INTRODUCTION TO MYCOBACTERIA

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This lecture will cover 3 groups of mycobacteria: *M. tuberculosis* complex, nontuberculous mycobacteria and *M. leprae*, the cause of leprosy. The *M. tuberculosis* complex is a group of closely-related organisms including *M. tuberculosis* and *M. bovis.* In Africa, *M. africanum* causes disease very similar to *M. tuberculosis* and is often not differentiated from *M. tuberculosis* in the laboratory. Nontuberculous mycobacteria are a broad group of other organisms that do not fall into either of the other 2 categories. These are often found in the environment but can also be pathogens.

Mycobacteria are neither gram-positive nor gram-negative. Although they are classified phylogenetically with the group Actinomycetales, a gram-positive group, they are not stained by the gram-stain. As we'll see, they have a specialized cell envelope different from gram-positives. **Acid-fast stains are used instead.**

Key Points: Physiology and Structure
1) Slow rate of replication
2) Lipid-rich outer layer of mycolic acids
3) Resistant to desiccation
4) Stained by acid-fast stains but not gram-stain

Common features of the structure and bacteriology of mycobacteria. These are non-spore forming and non-motile bacilli. **They are obligate aerobes and have a particularly slow rate of replication.** Rather than doubling every 20 or 30 min like *E. coli*, *M. tuberculosis* divides every 15 to 20 hours. Some—but not all—nontuberculous mycobacteria replicate more quickly, but even the fastest of these take several days to develop colonies on agar plates. The slowest doubling time among the mycobacteria (and among all medically important bacteria) belongs to *M. leprae* at 11 to 13 days.

Mycobacteria have a unique structure to their cell envelope, which bears some superficial resemblance to that of gram-negatives. **They have a waxy, lipid-rich outer layer made of mycolic acids that acts as a barrier to many small molecules.** Like the gram-negative outer membrane, porins form channels across this barrier. Inside the mycolic acid layer is a thin layer of peptidoglycan, like in the cell walls of other bacteria, but also a distinctive layer of arabinogalactan. This arrangement results in a periplasm-like space between the mycolic acids and cytoplasmic membrane.

The distinctive outer layer of mycolic acids confers several important properties. It imparts resistance to gram-staining since the dyes are not taken up well. Acid-fast stains, however, bind to the mycolic acids. The waxy coating also provides resistance against desiccation that is important in airborne transmission of tuberculosis. Finally, mycolic acid biosynthesis is the target of one of the most important drugs for treating tuberculosis, INH.

Mycobacteria are facultative intracellular pathogens. In discussing pathogenesis and the immune response, we are going to focus on tuberculosis because that is what we understand the best. **Their preferred niche is replication within macrophages.** Intracellular bacteria have to deal with the problem of how to avoid being killed by the host cell. **Some bacteria escape from the phagocytic vesicle into the cytoplasm. *M. tuberculosis* doesn't take this strategy, but stays within the vesicle and prevents the vesicle from fusing with lysosomes**, effectively arresting its maturation at the stage of an early phagocytic vesicle. Because tuberculosis is spread by an airborne route, it usually first encounters an alveolar macrophage, and is then carried to a regional lymph node.

Key Points: Pathogenesis and Immunity
1) Intracellular pathogen that replicates in macrophages
2) Triggers granulomatous inflammation
3) Granulomas often develop central necrosis with active mycobacterial replication
4) Cell-mediated immunity is required to control infection
5) Latent infections are frequent even in immunocompetent individuals

At the level of histopathology, the immune response to mycobacterial infections is dominated by granulomas. These characteristic structures are inflammatory aggregates of macrophages and T-cells. May contain multinucleated giant cells. These have traditionally been thought to represent a balance between the host and pathogen, with the host "walling off" the infection. Not unique to mycobacterial infections. Caseous ("cheese-like") necrosis often develops at the centers of tuberculous granulomas. Other mycobacteria are less likely to cause caseous necrosis. Viable bacteria may be in the necrotic core.

