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Antigenic Determinants of the Attachment Protein of *Mycoplasma pneumoniae* Shared by Other Pathogenic *Mycoplasma* Species

WALLACE A. CLYDE, JR.* AND PING C. HU

Departments of Pediatrics and Microbiology, University of North Carolina, Chapel Hill, North Carolina 27514

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In previous studies with hyperimmune rabbit antisera, we found evidence of serologic cross-reactivity among *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, and *Mycoplasma gallisepticum*. Because of certain biologic and morphologic similarities of these species, attempts were made to determine if this cross-reactivity related to the attachment protein (P1) of *M. pneumoniae*. Monoclonal and monospecific antibodies against P1 were used to probe proteins of the other species by immunoblotting. One of the P1 monoclonal antibodies was reactive with a smaller protein of *M. genitalium*; rabbit antiserum raised by immunization with P1 excised from a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel was found to react with a similar-sized protein of *M. gallisepticum*. These preliminary findings suggest antigenic sharing among the species examined; however, limitations of the methods used are discussed.

An important virulence component of *Mycoplasma pneumoniae* is a surface protein (named P1) which mediates initial attachment of the organisms to host cells (6, 8). Since several other pathogenic mycoplasma species parasitize cells in a similar fashion, the possibility that they share a P1-like substance was explored. Both monospecific and monoclonal antibodies against P1 protein were used to probe electrophoretically separated proteins of *Mycoplasma gallisepticum* and *Mycoplasma genitalium* seeking similar composition among the species. Evidence is presented that there is antigenic conservation among these species.

M. pneumoniae M129-B12 (ATCC 29342) was from our laboratory stock (12). *M. genitalium* G-37 (ATCC 33530), *M. gallisepticum* PG-31 (ATCC 19610), and *Mycoplasma pulmonis* PG-34 (ATCC 19612) were obtained from the American Type Culture Collection, Rockville, Md. *M. genitalium* was propagated in SP-4 medium (17) and the other species in Edward medium (3) contained in glass prescription bottles as previously described (7, 8). Organisms attaching firmly to glass (*M. pneumoniae* and *M. genitalium*) were harvested by rinsing confluent monolayers with phosphate-buffered saline (0.01 M, pH 7.2) followed by scraping of the cells into cold phosphate-buffered saline and pelleting by centrifugation (8). *M. gallisepticum* and *M. pulmonis*, which attach less avidly to glass, were washed and harvested by centrifugation. All samples were stored at −70°C until used.

Hyperimmune rabbit sera to each mycoplasma species were produced by conventional immunization regimens consisting of subcutaneous injection of whole washed organisms emulsified in complete Freund adjuvant followed by booster injections in incomplete Freund adjuvant (14). Monospecific antibodies against the *M. pneumoniae* P1 protein were prepared by immunization of a rabbit with this band cut from sodium dodecyl sulfate (SDS) gels after electrophoresis (1). The procedures described by Shulman et al. (16) were used to produce monoclonal antibodies against P1 protein. Briefly, splenic cells from BALB/c mice immunized with whole *M. pneumoniae* organisms were hybridized with myeloma cells SP2/0 Ag14 (ATCC CRL-1581). Hybrids producing antibodies against *M. pneumoniae* were screened with a solid-phase radioimmunoassay (11), and those specific to P1 were identified with "Western" blots in combination with a radioimmunobinding assay, using ^{125}I-labeled immunoglobulin G fraction of goat anti-mouse immunoglobulins as described previously (10). The procedures for SDS gel electrophoresis of mycoplasma proteins and preparation of Western blots were those used previously (10). ^{125}I-labeled immunoglobulins were prepared by the chloramine T method (4).

Our previous observations, using hyperimmune rabbit sera against *M. gallisepticum*, *M. genitalium*, and *M. pneumoniae* showed extensive interspecies serological cross-reactivity (5). This suggests phylogenetic relatedness among these species and raises the possibility that they have certain antigenic proteins in common. However, this does not necessarily imply that the cross-reactive substances are identical or that they have similar biologic functions in each case. In the present studies, refinement of serological cross-reactivity was conducted by using monospecific and monoclonal antibodies to the *M. pneumoniae* P1 protein to determine if antigenic determinants were shared by the other species.

Evaluation of a library of 23 distinct monoclonal antibodies against the P1 protein has shown that the collection recognizes at least five different epitopes of this high-molecular-mass protein (190 kilodaltons [kDa] [9]). Figure 1 shows the result of probing proteins of *M. gallisepticum* and *M. genitalium* with a pool of all 23 P1 monoclonal antibodies. *M. pulmonis* antigens were included as a control since our previous study showed no reactivity with hyperimmune rabbit antisera against the other species (5). A strong cross-reaction was seen only with a single protein of *M. genitalium* which had a molecular mass of about 100 kDa, while there was no recognition of similar proteins in *M. gallisepticum* or *M. pulmonis*. Further reaction of *M. genitalium* blots with individual P1 monoclonal antibodies showed that only 1 of the 23 was responsible for the cross-reaction demonstrated (Fig. 2).

The limitations of the Western blot technique must be recognized in interpreting these results. All of the mycoplasma proteins may not renature or transfer equally from SDS gels to nitrocellulose filters (9, 10). Also, the exact epitopic composition of P1 protein is still unknown and may

* Corresponding author.

