

# Legionella

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*Washington C. Winn, Jr.*

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## General Concepts

### Clinical Manifestations

The most common presentation of *Legionella pneumophila* is acute pneumonia (legionellosis); potentially any species of *Legionella* may cause the disease. Extrapulmonary disease (e.g., pericarditis and endocarditis) is rare. Less often, disease presents as a nonpneumonic epidemic, influenzalike illness called Pontiac fever.

### Structure, Classification, and Antigenic Types

*Legionella* species are Gram-negative bacilli. There are currently 39 species and 60 distinct antigenic types of *Legionella*.

### Pathogenesis

*Legionella* bacilli reside in surface and drinking water and are usually transmitted to humans in aerosols. The bacteria multiply intracellularly in alveolar macrophages. Recruited neutrophils and monocytes, as well as bacterial enzymes, produce destructive alveolar inflammation. Direct inoculation of surgical wounds by contaminated tap water has been described.

### Host Defenses

Nonspecific physical and inflammatory pulmonary defenses are important, but cell-mediated immunity is critical. Immunologically activated monocytes and macrophages restrict intracellular bacterial growth. The role of humoral immunity is unclear.

### Epidemiology

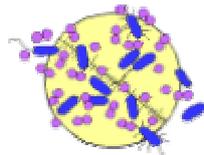
*Legionella* species are widespread in nature. Interactions with other environmental organisms may facilitate growth. Disease may be sporadic or epidemic and may occur in the community or in hospitals. People with compromised host defenses are at increased risk.

### Diagnosis

Legionellosis can be suspected clinically, but diagnosis can be confirmed only by laboratory testing. The preferred method is culturing on special charcoal-containing agar.

## Control

Decontamination of identified environmental sources is of primary importance for prevention. The drug of choice is erythromycin. Immunization works in experimental animals but has not been attempted in humans.

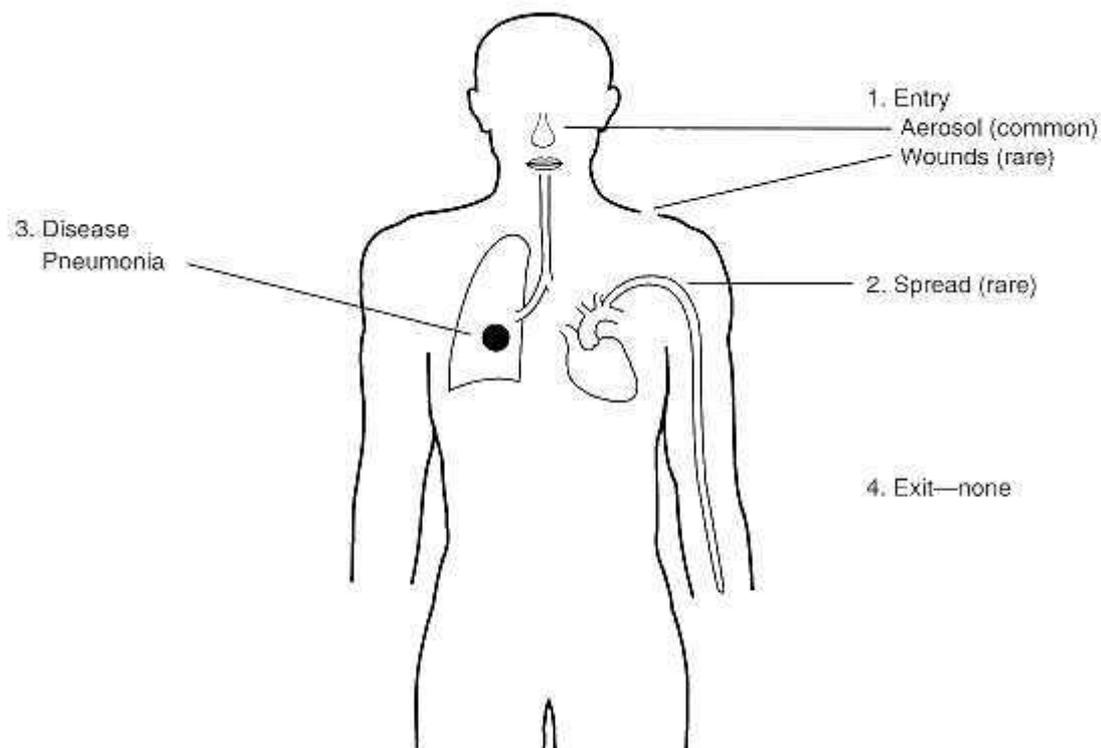


## INTRODUCTION

*Legionella* was first recovered from the blood of a soldier more than 50 years ago, but its importance as a human pathogen was not recognized until 1976, when a mysterious epidemic of pneumonia struck members of the Pennsylvania American Legion. The disease was dubbed Legionnaire's disease by the press. Within 6 months a bacterium, subsequently named *Legionella pneumophila*, had been isolated and definitively established as the agent, thanks to the efforts of many investigators from Pennsylvania and the Centers for Disease Control and Prevention in Atlanta. A general term for disease produced by *Legionella* species is legionellosis.

