The Use of Transgenic Animals in the Study of Colitis: The Link between Molecular Biology and Immunology

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I. Unit Introduction

During the summer of 2016, I had the opportunity to shadow Dr. Laurie Harrington, Boyoung Shin, and others in the department of Cell, Developmental, and Integrative Biology. During my time in lab, I was able to experience many immunological research techniques including flow cytometry, real-time PCR, and ELISA immunoassay. I also was able to learn much about the role of transgenic mice in immune research. I observed how they are maintained, utilized for experiments, and was able to learn about mouse standards of care. The experiments that I was involved with centered on the role of ST6Gal1 expression in the activation, function, and survival of CD4 T cells and the development of colitis in the mouse model.

My overall goal for this unit is to expose students to some lab techniques used to investigate immune diseases as well as connect what they already know about genetics and molecular biology to understanding how these techniques actually work. I also want to pique the interest of my students by examining the disease of colitis and showing them the real-world connection between the biology fundamentals learned in class and active research occurring right now.

My inspiration for the format of this unit came from an activity that I already do with my AP Biology students every year: *Human Biology: Autoimmune Disorders, Case Study on Rheumatoid Arthritis*. My students always enjoy doing this case study because they are guided through learning a new topic by finding the information on their own. This allows me to assume the role of guide and alternative resource instead of being the direct conduit of knowledge.

Therefore, all unfamiliar content knowledge is examined through the case study itself. An answer key is provided for teachers to monitor and check student's work. All instructions and lab procedures are also covered in the case study.

II. Science Background

Ulcerative Colitis (UC) is chronic inflammation of the large intestine (colon) resulting in the formation of open sores (ulcers). People living with this disease often experience abdominal pain and cramping, diarrhea, fatigue, and weight loss[1]. UC associated inflammation is the result of autoimmunity—the person's immune system has mistakenly attacked their own tissues. In order to understand the progression of this disease, many immunology researchers study the development and behavior of immune cells that are linked to inflammation.

One such class of immune cells are CD4 T cells. These cells are able to differentiate into T helper cells with various roles in the immune response[2]. One cell lineage, Th17, has been associated with inflammation because of its

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[1] Crohn's & Colitis Foundation http://www.crohnscolitisfoundation.org/what-are-crohns-and-colitis/what-is-ulcerative-colitis/
[2] Zhu, J., Yamane, H., and William, P.E. (2010). Differentiation of Effector CD4 T Cell Populations. Annu Rev immunol. 2010; 23: 445-489. Doi: 10.1146/annurev-immunol-030409-101212

release of the inflammatory cytokine IL-17[3]. The activation and proliferation of these cells often leads to the development of autoimmune diseases like UC[4].

In order to better understand how autoimmune diseases develop, researchers also look at other genetic determinants that may be involved in regulating the activity of these cells. *ST6Gal1* is a protein coding gene involved with regulating cell signaling pathways that lead to cell differentiation and apoptosis (programmed cell death)[5]. It has been found that overexpression of the ST6Gal1 enzyme occurs in undifferentiated stem cells[6] and that its activity can lead to apoptosis-resistant cells. It is possible that this gene may play a role in protecting inflammatory T cell types from cell death, thus increasing those cells' ability to cause inflammation[7,8].

To investigate this genetic link, transgenic animals are often used. As models of biological systems, scientists can easily manipulate aspects of their immune systems in order to determine the function of certain genes, proteins, or cell types[9]. The RAG-1-deficient mouse is optimal to use for this purpose because they do not have a functional immune system of their own[10]. Therefore, researchers can create the necessary conditions in which to test the function of *ST6Gal1*. Better understanding of this gene's role in causing inflammation could lead to the development of better treatments for diseases like UC.

III. Student Outcomes

- Biology Concepts
  Basics of the acquired immune system
  Factors affecting T cell differentiation
  Autoimmunity and autoimmune disorders
  The use of transgenic animals in research
  Biotechnology: ELISA and gel electrophoresis

- Recommended Placement
  This unit was designed to be taught in an AP Biology course after covering units on cell signaling, molecular biology, and the mammalian immune system; therefore this unit should be used in a course of equal or greater difficulty. However, some aspects may be adapted for a Pre-AP Biology course. A smaller class size (no more than 20 students) is also optimal. The unit can be adapted for a larger class, but please allow for additional time to cover the case study and lab activities.

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[3] Onishi, R. M., & Gaffen, S. L. (2010). Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology, 129(3), 311–321. http://doi.org/10.1111/j.1365-2567.2009.03240.x

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[5] ST6GAL1 ST6 beta-galactoside alpha-2,6-sialytransferase 1 [Homo sapiens (human)]. (2016). NCBI Gene ID:6480, updated 9-Oct-2016. https://www.ncbi.nlm.nih.gov/gene/6480

[6] Wang, Y. et al. (2015). Glycosyltransferase ST6GAL1 contributes to the regulation of pluripotency in human pluripotent stem cells. Scientific Reports, 5(13317). Doi:10.1038/srep13317

[7] Toscano, M.A. et al (2007). Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death. Nature Immunology, 8(8). pp. 825-834.

[8] Swindall, A.F. and Bellis, S.L. (2011). Sialylation of the Fas Death Receptor by ST6Gal-1 Provides Protection against Fas-mediated Apoptosis in Colon Carcinoma Cells. The Journal of Biological Chemistry, 286(26). Pp. 22982-22990. Retrieved from http://www.jbc.org/content/286/26/22982.full.pdf.

[9] Transgenic Mice. Learn.Genetics Genetic Science Learning Center. University of Utah. http://learn.genetics.utah.edu/content/science/transgenic/

[10] Belizario, J.E. (2009). Immunodeficient Mouse Models: An Overview. The Open Immunology Journal, 2, 79-85

- Skills Learned
  Students will learn or enhance their ability to perform detailed experiments involving hypothesis testing and technical lab skills like pipetting and maintaining a sterile lab environment. They will also be required to differentiate between reliable sources when gathering information from the internet. Enhancing science writing skills will be the focus of the final assessment of the unit which takes the form of a detailed lab report. The following science practices outlined in the AP Biology Curriculum Framework are the basis for skills enhanced through this unit:

  Science Practices
  SP 1: The student can use representations and models to communicate scientific phenomena and solve scientific problems.
  SP 3: The student can engage in scientific questioning to extend thinking or to guide investigations within the context of the AP course.
  SP 4: The student can plan and implement data collection strategies appropriate to a particular scientific question.
  SP 5: The student can perform data analysis and evaluation of evidence.
  SP 7: The student is able to connect and relate knowledge across various scales, concepts, and representations in and across domains.

- Relevance
  For students that are planning on entering the medical field or some type of cell biology research, this unit should provide more in-depth information on what they might encounter once they begin work in a lab or in a medical setting. Autoimmune disorders are the cause of many prevalent diseases that may affect students' families and friends, or even the students themselves. By exploring the fundamentals of immunology, they will hopefully have a better understanding of these diseases.

IV. Learning Objectives for AP Biology

- **LO 2.29**—The student can create representations and models to describe immune responses.
- **LO 3.7**—The student can justify the claim that humans can manipulate heritable information by identifying at least two commonly used technologies.
- **LO 3.33**—The student is able to use representations and appropriate models to describe features of a cell signaling pathway.
- **LO 3.34**—The student is able to construct explanations of cell communication through cell-to-cell direct contact or through chemical signaling.

V. Student Prior Knowledge and Skills

Students should have a working knowledge of molecular biology, basic biotechnology techniques, and basic laboratory skills. Cell signaling principles, cell membrane structure, as well as fundamental genetics principles are assumed knowledge in this unit.

VI.   Time Requirements

It is recommended that 7-9 days of double-period blocks (80-90 minutes) be set aside to cover this unit. More time may be needed to complete additional suggested activities. Please refer to section IX before establishing your timeline.

VII.   Materials and Equipment

- *Using Model Animals to Study Autoimmune Disorders* Teacher and Student Versions
- Bio Rad Biotechnology Explorer ELISA Immuno Explorer Kit (1662400EDU)[11]
- Bio Rad ELISA Kit Reagent Refill Pack (1662401EDU) [12]
- 20-200 µl adjustable volume micropipets (5)
- Pipet tips
- 96-well polystyrene flat-bottom cell culture microplates (5)
- Distilled water
- 15 ml centrifuge tubes
- 30 ml centrifuge tubes
- 1 L Graduate cylinder
- 1 L beaker
- 100 ml beakers
- Disposable 1 ml graduated pipettes
- Paper towels
- Marking pens
- Access to a refrigerator and ice

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[11] All Bio Rad Laboratory supplies can be ordered here:
http://www.bio-rad.com/en-us/education
[12] All Bio Rad Laboratory supplies can be ordered here:
http://www.bio-rad.com/en-us/education

VIII.   Case Study Advance Preparation

- Every student will need their own copy of the *Student Version* of the case study. This can be provided electronically or printed; however, it is important that the copies be in color if printed. You may choose to give the entire case study to students at the very beginning of the unit, or you can give them each individual part as the unit progresses each day.
- Students will need to work in pairs for the case study and groups of four to complete all lab activities.
- Every student will also need reliable access to the internet during the entire unit, both in the classroom and at home. If this is unavailable, copies of sources can be used (see the list of references to find sources that can be used).
- Please look ahead to the days 5 and 6 to plan for laboratory set-up.

