

From Horses to High School; Bringing Pigeon Fever into the Classroom

Mary Haus

Los Osos High School

6001 Milliken Ave.

Rancho Cucamonga, CA 91737

Mary_Haus@cjuhsd.k12.ca.us

Mentored by Dr. Roberta Pollock, Occidental College,

Department of Biology

Funded through the American Association of Immunologists

Table of Contents

Teacher Section	2
Overview	3
Science Background	3
Learning Objectives	4
Time Requirements	4
Advance Preparation	4
Materials and Equipment	5
Student Prior Knowledge	6
Student Expectations	6
Anticipated Results	6
Lesson Planning/Classroom Discussion	7
Keys	8
Student Section	28
Rationale Page	29
Sample Reading Quiz	31
General Immune System Guided Notes	32
Antibody/Isotyping Guided Notes	39
ELISA Guided Notes	44
Pigeon Fever Guided Notes	47
Attachments	50
Immune System Power Point	
Antibody/Isotyping Power Point	
Who's ELISA Power Point	
How is Pigeon Fever a Horse Disease Power Point	

TEACHER SECTION

Overview

Pigeon Fever is an equine disease caused by *Corynebacterium psuedotuberculosis*. It manifests itself in three forms; external abscesses, internal abscesses, and ulcerative lymphangitis. The hypothesis is that the different manifestations of the disease are due to differences in T_H1 versus T_H2 responses in infected horses. ELISA tests were used to determine the antibody response to phospholipase D (PLD), the antigen produced by the bacteria. Current diagnostic tools state that horses with internal abscesses have high titers to PLD and horses with external abscesses have low titers. Our results show that while most horses with internal abscesses show high antibody responses to PLD, many horses with external abscesses and some uninfected horses also show high titers to PLD. Another possible tool for diagnosing and studying this disease is by typing monoclonal antibodies to determine the class and subclasses of anti-PLD antibodies being produced to test whether T_H1 or T_H2 induced antibodies predominate. These monoclonal antibodies have been made by R. Pollock and her colleagues. Some of the monoclonal antibodies have also been typed. Additional typing of these antibodies will be done by an Advanced Placement Biology class.

Through this curriculum students will understand the basics of how the immune system works, the principles of an ELISA test, the differences between the five isotypes/classes of antibodies, and the research applications of an ELISA protocol in a real world environment. Through the laboratory exercises, students will see how antibodies react to antigens, and how the different types of antibody responses could indicate a particular immune response to a bacterial exposure.

This series of lessons is designed to be taught within the immune system unit of an Advanced Placement Biology Course. Students will be studying how the body responds to antigen exposure and how an ELISA can be used as a diagnostic tool. They will in turn use this information towards a contributing effort to an ongoing research project.

Science Background

There are five isotypes/classes of antibodies produced which include Immunoglobulin G (IgG), Immunoglobulin M (IgM), Immunoglobulin A (IgA), Immunoglobulin E (IgE), and Immunoglobulin D (IgD). Each type differs in its amino acid sequence within its constant region and gives unique structural and functional properties. Additionally, IgG is further divided into subclasses based on differences in their y-chains. These classes and subclasses of immunoglobins have different biological activities and can be used to determine the type of antibody that is produced during a specific immune response. By developing an ELISA test that identifies which isotypes are being produced by infected horses, we can potentially determine the type of antibody response which may be useful for diagnosing and studying different aspects of pigeon fever.

Learning Objectives

- Students will learn how the immune system works by completing a reading assignment with guided instruction pages.
- Students will observe the antibody response to specific antigens by completing an ELISA antibody test.
- Students will learn the importance of both positive and negative controls as well as the importance of multiple trials and reproducibility.
- Students will determine the different Isotypes produced in response to a specific antigen, PLD, (phospholipase D) by using a research developed ELISA antibody test.
- Students will apply their knowledge and findings to help ascertain whether different manifestations of Pigeon Fever are caused by different isotype releases in their immune response.
- Students will learn how an ELISA antibody test is used in disease detection and description.

Time Requirements

For single class periods, about 8 days will be needed to complete these lessons. If you are completing the immune component without the research data, it could be completed in 5 days.

- Day 1 – Pre-reading quiz and introduction to terminology
- Day 2 – Guided instruction covering general immune system information
- Day 3 – ELISA Pre-lab and protocol instruction
- Day 4 – ELISA lab
- Day 5 – Finish ELISA lab and drawing conclusions
- Day 6 – Pigeon Fever introduction and guided instruction
- Day 7 – Research ELISA
- Day 8 - Data collection and drawing conclusions

Advance Preparation

Prior to the first day of actual instruction, copies of all appropriate materials should be made. These include pre-lab assignments and lab protocols for both the ELISA antibody test and the Isotyping ELISA, guided notes pages for all PowerPoint presentations, pre-reading quiz, and any conclusions question sets assigned. To obtain a free copy of the ELISA antibody test protocols and student pages you must register at www.bio-rad.com and order a copy of the literature. Additionally, students should be given appropriate time to complete any outside of class work such as the reading assignment and pre-labs. Suggested timing would be to give all assignment due-dates and handouts at least one week in advance to allow for student completion.

- Day 1 –
 1. Assign specific pages of student text book, or supplemental material covering general immune system information. (ex. Campbell, *Biology* Eighth Edition, chapter 43, 2008)
 2. Write a short student quiz based on the reading assignment given. (See student section or keys for sample.)

- Day 2 –
 1. Load Power Point presentation.
 2. Copy guided notes for student use.
- Day 3 –
 1. Prepare student workstation kits for visual inspection and discussion.
 2. Load Power Point presentations.
 3. Copy guided notes for student use.
- Day 4 and 5 –
 1. Bio Rad advanced preparation instructions (page 62 – 65 of Immunoexplorer kit)
- Day 6 –
 1. Load Power Point presentation.
 2. Copy guided notes for instruction.
- Day 7 and 8 – Protocols still in development

Materials and Equipment

ELISA antibody test (for a class of 40 – 10 groups)

Biotechnology Explorer™ ELISA Immuno Explorer™ Kit (BioRad catalog #166-2400EDU, www.bio-rad.com)

Adjustable or fixed volume pipets – 1 per student group

Sterile distilled water

Ice

Gloves – 1 pair per student

Safety glasses – 1 per student

Lab aprons or coats – 1 per student

Paper towels

Isotyping ELISA (for a class of 40 – 10 groups) ***

Antigen, PLD

Sera samples with primary antibodies

Secondary antibody

Substrate

PBS

Tween

ELISA Plates (2 per class)

Micropipets and tips or fixed volume pipets

Saran wrap

Refrigerator for overnight storage

5 ml or 10 ml pipets

Marking pen

Paper towels

***this is a projected materials list as research protocol is still in development

Student Prior Knowledge

Before starting these lessons, students should have an understanding of:

- the structure and function of the cell membrane
- cell recognition strategies
- what is the function of a leukocyte
- what is the function of enzymes
- what is phagocytosis

Before starting these lessons, students should be able to:

- use pipets and micropipets
- accurately measure small amounts
- make detailed observations
- record observations and data precisely

Student Expectations

- Proficiently complete a pre-lesson reading quiz.
- Complete detailed notes on function of immune system and lab protocols.
- Complete ELISA antibody test, a data table, and draw conclusions on a lab sheet.
- Discuss Isotyping ELISA and applicable data, and speculate on how data collected fits in with a real world research project in progress.

Anticipated Results

ELISA antibody test

Results: Students should see the following results in their triplicate trials with “+” = positive for antibody, “-“ = negative for antibody:

Well Number	1	2	3	4	5	6	7	8	9	10	11	12
Result	+	+	+	-	-	-	-	-	-	+	+	+

Results in columns 1 through 6 should be the same for all student groups as these are the positive and negative controls, but columns 7 through 12 can vary. The results for 7 through 12 differ based on how the lab is set up for student use. A suggested possibility would be to set up 4 sample groups A,B,C,D and have two of these test positive and the other two test negative. Another possibility would be to allocate enough samples for each of the student groups. Have half of these be positive samples and half be negatives. Then, each individual can be assigned a different “patient identification” or “experimental case.” This second scenario would more closely represent the research ELISA that students may have an opportunity to perform.

Results may vary due to cross contamination of samples or failure to switch tips on micropipets when transferring samples. If samples are not stored correctly or washing is not thorough, results will also be compromised.

