

Chlamydiae: Genital, Ocular, Respiratory, and (Emerging) Cardiovascular Pathogens

Reading Assignments: (1) Text Chapter 27 (2) Handout: Plates 95, 96, and 98, In Kapit, W. and L.M. Elson. 1977. *The Anatomy Coloring Book*, Harper and Row Publishers, New York. (3) Gilligan, P.H., M.L. Smiley, and D.S. Shapiro. 1997. Case 3 In: *Cases in Medical Microbiology and Infectious Diseases* (2nd ed.), American Society for Microbiology, Washington, D.C. (4) Cunha, B. 1999. Patients 9, 12, 63. In: *Infectious Disease Pearls*, Hanley and Belfus, Inc., Philadelphia, PA., (5) Lutwick, L.E. “We Love You Conrad”, *Infect. Med.* 15: 230-232.

1. INTRODUCTION (Chlamydia – singular; Chlamydiae- plural)

Chlamydiae are small **bacteria** that are **obligate intracellular parasites**. They were once considered to be viruses, because they are filterable through a 0.45 um pore size filter. These pathogens exist have a unique developmental life cycle and cause a wide spectrum of human (and animal) diseases.

2. The Chlamydiae- structurally similar to Gram negative bacteria

A. Structure and Physiology

1. Chlamydiae are small nonmotile, nonpiliated cells that exist in two morphological forms – the **elementary body (EB)** and the **reticulate body (RB)** (see B below).

Both morphological forms have a double membrane organization:

- a. an **inner cytoplasmic membrane**
- b. a variable sized periplasmic space
- c. **no peptidoglycan** layer (even though they carry the genes for peptidoglycan synthesis and for penicillin binding proteins)
- d. an **outer membrane** containing **lipopolysaccharide** and **proteins**.

These two cell surface antigens are the immunodominant cell surface structures and define group and species-specific antigens. Diagnostic reagents used to detect chlamydiae are targeted at one of these two antigens:

- (1) **Shared group (genus) specific antigens**- lipopolysaccharides.
- (2) **Species-specific** or **serovar-specific** antigens- outer membrane proteins.

These antigens are detected by using monoclonal antibodies.

e. How can chlamydiae exist in nature without the rigidity of a cell wall

In the physiologically inactive, non-replicating **EB** (which is the infectious form), the major outer membrane protein is highly cross-linked to neighboring proteins by multiple cysteine disulfide bonds.

2. Sensitivity to Antibiotics

The RB (the intracellular replicative form) is sensitive to a variety of antibiotics, while the EB (that can exist outside the host cell) is not susceptible to antimicrobial agents.

3. Although chlamydiae were once thought to be viruses (because they pass through a 0.45 um filter), they contain **both DNA and RNA**, have **prokaryotic ribosomes**, **synthesize their own proteins, nucleic acids and lipids**. Their genome is circular

like other bacterial chromosomes.

4. Chlamydiae have an **obligate intracellular** life style because they are **energy parasites**. They **cannot synthesize ATP** and must rely on the cell for energy rich intermediates.
5. **Staining properties:**
 - a. Chlamydiae cannot be stained with Gram stain – why???
 - b. Their size challenges the limits of the light microscope
 - c. With Giemsa stain - EBs stain purple and RBs stain blue.
 - d. Mature intracellular **inclusions** stain dark purple with Giemsa's stain and brown with Lugol's iodine solution (because of the glycogen matrix surrounding the particles.)

B. Developmental Cycle

1. Two Morphologically Distinct Forms:
 - a. Small elementary body (EB) (0.3 - 0.4 um in diameter) – environmentally stable, metabolically inert, infectious particle with an electron dense nucleoid. Although chlamydiae do not replicate in the EB form, they are infectious; they can bind to receptors on host cells, stimulating uptake by the infected cell.
- b. **Large reticulate body (RB) (0.8 to 1.0 um in diameter)** – metabolically active, replicating form without an electron-dense nucleoid.

2. Growth Cycle

- a. **Attachment of the EB to a host epithelial cell.**

A limited number of cell types are infected including the non-ciliated columnar, cuboidal, or transitional epithelial cells on the mucous membranes of the urethra, endocervix, endometrium, fallopian tubes, rectum, respiratory tract, and conjunctivae. The LGV biovar replicates in mononuclear phagocytes present in the lymphatic system.
- b. **Organisms are internalized by receptor-mediated endocytosis** (taken into a vesicle derived from the host cell membrane).