A cell-mediated immune response—rather than a humoral response—is required to combat tuberculosis, as you would expect for an intracellular pathogen**.** A T_H1 type response is most effective. **Even in healthy individuals, however, eradication of the infection is often not complete.** We'll talk later about implications of this latent stage of infection. While *M. tuberculosis* replicates well in resting macrophages, it can be killed by activated macrophages. The importance of a strong cell-mediated immune response in combating tuberculosis is seen in patients with problems with their CD4+ T-cell responses such as HIV patients who suffer from a high rate of tuberculosis.

Cytokine signaling—particularly those cytokines important in a T_H1 immune response—plays a key role in fighting tuberculosis infections. $CD4^+$ T-cells that recognize *M. tuberculosis* and become activated produce interferon-gamma. This in turn activates macrophages to produce TNF-alpha and to kill bacteria within their phagosomes. Part of this is due to enhanced fusion of lysosomes with the vesicles. Clinical observations have confirmed the importance of both of these cytokines in human tuberculosis as well. We now have drugs that act to block TNF signaling (infliximab, etanercept, etc), which are used to treat inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. Patients treated with these medications, however, have a substantially increased risk of developing active tuberculosis. There are also patients described who have genetic defects in the receptor for INF-gamma and who have increased susceptibility to mycobacterial diseases.

Tuberculosis is one of the leading infectious causes of morbidity and mortality worldwide. About 2 billion people, or 1/3 of the world's population, are infected. Most of these infections are asymptomatic and fall into the category of LTBI. These people however have about a 10% lifetime risk of developing active tuberculosis. This translates to about 8 million new cases a year of active disease and 2 million deaths.

Tuberculosis is a disease that has been with humanity at least since the dawn of civilization. *M. tuberculosis* DNA has been amplified from the remains of a bison that died in North America about 17,500 years ago. Skeletal deformities characteristic of tuberculosis have been found in Egyptian mummies, and *M. tuberculosis* DNA has been isolated from about 1/3 of mummies studied from Egypt. In the New World, too, skeletons have been found with evidence of tuberculosis such as these vertebrae with lytic lesion from a Chilean skeleton from 1000 years ago. *M. tuberculosis* DNA was amplified from these bones.

In the more recent part of the pre-antibiotic era, tuberculosis accounted for ¼ of all deaths in Europe during the 17th and 18th centuries when it was known as "consumption". Victims included many prominent individuals. In 1849 a movement was begun to care for patients in dedicated facilities called sanatoria. Treatment in a sanatorium emphasized exposure to fresh air and sunlight removed from the large urban centers where tuberculosis was particularly common.

So did sanatorium treatment help individual patients in addition to slowing the spread of infection? Recent work has shown a mechanism by which the innate immune response against tuberculosis may have been enhanced by vitamin D and sunlight. A mycobacterial lipopeptide has been found to stimulate the heterodimeric TLR2/1 receptor, a pathogen-recognition receptor of the innate immune system. This receptor then increases expression of the vitamin D receptor on macrophages and increases expression of the enzyme that performs the final step in converting vitamin D to its active form ($1,25(OH)_2D_3$). Activation of the vitamin D receptor on macrophages triggers them to increase production of antimicrobial peptides and promotes killing of *M. tuberculosis*. This effect, however, requires adequate levels of the precursor $25(OH)D_3$, which is ultimately derived from either dietary sources or synthesis in the skin on exposure to UV light.

Sanatorium treatment may have helped check the spread of disease by isolating patients. This began, however, before tuberculosis was even known to be an infectious disease. **The modern era in the history of tuberculosis began in 1882 when Robert Koch identified *M. tuberculosis*** and demonstrated that it fulfilled his criteria for proving the etiology of a disease (which have become known as Koch's postulates). For this work he received the Nobel Prize in medicine in 1905.

Rates of tuberculosis declined during the first part of the 20th century, and then fell further after the introduction of the first effective antibiotics (streptomycin in 1946 and then over the 1950s and 1960s the drugs INH, rifampin, pyrazinamide and ethambutol that still are cornerstones of treatment today). **Rates of tuberculosis in the US continued to fall until the mid 1980s when the AIDS epidemic spurred a rise in the incidence again.** After the introduction of antiviral therapy for HIV the incidence of tuberculosis has again fallen.

