American Association of Immunologists
Teacher Summer Research Project and Curriculum Development
June 2012– May 2013

By:
Judy Birschbach
West Bend East High School

Exploring the link between regulatory T cells and tumor growth: A lesson in the immune system and cancer.

I. Unit Overview: Students will…
   a. Review prior knowledge (see section VIII)
   b. Identify new learning objectives (see section IV)
   c. Conduct literary research into the role of Regulatory T cells and their potential link to cancer and autoimmune disease.
   d. Practice common laboratory techniques: simulation of counting red blood cells in a hemocytometer
   e. Develop and present an immune system role play activity incorporating new knowledge of innate and acquired immunity, including the role of various B & T cells (focus on Tregs)

II. Wisconsin's Model Academic Standards for Science Addressed:
   a. B.12.4 Show how basic research and applied research contribute to new discoveries, inventions, and applications
   b. C.12.6 Present the results of investigations to groups concerned with the issues, explaining the meaning and implications of the results, and answer questions in terms the audience can understand
   c. C.12.7 Evaluate articles and reports in the popular press, in scientific journals, on television, and on the Internet, using criteria related to accuracy, degree of error, sampling, treatment of data, and other standards of experimental design
   d. F.12.2 Understand how cells differentiate and how cells are regulated

III.

f. Research and explain the specific role of regulatory T cells in immune homeostasis and predict what happens when regulatory T cells react appropriately versus inappropriately.
g. Define cancer, explain several potential causes of cancer, and draw conclusions about how both internal and external factors can lead to tumor growth and metastasis.

V. Time Requirements
a. 9 class periods (50 minutes each)
   i. Review, notes & lecture discussions: 2 class periods
   ii. Research Article and Questions: 1 class period, and time outside of class.
   iii. Counting Simulated Red Blood Cells: 1 class period
   iv. Role play discussion and preparation: 2 class periods
   v. Role play presentations: 1 class period
   vi. Summary and review, lab and role play analysis: 1 day
   vii. Summative assessment: 1 class period

VI. Advance Preparation
a. Prepare copies of the Gallimore article and scaffolding questions, 1 for each student.
b. Prepare materials for Counting Blood Cells lab:
   i. mix yeast, safranin O and DI water according to teacher notes included in lab kit.
c. Prepare role play figures/pictures and rubric 1 for each student, groups of 2-3 students.
d. Prepare powerpoint or other lecture/discussion notes on the Immune System. (Ch. 43 in Campbell Biology 7th Edition)

VII. Materials and Equipment
a. Campbell Biology 7th edition text, or comparable
b. Projection system for lecture notes (Smartboard or the like)
c. Review articles:
   i. Gallimore, Awen and Andrew Godkin. “Regulatory T cells and tumour immunity – observations in mice and men.” 2007. Blackwell Publishing. *Immunology,* 123, 157-163. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-

f. What is the function of the immune system?
g. What is cancer?

IX. Student Expectations and Anticipated Results
a. (Answer keys/Discussion points)

X. For Classroom Discussion:
a. Chapter 43 in Campbell Biology, 7th edition
b. Powerpoint lecture notes
c. Text book guided reading questions

Exploring the link between regulatory T cells and tumor growth: A lesson in the immune system and cancer.

Unit Background and Rationale:

Like any other system in our bodies, the immune system requires strict regulation in order to maintain homeostasis and prevent

