Chlamydia

Yechiel Becker

General Concepts

Clinical Manifestations

Ocular Infections: *Chlamydia trachomatis* causes trachoma and inclusion conjunctivitis. Trachoma is characterized by the development of follicles and inflamed conjunctivae. The cornea may become cloudy and vascularized; repeated infections are a common cause of blindness. Inclusion conjunctivitis is a milder inflammatory conjunctival infection with purulent discharge.

Genital Infections: Some *C trachomatis* strains cause genital infections, including nongonococcal urethritis in men and acute salpingitis and cervicitis in women. Other strains cause lymphogranuloma venereum, a venereal disease with genital lesions and regional lymph node involvement (buboes).

Respiratory Infections: *Chlamydia psittaci* usually causes an influenzalike illness called psittacosis. *Chlamydia pneumoniae* (TWAR organism) causes atypical pneumonitis in humans.

Structure, Classification, and Antigenic Types

Chlamydiae are obligate intracellular bacteria. They lack several metabolic and biosynthetic pathways and depend on the host cell for intermediates, including ATP. Chlamydiae exist as two stages: (1) infectious particles called elementary bodies and (2) intracytoplasmic, reproductive forms called reticulate bodies. The chlamydiae consist of three species, *C trachomatis*, *C psittaci*, and *C pneumoniae*. The first two contain many serovars based on differences in cell wall and outer membrane proteins. *Chlamydia pneumoniae* contains one serovar the TWAR organism.

Pathogenesis

Chlamydiae have a hemagglutinin that may facilitate attachment to cells. The cell-mediated immune response is largely responsible for tissue damage during inflammation, although an endotoxin-like toxin has been described.

Host Defenses

Antibodies develop during infection, but they do not prevent reinfection. The precise role of cell-mediated immunity is not known.
Epidemiology

Trachoma occurs worldwide and is prevalent in Africa and Asia. *Chlamydia trachomatis* usually is inoculated into the eye by contaminated fingers or fomites or, in neonates, by passage through an infected birth canal. Genital infections are spread venereally, and respiratory infections usually by inhalation. Psittacosis is acquired from infected birds.

Diagnosis

The clinical presentation is often diagnostic; the diagnosis may be confirmed by serology (complement fixation or microimmunofluorescence tests) on sera and/or tears.

Control

Tetracycline and erythromycin are the drugs of choice. Penicillin is not effective.

INTRODUCTION

The chlamydiae are a small group of nonmotile coccoid bacteria that are obligate intracellular parasites of eukaryotic cells. Chlamydial cells are unable to carry out energy metabolism and lack many biosynthetic pathways; therefore they are entirely dependent on the host cell to supply them with ATP and other intermediates. Because of their dependence on host biosynthetic machinery, the chlamydiae were originally thought to be viruses; however, they have a cell wall and contain DNA, RNA, and ribosomes and therefore are now classified as bacteria. The group consists of a single genus, *Chlamydia* (order Chlamydiales, class Chlamydiaceae). This genus contains the species *C trachomatis* and *C psittaci*, as well as a new organism, the TWAR organism, which has recently been proposed as a third species (*C pneumoniae*). All three species cause disease in humans. *Chlamydia psittaci* infects a wide variety of birds and a number of mammals, whereas *C trachomatis* is limited largely to humans. *Chlamydia pneumoniae* (TWAR organism) has been found only in humans.

Clinical Manifestations

*Chlamydia trachomatis* Infections

The diseases caused by chlamydiae are summarized in Table 39-1 and Figure 39-1.
Trachoma, a *C. trachomatis* infection of the conjunctival epithelial cells, results in subepithelial infiltration of lymphocytes, leading to the development of follicles. The infected epithelial cells contain cytoplasmic inclusion bodies. As a result of damage to the epithelial cells, fibroblasts and blood vessels invade the infected area, a pannus forms, and the cornea becomes vascularized and clouded. The eyelids become scarred and malformed, an abnormal inward growth of the eyelashes. Continual scraping of the cornea by the eyelashes leads to corneal opacification and blindness.
*Chlamydia trachomatis* also causes inclusion conjunctivitis, an eye disease of children and adults that is milder than trachoma. It consists of purulent conjunctivitis that heals spontaneously without scarring.

