Macrophage profiling of challenged, splenectomized mice: an advanced, secondary curriculum unit on cancer immunology

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Abstract

Macrophage profiling of challenged, splenectomized mice: an advanced, secondary curriculum unit on cancer immunology

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The American Association of Immunologists allows high school teachers to participate in research experiences aimed at contextualizing the often abstract, nuanced content they present to their students. Last summer, I investigated the phenotype of murine macrophages implicated in the termination of immune privilege and subsequent rejection of uveal tumors by splenectomized mice. Macrophages were characterized as M1 or M2 type based on their cytokine profiles, which was evaluated using RT-PCR analysis to determine the levels of cytokine transcripts in harvested macrophages and ELISA assays to assess the amount of secreted protein in cell culture supernatants. As a result of my work, I developed curriculum for use in advanced courses. Through readings, lectures, and class discussions, students will be introduced to the seminal concepts involved in the study of immunology and cancer biology. Online simulations and data analysis tools will be used to facilitate a deeper understanding of the procedures for harvesting cells from mice and quantifying transcription levels. Efforts will be made to develop an appropriate investigation involving either clonal selection or standard ELISA. The implications of these experiences and possible modifications for use with various student groups will be discussed.

Teacher Section

General Overview

The study of cell biology is integral to any survey and advanced –level, secondary Biology course. Investigating the mechanisms involved in cancer biology specifically enables students to contextualize some of the more difficult cellular mechanisms involved in cell signaling, communication, division, and death. This past year, the College Board selected the immune system as one of three main human body systems to be emphasized in the re-developed Advanced Placement (AP) course. Through my participation in the AAI Summer Research Program for Teachers, I have developed a unit consisting of series of lessons, readings, and activities that integrate the study of immunology in relation to cancer biology. While the lessons may be modified or abbreviated for use in a general Biology course, the design of the unit is primarily structured so that advanced-level students learn seminal immunological concepts and techniques through a study of the research investigation I participated in over the summer involving the potential role of macrophages in the ability of mice without spleens to reject the development of ocular tumors.

Science Background

This particular unit resulted from my experience in an ophthalmology/immunology research laboratory over a five week period. The particular project under study centered on the ability of a given mouse model to reject uveal (eye) tumors. Splenectomy (the removal of the spleen) had been shown to terminate ocular immune privilege, and it had recently been demonstrated that tumors are rejected in splenectomized mice by a process that requires various cells and molecules, including macrophages. In the project, we tested the hypothesis that the rejection of ocular tumors in splenectomized mice involves a switch in macrophages from a nontumoricidal and proangiogenic “M2” type to a tumoricidal and anti-angiogenic “M1” type. The macrophages examined were harvested from both the eye and peritoneal cavity of control and splenectomized mice.

This secondary unit is designed to connect elements involved in cell signaling, the immune system, and the development of cancer. While each of these concepts consists of an enormous amount of specific information, the extent to which the specific student audience is expected to develop an understanding of each will determine the nature of the science background required and the amount of prior knowledge students may have. At a minimum, students will be expected to understand the interaction between ligand and receptor molecules involved in signaling, the idea that there exist a variety of immune cells with respective functions, and the concept of tumor growth and/or rejection as defined by unregulated cell division. For advanced-level (AP) courses, students will be expected to compare/contrast a variety of signaling methods and cellular responses, differentiate, in detail, between macrophages, humoral and cell-mediated immunity, and describe how a particular macrophage profile may relate to the development/rejection of ocular tumors in mice.

A second component to the curriculum involves the lab techniques used by immunologists. General Biology students will be expected to explain the interaction between antigen and antibody and describe how such an interaction is used in a standard ELISA experiment. AP students will be expected to differentiate between a variety of immunological methods (such as quantitative PCR, flow cytometry, and ELISA) in terms of both procedure and purpose.

More specific information pertaining to the science background for the above content and experimental procedures in contained within the lesson documents themselves.