Memory cells
Clonal selection

Disposable Hemocytometer
montreal-biotech.com

Discussion/Analysis

Part A: Galimore Research Article Questions

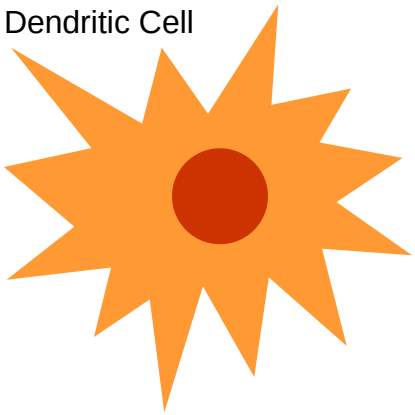
1. There are many different types of lymphocytes, including T cells. This article focuses on the role of a specific population called Treg cells. Before reading, review the different types of T cells and their different roles.
2. For better comprehension, get in the habit of looking up unfamiliar words. Look up and define the following terms fibrosarcoma, concomitant, ablating, CD4, CD8, CD25, *in vitro* and *in vivo*. Continue with additional unknown words throughout the article as needed.
3. According to Robert North's studies in the 1980s, why were T cells unable to stop tumor growth?
4. Summarize the correlation between rejection of tumor cells and regulatory T cell (Treg) activity.
5. What did the drug cyclophosphamide result in? How/why?
6. What is CD4? Why were CD4-depleting antibodies used? What was the result?
7. What is the most reliable marker used to identify Treg cells?
8. Look up on the internet: what population of T cells are CD8+ and help with tumor rejection?
9. In Shimizu's study, what happened to mice inoculated with tumor cells after the all the CD25+ cells (Tregs) were depleted?
10. What is meant by the "immune surveillance" concept? How did it become better supported in recent years?
11. Why do you think human studies are more restricted, making drawing a correlation between Treg function in mice and Treg function in humans difficult?
12. Even though immunosuppressive drugs are used widely for many diseases in humans, still the greatest increase in tumors is due to what other variable?
13. Studies indicate there's a positive correlation between better cancer patient outcomes and increased numbers of lymphocytes in their tumors (tumor-infiltrating lymphocytes, or TILs). What might this suggest about how your body responds to cancer?
14. Describe the concept of "Duke's staging" in reference to CRC?
15. What is the difficult question regarding lymphocyte reaction and tumor progression?
16. Summarize the conclusion of the Galon *et al* study.
17. What are 4 potential reasons for whsupporu -1.15 Td [(r)-1(eem)-2(i)-2(rn2(hi)-2(s)-e(n)-10(ru)-1-6(y antitumor CD8+ T [(rc)6(e)6(ll)]TJ 0 Tc 0 Tw ( )Tj [(r)3(e)-6(s)-1(pon)-10(ru)-"u Look up "downregula between CD4+ and CD8+ cells and paraphrase that statement.
19. What is the overall trend in Treg 1.5 0 volvemeeem regulating responruphctumors What is the implication of this obruvaton?
20. Determine what the term "ascites" means in reference to ovarian cancer.
21. In the 2004 Curiel study of 104 biopsiedmpumors, what conclusion did they draw 1 Tftwrun numbhi of Tregs and stage of the diserund patieeemurvival?
22.

Part B: Hemocytometry Analysis

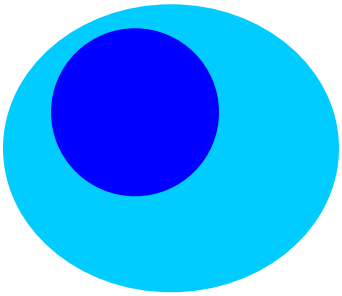
1. Calculate the number of red blood cells per mm^3 for each sample using the following equation:
   (sum of the 5 squares) x 5 x 10^4 = Number of cells in 1 mm^3
2. Is there a difference in the cell counts for each sample? Research the normal range for RBC's and propose a reason why the cell counts might not fit

Sample figures for Role Play activity

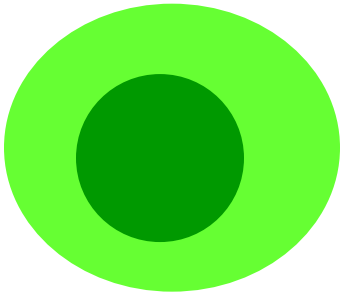
Dendritic Cell



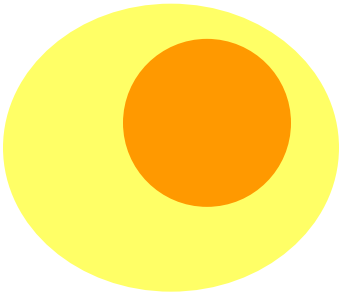
Cytotoxic T Cell



Helper T Cell



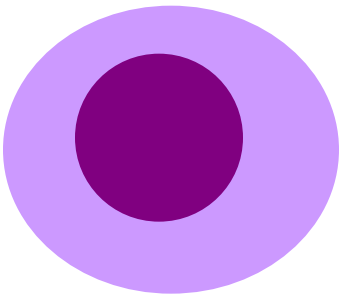
B Cell



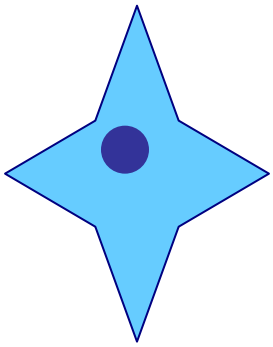
Antigen

Antibody

T $_{Reg}$ Cell



Cancer Cell



Appendix B
Textbook Alignment

Chapter 43: The Immune System
(Campbell Biology 7th Edition)

Background vocab – Define the following:

Microorganism

Pathogen

Antibiotic

Antibody

Antigen

How are microbes destroyed once they are phagocytized?

It is the job of phagocytes to identify and engulf foreign microoranisms. Why do we worry about the pathogenic threat of microbes?

