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Immunologic Response of Patients with Legionellosis against Major Protein-Containing Antigens of *Legionella pneumophila* Serogroup 1 as Shown by Immunoblot Analysis

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Major protein-containing antigens of *Legionella pneumophila* serogroup 1 were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis with rabbit antisera to 14 different *Legionella* species or serogroups. Fourteen bands were observed in immunoelectropherograms of whole-cell, sonicated cell, and heated cell preparations, seven of which appeared in the supernatant fluid from the heated cells and three of which were shown in an outer membrane fraction. Immunoblots of whole-cell antigen preparations of 14 *Legionella* species or serogroups revealed seven major *Legionella* proteins: antigens with molecular weights of 58,000, 79,000, and 154,000 were present in all *Legionella* sp. strains, antigens with molecular weights of 44,000 and 97,000 occurred in multiple species, and antigens with molecular weights of 14,000 and 25,000 were present only in *L. pneumophila* strains. All sera from 15 patients with culture-confirmed *L. pneumophila* serogroup 1 disease and 14 of 18 (78%) sera from serologically diagnosed patients reacted with the 58-kilodalton (kDa) common antigen. In contrast, less than one-half of the sera reacted with the *L. pneumophila*-specific proteins (14 and 25 kDa). Absorption of sera with *Escherichia coli* cells had no effect on their reactivity with the 58-kDa antigen, whereas absorption with *L. pneumophila* serogroup 1 cells removed reactivity. These data suggest that the 58-kDa antigen may prove useful in serodiagnostic tests for legionellosis.

The immune response to *Legionella pneumophila* serogroup 1 disease is multiple. Patients' antibodies can react to serogroup-specific, *Legionella* sp.-specific, and nonspecific surface antigens in various combinations (21), and this multiplicity of the immunologic response can confound attempts to determine the identity of the infecting strain serologically. Furthermore, the large number of *Legionella* species (n = 22) and serogroups (n = 33) currently recognized (17) makes diagnostic testing with all known serogroup antigens impractical. We used a soluble, heat-stable, serogroup-specific antigen and an antigen common to at least six *L. pneumophila* serogroups in an enzyme-linked immunosorbent assay (16). Test results compared favorably with results of the indirect immunofluorescence assay (IFA). A detailed chemical analysis of the enzyme-linked immunosorbent assay antigens was not done, but protein was detected in each.

Several investigators have used rabbit antisera to identify protein antigens in *L. pneumophila* serogroup 1 cells that are specific for the species and others that may be more broadly reactive (5–7, 10, 15). Some of these antigens could be useful in serodiagnostic tests for legionellosis if they were adequately sensitive and specific. The purpose of our study was to determine the immunologic response of patients with legionellosis to the major protein antigens of *Legionella* spp. and, from these data, to identify potentially useful enzyme-linked immunosorbent assay antigens.

MATERIALS AND METHODS

Strains and sera. *Legionella* sp. strains were taken from the stock culture collection of the Immunology Section, Centers for Disease Control: *L. pneumophila* serogroup 1 (strain Philadelphia 1), serogroup 2 (Togus 1), serogroup 3 (Bloomington 2), serogroup 4 (Los Angeles 1), serogroup 5 (Dallas 1), serogroup 6 (Chicago 2), serogroup 7 (Chicago 8), and serogroup 8 (Concord 3); *L. bozemanii* (WIGA), *L. dumoffii* (NY-23), *L. micdadei* (TATLOCK), *L. longbeachae* serogroup 1 (Long Beach 4), *L. wadsworthii* (81-716), and *L. feeleii* (WO-44C-C3). *Escherichia coli* O13:K11:H11 was obtained from the Biological Products Program, Centers for Disease Control. The same *Legionella* sp. strains were used to prepare electrophoresis samples and rabbit antisera. Formalinized whole-cell vaccines for antiserum production were prepared as described by Thacker et al. (18). Sera from patients with culture-documented *L. pneumophila* serogroup 1 pneumonia were generously donated by Paul Morgan, Veterans Administration Hospital, Little Rock, Ark., and by Christopher Bentsen, Wilmington Medical Center, Wilmington, Del. Additional sera, submitted to the Immunology Section from patients with suspected Legionnaires disease and with IFA titers showing seroconversion to ≥128, were from the Immunology Section collection. Convalescent-phase titers against *L. pneumophila* antigens for these patients ranged from 256 to ≥2,048. Normal human sera with titers of ≤64 were obtained from healthy volunteers.

Antigen preparation. Whole-cell, Formalin-killed, and heat-killed antigens were each prepared by harvesting the 48-h growth from a 2-[(2-amino-2-oxoethyl)-amino]ethanesulfonic acid-buffered, charcoal-yeast extract agar (BCYE) plate (that had been incubated in a sealed bag at 37°C) into 2 ml of sterile distilled water (whole cell and heat killed) or 10% Formalin in 0.01 M phosphate-buffered saline (PBS), pH 7.6 (Formalin-killed). The whole-cell and Formalin-killed suspensions were allowed to remain overnight at 4°C. The heat-killed antigen suspension was placed in a boiling-water bath for 15 min. All suspensions were then centrifuged at 2,000 × g for 15 min, and the cell pellets were each resuspended in 2 ml of 0.05 M Tris hydrochloride