IX.   Daily Unit Plans

Day 1.   Give students 15 minutes to complete task 1. Review student answers (about 15 minutes). Give students 10 minutes to compete task 2, reviewing answers for another 10 minutes. Task 3 should take students 15 minutes to complete. It may be useful to review students' work on this task with a desktop presenter like ELMO.

Day 2.   Administer AP Biology Free Response Question 2 from 2007 (form B). The question and grading rubric is available at AP Central (http://apcentral.collegeboard.com/apc/members/exam/exam_information/219291.html). Give students 20 minutes to respond to the question. This can be used as summative or formative assessment of parts 1 and 2. Take 10 minutes to review task 4. Task 5 and 6 should take students about 10 minutes with another additional 10 minutes for discussion. The rest of the class period can be used for students to work on task 7.

Day 3.   Review task 7 for the first 5 minutes of class. Give students 5 minutes to complete task 8 and review it for another 5 minutes. Give students 10 minutes to compete task 9[13] discussing answers for 10 minutes afterwards. Task 10 should take students 10 minutes to complete. Review task 10 for 10 minutes. Give students 10 minutes to complete task 11[14] and 12. Discuss task 11 and 12 for 10 minutes. Assign task 13 and 14 for homework.

Day 4.   Review task 13 and 14. Complete Part 8A as a class. Review the objectives and ask students to explain the purpose of each step. Walk students through tasks 15-18, allowing them time to generate responses and time to review their responses before continuing to the next task. Emphasize referring back to the

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[13] If you believe that your students would be interested in discussing more about the ethics of using animals in research, you can use this activity before completing part 5: https://med.nyu.edu/highschoolbioethics/sites/default/files/highschoolbioethics/AnimalEthics_Module.pdf. Please allow for additional time within the unit to complete this additional activity.

[14] If you have actual colon tissue histology slides available, and extension for task 11 could be have students use a microscope to view tissue and sketch their observations on the blank space on page 9 of their packets.

diagram in task 7 for clues to what might happen.   Especially for the Th2 cells, emphasize humoral vs. cell-mediated immunity.

For the discussion of task 16—Make sure to discuss the independent and dependent variables so students understand the importance of weight. Tamoxifen is used to induce ST6Gal1 overexpression, for the possible results clarify that all mice have the possibility to lose weight because of evidence from the previous histology slides, however, ST6Gal1 will be more severe because of its hypothesized role.

For task 18—A graphing calculator can be used to calculate standard deviation and standard error as well as Excel. If you do not wish for students to do this, just provide the numbers for them (the important thing is to make sure they understand the significance on the measurements—a handy explanation of this can be found here: www.bville.org/tfiles/folder427/ap_standard_deviation_and_standard_error.pptx).

At this point, an optional assignment would be for students to complete the virtual immunology lab found here: http://www.hhmi.org/biointeractive/immunology-virtual-lab. If you feel your students do not need this introductory activity, just assign reading pgs. 71-75 of the ELISA Immunoexplorer Kit Instruction Manual (make copies for students). They should complete the pre-lab questions for homework.

Day 5.   Review the pre-lab questions from Task 19. Students will perform Protocol III: ELISA Antibody Test from the ELISA Immunoexplorer Kit as a practice run before part 8B. They should complete the post-lab questions afterwards (make copies for students). Please read all instructor instructions provided by the kit to prepare the materials for this lab. This lab can be graded as a formative or summative assessment.

Day 6.   In part 8B, students will execute an experiment using ELISA to test for the presence of pro-inflammatory cells. They should use the information from the entire packet and their experience with the practice ELISA (day 5) to do this. Please approve each group's well plate plan prior to them beginning their experiment. Here's what to look for: the standard curve should occupy wells A1-A12, there should be all 8 mouse serum samples in triplicate, and the positive and negative controls should also be in triplicate. Once you have approved each design, students are free to begin. After their assays are complete, discuss the importance of using the standard curve to quantify the amount of IL-17 present in the mouse serum samples[15]. It may be helpful to have students take pictures of their results to refer back to while completing their lab reports.

Day 7.   Students should spend a few minutes viewing each other's data from the ELISA. Give students the lab report rubric and allow them to begin writing in their groups. If your students are unfamiliar with writing formal lab reports, you may choose to guide them through the process as a class by allowing them to write each section as a group.

X.   Laboratory Advance Preparation

Step 1. Label all tubes according to this chart.

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[15] For more information on how to use ELISA quantitatively, please visit: https://www.bio-rad-antibodies.com/an-introduction-to-elisa.html

	Tube Color	Number of Tubes	Label on Tube	Actual Contents	Simulated Contents	Amount to Add to Tube
Micro centrifuge tubes (2 mL)	Pink	5	1x PBS	1x PBS	1x PBS	2 mL
	Violet	5	+	Positive controls	1x 1µg/mL IL-17 (antigen)	0.5 mL
	Blue	5	-	Negative controls	1x PBS IL-17 negative serum	0.5 mL
	Green	5	PA	Primary antibody	1x anti-human IL-17 antibody	2.5 mL
	Orange	5	SA	Secondary antibody	1x anti-human immunoglobulin antibodies conjugated to HRP	2.5 mL
	Brown	5	SUM	Enzyme substrate	Enzyme substrate	2 mL
	Yellow	5	1x IL-17	Positive control	1 µg/mL IL-17 antigen for serial dilution	0.5 mL
	Yellow	5	Mouse #1	31.25 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #2	31.25 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #3	500 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #4	1000 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #5	500 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #6	250 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #7	250 ng/mL IL-17	Serum sample	0.5 mL
	Yellow	5	Mouse #8	1000 ng/mL IL-17	Serum sample	0.5 mL
Centrifuge tubes (50 mL)		1	1x antigen	1x antigen		
		1	1x primary antibody	1x primary antibody		
		1	1x secondary antibody	1x secondary antibody		

Step 2. Refer to page 111 in the Bio Rad Biotechnology Explorer ELISA Immuno Explorer Kit instruction manual. Follow steps 1 and 2, ignoring the creation of antigen A and B at the bottom of the table.

Step 3. To prepare the mouse serum samples, create a large serial dilution of 1x antigen starting with 3 mL of 1x antigen. Use additional large tubes for this.

Step 4. Dispense reagents for student workstations according to the amounts shown in the above table. Have these materials on ice before the class period begins.

XI.   Summative Assessment

See the ELISA Lab Report rubric for how student lab reports can be graded. A sample student lab report has been included in this unit as well.

XII.   References

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Using Model Animals to Study Autoimmune Disorders

Teacher Version

PART 1 BACKGROUND KNOWLEDGE REVIEW

Task 1

Use the table below to summarize what you have learned so far about the human immune system and to compare and contrast innate and acquired immunity.

Characteristic	Innate Immunity	Acquired Immunity
Definition	Set of defenses that humans have from birth and is the first line of defense against pathogens. Defends against a broad range of pathogens in a non-specific capacity.	The body's second line of defense. Defends against specific pathogens and enables the body to quickly mount a defense against a recurring exposure to the same pathogen. Often called "learned" immunity.
Types of Tissues and/or organs	Barrier defenses like skin, mucus, hair, and stomach acid; the circulatory system	Thymus and bone marrow; the circulatory system and lymphatic system
Types of Cells	Mast cells, macrophages, natural killer cells and other phagocytic cells	Antigen presenting cells, B cells and T cells, effector cells and memory cells
Molecules Involved	histamine, cytokines, the complement system and interferons, anti-microbial proteins	Cytokines and interleukins, immunoglobulins (antibodies), antigens

Task2

Use the table below to describe the two branches of the acquired immune system.

Characteristic	Humoral Response	Cell-Mediated Response
Cell Types	B cells—plasma cells and memory B cells	T cells—helper T cells, cytotoxic T cells, memory T cells
Role in the Immune Response	Secretes antibodies that interact with pathogens, easing their elimination (agglutination)	Interact directly with other cells to either activate them (helper T cells) or destroy them (infected cells, cancer cells, non-self tissues)
Labeled Diagram of Cell Receptor	Sample Receptor Diagram	Sample Receptor Diagram

Task 3

In the space below, outline the steps of the entire immune response starting with the initial exposure to a novel pathogen and ending with the creation of memory cells. Use arrows to indicate the flow of steps. Feel free to include diagramed drawings of specific cells to help illustrate your outline.

Sample Immune Response Diagram

PART 2 INTRODUCTION TO AUTOIMMUNITY

Task 4

Use the following website to answer the questions: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMHT0022033/

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1. Summarize the definition of autoimmune disease.

Diseases that occur as a result of the immune system attacking the body's own cells and tissues

2. Autoimmune diseases are chronic conditions. What does chronic mean in this sense?

The opposite of acute conditions, chronic conditions last a long time and can be present for years.

3. What are the causes of autoimmune diseases?

Most causes are unknown, but it is likely that a person's environment and genes interact to make some more susceptible to the development of autoimmune diseases.

4. Use the list provided at the following website to learn about two autoimmune diseases of your choice: https://www.aarda.org/disease-list/. Make sure to choose two that have an identified cause or causes. In the space below, summarize the symptoms and the causes of the symptoms for each chosen disease.

Answers will vary according to the student's chosen diseases

PART 3: INTRODUCTION TO COLITIS

Task 5

Visit the following website to answer the questions: http://www.ccfa.org/what-are-crohns-and-colitis/what-is-ulcerative-colitis/

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1. What is ulcerative colitis (UC)?

