INFECTION AND IMMUNITY, July 1983, p. 437–439
0019-9567/83/070437-03$02.00/0

Vol. 41, No. 1

# Demonstration of Antibodies to *Mycoplasma pneumoniae* Attachment Protein in Human Sera and Respiratory Secretions

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Received 29 November 1982/Accepted 8 April 1983

Antibodies specific to the attachment protein of *Mycoplasma pneumoniae* were demonstrated in sera and respiratory secretions of human patients. The results indicate that the attachment protein is a major immunogen.

*Mycoplasma pneumoniae* is one of the most common causes of human respiratory tract infections (6) and is the principal etiological agent of atypical pneumonia. It has been recognized for some time that *M. pneumoniae*, as well as other mycoplasma species, can attach firmly to various types of epithelium cells (16). Previous studies have indicated that attachment of *M. pneumoniae* to host respiratory epithelium is mediated through a specialized structure at one end of this filamentous organism (4, 5). Subsequently, we have demonstrated that a surface protein component, designated as P1 protein, is involved in this attachment process (10). Recently, we have provided direct evidence that P1 protein is localized at the surface of the terminal organelle of *M. pneumoniae* by using monoclonal antibodies specific for P1 protein (9). This observation was subsequently confirmed by Feldner et al. (7) and Baseman et al. (1), who used monoclonal antibody and monospecific antibody, respectively, against P1 protein. Baseman et al. claimed that the P1 protein, although mainly concentrated over the terminal structure, might also be scattered over other parts of the surface of *M. pneumoniae*. Despite this minor discrepancy, the importance of P1 in the pathogenesis of *M. pneumoniae* infection is now considered established.

The role of the humoral immune response in protection against *M. pneumoniae* infection has been well documented (13, 17), and surface proteins of *M. pneumoniae*, including P1 protein, have been suggested as possible vaccine candidates (8). To investigate this possibility, we conducted experiments to study the immunogenicity of the constituent proteins of *M. pneumoniae*. When Western blot transfer was combined with a radioimmunobinding assay (11), antibodies to seven immunogens, including P1 protein, were found in the sera of hamsters infected with *M. pneumoniae* by inhalation. We found that many more proteins were capable of stimulating antibodies in rabbits immunized parenterally with mycoplasmas. The present study extends this technique to human sera and respiratory secretions to ensure that P1 and other *M. pneumoniae* proteins are also immunogenic for humans.

*M. pneumoniae* M129 (ATCC 29342), was used in this study. Cultures were maintained in Hayflick medium supplemented with 20% agammaglobulin horse serum, 10% yeast dialysate, and penicillin (1,000 U/ml) (10). Monolayer cultures were grown in glass prescription bottles. After incubation at 37°C for 36 to 48 h, the medium was decanted, and the organisms were rinsed three times with phosphate-buffered saline (pH 7.2). The organisms were then scraped into the phosphate-buffered saline, pelleted by centrifugation (12,000 × *g* for 15 min), and processed for sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Electrophoresis of *M. pneumoniae* proteins on sodium dodecyl sulfate-polyacrylamide (10%) and Western blot transfer were the same as described previously (9). After transfer, the nitrocellulose blots were incubated with sera or respiratory secretions from human patients with culture-proven *M. pneumoniae* infection and then imaged with $^{125}$I-labeled goat anti-human immunoglobulin G (IgG), IgM, or secretory IgA (Cappel Laboratories, Cochranville, Pa.). Radioautographs were produced with Xomat-AR5 film (Kodak) at −80°C for 12 to 24 h with an intensifying screen (DuPont Co., Wilmington, Del.).

A total of 12 paired (acute and convalescent) serum samples were examined. Representative data are presented in Fig. 1. Despite marked variations in each individual's ability to recognize and respond to different mycoplasma antigens, all patients with high complement fixation or radioimmunoassay titers (Table 1) reacted with P1 protein (molecular weight, 190,000) and a second protein (molecular weight, 88,000).

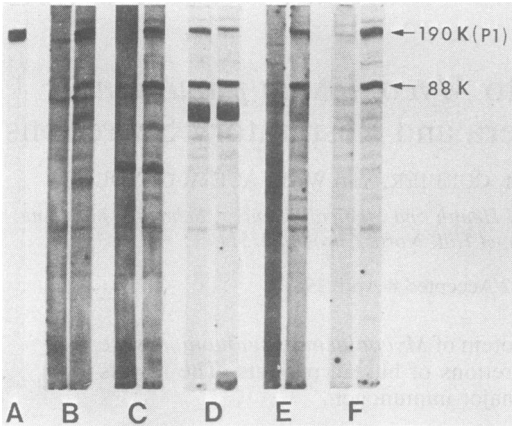


FIG. 1. Demonstration of antibodies to *M. pneumoniae* attachment protein (P1) in human sera. (A) A nitrocellulose blot was incubated with a monoclonal antibody (M128) which has been shown to be specific to P1 protein (9), followed by incubation with $^{125}$I-labeled rabbit anti-mouse IgG. (B to F) Nitrocellulose blots were incubated with paired acute and convalescent sera obtained from five patients and imaged with $^{125}$I-labeled goat anti-human IgG. Antibodies specific to P1 protein were demonstrated in all sera tested, indicating that it is a major immunogen in natural *M. pneumoniae* infection. Molecular weights were determined by coelectrophoresis of protein standards with known molecular weights.

