

COURSE: Medical Microbiology, MBIM 650/720 - Fall 2009

TOPIC *Chlamydia* and *Chlamydophila* (Chlamydia)

Lecture 54

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TEACHING OBJECTIVES:

1. To describe the developmental cycle of several members of the family Chlamydiaceae.
2. To describe the pathogenesis, epidemiology, and clinical syndromes associated with these bacteria.

SUPPLEMENTAL READING:

Murray *et al.* Medical Microbiology, 6th Ed., Chapter 46.

KEY WORDS:

Chlamydiaceae, *Chlamydia trachomatis*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, elementary bodies, reticulate bodies, inclusion, biovar, serovar, trachoma, inclusion conjunctivitis, lymphogranuloma venereum (LGV), reactive arthritis, psittacosis, ornithosis, TWAR agent

Chlamydia

The family Chlamydiaceae consists of two genera. The first genus, *Chlamydia*, has one species that causes human disease, *C. trachomatis*, which can cause urogenital infections, trachoma, conjunctivitis, pneumonia and lymphogranuloma venereum (LGV). The second genus, *Chlamydophila*, has two species, *C. pneumoniae*, which can cause bronchitis, sinusitis, pneumonia and possibly atherosclerosis and *C. psittaci*, which can cause pneumonia (psittacosis). Previously these three species were included in one genus called *Chlamydia*. It will take some time for the new nomenclature to be widely used.

Chlamydia are small obligate intracellular parasites and were once considered to be viruses. However, they contain DNA, RNA, and ribosomes and make their own proteins and nucleic acids and are now considered to be true bacteria. They possess an inner and outer membrane like gram-negative bacteria and LPS. They possess a cell wall structure but it is not the peptidoglycan characteristic of other bacteria. Although they synthesize most of their metabolic intermediates they are unable to make their own ATP and thus are energy parasites.

I. Physiology and Structure

A. Elementary bodies (EB) - EB are the small (0.3 - 0.4 μm), infectious, form of these bacteria.

They possess a rigid outer membrane that is extensively cross-linked by disulfide bonds. Because of their rigid outer membrane the elementary bodies are resistant to harsh environmental conditions encountered when the chlamydia are outside of their eukaryotic host cells. The elementary bodies bind to receptors on host cells and initiate infection. Most chlamydia infect columnar epithelial cells but some can also infect macrophages.

B. Reticulate bodies (RB) - RB are the non-infectious intracellular form of the chlamydia. They are the metabolically active replicating form of the chlamydia. They possess a fragile membrane lacking the extensive disulfide bonds characteristic of the EB.

C. Developmental cycle (**Figure 1** Source: Medical Microbiology, 6th Ed., Murray *et al.*, Figure 46-1). The EB bind to receptors on susceptible cells and are internalized by endocytosis and/or by phagocytosis. Within the host cell endosome the EB reorganize and become RB. The chlamydia inhibit the fusion of the endosome with the lysosomes and thus resist intracellular killing. The entire intracellular life cycle of the chlamydia occurs within the endosome. RB replicate by binary fission and reorganize into EB. The resulting inclusions may contain 100 - 500 progeny. Eventually the cells and inclusions lyse (*C. psittaci*) or the inclusion is extruded by reverse endocytosis (*C. trachomatis* and *C. pneumoniae*).

