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Absence of *Mycoplasma pneumoniae* Cytadsorption Protein P1 in *Mycoplasma genitalium* and *Mycoplasma gallisepticum*

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Polyclonal and monoclonal antibodies to *Mycoplasma pneumoniae* protein P1 were nonreactive with whole-cell or soluble preparations of *M. genitalium* and *M. gallisepticum*. However, radioimmunoprecipitation performed with hyperimmune rabbit sera raised against each mycoplasma species indicated antigenic cross-reactivity between *M. pneumoniae* and *M. genitalium*.

Several *Mycoplasma* species (*M. pneumoniae*, *M. genitalium*, and *M. gallisepticum*) share unique biological features. Each is flask-shaped and possesses specialized tiplike organelles that mediate adherence to sialic acid residues on a variety of eucaryotic cells (3, 4, 6–8, 18, 20, 21; J. G. Tully, D. Taylor-Robinson, D. L. Rose, R. M. Cole, and J. M. Bové, Int. J. Syst. Bacteriol., in press). Competition between *M. pneumoniae* and *M. gallisepticum* for sialic acid receptors on erythrocytes has also been reported (1). Additional evidence supports the role of mycoplasma proteins in this cytadsorption event (8–12). In *M. pneumoniae*, a group of cytadsorption-associated proteins has been identified by mutant and revertant analysis (11, 12), ligand-receptor binding assays (9), and biochemical and ultrastructural data (2). It appears that protein P1 (molecular weight 165,000 [165K]) clusters at the tip of virulent *M. pneumoniae* strains and, in association with a naplike structure (2), interacts with host cell membranes. P1-containing avirulent isogenic mutants do not concentrate P1 at the terminus, as determined by immunoferritin anti-P1 labeling and electron microscopy, suggesting a cooperative and essential function of accessory proteins (12) in the activation, lateral mobility, or anchoring of protein P1.

Because of the importance of P1 in *M. pneumoniae* cytadsorption and the similarities shared by the aforementioned mycoplasmas, including reported serological cross-reactivity between *M. pneumoniae* and *M. genitalium* (15, 19), we examined strains of *M. genitalium* and *M. gallisepticum* for the presence of P1 and other antigenically cross-reactive proteins to further analyze the common mechanisms of cytadsorption.

M. pneumoniae M129-B16 (16) and *M. genitalium* G-37 (20), both of which grow as glass-adherent colonies in Hayflick medium, were radiolabeled with [^{35}S]methionine as previously described (14). *M. gallisepticum* S6 (received from J. G. Tully, National Institute for Allergy and Infectious Diseases) was handled similarly, except that this microorganism adheres less avidly to glass, and centrifugation of broth cultures was necessary during the washing process. All mycoplasmas were stored as pellets at −70°C until used.

Hyperimmune rabbit sera directed against each mycoplasma strain were obtained by conventional immunization regimens, using subcutaneous and intramuscular injections of whole mycoplasmas in Freund complete adjuvant followed by booster injections in Freund incomplete adjuvant (2). Rabbit anti-P1 serum was obtained by immunization with polyacrylamide gel-purified protein (2). Monoclonal antibodies to *M. pneumoniae* protein P1 were produced by a modification of the procedure of Oi and Herzenberg (17). Spleen cells from immunized BALB/c female mice were fused with nonsecreting SP2/0-Ag14 BALB/c myeloma cells. Hybrids that were positively identified as secreting anti-*M. pneumoniae* antibody by an enzyme-linked immunosorbent assay (16a) were further analyzed for anti-P1 activity by a soluble-antigen radioimmunoprecipitation (RIP) assay (14). Briefly, detergent-solubilized [^{35}S]methionine-labeled mycoplasma preparations were incubated with test sera or monoclonal antibodies and precipitated with protein A-bearing *Staphylococcus aureus*. The labeled antigens were eluted and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography (5, 13). To analyze antibody-accessible proteins on mycoplasma surfaces, a whole-cell RIP was employed in which intact radiolabeled mycoplasmas were exposed to specific antibody probes before solubilization and antigen analysis (14).

The presence of protein P1 in the *M. genitalium* and *M. gallisepticum* strains was assessed by soluble-antigen and whole-cell RIPs (Fig. 1). Note that P1 was only precipitated in control *M. pneumoniae* preparations by polyclonal or monoclonal antibodies against P1. Possibly, cross-reactive epitopes occur among these *Mycoplasma* species that were not detected by the RIP method. One protein band (approximate molecular weight, 90K) (Fig. 1b, lanes D and E) was observed in the control RIP without antibody. We attempted to clarify a possible shared protein homology between these strains (Fig. 2). Hyperimmune anti-*M. genitalium* serum reacted strongly with *M. pneumoniae* proteins (Fig. 2a, lane C), consistent with previous reports of a serological relationship determined by complement fixation titers and growth metabolism inhibition tests (15). Antiserum against *M. pneumoniae* showed a significant but lower reactivity to *M. genitalium* (Fig. 2b, lane C), which correlates with the reduced cross-reactions observed between these strains in a metabolism inhibition assay (19). *M. gallisepticum* was nonreactive in both cases (Fig. 2a and b, lanes D).

