INFECTION AND IMMUNITY, Aug. 1987, p. 1830–1836
0019-9567/87/081830-07$02.00/0

Vol. 55, No. 8

The Principal Protein Antigens of Isolates of *Mycoplasma pneumoniae* as Measured by Levels of Immunoglobulin G in Human Serum Are Stable in Strains Collected over a 10-Year Period

AN CONG VU,[1] HJORDIS M. FOY,[2] FRANK D. CARTWRIGHT,[1] AND GEORGE E. KENNY[1]*

Department of Pathobiology[1] and Department of Epidemiology,[2] School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98195

Received 21 January 1987/Accepted 22 April 1987

To determine whether antigenic variation in protein antigens of *Mycoplasma pneumoniae* occurred over time, 12 isolates obtained from pneumonia patients over a 10-year period (1964 to 1974) were compared by immunoblotting (Western blotting) against acute and convalescent human serum samples obtained from the same patients. The strains selected were isolated from patients who had low anti-lipid complement-fixing antibody titers in their acute-phase serum samples and high titers in their convalescent-phase serum samples. The polypeptide composition of the strains was closely similar by protein staining even when compared with prototype FH-Liu. On immunoblotting, all strains showed five bands (170, 130, 90, 45, and 35 kilodaltons [kDa]) which were stained more intensely by convalescent-phase than by acute-phase specimens. A sixth band (62 kDa) was detected by the conjugate alone. In FH-Liu, one band (110 kDa) was prominently stained by convalescent-phase specimens; this band was much less apparent in all of the clinical isolates. Two isolates possessed an additional band (92 kDa) which was stained more prominently by some but not all convalescent-phase specimens. Because of its known antigenic relationships and culture similarities, *Mycoplasma genitalium* was used for comparison. More polypeptides of *M. genitalium* than of *M. pneumoniae* were recognized by acute-phase serum samples, and 4 of 12 convalescent-phase serum samples showed increases in antibodies to certain *M. genitalium* polypeptides. However, these reactive polypeptides did not correspond in molecular mass to polypeptides recognized in *M. pneumoniae*; thus the signature profile of human convalescent-phase specimens with *M. pneumoniae* was distinct. These five polypeptides, individually or in combination, are especially promising for use in detection of human serum antibodies by enzyme-linked immunosorbent assay because they were found in all *M. pneumoniae* isolates tested.

Mycoplasma pneumoniae is a frequent cause of acute respiratory illnesses and is estimated to be the etiologic agent of 10 to 20% of all outpatient pneumonias (1, 6, 12). Laboratory diagnosis of *M. pneumoniae* pneumonia is usually based upon a fourfold rise in titer demonstrated between acute- and convalescent-phase sera (26). Opportunities for isolation of the organism are few because clinical laboratories seldom offer isolation services for the organism. The complement fixation (CF) test (26) and the metabolic inhibition test (39, 41) are both based on the detection of antibodies to glycolipids of *M. pneumoniae* (40). These glyceroglycolipids cross-react with glycolipids found in various animal and even plant tissues (27). Furthermore, serum samples from patients with proven bacterial meningitis often show a fourfold rise in CF titer against *M. pneumoniae* glycolipids (28, 29, 38) which did not show any antibody increase by immunoblotting (29), indicating that the CF test can give false-positive reactions. It is to be expected that protein antigens provide for more specific serodiagnostic tests. Recent studies (16, 18, 19, 32) show that protein antigens of *M. pneumoniae* react with serum samples from humans or animals infected with *M. pneumoniae*. A surface protein, designated P1 (3, 15, 16), has been well characterized as an attachment protein of *M. pneumoniae* to host cells (3, 15, 17, 20, 21), and most serum samples from patients with *M. pneumoniae* pneumonia show an immune response to it (19, 32).

The primary purpose of our study was to define the *M. pneumoniae* protein antigens toward which humans consistently make an immunoglobulin G (IgG) response during culture-proven *M. pneumoniae* pneumonia. A second purpose of the study was to determine the degree of homogeneity or heterogeneity among *M. pneumoniae* strains observed over a 10-year period as detected by IgG antibodies in human serum samples. A third purpose was to search for cross-reacting antibodies to polypeptides of the closely related *Mycoplasma genitalium*.

MATERIALS AND METHODS

Selection of strains and serum samples from patients. *M. pneumoniae* strains were selected from isolates stored at −70°C which had been grown on artificial medium no more than twice since isolation from patients (12). The following criteria were used to select patients and their isolates: positive chest X ray confirming the diagnosis of pneumonia, an isolate of *M. pneumoniae*, a greater-than-fourfold antibody rise with a final anti-lipid CF titer of 64 or greater, and availability of paired serum samples of good quality and sufficient quantity (Table 1). Twelve isolates were selected from isolates obtained from 1964 to 1974 (including two in 1967 and two in 1974 but none in 1973). This process resulted in the selection of 12 patients (10 females and 2 males) ranging in age from 8 to 39 years. Isolates and serum samples were identified by study numbers used for the patients. Also included in the study as references were strain AP-1046, cloned three times in passage 6 from the patient (14) and strain FH-Liu (8), obtained from the National Institutes of

* Corresponding author.