Ulcerative colitis is a chronic disease of the large intestine, also known as the colon, in which the lining of the colon becomes inflamed and develops tiny open sores, or ulcers, that produce pus and mucous. The combination of inflammation and ulceration can cause abdominal discomfort and frequent emptying of the colon.

2. What is the cause of ulcerative colitis?

Ulcerative colitis is the result of an abnormal response by your body's immune system. The immune system mistakes food, bacteria, and other materials in the intestine for foreign or invading substances. When this happens, the body sends white blood cells into the lining of the intestines, where they produce chronic inflammation and ulcerations.

3. What is the difference between ulcerative colitis and Crohn's disease?

Crohn's disease can affect any part of the Gastrointestinal (GI) Tract, but ulcerative colitis affects only the colon. Additionally, while Crohn's disease can affect all layers of the bowel wall, ulcerative colitis only affects the lining of the colon.

4. Who is generally affected by ulcerative colitis?

About 700,000 Americans may be affected and men and women have an equal chance of being diagnosed. The disease is usually caught in a person's mid-30s. There may be a genetic factor involved since up to 20 percent of people diagnosed will also have a close relative affected. Whites of European descent and people of Jewish heritage are more likely to develop colitis.

5. Why do you think UC occurs during someone's mid-30s?

Answers will vary

Task 6

Visit the following website: http://www.mayoclinic.org/diseases-conditions/ulcerative-colitis/basics/symptoms/con-20043763. In the space below, make a list of symptoms associated with ulcerative colitis.

- Symptoms can vary depending on the severity of inflammation and its location
- Diarrhea, often with blood or pus
- Abdominal pain and cramping
- Rectal pain
- Rectal bleeding

- Urgency to defecate
- Weight loss
- Fatigue
- Fever
- In children, failure to grow

PART 4: CD4 T CELLS

Recall from your background knowledge that there are many types of cells involved with acquired immunity. One such branch of cells, the T cells, is directly involved in the cell-mediated response. A specific type of T cell, the CD4 T cell, has an intricate role in mediating the acquired immune system and its responses because it has the ability to differentiate into several types of T helper cells, or (Th) cells (Zhu, Yamane, & William, 2010). Also recall that T helper cells are able to influence the development and action of cytotoxic T cells, B cells, and memory cells.

You have now also explored a variety of autoimmune diseases, especially ulcerative colitis, and understand that these diseases are caused by the acquired immune system malfunctioning by recognizing the body's cells and tissues as foreign, thus mounting an attack on them. Inflammation and tissue damage is the result of this attack. Usually the immune system is able to avoid this self-intolerance because self-reactive T cells are destroyed in the thymus before being released into the body (Groux, et al., 1997). Therefore, much research of autoimmune diseases and their causes centers on the understanding of how CD4 T cell differentiation and regulation occurs.

It has been discovered that naïve CD4 T cells, when exposed to specific signals, may develop into different lineages of Th cells which includes Th1, Th2, Th17, and Treg (Zhu, Yamane, & William, 2010). These helper T cells then assume different roles dependent on what pathogenic signal triggered the differentiation.

Task 7

Use the internet to research the following terms from the diagram found here (scroll to 3 figures box and click on the first
image): https://www.researchgate.net/publication/43100136_The_cytokine_milieu_in_the_interplay_of_pathogenic_T h1Th17_cells_and_regulatory_T_cells_in_autoimmune_disease. Include the reference information that you used.

- APC
  Antigen-presenting cell such as macrophages that engulf and degrade pathogens
- MHC
  Major histocompatibility complex—MHC proteins are what carries antigens from a degraded pathogen to the surface of an APC (Alberts, Johnson, Lewis et al., 2002).
- TCR
  T-cell receptor—where the T cell binds to the MHC protein
- IFN-γ
  Soluble cytokine that triggers a cellular response to viral and microbial infections (**IFNG interferon gamma [*Homo sapiens* (human)]** from http://www.ncbi.nlm.nih.gov/gene/3458#bibliography)
- TGF-β
  Transforming growth factor, regulates cell growth, differentiation, and development (**TGF-β / Smad Signaling Pathway** from https://www.cellsignal.com/contents/science- pathway-research-stem-cell-markers/tgf-smad-signaling-pathway/pathways-tgfb)
- IL-17
  Interleukin that promotes inflammatory pathology in autoimmune diseases (Onishi & Gaffen, 2010)
- Foxp3
  Forkhead Box P3, transcriptional regulator crucial for the development and function of Treg. Allows the immune system to achieve suppressive function and stability. Mutations are associated with autoimmune diseases because Treg is not able to function properly (http://www.genecards.org/cgi-bin/carddisp.pl?gene=FOXP3)
- T-bet
  Master regulator transcription factor involved in the development of Th1 cells
- Peripheral tolerance
  The immune system's ability to tolerate non-pathogenic antigens that the body is constantly exposed to, helps avoid chronic cell activation and inflammation (Groux, et al., 1997)

Task 8

For each of the following cells, predict what might happen to a person if the cell malfunctioned or was not able to perform its job.

- Naïve CD4 cell
  Student responses should focus on the inability for T cells to differentiate (there will be no T cells at all), resulting in no acquired immunity
- Th1
  Student responses should predict the inability of the person to fight off bacteria or viruses. They could also mention possible autoimmune reactions
- Th2
  Student responses should predict the inability of the person to respond to parasites. They could also mention possible autoimmune reactions or impairment of B cells
- Th17
  Student responses should predict the inability of the person to respond to fungi. They should also mention the development of autoimmune disease or an increase in inflammation in the person's tissues.
- Treg
  Students should predict that the person will probably experience more cell activation and inflammation since these cells help to regulate other T cells

PART 5: TRANSGENIC AND KNOCKOUT MICE

Task 9

Visit the following website to answer questions http://www.whatisbiotechnology.org/science/transgenic

1. What is a transgenic animal?
Animals that have had a foreign gene deliberately inserted into their genome

2. How are they created?
"Such animals are most commonly created by the micro-injection of DNA into the pronuclei of a fertilized egg which is subsequently implanted into the oviduct of a pseudopregnant surrogate mother. This results in the recipient animal giving birth to genetically modified offspring. The progeny are then bred with other transgenic offspring to establish a transgenic line. Transgenic animals can also be created by inserting DNA into embryonic stem cells which are then micro-injected into an embryo which has developed for five or six days after fertilization, or infecting an embryo with viruses that carry a DNA of interest. This final method is commonly used to manipulate a single gene, in most cases this involves removing or 'knocking out' a target gene. The end result is what is known as a 'knockout' animal."

3. What is the importance of transgenic animals in research?

"Transgenic animals are routinely used in the laboratory as models in biomedical research. Over 95 per cent of those used are genetically modified rodents, predominantly mice. They are important tools for researching human disease, being used to understand gene function in the context of disease susceptibility, progression and to determine responses to a therapeutic intervention. Mice have also been genetically modified to naturally produce human antibodies for use as therapeutics."

4. What makes mice "the model of choice" for many investigations of human disease?

"There is extensive analysis of its completed genome sequence, but its genome is similar to the human. Moreover, physiologic and behavioral tests performed on mice can be extrapolated directly to human disease. Robust and sophisticated techniques are also easily available for the generic manipulation of mouse cells and embryos. Another advantage of mice is the fact that they have a short reproduction cycle."

Task 10

Visit http://learn.genetics.utah.edu/content/science/transgenic/ to learn about how knockout mice are created. Read about Dr. Mario Capecchi's work. In the space below, summarize the procedure for creating the OhNo knockout mice in eight steps. It may help to include drawings or diagrams in your steps.

---

1. isolate stem cells—gather embryonic stem cells from male brown mice with a normal OhNo gene

2. add inactive gene with a marker—add a copy of the mutated and inactive gene with a drug resistance marker gene

3. gene swapping—similar genes can swap places, the OhNo gene plus drug resistance marker gene will become incorporated into the genome, the normal version is displaced (homologous recombination)

4. drug addition—adding a drug will kill the cells that have not incorporated the OhNo plus drug resistance marker

5. chimeric mice—transplant stem cells into a white mouse embryo to create a chimera (identified by white and brown fur)

6. mate male chimera—some cells that have the OhNo gene may become reproductive cells and he will produce some brown offspring when mated with a white female

7. test and breed brown offspring—half of the brown offspring will have a copy of the inactive OhNo gene, these mice can be identified by DNA sequencing and then bred to each other

8. Knockout line established—one fourth of the offspring will have two copies of the mutant, inactive OhNo gene (remember Mendelian genetics?)

PART 6: USING A MOUSE MODEL TO STUDY COLITIS

Task 11

Visit http://www.ncbi.nlm.nih.gov/pubmed/1547488 to answer questions 1 and 2.

1. What are RAG-1-deficient mice?
"RAG-1-deficient mice have small lymphoid organs that do not contain mature B and T lymphocytes."

2. Why might these knockout mice be useful for investigating the functioning of the immune system?
Because these mice do not have a functional acquired immune system of their own, they are essentially a "blank slate" that can be used to model immune function *in vivo* (Belizario, 2009).

