Can We Save Them? Using ELISAs to Determine the IL-6 Levels in Septic Patients

Gina Castellanos Ellsworth
Haverhill High School
137 Monument Street
Haverhill, MA 01832
gina32882@gmail.com

Created with assistance from:
Daniel Remick, M.D.
and
Evan Chiswick, Ph.D.
Boston University Medical School
Funded by:
The American Association of Immunologists

Science Background

Sepsis is an increasing problem in hospitals across the United States. In 2000, there were 621,000 reported cases of sepsis, and that number nearly doubled in 2008 to 1,142,000.[1] From this group, 28% to 50% will die of sepsis.[2] Many pharmaceutical companies have developed potential therapies for septic patients, but none have been successful enough to make it past clinical trials.[3] Thus, much effort has been put into uncovering how sepsis works in order to design a better way to test and treat it.

Sepsis has been defined as the systemic inflammatory response to infection.[4] There are many key players that affect inflammation levels, one f(es)9(t S)13()-2(,)-7(o11(1(1(1(1( )11(b11(w)13(a)-11(y)

Student Outcomes

- Students will be able to analyze a case study and design an experiment to test their hypothesis.
- Students will be able to identify the functions of both the innate and adaptive cells of the immune system.
- Students will be able to summarize a journal article on cytokines.
- Students will be able to summarize an article on sepsis, and make suggestions for future research.
- Students will be able to create a standard curve of IL-6 samples.
- Students will be able to evaluate if their samples contain IL-6 by comparing to the standard curve.
- Students will be able to write a formal lab report, and analyze a classmate's report in a peer edit.

Learning Objectives

AP Biology Standards Addressed:

2.C.1. Organisms use feedback mechanisms to maintain their internal environments and respond to external environmental changes.

2.D.3. Biological systems are affected by disruptions to their dynamic homeostasis.

A. Disruption at the molecular and cellular level affects the health of the organism.

2.D.4. Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.

B. Mammals use specific immune responses triggered by natural or artificial agents. (1-6)

3.B.2. A variety of intercellular and intracellular signal transmissions mediate gene expression.

A. Signal transmission within and between cells mediates gene expression (cytokines)

3.D.2. Cells communicate with each other by direct contact with other cells or from a distance via chemical signaling.

A. Cells communicate by cell-cell contact (APCs : 7 F H) O O V

Next Generation Science Standards Addressed:

• Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly. (HS-LS1-3)

• Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS1-1)

• Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level. (HSLS1-2)
• Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system. (HS-LS1-3)
• Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects. (HS-LS4-2), (HS-LS4-4), (HS-LS4-5)

Daily Unit Plans (45-minute periods)

This unit is intended to be completed in about ten days for 45-minute class periods. Feel free to add/remove lessons as time or resources allow.

Day 1: Introduction to Immunology

Lesson Roadmap:

1. Teacher will assign students into groups of 4. (2 minutes)
2. Teacher will present Part 1 of the case study (project slides on board), and allow groups to discuss the case. (8 minutes)
3. Student groups will present their ideas. (5 minutes)
4. Teacher will present Part 2 of the case study (project slides on board), and allow groups to discuss the case. (8 minutes)
5. Student groups will present their ideas. (5 minutes)
6. Teacher will present Part 3 of the case study (project slides on board), and allow groups to discuss the case. (8 minutes)
7. Student groups will present their ideas. (5 minutes)
8. Teacher will reveal the outcome of the actual case studies and research. (2 minutes)

Day 2: Innate Defenses and Innate Cells of the Immune System

Lesson Roadmap:

1. Students will brainstorm parts of the body that are involved in immune system defenses. (5 minutes)
2. Teacher will lead the class in a discussion of innate defenses. (15 minutes)
3. Teacher will ask the class:
   What happens when pathogens slip by these innate defenses?
   What is the next step your body takes? (2 minutes)
4. Teacher will lead the class is a discussion of innate immune system cells. (23 minutes)

Day 3: Cellular/Specific Immunity

Lesson Roadmap:

1. Students will brainstorm 3 things that they know about T cells, B cells, and antibodies.
2. Teacher will lead the class in a discussion of T cells, B cells, and antibodies.

Day 4: Cytokines

Lesson Roadmap:

1. Teacher will briefly explain what cytokines are and hand out journal article.

2. Students and teacher will read the introduction to the journal article together, popcorn - style.
3. Students will break into groups of 3 and will be assigned one section of the article to summarize.
4. Students will present their summarizations round-robin style to the class. All students will complete a chart on cytokines during this section.
5. Students will research one cytokine used therapeutically in patients for homework.