Lesson Planning/Classroom discussion:

- Day 1 – Pre-reading quiz and introduction to terminology. (Note: Remember to have reading assigned prior to this lesson.)
 - Have available copies of reading quiz and hand out to students for individual completion. (See sample in student section.) When finished, either collect for personal scoring or pass for peer scoring. Review correct answers as a whole class to open the discussion of the immune system.
 - Review appropriate terminology (all definitions taken from Kuby or Campbell)
 - 1) Immunity – State of being resistant either by virtue of previous exposure (adaptive or acquired immunity) or as an inherent trait (innate immunity).
 - 2) Antigen/Immunogen – Any substance (usually foreign) that binds specifically to an antibody. Sometimes referred to as pathogen.
 - 3) Antibody/Immunoglobulin – a protein secreted by plasma cells that binds to a particular antigen.
 - 4) Phagocytosis – The uptake of particulate materials by cells via engulfment.
 - 5) Inflammatory Response – An innate immune defense triggered by physical injury or infection of tissue involving the release of substances that promote swelling, enhance the infiltration of white blood cells, and aid in tissue repair and destruction of invading pathogens
- Day 2 – Guided instruction covering general immune system information. Students should work on guided notes pages as discussion of immune system progresses.
- Day 3 – ELISA Pre-lab and protocol instruction.
 - Students should work on guided notes pages as discussion of antibodies/isotypes progresses.
 - Using prepared lab group bags/packets, have students identify the different pieces of equipment, i.e. 96 well plate, micropipet, etc.
 - If time permits, dry run the steps of the lab to be completed over the next two days.
- Day 4 – ELISA lab per kit instructions
- Day 5 – Finish ELISA lab and drawing conclusions per kit instructions
- Day 6 – Pigeon Fever guided instruction. Students should work on guided notes pages as discussion of disease and research ELISA progresses.
- Day 7 – protocols still in development
- Day 8 - protocols still in development

Keys:

Immunology Pre-reading Quiz – ch. 43

Answer each question in complete sentences unless otherwise specified:

1) What are the two basic parts of Innate Immunity in vertebrates?

First, there are barriers such as skin and mucus. Next, there are internal parts like macrophages.

2) Where are the pathogens that the humoral immune response act upon?

The pathogens are found in the blood.

3) Where are the pathogens that the cell mediated immune response act upon?

The pathogens are inside of body cells. They were “eaten” or engulfed through phagocytosis.

4) What two things can B cells give rise to?

B cells can give rise to plasma cells and to memory cells.

5) List the three types of T cells that are active in cell mediated immune responses.

The three types of T cells are cytotoxic T cells, helper T cells, and memory T cells.

6) Briefly describe the role of antibodies in immunity:

Antibodies bind to antigens to either inactivate them, or attract other cells (macrophages) to come and destroy them.

7) What are the three disruptions in immune system function?

The three disruptions include exaggerated response also known as allergies, self-directed response also known as autoimmune disease, or diminished response also known as immunodeficiency.

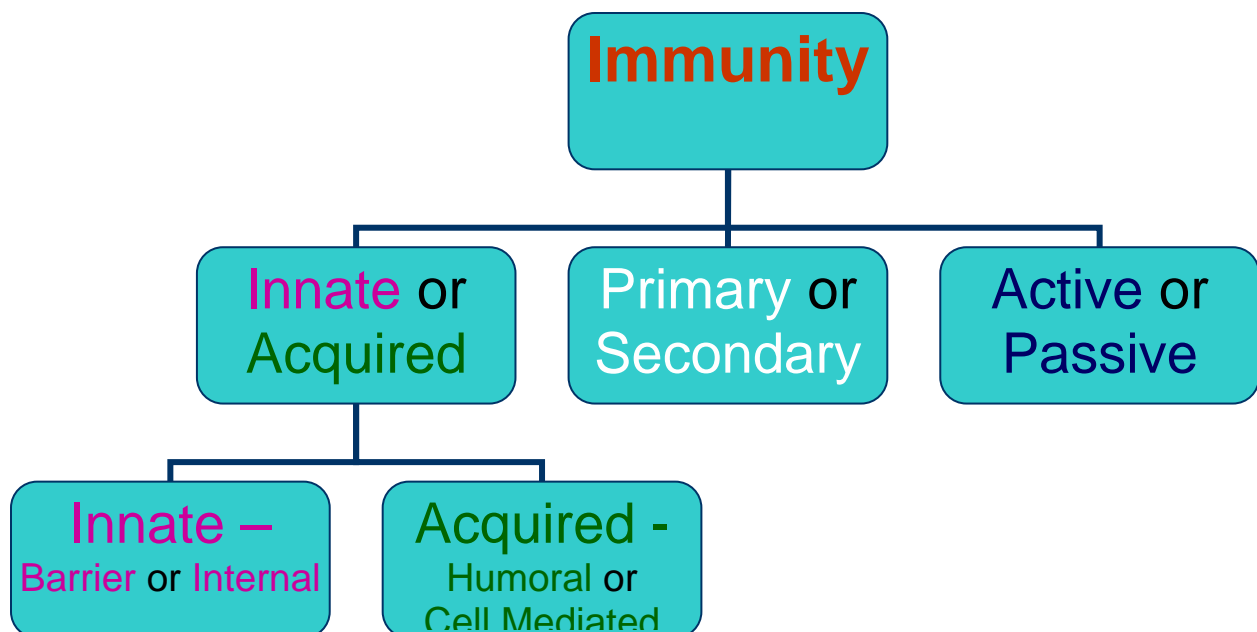
Welcome to the Immune System

I) General Description

- A) The immune system is the “great defender” of the body
- B) Consists of organs, tissues, cells, and molecules that help protect you from foreign invaders such as viruses and bacteria

II) 3 Basic Types of Immunity

- A) Innate or Acquired (adaptive) immunity
 - 1) Innate – barrier or internal defenses
 - 2) Acquired – Humoral or Cell Mediated response
- B) Primary or Secondary response
- C) Active or Passive immunity



III) Innate vs. Acquired

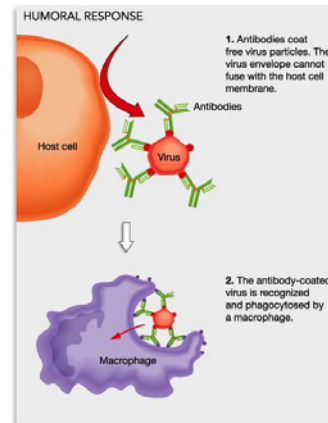
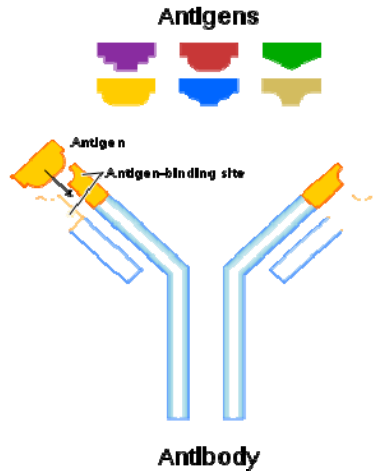
- A) Innate Immunity – a form of defense common to all animals that is active immediately upon exposure to pathogens and that is the same whether or not the pathogen has been encountered before. (Campbell, *Biology* Eighth ed., Pearson Benjamin Cummings, 2008)
- B) In other words, it is non specific. It responds the same way regardless of the invader.
- C) It can be further divided into barrier defenses or internal defenses
 - 1) barrier defenses include skin, mucus, and other secretions like saliva and tears.
 - a) skin – physical block and low pH to prevent growth of microbes
 - b) mucus – traps and can remove invaders
 - c) secretions – can contain conditions that pathogens can't survive in
 - 2) Internal Defenses include Phagocytes, Inflammation, Natural Killer Cells, and Antimicrobial Proteins
 - a) phagocytes are white blood cells that are responsible for ingesting and destroying foreign particles and dying cells. Types include:
 - 1. Neutrophils – eat and destroy

