

Basics of Immunology and the Ethics of Animal Research

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For the American Association of Immunologists

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Teacher Guide

Overview

This module is designed to present students with the following aspects of immunology:

- Infection of organisms by pathogens
- Organism immune response to pathogens
- Quantitative ELISA illustrating antibody production after infection
- Quantitative ELISA illustrating T cell IL-2 production *in vitro* in response to pathogen infected cells
- Use of knockout mice to study immune response *in vivo*
- Ethics of use of animals and stem cells in research

The setup of this module is such that it flows from activity to activity, with later activities building upon understanding gained from previous activities. However, teachers can decide how much of the curriculum they wish to cover and can stop after any activity. Each activity can be used independently, if desired. It is recommended when using the complete module that the placement be towards the end of the teaching year, after students have acquired knowledge on cell specialization, cell responses to stimuli, and the functions of the organ systems.

Through their participation in these activities, students will learn to quantify the production of specific antibodies and cytokines through the use of ELISA, including the production and use of standard curves. Students will then gain knowledge of two features of immunology and other areas of research, namely the use of knockout mice and stem cells. They will use this information to conduct library research and write a supported opinion paper indicating their stance on the use of animals, including humans, in research.

Science Background and Handouts

Stages of Immune Response¹

The human immune response can be divided up into different stages:

- Exposure to pathogen
- Pathogen crosses the epithelium
- Immediate cellular response in the dermis (Innate response)
- Adaptive immunity

In the first stage, the pathogen is present on the skin of the organism. Via cuts, broken skin, etc., the pathogen can cross the epithelium (second stage) into the dermis, where macrophages and dendritic cells respond to destroy the pathogen (third stage). Specific antigen-presenting cells (APC's), known as Langerhans cells, then travel to the lymph nodes and present the antigens to T-cells, thereby starting the process known as adaptive immunity. Activities in this module will be focused on mechanisms of adaptive immunity.

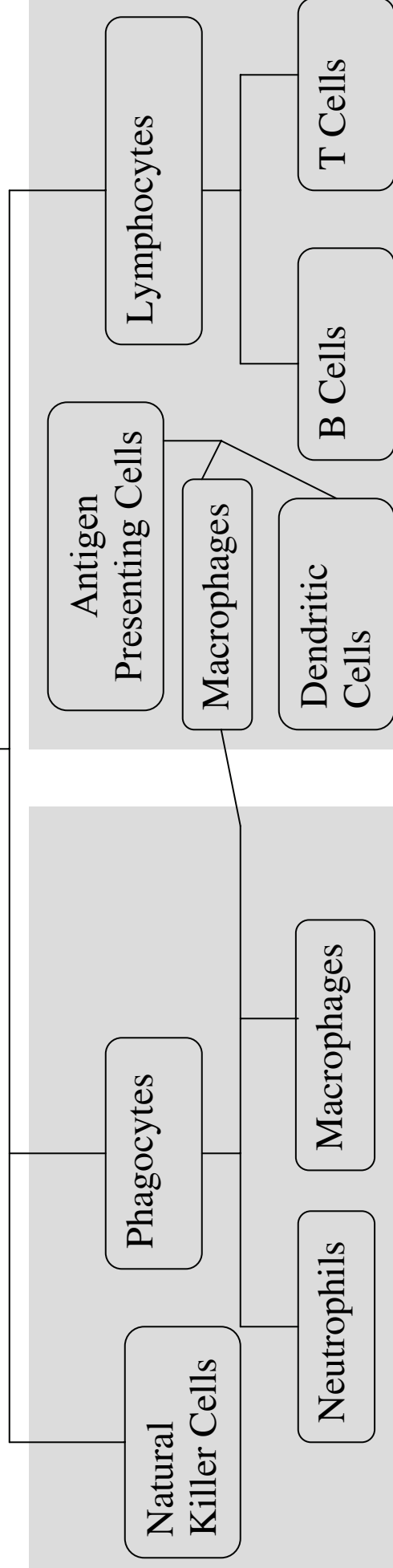
Cells Involved in Immunity

Several cells are involved in the immune response. Some cells are associated with innate immunity, including natural killer cells and different phagocytes, which are cells that engulf pathogens and infected cells. Other cells are associated with adaptive immunity, specifically T cells, B cells and antigen presenting cells. The figure on page 4 illustrates this organization of cells on a very basic level. Antigen presenting cells include dendritic cells, macrophages and other infected tissue cells. Note that leukocytes (white blood cells) make up the majority of the cell types involved in overall immune response.

¹ Janeway, Charles A., et al. Immunobiology. 5th ed. New York: Garland, 2001

Cells Involved in Immunity

Leukocytes
White Blood Cells



Innate Immunity

- Non-specific defense against pathogens
- Activate within hours of infection

Adaptive Immunity

- Highly specific cellular defense against a pathogen
- Full activation takes days

