In Search of Better Cures

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- Service - United States Public Health Service Centers for Disease Control (Atlanta, GA)
- Board Certified - Anatomic and Clinical Pathology
Case 1
“Legionnaires’ Disease”

- Presentation - Malaise, Myalgia, Headache
- 12-24 Hours - Fever, Chill, Prostration
- 24 Hours - Dry cough, Chest pain, X-ray evidence of consolidation
“Legionnaires Disease”
Additional Clinical Features

- Diarrhea
- Encephalopathy
- Relative bradycardia
- Renal failure

(182 LD cases, 28 deaths)
Legionella

• Common organism in water, especially warm, stagnant water.
• May be aerosolized by improperly maintained cooling towers.
• Also has been identified in plumbing systems, hot water tanks, and spas.
• Source in Philadelphia hotel never identified.
Treatment

- Legionella is resistant to beta-lactam and aminoglycoside antibiotics.
- Azythromycin, 5 – 10 days is effective and is the treatment of choice in children.
- Other antibiotics require 2 – 3 weeks of treatment. Immunocompromised patients require longer.
- Successful treatment depends on clinical suspicion.
Legionella Pneumonia Outcome

• Resolution
• Superinfection
• Organization with possible permanent impairment
• Death (Approximately 25% overall)
Case 2
Case Presentation: 2011

A 47 year old man at from a homeless shelter goes to a public clinic complaining of chronic cough and not feeling well. He does not remember the last time he held a fulltime job, and has lived on the street in several large cities for at least the last five years. It is difficult to take an accurate history because he seems to be talking to people not in the examination room. The patient remembers being told he had some sort of lung infection about a year ago, but the medicine made him sick and he moved on before taking all of the treatment.
Tuberculosis

- Caused by *Mycobacterium tuberculosis*
- Culture of sputum is the most common method for diagnosis. Several weeks may be required to grow organisms and obtain sensitivity patterns.
- PPD test determines only past exposure to the organism.
Primary Tuberculosis Infection

- Seen as an initial infection, usually in children.
- The initial focus of infection is often just a small subpleural nodule (granuloma), along with hilar lymph node involvement that is more prominent.
- Together, these initial lesions make up the “Ghon complex.”
- In nearly all immunocompetent persons, these granulomas resolve and there is no further spread.
Secondary Tuberculosis Infection

• Seen mostly in adults.
• It can result from a reactivation of a previous infection, particularly when health status declines (as in alcoholics).
• However, most cases probably result from reinfection.
• Typically, the upper lung lobes are most affected, and cavitation can occur.
Miliary Tuberculosis

• When resistance to infection is particularly poor, a “miliary” pattern of spread can occur.

• Grossly, there is a myriad of small millet seed (1-3 mm) sized granulomas, either in lung or in other organs.
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Tuberculosis

• Rarely in immunocompromised patients tuberculosis may cause a severe pneumonia (non-reactive tuberculosis).
Treatment for New Skin Test Conversion

• In general, Isoniazid for nine months.

• Decreases incidence off reactivation and secondary tuberculosis.

• Many factors go into determining whether to recommend treatment.
Secondary Tuberculosis Treatment

Six months total-
  Four drugs for two months
  Two drugs for four months

Long course is required due to slow growth rate of organism.

After treatment there is a 2 – 33% relapse rate.

Incomplete treatment encourages drug resistance.
Case 3
Ebola

- Abrupt onset
- Severe headache and malaise
- Diarrhea and vomiting
- Maculopapular rash and desquamation
- Gastrointestinal and pulmonary hemorrhage (Hemorrhagic fever)
Using molecular approaches to isolate new antibiotics

11/17/2011

Yanchang Wang

Department of Biomedical Sciences
College of Medicine
Florida State University
Medical Student Education

Class of 2012
Biomedical Research

Support:
- Florida State
- National Institute of Health
- Private foundation (ACS)

How to justify the support of our research by federal and state money?
Major research interest

- Budding Yeast Cell Cycle
  - G1
  - S phase
  - Telophase
  - Metaphase
  - Anaphase

(D.W. Hailey, Yeast Resource Center, University of Washington)

