Using differentiated instruction to teach immunological concepts to a diverse group of learners

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General Overview
Using differentiated instruction to teach immunological concepts to a diverse group of learners is a comprehensive curriculum unit designed to expose high school students to the immune system by using active hands-on and minds-on strategies. This curriculum unit includes a student created collaborative poster, a Biology Action Model (BAM) and a double diffusion test that demonstrates the interaction between antibodies and antigen. In addition, to gain an appreciation of science as an ongoing process of discovery and understanding students will also be introduced to the role of γδ T cells in wound healing and tissue repair, a relatively new understanding discovered in the Havran Lab at The Scripps Research Institute (TSRI). This unit will incorporate a variety of teaching strategies that engage students in active learning and will foster a better understanding of immunology.

Science Standards

California Physiology Standards
Organisms have a variety of mechanisms to combat disease. As a basis for understanding the human immune response:
   a. Students know the role of the skin in providing nonspecific defenses against infection.
   b. Students know the role of antibodies in the body’s response to infection.
   c. Students know how vaccination protects an individual from infectious diseases.
   d. Students know that there are important differences between bacteria and viruses with respect to their requirements for growth and replication, the body’s primary defenses against bacterial and viral infections, and effective treatments of these infections.
   e. Students know why an individual with a compromised immune system (for example, a person with AIDS) may be unable to fight off and survive infections by microorganisms that are usually benign.
   f. Students know the roles of phagocytes, B-lymphocytes, and T-lymphocytes in the immune system.

National Science Inquiry Standards
Engaging students in inquiry helps students develop
   a. Understanding of scientific concepts.
   b. An appreciation of "how we know" what we know in science.
   c. Understanding of the nature of science.
   d. Skills necessary to become independent inquirers about the natural world.
   e. The dispositions to use the skills, abilities, and attitudes associated with science.
Student Outcomes and Objectives

- Students will understand that science is a growing body of knowledge that serves to explain events in the natural world.
- Students will gain an understanding of the role of the immune system in combating disease.
- Students will be able to create a collaborative poster that demonstrates understanding of specific and non-specific defenses and the roles of different cells in the immune system.
- Students will use a model to simulate and explain a humoral response and a cell mediated response.
- Students will develop science literacy skills by using a structured approach to analyze a scientific journal article.

Science Background

The body's main defense against pathogens is the immune system which recognizes, attacks, destroys, and "remembers" pathogens that enter the body. It accomplishes this by making specialized cells that inactivate pathogens. For each different pathogen, the immune system will make cells that are specific to that pathogen. The function of the immune system is to fight infection and eliminate foreign invaders through the production of cells that inactivate foreign substances or cells. This process is called immunity.

There are two general categories of defense mechanisms against infection: nonspecific defenses and specific defenses. Most nonspecific defenses are like the outer walls of a fortress and serve as a barrier against pathogens that try and enter the body. Specific defenses are like an army that works within the body. They find and eliminate harmful pathogens that have managed to break through the body's nonspecific defenses.

Nonspecific defenses do not differentiate between one type of threat and another. These defenses include physical and chemical barriers. Your skin is the first line of defense. Few pathogens can infiltrate the layers of dead cells at the skin's surface. In addition to your skin you have many secretions of the body, including mucus, saliva, and tears, which contain lysozyme, an enzyme that breaks down the cell walls of many bacteria. In addition, oil and sweat glands in the skin produce an acidic environment that kills many bacteria.

If pathogens are able to enter your body, they can multiply quickly, releasing toxins into your tissues which may cause disease. When this happens, a second line of defense, the inflammatory response, is activated. Inflammation, which is often non-specific, is stimulated when injured cells release chemical factors that establish physical barriers against the spread of infection. The chemical factors make the infected area very sensitive to pain, cause vasodilatation, and attract phagocytes such as neutrophils.
If a pathogen is able to escape the body's nonspecific defenses, the immune system responds with a series of specific defenses that attack the disease-causing agent. An antigen is a substance that triggers an immune response. Viruses, bacteria, and other pathogens may serve as antigens that trigger responses by the immune system.

The cells of the immune system that recognize specific antigens are two types of lymphocytes: B lymphocytes (B cells) and T lymphocytes (T cells). B cells provide immunity against antigens and pathogens in the body fluids. This process is called humoral immunity. T cells provide a defense against abnormal cells and pathogens inside living cells. This process is called cell-mediated immunity.

When a pathogen invades the body, its antigens are recognized by a small subset of the body's B cells. These B cells grow and divide rapidly; producing large numbers of plasma cells that release antibody, subsequently some of the B cells become memory B cells.
Antibodies are proteins that recognize and bind to antigens. The antibodies are carried in the bloodstream to attack the pathogen that is causing the infection.

After an initial exposure to a pathogen, millions of memory B cells remain in the body capable of producing antibodies specific to that pathogen. These memory B cells prevent the pathogen from causing disease a second time. If the same antigen enters the body a second time, a secondary response occurs. The memory B cells divide rapidly, forming new plasma cells. The plasma cells produce the specific antibodies needed to destroy the pathogen.

An antibody is shaped like the letter Y and has two identical antigen-binding sites. Small differences in the amino acids affect the shapes of the binding sites. The shape of the binding site makes it possible for the antibody to recognize a specific antigen with a complementary shape. The different shapes give antibodies the ability to recognize a large variety of antigens. It is estimated that a healthy adult can produce about 100 million different types of antibodies.

In another form of immune response called cell mediated immunity the body is afforded mechanisms by which it can attack and destroy its own cells that have been infected by virus or have become cancer cells. In addition, helper T cells within the cell mediated immune response also play an important role in amplifying the humoral response and the production of antibody.

