INNATE (NON-SPECIFIC) IMMUNITY

OVERVIEW OF THE IMMUNE SYSTEM

We are constantly being exposed to infectious agents and yet, in most cases, we are able to resist these infections. It is our immune system that enables us to resist infections. The immune system is composed of two major subdivisions, the innate or non-specific immune system and the adaptive or specific immune system (Figure 1). The innate immune system is our first line of defense against invading organisms while the adaptive immune system acts as a second line of defense and also affords protection against re-exposure to the same pathogen. Each of the major subdivisions of the immune system has both cellular and humoral components by which they carry out their protective function (Figure 1). In addition, the innate immune system also has anatomical features that function as barriers to infection. Although these two arms of the immune system have distinct functions, there is interplay between these systems (i.e., components of the innate immune system influence the adaptive immune system and vice versa).
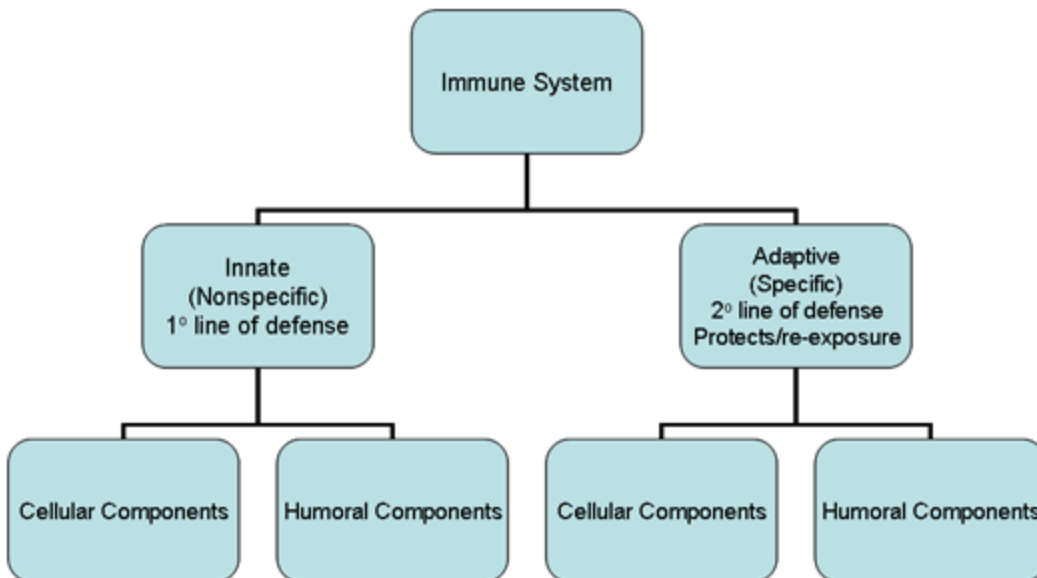

Figure 1

Overview of the immune system

Although the innate and adaptive immune systems both function to protect against invading organisms, they differ in a number of ways. The adaptive immune system requires some time to react to an invading organism, whereas the innate immune system includes defenses that, for the most part, are constitutively present and ready to be mobilized upon infection. Second, the adaptive immune system is antigen specific and reacts only with the organism that induced the response. In contrast, the innate system is not antigen specific and reacts equally well to a variety of organisms. Finally, the adaptive immune system demonstrates immunological memory. It "remembers" that it has encountered an invading organism and reacts more rapidly on subsequent exposure to the same organism. In contrast, the innate immune system does not demonstrate immunological memory.

All cells of the immune system have their origin in the bone marrow and they include myeloid (neutrophils, basophils, eosinpophils, macrophages and dendritic cells) and lymphoid (B lymphocyte, T lymphocyte and Natural Killer) cells (Figure 2), which differentiate along distinct pathways (Figure 3). The myeloid progenitor (stem) cell in the bone marrow gives rise to erythrocytes, platelets, neutrophils, monocytes/macrophages and dendritic cells whereas the lymphoid progenitor (stem) cell gives rise to the NK, T cells and B cells. For T cell development the precursor T cells must migrate to the thymus where they undergo differentiation into two distinct types of T cells, the CD4+ T helper cell and the CD8+ pre-cytotoxic T cell. Two types of T helper cells are produced in the thymus the TH1 cells, which help the CD8+ pre-cytotoxic cells to differentiate into cytotoxic T cells, and TH2 cells, which help B cells, differentiate into plasma cells, which secrete antibodies.
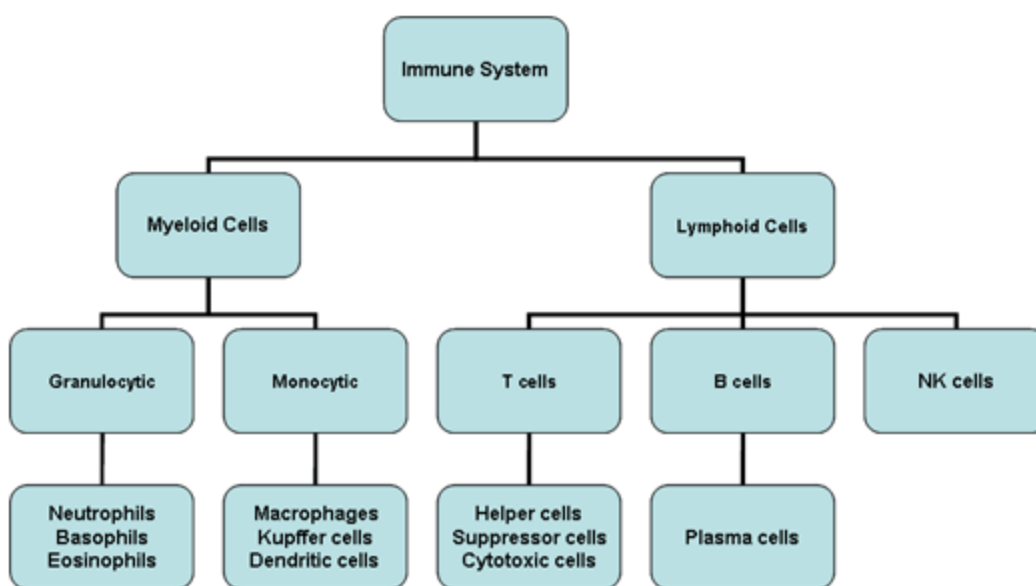

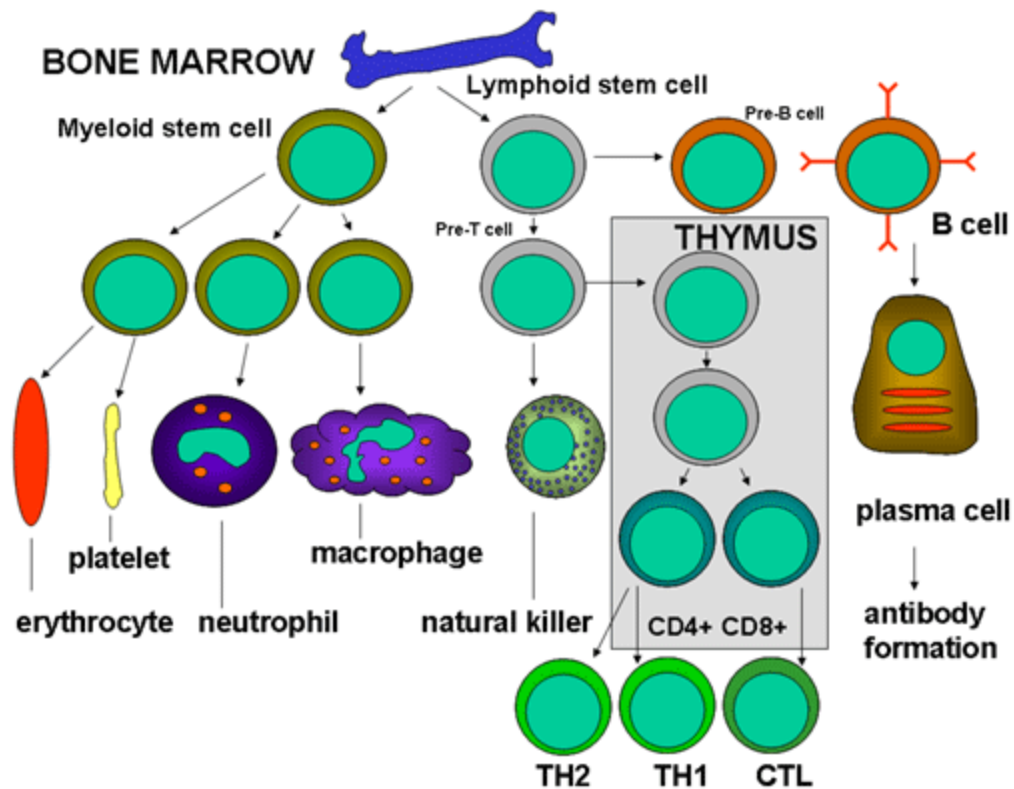
Figure 2

Cells of the immune system

Figure 3

Development of the cells of the immune system

The main function of the immune system is self/non-self discrimination. This ability to distinguish between self and non-self is necessary to protect the organism from invading pathogens and to eliminate modified or altered cells (e.g. malignant cells). Since pathogens may replicate intracellularly (viruses and some bacteria and parasites) or extracellularly (most bacteria, fungi and parasites), different components of the immune system have evolved to protect against these different types of pathogens. It is important to remember that infection with an organism does not necessarily mean diseases, since the immune system in most cases will be able to eliminate the infection before disease occurs. Disease occurs only when the bolus of infection is high, when the virulence of the invading organism is great or when immunity is compromised. Although the immune system, for the most part, has beneficial effects, there can be detrimental effects as well. During inflammation, which is the response to an invading organism, there may be local discomfort and collateral damage to healthy tissue as a result of the toxic products produced by the immune response. In addition, in some cases the immune response can be directed toward self tissues resulting in autoimmune disease.

Table 1	
Non-specific Immunity	**Specific Immunity**
Response is antigen-independent	Response is antigen-dependent
There is immediate maximal response	There is a lag time between exposure and maximal response
Not antigen-specific	Antigen-specific
Exposure results in no immunologic memory	Exposure results in immunologic memory

INNATE (NON-SPECIFIC) IMMUNITY

The elements of the innate (non-specific) immune system (Table 2) include anatomical barriers, secretory molecules and cellular components. Among the mechanical anatomical barriers are the skin and internal epithelial layers, the movement of the intestines and the oscillation of broncho-pulmonary cilia. Associated with these protective surfaces are chemical and biological agents.

Anatomical barriers to infections

Mechanical factors

The epithelial surfaces form a physical barrier that is very impermeable to most infectious agents. Thus, the skin acts as our first line of defense against invading organisms. The desquamation of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. Movement due to cilia or peristalsis helps to keep air passages and the gastrointestinal tract free from microorganisms. The flushing action of tears and saliva helps prevent infection of the eyes and mouth. The trapping effect of mucus that lines the respiratory and gastrointestinal tract helps protect the lungs and digestive systems from infection.

Chemical factors

Fatty acids in sweat inhibit the growth of bacteria. Lysozyme and phospholipase found in tears, saliva and nasal secretions can breakdown the cell wall of bacteria and destabilize bacterial membranes. The low pH of sweat and gastric secretions prevents growth of bacteria. Defensins (low molecular weight proteins) found in the lung and gastrointestinal tract have antimicrobial activity. Sweat also contains low molecular weight anti-microbial peptides that interact with bacterial cell membranes (including MRSA) in which they form a channel that allows the passage of water and ions, disrupting the transmembrane potential, leading to the death of the cell.

Surfactants in the lung act as opsonins (substances that promote phagocytosis of particles by phagocytic cells).

Biological factors

The normal flora of the skin and in the gastrointestinal tract can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces.

Humoral barriers to infection

The anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues the anatomical barriers are breached and infection may occur. Once infectious agents have penetrated tissues, another innate defense mechanism comes into play, namely acute inflammation. Humoral factors play an important role in inflammation, which is characterized by edema and the recruitment of phagocytic cells. These humoral factors are found in serum or they are formed at the site of infection.

Complement system

The complement system is the major humoral non-specific defense mechanism (see complement chapter). Once activated complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.

Coagulation   system
Depending on the severity of the tissue injury, the coagulation system may or may not be activated. Some products of the coagulation system can contribute to the non-specific defenses because of their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, beta-lysin, a protein produced by platelets during coagulation can lyse many Gram positive bacteria by acting as a cationic detergent.

Lactoferrin and transferrin

By binding iron, an essential nutrient for bacteria, these proteins limit bacterial growth.

Interferons
Interferons are proteins that can limit virus replication in cells.

Lysozyme
Lysozyme breaks down the cell wall of bacteria

.

Interleukin-1

Il-1 induces fever and the production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

Table 2. Physico-chemical barriers to infections		
System/Organ	**Active component**	**Effector Mechanism**
Skin	Squamous cells; Sweat	Desquamation; flushing, organic acids
GI tract	Columnar cells	Peristalsis, low pH, bile acid, flushing, thiocyanate
Lung	Tracheal cilia	Mucocialiary elevator, surfactant
Nasopharynx and eye	Mucus, saliva, tears	Flushing, lysozyme
Circulation and lymphoid organs	Phagocytic cells	Phagocytosis and intracellular killing
	NK cells and K-cell	Direct and antibody dependent cytolysis
	LAK	IL2-activated cytolysis
Serum	Lactoferrin and Transferrin	Iron binding
	Interferons	Antiviral proteins
	TNF-alpha	antiviral, phagocyte activation
	Lysozyme	Peptidoglycan hydrolysis
	Fibronectin	Opsonization and phagocytosis
	Complement	Opsonization, enhanced phagocytosis, inflammation

Cellular barriers to infection

Part of the inflammatory response is the recruitment of polymorphonuclear [eosinophiles](#) and macrophages to sites of infection. These cells are the main line of defense in the non-specific immune system.

Neutrophils

Polymorphonuclear cells (PMNs, figure 4) are recruited to the site of infection where they phagocytose invading organisms and kill them intracellularly. In addition, PMNs contribute to collateral tissue damage that occurs during inflammation.
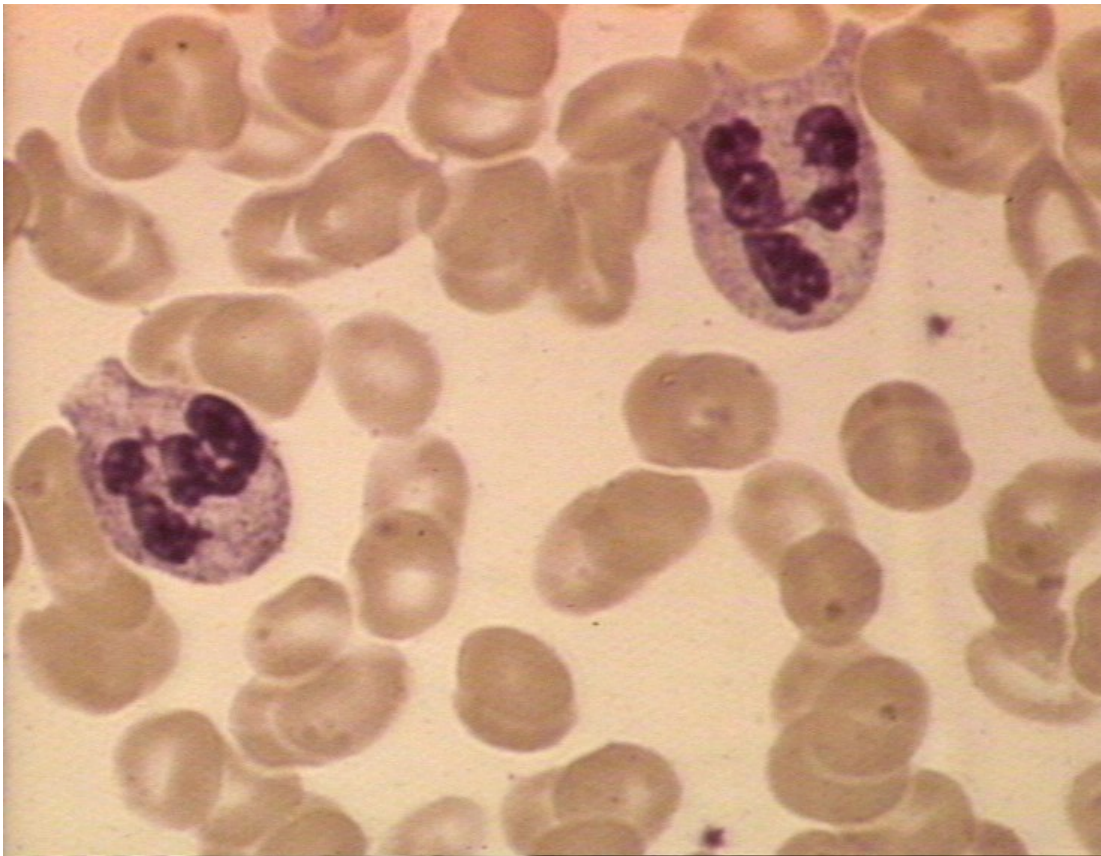

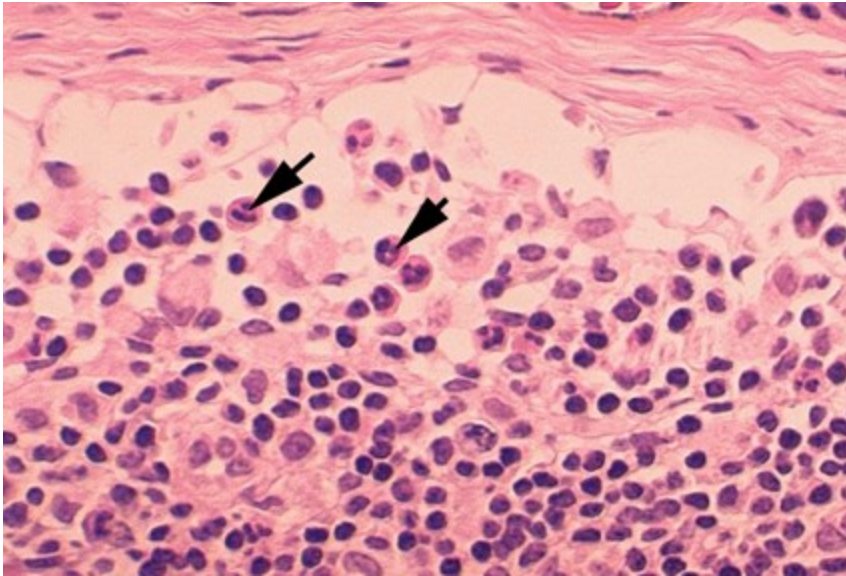Figure 4A Two neutrophils in blood film

Figure 4B Histopathology of lymphadenopathy due to infection by HIV-1. Subcapsular sinus. The sinus contains increased numbers of neutrophils.