microbes

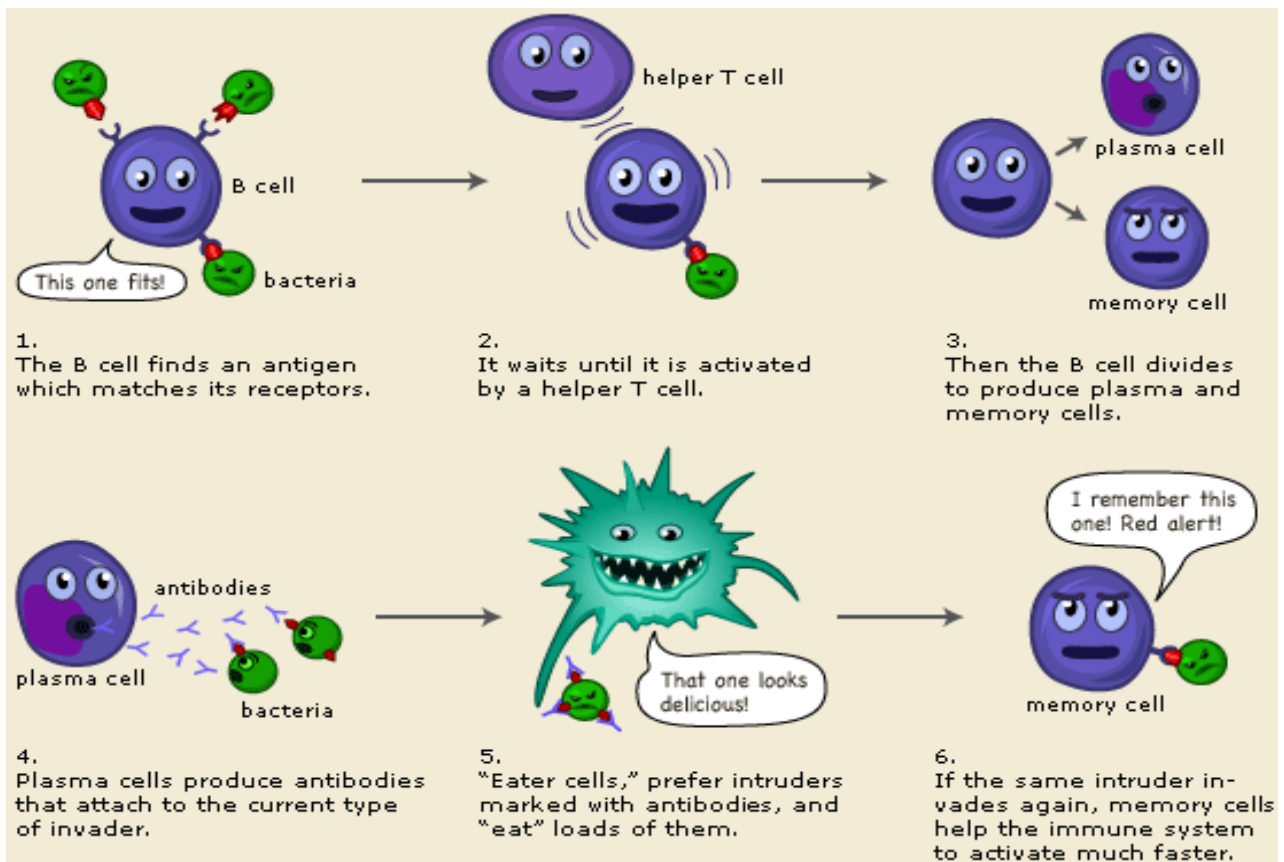
2. Macrophages – eat microbes and release cytokine molecules to help stimulate acquired immunity
 3. Eosinophils – release pathogen destructive and immune trigger molecules, (prostaglandins, growth factors, cytokines)
 4. Dendritic cells – antigen presenting cells which trigger acquired immunity
- b) Inflammation –
1. mast cells release histamine to change blood flow
 2. blood flow to area increases bringing more lymphocytes and proteins
 3. pus accumulates
 4. local or systemic fever
- c) Natural Killer Cells – attack cancerous cells and other cells that do not have a specific surface marker (“self” identifier)
- d) Antimicrobial Proteins
1. lysozymes – enzymes that break down microbe cell walls
 2. interferons – cell signalling

proteins that help stimulate
further immune responses

- D) Acquired Immunity – occurs after exposure to the foreign invader or pathogen
- E) Also called adaptive immunity and is highly specific in pathogen response
- F) Triggered by molecules from innate immune response – like cytokines from macrophages
- G) Can be further divided into humoral response or cell mediated response
 - 1) Humoral response attacks pathogens found in body fluids
 - a) involves primarily B cells
 - 1. type of lymphocytes
 - 2. divide to produce plasma cells and memory cells
 - 3. plasma cells mature and produce antibodies
 - b) specific antibodies recognize and attach to specific antigens on pathogens
 - c) Antibody attachment results in inactivation of pathogen or destruction from macrophages



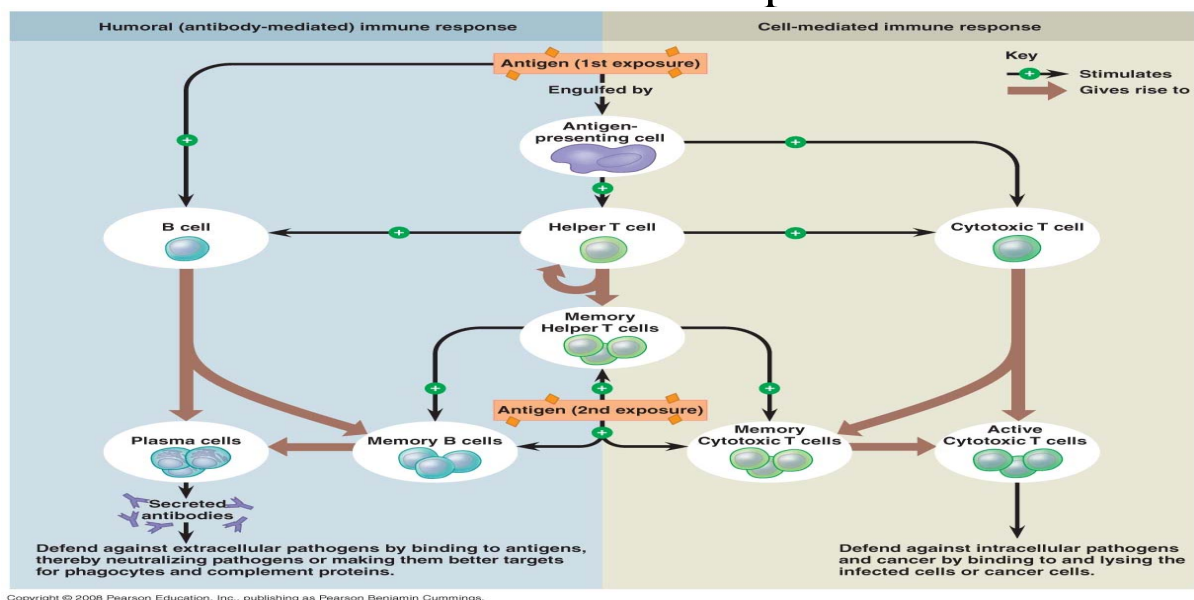
www.answers.com/topic/antibody www.uic.edu/.../bios100/lecturesf04am/lect23.htm



nobelprize.org/.../immunity/immune-detail.html

2) Cell mediated response attacks pathogens that have been ingested into cells through phagocytosis.

- a) involves T cells and stimulates B cells
 - 1. also a type of lymphocyte
 - 2. can attack bound antigens or secreted ones
- b) cytotoxic T cells
 - 1. when activated attach to antigen presenting cells (those that have ingested pathogen) and lyse the cells
 - 2. some become memory cytotoxic T's which stimulate both memory T's and memory B's after second exposure to pathogen
- c) Helper T cells are stimulated by antigen presenting cells and signal further immune response



IV) Primary or Secondary Response

- A) Primary response occurs the first time the system is exposed to a pathogen. B and T cells are activated, differentiated, and respond.
- B) Secondary Response occurs at successive exposures to the same pathogen. Memory cells trigger a much faster response.

V) Active or Passive Immunity

- A) Active immunity occurs within the individual (host) upon exposure to a pathogen.
 - 1) B and T cells become active.
 - 2) Immunity is essentially permanent.
- B) Passive immunity occurs when antibodies or activated T cells are transferred from an immune individual to another individual.
 - 1) Immunity is short term, a few months.
 - 2) Examples include transfer from mother to fetus, or direct injection.

