasma marginale*, or MAb 23/3.16.45, reactive with the *B. bovis* 42-kDa major merozoite surface protein, were used as negative controls (5, 12, 19). Polyclonal immune sera were from cattle recovered from infection with Mexico strain *B. bovis* (serum B102) or *B. bigemina* (serum B227) and shown to be protected against subsequent homologous challenge (9). Monospecific bovine antisera to *B. bigemina* p58 (B279, B277, B273, and B274) have been previously described (11). Sera from uninfected cattle (preimmune sera B102 and B258) were used as negative controls. Rabbit

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antibodies to the 14-mer synthetic peptide PLSLPNPY QLDAAF coupled to keyhole limpet hemocyanin with either glutaraldehyde or carbodiimide (2) were prepared by biweekly immunization with 1 mg of coupled antigen in saponin adjuvant for a total of four immunizations (2). Rabbit antisera against the conjugated 14-mer were tested for reactivity with the identical peptide (coupled to bovine serum albumin with glutaraldehyde) in an enzyme-linked immunosorbent assay (ELISA) (1), with native RAP-1 by Western blot (immunoblot) (5) using enhanced chemiluminescence detection per the manufacturers' protocol (Amersham Corporation, Arlington Heights, Ill.), and with the *B. bovis* merozoite surface by immunofluorescence (10).

**Immunoelectron microscopy.** *B. bovis* merozoites were harvested by differential centrifugation from microaerophilous stationary-phase culture (6). Free merozoites were fixed with 1% glutaraldehyde in 0.1 M phosphate buffer for 1 h at room temperature, washed in phosphate-buffered saline (PBS), dehydrated, and infiltrated with LR-white. Thin sections were mounted on nickel grids, etched with $NaIO_4$ saturated solution, washed 3 times with distilled water, and incubated on a drop of PBS buffer containing 5% goat serum for 15 min. The grids were then transferred to a drop of MAb BABB75, negative control MAb ANA22B1 (50 μg/ml in PBS–1% goat serum), rabbit antiserum to recombinant RAP-1 (rRAP-1) (23), or rabbit preimmune serum (diluted 1:50 in PBS–1% goat serum) and incubated overnight at 4°C. The grids were then washed 3 times in PBS–1% goat serum and incubated with either anti-mouse or anti-rabbit immunoglobulin G-gold complex (10-nm diameter; Amersham) for 1 h at room temperature. The grids were then washed in PBS, postfixed, stained with 7% uranyl acetate and lead citrate, and observed in a Hitachi 600 electron microscope.

**B-cell epitope mapping.** RAP-1 B-cell epitopes were first regionally localized by MAb reactivity with rRAP-1 subclones. Epitopes were then further defined by using synthetic peptides.

**(i) Recombinant plasmids.** Plasmid pBv60 contains a 1.9-kb insert in pBluescript encoding the complete *B. bovis* 60-kDa RAP-1 protein (23). The RAP-1 sequence has been previously published (23) (GenBank accession number M38218). Digestion with *Eco*RV resulted in a 3.8-kb segment, designated F1, that contained the first 825 bp of the 5′ region of pBv60. The second *Eco*RV restriction fragment, F2, contained the remaining 1,165 bp of pBv60. Both segments were purified by electroelution after 0.8% agarose gel electrophoresis (20). Fragment F1, encoding the first 235 amino acids of the amino terminus of pBv60, was religated with T4 ligase. F2, encoding the 330 amino acids of the carboxyl end of pBv60, was subcloned by ligation into the *Pst*I site of pBluescript after being blunt ended with T4 DNA polymerase. Plasmids F3 to F7 were produced by unidirectional deletion of pBv60 or F1 with the exonuclease III-mung bean nuclease system (Stratagene, La Jolla, Calif.) from either the T3 or the T7 promoter side. Recombinant *Escherichia coli*, XL-1 Blue transformed with plasmid subclones, was immunoscreened with MAbs as previously described (23). Positive colonies were purified, and plasmid DNA was sequenced by using T3, T7, or sequentially derived synthetic primers that initiated dideoxy chain reactions (21) (Sequenase Version 2 Kit; U.S. Biochemical Co., Cleveland, Ohio).

**(ii) Immunoprecipitations.** Recombinant polypeptides were labeled with [$^{35}$S]methionine by in vitro transcription and translation of plasmid subclones with a prokaryotic DNA-directed translation kit (Amersham). *B. bovis* or *B. bigemina* merozoite proteins were metabolically labeled with [$^{35}$S]methionine during in vitro culture (4, 10, 17). Radiolabeled antigen preparations were ultracentrifuged at 135,000 × *g* and sonicated prior to immunoprecipitation as previously described (4, 10, 17). Immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography as described previously (17).

