Using Markers for Inflammation to Diagnose Inflammatory Diseases

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Part I: Teacher Guide

I. Science Background

The human immune system protects the body from pathogens using innate and adaptive immunity. Innate immunity is non-specific, meaning that the response is immediate but not long lasting as with adaptive immunity. There are several major categories of innate immune responses including barriers, inflammation, and cellular responses. Barriers, such as skin and mucous membranes, provide the first line of defense against pathogens. These tissues cover the exterior of the body and line the tracts and entrances to the body's orifices. Pathogens cannot easily pass through barriers to gain entry into the more vulnerable interior of the human body. Inflammation is an important response to injury or invasion that includes symptoms such as redness, swelling, heat, pain, and increased mucus production. The causes of inflammation are often a result of immunological benefits such as increasing body temperature to kill pathogens, recruiting blood cells and platelets to injury sites, or indicating a painful stimulus. Despite the uncomfortable symptoms, the human body requires the inflammatory response to fight off pathogens. The cells of the innate immune system include natural killer cells, phagocytes, basophils, eosinophils, and mast cells. These cells act by lysing or engulfing harmful invaders that have made their way into the body.

Inflammatory diseases are very common as most diseases are associated with at least some level of inflammation. Some examples of inflammatory diseases include non-alcoholic fatty liver disease, irritable bowel disease, colorectal cancer (among many other types), atherosclerosis, appendicitis, and peritonitis. Many of these are the result of unwarranted responses by the immune system or the body's attempt to repair itself. Sometimes, the body accidentally attacks the wrong cells. Autoimmune disorders occur when the body attacks healthy cells. These include rheumatoid arthritis, Crohn's disease, type 1 diabetes, multiple sclerosis, lupus, and several others.

Diagnosing inflammatory diseases and autoimmune disorders can be difficult. Scientists use specific biotechnological tools including polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and Western blot analysis. PCR is a technique used to amplify specific sequences of DNA. This can be helpful in diagnosis as it allows for certain hallmark genes of inflammation to be more easily detected in human samples. ELISA can be used to detect the presence of proteins, antibodies, and hormones associated with inflammation. Western blot analysis is another technique for detecting the presence of specific proteins.

II. Student Outcomes

A. Concepts Covered in this Unit

Concepts covered include the immune system, innate immunity, inflammation, diseases and disorders of the immune system, transcription, translation, polymerase chain reaction (PCR), Western blot analysis, and enzyme-linked immunosorbent assay (ELISA).

B. Outcomes from the Next Generation Science Standards (NGSS)

The NGSS addressed by this unit include:

- Science and Engineering Practices
    - Developing and Using Models
    - Planning and Carrying Out Investigations
    - Constructing Explanations and Designing Solutions
- Disciplinary Core Ideas
    - Structure and Function
    - Inheritance and Variation of Traits

C. Course Placement

This unit is appropriate for high school Biology 1 when discussing biotechnology, Anatomy & Physiology during the lymphatic/immune system unit, and as part of the human body unit in AP Biology.

D. Relevance

Students will learn about the immune system, causes of inflammation, and the biotechnology that can be used to detect it in patients, use to primary sources to research inflammatory diseases, model PCR, Western blot analysis, and ELISA, and conduct mock experiments using these three tools.

Real-world implications for this curriculum include a better understanding of the functions and dysfunctions of the human body – students may suffer or may know someone who suffers from an inflammatory disease.

III. Learning Objectives

Students will describe how DNA is transcribed into mRNA and translated into proteins.

Students will explain what causes inflammation and the pros and cons of the effects it can have on the human body.

Students will name markers for inflammation and identify which biotechnological tool is used for detection.

Students will describe and model biotechnological tools used for detection of markers for inflammation (PCR, ELISA, and Western blot).

Students will design and carry out experiments using biotechnological tools (PCR, ELISA, and Western blot).

IV. Time Requirements

For block scheduling with approximately 90-minute classes, this unit will require six days.

- Day 1: Review of transcription/translation, DNA, proteins, and the functions of enzymes and introduction to PCR, Western blot, and ELISA
- Day 2: Innate Immunity
- Day 3: Inflammatory Disorders and Autoimmune Diseases
- Day 4: Virtual Labs (PCR, ELISA, Western blot)
- Day 5: ELISA wet lab and summative assessment workday
- Day 6: Summative assessment peer review

For 45-60 minute classes, plan for approximately 10-12 days. Split the content (Days 1-3) into six days, perform the ELISA wet lab and virtual lab on day seven, and utilize days nine and ten to perform the PCR and Western Blot virtual labs. Additional days can be added to provide students time in class for their summative project research and peer editing.

V. Advance Preparation

The following materials and recipes are required for the laboratory portion of this unit.