Standards

American Association for the Advancement of Science (Project 2061)
(http://www.project2061.org/publications/bsl/online/index.php?chapter=6#C4)

The immune system functions to protect against microscopic organisms and foreign substances that enter from outside the body and against some cancer cells that arise within.

Communication between cells is required to coordinate their diverse activities. Cells may secrete molecules that spread locally to nearby cells or that are carried in the bloodstream to cells throughout the body. Nerve cells transmit electrochemical signals that carry information much more rapidly than is possible by diffusion or blood flow.

The human body is a complex system of cells, most of which are grouped into organ systems that have specialized functions. These systems can best be understood in terms of the essential functions they serve for the organism: deriving energy from food, protection against injury, internal coordination, and reproduction.

Pennsylvania Science Standards
(http://www.pdesas.org/Standard/Views#0|0|707|0)

Explain how cells differentiate in multicellular organisms.

Recognize that systems within cells and multicellular organisms interact to maintain homeostasis.

Analyze how cells in different tissues/organs are specialized to perform specific functions.

The College Board –AP Biology
(http://apcentral.collegeboard.com/apc/public/repository/AP_BiologyCED_Effective_Fall_2012_lkd.pdf)

Growth and dynamic homeostasis of a biological system are influenced by changes in the system's environment.

- Plants, invertebrates and vertebrates have multiple, nonspecific immune responses.
- Vertebrate immune systems have nonspecific and nonheritable defense mechanisms against pathogens.
- Mammals use specific immune responses triggered by natural or artificial agents that disrupt dynamic homeostasis.

The student can create representations and models to describe immune responses.

Cells communicate by generating, transmitting and receiving chemical signals.

- Immune cells interact by cell-cell contact, for example, contact between antigen-presenting cells (APCs) and helper T-cells or APCs and killer T-cells.

A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas (http://www.nap.edu/catalog.php?record_id=13165#toc)

By the end of grade 12: Systems of specialized cells within organisms help them perform the essential functions of life, which involve chemical reactions that take place between different types of molecules, such as water, proteins, carbohydrates, lipids, and nucleic acids. Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level. Feedback mechanisms maintain a living system's internal conditions within certain limits and mediate behaviors, allowing it to remain alive and functional even as external conditions change within some range. Feedback mechanisms can encourage (through positive feedback) or discourage (negative feedback) what is going on inside the living system.

Student Outcomes and Objectives

The expected outcomes depend on the nature of the course and the level of the students.

For an advanced-level, AP Biology class, the students will be expected to:

1. Explain the relationship between the immune system and the development of cancer.
2. Delineate the main components of innate immunity in animals.
3. Compare/contrast the functions of the various cells of the adaptive immune system (with an emphasis on macrophages, B cells and T cells).
4. Describe the role of cytokines in the cell signaling and differentiate between their expression levels in M1 and M2 macrophages.
5. Identify the major procedural elements and functions of commonly used immunological techniques (quantitative PCR, flow cytometry, and ELISA)

6. Successfully perform a standard ELISA experiment (with a qualitative analysis of varying concentrations of antibody) and accurately depict the interaction between the components involved in the procedure.

For a general-level, survey course the students will be expected to:

1. Explain the basic structure and function of the cells of the immune system (neutrophils, macrophages, and lymphocytes).
2. Describe the three main mechanisms used to destroy pathogens in the body.
3. Summarize the process used by the immune system to remember pathogens.
4. Successfully complete a standard ELISA experiment and accurately depict the interaction between the components involved in the procedure.

Time Requirements and Advanced Preparation

The time requirements for the unit depend on the activities/lessons selected and the amount of prior knowledge students may have pertaining to the concepts under review. The maximum amount of time required to complete all of the components detailed below should be 10-12 class periods (50 minutes each).

