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Identification of Protein Antigens of *Legionella pneumophila* Serogroup 1

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Growth of *Legionella pneumophila* serogroup 1 in yeast extract broth was standardized, and protein profiles of detergent-solubilized cells were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Over 30 protein bands were identified, 6 of which were more prominent both in Coomassie brilliant blue-stained profiles and in fluorograms with intrinsically radiolabeled bacteria. These major proteins were 22,000 dalton (22K), 24K, 43K, 63K, 76K, and 78K. We found that the 24K and 63K major proteins were antigenic, as demonstrated both by radioimmunoprecipitation (RIP) of [^{35}S]methionine-labeled organisms and by immunoblotting with rabbit hyperimmune sera. In addition, both techniques detected antigens migrating at 58K, 72K, 76K, and 78K. The major 22K and 43K major proteins and antigens migrating at 25.5K, 29K, and 46K were only detected by radioimmunoprecipitation, whereas antigens of 19K, 48K, 53K, and 68K were detected only by immunoblotting. Cell-surface localization of the proteins was determined by a modified radioimmunoprecipitation assay designed to react specifically with surface antigens and by the use of hyperimmune sera that had been extensively preabsorbed with intact cells to deplete the sera of antibodies directed against surface components. The 22K, 24K, 43K, 63K, and 78K major proteins and several minor proteins were found to be located on the surface of *L. pneumophila* cells.

Legionella pneumophila is the causative agent of both Legionnaires disease and Pontiac fever. Since its recognition in 1976 (23), the immunologic response to the organism has been studied extensively both in animal models (6, 7) and in in vitro (16–20) systems; however, only a few studies have attempted to separate and characterize individual *L. pneumophila* antigens. Wong et al. (29) purified subcellular fractions of various *L. pneumophila* serogroups and showed that the cross-reactive antigen fractions were composed almost entirely of protein. Electrophoretic analysis determined that the cross-reactive fractions were heterogeneic, being composed of at least 5 major and 15 minor protein bands. In another study, crossed immunoelectrophoresis identified more than 80 *L. pneumophila* antigens, several of which were shown to be located on the surface (5). This technique, although highly sensitive, neither distinguishes proteins from other components nor characterizes antigens by molecular weights. Other reports of specific *L. pneumophila* proteins have identified a major 29,000-dalton (29K) outer membrane protein (9), a 47K flagellar subunit (10), and peptidoglycan-associated polypeptides (2). A preliminary report utilizing immunoblotting with acute and convalescent human sera identified several cross-reactive protein antigens among various *Legionella* species (M. S. Hindahl and B. H. Iglewski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C104, p. 329).

These reports notwithstanding, the role of individual *Legionella* proteins in pathogenesis is still undetermined. Our long-term research goal is to develop an understanding of the molecular immunobiology of *L. pneumophila* by focusing on the proteins. By employing methodology that has been previously used in the identification of protein antigens of various organisms (including *Haemophilus influenzae* [13, 14], *Mycoplasma pneumoniae* [22], *Treponema pallidum* [3], and *Trichomonas vaginalis* [1]), we identified *L. pneumophila* proteins that are recognized by the immunized host and have determined those antigens that are located on the surface of the bacterial cell.

MATERIALS AND METHODS

Source of materials. *Legionella pneumophila* serogroup 1 strain 130b was recovered during an outbreak of Legionnaires disease at Wadsworth Veterans Administration Hospital in Los Angeles and was sent to us by Paul Edelstein. Radioisotopes were obtained from Amersham Corp. (Arlington Heights, Ill.), protein A-bearing *Staphylococcus aureus* (IgG-Sorb) was obtained from The Enzyme Center, Inc. (Malden, Mass.), EN^{3}HANCE was obtained from New England Nuclear Corp. (Boston, Mass.), nitrocellulose and the peroxidase substrate and conjugated antibody were obtained from Bio-Rad Laboratories (Richmond, Calif.). Molecular weight standards (unlabeled) were obtained from Sigma Chemical Co. (St. Louis, Mo.), and ^{14}C-labeled protein standards were obtained from BRL (Gaithersburg, Md.). Conjugated fluorescent antibody was obtained from the Centers for Disease Control, Atlanta, Ga.

