

**The Immune System: Defenders of our Health
Who are "those guys anyways"?**

Betsy Withum
Coolidge Middle School
Reading, MA 01867
withum@hotmail.com

Mentored by Rachel McLoughlin, Ph.D.
Brigham & Women's Hospital
Harvard Medical School
Channing Lab

Funded by the American Association of Immunologists

Table of Contents

I. Overview	Pages 1
II. Science Background	Pages 2-10
III. Materials and Equipment	Page 11
IV. Time Requirements	Page 12
V. Student Prior Knowledge and Skills	Page 13
VI. Student Outcomes	Page 14
VII. Learning Objectives/ Mass. Curriculum Standards: 2006	Page 15-16
VIII. Daily Lesson Plans	Pages 17-27
IX. Student Handouts for Lesson Plans	Pages 28-50
X. Templates for ELISA Practice	Pages 51-53
XI. Rubric for Life of a Neutrophil	Page 54
XII. Reference List	Page 55

I. Overview

The human body is constantly being invaded by foreign substances, which are known as antigens. These antigens can cause a multitude of reactions from mild to fatal. How the human body discovers, interprets, identifies initially, reacts and then stores the information for future protection against these antigens is the function of the Immune System.

This curriculum unit will introduce students to the basics of the immune system. Students will learn how the different types of cells perform specific functions and understand how these cells will seek out and engulf bacteria. The content of this curriculum unit is designed to have as much student interaction or "hands-on" activities as possible. The need for the high level of interaction is imperative. The material being introduced is highly technical and specific. It is important for the students to be introduced to this information as they go through the activities. This should offer students an opportunity to grasp the basic idea that the immune system is a highly complex system with specific structures. These activities are designed to allow creativity while also requiring a higher level of intellectual demand. At the end of this unit, the students should develop a basic understanding of the types of cells in the human immune system and the cellular processes that occur to maintain a healthy body.

The series of lessons in this unit can be taught as an introduction to the various Kingdoms with the connection of Animalia and Monera in our world. This is also an excellent unit to reinforce laboratory skills, such as microscope skills, observation, and data collection. Students will use ELISA testing and analyze data as it relates to the human body and health. Another powerful connection for this unit is to use it in a unit on the human body systems. This unit specifically tailors itself well to the Immune System and how it functions to keep us healthy.

The ELISA test is done at the end of the unit to reinforce the concepts of the immune system as a highly complex system that works constantly to protect us. When the immune system is under attack, one goes to the doctor. At this point, this is where the students experience ends. By doing the ELISA testing and additional activities embedded within this unit, students will explore what is happening in the human body after visiting the doctor. The goal of this unit is to allow the students the opportunity to model the procedures of a professional lab. This will assist them to understand how the doctors diagnose patients and what happens in the human body during a sickness. Students of the middle school level tend to have difficulty understanding phenomenon that cannot be directly observed. Running a mock ELISA will allow the students to indirectly observe cellular level phenomenon.

II. Science Background

A. What is Immunology?

The human body is composed of millions of cells with each type of cell doing a specific job. All living things interact with each other on this planet. Some of these interactions are helpful. Some interactions go unnoticed and others are extremely harmful. If the interaction is harmful there is an intricate set of occurrences within the host or harmed organism that will happen. When this damaging state occurs in our bodies, many chemical and cellular changes occur that require an intricate blueprint of procedures. These complex and sometimes mysterious cellular procedures have become much more available for scientists and researchers to understand with advancements in modern technology.

Immunology is the study of how our body protects us from foreign or invading organisms, specifically viruses, bacteria, parasites and protozoan. In addition to the job of protection from outside agents, the immune system works to develop specific immune responses to identify our own aberrant cells.

When a person is exposed to a foreign invader, the body sets up an immune reaction. These reactions occur immediately in the body. The mechanisms of these reactions are the same whether it is a simple splinter or exposure to a deadly pneumonia bacterium. Each reaction will occur on a different scale during the attack. The body sends out its attack force to protect us from infection. The attack force consists of a specific chemical and cellular organism designed to do one job. This job is to seek out the bacteria, identify the bacteria, wage war, ingest the bacteria and protect us from future infection. These situations are happening constantly in the human body.

B. Bacterial Infection

Bacteria are unicellular micro-organisms and can be found in every habitat on earth. In our day to day operations, we are constantly exposed to different types of bacteria. The majority are harmless. When we encounter a pathogenic, a biological agent that can cause disease or illness, bacteria that gains entry into our bodies, it can cause an infection. Antibiotics are the main form of treatment against bacteria infection. However, some bacteria have become resistant to the antibiotics that are currently available. As a result of this resistance, the treatment methods are no longer effective. The body succumbs to bacterial infections at an alarming rate. One form of bacteria in particular, *Staphylococcus Aureus*, has been the annoyance of many hospitals. This particular species of bacteria can be found living in a large percentage of the human population with very few problems. The bacteria primarily live in the nasal passages of humans. Normally, they do not cause us any harm unless the protective barrier of the skin is broken. Bacteria gain entry into the body tissues. This is time when exposure to these bacteria can be extremely harmful.

C. How does the immune system react?

The mechanism by which the body starts its response to an attack by an invading organism is extremely sensitive. First, the protective layer of the skin has to be breached for the immune system to start its chemical response. Depending upon what chemicals are activated will then determine what cellular responses are initiated.

Our immune response has developed into a two-fold system. There are two ways for a reaction to occur. First, there is an immediate or the **"non-specific"** response. The non-specific response begins its defense immediately against invading organisms. This occurs no matter whether or not the body has ever been exposed to this agent before. The non-specific is also called the **"Innate Immune Response."** The second response is a time delay system, called the **"specific"** response. The specific response is dependent upon prior exposure to the invading substance to start the response. The prior exposure gives the system a chance to "identify" the attacker and mount a "specific" response against it. The time dependent specific response system is also known as, the **"Adaptive Immune Response."**

In a healthy individual, the innate response is initiated genetically and we carry it our entire lives. This system is continually ready to respond. The adaptive response develops over a lifetime of adaptation to specific pathogens. The reactions of the innate system are a prerequisite for the development and functionality of the adaptive system. These systems together provide a remarkably effective defense system. Even though we spend our lives surrounded by pathogenic microorganisms, we become ill infrequently. Many infections are dealt with quickly and efficiently by the innate system. Others need the reinforcement of the adaptive system to successfully "zap" the pathogen and give us lifelong immunological memory.

D. The Components of the Immune System

The immunological attack force has many participants. The initial line of defense is the protective layer of the skin. Our skin has evolved to be a barrier for entry of any pathogens into our bodies. Other components of defense include certain molecules found on the surface of the skin that starve the invader of essential nutrients along with tears, mucus, and saliva. These players all operate within the innate immune response.

At the cellular level there are many players. It all begins in the long bones, particularly the femur where the white blood cells or leukocytes are made. The bones of the human body contain material known as marrow. This marrow is a producer of blood cells. The leukocytes can be divided into two categories, the myloid, which are part of the innate immune system, and the Lymphoid, which are part of the adaptive immune system. The myloid cells include macrophages,

dendritic, neutrophils, basophils, eosinophils, and mast Cells. The lymphoid cells are T-Cells, B-Cells, and natural killer cells.

E. *The Family of White Blood Cells*

1. **Macrophage**

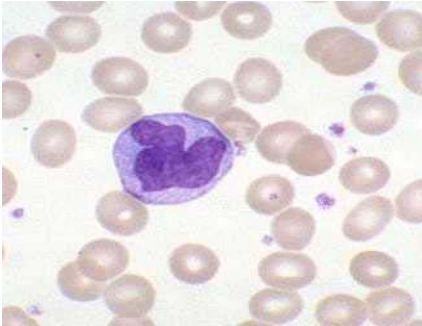


Figure 1: Macrophage image from:

http://www.bloodlines.stemcells.com/img/Metcalf_Fig10_4.gif

Macrophages play a crucial role in the immune system. The word macrophage comes from the greek word for "big eaters" *macros=large, phagein=eat*. They originate from monocytes which are circulating in the blood and then differentiate into macrophages within the tissue. They are primarily phagocytic cells. This means that they seek to engulf and digest the pathogen. They also act to stimulate other immune cells such as lymphocytes to respond to the pathogen. Macrophages function in both the innate and the adaptive immune responses.