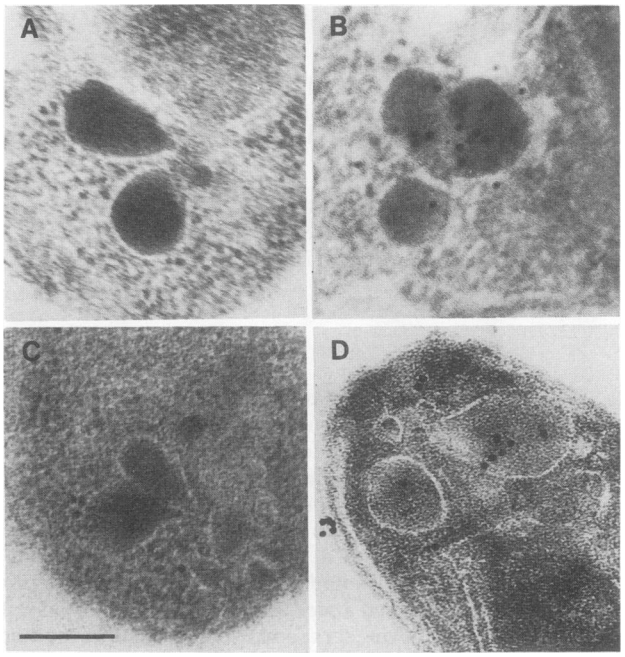


FIG. 1. Cellular localization of Bv60: (A) Reactivity with unrelated MAb; (B) reactivity with anti-Bv60 MAb BABB75; (C) reactivity with preimmune rabbit serum; (D) reactivity with rabbit anti-rBv60 serum. Bar, 1 μm.

**(iii) Synthetic peptides and peptide screening.** Synthetic peptides were prepared by using solid-phase FMOC chemistry in an Applied Biosystems 431-A Peptide Synthesizer according to the manufacturer's protocol. Reactivity of synthetic peptides with the MAbs was tested by immunoblotting on nitrocellulose filters and ELISA as described previously (1). ELISA results were considered positive when the optical density at 460 nm was threefold higher than that of negative controls.

**Sequence analysis.** Computer analysis of secondary structure was done using the Genetics Computer Group package, version 7.1 (3), on a VAX 11/785 computer.

## RESULTS

**Localization of Bv60 to the rhoptry.** The polar distribution of Bv60 in intraerythrocytic *B. bovis* merozoites and the punctuate surface exposure on free live merozoites suggested that Bv60 was present in the apical complex (4, 16, 19). To confirm the localization of Bv60 in apical organelles, glutaraldehyde-fixed *B. bovis* merozoites were incubated sequentially with monoclonal or monospecific antibodies and gold-labeled second antibodies. Bound immunogold complexes were detected by transmission electron microscopy. Specifically bound antibodies were found on rhoptries (Fig. 1B and D), on the merozoite surface in a focal pattern (Fig. 1D), and on erythrocyte stroma (data not shown). No

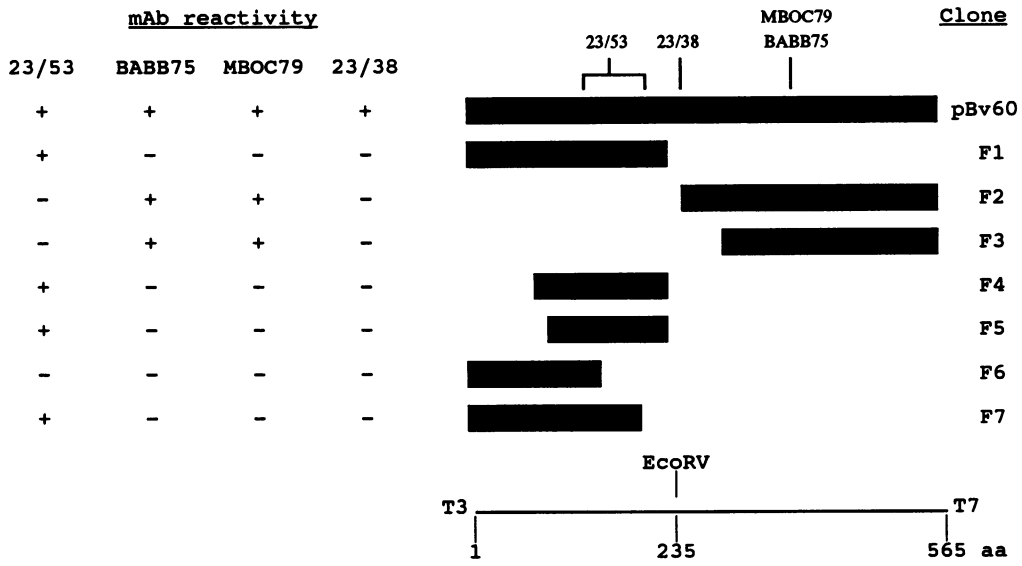


FIG. 2. Structure of pBv60 deletion clones F1 to F7 and their reactivities with MAbs 23/53.156, 23/38.120, BABB75A4, and MBOC79. The positions of the T3 and T7 promoters and the *Eco*RV site are indicated in the lower right.

