Mycobacteriology
William H. Benjamin, Jr.

Mycobacteria sp.

- Acid Fast Bacilli (AFB)
- Mycolic acids (C78 - 91)
- Waxes
- Obligate aerobes
- Slow growing
  - days to weeks to form colonies
  - 18 hour doubling time for *M. tuberculosis*
Identification of Acid Fast Bacilli

- *Mycobacterium sp.* - are identified by the acid fast stain
- Mycobacteria predate animal life
- 100 named Mycobacterial species
  - More than 40 have infected humans
    - AIDS
    - other immunocompromised

The Bacterial Cell Wall

Mycobacteria

<table>
<thead>
<tr>
<th>Gram-positive organism</th>
<th>Gram-negative organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipid bilayer</td>
<td>peptidoglycan</td>
</tr>
<tr>
<td>peptidoglycan</td>
<td>lipid + LPS</td>
</tr>
<tr>
<td>lipid + LPS</td>
<td>orins</td>
</tr>
<tr>
<td>MYCOLATE</td>
<td>acyl lipids</td>
</tr>
<tr>
<td>acyl lipids</td>
<td>LAM</td>
</tr>
<tr>
<td>abinogalactan</td>
<td></td>
</tr>
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</table>
Obligate Pathogenic Mycobacteria

• *Mycobacterium tuberculosis*
  – First bacteria shown to cause disease
  – 1882 Koch’s postulates

• *M. leprae*
  – causes Hansen’s disease or leprosy

*Mycobacterium tuberculosis*

• Humans are the only natural host

• 1/3 of the world population is infected

• 9.2 million cases of tuberculosis/year
  – disease

• 1.7 million deaths caused by tuberculosis/year
Prehistory of Tuberculosis

- 17,000 BPE (before present era) bison in Wyoming USA
  - IS6110 and Spoligotype confirmed *M. tuberculosis*
- 10,000 BPE in Germany skeletal evidence
- 3500 - 3000 BPE Egypt Potts disease
  - PCR positive for Mtb sequence
- 1300 BPE 8 year old Inca boy
  - Pott’s disease, AFB smear positive, IS6110 PCR positive

Fig. 1.3. Mean annual morbidity from pulmonary tuberculosis in England and Wales. Adapted from Kass (3), reprinted by permission of the *Journal of Infectious Diseases.*
Tuberculosis in the United States

U. S. Tuberculosis Cases

[Chart showing tuberculosis cases and deaths from 1980 to 2006 with data points for cases and deaths in different years.]
Trends in TB Cases in Foreign-born Persons, United States, 1986–2007*

* Unpublished provisional data, not for citation
Examples of high prevalence countries

<table>
<thead>
<tr>
<th></th>
<th>Namibia</th>
<th>Zambia</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population (million)</td>
<td>1.8</td>
<td>11.5</td>
<td>300</td>
</tr>
<tr>
<td>Tuberculosis cases</td>
<td>16,156</td>
<td>78,000</td>
<td>13,676</td>
</tr>
<tr>
<td>Tuberculosis rate/100,000</td>
<td>822</td>
<td>680</td>
<td>4.2</td>
</tr>
<tr>
<td>Tuberculosis death rate</td>
<td>61</td>
<td>?</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Transmission of Tuberculosis

- Transmission is by infectious droplet
  - droplet diameter 1 - 5 µm
  - droplet contains 1 - 3 bacilli
  - droplets settle 9 mm/min in still air
  - infectious dose is 5 - 200 infectious droplets
  - average patient exhales 1.25 infectious droplets/hour
  - some cases produce 150 - 200 infectious droplets/hour
Exposure to close contact 

Infection

Primary active TB 5%

Latent TB 95%

HIV+ 
5-10% per year

Never reactivate 90%

Reactivation TB 5% lifetime

Tuberculosis Risk Factors

• AIDS - CD$_4$ < 400
• Iatrogenic immunosuppression - corticosteroids
• Age
  – young
  – old
• Alcoholism/malnourishment
• Diabetes
• Genetics
Mantoux skin test
mm of induration

Significant Induration on Mantoux

- 15 mm  Always indicates infection
- 10 mm  population at risk
  - low income, minority races, IV drug users
  - foreign born - from high prevalence countries
  - Institution populations - prisons, nursing homes, mental institutions
  - silicosis, diabetes mellitus, malignancies, immunosuppressive agents
- 5 mm  Early, immunosuppressed
  - HIV positive or HIV risk factors HIV status unknown
  - Chest film consistent with old nonreactive tuberculosis
  - Recent close contact with infectious tuberculosis case
Types of Tuberculous disease

- Childhood tuberculosis
- “Adult” or reactivation disease
- Acute tuberculous pneumonia (AIDS)
- Miliary tuberculosis
- Cold abscess
- Addison’s disease (Adrenal insufficiency)

Types of Disease caused by *M. tuberculosis*
M. tuberculosis infection without disease

