



AAI High School Teachers Summer Research Program in Immunology

CD4+ T-cell differentiation: Using computer modeling to explore the management of the adaptive immune response to inflammation.

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I. Overview

Topics Addressed:

-Cell signaling, cytokines, inflammation, T-cell differentiation (T17 → TReg)

Objectives:

1. Students will use a computer simulation to explore the body's inflammatory response and potential treatments.
2. Students will adjust variables, magnitude of inflammation and treatment to carry out a virtual mouse study.
3. Students will code an extension to the virtual mouse study simulator.

Technical Skills:

-Students will learn to operate a virtual immunology simulator. After, the students will learn how to perform simple coding to extend on the simulator.

Placement:

-In an Anatomy and Physiology class, this unit would supplement the study of the immune system (usually toward the end of the year).

-In an AP Biology class, this unit would fulfill the study of the immune system, also at the end of the year.

Anatomy and Physiology Standards Correlation:

AP4.1 Demonstrate knowledge of the acquired immune response, particularly the function and differentiation of T-Cells.

AP4.2 Describe the mechanisms of the acute inflammatory response, its causes, and the role of chemical signaling molecules.

AP Biology Standard Correlation:

EK 2.D.4: Plants and animals have a variety of chemical defenses against infections that affect dynamic homeostasis.

- b. Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.
 2. In the cell-mediated response, cytotoxic T cells, a type of lymphocytic white blood cell, “target” intracellular pathogens when antigens are displayed on the outside of the cells.
 - Learning Objective 2.29: The student can create representations and models to describe immune responses. [See SP 1.1, 1.2]

II. Science Background

One in 200 Americans suffers from Inflammatory Bowel Disorder (IBD), resulting in chronic gastrointestinal distress and an increased risk of colon cancer. According to the Center for Disease Control (CDC), IBD accounts for over 700,000 physician visits and 100,000 hospitalizations in the US every year (CDC, 2011). About 1.4 million Americans suffer from IBD.

In contrast to innate immunity, where immune receptor presence is encoded in the genes, acquired (adaptive) immunity consists of a series of receptors and responses that evolve over the lifetime of the organism and that depend on environmental exposure. T-cells are a key subset of the acquired immune system. Their functions include recruitment of other immune cells and production of cytokines. For example, T helper 17 (Th17) cells produce interleukin 17 (IL-17) cytokines in the gut mucosa leading to inflammation, which sometimes in excessive amounts causes irritable bowel disease (IBD) or Crohn's disease.

PPAR γ is a Type II nuclear receptor that helps regulate metabolism and inflammation by promoting regulatory T cell (Treg) production and by limiting the autoimmune response of inflammation. During my lab experience, we observed that PPAR γ KO mice show a significant increase in disease activity compared to their WT counterparts due to a lack of Treg production (unpublished data). Since PPAR γ is a glitazone receptor, pioglitazone treatments have no effect on disease state in the KO mice leading to IBD.

Designed for educational use, NetLogo is a user-friendly Java programming interface that allows the programmer to run simulations based on data collected from mouse studies. The *in silico* model provides a visual response to the molecular process which can then be fine-tuned and altered to drive the next mouse study more efficiently.

References:

Carbo, A. et al. Systems modeling of molecular mechanisms controlling CD4+ T cell differentiation and phenotype plasticity, *pLOS Computational Biology*. In revision.

Center for Disease Control, (2011). Inflammatory Bowel Disease (IBD). Retrieved from <http://www.cdc.gov/ibd/>.

Wilensky, U. (1999). NetLogo. <http://ccl.northwestern.edu/netlogo/>. Center for Connected Learning and Computer-Based Modeling, Northwestern University, Evanston, IL.