Day 5: Sepsis

Lesson Roadmap:

1. Teacher will briefly lead the class in discussion of the previous night's homework.
2. Teacher will ask the class:
   What happens when wounds don't heal?
   Or when a surgeon mistakenly cuts an incorrect organ and doesn't suture it?
3. Teacher will briefly lead the class in a discussion on sepsis.
4. Students will read an article on sepsis.

Day 6: Online ELISA lab

1. Teacher will hand out the Student Guide for online ELISA lab.
2. Students will log into their computers and begin working on ELISA lab:
   http://www.hhmi.org/biointeractive/immunology-

- Phosphate Buffered Saline solution (PBS)
- Horseradish peroxidase
- Streptavidin
- TMB (3,3’,5,5’-tetramethylbenzidine)
-

Teacher Guide for ELISA Lab

1 Day before the Lab:

You will need to make: aliquots of IL-6 Standard, aliquot samples, Biotinylated Antibody solution/Horseradish Peroxidase-SA solution, sodium acetate solution, sulfuric acid solution, and wash buffer solution.

*This prep will take over an hour to complete. A well

Day 8: ELISA Lab

Teacher Guide for ELISA Lab

Day of the Lab:

1. Turn on and set incubator to 37°C.
2. Set up lab benches with two pre-coated well plate strips, samples and pre-made standard curve samples, biotinylated antibody aliquot/HRP, sulfuric acid, and wash buffer.
3. Also, place a tube rack, a p-20 and p-200 micropipette at each station, with pipette tips, and a waste beaker for used pipette tips.
4. Defrost a TMB aliquot at the beginning of each class, keep it in the dark (pocket or drawer) until use. When students are about to wash off biotinylated antibody/HRP-SA solution, mix the 125 L TMB with 12.5 mL of sodium acetate solution, and 2 L of 30% hydrogen peroxide. Aliquot 1 mL to each group.
5. Remind students to have their cell phones handy for photograph taking.
6. You might find that 5-10 minutes is not enough time to develop the color reagent from TMB--if so, have the next class take photographs of the previous class' data until the end of the day. The ideal time frame for TMB color development is 20-25 minutes.
7. Students will complete the ELISA lab per the protocol, in groups of two or three. If need be, one student will stay behind to take a picture of their group's finished plate.

Day 9: ELISA Analysis

1. Teacher will hand out ELISA Analysis Guide to students.
2. Students will complete the ELISA analysis, including the image processing using ImageJ software and making a standard curve graph in Microsoft Excel.
3. Students will write a rough draft of their lab reports for homework.

Day 10: Lab Report Peer Edier (f)-17* <</MCID 50 >>BDC /TT1 1 Tf -0h(x)9(c)B91 s

Student Handouts

Name:_____ Per:_____ Date:_____

Immunology Case Study

Scenario 1: What is happening in the wards in this hospital?

Design a study to test this hypothesis. Outline what you would do here. (Remember to think about controls and experimental design!)

Scenario 2: What happened to the doctor?

Name:_____ Per:_____ Date:_____

Cytokine Journal Article

Directions: Read your assigned section of the journal article written by Dr. Charles Dinarello.

Dinarello, CA. Eur. J. Immunol. 2007. 37: S34–45 Historical insights into cytokines

Write down, and look up the definitions of 5 unknown words from your section:

1.
2.
3.
4.
5.

Give a brief summary of your section here (DO NOT WRITE WORDS YOU DO NOT KNOW OR UNDERSTAND. FIND A SYNONYM FIRST.):

_____
_____
_____
_____

Section	Summary

Name:_____ Per:_____ Date:_____

Cytokine Homework

Directions: Choose a cytokine that is used therapeutically (given to patients as medicine).

Which cytokine did you choose?

Which disease is the cytokine used for?

What are some of the known side effects of taking this cytokine?

What is its success rate? (How often does it “cure” patients?)

Is it being researched for any clinical trials?

If you had this disease, would you take this cytokine? Why or why not?

Name:_____ Per:_____ Date:_____

Reading

Read “Researchers Struggle to Develop New Treatments for Sepsis” by M McKenna at Scientific American. 3.19.13

Summarize the article below in the first paragraph. Then, in your second paragraph, suggest what scientists and doctors should research next.