During cell-mediated immunity, T cells divide and differentiate into different types of cells such as: killer T cells (cytotoxic T cells), helper T cells, regulatory T cells, and memory T cells. Cytotoxic T cells induce apoptosis in body cells by
recognizing and interacting with epitopes of foreign antigen on the surface of virus infected cell. Type 1 helper T cells produce memory T cells which will cause a secondary response if the same antigen enters the body again. Type 2 helper T cells stimulate B cells into proliferation which causes an increase in antibody production within the humoral response. After a pathogen has been brought under control regulatory T cells release substances that shut down the killer T cells so that the body returns to homeostasis.
Implementation Guide

Rationale
The four activities within this unit are designed to give students opportunities to explore, learn, and peer teach concepts related to the general function of the immune system. The specific assignments are geared toward a general biology course but the strategies are applicable for honors or AP biology classes if the content is scaled up. The order in which they should be applied follows a scaffolding approach where students uncover new knowledge at each level and then use it to bridge their understandings to new learning.

Collaborative Group Poster
Collaborative posters are a great way to help students synthesize their understanding in a visual form with close reference to the text. Creating a poster encourages student’s creativity while assisting student’s self assessment via a rubric. This strategy is applicable to any topic as well as beneficial for students’ use as a means to express creativity and to familiarize students with this type of performance task throughout the year.

Time requirements
The collaborative poster is designed to be implemented over 2 hours. One hour for creating the poster and one hour for presentations.

Materials
Poster Paper
Markers
Text Books or Web based resources

Procedures
In this task, students will be assigned a portion of text from the immunology section of a biology textbook such as Biology by Miller and Levine or Biology 8th ed. by Campbell. This group activity can be scaled up or down depending on the level of the class such as regular, honors, or AP.

Concepts to be considered:
- Agents of disease
- How diseases are spread
- Non-specific defense
- Specific defense

Students are given time to think about how to represent the information from the text on a collaborative poster. Students are to discuss and reach a consensus on which images, quote, or original phrases are to be included in the collaborative poster.
As groups plan and create their poster, a rubric is essential to ensure that they discuss the text, stay on task, and use images to highlight main ideas rather than merely to decorate the poster. Each student in the team uses a single and distinct color marker, meaning each member uses a different color to represent his/her work on the poster. Each group member also signs the poster in his/her respective color when the group agrees that the poster is complete.

After the posters are complete, groups present the information to the whole class, or groups share the information in a gallery walk format.

Source: Quality Teaching for English Learners (West Ed website)

**Biology Action Modeling**

Biology action modeling is a strategy that allows students to participate in active learning where they manipulate a simple materials model that simulates a dynamic biological process. This approach to learning increases student accountability for their own learning and allows multiple opportunities for the teacher to check for understanding. In addition, the modeling will help students take an abstract concept and make it tangible and more concrete.

This action model demonstrates how humoral immunity and the cell mediated response function to combat disease. Students will be able to model the interaction between antigens, B-cells, T-Cells, and antibodies.

**Objective:**
Students will explore how antibodies and different lymphocytes help protect against pathogens. Students will be able to model the interaction between antigens, B-cells, and antibodies.

**Vocabulary:**
Specific defense  Memory B Cells  Cell Mediated
Antibody T Cells Response
Antigen Pathogens Immune response
B Cells Humoral Response

**Pre-requisite knowledge:**
Students should be familiar with the structure and function of various organelles. Students should know the process of protein synthesis. Students should be familiar with recurring themes such as homeostasis and form and function.

**Time Requirements:**
1. Preparation of model pieces and pre-lab questions - 1 hour
2. Modeling of processes - 1 hour
**Materials**
- Templates of Action Model (laminated or printed on card stock)
- Scissors
- Tape

**Directions about model:**
This modeling process is to be done by groups of 3-4 students. Students will use the textbook to read about the processes of humoral immunity and the cell mediated response. Then, the students complete the pre-lab questions. They will use this information to model the process by following the student hand out. Guiding questions for the groups should be used to monitor student progress and to check for understanding as students interact with the model.

**Guiding/Prompt Questions**
The following questions can be used during the modeling processes to assess whether the students are able to demonstrate mastery on each part of the Biology Action Model. Keep in mind that these are only suggested guiding questions and may be modified to suit the level of your students and your curriculum.

**Part A – Preparing and Labeling**
- Ask students to identify all major pieces of the model.
- Ask students to indicate where in the body these structures are found.

**Part B – Humoral Response**
- Ask students where in the body this process takes place.
- Ask students to model the process of antigen recognition step-by-step using relevant vocabulary.
- Ask students to summarize the importance of the variable region.
- Ask students to model how B cells become either a plasma cell or a memory B cell.
- Ask students to explain the importance of maintaining a population of memory B cells in circulation.
- Ask students to model how a secondary response differs from the initial humoral response and how this difference greatly reduces the chances of developing the disease a second time.

**Part C – Cell Mediated Response**
- Ask students where in the body this process takes place.
- Ask students how B Cells and T Cells differ in their antigen recognition.
- Ask students to model a macrophage engulfing and presenting antigen.
- Ask students to explain the role of each type of T cell.
Extension activity - Story Boarding
Have students create a story board of the process. Story boards are graphic organizers that illustrate an animated process as a series of images. Students draw the story board with six or more sections. For each section, students include a picture, label all the components involved, and include a description of what is happening within the process.

Double Diffusion Test
Lesson taken from
Source: Food Forensics - A case of mistaken Identity (Michael Grupe)

The double diffusion or Ouchterlony test purposefully follows the biology action model. In the action model, students simulate how antibody structure leads to the binding of antigen. This lab uses real antibody and antigen to demonstrate antibody specificity. Students will carry out the double diffusion test to investigate whether or not certain foods are made with egg products and how that can affect someone with an allergy to albumin.