- Human cell cycle
  - Mitosis

Human cell cycle
Modern biotechnology and drug screen

From an idea to new anti-TB antibiotics

Yanchang Wang (Florida State University)

Shuyi Si (Chinese Academy of Medical Sciences)

Idea → Screen strategy → Results
Antibiotics

on the morning of Friday, Sept. 28 1928
• Alexander Fleming observed the killing of staphylococci by a fungus (Penicillium notatum)

• Florey & Chain purified Penicillin (1940)
  - Three shared Nobel prize in 1945

• First use in a patient: 1942

• World War II: saved 12-15% of lives

In 1999, *Time* magazine named Fleming one of the 100 most important people of the 20th century for his discovery of penicillin.
Antibiotics

- Selman Waksman - Streptomycin (1943)
  - active against all Gram-negatives
  - first antibiotic against *M. tuberculosis* (TB)
  - extracted from *Streptomyces*
  - 20 other antibiotics, neomycin, actinomycin

Sleman Waksman
Nobel prize 1952

Waksman institute of Microbiology
Antibiotic

• Antibiotics are drugs used to treat infections caused by bacteria, fungi, and virus.

• Antibiotics could be products of microorganisms and synthetic or semi-synthetic compounds

• Key: it needs to kill the microbial cell and not be toxic to normal healthy human cells.
Bacteria and human cells
Principle of the isolation of antibiotics
The key difference between human cells and bacteria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>bacterial Cell</th>
<th>human Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>size</td>
<td>average size 1-10 μm</td>
<td>average size 10-100 μm</td>
</tr>
<tr>
<td>nucleus</td>
<td>nucleoid (no membrane)</td>
<td>membrane bound</td>
</tr>
<tr>
<td>chromosomes</td>
<td>single circular loop of naked DNA</td>
<td>linear, arranged with histones</td>
</tr>
<tr>
<td>organelles</td>
<td>absent</td>
<td>present, vary with cell function</td>
</tr>
<tr>
<td>ribosomes</td>
<td>present as smaller 70S</td>
<td>present as larger 80S</td>
</tr>
<tr>
<td>cell wall</td>
<td>present</td>
<td>absent</td>
</tr>
</tbody>
</table>
Antibiotic Mechanisms of Action

- Cell wall synthesis
  - Beta-lactams
  - Vancomycin
  - Isoniazid
  - Ethambutol
  - Cycloserine
  - Ethionamide
  - Bacitracin
  - Polymyxin

- DNA replication
  - Quinolones
  - Metronidazole

- RNA synthesis
  - Rifampin
  - Rifabutin

- DNA

- mRNA

- Ribosomes

- Translation
  - Protein synthesis (50S ribosome)
    - Chloramphenicol
    - Macrolides
    - Clindamycin
    - Streptogramins
  - Protein synthesis (30S ribosomes)
    - Aminoglycosides
    - Tetracyclines
    - Oxazolidinone

- Translation

- Antimetabolites
  - Sulfonamides
  - Dapsone
  - Trimethoprim
  - Para-aminosalicylic acid

- Alteration of Cell Membrane
  - Polymyxins
  - Bacitracin
  - Neomycin
Ribosome and protein synthesis

Replication (DNA) → Transcription (RNA synthesis) → mRNA → Translation (protein synthesis) → Ribosome → Protein
Ribosome as a target for antibiotics

- **Aminoglycosides**: Block the initiation of translation and causes the misreading of mRNA.
- **Tetracyclines**: Block the attachment of tRNA to the ribosome.
- **Macrolides**: Prevent the continuation of protein synthesis.
- **Chloramphenicol**: Prevents peptide bonds from being formed.
- **Lincosamides**: Prevent the continuation of protein synthesis.
- **Streptogramins**: Each interferes with a distinct step of protein synthesis.
- **Oxazolidinones**: Interfere with the initiation of protein synthesis.
Streptomyces and antibiotics

- Cefoxitin
- Chloramphenicol
- Daptomycin
- Lincomycin
- Neomycin
- Puromycin
- Rifamycin
- Streptomycin
- Tetracycline
- Vancomycin
Old and modern days for biomedical research

1989

- New technology in molecular biology
- Genome database
- Compound library

2011
New anti-tuberculosis (anti-TB) antibiotics are needed

- 2 million people die of TB

Available antibiotics:
- Rifampin: RNA synthesis, 1967
- Isoniazid: Cell wall, 1952