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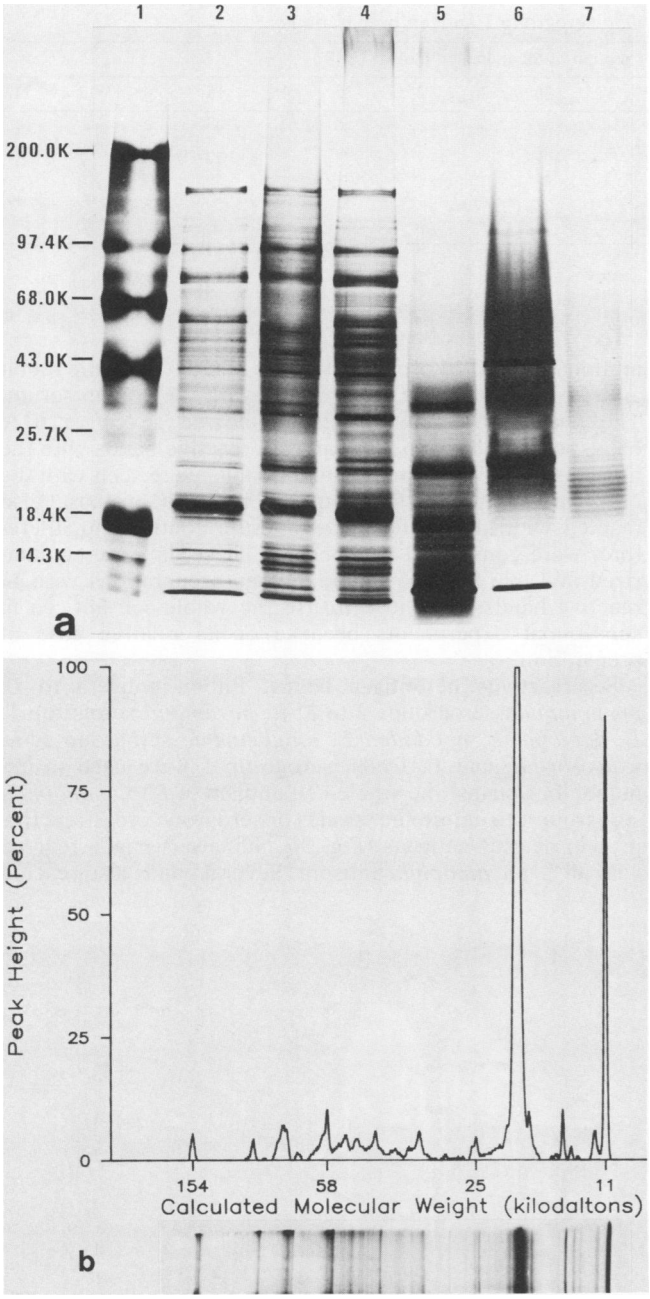

FIG. 1. Silver-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles of *L. pneumophila* serogroup 1 proteins. (a) Protein standards of known molecular weight (lane 1), whole-cell (lane 2), sonicated cell (lane 3), heat-killed (lane 4), heated supernatant (lane 5), outer membrane fraction (lane 6), and Formalin-killed (lane 7) antigen preparations. Twelve to fourteen prominant bands appeared in the whole-cell, sonicated cell, and heat-killed preparations. Six appeared in the heated supernatant preparation, and three appeared in the outer membrane fraction antigen (see Table 1 for summary). (b) Densitometric trace of whole-cell antigen.

buffer, pH 8.0. An additional antigen (heated supernatant) was prepared by concentrating the supernatant fluid from the heated cell suspension. The stock cellular antigens were diluted to a concentration that gave a percent transmittance of 11 ± 1 at 420 nm (1-cm-diameter cell; Coleman Jr. spectrophotometer). The sonicated cell antigen was prepared from the 48-h growth of two BCYE plates which was harvested into 4 ml of sterile distilled water. Cells were centrifuged and washed twice by centrifugation in 10 ml of PBS, pH 7.6. The cells were resuspended to the original volume in PBS and then were sonicated for 10 1-min bursts at 60% of maximum setting (Bronwill Biosonik IV; VWR Scientific, San Francisco, Calif.), with a 1-min cool down period between each burst. The whole sonic extract was used as an antigen.

Outer membrane fraction. Outer membrane fraction was prepared by the method of Bolin and Wolf-Watz (2), with slight modifications. The 48-h growth on 100 BCYE plates was harvested in 2 ml of sterile distilled water, and the suspension was filtered through sterile gauze. The cells were packed by centrifugation at 2,000 × *g* for 30 min. The cell pellet was resuspended at a 1:10 ratio in buffer consisting of 10 mM Tris hydrochloride, 5 mM EDTA, and 1 mM 2-mercaptoethanol. The suspension was sonicated at 75% of maximum setting for eight 15-s bursts, with a 15-s cool down period between each burst. To remove cell debris, the suspension was centrifuged at 27,000 × *g* for 25 min. The supernatant fluid was then centrifuged at 44,000 × *g* for 137 min at 4°C. The pellet, which contained the membrane fraction, was resuspended in 0.5% Sarkosyl (Sigma Chemical Co., St. Louis, Mo.), and the suspension was held at 4°C overnight. The Sarkosyl suspension was centrifuged as before and the pellet was resuspended in Sarkosyl, held overnight at 4°C, and centrifuged as above. The outer membrane fraction pellet was resuspended in 2 ml of deionized water and stored at −70°C.