This lesson can be used with high school students and can be adapted to any level (biology or advanced biology) in association with the study of biochemistry, cell biology, health, or physiology. It can be used in association with topics such as allergies, food safety, or antigen-antibody precipitation. As a result of completing this lesson, students will be able to answer questions like these: "Why am I allergic to some things but not to others?" or "Why does clotting occur when incompatible blood types are mixed?"

Background Information
The major concept of this lesson is the specificity of the reaction between an antibody and an antigen. Antibodies are proteins produced by cells of the immune system in response to the exposure of an individual to a foreign substance (an antigen). This concept will be illustrated through the use of an experimental procedure called a double diffusion assay.

This assay is based on the formation of a precipitate (precipitin line) when an antibody reacts with its specific antigen. In this test, often called the Ouchterlony test, antibody and possible antigens are placed in wells in agar plates and allowed to diffuse toward one another. The antibody is placed in a center well and antigens (specific or nonspecific) are placed in surrounding wells. When an antibody and its specific antigen meet one another and are at the proper concentrations, the precipitate will form a visible white line between the two wells. This line is called a precipitin line.
In the picture below, a precipitin line can be seen between the center and outer wells. The fact that the line is continuous indicates that all wells contain the same antigen. Antibodies and antigens that are not complementary will diffuse past one another in the agar and will not form a precipitate.

The scenario for this lesson is centered on hypersensitivity to environmental antigens that are generally not particularly harmful (e.g., pollen, dust mite excrement, mold, drugs, food, etc.). In these situations, the immune system reacts to these antigens by producing a type of antibody known as immunoglobulin E (IgE). IgE antibodies trigger the release of histamine by mast cells, which then leads to typical allergic symptoms. An extreme response is called an anaphylactic response.

**Materials for experiments 1 and 2 (per class and per team of students):**
* Anti-chicken egg albumin (Sigma Chemical Co.)
* 2 - 1.5% agarose plates
* 6 mm diameter soda straw
* Toothpick
* Glass marking pen
* Small quantities of:
  o Raw egg white (diluted 1:625)
  o Uncooked egg-enriched pasta (1:40)
  o Uncooked egg-free pasta (1:40)
* Samples of various foods:
  o some positives (egg-containing) like mayonnaise (1:10), custard (1:10), pasta (1:40), baked items (1:10), egg white
  o some negatives (without egg) like sugar, salt, milk, beef broth, molasses, etc.

The dilution is not critical on negatives.
**Preparation:**
Most of these materials are cheap and easily obtainable. Dilutions can be made days in advance and stored until needed. Plates should be made several days prior to use to allow proper drying. Antibody is the biggest expense but a little bit goes a long way. (2 ml supplies 50 teams of 2) Out-of-date antibody would be cheaper and would still work for these experiments. Antibody (Anti-Chicken Egg Albumin) from Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 Stock # C-6534 2 ml is about $50. Total prep time is about 2 hours.

**Time Requirements**
This lesson is designed for four 50-minute periods. Activities can be reorganized to fit your schedule.

Day 1:
* Introduction and exploration
* Class discussion of allergies

Day 2:
* Gather/discuss data
* Observe, record, discuss results of experiment 1
* Concepts presented
* Mystery read by students

Day 3:
* Design experiment to solve mystery and set-up of experiment 2

Day 4:
* Gather/discuss data
* Follow-up
* Observe, record, discuss results of experiment
* Form conclusion (solve mystery)
* Return to day 1 questions
LESSON
Day 1:
A quick discussion during which students are questioned about their allergies, symptoms, and how their allergies compare to the allergies of family members or friends. This discussion is not intended to result in answers, but rather to stimulate interest. Highlight that different people are allergic to different and specific substances. This will be explained later by antigen-antibody specificity.

Experiment 1:
Fooling with Food is a chance for students to explore the interaction between various foods (some negative and some positive) and Reagent A (the antibody). The figure below shows the relative position of wells to be cut in the agar plates with the straws. Toothpicks are good for removing the plugs from the wells. Extra plates and colored water can be used first for students to practice loading the wells. Only 1-2 drops with a fine-tipped pipettes is needed per well.

1. Cut wells in agar using a template (teacher prepared) under the plate as a guide.
2. Remove plugs and label wells and plate.
3. Students select six different foods to load in the six outer wells. Give students about 10 foods to choose from so there is variation in selections. Different pipettes should be used for each food. Foods should not spill over edge of wells.
4. Reagent A is placed in the center well. (I suggest that the teacher does this because of expense.)
5. Plates can be stored overnight in a flat position at room temperature.

Day 2:
1. Let students find precipitin lines. Tell them only that they may have to hold plates up to a light or toward a window. Faint white lines will be seen by someone. Then, others will see.
2. Compile a list of positive foods, and students will quickly see that all are egg-containing.
3. Teacher can now discuss specific interaction between antibody (reagent A) and antigen (albumin in egg). Terms can be presented at this time. Basic (forked) structure of antibodies that allows for cross-linkage and formation of precipitate can also be discussed.

Day 3:
Experiment 2:
Students (or teacher) can design a test to answer the question posed in the mystery. Make sure both positive and negative controls are included. With six wells you could test the unknown (egg-enriched pasta), egg-enriched pasta (+ control), egg-free pasta (- control), egg white - 1:625 dilution and 1:3125 dilution (+ controls that show a range of concentrations that will result in formation of a
precipitin line), saline solution (- control that is used for all dilutions).

