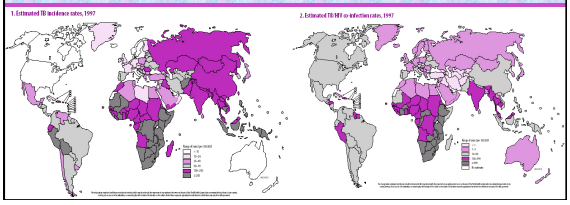
Life Within a Phagocyte:
Mycobacterium tuberculosis

Tuberculosis - The White Plague

- Tuberculosis: "White Plague" of 17th and 18th centuries. Nearly 100% of Europeans infected.
- 1882: Robert Koch identified *Mycobacterium tuberculosis* as the causative agent of tuberculosis.
- 1890: Tuberculin Skin Test developed. Used cell wall materials (called purified protein derivative or PPD) to detect immune response.
- TB caused by 4 species of Mycobacteria: *M. tuberculosis*, *M. bovis*, *M. microti* and *M. africanum*.

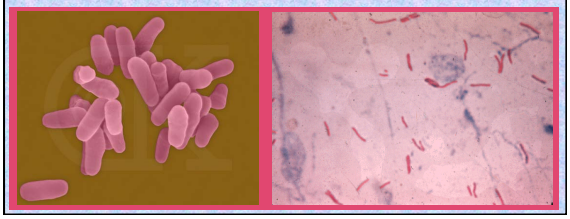
Incidence of Tuberculosis

- Nearly 1/3 of world population infected.
  - 1.7 billion people carry TB. 5 to 10% develop active TB.
  - Advanced TB: 50% fatality. 3 million deaths/year.
  - 1/3 AIDS patients infected. Fatality rate >80%.
- TB rates in US: decreased steadily until 1985, then started increasing. Reasons:
  - AIDS patients: extremely susceptible.
  - Development of drug resistant strains. Treatment long with many side effects. If not completed, resistance develops.



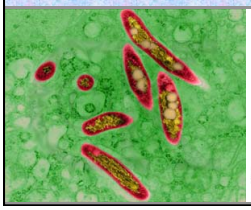
Mycobacterium tuberculosis

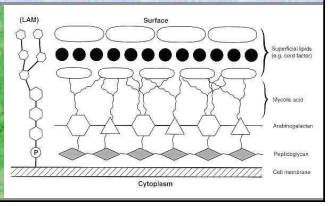
- *Mycobacterium tuberculosis:*
  1. Large, nonmotile, rod-shaped bacterium classified Gram+ (difficult to stain). Visualized with acid fast stain.
  2. Obligate aerobe. Disease always in well-aerated, upper lobes of lungs.
  3. Facultative intracellular parasite. Grows in macrophages.
  4. Very slow generation time, 15-20 hours *in vivo*, days *in vitro*.



Cell Wall Structure of *Mycobacterium tuberculosis*

- Mycobacteria: very high lipid content, >40% lipid.
- Lipid source: cell wall. 3 lipids, mycolic acid, cord factor, and wax D.
- Properties (due to lipids):
  1. Difficult to stain.
  2. Resistance to antibiotics.
  3. Resistance to killing by lysosomal components.
  4. Resistance to toxic oxygen radicals (able to live in macrophage).
  5. Resistant to lysis by complement.





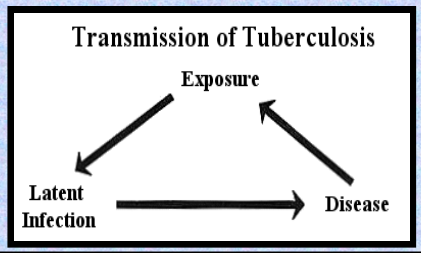
Virulence Factors of *M. tuberculosis*

- *M. tuberculosis:* no toxins or enzymes that damage host cells. Virulence due to cell wall and ability to prevent killing after phagocytosis.