2. **Neutrophils**

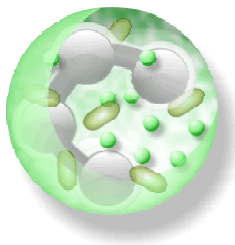


Figure 2: Neutrophils image from:

<http://www.blobs.org/science/cells/neutrophil.gif>

The Neutrophil is the most common type of white blood cell. This commonality makes them particularly useful in fighting any threat to the body. The Neutrophil is a bit of a sleuth. It follows a chemical trail by the process of chemotaxis or "smelling" the chemical. It consumes or "eats" the bacteria by a

process called phagocytosis. Neutrophils are multi-nucleated, meaning they have more than one nucleus. They are key players in the innate immune system.

3. Eosinophils

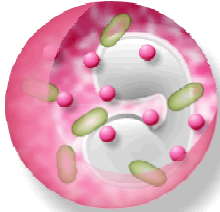


Figure 3: Eosinophils image from:

<http://www.blobs.org/science/article.php?article=16>

The Eosinophil functions to control parasitic infections and allergic reactions. However, these are also the cells that can cause problems for you. If they start producing chemicals, problems can occur in the airways to cause disorders such as asthma. Eosinophils are part of the innate immune system and make up about 1-5% of the total white blood cells.

4. Basophil

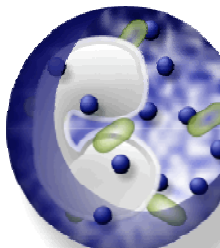


Figure 4: Basophils image from:

<http://www.blobs.org/science/article.php?article=16>

The Basophil is an extremely rare blood cell, representing approximately 0.3% of blood cells. They are involved with allergies. They contain the chemical element histamine, which causes the symptoms of an allergic attack.

5. Dendritic Cell

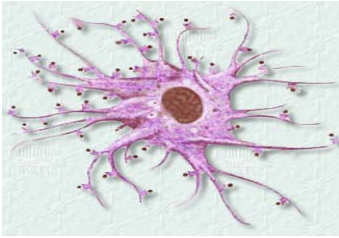


Figure 5: Dendritic cell image from <http://www.immusystems.de/assets/images/dendriticcell2.png>

The main function of the dendritic cell is to process foreign antigen material and present it on the surface to other cells of the immune system. They tend to migrate to the lymphoid tissues where they interact with T and B cells to shape the adaptive immune system. Dendritic cells are present in small quantities in tissue that are in contact with the external environment, such as tissues of the skin, lining of the nose, lungs and digestive tract.

6. T-Cell

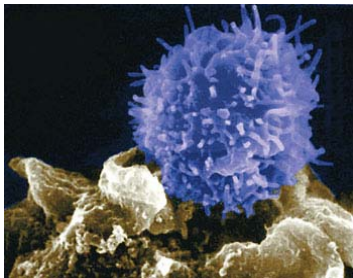


Figure 6: T-Cell image from: <http://www.lbl.gov/Publications/Currents/Archive/view-assets/Oct-03-2003/t-cell2.jpg>

The T-Cell develops in the thymus gland. This is why it is called the T-Cell. T-cell has many functions. It forms part of the adaptive immune response. There are numerous classes of T-cell that have different roles to play in the specific immune response. T-helper cells secrete specific proteins called cytokines that facilitate or "help" the immune response. Cytotoxic T-cells destroy virally infected cells and tumors. T-cells retain memory of an invading foreign antigen and help body respond to future infections.

7. B-Cell

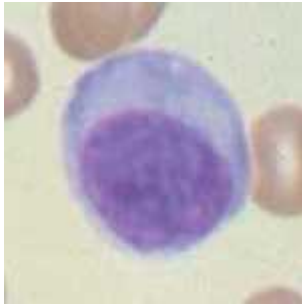


Figure 7: B-Cell image from <http://upload.wikimedia.org/wikipedia/commons/1/11/Plasmacell.jpg>

The B-Cells are produced in the bone marrow. This is why it is called the B-cell. They circulate in the blood and lymphatic system where they perform immune surveillance. This means that the B-cell looks for any foreign substance which may have invaded the body. Their principal function is to produce antibodies. The antibodies recognize invading foreign antigens and assist in their destruction.

8. Natural Killer Cells

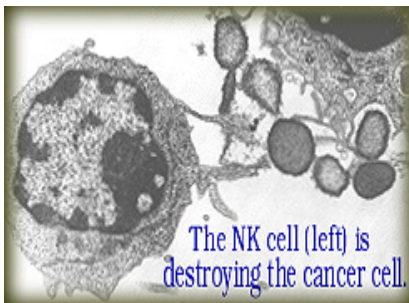


Figure 8: Natural Killer Cell image from <http://www.discovertransferfactor.com/NKcell.jpg>

The Natural Killer Cell plays a major role in the rejection of tumors and virally infected cells. They bind to their target and secrete their lethal cocktail. This bursts the target cell's membrane. They are a major component of the innate immune system.

F. The key player in the innate immune response: The life of the Neutrophil

Initially, the skin with its mucous, salt, hair and dense structure protects us from foreign pathogens. However, there are times when a pathogen can make entry into the body. This is when the battle begins. Initially, an innate immune response

occurs. The process by which the neutrophils are recruited to the site of infection is one of the most important functions of the innate immune system.

The neutrophil is a unique cell. It has a very specific function to seek out and kill bacteria. The neutrophil develops a multinucleated structure deep within the marrow of the long bones. They are abundant in blood, but are not present in healthy tissue. They are recruited to the site of infection by a process known as **chemotaxis**. This generally occurs in response to the production of **chemokines** at the infection site. Once they reach the site of the invading organism, the neutrophils engulf and destroy them in a process known as **phagocytosis**. When this is occurring, it is visible to the naked eye. We see "pus" or a combination of deceased bacteria and neutrophils. The pus is where the "battle" is taking place. If the neutrophil does not have the opportunity to fulfill its phagocytic role of defense, it must undergo a programmed death known as **apoptosis**. The life span, or time to respond and battle, is approximately 3 days.

1. Chemotaxis

Chemotaxis is the process of cell movement towards its target site. The neutrophil is the first type of cell to be recruited to the site of infection by the generation of a chemotactic gradient at the infection site. The neutrophil's success at reaching the site of infection depends upon this chemotactic gradient produced locally at the infection site. This gradient can be activated by a "chemotactic" substance that the invading bacteria itself produces directly. An example of a chemotactic substance produced by bacteria is fMLP. This is a potent chemotactic factor that recruits the neutrophil. The gradient can also be achieved by the production of **chemokines** by the cells present locally at the site of the infection. For example if a cut in the skin gets infected, the cells which make up the skin or i.e. keratinocytes will can produce the chemokines. Chemokines are powerful chemicals that serve to guide specific cells to the site of infection. Specific chemokines recruit specific cells from the blood stream. There are two main families of chemokines *CXC* and *CC*.

Chemokine family	Target cell for recruitment	Examples
<i>CXC</i>	Neutrophils	<i>CXCL8</i>
<i>CC</i>	Monocytes, T-cells	<i>CCL2</i>

Table 1: Chemokines.....

The level of *CXC* chemokines produced at the site of infection can vary depending on various factors such as the type of invading organism and the strength of the body's immune system. High levels of *CXC* chemokines will recruit high levels of neutrophils. Low level of *CXC* chemokines will recruit low levels of neutrophils. This should translate into an efficient and a non efficient clearance of the invading bacteria respectively.

The immunological test which we will be studying in this unit is called an ELISA. We will use it to measure the levels of CXC chemokine present at an infection site as a reflection of immunological response to bacterial infection

2. Phagocytosis

Now that the neutrophils have been recruited to the site of infection, they can begin their job of destruction. The neutrophil begins to recognize the bacteria through a series of cell surface receptors. Once identification is made by the neutrophil, the process of phagocytosis begins. This is an active process. The pathogen becomes engulfed by the neutrophil and a phagosome is formed inside the cell which is the site for killing the bacteria. The neutrophil release a variety of toxic products into the phagosome. Most notably, nitric oxide (NO) and hydrogen peroxide (H₂O₂) are directly toxic to bacteria. These toxic products are known as reactive oxygen species and the generation of these products is known as "respiratory burst".

3. Apoptosis

Once the bacteria are destroyed inside the neutrophil, it has fulfilled its obligation and it then dies by a process known as "apoptosis". The process of apoptosis is known as programmed cell death. As mentioned previously the neutrophil has a very short life. From the moment it was formed, it was programmed to undergo eventual cell death. Apoptosis involves a series of biochemical events inside the cell. It results in the safe disposal of the dead neutrophil which contains dead bacterial particles. This allows for no further damage to surrounding healthy tissue.

