Unit 6:
Adaptive Immunity

Adaptive Immunity (Humoral Immunity; Cell-Mediated Immunity; Immunodeficiency; Hypersensitivity)

ADAPTIVE IMMUNITY:
AN OVERVIEW OF INNATE AND ADAPTIVE IMMUNITY

Adaptive Immunity

An Overview of Innate and Adaptive Immunity

Fundamental Statement for this Softchalk Lesson:

1. The body has two immune systems: the innate immune system and the adaptive immune system.
2. Innate immunity is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe.
3. Innate immunity is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.
4. Immediate innate immunity begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood and in extracellular tissue fluids.
5. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs.
6. Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen.
7. Adaptive immunity is the immunity one develops throughout life.
8. An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes.
9. The actual portions or fragments of an antigen that react with antibodies and lymphocyte receptors are called epitopes.

Common Course Objectives

1. Cite the differences between innate and adaptive (acquired) immunity
2. Identify places where innate and adaptive immunity intersect.

Detailed Learning Objectives

1**. Compare adaptive (acquired) immunity with innate immunity.

2*. Compare immediate innate immunity with early induced innate immunity.

3. Define the following:

a*. pathogen-associated molecular patterns (PAMPs)
b*. pattern-recognition receptors (PRRs)
c*. antigen
d*. immunogen
e*. epitope.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

An Overview of Innate and Adaptive Immunity

As mentioned in Unit 5, the body has two immune systems: innate immunity and adaptive immunity.

Unit 5 dealt with innate immunity. In Unit 6 we will cover adaptive immunity. Let's first again briefly compare acquired and innate immunity.

1. Innate Immunity

Innate immunity is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. **This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection**. Innate immunity can be divided into immediate innate immunity and early induced innate immunity.

a. Immediate innate immunity

Immediate innate immunity **begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules** that **circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells**. These include:

- antimicrobial enzymes and peptides;
- complement system proteins; and
- anatomical barriers to infection, mechanical removal of microbes, and bacterial antagonism by normal body microbiota.

These preformed innate defense molecules will be discussed in greater detail later in this unit.

b. Early induced innate immunity

Early induced innate immunity **begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs**. These recruited defense cells include:

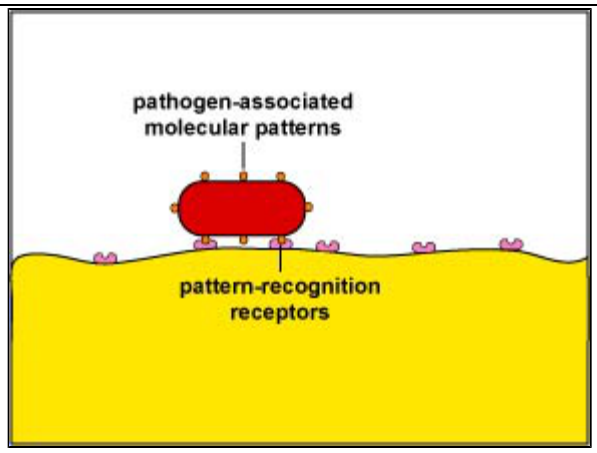
- phagocytic cells: leukocytes such as neutrophils, eosinophils, and monocytes; tissue phagocytic cells in the tissue such as macrophages;
- cells that release inflammatory mediators: inflammatory cells in the tissue such as macrophages and mast cells; leukocytes such as basophils and eosinophils; and
- natural killer cells (NK cells).

Unlike adaptive immunity, innate immunity does not recognize every possible antigen. Instead, it is **designed to recognize molecules shared by groups of related microbes that are essential for the survival of those organisms** and are not found associated with mammalian cells. These unique microbial molecules are called **pathogen-associated molecular patterns** or PAMPS and include LPS from the gram-negative cell wall, peptidoglycan and lipotechoic acids from the gram-positive cell wall, the sugar mannose (a terminal sugar common in microbial glycolipids and glycoproteins but rare in those of humans), bacterial and viral unmethylated CpG DNA, bacterial flagellin, the amino acid *N*-formylmethionine found in bacterial proteins, double-stranded and single-stranded RNA from viruses, and glucans from fungal cell walls. In addition, unique molecules displayed on stressed, injured, infected, or transformed human cells also act as PAMPS. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

Most body defense cells have pattern-recognition receptors or PRRs for these common PAMPS **(see Fig. 1)** and so there is an **immediate response** against the invading microorganism. Pathogen-associated molecular patterns can also be recognized by

a series of soluble pattern-recognition receptors in the blood that function as opsonins and initiate the complement pathways. In all, the innate immune system is thought to recognize approximately 10^3 of these microbial molecular patterns.

Fig. 1: Illustration of Pathogen-Associated Molecular Patterns Binding to Pattern-Recognition Receptors on Defense Cells



Glycoprotein molecules known as pattern-recognition receptors are found on the surface of a variety of body defense cells. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, flagellin, pilin, and bacterial DNA. There are also pattern-recognition molecules for viral double-stranded RNA (dsRNA) and fungal cell walls components such as lipoteichoic acids, glycolipids, mannans, and zymosan. Many of these pattern recognition receptors are known as toll-like receptors.

Flash animation illustrating the PAMP LPS binding to its PRR TLR-4

html5 version of animation for iPad illustrating the PAMP LPS binding to its PRR TLR-4

1) Gram-negative bacteria release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall.
2) The LPS binds to a pair of TLR-4s on defense cells such as macrophages and dendritic cells. LPS also binds to LPS-binding protein in the plasma and tissue fluid. The LPS-binding protein promotes the binding of LPS to the CD14 receptors. At that point the LPS-binding protein comes off and the LPS-CD14 bind to TLR-4.
3) The binding of LPS to TLR-4 enables regulatory molecules within the cell to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines.

For more information: Preview of pathogen-associated molecular patterns (PAMPs)

For more information: Preview of pattern-recognition receptors (PRRs)

For More Information: Preview of leukocytes

Examples of innate immunity include anatomical barriers, mechanical removal, bacterial antagonism, antigen-nonspecific defense chemicals, the complement pathways, phagocytosis, inflammation, fever, and the acute-phase response. In this current unit we will look at each of these in greater detail.

Concept Map for Innate Versus Adaptive Immunity

2. Adaptive Immunity

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen. **This is the immunity one develops throughout life**.

During adaptive immunity, antigens are transported to lymphoid organs where they are recognized by naive B-lymphocytes and T-lymphocytes. These activated B- and T-lymphocytes subsequently proliferate and differentiate into effector cells.

An **antigen** is defined as **a substance that reacts with antibody molecules and antigen receptors on lymphocytes**. An **immunogen** is **an antigen that is recognized by the body as nonself and stimulates an adaptive immune response**. For simplicity we will use the term antigen when referring to both antigens and immunogens. The actual portions or fragments of an antigen that react with antibodies and lymphocyte receptors are called **epitopes**.

For more information: Preview of antigens, epitopes, and immunogens

For more information: Preview of antibodies

The body recognizes an antigen as foreign when **epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope**. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

It is estimated that the human body has the ability to recognize 10^7 or more different epitopes and make up to 10^9 different antibodies, each with a unique specificity. In order to recognize this immense number of different epitopes, the body produces 10^7 or more distinct clones of both B-lymphocytes and T-lymphocytes, each with a unique B-cell receptor or T-cell receptor. Among this large variety of B-cell receptors and T-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, any antigen the immune system eventually encounters. With the adaptive immune responses, the body is able to **recognize any conceivable antigen it may eventually encounter**.

The downside to the specificity of adaptive immunity is that only a few B-cells and T-cells in the body recognize any one

epitope. **These few cells then must rapidly proliferate in order to produce enough cells to mount an effective immune response** against that particular epitope, and that **typically takes several days**. During this time the pathogen could be causing considerable harm, and that is why innate immunity is also essential.

For more information: Preview of B-Lymphocytes
For more information: Preview of T4-Lymphocytes
For more information: Preview T8-Lymphocytes

Flash animation showing epitopes reacting with specific B-cell receptor on a B-lymphocytes
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing epitopes reacting with specific B-cell receptor on a B-lymphocytes
B-lymphocytes have B-cell receptors (BCRs) corresponding to the specific antibody molecule they are genetically programmed to make. These BCRs recognize epitopes of antigens having a complementary shape. Different B-lymphocytes are programmed to produce different BCRs, each specific for a unique epitope.

Flash animation showing epitopes reacting with a specific TCR on a T8-lymphocyte
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing epitopes reacting with a specific TCR on a T8-lymphocyte
Naive T8-lymphocytes via the unique T-cell receptors and CD8 molecules on their surface recognize peptide epitopes from endogenous antigens bound to MHC-I molecules on antigen presenting cells (APCs). Different T-cell receptors recognize different epitopes.

Adaptive immunity usually improves upon repeated exposure to a given infection and involves the following:

- antigen-presenting cells (APCs) such as macrophages and dendritic cells;
- the activation and proliferation of antigen-specific B-lymphocytes;
- the activation and proliferation of antigen-specific T-lymphocytes; and
- the production of antibody molecules, cytotoxic T-lymphocytes (CTLs), activated macrophages, and cytokines.

Acquired immunity includes both humoral immunity and cell-mediated immunity and will be the topic of Unit 6.

Concept Map for Innate Versus Adaptive Immunity

TPS Questions

Self Quiz for an Overview of Innate and Adaptive Immunity

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ADAPTIVE IMMUNITY:
INTRODUCTION: ANTIGENS AND EPITOPES

Adaptive Immunity

Antigens and Epitopes

Fundamental Statements for this Softchalk Lesson:

1. An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes.
2. An immunogen is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response.
3. Chemically, antigens are large molecular weight proteins and polysaccharides.
4. The actual portions or fragments of an antigen that react with receptors on B-lymphocytes and T-lymphocytes, as well as with free antibody molecules, are called epitopes.
5. The size of an epitope is generally thought to be equivalent to 5-15 amino acids or 3-4 sugar residues.
6. Polysaccharides antigens usually have many epitopes but all of the same specificity.
7. Proteins antigens usually have many epitopes of different specificities.
8. Immune responses are directed against many different epitopes of many different antigens of the same microbe.
9. The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope.
10. The antigen receptors on the cytoplasmic membrane of B-lymphocytes are called B-cell receptors and are actually antibody molecules made by that cell and anchored to the outer surface of its cytoplasmic membrane and is composed of composed of four interconnected glycoprotein chains.
11. The receptors on the membrane of T-lymphocytes are called T-cell receptors or TCRs and are composed of just two glycoprotein chains.
12. During its development, each different B-lymphocyte and T-lymphocyte becomes genetically programmed to produce a B-cell receptor or T-cell receptor with a unique three-dimensional shape.
13. The body produces 107 or more distinct clones of both B-lymphocytes and T-lymphocytes, each with a unique B-cell receptor or T-cell receptor and with this large variety of B-cell receptors and T-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, any antigen the immune system eventually encounters.
14. In terms of infectious diseases, microbial structures and microbial toxins act as antigens.
15. Certain noninfectious materials also act as antigens, including allergens, foreign tissues and cells from transplants and transfusions, and the body's own cells that the body fails to recognize as "normal self," such as cancer cells, infected cells, and cells involved in autoimmune diseases.
16. Endogenous antigens are antigens found within the cytosol of human cells such as viral proteins, proteins from intracellular bacteria, and tumor antigens.
17. Exogenous antigens are antigens that enter from outside the body, such as bacteria, fungi, protozoa, and free viruses.
18. Autoantigens are any of an organism's own antigens (self-antigens) that stimulate an autoimmune reaction.

Common Course Objective

1. Define antigen, immunogen, and epitope and predict what types of molecules can serve as antigens.

2. Compare the sources of exogenous, endogenous and autoantigens.

3. Compare a hapten to an antigen.

4. Briefly describe how the body recognizes an antigen as foreign and compare B-cell receptors and T-cell receptors in terms of how they recognize epitopes of an antigen.

Detailed Learning Objectives

1*. Define antigen and immunogen.

2*. State what antigens are composed of chemically.

3. List 3 characteristics an antigen must have to be immunogenic.

4*. Define epitope.

5*. Briefly describe how the body recognizes an antigen as foreign.

6*. Compare B-cell receptors and T-cell receptors in terms of how they recognize epitopes.

7*. In terms of infectious diseases, list 2 categories of microbial materials that may act as an antigen.

8*. List 3 groups of noninfectious materials that may act as an antigen.

9*. Define the following:

a. endogenous antigen
b. exogenous antigen
c. autoantigen
d. hapten

(*) = Common theme throughout the course

TPS Questions

Antigens and Epitopes

An **antigen** is defined as **a substance that reacts with antibody molecules and antigen receptors on lymphocytes**. An **immunogen** is **an antigen that is recognized by the body as non-self and stimulates an adaptive immune response**. For simplicity, both antigens and immunogens are usually referred to as antigens.

To be immunogenic, an antigen must possess three characteristics:

- be of **high molecular weight**,
- **exhibit chemical complexity**, and
- **exhibit foreignness** (recognized as non-self by the body).

1. Chemical nature of antigens

Chemically, antigens are large molecular weight **proteins** (including conjugated proteins such as glycoproteins, lipoproteins, and nucleoproteins) and **polysaccharides** (including lipopolysaccharides). These protein and polysaccharide antigens are found on the surfaces of viruses and cells, including microbial cells (bacteria, fungi, protozoans) and human cells.

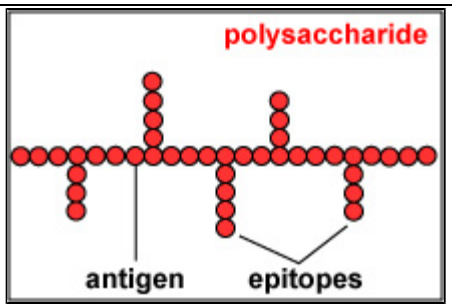
2. Epitopes of an antigen

The actual portions or fragments of an antigen that react with receptors on B-lymphocytes and T-lymphocytes, as well as with free antibody molecules, are called **epitopes** or antigenic determinants. The size of an epitope is generally thought to be equivalent to **5-15 amino acids** or **3-4 sugar residues**.

Some antigens, such as **polysaccharides**, usually have **many epitopes**, but all of the **same specificity**. This is because polysaccharides may be composed of hundreds of sugars with branching sugar side chains, but usually contain only one or two different sugars. As a result, most "shapes" along the polysaccharide are the same **(see Fig. 1)**.

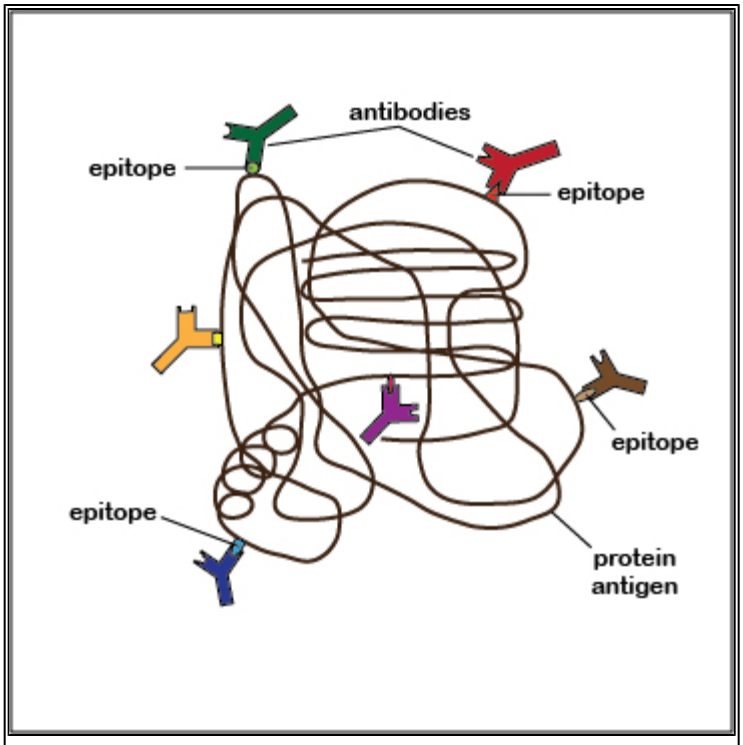
Other antigens such as **proteins** usually have **many epitopes of different specificities**. This is because proteins are usually hundreds of amino acids long and are composed of 20 different amino acids. **Certain amino acids are able to interact with other amino acids in the protein chain and this causes the protein to fold over upon itself and assume a complex three-dimensional shape**. As a result, there are many different "shapes" on the protein **(see Fig. 2)**. That is why proteins are more immunogenic than polysaccharides; they are chemically more complex.

Fig. 1: Epitopes of an Antigen (Polysaccharide)



Polysaccharides have many epitopes but of similar specificities.

Fig. 2: Epitopes of an Antigen (Protein)

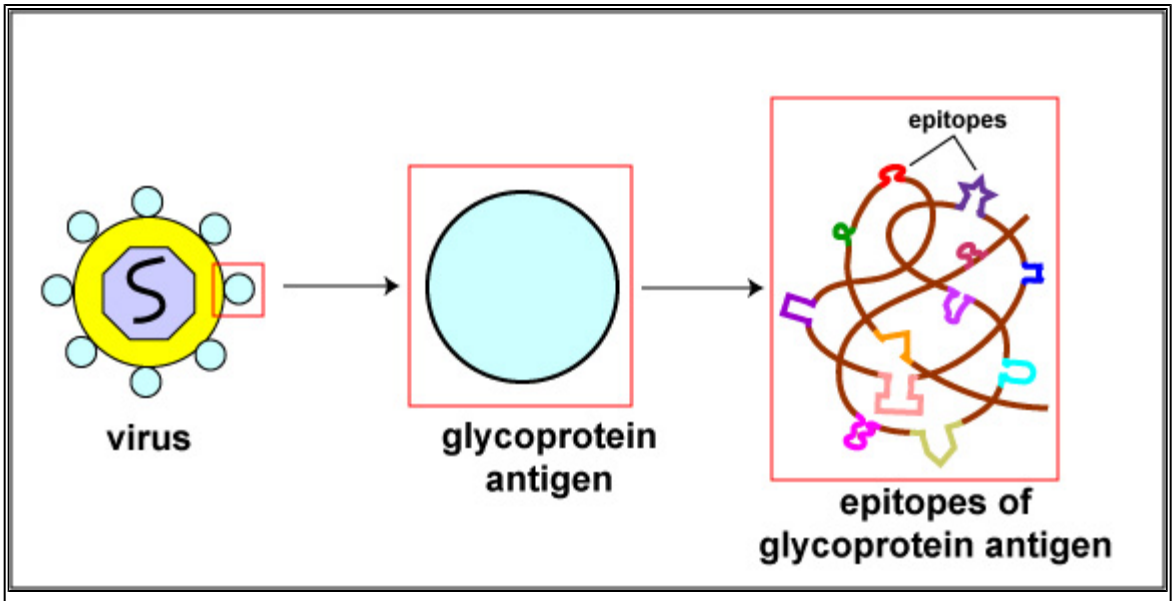


Proteins have many epitopes of different specificities. During humoral immunity, antibodies are made to fit each epitope of each antigen.

A microbe, such as a single bacterium, has many different proteins (and polysaccharides) on its surface that collectively

form its various structures, and each different protein may have many different epitopes. Therefore, **immune responses are directed against many different epitopes of many different antigens of the same microbe**. (For example, a bacterial cell wall alone may contain over 100 different epitopes.) Even simple viruses possess many different epitopes **(see Fig. 3)**.

Fig. 3: Antigens and Epitopes of a Virus



Each different protein and glycoprotein of a virus constitutes a different antigen. Each different antigen contains a number of different epitopes.

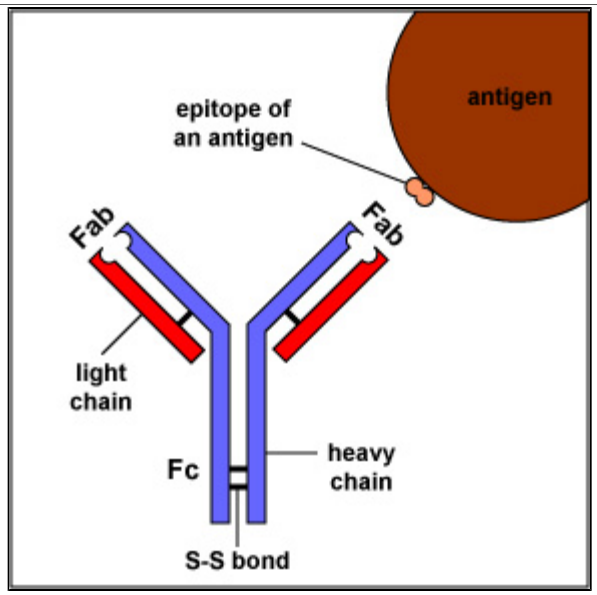
3. Recognizing an antigen as foreign

As we saw earlier in Unit 5, the B-lymphocytes and T-lymphocytes are the cells that carry out the immune responses. **The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope** (similar to interlocking pieces of a puzzle).

a. **B-cell receptors**

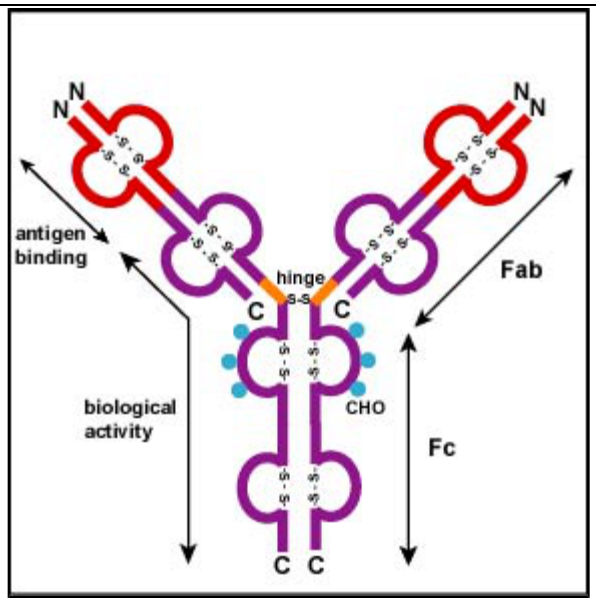
The antigen receptors on the cytoplasmic membrane of **B-lymphocytes** are called **B-cell receptors** and are actually antibody molecules made by that cell and anchored to the outer surface of its cytoplasmic membrane. As will be seen in a later section, antibodies are "Y"-shaped macromolecules composed of **four glycoprotein chains** connected to one another by disulfide (S-S) bonds and noncovalent bonds **(see Fig. 4)**. Additional S-S bonds fold the individual glycoprotein chains into a number of distinct globular domains **(see Fig. 5)**.

Fig. 4: The antibody IgG



The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody.

Fig. 5: Folding Domains of an Antibody

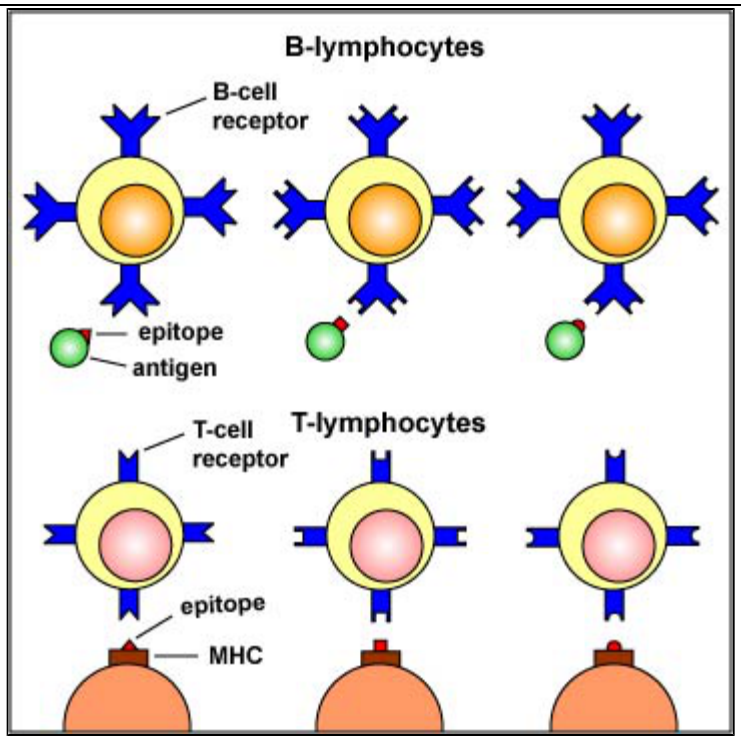


The Fab portion of the antibody has the complementarity-determining regions (red) providing specificity for binding an epitope of an antigen. The Fc portion (purple) directs the biological activity of the antibody. (S-S = disulfide bond; N = amino terminal of glycoprotein; C = carboxy terminal of glycoprotein; CHO = carbohydrate.)

The two tips of the "Y" are referred to as the **Fab portions** of the antibody **(see Fig. 4 and Fig. 5)**. The first 110 amino acids or first domain of both the heavy and light chain of the Fab region of the antibody **provide specificity for binding an epitope on an antigen**. Because they recognize molecular shapes that occur as a result of the 3-dimensional folding of an antigen, **B-cell receptors can bind directly to epitopes on peptide, protein, polysaccharide, nucleic acid, and lipid antigens**.

The bottom part of the "Y", the C terminal region of each glycoprotein chain, is called the **Fc portion**. The Fc portion has a **constant amino acid sequence** that defines the class and subclass of each antibody. The terminal portion of the Fc region of the B-cell receptor is the part that becomes anchored to the cytoplasmic membrane of B-lymphocyte **(see Fig. 6)**.

Fig. 6: Epitope-Specific Receptors on the Surface of B- and T-Lymphocytes

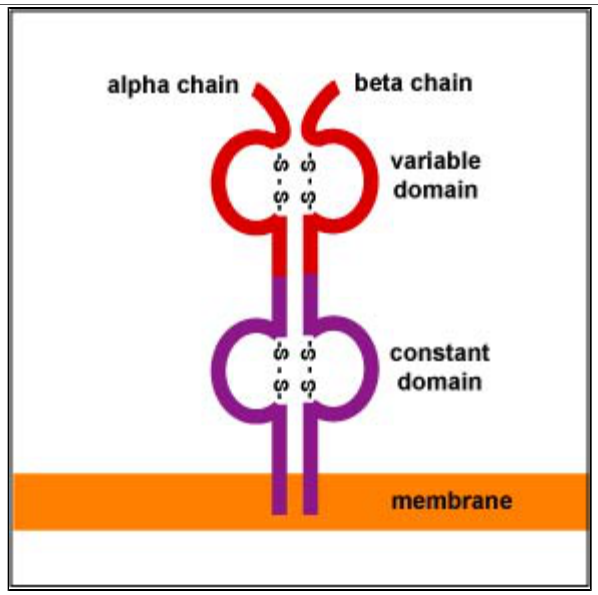


B-lymphocytes have B-cell receptors (sIg) that recognize epitopes directly on antigens. T-lymphocytes have TCR molecules that recognize epitopes only after they have been placed on the cells of the body by way of MHC molecules.

b. **T-cell receptors**

The receptors on the membrane of **T-lymphocytes** are called **T-cell receptors** or **TCRs**. They are analogous to the B-cell receptor, but are composed of just two glycoprotein chains, each having a variable domain and a constant domain **(see Fig. 7**).

Fig. 7: Domains of the T-Cell Receptor



Most T-cell receptors are composed of two polypeptide chains, an alpha chain and a beta chain. The variable domains of the alpha and beta chains (red) provide specificity for binding a peptide bound to a MHC molecule. The terminus of the constant domains (purple) anchor the receptor to the cytoplasmic membrane of the T-lymphocyte. (S-S = disulfide bond).

Unlike B-cell receptors that can directly bind to epitopes on antigens, the **T-cell receptor** or **TCR** of most T4-lymphocytes and T8-lymphocytes **can only recognize peptide epitopes from protein antigens presented by the body's own cells by way of special molecules called MHC molecules** as seen in **Fig. 6**. The terminal portion of the variable domains **provides specificity for binding peptides of protein antigens** after the protein has been unfolded, broken into peptides, and bound to a MHC molecule, while the teminus of the constant region becomes anchored to the cytoplasmic membrane of the T-lymphocyte.

The TCR of $CD4^-CD8^-$ T-lymphocytes and non-MHC restricted $CD4^+$ and $CD8^+$ lymphocytes can recognize epitopes of lipid or glycolipid antigens after they have been attached to CD1 molecules on antigen-presenting cells or in some cases, epitopes directly on antigens.

Since the immune system of the body has no idea as to what antigens it may eventually encounter, it has evolved a system that possesses the capability of responding to epitopes of any conceivable antigen. During its development, **each different B-lymphocyte and T-lymphocyte becomes genetically programmed to produce a B-cell receptor or T-cell receptor with a unique three-dimensional shape (see Fig. 6 above)**.

It is estimated that the human body has the ability to recognize 10^7or more different epitopes and make up to 10^9 different antibodies, each with a unique specificity. In order to recognize this immense number of different epitopes, **the body produces 10^7 or more distinct clones of both B-lymphocytes and T-lymphocytes, each with a unique B-cell receptor or T-cell receptor.** Among this large variety of B-cell receptors and T-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, any antigen the immune system eventually encounters. **With the adaptive immune responses, the body is able to recognize any conceivable antigen it may eventually encounter**.

Flash animation of epitopes reacting with specific B-cell receptors on B-lymphocytes

html5 version of animation for iPad showing epitopes reacting with specific B-cell receptor on a B-lymphocytes.

B-lymphocytes have B-cell receptors (BCRs) corresponding to the specific antibody molecule they are genetically programmed to make. These BCRs recognize epitopes of antigens having a complementary shape. Different B-lymphocytes are programmed to produce different BCRs, each specific for a unique epitope.

Flash animation showing epitopes reacting with a specific TCR on a T8-lymphocyte.

html5 version of animation for iPad showing epitopes reacting with a specific TCR on a T8-lymphocyte.

Naive T8-lymphocytes via the unique T-cell receptors and CD8 molecules on their surface recognize peptide epitopes from endogenous antigens bound to MHC-I molecules on antigen presenting cells (APCs). Different T-cell receptors recognize different epitopes.

For more information: Preview of B-Lymphocytes

For more information: Preview of T4-Lymphocytes

For more information: Preview of T8-Lymphocytes

4. Substances that act as antigens

In terms of infectious diseases, the following may act as antigens:

a. **microbial structures**, such as bacterial and fungal cell walls, protozoan cell membranes, bacterial and fungal capsules, microbial flagella, bacterial pili, viral capsids, viral envelope-associated glycoproteins, etc.; and

b. **microbial toxins**

Certain **non-infectious materials** may also act as antigens if they are recognized as "nonself" by the body. These include:

a. **allergens**, including dust, pollen, hair, foods, dander, bee venom, drugs, and other agents causing allergic reactions;

b. **foreign tissues and cells** from transplants and transfusions; and

c. **the body's own cells that the body fails to recognize as "normal self,"** such as cancer cells, infected cells, and autoantigens involved in autoimmune diseases.

There are three broad categories of antigens: endogenous antigens, exogenous antigens, and autoantigens.

1. **Endogenous antigens are proteins found within the cytosol of human cells.** Examples of endogenous antigens

include:

a. viral proteins produced during viral replication;
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication;
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells;
d. tumor antigens produced by cancer cells; and
e. self-peptides from host cellular proteins.

2. Exogenous antigens are **antigens that enter from outside the body**, such as bacteria, fungi, protozoa, and free viruses. These exogenous antigens enter macrophages, dendritic cells, and B-lymphocytes through phagocytosis or pinocytosis.

3. **Autoantigens are any of an organism's own antigens (self-antigens) that stimulate an autoimmune reaction**, that is humoral immunity or cell-mediated against self.

A **hapten** is a small molecule that by itself is not immunogenic but can act as an antigen when it binds to a larger protein molecule. The hapten then acts as an epitope on the protein. For example with penicillin and poison ivy allergies, the penicillin molecules and the oil urushiol from the poison ivy plant function as haptens, binding to tissue proteins to form an antigen and stimulating an allergic immune response.

TPS Questions

Concept Map for Antigens and Epitopes

Self Quiz for Antigens and Epitopes

Quiz Group

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ADAPTIVE IMMUNITY:
IMPORTANT CELLS AND MOLECULES: MHC MOLECULES

Adaptive Immunity

Important Cells and Molecules for Adaptive Immunity: MHC Molecules

Fundamental Statements for this Softchalk Lesson:

1. MHC molecules enable T-lymphocytes to recognize epitopes and discriminate self from non-self.
2. T-cell receptors (TCRs) of T-lymphocytes can only recognize epitopes - typically short chains of amino acids called peptides - after they are bound to MHC molecules.
3.MHC-I presents epitopes to T8-lymphocytes; MHC-II presents epitopes to T4-lymphocytes.
4. MHC-I molecules are designed to enable the body to recognize infected cells and tumor cells and destroy them with cytotoxic T-lymphocytes or CTLs. (CTLs are effector defense cells derived from naïve T8-lymphocytes.)
5.MHC-I molecules are made by all nucleated cells in the body; bind peptide epitopes typically from endogenous antigens; present MHC-I/peptide complexes to naive T8-lymphocytes and cytotoxic T-lymphocytes possessing a complementary-shaped T-cell receptor or TCR.
6. Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.
7. Endogenous antigens are proteins found within the cytosol of human cells and include viral proteins produced during viral replication, proteins produced by intracellular bacteria, proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells, and tumor antigens produced by cancer cells.
8. During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins are degraded into a variety of peptide epitopes by cylindrical organelles called proteasomes. The resulting peptide epitopes are then attached to MHC-I molecules that are then transported to the surface of that cell.
9. Exogenous antigens are antigens that enter from outside the body such as bacteria, fungi, protozoa, and free viruses.
10. MHC-II molecules are made by antigen-presenting cells or APCs, such as dendritic cells, macrophages, and B-lymphocytes; bind peptide epitopes typically from exogenous antigens; and present MHC-II/peptide complexes to naive T4-lymphocytes or effector T4-lymphocytes that have a complementary shaped T-cell receptor or TCR.
11. Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of endogenous antigens to MHC-II molecules for eventual presentation to naive T4-lymphocytes.
12. Exogenous antigens enter antigen-presenting macrophages, dendritic cells, and B-lymphocytes through phagocytosis, and are engulfed and placed in a phagosome where protein antigens from the microbe are degraded by proteases into a series of peptides. These peptides are then attached to MHC-II molecules that are then put on the surface of the APC.

Common Course Objectives

1. Contrast the MHC-I and MHC-II systems in terms of how they process antigens for recognition by T-cells.
2. Explain the role of T-cells and B-cells in acquired immunity.

Detailed Learning Objectives

1*. State which body cells display MHC-I surface molecules and which cells normally display MHC-II surface molecules.

2*. Define endogenous antigen and exogenous antigen and state which class of MHC molecule primarily binds each.

3*. State which type of T-lymphocyte recognizes epitopes from protein antigens on MHC-I molecules and which type

recognizes epitopes from protein antigens on MHC-II molecules.

4*. State the role of proteasomes in binding of peptides from endogenous antigens by MHC-I molecules.

5*. State the role of lysosomes in binding of peptides from exogenous antigens by MHC-II molecules.

(*) = Common theme throughout the course

TPS Questions

TPS Questions

Important Cells and Molecules for Adaptive Immunity: MHC Molecules

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

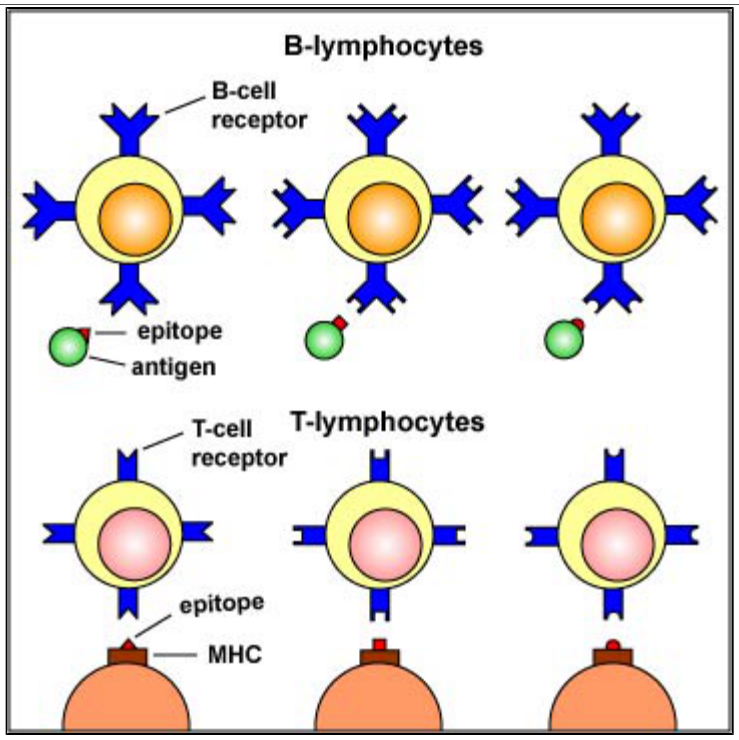
1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen *(def)* and is mediated by B-lymphocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of MHC molecules in adaptive immune responses.

MHC molecules enable T-lymphocytes to recognize epitopes of antigens and discriminate self from non-self. Unlike B-cell receptors on B-lymphocytes that are able to directly bind epitopes on antigens, the T-cell receptors (TCRs) of T-lymphocytes can only recognize epitopes - typically short chains of amino acids called peptides - after they are bound to MHC molecules **(see Fig. 1)**.

Fig. 1: Epitope-Specific Receptors on the Surface of B- and T-Lymphocytes



B-lymphocytes have B-cell receptors that recognize epitopes directly on antigens. T-lymphocytes have TCR molecules that recognize epitopes only after they have been placed on cells of the body by way of MHC molecules.

The MHC genes are the most polymorphic genes in the human genome, possessing many alleles for each gene. The MHC genes are co-dominantly expressed so that an individual expresses the alleles inherited from each parent. In this way, the number of MHC molecules that bind peptide for presentation to T-lymphocytes is maximized. In addition, each MHC molecule is able to bind a wide variety of different peptides, both self-peptides and foreign peptides.

There are two classes of MHC molecules: MHC-I and MHC-II.

1. MHC-I molecules present epitopes to T8-lymphocytes.
2. MHC-II molecules presents epitopes to T4-lymphocytes.

The expression of MHC molecules is increased by cytokines produced during both innate immune responses and adaptive immune responses. Cytokines such as interferon-alpha, interferon-beta, interferon-gamma, tumor necrosis factor increase the expression of MHC-I molecules, while interferon-gamma is the main cytokine to increase the expression of MHC-II molecules.

a. MHC-I molecules

MHC-I molecules are designed to enable the body to recognize infected cells and tumor cells and destroy them with cytotoxic T-lymphocytes or CTLs. CTLs are effector defense cells derived from naive T8-lymphocytes.

MHC-I molecules are:

1. **Made by all nucleated cells** in the body.
2. Possess a deep groove that can **bind peptide epitopes**, typically 8-11 amino acids long, **typically from endogenous antigens**.

3. Present MHC-I/peptide complexes to naive T8-lymphocytes and cytotoxic T-lymphocytes possessing a complementary-shaped T-cell receptor or TCR.

4. Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.

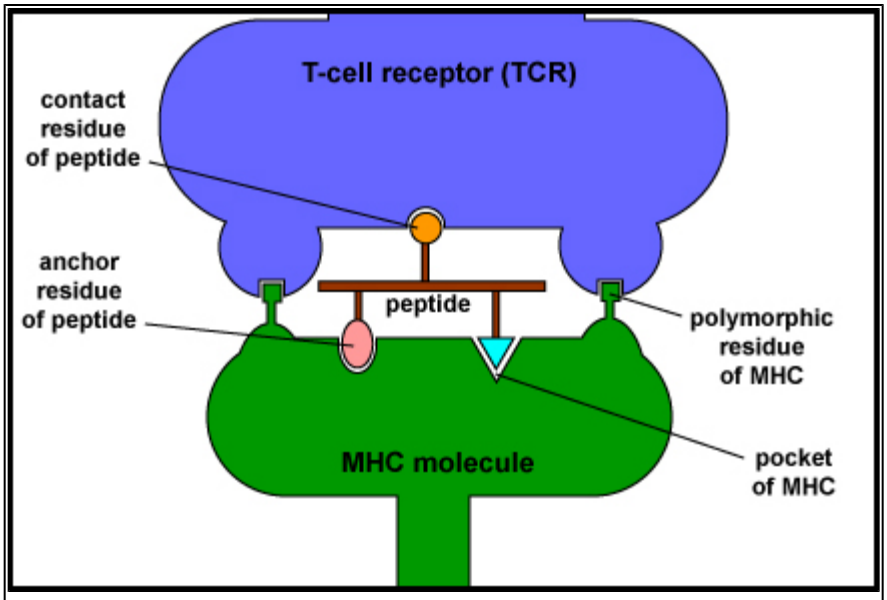
Endogenous antigens are proteins found within the cytosol of human cells. Examples of endogenous antigens include:

1. **Viral proteins produced during viral replication**;
2. **Proteins produced by intracellular bacteria** such as Rickettsias and Chlamydias during their replication;
3. **Proteins that have escaped into the cytosol from the phagosome of phagocytes** such as antigen-presenting cells;
4. **Tumor antigens produced by cancer cells**; and
5. **Self-peptides from host cellular proteins**.

During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins are degraded into a variety of peptide epitopes by cylindrical organelles called **proteasomes**. The body's own cytosolic proteins are also degraded into peptides by proteasomes.

These peptide epitopes are then attached to a groove of MHC-I molecules that are then transported to the surface of that cell where they can be recognized by a **complementary-shaped T-cell receptor (TCR) and a CD8 molecule**, a co-receptor, **on the surface of** either a naive T8-lymphocyte or a cytotoxic T-lymphocyte (CTL). The TCRs recognize both the foreign peptide antigen and the MHC molecule **(see Fig. 2)**. TCRs, however, will not recognize self-peptides bound to MHC-I. As a result, normal cells are not attacked and killed.

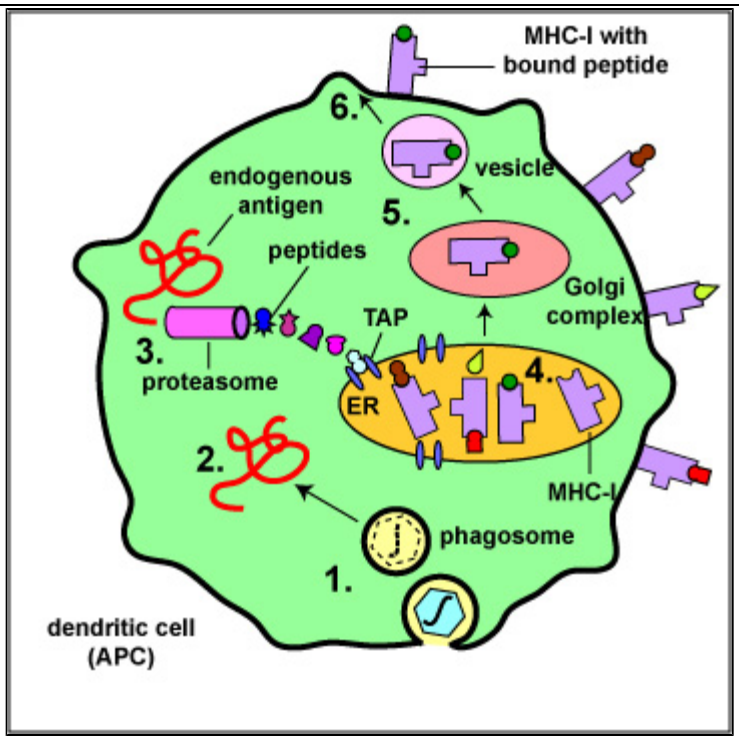
Fig. 2: MHC Molecule with Bound Peptide Binding to a Complementary T-Cell Receptor



This illustration shows a T-cell receptor (TCR) on the surface of a T-lymphocyte recognizing two polymorphic residues of a MHC molecule and one residue of its bound peptide epitope.

MHC-I molecule with bound peptide on the surface of **antigen-presenting dendritic cells (see Fig. 3)** can be **recognized by a complementary-shaped TCR/CD8** on the surface of **a naive T8-lymphocyte** to initiate cell-mediated immunity **(see Fig. 4)**. (Certain dendritic cells, as discussed later, can also cross-present exogenous antigens to MHC-I molecules.)

Fig. 3: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Dendritic Cell



Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

Dendritic cells bind epitopes from endogenous antigens to MHC-I molecules and present them to naive T8-lymphocytes in order to activate these naive T8-lymphocytes.

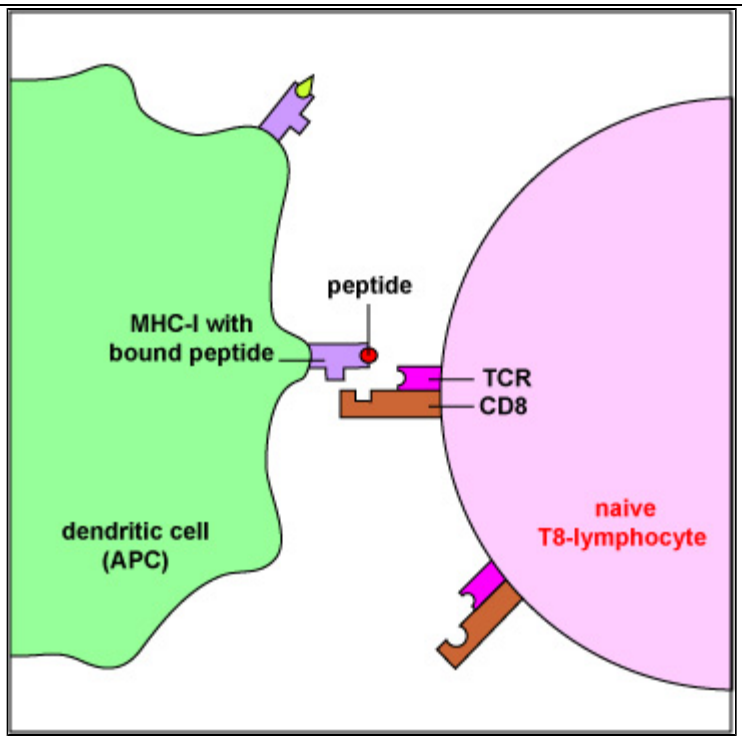
Key

1. Antigens are engulfed by dendritic cells and placed in a phagosome. Some of the proteins escape from the phagosome into the cytosol of the dendritic cell where they become endogenous antigens.
2. These endogenous antigens pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by naive T8-lymphocytes by way of TCRs and

CD8 molecules having a complementary shape.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.)

Fig. 4: An Antigen-Presenting Dendritic Cell Presenting MHC-I with Bound Peptide to a Naive T8-lymphocyte having a Complementary T-Cell Receptor



Antigen-presenting dendritic cells produce both MHC-I and MHC-II molecules. These APCs can phagocytose infected cells and tumor cells, place them in phagosomes, and degrade them with lysosomes. During this process, some of the proteins escape from the phagosome into the surrounding cytosol. Here they can be degraded into peptides by proteasomes, bound to MHC-I molecules, and placed on the surface of the dendritic cell. Now the peptide/MHC-I complexes can be recognized by a naive T8-lymphocyte having a complementary shaped T-cell receptor (TCR) and CD8 molecule. This activates the naive T8-lymphocyte enabling it to eventually proliferate and differentiate into cytotoxic T-lymphocytes (CTLs).

Flash animation of MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from

endogenous antigens by an antigen-presenting cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs. These CTLs are effector cells derived from T8-lymphocytes during cell-mediated immunity. However, in order to become CTLs, naive T8-lymphocytes must become activated by cytokines produced by antigen-presenting cells (APCs). This interaction between APCs and naive T8-lymphocytes occurs primarily in the lymph nodes, the lymph nodules, and the spleen. The process can be summarized as follows:

1. In addition to microbes, dendritic cells and macrophages also engulf and degrade infected cells, tumor cells, and the remains of killed infected and tumor cells. It is thought that in this manner, endogenous antigens from other cells are able to enter the APC. During phagocytosis, some proteins are released from the phagosomes into the cytosol of the APC.
2. These cytosolic proteins then pass through proteasomes, where proteases and peptidases chop the protein up into a series of peptides, typically 8-11 amino acids long.
3. A transporter protein called TAP located in the membrane of the cell's endoplasmic reticulum then transports these peptide epitopes into the endoplasmic reticulum where they bind to the grooves of various newly made MHC-I molecules.
4. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles .
5. The exocytic vesicles carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface.

The MHC-I molecules with bound peptide on the surface of the APCs can now be recognized by naive T8-lymphocytes possessing TCRs and CD8 molecules with a complementary shape. This recognition of the peptide epitope by the TCR serves as a first signal for activating the naive T8-lymphocyte for cell-mediated immunity function.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.)

Flash animation of a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an antigen-presenting dendritic cell.

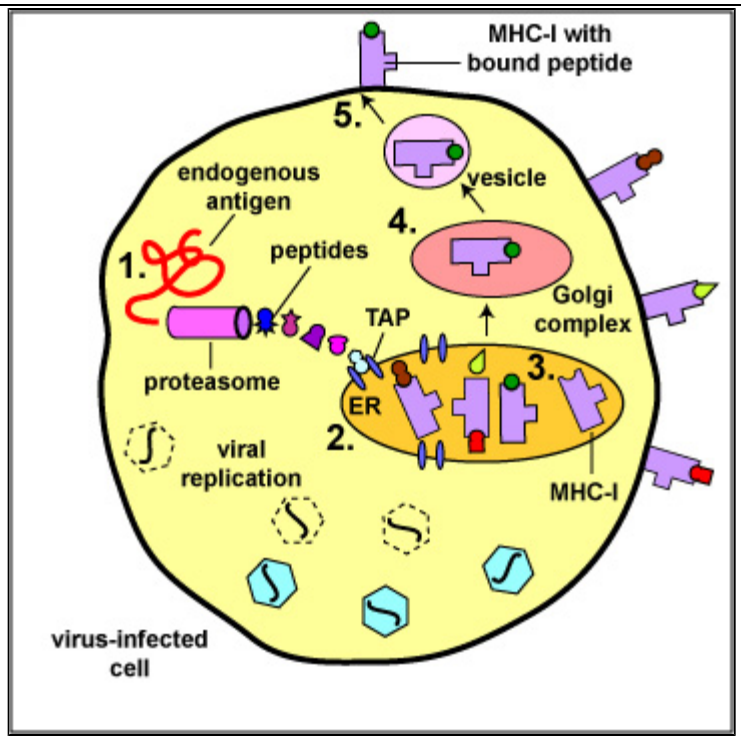
html5 version of animation for iPad showing a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an antigen-presenting dendritic cell.

A naive T8-lymphocyte uses its TCR and CD8 molecules to bind to complementary shaped MHC-I molecule with attached peptide epitope on an antigen-presenting dendritic cell. As naive T8-lymphocytes migrate through lymphoid tissues, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding surface receptors

such as ICAM-1, ICAM-2 and CD58 on dendritic cells. This transient binding allows time for the TCRs on the T8-lymphocyte to sample large numbers of MHC-I/peptide complexes on the dendritic cells.

MHC-I molecule with bound peptide on the surface of infected cells and tumor cells (see Fig. 5) can be recognized by a complementary-shaped TCR/CD8 on the surface of a cytotoxic T-lymphocyte or CTL to initiate destruction of the cell containing the endogenous antigen (see Fig. 6). (CTLs are effector cells derived from naive T8-lymphocytes.)

Fig. 5: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Virus-Infected Cell



Endogenous antigens are those being produced within the cytosol of the cells of the body. Examples include:

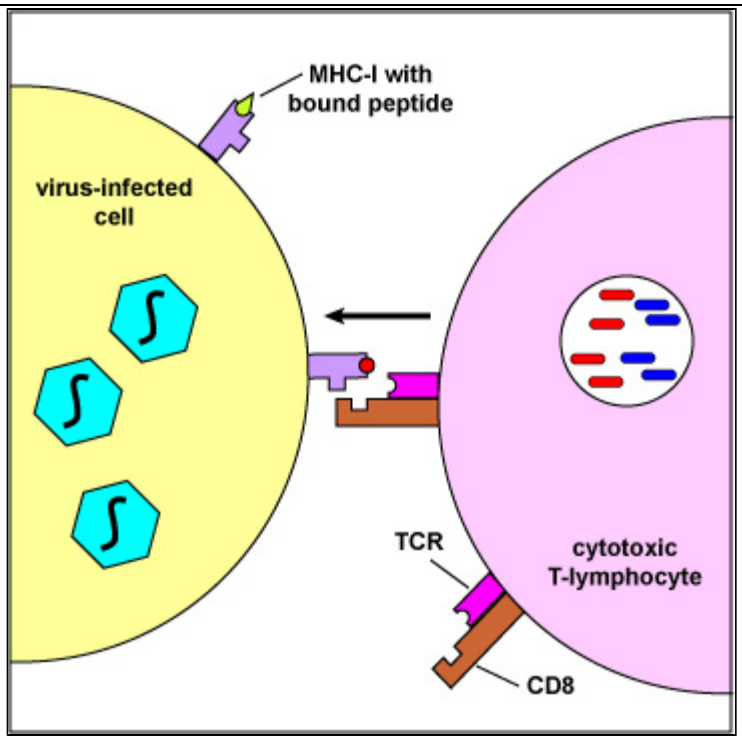
a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

Key

1. During viral replication within the host cell, endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

Fig. 6: A Cytotoxic T-lymphocyte Recognizing a Virus-Infected Cell



Endogenous antigens are those being produced within the cytosol of the cells of the body. Examples include proteins from replicating viruses, proteins from intracellular bacteria, and tumor antigens. The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

Flash animation of MHC-I molecules binding epitopes from endogenous antigens in an infected cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from endogenous antigens in an infected cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. Endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

Flash animation of a CTL triggering apoptosis by way of perforins and granzymes.

html5 version of animation for iPad showing a CTL triggering apoptosis by way of perforins and granzymes.

Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.)

Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate DNase enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash Animation of CTL-induced apoptosis of a virus-infected cell.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing CTL-induced apoptosis of a virus-infected cell.
Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms: • Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and degrade both the target cell's nucleoprotein and microbial DNA within the cell. • Granzymes cleave a variety of other cellular substrates that contribute to cell death. • The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can increase the permeability of the infected cell and contribute to cell death. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis. A granule called granulysin can also alter the permeability of both miocrobial and host cell membranes. This animations shows destruction of both the cytoskeleton and nucleoprotein of the infected cell. As the infected cell breaks up into apoptotic fragments, the fragments are subsequently removed by phagocytes. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells.

MHC-I molecules are coded for by three MHC-I genes, *HLA-A*, *HLA-B*, and *HLA-C*. As mentioned above, however, there are many different alleles for each gene that a person inherits. In this way, the number of MHC-I molecules that bind peptides for presentation to T-8 lymphocytes is maximized. The expression of MHC-I molecules on all cell types is increased by the cytokines interferon-alpha (IFN-a) and interferon-beta (IFN-ß).

Concept Map for MHC Molecules

TPS Question: MHC-I Molecules

b. MHC-II molecules

MHC-II molecules **are designed to enable T4-lymphocytes to recognize epitopes** of exogenous antigens and discriminate self from non-self.

MHC-II molecules are:

1. **Made by antigen-presenting cells or APCs**, such as dendritic cells, macrophages, and B-lymphocytes.

2. Possess a deep groove that can **bind peptide epitopes**, often 10-30 amino acids long but with an optimum length of 12-16 amino acids, **typically from exogenous antigens**. The peptides interact along their entire length with the groove.

3. Present MHC-II/peptide complexes to naive T4-lymphocytes or effector T4-lymphocytes that have a complementary

shaped T-cell receptor or TCR.

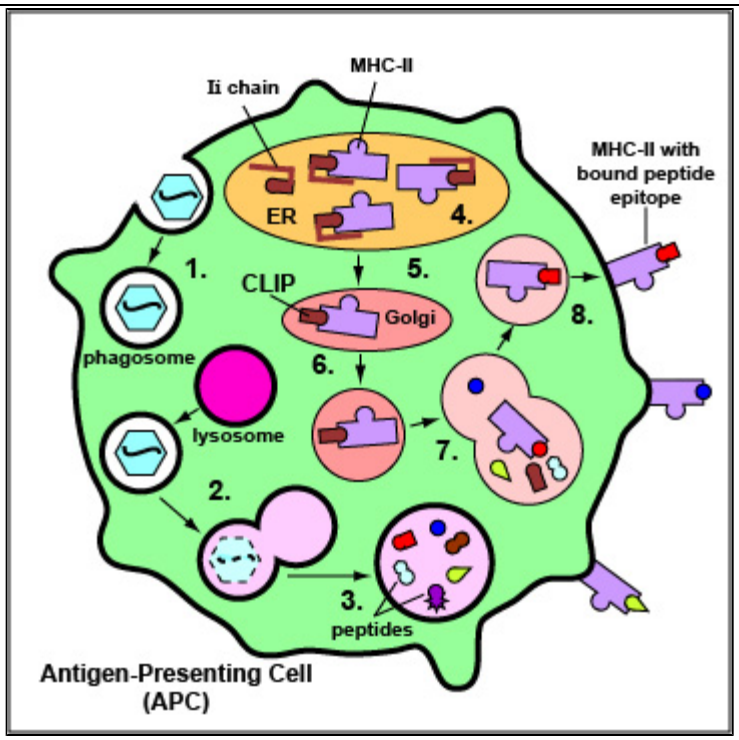
4. Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of endogenous antigens to MHC-II molecules for eventual presentation to naive T4-lymphocytes .

Exogenous antigens are **antigens that enter from outside the body**, such as bacteria, fungi, protozoa, and free viruses. These exogenous antigens enter macrophages, dendritic cells, and B-lymphocytes through phagocytosis. The microbes are engulfed and placed in a phagosome which then fuses with **lysosomes**. Following this fusion, **the phagolysosome becomes acidified**. Acidification, in turn, **activates the proteases within the phagolysosome enabling protein antigens** from the microbe **to be degraded into a series of short peptides**. These peptide epitopes are then attached to MHC-II molecules and are then transported to the surface of the antigen-presenting cell **(see Fig. 7)**. (Certain dendritic cells, as discussed later, can also cross-present endogenous antigens to MHC-II molecules.)

Some pathogens, such as *Mycobacterium tuberculosis, Mycobacterium leprae*, and *Leishmania*, are able to grow in the endocytic vesicles of macrophages without being killed by lysosomes. These macrophages can, however, become activated by T4-effector lymphocytes called T_H1 cells and subsequently use intravesicular proteases to degrade the proteins from these pathogens into peptides for presentation to MHC-II molecules that pass through on their way to the cell surface.

Here the **MHC-II molecules with bound peptides can be recognized by a complementary-shaped T-cell receptor and a CD4 molecule**, a co-receptor, **on the surface of a T4-lymphocyte (see Fig. 8)** . T4-lymphocytes are the cells the body uses to regulate both humoral immunity and cell-mediated immunity.

Fig. 7: Binding of Peptide Epitopes from Exogenous Antigens to MHC-II Molecules



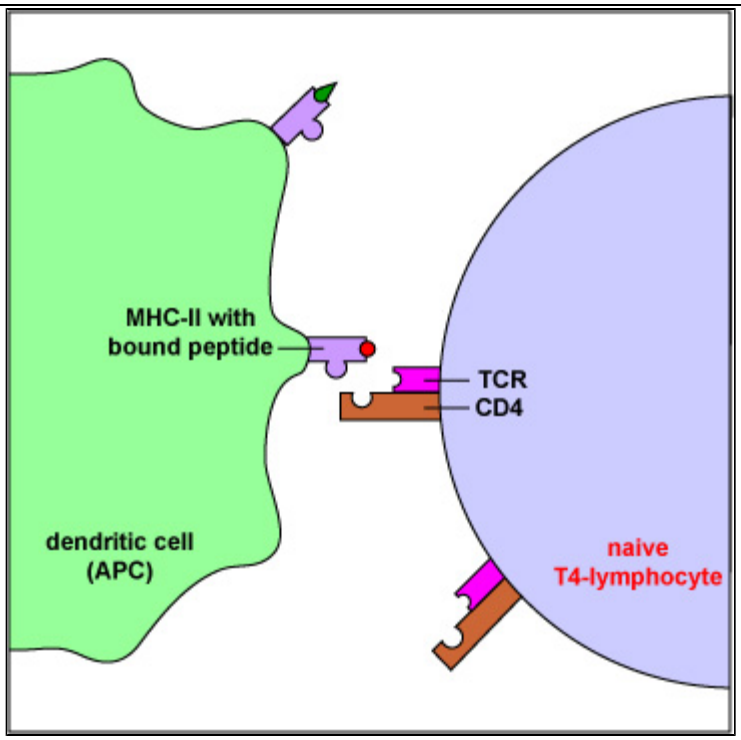
Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (macrophages, dendritic cells, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

Key

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of endogenous antigens to MHC-II molecules for eventual presentation to naive T4-lymphocytes.)

Fig. 8: A T4-Lymphocyte Recognizing Epitope/MHC-II on an Antigen-Presenting Dendritic Cell



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting dendritic cells through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a

series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

Flash animation of MHC-II molecules binding epitopes from exogenous antigens.

html5 version of animation for iPad showing MHC-II molecules binding epitopes from exogenous antigens.

Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (dendritic cells, macrophages, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides by proteases and peptidases.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
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8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of endogenous antigens to MHC-II molecules for eventual presentation to naive T4-lymphocytes.)

Flash animation of a naive T4-lymphocyte recognizing epitopes bound to MHC-II molecules on an APC.

html5 version of animation for iPad showing a naive T4-lymphocyte recognizing epitopes bound to MHC-II molecules on an APC.

A naive T4-lymphocyte uses its TCR and CD4 molecules to bind to complementary shaped MHC-II molecule with attached peptide epitope on an antigen-presenting dendritic cell. As naive T4-lymphocytes migrate through the cortical region of lymph nodes, they use surface

cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding receptors such as ICAM-1, ICAM-2 and CD58 on the surface of dendritic cells. This transient binding allows time for the TCRs on the T4-lymphocyte to sample large numbers of MHC-II/peptide complexes on the antigen-presenting dendritic cells.

MHC-II molecules are coded for by three MHC-II genes, *HLA-DR*, *HLA-DP*, and *HLA-DQ*. Interferon-gamma (IFN- ?) increases the expression of both MHC-I and MHC-II molecules.

Concept Map for MHC Molecules

TPS Question: MHC-II Molecules

For More Information: Preview of B-Lymphocytes

For More Information: Preview of T4-Lymphocytes

For More Information: Preview of T8-Lymphocytes

For More Information: Preview of antigen-presenting cells

Self Quiz for MHC Molecules

Return to Unit 5 and 6 Table of Contents

Return to Softchalk Lessons Table of Contents

ADAPTIVE IMMUNITY:

ADAPTIVE IMMUNITY:
IMPORTANT CELLS AND MOLECULES: ANTIGEN-PRESENTING CELLS (APCs)

Adaptive Immunity

Important Cells and Molecules for Adaptive Immunity: Antigen-Presenting Cells (APCs)

Fundamental Statements for this Softchalk Lesson:

1. Antigen-presenting cells (APCs) include dendritic cells, macrophages, and B-lymphocytes.
2. APCs express both MHC-I and MHC-II molecules and serve two major functions during adaptive immunity: they capture and process antigens for presentation to T-lymphocytes, and they produce signals required for the proliferation and differentiation of lymphocytes.
3. Most dendritic cells are derived from monocytes and are referred to as myeloid dendritic cells and are located under the surface epithelium of the skin, the mucous membranes of the respiratory tract, genitourinary tract, and the gastrointestinal tract, and throughout the body's lymphoid tissues and in most solid organs.
4. The primary function of dendritic cells is to capture and present protein antigens to naive T-lymphocytes which enables the naïve T4-lymphocytes or T8-lymphocytes to become activated, proliferate, and differentiate into effector cells.
5. Naïve lymphocytes are B-lymphocytes and T-lymphocytes that have not yet reacted with an epitope of an antigen.
6. Dendritic cells use MHC-II molecules to present protein antigens to naïve T4-lymphocytes and MHC-I molecules to present protein antigens to naïve T8-lymphocytes.
7. When monocytes leave the blood and enter the tissue, they become activated and differentiate into macrophages.
8. When functioning as APCs, macrophages capture and present peptide epitopes from exogenous antigens to effector T4-lymphocytes.
9. Effector lymphocytes are lymphocytes that have encountered an antigen, proliferated, and matured into a form capable of actively carrying out immune defenses.
10. B-lymphocytes mediate antibody production.
11. When functioning as APCs, B-lymphocytes are able to capture and present peptide epitopes from exogenous antigens to effector T4-lymphocytes.
12. To activate naïve T4-lymphocytes, dendritic cells engulf exogenous antigens, place them in a phagosome, degrade protein antigens into peptides via lysosomes, bind those peptides to MHC-II molecules and transport them to the surface of the dendritic cell where they can be recognized by the T-cell receptors and CD4 molecules of naïve T4-lymphocytes.
13. To activate naïve T8-lymphocytes, dendritic cells degrade endogenous protein antigens into peptides via their proteasomes, bind those peptides to MHC-I molecules and transport them to the surface of the dendritic cell where they can be recognized by the T-cell receptors and CD8 molecules of naïve T8-lymphocytes.

Common Course Objectives

1. Briefly describe how the body recognizes an antigen as foreign and compare B-cell receptors and T-cell receptors in terms of how they recognize epitopes of an antigen.
2. Contrast the MHC-I and MHC-II systems in terms of how they process antigens for recognition by T-cells.
3. Explain the role of antigen presenting cells in the adaptive immune responses.

Detailed Learning Objectives

1**. Describe the overall function of antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B-

lymphocytes in terms of the following:

a. how they "process" exogenous antigens

b. how they "process" endogenous antigens

b. the types of MHC molecule to which they attach peptides

c. the role of proteasomes in the binding of peptides from endogenous antigens by MHC-I molecules.

d. the role of lysosomes in the binding of peptides from exogenous antigens by MHC-II molecules.

e. the types of cells to which they present peptides

2*. Name the primary type of cell that functions as an antigen-presenting cell to naive T4-lymphocytes and naive T8-lymphocytes.

3. State the role of T4-effector cells in activating macrophages.

4. State the role of T4-effector cells in the proliferation and differentiation of activated B-lymphocytes.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

Important Cells and Molecules for Adaptive Immunity: Antigen-Presenting Cells (APCs)

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen *(def)* and is mediated by B-lymphocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of antigen-presenting cells in adaptive immune responses.

Antigen-presenting cells or APCs include dendritic cells, macrophages, and B-lymphocytes. APCs express both MHC-I and MHC-II molecules and serve two major functions during adaptive immunity:

1. they capture and process antigens for presentation to T-lymphocytes, and

2. they produce signals required for the proliferation and differentiation of lymphocytes.

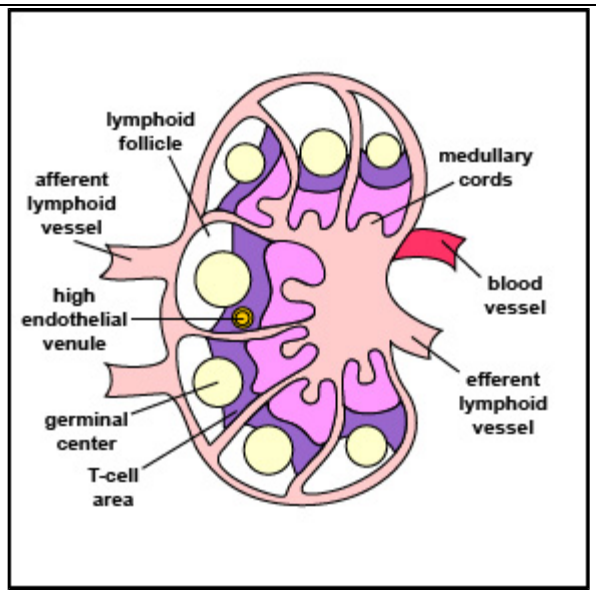
We will now take a closer look at our three primary groups of APCs: dendritic cells, macrophages, and B-lymphocytes.

a. Dendritic Cells

As learned in Unit 5, most dendritic cells are derived from monocytes and are referred to as myeloid dendritic cells. They are located under the surface epithelium of the skin and the surface epithelium of the mucous membranes of the respiratory tract, genitourinary tract, and the gastrointestinal tract. They are also found throughout the body's lymphoid tissues and in most solid organs.

In these locations, in their immature form, they are attached by long cytoplasmic processes. Upon capturing antigens through pinocytosis and phagocytosis and becoming activated by inflammatory cytokines, the dendritic cells detach from their initial site, enter lymph vessels, and are carried to regional lymph nodes **(see Fig. 1)**. Activation of the dendritic cell promotes its expression chemokine receptor CCR7 that enables the dendritic cell to migrate towards the chemokine CCL21 produced by lymphoid tissues. By the time the dendritic cells enter the lymph nodes, they have matured and are now able to present antigen epitopes to the ever-changing populations of **naive T8-lymphocytes** and **naive T4-lymphocytes** located in the T-cell area of the lymph nodes.

Fig. 1: Structure of a Lymph Nodes



Antigens enter lymph nodes through afferent lymphoid vessels. Antigen-presenting dendritic cells, B-Antigens enter lymph nodes through afferent lymphoid vessels. Antigen-presenting dendritic cells enter the lymph node through afferent lymphatic vessels while naive B-lymphocytes, and naive T-lymphocytes enter through high endothelial venules. Non-activated and effector lymphocytes leave the lymph node through efferent lymphatic vessels. Naive B-lymphocytes become activated, proliferate, and differentiate into plasma cells in the germinal centers of lymphoid follicles while naive T-lymphocytes become activated, proliferate and differentiate into T-effector lymphocytes in the T-cell area.

The primary function of dendritic cells, then, is to capture and present protein antigens to naive T-lymphocytes. (Naive lymphocytes are those that have not yet encountered an antigen.) Since dendritic cells are able to express both MHC-I and MHC-II molecules, they are able to present antigens to both naive T8-lymphocytes and naive T4-lymphocytes.

These interactions enable the naive T4-lymphocyte or T8-lymphocyte to become activated, proliferate, and differentiate into effector lymphocytes. (Effector lymphocytes are lymphocytes that have encountered an antigen, proliferated, and matured into

a form capable of actively carrying out immune defenses.)

1. MHC-II presentation of protein antigens to naive T4-lymphocytes

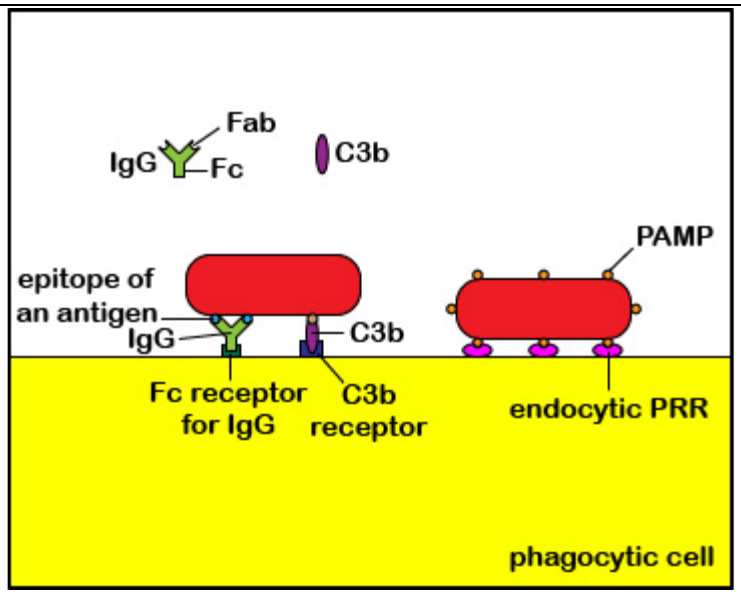
a. MHC-II presentation of exogenous antigens to **naive T4-lymphocytes**

Immature dendritic cells take in protein antigens for attachment to MHC-II molecules and subsequent presentation to naive T4-lymphocytes by:

1. Receptor-mediated phagocytosis, e.g., PAMPs binding to endocytic PRRs, IgG or C3b attachment of microbes to phagocytes during opsonization **(see Fig. 2)**.

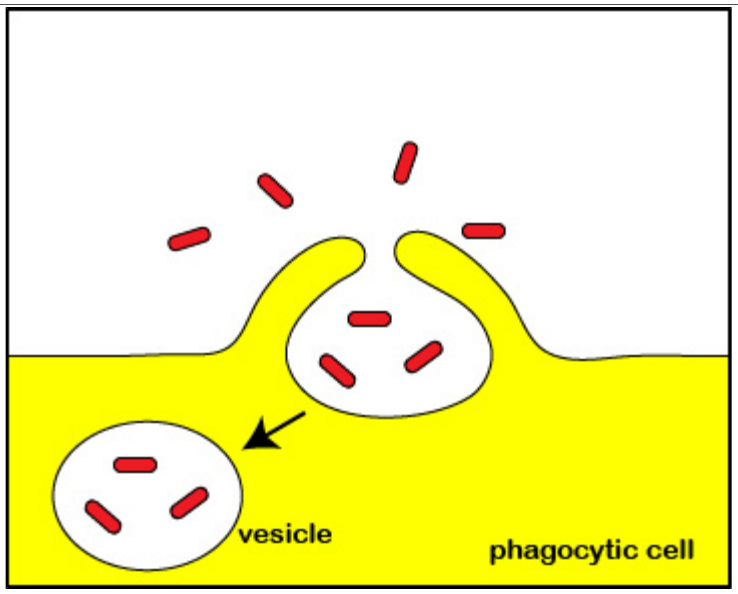
2. Macropinocytosis, a process where large volumes of surrounding fluid containing microbes are engulfed. This also enables dendritic cells to take in some encapsulated bacteria that might resist classical phagocytosis **(see Fig.3)**.

Fig. 2: Receptor-Mediated Phagocytosis



Receptor-mediated phagocytosis is the process whereby microbes are bound to phagocytic cells by means of molecules such as PAMPs, IgG, and C3b.

Fig. 3: Macropinocytosis



Macropinocytosis is a process whereby large volumes of fluid which may contain microbes are engulfed during pinocytosis.

The binding of microbial PAMPs to the PRRs of the immature dendritic cell activates that dendritic cell and promotes production of the chemokine receptor CCR7 that directs the dendritic cell into local lymphoid tissue. Following maturation, the dendritic cell can now present protein epitopes bound to MHC molecules to all the various naive T-lymphocytes passing through the lymphoid system (See **Fig. 4A** and **Fig. 4B**).

