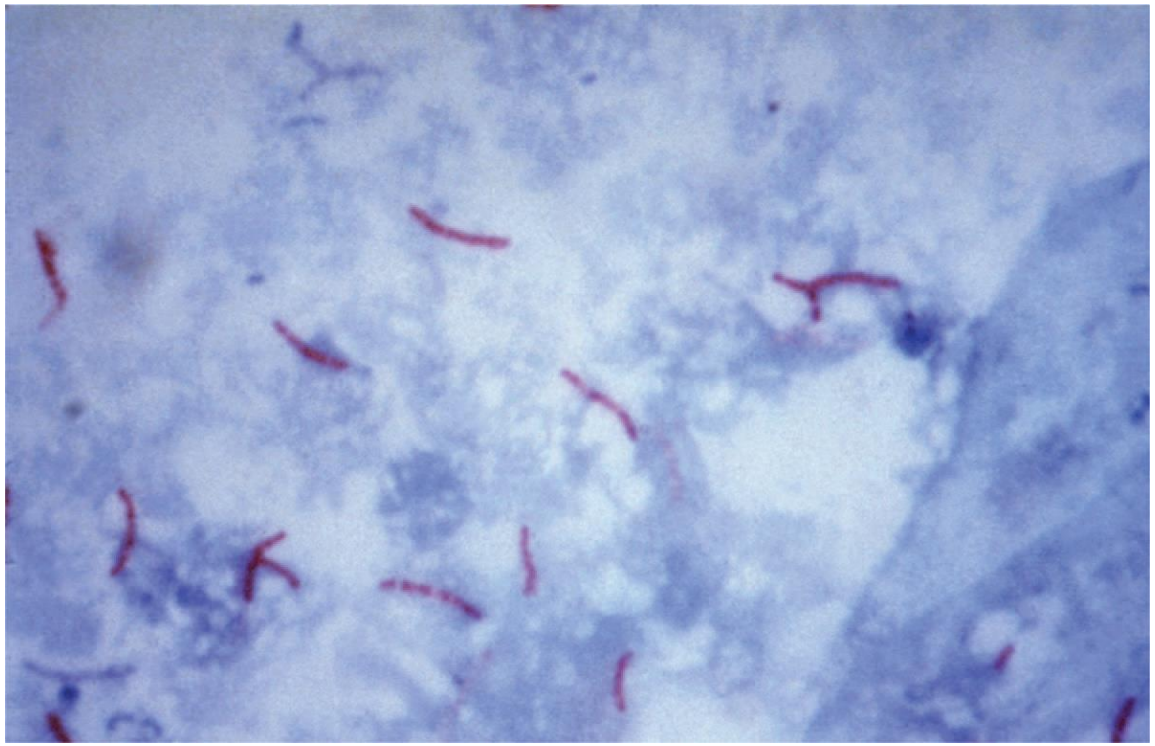
Practical lesson 16 : Microbiological diagnosis of tuberculosis and actinomycosis. Microbiological diagnosis of infections caused by pathogenic spirochetes



MYCOBACTERIUM

Mycobacteria are aerobic, acid-fast bacilli (rods) .They are neither gram-positive nor gram-negative.They are virtually the only bacteria that are acid-fast. The term acid-fast refers to an organism's ability to retain the carbolfuchsin stain despite subsequent treatment with an ethanol–hydrochloric acid mixture. The high lipid content of their cell wall makes mycobacteria acid-fast.The major pathogens are Mycobacterium tuberculosis, the cause of tuberculosis, and Mycobacterium leprae, the cause of leprosy.

MYCOBACTERIUM TUBERCULOSIS

Disease

This organism causes tuberculosis. Worldwide, M. tuberculosis causes more deaths than any other single microbial agent. Approximately one-third of the world's population is infected with this organism. Each year, it is estimated that 1.7 million people die of tuberculosis and that 9 million new cases occur. An estimated 500,000 people are infected with a multidrug-resistant strain of M. tuberculosis

Important Properties

Mycobacterium tuberculosis grows slowly (i.e., it has a doubling time of 18 hours, in contrast to most bacteria, which can double in number in 1 hour or less). Because growth is so slow, cultures of clinical specimens must be held for 6 to 8 weeks before being recorded as negative. Mycobacterium tuberculosis can be cultured on bacteriologic media, whereas M. leprae cannot. Media used for its growth (e.g.,Löwenstein-Jensen medium) contain complex nutrients (e.g., egg yolk) and dyes (e.g., malachite green). The dyes inhibit the unwanted normal flora present in sputum samples. Mycobacterium tuberculosis is an obligate aerobe; this explains its predilection for causing disease in highly oxygenated tissues such as the upper lobe of the lung and the kidney. The acid-fast property of M. tuberculosis (and other mycobacteria) is attributed to long-chain (C78–C90) fatty acids called mycolic acids in the cell wall. Cord factor (trehalose dimycolate) is correlated with virulence of the organism. Virulent strains grow in a characteristic“serpentine” cordlike pattern, whereas avirulent strains do not.