Key Points: Epidemiology
1) About 1/3 of the world's population is latently infected with tuberculosis.
2) Tuberculosis in the U.S. increased with the AIDS epidemic.
3) Drug resistance is an increasing problem.
4) Resistance to isoniazid and rifampin—two key drugs for fighting TB—constitutes multi-drug resistance (MDR).
5) Risk factors include poverty, malnutrition, homelessness and institutional settings (including the medical system).
6) Transmission by inhalation of aerosol droplet nuclei which remain airborne for prolonged periods.

Despite progress over the past 15 years, large problems remain. 10 to 15 million people in the US have latent infection and are at risk for reactivation. With aging of the population and increasing use of immunosuppressive drugs the risk of reactivation increases. **Drug resistance (MDR and XDR TB) has become an increasing problem.** And global levels of infection and disease remain extremely high with 2 million deaths per year especially in areas hardest hit by the HIV epidemic. It's worth pulling up this WHO map again to emphasize how the distribution of tuberculosis closely resembles that of HIV.

The burden of tuberculosis is not evenly distributed in the US or world populations. Many of the risk factors depicted in this 1920 public health poster from France in the wake of Wolrd War I remain relevant today. Among these **risk factors are urban poverty, malnutrition, crowding, homelessness and alcoholism**. You are all in the process of joining a high-risk group as well with careers as health care workers. **Rates are also high in the prison system and among immigrants from areas with high levels of endemic tuberculosis transmission.**

Tuberculosis is spread by an airborne route. With coughing, tiny droplets are aerosolized from deep within the lung. **These droplets are small enough that their liquid evaporates before they settle, leaving what is known as droplet nuclei that can remain suspended in the air for hours.** In contrast, many other respiratory infections such as influenza are spread by larger droplets of respiratory secretions that settle rapidly. Because the waxy mycolic acid coat provides resistance to desiccation, *M. tuberculosis* can survive this process. In the healthcare setting, this mode of spread means that patients need to be isolated in rooms where the airflow doesn't mix with the rest of the ward. When working with patients**, hospital personnel need to use an N95 respirator mask—which provides a tight seal—rather than a simple surgical mask.**

Clinical disease with tuberculosis usually falls into distinct stages of primary infection, latency and reactivation. The organism is spread by deposition of small aerosolized particles deep into the lung to the level of the alveoli. There it infects resident alveolar macrophages and recruits new macrophages which are also infected. It is then carried to regional lymph nodes and can spread throughout the body through the bloodstream and lymphatics. **Often there is no clinical evidence of the primary infection, which is controlled with the development of a cell-mediated adaptive immune response. A positive tuberculin skin test to the PPD reagent may be the only evidence of infection.**

Key Points: Clinical Diseases (Natural History I)
1) Stages of primary infection, latency and reactivation
2) Patients with LTBI (latent infection) are asymptomatic and not contagious, but have risk of reactivation.
3) Reactivation especially with aging and weakened immunity.
4) Apices of the lungs are common sites for reactivation.
5) Necrosis causes cavities with high densities of mycobacteria.

So long as an effective immune response keeps the infection in check, the patient remains asymptomatic and is considered to have latent tuberculosis infection (LTBI). No evidence of active disease by symptoms or on chest x-ray. The x-ray may show calcifications at the initial site of pulmonary infection (known as a Ghon focus) or in the mediastinal lymph nodes (Ranke complex) or both, which by themselves don't indicate active disease. **These patients are not contagious.**

Latent infection however can reactivate, which happens especially with aging, at times of physical stress and when the immune system is weakened. During the first year after infection there is a 3-4% risk of reactivation (in young children, this risk of early reactivation is substantially higher). **Over a lifetime, the risk of reactivation is about 5-15%.** To highlight the importance of immune control, patients with AIDS have an 8% annual risk of reactivation, and nearly all with latent infection will progress to active disease eventually without antiretroviral therapy.