Fig. 4A: Immature Dendritic Cells Engulfing and Processing Microbes and Carrying them to Regional Lymph Nodes, Part 1

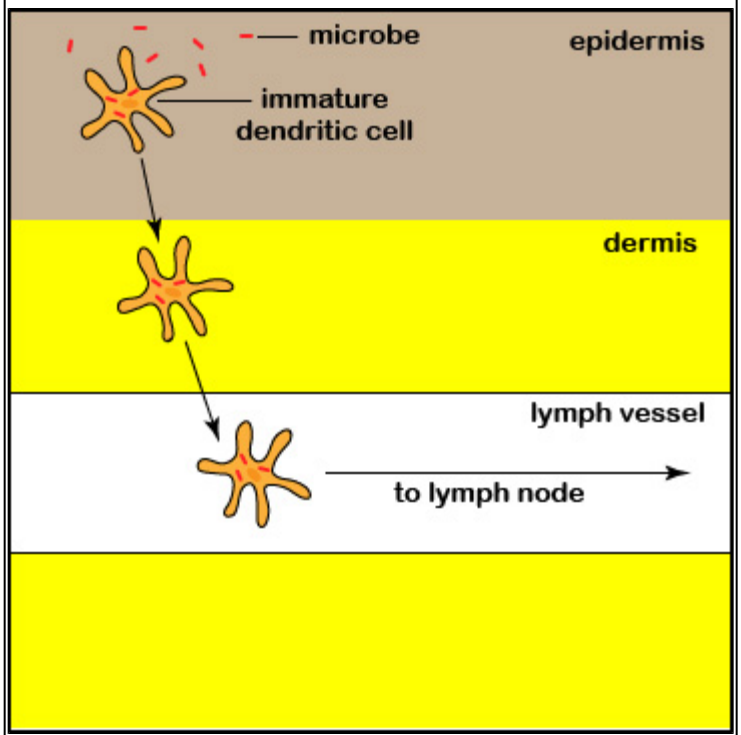
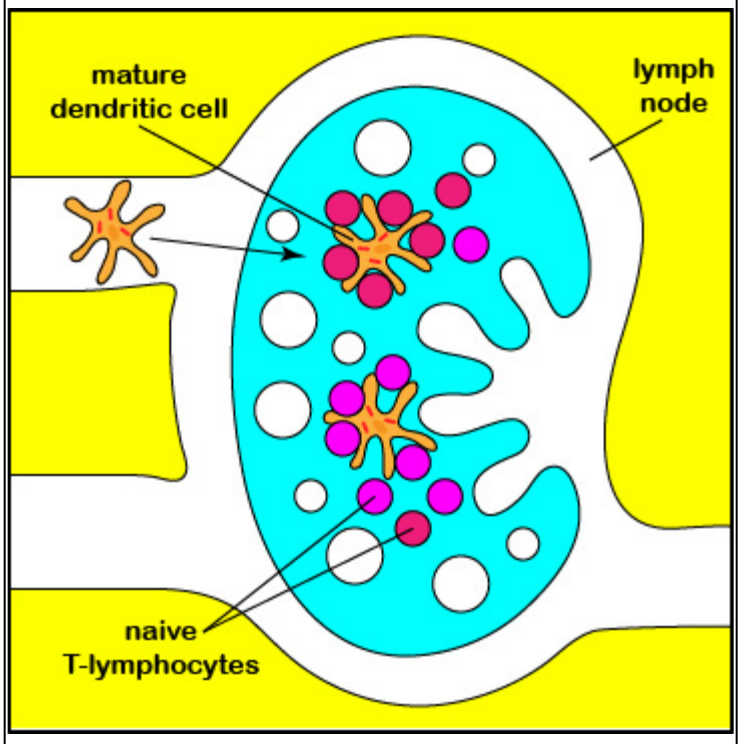


Fig. 4B: Immature Dendritic Cells Engulfing and Processing Microbes and Carrying them to Regional Lymph Nodes, Part 2

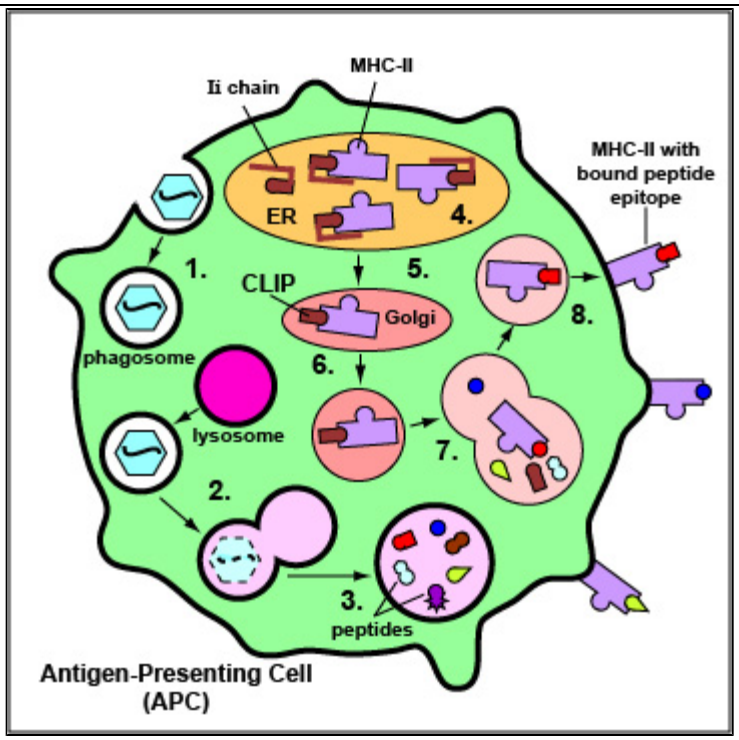


The binding of microbial PAMPs to the PRRs of the immature dendritic cell activates that dendritic cell and promotes

production of the chemokine receptor CCR7 that directs the dendritic cell into local lymphoid nodes. Following maturation, the dendritic cell can now present protein epitopes bound to MHC molecules to all the various naive T-lymphocytes passing through the lymphoid system.

The MHC-II molecules bind peptide epitopes from exogenous antigens and place them on the surface of the dendritic cell **(see Fig. 5)**. Here the MHC-II/peptide complexes can be recognized by complementary shaped T-cell receptors (TCRs) and CD4 molecules on naive T4-lymphocytes **(see Fig. 6)**.

Fig. 5: Binding of Peptide Epitopes from Exogenous Antigens to MHC-II Molecules



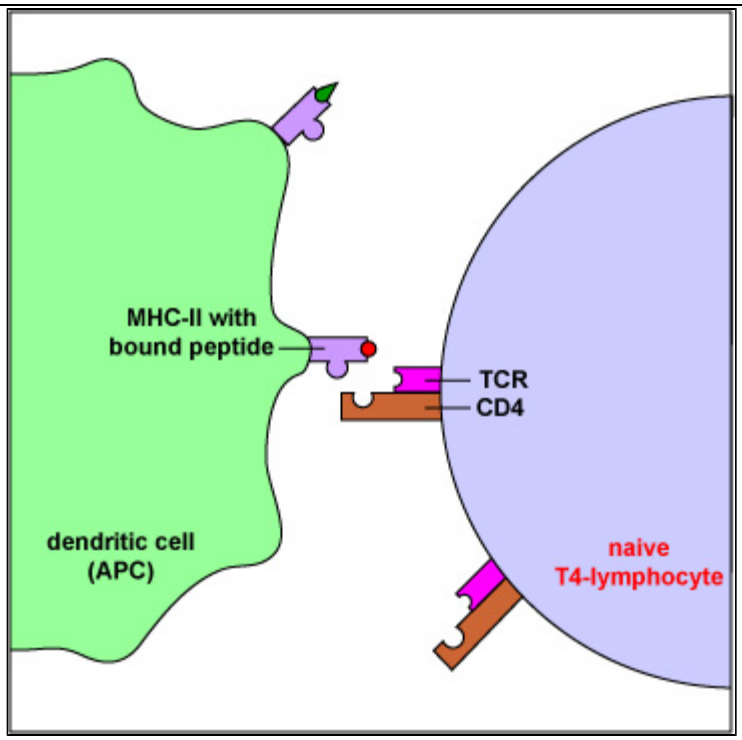
Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (macrophages, dendritic cells, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can

be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of endogenous antigens to MHC-II molecules for eventual presentation to naive T4-lymphocytes.)

Fig. 6: A Naive T4-Lymphocyte Recognizing Epitope/MHC-II on an Antigen-Presenting Dendritic Cell



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting dendritic cells through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

Flash animation of MHC-II molecules binding epitopes from exogenous antigens.

html5 version of animation for iPad showing MHC-II molecules binding epitopes from exogenous antigens.

Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (dendritic cells, macrophages, and B-lymphocytes) through phagocytosis. The

microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
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(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of endogenous antigens to MHC-II molecules for eventual presentation to naive T4-lymphocytes.)

Flash animation of a naive T4-lymphocyte recognizing epitopes bound to MHC-II molecules on an antigen-presenting dendritic cell.

html5 version of animation for iPad showing a naive T4-lymphocyte recognizing epitopes bound to MHC-II molecules on an antigen-presenting dendritic cell.

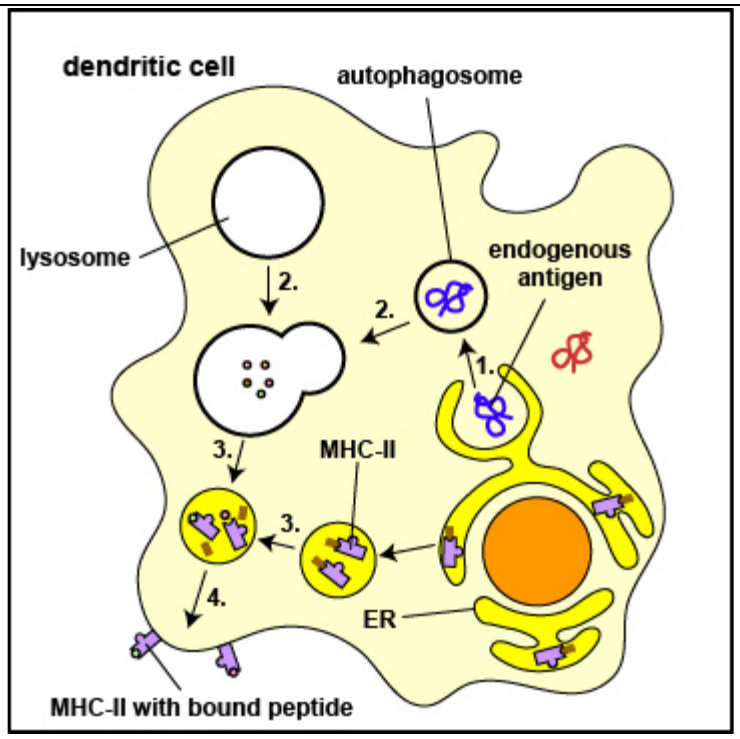
A naive T4-lymphocyte uses its TCR and CD4 molecules to bind to complementary shaped MHC-II molecule with attached peptide epitope on an antigen-presenting dendritic cell. As naive T4-lymphocytes migrate through the cortical region of lymph nodes, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding receptors such as ICAM-1, ICAM-2 and CD58 on the surface of dendritic cells. This transient binding allows time for the TCRs on the T4-lymphocyte to sample large numbers of MHC-II/peptide complexes on the antigen-presenting dendritic cells.

b. MHC-II cross-presentation of endogenous antigens to naive T4-lymphocytes

While most dendritic cells present exogenous antigens to naive T4-lymphocytes, certain dendritic cells are capable of cross-presentation of endogenous antigens to naive T4-lymphocytes. In this way, T4-lymphocytes can play a role in defending against both exogenous and endogenous antigens. This is done via autophagy, the cellular process whereby the cell's own cytoplasm is taken into specialized vesicles called autophagosomes **(see Fig. 7)**. The autophagosomes subsequently fuse with lysosomes containing proteases that will degrade the proteins in the autophagosome into peptides. From here, the peptides are transported into the vesicles containing MHC-II molecules where they can bind to the MHC-II groove, be transported to the surface of the dendritic cell, and interact with the TCRs and CD4 molecules of naive T4-lymphocytes **(see Fig .7)**.

Fig. 7: Cross-Presentation of Endogenous Antigens to MHC-II Molecules

by a Dendritic Cell Through Autophagy



Endogenous antigens can be cross-presented to MHC-II molecules by certain dendritic cells through a cellular process called autophagy.

1. Cellular cytoplasm containing endogenous antigens is engulfed and placed in a autophagosome.
2. After a lysosome fuses with the autophagosome, these endogenous proteins are degraded by lysosomal proteases into peptides.
3. The peptides are directly transfered from the autophagolysosome to the vesicles containing the MHC-II molecules.
4. The MHC-II molecules with bound peptides are then transported to the surface of the dendritic cell where they can be presented to naive T4-lymphocytes.

2. MHC-I presentation of protein antigens to naive T8-lymphocytes

Immature dendritic cells take in protein antigens for attachment to MHC-I molecules and subsequent presentation to naive T8-lymphocytes.

a. **MHC-I presentation of endogenous antigens to naive T8-lymphocytes**

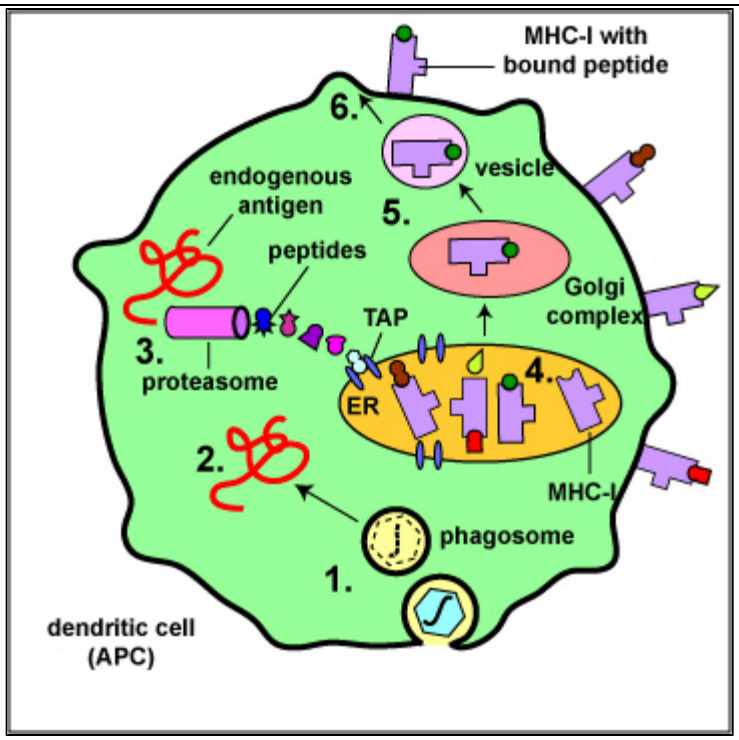
During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins are degraded into a variety of peptide epitopes by cylindrical organelles called proteasomes. The body's own cytosolic proteins are also degraded into peptides by proteasomes.

These peptide epitopes are then attached to a groove of MHC-I molecules that are then transported to the surface of that cell where they can be recognized by a complementary-shaped T-cell receptor (TCR) and a CD8 molecule, a co-receptor, on the surface of either a naive T8-lymphocyte or a cytotoxic T-lymphocyte (CTL). The TCRs recognize both the foreign peptide antigen and the MHC molecule. TCRs, however, will not recognize self-peptides bound to MHC-I. As

a result, normal cells are not attacked and killed.

MHC-I molecule with bound peptide on the surface of antigen-presenting dendritic cells **(see Fig. 8)** can be recognized by a complementary-shaped TCR/CD8 on the surface of a naive T8-lymphocyte to initiate cell-mediated immunity **(see Fig. 9)**.

Fig. 8: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Dendritic Cell



Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

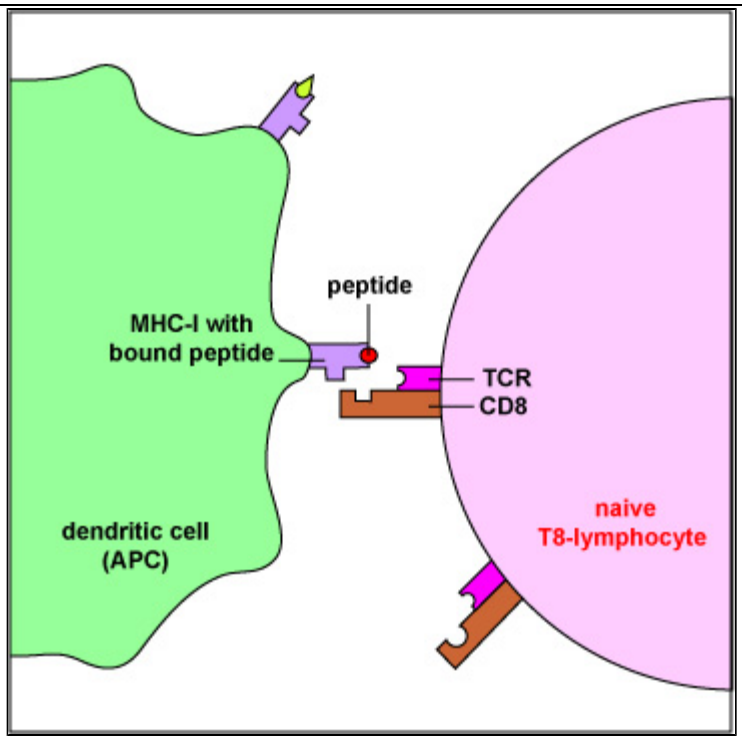
a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

Dendritic cells bind epitopes from endogenous antigens to MHC-I molecules and present them to naive T8-lymphocytes in order to activate these naive T8-lymphocytes.

1. Antigens are engulfed by dendritic cells and placed in a phagosome. Some of the proteins escape from the phagosome into the cytosol of the dendritic cell where they become endogenous antigens.
2. These endogenous antigens pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an

exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by naive T8-lymphocytes by way of TCRs and CD8 molecules having a complementary shape.

Fig. 9: An Antigen-Presenting Dendritic Cell Presenting MHC-I with Bound Peptide to a Naive T8-lymphocyte having a Complementary T-Cell Receptor



Antigen-presenting dendritic cells produce both MHC-I and MHC-II molecules. These APCs can phagocytose infected cells and tumor cells, place them in phagosomes, and degrade them with lysosomes. During this process, some of the proteins escape from the phagosome into the surrounding cytosol. Here they can be degraded into peptides by proteasomes, bound to MHC-I molecules, and placed on the surface of the dendritic cell. Now the peptide/MHC-I complexes can be recognized by a naive T8-lymphocyte having a complementary shaped T-cell receptor (TCR) and CD8 molecule. This activates the naive T8-lymphocyte enabling it to eventually proliferate and differentiate into cytotoxic T-lymphocytes (CTLs).

Flash animation of MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,

c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs. These CTLs are effector cells derived from T8-lymphocytes during cell-mediated immunity. However, in order to become CTLs, naive T8-lymphocytes must become activated by cytokines produced by antigen-presenting cells (APCs). This interaction between APCs and naive T8-lymphocytes occurs primarily in the lymph nodes, the lymph nodules, and the spleen. The process can be summarized as follows:

1. In addition to microbes, dendritic cells and macrophages also engulf and degrade infected cells, tumor cells, and the remains of killed infected and tumor cells. It is thought that in this manner, endogenous antigens from other cells are able to enter the APC. During phagocytosis, some proteins are released from the phagosomes into the cytosol of the APC.
2. These cytosolic proteins then pass through proteasomes, where proteases and peptidases chop the protein up into a series of peptides, typically 8-11 amino acids long.
3. A transporter protein called TAP located in the membrane of the cell's endoplasmic reticulum then transports these peptide epitopes into the endoplasmic reticulum where they bind to the grooves of various newly made MHC-I molecules.
4. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles .
5. The exocytic vesicles carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface.

The MHC-I molecules with bound peptide on the surface of the APCs can now be recognized by naive T8-lymphocytes possessing TCRs and CD8 molecules with a complementary shape. This recognition of the peptide epitope by the TCR serves as a first signal for activating the naive T8-lymphocyte for cell-mediated immunity function.

Flash animation of a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an antigen-presenting dendritic cell.

html5 version of animation for iPad showing a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an antigen-presenting dendritic cell.

A naive T8-lymphocyte uses its TCR and CD8 molecules to bind to complementary shaped MHC-I molecule with attached peptide epitope on an antigen-presenting dendritic cell. As naive T8-lymphocytes migrate through lymphoid tissues, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding surface receptors such as ICAM-1, ICAM-2 and CD58 on dendritic cells. This transient binding allows time for the TCRs on the T8-lymphocyte to sample large numbers of MHC-I/peptide complexes on the dendritic cells.

b. **MHC-I cross-presentation of exogenous antigens to naive T8-lymphocytes**

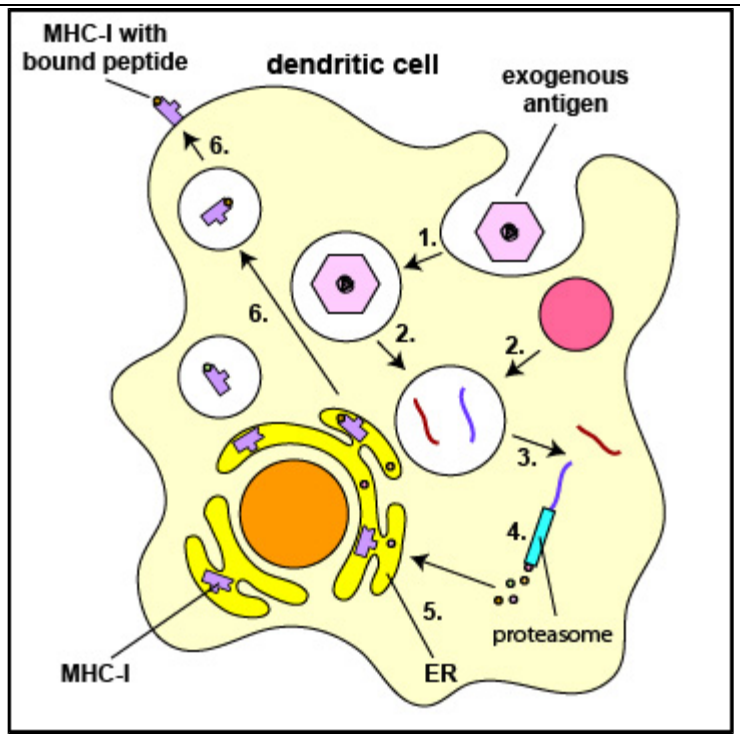
While most dendritic cells present endogenous antigens to naive T8-lymphocytes, certain dendritic cells are capable of cross-presentation of exogenous antigens to naive T8-lymphocytes. In this way, T8-lymphocytes can play a role in

defending against both exogenous and endogenous antigens. There are two proposed mechanisms for cross-presentation of exogenous antigens to T8-lymphocytes:

1. The dendritic cell engulfs the exogenous antigen and places it in a phagosome which then fuses with a lysosome to form a phagolysosome. The antigen is partially degraded in the phagolysosome where proteins are translocated into the cytoplasm where they are processed into peptides by proteasomes, enter the endoplasmic reticulum, and are bound to MHC-I molecules **(see Fig. 10)**.

2. The dendritic cell engulfs the exogenous antigen and places it in a phagosome which then fuses with a lysosome to form a phagolysosome. The protein antigens are degraded into peptides within the phagolysosome which then directly fuses with vesicles containing MHC-I molecules to which the peptides subsequently bind **(see Fig. 11)**.

Fig. 10: Cross-Presentation of Exogenous Antigens to MHC-I Molecules by a Dendritic Cell: Translocation of proteins in the Phagolysosome into the Cytoplasm

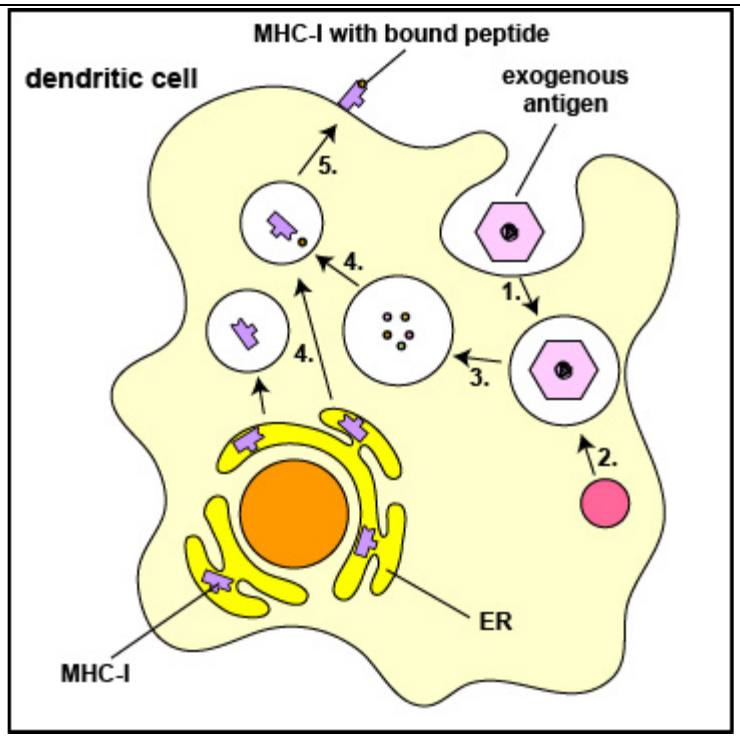


Exogenous antigens can be cross-presented to MHC-I molecules by certain dendritic cells. One proposed mechanism is via the translocation of proteins from the phagosome into the cytoplasm of the dendritic cell.

1. An exogenous antigen is engulfed and placed in a phagosome.
2. A lysosome fuses with the phagosome forming a phagolysosome.
3. Some proteins from the partially degraded exogenous antigen within the phagolysosome are translocated into the cytoplasm of the dendritic cell.
4. These proteins are then degraded into peptides by a proteasome.
5. The peptides enter the endoplasmic reticulum (ER) where they bind to MHC-I molecules.
6. The MHC-I/peptide complexes are then transported to the surface of the dendritic cell where they can be presented to naive T8-lymphocytes.

Fig. 11: Cross-Presentation of Exogenous Antigens to MHC-I Molecules by a Dendritic Cell: Transfer of Peptides from

Phagolysosome to Vesicles Containing MHC-I Molecules



Exogenous antigens can be cross-presented to MHC-I molecules by certain dendritic cells. One proposed mechanism is via the direct transfer of peptides from the phagolysosome into the vesicles containing the MHC-I molecules in the dendritic cell.

1. An exogenous antigen is engulfed and placed in a phagosome.
2. After a lysosome fuses with the phagosome forming a phagolysosome.
3. Proteins from the exogenous antigen are degraded by lysosomal proteases into peptides.
4. The peptides are directly transfered from the phagolysosome to the vesicles containing the MHC-I molecules.
5 . The MHC-I molecules with bound peptides are then transported to the surface of the dendritic cell where they can be presented to naive T8-lymphocytes.

In addition, **dendritic cells are very susceptible to infection by many different viruses. Once inside the cell, the viruses become endogenous antigens in the cytosol**. Once in the cytosol, the viral proteins from the replicating viruses are degraded into peptides by proteasomes where they subsequently bind to MHC-I molecules.

The binding of microbial PAMPs to the PRRs of the immature dendritic cell activates that dendritic cell and promotes production of the chemokine receptor CCR7 that directs the dendritic cell into local lymphoid tissue. Following maturation, the dendritic cell can now present protein epitopes bound to MHC molecules to all the various naive T-lymphocytes passing through the lymphoid system.

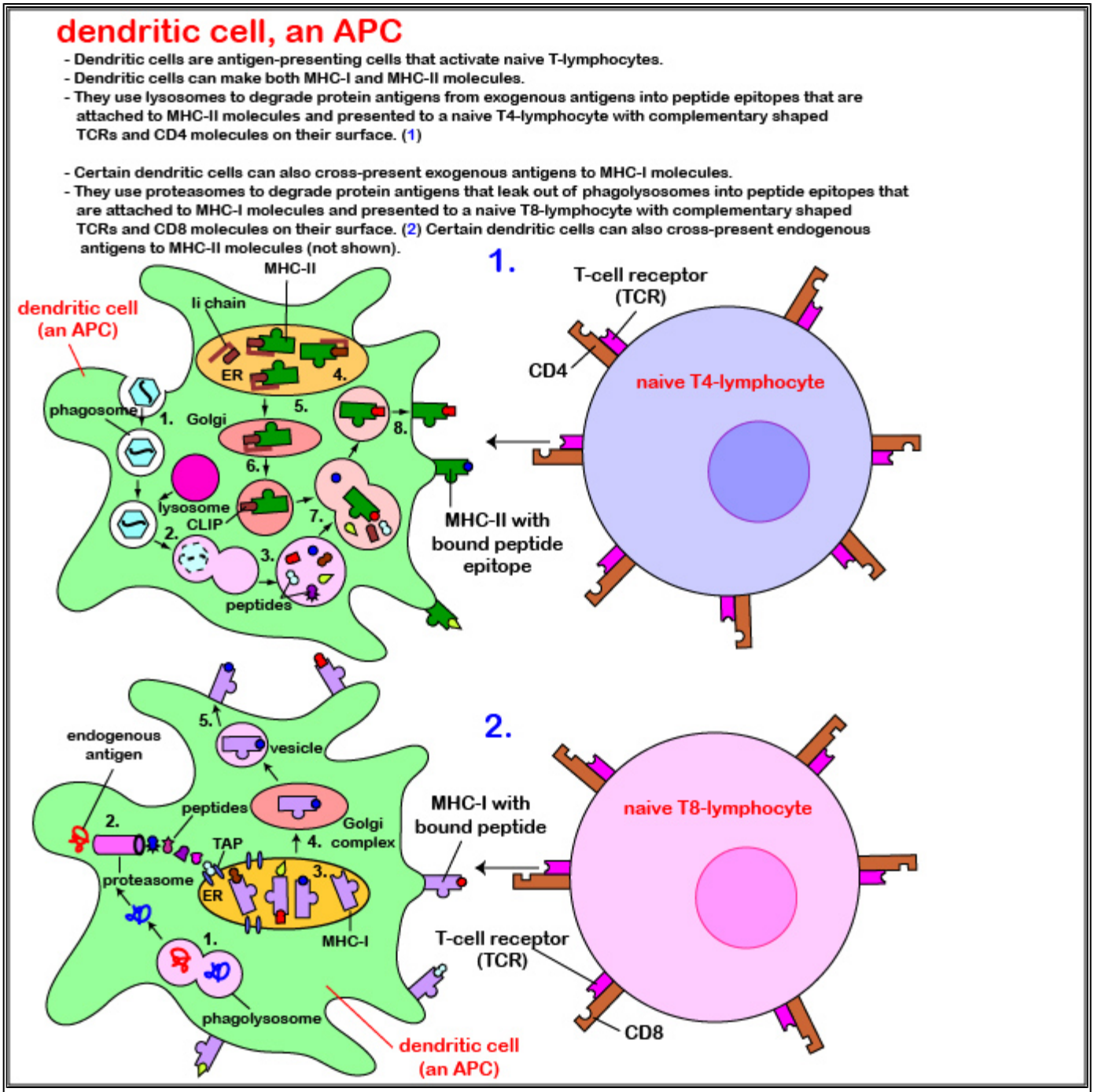
To view an electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #1 see the Web page for the University of Illinois College of Medicine.

To view an electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #2 see the Web page for the University of Illinois College of Medicine.

TPS Questions

For a Summary of Key Surface Molecules and Cellular Interactions of Antigen-Presenting Dendritic Cells, see Fig. 12.

Fig. 12: A Summary of Key Surface Molecules and Cellular Interactions of Antigen-Presenting Dendritic Cells



Concept Map for Antigen-Presenting Cells (APCs)

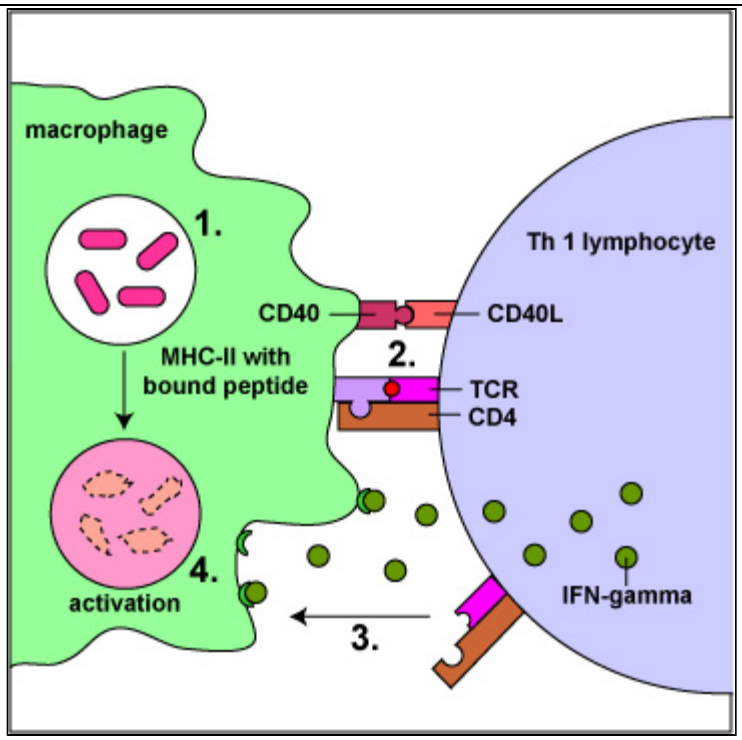
b. Macrophages

When monocytes leave the blood and enter the tissue, they become activated and differentiate into macrophages. Those that have recently left the blood during inflammation and move to the site of infection are sometimes referred to as wandering macrophages. In addition, the body has macrophages already stationed throughout the tissues and organs of the body. These are sometimes referred to as fixed macrophages. Many fixed macrophages are part of the mononuclear phagocytic (reticuloendothelial) system. They, along with B-lymphocytes and T-lymphocytes, are found supported by reticular fibers in lymph nodules, lymph nodes, and the spleen where they filter out and phagocytose foreign matter such as microbes. Similar cells derived from stem cells, monocytes, or macrophages are also found in the liver (Kupffer cells), the kidneys (mesangial cells), the brain (microglia), the bones (osteoclasts), the lungs (alveolar macrophages), and the gastrointestinal tract (peritoneal macrophages).

The primary function of macrophages, then, is to capture and present protein antigens to effector T-lymphocytes. (Effector lymphocytes are lymphocytes that have encountered an antigen, proliferated, and matured into a form capable of actively carrying out immune defenses.)

The MHC-II molecules bind peptide epitopes from exogenous antigens and place them on the surface of the macrophages. Here the MHC-II/peptide complexes can be recognized by complementary shaped T-cell receptors (TCRs) and CD4 molecules on an effector T4-lymphocytes (**see Fig.13)**.

Fig. 13: Activation of a Macrophage by a T_H1 Lymphocyte



1. Engulfed bacteria inside a phagosome or a phagolysosome.
2. An activated T_H1 lymphocyte binds to a peptide/MHC-II complex on a macrophage by way of its TCR and CD4 molecule. Co-stimulatory molecules such as CD40L on the T_H1 cell then bind to CD40 on a macrophage.

3. This triggers the T_H1 lymphocyte to secrete the cytokine interferon-gamma (IFN-Î³) that binds to IFN-Î³ receptors receptors on the macrophage.
4. The IFN-Î³ activates the macrophage enabling it to produce more hydrolytic lysosomal enzymes, nitric oxide, and toxic oxygen radicals that destroy the microorganisms within the phagosomes and phagolysosomes.

Effector T4-lymphocytes called T_H1 cells coordinate immunity against intracellular bacteria and promote opsonization by macrophages.

1. **They produce cytokines such as interferon-gamma (IFN-?) that promote cell-mediated immunity against intracellular pathogens**, **especially by activating macrophages that have either ingested pathogens or have become infected with intracellular microbes** such as *Mycobacterium tuberculosis, Mycobacterium leprae, Leishmania donovani*, and *Pneumocystis jiroveci* that are able to grow in the endocytic vesicles of macrophages. Activation of the macrophage by T_H1 cells greatly enhances their antimicrobial effectiveness **(see Fig. 13)**.

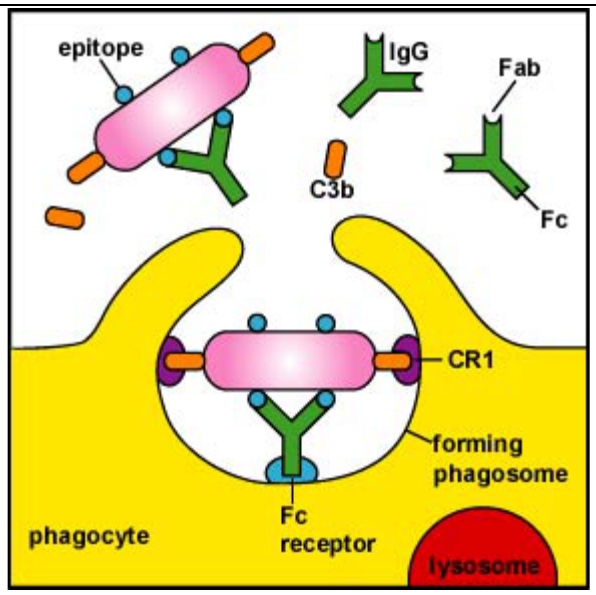
2. They produce cytokines that promote the **production of opsonizing and complement activating IgG that enhances phagocytosis (see Fig. 14)**.

3. They produce receptors that bind to and kill chronically infected cells, releasing the bacteria that were growing within the cell so they can be engulfed and killed by macrophages.

4. They **produce cytokines such as tumor necrosis factor-alpha (TNF-a) that promote diapedesis** of macrophages.

5. They produce the chemokine CXCL2 to attract macrophages to the infection site.

Fig. 14: Opsonization (Enhanced Attachment)



The Fab portion of the antibody IgG binds to epitopes of an antigen such as this bacterium. The Fc portion of IgG then binds to Fc receptors on phagocytes for opsonization or enhanced attachment. Once attached to the phagocyte the microbe can be engulfed more efficiently and placed in a phagosome. Complement pathway proteins such as C3b and C4b can also function as opsonins.

Flash animation of the activation of a macrophage by a T_H1 cell.

html5 version of animation for iPad showing the activation of a macrophage by a T_H1 cell.

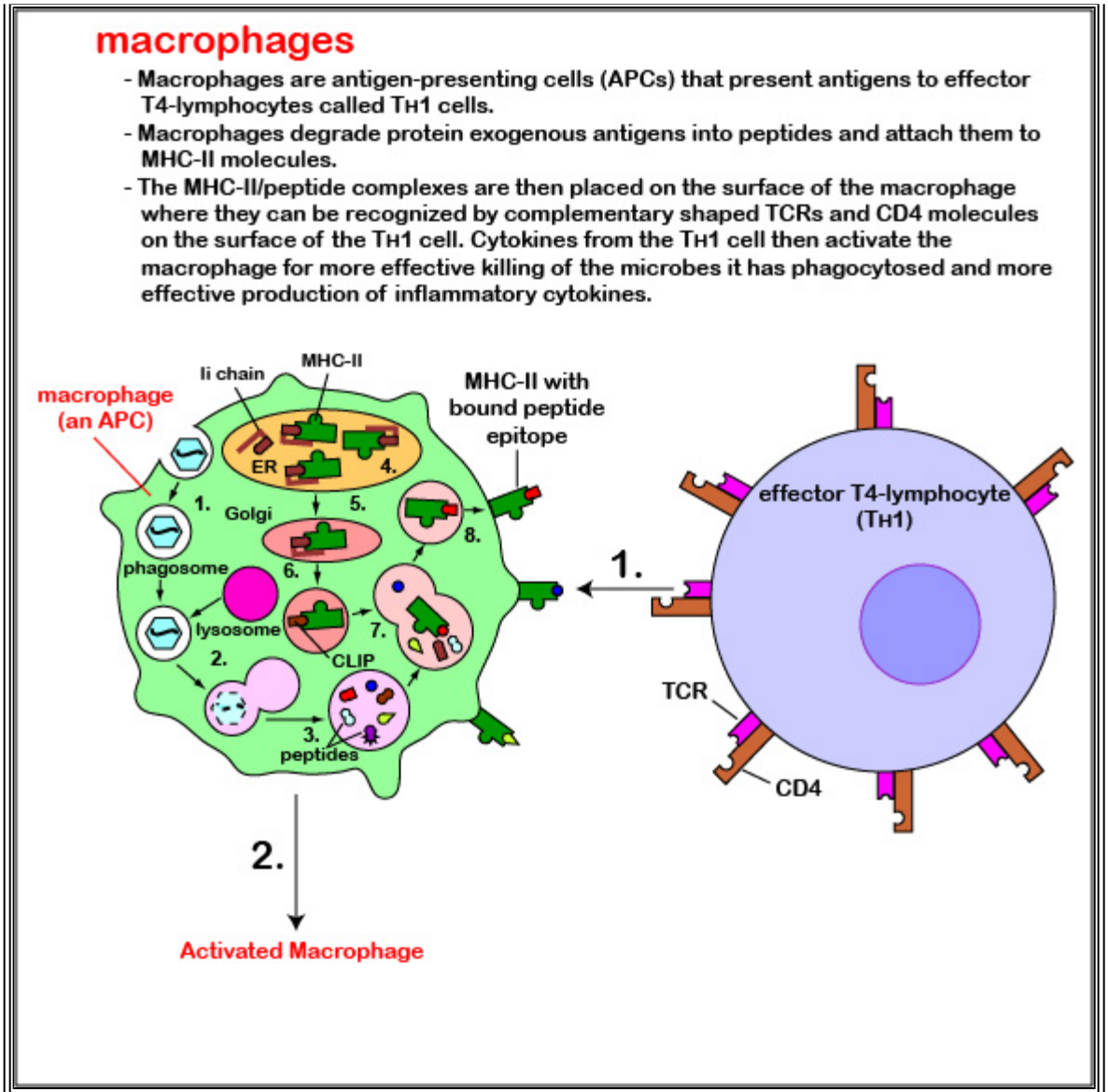
A major function of T_H1 cells is to both promote phagocytosis of microbes and the killing of intracellular microbes.

1. Bacteria are engulfed by a macrophage and placed in a phagosome. A lysosome fuses with the phagosome forming a phagolysosome.
2. An activated T_H1 lymphocyte binds to a peptide/MHC-II complex on a macrophage by way of its TCR and CD4 molecule.
3. Co-stimulatory molecules such as CD40L on the T_h1 cell then bind to CD40 on a macrophage.
4. The T_H1 lymphocyte secretes the cytokine interferon-gamma (IFN-Î³) that binds to IFN-Î³ receptors receptors on the macrophage.
5. The IFN-Î³ activates the macrophage enabling it to produce more hydrolytic lysosomal enzymes, nitric oxide, and toxic oxygen radicals that destroy the microorganisms within the phagosomes and phagolysosomes.

There is growing evidence that **monocytes and macrophages can be "trained" by an earlier infection to do better in future infections, that is, develop memory**. It is thought that microbial pathogen-associated molecular patterns (PAMPs) binding to pattern-recognition (PRRs) on monocytes and macrophages triggers the cell's epigenome to reprogram or train that cell to react better against new infections.

For a Summary of Key Surface Molecules and Cellular Interactions of Antigen-Presenting Macrophages, see Fig. 15.

Fig. 15: A Summary of Key Surface Molecules and Cellular Interactions of Antigen-Presenting Macrophages



Concept Map for Antigen-Presenting Cells (APCs)

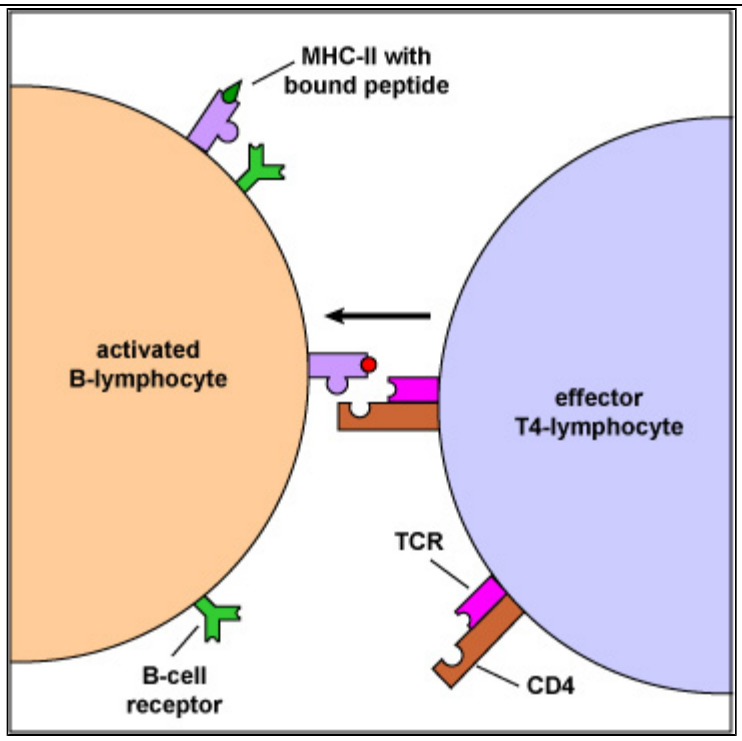
c. B-lymphocytes

Like all lymphocytes, B-lymphocytes circulate back and forth between the blood and the lymphoid system of the body. B-lymphocytes are able to capture and present peptide epitopes from exogenous antigens to effector T4-lymphocytes.

The MHC-II molecules bind peptide epitopes from exogenous antigens and place them on the surface of the B-lymphocytes. Here the MHC-II/peptide complexes can be recognized by complementary shaped T-cell receptors (TCRs) and CD4 molecules on an effector T4-lymphocytes **(see Fig. 16)**.

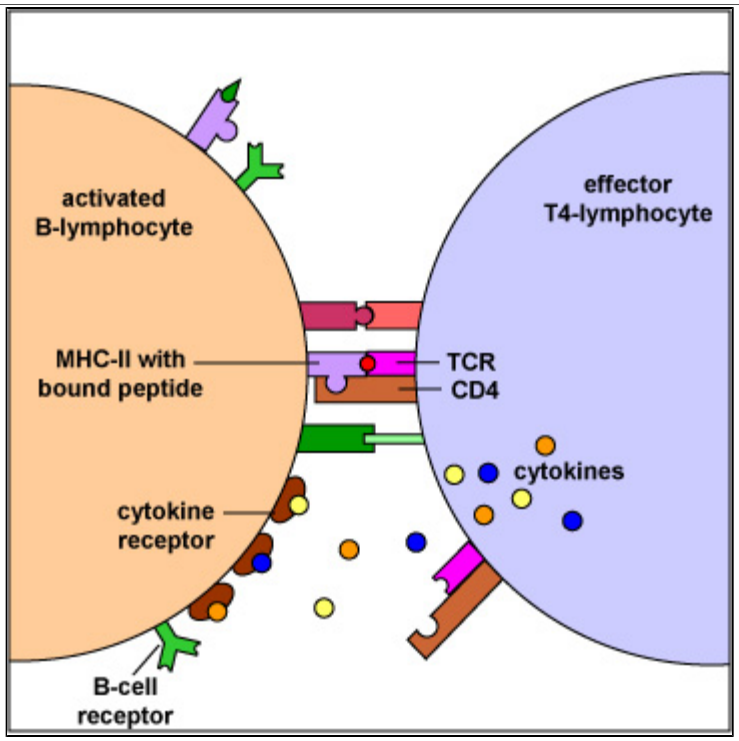
This interaction eventually triggers the effector T4-lymphocyte to produce and secrete various cytokines that enable that B-lymphocyte to proliferate and differentiate into antibody-secreting plasma cells **(see Fig. 17)**.

Fig. 16: An Effector T4-Lymphocyte Recognizing Epitope/MHC-II on an activated B-Lymphocyte



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens bind to B-cell receptors and enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II milecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize the peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

Fig. 17: An Effector T4-Lymphocyte Recognizing Epitope/MHC-II on an activated B-Lymphocyte and Producing Cytokines to Trigger Proliferation and Differentiation



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens bind to B-cell receptors and enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II milecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize the peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

This enables the effector T4-lymphocytes to produce cytokines such as interleukin-2 (IL-2) , interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-6 (IL-6). Collectively these cytokines:

1) Enable activated B-lymphocytes to proliferate;
2) Stimulate activated B-lymphocytes to synthesize and secrete antibodies;
3) Promote the differentiation of B-lymphocytes into antibody-secreting plasma cells; and
4) Enable antibody producing cells to switch the class or isotypeof antibodies being produced.

Flash animation of the binding of peptide epitopes to MHC-II molecules by a B-lymphocyte.

html5 version of animation for iPad showing the binding of peptide epitopes to MHC-II molecules by a B-lymphocyte

Exogenous antigens are those from outside cells of the body. Examples include bacteria, free

viruses, yeasts, protozoa, microbial proteins and polysaccharides, and toxins. These exogenous antigens bind to B-cell receptors either directly, or, more commonly, from the surface of either specialized macrophages or follicular dendritic cells (FDCs) located in the lymph nodes and the spleen. The antigens then enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Epitopes of exogenous antigens, such as viruses, bind to a complementary shaped B-cell receptor on a B-lymphocyte. The antigen is engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by effector T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Flash animation of an effector T4-lymphocyte recognizing epitopes bound to MHC-II molecules on a B-lymphocyte.

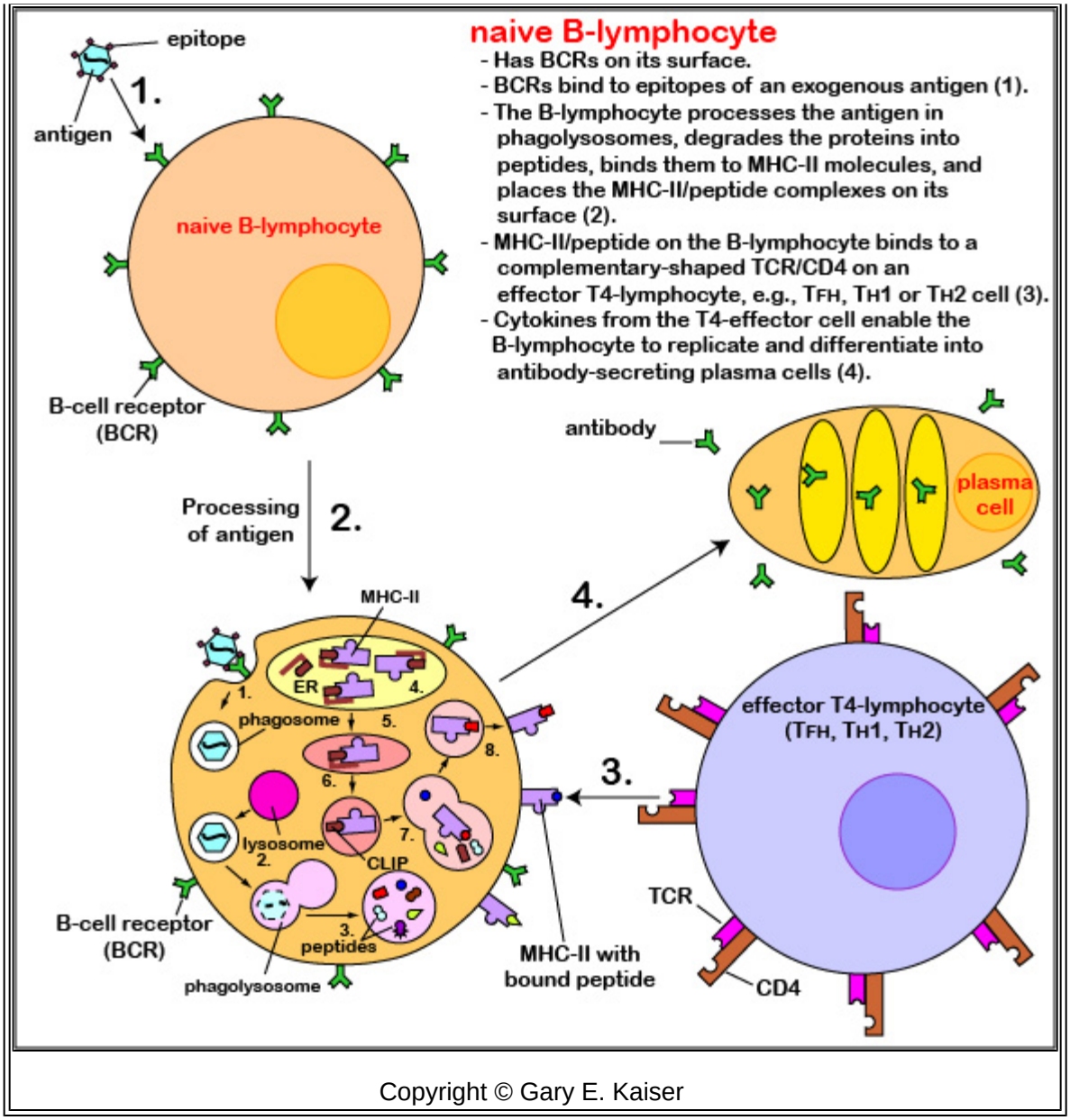
html5 version of animation for iPad showing an effector T4-lymphocyte recognizing epitopes bound to MHC-II molecules on a B-lymphocyte.

An effector T4-lymphocyte, such as a T_{FH} cell, use its TCRs and CD4 molecules to bind to a complementary shaped MHC-II molecules with attached peptide epitope on an activated B-lymphocyte. This interaction, along with the binding of co-stimulatory molecules such as CD40 and B7 on the B-lymphocyte with their complementary ligands CD40L and CD28 on the effector T4-lymphocyte triggers the T4-lymphocyte to produce cytokines that enable the activated B-lymphocyte to proliferate, differentiate into antibody-secreting plasma cells, and switch classes of the antibodies being made.

For a Summary of Key Surface Molecules and Cellular Interactions of Antigen-Presenting B-Lymphocytes, see Fig. 18.

Fig. 18: A Summary of Key Surface Molecules and Cellular Interactions of Antigen-Presenting B-Lymphocytes

ADAPTIVE IMMUNITY:



Concept Map for Antigen-Presenting Cells (APCs)

For more information: Preview of B-lymphocytes

For more information: Preview of T4-lymphocytes

For more information: Preview of T8-lymphocytes

For more information: Review of MHC molecules

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Return to Softchalk Lessons Table of Contents

ADAPTIVE IMMUNITY:
IMPORTANT CELLS AND MOLECULES: T4-LYMPHOCYTES

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Important Cells and Molecules for Adaptive Immunity: T4-Lymphocytes (T4-Cells, CD4+ Cells)
Common Course Objectives
Detailed Learning Objectives

Adaptive Immunity

Important Cells and Molecules for Adaptive Immunity: T4-Lymphocytes (T4-Cells, $CD4^+$ Cells)

Fundamental Statements for this Softchalk Lesson:

1. T-lymphocytes refer to lymphocytes that are produced in the bone marrow but require interaction with the thymus for their maturation .
2. The primary role of T4-lymphocytes is to regulate the body's immune responses through the production of cytokines.
3. T4-lymphocytes display CD4 molecules and T-cell receptors (TCRs) on their surface.
4. The TCR on T4-lymphocytes, in cooperation with CD4, typically bind peptides from exogenous antigens bound to MHC-II molecules.
5. During its development, each T4-lymphocyte becomes genetically programmed to produce a TCR with a unique specificity that is able to bind an epitope/MHC-II complex on an APC such as a dendritic cell, a macrophage, or a B-lymphocyte possessing a corresponding shape.
6. To become activated, naive T4-lymphocytes migrate through lymph nodes where the TCRs on the T4-lymphocyte are able to sample large numbers of MHC-II/peptide complexes on the antigen-presenting dendritic cells for ones that "fit", thus enabling activation of that naïve T4-lymphocyte.
7. After activation, the dendritic cell produces cytokines that contribute to proliferation of the T4-lymphocytes and their differentiation into effector T4-lymphocytes, the cells the body uses to regulate both humoral immunity and cell-mediated immunity through the cytokines they produce.
8. Some of the T4-lymphocytes differentiate into circulating T4-memory cells that enable a more 9. rapid and greater production of effector T4-lymphocytes upon subsequent exposure to the same antigen.
10. Functionally, there are many different types or subpopulations of effector T4-lymphocytes based on the cytokines they produce. Immune reactions are typically dominated by five primary types: TH1 cells, TH2 cells, TH17 cells, Treg cells, and TFH cells.
11. CD4 TH1 cells coordinate immunity against intracellular bacteria and promote opsonization.
12. CD4 TH2 cells coordinate immunity against helminths and microbes that colonize mucous membranes.

ADAPTIVE IMMUNITY:
IMPORTANT CELLS AND MOLECULES: T8-LYMPHOCYTES

Adaptive Immunity

Important Cells and Molecules for Adaptive Immunity: T8-Lymphocytes (T8-Cells, $CD8^+$ Cells)

Fundamental Statements for this Softchalk Lesson:

1. T-lymphocytes refer to lymphocytes that are produced in the bone marrow but require interaction with the thymus for their maturation.

2. The primary role of T8-lymphocytes is to kill infected cells and tumor cells by inducing apoptosis of those cells.
3. Once naive T8-lymphocytes are activated by dendritic cells, they proliferate and differentiate into T8-effector lymphocytes called cytotoxic T-lymphocytes (CTLs) that bind to and kill infected cells and tumor cells.
4. T8-lymphocytes display CD8 molecules and T-cell receptors (TCRs) on their surface.
5. The TCR on T8-lymphocytes, in cooperation with CD8, typically bind peptides from endogenous antigens bound to MHC-I molecules.
6. During its development, each T8-lymphocyte becomes genetically programmed, by gene-splicing reactions similar to those in B-lymphocytes and T4-lymphocytes, to produce a TCR with a unique shape capable of binding epitope/MHC-I complex with a corresponding shape.
7. To become activated, naive T8-lymphocytes migrate through lymph nodes where the TCRs on the T8-lymphocyte are able to sample large numbers of MHC-I/peptide complexes on the antigen-presenting dendritic cells for ones that "fit", thus enabling activation of that naïve T8-lymphocyte.
8. After activation, the dendritic cell produces cytokines that contribute to proliferation of the T8-lymphocytes and their differentiation into effector T4-lymphocytes called cytotoxic T-lymphocytes (CTLs) that are able to bind to and kill infected cells and tumor cells displaying the same peptide/MHC-I complex on their surface.
9. Some of the T8-lymphocytes differentiate into circulating T8-memory cells that enable a more rapid and greater production of CTLs upon subsequent exposure to the same antigen.
10. During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins in the cytosol of that cell are degraded into a variety of peptide epitopes by cylindrical organelles called proteasomes.
11. As these various endogenous antigens pass through proteasomes, proteases and peptidases chop the protein up into a series of peptides that are transported into the endoplasmic reticulum where they bind to newly made MHC-I molecules.
12. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles that carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface.
13. CTLs are, by way of their TCRs and CD8 molecules, are then able to recognize infected cells and tumor cells displaying MHC-I molecules with bound peptides on their surface. This sends a signal that triggers the release of the perforins/granzymes/granulysin complexes from the CTL to destroy the infected cell or tumor cell through apoptosis.
14. The perforin molecules may put pores in the membrane of the target cell allowing the granzymes to directly enter the cytosol, and certain granzymes activate the caspase enzymes that lead to apoptosis of the infected cell or tumor cell by destroying the cytoskeleton of the cell and degrading both the target cell's nucleoprotein and any microbial DNA within the cell.

Common Course Objectives

1. Contrast the MHC-I and MHC-II systems in terms of how they process antigens for recognition by T-cells.
2. Explain the role of antigen presenting cells in the adaptive immune system.

3. State where in the body antigen-presenting cells, naïve B-cells, and naive T-cells come together to initiate adaptive immunity.
4. Explain how TCR and BCR diversity is achieved.
5. Compare and contrast how T4-cells and T8-cells are activated.
6. Describe how activated cytotoxic T-cells kill target cells.

Detailed Learning Objectives

1*. Describe the overall function of T8-lymphocytes and their activation in terms of the following:

a. the role of their TCRs and CD8 molecules

b. how they are activated by antigen-presenting dendritic cells

c. the type of effector cells into which activated T8-lymphocytes differentiate

d. what CTLs recognize on infected cells and tumor cells

e. how CTLs kill infected cells and tumor cells

2*. State the overall function of T8-lymphocytes in adaptive immunity.

(*) = Common theme throughout the course

TPS Questions

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of T8-lymphocytes in adaptive immune responses.

Important Cells and Molecules for Adaptive Immunity: T8-Lymphocytes (T8-Cells, $CD8^+$ Cells)

The primary role of T8-lymphocytes is to kill infected cells and tumor cells by inducing apoptosis of those cells. Once naive T8-lymphocytes are activated by dendritic cells, they proliferate and differentiate into T8-effector lymphocytes called cytotoxic T-lymphocytes (CTLs) that bind to and kill infected cells and tumor cells.

T8-lymphocytes are T-lymphocytes displaying a surface molecule called **CD8**. T8-lymphocytes also have on their surface, **T-cell receptors or TCRs** similar to those on T4-lymphocytes. The TCR on T8-lymphocytes, in cooperation with CD8, **bind peptides from endogenous antigens bound to MHC-I molecules**.

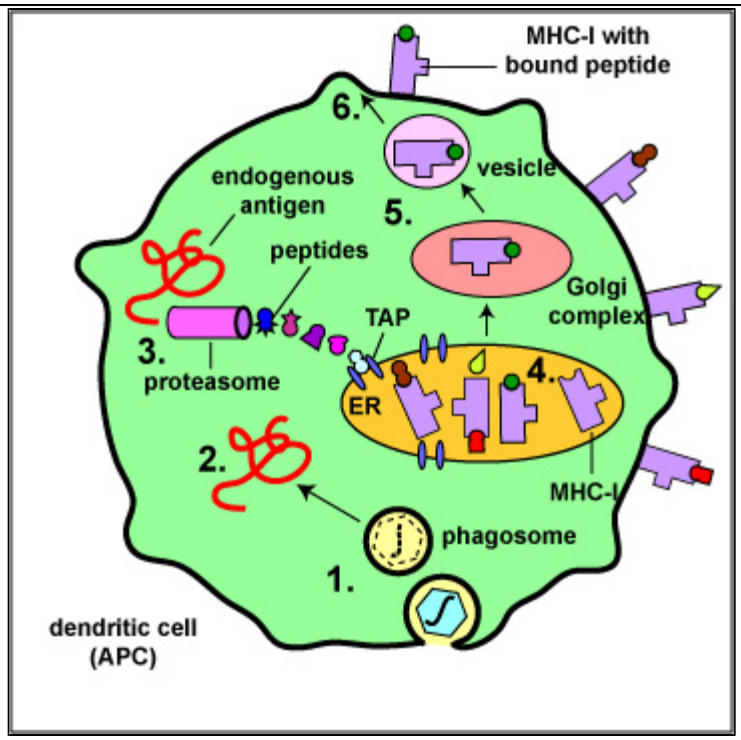
During its development, **each T8-lymphocyte becomes genetically programmed**, by gene-splicing reactions similar to those in B-lymphocytes and T4-lymphocytes, **to produce a TCR with a unique shape** capable of **binding epitope/MHC-I complex** with a corresponding shape. It is estimated that the human body has the ability to recognize 10^7 or more different epitopes. In order to recognize this immense number of different epitopes, the body produces 10^7 or more distinct clones of T-lymphocytes, each with a unique T-cell receptor. In this variety of T-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, peptides of any antigen the immune system eventually encounters.

1. Activation of a naive T8-lymphocyte by a dendritic cell

One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs

These CTLs are effector cells **derived from naive T8-lymphocytes** during cell-mediated immunity. However, in order to become CTLs, naive T8-lymphocytes must become activated by dendritic cells as shown in **Fig. 1** and **Fig. 2**.

Fig. 1: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Dendritic Cell



Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. Viral proteins produced during viral replication,
b. Proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. Proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. Tumor antigens produced by cancer cells, and
e. Self peptides from human cell proteins.

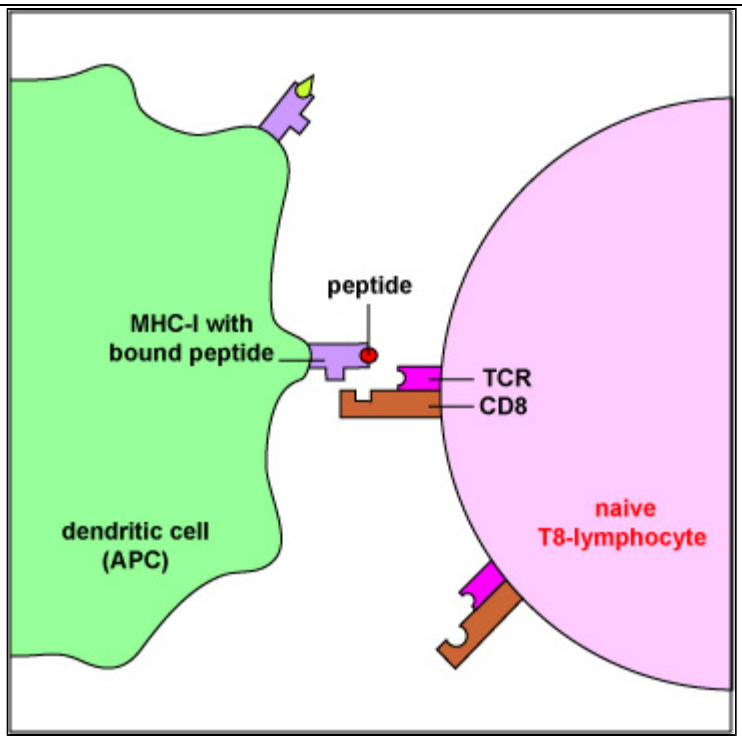
Dendritic cells bind epitopes from endogenous antigens to MHC-I molecules and present them to naive T8-lymphocytes in order to activate these naive T8-lymphocytes.

1. Antigens are engulfed by dendritic cells and placed in a phagosome. Some of the proteins escape from the phagosome into the cytosol of the dendritic cell where they

become endogenous antigens.
2. These endogenous antigens pass through proteasomes where they are degraded into a series of peptides.
3. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
4. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
5. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
6. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by naive T8-lymphocytes by way of TCRs and CD8 molecules having a complementary shape.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.)

Fig. 2: An Antigen-Presenting Dendritic Cell Presenting MHC-I with Bound Peptide to a Naive T8-lymphocyte having a Complementary T-Cell Receptor



Antigen-presenting dendritic cells produce both MHC-I and MHC-II molecules. These APCs can phagocytose infected cells and tumor cells, place them in phagosomes, and degrade them with lysosomes. During this process, some of the proteins escape from the phagosome into the surrounding cytosol. Here they can be degraded into peptides by proteasomes, bound to MHC-I molecules, and placed on the surface of the dendritic cell. Now the peptide/MHC-I complexes can be recognized by a naive T8-lymphocyte having a complementary shaped T-cell receptor (TCR) and CD8 molecule. This activates the naive T8-lymphocyte enabling it to eventually proliferate and differentiate into cytotoxic T-lymphocytes (CTLs).

Flash animation of MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting dendritic cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting dendritic cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. Viral proteins produced during viral replication,
b. Proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. Proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting dendritic cells,
d. Tumor antigens produced by cancer cells, and
e. Self peptides from human cell proteins.

One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs. These CTLs are effector cells derived from naive T8-lymphocytes during cell-mediated immunity. However, in order to become CTLs, naive T8-lymphocytes must become activated by cytokines produced by antigen-presenting dendritic cells. This interaction between dendritic cells and naive T8-lymphocytes occurs primarily in the lymph nodes, the lymph nodules, and the spleen. The process can be summarized as follows:

1. In addition to microbes, dendritic cells and macrophages also engulf and degrade infected cells, tumor cells, and the remains of killed infected and tumor cells. It is thought that in this manner, endogenous antigens from other cells are able to enter the dendritic cell. During phagocytosis, some protein antigens from the engulfed exogenous antigens are translocated from the phagosomes or phagolysosome of the dendritic cell to the cytosol where they become "endogenous" antigens.
2. These cytosolic protein antigens then pass through proteasomes, where proteases and peptidases chop the protein up into a series of peptides.
3. A transporter protein called TAP located in the membrane of the cell's endoplasmic reticulum then transports these peptide epitopes into the endoplasmic reticulum where they bind to the grooves of various newly made MHC-I molecules.
4. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles .
5. The exocytic vesicles carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface.

The MHC-I molecules with bound peptide on the surface of the APCs can now be recognized by naive T8-lymphocytes possessing TCRs and CD8 molecules with a complementary shape. This recognition of the peptide epitope by the TCR serves as a first signal for activating the naive T8-lymphocyte for cell-mediated immunity function.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.)

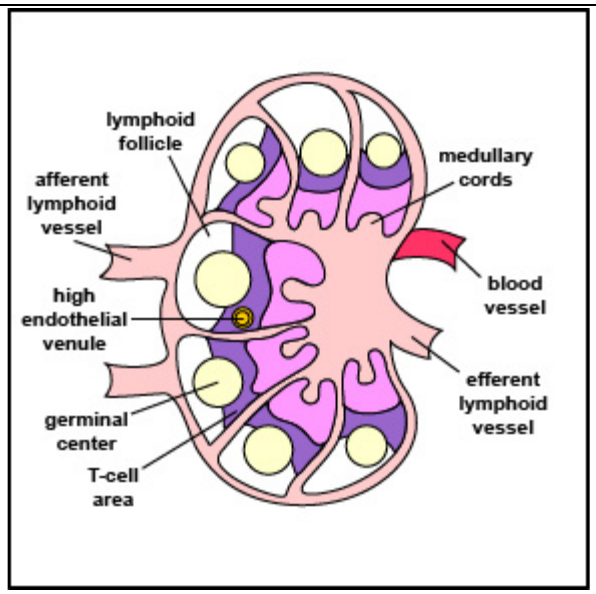
To view an electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #1 see the Web page for the University of Illinois College of Medicine.

To view an electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #2 see the Web page for the University of Illinois College of Medicine.

Certain dendritic cells are capable of cross-presentation of exogenous antigens to naive T8-lymphocytes. In this way, T8-lymphocytes can play a role in defending against both exogenous and endogenous antigens.

Naive T-lymphocytes circulate in the blood. In response to chemokines produced by lymphoid tissues, they leave the vascular endothelium in regions called high endothelial venules and enter lymph nodes **(see Fig. 3)** or other lymphoid tissues, a process called diapedesis.

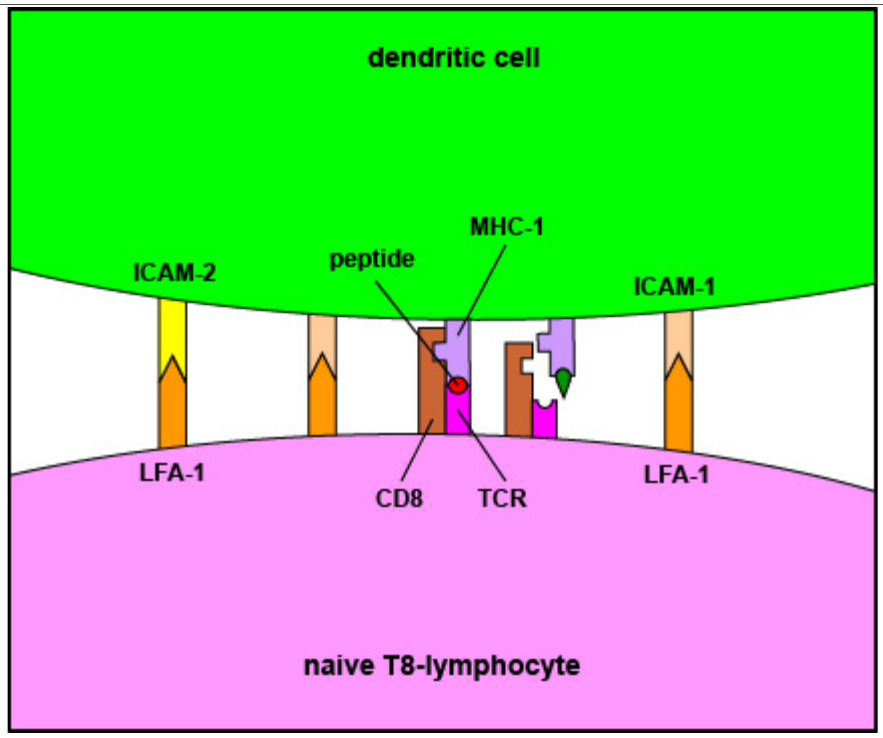
Fig. 3: Structure of a Lymph Nodes



Antigens enter lymph nodes through afferent lymphoid vessels. Antigen-presenting dendritic cells enter the lymph node through afferent lymphatic vessels while naive B-lymphocytes, and naive T-lymphocytes enter through high endothelial venules. Non-activated and effector lymphocytes leave the lymph node through efferent lymphatic vessels. Naive B-lymphocytes become activated, proliferate, and differentiate into plasma cells in the germinal centers of lymphoid follicles while naive T-lymphocytes become activated, proliferate and differentiate into T-effector lymphocytes in the T-cell area.

As naive T8-lymphocytes migrate through the cortical region of lymph nodes, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding receptors such as ICAM-1, ICAM-2 and CD58 on the surface of dendritic cells. This transient binding allows time for the TCRs on the T8-lymphocyte to sample large numbers of MHC-I/peptide complexes on the antigen-presenting dendritic cells **(see Fig. 4)**.

Fig. 4: Transient binding of T8-Lymphocytes to Dendritic Cells



As naive T8-lymphocytes migrate through the cortical region of lymph nodes, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding receptors such as ICAM-1, ICAM-2 and CD58 on the surface of dendritic cells. This transient binding allows time for the TCRs on the T8-lymphocyte to sample large numbers of MHC-I/peptide complexes on the antigen-presenting dendritic cells.

Flash animation of the activation of a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an antigen-presenting dendritic cell.

html5 version of animation for iPad showing the activation of a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an antigen-presenting dendritic cell.

A naive T8-lymphocyte uses its TCR and CD8 molecules to bind to complementary shaped MHC-I molecule with attached peptide epitope on an antigen-presenting dendritic cell. As naive T8-lymphocytes migrate through lymphoid tissues, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding surface receptors such as ICAM-1, ICAM-2 and CD58 on dendritic cells. This transient binding allows time for the TCRs on the T8-lymphocyte to sample large numbers of MHC-I/peptide complexes on the dendritic cells.

Those naive T8-lymphocytes not activated by epitopes of antigens on the dendritic cells exit the lymph node (or other lymphoid tissue) and eventually re-enter the bloodstream. However, if a TCR and CD8 molecule of the naive T8-lymphocyte detects a corresponding MHC-I/peptide complex on a mature dendritic cell, this will send a first signal for the activation of that naive T-lymphocyte. Next, a second signal that promotes survival of that T-lymphocyte is sent when co-stimulatory molecules such as B7.1 and B7.2 on the dendritic cell bind to CD28 molecules on the T8-lymphocyte. Finally, the dendritic cell produces cytokines such as interleukin-6 (IL-6), IL-4, IL-12, and T-cell growth factor-beta (TGF-ß) that contribute to proliferation of the T8-lymphocytes and their differentiation into **effector T8-lymphocytes** called cytotoxic T-lymphocytes (CTLs) that are able to bind to and kill infected cells and tumor cells displaying the same peptide/MHC-I complex on their surface. (Activated T8-lymphocytes remain in the lymph node as they proliferate (clonal expansion) and only leave the lymphoid tissues and re-enter the bloodstream after they have differentiated into CTLs.)

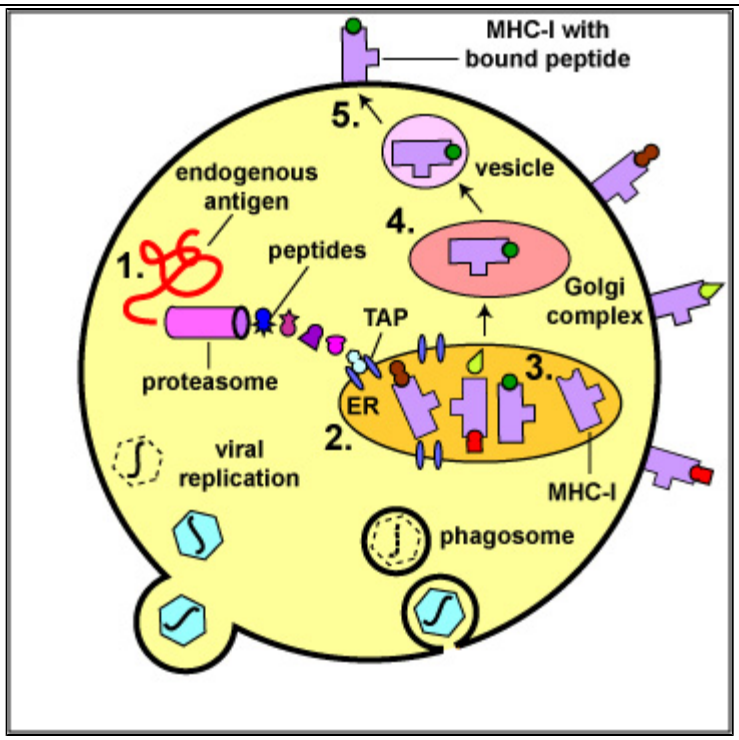
While activated T8-lymphocytes produce interleukin-2 (IL-2) as well as a high-affinity IL-2 receptor themselves, in most cases it is the IL-2 produced by effector T4-lymphocytes that enables cell proliferation and formation of a clone of thousands of identical T8-lymphocytes after several days. IL-2 also contributes to survival of those activated T8-lymphocytes and their differentiation into T8-effector cells called a cytotoxic T-lymphocytes or CTLs.

CTLs leave the secondary lymphoid organs and enter the bloodstream where they can be delivered anywhere in the body via the circulatory system and the inflammatory response. In addition, some of the T8-lymphocytes **differentiate into circulating T8-memory cells**. Circulating T8-memory cells allow for **a more rapid and greater production of CTLs upon subsequent exposure to the same antigen**.

2. Marking an infected cell or tumor cell for destruction by cytotoxic T-lymphocytes (CTLs)

During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins in the cytosol of that cell are degraded into a variety of peptide epitopes by cylindrical organelles called **proteasomes**. Other endogenous antigens such as proteins released into the cytosol from the phagosomes of antigen-presenting cells, such as macrophages and dendritic cells as well, as a variety of the human cell's own proteins (self-proteins) are also degraded by proteasomes. As these various endogenous antigens pass through proteasomes, **proteases and peptidases chop the protein up into a series of peptides**, typically 8-11 amino acids long **(see Fig. 5)**.