Maintenance of organisms. *L. pneumophila* organisms were maintained in guinea pigs and were used to infect BALB/c mice (7). Lungs from infected mice were used to inoculate buffered charcoal yeast extract (BCYEα) agar (8); *L. pneumophila* organisms were collected after 3 to 4 days, when visible colonies were present. Organisms used in this study were passaged no more than three times, were of single cell origin, and were free of bacterial contamination (confirmed by inoculating samples onto Luria-Bertani plates). The identity of the isolate was confirmed by the direct fluorescent-antibody technique.

Growth and radiolabeling. Kinetics of growth were determined by inoculating organisms into 20 ml of yeast extract broth (YEB [26]; 10 g of yeast extract, 0.4 g of L-cysteine hydrochloride, 0.25 g of ferric pyrophosphate [pH 6.9]) and the optical density at 660 nm (OD_{660}) was monitored at

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hourly intervals. To minimize accumulation of superoxide radicals, which are reported to have a toxic effect on growing cells (15), the following steps were taken: (i) culture medium was sterilized by filtration through a 0.2-μm Millipore filter instead of by autoclaving, (ii) cysteine was added just before inoculation, and (iii) the media were protected from light during bacterial growth. Cells were radiolabeled by adding 0.1 mCi of [^{35}S]methionine to the medium at the time of inoculation.

Viable counts were determined by removing samples at various times during growth, diluting them in phosphate-buffered saline (PBS), and plating them onto BCYEα plates. When CFUs were counted 3 to 4 days later, we found that an OD_{660} of 0.8 corresponded to ca. 8×10^9 CFU/ml. Cellular morphology was also determined at each time point by microscopy after the cells were stained with 0.8% malachite green.

Preparation of sera. Organisms from infected mice were passaged once on BCYEα plates, harvested from plates at 48 h, and killed by incubating overnight at 22°C in 2% Formalin. After washing in 0.01 M PBS (pH 7.2), the Formalin-killed cells were suspended to an OD_{550} of ~1.4 in PBS and were injected subcutaneously (2-ml dose) into two New Zealand rabbits at biweekly intervals. Sera were collected 7 weeks later, heat inactivated (56°C, 30 min), and centrifuged at 100,000 × *g* for 60 min to remove preexistent immune complexes. Pooled sera were used in all experiments. Control sera (preimmune) were obtained before inoculation and treated similarly. To selectively remove antibodies directed at surface antigens, washed cells that had been harvested from 100 ml of YEB (OD_{660} 0.8 to 1.0) were divided into five equal aliquots, centrifuged, and then sequentially incubated with 0.5 ml of hyperimmune rabbit sera at 4°C for 90 min each in the presence of 1 mM phenylmethylsulfonyl fluoride. The resultant sera, now depleted of antibodies to surface components, were centrifuged to remove any remaining bacterial cells and utilized in radioimmunoprecipitation experiments. Antisera to live *L. pneumophila* organisms were raised in two New Zealand rabbits by three subcutaneous inoculations of 1×10^{10}, 5×10^9, and 2.5×10^9, respectively, over a 2-month period and treated as described above.

Gel electrophoresis. Polyacrylamide gel electrophoresis (PAGE) was carried out in the presence of sodium dodecyl sulfate (SDS) under reducing conditions, utilizing the discontinuous system of Laemmli (21). The acrylamide concentrations employed for the separating and stacking gels were 10 and 5%, respectively. Samples were prepared for electrophoresis by boiling for 5 min in an equal volume of 2× sample buffer containing 4% (vol/vol) SDS, 10% (vol/vol) β-mercaptoethanol, and 20% (vol/vol) glycerol in 0.025 M Tris (pH 6.8). Bromophenol blue was added as a tracking dye, and electrophoresis was performed at a constant current of 30 mA per gel. Apparent molecular weights were determined by standard methods with known molecular weight standards. Densitometer tracings were performed in an ORTEC 4310 densitometer (Oak Ridge, Tenn.).

TCA precipitation. To obtain a profile of the total cell protein, a volume of 10% trichloroacetic acid (TCA) was added to an equal volume of washed cells and incubated at 4°C for 3 h. The precipitate was washed three times in cold PBS, resolved by SDS-PAGE, and then either stained with Coomassie brilliant blue or processed for fluorography with En^{3}Hance.