- Inhalation of infectious droplet
- Hilar and peribronchiolar lymph nodes
  - 4 - 6 weeks
- Lymphohematogenous dissemination
  - 6 - 8 weeks
- Tubercle formation
- Granulomatous inflammation: caseous necrosis
- Dystrophic calcification (Ghon complex)

Diagnosis of Tuberculosis

- AFB smear
- Tuberculin skin test (Mantoux test)
- Chest radiograph
- AFB culture
Tuberculosis prevention

• Environmental - decrease exposure
  – Avoid crowded conditions
  – Air changes
  – UV irradiation

• Chemoprophylaxis – after positive skin test
  – INH - after Tuberculin skin test conversion
    • 6 months of daily oral INH

• BCG vaccine (Bacille-Calmette-Guerin)
  – Causes positive skin test
  – Used in much of the world, except US

M. tuberculosis direct smear
Environmental Resistance of *M. tuberculosis*

- Survives drying
- Susceptible to UV irradiation (2 hours in sunlight)
- Resistant to many disinfectants
  - susceptible to chlorine and phenols
- Pasteurization kills (62°C 30 min or 71.7°C 15 sec)
- HEPA filters

Germicidal Ultraviolet Light

- UV light at 254 nm is effective in killing infectious agents
  - In air ducts
  - In upper room irradiation
- Also used in biological safety cabinets
  - Demonstration of effectiveness of killing *M. tuberculosis* on Middlebrook 7H11 plate
UV Exposed Culture Plate

0 min 1 min

AFB Cultures at UAB

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Cultures</th>
<th>Positive Cultures</th>
<th>Positive for Mtb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>2637</td>
<td>80 (3.0%)</td>
<td>42 (1.6%)</td>
</tr>
<tr>
<td>1989</td>
<td>2698</td>
<td>76 (2.8%)</td>
<td>23 (0.9%)</td>
</tr>
<tr>
<td>1990</td>
<td>2848</td>
<td>126 (4.4%)</td>
<td>24 (0.8%)</td>
</tr>
<tr>
<td>1991</td>
<td>2931</td>
<td>120 (4.1%)</td>
<td>23 (0.8%)</td>
</tr>
<tr>
<td>1992</td>
<td>3226</td>
<td>145 (4.5%)</td>
<td>35 (1.1%)</td>
</tr>
<tr>
<td>1993</td>
<td>3418</td>
<td>177 (5.2%)</td>
<td>39 (1.1%)</td>
</tr>
<tr>
<td>1994</td>
<td>4330</td>
<td>259 (5.9%)</td>
<td>89 (2.1%)</td>
</tr>
<tr>
<td>1995</td>
<td>4126</td>
<td>219 (5.3%)</td>
<td>98 (2.4%)</td>
</tr>
<tr>
<td>1996</td>
<td>3970</td>
<td>224 (5.6%)</td>
<td>82 (2.1%)</td>
</tr>
<tr>
<td>1997</td>
<td>4389</td>
<td>308 (7.0%)</td>
<td>114 (2.7%)</td>
</tr>
<tr>
<td>1998</td>
<td>5234</td>
<td>293 (5.6%)</td>
<td>77 (1.5%)</td>
</tr>
<tr>
<td>1999</td>
<td>4984</td>
<td>256 (5.1%)</td>
<td>73 (1.5%)</td>
</tr>
<tr>
<td>2000</td>
<td>4932</td>
<td>278 (5.6%)</td>
<td>99 (2.0%)</td>
</tr>
<tr>
<td>2001</td>
<td>5609</td>
<td>271 (4.8%)</td>
<td>69 (1.2%)</td>
</tr>
<tr>
<td>2002</td>
<td>5228</td>
<td>223 (4.2%)</td>
<td>50 (1.0%)</td>
</tr>
<tr>
<td>2003</td>
<td>4626</td>
<td>275 (5.9%)</td>
<td>57 (1.2%)</td>
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<tr>
<td>2004</td>
<td>4025</td>
<td>252 (6.2%)</td>
<td>29 (0.7%)</td>
</tr>
<tr>
<td>2005</td>
<td>3972</td>
<td>230 (5.8%)</td>
<td>31 (0.8%)</td>
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<tr>
<td>2006</td>
<td>4742</td>
<td>248 (5.2%)</td>
<td>38 (0.8%)</td>
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### Diagnostic *M. tuberculosis* Cultures

<table>
<thead>
<tr>
<th></th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>32</td>
<td>40</td>
<td>24</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Days to growth</td>
<td>13.6</td>
<td>15.5</td>
<td>16</td>
<td>14</td>
<td>17.6</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Days to Identification</td>
<td>16.8</td>
<td>19.8</td>
<td>21.3</td>
<td>17</td>
<td>21</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>AFB positive Respiratory</td>
<td>17/28</td>
<td>20/28</td>
<td>14/20</td>
<td>12/15</td>
<td>7/12</td>
<td>5/7</td>
<td>6/11</td>
</tr>
<tr>
<td>Positive AFB M. tuberculosis</td>
<td>17/35</td>
<td>20/31</td>
<td>14/34</td>
<td>12/28</td>
<td>7/29</td>
<td>5/22</td>
<td>6/18</td>
</tr>
</tbody>
</table>