gold label was associated with the parasites when control MAbs or preimmune rabbit serum was used under the same conditions (Fig. 1A and C). On the basis of its subcellular localization, Bv60 was redesignated RAP-1.

**RAP-1 B-cell epitopes bound by surface-reactive MAbs.** To map surface-exposed B-cell epitopes, radiolabeled in vitro transcription and translation products from three derivative subclones of pBv60, designated F1, F2, and F3 (Fig. 2), were immunoprecipitated with surface-reactive MAbs 23/53.156, 23/38.120, BABB75A4, and MBOC79. Subclone F1 encodes aa 1 to 235, F2 encodes aa 235 to 565, and F3 encodes aa 273 to 565. Following regional localization of B-cell epitopes to sequences encoded by F1, F2, or F3 subclones, MAb reactivities with additional derivative subclones (F4, aa 28 to 235; F5, aa 58 to 235; F6, aa 1 to 145; and F7, aa 1 to 218) and synthetic peptides spanning these regions were used to more precisely define epitopes. Results for each MAb are presented below.

**(i) Surface-exposed B-cell epitope recognized by MAbs MBOC79 and BABB75.** MAbs MBOC79 and BABB75, previously shown to bind the same or a similar surface epitope (16), immunoprecipitated in vitro-translated products of the predicted molecular size from plasmids F2 (aa 235 to 565) and F3 (aa 273 to 565) but did not specifically immunoprecipitate any F1 products (Fig. 2 and 3A). Thus, the epitope recognized by these two MAbs was between aa 236 and 565. Synthetic peptides P8 and P9, with overlapping amino acid sequences that correspond to a sequence exactly duplicated twice in RAP-1 (in the region between aa 372 and 408) (Table 1), reacted in ELISA with MAbs BABB75 and MBOC79, indicating that the sequence PTKEFFREA (aa 386 to 396) contains a surface-exposed B-cell epitope. The peptides were also reactive with these MAbs by immunoblot and were able to inhibit the binding of MAbs to rRAP-1 (data not shown).

**(ii) Surface-exposed B-cell epitope recognized by MAb 23/38.120.** MAb 23/38.120 did not immunoprecipitate any in vitro-translated product from plasmids F1, F2, and F3 but immunoprecipitated the intact native and rRAP-1 (Fig. 2 and 3A), suggesting that the epitope recognized by this MAb was encoded by the region contained in the *Eco*RV restriction site. Peptides P6 and P7, encoded by sequences overlapping the internal *Eco*RV site (Table 1), reacted in ELISA exclusively with MAb 23/38.120, indicating that the B-cell epitope was contained within the peptide TQLTSSYEDYML (aa 254 to 265), common to both P6 and P7.

**(iii) Surface-exposed B-cell epitope recognized by MAb 23/53.156.** MAb 23/53.156 specifically immunoprecipitated in vitro translation products of the predicted molecular size from plasmid F1 (aa 1 to 235) but did not specifically immunoprecipitate any F2 and F3 products (Fig. 2 and 3A). To further localize the epitope, plasmids F4 to F7 were prepared by unidirectional deletion of F1 with exonuclease III (Fig. 2). Bacteria containing plasmids F4, F5, and F7, but not F6, expressed an epitope specifically recognized by MAb 23/53.156 in colony lift assays (data not shown). MAb reactivity was confirmed by the immunoprecipitation of in vitro transcription and translation products of plasmid F7 (aa 1 to 218) but not F6 (Fig. 3B). Sequence present in plasmid F7, but not in F6, localized the epitope to aa 145 to 218. However, none of the synthetic peptides (P1, P2, P3, and P4) overlapping this entire region reacted with MAb 23/53.156 in immunoblots or ELISA (Table 1).

**(iv) Localization of MAb-reactive surface-exposed B-cell epitopes in relation to interspecies conserved domains of RAP-1.** Analysis of hydrophobicity profiles (data not shown) indicated that the MAb-reactive surface-exposed B-cell epitopes are located in areas of predicted high antigenicity. None of the surface-exposed B-cell epitopes mapped to the region containing the 14-residue oligopeptide (aa 121 to 134) strictly conserved between RAP-1 and *B. bigemina* p58, the region containing four strictly conserved cysteines (aa 80 to 105), or other shorter oligopeptides present in the highly conserved amino-terminal 300-amino-acid block.