Fig. 5: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules



Endogenous antigens are those located within the cytosol of cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells

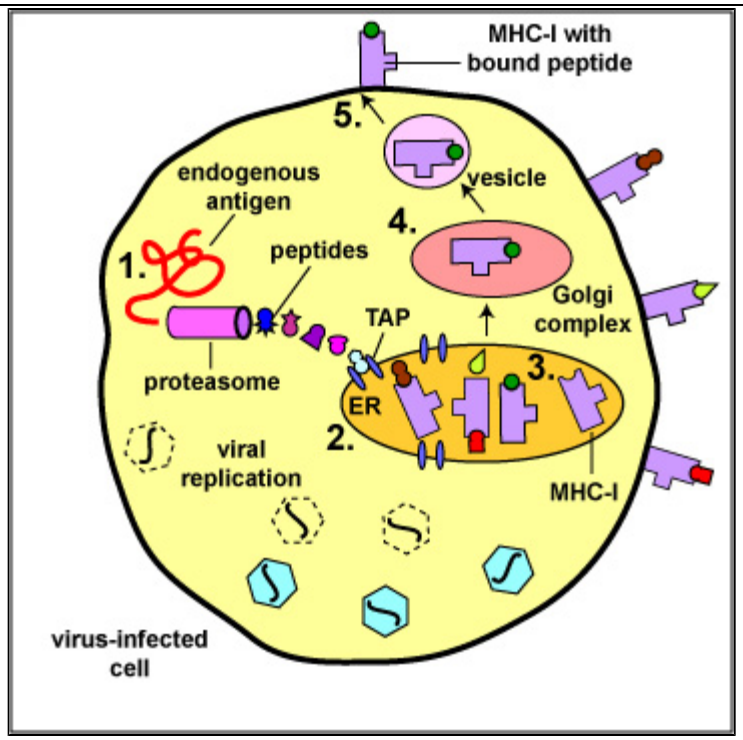
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. Endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

A transporter protein called **TAP** located in the membrane of the cell's endoplasmic reticulum then **transports these peptide epitopes into the endoplasmic reticulum where they bind to the grooves of various newly made MHC-I molecules**. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles. The **exocytic vesicles carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface (see Fig. 6)**. A single cell may have up to 250,000 molecules of MHC-I with bound epitope on its surface.

Fig. 6: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Virus-Infected Cell



Endogenous antigens are those located within the cytosol of the cells of the body. Examples

include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. During viral replication within the host cell, endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

Flash animation of MHC-I molecules binding epitopes from endogenous antigens in an infected cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from endogenous antigens in an infected cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
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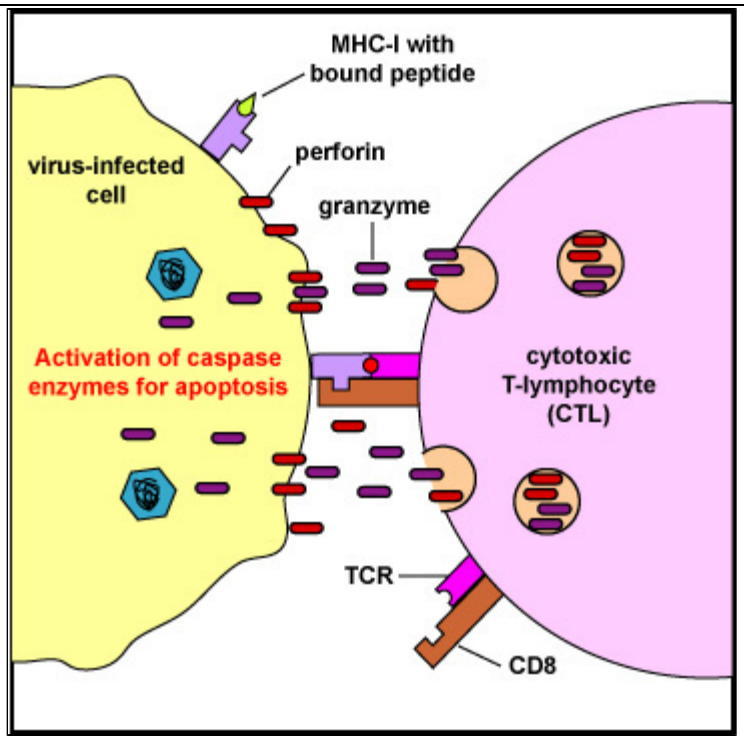
3. CTLs binding to infected cells or tumor cells and inducing apoptosis

CTLs are, by way of their TCRs and CD8 molecules, able to recognize infected cells and tumor cells displaying MHC-I molecules with bound peptides on their surface **(see Fig. 7)** and destroy them through apoptosis, a programmed cell suicide.

Apoptosis involves a complex of intracellular granules. This complex of granules in a protected state including:

1. Pore-forming proteins called perforins;
2. Proteolytic enzymes called granzymes; and
3. A proteoglycan called granulysin.

Fig. 7: Cytotoxic T-lymphocyte (CTL)-Induced Apoptosis of a Virus-Infected Cell



Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the caspase enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes.

When the TCR and CD8 of the CTL binds to the MHC-I/epitope on the surface of the virus-infected cell or tumor cell **(see Fig. 7)**, this sends a signal through a CD3 molecule which triggers the release of the perforins/granzymes/granulysin

complexes from the CTL.

The exact mechanism of entry of the granzymes into the infected cell or tumor cell is still debated. It is, however, dependent on perforins. Possibilities include:

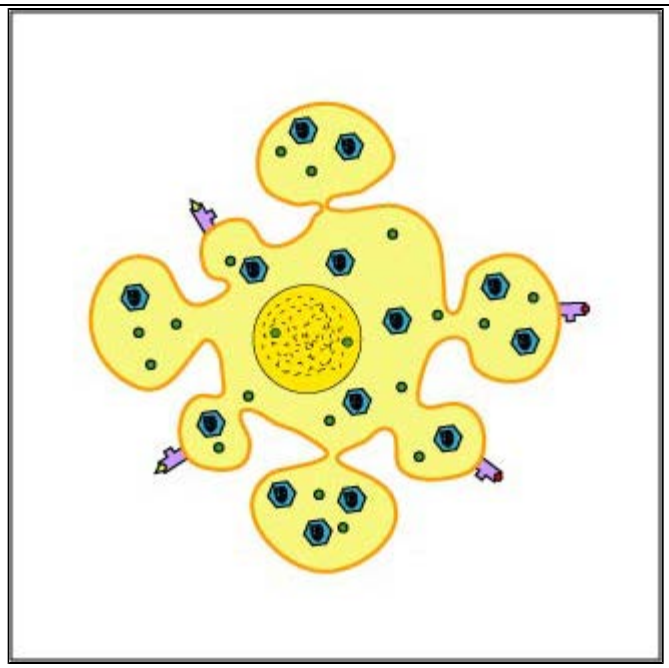
1. The perforins/granzymes/granulysin complex may be taken into the target cell by receptor-mediated endocytosis. The perforin molecules may then act on the endosomal membrane allowing granzymes to enter the cytosol.

2. The perforin molecules may put pores in the membrane of the target cell allowing the granzymes to directly enter the cytosol **(see Fig. 7)**.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

1. **Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell**. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and nucleases that degrade both the target cell's nucleoprotein and any microbial DNA within the cell **(see Fig. 8**).
2. Granzymes cleave a variety of other cellular substrates that contribute to cell death.
3. The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can **increase the permeability of the infected cell and contribute to cell death**. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis.
4. Granulysin has antimicrobial actions and can also induce apoptosis.

Fig. 8: CTL-Induced Apoptosis of a Virus-Infected Cell



Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the perforin pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. This prevents the release of any mature viruses that might occur if the infected cell was lysed.

Electron micrograph of a CTL binding to a tumor cell.

Electron micrograph showing a killed tumor cell.

Flash animation of a CTL triggering apoptosis by way of perforins and granzymes.

html5 version of animation for iPad showing a CTL triggering apoptosis by way of perforins and granzymes.

Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.)

Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation of CTL-induced apoptosis of a virus-infected cell.

html5 version of animation for iPad showing CTL-induced apoptosis of a virus-infected cell.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

- Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell. The caspases are proteases that destroy the protein structural scaffolding of the cell (the cytoskeleton) and degrade both the target cell's nucleoprotein and microbial DNA within the cell.
-Granzymes cleave a variety of other cellular substrates that contribute to cell death.
-The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can increase the permeability of the infected cell and contribute to cell death. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis. A granule called granulysin can also alter the permeability of both miocrobial and host cell membranes.

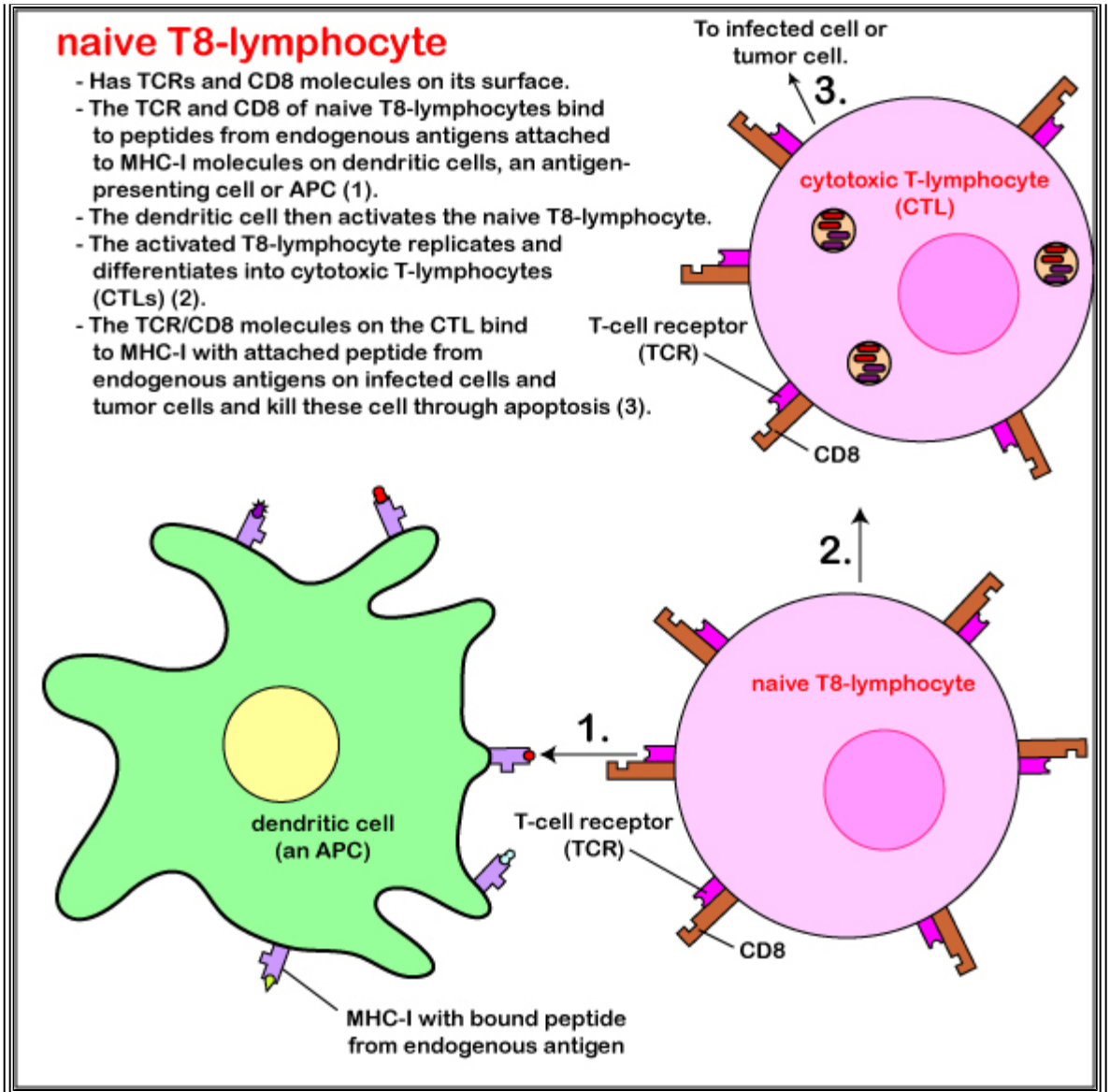
This animations shows destruction of both the cytoskeleton and nucleoprotein of the infected cell. As the infected cell breaks up into apoptotic fragments, the fragments are subsequently removed by phagocytes. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells.

Concept Map for T-lymphocytes

TPS Questions

For a Summary of Key Surface Molecules and Cellular Interactions of Naive T8-Lymphocytes, see Fig. 9.

Fig. 9: A Summary of Key Surface Molecules and Cellular Interactions of Naive T8-Lymphocytes



For more information: Review of MHC molecules
For more information: Review of antigen-presenting cells
For more information: Preview of cytotoxic T-lymphocytes

Self Quiz for T8-Lymphocytes (T8-Cells, $CD8^+$ Cells)

Quiz Group

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

ADAPTIVE IMMUNITY:
IMPORTANT CELLS AND MOLECULES: B-LYMPHOCYTES

Adaptive Immunity

Important Cells and Molecules for Adaptive Immunity: B-Lymphocytes

Fundamental Statements for this Softchalk Lesson:

1. B-lymphocytes are responsible for the production of antibody molecules during adaptive immunity.
2. Antibodies are critical in removing extracellular microorganisms and toxins.
3. B-lymphocytes refer to lymphocytes that are produced in the bone marrow and require bone marrow stromal cells and their cytokines for maturation.
4. During its development, each B-lymphocyte becomes genetically programmed to produce an antibody molecule with a unique 3-dimensional shape capable of binding a specific epitope of an antigen, and puts molecules of that antibody on its surface that function as B-cell receptors or BCRs.
5. Naive B-lymphocytes can be activated by both T-dependent antigens and T-independent antigens.
6. In order for naive B-lymphocytes to proliferate, differentiate, and mount an antibody response against T-dependent antigens, such as most proteins, these B-lymphocytes must interact with effector T4-lymphocytes called TFH cells.
7. The first signal for the activation of a naive B-lymphocyte occurs when BCRs on the surface of the B-lymphocyte bind epitopes of antigens having a corresponding shape.
8. Once bound to the BCR, the antigen is engulfed, placed in a phagosome, and degraded with lysosomes. During this process, protein antigens are broken down into a series of peptide epitopes, bind to MHC-II molecules, and are transported to the surface of the B-lymphocyte.
9. The T-cell receptors and CD4 molecules on TFH cells bind to the MHC-II molecules with bound peptide epitope on the B-lymphocyte which enables the TFH cells to produce cytokines that collectively enable the B-lymphocytes to proliferate, synthesize and secrete antibodies, differentiate into antibody-secreting plasma cells, and switch the class of antibodies being produced.
10. By way of a mutation process called affinity maturation, activated B-lymphocytes are able over time to "fine-tune" the shape of the antibody for better fit with the original epitope.
11. During the proliferation and differentiation that follows lymphocyte activation, some of the B-lymphocytes stop replicating and become circulating, long-lived memory cells that will initiate a rapid, heightened secondary response against that antigen if it again enters the body.
12. T-independent (TI) antigens are usually large carbohydrate and lipid molecules with multiple, repeating subunits. B-lymphocytes mount an antibody response to T-independent antigens without the requirement of interaction with effector T4-lymphocytes, but the resulting antibody molecules are generally of the IgM isotype only and do not give rise to a memory response.

Common Course Objectives

1. State where in the body antigen-presenting cells, naïve B-cells, and naive T-cells come together to initiate adaptive immunity.
2. Explain how B-cells are activated.
3. Describe how T-cells and B-cells acquire memory.

Detailed Learning Objectives

1*. Describe the overall function of B-lymphocytes and their activation by T-dependent antigens in terms of the following:

a. the antigen receptor on their surface

b. how they "process" exogenous antigens

c. the type of MHC molecule to which they attach peptides

d. the role of lysosomes in binding of peptides from exogenous antigens by MHC-II molecules.

e. the type of cell to which they present peptides

f. the types of cells into which activated B-lymphocytes differentiate

(*) = Common theme throughout the course

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of B-lymphocytes in adaptive immune responses.

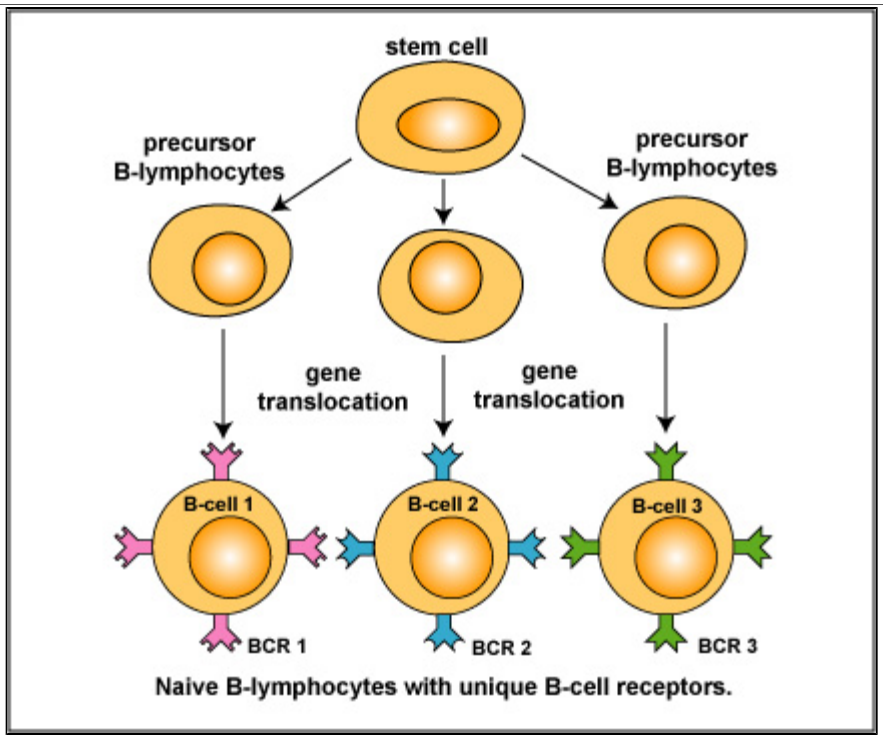
Important Cells and Molecules for Adaptive Immunity: B-Lymphocytes

B-lymphocytes are responsible for the production of antibody molecules during adaptive immunity. Antibodies are critical in removing extracellular microorganisms and toxins.

B-lymphocytes refer to lymphocytes that are produced in the bone marrow and require bone marrow stromal cells and their cytokines for maturation.

During its development, each B-lymphocyte becomes genetically programmed through a series of gene-splicing reactions to produce an antibody molecule with a unique specificity - a specific 3-dimensional shape capable of binding a specific epitope of an antigen **(see Fig. 1)**.

Fig. 1: B-Lymphocyte Precursors Making B-Cell Receptors (BCRs)

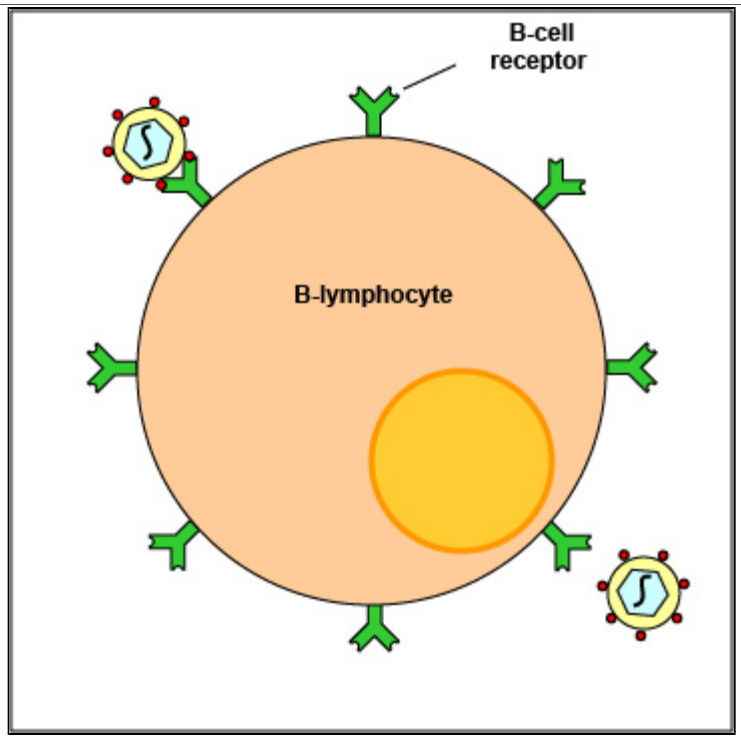


During its development, each B-lymphocyte becomes genetically programmed, through a process called gene translocation, to make a unique B-cell receptor. Molecules of that B-cell receptor are placed on its surface where it can react with epitopes of an antigen.

It is estimated that the human body has the ability to recognize 10^7 or more different epitopes and make up to 10^9 different antibodies, each with a unique specificity. In order to recognize this immense number of different epitopes, the body produces 10^7 or more distinct clones of B-lymphocytes, each with a unique B-cell receptor or BCR. In this variety of B-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, any antigen the immune system eventually encounters.

Typically, over 100,000 **identical molecules of that unique antibody are placed on the surface of the B-lymphocyte where they can function as B-cell receptors capable of binding specific epitopes of a corresponding shape (see Fig. 2)**. Naive B-lymphocytes can be activated by both T-dependent antigens and T-independent antigens.

Fig. 2: A B-lymphocyte Recognizing Epitopes of a Virus by way of B-cell Receptors



Epitopes of the virus bind to a B-lymphocyte by way of a specific B-cell receptor.

Flash animation of epitopes reacting with specific B-cell receptors on B-lymphocytes.

html5 version of animation for iPad showing epitopes reacting with specific B-cell receptors on B-lymphocytes.

B-lymphocytes have B-cell receptors (BCRs) corresponding to the specific antibody molecule they are genetically programmed to make. These BCRs recognize epitopes of antigens having a complementary shape. Different B-lymphocytes are programmed to produce different BCRs, each specific for a unique epitope.

1. Activation of naive B-lymphocytes by T-dependent antigens

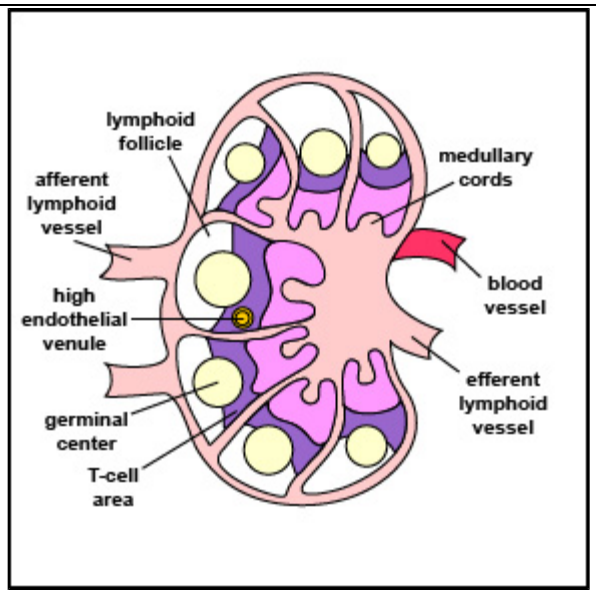
In order for naive B-lymphocytes to proliferate, differentiate, and mount an antibody response against T-dependent antigens

, such as most **proteins**, **these B-lymphocytes must interact with effector T4-lymphocytes** called T_{FH} cells. All classes of antibody molecules can be made against T-dependent antigens and there is usually a memory response against such antigens.

B-Lymphocytes and T4-lymphocytes encounter antigens in secondary lymphoid organs such as the lymph nodes and the spleen. Using a lymph node as an example **(see Fig. 3A)**, soluble antigens, such as microbial polysaccharides and

proteins and toxins, as well as microbes such as bacteria and viruses, enter the lymph node through afferent lymphatic vessels. By this time, complement pathway activation has coated these soluble antigens or microbes with opsonins such as C3b, which in turn can be degraded to C3d.

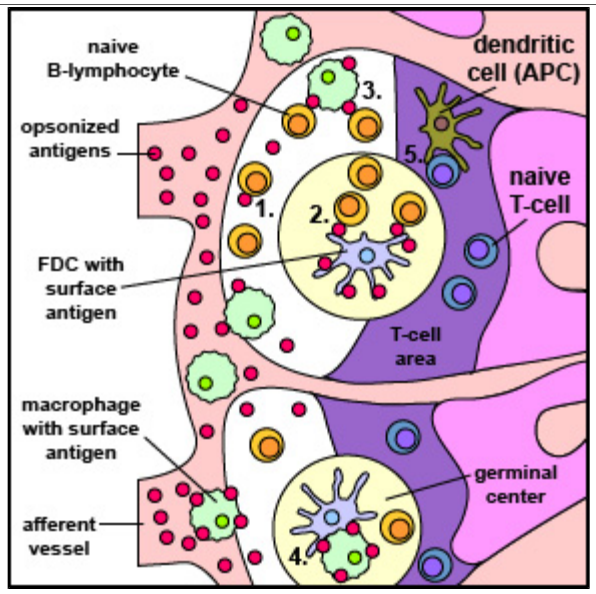
Fig. 3A: Structure of a Lymph Nodes



Antigens enter lymph nodes through afferent lymphoid vessels. Antigen-presenting dendritic cells, B-lymphocytes, and T-lymphocytes enter through high endothelial venules. Non-activated and effector lymphocytes leave the lymph node through efferent lymphatic vessels. Naive B-lymphocytes become activated, proliferate, and differentiate into plasma cells in the germinal centers of lymphoid follicles while naive T-lymphocytes become activated, proliferate and differentiate into T-effector lymphocytes in the T-cell area.

Located within the lymphoid tissues are specialized macrophages and specialized dendritic cells called **follicular dendritic cells (FDCs)**. These macrophages have poor endocytic ability and produce few lysosomes. The FDCs are nonphagocytic. Both cell types, however, have complement receptors called CR1 and CR2 that bind to the C3b and C3d, enabling the antigens and microbes to stick to the surface of the macrophages and FDCs. However,because of the poor endocytic ability of the macrophages and the lack of endocytosis by the FDCs, the antigens and microbes are not engulfed but rather remain on the surface of the cells. In addition, the macrophages can transfer their bound antigens or microbes to FDCs **(see Fig. 3B)**. Here the antigens and microbes in the lymph node can bind to complementary-shaped BCRs on naive B-lymphocytes directly, by way of macrophages, or via the FDCs **(see Fig. 3B)**.

Fig. 3B: A B-lymphocyte Recognizing Antigens on the Surface of Specialized Macrophages and Follicular Dendritic Cells (FDCs) Located in Lymph Nodes



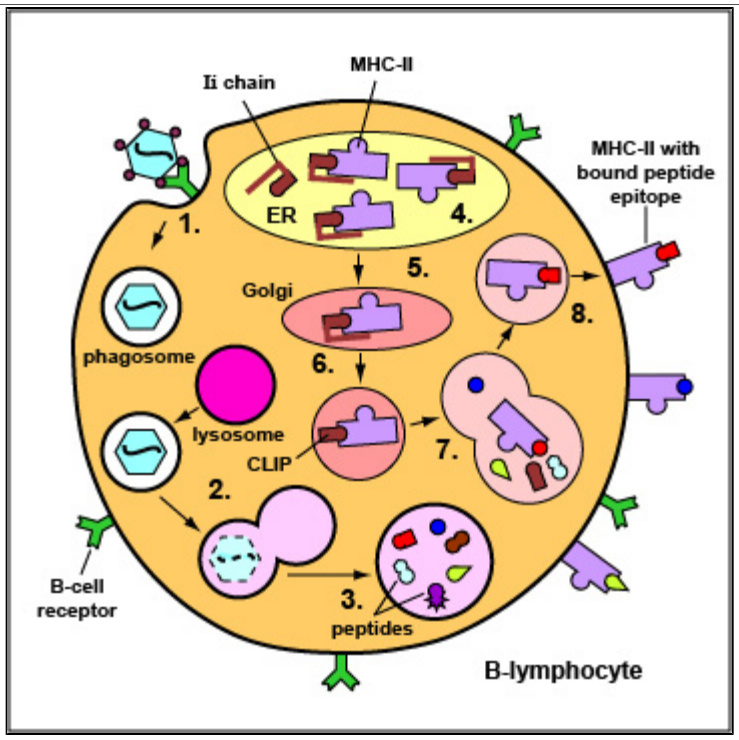
Opsonized antigens (those coated with C3b and C4b from the complement pathways) enter a lymph node through afferent lymphoid vessels. These opsonized antigens bind to and remain on the surface of specialized macrophages and follicular dendritic cells (FDCs). In addition, macrophages can transfer antigens to FDCs (see 4. above). Using their B-cell receptor (BCR), naive B-lymphocytes are able to recognize antigens directly (see 1. above), or more commonly, on the surface of FDCs (see 2. above), or on the surface of macrophages (see 3. above) in the germinal centers and lymphoid follicles of the lymph node. Meanwhile, naive T-lymphocytes are being activated by antigen-presenting dendritic cells in the T-cell areas of the lymph node (see 5. above). T4-effector cells and activated B-lymphocytes then interact with one another at the interface between the germinal centers and the T-cell areas.

Circulating naive B-lymphocytes, as a result of chemotaxis, enter lymph nodes through high endothelial venules. Any naive B-lymphocyte that bind antigens become activated and remain in the lymphoid nodes to proliferate and differentiate. Any B-lymphocytes not activated leave the lymphoid node through efferent lymphatic vessels and are returned to the bloodstream.

The first signal for the activation of a naive B-lymphocyte occurs when **BCRs** on the surface of the B-lymphocyte **bind epitopes of antigens having a corresponding shape**. A second signal is also needed for the activation of the naive B-lymphocyte. This is provided when the complement protein C3d on the microbial surface or soluble antigen binds to a complement receptor called CR2 on the surface of the naive B-lymphocyte.

Once bound, **the antigen is engulfed, placed in a phagosome, and degraded with lysosomes**. During this process, **protein antigens are broken down into a series of peptide epitopes**. These peptides eventually **bind to grooves in MHC-II molecules** that are then **transported to the surface of the B-lymphocyte (see Fig. 4)**.

Fig. 4: Binding of Peptide Epitopes from Exogenous Antigens to MHC-II Molecules by a B-Lymphocyte



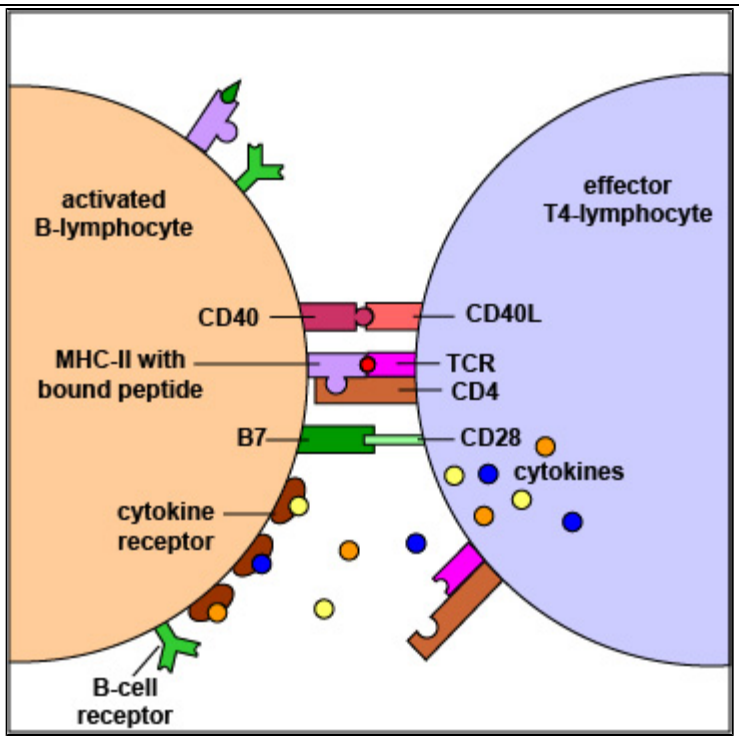
Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens bind to B-cell receptors and enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Epitopes of exogenous antigens, such as viruses, bind to a complementary shaped B-cell receptor on a B-lymphocyte. The antigen is engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane of the B-lymphocyte where they become anchored. Here, the peptide and MHC-II complexes can be recognized by effector T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Meanwhile, naïve T4-lymphocytes are being activated by epitopes of antigens bound to MHC-II molecules on antigen-presenting dendritic cells in the T-cell area of the lymph node and subsequently proliferate and differentiate into T4-effector lymphocytes such as T_{FH} cells which remain in the lymph node.The T-cell receptors and CD4 molecules on T_{FH} cells bind to the MHC-II molecules with bound peptide epitope on the B-lymphocyte. The binding of co-receptor molecules such as CD40L

and CD28 on the surface of the effector T4-lymphocyte to the corresponding molecules CD40 and B7 on the surface of the B-lymphocyte further contribute to the interaction between these two cells **(see Fig. 5)**. This enables the T_{FH} cells to produce cytokines such as interleukin-2 (IL-2) , interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-6 (IL-6) **(see Fig. 5)**.

Fig. 5: An Effector T4-Lymphocyte Recognizing Epitope/MHC-II on an activated B-Lymphocyte and Producing Cytokines to Trigger Proliferation and Differentiation



An effector T4-lymphocyte, such as a T_{FH} cell, use its TCRs and CD4 molecules to bind to a complementary shaped MHC-II molecules with attached peptide epitope on an activated B-lymphocyte. This interaction, along with the binding of co-stimulatory molecules such as CD40 and B7 on the B-lymphocyte with their complementary ligands CD40L and CD28 on the effector T4-lymphocyte triggers the T4-lymphocyte to produce cytokines that enable the activated B-lymphocyte to proliferate, differentiate into antibody-secreting plasma cells, and switch classes of the antibodies being made.

Flash animation of the binding of peptide epitopes to MHC-II molecules by a B-lymphocyte.

html5 version of animation for iPad showing the binding of peptide epitopes to MHC-II molecules by a B-lymphocyte

Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, microbial proteins and polysaccharides, and toxins. These exogenous antigens bind to B-cell receptors either directly, or, more commonly, from the surface of either specialized macrophages or follicular dendritic cells (FDCs) located in the lymph nodes and the spleen. The antigens then enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Epitopes of exogenous antigens, such as viruses, bind to a complementary shaped B-cell receptor on a B-lymphocyte. The antigen is engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by effector T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Flash animation of an effector T4-lymphocyte recognizing epitopes bound to MHC-II molecules on a B-lymphocyte.

html5 version of animation for iPad showing an effector T4-lymphocyte recognizing epitopes bound to MHC-II molecules on a B-lymphocyte.

An effector T4-lymphocyte, such as a T_{FH} cell, use its TCRs and CD4 molecules to bind to a complementary shaped MHC-II molecules with attached peptide epitope on an activated B-lymphocyte. This interaction, along with the binding of co-stimulatory molecules such as CD40 and B7 on the B-lymphocyte with their complementary ligands CD40L and CD28 on the effector T4-lymphocyte triggers the T4-lymphocyte to produce cytokines that enable the activated B-lymphocyte to proliferate, differentiate into antibody-secreting plasma cells, and switch classes of the antibodies being made.

Collectively these cytokines:

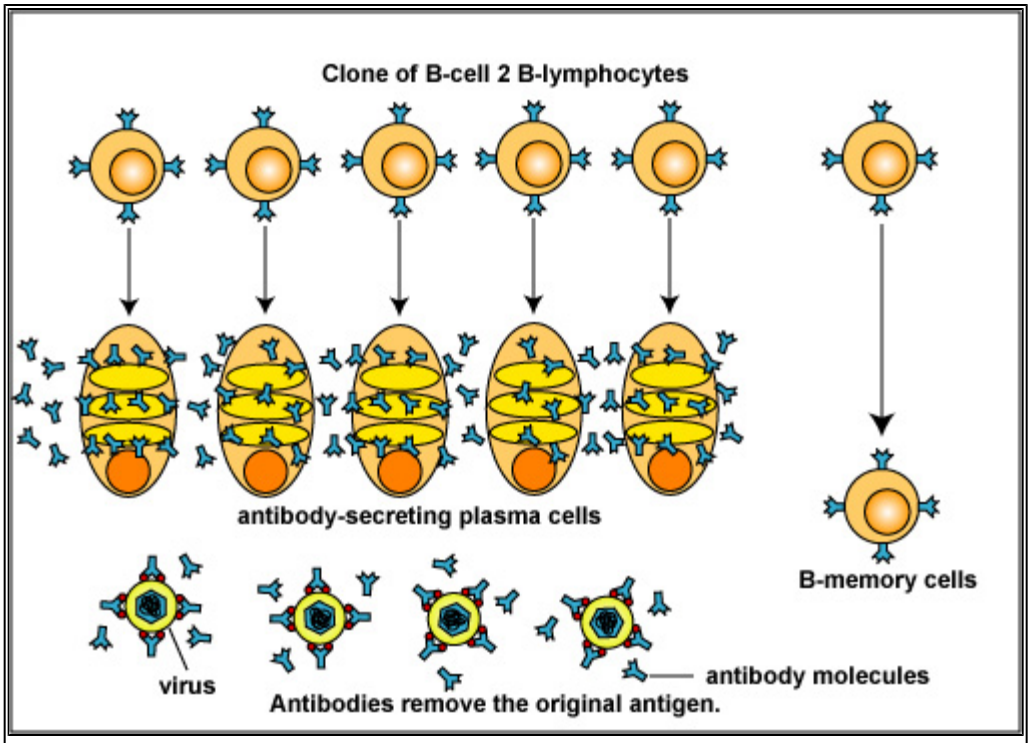
a. **Enable activated B-lymphocytes to proliferate**.

b. **Stimulate activated B-lymphocytes to synthesize and secrete antibodies**.

c. **Promote the differentiation of B-lymphocytes into antibody-secreting plasma cells**. **See Fig. 6** .

d. Enable antibody producing cells to switch the class or isotype of antibodies being produced.

Fig. 6: Differentiation of B-lymphocytes into Plasma Cells and B-Memory Cells



The B-lymphocytes now differentiate into plasma cells that secrete large quantities of antibodies that fit the original epitope. Some B-lymphocytes differentiate into B-memory cells capable of anamnestic response.

Effector T4-lymphocytes also enable B-lymphocytes to undergo **affinity maturation** through a high rate of somatic mutation. This **allows the B-lymphocytes to eventually "fine-tune" the shape of the antibody for better fit with the original epitope**. After mutation, some antibodies fit better, some worse. To select for B-lymphocytes displaying antibodies with a better fit, the variant B-lymphocytes interact with cells called follicular dendritic cells (FDCs) in the germinal centers of the secondary lymphoid organs. The FDCs display the same antigens that activated the original B-lymphocyte. **If the B-lymphocytes have high affinity antibodies for the antigen on the FDC, they are selected to survive. Those B-lymphocytes with low affinity antibodies undergo apoptosis.**

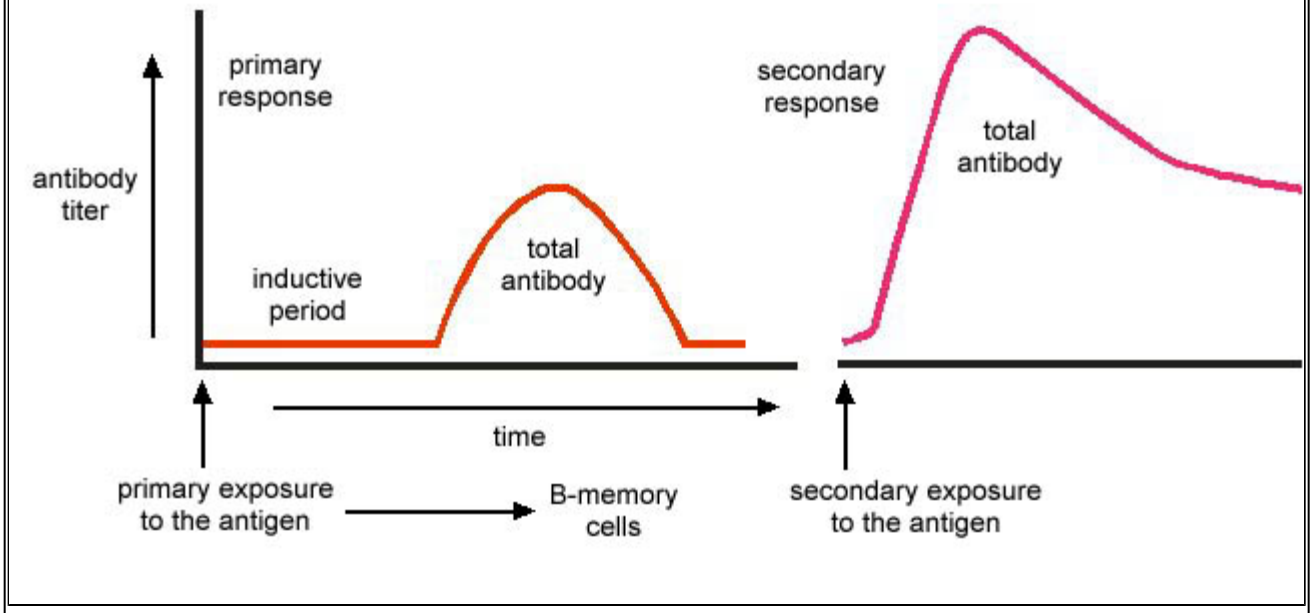
With the exception of T_{FH} cells which remain in the germinal centers of the lymph nodes and spleen, **progeny of the activated B-lymphocytes and T4 effector lymphocytes leave the secondary lymphoid organs and migrate to tissues** where they continue to respond to the invading antigen as long as it is present.

In the case of **systemic infections or vaccinations** where the antigens enter the bloodstream, plasma cells migrate to the bone marrow where antibodies can be produced for decades. After the antibodies are secreted by the plasma cells, they are found dissolved in the blood plasma and lymph. From here they can be delivered anywhere in the body via the circulatory system and the inflammatory response. In the case of **infections of the mucous membranes**, however, plasma cells only enter the mucous membranes where antibodies are only produced for a few months to a year or so.

During the proliferation and differentiation that follows lymphocyte activation, some of the B-lymphocytes stop replicating and become circulating, long-lived memory cells. **Memory cells** are capable of what is called anamnestic response or "memory", that is, they "remember" the original antigen. If that same antigen again enters the body while the B-memory cells (and T4-memory cells) are still present, these memory cells will **initiate a rapid, heightened secondary response against that antigen (see Fig. 7)**. This is why the body sometimes develops a permanent immunity after an infectious disease and is also

the principle behind immunization.

Fig. 7: Anamnestic Response



After the primary exposure to an antigen, there is an inductive period of generally several days to a week when no measurable antibodies are detected in the serum. This is the period when the antigen is being exposed to immunocompetent cells, being processed by APCs, clonal selection and clonal expansion are taking place, and B-lymphocytes are differentiating into plasma cells and B-memory cells. Because of the memory cells, however, a second exposure to the same antigen results in more antibodies being made faster for a longer period of time

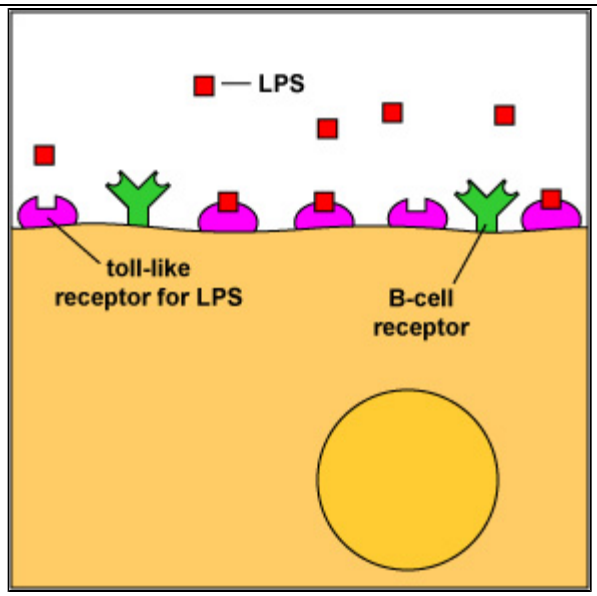
For More Information: Preview of anamnestic response

2. Activation of B-lymphocytes by T-independent antigens

T-independent (TI) antigens are usually large **carbohydrate and lipid molecules** with multiple, repeating subunits. **B-lymphocytes mount an antibody response to T-independent antigens without the requirement of interaction with effector T4-lymphocytes**. Bacterial LPS from the Gram-negative cell wall and capsular polysaccharides are examples of TI antigens. The resulting antibody molecules are generally of the IgM isotype and do not give rise to a memory response. There are two basic types of T-independent antigens: TI-1 and TI-2.

a. **TI-1 antigens** are pathogen-associated molecular patterns or PAMPS such as **lipopolysaccharide (LPS)** from the outer membrane of the gram-negative cell wall and **bacterial nucleic acid**. These antigens **activate B-lymphocytes by binding to their specific pattern-recognition receptors**, in this case toll-like receptors, rather than to B-cell receptors **(see Fig. 8)**. Antibody molecules generated against TI-1 antigens are often called "natural antibodies" because they are always being made against bacteria present in the body.

Fig. 8: Activation of a B-lymphocyte by way of LPS Binding to Toll-like Receptors



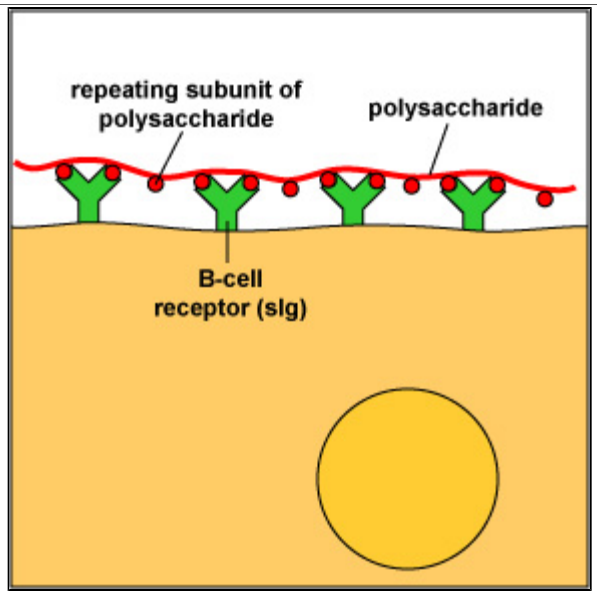
TI-1 antigens include lipopolysaccharide (LPS) from the outer membrane of the gram-negative cell wall and bacterial nucleic acid. These antigens activate B-lymphocytes by binding to their specific toll-like receptors rather than to B-cell receptors. Binding of LPS to its toll-like receptor activates chemical signals that lead to transcription of antibody and cytokine genes.

For More Information: Review of pathogen-associated molecular patterns (PAMPs)

For More Information: Review of pattern-recognition receptors (PRRs)

b. **TI-2 antigens**, such as **capsular polysaccharides**, are molecules with multiple, repeating subunits. These repeating subunits **activate B-lymphocytes by simultaneously cross-linking a number of B-cell receptors (see Fig. 9)**.

Fig. 9: Activation of a B-lymphocyte through the Cross Linking of B-Cell Receptors by TI-2 Antigens



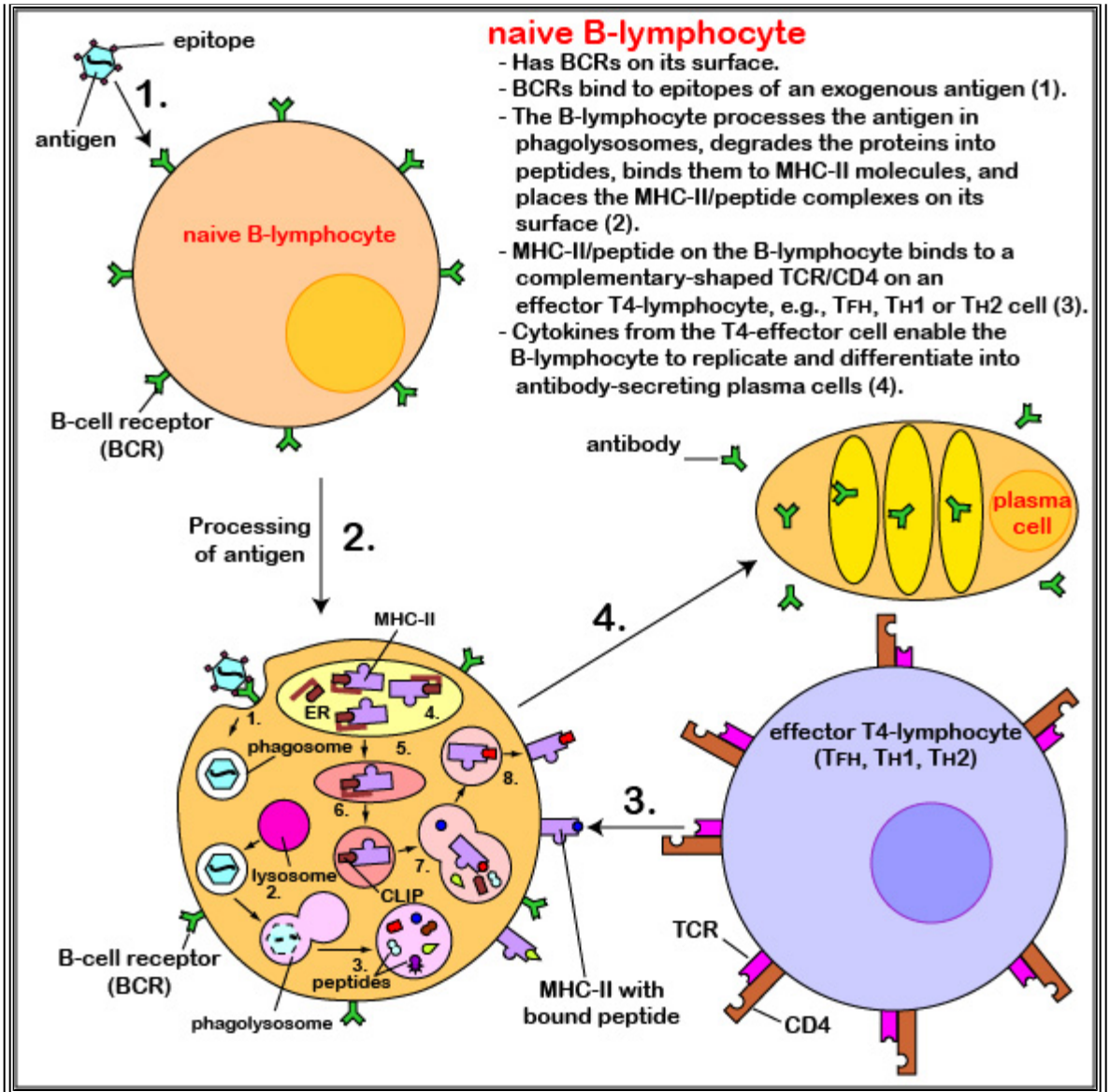
TI-2 antigens, such as capsular polysaccharides, are molecules with multiple, repeating subunits. These repeating subunits activate B-lymphocytes by simultaneously cross-linking a number of B-cell receptors. This cross-linking activates chemical signals that lead to transcription of antibody and cytokine genes.

For More Information: Review of T4-lymphocytes

For More Information: Review of MHC molecules

For a Summary of Key Surface Molecules and Cellular Interactions of Naive B-Lymphocytes, see Fig. 10.

Fig. 10: A Summary of Key Surface Molecules and Cellular Interactions of Naive B-Lymphocytes



Concept Map for B-lymphocytes

Self Quiz for B-Lymphocytes

Quiz Group

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ADAPTIVE IMMUNITY:
IMPORTANT CELLS AND MOLECULES: NK CELLS

Adaptive Immunity

Important Cells and Molecules for Adaptive Immunity: Natural Killer Cells (NK Cells)

Fundamental Statements for this Softchalk Lesson:

1. Natural Killer (NK) cells are able to recognize infected cells, cancer cells, and stressed cells and kill them. In addition, they produce a variety of cytokines, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses.
2. NK cells play a role in adaptive immune responses by way of antibody-dependent cellular cytotoxicity or ADCC where they bind to and kill cells to which antibody molecules have bound.
3. During ADCC, the Fab portion of the antibody binds to epitopes on the "foreign" cell. The NK cell then binds to the Fc portion of the antibody and the NK cell is then able to contact and kill the cell by inducing a programmed cell suicide called apoptosis.
4. During innate immunity, NK cells use a dual receptor system in determining whether to kill or not kill human cells.
5. When body cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell.
6. The first receptor, called the killer-activating receptor, can bind to these stress-induced molecules, and this sends a positive signal that enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal.
7. The second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are usually present on all nucleated human cells. If MHC-I molecules/self peptide complexes are expressed on the cell, the killer-inhibitory receptors on the NK cell recognize this MHC-I/peptide complex and sends a negative signal that overrides the original kill signal and prevents the NK cell from killing the cell to which it has bound.
8. NK cells kill their target cells by inducing apoptosis, a programmed cell suicide.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1*. Briefly describe how NK cells bind to and kill infected cells and tumor cells through ADCC.

2*. Briefly describe how NK cells recognize and kill infected cells and tumor cells that suppress MHC-I production.

(*) = Common theme throughout the course

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen and is

mediated by B-lymphocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of NK cells in adaptive immune responses.

Important Cells and Molecules for Adaptive Immunity: Natural Killer Cells (NK Cells)

NK cells are another group of cytolytic lymphocytes that are distinct from B-lymphocytes and T-lymphocytes, and participate in both innate immunity and adaptive immunity. NK cells are lymphocytes that lack B-cell receptors and T-cell receptors. They are **designed to kill certain mutant cells and virus-infected cells** in one of two ways:

1. **NK cells kill cells to which antibody molecules have attached** through a process called **antibody-dependent cellular cytotoxicity (ADCC)** as shown in **Slideshow Fig. 1**, **Fig. 2**, and **Fig. 3** . The Fab portion of the antibody binds to epitopes on the "foreign" cell. The NK cell then binds to the Fc portion of the antibody. The NK cell is then able to contact the cell and by inducing a programmed cell suicide called apoptosis.

Flash animation of ADCC contact by NK cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing ADCC contact by NK cells.
The Fab portion of the antibody IgG binds to epitopes on the "foreign" cell. The NK then releases pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.) Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation of apoptosis by NK cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing apoptosis by NK cells.
NK cells release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell,

this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

2. As discussed in Unit 5 under innate immunity, **NK cells are also able to kill cells lacking MHC-I molecules on their surface**.

NK cells are important in innate immunity because they are able to recognize infected cells, cancer cells, and stressed cells and kill them. In addition, they produce a variety of cytokines, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses. For example, through cytokine production NK cells also suppress and/or activate macrophages , suppress and/or activate the antigen-presenting capabilities of dendritic cells, and suppress and/or activate T-lymphocyte responses.

NK cells use a dual receptor system in determining whether to kill or not kill human cells. When cells are either **under stress, are turning into tumors, or are infected**, **various stress-induced molecules** such as MHC class I polypeptide-related sequence A (MICA) and MHC class I polypeptide-related sequence B (MICB) **are produced and are put on the surface of that cell**.

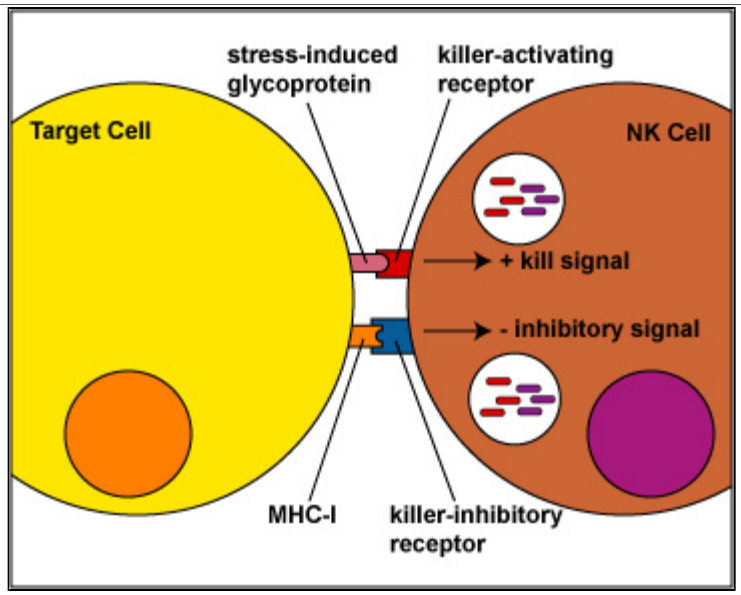
The first receptor, called the **killer-activating receptor**, **can bind to these stress-induced molecules, and this sends a positive signal that enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal**.

This second receptor, called the **killer-inhibitory receptor**, **recognizes MHC-I molecules that are usually present on all nucleated human cells**. MHC-I molecules, produced by all nucleated cells in the body, possess a deep groove that can bind peptides from proteins found within the cytosol of human cells, transport them to the surface of that cell, and display the MHC-!/peptide complex to receptors on cytotoxic T-lymphocytes or CTLs. If the MHC-I molecules have peptides from the body's own proteins bound to them, CTLs do not recognize those cells as foreign and the cell is not killed. If, on the other hand, the MHC-I molecules have peptides from viral, bacterial, or mutant proteins bound to them, CTLs recognize that cell as foreign and kill that cell. (CTLs will be discussed in greater detail in Unit 6.)

For more information: Preview of CTLs

If MHC-I molecules/self peptide complexes are expressed on the cell, the killer-inhibitory receptors on the NK cell recognize this MHC-I/peptide complex and sends a negative signal that overrides the original kill signal and prevents the NK cell from killing the cell to which it has bound (see Fig. 4).

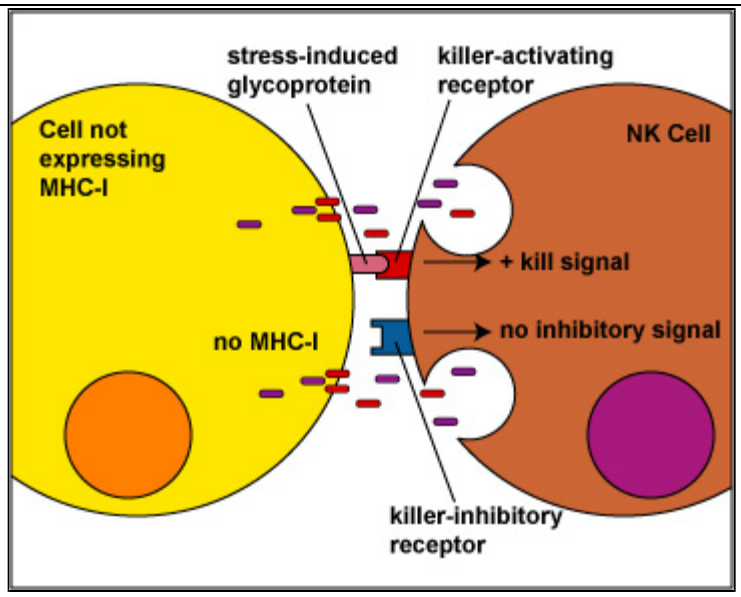
Fig. 4: NK Cell Interacting with a Normal Body Cell



NK cells use a dual receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Viruses, stress, and malignant transformation, however, can often interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound (see Fig. 5).

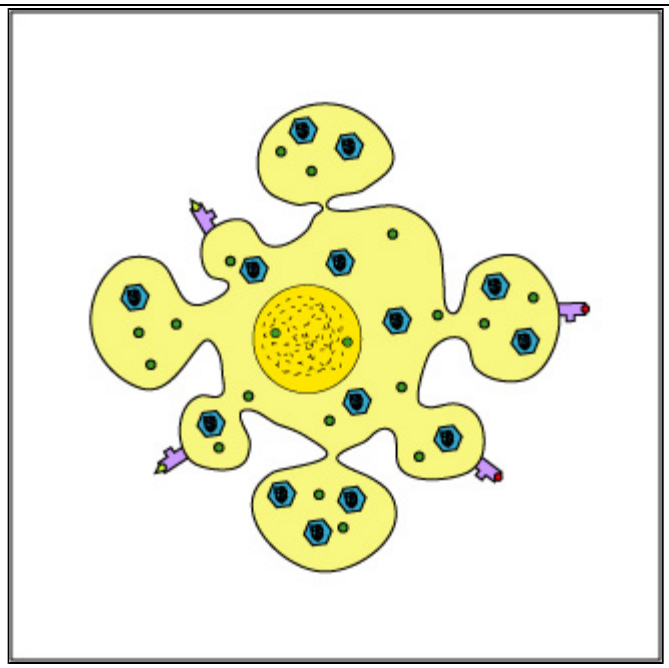
Fig. 5: NK Cell Interacting with a Virus-Infected Cell or a Mutant Cell Not Expressing MHC-I Molecules



Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

The NK cell then r**eleases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines**. Granzymes pass through the pores and activate the enzymes that **lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation**. As a result, the cell breaks into fragments that are subsequently removed by phagocytes **(see Fig. 6)**. Perforins can also sometimes result in cell lysis.

Fig. 6: Apoptosis by NK Cells



Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound. The NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Flash animation of a NK cell interacting with a normal body cell.

html5 version of animation for iPad showing a NK cell interacting with a normal body cell.
NK cells use a dual receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Flash animation of a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.
html5 version of animation for iPad showing a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.
Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

In addition, **NK cells produce a variety of cytokines**, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses. For example, through cytokine production NK cells also suppress and/or activate macrophages, suppress and/or activate the antigen-presenting capabilities of dendritic cells, and suppress and/or activate T-lymphocyte responses.

Concept Map for iNKT and NK Cells
For more information: Review of B-lymphocytes
For more information: Review of T4-lymphocytes
For more information: Review of T8-lymphocytes

Self Quiz for Natural Killer Cells (NK Cells)

Quiz Group

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ADAPTIVE IMMUNITY:
THE LYMPHOID SYSTEM

Adaptive Immunity

The Lymphoid System

Fundamental Statements for this Softchalk Lesson:

1. The body uses the lymphoid system to enable lymphocytes to encounter antigens and it is here that adaptive immune responses are initiated.
2. The lymphoid system consists of primary lymphoid organs, secondary lymphoid organs, and lymphatic vessels.
3. The bone marrow and the thymus constitute the primary lymphoid organs.
4. While both B-lymphocytes and T-lymphocytes are produced from stem cells in the bone marrow, B-lymphocytes mature in the bone marrow and T-lymphocytes migrate to the thymus to mature.
5. After maturation, both naive B-lymphocytes and naive T-lymphocytes circulate between the blood and the secondary lymphoid organs.
6. Adaptive immune responses require antigen-presenting cells, such as macrophages and dendritic cells, and ever changing populations of B-lymphocytes and T- lymphocytes. These cells gather to detect and present antigens in secondary lymphoid organs.
7. The secondary lymphoid organs include highly organized lymphoid organs such as lymph nodes and the spleen, as well as less organized accumulations of lymphoid organs scattered strategically throughout the body.
8. Lymphatic vessels are responsible for flow of lymph within the lymphoid system and are a part of the body's fluid recirculation system. The lymph flows through regional lymph nodes and eventually enters the circulatory system at the heart to maintain the fluid volume of the circulation.

Common Course Objectives

1. State where in the body antigen-presenting cells, naïve B-cells, and naivety-cells come together to initiate adaptive immunity.

Detailed Learning Objectives

1*. Compare and give examples of the following:

a. primary lymphoid organs

b. secondary lymphoid organs

2. Define the following:

a. plasma

b. tissue fluid

c. lymph

d. lymph vessels

e. MALT

3*. Briefly describe the importance of the lymphoid system in adaptive immune responses and how microbes and other

antigens encounter naive B-lymphocytes and T-lymphocytes.

(*) = Common theme throughout the course

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.

2. Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of the lymphoid system in adaptive immune responses.

The Lymphoid System

The Lymphoid System

The body uses the lymphoid system to enable lymphocytes to encounter antigens and it is here that adaptive immune responses are initiated. The lymphoid system consists of primary lymphoid organs, secondary lymphoid organs, and lymphatic vessels.

a. Primary lymphoid organs

The **bone marrow** and the **thymus** constitute the primary lymphoid organs. Both B-lymphocytes and T-lymphocytes are produced from stem cells in the bone marrow. **B-lymphocytes mature in the bone marrow while T-lymphocytes migrate to the thymus and mature there**. After maturation, both naive B-lymphocytes and naive T-lymphocytes circulate between the blood and the secondary lymphoid organs.

b. Lymphatic vessels

Lymphatic vessels are responsible for flow of lymph within the lymphoid system and are a part of the body's fluid recirculation system. The liquid portion of the blood, called **plasma**, constantly leaks out of capillaries to deliver oxygen and nutrients to cells of the surrounding tissue. Once in the tissue, the plasma is now called **tissue fluid**. While most of this tissue fluid re-enters capillaries and is returned directly to the bloodstream, some fluid enters lymph vessels as **lymph**. The lymph flows through regional lymph nodes and eventually enters the circulatory system at the heart to maintain the fluid volume of the circulation.

c. Secondary lymphoid organs

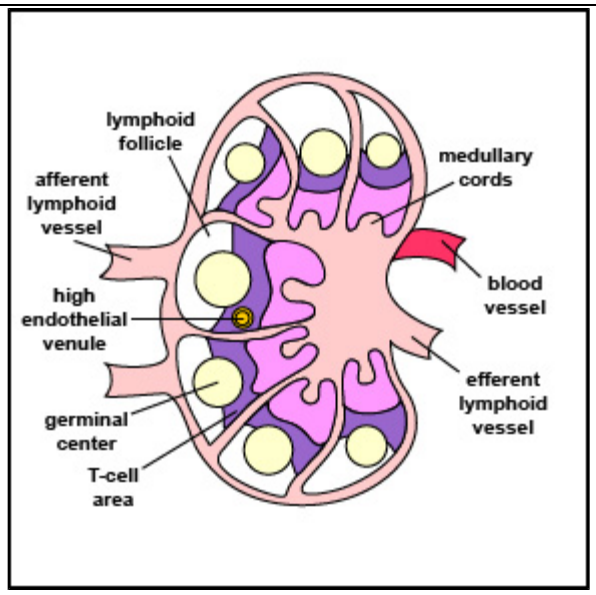
Adaptive immune responses require antigen-presenting cells, such as macrophages and dendritic cells, and ever changing populations of **B-lymphocytes** and **T- lymphocytes**. **These cells gather to detect and present antigens in secondary lymphoid organs**.

The secondary lymphoid organs include highly organized lymphoid organs such as **lymph nodes and the spleen**, as well as less organized accumulations of lymphoid organs scattered strategically throughout the body.

Lymph nodes (see Fig. 1) contain many reticular fibers that support fixed macrophages and dendritic cells as well as ever changing populations of circulating B-lymphocytes and T-lymphocytes. When microorganisms and other antigens **enter tissues**, they are **transported by tissue fluid into the lymph vessels**. Lymph vessels, in turn, carry these antigens, now in the lymph, to regional lymph nodes. In addition, **immature dendritic cells** located under the surface epithelium of the skin and the surface epithelium of the mucous membranes of the respiratory tract, genitourinary tract, and the gastrointestinal tract **capture antigens through pinocytosis and phagocytosis**. The dendritic cells detach from their initial site, enter lymph vessels, and are **carried to regional lymph nodes**. Here the microbes and other antigens in the lymph **encounter changing populations of B-lymphocytes**, are **filtered out and phagocytosed by the fixed**

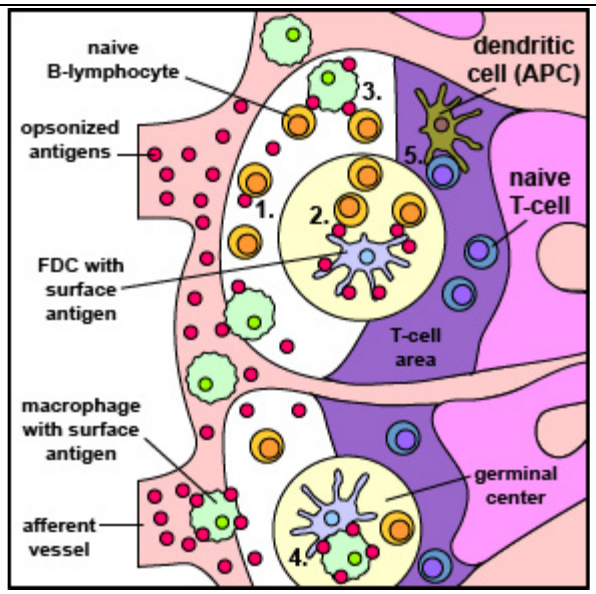
macrophages and dendritic cells, and are **presented to changing populations of T-lymphocytes (see Fig. 2)**. **Approximately 25 billion different lymphocytes migrate through each lymph node every day**.

Fig. 1: Structure of a Lymph Nodes



Antigens enter lymph nodes through afferent lymphoid vessels. Antigen-presenting dendritic cells enter the lymph node through afferent lymphatic vessels while naive B-lymphocytes, and naive T-lymphocytes enter through high endothelial venules. Non-activated and effector lymphocytes leave the lymph node through efferent lymphatic vessels. Naive B-lymphocytes become activated, proliferate, and differentiate into plasma cells in the germinal centers of lymphoid follicles while naive T-lymphocytes become activated, proliferate and differentiate into T-effector lymphocytes in the T-cell area.

Fig. 2: B-lymphocyte and T-Lymphocytes Recognizing Antigens in a Lymph Node

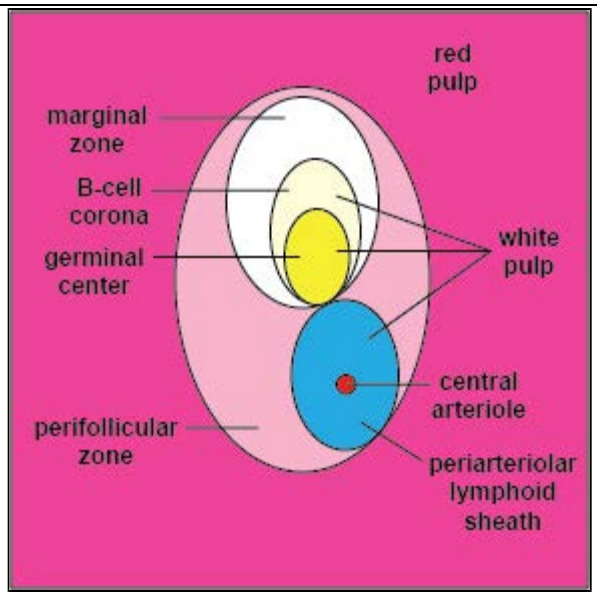


Opsonized antigens (those coated with C3b and C4b from the

complement pathways) enter a lymph node through afferent lymphoid vessels. These opsonized antigens bind to and remain on the surface of specialized macrophages and follicular dendritic cells (FDCs). In addition, macrophages can transfer antigens to FDCs (see 4. above). Using their B-cell receptor (BCR), naive B-lymphocytes are able to recognize antigens directly (see 1. above), or more commonly, on the surface of FDCs (see 2. above), or on the surface of macrophages (see 3. above) in the germinal centers and lymphoid follicles of the lymph node. Meanwhile, naive T-lymphocytes are being activated by antigen-presenting dendritic cells in the T-cell areas of the lymph node (see 5. above). T4-effector cells and activated B-lymphocytes then interact with one another at the interface between the germinal centers and the T-cell areas.

Like the lymph nodes, the **spleen** contains many reticular fibers that support fixed macrophages and dendritic cells as well as ever changing populations of circulating B-lymphocytes and T-lymphocytes. **When microorganisms and other antigens enter the blood, they are transported by the blood vessels to the spleen**. Most of the spleen is referred to as red pulp. This area is involved in the disposal of old red blood cells. Scattered throughout the spleen are isolated areas called the **white pulp (see Fig. 3)**. Here antigens in the blood **encounter macrophages, dendritic cells, and ever-changing populations of B-lymphocytes** and **T-lymphocytes**.

Fig. 3: Section of a Spleen Showing Red Pulp and White Pulp



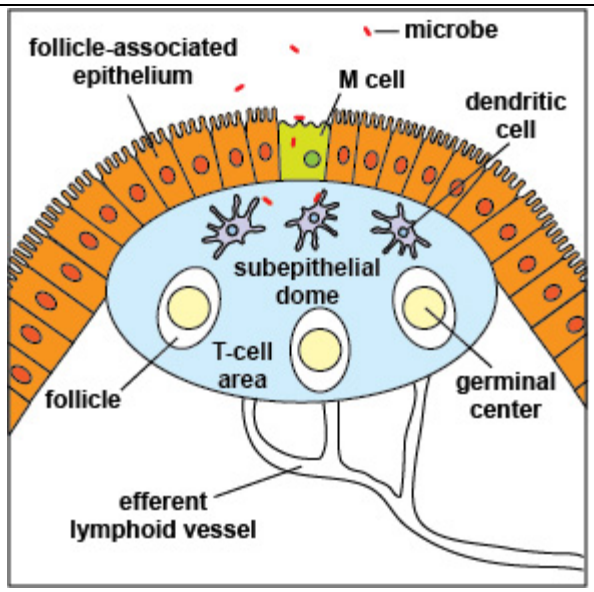
The red pulp makes up the majority of the spleen. This is where old red blood cells are destroyed. Scattered throughout the spleen are areas of white pulp where microbes, cells, and antigens encounter macrophages, dendritic cells, and changing populations of B-lymphocytes and T-lymphocytes. Soluble antigens, blood-borne microbes, and antigen-antibody complexes are filtered out of the blood and phagocytosed by immature dendritic cells and macrophages within the marginal zone. After maturation, dendritic cells migrate to the periphery of the periarteriolar lymphoid sheath, The T-cell area of the white pulp, and present antigens bound to MHC molecules to the TCRs of T-lymphocytes. Secondary follicles consisting of germinal centers surrounded by a B-cell corona are where B-lymphocytes encounter microbes and soluble antigens.

Mucosal surfaces within the body, the most common sites of microbial invasion, are protected by the mucosal immune system consisting of the **mucosa-associated lymphoid tissue** or **MALT**, an extensive diffuse system of small concentrations of lymphoid tissue found in various sites of the body such as the gastrointestinal tract, thyroid, breast, lung,

salivary glands, eye, and skin. **MALT is populated by loose clusters of T-lymphocytes, B-lymphocytes, plasma cells, activated T_H cells, and macrophages**. MALT can be subdivided into:

- **GALT (gut-associated lymphoid tissue**, such as the **Peyer's patches (see Fig. 4)** in the lining of the small intestines, as well as the **adenoids, tonsils, and appendix**)
- **BALT (bronchial-associated lymphoid tissue in the bronchi)**
- **SALT (skin-associated lymphoid tissue beneath the epidermis)**
- **NALT (nose-associated lymphoid tissue)**
- **LALT (larynx-associated lymphoid tissue)**
- **CALT (conjunctiva-associated lymphoid tissue in the eye)**

Fig. 4: Diagram of a Peyer's Patch



Peyer's patches are part of the mucosa-associated lymphoid tissue (MALT) in the small intestines. Microbes and antigens enter through specialized epithelial cells called microfold (M) cells. Changing populations of naive B-lymphocytes and naive T-lymphocytes enter the Peyer's patch via blood vessels with B-lymphocytes entering the follicles and germinal centers and T-lymphocytes entering the T-cell area. Dendritic cells engulf and process antigens and present them by way of MHC molecules to the TCRs of naive T-lymphocytes.

As can be seen, no matter how microbes and other antigens enter the body, they will eventually encounter the lymphoid system to initiate adaptive immune responses.

To view a diagram of the lymphatics system, see the Innerbody.com Webpage.