Solubilization. Cells were harvested from 20 ml of YEB (OD_{660}, 0.8 to 1.0), washed three times in 30 ml of PBS, and suspended in 5 ml of a detergent solution containing 1% (vol/vol) Triton X-100, 0.2% (wt/vol) sodium deoxycholate, 0.1% (wt/vol) SDS, and 10 mM EDTA in 10 mM Tris-hydrochloride (pH 7.8) (TDSET). Lysozyme (0.1 mg/ml) and (1 mM) phenylmethylsulfonyl fluoride were added, and the suspension was incubated at 37°C for 60 min. Insoluble material was removed by centrifugation at 150,000 × *g* for 45 min.

RIP assay. RIP assays were performed with either presolubilized organisms (PS-RIP) or whole cells (WC-RIP) in accordance with the methods and terminology employed by Hansen et al. (14). In the PS-RIP, detergent-solubilized organisms containing ca. 10^6 cpm of [^{35}S]methionine were incubated with 20 μl of sera for 15 min at 37°C and for at least 45 min at 4°C. IgG-Sorb (150 μl) in TDSET was incubated with the antigen-antibody mixture for 60 min at 30°C, followed by three washes with TDSET.

The WC-RIP was performed with 1 ml of washed cells (containing 3×10^6 to 5×10^6 cpm) that had been incubated with 100 μl of sera for 90 min at 4°C. Cells were washed to remove unbound antibodies and then solubilized in 0.6 ml of TDSET-lysozyme before incubation with IgG-Sorb (as described for PS-RIP).

To test for nonspecific binding of antigens to IgG-Sorb, we performed control RIP assays substituting PBS for sera.

Immunoblot. TDSET-solubilized cells were resolved by SDS-PAGE and transferred to nitrocellulose sheets by the method of Towbin et al. (28). The nitrocellulose was incubated for 60 min in Tween-Tris-buffered saline (TTBS:0.05% [vol/vol] Tween 20 in 20 mM Tris, 0.5 M NaCl, [pH 7.5]) to block unreacted sites and then incubated for at least 60 min with antisera that had been diluted 1:1,000 in TTBS. The nitrocellulose sheets were washed in TTBS and then incubated 60 min with peroxidase-conjugated goat anti-rabbit serum diluted 1:2,000 in TTBS. After addition of the substrate solution (containing 4-chloro-1-naphthol and hydrogen peroxide), color development was seen at the sites of antibody binding. The reaction was halted by immersing the strips in deionized water. Molecular weight standards and total protein profiles were visualized by staining the nitrocellulose strips with amido black (0.2% in 40% methanol–10% acetic acid) and destained with 70% methanol–2% acetic acid. All reactions were carried out at room temperature on a rotating platform.

RESULTS

Growth characteristics. The growth kinetics of *L. pneumophila* 130b in YEB are shown in Fig. 1. Cultures were routinely inoculated to give an initial OD_{660} of 0.1 to 0.15. The typical culture entered the logarithmic phase of growth 10 to 12 h after inoculation when the doubling time, as determined by monitoring the OD_{660}, was found to be 4 h. The stationary phase was reached after ~22 h (OD_{660}, ~1.0). Morphology and viability were determined at the early-, mid-, and late-log phases. During the early log phase (OD_{660}, 0.3), the bacteria formed short chains of up to 10 cells; by mid- (OD_{660}, 0.6) and late-log phase (OD_{660}, 0.9), they were found to be almost exclusively in the form of discrete cells.

In all further studies, cells were obtained from cultures that were harvested at an OD_{660} of 0.8 to 1.0 (0.8×10^{10} to 1×10^{10} cells).

Identification of major *L. pneumophila* proteins. Protein profiles of whole *L. pneumophila* organisms were resolved in an SDS-PAGE system with cells that had either been precipitated with 10% TCA or solubilized with TDSET-lysozyme. Profiles prepared by either method revealed the presence of approximately 30 bands, 5 of which were