Innate Response

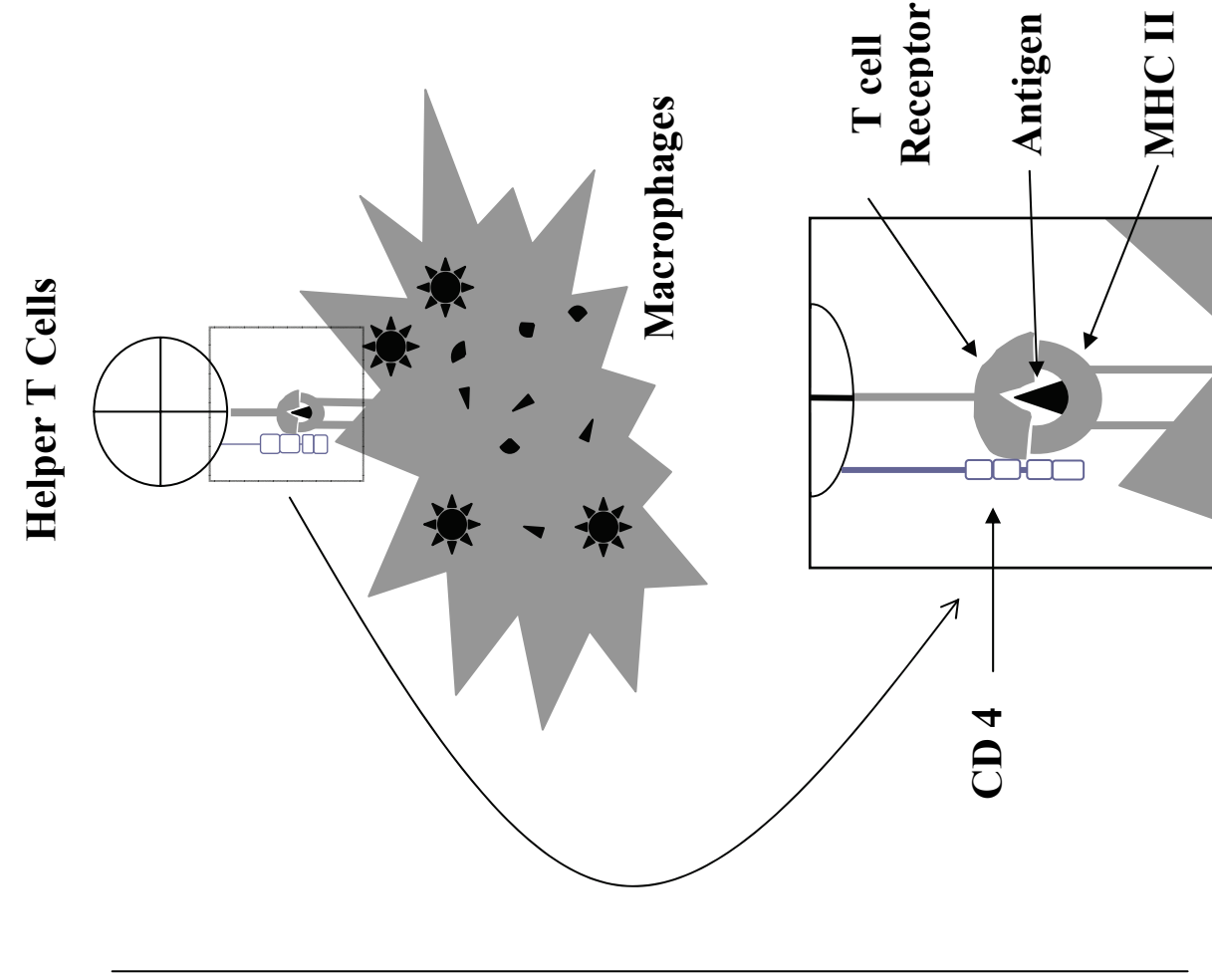
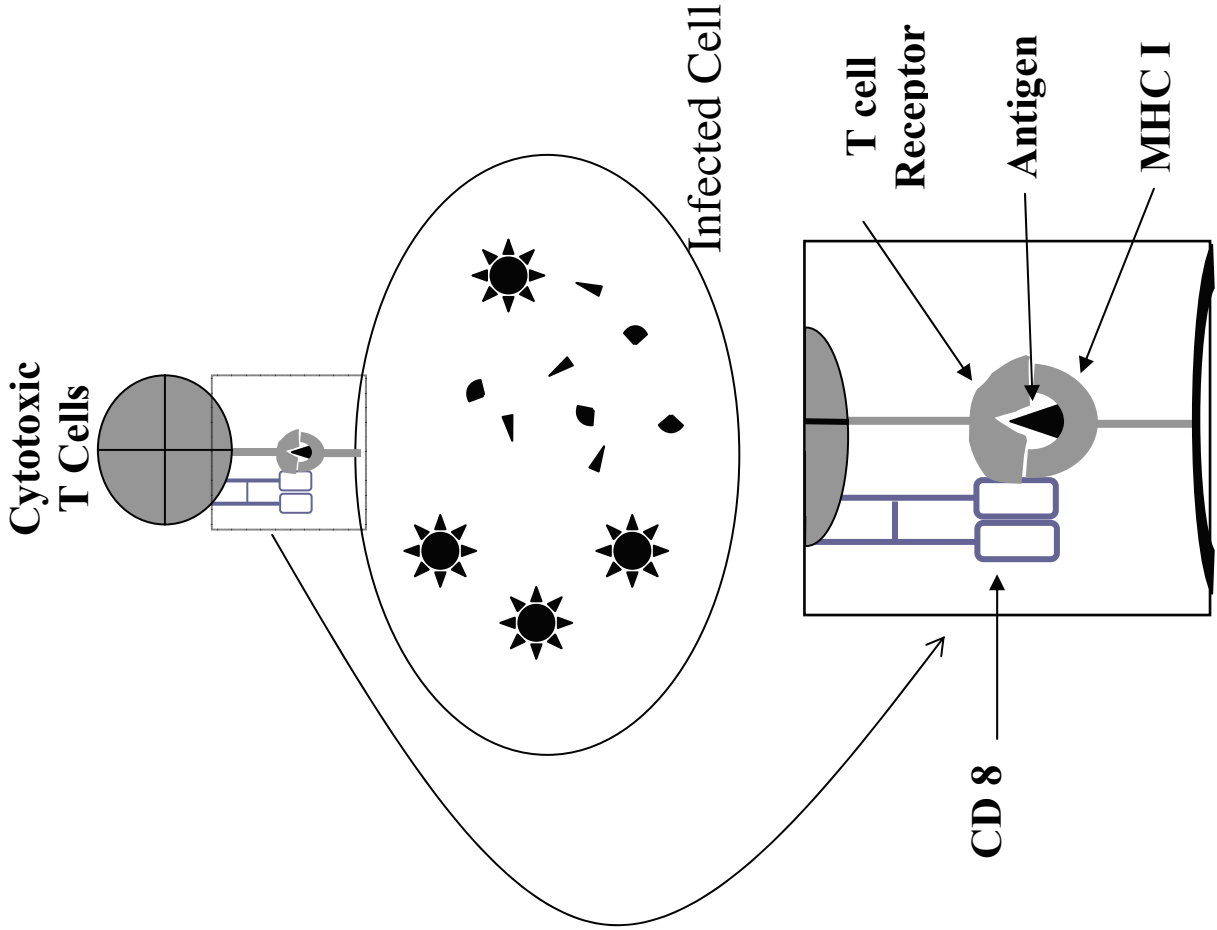
The innate response is the first response in the body's fight against a pathogen. In the innate response, the macrophages and dendritic cells work to identify and destroy any pathogens encountered. The signals prompting the innate response are general, identifying either self or non-self. Quick when compared to adaptive immunity, the innate response to a pathogen is activated within hours of exposure to the pathogen.

Adaptive Immunity

Adaptive immunity is the more aggressive and more specific stage of the immune response. In adaptive immunity, a pathogen is sought out and destroyed through a complex pathway of activated cells specifically designed to combat that particular pathogen.

There are two separate pathways in this response. The first is called the cell-mediated response. In the cell mediated response, body cells that are infected with a pathogen break down the pathogen into pieces that are called antigens. These antigens are then presented to cells in a molecule called the major histocompatibility complex (MHC). There are two types of MHC's. MHC I is present on the surface of body cells, and can form complexes with antigens from pathogens that have been broken down within the cell. Once the antigen-MHC 1 complex is presented on the surface of the infected cell, it can be recognized by cytotoxic T cells. The T cell that binds to the complex has a receptor that is specific for that particular antigen-MHC I complex. The cytotoxic T cell also has a molecule called CD 8 on its surface. When the antigen-MHC I complex, the T cell receptor and CD 8 bind together, this stimulates the cytotoxic T cell. This construct is shown in the figure on page 6. The second type of major histocompatibility complex is called MHC II. It is found on the surface of antigen presenting cells (APC), and presents the antigen to helper T cells. Helper T cells have the CD 4 molecule on their surface, which binds to antigen-MHC II complexes and activates the helper T cell. This interaction is shown on the right side of page 6.