**RAP-1 B-cell epitopes encoded by conserved sequences. (i) Reactivity of RAP-1 and p58 mAbs.** RAP-1 surface-reactive MAbs 23/53.156, 23/38.120, MBOC79, and BABB75 were unreactive with any *B. bigemina* metabolically labeled protein in immunoprecipitation (Fig. 4) and were unreactive by immunofluorescence (data not shown), confirming a lack of

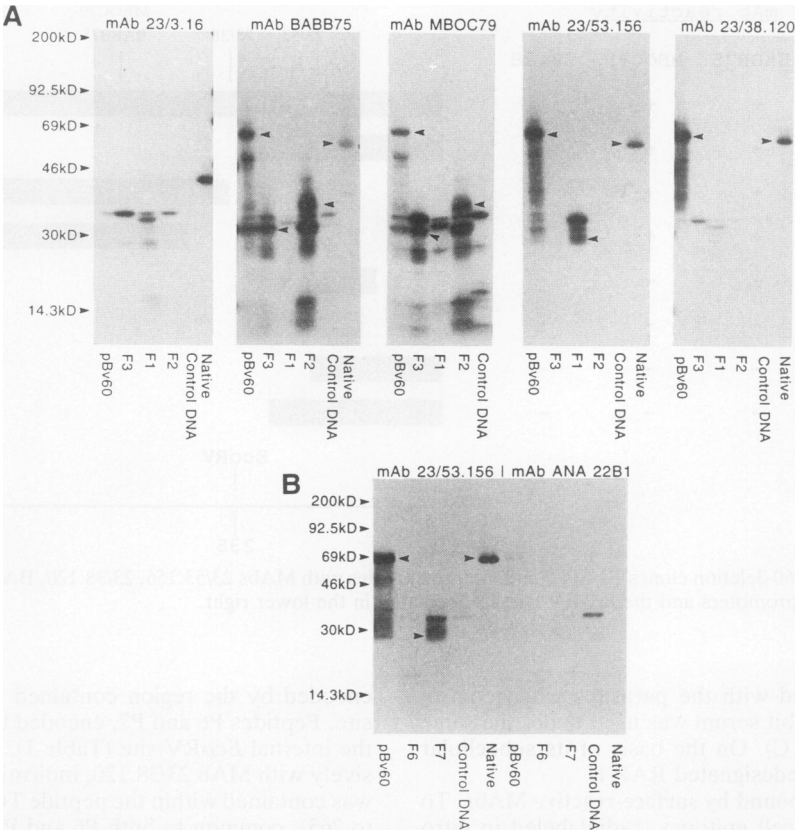


FIG. 3. (A) Immunoprecipitation of [$^{35}$S]methionine-labeled products of pBv60 deletion mutants F1, F2, and F3, control DNA, and [$^{35}$S]methionine-labeled native RAP-1 with negative control MAb 23/3.16 and MAbs BABB75, MBOC79, 23/53.156, and 23/38.120. Molecular size markers are indicated on the left. Arrowheads designate polypeptides of the predicted molecular size specifically immunoprecipitated by each MAb as compared with negative controls. (B) Immunoprecipitation of [$^{35}$S]methionine-labeled in vitro translation products of plasmids F6 and F7 with MAb 23/53.156 and negative control MAb ANA22B1. Molecular size markers are indicated on the left. Arrowheads designate polypeptides of the predicted molecular size specifically immunoprecipitated by each MAb as compared with negative controls.

interspecies conservation of these three epitopes (Fig. 4). In addition, MAb 14/16.1.7, reactive with *B. bigemina* p58, did not immunoprecipitate any *B. bovis* metabolically labeled protein (Fig. 4) or cross-react by immunofluorescence (data not shown), indicating that the epitope bound by this MAb was also not encoded by conserved sequences.