## Clinical Manifestations

The clinical manifestations of *Legionella* infections are primarily respiratory (Fig. 40-1). Two very different kinds of respiratory illness may result from infection; the reasons for this dichotomy are not understood (Table 40-1). The most common presentation is acute pneumonia, which varies in severity from mild illness that does not require hospitalization (walking pneumonia) to fatal multilobar pneumonia. Typically, patients have high, unremitting fever and cough but do not produce much sputum. Extrapulmonary symptoms, such as headache, confusion, muscle aches, and gastrointestinal disturbances, are common. Most patients respond promptly to appropriate antimicrobial therapy, but convalescence is often prolonged (lasting many weeks or even months).



**FIGURE 40-1 Pathogenesis of legionellosis.**

**TABLE 40-1 Clinical Manifestations of *Legionella* Infections**

Disease	Pneumonia	Occurrence	Attack Rate	Incubation Period	Species Implicated
Legionnaire's disease	Almost always	Epidemic, sporadic, nosocomial, community	Low	Long(days)	All, especially <i>L pneumophila</i> and <i>L micdadei</i>
Pontiac fever	Never	Epidemic community	High	Short (hours)	<i>L pneumophila</i> , <i>L ansia</i> , <i>L micdadei</i> , <i>L feelei</i>
Disseminated infection	Usually	Rare			<i>L pneumophila</i>
Primary Wound infection	Rarely	Sporadic, nosocomial	Rare		<i>L pneumophila</i> , <i>L dumoffii</i>

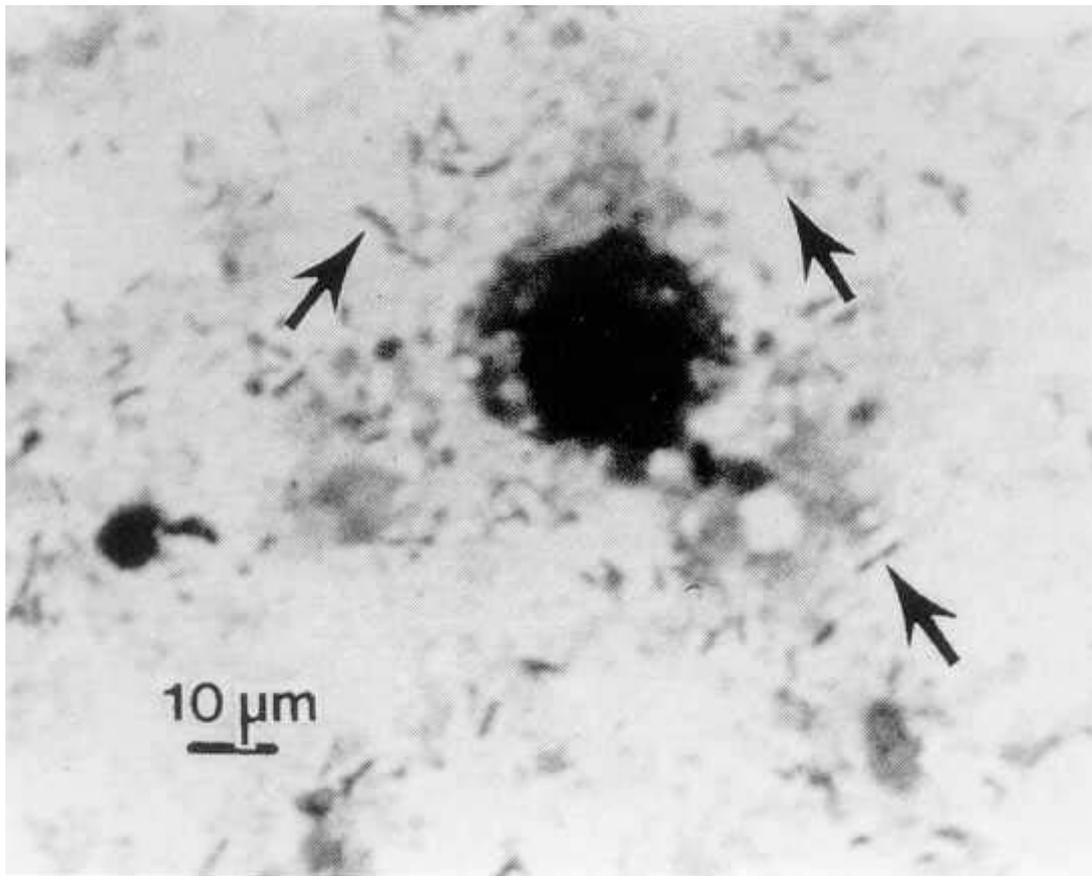
The second form of respiratory illness is called Pontiac fever after the city in Michigan where the first epidemic was recognized. This uncommon manifestation of infection resembles acute influenza, including fever, headache, and severe muscle aches. It is self-limited, and convalescence is uneventful.

Bacteremia occurs during *Legionella* pneumonia, and symptomatic infection outside the lungs occasionally develops. Under special conditions, bacteria introduced through portals other than the lungs, such as surgical wounds, may cause disease.

### Structure

*Legionella* cells are thin, somewhat pleomorphic Gram-negative bacilli that measure 2 to 20  $\mu\text{m}$  (Fig. 40-2). Long, filamentous forms may develop, particularly after growth on the surface of agar. Ultrastructurally, *Legionella* has the inner and outer membranes

typical of Gram-negative bacteria. It possesses pili (fimbriae), and most species are motile by means of a single polar flagellum.



**FIGURE 40-2** Impression smear from the lung of a patient fatally infected with *L. pneumophila* serogroup 1, demonstrating many thin, Gram-negative bacilli (arrows). These bacteria stain less intensely with safranin than do enteric bacilli.