RAG-1-deficient mice or "knockouts" (RAG -/-) will develop colitis when injected with naïve T cells. A researcher can then investigate the effects on the mouse's colon tissue by taking samples of the tissue and viewing them under a microscope. The following is a histology slide of normal colon tissue:

https://embryology.med.unsw.edu.au/embryology/index.php/File:Colon_histology_002.jpg

Task 12

Examine the following histology slides of mouse colon tissue. The labels on each slide show to what each mouse has been exposed. Compare and contrast them by writing your observations in the space provided. Which do you think is most like normal colon tissue and which looks like the colon tissue taken from a mouse suffering from colitis?

RAG-1 KO + PBS 10x. The Harrington Lab, UAB
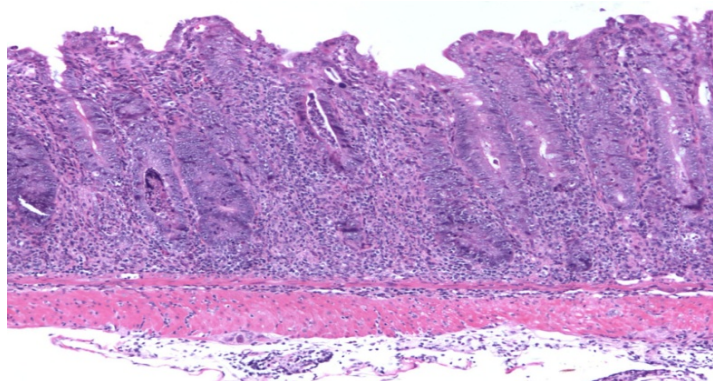
Observations:

Observations should center on similarities between the structure of this tissue and the structure of the normal colon tissue in figure 2. This mouse does not suffer from inflammation or colitis.
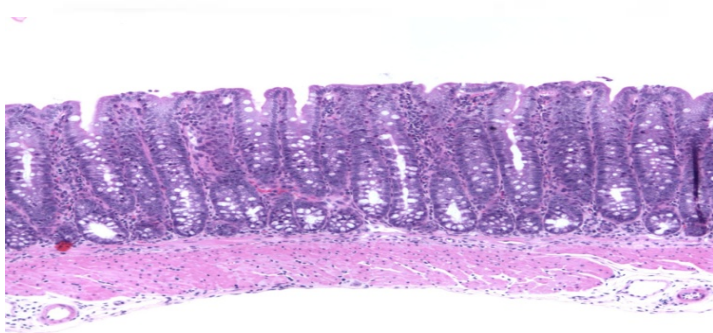
RAG-1 KO + Tbet KO naïve CD4 T cells 8 weeks post-transfer 10x. The Harrington Lab, UAB

Observations:

Observations should center on similarities between the structure of this tissue and the structure of the normal colon tissue in figure 2 and the Rag-1 KO + PBS tissue. Students may note that Tbet is also not active in these mice (Tbet is associated with Th1 cells and inflammation). The mouse probably does not suffer from colitis.

RAG-1 KO + WT naïve CD4 T cells 8 weeks post-transfer 10x. The Harrington Lab, UAB

Observations:

Observations should note that the tissue is very different from the normal colon tissue and the crypts are almost indistinguishable. This tissue is indicative of inflammation. Also, students should notice that these mice have received wild type (WT) naïve CD4 T cells which they should recall can differentiate into pro-inflammatory T cells. This mouse may suffer from colitis.

Task 13

Recall the information that you gathered about colitis from task 5 and 6. Would a mouse suffering from colitis show the same symptoms as humans? Use the table below to make predictions about a mouse's appearance and behavior if it was suffering from colitis. In other words, how could you determine by observation if the mouse was sick?

Characteristic	Normal Mouse	Sick Mouse	How would you know?
Appearance	coat is shiny and thick	Coat is dull and hair loss may be visible	Record observations on a regular basis (maybe daily)
Weight	Weight is normal for an adult (16-22 grams depending on age and sex)	Continuous weight loss instead of gain	Mass mouse regularly and record weights
Movement	Active movement around cage and interacts with cage mates	Seems lethargic and does not scurry when approached by experimenter	Keep a record of activity, check during the day and at night
Feeding/Drinking	Eats and drinks mostly at night, but regularly	Eating and drinking is reduced	Measure food and water intake daily by massing food and recording the volume of water consumed
Sleeping	Sleeps mainly during the day and is more active at night	May sleep more often	Check activity during the day to see if sleeping is occurring
Digestion/Excretion	Pellets are small, hard, and round	Signs of loose stool (diarrhea) may be present	Observations from the cage environment

PART 7: THE ROLE OF ST6GAL1 IN CELL SIGNALING

ST6GAL1 is an enzyme that catalyzes the transfer of sialic acid onto galactose-containing substrates and is involved in modifying carbohydrates and antigens found on the surface of cells (ST6GAL1, 2016). Remember that in cell signaling pathways, modification of surface proteins and carbohydrates influences the cell's ability to receive or send signals. Thus, this enzyme has been implicated in many cell signaling pathways, two of which are described below.

Stem Cells
Stem cells are found in human embryos and, rarely, in adult tissues. Recall that stem cells are pluripotent, meaning they are able to give rise to several different types of cells. ST6GAL1 has been shown to play a significant role in regulating the differentiation of human stem cells: undifferentiated stem cells produce a high amount of ST6GAL1 compared to differentiated cells (Wang et al, 2015). Also, loss of sialylation (the action of adding sialic acid) on stem cell membranes leads to cell differentiation; this further suggests that ST6GAL1 activity is crucial to regulating the pluripotent state (Wang et al, 2015).

Cancer

Remember that cancer is caused by mutations in genes that control the cell cycle. This results in unchecked cell growth. The spread of these mutated cells and tumor development likely follows if these cells can evade detection by the body's immune system. In general, cell growth and movement are influenced heavily by protein modification (changes to proteins that happen after translation of mRNA that affect folding and stability) of cell-surface proteins (Antony et al, 2014). ST6GAL1 modifies proteins by adding sialic acid. Increased invasiveness of certain cancers can be caused by the upregulation of ST6GAL1 because the enzyme can stimulate receptors that cause increased cell motility (Antony et al, 2014).

Apoptosis

Studies have also shown ST6GAL1 to be a regulator of several cell death pathways, influencing apoptosis (programmed cell death). Some of its enzymatic activity on certain cell death receptors can reduce apoptotic signaling, thus reducing or preventing cell death (Swindall and Bellis, 2011). If cells are protected from undergoing apoptosis, they are more likely to become cancerous.

Task 14

Using what you now know about the role of ST6GAL1 in cell signaling from the reading above, answer the questions below.

---

1. Formulate a prediction about the levels of ST6GAL1 activity in naïve T cells versus differentiated T cells. Include information from the reading to support your prediction.

Because ST6GAL1 activity seems to be linked to undifferentiated cell types, it should be higher in naïve T cells. Differentiated T cells would show lower or no activity.

2. Suppose Th17 cells had an increase in ST6GAL1 expression. What effect could this have on the cell's ability to resist apoptosis? Would this increase the amount of inflammation caused by these cells? Why or why not? Include information from the reading to support your prediction.

Because ST6GAL1 can protect cells from apoptosis, an increase of its expression would likely increase the longevity of Th17 cells. Earlier, we saw that Th17 cells are linked to the inflammation associated with autoimmune diseases. If Th17 cells are protected from apoptosis, more of the cells would be alive at any given time. This would increase their pathogenicity and inflammation may increase (Toscano et al, 2007).

3. Hypothesize about the role of ST6Gal1 and diseases like colitis. In other words, is there a possible connection between ST6Gal1 and autoimmunity?

Student responses should display understanding of the role of ST6Gal1 in cell differentiation and avoidance of apoptosis. They should predict that ST6Gal1 expression could be high in individuals with colitis or other inflammatory autoimmune diseases where effector T cell populations tied to inflammation are high because the enzyme could help keep these populations of cells from dying.