For more information: Review of B-lymphocytes
For more information: Review of T4-Lymphocytes
For more information: Review of T8-lymphocytes
For more information: Review of antigen-presenting

cells

Self Quiz for the Lymphoid System

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

ADAPTIVE IMMUNITY:
AN OVERVIEW OF THE STEPS INVOLVED IN THE ADAPTIVE IMMUNE REPONSES

Adaptive Immunity

An Overview of the Steps Involved in the Adaptive Immune Responses

Common Course Objectives

1. State what is meant by immunologic "memory" in acquired immunity and describe why there is able to be a heightened secondary response upon subsequent exposure to an antigen.
2. Explain the role of T-cells and B-cells in acquired immunity.
3. Briefly describe how the body recognizes an antigen as foreign and compare B-cell receptors and T-cell receptors in terms of how they recognize epitopes of an antigen.
4. Contrast the MHC-I and MHC-II systems in terms of how they process antigens for recognition by T-cells.
5. Explain the role of antigen presenting cells in the adaptive immune responses.
6. State where in the body antigen-presenting cells, naïve B-cells, and naive T-cells come together to initiate adaptive immunity.
7. Briefly describe how the body recognizes an antigen as foreign and compare B-cell receptors and T-cell receptors in terms of how they recognize epitopes of an antigen.
8. Compare and contrast how T4-cells and T8-cells are activated.
9. Compare and contrast how T4-cells and T8-cells are activated.
10. Explain how B-cells are activated.
11. Briefly describe the process of clonal selection and clonal expansion.

(*) = Common theme throughout the course

Detailed Learning Objectives

1. List the 5 general steps involved in the immune responses in their correct order.

2. State where antigens may encounter APCs, B-lymphocytes, and T-lymphocytes if they enter the following:

   a. the blood

   b. tissues

   c. the respiratory tract

   d. the gastrointestinal tract

   e. the genitourinary tract

3. Briefly describe how the receptor molecules on the surface of naive B-lymphocytes, T4-helper lymphocytes, and T8-lymphocytes eventually recognize or bind epitope, indicating the roles of BCR, TCR, CD4, CD8, MHC-I, and MHC-II molecules in lymphocyte activation.

4. State the overall function of T4-effector lymphocytes and the importance behind rapid proliferation of activated lymphocytes.

5. State what types of effector cells the proliferating B-lymphocytes and T8-lymphocytes differentiate into in order to destroy or neutralize the antigen.

6. Define cytokine.

7. State the function of memory cells.

8. State what is meant by immunologic tolerance.

(*) = Common theme throughout the course

Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to remove a specific antigen. This is the immunity one develops throughout life. There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. **Humoral immunity**: humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes.

2. **Cell-mediated immunity**: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes.

We will now take a look at the roles of T4-lymphocytes in adaptive immune responses.

An Overview of the Steps Involved in the Adaptive Immune Responses

Whether humoral immunity or cell-mediated immunity, there are several **general steps involved in the immune responses**.

1. The antigen must encounter the B-lymphocytes, T-lymphocytes, and antigen-presenting cells (APCs) capable of carrying out an adaptive immune response.

Fundamental Statement for this Step:

1. Antigens encounter the APCs, B-lymphocytes, and T-lymphocytes in the secondary lymphoid organs of the lymphoid system.

Antigens encounter the antigen-presenting cells (APCs), B-lymphocytes, and T-lymphocytes in the secondary lymphoid organs of the lymphoid system. Tissue fluid carries antigens to lymph nodes, blood carries antigens to the spleen, and immature dendritic cells under the skin and mucosal epithelium carry antigens to regional lymph nodes. Here they encounter ever changing populations of naive B-lymphocytes, T4-lymphocytes, and T8-lymphocytes as they circulate back and forth between the blood and the lymphatics.

a. Antigens that enter through the **bloodstream**, encounter the APCs, B-lymphocytes, and T-lymphocytes in the **spleen**; **see Fig. 1**.

b. Antigens that enter through the **tissue**, are picked up by tissue fluid, enter the lymph vessels, and are carried to the lymph nodes where they encounter APCs, B-lymphocytes, and T-lymphocytes; **see Fig. 2**.

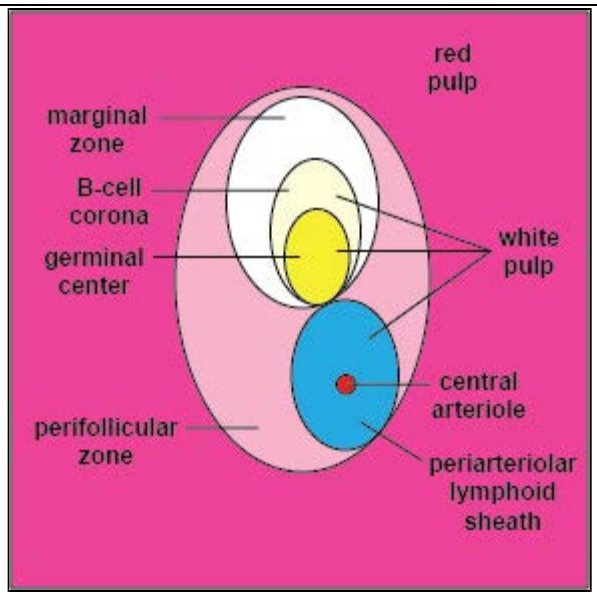
c. Antigens that enter the **respiratory tract**, encounter APCs, B-lymphocytes, and T-lymphocytes in the **tonsils** and the **mucosa-associated lymphoid tissue** (MALT), including the bronchial-associated lymphoid tissue (BALT), the nose-associated lymphoid tissue (NALT), and the larynx-associated lymphoid tissue (LALT).

d. Antigens that enter the **intestinal tract**, encounter APCs, B-lymphocytes, and T-lymphocytes in the **Peyer's patches (see Fig. 3)** and other **gut-associated lymphoid tissues** (GALT).

e. Antigens that enter the **genitourinary tract** , encounter APCs, B-lymphocytes, and T-lymphocytes in the **mucosa-associated lymphoid tissue** (MALT) found there.

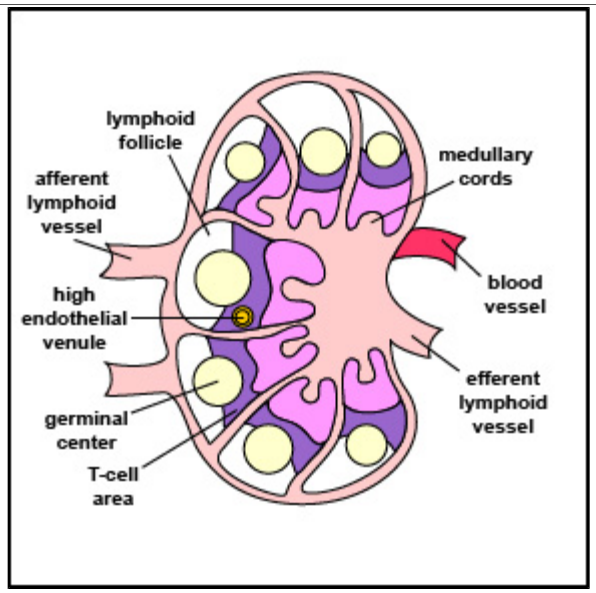
f. Finally, antigens that penetrate the **skin**, encounter APCs, B-lymphocytes, and T-lymphocytes of the **skin-associated lymphoid tissue** (SALT).

Fig. 1: Section of a Spleen Showing Red Pulp and White Pulp



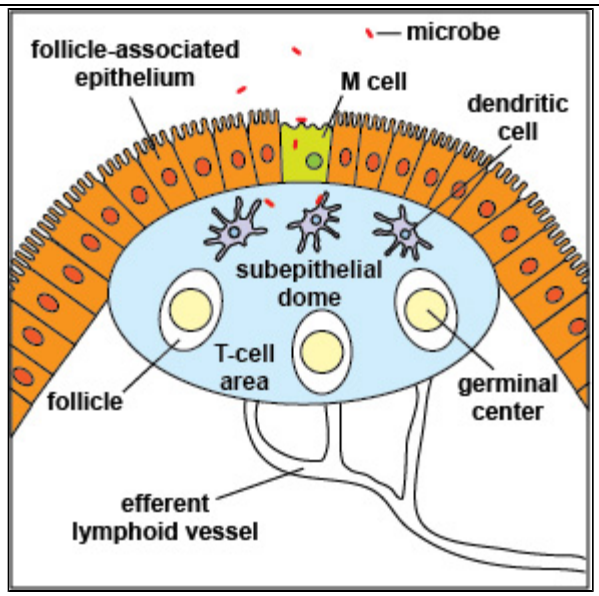
The red pulp makes up the majority of the spleen. This is where old red blood cells are destroyed. Scattered throughout the spleen are areas of white pulp where microbes, cells, and antigens encounter macrophages, dendritic cells, and changing populations of B-lymphocytes and T-lymphocytes. Soluble antigens, blood-borne microbes, and antigen-antibody complexes are filtered out of the blood and phagocytosed by immature dendritic cells and macrophages within the marginal zone. After maturation, dendritic cells migrate to the periphery of the periarteriolar lymphoid sheath, The T-cell area of the white pulp, and present antigens bound to MHC molecules to the TCRs of T-lymphocytes. Secondary follicles consisting of germinal centers surrounded by a B-cell corona are where B-lymphocytes encounter microbes and soluble antigens.

Fig. 2: Structure of a Lymph Nodes



Antigens enter lymph nodes through afferent lymphoid vessels. Antigen-presenting dendritic cells enter the lymph node through afferent lymphatic vessels while naive B-lymphocytes, and naive T-lymphocytes enter through high endothelial venules. Non-activated and effector lymphocytes leave the lymph node through efferent lymphatic vessels. Naive B-lymphocytes become activated, proliferate, and differentiate into plasma cells in the germinal centers of lymphoid follicles while naive T-lymphocytes become activated, proliferate and differentiate into T-effector lymphocytes in the T-cell area.

Fig. 3: Diagram of a Peyer's Patch



Peyer's patches are part of the mucosa-associated lymphoid tissue (MALT) in the small intestines. Microbes and antigens enter through specialized epithelial cells called microfold (M) cells. Changing populations of naive B-lymphocytes and naive T-lymphocytes enter the Peyer's patch via blood vessels with B-lymphocytes entering the follicles and germinal centers and T-lymphocytes entering the T-cell area.

Dendritic cells engulf and process antigens and present them by way of MHC molecules to the TCRs of naive T-lymphocytes.

For more information: Review of the lymphoid system

Concept Map for The General Steps in Adaptive Immunity

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Quiz Group

2. Naive B-lymphocytes, T4-lymphocytes, and T8-lymphocytes must recognize epitopes of an antigen by means of antigen-specific receptor molecules on their surface and become activated. This is known as clonal selection.

Fundamental Statements for this Step:

1. Dendritic cells bind peptide epitopes to MHC-II molecules to enable them to be recognized by complementary shaped T-cell receptors (TCR) and CD4 molecules on naive T4-lymphocyte.
2. Dendritic cells bind peptide epitopes to MHC-I molecules to enable them to be recognized by complementary shaped T-cell receptors (TCR) and CD8 molecules on naive T8-lymphocytes.
3. These interactions are required to enable the T4-lymphocyte or T8-lymphocyte to become activated, proliferate, and differentiate into effector cells.
4. Naive T4-lymphocytes have T cell receptors (TCRs) that, in cooperation with CD4 molecules, bind to MHC-II molecules with attached epitope from an antigen found on the surface of an antigen-presenting dendritic cell.
5. Naive T8-lymphocytes have T cell receptors (TCRs) that, in cooperation with CD8 molecules, bind to MHC-I molecules with attached epitope from an antigen found on the surface of antigen-presenting dendritic cells.
6. Most proteins are T-dependent antigens. In order for naive B-lymphocytes to proliferate, differentiate and mount an antibody response against T-dependent antigens, these B-lymphocytes must interact with effector T4-lymphocytes.
7. Specialized macrophages and specialized dendritic cells called FDCs are located in the lymphoid tissues. Antigens and microbes are are found on the surface of these FDCs and macrophages which present them to complementary-shaped BCRs on naive B-lymphocytes.
7. A few antigens are called T-independent antigens. T-independent (TI) antigens are usually large carbohydrate and lipid molecules with multiple, repeating subunits. B-lymphocytes mount an antibody response to T-independent antigens without the requirement of interaction with effector T4-lymphocytes but the antibody response is much more limited than with T-dependent antigens.

a. The role of antigen-presenting dendritic cells

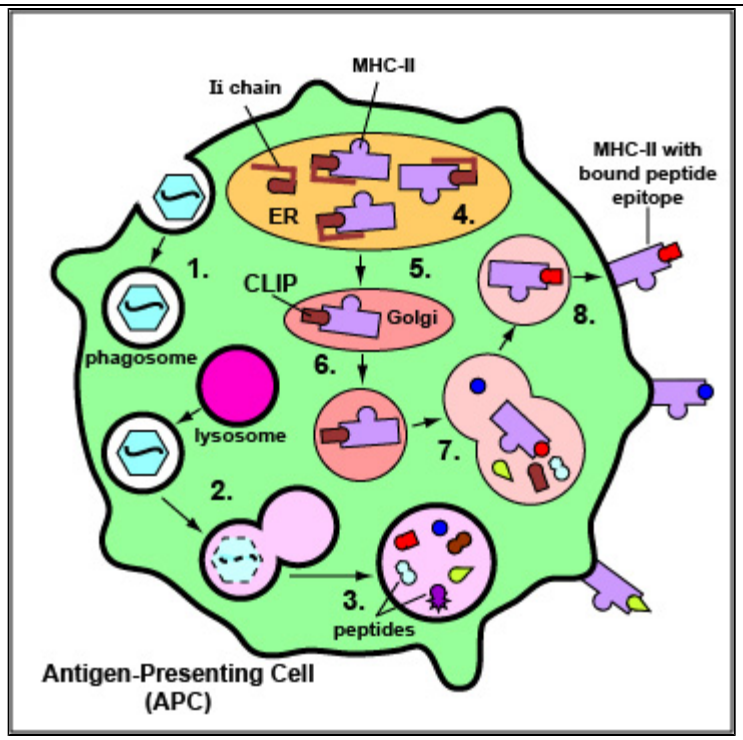
The primary function of dendritic cells is to capture and present epitopes of protein antigens to naive T-lymphocytes.

1. Dendritic cells bind peptide epitopes to MHC-II molecules **(see Fig. 4)** to enable them to be recognized by complementary shaped T-cell receptors (TCR) and CD4 molecules on naive T4-lymphocyte.

2. Dendritic cells bind peptide epitopes to MHC-I molecules **(see Fig. 5)** to enable them to be recognized by complementary shaped T-cell receptors (TCR) and CD8 molecules on naive T8-lymphocytes.

These interactions are required to enable the T4-lymphocyte or T8-lymphocyte to become activated, proliferate, and differentiate into effector cells.

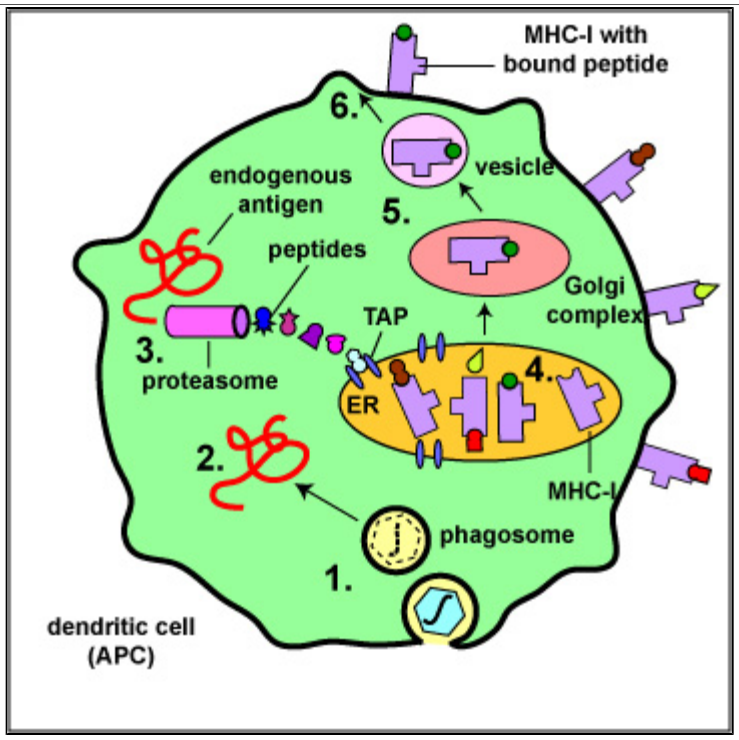
Fig. 4: Binding of Peptide Epitopes from Exogenous Antigens to MHC-II Molecules



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (macrophages, dendritic cells, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Fig. 5: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Dendritic Cell



Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. Viral proteins produced during viral replication,
b. Proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. Proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. Tumor antigens produced by cancer cells, and
e. Self peptides from human cell proteins.

Dendritic cells bind epitopes from endogenous antigens to MHC-I molecules and present them to naive T8-lymphocytes in order to activate these naive T8-lymphocytes.

1. Antigens are engulfed by dendritic cells and placed in a phagosome. Some of the proteins escape from the phagosome into the cytosol of the dendritic cell where they become endogenous antigens.
2. These endogenous antigens pass through proteasomes where they are degraded into a series of peptides.
3. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
4. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
5. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
6. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by naive T8-lymphocytes by way of TCRs and CD8 molecules having a complementary shape.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.)

Flash animation of MHC-II molecules binding epitopes from exogenous antigens.

html5 version of animation for iPad showing MHC-II molecules binding epitopes from exogenous antigens.

Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (dendritic cells, macrophages, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. The MHC-II molecules with bound Ii chain are now transported to the Golgi complex, and placed in vesicles.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The Ii chain is removed and the peptides are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Flash animation of MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting dendritic cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from endogenous antigens by an antigen-presenting dendritic cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. Viral proteins produced during viral replication,
b. Proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. Proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting dendritic cells,
d. Tumor antigens produced by cancer cells, and
e. Self peptides from human cell proteins.

One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs. These CTLs are effector cells derived from naive T8-lymphocytes during cell-mediated immunity.

However, in order to become CTLs, naive T8-lymphocytes must become activated by cytokines produced by antigen-presenting dendritic cells. This interaction between dendritic cells and naive T8-lymphocytes occurs primarily in the lymph nodes, the lymph nodules, and the spleen. The process can be summarized as follows:

1. In addition to microbes, dendritic cells and macrophages also engulf and degrade infected cells, tumor cells, and the remains of killed infected and tumor cells. It is thought that in this manner, endogenous antigens from other cells are able to enter the dendritic cell. During phagocytosis, some protein antigens from the engulfed exogenous antigens are translocated from the phagosomes or phagolysosome of the dendritic cell to the cytosol where they become "endogenous" antigens.
2. These cytosolic protein antigens then pass through proteasomes, where proteases and peptidases chop the protein up into a series of peptides.
3. A transporter protein called TAP located in the membrane of the cell's endoplasmic reticulum then transports these peptide epitopes into the endoplasmic reticulum where they bind to the grooves of various newly made MHC-I molecules.
4. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles .
5. The exocytic vesicles carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface.

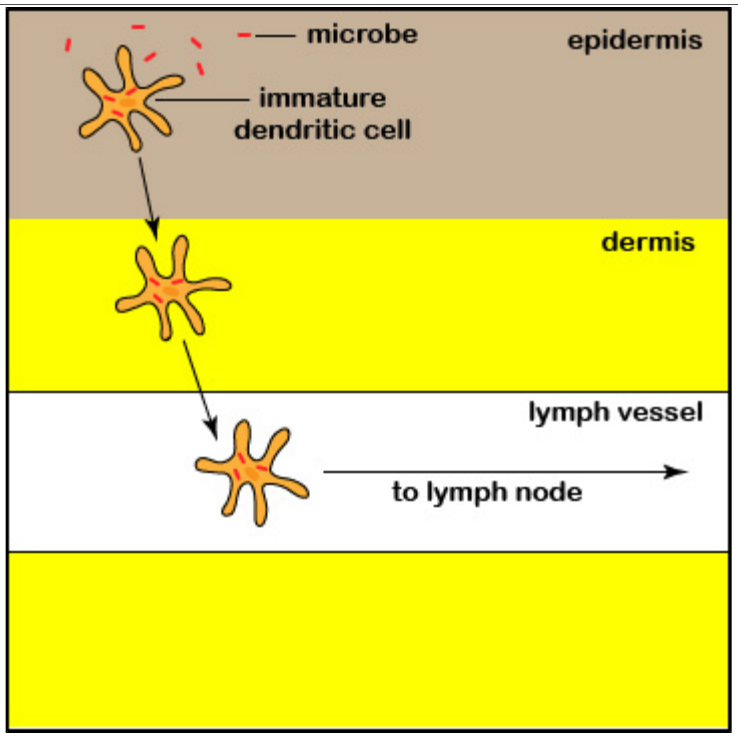
The MHC-I molecules with bound peptide on the surface of the APCs can now be recognized by naive T8-lymphocytes possessing TCRs and CD8 molecules with a complementary shape. This recognition of the peptide epitope by the TCR serves as a first signal for activating the naive T8-lymphocyte for cell-mediated immunity function.

(Through the process of cross-presentation, some antigen-presenting dendritic cells can cross-present epitopes of exogenous antigens to MHC-I molecules for eventual presentation to naive T8-lymphocytes.)

Most dendritic cells are derived from monocytes and are referred to as myeloid dendritic cells. They are located under the surface epithelium of the skin and the surface epithelium of the mucous membranes of the respiratory tract, genitourinary tract, and the gastrointestinal tract. They are also found throughout the body's lymphoid tissues and in most solid organs.

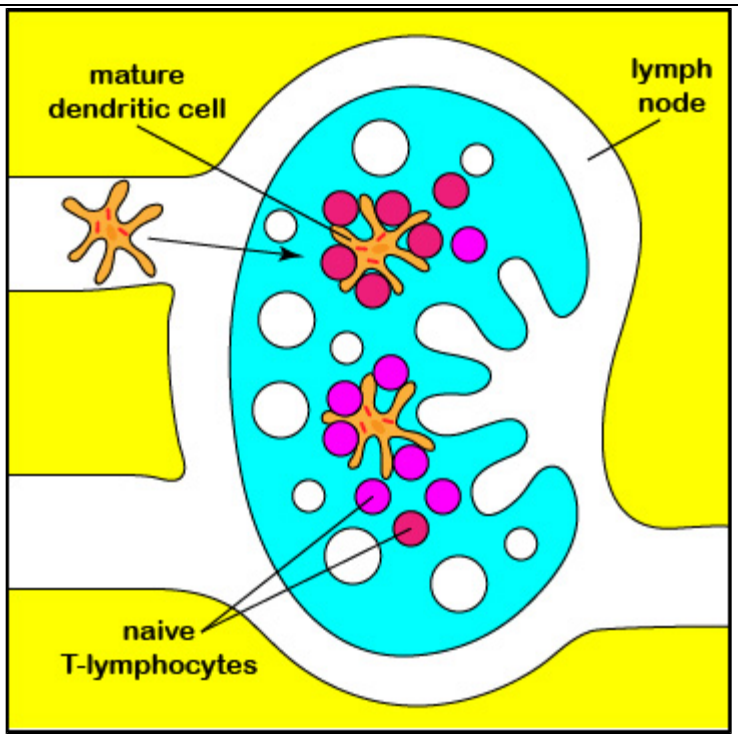
Upon capturing antigens through pinocytosis and phagocytosis and becoming activated by proinflammatory cytokines, the dendritic cells detach from the epithelium, enter lymph vessels, and are carried to regional lymph nodes **(see Fig. 6)**. By the time they enter the lymph nodes, they have matured and are now able to present antigen to the ever changing populations of naive T-lymphocytes located in the T-cell area of the lymph nodes **(see Fig. 7)**.

Fig. 6: Immature Dendritic Cells Engulfing and Processing Microbes and Carrying them to Regional Lymph Nodes, Part 1



The binding of microbial PAMPs to the PRRs of the immature dendritic cell activates that dendritic cell and promotes production of the chemokine receptor CCR7 that directs the dendritic cell into local lymphoid nodes. Following maturation, the dendritic cell can now present protein epitopes bound to MHC molecules to all the various naive T-lymphocytes passing through the lymphoid system.

Fig. 7: Immature Dendritic Cells Engulfing and Processing Microbes and Carrying them to Regional Lymph Nodes, Part 2



The binding of microbial PAMPs to the PRRs of the immature dendritic cell activates that dendritic cell and promotes production of the chemokine receptor CCR7 that directs the dendritic cell into local lymphoid nodes. Following maturation, the dendritic cell can now present protein epitopes bound to MHC molecules to all the various naive T-lymphocytes passing through the lymphoid system.

To view an electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #1 see the Web page for the University of Illinois College of Medicine.

To view an electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #2 see the Web page for the University of Illinois College of Medicine.

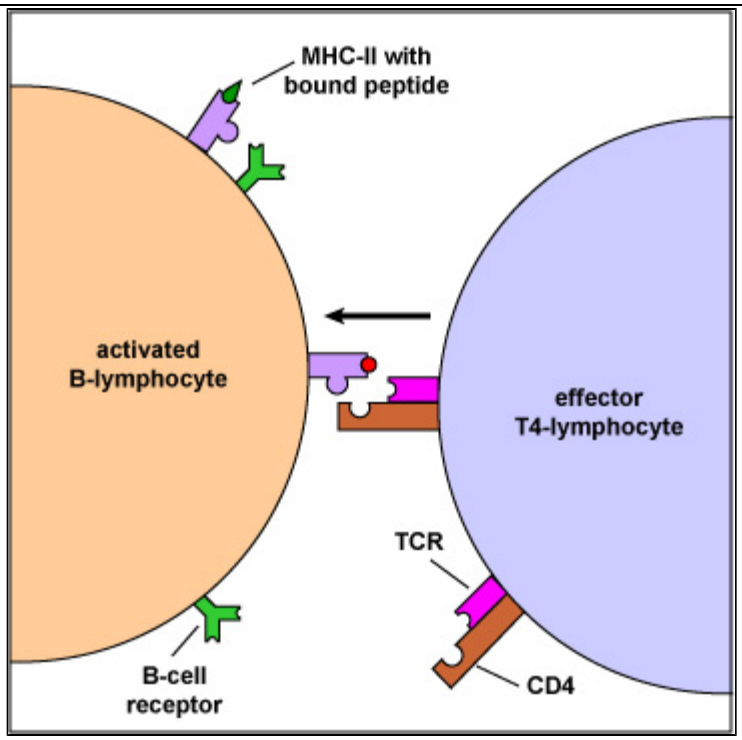
For more information: Review of antigen-presenting cells

b. Naive T4-helper lymphocytes recognizing peptide epitopes

Naive T4-lymphocytes circulate in the blood. In response to chemokines produced by lymphoid tissues, they leave the vascular endothelium in regions called high endothelial venules and enter lymph nodes or other secondary lymphoid tissues, a process called diapedesis.

Naive T4-lymphocytes have T-cell receptors **(TCRs) that, in cooperation with CD4 molecules**, bind to **MHC-II molecules with attached epitope from an antigen** found on the surface of an antigen-presenting dendritic cells; **(see Fig. 8)**. Each T4-lymphocyte is genetically programmed to make a unique TCR. The TCR recognizes the peptide while the CD4 molecule recognizes the MHC-II molecule.

Fig. 8: An Effector T4-Lymphocyte Recognizing Epitope/MHC-II on an activated B-Lymphocyte



Exogenous antigens are those from outside cells of the body. Examples

include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens bind to B-cell receptors and enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize the peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules. The subset of effector T4-lymphocytes that normally interact with activated B-lymphocytes to promote B-lymphocyte proliferation and differentiation are called Th2 lymphocytes.

Flash animation of a naive T4-lymphocyte recognizing epitopes on MHC-II via its TCR and CD4.

html5 version of animation for iPad showing a naive T4-lymphocyte recognizing epitopes on MHC-II via its TCR and CD4.

Naive T4-lymphocytes via the unique T-cell receptors and CD4 molecules on their surface recognize peptide epitopes from exogenous antigens bound to MHC-II molecules on antigen presenting dendritic cells. Different T-cell receptors recognize different epitopes.

For more information: Review of MHC molecules

For more information: Review of antigen-presenting cells

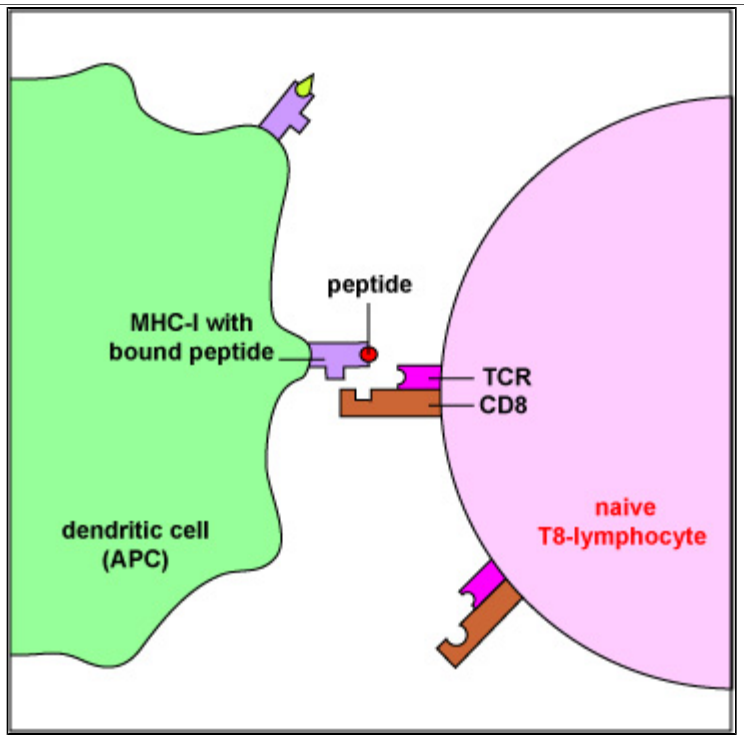
For more information: Review of T4-lymphocytes

c. Naive T8-lymphocytes recognizing peptide epitopes

Naive T8-lymphocytes circulate in the blood. In response to chemokines produced by lymphoid tissues, they leave the vascular endothelium in regions called high endothelial venules and enter lymph nodes or other secondary lymphoid tissues, a process called diapedesis.

Naive T8-lymphocytes have T-cell receptors **(TCRs) that, in cooperation with CD8 molecules**, **bind to MHC-I molecules with attached epitope from an antigen** found on the surface of antigen-presenting dendritic cells **(see Fig. 9)**. Each T8-lymphocyte is genetically programmed to make a unique TCR. The TCR recognizes the peptide while the CD8 molecule recognizes the MHC-I molecule.

Fig. 4: An Antigen-Presenting Dendritic Cell Presenting MHC-I with Bound Peptide to a Naive T8-lymphocyte having a Complementary T-Cell Receptor



Antigen-presenting dendritic cells produce both MHC-I and MHC-II molecules. These dendritic cells can phagocytose infected cells and tumor cells, place them in phagosomes, and degrade them with lysosomes. During this process, some of the proteins escape from the phagosome into the surrounding cytosol. Here they can be degraded into peptides by proteasomes, bound to MHC-I molecules, and placed on the surface of the dendritic cell. Now the peptide/MHC-I complexes can be recognized by a naive T8-lymphocyte having a complementary shaped T-cell receptor (TCR) and CD8 molecule. This activates the naive T8-lymphocyte enabling it to eventually proliferate and differentiate into cytotoxic T-lymphocytes (CTLs).

Flash animation of a naive T8-lymphocyte recognizing epitopes on MHC-I via its TCR and CD8.

html5 version of animation for iPad showing a naive T8-lymphocyte recognizing epitopes on MHC-I via its TCR and CD8.

A naive T8-lymphocyte uses its TCR and CD8 molecules to bind to complementary shaped MHC-I molecule with attached peptide epitope on an antigen-presenting dendritic cell. As naive T8-lymphocytes migrate through lymphoid tissues, they use surface cell adhesion molecules such as LFA-1 and CD2 to bind transiently to corresponding surface receptors such as ICAM-1, ICAM-2 and CD58 on dendritic cells. This transient binding allows time for the TCRs on the T8-lymphocyte to sample large numbers of MHC-I/peptide complexes on the dendritic cells.

For more information: Review of MHC molecules

For more information: Review of antigen-presenting cells

For more information: Review of T8-lymphocytes

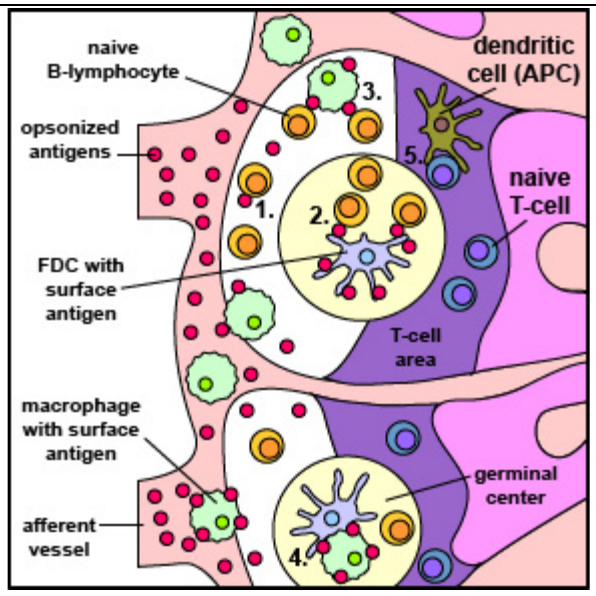
d. Naive B-lymphocytes recognizing epitopes of antigens

Most proteins are **T-dependent antigens**. In order for naive B-lymphocytes to proliferate, differentiate and mount an antibody response against T-dependent antigens, these B-lymphocytes **must interact with effector T4-lymphocytes**. **All classes or isotypes of antibody molecules can be made against T-dependent antigens and there is usually a memory response against such antigens**.

Naive B-Lymphocytes encounter antigens in secondary lymphoid organs such as the lymph nodes and the spleen. Using a lymph node as an example, **soluble antigens**, **such as microbial polysaccharides and proteins and toxins, as well as microbes such as bacteria and viruses, enter the lymph node** through afferent lymphatic vessels. By this time, **complement pathway activation has coated these soluble antigens or microbes with opsonins such as C3b, which in turn can be degraded to C3d**.

Located within the lymphoid tissues are **specialized macrophages and specialized dendritic cells called follicular dendritic cells (FDCs)**. These macrophages have poor endocytic ability and produce few lysosomes. The FDCs are nonphagocytic. Both cell types, however, **have complement receptors** called CR1 and CR2 that bind to the C3b and C3d, **enabling the antigens and microbes to stick to the surface of the macrophages and FDCs**. However,because of the poor endocytic ability of the macrophages and the lack of endocytosis by the FDCs, **the antigens and microbes are not engulfed but rather remain on the surface of the cells**. In addition, the macrophages can transfer their bound antigens or microbes to FDCs **(see Fig. 10)**. **Here the antigens and microbes in the lymph node can bind to complementary-shaped B-cell receptors or BCRs on naive B-lymphocytes directly, by way of macrophages, or via the FDCs (see Fig. 10)**.

Fig. 10: B-lymphocyte and T-Lymphocytes Recognizing Antigens in a Lymph Node



Opsonized antigens (those coated with C3b and C4b from the complement pathways) enter a lymph node through afferent lymphoid vessels. These opsonized antigens bind to and remain on the surface of specialized macrophages and follicular dendritic cells (FDCs). In addition, macrophages can transfer antigens to FDCs (see 4. above). Using their B-cell receptor (BCR), naive B-lymphocytes are able to recognize antigens directly (see 1. above), or more commonly, on the surface of FDCs (see 2. above), or on the surface of macrophages (see 3. above) in

the germinal centers and lymphoid follicles of the lymph node. Meanwhile, naive T-lymphocytes are being activated by antigen-presenting dendritic cells in the T-cell areas of the lymph node (see 5. above). T4-effector cells and activated B-lymphocytes then interact with one another at the interface between the germinal centers and the T-cell areas.

Flash animation of the binding of peptide epitopes to MHC-II molecules by a B-lymphocyte.

html5 version of animation for iPad showing the binding of peptide epitopes to MHC-II molecules by a B-lymphocyte.

Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, microbial proteins and polysaccharides, and toxins. These exogenous antigens bind to B-cell receptors either directly, or, more commonly, from the surface of either specialized macrophages or follicular dendritic cells (FDCs) located in the lymph nodes and the spleen. The antigens then enter B-lymphocytes through endocytosis. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the B-lymphocyte. Effector T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

1. Epitopes of exogenous antigens, such as viruses, bind to a complementary shaped B-cell receptor on a B-lymphocyte. The antigen is engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. As the MHC-II molecules with bound Ii chain are transported to the Golgi complex, the Ii is cleaved, leaving a short peptide called CLIP in the groove of the MHC molecule.
6&7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The CLIP peptide is removed from the MHC=II molecules and the peptide epitopes are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by effector T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Flash animation of an effector T4-lymphocyte recognizing epitopes bound to MHC-II molecules on a B-lymphocyte.

html5 version of animation for iPad showing an effector T4-lymphocyte recognizing epitopes bound to MHC-II molecules on a B-lymphocyte.

An effector T4-lymphocyte, such as a T_{FH} cell, use its TCRs and CD4 molecules to bind to a complementary shaped MHC-II molecules with attached peptide epitope on

an activated B-lymphocyte. This interaction, along with the binding of co-stimulatory molecules such as CD40 and B7 on the B-lymphocyte with their complementary ligands CD40L and CD28 on the effector T4-lymphocyte triggers the T4-lymphocyte to produce cytokines that enable the activated B-lymphocyte to proliferate, differentiate into antibody-secreting plasma cells, and switch classes of the antibodies being made.

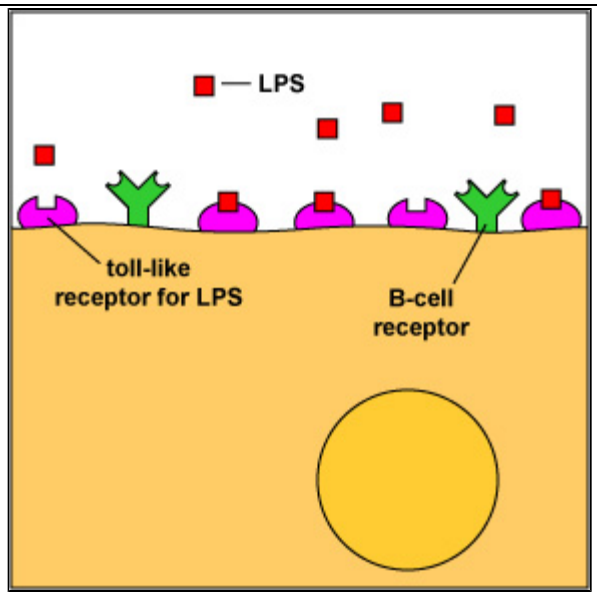
For more information: Review of B-lymphocytes

For more information: Review of antigen-presenting cells

A few antigens are called T-independent antigens. T-independent (TI) antigens are usually large **carbohydrate and lipid molecules** with multiple, repeating subunits. **B-lymphocytes mount an antibody response to T-independent antigens without the requirement of interaction with effector T4-lymphocytes**. Bacterial lipopolysaccharide (LPS) from the Gram-negative cell wall and capsular polysaccharides are examples of TI antigens. **The resulting antibody molecules are generally of the IgM isotype and do not give rise to a memory response**. There are two basic types of T-independent antigens: TI-1 and TI-2.

1. **TI-1 antigens** are pathogen-associated molecular patterns or PAMPS such as **lipopolysaccharide (LPS)** from the outer membrane of the Gram-negative cell wall and **bacterial nucleic acid**. These antigens **activate B-lymphocytes by binding to their specific pattern-recognition receptors**, in this case toll-like receptors, rather than to B-cell receptors **(see Fig. 11)**. Antibody molecules generated against TI-1 antigens are often called "natural antibodies" because they are always being made against bacteria present in the body.

Fig. 11: Activation of a B-lymphocyte by way of LPS Binding to Toll-like Receptors



TI-1 antigens include lipopolysaccharide (LPS) from the outer membrane of the gram-negative cell wall and bacterial nucleic acid. These antigens activate B-lymphocytes by binding to their specific toll-like receptors rather than to B-cell receptors. Binding of LPS to its toll-like receptor activates chemical signals that lead to transcription of antibody and cytokine genes.

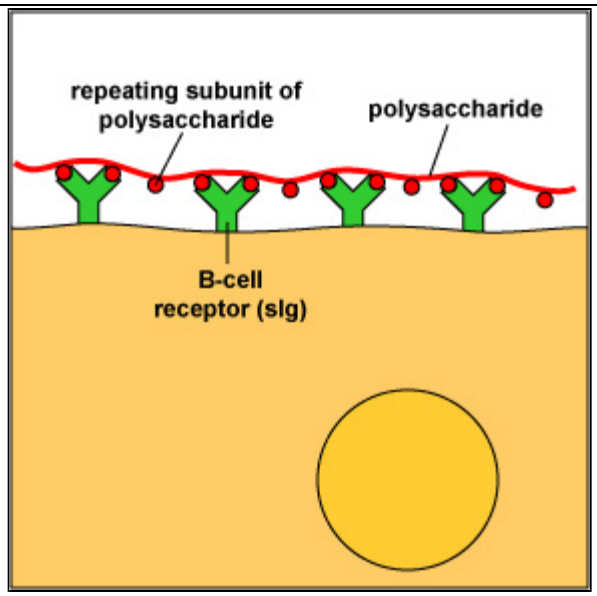
For more information: Review of pathogen-associated molecular

patterns (PAMPs)

For more information: Review of pattern-recognition receptors (PRRs)

2. **TI-2 antigens**, such as **capsular polysaccharides**, are molecules with multiple, repeating subunits. These repeating subunits **activate B-lymphocytes by simultaneously cross-linking a number of B-cell receptors (see Fig. 12)**.

Fig. 12: Activation of a B-lymphocyte through the Cross Linking of B-Cell Receptors by TI-2 Antigens



TI-2 antigens, such as capsular polysaccharides, are molecules with multiple, repeating subunits. These repeating subunits activate B-lymphocytes by simultaneously cross-linking a number of B-cell receptors. This cross-linking activates chemical signals that lead to transcription of antibody and cytokine genes.

Those naive B-lymphocytes not activated by epitopes of antigens exit the lymph node or other lymphoid tissue and eventually re-enter the bloodstream.

Concept Map for The General Steps in Adaptive Immunity

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Quiz Group

3. After the naive B-lymphocytes, T4-lymphocytes, and T8-lymphocytes bind their corresponding epitopes, they must proliferate into large clones of identical cells in order to mount a successful immune response against that antigen. This is known as

clonal expansion.

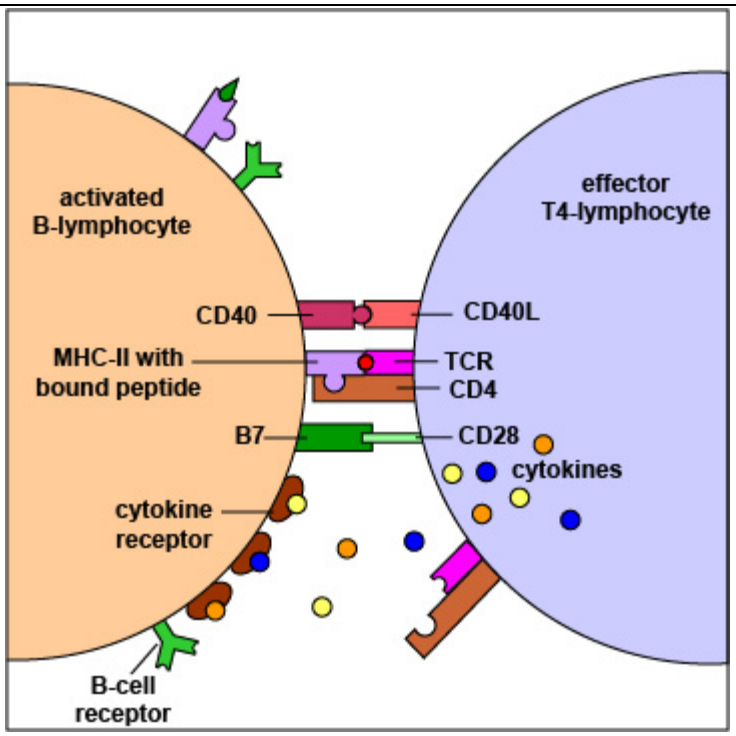
Fundamental Statements for this Step:

1. With the exception of T-independant antigens, naive B-lymphocytes must be stimulated to proliferate by means of cytokines called interleukins produced primarily by effector T4- lymphocytes such as T_{FH} cells.

2. In the case of T4-lymphocytes and T8-lymphocytes, dendritic cells produces cytokines that contribute to proliferation of the activated T-lymphocytes. CD28-dependent co-stimulation of the T4-lymphocyte also stimulates it to synthesize the cytokine interleukin-2 (IL-2) as well as a high-affinity IL-2 receptor. The binding of IL-2 to its high affinity receptor allows for cell proliferation and formation of a clone of thousands of identical T-lymphocytes after several days.

With the exception of T-independant antigens, the naive B-lymphocytes that were activated in step 2 above must be **stimulated to proliferate by means of cytokines** called interleukins (such as IL-2, IL-4, IL-5, Il-6, and IL-10) **produced primarily by effector T4- lymphocytes such as T_{FH} cells (see Fig. 13)**.

Fig. 13: An Effector T4-Lymphocyte Recognizing Epitope/MHC-II on an activated B-Lymphocyte and Producing Cytokines to Trigger Proliferation and Differentiation



An effector T4-lymphocyte, such as a T_{FH} cell, use its TCRs and CD4 molecules to bind to a complementary shaped MHC-II molecules with attached peptide epitope on an activated B-lymphocyte. This interaction, along with the binding of co-stimulatory molecules such as CD40 and B7 on the B-lymphocyte with their complementary ligands CD40L and CD28 on the effector T4-lymphocyte triggers the T4-lymphocyte to produce cytokines that enable the activated B-lymphocyte to proliferate, differentiate into antibody-secreting plasma cells, and switch classes of the antibodies being made.

Flash animation of an effector T4-lymphocyte interacting with an activated B-lymphocyte.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing an effector T4-lymphocyte interacting with an activated B-lymphocyte.
An effector T4-lymphocyte, such as a T_{FH} cell, use its TCRs and CD4 molecules to bind to a complementary shaped MHC-II molecules with attached peptide epitope on an activated B-lymphocyte. This interaction, along with the binding of co-stimulatory molecules such as CD40 and B7 on the B-lymphocyte with their complementary ligands CD40L and CD28 on the effector T4-lymphocyte triggers the T4-lymphocyte to produce cytokines that enable the activated B-lymphocyte to proliferate, differentiate into antibody-secreting plasma cells, and switch classes of the antibodies being made.

For more information: Review of T4-lymphocytes

In the case of T4-lymphocytes and T8-lymphocytes, **dendritic cells produces cytokines** such as interleukin-6 (IL-6), IL-4, IL-12, and T-cell growth factor-beta (TGF-ß) **that contribute to proliferation of the activated T-lymphocytes**. CD28-dependent co-stimulation of the T4-lymphocyte also stimulates it to synthesize the cytokine **interleukin-2 (IL-2) as well as a high-affinity IL-2 receptor**. **The binding of IL-2 to its high affinity receptor allows for cell proliferation and formation of a clone of thousands of identical T-lymphocytes after several days**.

It is thought that in most immune responses, only around 1/1000 to 1/10,000 lymphocytes will have a receptor capable of binding the initiating antigen. Thus, proliferation allows the **production of clones of thousands of identical lymphocytes** having specificity for the original antigen. **This is essential to give enough cells to mount a successful immune response against that antigen**.

GIF Animation showing proliferation of a B-lymphocyte
Copyright © Gary E. Kaiser
GIF Animation showing proliferation of a T4-lymphocyte
Copyright © Gary E. Kaiser
GIF Animation showing proliferation of a T8-lymphocyte
Copyright © Gary E. Kaiser

Concept Map for The General Steps in Adaptive Immunity

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4. The large clones of identical B-lymphocytes, T4-lymphocytes, and T8-lymphocytes now differentiate into effector cells capable of directing body defenses against the

original antigen resulting in its destruction or neutralization.

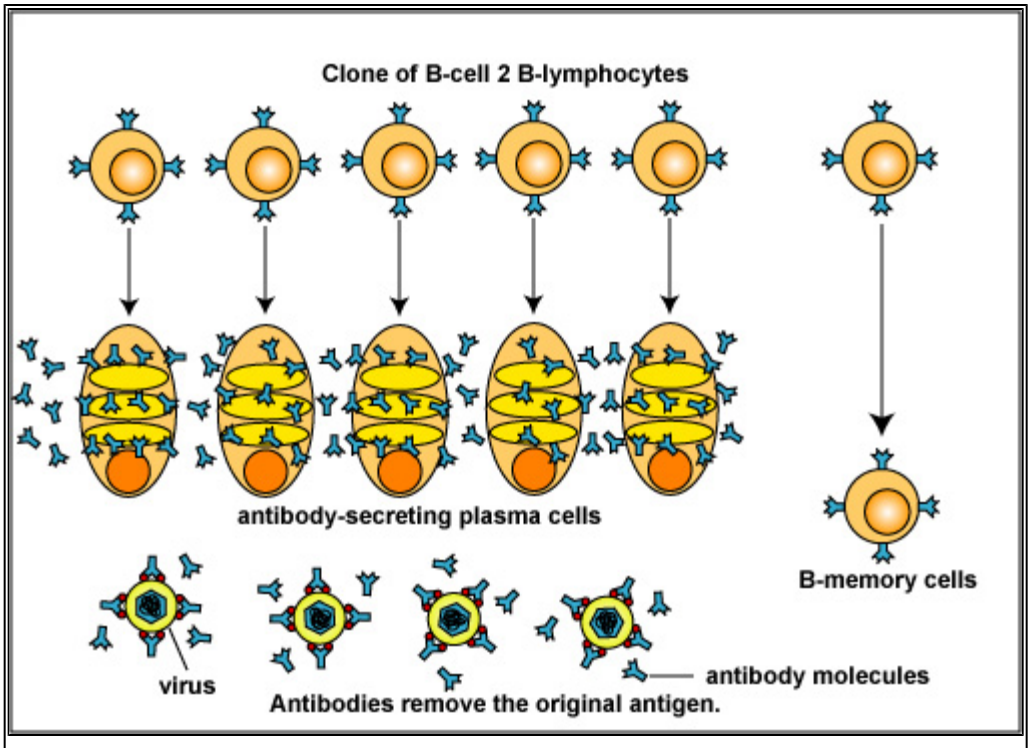
Fundamental Statements for this Step:

1. Cytokines produced by dendritic cells and T4-effector lymphocytes enable the clones of B-lymphocytes and T-lymphocytes above to differentiate into effector cells.
2. In the case of humoral immunity, B-lymphocytes differentiate into effector cells called plasma cells. These cells synthesize and secrete vast quantities of antibodies capable of reacting with and eliminating or neutralizing the original antigen.
3. T4-lymphocytes differentiate into T4-effector lymphocytes. Functionally, there are many different types or subpopulations of effector T4-lymphocytes based on the cytokines they produce. Examples include T_H1 cells, T_H2 cells, T_H17 cells, T_{reg} cells, and T_{FH} cells.
4. In the case of cell-mediated immunity , the T8-lymphocytes differentiate into cytotoxic T-lymphocytes (CTLs) capable of destroying body cells having the original epitope on their surface, such as viral infected cells, bacterial infected cells, and tumor cells by inducing apoptosis.
5. Antibodies, cytokines, activated macrophages, and cytotoxic T-lymphocytes eventually destroy or remove the antigen.

Cytokines produced by dendritic cells and T4-effector lymphocytes enable the clones of B-lymphocytes and T-lymphocytes from step 3 above to differentiate into effector cells.

a. In the case of **humoral immunity**, B-lymphocytes differentiate into effector cells called **plasma cells**. These cells synthesize and secrete vast quantities of **antibodies** capable of reacting with and eliminating or neutralizing the original antigen **(see Fig. 14)**.

Fig. 14: Differentiation of B-lymphocytes into Plasma Cells and B-Memory Cells



The B-lymphocytes now differentiate into antibody-secreting B-lymphocytes and plasma cells that secrete large quantities of antibodies that fit the original epitope. Some B-lymphocytes differentiate into B-memory cells capable of anamnestic response.

GIF Animation showing proliferation of a B-lymphocyte and its differentiation into an effector cell

For more information: Review of B-lymphocytes

b. **T4-lymphocytes differentiate into T4-effector lymphocytes**. Functionally, there are **many different types or subpopulations of effector T4-lymphocytes based on the cytokines they produce**. Immune reactions are typically dominated by five primary types: T_H1 cells, T_H2 cells, T_H17 cells, T_{reg} cells, and T_{FH} cells.

1. **CD4 T_H1 cells: Coordinate immunity against intracellular bacteria and promote opsonization**. They:

   a. Produce cytokines such as interferon-gamma (IFN-?) that **promote cell-mediated immunity against intracellular pathogens, especially by activating macrophages that have either ingested pathogens or have become infected with intracellular microbes** such as *Mycobacterium tuberculosis, Mycobacterium leprae, Leishmania donovani*, and *Pneumocystis jiroveci* that are able to grow in the endocytic vesicles of macrophages. **Activation of the macrophage by T_H1 cells greatly enhances their antimicrobial effectiveness**.

   b. They produce cytokines that **promote the production of opsonizing antibodies that enhance phagocytosis (see Fig. 15)**.

   c. **Produce receptors that bind to and kill chronically infected cells, releasing the bacteria that were growing within the cell so the can be engulfed and killed by macrophages**.

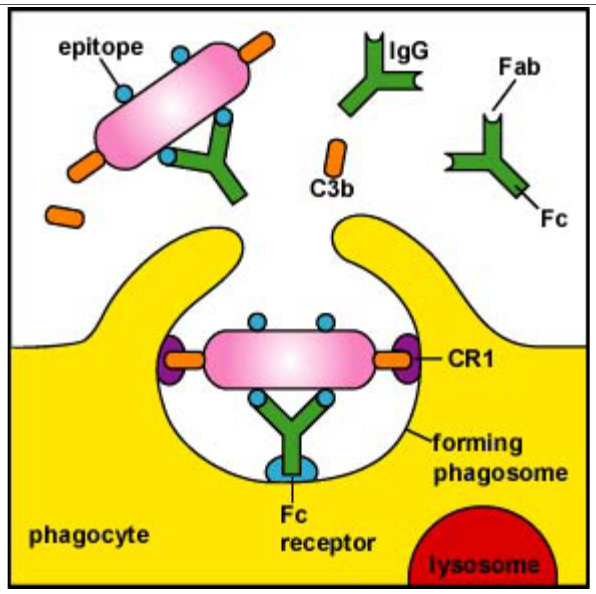
   d. Produce the cytokine interleukin-2 (IL-2) that **induces T-lymphocyte proliferatio**n.

   e. Produce cytokines such as tumor necrosis factor-alpha (TNF-a) that **promote diapedesis of macrophage**s.

   f. Produces the chemokine CXCL2 to **attract macrophages to the infection site**.

   g. Produce cytokines that block the production of T_H2 cells.

Fig. 15: Opsonization (Enhanced Attachment)



The Fab portion of the antibody IgG binds to epitopes of an antigen such as this bacterium. The Fc portion of IgG then binds to Fc receptors on phagocytes for opsonization or enhanced attachment. Once attached to the phagocyte the microbe can be engulfed more efficiently and placed in a phagosome. Complement pathway proteins such as C3b and C4b can also function as opsonins.

2. CD4 T_H2 cells: Coordinate immunity against helminths and microbes that colonize mucous membranes.

a. Produce the cytokine interleukin-4 (IL-4) that **promotes the production of the antibody isotype IgE in response to helminths and allergens**. **IgE is able to stick eosinophils to helminths for extracellular killing** of the helminth **(see Fig. 16)**; it also **promotes many allergic reactions**.

b. Produce cytokines that **attract and activate eosinophils and mast cells**.

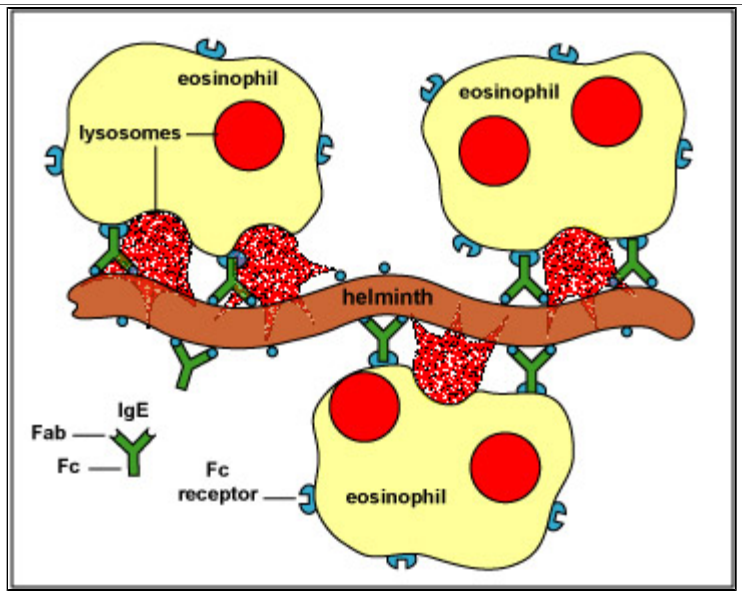
c. **Promote the production of antibodies that neutralize microbes (see Fig. 17) and toxins (see Fig. 18) preventing their attachment to host cell**s.

d. Produce cytokines that function as **B-lymphocyte growth factors** such as IL-4, IL-5, IL-9. and IL-13.

e. Produce interleukin-22 (IL-22) that **promotes the removal of microbes in mucosal tissues**.

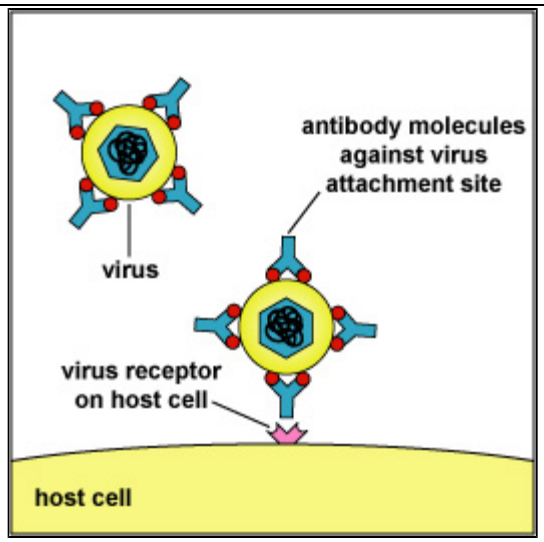
f. Produce cytokines that block the production of T_H1 cells.

Fig. 16: Opsonization of a Helminth by IgE and Eosinophils



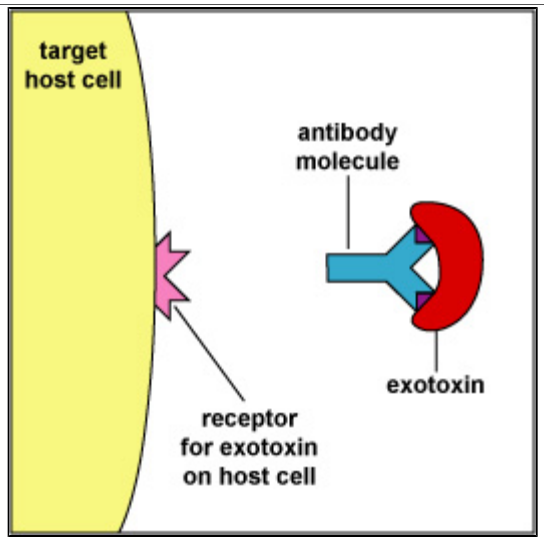
A major function of the cytokines produced by T_H2 cells is to enable B-lymphocytes to activate eosinophils and produce increased amounts of a class of antibodies called IgE against helminths (parasitic worms) and arthropods. IgE act as an opsonizing antibody to stick phagocytic eosinophils to helminths for extracellular killing of the helminths. The Fab portion of IgE reacts with epitopes on the helminth while the Fc portion binds to Fc receptors of activated eosinophils. The lysosomal proteases of eosinophils are able to destroy the tough integument of helminths. IgE also promotes inflammation to recruit phagocytes.

Fig. 17: Neutralization of Viruses



The Fab portion of the antibodies made against epitopes of the virus attachment site blocks the virus from adsorbing to the receptor site on the host cell membrane. As a result, the virus can not penetrate and replicate.

Fig. 18: Neutralization of Exotoxins



The Fab portion of the antibodies made against epitopes of the binding site of an exotoxin blocks the exotoxin from binding to the exotoxin receptor on the host cell membrane. As a result, the toxin can not enter the cell and cause harm.

3. CD4 T_H17 cells: Promote a local inflammatory response to stimulate a strong neutrophil response and promote the integrity of the skin and mucous membranes.

Produce cytokines like interleukin-17 (IL-17) and interleukin-6 (IL-6) that **trigger local epithelial cells and fibroblasts to produce chemokines that recruit neutrophils to remove extracellular pathogens**.

4. CD4 T_{reg} cells: Suppress immune responses.

a. Produce inhibitory cytokines such as Interleukin-10 (IL-10) and TGF-ß that **help to limit immune responses and prevent autoimmunity by suppressing T-lymphocyte activity**.

b. **Promoting anamnestic response (immunologic memory) to resist repeat infections by the same microbe**.

c. **Protecting beneficial normal flora in the intestines from being destroyed by the immune system**.

d. **Aiding in sustaining pregnancy** so that the immune system doesn't recognize a fetus as foreign and try to destroy it.

e. **Controlling established inflammation** in tissues.

5. T_{FH} cells: Promote humoral immunity by stimulating antibody production and antibody isotype switching by B-lymphocytes.

a. T follicular helper cells (T_{FH} cells) are located in lymphoid follicules.

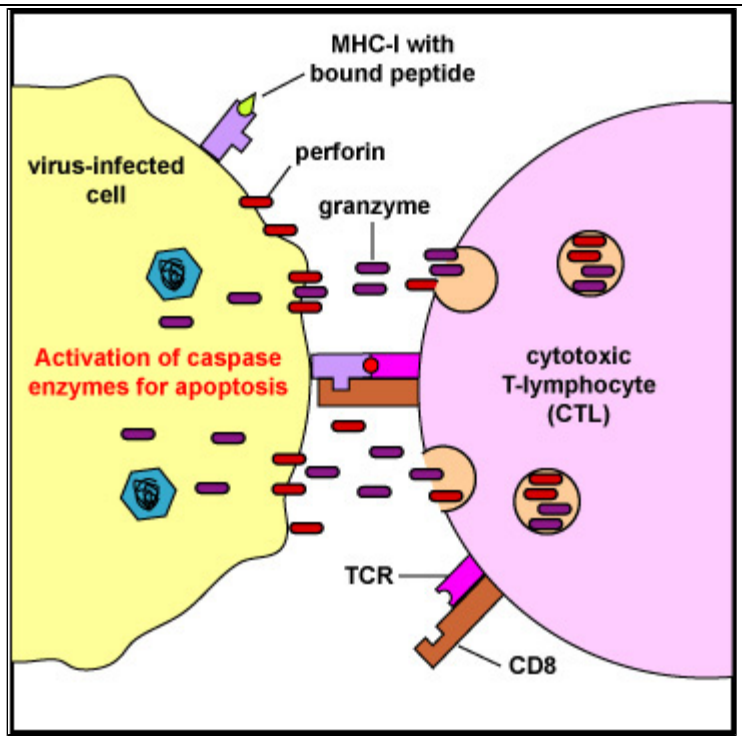
b. T_{FH} cells are now **thought to be the primary effector T-lymphocytes that stimulate antibody production and isotype switching by B-lymphocytes**. They are **able to produce cytokines that are characteristic of both T_H2 cells and T_H1 cells**.

c. T_{FH} cells producing (IFN-?) promote the production of opsonizing antibodies; those producing IL-4 promote the production of IgE.

c. In the case of **cell-mediated immunity**, the T8-lymphocytes differentiate into **cytotoxic T-lymphocytes (CTLs)** capable

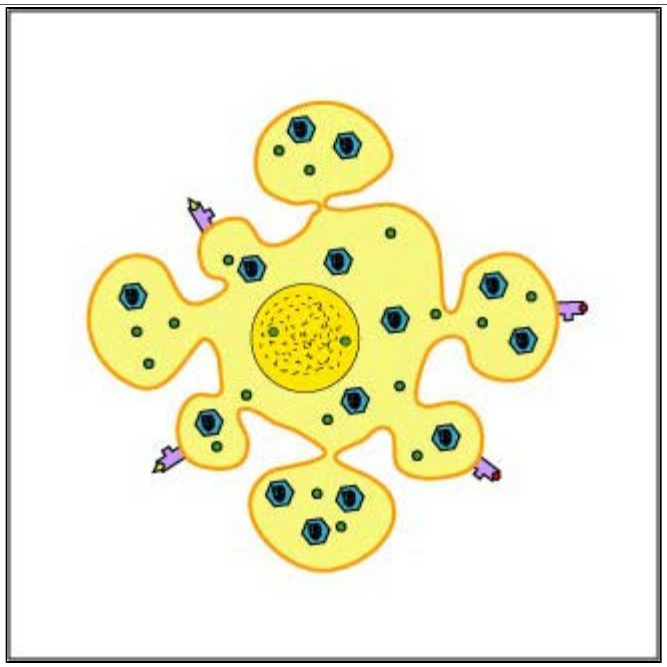
of destroying body cells having the original epitope on their surface, such as viral infected cells, bacterial infected cells, and tumor cells. They do this by inducing apoptosis, a programmed cell suicide (**see Fig. 19** and **Fig. 20**). T-lymphocytes also secrete various **cytokines** that participate in various aspects of adoptive and innate immunity.

Fig. 19: Cytotoxic T-lymphocyte (CTL)-Induced Apoptosis of a Virus-Infected Cell



Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the caspase enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes.

Fig. 20: CTL-Induced Apoptosis of a Virus-Infected Cell



Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the perforin pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. This prevents the release of any mature viruses that might occur if the infected cell was lysed.

GIF Animation showing proliferation of a T8-lymphocyte and its differentiation into an effector cell.

Flash animation showing induction of apoptosis by way of perforins and granzymes.

html5 version of animation for iPad showing induction of apoptosis by way of perforins and granzymes.

Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.)

Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles

and mediators of inflammation, are not released as they are during cell lysis.

Flash animation of CTL-induced apoptosis of a virus-infected cell.

html5 version of animation for iPad showing CTL-induced apoptosis of a virus-infected cell.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

- Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and degrade both the target cell's nucleoprotein and microbial DNA within the cell.
- Granzymes cleave a variety of other cellular substrates that contribute to cell death.
- The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can increase the permeability of the infected cell and contribute to cell death. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis. A granule called granulysin can also alter the permeability of both miocrobial and host cell membranes.

This animations shows destruction of both the cytoskeleton and nucleoprotein of the infected cell. As the infected cell breaks up into apoptotic fragments, the fragments are subsequently removed by phagocytes. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells.

For more information: Review of T8-lymphocytes

For more information: Preview of cytotoxic T-lymphocytes (CTLs)

Progeny of the original lymphocytes **leave the secondary lymphoid organs and migrate to tissues** where they continue to respond to the invading antigen.

Antibodies, cytokines, activated macrophages, and cytotoxic T-lymphocytes eventually destroy or remove the antigen. Antibodies and cytokines amplify defense functions and collaborate with cells of the innate immune system, such as phagocytes and NK cells, as well as with molecules of the innate immune system, such as those of the complement system and the acute phase response. Cytotoxic T-lymphocytes (CTLs) destroy body cells having the original epitope on their surface, e.g., viral infected cells, bacterial infected cells, and tumor cells. Cytokines also amplify innate immune defenses such as inflammation, fever, and the acute phase response.

Concept Map for The General Steps in Adaptive Immunity

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Quiz Group

5. Some of the B-lymphocytes, T4-lymphocytes, and T8-lymphocytes differentiate into long-lived, circulating memory cells.

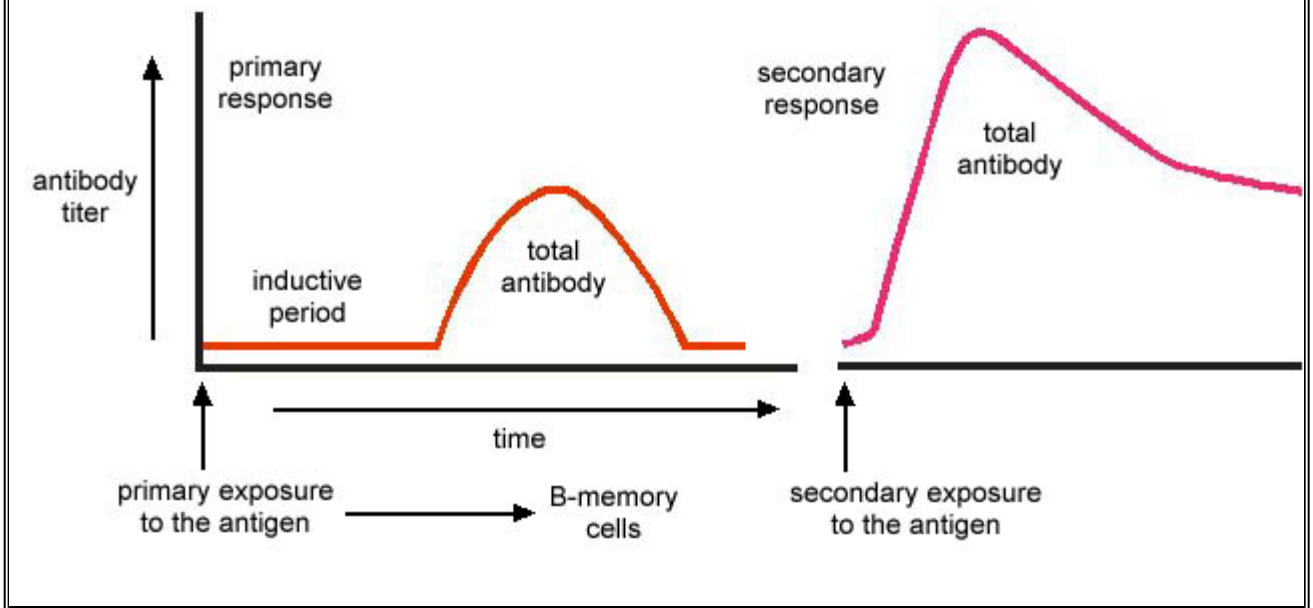
Fundamental Statements for this Step:

1. During the proliferation and differentiation that follows lymphocyte activation, some of the B-lymphocytes and T-lymphocytes stop replicating and become circulating, long-lived memory cells.
2. Memory cells are capable of what is called anamnestic response or "memory", that is, they "remember" the original antigen. If that same antigen again enters the body while the memory cells are still present, these memory cells will initiate a rapid, heightened secondary response against that antigen.

During the proliferation and differentiation that follows lymphocyte activation, some of the B-lymphocytes and T-lymphocytes stop replicating and become circulating, long-lived memory cells. **Memory cells** are capable of what is called anamnestic response or "memory", that is, they "remember" the original antigen. If that same antigen again enters the body while the memory cells are still present, these memory cells will **initiate a rapid, heightened secondary response against that antigen** (see **Fig. 14** and **Fig. 21**).

This is why the body sometimes develops a permanent immunity after an infectious disease and is also the **principle behind immunization**.

Fig. 21: Anamnestic Response



After the primary exposure to an antigen, there is an inductive period of generally several days to a week

when no measurable antibodies are detected in the serum. This is the period when the antigen is being exposed to immunocompetent cells, being processed by APCs, clonal selection and clonal expansion are taking place, and B-lymphocytes are differentiating into plasma cells and B-memory cells. Because of the memory cells, however, a second exposure to the same antigen results in more antibodies being made faster for a longer period of time.

For more information: Preview of anamnestic response (memory)

Concept Map for The General Steps in Adaptive Immunity

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Immune Regulation

Fundamental Statements for this Process:

1. The immune responses are carefully regulated by a variety of mechanisms. They are turned on only in response to an antigen and are turned off once the antigen has been removed.
2. The immune responses are also able to discriminate between self and non-self in order to prevent autoimmune tissue damage.
3. During the random gene-splicing reactions mentioned earlier, some lymphocytes are bound to produce receptors that fit the body's own proteins and polysaccharides. The body develops immunologic tolerance to these self antigens by triggering apoptosis in self-reactive lymphocytes.
4. Alternately, immature B-lymphocytes with self-reactive B-cell receptors may be stimulated to undergo a new gene rearrangement to make a new receptor that is no longer self-reactive. This process is called receptor editing.
5. Some autoreactive T-lymphocytes are able to slip through the system but a group of T4-effector lymphocytes called T_{reg} cells are able to suppress their action.
7. If there is a breakdown in this normal elimination or suppression of self-reacting cells, autoimmune diseases may develop.

The immune responses are **carefully regulated** by a variety of mechanisms. They are turned on only in response to an antigen and are turned off once the antigen has been removed.

The immune responses are also able to **discriminate between self and non-self** in order to prevent autoimmune tissue damage. During the random gene-splicing reactions mentioned earlier, some lymphocytes are bound to produce receptors that fit the body's own proteins and polysaccharides. Through mechanisms that are not fully understood, the body develops **immunologic tolerance** to these self antigens. In other words, the immune system becomes tolerant of the body's own

molecules.

During lymphocyte development, the body **eliminates self-reactive lymphocytes**. **Self-reactive B-lymphocytes undergo negative selection**. Since the bone marrow, where the B-lymphocytes are produced and mature, is normally free of foreign substances, any B-lymphocytes that bind substances there must be recognizing "self" and are eliminated by **apoptosis**, a programmed cell suicide. Apoptosis results in the activation of proteases within the target cell which then degrade the cell's structural proteins and DNA. Alternately, **immature B-lymphocytes with self-reactive B-cell receptors may be stimulated to undergo a new gene rearrangement to make a new receptor that is no longer self-reactive. This process is called receptor editing**.

This negative selection also occurs in secondary lymphoid organs whenever a T-dependent B-lymphocyte binds to an antigen but is then unable to react with its specific T-4 lymphocyte because the T4-lymphocyte does not recognize that antigen as foreign.

Self-reactive T-lymphocytes undergo both negative selection and positive selection. Positive selection occurs in the thymus and eliminates T-lymphocytes that cannot recognize MHC molecules. Because T4-lymphocytes and T8-lymphocytes can only recognize peptide epitopes bound to MHC molecules, any T-lymphocytes that cannot recognize MHC molecules fail this positive selection, do not develop any further, and are eventually eliminated. Then, each T-lymphocyte that passes positive selection by being able to recognize a MHC molecule must undergo negative selection. Any T-lymphocytes recognizing "self" peptides bound to MHC molecules are eliminated by apoptosis. Like with B-lymphocytes, this negative selection also occurs in secondary lymphoid organs whenever a T-lymphocyte binds to a peptide on a MHC molecule but is then unable to react with its specific T-4 lymphocyte because the T4-lymphocyte does not recognize that peptide as foreign.

Some autoreactive T-lymphocytes are able to slip through the system but a group of T4-effector lymphocytes called T_{reg} cells are able to suppress their action. If there is a breakdown in this normal elimination or suppression of self-reacting cells, **autoimmune diseases** may develop.

We will now look at the various events discussed above in greater detail as they apply to both humoral immunity and cell-mediated immunity with special emphasis on infectious diseases. Keep in mind that some infectious agents live outside human cells (e.g., most bacteria), a few live inside the phagosomes and lysosomes of human cells through which they enter (e.g., *Mycobacterium tuberculosis, Mycobacterium leprae*), and others live in the fluid interior of human cells (e.g., viruses, Rickettsias, and Chlamydias). Through a combination of humoral immunity and cell-mediated immunity, all types of infectious agents, as well as many types of tumor cells, may be eliminated from the body.

Self Quiz for An Overview of the Steps Involved in the Adaptive Immune Responses

Quiz Group

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ADAPTIVE IMMUNITY
HUMORAL IMMUNITY: ANTIBODY STRUCTURE

Adaptive Immunity

Humoral Immunity: Antibodies Structure

Fundamental Statements for this Softchalk Lesson:

1. ***Humoral Immunity refers to the production of antibody molecules in response to an antigen.***
2. Humoral immunity is most effective microbes or their toxins located in the extracellular spaces of the body.
3. Antibodies or immunoglobulins are specific glycoprotein configurations produced by B-lymphocytes and plasma cells in response to a specific antigen and capable of reacting with that antigen.
4. There are 5 classes or isotypes of human antibodies or immunoglobulins: IgG, IgM, IgA, IgD, and IgE.
5. The simplest antibodies, such as IgG, IgD, and IgE, are "Y"-shaped macromolecules called monomers and are composed of four glycoprotein chains: two identical heavy chains and two identical light chains.
6. The two tips of the "Y" monomer are referred to as the antigen-binding fragments or Fab portions of the antibody and these portions provide specificity for binding an epitope on an antigen.
7. Early in its development, each B-lymphocyte becomes genetically programmed through a series of gene-splicing reactions to produce a Fab with a unique 3-dimensional shape capable of fitting some epitope with a corresponding shape.
8. The Fc portion only becomes biologically active after the Fab component has bound to its corresponding antigen. Biological activated include activating the complement pathways, and binding to receptors on phagocytes and other defense cells to promote adaptive immunity.
9. IgM is a pentamer, consisting of 5 monomers joined at their Fc portions.
10. IgA is a dimer, consisting of 2 monomers joined at their Fc portions.