Pathogenesis

Primary tuberculosis can heal by fibrosis, can lead to progressive lung disease, can cause bacteremia and miliary tuberculosis, or cause hematogenous dissemination resulting in no immediate disease but with the risk of reactivation in later life.Mycobacterium tuberculosis produces no exotoxins and does not contain endotoxin in its cell wall. In fact, no mycobacteria produce toxins. The organism preferentially infects macrophages and other reticuloendothelial cells. Mycobacterium tuberculosis survives and multiplies within a cellular vacuole called a phagosome. The organism produces a protein called “exported repetitive protein” that prevents the phagosome from fusing with the lysosome, thereby allowing the organism to escape the degradative enzymes in the lysosome. Lesions are dependent on the presence of the organism and the host response. There are two types of lesions: (1) Exudative lesions, which consist of an acute inflammatory response and occur chiefly in the lungs at the initial site of infection. (2) Granulomatous lesions, which consist of a central area of giant cells containing tubercle bacilli surrounded by a zone of epithelioid cells. These giant cells, called Langhans’ giant cells, are an important pathologic finding in tuberculous lesions. A tubercle is a granuloma surrounded by fibrous tissue that has undergone central caseation necrosis.

Transmission & Epidemiology

Mycobacterium tuberculosis is transmitted from person to person by respiratory aerosols produced by coughing. The source of the organism is a cavity in the lung that has eroded into a bronchus. The portal of entry is the respiratory tract and the initial site of infection is the lung. In tissue, it resides chiefly within reticuloendothelial cells (e.g., macrophages). Macrophages kill most, but not all, of the infecting organisms. The ones that survive can continue to infect other adjacent cells or can disseminate to other organs. Humans are the natural reservoir of M. tuberculosis. Although some animals, such as cattle, can be infected, they are not the main reservoir for human infection. Most transmission occurs by aerosols generated by the coughing of “smear-positive” people (i.e., those whose sputum contains detectable bacilli in the acid-fast stain). However, about 20% of people are infected by aerosols produced by the coughing of “smear-negative” people. In the United States, tuberculosis is almost exclusively a human disease. In developing countries, Mycobacterium bovis also causes tuberculosis in humans. Mycobacterium bovis is found in cow’s milk, which, unless pasteurized, can cause gastrointestinal tuberculosis in humans. The disease tuberculosis occurs in only a small number of infected individuals. In the United States, most cases of tuberculosis are associated with reactivation in elderly, malnourished men. The risk of infection and disease is highest among socioeconomically disadvantaged people, who have poor housing and poor nutrition.

Treatment & Resistance

Multidrug therapy is used to prevent the emergence of drug-resistant mutants during the long (6- to 9-month) duration of treatment. (Organisms that become resistant to one drug will be inhibited by the other.) Isoniazid (INH), a bactericidal drug, is the mainstay of treatment. Treatment for most patients with pulmonary tuberculosis is with three drugs: INH, rifampin, and pyrazinamide. INH and rifampinare given for 6 months, but pyrazinamide treatment is stopped after 2 months. A somewhat different regimen can also be used. A convenient way to remember that regimen is to give four drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) for 2 months and two drugs (isoniazid and rifampin) for 4 months.