The following is a suggested timeline for use with advanced-level (AP) students. Specific readings, links to simulations, and lab ordering information (with a more detailed description of their content) are contained in the materials and equipment section.

A. Introduction to Cancer Biology (Website) and Review of Prior Concepts (Web Readings)—one class period
    a. The teacher should be sure to have internet access for class. The teacher can either move through the cancer tutorial with the students or have them complete it independently.

B. Relating Immunology to Cancer (Articles and online video) – one class period
    a. The teacher should be sure to have enough copies of article to share with students. This reading could be assigned for homework with guiding questions to facilitate class discussion.
    b. The teacher will need to access to the internet to view the MIT video.

C. Macrophage Profiling (Article) – one class period
    a. The teacher should be sure to have enough copies of article to share with students. This reading could be assigned for homework with guiding questions to facilitate class discussion.
    b. This article provides a good opportunity to review previously addressed concepts in cell signaling.

D. Immunity Content Sharing (Prezi) – two class periods

a. The teacher should be sure to have internet accessibility to use the Prezi. Students may need time at home to review concepts discussed in the lectures.

E. Immunology Laboratory Protocols (Online simulations and videos) – three class periods
   a. The teacher should be sure to have internet access for these simulations/animations. Students can view/complete any of these for homework (with appropriate guided questions). The particular sequence of the following simulations/labs is designed to move the students through the particular research work from my summer experience.
   b. Peritoneal extraction video (20 minutes with discussion)
   c. Flow cytometry simulation (30 minutes with discussion)
   d. Quantitative PCR video (30 minutes with discussion)
   e. Virtual ELISA lab (one class period)

F. Standard ELISA Lab – two class periods
   a. The ELISA lab takes at least to class periods. There will be specific pre-lab work required of the instructor to ensure students complete the lab work in two days.
   b. All lab materials/kits should be ordered and checked for accuracy at least two weeks prior to the lab experience. The two kits referenced in the unit were purchased from EDVOTEK® and can be found at: www.edvotek.com (Kit # 269- general Biology and Kit # 278- advanced-level (AP)

G. Review and Summative Assessment – two class periods

Materials and Equipment

The following annotated list contains links and descriptions for the teaching materials used throughout the complete unit. It is recommended that teachers follow the prescribed order only if they intend to incorporate all of the lesson/activities into a coherent unit modeled after the singular, research experience profiling macrophages in laboratory mice. Otherwise, teachers can move through the resources in an order more aligned with their particular teaching situations.

I. Introductory events

   a. Cancer Biology
      i. http://www.insidecancer.org/
         1. This link provides background information for the development of cancer. The section entitled, “Hallmarks of Cancer,” provides a general overview of cancer biology. The section entitled, “Pathways to Cancer,” relates cell signaling mechanisms to unmitigated cell division; a particularly useful connection to content taught throughout the year.

   b. Other mechanisms of tumor development (as described earlier in the year in class)
      i. http://webhost.bridgew.edu/fgorga/ras/ras_intro.htm
         1. This website provides a summary of the Ras protein and its role as a signaling molecule. Students can relate mutations in Ras to a loss of signaling in cell growth and division.
      ii. http://www.ncbi.nlm.nih.gov/pubmed/8695238

1. This brief abstract introduces students to the purported role of free radicals in the development of cancer. If cell respiration has been previously covered in the course, students could use this content to review the mechanisms involved in the electron transport chain that generate free radicals.

c. Immunology and cancer
    i. http://www.cancerresearch.org/news/2011/march/new-perspectives-on-cancer-and-the-immune-system
        1. In this web article three Cancer Research Institute scientists describe how advances in the field of tumor immunology have revealed a complex and paradoxical relationship between cancer and the immune system, and discuss how a growing understanding of this relationship is providing a scientific foundation for new therapies capable of unleashing the immune system's protective powers against cancer.
    ii. http://ki.mit.edu/research/immune/
        1. This web video from MIT discusses current research underway to engineer the immune system to better detect and interfere with tumor development.

