Part III: Basic Immunology

Introduction: This is an introductory unit on immunology. Important topics addressed include bacterial pathogens, vaccines, antibiotics, and cells of the immune system. After completing this unit it is still important to address viral pathogens through a viral disease system such as influenza or HIV (both of which are recommended as they are diseases addressed by many social studies curricula.)

Overview: Concepts addressed

- Definitions of pathogen, antigen infection, disease, immunity (natural and acquired), antibodies, antibiotics, vaccines, virulence, and bacterial plasmids
- Contributions by Louis Pasteur and Robert Koch in bacterial disease research
- The history of antibiotics and of vaccine development
- Extension computer activity for advanced placement students will introduce them to Pubmed resources, Genomics and the use of BLAST searches

Student prior knowledge and skills required

- Should follow a unit on classification as students should have a basic understanding of what bacteria are
- The extension activity requires a good understanding of DNA sequencing and protein synthesis
- Basic computer skills are required for the extension activity as students will be working with multiple windows open at the same time and copying and pasting information.

Time required

- 3 – 40 min. class periods depending on the pace of the instructor and reading level of the students
- extension computer activity should take about 2 – 40 min. class periods

Advanced Preparation

- Student activity #1 requires a copy of the ASM News article Mikesell, Perry et. Al. “Plasmids, Pasteur, and Anthrax” VOL. 49, NO. 7, 1983
- Extension activity requires a computer lab with internet access

What is expected from students

- Student activity #1 involves students filling in guided notes sheets of definitions for immunity
- Student activity #2 requires students read the “Plasmids, Pasteur, and Anthrax” and answering related questions
- Student activity #3 has students reading “History of antibiotics” and answering related questions.
- Extension activity requires students to follow a series of steps using an online database to search for a sequence of bases and doing a BLAST search to find out if any other organisms contain similar sequences

Assessment

- Each of the activities should be collected and graded and a final assessment should include a vocabulary quiz on frequently used terms.

Lesson 4: Treating bacterial disease

Objectives:
- Students will be able to define pathogen, immunity (natural and acquired), antigen, disease, antibiotic, and vaccine.
- Students will be exposed to the concept of immunological specificity.
- Students will gain a historical perspective of disease treatment by learning about the first antibiotics and vaccinations developed.

Introduction and Input:
- Bacillus anthracis is a bacterium that causes a disease called anthrax.
- It is a very old disease that has been described in history since biblical times.
- Robert Koch conclusively proved that the bacteria caused anthrax by using a series of steps that has come to be known as Koch's Postulates.
- Louis Pasteur developed a vaccine that protected against anthrax in 1881 (or was it W.S. Greenfield in England?)
- It is a large, gram-positive, spore forming rod-shaped bacteria that can be grown under aerobic and anaerobic conditions
- Phenotypically and genotypically it is very similar to B. cereus and B. thuringiensis.
- Primarily a disease of cattle, sheep, goats, and wild animals, but humans can be infected by diseased animal products.

Student activity #1: Have students fill in the guided note sheet of definitions for immunity attached

Student activity #2: Students read "Plasmids, Pasteur, and Anthrax" and answer the questions using information from the article

Student activity #3: Students read "History of antibiotics" and answer the questions using information from the article.

Student Activity #1 Guided notes
Teacher's guide

Definitions for immunity

1) **Pathogen** = *A disease causing organism*
Examples: *influenza virus, HIV virus, measles virus, tuberculosis bacilli, and anthrax bacilli*

2) **Antigen =** *The part of a pathogen that will cause an immune response*
Examples: *a protein on the HIV virus, a sugar on the cell wall of bacteria*

3) **Infection =** *occurs when an organism is exposed to a pathogen*

4) **Disease =** *specific symptoms that an organism often has when infected with a specific pathogen.(Note: you can be infected without developing a disease)*

5) **Immunity** = *The ability of an organism to prevent a pathogen from causing a disease*

Natural (innate) immunity = *refers to a type of immunity that* ***nonspecifically*** *prevents pathogens from entering a body or destroys pathogens* Examples of natural immunity = *skin acts as a barrier to prevent infection, some white blood cells (macrophages and neutrophils) kill anything that looks foreign*