Gel electrophoresis and immunoblot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out by the discontinuous method of Neville and Glossmann (14) as modified by Tsang et al. (19), except that prestained molecular weight standards (Bethesda Research Laboratories, Gaithersburg, Md.) were used. After completion of electrophoresis, protein-containing bands were revealed with the silver stain method (13) or were transferred to nitrocellulose sheets for subsequent immunologic analysis.

The procedure for immunoblot analysis was described previously (19) with the exception that rabbit antisera were diluted 1:1,000 and human sera were diluted 1:250 or 1:500 in buffer containing 5.0% bovine serum albumin, 3% Tween 20, and 0.5% gelatin in PBS, pH 7.6. Horseradish peroxidase conjugates were diluted 1:1,000 (polyvalent anti-human) or 1:500 (anti-rabbit immunoglobulin G) and were allowed to react for 2.5 h. Immunoblots and silver-stained gels were scanned with a laser-scanning densitometer (Biomed Instruments, Inc., Fullerton, Calif.). The data were captured and integrated with the Biomed Auto-Stepover and Videophoresis II program on an Apple computer. Molecular weights were calculated from a calibration curve obtained by fitting a modified hyperbola to the set of known standards (B. D. Plikaytis, G. M. Carlone, P. Edmonds, and L. W. Mayer, Anal. Biochem., in press).

Enzyme digestion of whole-cell antigen and absorption of antisera. To further characterize antigens relevant to the immunologic response, the use dilution of the whole-cell antigen was incubated at 37°C for 24 h with 4 mg (final concentration) of proteinase K (Sigma Chemical Co.) per ml in PBS, pH 7.2. Antisera were absorbed at a ratio of 1:5, cells/serum, with *E. coli* O13-K11-H11 or Philadelphia 1 at 37°C for 2 h and then at 4°C overnight.

RESULTS

***L. pneumophila* serogroup 1 protein profiles.** Silver-stained electropherograms of the six antigen preparations of *L.*

TABLE 1. Protein profiles of *L. pneumophila* serogroup 1 antigen preparations

Antigen prepn	Presence of protein of the indicated mol wt ($\times 10^3$)															
	154	97[a]	79[a]	58[a]	47	44[a]	37	36	26	25[a]	20	18	15	14	12	11[a]
Whole cell	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+
Sonicated cell	+	+	+	−	−	+	+	+	−	+	+	−	+	+	+	+
Heated cell	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+
Heated supernatant	−	−	−	−	−	−	+	−	−	+	−	+	−	+	+	+
Outer membrane fraction	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−

[a] Major proteins.

pneumophila serogroup 1, strain Philadelphia 1, are shown in Fig. 1a and summarized in Table 1. A densitometric trace used to determine molecular weights compared with known standards is illustrated in Fig. 1b. The whole-cell, sonicated cell, and heat-killed preparations each showed 12 to 14 prominent bands, 6 of which were also seen with the heated supernatant preparation. Only three major bands showed in the outer membrane fraction antigen, and no predominant bands were seen in the Formalin-killed antigen. The stepladder-like appearance of the latter preparation is usually attributed to nonprotein substances, and this preparation was not used in subsequent experiments.

***L. pneumophila* serogroup 1 immunoblot profiles.** The remaining five antigen preparations were subjected to electrophoresis, electroeluted onto nitrocellulose, and allowed to react with *L. pneumophila* serogroup 1 rabbit antiserum (Fig. 2a) and with serum from a patient with culture-confirmed Legionnaries disease (Fig. 2b). From these figures and from other experiments not shown, we found the following. The whole-cell, sonicated cell, and heat-killed preparations showed 14 prominent bands reactive with the rabbit antiserum; 13 of these also reacted with the human serum. The heated supernatant antigen showed seven reactive bands with rabbit antiserum and six reactive bands with the human serum. Three predominant bands were seen with the outer membrane fraction antigen, but two of these also reacted with the normal human serum control and, therefore, were considered nonspecific. These data are summarized in Table 2. Of the three antigen preparations with 14 reactive bands, we chose to use the whole-cell antigen in subsequent experiments because of its relative ease of preparation.

Seroreactivity of antigen bands. Rabbit antisera to *L. pneumophila* serogroups 2 to 8, *L. bozemanii* serogroup 1, *L. dumoffii*, *L. micdadei*, *L. longbeachae* serogroup 1, *L. wadsworthii*, and *L. feeleii* serogroup 1 were used in immunoblots against the whole-cell antigen of *L. pneumophila* serogroup 1 to determine species or serogroup cross-reactive protein marker antigens (Fig. 3). Numerous bands reacted with all *L. pneumophila* antisera. Several bands also reacted