Figure 4C

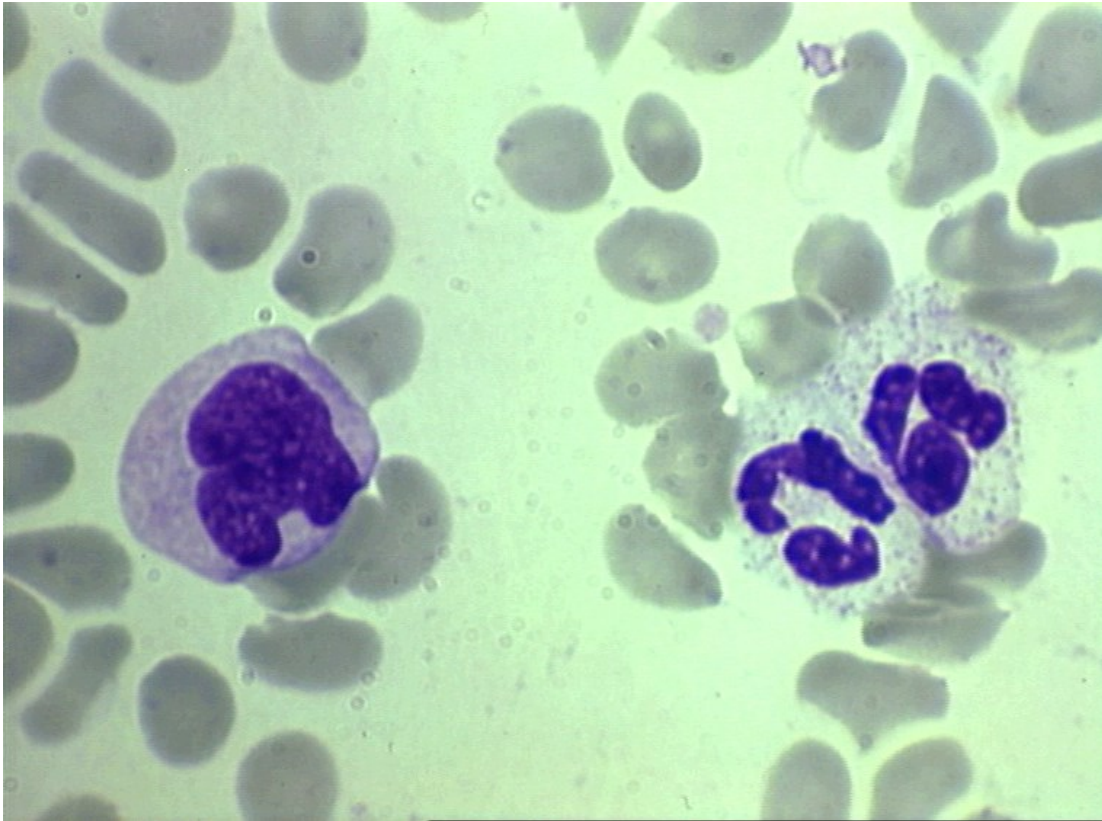Neutrophil - electron micrograph. Note the two nuclear lobes and the azurophilic granules

Blood film showing a monocyte (left) and two neutrophils

Macrophages

Tissue macrophages (figure 5, 6, 7) and newly recruited monocytes (figure 4 and 8), which differentiate into macrophages, also function in phagocytosis and intracellular killing of microorganisms. In addition, macrophages are capable of extracellular killing of infected or altered self target cells. Furthermore, macrophages contribute to tissue repair and act as antigen-presenting cells, which are required for the induction of specific immune responses.

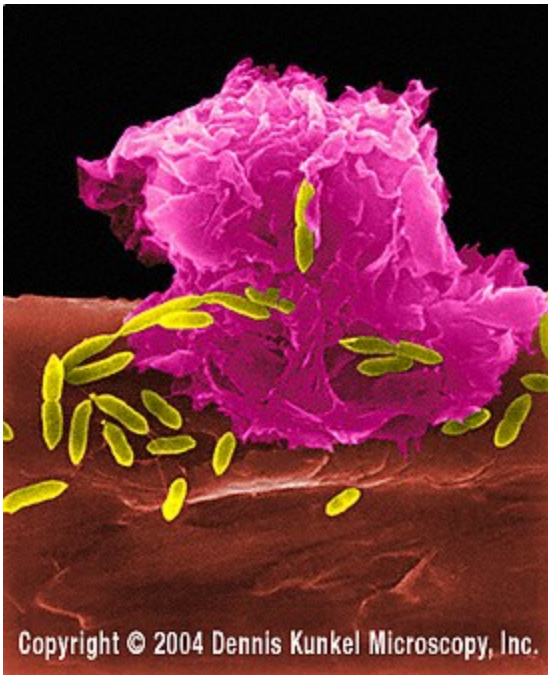Figure   5

Macrophage Attacking E.coli (SEM x8,800)

Figure 6

Alveolar (Lung) Macrophage Attacking E. coli (SEM x10,000)

Natural killer (NK) and lymphokine activated killer (LAK) cells

NK and LAK cells can nonspecifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in nonspecific immunity to viral infections and tumor surveillance.

Eosinophils

Eosinophils (figure 6a and b) have proteins in granules that are effective in killing certain parasites.
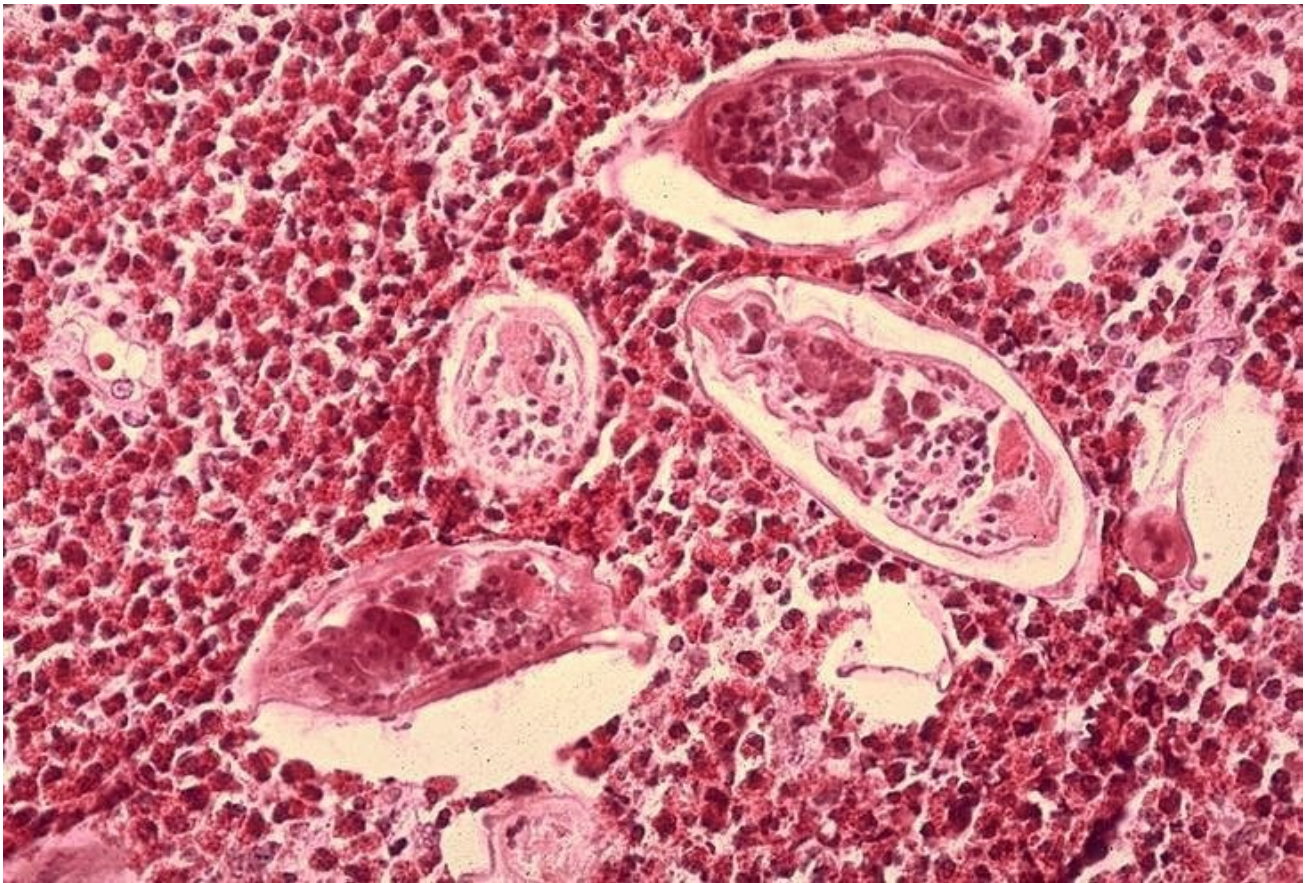
Figure 6A Eosinophil in blood film

Figure 6B

Histopathology of bladder shows eggs of Schistosoma haematobium surrounded by intense infiltrates of eosinophils

PHAGOCYTOSIS AND INTRACELLULAR KILLING

Phagocytic cells

Neutrophiles/Polymorphonuclear cells

PMNs are motile phagocytic cells that have lobed nuclei. They can be identified by their characteristic nucleus or by an antigen present on the cell surface called CD66. They contain two kinds of granules the contents of which are involved in the antimicrobial properties of these cells. The primary or azurophilic granules, which are abundant in young newly formed PMNs, contain cationic proteins and defensins that can kill bacteria, proteolytic enzymes like elastase, and cathepsin G to breakdown proteins, lysozyme to break down bacterial cell walls, and characteristically, myeloperoxidase, which is involved in the generation of bacteriocidal compounds. The second type of granule found in more mature PMNs is the secondary or specific granule. These contain lysozyme, NADPH oxidase components, which are involved in the generation of toxic oxygen products, and characteristically lactoferrin, an iron chelating protein and B12-binding protein.

Monocytes/Macrophages

Macrophages are phagocytic cells that have a characteristic kidney-shaped nucleus. They can be identified morphologically or by the presence of the CD14 cell surface marker. Unlike PMNs they do

not contain granules but they have numerous lysosomes which have contents similar to the PNM granules.
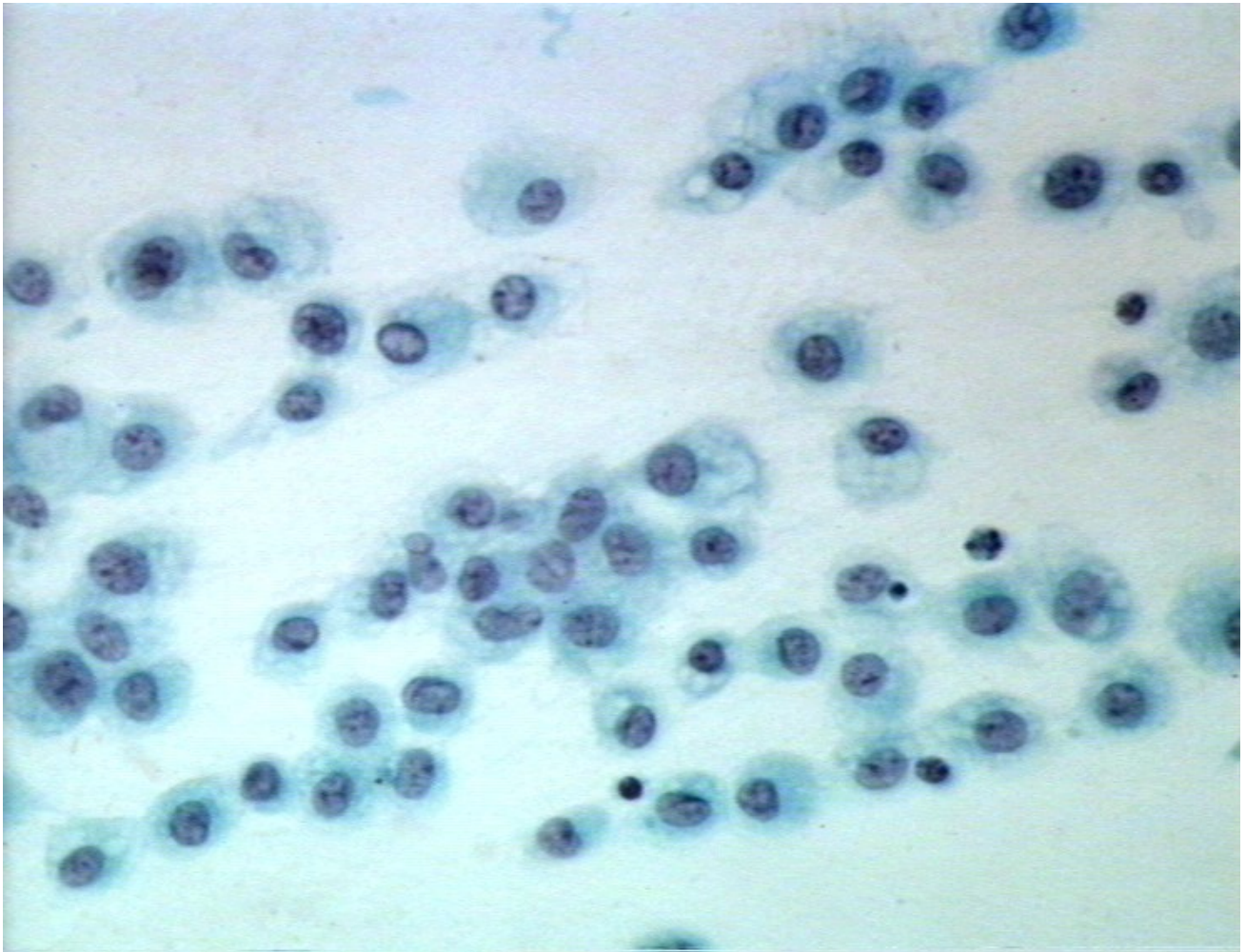

Figure 7

Histiocytes - Long lived resident macrophage found within tissues

Figure 8 Monocyte with ingested malaria parasite.

Response of phagocytes to infection

Circulating PMNs and monocytes respond to danger (SOS) signals generated at the site of an infection. SOS signals include N-formyl-methionine containing peptides released by bacteria, clotting system peptides, complement products and cytokines released from tissue macrophages that have encountered bacteria in tissue. Some of the SOS signals stimulate endothelial cells near the site of the infection to express cell adhesion molecules such as ICAM-1 and selectins which bind to components on the surface of phagocytic cells and cause the phagocytes to adhere to the endothelium. Vasodilators produced at the site of infection cause the junctions between endothelial cells to loosen and the phagocytes then cross the endothelial barrier by "squeezing" between the endothelial cells in a process called diapedesis (Figure 9). Once in the tissue spaces some of the SOS signals attract phagocytes to the infection site by chemotaxis (movement toward an increasing chemical gradient). The SOS signals also activate the phagocytes, which results in increased phagocytosis and intracellular killing of the invading organisms.
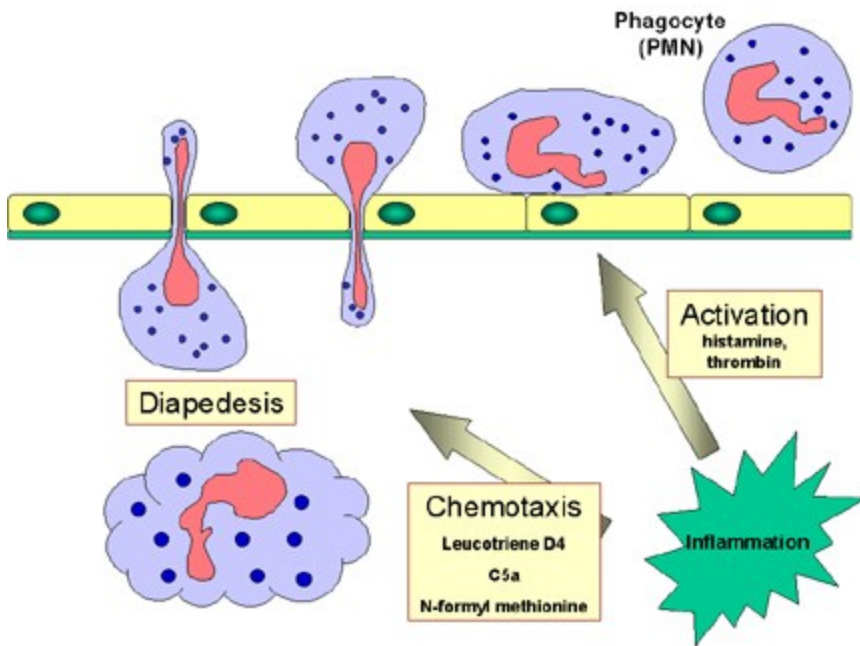
Figure 9 Chemotactic response to inflammatory stimulus

Initiation of Phagocytosis (Figure 10)

Phagocytic cells have a variety of receptors on their cell membranes through which infectious agents bind to the cells. These include:

Fc receptors

Bacteria with IgG antibody on their surface have the Fc region exposed and this part of the Ig molecule can bind to the receptor on phagocytes. Binding to the Fc receptor requires prior interaction of the antibody with an antigen. Binding of IgG-coated bacteria to Fc receptors results in enhanced phagocytosis and activation of the metabolic activity of phagocytes (respiratory burst).

Complement receptors

Phagocytic cells have a receptor for the 3rd component of complement, C3b. Binding of C3b-coated bacteria to this receptor also results in enhanced phagocytosis and stimulation of the respiratory burst.

Scavenger receptors

Scavenger receptors bind a wide variety of polyanions on bacterial surfaces resulting in phagocytosis of bacteria.

Toll-like receptors

Phagocytes have a variety of Toll-like receptors (Pattern Recognition Receptors or PRRs) which recognize broad molecular patterns called PAMPs (pathogen associated molecular patterns) on

infectious agents. Binding of infectious agents via Toll-like receptors results in phagocytosis and the release of inflammatory cytokines (IL-1, TNF-alpha and IL-6) by the phagocytes.
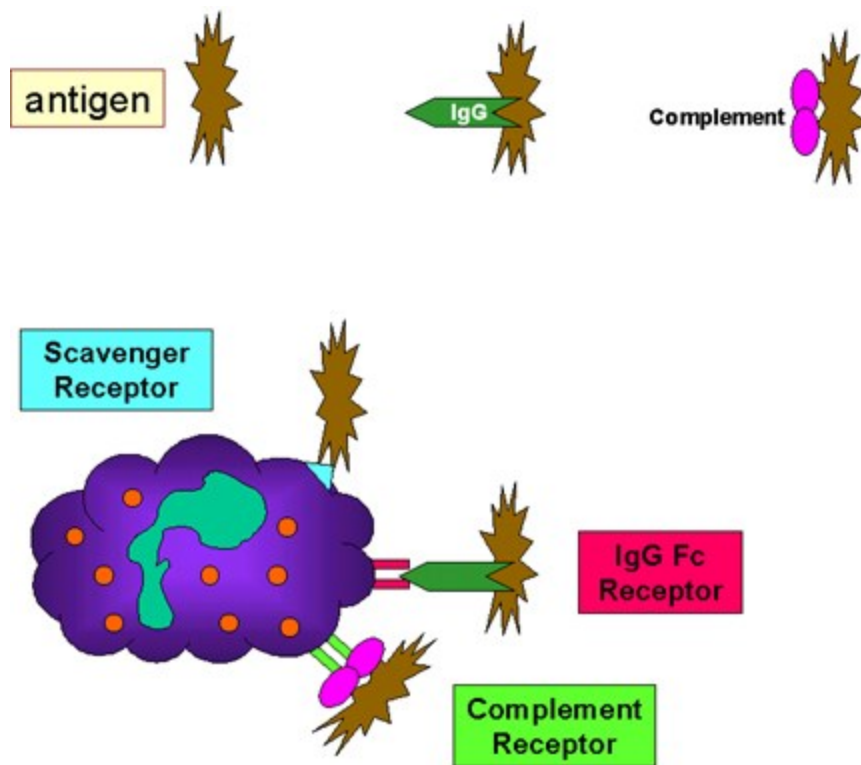

Figure 10 Adherence of bacteria via receptors

Phagocytosis

After attachment of a bacterium, the phagocyte begins to extend pseudopods around the bacterium. The pseudopods eventually surround the bacterium and engulf it, and the bacterium is enclosed in a phagosome. During phagocytosis the granules or lysosomes of the phagocyte fuse with the phagosome and empty their contents. The result is a bacterium engulfed in a phagolysosome which contains the contents of the granules or lysosomes.

Respiratory burst and intracellular killing

During phagocytosis there is an increase in glucose and oxygen consumption which is referred to as the respiratory burst. The consequence of the respiratory burst is that a number of oxygen-containing compounds are produced which kill the bacteria being phagocytosed. This is referred to as oxygen-dependent intracellular killing. In addition, bacteria can be killed by pre-formed substances released from granules or lysosomes when they fuse with the phagosome. This is referred to as oxygen-independent intracellular killing.