Problems:
- More people are infected (AIDS)
- The appearance of drug resistant strains
50S Structure of Ribosome

L12 protein Interacts with L10
Bacteria and human cells show similar but different L12 protein sequence

L12 protein sequence alignment

| Human       | MPPKFDPNEIKVVYLRCTGGEVG---ATSALAPKIGPLGLIEVVPVSAALSALIIKALKKEPPR |
| TB          | -MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAPVAVAAAGAAPAGAAVEAAE |
| Human       | DRKKQKNKHSGNITFDEIVNIARQMRHRSLARELSGTIKEILGTAGQVGNCNDGRHPHD |
| TB          | QSEFDVILEAAG---DKKIGVIKVREIVSGLGLKEAKDLVDGAPKPLEKVAKEAAD |
| Human       | IIDDINSGAVECPAS |
| TB          | AKAKLEAAGATTVK |
|             | .::*:...  ... |
We can see protein-protein interaction

-Yeast Two-hybrid Assay

- Growth on Histidine and adenine dropout plates
- Color assay for LacZ
If a compound disrupts the interaction between X and Y, we can see it!

**Two-hybrid activation**

- **BD**
- **AD**
- **Y**
- **Transcription**

**Histidine Adenine LacZ**

**No compounds:**
- • Growth on His⁻ Ade⁻: Yes
- • Color assay LacZ: Yes

**A compound**

**When compounds present:**
- • Growth on His⁻ Ade⁻: No
- • Color assay LacZ: No
Isolation of new anti-TB antibiotics targeting ribosomal L12-L10 interaction

Wang: Tallahassee, US 
Knows yeast two-hybrid

Si: Beijing China  
Knows TB ribosome
Screen procedure

1. To see L12-L10 interaction.

2. To isolate compounds that disrupt L12-L10 interaction.

3. These compounds will kill TB.

4. These compounds should be less toxic to human cells.
To see L12-L10 interaction

His\(^{-}\) Ade\(^{-}\)  LacZ color change

B

C

$\beta$-gal Activity (Units)
A compound inhibits the growth of yeast cells in Ade- His- medium.
T766 and T054 inhibit the color change

D

![Graph showing β-gal activity vs. T766 concentration (μg/ml)]

E

![Graph showing β-gal activity vs. T054 concentration (μg/ml)]
Surface Plasmon Resonance (SPR)
L12 interacts with L10
T766 interacts with L12 but not L10
T766 and T054 disrupt L12-L10 interaction
Do the identified compounds inhibit TB growth?

Minimal Inhibitory Concentration (MIC)

32 µg/ml 16 µg/ml 8 µg/ml 4 µg/ml 2 µg/ml 1 µg/ml

Sub-culture to agar medium

MIC = 8 µg/ml
Yes, T766 and T054 can kill TB

The MICs of T766 and T054 against various *M. tuberculosis* strains

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>H37Rv</td>
<td>STB-960</td>
<td>MDR-699</td>
</tr>
<tr>
<td>T766</td>
<td>0.312</td>
<td>0.25</td>
<td>4</td>
<td>64</td>
</tr>
<tr>
<td>T054</td>
<td>1.25</td>
<td>1.0</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.156</td>
<td>0.125</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0.156</td>
<td>0.125</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>1.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

STB, clinical sensitive strain of *M. tuberculosis*; MDR, multidrug resistance; XDR, extensively drug resistance. Isoniazid and Rifampin were used as reference drugs. STB-960, MDR-699 and XDR-83 strains were all clinical isolates.
1. Established a system to detect L12-L10 interaction (yeast two-hybrid)

2. Isolated T766 and T054 (high throughput screen)

3. T766 and T054 disrupt L12-L10 interaction

4. T766 and T054 kill TB, but not other bacteria

5. T766 and T054 inhibit ribosome-mediated protein synthesis

6. T766 exhibit low toxicity to mice (LD50 = 650mg/ml)

7. Further analysis.............
Alzheimer's disease
ADHD
Obesity
Sleep disorder
Others
Acknowledgement:

Dr. Kerry Maddox