Common Course Objectives

1. Define antibody and state the functions of the Fab and the Fc portions of an antibody.

Detailed Learning Objectives

1*. Define antibody.

2*. In terms of infectious disease, state what humoral immunity is most effective against.

(*) = Common theme throughout the course

Humoral Immunity: Antibody Structure

Humoral Immunity refers to the production of **antibody molecules in response to an antigen**. These antibody molecules

circulate in the plasma of the blood and enter tissue and organs via the inflammatory response. Humoral immunity is **most effective microbes or their toxins located in the extracellular spaces of the body**.

Antibodies or immunoglobulins are specific glycoprotein configurations produced by B-lymphocytes and plasma cells **in response to a specific antigen** and **capable of reacting with that antigen**.

In this section we will look at the structure of antibodies.

Antibody Structure

There are 5 classes or isotypes of human antibodies:

a. immunoglobulin G (IgG),

b. immunoglobulin M (IgM),

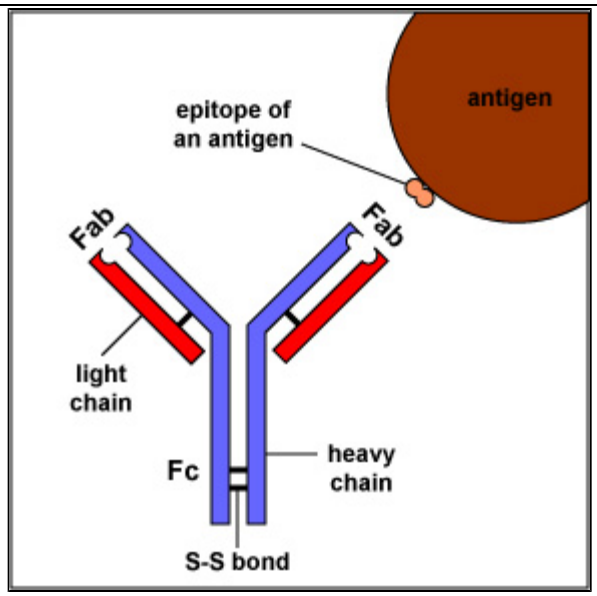
c. immunoglobulin A (IgA),

d. immunoglobulin D (IgD), and

e. immunoglobulin E (IgE).

The simplest antibodies, such as IgG, IgD, and IgE, are "Y"-shaped macromolecules called **monomers**. A monomer is composed of **four glycoprotein chains**: two identical **heavy chains** and two identical **light chains**. The two heavy chains have a high molecular weight that varies with the class of antibody. The light chains come in two varieties: kappa or lamda and have a lower molecular weight than the heavy chains. The four glycoprotein chains are connected to one another by disulfide (S-S) bonds and non-covalent bonds **(see Fig. 1)**.

Fig. 1: Structure of IgG

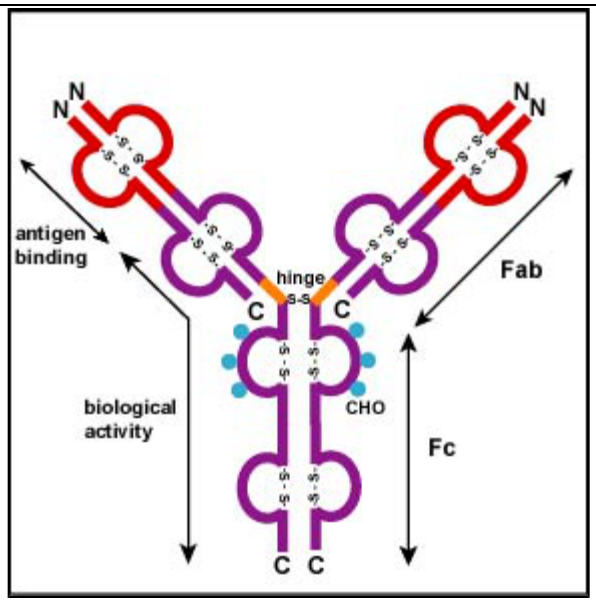


The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody.

For more information: Preview of the five classes of human antibodies

Additional S-S bonds fold the individual glycoprotein chains into a number of distinct globular domains **(see Fig. 2)**. The area where the top of the "Y" joins the bottom is called the **hinge**. This area is flexible to enable the antibody to bind to pairs of epitopes various distances apart on an antigen.

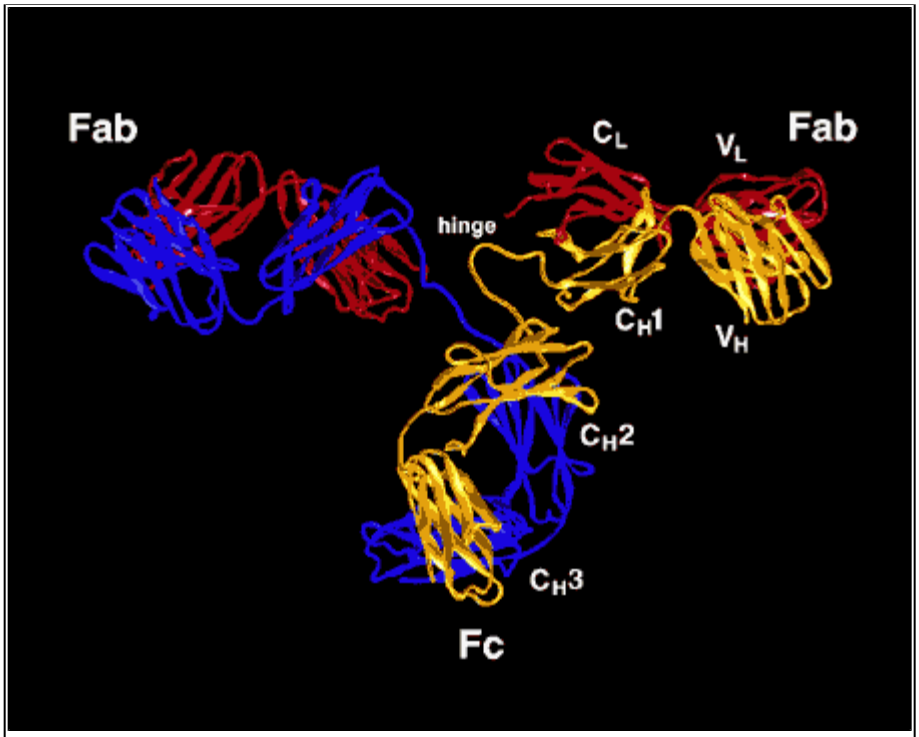
Fig. 2: Folding Domains of an Antibody



The Fab portion of the antibody, the first domain of the antibody shown in red has the complementarity-determining regions providing specificity for binding an epitope of an antigen. The Fc portion, shown in purple directs the biological activity of the antibody. (S-S = disulfide bond; N = amino terminal of glycoprotein; C = carboxy terminal of glycoprotein; CHO = carbohydrate.

The two tips of the "Y" monomer are referred to as the antigen-binding fragments or **Fab portions** of the antibody (see **Fig. 1**, **Fig. 2**, and **Fig. 3**). The first 110 amino acids or first domain of both the heavy and light chain of the Fab region of the antibody **provide specificity for binding an epitope on an antigen**.The amino acid sequence of this first domain of both the height chain and the heavy chain shows tremendous variation from antibody to antibody and constitutes the variable region (V region). This is because **each B-lymphocyte, early in its development, becomes genetically programmed through a series of gene-splicing reactions to produce a Fab with a unique 3-dimensional shape capable of fitting some epitope with a corresponding shape**.

Fig. 3: Ribbon Drawing of the Antibody Molecule IgG2a

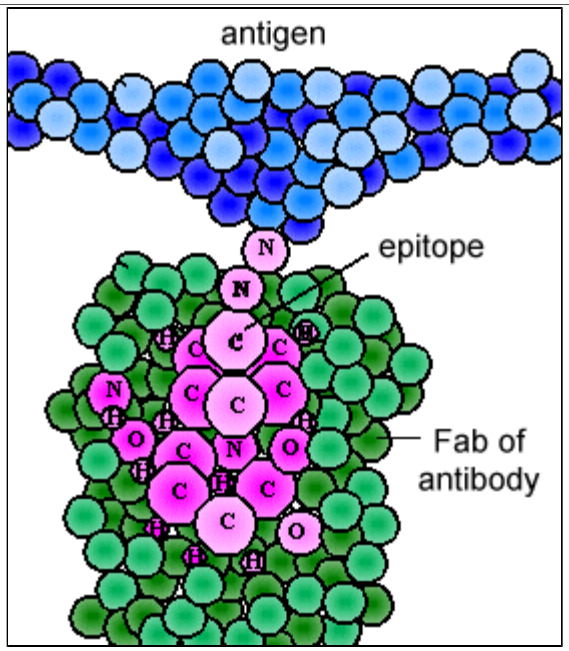


A ribbon drawing of the first intact antibody molecule ever crystallized (IgG2a). The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody.

GIF animation of a rotating antibody (IgG).

The various genes the cell splices together determine the order of amino acids of the Fab portion of both the light and heavy chain; the amino acid sequence determines the final 3-dimensional shape **(see Fig. 4)**. Therefore, different antibody molecules produced by different B-lymphocytes will have different orders of amino acids at the tips of the Fab to give them unique shapes for binding epitope. The antigen-binding site is large enough to hold an epitope of about 5-7 amino acids or 3-4 sugar residues. Epitopes bind to the Fab portion of the antibody by reversible, non-covalent bonds.

Fig. 4: Epitope of an Antigen Binding to Fab of an Antibody

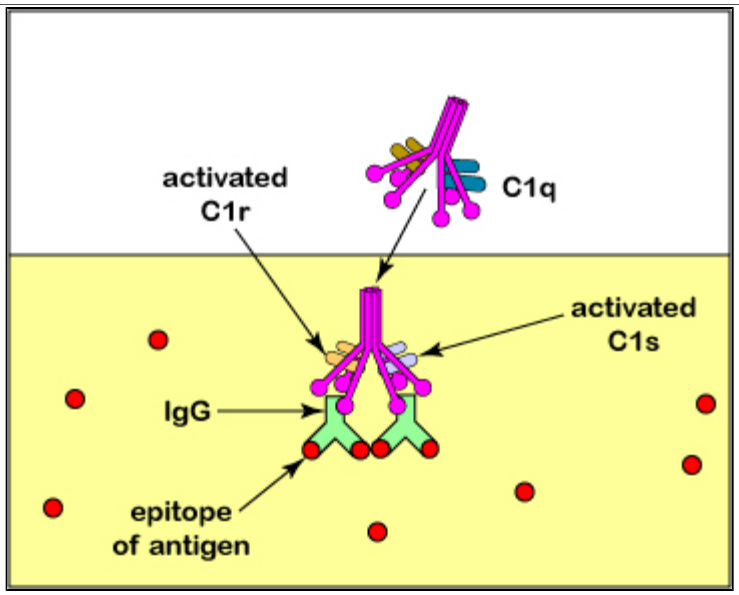


An epitope of an antigen fits into a 3-dimensional groove formed by the Fab portion light and heavy chains of the antibody.

The bottom part of the "Y", the C terminal region of each glycoprotein chain, is called the **Fc portion**. The Fc portion, as well as one domain of both the heavy and light chain of the Fab region has a **constant amino acid sequence** and is referred to as the constant region (C region) of the antibody and defines the class and subclass of each antibody. The Fc portion is responsible for the **biological activity** of the antibody (see **Fig. 1**, **Fig. 2**, and **Fig. 3**),however, the Fc portion only becomes biologically active after the Fab component has bound to its corresponding antigen. Depending on the class and subclass of antibody, biological activities of the Fc portion of antibodies include the ability to:

- **Activate the classical complement pathway** (IgG & IgM); s**ee Fig. 5**.
- Activate the lectin complement pathway and the alternative complement pathway (IgA)
- **Bind to receptors on phagocytes** (IgG); **see Fig. 6**.
- **Bind to receptors on mast cells, basophils, and eosinophils** (IgE); **see Fig 7** and **Fig. 8**.
- **Bind to receptors on NK cells** (IgG); **see Fig. 9**.
- Determine the tissue distribution of the antibodies, that is, to what tissues types the antibody molecules are able to go.

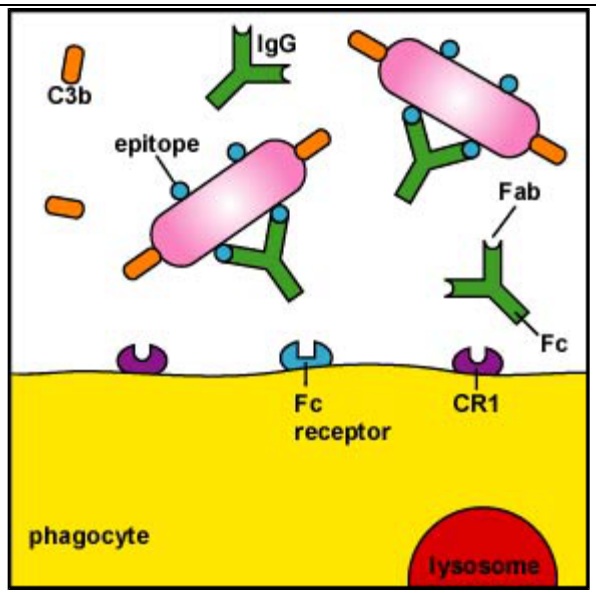
Fig. 5: Activation of C1 during the Classical Complement Pathway



The Fab of 2 molecules of IgG or 1 molecule of IgM bind to epitopes on an antigen. C1, consisting of C1q, C1r, and C1s then binds to the Fc portion of the antibodies. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b .

C1 is also able to directly bind to the surfaces of some pathogens as well as with the C-reactive protein (CRP) that is produced during the acute phase response of innate immunity.

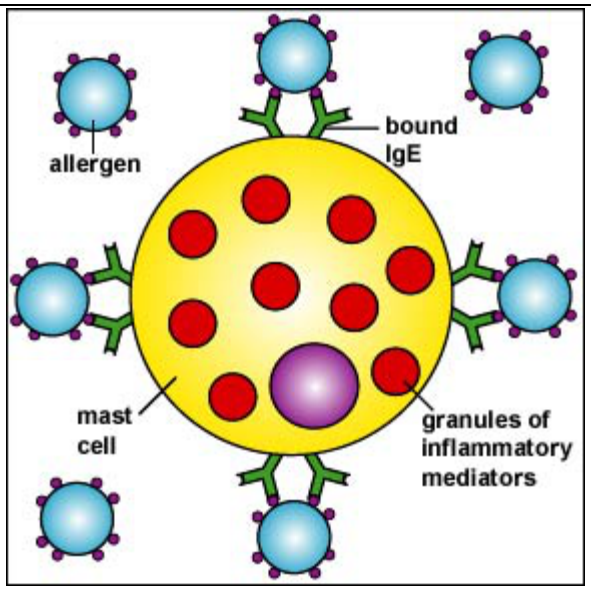
Fig. 6: Opsonization (Enhanced Attachment)



The Fab portion of IgG binds to epitopes of an antigen. The Fc portion can now attach the antigen to Fc receptors on phagocytes for enhanced attachment. This is especially important against

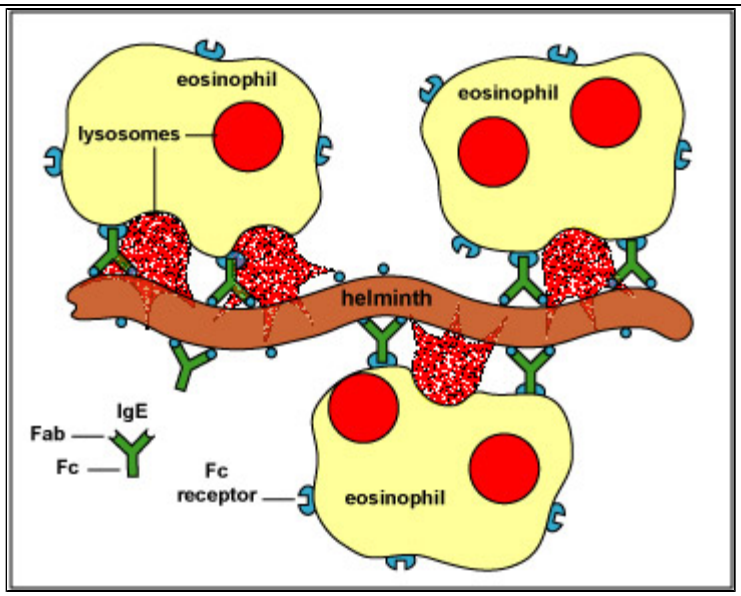
encapsulated microbes. C3b and C4b from the complement pathways can also attach antigens to phagocytes.

Fig. 7: Type-I Hypersensitivity



Allergen cross reacting with IgE on mast cell.

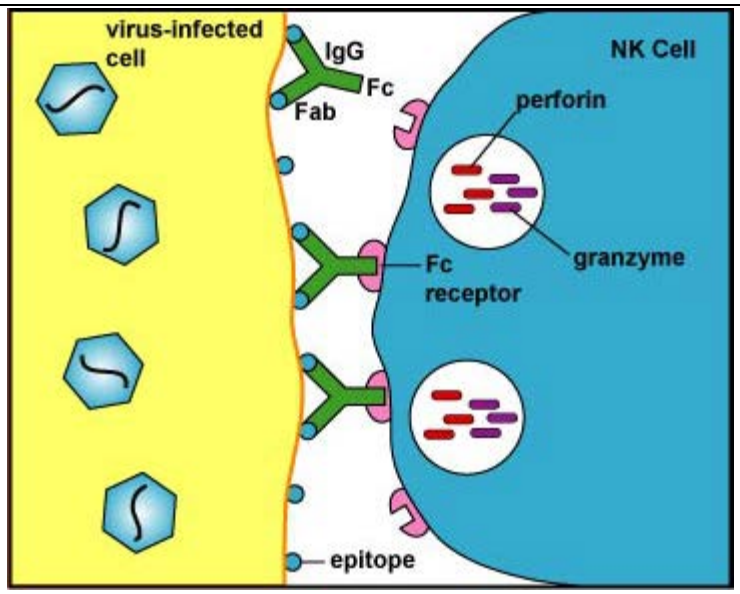
Fig. 8: Opsonization of a Helminth by IgE and Eosinophils



A major function of the cytokines produced by T_H2 cells is to enable B-lymphocytes to activate eosinophils and produce increased amounts of a class of antibodies called IgE against helminths (parasitic worms) and arthropods. IgE act as an opsonizing antibody to stick phagocytic eosinophils to helminths for extracellular killing of the helminths. The Fab portion of IgE reacts

with epitopes on the helminth while the Fc portion binds to Fc receptors of activated eosinophils. The lysosomal proteases of eosinophils are able to destroy the tough integument of helminths. IgE also promotes inflammation to recruit phagocytes.

Fig. 9: Destruction of Virus-Infected Cells by NK Cells through Antibody-Dependent Cellular Cytotoxicity (ADCC)



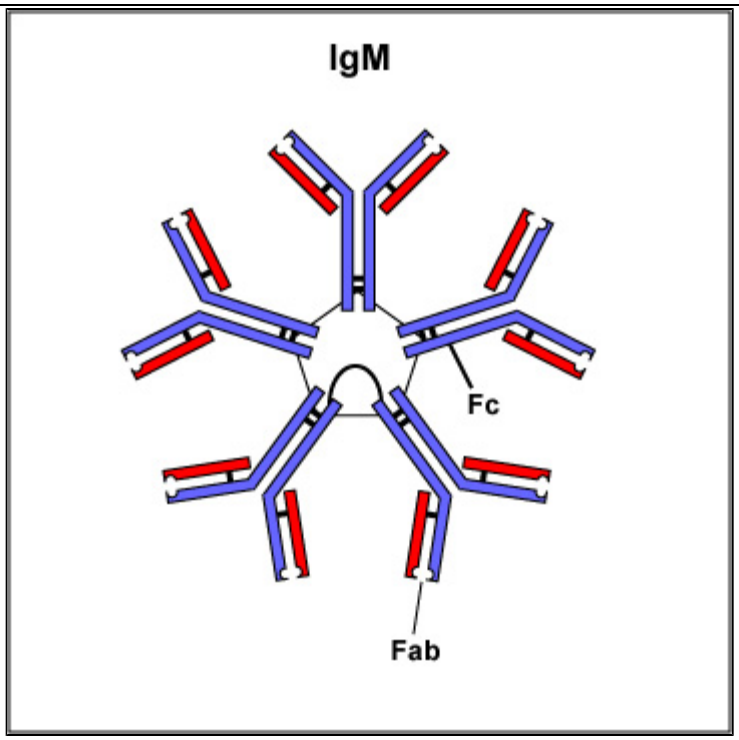
The Fab portion of the antibody binds to epitopes on the foreign cell. The NK cell then binds to the Fc portion of the antibody. The NK cell is then able to contact the cell and release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

For more information: Review of the complement pathways
For more information: Preview of opsonization
For more information: Preview of antibody-dependent cellular cytotoxity (ADCC)

Individual "Y"-shaped antibody molecules are called monomers and can bind to two identical epitopes. Antibodies of the classes **IgG, IgD, and IgE are monomers**.

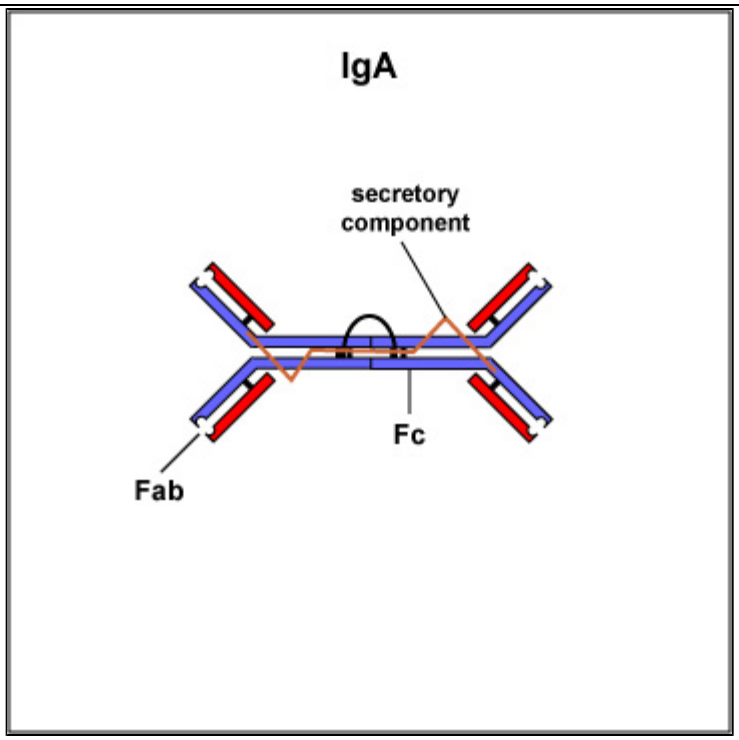
Two classes of antibodies are more complex. **IgM (see Fig. 10)** is a **pentamer**, consisting of 5 "Y"-like molecules connected at their Fc portions by a "J" or joining chain. **Secretory IgA (see Fig. 11)** is a **dimer** consisting of 2 "Y"-like molecules connected at their Fc portions by a "J" chain and stabilized to resist enzymatic digestion in body secretions by means of a secretory component.

Fig. 10: Structure of IgM



IgM is a pentamer and, therefore, has 10 Fab sites.

Fig. 11: Structure of Secretory IgA



Secretory IgA is a dimer and has 4 Fab sites. A secretory component helps protect it from digestion in body secretions.

Self Quiz for Antibody Structure

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ADAPTIVE IMMUNITY
HUMORAL IMMUNITY: THE 5 CLASSES (ISOTYPES) OF HUMAN ANTIBODIES

Adaptive Immunity

Humoral Immunity: The 5 Classes (Isotypes) of Human Antibodies

Fundamental Statements for this Softchalk Lesson:

1. IgG makes up approximately 80% of the serum antibodies, is a monomer with 2 Fab sites. The Fc portion can activate the classical complement pathway, bind to macrophages and neutrophils to enable opsonization, bind to NK cells to promote ADCC, and can cross the placenta.
2. IgM makes up approximately 13% of the serum antibodies, is the first antibody produced during an immune response, is found mainly in the blood, and is a pentamer with 10 Fab sites. The Fc portion can activate the classical complement pathway. Monomeric forms of IgM are found on the surface of B-lymphocytes as B-cell receptors.
3. IgA makes up approximately 6% of the serum antibodies, is a dimer with 4 epitope-binding sites and is found mainly in body secretions as secretory IgA (sIgA) where it protects internal body surfaces exposed to the environment by blocking the attachment of bacteria and viruses to mucous membranes.
4. The Fc portion of secretory IgA binds to components of mucous and contributes to the ability of mucous to trap microbes, and can bind to macrophages and neutrophils to enable opsonization, and can activate the lectin complement pathway and the alternative complement pathway.
5. IgD makes up approximately 0.2% of the serum antibodies, is a monomer with 2 Fab sites, is found on the surface of B-lymphocytes as a B-cell receptor, and may play a role in eliminating B-lymphocytes generating self-reactive autoantibodies.
6. IgE makes up about 0.002% of the serum antibodies, is a monomer with 2 Fab sites, and is made in response to parasitic worms (helminthes) and arthropods. It is also often made in response to allergens. The Fc portion of IgE can bind to mast cells and basophils (see Fig. 8) where it mediates many allergic reactions, and the Fc portion of IgE made against parasitic worms can bind to eosinophils enabling opsonization. IgE may also protect external mucosal surfaces by promoting inflammation.

Common Course Objectives

1. Define antibody and state the functions of the Fab and the Fc portions of an antibody.
2. Know the functions and different classes of antibodies.

Detailed Learning Objectives

1*. State which classes (isotypes) of human antibodies possess the following characteristics:

a. are monomers
b. is a pentamer
c. is a dimer
d. activates the classical complement pathway by its Fc portion
e. binds to macrophages and neutrophils by its Fc portion
f. binds to NK cells by its Fc portion
g. crosses the placenta
h. functions as a B-cell receptor
i. the first antibody produced during an adaptive immune response
j. binds to components of mucous by its Fc portion

k. found mainly in body secretions
l. binds to mast cells and basophils by its Fc portion and promotes inflammation, coughing, sneezing, vomiting, and allergic reactions
m. binds to eosinophils by its Fc portion and promotes the removal of parasitic worms and arthropods

2. Match the antibody isotype with its description.

(*) = Common theme throughout the course

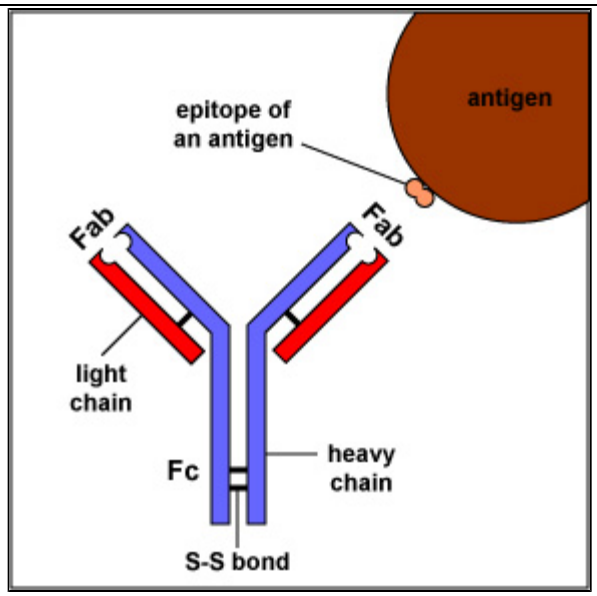
Humoral Immunity: The 5 Classes (Isotypes) of Human Antibodies

There are 5 classes or isotypes of human antibodies:

a. IgG (Immunoglobulin G; 4 subclasses, IgG1-4)

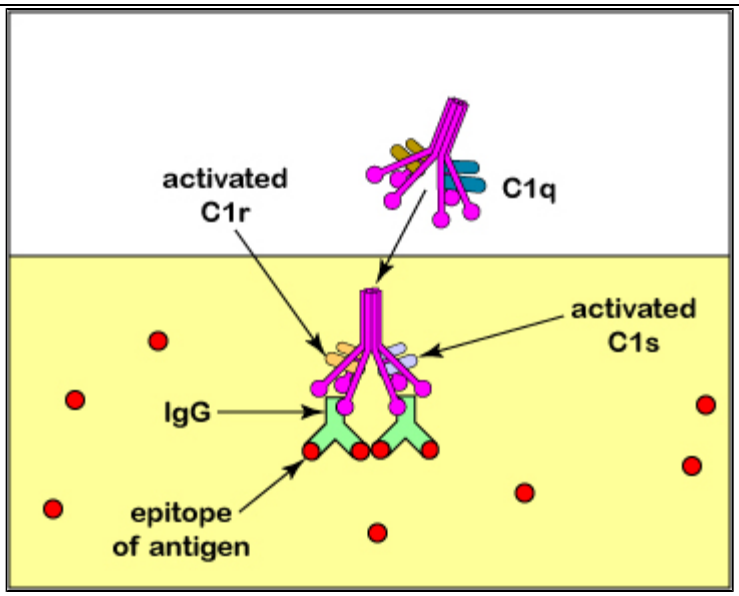
- IgG makes up approximately 80% of the serum antibodies.
- IgG has a half-life of 7-23 days depending on the subclass.
- IgG is a monomer and has **2 epitope-binding sites (see Fig. 1)**.
- The Fc portion of IgG can **activate the classical complement pathway (see Fig. 2)**.
- The Fc portion of IgG can **bind to macrophage and neutrophils** for opsonization (enhanced attachment) **(see Fig. 3)**.
- The Fc portion of IgG can **bind to NK cells** for antibody-dependent cytotoxicity or ADCC **(see Fig. 4)**.
- The Fc portion of IgG enables it to **cross the placenta**. (IgG is the only class of antibody that can cross the placenta and enter the fetal circulation.)
- Feedback inhibition of B-lymphocyte activation. High levels of IgG feedback to B-lymphocytes to prevent their activation in order to turn off antibody production.

Fig. 1: Structure of IgG



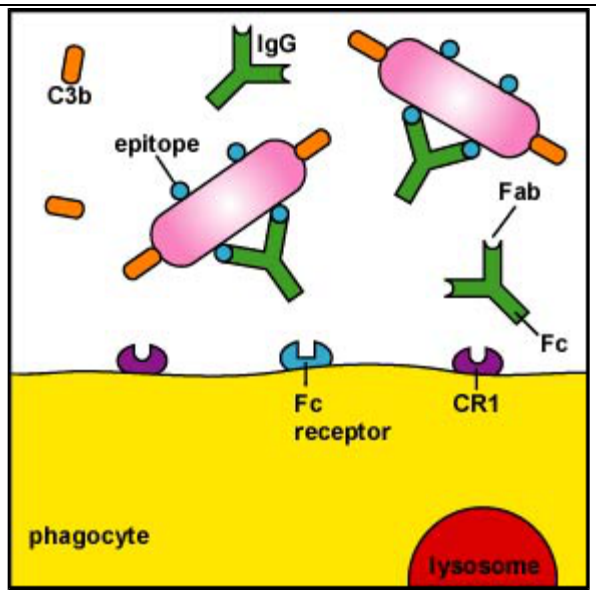
The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody.

Fig. 2: Activation of C1 during the Classical Complement Pathway



The Fab of 2 molecules of IgG or 1 molecule of IgM bind to epitopes on an antigen. C1, consisting of C1q, C1r, and C1s then binds to the Fc portion of the antibodies. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b.

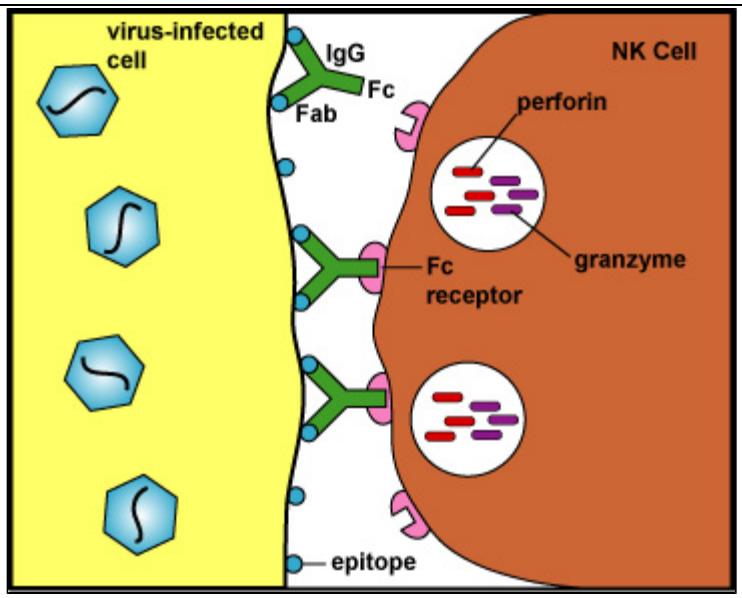
Fig. 3: Opsonization (Enhanced Attachment)



The Fab portion of IgG binds to epitopes of an antigen. The Fc portion can now attach the antigen to Fc receptors on phagocytes for enhanced attachment. This is especially important against

encapsulated microbes. C3b and C4b from the complement pathways can also attach antigens to phagocytes.

Fig. 4: Destruction of Virus-Infected Cells by NK Cells through Antibody-Dependent Cellular Cytotoxicity (ADCC)



The Fab portion of the antibody binds to epitopes on the foreign cell. The NK cell then binds to the Fc portion of the antibody. The NK cell is then able to contact the cell and release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

For more information: Review of the complement pathways

For more information: Preview of opsonization

For more information: Preview of antibody-dependent cellular cytotoxity (ADCC)

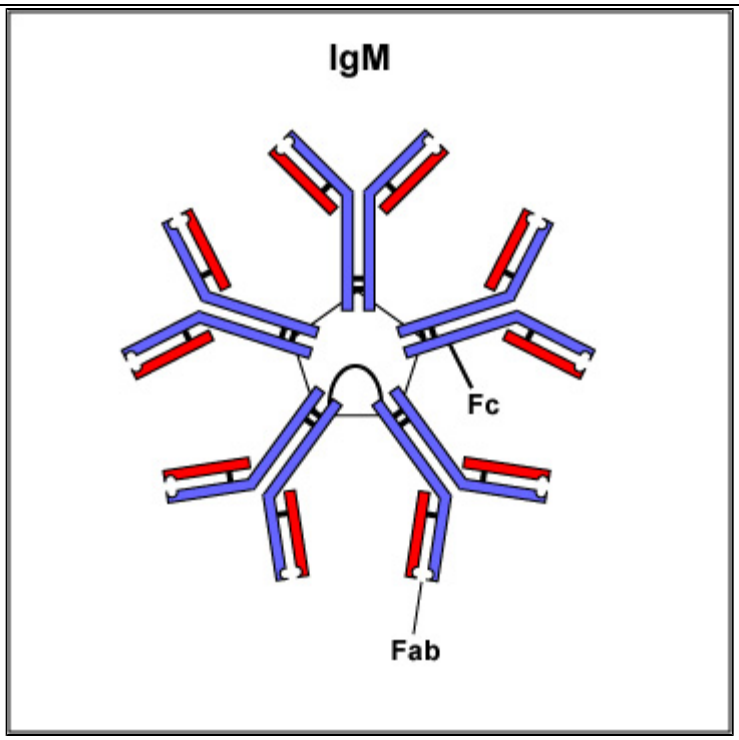
b. IgM (Immunoglobulin M)

- IgM makes up approximately 13% of the serum antibodies and is **the first antibody produced during an immune response**.
- IgM is found mainly in the bloodstream rather than in the intracellular spaces of tissues where it can control infections in the blood.

IgM has a half-life of about 5 days.

- IgM is a pentamer and has **10 epitope-binding sites (see Fig. 5)**.
- The Fc portions of IgM are able to **activate the classical complement pathway**. IgM is the most efficient class of antibody for activating the classical complement pathway.
- Monomeric forms of IgM are found on the surface of B-lymphocytes as **B-cell receptors**.

Fig. 5: Structure of IgM

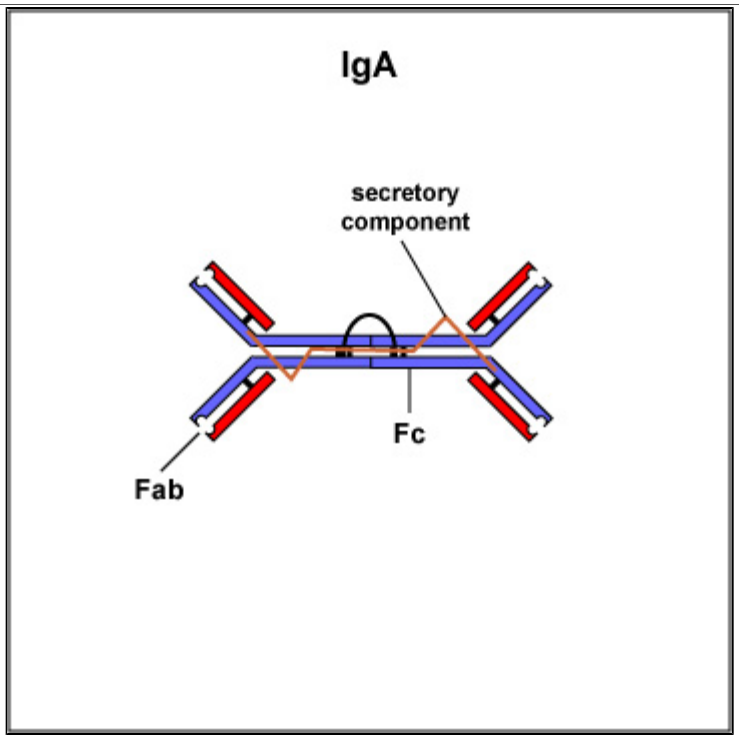


IgM is a pentamer and, therefore, has 10 Fab sites.

c. IgA (Immunoglobulin A; 2 subclasses, IgA1-2)

- IgA makes up approximately 6% of the serum antibodies where it has a half-life of approximately 6 days.
- IgA is found **mainly in body secretions** (saliva, mucous, tears, colostrum and milk) as **secretory IgA (sIgA)** where it **protects internal body surfaces exposed to the environment by blocking the attachment of bacteria and viruses to mucous membranes**. While only 6% of the antibodies in the serum are IgA, secretory IgA is the most immunoglobulin produced.
- IgA is made primarily in the mucosal-associated lymphoid tissues (MALT).
- IgA appears as a dimer of 2 "Y"-shaped molecules and has **4 epitope-binding sites** and a secretory component to protect it from digestive enzymes in the secretions **(see Fig. 6)**.
- The **Fc portion of secretory IgA binds to components of mucous** and contributes to the ability of mucous to trap microbes.
- The **Fc portion of secretory IgA can bind to macrophages and neutrophils** for opsonization (enhanced attachment).
- IgA can activate the lectin complement pathway and the alternative complement pathway.

Fig. 6: Structure of Secretory IgA



Secretory IgA is a dimer and has 4 Fab sites. A secretory component helps protect it from digestion in body secretions.

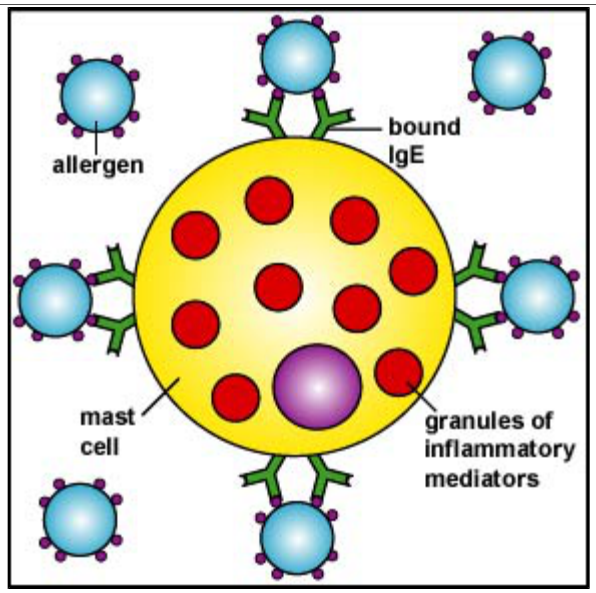
d. IgD: (Immunoglobulin D)

- IgD makes up approximately 0.2% of the serum antibodies.
- IgD is a monomer and has **2 epitope-binding sites**.
- IgD is found on the surface of B-lymphocytes (along with monomeric IgM) as a **B-cell receptor** where it may control of B-lymphocyte activation and suppression.
- IgD may **play a role in eliminating B-lymphocytes generating self-reactive autoantibodies**.

e. IgE (Immunoglobulin E)

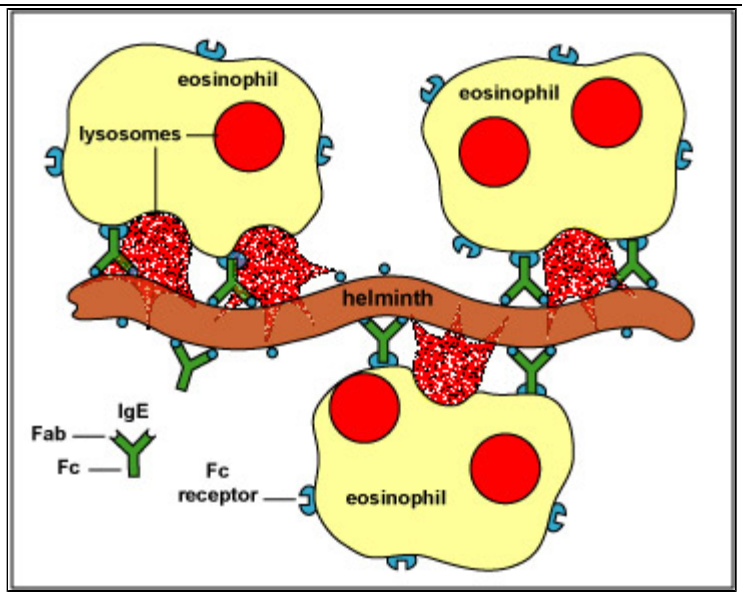
- IgE makes up about 0.002% of the serum antibodies with a half-life of 2 days.
- Most IgE is tightly bound to basophils and mast cells via its Fc region.
- IgE is a monomer and has **2 epitope-binding sites**.
- IgE is made in response to parasitic worms or (helminths) and arthropods. It is also often made in response to allergens. (Allergens are antigens causing allergic reactions.)
- IgE may protect external mucosal surfaces by **promoting inflammation**, enabling IgG, complement proteins, and leucocytes to enter the tissues, as well as by **triggering coughing, sneezing, and vomiting** for mechanical removal of microbes and toxins. .
- The Fc portion of IgE can **bind to mast cells and basophils** where it mediates many **allergic reactions**. Cross linking of cell-bound IgE by antigen triggers the release of vasodilators for an inflammatory response **(see Fig 7)**.
- The Fc portion of IgE **made against parasitic worms and arthropods can bind to eosinophils enabling opsonization (Fig. 8)**. This is a major defense against parasitic worms and arthropods.

Fig. 7: Type-I Hypersensitivity



Allergen cross reacting with IgE on mast cell.

Fig. 8: Opsonization of a Helminth by IgE and Eosinophils



A major function of the cytokines produced by T_H2 cells is to enable B-lymphocytes to activate eosinophils and produce increased amounts of a class of antibodies called IgE against helminths (parasitic worms) and arthropods. IgE act as an opsonizing antibody to stick phagocytic eosinophils to helminths for extracellular killing of the helminths. The Fab portion of IgE reacts with epitopes on the helminth while the Fc portion binds to Fc receptors of activated eosinophils. The lysosomal proteases of eosinophils are able to destroy the tough integument of helminths. IgE also promotes inflammation to recruit phagocytes.

For more information: Preview of IgE-mediated hypersensitivity (Type-I)

Each day an average adult produces approximately three grams of antibodies, about two-thirds of this IgA.

Self Quiz for the 5 Classes (Isotypes) of Human Antibodies

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ADAPTIVE IMMUNITY
HUMORAL IMMUNITY: GENERATION OF ANTIBODY DIVERSITY

Adaptive Immunity

Humoral Immunity: Generation of Antibody Diversity

Fundamental Statements for this Softchalk Lesson:

1. The adaptive immune responses have evolved a system that possesses the capability of responding to any conceivable antigen the body might eventually encounter through a process called gene translocation.
2. Gene translocation is a type of gene-shuffling process where various different genes along a chromosome are cut out of one location and joined with other genes along the chromosome to create a maximum number of different B-cell and T-cell receptors.
3. Each B-lymphocyte becomes genetically programmed to produce an antibody functioning as a B-cell receptor (BCR) having a unique shaped Fab.
4. The variable portion of both the heavy and light chain of the antibody is coded for by multiple genes and there are multiple forms of each one of these variable genes.
5. Through random gene translocations, any combination of the multiple forms of each gene can join together resulting in thousands of possible gene combinations. This is known as combinatorial diversity.
6. During gene translocation, specialized enzymes in the B-lymphocyte cause splicing inaccuracies wherein additional nucleotides are added or deleted at the various gene junctions and this change in the nucleotide base sequence generates even greater diversity in Fab shape. This is called junctional diversity .
7. As B-lymphocytes proliferate, they undergo affinity maturation, a process that "fine tunes" the shape of the Fab epitope binding site through a high rate of somatic hypermutation. This creates a great opportunity for selection of variant B-lymphocytes with even better fitting antigen-binding sites that fit the epitope more precisely.
8. Immature B-lymphocytes with self-reactive B-cell receptors may be stimulated to undergo a new gene rearrangement to make a new receptor that is no longer self-reactive through a process called receptor editing. Alternately, self-reactive B-lymphocytes can also undergo negative selection whereby any B-lymphocytes that bind substances recognized as "self" and are eliminated by apoptosis.

Common Course Objectives

1. Define antibody and state the functions of the Fab and the Fc portions of an antibody.
2. Explain how TCR and BCR diversity is achieved.

Detailed Learning Objectives

1*. Define antibody.

2*. Define gene translocation and relate it to each B-lymphocyte being able to produce an antibody with a unique shaped Fab.

3. Define the following:

a. combinatorial diversity

b. junctional diversity

c. affinity maturation

(*) = Common theme throughout the course

Humoral Immunity: Generation of Antibody Diversity

Humoral Immunity refers to the production of **antibody molecules in response to an antigen**. These antibody molecules circulate in the plasma of the blood and enter tissue and organs via the inflammatory response. Humoral immunity is **most effective microbes or their toxins located in the extracellular spaces of the body**.

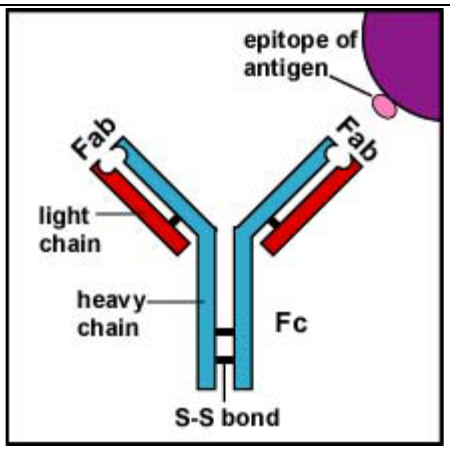
Antibodies or immunoglobulins are specific glycoprotein configurations produced by B-lymphocytes and plasma cells **in response to a specific antigen** and **capable of reacting with that antigen**.

In this section we will look at the generation of antibody diversity by B-lymphocytes.

As mentioned earlier, the immune system of the body has no idea as to what antigens it may eventually encounter. Therefore, it has evolved a system that possesses the capability of responding to any conceivable antigen. **The immune system can do this because both B-lymphocytes and T-lymphocytes have evolved a unique system of gene-splicing called gene translocation**, a type of gene-shuffling process where **various different genes along a chromosome are cut out of one location and joined with other genes along the chromosom**e.

To demonstrate this gene translocation process, we will look at how each B-lymphocyte becomes **genetically programmed to produce an antibody functioning as a B-cell receptor (BCR) having a unique shaped Fab**. As mentioned above, the Fab portion of an antibody is composed of 2 protein chains: a heavy and a light **(see Fig. 1)**.

Fig. 1: Structure of the Antibody IgG



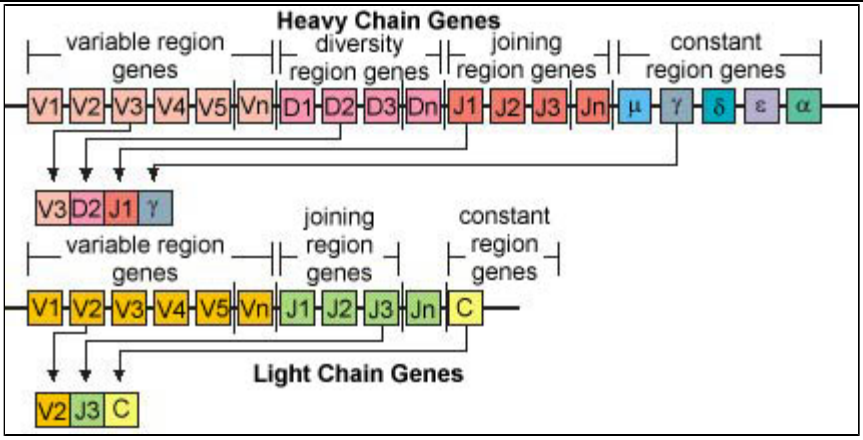
The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody.

The **variable heavy chain portion of the Fab** is coded for by a combination of **3 genes**, called **VH** (variable heavy), **DH** (diversity heavy), and **JH** (joining heavy). The **variable light chain portion of the Fab** consists of **either a kappa chain or a lambda chain** coded for by a combination of **2 genes**, **VL** (variable light) and **JL** (joining light). In the DNA of each B-lymphocyte there are **multiple forms of each one of these variable determinant genes**. Although the exact number of each gene isn't known and varies from person, there are approximately 38-46 VH genes; 23 DH genes; 6 JH genes; 34-38 kappa VL genes; 5 kappa JL genes; 29-33 lambda VL genes; and 4-5 lambda JL genes.

While a person inherits alleles for the various V(D)J genes from each parent, an individual B-lymphocyte will only express an inherited allele set from one parent. This increases a greater diversity of antibodies in that individual.

Through random gene translocation, any combination of the multiple forms of each gene can join together (see Fig. 2) resulting in thousands of possible gene combinations. This is known as **combinatorial diversity**.

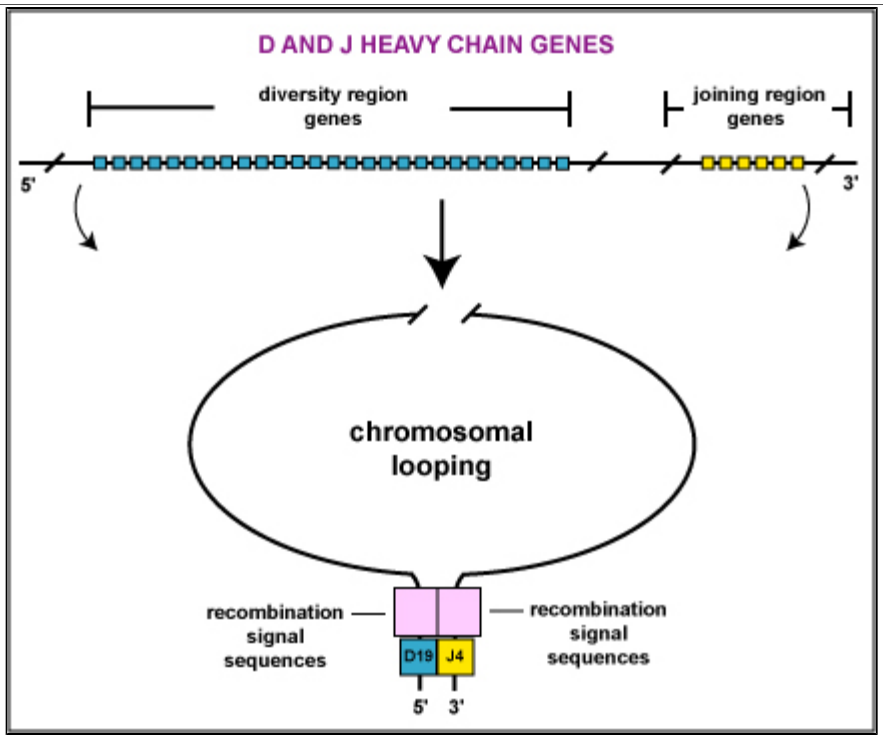
Fig. 2: Gene Translocation



Gene translocation determines the amino acid sequence of the variable portion of the heavy and light chains that form the Fab of an antibody molecule. The variable portion of the heavy chain is determined after one of the multiple genes of the VH region join with one of the genes of the DH region and one of the genes of the JH region. Likewise, the variable portion of the light chain is determined after one of the multiple genes of the VL region joins with one of the genes of the JL region. Although the exact number of each gene is not known and varies from person, there are approximately 38-46 VH genes; 23 DH genes; 6 JH genes; 34-38 kappa VL genes; 5 kappa JL genes; 29-33 lambda VL genes; and 4-5 lambda JL genes.

Gene translocation of the V(D)J genes is initiated when an enzyme called V(D)J recombinase recognizes recombination signal sequences located at the 3' end of V genes, the 5' end of J genes, and both ends of D genes. As a result, the **chromosome forms a loop allowing different genes from different regions along the chromosome to align (see Fig. 3)**. In the heavy chain any J-heavy gene and any D-heavy gene align and bind together as the **genes are cut from one location and pasted into another**. Subsequently, any one of the V-heavy genes is attached to this DJ segment. In the light chain, chromosomal looping enables any V-light gene to attach to any J-light gene.

Fig. 3: Chromosomal Looping of V(D)J Genes



Gene translocation of the V(D)J genes is initiated when an enzyme called V(D)J recombinase recognizes recombination signal sequences located at the 3-prime end of V genes, the 5-prime end of J genes, and both ends of D genes. As a result, the chromosome forms a loop allowing different genes from different regions along the chromosome to align.In the heavy chain any J-heavy gene and any D-heavy gene align and bind together as the genes are cut from one location and pasted into another. Subsequently, any one of the V-heavy genes is attached to this DJ segment. In the light chain, chromosomal looping enables any V-light gene to attach to any J-light gene.

Flash animation showing gene translocation and combinatorial diversity.

html5 version of animation for iPad showing gene translocation and combinatorial diversity.

The variable heavy chain portion of the Fab is coded for by a combination of 3 genes, called VH, DH, and JH. The variable light chain portion of the Fab consists of either a kappa chain or a lambda chain coded for by a combination of 2 genes, VL and JL. In the DNA of each B-lymphocyte there are multiple forms of each one of these variable determinant genes. Although the exact number of each gene isn't known and varies from person, there are approximately 38-46 VH genes; 23 DH genes; 6 JH genes; 34-38 kappa VL genes; 5 kappa JL genes; 29-33 lambda VL genes; and 4-5 lambda JL genes.

Through random gene-splicing, any combination of the multiple forms of each gene can join together resulting in thousands of possible gene combinations. This is known as combinatorial diversity.

Additionally, specialized enzymes in the B-lymphocyte cause splicing inaccuracies where additional nucleotides are added or deleted at the various gene junctions to

generate a great deal of further diversity. This is called junctional diversity but is not shown in this animation.

During gene translocation, specialized enzymes in the B-lymphocyte cause **splicing inaccuracies wherein additional nucleotides are added or deleted at the various gene junctions**. This change in the nucleotide base sequence generates even greater diversity in Fab shape. This is called **junctional diversity**.

Furthermore, as B-lymphocytes proliferate, they undergo **affinity maturation, a process that "fine tunes" the shape of the Fab epitope binding site**. This is because the immunoglobulin V genes of B-lymphocytes have a mutation rate between 1000 to 10,000 times greater than other human genes in the body. This **somatic hypermutation creates a great opportunity for selection of variant B-lymphocytes with even better fitting antigen-binding sites that fit the epitope more precisely**. The longer and more tightly the antigen binds to the B-cell receptor, the greater the chance that B-lymphocyte has of surviving and replicating. In other words, **the "fit" of the antibody can be improved over time**. Affinity maturation occurs in the germinal centers of the lymph nodes.

Most likely humans produce a**t least 10^{11} different shaped BCRs**. Keep in mind that the 3-dimensional shape of a protein is ultimately determined by the sequence of its amino acids and the sequence of amino acids is determined by the order of nitrogenous bases in the genes coding for that protein. Between combinatorial diversity, junctional diversity, and affinity maturation, there are probably billions of possible gene combinations and rearrangements that can code for the Fab portions of an antibody. Chances are, then, **each B-lymphocyte will carry out a unique series of gene translocations and be able to produce an antibody with a unique shaped epitope-binding site**.

Because gene translocation is a random process, some immature B-lymphocytes do wind up making B-cell receptors that fit the body's own antigens. Immature B-lymphocytes with self-reactive B-cell receptors may be stimulated to **undergo a new gene rearrangement to make a new receptor that is no longer self-reactive**. Recognition of self antigen can reactivate genes that allow the B-lymphocyte to carry out new light chain V-J recombinations and enabling that cell to express a new B-cell receptor. This process is called **receptor editing**.

Alternately, **self-reactive B-lymphocytes can also undergo negative selection**. Since the bone marrow, where the B-lymphocytes are produced and mature, is normally free of foreign substances, any B-lymphocytes that bind substances there must be recognizing "self" and are eliminated by **apoptosis**, a programmed cell suicide. Apoptosis results in the activation of proteases within the target cell which then degrade the cell's structural proteins and DNA.

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ADAPTIVE IMMUNITY
HUMORAL IMMUNITY: CLONAL SELECTION AND CLONAL EXPANSION

Adaptive Immunity

Humoral Immunity: Clonal Selection and Clonal Expansion

Fundamental Statements for this Softchalk Lesson:

1. Each naïve B-cell becomes genetically programmed to make an antibody with a unique antigen-binding site (Fab) through a series of gene translocations, and molecules of that antibody are put on its surface to function as the B-cell receptor.
2. When an antigen encounters the immune system, its epitopes eventually will react only with B-lymphocytes with B-cell receptors on their surface that more or less fit and this activates those B-lymphocytes. This process is known as clonal selection.
3. Cytokines produced by activated T4-helper lymphocytes enable those activated B-lymphocytes to rapidly proliferate to produce large clones of thousands of identical B-lymphocytes.
4. In this way, even though only a few B-lymphocytes in the body may have an antibody molecule able to fit a particular epitope, eventually eventually many thousands of cells are produced with the right specificity. This is referred to as clonal expansion.

Common Course Objectives

1. Briefly describe the process of clonal selection and clonal expansion.

Detailed Learning Objectives

1.* Briefly describe the process of clonal selection and clonal expansion.

(*) = Common theme throughout the course

Humoral Immunity: Clonal Selection and Clonal Expansion

Humoral Immunity refers to the production of **antibody molecules in response to an antigen**. These antibody molecules circulate in the plasma of the blood and enter tissue and organs via the inflammatory response. Humoral immunity is **most effective microbes or their toxins located in the extracellular spaces of the body**.

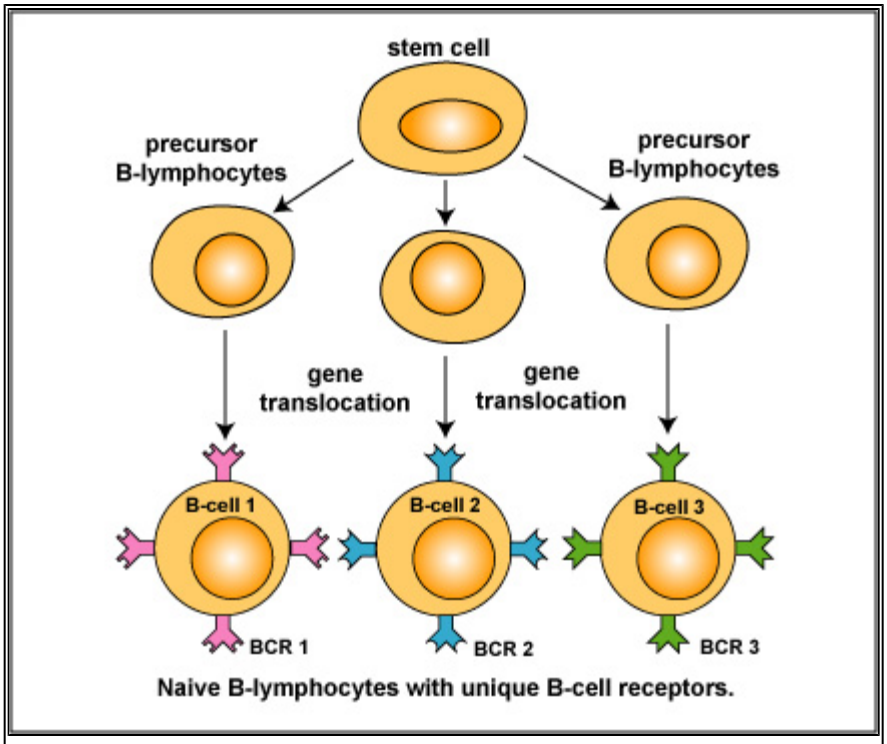
Antibodies or immunoglobulins are specific glycoprotein configurations produced by B-lymphocytes and plasma cells **in response to a specific antigen** and **capable of reacting with that antigen**.

In this section we will look at clonal selection and clonal expansion of lymphocytes.

As mentioned above, during early differentiation of naive B-lymphocytes in the bone marrow, **each B-lymphocyte becomes genetically programmed to make an antibody with a unique antigen-binding site (Fab)** through a series of gene translocations, and **molecules of that antibody are put on its surface to function as the B-cell receptor (see Fig. 1)** . When an antigen encounters the immune system, its epitopes eventually will react only with B-lymphocytes with B-cell receptors on their surface that more or less fit and this activates those B-lymphocytes. This process is known as **clonal**

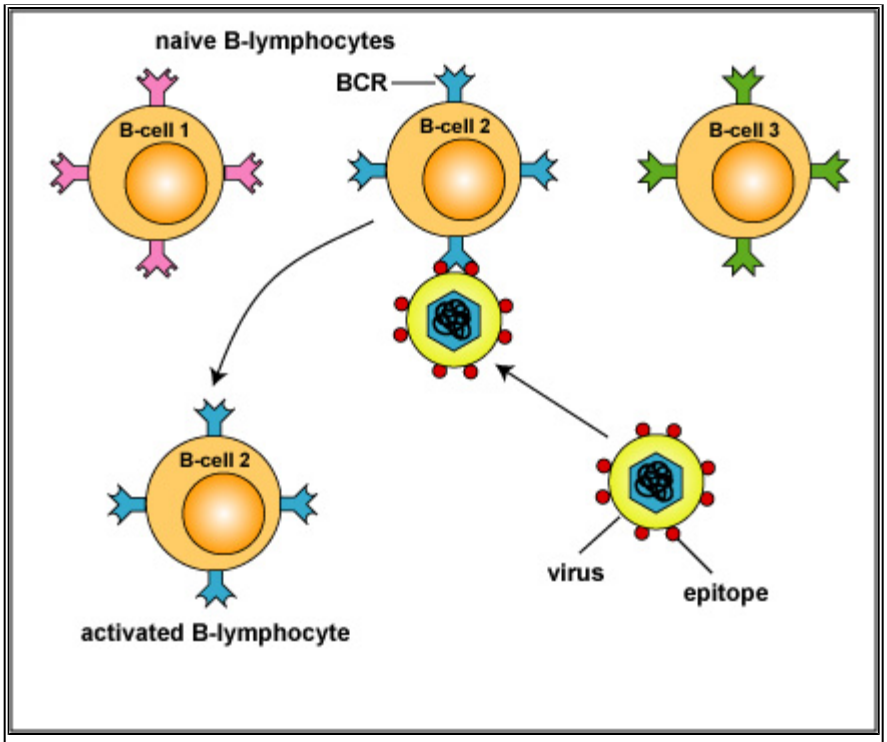
selection (see Fig. 2).

Fig. 1: Clonal Selection, Step-1



During its development, each B-lymphocyte becomes genetically programmed, through a process called gene translocation, to make a unique B-cell receptor. Molecules of that B-cell receptor are placed on its surface where it can react with epitopes of an antigen.

Fig. 2: Clonal Selection, Step-2

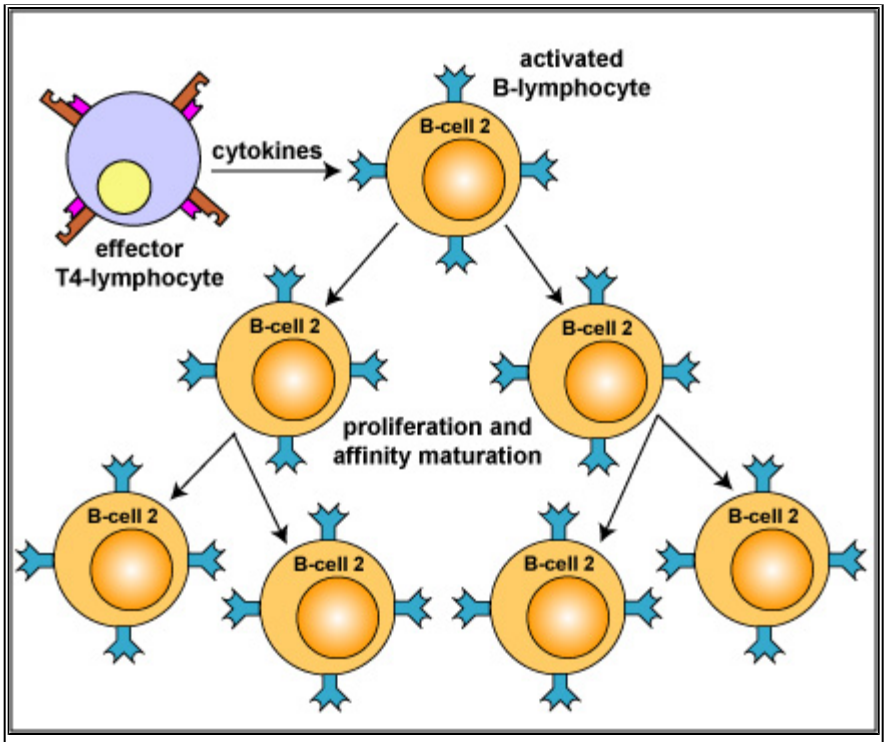


A B-lymphocyte with an appropriately fitting B-cell receptor can now react with epitopes of an antigen having a corresponding shape. This activates the B-lymphocyte.

For more information: Review of B-lymphocytes

Cytokines produced by effector T4-helper lymphocytes **enable those activated B-lymphocytes to rapidly proliferate to produce large clones of thousands of identical B-lymphocytes**. In this way, even though only a few B-lymphocytes in the body may have an antibody molecule able to fit a particular epitope, eventually many thousands of cells are produced with the right specificity. This is referred to as **clonal expansion (see Fig. 3)**.

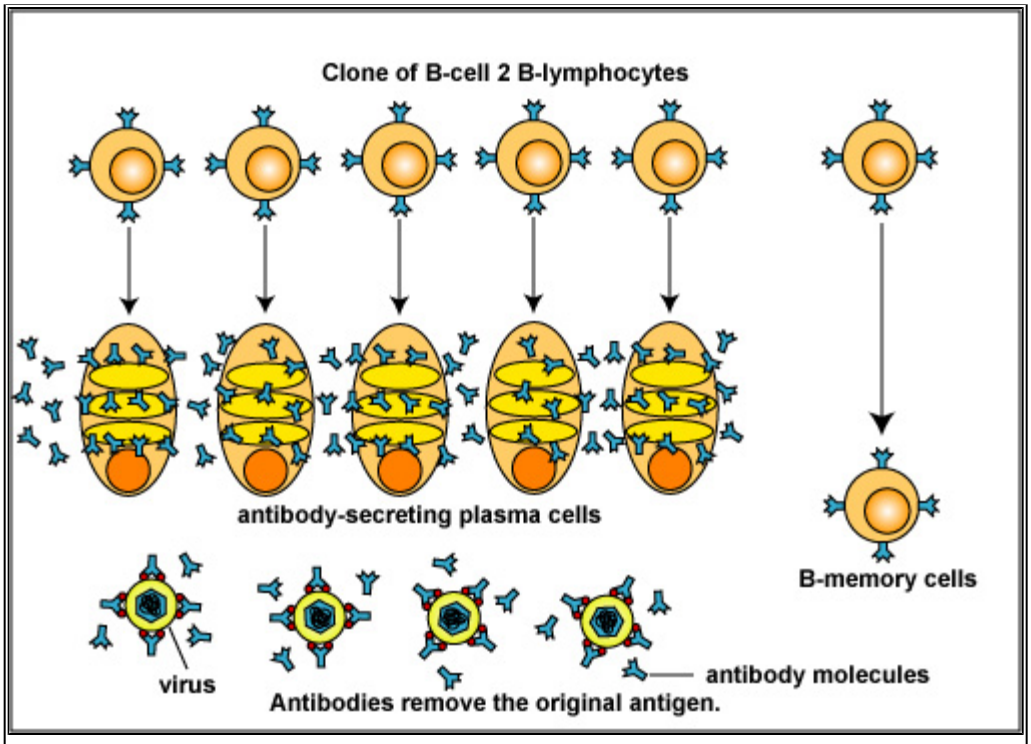
Fig. 3: Clonal Expansion



Cytokines from an effector T4-lymphocyte now enable the activated B-lymphocyte to proliferate into a large clone of identical B-lymphocytes. During this time, "fine-tuning" of the B-cell receptor occurs through affinity maturation.

Furthermore, as the B-lymphocytes proliferate, they undergo **affinity maturation** as a result of somatic hypermutations. This allows the B-lymphocytes to **"fine-tune" the shape of the antibody** for better fit with the original epitope. B-lymphocytes having better fitting B-cell receptor on their surface bind epitope longer and more tightly allowing these cells to selectively replicate. Eventually these variant B-lymphocytes differentiate into **plasma cells** *(def)* that synthesize and secrete vast quantities of antibodies that have Fab sites which fit the original epitope very precisely **(see Fig. 4)**. It generally takes 4-5 days for a naive B- lymphocyte that has been activated to complete clonal expansion and differentiate into effector B-lymphocytes.

Fig. 4: Differentiation of B-lymphocytes into Plasma Cells and B-Memory Cells



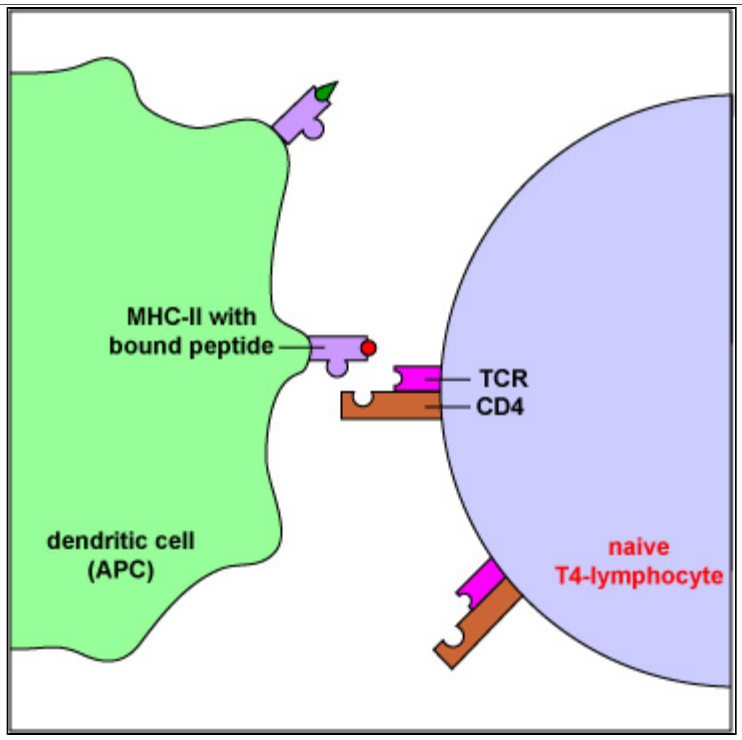
The B-lymphocytes now differentiate into antibody-secreting B-lymphocytes and plasma cells that secrete large quantities of antibodies that 'fit' the original epitope. Some B-lymphocytes differentiate into B-memory cells capable of anamnestic response.

A single activated B-lymphocyte can, within seven days, give rise to approximately 4000 antibody-secreting cells. Over 2000 antibody molecules can be produced per plasma cell per second for typically up to four to five days. The B-memory cells that eventually form also have these high affinity antibodies on their surface.

GIF animation showing clonal selection of B-lymphocytes

As with naive B-lymphocytes, during its development, **each naive T4-lymphocyte becomes genetically programmed** by gene-splicing reactions similar to those in B-lymphocytes, **to produce a TCR with a unique specificity**. Identical molecules of that TCR are placed on its surface where they are **able to bind an epitope/MHC-II complex on an antigen-presenting dendritic cell with a corresponding shape (see Fig. 5)**. This is **clonal selection** of the T4-lymphocytes that are required for the body's response to T-dependent antigens.

Fig. 5: A Naive T4-Lymphocyte Recognizing Epitope/MHC-II on an Antigen-Presenting Dendritic Cell



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting dendritic cells through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the dendritic cell. Naive T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

In response to cytokines, these activated T4-lymphocytes now rapidly proliferate and differentiate into effector T4-lymphocytes. This is **clonal expansion** of the T4-lymphocytes.

Before an antigen enters the body, the number of naive T4-lymphocytes specific for any particular antigen is between 1 in 10^5 to 10^6 lymphocytes. After antigen exposure, the number of T4-lymphocytes specific for that antigen may increase to 1 in 100 to 1000 lymphocytes.

For more information: Review of T4-lymphocytes

For more information: Review of antigen-presenting cells

For more information: Review of MHC molecules

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ADAPTIVE IMMUNITY
HUMORAL IMMUNITY: ANAMNESTIC RESPONSE (MEMORY)

Adaptive Immunity

Humoral Immunity: Anamnestic Response (Memory)

Fundamental Statements for this Softchalk Lesson:

1. As a result of B-lymphocytes recognizing T-dependent antigens (proteins) during humoral immunity, numerous circulating B-memory cells and T4-memory cells develop which possess anamnestic response or memory.
2. A subsequent exposure to that same antigen results in a more rapid production of antibodies that are produced in greater amounts for a longer period of time.
3. The primary response to a new antigen generally peaks at 5 - 10 days.
4. Because of the numerous circulating B-memory cells and T4-memory cells from the primary response, the secondary anamnestic response peaks in only 1 - 3 days.
5. In the case of systemic infections and most vaccinations, many of the plasma cells migrate to the bone marrow where they may continue to secrete antibodies for months or years after the antigen has been eliminated.
6. Plasma cells produced in the mucous membranes generally remain in the mucous membranes and secrete antibodies for only around a year. Therefore, anamnestic response is better at preventing systemic infections than preventing mucosal infections.

Common Course Objectives

1. Describe how T-lymphocytes and B-lymphocytes acquire memory.

Detailed Learning Objectives

1.* In terms of humoral immunity, state what is meant by anamnestic response and discuss its role in immune defense.

2. Briefly describe **why** there is a heightened secondary response during anamnestic response.

(*) = Common theme throughout the course

Humoral Immunity: Anamnestic Response (Memory)

Humoral Immunity refers to the production of **antibody molecules in response to an antigen**. These antibody molecules circulate in the plasma of the blood and enter tissue and organs via the inflammatory response. Humoral immunity is **most effective microbes or their toxins located in the extracellular spaces of the body**.

Antibodies or immunoglobulins are specific glycoprotein configurations produced by B-lymphocytes and plasma cells **in response to a specific antigen** and **capable of reacting with that antigen**.

