IDENTIFICATION OF P1 GENE DOMAIN CONTAINING EPITOPE(S) MEDIATING MYCOPLASMA PNEUMONIAE CYTADHERENCE

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Mycoplasma pneumoniae is the causative agent of primary atypical pneumonia in humans. The intimate association between M. pneumoniae and the host cell surface is prerequisite for the development and persistence of disease (1, 2). Protein P1 has been designated the major adhesin mediating the attachment of M. pneumoniae to respiratory epithelium (3-6). M. pneumoniae organisms that lack P1 or are unable to densely cluster and properly position the adhesin at the mycoplasma tip are avirulent (3, 7, 8). We recently cloned and sequenced the cytadhesin P1 gene and deduced the protein sequence to determine the primary structure of P1 and assist in identification of its functional domains (9).

In this report a λgt11 recombinant DNA expression library of M. pneumoniae is constructed and used to characterize the P1 domain involved in cytadherence. Clones expressing P1 epitopes were identified by screening the library with two categories of anti-P1 mAbs known to block M. pneumoniae attachment to erythrocytes and respiratory epithelium. Clones were sequenced and their position located in the P1 structural gene. Since adhesin P1 is a major immunogen of M. pneumoniae (10), these clones were also tested for their reactivity with acute and convalescent sera from M. pneumoniae-infected patients. This experimental approach permits the identification of antigenic and biofunctional determinants of adhesin P1.

Materials and Methods

λgt11 DNA arms and phage extracts were purchased from Promega Biotech (Madison, WI). Enzymes used for constructing the genomic library were from New England Biolabs (Beverly, MA); restriction enzymes were from Bethesda Research Laboratories (Gaithersburg, MD).

M. pneumoniae strain M129-B16 genomic DNA was sheared and ligated to expression vector λgt11 according to the procedures described by Young and Davis (11, 12). The genomic library was screened with a pool of two anti-P1 mAbs designated 5B8 and 6E7, which recognize distinct epitopes and block M. pneumoniae cytadherence (13). The recombinant phages were grown and DNA was extracted as described by Maniatis et al. (14). The inserts from the different clones were subcloned into M13mp19 phage and sequenced by the dideoxy chain termination method of Sanger et al. (15).

M. pneumoniae genomic DNA was cut with different restriction enzymes and transferred to nitrocellulose (NC) filters according to Southern (16). Hybridizations were performed

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FIGURE 1. Immunoblot of adhesin-related fusion proteins using anti-P1 mAbs. (Lane A) Total M. pneumoniae proteins reacted with a pool of the two mAbs designated 5B8 and 6E7 (see text). (Lane B) The β-galactosidase protein reacted with an mAb to β-galactosidase (Promega Biolab). (Lanes C and D) Clones P1-7 and P1-9, respectively, reacted with mAb 6E7. (Lane E) Clone P1-10 reacted with mAb 5B8.

at 68°C (14). Recombinant proteins from individual clones were transferred from plate lysates as described by Trevino et al. (17). Samples were electrophoresed on a 5% polyacrylamide gel and then transferred to NC paper for immunoblotting (18). Also, recombinant proteins were transferred to NC paper using both immunophagoc and Western blot methodologies. Phages were dotted at two different concentrations on a lawn of Y1090 strain of Escherichia coli and plates were incubated at 42°C for 3.5 h. Then, an NC filter (HAHY; Millipore, Bedford, MA) previously saturated with 10 mM isopropyl-β-D-thio galactopyranoside (IPTG) was overlaid on individual plates and incubation was continued at 37°C overnight. Filters were removed and processed as described by Young and Davis (11, 12). These filters were treated with sera from M. pneumoniae-infected patients kindly provided by Dr. Joseph Tully (National Institute of Allergy and Infectious Diseases, Frederick, MD).

Results and Discussion

A pool of two anti-P1 mAbs directed against unique M. pneumoniae epitopes involved in cytadherence was used to probe a λgt11 library of M. pneumoniae genomic DNA. 10 independent clones that produced strong signals were isolated. Eight of the clones reacted with both mAbs; one clone, P1-7, reacted only with mAb 6E7 and another clone, P1-10, reacted only with mAb 5B8. Fusion proteins expressed by these clones were analyzed by Western blot. As shown in Fig. 1, representative clones produced fusion proteins larger than the control λgt11 β-galactosidase protein. However, except for clone P1-7, the size of the fusion proteins is much smaller than what would be expected from the cloned DNA inserts (Fig. 2A). This early termination is probably due to the presence of the TGA codon at position 4557, which M. pneumoniae uses for tryptophan in contrast to E. coli, which reads UGA as a stop signal (9, 19). By comparing the sequence of these clones with the P1 gene sequence (9), we found that each mapped near the COOH-terminal region of the P1 gene.