II. Immunology content

a. Cells of the immune system
    i. http://www.sciencedaily.com/releases/2009/08/090810174303.htm
        1. This article introduces students to the relationship between macrophages (a component of the immune system) and metastasis. In my particular class, students were first exposed to my research experience earlier on in the year thereby enabling me to use this article to begin to review their prior knowledge.
    ii. http://bloodjournal.hematologylibrary.org/content/108/2/408.full
        1. This article differentiates between M1 and M2 macrophages as they relate to tumor promotion or rejection. Students are introduced to cytokines and their respective profiles in the two types of macrophages. I use this article to make explicit the hypothesis we were testing for between control (which developed tumors) and splenectomized (which rejected tumors) mice.

b. Basics of the immune system
    i. http://prezi.com/nvaoowj3p8jz/ap-bio-physiology-4-immunity/
        1. This Prezi was developed by David Knuffke. I use it to explain the various components of the mammalian immune system (innate vs. acquired and humoral vs. cell-mediated). It contains the majority of the content required for the AP exam.
    ii. http://www.interactive-immunity.org/
        1. This website provides information on the immune system for general-level Biology students.
        2. See the appendix for a note sheet for this site.

III. Laboratory protocols (in order of research experience from summer project)

a. Extraction of macrophages
   i. http://www.jove.com/video/1488/isolation-of-mouse-peritoneal-cavity-cells
      1. This online video walks students through the procedure used for isolating peritoneal macrophages from mice.

b. Flow cytometry
   i. http://www.unsolvedmysteries.oregonstate.edu/flow_01
      1. This online simulation introduces students to the concepts of flow cytomery and provided a case study for analyzing experimental results. I mentioned to the students that this particular technique was used to isolate macrophages from homogenized eye tissue in the lab.

c. Quantitative PCR
   i. https://www.youtube.com/watch?v=QVeVIM1yRMU
      1. This animation depicts the reactions taking place in a quantitative RT-PCR experiment
   ii. https://www.youtube.com/watch?v=TkCBcL_xUUs&feature=related
      1. This video shows the steps used to set up a quantitative RT-PCR experiment.
   iii. It should be noted here that this particular technique is perhaps the most difficult for most students to comprehend. Therefore, I limited my lesson to teaching them the fundamentals of the procedure (in comparison to traditional PCR) and its purpose in reference to the summer research project (to quantify the RNA levels of various cytokines from isolated macrophages).

d. Virtual ELISA Lab
   i. http://media.hhmi.org/biointeractive/vlabs/immunology/index.html
      1. This virtual lab guides students through the set-up and interpretation/analysis of an ELISA experiment.
      2. I explain to the student that this lab exercise was used to measure protein levels of various cytokines in cell supernatants (to compare with RNA levels quantified through RT-PCR).
      3. See the appendix for a note sheet for this assignment.

e. ELISA Wet Lab
   i. www.edvotek.com (Kit # 278) – Advanced (AP) level
      1. The objective of this experiment is to perform and master the concepts and methodology involved with enzyme linked immunosorbent assays. This ELISA experiment is designed to detect circulating IgG directed toward an antigen. Observations in this experiment include specificity of antibodies, the effect of dilution on ELISA reactions, color development and quantification.
   ii. www.edvotek.com (Kit # 269) – General-level Biology

1. This experiment introduces concepts and methods of enzyme linked immunosorbent assays.

End of Unit Possible Assessments

f. See the appendix for unit assessments for AP and general-level Biology
   i. Immune Cell Notes: Intended for use with general-level Biology courses as students work through http://www.interactive-immunity.org/
   ii. ELISA Virtual Lab Questions/notes: Intended for advanced-level students to complete as they work through the simulation at http://media.hhmi.org/biointeractive/vlabs/immunology/index.html
   iii. Post Lab Questions for Quantitative ELISA: To be completed following www.edvotek.com (Kit # 278).
   iv. Post Lab Questions for Introductory ELISA: To be completed following www.edvotek.com (Kit # 269).
   v. AP Biology Test Three (Immunology): This is a full-length test with questions derived from the all of the above teaching materials, including the readings, Immune System Prezi, simulations, and ELISA lab.
   vi. Biology Quiz (Immunity): This document provides sample questions taken from the general-level Biology test which included immunology as one of several topics.