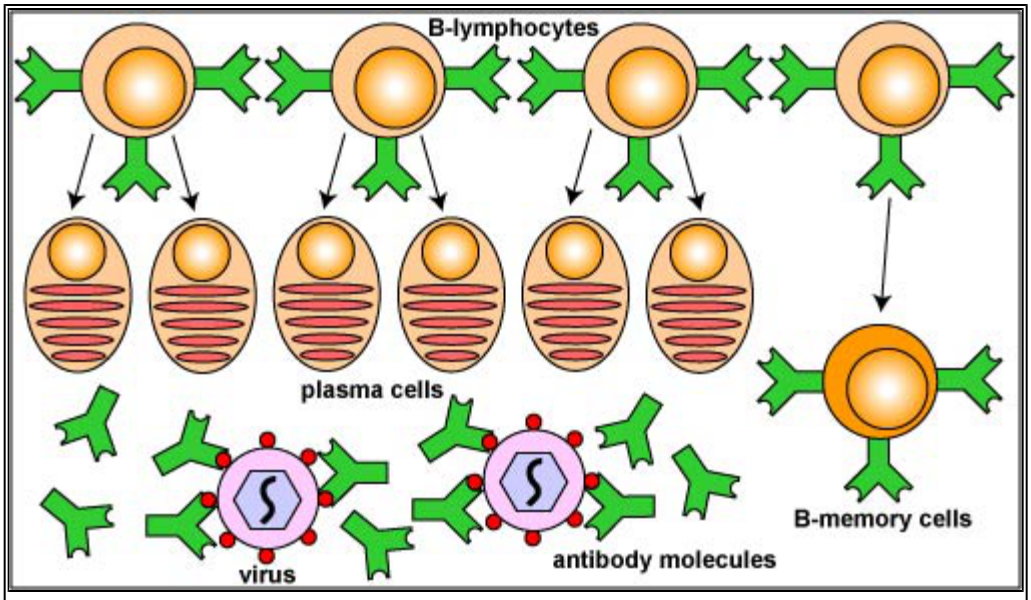
In this section we will look at clonal selection and clonal expansion of lymphocytes.

As a result of B-lymphocytes recognizing T-dependent antigens (proteins) during humoral immunity, numerous circulating **B-memory cells** and T4-memory cells develop **(see Fig. 1**) which possess **anamnestic response** or memory. (During cell-

mediated immunity, T8-memory cells also develop.) A subsequent exposure to that same antigen results in:

- **A more rapid production of antibodies;**
- **Produced in greater amounts; and**
- **Produced for a longer period of time**.

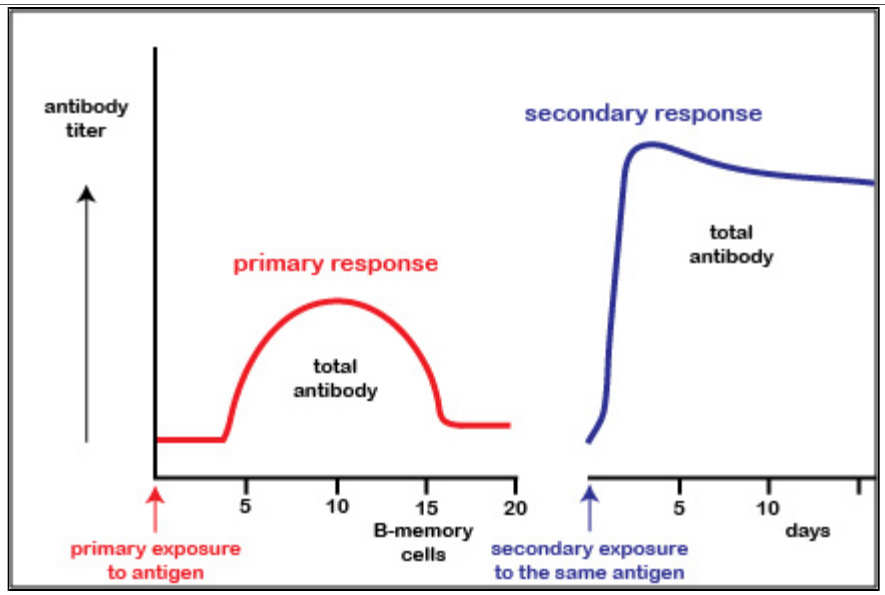
Fig. 1: Differentiation of B-lymphocytes into Plasma Cells and B-Memory Cells



The B-lymphocytes now differentiate into plasma cells that secrete large quantities of antibodies that fit the original epitope. Some B-lymphocytes differentiate into B-memory cells capable of anamnestic response.

The **primary response to a new antigen generally peaks at 5 - 10 days**. IgM is nade first later to be replaced by IgG. Because of the numerous circulating B-memory cells and T4-memory cells from the primary response, however, the **secondary anamnestic response peaks in only 1 - 3 days (see Fig. 2**). There is an increase in the amount of IgG made and under certain conditions, IgA or IgE may be made.

Fig. 2: Anamnestic Response



After the primary exposure to an antigen, there is an inductive period of generally several days to a week when no measurable antibodies are detected in the serum. This is the period when the antigen is being exposed to immunocompetent cells, being processed by APCs, clonal selection and clonal expansion are taking place, and B-lymphocytes are differentiating into plasma cells and B-memory cells. Because of the memory cells, however, a second exposure to the same antigen results in more antibodies being made faster for a longer period of time, as shown in the secondary response.

For more information: Review of the five classes of human antibodies

Because of clonal expansion and affinity maturation, there is now a **pool of B-memory cells having the "fine-tuned" B-cell receptors** on their surface. The pool of B-memory cells migrate to lymph nodes, to mucosal tissue, and circulate in the blood waiting to encounter the original antigen if it again enters the body. B-memory cells have a long life and also replicate and produce antibodies periodically when they are exposed to persisting epitope remaining on the surface of follicular dendritic cells in the lymphoid organs. In addition to the B-memory cells, a pool of **circulating T4-effector memory cells** (CD4 TEM cells), as well **T4 tissue resident memory cells** (CD4 TRM cells) located in the mucosa enable an **accelerated helper function**.

For more information: Review of affinity maturation

This memory response applies to T-dependent antigens. In the case of the T-independent antigens, there is usually no anamnestic response.

In the case of systemic infections and most vaccinations, many of the plasma cells migrate to the bone marrow where they may continue to secrete antibodies for months or years after the antigen has been eliminated. Plasma cells produced in the mucous membranes generally remain in the mucous membranes and secrete antibodies for only around a year.

Memory is better in preventing systemic infections than preventing mucosal infections because infections limited to the mucous membranes generally do not provide enough time for the development of effector cells such as plasma cells, effector T4-lymphocytes, and cytotoxic T-lymphocytes from the activated memory cells.

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: OPSONIZATION

Adaptive Immunity

Ways in which Antibodies Protect the Body: Opsonization

Fundamental Statements for this Softchalk Lesson:

1. Opsonization, or enhanced attachment, refers to the antibody molecules IgG and IgE, the complement proteins C3b and C4b, and other opsonins attaching antigens to phagocytes.
2. The Fab portions of the antibody IgG react with epitopes of the antigen. The Fc portion of IgG can then bind to neutrophils and macrophages thus sticking the antigen to the phagocyte. The Fc portion of secretory IgA can also bind to macrophages and neutrophils for opsonization.
3. IgG and IgM can activate the classical complement pathway and C3b or C4b can stick the antigen to phagocytes.
4. IgE is made against parasitic worms (helminths) and arthropods. The Fab portions of IgE bind to epitopes on the helminth or arthropod while the Fc portion binds to receptors on eosinophils enabling opsonization.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1*. **Discuss** how antibodies protect the body by way of opsonization. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

2. Briefly describe 2 different ways bacteria may resist opsonization.

(*) = common theme throughout the course

TPS Questions: Opsonization

Ways in which Antibodies Protect the Body: Opsonization

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans

9. Promoting an Inflammatory Response

In this section we will look at opsonization.

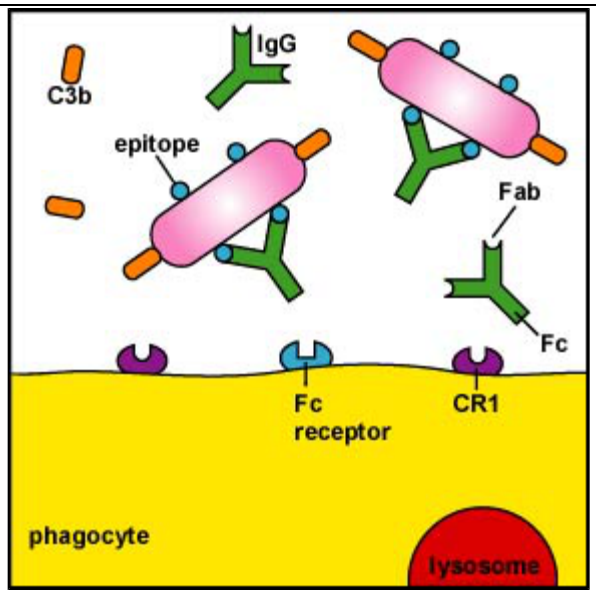
Opsonization

Opsonization , or enhanced attachment, refers to the antibody molecules IgG and IgE, the complement proteins C3b and C4b, and other opsonins *(def)* attaching antigens to phagocytes. This results in a much more efficient phagocytosis.

A. Opsonization with IgG, C3b, and C4b

1. The process starts with antibodies of the isotype **IgG, IgA, or IgM** being made against a surface antigen of the organism or cell to be phagocytosed. The **Fab portions of the antibody react with epitopes of the antigen**. The **Fc portion of IgG** (but not IgM) can then bind to receptors on neutrophils and macrophages thus sticking the antigen to the phagocyte **(see Fig. 1)**. **The Fc portion of secretory IgA** can also bind to macrophages and neutrophils for opsonization.

Fig. 1: Opsonization (Enhanced Attachment)



The Fab portion of IgG binds to epitopes of an antigen. The Fc portion can now attach the antigen to Fc receptors on phagocytes for enhanced attachment. This is especially important against encapsulated microbes. C3b and C4b from the complement pathways can also attach antigens to phagocytes

Flash animation illustrating enhanced attachment by way of IgG.

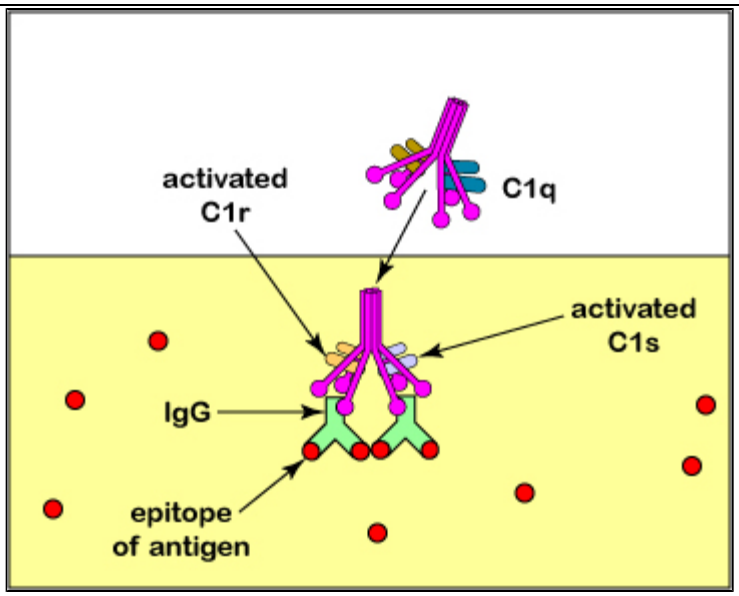
html5 version of animation for iPad showing enhanced attachment by way of IgG.

The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes.

2. Alternately, **IgG, IgA, and IgM** can activate the complement pathways **(see Fig. 2)** and **C3b or C4b** can stick the antigen to phagocytes **(see Fig. 1)**. Like IgG, **C3b,** and to a lesser extent **C4b**, **can function as opsonins**, that is, they can attach

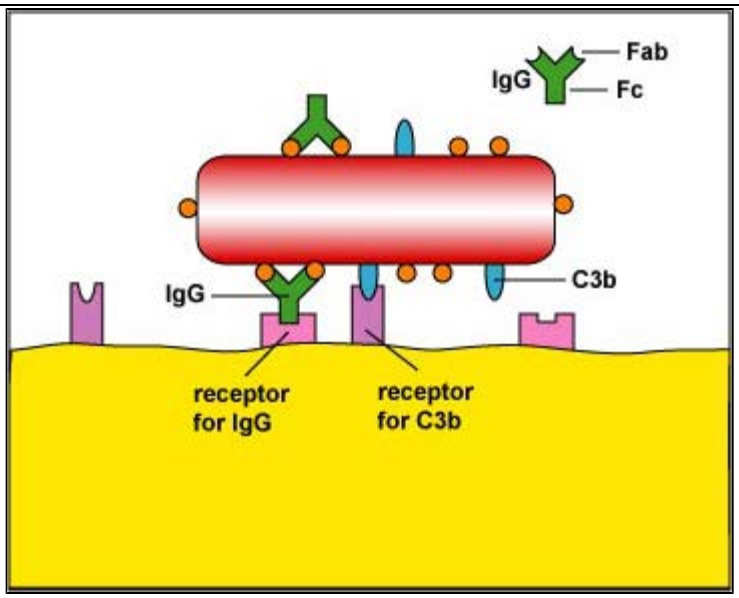
antigens to phagocytes. One portion of the C3b binds to proteins and polysaccharides on microbial surfaces; another portion **attaches to CR1 receptors on phagocytes, B-lymphocytes, and dendritic cells for enhanced phagocytosis (see Fig. 3)**. (Remember that C3b and C4b are also produced during the alternative complement pathway and the lectin pathway.) Activation of the complement pathway also promotes inflammation to bring phagocytes and defense chemicals from the bloodstream to the infection site as discussed later under this topic.

Fig. 2: Activation of C1 during the Classical Complement Pathway



The Fab of 2 molecules of IgG or 1 molecule of IgM bind to epitopes on an antigen. C1, consisting of C1q, C1r, and C1s then binds to the Fc portion of the antibodies. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b.

Fig. 3: Enhanced Attachment of Bacteria to Phagocytes



One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The "tips" of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The "stalk" of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

Flash animation showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.

html5 version of animation for iPad showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.

During the complement pathways, complement proteins such as C3a, C3b, C4a, C4b, and C5a are produces. These all play a role in inflammation and phagocytosis. C5a, C3a, and C4a stimulate mast cells to release histamine and other vasoactive agents to promote inflammation and diapedesis. C5a also functions as a chemoattractant for phagocytes. Most C3b and C4b binds to antigens on the microbial surface.The phagocytes are then able to bind to the C3b attached to the surface of the microorganism allowing for opsonization (enhanced attachment).

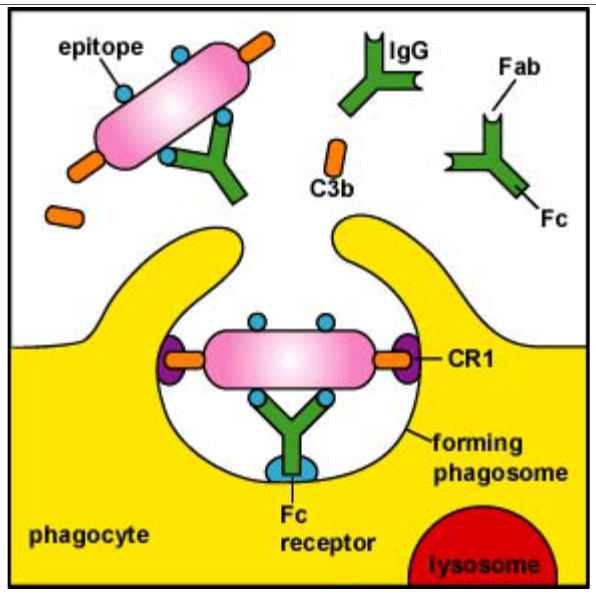
Actually, C3b molecule can bind to pretty much any protein or polysaccharide. **Human cells**, however, produce **Factor H** that binds to C3b and **allows Factor I to inactivate the C3b**. On the other hand, **substances such as LPS on bacterial cells facilitate the binding of Factor B to C3b** and this **protects the C3b from inactivation by Factor I**. In this way, C3b does not interact with our own cells but is able to interact with microbial cells.

For more information: Review of the five classes of human antibodies

For more information: Review of the complement pathways

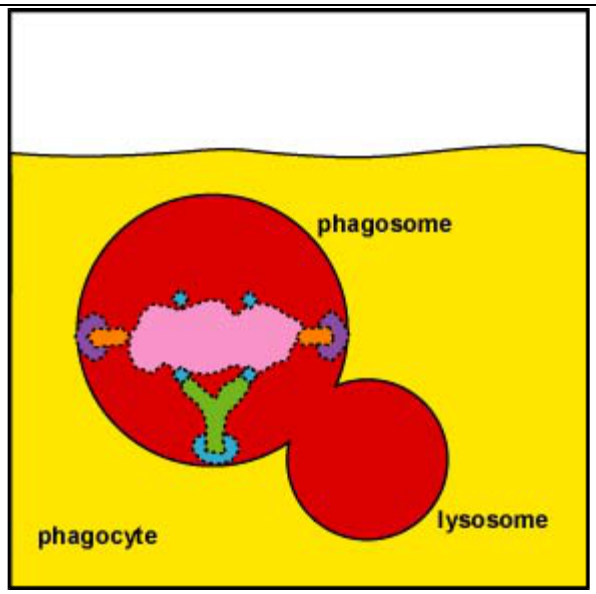
Attachment then promotes destruction of the antigen. Microorganisms are placed in phagosomes **(see Fig. 4)** where they are ultimately digested by lysosomes **(see Fig. 5)**.

Fig. 4: Formation of a Phagosome



Once attached to the phagocyte by way of IgG and/or C3b, the microbe can be engulfed more efficiently and placed in a phagosome.

Fig. 5: A Lysosome Fusing with a Phagosome



A lysosome fuses with the phagosome and the microbe is digested.

Flash animation of opsonization and intracellular destruction.

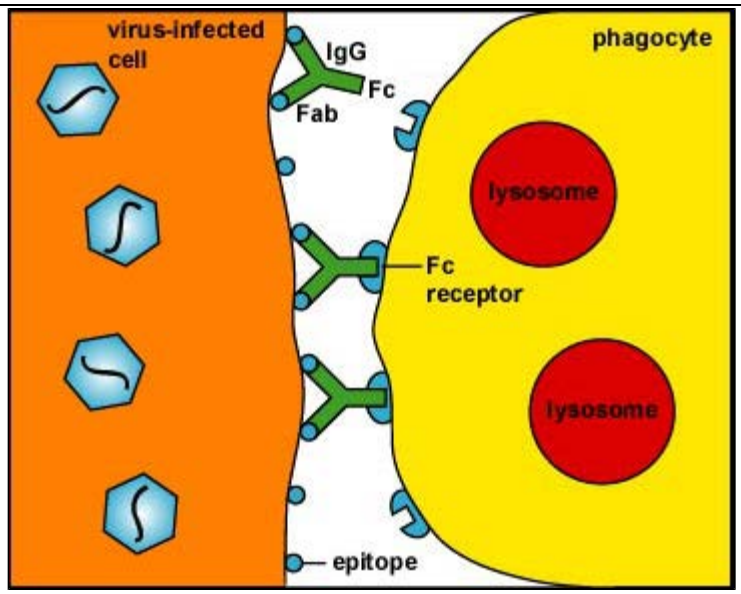
html5 version of animation for iPad showing opsonization and intracellular destruction.

Enhanced attachment is the attachment of microbes to phagocytes by way of

molecules such as the antibody molecule IgG or proteins produced during the complement pathways called C3b and C4b. Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and place it in a vesicle called a phagosome. Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed.

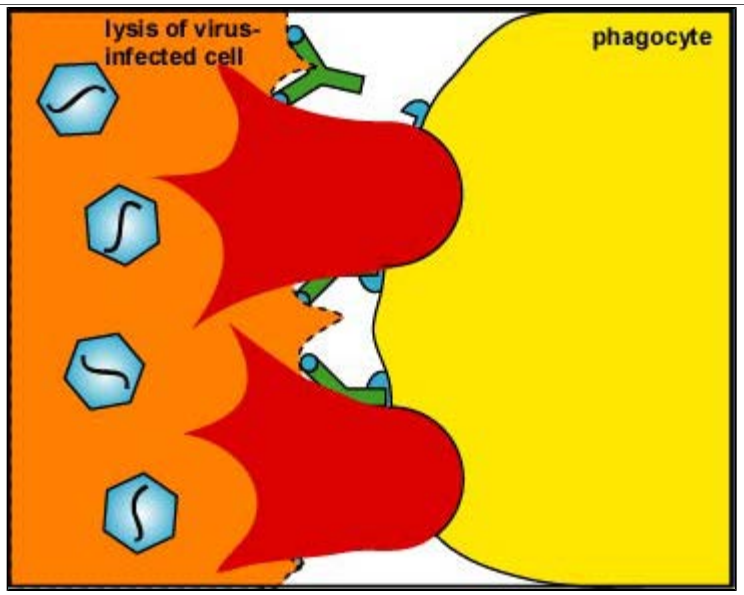
If the antigen is a cell too large to be ingested - such as virus-infected host cells, transplant cells, and cancer cells - the phagocyte empties the contents of its lysosomes directly on the cell for extracellular killing (see **Fig. 6** and **Fig. 7**).

Fig. 6: Sticking of Phagocytes to Larger Cells by Opsonization, Step-1



The Fab portions of IgG or molecules of C3b bind to epitopes on human cells recognized as foreign, such as virus-infected cells, tumor cells, or cells involved in autoimmune reactions. This enables phagocytes to attach to the cell by way of their Fc and C3b receptors.

Fig. 7: Sticking of Phagocytes to Larger Cells by Opsonization, Step-2



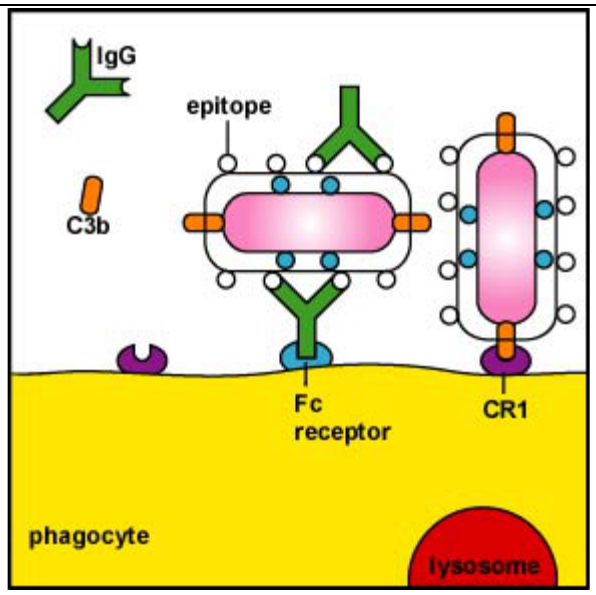
The phagocytes now destroy the cell by discharging their lysosomal contents.

Flash animation of opsonization and extracellular destruction.

html5 version of animation for iPad showing opsonization and extracellular destruction.

Opsonization is especially important against microorganisms with antiphagocytic structures such as capsules since opsonizing antibodies made against the capsule are able to stick capsules to phagocytes **(see Fig. 8)**. In vaccines against pneumococccal pneumonia and *Haemophilus influenzae* type b, it is capsular polysaccharide that is given as the antigen in order to stimulate the body to make opsonizing antibodies against the encapsulated bacterium.

Fig. 8: Opsonization of an Encapsulated Bacterium



The Fab portion of IgG binds to epitopes of a capsule. The Fc portion can now attach the antigen to Fc receptors on phagocytes for enhanced attachment. C3b and C4b from the complement pathways can also attach capsules to phagocytes. This is a major defense against encapsulated microbes.

Flash animation showing phagocytosis of an encapsulated bacterium through opsonization.

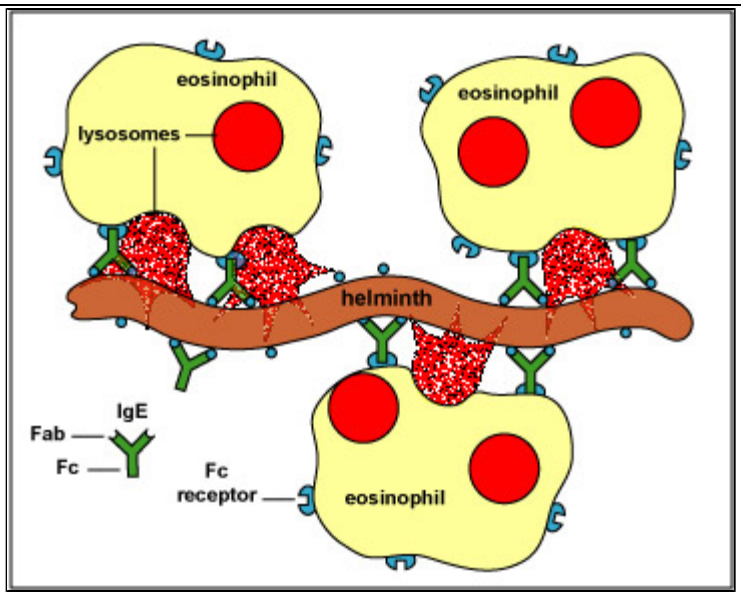
html5 version of animation for iPad showing phagocytosis of an encapsulated bacterium through opsonization.

The Fab portion of IgG binds to epitopes of a capsule. The Fc portion can now attach the capsule to Fc receptors on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the encapsulated bacterium can be engulfed more efficiently and placed in a phagosome.

B. Opsonization with IgE and the promotion of inflammation

The antibody isotype IgE is made against parasitic worms (helminths) and arthropods. The Fab portions of IgE bind to epitopes on the helminth or arthropod while the Fc portion binds to receptors on eosinophils enabling opsonization. In other words, IgE sticks phagocytic eosinophils to helminths and arthropods for the extracellular killing of that organism **(see Fig. 9)**.

Fig. 9: Opsonization of a Helminth by IgE and Eosinophils

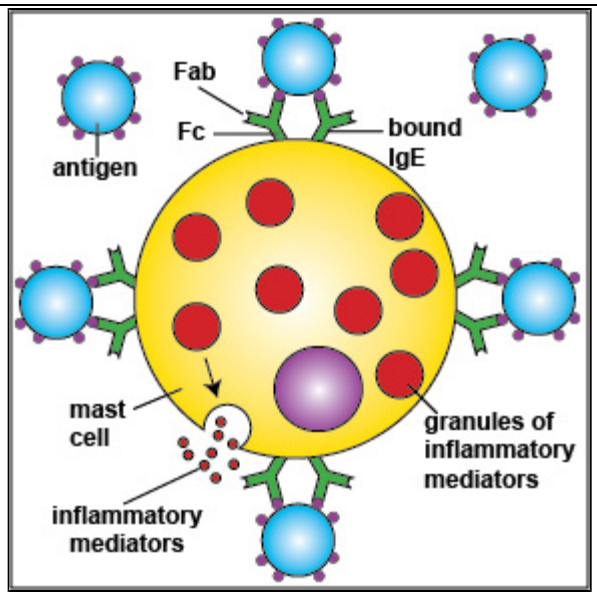


A major function of the cytokines produced by T_H2 cells is to enable B-lymphocytes to activate eosinophils and produce increased amounts of a class of antibodies called IgE against helminths (parasitic worms) and arthropods. IgE act as an opsonizing

antibody to stick phagocytic eosinophils to helminths for extracellular killing of the helminths. The Fab portion of IgE reacts with epitopes on the helminth while the Fc portion binds to Fc receptors of activated eosinophils. The lysosomal proteases of eosinophils are able to destroy the tough integument of helminths. IgE also promotes inflammation to recruit phagocytes.

The Fc portion of IgE also binds to receptors on mast cells and basophils to trigger the release of inflammatory mediators **(see Fig. 10)** . The inflammatory response then enables phagocytes and defense chemicals to leave the bloodstream and go to the infected site as will be discussed later under this topic.

Fig. 10: IgE Binding to Mast Cells and Basophils and Promoting an Inflammatory Response



The Fc portion of IgE is able to bind to receptors on mast cells and basophils. The cross-binding of antigens to the Fab portion of IgE triggers the release of inflammatory mediators. The resulting inflammatory response subsequently delivers defense cells and defense molecules from the bloodstream to the infection site.

Concept Map for Ways in which Antibodies Protect the Body

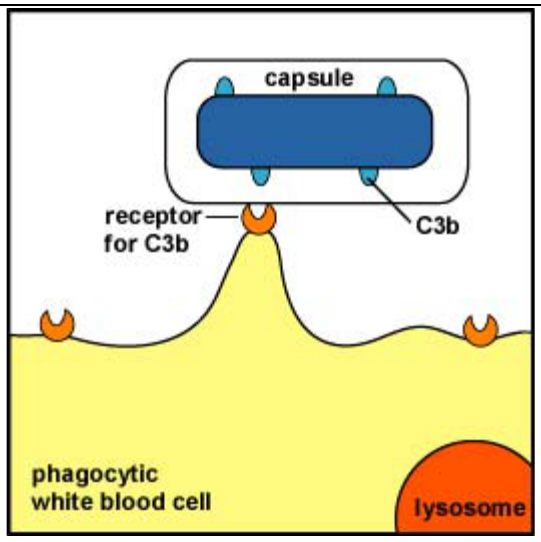
TPS Questions: Opsonization

C. How Bacteria Resist Attachment to Phagocytes

As we learned in Unit 3, some bacteria by means of the activities described below are **able to resist phagocytic attachment.** For example:

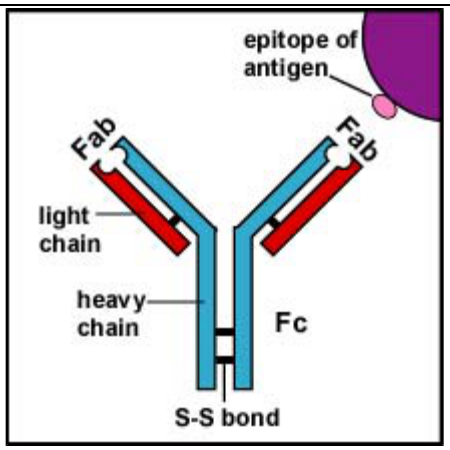
- An outer membrane molecule of *Neisseria gonorrhoeae* called Protein II and the M-protein of *Streptococcus pyogenes* allow these bacteria to be more resistant to phagocytic engulfment. The M-protein of *S. pyogenes*, for example, binds factor H of the complement pathway and this leads to the degradation of the opsonin C3b by factor I and the formation of C3 convertase.
- Some capsules simply cover the C3b that does bind to the bacterial surface and prevent the C3b receptor on phagocytes from making contact with the C3b **(see Fig. 11)**. This is seen with the capsule of *Streptococcus pneumoniae*.
- Capsules can also resist unenhanced attachment by preventing the glycoprotein receptors on phagocytes from recognizing the bacterial cell wall components and mannose-containing carbohydrates.
- *S. pneumonia* activates the classical complement pathway, but resists C3b opsonization, and complement causes further inflammation in the lungs.
- *Neisseria meningitidis* has a capsule composed of sialic acid *while Streptococcus pyogenes* has a capsule made of hyaluronic acid. Both of these polysaccharides closely resemble carbohydrates found in human tissue polysaccharides and because they are not recognized as foreign by the lymphocytes that carry out the immune responses, antibodies are not made against these capsules.
- Some bacteria are able to coat themselves with host proteins such as fibronectin, lactoferrin, or transferrin. This prevents antibody molecules from binding to epitopes on the bacterial surface.
- *Staphylococcus aureus* produces protein A while *Streptococcus pyogenes* produces protein G. Both of these proteins bind to the Fc portion of antibodies, the portion that normally binds to receptors on phagocytes **(see Fig. 12)**. In this way the bacteria become coated with antibodies in a way that does not result in opsonization **(see Fig. 13)**.
- *Streptococcus pyogenes* produces Mac proteins that are able to bind to the receptors on phagocytes to which IgG and C3b normally attach (see **Fig. 14**.and **Fig. 15)**. This blocks opsonization.
- Pathogenic *Yersinia*, such as the one that causes plague, contact phagocytes and, by means of a type III secretion system, deliver proteins which depolymerize the actin microfilaments needed for phagocytic engulfment into the phagocytes. Another *Yersinia* protein degrades C3b and C5a.

Fig. 11: Bacterial Capsule Preventing C3b Receptors on Phagocytes from Binding to C3b Attached to a Bacterial Cell Wall



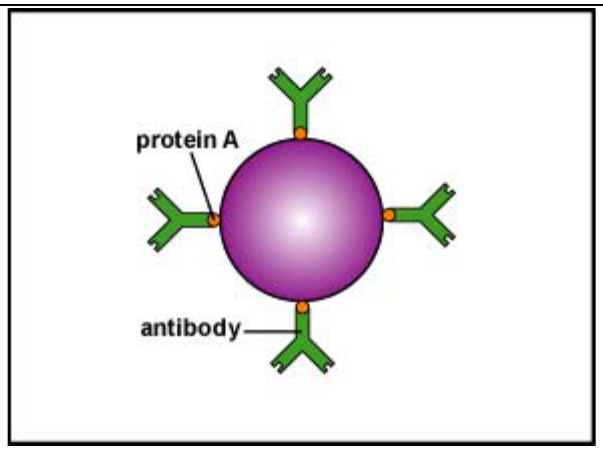
In some bacteria, the capsule covers the opsonin C3b bound to the bacterial cell wall so that it cannot bind to C3b receptors (called CR1) on the surface of phagocytes.

Fig. 12: Structure of the Antibody IgG



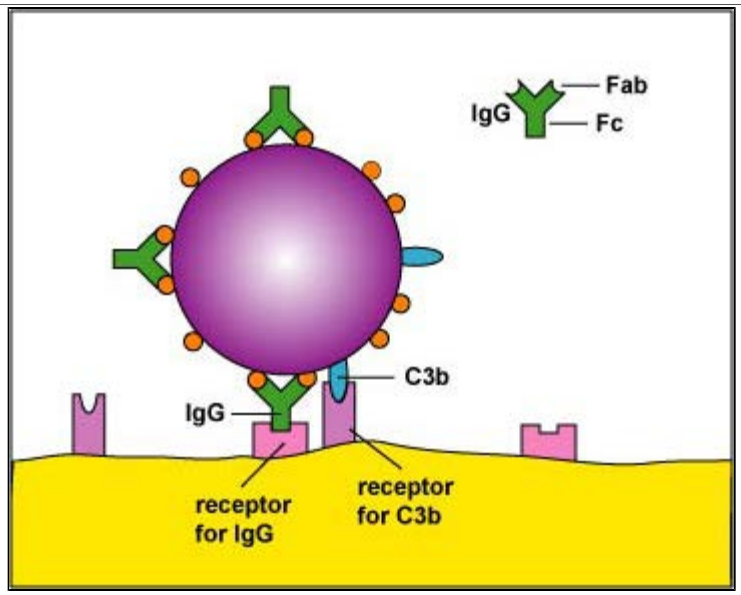
The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. Antibodies are composed of 4 protein chains: 2 identical heavy chains and 2 identical light chains. Disulfide (S-S) bonds join the protein chains together.

Fig. 13: *Staphylococcus aureus* Resisting Opsonization via Protein A



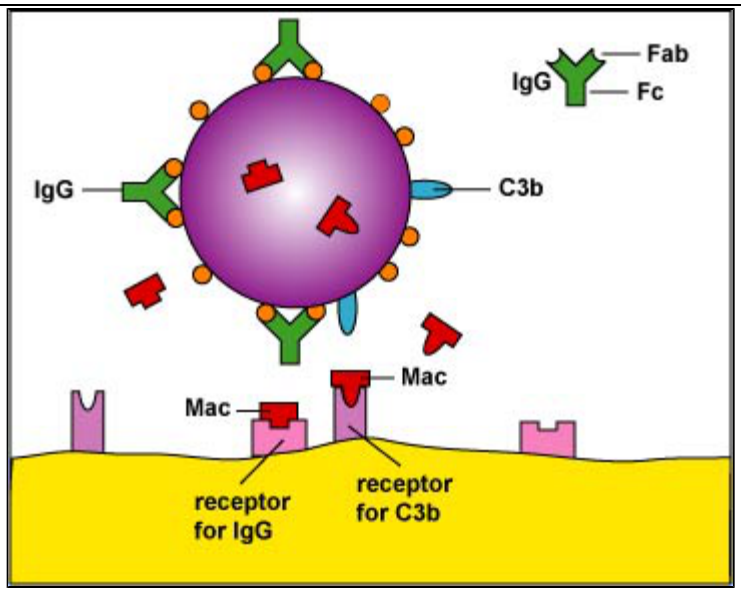
The Fc portion of the antibody IgG, the portion that would normally binds to Fc receptors on phagocytes, instead binds to protein A on *Staphylococcus aureus*. In this way the bacterium becomes coated with a protective coat of antibodies that do not allow for opsonization.

Fig. 14: Enhanced Attachment of *Streptococcus pyogenes* to Phagocytes



One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The tips of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The stalk of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

Fig. 15: *Streptococcus pyogenes* Resisting Opsonization with Mac Proteins



Mac proteins produced by *Streptococcus pyogenes* bind to IgG

receptors and C3b receptors on phagocytes. This blocks opsonization by preventing C3b and the Fc portion of IgG from attaching to their corresponding receptors.

For more information: Review of the ability to resist phagocytic engulfment

Self Quiz for Opsonization

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: MAC CYTOLYSIS

Adaptive Immunity

Ways in which Antibodies Protect the Body: MAC Cytolysis

Fundamental Statements for this Softchalk Lesson:

1. The Fab portion of IgG or IgM reacts with the epitopes on the membrane and the Fc portion of the antibody then activates the classical complement pathway. C5b6789n (the membrane attack complex or MAC) then puts holes in the membrane.
2. In the case of bacteria, MAC can put holes in the outer membrane and possibly the cytoplasmic membrane of the Gram-negative cell wall causing lysis.
3. In the case of enveloped viruses, MAC can damage the viral envelope.
4. In the case of human cells recognized as nonself - virus-infected cells, transplanted cells, transfused cells, cancer cells- the MAC causes direct cell lysis.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* Discuss how antibodies protect the body by way of MAC cytolysis. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

2. State specifically how MAC cytolysis protects against the following:

a. Gram-negative bacteria
b. human cells recognized as nonself
c. enveloped viruses

3. Describe one way Gram-negative bacteria may resist cytolysis.

(*) = common theme throughout the course

Ways in which Antibodies Protect the Body: MAC Cytolysis

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells

7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans
9. Promoting an Inflammatory Response

In this section we will look at MAC cytolysis.

The process starts with the antibody isotypes **IgG or IgM** being made against **epitopes on membranes**. The **Fab portion** of IgG or IgM reacts with the epitopes on the membrane and the **Fc portion** of the antibody then activates the classical complement pathway. **$C5b6789_n$, the membrane attack complex or MAC** then puts holes in the membrane. (Remember that MAC is also produced during the alternative complement pathway and the lectin pathway as was discussed in Unit 5.)

a. In the case of **bacteria**, MAC can put holes in the **outer membrane and possibly the cytoplasmic membrane of the Gram-negative cell wall** causing lysis **(see Slideshow Figs. 1 and 2)**.

Flash animation showing cytolysis of a Gram-negative bacterium by MAC.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing cytolysis of a Gram-negative bacterium by MAC.
The Fab portion of IgG or IgM binds to epitopes on the outer membrane of the gram-negative cell wall. This activates the complement pathway enabling the membrane attack complex (MAC) to insert into the outer membrane and possibly the cytoplasmic membrane causing the bacterium to lyse.

b. In the case of **enveloped viruses,** the MAC can damage the viral envelope (**see Slideshow Figs. 3 and 4**).

Flash animation showing damage to a viral envelope by MAC.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing damage to a viral envelope by MAC.
The Fab portion of IgG or IgM binds to epitopes on the viral envelope. This activates the complement pathway enabling the membrane attack complex (MAC) to insert into and damage the viral envelope causing viral inactivation.

c. In the case of **human cells recognized as nonself, such as** virus-infected cells, transplanted cells, transfused cells, and cancer cells, the MAC causes direct cell lysis (**see Slideshow Figs. 5 and 6**).

Flash animation showing cytolysis of an infected human cell by MAC.

html5 version of animation for iPad showing cytolysis of an infected human cell by MAC.

Typically to activate the classical complement pathway, IgG or IgM is made in response to an antigen. The Fab portion of IgG (2 molecules) or IgM (1 molecule) reacts with epitopes of that antigen. This alters the shape of the Fc portion of the antibody. A protein called C1q is then able to bind to the Fc portion of antigen-bound IgG or IgM after which C1r and C1s attach. Collectively, these three proteins form C1, the first enzyme of the pathway. At the end of the complement pathway, C5b6789n, or the membrane attack complex (MAC), puts pores into the lipid bilayer membrane of the infected cell causing its lysis.

Concept Map for Ways in which Antibodies Protect the Body

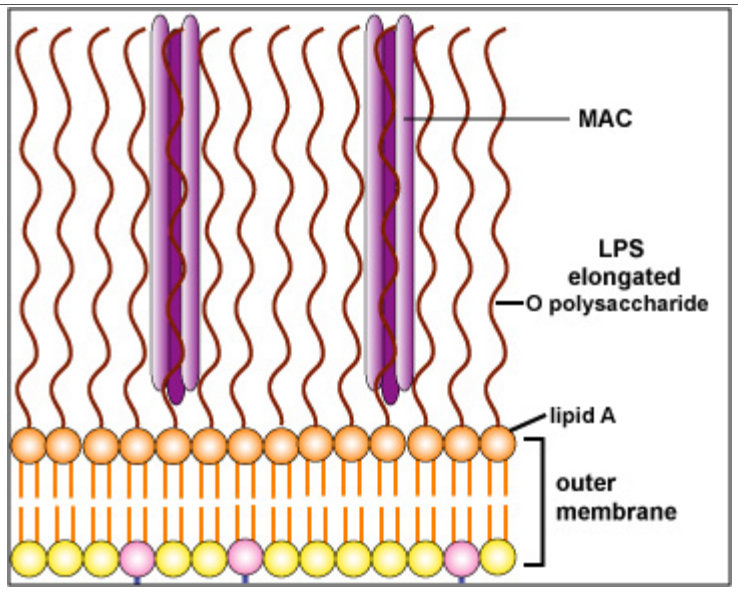
For more information: Review of the five classes of human antibodies

For more information: Review of the complement pathways

However, as learned in Unit 3, some bacteria by means of the activities described below **are more resistant to MAC lysis**.

- The LPS of the cell wall is the principle target for complement in Gram-negative bacteria by activating the alternative complement pathway and serving as a binding site for C3b as well as the site for formation of MAC. Some Gram-negative bacteria attach sialic acid to the LPS O antigen and this prevents the formation of the complement enzyme C3 convertase that is needed for the eventual formation of all the beneficial complement proteins such as C3b, C5a, and MAC. Blood-invasive strains of *Neisseria gonorrhoeae* as well as *Bordetella pertussis* and *Hemophilus influenzae* are examples of Gram-negative bacteria that are able to alter their LPS in this manner.
- Some Gram-negative bacteria, such as *Salmonella* lengthen the LPS O antigen side chain **(see Fig. 7)** and this prevents the formation of MAC. *Neisseria meningitidis* and Group B Streptococcus, on the other hand, produces capsular polysaccharides composed of sialic acid and as mentioned above, sialic acid prevents MAC lysis.

Fig. 7: Elongation of O-Polysaccharide Preventing the Insertion of MAC into the Cell Wall of Gram-Negative Bacteria



Some gram-negative bacteria lengthen the LPS O-polysaccharide preventing MAC from lysing the bacterium.

- An outer membrane molecule of *Neisseria gonorrhoeae* called Protein II binds factor H of the complement pathway and this leads to the degradation of the opsonin C3b by factor I and the formation of C3 convertase. Without C3 convertase, no MAC is produced.

For more information: Review of the ability to resist phagocytic destruction and complement

Self Quiz for MAC Cytolysis

Quiz Group

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: ADCC

Adaptive Immunity

Ways in which Antibodies Protect the Body: Antibody-Dependent Cellular Cytotoxicity (ADCC)

Fundamental Statements for this Softchalk Lesson:

1. NK cells are capable of antibody-dependent cellular cytotoxicity or ADCC.
2. When IgG is made against epitopes on "foreign" membrane-bound cells, such as virus-infected cells and cancer cells, the Fab portions of the antibodies react with epitopes on the "foreign" cell and then NK cells bind to the Fc portion of the antibody.
3. The NK cell then releases pore-forming proteins called perforins and proteolytic enzymes called granzymes.
4. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell and the cell breaks into fragments that are subsequently removed by phagocytes.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* Discuss how antibodies protect the body by way of ADCC by NK cells. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

(*) = common theme throughout the course

TPS Questions: ADCC

Ways in which Antibodies Protect the Body: Antibody-Dependent Cellular Cytotoxicity (ADCC)

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses

6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans.
9. Promoting an Inflammatory Response

In this section we will look at antibody-dependent cellular cytotoxicity (ADCC) by NK cells.

NK cells are capable of antibody-dependent cellular cytotoxicity or ADCC. NK cells have receptors on their surface for the Fc portion of certain subclasses of IgG. When the antibody IgG is made against epitopes on "foreign" membrane-bound cells, such as virus-infected cells and cancer cells, the Fab portions of the antibodies react with the "foreign" cell. The NK cells then bind to the Fc portion of the antibody.

The NK cell then releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation (see Fig. 1A and Fig. 2). As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis. (When NK cells are carrying out ADCC, they are sometimes also referred to as killer cells.)

The NK cell then releases pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation **(see slideshow Figs. 1, 2, and 3)**. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis. (When NK cells are carrying out ADCC, they are sometimes also referred to as killer cells.)

Slideshow Activity

Flash animation of ADCC contact by NK cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing ADCC contact by NK cells.
The Fab portion of the antibody IgG binds to epitopes on the "foreign" cell. The NK then releases pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.) Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation of apoptosis by NK cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing apoptosis by NK cells.
NK cells release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Concept Map for Ways in which Antibodies Protect the Body

TPS Questions: ADCC

Self Quiz for Antibody-Dependent Cellular Cytotoxicity (ADCC)

Quiz Group

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: NEUTRALIZATION OF EXOTOXINS

Adaptive Immunity

Ways in which Antibodies Protect the Body: Neutralization of Exotoxins

Fundamental Statements for this Softchalk Lesson:

1.For an exotoxin to cause harm it must first bind to receptors on a susceptible host cell.
2. Antitoxin antibodies are made against microbial exotoxins. The Fab portion binds to the exotoxin molecules before they can interact with host target cells and thus neutralizes the toxin.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* Discuss how antibodies protect the body by way of neutralizing exotoxins. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

2. Describe how the ability of bacteria to sense their own population density, communicate with each other by way of secreted factors (cell-to-cell signaling), and behave as a population rather than as individual bacteria most likely plays an important role in pathogenicity for many bacteria.

(*) = common theme throughout the course

Ways in which Antibodies Protect the Body: Neutralization of Exotoxins

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

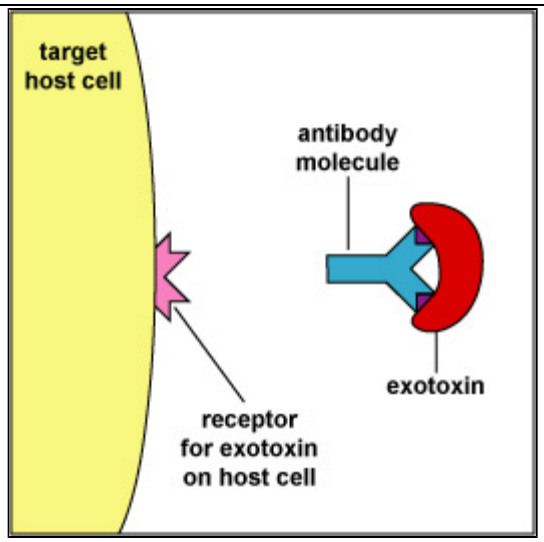
1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans.
9. Promoting an Inflammatory Response

In this section we will look at neutralization of exotoxins.

For an exotoxin to cause harm **it must first bind to receptors on a susceptible host cell**.

Antitoxin antibodies are made against microbial **exotoxins**. The Fab portion binds to the exotoxin molecules before they can interact with host target cells and thus **neutralizes** the toxin **(see Fig. 1)**. IgG neutralizes toxins in tissues while IgA neutralizes toxins at mucosal surfaces within the body.

Fig. 1: Neutralization of Exotoxins



The Fab portion of the antibodies made against epitopes of the binding site of an exotoxin blocks the exotoxin from binding to the exotoxin receptor on the host cell membrane. As a result, the toxin can not enter the cell and cause harm.

Flash animation showing neutralization of an exotoxin.

html5 version of animation for iPad showing neutralization of an exotoxin.

The Fab portion of the antibodies made against epitopes of the binding site of an exotoxin blocks the exotoxin from binding to the host cell membrane. As a result, the toxin can not enter the cell and cause harm.

Concept map for Ways in Which Antibodies Protect the Body

For more information: Review of exotoxins

For more information: Review of type 1 toxins

For more information: Review of type 2 toxins

For more information: Review of type 3 toxins

However, as learned in Unit 2, many Gram-negative and Gram-positive are able to sense their own population density, communicate with each other by way of secreted factors, and behave as a population rather than as individual bacteria. This is referred to as **quorum sensing** and most likely plays an important role in pathogenicity for many bacteria.

- For example, *Pseudomonas aeruginosa* causes severe nosocomial infections, chronic infections in people with cystic fibrosis, and potentially fatal infections in those who are immunocompromised. Its virulence depends on the secretion of a variety of harmful exotoxins and enzymes. If there was an isolated production of these virulence toxins and enzymes by a small number of *Pseudomonas*, the body's immune responses would most likely be able effectively neutralize these harmful agents with antibodies. However, through a coordination of the expression of the genes coding for these toxins and enzymes by the entire population of bacteria, *P. aeruginosa* appears to only secrete these extracellular virulence factors when the density of bacteria is large enough that they can be produced at high enough levels to overcome body defenses.

For more information: Review of quorum sensing

Self Quiz for Neutralization of Exotoxins

Quiz Group

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY

Adaptive Immunity

Ways in which Antibodies Protect the Body: Neutralization of Viruses

Fundamental Statements for this Softchalk Lesson:

1. In order for viruses to infect a cell and replicate, they must first adsorb to receptors on the host cell's plasma membrane.
2. Antibodies are made against viral capsids or envelope glycoproteins where the Fab portion binds to and covers the viral attachment molecules. This prevents viral adsorption to host cells.
3. Neutralizing antibodies are especially important in preventing viral reinfection.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* Discuss how antibodies defend the body by way of neutralizing viruses. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

2. Briefly describe 2 different ways viruses may resist neutralizing antibodies.

(*) = common theme throughout the course

TPS Question: Neutralization of Viruses

Ways in which Antibodies Protect the Body: Neutralization of Viruses

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells

4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans
9. Promoting an Inflammatory Response

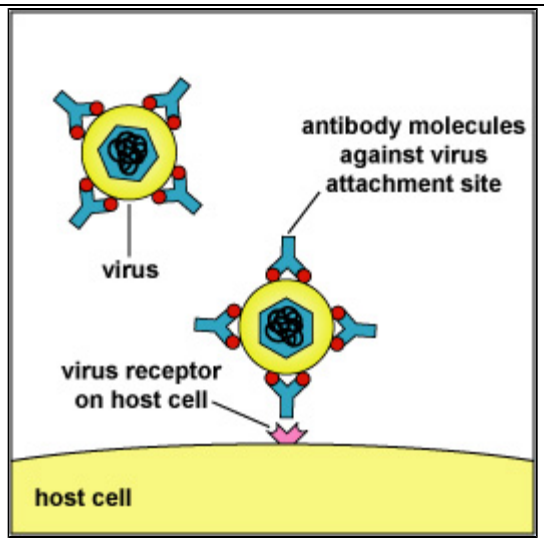
In this section we will look at neutralization of viruses.

In order for viruses to infect a cell and replicate, they must first adsorb to receptors on the host cell's plasma membrane.

For more information: Review of the productive life cycle of animal viruses

Antibodies are made against viral capsids or envelope glycoproteins where the Fab portion binds to and covers the viral attachment molecules. **This prevents viral adsorption to host cells (see Fig. 1)**. Neutralizing antibodies are especially important in **preventing viral reinfection**. IgG neutralizes viruses in tissues while IgA neutralizes viruses at mucosal surfaces within the body.

Fig. 1: Neutralization of Viruses



The Fab portion of the antibodies made against epitopes of the virus attachment site blocks the virus from adsorbing to the receptor site on the host cell membrane. As a result, the virus can not penetrate and replicate.

Flash animation showing neutralization of a virus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing neutralization of a virus.

The Fab portion of the antibodies made against epitopes of the virus attachment site blocks the virus from adsorbing to the receptor site on the host cell membrane. As a result, the virus can not penetrate and replicate.

Concept Map for Ways in which Antibodies Protect the Body

However, as learned in Unit 4, some viruses by means of the activities described below **are able to overcome this antibody defense**.

- The influenza viruses undergo what is called antigenic drift and antigenic shift. With antigenic drift, mutations cause a gradual change in the hemagglutinin antigen that adsorbs to receptors on host cells. Antigenic shift is caused by a human influenza virus acquiring a new genome segment from an influenza virus capable of infecting other animals such as a ducks or swine. This new genome segment causes a major change in the hemagglutinin antigen. Antibodies made against the original human influenza virus can no longer bind to the new strain of virus or stick the virus to phagocytes.
- Likewise HIV, because of its high rate of mutation and its intracellular recombination with other strains of HIV, as mentioned earlier in this unit, produces altered gp120 to which antibodies made against the earlier strains of HIV can no longer bind.
- The hepatitis C virus (HCV) frequently, through mutation, produces viral variants ("escape mutants") to resist antibodies.

TPS Question: Neutralization of Viruses

For more information: Review of pathogenicity of animal viruses

Quiz Group

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: PREVENT BACTERIAL ADHERENCE

Adaptive Immunity

Ways in which Antibodies Protect the Body: Prevent Bacterial Adherence

Fundamental Statements for this Softchalk Lesson:

1. Bacteria resist physical removal by means of pili, cell wall adhesin proteins, and/or biofilm-producing capsules.
2. The binding of the Fab portion of the antibody to the adhesive tip of the pili, the cell wall adhesins, or the capsular molecules prevents the bacteria from adhering to and colonizing host cells.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* **Discuss** how antibodies protect the body by way of preventing bacterial adherence to host cells. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

2. Briefly describe 2 different ways bacteria may resist antibodies that block bacterial adherence to host cells.

(*) = common theme throughout the course

TPS Questions: Prevent Bacterial Adherence

Ways in which Antibodies Protect the Body: Prevent Bacterial Adherence

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans

9. Promoting an Inflammatory Response

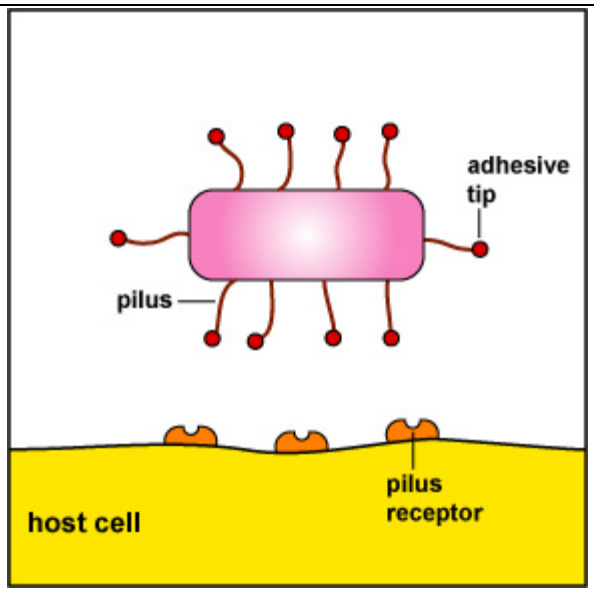
In this section we will look at preventing bacterial adherence to host cells.

One of the body's innate defenses is the ability to physically remove bacteria from the body through such means as the constant shedding of surface epithelial cells from the skin and mucous membranes, the removal of bacteria by such means as coughing, sneezing, vomiting, and diarrhea, and bacterial removal by bodily fluids such as saliva, blood, mucous, and urine. **Bacteria may resist this physical removal producing pili, cell wall adhesin proteins, and/or biofilm-producing capsules**.

For more information: Review of bacterial adherence

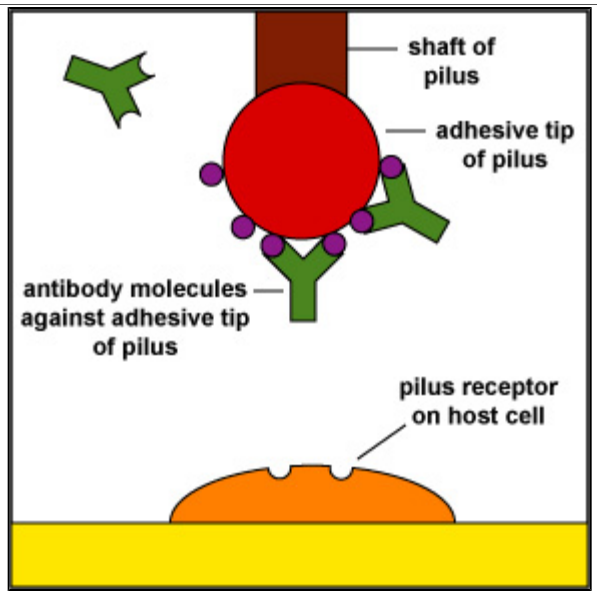
Antibodies are made against **pili**, **capsules**, and **cell wall adhesins**. The binding of the Fab portion of the antibody to the adhesive tip of the pili, the cell wall adhesins, or the capsular molecules prevents the bacteria from adhering to and colonizing host cells (see **Fig. 1** and **Fig. 2**.) IgG blocks adherence of bacteria in tissues while IgA blocks adherence of bacteria at mucosal surfaces within the body.

Fig. 1: Bacterial Adherence Via Pili



The Fab portion of the antibodies made against epitopes of adherence structures such as pili bind and block the bacteria from adhering to the host cell membrane. As a result, the bacteria may be flushed away. Antibody molecules made against the adhesive tip of pili block the adhesive tip from binding to receptors on host cell membranes.

Fig. 2: Blocking Bacterial Adherence with Antibody Molecules



Antibody molecules made against the adhesive tip of pili block the adhesive tip from binding to receptors on host cell membranes. This prevents the bacterium from colonizing the host cells.

Flash animation showing antibodies blocking bacterial adherence to host cell.

html5 version of animation for iPad showing antibodies blocking bacterial adherence to host cell.

The Fab portion of the antibodies made against epitopes of adherence structures such as cell wall adhesins bind and block the bacteria from adhering to receptors on the host cell membrane. As a result, the bacteria are unable to colonize and may be flushed away.

Concept Map for Ways in which Antibodies Protect the Body

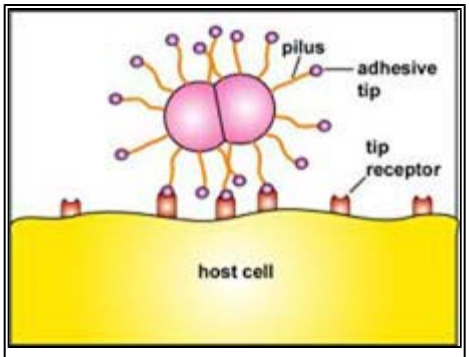
TPS Questions: Prevent Bacterial Adherence

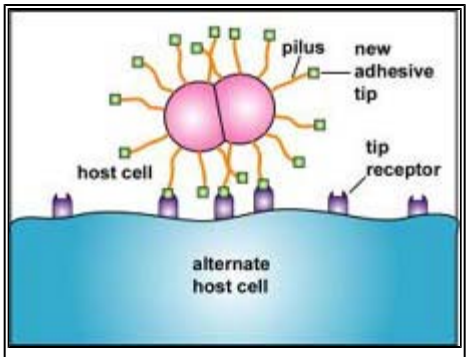
However, as learned in Unit 3, some bacteria by means of the activities described below **are able to overcome this antibody defense**.

Some bacteria can produce immunoglobulin proteases which can degrade the protective IgA found in mucus. Examples include bacteria that colonize the mucous membranes such as *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Helicobacter pylori* ,*Shigella flexneri* and enteropathogenic *Escherichia coli*.

- Another way certain bacteria can evade antibodies is by changing the adhesive tips of their pili as seen with *Neisseria gonorrhoeae* **(see Fig. 3)**. Bacteria can also vary other surface proteins so that antibodies already made will no longer "fit."

Fig. 3: Bacteria Altering the Adhesive Tips of Their Pili





By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

For More Information: Bacteria Resisting Adaptive Immunity

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: AGGLUTINATION OF MICROBES

Adaptive Immunity

Ways in which Antibodies Protect the Body: Agglutination of Microbes

Fundamental Statements for this Softchalk Lesson:

1. Agglutination is mainly a function of antibodies with multiple reactive Fab sites such as IgM and IgA.
2. The Fab portion of the antibodies links microorganisms together (causes them to agglutinate) so they can be phagocytosed more effectively.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* Discuss the how antibodies protect the body by agglutinating microorganisms. (Include what classes or isotypes of immunoglobulins are involved, the role of the Fab portion of the antibody, the role - if any - of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

(*) = common theme throughout the course

Ways in which Antibodies Protect the Body: Agglutination of Microbes

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

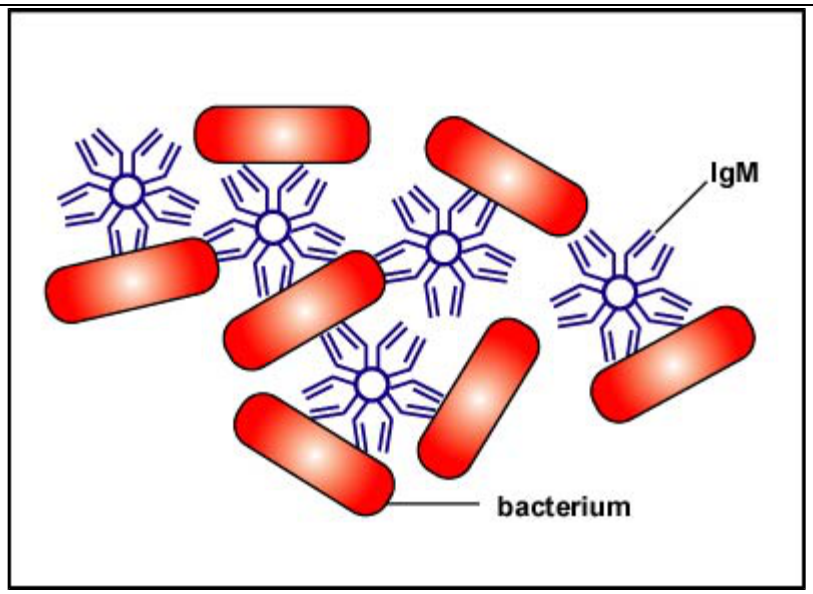
1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans
9. Promoting an Inflammatory Response

In this section we will look at antibody-induced agglutination of microorganisms.

Agglutination is mainly a function of antibodies with multiple reactive Fab sites such as **IgM and IgA**. The Fab portion of the

antibodies **links microorganisms together** (causes them to agglutinate) so they can be **phagocytosed** more effectively **(see Fig. 1)**.

Fig. 1: Antibody-Induced Agglutination of Microorganisms



The multiple Fab portions of IgM link microorganism together so out of the lymph and blood and phagocytosed more effectively.

For more information: Review of the five classes of human antibodies

Concept Map for Ways in which Antibodies Protect the Body

Show/hide comprehension question...

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: IMMOBILIZATION OF BACTERIA AND PROTOZOANS

Adaptive Immunity

Ways in which Antibodies Protect the Body: Immobilization of Bacteria and Protozoans

Fundamental Statements for this Softchalk Lesson:

1. Flagella and cilia are organelles of locomotion and enable motile microorganisms to move towards or away from environmental molecules through a process called taxis.
2. Antibodies are made against the flagella of motile bacteria or the flagella or cilia of motile protozoans.
3. The Fab portions of the antibodies bind to these locomotor organelles and arrest the organism's movement blocking its ability to spread.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* **Discuss** how antibodies protect the body by immobilizing bacteria and protozoans. (Include the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

(*) = common theme throughout the course

Ways in which Antibodies Protect the Body: Immobilization of Bacteria and Protozoans

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans
9. Promoting an Inflammatory Response

In this section we will look at the immobilization of bacteria and protozoans.

Flagella and cilia are organelles of locomotion and enable motile microorganisms to move towards or away from environmental

molecules through a process called taxis. The mucosal surfaces of the bladder and the intestines constantly flush bacteria away in order to prevent colonization. Motile bacteria that can swim chemotactically toward mucosal surfaces may have a better chance to make contact with the mucous membranes, attach, and colonize.

For more information: Review of bacteria using motility to contact host cells

Antibodies are made against the flagella of motile bacteria or the flagella or cilia of motile protozoans. The Fab portions of the antibodies bind to these locomotor organelles and arrest the organism's movement blocking its ability to spread.

Concept Map for Ways in which Antibodies Protect the Body

Show/hide comprehension question...

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ADAPTIVE IMMUNITY
WAYS IN WHICH ANTIBODIES PROTECT THE BODY: PROMOTING AN INFLAMMATORY RESPONSE

Adaptive Immunity

Ways in which Antibodies Protect the Body: Promoting an Inflammatory Response

Fundamental Statements for this Softchalk Lesson:

1.IgG and IgM can activate the classical complement pathway and C5a, C3a, and C4a can trigger inflammation.
2. IgA can activate the lectin complement pathway and the alternative complement pathway and C5a, C3a, and C4a can trigger inflammation.
3. IgE can bind to mast cells and basophils and trigger the release of inflammatory mediators.

Common Course Objectives

1. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1.* Describe two different ways antibodies defend the body by promoting an inflammatory response and state the importance of inflammation. (Include the role of the Fab portion of the antibody, the role, if any, of the Fc portion of the antibody, and the role of any complement proteins, if any, involved.)

(*) = common theme throughout the course

Ways in which Antibodies Protect the Body: Promoting an Inflammatory Response

The antibodies produced during humoral immunity ultimately defend the body through a variety of different means. These include:

1. Opsonization
2. MAC Cytolysis
3. Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
4. Neutralization of Exotoxins
5. Neutralization of Viruses
6. Preventing Bacterial Adherence to Host Cells
7. Agglutination of Microorganisms
8. Immobilization of Bacteria and Protozoans
9. Promoting an Inflammatory Response

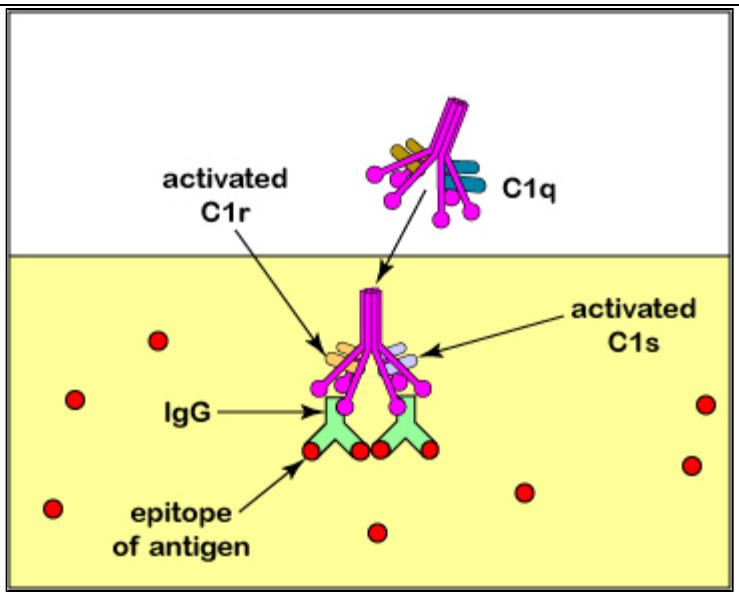
In this section we will look at how antibodies can promote an inflammatory response.

Antigen-antibody reactions can also promote an inflammatory response

a. IgG and IgM can activate the classical complement pathway

As learned under innate immunity in Unit 5, the classical complement pathway is primarily activated when a complement protein complex called C1 interacts with the Fc portion of the antibody molecule isotypes IgG or IgM after they have bound to their specific antigen via their Fab portion **(see Fig. 1)**. C1 is also able to directly bind to the surfaces of some pathogens.

Fig. 1: Activation of C1 during the Classical Complement Pathway



The Fab of 2 molecules of IgG or 1 molecule of IgM bind to epitopes on an antigen. C1, consisting of C1q, C1r, and C1s then binds to the Fc portion of the antibodies. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b.

The C1 complex is composed of three complement proteins called C1q, C1r, and C1s.

- The C1q is the portion of the C1 complex that binds to the antibodies or the microbe.
- The binding of C1q activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and C2 into C2a and C2b and begin the classical complement pathway.

Flash animation showing assembly of C1 during the classical complement

pathway.

html5 version of animation for iPad showing assembly of C1 during the classical complement pathway.

The C1 complex is composed of three complement proteins called C1q, C1r, and C1s. The C1q is the portion of the C1 complex that can bind to the Fc portion of the antibodies IgG or IgM, certain microbes, or the C-reactive protein (CRP) from the acute phase response.

In the case of antibodies, the Fab portions of 2 molecules of IgG (or 1 molecule of IgM) bind to their corresponding epitopes on an antigen. Binding of the IgG to the epitopes activates the Fc portion of the IgG enabling C1q of the C1 complex to bind. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b.

The beneficial results of the activated complement proteins include :

1. **Triggering inflammation**: C5a>C3a>C4a.

2. **Chemotactically attracting phagocytes to the infection site**: C5a;

3. **Promoting the attachment of antigens to phagocytes via enhanced attachment or opsonization**: C3b>C4b (discussed earlier under opsonization);

4. **Causing the lysis of Gram-negative bacteria, viral envelopes, and human cells displaying foreign epitopes** (discussed earlier under MAC cytolysis).

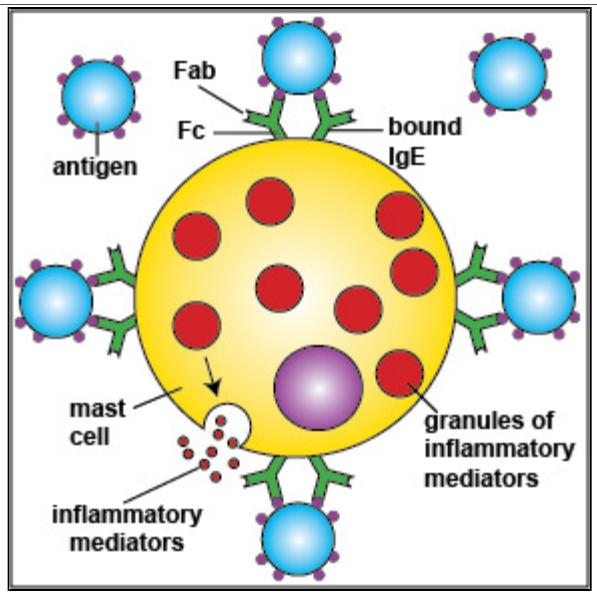
b. IgA can activate the lectin complement pathway and the alternative complement pathway and C5a, C3a, and C4a can trigger inflammation.

For more information: Review of the complement pathways

c. IgE can bind to mast cells and basophils and trigger the release of inflammatory mediators.

The Fc portion of IgE can **bind to receptors on mast cells and basophils**. Cross linking of the cell-bound IgE by antigen triggers the release of vasodilators and other inflammatory mediators for an inflammatory response **(see Fig 2)**.

Fig. 2: IgE Binding to Mast Cells and Basophils and Promoting an Inflammatory Response



The Fc portion of IgE is able to bind to receptors on mast cells and basophils. The cross-binding of antigens to the Fab portion of IgE triggers the release of inflammatory mediators. The resulting inflammatory response subsequently delivers defense cells and defense molecules from the bloodstream to the infection site.

As learned under inflammation in Unit 5, most of the body defense elements are located in the blood and **inflammation is the means by which body defense cells and defense chemicals leave the blood and enter the tissue around the injured or infected site**.

The inflammatory response produces vasodilators that increase capillary permeability. As a result of this increased permeability:

a. **Plasma flows out of the blood into the tissue.**

Beneficial molecules in the plasma include:

1. **Clotting factors**. Tissue damage activates the coagulation cascade causing fibrin clots to form to localize the infection, stop the bleeding, and chemotactically attract phagocytes.
2. **Antibodies**. These help remove or block the action of microbes through a variety of methods described in this section.
3. **Proteins of the complement pathways**. These, in turn: 1) stimulate more inflammation (C5a, C3a, and C4a), 2) stick microorganisms to phagocytes (C3b and C4b), 3) chemotactically attract phagocytes (C5a), and 4) lyse membrane-bound cells displaying foreign antigens (membrane attack complex or MAC).
4. **Nutrients.** These feed the cells of the inflamed tissue.
5. **Lysozyme**, cathelicidins, phospholipase A_2, and **human defensins**. Lysozyme degrades peptidoglycan. Cathelicidins are cleaved into two peptides that are directly toxic to microbes and can neutralize LPS from the gram-negative bacterial cell wall. Phospholipase A_2 hydrolizes the phospholipids in the bacterial cytoplasmic membrane. Human defensins put pores in the cytoplasmic membranes of many bacteria. Defensins also activate cells involved in the inflammatory response.
6. **Transferrin**. Transferrin deprives microbes of needed iron.

b. **Leukocytes enter the tissue through a process called diapedesis** or **extravasation**.

Benefits of diapedesis include:

1. **Increased phagocytosis.** Phagocytes such as neutrophils, monocytes that differentiate into macrophages when they enter the tissue, and eosinophils are phagocytic leukocytes.
2. More **vasodilation.** Basophils, eosinophils, neutrophils, and platelets enter the tissue and release or stimulate the production of vasoactive agents that promote inflammation.
3. **Cytotoxic T-lymphocytes (CTLs)**, effector T4-cells, **and NK cells** enter the tissue to kill cells such as infected cells and cancer cells that are displaying foreign antigens on their surface (discussed in Unit 6).

For more information: Review of inflammation

Concept Map for Ways in which Antibodies Protect the Body

Quiz Group

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ADAPTIVE IMMUNITY
ACTIVE AND PASSIVE IMMUNITY

Adaptive Immunity

Active and Passive Immunity

Fundamental Statements for this Softchalk Lesson:

1. During passive immunity, the body receives antibodies made in another person or animal and the immunity is short-lived.
2. During active immunity, antigens enter the body and the body responds by making its own antibodies and B-memory cells. In this case, immunity is longer lived although duration depends on the persistence of the antigen and the memory cells in the body.
3 . Active naturally acquired immunity refers to the natural exposure to an infectious agent or other antigen by the body. The body responds by making its own antibodies.
4. There are two examples of passive naturally acquired immunity: The placental transfer of IgG from mother to fetus during pregnancy that generally lasts 4 to 6 months after birth; and The IgA and IgG found in human colostrum and milk of babies who are nursed.
5 . Active artificially acquired immunity refers to any immunization with an antigen.
6. During artificially acquired active immunity, one is immunized with one or more of the following: attenuated microbes, killed organisms, fragmented microorganisms, or antigens produced by recombinant DNA technology, or toxoids.
7. Passive artificially acquired immunity refers to the injection of antibody-containing serum, or immune globulin (IG), from another person or animal. Since the body is not making its own antibodies and memory cells are not produced, passive artificially acquired immunity is short lived and offers only immediate, short term protection.
8. When a critical portion of a community becomes immunized against a particular infectious disease, most members of the community - including those who were not immunized - are protected against that disease because there is little opportunity for an outbreak. This is known as herd immunity or community immunity.

Common Course Objectives

1. Differentiate between active and passive immunity.
2. Explain why vaccination protects against diseases.
3. Recall the sources of antigen used in vaccines.
4. Explain why boosters are given for vaccines.
5. Explain herd immunity and how it can protect selective populations of people.

Detailed Learning Objectives

1. Differentiate between active immunity and passive immunity.
2. Give an example of naturally acquired active immunity.
3. Give two examples of naturally acquired passive immunity and state why this is important to newborns and infants.
4*. Define and give at least one example of artificially acquired active immunity.
5*. Define and give at least one example of artificially acquired passive immunity.
6*. List 3 different forms of antigen that may be used for artificially acquired active immunity and state 2 common examples of each.
7. State what DTaP stands for and what specifically is being injected with the DTaP vaccine.
8. Briefly compare active immunization with passive immunization in terms of tetanus prophylaxis.
9. Define adjuvant.