PART 8: THE INVESTIGATION

Now that you have discovered a possible link between ST6Gal1 and the disease colitis, you are going to perform a few experiments to test your ideas. Remember that in immunological research it is often helpful to use transgenic mice as models for investigating disease. You will begin the investigation by first following an experiment that induces colitis in mice and manipulates ST6Gal1 expression then observes the mice for signs of disease (Part A). Then you will analyze blood taken from those mice to test for the presence and quantity of pro-inflammatory cells by using an ELISA (enzyme-linked immunosorbent assay) (Part B).
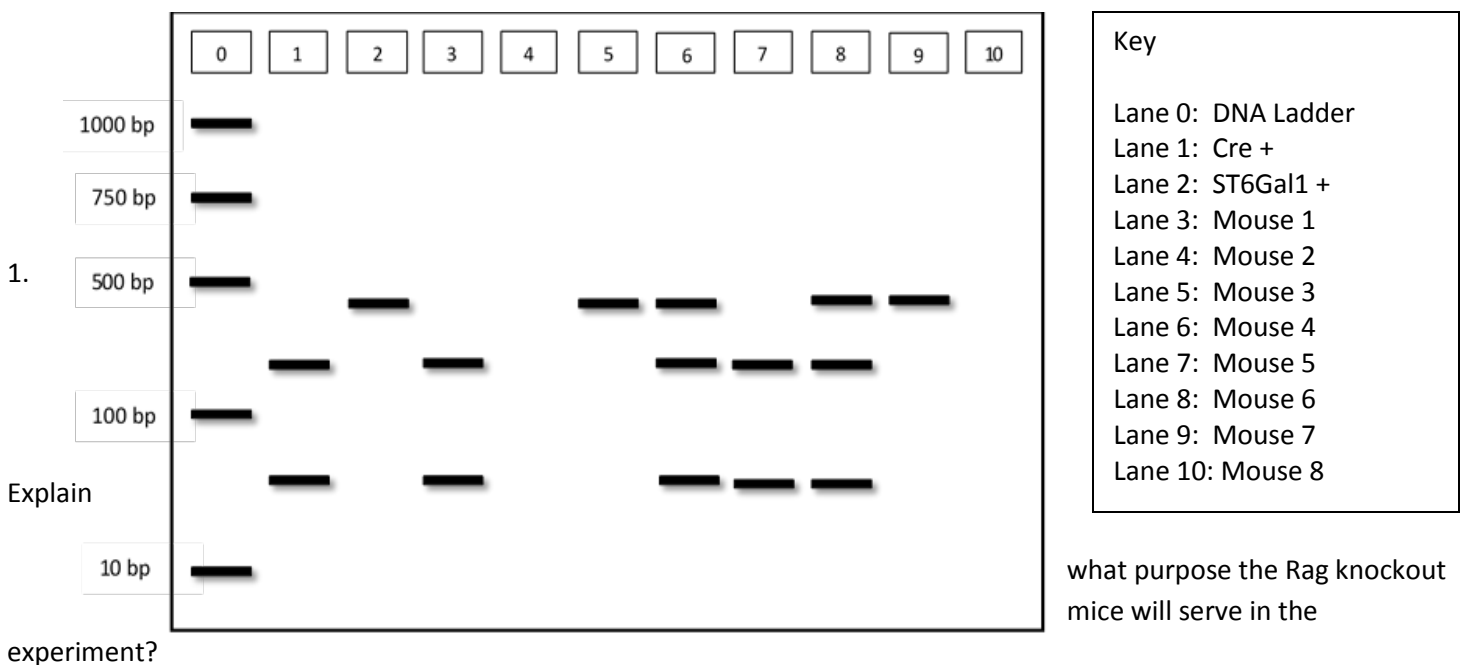
Part A—Disease Development in the Mouse Model

Objectives:
1. *Chose specific mice based on their genotype from gel electrophoresis results*
2. *Isolate naïve CD4 T cells from wild type (WT) mice and mice that over express ST6Gal1 (ST6Gal1 OE)*
3. *Transfer these isolated cells into Rag knockouts to induce colitis*
4. *Some mice will receive drugs to induce ST6Gal1 expression*
5. *Observe all test mice for signs of colitis by weighing them daily*

Task 15

The following gel electrophoresis results shows several mice that could potentially be used for this experiment. Mice that have both the *Cre* and *ST6Gal1* gene are able to be induced for ST6Gal1 protein expression. Mice that have neither or just one, cannot. Analyze the gel and determine which mice you would select for this experiment and justify your choice by answering the questions below.

1.

Explain

Key

Lane 0: DNA Ladder
Lane 1: Cre +
Lane 2: ST6Gal1 +
Lane 3: Mouse 1
Lane 4: Mouse 2
Lane 5: Mouse 3
Lane 6: Mouse 4
Lane 7: Mouse 5
Lane 8: Mouse 6
Lane 9: Mouse 7
Lane 10: Mouse 8

what purpose the Rag knockout mice will serve in the

experiment?

Because these mice do not have a functional acquired immune system of their own, they are essentially a "blank slate" that can be used to model immune function *in vivo* (Belizario, 2009). By transferring the wild type cells and ST6Gal1 OE cells into these knockouts, we can observe the results in an animal that has no immune cells except the ones we give it.

2. Which mice would you choose to use for the experimental group for ST6Gal1 overexpression?
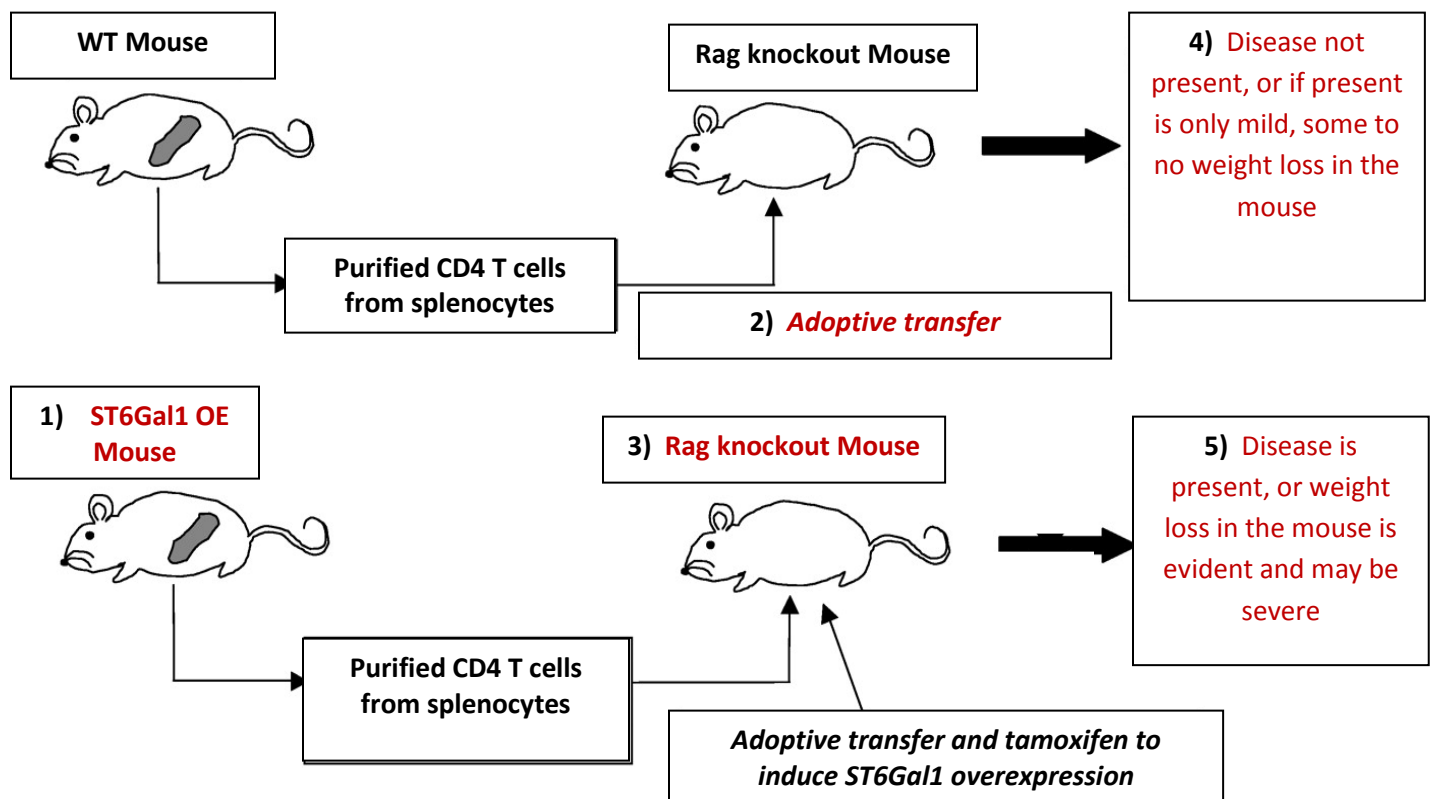
Mouse 4 and 6 because they have both genes required (*Cre* and *St6Gal1*)

3. Which mice would you choose to use for the control group?

Mouse 2 and 8 because they do not have either gene and are WT (wild type).

Task 16

Now that mice have been chosen, naïve CD4 T cells will be isolated and then transferred into the Rag knockouts. Using the diagram below, fill in the missing parts in the procedure that should be used to accomplish this (numbers 1-3). Complete the diagram by making a prediction about the experimental group and the control group (numbers 4 and 5).

WT Mouse		Rag knockout Mouse		4) Disease not present, or if present is only mild, some to no weight loss in the mouse

Purified CD4 T cells from splenocytes

2) *Adoptive transfer*

1) **ST6Gal1 OE Mouse**		3) **Rag knockout Mouse**		5) Disease is present, or weight loss in the mouse is evident and may be severe

Purified CD4 T cells from splenocytes

Adoptive transfer and tamoxifen to induce ST6Gal1 overexpression


Task 17

After mice received the naïve CD4 T cells as shown in the previous diagram, they were observed for several weeks. The data for each mouse is shown in the table below. Calculate the percent change in mass for each mouse and round your answer to the nearest tenth. Calculate the mean, standard deviation, and standard error of the mean for the control and experimental group. Round all decimals to the hundredths place.

