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Mycobacterium tuberculosis

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I. Introduction

A. History
    1. Evidence for spinal TB in Egyptian mummies and pre-Columbian remains
    2. Not a significant problem until the 17th and 18th centuries as urbanization and crowding in unventilated living conditions increased
    3. By the 19th century with industrialization, TB caused one quarter adult deaths in Europe
    4. Germ theory of diseases and discovery of TB bacillus by Koch

B. Pre-antibiotic era
    1. Sanatorium regimens and rest
    2. Recognition of importance of cavitary disease- collapse therapy
    3. Fresh air and sunshine-rooftops and solaria

C. Antibiotics
    1. 1946 –Streptomycin
    2. Rapid development of failure with monotherapy-PAS
    3. INH- the magic bullet-1952
    4. Rifampin and short course treatment era-1970

D. Rising incidence in world:
Failure of public health=failure of political will since Rx to cure costs $12 per person; all drugs off patent

II. Epidemiology

A. World wide: WHO Maps: Estimated incidence vs. case notifications
1. *M. tuberculosis* infects one third world's population
Causes 8 million new cases active disease annually
2. Causes 2 million deaths= 2nd only to HIV as cause of death from infectious agent world wide among adults
3. HIV/TB relationship has exacerbated problem with TB increasing in areas with high AIDS incidence- Especially sub-Saharan Africa

4. Absolute numbers of cases of TB highest in Asia as population density highest there but case rates highest in sub-Saharan Africa: 300 per 100,000 estimated incidence rates in sub-Saharan Africa vs. 100-299 per 100,000 in Asia

B. Developed World- Europe

1. Downward trend in incidence even before advent of antibiotics
2. 10% infected people develop active disease and mainly cavitary cases infectious; only 50% cases are cavitary
3. Each cavitary case needs to infect 20 to maintain constant rate of cases
4. Data from Pre-WW2 Holland shows 1 infectious case produced 13 new infections
5. Annual decrease in mortality and morbidity of 4%-6% in developed countries between 1900 and WW2:
   a. Progressively higher natural residual resistance in those who had survived infection
   b. Better living conditions less conducive to airborne spread.
6. Advent of antibiotics late 1940s (Streptomycin) and INH in 1952: Tuberculosis is curable

C. United States

1. Decline steady until 1984 when slowly increasing incidence
2. Causes- Neglect of TB control programs; Increase in urban homelessness and resultant crowding into homeless shelters; Advent of AIDS epidemic among this population
3. Currently, restored TB control program funding and decreasing crowding of homeless leaves background rates high among immigrants from high prevalence countries
4. One half cases in US are now among foreign born; Dramatic change between 1993 and 2003; New York, New England, west coast states all have greater than 50% cases foreign born in 2003; 300 per 100,000 estimated incidence rates (maps)

III. Microbiology

A. *M.tuberculosis* complex includes several species, all probably derived from a soil bacterium:

1. *Mycobacterium tuberculosis*
2. *Mycobacterium bovis*- unpasteurized milk; recent rash of cases in US among immigrants who have favorite cheeses made from unpasteurized milk sent them from home, especially Mexico and Dominican Republic
3. *Mycobacterium bovis*-BCG= used to treat bladder cancer
4. *Mycobacterium africanum* and *Mycobacterium canetti*= rare causes of tuberculosis in Africa
5. *Mycobacterium microti*= pathogen for rodents

B. Organism characteristics

1. Aerobic, non-motile, non-spore forming bacillus
2. High cell wall content of high molecular weight lipids- mycolic acid
3. SLOW GROWTH RATE
   a. generation time of 20 hours vs *E.coli* generation time of 20 minutes

b. 3-8 weeks before growth on solid media;
c. implications for length of treatment for complete sterilization compared with most bacterial pathogens

