Bio119
Person-Person Transmission

Mycobacterium tuberculosis
- CHARACTERISTICS
  - Gram positive cell wall, unusually high glycolipid (mycolic acid)
  - Special stain (acid-fast stain is required)
  - Rod shape, Non-motile
  - Aerobic, very slow growth in culture
  - Capable of long-term survival in the human body (persistence)

Tuberculosis (TB)
- Epidemic disease in 1800s in the US and Europe. TB accounted for 1/4 deaths in major European cities
- Incidence declined in developed countries
  - 1950's: Cure was discovered for TB as well as inexpensive screening tests. US implemented strict public health program.
    - Screen population to see who had disease
    - Require people who tested positive to accept treatment. Heavily monitored.
  - 1980's: Public Health measures dismantled
    - Immigrants, crowded shelters, drug resistance.
- TB remains one of the top three killers (malaria and HIV)
  - ~ 2 billion people are infected. ~ 8,000,000 new cases a year
  - 6% of all infant deaths, 20% of adult deaths

Topics:
- Person-Person Transmission: the disease goes from person to person without a significant environmental reservoir.
- **Mycobacterium tuberculosis** -- Airborne
- **Helicobacter pylori** -- Direct Contact
  - Disease
  - Virulence factors
Tuberculosis (TB)

- TB is transmitted via airborne particles
- Humans with TB are the reservoir of the disease
- Small droplets (1-10 bacteria) generated via coughing, sneezing, or simply speaking
- Must get all the way to the alveoli
- Low ID50

Symptoms of TB

- Fever, coughing (bloody sputum—materials coughed up from the lungs), weight loss and loss of energy.
- Irreversible lung destruction
- The bacteria may escape from the lungs and enter the bloodstream.
- Systemic form of the disease is fatal.

Tuberculosis: the disease

1. Bacterium is inhaled into lung
2. Taken up by macrophages. Human immune cells that take up foreign objects (e.g. bacteria) and destroy them.
3. Mtb is not easily destroyed and can survive inside of macrophages

After Mtb is inside the Macrophage, there can be a couple of outcomes.

- 90% of Infections: macrophages control infection so there is minimal bacterial replication. To control infection, the body may make a tubercle
- still skin-test (aka tuberculin or PPD) positive
- Immune cells are attracted to the area and accumulate around the sites where the bacteria are growing. Acts to wall off the Mtb into a structure called a tubercle.
Tubercles

---May contain live bacteria (can’t tell).
• With just a few bacteria they have a thick cheese-like consistency and are called “caseous necrosis”.
  – The bacteria may still be alive in here, and may live for decades. Called latency.
  • 2-23% of latent infections reactivate.
• As bacteria continue to divide, the tubercles become more liquid. Easier to cough up bacteria, and easier for the bacteria to spread within body too. This situation is bad.

TB Lungs

• Top lung has a solid caseous tubercle.

• Bottom lung has fluid-filled cavities.

Latent Infection

• Definition: An infection that is dormant but could reactivate.
• Occurs in people who have a tubercle.
• Bacteria can come out of a tubercle and cause tuberculosis-called reactivation disease.
• Mtbt survive for decades before reactivating.

TB: Detection, treatment, control

• Diagnosis with active TB is based on symptoms (coughing mostly, fever, malaise, and how your lung looks).
• Treatment: antibiotics. The most common is isoniazid, and ethambutol. Prevents synthesis of lipids in the cell wall (mycolic acids).
• Tuberculin/PPD test is used to detect whether you’ve ever been infected.
Purified Protein Derivative (PPD) Test

- Crude extract of mycobacterial proteins injected under your skin.
- If you have been infected, this injection elicits a localized immune reaction. Seen as hardening and swelling.
- Doesn’t mean you have active infection—you would still have to have an x-ray after this.

Virulence Factors

- Ability to survive in lung macrophages
- Cell wall components that elicit damage to tissue
- Ability to survive for decades in walled-off lesions

- Difficult to study
- Only recently amenable to genetic studies
  - Molecular basis of virulence is difficult to determine
- Whole genome of the pathogen is sequenced
  - Microarray Technology and Whole genome transcriptome (quantify all mRNAs encoded by the genomes of bacteria) analysis is used to study the interaction of Mycobacterium tuberculosis with macrophages.

Microarrays and the Transcriptome

- Microarrays are genes or gene fragments attached to a solid support in a known pattern.
- These arrays can be used to hybridize to mRNA and analyzed to determine patterns of gene expression.
- The arrays are large enough and dense enough that the transcription pattern of the entire genome (the transcriptome) can be analyzed.

