Rickettsia, Chlamydia, Mycoplasma, Legionella, and Gardnerella

UZHGOROD NATIONAL UNIVERSITY
MEDICAL FACULTY
MICROBIOLOGY, VIROLOGY, IMMUNOLOGY WITH THE COURSE OF INFECTIOUS DISEASE
• Classification – Family Rickettsiaceae:

• 1. *Rickettsia* (11 species) -- obligate intracellular parasites which do not multiply within vacuoles and do not parasitize white blood cells.

• 2. *Ehrlichia* (2 species) - obligate intracellular parasites which do not multiply within vacuoles but do parasitize white blood cells.

• 3. *Coxiella* (1 species) -- obligate intracellular parasite which grows preferentially in vacuoles of host cells.

• 4. *Bartonella* (3 species) -- intracellular parasite which attacks the red blood cell.

• But with 3 medically important generations:
  • *Rickettsia*
  • *Coxiella*
  • *Rochlimaea*
Classification of rickettsia and diseases it cause
Morphology and cultural characteristics

• The structure of the typical rickettsia is very similar to that of Gram-negative bacteria. The typical envelope consists of three major layers: an innermost cytoplasmic membrane, a thin electron dense rigid cell wall and an outer layer. The outer layer resembles typical membranes in its chemical composition and its trilaminar appearance.

• The cell wall is chemically similar to that of Gram-negative bacteria in that it contains diaminopimelic acid and lacks teichoic acid. Intracytoplasmic invaginations of the plasma membrane (mesosomes) and ribosomes are also seen. There are no discrete nuclear structures.
• **Key points:**

  • All are obligate intracellular parasites (except *Rochlimaea*) that can grow in both phagocytic and nonphagocytic cells.
  
  • *Rochlimaea* can be cultivated on artificial media containing blood
  
  • Others are grown in embryonated eggs or tissue culture
  
  • Cultivation is costly and hazardous because aerosol transmission can easily occur
  
  • Small, pleomorphic coccobacilli
  
  • Gram stain poorly, but appear to be G-
  
  • Stain readily with Giemsa
  
  • All, except *Coxiella*, are transmitted by arthropod vectors.
Diagnosis/serological tests

• Presumptive laboratory diagnosis is based on the finding of rickettsial-like organisms in tissue or blood. Although the organisms are gram-negative, they only weakly take the counter stain, safranin. Therefore, special staining procedures are used. Infected tissue may be stained with:

  1. **Macchiavello stain**--organisms are bright red against the blue background of the tissue.

  2. **Castaneda stain**--blue organisms against a red background.

  3. **Giemsa stain**--bluish purple organisms.
Diagnosis/serological tests

- Confirmative diagnosis is based on a serological reaction (Weil-Felix reaction) in which the titer of the agglutinins in the patient's serum against the *Proteus* strains OX-19, OX-2 and OX-K are determined. These *Proteus* strains have no etiological role in rickettsial infections, but appear to share antigens in common with certain rickettsia. These antigens are alkali stable polysaccharide haptens which are distinct from the group-specific and type-specific antigens. In interpreting the results, it must be kept in mind that *Proteus* infections are fairly common (especially in the urinary tract) and that they, too, may evoke antibodies to the *Proteus*-OX strains.

- This test is usually positive seven days after the initial infection. A more specific complement fixation test is available but does not show positive results until 14 days into the infection. The indirect fluorescent antibody test is also useful for the detection of IgM and IgG antibodies against rickettsia. In fact, this is the diagnostic test of choice for ehrlichiosis.
Diagnosis/serological tests

- Direct detection of *Rickettsia* in tissues (Giemsa stain or direct fluorescent antibody test)
  Weil-Felix reaction – in certain rickettsial infections (typhus group) antibodies are formed that will agglutinate OX strains of *Proteus vulgaris*.

- This is used as a presumptive evidence of typhus group infection, however, the test is not very sensitive or specific and many false positives occur.
• Agglutination or complement fixation tests using specific Rickettsial antigens are better serological diagnostic tests.

**Virulence factors:**

- *Rickettsial sp.*
  - Induced phagocytosis
  - Recruitment of actin for intracellular spread

- *Coxiella burnetti*
  - Is resistant to lysosomal enzymes
Rickettsia cell-to-cell spread
Rickettsia cell-to-cell spread

Rickettsia normally multiply by transverse binary fission. Under poor nutritional conditions, the rickettsia cease dividing and grow into long filamentous forms, which subsequently undergo rapid and multiple division into the typical short rod forms when fresh nutrient is added. Immediately after division, the rickettsia engage in extensive movements through the cytoplasm of the cell.