Interactions between Antigen Presenting Cells and T Cells

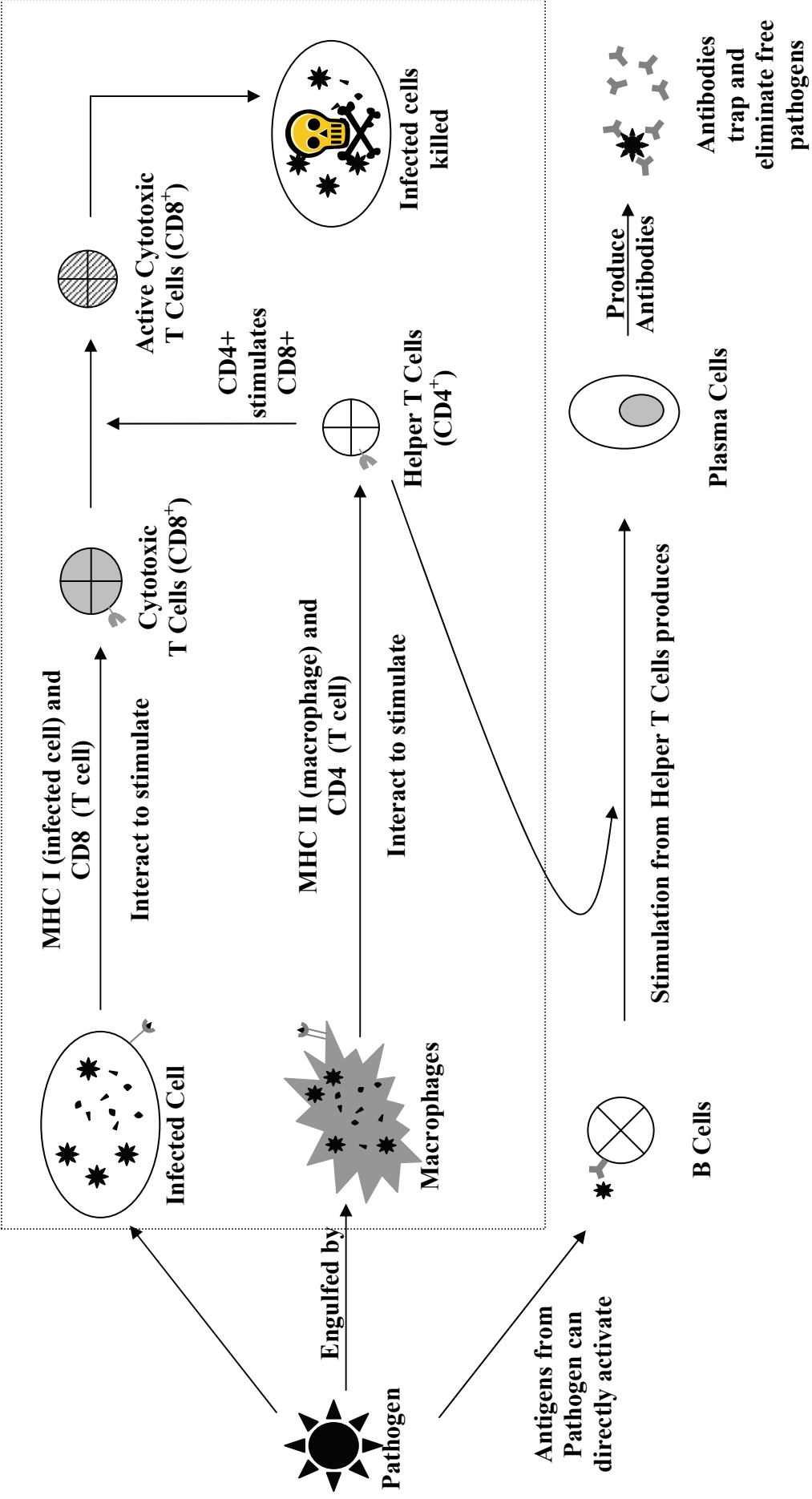


These interactions between T cells and the infected cells or APC's make up only a part of adaptive immunity. The second part of adaptive immunity involves B cells and is called humoral immunity. These B cells are activated when an B cell receptor, which is a membrane-bound form of an antibody, binds to an antigen directly, without the need of an MHC molecule. The binding of the B cell receptor and the antigen activates the B cell, which then becomes a plasma cell. This plasma cell produces antibodies that bind to the pathogen and mark it for attack by other immune cells in the body. Helper T cells that have been activated by the same antigen are a necessary part of B cell activation. The figure on page 8 shows the interactions involved in adaptive immunity, including the role of the helper T cells in both humoral and cell-mediated immunity.

Memory

One impressive result of adaptive immunity is the formation of memory cells. Memory cells are B cells, helper T cells and cytotoxic T cells, specific to a particular antigen, that remain in the immune system even after the pathogen has been cleared in the first response. These memory cells lay in wait for a second exposure to the same pathogen. This time, when the pathogen enters the body, the memory cells jump to quick action, producing antibodies and killing infected cells. It is strong and fast, and often, the pathogen is cleared without the organism even being aware of the infection.

How Adaptive Immune Response Eliminates Pathogens



Items in the dashed box above are components of the cell-mediated response.
 Items outside the box are part of the humoral response

Interleukin 2 and the Case for Knockout Mice

In order to test the function of different gene products *in vivo*, we study organisms that have a defective or missing copy of that gene. This allows scientists to compare experiments in organisms with and without the gene being studied. Organisms with the defective or missing genes are called knockouts. Knockout mice are used extensively in immunology research.

Many knockout mice strains are available for studies. In some cases, the use of knockout mice for *in vivo* studies has supported previous *in vitro* experiments. However, sometimes the opposite is true, as in the case of interleukin 2 (IL-2) and T cells.

IL-2 is a T-cell growth factor (TCGF). It causes marked proliferation of T cells *in vitro* and is still used for that purpose. As a result, many therapies were developed to use IL-2 in an effort to increase T cell numbers *in vivo*, such as in cancer and AIDS treatments. Later studies with knockout mice show that *the in vivo* role of IL-2 is not to enhance proliferation, but rather to regulate the production of regulatory T cells that suppress self-immunity.² Hence, *in vitro* effects do not necessarily correlate to *in vivo* situations. The use of knockout mice has been instrumental in our understanding of *in vivo* processes, including those of the immune system.

