TEACHING OBJECTIVES

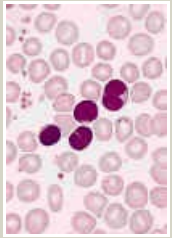
To know the general morphology and physiology the organisms

To know epidemiology and clinical symptoms

To understand the mechanisms pathogenesis

To know the diagnostic, therapeutic and preventive procedures

Photomicrograph of *Bordetella (Haemophilus) pertussis* bacteria using Gram stain technique. CDC



BLOOD LYMPHOCYTOSIS IN A PATIENT WITH

BACTERIOLOGY - CHAPTER EIGHTEEN

BORDETELLA, HAEMOPHILUS AND LEGIONELLA

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BORDETELLA

Bordetella pertussis is the only organism of major clinical significance within this genus; it causes whooping cough in infants and young children. However, a closely related organism, *B. parapertussis* can also cause a milder form of bronchitis. *B. bronchosepticus*, another member of the genus *Bordetella*, is the causative agent of respiratory diseases in cats and swine, but can cause broncho-pulmonary symptoms in severely immunosupressed individuals.

Bordetella pertussis

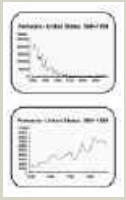
Morphology and physiology

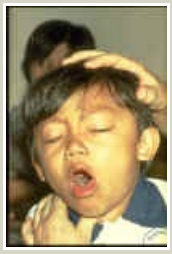
B. pertussis is an extremely small, strictly aerobic, Gram negative, non-motile cocobacillus (short rod). Compared to other *Bortdetella* species, *B. pertussis* does not grow on common laboratory media and can be distinguished from *B. parapertussis* in that *B. pertussis* is oxidase positive but urease negative, while *B. parapertussis* is oxidase negative and urease positive. *B. bronchosepticus* is positive for both enzymes.

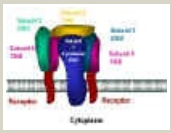
Epidemiology and symptoms

Most of the patients with whooping cough are less than a year old although older children may also get the disease. The severity of disease is also age-related. The organism, contained in aerosol droplets, gains access via inhalation and colonizes the bronchial ciliary epithelial cells. After a week to 10 days of incubation period, mild symptoms of rhinitis, mild cough and sneezing occur (catarrhal stage) which last 1-2 weeks. Further proliferation of the organism compromise ciliary function and is accompanied by increased frequency and intensity of symptoms. This leads to the paroxysmal stage, characterized by paroxysms of cough followed by a prolonged and distressing inspiratory gasp (whoop). The cough, which recurs at variable intervals and often every few minutes, may last for 2-3 weeks. The cough interferes with oral intake, and the swallowed mucus may induce vomiting, resulting in severe dehydration and weight loss. Hypoxia during prolonged attacks may lead to seizure, hypoxic encephalopathy or coma. The cough episodes slowly decrease and there is gradual recovery over 3-16 weeks (convalescent stage). Pneumonia (due to *B. pertussis* or other bacterial pathogens), otitis media, rectal prolapse and meningo-encephalitis are among the secondary

PERTUSSIS. The lymphocytes in this blood smear from an 18-month-old child with a Bordetella pertussis infection have lobulated nuclei. Lymphocytosis is characteristic of this disorder and the lymphocyte morphology is often atypical. The cytology of the cells could be mistaken for neoplastic lymphocytes. (Wright-Giemsa stain) © The Johns Hopkins Autopsy Resource (JHAR). Image Archive.


Pertussis in the US, 1940-1999 CDC


This child has pertussis. It is difficult for him to stop coughing and to get air. Coughing spasms with a "whooping" sound that follows the cough are typical. The sound means child is trying to catch his breath before the next round of coughing © WHO


Binding of pertussis toxin to cell membrane

VIDEO

Baby with pertussis
Infant with pertussis
Toddler with pertussis
Child with pertussis

Courtesy of California Department of Health Services and Healthy Nevadans 2000, Nevada State Health Division and Immunization Action Coalition
Real Video

complications.

Pathogenesis

The symptoms following the infection are due to many factors. In addition to the attachment to and growth on ciliated cells, the organism produces a number of exotoxins which contribute to these symptoms.

Pertussis toxin (pertussigen)

Pertussis toxin is an oligopeptide AB-type exotoxin that is the major cause of pertussis (abnormal cough). It causes T cell lymphocytosis and has adjuvant properties. It also causes hypoglycemia, increased IgE synthesis, and increased histamine and endotoxin sensitivity. The organism inhibits many leukocyte functions, including chemotaxis, phagocytosis and respiratory burst and impairs NK cell killing. It also contributes to bacterial binding to ciliated epithelial cells. It exerts many of its effects by covalent addition of ADP-ribose to the GTP binding Gi protein and thereby preventing the deactivation of adenylate cyclase. This results in the accumulation of large amounts of cAMP which leads to increased mucus secretion and interferes with many cellular functions.

