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

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Received 6 November 1998/Returned for modification 19 January 1999/Accepted 6 April 1999

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 enterica. 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, 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. enterica.

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 Mycoplasma; 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 recent years for its use in 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 insoluble lipoproteins (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 Y. enterocolitica O:9 (7).

The Brucella outer membrane contains three major proteins with molecular masses ranging from 25 to 37, 34 to 36, and 38 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,
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, 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 control MAbs were 3D6, specific for peptidoglycan (6), and its counterparts. The anti-Omp10 MAbs gave no positive reactions in immunoblotting and reacted only weakly with B. abortus.

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. anthrophi. 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. anthrophi 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.

We thank J. M. Verger and M. Grayon for supplying the strains. We also thank J. N. Limet for technical support.

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![TABLE 1. Binding to MAbs to Brucella and related bacteria in ELISA](image)

| Specificity | MAb                  | Absorbance of MAbs binding at dilution of 1/2 |
|------------|----------------------|---------------------------------------------|
|            | B. abortus  | O. anthrophi 3301 | O. anthrophi 3331 | P. rubiacearum | R. leguminosarum | A. tumefaciens | Y. enterocolitica O:9 | E. coli O:157 | S. urbana |
| Omp10      | A68/07G11/C10      | 2.320                                      | ——                      | ——                  | ——                  | ——                  | ——                  | ——                  | ——                  |
| Omp16      | A68/04G01/C06      | 2.243                                      | 2.591                  | 2.349              | 2.860              | 2.860              | 2.860              | 2.860              | 2.860              |
| Omp19      | A68/25H10/A05      | 2.119                                      | 1.325                  | 1.192              | 1.040              | ——                  | ——                  | ——                  | ——                  |
| DnaK       | A53/09G03/D02      | 2.338                                      | 0.913                  | 2.128              | 2.674              | 2.860              | 2.108              | 2.860              | 2.860              |

* — nonsignificant binding (absorbance below 0.5).

** — PG, peptidoglycan.

**3**

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. anthrophi (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).
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