References:

- Campbell, *Biology, 8th edition*, 2008, Pearson Benjamin Cummings
- http://en.wikipedia.org/wiki/Immune_system
- Kuby *Immunology, 6th edition*, 2007, W.H. Freeman

Antibodies and Isotypes

I) What is an antibody

- A) Definition – A protein consisting of two identical heavy chains and two identical light chains, that recognizes a particular antigen and facilitates the clearance of that antigen (Kuby Immunology, 6th edition, 2007, W.H. Freeman)
- B) Antibodies are also referred to as immunoglobulins.

II) Antibody Structure

- A) Antibody basic structure consists of 2 heavy chains plus two light chains (H₂L₂).

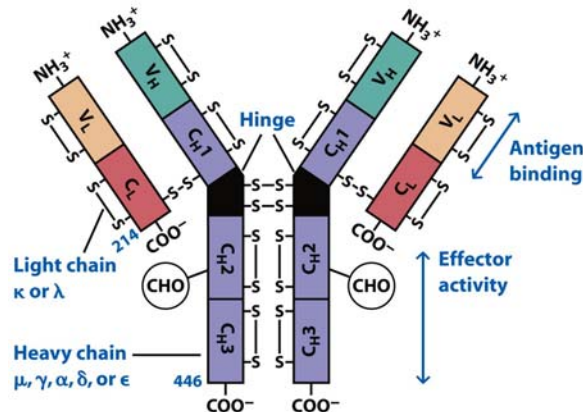
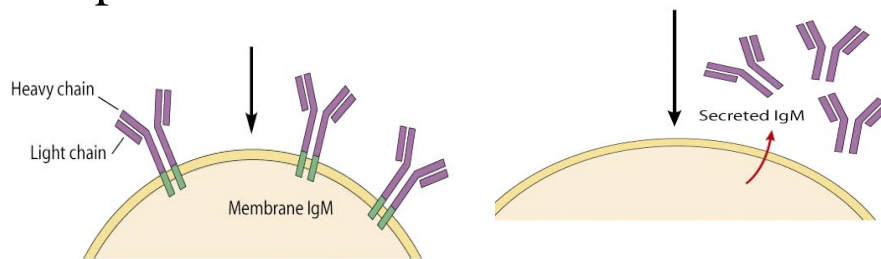


Figure 4-8
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W. H. Freeman and Company

III) General Occurrence of Antibodies

- A) There are two types of antibodies:
 - 1) Membrane bound antibodies that are attached to B cells.

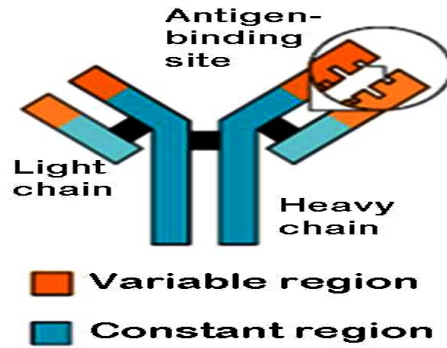
2) Secreted antibodies that are secreted by plasma cells.



Copyright © 2009 Pearson Education, Inc.

IV) Antibody Function

- A) Both types of antibodies identify and attach to foreign substances in the body.
- B) Foreign substance can include bacteria and viruses and are referred to as antigens.
- C) By attaching to the antigen it marks it for other parts of the immune system to act on, or inactivates the antigen directly.
(i.e. binding the part of the antigen that allows it to infect cells)
- D) Attachment of antibodies to antigens is specific - each antibody can only attach to one particular antigen (think lock-and-key model).
- E) This results from a small variable region on the tip of the antibody.
- F) The high variety of antibodies allows for an equal high variety of antigens that can be neutralized.



V) Isotypes - Background

- A) In addition to variable regions, the constant region can have different types of heavy chains.
- B) These determine the isotypes of antibodies that exist.
- C) Definition: An antibody class, which is determined by the constant region sequence of the heavy chain.

(Kuby Immunology, 6th edition, 2007, W.H. Freeman)

VI) Isotypes

- A) There are five isotypes/classes of antibodies produced in mammals each having unique structural and functional properties.
 - Immunoglobulin G (IgG)
 - Immunoglobulin M (IgM)
 - Immunoglobulin A (IgA)
 - Immunoglobulin E (IgE)
 - Immunoglobulin D (IgD)

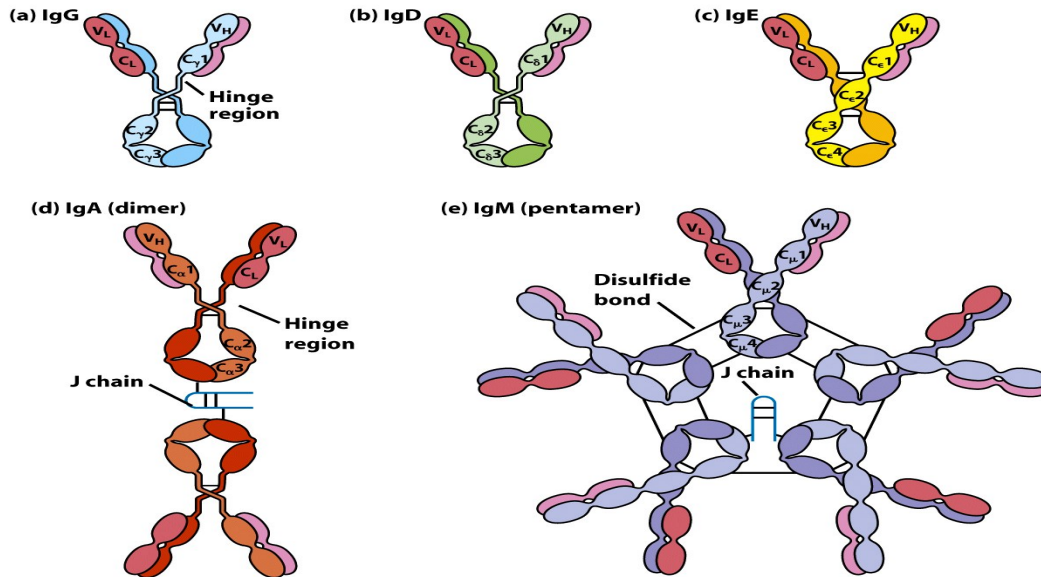


Figure 4-17
Kuby IMMUNOLOGY, Sixth Edition
 © 2007 W. H. Freeman and Company

- B) Certain classes have additional subclasses based on small differences in their heavy chains.
- C) These classes and subclasses of immunoglobulins have different biological activities and can be used to determine the type of antibody that is produced during a specific immune response.

VI) Isotypes – general functions

Names	Types	Description
IgG	4	Provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to fetus.
IgM	1	Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG
IgA	2	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.
IgE	1	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy . Also protects against parasitic worms.
IgD	1	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. Its function is less defined than other isotypes.

<http://schools-wikipedia.org/wp/a/Antibody.htm>

VII) Isotypes – diagnostic use

- A) By developing an ELISA test that identifies which isotypes are being produced during an infection, we can potentially determine the type of antibody response.

B) This may be useful for diagnosing and studying different aspects of an illness such as pigeon fever.

Who's ELISA?

I) Actually, it should be what's an ELISA?

A) Stands for: Enzyme-Linked Immunosorbent Assay

B) Definition: It is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

(<http://en.wikipedia.org/wiki/ELISA>)

C) ELISAs always include controls, positive and negative, many times both.



D) Purpose: ELISA testing can be done for pregnancy testing, disease detection, detecting illegal drug use, and many other uses in both medicinal and agricultural fields

E) Research example: developing an ELISA test that identifies which isotypes are being produced by infected horses. This may be useful for diagnosing and studying different aspects of pigeon fever

II) Isotyping ELISA – Methods (using research example)

1. Coat 96 well plate with phospholipase D, (the major antigen and exotoxin produced by the bacteria)
2. Blocking with detergent
3. Load the samples:
 HY...= hybridomas
 BSA = buffer
 NRS = Normal Rabbit Serum

HYA23	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA23	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYD5	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYD5	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA24	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA24	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA27	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA27	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda

4. Add the detecting antibody for 8 different mouse classes/subclasses (isotypes).
5. Each detecting antibody is conjugated to horse radish peroxidase.
6. Add substrate and measure color change – can be done visually or with a spectrophotometer.