*Chlamydia trachomatis* also causes sexually transmitted genital and rectal infections. The frequency of *C. trachomatis* infections in men may equal or exceed the frequency of gonorrhea. Nongonococcal urethritis, epididymitis, and proctitis in men can result from infection with *C. trachomatis*. Superinfection of gonorrhea patients with *C. trachomatis* also occurs. Acute salpingitis and cervicitis in young women can be caused by a *C. trachomatis* infection ascending from the cervix. A high rate genital tract coinfection by *C. trachomatis* in women with gonorrhea has been reported. *Chlamydia trachomatis* was isolated from the fallopian tubes of infected women. In one report *C. trachomatis* elementary bodies attached to spermatozoa were recovered from the peritoneal cavity of patients with salpingitis.

Neonates exposed to *C. trachomatis* in an infected birth canal may develop acute conjunctivitis within 5 to 14 days. The disease is characterized by marked conjunctival erythema, lymphoreticular proliferation, and purulent discharge. Untreated infections can develop into pneumonitis; this type of pneumonitis occurs only during the first 4 to 6 months of life.

Recently, *C. trachomatis* has been suspected of causing lower respiratory tract infections in adults, and several cases of *C. trachomatis* pneumonia have been reported in immunocompromised patients from whom the pathogen was isolated. Evidence also indicates that *C. trachomatis* may cause pneumonia or bronchopulmonary infections in immunocompetent persons.

Polyarthritis in lambs, calves, and possibly humans also may be caused by *C. trachomatis*.

Lymphogranuloma venereum is a human venereal disease caused by *C. trachomatis* strains different from the strains that cause trachoma (Table 39-1). The disease usually occurs in men and involves inguinal lymphadenopathy. Signs of lymphogranuloma venereum appear a few days after venereal exposure. The initial lesions, or vesicles, appear in the urogenital tract in men and women. If the disease does not heal spontaneously, regional lymph nodes become involved.

**Chlamydia psittaci** Infections

*Chlamydia psittaci* infects birds through the respiratory tract. Humans exposed to dead or living infected birds may develop fever, a mild influenzalike disease, or toxic fulminating pneumonitis after an incubation period of 2 to 4 weeks. *Chlamydia psittaci* can cause pneumonia in cats and sheep as well as in humans. Other strains of *C. psittaci* can cause abortions in animals.

**TWAR Organism Infections**

Recently, a new *Chlamydia* strain (designated *C. pneumoniae* serovar TWAR organism) that spreads from person to person in human populations was reported to cause outbreaks of respiratory tract infections in immunocompetent persons.
Latent *Chlamydia* Infections

Latent and inapparent infections of humans, other mammals, and birds are sometimes caused by chlamydiae. The agents of lymphogranuloma venereum, for example, may persist in infected humans for years before the disease becomes apparent. Individuals may develop acute trachoma years after leaving areas endemic for trachoma.