III. Student Outcomes

In a high school Anatomy and Physiology or AP Biology class, students will perform scientific research on inflammation in chronic inflammation diseases. Then, they will write program code to build on an existing computer model. The model will include the following: new treatments for inflammation, inflammation in other body systems from various triggers or disease, inflammation in mice with genetic modifications, or the response of other types of adaptive immune cells. The program will serve to both introduce and extend students' interest and knowledge of adaptive immunity.

IV. Learning Objectives

1. Students will use a computer simulation to identify the cytokines and immune cells involved in the acute inflammatory response and T-Cell differentiation.
2. Students will design and conduct a virtual mouse study to examine how magnitude of inflammation is introduced and volume of treatment as administered affect the adaptive immune response.

V. Time Requirements

~Two weeks:

- a. Five 90-minute blocks
- b. Ten 45-minute periods

VI. Advance Preparation

Before downloading NetLogo 3D, be sure to check your district policy on downloading software from the Internet to student computers (or computers in a computer lab). Teachers and all students will need to download the NetLogo 3D software from the NetLogo website: <http://ccl.northwestern.edu/netlogo/download.shtml>. You simply enter your name, affiliation, and email and click on the “Download” button. Follow the prompts to complete the download to your computer. The software is free and supported by Northwestern University. You will not receive any emails or advertisements for downloading this educational software. The download takes about 5-10 minutes depending on processing and server speed.

VII. Materials and Equipment

Each student (or pair) will need a computer with NetLogo installed. Once the software is installed, and files are downloaded, the computers do not need an Internet connection. The software is free to download.

VIII. Student Prior Knowledge and Skills

Students should have a working understanding of the immune system, specifically the similarities and differences between the innate and acquired immune systems (including cells involved). Students should have a basic understanding of how computer simulators work, including their strengths and limitations in running a virtual study.

