Susceptibility of LSH Hamsters to Intraperitoneal Inoculation with Legionella pneumophila

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ABSTRACT

The guinea pig is the most widely used animal model in the study of Legionellosis. The hamster should also be considered, since it acquires virtually no spontaneous epidemic lung infection, possesses similar cellular immune components observed in other mammals, has a normal body temperature identical to that of man, and is readily available for laboratory investigation.

We studied the pathologic findings of four 12 week old inbred London School of Hygiene (LSH) hamsters inoculated intraperitoneally with 0.2 ml of $10^9$ organisms per ml suspension of a viable Philadelphia 1 strain of Legionella pneumophila. Four LSH hamsters (control group) received 0.2 ml of sterile phosphate buffered saline, intraperitoneally.

All animals of the test group became clinically ill and two of the four spontaneously expired on days 1 and 2 after inoculation. The remainder were sacrificed on day 3. In three out of four animals of the test group, a suppurative peritonitis and an interstitial pneumonitis were observed. It was characterized by infiltrates of neutrophils and macrophages. The test group also exhibited acute splenitis, including microabscesses, and two of four test animals showed hepatic congestion, vacuolization of hepatocytes, and microabscesses. None of the controls appeared sick or died after three days, and neither gross nor microscopic lesions were found at autopsy. Culture results documented L pneumophila in lung and spleen of all test animals and the absence of organisms in the control group.

Hence, the LSH hamster is rapidly infected with the Philadelphia 1 strain of L pneumophila given intraperitoneally, and pathological changes can be readily observed. The findings of our study add hamsters to the list of animals susceptible to intraperitoneal infection by L pneumophila.

Introduction

The ability of Legionella pneumophila to produce infections has been documented in a variety of animals. The guinea pig is the most widely tested animal, but some laboratories use rats,
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mice, rabbits, and monkeys. Routes of inoculation include intraperitoneal, intratracheal, subcutaneous, respiratory aerosolization, and intranasal inhalation (nose drops). Patton performed a study evaluating the relative susceptibility of *L. pneumophila* in different animals. By intraperitoneal inoculation, highly susceptible species were the Hartley strain of guinea pig, Sherman rat, and Mongolian gerbil. Moderate to low susceptibility occurred in the Institute for Cancer Research (ICR) mouse, Syrian hamster, and New Zealand white rabbit. The white leghorn chicken, coturnix quail and corneaux pigeon were not susceptible. Collins demonstrated a high prevalence of antibody to *L. pneumophila* in horses, suggesting that horses are commonly infected with Legionella. Other animals such as cattle, sheep, pigs, and goats exhibit antibodies to Legionella.

The pathologic findings are described of LSH hamsters inoculated intraperitoneally with viable Philadelphia 1 strain of *L. pneumophila*, and the utility of the hamster as an animal model for further study of legionellosis is discussed.

**Materials and Methods**

Eight pathogen free, 12 week old inbred London School of Hygiene (LSH) hamsters were utilized. Four of these hamsters (test group) were inoculated intraperitoneally with 0.2 ml of a 10⁹ organisms per ml suspension of a viable Philadelphia 1 strain of *L. pneumophila*. The microbe, obtained from the Department of Health, Commonwealth of Pennsylvania, Bureau of Laboratories, was grown for at least one year on charcoal yeast agar, passed one time in embryonated hen’s eggs, suspended in sterile phosphate buffered saline (PBS), and tested for virulence in pathogen free male Hartley guinea pigs. Four LSH hamsters (negative controls) received 0.2 ml of sterile PBS intraperitoneally. Each animal was housed in a separate cage in a well-ventilated biocontainment area, and each was examined daily for signs of illness.

From day 1 to day 3 after inoculation, when clinical signs of illness developed (ruffled fur, lethargy, watery eyes) hamsters from each test group were autopsied. Samples of lung and spleen were taken for culture using charcoal yeast extract agar, blood agar, eosin methylene blue agar, and thryogocylate broth to confirm the presence of *L. pneumophila* and the absence of bacterial contamination. In addition to the previous tissues, the liver, kidney and trachea were evaluated by direct immunofluorescence for presence of *L. pneumophila*. The fluorescence conjugated antiserum to *L. pneumophila* was prepared in our laboratory by inoculation of *L. pneumophila* into rabbits. Samples of these tissues were fixed in four percent buffered formaldehyde solution and processed for routine light microscopy. The slides were stained with hematoxylin and eosin to detect lesions and by the Dieterle method to visualize the bacteria.

**Results**

Within one day after inoculation, all animals given *L. pneumophila* appeared to be sick; they had ruffled fur and runny noses. Two test animals expired spontaneously on days 1 and 2 after inoculation, and the other two animals were sacrificed on day 3. A suppurative peritonitis and an interstitial pneumonitis, observed in three out of four infected animals, was characterized by alveolar septal edema and a diffuse, hemorrhagic peribronchiolar and interstitial consolidation by macrophages and neutrophils (figures 1 and 2). All infected animals exhibited acute splenitis (figure 3), including microabscesses and sinus histiocytosis. The livers in two of four test animals showed marked congestion, vac-
uolization of hepatocytes, and occasional microabscesses (figure 4). None of the controls expired or appeared sick after three days. Each was sacrificed on day 3 for complete autopsy, and neither gross nor microscopic lesions were detected.

Culture results (table I) documented $L$ pneumoniae in lung and spleen of all test animals and the absence of organisms in the controls.

Direct fluorescence antibody test (DFA) in table I showed $L$ pneumoniae in lung, spleen, and liver in all test animals, and in kidneys in three of the four. Controls were negative for $L$ pneumoniae by DFA.

Discussion

The LSH strain of hamster is rapidly
infected with the Philadelphia 1 strain of *L pneumophila* given intraperitoneally, and pathological changes can be readily observed. Suppurative peritonitis, acute splenitis and pneumonitis were found in three of four infected animals receiving the inoculum, $-2 \times 10^8$ organisms. By contrast, a study by Rolstad on rats injected intraperitoneally with the Philadelphia 1 strain of *L pneumophila* revealed no pathological changes except for splenomegaly; however, injection of the LD-8 strain of *L pneumophila* in rats produced pathological changes in the liver, spleen, and lung.

The results contained herein indicate...
that the hamster is susceptible to *L. pneumophila*. Tests employing other doses and strains of *L. pneumophila*, and other routes of inoculation, seem warranted. In addition, species variation among hamsters to infection with filariasis has been reported between inbred LSH strain and random bred LAKZ strain hamsters. Further studies using *L. pneumophila* in various hamster strains are needed to establish the same possibility.

The hamster is a useful animal to study a variety of infections. First, it acquires virtually no spontaneous epidemic lung infection. Second, unlike mice and rats, its cellular immune components are similar to those observed in other mammals. Third, unlike the mouse, the normal body temperature of the hamster is the same as that of man. The results of the present study indicate that the hamster is a potentially useful mode for the study of legionellosis, although additional work seems warranted.

The findings of our study add to the list of animal models suitable for evaluation of intraperitoneal infection with Legionnaires' disease. The hamster model has merit owing to its availability, practicality, and suitability for laboratory investigation. The histological lesions resemble those seen in humans.

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**References**