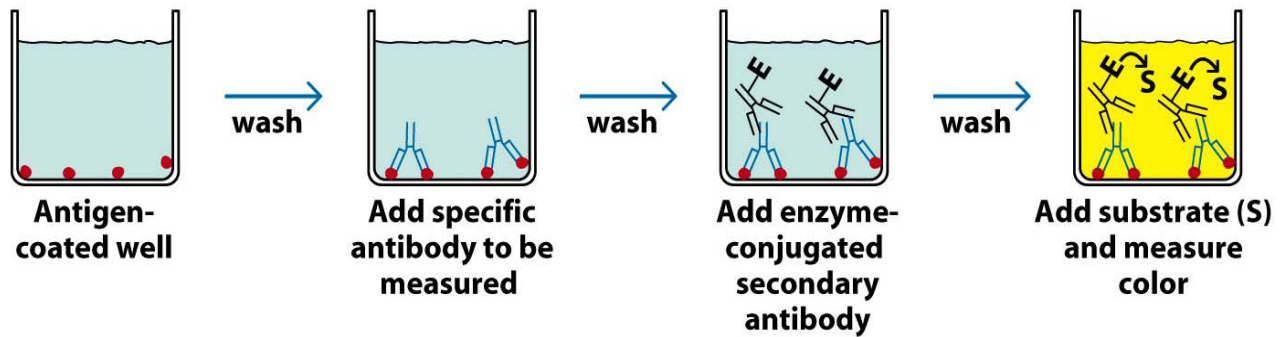


Figure 6-10a
 Kuby IMMUNOLOGY, Sixth Edition
 © 2007 W. H. Freeman and Company

III) Isotyping ELISA – Sample Results

- A) Color change = positive result for the specific antibody
- B) Clear = negative result for the antibody
- D) For the research ELISA, a positive result indicates a particular isotype of antibody was produced in response to the antigen.
- E) This result can be useful in determining the type of immune response for the infected individuals.

How is “pigeon fever” a horse disease?

I) “Pigeon Fever” Introduction

A) Aka “Dryland Distemper”

B) Caused by the intracellular bacterium

Corynebacteria pseudotuberculosis

1) Major antigen and toxin is phospholipase D (PLD),

2) PLD is an enzyme that weakens cellular membranes allowing bacteria to enter body cells and escape from blood vessels.

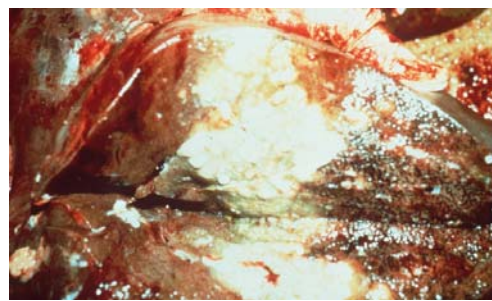
C) Manifests as three forms:

1) External abscesses

2) Internal abscesses

3) Ulcerative lymphangitis

II) “Pigeon Fever” – pictures



1) external abscess 2) internal abscess



3) ulcerative lymphangitis

III) “Pigeon Fever” Name Origin:

- A) The name “pigeon fever” comes from the external abscess manifestation.
- B) A horse’s chest swells up and looks like a pigeon’s chest.

IV) “Pigeon Fever” Prevalence

- A) 90% survival rate for horses with external abscesses
- B) 40+% fatality rate for horses with internal abscesses
- C) Fatality rate for ulcerative lymphangitis is unknown as it occurs so rarely.

V) “Pigeon Fever” Diagnosis

- A) Current diagnosis relies on culturing pus from external abscesses, and serology testing plus ultrasound for internal abscesses.

- B) Treatment options are variable and debatable.
- 1) Some veterinarians suggest antibiotic course.
 - 2) Others feel that antibiotic usage delays resolution of external abscesses.
 - 3) All agree that antibiotics are essential for treating internal abscesses.

VI) “Pigeon Fever” Research Goals

- A) To study and describe the equine immune response to *Corynebacterium pseudotuberculosis* (isotyping)
- B) Determine if the different manifestations of the disease are due to a difference in the T_H1 versus T_H2 response (cytokines).
- C) Long term – the possible development of a diagnostic field test and vaccine for the disease

STUDENT SECTION

Name: _____

Date: _____

Rationale Page – What is important about the immune system?

As humans going about our daily lives, we don't often think about the fact that we, as mammals, are actually in the minority of organisms. Surrounding us are innumerable microbiotic organisms and even non-living things that are potentially lethal to us. If it wasn't for our immune system, we could quickly succumb to these unseen invaders. Over the next few days, we will examine the complex and amazing immune system, gain an understanding of how a specific scientific tool can help us diagnose and describe infections and other medicinal scenarios, and observe how this tool is being used to study a difficult disease in a fellow mammal, the horse.

Introduction:

Pigeon Fever is an equine disease caused by *Corynebacterium pseudotuberculosis*. It manifests itself in three forms; external abscesses, internal abscesses, and ulcerative lymphangitis. The hypothesis is that the different manifestations of the disease are due to differences in T_H1 versus T_H2 responses in infected horses. ELISA tests were used to determine the antibody response to phospholipase D (PLD), the antigen produced by the bacteria. Current diagnostic tools state that horses with internal abscesses have high titers to PLD and horses with external abscesses have low titers. Our results show that while most horses with internal abscesses show high antibody responses to PLD, many horses with external abscesses and some uninfected horses also show high titers to PLD. Another possible tool for diagnosing and studying this disease is by typing monoclonal antibodies to determine the class and subclasses of anti-PLD antibodies being produced.

Background Information

There are five isotypes/classes of antibodies produced which include Immunoglobulin G (IgG), Immunoglobulin M (IgM), Immunoglobulin A (IgA), Immunoglobulin E (IgE), and Immunoglobulin D (IgD). Each type differs in its amino acid sequence within its constant region and gives unique structural and functional properties. Additionally, IgG is further divided into subclasses based on differences in their y-chains. These classes and subclasses of immunoglobulins have different biological activities and can be used to determine the type of antibody that is produced during a specific immune response. By developing an ELISA test that identifies which

isotypes are being produced by infected horses, we can potentially determine the type of antibody response which may be useful for diagnosing and studying different aspects of pigeon fever.

Terminology

- 1) Immunity – State of being resistant either by virtue of previous exposure (adaptive or acquired immunity) or as an inherent trait (innate immunity).
- 2) Antigen/Immunogen – Any substance (usually foreign) that binds specifically to an antibody. Sometimes, it is referred to as pathogen.
- 3) Antibody/Immunoglobulin – a protein secreted by plasma cells that binds to a particular antigen.
- 4) Phagocytosis – The uptake of particulate materials by cells via engulfment.
- 5) Inflammatory Response – An innate immune defense triggered by physical injury or infection of tissue involving the release of substances that promote swelling, enhance the infiltration of white blood cells, and aid in tissue repair.

Name: _____

Date: _____

Immunology Pre-reading Quiz – ch. 43

Answer each question in complete sentences unless otherwise specified:

- 1) What are the two basic parts of Innate Immunity in vertebrates?**

- 2) Where are the pathogens that the humoral immune response act upon?**

- 3) Where are the pathogens that the cell mediated immune response act upon?**

- 4) What two things can B cells give rise to?**

- 5) List the three types of T cells that are active in cell mediated immune responses.**