It is ironic that *Legionella* species are sometimes referred to as fastidious bacteria, because they may grow luxuriantly in tap water and can multiply in the usually hostile environment of phagocytic cells. They are fastidious only in regard to the media commonly used in laboratories. The primary growth factor required is L-cysteine, a nutrient that is also essential for *Francisella tularensis*. Ferric iron is also essential, and other compounds are necessary for optimal growth. Energy is derived from amino acids rather than carbohydrates.

### **Classification and Antigenic Types**

Molecular characterization of strains isolated from patients in Pennsylvania led to the creation of a new family of bacteria, Legionellaceae, as well as a new genus, *Legionella*. The genus now has 39 species, defined by studies of DNA homology. Only one genus within the family has been recognized. Immunologic diversity within species is reflected in the creation of serogroups (Table 40-2). *Legionella pneumophila* holds the record, with 14 distinct serologic types. Important antigens include outer membrane proteins, some of which are species specific antigens, and the lipopolysaccharide that is the major serogroup specific antigen. Strains may be divided into subtypes by antigenic

analysis, using panels of monoclonal antibodies, or by characterization of bacterial enzyme systems. These increasingly fine distinctions can be very valuable for epidemiologic study but do not affect clinical decisions.

**TABLE 40-2 Pathogenic Legionella Species Isolated from Humans<sup>a</sup>**

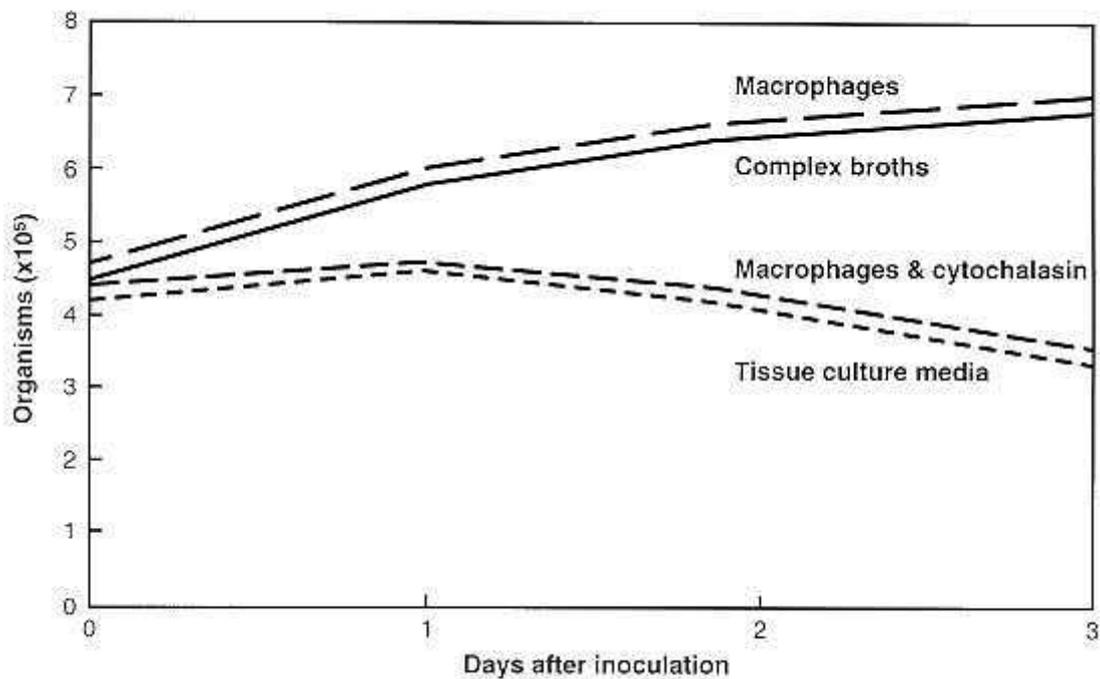
Species	Number of Serogroups
<i>L. pneumophila</i>	14
<i>L. micdadei</i>	1
<i>L. dumoffii</i>	1
<i>L. bozemanii</i>	2
<i>L. gormanii</i>	1
<i>L. feelei</i>	2
<i>L. hackelliae</i>	2
<i>L. israelensis</i>	1
<i>L. jordanis</i>	1
<i>L. sainthelensi</i>	2
<i>L. longbeachae</i>	2
<i>L. maceachernii</i>	1
<i>L. oakridgensis</i>	1
<i>L. wadsworthii</i>	1
<i>L. birminghamensis</i>	1
<i>L. cincinnatiensis</i>	1
<i>L. anisa</i>	1
<i>L. tucsonensis</i>	1
<i>L. lansingensis</i>	1

<sup>a</sup>Species isolated from the environment only: *Legionella cherrii*, *L. erythra*, *L. jamestownensis*, *L. parisiensis*, *L. shakespearei*, *L. santicrucis*, *L. steigerwaltii*, *L. adelaidensis*, *L. fairfieldensis*, *L. brunensis*, *L. moravica*, *L. quinlivianii*, *L. gratiana*, *L. quateirensis*, *L. nautarum*, *L. worsteiensis*, *L. londiniensis*, *L. geestiana*, *L. rubrilucens*, *L. spiritensis*.