IX. What Is Expected from Students

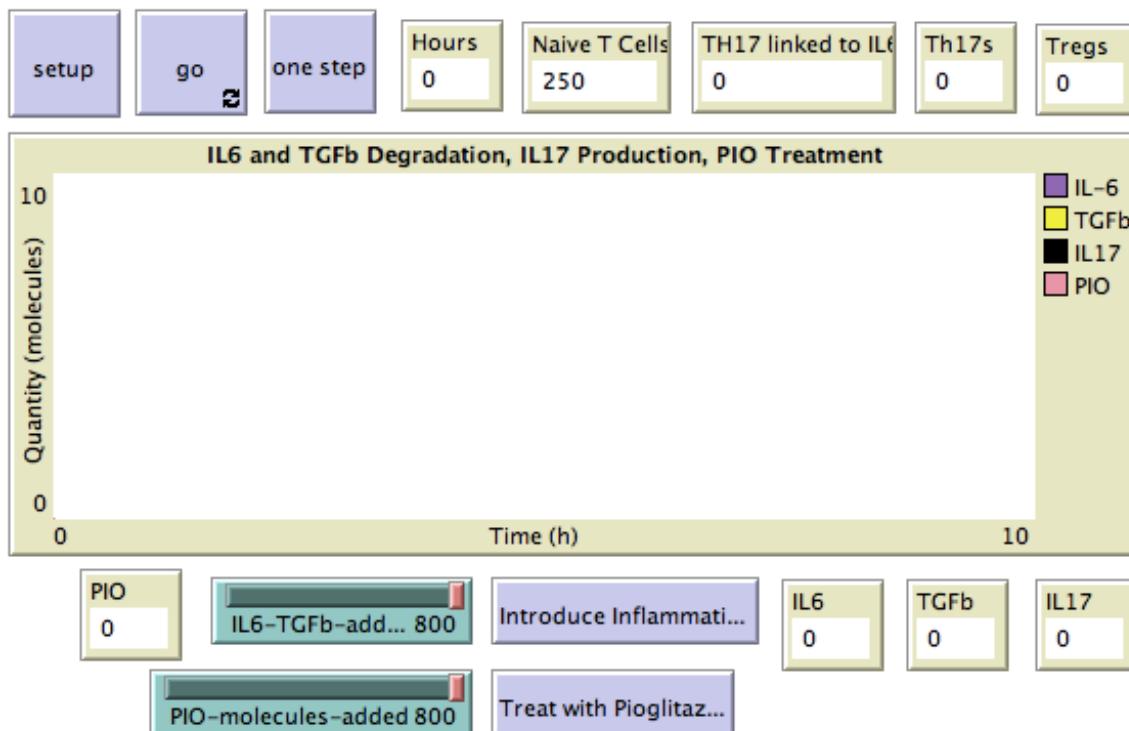
5E Model Lesson Plan Overview

See below for detailed descriptions for each stage of the lesson

Engage	Students will watch the results of the wildtype and knockout (PPARg) mouse study in NetLogo 3D and discuss the differences, components involved, etc.
Explore	Students will design and conduct a virtual mouse study using the teacher-designed interactive NetLogo 3D file
Explain/ Elaborate	Students will conduct research on a different lymphocyte, different disease, etc. and code an extension to the original program using the literature as their guide
Evaluate	Students will present their new programs to the class using the student-designed rubric

Engage: Students will watch the teacher's NetLogo simulation from the PPARg mouse study conducted at VBI last summer. The teacher will explain each visual component (IL-17, Pioglitazone, etc.) and play the video. Then, students will write in their journal to explain the visual using the terms and concepts relating to inflammation from the day before.

Explore: Students, working in pairs, will use interactive teacher-designed interface (see below) based on actual WT mouse-study data to explore how different levels of disease (magnitude of inflammation) will affect the immune response of the organism (levels of Th17 differentiation and cytokine production) and how different levels of pioglitazone treatment affect disease activity and response. Students will design and conduct a virtual study: identifying variables, collecting data, interpreting results, and sharing conclusions with their peers.



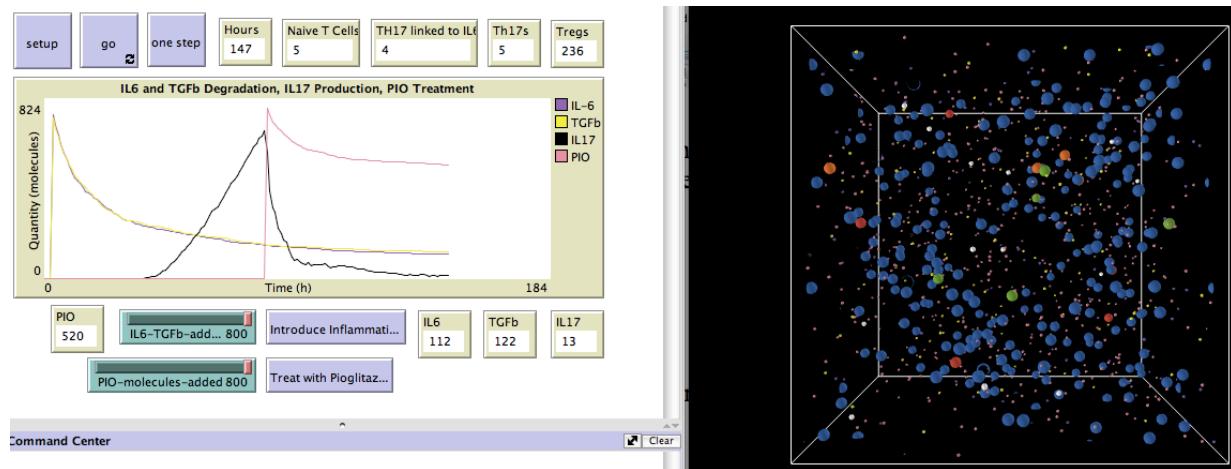
Formative Evaluation: Students will informally present their study design and findings in a group of 4 following a class created rubric to determine project score and decide follow-up instruction for the teacher. This formative assessment for the teacher enables the teacher to adjust instruction for what mini-lecture, coding assistance, or programming skills need to be addressed before an “extend” activity. Also, it gives the chance for students to confront misunderstandings regarding the original program and its capabilities.

