

Biology 067: Emerging Diseases Midterm KEY

Thursday, October 16, 2003

Instructions:

1. For each question, numbers in brackets indicate the number of points. The **relative number of points** should give you a rough idea of **how much time to spend** per question.
 2. Read through the **entire** question **carefully before starting to write** your answer. The **space** that has been left between questions **roughly approximates** how long your answers will be, depending on the size of your handwriting. You should also **feel free** to use **PICTURES** to **help explain ideas** when **appropriate**.
 3. Aim for **CLEAR, CONCISE, COMPLETE** answers.
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1. (15 pts total) Exposure, infection and disease

You are watching the "Influenza, 1918" video with your grandparents. When the epidemiologist Dr. Shirley Fannin says "You **can't barrier** yourself from **being exposed** [to influenza], because the **person who looks healthy** may be the one **spreading the disease**," your grandparents are confused. **They ask you** the following **questions**, which **you proceed to answer**:

A. (3 pts) What does Dr. Fannin mean by "**spreading the disease**?" What exactly is **being spread** from person to person?

She means that the microbe that causes the disease (in this case influenza virus) gets transmitted from one person to another person.

B. (6 pts) What is the **difference** between being "**exposed**" and being "**infected**?"

When one is "exposed" to an infectious agent, one has had contact with that microbe, although it has not necessarily multiplied in one's body. When one is "infected," the microbe has actually started to multiply in the body.

C. (6 pts) How can a person who **appears healthy** "**spread a disease**?" At what stage of disease is this type of **spread likely to happen**?

There is a delay between when a microbe starts growing/multiplying in your body and when that infection causes enough damage to the body to cause symptoms of disease. During this incubation period (sub-clinical phase), people who appear healthy can transfer the infectious agent to other potential hosts because they won't get treatment or change their behavior to avoid transmission, since they don't know they are infected.

2. (18 pts total) Identifying disease causes in the 19th century

In the early 1890's, Dr. Friedrich Johann Pfeiffer was studying **influenza** and he isolated a bacterium he called "*Hemophilus influenzae*" he thought caused the disease.

A. (12 pts) Assuming he listened to Robert Koch's proposal for identifying causative agents, what experimental steps do you think Pfeiffer would have taken to identify his bacterium?

1. Take diseased tissue (lung sample or swab from throat/nose) and transfer to solid nutrient medium to allow growth of potential infectious agent.
2. Produce pure culture of bacterium on the plate and look at it with a light microscope.
3. Transfer the pure culture into an animal and show that the animal has the same symptoms as the human cases.
4. Re-isolate the bacterium from the animal and show that it is the same as the one from the human (growth characteristics on the plate, form visible by microscopy).
5. All people with flu should have this organism present in their body (Pfeiffer's bacterium failed this test).

B. (6 pts) As it turned out, Pfeiffer misidentified the cause of influenza. What is the real causative agent for influenza and what would you do differently to identify this agent and why (assuming you had all the right experimental tools)?

Influenza is caused by an RNA virus. Since viruses require host cells to multiply, in step 1 rather than transferring the diseased tissue to solid medium, I would put it either in an animal or in cultured cells in a dish to allow the virus to multiply. I would also have to use an electron microscope to see the virus in steps 2&4, since viruses are smaller than bacteria and are not visible by light microscopy.

3. (67 pts total) Anthrax, antibiotics and immunity

Twin sisters were born and raised on a dairy farm in Huxley, Iowa. Helen moved to Minneapolis after college and Imogene stayed in Huxley to run the family farm. In 1990, Imogene noticed an **infected cut** on her arm but **waited several days** to go to the doctor. After **taking a sample** for future study, the **doctor diagnosed the infection as cutaneous (skin) anthrax** and prescribed a 60-day course of **ciprofloxacin** (an **antibiotic**), which **completely cleared the infection**.

A. (6 pts) What is an **antibiotic** and **how** does it help clear an infection?

An antibiotic is a molecule that binds to a bacterial protein (or other molecule) and stops it from functioning. Since the bacterium requires the protein to grow and multiply, the antibiotic will kill the bacterium and therefore clear the infection.

B. (6 pts) If the infection on Imogene's arm had been diagnosed as a smallpox infection, would ciprofloxacin have been used to treat her? Why or why not?

No. Antibiotics like ciprofloxacin only work on bacteria, not on viruses like the one that causes smallpox. Ciprofloxacin works by binding specifically to a particular bacterial protein. Since viruses have different proteins than bacteria, the antibiotic will not be able to bind to and inhibit a viral protein and therefore the virus will not be killed by the antibiotic.

