Immunology Overview:
From textbook to application

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- Students will learn how tumor cells avoid destruction by immune cells, and how current technology allows us to use a personalized medicine approach to ‘reawaken’ immune cells and target them to tumor cells.

b. Educational Standards:

Next Generation Science Standards (NGSS)

- Develop and use a model based on evidence to illustrate the relationships between systems or between components of a system (HS-LS-1-2)
- 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. (HSLS1-1)
- 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. (HS-LS1-2)
- Construct an explanation based on evidence for how natural selection leads to adaptation of populations. (HS-LS4-4)
- Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in n1 Tm[Slm 1 2348Tm 0.0471s8m)-3(i)10(n)-3( )]TJETBT1go im[fa)-554

- Describe how B and T cells that recognize self antigens are eliminated, and how defects in the process lead to many common autoimmune disorders
- Compare the primary and secondary immune responses, and explain how they relate to immunological memory and are the basis for vaccines

Read a case study on host-pathogen interactions, and use evolutionary principles to explain how both pathogens and tumor cells can manipulate components of the immune system to facilitate their propagation.

Read an article on how an emerging anti-cancer therapy involves disrupting interactions between tumor cells and T cells that inhibits T cell function.

Carry out ELISA to assess PD-L1 expression in samples representing tumor cells from fictional patients. Students will compare their results to a standard curve and make a recommendation as to whether each patient is a good candidate for treatment with a monoclonal antibody that blocks the interaction between PD-L1 and PD-1.

V. Teacher Guide

A. Student Prior Knowledge and Skills

	symptoms relate to each part of the response	
2	Review the roles	

	huge diversity of Abs can be made from a limited repertoire of genes.	
5	Compare and contrast humoral vs. cellular immunity, roles of specific effector cells. In groups: AP Biology POGIL-Immune System (Flinn Scientific)	Pre-reading assignment: Concept 35.3 *Campbell Biology In Focus* This section discusses humoral vs cellular responses as well as diseases associated with immune dysfunction.
6	Case study: Immune Evasion (From NCCSTS) Functions as excellent review for students to tie everything together, as well as to consider ways in which pathogens can evade the immune system	
7	Students read press release from the 2018 Nobel Prize in Physiology or Medicine, which describes the research behind immune checkpoint inhibition therapies, answer questions Notes: The immune system and cancer-what *should* happen, how tumor cells can evolve to avoid destruction, advent of immunotherapies.	Read Introduction to ELISA lab, answer pre-lab questions.
8	Notes: PD1 signaling as a potential drug target PD-L1 ELISA	Complete analysis questions for homework.

Teacher Instructions for ELISA Lab

This lab is based on using the Bio

Day of lab

1. Distribute tubes of reagents to each group (5 minutes)
3. Dispense wash buffer to each group's beaker. (5 minutes)
4. For patients 1 and 4, aliquot 0.25 ml 1x antigen to each group's tubes (10 minutes)
5. For patients 2 and 3, aliquot 0.25 ml PBS to each group's tubes (10 minutes)

Instructions: LAL Endotoxin Lab

This lab is based on the ToxinSensor Chromogenic LAL Endotoxin Kit (GenScript, catalog # L00350, $180.00 for 32 reactions, enough for 9 student groups). A cheaper alternative is the ToxinSensor GelClot Endotoxin Assay Kit (GenScript, catalog #L00351, 103.00 for 40 reactions). The GelClot kit is non-quantitative, simply giving a +/- result of whether the toxin concentration exceeds 0.25 EU/ml. A positive result in this assay is simply coagulation of the sample-if you do not have access to a spectrophotometer, this would this would be a good alternative.

Materials required (aside from those provided in kit)

Water bath or heat block adjustable to 37 C

3. Reconstitute Color-stabilizer #1 in 10ml Buffer S. Refrigerate.
4. Reconstitute Color-stabilizer #2 and #3 by adding 10ml endotoxin-free water. Refrigerate until day of lab.
5. Dissolve lyophilized endotoxin standard by adding 2 ml endotoxin-free water. Mix thoroughly with repeated vortexing. Stable for 1 week in refrigerator (Do Not Freeze).
6. This endotoxin stock solution is 5EU/ml. Dilute to 1EU/ml by adding 200 µl of this stock to 800 µl water. Refrigerate.

2 days prior to lab (90 minutes)

1. Make a dilution series using the 1 EU/ml stock to generate standards of 0.1, 0.05, 0.25, and 0.01 EU/ml. A flow chart is provided in the kit instructions. These will be used to generate a standard curve.
2. Prepare Sample 1 stock by mixing 100 µl of the 1 EU/ml stock with 1.4ml water. This will yield a concentration of 0.075 EU/ml.
3. Prepare Sample 2 stock by mixing 100 µl of the Sample 1 stock with 1.4 ml water. This will yield a concentration of 0.0075 EU/ml.
4. Carry out the LAL Chromogenic protocol to determine the absorbances for each standard stock that you prepared in Step 1

1 day prior to lab (45 minutes)

1. For each group, label 3 tubes “S1”, “S2”, and “B”, and add 100 µl of Sample 1 stock, Sample 2 stock, or water, respectively. Refrigerate.
2. For each group, label 4 tubes: "LAL", Stop #1, Stop #2, and Stop #3"
3. Add 350 µl LAL stock to the LAL tube, and 1.7ml of the appropriate stop solution to the other tubes.
4. Set out student work stations (pipets, tips, waste containers, etc.)
5. Set out vortexers
6. Set up water bath-turn on.