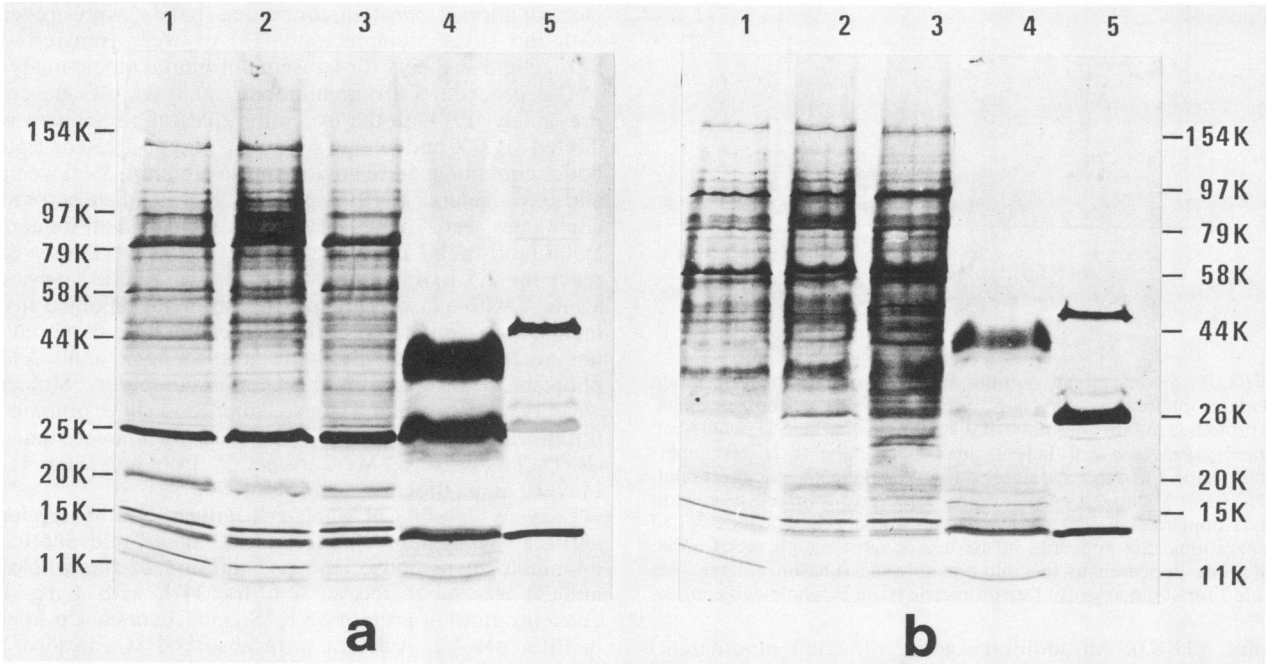

FIG. 2. Immunoblot profiles of *L. pneumophila* serogroup 1 antigens with rabbit antiserum (a) and culture-confirmed human serum (b). Lanes 1 to 5, respectively, are whole-cell, sonicated cell, heat-killed, heated supernatant, and outer membrane fraction antigen preparations. The numbers on the left (a) or right (b) represent molecular weights of the major antigens, as calculated from the corresponding position of prestained standards. Thirteen to fourteen prominant bands appeared with human or rabbit sera, respectively, in the whole-cell, sonicated cell, and heat-killed preparations, six to seven appeared in the heated supernatant preparation, and three appeared in the outer membrane fraction antigen (see Table 2 for summary).

TABLE 2. Immunoblot profiles of *L. pneumophila* serogroup 1 antigen preparations

Antigen prepn	Presence of antigen of the indicated mol wt ($\times 10^3$)																
	154[a]	97[a]	79[a]	58[a]	47	46–36	44[a]	37	36	26	25[a]	20	18	15	14	12	11[a]
Whole cell	+[b]	+	+	+	NA[c]	NA	+	+	+	+	+	+	NA	+	+	+	+
Sonicated cell	+	+	+	+	NA	NA	+	+	+	+	+	+	NA	+	+	+	+
Heated cell	+	+	+	+	NA	NA	+	+	+	+	+	+	NA	+	+	+	+
Heated supernatant	NA	NA	NA	NA	NA	+	NA	+	NA	NA	+	NA	−[d]	+	+	+	+
Outer membrane fraction	NA	NA	NA	NA	+[e]	NA	NA	NA	NA	+[e]	NA	NA	NA	NA	+	−	NA

[a] Major antigens.
[b] +, Reactive with either rabbit antiserum or human serum.
[c] NA, Not applicable.
[d] −, Nonreactive with rabbit or human sera.
[e] Also reacted with normal human serum.

with antisera to other *Legionella* species. The molecular weight of the most notable interspecies cross-reactive antigen was 58,000. The most prominent cross-reactive antigen among *L. pneumophila* serogroups was the 25-kilodalton (kDa) antigen.

Figure 4a shows the reverse antigen-antibody immunoblot reaction. Whole-cell antigens were prepared from strains representing 14 *Legionella* species or serogroups, and bands were probed with unabsorbed rabbit antiserum to *L. pneumophila* serogroup 1. Again, the most prominent cross-reactive band among the *L. pneumophila* antigens was the 25-kDa antigen. Cross-reactive bands in the other *Legionella* species were weak or absent. In contrast, human sera cross-reacted strongly to multiple antigens in all 14 *Legionella* sp. strains. Seven of the bands appeared most frequently among the human sera tested. Two were prominent among the *L. pneumophila* serogroups only, three were detected on blots of all legionellae tested, and the remaining two were present among several *Legionella* species. The 58-kDa antigen was especially prominent. Figure 4b shows a representative example. Absorption of the serum with *E. coli* cells had no effect on its reactivity with the 58-kDa band, whereas absorption with Philadelphia 1 cells removed all reactivity to this antigen. Therefore, the 58-kDa antigen is likely a cell surface component and could provide a specific *Legionella* sp. common antigen for serologic tests.