III. Materials and Equipment required for this unit.

A. *Specific Science Equipment*

-ELISA kit purchasable from Carolina Supply \$90.00/32 students*
www.carolina.com, catalogue # 211248

(This kit was the most appropriate for middle school, but there are other supply houses that distribute ELISA's) such as:

1. Bio-Rad at www.biorad.com, catalogue. # 166-2400EDU. Pricing for this kit requires a school/teacher to register and login.
2. Edvotek at www.edvotek.com, catalogue # 269 per 10 groups. Full brochure is downloadable at www.edvotek.com/pdf/269.pdf
3. SK & Boreal at www.sciencekit.com, catalogue #www0175083 per 10 groups of students.

- Microscopes
- Blank slides
- Prepared slides of bacteria (if difficulty preparing own slides)
- Petri Dishes
- Pipettes (eyedroppers)
- Agar
- Computer with internet access
- Illustrations/Examples of living kingdoms for comparison

* Price is based on 2007 catalog.

B. *General Class Supplies*

- general arts & crafts supplies available at any crafts store
- colored paper
- scissors
- glue
- tape
- markers, colored pencils

IV. Time Requirements

1. It should be anticipated that the unit will take approximately 10-12 single (50 minute) class periods. This is variable depending upon the prior knowledge and skills of your students. In particular, students should be familiar with cells, cell types and cell organelles.
2. Each activity is designed to be 1-2 periods long. It is assumed there will be assistance for the students on IEP or 504's as the language/terminology can be a bit overwhelming. If the terms become too overwhelming feel free to adjust the terms.
3. Also, it is expected that there is a fair amount of comfort working in the lab with microscopes, making slides, Petri dishes, cultures, observing with patience and recording observations.
4. It would be advisable to have small, frequent assessments throughout the unit as there are many complex terms. These assessments can be vocabulary and open response quizzes. The primary goal of your assessments is to monitor what your students are understanding and what you need to do next. A general rubric for the neutrophil project has been included. You may want to tailor this rubric to the project that you select for your students. Other examples of projects include PowerPoint presentations, story books, or posters.

V. Student Prior Knowledge and Skills

It should be anticipated that the students have an operative knowledge of cells, types of cells and basic cellular structure. They should know that different types of cells form and the formation tends to determine cellular function. Students should be comfortable with the systems of the human body, specifically the circulatory and the skeletal system. Since this unit involves the immune system, students should know which components are in blood and the difference between white and red blood cells.

Basic laboratory skills are required for this unit. This includes the ability to read and follow multi-step directions, the ability to operate general laboratory equipment and properly measure with graduated cylinders, eyedroppers, and triple beam balances. The development of good laboratory habits is an extremely important aspect of middle school laboratory work. The majority of work in this unit will involve microscopes, so familiarity with proper handling, setting up, preparing slides, focusing, observing and identifying structures is extremely important.

VI. Student Outcomes

In a healthy immune system the maintenance of defense is a complex system of checks and balances among the white blood cells and the infecting structure. Upon infection, the chemical messenger, chemokines, send out messages to the recruit the neutrophils for action. The neutrophils now charged for action, race to the site of infection and perform phagocytosis or "eat" the bacteria. The infected site is now clear of infection. The battlefield is left with dead neutrophils and bacteria cells. We call this, "pus". Any remaining neutrophils are naturally programmed to die or undergo "apoptosis". If the neutrophils do not go through apoptosis, they would start attacking the organisms good cells. This leads to a whole new series of problems under the title of "autoimmune diseases".

The ELISA test is the standard lab procedure to measure the amount of specific antigen or antibodies. The ELISA test takes time and requires many procedures or protocols. In the classroom setting, the time requirements are not available. Given the time constraints, a modified ELISA can be run to show the basics of this technique.

This curriculum is looking at the "process" of the ELISA without the specificity of lab protocols. We are looking at a simple color change to show the different antigens or antibodies produced. The ELISA that the students will follow will be completed in one class period. They will look for a color change to conclude if the patient is experiencing an immune response to a pathogen. With the results of this test, the students should be able to make the connection between bacterial or some foreign invader and the efficiency of a healthy immune system. The activities in this unit lend themselves wonderfully to discussions of the lifestyle choices teenagers make regarding cleanliness and the sharing of personal items, such as drinking straws, makeup, hairbrushes, or clothing.

VII. Learning Objectives and State Standards

A. *Learning Objectives*

- Students will learn about Bacteria as unique living organisms through observations using microscopes, prepared slides and cultures taken from their surroundings.
- Students will learn to take samples and prepare these on an agar Petri dish
- Students will become familiar with the characteristics of bacteria based upon criteria of shape, prokaryotic, healthy and unhealthy species
- Students will learn the role bacteria play in a healthy system and the role bacteria play to make one sick.
- Students will learn what the immune system is and how it functions to keep us healthy.
- Students will learn what cells (neutrophils) and chemical messengers are involved in the healthy system.
- Students will understand the cellular process that occur in the body to maintain health.
- Students will conduct research and create models of their research
- Students will apply their knowledge of the immune system by performing an experiment using a purchased ELISA kit.
- Students will be able to identify a system that is positive/negative for chemokines.

B. *Connections of the Immune System to the Mass. State Curriculum Standards as of Spring 2006*

- In grades 6-8, the emphasis changes from observation and description of individual organisms to the development of a more connected view of biological systems. Students in these grades begin to study biology at the microscopic level, without delving into the biochemistry of cells. They learn that organisms are composed of cells and that some organisms are unicellular and must therefore carry out all of the necessary processes for life within that single cell. Other organisms, including human beings, are multicellular, with cells working together. Students should observe that the cells of a multicellular organism can be physically very different from each other, and should relate that fact to the specific role that each cell has in the organism (specialization). For example, cells of the eye or the skin or the tongue look different and do different things. Students in these grades also examine the hierarchical organization of multicellular organisms and the roles and relationships that organisms occupy in an ecosystem. As is outlined in the *National Science Education Standards*, students in grades 6-8 should be exposed in a general way to the systems of the human body, but are not expected to develop a detailed understanding at this grade level. They should develop the understanding that the human body has organs, each of

which has a specific function of its own, and that these organs together create systems that interact with each other to maintain life.

At the macroscopic level, students focus on the interactions that occur within ecosystems. They explore the interdependence of living things, specifically the dependence of life on photosynthetic organisms such as plants, which in turn depend upon the sun as their source of energy. Students use mathematics to calculate rates of growth, derive averages and ranges, and represent data graphically to describe and interpret ecological concepts.

Learning standards for grades 6-8 fall under the following eight subtopics: *Classification of Organisms; Structure and Function of Cells; Systems in Living Things; Reproduction and Heredity; Evolution and Biodiversity; Living Things and Their Environment; Energy and Living Things; and Changes in Ecosystems Over Time.*

C. Assessment of Learning Objectives

- The assessment of the curriculum will be done via the **Life of the Neutrophil Project** and the **ELISA**.
- As the students complete the worksheets, as available in the handout section, it is recommended that these be given value (check, check plus or check minus).
- The rubric for the project is dependent upon the value one feels is necessary to assign.

VIII. Daily Lessons Plans

Class 1 Lesson Plan: Introductory Discussion

Materials and Equipment:

- Students will need notebook, pen/pencil

Teachers Notes:

This is a discussion of what the students know and don't know about the immune system. Let them try to give you and each other as much information as possible. Record the students' responses on the board during the discussion in class. Have the students take notes from the board into their notebook.

Discussion questions can include:

How do you feel when you are sick?

What happens to your body when are sick?

What makes you feel "better"?

What is the Immune System?

Where is it found?

What does it your immune system do for you?

How do you know if you have an Immune System?

How can you tell when your immune system is working?

At this point you may want to direct the discussion toward the types of immune responses, such as a visible wound healing, pus, fever, feeling tired, sore throat, coughing, body aches, etc.

What are the parts of your Immune System?

Where would you go in school if you feel sick?

What does the school nurse or doctor do when you go to the office?

Why does the doctor feel your neck, take your temperature and blood pressure?

Class 2 Lesson plan: Internet search for terms of the Immune System**Materials and Equipment**

-**Handout 1: My Immune System- Terms to be researched**

- colored pencils

-computer with Internet access

(If computer access is unavailable, you can supply library resources such as encyclopedia sets or textbooks for definitions.)

-Here is a starting point of potential websites that are readable for middle school:

http://www.kidshealth.org/parent/general/body_basics/immune.html

http://www.kidshealth.org/teen/your_body/body_basics/immune.html

www.wikipedia.org

Teachers Notes:

1. Students will be given vocabulary that should be researched with supervision. It is suggested, if available, to use the internet as this will give the most up to date information. Make sure that the students are not "copying" definitions as they can be a bit overwhelming for some.
2. A key concern is the websites that the students will use. Some can be quite difficult for middle school students. If possible try to have biology textbooks available to use instead of the internet, it might simplify definitions for the students.
3. The students should define the terms in their "own words" and include illustrations. The illustrations can be from the internet or depending upon the skill of the student, they may be hand drawn. Middle school students like to draw so a supply of colored pencils, markers, etc. is helpful.
4. Be open to the students illustrations and check to see if the illustrations shows the students understanding of the terms.