TABLE 1. Characteristics of patients, serum samples, and *M. pneumoniae* strains used in this study

Strain and patient no.	Yr isolated	Patient		Antilipid CF titer		Lane no.[a]
		Age (yr)	Sex	Acute phase	Convalescent phase	
597	1964	8	F	4	64	1
1798	1965	15	M	2	256	2
3519	1966	39	F	2	256	3
4504	1967	9	F	2	256	4
5555	1967	12	F	2	256	5
6284	1968	30	F	4	128	6
6644	1969	13	F	2	64	7
7870	1970	39	M	8	256	8
9607	1971	11	F	2	128	9
11544	1972	36	F	4	128	10
14366	1974	12	F	2	128	11
15947	1974	33	F	2	128	12
FH-Liu	1961					13
AP-1046	1966					14

[a] Lane numbers are used for identification of strains in Fig. 1 to 4.

Health, which is known to have been grown for at least 200 passages in vitro. The paired human specimens had been stored at −20°C. The convalescent-phase serum samples had been collected 22 to 37 days after the acute-phase samples.

Cultivation of organisms. Strains of *M. pneumoniae* were grown in a dialysate broth (22) derived from soy-peptone (Hy Soy; Sheffield Products, Memphis, Tenn.) and autoclaved fresh yeast (Fleishman's active dry yeast; Nabisco Brands Inc., East Hanover, N.J.), supplemented with 10% agamma horse serum (Alpha Gamma Laboratories, Sierra Madre, Calif.) and 100 U of penicillin per ml. Cultures were incubated at 37°C in 800-ml volumes in 32-oz (946-ml) bottles with agitation from a slowly turning spin bar. Cultures (2.4 liters for each strain) were harvested when they became hazy and their pH began to change. Pellets were washed three times in 5 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid]-buffered saline, suspended at a 10^3-fold-higher concentration, and stored in four aliquots at −70°C until used. No more than three additional passages ensued before the organisms were harvested; thus, the isolates had been passed a total of five times. Protein was determined by the method of Bradford (5) on samples of sonicated *M. pneumoniae* with bovine serum albumin as standard.

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Laemmli (31) on 1.5-mm-thick gels made of 8 and 5% acrylamide in the separating and stacking gels, respectively. Each *M. pneumoniae* antigen was boiled for 5 min in sample buffer containing 1% 2-mercaptoethanol, and 10 μg of protein from each sample was loaded on each 5.4-mm-wide well. All strains were then electrophoresed on the same gel until the tracking dye was 1 cm above the bottom of the gel. The gels were either stained overnight with Coomassie brilliant blue (0.05%) for protein or used for Western blotting.

Immunoblotting (Western blot). *M. pneumoniae* proteins were transferred onto nitrocellulose paper by the method of Towbin et al. (42). Each run was performed overnight at 120 mA in a Hoeffer transblot unit. Unoccupied sites on the blots were saturated by a blocking buffer containing 0.25% gelatin and 0.1% Tween 20. The nitrocellulose sheets, each bearing transferred materials from all 14 strains, were exposed to each human serum sample (1:100 dilution) for 1 h. Unbound materials were removed by extensive washing for 30 min in phosphate-buffered saline containing 0.1% Tween 20. Horseradish peroxidase-labeled goat anti-human IgG antiserum (affinity purified, gamma chain specific; Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted 1:100 was then allowed to react with the bound human IgG on the blots for 1 h. Control blots were developed directly with the conjugated serum (also at 1:100 dilution) without prior exposure to human serum. After the blots were extensively washed with phosphate-buffered saline–0.1% Tween 20, the color-developing solution (0.05% H_2O_2, 0.05% 4-chloro-1-naphthol) was added for color development for 15 min. The stained blots were then washed in distilled water, air dried, and stored away from light.

Molecular weight determination. Calibration curves for molecular weight estimation of bands on gels or blots were established by using different sets of standard molecular weight markers run on gels made of various concentrations of acrylamide (6, 8, and 10%). Molecular weights were estimated by plotting relative electrophoretic mobilities (R_f) on the appropriate calibration curve.

Identification and scoring of bands on immunoblots. Bands of interest revealed by blots were identified on the gel by their relative electrophoretic mobility. An arbitrary scoring system was used to estimate the importance of polypeptides reacting to human serum antibodies. A score of − (nonreacting), 1, 2, 3, 4, or 5 was assigned to each band according to the intensity (darkness) and the area of the antibody-stained band (Table 2).

Controls. The following two controls were used: (i) *M. genitalium* G37 (43) grown in agamma horse serum (24) and (ii) *M. pneumoniae* AP-1046 grown in rabbit serum. The culture conditions for controls were similar to those described above, except that 5.6 mM $NaHCO_3$ was included in the medium for the *M. genitalium* strain and these cultures were equilibrated with 2.5% CO_2 in air for 2 days before being incubated with agitation until the culture became hazy and the pH began to change.