IV. Pathogenesis

A. Transmission: Lungs are the portal of entry except *M. bovis* in unpasteurized dairy products from other countries

1. Inhalation of droplet nuclei (bacillus 5 microns): from infectious person with active pulmonary tuberculosis, NOT just positive PPD
   a. cough: most efficient at 3000 infectious droplet nuclei per cough
   b. talking: similar quantity over 5 minutes
   c. sneezing more efficient than coughing; singing intermediate between talking and coughing.
   d. Inoculum size relevant: Autopsy suite transmissions; cutting through lung tissue aerosolized millions of bacilli; PPD conversion and progression to active tuberculosis astonishingly high
   e. Virulence of strain: Kentucky outbreak after minimal contact with index patient
   f. Bacillus remains alive and infectious in air for long period; ventilation key in preventing transmission; isolation of patient and mandated number of air exchanges in hospital rooms
2. Primary infection: BEFORE IMMUNE RESPONSE:
   a. Bacillus reaches alveoli
   b. replicates extracellularly in alveolar space and intracellularly in alveolar macrophage
   c. lack of immediate host immune response:
      alveolar macrophage ingests TB bacillus; bacillus sits in phagosome; phagosome normally incorporates proton-ATPase into membrane leading to decreased pH and acidification within phagosome; acidified phagosome then normally fuses with cell lysosome, exposing organism to lysosome's toxic enzymes

   BUT MTB prevents insertion of proton-ATPase into phagosome so phagosome never gets acidified and never merges with lysosome
   d. MTB multiplies for weeks, both in initial focus in alveolar macrophages and in cells transported lymphohematogenously throughout body:
   e. metastatic foci well established in regional nodes (hilar, mediastinal) and then to tissues which retain bacilli and favor multiplication:
      apical posterior areas of lungs
      lymph nodes in neck
      kidneys
      epiphyses of long bones
      vertebral bodies
      juxtaependymal meningeal areas adjacent to subarachnoid space

*****These will be areas of reactivation disease in future as organisms seeded remain alive but dormant once immune response occurs

*****Reactivation can occur in any one of these areas of the body with or without reactivation in others i.e. TB meningitis or "scrofula" with no pulmonary TB

B. Development of immune response: **MUST HAVE INTACT CELLULAR IMMUNE SYSTEM, INCLUDING CD4 CELLS**

1. 6-12 weeks after initial infection
2. alveolar macrophage infected with *M. tuberculosis* releases interleukins 12 and 18
3. These attract and stimulate T lymphocytes (mainly CD4): all people have native population of CD4 cells which can recognize mycobacterial antigens which have been processed and presented by macrophages
4. CD4 cell meets mycobacterial antigen presented by macrophage and becomes activated= transformed
   -- Transformed CD4 cell proliferates and produces clone of similarly reactive T lymphocytes
5. When population of activated lymphocytes is large enough, get cutaneous delayed reaction to tuberculin=tissue hypersensitivity: Positive PPD (Implications in AIDS patients with low CD4 cells: Cannot perform this process so no positive PPD response to tuberculin)
6. Meanwhile, CD4 cells release interferon gamma
   -- Interferon gamma stimulates additional macrophage phagocytosis AND interferon gamma stimulates macrophage to secrete regulatory factors including tumor necrosis factor alpha (TNF alpha).
7. TNF alpha increases macrophages ability to kill *M. tuberculosis* and is required for granuloma formation

Lack of TNF alpha results in inability to control initial TB infection as well as reactivation of latent organisms:

a.Murine experiments:

-Blockade of TNF alpha resulted in reactivation, high bacillary burden, persistent tuberculosis and death

-TNF alpha knock-out mice infected with *M. tuberculosis* followed similar course

b. Anti-TNF alpha agents for rheumatoid arthritis and auto-immune disorders cause reactivation of tuberculosis

C. Pathology: Tissue response depends on activation of macrophages with secretion of lytic enzymes which cause tissue necrosis

-Epithelioid cells= highly stimulated macrophages

-Langhans giant cell=fused macrophages oriented around tuberculosis antigen with multiple nuclei lined up peripherally

1. Small antigen load and high tissue hypersensitivity produce organization of lymphocytes, macrophages, Langhans giant cells, fibroblasts and result in granuloma

   a. Granuloma=successful tissue reaction resulting in containment of infection, healing with fibrosis, encapsulation and scar formation