Prevention

The incidence of tuberculosis began to decrease markedly even before the advent of drug therapy in the 1940s. This is attributed to better housing and nutrition, which have improved host resistance. At present, prevention of the spread of the organism depends largely on the prompt identification and adequate treatment of patients who are coughing up the organism. The use of masks and other respiratory isolation procedures to prevent spread to medical personnel is also important. Contact tracing of individuals exposed to patients with active pulmonary disease who are coughing should be done. An important component of prevention is the use of the PPD skin test to detect recent converters and to institute treatment for latent infections as described earlier. Groups that should be screened with the PPD skin test include people with HIV infection, close contacts of patients with active tuberculosis, low-income populations, alcoholics and intravenous drug users, prison inmates, and foreignborn individuals from countries with a high incidence of tuberculosis

Mycobacterium Leprae

Important Properties

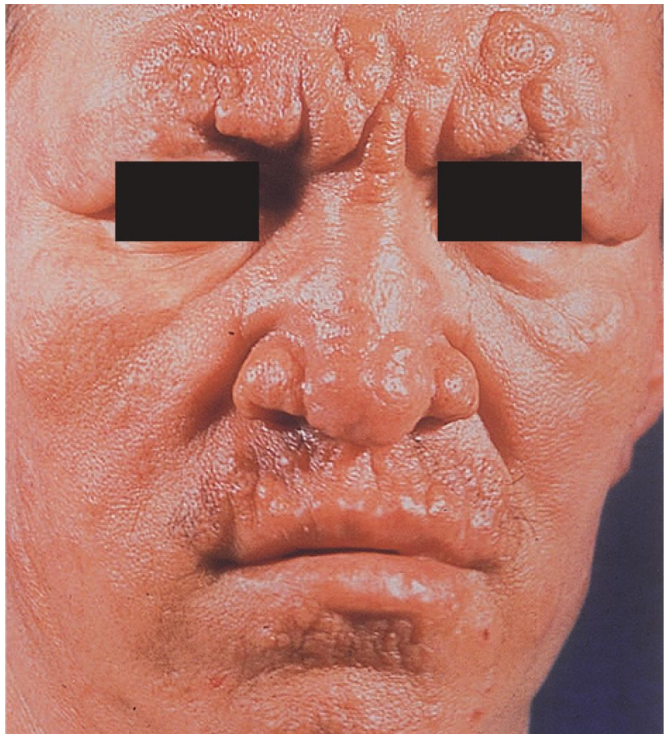
Mycobacterium leprae has not been grown in the laboratory, either on artificial media or in cell culture. It can be grown in experimental animals, such as mice and armadillos. Humans are the natural hosts, although the armadillo appears to be a reservoir for human infection in the Mississippi Delta region where these animals are common. In view of this, leprosy can be thought of as a zoonotic disease, at least in certain southern states, such as Louisiana and Texas. The optimal temperature for growth (30°C) is lower than body temperature; therefore, M. leprae grows preferentially in the skin and superficial nerves. It grows very slowly, with a doubling time of 14 days. This makes it the slowest-growing human bacterial pathogen. One consequence of this is that antibiotic therapy must be continued for a long time, usually several years.

Transmission

Infection is acquired by prolonged contact with patients with lepromatous leprosy, who discharge M. leprae in large numbers in nasal secretions and from skin lesions. In the United States, leprosy occurs primarily in Texas, Louisiana, California, and Hawaii. Most cases are found in immigrants from Mexico, the Philippines, Southeast Asia, and India. The disease occurs worldwide, with most cases in the tropical areas of Asia and Africa. The armadillo is unlikely to be an important reservoir because it is not found in many areas of the world where leprosy is endemic.