**Structure**

The chlamydiae exist in nature in two forms: (1) a nonreplicating, infectious particle called the elementary body (EB), 0.25 to 0.3 µm in diameter, that is released from ruptured infected cells and can be transmitted from one individual to another (*C. trachomatis*, *C. pneumoniae*) or from infected birds to humans (*C. psittaci*), and (2) an intracytoplasmic form called the reticulate body (RB), 0.5 to 0.6 µm in diameter, that engages in replication and growth (Fig. 39-2 and 39-3). The elementary body, which is covered by a rigid cell wall, contains a DNA genome with a molecular weight of 66 X 10^7 (about 600 genes, one-quarter of the genetic information present in the DNA of *Escherichia coli*). A cryptic DNA plasmid (7,498 base pairs) is also found. It contains an open reading frame for a gene involved in DNA replication. In addition, the elementary body contains an RNA polymerase responsible for the transcription of the DNA genome after entry into the host cell cytoplasm and the initiation of the growth cycle. Ribosomes and ribosomal subunits are present in the elementary bodies. Throughout the developmental cycle, the DNA genome, proteins, and ribosomes are retained in the membrane-bound prokaryotic cell (reticulate body).
FIGURE 39-2 (A) Electron micrograph of *C trachomatis* inclusion body in cytoplasm (C) of infected cell. Part of the nucleus (N) and mitochondria (M) can also be seen. (B) Enlarged view of inclusion body showing elementary bodies (E.B.) and reticulate (initial) bodies (I.B.). (Courtesy of Y. Becker, Jerusalem, Israel.)

FIGURE 39-3 Developmental cycle of *C psittaci* in L cells (mouse fibroblasts). (A) Bar, 1 µm. At 2.5 hours after infection, the figure shows an elementary body (arrow) that has just begun to differentiate into a reticulate body (X 36,000). (B) Twelve hours after infection (X 23,000). (C) Twenty hours after infection (X 23,000) (D) Thirty hours after infection (X 23,000). (From Tribby II E, Friis RR, Moulder JW: Effect of chloramphenicol, rifampicin, and nalidixic acid on Chlamydia psittaci growing in L cells. J Infect Dis 127:158, 1973, with permission.)

A complex series of events occurs during the developmental cycle of chlamydiae. These and the effects on the host cell are summarized in Figure 39-4 and Table 39-2. Studies on the growth cycle of *C trachomatis* and *C psittaci* in cell cultures in vitro revealed that the infectious elementary body develops into a noninfectious reticulate body (RB) within a cytoplasmic vacuole in the infected cell. There is an eclipse phase of about 20 hours after entry of the elementary body into the infected cell, during which the infectious particle develops into a reticulate body. In these structures the chlamydial genome is transcribed into RNA, proteins are synthesized, and the DNA is replicated. The reticulate body divides by binary fission to form particles which, after synthesis of the outer cell wall, develop into new infectious elementary body progeny. The yield of chlamydial elementary bodies is maximal 36 to 50 hours after infection.
FIGURE 39-4 Developmental cycle of the chlamydiae.
<table>
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<th>Stage 1. Dormant phase.</th>
<th>Host cell can be in any phase of its growth cycle at infection.</th>
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<td>Elementary bodies (EBs) have no or little metabolic activity; contain a DNA genome (ca. 600 genes), plasmid DNA (ca. 8 kbp), ribosomal subunits, cytoplasm, cell membrane, and cell wall containing a major outer membrane protein (MOMP), three cysteine-rich outer membrane proteins (60 to 62, 15 and 74 kDa), and two cell-binding proteins (31 and 18 kDa).</td>
<td>Duration: 0-12 h after infection.</td>
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<td>EBs adsorb to host cell membrane. The organization of the EB cell wall changes (deficiency of the cysteine rich proteins; no change in MOMP). EBs adsorb to host cells and utilize glucose-6 phosphate as substrate. EBs utilize mitochondrial functions. DNA in EBs changes conformation from compact organization to loose arrangement, possibly for transcriptional processes. DNA-dependent RNA polymerase molecules attached to DNA genome, are activated, and transcribe the genome. At this stage, EB development becomes sensitive to rifampin. Protein synthesis with existing ribosomes begins in EBs. Low level of DNA synthesis in EB is detected since development is sensitive to 5 x 10^{-4} M hydroxyurea. EB mass increases because of macromolecule synthesis.</td>
<td>Cell forms vacuole around EBs. Mitochondria support EB development. Cellular enzyme in glucose metabolic pathway is utilized by EB.</td>
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Classification, and Antigenic Types

Several distinct antigenic components have been recognized in *C. trachomatis* and *C. psittaci*, some group specific and others species specific. Detergents have been used to extract antigens from elementary bodies and reticulate bodies. *Chlamydia pneumoniae* (TWAR organism) is serologically unique and differs from *C. trachomatis* species and all *C. psittaci* strains tested.