Materials for Advance Prep:

- Gloves
- Safety Goggles/Glasses
- Lab Apron/Coat
- Pipettes
- Pipette Tips
- Beaker
- Graduated Cylinder
- Test Tubes
- Test Tube Racks
- Distilled Water
- Baking Soda
- Phenolphthalein
- Red, blue, and yellow food coloring

Recipes for Advance Prep:

- "Human Samples" for ELISA
    - Prepare and label 5 separate test tubes
    - Add 10mL distilled water to each test tube
    - Randomly select one test tube (make note of which one)
    - Pour the contents of the selected test tube into the beaker and add ¼ teaspoon of baking soda
    - Place the magnetic stir bar into the beaker and place on the stirrer until baking soda is completely dissolved
    - Pour the contents of the beaker back into the labeled test tube

    *Set-up is for one class of ~24 students in 6 groups of 4

VI. Materials and Equipment for Students

- Gloves
- Safety Goggles/Glasses
- Lab Aprons/Coats
- P200 Pipette (21μL-200μL)
- P1000 Pipette (201μL-1000μL)
- Pipette tips for each pipette type
- 1.5mL Eppendorf tubes (5 per student group)
- Beaker
- Graduated Cylinder
- Stir Bar
- Stirrer
- Test Tubes (5)
- Test Tube Rack
- Distilled Water
- Baking Soda
- Phenolphthalein
- Prepared "Human Samples" for ELISA
- 96-well plates (1 per student group)
- Computers with internet access (at least one per every 2-3 students)
- Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)
- ELISA Virtual Lab (http://www.hhmi.org/biointeractive/immunology-virtual-lab)
- Western Blot Analysis Virtual Lab (http://vlab.amrita.edu/?sub=3&brch=187&sim=1331&cnt=1)

VII. Student Prior Knowledge and Skills

Students should have a basic understanding of DNA, RNA, transcription, translation, protein synthesis, and enzyme function. A working knowledge of the innate immune system will benefit students as they design experiments as part of the summative assessment. It will also be necessary for students to know how to use a pipette and practice lab safety.

VIII. Daily Unit Plans

- Day 1: Review of transcription/translation, DNA, proteins, and the functions of enzymes and introduction to PCR, Western blot, and ELISA
    - PowerPoint and notes
- Day 2: Innate Immunity
    - PowerPoint and notes
    - Inflammation Worksheet
- Day 3: Inflammatory Disorders and Autoimmune Diseases
    - PowerPoint and notes
- Day 4: Virtual Labs (PCR, ELISA, Western blot)
    - Complete virtual labs using a computer or tablet
- Day 5: ELISA wet lab and summative assessment workday
    - Background and safety procedures
    - ELISA Lab
    - Research for summative assessment
- Day 6: Summative assessment peer review
    - Evaluate a peer using the rubric

XI. Summative Assessment

Students will select an inflammatory disorder of their choice and design an experiment to detect the inflammation markers in patient samples. Alternately, students can be assigned specific disorders to research. Students should create a PowerPoint or Keynote presentation that provides the rationale, materials, methods, anticipated results, and real-world implications of their proposed experiment(s).

Summative Assessment Rubric:

	0 - Poor	5 - Fair	10 - Excellent
Disease Selection	The student does not select an inflammatory disorder or autoimmune disease.		The student selects an appropriate inflammatory disorder or autoimmune disease.
Experimental Rationale	The student does not include the section or the rationale is not complete or coherent.	The section is incomplete or contains a few errors.	The section is complete and the student provides adequate evidence to support their rationale.
Materials Section	The student does not include the section or the section is not complete or correct.	The section is incomplete or contains a few errors.	The section is complete and the student provides a detailed list of materials.
Methods Section	The student does not include the section or the section is not complete or correct.	The section is incomplete, contains a few errors, or lacks adequate detail.	The section is complete and the student provides detailed methods.
Anticipated Results	The student does not include the section or the section is not complete or correct.	The section is incomplete or contains a few errors.	The section is complete and the student provides evidence to support their results.
Real-World Application	The student does not include the section or the section is not complete or correct.	The section is incomplete or contains a few errors.	The section is complete and the student provides relevant real-world applications.
Design	The PowerPoint/Keynote presentation has 5 or more grammatical errors, is not visually pleasing, and is not coherent.	The PowerPoint/Keynote presentation has 1-4 grammatical errors, is somewhat visually pleasing, and is mostly coherent.	The PowerPoint/ Keynote presentation has no grammatical errors, is visually pleasing, and is coherent.
Sources	The student includes 0-1 sources are does not cite sources properly.	The student includes 2-4 properly cited sources.	The student includes 5 or more properly cited sources.