1. Binds to complement and Fc receptors on macrophage. Induce receptor-mediated endocytosis. No phagosome or phagolysosome formed.
2. Intracellular growth: effective for evading antibodies and complement.
3. If phagocytized: inhibits phagosome-lysosome fusion.
4. Prevents acidification of phagolysosome: inhibits killing.
5. Down-regulates production of oxygen radicals: inhibits killing.
6. Slow growth: immune system may not recognize or may not be sufficient to eliminate. Bacteria have prolonged period to grow before defense systems act.
7. High lipid concentration: limits permeability, provides resistance to antimicrobial agents, to acidic and alkaline compounds (in lysosomes or extracellular environment), and to lysis by complement or lysozyme.
8. Cord factor (lipid): somewhat toxic to cells and inhibits PMN migration.
9. Antigen 85 complex: Secreted proteins that bind fibronectin. Walls off bacteria from immune system (wall of fibroblasts form around infected cells).

Tuberculosis - Extremely Contagious

- Infectious dose: 1 to 10 bacteria. Shed in aerosol droplets.
- Each person with active tuberculosis: Infects 10 to 200 more individuals.
  - Example: School bus driver. Active disease for several months (prior to diagnosis). 32% of children (238 total) infected. 57% on bus for longest times got TB.
- Survives long periods in environment.

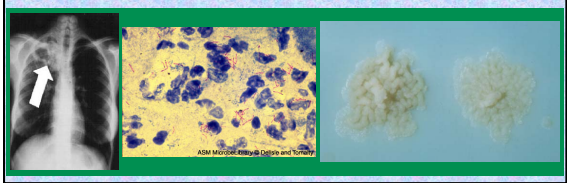


Signs and Symptoms of TB

- Symptoms:
  - Prolonged cough.
  - Weight loss.
  - Fever.
  - Night sweats.
  - Weak or tired.
  - Chest pain.
  - Coughing up blood.

Diagnosis of TB Disease

- Chest X-ray: calcified lesions.
- Acid fast stain of sputum. Single positive organism considered suspicious for active TB.
- Culture in lab: Requires 6 weeks.
- Measuring niacin production and PCR also used for ID.

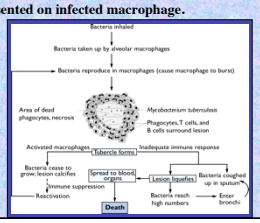


TB Infection Versus TB Disease

- **TB infection (without disease): *M. tuberculosis* present but inactive. Increased chance of developing active TB. Treatment prescribed (in US).**
- **Characteristics of infection:**
  - **Positive tuberculin test.**
  - **Normal chest X-ray.**
  - **Negative sputum and culture results.**
  - **No symptoms.**
  - **Not infectious.**
- **TB disease: *M. tuberculosis* active. May occur in short time period or many years later. Causes permanent damage/death. Treatment required.**
- **Characteristics of disease:**
  - **Positive tuberculin test.**
  - **Chest X-ray shows lung lesions.**
  - **Positive sputum and cultures results.**
  - **Symptoms.**
  - **Infectious.**

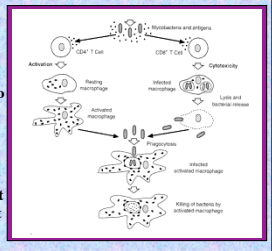
Progression of TB

- **Slow growth of *M. tuberculosis* makes development of active TB slow process. Weeks to months before diagnosed.**
- **With 90-95% infected, immune system stops progression.**
- **Stages:**
  - **Stage 1 - Infection: Aerosol droplets inhaled. Bacteria enter alveolar macrophage (phagocytosis or receptor-mediated endocytosis).**
  - **Stage 2 - Initial multiplication: Bacteria multiply until macrophage bursts. Other macrophages take up *M. tuberculosis* but cannot kill. Bacterial growth continues.**
  - **Stage 3 - Lymphocyte Infiltration: Lymphocytes (T-cells most important) recognize TB antigens presented on infected macrophage.**



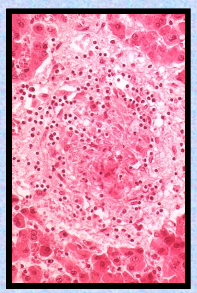
T Cell Activation

- **Activated T-cells produce cytokines including gamma interferon (IFN).**
- **IFN activates macrophages. Macrophage can now destroy *M. tuberculosis*.**
- **Cytotoxic T cells formed. Recognize infected macrophage. Apoptosis induced. High lipid content protects bacteria. Bacteria released.**
- **Patient develops positive skin test due to T-cell mediated immune (CMI) response.**
- **CMI essential for halting disease.**
- **Antibody ineffective. Organism primarily intracellular, antibody cannot reach. When extracellular, lipid content makes resistant to antibody-mediated complement activation.**