Pathogenesis

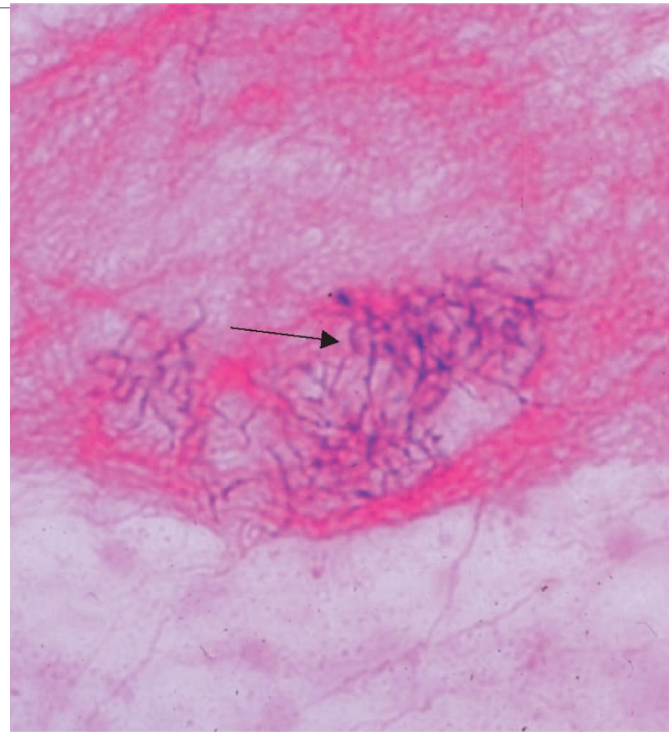
The organism replicates intracellularly, typically within skin histiocytes, endothelial cells, and the Schwann cells of nerves. The nerve damage in leprosy is the result of two processes: damage caused by direct contact with the bacterium and damage caused by CMI attack on the nerves. There are two distinct forms of leprosy—tuberculoid and lepromatous—with several intermediate forms between the two extremes. (1) In tuberculoid (also known as paucibacillary) leprosy, the CMI response to the organism limits its growth, very few acid-fast bacilli are seen, and granulomas containing giant cells form. The nerve damage seems likely to be caused by cell-mediated immunity as there are few organisms and the CMI response is strong. The CMI response consists primarily of CD4-positive cells and a Th-1 profile of cytokines, namely, interferon-γ, interleukin-2, and interleukin-12. It is the CMI response that causes the nerve damage seen in tuberculoid leprosy. The lepromin skin test result is positive. The lepromin skin test is similar to the tuberculin test (see earlier). An extract of M. leprae is injected intradermally, and induration is observed 48 hours later in those in whom a CMI response against the organism exists. (2) In lepromatous (also known as multibacillary) leprosy, the cell-mediated response to the organism is poor, the skin and mucous membrane lesions contain large numbers of organisms, foamy histiocytes rather than granulomas are found, and the lepromin skin test result is negative. The nerve damage seems likely to be caused by direct contact as there are many organisms and the CMI response is poor.



ACTINOMYCETES

Actinomycetes are a family of bacteria that form long, branching filaments that resemble the hyphae of fungi. They are gram-positive, but some (such as Nocardia asteroides) are also weakly acid-fast rods**.**

Nocardia asteroides—Gram stain. Arrow points to area of filaments of gram-positive rods.



Actinomyces Israeli

Disease

Actinomyces israelii causes actinomycosis.

Important Properties & Pathogenesis

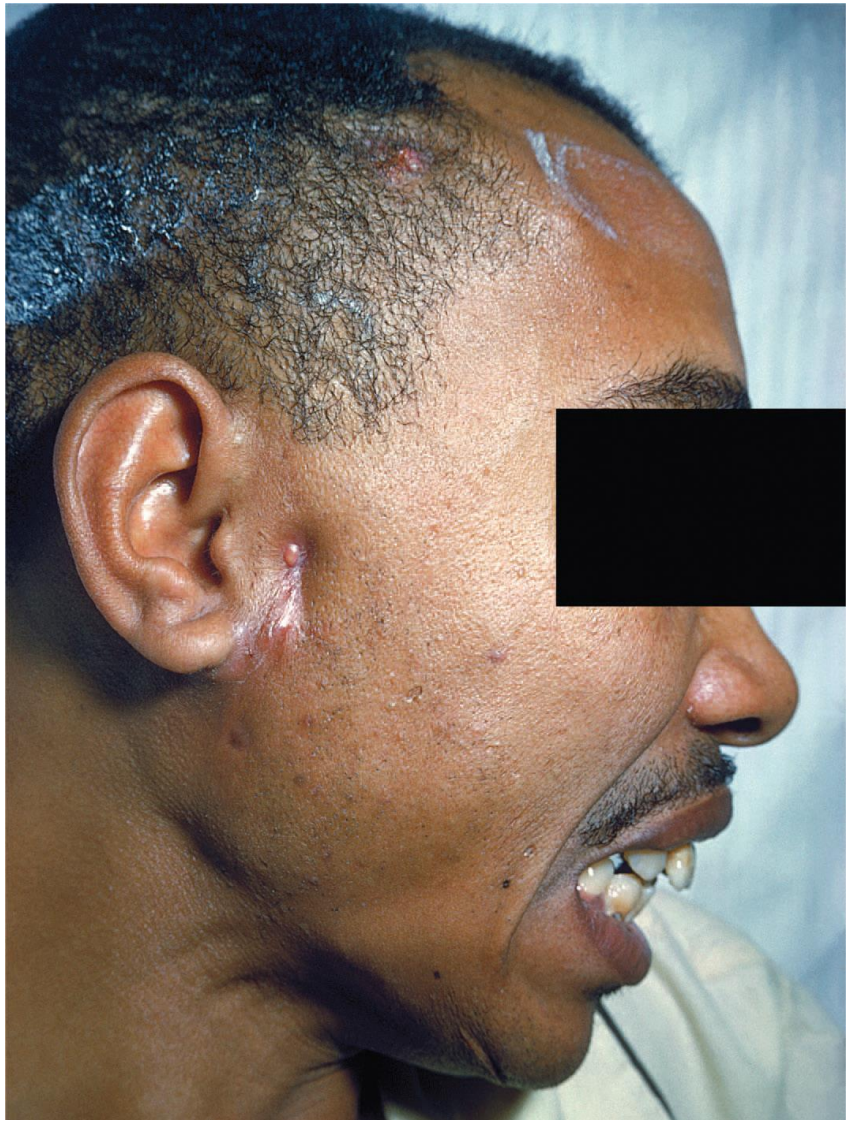
Actinomyces israelii is an anaerobe that forms part of the normal flora of the oral cavity. After local trauma such as a broken jaw or dental extraction, it may invade tissues, forming filaments surrounded by areas of inflammation.