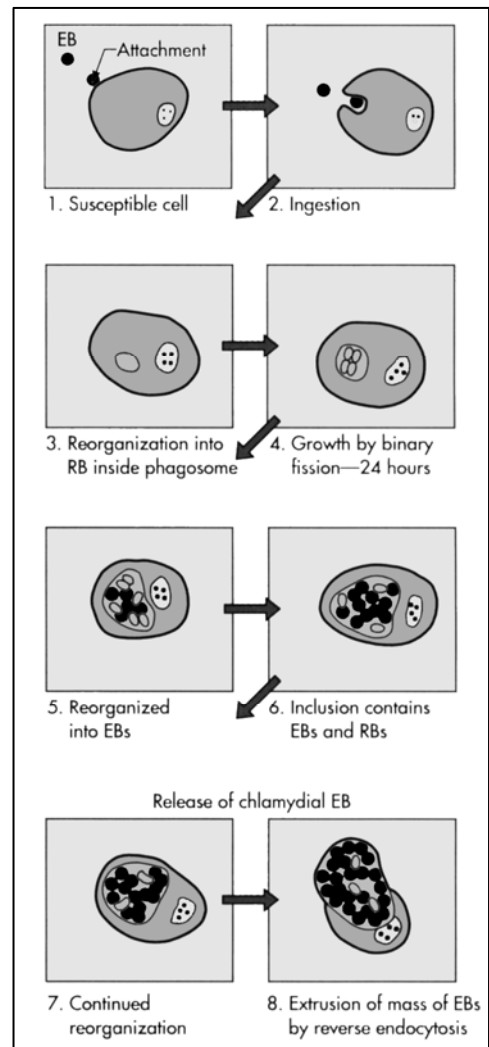


Figure 1. Developmental cycle of *Chlamydia trachomatis*

II. *Chlamydia trachomatis* - *C. trachomatis* is the causative agent of *trachoma*, oculogenital disease, infant pneumonia and lymphogranuloma venereum.

A. Biovars - *C. trachomatis* has a limited host range and only infects human epithelial cells (one strain can infect mice). The species is divided into three biovars (biological variants): trachoma, LGV and mouse pneumonitis (mouse model of infection).

- B. Serovars - The human biovars have been further subdivided into several serovars (serological variants) that differ in their major outer membrane proteins and which are associated with different diseases.

Serovars	Diseases
A, B, Ba, C	Trachoma (trachoma biovar)
D - K	Urogenital tract disease (trachoma biovar)
L1, L2, L2a, L2b, L3	Lymphogranuloma venereum (LGV biovar)

- C. Pathogenesis and Immunity - *C. trachomatis* infects non-ciliated columnar epithelial cells. The organisms stimulate the infiltration of polymorphonuclear cells and lymphocytes, which leads to lymphoid follicle formation and fibrotic changes. The clinical manifestations result from destruction of the cells and the host inflammatory response. Infection does not stimulate long lasting immunity and reinfection results in an inflammatory response and subsequent tissue damage.
- D. Epidemiology

1. Ocular infections

- a. *C. trachomatis* (biovar: trachoma) is found worldwide primarily in areas of poverty and overcrowding. The overall incidence is unknown but it is estimated that ~80 million people are infected worldwide and 7 - 9 million people are blind or visually impaired as a consequence. *C. trachomatis* biovar: trachoma is endemic in Africa, the Middle East, India and Southeast Asia. In the United States American Indians are most commonly infected. Infections occur most commonly in children. The organism can be transmitted by droplets, hands, contaminated clothing, flies, and by passage through an infected birth canal.

2. Genital tract infections

- a. *C. trachomatis* (biovar: trachoma) is the most common sexually transmitted bacterial disease in the United States (4 million new cases each year) and 50 million new cases occur yearly worldwide.

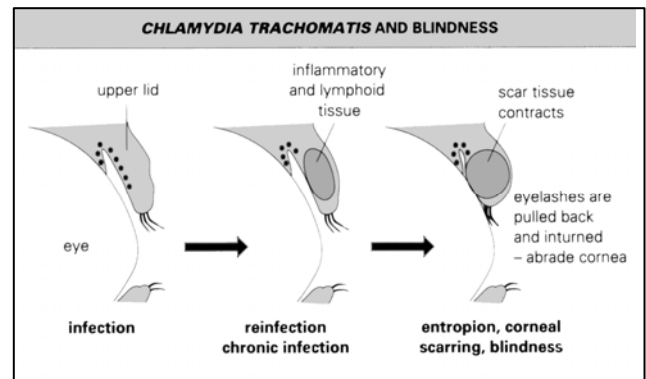


Figure 3. Pathogenesis of trachoma

- b. *C. trachomatis* (biovar: LGV) is a sexually transmitted disease that occurs sporadically in the United States but is more prevalent in Africa, Asia and South America. Humans are the only natural host. Incidence is 300 - 500 cases per year in the United States primarily in male homosexuals.