2. Large antigen load and high tissue hypersensitivity produce few or no epithelioid clls or Langhans cells, disorganized lymphocytes, macrophages and polys and result in necrosis and caseation
a.-caseous material is acellular and inhibits multiplication of organisms due to its pH and oxygen tension but is inherently unstable, liquefies and discharges through the bronchial tree
b.this discharge produces a cavity in which TB bacillus multiplies to make population 5-6 logs greater than in noncavitary lesions
3. Large or small antigen load with no tissue hypersensitivity produces few cells (polys and mononuclear cells) and huge numbers of bacilli=seen in AIDS patients with low CD4 counts
-implications for post treatment appearance of lung and chest x-ray in AIDS patients- lack of fibrosis or granuloma

V. Clinical Syndromes

A. Primary Infection with Resolution: 85% of cases
1. Patient asymptomatic or mild viral syndrome
2. Enlargement of hilar and peribronchial nodes if chest x-ray taken at time
3. Calcified granuloma on Chest x-ray=evidence that tuberculosis infection successfully contained
4. Development of positive PPD 6-12 weeks later

B. Primary Infection with progression
1. Progressive Primary Disease
a. very young children (below age 5): unable to resolve infection; local progression with mid or lower lung field pneumonitis initially, then dissemination, miliary pattern in lungs and frequent CNS involvement
-almost always in developing world countries where tuberculosis remains endemic
b. tuberculous pleurisy: hypersensitivity reaction to small number of organisms which reach pleura in primary infection; exudative pleural effusion, culture negative as very few organisms present; 90% resolve spontaneously but WW II studies of soldiers showed 65% relapse to active TB (pre-antibiotic era); TB pleurisy should be treated
2. Primary infection in adolescence and young adulthood results in “adult type” upper lobe cavitary disease
a. epidemiologic data shows puberty influences tendency to apical cavitation soon after initial infection;
b. Data shows 23% of those infected between 15-19, 13% of those infected between ages 20-24, 4% of those infected from 25-29 develop cavitary disease
c. Only 2% of those infected after 30 do so.
3. AIDS nosocomial outbreaks of tuberculosis: AIDS wards, homeless shelters and prisons
a. undiagnosed patient with active pulmonary tuberculosis hospitalized in AIDS ward or shelter for AIDS patients; all patients have CD4 <50
b. index patient coughs and infects other AIDS patients

c. AIDS patients with no cellular immune function cannot mobilize CD4 and macrophages to contain or kill bacillus

d. rapid dissemination and death if untreated; blood cultures positive for *M.tuberculosis*

e. MDR (multi-drug resistance) outbreaks killed many as resistance not monitored

C. Reactivation: 10-15% of those infected

1. Persistence of viable organisms following containment of initial infection
2. Disease occurs years after infection when cellular immune response no longer able to contain MTB:

a. Iatrogenic (transplant patients, immunosuppressive Rx for connective tissue disorders)

b. Immunocompromising diseases (AIDS, malignancies, end stage renal disease, cirrhosis)

c. Malnutrition

d. Old age: Hypersensitivity and cellular immunity wane with age

e. Unknown- possibly hormonal, stress (immigrants)

3. Pulmonary location most frequent site of reactivation: 85%

a. Posterior aspect of upper lobe is focus where reactivation begins
-location attributed to increased oxygen in apices and MTB's aerophilia
-other possibility= deficient lymphatic flow at apices resulting in retention of bacillary antigen; with hypersensitivity get necrosis

b. localized pneumonitis, inflammatory response produces fibrin rich exudates into alveoli, caseating necrosis, liquefaction

c. drainage into bronchial tree with cavity formation

d. cavity favors bacillary multiplication to huge amounts: 5-6 logs greater than # of organisms in non-cavitary lesions: $10^9 – 10^{10}$ organisms/gram tissue

e. cavitary disease is most contagious as cough aerosolizes hundreds of thousands of organisms

f. implications for development of drug resistance

4. Viable organisms remain alive, dormant for years in all sites to which disseminated during primary infection: extrapulmonary tuberculosis

a. lymph nodes: scrofula; most frequent form of extra pulmonary TB
-usually cervical or supraclavicular chain
-biopsy and culture essential (fine needle aspirate usually smear and culture neg.)