Clinical Findings

The typical lesion of actinomycosis appears as a hard, nontender swelling that develops slowly and eventually drains pus through sinus tracts. Hard, yellow granules (sulfur granules) composed of a mass of filaments are formed in pus. In about 50% of cases, the initial lesion involves the face and neck; in the rest, the chest or abdomen is the site. Pelvic actinomycosis can occur in women who have retained an intrauterine device for a long period of time. Actinomyces israelii and Arachnia species are the most common causes of actinomycosis in humans. The disease is not communicable.

Laboratory Diagnosis

Diagnosis in the laboratory is made by (1) seeing grampositive branching rods, especially in the presence of sulfur granules; and (2) seeing growth when pus or tissue specimens are cultured under anaerobic conditions. Organisms can be identified by immunofluorescence. Note that in contrast to N. asteroides (see later), Actinomyces is not acid-fast. There are no serologic tests.



Actinomycosis. Note inflamed lesion with small sinus tract opening anterior to right ear. Yellowish “sulfur granule” can be seen at the opening

Treatment & Prevention

Treatment consists of prolonged administration of penicillin G, coupled with surgical drainage. There is no significant resistance to penicillin G. No vaccine or prophylactic drug is available.

Disease : Nocardia asteroides causes nocardiosis.

Important Properties & Pathogenesis

Nocardia species are aerobes and are found in the environment, particularly in the soil. In immunocompromised individuals, they can produce lung infection and may disseminate. In tissues, Nocardia species are thin, branching filaments that are gram-positive on Gram stain. Many isolates of N. asteroides are weakly acid-fast (i.e., the staining process uses a weaker solution of hydrochloric acid to decolorize than that used in the stain for mycobacteria). If the regular-strength acid is used, N. asteroides will decolorize.

Clinical Findings

Nocardia asteroides typically causes either pneumonia, lung abscess with cavity formation, lung nodules, or empyema. From the lung, the organism can spread to various organs, notably the brain, where it causes brain abscess. Disease occurs most often in immunocompromised individuals, especially those with reduced cell-mediated immunity. Nocardia brasiliensis, a different species of Nocardia, causes skin infections in the southern regions of the United States and mycetoma, usually in tropical regions.

Laboratory Diagnosis

Diagnosis in the laboratory involves (1) seeing branching rods or filaments that are gram-positive or weakly acid-fast in an acid-fast stain and (2) seeing aerobic growth on bacteriologic media in a few days.

Treatment & Prevention

Treatment is with trimethoprim-sulfamethoxazole. Surgical drainage may also be needed. Occasional drug

SPIROCHETES

Three genera of spirochetes cause human infection: (1)Treponema, which causes syphilis and the nonvenereal treponematoses; (2) Borrelia, which causes Lyme disease and relapsing fever; and (3) Leptospira, which causes leptospirosis. Spirochetes are thin-walled, flexible, spiral rods. They are motile through the undulation of axial filaments that lie under the outer sheath. Treponemes and leptospirae are so thin that they are seen only by dark field microscopy, silver impregnation, or immunofluorescence. Borreliae are larger, accept Giemsa and other blood stains, and can be seen in the standard light microscope.

Treponema

1. Treponema pallidum

Treponema pallidum causes syphilis.