The most common site for reactivation is the lungs, and within the lungs tuberculosis has a predilection for the upper lobes. There it causes caseous necrosis and these **necrotic regions coalesce to form cavities.** Within cavities, *M. tuberculosis* replicates to concentrations 5-6 orders of magnitude higher than it achieves in the absence of cavitation. These cavities erode into bronchi and release their contents into the airway with spread of the infection to other areas of the lung. **Patients with cavitary tuberculosis are highly infectious.** Rupture into the airway can seed endobronchial tuberculosis as well, which is a focus of infection within a large airway. This can compress the airway and be mistaken for bronchogenic carcinoma. It isn't known with certainty why tuberculosis favors the apices of the lung. Theories include that hyperoxic conditions at the lung apex promotes growth of this obligate aerobe and that deficient lymphatic flow in the apices may increase the retention of bacillary antigens and trigger a more robust immune response to cause necrosis and cavitation.

Symptoms of pulmonary tuberculosis include respiratory tract symptoms such as cough, sputum production (often blood-tinged), and shortness of breath. In addition, systemic symptoms are often prominent including fever, chills, night-sweats, fatigue and weight loss.

Key Points: Clinical Diseases (Natural History II)
1) Pulmonary TB causes prominent systemic symptoms in addition to pulmonary findings.
2) Severe, progressive disease results if the initial immune response does not contain the infection.
3) Extrapulmonary manifestations of tuberculosis are less common but include meningitis, gastrointestinal infection, lymphadentitis, genitourinary disease and skeletal tuberculosis.
4) Tuberculin skin test (TST) measures cell-mediated delayed hypersensitivity reaction to tuberculosis.

Tuberculosis, however, does not always follow the pattern of primary infection, latency and reactivation. If the immune response does not fully contain the initial infection, patients can develop a **primary progressive tuberculous pneumonia** without a period of latency. A further extreme with the bacterium truly overwhelming the immune system is **miliary tuberculosis, which is progressive, disseminated hematogenous tuberculosis**. In the lungs miliary tuberculosis often causes a diffuse, millet seed-like infiltrate like in this chest x-ray of an infant who also has a lobar pneumonia in the right upper lobe. Miliary disease, however, is a systemic process not limited to the lungs (sometimes pulmonary changes are absent) and is often fatal.

Manifestations of tuberculosis are diverse and can affect most systems. It can spread directly along mucosal surfaces after rupture from pulmonary cavities. This can lead **to laryngeal tuberculosis** (which is also highly contagious) and **gastrointestinal disease**. Gastrointestinal disease used to also be frequently associated with ingestion of milk containing *M. bovis*, but pasteurization has greatly reduced this type of disease. Tuberculosis can also spread directly from the lungs to the pleural space. During the initial infection, there is often silent lymphohematogenous seeding of distant organs where disease can also reactivate. These extrapulmonary processes can include **meningitis, lymphadenitis (scrofula), renal tuberculosis and skeletal tuberculosis** (often of the spine, where it is called Pott's disease).

Testing for tuberculosis is imperfect even today. **The most widely used test is still the tuberculin skin test (TST)**, which tests for a delayed-type hypersensitivity reaction to a rather crude preparation of antigens from *M. tuberculosis*. Koch first developed the original tuberculin reagent for use in skin testing by making an extract of boiled *M. tuberculosis*. Today what is used is called purified protein derivative (PPD) and is simply a protein precipitate of tuberculin. The most common TST method is the Mantoux test that involves intracutaneous inoculation of 5 tuberculin units of PPD. This needs to generate a wheal. **48-72 hours later, the test is read by measuring the diameter of any region of induration that forms at the site.**

If a person has been infected, the TST result generally converts to positive between 3-9 weeks after exposure. TST reactivity may be maintained for many years, but can revert to negative with age and declining cellular immune function. **A positive test can reflect either latent or active infection.** In the absence of clinical symptoms or a positive chest x-ray, a reactive TST indicates LTBI.

The TST is far from perfect. False positives can arise from cross-reactivity with environmental mycobacterium (remember the PPD reagent is a broad mix of proteins from *M. tuberculosis*, some of which are shared with other mycobacteria). **Recent vaccination with BCG** (a live, attenuated vaccine to be discussed later) **also can give false positive results.** Because the test measures an immune reaction to tuberculous antigens, **defect in cell-mediated immunity can produce a falsely negative result**. This can occur in patients who are malnourished or have chronic diseases. Even patients sufficiently weakened by infection with tuberculosis itself can have a negative result. HIV also frequently causes a false negative TST. (It is estimated that up to 20% of patients with tuberculosis may have a negative TST). So your index of suspicion needs to remain high in the appropriate clinical setting even if the TST is negative.