The fact that clone P1-7 reacts only with mAb 6E7 was helpful in studying P1 epitopes involved in cytadherence. As shown in Fig. 2B, clone P1-7 starts at position 4067 and ends at position 4185; clone P1-9 starts at position 4148 and extends beyond the end of the P1 gene. These two clones share a stretch of nucleotides between positions 4148 and 4185. This fragment codes for 13 amino acids and contains the epitope reactive with the cytadherence-blocking mAb.
FIGURE 2. (A) Location of the 10 XgtI clones within the P1 structural gene. The predicted fusion protein size and DNA insert size of each clone are given. Molecular mass values of the M. pneumoniae fusion proteins were calculated by subtracting the value of the β-galactosidase protein (116 kD). The location and dimension of the insert size are shown (—). As described in text, a TGA stop codon exists just downstream from the EV site. (B) Gene sequence and deduced protein sequence of the epitope involved in cytadherence by M. pneumoniae. The 13 amino acids within which the epitope is located are underlined. (▼) Start of clone P1-7; (▲) end of clone P1-7; (*) start of clone P1-9; (▼) start of clone P1-10. The stop codon is indicated by the box.

6E7. Also, clone P1-10 starts at position 4202 and extends beyond the P1 gene. This clone is nonreactive with mAb 6E7 yet shares a stretch of nucleotides that overlap with clone P1-9 further demarcating the 13 amino acid cytadherence-
related epitope. Southern blot analysis of *M. pneumoniae* DNA that was cut with different restriction enzymes and hybridized to clone P1-7 shows that the stretch of 13 amino acids occurs only once in the genome (Fig. 3) clearly demonstrating the single copy nature of this cytadherence-related epitope in *M. pneumoniae*.

Studies have shown that adhesin P1 is highly immunogenic (4, 10), and patients infected with *M. pneumoniae* elicit antibodies to the P1 adhesin (10). Since the isolated clones express P1 epitopes implicated as mediators of the cytadherence event, we analyzed these clones for reactivity with patient sera at early and late stages of infection. When the immunophage or Western blot methodologies were used, all 10 clones that produced fusion proteins detected by the anti-P1 mAbs (Fig. 2A) also reacted with acute and convalescent sera of *M. pneumoniae*–infected patients (Fig. 4). These fusion proteins were nonreactive with normal human sera.

This experimental approach has permitted the localization of a key domain of adhesin P1 that mediates the recognition event between virulent *M. pneumoniae* and respiratory epithelium. It is interesting that the COOH terminus of the P1 protein is proline rich (13 of the last 26 amino acids are proline), which may anchor the COOH-terminal end in the membrane (20). Adjacent to these sequences is the biofunctionally related, highly immunogenic domain of the *M. pneumoniae* adhesin. It appears that the cytadherence-related P1 epitopes de-
scribed here will be useful for the serodiagnosis of patients infected with *M. pneumoniae*. In addition, a synthetic peptide that includes the 13 amino acid sequence and neighboring regions should be considered in the development of rational vaccine candidates and antibody-capture formats for rapid diagnosis.

**Summary**

A genomic library of *Mycoplasma pneumoniae* was constructed by cloning sheared genomic DNA into the expression vector Agt11. Recombinant clones were screened using anti-*M. pneumoniae* mAbs reactive with adhesin P1 epitopes that mediate cytadherence. 10 clones with different size inserts were isolated. These clones possessed P1 sequences localized to the COOH terminus of the P1 gene. All clones produced fusion proteins that reacted with acute and convalescent sera of patients infected with *M. pneumoniae*.

Interestingly, one clone, P1-7, contained an epitope that was confined to a region of 13 amino acids present in the *M. pneumoniae* genome as a single copy. The identification of this cytadherence-related epitope permits the production of a synthetic peptide that can be used as a rational vaccine candidate and serodiagnostic probe.

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**References**