Important Properties

Treponema pallidum has not been grown on bacteriologic media or in cell culture. Nonpathogenic treponemes, which are part of the normal flora of human mucous membranes, can be cultured. Treponema pallidum grows very slowly. The medical importance of that fact is that antibiotics must be present at an effective level for several weeks to kill the organisms and cure the disease (see “Treatment” section later). For example, benzathine penicillin is the form of penicillin used to treat primary and secondary syphilis because the penicillin is released very slowly from this depot preparation, and bactericidal concentrations are present for weeks after administration of the antibiotic. The antigens of T. pallidum induce specific antibodies, which can be detected by immunofluorescence or hemagglutination tests in the clinical laboratory. They also induce nonspecific antibodies (reagin),1 which can be detected by the flocculation of lipids (cardiolipin) extracted from normal mammalian tissues (e.g., beef heart). Both specific antitreponemal antibody and nonspecific reagin are used in the serologic diagnosis of syphilis.

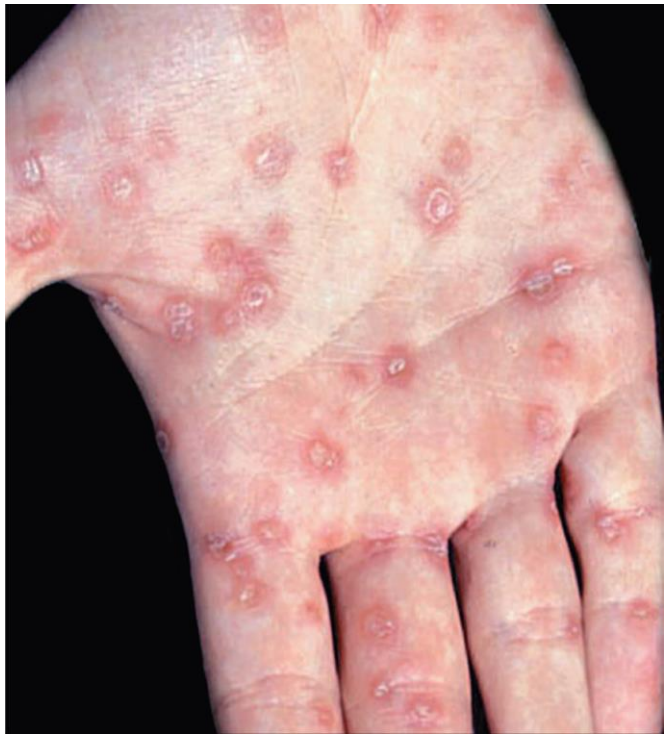
Transmission & Epidemiology

Treponema pallidum is transmitted from spirochetecontaining lesions of skin or mucous membranes (e.g.,genitalia, mouth, and anus) of an infected person to other persons by intimate contact. It can also be transmitted from pregnant women to their fetuses. Rarely, blood for transfusions collected during early syphilis is also infectious. Treponema pallidum is a human organism only.There is no animal reservoir.Syphilis occurs worldwide, and its incidence is increasing.Many cases are believed to go unreported, which limits public health efforts. There has been a marked increase in incidence of the disease in men who have sex with men in recent years.

Palmar lesions of secondary syphilis. Note the papulosquamous lesions on the right palm. Palmar lesions are typically Bilateral.

Laboratory Diagnosis

There are three important approaches. Microscopy Spirochetes are demonstrated in the lesions of primary or secondary syphilis, such as chancres or condylomata lata, by dark field microscopy or by direct fluorescent antibody (DFA) test. They are not seen on a Gram-stained smear. In biopsy specimens, such as those obtained from the gummas seen in tertiary syphilis, histologic stains such as silver stain or fluorescent antibody can be used.



Treatment

Penicillin G is effective in the treatment of all stages of syphilis. A single injection of benzathine penicillin G (2.4 million units) can eradicate T. pallidum and cure early (primary and secondary) syphilis. Note that benzathine penicillin is used because the penicillin is released very slowly from this depot preparation. Treponema pallidum grows very slowly, which requires that the penicillin be present in bactericidal concentration for weeks. If the patient is allergic to penicillin, doxycycline can be used but must be given for prolonged periods to effect a cure. In neurosyphilis, high doses of aqueous penicillin G are administered because benzathine penicillin penetrates poorly into the central nervous system. No resistance to penicillin has been observed. However, strains resistant to azithromycin have emerged.