The outer chlamydial cell wall contains several antigenic proteins, including a 40-kilodalton (kDa) major outer membrane protein (MOMP), a 60- to 62-kDa and 15-kDa, cysteine-rich proteins, a 74 kDa species-specific protein, and 31- and 18-kDa eukaryotic cell-binding proteins, which share the same primary sequence.

Hyperimmune mouse antiserum against the 40-kDa MOMP protein from serotype L2 reacted with elementary bodies of *C. trachomatis* serotypes Ba, E, D, K, L1, L2, and L3 during indirect immunofluorescence but failed to react with serotypes A, B, C, F, G, H, I, and J or with *C. psittaci*. Indeed, cloning and sequencing of the *C. trachomatis* MOMP gene revealed the same number of amino acids for serovars L2 and B, while the MOMP gene of serovar C contained codons for three additional amino acids. The antigenic diversity of the chlamydial MOMP was reflected in four sequence-variable domains, two of which are candidates for the putative type-specific antigenic determinants. The basis for MOMP differences among *C. trachomatis* serovars were clustered nucleotide substitutions for closely related serovars and insertions and deletions for distantly related serovars. When MOMP is inserted into the outer elementary body envelope,
exposed domains of MOMP serve as both serotyping and protective antigenic determinants. Predominantly conserved regions of C and B serotypes are interspersed with short variable domains.

Three monoclonal antibodies that recognize epitopes on cysteine-rich membrane proteins interact with all 15 human *C. trachomatis* serotypes, establishing the species specificity of this antigen. Monoclonal antibodies to the 15-kDa cysteine-rich protein showed biovar specificity and species specificity. The 60- to 62-kDa and 15-kDa cysteine-rich proteins are highly immunogenic in the natural infection, but the antibodies do not neutralize the infectivity of *C. trachomatis* elementary bodies.

**Pathogenesis**

**Spread of Agents**

Human diseases caused by chlamydiae can be divided into two types: (1) chlamydial agents transmitted by direct contact (*C. trachomatis* genital and ocular infections, *C. pneumoniae* ocular infection) and (2) chlamydial agents that are transmitted by the respiratory route (*C. psittaci* and *C. pneumoniae*).

The spread of *C. trachomatis* from person to person may cause trachoma, inclusion conjunctivitis, or lymphogranuloma venereum. Transmission of *C. trachomatis* from the urogenital tract to the eyes and vice versa occurs via contaminated fingers, towels, or other fomites and, in neonates, by passage through an infected birth canal. These diseases appear in an epidemic form in populations with low standards of hygiene. *Chlamydia trachomatis* genital infections are sexually transmitted. *Chlamydia psittaci* is transmitted from infected birds or animals to humans through the respiratory tract. *Chlamydia pneumoniae* spreads from infected individuals by respiratory tract infections but is not sexually transmitted.