- 6) Briefly describe the role of antibodies in immunity:**

- 7) What are the three disruptions in immune system function?**

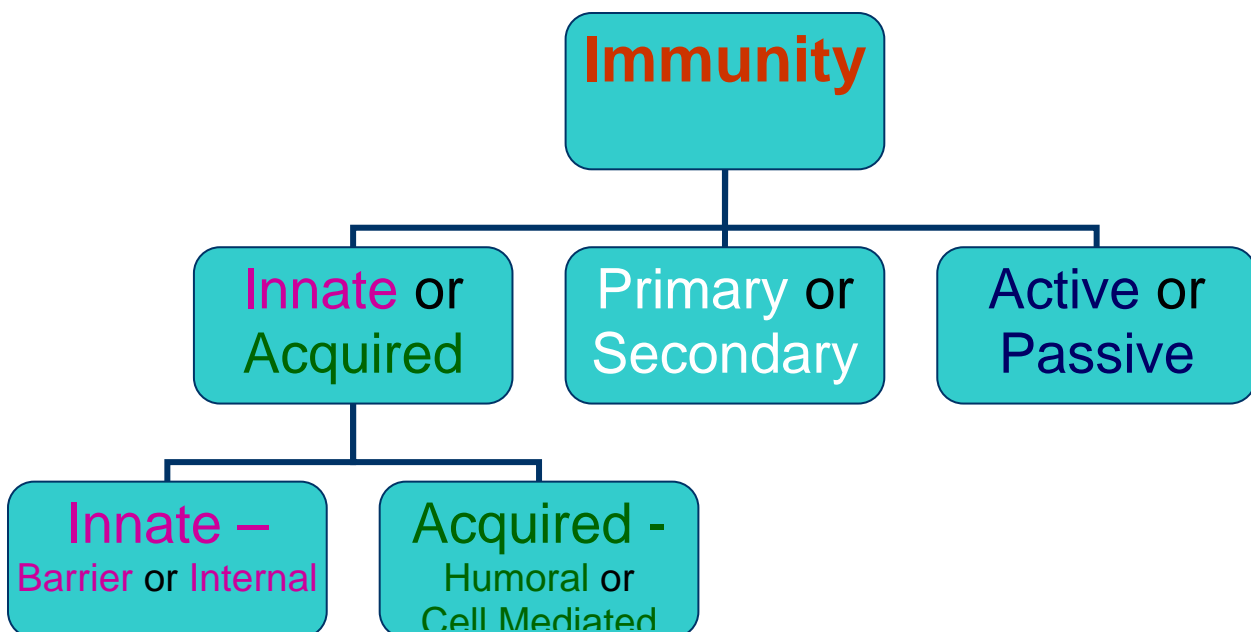
Welcome to the Immune System

I) General Description

- A) The immune system is the “_____” of the body
- C) Consists of _____, _____, _____, and _____ that help protect you from foreign _____ such as viruses and bacteria

II) 3 Basic Types of Immunity

- A) Innate or Acquired (adaptive) immunity
 - 1) _____ – barrier or internal defenses
 - 2) _____ – Humoral or Cell Mediated response
- B) _____ or Secondary response
- C) Active or _____ immunity



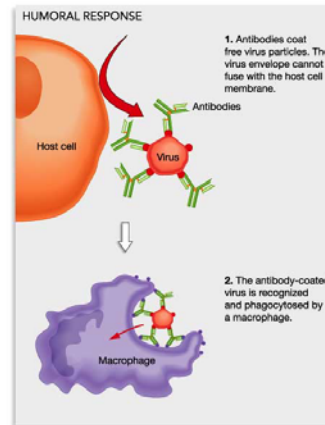
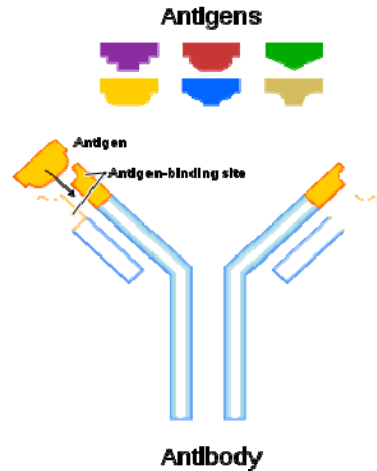
III) Innate vs. Acquired

- A) _____ Immunity – a form of defense common to all animals that is active _____ upon exposure to pathogens and that is the _____ whether or not the pathogen has been encountered before. (Campbell, *Biology* Eighth ed., Pearson Benjamin Cummings, 2008)
- B) In other words, it is _____ _____. It responds the same way regardless of the invader.
- C) It can be further divided into _____ defenses or _____ defenses.
- 1) _____ defenses include skin, mucus, and other secretions like saliva and tears.
 - a) _____ – physical block and low pH to prevent growth of microbes
 - b) _____ – traps and can remove invaders
 - c) _____ – can contain conditions that pathogens can't survive in
 - 2) _____ Defenses include Phagocytes, Inflammation, Natural Killer Cells, and Antimicrobial Proteins
 - a) _____ are white blood cells that are responsible for ingesting and destroying foreign particles and dying cells. Types include:
 1. _____ – eat and destroy microbes

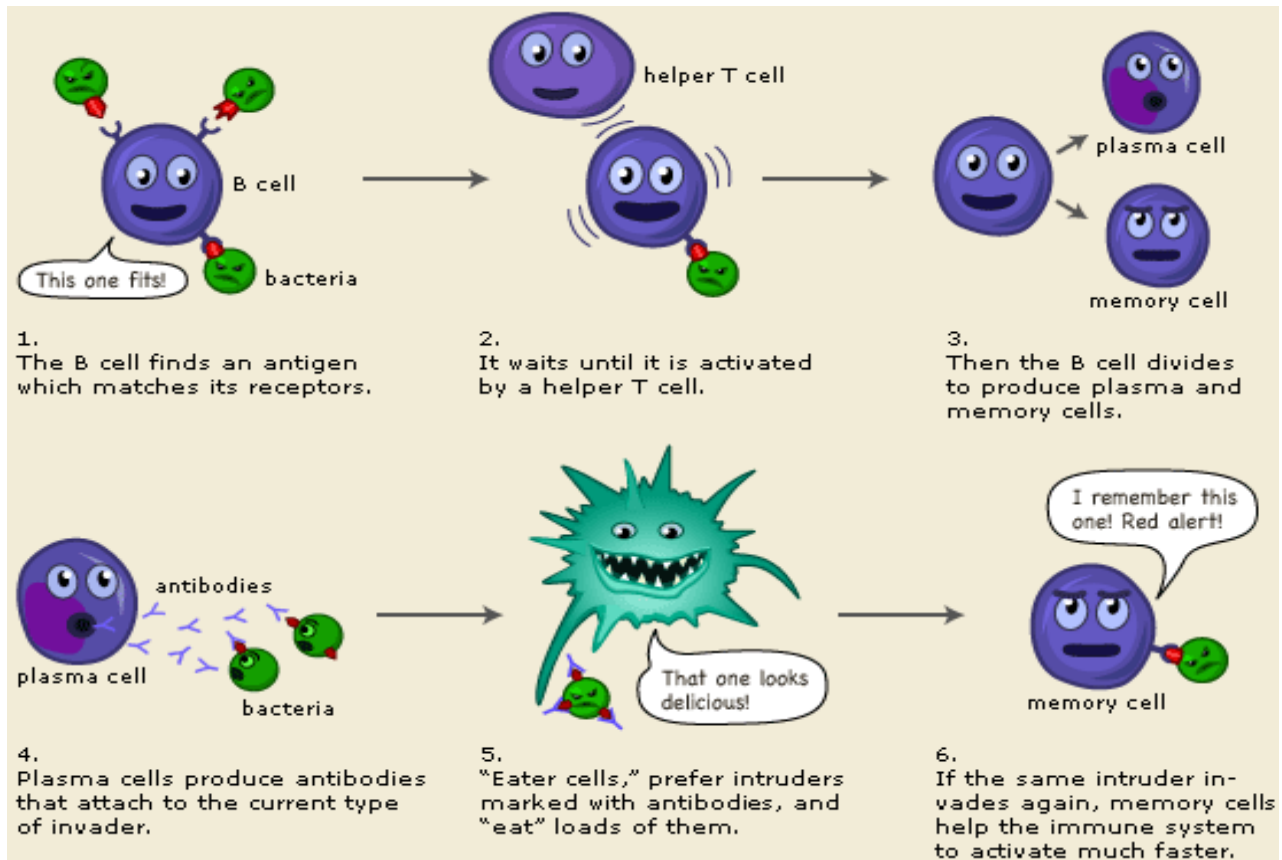
3. _____ – eat microbes and release cytokine molecules to help stimulate acquired immunity
 3. _____ – release pathogen destructive and immune trigger molecules, (prostaglandins, growth factors, cytokines)
 5. _____ _____ – antigen presenting cells which trigger acquired immunity
- b) Inflammation –
1. _____ cells release _____ to change blood flow
 2. blood flow to area _____ bringing more _____ and _____
 3. _____ accumulates
 4. local or systemic _____
- c) Natural Killer Cells – attack _____ cells and other cells that do not have a specific surface marker (_____ identifier)
- d) Antimicrobial Proteins
1. _____ – enzymes that break down microbe cell walls
 3. _____ – cell signalling proteins that help stimulate

further immune responses

- D) _____ Immunity – occurs after exposure to the foreign invader or pathogen
- E) Also called _____ immunity and is highly _____ in pathogen response
- F) Triggered by _____ from innate immune response – like _____ from macrophages
- G) Can be further divided into humoral response or cell mediated response
 - 1) _____ response attacks pathogens found in body fluids
 - a) involves primarily _____
 1. type of _____
 2. divide to produce _____ cells and _____ cells
 3. plasma cells mature and produce _____
 - b) specific antibodies recognize and attach to specific _____ on pathogens
 - d) Antibody attachment results in _____ of pathogen or _____ from macrophages