Total: _____/80

List of Possible Inflammatory Disorders/Autoimmune Diseases:

- Ankylosing Spondylitis
- Osteoarthritis
- Asthma
- Atherosclerosis
- Colitis
- Dermatitis
- Diverticulitis
- Irritable Bowel Syndrome (IBS)
- Fibromyalgia
- Hepatitis
- Nephritis
- Non-Alcoholic Fatty Liver Disease
- Type I Diabetes
- Rheumatoid Arthritis (RA)
- Psoriasis
- Multiple Sclerosis (MS)
- Lupus
- Crohn's Disease
- Ulcerative Colitis
- Sjogren's Syndrome
- Hashimoto's Thyroiditis
- Vasculitis
- Pernicious Anemia
- Myasthenia Gravis
- Celiac Disease
- Grave's Disease
- Addison's Disease

Part II: Student Section

I. Notes

MOLECULAR GENETICS AND BIOTECHNOLOGY

I. Deoxyribonucleic acid (DNA)

- DNA is the genetic _____ for life
  - Made up of monomers called _____
    - Sugar-phosphate backbone and _____

II. The Central Dogma

- DNA is transcribed into mRNA through a process called _____
- mRNA is then translated into amino acid chains through a process called _____

III. Proteins

- Proteins are important for body tissue _____, acting as _____ to catalyze chemical reactions, and forming _____

IV. Polymerase Chain Reaction (PCR)

- A technique used to quickly _____ DNA and amplify specific _____ to a measurable point

V. Western blot Assay

- Used to detect specific proteins based on their _____
- Uses specific _____ to bind to proteins of interest
- Can be _____ using a secondary antibody that binds to the specific primary antibody

VI. Enzyme-linked immunosorbent assay (Elisa)

- Used to _____ antibodies, hormones, and proteins
- _____ help identify substances present in a sample

INNATE IMMUNITY

I. Non-Specific Defenses

- What are the main differences between Non-Specific and Specific Body Defenses?
    - Non-specific defenses mount an attack on _____ substances, no matter what they are.
    - Specific defenses mount an attack against _____ substances.
- _____ are harmful or disease-causing microorganisms.

II. Barriers to Entry

- Skin:
    - Your skin is impermeable as long as it is _____
    - There are two ways the skin can kill bacteria:
        - ____ of skin inhibits bacterial growth
        - _____ contains chemicals that are toxic to bacteria
- Mucous Membrane:
    - Examples of cavities that are lined by a mucous membrane:

      _____

      _____ tracts.
    - Saliva contains _____ which kill bacteria
    - Mucous membranes are the _____ place that microbes gain entry because they are _____

III. Cell and Chemical Defenses

1. Phagocytic Cells:

- _____ foreign particles
- _____ and _____ are types of phagocytic cells

2. Natural Killer Cells:

- Natural Killer Cells are _____ phagocytic

- They _____ (or burst open) foreign cells

IV. Inflammatory Response

- Inflammation is _____
- Four symptoms of the inflammatory response:
    - _____, _____, _____, _____
- Interferons:
    - When a cell is invaded by a virus, it releases small proteins called, _____.
    - How do interferons work?
        - Virus-_____ cells release interferons. These interferons bind to receptors on _____ cells. This hinders the virus' ability to multiply within the cell.
- Fever:
    - How can a mild to moderate fever aid the body when it is under a bacterial or viral attack?
        - Bacteria live at a very _____ temperature. A fever produces temperatures that can _____ bacteria.
        - A fever makes _____ and _____ less available in the body. Viruses need these two minerals to _____.
- Pus:
    - Made up of dead _____, dead _____, and dead _____

INFLAMMATORY DISORDERS AND AUTOIMMUNE DISEASES

I. Inflammatory disorders

- Disorders that cause or are the result of _____ inflammation
- Results in chronic _____, redness, swelling, stiffness, and tissue _____

Ankylosing Spondylitis
Osteoarthritis
Asthma
Atherosclerosis
Colitis
Dermatitis
Diverticulitis
Irritable Bowel Syndrome (IBS)
Fibromyalgia
Hepatitis
Nephritis
Non-Alcoholic Fatty Liver Disease

II. Autoimmune diseases

- An autoimmune disease occurs when the immune system mistakenly _____ the body
  - Normally, the immune system can tell the difference between foreign invaders and the body's _____

Type I Diabetes
Rheumatoid Arthritis (RA)
Psoriasis
Multiple Sclerosis (MS)
Lupus
Crohn's Disease
Ulcerative Colitis
Sjogren's Syndrome
Hashimoto's Thyroiditis
Vasculitis
Pernicious Anemia
Myasthenia Gravis
Celiac Disease
Grave's Disease
Addison's Disease

II. Formative Assessment

Name: _____ Date: _____ Period: _____

Inflammation: The Good, the Bad, and the Ugly

1. Define innate immunity.

2. What is inflammation?

3. What are some visible signs and symptoms of inflammation in the human body?

4. How could you determine if someone has inflammation of an internal organ?

5. Read the following medical cases and decide if inflammation is helping or harming the patients. Write your choice (helpful or harmful) and defend your answer in the "Initial Thoughts" column in the table below. At the end of the unit, you will revisit your answers and correct them in the "Final Thoughts" column if necessary.