Explain and Elaborate: Students will first complete the NetLogo programming tutorial (found at <http://ccl.northwestern.edu/netlogo/>) to learn the basics of working with agent-based modeling software. Next, students will start with the teacher-designed interface (see above) and code their own extensions based on their disease research. Students may choose to incorporate a different lymphocyte (B, T1, T2, etc.), a different magnitude of disease response, a different treatment, or even a different disease (Crohn’s or something researched before) by implementing different buttons, slider bars, lymphocyte and/or cytokine counters, and/or response times. All students will share their results with a partner. At this stage, students will “trade-and-trial” each other’s programs.

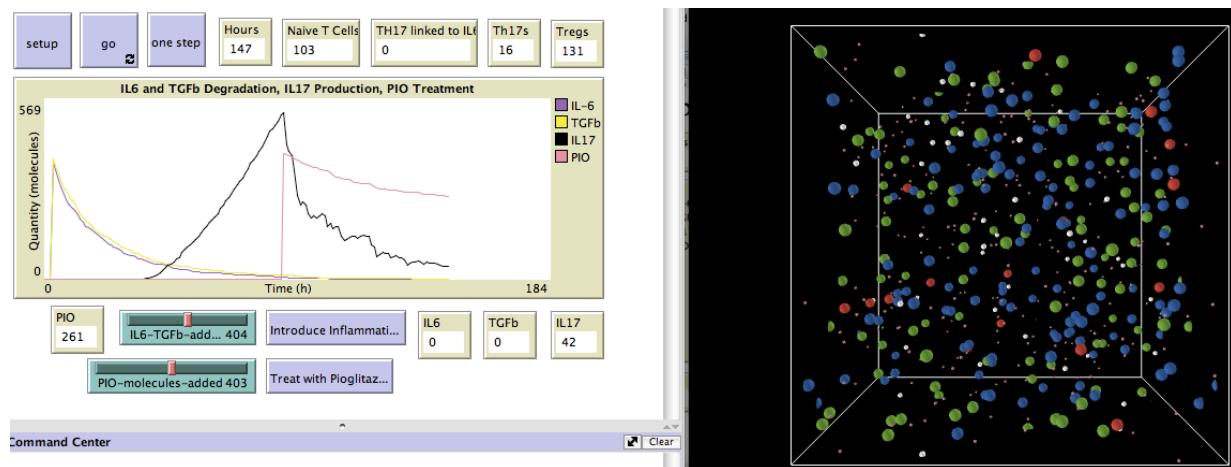
Evaluate: Finally, the students will present their programs to the class following the student-designed “Program Design and Presentation Rubric”. As a class, we will decide on 5 key criteria that all projects will address. Then, we will list things that must be present to receive full credit in the “4” column. A sample rubric is provided in the “assessment” section with some possible criteria and “must-haves” listed.

X. Anticipated Results

Sample Data 1: If a student does not adjust the magnitude of inflammation introduced (IL6-TGFb slider bar) or the magnitude of treatment given (PIO-molecules slider bar) and allows for the acute inflammatory response to occur (IL-17 black line in graph) before administering treatment (hitting “Treat with Pioglitazone” button), the results will resemble these below. Notice how a vast majority of the T-17 cells (green spheres) have differentiated into T-Reg cells (blue), bringing inflammation down to nearly zero in a short time.



Sample Data 2: If a student cuts the inflammation introduced by half (400 molecules) and cuts the treatment dosage in half (400 molecules), the results would resemble these. Notice that after the same amount of time as before (147 hours), there are still many T-17 cells that have undifferentiated, leaving a lingering inflammatory response.