Student Section

(This section is developed for advanced-level/AP Biology. It should be modified accordingly for other levels of instruction)

Unit Rationale

In this unit, we will relate the ideas from our previous cell biology discussions to the study of immunology. In particular, we will use a current research investigation underway at the University of Pittsburgh's Ear and Eye Hospital to learn about the structure and function of the various immune cells, the mechanisms involved in immune signaling, and the role that the immune system may play in the development of cancer. Additionally, we will explore various lab applications used commonly in the field of immunology and complete the unit by performing a wet lab on one of these applications.

The unit consists of a series of preliminary readings from online periodicals and websites, a detailed Prezi with the majority of the content needed to understand the research project, a collection of animations and simulations used to introduce the lab applications, and the culminating ELISA wet lab. At the conclusion of the unit, there will be a full-length exam, and students will submit questions pertaining to the analysis of the ELISA experiment.

Research Project Rationale

This particular unit resulted from my experience in an ophthalmology/immunology research laboratory over a five week period. The particular project under study centered on the ability of a given mouse model to reject uveal (eye) tumors. Splenectomy (the removal of the spleen) had been shown to terminate ocular immune privilege, and it had recently been demonstrated that tumors are rejected in splenectomized mice by a process that requires various cells and molecules, including macrophages. In the project, we tested the hypothesis that the rejection of ocular tumors in splenectomized mice involves a switch in macrophages, a particular type of immune cell, from a nontumoricidal and proangiogenic "M2" type to a tumoricidal and anti-angiogenic "M1" type. The macrophages examined were harvested from both the eye and peritoneal cavity of control and splenectomized mice.

Articles and Websites

We will begin our study of the immune system by examining content previously discussed this year in our larger Cell Biology unit. The following websites and articles will guide our class work. The general objectives for each reading are described below.

A. Cancer Biology
    i. http://www.insidecancer.org/
        1. Read the section entitled, "Hallmarks of Cancer," and record some notes on general overview of cancer biology. Read the section

entitled, “Pathways to Cancer,” and explain how cell signaling mechanisms relate to unmitigated cell division.

B. Other mechanisms of tumor development
    i. http://webhost.bridgew.edu/fgorga/ras/ras_intro.htm
        1. Using the information from this reading, describe how mutations in Ras relate to a loss of signaling in cell growth and division.
    ii. http://www.ncbi.nlm.nih.gov/pubmed/8695238
        1. This brief abstract introduces students to the purported role of free radicals in the development of cancer. Elaborate on how the mechanisms involved in the electron transport chain generate free radicals.

C. Immunology and cancer
    i. http://www.cancerresearch.org/news/2011/march/new-perspectives-on-cancer-and-the-immune-system
        1. Relate the development of cancer to the functioning of the immune system and explain how this relationship is being considered for treating certain cancers.
    ii. http://ki.mit.edu/research/immune/
        1. Explain the current research underway to engineer the immune system to better detect and interfere with tumor development.

D. Cells of the immune system
    i. http://www.sciencedaily.com/releases/2009/08/090810174303.htm
        1. How are macrophages involved in metastatic cancer?
    ii. http://bloodjournal.hematologylibrary.org/content/108/2/408.full
        1. Differentiates between M1 and M2 macrophages as they relate to tumor promotion or rejection. What are the respective cytokine profiles of each? How do these profiles relate to the experimental hypothesis concerning control and splenectomized mice?