Acquired (adaptive) immunity = *immunity that involves killing* ***specific targets*** *and remembering how to kill those targets in the future.* Examples of acquired immunity = 1) *get infected-get sick-develp cells that kill only the specific target antigen- maintain memory to prevent getting sick from the same thing in the future 2) get immunized with a harmless form of antigen – develop cells that kill only specific target antigen – maintain memory to prevent getting sick from the same antigen in the future*

6) **Antibodies =** *Proteins found in the blood made by white blood cells that bind to antigens making them harmless. NOTE: antibodies are specific and bind only to the antigen that the organism was exposed to*

7) **Antibiotics =** *chemicals that kill bacteria (often produced by fungi)*

8) **Vaccines =** *harmless forms of antigen that trick your body into thinking that it has a disease→ body responds giving it acquired immunity (Note: vaccines can be made to prevent viral diseases as well as bacterial diseases)*

9) **Bacterial plasmids =** *circular form of DNA found in bacteria that carries extra genes, not found in all strains of a bacterial species. (can carry genes for antibiotic resistance or virulence (disease causing))(can be easily transferred from one bacteria to another (naturally and manmade))*

10) **Virulence =** *describes the ability to transmit disease. A virulent strain of a bacteria is a strain that can cause disease.*

Teacher's guide

Student Activity #2 Answer the following questions based on the information in the attached article "Plasmids, Pasteur, and Anthrax"

1) Name 3 things that have led to the decline of anthrax in humans in the US during the 1900's.

Vaccines for people who are at risk of exposure

Better working conditions

Less exposure to contaminated animal products

2) In 1877 Robert Koch was the first to demonstrate that *a specific bacteria was responsible for a particular disease.*

3) Louis Pasteur showed that organisms grown at high temperatures have *decreased virulence* and were capable of producing *immunity*

Pasteur wanted to know why bacteria grown at elevated temperatures was no longer virulent (disease causing). Thanks to advances in molecular biology and biotechnology, we now know the answer to this question.

4) Why can't heat attenuated bacteria cause disease? *The heat destroys a DNA plasmid that produces the toxin that makes people sick.*

"The History of Antibiotics"

What are antibiotics? Antibiotics are chemicals that will stop the growth of or kill certain types of bacteria. It is important to realize that different types of chemicals kill different types of bacteria. Therefore, an antibiotic that is good for one type of bacterial infection may have no affect on a different type of bacteria. In general antibiotics have no affect on viral infections and therefore should not be prescribed for things like the common cold or the flu, which are caused by viruses. The more we use antibiotics the more we select for antibiotic resistant strains of bacteria.

Many different types of fungi produce antibiotics. Scientists are constantly searching for new chemicals to use in the fight against pathogens. Many drug companies have research teams collecting rare plants, microbes and fungi from the rainforest and the ocean in hopes of finding and producing new antibiotics. Loss of biodiversity in these areas due to pollution, deforestation, and habitat destruction could mean the loss of millions of potential antibiotics and wonder drugs.

The first mass-produced antibiotic was discovered in 1929, by Alexander Fleming. He was studying staphylococcus bacteria in the lab by growing it up in petri dishes. Quite accidentally one of the plates became contaminated with a green fungus (similar to the green mold that grows on bread). He noticed that the bacteria couldn't grow in the presence of the mold. Many years later a chemical produced by that penicillium mold was mass-produced and used to treat a wide variety of diseases caused by bacteria.

Antibiotics, considered the wonder drugs of the 20th century, are becoming alarmingly less effective in the treatment of diseases. Many of the bacteria that were once killed by antibiotics have developed resistance to the antibiotics. In order to understand how bacteria have been able to adapt to changes in their environment, we must review a little of what we know about evolution. Evolution is a change in a population over time that occurs because:

- There is variation within a population
- The organisms best adapted to the environment will survive
- The survivors of a generation will reproduce and pass those favorable genes on to the next generation

Now if we put that into the context of a bacterial colony growing in the presence of an antibiotic, we realize that only the 1 or 2 bacteria that are naturally resistant to the antibiotic will survive and live to repopulate the next generation of bacteria. Because bacteria reproduce so quickly, if one has resistance, it can become many in a relatively short period of time.