Sensitivity of antigen bands as markers of human infection. Immunoblot profiles were determined against the *L. pneumophila* serogroup 1 whole-cell antigen with sera from 15 patients with culture-confirmed Legionnaires disease and 18 additional patients with diagnostic IFA titers. The 154-kDa antigen gave variable results on repeated experiments and was usually present in both acute- and convalescent-phase sera; therefore, it was considered nonspecific. Figure 5 shows the distribution of reactivity in the convalescent-phase sera to each of the six remaining prominent bands. The 58-kDa common antigen detected all of the culture-confirmed cases and 14 of 18 (78%) of the additional seropositive cases. *L. pneumophila*-specific antigens detected 27 to 40% of the cases. That all six antigens were protein was confirmed by their absence on immunoblots after protease digestion. Table 3 provides a summary of these data.

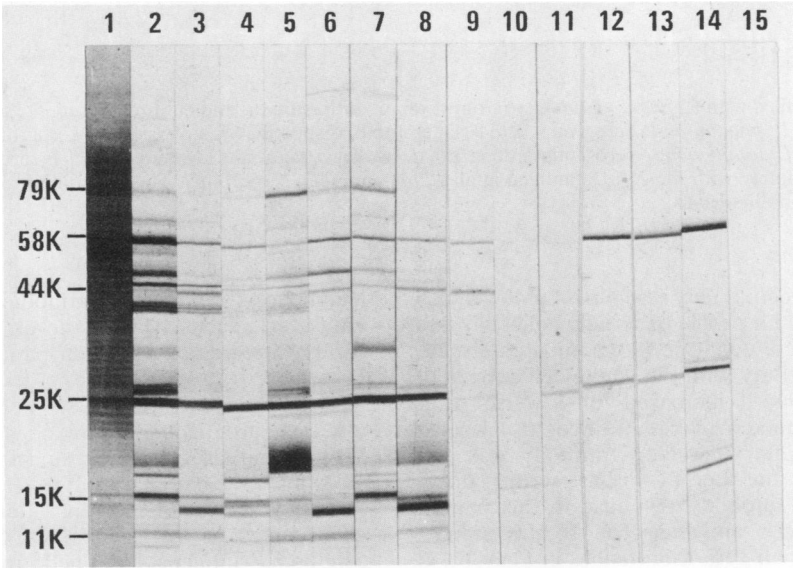

FIG. 3. Immunoblot profiles with *L. pneumophila* serogroup 1 whole-cell antigen preparation and rabbit antisera to *L. pneumophila* serogroups 1 to 8 (lanes 1 to 8, respectively), *L. bozemanii* serogroup 1 (lane 9), *L. dumoffii* (lane 10), *L. micdadei* (lane 11), *L. longbeachae* serogroup 1 (lane 12), *L. wadsworthii* (lane 13), and *L. feeleii* serogroup 1 (lane 14); and normal rabbit serum (lane 15). The most notable interspecies cross-reactive antigen was the 58-kDa antigen; the most notable *L. pneumophila*-reactive antigen was the 25-kDa antigen.