Oxygen-dependent myeloperoxidase-independent intracellular killing (Figure11A)

During phagocytosis glucose is metabolized via the [pentose monophosphate shunt](#) and NADPH is formed. Cytochrome B which was part of the specific granule combines with the plasma membrane NADPH oxidase and activates it. The activated NADPH oxidase uses oxygen to oxidize the NADPH. The result is the production of superoxide anion. Some of the superoxide anion is converted to H2O2 and singlet oxygen by superoxide dismutase. In addition, superoxide anion can react with H2O2 resulting in the formation of hydroxyl radical and more singlet oxygen. The result of all of these reactions is the production of the toxic oxygen compounds superoxide anion (O_2-), H_2O_2, singlet oxygen ($^1O_2$) and hydroxyl radical (OH•).

$$\frac{Glucose + NADP^+}{G\text{-}6\text{-}P\text{-}dehydrogenase} \longrightarrow Pentose\text{-}P + NADPH$$

$$\frac{NADPH + O_2}{Cytochrome_{\,b558}} \longrightarrow NADP^+ + O_2^-$$

$$\frac{2O_2^- + 2H^+}{Superoxide\ dismutase} \longrightarrow H_2O_2 + {}^1O_2$$

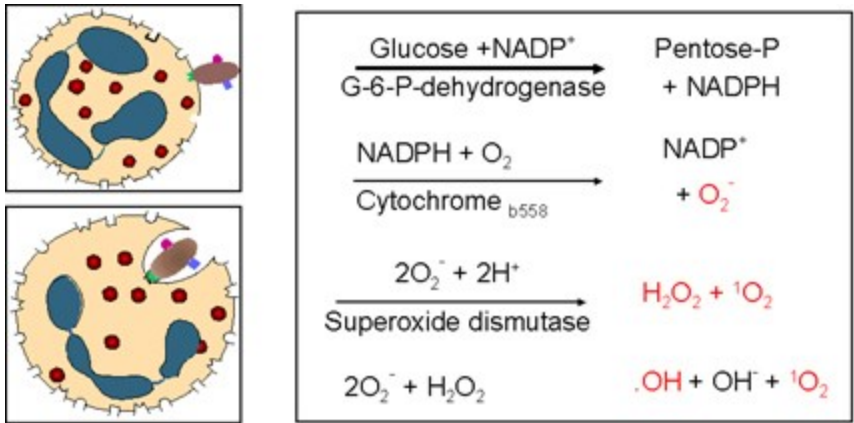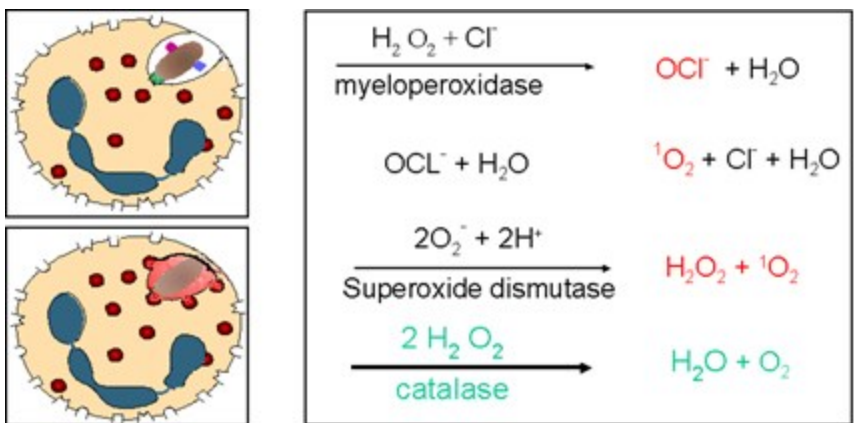$$2O_2^- + H_2O_2 \longrightarrow \cdot OH + OH^- + {}^1O_2$$

Figure11

A. Respiratory burst: Oxygen-dependent, myeloperoxidase-independent reactions

Oxygen-dependent myeloperoxidase-dependent intracellular killing (Figure 11B)

As the azurophilic granules fuse with the phagosome, myeloperoxidase is released into the phagolysosome. Myeloperoxidase utilizes H_2O_2 and halide ions (usually Cl-) to produce hypochlorite, a highly toxic substance. Some of the hypochlorite can spontaneously break down to yield singlet oxygen. The result of these reactions is the production of toxic hypochlorite (OCl-) and singlet oxygen ($^1O_2$).

$$\frac{H_2O_2 + Cl^-}{myeloperoxidase} \longrightarrow OCl^- + H_2O$$

$$OCL^- + H_2O \longrightarrow {}^1O_2 + Cl^- + H_2O$$

$$\frac{2O_2^- + 2H^+}{Superoxide\ dismutase} \longrightarrow H_2O_2 + {}^1O_2$$

$$\frac{2\,H_2O_2}{catalase} \longrightarrow H_2O + O_2$$

B. Respiratory burst: Oxygen-dependent, myeloperoxidase-dependent reactions

Detoxification reactions (Table 3)

PMNs and macrophages have means to protect themselves from the toxic oxygen intermediates. These reactions involve the dismutation of superoxide anion to hydrogen peroxide by superoxide dismutase and the conversion of hydrogen peroxide to water by catalase.

Table 3	
Reaction	**Enzyme**
$H_2O_2 + Cl^- \rightarrow OCl^- + H_2O$ $OCl^- + H_2O \rightarrow {}^1O_2 + Cl^- + H_2O$	Myeloperoxidase
$2O_2 + 2H^+ \rightarrow O_2^- + H_2O_2$	Superoxide dismutatse
$H_2O_2 \rightarrow H_2O + O_2$	Catalase

Oxygen-independent intracellular killing (table 4)

In addition to the oxygen-dependent mechanisms of killing there are also oxygen–independent killing mechanisms in phagocytes: cationic proteins (cathepsin) released into the phagolysosome can damage bacterial membranes; lysozyme breaks down bacterial cell walls; lactoferrin chelates iron, which deprives bacteria of this required nutrient; hydrolytic enzymes break down bacterial proteins. Thus, even patients who have defects in the oxygen-dependent killing pathways are able to kill bacteria. However, since the oxygen-dependent mechanisms are much more efficient in killing, patients with defects in these pathways are more susceptible and get more serious infections.

Table 4. Oxygen-independent mechanisms of intracellular killing	
Effector Molecule	**Function**
Cationic proteins (including cathepsin)	Damage to microbial membranes
Lysozyme	Splits mucopeptide in bacterial cell wall
Lactoferrin	Deprives proliferating bacteria of iron
Proteolytic and hydrolytic enzymes	Digestion of killed organisms

NITRIC OXIDE-DEPENDENT KILLING

Binding of bacteria to macrophages, particularly binding via Toll-like receptors, results in the production of TNF-alpha, which acts in an autocrine manner to induce the expression of the inducible nitric oxide synthetase gene (i-nos) resulting in the production of nitric oxide (NO) (figure 12). If the cell is also exposed to interferon gamma (IFN-gamma) additional nitric oxide will be produced (figure 12). Nitric oxide released by the cell is toxic and can kill microorganism in the vicinity of the macrophage.
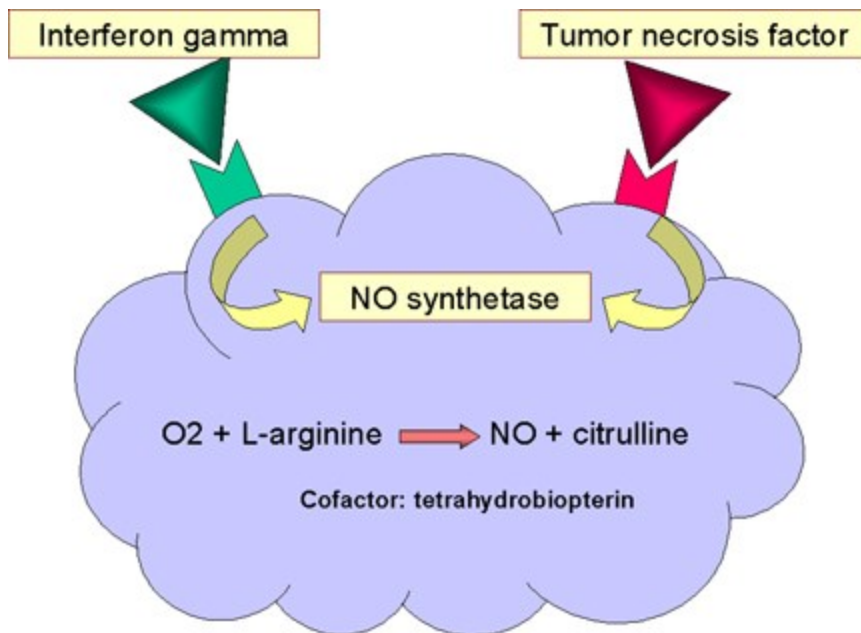

Figure 12 Nitric oxide-dependent killing

NON-SPECIFIC KILLER CELLS

Several different cells including NK and LAK cells, K cells, activated macrophages and eosinophils are capable of killing foreign and altered self target cells in a non-specific manner. These cells play an important role in the innate immune system.

NK and LAK cells

Natural killer (NK) cells are also known as large granular lymphocytes (LGL) because they resemble lymphocytes in their morphology, except that they are slightly larger and have numerous granules. NK cells can be identified by the presence of CD56 and CD16 and a lack of CD3 cell surface markers. NK cells are capable of killing virus-infected and malignant target cells but they are relatively inefficient in doing so. However, upon exposure to IL-2 and IFN-gamma, NK cells become lymphokine-activated killer (LAK) cells, which are capable of killing malignant cells. Continued exposure to IL-2 and IFN-gamma enables the LAK cells to kill transformed as well as malignant cells. LAK cell therapy is one approach for the treatment of malignancies.

How do NK and LAK cells distinguish a normal cell from a virus-infected or malignant cell? NK and LAK cells have two kinds of receptors on their surface – a killer activating receptor (KAR) and a killer inhibiting receptor (KIR). When the KAR encounters its ligand, a killer activating ligand (KAL) on the

target cell the NK or LAK cells are capable of killing the target. However, if the KIR also binds to its ligand then killing is inhibited even if KAR binds to KAL. The ligands for KIR are MHC-class I molecules. Thus, if a target cell expresses class I MHC molecules it will not be killed by NK or LAK cells even if the target also has a KAL which could bind to KAR. Normal cells constitutively express MHC class I molecules on their surface, however, virus infected and malignant cells down regulate expression of class I MHC. Thus, NK and LAK cells selectively kill virus-infected and malignant cells while sparing normal cells.
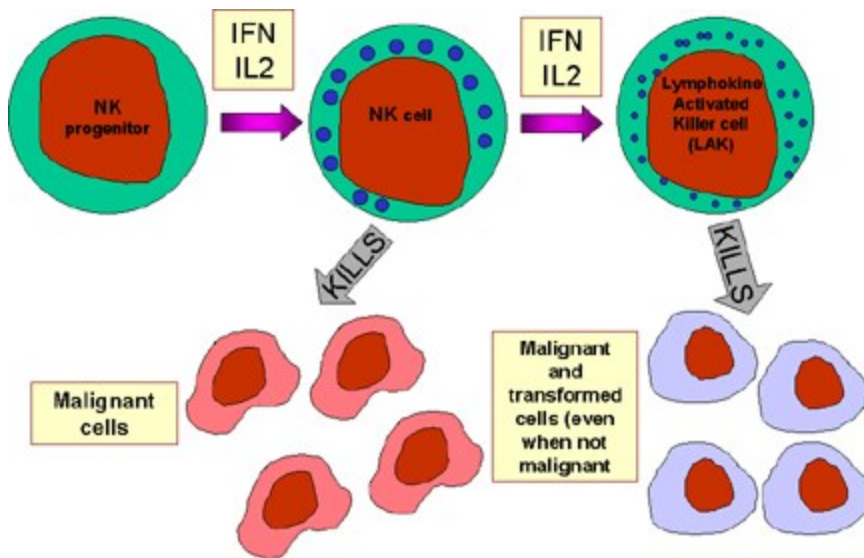

Figure 13 NK cells and their activation

K cells (Figure 14)

Killer (K) cells are not a morphologically distinct type of cell. Rather a K cell is any cell that mediates antibody-dependent cellular cytotoxicity (ADCC). In ADCC antibody acts as a link to bring the K cell and the target cell together to allow killing to occur. K cells have on their surface an Fc receptor for antibody and thus they can recognize, bind and kill target cells coated with antibody. Killer cells which have Fc receptors include NK, LAK, and macrophages which have an Fc receptor for IgG antibodies and eosinophils which have an Fc receptor for IgE antibodies.

NK cell   Target cell
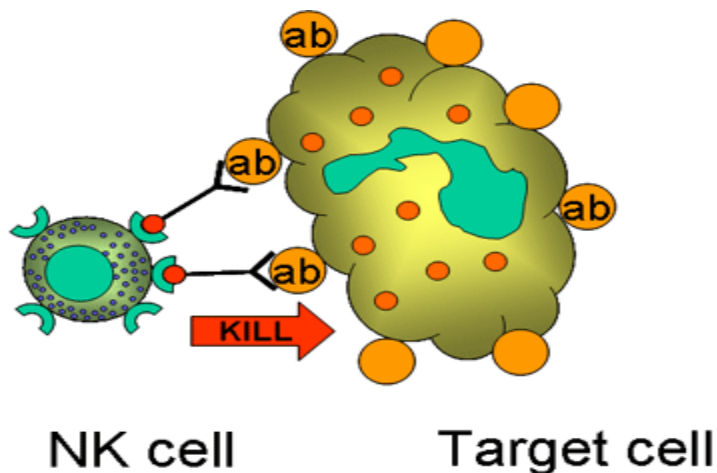
Figure 14

Killing of opsonised target by by K cell

All components of the non-specific immune system are modulated by products of the specific immune system, such as interleukins, interferon-gamma, antibody, etc.

Table 5. Characteristics of cells involved in non-specific resistance					
	Identifying marker(s) and/or function				
Effector cell	**CD3**	**Ig**	**Fc**	**CD**	**Phagocytosis**
Neutrophil	-	-	IgG	CD67	+
Macrophage	-	-	IgG	CD14	+
NK cell	-	-	IgG	CD56 & 16	-
K-cells	-	-	IgG	?	-
LAK cell	-	-	?	?	?
Eosinophil	-	-	IgE	CD67	-

At this time you should know the following:

1. Differences between the non-specific and specific immune functions

2. Humoral components of the non-specific immune system and their action

3. Cellular components of the non-specific immune function and their action

4. Pathways of intracellular killing of bacteria by phagocytes and their characteristic features

5. Effect of humoral components such as interferon, TNF, IL-2, complement etc. on cellular components of the non-specific immune system

ACQUIRED (SPECIFIC) IMMUNITY

INTRODUCTION

Adaptive immunity is created after an interaction of lymphocytes with particular foreign substances which are recognized **specifically** by those lymphocytes. This recognition process triggers proliferation and maturation of the lymphocytes which in the case of **B lymphocyte** results in the **secretion** of **antibodies** and the **"memorizing"** of that particular agent in a process called the **primary immune response**. **On the second contact** with the same agent the **magnitude** of the response is increased as a result of the **more rapid** and more **abundant** production of specific antibodies: a process called **secondary immune response**.

The adaptive immune response is a more highly developed system than the innate immune system. It includes not only humoral immunity but also cellular immunity, the production of specific-lymphocytes. As its name implies, acquired immunity is a consequence of an encounter with a foreign substance. The first encounter with a foreign substance that has penetrated the body triggers a chain of events that induces an immune response with specificity against the foreign substance. Although an individual is genetically endowed with the capacity to mount an immune response against a certain substance, acquired immunity is usually exhibited only after an initial encounter with the substance. Thus acquired immunity develops only after exposure to, or **immunization** with, a given substance. Development of the adaptive immunity requires specific **immune responses**.

IMMUNE RESPONSE

When an individual exposed to non-self substance either by injection or infection, a complex series of events are created:

a. An **antigen-presenting cell** (usually a macrophage) processes the antigen and presents it to the **lymphoid cells** of the immune system.

    (1) For a **successful immune response** to occur, the **processed antigen** (specifically, its **epitope**) must be **presented to lymphocytes** in association with a glycoprotein encoded by genes of the major histocompatibility complex (MHC).

    (2) This requirement for effective cell interaction is called **MHC restriction**.

b. The lymphoid cells recognize that particular epitope and acquire the ability to react with it.

c. The result of these consequences of events is the **activation of antigen-specific B and T cells,** causing them to proliferate and mature.

The consequences of the initial interaction between lymphocytes and their homologous epitopes are far-reaching.

d. A subsequent exposure to antigen will induce some **B lymphocytes** (memory B cells) to proliferate and differentiate into **antibody-secreting plasma cells.**

    (1) These **active plasma cells** secrete their specific antibody in large amounts when they contact antigen a second time, a phenomenon known as **anamnesis.**

    (2) The **secreted antibodies** react specifically with the antigen that originally induced the B cell to proliferate. The potential exists to produce an extremely large (> 100,000) variety of different, specifically reactive, antibodies.

e. Some **T lymphocytes** (memory T cells) are induced to differentiate and proliferate to form mature progeny that will be triggered to release biologically active metabolites when they contact antigen a second time.

The immune response is under highly complex genetic control. Most of the genes that code for chain segments of the immunoglobulin molecule or the T cell receptor are polycistronic (present in the cell in many forms). The process of **DNA re-arrangement and deletion,** followed by RNA splicing, selects alleles that code for a particular **immunologic specificity**.

# CELLULAR ACQUIRED IMMUNITY E

The cells participate in the immune response arise from pluripotent stem cells through two main lines of differentiation. All acquire instructions from the T-lymphocyte called T-helper cells. These cells are:

● the lymphoid lineage produces lymphocytes
● the myeloid lineage produces phagocytes (monocytes, macrophages, and neutrophils) and other cells.

● the accessory cells which include:

A. Antigen presenting cells (APCs) present antigen to T cells.
B. Mast cells which is structurally and functionally similar to basophils
C. Endothelial cells, which can recognize certain lymphocytes by expressing some surface molecules, and through this way, these cells can control the distribution of the lymphocytes.
D. Platelets which is one of the major components of the clotting system and inflammation.

LYMPHOCYTES

Lymphocytes are produced in the central lymphoid or primary lymphoid organs (Thymus and adult bone marrow), some of these cells migrate to the secondary lymphoid organs, such as tonsil, lymph nodes, spleen, and mucosa-associated lymphoid tissues. The lymphocytes represent about 20% of the circulating human leukocytes. The lymphoid memory cells are of long-lived cells, they can circulate for several years or even the lifetime of the individual. The morphology of the lymphocytes is heterogeneous. They have different sizes range from 6-10 micro-meters. The differences also can be recognized in the nuclear to cytoplasmic ratio (N: C ratio), the presence or absence of the azurophilic granules, and the nuclear shape.

Two types of lymphocytes can be seen in the conventional blood smear stained with Giemsa stain. One population is relatively small with high N:C ratio, and **a** granular lymphocytes. The other population shows a low N:C ratio, with intracytoplasmic azurophilic granules, these are called (LGL) large granular lymphocytes **(Figure 1-1, 2,3, and 4)**.
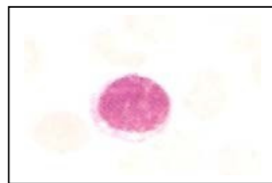

Figure 1-1 Morphological heterogeneity of lymphocytes. The small lymphocyte has no granules, around nucleus nada high N:C ration.
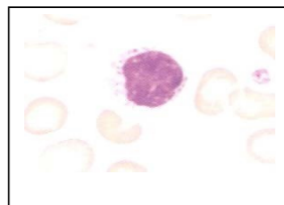
Figure 1-2 Morphological heterogeneity of lymphocytes. The large granular lymphocytes has a lower N:C ration, indented nucleus and azurophilic granules in the cytoplasm.