Complement fixation titers were determined by procedures previously described by Kenny and Grayston (12). The solid-phase radioimmunoassay was developed in our laboratory, and details will be published elsewhere (P. C. Hu, D. A. Powell, F. Albright, D. E. Gardner, A. M. Collier, and W. A. Clyde, Jr., J. Clin. Lab. Immunol., in press). These results are consistent with our previous observations in infected animals (9) that P1 protein is a major immunogen. The role of the 88,000-dalton protein is not known at the present time.

Although Taylor and Taylor-Robinson (16) have shown that colonization of *Mycoplasma pulmonis* in the mouse respiratory tract could be suppressed by systemic antibody, it has been thought that local immune mechanisms, including antibodies in the lung, may play a more important role in the prevention of *M. pneumoniae* disease (2, 3). Biberfeld and Sterner (2) used an indirect immunofluorescence test to detect *M. pneumoniae* antibody in sputum samples. IgA antibodies were present in all 31 samples with detectable antimycoplasma activity: IgG antibodies were present in 24 samples, IgM in 13 samples. Local synthesis of secretory IgA and transudation of serum antibodies into bronchial secretions were thought to be the source of *M. pneumoniae* antibodies found in respiratory tract secretions. To evaluate the role of P1

TABLE 1. Complement fixation and radioimmunoassay titers in human mycoplasmal pneumonia sera

| Patient code[a] | Date serum obtained | Complement fixation titer | Radioimmunoassay titer |
|---|---|---|---|
| B | 10–06–79 | 2 | 7,663 |
| | 10–31–79 | 128 | 25,850 |
| C | Pre | 4 | 3,421 |
| | 08–20–80 | 256 | 27,336 |
| D | 10–20–80 | 8 | 12,296 |
| | 11–01–80 | 32 | 12,219 |
| E | 11–19–80 | <2 | 4,060 |
| | 12–05–80 | 512 | 28,520 |
| F | 12–04–80 | 8 | 12,197 |
| | 01–07–81 | 64 | 19,886 |

[a] Patient designations correspond to the letters in Fig. 1.

[b] The technical details of the radioimmunoassay have been described elsewhere (P. C. Hu, D. A. Powell, F. Albright, D. E. Gardner, A. M. Collier, W. A. Clyde, Jr., J. Clin. Lab. Immunol., in press).

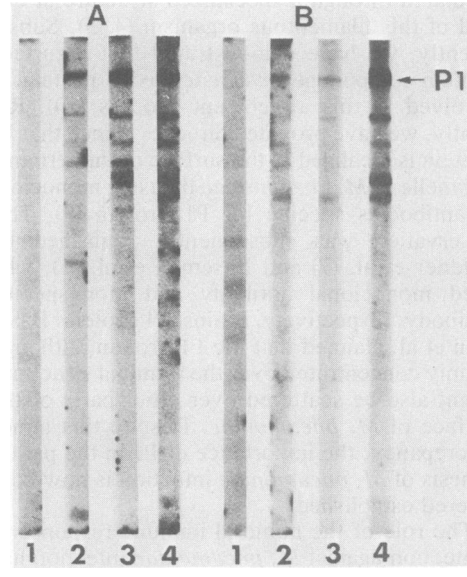


FIG. 2. Demonstration of antibodies to *M. pneumoniae* attachment protein (P1) in human respiratory secretions. Nitrocellulose blots were incubated with human sputa or nasal wash and imaged with $^{125}$I-labeled goat anti-human IgG (A) or secretory IgA (B). 1, Nasal wash from a patient diagnosed with a negative mycoplasma infection; 2 and 3, sputum samples from patients with culture-proven *M. pneumoniae* infection; 4, nasal wash from a noninfected but heavily exposed laboratory investigator (P.C.H.). Results indicate that both IgG and secretory IgA antibodies specific to the attachment protein were present in the tested samples.

protein as an immunogen in the respiratory tract, respiratory secretions were examined for the presence of antibodies specific to P1 protein. As shown in Fig. 2, both IgG and secretory IgA specific for P1 protein could be observed in either sputum or nasal washes from all subjects examined. It is possible that antibodies specific to P1 protein may play a role(s) other than that of antiattachment in the respiratory tract (7). Powell and Clyde (16) reported that phagocytosis of *M. pneumoniae* by alveolar macrophages can be greatly enhanced by specific antibodies. In addition, monoclonal antibodies specific to P1 protein have been shown to inhibit the hemabsorption activity and motility of *M. pneumoniae* (7).

In conclusion, the present studies have demonstrated that patients with natural infection uniformly react to the attachment protein of *M. pneumoniae* by producing antibodies against it in their blood and respiratory secretions. Identification of this surface protein, which stimulates immunity, suggests the possibility of its use as a vaccine to prevent *M. pneumoniae* disease.

This work was supported in part by Public Health Service grant HL-19171 from the National Institutes of Health and by Cooperative Agreement CR 807392 from the U.S. Environmental Protection Agency.

We thank Susan Stedman for her secretarial assistance.

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