Detection of *Mycoplasma pneumoniae* Adhesin (P1) in the Nonhemadsorbing Population of Virulent *Mycoplasma pneumoniae*

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Received 21 March 1985/Accepted 7 May 1985

*Mycoplasma pneumoniae* organisms possessing a hemadsorbing-negative (HA−) phenotype comprise more than 50% of the population of virulent *M. pneumoniae* cultures. Monoclonal antibody to P1, the major adhesin of *M. pneumoniae* reacts with this HA− mycoplasma fraction based upon radioimmunoprecipitation and immunoblotting. Demonstration of P1 in the entire mycoplasma population suggests that topological organization of this adhesin in the membrane or the physiological state of the mycoplasmas may determine hemadsorbing capabilities.

The adherence of *Mycoplasma pneumoniae* to host cell membranes appears to be mediated by a protein adhesin designated P1 (3, 7–10). This membrane protein is surface localized and trypsin sensitive (3, 5, 7, 8). The absence of P1 and other specific proteins of *M. pneumoniae* as determined by mutant analysis (11) and chemical and immunological characterization (9, 10) is related to loss of hemadsorption capabilities. When virulent, hemadsorbing-positive (HA+) *M. pneumoniae* was grown in broth culture and tested for adherence capacity, only a subpopulation (varying from 7 to 30%) of the *M. pneumoniae* culture cytadsorbed (1, 2). We initiated experiments to determine whether the hemadsorbing-negative (HA−) population was deficient in P1.

Virulent HA+ *M. pneumoniae* parent strain M129-B25C was grown in 32-oz (~1-liter) glass bottles containing 70 ml of Hayflick medium (6). After 72 h the medium was changed, fresh medium (prewarmed to 37°C) was added, and cultures were incubated for an additional 24 h. Mycoplasmas were metabolically labeled with [35S]methionine (250 μCi) for 90 min, followed by a 30-min chase in fresh unlabeled medium (10). Glass-attached *M. pneumoniae* organisms were scraped into 10 ml of phosphate-buffered saline, centrifuged for 10 min at 12,000 × g at 4°C, suspended in buffer, and used directly in the hemadsorption assay (10). To remove the HA+ mycoplasmas, *M. pneumoniae* organisms (1.5 ml of a suspension of 0.2 mg of mycoplasma protein per ml) were incubated with erythrocyte (RBC) suspensions (1 ml of a 10% suspension) of human O blood type or sheep cells for 30 min at 37°C. The mycoplasma-RBC mixture was centrifuged for 10 min at 300 × g, and the supernatant (which included the HA+ mycoplasmas) was reincubated with two additional RBC preparations as outlined above. Mycoplasmas which remained in the supernatant after the third RBC adsorption were collected by centrifugation (12,000 × g, 20 min at 4°C). The resultant mycoplasma pellet was solubilized and tested in the soluble antigen radioimmunoprecipitation assay as previously described (14), using a monoclonal antibody (immunoglobulin G) to protein P1 (13). Protein profiles were displayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the methods of Laemmli (12) and Bonner and Laskey (4).

About 30% of the total mycoplasma population was removed by the first RBC adsorption. The second and third mycoplasma-RBC incubations removed a much smaller fraction, about 5 and 1% of the remaining mycoplasmas, respectively. Thus, approximately 40% of the total radiolabeled mycoplasmas hemadsorbed. This value is slightly higher than those indicated in other reports and may be attributed to the increased ratio of RBCs to mycoplasmas used in this procedure and the successive exposures of mycoplasmas to RBC suspensions.

The electrophoretic protein profiles of the HA− mycoplasmas were compared to those obtained when this fraction cultured in the absence of RBCs was subjected to electrophoresis. The profiles of the unfractionated mycoplasma population (lanes a, b) and the hemadsorbing-negative population (lane c) are compared. The major protein bands with molecular weights of 25-27 k, 43 k, 68 k, and 92.5 k appear in both the unfractionated and the hemadsorbing-negative populations, whereas the P1 protein of 200 k is present only in the unfractionated population.

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FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis fluorogram of *M. pneumoniae* HA− fraction. Virulent M. pneumoniae organisms metabolically labeled with [35S]methionine were adsorbed three times with human or sheep RBC preparations. Protein profiles of mycoplasmas which did not adhere to human RBCs (lane a) or sheep RBCs (lane b) are compared to the original nonfractionated population (lane c).

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M. pneumoniae protein P1 is the major adhesin of M. pneumoniae. This protein is densely clustered at the tip region of virulent M. pneumoniae, mediating adherence of mycoplasmas to host cells (3, 5, 8). It has also been shown that P1 is distributed in less-dense foci along the M. pneumoniae unit membrane (3). Further experiments are warranted to elucidate the distribution pattern of P1 on the membranes of the HA- fraction of virulent M. pneumoniae populations.

LITERATURE CITED