Obviously, there are numerous combinations of variables (time, magnitude of inflammation, magnitude of treatment, timing of treatment), so results will vary. There is typically a lot more troubleshooting involved in the coding of the extension program than in the operation of the initial interactive student interface.

XI. Classroom Discussion

There are a couple of places during the unit where class discussion is beneficial. Those points are listed below with corresponding questions.

Point in Lesson	Potential Discussion Questions
Following the “Engage” activity	-Ask any or all of the “Engage” questions
Following the “Explore” activity	-Ask any or all of the “Explore” questions
Throughout the “Explain and Elaborate” section	-Ask individual students why they have chosen to explore certain criterion or how they plan to control for others, what data they expect to collect and why
Throughout entire unit	-Ask students about the role of T-Cells in the immune response and why we are trying to negate it -Ask students how this exploration of the acquired immune response is different from the innate immune system

XII. Assessment

To assess the student extension programs and their corresponding presentations, the class can develop a student-created rubric (see below). The students first decide on the major criteria to be evaluated; the teacher will list these in the first column in bold. Next, the students will list the items that should be present in each criterion to get full credit, listed under the “4” points column.

Program Design and Presentation Rubric SAMPLE (20 pts)

Criteria	4 points	3 pts	2 pts	1 pt
Physiology Content	- research-based = scientific literature - accurate use of content - addresses immune response to inflammation	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
Coding	- clean - sensible, effective programming notes - linear build - free of coding errors	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
Program execution	- user-friendly - true to storyboard - free of glitches - controls for user error - slider bars / counters / etc. accurate, functional	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
Trade and Trial	- evidence of peer editing (program notes) - peer advice taken and enacted - errors troubleshooted	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
Poster and Oral Presentation	- PPT template filled out completely - students knowledgeable of all aspects of program - sample screenshots provided - headings clear and accurate - organized	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
Final Score _____ / 20 x 100% = _____				

Reflection Scoring Rubric (12 points total)

- Explanation of inflammation is accurate: _____ / 3
 - Metaphor is clear and accurate: _____ / 3
 - All cytokines and cells accurately addressed: _____ / 3
 - Explanation of treatment clear and accurate: _____ / 3
- Total: _____ / 12

STUDENT SECTION

Name: _____
Name: _____

CD4+ T-cell differentiation: Using computer modeling to explore the management of the adaptive immune response to inflammation

Objectives:

1. Students will explore the cytokines and immune cells involved in the acute inflammatory response in humans.
2. Students will design and conduct a virtual mouse study to examine how magnitude of inflammation introduced and volume of treatment administered affect the adaptive immune response.

Materials:

- One laptop per student with the NetLogo 3D 5.0.2 software installed.
- Access to the following files posted on Blackboard:

1. Th17diffPIOppargWTsb.nlogo3d
2. Th17diffPIOppargKOsb.nlogo3d
3. Th17diffPIOppargWTsbSTUDENT.nlogo3d

Background: SEE COMMENTS IN TEACHER SECTION AND MODIFY

According to the Center for Disease Control (CDC), inflammatory bowel disease (IBD) accounts for over 700,000 physician visits and 100,000 hospitalizations in the US every year. About 1.4 million Americans suffer from IBD.

In contrast to innate immunity, where immune receptor presence is encoded in the genes, acquired (adaptive) immunity consists of a series of receptors and responses that evolve over the lifetime of the organism and that depend on environmental exposure. T-cells are a key subset of the acquired immune system. Their function is to recruit other immune cells and to trigger cytokine release. For example, T helper 17 (Th17) cells produce interleukin 17 (IL-17) cytokines in the gut mucosa leading to inflammation, which sometimes in excessive amounts causes irritable bowel disease (IBD) or Crohn's disease. One in 200 Americans suffers from Inflammatory Bowel Disorder (IBD), resulting in chronic gastrointestinal distress and an increased risk of colon cancer.