Table 1. Observations of mice 6 weeks post-transfer

	Physical Observations at the end of week 6	Mass (g)						Percent Change in Mass
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	
Rag KO + WT								
1	Generally healthy	21.6	22.0	21.9	21.8	21.2	21.5	-0.5
2	Generally healthy	21.5	21.6	21.1	22.0	21.4	21.3	-0.9
3	Healthy, but signs of hair loss and some bites	18.0	18.1	18.2	17.9	17.8	17.9	-0.6
4	Signs of dehydration and reduced activity	19.9	19.0	19.2	18.9	19.1	19.0	-4.5
5	Generally healthy	20.5	20.0	19.9	19.7	19.9	20.2	-1.5
6	Generally healthy	17.4	18.0	17.9	17.5	17.6	17.2	-1.1
7	Signs of diarrhea and distress	18.1	18.0	17.5	17.4	17.3	17.3	-4.4
8	Generally healthy	17.6	17.7	17.9	17.6	17.5	17.7	0.6
				$SE_{\bar{x}}$= 0.66		S= 1.85		$\bar{X}$= -1.6
Rag KO + ST6Gal1 OE								
1	Signs of hair loss and diarrhea	17.1	17.4	17.5	17.0	16.9	16.2	-5.3
2	Signs of hair loss and diarrhea	16.3	16.3	16.3	16.2	16.0	15.3	-6.1
3	Signs of hair loss and does not respond to touch	21.1	21.2	21.0	19.0	19.2	18.9	-10.4
4	Signs of hair loss and diarrhea no response to touch	20.6	20.1	20.0	19.7	19.2	17.9	-13.1
5	Signs of hair loss and diarrhea and reduced activity	19.8	19.9	18.5	18.0	17.8	17.4	-12.1
6	Signs of hair loss and diarrhea and is lethargic	16.9	17.4	16.3	16.1	15.4	15.1	-10.7
7	Signs of hair loss and diarrhea	18.5	17.9	17.4	17.2	16.8	16.7	-9.7
8	Signs of hair loss and diarrhea and is lethargic	18.8	18.2	17.4	17.0	16.7	15.9	-15.4
				$SE_{\bar{x}}$= 1.18		S= 3.34		$\bar{X}$= -10.38
Task 18								

Create a bar graph of the mean of each group and include error bars to indicate standard error. Using the data from the table and your graph, write a paragraph outlining your conclusions. Make sure to reference your predictions from task 16.

Average Weight Loss of Mice
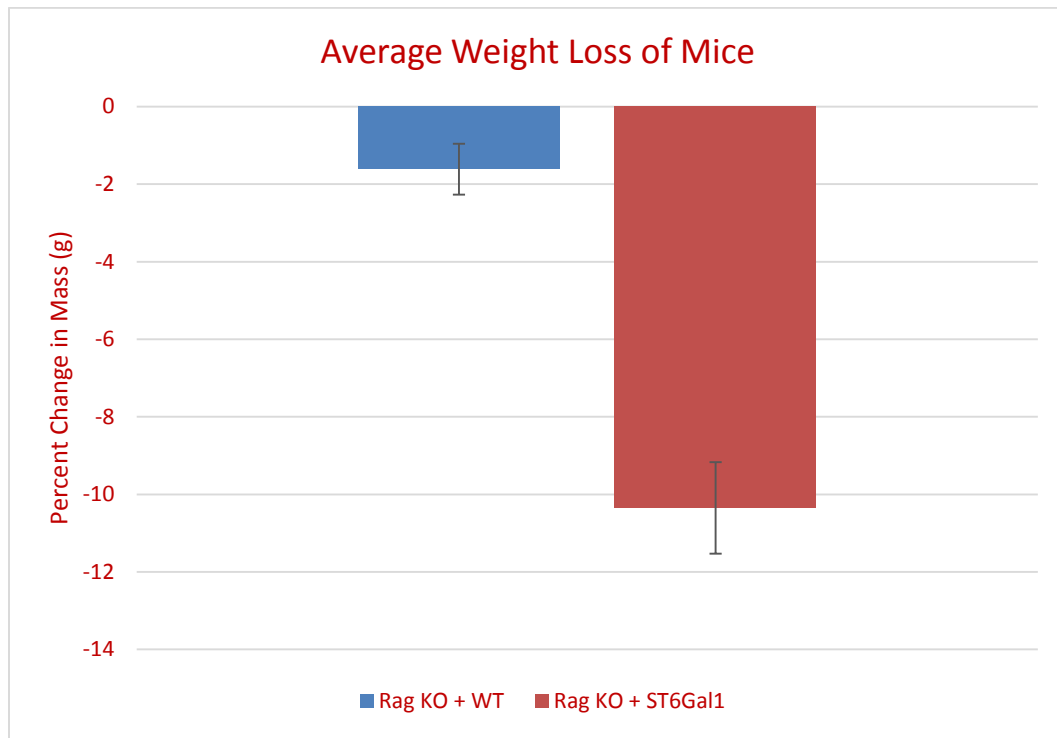
Error bars indicate standard error. WT Mice (mean=-1.61, *S*=1.85), ST6Gal1 OE Mice (mean=-10.38, *S*=3.34)

Student answers should include the following:

- The mice injected with tamoxifen (ST6Gal1 OE) mice had significantly more weight loss than the WT mice.
- Because the error bars do not overlap, we can trust that this data is significantly different.
- ST6Gal1 OE mice also appeared less healthy than WT mice in the 6 weeks qualitative observations.
- This data points to the development of more severe UC in the treatment mice compared to the control mice.
- This confirms the predictions made in task 16.

Part B—Using ELISA to Detect Inflammatory Cytokines in the Mouse Model

*please see *X. Laboratory Advance Preparation* section in the teacher's guide for instructions on lab set-up

Objectives:

1. *Read the student manual introduction for ELISA and answer the pre-lab questions*
2. *Complete a practice ELISA assay and answer the post-lab questions*
3. *Determine an experimental design for using ELISA to test for inflammatory cytokines*
4. *Execute your experimental design*
5. *Prepare a lab report to communicate your findings*

The eight ST6Gal1 OE mice have had serum samples taken from the blood at the last week of weighing them. You will be testing the serum for the presence of IL-17 by performing another ELISA.

Materials:

Microcentrifuge tubes

- Positive control (+)
- Negative control (-)
- Sample of known concentration (1xIL-17)
- Primary antibody-1xanti-human IL-17 antibody (PA)
- Secondary antibody-1xanti-human immunoglobulin antibodies+HRP (SA)

- Enzyme substrate (SUB)
- Mouse serum samples (#1-#8)

96-well plate

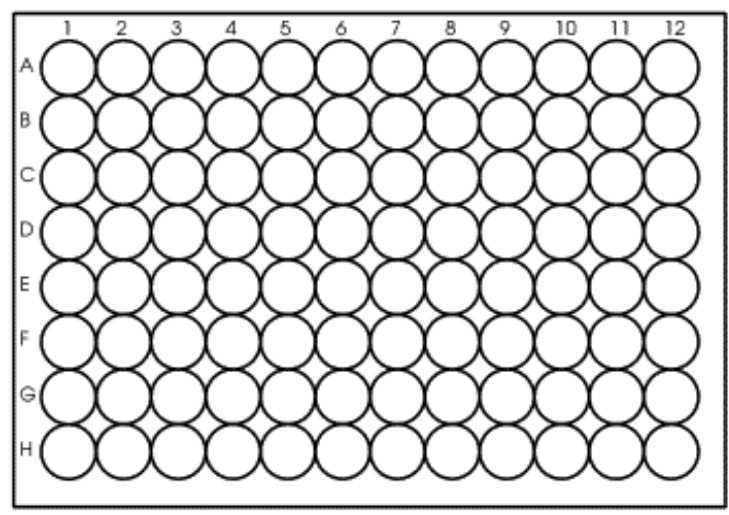70-80 mL of was buffer in beaker

Paper towels

Waste container

Instructions:

1. Plan your assay by labeling the well plate diagram with the following:

- Serial dilution to create a standard curve (12 wells)
- Positive and negative controls
- Mouse serum samples (#s 1-8, 3 trials each)
- Your instructor must approve your well plate before you can begin.

2. Create serial dilution by following these steps:

- Use a pipet to add 100 μL of 1xPBS to wells A2-A12.
- Add 200 μL of 1xIL-17 to well A1.
- Pipet 100 μL out of well A1 and transfer it to well A2. Pipet up and down three or four times to sufficiently mix.
- Using the same pipet tip, transfer 100 μL from well A2 to A3, repeating the mixing from the previous step.
- Continue in this fashion until well A11. Discard the 100 μL of solution from well A11 into a waste container.

3. Transfer 100 μL of positive and negative controls from the appropriate tubes as laid out by your plan.

4. Use fresh pipets to transfer 100 μL of each of your team's samples into the wells laid out by your plan.

5. Wait for 5 minutes to allow binding of the cytokine to the well plate.

6. Wash the unbound solution out of the wells by draining the plate onto a stack of paper towels. Next, fill all wells will wash buffer, taking care to prevent spillage into neighboring wells.

7. Discard the wash buffer onto the paper towels. Make sure to tap the plate to sufficiently empty all wells.

8. Repeat washing step once more.

9. Using a fresh pipet tip, transfer 100 μL of primary antibody into all wells. Wait 5 minutes for binding.

10. Repeat washing step two times.

11. Using a fresh pipet tip, transfer 100 μL of secondary antibody into all wells. Wait 5 minutes for binding.

12. Repeat washing *three* times.

13. Transfer 100 μL of enzyme substrate (SUB) into all wells.

14. Wait 5 minutes before observing the results. Take the plate to a well-lit area and take a picture of your results.

Using ELISA to Detect Inflammatory Cytokines in Mouse Model

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Abstract

Ulcerative colitis is an autoimmune disease that affects millions. T-helper 17 cells are heavily involved in cell-mediated immune responses and secrete IL-17, an inflammatory cytokine that has been linked to autoimmune disorders. It has been hypothesized that an overexpression of the gene *ST6Gal1* could promote the longevity of these pro-inflammatory cells. This study utilizes ELISA to ascertain the relative concentrations of IL-17 in two groups of mice with ulcerative colitis—one group is made to overexpress ST6Gal1 enzyme. It was hypothesized that ST6Gal1-OE mice have the worst observed symptoms of colitis because of increased concentration of IL-17 due to this overexpression. A serial dilution, positive controls, and negative controls were used to compare the concentrations of eight mouse samples and draw conclusions. It was found that mice 4 and 8 had the most severe cases of colitis and had the highest concentrations of IL-17, along with mouse 3, with moderate colitis. This supports the hypothesis that IL-17 concentrations are proportional to the severity of ulcerative colitis. These results may not be completely sound, as it was shown that the ELISA experienced some contamination in the negative control wells. This has implications on ulcerative colitis in that if concentrations of IL-17 can be lowered, symptoms can be considerably lessened and the overall quality of life of the subject improved.