Classes 3, 4, 5 Lesson plan: Science Background on the Immune System

Materials needed:

- Handout 2:** Science Background Information - The Immune System A-F
- Handout 3a:** Components of Immune system note taking chart
- Handout 3b:** The Key Player: the Neutrophil note taking chart
- Images of leukocytes that the students should obtain as a homework assignment or if possible they can be purchase separately. (A good source is Carolina Biological at www.carolina.com. See note 3 for further reference.)

Teachers Notes:

1. Make copies of the **Science Background Handout 2** (color copies if possible) for each student to keep in their notebook and to use as future reference

2. In class read the Science Background as a group, use the leukocyte pictures to compare/contrast actual microscopic images from artistic. If you are not able to obtain good color pictures it might be a great idea to challenge the students for **HOMEWORK** to get pictures that can be brought in and then placed around the room for display. Discuss form and function of cells. Stop periodically to explain and answer any questions.

3. One source of Leukocyte images can be obtained from Carolina Biological, This is a wall mount that is approximately \$185.50. item #56-6835. These prices are from the 2006/2007 catalogue, check the updated catalogue at www.carolina.com and search item #566835 Human Blood Cell Types.

4. While the class is reading/discussing stop periodically to have the students to highlight the reading and fill in the definitions on the note taking chart, **Handout 3**, that has been provided.

5. After reading the background go back to the **Handout 3** and go over the definitions with the class to ensure accuracy and comprehension.

6. There are many excellent summarizing activities one can do to check in on not only understanding of the concepts so far, but the depth of understanding. This is a great time to check for and misunderstandings also. Possible ideas: students can develop "a dictionary of terms that include not only def. but pictures, a Jeopardy style game can be played, if available students can create brochures of the terms using a publisher program, etc.

Class 6 Lesson Plan: Webquest of the chemokine/neutrophil connection**Part 1****Materials Needed:**

- Computers with internet access
- Some reward ie. Homework passes, extra credit, tickets for prizes, etc.

Teachers Notes:

1. There are many sites available to the students that demonstrate the **chemokine/neutrophil** connection. Explain to the students the difficulty in getting these images as the **neutrophil** is invisible to the naked eye and that **chemokines** are chemicals/molecules that cause a reaction.

2. Let the students explore these sites and then challenge them to find others that illustrate this connection or show neutrophils, (leukocytes). I tend to make a competition as to who can find the best, the most colorful, the coolest, the grossest, and whatever categories you find appropriate. I give the students tickets that can be later redeemed for prizes, but a homework pass, extra credit etc.

3. A starting site that demonstrates this wonderfully can be found at this address:

1. You tube.com

2. Type the following two images into the search

a. <http://www.youtube.com/watch?v=ZUUfdP87Ssg>
(neutrophil undergoing chemotaxis)

b. <http://www.youtube.com/watch?v=fpOxgAU5fFQ>
(neutrophil initiating phagocytosis)

Class 7 The Neutrophil/Chemokine Connection

Materials Needed:

- Handout 3c The Neutrophil/Chemokine Connection Chart
- Notes from Handout 3a The Components of the Immune System

Teachers Notes:

1. The following chart has the chemokine (chemical messenger) levels pre-determined. The students have to be able to make the connection between high, medium and low levels of chemokines to the levels of neutrophils and bacteria. This is done by drawing in the chart with the amount of neutrophil and bacteria based upon the chemokine levels.

2. The students should fill out their chart and answer the questions. This could be done as a homework activity and discussed the next day.

High Chemokines → High Neutrophils → Low count of Bacteria

Medium Chemokines → Medium Neutrophil → Medium count of Bacteria

Low Chemokines → No Neutrophil → No Bacteria present

3. The analysis questions make the student aware of the immune response and the indicators that the doctor/laboratory will look for before making a diagnosis.

Class 8 Lesson Plan: The Life of the Neutrophil**Materials Needed:**

- arts and craft supplies (colored paper, markers, glue scissors, various sizes of paper, etc)
- Handout 4 The Life of the Neutrophil Assignment**
- Handouts 1-3 as reference**
- Rubric Handout for final project**

Teachers Notes:

1. After viewing the images of the chemokine/neutrophil connection, reading the key players of immunology, the summative assignment will be an "arts and crafts" assignment. This will give the students a chance to show that they have made the neutrophil/chemokine connection.
2. The students will demonstrate visually their interpretation of the neutrophil and how this works in the body's immune system. The students can demonstrate this through a large variety of formats. It is also a good way to check the progress of their understanding.
3. Be open to all levels of interpretation, with the expectation that *SOME* connection will be made that there is both a chemical and cellular component to "staying healthy"
4. The rubric has been attached but the values have been left blank as this will allow values to be placed as the teacher feels is appropriate.

Class 9 Lesson Plan: The ELISA test- General Background

Materials Needed:

- Handout 5: Science background information - ELISA
- Handout 6: Note taking chart on ELISA terms

Teachers Notes:

1. At this time, there should be an excellent general understanding of the immune system and the structures involved in our health. The next couple of assignments will involve the diagnostic tool used by scientists, lab technicians, doctors, etc. to indicate the level at which the immune system is performing.
This level of performance is indicative of your health, whether you are sick from a bacterial invasion or other intruder. The students will be modeling this test initially with a website and paper models before the "real" test is done in class.
2. It is highly recommended that the ELISA reading is done in class together as some of the terminology may be cumbersome. It will also ensure that the key concepts as written on Handout 6 are correctly described.

Class 10 Lesson Plan: The ELISA Website**Materials needed:**

-Computer with internet access. If computers are limited it is possible to work in pairs, ideally it is best to work individually.

-Website: www.biorad.com

1. Search within the biorad site: ELISA Immuno Kit
2. Click on the link for Interactive Immuno Explorer Animation

-Handout 7: Web/Computerized The ELISA

Teachers Notes:

1. The ELISA or Enzyme-Linked ImmunoSorbent Assay is a biochemical technique used in immunology to detect the presence of an antibody or and antigen in a sample. It has been used as a diagnostic tool in medicine and plant pathology. When the ELISA is administered, an unknown amount of antigen is affixed to a surface. Then, a specific antibody is washed over the surface to bind with the antibody. The antibody carries a specific enzyme that has a specific substance added. That is a "marker" that will allow the antigen to be observed. This is usually done so that it will appear under fluorescence.

2. The ELISA that the students will be using will not fluoresce; rather there will be a color change. The degree of color change will indicate the amount of antigen in the sample. There are many steps (protocols) when conducting an ELISA.

3. The web site by Biorad, has a wonderful simulation of the steps. This is illustrated with detailed descriptions. It would be helpful if the students go through once before you hand out the questions.

Class 11 Lesson Plan: Pre- ELISA Practice**Materials Needed:**

- Handout 8:** The ELISA Protocol Practice
- Pencil and Paper
- Scissors
- Glue
- Notes of the Immune System as reference

Teachers Notes:

1. Before the students perform their ELISA, they will practice the steps involved. You may want to make multiple copies of the templates as it may be helpful for the students to glue each step and label what is happening. They can make an **ELISA Book** that has each step with explanation on a separate page.
2. They should be able to see what is happening during the ELISA by using their models of the antigens, antibodies, etc. The ELISA does not allow the students to visually see the process, only the end results.
3. It is sometimes difficult for the more concrete thinkers to make the connection between the visible and the invisible.
4. This checklist and diagrams should be kept in their notebook to use as an additional reference when they are performing the actual ELISA.

Class 12 Lesson Plan: Running the ELISA

Materials Needed:

- Large Wall chart for results (This can be done hand drawn on the board or on a poster taped on the wall)
- Handout 9: Student Procedures
- Handout 10: ELISA Analysis Results
- Handout 11: ELISA Summary and Conclusions
- ELISA kits Purchased from the Carolina Biological.

The ELISA Kit information:

1. The kit contains enough supplies for 32 students working in pairs. It will contain all supplies necessary and as of this date it was available for +/- \$100.00. These prices reflect the 2006/2007 prices. The kit can be tailored in a variety of ways to fit whatever your needs regarding the immune system.
2. A great pre-reference to assure that the kit will fit your needs, can be investigated at www.carolina.com with ELISA in the search box. The site is quite comprehensive and should answer any questions or concerns regarding the ELISA.