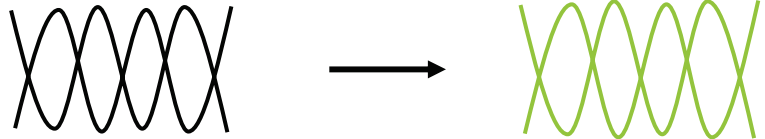
The production of different strains of knockout mice is quite elegant. First, the gene for the specific gene product desired is cloned and altered so that the targeted gene is non-functional. This gene is then inserted into embryonic stem cells (ES cells). These modified ES cells are inserted into blastocysts, where they are capable of developing into any of the body's many cell types, including germ cells for reproduction. The blastocysts are implanted into a pseudopregnant female mouse. Some of the offspring will be chimeras, where the modified ES cells in the blastocysts developed along with non-modified cells that were already in the original blastocyst. This chimeric mouse has

² Nelson, Brad H. "IL-2, Regulatory T Cells and Tolerance." Journal of Immunology 172 (2004): 3983-3988.

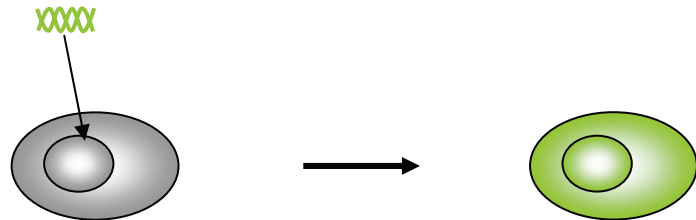
some tissues (including germ cells) with the modified gene, and some tissues without the gene. Chimeric mice are then bred to produce mice whose tissues all have the modified gene. These knockout mice can then be used for *in vivo* studies to determine the role of the non-functional gene. A visual representation of the process is offered on page 11.

How to Create Knockout Mice

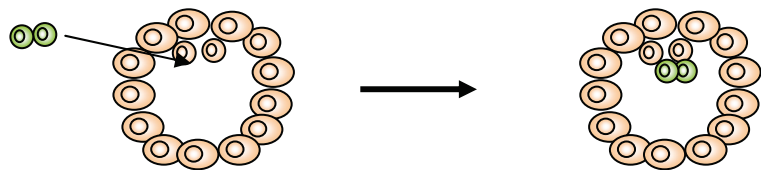
- Clone and alter the target gene, making it non-functional



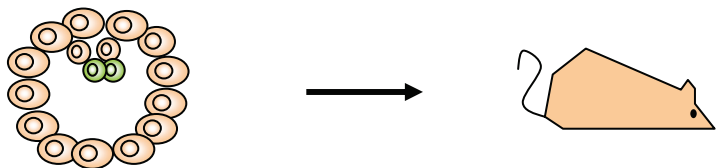
- Insert non-functional gene into embryonic stem (ES) cells



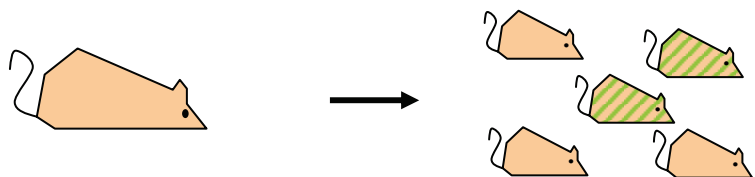
- Insert ES cells into a mouse blastocyst



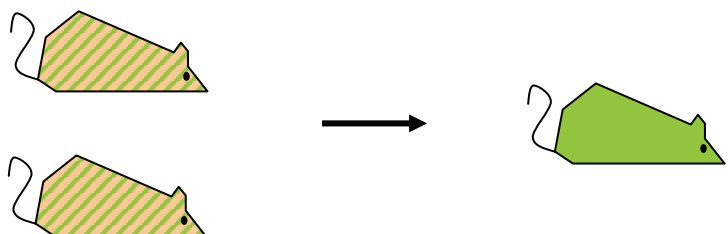
- Insert Blastocyst into pseudopregnant mouse



- Select offspring with some tissues containing the knockout gene



- Inbreed mice to produce knockout mice with the defective gene in all tissues



Stem Cells³

Embryonic Stem Cells

Embryonic stem cells are used to create knockout mice. They are also the source of much controversy as the use of embryonic stem in research for humans has hit the media forefront. In order to form a more educated opinion about the use of embryonic stem cells in current and future research, the general public needs a better understanding of what they are and their potential uses.

Most embryonic stem cells are taken from embryos that are formed as a result of in vitro fertilization. The stem cells are located in a mass of cells inside the blastocyst. These cells can be removed from the young embryo and cultured in vitro to produce cell lines that can reproduce large numbers of stem cells for use in research and therapies.

The most important characteristic of embryonic stem cells is that they can develop into any cell type of the organism from which they are derived. This also presents a great challenge to scientists, who must not only determine how to get the stem cells to change into the type of cell desired, but also how to keep them from changing into non-desired cell types.

Adult Stem Cells

Adult stem cells are cells that are found throughout the body of an organism that have the ability to change into a variety of cell types when the need arises. These cells can be sparked to specialize in response to injury or loss of particular cell types. Adult stem cells have been found in many major organs of the body, including the skin, brain and heart. The use of adult stem cells causes less media controversy, but there are limits to their usefulness.

Adult stem cells have similar characteristics to embryonic stem cells, but have distinct limitations that make them less desirable for research. First, adult stem cells are found in small numbers throughout the organism. Couple this with the fact that they are difficult to culture, and their use as a therapy is limited, since many cells are needed for these treatments. Second, unlike embryonic stem cells, adult stem cells are limited in the types

³ The information on stem cells presented here has been taken from the following source
Weiss, R. (2005, July) The Power to Divide. National Geographic, pp. 2-27.

of cells into which they can develop. A prime example is stem cells isolated from umbilical cord blood. These stem cells are only capable of developing into the various blood cells types (including the lymphocytes of the immune system), but not into any other cell type. Therefore, their use in therapies is limited.

Ethics in Animal Research

The use of animals in experimental research is the source of much controversy. Students themselves are often very clear about which side of the argument they support. However, what is generally lacking is the background information students need to effectively defend their decisions about the topics. For example, students in my classes are very opinionated about the subject of dissection in biology classes, but often cannot articulate exactly why they are for or against it other than to say, “They’re just animals!” or “It’s cruel!” No matter which side of the argument the students choose, the goal of this section of the module is to challenge them to explore and defend their opinions on the ethics of animal experimentation.

In February, 1997, *Scientific American* published 3 articles that can be used to start the debate on the use of animals in experimental research. Their reference information is found below:

- Mukerjee, Madhusree “Trends in Animal Research” *Scientific American* February, 1997: 86-93
- Botting, Jack H. , and Adrian R. Morrison “Animal Research is Vital to Medicine” *Scientific American* February, 1997: 83-85
- Barnard, Neal D. and Stephen R. Kaufman “Animal Research is Wasteful and Misleading” *Scientific American* February, 1997: 80-82

Student Outcomes

The material in this module has been designed to teach and challenge students in the following areas:

- Process of immunity
- Major cells involved in adaptive immunity and their functions
- Use of ELISA to study immune response
- Understand arguments for and against *in vivo* animal studies.