E. Basics of the immune system
    i. http://prezi.com/nvaoowj3p8jz/ap-bio-physiology-4-immunity/
        1. This Prezi was developed by David Knuffke. After reviewing the information in the Prezi, you should be able to:
            a. Differentiate between innate and acquired immunity.
            b. Differentiate between humoral and cell-mediated immunity.
            c. Provide examples for each of the above.
            d. Explain clonal selection and antigen presentation.

Animations and Simulations

The following animation and simulations are used to introduce and explain the laboratory protocols used to compare the macrophage profiles (M1 and M2) in control and splenectomized mice. They are listed below in the order they were performed.

A. Extraction of macrophages
   ii. http://www.jove.com/video/1488/isolation-of-mouse-peritoneal-cavity-cells
      1. This online video explains the procedure used for isolating peritoneal macrophages from mice.

B. Flow cytometry
   iii. http://www.unsolvedmysteries.oregonstate.edu/flow_01
      1. This online simulation explains the concepts of flow cytometry and provides a case study for analyzing experimental results. This particular application was used to isolate macrophages from the eyes of mice.

C. Quantitative PCR
   iv. https://www.youtube.com/watch?v=QVeVIM1yRMU
      1. This animation depicts the reactions taking place in a quantitative RT-PCR experiment, a method used measure the RNA levels of specific genes.
   v. https://www.youtube.com/watch?v=TkCBcL_xUUs&feature=related
      1. This video shows the steps used to set up a quantitative RT-PCR experiment. How does RT-PCR compare with traditional PCR, as discussed earlier in the year?

D. Virtual ELISA Lab
   vi. http://media.hhmi.org/biointeractive/vlabs/immunology/index.html
      1. This virtual lab simulates the set-up and interpretation/analysis of an ELISA experiment, used to measure the amount of protein levels in solution. We used this lab to measure protein levels of various cytokines in cell supernatants (to compare with RNA levels quantified through RT-PCR and verify if the RNA was actually expressed.)
      2. There is a separate handout to complete with this assignment.

ELISA Wet Lab

Your teacher will provide you with the specific protocol for the ELISA lab. In this lab, you will detect circulating IgG directed toward two antigens. Observations in this experiment include specificity of antibodies, the effect of dilution on ELISA reactions, color development and quantification. There is a separate handout to complete for this assignment.

Appendix

Immune Cells Notes

Complete the following, using the information from the link, “Interactive Immunity Site” on the course blog. You will only need the information from Part I. All about immunity.

The Basics of the Immune System

1. Identify the various organ systems involved in the immune response.
2. Differentiate between erythrocytes and leukocytes.
3. Compare the function of neutrophils, macrophages, and lymphocytes.
4. Describe the various mechanisms used to destroy pathogens in the body.

How the Immune System Works

5. What are antigen receptors? How do they differ between B and T cells?
6. Explain the general process used by the immune system to remember pathogens.

Where the Cells of the Immune System are Made

7. Differentiate between the location of T cell generation and where the other cells of the immune system are produced.
8. What are the functions of the lymph nodes, lymph vessels, and the spleen?
9. What are chemokines and cell adhesion molecules?
10. What role do cytokines play in immune cell signaling?

ELISA Virtual Lab Questions/Notes (Pre-lab)

Complete the ELISA virtual lab on the course blog and answer the following questions:

1. What does ELISA stand for?

2. Which condition(s) could result in false positive and false negative results?

3. Visually represent the interaction between the target antigen, primary antibody, secondary antibody (with enzyme), and substrate.

4. What molecule has been applied to the immunoassay prior to the lab? Why (what molecule are you testing for the presence of)?

5. What will serve as the positive and negative control for the experiment?

6. From which species is the secondary antibody derived?

7. What is the enzyme used in the lab to detect the presence of conjugates (attached antibody)?

8. What are you looking for to indicate a positive result?

9. Which general procedures (throughout the lab) are used to ensure that the respective antibodies attach sufficiently and to reduce unspecific binding?