E. Clinical Syndromes

1. Trachoma (**Figure 3** Source: Medical Microbiology, Mims, *et al.* Figure 21.4) - Chronic infection or repeated reinfection with *C. trachomatis* (biovar: trachoma) results in inflammation and follicle formation involving the entire conjunctiva. Scarring of the conjunctiva causes turning in of the eyelids and eventual scarring, ulceration, and blood vessel formation in the cornea, resulting in blindness. The name trachoma comes from “trakhus” meaning rough, which characterizes the appearance of the conjunctiva. Inflammation in the tissue also interferes with the flow of tears which is an important antibacterial defense mechanisms. Thus, secondary bacterial infections occur.
2. Inclusion conjunctivitis - Inclusion conjunctivitis is caused by *C. trachomatis* (biovar: trachoma) associated with genital infections (serovars D - K). The infection is characterized by a mucopurulent discharge, corneal infiltrates and occasional corneal vascularization. In chronic cases corneal scarring may occur. In neonates infection results from passage through an infected birth canal and becomes apparent after 5 - 12 days. Ear infection and rhinitis can accompany the ocular disease.
3. Infant pneumonia - Infants infected with *C. trachomatis* (biovar: trachoma; serovars: D - K) at birth can develop pneumonia. The children develop symptoms of wheezing and cough but not fever. The disease is often preceded by neonatal conjunctivitis.
4. Ocular lymphogranuloma venereum - Infection with the LGV serovars of *C. trachomatis* (biovar: LGV) can lead to oculoglandular conjunctivitis. In addition to the conjunctivitis patients also have an associated lymphadenopathy.

5. Urogenital infections - In females the infection is usually (80%) asymptomatic but symptoms can include cervicitis, urethritis, and salpingitis. Postpartum fever in infected mothers is common. Premature delivery and an increased rate of ectopic pregnancy due to salpingitis can occur. In males the infection is usually (75%) symptomatic (**Figure 4** Source: Medical Microbiology, 5th Ed., Murray *et al.*, Figure 47-4). After a 3 week incubation period patient's may develop urethral discharge, dysuria and pyuria.

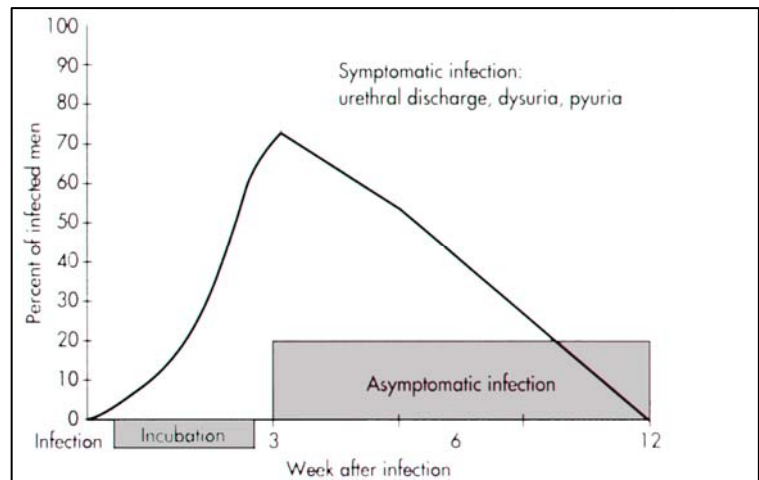


Figure 4. Time course of *C. trachomatis* urogenital infection in males

Approximately 35 - 50% of nongonococcal urethritis is due to *C. trachomatis* (biovar: trachoma). Postgonococcal urethritis also occurs in men infected with both *Neisseria*

gonorrhoeae and *C. trachomatis*. The symptoms of chlamydial infection occur after treatment for gonorrhea because the incubation time is longer.