Prevention

Prevention depends on early diagnosis and adequate treatment, use of condoms, administration of antibiotic after suspected exposure, and serologic follow-up of infected individuals and their contacts. The presence of any sexually transmitted disease makes testing for syphilis mandatory, because several different infections are often transmitted simultaneously. There is no vaccine against syphilis.

Borrelia

Borrelia species are irregular, loosely coiled spirochetes that stain readily with Giemsa and other stains. They can be cultured in bacteriologic media containing serum or tissue extracts. They are transmitted by arthropods. They cause two major diseases, Lyme disease and relapsing fever.

1. Borrelia burgdorferi

Disease

Borrelia burgdorferi causes Lyme disease (named after a town in Connecticut). Lyme disease is also known as Lyme borreliosis. Lyme disease is the most common tick-borne disease in the United States. It is also the most common vector-borne disease in the United States. Approximately 20,000 cases each year are reported to the Centers for Disease Control and Prevention, and that number is thought to be significantly less than the actual number.

Important Properties

Borrelia burgdorferi is a flexible, motile spirochete that can be visualized by dark field microscopy and by Giemsa and silver stains. It can be grown in certain bacteriologic media, but routine cultures obtained from patients (e.g., blood, spinal fluid) are typically negative. In contrast, culture of the organism from the tick vector is usually positive.

Transmission & Epidemiology

Borrelia burgdorferi is transmitted by tick bite. The tick Ixodes scapularis is the vector on the East Coast and in the Midwest; Ixodes pacificus is involved on the West Coast. The organism is found in a much higher percentage of I. scapularis (35%–50%) than I. pacificus (approximately 2%) ticks. This explains the lower incidence of disease on the West Coast. The main reservoir of the organism consists of small mammals, especially the white-footed mouse, upon which the nymphs feed.4 Large mammals, especially deer, are an obligatory host in the tick's life cycle but are not an important reservoir of the organism. The nymphal stage of the tick transmits the disease more often than the adult and larval stages do. Nymphs feed primarily in the summer, which accounts for the high incidence of disease during the months of May to September. The tick must feed for 24 to 48 hours to transmit an infectious dose. This implies that inspecting the skin after being exposed can prevent the disease. However, the nymphs are quite small and can easily be missed. There is no human-to-human spread.

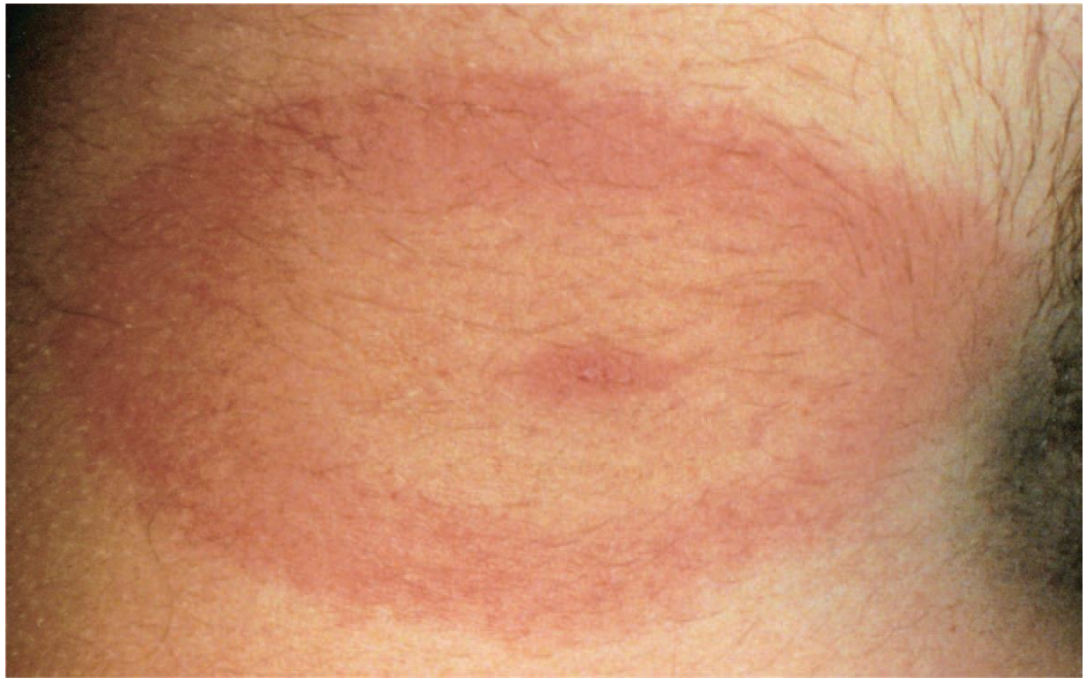
Ixodes tick. Nymph form of tick with head buried in skin surrounded by an erythematous macular rash.