Day of lab

1. Set out student reagents in ice buckets.

Why Do I Feel Better? How do Aspirin, Advil, and Aleve relieve pain?

Introduction: We've all been there-a bruise from soccer practice, aching arms the day after hitting the weight room, that random stress headache that hits after staying up late ~~playing video games~~ studying. Whatever the cause, you may have found yourself reaching for a pain reliever. Whether you reached for something old-school (aspirin), or a newer product, such as Aleve, you likely took something called a Non-Steroidal Anti-Inflammatory Drug (NSIAD). As implied by the name, these drugs affect inflammation in your body. But how? Your task is to find out…

Assignment: Your job is to determine the mechanism of function for NSAIDs.

The passage at the following link will be helpful:

https://www.intechopen.com/books/nonsteroidal-anti-inflammatory-drugs/mechanism-of-action-of-nonsteroidal-anti-inflammatory-drugs.

Answer the following questions (you are not limited to the above site in finding this information).

1. What was the first NSAID used by people?
2. In general, what are the most common symptoms alleviated by taking an NSAID?
3. Briefly describe the functions of:
    - **a.** B0 0 1 0 1 0 1 0 1 02Tkc 1 1Non

Immune Checkpoint Therapy

Last year, two scientists, James Allison and Tasuku Honjo, won the Nobel Prize in Physiology or Medicine. Their research into the functioning of the human immune system has led to the development of exciting new cancer treatments where the patient's own immune system is activated to attack and destroy tumors. For some types of cancer, these therapies have resulted in dramatic, long-term remission of cancer. To understand how this works, read the article at the site below, and answer the questions below the link.

Link: https://www.nobelprize.org/prizes/medicine/2018/press-release/

Analysis Questions

1. Why is it essential that the function of immune cells like T cells is subject to both positive and negative regulation?

2. What can result if the immune system is excessively active?

3. What are the normal functions of the CTLA-4 and PD1 proteins?

4. What was Dr. Allison's hypothesis for the experiment in which he tested the effect of CTLA-4 blockade on mice with tumors? What did he use to block the function of CTLA-4?

4. Based on what you know about antibodies, would an antibody against CTLA-4 only bind to CTLA-4, or would it bind to other proteins (such as PD1) as well? Explain.

5. What side effects can result from this type of therapy?

PD-L1 ELISA Lab

Background

5. What is the role of HRP (Horseradish Peroxidase) in this assay?

6. Draw labeled diagram showing an interaction between an antigen, a primary antibody, and a secondary antibody

PD-L1 ELISA Lab-Data and Analysis

Addendum to Laboratory Procedure:

Each table group will be testing 4 patient samples-each sample is in a numbered yellow tube in your ice bucket. Instead of recording your initials on the tubes in your 12-well strip, use the numbers from the patient tubes as follows:

Strip 1: Tubes 7,8,9: Patient 1
Tubes 10,11,12: Patient 2

Strip 2: Tubes 7,8,9: Patient 3
Tubes 10,11,12: Patient 4

Carry out the remaining steps in the protocol as described

Following Step 14, you should observe a color change in some of the tubes. Record your results in the table below. Record a "-" for no color change, and a "+" for a blue color change. If you feel any of the lanes are a darker blue than others, you can use ++" for a more intense blue color change.

Table 1: Results of PD-L1 ELISA

Strip	1	2	3	4	5	6	7	8	9	10	11	12
1												

Analysis

1. Tubes 1-3 in each strip were positive controls-tests that should be positive, if all of the reagents in the kit are all functioning properly. What antigen do they all contain?

2. Tubes 4-6 are negative controls--tests that should be negative, due to the lack of any added antigen. What could be a possible reason that you could get a positive signal in one of these tubes?

3. Suppose that your patient samples all came up negative, but so did your positive controls. What could you conclude about whether your patient samples express the antigen?

4. Suppose that you had one or more patient samples that were positive for antigen (changed color), but 1 or more of your negative control tubes also changed color. What could you conclude about whether your patient samples express the antigen?

5. Based on your results, whic

Blue-Bloods: How Horseshoe Crabs Save Lives

response that can quickly lead to organ failure. In response to infection, particularly when the pathogen enters the bloodstream, the body releases pro-inflammatory molecules into the blood to combat the infection. Those chemicals trigger widespread inflammation, which leads to blood clots and leaky blood vessels. As a result, blood flow is impaired, and that deprives organs of nutrients and oxygen and leads to organ damage. In severe cases, one or more organs fail. In the worst cases, blood pressure drops, the heart weakens, and the patient spirals toward septic shock. Once this happens, multiple organs—lungs, kidneys, and liver—may quickly fail, and the patient can die. The progression from mild symptoms of infection to total collapse of multiple organ systems and death can be astonishingly fast. For example, Jim Henson, creator of the Muppets, began feeling flu-like symptoms on a Saturday, was admitted to a major New York Hospital on Tuesday morning, and died less than 20 hours later.