Supplies in each kit:

- 4 pipettes labeled
- 1 microtiter plate with specific group A-G labeled
- 4 different colored tubes labeled for each group
- antigens: 1st antibody, 2nd antibody, simulated chromogen
- purple and green colored pencils

Procedure:

1. Each group should be no more than 3-4 students. There will be a total of 8 kits. The kits are labeled as patient A-H.
2. The students should not reuse the pipettes as this will cause a cross contamination of the results.
3. In the different colored tubes, fill 1ml of simulated antigen, 1st antibody, 2nd antibody and simulated chromogen.
4. Label each group's tubes as A-H. Each letter represents patient A-F and the group with G represent the positive control while the group with the H represent the negative control.
5. It is suggested that you have a chart somewhere in the classroom with the color change written down along with a description as to what the color change represents. Dark purple = positive, light purple=false positive and light green=negative.
6. Have the students fill in their data table using colored pencils from the results of their lab.

IX. Student Handouts for Daily Lesson plans

Handout 1.....My Immune System

Handout 2.....Immunology Science Background

Handout 3a.....The Components of the immune system note taking chart

Handout 3b.....The Life of the Neutrophil Note taking chart

Handout 3c.....Neutrophil/Chemokine Chart with Analysis

Handout 4.....Life of the Neutrophil Project

Handout 5..... ELISA Science Background

Handout 6.....ELISA note taking chart

Handout 7.....Web/Computerized ELISA

Handout 8.....The ELISA Protocol Practice

Handout 9..... The ELISA: Student Procedures

Handout 10.....Analysis of your ELISA results

Handout 11.....Summary and Conclusions

Handout 12.....Templates for ELISA Practice

Handout 13.....Rubric for Neutrophil Project

Handout 1: My Immune System

In our new unit we will be studying the way that your body protects you from harmful substances. This unit has **new terminology** that you will be using daily in our class discussions. **Your job now will be to research these terms and then illustrate each new term based upon your definition.** Remember, these definitions should be rephrased into your "own words."

New Term	Definition	Illustration
Immune System		
Innate		
Adaptive		
Bone Marrow		
B-Cell		
T-Cell		
Natural Killer		
Neutrophil		
Macrophage		
Dendritic Cell		

Handout 2: Science Background

A. What is Immunology?

The human body is composed of millions of cells with each type of cell doing a specific job. All living things interact with each other on this planet. Some of these interactions are helpful. Some interactions go unnoticed and others are extremely harmful. If the interaction is harmful there is an intricate set of occurrences within the host or harmed organism that will happen. When this damaging state occurs in our bodies, many chemical and cellular changes occur that require an intricate blueprint of procedures. These complex and sometimes mysterious cellular procedures have become much more available for scientists and researchers to understand with advancements in modern technology.

Immunology is the study of how our body protects us from foreign or invading organisms, specifically viruses, bacteria, parasites and protozoan. In addition to the job of protection from outside agents, the immune system works to develop specific immune responses to identify our own aberrant cells.

When a person is exposed to a foreign invader, the body sets up an immune reaction. These reactions occur immediately in the body. The mechanisms of these reactions are the same whether it is a simple splinter or exposure to a deadly pneumonia bacterium. Each reaction will occur on a different scale during the attack. The body sends out its attack force to protect us from infection. The attack force consists of a specific chemical and cellular organism designed to do one job. This job is to seek out the bacteria, identify the bacteria, wage war, ingest the bacteria and protect us from future infection. These situations are happening constantly in the human body.

B. Bacterial Infection

Bacteria are unicellular micro-organisms and can be found in every habitat on earth. In our day to day operations, we are constantly exposed to different types of bacteria. The majority are harmless. When we encounter a pathogenic, a biological agent that can cause disease or illness, bacteria that gains entry into our bodies, it can cause an infection. Antibiotics are the main form of treatment against bacteria infection. However, some bacteria have become resistant to the antibiotics that are currently available. As a result of this resistance, the treatment methods are no longer effective. The body succumbs to bacterial infections at an alarming rate. One form of bacteria in particular, *Staphylococcus Aureus*, has been the annoyance of many hospitals. This particular species of bacteria can be found living in a large percentage of the human population with very few problems. This bacteria primarily lives in the nasal passages of humans. Normally, they do not cause us any harm unless the protective barrier of the skin is broken. Bacteria gain entry into the body tissues. This is time when exposure to this bacteria can be extremely harmful.

C. How does the immune system react?

The mechanism by which the body starts its response to an attack by an invading organism is extremely sensitive. First, the protective layer of the skin has to be breached for the immune system to start its chemical response. Depending upon what chemicals are activated will then determine what cellular responses are initiated.

Our immune response has developed into a two-fold system. There are two ways for a reaction to occur. First, there is an immediate or the **"non-specific"** response. The non-specific response begins its defense immediately against invading organisms. This occurs no matter whether or not the body has ever been exposed to this agent before. The non-specific is also called the **"Innate" Immune Response.** The second response is a time delay system, called the **"specific"** response. The specific response is dependent upon prior exposure to the invading substance to start the response. The prior exposure gives the system a chance to "identify" the attacker and mount a "specific" response against it. The time dependent specific response system is also known as, the **"Adaptive Immune Response."**

In a healthy individual, the innate response is initiated genetically and we carry it our entire lives. This system is continually ready to respond. The adaptive response develops over a lifetime of adaptation to specific pathogens. The reactions of the innate system are a prerequisite for the development and functionality of the adaptive system. These systems together provide a remarkably effective defense system. Even though we spend our lives surrounded by pathogenic microorganisms, we become ill infrequently. Many infections are dealt with quickly and efficiently by the innate system. Others need the reinforcement of the adaptive system to successfully "zap" the pathogen and give us lifelong immunological memory.

D. The Components of the Immune System

The immunological attack force has many participants. The initial line of defense is the protective layer of the skin. Our skin has evolved to be a barrier for entry of any pathogens into our bodies. Other components of defense include certain molecules found on the surface of the skin that starve the invader of essential nutrients along with tears, mucus, and saliva. These players all operate within the innate immune response.

At the cellular level there are many players. It all begins in the long bones, particularly the femur where the white blood cells or leukocytes are made. The bones of the human body contain material known as marrow. This marrow is a producer of blood cells. The leukocytes can be divided into two categories, the myloid, which are part of the innate immune system, and the Lymphoid, which are part of the adaptive immune system. The myloid cells include macrophages,

dendritic, neutrophils, basophils, eosinophils, and mast Cells. The lymphoid cells are T-Cells, B-Cells, and natural killer cells.

E. *The Family of White Blood Cells*

1. **Macrophage**

Macrophages play a crucial role in the immune system. The word macrophage comes from the greek word for "big eaters" *macros=large, phagein=eat*. They originate from monocytes which are circulating in the blood and then differentiate into macrophages within the tissue. They are primarily phagocytic cells. This means that they seek to engulf and digest the pathogen. They also act to stimulate other immune cells such as lymphocytes to respond to the pathogen. Macrophages function in both the innate and the adaptive immune responses.

2. **Neutrophils**

The Neutrophil is the most common type of white blood cell. This commonality makes them particularly useful in fighting any threat to the body. The Neutrophil is a bit of a sleuth. It follows a chemical trail by the process of chemotaxis or "smelling" the chemical. It consumes or "eats" the bacteria by a process called phagocytosis. Neutrophils are multi-nucleated, meaning they have more than one nucleus. They are key players in the innate immune system.

3. **Eosinophils**

The Eosinophil functions to control parasitic infections and allergic reactions. However, these are also the cells that can cause problems for you. If they start producing chemicals, problems can occur in the airways to cause disorders such as asthma. Eosinophils are part of the innate immune system and make up about 1-5% of the total white blood cells.

4. **Basophil**

The Basophil is an extremely rare blood cell, representing approximately 0.3% of blood cells. They are involved with allergies. They contain the chemical element histamine, which causes the symptoms of an allergic attack.

5. **Dendritic Cell**

The main function of the dendritic cell is to process foreign antigen material and present it on the surface to other cells of the immune system. They tend to migrate to the lymphoid tissues where they interact with T and B cells to shape the adaptive immune system. Dendritic cells are present in small quantities in tissue that are in contact with the external environment, such as tissues of the skin, lining of the nose, lungs and digestive tract.

6. T-Cell

The T-Cell develops in the thymus gland. This is why it is called the T-Cell. T-cell has many functions. It forms part of the adaptive immune response. There are numerous classes of T-cell that have different roles to play in the specific immune response. T-helper cells secrete specific proteins called cytokines that facilitate or "help" the immune response. Cytotoxic T-cells destroy virally infected cells and tumors. T-cells retain memory of an invading foreign antigen and help body respond to future infections.