Resting blood T lymphocytes

These cells can be either LGL or small lymphocytes. The majority (>90%) of T helper cells and proportion (>65%) of cytotoxic T (Tc) cells are of the small type(non-granular with a high N:C ratio). They also contain in their cytoplasm a structure called **Gall body** (a cluster of primary lysosomes associated with a lipid droplet). The rest of these cells of LGL morphology with primary lysosomes dispersed in the cytoplasm and a well developed Golgi apparatus. The gamma/delta or TCR-1⁺ lymphocytes population is another subset of T cells with LGL morphology.
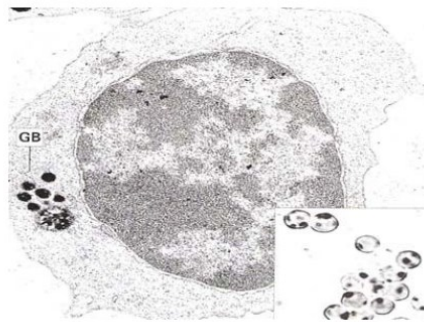
Figure 1-3 Ultrasturcture of non-granular T cell. This electron micrograph shows the **Gall body (GB)** that is characteristic of the majority of resting T cells. It consists of primary lymsosomes and a lipid droplet. This structure is also seen as a single "spot" after staining for non-specific esterases in light microscopy.

Figure 1-4 Ultrasturcture of T cells with granular morphology. These cells characteristically have electron dense peroxide .negative granules (primary lysosomes, pl), scattered throughout the cytoplasm, with some close to the Golgi apparatus (GA). There are many mitochondria (M) present.
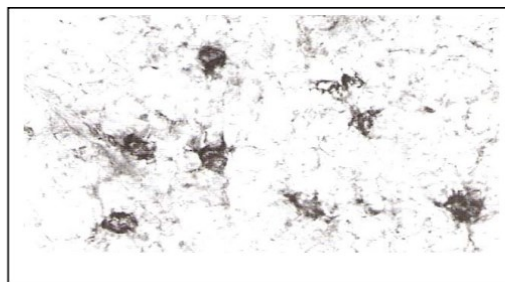

Figure 1-5 dendritic morphology of γδ T cells in the tonsil. This T-cell population is predominantly localized into intermolecular T cell-dependent zones. Note the dendritic morphology of the cells. Anti-γδ T cell mAb and immunoperoxidase.

Resting blood B lymphocytes

These cells do not show Gall body or LGL morphology, and contain scattered single ribosomes in their cytoplasm, when activated, the rough endoplasmic reticulum are recognized. B cell stained for antibody **(Figure 1-6, 7, 8)**.
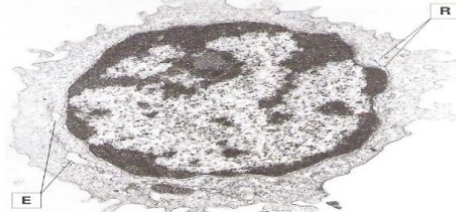
Figure 1-6 Ultrasturcture of resting B cells. These cells have no Gall body or granules. Scattered ribosomes (R) and isolated strands of rough endoplasmic reticulum (E) are seen in the cytoplasm. Development of Golgi-lysosomal system in the B cell occurs on activation.

Figure 1-7 Ultrasturcture of B cell blasts. The main feature of activated B cells is the development of the machinery for immunoglobulin synthesis. This includes rough endoplasmic reticulum (E), free polyribosome and the Golgi apparatus (GA), which is involved in glycosylation of the immunoglobulins.
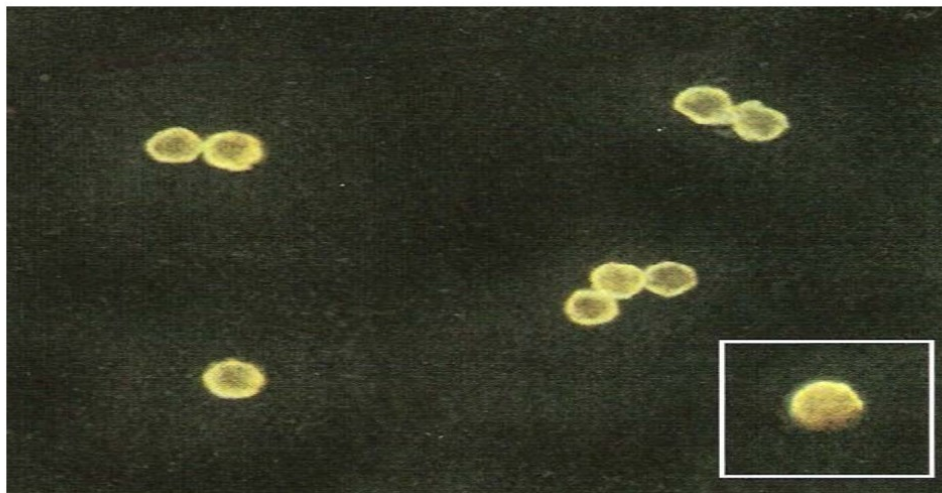
Figure 1-8 B cells stained for surface immunoglobulin. B cells stained with fluorescent anti-IgM in the cold show a surface ring-like pattern under UV light. A polar redistribution (capping) is seen when the cells are incubated at 37° C in the presence of the antibody (inset).

MONONUCLEAR PHAGOCYTES

The phagocytic tissue macrophages, together with endothelial cells, were previously termed as "the reticuloendothelial system (RES). These cells perform two main functions:

● Professional phagocytic macrophages, which remove particulate antigens.
● Antigen-presenting cells (APCs), which bind, process, and present antigen to **T cells.**

ANTIGEN- PRESENTING   CELLS (APCs)

These cells are rich in class II MHC molecules, which are important for presenting antigen to TH cells. APCs are a heterogeneous population of leukocytes. Cells other than leukocytes such as endothelial or epithelial cells can be classified as APCs. These cells can presents antigens to T cells when they stimulated with cytokines. APCs are found mainly in lymph nodes e.g., Follicular dendretic cells **(Figure 1-9)**, and in skin e.g., Langerhans'cells **(Figure 1-10)**, thymus, and spleen.
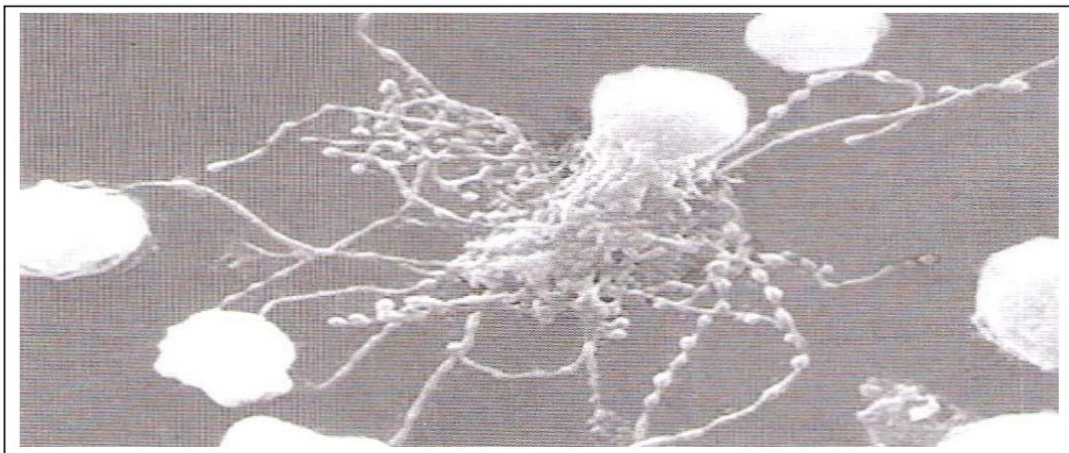
Figure 1-9 Follicular dendritic cells (FDC). An isolated follicular dendritic cell from the lymph node, the FDC is of intermediate maturity with smooth filiform dendrites typical of young FDCs, and beaded dendrites which participate in the formation of incosomes of mature FDCs. The adjacent small white cells are lymphocytes.

The characteristic properties of the adaptive immunity

1. The ability to **distinguish** self from **non-self**.
2. The **induction phase** which is the time required for lymphocytes to proliferate and maturate into antibody-secrete lymphokines and cytotoxins.
3. Specificity developed when the immune system selects antibodies and lymphocytes for responding to their antigens.
4. An **immunologic memory** that allows sensitized lymphocytes to remember their recognized antigen and respond to them later producing elements of both cellular and humoral immunity.

Lymphatic Organs

The **lymphatic organs** are those organs in which maturation, differentiation, and proliferation of lymphocytes take place. **Lymphocytes** are derived from the pluripotential **haematopoietic bone marrow stem cells,** which give rise to all blood cells. The **erythroid** and **myeloid** cells, which differentiate into erythrocytes and granulocytes, are derived from these stem cell progenitors. Lymphoid progenitor cells differentiate into lymphocytes.

The lymphoid organs are generally divided into two categories: the **primary or central, lymphoid organs** are those in which the maturation of T and **B** lymphocytes into antigen recognizing lymphocytes takes place. The **secondary lymphoid organs** are those organs in which antigen driven proliferation and differentiation take place.

Primary lymphoid organs

There are two major primary lymphoid organs, one in which the T cells develop and the other in which the B cells develop. The **Bone marrow** and the **Thymus gland** is illustrated in **(Figure 1-11)**. The thymus gland is a bilobed structure, derived from the endoderm of the third and fourth pharyngeal pouches. It reaches its maximum size around birth, after which it begins to decrease in size and undergoes atrophy with aging.
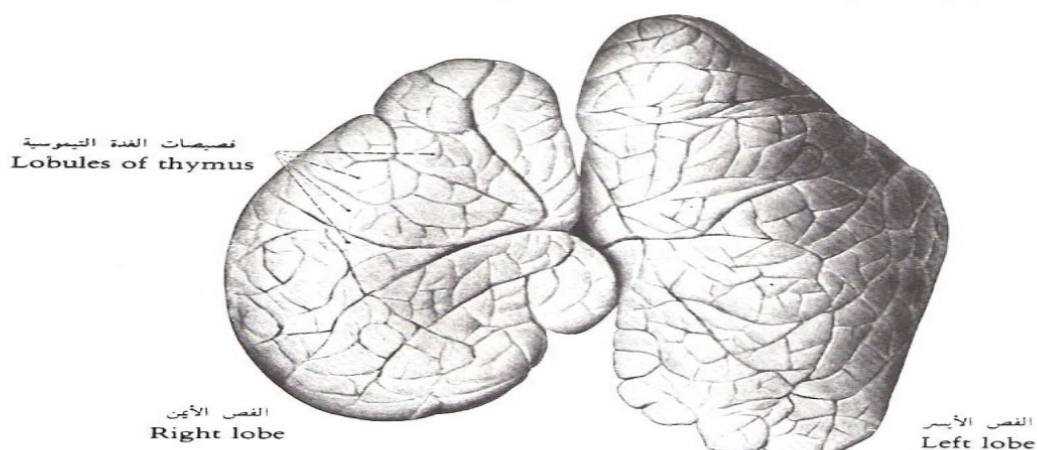

فصيصات الغدة التيموسية
Lobules of thymus

الفص الأيمن
Right lobe

الفص الأيسر
Left lobe

Figure 1-11. A diagrammatic representation of right and left lobes of the thymus gland.

Secondary lymphoid organs

These represent the locations where the pathogenic recognition occurs, these organs are; the spleen (Figure 1-2), lymph node (Figure 1-12, 13, and 14), and the Peyer's patches (Figure 1-4) which is considered as a mucosal associated Tissues (MALT).
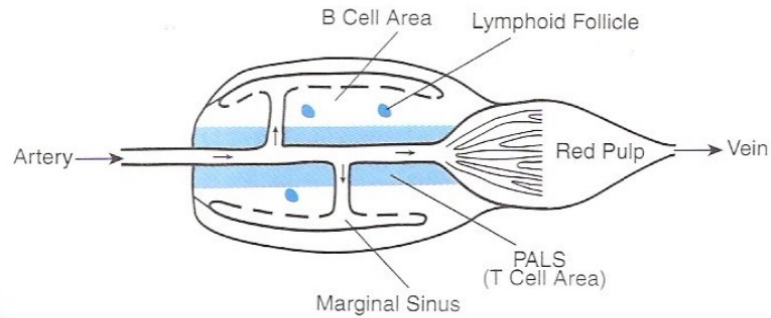
Figure 1-12 Spleen is an example of secondary lymphoid organs
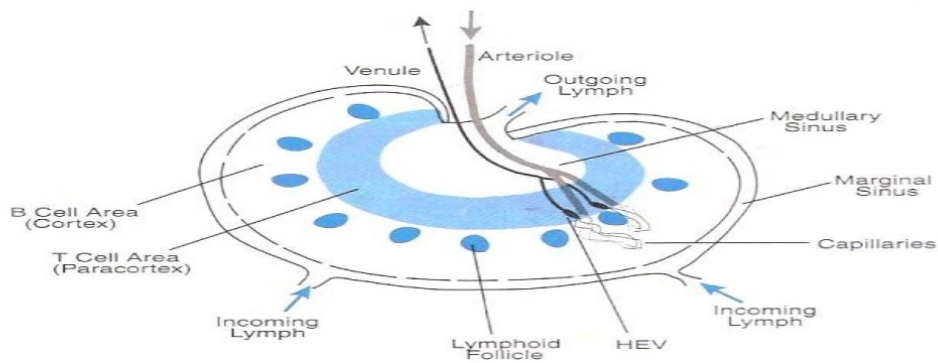
Figure 1-13 Lymph node is an example of the secondary lymphoid organs
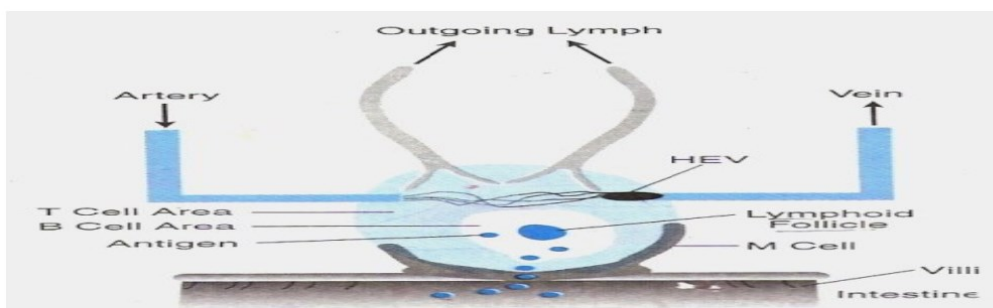
Figure 1-14 Peyer's patches is an example of the MALT (Mucosal Associated Lymphoid Tissues)

Humoral (non-cellular) immunity

Primarily involves **b**ursa- or **b**one marrow-derived **(B) lymphocytes, or B cells.**

(1) The B cell expresses specific immunoglobulin on its surface.

(2) When this surface immunoglobulin **interacts (meet)** with its matching (homologous) **antigen**, the **B** cell is **triggered** to "**proliferate**" and "**differentiate**" into <u>plasma cell</u> **[antibody producing cell (APC)]** which excrete vast quantities of **immunoglobulins (Figure 1-15).**

(a) These produced immunoglobulins are **specific** for the same antigen (non-self) that originally triggered the **B lymphocyte.**

(b) Immunoglobulins, as proteins in the plasma fraction of the blood, comprise the **humoral (soluble)** components of the <u>**specific**</u> immune system.
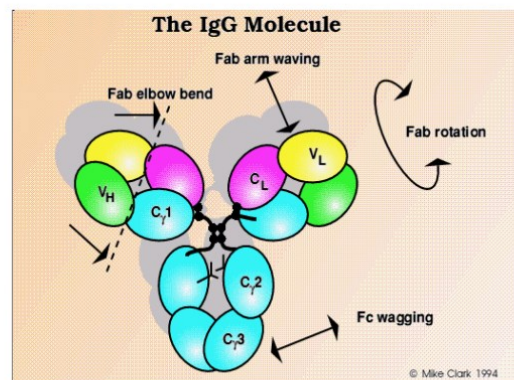

Figure 15- An example of acquired humoral immunity is the IgG antibody.

PHASES OF HUMORAL IMMUNE RESPONSE

A. The primary and secondary immune responses

   1. Characteristics

     a. The primary immune response occurs following the **first exposure to antigen** and produces a relatively <u>**small amount of antibody.**</u>
     b. If a sufficient length of time elapses after the primary antigenic stimulation, the antibody level will <u>decrease markedly.</u>
     c. However, subsequent exposure to even a small amount of antigen will evoke an **anamnestic response** (also called **booster response,** <u>**memory response**</u>, or **secondary immune response**).

     (1) The anamnestic response consists of a **rapid proliferation of plasma cells,** with the concomitant production of <u>**large amounts of specific antibody.**</u>
     (2) The anamnestic response occurs because a large population of <u>**memory B and T cells**</u> are recruited into the humoral immune response.

     (a) These memory cells are produced during the initial exposure to the antigen.
     (b) The memory cells are precursors of the Th cells and plasma cells, and represent another product of the collaboration between T cells and B cells **(Figure 1-16)**.

Figure 1-16. Schematic representation exhibiting human cellular events in the primary and secondary (anamnestic) antibody responses.

2. Immunoglobulin class switching

a. The IgM-IgG switch

(1) In the **primary immune response**, the immunoglobulin produced is mainly **IgM. Subsequent exposures** to antigen will cause the response to **shift to IgG** production.

(2) This change occurs within individual plasma cells; it is not the result of recruitment of new IgG-producing cell to replace effete IgM-producing plasma cells.

(3) The individual plasma cell spices out the μ constant region gene complex and replaces it with a γ3, γ1, or another constant region gene.

(4) The entire light (L) chain gene complex and the variable, diversity, and joining segments of the heavy (H) chain remain intact. Thus, the antigenic specificity of the plasma cell and its immunoglobulins is not changed.

b. Class switching to IgA, IgD, or IgE takes place by similar splicing processes.

Acquired immunity is not always protective, but some times offensive

Examples:

1. Hypersensitivity reactions.
2. Autoimmunity reactions.
3. Immunodeficiency diseases.
4. Transplantation rejections.

ANTIGENS

DEFINITIONS

Immunogen
A substance that induces a specific immune response.

Antigen (Ag)

A substance that reacts with the products of a specific immune response.

Hapten
A substance that is non-immunogenic but which can react with the products of a specific immune response. Haptens are small molecules which could never induce an immune response when administered by themselves but which can when coupled to a carrier molecule. Free haptens, however, can react with products of the immune response after such products have been elicited. Haptens have the property of antigenicity but not immunogenicity.

Epitope or Antigenic Determinant

That portion of an antigen that combines with the products of a specific immune response.

Antibody (Ab)

A specific protein which is produced in response to an immunogen and which reacts with an antigen.

FACTORS INFLUENCING IMMUNOGENICITY

Contribution of the Immunogen

Foreignness
The immune system normally discriminates between self and non-self such that only foreign molecules are immunogenic.

Size
There is not absolute size above which a substance will be immunogenic. However, in general, the larger the molecule the more immunogenic it is likely to be.

Chemical Composition

In general, the more complex the substance is chemically the more immunogenic it will be. The antigenic determinants are created by the primary sequence of residues in the polymer and/or by the secondary, tertiary or quaternary structure of the molecule.

Physical form

In general particulate antigens are more immunogenic than soluble ones and denatured antigens more immunogenic than the native form.

Degradability

Antigens that are easily phagocytosed are generally more immunogenic. This is because for most antigens (T-dependant antigens, see below) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

Contribution of the Biological System

Genetic Factors

Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (*i.e.* responders and non-responders). The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells or they may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

Age

Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to mount and immune response in response to an immunogen.

Method of Administration

Dose

The dose of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.

Route

Generally the subcutaneous route is better than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response

Adjuvants

Substances that can enhance the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

CHEMICAL NATURE OF IMMUNOGENS

Proteins

The vast majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens.

Polysaccharides

Pure polysaccharides and lipopolysaccharides are good immunogens.

Nucleic Acids

Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins.