The EB modifies the vesicle so that (1) the pH is maintained above 6.2, and (2) the vesicle cannot fuse with the lysosome.
- c. **Conversion of EBs into RBs** (by 9 hrs after infection).
- d. **Growth of the RBs by binary fission.** The RBs grows in size and divide by binary fission. As the RBs grow, the vesicle membrane expands to accommodate 200-1000 organisms. These membrane-bound structures, which are visible through the light microscope, are called **inclusions**. During this stage, genus specific chlamydial antigens become associated with the host cell membrane
- e. Since RBs are fragile, and cannot survive in the environment, over the next 24

hours, **RBs are reorganized into EBs** by binary fission (Included in this process is cross linking of the outer membrane proteins to impart rigidity to the cells). RBs and EBs appear together, but with continued divisions, all the RBs are replaced by EBs.

f. Infectious EBs are released by lysis of the host cell.

Note: There is evidence that maturation of EBs from RBs may be impaired by host factors such as alpha interferon, resulting in persistent infections of host cells.

D. Characteristics of the Host-Pathogen Relationship

Infections may be acute or chronic. Chronic infections are common. Spread from one species to another (such as in psittacosis) more frequently leads to disease. The host's immune response to infection by chlamydiae is thought to be responsible for the pathology. A delayed type- hypersensitivity response to specific chlamydial antigens may produce the scarring seen in trachoma or in PID.

The host mounts a humoral immune response but antibody is ineffective in reaching the intracellular organisms. The pathogen persists in the presence of high antibody titers.

E. Classification – Three species are currently recognized (see Table 27.1 in the Text)

1. *C. trachomatis*- subdivided into different serotypes (serovars)

- a. **A-C** – trachoma
- b. **D-K** – STD's, conjunctivitis and pneumonia in newborns
- c. **L1, L2, L3** – the systemic diseases - lymphogranuloma venereum
2. ***C. psittaci*** – psittacosis (parrot fever, ornithosis)
3. ***C. pneumoniae*** – acute respiratory disease and atherosclerosis ?

3. Chlamydia trachomatis

A. Epidemiology

1. Only reservoir is humans
2. STD's maintained within the population by asymptomatic **infection of women and men.**

B. Infections of the Eye - Serovars A-C – Trachoma; Serovars D-K–conjunctivitis

1. The Conjunctiva - the delicate mucous membrane that lines the

eyelids and covers the exposed surface of the sclera.

- a. Nonspecific Defenses of the Eyes? (Review “Pseudomonas Can

Thwart the Eye's Multitiered Infection Defenses” – Lecture 12 and Lecture 3)

1. Mechanical action of blinking
2. Washing action of tears
3. Antimicrobial properties of tears – lysozyme, lactoferrin, and sIgA
4. Corneal cells regularly slough off surface cells; are resistant to invasion except on the basolateral side.

5. Corneal cells produce and export defensins (antibacterial proteins) in response to LPS
- b. The eye is potentially vulnerable because the conjunctiva is covered by the eyelids, creating a warm moist enclosed environment in which contaminating microbes can set up a focus of infection.

2. Trachoma (Gr. *trachoma* roughness)– *C. trachomatis* Serovars A-C

- a. **The most important eye infection in the world.** About **500 million** people have trachoma and **seven million** people in the world are blind because of the disease.
- b. Trachoma was known in ancient Egypt 4000 years ago and tweezers to remove in-turned eyelashes have been found in royal tombs.
- c. Transmission – direct contact with contaminated flies, fingers and towels. Most prevalent in Africa, Asia, and the Mediterranean basin, where hygiene is poor and water is scarce. It is preventable by regular washing of hands and face with a towel that is not shared by other people.
- d. Pathogenesis – chronic evolution
 1. Onset - conjunctivitis with symptoms of lacrimation, mucopurulent discharge.
 2. Chronic inflammation of the eyelids with increased vascularization of the corneal conjunctiva, formation of lymphoid follicles, and the infiltration of granulation tissue
 3. Entropion, severe corneal scarring, and conjunctival deformities (Entropion – the turning inward of the margin of the eyelid resulting in continual trauma to the cornea because of the inwardly directed lashes causing ulceration, opacities, and visual impairment.)
 4. Blindness 15-20 years after infection.