Experiment 2 is also a double-diffusion test as was experiment 1. Now, however, students know that they are putting different antigens in the outer wells and antibody in the center well. They know why there will be positive results and why there will be negative results so they can predict which wells will have precipitin lines. Again, store plates at room temperature until the following day.

Day 4:
Follow-up:
Results are observed and compared to predictions. A conclusion is formed about Stan's Salad Saga (of course, Stan is innocent).

Now, you can refer back to your original discussion about allergies and answer some of the unanswered questions from day 1. Other uses of specificity can also be discussed at this time. Many home pregnancy tests use an antibody to detect the presence of human chorionic gonadotropin (HCG) that is present in a woman's urine during pregnancy. The test for HIV also involves formation of an antibody-antigen complex.

**Role of Gamma Delta T cells**

Students will be exposed to contemporary research, lab techniques, and recent discoveries in immunology through structured interaction with a scientific journal article. The article is one that comes out of the Havran Lab and discusses the role of gamma delta T cells in wound healing(1). Students will Interpret graphics, annotate a text, and reach their own conclusions about the function of gamma delta T cells in mice and possibly humans.


**Time Requirements**

This activity can be completed in two instructional hours. Day one is for students to interact with images of experimental outcomes to make predictions and observations of the results. On day two, students will jig saw the scientific journal article. By forming home groups and expert groups, students will annotate their part of the text in the expert group and then share their learning with their home group.

**Interpreting Graphics**

Students are familiar with textbook graphics, but interpreting images and graphs from a scientific journal affords them a new opportunity to explore recent discoveries and remind them of the nature of science as a process.
Procedures

Interpreting Graphics - Students will interpret a set of 2 images and 1 graph from a scientific journal. Students will answer the guiding questions on their hand out regarding what they think is the result of the experiment, and they will note any observations. Then, students will be given the descriptions of the experiments and compare them to the conclusions of the actual results.

Annotating the text- Students are placed in expert groups where they will annotate a section of the journal using the following criteria.

1. Circle unknown words. As you read, circle each word you come across that is unfamiliar. You may need to come back and reread the sentences before and after the word to fully understand the meaning of the word.

2. Mark definitions. Underline, highlight or circle sentences that provide you with a definition. It is useful to write "def" in the margin so you can locate the definition quickly. Also mark sentences that provide examples by marking an "X" next to the sentence.

3. Number lists of ideas. Writers provide support points to back up their main idea. Write a number in the margin next to each support point or lists of points that clarify the main idea.

4. Make notes to yourself in the margins. As you read, write any questions or comments that crop up in your mind in the margin next to the passage.

5. Place a check or star next to important passages. This is extremely helpful when taking a test that requires you to read a passage, because the questions that follow the reading will most likely refer back to these points.

6. Keep it simple. Remember, you are trying to connect with the reading in some way. Use the tools that work best for you.


An example follows:
A Role for Skin γδ T Cells in Wound Repair

Julie Jameson,1 Karen Ugarte,1 Nicole Chen,1 Pia Yachi,1 Elaine Fuchs,2 Richard Boismenu,1 Wendy L. Havran1*

γδ T Cells

γδ T cell receptor–bearing dendritic epidermal T cells (DETCs) found in murine skin recognize antigen expressed by damaged or stressed keratinocytes. Activated DETCs produce keratinocyte growth factors (KGFs) and chemokines, raising the possibility that DETCs play a role in tissue repair. We performed wound healing studies and found defects in keratinocyte proliferation and tissue reepithelialization in the absence of wild-type DETCs. In vitro skin organ culture studies demonstrated that adding DETCs or recombinant KGF restored normal wound healing in γδ DETC-deficient skin. We propose that DETCs recognize antigen expressed by injured keratinocytes and produce factors that directly affect wound repair.

1. Gamma Delta T cells are found in epithelial tissues.
2. DETCs respond to damaged or stressed keratinocytes.
3. DETCs will then produce chemokines that may assist in wound repair.

° T cells compose a major T cell component in epithelial tissues (1, 2). The tight correlation between T cell receptor (TCR) V gene segment usage and tissue localization suggests a highly specialized function. γδ TCR-bearing DETCs found in murine skin produce cytokines and proliferate in response to damaged or stressed keratinocytes (3), indicating a functional interaction between these two neighboring cell types in vivo. DETCs produce KGF-1, also called fibroblast growth factor-7 (FGF-7), following stimulation through the γδ TCR (4). Both FGF-7 and KGF-2 (FGF-10) bind the FGFR2-IIIb receptor and have been implicated in wound healing (5–15). To directly test the potential role of γδ T cells in wound repair, we set up wound healing studies in mice lacking γδ DETCs.

Location, morphology, and density of DETCs were evaluated after wounding of C57BL/6 mouse ear skin (16) (Fig. 1, A through C). Twenty-four to 48 hours after full-thickness wounding, DETCs located around the wound exhibited a change in morphology characterized...
Student Handout Section
Name ___________________________________ Date _________ Period_____

Immune System Collaborative Posters

Introduction: You and your group will create a poster through a collaborative effort that demonstrates understanding of the immune system. The poster will show what you have learned through your reading and investigations.

Topic or page numbers assigned by your teacher ______________________________

Directions: Working in groups of 3-4 students you will create a poster that will include the following elements

1. Title for your poster
   a. Must be more than one word
   b. Must include the concept you were assigned (be creative)

2. Concept map
   a. All members must agree on a concept map.
   b. Should include key vocabulary and main idea words discussed in readings and investigations.

