Identification and Characterization of a 14-Kilodalton *Brucella abortus* Protein Reactive with Antibodies from Naturally and Experimentally Infected Hosts and T Lymphocytes from Experimentally Infected BALB/c Mice

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A low-molecular-weight recombinant *Brucella abortus* protein reactive with antibodies from a variety of naturally and experimentally infected hosts and T lymphocytes from experimentally infected mice was identified and given the designation BA14K. The gene encoding BA14K was cloned and characterized, and the predicted amino acid sequence of this immunoreactive protein showed no significant homology with previously described proteins. Sequences homologous to the cloned fragment encoding BA14K were identified by Southern blot analysis of genomic DNAs from representatives of all of the currently recognized *Brucella* species. Studies employing BA14K should contribute to our efforts to better understand the antigenic specificity of protective immunity to brucellosis.

*Brucella* spp. are gram-negative, facultative intracellular bacterial pathogens which cause abortion and infertility in numerous domestic and wild mammals, as well as a disease known as undulant fever in humans (1). People become infected through direct contact with infected animals or animal products (16). Consequently, brucellosis in animals used for food is not only a serious economic problem but also a potential public health hazard. Attenuated live vaccines, such as *S19*, which until recently was the vaccine used in cattle in the United States, and *Brucella melitensis* Rev1, which is used in sheep and goats in other parts of the world, have been used successfully in eradication and control programs (1). Although these vaccines have been invaluable components of eradication programs, there are significant problems associated with their use. These include the virulence of S19 and Rev1 for humans (24), the potential for abortion when these strains are used in pregnant animals (2, 17), and the development of agglutinating antibodies in animals vaccinated as adults which are indistinguishable from those elicited by natural infection (17). Clearly, the construction of brucellosis vaccines lacking these undesirable properties would be of great benefit to both veterinary medicine and human medicine.

Similarly to other infections caused by facultative intracellular pathogens, the induction of specific cell-mediated immunity is required for effective clearance of *Brucella* infections (4, 22). Unfortunately, the nature and antigenic specificity of protective cellular immunity against brucellosis are unclear (13). Therefore, the identification of *Brucella* cellular components which contribute to the induction of protective responses in the host will be an important step in designing improved vaccines. The cloning and characterization of genes encoding immunoreactive *Brucella* proteins will provide a useful source of antigen for immunologic assays and subunit immunization studies. It will also facilitate the construction of replicating antigen delivery systems such as those based on salmonellae (7) and vaccinia virus (15). Studies employing both purified subunit preparations and live, recombinant antigen delivery systems should allow a comprehensive evaluation of the relative importance of specific *Brucella* proteins in eliciting protective immunity.

In an attempt to identify *Brucella* proteins capable of inducing protective immune responses, a collection of recombinant *Escherichia coli* clones expressing *Brucella* proteins reactive in immunoblot assays with sera from a variety of experimentally and naturally infected hosts was assembled (18). One of these clones, which was designated IV-4, produced a recombinant *Brucella* protein with an apparent molecular mass of 14 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. Restriction enzyme analysis of the plasmid encoding the recombinant 14-kDa protein, which was designated pBA44, revealed the presence of a 1.8-kb insert. For ease of communication, the recombinant *Brucella* protein produced by clone IV-4 was designated BA14K.