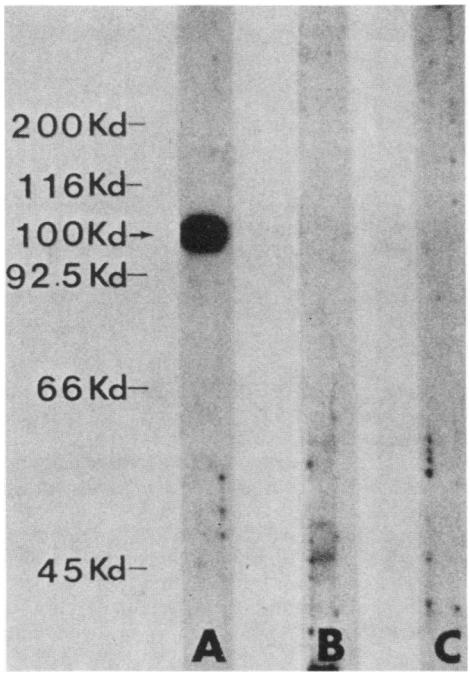

FIG. 1. Western blots of electrophoresed proteins from 7% SDS gels of (A) *M. genitalium*, (B) *M. gallisepticum*, and (C) *M. pulmonis* reacted with a pool of 23 monoclonal antibodies against the P1 attachment protein of *M. pneumoniae*. A single protein in *M. genitalium* shows a strongly positive reaction, while the other two are completely negative. Molecular standards: myosin, 200 kDa; galactosidase, 116 kDa; phosphorylase B, 92.5 kDa; bovine serum albumin, 66 kDa; and ovalbumin, 45 kDa.

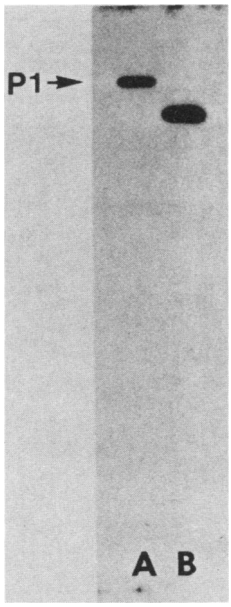

FIG. 2. Western blots of electrophoresed proteins from a 10% SDS gel of (A) *M. pneumoniae* and (B) *M. genitalium* reacted with a single monoclonal antibody, M-525, which was the only one responsible for the reaction shown in Fig. 1. The other 22 monoclonal antibodies tested were negative. The *M. genitalium* protein recognized by M-525 has an estimated molecular mass of 100 kDa.

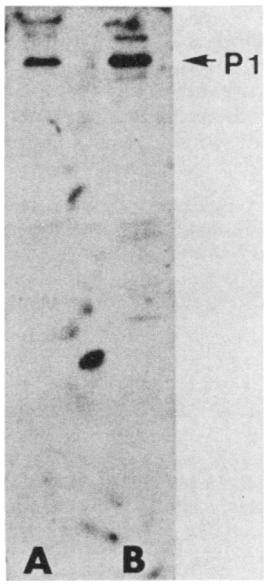

FIG. 3. Western blots of electrophoresed proteins from a 10% SDS gel of (A) *M. gallisepticum* and (B) *M. pneumoniae* reacted with a monospecific antibody against the P1 attachment protein of *M. pneumoniae*. A strong reaction is shown against a single protein of *M. gallisepticum* which has essentially the same molecular weight as that of the P1 protein.

not be fully recognized with the monoclonal antibodies on hand. To extend these observations, a monospecific antibody to P1 protein was used as another probe, with the rationale that this should contain a more complete representation of the epitopes that are present. The monospecific antibody which reacts only with the P1 but not other proteins of *M. pneumoniae* also cross-reacted with a protein of nearly identical size in *M. gallisepticum* (Fig. 3). These results indicate that the monoclonal antibody library does not recognize all available epitopes of the P1 protein. In addition, failure of the monospecific antibody to react with *M. genitalium* (data not shown) suggests that some denaturation of the P1 antigenic composition occurred during SDS treatment, electrophoretic separation, or renaturation of protein. Thus, in these experiments more information on antigenic relationships among the mycoplasmas examined was obtained by using a combination of monospecific and monoclonal antibody preparations as opposed to either type alone.

The species of pathogenic mycoplasmas studied in this report share a number of biologic properties, although they differ markedly in guanine plus cytosine content (15). All ferment carbohydrates, hemolyze erythrocytes, and reduce triphenyl tetrazoleum to formazan. They also are known to attach to a variety of host cells by differentiated subcellular organelles. In the case of *M. pneumoniae*, *M. gallisepticum*, and *M. genitalium* (6, 13, 18), but not *M. pulmonis*, these organelles are covered with peplomerlike protrusions of the unit membrane. These similarities prompted our search for conserved antigens among the species. A previous effort by others to do this was unsuccessful, probably because of the limited number of monoclonal antibodies used (2).

While the cross-reactions shown between P1 protein of *M. pneumoniae*, a similar-molecular-weight protein of *M. gallisepticum* and a smaller protein of *M. genitalium* suggest that similar functions may be served in all three species. However, the present data do not establish this point.

Further experiments are necessary to show that the cross-reacting proteins of *M. gallisepticum* and *M. genitalium* are located on the membrane surface, that they are found only on the attachment organelle, and that the attachment activity can be blocked with specific monoclonal antibodies, as has been accomplished with *M. pneumoniae* (6).

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