## Pathogenesis

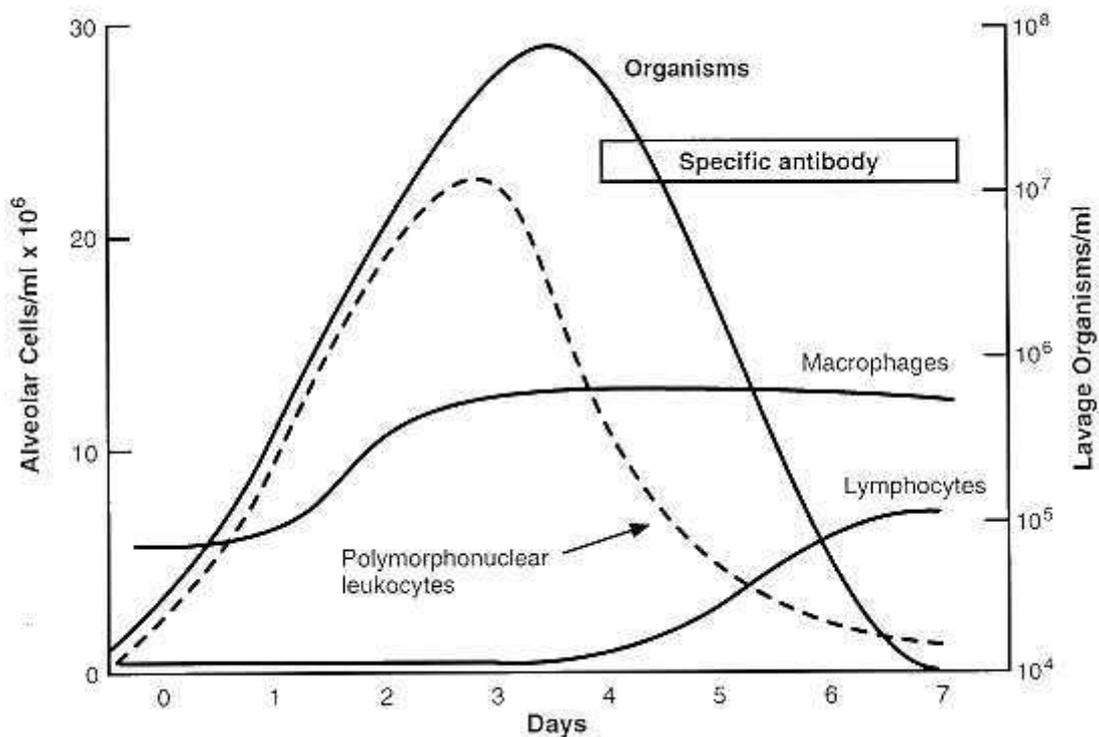
The pathogenesis of *Legionella* infections begins with a supply of water containing virulent bacteria and with a means for dissemination to humans (Fig. 40-1). Person-to-person transmission has never been demonstrated, and *Legionella* is not a member of the bacterial flora of humans.

Infection begins in the lower respiratory tract. Alveolar macrophages, which are the primary defense against bacterial infection of the lungs, engulf the bacteria; however, *Legionella* is a facultative intracellular parasite and multiplies freely in macrophages (Fig. 40-3). The bacteria bind to alveolar macrophages via the complement receptors and are engulfed into a phagosomal vacuole. However, by an unknown mechanism, the bacteria block the fusion of lysosomes with the phagosome, preventing the normal acidification of the phagolysosome and keeping the toxic myeloperoxidase system segregated from the susceptible bacteria. The bacilli multiply within the phagosome. Thus, a cellular compartment that should be a death trap instead becomes a nursery. Eventually, the cell is destroyed, releasing a new generation of microbes to infect other cells.