By completing activities 1-3 of this module, the teacher will guide the students step by step through a simulation of infection and the subsequent immune response. The remaining activities address the methods and procedures behind our knowledge of the process of immunity and allow students to explore their own position regarding the ethics of their use.

Learning Objectives

After completion of each activity in this module, students will meet the objectives outlined below.

Activity 1

Students will be able to (SWBAT):

- determine the infectious agent (pathogen) by identifying the infected and determining commonalities
- explain the various stages of the immune response.

Activities 2 and 3

SWBAT:

- Use serial dilutions to produce different concentrations of antibody
- Use ELISA to produce a standard curve for known concentrations of antibody and cytokine production.
- Determine the concentrations of antibody samples from “exposed patients” through comparison with a standard curve

- Diagnose “patient” exposure based on knowledge of immune response (e.g. no exposure, first exposure, second exposure)

Activity 4

SWBAT

- Explain the process used to create knockout mice
- compare and contrast embryonic stem cells and adult stem cells.

Activity 5

SWBAT:

- Explain the role of the 3R’s of animal research
- Formulate an educated opinion regarding the use of animal models in experimental research
- Support his/her opinion using scientific literature
- Investigate and evaluate opinions and literature that is contrary to their own opinions.

Student Activities

Activity 1 – Model Exposure to Pathogen

Rationale

This activity is an exercise in understanding the ease at which pathogens can be transferred to the skin. This activity is taken, with elaboration, from suggestions made on the Glo Germ and Educational Innovations websites.

The goal of this activity is to illustrate the first event in the body's immune response: exposure to a pathogen. Students are exposed to a "pathogen" through contact with a plush toy. Using reasoning skills, students determine the source of the pathogen.

After completion of the activity, the teacher should teach the stages of immune response, including innate and adaptive immunity. In addition to the handouts on this subject found in the Teacher Guide, the following web sites have information and/or animations that may help the teacher with explanation of these concepts:

- **Doc Kaiser's Microbiology Homepage: Lecture Unit III: The adaptive immune system.** This site has detailed information of the response of the adaptive immune system to a viral pathogen. It includes animations.
<http://www.cat.cc.md.us/courses/bio141/lecguide/unit3/index.html>
- **Cells Alive!** This site has a variety of images and animations of the different cells of the immune system that you can view directly from the site. It explains the immune response to splinters, allergens and HIV. An online quiz is included. You can also purchase downloadable clips as part of their "Immune System Collection".
<http://www.cellsalive.com/index.htm>

Time Requirements:

This activity requires 15-30 minutes of preparation time for the teacher. Class time needed for activity and associated lecture: 2-3 single periods, 2 blocks.

Materials:

- Glo-Germ Kit (available from www.glogerm.com or Educational Innovations – www.teachersource.com)
- 3 or 4 GIANT microbes (Educational Innovations, www.teachersource.com), or other small plush toys
- Corn Starch
- Zipper-close bags large enough to hold the toys

Procedure

Prior to class hour:

1. Dust one microbe toy lightly with Glo-Germ powder. This toy will be the infected item.
2. Dust other microbes with cornstarch.
3. Place all microbes into their own plastic bags (labeled “A”, “B”, etc.), making a note of which microbe is infected.

During class period:

1. Divide class into as many teams as you have microbes.
2. Give each team a bag containing a microbe toy. Indicate to students that they are to remove the toys from the bags, and look them over. Ask students to remember which toys they touch.
3. Have the students toss the microbes to members of another team. Give students a moment to look over the microbes.
4. Repeat step 3 until all teams have had the opportunity to observe 3 microbes.
5. Have students return microbes to their bags and collect them.
6. Explain to students that they have been exposed to a “pathogen” that can enter their bodies easily through broken skin.
7. Test each student’s hands for exposure using UV light. (contained in kit)
8. Have students fill in the chart from p. 19, indicating each student’s name, the degree of exposure, and the list of microbes with which the student came in direct contact (touched).
9. Have students use information in their data tables to conclude which microbe was the source of their exposure.

Possible Post Activity Questions:

1. Which toy was contaminated with the “pathogen”?
2. How were you able to trace the infection back to that toy?
3. What percentage of students in the class was infected?
4. What could you have done to reduce your chances of being infected?

Activity 2 – ELISA to Demonstrate in vivo antibody response to pathogen

Rationale

This activity is designed to show students how to quantify the degree of immune response to a pathogen. Persons who have been exposed to a pathogen for the first time (primary exposure) will have a lower titer (concentration) of antibodies in their system. Persons who are experiencing a secondary exposure (additional exposures after the primary exposure) will have a higher antibody titer in response to the pathogen.

Students will use a commercially available ELISA kit to determine the amount of antibody produced in response to the pathogen. With the data they get from this lab, students will create a standard curve using known antibody titers, and use the curve to determine the type of immune response from 2 different individuals.

Time Requirements:

Teacher preparation time for this activity is 1.5-2 hours.

This activity can be run in 2-3 periods (2 blocks).

Single periods

As suggested in the kit, have students perform steps up to the coating of the antigen in the wells and rinsing, then have them continue with the assay on day 2 of the activity. Day 3 is then used for graphing and data analysis.

Block classes

Have students perform the ELISA as directed in the kit on day 1. Day 2 can then be used for graphing and data analysis. You can reduce time constraints on day 1 by splitting each group in two: Student 1 works to coat the antigen in the wells. Meanwhile, the remaining students do the antibody dilutions. Once finished, both teams work together to add the antibodies and develop the substrate.

Extending Materials

Each kit comes with enough materials for 6 lab groups to test two different antigens.

You can extend this to 12 lab groups by having 6 groups run the ELISA using antigen 1 with corresponding antibodies, while the other 6 groups use antigen 2. Plates supplied

with the kit can be easily cut with sharp scissors to create plates with 4 rows and 6 columns for extension to 12 lab groups. When aliquoting materials, reduce the volume of aliquots by one half, increasing the number of aliquots to 12 for each reagent.

Materials

- Edvotek Quantitative ELISA Kit (available from Wards Scientific – www.wardsci.com)
- distilled or deionized water
- incubator/oven set for 37°C
- refrigerator for storing perishable reagents.
- 96-well plate handout (at end of Activity 3 – for student/teacher use for clarifying what is in, or should be added to, each well.)
- Types of ELISA handout (after Activity 3)

Procedure

Prior to class hour

1. Teacher should prepare the PBS, antigens, antibodies and other reagents according to the instructions that come with the kit.
2. Make the following dilutions of Ab 1 and Ab 2 using the recipe in the table:

Titer from infected person	Recipe
A. 1:200	Take 220µl (4 drops) of 1:100 antibody; add it to 220µl (4 drops) PBS
B: 1:2000	Take 50 µl (1 drops) of 1:200 antibody; add it to 450µl (9 drops) PBS

3. All perishable materials should be stored in the refrigerator until use.

The 1:2000 antibody titer (B) represents a person who is experiencing a primary infection to the pathogen (the person has been exposed to the pathogen for the first

time). The 1:200 antibody titer (A) represents a person who is experiencing a secondary infection (person has previously been exposed to the pathogen).