Adenylate cyclase toxin

This exotoxin penetrates the host cells, is activated by calmodulin and catalyzes the conversion of ATP to cAMP. Like pertussigen, it also inhibits phagocyte and NK cell functions. However, in contrast with pertussigen, the cAMP increase caused by this toxin is short-lived.

Tracheal cytotoxin

This is a peptidoglycan-like molecule (monomer) which binds to ciliated epithelial cells, thus interfering with ciliary movement. In higher concentrations, it causes ciliated epithelial cell extrusion and destruction. The destruction of these cells contributes to pertussis.

Dermonecrotic (heat-labile) toxin

Dermonecrotic toxin is a very strong vaso-constrictor and causes ischemia and extravasation of leukocytes and, in association with tracheal cytotoxin, causes necrosis of the tracheal tissue.

Filamentous haemagglutinins (agglutinogens)

These are not exotoxins but are filament-associated lipo-oligo-saccharides which are implicated in the binding of the organism to ciliated epithelial cells. Antibodies against these molecules are protective, probably by preventing bacterial attachment.

Lipopolysaccharide (LPS)

Like LPS of other gram negative bacteria, these endotoxins cause a number of patho-physiolocigal effects. When released in relatively large quantities following bacterial cell lysis, they cause irreversible shock and cardiovascular collapse. In smaller quantities, they activate a variety of inflammatory mediators (TNF, IL1, IL6, prostaglandins, *etc.*) and generate complement activation products.

Diagnosis

Symptoms are characteristic. Laboratory diagnosis is made by obtaining a nasopharyngeal aspirate and primary culture on Bordet-Gengou medium (potato-glycerol-blood agar). Growth is inhibited by peptones, unsaturated fatty acids, sulphides, *etc.* found in ordinary media. The organism grows as small transparent hemolytic colonies. It can be serologically distinguished from *B. parapertussis* and *B. bronchosepticus*.

Prevention and treatment

A killed whole bacterial vaccine is normally administered as DPT combination. An acellular vaccine consisting of filamentous hemagglutinins and detoxified pertussigen is also available and is recommended for booster shots. Erythromycin is the current drug of choice.

Incidence of H. influenzae non-type b invasive disease among children <5 years of age, 1996. CDC/Barbara Rice ber2@cdc.gov

HAEMOPHILUS

The genus *Haemophilus* contains many species but *H. influenzae* is the most common pathogen. Other species of *Haemophilus* that are of clinical importance to immuno-competent humans are *H. ducreyi* (causes chancroid: an STD), *H. influenzae aegyptius* (associated with conjunctivitis and Brazilian purpuric fever) and *H. parainfluenzae* (a rare cause of pneumonia and endocarditis). There are several species of *Hemophilus* that are normal flora, but may be pathogenic in immuno-compromised hosts. The capsulated strain of *H. influenzae* (type b) is most virulent, although some non-encapsulated (non typable) strains are also pathogenic.

Haemophilus influenzae

Morphology and physiology

H. influenzae is a small Gram negative bacillus which can be grown on chocolate agar (heated blood) and requires hemin (factor X) and nicotinamide adenine dinucleotide (NAD^+:factor V) for growth which is enhanced by high CO_2 concentration (5%). It does not grow on normal blood agar. The factor V and factor X requirement can be used to distinguish between *H. influenzae* which requires both, *H. parainfluenzae* which requires factor V only and *H. ducreyi* which requires factor X only. *H. influenzae* are divided into several strains on the basis of capsular polysaccharides (a-f) or the absence of a capsule (non-typable).