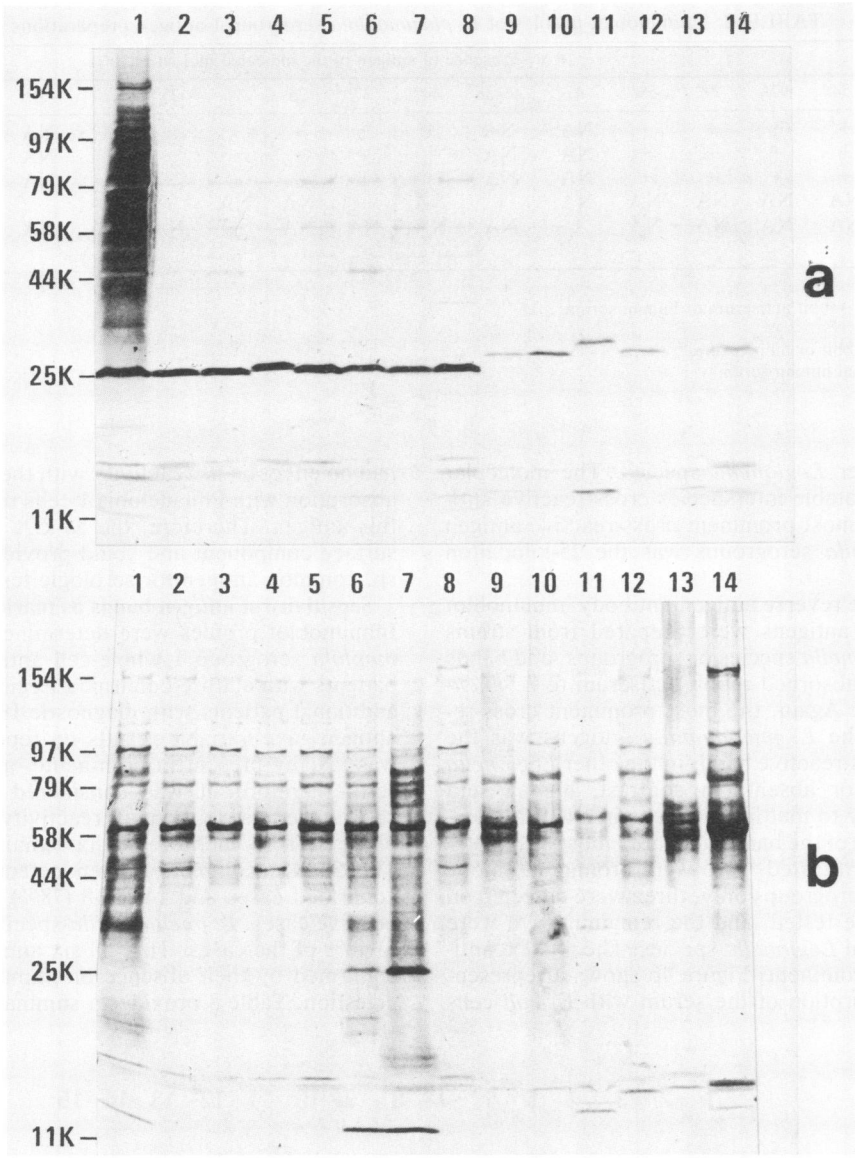

FIG. 4. Immunoblot profiles with *L. pneumophila* serogroup 1 rabbit antiserum (a) and culture-documented human serum (b). Whole-cell antigen preparations were : *L. pneumophila* serogroups 1 to 8 (lanes 1 to 8, respectively), *L. bozemanii* serogroup 1 (lane 9), *L. dumoffii* (lane 10), *L. micdadei* (lane 11), *L. longbeachae* serogroup 1 (lane 12), *L. wadsworthii* (lane 13), and *L. feeleii* serogroup 1 (lane 14). The rabbit antiserum reacted predominately with the 25-kDa antigen in all *L. pneumophila* serogroups. The most notable reaction of the human serum was to the 58-kDa antigen in all species.

Duration and diversity of antibody response of patients with legionellosis. An immunoblot profile of serially collected sera from a patient with culture-confirmed Legionnaires disease is shown in Fig. 6. Reactivity with the whole-cell antigen of *L. pneumophila* serogroup 1 increased for 3 weeks after onset of symptoms, reached a maximum level at 4 weeks, and gradually declined after 5 weeks. Antibody was still detectable after 52 days, the time of the last serum collection. The major specific antibody response in this patient was to the 58- and 79-kDa antigens. The 26-kDa antigen reacted with normal human sera (not visible in figure) and with bovine serum albumin. The 154-kDa antigen was considered nonspecific for reasons stated above. The diversity of antibody responses among different patients is illustrated in Fig. 7. Each of four patients with culture-confirmed legionellosis developed antibodies to the 58-kDa antigen. However, reactivity with the other major *L. pneumophila* bands varied among the four patients.

DISCUSSION

We determined sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein and immunoblot profiles of six different antigen preparations of *L. pneumophila* serogroup 1 and whole-cell antigen preparations of 14 different *Legionella* species or serogroups. Of the seven major protein-containing antigens identified, the 58-, 79-, and 154-kDa antigens were present in all *Legionella* sp. strains; the 44- and 97-kDa antigens were found in multiple (but not all) species, and the 11- and 25-kDa antigens appeared to be *L. pneumophila* specific. Maximal reactivity of patients' sera