3. Sum It Up
   a. A 20 word summary of your section of the immune system reading.
   b. Whole group must agree on the Sum It Up

4. Illustrations
   a. 2 or more drawings or diagrams that illustrate your key learning about the immune system. Illustrations should be accompanied by a short explanation.

IMPORTANT: Each student is to select a colored marker and only use that color. You will sign your poster with that marker when the poster is complete. Review the rubric as you will self assess your work when you are complete. Be prepared to present the poster to the class or set it up for a gallery walk.

Group Members:
____________________________________
____________________________________
____________________________________
____________________________________
Collaborative Poster Rubric

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<th>Exceeds Expectations</th>
<th>Meets Expectations</th>
<th>Needs Revision</th>
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<tbody>
<tr>
<td><strong>Content and Ideas</strong></td>
<td>• Chart clearly shows the purpose and critical attributes of Independent Learning and the key features.</td>
<td>• Chart shows the purpose and critical attributes of Independent Learning and most key features.</td>
<td>• Chart demonstrates few critical attributes of Independent Learning.</td>
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<td></td>
<td>• Examples given clearly represent meaningful, relevant tasks that promote learning.</td>
<td>• Examples given represent meaningful, relevant tasks that promote learning.</td>
<td>• Examples given are rote, repetitive, at inappropriate level, or do not promote learning.</td>
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<tr>
<td></td>
<td>• Definition is clear, comprehensive, and comprehensible.</td>
<td>• Definition is clear and comprehensible.</td>
<td>• Definition is comprehensible but addresses only a part of Independent Learning.</td>
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<tr>
<td></td>
<td>• Illustration clearly represents the critical attributes of Independent Learning.</td>
<td>• Illustration represents most critical attributes of Independent Learning.</td>
<td>• Illustration is not clearly related to Independent Learning.</td>
</tr>
<tr>
<td></td>
<td>• Illustration demonstrates original thinking.</td>
<td>• Illustration does not demonstrate original thinking.</td>
<td>• Illustration is copied.</td>
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<tr>
<td><strong>Presentation and Organization</strong></td>
<td>• Chart is neat and easy to understand.</td>
<td>• Chart is comprehensible, may be messy.</td>
<td>• Poster is not neat or easy to understand.</td>
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<td>• Chart is complete</td>
<td>• Chart is complete.</td>
<td>• Chart is incomplete.</td>
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<tr>
<td><strong>Collaboration</strong></td>
<td>• All members of the group contributed ideas in relatively equal amounts.</td>
<td>• All members of the group contributed something to the chart.</td>
<td>• One member dominated or some members of the group did not contribute.</td>
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http://professional-development-d.springdale.schoolfusion.us
Biology Action Model

Purpose: To model the processes of humoral immunity and the cell mediated response.

Introduction: Your body combats pathogens that flow in your blood though humoral immunity by creating antibodies which bind to and help eliminate pathogens. If your cells get infected with virus or become cancerous, your body can kill off those cells through the process of cell mediated response.

Complete the following pre-lab questions:
1. What is the function of the immune system?
2. What are the body's non-specific defenses against invading pathogens?
3. How are antigens related to antibodies?
4. Describe what happens when pathogens enter the bloodstream.
5. Describe what happens when pathogens such as viruses are able to infect a host cell.

Procedure:

1. Cut out the model pieces and vocabulary words from the templates.
2. Review your text to study and trace the steps in each of the processes.
3. Use the modeling pieces to perform the following tasks, be sure to show your progress to your teacher at each step.
   a. Label all parts of your model using the vocabulary terms.
   b. Model the process of Humoral immunity and orally explain the process to your group members until all are able to explain the process.
   c. Model the process of the cell mediated response and orally explain the process to your group members until all are able to explain the process.

Extension Activity:

Create a story board of the process. Story boards are graphic organizers that illustrate an animated process as a series of images. Draw a story board with six or more sections. For each section, include a picture, label all the components involved, and include a brief description of what is happening in the process.
<table>
<thead>
<tr>
<th></th>
<th>Beginning</th>
<th>Developing</th>
<th>Accomplished</th>
<th>Exemplary</th>
<th>Score</th>
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<td>Part A – Preparing and Labeling Model</td>
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<td>Part B – Humoral Response</td>
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<td>Part C - Cell mediated response</td>
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B Cells
Plasma Cells

Antibodies
Macrophage
Displaying
Antigen
Infected Cell
Vocabulary Terms

Plasma Cell  T Cell
Memory B Cell  Infected Cell
B Cell  Helper T Cell
Antibody  Killer T Cell
Antigen  Suppressor T Cell
Binding Site
Macrophage
Double Diffusion Lab

Introduction:

You have modeled how antibody and antigen interact and you understand that the antibody binding sites are very specific. Now, you will carry out a lab that will use real antibody and antigen to demonstrate this specificity. This assay is based on the formation of a precipitate (precipitin line) when an antibody reacts with its specific antigen. In this test, often called the Ouchterlony test, antibody and possible antigens are placed in wells in agar plates and diffuse toward one another. The antibody is placed in a center well and antigens (specific or nonspecific) are placed in surrounding wells. When an antibody and its specific antigen meet one another and they are at the proper concentrations, the precipitate will form a visible white line between the two wells.

Procedures:

1. Obtain a plate with the agarose gel and create six holes like in the image above.
2. Have your teacher place the antibody in the center well.
3. Place different substances that have been diluted in the outer wells.
4. If the substances contained egg product, they will form a precipitin line as shown above.
5. On a separate sheet of paper record which of the foods contained egg products.
6. Read the Mystery Story and then design an experiment to test whether or not the person had any food that contained egg product.
7. Present your procedures to your teacher. Once your teacher approves your procedures, carry out your experiment and present your results.
Experimental Design Worksheet

1. What is the question/problem you want to investigate? What is your testable hypothesis? What is/are your expected outcome(s) if your hypothesis is supported?