3. Conjunctivitis (Serotypes D-K)

- a. **Neonatal conjunctivitis** of the newborn – begins mucopurulent conjunctivitis 7-12 days after delivery. 20-50% of infants born to infected mothers will acquire the infection. Resolves with antibiotic treatment. If left untreated, over time the infection may progress to one that is similar to trachoma.
- b. Also causes **conjunctivitis in adults** – source of infection usually self-inoculation from genital secretions.

C. STD's (*C. trachomatis* serotypes D-K and L1, L2, L3)

1. Serotypes D-K
 - a. **STDs** *C. trachomatis* is the most common STD in the United States (estimated 4 million cases each year). 5-15% prevalence in sexually active adolescents, regardless of socioeconomic status. (Because of the risks and complications associated with this infection, CDC has

recommended that all sexually active adolescent young women undergoing a pelvic examination receive routine screening for chlamydia.

ACTIVE SURVEILLANCE

b. Causes:

1. Lower and upper reproductive tract infections.
 - a. 50% of **nongonococcal urethritis** in men (See Text “Mr. C.”, pp.261-262 and Case 9 – Reading Assignment (4) – compares symptoms of gonococcal and nongonococcal urethritis.)
 - b. 80% of women are asymptomatic; if symptoms are present – mucopurulent cervicitis. Because genital tract infections with *C. trachomatis* are frequently asymptomatic, this pathogen is a **major cause of PID, ectopic pregnancy, and infertility** among women. (See Reading Assignment (3).
 - c. Perinatal transmission to infants can cause neonatal conjunctivitis and pneumonia.
 - d. Presence of multiple etiological agents is probable (gonococci, treponemes, trichomonads, herpes, etc.) *C. trachomatis* present in 50% of cases where gonococci are isolated.
 - e. Diagnosis – see below
 - f. Treatment of choice – azithromycin – concentrates intracellularly

2. Lymphogranuloma venereum (Serotypes L1, L2, L3) (See Patient 12- Reading Assignment (4))

- a. Endemic in the Caribbean, Africa, Southeast Asia, India, and South America.
- b. An STD with local spread to the regional lymph nodes
 1. After a 1-4 week incubation period – primary lesion appears at the site of infection (penis, urethra, vaginal wall, cervix, vulva). Often overlooked because it is small, painless, inconspicuous and heals rapidly.
 2. Second stage of infection – several weeks to months later- inflammation and swelling of the lymph nodes draining the site of initial infection (often the inguinal and femoral nodes). The inguinal nodes become swollen and painful forming **buboes** that gradually enlarge and can rupture forming **draining fistulas**. (**Bubo** – an enlarged and inflamed lymph node, particularly in the axilla or groin, due to such infections as plague, syphilis, lymphogranuloma venereum, and tuberculosis). (**Fistula** – an abnormal passage between two internal organs, or leading from an internal organ to the surface of the body.)

Systemic symptoms include fever, chills, anorexia, headache, myalgias, and arthralgias. Proctitis is common in women and results from lymphatic spread from the cervix or vagina. Prostatitis in men develops after anal intercourse or from lymphatic spread from the

urethra.

3. Untreated disease may resolve with spontaneous healing after several months or it may progress to a chronic ulcerative stage in which genital ulcers, fistulas, strictures, or genital elephantiasis develops.

- c. Diagnosis and Treatment – clinical appearance and by isolation of *C. trachomatis* from bubo pus. Treatment – oral doxycycline.

3. Control of Chlamydial STDs

- a. Prompt treatment of infected individuals **and** their sexual partners
- b. Safe sexual practices
- c. No vaccines

D. Laboratory Diagnosis of *C. trachomatis*

THREE GENERATIONS OF DIAGNOSTIC TESTS FOR CHLAMYDIA- In search of a sensitive and specific test to detect *C. trachomatis* in patient specimens.

