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Brucella Outer Membrane Lipoproteins Share Antigenic Determinants with Bacteria of the Family *Rhizobiaceae*

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Brucellae have been reported to be phylogenetically related to bacteria of the family *Rhizobiaceae*. In the present study, we used a panel of monoclonal antibodies (MAbs) to *Brucella* outer membrane proteins (OMPs) to determine the presence of common OMP epitopes in some representative bacteria of this family, i.e., *Ochrobactrum anthropi*, *Phyllobacterium rubiacearum*, *Rhizobium leguminosarum*, and *Agrobacterium tumefaciens*, and also in bacteria reported to serologically cross-react with brucella, i.e., *Yersinia enterocolitica* O:9, *Escherichia coli* O:157, and *Salmonella urbana*. In particular, most MAbs to the *Brucella* outer membrane lipoproteins Omp10, Omp16, and Omp19 cross-reacted with *O. anthropi* and *P. rubiacearum*, which are actually the closest relatives of brucellae. Some of them also cross-reacted, but to a lower extent, with *R. leguminosarum* and *A. tumefaciens*. The putative Omp16 and Omp19 homologs in these bacteria showed the same apparent molecular masses as their *Brucella* counterparts. None of the antilipoprotein MAbs cross-reacted with *Y. enterocolitica* O:9, *E. coli* O:157, or *S. urbana*.

Brucellae are gram-negative, facultative, intracellular bacteria that can infect humans and many species of animals. Six species are recognized within the genus *Brucella*: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* (7). These classifications are based mainly on their differences in pathogenicity and host preference (7). The *Brucella* species constitute a very homogeneous group, as shown by their antigenic relatedness and by DNA-DNA hybridization studies (>90% DNA homology for all species) (8, 9, 25). On the basis of the 16S rRNA sequence, brucellae have been shown to belong to the family *Rhizobiaceae* (27). This family includes plant and animal pathogens, such as *Agrobacterium*, *Bartonella*, and *Brucella*, that are characteristically associated pericellularly or intracellularly with eukaryotic cells; plant endosymbionts, such as *Rhizobium* and *Phyllobacterium*; soil inhabitants, such as *Mycoplana*; and isolates from soil and from human clinical specimens, such as *Ochrobactrum* (14, 18, 19). Among all these bacteria, *Ochrobactrum anthropi* is the closest known relative of brucellae (14, 24, 27). This bacterium has gained interest in the past few years because of its isolation from immunocompromised hosts (1, 11–13). Recent reports have also described immunological cross-reactions between *Brucella* spp. and *O. anthropi* (23, 24). The antigens containing common epitopes were described as rough lipopolysaccharide and soluble and membrane proteins of unknown nature (23, 24). Since *O. anthropi* constitutes a heterogeneous group of bacteria on the basis of classical phenotypical characterization and DNA-DNA hybridization studies, further subdivision of the genus into two species, *O. anthropi* and *O. intermedium*, has recently been proposed (24). The latter, new species name has been suggested because of a closer genetic and antigenic relationship with brucellae than with *O. anthropi* (24). Additionally, brucellae also share epitopes, mainly on the smooth lipopolysaccharide (S-LPS), with bacteria reported earlier to serologically cross-react with *Brucella*, of which the most important is *Yersinia enterocolitica* O:9 (7).

The *Brucella* outer membrane contains three major proteins with molecular masses ranging from 25 to 27, 31 to 34, and 36 to 38 kDa (2, 6). The largest protein has been identified and characterized as a porin (10, 17). The genes coding for these proteins have been cloned and sequenced, and the current names for these outer membrane proteins (OMPs) are Omp25, Omp31, and Omp2b, respectively (4, 5, 17). The other OMPs identified so far by use of monoclonal antibodies (MAbs) are less abundant (minor) proteins with molecular masses of 10, 16.5, 19, and 89 kDa (2). Gene cloning, the predicted amino acid sequences, and the presence of particular protein motifs have identified the 10-, 16.5-, and 19-kDa OMPs as outer membrane lipoproteins (21, 22). The current names for these OMPs are Omp10, Omp16, and Omp19, respectively (21, 22). Omp16 actually belongs to the peptidoglycan-associated lipoprotein family of proteins found in many gram-negative bacteria (22). Homologs of Omp10 and Omp19 have not yet been reported for other bacteria. All of these proteins have been found as immunogenic proteins in infected cattle, sheep, and goats (3, 15, 16, 21, 28).

In the present study, we used MAbs to analyze the occurrence of epitopes common to *Brucella* OMPs in phylogenetically related bacteria of the family *Rhizobiaceae* and reported S-LPS-cross-reacting bacteria as well. The importance of the epitopes recognized by the MAbs in the antibody responses of *Brucella*-infected cattle and sheep has been previously shown by competitive enzyme-linked immunosorbent assay (ELISA) with these MAbs (3, 28). The occurrence of common epitopes could explain some of the serologic protein cross-reactivities reported between *Brucella* and *Ochrobactrum* (23, 24). In addition, the present study also led to the identification of new homologous proteins within the family *Rhizobiaceae*.