2. What DATA do you need to obtain in order to test your hypothesis? Indicate units of the actual measurements and also how the data would be summarized and/or normalized (i.e., mean ± SD, %, mm/sec, etc.).

3. What is/are the TREATMENT(S)? Be specific with quantitative parameters (concentrations, etc.). Give some thought here as to what kind of statistical analysis you'll perform. (It is prudent to make sure you can analyze the data later using routine statistics.)

4. What is/are your CONTROL(S) for the variable(s) being tested? What will each control tell you?

5. What is one REPLICATE in your experiment? How many replicates (e.g., measurements, observations, trials) in each treatment level and control will there be? Consider how long it takes to get one observation and how much time you have.

6. Outline the step-by-step procedure you'll use to obtain a single measurement or observation, and be sure to specify all the quantitative parameters (how much, how long, when, what dose, etc.) and the equipment used for each step. This must be precise and clear enough that anyone can do it with a consistent level of accuracy and complete enough for anyone to replicate your experiment with comparable equipment.

7. How will your data be summarized, analyzed, and presented? Show relevant calculations (e.g., normalization of data, etc.) and indicate the statistical tests you'll employ. For graphic presentation, indicate the type of graph and the variables to be plotted. For Prism users, consider how the data sheet will be set up to facilitate analysis of the data.

8. State any assumptions that you are making in doing this experiment and justify them, i.e., explain your rationale for making them. How will know if your assumptions are not met?

9. List all materials that you will need including any that have not been provided already.
Mystery:

Stan's Salad Saga

As Stan lay in his hospital bed, red, swollen and gasping for breath, he agonized over the cause of the near life-threatening reaction he had suffered. All of his adult life he had known of his allergy to eggs. His physician had made abundantly clear to him the severity of the reaction that he could expect if he included eggs in his diet. Now, he was suffering from the very symptoms that had been predicted. He wasn't allergic to lots of different things. Eggs were the only substance that could have brought him to this extreme condition. Now, he faced a multi-thousand dollar hospital bill, and his insurance agent was placing the blame on him. The company would refuse to pay if Stan was shown to have been negligent. He had been far too careful to have made a mistake on his own. He had to somehow convince his agent that he was not at fault. Someone else was responsible for his being here! For the benefit of both his insurance agent, Carl, and his allergist, Judy, he recapped the activities prior to this onset of anaphylactic shock.

It had been a typical day with the exception of his departure time for work. Running late, he had not had time to eat breakfast or make his lunch. He grabbed an apple on his way out the door. When the lunch hour came, he went to the nearest branch of a local grocery chain to get a salad at the salad bar. The pasta salad looked particularly appealing that day. Conscientiously, Stan asked the salad technician whether any eggs were used in the salad. He was assured that the salad was egg-free. Stan's decision was made. His wife would be pleased that he was avoiding his usual high cholesterol diet. Stan had walked to the park to eat his lunch and that was when the crisis began. After eating only three or four bites of lunch, he began to experience a burning sensation in his ears, and he had trouble breathing. A police officer who happened to be nearby noticed his difficulty and made a 911 emergency call. That is how Stan ended up in the hospital.

Knowing that Stan was not allergic to anything else that he had eaten, the contents of the pasta salad became the immediate focus of the allergist's attention. A sample had been brought into the hospital by an alert paramedic. In addition to the pasta, it had contained tomatoes, onions, black olives and an oil and vinegar dressing. Since all the other ingredients clearly did not contain egg, the only possible source of egg was the pasta itself. The salad technician had told Stan there was no egg in the salad. Had a mistake been made? Had egg-enriched pasta been used? Or had Stan eaten something else?

You are the lab technician asked to test for the presence of egg in the pasta. Your evidence might place responsibility on the grocery store, in which case the insurance company will pay Stan's medical bills. Or you will show no evidence of egg in the pasta and Stan will be handed the blame and will be forced to pay for his negligence.
Role of Gamma Delta T Cells

Introduction:
Science is a process of continuing to build a body of knowledge in order to understand the natural world. Immunology works in the same way; new discoveries lead to better understanding and explanations about how the body works to defend itself, maintain proper functioning, and repair tissue. In this activity, you will interpret and analyze a scientific journal article that discusses the role of gamma delta T cells, a small subset of the T cell population.

Procedure:

1. In your groups, compare the side-by-side images taken from the experiments.
2. On a separate sheet of paper make any observations about the images particularly looking for similarities and differences.
3. Your teacher will then hand out the following journal article: A Role for Skin Gamma Delta T Cells in Wound Repair. Read and annotate the journal article using the following criteria.
   a. Circle unknown words. As you read, circle each word you come across that is unfamiliar. You may need to come back and reread the sentences before and after the word to fully understand the meaning of the word.
   b. Mark definitions. Underline, highlight or circle sentences that provide you with a definition. It is useful to write "def" in the margin so you can locate the definition quickly. Also mark sentences that provide examples by marking an "X" next to the sentence.
   c. Number lists of ideas. Writers provide support points to back up their main idea. Write a number in the margin next to each support point or lists of points that clarify the main idea.
   d. Make notes to yourself in the margins. As you read, write any questions or comments that crop up in your mind in the margin next to the passage.
   e. Place a check or star next to important passages. This is extremely helpful when taking a test that requires you to read a passage, because the questions that follow the reading will most likely refer back to these points.
   f. Keep it simple. Remember, you are trying to connect with the reading in some way. Use the tools that work best for you.
Images for Group Discussion

Non wounded  Wounded

(Jameson 2002)

C57BL/B6  TCR δ-/-

(Jameson 2002)

![Graph showing wound closure over days post-wounding for C57BL/B6 and TCR δ-/- mice with statistical significance indicated by asterisks.]