How microarrays are made: spotted microarrays

- DNA mechanically placed on glass slide
- Need to deliver nanoliter to picoliter volumes
  - Too small for normal pipetting devices
- Robot “prints,” or “spots,” DNA in specific places
DNA spotting I

- DNA spotting usually uses multiple pins
- DNA in microtiter plate
- DNA usually PCR amplified
- Oligonucleotides can also be spotted

Commercial DNA spotter

Microarray analysis
Analysis of data from a microarray experiment

Transcriptional Adaptation of *Mycobacterium tuberculosis* within Macrophages

- Changes in the transcriptome suggest that MTB adapts to this environment by the induction of fatty acid–degrading enzymes.
  - *M. tuberculosis* grows inside macrophages. What is its carbon source?
  - Induction of genes involved in the degradation of fatty acids suggest that fatty acids furnish carbon and energy
  - How can you test this hypothesis experimentally?

Isocitrate lyase (ICL) is required for MTB survival in vivo

- Two genes *icl1* or *icl2*, the genes that encode ICL1 and ICL2, respectively.
- Single deletions had little effect on bacterial growth in macrophages and mice, deletion of both genes resulted in complete impairment of intracellular replication and rapid elimination from the lungs.
- Chemical inhibitors of fatty acid–degradation pathway could be important for treatment of TB.
Comparative genomics of BCG vaccines by whole-genome DNA microarray

- Bacille Calmette-Guérin (BCG) vaccines are live attenuated strains of Mycobacterium bovis administered to prevent tuberculosis.
- Efficacy is variable.
- To better understand the differences between M. tuberculosis, M. bovis, and the various BCG daughter strains, their genomic compositions were studied by performing comparative hybridization experiments on a DNA microarray.
- Eleven regions (encompassing 91 open reading frames) of H37Rv were found that were absent from one or more virulent strains of M. bovis. Evidence for the ongoing evolution of BCG strains since their original derivation. (Behr et. al. Science. 1999 May 28;284(5419):1520-3)

Summary--Mtb

- Slow-growing bacterium
- Very common
- Infects the lungs and lives inside of macrophages
- As a response, we try to wall off the bacterium into a tubercle—layers of cells and connective tissue around bacteria.
- Tubercles can contain live bacteria (or dead) so the bacteria may reactivate and cause infection
- PPD test is composed of mycobacterial proteins and tells whether a person has ever been infected

Helicobacter pylori

- CHARACTERISTICS
  - Gram-negative curved rod
  - Motile due to polar flagella
  - Microaerophile

- DISEASE
  - Can cause ulcers in the stomach and in the first portion of the intestine (duodenum)—collectively called “Peptic Ulcers”
  - Ulcers are associated with development of gastric cancer

- RESERVOIR
  - Human stomach
In the endoscopic views below, the normal appearance of the pylorus is seen at the left, with the first portion of the duodenum at the right.

An acute duodenal ulcer is seen in two views on upper endoscopy in the panels below.

Historically ulcers were thought to be caused by stress
1. Before 1985 or so, the cause of ulcers was unknown
   Used to be thought of as caused by excess acid that was brought about by stress or diet
2. Result—usually if you had an ulcer the symptoms came and went, but it never really went away.

For a long time (since 1940) people had observed bacteria in the ulcer craters---Cause or random?

• If a bacteria is in the ulcer, did it cause it?

• Should there be bacteria in your body? In your stomach?
So when the scientists saw the bacteria in the ulcer, they made two hypotheses:

- Hypothesis 1: something else formed the ulcer, and then the bacteria came along and grew there

- Hypothesis 2: The bacteria formed the ulcer, and then it continued to live there

- How do you figure out which is the truth?

**Koch Postulates**

1. The organism must be present in every case of the disease but absent from healthy individuals.
2. The suspected organism must be isolated and grown in pure culture on artificial media.
3. Inoculation of this pure culture in experimental animals the same disease must result.
4. The same organism must be recovered from the diseased animals

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**Do the bacteria in the ulcers cause them? (I)**

1. Take stomach samples

2. Try to grow bacteria in the lab
   - Conditions and Food needed by bacteria

3. Difficulties

4. But now we know this bacterium as *H. pylori*

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**Do the bacteria in the ulcers cause them? (II)**

**Will lab bacteria infect animals?**

- Bacterial sample--force feed it into the stomach of an animal that doesn't have the bacterium.

- What animal to use?
Do the bacteria in the ulcers cause them? (III) Another animal...

So one of the researchers, an Australian Doctor named Barry Marshall decided to take the stomach bacteria himself.

1. He went into the hospital and got checked out to make sure he didn’t have the bacteria and to make sure he didn’t already have an ulcer—he didn’t.
2. Then he drank a culture of 1 billion of the bacteria.
3. And he waited to see what would happen.

Barry Marshall...

Early...
- His stomach gurgled a lot.
- After one week, he had stomach pain and vomiting.
- He was irritable and had putrid breath.