*C. burnetii* differs from other rickettsia in that it is enclosed in a persistent vacuole during growth and division. Six to ten daughter cells will form within a host cell before the cell ruptures and releases them.
Rickettsia rickettsii
Rickettsia pathogenesis

- In their arthropod vectors, the rickettsia multiply in the epithelium of the intestinal tract; they are excreted in the feces, but occasionally gain access to the arthropods salivary glands. They are transmitted to man, via the arthropod saliva, through a bite. In their mammalian host, they are found principally in the endothelium of the small blood vessels, particularly in those of the brain, skin and heart.

- Hyperplasia of endothelial cells and localized thrombus formation lead to obstruction of blood flow, with escape of RBC's into the surrounding tissue. Inflammatory cells also accumulate about affected segments of blood vessels. This angiitis appears to account for some of the more prominent clinical manifestations, such as petechial rash, stupor and terminal shock. Death is ascribed to damage of endothelial cells, resulting in leakage of plasma, decrease in blood volume, and shock.
It is assumed that the observed clinical manifestations of a rickettsial infection are due to production of an endotoxin, although this endotoxin is quite different in physiological effects from that produced by members of the Enterobacteriaceae. This is inferred, although the toxin has not been isolated, from these facts:

1. IV-injected rickettsia cause rapid death in experimental animals.
2. UV-irradiation of rickettsia diminished their infectivity without reducing toxicity.
3. The use of anti-rickettsial drugs does not prevent rapid death in experimental animals.
4. Antiserum specific for cell wall antigens of the rickettsia prevents the toxic effect.
• Clinical significance – the diseases caused by Rickettsia are all characterized by fever, headache, myalgias, and usually a rash.

• Typhus fevers – incubation is 5-18 days.

• Symptoms include a severe headache, chills, fever, and after a fourth day, a maculopapular rash caused by subcutaneous hemorrhaging as Rickettsia invade the blood vessels.

• The rash begins on the upper trunk and spread to involve the whole body except the face, palms of the hands, and the soles of the feet.

• The disease lasts about 2 weeks and the patient may have a prolonged convalescence.

• Two types of typhus may occur: epidemic and murine
• Epidemic typhus – caused by R. prowazekii and transmitted by human lice as it bites and defecates in the wound.

• This occurs in crowded areas causing epidemics. Mortality rates are high in untreated cases. Following an initial attack, some individuals may harbor the organism of a latent infection with occasional relapses = Brill-Zinsser disease

• Endemic typhus – caused by R. typhi and transmitted to man by rat fleas.

• The disease occurs sporadically, but is clinically the same, but less severe than epidemic typhus.
• *Rickettsia* responsible for spotted fevers

• Rocky mountain spotted fever – caused by *R. rickettsii* and transmitted by ticks that must remain attached for hours in order to transmit the disease.

• An incubation of 2-6 days is followed by a severe headache, chills, fever, aching, and nausea.

• After 2-6 days a maculopapular rash develops, first on the extremities, including palms and soles, and spreading to the chest and abdomen.

• If left untreated, the rash will become petechial with hemorrhages in the skin and mucous membranes due to vascular damage as the organism invades the blood vessels.

• Death may occur during the end of the second week due to kidney or heart failure.
Rocky mountain spotted fever
Rocky mountain spotted fever
• Rickettsial pox – caused by *R. akari* and transmitted by a mouse mite.

• After a 1-2 day incubation a papule develops at the entry site and within 1-2 weeks a fever, malaise, and headache develop followed by a rash.

• The disease is mild and usually not fatal.

• Q fever – caused by *Coxiella burnetii*. The infection is acquired by inhalation of infectious material.

• After an incubation of 14-26 days there is a sudden onset of fever, chills, and headache, but no rash.

• The disease is characteristically an atypical pneumonia lasting 5-14 days with a low mortality rate.
• Trench fever – caused by Rochalimaea quintana and transmitted by body lice. Was a major problem during WW I and WW II.

• After an incubation of 6-22 days, the patient experiences a headache, exhaustion, leg pains, and a high, relapsing fever.