Name:_____ Per:_____ Date:_____

HHMI Virtual Immunology Prelab

Directions: Answer the questions below, using a textbook or internet resources to help. This MUST BE FINISHED by _____at the beginning of class. You will NOT be able to complete the lab if you do not do the following questions:

What is a centrifuge?

What is an antibody?

What is an antigen?

Why do scientists use antibodies to recognize proteins?

What does it mean if someone's blood tests positive for a specific antibody?

What is an ELISA? Explain what it detects in the blood or cell extract.

What is a primary antibody?

What is a secondary antibody?

Why must a secondary antibody be from a different animal than the primary?

What is Horseradish Peroxidase? (HRP)

Why must a scientist incubate their ELISA samples with secondary antibody, and then wash the plate?

What is a spectrophotometer?

Why would a scientist use a spectrophotometer at the end of an ELISA?

What is a serial dilution?

What is Systemic Lupus Erythematosis (SLE)?

What is PBS (phosphate-buffered saline)?

Do you think ELISA is a good method for testing the presence of disease? Why or why not?

6. What wavelength do you use to observe the color on the plate? Why?

7. Record your results here: (Draw the piece of the microtiter plate that has samples in it, and shade appropriately.)

8. Were you successful at performing your first ELISA? Describe which mistakes you made if you were not (the program provides a checklist at the end).

Name:_____ Per:_____ Date:_____

Pipetting Practice and Setting up Samples/Standard Curve

For this lab, you will be using a p-20 and a p-200 micropipettor. Please observe your instructor's proper use and care of the micropipettor.

1.

Name:_____ Per:_____ Date:_____

ELISA OF INTERLEUKIN-6

Make sure that the following items are on your benchtop:

- samples you made yesterday
- Pipettes, tips, and waste container
- ELISA test strip taped to the benchtop
- Paper towels
- Solutions: Wash buffer, Antibody/HRP solution, TMB solution.

1. Go to the goggle box and put on a pair of goggles and put on an appropriate-sized pair of rubber gloves.
2. Label the edge of your strip with your name, and add 50 of your standard curve to each well:
   Top well =Tube A
   2nd well =Tube B
   3rd well = Tube C
   4th well = Tube D
   5th well = Tube E
   6th well = Tube F
   7th well = Tube P1
   8th well = Tube P2
3. Place your strip in a white plastic carrier in the incubator. Allow it to sit for 15 minutes.
4. Remove strip from the incubator. Dump out samples onto the paper towels. Add 100 L of wash buffer to each well, tap gently, and dump onto paper towels. Repeat the wash 3 more times.
5. Add 50 L of Antibody/HRP solution to all wells. Put it in the incubator for 15 minutes.
6. Remove from incubator, dump antibody out onto paper towels. Add 100 L of wash buffer to each well, tap gently, and dump onto paper towels. Repeat the wash 3 more times.
7. Add 100 L of TMB solution to each well, cover with foil, and set the timer for 20 minutes.
8. If time remains in the period, take a photograph of your strip once the timer beeps, or before the bell rings.
9. Remove your goggles and gloves, and WASH YOUR HANDS!!!

PS: You may be asked to take a photograph of the previous class' data and upload it to the Google Classroom website. Thank you for helping out!

Name:_____ Per:_____ Date:_____

Anatomy and Physiology Lab Report

Criteria for Lab Report	Points allotted	Points earned
Problem, heading and format -Name, Date, and period number on the top left of the first page -Sections of the lab report are clearly labeled starting with the Problem (the question your lab is trying to answer) through the conclusion	5	
Abstract - Brief summary of the experiment (5 - 6 sentences) - Includes introduction, hypothesis, methods, hypothesis supported or not	10	
Purpose - 1 to 2 sentences explaining the purpose	5	
Hypothesis & Prediction -If …. then… because… statement - Addresses the problem	5	
Materials -Listed w/ Amounts of each material used	5	
Methodology (procedures) -Specific description (either list of steps or in paragraph form) -Safety precautions -Including how to manipulate data (if statistics are used)	5	
Data Table(s) - In an organized chart/table -Several trials	10	
Graph (computer generated) - Correct type of graph -Title graph and label axis - Correct info on the axis - Answers the questions asked	10	

Name:_____ Per:_____ Date:_____

Peer Edit Sheet

1. Read each section of the lab report.