RESULTS

Protein profiles of *M. pneumoniae* strains. Twelve *M. pneumoniae* strains isolated in Seattle from patients with proven *M. pneumoniae* pneumonia during the 10-year period from 1964 to 1974 were compared by their protein profiles and their immunostaining patterns with human serum antibodies obtained from these patients (Table 1). Seattle standard strain AP-1046 (14) and the prototypic strain FH-Liu (8) also were included as references. When all the strains were analyzed together by SDS-PAGE under reducing conditions, their protein profiles were almost identical (Fig. 1). The 12 isolates and the reference strains yielded essentially the same number of polypeptides on the gel, and these polypeptides did not show any appreciable variation in their apparent molecular weight from one strain to another. The only observable dissimilarity between these protein profiles appeared to be a slight variation in the density of the band with an apparent molecular mass of 70 kilodaltons (kDa) (Fig. 1).

Immunoblot patterns of human serum samples. Each convalescent-phase serum sample produced nearly identical patterns when tested with any of the 14 strains (Fig. 2 and 3). Although certain samples reacted with few polypeptides (Fig. 2) and others reacted with many (Fig. 3), the 12 convalescent-phase samples consistently showed increased antibodies to five bands of molecular mass ca. 170, 130, 90, 45, and 35 kDa (Table 2). The molecular masses of these

TABLE 2. Relative reactivity of 12 paired human serum samples to different *M. pneumoniae* polypeptides

Patient and serum no.	Reactivity[a] to polypeptide of molecular size (kDa):															
	170		130		110		92		90		62		45		35	
	A[b]	C[b]	A	C	A	C	A	C	A	C	A	C	A	C	A	C
597	–	5	–	1	–	–	–	–	–	2	1	1	–	1	–	1
1798	1	5	–	2	–	1	–	–	–	3	1	1	–	1	–	1
3519	1	5	1	2	1	1	–	1	1	2	1	1	1	1	1	1
4504	–	5	–	1	–	1	–	–	–	4	1	1	–	1	–	1
5555	–	2	–	1	–	1	–	–	–	3	1	1	–	1	–	1
6284	–	5	–	2	–	–	–	–	–	1	1	1	1	1	–	1
6644	–	5	–	2	–	–	–	1	–	2	1	1	–	1	–	1
7870	–	5	–	2	–	1	–	1	–	3	–	1	–	1	–	1
9607	–	5	–	2	–	1	–	1	–	4	1	1	–	1	–	1
11544	–	5	–	2	–	1	–	1	–	4	–	1	–	1	–	1
14366	–	5	–	2	–	1	–	–	–	4	1	1	–	1	–	1
15947	1	5	–	2	–	1	–	1	3	3	3	1	1	2	1	2
Total score	3	60	1	21	1	9	0	6	4	35	12	12	3	13	2	13

[a] –, Nonreactive; 1 to 5, increasing degree of reactivity as estimated by darkness or area of the band.
[b] A, Acute-phase serum sample; C, convalescent-phase serum sample.

polypeptides were determined by their relative electrophoretic mobility (arrows on Fig. 1). Although the strains in lanes 6 and 11 were similar on protein stain (Fig. 1), they stained less well with antibody (Fig. 2 and 3).

The strongest antibody staining was consistently observed with the 170- and 90-kDa bands (Fig. 2 and 3; Table 2). The 170-kDa polypeptide appears to correspond to P1 (19, 32). Some (6 of 12) but not all convalescent-phase serum samples recognized a 92-kDa band which stained more intensely in strains 3519 and 11544 (Fig. 3, lanes 3 and 10) than in the remaining strains. The high-passage prototypic strain FH-Liu, in addition to the common antibody staining profile, had a 110-kDa polypeptide which was stained more prominently by all convalescent-phase serum samples than was the similar polypeptide in any other strain (thin arrows in Fig. 2 and 3, lane 13).

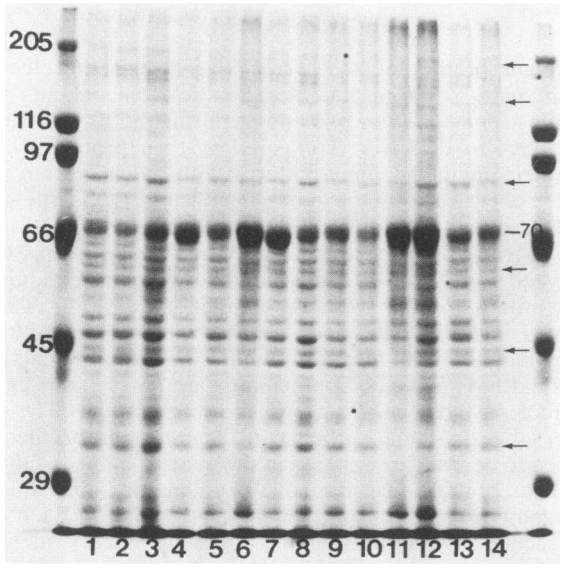

FIG. 1. SDS-PAGE protein profile of 12 isolates and two reference strains of *M. pneumoniae* as stained with Coomassie brilliant blue. The extreme left and right lanes represent the molecular weight markers. Arrows indicate locations and probable identity of common polypeptides recognized by all 12 human convalescent-phase serum samples. Lane numbers are identified in Table 1.