PPAR γ is a Type II nuclear receptor that helps regulate metabolism and inflammation by promoting regulatory T cell (Treg) production and by limiting the autoimmune response of inflammation. PPAR γ KO mice show a significant increase in disease activity compared to their WT counterparts due to a lack of Treg production. Since PPAR γ is a glitazone receptor, pioglitazone treatments have no effect on disease state in the KO mice leading to IBD.

Part I: Engage

Open the NetLogo 3D 5.0.2 program. Open the “Th17diffPIOppargWTsb.nlogo3d” file that your teacher has posted for you on Blackboard. This is a visual representation of the wild-type data collected from an actual mouse study run at the Virginia Bioinformatics Institute (VBI) in Summer 2012. Click “setup” and “run”. Take notes as you watch the simulation.

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Now open the “Th17diffPIOppargKOsb.nlogo3d” file. This is the knockout data collected from that same mouse study. Here, the gene that encodes for the PPAR γ receptor has been knocked out. Click “setup” and “run”. Take notes as you watch.

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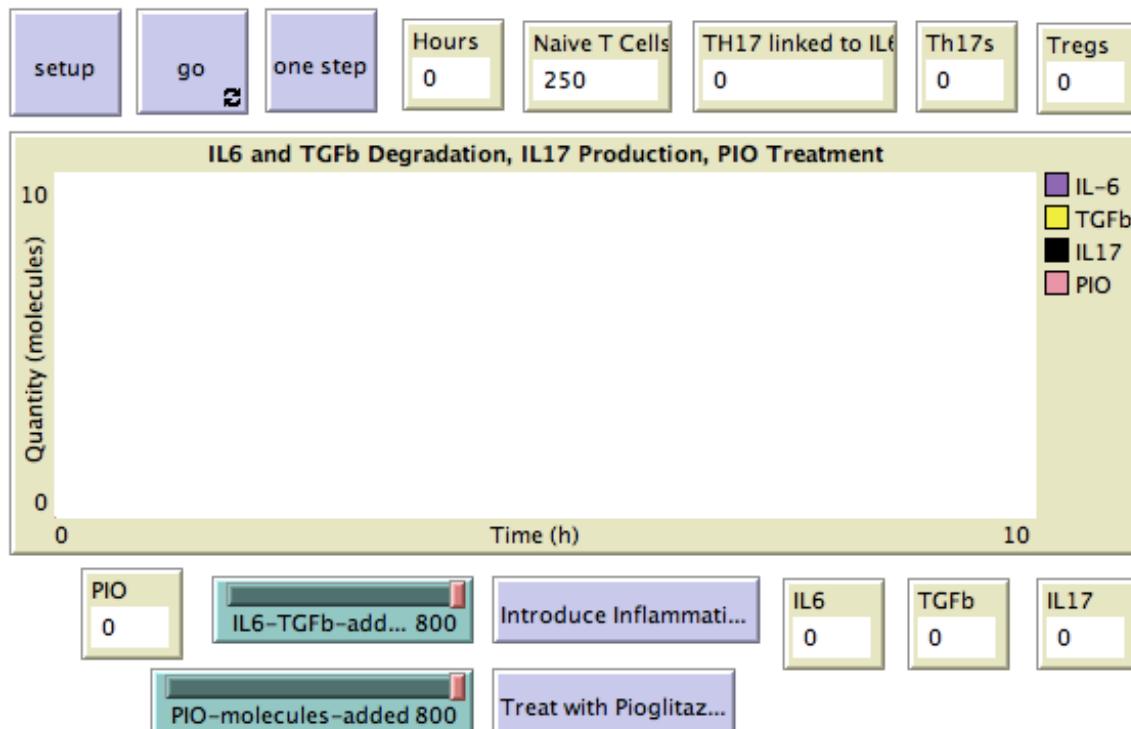
Engage Questions:

1. What are the differences in the two files?
2. What cytokines are involved? Identify and define each.
3. What cells are involved? Identify and define each.
4. What were the variables involved in this study?
5. What do you think were the constants in this study?
6. What data was collected in this study?
7. What was the possible conclusion you could make?