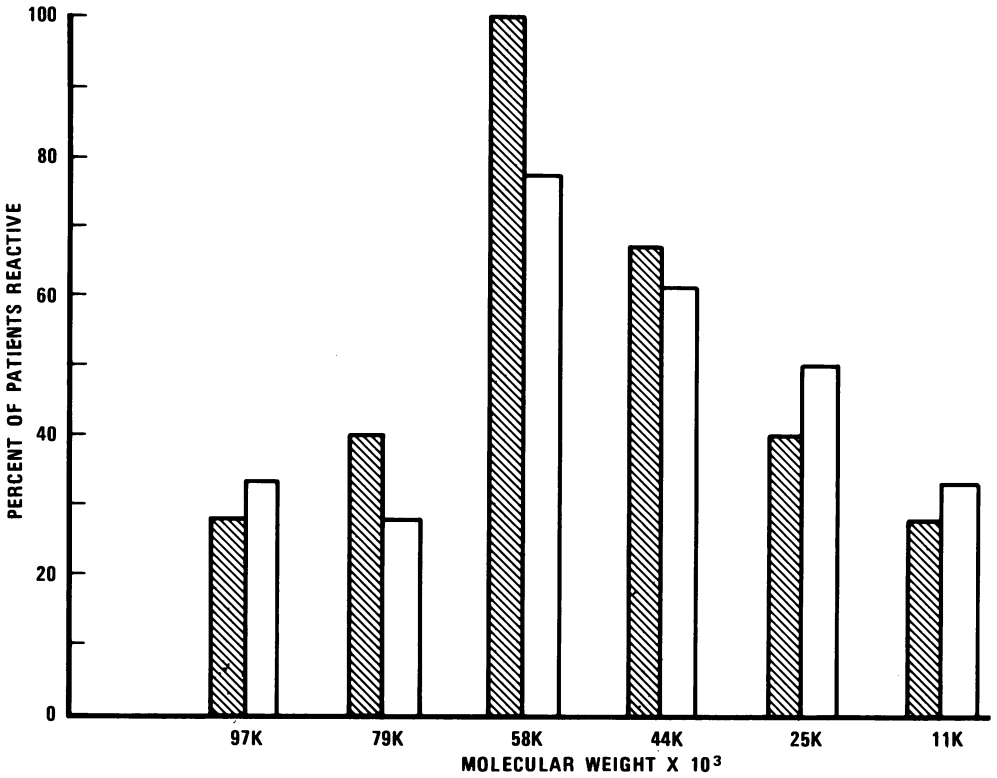

FIG. 5. Patient response to marker antigens as determined by immunoblot analysis of *L. pneumophila* serogroup 1 whole-cell antigen preparation. Sera were from culture-documented, *L. pneumophila* serogroup 1-infected patients (shaded bars; $n = 15$) and from patients with diagnostic IFA titers against *L. pneumophila* serogroup 1 (clear bars; $n = 18$). All culture-documented patients and 78% of the additional seropositive patients reacted with the 58-kDa antigen.

from cases of culture-confirmed or serologically diagnosed legionellosis was to the 58-kDa antigen that was common to all legionellae. Reactivity to the *L. pneumophila*-specific proteins varied from 27 to 40% in the patients' sera. Sera from all patients reacted to at least one of the major antigens, but in various and unpredictable combinations.

Our observations with protein-containing antigen markers are similar to those we obtained with heat-killed IFA antigens in the duration and multiplicity of antibody response (20–22). Antibodies were shown to persist post-onset for months, or even years, and could either be serogroup specific or show reactivity to common *Legionella* sp. antigens. The IFA may measure antibodies to both protein and glycolipid determinants, as several studies suggested that the serogroup 1-specific antigen of *L. pneumophila* is composed of a lipid-protein-polysaccharide complex (8, 12, 23). We could not determine glycolipid reactivity in the present experiments since proteins are primarily detected by this procedure, although the presence of protein-glycolipid complexes cannot be ruled out. Also undetermined is the relationship of our antigen markers to those described for *L. pneumophila* serogroup 1 by other investigators. In one study, rabbit antisera reacted in crossed-immunoelectrophoresis analyses with 85 antigens released by sonification; 7 were heat-stable, surface antigens with both serogroup-specific and common antigen reactivity (4). Other studies showed the presence of 28-kDa (9), 38.5-kDa (1), and 29-kDa (5, 11) proteins that were associated with the peptidoglycan in outer membrane (5, 9, 11) or cell wall (1) preparations of *L. pneumophila* serogroup 1. A 29-kDa antigen was described as an *L. pneumophila* species-specific antigen (10) and a 24-kDa outer membrane protein was described as a genus-specific antigen (3). A 19-kDa protein antigen, cloned in *E. coli*, reacted with rabbit antisera to *L. pneumophila* serogroup 1 (6, 7), as did seven other *L. pneumophila* serogroup 1 proteins found on immunoblots by these investigators (15). Our studies may not be directly comparable to theirs since we used different methods of antigen preparation. Furthermore, we used antisera to and strains of 14 different *Legionella* species or serogroups to determine the

TABLE 3. Characteristics of *Legionella* sp. antigens reactive with human sera

Mol wt ($\times 10^3$)	Reactivity	Degraded by protease	No. (%) reactive with patients' sera	
			Culture confirmed ($n = 15$)	Serologically diagnosed ($n = 18$)
154	Genus[a]	Yes	Variable	Variable
97	Several species[b]	Yes	4 (27)	6 (33)
79	Genus	Yes	6 (40)	5 (28)
58	Genus	Yes	15 (100)	14 (78)
44	Several species[c]	Yes	10 (67)	11 (61)
25	*L. pneumophila*	Yes	6 (40)	9 (50)
11	*L. pneumophila*	Yes	4 (27)	6 (33)

[a] Detected in *L. pneumophila* serogroups 1 to 8, *L. bozemanii* serogroup 1, *L. dumoffii*, *L. longbeachae* serogroup 1, *L. wadsworthii* serogroup 1, and *L. feeleii* serogroup 1.

[b] Detected in *L. pneumophila* serogroups 1 to 8, *L. dumoffii*, *L. longbeachae* serogroup 1, and *L. feeleii* serogroup 1.

[c] Detected in *L. pneumophila* serogroups 1 to 8, *L. longbeachae* serogroup 1, and *L. feeleii* serogroup 1.