In contrast to the convalescent-phase serum samples, the great majority (11 of 12) of the acute-phase serum samples did not produce any distinctive antibody staining pattern (Fig. 4). Only acute-phase serum sample 15947 showed a fairly strong reactivity with the 90-kDa band (Table 2). Of 12 acute-phase samples, 10 reacted with a 62-kDa band found in all 14 strains. Two acute-phase samples did not react at all with any *M. pneumoniae* polypeptide on the blot, and the remaining samples reacted faintly with the principal bands (170, 130, 90, 45, and 35 kDa; Table 2).

Scoring of immune response to individual polypeptides. The reactivity of all acute- and convalescent-phase serum samples with common *M. pneumoniae* polypeptides was deter-

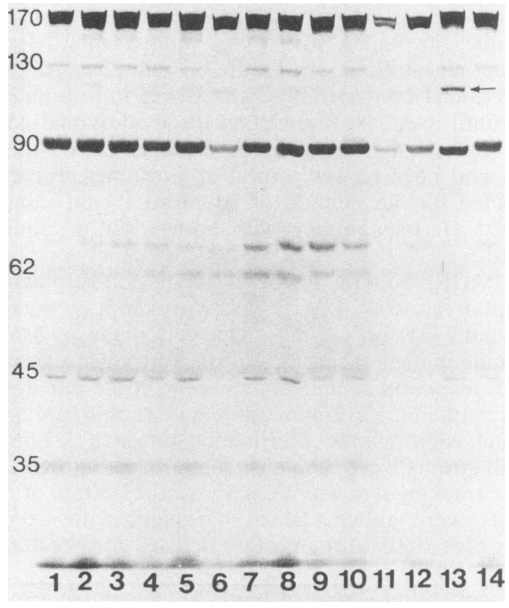

FIG. 2. Western blot of 14 *M. pneumoniae* strains stained with human convalescent-phase serum sample 11544 to illustrate the staining properties of moderately reactive human serum. Numbers on the left-hand side correspond to the approximate molecular masses (in kilodaltons) of the bands commonly seen by all 12 convalescent-phase samples. The arrow in lane 13 indicates the darkly stained 110-kDa band in the prototypic strain FH-Liu. For strain identification, see Table 1.

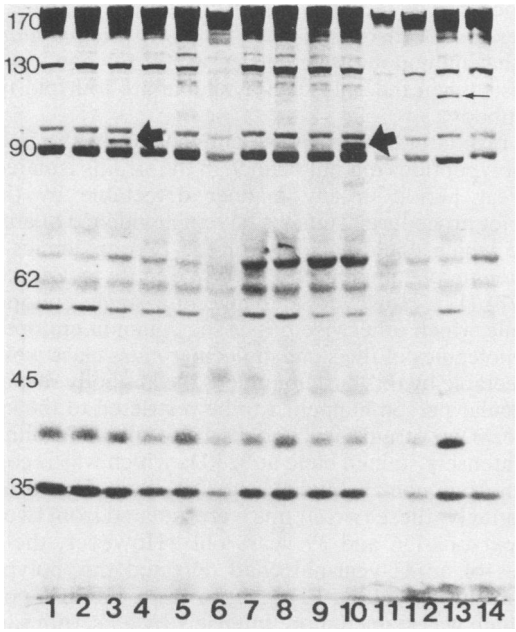

FIG. 3. Immunoblot of the *M. pneumoniae* strains stained with human convalescent-phase serum sample 9607 to illustrate the staining properties of strongly reactive human serum and to show the dark 92-kDa band in strains isolated from patients 3519 and 11544 (thick arrows in lanes 3 and 10). The thin arrow in lane 13 points toward the more prominently reacting 110-kDa band unique to the FH-Liu strain. For strain identification, see Table 1.

mined by using the scoring system mentioned in Materials and Methods (Table 2). Since each sample produced nearly identical profiles against all 14 strains in terms of the number and intensity of the six major bands, it was possible to assign

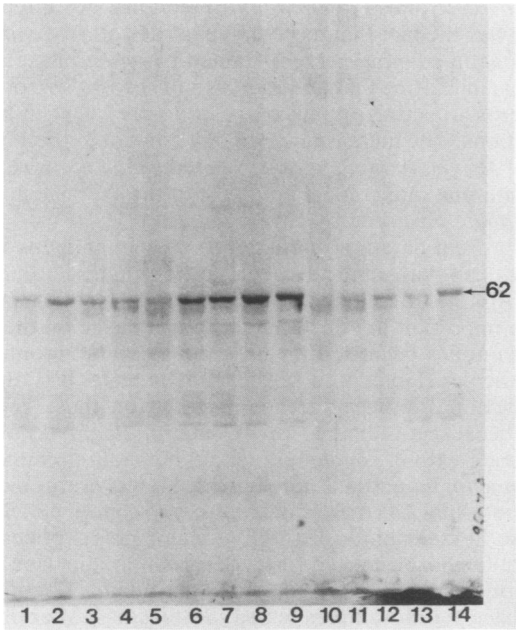

FIG. 4. Typical immunoblot of the *M. pneumoniae* strains stained with human acute-phase serum sample 6644. The arrow indicates the 62-kDa band which reacted with most (10 of 12) acute-phase samples.