### Number of Patients Each Species of Mycobacteria Were Isolated from at UAB 1990 to 2007

- *M. avium cx* 771
- *M. tuberculosis* 441
- *M. fortuitum* 391
- *M. kansasii* 132
- *M. abscessus* 115
- Rapid pigmented 19
- *M. xenopi* 18
- *M. lentiflavum* 18
- *M. marinum* 13
- *M. scrofulaceum* 12
- *M. mucogenicum* 8

- *M. szulgai* 5
- *M. gastri* 3
- *M. brumae* 3
- *M. triplex* 3
- *M. austroafricanum* 2
- *M. smegmatis* 2
- *M. flavescens* 1
- *M. haemophilum* 1
- *M. thermoresistable* 1
- *M. peregrinum* 1
- *M. simiae* 1
Microbiological Diagnosis of Tuberculosis

- Digestion - mucolytic agents
- Decontamination
- Concentration
- Acid fast Stain
- Cultivation of Mycobacteria
  - solid media
  - liquid culture
- Anti-Mycobacterial susceptibilities
Decontamination and Concentration of AFB Cultures

- Mucolytic agent (N-acetyl-L-cysteine)
- 2% NaOH
  - 1% oxalic acid
- Centrifuge 3000 x g for 30 min.
**Mycobacteria Culture Media**

- **Solid media** - 21 - 26 days to detection
  - Loewenstein-Jensen (egg based)
  - Middlebrook 7H11 (agar based)

- **Liquid media** - 8 to 14 days to detection
  - BACTEC 460 (\(^{14}\text{CO}_2\) release from \(^{14}\text{C}\) palmitic acid)
  - MB/BacT (CO\(_2\) production)
  - ESP (O\(_2\) utilization - pressure change)
  - MIDGIT (O\(_2\) utilization - quenching of fluorescence)

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**Colony Morphology of**

*M. gordonae and M. tuberculosis*
Liquid Culture Systems

Growth in Liquid Medium:
*M. tuberculosis* and *M. fortuitum*
Gram Stain of *M. tuberculosis* cords

*M. Kansasii* concentrated smear
Identification of Mycobacteria

- Biochemical tests
  - 2 to 3 weeks
- GenProbe - DNA - RNA hybridization
  - 2 hours
- HPLC - high performance liquid chromatography
  - 1 hour

Hybridization Protection Assay (GenProbe)

1. Denatured (heat)
2. Acridinium-labeled probe
3. Alkaline hydrolysis
4. Inactivated probe
5. Substrate
6. Light
**Mycobacterium avium** complex (MAC)

- found in soil and water - tap water
- transmission through either respiratory or GI tract
- pulmonary disease like tuberculosis
- disseminated disease in AIDS patients
  - 50% of autopsies
- resistant to many anti-mycobacterial drugs
- slow growing non-pigmented colonies

**Other Important MOTT**

- **M. kansasii** - Photochromogen
  - Tuberculosis like disease
- **M. marinum** - Photochromogen
  - Found in water - fish tanks and surface water
  - 30 to 33°C optimum temperature
- **M. scrofulaceum** -
  - granulomatous cervical lymphadenitis in children
- **M. fortuitum - M. chelonei** complex
  - Rapid growers - colonies in less than 7 days
  - Skin infections, pulmonary disease
M. marinum
lymphocutaneous

- Fresh or salt water or no water exposure
- Photochromogen
- 1-2 patients/year at UAB
- 9 finger patients
- 30°C optimum temperature
- colonies form in 10-14 days
**Mycobacterium leprae**

- Hansen’s disease
- humans and armadillos are the only natural hosts
- 12 million cases worldwide
- 6,000 registered cases in US, 112 - 350 cases/year
- transmitted by inhalation or skin contact with contaminated respiratory secretions of lepromatous patients
- incubation period is 3 months to 3 years
Clinical Types of Leprosy

• Tuberculoid leprosy
  – intact cell mediated response to *M. leprae*
  – organisms rare in tissue
  – organisms grow in nerves in cooler parts of the body
  – cutaneous loss of sensation - nerve damage due to cell mediated immunity

Leprosy

• diagnosis
  – does not grow on artificial media
  – will grow in nude mice or armadillo
  – AFB stain of nasal secretions
  – lepromin test - skin test

• treatment
  – dapsone and rifampicin - at least 1 year

• prevention
  – isolation of acute lepromatous cases
  – vaccines under development
Lepromatous Leprosy

- depressed CMI response specific for *M. leprae*
- bacteremia with localization in nerves and skin
- high numbers of organisms in macrophages
- less loss of nerve function
- leonine facies
- other organs involved - testes, spleen and liver
Tuberculoid leprosy

- Non-progressive disease
- intact cell mediated response
- organisms rare in tissue macular lesions predominate
- organisms invade nerves and form granulomas
- cutaneous loss of sensation - nerve damage due to CMI