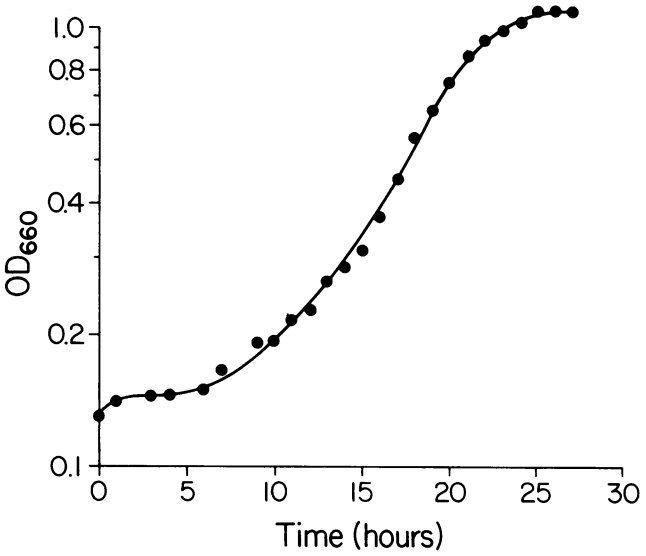

FIG. 1. Growth kinetics of growth of *Legionella pneumophila* 130b in YEB. Organisms isolated from infected BALB/c mice were passaged on charcoal yeast extract agar and inoculated into YEB. Growth was determined by monitoring OD_{660} at hourly intervals.

consistently more prominent in Coomassie brilliant blue-stained gels (Fig. 2). The major proteins (indicated with asterisks) were approximately 22K, 24K, 43K, 63K, and 76K to 78K. Samples from similarly grown cultures that were solubilized on different days gave highly reproducible protein profiles. No major differences were noted between the TCA-precipitated and TDSET-solubilized preparations.

Fluorograms of ^{35}S-labeled cells showed protein profiles very similar to those in the Coomassie brilliant blue-stained gels, except that the diffuse band migrating at 76K to 78K resolved as two distinct bands of 76K and 78K proteins (Fig. 3). Over 90% of the radioactivity present in the washed bacteria was solubilized by the TDSET solution, confirming that the detergent system was highly efficient at extracting *L. pneumophila* proteins.

Identification of *L. pneumophila* antigens. The results of the RIP performed with presolubilized cells are shown in Fig. 4 (lanes B through D). Hyperimmune sera detected four major protein antigens, three of which comigrated with the major proteins seen at 22K, 24K, and 63K (Fig. 4, lane B); the fourth major antigen was found to be 46K. Other minor antigens were found to migrate at 25.5K, 29K, 43K, 58K, 72K, 76K, and 78K. Preimmune sera detected the 25.5K, 43K, 46K, and 63K proteins (Fig. 4, lane D).

The 24K and 63K antigens were prominent in the immunoblot when hyperimmune sera were used as a probe (Fig. 5, lane B); the 76K and 78K antigens were also detected. In this assay, however, the 22K and 43K proteins were not prominent either in the amido black-stained profile (Fig. 5, lane A) or in the immunoblot (Fig. 5, lane B). To ensure that Formalin treatment of the organisms used for immunization had not altered any major immunogens, we repeated the immunoblots employing sera raised against live *L. pneumophila* organisms (Fig. 5, lane C). Once again, the major 24K and 63K antigens and several previously identified weaker antigens (58K, 72K, 76K, and 78K) were detected with antisera to live organisms. Four other antigens migrating at 19K, 48K, 53K, and 68K were detected with these sera but not with antisera to Formalin-killed cells. Preimmune sera detected the major 63K protein and a minor antigen at 48K (Fig. 5, lane D).

Identification of major surface antigens. When the PS-RIP was performed with hyperimmune sera that had been extensively preabsorbed with intact *L. pneumophila* cells to deplete the sera of surface-directed antibodies, reactivity to all the antigens was totally removed (Fig. 4, lane C), with the exception of residual activity to the 24K protein. These results suggest that all of these antigens are accessible to antibody on the intact cell. To corroborate these results, we performed a WC-RIP, a procedure designed to identify only surface-located antigens by reacting the antibody with the whole cell before solubilization (14). The reaction with hyperimmune rabbit sera is shown in Fig. 6, lane B. Most prominent were the 22K and 24K proteins, whereas the 25.5K, 29K, 43K, 46K, 63K, and 78K proteins were weakly precipitated (Table 1). Preimmune sera detected only the 25.5K protein (Fig. 6, lane C).

