Cell Communication

Teresa Burke
Westford Academy
30 Patten Road
Westford, MA 01886
tburke@westfordk12.ma.us
Mentored by William Cruikshank
Boston University Medical School-Immunology
Funded through the American Association of Immunologists

"Can You Hear Me Now?" Cell communication

Dawn Martell
Wilmington High School
159 Church Street
Wilmington, MA 01887
dmartell@wilmington.k12.ma.us
Mentored by Dr. William Cruikshank,
Boston University Medical School-Immunology
Funded through the American Association of Immunologists

Table of Contents

Teacher Preparation Section

Teacher Preparation Section

Overview

Cell communication and homeostasis is an integral process by which the body maintains functions. Sustaining life depends on a quick and effective response from the immune system in recognition and reaction to foreign invaders. In order to maintain homeostasis of the immune system, several positive and negative feedback loops are constantly regulating the human body.

In this laboratory investigation, students will have the opportunity to learn how the immune system builds its arsenal to defend itself from foreign invaders. Students will also gain the understanding of how the lymphatic System facilitates immune responses. A particular focus in this unit will include the orig

Massachusetts Curriculum Frameworks:

Content Standard
Standard 4-Anatomy and Physiology:

2.6 Describe the cell cycle and the process of Mitosis in the formation of new cells.

- A brief informational session on the cloning, proliferation of new and the activation of APCs/macrophages and helper T cells.

4.7 Recognize that communication among cells is required for coordination of body functions.

- Discussion session on cell signaling

4.8 Recognize that the body's systems interact to maintain homeostasis. Describe the basic function of a physiological feedback loops.

- Discussion on the importance of feedback loops to maintain homeostasis in the body will take place

Scientific Inquiry Skills Standard*:

SIS1. Make observations, raise questions, and formulate hypotheses.

SIS2. Design and conduct scientific investigations.

SIS3. Analyze and interpret results of scientific investigations.

SIS4. Communicate and apply the results of scientific investigations.

*For specifics see Massachusetts Curriculum Frameworks Learning Standards

Science Background:

Chromatography is a process used to separate molecules. There are many types of chromatography, such as gas, ion exchange, electrophoresis, or paper. Below is a brief description of the more common types of chromatography techniques used. These descriptions can be used as an introduction for the students before beginning the laboratory process of chromatography in the separation of simulated human blood.

Gas chromatography is usually the separation of a liquid or polymer using an un-reactive gas, which is contained inside a glass or metal tube (column). The gas is separated by different rates due to its chemical and physical properties and how these gases interact with the stationary phase (column). These interactions cause the gases to exit the column at different rates, which is electronically detected determining the amount of time each component reaches the outlet.

Ion-exchange chromatography is used

Ouchterlony Technique

The Ouchterlony technique is an immunoprecipitation assay in which chemicals migrate through a medium and when these chemicals react with one another form a precipitation band at the point of contact. This exploration is to determine if the chemoattractant-interleukin 16 (IL-16) and macrophage inflammatory proteins (MIP) influence the migration of CD4 Helper T cells, cheek cells and an unknown sample. CD4 Helper T cells become Th1 and Th2 cells in mediated responses. One of the following cells, either helper T cells, cheek cells or the unknown will migrate through the plate towards a specific chemokine, the point where the cells and the chemokine meet, a precipitation band will form.

Students will be able to observe the precipitation band that represents the interaction of cytokines (chemokines) acting on target cells by binding to their specific membrane receptors in a simulated inflammation response. Students will be able to identify the unknown cell solution by matching the band to the known cell type demonstrating cell-specific chemokine to cell migration and cell signaling.

Immune Response

When a foreign invader enters the body, the immune system is activated. Lymphocytes, T cells, are derived in the bone marrow. Then, T cells travel to the thymus gland where they mature and acquire the antigen specificity. This enables them to respond during the inflammatory process. T cells are mainly involved in cell-mediated immunity. Their function is to attack cells that are infected by microbes and viruses or cells that are recognized as foreign such as cancer cells. After T-cells have matured, they move into the blood and lymph tissue and circulate throughout the body looking for invaders. T-cells generate cytokines, also called lymphokines, which are proteins that are important in cell communication. During an immune response, T cells produce a number of different lymphkines that function to activate other T cells, B-cells, NK-Natural Killer cells, or macrophages. Some of the lymphokines, such as IL-16 and MIP act to recruit the other cells to the site of inflammation. Other lymphokines activate the cells to facilitate their role in the inflammatory process. T-cell activation of B lymphocytes results in production of antibodies. This can further enhance the immune response and serve to act as an early response mechanism for future encounters with the same pathogen. Some of the cytokines produced by the T cells also act to regulate the immune response and help to resolve inflammation once the pathogen has been destroyed.