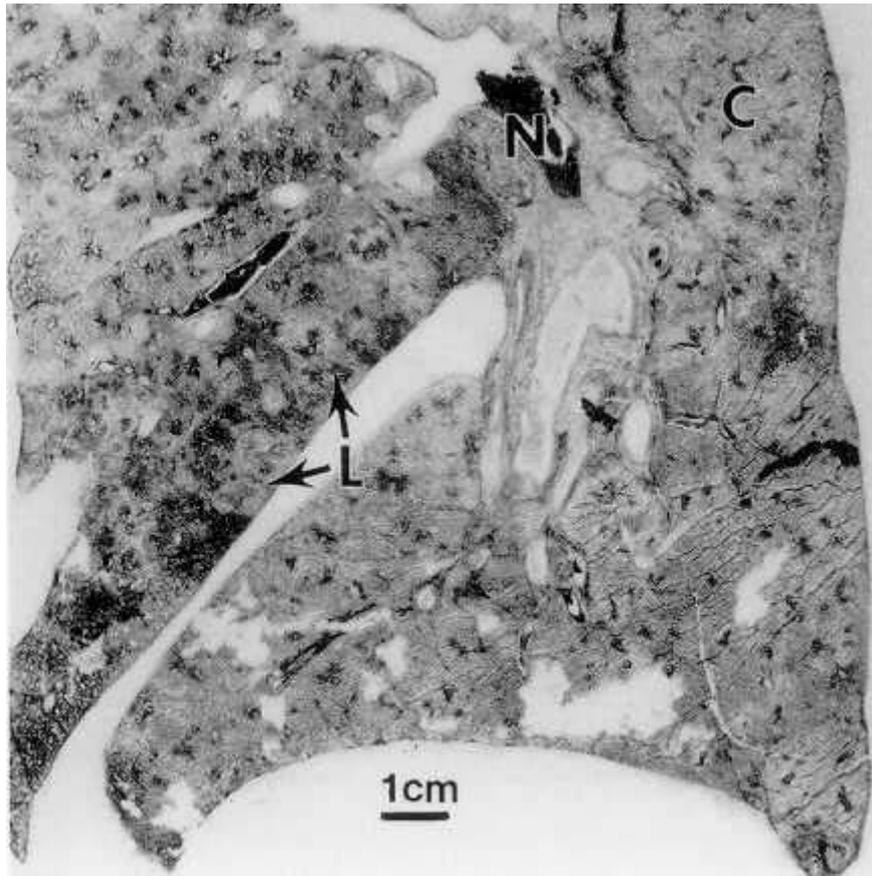


**FIGURE 40-3 Growth of *Legionella* cells in vitro. This facultative intracellular pathogen grows well in complex broths that provide all necessary nutrients.** The usual tissue culture media, which are adequate to support the growth of human and animal cells, cannot support the growth of *Legionella* cells. The bacteria also grow well within alveolar macrophages that have been maintained in cell culture. If phagocytosis is prevented by treatment with cytochalasin, however, the bacteria are denied access to the intracellular environment and growth does not occur.

Bacterial growth, activation of the complement system, and/or the death of alveolar macrophages produce powerful chemotactic factors that elicit an influx of monocytes and polymorphonuclear neutrophils (Fig. 40-4). Leaky capillaries allow the transudation of serum and deposition of fibrin in the alveoli. The result is a destructive pneumonia that obliterates the air spaces and compromises respiratory function (Fig. 40-5). Dissemination of bacteria to sites outside the lung occurs at least partially via macrophages, but only rarely does an inflammatory response develop.



**FIGURE 40-4 Inflammatory response in experimental *Legionella* pneumonia.** Alveolar macrophages are the only resident cells in the air spaces of the lungs. Exponential bacterial growth begins soon after infection at a time when only macrophages are present; bacteria are most numerous 3 to 4 days later, but have virtually disappeared by the end of the first week. Infection elicits a large influx of polymorphonuclear leukocytes, followed by monocytes from the peripheral blood. Fluid exudation into the alveoli follows the pattern of the polymorphonuclear leukocytes. Specific immunologic responses (i.e., antibody and lymphocyte influx) are detectable 4 to 5 days after infection.



**FIGURE 40-5 Acute *L pneumophila* pneumonia. Papermounted whole-lung section, unstained.** The air spaces are filled with fibrin and inflammatory cells. The consistency of the completely consolidated lower lobe (C) resemble that of an adjacent hilar lymph node (N). The lobular nature of the process is accentuated by gray carbon accumulation around the terminal bronchioles in the centers of the lobules (L).

The symptoms of *Legionella* infection undoubtedly result from a combination of physical interference with oxygenation of blood, ventilation-perfusion imbalance in the remaining lung tissue, and release of toxic products from bacteria and inflammatory cells. Bacterial factors include a protease that may be responsible for tissue damage. Cellular factors include interleukin-1, which produces fever after it is released from monocytes, and tumor necrosis factor, which may be responsible for some of the systemic symptoms.

Virulence appears to be multifactorial. An outer membrane protein that functions as a metalloprotease and a cytoplasmic membrane heat-shock protein elicit protective immune responses, but are not essential for expression of virulence. A gene that encodes a 29 Kd protein and plays a role in cellular infection has been identified. Mutations of the gene are associated with decreased virulence.

### **Host Defenses**

Risk factors for legionnaire's disease include conditions that compromise both the specific and non-specific defenses. The fact that patients with chronic heart and lung disease are at increased risk of developing serious *Legionella* pneumonia suggests that

the integrity of physical clearance mechanisms, such as the mucociliary escalator of the tracheobronchial tree, is an important element of the defenses. Nonimmunologic antibacterial factors normally found in respiratory secretions, such as lactoferrin or lysozyme, may also play a role.