During class: Students should perform the investigation as written in the instruction packet that comes with the kit. For 12 groups, students working with antigen 1 follow instructions for rows A-D; those with antigen 2 follow the instructions for rows E-H. Have students highlight the instructions they need to follow prior to the class period. In order test the level of infection of persons A and B, have students mark off 4 wells in the PBS row (Row C or E). Antigen coating continues as is written in the instructions. For antibody testing, instead of using PBS in these wells, Antibody titer A will be used in 2 of the wells. Antibody titer B will be used in the last 2 wells.

Once students have completed the lab and added the stop solution, the wells can be read in a standard plate reader or a spectrophotometer. Students graph data from the standards rows (A, B, G or H) and use it to determine the type of infection (primary or secondary) of persons A and B. In the event you do not have a spectrophotometer with plate reader, sample data is included below:

Sample data from the EDVOTEK Kit

In the event you do not have access to a plate reader, or if student data is less than ideal, the following data can be used. In the case of adjusted absorbance, the adjusted absorbance has the average absorbance in wells in PBS rows (C and E) subtracted out.

Concentration	Arbitrary Units	adjusted abs.
1:100	1000	0.605
1:400	250	0.502
1:1600	62.5	0.453
1:6400	15.625	0.428
1:25600	3.90625	0.425
1:102400	0.976563	0.265

This same data, using the “Arbitrary Units” column, can be used in Activity 3.

There are questions that can be answered in the manual for the ELISA activity from Edvotek.

Additional questions to be answered:

1. What type of immune response is observed for Person A? Person B? How do you know?
2. How specific to a given antigen is the ELISA assay?
3. An ELISA assay of this type is used to test for the HIV virus. Explain how false positives or false negatives might result.

Activity 3 – Elisa to determine in vitro IL-2 Production

Rationale

This activity is designed to show students how to quantify the level of cytokine production in infected individuals. Persons who have a normal immune response will secrete a higher concentration of cytokines than those people with compromised immune systems.

Students will use a commercially available ELISA kit to determine the amount cytokine (IL-2) produced in response to the pathogen exposure. With the data they get from this lab, students will create a standard curve using known cytokine concentrations, and use the curve to determine whether the individuals involved have compromised immune systems.

Time Requirements:

Teacher preparation time for this activity is 1.5-2 hours.

This activity can be run in 2-3 periods (2 blocks).

Single periods

As suggested in the kit, have students perform steps up to the coating of the antigen in the wells and rinsing, then have them continue with the assay on day 2 of the activity. Day 3 is then used for graphing and data analysis.

Block classes

Have students perform the ELISA as directed in the kit on day 1. Day 2 can then be used for graphing and data analysis. You can reduce time constraints on day 1 by splitting each group in two: Student 1 works to coat the antibody in the wells. Meanwhile, the remaining students do the antigen dilutions. Once finished, both teams work together to complete the lab.

Extending Materials

Materials can be extended to 12 groups using the same extension instructions as described in Activity 2.

Materials

- Edvotek Quantitative ELISA Kit (available from Wards Scientific – www.wardsci.com)
- Distilled or deionized water
- Incubator/oven set for 37°C
- Refrigerator for storing perishable reagents.
- 96-well plate handout (at end of this activity – for student/teacher use for clarifying what is in, or should be added to, each well.)
- Types of ELISA handout

Procedure

Prior to class hour

1. Teacher should prepare the PBS, antigens, antibodies and other reagents according to the instructions that come with the kit. In order to simulate a sandwich ELISA,

2. Make the following dilutions of Ab 1 and Ab 2 using the recipe in the table:

Cytokine Concentration from infected person	Recipe
A. 500 units	Take 220 μ l (4 drops) of 1:100 antibody; add it to 220 μ l (4 drops) PBS
B: 50 units	Take 50 μ l (1 drops) of 1:200 antibody; add it to 450 μ l (9 drops) PBS

3. Make the following changes to the labels of aliquots:

A.	Label Ag 1 aliquots “Ab 1”
B.	Label Ag 2 aliquots “Ab 2”
C.	Label Ab 1 aliquots “Ag 1”
D.	Label Ab 2 Aliquots “Ag 2”
E.	Use correction fluid to make the corresponding changes to the student version of the experimental procedures.

4. All perishable materials should be stored in the refrigerator until use.

Person A represents a person who has normal cytokine production. Person B represents a person who has low cytokine production due to an immune deficiency.

During class: Students should perform the investigation as written in the instruction packet that comes with the kit. For 12 groups, students working with “antibody 1” follow instructions for rows A-D; those with “antibody 2” follow the instructions for rows E-H. Have students highlight the instructions they need to follow prior to the class period. In order test the level of cytokine production of persons A and B, have students mark off 4 wells in the PBS row (Row C or E). Antibody coating continues as is written in the instructions. For cytokine testing, instead of using PBS in these wells, cytokine concentration A will be used in 2 of the wells. Cytokine concentration B will be used in the last 2 wells.

Once students have completed the lab and added the stop solution, the wells can be read in a standard plate reader or a spectrophotometer. Students graph data from the standards rows (A, B, G or H) and use it to determine the immune system condition (normal or compromised) of persons A and B. In the event you do not have a spectrophotometer with plate reader, use the Arbitrary units and adjusted absorption data from Activity 2.

Questions:

1. Which person showed a compromised immune system? Explain how you know this.
2. Compare and contrast the standard ELISA assay and the sandwich ELISA assay.
3. Why do you think the standard ELISA assay is used to determine HIV infection instead of the sandwich ELISA assay?