6. Reactive arthritis (Reiter syndrome) - is a triad of symptoms that include conjunctivitis, polyarthritis and genital inflammation. The disease is associated with HLA-B27. Approximately 50 - 65% of patient have an acute *C. trachomatis* infection at the onset of arthritis and greater than 80% have serological evidence for *C. trachomatis* infection. Other infections (shigellosis or *Yersinia enterocolitica*) have also been associated with reactive arthritis.
 7. Lymphogranuloma venereum (*C. trachomatis* biovar: LGV) - The primary lesion of LGV is a small, painless, and inconspicuous vesicular lesion, that appears at the site of infection. The patient may also experience fever, headache, and myalgia. The second stage of the disease presents as a marked inflammation of the draining lymph nodes. The enlarged nodes become painful "buboes" that can eventually rupture and drain. Fever, headache and myalgia can accompany the inflammation of the lymph nodes. Proctitis is common in females with LGV resulting from lymphatic drainage from the cervix or vagina. Proctitis in males results from anal intercourse or from lymphatic spread from the urethra. Course of the disease is variable but it can lead to genital ulcers or elephantiasis due to obstruction of the lymphatics.
- F. Laboratory diagnosis - There are several laboratory tests for diagnosis of *C. trachomatis* but the sensitivity of the tests will depend on the nature of the disease, the site of specimen collection and the quality of the specimen. Since chlamydia are intracellular parasites, swabs of the involved sites rather than exudate must be submitted for analysis. It is estimated that as many as 30% of the specimens submitted for analysis are inappropriate.
1. Cytology - Examination of stained cell scrapings for the presence of inclusion bodies has been used for diagnosis but this method is not as sensitive as other methods.
 2. Culture - Culture is the most specific method for diagnosis of *C. trachomatis* infections. Specimens are added to cultures of susceptible cells and the infected cells are examined for the presence of iodine-staining inclusion bodies (See the figure in Medical Microbiology, 6th Ed., Murray *et al.*, Figure 46-5, page 447). Iodine stains glycogen in the inclusion bodies. The presence of iodine-staining inclusion bodies is specific for *C. trachomatis* since the inclusion bodies of the other species of chlamydia do not contain glycogen and stain with iodine.
 3. Antigen detection - Direct immunofluorescence and ELISA kits that detect the group specific LPS or strain specific outer membrane proteins are available for diagnosis. Neither is as good as culture, particularly with samples containing few organisms (*e.g.* asymptomatic patients).
 4. Serology - Serological tests for diagnosis is of limited value in adults, since the tests do not distinguish between current and past infections. Detection of high titer IgM antibodies is indicative of a recent infection. Detection of IgM antibodies in neonatal infection is useful.

5. Nucleic acid probes - Three new tests based on nucleic acid probes are available. These tests are sensitive and specific and may replace culture as the method of choice.

G. Treatment and prevention - Tetracyclines, erythromycin and sulfonamides are used for treatment but they are of limited value in endemic areas where reinfection is common. Vaccines are of little value and are not used. Treatment coupled with improved sanitation to prevent reinfection is the best way to control infection. Safe sexual practices and prompt treatment of symptomatic patients and their sexual partners can prevent genital infections.

III. *Chlamydophila psittaci* - *C. psittaci* is the causative agent of psittacosis (parrot fever). Although the disease was first transmitted by parrots, the natural reservoir for *C. psittaci* can be any species of bird. Thus, the disease has also been called ornithosis from the Greek word for “bird.”

A. Pathogenesis - The respiratory tract is the main portal of entry. Infection is by inhalation of organisms from infected birds or their droppings. Person-to-person transmission is rare. From the lungs the organisms enter the blood stream and are transported to the liver and spleen. The bacteria replicate at these sites where they produce focal areas of necrosis. Hematogenous seeding of the lungs and other organs then occurs. A lymphocytic inflammatory response in the alveoli and interstitial spaces leads to edema, infiltration of macrophages, necrosis and sometimes hemorrhage. Mucus plugs may develop in the alveoli causing cyanosis and anoxia.