Recruitment of immune cells is a selective process. Each chemoattractant protein, such as IL-16 or MIP, binds to a specific receptor. Cells can only respond if they express the appropriate receptor. Therefore, at sits of inflammation where only IL is produced, only cells that express the IL-16 receptor will be recruited. Cells that express the MIP receptor will not be recruited. In this fashion, the types of recruiting

cytokines produced influence the type of cells that are recruited. Therefore, different types of inflammation can occur.

The two primary types of T-cells are CD8T and CD4T. CD8T cells are cytotoxic T cells and killer cells. They respond to foreign antigens on cell surfaces and eliminates

- 2ml Sudan IV reagent
- 1ml 70% isopropyl alcohol

Simulated Cell Samples

- Well A-water
- Well B-Th_1 cell (2M CaCl ~3 drops)
- Well C-Th_2 (.25 NaOH ~3 drops), Th2 cell sample .4M NaOH, 30 drops=.5ml
- Well D-cheek cell (water-control)
- Well E-unknown-Elution from Column Chromatography
- Plate A-chemokine in center, IL-16, 2M K_2HPO_4 either di/tri basic (3 drops)
- Plate B-chemokine in center, MIP, phenolphthalein (3 drops)
- 2ml of Sudan IV reagent
- Run sample (blood) 1M NaOH

This simulated blood suspension will be placed at the teacher station. In this solution, the NaOH will simulate Th2 cells and CaCl will simulate Th1 cells. This activity is used to initiate the attraction of specific cells to selective chemokines. Each group will need to aliquot 10 ml of the blood solution into a 10 ml conical tube (6 conical tubes per class) and bring it back to their lab station.
Do not dispose to the NaOH and the CaCl solutions. They will both be used in the lab.

Simulated Cell Samples

Clearly label six 250 ml Erlenmeyer flasks as follows and place the fill with the corresponding solutions:
Flask 1: Label-**Th1 Cells**. Fill with the remainder of the 2M CaCl solution
Flask 2: Label-**Th2 Cells.** Fill with the remainder of the .25M NaOH solution
Flask 3: Label- **Human Cheek Cells.** Fill with ~200 ml of distilled water
Flask 4: Label- **Water.** Fill with ~200 ml of distilled water
Flask 5: Label-**Chemokine-IL16.** 200 ml of 2M Potassium Phosphate Tribasic solution. To make this solution add 84.9g of potassium phosphate tribasic to 100ml of distilled water, mix thoroughly and bring volume to 200ml
Flask 6: Label-**Chemokine-MIP.** ~100 ml of phenolphthalein

These solutions will be set up on the teacher station. Each group will receive six 1.5ml microtubes. Each group will label the microtubes with each of the four solutions listed above. The groups will aliquot 1ml of each of the above solutions in the designated microtube and bring the microtubes back to their workbench.

2% Agar plates

Preparation for the Ouchterlony plates may be done by the teacher previous to the lab or may be done by the students depending on time restrictions.

- Boil 1 L of distilled water
- Add 20 g of agar to the boiling water and stir until completely dissolved
- Pour hot agar into plates (~25 ml-until agar covers the bottom of the plate completely)
- Allow plates to solidify

If students have the time to make their own

- Hose clamp

Ouchterlony plate technique

This technique is used to demonstrate cell migration to specific chemokines. When certain immune cells are exposed to chemokines, the T cells, with the corresponding protein receptors, will migrate towards these protein signals. The potassium phosphate tribasic will simulate the chemokine MIP and phenolphtha

- Six markers
- Thirty-six 1.5 microtubes-six for each group
- Electronic scale

otal costs

iment in not costly and many of the supplies are found in most high school laboratories.