10. In artificially acquired immunity, active immunization is preferred over passive immunization. Explain why.
11. Describe what is meant by herd immunity (community immunity).

(*) = common theme throughout the course

TPS Question: Active and Passive Immunity

Active and Passive Immunity

Immunity may be passive or active:

During **passive immunity**, **antibodies made in another person or animal enter the body** and the immunity is short-lived.

In the case of **active immunity**, **antigens enter the body** and the body responds by making its own antibodies and B-memory cells. In this case, immunity is longer lived although duration depends on the persistence of the antigen and the memory cells in the body.

Both passive and active immunity can be either naturally or artificially acquired.

Show/hide comprehension question...

Naturally Acquired Immunity

a. Active Naturally Acquired Immunity

Active naturally acquired immunity refers to the natural exposure to an infectious agent or other antigen by the body. The body responds by making its own antibodies.

b. Passive Naturally Acquired Immunity

There are two examples of passive naturally acquired immunity:

1. The **placental transfer of IgG** from mother to fetus during pregnancy. These antibodies generally last 4 to 6 months following birth. The immune responses reach full strength at about age 5.

2. The **IgA and IgG found in human colostrum and milk** of babies who are nursed. In addition to the IgA and IgG, human milk also contains:

- Oligosaccharides and mucins that adhere to bacteria and viruses to interfere with their attachment to host cells;
- Lactoferrin to bind iron and make it unavailable to most bacteria;
- B_{12} binding protein to deprive bacteria of needed vitamin B_{12};
- Bifidus factor that promotes the growth of *Lactobacillus bifidus*, normal flora in the gastrointestinal tract of infants that crowds out harmful bacteria;
- Fibronectin that increases the antimicrobial activity of macrophages and helps repair tissue damage from infection in the gastrointestinal tract;
- Gamma-interferon, a cytokine that enhances the activity of certain immune cells;
- Hormones and growth factors that stimulate the baby's gastrointestinal tract to mature faster and be less susceptible to infection;
- Lysozyme to break down peptidoglycan in bacterial cell walls.

According to the Centers for Disease Control and Prevention (CDC), **breast-fed infants have a lower incidence of gastrointestinal infections, ear infections, atopic dermatitis, respiratory infections, urinary tract infections,**

meningitis, type 2 diabetes, and sudden infant death syndrome. Benefits to the mother include a decreased risk of breast cancer, ovarian cancer, and type 2 diabetes, as well stopping post-birth bleeding and temporarily suppressing ovulation. It may also be associated with a reduced risk of pediatric overweight.

Concept Map for Active and Passive Immunity

Quiz Group

Artificially Acquired Immunity

a. Active artificially acquired immunity

Active artificially acquired immunity refers to any immunization with an antigen. By giving a safe form of the antigen artificially, the body will produce its own antibodies and, more importantly, develop circulating, long-lived **B-memory cells** with high affinity B-cell receptors on their surface. If at a later date the body is again exposed to that same antigen, **the memory cells will cause immediate and rapid production of the appropriate antibodies for protection.**

CDC Recommended Immunization Schedules for Persons Aged 0 Through

18 Years, UNITED STATES, 2015

With artificially acquired active immunity, one is immunized with one or more of the following:

1. Attenuated microbes

Attenuated microbes are **living, non-virulent strains** of a microbe. Viruses are attenuated by growing them in non-human cells until they mutate and adapt to the non-human host. In the process, they lose virulence for humans. Viruses can also be attenuated using recombinant DNA techniques to either mutate or delete virulence genes in the viral genome.

Attenuated viral vaccines tend to be immunologically quite effective since the viruses can multiply slowly in the body, thus **increasing the amount and persistence of the antigen for a greater antibody response**. In addition, attenuated viruses enter the cytosol of cells and peptides from viral antigens can be presented by MHC-I molecules to **activate naive T8-lymphocytes and stimulate the production of cytotoxic T-lymphocytes (CTLs)**. Living attenuated microbes can, however, sometimes be **potentially dangerous to highly immunosuppressed individuals** in whom they may cause opportunistic infections.

Examples of vaccines that contain **attenuated microbes** include:

- The MMR vaccine containing attenuated measles, mumps, and rubella viruses;
- The MMRV vaccine containing attenuated measles, mumps, rubella viruses and varicella zoster (chickenpox) viruses;
- The TOPV or trivalent oral polio vaccine containing attenuated poliomyelitis viruses types 1, 2, and 3;

The yellow fever vaccine containing attenuated yellow fever viruses;
- The Var or varicella zoster virus vaccine containing attenuated varicella zoster viruses.

The body responds by producing antibodies that block viral adsorption to host cells.

Flash animation showing the neutralization of a virus by antibodies
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing neutralization of a virus.
The Fab portion of the antibodies made against epitopes of the virus attachment site blocks the virus from adsorbing to the receptor site on the host cell membrane. As a result, the virus can not penetrate and replicate.

2. Killed organisms, fragmented microorganisms, or antigens produced by recombinant DNA technology

a. Examples of vaccines containing **killed or inactivated microbes** include:

- The IPV or inactivated poliomyelitis vaccine containing inactivated poliomyelitis viruses types 1, 2, and 3;
- The rabies vaccines containing whole, killed rabies viruses;
- The influenza vaccines consist of inactivated influenza viruses, either whole or broken down;
- The hepatitis A vaccine containing inactivated hepatitis A virus;
- RV1, an attenuated strain of a human rotavirus. Rotaviruses are the most common cause of gastroenteritis in children.

b. Examples of vaccines containing **fragments of microorganisms** include the immunizations for:

- Meningococcal meningitis; contains capsular polysaccharide from 4 strains of *Neisseria meningitidis*;
- Pneumococcal pneumonia; PCV13 containing capsular material from the 13 most serious strains of *Streptococcus pneumoniae* in children conjugated to diphtheria toxoid protein; PCV 23 containing capsular material from the 23 most serious strains of *S. pneumoniae* in adults conjugated to diphtheria toxoid protein;
- *Hemophilus influenzae* type b containing capsular polysaccharide from *H. influenzae* type B conjugated to protein (either diphtheria toxoid or an outer membrane protein from *Neisseria meningitidis*).

These vaccines contain **polysaccharide capsular material** from the bacteria, usually **conjugated to protein for greater immunogenicity**. The body responds by producing opsonizing antibodies against the capsule.

Flash animation showing phagocytosis of an encapsulated bacterium through opsonization.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing phagocytosis of an encapsulated bacterium through opsonization.
The Fab portion of IgG binds to epitopes of a capsule. The Fc portion can now attach the capsule to Fc receptors on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the encapsulated bacterium can be engulfed more efficiently and placed in a phagosome.

While the B-cell receptors of B-lymphocytes can recognize epitopes on polysaccharides, T4-lymphocytes can only recognize peptide epitopes bound to MHC-II molecules. The protein conjugate added to the polysaccharide in the vaccine is degraded into peptides and bound to MHC-II molecules by APCs. They then present the peptide to the TCRs on T4-lymphocytes for their activation. In this way the cytokines produced by the activated T4-lymphocytes become available for use by the B-lymphocytes sensitized to the polysaccharide component of the vaccine.

c. Examples of vaccines **produced by recombinant DNA technology** include:

- The hepatitis B vaccine, the first human vaccine produced by recombinant DNA technology, contains hepatitis B virus surface antigen (HBsAG);
- The acellular pertussis part of the diphtheria, tetanus, and acellular pertussis vaccine (DTaP) containing diphtheria toxoid, tetanus toxoid, and antigens from the whooping cough bacterium *Bordetella pertussis* (Acellular pertussis vaccines contain inactivated pertussis toxin (PT) and may contain one or more other bacterial components (e.g., filamentous hemagglutinin [FHA], an outer-membrane protein; pertactin [Pn], and fimbriae [Fim] types 2 and 3);
- The vaccine against Lyme disease;
- Gardasil, a vaccine against human papilloma virus (HPV) types 6, 11 that cause about 90% of genital warts, and types 16, and 18 responsible for around 70% of cervical cancer in the US; and Cervarix, a vaccine against HPV types 16 and 18. Both contain recombinant L1 capsid protein from the different strains of HPV;
- RV5, an oral vaccine against human rotavirus gastroenteritis. Capsid proteins from human rotaviruses have been expressed on the surface of harmless non-human rotavirus strains.

3. Toxoid

A toxoid is an exotoxin treated so as to be non-poisonous but still immunogenic. Examples of vaccines containing toxoids include:

- The diphtheria and tetanus components of the DTaP and Td vaccines.

The body responds by **making antibodies capable of neutralizing the exotoxin**.

Flash animation showing neutralization of an exotoxin.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing neutralization of an exotoxin.
The Fab portion of the antibodies made against epitopes of the binding site of an exotoxin blocks the exotoxin from binding to the host cell membrane. As a result, the toxin can not enter the cell and cause harm.

The antigen may be adsorbed to an **adjuvant**, a substance such as aluminum hydroxide or aluminum phosphate that is not immunogenic but enhances the immunogenicity of antigens.

Routine immunization practices protect more than just the individuals receiving the vaccine. When a critical portion of a community becomes immunized against a particular infectious disease, most members of the community - including those who were not immunized - are protected against that disease because there is little opportunity for an outbreak. This is known as **herd immunity or community immunity**.

b. Passive artificially acquired immunity

Passive artificially acquired immunity refers to the injection of antibody-containing serum, or immune globulin (IG) , from another person or animal.

Since the body is not making its own antibodies and memory cells are not produced, **passive artificially acquired immunity is short lived** and offers only immediate, short term protection. Also, **the injection of serum during passive immunization carries a greater risk of allergic reactions** than the injection of antigens during active immunization. These allergic reactions are referred to as **serum sickness** and will be discussed later under hypersensitivities.

Examples include:

- The use of pooled adult human immune globulin (IG) to prevent hepatitis A and measles and to prevent infections in people with certain immunodeficiency diseases;
- Human HBIG to prevent hepatitis B in those not actively immunized with the HepB vaccine;
- Human TIG to prevent tetanus in those not actively immunized with the DTP, DTaP, or Td vaccines;

- RhoGAM to prevent Rh hemolytic disease of newborns;
- VZIG to prevent varicella;
- CMV-IGIV to prevent cytomegalovirus infections in highly immunosuppressed individuals;
- RIG to prevent rabies, given concurrently with active immunization with the rabies vaccine;
- The antisera used for botulism; and
- IVIG (intravenous immune globulin), now being used to reduce infections in people with certain immunosuppressive diseases such as primary immunodeficiency syndrome and chronic lymphocytic leukemia as well as to treat certain autoimmune diseases such as immune thrombocytopenia purpura (ITP) and Kawasaki disease.

Tetanus provides a nice example of how active immunization (DTaP) and passive immunization (TIG) may be used in preventing a disease **(see Fig. 3)**.

Fig. 3: Tetanus prophylaxis in Routine Wound Management

History of tetanus toxoid doses	Clean, minor wound		All other wounds *(1)*	
	Td *(2)*	TIG *(3)*	Td	TIG
Unknown or < 3	Yes	No	Yes	Yes
Three or more	No *(4)*	No	No *(5)*	No

*(1)*Such as, but not limited to, wounds contaminated with dirt, feces, soil, saliva, etc.: puncture wounds, avulsions, and wounds resulting from missiles, crushing, burns, and frostbite.
(2) Tetanus toxoid, diphtheria toxoid (active immunization).
(3) Tetanus Immune Globulin (passive immunization).
(4) Yes, if more than 10 years since last dose.
(5) Yes, if more than 5 years since last dose. (More frequent boosters are not needed and can accentuate side effects.)

There is also some early evidence that immunization may be of value in the **treatment of some infections** as well as in their prevention, possibly by **supercharging the immune system** of those already infected. Vaccine therapies in various stages of testing include those against diseases such as herpes, leprosy, tuberculosis, and hepatitis B.

Concept Map for Active and Passive Immunity

TPS Question: Active and Passive Immunity

Quiz Group

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ADAPTIVE IMMUNITY
CELL-MEDIATED IMMUNITY: AN OVERVIEW

Adaptive Immunity

Cell-Mediated Immunity: An Overview

Fundamental Statements for this Softchalk Lesson:

1. Cell-mediated immunity (CMI) is an immune response that does not involve antibodies but rather involves the activation of macrophages and NK-cells, the production of antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.
2. Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in destroying virus-infected cells, intracellular bacteria, and cancers.
3. In a manner similar to B-lymphocytes, T-lymphocytes are able to randomly cut out and splice together different combinations of genes along their chromosomes through a process called gene translocation. This is known as combinatorial diversity and results in each T-lymphocyte generating a unique T-cell receptor (TCR).
4. During gene translocation, specialized enzymes in the T-lymphocyte cause splicing inaccuracies wherein additional nucleotides are added or deleted at the various gene junctions. This change in the nucleotide base sequence generates even greater diversity in the shape of the TCR. This is called junctional diversity.
5. As a result of combinatorial diversity and junctional diversity, each T-lymphocyte is able to produce a unique shaped T-cell receptor (TCR) capable of reacting with complementary-shaped peptide bound to a MHC molecule.
6. As a result of T-lymphocytes recognizing epitopes of protein antigens during cell-mediated immunity, numerous circulating T8-memory cells and T4-memory cells) develop which possess anamnestic response or memory.
7. A subsequent exposure to that same antigen results in a more rapid and longer production of cytotoxic T-lymphocytes (CTLs), and a more rapid and longer production of T4-effector lymphocytes.
8. When an antigen encounters the immune system, epitopes from protein antigens bound to MHC-I or MHC-II molecules eventually will react with a naive T4- and T8-lymphocyte with TCRs and CD4 or CD8 molecules on its surface that more or less fit and this activates that T-lymphocyte. This process is known as clonal selection.
9. Cytokines produced by effector T4-helper lymphocytes enable the now activated T4- and T8-lymphocyte to rapidly proliferate to produce large clones of thousands of identical T4- and T8-lymphocytes. This is referred to as clonal expansion.

Common Course Objectives

1. Explain how TCR and BCR diversity is achieved.
2. Describe how T-cells and B-cells acquire memory.
3. Briefly describe the process of clonal selection and clonal expansion.

Detailed Learning Objectives

1. Briefly compare humoral immunity with cell-mediated immunity.

2.* Define cell-mediated immunity and state what it is most effective against.

3.* State three different ways by which cell-mediated immunity protects the body.

4.* Define gene translocation and relate it to each T-lymphocyte being able to produce T-cell receptor with a unique shape.

5. Define the following:

a. combinatorial diversity

b. junctional diversity

6*. In terms of cell-mediated immunity, state what is meant by anamnestic response and discuss its role in immune defense.

7. Briefly describe why there is a heightened secondary response during anamnestic response.

(*) = common theme throughout the course

Cell-Mediated Immunity: An Overview

Cell-mediated immunity (CMI) is an immune response that **does not involve antibodies** but rather involves **the activation of macrophages and NK cells, the production of antigen-specific cytotoxic T-lymphocytes**, **and the release of various cytokines** in response to an antigen. Cellular immunity protects the body by:

1. Activating **antigen-specific cytotoxic T-lymphocytes (CTLs)** that are able to **destroy body cells displaying epitopes of foreign antigen on their surface**, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;

2. **Activating macrophages and NK cells, enabling them to destroy intracellular pathogens**; and

3. Stimulating cells to **secrete a variety of cytokines** that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in **destroying virus-infected cells, intracellular bacteria, and cancers. It also plays a major role in delayed transplant rejection.**

1. Generation of T-cell receptor (TCR) diversity through gene translocation

As mentioned earlier, the immune system of the body has no idea as to what antigens it may eventually encounter. Therefore, it has evolved a system that possesses the capability of responding to any conceivable antigen. The immune system can do this because both B-lymphocytes and T-lymphocytes have evolved a unique system of gene-splicing called **gene translocation** , a type of gene-shuffling process where various different genes along a chromosome move and join with other genes from the chromosome.

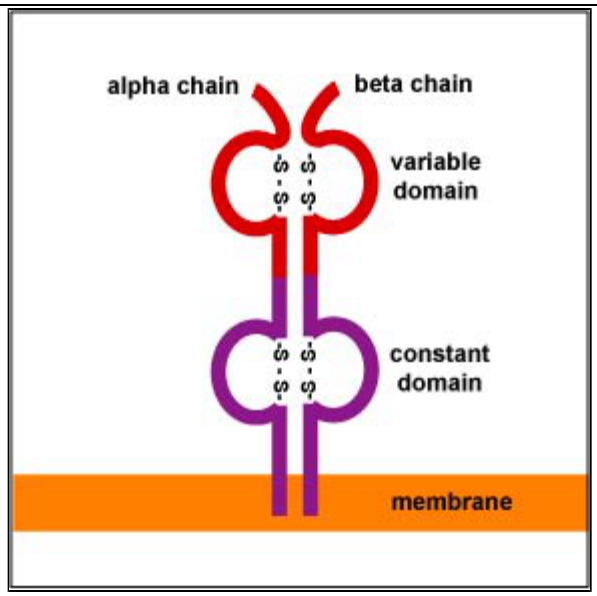
To demonstrate this gene translocation process, we will look at how each T-lymphocyte becomes genetically programmed to produce a T-cell receptor (TCR) having a unique shape to fit a specific epitope.

In a manner similar to B-lymphocytes, T-lymphocytes are able to cut out and splice together different combinations of genes along their chromosomes. **Through random gene translocation, any combination of the multiple forms of each gene can join together**.This is known as combinatorial diversity.

The T-cell receptors or TCRs **(see Fig. 1)** of most T-lymphocytes involved in adaptive immunity consist of an alpha (a) and a beta (ß) chain. There are 70-80 different V genes and 61 different J genes that code for the variable portion of the a chain of

the TCR. Likewise, there are 52 $V_{ß}$ genes, 1 $D_{ß1}$ gene, 1 $D_{ß2}$ gene, and 6-7 $J_{ß}$ genes that can recombine to form the variable portion of the TCR.

Fig. 1: Illustration of the T-Cell Receptor



Most T-cell receptors are composed of two polypeptide chains, an alpha (a) chain and a beta (ß) chain. The variable domains of the a and ß chains (red) provide specificity for binding a peptide bound to a MHC molecule. The terminus of the constant domains (purple) anchors the receptor to the cytoplasmic membrane of the T-lymphocyte. (S-S = disulfide bond).

During gene translocation, specialized enzymes in the T-lymphocyte cause **splicing inaccuracies wherein additional nucleotides are added or deleted at the various gene junctions**. This change in the nucleotide base sequence generates even greater diversity in Fab shape. This is called junctional diversity. Unlike the BCR, somatic hypermutation does not occur during the production of the TCRs.

As a result of combinatorial diversity and junctional diversity, each T-lymphocyte is able to produce a unique shaped T-cell receptor (TCR) capable of reacting with complementary-shaped peptide bound to a MHC molecule.

2. Anamnestic Response (Memory)

As a result of T-lymphocytes recognizing epitopes of protein antigens during cell-mediated immunity, numerous circulating **T8-memory cells** and T4-memory cells develop which possess **anamnestic response** or memory. These T-memory cells can persist for the remainder of a person's life.

Effector memory T-cells (T_{EM} cells) circulate in the blood whereas tissue resident memory T-cells (T_{RM} cells) are found within the epithelium of the skin and mucous membranes. CD8 T_{RM} cells are typically activated by viral antigens and subsequently produce inflammatory cytokines that trigger an innate immune response for nonspecific antiviral activity. CD4 T_{RM} cells are found in clusters surrounding macrophages in the mucosa. Unlike T_{EM} cells, T_{RM} cells do not circulate in the blood and are not replenished from the blood. They remain in peripheral tissues.

A subsequent exposure to that same antigen results in:

- **A more rapid and longer production of cytotoxic T-lymphocytes (CTLs); and**
- **A more rapid and longer production of T4-effector lymphocytes**
- **Triggering of nonspecific innate immune responses**.

3. Clonal Selection and Clonal Expansion

As mentioned above, during early differentiation of naive T-lymphocytes in the thymus marrow, **each T4-lymphocyte and each T8-lymphocyte becomes genetically programmed to make a T-cell receptor or TCR with a unique shape** through a series of gene translocations, and **molecules of that TCR are put on its surface of that T-lymphocyte to function as its epitope receptor**. When an antigen encounters the immune system, epitopes from protein antigens bound to MHC-I or MHC-II molecules eventually will react with a naive T4- and T8-lymphocyte with TCRs and CD4 or CD8 molecules on its surface that more or less fit and this activates that T-lymphocyte. This process is known as **clonal selection**.

Cytokines produced by effector T4-helper lymphocytes **enable the now activated T4- and T8-lymphocyte to rapidly proliferate to produce large clones of thousands of identical T4- and T8-lymphocytes**. In this way, even though only a few T-lymphocytes in the body may have TCR molecule able to fit a particular epitope, eventually many thousands of cells are produced with the right specificity. This is referred to as **clonal expansion**. These cells then differentiate into effector T4-lymphocytes and cytotoxic T-lymphocytes or CTLs.

Cellular immunity is also the mechanism behind **delayed hypersensitivity** (discussed later in this unit). Delayed hypersensitivity is generally used to refer to the harmful effects of cell-mediated immunity (tissue and transplant rejections, contact dermatitis, positive skin tests like the PPD test for tuberculosis, granuloma formation during tuberculosis and deep mycoses, and destruction of virus-infected cells).

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ADAPTIVE IMMUNITY
CELL-MEDIATED IMMUNITY: ACTIVATING ANTIGEN-SPECIFIC CYTOTOXIC T-LYMPHOCYTES (CTLs)

Adaptive Immunity

Cell-Mediated Immunity: Activating Antigen-Specific Cytotoxic T-Lymphocytes (CTLs)

Fundamental Statements for this Softchalk Lesson:

1. Cell-mediated immunity (CMI) is an immune response that does not involve antibodies but rather involves the activation of macrophages and NK-cells, the production of antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen.
2. Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells.
3. One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs, effector cells derived from naïve T8-lymphocytes during cell-mediated immunity.
4. The TCRs and CD8 molecules on the surface of naive T8-lymphocytes are designed to recognize peptide epitopes bound to MHC-I molecules on antigen-presenting cells (APCs).
5. During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins (endogenous antigens) in the cytosol of that cell are degraded into a variety of peptide epitopes by cylindrical organelles called proteasomes.
6. These peptide epitopes bind to MHC-I molecules being synthesized in the endoplasmic reticulum which are eventually transported to the cytoplasmic membrane of that cell.
7. During cell-mediated immunity, MHC-I molecule with bound peptide on the surface of infected cells and tumor cells can be recognized by a complementary-shaped TCR/CD8 on the surface of a cytotoxic T-lymphocyte (CTL) to initiate destruction of the cell containing the endogenous antigens.
8. When the TCR and CD8 of the CTL binds to the MHC-I/epitope on the surface of the virus-infected cell or tumor cell, this triggers the release of cytotoxic perforins/granzymes/ granulysin granules from the CTL that lead to apoptosis, a programmed cell suicide of that cell.
9. Cell death by apoptosis does not result in the release of cellular contents such as inflammatory mediators or viruses as occurs during immune-induced cell lysis.
10. During apoptosis, the cell breaks into membrane-bound apoptotic fragments that are subsequently removed by macrophages.

Common Course Objectives

1. Compare and contrast how T4-cells and T8-cells are activated.

2. Describe how activated cytotoxic T-cells kill target cells.

Detailed Learning Objectives

1.* In terms of the role of cytotoxic T-lymphocytes (CTLs) in body defense:

   a. State from what cells cytotoxic T-lymphocytes are derived.

   b. Describe how they can react with and destroy virus-infected cells, cells containing intracellular bacteria, and cancer cells without harming normal cells. (Indicate the role of following: TCR, CD4, MHC-I, and peptides from endogenous antigens.)

c. State the mechanism by which cytotoxic T-lymphocytes kill the cells to which they bind. (Indicate the role of the following: perforins, granzymes, caspases, and macrophages in the process.)

2. Briefly describe two ways certain viruses may evade cell-mediated immunity.

(*) = common theme throughout the course

TPS Questions: Cytotoxic T-lymphocytes (CTLs)

Cell-Mediated Immunity: Activating Antigen-Specific Cytotoxic T-Lymphocytes (CTLs)

Cell-mediated immunity (CMI) is an immune response that **does not involve antibodies** but rather involves **the activation of macrophages and NK cells, the production of antigen-specific cytotoxic T-lymphocytes**, **and the release of various cytokines** in response to an antigen. Cellular immunity protects the body by:

1. Activating **antigen-specific cytotoxic T-lymphocytes (CTLs)** that are able to **destroy body cells displaying epitopes of foreign antigen on their surface**, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;

2. **Activating macrophages and NK cells, enabling them to destroy intracellular pathogens**; and

3. Stimulating cells to **secrete a variety of cytokines** that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in **destroying virus-infected cells, intracellular bacteria, and cancers. It also plays a major role in delayed transplant rejection.**

In this section we will look at how cell-mediated immunity helps to defend the body by way of cytotoxic T-lymphocytes.

A. Marking an Infected Cell or a Tumor Cell for Destruction by Cytotoxic T-Lymphocytes (CTLs)

One of the body's major defenses against viruses, intracellular bacteria, and cancers is the destruction of infected cells and tumor cells by cytotoxic T-lymphocytes or CTLs. These CTLs are effector cells **derived from naive T8-lymphocytes** during cell-mediated immunity. Both T8-lymphocytes and CTLs produce T-cell receptors or TCRs and CD8 molecules that are anchored to their surface.

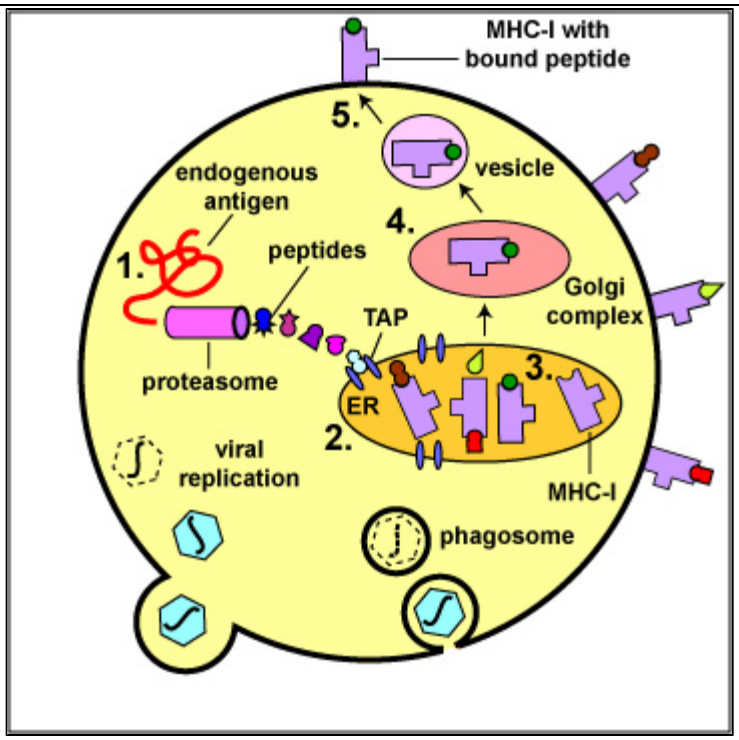
a. The TCRs and CD8 molecules on the surface of naive T8-lymphocytes are designed to recognize peptide epitopes bound to MHC-I molecules on antigen-presenting cells or APCs.

b. The TCRs and CD8 molecules on the surface of cytotoxic T-lymphocytes (CTLs) are designed to recognize peptide epitopes bound to MHC-I molecules on infected cells and tumor cells.

During the replication of viruses and intracellular bacteria within their host cell, as well as during the replication of tumor cells, viral, bacterial, or tumor proteins in the cytosol of that cell are degraded into a variety of peptide epitopes by cylindrical organelles called **proteasomes**. Other endogenous antigens such as proteins released into the cytosol from the phagosomes of antigen-presenting cells, such as macrophages and dendritic cells as well, as a variety of the human cell's own proteins (self-proteins) are also degraded by proteasomes. As these various endogenous antigens pass through proteasomes,

proteases and peptidases chop the protein up into a series of peptides, typically 8-11 amino acids long **(see Fig. 1)**.

Fig. 1: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules



Endogenous antigens are those located within the cytosol of cells of the body. Examples include:

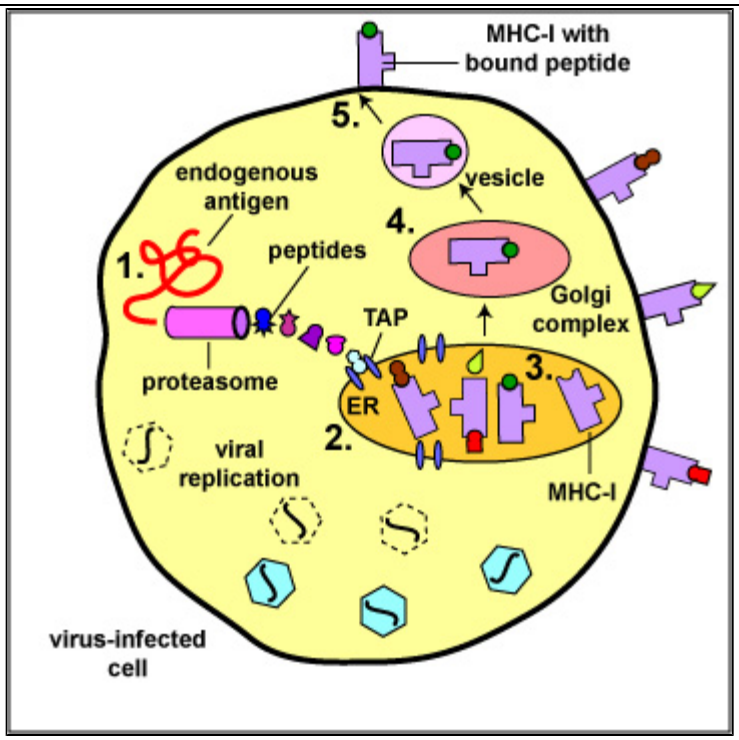
a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. Endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

A transporter protein called **TAP** located in the membrane of the cell's endoplasmic reticulum then **transports these peptide epitopes into the endoplasmic reticulum where they bind to the grooves of various newly made MHC-I molecules**. The MHC-I molecules with bound peptides are then transported to the Golgi complex and placed in exocytic vesicles. The **exocytic vesicles carry the MHC-I/peptide complexes to the cytoplasmic membrane of the cell where they become anchored to its surface (see Fig. 2)**. A single cell may have up to 250,000 molecules of MHC-I with bound epitope on its surface.

Fig. 2: Binding of Peptide Epitopes from Endogenous Antigens to MHC-I Molecules by a Virus-Infected Cell



The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

1. During viral replication within the host cell, endogenous antigens, such as viral proteins, pass through proteasomes where they are degraded into a series of peptides.
2. The peptides are transported into the rough endoplasmic reticulum (ER) by a transporter protein called TAP.
3. The peptides then bind to the grooves of newly synthesized MHC-I molecules.
4. The endoplasmic reticulum transports the MHC-I molecules with bound peptides to the Golgi complex.
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

Flash animation of MHC-I molecules binding epitopes from endogenous antigens in an infected cell.

html5 version of animation for iPad showing MHC-I molecules binding epitopes from endogenous antigens in an infected cell.

Endogenous antigens are those located within the cytosol of the cells of the body. Examples include:

a. viral proteins produced during viral replication,
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication,
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells
d. tumor antigens produced by cancer cells,
e. and self peptides from human cell proteins.

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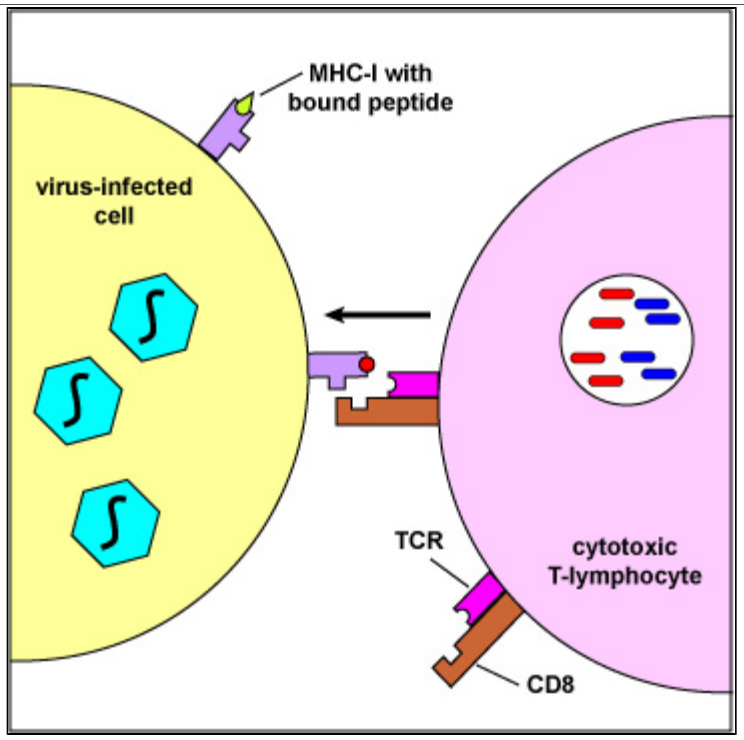
5. The Golgi complex, in turn, transports the MHC-I/peptide complexes by way of an exocytic vesicle to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-I/peptide complexes can be recognized by CTLs by way of TCRs and CD8 molecules having a complementary shape.

During cell-mediated immunity, **MHC-I molecule with bound peptide on the surface of** infected cells and tumor cells **can be recognized by a complementary-shaped TCR/CD8 on the surface of** a cytotoxic T-lymphocyte (CTL) to initiate destruction of the cell containing the endogenous antigen **(see Fig. 3)**.

Fig. 3: A Cytotoxic T-lymphocyte Recognizing a Virus-Infected Cell



Endogenous antigens are those being produced within cells of the body. Examples include proteins from replicating viruses, proteins from intracellular bacteria, and tumor antigens. The body marks infected cells and tumor cells for destruction by placing peptide epitopes from these endogenous antigens on their surface by way of MHC-I molecules. Cytotoxic T-lymphocytes (CTLs) are then able to recognize peptide/MHC-I complexes by means of their T-cell receptors (TCRs) and CD8 molecules and kill the cells to which they bind.

Animation illustrating the MHC-I system marking an infected cell for destruction and its subsequent killing by CTLs.

Courtesy of HHMI's Biointeractive.

Movie of a CTL inducing apoptosis of an infected cell.

Courtesy of HHMI's Biointeractive.

Concept Map for Ways in which Cell-Mediated Immunity Protects the Body

For more information: Review of MHC molecules

For more information: Review of T8-lymphocytes

B. Cytotoxic T-Lymphocyte (CTL) Destruction of Body Cells Displaying Epitopes of Foreign Antigen on their Surface

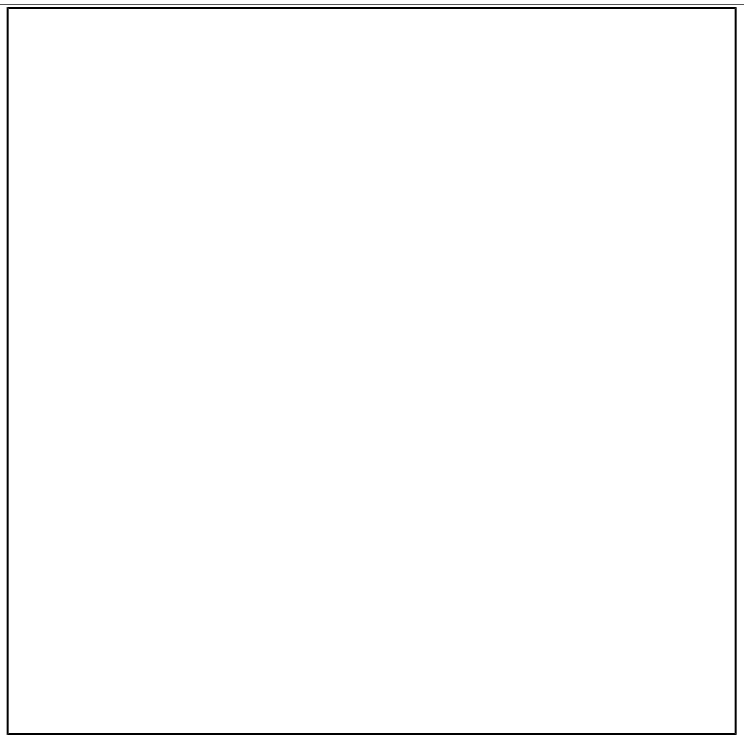
The **cytotoxic T-lymphocytes (CTLs)** produced during cell-mediated immunity are designed to remove body cells displaying "foreign" epitope, such as virus-infected cells, cells containing intracellular bacteria, and cancer cells with mutant surface proteins. The CTLs are able to kill these cells by inducing a **programmed cell death known as apoptosis**.

Using virus-infected cells as an example, the CTLs circulate throughout the body where they encounter virus-infected cells and induce apoptosis. This involves involves a complex of intracellular cytotoxic granules containing:

1. Pore-forming proteins called perforins;
2. Proteolytic enzymes called granzymes; and
3. Granulysin.

When the TCR and CD8 of the CTL binds to the MHC-I/epitope on the surface of the virus-infected cell or tumor cell **(see Fig. 4)**, this sends a signal through a CD3 molecule which triggers the release of the cytotoxic perforins/granzymes/granulysin granules from the CTL.

Fig. 4: Cytotoxic T-lymphocyte (CTL)-Induced Apoptosis of a Virus-Infected Cell



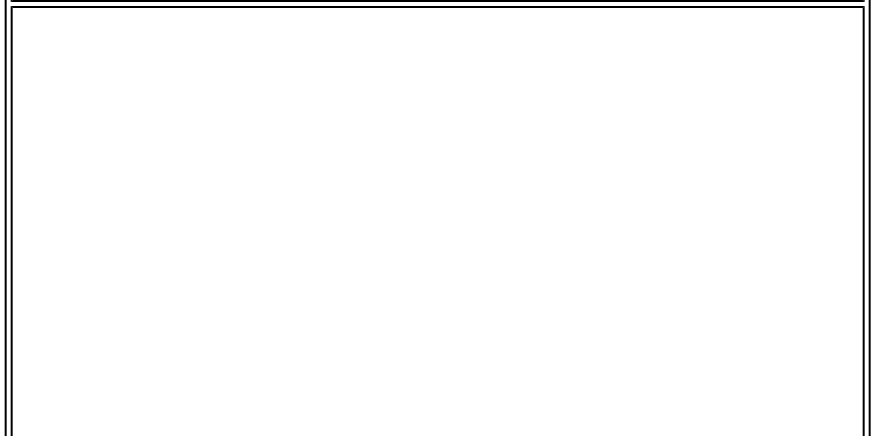
Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the caspase enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes.

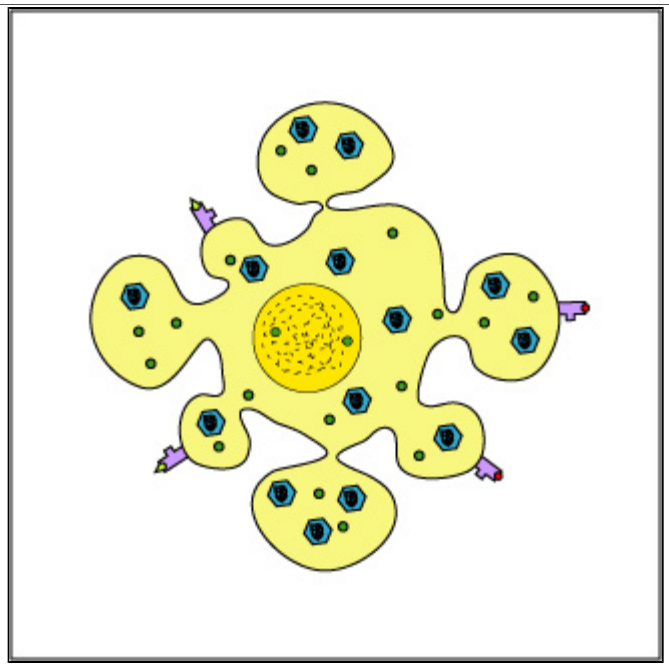
The exact mechanism of entry of the granzymes into the infected cell or tumor cell is still debated. It is, however, dependent on perforins. Possibilities include:

1. The perforins/granzymes/granulysin complex may be taken into the target cell by receptor-mediated endocytosis. The perforin molecules may then act on the endosomal membrane allowing granzymes to enter the cytosol.

2. The perforin molecules may put pores in the membrane of the target cell allowing the granzymes to directly enter the cytosol **(see Fig. 5)**.

Fig. 5: CTL-Induced Apoptosis of a Virus-Infected Cell





Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the perforin pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. This prevents the release of any mature viruses that might occur if the infected cell was lysed.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

1. **Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell**. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and nucleases that degrade both the target cell's nucleoprotein and any microbial DNA within the cell **(see Fig. 5)**.
2. Granzymes cleave a variety of other cellular substrates that contribute to cell death.
3. The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by the membrane attack complex (MAC) of the complement pathways. This can **increase the permeability of the infected cell and contribute to cell death**. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis.
4. Granulysin has antimicrobial actions and can also induce apoptosis.

Flash animation of a CTL triggering apoptosis by way of perforins and granzymes.

html5 version of animation for iPad showing a CTL triggering apoptosis by way of perforins and granzymes.

Binding of the CTL to the infected cell triggers the CTL to release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.)

Apoptosis occurs when certain granzymes activate a group of protease enzymes called

caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation showing CTL-induced apoptosis of a virus-infected cell.

html5 version of animation for iPad showing CTL-induced apoptosis of a virus-infected cell.

Killing of the infected cell or tumor cell by apoptosis involves a variety of mechanisms:

1. Certain granzymes can activate the caspase enzymes that lead to apoptosis of the infected cell. The caspases are proteases that destroy the protein structural scaffolding of the cell - the cytoskeleton - and degrade both the target cell's nucleoprotein and microbial DNA within the cell.
2. Granzymes cleave a variety of other cellular substrates that contribute to cell death.
3. The perforin molecules may also polymerize and form pores in the membrane of the infected cell, similar to those produced by MAC. This can increase the permeability of the infected cell and contribute to cell death. If enough perforin pores form, the cell might not be able to exclude ions and water and may undergo cytolysis. A granule called granulysin can also alter the permeability of both miocrobial and host cell membranes.

This animations shows destruction of both the cytoskeleton and nucleoprotein of the infected cell. As the infected cell breaks up into apoptotic fragments, the fragments are subsequently removed by phagocytes. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells.

Electron micrograph of a CTL binding to a tumor cell.

Electron micrograph showing a killed tumor cell.

Animation illustrating the MHC-I system marking an infected cell for destruction and its subsequent killing by CTLs.

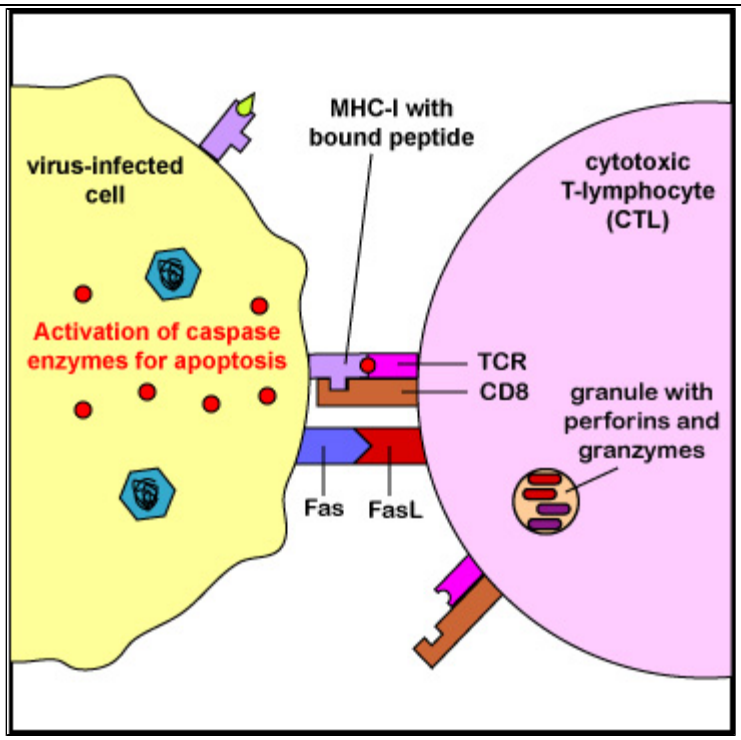
Courtesy of HHMI's Biointeractive.

Movie of a CTL inducing apoptosis of an infected cell.

Courtesy of HHMI's Biointeractive.

CTLs can also trigger apoptosis through FasL/Fas interactions. Activated lymphocytes express both death receptors called Fas and Fas ligand or FasL **(see Fig. 6)** on their surface. This FasL/Fas interaction triggers an intracellular transduction that activates the caspase enzymes that lead to apoptosis. In this way, CTLs can kill other lymphocytes and terminate lymphocyte proliferation after the immune responses have eradicated an infection.

Fig. 6: A Cytotoxic T-Lymphocyte (CTL) Inducing Apoptosis by Fas/FasL Interaction



A cytotoxic T-lymphocyte (CTL), by way of its TCR and CD8, can bind to MHC-I/epitope on a virus-infected and cause apoptosis by way of perforins and granzymes. In addition, a Fas ligand (FasL) on the CTL can bind to a Fas molecule on the surface of many cell types and activate the enzymes that lead to apoptosis. Most likely this mechanism is used to remove effector lymphocytes once they are no longer needed after an adaptive immune response.

Death by apoptosis does not result in the release of cellular contents such as inflammatory mediators or viruses as occurs during immune-induced cell lysis. Instead, the cell breaks into membrane-bound apoptotic fragments that are subsequently removed by macrophages. This reduces inflammation and also prevents the release of viruses that have assembled within the infected cell and their spread into uninfected cells. Since the CTLs are not destroyed in these reactions, they can function over and over again to destroy more virus-infected cells.

Concept Map for Ways in which Cell-Mediated

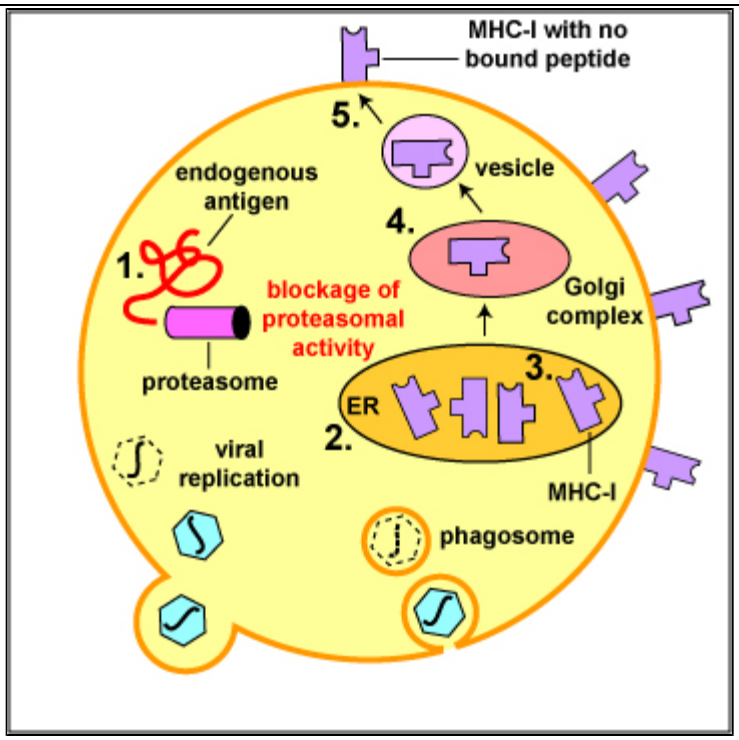
Immunity Protects the Body

TPS Questions: Cytotoxic T-lymphocytes (CTLs)

As with humoral immunity, certain microbes are able to evade to some degree cell-mediated immunity:

- Epstein-Barr virus (EBV) and cytomegalovirus (CMV) inhibit proteasomal activity so that viral proteins are not degraded into viral peptides **(see Fig. 7A)**.

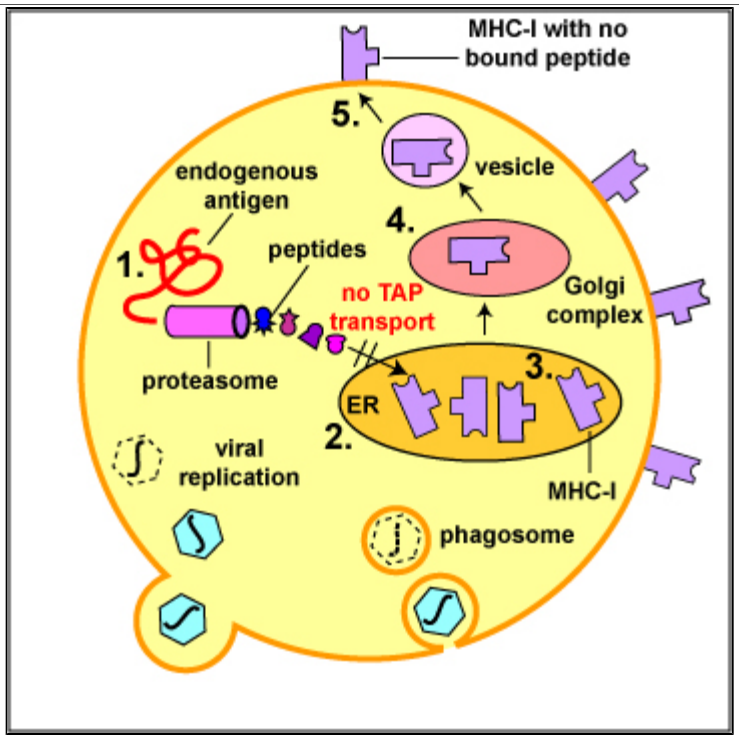
Fig. 7A: Inhibition of Proteasome Activity



Epstein-Barr virus (EBV) and cytomegalovirus (CMV) inhibit proteasomal activity so that viral proteins are not degraded into viral peptides.

- Herpes simplex viruses (HSV) can block the TAP transport of peptides into the endoplasmic reticulum **(see Fig. 7B)**.

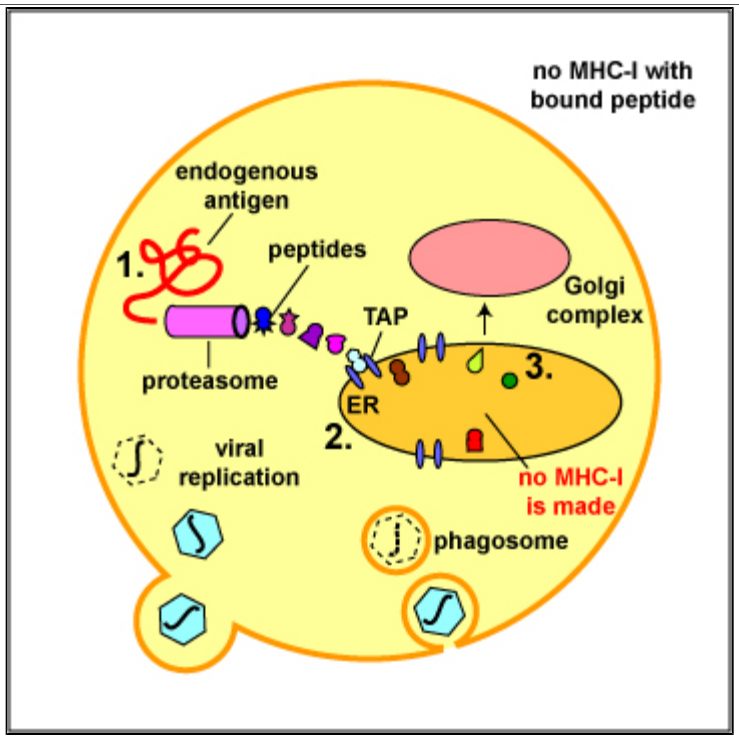
Fig. 7B: Blockage of TAP Transport of Peptides into the Endoplasmic Reticulum



Herpes simplex viruses (HSV) can block the TAP transport of peptides into the endoplasmic reticulum.

- Numerous viruses, such as the cytomegalovirus (CMV) and adenoviruses can block the formation of MHC-I molecules by the infected cell. As a result, no viral peptide is displayed on the infected cell and the CTLs are no longer able to recognize that the cell is infected and kill it **(see Fig. 7C)**.

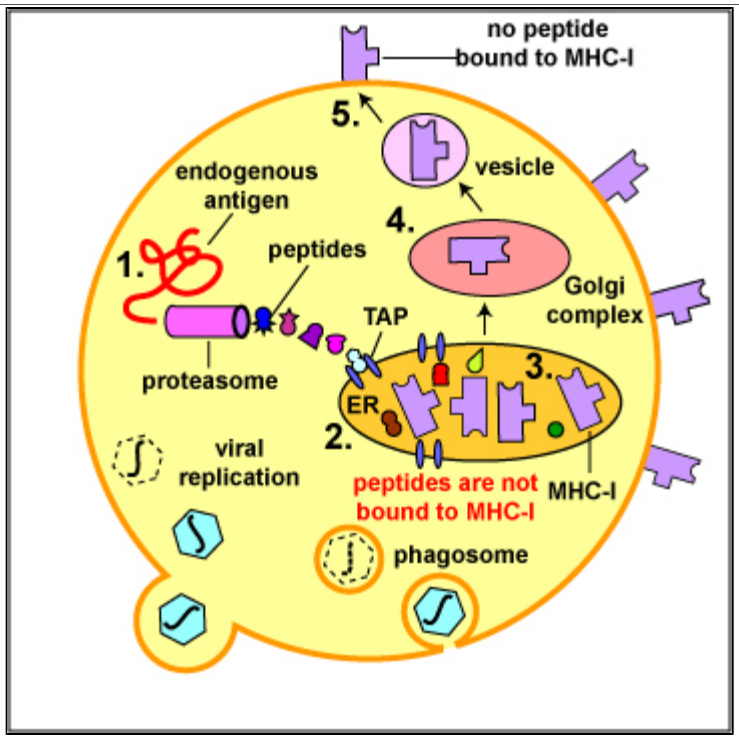
Fig. 7C: Suppression of the Synthesis of MHC-I Molecules



Numerous viruses, such as the cytomegalovirus (CMV) and adenoviruses can block the formation of MHC-I molecules by the infected cell. As a result, no viral peptide is displayed on the infected cell and the CTLs are no longer able to recognize that the cell is infected and kill it.

- Epstein-Barr virus (EBV) down regulates several host proteins involved in attaching viral epitopes to MHC-I molecules and displaying them on the host cell's surface **(see Fig. 7D)**.

Fig. 7D: Blockage of the Binding of Peptide Epitopes from Viruses to MHC-I Molecules



Epstein-Barr virus (EBV) down regulates several host proteins involved in attaching viral epitopes to MHC-I molecules and displaying them on the surface of the host cell. As a result, no viral peptide is displayed on the infected cell and the CTLs are no longer able to recognize that the cell is infected and kill it.

- Adenoviruses and Epstein-Barr Viruses (EBV) code for proteins that blocks apoptosis, the programmed cell suicide mechanism triggered by various defense mechanisms in order to destroy virus-infected cells.

Self Quiz for Activating Antigen-Specific Cytotoxic T-Lymphocytes (CTLs)

Quiz Group

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

ADAPTIVE IMMUNITY
CELL-MEDIATED IMMUNITY: ACTIVATING MACROPHAGES AND NK CELLS

Adaptive Immunity

Cell-Mediated Immunity: Activating Macrophages and NK Cells

Fundamental Statements for this Softchalk Lesson:

1. Effector T4-lymphocytes called T_H1 cells coordinate immunity against intracellular bacteria and promote opsonization by macrophages.
2. Cytokines produced by T_H1 cells promote cell-mediated immunity against intracellular pathogens by activating macrophages and enhancing their antimicrobial effectiveness, increasing the production of opsonizing and complement activating IgG that enhances phagocytosis, and promoting diapedesis and chemotaxis of macrophages to the infection site.
3. Activation of natural killer T-lymphocytes (NKT cells) produces large amounts of IFN-gamma to activate macrophages.
4. Cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) produced by T_H1 lymphocytes activate NK cells.
5. Activated NK cells kill cells to which antibody molecules have attached through a process called antibody-dependent cellular cytotoxicity (ADCC).
6. Activated NK cells also use a duel receptor system in determining whether to kill or not kill cells such as cancer cells and infected cells that are displaying stress molecules and are not producing MHC-I molecules.
7. NK cells kill infected cells and cancer cells by inducing apoptosis, a programmed cell suicide.

Common Course Objectives

1. Explain the role of macrophages, dendritic cells, and mast cells in immunity.
2. Describe the role of NK cells in innate and adaptive immunity.

Detailed Learning Objectives

1. Describe how T_H1 effector cells are able to interact with and activate macrophages.

2*. Describe how NK cells are able to recognize and destroy infected cells and cancer cells lacking MHC-I molecules.

(*) = common theme throughout the course

Cell-Mediated Immunity: Activating Macrophages and NK Cells

Cell-mediated immunity (CMI) is an immune response that **does not involve antibodies** but rather involves **the activation of macrophages and NK cells, the production of antigen-specific cytotoxic T-lymphocytes**, **and the release of various cytokines** in response to an antigen. Cellular immunity protects the body by:

1. Activating **antigen-specific cytotoxic T-lymphocytes (CTLs)** that are able to **destroy body cells displaying epitopes of foreign antigen on their surface**, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;

2. **Activating macrophages and NK cells, enabling them to destroy intracellular pathogens**; and

3. Stimulating cells to **secrete a variety of cytokines** that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in **destroying virus-infected cells, intracellular bacteria, and cancers. It also plays a major role in delayed transplant rejection.**

In this section we will look at how cell-mediated immunity helps to defend the body by way of activating macrophages and NK cells.

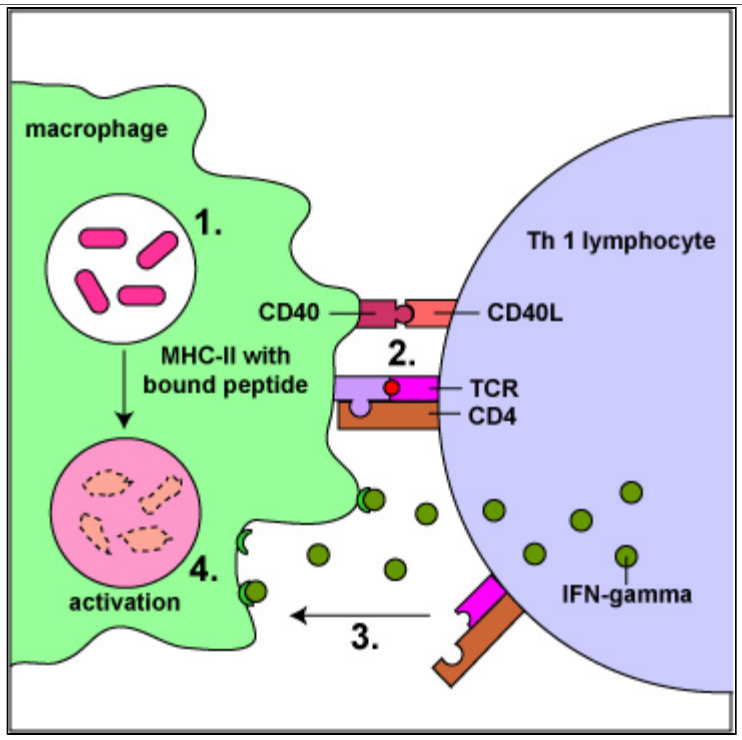
After interacting with APCs, some naive T4-lymphocytes differentiate into a subset of effector cells called T_H1 cells. T_H1 cells function primarily to **promote phagocytosis of microbes and the killing of intracellular microbes.**

a. Activation of Macrophages

Effector T4-lymphocytes called T_H1 cells coordinate immunity against intracellular bacteria and promote opsonization by macrophages.

1. **They produce cytokines such as interferon-gamma (IFN-?) that promote cell-mediated immunity against intracellular pathogens**, especially by activating macrophages that have either ingested pathogens or have become infected with intracellular microbes such as *Mycobacterium tuberculosis, Mycobacterium leprae, Leishmania donovani*, and *Pneumocystis jiroveci* that are able to grow in the endocytic vesicles of macrophages. **Activation of the macrophage by T_H1 cells greatly enhances their antimicrobial effectiveness (see Fig. 1)**.

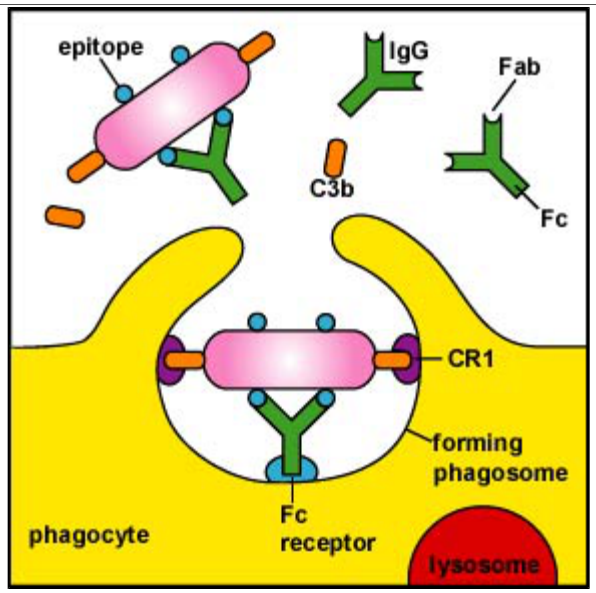
Fig. 1: Activation of a Macrophage by a T_H1 Lymphocyte



1. Engulfed bacteria inside a phagosome or a phagolysosome.
2. An activated T_H1 lymphocyte binds to a peptide/MHC-II complex on a macrophage by way of its TCR and CD4 molecule. Co-stimulatory molecules such as CD40L on the T_H1 cell then bind to CD40 on a macrophage.
3. This triggers the T_H1 lymphocyte to secrete the cytokine interferon-gamma (IFN-Î³) that binds to IFN-Î³ receptors receptors on the macrophage.
4. The IFN-Î³ activates the macrophage enabling it to produce more hydrolytic lysosomal enzymes, nitric oxide, and toxic oxygen radicals that destroy the microorganisms within the phagosomes and phagolysosomes.

2. They produce cytokines that **promote the production of increases the production of opsonizing and complement activating IgG that enhances phagocytosis (see Fig. 2)**.

Fig. 2: Illustration of Opsonization



The Fab portion of the antibody IgG binds to epitopes of an antigen such as this bacterium. The Fc portion of IgG then binds to Fc receptors on phagocytes for opsonization or enhanced attachment. Once attached to the phagocyte the microbe can be engulfed more efficiently and placed in a phagosome. Complement pathway proteins such as C3b and C4b can also function as opsonins.

3. They **produce receptors that bind to and kill chronically infected cells**, releasing the bacteria that were growing within the cell so they can be engulfed and killed by macrophages.

4. They produce cytokines such as tumor necrosis factor-alpha (TNF-a) that **promote diapedesis of macrophages**.

5. They **produce the chemokine** CXCL2 **to attract macrophages to the infection site**.

Activated natural killer T-lymphocytes (NKT cells) also produce large amounts of IFN-gamma to activate macrophages.

Flash animation of a macrophage processing an antigen for presentation to a TH1 cell.

html5 version of animation for iPad showing a macrophage processing an antigen for presentation to a T_H1 cell.

1. Exogenous antigens are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
5. The MHC-II molecules with bound Ii chain are now transported to the Golgi complex, and placed in vesicles.
6. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes.
7. The Ii chain is removed and the peptides are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane

where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

Flash animation of the activation of a macrophage by a T_H1 cell.

html5 version of animation for iPad showing the activation of a macrophage by a T_H1 cell.

A major function of T_h1 cells is to both promote phagocytosis of microbes and the killing of intracellular microbes.

1. Bacteria are engulfed by a macrophage and placed in a phagosome. A lysosome fuses with the phagosome forming a phagolysosome.
2.An activated T_h1 lymphocyte binds to a peptide/MHC-II complex on a macrophage by way of its TCR and CD4 molecule.
3.Co-stimulatory molecules such as CD40L on the T_h1 cell then bind to CD40 on a macrophage.
4. The T_h1 lymphocyte secretes the cytokine interferon-gamma (IFN-gamma) that binds to IFN-gamma receptors receptors on the macrophage.
5. The IFN-gamma activates the macrophage enabling it to produce more hydrolytic lysosomal enzymes, nitric oxide, and toxic oxygen radicals that destroy the microorganisms within the phagosomes and phagolysosomes.

Activation of macrophages:

a. **Increases their production of toxic oxygen radicals, nitric oxide, and hydrolytic lysosomal enzymes enabling the killing of microbes within their phagolysosomes**.

b. **Causes the macrophages to secrete cytokines such as TNF-a, IL-1, and IL-12. TNF-a and IL-1 promote inflammation to recruit phagocytic leukocyte**s. **IL-12 enables naive T4-lymphocytes to differentiate into T_H1 cells**.

c. Increases the production of B7 co-stimulator molecules and MHC-1 molecules by macrophages for increased T-lymphocyte activation.

Concept Map for Ways in which Cell-Mediated Immunity Protects the Body

For more information: Review of macrophages

For more information: Review of T4-lymphocytes

b. Activation of NK Cells

Cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) produced by T_H1 lymphocytes activate NK cells.

NK cells are another group of cytolytic lymphocytes, distinct from B-lymphocytes and T-lymphocytes, that participate in both innate immunity and adaptive immunity. NK cells are lymphocytes that lack B-cell receptors and T-cell receptors. They are **designed to kill certain mutant cells and virus-infected cells** in one of two ways:

1. **NK cells kill cells to which antibody molecules have attached** through a process called **antibody-dependent cellular cytotoxicity (ADCC)** as shown in **Slideshow Fig. 3A, Fig. 3B, and Fig. 3C**. The Fab portion of the antibody binds to epitopes on the "foreign" cell. The NK cell then binds to the Fc portion of the antibody. The NK cell is then able to contact the cell and by inducing a programmed cell suicide called apoptosis.

Slideshow Activity

Flash animation of ADCC contact by NK cells
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing ADCC contact by NK cells.
The Fab portion of the antibody IgG binds to epitopes on the foreign cell. The NK then releases pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. (Alternately, the granzymes and perforins may enter by endocytosis and the perforins then promote the release of the granzymes from the endocytic vesicle into the cytoplasm.) Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Flash animation of apoptosis by NK cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing apoptosis by NK cells.
NK cells release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

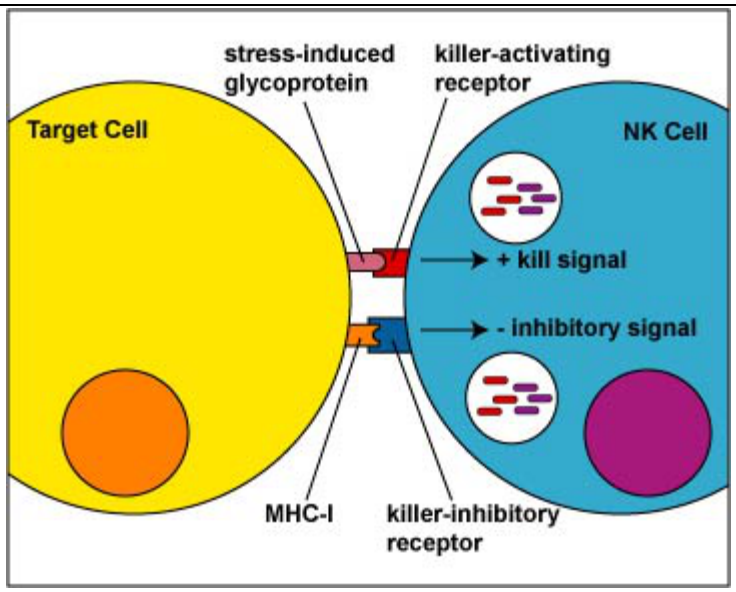
2. NK cells to use a **duel receptor system** in determining whether to kill or not kill human cells. **When cells are either under stress, are turning into tumors, or are infected, various molecules such as MICA and MICB are produced**

and are put on the surface of that cell.

The first receptor, called the **killer-activating receptor**, can **bind to various stress molecules such as MICA and MICB** that are produced and are put on the surface of that cell, and this **sends a positive signal that enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal**.

This second receptor, called the **killer-inhibitory receptor**, **recognizes MHC-I molecules that are also usually present on all nucleated human cells**. **If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell (see Fig. 4)**.

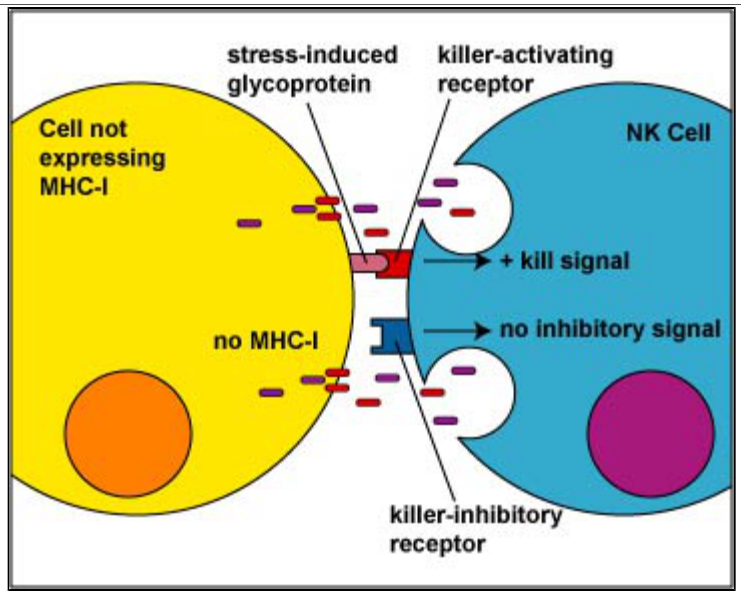
Fig. 4: NK Cell Interacting with a Normal Body Cell



NK cells appear to use a duel receptor system in determining whether to kill or not kill human cells. NK cells appear to use a duel receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound (see Fig. 7).

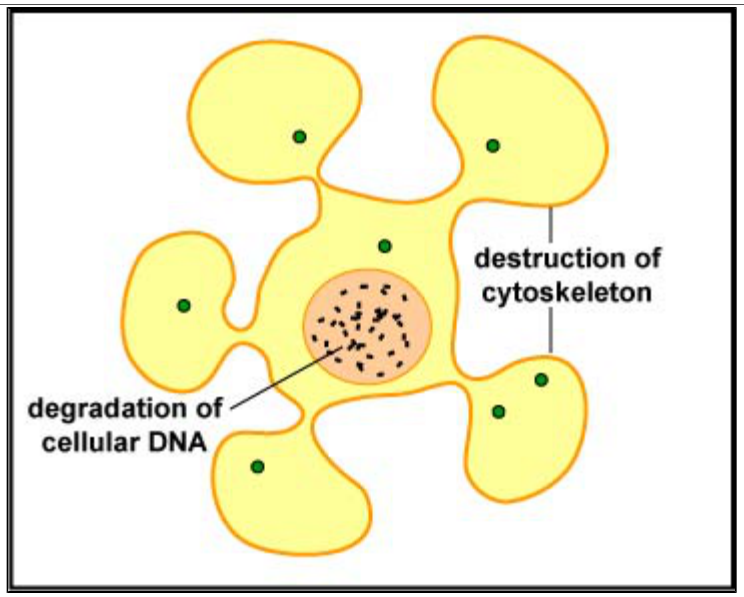
Fig. 5: NK Cell Interacting with a Virus-Infected Cell or a Mutant Cell Not Expressing MHC-I Molecules



When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. In addition, viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

The NK cell releases **pore-forming proteins called perforins and proteolytic enzymes called granzymes**. **Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation**. As a result, the cell breaks into fragments that are subsequently **removed by macrophages (see Fig. 8)**. Perforins can also sometimes result in cell lysis. The distinction between causing apoptosis versus causing cell lysis is important because lysing a virus-infected cell would only release the virions, whereas apoptosis leads to destruction of the virus inside.

Fig. 6: Apoptosis by NK Cells



Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound. The NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Flash animation of a NK cell interacting with a normal body cell.

html5 version of animation for iPad showing a NK cell interacting with a normal body cell.

NK cells appear to use a duel receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Flash animation of a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

html5 version of animation for iPad showing a NK cell interacting with a virus-

infected cell or tumor cell not expressing MHC-I molecules.

Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Flash animation of apoptosis by NK cells.

html5 version of animation for iPad showing apoptosis by NK cells.

NK cells release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

NK cells also produce a variety of cytokines, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses. For example, through cytokine production NK cells also suppress and/or activate macrophages, suppress and/or activate the antigen-presenting capabilities of dendritic cells, and suppress and/or activate T-lymphocyte responses.

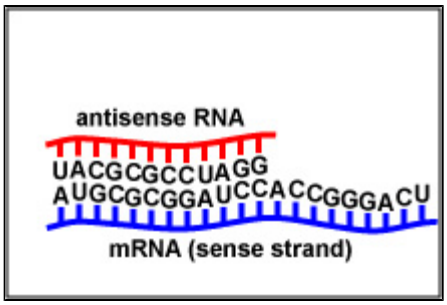
Concept Map for Ways in which Cell-Mediated Immunity Protects the Body

As with humoral immunity, certain microbes are able to evade to some degree NK cells:

- The cytomegalovirus (CMV) can also trigger its host cell to produce altered MHC-I molecules that are unable to bind viral epitopes, and, therefore, are not recognized by CTLs. However, NK cells are also unable to kill this infected cell because it is still displaying "MHC-I molecules" on its surface.

- CMV also produces microRNAs (miRNAs), small non-coding RNA molecules that down-regulates the production of stress-induced proteins that the killer-activating receptor of NK cells first recognizes. The miRNAs do this by binding to the host cell's mRNA coding for stress-induced proteins **(see Fig. 7)**. Without this binding there is no kill signal by the NK cell.

Fig. 7: Antisense RNA (microRNA or miRNA)



When an antisense RNA (microRNA or miRNA) that is complementary to a mRNA coding for a particular protein or enzyme binds to the mRNA by complementary base pairing, that mRNA cannot be translated and the protein or enzyme is not made.

- Cytomegalovirus (CMV) and herpes simplex type 1 virus (HSV-1) produce microRNAs (miRNAs), small non-coding RNA molecules that block protein involved in apoptosis, a programmed cell suicide. The miRNAs do this by binding to the host cell's mRNA coding for apoptosis-inducing proteins **(see Fig. 7)**.

Self Quiz for Activating Macrophages and NK Cells

Quiz Group

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

ADAPTIVE IMMUNITY
CELL-MEDIATED IMMUNITY: SIMULATING CELLS TO SECRETE CYTOKINES

Adaptive Immunity

Cell-Mediated Immunity: Stimulating Cells to Secrete Cytokines

Fundamental Statements for this Softchalk Lesson:

1. Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems.
2. Cytokines are pleiotropic, meaning that a particular cytokine can act on a number of different types of cells rather than a single cell type.
3. Cytokines are redundant, meaning that a number of different cytokines to carry out the same function.
4. Cytokines are multifunctional, meaning the same cytokine is able to regulate a number of different functions.
5. There are three functional categories of cytokines: Cytokines that regulate innate immune responses; cytokines that regulate adaptive Immune responses; and cytokines that stimulate hematopoiesis.
6. Type I interferons provide an early innate immune response against viruses. Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

Common Course Objectives

1. Explain what cytokines are and their role in immunity.
2. Recall the 4 different types of cytokines and predict what type of response would occur when released.

Detailed Learning Objectives

1.* Define cytokine and explain what is meant by "cytokines are pleiotropic, redundant, and multifunctional."

2. Name 3 cytokines that regulate innate immune responses by triggering an inflammatory response.

3.* Name the group of cytokines that regulates innate immunity by preventing translation of viral mRNA and by degrading both viral and host cell RNA.

3. Name 4 cytokines that regulate adaptive immune responses.

4. Name 2 cytokines that stimulate hematopoiesis.

(*) = common theme throughout the course

Cell-Mediated Immunity: Stimulating Cells to Secrete Cytokines

Cell-mediated immunity (CMI) is an immune response that **does not involve antibodies** but rather involves **the activation**

of macrophages and NK cells, the production of antigen-specific cytotoxic T-lymphocytes, **and the release of various cytokines** in response to an antigen. Cellular immunity protects the body by:

1. Activating **antigen-specific cytotoxic T-lymphocytes (CTLs)** that are able to **destroy body cells displaying epitopes of foreign antigen on their surface**, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens;

2. **Activating macrophages and NK cells, enabling them to destroy intracellular pathogens**; and

3. Stimulating cells to **secrete a variety of cytokines** that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in **destroying virus-infected cells, intracellular bacteria, and cancers. It also plays a major role in delayed transplant rejection.**

In this section we will look at how cell-mediated immunity helps to defend the body by way of activating macrophages and NK cells.