b. meninges: rupture of subependymal tubercle into subarachnoid space (distinct from meningitis in young children following dissemination as discussed above)
-meningitis most severe at base of brain causing thick gelatinous exudate; affects cranial nerves as they exit
-CSF exam essential to make diagnosis: low glucose; elevated protein; lymphocytic pleocytosis

c. bones: one third involve spine=Pott's disease; hematogenous spread, contiguous disease, lymphatic spread from pleural disease
-early focus is anterior part of vertebral body; spreads to disk and then to adjacent vertebra; X-ray shows anterior wedging of 2 adjacent vertebral bodies and

destruction of disk; tender spine prominence on exam=gibbus

VI Diagnosis

A**.** Symptoms

1. Systemic symptoms non-specific: fever, fatigue, night sweats, weight loss
2. Pulmonary symptoms: cough, productive or dry-most patients have cough but may be ignored by patient for weeks
3. Hemoptysis
   a. mild-moderate, chronic blood streaking: results from caseous sloughing or endobronchial erosion; seen in advanced disease
   b. sudden massive hemoptysis= erosion of pulmonary artery=only TB emergency (Rasmussen's aneurysm)

B. Diagnostic procedures: **SPUTUM**: staining, cultures and molecular diagnostics

1. Acid fast stain: Acid fast implies mycobacterial species although nocardia is weakly acid fast; many other species besides *M. tuberculosis* complex will all be AFB positive (*Mycobacterium avium, kansasii, abscessus, chelonae*)
   a. Ziehl-Neelsen stain=fixed smear covered with carbol-fuchsin, heated, rinsed, decolorized with acid alcohol; Kinyoun stain is similar but heating unnecessary
   b. Fluorochrome stain with phenol-auramine or auramine-rhodamine; modified acid alcohol step and potassium permanganate counterstaining; fluorescent mycobacteria visible with 20 or 40X magnification

SMEAR POSITIVITY MEANS AT LEAST 10,000 ORGANISMS/mL SPUTUM

2. Culture: Gold Standard. Now available in most of world via WHO reference labs
   a. Solid media= Lowenstein Jensen (egg based) or Middlebrook 7H11 (agar based): can detect colony morphology, mixed infections; can detect 10-100 organisms/mL; 3-8 weeks incubation to detect organisms
   b. Liquid broth= Middlebrook 7H12, BACTEC systems; 1-3 weeks of incubation to detect organisms

CULTURE NECESSARY TO DETERMINE DRUG SUSCEPTIBILITIES

3. Nucleic acid amplification- can detect *M. tuberculosis* complex in fresh sputum: developed world technology: too costly for resource poor countries
   a. sensitivity intermediate between acid fast smear and culture
   b. AFB smear is negative, nucleic acid amplification is 40-77% sensitive
   c. AFB smear is positive, nucleic acid amplification is 95% sensitive and nearly 100% specific
4. DNA fingerprinting: Molecular epidemiologic tool: RFLP (Restriction fragment length polymorphism); also developed world technology
   a. Restriction endonuclease produces DNA fragments; separate fragments by electrophoresis; probe to repetitive DNA sequence=Insertion sequence (IS)6110 numerous copies of IS6110 present in *M.tuberculosis* chromosome at highly variable locations
   b. Utility in identifying transmission from person to person; distinguishing endogenous reactivation from exogenous reinfection in recurrent TB; laboratory cross-contamination

C. Chest X-ray: Luxury of developed world technology

1. Upper lobe infiltrate with or without cavity: apical or sub-apical
   a. most common in reactivation disease in intact immune system

b. radiologic extent of disease reflects tissue damage
c. tissue damage reflects host's ability to have hypersensitivity reaction

2. Hilar adenopathy with or without infiltrates
a. most common in AIDS patients
b. reflects minimal cellular immune response

3. Pleural effusion; always exudative
a. seen in post-primary infection as above
-scant organisms=hypersensitivity tissue reaction;
-smear never positive; culture may be positive 25%
b. seen as complication of reactivation pulmonary tuberculosis
-more likely to have organisms
-smear positive 50%; culture positive 60%