Lipids
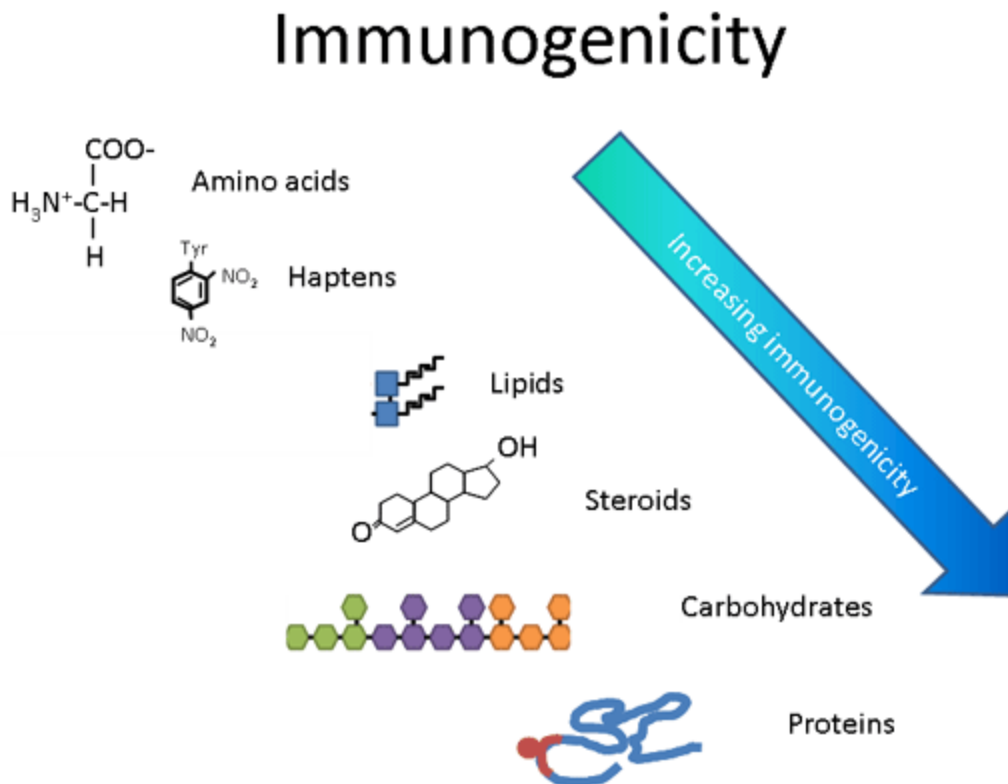In general lipids are non-immunogenic, although they may be haptens.

See figure 1a.

Figure 1a

Immunogenicity of biological molecule

TYPES OF ANTIGENS

T-independent Antigens

T-independent antigens are antigens which can directly stimulate the B cells to produce antibody without the requirement for T cell help In general, polysaccharides are T-independent antigens. The responses to these antigens differ from the responses to other antigens.

Properties of T-independent antigens

Polymeric structure

These antigens are characterized by the same antigenic determinant repeated many times as illustrated in Figure 1b.
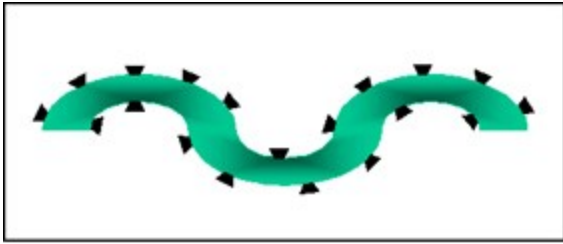
Figure 1b

In an antigen, the same antigenic determinant repeated many times

Polyclonal activation of B cells

Many of these antigens can activate B cell clones specific for other antigens (polyclonal activation). T-independent antigens can be subdivided into Type 1 and Type 2 based on their ability to polyclonally activate B cells. Type 1 T-independent antigens are polyclonal activators while Type 2 are not.

Resistance to degradation

T-independent antigens are generally more resistant to degradation and thus they persist for longer periods of time and continue to stimulate the immune system.

T-dependent Antigens

T-dependent antigens are those that do not directly stimulate the production of antibody without the help of T cells. Proteins are T-dependent antigens. Structurally these antigens are characterized by a few copies of many different antigenic determinants as illustrated in the Figure 2.
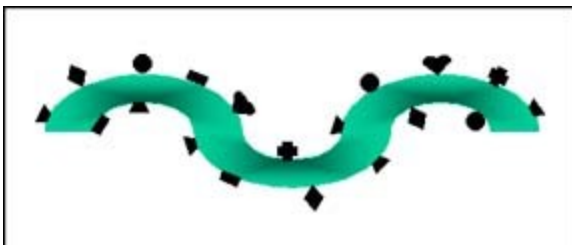

Figure 2

T-dependent antigens are characterized by a few copies of many different antigenic determinants

HAPTEN-CARRIER CONJUGATES

Definition
Hapten-carrier conjugates are immunogenic molecules to which haptens have been covalently attached. The immunogenic molecule is called the carrier.

Structure
Structurally these conjugates are characterized by having native antigenic determinants of the carrier as well as new determinants created by the hapten (haptenic determinants) as illustrated in the Figure 3. The actual determinant created by the hapten consists of the hapten and a few of the adjacent

residues, although the antibody produced to the determinant will also react with free hapten. In such conjugates the type of carrier determines whether the response will be T-independent or T-dependent.
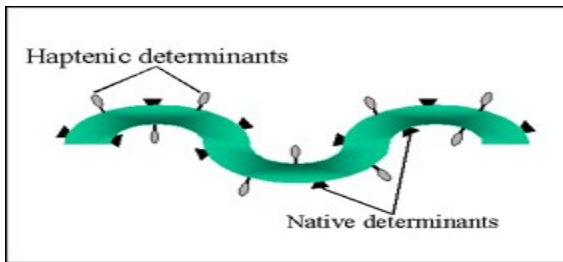

Figure 3

Hapten-carrier conjugates have native antigenic determinants of the carrier as well as new determinants of the hapten

ANTIGENIC DETERMINANTS

Determinants recognized by B cells

Composition
Antigenic determinants recognized by B cells and the antibodies secreted by B cells are created by the primary sequence of residues in the polymer (linear or sequence determinants) and/or by the secondary, tertiary or quaternary structure of the molecule (conformational determinants).

Size
In general antigenic determinants are small and are limited to approximately 4-8 residues. (amino acids and or sugars). The combining site of an antibody will accommodate an antigenic determinant of approximately 4-8 residues.

Number
Although, in theory, each 4-8 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much lower than what would theoretically be possible. Usually the antigenic determinants are limited to those portions of the antigen that are accessible to antibodies as illustrated in the Figure 4 (antigenic determinants are indicated in black).
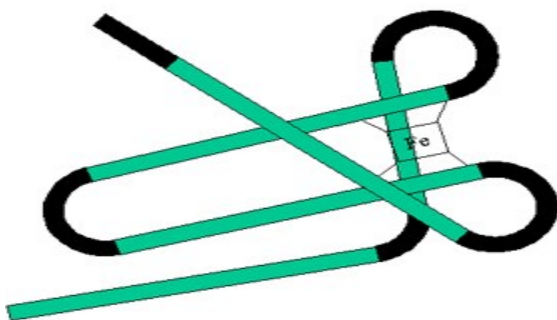

Figure 4

Antigenic determinants are usually limited to those portions of the antigen that are accessible to antibodies shown in black for this iron-containing protein

Determinants recognized by T cells

Composition

Antigenic determinants recognized by T cells are created by the primary sequence of amino acids in proteins. T cells do not recognize polysaccharide or nucleic acid antigens. This is why polysaccharides are generally T-independent antigens and proteins are generally T-dependent antigens. The determinants need not be located on the exposed surface of the antigen since recognition of the determinant by T cells requires that the antigen be proteolytically degraded into smaller peptides. Free peptides are not recognized by T cells, rather the peptides associate with molecules coded for by the major histocompatibility complex (MHC) and it is the complex of MHC molecules + peptide that is recognized by T cells.

Size

In general antigenic determinants are small and are limited to approximately 8-15 amino acids.

Number

Although, in theory, each 8-15 residues can constitute a separate antigenic determinant, in practice, the number of antigenic determinants per antigen is much less than what would theoretically be possible. The antigenic determinants are limited to those portions of the antigen that can bind to MHC molecules. This is why there can by differences in the responses of different individuals.

SUPERANTIGENS

When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 10^4 -10^5) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called superantigens (Figure 5).

Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock). Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well.

The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

Superantigens

• Definition

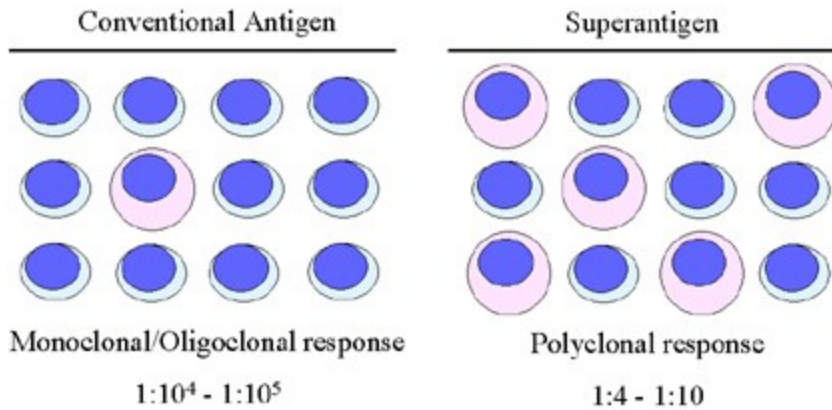| Conventional Antigen	Superantigen
Monoclonal/Oligoclonal response	Polyclonal response
$1:10^4 - 1:10^5$	$1:4 - 1:10$

Figure 5

Superantigens activate a large fraction of T cells in contrast to conventional T-dependent antigens

Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock). Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well.

The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

DETERMINANTS RECOGNIZED BY THE INNATE IMMUNE SYSTEM

Determinants recognized by components of the innate (nonspecific) immune system differ from those recognized by the adaptive (specific) immune system. Antibodies, and the B and T cell receptors recognize discrete determinants and demonstrate a high degree of specificity, enabling the adaptive immune system to recognize and react to a particular pathogen. In contrast, components of the innate immune system recognize broad molecular patterns found in pathogens but not in the host. Thus, they lack a high degree of specificity seen in the adaptive immune system. The broad molecular patterns recognized by the innate immune system have been called PAMPS (pathogen associated molecular patterns) and the receptors for PAMPS are called PRRs (pattern recognition receptors). A particular PRR can recognize a molecular pattern that may be present on a number of different pathogens enabling the receptor to recognize a variety of different pathogens. Examples of some PAMPs and PRRs are illustrated in Table1.

Table 1 Examples of pathogen associated molecular patterns and their receptors		
PAMP	PRR	Biological Consequence of Interaction
Microbial cell wall components	Complement	Opsonization, Complement activation
Mannose-containing carbohydrates	Mannose-binding protein	Opsonization Complement activation
Polyanions	Scavenger receptors	Phagocytosis
Lipoproteins of Gram + bacteria Yeast cell wall components	TLR-2 (Toll-like receptor 2)	Macrophage activation, secretion of inflammatory cytokines
Double stranded RNA	TLR-3	Production of interferon (antiviral)
LPS (lipopolysaccharide of Gram negative bacteria)	TLR-4	Macrophage activation, secretion of inflammatory cytokines
Flagellin (bacterial flagella)	TLR-5	Macrophage activation, secretion of inflammatory cytokines
U-rich Single stranded viral RNA	TLR-7	Production of interferon (antiviral)
CpG containing DNA	TLR-9	Macrophage activation, secretion of inflammatory cytokines

COMPLEMENT

COMPLEMENT FUNCTIONS

Historically, the term complement (C) was used to refer to a heat-labile serum component that was able to lyse bacteria (activity is destroyed (inactivated) by heating serum at 56 degrees C for 30 minutes). However, complement is now known to contribute to host defenses in other ways as well. Complement can opsonize bacteria for enhanced phagocytosis; it can recruit and activate various cells including polymorphonuclear cells (PMNs) and macrophages; it can participate in regulation of antibody responses and it can aid in the clearance of immune complexes and apoptotic cells. Complement can also have detrimental effects for the host; it contributes to inflammation and tissue damage and it can trigger anaphylaxis.

Complement comprises over 20 different serum proteins (see Table 1) that are produced by a variety of cells including, hepatocytes, macrophages and gut epithelial cells. Some complement proteins bind to immunoglobulins or to membrane components of cells. Others are proenzymes that, when activated, cleave one or more other complement proteins. Upon cleavage some of the complement proteins yield fragments that activate cells, increase vascular permeability or opsonize bacteria.

Table 1. Proteins of the Complement system			
Classical Pathway	**Lectin Pathway**	**Alternative Pathway**	**Lytic Pathway**
Activation Proteins: C1qrs, C2, C3, C4 Control Proteins: C1-INH, C4-BP	Mannan binding protein (MBP), mannan-asociated serine protease (MASP, MASP2)	C3, Factors B & D*, Properdin (P) Factors I* & H, decay accelerating factor (DAF), Complement receptor 1(CR1), *etc*.	C5, C6, C7, C8, C9 Protein S

Components underlined acquire enzymatic activity when activated.

Components marked with an asterisk have enzymatic activity in their native form.

PATHWAYS OF COMPLEMENT ACTIVATION

Complement activation can be divided into four pathways (figure 1): the classical pathway, the lectin pathway, the alternative pathway and the membrane attack (or lytic) pathway. Both classical and alternative pathways lead to the activation of C5 convertase and result in the production of C5b which is essential for the activation of
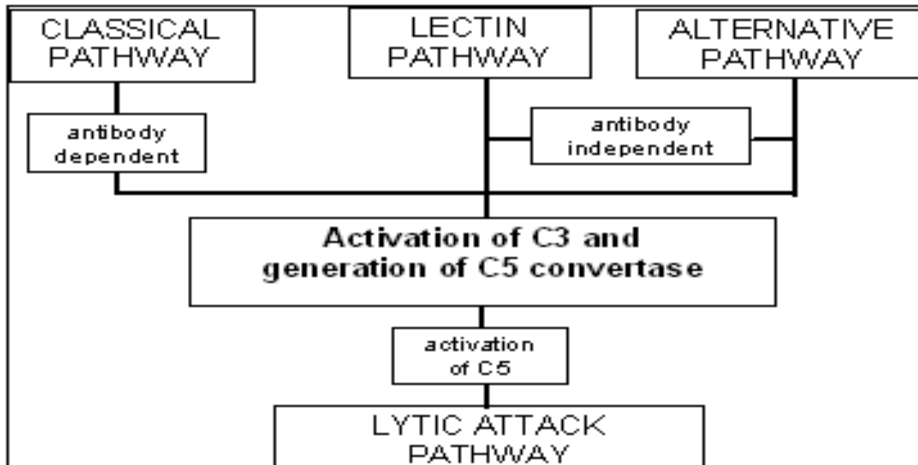
the membrane attack pathway.

Figure 1. Pathways of complement activation

Classical Pathway (Figure 2)

C1 activation

C1, a multi-subunit protein containing three different proteins (C1q, C1r and C1s), binds to the Fc region of IgG and IgM antibody molecules that have interacted with antigen. C1 binding does not occur to antibodies that have not complexed with antigen and binding requires calcium and magnesium ions. (*N.B.* In some cases C1 can bind to aggregated immunoglobulin [e.g. aggregated IgG] or to certain pathogen surfaces in the absence of antibody). The binding of C1 to antibody is via C1q and C1q must cross link at least two antibody molecules before it is firmly fixed. The binding of C1q results in the activation of C1r which in turn activates C1s. The result is the formation of an activated "C1qrs", which is an enzyme that cleaves C4 into two fragments C4a and C4b.

C4 and C2 activation (generation of C3 convertase)

The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated "C1qrs" also cleaves C2 into C2a and C2b. C2a binds to the membrane in association with C4b, and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b.
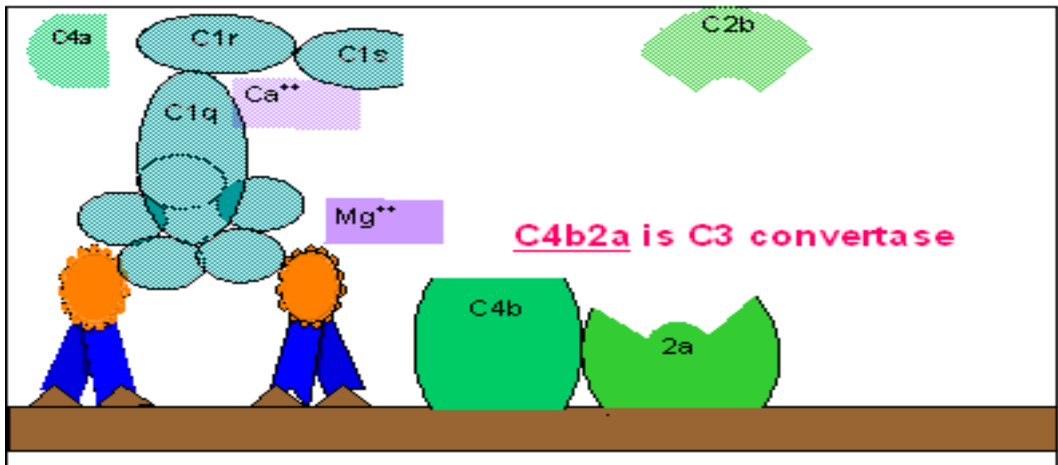
Figure 2. Generation of C3 convertase in the classical pathway

C3 activation (generation of C5 convertase)

C3b binds to the membrane in association with C4b and C2a, and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the classical pathway.

Figure 3. Generation of C5 convertase in the classical pathway
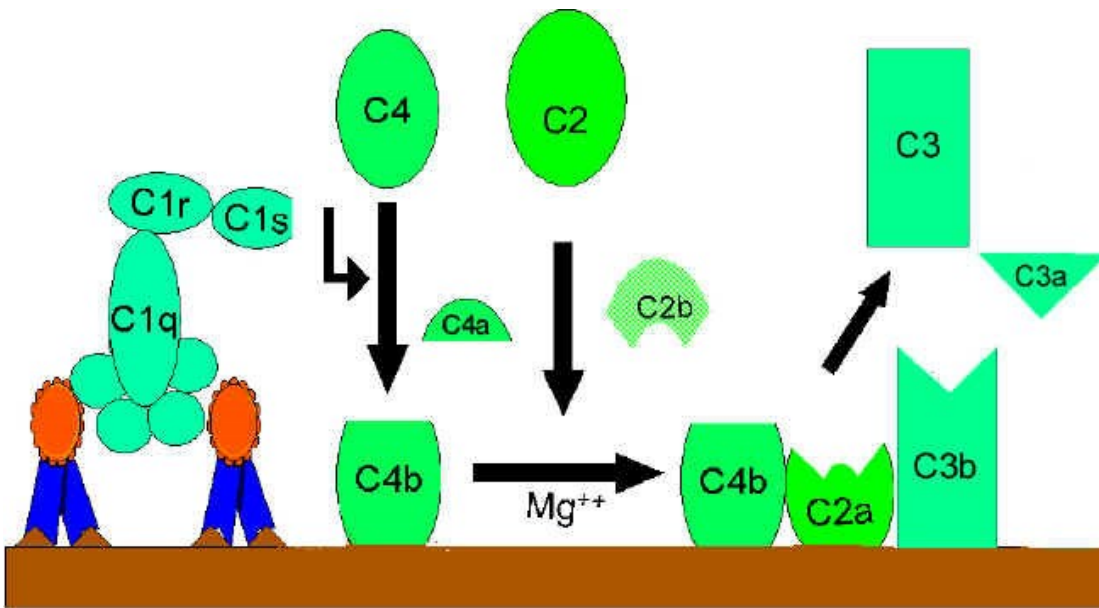
C Activation of C3 by the classical pathway

Several of the products of the classical pathway have potent biological activities that contribute to host defenses. Some of these products may also have detrimental effects if produced in an unregulated manner. Table 2 summarizes the biological activities of classical pathway components.