Keywords: Ulcerative colitis, IL-17, ST6Gal1, ELISA

Using ELISA to Detect Inflammatory Cytokines in Mouse Model

Ulcerative colitis is a disease that causes chronic inflammation and ulcers in the colon. The exact cause of ulcerative colitis is unknown, although it is thought to be brought on by a malfunction of the immune system ("Ulcerative Colitis," 2014). This lab utilizes ELISA to test serum samples of mice with ulcerative colitis for the presence of Interleukin-17 (IL-17), a glycoprotein that has been found at high levels in several autoimmune disorders (Gaffen, 2008). Th17 cells are heavily involved in cell-mediated immune responses and secrete IL-17 (Ouyang, Kolls, & Zheng, 2008). The samples used in the ELISA were taken from mice with overexpressed *ST6-Gal1* genes, which have also been linked to autoimmune disorders (Lauc et al., 2013), such as ulcerative colitis. Liu, Swindall, Keterson, Schoeb, Bullard, and Bellis (2011) hypothesized that the *ST6-Gal1* gene has influence over the rate of apoptosis in immune cells, often decreasing apoptosis and causing increased inflammation. With the apoptosis slowed down or halted, a larger number of T-helper 17 (Th17) cells can build up, resulting in a higher concentration of IL-17. The primary goal of this lab is to ascertain the concentrations of IL-17 found in mice with overexpressed *ST6-Gal1* genes and determine which mice have the most severe cases of colitis based on that data. The mice labeled four and eight had the most weight loss and noticeable symptoms of ulcerative colitis in the period of observation of previous research, and were concluded to have the most severe cases of the disease. For this reason, it is hypothesized that those mice will show the highest concentrations of IL-17.

Methods

Participants

The eight mice selected for this lab were all confirmed to have an overexpression of the *ST6-Gal1* gene and after several weeks had noticeable symptoms of ulcerative colitis, such as weight loss, dehydration, hair loss, and diarrhea.

Materials

Several microcentrifuge tubes of a negative control solution, a positive control solution, and 1X of IL-17 were used. Phosphate buffered saline (PBS)**,** primary antibody (PA)**,** secondary antibody (SA)**,** and enzyme substrate (SUB) were also used as well as eight serum samples from the mice with ulcerative colitis.

Procedure

First the well plate was divided into sections as shown in Figure 1 in the Appendix. Then a serial dilution was created by using a pipet to add 100 µL of PBS to wells A2-A12, adding 200 µL of IL-17 to well A1, pipetting 100 µL out of A1 and transferring it to well A2. Solutions were pipetted up and down three times to sufficiently mix. Then the same pipette was used to transfer 100 µL from well A2 to A3, repeating the mixing from the previous step, and continuing in this fashion until well A11, then 100µL from A11 was discarded into a waste container. Once the serial dilution was complete, 100 µL of positive and negative controls were transferred into the designated wells. After that, fresh pipets were used to transfer 100 µL of the mouse samples into the appropriate wells. After waiting five minutes to allow for the binding of the cytokine to the well plate, unbound solution was washed out of the well by draining the plate onto a stack of paper towels, and tapping it against the towels. Next, the used wells were filled with wash buffer, taking care to prevent spillage into neighboring wells. Then the wash buffer was discarded onto the paper towels, while the bottom was tapped to ensure all the wells were emptied. The entire washing processes was repeated once more before 100 µL of PA was transferred into all used wells using a fresh pipet tip. Once again there was a five-minute wait time to allow for binding. The washing process was then repeated twice before using a fresh pipet tip to transfer 100 µL of SA into all used wells, and waiting five minutes for it to bind. The washing process was then repeated three times. Finally, a fresh pipet tip was used to transfer 100 µL of SUB into all used wells, and the results appeared after about ten minutes. The well plate was then moved to an area of good lighting, on top of a white surface and photographed.

Results

As shown in Figure 2 of the Appendix, the serial dilution turned out well, producing a clear and visible gradient. The results of the ELISA showed mice three, four, and eight to have the darkest indicator color, revealing those mice to have the highest concentrations of IL-17 at about 250 ng/ml. The mice with the lowest concentrations of IL-17 are six and seven, with concentrations at about 31.25 ng/ml. The positive control turned out well and matched the 500 ng/ml well, but the negative control was contaminated and corresponded to about 0.98 ng/ml.

Discussion

The hypothesis is partially supported by the results. The results of the ELISA indicated that mice four and eight had the highest concentrations of IL-17, which supports the hypothesis. However, it also shows that mouse three had roughly the same concentration of IL-17 as either of those. While mouse three did show severe symptoms of ulcerative colitis, such as hair loss and no response to touch, mice six and five showed more weight loss, which is why mouse three was not included in the hypothesis. It is possible that mouse three shows fewer outward symptoms than most mice, giving the resulting data. It is also possible that the wells were contaminated. A primary error made in this lab was the contamination of the wells. As shown in Figure 2, the negative control is slightly blue, indicating that IL-17 somehow bonded to that well. This was likely a result of splashing when emptying out the well plate between washes. Other possible errors are not waiting the precise amount of time for the cytokine to bind and slight fluctuations in the volume of solution in the wells. It is unsurprising that the mice with the most severe cases of colitis were found to have the highest concentrations of IL-17. Those mice would have the most swelling and inflammation, a result of high IL-17 concentrations. This has implications on ulcerative colitis in that if concentrations of IL-17 can be lowered, symptoms can be considerably lessened and the overall quality of life of the subject improved. This also has implications in terms of most autoimmune disorders in that if there were a way to induce a stable rate of apoptosis in Th17 cells, even with over expressed *ST6-Gal1* genes, the concentration of IL-17 could be lowered and swelling and irritation in a subject significantly decreased. Possible extensions of this lab include looking at the

concentration of IL-17 in other autoimmune disorders, more closely examining the structure and function

of Th17, and investigating the concentration of IL-17 in other mammals with ulcerative colitis.

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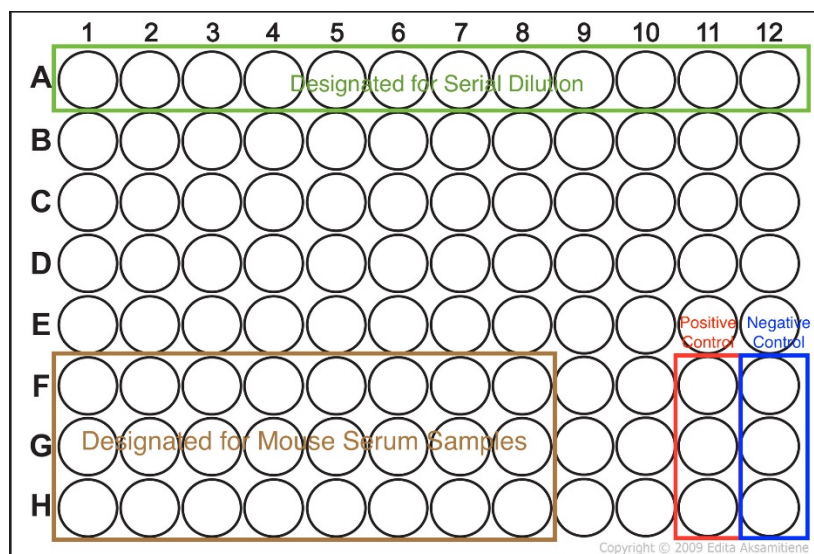
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Appendix

Figure 1. The ELISA was planned according to this layout. Serial dilution, mouse sample, and positive
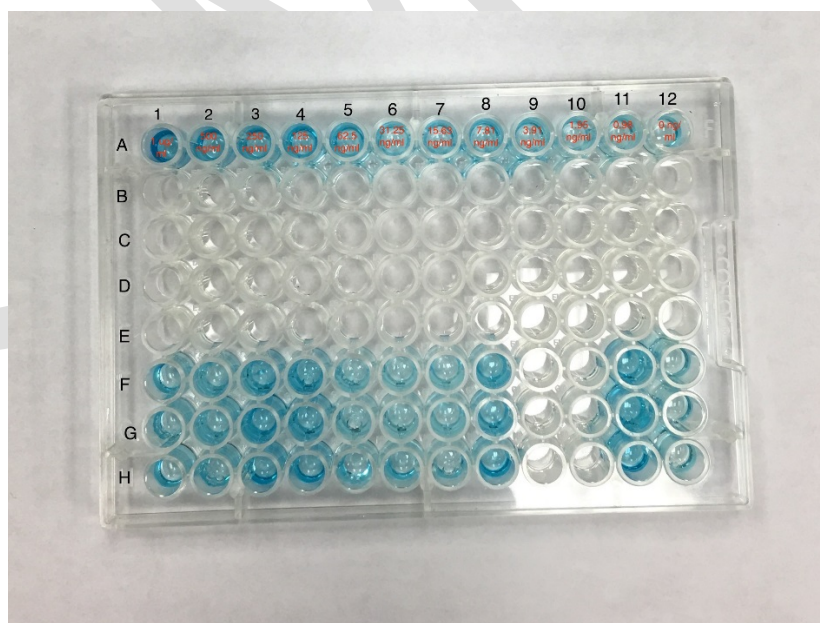
(+) and negative (-) are labeled.