TABLE 1. Amino-acid sequence, position, and MAb reactivity of RAP-1 synthetic peptides P1 to P9

| Peptide | Position[a] | Sequence | MAb reactivity |
|---|---|---|---|
| P1 | 146–169 | KNSVKREWLRFRNGANHGDYHYFV | None |
| P2 | 163–187 | GDYHYRVTGLLNNNVVHEEGTTDVE | None |
| P3 | 178–202 | VHEGTTDVEYLVNKVLYMATMNYK | None |
| P4 | 193–219 | VLYMATMNYKTYLTVNSMNAKFFNRFS | None |
| P5 | 236–252 | IIRWNVPEDFEERSIER | None |
| P6 | 241–262 | VPEDFEERSIERITQLTSSYEDYML[b] | 23/38.120 |
| P7 | 254–273 | TQLTSSYEDYMLTQIPTLSK[b] | 23/38.120 |
| P8 | 372–394 | PQVTKHFFDENIGQPTKEFFREA[c] | MBOC79, BABB75 |
| P9 | 386–408 | PTKEFFREAPQATKHFLDENIGA[c] | MBOC79, BABB75 |

[a] Position of amino acid residues in RAP-1.
[b] Underlined sequence is common to P6 and P7.
[c] Underlined sequence is common to P8 and P9.

**(ii) Reactivity of antibodies in bovine immune sera.** Bovine *B. bovis* immune serum (B102) specifically immunoprecipitated [$^{35}$S]methionine-labeled rRAP-1 and [$^{35}$S]methionine-labeled in vitro-translated products from plasmids F1 and F2, indicating that B-cell epitopes recognized during the course of natural infection are present in these regions (Fig. 5). In contrast, heterologous bovine *B. bigemina* immune serum (B227) did not immunoprecipitate whole rRAP-1 or F2 products but did immunoprecipitate F1 products containing the conserved motifs (Fig. 5). Serum antibodies from cattle hyperimmunized with *B. bigemina* p58, strongly bound p58 in ELISA (11) and immunoprecipitations (Fig. 6), but only two of the four sera (B274 and B277) tested reacted, and did so weakly, with the heterologous RAP-1 protein (Fig. 6). One of the sera (B274) also immunoprecipitated a lower-molecular-weight polypeptide (Fig. 6). The other two hyperimmune anti-p58 bovine sera (B279 and B274) were unreactive with native RAP-1 (Fig. 6). Consistent with the reactivity of heterologous *B. bigemina* immune serum, monospecific anti-*B. bigemina* p58 sera immunoprecipitated F1 products containing the conserved motifs but not whole rRAP-1 or F2 products (Fig. 5).

**(iii) Reactivity of the 14-amino-acid peptide common to RAP-1 and p58.** A 14-amino-acid synthetic peptide, PLSLP NPYQLDAAF (aa 121 to 134), representing the longest continuous peptide sequence common to *B. bovis* RAP-1 and

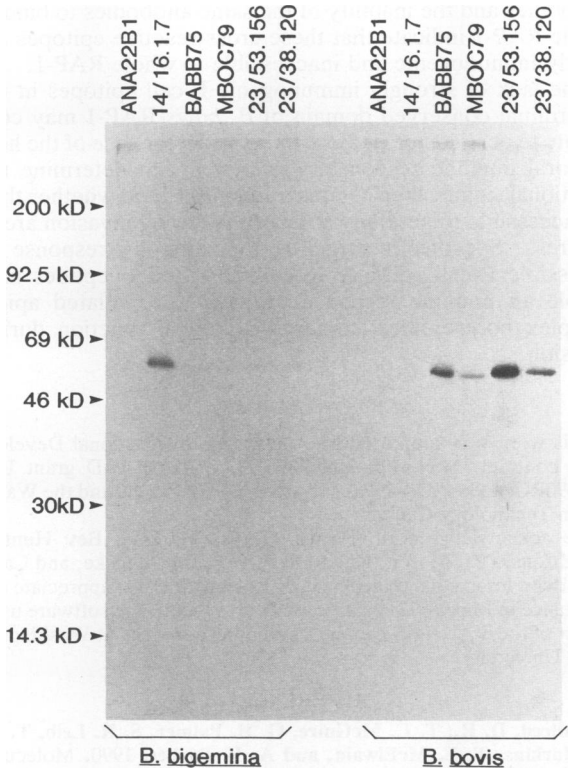


FIG. 4. Immunoprecipitation of [$^{35}$S]methionine-labeled *B. bigemina* and *B. bovis* antigens with negative control MAb ANA22B1, anti-p58 MAb 14/16.1.7, and anti-RAP-1 MAbs BABB75, MBOC79, 23/53.156, and 23/38.120.