Clearly, considerable antigenic cross-reactivity exists between *M. pneumoniae* and *M. genitalium*, and yet the absence of P1 in *M. genitalium*, coupled with the appearance of strongly cross-reactive but distinct proteins in heterolo-

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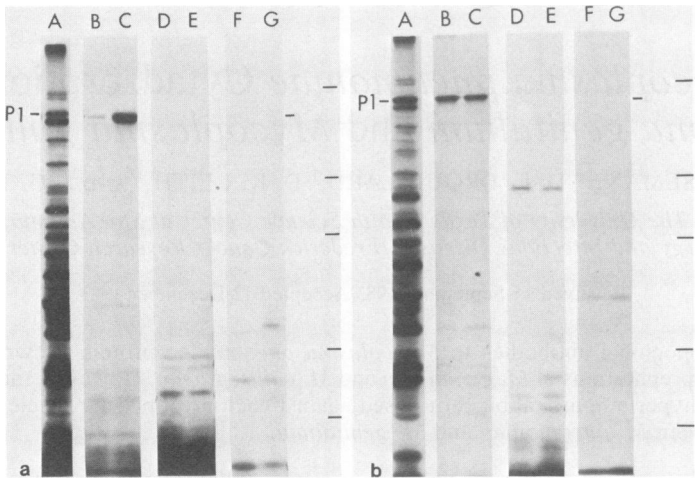

FIG. 1. Detection of P1 by (a) soluble-antigen RIP and (b) whole-cell RIP. Lanes: A, labeled *M. pneumoniae* total protein profile; B, D, and F, labeled preparations of *M. pneumoniae*, *M. genitalium*, and *M. gallisepticum*, respectively, incubated with polyclonal antibody to P1 (2); C, E, and G, labeled preparations of *M. pneumoniae*, *M. genitalium*, and *M. gallisepticum*, respectively, incubated with a pool of four individual monoclonal antibodies to P1. Molecular weight markers are indicated at the right (top to bottom: 200K, 92.5K, 68K, 43K, 25.7K). The weakly visible non-P1 protein bands were observed in control RIPs without antibody and represent nonspecific background.

gous RIPs (i.e., compare lanes B and C, Fig. 2a, and lanes B and C, Fig. 2b), support the classification of *M. genitalium* as a separate species, as previously proposed (19, 20; Tully et al., in press). Furthermore, several experimental precautions should be addressed. A highly cross-reactive antigenic relationship was observed between *M. pneumoniae* and *M. genitalium* when *M. genitalium* antiserum was used (Fig. 2a, lanes B and C), but reduced cross-reactivity was detected with antiserum raised against *M. pneumoniae* (Fig. 2b, lanes B and C). These results underscore the experimental limitations of low- versus high-affinity hyperimmune sera in resolving protein homologies between related species.

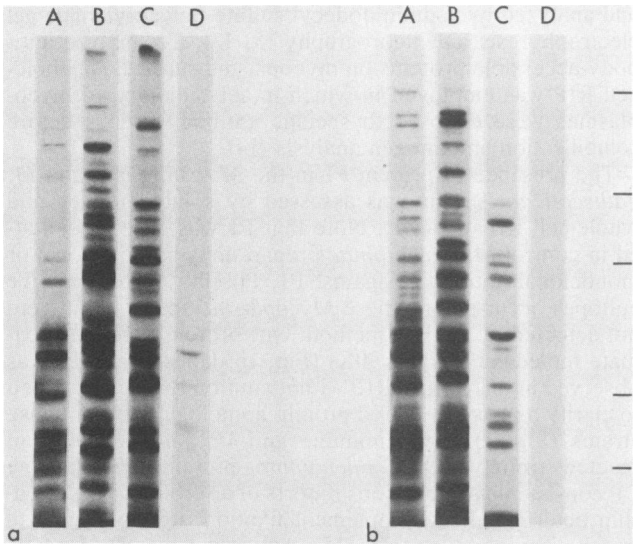

FIG. 2. Comparison of soluble-antigen RIP with [^{35}S]methionine-labeled mycoplasmas and hyperimmune rabbit serum against *M. genitalium* and *M. pneumoniae*. (a) Total *M. genitalium* protein profile (A) and RIP of intrinsically labeled *M. genitalium*, *M. pneumoniae*, and *M. gallisepticum* (B, C, and D, respectively) incubated with hyperimmune anti-*M. genitalium* serum. (b) Total *M. pneumoniae* protein profile (A) and RIP of intrinsically labeled *M. pneumoniae*, *M. genitalium*, and *M. gallisepticum* (B, C, and D, respectively) incubated with hyperimmune anti-*M. pneumoniae* serum.

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