Possible substitutions

For chemicals, most chemicals that can react in a double-replacement reaction will be suitable. Potassium Phosphate tribasic can be substituted for monobasic or dibasic. Small Petri dishes can be used in replace of larger ones. Instead of plastic disposable pipettes for creating holes in the agar, plastic straws can be used.

Safety

Normal safety precautions should be taken including: goggles, aprons, and insulated mitts. All chemicals can be disposed of in the trash receptacles and in the drain with dilutions of water to follow.

Student Knowledge and Skills

After this laboratory investigation, students will have a greater understanding of how the immune system functions, be able to explain the sequence of events in response to foreign invaders and how the body maintains of homeostasis.

Students will be familiar with measurements of volumes, such as the skills needed to micropipette. They will understand chromatography column and be able to contrast the difference between types of chromatography. Students will be able to perform and understand the Ochterlony essay.

Misconceptions of immune system

The following are a few misconceptions of the immune system which students may be unaware of, these misconceptions may be used for an opening discussion between teacher and students or may be used as a group activity to evaluate students preknowledge on their understanding of the immune system.

Does receiving the flu shot give you the flu?

When one receives the flu shot, they receive an inactive part or dead virus. The part of the flu that makes you sick is "turned off". The inactive part or dead virus stimulates antibody production in the body. When one does encounter this virus antibodies are already present to "fight" this virus. A person who has severe allergies to eggs should not receive the flu shot because eggs are used to create the flu vaccine.

Does cold weather cause one to catch a cold?

Cold weather does not cause a viral infection. There are over 200 different known viruses. Viruses are transmitted from an infected person usually via airborne, shaking of hands, contact with a phone or door handles.

Can one get arthritis from cracking their knuckles?

Cracking the knuckles does not give you arthritis. Cracking the knuckles causes the two joints to be pulled apart. The synovial fluid located within this joint fills more of the space. This decreases the fluid and dissolved gases, these gases leave the joint as tiny bubbles. It is the popping of the gas bubbles that one hears. Continuous cracking of the knuckles can injure the joint and weaken the fingers.

Student Expectations

- Students will research the functions of Th1, Th2 cells, chemokines IL-16 and MIP. This information will be placed within the introduction of their laboratory report.
- Students will write a scientific hypothesis for both procedures, column chromatography and Ouchterlony Plates
- Students will complete a formal laboratory report.
- Students will draw and interpret the results from Ouchterlony plates and complete analysis questions from laboratory exploration
- Students will create a cartoon drawing of cell communication within the immune system.
- Students will complete the CD activity sheet.
- Students will take an assessment test.

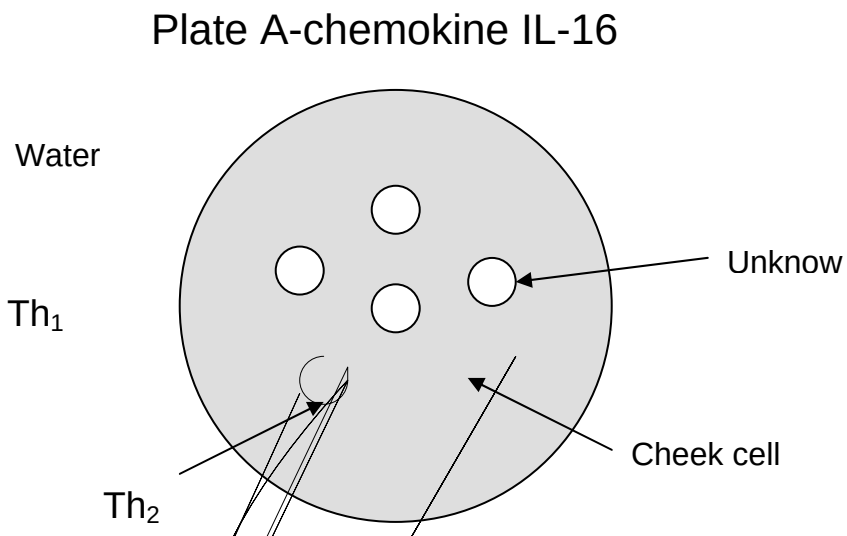
Anticipated Results

During the column chromatography process the simulated red blood cells will adhere to the polyester fiber located in the column. The simulated white cells will elude from the column. The migration of the solutions in the Ochterlony plates takes approximately 30-40 minutes before results will appear.