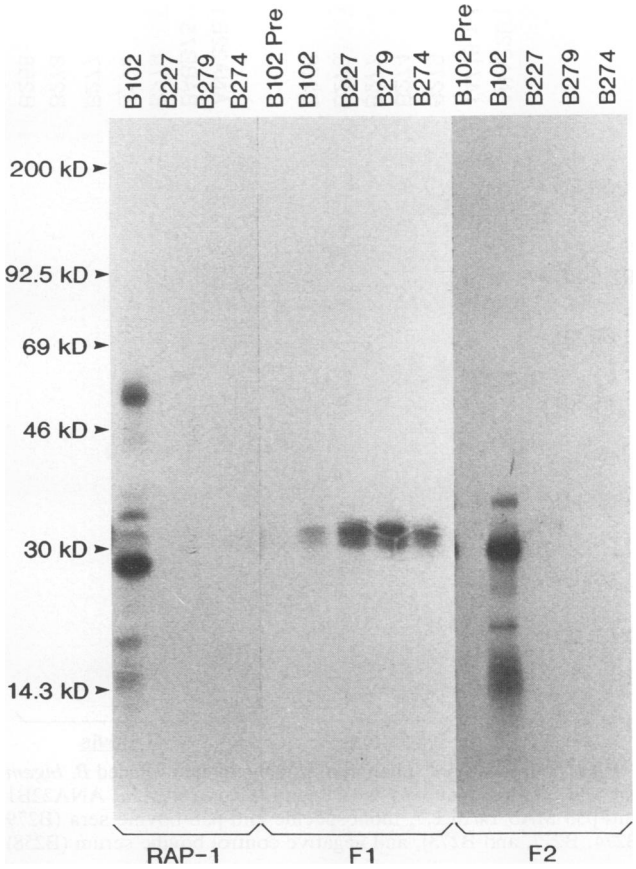


FIG. 5. Immunoprecipitation of [$^{35}$S]methionine-labeled rRAP-1 and products of pBv60 deletion mutants F1 and F2 with immune bovine antiserum to *B. bovis* (B102) or *B. bigemina* (B227), monospecific *B. bigemina* p58 antisera (B274 and B279), and negative control serum (B102 preimmune).

*B. bigemina* p58, was tested for reactivity with bovine serum antibodies. Only one of seven immune sera from cattle that had recovered from infection with *B. bovis* or *B. bigemina* contained antibodies reactive with the 14-mer in ELISA (data not shown). Bovine immune serum B102, from an animal that had recovered from *B. bovis* infection, reacted in ELISA with the 14-mer at a dilution of 1:4. Bovine serum antibodies monospecific for *B. bigemina* p58 were also weakly reactive in ELISA (data not shown). Rabbit antibodies prepared against the conserved 14-mer strongly bound the peptide in ELISA ($10^4$ titer) but failed to recognize any *B. bovis* protein in immunoblots and were not reactive with live or fixed *B. bovis* merozoites when tested by immunofluorescence (data not shown).

## DISCUSSION

*B. bovis* Bv60 was previously shown to be a merozoite apical membrane protein with surface-exposed epitopes conserved among all *B. bovis* strains analyzed (16, 19, 23). As shown by immunoelectron microscopy, MAbs reactive with these surface-exposed epitopes bound the merozoite membrane, as expected, but were also present on rhoptries and erythrocyte stroma. This pattern of localization is similar to that described for *Plasmodium* rhoptry proteins (18) and has led us to redesignate Bv60 *B. bovis* RAP-1.

Previous work has shown that RAP-1 has significant sequence similarity to several other apical complex proteins from related protozoa (22). The most significant conservation is with p58, the RAP-1 homolog of *B. bigemina* (22). While *B. bovis* RAP-1 and *B. bigemina* p58 have divergent sequences in their C-terminal halves, the high degree of sequence identity in the first 300 aa suggests conservation of a critical function. We hypothesize that accessible B-cell epitopes encoded by the conserved sequences may induce antibodies that block function. Thus, mapping of the RAP-1 B-cell epitopes bound by surface-reactive MAbs and immune bovine serum antibodies allowed direct comparison of the conserved sequences with major RAP-1 B-cell epitopes.

The continuous B-cell epitope recognized by surface-reactive MAbs BABB75A4 and MBOC79 is included in the sequence PTKEFFREA (aa 386 to 396), which is located in a predicted hydrophilic region of RAP-1. This sequence, with a few conservative substitutions, is repeated eight times in RAP-1 (23) and is part of a degenerate repetitive element located in the C terminus that is not conserved in *B. bigemina* p58 (22). Interestingly, sequence analysis using the program BESTFIT indicated a significant degree of sequence conservation (60% similarity and 24% identity; quality score, 6.15) between the erythrocyte-binding domain of *Plasmodium falciparum* EBA-1 (14) and a C-terminal region of RAP-1, including a short sequence present in the repeats (aa 492 to 546).