Case #1: Susan has swollen, achy joints. She is active, but is always careful not to put stress on her joints during exercise. She recently tested positive for markers of Rheumatoid Arthritis.

Case #2: Billy was playing on the playground when he tripped and twisted his ankle. His ankle became swollen and felt warm to the touch.

Case #3: Gretchen was chopping onions in her kitchen when the knife slipped. She accidently cut her finger and it began to bleed. Once the bleeding stopped, she noticed that the area around the cut was red and felt warm and tingly.

Case #4: Maria fell off of her bicycle and scraped her knee. She did not bleed much, but her knee was red, warm, and excreted a straw-colored fluid. After a few hours, she noticed that a scab had formed.

Case #5: Larry went to the emergency room with severe pain in his lower right abdomen. Doctors determined that he had appendicitis and needed surgery to have his appendix removed immediately.

Case Number	Helpful or Harmful?	Initial Thoughts	Final Thoughts

#1			
#2			
#3			
#4			
#5			

III. Laboratory and Virtual Experiments

A. Rationale

- Context

- It is highly likely that you or someone you know has an inflammatory disorder or autoimmune disease. These are sometimes viewed as "invisible illnesses," since they don't always present outward symptoms that you can see. It is important to know what is happening to the body. Understanding how your body fights off pathogens will help you understand how to prevent getting sick.

- Introduction
  - Even though it can hurt, inflammation is usually a good thing! It is a response to pathogens that are invading your body and helps to fight off infections. Sometimes, your body might not be able to properly control its inflammatory response. This can lead to pain, swelling, and even rupture of some organs (think appendicitis). Many autoimmune diseases result in uncontrollable inflammation. Markers for inflammation can be detected using biotechnological tools to determine if a patient is experiencing excessive inflammation.

- Overview of Labs
  - You will be exploring three types of biotechnology that help scientists and doctors diagnose patients with inflammatory disorders, autoimmune diseases, and many other ailments.

- Techniques Used
  - Polymerase Chain Reaction (PCR) – detection of genes that may be turned on or off in patients with inflammatory diseases
  - Enzyme-Linked ImmunoSorbent Assay (ELISA) – detection of proteins in the blood
  - Western Blot Analysis – detection of proteins in the blood

- Lab Equipment
  - Pipettes
  - Computers or tablets with internet access

B. Materials

- Pen or pencil
- Gloves
- Safety Goggles/Glasses
- Lab Apron/Coat
- P200 Pipette (21µL-200µL)
- P1000 Pipette (201µL-1000µL)
- Pipette tips for each pipette type
- 1.5mL Eppendorf tubes (5 per group)
- "Human Samples" for ELISA

- 96-well plates (1 per group)
- Computers with internet access (at least one per every 2-3 students)
    - Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)
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C. Procedures

1. Obtain Eppendorf tubes containing the 5 patient samples.
2. Clearly label wells 1-5 on the top of the 96-well plate.
3. Use the P1000 pipette to load 500µL of patient sample into the first well.
4. Discard the P1000 pipette tip and replace with a clean tip.
5. Repeat steps 2 and 3 until all samples have been loaded into the plate.
6. Use a P200 pipette to load 200µL of the detection solution to the first well containing a patient sample.
7. Make note of any color changes that occur in the data table.

D. Data Collection

- Use the table below for data collection.
    - Describe the color of the sample in the first column.
    - If the sample turns pink, the patient has the disease. Put a checkmark in the Diseased column if the patient is diseased or in the Not Diseased column if the patient is healthy.
- Determine which patient(s) have the diseases based on the data collected

Sample #	Color Description	Diseased	Not Diseased
1			
2			
3			
4			
5			

E. Discussion/Analysis Questions

1. What changed in the samples of the patient(s) with the disease?

2. What does the color change indicate is present in the sample?

3. What is an enzyme?

4. What is a substrate? What acts as the substrate in this lab?

5. Extension questions
    - Is this a cost-effective diagnostic tool?

    - Sometimes there are false-positive results. How do you think this could occur in the experiment?

IV. Summative Assessment

You will select an inflammatory disorder or autoimmune disease and design an experiment to detect the inflammation markers in patient samples. You should create a PowerPoint or Keynote presentation that provides the rationale, materials, methods, anticipated results, and real-world implications of your proposed experiment(s). Use the attached rubric to create your presentation.

Rubric:

	0 - Poor	5 - Fair	10 - Excellent
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