Table 2. Biological Activity of classical pathway products	
Component	**Biological Activity**
C2b	**Prokinin**; cleaved by plasmin to yield kinin, which results in edema
C3a	**Anaphylotoxin**; can activate basophils and mast cells to degranulate resulting in increased vascular permeability and contraction of smooth muscle cells, which may lead to **anaphylaxis**
C3b	**Opsonin**; promotes phagocytosis by binding to complement receptors Activation of phagocytic cells
C4a	**Anaphylotoxin** (weaker than C3a)
C4b	**Opsonin**; promotes phagocytosis by binding to complement receptors

If the classical pathway were not regulated there would be continued production of C2b, C3a, and C4a. Thus, there must be some way to regulate the activity of the classical pathway. Table 3 summarizes the ways in which the classical pathway is regulated.

Table 3. Regulation of the Classical Pathway	
Component	**Regulation**
All	**C1-INH**; dissociates C1r and C1s from C1q
C3a	**C3a inactivator (C3a-INA;Carboxypeptidase B)**; inactivates C3a
C3b	**Factors H and I;** Factor H facilitates the degradation of C3b by Factor I
C4a	**C3-INA**
C4b	**C4 binding protein(C4-BP) and Factor I**; C4-BP facilitates degradation of C4b by Factor I; C4-BP also prevents association of C2a with C4b thus blocking the formation of C3 convertase

The importance of C1-INH in regulating the classical pathway is demonstrated by the result of a deficiency in this inhibitor. C1-INH deficiencies are associated with the development of hereditary angioedema.

Lectin Pathway

The lectin pathway (figure 3) is very similar to the classical pathway. It is initiated by the binding of mannose-binding lectin (MBL) to bacterial surfaces with mannose-containing polysaccharides (mannans). Binding of MBL to a pathogen results in the association of two serine proteases, MASP-1 and MASP-2 (MBL-associated serine proteases). MASP-1 and MASP-2 are similar to C1r and C1s, respectively and MBL is similar to C1q. Formation of the MBL/MASP-1/MASP-2 tri-molecular complex results in the activation of the MASPs and subsequent cleavage of C4 into C4a and C4b. The C4b fragment binds to the membrane and the C4a fragment is released into the microenvironment. Activated MASPs also cleave C2 into C2a and C2b. C2a binds to the membrane in association with C4b and C2b is released into the microenvironment. The resulting C4bC2a complex is a C3 convertase, which cleaves C3 into C3a and C3b. C3b binds to the membrane in association with C4b and C2a

and C3a is released into the microenvironment. The resulting C4bC2aC3b is a C5 convertase. The generation of C5 convertase is the end of the lectin pathway.

The biological activities and the regulatory proteins of the lectin pathway are the same as those of the classical pathway.
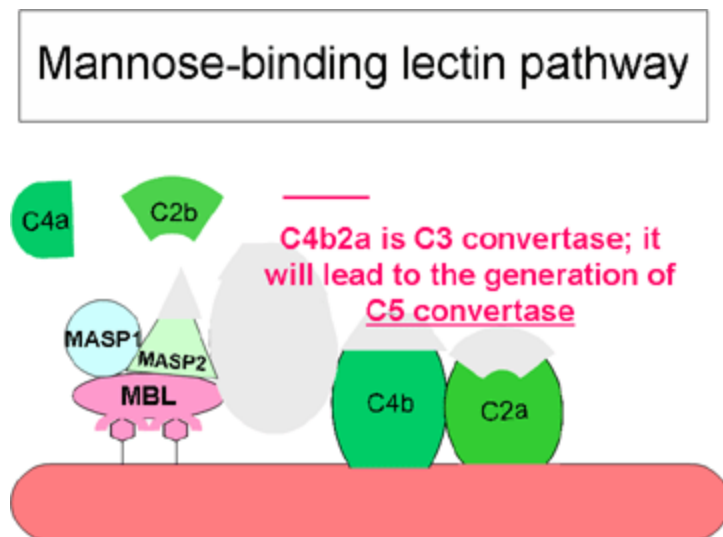

Figure 3 Lectin-initiated pathway

Alternative Pathway

The alternative pathway begins with the activation of C3 and requires Factors B and D and Mg^{++} cation, all present in normal serum.

Amplification loop of C3b formation (Figure 4)

In serum there is low level spontaneous hydrolysis of C3 to produce C3i. Factor B binds to C3i and becomes susceptible to Factor D, which cleaves Factor B into Bb. The C3iBb complex acts as a C3 convertase and cleaves C3 into C3a and C3b. Once C3b is formed, Factor B will bind to it and becomes susceptible to cleavage by Factor D. The resulting C3bBb complex is a C3 convertase that will continue to generate more C3b, thus amplifying C3b production. If this process continues unchecked, the result would be the consumption of all C3 in the serum. Thus, the spontaneous production of C3b is tightly controlled.
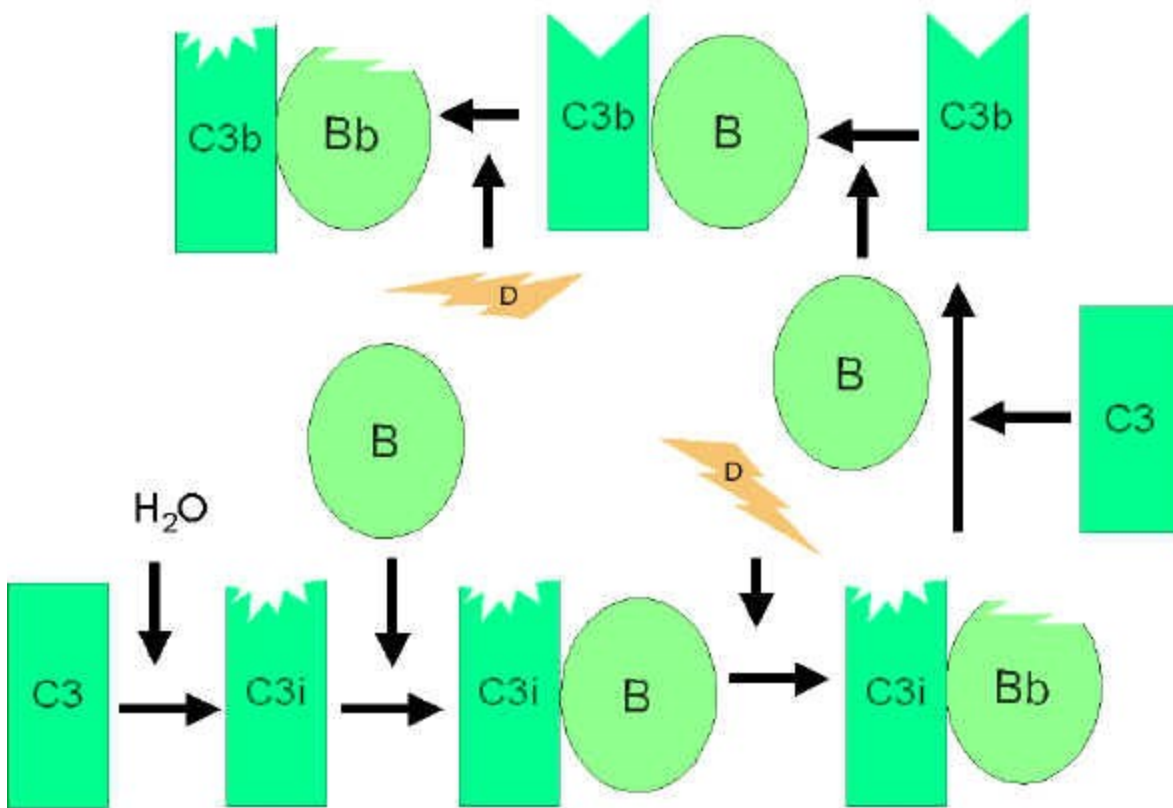
Figure 4 Spontaneous activation of C3 (C3 tick-over)

Control of the amplification loop (Figures 5 and 6)

As spontaneously produced C3b binds to autologous host membranes, it interacts with DAF (decay accelerating factor), which blocks the association of Factor B with C3b thereby preventing the formation of additional C3 convertase. In addition, DAF accelerates the dissociation of Bb from C3b in C3 convertase that has already formed, thereby stopping the production of additional C3b. Some cells possess complement receptor 1 (CR1). Binding of C3b to CR1 facilitates the enzymatic degradation of C3b by Factor I. In addition, binding of C3 convertase (C3bBb) to CR1 also dissociates Bb from the complex. Thus, in cells possessing complement receptors, CR1 also plays a role in controlling the amplification loop. Finally, Factor H can bind to C3b bound to a cell or in the in the fluid phase and facilitate the enzymatic degradation of C3b by Factor I. Thus, the amplification loop is controlled by either blocking the formation of C3 convertase, dissociating C3 convertase, or by enzymatically digesting C3b. The importance of controlling this amplification loop is illustrated in patients with genetic deficiencies of Factor H or I. These patients have a C3 deficiency and increased susceptibility to certain infections.
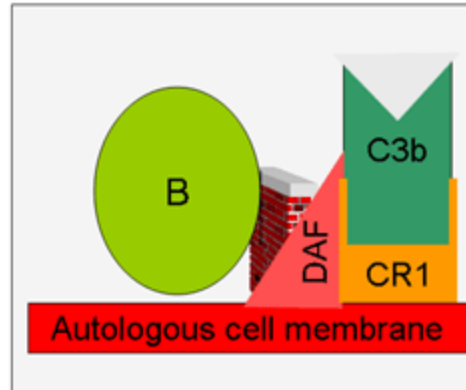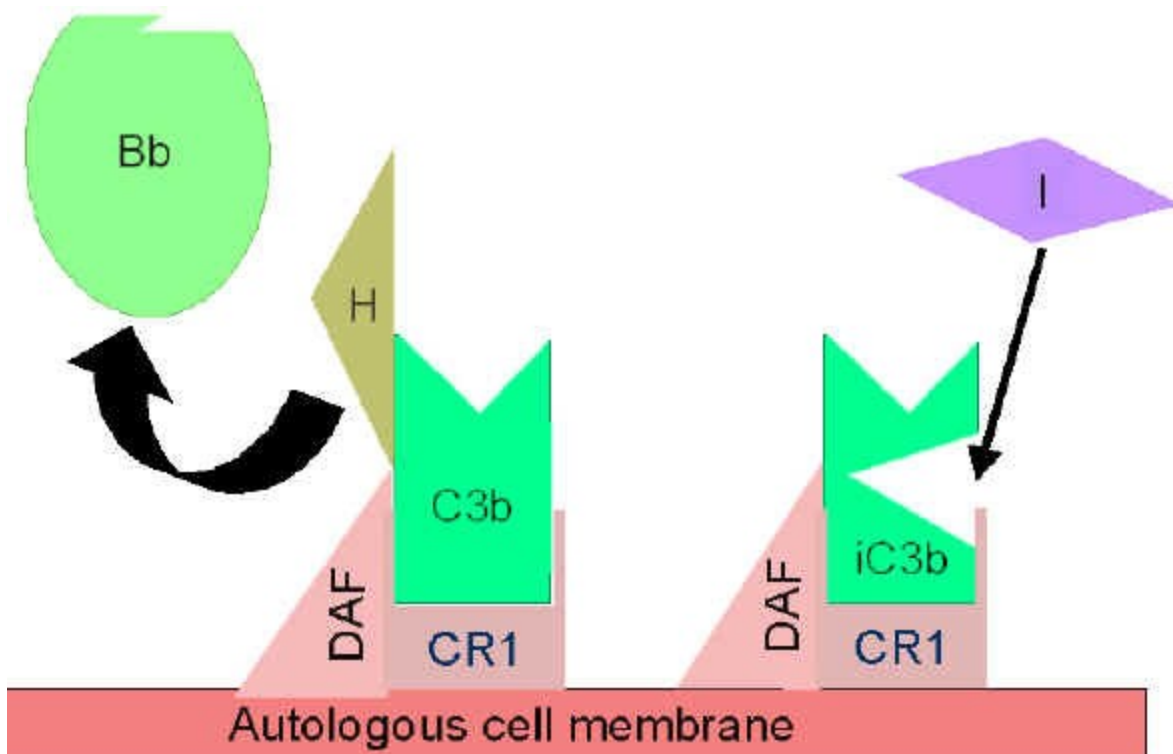
Figure 5 Regulation of activated C3 by DAF

Figure 6 Regulation of activated C3 by Cr1

Stabilization of C convertase by activator (protector) surfaces (Figure 7)

When bound to an appropriate activator of the alternative pathway, C3b will bind Factor B, which is enzymatically cleaved by Factor D to produce C3 convertase (C3bBb). However, C3b is resistant to degradation by Factor I and the C3 convertase

is not rapidly degraded, since it is stabilized by the activator surface. The complex is further stabilized by properdin binding to C3bBb. Activators of the alternate pathway are components on the surface of pathogens and include: LPS of Gram-negative bacteria and the cell walls of some bacteria and yeasts. Thus, when C3b binds to an activator surface, the C3 convertase formed will be stable and continue to generate additional C3a and C3b by cleavage of C3.
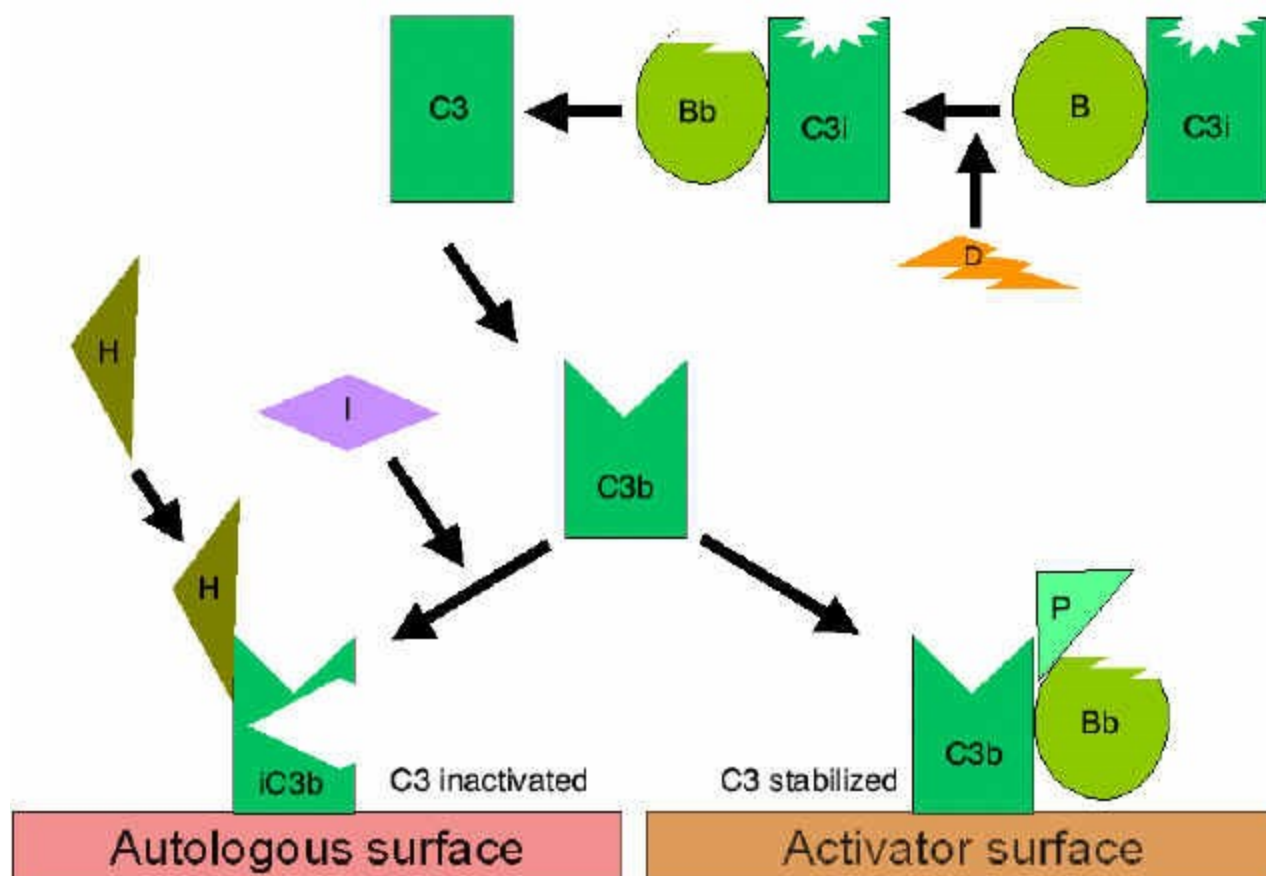

Figure 7 Stabilization of C3 convertase

Generation of C5 convertase (Figure 10)

Some of the C3b generated by the stabilized C3 convertase on the activator surface associates with the C3bBb complex to form a C3bBbC3b complex. This is the C5 convertase of the alternative pathway. The generation of C5 convertase is the end of the alternative pathway. The alternative pathway can be activated by many Gram-negative (most significantly, *Neisseria meningitidis* and *N. gonorrhoea*), some Gram-positive bacteria and certain viruses and parasites, and results in the lysis of these organisms. Thus, the alternative pathway of C activation provides another means of protection against certain pathogens before an antibody response is mounted. A deficiency of C3 results in an increased susceptibility to these organisms. The

alternate pathway may be the more primitive pathway and the classical and lectin pathways probably developed from it.
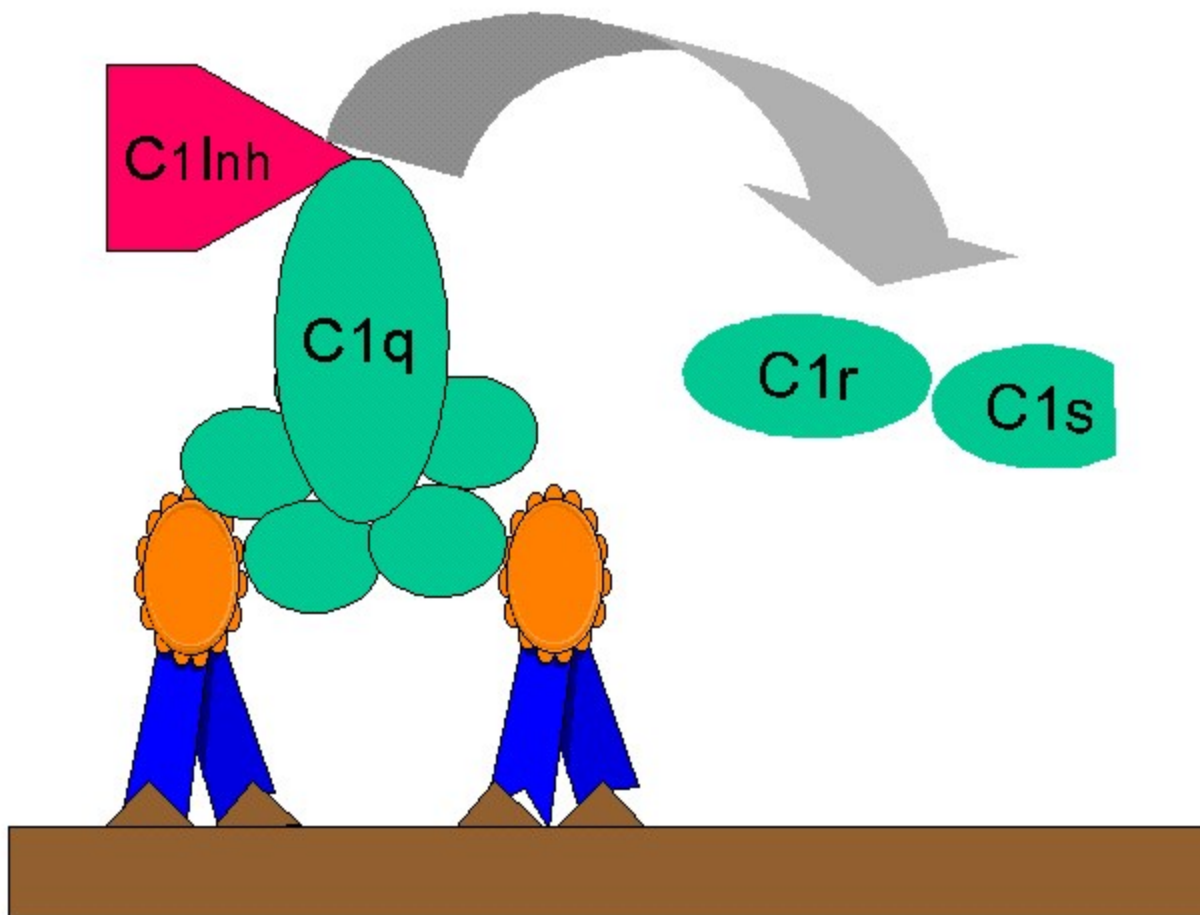

Figure 10 Regulation of C1rs (C4 convertase) by C1-INH

Remember that the alternative pathway provides a means of non-specific resistance against infection without the participation of antibodies and hence provides a first line of defense against a number of infectious agents.