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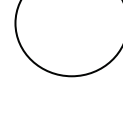
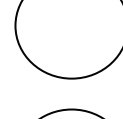
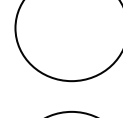
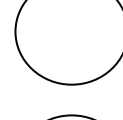
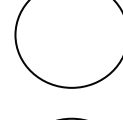
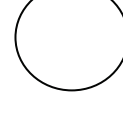
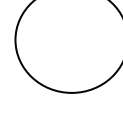
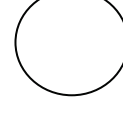
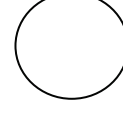
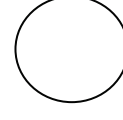
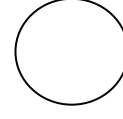
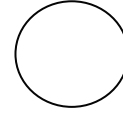
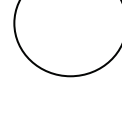
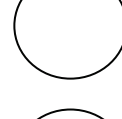
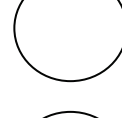
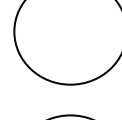
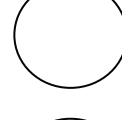
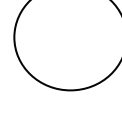
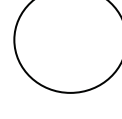
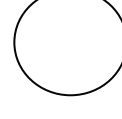
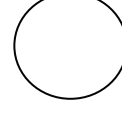
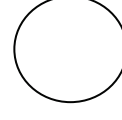
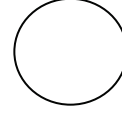
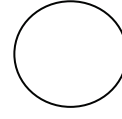
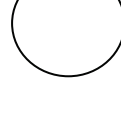
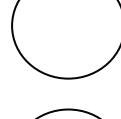
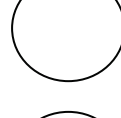
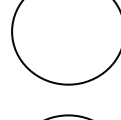
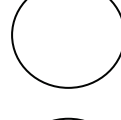
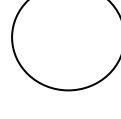
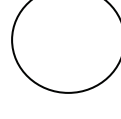
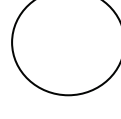
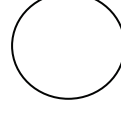
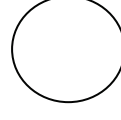
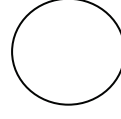
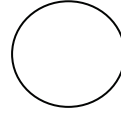
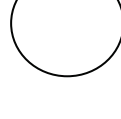
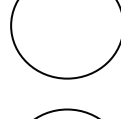
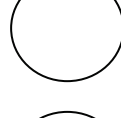
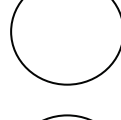
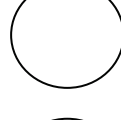
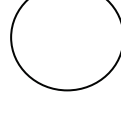
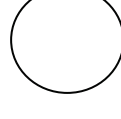
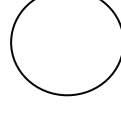
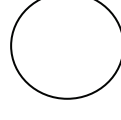
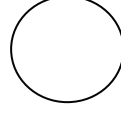
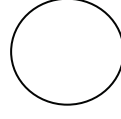
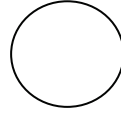
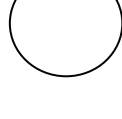
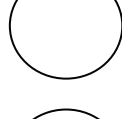
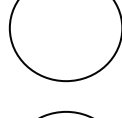
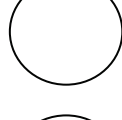
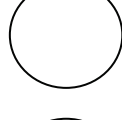
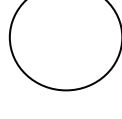
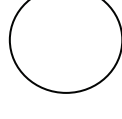
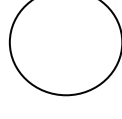
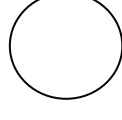
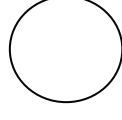
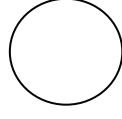
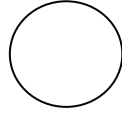
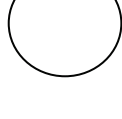
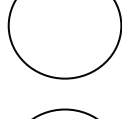
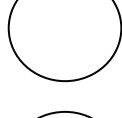
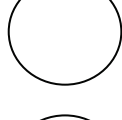
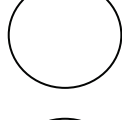
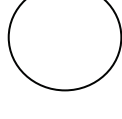
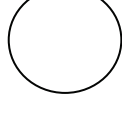
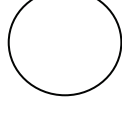
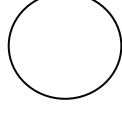
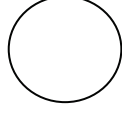
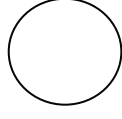
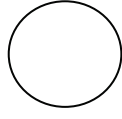
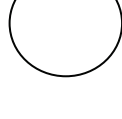
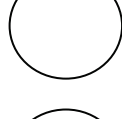
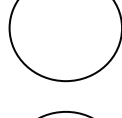
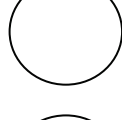
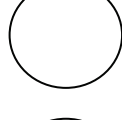
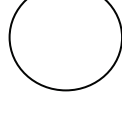
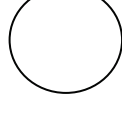
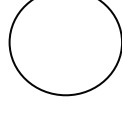
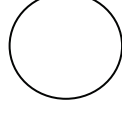
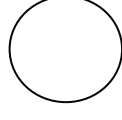
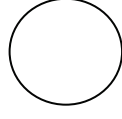
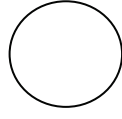
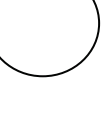
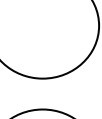
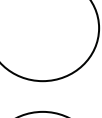
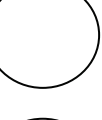
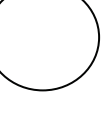
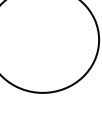
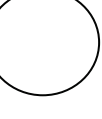
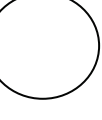
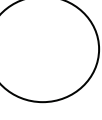
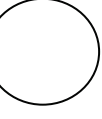
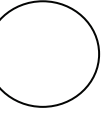
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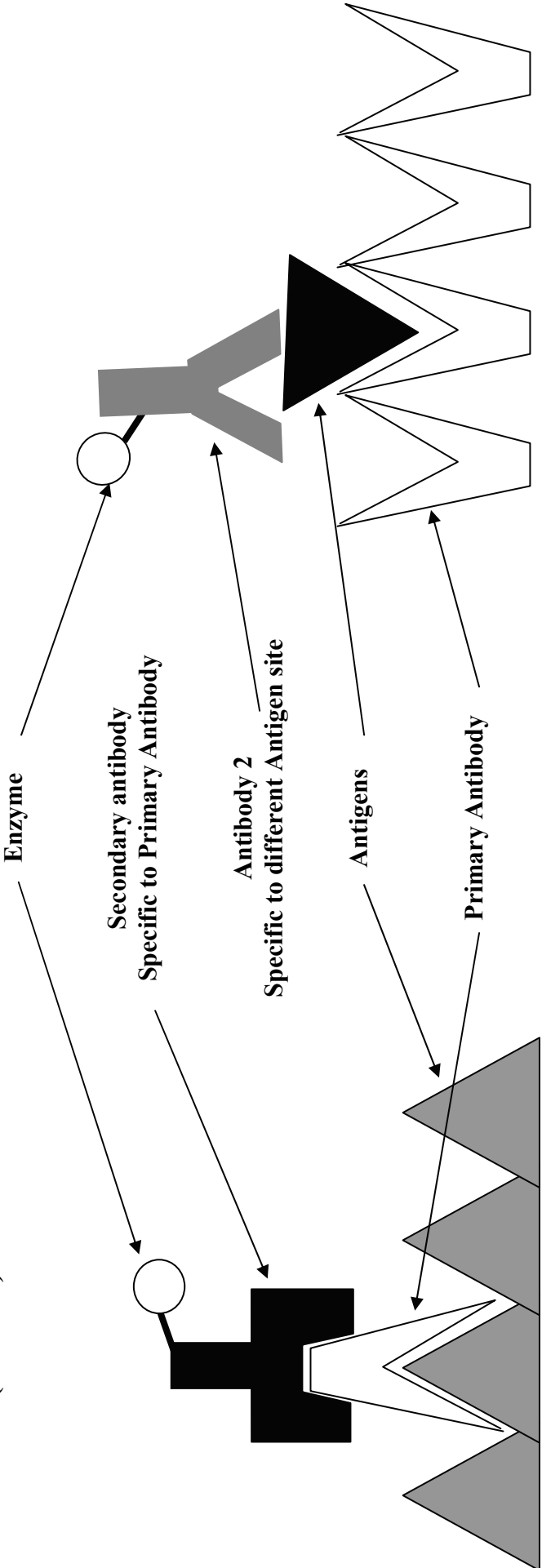
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Types of ELISA Assays