Post Lab Questions for Quantitative ELISA (from Edvotek Instructor's Manual)

1. To what do antibodies respond?

2. Can an antibody act as an antigen? Explain.

3. Is a positive result always visualized as a brown color in the ELISA assay?

Post Lab Questions for Introduction to ELISA (from Edvotek Instructor's Manual)

1. What is the effect of not including the antigen or the primary antibody in the reaction?

2. Why is the washing of the wells a necessary step in the process?

3. Do you think nucleic acids can be detected by ELISA? Why or why not?

AP Biology Test Three
Immunology

Part I. Multiple Choice (15) (approximately 12 minutes)

1. The innate immunity that protects a person digging in a garden from developing a microbial infection includes all of the following except
    a. Lymphocytes
    b. Skin
    c. acidic secretions
    d. mucous membranes

2. Which of the following is not a part of an antibody molecule?
    a. The epitope
    b. The constant region
    c. the light chain
    d. the heavy chain

3. The clonal selection theory is an explanation for how
    a. A single type of stem cell can produce both red and white blood cells
    b. Constant and variable regions can be rearranged to match a given antigen
    c. An antigen can provoke development of specific cells, resulting in increased antibody production
    d. Macrophages can present antigen to specific T and B cells

4. Which of the following is not involved in both antibody and cell-mediated immunity?
    a. Plasma cells
    b. Helper T cells
    c. macrophages
    d. memory cells

5. The lymphatic system includes
    a. Spleen and lymph nodes
    b. Adenoids and tonsils
    c. appendix and portions of the small intestine
    d. all of the above

6. If a person's bone marrow were destroyed by radiation, which of the following cells could not be produced?
    a. B cells
    b. T cells
    c. macrophages
    d. all of the above

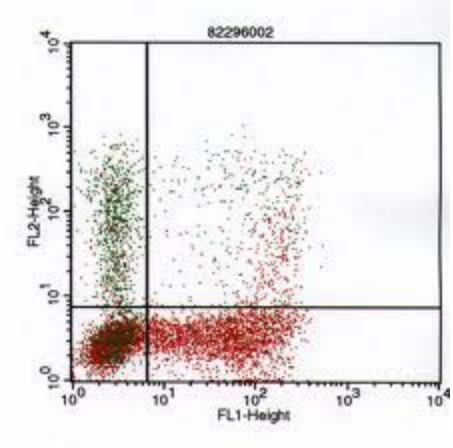
7. Both lysozyme (secreted in the mucous) and cytotoxic T cells
    a. Kill cells through a variety of chemical interactions
    b. Kill cells by only inducing apoptosis
    c. Are part of innate immunity
    d. Are involved in cell-mediated responses

8. Which of the following would be the correct order/sequence for an ELISA test designed to measure the levels of a given protein (i.e. IL12) in various samples?
    i. IL12 antibody pre-coated/attached
    ii. Substrate added
    iii. Addition of sample
    iv. Enzyme-linked antibody added
        a. i, iii, iv, ii
        b. i, ii, iv, iii
        c. i, iii, ii, iv
        d. i, iv, iii, ii

9. M1 macrophages are characterized as expressing high levels of IL12 and rejecting tumors. M2 macrophages are characterized as expressing high levels of IL10 and promoting tumor growth. If macrophages are implicated in the ability of an organism to accept or reject ocular tumors, then quantitative PCR analysis of harvested macrophages from control and splenectomized (splnx) mice should yield
    a. Low levels of IL10 in both
    b. Low levels of IL10 for the control and high levels of IL12 for the splnx mice
    c. High levels of IL10 for the control and high levels of IL12 for the splnx mice
    d. High levels of IL10 for the control and low levels of IL12 for the splnx mice