DISCUSSION

This study was performed to identify and characterize the major protein antigens of *L. pneumophila* serogroup 1. To accomplish this characterization, it was necessary to devise a reliable system for growing and quantitating *L. pneumophila* cells, as well as to select a detergent system that would

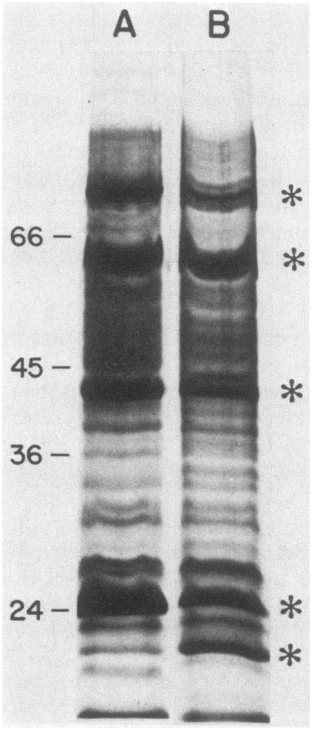

FIG. 2. Comparison of total protein profiles of *L. pneumophila* by SDS-PAGE after staining with Coomassie brilliant blue. The proteins were either precipitated with 10% TCA (lane A) or solubilized with lysozyme and a detergent solution containing Triton X-100, deoxycholate, and SDS in Tris-EDTA (TDSET) (lane B). The asterisks represent the major (most intensely stained) proteins with apparent molecular weights 22,000, 24,000, 43,000, 63,000, and 76,000 to 78,000. Molecular weight standards represent the following proteins: bovine albumin, 66,000; egg albumin, 45,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; trypsinogen, 24,000; and β-lactoglobulin, 18,400.

effectively solubilize the cells, yet still permit antibody-antigen interactions.

We found that *L. pneumophila* grew well in YEB, yielding 10^{10} cells after 24 h of growth, which is greater than that reported by Ristroph et al. (2×10^9 cells after 40 h) for a different serogroup 1 strain (26). In the present study, the cells were always seen by phase-contrast microscopy to be motile and were typically found in the form of discrete organisms at the time of harvesting and in short chains during the early phase of growth (OD_{660}, 0.3). In contrast to previous studies, we did not find the organisms to be filamentous during growth (25), nor did we detect the presence of extracellular debris (26).

In our characterization of *L. pneumophila* proteins, we used a detergent system that has been described previously for the solubilization of other organisms, including *Haemophilus influenzae* (13, 14) and *Mycoplasma pneumoniae* (22). In our study, the addition of 100 μg of lysozyme per ml was found to improve the efficiency of the solubilization. TCA precipitation and TDSET solubilization of *L. pneumophila* yielded similar and highly reproducible patterns, which differed only in the intensity of some of the bands. We found that six bands were consistently stained or radiolabeled more intensely than the others; these proteins migrated at 22K, 24K, 43K, 63K, 76K, and 78K.

To investigate which of the *L. pneumophila* proteins were immunogenic, we utilized two complementary immunoassays with rabbit hyperimmune sera. In the first, the PS-RIP, antibodies react with solubilized bacterial proteins in liquid phase under conditions that may not totally disaggregate all complexes (which could include coprecipitated nonantigenic proteins) until the final detection step. In the second assay, the immunoblot, antibodies detect only those proteins that remain antigenic after denaturation, electrophoresis, and transfer to the solid (nitrocellulose-bound) phase. Despite the different reaction conditions, we found that the 24K, 63K, 76K, and 78K major proteins were consistently present in both assays. However, the 22K and the 46K proteins, which were strongly precipitated in the RIP, were not detected in the immunoblot. The 24K and the 63K major proteins were consistently prominent on the amido black-stained profile, whereas the presence of the 22K and 43K major proteins was variable. This may reflect differences in their ability to be transferred to the nitrocellulose.

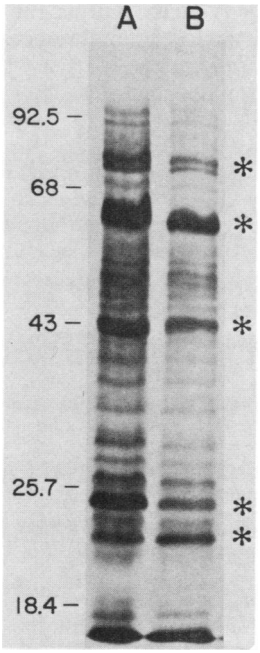

FIG. 3. Comparison of total protein profiles of *L. pneumophila* by SDS-PAGE. Gels were processed by fluorography after the cells had been radiolabeled with [^{35}S]methionine. The proteins were either precipitated with 10% TCA (lane A) or solubilized with TDSET (lane B). The asterisks denote the major *L. pneumophila* proteins. Molecular weight standards represent the following proteins: phosphorylase B, 92,500; bovine serum albumin, 68,000; ovalbumin, 43,000; α-chymotrypsinogen, 25,700; and β-lactoglobulin, 18,400.