Immunoassay Results:

- All the cells and chemokines will permeate through the agar. Only specific cells that are attracted to their exact chemokines will react causing a precipitation band.
- Results of Plate A: As the chemokine-IL-16

Results of Plate A: IL-16 and unknown cells=Th1,



Results of Plate B: MIP and unknown cells=Th2

Classroom Discussion

- Demonstration of the activation of APC, chemokines and helper T cells
- Function of the immune system
- Review terminology
    - Specifically macrophage, helper T cells and chemokines
- Discuss the differences in the precipitation bands from Plate A and Plate B
    - Different chemicals represent simulated chemokines and cells, as each react with a specific chemical causing a color change take place
- Discuss specific chemokine functions and their attachment to specific cell surface receptors
- Discuss the advantages and disadvantages of having a healthy and unhealthy immune system

Assessment

- Classroom discussion and, feedback from students during question and answer sessions during daily lessons and after laboratory exploration
- Formal laboratory report:
    - Introduction-research on function of the immune system, including chemokines-MIP and IL-16, macrophages, APC cells and helper T 1&2 cells
    - Analysis questions
    - A final statement that includes data collection and laboratory results
- Animated Drawing that models the response of the immune system to a foreign invader.
- Teacher observations of students during preparation and laboratory procedures
- Activity sheet to follow along using the CD on the immune system
- Student Test

Student Section

Name:_____Date:_____Class:_____

“Can You Hear Me Now?”

Name:_____Date:_____Class:_____

“Can You Hear Me Now?”

Laboratory Procedure

Ouchterlony Plate

Introduction

Cell communication and homeostasis is an integral part of maintaining normal body function. Sustaining life depends on a quick response from the immune system in the recognition and reaction to foreign invaders. Continual enhancement of the immune system returns the body to homeostasis via feedback loops.

Through this laboratory investigation you will have a better understanding of how the lymphatic system facilitates immune responses. Lymphocyte T cells chemically communicate between specific cells to destroy cells that contain foreign invaders by releasing cytotoxins. You will describe the role of Antigen Presenting Cells (APC), the role and the origin of chemokines and that specific chemokines that attract and activate certain helper T cells. These helper T cells in return stimulate self-growth and activate the division of more helper T cells. The newly produced helper T cells proliferate memory cells through a positive feedback loop. The activation of helper T cells also helps to trigger B cells. The B cells manufacture antibodies which in turn activate Cytotoxic T cells during the immune re471.54089 Tm(9cof 12 actur.48o(lps to 33m(10.0002 Tc 0.039

7. Using the insulated gloves, place the flask into the microwave and cook it for 10-15 second intervals or until all particles are dissolved (approximately 60-90 seconds). The solution should be a golden brown. **Do not let the agar boil over.**
8. Let the agar cool for 3 minutes.
9. Using the insulated gloves, carefully pour the liquid agar into the base of the Petri dish. Leave approximately 2mm of space at the top of the dish. Do the same procedure for the second Petri dish.
10. Cover the Petri dishes and let them stand until the liquid has solidified.
11. Then, place the Petri dishes in the appropriate location, which is specified by the teacher, until your next class.
12. Clean and return all materials to their proper place.
13. If time permits, you will practice making wells in the pre-made agar plates.

Part 3:
Running Immunoassay

Materials:	**Teacher Station:**
Petri Dish from Part 2	1 tube each of the following cell samples & chemokines
Scissors	Unknown
13 Pasteur Pipettes	Water
Marker	Cheek
Metric Ruler	Th1
Calculator	Th2
Conical tube	IL-16-chemokine
	MIP-chemokine
	Microtube with plasma

Procedure

1. Gather all materials.
2. Label one plate A and the other B. Also label each plate with your initials.
3. Place the assay plate (agar plate) on top of the template below and remove the cover.
4. Using the scissors cut 5cm of the very bottom of one of the Pasteur pipette.
5. Squeeze the bulb of the pipette.
6. Then, carefully place the tip of the pipette over the circle labeled water.
7. Carefully push down and twist the pipette until it hits the bottom of the plate.
8. Then, carefully remove the pipette. The pipette should contain a small circle of agar leaving a small hole in the agar plate.
9. Using the same Pasture pipette, follow the same procedures (steps 4-8) for the remaining wells.
10. See template below for each well.
11. To keep from cross-contamination, use a new pipette to fill each well with the specific cells and chemokine. Do not overflow the well and do not let the tip of the pipette touch the bottom of the agar plate. See the chart on the next page.