Key Points: Clinical Diseases (Testing)
1) Tuberculin skin test (TST) measures a cell-mediated delayed hypersensitivity reaction to tuberculosis, but is subject to false positives and negatives.
2) IGRA testing is more specific but still may not be positive in patients with weakened immunity.
3) Growth in culture is slow. Acid-fast staining of a sputum smear may give preliminary information before culture.

A more specific test has recently been developed. **Interferon-gamma release assays (IGRAs) still measure the immune response as an indication of infection, but rather than using the broad protein mix of the PPD they use 2 small, immunogenic peptides that are secreted by *M. tuberculosis*.** T-cells in the patient's blood are stimulated ex vivo with these peptides and the production of the cytokine interferon-gamma is measured**. Because most environmental mycobacteria don't produce these peptides, there is less cross-reactivity with nontuberculous mycobacteria. The IGRAs are also not affected by BCG vaccination because the locus encoding these peptides is missing in BCG.** A weakened immune response, however, may still prevent a meaningful result.

Diagnosis can also be made by identification of the organism in the laboratory. Here testing is limited by the slow growth rate of the organism. Growth requires special media such as Löwenstein-Jensen medium. **It may take 2-3 weeks to grow colonies on a plate**, although modern laboratory systems may detect growth in liquid media more rapidly. Once an organism begins to grow, a DNA probe is often used to identify an isolate quickly. Acid-fast staining of can provide an initial indication of likely mycobacterial infection and may give an idea of the density of infection.

Acid-fast stains (Ziehl-Neelsen or Kinyon modified acid-fast stain) **work by binding of carbol fuchsin to mycolic acids coating the bacteria.** Even with active disease, bacteria may be rare on a smear. Need to look closely and carefully. To facilitate the identification of rare acid-fast bacilli on smears with lots of other background material, fluorochrome stains have been developed. These stains with either phenolic auramine or auramine-rhodamine bind to mycolic acids (like earlier acid-fast staining).

Molecular detection of tuberculosis is now also available using nucleic acid amplification. Among these, the Xpert MTB/RIF assay uses heminested real-time PCR of the bacterial *rpoB* gene to detect *M. tuberculosis*, and is also able to detect mutations associated with rifampin resistance. Sensitivity of this test has been reported to be as high as 98.2% in smear-positive cases and 72.5% in smear-negative cases.

There are 2 main principles to keep in mind in treating tuberculosis:
1) Prolonged courses of therapy are required.
2) Drug resistance evolves readily in patients who are on monotherapy (or who are already resistant to all but one of the drugs in their regimen) or who are non-adherent to their medications.

Key Points: **Treatment**, Prevention and Control
1) Treatment is prolonged (usually at least 6 months).
2) Multi-drug therapy is required to prevent development of resistance.
3) First-line drugs for TB:
    Isoniazid—inhibits mycolic acid synthesis
    Rifampin—inhibits RNA polymerase
    Pyrazinamide—inhibits recycling of stalled ribosomes (trans-translation)
    Ethambutol—inhibits arabinogalactan synthesis
4) Toxicities of therapy include hepatitis (INH, RIF, PZA), drug interactions (RIF), joint pain (PZA) and color vision impairment (EMB).
5) Sputum cultures may remain positive for several weeks on therapy to which the organism is sensitive. Follow-up sputum cultures over several months are needed to monitor response to treatment.
6) LTBI treatment consists of a single drug (INH) for 9 months to prevent reactivation.
7) Resistance to first-line drugs is increasing. Multi-drug resistance (MDR) is defined as resistance to both INH and RIF.