**Chlamydial Diseases**

Chlamydial agents are intracytoplasmic obligate parasites of mammalian cells and can damage infected cells in tissues. The elementary bodies are infectious particles that can be transmitted from the infected tissues to uninfected tissues in the same person (transfer of *C. trachomatis* elementary bodies from an infected genital tract to the eyes and vice versa) or from a person with atypical pneumonia (caused by *C. psittaci* or *C. pneumoniae*) to healthy individuals (respiratory release of elementary bodies). In the infected individuals the chlamydial agent causes tissue damage and induction of interleukin-1α, interleukin-1β, and tumor necrosis factor alpha, which are cytokines involved in the inflammation process. Ocular infections by *C. trachomatis* and sometimes *C. pneumoniae* strains cause acute purulent conjunctivitis either due to infection of the neonate during passage through the birth canal or due to subsequent infections leading to scarring of the conjunctiva and to blindness subsequent to mucopurulent follicular conjunctivitis. *Chlamydia trachomatis* infection also spreads through sexual contact when urethritis or cervicitis is present. The genital tract infection serves as a source of infectious elementary bodies for the eyes.
The recently recognized *C pneumoniae* isolates cause mild to severe pneumonia, prolonged bronchitis, pharyngitis, sinusitis, and a febrile illness in humans. The agent does not cause death in patients without complications.

**Host Defenses**

**Nonspecific Responses**

Infections with chlamydial agents evoke responses from the blood vessels (ocular trachoma), connective tissue (scars in *C trachomatis* infections), and lymphocyte infiltration (pannus). Chlamydia infections are characterized by chronic inflammation. The mechanisms that trigger migration of lymphocytes or connective tissue to the site of *C trachomatis* infection in the eyes are not known. However, coculture of *C trachomatis* (serovar L2) with human blood monocytes induced the production of interleukin-1, an important mediator of inflammation and scarring. Interleukin-1α and interleukin-1β can be induced in human monocytes by *C trachomatis* lipopolysaccharide. Release of angiogenesis factors from infected cells may cause proliferation of blood vessels in the infected eye. Fever accompanies *C psittaci* pneumonitis. It was reported that tumor necrosis factor is induced by *C trachomatis* infection in athymic nude mice.

Cultured chlamydiae are sensitive to interferon, which is produced by cultured cells infected with chlamydiae.

**Immune Response in Humans**

All chlamydial infections induce IgM, IgG, IgA, and IgE antibodies, but these antibodies do not prevent reinfection. Although secretions from trachomatous eyes contain specific antitrachoma IgG and IgA antibodies, these antibodies do not impede the infection. Moreover, antibodies that bind to *C trachomatis* elementary bodies do not impair their infectivity in cell cultures. However, the addition of anti-gamma globulin to antibody-treated elementary bodies neutralizes their infectivity. Monoclonal antibodies to proteins in the outer elementary body envelope were reported to neutralize elementary body infectivity. Most patients with *C trachomatis* infections have antibodies that react with the *C trachomatis* cell wall proteins. Sera from individuals with genital infections caused by *C trachomatis* also reacted with the 60- to 62-kDa cysteine-rich proteins of all the *C trachomatis* serotypes. The precise role of cell-mediated immunity is not known.

**Epidemiology**

Trachoma is still prevalent in Africa and Asia (more than 500 million people are estimated to have the disease), and sporadic cases occur all over the world. The disease flourishes in hot, dry areas where there is a shortage of water and where standards of hygiene are low. The agent is spread to the eyes by flies, dirty towels, fingers, or cosmetic eye pencils. The initial infection usually occurs in childhood, and the active disease eventually appears (mostly by 10 to 15 years of age). Trachoma may leave a residuum of permanent lesions that can lead to blindness. *Chlamydia trachomatis* also resides in the genital tract, cervix, and urethra of adults, and genital infection is spread sexually. Lymphogranuloma venereum persists in the genital tract of infected persons.
Because *C. trachomatis* is able to infect both the eyes and the urogenital tract, antitrachoma campaigns involving only ocular treatments are futile.

*Chlamydia psittaci*, the cause of psittacosis in birds and occasionally in humans, is carried by wild and domestic birds, including poultry. The severity of psittacosis in humans has been considerably reduced by the susceptibility of *C. psittaci* to antibiotics.

*Chlamydia pneumoniae* spreads in human populations by respiratory tract infections. It is the agent of atypical pneumonia in hospitalized patients as well as in young individuals with an acute respiratory disease. It has caused epidemics in Scandinavia. Studies of the prevalence of antibodies to *C. pneumoniae* in humans around the world showed that it also prevails in Japan, Panama, and North America.