7. B-Cell

The B-Cells are produced in the bone marrow. This is why it is called the B-cell. They circulate in the blood and lymphatic system where they perform immune surveillance. This means that the B-cell looks for any foreign substance which may have invaded the body. Their principal function is to produce antibodies. The antibodies recognize invading foreign antigens and assist in their destruction.

8. Natural Killer Cells

The Natural Killer Cell plays a major role in the rejection of tumors and virally infected cells. They bind to their target and secrete their lethal cocktail. This bursts the target cell's membrane. They are a major component of the innate immune system.

F. The key player in the innate immune response: The life of the Neutrophil

Initially, the skin with its mucous, salt, hair and dense structure protects us from foreign pathogens. However, there are times when a pathogen can make entry into the body. T and this is when the battle begins. Initially, an innate immune response occurs. The process by which the neutrophils are recruited to the site of infection is one of the most important functions of the innate immune system.

The neutrophil is a unique cell. It has a very specific function to seek out and kill bacteria. The neutrophil develops a multinucleated structure deep within the marrow of the long bones. They are abundant in blood, but are not present in healthy tissue. They are recruited to the site of infection by a process known as **chemotaxis**. This generally occurs in response to the production of **chemokines** at the infection site. Once they reach the site of the invading organism, the neutrophils engulf and destroy them in a process known as **phagocytosis**. When this is occurring, it is visible to the naked eye. We see "pus" or a combination of deceased bacteria and neutrophils. The pus is where the "battle" is taking place. If the neutrophil does not have the opportunity to fulfill its phagocytic role of defense, it must undergo a programmed death known as **apoptosis**. The life span, or time to respond and battle, is approximately 3 days.

Chemotaxis

Chemotaxis is the process of cell movement towards its target site. The neutrophil is the first type of cell to be recruited to the site of infection by the generation of a chemotactic gradient at the infection site. The neutrophil's success at reaching the site of infection depends upon this chemotactic gradient produced locally at the infection site. This gradient can be activated by a "chemotactic" substance that the invading bacteria itself produces directly. An example of a chemotactic substance produced by bacteria is fMLP. This is a potent chemotactic factor that recruits the neutrophil. The gradient can also be achieved by the production of **chemokines** by the cells present locally at the site of the infection. For example if a cut in the skin gets infected, the cells which make up the skin or keratinocytes will produce the chemokines. Chemokines are powerful chemicals that serve to guide specific cells to the site of infection. Specific chemokines recruit specific cells from the blood stream. There are two main families of chemokines CXC and CC.

Chemokine family	Target cell for recruitment	Examples
CXC	Neutrophils	CXCL8
CC	Monocytes, T-cells	CCL2

Table 1: Chemokines.....

The level of CXC chemokines produced at the site of infection can vary depending on various factors such as the type of invading organism and the strength of the body's immune system. High levels of CXC chemokines will recruit high levels of neutrophils. Low level of CXC chemokines will recruit low levels of neutrophils. This should translate into an efficient and a non efficient clearance of the invading bacteria respectively.

The immunological test which we will be studying in this unit is called an ELISA. We will use it to measure the levels of CXC chemokine present at an infection site as a reflection of immunological response to bacterial infection

2. Phagocytosis

Now that the neutrophils have been recruited to the site of infection, they can begin their job of destruction. The neutrophil begins to recognize the bacteria through a series of cell surface receptors. Once identification is made by the neutrophil, the process of phagocytosis begins. This is an active process. The pathogen becomes engulfed by the neutrophil and a phagosome is formed inside the cell which is the site for killing the bacteria. The neutrophil release a variety of toxic products into the phagosome. Most notably, nitric oxide (NO) and hydrogen peroxide (H₂O₂) are directly toxic to bacteria. These toxic products are known as

reactive oxygen species and the generation of these products is known as "respiratory burst".

3. Apoptosis

Once the bacteria are destroyed inside the neutrophil, it has fulfilled its obligation and it then dies by a process known as "apoptosis". The process of apoptosis is known as programmed cell death. As mentioned previously the neutrophil has a very short life. From the moment it was formed, it was programmed to undergo eventual cell death. Apoptosis involves a series of biochemical events inside the cell. It results in the safe disposal of the dead neutrophil which contains dead bacterial particles. This allows for further damage to surrounding healthy tissue.

Handout 3a: The components of the immune system note-taking chart

As we read the background, fill in the chart from each section with the key terms and concepts.

Definition	Descriptions of structures/concepts
What is immunology?	
What is a bacterial Infection?	
How does the body respond to infection?	
Macrophage	
Neutrophil	
Basophil	
Eosoniphil	
Mast Cell	
T-Cell	
B- Cell	

Handout 3b. The key player: The Neutrophil note-taking chart

As we read the background, fill in the chart from each section with the key terms and concepts.

Term to be defined	Definition/Description
Neutrophil	
Multinucleated	
Pathogens	
Chemotaxis	
Chemokines	
Phagocytosis	
Apoptosis	

Handout 3c The Chemokine/Neutrophil Connection

When the body suspects that there has been an invasion by foreign substance, **chemokines**, or chemical messengers, gather their forces and send a signal to the neutrophils, or white blood cells. The neutrophils increase in number and go to the wound or site of infection. This is where the neutrophils will begin their attack.

1. Using your knowledge of how the body reacts to chemokines, determine (approximately) how many Neutrophils and Bacteria are in each box based upon the Chemokine values.
2. Now, using your symbols for Chemokines, Neutrophils and Bacteria, fill in the chart according to the bodies response and the reaction of the bacteria.
3. Use the chart to answer the questions based upon the amounts of Chemokines, Neutrophils and Bacteria that are on your chart.

Chemokine amount	Neutrophil amount	Bacteria amount
High		
Medium		
None		

Analysis questions. Answer each question in a COMPLETE SENTENCE.

1. Describe the function of

a. chemokines

b. neutrophils

c. bacteria

2. What happens to the number of neutrophils as the level of chemokines increase or decrease?

3. How do these levels of chemokines/neutrophil affect the bacteria levels?

Handout 4: The Life of a Neutrophil

Now that you have become experts on the Immune System and its job to keep the body healthy, we have to show our expertise.

This assignment will require you to model the life cycle of a neutrophil from its beginning to its death. This can be done in just about any way you want as long as it covers the entire life cycle of the neutrophil. Below is list of possible ideas to use for your project. If you have an idea that is not listed, please see me before you begin your project for approval.

However you decide to present your neutrophil life story, it needs to cover all stages of the neutrophil's life that we have discussed. Use your class notes, handouts, textbook, etc. to assist you. You must include a beginning, fighting a bacterium and finally death. Detailed descriptions are extremely necessary.

Possible Ideas:

Childrens story book

Picture book

Flip Book

Wanted poster

Biography

Diarama

Play/Skit

Model(s)

Poster

Game board

Painting

Clay models

Food (?) representations

Edible cells (neutrophil, leukocytes etc.)

Pamphlet

Brochure

Handout 5: ELISA Science Background

The **ELISA** (enzyme-linked immunosorbent assay) is a specific test used in immunology to detect the presence of a specific protein in a sample of material. ELISAs are used as a diagnostic tool in medicine, in plant pathology and as a quality control test in many industries. The test is based on the principle that **antibodies** can recognize **antigen targets**. This attachment occurs with great specificity to form the "antigen/antibody complexes". The antibodies have **enzymes** attached to them, which are then reacted with a chemical to cause a color change that can be visibly detected.

Antibodies are Y shaped proteins found naturally in the body fluids of all mammals. Their job is to recognize foreign molecules. They are capable of doing this because of a small region on the tip of the Y which acts as the recognition site for the target antigen only.

Antigens are any molecules that can stimulate an immune response. Antigens are typically proteins. They can come from outside the body like parts of bacteria, viruses, or allergens, such as pollen. Also, proteins which are inside the body can cause an immune response. These are referred to as antigens.

Enzymes are proteins that speed up a chemical reaction. If you take a molecule and add an enzyme to it, the enzyme can convert this molecule to a different molecule. Some chemical molecules can be colorless, but when you add an enzyme to them, the enzyme changes them into a different molecule that has a color. This is why we can use enzymes in our ELISA test.

Antibodies, antigens and enzymes can be manufactured for use in immunology research such as in an ELISA. ELISA can be used to measure the levels of antigen or antibody in a sample.