• A roseolar rash occurs and the patient usually recovers.
Treatment/antimicrobial therapy

• Chloramphenicol or tetracycline

• Wear protective clothing and use insect repellents.
Chlamydia
Classification of chlamydia and diseases it causes
The chlamydia fall into two main ecological groups. In the first group, are the agents causing trachoma, inclusion conjunctivitis, and lymphogranuloma venereum, which seem to infect man only. In the second group, are those agents transmitted to man as zoonotic infections. About 100 species of birds are naturally infected with chlamydia. This includes 71 species of parrots as well as finches, pigeons, chickens, ducks, turkeys and seabirds. The chlamydia are thought to have evolved in the following way:

- Gram-negative cocci
- Facultative intracellular parasites of mammals
- Protochlamydiae
- Gram-negative cocci
- Obligate intracellular parasites
- Host range restricted to rodents
- Restricted virulence (compact inclusions)
- Folates synthesized (sulfonamide susceptible)
- Glycogen synthesized and deposited in inclusions
<table>
<thead>
<tr>
<th>Subgroup A</th>
<th>Subgroup B</th>
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<tbody>
<tr>
<td>Mammalian parasites</td>
<td>Primarily bird parasites</td>
</tr>
<tr>
<td>Compact inclusions</td>
<td>Diffuse inclusions</td>
</tr>
<tr>
<td>Glycogen synthesized</td>
<td>Glycogen not synthesized</td>
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<tr>
<td>Folates synthesized</td>
<td>Folates not synthesized</td>
</tr>
<tr>
<td>Sensitive to D-cycloserine</td>
<td>Resistant to D-cycloserine</td>
</tr>
<tr>
<td>Restricted host range</td>
<td>Broadening of host range</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>Chlamydia psittaci</td>
</tr>
<tr>
<td>Seven strains which are probably distinct species</td>
<td>Ten strains which are probably distinct species</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>Only one serotype has been identified</td>
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</table>
• Classification – order Chlamydiales – contains one medically important genus – *Chlamydia*

• Are obligate intracellular parasites

• Cell walls are similar to the cell walls of G- B, but lack muramic acid

• Have a complex developmental cycle
  • The infectious form is called an elementary body (EB) which is circular in form and is taken into the cell by induced phagocytosis.
  • Inside the phagocytic vesicle replication takes place
• Over the next 6-8 hours, the EB reorganizes into the noninfectious, but metabolically active reticulate body (RB) which is larger and less dense than the EB.

• For 18-24 hours the RB synthesized new materials and divides by binary division to form inclusion bodies that reorganize and condense into EBs.

• Between 48-72 hours, the cell lyases and releases the EB which begin the cycle again.
• Are energy parasites that use ATP produced by the host cell

• A Giemsa stain can be used to visualize chlamydial inclusions in tissues.

• Identification:
  • Direct methods – stain tissues with Giemsa or use a direct fluorescent antibody technique.
  • The most sensitive method is to culture the organisms in tissue cultures and then stain the infected tissue culture cells
Chlamydia in tissues
Chlamydia inclusion bodies
Laboratory diagnosis is made by one or more of the following:

1. Isolation of the organism from infected tissue. The tissue is inoculated into the yolk sac of seven-day chick embryos or in McCoy human cells.

2. Characteristic cytoplasmic inclusion bodies infected cells.

3. Serological diagnosis:
   a. Microimmunofluorescent tests in tears of patients with eye infections for the presence of anti-chlamydia antibody. In neonatal conjunctivitis and early trachoma, direct immunofluorescence of conjunctive cells with fluorescein-conjugated monoclonal antibody is sensitive and specific.
   b. Delayed-type skin reaction (type IV hypersensitivity) to killed organisms in genitourinary infections (Frei test).
   c. Rising titer of antibody against the chlamydial family antigen in lung infections. This accomplished with the complement fixation test or the fluorescent antibody test.
• A complement fixation serological test is available as are DNA based tests.

• Virulence factors:
  • Toxicity from attachment and penetration

• Clinical significance:
  • Chlamydia trachomatis – serotypes A-K and L_1,2,3_; the serotype determines the clinical manifestation.
  • Genital tract infection (serotypes D-K) – is the major cause of nongonococcal urethritis; is sexually transmitted and frequently found concomitantly with *N. gonorrhoeae*
  • In males symptoms include urethritis, dysuria and it sometimes progresses to epididymitis
• Subgroup A organisms primarily infect the mucous membranes of the eye or the genitourinary tract of humans. Subgroup B organisms, although primarily parasites of birds, can be transmitted to man where they cause a lung infection.

• The mechanism by which chlamydia cause disease or injure cells is unknown. Chlamydial infections of mucous membranes cause damage to tissues deep in the epithelial layer; for example, in trachoma, scarring of the tarsal plate occurs frequently. There is some evidence that a toxin is produced.
• In females symptoms include mucopurulent cervical inflammation which can progress to salpingitis and PID.