The RAP-1 surface-exposed epitope defined by MAb 23/38.120 is also continuous and is contained in the peptide TQLTSSYEDYML (aa 254 to 265). This sequence is located in a surface-exposed and highly antigenic domain and, again,

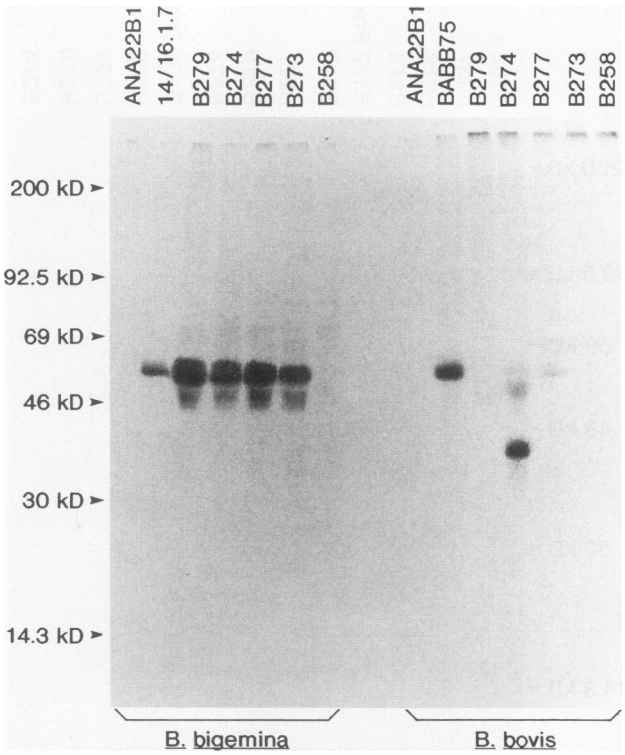


FIG. 6. Immunoprecipitation of [$^{35}$S]methionine-labeled *B. bigemina* and *B. bovis* antigens with negative control MAb ANA22B1, anti-p58 MAb 14/16.1.7, monospecific anti-p58 bovine sera (B279, B274, B277, and B273), and negative control bovine serum (B258).

is not conserved in *B. bigemina* p58. MAb 23/53.156 recognized a surface-exposed B-cell epitope between aa 145 and 218. Overlapping synthetic peptides, representing all predicted continuous epitopes to which conserved motifs would contribute, failed to react with MAb 23/53.156, indicating that none of these sequences contained an appropriate linear B-cell epitope. We hypothesize that MAb 23/53.156 recognizes a discontinuous or conformationally dependent epitope located in part within the conserved domain. However, conserved sequences do not appear to contribute significantly to this B-cell epitope, as MAb 23/53.156 did not bind native *B. bigemina* p58.

Computer-aided analysis of RAP-1 sequences predicts that the regions strictly conserved with p58 are located in the less hydrophilic and poorly immunogenic regions of the polypeptides. The following observations are consistent with this prediction and indicate that the major RAP-1 B-cell epitopes inducing antibodies in cattle and reactive with surface-binding MAbs are distinct from the conserved motifs: (i) linear sequences encoding surface-exposed B-cell epitopes defined by surface-reactive MAbs do not include conserved amino acids, (ii) serum antibodies from cattle immune to *B. bigemina* or hyperimmunized with *B. bigemina* p58 are not cross-reactive with whole RAP-1, and (iii) *B. bovis* immune serum and monospecific anti-p58 antibodies only weakly recognize a 14-amino-acid sequence representing the longest region of conservation between RAP-1 and p58. Heterologous antibody reactivity with truncated *B. bovis* RAP-1 containing primarily the conserved region indicates that conserved motifs may contribute to one or more B-cell epitopes. However, the weak binding of heterologous antibodies and the inability of the same antibodies to bind to whole RAP-1 indicate that these cross-reactive epitopes are poorly immunogenic and inaccessible in whole RAP-1.

The lack of strongly immunogenic B-cell epitopes in the N-terminal conserved domain of *B. bovis* RAP-1 may contribute to persistence of these parasites in the face of the host humoral immune response. Experiments to determine the functional significance of conserved motifs and whether they are accessible to antibody at any time during invasion are in progress. Specifically targeting the antibody response to accessible B-cell epitopes in conserved oligopeptides may enable an immune response to RAP-1, or related apical complex polypeptides, to block a critical function during invasion.