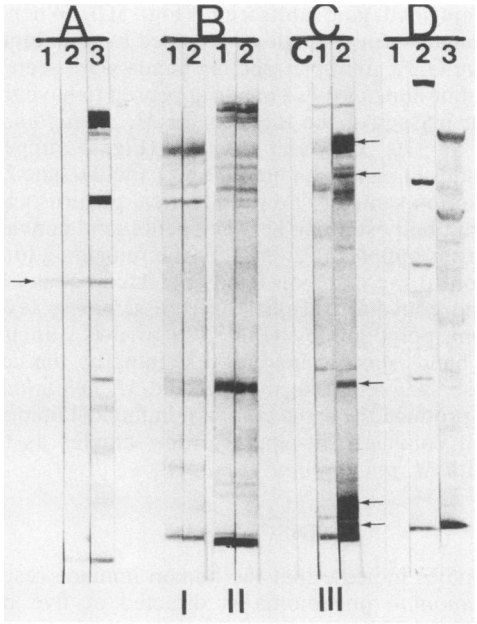

FIG. 5. Comparison of immunostaining of *M. pneumoniae* and *M. genitalium* with paired human serum samples. (A) Immunoblots of *M. pneumoniae* stained with conjugated goat anti-human IgG antiserum alone (lane 1), acute-phase human serum (lane 2), and convalescent-phase human serum (lane 3) to show nonspecific staining of the 62-kDa band (arrow). (B and C) Immunoblots of *M. genitalium* stained with paired human serum samples (acute phase [lanes 1] and convalescent phase [lanes 2]) 1798 (I), 3519 (II), and 11544 (III). Lane C (control) in group III was stained with conjugated goat anti-human IgG antiserum alone. In panel C, arrows indicate the polypeptides of *M. genitalium* toward which an increase in antibody level was observed. (D) Immunoblots of *M. pneumoniae* (lane 2) and *M. genitalium* (lane 3) stained with human convalescent-phase serum sample 11544 to contrast the characteristic signature of human serum antibodies specific for *M. pneumoniae* antigens with the pattern they produced in the *M. genitalium* antigens. Note the absence of P1 in panel D, lane 3 (P1 is the top band in panel D, lane 2). Panel D, lane 1, shows that the nonspecific staining of the 62-kDa band by conjugated goat anti-human IgG antiserum also occurred with *M. pneumoniae* grown in rabbit serum.

one overall score for each serum reactivity to an individual polypeptide. The sum of scores at the bottom of Table 2 is therefore representative of the collective reactivity of all 12 serum samples of each category (acute or convalescent phase) against each individual *M. pneumoniae* polypeptide. Absolute total scores could be used as an indicator of the strength of human antibody response to a particular polypeptide, whereas differences between acute- and convalescent-phase scores reflect the magnitude of antibody rise in paired human serum samples. Because of the intensity of staining, the highest scores were found for the 170-kDa band and the second- and third-highest scores were found for the 90- and 130-kDa bands, respectively. Clear-cut differential scores are seen with two (45 and 35 kDa) of the remaining three common polypeptides. The 62-kDa band appeared to react equally well with acute- and convalescent-phase serum samples.

Controls. In the control conjugate blot, a 62-kDa polypeptide of *M. pneumoniae* stained with the conjugated goat anti-human IgG serum (Fig. 5A) regardless of the nature of the serum used for growing the antigen (rabbit or agamma horse). No *M. genitalium* antigen stained directly with the

same conjugated goat antiserum (Fig. 5C). When the 12 paired human serum samples were used to stain blots of *M. genitalium* G-37, multiple reacting bands were seen with all samples, but none of these bands appeared to have the same molecular masses of the three major *M. pneumoniae* polypeptides at 170, 130, and 90 kDa (Fig. 5, upper three polypeptides in panel A, lanes 3, and panel D, lane 2). Eight pairs of serum samples showed identical patterns and intensities of antibody staining between acute- and convalescent-phase serum samples (Fig. 5B). In the remaining four pairs, in addition to the common bands detected equally by both acute- and convalescent-phase serum samples, several *M. genitalium* polypeptides (Fig. 5C, arrows), including a 110-kDa band, showed increased staining by the convalescent-phase sample. In no instance did *M. genitalium* polypeptides produce the same common immunostaining pattern of human convalescent-phase serum samples as that obtained with *M. pneumoniae* (Fig. 5D).