Later...
Then, about 10 and 14 days later, doctors examined his stomach and saw that he had the symptoms of starting to get an ulcer. And, when the scraped some of the stomach out and tried to grow the bacteria, they found the same bacteria.

Koch Postulates -- did they do them all?

1. The organism must be present in every case of the disease but absent from healthy individuals. ✔
2. The suspected organism must be isolated and grown in pure culture on artificial media. ✔
3. Inoculation of this pure culture in experimental animals the same disease must result. ✔
4. The same organism must be recovered from the diseased animals. ✔

H. pylori is found in 50% of people worldwide; 30-40% of US population.

Although the prevalence is huge, only about 10-20% of those infected develop ulcer disease.
- This implies that there are additional factors that contribute to whether a person gets an ulcer or not.
- HP is more considered a risk factor.
Transmission: oral-oral or fecal-oral?

- Comes out of infected people from both ends
- Can be found in water
- Most people get *H. pylori* as a child
- Families tend to all have the same *H. pylori* suggesting there is transmission within the family

Other *Helicobacter* species infect other animal species and cause ulcers

- *Helicobacter mustelae* = ferrets
- Other *Helicobacters* for cheetahs, whales, dolphins, cats/dogs.

*H. Pylori* Virulence Factors

- Motility and Chemotaxis
  - *H. Pylori* is sensitive to low pH
    - Does not colonize lumen (low pH)
    - Colonize mucosal surfaces
    - Uses motility to reach the mucus layer
    - Uses chemotaxis to reach the lower part of the stomach, antrum.

- Urease
  - Protein that cleaves urea into CO2 and NH3, helps buffer the bacterium from acid.
  - Virulence Factor that help *H. pylori* to survive in the stomach
  - Urease negative mutants unable to colonize and did not produce ulcers
**H. Pylori Virulence Factors**

- Eliciting an inflammatory response
  - Vacuolating cytotoxin

- Type IV secretion system
  - Virulent H. pylori isolates harbour the cag (cytotoxin-associated genes) pathogenicity island, a 40 kb stretch of DNA that encodes components of a type IV secretion system (T4SS).
  - This T4SS forms a pilus for the injection of virulence factors into host target cells such as the CagA oncoprotein.

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**Ulcer diagnosis**

First indication is symptoms
- both gastric and duodenal ulcers feel the same
- mild-moderate abdominal pain (gnawing or burning)
  - commonly in middle of night, but rarely first thing in the morning
  - discomfort relieved by antacid/food
  - If the ulcer perforates, the pain is severe

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**Diagnostics for H. pylori**

Diagnostics are required to confirm ulcer

- First choice is endoscopy—when you place a scope down the throat and into the stomach and look for ulcers. Can also take a snippet of tissue—biopsy—to analyze for cancer

  1. Plate the biopsy sample to grow *H. pylori*
  2. Test the biopsy sample for urease
  3. Test blood to see if patient is making an immune reaction—antibodies—to *H. pylori*
  4. Urea breath test

\[
	ext{Urea} \rightarrow 
\text{NH}_3 + \text{CO}_2
\]
Treatment is Antibiotics
Lots of possible antibiotics—for this organism, it is recommended that doctors give 2 antibiotics and 1 antacid
One possible combination:
• Amoxicillin—inhibits the making of the bacterial cell wall
• Clarithromycin—inhibits the making of bacterial proteins
• Lansoprazole—antacid
80-90% of patients will become cured of HP, and ulcers, after taking this regime for two weeks

Ulcers can be caused by several things so the next step is figuring out the cause
• The two most common causes are:
  – 1) H. pylori
  – 2) Taking too many non-steroidal anti inflammatory drugs like advil, tylenol.

Summary--H. pylori
• Gram - bacterium.
• Discovered by Barry Marshall by fulfilling Koch’s postulates on himself. Nobel Prize.
• Infection leads to ulcers and gastric cancer.
• People with ulcers and H. pylori are treated with antibiotics—usually works.
• Virulence factors include: urease, motility, ability to eliciting an inflammatory response

Study Questions
1. For Mycobacterium tuberculosis and Helicobacter pylori, know what (i) disease(s) they cause, (ii) how they are transmitted and the steps in causing disease, (iii) treatment, (iv) any virulence factors we discussed in class, (v) general microbiological features of the organisms.
2. After a person inhales Mtb, there are a couple of possible outcomes—what are these and how do they differ? Why does one happen versus the others?
3. Why would it be a bad idea to antibiotic-treat everyone with a positive PPD/tuberculin test? Be sure to answer what the PPD test tells you.
4. What is different about the cell wall of Mycobacteria as compared to other bacteria? What properties would this difference confer on the Mycobacteria?
5. How does H. pylori fulfill or not fulfill Koch’s postulates?
6. What would happen if you eliminated each of the H. pylori virulence factors?
7. Why don’t we just treat everyone with an ulcer with antibiotics?