Cytokines are **low molecular weight, soluble proteins that function as chemical messengers for regulating the innate and adaptive immune systems**. They are produced by virtually all cells involved in innate and adaptive immunity, but especially by T helper (T_H) lymphocytes. The activation of cytokine-producing cells triggers them to synthesize and secrete their cytokines. The **cytokines**, in turn, are then able to **bind to specific cytokine receptors on other cells of the immune system and influence their activity in some manner**.

Cytokines are pleiotropic, redundant, and multifunctional.

- **Pleiotropic** means that a particular cytokine can act on a number of different types of cells rather than a single cell type.
- **Redundant** refers to to the ability of a number of different cytokines to carry out the same function.
- **Multifunctional** means the same cytokine is able to regulate a number of different functions.

Some cytokines are antagonistic in that one cytokine stimulates a particular defense function while another cytokine inhibits that function. Other cytokines are synergistic wherein two different cytokines have a greater effect in combination than either of the two would by themselves.

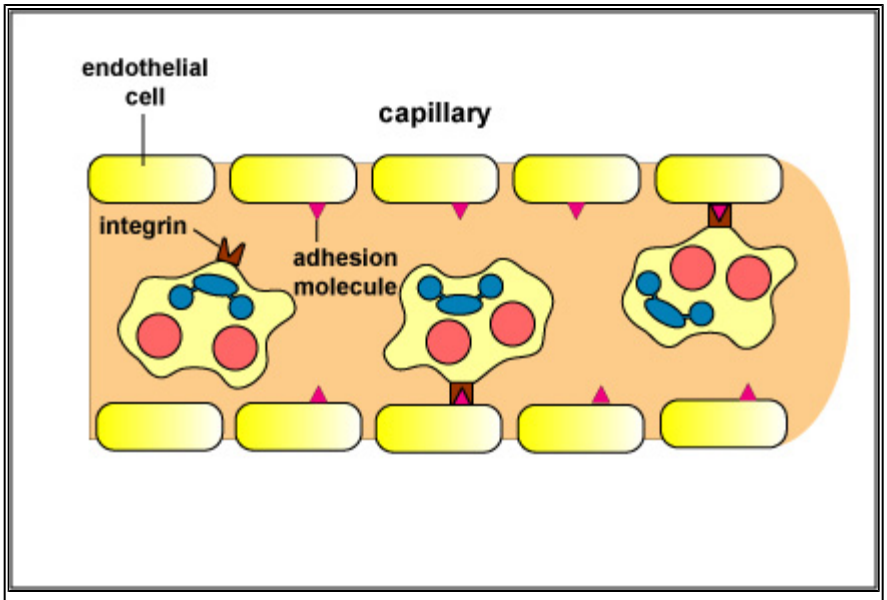
There are **three functional categories of cytokines**:

1. Cytokines that **regulate innate immune responses**,
2. Cytokines that **regulate adaptive immune responses**, and
3. Cytokines that **stimulate hematopoiesis**.

a. Cytokines that Regulate Innate Immunity

Cytokines that regulate innate immunity are produced primarily by mononuclear phagocytes such as macrophages and dendritic cells, although they can also be produced by T-lymphocytes, NK cells, endothelial cells, and mucosal epithelial cells. They are produced primarily in response to pathogen-associated molecular patterns (PAMPs) such as LPS, peptidoglycan monomers, teichoic acids, unmethylated cytosine-guanine dinucleotide or CpG sequences in bacterial and viral genomes, and double-stranded viral RNA. Cytokines produced in response to pattern-recognition receptors (PRRs) on cell surfaces, such as the inflammatory cytokines IL-1, IL-6, IL-8, and TNF-alpha, mainly act on leukocytes and the endothelial cells that form blood vessels in order to promote and control early inflammatory responses **(see Fig. 1)**. Cytokines produced in response to PRRs that recognize viral nucleic acids, such as type I interferons, primarily block viral replication within infected host cells **(see Slideshow Figs. 2A and 2B)**.

Fig. 1: Diapedesis During Inflammation



Integrins on the surface of the leukocyte bind to adhesion molecules on the inner surface of the vascular endothelial cells. The leukocytes flatten out and squeeze between the endothelial cells to leave the blood vessels and enter the tissue. The increased capillary permeability also allows plasma to enter the tissue.

Slideshow Activity

Examples include:

1. **Tumor necrosis factor-alpha (TNF-a)**

TNF-a is the principle cytokine that mediates acute inflammation. In excessive amounts it also is the principal cause of systemic complications such as the shock cascade. Functions include acting on endothelial cells to stimulate inflammation and the coagulation pathway; stimulating endothelial cells to produce selectins and ligands for leukocyte integrins **(see Fig. 1)** during diapedesis; stimulating endothelial cells and macrophages to produce chemokines that contribute to diapedesis, chemotaxis, and the recruitment of leukocytes; stimulating macrophages to secrete interleukin-1 (IL-1) for redundancy; activating neutrophils and promoting extracellular killing by neutrophils; stimulating the liver to produce acute phase proteins, and acting on muscles and fat to stimulate catabolism for energy conversion. In addition, TNF is cytotoxic for some tumor cells; interacts with the hypothalamus to induce fever and sleep; stimulates the synthesis of collagen and collagenase for scar tissue formation; and activates macrophages. TNF is produced by monocytes, macrophages, dendritic cells, T_H1 cells, and other cells.

2. **Interleukin-1 (IL-1)**

IL-1 function similarly to TNF in that it mediates acute inflammatory responses. It also works synergistically with TNF to

enhance inflammation. Functions of IL-1 include promoting inflammation; activating the coagulation pathway, stimulating the liver to produce acute phase proteins, catabolism of fat for energy conversion, inducing fever and sleep; stimulates the synthesis of collagen and collagenase for scar tissue formation; stimulates the synthesis of adhesion factors on endothelial cells and leukocytes **(see Fig. 1)** for diapedesis; and activates macrophages. IL-1 is produced primarily by monocytes, macrophages, dendritic cells, endothelial cells, and some epithelial cell.

3. **Chemokines**

Chemokines are a group of cytokines that enable the **migration of leukocytes from the blood to the tissues at the site of inflammation**. They increase the affinity of integrins on leukocytes for ligands on the vascular wall (see Fig. 1) during diapedesis, regulate the polymerization and depolymerization of actin in leukocytes for movement and migration, and function as chemoattractants for leukocytes. In addition, they trigger some WBCs to release their killing agents for extracellular killing and induce some WBCs to ingest the remains of damaged tissue. Chemokines also regulate the movement of B-lymphocytes, T-lymphocytes, and dendritic cells through the lymph nodes and the spleen. When produced in excess amounts, chemokines can lead to damage of healthy tissue as seen in such disorders as rheumatoid arthritis, pneumonia, asthma, adult respiratory distress syndrome (ARDS), and septic shock. Examples of chemokines include IL-8, MIP-1a, MIP-1b, MCP-1, MCP-2, MCP-3, GRO-a, GRO-b, GRO-g, RANTES, and eotaxin. Chemokines are produced by many cells including leukocytes, endothelial cells, epithelial cells, and fibroblasts.

4. I**nterleukin-12 (IL-12)**

IL-12 is a primary mediator of early innate immune responses to intracellular microbes. It is also an inducer of cell-mediated immunity. It functions to stimulate the synthesis of interferon-gamma by T-lymphocytes and NK cells; increases the killing activity of cytotoxic T-lymphocytes and NK cells; and stimulates the differentiation of naive T4-lymphocytes into interferon-gamma producing T_H1 cells. It is produced mainly by macrophages and dendritic cells.

5. **Type I Interferons**

Interferons modulate the activity of virtually every component of the immune system. **Type I interferons** include 13 subtypes of interferon-alpha, interferon-beta, interferon omega, interferon-kappa, and interferon tau. (There is only one **type II interferon**, interferon-gamma, which is involved in the inflammatory response.)

The most powerful stimulus for type I interferons is the binding of viral DNA or RNA to toll-like receptors TLR-3, TLR-7, and TLR-9 in endosomal membranes.

a. TLR-3 - binds double-stranded viral RNA;
b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
c. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommom or masked in human DNA and RNA.

Flash animation showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.
In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns or PAMPS and they bind to pattern recognition receptors called toll-like receptors (TLRs) found on host defense cells. For example, most viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide sequences (a cytosine lacking a methyl or CH_3 group and located adjacent to a guanine). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. In addition, most viruses produce unique double-stranded viral RNA, and some viruses produce uracil-rich single-stranded viral RNA during portions of their life cycle. The binding of these unique viral molecules bind to the endodsomal TLRs of defense cells such as macrophages and dendritic cells triggers the production of antiviral cytokines called type I

interferons that are able to block viral replication.

a. TLR-3 - binds double-stranded viral RNA;
b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
c. TLR-8 - binds single-stranded viral RNA;
d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommom or masked in human DNA and RNA.

Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

Signaling pattern recognition receptors located in the cytoplasm of cells such as RIG-1 and MDA-5 also signal synthesis and secretion of type-I interferons.

For More Information: Review of pathogen-associated molecular patterns (PAMPs)

For More Information: Review of pattern-recognition receptors (PRRs)

Type I interferons, produced by virtually any virus-infected cell, provide an early innate immune response against viruses. **Interferons induce uninfected cells to produce enzymes capable of degrading mRNA**. These enzymes **remain inactive until the uninfected cell becomes infected with a virus**. At this point, the enzymes are activated and begin to **degrade both viral and cellular mRNA**. This not only **blocks viral protein synthesis**, it also eventually **kills the infected cell (see Slideshow Figs 2A and 2B)**. In addition, type I interferons also cause infected cells to produce enzymes that interfere with transcription of viral RNA or DNA. They also promote body defenses by enhancing the activities of CTLs, macrophages, dendritic cells, NK cells, and antibody-producing cells.

Slideshow Activity

GIF animation showing the antiviral nature of interferon.

Type I interferons also induce MHC-I antigen expression needed for recognition of antigens by cytotoxic T-lymphocytes; augment macrophage, NK cell, cytotoxic T-lymphocytes, and B-lymphocyte activity; and induce fever. Interferon-alpha is produced by T-lymphocytes, B-lymphocytes, NK cells, monocytes/macrophages; interferon-beta by virus-infected cells, fibroblasts, macrophages, epithelial cells, and endothelial cells.

6. Interleukin-6 (IL-6)

IL-6 functions to stimulate the liver to produce acute phase proteins; stimulates the proliferation of B-lymphocytes; and increases neutrophil production. IL-6 is produced by many cells including T-lymphocytes, macrophages, monocytes, endothelial cells, and fibroblasts.

7. Interleukin-10 (IL-10)

IL-10 is an inhibitor of activated macrophages and dendritic cells and as such, regulates innate immunity and cell-mediated

immunity. IL-10 inhibits their production of IL-12, co-stimulator molecules, and MHC-II molecules, all of which are needed for cell-mediated immunity. IL-10 is produced mainly by macrophages, and T_H2 cells.

8. Interleukin 15 (IL-15)

IL-15 stimulates NK cell proliferation and proliferation of memory T8-lymphocytes. IL-15 is produced by various cells including macrophages.

9. Interleukin-18 (IL-18)

IL-18 stimulates the production of interferon-gamma by NK cells and T-lymphocytes and thus induces cell-mediated immunity. It is produced mainly by macrophages.

b. Cytokines that Regulate Adaptive Immune Responses (Humoral Immunity and Cell-Mediated Immunity)

Cytokines that regulate adaptive immunity are produced primarily by T-lymphocytes that have recognized an antigen specific for that cell. These cytokines function in the proliferation and differentiation of B-lymphocytes and T-lymphocytes after antigen recognition and in the activation of effector cells.

Examples include:

1. **Interleukin-2 (IL-2)**

IL-2 is a growth factor for NK cells and antigen-stimulated T-lymphocytes and B-lymphocytes. IL-2 also increases the killing ability of NK cells; increases the synthesis of other cytokines; increases Fas-mediated apoptosis; and stimulates antibody synthesis by B-lymphocytes. IL-2 is produced mainly by T4-lymphocytes and to a lesser extent T8-lymphocytes.

2. **Interleukin-4 (IL-4)**

IL-4 is a major stimulus for production of the antibody isotype IgE and the development of T_H2 cells for defense against helminths and arthropods. It also antagonizes the effects of interferon-gamma and thus inhibits cell-mediated immunity. IL-4 is produced mainly by T_H2 cells and mast cells.

3. **Interleukin-5 (IL-5)**

IL-5 is a growth and activating factor for eosinophils as a defense against helminths and arthropods. It also stimulates the proliferation and differentiation of antigen-activated B-lymphocytes and the production of **IgA. IL-5 is produced mainly by T_H2 cells.**

4. Interferon-gamma (IFN-?)

Interferons modulate the activity of virtually every component of the immune system. **Type I interferons** include more than 20 types of interferon-alpha, interferon-beta, interferon omega, and interferon tau. There is only one **type II interferon**, interferon-?. Type II interferon is produced by activated T-lymphocytes as part of an immune response and functions mainly to promote the activity of the components of the cell-mediated immune system such as CTLs, macrophages, and NK cells.

IFN-? is the principal cytokine for activating macrophages. It also induces the production of MHC-I molecules, MHC-II molecules, and co-stimulatory molecules by APCs in order to promote cell-mediated immunity and activates and increases the antimicrobial and tumoricidal activity of monocytes, macrophages, neutrophils, and NK cells. IFN-? stimulates the differentiation of naive T4-lymphocytes into T_H1 cells and inhibits the proliferation of T_H2 cells; stimulates the production of

IgG subclasses that activate the complement pathway and promote opsonization; and augments or inhibits other cytokine activities. IFN-gamma is produced primarily by T_H1 cells, $CD8^+$ cells, and NK cells.

5. **Transforming growth factor-beta (TGF-ß)**

TGF-ß functions to inhibit the proliferation and effector function of T-lymphocytes; inhibit the proliferation of B-lymphocytes; and inhibits macrophage function. It also promotes tissue repair. TGF-beta is produced by T-lymphocytes, macrophages, and other cells.

6. **Lymphotoxin (LT)**

LT plays a role in the recruitment and activation of neutrophils and in lymphoid organogenesis. Being chemically similar to TNF, LT is also a mediator of acute inflammatory responses. LT is made by T-lymphocytes.

7. **Interleukin-13 (IL-13)**

IL-13 increases the production of IgE by B-lymphocytes, inhibits macrophages, and increases mucus production. IL-13 is made primarily by T_H2 cells.

c. Cytokines that Stimulate Hematopoiesis

Produced by bone marrow stromal cells, these cytokines stimulate the growth and differentiation of immature leukocytes.

Examples include:

1. **Colony-stimulating factors (CSF)**

Promote the production of colonies of the different leukocytes in the bone marrow and enhance their activity. Examples include granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), and macrophage colony stimulating factor (M-CSF). In addition to their role in promoting production of leukocyte colonies, the CSFs also appear to promote their function. For example, when GM-CSF binds to receptors on neutrophils, eosinophils, and monocytes, it activates these cells and inhibits their apoptosis. GM-CSF increases adhesion of these cells to capillary walls during diapedesis, enhances their phagocytosis and extracellular killing, and increases both superoxide anion generation and antibody-dependent cytotoxicity. The various CSFs are produced by T-lymphocytes, macrophages, and other cells.

2. **Stem cell factor**

Stem cell factor makes stem cells in the bone marrow more responsive to the various CSFs. It is made mainly by bone marrow stromal cells.

3. **Interleukin-3 (IL-3)**

IL-3 supports the growth of multilineage bone marrow stem cells. IL-3 is made primarily by T-lymphocytes.

4. **Interleukin-7 (IL-7)**

IL-7 plays a role in the survival and proliferation of immature B-lymphocyte and T-lymphocyte precursors. IL-7 is produced mainly my fibroblasts and bone marrow stromal cells.

Some viruses cause infected host cells to secrete molecules that bind and tie up cytokines, preventing them from binding to normal cytokine receptors on host cells.

- Poxviruses cause infected host cells to secrete molecules that bind interleukin-1 (IL-1) and interferon-gamma (IFN-?).
- Cytomegaloviruses (CMV) cause infected host cells to secrete molecules that bind chemokines.

Self Quiz for Stimulating Cells to Secrete Cytokines

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ADAPTIVE IMMUNITY
IMMUNODEFICIENCY: PRIMARY IMMUNODEFICIENCY

Adaptive Immunity

Immunodeficiency: Primary Immunodeficiency

Fundamental Statements for this Softchalk Lesson:

1. Immunodeficiency results in an inability to combat certain diseases.
2. A primary immunodeficiency is usually an immunodeficiency that one is born with.
3. Conventional primary immunodeficiencies are rare recessive genetic defect in the immune responses that involved the development of B-lymphocytes, T-lymphocytes, or both and resulted in multiple, recurrent infections during infancy. Depending on the disorder, the lymphocytes in question were either completely absent, present in very low levels, or present but not functioning normally.
4. Conventional primary immunodeficiencies include B-lymphocyte disorders, T-lymphocyte disorders, Severe combined immunodeficiency disease or SCID,and innate immunity disorders.
5. B-lymphocyte disorders may result in greatly decreased humoral immunity but cell-mediated immunity, mediated by T-lymphocytes, remains normal.
6. T-lymphocyte disorders may result in little or no cell-mediated immunity if the disorder involves T8-lymphocytes and/or T4-helper lymphocytes. There may also be decreased humoral immunity if there is a disorder involves T4-helper lymphocytes.
7. Severe combined immunodeficiency disease deficiencies affect both humoral immunity and cell-mediated immunity may result in a defect in both B-lymphocytes and T-lymphocytes, or just T-lymphocytes in which case the humoral deficiency is due to the lack of T4-helper lymphocytes.
8. Innate immunity disorders are due to defects in genes that play a role in innate immune responses.
9. Novel primary immunodeficiencies include a multitude of common, less severe primary immunodeficiencies involving just one or more of the huge number of genes involved in the immune responses resulting in the decreased ability to combat just a single type of infection or a narrow range of infections.

Common Course Objectives

1. Compare and contrast primary immunodeficiencies, secondary immunodeficiencies, and novel immunodeficiencies.

Detailed Learning Objectives

1. Define primary immunodeficiency.
2. Compare and contrast conventional and novel primary immunodeficiencies.
3. Name 4 categories of conventional immunodeficiencies and give an example of each.

Immunodeficiency: Primary Immunodeficiency

Immunodeficiency results in an inability to combat certain diseases and may be of two types: primary or secondary. **Primary immunodeficiency** is usually an immunodeficiency that one is born with. In the case of **secondary immunodeficiency**, one

is born with normal immune responses but some secondary factor or occurrence causes a decrease in immune responses.

In this section we will look at primary immunodeficiencies.

A primary immunodeficiency is usually an immunodeficiency that one is born with. Until recently, primary immunodeficiencies were defined as a rare recessive genetic defect in the immune responses that involved the development of B-lymphocytes, T-lymphocytes, or both and resulted in multiple, recurrent infections during infancy. Depending on the disorder, the lymphocytes in question were either completely absent, present in very low levels, or present but not functioning normally. These disorders represent the **conventional immunodeficiencies**.

However, based on our increased understanding of the human genome and immune responses it now appears that there are a multitude of common, less severe primary immunodeficiencies involving just one or more of the huge number of genes involved in the immune responses. These so called **novel primary immunodeficiencies** involve the decreased ability to combat just a single type of infection or a narrow range of infections.

1. Conventional Immunodeficiencies

The conventional primary immunodeficiencies were grouped as follows:

a. B-lymphocyte Disorders

In the case of B-lymphocyte disorders, there may be greatly decreased humoral immunity but cell-mediated immunity, mediated by T-lymphocytes, remains normal.

1. **Agammaglobulinemias**

Few if any antibodies are produced and there are reduced B-lymphocyte numbers. The person is very susceptible to recurrent infections by common pyogenic bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria meningitidis,* and *Hemophilus influenzae.Th ese* bacteria have antiphagocytic capsules that are normally eliminated by antibodies through opsonization.

Examples include:

- X-linked agammaglobulinemia
- Autosomal recessive agammaglobulinemia.

2. **Hypogammaglobulinemias/Isotype Defects**

Hypogammaglobulinemias /isotype defects result in decreased general antibody production or decrease production of a single isotype of antibody.

Examples include:

- IgG2 subclass deficiency. A person is unable to produce the subclass of IgG called IgG2 but can produce other classes of antibodies. There is increased susceptibility to bacterial infections.
- Selective IgA deficiency. A person is unable to make IgA but can produce other classes of antibodies. There is increased susceptibility to bacterial infections and certain protozoan infections.
- Combined Variable Immunodeficiency (CVID). Hypogammaglobulinemia with normal or decreased numbers of B-lymphocytes.

For more information: Review of B-lymphocytes

More severe forms such as agammaglobulinemia are treated with artificially-acquired passive immunization - periodic injections of large amounts of immune globulin (IG or IVIG).

b. T-lymphocyte Disorders

In the case of T-lymphocyte disorders, there is little or no cell-mediated immunity if the disorder involves T8-lymphocytes and/or T4-lymphocytes. There may also be decreased humoral immunity if there is a disorder involves T4-lymphocytes.

Examples include:

1. **MHC Expression Defects**

- MHC-I deficiency. Decreased levels of MHC-I production and reduced T8-lymphocyte numbers.
- Bare lymphocyte syndrome. Decreased levels of MHC-II, decreased numbers of T4-lymphocytes, and decreased T4-dependent antibody production by B-lymphocytes.

2. **T-Lymphocyte Signaling Defects**

- Wiskott-Aldrich syndrome. Defective T-lymphocyte activation and defective leukocyte mobility.
- Proximal TCR signaling defects. Defective cell-mediated immunity and defective T4-dependent antibody production by B-lymphocytes.

3. **Familial Hemophagocytic Lymphohistiocytosis**

- Perforin deficiencies. Defective cytotoxic T-lymphocytes (CTL) and NK cell function; uncontrolled activation of macrophages and CTLs.
- Granule fusion defects. Defective CTL and NK cell function; uncontrolled activation of macrophages and CTLs.
- X-linked lymphoproliferative syndrome. Defective CTL and NK cell function; uncontrolled activation of macrophages and CTLs. Uncontrolled Epstein-Barr virus - induced B-lymphocyte proliferation.

For more information: Review of MHC molecules

For more information: Review of T4-lymphocytes

For more information: Review of T8-Lymphocytes

c. Combined B- and T-lymphocyte Disorders (Severe Combined Immunodeficiency Disease or SCID)

Severe combined immunodeficiency disease or SCID affects both humoral immunity and cell-mediated immunity. There is a defect in both B-lymphocytes and T-lymphocytes, or just T-lymphocytes in which case the humoral deficiency is due to the lack of T4-lymphocytes.

1. **Cytokine-Signaling Defects**

- Autosomal recessive SCID. Shows a marked decrease in T-lymphocytes but normal to increased levels of B-lymphocytes. There is reduced antibody levels due to the lack of T4-helper lymphocytes.

X-linked recessive SCID. Shows a marked decrease in T-lymphocytes but normal to increased levels of B-lymphocytes. There is reduced antibody levels due to the lack of T4-helper lymphocytes.

2. **Defects in Nucleotide Salvage Pathways**

- PNP deficiency. Shows a progressive decrease in both T-lymphocytes, B-lymphocytes, and NK cells, as well as reduced antibody levels.
- ADA deficiency. Shows a progressive decrease in both T-lymphocytes, B-lymphocytes, and NK cells, as well as reduced antibody levels.

3. **Defects in V(D)J Recombination (Combinatorial Diversity)**

- RAG1 or RAG2 deficiency. Shows an absence or deficiency of both T-lymphocytes and B-lymphocytes, as well as reduced antibody levels.
- ARTEMIS defects. Shows an absence or deficiency of both T-lymphocytes and B-lymphocytes, as well as reduced antibody levels.

4. D**efective Thymus Development**

The thymus is needed for the development of T-lymphocytes from stem cells.

- DiGeorge syndrome. Shows decreased levels of T-lymphocytes, normal levels of B-lymphocytes, and reduced antibody levels.
- Defective pre-TCR checkpoint. Shows decreased levels of T-lymphocytes, normal or reduced levels of B-lymphocytes, and reduced antibody levels.

For more information: Review of cytokines
For more information: Review of V(D)J genes

d. Innate Immunity Disorders

- Chronic granulomatous disease. No oxygen-dependant killing pathway in phagocytes. Recurrent intracellular bacterial and fungal infections.
- Leukocyte adhesion deficiencies. Defective leukocyte adhesion, diapedesis and migration. Recurrent bacterial and fungal infections.
- Chediak-Higashi syndrome. Defective vesicle fusion and lysosomal function in neutrophils, dendritic cells, macrophages and other cells. Recurrent infections by pyogenic bacteria.

2. Novel Immunodeficiencies

While the rare conventional primary immunodeficiencies mentioned above are still very important, based on our increased understanding of the human genome and immune responses it now appears that there are a multitude of common, less severe primary immunodeficiencies.

These so called novel primary immunodeficiencies relate to an individual's own unique genetics and can involve one or more of many immunity genes, ranging from any of the huge number of genes conferring protective immunity in general, to individual

genes conferring specific immunity to a single pathogen.

It is now thought that almost every person suffers from one form of primary immunodeficiency or another. Unlike the classical primary immunodeficiencies, however, these primary immunodeficiencies involve the decreased ability to combat just a single type of infection or a narrow range of infections. Examples include:

- Disorders of the interleukin-12/interferon-gamma pathway appear to make individuals more susceptible to *Mycobacterium* and *Salmonella* infections.
- Disorders of the TLR-3 pathway makes individuals more susceptible to herpes simplex virus encephalitis.
- Disorders of the toll-interleukin 1 receptor/nuclear factor kappa B pathway makes individuals more susceptible to staphylococcal and pneumococcal infections.
- Disorders of properdin and terminal components of the complement pathways make individuals more susceptible to *Neisseria* infections.
- People with chronic sinusitis that does not respond well to treatment have decreased activity of TLR-9 and produce reduced levels of human beta-defensin 2, as well as mannan-binding lectin needed to initiate the lectin complement pathway.

For more information: Review of the complement pathways
For more information: Review of pattern-recognition receptors

Self Quiz for Primary Immunodeficiency

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ADAPTIVE IMMUNITY
IMMUNODEFICIENCY: SECONDARY IMMUNODEFICIENCY

Adaptive Immunity

Immunodeficiency: Secondary Immunodeficiency

Fundamental Statements for this Softchalk Lesson:

1. A secondary immunodeficiency is one in which a person is born with normal immune responses but some secondary factor or occurrence causes a decrease in immune responses.
2. Causes of secondary immunodeficiencies include malnutrition, some viruses such as HIV, irradiation, cytotoxic drugs used in cancer chemotherapy, anti-inflammatory steroids, leukemias, aging, and removal of the spleen.
3. HIV infects and destroys T4-lymphocytes and when the body becomes unable to replace the T4-lymphocytes as fast as they are being destroyed, secondary immunodeficiency results..

Common Course Objectives

1. Compare and contrast primary immunodeficiencies, secondary immunodeficiencies, and novel immunodeficiencies.

Detailed Learning Objectives

1. State what is meant by secondary immunodeficiency and list 4 possible contributing factors.
2. Briefly give at least four mechanisms of HIV-induced immunodeficiency.

Immunodeficiency: Secondary Immunodeficiency

Immunodeficiency results in an inability to combat certain diseases and may be of two types: primary or secondary. **Primary immunodeficiency** is usually an immunodeficiency that one is born with. In the case of **secondary immunodeficiency**, one is born with normal immune responses but some secondary factor or occurrence causes a decrease in immune responses.

In this section we will look at secondary immunodeficiencies.

Secondary Immunodeficiency

In the case of secondary immunodeficiency, **one is born with normal immune responses but some secondary factor or occurrence causes a decrease in immune responses**. Secondary immunodeficiency is induced by factors such as:

- Malnutrition. Inhibits lymphocyte maturation and function.
- Some viruses, e.g., HIV. Depletes T4-lymphocytes.
- Irradiation - exposure to X-rays and gamma rays. Causes a decreased production of lymphocyte precursors in the bone marrow.
- Cytotoxic drugs such as many used in cancer chemotherapy. Causes a decreased production of lymphocyte precursors

in the bone marrow.
- Corticosteroids – anti-inflammatory steroids. Damages lymphocytes.
- Leukemias, cancers of the lymphoid system, metastases. Reduces areas for lymphocyte development.
- Aging. Adaptive immunity, especially cell-mediated immunity, tends to diminish with aging.
- Removal of the spleen. Decreased ability to remove microbes that enter the blood.

A secondary immunodeficiency of current notoriety is of course **Acquired Immunodeficiency Syndrome or AIDS**, a secondary immunodeficiency caused by Human Immunodeficiency Virus (HIV). **HIV, via its gp120, primarily infects cells with CD4 molecules and chemokine receptors on their surface**, namely, T4-lymphocytes, macrophages, and dendritic cells. The median incubation period for AIDS is around 10 years.

During **early or acute HIV infection** the virus primarily **infects and destroys memory T4-lymphocytes which express the chemokine receptor CCR5 and are very abundant in mucosal lymphoid tissues**. Here **HIV also encounters the dendritic cells located throughout the epithelium of the skin and the mucous membranes** where in their immature form called Langerhans cells they are attached by long cytoplasmic processes. The envelope glycoproteins gp41 and gp120 of HIV contain mannose-rich glycans that bind to mannan-binding proteins (pattern recognition receptors; also called lectin receptors) on the dendritic cells.

Upon capturing antigens through pinocytosis and phagocytosis and becoming activated by pro-inflammatory cytokines, **the dendritic cells detach from the epithelium, enter lymph vessels, and are carried to regional lymph nodes**. By the time they enter the lymph nodes, the dendritic cells have matured and are now able to present antigens of HIV to naive T-lymphocytes located in the the lymph nodes in order to **induce adaptive immune responses**.

At this point the infection has **transitioned from the acute phase to the chronic phase**. The chronic phase of HIV infection is characterized by viral dissemination, viremia, and induction of adaptive immune responses. The **viremia allows the viruses to spread and infect T4-helper lymphocytes, macrophages, and dendritic cells found in peripheral lymphoid tissues**.

During the **chronic phase** of HIV infection, **the lymph nodes and the spleen become sites for continuous viral replication and host cell destruction**. During most of this phase, **the immune system remains active and competent and there are few clinical symptoms. A steady state-infection generally persists where T4-lymphocyte death and T4-lymphocyte replacement by the body are in equilibrium**. In a person infected with HIV, somewhere between one and two billion of these T4-cells die each day as a result of HIV infection and must be replaced by the body's lymphopoietic system in the bone marrow. It is estimated that 10 billion virions are produced and cleared in an infected individual each day. However, **the enormous turnover of T4-lymphocytes eventually exhausts the lymphopoietic system and it becomes unable to replace the T4-cells being destroyed**. A variety of mechanisms then eventually **lead to immunodeficiency**.

Mechanisms of HIV-induced immunodeficiency include:

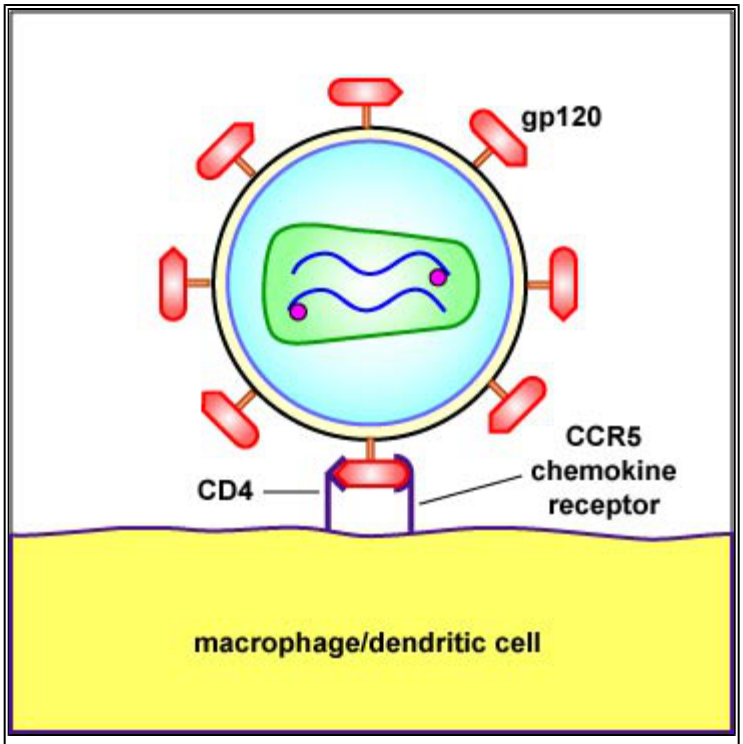
- Direct HIV-induced cytopathic effect on infected T4-lymphocytes. This can occur through:
  - Increased cell permeability as a result of gp41 expression in the host cell membrane and viral release by budding;
  - Inhibition of host cell protein synthesis as a result of viral replication within the infected cell; and
  - Fusion of infected T4-cells with numerous uninfected T4-cells resulting in syncytia formation.
- Killing of HIV-infected T4-cells by cytotoxic T-lymphocytes or CTLs.
- Killing of HIV-infected T4-cells by antibody-dependent cytotoxicity or ADCC.
- Apoptosis of T4-cells as a result of chronic activation by HIV and by cytokines.
- Shedding of gp120 molecules by HIV. This subsequently triggers a series of events that cause the adaptive immune system to become less and less effective, primarily by altering the normal balance of immunoregulatory T_H1 and T_H2 cells in the body.
- Impaired function of HIV infected macrophages and dendritic cells.

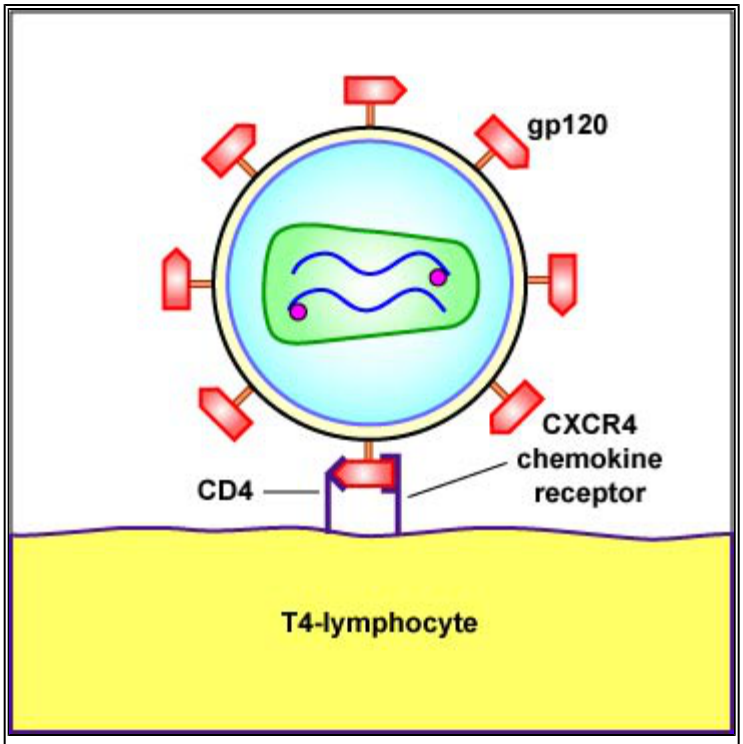
For more information: Review of the life cycle of HIV

To further complicate problems, during the replication of HIV the **reverse transcriptase of HIV exhibits a high error rate as it transcribes the RNA genome into DNA**. As a result, HIV readily mutates to become more immunoresistant, more drug

resistant, and able to change the preferred cell type it is able to infect, , e.g., M-tropic to T-tropic as shown in **Fig. 1**.

Fig. 1: Affinity of HIV for Different Immune Cells





In early phase HIV infection, initial viruses are M-tropic. Their envelope glycoprotein gp120 is able to bind to CD4 molecules and chemokine receptors called CCR5 found on macrophages. In late phase HIV infection, most of the viruses are T-tropic, having gp120 capable of binding to CD4 and CXCR4 found on T4-lymphocytes.

Progression to AIDS is marked by a viral load that progressively increases in number while the immune system weakens as a result of the destruction of increasing numbers of T4-lymphocytes and the inability of the body to continually replace these destroyed cells. The loss of T4-helper lymphocytes **leads to a marked decline in cells called cytotoxic T-lymphocytes (CTLs)**, the primary cells the body's immune responses use to destroy virus-infected cells. Once a person progresses to full-blown AIDS he or she becomes susceptible to a variety of opportunistic infections by:

- bacteria such as *Mycobacterium avium* complex (MAC), *Salmonella*, and *Nocardia*;
- protozoa such as *Cryptosporidium* and *Toxoplasma*;
- viruses such as cytomegalovirus (CMV), herpes simplex viruses types 1 and 2 (HSV-1, HSV-2), and varicella zoster virus (VZV);
- *Candida, Cryptococcus, Coccidioides, Histoplasma*, and *Pneumocystis*.

There is also an increased incidence of tumors, such Epstein-Barr virus-associated B-cell lymphomas, other lymphomas, cervical cancer, and Kaposi's sarcoma. Wasting syndrome and encephalopathy are also common.

Self Quiz for Secondary Immunodeficiency

Quiz Group

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ADAPTIVE IMMUNITY
IMMEDIATE HYPERSENSITIVITY: TYPE-I (IgE MEDIATED)

Adaptive Immunity

Immediate Hypersensitivity: Type I (IgE Mediated or Anaphylactic-Type)

Fundamental Statements for this Softchalk Lesson:

1. Immediate hypersensitivities refer to humoral immunity (antigen/antibody reactions) causing harm.
2. During Type I (IgE mediated or anaphylactic-type) hypersensitivity, IgE is made in response to an allergen.
3. In allergic individuals, the levels of IgE may be thousands of times higher than in those without allergies.
4. The Fc portion of IgE binds to the surface of mast cells and basophils and when the allergen subsequently cross-links the Fab portions of the mast cell-bound IgE, this triggers the release of inflammatory mediators such as histamine release by the mast cell, as well as the synthesis of other inflammatory mediators such as platelet-activating factor, leukotrienes, bradykinins, prostaglandins, and cytokines that contribute to inflammation.
5. The inflammatory agents then lead to dilation of blood vessels (redness or erythema, increased capillary permeability (swelling or edema), constriction of bronchial airways (wheezing and difficulty in breathing), stimulation of mucous secretion (congestion of airways), and stimulation of nerve endings (itching and pain in the skin).
6. In a systemic anaphylaxis, the allergin is usually picked up by the blood and the reactions occur throughout the body and can lead to shock. Examples include severe allergy to insect stings, drugs, and antisera.
7. With a localized anaphylaxis, the allergin is usually found localized in the mucous membranes or the skin. Examples include allergy to hair, pollen, dust, dander, feathers, and food.
8. Type I hypersensitivity is treated symptomatically with anti-inflammatory agents such antihistamines and epinephrine.
9. Desensitization shots (allergy shots) are thought to stimulate the production of IgG and IgA which then act as blocking antibodies to bind and neutralize much of the allergen in secretions before it can bind to the deeper cell-bound IgE on the mast cells in the connective tissue.
10. Monoclonal antibodies that have been made against the Fc portion of human IgE have also been used in treatment. They block the attachment of the IgE to the Fc receptors on mast cells and basophils and the subsequent release of histamine by those cells upon exposure to allergen.

Common Course Objectives

1. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1. Describe the mechanism for Type I (IgE-mediated) hypersensitivity and give 3 examples. State how they are treated symptomatically.

2. Describe how desensitization (allergy) shots work to lessen the severity of Type I hypersensitivities.

3. Briefly describe how monoclonal antibodies against the Fc portion of IgE may someday be used to prevent Type I allergies.

4. When a person has hay fever, common symptoms include runny eyes, runny nose, swollen sinuses, and difficulty in breathing. In terms of humoral immunity, discuss the mechanism behind these symptoms. Also state the reason for giving antihistamines.

When the immune systems cause harm to the body, it is referred to as a hypersensitivity. There are two categories of adaptive hypersensitivities: immediate hypersensitivity and delayed hypersensitivity. **Immediate hypersensitivities** refer to **humoral immunity** (antigen/antibody reactions) causing harm; **delayed hypersensitivities** refer to **cell-mediated immunity** (cytotoxic T-lymphocytes. macrophages, and cytokines) leading to harm.

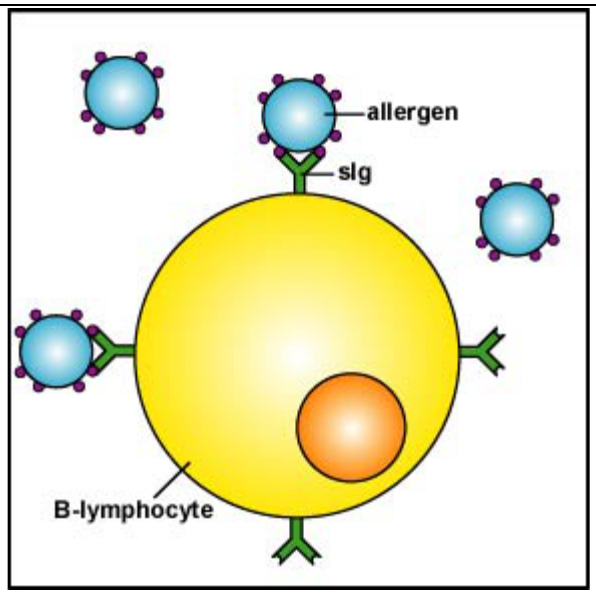
There are 3 types of immediate hypersensitivities that depend on the interaction of antigens with antibodies: Type I, Type II, Type III, and Type V.

In this section we will look at **Type I** immediate hypersensitivities.

Immediate Hypersensitivity: Type I (IgE Mediated or Anaphylactic-Type)

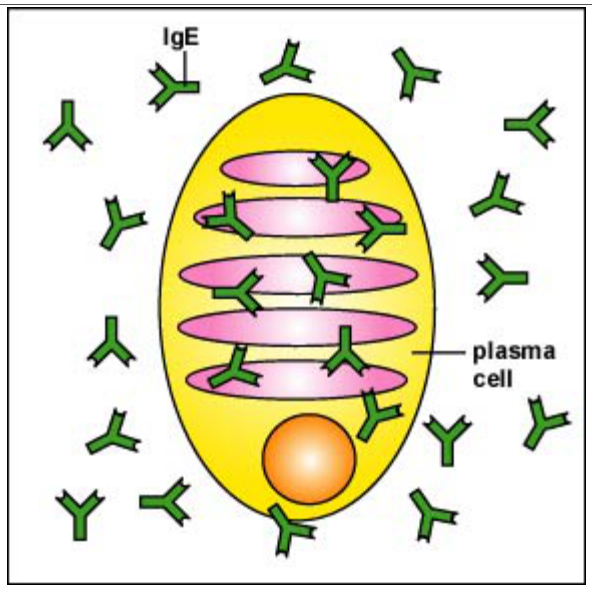
Mechanism : Type I (IgE mediated or anaphylactic-type) hypersensitivity is the most common type of hypersensitivity, seen in about 20% of the population. IgE is made in response to an allergen **(see Fig. 1 and Fig. 2)**. In allergic individuals, the levels of IgE may be thousands of times higher than in those without allergies. Possibly this is due to a higher number of T_H2 cells which produce IL-4, a cytokine that can increase production of IgE, and a lower number of T_H1 cells that produce gamma-interferon, a cytokine that decreases IgE production.

Fig. 1: Type-I Hypersensitivity, Step-1



The allergen enters the body and is recognized by sIg on a B-lymphocyte. The B-lymphocyte then proliferates and differentiates into plasma cells.

Fig. 2: Type-I Hypersensitivity, Step-2

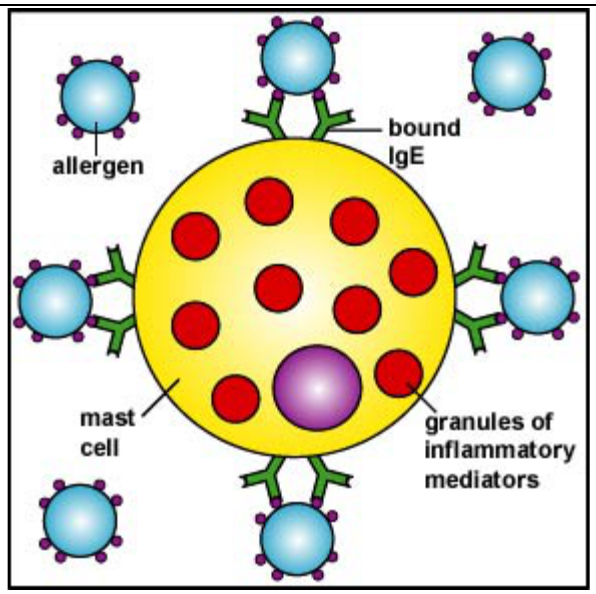


The plasma cells produce and secrete IgE which binds to receptors on mast cells and basophils.

GIF animation showing production of IgE in response to an allergen.

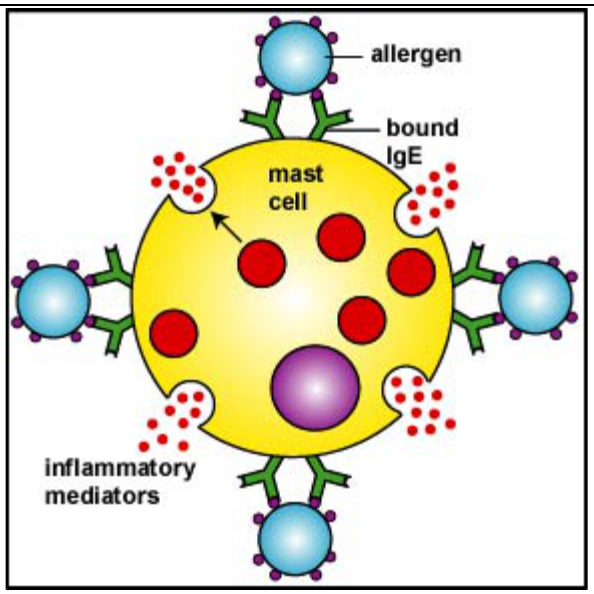
The Fc portion of IgE binds to the surface of mast cells and basophils (see Fig. 3). When the allergen cross-links the Fab portions of the mast cell-bound IgE, this triggers **histamine release** by the mast cell, a process called degranulation, and the **synthesis of other inflammatory mediators** such as platelet-activating factor, leukotrienes, bradykinins, prostaglandins, and cytokines that contribute to inflammation **(see Fig. 4)**. These agents cause the **early phase** of allergic reactions that appears within minutes after exposure to the antigen.

Fig. 3: Type-I Hypersensitivity, Step-3



Allergen cross reacting with IgE on mast cell.

Fig. 4: Type-I Hypersensitivity, Step-4



The next time the allergen enters the body, it cross-links the Fab portions of the IgE bound to the mast cell. This triggers the mast cell to degranulate, that is, release its histamine and other inflammatory mediators. The inflammatory mediators are now able to bind to receptors on target cells which leads to dilation of blood vessels, constriction of bronchioles, excessive mucus secretion, and other symptoms of allergy.

Flash animation showing the mechanism behind Type-1 hypersensitivity.

html5 version of animation for iPad showing the mechanism behind Type-1 hypersensitivity.

IgE is made in response to an allergen and binds to Fc receptors on mast cells and basophils. The next time the allergen enters the body, it cross-links the Fab portions of the IgE bound to the mast cell. This triggers the mast cell to degranulate, that is, release its histamine and other inflammatory mediators. The inflammatory mediators are now able to bind to receptors on target cells which leads to dilation of blood vessels, constriction of bronchioles, excessive mucus secretion, and other symptoms of allergy.

For more information: Review of the five classes of human antibodies

For more information: Review of T_H1 and T_H2 cells

For more information: Review of inflammation

Late phase allergic reactions may begin several hours after exposure to antigen. It is thought that basophils play a major role here. Cell-bound IgE on the surface of basophils of sensitive individuals binds a substance called histamine releasing factor (possibly produced by macrophages and B-lymphocytes) causing further histamine release.

The inflammatory agents released or produced cause the following:

a. **Dilation of blood vessels**. This causes **local redness** (erythema) at the site of allergen delivery. If dilation is widespread, this can contribute to **decreased vascular resistance**, a drop in blood pressure, and **shock**.

b. **Increased capillary permeability**. This causes **swelling of local tissues** (edema). If widespread, it can contribute to **decreased blood volume** and **shock**.

c. **Constriction of bronchial airways**. This leads to **wheezing and difficulty in breathing**.

d. **Stimulation of mucous secretion**. This leads to **congestion of airways**.

e. **Stimulation of nerve endings**. This leads to **itching and pain in the skin**.

In a **systemic anaphylaxis**, the allergin is usually picked up by the blood and the reactions occur throughout the body. Examples include severe allergy to insect stings, drugs, and antisera. With a **localized anaphylaxis**, the allergin is usually found localized in the mucous membranes or the skin. Examples include allergy to hair, pollen, dust, dander, feathers, and food.

Type I hypersensitivity is treated symptomatically with such agents as:

a. **Epinephrine**. Epinephrine relaxes smooth muscle, constricts blood vessels, and stimulates the heart. It is used for severe systemic reactions.

b. **Histamine H1-receptor antagonists**. **Antihistamines** block the binding of histamine to histamine H1-receptors on target cells, eg, loratadine, fexofenadine, cetirizine.

c. **Beta2- agonists**. Increase cyclic AMP levels leading to relaxation of bronchial smooth muscles and inhibit mast cell degranulation, eg, albuterol, salmeterol, formoterol.

d. **Leukotriene receptor antagonists**. Block smooth muscle constriction, eg, pranlukast.

e. **Sodium cromoglycate**. Sodium cromoglycate prevents mast cells from releasing histamines.

f. **Nasally administered steroids**. Corticosteroids are potent antiinflammatory agents.

Severity may be reduced by **desensitization shots** (allergy shots). It is thought that when very dilute allergen is given by injection, it stimulates the production of IgG and IgA. IgG and IgA then act as **blocking antibodies** to bind and neutralize much of the allergen in secretions before it can bind to the deeper cell-bound IgE on the mast cells in the connective tissue. The shots also appear to suppress production of IgE by inducing tolerance and/or by activating T8-suppressor cells.

A new experimental approach to treating and preventing Type-I hypersensitivity involves giving the person with allergies injections of **monoclonal antibodies that have been made against the Fc portion of human IgE**. This, in turn, blocks the attachment of the IgE to the Fc receptors on mast cells and basophils and the subsequent release of histamine by those cells upon exposure to allergen. In addition, the anti-IgE binds to IgE-producing B-lymphocytes causing apoptosis. The monoclonal antibody is a humanized hybrid molecule consisting of a mouse binding (Fab) portion attached to a human constant (Fc)

portion and is known as rhuMab (recombinant human monoclonal antibody).

Flash animation showing the use of monoclonal antibodies to block the attachment of IgE to mast cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the use of monoclonal antibodies to block the attachment of IgE to mast cells.
A new approach to treating and preventing Type-I hypersensitivity involves giving the person with allergies injections of monoclonal antibodies that have been made against the Fc portion of human IgE. This, in turn, blocks the attachment of the IgE to the Fc receptors on mast cells and basophils and the subsequent release of histamine and other inflammatory mediators by those cells upon subsequent exposure to allergen.

Self Quiz for Type I (IgE Mediated or Anaphylactic-Type)

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ADAPTIVE IMMUNITY
IMMEDIATE HYPERSENSITIVITY: TYPE II (ANTIBODY-DEPENDENT CYTOTOXICITY)

Adaptive Immunity

Immediate Hypersensitivity: Type II (Antibody-Dependent Cytotoxicity)

Fundamental Statements for this Softchalk Lesson:

1. During type II (antibody-dependent cytotoxicity) hypersensitivity, either IgG or IgM is made against normal self antigens as a result of a failure in immune tolerance, or a foreign antigen resembling some molecule on the surface of host cells enters the body and IgG or IgM made against that antigen then cross reacts with the host cell surface.
2. The binding of these antibodies to the surface of host cells then leads to opsonization of the host cells, membrane attack complex (MAC) lysis of the cells, and antibody-dependent cellular cytotoxicity (ADCC) destruction of the host cells.
3. Examples include AB and Rh blood group reactions and autoimmune diseases such as rheumatic fever, acute glomerulonephritis, myasthenia gravis, and multiple sclerosis.

Common Course Objectives

1. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1. Describe the mechanism for Type II (antibody-dependent cytotoxicity) hypersensitivity and give 2 examples.

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When the immune systems cause harm to the body, it is referred to as a hypersensitivity. There are two categories of adaptive hypersensitivities: immediate hypersensitivity and delayed hypersensitivity. **Immediate hypersensitivities** refer to **humoral immunity** (antigen/antibody reactions) causing harm; **delayed hypersensitivities** refer to **cell-mediated immunity** (cytotoxic T-lymphocytes. macrophages, and cytokines) leading to harm.

There are 3 types of immediate hypersensitivities that depend on the interaction of antigens with antibodies: Type I, Type II, Type III, and Type V.

In this section we will look at **Type II** immediate hypersensitivities.

Immediate Hypersensitivity: Type II (Antibody-Dependent Cytotoxicity)

Mechanism:

During type II (antibody-dependent cytotoxicity) hypersensitivity, either **IgG or IgM is made against normal self antigens** as a result of a failure in immune tolerance, or a **foreign antigen resembling some molecule on the surface of host cells** enters the body and IgG or IgM made against that antigen then cross reacts with the host cell surface. The binding of these antibodies to the surface of host cells then leads to:

a. **Opsonization of the host cells** whereby phagocytes stick to host cells by way of IgG, C3b, or C4b and discharge their lysosomes **(see slide show Fig. 1A and Fig. 1B).**

Slideshow Activity

Flash animation showing opsonization of cells during Type-II hypersensitivity.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing opsonization of cells during Type-II hypersensitivity.
IgG reacts with epitopes on the host cell membrane. Phagocytes then bind to the Fc portion of the IgG and discharge their lysosomes.

b. **Activation of the classical complement pathway causing membrane attack complex** (**MAC) lysis of the cells (see slide show Fig. 2A and Fig. 2B**).

Slideshow Activity

Flash animation showing MAC lysis of cells during Type-II hypersensitivity.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing MAC lysis of cells during Type-II hypersensitivity.
After IgG or IgM reacts with epitopes on the host cell membrane and activates the classical complement pathway, the membrane attack complex (MAC) causes lysis of the cell.

c. **Antibody-dependent cellular cytotoxicity** (**ADCC) destruction of the host cells** whereby NK cells attach to the Fc portion of the antibodies. The NK cell then release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. **(see Slideshow Fig. 3A , Fig. 3B, and Fig. 3C)**.

Slideshow Activity

Flash animation showing ADCC destruction of cells during Type-II hypersensitivity.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing ADCC destruction of cells during Type-II hypersensitivity.
Antibodies react with "self" epitopes on the host cell membrane and NK cells bind to the Fc of the antibodies. The NK cell is then able to contact the cell and release pore-

forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Flash animation showing apoptosis of cells during Type-II hypersensitivity.

html5 version of animation for iPad showing apoptosis of cells during Type-II hypersensitivity.

NK cells release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

For more information: Review of the five classes of human antibodies

For more information: Review of opsonization

For more information: Review of MAC cytolysis

For more information: Review of ADCC

Examples include:

- AB and Rh blood group reactions;
- autoimmune diseases such as:
  - rheumatic fever where antibodies result in joint and heart valve damage;
  - idiopathic thrombocytopenia purpura where antibodies result in the destruction of platelets;
  - myasthenia gravis where antibodies bind to the acetylcholine receptors on muscle cells causing faulty enervation of muscles;
  - Goodpasture's syndrome where antibodies lead to destruction of cells in the kidney;
  - multiple sclerosis where antibodies are made against the oligodendroglial cells that make myelin, the protein that forms the myelin sheath that insulates the nerve fiber of neurons in the brain and spinal cord; and
- some drug reactions.

Type II hypersensitivity also participates in early transplant rejections.

Self Quiz for Type II (Antibody-Dependent Cytotoxicity)

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ADAPTIVE IMMUNITY
IMMEDIATE HYPERSENSITIVITY: TYPE III HYPERSENSITIVITY (IMMUNE COMPLEX-MEDIATED)

Adaptive Immunity

Immediate Hypersensitivity: Type III (Immune Complex Mediated)

Fundamental Statements for this Softchalk Lesson:

1. Type III (immune complex-mediated) hypersensitivity is caused when soluble antigen-antibody (IgG or IgM) complexes, which are normally removed by macrophages in the spleen and liver, form in large amounts and overwhelm the body.
2. These small complexes lodge in the capillaries, pass between the endothelial cells of blood vessels - especially those in the skin, joints, and kidneys - and become trapped on the surrounding basement membrane beneath these cells.
3. The antigen/antibody complexes then trigger excessive activation of the classical complement pathway leading to a massive inflammatory response, influx of neutrophils with extracellular killing of body tissue, MAC lysis of tissue, and aggregation of platelets and macrophages.
4. Examples include Serum sickness, autoimmune acute glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosis.

Common Course Objectives

1. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1. Describe the mechanism for Type III (immune complex-mediated) hypersensitivity and give 2 examples.

When the immune systems cause harm to the body, it is referred to as a hypersensitivity. There are two categories of adaptive hypersensitivities: immediate hypersensitivity and delayed hypersensitivity. **Immediate hypersensitivities** refer to **humoral immunity** (antigen/antibody reactions) causing harm; **delayed hypersensitivities** refer to **cell-mediated immunity** (cytotoxic T-lymphocytes. macrophages, and cytokines) leading to harm.

There are 3 types of immediate hypersensitivities that depend on the interaction of antigens with antibodies: Type I, Type II, Type III, and Type V.

In this section we will look at **Type II** immediate hypersensitivities.

Immediate Hypersensitivity: Type III (Immune Complex Mediated)

Mechanism : Type III (immune complex-mediated) hypersensitivity is caused when **soluble antigen-antibody (IgG or IgM) complexes**, which are normally removed by macrophages in the spleen and liver, **form in large amounts** and overwhelm the body **(see Slideshow Fig. 1A)**. These small complexes **lodge in the capillaries**, pass between the endothelial cells of blood vessels - especially those in the skin, joints, and kidneys - and become trapped on the surrounding basement membrane beneath these cells **(see Slideshow Fig. 1B)**. **The antigen/antibody complexes then activate the classical complement pathway (see Slideshow Fig. 1C)**.

This may cause:

a. **Massive inflammation**, due to complement protein C5a triggering mast cells to release inflammatory mediators;

b. **Influx of neutrophils**, due to complement protein C5a , resulting in neutrophils discharging their lysosomes and causing tissue destruction through extracellular killing and causing further inflammation **(see Slideshow Fig. 1D and Fig. 1E)**;

c. **MAC lysis** of surrounding tissue cells, due to the membrane attack complex, $C5_b6789_n$;

d. **Aggregation of platelets**, resulting in more inflammation and the formation of microthrombi that block capillaries; and

e. Activation of macrophages, resulting in production of inflammatory cytokines and extracellular killing causing tissue destruction.

This can **lead to tissue death and hemorrhage**.

Slideshow Activity

GIF animation showing inflammation and tissue death during Type-III hypersensitivity.

For more information: Review of the five classes of human antibodies

For more information:Review of the complement pathways

Examples include:

- Serum sickness, a combination type I and type III hypersensitivity;
- Autoimmune acute glomerulonephritis;
- rheumatoid arthritis;
- Systemic lupus erythematosis;
- Some cases of chronic viral hepatitis; and
- The skin lesions of syphilis and leprosy.

Self Quiz for Type III (Immune Complex Mediated)

Quiz Group

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ADAPTIVE IMMUNITY
IMMEDIATE HYPERSENSITIVITY: Type V (STIMULATORY HYPERSENSITIVITY)

Adaptive Immunity

Immediate Hypersensitivity: Type V (Stimulatory Hypersensitivity)

Fundamental Statements for this Softchalk Lesson:

1. During type V (stimulatory hypersensitivity) antibodies are made against a particular hormone receptor of a hormone-producing cell leading to the overstimulation of those hormone-producing cells.
2. An example is Graves' disease where antibodies are made against thyroid-stimulating hormone receptors of thyroid cells.

Common Course Objectives

1. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1. Describe the mechanism for Type V (Stimulatory) hypersensitivity and give an example.

When the immune systems cause harm to the body, it is referred to as a hypersensitivity. There are two categories of adaptive hypersensitivities: immediate hypersensitivity and delayed hypersensitivity. **Immediate hypersensitivities** refer to **humoral immunity** (antigen/antibody reactions) causing harm; **delayed hypersensitivities** refer to **cell-mediated immunity** (cytotoxic T-lymphocytes. macrophages, and cytokines) leading to harm.

There are 3 types of immediate hypersensitivities that depend on the interaction of antigens with antibodies: Type I, Type II, Type III, and Type V.

In this section we will look at **Type II** immediate hypersensitivities.

Immediate Hypersensitivity: Type V (Stimulatory Hypersensitivity)

Mechanism: During type V hypersensitivity (stimulatory hypersensitivity) antibodies are made against a particular hormone receptor on a hormone-producing cell. This leads to the overstimulation of those hormone-producing cells.

- An example is Graves' disease where antibodies are made against thyroid-stimulating hormone receptors of thyroid cells. The

binding of the antibodies to the TSH receptors results in constant stimulation of the thyroid leading to hyperthyroidism.

Show/hide comprehension question...

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ADAPTIVE IMMUNITY
DELAYED HYPERSENSITIVITY: TYPE IV HYPERSENSITIVITY

Adaptive Immunity

Delayed Hypersensitivity: Type IV Hypersensitivity

Fundamental Statements for this Softchalk Lesson:

1. During delayed hypersensitivity,T8-lymphocytes become sensitized to an antigen and differentiate into cytotoxic T-lymphocytes (CTLs) while effector T4-lymphocytes become sensitized to an antigen and produce cytokines.
2. CTLs, cytokines, eosinophils, and/or macrophages then cause harm rather than benefit.
3. Examples include the cell or tissue damage done during diseases like tuberculosis, leprosy, smallpox, measles, herpes infections, candidiasis, and histoplasmosis, the skin test reactions seen for tuberculosis and other infections, contact dermatitis like poison ivy, type-1 insulin-dependent diabetes where CTLs destroy insulin-producing cells, multiple sclerosis, where T-lymphocytes and macrophages secrete cytokines that destroy the myelin sheath that insulates the nerve fibers of neurons, and Crohn's disease and ulcerative colitis.

Common Course Objectives

1. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1. Describe the mechanism for Type IV (delayed) hypersensitivity and give 2 examples.

When the immune systems cause harm to the body, it is referred to as a hypersensitivity. There are two categories of adaptive hypersensitivities: immediate hypersensitivity and delayed hypersensitivity. **Immediate hypersensitivities** refer to **humoral immunity** (antigen/antibody reactions) causing harm; **delayed hypersensitivities** refer to **cell-mediated immunity** (cytotoxic T-lymphocytes. macrophages, and cytokines) leading to harm.

In this section we will look at **Type IV** or **delayed hypersensitivitie**s.

Delayed Hypersensitivity: Type IV Hypersensitivity

Delayed hypersensitivity **is cell-mediated** rather than antibody-mediated.

Mechanism:

Delayed hypersensitivity is the same mechanism as cell-mediated immunity. **T8-lymphocytes become sensitized to an antigen and differentiate into cytotoxic T-lymphocytes** or CTLs **while effector T4-lymphocytes become sensitized to an antigen and produce cytokines**. CTLs, cytokines, eosinophils, and/or macrophages then cause harm rather than benefit **(see Slideshow Fig. 1A and Fig. 1B)**.

1. **CTLs** use their TCR/CD8 to bind to peptide epitopes bound to MHC-I on infected cells or normal cells having cross-reacting epitopes and kill them through apoptosis.

2. **T_H1 cells** activate macrophages causing the production of inflammatory cytokines and extracellular killing by the macrophages leading to tissue damage.

3. **T_H2 cells** produce interleukin-4 (IL-4) and interleukin-5 (IL-5) to promote extracellular killing by eosinophils and causing tissue damage.

Slideshow Activity

Examples include:

- The cell or tissue damage done during diseases like tuberculosis, leprosy, smallpox, measles, herpes infections, candidiasis, and histoplasmosis;
- The skin test reactions seen for tuberculosis and other infections;
- Contact dermatitis like poison ivy;
- Type-1 insulin-dependent diabetes where CTLs destroy insulin-producing cells;
- Multiple sclerosis, where T-lymphocytes and macrophages secrete cytokines that destroy the myelin sheath that insulates the nerve fibers of neurons;
- Crohn's disease and ulcerative colitis; and
- Psoriasis.

Delayed hypersensitivity also plays a major role in chronic transplant rejection as a result of CTL destruction of donor cells (host versus graft rejection) or recipient cells (graft versus host rejection). Immunosuppressive drugs such as cyclosporin A or FK-506 (Tacrolimus) are given in an attempt to prevent rejection. Both of these drugs prevent T-lymphocyte proliferation and differentiation by inhibiting the transcription of IL-2.

For more information: Review of T_H1 and T_H2 cells
For more information: Review of cytotoxic T-lymphocytes (CTLs)

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ADAPTIVE IMMUNITY
SUPERANTIGENS

Adaptive Immunity

Superantigens

Fundamental Statements for this Softchalk Lesson:

1. Conventional antigens are only recognized by specific T4-cells having a TCR with a corresponding shape.
2. Superantigens are unusual bacterial toxins that interact with exceedingly large numbers of T4-lymphocytes.
3. Activation of very large numbers of T4-lymphocytes results in the secretion of excessive amounts of a cytokine called interleukin-2 (IL-2).
4. Excess stimulation of IL-2 secretion can also lead to production of inflammatory and can lead to the same endothelial damage, acute respiratory distress syndrome, disseminated intravascular coagulation, shock, and multiple organ system failure seen with PAMP-induced inflammation.
5. Examples of superantigens include toxic shock syndrome toxin-1 (TSST-1), Streptococcal pyrogenic exotoxins (SPE), Staphylococcal enterotoxins (SE), and enterotoxogenic E. coli (ETEC) enterotoxin.

Common Course Objectives

1. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1*. Define superantigen.

2. Briefly describe the mechanism by which superantigens cause harm to the body.

3. Name 2 superantigens and give an example of a bacterium that produces each.

(*) = Common theme throughout the course

As was learned earlier under Bacterial Pathogenicity, **superantigens are type I toxins that can trigger a harmful immune response**.

Exotoxins are **toxins**, often proteins in nature, **secreted from a living bacterium** but also **released upon bacterial lysis**. In addition, some bacteria use a type 3 secretion system or a type 4 secretion system to **inject toxins directly into human cells**. There are three main types of exotoxins:

1. **Superantigens (Type I toxins)**,
2. **Exotoxins that damage host cell membranes (Type II toxins)**
3. **A-B toxins and other toxin that interfere with host cell function (Type III toxins)**.

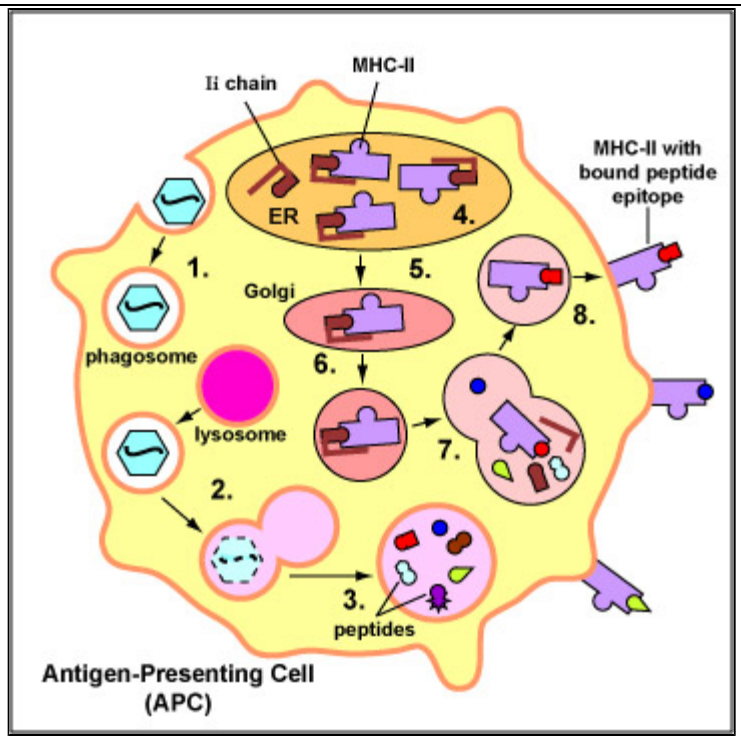
We will look at superantigens and their role in hypersensitivity.

Adaptive Immunity: Superantigens (Type 1 Toxins)

Superantigens are unusual bacterial toxins that **interact with exceedingly large numbers of T4-lymphocytes**. They bind to the surface of the target cell but do not enter the cell.

Conventional antigens are engulfed by antigen presenting cells (APCs), degraded into epitopes, bind to the peptide groove of MHC-II molecules, and are put on the surface of the APC **(see Fig. 1)**. Here they are **recognized by specific T4-lymphocytes having a TCR with a corresponding shape (see Fig. 2)**.

Fig. 1: Binding of Peptide Epitopes from Exogenous Antigens to MHC-II Molecules



Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (macrophages, dendritic cells, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

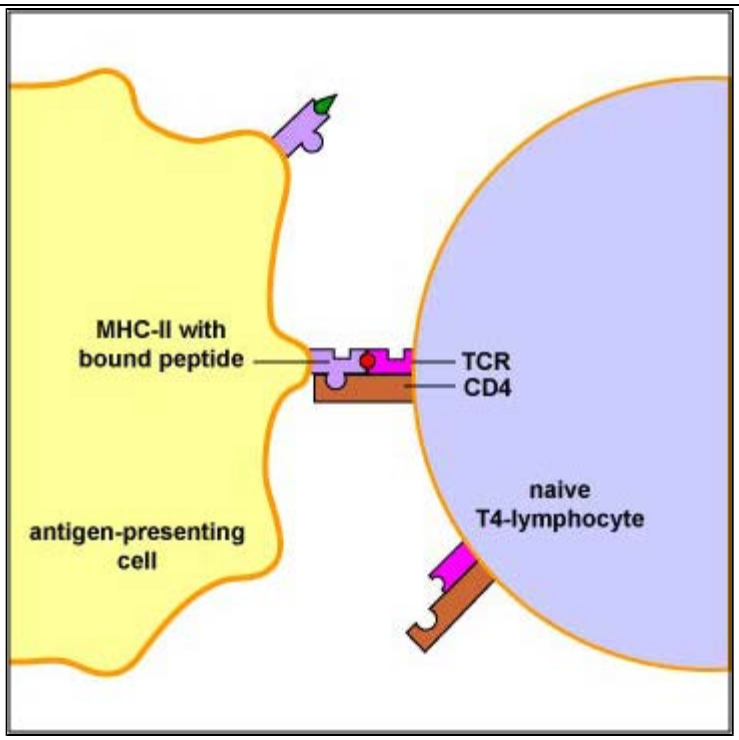
1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invarient chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.

5&6. The MHC-II molecules with bound Ii chain are now transported to the Golgi complex, and placed in vesicles.

7. The vesicles containing the MHC-II molecules fuse with the peptide-containing

phagolysosomes. The Ii chain is removed and the peptides are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.

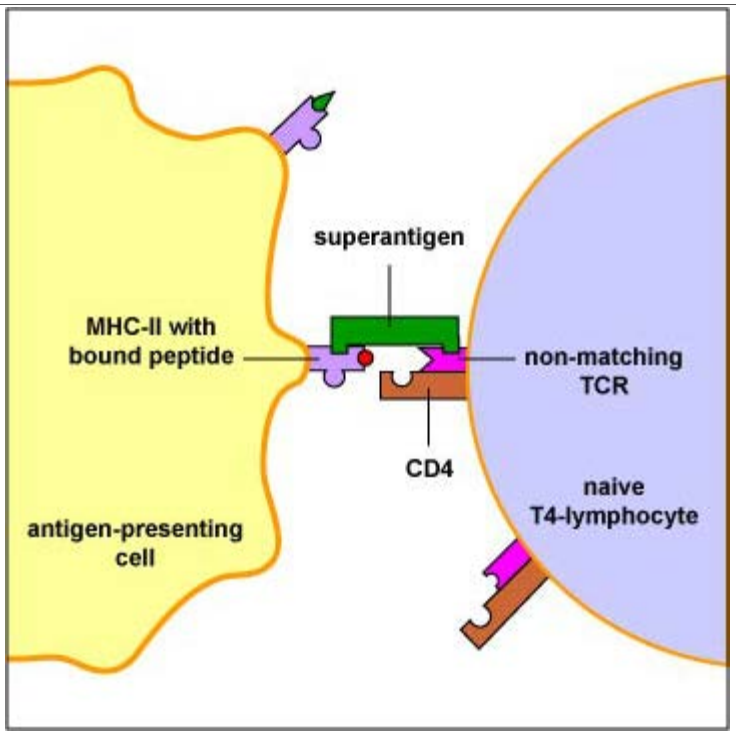
Fig. 2: Binding of T4-Lymphocytes to Conventional Antigens



Conventional antigens are only recognized by specific T4-lymphocytes having a TCR with a shape that corresponds to a peptide of that antigen bound to MHC-II molecules.

Superantigens, however, **bind directly to the outside of MHC-II molecules and activate large numbers of T4-lymphocytes (see Fig. 3)**. This activation of very large numbers of T4-lymphocytes results in the **secretion of excessive amounts of a cytokine called interleukin-2 (IL-2)** as well as the activation of self-reactive T-lymphocytes. The normal response to a conventional antigen results in the activation of maybe 1 in 10,000 T-lymphocytes; superantigens can activate as many as 1 in 5 T-lymphocytes.

Fig. 3: Binding of Superantigens



Conventional antigens are only recognized by specific T4-lymphocytes having a TCR with a shape that corresponds to a peptide of that antigen bound to MHC-II molecules. Superantigens, on the other hand, bind directly to the outside of MHC-II molecules and the TCRs and activate many T4-lymphocytes. A specific TCR is not required for activation.

Production of high levels of IL-2 can result in circulation of IL-2 in the blood leading to symptoms such as fever, nausea, vomiting, diarrhea, and malaise. However, **excess stimulation of IL-2 secretion can also lead to production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), inflammatory chemokines such as IL-8, and platelet-activating factor (PAF)**, and can lead to the same endothelial damage, acute respiratory distress syndrome, disseminated intravascular coagulation, shock, and multiple organ system failure seen above with LPS and other bacterial cell wall factors. Activation of self-reactive T-lymphocytes can also lead to autoimmune attack.

For more information: Review of the shock cascade

For more information: Preview of inflammation

The following are examples of superantigens.

1. **Toxic shock syndrome toxin-1 (TSST-1)**, produced by some strains of *Staphylococcus aureus.* This exotoxin causes toxic shock syndrome (TSS). Excessive cytokine production leads to fever, rash, and shock.

2. **Streptococcal pyrogenic exotoxin (Spe)**, produced by rare invasive strains and scarlet fever strains of *Streptococcus pyogenes* (the group A beta streptococci). *S pyogenes* produces a number of SPEs that are cytotoxic, pyrogenic, enhance

the lethal effects of endotoxins, and contribute to cytokine-induced inflammatory damage. SPEs are responsible for causing streptococcal toxic shock syndrome (STSS) whereby excessive cytokine production leads to fever, rash, and triggering the shock cascade. The SPEs also appear to be responsible for inducing necrotizing fasciitis, a disease that can destroy the skin, fat, and tissue covering the muscle (the fascia). SPE B is also a precursor for a cysteine protease that can destroy muscles tissue.

3. **Staphylococcal enterotoxins (SE)**, produced by many strains of *Staphylococcus aureus.* These exotoxins cause staphylococcal food poisoning. Excessive Il-2 production results in fever, nausea, vomiting,and diarrhea. The vomiting may also be due to these toxins stimulating the vagus nerve in the stomach lining that controls vomiting.

4. **ETEC enterotoxin**, produced by enterotoxogenic *E. coli* (ETEC), one of the most common causes of traveler's diarrhea.

Self Quiz for Superantigens

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