10. A cell preparing to undergo replication would be predicted to have
    a. Higher levels of ras-GTP complexes and activated downstream (subsequent) expression of other genes
    b. Higher levels of ras-GDP complexes and activated downstream (subsequent) expression of other genes
    c. Higher levels of ras-GTP complexes and inhibited downstream (subsequent) expression of other genes
    d. Higher levels of ras-GDP complexes and inhibited downstream (subsequent) expression of other genes

11. A single cell suspension is treated with two different fluorochromes that recognize two different membrane proteins. Macrophages and other monocytes do not contain either of those proteins. Following flow cytometry, signal from which of the following quadrants indicates the presence of monocytes?
    a. Lower left
    b. Lower right
    c. Upper left
    d. Upper right



Sample Flow Cytometry Result

12. Which of the following is not part of antigen presentation?
    a. T-cell antigen receptors
    b. Pathogen presenting MHCs
    c. Displayed antigen fragments
    d. Pathogen specific antibodies

13. Which of the following is not a shared characteristic of humoral and cell-mediated acquired immunity?
    a. The production of secreted antibodies into the plasma
    b. Antigen-binding receptors on lymphocytes
    c. Possible stimulation by helper T-cells
    d. The production of memory cells

Part II. Short Answer (1) and Free-response (1) (approximately 25 minutes)

14. Several common experimental protocols are used in the field of immunology. For any ONE of the following, discuss both the purpose of AND the steps involved in carrying out the selected protocol.

Quantitative PCR
Flow cytometry
ELISA

15. An important defense against diseases in vertebrate animals is the ability to eliminate, inactivate, and destroy foreign substances and organisms. Explain how the immune system achieves any THREE of the following:

Provides an immediate non-specific immune response
Activates T and B cells in response to an infection
Responds to the second exposure to the same infectious agent
Recognizes self from non-self

Biology Quiz
Immunity

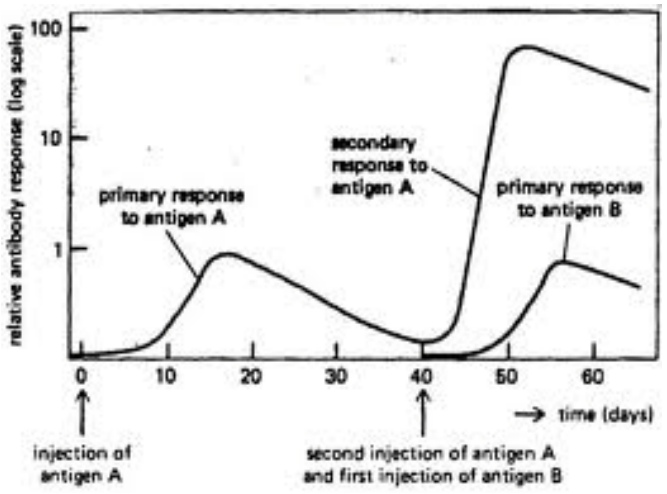
Part I. Multiple Choice

1. Which of the following immune cell types is incorrectly paired with its function?
    a. Macrophages: able to swallow complete pathogens
    b. B-cell lymphocytes: involved in antibody production
    c. Neutrophils: aids in the production of memory cells
    d. T-cell lymphocytes: able to kill infected cells

2. Which of the following statements accurately describes antigen receptors and their role in immunity?
    a. They are located on both types of lymphocytes (B and T)
    b. The same antigen often recognizes multiple pathogens
    c. B and T cell antigen receptors have similar shapes
    d. They are not required for T lymphocytes to begin to initiate a response to an infection

Part II. Short Answer

3. Refer to the following diagram to answer the questions that follow:



    a. Which specific cells of the immune system are most active in fighting against antigen A between 0 and 10 days? Why?
    b. Which specific cells of the immune system are responsible for the observed result between 10 and 20 days? Why?
    c. Explain the reason for the difference between the observed responses recorded following the injection of antigen A on day 0 and day 40.