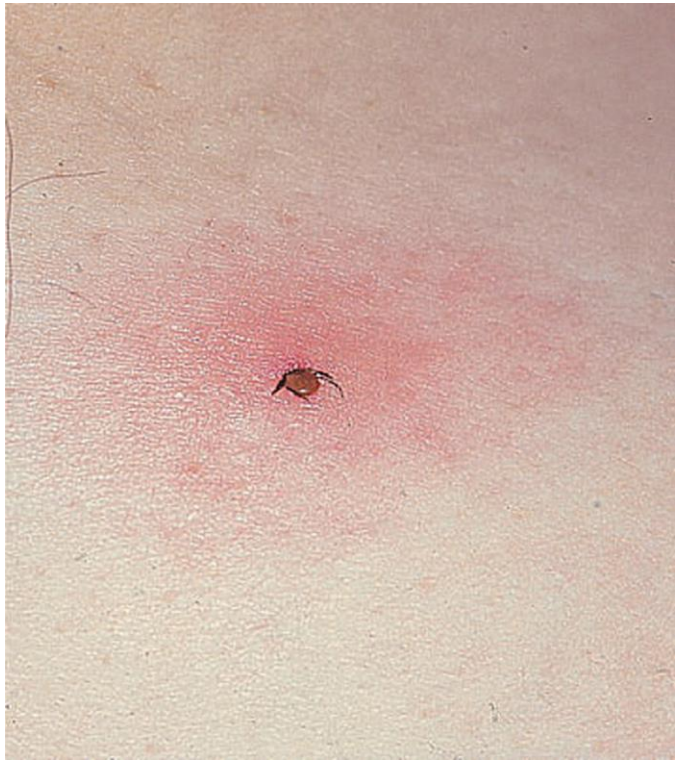
Pathogenesis

Pathogenesis is associated with spread of the organism from the bite site through the surrounding skin followed by dissemination via the blood (bacteremia) to various organs, especially the heart, joints, and central nervous system. No exotoxins, enzymes, or other important virulence factors have been identified. Note that the organism must adapt to two markedly different hosts, the tick and the mammal (either mice or humans). It does so by changing its outer surface protein (OSP). These OSPs vary antigenically within humans. Multiple episodes of Lyme disease are due to reinfection, rather than relapse caused by reactivation of the organism. There is no evidence for a latent stage of B. burgdorferi.

Clinical Findings

The clinical findings have been divided into three stages; however, this is a progressive disease, and the stages are not discrete. In stage 1 (early localized stage), the most common finding is erythema chronicum migrans (also called erythema migrans), an expanding, erythematous, macular rash that often has a "target" or "bull's eye" appearance.The rash appears between 3 and 30 days after the tick bite. Both the tick bite and the rash are painless and nonpruritic.





Laboratory Diagnosis

Although the organism can be grown in the laboratory, cultures are rarely positive and hence are usually not performed. The diagnosis is typically made serologically by detecting either IgM antibody or a rising titer of IgG antibody with an enzyme-linked immunosorbent assay (ELISA) or an indirect immunofluorescence test. IgM is typically detectable 2 weeks after infection and peaks at 3 to 6 weeks. Serologic tests done before 2 weeks are likely to yield negative results. Thirty days after infection, tests for IgG are more reliable. Unfortunately, there are problems with the specificity and sensitivity of these tests because of the presence of cross-reacting antibodies against spirochetes in the normal flora. A positive test result should be confirmed with a Western blot (immunoblot) analysis. In addition, patients treated early in the disease may not develop detectable antibodies. A polymerase chain reaction (PCR) test that detects the organism's DNA is also available.

Treatment & Prevention

The treatment of choice for stage 1 disease or other mild manifestations is either doxycycline or amoxicillin. Amoxicillin should be used in pregnant women and young children, as doxycycline is contraindicated. For more severe forms or late-stage disease, ceftriaxone is recommended. There is no significant antibiotic resistance. Prevention involves wearing protective clothing and using insect repellents. Examining the skin carefully for ticks is also very important, because the tick must feed for 24 to 48 hours to transmit an infective dose