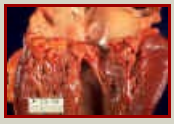
Haemophilus influenzae - coccobacillus prokaryote (dividing); causes meningitis in children, pneumonia, epiglottitis, laryngitis, conjunctivitis, neonatal infection, otitis media (middle ear infection) and sinusitis in adults (SEM x 64,000) © Dennis Kunkel Microscopy, Inc. Used with permission

Epidemiology and symptoms

H. influenzae causes a variety of clinical symptoms some of which may depend on the presence of the bacterial capsule. Until the availability of the Hib vaccine, the type-b *H. influenzae* was the main cause of meningitis in children between 6 months and 5 years, although older children, adolescents and adults can also be infected. The infection initially causes a runny nose, low grade fever and headache (1-3 days). Due to its invasive nature the organism enters the circulation and crosses the blood-brain barrier, resulting in a rapidly progressing meningitis (stiff neck), convulsions, coma and death. Timely treatment may prevent coma and death, but the patient may still suffer from deafness and mental retardation. Type-b *H. influenzae* may also cause septic arthritis conjunctivitis, cellulitis, and epiglottitis, the latter results in the obstruction of the upper airway and suffocation. *H. influenzae* of other types may rarely cause some of the symptoms listed above. Non-typable strains of *H. influenzae* are the second commonest cause of otitis media in young children (second to *Streptococcus pneumoniae)*. In adults, these organisms cause pneumonia, particularly in individuals with other underlying pulmonary infections. These organisms also cause acute or chronic sinusitis in individuals of all ages.

Clinical symptoms of infection by Haemophilus

This child has swollen face due to Hib infection. The tissue under the skin covering the jaw and cheek is infected. Infection is spreading into her face. She is probably very sick Courtesy of Children's Immunization Project, St. Paul, MN



Gross pathology of subacute bacterial endocarditis

Pathogenesis

The exact mechanism of pathogenesis is not known but the presence of capsule, which is anti-phagocytic, is a major factor in virulence. Type-b *H. influenzae* are more invasive and pathogenic than other strains. The lipopolysaccharide is responsible for the inflammatory process. The organisms also produce IgA1-specific protease which may aid their mucosal colonization.

Diagnosis

Presumptive diagnosis is based on history, physical examination and symptoms. Blood cultures are positive in more than 50% of symptomatic patients, except those with conjunctivitis. Polyribitol phosphate (PRP), a component of the capsular polysaccharide is present in the serum, cerebrospinal fluid (CSF) and concentrated urine of more than 95% of *H. influenzae*-b meningitis cases. Gram-negative cocobacilli can be found in the CSF in more than 80% of meningitis cases. Some Gram-stained preparations may be useful in rapid diagnosis of septic arthritis and lower respiratory diseases.

Treatment and prevention

Unless prompt treatment is initiated, *H. influenzae*-b meningitis and epiglotitis are almost 100% fatal. Due to common resistance to ampicillin and some resistance to chloramphenicol, cephalosporin, which penetrates the blood brain

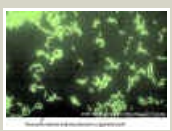
involving mitral valve. Left ventricle of heart has been opened to show mitral valve fibrin vegetations due to infection with Haemophilus parainfluenzae. Autopsy. CDC/Dr. Edwin P. Ewing, Jr. epe1@cdc.gov


Countries implementing routine childhood Hib immunization © WHO


Legionella pneumophila multiplying inside a cultured cell. Multiple intracellular bacilli, including dividing bacilli, are visible in longitudinal and cross section. Transmission electron micrograph. CDC/Dr. Edwin P. Ewing, Jr.


Legionella pneumophila. Rod-Shaped Bacterium (SEM x22,810) © Dennis Kunkel Microscopy, Inc. Used with permission


Legionella growing on an agar plate with enriched nutrients and charcoal. The iridescent sheen of the colonies as well as the apparent "cut-glass" appearance is characteristic of this species. A confirmed identification would be made by direct fluorescent antibody (DFA) technique. © Gloria J. Delisle and Lewis Tomalty, Queens University, Kingston, Ontario Canada and The MicrobeLibrary


DFA technique to detect the Legionella antigen directly in patient specimens. Respiratory tract specimens are spread on a glass slide. A monoclonal antibody to Legionella that is tagged with a fluorescein dye is added to the slide. If the antigen is present, the antibody will bind and the outline of the bacilli can be detected by

barrier, is the antibiotic of choice in these cases. Other diseases caused by this organism can be treated with ampicillin (if susceptible) or choice of trimethoprim-sulphamethoxazol, tetracyclin and cefaclor.

Hib-C vaccine which consists of capsular PRP conjugated to tetanus toxoid has been used successfully to provide protection and is a part of the recommended routine vaccination schedule.

Haemophilus ducreyi

This is a significant cause of genital ulcers (chancroid) in Asia and Africa but, is seen less commonly in the United States. The incidence is approximately 4000-5000 per year with clusters found in California, Florida, Georgia and New York. The infection is asymptomatic in women but about a week following sexual transmission to a man, it causes appearance of a tender papule with erythematous base on the genitalia or the peripheral area. The lesion progresses to become a painful ulcer with inguinal lymphadenopathy. The *H. ducreyi* lesion (chancroid) is distinguished from a syphilitic lesion (chancre) in that it is a comparatively soft lesion. The organism is more fastidious than *H. influenzae* but can be grown on chocolate agar, supplemented with IsovitaleX in 5%-10% CO_2 atmosphere and the growth can be detected in 2-4 days.