The first-line drugs for treatment of tuberculosis are an aging combination that were all introduced between 1952 and 1966**. These first-line drugs are isoniazid, rifampin, pyrazinamide and ethambutol.**

Isoniazid (INH) inhibits bacterial fatty acid synthases and blocks mycolic acid biosynthesis. This drug is highly active against replicating *M. tuberculosis*. Toxicities include hepatitis (which is a side-effect shared by several first-line drugs) and peripheral neuropathy.

Rifampin (RIF) inhibits bacterial RNA polymerase. Its most distinctive side effect is turning bodily fluids (urine, tears, etc.) orange. It also causes hepatitis and strongly induces hepatic P-450 cytochrome oxidases, resulting in interactions with the metabolism of many other drugs.

Pyrazinamide (PZA) has recently been shown to target the 30S ribosomal protein RpsA. It inhibits a process known as trans-translation by which stalled ribosomes are rescued. **PZA appears to be active against semi-dormant organisms.** The course of therapy may need to be extended from 6 to 9 months if PZA is not used. Toxicities include hepatitis and (in up to 40% of patients) a non-gouty polyarthralgia.

Ethambutol (EMB) inhibits synthesis of arabinogalactan for the cell wall. EMB has a distinctive toxicity in causing retrobulbar neuritis, which is often manifested by impairment in color vision.

Treatment should always use at least 3 different drugs to which the bacterium is sensitive. Because of the possibility of resistance, that means **most patients are initially started on at least 4 drugs until susceptibility information is available.** In general, treatment of chronic infections and of bacteria that replicate slowly tends to take much longer than treatment of acute, pyogenic infections with rapidly-dividing bacteria that are more metabolically active. Tuberculosis is no exception to this rule. The most common treatment regimen for pulmonary tuberculosis uses all 4 first-line drugs for 2 months followed by INH and RIF for an additional 4 months. Because non-adherence with medications greatly increases the risk of disease relapse and drug resistance, a key part of therapy has become Directly Observed Therapy (DOT) in which health professional monitors patients taking their doses of medication in the community.

Patients may continue to have positive sputum cultures for weeks on appropriate treatment, but by 2 months 80% should have turned negative. Patients with cultures still positive at 4 months are considered treatment failures. Three consecutive negative sputum AFB smears is used as a marker for patients no longer being contagious.

Latent tuberculosis infection (LTBI) carries a 5-15% risk of progression to active disease, and treatment at the stage of LTBI can prevent this. Treatment is therefore generally recommended for persons with LTBI. LTBI is the one exception to the rule requiring multiple-drug regimens for tuberculosis. **The preferred treatment is a 9-month course of INH monotherapy.** A 6-month course of rifampin may sometimes be used as an alternative. Risk of hepatotoxicity from INH increases over age 35.

Drug resistance has become an increasing problem. 8.6% of *M. tuberculosis* isolates in the US are resistant to INH even before a patient starts therapy. More concerning is **multiply drug-resistant (MDR) tuberculosis, which is defined as resistance to both INH and rifampin**. These drugs are among the most effective against tuberculosis, and resistance to both makes treatment more challenging. This rate is currently 1.2% nationally but can be regionally higher (and was over 20% in New York City during part of the 1990s). **Recently strains have also emerged that are extensively drug resistant (XDR).** To be classified as XDR, as strain must be resistant to INH and rifampin (MDR) as well as to a fluoroquinolone and one of the second-line injectable agents amikacin, kanamycin or capreomycin.

Many drugs are used as second-line agents for the treatment of tuberculosis, but these drugs tend to be less effective than first-line agents. Treatment of MDR tuberculosis with second-line drugs requires even more prolonged courses of therapy. A new drug, bedaquiline (Sirturo), which affects the proton pump for ATP synthase, was recently approved and may eventually become the first new first-line drug in 50 years.