• Inclusion conjunctivitis – this occurs in both newborns and adults and a genital tract infection is the source of the infection (serotypes D-K); is a benign, self-limited conjunctivitis which heals with no scarring

• Newborns – are infected during the birth process and the infection manifests 1-2 weeks after birth as a mucopurulent discharge that lasts 2 weeks and then subsides.

• Some may develop an afebrile, chronic pneumonia
• In adults – causes an acute follicular conjunctivitis with little discharge.

• Trachoma (serotypes A-C) – is the single, greatest cause of blindness in underdeveloped countries.

• Transmission is by direct contact and in poor, less developed countries children may be infected in the first three months of life.

• Chronic infection and reinfection are common and result in conjunctival scarring and corneal vascularization.

• The scars contract causing the upper lid to turn in so that the eyelashes cause corneal abrasions.

• This leads to secondary bacterial infections and results in blindness.
Trachoma
- Lymphogranuloma venereum (serotypes $L_{1,2,3}$) is a venereal disease that occurs in poor, tropical areas.

- Upon infection, widespread dissemination takes place and a primary, painless lesion (either a vesicle or an ulcer) occurs at the site of entry within a few days.

- This heals with no scarring.

- A secondary stage occurs 2-6 weeks later with symptoms of regional suppurative lymphadenopathy (buboes) that may drain for a long time and be accompanied by fever and chills.

- Arthritis, conjunctival, and CNS symptoms may also occur.

- A tertiary stage may occur and is called the urethrogenital perineal syndrome.

- This is characterized by structural changes such as non-destructive elephantiasis of the genitals and rectal stenosis.
Chlamydia psittaci – naturally infects avian species and non-primate animals causing mild to severe illness.

In man causes psittacosis (ornithosis) and is acquired by contact with an infected animal.

Infection can range from subclinical to fatal pneumonia.

Most commonly causes an atypical pneumonia with fever, chills, dry cough, headache, sore throat, nausea, and vomiting.
• Treatment/antimicrobial susceptibility

• *C. trachomatis* –
  
  *Trachoma* – systemic tetracycline, erythromycin; long term therapy is necessary

• Genital tract infections and conjunctivitis – tetracyclines and erythromycin

• *C. psittaci* – same as above
Chlamydia exhibit low pathogenicity except in a compromised host. The chlamydial diseases are relatively easy to treat, but present two problems.

1. Latency of infection--infections may remain latent or sub-clinical for years.

2. Susceptibility of compromised host to reinfection--the compromised host usually remains compromised because of genetic and/or environmental factors and becomes reinfected.

3. Minimal symptomology

*Chlamydia trachomatis* - doxycycline or azithromycin  
*Chlamydia pneumonia* - doxycycline or azithromycin or erythromycin  
*Chlamydia psittaci* - doxycycline or erythromycin
Mycoplasma
Classification of mycoplasma and diseases it causes
• Classification – order Mycoplasmatales; family Mycoplasmataceae; 2 medically important genera
  • Mycoplasma
  • Ureoplasma

• Three common clinical isolates – *M. pneumoniae*, *M. hominis*, and *U. urealyticum*

• Morphology and cultural characteristics
  • Do not possess the distinctive cell wall of bacteria
• Plasma membrane is the outermost part of the organism and is unique in bacteria in that it has a high content of sterols that act to prevent osmotic lysis
• Very small in size (too small to be seen with an ordinary light microscope) and highly pleomorphic
• Don’t stain with a Gram’s stain
• Non-motile
• May possess a capsule
• Although some are free living, most are closely adapted parasites
• Grow on media enriched with serum (need cholesterol)
• Grow beat at 35-37°C either aerobically or anaerobically
• *M. pneumoniae* grows in 5-14 days, *M. hominis* in 2-4 days, and *U. urealyticum* in 24-28 hours.
• *M. pneumoniae* colonies resemble fried eggs and can be stained with Dienes stain (they stain blue)
• Identification
• *M. pneumonia* - isolation in culture
The laboratory diagnosis of mycoplasma infection can be accomplished by:

1. Culturing the organism from sputum, mucous membrane swabbings or other specimens by direct inoculation into liquid or solid media containing serum, yeast extract and penicillin to inhibit contaminating bacteria. Colonies will become detectable in one to three weeks. They stain intensely with neutral red or tetrazolium or methylene blue.

   The organism can be presumptively identified by its hemabsorption or B-hemolysis of guinea pig red blood cells. It is conclusively identified by staining its colonies with homologous fluorescein-labelled antibody.