The **direct ELISA** is used to detect the presence or absence of antigens. This type of ELISA can be used in the production of foods to detect the presence of food allergens such as peanuts in certain products. The indirect ELISA with modifications will be the type used in class. We will investigate in a simulation. None of the products are from actual patients. The unknown sample in your case is from an infected patient. You add the chemokine to a **microtiter plate**. A specific **primary antibody** is then added to detect the presence of your chemokine antigen. The primary antibody will recognize the antigen of interest because of the specific detection region on the tips of the Y. The primary antibody binds tightly to your chemokine antigen. Now, you add a **secondary antibody**. The secondary antibody is designed to recognize the primary antibody that is bound to the antigen. The

secondary antibody is special because it has an **enzyme** attached to it. Now, you have a structure consisting of antigen bound to primary antibody bound to secondary antibody that also has an enzyme attached to it. Next, add the **enzyme substrate**, such as Chromagen. Chromagen is a chemical that reacts with the enzyme and causes a color change. The greater the color change means that more enzyme is present. This also means that more secondary antibody is present and that more primary antibody is present too. Ultimately, it suggests that there is more antigen, or chemokine, present in the test samples.

Handout 6: ELISA definitions note taking chart

Structure	Definitions/descriptions with illustrations where appropriate
The ELISA	
Antibody	
Antigen	
Enzymes	
Direct ELISA	
Further terms	

5. Why is the well washed?

6. What is added to the "bound antigen"?

7. What do you notice about the primary (first) antibodies that were added?

8. Why is there a 2nd wash?

9. What is an "Enzyme linked Secondary Antibody"? What is it supposed to do?

10. How is secondary shape different from the primary antibody?

11. What is attached to the secondary antibody?

12. There is now a 3rd wash, why?
13. Enzyme substrate or color change chemical is added last.
14. What does this bind to?
15. What happens to the solution when this binding or connection occurs?
16. What does this color change tell us?
17. What level do these reactions occur:
 - a. visual, easy to see with the naked eye
 - b. magnifying glass
 - c. microscopic, not visible to the naked eye
18. Draw the symbol for:
 - Antigen
 - Primary Antibody
 - Secondary Antibody
 - Enzyme substrate

Handout 8: The ELISA PROTOCOL Practice

You will be conducting an ELISA test soon. The ELISA will be at the microscopic level. It will not be visible to the naked eye. This test will require many steps for you to follow. Before we do the actual test, we will practice the steps using a scientific model.

You will need to one cut out of each model for each pair of students :

(Have plenty of extras available for students if manipulating the scissors is an issue and also for any that are lost or cut incorrectly).

1. microtiter plates with wells
2. antigens/chemokine
3. primary antibodies
4. secondary antibodies with enzyme
5. chromogen or color change enzymes

The Process

Check off each step as you complete it.

- _____ 1. Cut out each model and place them in a secure area.
- _____ 2. In two wells of the microtiter plate, place the antigens or chemokines that will come from the patient.
- _____ 3. In each well place the primary antibody is seeking chemokines.
- _____ 4. Add 3 secondary antibodies which are connected to the enzyme to each well. The secondary antibody will bind with the primary antibody.
- _____ 5. Add 3 chromogen. Chromogen will react with the enzyme and cause a color change.
- _____ 6. In each of the wells you may have noticed that some of the symbols seem to have shapes that "fit" together. When you find these patterns, attach your symbols together.

Handout 9 The ELISA Student Procedures. Check off as you complete.

1. You will need ONE box per group. _____

2. Make sure you have in your box the following supplies: _____
 - 4 pipettes (eyedroppers)
 - 4 tubes marked antigen, 1st antibody, 2nd antibody and chromogen.
 - Microtiter plate marked according to patient ID

3. Remember that Patient A will use only wells A and patient B will only use wells B and Patient C will use wells C etc. This is for all Patients A-H. _____

4. You will only use columns 1, 2 and 3. _____

5. Using a CLEAN pipette, place 3 drops of unknown Patient Antigen into columns 1,2 and 3 of your specific Patient ID. _____

6. Discard the pipette. DO NOT USE IT AGAIN. _____

7. Using a CLEAN pipette, place 3 drops of your 1st antibody samples into your Patient wells. _____

8. Discard the pipette. DO NOT USE IT AGAIN. _____

9. Using a CLEAN pipette, place 3 drops of 2nd antibody into your Patient wells. _____

10. Discard the pipette. DO NOT USE IT AGAIN. _____

11. Using a CLEAN pipette, place 3 drops of **chromogen** into your patient wells. _____

12. Incubate your microtiter plate. (5-10 minutes)

Handout 10: Analysis of your results

1. Observe the color.
2. Record the patient's color change on the board.
3. Using colored pencils, fill in your data charts after all of the patients' data have been recorded on the board.
4. Determine the chemokine, neutrophil and bacteria levels based on the color change.

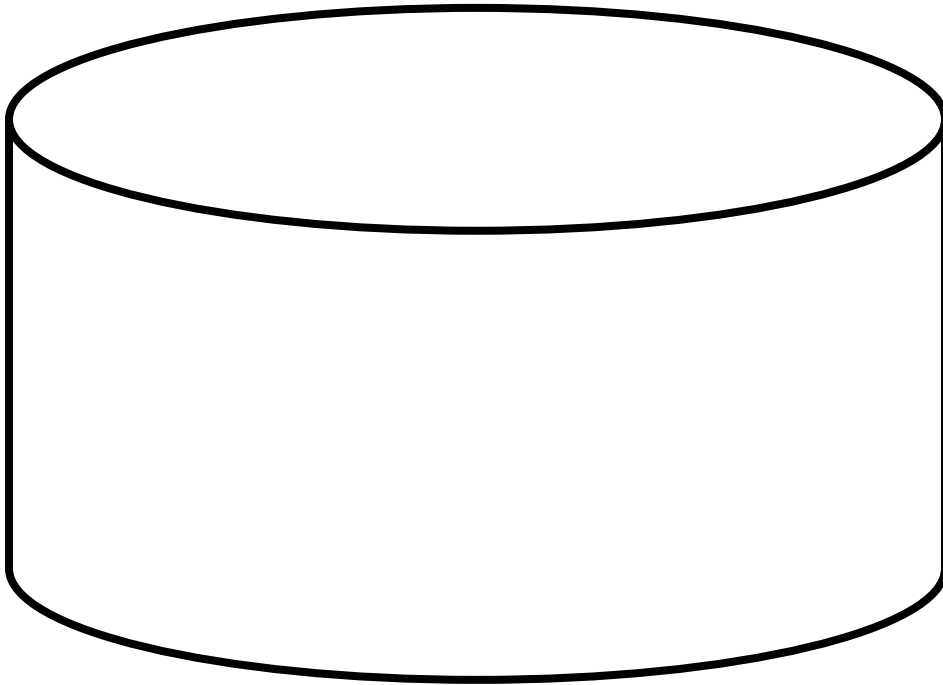
ELISA Data Chart

Patient	Columns			Levels		
	1	2	3	Chemokines	Neutrophils	Bacteria
A						
B						
C						
D						
E						
F						
G						
H						

Handout 11: Summary and Conclusions

Answer each question in a COMPLETE SENTENCE.

1. Why did you perform three identical tests for each patient?
2. Why did you add the 1st antibody to the antigen?
3. Why did you add the 2nd antibody to the antigen?
4. Why did you add the chromogen to the antigen?
5. Why was it necessary to use a clean pipette each time?
6. How would you know if the patient was fighting the infection well?
7. What does the lighter color purple indicate?
8. What could be some possible reasons for the light purple?