Unfortunately the widespread and often improper use of antibiotics over the past few decades has resulted in a serious problem. Many bacteria have become resistant to antibiotics that once killed them. Resistant bacteria make the treatment of infections very difficult. Ear infections in children, sinus infections, tuberculosis, and bacterial pneumonia are a few of the illnesses and conditions that have been affected by the threat of bacterial resistance. First choice and often second choice antibiotic therapies are no longer effective leading to fewer options for patients with dangerous and potentially life threatening diseases.

www.infectionspotlight.com/resistance/BacterialResistanceABriefHistory.jsp

Student Activity #3 Answer the following questions based on the information in the attached article "History of Antibiotics"

1) What do antibiotics do?
   Kill bacteria or prevent them from growing

2) How are antibiotics obtained?
   By isolating chemicals from plants, fungi, and microorganisms

3) Why are drug companies buying up portions of the rainforest?
   In hopes of finding new antibiotics from the huge variety of organisms found in rainforest ecosystems

4) What did Alexander Fleming discover?
   The antibacterial properties of penicillin

5) What are 3 things that must occur in order for an evolutionary change to occur in a population?
   a) variation within the population

   b) survival of the fittest

   c) reproduction of the fittest

Lesson #4 Extension for AP Biology
Scientific research: Anthrax sample exercise computer lab

Objective: This exercise was designed to teach students the importance of computers in analyzing, finding, and using data produced by the Human Genome Project.

- Students will use PubMed to gain information about an organism (Bacillus anthracis),
- will use the NCBI (National Center for Biotechnology Information) homepage to find out about and access DNA sequences from that organism,
- and will do a BLAST search to see if there are any other organisms with similar sequences.

After completing this activity students should have a greater appreciation and improved understanding of genetic diversity.

Procedure:

Part 1

Scientists often begin researching a topic by searching PubMed. PubMed is a free online service that can be used to browse online scientific journal references and abstracts. All of the information in the journal articles in the PubMed database are considered reliable because they have been peer reviewed. (Nothing gets published in a scientific journal unless it has been reviewed and scrutinized by a team of scientists in the field.) **In this exercise you will try to access information about the virulence of the species of bacteria responsible for causing anthrax.**

Question: What can you find out about the pathogenicity and virulence of Bacillus anthracis?

1) We will want to consult the primary literature to answer this question, so we will use PubMed to browse online journal references and abstracts.
2) Go to the NCBI (National Center for Biotechnology Information homepage on the web (www.ncbi.nlm.nih.gov/)
3) Click on the menu item that says ‘PubMed’ (you will find it at the top of the page just over the search box)
4) Enter ‘anthrax virulence’ into the text entry box next to ‘Search PubMed for’ and click ‘Go’.
5) A list of your results will come up. We want something that looks like it has something to do with the cause of anthrax virulence.
6) Click on one of the articles to see an abstract of the paper.
7) Repeat the exercise using different search terms such as ‘anthrax toxins’, etc…

You’ll soon find out that the more you learn about the topic – the more questions you have. You can now narrow your search to ask more specific queries.

After reading several abstracts you have obtained using PubMed, you should have some idea of how scientists search the literature for published information.

What did you learn about anthrax virulence?

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Part 2: (Scientists can also learn about the taxonomy of different organisms by using the NCBI homepage. When working with an organism it is essential to know as much as possible about that organism's phylogeny (evolutionary tree). Similar organisms have similar characteristics and similar genetic sequences.)

Question: What can you learn about the taxonomy of B. anthracis?

1) go to the NCBI homepage (www.ncbi.nlm.nih.gov/)
2) Click on the menu item that says 'Taxonomy'
3) Enter 'Bacillus anthracis' in the empty text box and click 'go' to initiate the search
4) A results page will come up listing the bacteria. Click on the blue link on the name of the bacteria to go to the page for Bacillus anthracis.
5) The lineage section at the top of the page describes the phylogeny of the bacteria. Click on the last subgroup 'Bacillus cereus group'.
6) This will show you a list of closely related bacteria species. Clicking on the names of the bacteria will bring up additional genetic information, including the DNA sequence if it has been done.
7) Notice that one of the bacterial species that resembles Bacillus anthracis is Bacillus thuringiensis (Bt). So what????