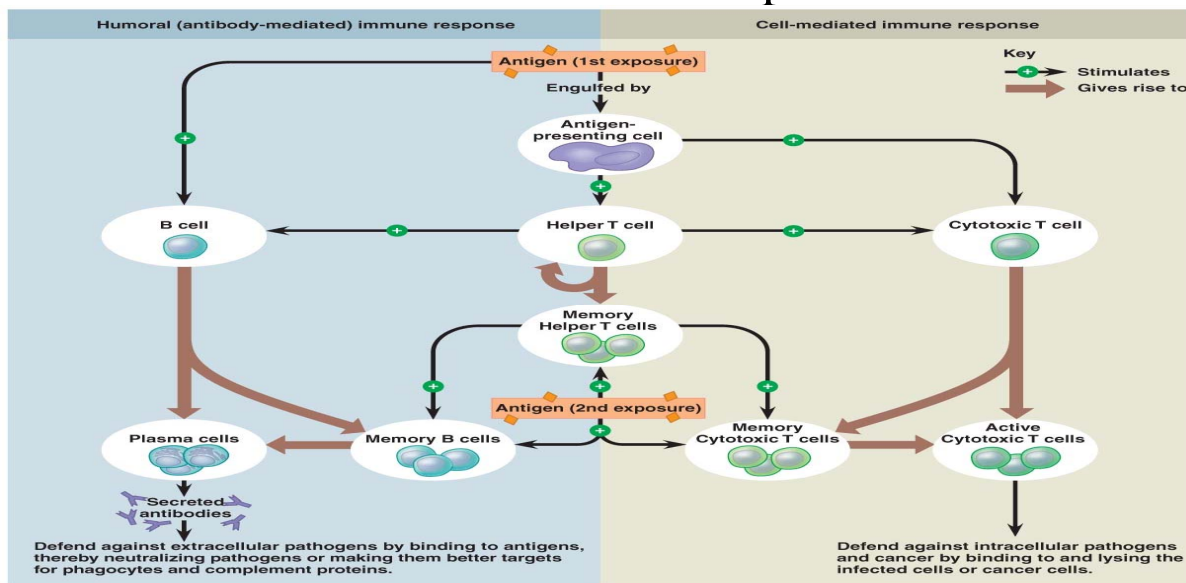
www.answers.com/topic/antibody www.uic.edu/.../bios100/lecturesf04am/lect23.htm



nobelprize.org/.../immunity/immune-detail.html

2) _____ response attacks pathogens that have been _____ into cells through _____

- a) involves _____ and stimulates B cells
1. also a type of lymphocyte
 2. can attack _____ antigens or secreted ones
- b) _____ T cells
1. when _____ attach to antigen _____ cells (those that have ingested pathogen) and _____ the cells
 2. Some become _____ cytotoxic T's which stimulate both memory T's and memory B's after _____ exposure to pathogen.
- c) Helper T cells are _____ by antigen presenting cells and _____ further immune response.



IV) Primary or Secondary Response

- A) Primary response occurs the _____ time the system is _____ to a pathogen. B and T cells are _____, differentiated, and _____.
- B) Secondary Response occurs at _____ exposures to the same pathogen. Memory cells trigger a much _____ response.

V) Active or Passive Immunity

- A) Active immunity occurs _____ the individual (_____) upon exposure to a pathogen.
- 1) B and T cells become _____.
 - 2) Immunity is essentially _____.
- B) Passive immunity occurs when antibodies or activated T cells are _____ from an _____ individual to _____ individual.
- 1) Immunity is _____ term, a few months.
 - 2) Examples include _____ from mother to fetus, or _____ injection.

References:

- Campbell, *Biology*, 8th edition, 2008, Pearson Benjamin Cummings
- http://en.wikipedia.org/wiki/Immune_system
- Kuby *Immunology*, 6th edition, 2007, W.H. Freeman

Antibodies and Isotypes

I) What is an antibody

- A) Definition – A _____ consisting of two identical _____ chains and two identical _____ chains, that _____ a particular _____ and facilitates the _____ of that antigen (Kuby Immunology, 6th edition, 2007, W.H. Freeman).
- B) Antibodies are also referred to as _____.

II) Antibody Structure

- A) Antibody basic structure consists of 2 heavy chains plus two light chains (H₂L₂).

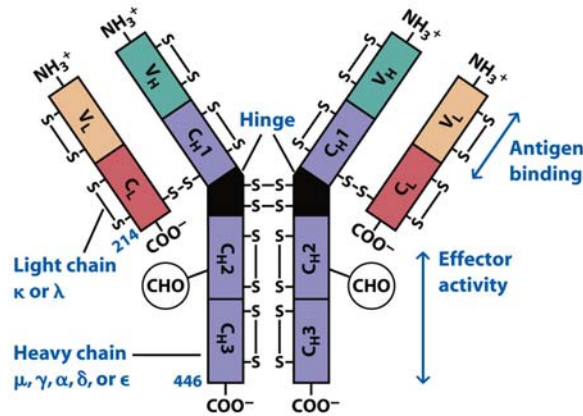
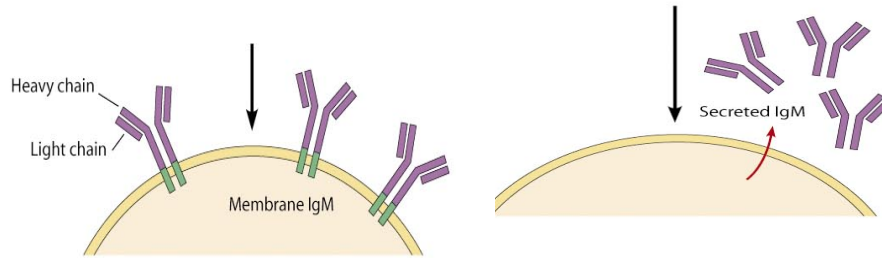


Figure 4-6
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W. H. Freeman and Company

III) General Occurrence of Antibodies

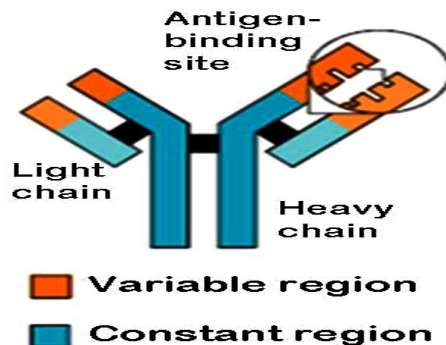
- A) There are _____ types of antibodies:
- 1) _____ bound antibodies that are attached to B cells.
 - 2) _____ antibodies that are secreted by plasma cells.



Copyright © 2009 Pearson Education, Inc.

IV) Antibody Function

- A) Both types of antibodies _____ and _____ to _____ substances in the body.
- B) Foreign substance can include bacteria and viruses and are referred to as _____
- C) By attaching to the antigen it _____ it for other parts of the immune system to _____ on, or _____ the antigen directly. (i.e. _____ the part of the antigen that allows it to _____ cells)
- D) Attachment of antibodies to antigens is _____ - each antibody can only attach to _____ particular antigen (think lock-and-key model).
- E) This results from a small _____ region on the tip of the antibody.
- F) The high variety of antibodies _____ for an equally high variety of antigens that can be _____.



V) Isotypes - Background

- A) In addition to variable regions, the _____ region can have different _____ of heavy chains.
- B) These determine the _____ of antibodies that exist.
- C) Definition: An antibody _____, which is determined by the constant region sequence of the heavy chain.

(Kuby Immunology, 6th edition, 2007, W.H. Freeman)

VI) Isotypes

- A) There are five isotypes/classes of antibodies produced in mammals each having unique _____ and _____ properties.