Exactly why the body's immune response spirals out of control in these cases is uncertain, but one thing is clear-once started, septic shock can be very difficult to stop, even if the original infection is cleared with antibiotics. Physicians therefore place heavy emphasis on early detection of sepsis-studies show that for every hour of delay in starting treatment, the chance of death can increase be as much as 8%.*

*http://www.ncbi.nlm.nih.gov/pubmed/16625125

Video: We will watch the following Khan Academy video on the physiology and symptoms of septic shock (https://www.youtube.com/watch?v=-bt-H5VQl5E). Following the video, answer the following questions:

1. White blood cells release molecules such as nitrous oxide in response to encountering a pathogen. What are the two effects on blood vessels of molecules like nitrous oxide?

2. Under normal conditions (an infection in the peripheral tissues, not in the blood), what is the purpose of nitrous oxide release?

3. How do these effects (from question 1) contribute to low blood pressure?

4. Describe how cytotoxic molecules released by white blood cells contribute to septic shock.

5. What is the cause of ARDS (Acute Respiratory Distress Syndrome)?

6. People experiencing septic shock are typically given large volumes of IV fluids. What is the purpose of this?

Sepsis is a leading cause of death in hospitals

Sepsis is a major challenge in hospitals, where it's one of the leading causes of death. It is also a main reason why people are readmitted to the hospital. There are many contributing factors to this. First, many patients are already immunocompromised in some way-they many already have an infection, or their immune systems may be weakened by coping with injuries and/or other illnesses. Also, invasive medical procedures such as insertion of IV's, breathing tubes, catheters, and other medical equipment can introduce bacteria into the bloodstream and bring on the condition. In addition to the possibility of bacteria being introduced into the body on equipment, hospitals must also be diligent about possible bacterial contamination of any fluids (such as saline, medicinal drugs, chemotherapy agents, etc.) that enter the patient's blood.

Even worse, we must be concerned not only about contamination with live bacteria, but also with certain bacterial compounds called **endotoxins** that can be released by some types of bacteria. The most common endotoxin is called lipopolysaccharide (LPS), a carbohydrate component of the cell walls of some bacteria. LPS is recognized by receptor proteins on many immune cells (see diagram to right) Humans are extremely sensitive to LPS-a dose of 50 micrograms (a microgram is 1 millionth of a gram) can induce sepsis by itself in the absence of any infection. How do we ensure that our medicines and medical devices are free of both pathogens and endotoxins?

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phttps://www.creative-biolabs.com/drug-discovery/therapeutics/lps-induced-rodent-sepsis-model.htm

Enter *Limulus*

The humble horseshoe crab, *Limulus polyphemus*, would seem an unlikely source of help in our fight against bacteria. A marine relative of spiders and scorpions, horseshoe crabs lack an adaptive immune system altogether. Not only that, but they utilize a compound called hemocyanin to carry oxygen, which gives horseshoe crab blood a ot only 390055≥( )-4

and this extract is mixed with the fluid to be tested. Formation of a thick gel indicates the presence of hazardous bacterial toxins. Approximately half a million horseshoe crabs are captured, bled, and released each year to support this testing. Not surprisingly, being removed from the ocean for several hours and having a significant proportion of your blood removed (20-40%) is stressful to these animals-leading to a mortality rate of at least 20%. Fortunately, new techniques involving the *in vitro* production and purification of the relevant horseshoe crab proteins are beginning to enter the market, but for now, the traditional LAL test is still the gold standard for ensuring the safety of medical and

Procedure

a. **Wear nitrile gloves whenever handling the vials or pipettes.**
b. **Use a fresh pipet tip each time**

1) You will be testing 2 samples, plus a “blank” (water known to be LPS-free). Label your 3 vials “S1”, “S2”, and “B”

   Use a micropipette to add 100 µl of each test sample into a separate endotoxin-free vial. Add 100 µl of LPS-free water to a third vial.

2) Vortex each vial for 30 seconds.

3) Add 100 µl of reconstituted LAL to each vial. Cap the vials and mix well by swirling gently.

4) Place all vials in the 37°C water bath. Incubate **10 minutes.**

5) After incubation, add 100 µl of reconstituted chromogenic substrate solution to each vial. Cap the vials and swirl gently to mix well. **Do not shake or vortex to avoid foaming.** Incubate at 37°C for **6 minutes**.

6) Add 500 µl of reconstituted C TJrepA)

*(Absorbance of sample-absorbance of blank)

** The amount of LPS in a sample is reported in Endotoxin Units (EU). 1 EU=1 picogram