B. Epidemiology - Approximately 50 - 100 cases of psittacosis occur annually in the United States with most infections occurring in adults. The organism is present in tissues, feces and feathers of infected birds that are symptomatic or asymptomatic. Veterinarians, zoo keepers, pet shop workers and poultry processing workers are at increased risk for developing the disease.

C. Clinical Syndromes (**Figure 5** Source: Medical Microbiology, 5th Ed., Murray *et al.*, Figure 47-7) - The illness develops after an incubation time of 7 - 15 days. Symptoms include fever, chills, headache, a nonproductive cough and a mild pneumonitis. In uncomplicated cases the disease subsides by 5-6 weeks after infection. Asymptomatic infections are common. In complicated cases convulsions, coma, and death (5% mortality rate) can occur. Other complications include carditis, hepatomegaly, and splenomegaly.

D. Laboratory diagnosis - Laboratory diagnosis is based on serological tests. A four-fold rise in titer in paired samples in a complement fixation test is indicative of infection.

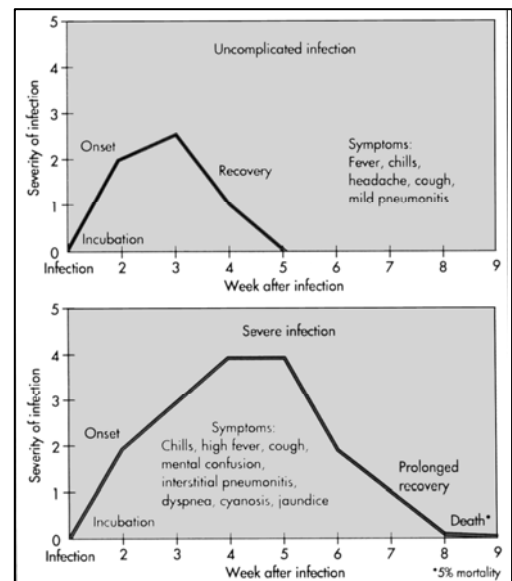


Figure 5. Time course of *C. psittaci* infections.

- E. Treatment and prevention - Tetracycline or erythromycin are the antibiotics of choice. Control of infection in birds by feeding of antibiotic supplemented food is employed. No vaccine is available.

IV. *Chlamydophila pneumoniae* - is the causative agent of an atypical pneumonia (walking pneumonia) similar to those caused by *Mycoplasma pneumoniae* and *Legionella pneumoniae*. In addition it can cause a pharyngitis, bronchitis, sinusitis, and possibly atherosclerosis. The organism was originally called the TWAR strain from the names of the two original isolates - Taiwan (TW-183) and an acute respiratory isolate designated AR-39. It is now considered a separate species of chlamydia.

- A. Pathogenesis - The organism is transmitted person-to-person by respiratory droplets and causes bronchitis, sinusitis, and pneumonia.
- B. Epidemiology - The infection is common with 200,000 - 300,000 new cases reported annually, mostly in young adults. Although 50% of people have serological evidence of infection most infections are asymptomatic or mild. The disease is most common in military bases and college campuses (crowding). No animal reservoir has been identified.

Potential link to atherosclerosis: One report (June 1996) in the *Journal of the American College of Cardiology* documented a high incidence of *C. pneumoniae* in the arteries of patients with atherosclerosis (79% compared with 4% in the control group). Previous reports showed a high association between presence of antibodies to *C. pneumoniae* in serum of patients with atherosclerosis as well as the presence of the organisms in the coronary and carotid arteries. There are many publications (~900) concerned with this association.

- C. Clinical Syndrome - Symptoms include pharyngitis, bronchitis, a persistent cough and malaise. More severe infections can result in pneumonia, usually of a single lobe.
- D. Laboratory diagnosis - Culture is difficult so serological tests are most common. A four-fold rise in titer in paired samples is diagnostic.
- E. Treatment and prevention - Tetracycline and erythromycin are the antibiotics of choice. No vaccine is available.