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

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

VI. Materials and Equipment for Students

- Gloves
- Safety Goggles/Glasses
- Lab Aprons/Coats
- P200 Pipette (21 L-200 L)
- P1000 Pipette (201 L-1000 L)
- 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/)

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.

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

- 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

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

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

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

#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
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- W í ì ì ì W] ‰ š ší ì ì-ì î.ì.í>..• >
- 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/)
  - ELISA Virtual Lab (http://www.hhmi.org/biointeractive/immunology-virtual-lab)
  - Western Blot Virtual Lab (http://vlab.amrita.edu/?sub=3&brch=187&sim=1331&cnt=1)

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. h • š Z W í ì ì ì ‰] ‰ š š š } o } ñ ì ì … > } (‰ š] v š • u ‰ o] v š }
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. h • W î ì ì ‰] ‰ š š š } o } î ì ì … > } (š Z š š] } v • } o µ š] } v š } 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.

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

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 2TjET	