1. FIRST GENERATION TEST: Isolation of the organism in cell culture (was the “gold standard” for diagnosis of chlamydial infections). Takes the patient’s specimen (which may only have a few chlamydiae present and amplifies the number of organisms by allowing them to grow in tissue culture cells.
 - a. Inoculate specimens into appropriate cell lines in vitro (McCoy, HeLa-299, Buffalo green monkey kidney cells). Allow the chlamydia to grow and form inclusions.
 - b. Stain the cells with Giemsa stain or by using direct fluorescent antibody to visualize the inclusions under the light microscope.
 - c. Cell culture – 70-85% sensitive
 - d. All the newer tests are compared to “the gold standard”, of culture, but culture is now less sensitive than the newer molecular amplification methods.
2. SECOND GENERATION TESTS: Direct detection of Chlamydial cell surface antigens in clinical specimens (without amplifying the number of organisms present in the sample)
 - a. Detection of chlamydial antigens in clinical specimens by **direct fluorescent antibody (FA) testing** or **ELISA**. In both assays, **monoclonal antibodies** against LPS or the MOMP have been used.
 1. Antibody tests that target LPS are less specific because antigenic determinants on LPS are shared with other bacteria, particularly those in fecal specimens.
 2. Advantage of Direct FA - quality of the specimen can be judged because the specimen is visualized while looking for chlamydial inclusions.

3. Advantage of ELISA- can be done in a microtiter plate and automated. (Quality of specimen – i.e. columnar epithelial cells- cannot be judged.)
- b. Advantages over the “gold standard” of culture – more rapid than culture, they do not require viable cells, and multiple samples can be run.
- c. Disadvantages - The **sensitivity** of either of these methods is less than that of cell culture - especially if specimens from asymptomatic patients are used (because relatively few chlamydiae may be present). ELISA tests require 10^4 - 10^5 elementary bodies be present in the specimen.

3. THIRD GENERATION TESTS: Direct Detection of Chlamydial Nucleic Acid in Patient Specimens – with or without amplification

- a. Based on detection of species-specific sequences of **16s rRNA** (10-20,000 copies/cell) or **target sequences on plasmid DNA** (there are several copies of the plasmid in the cell) .
- b. Two Types of Nucleic Acid Tests:
 1. **Without Amplification of the Target Nucleic Acids** (requires 10^4 elementary bodies)
 2. **With Amplification of Target Nucleic Acids** by Polymerase Chain Reaction (PCR) or by Ligase Chain Reaction (LCR) – both can detect 1 elementary body and have a clinical sensitivity of 85-90%.
 - a. The amplification tests are so sensitive they may redefine the “gold standard” for chlamydial diagnostic tests.
 - b. They are being used to screen **urine samples** (a noninvasive sample) for both Chlamydia and gonorrhea.
- c. Concerns/ problems with the amplified tests:
 1. False negative – because of inhibitors present in urine (nitrites, hemoglobin, and crystals).
 2. False positive – because of contamination or non viable organisms. (Since these tests cannot differentiate between living and dead organisms, they cannot be used for tests of cure.)
- d. Used in several studies to screen large numbers of urine samples and to determine the prevalence of asymptomatic infections. Prevalence rates of 4-6% have been found. In one study in which they screened for Chlamydia and treated those with infection, the rate of PID disease decreased from 18%-8%.

2. Serology – Detection of Chlamydial Antibody – not generally useful for STDs; used for *C. psittaci*)

4. *Chlamydia pneumoniae* (formerly known as an atypical chlamydia -the TWAR agent- Taiwan Acute Respiratory strains)

- A. History:** First isolated from the conjunctiva of a child in Taiwan. Originally considered to be a psittacosis strain because of the morphology of the inclusions produced in cell culture. Subsequently, TWAR strain shown to be a distinct species from either *C. trachomatis* or *C. psittaci*.
- B. Strictly a human pathogen;** no animal reservoir identified.
- C. Transmission** – person-to-person via respiratory droplets.
- D. Only a single serotype** identified.
- E. Diseases:**
 - 1. **An important cause of primary atypical pneumonia in young adults** (see Patient 63 – Reading Assignment (4)), such as college students and members of the military.
 - a. Infection with *C. pneumoniae* is common. Estimated 400,000 cases of *C. pneumoniae* pneumonia are reported annually. More than 50% of the population have serological evidence of past infection.
 - b. Most infections are asymptomatic or mild and include a persistent cough and malaise. Atypical pneumonia usually involves a single lobe of the lung. These infections cannot be differentiated from other cases of **atypical pneumonias** (see below).

WHAT IS PRIMARY ATYPICAL PNEUMONIA?

When effective antibiotic treatment for *S. pneumococci* (the cause of pneumococcal pneumonia) became available, a significant proportion of cases of pneumonia failed to respond to this treatment and were labeled as **primary atypical pneumonia**.

- 1. “Primary” because the pneumonia was occurring as a new event (not secondary to influenza, for example)
- 2. “Atypical” because (1) *S. pneumoniae* could not be isolated from the sputum of the patients, (2) the symptoms were often general as well as respiratory, and (3) because the patients did not respond to penicillin or ampicillin.