(Jameson 2002)
of the spizzle ligand for the Drosophila Toll receptor that is generated via a serine protease cascade (24). In mature flies, Toll also functions in immunity to fungal pathogens, and dysregulation of this pathway, due to the absence of the Spre43Ac protease inhibitor, also results in necrosis (25).

Generating a mature ligand is unlikely to be the sole function of Req3, because req3 mutants do not exhibit an HR when infiltrated with intercellular fluid preparations that contain Avr2 isolated from infected sonata plants (2).

Alternatively, Req3 could process Cf2 or another plant protein. Differences in the substrate specificity or activity of Req3m and Req3l might explain why Req3m induces Avr2-independent necrosis. An extracellular protease is also required for brassinosteroid perception in Arabidopsis (26). Overexpression of the serine carboxypeptidase IBS1 suppresses extracellular domain mutants of the BRI1 LRR-receptor kinase. IBS1 may process a protein that forms part of the BRI1 ligand (26). Alternatively, Req3 might be a plant defense component that is inhibited by Avr2. Avr2 could also inhibit other cysteine proteases, either by binding to or by modifying them. Whether Req3 has a role in defense is not established. The C2 domain motif does not appear more susceptible to C. falvum than CfD, but tomato encodes many different cysteine proteases. It is interesting that the C. falvum Avp9 protein shows significant structural homology to several protease inhibitors (27).

It is possible that Avr2 and Req3 together constitute a complex ligand that is recognized by Cf-2. It has been suggested that R proteins act as “guards” for specific proteins targeted by pathogen Avr proteins during infection (1, 2). Cf-2 may guard Req3 and trigger a defense response upon perception of an Req3/Cf2 complex. In req1 mutants, no Avr2-independent signaling would occur either because no Req3/Cf-2 complex is formed or because the complex does not activate defense signaling. A subtle structural difference in Req3m (Fig. 1A) may result in activation of an Avr2-independent response upon binding to Cf-2.

With the recent isolation of the Avr2 gene (28), it will be possible to determine whether the Avr2, Req3, and Cf2 proteins can interact. This should further increase our understanding of the molecular mechanism of ligand perception by this unique class of R proteins.

References and Notes

A Role for Skin γδ T Cells in Wound Repair
Julie Jamerson, Karen Ugarte, Nicole Chen, Pia Yachi, Eline Fuchs, Richard Boismer, Wendy L. Havran

γδ T cell receptor-bearing dendritic epidermal T cells (DETCs) found in murine skin recognize antigen expressed by damaged or stressed keratinocytes. Activated DETCs produce keratinocyte growth factors (KGFs) and chemokines, raising the possibility that DETCs play a role in tissue repair. We performed wound healing studies and found defects in keratinocyte proliferation and tissue reepithelialization in the absence of wild-type DETCs. In vitro skin organ culture studies demonstrated that adding DETCs or recombinant KGF restored normal wound healing in γδ T cell-deficient skin. We propose that DETCs recognize antigen expressed by injured keratinocytes and produce factors that directly affect wound repair.

References and Notes
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**Fig. 1.** γδ DETCs participate in wound repair. (A through C) Detection of DETCs in epidermal sheets isolated from C57BL/6 (B6) mice with a γδ TCR-specific mAb (PE-GL3; Pharmingen, La Jolla, CA) (16, 27, 28). Arrows indicate the wound edge. (A) Non-wounded (magnification ×200). Day 2 after wounding (B) ×200 and (C) ×100. Data are representative of wounds examined from at least 10 mice. (D) Photographic images of excision wounds from B6 and TCRδ−/− mice 2 days after wounding. (E) Wound closure kinetics in wild-type (A) and TCRδ−/− mice (B). Data are representative of at least three experiments. **P < 0.005 and *P < 0.05 using an unpaired 2-tailed Student t-test.

**Fig. 2.** γδ DETCs affect epidermal thickening and keratinocyte proliferation in wounded tissue. Detection of proliferating cells in wounded tissue by BrdU labeling in wild-type (A and C) and TCRδ−/− (B and D) mice 3 days after wounding (16). Wound edge is marked with arrow (↓) (A and B) ×100 and (C and D) ×200 magnification. Representative BrdU-positive cells are marked by arrows: e, epidermis; h, hair follicles; and Es, eschar. (E) Quantification of BrdU-positive cells in B6 (A) and TCRδ−/− (B) skin. At each time point, BrdU-positive cells were quantified from at least six individual mice per strain (16). (F) BrdU incorporation at the wound site 5 days after wounding in wild-type, TCRδ−/−, OT-1 Rag−/−, and Rag−/− mice. No significant differences were observed between OT-1 Rag−/−, Rag−/−, and TCRδ−/− mice. **P < 0.005 and *P < 0.01 using an unpaired 2-tailed Student t-test compared to B6 mice.

by a partial loss of the distinctive dendritic shape, although no significant change in DETC density was observed (Fig. 1, B and C). Five days after wounding, the DETCs at the wound margins began to regain their dendritic morphology (17). DETCs that were distant from the wound retained their normal shape.