There is a vaccine (BCG) against tuberculosis, but its efficacy is relatively poor. This is a live, attenuated bacterial strain known as Bacille Calmette-Guérin after its developers. It was derived from an isolate of *M. bovis* (in the *M. tuberculosis* complex and which itself can cause disease in humans) through extensive in vitro passaging from 1908 until 1921. It was grown in mixture of beef bile and potatoes and transferred to fresh broth every 3 weeks. After this process, the resulting BCG strain was substantially attenuated in virulence and tested as a vaccine. Samples of BCG were distributed around the world and served as the seed cultures for stocks of vaccines made and used in different countries. These stocks have been propagated independently and have diverged from one another. Now that we have the ability to analyze bacterial genomes, researchers have gone back and compared these modern BCG strains with *M. bovis* to determine what changes caused the decrease in virulence. A number of regions of the *M. bovis* chromosome were found to be deleted in the BCG strains, but only a single deletion was common to all the BCG strains. This deletion appears to have occurred during the original in vitro passage and attenuation. The deleted region (RD1) encodes a peptide secretion system as well as 2 small peptides that are secreted by both *M. bovis* and *M. tuberculosis*. This secretion system has been shown to be important for full virulence of *M. tuberculosis*. The 2 peptides (ESAT-6 and CFP-10) are the ones that we encountered earlier that are used to stimulate patient T-cells in the IGRAs. The deletion of these peptides in BCG is what makes these interferon-gamma release assays specific for *M. tuberculosis* (or *M. bovis*) infection in patients who have been vaccinated with BCG.

So why does tuberculosis remain a problem despite the availability of a vaccine? Unfortunately BCG provides very limited protection. It does decrease the risk of severe disease in young children and decreases the risk of tuberculous meningitis. But it does not prevent these things entirely and has relatively little impact on the development of typical pulmonary reactivation disease. Furthermore, the attenuation of virulence in BCG is incomplete and it can cause disease, especially in immunocompromised hosts. Children with SCID (severe combined immunodeficiency) can develop disseminated infection with BCG**. For these reasons and because its use makes tuberculin testing more difficult to interpret, the US has chosen not to use BCG. Most of the rest of the world, however, routinely vaccinates children with BCG.** The impact of vaccination on TST status wanes with time and is not supposed to be taken into account in interpreting TST results.

Key Points: Treatment, **Prevention and Control**
1) Bacille Calmette-Guérin (BCG) is a live-attenuated vaccine for tuberculosis.
2) BCG provides rather limited protection against TB and can give a false-positive TST.
3) BCG is not given in the US, but vaccination is routine in most other countries.
4) Public health measures such as DOT and contact tracing are essential for control of tuberculosis.

The nontuberculous mycobacteria are a broad group that includes all the mycobacteria except the *M. tuberculosis* complex and *M. leprae*. The NTM are commonly referred to as "atypical" mycobacteria. They are most commonly divided into slow-growers and rapid-growers, though these terms are clearly relative since even the rapid-growers may take up to a week to produce colonies on a plate. They are also classified into 4 Runyon groups based on pigment production, although these groupings are rarely used clinically. Photochromogens produce pigment when grown in the light, while scotochromogens produce pigment also when grown in the dark. We will talk only about some representative species listed in the table.

Nontuberculous mycobacteria are frequently found in the environment. They are in soil, water (even tap water), plant material, etc. A few environmental associations are noteworthy. *M. avium* complex is known for being found in hot tubs and may be found in milk even despite pasteurization. *M. marinum* grows at lower temperatures but not 37°C and is found in both fresh and salt water. It causes a version of tuberculosis in fish, but in humans tends to infect abrasions sustained while swimming or working with fish tanks. Infections tend to be on the skin and extremities where temperatures are lower.

Diseases caused by NTM can be diverse; we'll cover only some here. **Pulmonary disease can be caused by *M. avium* complex and *M. kansasii*** (and others). Symptoms typically consist of chronic cough, fatigue and weight loss, often without fever. These patients may have normal immune systems. **In immunocompromised hosts** (particularly HIV patients with CD4 counts less than 50) **MAC can cause a severe, disseminated disease** characterized by fever, weight loss and anemia, often with diarrhea and enlargement of the liver and spleen. Respiratory symptoms tend not to be prominent.

Other diseases caused by NTM include a hypersensitivity pneumonitis caused by chronic exposure to aerosolized MAC antigens ("hot tub lung"). This is not an invasive infection, but rather an immunologic reaction. In young children, MAC and *M. scrofulaceum* can cause **cervical lymphadenitis**. NTM also cause **cutaneous infections** such as this ulcer caused by *M. marinum* on the back of a hand, but rapid-growers can also cause this sort of ulceration. In the tropics and Australia, *M. ulcerans* is an important pathogen causing a chronic, disfiguring ulcer known as Buruli ulcer.