The strains studied that belong to the family *Rhizobiaceae* were *O. anthropi* 3301 (proposed as a reference strain for *O. intermedium*), *O. anthropi* 3331, *Phyllobacterium rubiacearum* Pr1, *Rhizobium leguminosarum* R11, and *Agrobacterium tumefaciens* At1 (26). The S-LPS-cross-reacting bacteria were *Y. enterocolitica* O:9 strain Ye8, *Escherichia coli* O:157 strain Ec2,

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TABLE 1. Binding to MAbs to *Brucella* and related bacteria in ELISA[a]

Specificity	MAb	Absorbance of MAb binding at dilution of 1/2								
		B. abortus	*O. anthropi* 3301	*O. anthropi* 3331	*P. rubiacearum*	*R. leguminosarum*	*A. tumefaciens*	*Y. enterocolitica* O:9	*E. coli* O:157	*S. urbana*
Omp10	A68/07G11/C10	2.320	—[a]	—	—	—	—	—	—	—
	A68/08E07/B11	2.303	1.060	1.008	0.992	1.067	—	—	—	—
Omp16	A68/04G01/C06	2.243	2.591	2.349	2.860	2.860	—	—	—	—
	A76/08C03/G03	2.312	2.604	2.233	—	0.536	0.869	—	—	—
Omp19	A68/25H10/A05	2.119	1.325	1.192	1.040	—	—	—	—	—
	A76/05C10/A08	2.100	1.358	1.254	0.987	—	—	—	—	—
	A76/10D03/H02	2.260	2.179	2.227	1.452	0.667	—	—	—	—
	A76/02A06/H10	1.516	—	—	—	—	—	—	—	—
DnaK	A53/09G03/D02	2.338	0.913	2.128	2.674	—	—	—	—	—
PG[b]	3D6	2.295	2.554	1.693	1.874	2.860	2.108	2.860	2.860	2.860

[a] —, nonsigificant binding (absorbance below 0.5).
[b] PG, peptidoglycan.

and *Salmonella urbana* Su1 (26). *B. abortus* 544 (biovar 1) was used as a reference. Strains were grown on tryptic soy agar (Gibco BRL) supplemented with 0.1% (wt/vol) yeast extract (Difco) at 37°C. *R. leguminosarum* was cultured in tryptone-yeast medium at 30°C (20). MAbs used were those of previous studies (2, 3, 6, 21, 22, 26, 28), and they were used as hybridoma culture supernatants (twofold diluted in ELISA and immunoblotting).

The occurrence of cross-reacting epitopes was first screened by ELISA, performed as described previously (2, 5, 28). Microtiter plates were coated with bacterial suspensions in phosphate-buffered saline at an absorbance (600 nm) of 1.0. To improve accessibility of OMPs, bacteria were sonicated prior to coating (5). MAbs were used at a dilution of 1/2. Positive control MAbs were 3D6, specific for peptidoglycan (6), and A53/09G03/D02, specific for DnaK, previously shown to cross-react with *O. anthropi* and *P. rubiacearum* (26).

In particular, most MAbs to the outer membrane lipoproteins Omp10, Omp16, and Omp19 cross-reacted in ELISA with both *O. anthropi* 3301 and 3331 and *P. rubiacearum* (Table 1). Fewer MAbs against the three OMPs reacted with *R. leguminosarum*, and only one MAb, against Omp16, reacted weakly with *A. tumefaciens*. None of these MAbs reacted with the S-LPS-cross-reacting bacteria *Y. enterocolitica* O:9, *E. coli* O:157, and *S. urbana*. The MAb bindings observed correlated with the genetic closeness to brucellae. However, there was no significant difference in MAb bindings between *O. anthropi* 3301 (proposed as *O. intermedium*) and *O. anthropi* 3331.

In immunoblotting after sodium dodecyl sulfate-polyacrylamide gel electrophoresis, performed as described previously (2, 28), the anti-Omp16 MAbs reacted strongly with *O. anthropi* 3301 and 3331, *P. rubiacearum*, and *R. leguminosarum* and weakly with *A. tumefaciens*, thus confirming the ELISA results (Fig. 1). The anti-Omp19 MAbs reacted strongly only with *O. anthropi* and *P. rubiacearum*, which is also in accordance with the ELISA results. The putative Omp16 and Omp19 homologs detected by the MAbs in these bacteria showed the same apparent molecular masses as their *Brucella* counterparts. The anti-Omp10 MAbs gave no positive reactions in immunoblotting and reacted only weakly with *B. abortus*, which was used as the control (Fig. 1).

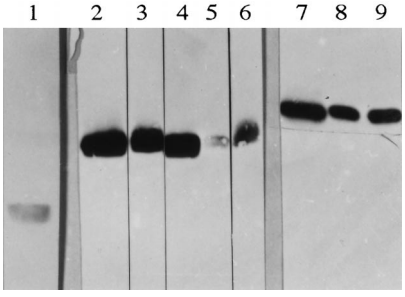

FIG. 1. Reactivity in immunoblotting of anti-Omp10 (lane 1), anti-Omp16 (lanes 2 to 6), and anti-Omp19 MAbs (lanes 7 to 9) after sodium dodecyl sulfate-polyacrylamide gel electrophoresis of *B. abortus* 544 (lanes 1, 2, and 7), *O. anthropi* (strains 3301 and 3331 gave the same result) (lanes 3 and 8), *P. rubiacearum* (lanes 4 and 9), *A. tumefaciens* (lane 5), and *R. leguminosarum* (lane 6).

In conclusion, the present study showed the presence of epitopes cross-reactive with *Brucella* outer membrane lipoproteins on genetically related bacteria, of which the most important is *O. anthropi*. Of particular interest are the lipoproteins Omp10 and Omp19, not yet reported for other bacteria. Thus, these proteins could constitute a new family of OMPs specifically encountered in *Rhizobiaceae*. As suggested by Velasco et al. (23), the immunoresponse of *Brucella*-infected hosts to protein antigens may not necessarily be specific for brucellae, and the presence of *O. anthropi* or related bacteria may explain previously described reactivities to OMPs in healthy animals (16). The outer membrane lipoproteins Omp10, Omp16, and Omp19 are the first identified among these OMPs.

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