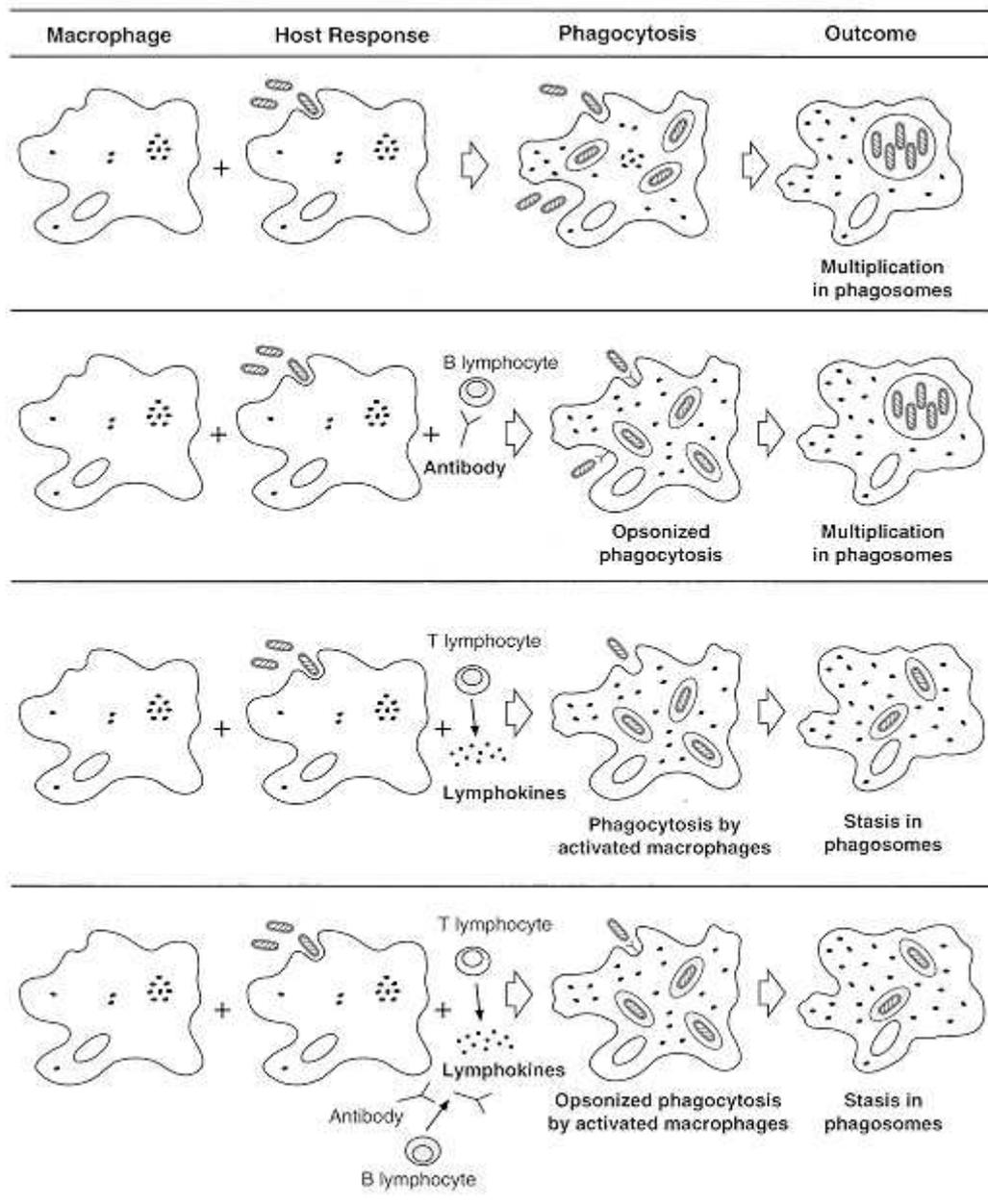
Inflammatory cell defenses play both positive and negative roles. The human alveolar macrophage and its relative, the recruited blood monocyte, abdicate their normal roles as primary antibacterial defenses in *Legionella* infections. The major reason why mice are very resistant to experimental *Legionella* pneumonia is probably that their alveolar macrophages do not support intracellular bacterial growth.

The role of polymorphonuclear leukocytes is less clear. These cells do not support bacterial growth in vitro and are only minimally bactericidal. Treatment with cytokines such as gamma interferon marginally increases their bactericidal activity. Neutropenia is not an important risk factor.

The most impressive risk factors for human disease are various types of immunosuppression. In a small outbreak of disease caused by contaminated nebulizers, pneumonia developed most often in patients being treated with corticosteroids.

The critical component of the immune system in resistance to legionellosis has not yet been pinpointed. Attention has focused on cell-mediated immunity because *Legionella* is a facultative intracellular pathogen. Infected patients produce a cell-mediated immune response that can be detected by measuring lymphocyte blastogenesis after exposure to *Legionella* antigen. Lymphocytes appear in the air spaces of experimentally infected animals about 5 days after an acute infection (Fig. 40-4). In contrast to naive alveolar macrophages, which are permissive for intracellular bacterial growth, activated alveolar macrophages or peripheral blood monocytes restrict bacterial multiplication in vitro (Fig. 40-6). The macrophages can be activated by treatment with lymphokines produced by specifically stimulated lymphocytes. Gamma interferon, which can substitute for the lymphokines, is an important mediator. It has been suggested that restriction of entry of iron (an important growth factor for *Legionella*) into the phagosome inhibits intracellular growth.

The role of humoral immunity is less clear. Antibody in all immunoglobulin classes is made after human or experimental infection. This antibody serves an opsonizing function in vitro, facilitating the phagocytosis of bacteria by polymorphonuclear leukocytes, macrophages, and monocytes. Antibody does not kill most strains of *Legionella*; however, so that the outcome of the interaction depends on the capabilities of the phagocytic cell (Fig. 40-6). The classic pathway of the complement system is activated by *L pneumophila*, enhancing phagocytosis still further. *Legionella micdadei* activates the alternative complement pathway as well, so that opsonization of this species can occur even before an immunologically specific antibody response is mounted. One can construct scenarios from in vitro data in which antibody is deleterious as well as helpful. Experimental studies with animals support a protective role for antibody.