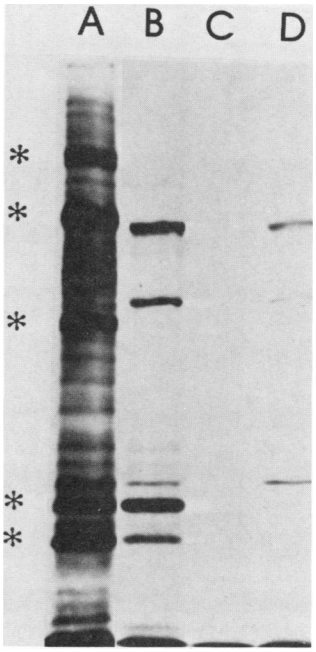

FIG. 4. Antigenic analysis of solubilized *L. pneumophila* proteins. Cells were grown in the presence of [^{35}S]methionine, detergent solubilized, and used to generate a total protein profile (lane A) or an RIP profile performed with hyperimmune rabbit anti-*L. pneumophila* sera (lane B), hyperimmune sera absorbed extensively with intact organisms (lane C), or preimmune rabbit sera (lane D). Asterisks denote major *L. pneumophila* proteins.

Although Formalin-killed cells have been used routinely to produce hyperimmune sera, such treatment has been reported to alter the immunogenicity of surface antigens in certain systems (24). We therefore repeated immunoblots utilizing antisera raised against live organisms. These antisera detected the 24K and 63K major protein antigens, the 58K, 72K, 76K, and 78K antigens seen in the PS-RIP, and four previously unidentified antigens of 19K, 48K, 53K, and 68K. We also performed RIPs utilizing sera raised against live organisms and detected a previously nonprecipitated 19K weight protein (data not shown), further suggesting that the immunogenicity of this protein is sensitive to Formalin treatment. This 19K antigen appears to be the same *L. pneumophila* protein antigen that was strongly expressed on the surface in recombinant strains of *Escherichia coli* (11, 12) and subsequently shown to possess Formalin-sensitive epitopes (12).

In repeat experiments, a relatively high background was consistently seen when blots were performed with sera that had been raised against Formalin-killed organisms compared

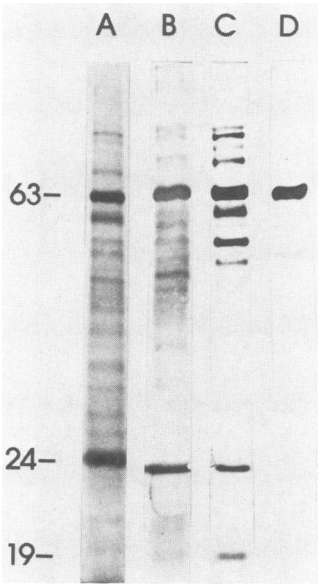

FIG. 5. Immunoblot analysis of *L. pneumophila* antigens. SDS-PAGE of unlabeled cells was followed by electrophoretic transfer to nitrocellulose paper which was then stained with amido black (lane A) or reacted with hyperimmune rabbit sera that had been raised against Formalin-killed cells (lane B) or live cells (lane C), or with preimmune sera (lane D). The numbers on the left represent the molecular weights ($\times 10^3$) of three major *L. pneumophila* antigens as calculated from amido black-stained molecular weight markers that were electrophoresed and blotted with the antigens.