Enzyme-Linked Immunosorbant Assay (ELISA)

Sandwich ELISA – A variation



Activity 4 – Knockout Mice and Stem Cells

Note: The teacher should directly teach this section of the protocol. This is the only section of the module that does not include a hands-on or research activity.

Important Resources

- Janeway, C. A., et al. Immunobiology. 5th ed. New York: Garland Publishing, 2001.
This source has information about knockout mice and how they are produced.
- Weiss, R. (2005, July) The Power to Divide. National Geographic, pp. 2-27. This is a well-written article that explains what stem cells are, as well as their potential uses. Also included is what countries are doing the most research and why. More information included on their web site, at nationalgeographic.com/magazine/0507
- <http://stemcells.nih.gov/index.asp> . This is a web site about stem cells maintained by the National Institutes of Health. It includes a section on ethics of stem cells use, as well as links to other sites that discuss the topic.

Activity 5 – Ethics of Animal Research

Rationale

Many students have an opinion regarding the use of animals in clinical research. However, many students are unable to back up their opinion with research. This project encourages students to explore the reasoning behind their own opinions as well as the opinions of those people on the opposite end of the argument.

Materials:

- Computer and Internet Access
- Copies of Scientific American articles listed in Teacher Resources section.
- Other library resources, such as encyclopedias, journals, etc.

Ethics of Animal Research Paper

Animals are used many types of research, from testing of cosmetics to stem cell research. There are particular guidelines as to how, why and when animals can be used for research purposes. These guidelines are designed to deal with the ethics of animal research.

Ethics is “the discipline dealing with what is good and bad and with moral duty and obligation.”⁴ Basically it is an investigation of what can be done in research and what cannot be done based on a general evaluation of right and wrong. This is why there is a lot of argument around the topic of animal research. Who gets to decide what is right or wrong? What is the basis of their decision? What if I don’t agree with the decision that is made? What if I do?

Answering the question, “Is animal research ethical?” is not easy. Some say yes, some say no. The assignment is for students to decide for themselves. Using journal articles, they are going to develop their opinions of the use of animals for scientific research. As teachers, you may have your own ideas for how to arrange your time and how to encourage quality writing from your students. The following handouts and suggested websites are merely the suggestions of the author.

⁴ “ethic” Merriam-Webster's Collegiate Dictionary. 10th ed. 1998.

Ethics of Animal Research:

A Research Paper:

The following information should be included in your paper on animal research. The supporting evidence both for and against your opinion must be based on research you do using library resources and the journals supplied in class.

- **Introductory statements:** Discuss the current state of animal research. Look for facts regarding numbers of animals, current policies, etc.
- **Opinion statements:** What is your opinion of the ethics of using animals for scientific research?
- **Arguments in support of opinion:** What are your reasons for your opinion? What support do you have for these reasons? (Use library research to find evidence to support your opinion.)
- **Arguments against opinion:** What are the reasons (from your library research) others have for not agreeing with you? Explain what influence these facts have on your own opinions.
- **Conclusion statement:** Restate your position on the use of animals in research and summarize your research evidence.

Possible topics:

- Are the 3 R's enough?
 - Mouse, Dog, Monkey, or Humans: Are some animals off-limits for research?
 - A mouse and me: Are the results "close enough"?
 - Knockout mice vs. Knockout Humans: Is embryonic stem cell research ever ethical?
 - Searching for a cure: Justifications of animal research.
 - When the results don't apply: Arguments against animal research.
-

The Three R's of Animal Research

Reduce

**Carefully plan experiments so that
only the animals needed are used**

Refine

**Make sure experiment protocols
eliminate animal pain and suffering**

Replace

Develop non-animal models and assays

Research Paper Timeline:

Due Date	What is due
	<ul style="list-style-type: none"> • Theme Statement Memo • 2 source cards and 8 note cards (4 for each source card)
	<ul style="list-style-type: none"> • minimum of 6 source cards • minimum of 20 note cards (3-5 cards per source card) • (26 cards total) Note cards should be organized by topic.
	<ul style="list-style-type: none"> • Outline/Graphic Organizer • Bibliography in MLA format • Abstract (hand-written)
	<ul style="list-style-type: none"> • Rough Draft 1, hand-written – minimum of 8 hand-written pages (one-sided, single-spaced). Rough draft must be edited by a peer for spelling, grammar, punctuation and flow.
	Typing Day
	Typing Day
	Final Draft due (typed)

Theme Statement Memo

To: _____

From: _____

Date: _____

Subject: Theme statement

In the space below, write the theme that will be the topic of your paper and explain why you chose this topic. Your response must be given in a full paragraph to receive credit. Anything else will be returned as incomplete.

Theme Statement Memo

To: _____

From: _____

Date: _____

Subject: Theme statement

In the space below, write the theme that will be the topic of your paper and explain why you chose this topic. Your response must be given in a full paragraph to receive credit. Anything else will be returned as incomplete.

Good information on all aspects of how to write a paper:

- Purdue's Online Writing Lab (OWL) – provides handouts in a variety of topics, including topic sentences, punctuation, grammar, etc.
<http://owl.english.purdue.edu/lab/>
- Meriwether, N. W. Successful Research Papers in 12 Easy Steps. Westerville: Glencoe McGraw-Hill, 2002
- Trimmer, Joseph F. A Guide to MLA Documentation 5ed. Boston: Houghton Mifflin Co. 1999
- Concordia University Style Guides – provide pdf files of a variety of different styles, including MLA and APA. <http://library.concordia.ca/help/howto/citations.html>