DISCUSSION

Our studies indicate that the human immune response in *M. pneumoniae* pneumonia is directed at five principal polypeptides of *M. pneumoniae* and that the antigenic specificities of these polypeptides did not change in strains collected over a 10-year period. Although certain human convalescent-phase serum samples showed responses to 16 to 20 polypeptides of *M. pneumoniae* (Fig. 3), all human convalescent-phase samples tested consistently showed antibody increases to five polypeptides with molecular masses of 170, 130, 90, 45, and 35 kDa. In addition, polypeptides of 110 and 92 kDa were recognized by some but not all serum samples. The acute-phase samples, initially selected for low anti-lipid CF antibody titers, showed no antibodies or low levels of antibodies to the five major polypeptides as judged by intensity of the staining reactions (Table 2; Fig. 4). One polypeptide, with a molecular mass of 62 kDa, reacted with the conjugated antiserum alone, a reaction also observed in organisms grown in rabbit serum, indicating that it was not a horse serum contaminant. It did not appear to be immunogenic for humans either, since the intensity of antibody staining of this polypeptide was essentially the same with acute- and convalescent-phase samples. Possibly, this polypeptide binds the IgG molecule nonspecifically.

The general reactivity pattern of human sera to *M. pneumoniae* on immunoblot has been seen by others (16, 18, 19, 32), and most studies have focused on the prominent and well characterized adhesin P1. Estimates of the molecular masses of the P1 protein have differed: 170 kDa by us, 190 kDa by Hu et al. (18), 165 kDa by Baseman et al. (3), and 168 kDa by Jacobs et al. (19). These differences may reflect the difficulties in determining molecular masses of large polypeptides by SDS-PAGE. We used 6% gels for our estimate.

Although there has been considerable speculation concerning possible complications of *M. pneumoniae* infections such as neurological diseases (2, 9, 28, 34, 35, 37), most data available are from serological studies with the glycolipid antigens of *M. pneumoniae*, which are known to cross-react (23, 27). Samples from persons with proven bacterial meningitis were shown to have significant CF antibody titers to *M. pneumoniae* (28), but such samples do not show antibody increase to its protein antigens by immunoblot (29), indicating that the anti-lipid antibody titer increases were not a result of *M. pneumoniae* infection. The frequent occurrence of *M. pneumoniae* infections in the general population (1, 10, 12, 13) also suggests that dual infection with other organisms could occur. The fact that human convalescent-phase serum samples give a distinct pattern with *M. pneumoniae* indicates that immunoblotting can be useful for confirming the diagnosis when the only data available are fourfold rises in CF antibody.

Our results indicate that the antigenicity of the five principal polypeptides did not change in the strains isolated over a 10-year period in any manner detectable by the immunoblot procedure. Thus we have no evidence of antigenic change which could account for the two epidemics of *M. pneumoniae* pneumonia observed in Seattle in 1965 to 1966 and 1974 (12). However, a change in a single epitope on a molecule which otherwise possesses common epitopes with other molecules of the same molecular mass class would not be detectable by this system, unless the antibody response of a particular person happened to be restricted to the specific epitope. Two strains isolated in 1966 and 1972 showed a more intensely stained band at 92 kDa which was recognized by some but not all human sera (compare Fig. 2 and 3). Interestingly, these two strains were isolated from two of the older persons (36 and 39 years old). However, the serum samples of an 11-year-old child detected this polypeptide also. In addition, strain FH-Liu showed a 110-kDa polypeptide which was stained more intensely by all serum samples. Even so, any of these strains of *M. pneumoniae* could be used to identify *M. pneumoniae* infections by immunoblotting, because all show the same five signature polypeptides. This result is not surprising in view of the close genetic relatedness observed with strains of *M. pneumoniae* (7).

M. genitalium was used as a control because it is known to cross-react with *M. pneumoniae* both in protein and in lipid antigens (24, 33). Furthermore, because of cross-reactivity between these organisms, antibodies from infection with one would certainly confuse the interpretation of serological results in the absence of an isolate of the organism. Since human serum samples gave a signature with *M. pneumoniae* distinctly different from that with *M. genitalium*, immunoblotting could be used as a means of distinguishing *M. pneumoniae* from *M. genitalium* infections. An interesting finding was the fact that more polypeptides of *M. genitalium* than of *M. pneumoniae* were stained by acute-phase serum samples, indicating that antibodies to *M. genitalium* or some other cross-reacting organism are quite prevalent in human populations. The increase in antibody content to the 110-kDa band of *M. genitalium* might be related to the cross-reaction seen with one monoclonal antibody to the P1 protein of *M. pneumoniae* observed by Clyde and Hu (11).

The present data are restricted to protein antigens whose epitopes can withstand the denaturation that occurs during SDS-PAGE and immunoblotting. Clearly, labile epitopes may be important in infections, but we focused on the more stable epitopes because they have considerable promise for use in the development of serodiagnostic tests. It is unlikely that glycolipid antigens are represented on these gels and blots, because antibodies to *M. neurolyticum* (known to cross-react with *M. pneumoniae*) did not stain immunoblots of *M. pneumoniae* (data not shown). However, an increase in the anti-lipid CF titer appeared to correlate well with the increase in stainability of the five major polypeptides. The data are further restricted to IgG-class antibodies and to the polypeptides commonly recognized by all the 12 convalescent-phase serum samples tested. Studies of IgM antibodies were not attempted because the samples had been stored for 10 to 20 years.