To determine whether DETCs participate in wound repair, wild-type and TCRδ−/− mice received full-thickness wounds in their back skin (16), and the rate of wound closure was assessed over a 20-day period. Clear differences in wound size and rate of healing were evident when wild-type and TCRδ−/− wounds were compared on each day after wounding, as shown for day 2 (Fig. 1D). TCRδ−/− mice had a 2- to 3-day delay in wound closure relative to wild-type mice (Fig. 1E). Histological analysis of full-thickness wounds revealed reduced epidermal hyperthickening in the TCRδ−/− mice, relative to wild-type mice (Fig. 2, A through D), suggesting that keratinocyte proliferation was impaired in the absence of γδ DETCs. To test this more directly, we injected mice with BrdU at various times after wounding (16). Significantly fewer BrdU-positive epidermal cells were detected from the wound edge to the wound center in TCRδ−/− compared to wild-type mice (Fig. 2, C through E). Together, these data indicate that γδ T cells play a role in keratinocyte proliferation and reepithelialization during wound healing.

DETCs are activated through the canonical Vγ3Vδ1 TCR by antigen expressed by stressed keratinocytes. Mice that lack the canonical Vγ3 chain have DETCs with a TCR that retains the original Vγ3 conformation and antigen specificity (18), emphasizing the functional relevance of this TCR. To determine if DETC activation in response to wounding requires Vγ3Vδ1, we utilized Vγ1Vδ1 transgenic mice (19) on a Rag−/− background. In OT-1 mice, all T cells, including DETCs, express an identical Vα2Vδ2 TCR that recognizes an ovalbumin peptide complexed with I-Ak (19). BrdU incorporation in the wound was diminished (Fig. 2F) and wound closure delayed (17) in OT-1 compared to wild-type mice in a manner similar to that observed in TCRδ−/− mice.
We conclude that DETCs contribute to wound repair via specific recognition of antigens mediated through the Vγ3Vδ8 TCR. γδ DETCs do not constitutively produce FGF-5 but require activation through the TCR (4). Furthermore, keratinocyte proliferation mediated by γδ DETCs was inhibited by a FGF2-βIIb neutralizing peptide (20), indicating that KGFs are the major epithelial growth factors produced by DETCs (20). To determine if γδ DETCs are activated to produce FGF-10 in response to tissue injury, FGF-7 expression was examined in full-thickness wounds (15) (Fig. 3A). FGF-7 mRNA was detected in DETCs isolated from the wound area but not from nonwounded skin. In addition, mRNA encoding other factors including TNF-α and IFN-γ was expressed by DETCs following wounding (17). The data suggest that DETCs in wounded skin are activated by neighboring, damaged keratinocytes and play a role in wound repair by expressing KGFs and cytokines.

Because no significant wound healing defect has been observed in FGF-7−/− mice (15, 21), we examined whether γδ DETCs also produced FGF-10. The importance of FGF-10 in wound healing cannot be studied in FGF-10−/− mice as they die shortly after birth (22, 23). Because the DETC cell line 7-17 produced FGF-10 upon TCR stimulation, it was possible that DETCs might participate in wound healing by production of FGF-10. Consistent with this, FGF-10 was detected in wounded skin obtained from wild-type and FGF-7−/− mice, but not in the epidermis of wounded TCRδ−/− mice, indicating that DETCs were the key source of FGF-10 in the wounded epidermis 2 days after wounding (Fig. 3B).

A skin organ culture (SOC) assay was used to further characterize the contribution of γδ DETC-produced factors to wound repair and keratinocyte proliferation (16, 24, 25). The in vivo finding that TCRδ−/− mouse skin showed delayed kinetics of wound closure when compared with wild-type mouse skin could be reproduced in the SOC assay (Fig. 3C). Significantly, addition of FGF-10 normalized the wound closure rate and BrdU incorporation observed with TCRδ−/− skin (Fig. 3D). Skin from OT-1 Rag2−/− and Rag2−/− mice exhibited similar delays in wound closure as TCRδ−/− skin in SOC (22). Addition of Con A-activated 7-17 cells to the culture of wounded TCRδ−/− skin restored proliferation to wild-type levels (Fig. 3E). Together, these data indicate that the defect in keratinocyte proliferation and wound closure in TCRδ−/− mice can be attributed to a lack of KGFs produced by DETCs.

Wound healing is a complex process that involves epithelial cell proliferation, granulation tissue deposition, and inflammatory cell recruitment. Many cell types are involved in this process; however, the role of resident DETCs in wound repair has been neglected. Here, we demonstrate that mice lacking DETCs have a significant delay in wound healing and impaired epidermal cell proliferation.

We have proposed that DETCs recognize antigen expressed on neighboring epithelial cells following injury or disease (3), and participate in tissue repair through the local production of factors including FGF-7 and FGF-10. DETCs are considered a prototype intrap epithelial γδ T cell population, raising the possibility that participation in tissue repair is a conserved function shared by intraepithelial γδ T cells in different epithelial tissues. Indeed, we have recently shown that intestinal intraepithelial γδ T cells protect the intestinal mucosa from damage (26). Future studies of epithelial diseases such as inflammatory bowel disease, asthma, and wound healing will need to consider the role of intrapithelial γδ T cells in disease progression and tissue repair as well as in design of treatment strategies.

References and Notes
16. Supplementary details of experimental procedures are available on Science Online at www.sciencemag.org/content/full/296/S687/47DC1.
17. J. M Amano et al., unpublished observations.
30. We thank S. Werner for providing IGF-10 adenosine, and S. T. C. C. for GR-17877, mice, and A. A. M. S. for recombinant IGF-10. We also thank N. Gardner, T. Chen, D. L. Plage, E. Wolters, and K. Tomlin for advice and review of the manuscript. This work was supported by NIH grants AI06556 and AI27751 (W.H.) and ST23AA0575-11 (W.L.H.). W.L.H. is a Scholar of the Leukemia and Lymphoma Society. This manuscript is in preparation from the Scripps Research Institute.
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