Diagnosis of infections with NTM generally requires culturing the organism in the proper clinical context. Since these are common environmental organisms, they may frequently be found as incidental non-pathogens in cultures obtained from non-sterile sites, so the clinical context is important in interpreting culture results.

Treatment of infections with NTM, as with tuberculosis, generally requires regimens of multiple drugs and prolonged courses of treatment. Prevention is usually not feasible since most encounters between humans and these organisms don't result in infection. One exception is that antibiotic prophylaxis is given to HIV patients who have CD4 counts less than 50 in order to prevent disseminated MAC disease.

Key Points: Nontuberculous Mycobacteria (NTM)
1) Encompasses a broad range of mycobacteria except *M. tuberculosis* complex and *M. leprae*.
2) Prevalent in environmental sources such as soil, vegetation and water.
3) Some grow faster than *M. tuberculosis*, but still slower than many other pathogens.
4) Diseases include pulmonary infections, disseminated infections (immunocompromised hosts), cervical lymphadenitis and cutaneous ulcers.
5) Treatment requires prolonged courses of several drugs.

The last major pathogen to discuss among the mycobacteria is *M. leprae*, which causes leprosy. This bacterium was discovered by Armauer Hansen in 1874, 8 years before Koch discovered *M. tuberculosis*. Leprosy is also known as Hansen's disease, and this term is sometimes preferred due to the stigma associated with the term leprosy. While Koch became renowned for his demonstration that bacteria grown in pure culture could cause tuberculosis, **Hansen—and all investigators to this day—have been unable to culture *M. leprae* in vitro**. The bacterium grows best at lower temperatures and infects armadillos in the wild. In the lab it can be cultured in the cool footpads of mice. In this model system its **doubling time is estimated at 11 to 13 days**, which is the slowest of all the medically-relevant bacteria.

Leprosy remains a significant problem worldwide with about 250,000 new cases per year, concentrated in the tropics. In the US, 100 to 200 cases are diagnosed per year, mostly among immigrants from endemic regions. The bacterium grows in areas of the body with lower temperature such as the skin and replicates well in the cool nasal mucosa. **Transmission is primarily by contact with nasal secretions or respiratory droplets.** It has been suggested that biting insects might also play a role in transmission.

With its preference for lower temperatures, the bacterium replicates primarily in the skin, nasal mucosa and distal extremities. It causes peripheral sensory nerve damage and infiltrative skin lesions. Disease from leprosy is divided into 2 broad categories based on the balance between bacterial replication and the immune response**. Multibacillary or lepromatous disease is characterized by lesions with many bacteria and few lymphocytes. These patients appear to mount primarily a T_H2 type immune response, which as with tuberculosis, is less effective in controlling mycobacterial disease.** These patients lack well-formed granulomas and develop more severe disease. They develop the characteristic facial appearance of leprosy with infiltration and thickening of the loose skin on the lips, ears and forehead as well as damage to the nasal bones and septum. **In contrast, paucibacillary or tuberculoid leprosy is characterized by lesions with few bacteria and many lymphocytes. These patients mount a more effective T_H1 type immune response and have well-formed granulomas and less severe disease.**

Leprosy is diagnosed and categorized by history, exam and biopsy of skin lesions. **Effective drug treatments are available and like with other mycobacterial diseases require multiple-drug regimens.** These involve combinations of rifampin, dapsone and clofazimine. Once again, monotherapy leads to resistance. Treatment lasts for years. The WHO in an effort to combat the disease now offers free medications to all patients in countries where leprosy is endemic.

Key Points: *Mycobacterium leprae* and Leprosy
1) Grows extremely slowly and cannot be cultured in vitro.
2) Transmission is by nasal secretions or respiratory droplets.
3) Causes either multibacillary (lepromatous) or paucibacillary (tuberculoid) leprosy depending on the efficacy of the host immune response.
4) Therapy with rifampin, dapsone and clofazimine is effective but takes years.