2. Quantitation of the patient antibody response to mycoplasma by complement fixation tests on acute and convalescent serum. Cold agglutinins to human O erythrocytes may also be measured.
Mycoplasma colonies with Diene’s stain
• Ability of colonies to hemolyze guinea pig RBCs
• Rise in specific antibody titer
• Cold agglutinin test – a nonspecific test in which the patient produces cold reacting antibodies that agglutinate type O human RBCs at 4\(^0\) C, but not at 37\(^0\) C

A single titer of 1:128 is significant and occurs in 7 days and disappears in 6 weeks.

• M. hominis
  • Isolation in culture
  • No hemolysis of guinea pig RBCs

• U. urealyticum
  • Urease production
• Virulence factors
  • Not invasive and simply colonize cell surfaces through specific binding
  • Damage to host tissues may be due to toxic metabolic products

• Clinical significance
  • M. pneumonia – the major cause of primary, atypical pneumonia (walking pneumonia)
• Transmitted by droplet infection
  • After a 2-3 week incubation, the disease begins as a mild, upper respiratory tract infection and progresses to fever, headache, malaise, and a dry cough which is usually mild and self-limited.
  • 3-10% develop clinically apparent pneumonia with occasional complications of arthritis, rashes, cardiovascular problems, or neurological problems.
• Genital tract infections - caused by *M. hominis* and *U. urealyticum* which may also be found as part of the NF in the genital tract
• May cause nongonococcal urethritis, PID, post-partum fever, infertility, stillbirth, spontaneous abortion, and acute urethral syndrome

• Treatment
  
  • M. pneumonia – tetracycline or erythromycin
  
  • Genital infections - tetracycline
• Primary atypical pneumonia is usually self-limiting and does not require antibiotic treatment. However, if antibiotics are needed, the drug of choice is one of the macrolide antibiotics:

  - Azithromycin
  - Clarithromycin
  - Dirithromycin
  - Erythromycin

• Urogenital diseases may be treated with:

  - Metronidazole (except during the first trimester of pregnancy)
  - Clindamycin
• Classification – family Legionellaceae with more than 21 species. We will only discuss *L. pneumophila*

• Morphology/cultural characteristics
  - Small, G- pleomorphic rods that stain very poorly
  - Motile
  - Requires cysteine for growth and, therefore, won’t grow on ordinary lab media
  - The best media for primary isolation is buffered charcoal yeast extract with alpha keto glutarate (BCYEα).
    - This can made selective by the addition of cefamandole, anisomycin, and polymyxin B.
Legionella in BCYEα
• Growth in enhanced by incubation in a candle jar or in 2.5% CO₂
• Colonies are pinpoint with a ground-glass appearance

Diagnosis
• Inoculate BCYEα and CBA and look for growth versus no growth
• Are relatively inert and nonfermentative
• Catalase +
• Direct fluorescent antibody testing
DFA stain of Legionella
• Virulence factors
  • Inhibit phagosome-lysosome fusion which allows for intracellular growth
• Endotoxin
  • Inhibit generation of bacteriocidal substances in phagocytic cells (peroxide)
• Hemolysin
• Collagenase
• Clinical significance
• Acute pneumonia – Legionnaire’s disease
  • Airborne transmission with an incubation of 2-10 days
  • Symptoms include fever, chills, malaise, myalgia, headache, dry cough, vomiting, diarrhea, and abdominal and chest pain. Hospitalization is usually required in 3-5 days.
  • Without antibiotics, the fatality rate is as high as 15%
  • The disease occurs more in males over 60 years of age and in the immunocompromised
  • The reservoir of infection is often in the cooling towers of air conditioning systems and in hot water lines as well as in soil and water
• Pontiac fever – an acute, self-limited febrile illness with an incubation of 24-36 hours. Symptoms include a high fever, chills, malaise, myalgia, and headache which lasts 2-5 days

• Treatment/antimicrobial susceptibility
  • Erythromycin, rifampin, or tetracycline
Gardnerella vaginalis
• Classification – included under facultatively anaerobic G-B

• Morphology/cultural characteristics

  • A direct wet mount may reveal many characteristic “clue cells” = desquamated epithelial cells with attached organisms

• Gram variable

• Growth on CBA
Clue cells
On nonselective vaginalis agar which contains human blood, produces diffuse, beta hemolytic colonies

- Growth in 5-10% CO$_2$ for 48 hours

**Diagnosis**
- Observation of clue cells on direct wet mount
- Beta hemolysis on vaginalis agar
- Gram variable
- Catalase-
• Clinical significance

• May be part of the NF in the vagina. Acts synergistically with anaerobic bacteria to cause a bacterial vaginosis characterized by a thin, milky vaginal discharge with a fishy odor upon KOH addition.

• May be involved in maternal and neonatal septicemia