Complete the following table regarding Phagocytic Cells:

Type of White Blood Cell	Abundance	Location

What kinds of cells do Natural Killer (NK) cells target?

Discuss the roles of Helper T cells, Cytotoxic T cells, and B cells. Include CD4 and CD8 in your discussion.

Antibody Classes

	Roles	Special Features/Misc.
IgM		
IgG		
IgA		
IgE		
IgD		

Disposal of Antigens: what is a “MAC”

Compare and contrast Active vs. Passive immunity. Include vaccinations in your discussion.

Appendix C

Formative Assessment

Name: _____ Class: _____ Date: _____

Cells and Systems Test: Endocrine, Nervous, Immune

Multiple Choice
Identify the letter of the choice that best completes the statement or answers the question.

____ 1. Based on their effects, which pair below could be considered antagonistic?
   a. insulin and glucagon
   b. growth hormone and prolactin
   c. endocrine and exocrine glands
   d. hormones and target cells

____ 2. Why is it that some body cells respond differently to the same peptide hormones?
   a. Different target cells have different genes
   b. Each cell knows how it fits into the body's master plan.
   c. the response to the peptide is determined by the type of receptor molecule that the hormone binds to and the resulting action in the cell
   d. The circulatory system regulates responses to hormones by routing the hormones to specific targets.

____ 3. How does a steroid hormone initiate a response in target cells?
   a. by binding to cell membrane receptors
   b. through release outside the body
   c. by causing a negative feedback mechanism
   d. by binding to receptor proteins present inside of the cell

____ 4. The endocrine system and the nervous system are structurally related. Which of the following cells best illustrates this relationship?
   a. a neuron in the spinal cord
   b. a steroid-producing cell in the adrenal cortex
   c. a neurosecretory cell in the hypothalamus
   d. a brain cell in the cerebral cortex

____ 5. If a person drinks a large amount of water in a short period of time, he or she may die from water toxicity. ADH can help prevent water retention through interaction with target cells in the
   a. anterior pituitary.
   b. posterior pituitary.
   c. adrenal gland.
   d. kidney.

____ 6. Iodine is necessary in the production of Thyroxine. Which gland requires iodine to function properly?
   a. ovaries and testes
   b. adrenal
   c. thyroid
   d. pancreas

The question below refers to the following information.

In an experiment, rats' ovaries were removed immediately after impregnation and then the rats were divided into two groups. Treatments and results are summarized in the table below.

	Group 1	Group 2
Daily injections of progeterone (milli grams)	0.25	2.0
Percentage of rats that carried fetuses to birth	0	100

____ 7. The results most likely occurred because progesterone exerts an effect on the

a. general health of the rat.

For the following 2 questions,

For the questions below, match the following answers with the phrase that best describes them.

A. cytotoxic T cells
B. B cells
C. helper T cells
D. macrophages

____ 23. These cells are involved in cell-mediated immunity and destroy virally infected cells.

a. A
b. B
c. C
d. D

____ 24. These cells are involved in humoral immunity, and release antibodies to target antigens in bodily fluids.

a. A
b. B
c. C
d. D

Cells and Systems Test: Endocrine, Nervous, Immune
Answer Section

MULTIPLE CHOICE

1. ANS: A TOP: Concept45.2
2. ANS: C TOP: Concept45.2
3. ANS: D TOP: Concept45.2
4. ANS: C TOP: Concept45.3
5. ANS: D TOP: Concept45.3
6. ANS: C TOP: Concept45.4
7. ANS: C TOP: Concept45.4
8. ANS: B TOP: Concept45.4
9. ANS: B TOP: Concept45.4
10. ANS: A TOP: Concept45.4
11. ANS: D TOP: Concept48.2
12. ANS: A TOP: Concept48.3
13. ANS: E TOP: Concept48.3
14. ANS: A TOP: Concept48.4
15. ANS: B TOP: Concept48.4
16. ANS: D TOP: Concept48.4
17. ANS: B TOP: Concept48.5
18. ANS: D
19. ANS: A TOP: Concept43.1
20. ANS: A TOP: Concept43.1

21. ANS: A TOP: Concept43.1
22. ANS: C TOP: Concept43.1
23. ANS: A TOP: Concept43.3
24. ANS: B TOP: Concept43.3
25. ANS: D TOP: Concept43.3
26. ANS: B TOP: Concept43.3
27. ANS: D TOP: Concept43.5
28. ANS: B TOP: Concept43.5

35. Which of the following best describes current research findings on the possible link between Treg cells and tumors?
    a. Numbers of Tregs have been positively correlated with tumor progression in mice
    b. Treg malfunction causes cancer
    c. Lower numbers of Tregs are correlated with more advanced stages of cancer
    d. Tr