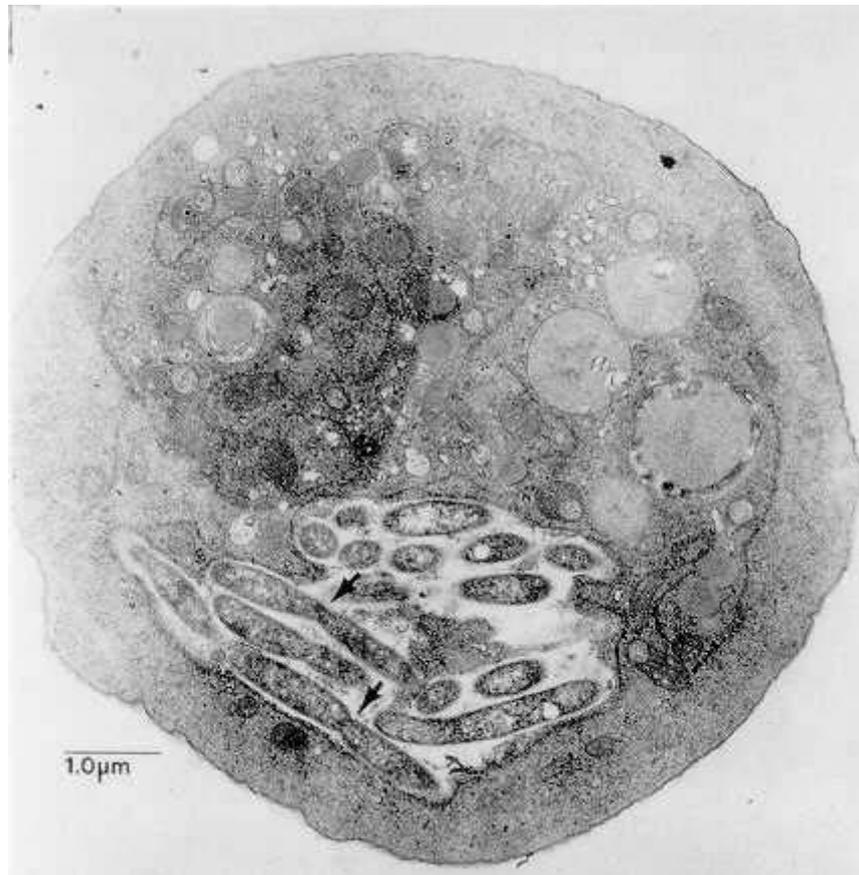
**FIGURE 40-6** Multiplication of *Legionella* is inhibited in activated macrophages, whereas growth in normal macrophages provides a preferred environment, enhancing bacterial growth.

## Epidemiology

The epidemiology of *Legionella* infections is a complex equation that is composed of the aquatic environment (including representatives of multiple microbial phyla, humans, mechanical devices and medical facilities), dissemination from the environment to the host, and host susceptibility. The complexity of the environmental interactions rivals those of viral and parasitic infections.

The only documented source of *Legionella* species is water, particularly the surface waters of rivers and lakes and drinking water. *Legionella* does not multiply in sterile tap water, but the addition of free-living amoebae results in growth of *Legionella* in vitro.

The relationship with a variety of amoebae is particularly interesting. These single-cell animals, which can be viewed as nature's macrophages, occur in the same aquatic environment as do *Legionella* organisms (Fig. 40-7) and support the intracellular growth of *Legionella* in much the same way. Another factor that favors the survival of *Legionella* in natural or treated waters is its relative resistance to the effects of chlorine and heat; *Legionella* can find refuge in relatively inhospitable environments such as hot-water tanks.



**FIGURE 40-7** Electron micrograph showing *L pneumophila* serogroup 1 in the process of dividing (arrows) within a vesicle of an amoeba (*Hartmanella veriformis*) cell. (X 18,500.) (Courtesy of Barry S. Fields, Centers for Disease Control.)

The second factor in the epidemiology equation is dissemination of bacteria from the environment to the host. In most cases the link is an aerosol of water contaminated with the organisms. Evaporative condensers and cooling towers are proven sources of outdoor infection. Indoors, nebulizers and humidifiers filled with contaminated drinking water have disseminated *Legionella* to susceptible patients. The automatic misting devices that keep supermarket produce fresh have even been fingered as culprits in outbreaks of pneumonia. Aerosols are produced in numerous ways in our environment, from taking a shower to flushing the toilet. An epidemic of Pontiac fever caused by *Legionella anisa* was associated with an ornamental fountain in a public place. Clusters of *Legionella* pneumonia have occurred after exposure to whirlpool spas in hotels or cruise ships. In most cases we do not know the source of the infection. Direct infection of surgical wounds has been linked to washing of patients with tap water that harbored pathogenic *Legionella* organisms.

The final factor in the equation is the susceptibility of patients. The diseases and conditions that serve as risk factors are concentrated in health care facilities, so it should be no surprise that many of the epidemics of disease have been nosocomial.

*Legionella* infections may be sporadic or epidemic, community acquired or nosocomial. There is great geographic variation in the frequency of infection even within communities, presumably reflecting the presence of suitable aquatic environments and susceptible subjects. Both sporadic and epidemic cases are more common during summer than winter months, perhaps because of increased use of air-cooling equipment that generates aerosols.

## Diagnosis

There are no reliable distinguishing clinical features of *Legionella* pneumonia, so the diagnosis must come from the laboratory. Some clinical features suggest legionnaire's disease; however, and should prompt the selection of appropriate laboratory tests (Table 40-3). The diagnosis is confirmed in the laboratory by culture, demonstration of bacterial antigen in body fluids, or detection of a serologic response.