To further examine the extent of the reactivity of BA14K with sera from naturally and experimentally infected hosts, cell lysates of recombinant *E. coli* DH5α [F− ϕ80d lacZM15Δ (lacZYA-argF)U169 deoR recA1 endA1 thi-1 gryA96 relA1] carrying pBA44 and pUC9 were prepared and subjected to SDS-PAGE and immunoblot analysis by previously described methods (19, 20). Affinity-purified, species-specific anti-immunoglobulin G (IgG) horseradish peroxidase conjugates (Sigma Chemical Co., St. Louis, Mo.) were employed in these experiments. IgG-type antibodies reactive with BA14K were detected in sera from naturally infected dogs (Fig. 1), cattle (data not shown), and humans
Brucella canis
concentrations of 0.5, 1, and 2
lymphocyte-enriched splenocytes by previously described
says were performed on pooled, single-cell suspensions of T-
were aseptically removed, and lymphocyte transformation as-
were euthanized with a halothane overdose. Their spleens
weeks postinfection, five mice from each experimental group
as immunogens. T lymphocytes reactive with BA14K were de-
cyttes from mice infected with each of the Brucella strains used
as immunogens. Thus, detection of BA14K-reactive T lymphocytes in mice infected with both vir-
cules and vaccine strains suggests that further investigation of
the immunoreactive nature of this protein along with its ca-
pacity to induce protective immune responses is justified.
Western blot analysis showed that expression of BA14K was
isopropyl-β-D-thiogalactopyranoside inducible in E. coli
JM1109 [Δ(lacl-proAB) (F’ traD36 proAB lacI ZAM15) c14- (mcrA) recA1 endA1 hsdR17 (F− mcrB mcrC) supE44 thi-1 gyrA96
relA1], suggesting that the cloned gene encoding BA14K was
expressed from the lac promoter resident in pUC9 (data not shown).
By employing the dideoxynucleotide-based procedures
described by Sanger et al. (21), nucleotide sequence analysis of the 1.8-kb insert in pBA44 confirmed that BA14K
was produced in E. coli as a fusion composed of the C-terminal
133 amino acids of a native Brucella protein fused to the N-
terminal 13 amino acids of the α-subunit of β-galactosidase
encoded by pUC9.
The complete gene encoding the native Brucella protein was
cloned in the following manner. Southern blot analysis of B.
abortus 2308 genomic DNA employing a 1.5-kb HindIII frag-
ment from pBA44 which encompassed the BA14K coding re-
and the HindIII site from the multiple cloning site of
pUC9 was used to identify a HindIII fragment of approxi-
(not shown) and also in sera obtained from BALB/c mice
and goats experimentally infected with B. abortus 2308 (data
not shown). The detection of IgG-type antibodies specific for
BA14K in sera from these hosts is relevant for several reasons.
First, cattle, goats, dogs, and humans are important natural
hosts for Brucella infections (1, 16), and mice represent a
well-established model for both human (6, 25) and ruminant
hosts for B. abortus infections (1, 16), and mice represent a
chronic
infections (1, 16), and mice represent a
effective host clearance of infection. Thus, detection of
BA14K-reactive T lymphocytes in mice infected with both vir-
ulent and vaccine strains suggests that further investigation of
the immunoreactive nature of this protein along with its ca-
pacity to induce protective immune responses is justified.
coding the C-terminal 132 amino acids of a protein showing limited homology with the lactaldehyde reductase protein FucO of *E. coli* (5) (GenBank accession no. M31059) ends approximately 172 nucleotides upstream of the predicted start codon of the BA14K coding sequence, with no detectable consensus promoter elements in the intervening sequences. This organization suggests that the gene encoding the immunoreactive *Brucella* protein is the last component of an operon. Further characterization of the upstream nucleotide sequence in pRLF6 has not been performed.

Although the unprocessed form of the immunoreactive *Brucella* protein has a predicted molecular mass of approximately 17 kDa, computer-assisted analysis of its amino acid sequence employing the Protein Analysis Toolbox suite of programs (MacVector 6.0; Oxford Molecular Group, Campbell, Calif.) suggests that the first 26 amino acids of this polypeptide form a potential signal sequence for export. Removal of the predicted leader sequence would result in the production of a protein of approximately 14,200 Da and with a pI of 11.25, which is consistent with the mobility of BA14K detected by SDS-PAGE and Western blot analysis. Therefore, we have retained the designation BA14K for this protein. Amino acids 83 to 98 of the unprocessed form of BA14K form a potential transmembrane domain; thus, it appears likely that this protein is associated with the bacterial cell envelope; however, further biochemical characterization will be required to confirm this subcellular localization. No homology to previously deposited protein sequences in the SWISS-PROT database could be detected when the predicted BA14K amino acid sequence was evaluated with the FSTPSCAN program from PCGENE (IntelliGenetics, Mountain View, Calif.) or when this sequence was compared with multiple protein sequence databases by using the BLAST algorithm (3). This latter service was provided by the National Center for Biotechnology Information.

The results presented here indicate that the *B. abortus* immunoreactive protein BA14K and the deduced amino acid sequence of this protein. Underlining, putative ribosome-binding site; asterisks, stop codons; double daggers, fusion site between the reactive protein BA14K and the deduced amino acid sequence of this protein. A dot above every triangle, the predicted signal peptidase cleavage site; dashed arrows, inverted repeat, which may serve as a rho-independent terminator. A dot above every nucleotide is present.

![Image](https://via.placeholder.com/150)

**FIG. 3.** Nucleotide sequence of the gene encoding the *B. abortus* immunoreactive protein BA14K and the deduced amino acid sequence of this protein. Underlining, putative ribosome-binding site; asterisks, stop codons; double daggers, fusion site between the reactive protein BA14K and the deduced amino acid sequence of this protein. A dot above every triangle, the predicted signal peptidase cleavage site; dashed arrows, inverted repeat, which may serve as a rho-independent terminator. A dot above every nucleotide is present.
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