Figure 2. This shows the ELISA results. The serial dilution displayed a clear gradient. Since mouse 3, 4, and 8 are the darkest, they had the most concentration of IL-17. Negative controls show signs of contamination.

ELISA Formal Lab Report Rubric

Student Name: _____   **Date:** _____

Category	Point Value	1 0-60%	2 60-75%	3 75-90%	4 90-100%	Points Earned
Title Page	5	Title does not at all reflect the main idea or is not included. Author's name and names of contributors are not included. **(0-2 pts)**	Title does not summarize the main idea of the project accurately. Author's name and names of contributors are included. **(3 pts)**	Title somewhat summarizes the main idea of the project accurately. Author's name and names of contributors are included. **(4 pts)**	Title summarizes the main idea of the project accurately. . Author's name and names of contributors are included. **(5 pts)**	
Introduction						
Background Information	20	Discussion of related literature is inadequate or missing. Discussion of the importance of the research problem is inadequate or missing. **(0-11 pts)**	Discussion of related literature is missing details relevant to the study and the understanding of the research problem. Discussion of the importance of the research problem is inadequate. **(12-14 pts)**	Discussion of related literature is adequate. Discussion of the importance of the research problem is adequate. **(15-17 pts)**	Discussion of related literature is comprehensive. Discussion of the importance of the research problem is thorough. **(18-20 pts)**	
Purpose	10	Approach to solving the problem is not explained. **(0-6 pts)**	Approach to solving the problem is weakly explained. **(7 pts)**	Approach to solving the problem is explained, but may be missing important aspects. **(8 pts)**	Approach to solving the problem is explained in detail. **(9-10 pts)**	
Statement of Hypothesis(es)	10	Variables are not defined. An incomplete statement of the hypothesis(es) is made or is missing. Explanation of how the hypothesis and experimental design relates to the problem is not included. **(0-6 pts)**	Variables may or may not be defined. An incomplete statement of the hypothesis(es) is made. Explanation of how the hypothesis and experimental design relates to the problem is not included. **(7 pts)**	Variables are defined and a formal statement of the hypothesis(es) is made. Explanation of how the hypothesis and experimental design relates to the problem is not included. **(8 pts)**	Variables are defined and a formal statement of the hypothesis(es) is made. Explanation of how the hypothesis and experimental design relates to the problem is included. **(9-10 pts)**	

Methods						
Materials	10	Participants, subjects, experimental groups, locations, etc. are not described. Specialized materials and apparatuses are not included (lists of common lab equipment are not necessary). **(0-6 pts)**	Participants, subjects, experimental groups, locations, etc. are minimally described. Specialized materials and apparatuses may or may not be included (lists of common lab equipment are not necessary). **(7 pts)**	Participants, subjects, experimental groups, locations, etc. are somewhat described. Specialized materials and apparatuses are included (lists of common lab equipment are not necessary). **(8 pts)**	Participants, subjects, experimental groups, locations, etc. are defined and described in detail. Specialized materials and apparatuses are included (lists of common lab equipment are not necessary). **(9-10 pts)**	
Procedure	20	Procedure is lacking important details and would be difficult to replicate. Does not Include a description of any specialized technique, test, etc. used. **(0-11 pts)**	Procedure is somewhat detailed and might be able to be replicated. Does not Include a description of any specialized technique, test, etc. used. **(12-14 pts)**	Procedure is somewhat detailed and might be able to be replicated. Includes a description of any specialized technique, test, etc. used. **(15-17 pts)**	Procedure is very detailed and would be easy to replicate. Includes a description of any specialized technique, test, etc. used. **(18-20 pts)**	
Results						
Summary of Data	20	Major findings relevant to the discussion are not summarized. Baseline data (control group) is not available (if applicable). References to tables and figures (if included) are not made. **(0-11 pts)**	Major findings relevant to the discussion are summarized weakly. Baseline data (control group) is not available (if applicable). References to tables and figures (if included) may or may not be made. **(12-14 pts)**	Most major findings relevant to the discussion are summarized. Baseline data (control group) may or may not be available (if applicable). References to tables and figures (if included) are made. **(15-17 pts)**	All major findings relevant to the discussion are summarized. Baseline data (control group) is available (if applicable). References to tables and figures (if included) are made. **(18-20 pts)**	
Mathematical or Statistical Methods	15	Data analysis, if present, is insufficient. No information is available for the reader to understand the analyses conducted. **(0-8 pts)**	Data analysis is present, but lacks depth and detail. Insufficient information is available for the reader to understand the analyses conducted. **(9-10 pts)**	Data analysis is present, but may not be accurate, unbiased, and complete. Sufficient information is available for the reader to understand the analyses conducted. **(11-12 pts)**	Data analysis is accurate, unbiased, and complete. Sufficient information is available for the reader to understand the analyses conducted. **(13-15 pts)**	

Discussion					
Discussion of Hypothesis(es)	15	No statement(s) of support or nonsupport for the hypothesis(es) is/are made. Post hoc explanations are not made for statements of nonsupport. **(0-8 pts)**	Statement(s) of support or nonsupport for the hypothesis(es) is/are made, but lack detail. Post hoc explanations are not made for statements of nonsupport. **(9-10 pts)**	Clear statement(s) of support or nonsupport for the hypothesis(es) is/are made. Post hoc explanations are not made for statements of nonsupport. **(11-12 pts)**	Clear statement(s) of support or nonsupport for the hypothesis(es) is/are made. Post hoc explanations are made for statements of nonsupport. **(13-15 pts)**	
Evaluation and Interpretation of Results	25	Similarities and differences between results and previous findings are not used to contextualize, confirm, and clarify conclusions. Sources of potential bias, errors, and other limitations are not discussed and alternative explanations are not provided. **(0-14 pts)**	Similarities and differences between results and previous findings may be used to contextualize, confirm, and clarify conclusions, but lack depth. Sources of potential bias, errors, and other limitations are not discussed and alternative explanations are not provided. **(15-18 pts)**	Similarities and differences between results and previous findings may be used to contextualize, confirm, and clarify conclusions, but lack depth. Sources of potential bias, errors, and other limitations may be discussed and alternative explanations may be provided. **(19-21 pts)**	Similarities and differences between results and previous findings are used to contextualize, confirm, and clarify conclusions. Sources of potential bias, errors, and other limitations are discussed and alternative explanations are provided. **(22-25 pts)**	
Implications and Importance of Results	15	Generalizability (external validity) of results are not discussed. Reasoned and justifiable commentary on the importance of findings are not included. Theoretical, clinical, or practical significance of outcomes may or may are not discussed. Possible extensions of the study are not suggested. **(0-8 pts)**	Generalizability (external validity) of results are weakly discussed. Reasoned and justifiable commentary on the importance of findings are not included. Theoretical, clinical, or practical significance of outcomes may or may are not discussed. Possible extensions of the study are not suggested. **(9-10 pts)**	Generalizability (external validity) of results are somewhat discussed. Reasoned and justifiable commentary on the importance of findings may or may not be included. Theoretical, clinical, or practical significance of outcomes may or may not be discussed. Possible extensions of the study may or may not be suggested. **(11-12 pts)**	Generalizability (external validity) of results are discussed. Reasoned and justifiable commentary on the importance of findings is included. Theoretical, clinical, or practical significance of outcomes are discussed. Possible extensions of the study are suggested. **(13-15 pts)**	
References	10	An insufficient amount of	An insufficient amount of	A minimal amount of	A sufficient amount of	

		resources are used. Some references are inappropriate for the research topic. Some references have not been cited in the body of the paper. **(0-6 pts)**	resources are used. Some references are inappropriate for the research topic. All references have been cited in the body of the paper. **(7 pts)**	resources are used. References are appropriate for the research topic. All references have been cited in the body of the paper. **(8 pts)**	resources are used. References are appropriate for the research topic. All references have been cited in the body of the paper. **(9-10 pts)**	
Readiness of Manuscript						
Grammar and Mechanics	30	Paper has numerous grammatical, spelling, and punctuation errors. **(0-17 pts)**	Paper has some grammatical, spelling, and punctuation errors. **(18-21 pts)**	Paper has few grammatical, spelling, and punctuation errors. **(22-26 pts)**	Paper is free of grammatical, spelling, and punctuation errors. **(27-30 pts)**	
Writing Style	30	Writing is irregular and hard to follow. Writing does not show much understanding of related ideas and content **(0-17 pts)**	Writing is mediocre. Some major indications of misunderstanding are present. **(18-21 pts)**	Writing is flowing and easy to follow. Some minor indications of misunderstanding are present. **(22-26 pts)**	Writing is flowing and easy to follow. Writing shows superior understanding of related ideas and content. **(27-30 pts)**	
					Total Points 235	
					Final Grade _____/100	

Additional Comments:

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