12. Place the covers on Petri dishes.
13. Observe, draw, and record results.
14. Clean your lab station and replace all materials to their proper place.

Place drawing below:

Analysis Questions:

1. What is the purpose of using the water?

2. Was there any reaction between the chemokine and cells? If so explain this reaction.

3. Which type of cell represents the unknown for Plate A? For Plate B? How do know?

Animated Drawing of an Immune Response

Name:_____Date:_____Class:_____

Introdcution

As we begin the study of cell communication, feedback loops and homeostasis of the immune system, you and a partner will create an animation drawing. As cartoonists you will create a drawing that simulates an inflammation response to a foreign invader that has entered your body.

Part I

Pre-drawing

For the first part of the assignment, you will have 20 minutes to brainstorm some ideas that you may use for your first drawing. List these ideas below. Then using the list you created, you are to sketch or draw, and label your drawing. Then, give a brief explanation about the drawing. White drawing paper and colored pencils will be provided. After finishing your drawing place your names on the back and tape the drawing on the board. See an example on the next page.

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

Part II

Post-drawing

Immunology Animated Drawing

Cell communication and homeostasis is an integral part of maintaining normal body function. Sustaining life depends on a quick response from the immune system in the recognition and reaction to foreign invaders. This along with continual enhancement returns the body to homeostasis via feedback loops.

Using the knowledge you have acquired from classroom discussions and the laboratory investigations, you and your partner will create at least a three-frame animated drawing to show how the immune system simulates an inflammation response to a foreign invader, and how the immune system responds to this foreign invader. Your animated drawing must demonstrates the

Animated Drawing Example

Figure 3, Photography by Dawn Martell

Rubric for the Animated Drawing

Graphics

- Colorful & neat
- Creative
- Attention-getting
- Graphics create interest for reader

Name:_____Date:_____Class:_____

Pre/post quiz for the Immunology unit

1. `An antigen is a (n)

- a. protein molecule that helps defend the body against disease.
- b. type of white blood cell.
- c. invading virus or bacterium.
- d. foreign molecule that evokes an immune response.
- e. body cell attacked by an invading microorganism.

2. Your lymphatic system fights infection and

- a. delivers foo001 Tm(a. d)Tj10.02 11.24751 563.70001 Tm82 0 0 10.02 2068C 8455 n(f.y6/CS190002 Tm5 water toers)Tj

6. A clone of lymphocytes

a. produces different anti

11. Bacteria in body fluids are attacked by

- a. antibodies from B cells.
- b. cytotoxic T cells.
- c. interferons.
- d. helper T cells.
- e. antigens.

12. What do the antibodies secreted by plasma cells (the effector cells of humoral immunity) do to attack their targets?

- a. activate complement to punch holes in them
- b. clump cells together so that phagocytes can ingest them
- c. cause antigen molecules to settle out of solution
- d. attach to antigens and detoxify them
- e. all of the above

13. Which of the following is *not* present until after the primary immune response occurs?

- a. memory cells
- b. macrophages
- c. helper T cells
- d. complement proteins
- e. antigens

14. The body produces antibodies co

16. The idea behind vaccination is to induce _____ without the vacci

Name:_____Date:_____Class:_____

CD Review question sheet

As you watch chapter 24a on the interactive CD, answer the following questions.

9. What chemical signal enhances the activation of T- cells and what type of cells secrete these signals?

10. What does IL-2 stimulate?

11. How do cytotoxic T-cells work?

12. What is the purpose of perforin?

Campbell, Reece, Mitchell, Interactive Study Partner for Biology, The Immune System, Fifth Edition, CD-ROM, San Francisco, CA, Pearson, Benjamin Cummings, 2006.

References

Banerjee, Ena Ray, et. al., “ 4 and 2 intergins have nonredundant roles for asthma development, but for optimal allergen sensitization only 4 is critical.” Experimental Hematology, 35(2007): 605-617.

Bernhagen, Jurgen; et.al, “MIF is a noncogna