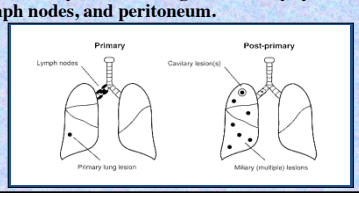
Pathology Due to Cell-mediated Immunity in TB

- CMI necessary to control *M. tuberculosis* infection, but also responsible for pathology in tuberculosis.
  - Activated macrophages release lytic enzymes and oxygen radicals. Causes tissue damage.
  - Activated macrophages and T-cells secrete cytokines including IL-l, TNF, and gamma IFN. Induce fever and weight loss.
  - Inflammation and immune response cause formation of granuloma (in TB, called tubercle).
    - Granulomas: chronic inflammatory lesions characterized by large numbers of cells (macrophages, lymphocytes, fibroblasts, giant cells). Some degrade and some repair tissues.
  - Tubercle surrounded by fibroblasts. Center progresses to "caseation necrosis" (semi-solid or "cheesy" consistency).
  - *M. tuberculosis* cannot multiply because of low pH and anoxic environment. Can persist within tubercle (becomes latent).



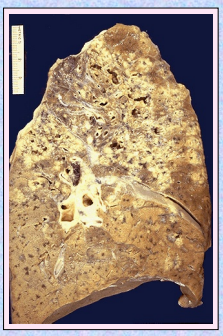
Progression of TB

- Stage 4 - Tubercle Growth and Dissemination of *M. tuberculosis*: Activated macrophages surround tubercles, limit spread of disease. Other macrophages not activated. *M. tuberculosis* infects and tubercle grows.
- If airway penetrated, organisms can leave tubercle and spread in lung.
- If blood stream penetrated, *M. tuberculosis* spreads systemically resulting in milliary tuberculosis (extrapulmonary tuberculosis). Milliary TB usually involves the genitourinary system, bones, joints, lymph nodes, and peritoneum.



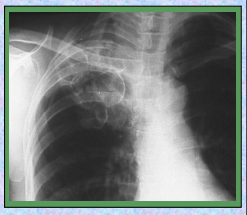
Progression of TB

- Stage 5 - Liquefaction: Centers of tubercles liquefy, environment allows *M. tuberculosis* growth. Organism rapidly multiplies extracellularly.
- Walls of bronchi become necrotic and rupture. Cavities form in lung tissue. Allows spread to other parts of lung.



Progression of TB

- 5-10% infected with *M. tuberculosis* progress to disease. Smaller percentage develop advanced TB.
- Host's CMI controls infection at some point.
- As primary lesion heals, becomes fibrous and calcifies. Lesion may never subside and is visible with chest X-ray.
- Small lesions formed from systemic spread contain a few *M. tuberculosis*.
- Lesions can calcify but continue to contain viable organisms (protected from immune response). Sites for reactivation of TB if immune response repressed.



Treatment of Tuberculosis

- 3 to 4 antibiotics (isoniazid, ethambutol, rifampin, and pyrazinamide) daily for 6 to 9 months.
- Antibiotics target different stages of infection. Most are tuberculosis specific.
- Side effect: nausea.
- Multi-drug resistance: Skipping medications (side effects) or not completing treatment causes development of drug resistantance.
  - Extremely virulent bacterial strains developing: W strain, 367 cases in New York City between 1990 and 1993. 83% fatal and death occurred in 1 to 4 months (usually tuberculosis is slow disease with fatal outcomes taking years, not months).

Vaccination

- A strain of *M bovis*, called Bacillus de Calmette et Guerin (BCG, named after French microbiologists) has been used in ~120 countries as vaccine to prevent clinical tuberculosis.
- Vaccination is only way to control TB in developing countries.
- Effectiveness varies from 0 to 85 percent. Unknown local environmental or host factors affect success of vaccination.
- BCG not used in US because it causes skin test to become positive.