In 1991, Helen was working in the central Minneapolis post office when she discovered a letter that was leaking a fine, white powder. She was immediately **treated** with the **new anthrax anti-toxin** called "Abthrax" (in combination with antibiotics). This **anti-toxin** consists of **purified human antibody** that recognizes the "A" subunit of the anthrax toxin.

C. (12 pts) Describe what Abthrax would do in the body to help prevent the damage caused by *B. anthracis*. Highlight how this process differs from antibiotic treatment.

Since Abthrax is an antibody that can bind to anthrax toxin, it could block the ability of that toxin to bind to host macrophages. Without binding of the "A" subunit to macrophages, the other two parts of the toxin wouldn't be able to bind and the toxin wouldn't be able to get into the macrophages and cause them to send signals that result in damage to the host. Therefore, rather than killing the bacterium itself by blocking an essential protein within the anthrax cell, this antibody stops a free bacterial protein from causing host cells to damage the body.

Later laboratory analysis revealed that the powder was talcum powder and contained **no** *Bacillus anthracis*. In 1996 Helen retired from the post-office and moved back to the family farm. When a few cows on the farm became terribly sick, the sisters worked side-by-side to care for the animals. Shortly thereafter, Helen developed a severe case of cutaneous anthrax whereas Imogene showed **no** symptoms of infection.

D. (18 pts) Assuming both sisters were exposed to *B. anthracis* at **this** time, why would Imogene be immune to infection while Helen was susceptible? Explain the differences in the cells of their immune systems and why Abthrax treatment did **not** confer lasting immunity.

When Imogene was infected with anthrax in 1990, the B cells in her body that had antibodies that bound specifically to *B. anthracis* were activated. In addition to producing free antibody to bind to the bacterium, this activation created memory B cells. Also, helper T cells specific for *B. anthracis* recognized bacterial antigens presented by phagocytes on MHC class II molecules, helped activate the B cells, and memory helper T cells were created. Therefore, when Imogene was re-exposed her memory B and T cells specific for anthrax were able to be very quickly activated to produce lots of antibodies to fight the bacteria, ridding the body of the bacteria before symptoms appeared.

Since Helen only received purified antibody and was never previously infected by *B. anthracis* (only exposed to talcum powder), in 1991 she had no activation of *B. anthracis*-specific B- and helper T-cells. Therefore no memory cells were formed that could act upon her later exposure to the bacterium. It's the memory cells, not the antibody itself that confers lasting immunity. The Abthrax antibodies would only last 2-3 weeks in Helen's body, certainly not surviving until 1996.

Helen was immediately given ciprofloxacin, but the infection persisted until they switched to a different antibiotic. Worried about the emergence of a ciprofloxacin-resistant *B. anthracis* strain, researchers used PCR and DNA sequencing to determine gene sequences for the strains that infected Imogene (in 1990) and Helen (in 1996). They only found **one** gene whose sequence differed between the two strains, as shown on the next page.

E. (15 pts) Predict the amino acid sequence of the proteins encoded by the different bacteria, using the transfer RNAs shown below.













Imogene's strain

Protein: **Asp Val Ser Ile Val Asp**
 GAU UAU UCU AUC UAU GAU mRNA
 CTA ATA AGA TAG ATA CTA DNA
 GAT TAT TCT ATC TAT GAT

Helen's strain

Protein: **Asp Val Phe Ile Val Asp**
 GAC UAU UUU AUC UAU GAU mRNA
 CTG ATA **AAA** TAG ATA CTA DNA
 GAC TAT **TTT** ATC TAT GAT

transfer RNAs

Phe  AAA	Asp  CUA	Leu  GAC	Ile  UAG	amino acid
Ser  AGA	Asp  CUG	Leu  GAU	Ile  UAU	amino acid
Val  AUA			Arg  UCU	amino acid
Sto  AUC			Lys  UUU	amino acid

F. (10 pts) Circle the DNA mutation that you think is responsible for the antibiotic-resistance of Helen's strain and explain your reasoning.

Although there are two mutations in the DNA (in the third nucleotide of the first codon and in the second nucleotide of the third codon), only the second mutation caused a difference in the amino acid sequence of the protein. Since antibiotics work by binding to and stopping a bacterial protein (see part A), only mutations that change the protein sequence can lead to antibiotic resistance, by changing the binding site for the drug and not allowing it to bind or block protein function.