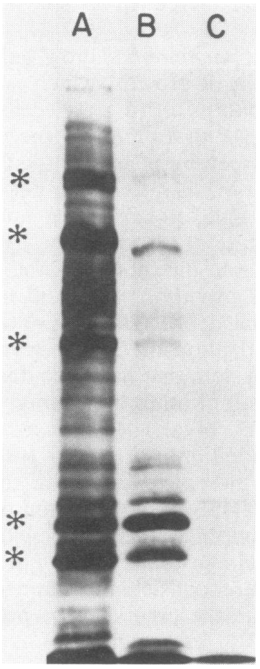

FIG. 6. Identification of *L. pneumophila* surface protein antigens. Washed cells labeled with [^{35}S]methionine were used to generate a total protein profile (lane A) and WC-RIP profiles with hyperimmune sera (lane B) and preimmune sera (lane C). Asterisks denote major *L. pneumophila* proteins.

with sera raised against live organisms (Fig. 5, lane B versus lane C). Because this background was absent in RIPs (suggesting that it is not protein in nature) and in immunoblots prepared from cells of other *L. pneumophila* serogroups (E. Pearlman, N. C. Engleberg, and B. I. Eisenstein, manuscript in preparation), we suspect that antibodies against serogroup-specific, nonprotein antigens were more abundant in the sera raised against Formalin-killed cells than in the sera raised against live cells and that these antibodies may give the impression of nonspecific background by reacting with nonprotein antigens that migrate more diffusely in the gel.

Since surface proteins of gram-negative bacteria have been implicated in various aspects of the pathogenic process, including attachment to mucosal surfaces, antibody binding, and phagocytosis (for a review, see reference 4), we determined those *L. pneumophila* protein antigens that were exposed on the surface. In the present study two different techniques were employed to identify such protein antigens: a modified (whole cell) RIP assay, which reacts immune sera with intact cells before solubilization of the antigen-antibody complexes (14), and a complementary RIP assay, which utilizes immune sera that has been preabsorbed with intact cells to remove specifically those antibodies directed against surface antigens. The results of these assays (Table 1) indicate that there are a number of surface-expressed protein antigens, the most prominent of which migrated at 22K and 24K. The 25.5K, 46K, and 63K surface-expressed antigens were also recognized by preimmune sera, suggesting that similar immunogens had been previously encountered by the rabbits. An equivalent absorption with intact *E. coli* cells did not reduce reactivity to the 22K or 24K antigen, but did remove reactivity to the 46K and 63K antigens (data not shown). This observation suggests that *E. coli* possesses surface antigens that cross-react with the 46K and 63K *L. pneumophila* antigens and confirms the specificity of the absorption method since it did not reduce reactivity to other major *L. pneumophila* antigens. Cross-reactivity with other organisms (e.g., common enterics) may explain why antibodies to these antigens (and the 25.5K) were present in the preimmune sera.

In the present study, sera from immunized rabbits were utilized to identify *L. pneumophila* protein antigens. The role that these antigens have in the pathogenesis of the organism has not yet been determined, but if antibodies play

TABLE 1. Comparison of surface-localized *L. pneumophila* protein antigens detected by PS-RIP and WC-RIP assays

Molecular weights of protein antigens[a]	Relative intensity of protein bands for the following assays with the indicated sera[b]:		
	PS-RIP		WC-RIP
	Immune	Absorbed[c]	Immune
22,000	+++	–	+++
24,000	++++	+/–	++++
25,500	+	–	+
29,000	+/–	–	+
43,000	+/–	–	+
46,000	+++	–	+
63,000	+++	–	++
78,000	+/–	–	+

[a] An antigen with a molecular weight of 19,000 was detected in immunoblots and in RIPs utilizing sera raised against live organisms and subsequently shown to be surface oriented (see text).

[b] The number of plus signs indicates the relative intensity of bands based on densitometer tracings.

[c] Rabbit anti-*L. pneumophila* sera after extensive absorption with intact *L. pneumophila* cells.

a major role in the defense against *L. pneumophila* infection, then the antigens that are recognized may be important in pathogenesis. Several lines of evidence support a role for humoral immunity in defense against disease caused by this organism: (i) Immune sera confer protection against lethal infection with *L. pneumophila* in a rat model system (27) and a BALB/c mouse system (7; D. Drutz, personal communication). (ii) In a recently published report on the kinetics of *L. pneumophila* infection in the guinea pig model, it was shown that the onset of pathogen elimination coincides with the appearance of an antibody response (6). (iii) In vitro studies with human monocyte cultures have demonstrated that immune sera reverse *L. pneumophila*-mediated inhibition of phagosome-lysosome fusion (17). Work at present is being directed at establishing the relationship between the cloned *L. pneumophila* proteins (11, 12) and the major antigens identified in this study. Eventually, we hope to evaluate the role that each of these proteins plays in the immunopathogenesis of both experimental and human infection with *L. pneumophila*.

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