Many gram negative and some gram positive bacteria, certain viruses, parasites, heterologous red cells, aggregated immunoglobulins (particularly, IgA) and some other proteins (e.g. proteases, clotting pathway products) can activate the alternative pathway. One protein, cobra venom factor (CVF), has been extensively studied for its ability to activate this pathway.

Membrane Attack (Lytic) Pathway (figure 9)

C5 convertase from the classical (C4b2a3b), lectin (C4b2a3b) or alternative (C3bBb3b) pathway cleaves C5 into C5a and C5b. C5a remains in the fluid phase and the C5b rapidly associates with C6 and C7 and inserts into the membrane.

Subsequently C8 binds, followed by several molecules of C9. The C9 molecules form a pore in the membrane through which the cellular contents leak and lysis occurs. Lysis is not an enzymatic process; it is thought to be due to physical damage to the membrane. The complex consisting of C5bC6C7C8C9 is referred to as the membrane attack complex (MAC).

C5a generated in the lytic pathway has several potent biological activities. It is the most potent anaphylotoxin. In addition, it is a chemotactic factor for neutrophils and stimulates the respiratory burst in them and it stimulates inflammatory cytokine production by macrophages. Its activities are controlled by inactivation by carboxypeptidase B (C3-INA).

Some of the C5b67 complex formed can dissociate from the membrane and enter the fluid phase. If this were to occur it could then bind to other nearby cells and lead to their lysis. The damage to bystander cells is prevented by Protein S (vitronectin). Protein S binds to soluble C5b67 and prevents its binding to other cells.
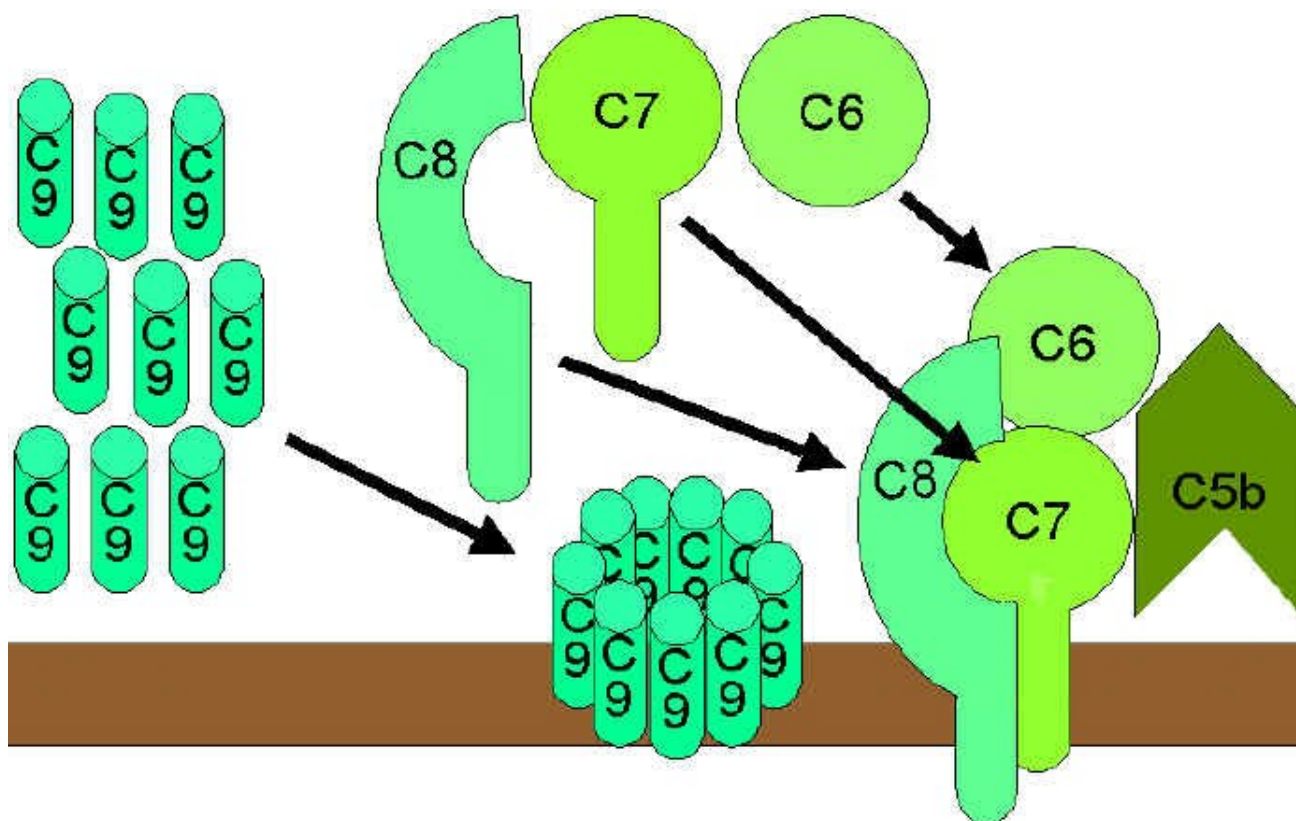

Figure 9 The lytic pathway

BIOLOGICALLY ACTIVE PRODUCTS OF COMPLEMENT ACTIVATION

Activation of complement results in the production of several biologically active molecules which contribute to resistance, anaphylaxis and inflammation.

Kinin production

C2b generated during the classical pathway of C activation is a prokinin which becomes biologically active following enzymatic alteration by plasmin. Excess C2b production is prevented by limiting C2 activation by C1 inhibitor (C1-INH) also known as serpin which displaces C1rs from the C1qrs complex (Figure 10). A genetic deficiency of C1-INH results in an overproduction of C2b and is the cause of hereditary angioneurotic edema. This condition can be treated with Danazol which promotes C1-INH production or with ε-amino caproic acid which decreases plasmin activity.

Anaphylotoxins

C4a, C3a and C5a (in increasing order of activity) are all anaphylotoxins which cause basophil/mast cell degranulation and smooth muscle contraction. Undesirable effects of these peptides are controlled by carboxypeptidase B (C3a-INA).

Chemotactic Factors

C5a and MAC (C5b67) are both chemotactic. C5a is also a potent activator of neutrophils, basophils and macrophages and causes induction of adhesion molecules on vascular endothelial cells (figure 12).
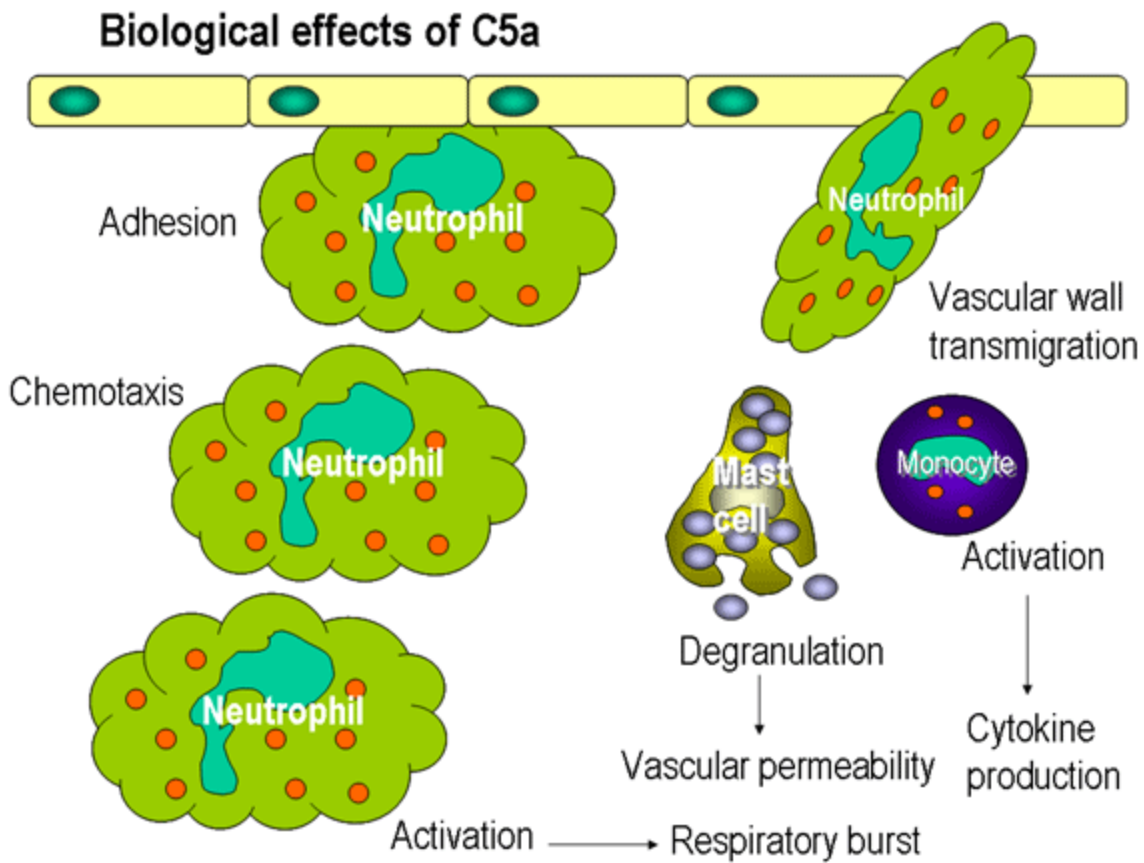
Figure 12 Biological effects of C5a

Opsonins

C3b and C4b in the surface of microorganisms attach to C-receptor (CR1) on phagocytic cells and promote phagocytosis (figure 11).
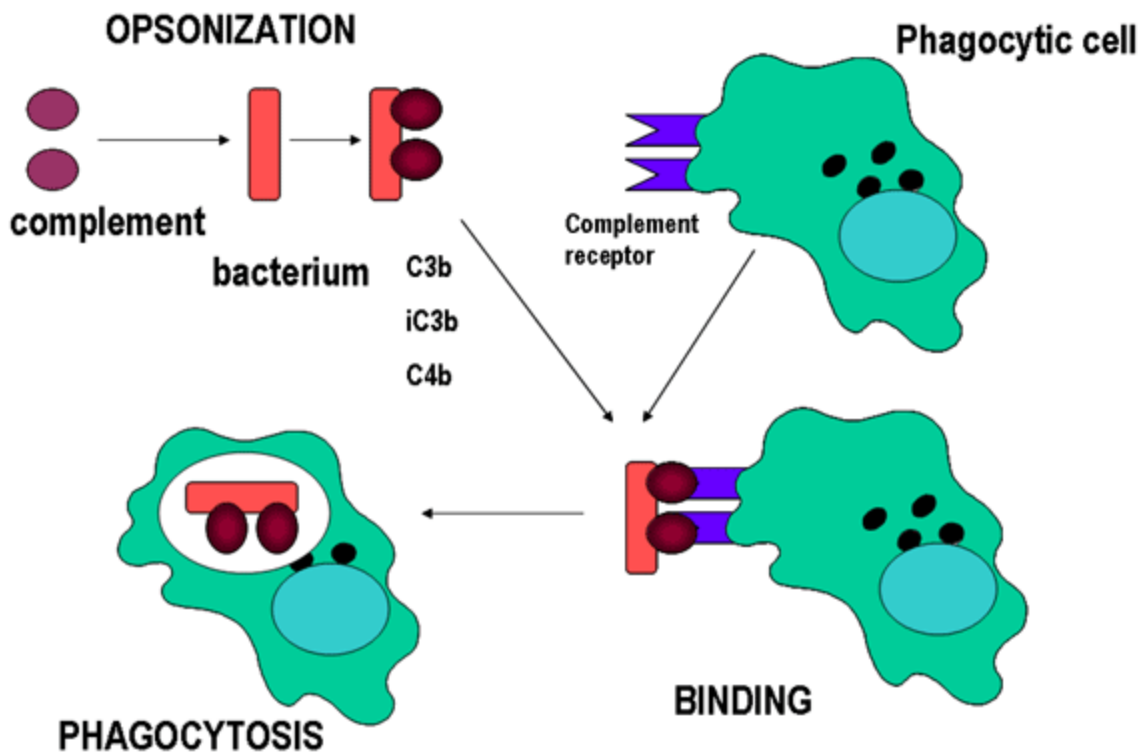
Figure 11 Complement proteins bind to the surface of microorganisms and promote phagocytosis via complement receptors

Other Biologically active products of C activation

Degradation products of C3 (iC3b, C3d and C3e) also bind to different cells by distinct receptors and modulate their functions.

In summary, the complement system takes part in both specific and non-specific resistance and generates a number of products of biological and pathophysiological significance (Table 4).

There are known genetic deficiencies of most individual C complement components, but C3 deficiency is most serious and fatal. Complement deficiencies also occur in immune complex diseases (e.g., SLE) and acute and chronic bacterial, viral and parasitic infections.

Table 4. Activities of Complement Activation Products and their Control Factors

Fragment	Activity	Effect	Control Factor (s)
C2a	Prokinin, accumulation of fluids	Edema	C1-INH
C3a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis	C3a-INA
C3b	Opsonin, phagocyte activation	Phagocytosis	Factors H and I
C4a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (least potent)	C3a-INA
C4b	Opsonin	Phagocytosis	C4-BP and Factor I
C5a	Basophil and mast cells degranulation; enhanced vascular permeability, smooth muscle contraction	Anaphylaxis (most potent)	C3a-INA
	Chemotaxis, stimulation of respiratory burst, activation of phagocytes, stimulation of inflammatory cytokines	Inflammation	
C5bC6C7	Chemotaxis	Inflammation	Protein S (vitronectin)
	Attaches to other membranes	Tissue damage	

able 5. Complement deficiencies and disease

Pathway/Component	Disease	Mechanism

Classical Pathway		
C1INH	Hereditary angioedema	Overproduction of C2b (prokinin)
C1, C2, C4	Predisposition to SLE	Opsonization of immune complexes help keep them soluble, deficiency results in increased precipitation in tissues and inflammation
Lectin Pathway		
MBL	Susceptibility to bacterial infections in infants or immunosuppressed	Inability to initiate the lectin pathway
Alternative Pathway		
Factors B or D	Susceptibility to pyogenic (pus-forming) bacterial infections	Lack of sufficient opsonization of bacteria
C3	Susceptibility to bacterial infections	Lack of opsonization and inability to utilize the membrane attack pathway
C5, C6, C7 C8, and C9	Susceptibility to Gram-negative infections	Inability to attack the outer membrane of Gram-negative bacteria
Properdin (X-linked)	Susceptibility meningococcal meningitis	Lack of opsonization of bacteria
Factors H or I	C3 deficiency and susceptibility to bacterial infections	Uncontrolled activation of C3 via alternative pathway resulting in depletion of C3

You have learned

The proteins of the complement system

The differences and similarities among the different pathways of C3 activation

The significance of the different pathways in specific and nonspecific immunity

The role of different complement activation products in amplification of nonspecific and specific immunity and inflammation

IMMUNOGLOBULINS - STRUCTURE AND FUNCTION

DEFINITION

Immunoglobulin (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field (Figure 1).
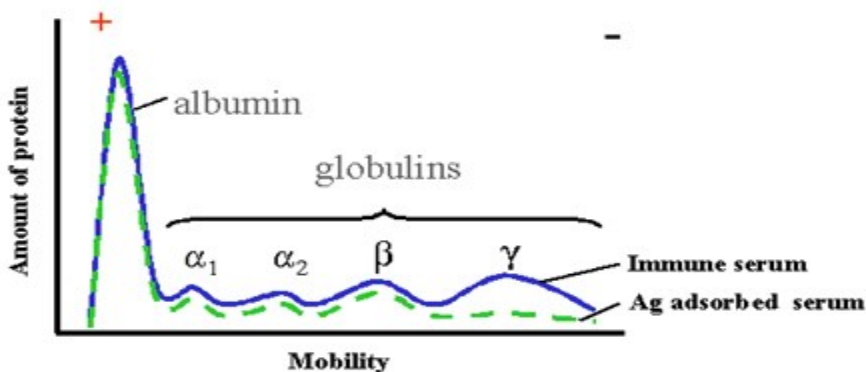

Figure 1
Electrophoretic separation of serum proteins

GENERAL FUNCTIONS OF IMMUNOGLOBULINS

Antigen binding

Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

Effector Functions

Frequently the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

- Fixation of complement - This results in lysis of cells and release of biologically active molecules (see chapter two)
- Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function.

Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn

BASIC STRUCTURE OF IMMUNOGLOBULINS

The basic structure of the immunoglobulins is illustrated in figure 2. Although different immunoglobulins can differ structurally, they all are built from the same basic units.

Heavy and Light Chains
All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

Disulfide bonds

Inter-chain disulfide bonds
The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

Intra-chain disulfide binds
Within each of the polypeptide chains there are also intra-chain disulfide bonds.

Variable (V) and Constant (C) Regions
When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the:

Light Chain - V_L (110 amino acids) and C_L (110 amino acids)

Heavy Chain - V_H (110 amino acids) and C_H (330-440 amino acids)

Hinge Region
This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

Domains
Three dimensional images of the immunoglobulin molecule show that it is not straight as depicted in figure 2A. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond (figure 2B-D). These regions are called domains.

Light Chain Domains - V_L and C_L

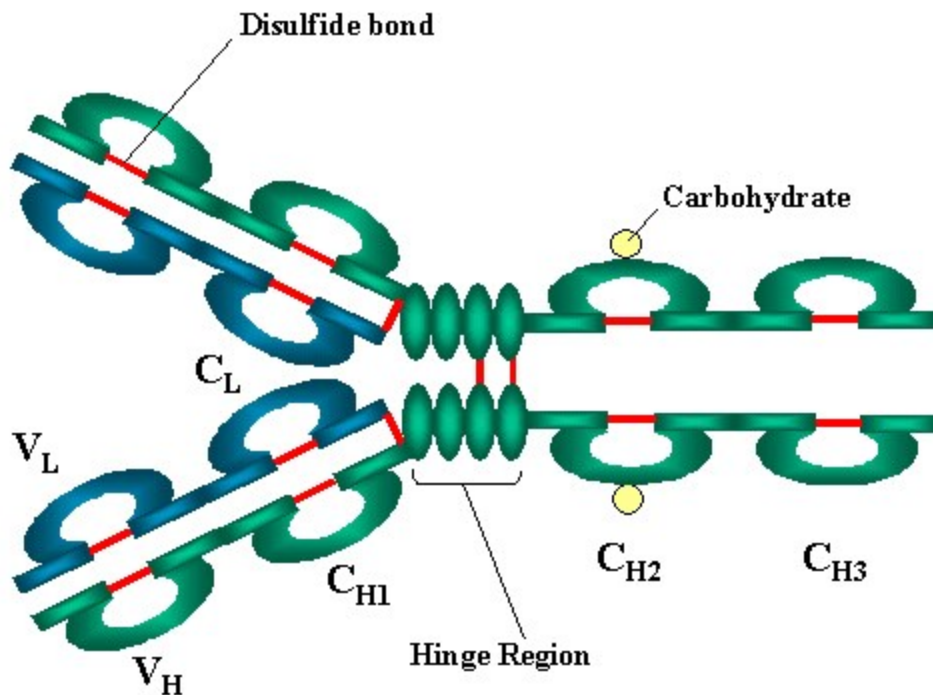Heavy Chain Domains - V_H, C_{H1} - C_{H3} (or C_{H4})

Figure 2A
The basic structure of immunoglobulins

Hinge Region
This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

Domains
Three dimensional images of the immunoglobulin molecule show that it is not straight as depicted in figure 2A. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond (figure 2B-D). These regions are called domains.