Because it is known that the use of antigenic mixtures such as disrupted whole organisms in an enzyme-linked

immunosorbent assay (ELISA) may permit only low antibody titers (25), we plan to apply these results to the selection of appropriate antigens to purify for use in ELISA. Our data and those of others (3, 4, 15, 16, 18, 20, 30) suggest the following for improvement of ELISA for *M. pneumoniae* infections. Specific tests could be devised that involve the use of the 170-, 130-, or 90-kDa polypeptide. Because of its reactivity with conjugates, the 62-kDa polypeptide should be excluded from the antigens to be used for ELISA. The two lower-molecular-weight antigens, 45 and 35 kDa, might not be good choices, because it is not clear that they can be distinguished from antigens of *M. genitalium* (Fig. 5). In addition to the cross-reaction between certain monoclonal antibodies to P1 with an *M. genitalium* polypeptide of 110 kDa (11), Morrison-Plummer et al. (36), using monoclonal antibodies, have shown a cross-reaction between P1 and a 140-kDa component of *M. genitalium*. Both findings suggest a need for caution in using P1 as an antigen for ELISA.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grants AI06720 and AI12192 from the National Institute of Allergy and Infectious Diseases and by Public Health Training grant 702605335 from the National Institutes of Health.

We thank S. Razin for helpful criticism of the manuscript.

LITERATURE CITED

1. **Alexander, E. R., H. M. Foy, and G. E. Kenny.** 1966. Pneumonia due to *Mycoplasma pneumoniae*. N. Engl. J. Med. **275:** 131–136.
2. **Assaad, F., R. Gispen, and M. Kleemola.** 1980. Neurological diseases associated with viral and *Mycoplasma pneumoniae* infections. Bull. W.H.O. **58:**297–311.
3. **Baseman, J. B., R. M. Cole, D. C. Krause, and D. K. Leith.** 1982. Molecular basis for cytadsorption of *Mycoplasma pneumoniae*. J. Bacteriol. **151:**1514–1522.
4. **Baseman, J. B., D. L. Drouillard, D. K. Leith, and J. G. Tully.** 1984. Absence of *Mycoplasma pneumoniae* cytadsorption protein P1 in *Mycoplasma genitalium* and *Mycoplasma gallisepticum*. Infect. Immun. **43:**1103–1106.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Cassel, G. H., and B. C. Cole.** 1981. Mycoplasmas as agents of human disease. N. Engl. J. Med. **304:**80–89.
7. **Chandler, D. F., S. Razin, E. B. Stephens, R. Harasawa, and M. F. Barile.** 1982. Genomic and phenotypic analysis of *Mycoplasma pneumoniae* strains. Infect. Immun. **38:**604–609.
8. **Chanock, R. M., L. Hayflick, and M. F. Barile.** 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Natl. Acad. Sci. USA **48:**41–49.
9. **Clyde, W. A., Jr.** 1980. Neurological syndromes and mycoplasma infection. Arch. Neurol. **37:**65–66.
10. **Clyde, W. A., Jr.** 1983. *Mycoplasma pneumoniae* respiratory disease symposium: summation and significance. Yale J. Biol. Med. **56:**523–527.
11. **Clyde, W. A., Jr., and P. C. Hu.** 1986. Antigenic determinant of the attachment protein of *Mycoplasma pneumoniae* shared by other pathogenic *Mycoplasma* species. Infect. Immun. **51:**690–692.
12. **Foy, H. M., G. E. Kenny, and M. K. Cooney.** 1979. Long term epidemiology of infections with *Mycoplasma pneumoniae*. J. Infect. Dis. **139:**681–687.
13. **Foy, H. M., G. E. Kenny, and R. MacMahan.** 1970. *Mycoplasma pneumoniae* in an urban area. J. Am. Med. Assoc. **214:**1666–1672.
14. **Gale, J. L., and G. E. Kenny.** 1970. Complement dependent killing of *Mycoplasma pneumoniae* by antibody. J. Immunol. **104:**1175–1183.
15. **Hu, P. C., R. M. Cole, Y. S. Huang, and A. M. Collier.** 1982. *Mycoplasma pneumoniae* infection: role of a surface protein in the attachment organelle. Science **216:**313–314.
16. **Hu, P. C., C. H. Huang, A. M. Collier, and W. A. Clyde, Jr.** 1982. Demonstration of antibodies to *Mycoplasma pneumoniae* attachment protein in human sera and respiratory secretions. Infect. Immun. **41:**437–439.
17. **Hu, P. C., Y. S. Huang, A. M. Collier, and W. A. Clyde, Jr.** 1985. Demonstration of multiple antigenic determinants on *Mycoplasma pneumoniae* attachment protein by monoclonal antibodies. Infect. Immun. **50:**292–296.
18. **Hu, P. C., Y. S. Huang, J. A. Graham, and D. E. Gardner.** 1981. Identification of immunogens of *Mycoplasma pneumoniae* by protein blotting. Biochem. Biophys. Res. Commun. **103:**1363–1370.
19. **Jacobs, E., A. Bennewitz, and W. Bredt.** 1986. Reaction pattern of human anti-*Mycoplasma pneumoniae* antibodies in enzyme-linked immunosorbent assays and immunoblotting. J. Clin. Microbiol. **23:**517–522.
20. **Kahane, I., S. Tucker, and J. B. Baseman.** 1985. Detection of *Mycoplasma pneumoniae* adhesin in the nonhemadsorbing population of virulent *M. pneumoniae*. Infect. Immun. **49:**457–458.
21. **Kahane, I., S. Tucker, D. K. Leith, J. Morrison-Plummer, and J. B. Baseman.** 1985. Detection of the major adhesin P1 in Triton shells of virulent *M. pneumoniae*. Infect. Immun. **50:** 944–946.
22. **Kenny, G. E.** 1967. Heat-lability and organic solvent-solubility of *Mycoplasma* antigens. Ann. N.Y. Acad. Sci. **143:**676–681.
23. **Kenny, G. E.** 1979. Antigenic determinants, p. 352–358. *In* M. F. Barile and S. Razin (ed.), The mycoplasmas, vol. 1. Academic Press, Inc., New York.
24. **Kenny, G. E., and F. D. Cartwright.** 1984. Immunoblotting for determination of antigenic specificities of antibodies to the Mycoplasmatales. Isr. J. Med. Sci. **20:**908–911.
25. **Kenny, G. E., and C. L. Dunsmoor.** 1983. Principles, problems, and strategies in the use of antigenic mixtures for the enzyme-linked immunosorbent assay. J. Clin. Microbiol. **17:**655–665.
26. **Kenny, G. E., and J. T. Grayston.** 1965. Eaton pleuropneumonia-like organism (*Mycoplasma pneumoniae*) complement-fixing antigen: extraction with organic solvents. J. Immunol. **95:**19–25.
27. **Kenny, G. E., and R. M. Newton.** 1973. Close serological relationship between glycolipids of *Mycoplasma pneumoniae* and glycolipids of spinach. Ann. N.Y. Acad. Sci. **225:**54–61.
28. **Kleemola, M., and H. Kayhty.** 1982. Increase in titers of antibodies to *Mycoplasma pneumoniae* in patients with purulent meningitis. J. Infect. Dis. **146:**284–288.
29. **Kleemola, M., H. Kayhty, and R. Raty.** 1983. Reply. Presence of antibodies to *Mycoplasma pneumoniae* in patients with bacterial meningitis. J. Infect. Dis. **148:**363–365.
30. **Krause, D. C., and J. B. Baseman.** 1983. Inhibition of *Mycoplasma pneumoniae* hemadsorption and adherence to respiratory epithelium by antibodies to a membrane protein. Infect. Immun. **39:**1180–1186.
31. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
32. **Leith, D. K., L. B. Trevino, J. G. Tully, and J. B. Baseman.** 1983. Host discrimination of *Mycoplasma pneumoniae* proteinaceous immunogens. J. Exp. Med. **157:**502–514.
33. **Lind, K., B. O. Lindhardt, H. J. Schutten, J. Blom, and C. Christiansen.** 1984. Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. J. Clin. Microbiol. **20:**1036–1043.
34. **Lind, K., H. Zoffman, and S. O. Larsen.** 1979. *Mycoplasma pneumoniae* infection associated with affection of the central nervous system. Acta Med. Scand. **205:**325–332.
35. **Michel, D., B. Laurent, and R. Granouillet.** 1981. Infections aigues recentes et parfois persistantes à *Mycoplasma pneumoniae*, associées à des manifestations neurologiques. Discussion des liens de causalité. Rev. Neurol. (Paris) **137:**393–413.
36. **Morrison-Plummer, J., A. Lazell, and J. B. Baseman.** 1987. Shared epitopes between *Mycoplasma pneumoniae* major ad-