**Diagnosis**

Most diseases caused by the chlamydiae are diagnosed on the basis of their clinical manifestations. Eye damage caused by *C. trachomatis* is typical, as are the vesicles in the infected urogenital tract. Diagnosis of pneumonitis requires laboratory testing.

*Chlamydia trachomatis* can be identified microscopically in scrapings from the eyes or the urogenital tract. Inclusion bodies in scraped tissue cells are identified by iodine staining of glycogen present in the cytoplasmic vacuoles in infected cells. To isolate the agent, cell homogenates that contain the chlamydial elementary bodies are centrifuged onto the cultured cells (e.g., irradiated McCoy cells). After incubation, typical cytoplasmic inclusions are seen in the cells stained with Giemsa stain or iodine. Staining with iodine can distinguish between inclusion bodies of *C. trachomatis* and *C. psittaci*, as only the former contain glycogen. Each chlamydial agent can also be identified by using specific immunofluorescent antibodies prepared against either *C. trachomatis* or *C. psittaci*. Homogenates or exudates of infected tissues also have been used to isolate the agent in the yolk sac of embryonated eggs.

Sera and tears from infected humans are used to detect anti-*Chlamydia* antibodies by the complement fixation or microimmunofluorescence tests. The latter is useful for identifying specific serotypes of *C. trachomatis*; however, even detection of anti-*C. trachomatis* antibodies of the IgM class cannot be used diagnostically for genital infections, because similar antibodies are found in *Chlamydia*-negative patients. Fluorescent monoclonal antibodies are used to stain *C. trachomatis* elementary bodies in urethral and cervical exudates.

It is possible to diagnose *C. trachomatis* in tissue biopsy specimens by *in situ* DNA hybridization with cloned *C. trachomatis* DNA probes. DNA from *C. trachomatis* isolates can be examined by restriction endonuclease analysis. The DNA cleavage pattern of *C. trachomatis* isolates differs greatly from that of DNA from *C. psittaci* isolates. DNAs of the agents of trachoma and lymphogranuloma venereum differ in their cleavage patterns, and this allows identification of the biovars.

*Chlamydia pneumoniae* DNA has 10 percent homology with *C. trachomatis* or *C. psittaci*; *C. pneumoniae* isolates have 100 percent homology. *Chlamydia pneumoniae* isolates can be diagnosed by hybridization with a specific DNA probe that does not hybridize to other chlamydiae. Two additional serologic tests are in use: the
microimmunofluorescence test with *C pneumoniae*-specific elementary body antigen, and the complement fixation test, which measures *Chlamydia* antibodies.

**Control**

Attempts to use *C trachomatis* vaccines for prophylaxis and treatment of trachoma have failed. The course of trachoma is more severe in immunized than in nonimmunized individuals. Specific anti-*Chlamydia* antibodies fail to neutralize chlamydial elementary bodies *in vivo*.

Tetracycline and erythromycin are the antibiotics commonly used to treat chlamydial infections in humans. Penicillin is not effective. Patients with trachoma have been treated effectively with erythromycin, rifampin, sulfonamides, chloramphenicol, and tetracyclines. Repeated treatment cycles of long-acting sulfonamides also have been used in local or systemic treatment of trachoma infections. In trachoma patients with trichiasis, corrective surgery is necessary. Patients with inclusion conjunctivitis usually are not treated, because the infection is self-limiting and relatively mild.

Tetracyclines or sulfonamides sometimes are effective in patients with lymphogranuloma venereum, but treatment does not always improve the condition. Tetracycline treatment of gonorrhea in patients infected with gonococci or *Chlamydia* is more effective against postgonococcal urethritis than is treatment with penicillin.

**REFERENCES**


Beatty WL, Morrison RP, Byrne GI. Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis. Microbiol Rev 58:686, 1994