4. Miliary- from description of pathologic lesions as "millet seeds"; chest x-ray shows 0.5-1.0 mm nodules
a. following childhood infection and progression as discussed above
b. immunocompromised: alcoholism, cirrhosis, rheumatologic diseases, treatment with immunosuppressive agents;
-diagnosis difficult; may have multiple organ involvement with millet seeds granulomas in tissues
-transbronchial bx=highest yield for diagnosis

5. Atypical infiltrates

VII Treatment

A. General Principles

1. Always use at least 2 drugs; usually begin with 3 or 4 pending sensitivities
a. natural incidence of spontaneous drug resistance: 1 in 10^5 organisms resistant to each drug;
b. bacilli resistant to 1 drug will be killed by other drug
c. natural resistance to 2 drugs spontaneously 1 in 10^{10} or 1 in 10^{11}

2. Prolonged length necessary: 6-9 months if organism pan-sensitive

3. Directly Observed Therapy for all patients
a. No one is 100% compliant regardless of age, sex, race, education
b. Daily treatment for first 2 months; intermittent with adjusted doses for continuation phase of 4-7 months depending on regimen

B. Drugs: **ALL GIVEN ONCE DAILY TOGETHER: NEVER DIVIDE DOSES**

1. Isoniazid=INH; bactericidal against dividing organisms
-hepatitis: Chemical (20%) vs clinical (age related:<35=0.3%; >65=4%)

2. Rifampin=RMP=bactericidal; Enables short course treatment (6-9 months vs 18-24 months with non-RMP regimens
-drug-drug interactions:RMP is potent inducer of hepatic microsomal enzymes: cytochrome p450

3. Pyrazinamide=PZA; Enables shortening of regimen from 9 months to 6 months

4. Ethambutol=EMB: Used in drug resistance and situations where INH or RMP cannot be used (INH hepatotoxicity; RMP drug-drug interactions)

VIII Prophylaxis: Latent tuberculosis infection (LTBI)

A. Targeted testing: PPD is NOT a general screen; Only use PPD for patients at high risk of developing active tuberculosis
   1. Immunocompromised: HIV infected, chemotherapy patients, patients undergoing organ transplant, patients on immunosuppressive Rx for autoimmune diseases, rheumatoid arthritis
   2. Close contacts of infectious case (household or close working quarters)
   3. Previously untreated patients with Chest X-ray evidence of old fibrotic changes -not just calcified granuloma
   4. Recent immigrants (in US < 5 years) from endemic areas (see map at beginning)
   5. People who work in institutions where TB exposure likely: hospitals, nursing homes, homeless shelters, prisons

B. Positive PPD: Purified Protein Derivative= protein extract derived from=skin test *M.tuberculosis*; contains multiple antigens
   1. Definition of positive PPD
      a. 5 mm: HIV infected, close contacts of infectious case, chest x-ray evidence of old disease (fibrotic scarring, not just calcified granuloma)
      b. 10mm: patients from endemic areas of tuberculosis
   2. Booster phenomenon: 2 step testing essential for all those >55 whose exposure/infection in distant past and for those with BCG

C. Treatment: Only for those at high risk of reactivation (see above): INH for 9 months

D. New tests: Need to know if reactivity represents tuberculosis infection, not BCG: Enzyme-linked immunospot (ELISPOT): T-cell based assay from blood;
   - M.*tuberculosis* genes not present in *M.bovis* BCG produce antigen to which T-cell reacts
   - 1 tube of blood needed: not feasible in resource poor settings
   - Useful in outbreaks for contact investigations- UK school outbreak showed greater sensitivity and specificity than PPD

IX Prevention: BCG

Most widely used and most controversial vaccine in the world

A. What is it?
   1. *M. bovis* strain attenuated through serial passage
   2. No standardized strain or procedure to make one

B. Does it work?
   1. Largest study: India= no protection from TB infection
   2. Other studies: England= protection from TB infection
   3. Prevalence of non tuberculous mycobacteria in given region may interfere
   4. Background prevalence of tuberculosis determines utility

C. Who uses it?
   -All agree that it is highly effective for infants and small children in preventing dissemination and meningitis when infected by *M. tuberculosis*
   -Newborns vaccinated in all high prevalence areas of world shown on first map