## ACKNOWLEDGMENTS

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## REFERENCES

1. **Allred, D. R., T. C. McGuire, G. H. Palmer, S. R. Leib, T. H. Harkins, T. F. McElwain, and A. F. Barbet.** 1990. Molecular basis for surface antigen polymorphisms and conservation of a neutralization-sensitive epitope in *Anaplasma marginale*. Proc. Natl. Acad. Sci. USA **87**:3220–3224.
2. **Coligan, J. E., A. M. Kruisbeek, D. H. Margulies, E. T. Shevach, and W. Strober.** 1992. Current protocols in immunology. John Wiley & Sons, New York.
3. **Devereaux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
4. **Goff, W. L., W. C. Davis, G. H. Palmer, T. F. McElwain, W. C. Johnson, J. F. Bailey, and T. C. McGuire.** 1988. Identification of *Babesia bovis* merozoite surface antigens by using immune bovine sera and monoclonal antibodies. Infect. Immun. **56:** 2363–2368.
5. **Hines, S. A., T. F. McElwain, G. M. Buening, and G. H. Palmer.** 1989. Molecular characterization of *Babesia bovis* merozoite surface proteins bearing epitopes immunodominant in protected cattle. Mol. Biochem. Parasitol. **37**:1–10.
6. **Levy, M. G., and M. Ristic.** 1980. *Babesia bovis*: continuous cultivation in a microaerophilous stationary phase culture. Science **207**:1218–1220.
7. **Mahoney, D. F., J. D. Kerr, B. V. Goodger, and I. G. Wright.** 1979. The immune response of cattle to *Babesia bovis*. Studies on the nature and specificity of protection. Int. J. Parasitol. **9**:297–306.
8. **Mahoney, D. F., I. G. Wright, and B. V. Goodger.** 1979. Immunity in cattle to *Babesia bovis* after single infections with parasites of various origins. Aust. Vet. J. **55**:10–12.
9. **McElwain, T. F., G. H. Palmer, W. L. Goff, and T. C. McGuire.** 1988. Identification of *Babesia bigemina* and *Babesia bovis* merozoite proteins with isolate- and species-common epitopes recognized by antibodies in bovine immune sera. Infect. Immun. **56**:1658–1660.
10. **McElwain, T. F., L. E. Perryman, W. C. Davis, and T. C. McGuire.** 1987. Antibodies define multiple proteins with epitopes exposed on the surface of live *Babesia bigemina* merozoites. J. Immunol. **138**:2298–2304.
11. **McElwain, T. F., L. E. Perryman, A. J. Musoke, and T. C. McGuire.** 1991. Molecular characterization and immunogenicity of neutralization-sensitive *Babesia bigemina* merozoite surface protein. Mol. Biochem. Parasitol. **47**:213–222.

12. **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* detected with monoclonal antibodies. Infect. Immun. **45**:697–700.
13. **Mishra, V. S., E. B. Stephens, J. B. Dame, L. E. Perryman, T. C. McGuire, and T. F. McElwain.** 1991. Immunogenicity and sequence analysis of recombinant p58: a neutralization sensitive, antigenically conserved *Babesia bigemina* merozoite surface protein. Mol. Biochem. Parasitol. **47**:207–212.
14. **Orlandi, P. A., B. K. L. Sim, J. D. Chulay, and J. D. Haynes.** 1990. Characterization of the 175 kilodalton erythrocyte binding antigen of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **40**:285–294.
15. **Palmer, D. A., G. M. Buening, and C. A. Carson.** 1982. Cryopreservation of *Babesia bovis* for in vitro cultivation. Parasitology **84**:567–571.
16. **Palmer, G. H., T. F. McElwain, L. E. Perryman, W. C. Davis, D. W. Reduker, D. P. Jasmer, V. Shkap, E. Pipano, W. L. Goff, and T. C. McGuire.** 1991. Strain variation of *Babesia bovis* merozoite surface-exposed epitopes. Infect. Immun. **59**:3340–3342.
17. **Palmer, G. H., and T. C. McGuire.** 1984. Immune serum against *Anaplasma marginale* initial bodies neutralizes infectivity for cattle. J. Immunol. **133**:1010–1015.
18. **Perkins, M. E.** 1992. Rhoptry organelles of apicomplexan parasites. Parasitology Today **8**:28–32.
19. **Reduker, D. W., D. P. Jasmer, W. L. Goff, L. E. Perryman, W. C. Davis, and T. C. McGuire.** 1989. A recombinant surface protein of *Babesia bovis* elicits bovine antibodies that react with live merozoites. Mol. Biochem. Parasitol. **35**:239–248.
20. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
22. **Suarez, C. E., T. F. McElwain, E. B. Stephens, V. S. Mishra, and G. H. Palmer.** 1991. Sequence conservation among merozoite apical complex proteins of *Babesia bovis*, *Babesia bigemina* and other apicomplexa. Mol. Biochem. Parasitol. **49**:329–332.
23. **Suarez, C. E., G. H. Palmer, D. P. Jasmer, S. A. Hines, L. E. Perryman, and T. F. McElwain.** 1991. Characterization of the gene encoding a 60-kilodalton *Babesia bovis* merozoite protein with conserved and surface exposed epitopes. Mol. Biochem. Parasitol. **46**:45–52.