Handout 12: Templates for ELISA practice**Well of Microtiter plate**

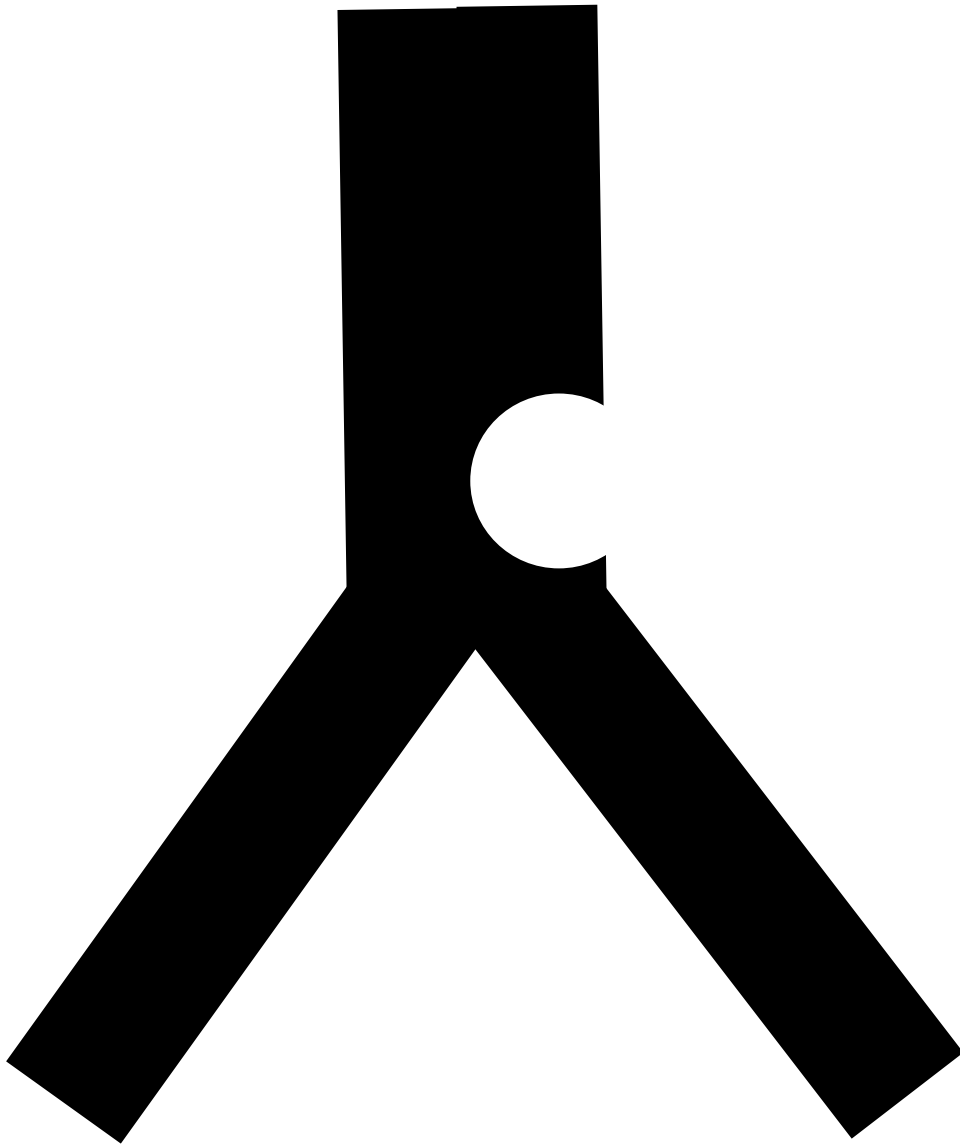
Chemokine

Chemokine

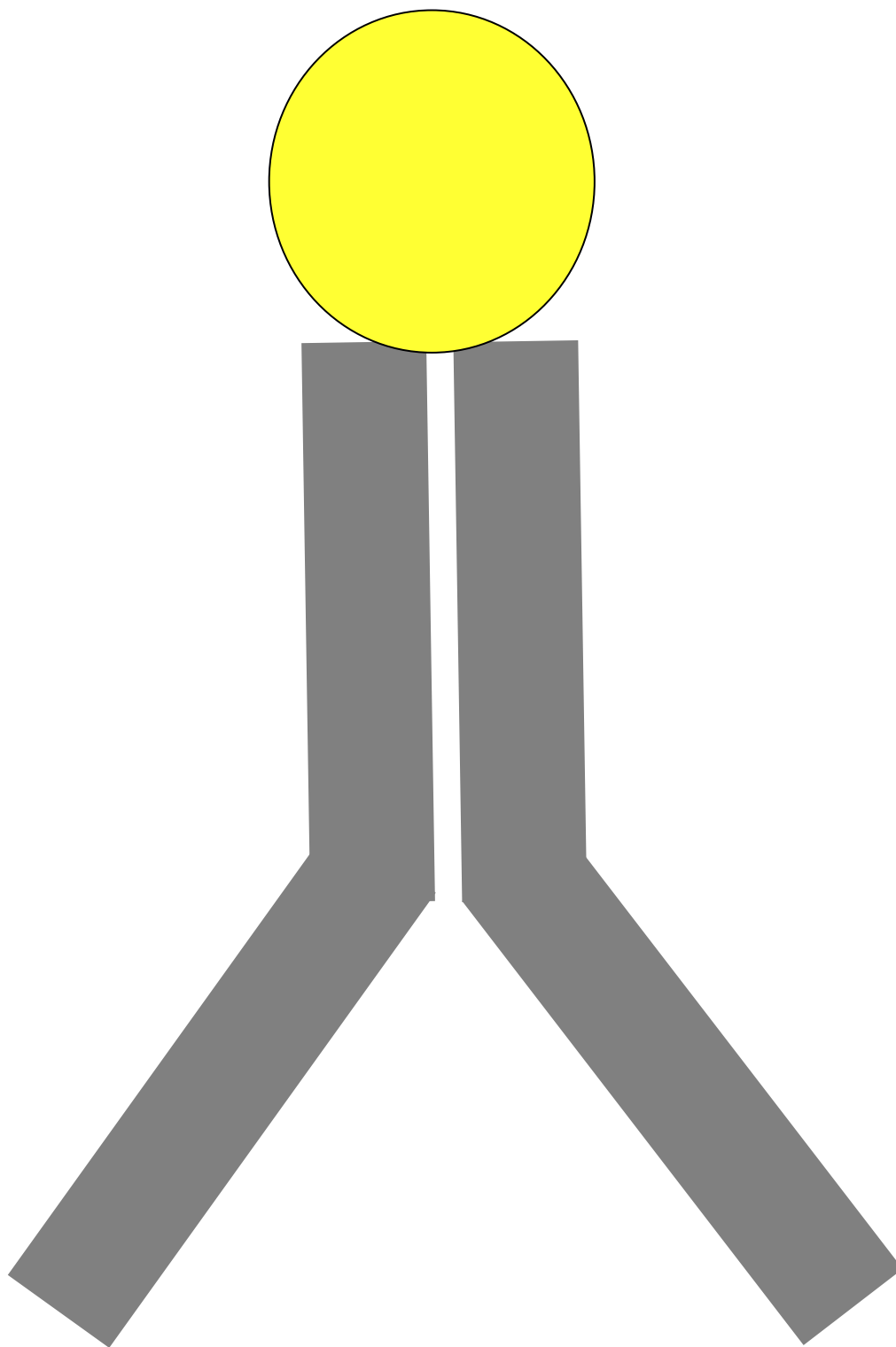
Chemokine

Antigens/Chemokines

Primary Antibody






Secondary Antibody



Handout 13

Rubric: Life of the Neutrophil Project

Element	Possible Points	Earned Assessment	
		Self	Teacher
Focus The topic is very clear when you first look at it.			
Main Ideas The main ideas are appropriate to the topic and are presented correctly.			
Supporting Details Appropriate and accurate details support each main idea.			
Purpose The purpose of the poster is clearly accomplished.			
Drawings and Illustrations All illustrations, photographs, and drawings add to the purpose and interest of the poster.			
Mechanics (C-U-P-S) There are no errors in capitalization, usage, punctuation, or spelling.			
Layout and Design The overall organization, design, use of color, and use of space help to make the poster interesting and to communicate the message.			
Creativity The poster is highly original and creative.			
Neat and Presentable The poster is very neat and presentable.			
Total:			

Did I do my best work?		
 TERRIFIC	 OK	 NEEDS WORK

XII. Reference List

- "B-Cell." [www.wikipedia.org](http://en.wikipedia.org/wiki/B-cell). 8 Jan. 2008. 17 Jan. 2008 <<http://en.wikipedia.org/wiki/B-cell>>.
- Metcalf, Donald. "Macrophage." *Blood Lines*. 2007. Alpha Med Press. 17 Jan. 2008 <http://www.bloodlines.stemcells.com/img/Metcalf_Fig10_4.gif>.
- "Neutrophil." <http://www.blobs.org>. 2004. 17 Jan. 2008 <<http://www.blobs.org/science/article.php?article=16>>.
- Sheppard, Tim. "What are white blood cells?" <http://www.blobs.org>. 2004/2005. 17 Jan. 2008 <<http://www.blobs.org/science/article.php?article=16>>.
- "T-Cell." <http://www.lbl.gov/>. 23 Oct. 2003. 17 Jan. 2008 <<http://http://www.lbl.gov/Publications/Currents/Archive/view-assets/Oct-03-2003/t-cell2.jpg>>.
- "Transfer Factor & the Natural Killer Cells ." www.discovertransferfactor.com. 2006/2007. 17 Jan. 2008 <<http://http://www.discovertransferfactor.com/NKcell.jpg>>.
- "Welcome to the Immune System." <http://www.immusystems.de/>. 17 Jan. 2008 <<http://http://www.immusystems.de/assets/images/dendriticcell2.png>>.