Part II: Explore

Now you will explore an interactive version of the simulator. You will make adjustments to find out how different variables (e.g. time, magnitude) alter the results in the virtual mouse study.

1. Open the NetLogo 3D 5.0.2 program. Open the “Th17diffPIOppargWTsbSTUDENT.nlogo3d” file posted on Blackboard. You will see an interface like the one shown here.



2. Working with a partner, use the program to observe the function of each slider and button. As you observe, answer the questions below.
 - a. What is the inflammation? How is it identified in the graph?
 - b. What is the treatment? How is it identified in the graph?
 - c. What happens following treatment?
 - d. What happens when you change the magnitude of inflammation?
 - e. What happens when you change the magnitude of treatment?
 - f. How is the data presented in this simulator?
 - g. What do the different color dots and spheres in the 3D model represent?
 - h. How do the large spheres change as the program progresses?

3. With that same partner, conduct a virtual mouse study using the simulator. Complete the components listed below.
 - a. Problem statement:
 - b. Hypothesis:
 - c. Independent Variable:
 - d. Procedure (include specific times and magnitudes):
 - e. Results (copy and paste a screenshot here):
 - f. Conclusion:
 - g. Teacher sign-off to continue to next part: _____.

Part III: Explain and Elaborate

Now that you had the chance to present and build on your knowledge foundation by creating an extension to the original program, you will present your updated program to the class.

1. For homework, you will complete the NetLogo programming tutorial (found at <http://ccl.northwestern.edu/netlogo/>) to learn the basics of working with agent-based modeling software.
2. In class, you will start with the teacher-designed interface that you used in the Explore section and code your own extensions based on your disease research. You may choose to incorporate a different lymphocyte (B, T1, T2, etc.), a different magnitude of disease response, a different treatment, or even a different disease by implementing different buttons, slider bars, lymphocyte and/or cytokine counters, and/or response times. Fill out the criteria below.
 - a. Problem statement:
 - b. Hypothesis:
 - c. Independent Variable:
 - d. Procedure (include specific times and magnitudes):
 - e. Results (copy and paste a screenshot here):
 - f. Conclusion:
3. Once you have a working program, you will share your results with a partner to “trade-and-trial” each other’s programs.

Part IV: Evaluate

Finally, you will present your programs to the class.

- a. I will grade you on the following student-designed “Program Design and Presentation Rubric.”
- b. As a class, we will decide on 5 key criteria that all projects will address.
- c. We will then list things that must be present in each criterion to receive full credit in the “4” column. See the blank rubric below.

Program Design and Presentation Rubric (20 pts)

Criteria	4 points	3 pts	2 pts	1 pt
1. _____	- - - - -	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
2. _____	- - - - -	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
3. _____	- - - -	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
4. _____	- - - - -	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
5. _____	- - - - -	Some of these aspects present	Few of these aspects present	Very few or none of these aspects present
Final Score _____ / $20 \times 100\% =$ _____				

Reflection: In addition to receiving your Program and Presentation Score, you will also be assessed by completing the following reflection prompt.

A family member has just been diagnosed with a chronic inflammatory bowel disease. The doctor tried to explain what was happening, but it was full of medical terminology and left your relative with more questions than answers. In your own layman's terms and using a clear metaphor, explain the situation to your relative. Address the following: the cause of the inflammation, all of the chemicals and cells involved, and how the treatment works to reduce the inflammation.

Reflection Scoring Rubric (12 points total):

1. Explanation of inflammation is accurate: _____ / 3
 2. Metaphor is clear and accurate: _____ / 3
 3. All cytokines and cells accurately addressed: _____ / 3
 4. Explanation of treatment clear and accurate: _____ / 3
- Total: _____ / 12