The causes of atypical pneumonia include:

- | | |
|---------------------------------|----------------------------------|
| 1. <i>Mycoplasma pneumoniae</i> | 4. <i>Legionella pneumophila</i> |
| 2. <i>Chlamydia pneumoniae</i> | 5. <i>Coxiella burnetii</i> |
| 3. <i>Chlamydia psittaci</i> | |

2. Other Clinical Conditions Now Associated With *C. pneumoniae*:

- a. There is evidence that prolonged exposure to *C. pneumoniae* may be causally associated with wheezing, asthmatic bronchitis, and adult onset asthma.
- b. Association of *C. pneumoniae* with coronary artery disease?
 - 1. What is the evidence linking *C. pneumoniae* with atherosclerosis?
 - 2. What are persistent Chlamydiae?
 - 3. How might chlamydial infection contribute to development of atherosclerosis (i.e. proposed pathogenesis)?

4. How will the determination of causality ultimately be determined?
5. *Chlamydia psittaci* (See Reading Assignment (5) - "We Love You Conrad")
 - A. The etiological agent of psittacosis (parrot fever) or ornithosis (associated with birds other than parrots)
 - B. Natural reservoirs
 1. Birds
 - a. Virtually any species of birds can be infected. About 70% of humans are infected from caged psittacine birds such as parrots, macaws, cockatiels, and parakeets. Other caged birds associated with human disease include pigeons, doves, and mynah birds.
 - b. In birds, the disease is called avian chlamydiosis (AC). In birds, *C. psittaci* can cause asymptomatic infections or can result in an acute, subacute, or chronic clinical disease. Birds can appear healthy but be carriers of the disease. Latent, asymptomatic carriage may become overt disease when birds are stressed from overcrowding, unsanitary conditions, concurrent infections, or shipping. Clinical signs in birds: lethargy, anorexia, ruffled feathers, a serous or mucopurulent ocular or nasal discharge, diarrhea and excretion of green-yellow urates. Birds may die soon after onset of illness, or as the disease progresses, can become emaciated and dehydrated before death. Whether the bird exhibits clinical signs of illness or dies depends on the species of bird, virulence of the strain, infectious dose, stress factors, age and treatment given.

2. Mammals – see below

C. Transmission from birds to humans

1. *C. psittaci* is present in the blood, tissues, feces, and feathers of infected birds (that may appear either ill or healthy). Nasal and fecal contents, which dry on the feathers, cause infectious dust aerosols which are highly infectious. Other means of exposures include bird bites, mouth-to-beak contact, and handling infected birds' plumage and tissues. Even brief exposure can lead to infection; some patients may not even remember having any contact with birds.
2. Mammals occasionally transmit disease to humans. Certain strains of *C. psittaci* infect sheep, goats, and cattle causing chronic infection of the reproductive tract, placental insufficiency, and abortion. Strains can be transmitted to humans when they are exposed to the birth fluids and placentas of infected animals.

3. Person-to-person spread is rare but can occur.

D. Occupational risks include – veterinarians, zookeepers, pet shop workers, employees in poultry processing plants (see case study below).

E. Disease in Humans (Psittacosis, Ornithosis)

1. Incubation period 5-14 days.
2. Severity of the disease ranges from inapparent illness to systemic illness with severe pneumonia.
3. Symptoms include a sudden onset of headache, high fever and chills.
4. Patients usually develop a nonproductive cough that can be accompanied by breathing difficulty and a tight chest.
5. Lobar or interstitial pneumonia may be seen. Patients at autopsy show alveoli spaces with an exudate containing erythrocytes, PMN, and mononuclear cells.
6. The spleen is often enlarged and hepatitis with jaundice may be seen.
7. Severe cases may progress to endocarditis, myocarditis, arthritis, keratoconjunctivitis, and encephalitis. Death is usually the result of cardiac and respiratory insufficiency.
8. Before the advent of antibiotics, death rates were 20%. This has been reduced to 1% with **tetracycline** – the drug of choice for treatment.

F. Laboratory Diagnosis

1. Diagnosis is usually by **serology** (using acute and convalescent sera samples); either complement fixation or immunofluorescence is used.
2. *C. psittaci* can be isolated from blood and respiratory secretions - not done because specimens are too dangerous to handle in the laboratory without adequate facilities.