Read the article "Friend or Foe" to find out why this may be of concern.

1) What is Bt used for and why? *It is used as an insecticide because it produces toxins that kill insects*
2) What makes Bt, B. cereus, and B. antrhacis different? *Their chromosomal DNA is the same but they have or are missing different plasmids*
3) What conditions are necessary for plasmids to be swapped? *Bacilli must be in the growth phase*
4) Now try a google search (to look for more general information) to find out if and why they are spraying Bacillus thuringiensis in the Adirondacks.
   What did you find? *Hopefully students will realize that they are spraying to kill black flies. I chose the Adirondacks because that is where we live I would suggest asking them to find it in their area.*

Part 3 Genomics is the comprehensive study of whole sets of genes and their interactions. Plasmids are extra pieces of bacterial genes that are not part of the chromosomes. They can replicate on their own and are capable of jumping from one organism to another. If you were a scientist that had identified a plasmid found in B. anthracis that seemed to be linked to virulence you might want to take a closer look at the genes and find out if any other types of bacteria had the same types of sequences.

In this part of the lab you will use an online database to search for the sequence of bases on the B. anthracis virulence plasmid pX02. You will then do a BLAST search to find out if any other organisms contain the same plasmid.

1) Go to the NCBI home page (www.ncbi.nlm.nih.gov/)
2) In the drop down box next to search select 'genome' then type in 'Bacillus anthracis virulence plasmid pX01'
3) You should get some references – select the blue reference # for the complete sequence for the plasmid (pX01)
4) If you are lucky you should now see a map of all of the genes on the plasmid –Let's look at one of the genes on the plasmid by selecting (pX01-142).
5) You should now see a blue line underneath what looks like a ruler representing the the gene you selected – select the entire sequence (by clicking on the blue line and then clicking on the + sign that should appear to the right) until the entire blue line is selected.
6) You will now see the sequence of bases that make up the gene on plasmid X01.
7) Notice the letters that appear below the bases. What do you think they represent?
   The amino acids that each codon codes for.

Now we can see if any other organisms have the same gene by doing something called a BLAST search.

8) First we need to be able to copy the sequence. In order to do this we must go up into the search box at the top of the page and change the 'Display' to "FASTA" then select 'text' in the dropdown list next to 'send to' then select send to.
9) Now you should see only the bases. Highlight the bases only by clicking and dragging and then select Edit Copy (make sure you don't highlight the first line which is a description of the sequence – just the bases)
10) Go back to the NCBI homepage. (www.ncbi.nlm.nih.gov/)
11) Finally we are ready to do a BLAST search!!! Select BLAST at the top of the page
12) Select 'Standard nucleotide-nucleotide blast'
13) In the Search box paste your sequence (under Options Expect change to 0.01) Then at the bottom of the page hit BLAST.
14) You will now have to wait while your computer searches all the databases for DNA sequences that are the same or similar to the one you are looking for.

RESULTS

You should end up with a list of hits after a few minutes. In order to look more closely at the sequences that matched click on one of them.

- Look at the number of identities: this will tell you what percent of the sequence is the same and what percent is different.
- Plus/plus means that the strands both read in the same direction
- Plus/minus means that they read in opposite direction
- You can also look at the bases together and see where they do and don’t match up

Student Activity #1 Guided notes
Student's guide page 1

Definitions for immunity

1) **Pathogen =**
Examples:

2) **Antigen =**
Examples:

3) **Infection =**

6) **Disease =**

7) **Immunity** =

Natural (innate) immunity =

Examples of natural immunity =

Acquired (adaptive) immunity =

Examples of acquired immunity = 1)

6) Antibodies =

11) Antibiotics =

12) Vaccines =

13) Bacterial plasmids =

14) Virulence =

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    d)

    e)

    f)