hesin protein P1 and a 140-kilodalton protein of *Mycoplasma genitalium*. Infect. Immun. **55**:49–56.
37. **Ponka, A.** 1979. The occurrence and clinical picture of serologically verified *M. pneumoniae* infections with emphasis in central nervous system, cardiac, and joint manifestations. Ann. Clin. Res. **11**(Suppl. 24):9–60.
38. **Ponka, A., T. Ponka, and S. Sarna.** 1981. Questionable specificity of lipid antigen in the *Mycoplasma pneumoniae* complement fixation test in patients with extrapulmonary manifestations. J. Infect. **3**:332–338.
39. **Senterfit, L. B., and K. E. Jensen.** 1966. Antimetabolic antibodies to *Mycoplasma pneumoniae* measured by tetrazolium reduction inhibition. Proc. Soc. Exp. Biol. Med. **122**:786–790.
40. **Senterfit, L. B., J. D. Pollack, and N. L. Somerson.** 1972. Antibodies to *Mycoplasma pneumoniae*: correlation of complement fixation and tetrazolium reduction inhibition tests. Proc. Soc. Exp. Biol. Med. **140**:1294–1297.
41. **Taylor-Robinson, D., R. H. Purcell, D. C. Wong, and R. M. Chanock.** 1966. A colour test for the measurement of antibody to certain mycoplasma species based upon the inhibition of acid production. J. Hyg. **64**:91–104.
42. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
43. **Tully, J. G., D. Taylor-Robinson, R. M. Cole, and D. L. Rose.** 1983. *Mycoplasma genitalium*, a new species from the human genital tract. Int. J. Syst. Bacteriol. **33**:387–396.