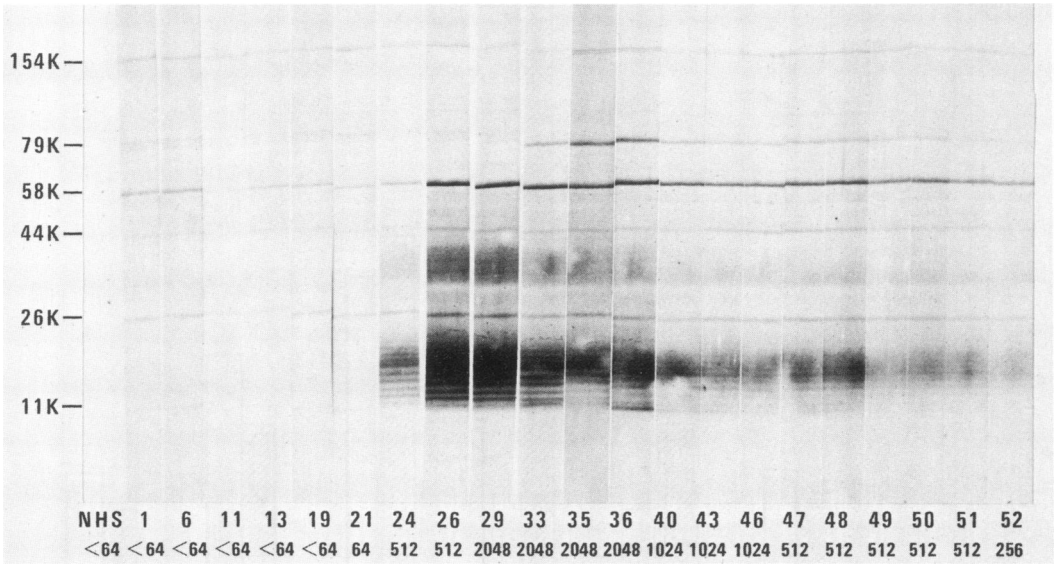

FIG. 6. Duration of antibody response of a patient with culture-confirmed *L. pneumophila* serogroup 1 disease as determined by immunoblot analysis with *L. pneumophila* serogroup 1 whole-cell antigen. Lanes are designated NHS (normal human serum) or as number of days after onset of symptoms. IFA titer of each serum is indicated under the day of serum collection. The major specific antibody response was to the 58- and 79-kDa antigens (see text).

specificity of protein markers and sera from legionellosis patients to determine the relevance of these markers in the immunologic response to infection.

The response detected in sera from 33 patients suggests that the 58-kDa protein antigen is a prime candidate as a specific antigen for the serologic confirmation of legionellosis. That sera absorbed with *E. coli* cells reacted with the 58-kDa antigen and sera absorbed with *L. pneumophila* serogroup 1 cells did not react with it suggests that the antigen is specific for *Legionella* spp. Advantages of using the 58-kDa antigen include the likelihood of detecting disease caused by a variety of species and serogroups and the

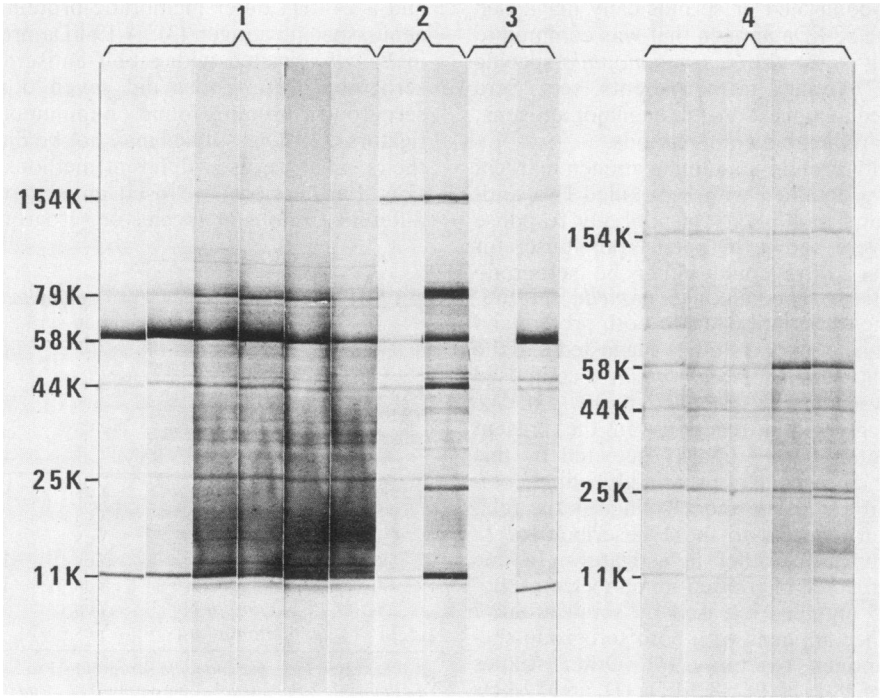

FIG. 7. Diversity of antibody response among four patients with culture-confirmed *L. pneumophila* serogroup 1 infection as determined by immunoblot analysis with *L. pneumophila* serogroup 1 whole-cell antigen. For each of the four patients, as indicated by brackets, lanes from left to right are in increasing order of serum collection date. Although reactivity with most of the *L. pneumophila* antigens varied among the four patients, each had a serum that reacted with the 58-kDa antigen.

apparent high sensitivity of testing with this antigen. All patients with culture-confirmed *L. pneumophila* serogroup 1 disease showed antibodies bound to the 58-kDa protein. Ancillary data, not presented in this study, showed that sera from patients with culture-documented *L. pneumophila* serogroup 6 and *L. bozemanii* serogroup 1 disease also reacted with the 58-kDa antigen. Further studies are in progress to confirm these observations.

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