**TABLE 40-3 Clinical Clues to *Legionella* Pneumonia**

Clue	Example
Patient's history and physical examination	Family, friends, or associates with similar infection and similar exposure
Presence of an epidemic or documented source of infection	
Prominent neurologic or gastrointestinal symptoms	Pneumonia with confusion, nausea, and vomiting
Nonresponse to aminoglycoside or $\beta$ -lactam antibiotics	Worsening condition after 5 days on antibiotics
Patient's laboratory data	
Gram stain of sputum with many neutrophils but no bacteria	Laboratory report showing many neutrophils and few normal flora or no bacteria
Nodular peripheral infiltrates in chest radiograph	Progression of unilateral opacities to bilateral nodular infiltrates over several days

The preferred diagnostic method is culturing, because it is both sensitive and specific; however, appropriate specimens are not always available. The laboratory must be alerted to the possibility of legionellosis, because specially designed media must be used. The medium of choice is buffered charcoal-yeast extract -  $\alpha$ -ketoglutarate medium. This medium contains yeast extract, iron, L-cysteine, and  $\alpha$ -ketoglutarate for bacterial growth; activated charcoal to inactivate toxic peroxides that develop in the media; and buffer with a pK at pH 6.9, the optimum for growth of *Legionella* organisms. Addition of albumin to the media may further facilitate growth of species other than *L pneumophila*. For contaminated specimens such as sputum, antibiotics should be added. Morphologically distinctive bacterial colonies can usually be detected within 3 to 5 days and identified presumptively as *Legionella* species if the isolated bacteria depend on cysteine for growth. The identification can be confirmed by specific immunologic typing of the isolated bacteria or, in problematic cases, by molecular analysis.

Direct detection of bacterial antigen in clinical specimens is potentially much faster than culturing. Unfortunately, direct immunofluorescence detection of *Legionella* antigen in

respiratory specimens is neither sensitive nor specific enough to warrant general use. A commercially available radioimmunoassay for bacterial antigen in urine is satisfactory, but is available only for serogroup 1 of *L pneumophila* and requires the use and disposal of radiochemicals.

Serologic diagnosis is moderately sensitive and reasonably specific. It should be considered as an adjunct to diagnosis by culture. Indirect immunofluorescence has been used most frequently.

It is important to use an assay that detects IgM and IgG. The advantages of serologic diagnosis are that it is performed on easily obtained blood specimens and can detect mild or even asymptomatic infection. The major disadvantage of the technique is that paired acute and convalescent-phase sera are essential. The convalescent-phase specimen must be obtained at least 6 weeks after onset of the infection, by which time the physician and patient have often lost interest in the enterprise. Furthermore, in non-epidemic situations when the prevalence of disease is low, cross-reactions with other bacteria may create an unacceptable low predictive value for a positive test. Cross-reactions among *Legionella* species and serogroups make assignment of a species-specific diagnosis impossible on serologic grounds.

## Control

Because the interactions between *Legionella* organisms, the environment, and the host are so complex, the incidence of disease may be controlled in several ways. If an aquatic source of infection can be found, elimination of *Legionella* from the source is an effective control mechanism. This genus is so common in water systems that molecular analysis of environmental and clinical stains is often helpful in pinpointing the source. Unfortunately, decontamination can be expensive. The two most common means of eradicating *Legionella* are periodic superheating of water with attendant dangers of scalding, and continuous chlorination, which accelerates deterioration of plumbing systems unless carefully monitored. Even "chlorinated" drinking water must be treated because the levels of chlorine decrease with increasing distance from the distribution center, particularly in hot water. Constant vigilance must be maintained to prevent return of the unwanted pathogens. Elimination of *Legionella* spp. from all environmental sites is unlikely ever to be accomplished.

Immunization has been proposed as a means of preventing *Legionella* infection in susceptible populations. This approach works in experimental animals but has not been attempted in humans.

Pontiac fever does not require antimicrobial therapy. The preferred drug for symptomatic *Legionella* infections is erythromycin. If the patient is seriously ill, it is important to deliver the antibiotic intravenously at first; subsequently, oral therapy may be used. Rifampin is sometimes added as a second antibiotic in seriously ill patients. Retrospective analysis of the antibiotics used to treat the Pennsylvania legionnaires suggests that erythromycin was the most effective agent. If these patients had been hospitalized in major medical centers, they would undoubtedly have received the latest antimicrobial agents and it might have taken us years to determine that erythromycin is the drug of choice. Experimentally, both erythromycin and rifampin inhibit the growth of *Legionella* organisms in infected macrophages, but do not kill the bacteria.

Prophylactic antibiotic therapy may be useful for patients at high risk of serious disease, such as transplant recipients, when a documented epidemic is occurring.

It may appear that we are defenseless against *Legionella* infection, because the most effective type of host defense shows only very modest bactericidal abilities in vitro. In fact most infections are subclinical, and mortality is low in patients who are not immunocompromised. Similarly, even susceptible experimental animals survive infection unless moderately large doses of bacteria are given. The defense mechanisms probably function better in vivo than in vitro. The action of host defenses may also be additive in vivo. One can construct a scenario by which bacteria are increasingly phagocytosed by cells that do not permit bacterial growth. The net result is a decreasing number of extracellular bacteria and hence a decreased source of infection for a decreasing population of permissive cells. Obsolescent inflammatory cells in the lungs are removed by the mucociliary escalator and expectorated as sputum. Therefore, the infection may begin with a bang, but it ends in most cases with a whimper.

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