Isolation of *Chlamydia pneumoniae* from the Lungs of Patients Infected with the Human Immunodeficiency Virus

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*Chlamydia pneumoniae* is being recognized as a common cause of respiratory tract infections. Bronchoalveolar lavage specimens from human immunodeficiency virus-infected patients were examined by culture for this pathogen. Of 50 specimens examined, 5 (10%) were positive for *C. pneumoniae*. Four of these (80%) were also positive for other pathogens frequently implicated as causes of respiratory disease in this patient population. *C. pneumoniae* may frequently inhabit the respiratory tracts of human immunodeficiency virus-infected individuals.

Since the mid-1980s a body of literature has emerged describing outbreaks of respiratory tract infections caused by a species of chlamydia distinct from *Chlamydia trachomatis* and *Chlamydia psittaci* recently designated as *Chlamydia pneumoniae* (5). Species-specific serologic studies have strongly suggested that this organism is the etiologic agent responsible for infection in populations as diverse as young, otherwise healthy military recruits and hospitalized patients with chronic diseases (4, 8). Despite this accumulated experience, little epidemiologic information is available based upon the isolation of this organism by culture. This technique may be particularly important in patients for whom the antibody response is unreliable (e.g., patients with AIDS).

The response of immunocompromised patients to chlamydial species has not been fully explored. Information is scant on the mechanisms of resistance to chlamydial infections in humans. Humoral response, both immunoglobulin G (IgG) and IgM, has been well characterized for patients with *C. trachomatis* infections, although its role in the evolution of such processes is unclear (11). The role of cell-mediated immunity in protecting against these intracellular pathogens, although clearly demonstrated in animal models, has not been readily shown in humans (14, 15).

The potential for *C. pneumoniae* to act as a respiratory pathogen in human immunodeficiency virus (HIV)-infected patients deserves study. We obtained bronchoalveolar lavage specimens from randomly selected HIV-infected patients undergoing bronchoscopy for the diagnosis of unexplained pulmonary processes. These specimens were cultured for *C. pneumoniae*.

From September 1988 through March 1989, bronchoalveolar lavage was performed on 64 adult patients with pneumonitis of unclear etiology. Fifty patients had documented HIV infection, of which 41 (82.0%) had AIDS as defined by the classification system of the Centers for Disease Control (1). Nine HIV-infected patients did not meet AIDS-defining criteria. The remaining 14 patients had risk factors for HIV infection but were not tested.

Specimens from bronchoalveolar lavage were sent for routine bacterial culture, fungal culture, mycobacterial culture, viral culture, mycoplasmal culture, and methenamine silver and Gram Weigert staining, as well as cytologic examination with the Papanicolaou stain.

A portion of each specimen was stored in chlamydia transport medium (sucrose-phosphate buffer with 10% fetal bovine serum and antibiotics) at −70°C. At a later date specimens were inoculated onto 24-well microtiter plates containing HeLa 229 cell monolayers pretreated with DEAE-dextran. These were then centrifuged at 1,700 × g for 1 h. After 72 h of incubation at 37°C, the infected monolayers were scraped and the cells were sonicated and centrifuged at 500 × g. The supernatant was again inoculated onto new HeLa 229 cell monolayers. This procedure was repeated four times for each specimen. Cell layers separately prepared in 96-well microtiter plates at each passage were examined with genus-specific fluorescein-conjugated monoclonal antibody (Pathfinder Chlamydia Culture Confirmation System; Kallestad Diagnostics, Chaska, Minn.), specific anti-*C. trachomatis* monoclonal antibody (Microtrak Chlamydia trachomatis Culture Confirmation Test; Syva Co., Palo Alto, Ca.), and specific anti-*C. pneumoniae* antibody (Washington Research Foundation, Seattle).

Sera from these patients were not available for study. Of the 50 specimens, 5 (10%) from HIV-infected patients were positive by culture for *C. pneumoniae*. Four specimens required at least two passages to yield discernible inclusions. The remaining positive specimen took three passages. Positive specimens had from 2 to 10 inclusions per well and stained brightly with both genus-specific and *C. pneumoniae* species-specific monoclonal antibody. None of these positive specimens stained with monoclonal antibody specific for *C. trachomatis*. Four of the five patients were found to have other pulmonary pathogens: two had *Pneumocystis carinii* and two had *Mycobacterium tuberculosis*. Culture of a specimen from one patient grew herpes simplex virus, but neither the clinical presentation nor results of cytoclogic studies suggested this as a pulmonary pathogen. Patients with *M. tuberculosis* and *P. carinii* received appropriate therapy for those infections. All except one of the five patients with *C. pneumoniae* documented by culture recovered and were discharged. One patient died as a result of *Enterobacter cloacae* sepsis. Four of the five specimens were from patients with AIDS. The fifth patient had stage IV disease.
C-2 HIV infection, as defined by the classification system of the Centers for Disease Control. None of these patients received antimicrobial agents known to have significant activity against *C. pneumoniae* (2, 10). One patient received pentamidine, and three patients received antimycobacterial agents (i.e., isoniazid, rifampin, and pyrazinamide). The activities of these substances against *C. pneumoniae* have not been investigated. There were no identifiable routine physical, historical, or laboratory data which could distinguish patients with *C. pneumoniae* from the remainder of the patients studied.

Of the 14 patients without documented HIV infection, 1 patient was also positive by culture for *C. pneumoniae*.

We feel that finding *C. pneumoniae* in the bronchoalveolar lavage fluid of 10% of our HIV-infected patients was significant. Concomitant serologies would have been useful, although they are frequently difficult to interpret in HIV-infected patients. Most of our patients had other documented respiratory pathogens and seemed to respond clinically to therapy not specifically directed against *C. pneumoniae*. Thus, the role of this organism in the pathologic process remains unclear.

Evidence indicates that *C. pneumoniae* is a frequent cause of respiratory tract infections in humans. In previous epidemiologic studies done in various countries, the prevalence of antibody to *C. pneumoniae* ranged anywhere from 40 to 70% (3). These seropositive rates appeared to increase with increasing age (16). Yet, how much of this reflects a history of symptomatic infection is not certain. Asymptomatic genital infection with *C. trachomatis* is well described. We have documented asymptomatic nasopharyngeal infection with *C. pneumoniae* with and without seroconversion (6).

Patients with AIDS are at risk for infection with an ever-widening range of microorganisms. Yet, chlamydia have not been implicated as a common cause of infection in these patients. Although *C. trachomatis* has been documented to cause pneumonia rarely in certain immunocompromised adults, including those with leukemia (7) and bone marrow recipients (12), only one well-documented case has been described in a patient with AIDS (9). Moncada et al. (13) isolated *C. trachomatis* in only 3 of 1,316 bronchoscopy specimens obtained from patients with AIDS. No cases of *C. pneumoniae* infection in patients with AIDS have been reported.

Since chlamydiae are obligate intracellular parasites, isolation of the organism suggests infection and not simply colonization. The isolation of *C. pneumoniae* from the lungs of these HIV-infected patients may not imply an etiologic role for this organism in their pulmonary processes. Nor can we be certain about its possible role as an agent that modifies other infectious processes (e.g., superinfection). Additional information on the prevalence of infection with *C. pneumoniae* in the general population documented by culture along with concomitant serologic studies is needed before the true value of our findings can be appreciated.

**REFERENCES**