Haemophilus influenzae aegyptius

This bacterium, previously known as *H. aegyptius,* causes an opportunistic organism which can result in a fulminant pediatric disease (Brazilian purpuric fever) characterized by an initial conjunctivitis, followed by an acute onset of fever, accompanied by vomiting and abdominal pain. Subsequently, the patient develops petechiae, purpura, shock and may face death. The pathogenesis of this infection is poorly understood. The growth conditions for this organism are the same as those for *H. influenzae*.

Both *H. ducreyi* and *H. influenzae aegyptius* can be treated with erythromycin.

LEGIONELLA

In 1976, *Legionella pneumophila* was recognized as a newly described pathogen after an outbreak of pneumonia among a group of Legionnaires at a convention in Philadelphia. The disease was subsequently referred to as Legionnaires' disease. Another flu-like form of the disease is referred to as Pontiac fever. *L. pneumophila* is now recognized as a ubiquitous aquatic saprophyte which causes epidemics and sporadic infections. The organisms are spread via aerosols and no person to person transmission has been reported.

Legionellae are facultative intracellular pathogens, which stain poorly as Gram negative rods. The causative agent was not recognized previously, since it does not grow on conventional agar such as sheep blood agar. Nowadays *L. pneumophila* is cultured on medium that contains iron and cysteine which are vital for growth (e.g. charcoal yeast extract agar). However, primary isolation is still difficult from clinical specimens.

Organisms of Clinical Importance

After recognition of their unique culture characteristics, a large number of other species of *Legionella* were isolated from environmental and clinical samples. These organisms are only occasional causes of human disease and the vast bulk of legionellosis is caused by *Legionella pneumophila* (most are serogroup 1 and 6).

The second most common cause of pneumonia is *Legionella micdadei*. This organism also stains weakly acid fast on primary isolation, but loses this property *in vitro*. This does not mean that it is anyway related to the Mycobacteria.

Microbiology

Legionellae are poorly staining Gram negative rods which are identified by growth on buffered charcoal yeast extract (BCYE), and require L-cysteine and iron for growth. The organisms are fairly slow growing requiring 3 to 7 days at 35 degrees. Colonies are small with a ground glass appearance.

The Center for Disease Control (CDC) lists four tests for the identification of Legionnaires' disease:

- Culture

microscopy under UV light.

- Urine antigen
- Paired serology
- Direct fluorescent antibody stain.

PCR tests for *L. pneumophila* in clinical specimens are available; however the CDC does not recommend the routine use of genetic probes or PCR for detection in clinical samples.

Public Health

Legionella pneumophila is an organism that resides in the environment in pools of stagnant water worldwide. It is found as an intra-cellular agent within protozoa and a component of biofilms. Legionnaires' disease is recognized as a sporadic infection, often associated with travel, an epidemic disease of community-acquired pneumonia and a nosocomial infection. It often infects hot water towers and air conditioning systems. When found in buildings, anti-bacterial treatment of the water supply is recommended. One recently identified source of *Legionella* infections is the water used in car windscreen washers, the reservoirs being warmed by the car engine. The use of windscreen washer fluid (which contains methanol) solves this problem.

The organism is transmitted in contaminated air but not spread person-person. Legionellosis is listed as one of the Nationally Notifiable Diseases by the Centers for Disease Control.

Clinical Presentation

Legionellae present as two distinct clinical diseases. The first is Legionnaires' disease, a typical pneumonia with an incubation period of 2 to 10 days. The mortality rate is as low as 20 % for healthy individuals and as high as 75% for the immune compromised persons. Legionnaires' disease is treated with erythromycin. The second form of disease presentation is Pontiac Fever. This illness has an incubation period of 1 to 2 days and is self-limiting with flu-like symptoms and no reported mortality.

Pathogenesis

Pathogenesis of *Legionellae* species requires the organism be phagocytosed into monocytes via complement receptors. Once inside the monocytes, the bacteria prevent phagosome-lysosome fusion and proceed to replicate until they lyse the phagosome which leads to apoptosis of the monocyte and release of the bacteria. Humoral immunity has little effect and the sensitized T helper (TH1) cells are required to activate the infected cells. Interferon- gamma is also critical to the elimination of *Legionellae*.

 Return to the Bacteriology Section of Microbiology and Immunology On-line

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