- Immunoglobulin G (IgG)
- Immunoglobulin M (IgM)
- Immunoglobulin A (IgA)
- Immunoglobulin E (IgE)
- Immunoglobulin D (IgD)

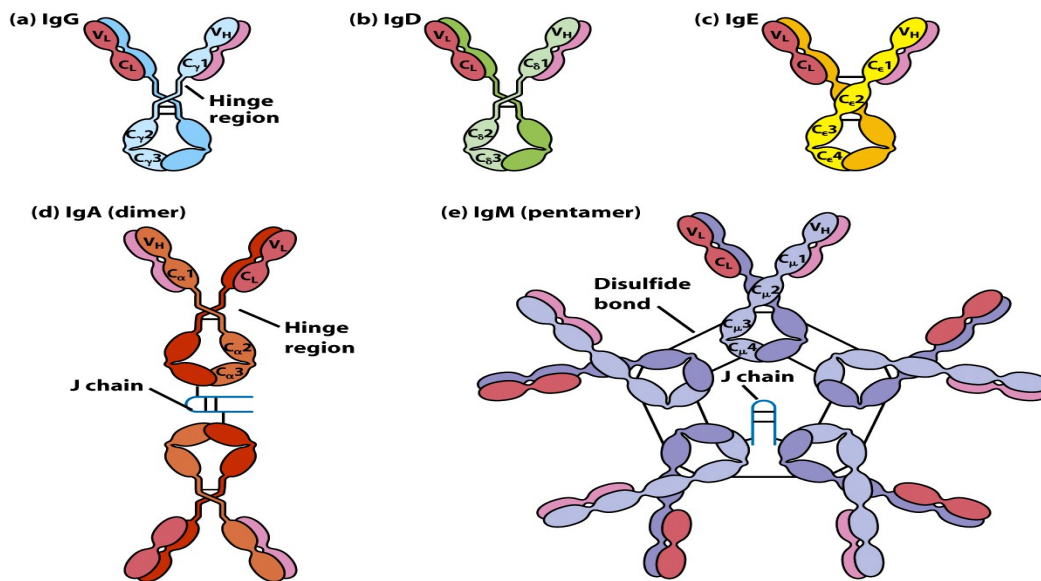


Figure 4-17
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W. H. Freeman and Company

- B) Certain classes have additional _____ based on _____ differences in their heavy chains.
- C) These classes and subclasses of immunoglobulins have different biological _____ and can be used to _____ the type of antibody that is produced _____ a specific _____ response.

VI) Isotypes – general functions

Names	Types	Description
	4	Provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to fetus.
IgM		Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG
	2	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.
IgE		Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy . Also protects against parasitic worms.
	1	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. Its function is less defined than other isotypes.

<http://schools-wikipedia.org/wp/a/Antibody.htm>

VII) Isotypes – diagnostic use

- A) By developing an ELISA test that _____ which isotypes are being produced during an _____, we can _____ determine the type of antibody response.
- B) This may be useful for _____ and studying different aspects of an _____, such as pigeon fever.

Who's ELISA?

I) Actually, it should be what's an ELISA?

- A) _____ for: Enzyme-Linked Immunosorbent Assay
- B) Definition: It is a biochemical _____ used mainly in immunology to _____ the presence of an _____ or an _____ in a sample.
(<http://en.wikipedia.org/wiki/ELISA>)
- C) ELISAs always include _____, positive and negative, many times _____



- D) _____: ELISA testing can be done for _____ testing, disease _____, detecting illegal drug use, and many other uses in both _____ and _____ fields
- F) _____ example: developing an ELISA test that identifies which _____ are being produced by _____ horses. This may be useful for diagnosing and studying different _____ of pigeon fever

II) Isotyping ELISA – Methods (using research example)

2. _____ 96 well plate with phospholipase D, (the major antigen and exotoxin produced by the bacteria)
2. _____ with detergent
3. Load the samples:
 - _____ = hybridomas
 - _____ = buffer
 - _____ = Normal Rabbit Serum

HYA23	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA23	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYD5	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYD5	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA24	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA24	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA27	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda
HYA27	BSA	NRS	IgG1	IgG2A	IgG2B	IgG3	IgA	IgM	kappa	lambda

4. Add the _____ antibody for 8 different mouse classes/subclasses (isotypes)
5. Each detecting antibody is _____ to horse radish peroxidase (an enzyme)
7. Add substrate and measure _____ change – can be done _____ or with a spectrophotometer

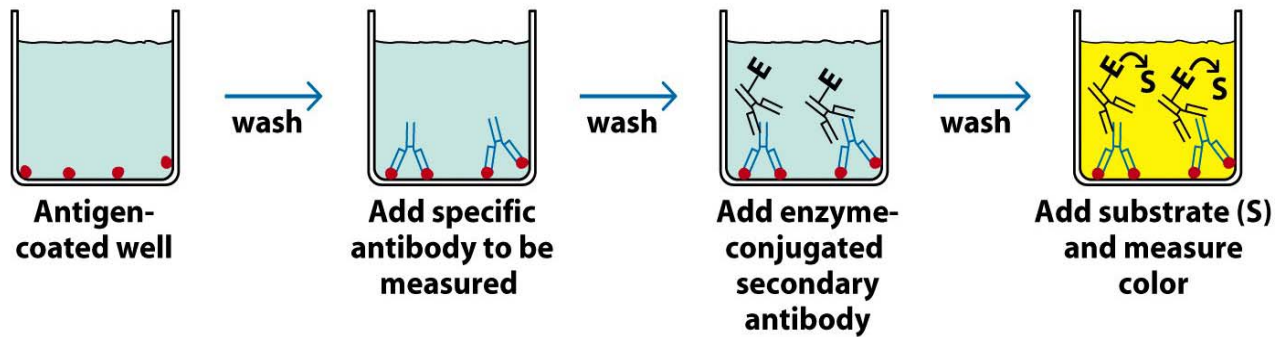


Figure 6-10a
Kuby IMMUNOLOGY, Sixth Edition
 © 2007 W.H. Freeman and Company

III) Isotyping ELISA – Sample Results

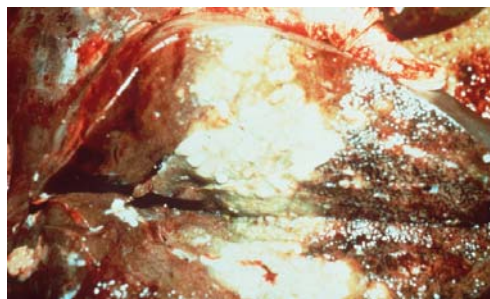
- A) Color change = _____ result for the specific antibody
- C) Clear = _____ result for the antibody
- D) For the research ELISA a positive result indicates a _____ isotype of antibody was _____ in response to the antigen
- F) This result can be useful in determining the _____ of immune _____ for the infected individuals.

How is “pigeon fever” a horse disease?

I) “Pigeon Fever” Introduction

- A) Aka “Dryland _____”
- B) Caused by the _____ bacterium
Corynebacteria pseudotuberculosis
 - 1) Major antigen and _____ is phospholipase D (PLD),
 - 2) PLD is an _____ that _____ cellular membranes _____ bacteria to enter body cells and _____ from blood vessels.
- C) Manifests as three forms:
 - 1) _____ abscesses
 - 2) _____ abscesses
 - 3) Ulcerative lymphangitis

III) “Pigeon Fever” – pictures



1) _____

2) _____



3) _____

III) “Pigeon Fever” Name Origin:

- A) The name “pigeon fever” comes from the _____ abscess manifestation.
- B) A horse’s chest _____ up and _____ like a pigeon’s chest.

IV) “Pigeon Fever” Prevalence

- A) 90% _____ rate for horses with external abscesses
- B) 40+% _____ rate for horses with internal abscesses
- C) Fatality rate for ulcerative lymphangitis is _____ as it occurs so rarely.

V) “Pigeon Fever” Diagnosis

- A) Current diagnosis relies on _____ pus from external _____, and serology testing plus ultrasound for _____ abscesses.

- B) Treatment options are _____ and debatable.
- 1) Some veterinarians suggest _____ course.
 - 2) Others feel that antibiotic usage _____ resolution of external abscesses.
 - 3) All agree that antibiotics are _____ for treating internal abscesses.

VI) “Pigeon Fever” Research Goals

- A) To _____ and _____ the equine immune response to *Corynebacterium pseudotuberculosis* (_____)
- B) Determine if the different manifestations of the disease are due to a difference in the T_H1 versus T_H2 response (_____).
- C) Long term – the possible development of a diagnostic _____ and _____ for the disease.