NOTES

Ultrastructural Localization and Protective Activity of a High-Molecular-Weight Antigen Isolated from Legionella pneumophila

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Immunoperoxidase labeling showed that the F-1 antigen of Legionella pneumophila is located on the bacterial cell surface. Protection against lethal intraperitoneal challenge with serogroup 1 L. pneumophila was induced in guinea pigs by heat-killed cells and F-1 antigen from serogroup 1, but not by heat-killed cells or F-1 antigens from serogroup 2, 3, or 4.

Legionella pneumophila has been identified as the etiologic agent of an acute pneumonia in humans (8). Six serogroups have been identified (10), with most of the clinical isolates belonging to serogroup 1 (9). Although considerable efforts have been made to elucidate the serology and epidemiology of Legionnaires disease, little is currently known about the mechanism of pathogenesis of infections caused by L. pneumophila or the mechanisms of immunity responsible for protection against infections caused by L. pneumophila. Mueller (11) has reported the production of serum proteases by L. pneumophila, and Friedman et al. (5) have shown that culture filtrates of L. pneumophila contain a cytotoxin for Chinese hamster ovary cells. However, the role of these two toxins in the pathogenesis of L. pneumophila infections remains unresolved. Johnson et al. (6, 7) have shown that L. pneumophila produces a high-molecular-weight antigen (F-1) which is responsible for the serogroup specificity of L. pneumophila. This high-molecular-weight antigen may be involved in the virulence of the organism since uptake of L. pneumophila cells by rat alveolar and mouse peritoneal macrophages appears to be dependent on the presence of serogroup-specific antisera (7). The F-1 antigen was found to be the major antigen detected by both the indirect immunofluorescence and microagglutination assays (6).

The purpose of the present study was to determine the ultrastructural location of the F-1 antigens in L. pneumophila and to determine whether protective activity could be induced by the F-1 antigens against lethal challenge with serogroup 1 organisms.

L. pneumophila serogroups 1, 2, 3, and 4 were obtained from the Center for Disease Control, Atlanta, Ga. Bacteria were grown on charcoal-yeast extract agar (3) in a humidified incubator at 35°C. After confluent growth was obtained, cells were harvested from the surface of the medium with sterile saline (0.85%). The F-1 antigens of L. pneumophila serogroups 1, 2, 3, and 4 were isolated as previously described (7).

Immunoperoxidase staining was performed as described by Bohn (1), except that the fixed bacteria were preincubated with 5% bovine serum albumin before incubation with antiserum. This reduced nonspecific antibody attachment. Antisera to the F-1 antigens were prepared by intramuscular immunization of rabbits with antigens suspended in complete Freund adjuvant. Each rabbit received an initial injection of 2 mg of antigen followed by a booster immunization at 3 weeks. Serum was obtained from the lateral ear vein 2 weeks after the booster immunization.

Groups of four Hartley strain guinea pigs received two subcutaneous injections of 0.5 ml (0.5 mg/ml) of F-1 antigen of serogroups 1, 2, 3, or 4 suspended in complete Freund adjuvant. Booster injections were given 3 weeks later. Immunization with heat-killed cells (101°C for 1 h) was performed by subcutaneous injection of 0.5 ml of a suspension of heat-killed cells in complete Freund adjuvant (ca. 10⁶ cells per 0.5 ml) into guinea pigs. Booster immunizations were given three weeks later. Before challenge, serum was obtained from one guinea pig in each F-1-immunized group to determine antibody titers by microagglutination (2). At 2 weeks after the second injection the guinea pigs were challenged intraperitoneally with 100 50% lethal doses of serogroup 1 organisms. Virulent serogroup 1 L. pneumophila was obtained from a stock guinea
pig spleen suspension plated on charcoal-yeast extract. The 50% lethal dose of *L. pneumophila* serogroup 1 was $1.5 \times 10^5$ colony-forming units, as determined by the method of Reed and Muench (13).

The ultrastructural localization of the serogroup 1 F-1 antigen on the surface of the organism and the specificity of the serogroup 1 F-1 antigen for serogroup 1 cells are shown in Fig. 1A and B. Fig. 1A shows that immunoperoxidase-labeled antibody to the F-1 antigen isolated from serogroup 1 cells was located on the bacterial cell surface of serogroup 1 organisms. Fig. 1B shows that the antibody to the F-1 fraction of serogroup 2 organisms did not react with the cell surface of serogroup 1 organisms. Additional experiments showed that antibody to F-1 antigens isolated from serogroups 3 and 4 did not react with serogroup 1 cells.

The protective activity of F-1 fractions isolated from the four serogroups of *L. pneumophila* and heat-killed cells against challenge with serogroup 1 organisms is shown in Table 1. Immunization of guinea pigs with the serogroup 1 F-1 antigen cells resulted in the development of antibody to the homologous antigen and protected guinea pigs against challenge with 100 50% lethal doses of the serogroup 1 organism. In contrast, although guinea pigs inoculated with F-1 antigens from serogroups 2, 3, and 4 developed antibody titers to the homologous antigen, they failed to develop antibody to serogroup 1 organisms, and little or no protection against challenge with serogroup 1 cells was observed. The specificity of protection observed in guinea pigs immunized with F-1 antigens was also seen in guinea pigs immunized with heat-killed cells. Guinea pigs immunized with heat-killed serogroup 1 cells were protected against challenge with serogroup 1 cells. However, guinea pigs immunized with heat-killed serogroup 2 or 3 cells showed no protection against challenge.

**Fig. 1.** Localization of the F-1 antigen of *L. pneumophila* and demonstration of the serogroup specificity of antisera to the F-1 antigen by immunoperoxidase labeling. A, Reaction of serogroup 1 antiserum with serogroup 1 cells; the immunoperoxidase label is associated with the cell surface. B, Reaction of serogroup 2 antiserum with serogroup 1 cells; the serogroup 2 antiserum does not react with the serogroup 1 cells.
with serogroup 1 organisms. Since previous studies have shown that guinea pigs (12) and rabbits (7) infected with L. pneumophila respond with serogroup-specific antibodies, extensive serological studies were not done with serum from guinea pigs immunized with heat-killed cells. However, one guinea pig immunized with heat-killed serogroup 2 organisms had a significant microagglutination titer to the homologous antigen, but not to the serogroup 1 antigen (Table 1).

The results of this investigation show that the F-1 antigen is located on the surface of the cell and confirms previous observations (6, 7) that the F-1 antigen is the serogroup-specific antigen of L. pneumophila. Although the F-1 antigen is located on the cell surface, it is easily removed by gently washing the cells with saline (6, 7). The F-1 antigen isolated by this procedure may be associated with the blebs and rods originating from the outer membrane, as described by Flesher et al. (4). The finding that protection against challenge with serogroup 1 cells is most efficiently induced by heat-killed serogroup 1 cells or serogroup 1 F-1 antigen is consistent with our previous observation that serogroup-specific antiserum is required for phagocytosis of L. pneumophila by peritoneal and alveolar macrophages (7). The finding that guinea pigs immunized with F-1 antigens respond with significant antibody titers to the homologous antigen as determined by microagglutination titers further suggests that serogroup-specific anti-body may be necessary to protect against challenge with L. pneumophila. However, further studies will be necessary to elucidate the mechanism of immunity induced by the F-1 antigen and heat-killed cells. The protective activity of the serogroup 1 F-1 antigen against challenge with the serogroup 1 organism further suggests that the F-1 antigen may be a virulence factor of L. pneumophila.

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### LITERATURE CITED