Light Chain Domains - V_L and C_L

Heavy Chain Domains - V_H, C_{H1} - C_{H3} (or C_{H4})

Oligosaccharides
Carbohydrates are attached to the C_{H2} domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

STRUCTURE OF THE VARIABLE REGION

Hypervariable (HVR) or complementarity determining regions (CDR)

Comparisons of the amino acid sequences of the variable regions of immunoglobulins show that most of the variability resides in three regions called the hypervariable regions or the complementarity determining regions as illustrated in figure 3. Antibodies with different specificities (i.e. different combining sites) have different

complementarity determining regions while antibodies of the exact same specificity have identical complementarity determining regions (*i.e.* CDR is the antibody combining site). Complementarity determining regions are found in both the H and the L chains.

Structure of the Variable Region

- Hypervariable (HVR) or complimentarity determining regions (CDR)
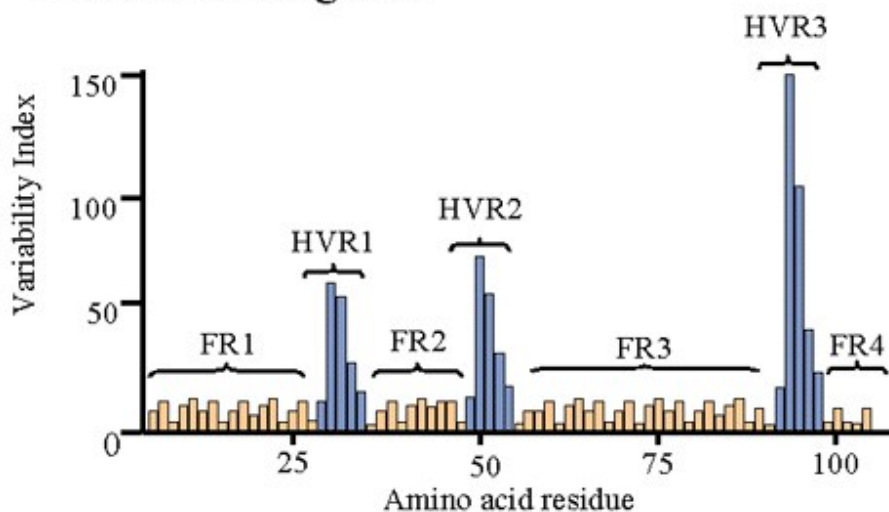- Framework regions

Figure 3
Structure of the variable region framework regions

Framework regions

The regions between the complementarity determining regions in the variable region are called the framework regions (figure 3). Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain variable regions can be divided into groups and subgroups. These represent the products of different variable region genes.

IMMUNOGLOBULIN FRAGMENTS: STRUCTURE/FUNCTION RELATIONSHIPS

Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating structure/function relationships in immunoglobulins.

Fab
Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond Figure 4. This results in the formation of two identical fragments that contain the light chain and the V_H and C_{H1} domains of the heavy chain.

Antigen binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. Each Fab fragment is monovalent whereas the original molecule was divalent. The combining site of the antibody is created by both V_H and V_L. An antibody is able to bind a particular antigenic determinant because it has a particular combination of V_H and V_L. Different combinations of a V_H and V_L result in antibodies that can bind a different antigenic determinants.

Fc

Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a C_{H2} and C_{H3} domain. This fragment was called Fc because it was easily crystallized.
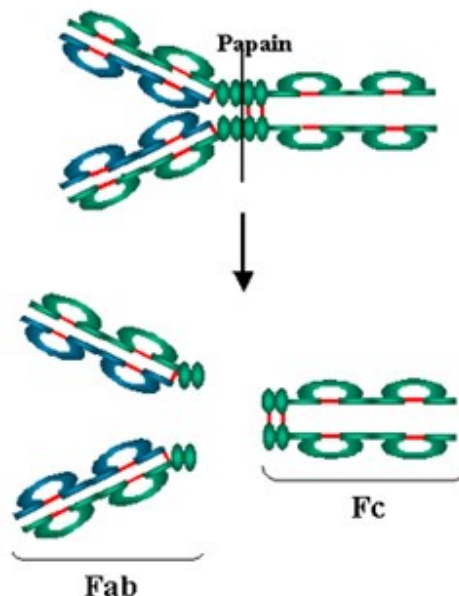

Figure 4 Immunoglobulin fragments: Structure/function relations

Effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule. Different functions are mediated by the different domains in this fragment (figure 5). Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen; however, there are exceptions to this rule.

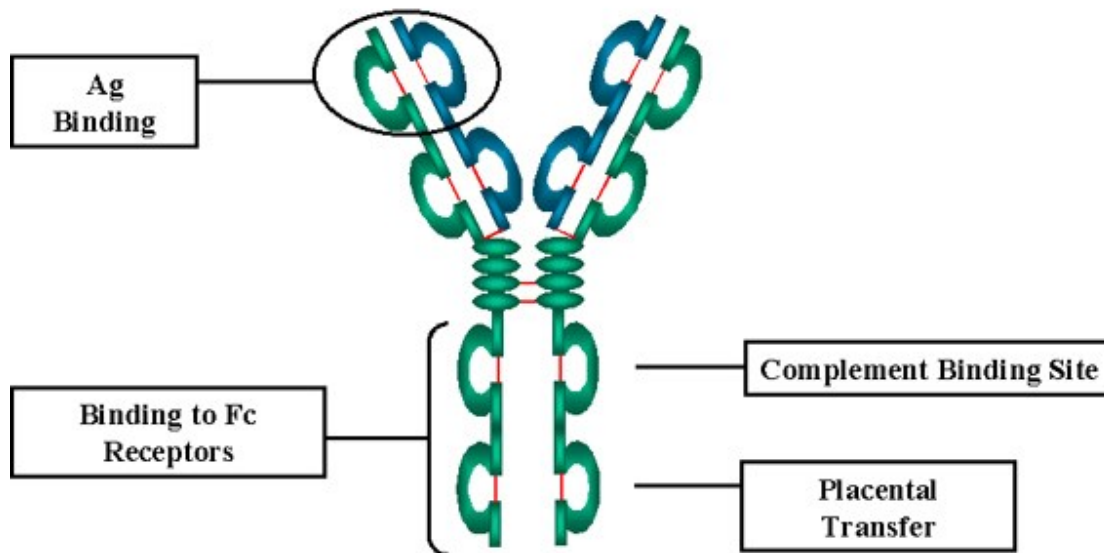# Immunoglobulin Fragments: Structure/Function Relationships

Figure 5
Immunoglobulin fragments: Structure function relationships

F(ab')$_2$

Treatment of immunoglobulins with pepsin results in cleavage of the heavy chain after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites (figure 6). This fragment was called F(ab')$_2$ because it is divalent. The Fc region of the molecule is digested into small peptides by pepsin. The F(ab')$_2$ binds antigen but it does not mediate the effector functions of antibodies.

Immunoglobulin Fragments: Structure/Function Relationships

- Fab
  - Ag binding
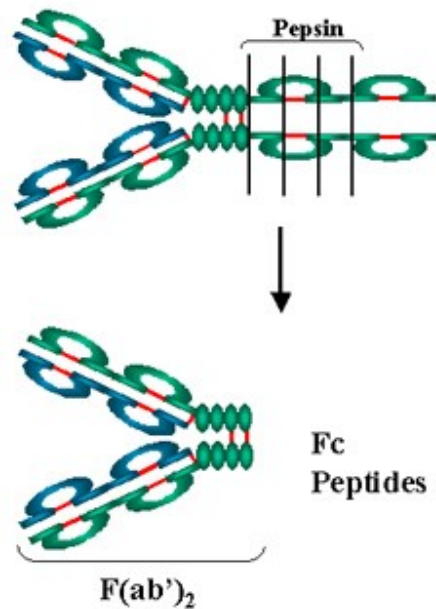- Fc
  - Effector functions
- F(ab')₂

Figure 6
Immunoglobulin fragments: Structure/function relationships

HUMAN IMMUNOGLOBULIN CLASSES, SUBCLASSES, TYPES AND SUBTYPES

Immunoglobulin classes

The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means (*i.e.* by the use of antibodies directed to these differences).

- IgG - Gamma heavy chains
- IgM - Mu heavy chains
- IgA - Alpha heavy chains
- IgD - Delta heavy chains
- IgE - Epsilon heavy chains

Immunoglobulin Subclasses

The classes of immunoglobulins can de divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a subclass will have very similar heavy chain constant region amino acid sequences. Again these differences are most commonly detected by serological means.

- IgG Subclasses

- IgG1 - Gamma 1 heavy chains
- IgG2 - Gamma 2 heavy chains
- IgG3 - Gamma 3 heavy chains
- IgG4 - Gamma 4 heavy chains

- IgA Subclasses

- IgA1 - Alpha 1 heavy chains
- IgA2 - Alpha 2 heavy chains

Immunoglobulin Types
Immunoglobulins can also be classified by the type of light chain that they have. Light chain types are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means.

Kappa light chains

Lambda light chains

Immunoglobulin Subtypes
The light chains can also be divided into subtypes based on differences in the amino acid sequences in the constant region of the light chain.

Lambda subtypes

- Lambda 1
- Lambda 2
- Lambda 3
- Lambda 4

Nomenclature
Immunoglobulins are named based on the class, or subclass of the heavy chain and type or subtype of light chain. Unless it is stated precisely, you should assume that all subclass, types and subtypes are present. IgG means that all subclasses and types are present.

Heterogeneity
Immunoglobulins considered as a population of molecules are normally very heterogeneous because they are composed of different classes and subclasses each of which has different types and subtypes of light chains. In addition, different immunoglobulin molecules can have different antigen binding properties because of different V_H and V_L regions.

STRUCTURE AND SOME PROPERTIES OF IG CLASSES AND SUBCLASSES

IgG

Structure
The structures of the IgG subclasses are presented in figure 7. All IgG's are monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.
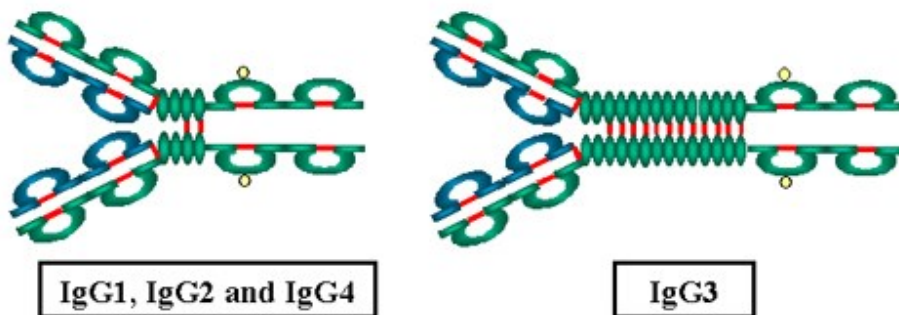
IgG

- Structure
  - Monomer (7S)

IgG1, IgG2 and IgG4   IgG3

Figure 7 IgG Structure

Properties

IgG is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.

- IgG is the major Ig in serum - 75% of serum Ig is IgG
- IgG is the major Ig in extra vascular spaces
- Placental transfer - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by a receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally well; IgG2 does not cross well.
- Fixes complement - Not all subclasses fix equally well; IgG4 does not fix complement
- Binding to cells - Macrophages, monocytes, PMNs and some lymphocytes have Fc receptors for the Fc region of IgG. Not all subclasses bind equally well; IgG2 and IgG4 do not bind to Fc receptors. A consequence of binding to the Fc receptors on PMNs, monocytes and macrophages is that the cell can now internalize the antigen better. The antibody has prepared the antigen for eating by the phagocytic cells. The term opsonin is used to describe substances that enhance phagocytosis. IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions.

IgM

Structure

The structure of IgM is presented in figure 8. IgM normally exists as a pentamer (19S immunoglobulin) but it can also exist as a monomer. In the pentameric form all heavy chains are identical and all light chains are identical.

Thus, the valence is theoretically 10. IgM has an extra domain on the mu chain (C_{H4}) and it has another protein covalently bound via a S-S bond called the J chain. This chain functions in polymerization of the molecule into a pentamer.
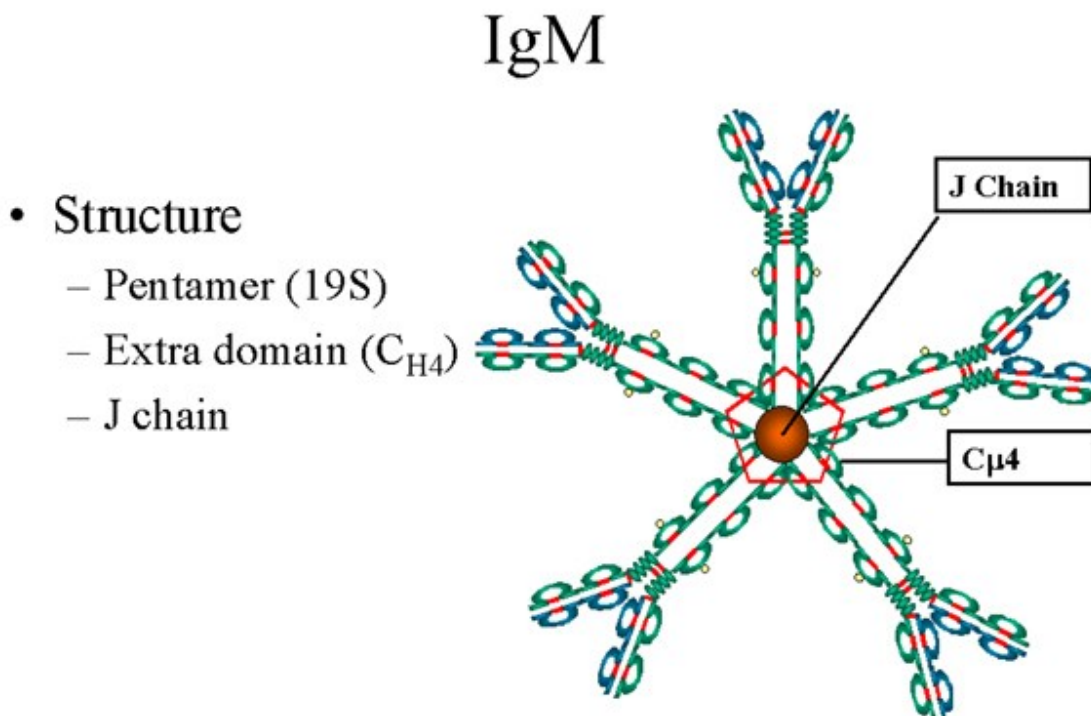
IgM

- Structure
  - Pentamer (19S)
  - Extra domain (C_{H4})
  - J chain

Figure 8
Pentameric serum IgM structure

Properties

- IgM is the third most common serum Ig.
- IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.
- As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms.
- As a consequence of its structure, IgM is also a good agglutinating Ig . Thus, IgM antibodies are very good in clumping microorganisms for eventual elimination from the body.
- IgM binds to some cells via Fc receptors.
- B cell surface Ig
  Surface IgM exists as a monomer and lacks J chain but it has an extra 20 amino acids at the C-terminus to anchor it into the membrane (figure 9). Cell surface IgM functions as a receptor for antigen on B cells. Surface IgM is noncovalently associated with two additional proteins in the membrane of the B cell called Ig-alpha and Ig-beta as indicated in figure 10. These additional proteins act as signal transducing molecules since the cytoplasmic tail of the Ig molecule itself is too short to transduce a signal. Contact between surface immunoglobulin and an antigen is required before a signal can be transduced by the Ig-alpha and Ig-beta chains. In the case of T-independent antigens, contact between the antigen and surface immunoglobulin is sufficient to activate B cells to differentiate into antibody secreting plasma cells. However, for T-dependent antigens, a second signal provided by helper T cells is required before B cells are activated.

IgM

- Structure
- Properties
  - 3rd highest serum Ig
  - First Ig made by fetus and B cells
  - Fixes complement
  - Agglutinating Ig
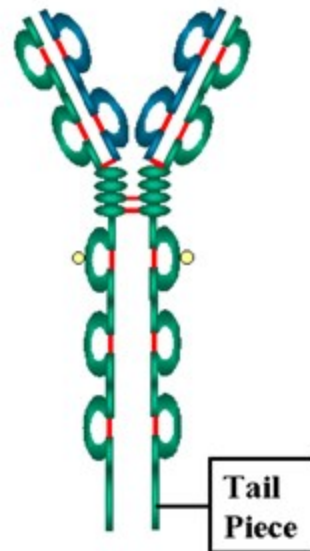 - Binds to Fc receptors
  - B cell surface Ig

Tail Piece

Figure 9
Cell surface IgM structure

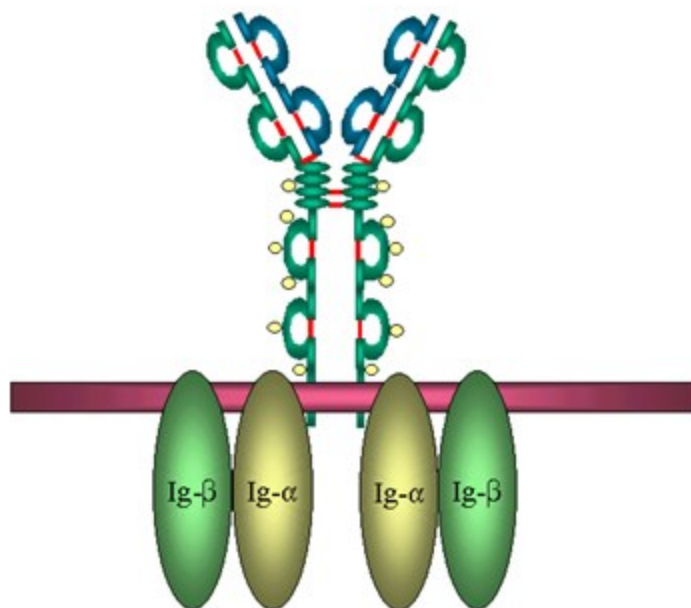# B Cell Antigen Receptor (BcR)

Ig-β | Ig-α | Ig-α | Ig-β

Figure 10
B cell antigen receptor (BcR

IgA

Structure
Serum IgA is a monomer but IgA found in secretions is a dimer as presented in Figure 11. When IgA exits as a dimer, a J chain is associated with it.

When IgA is found in secretions is also has another protein associated with it called the secretory piece or T piece; sIgA is sometimes referred to as 11S immunoglobulin. Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions (Figure 12). The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

IgA

- Structure
  - Serum - monomer
  - Secretions (sIgA)
    - Dimer (11S)
    - J chain
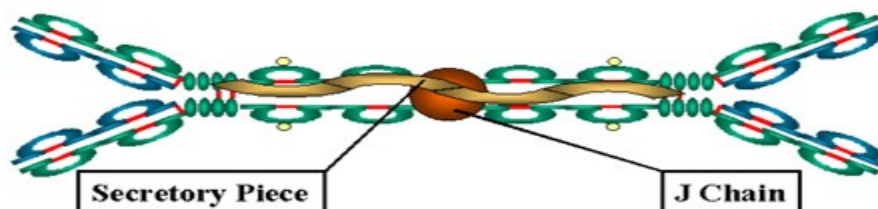   - Secretory component

Secretory Piece   J Chain
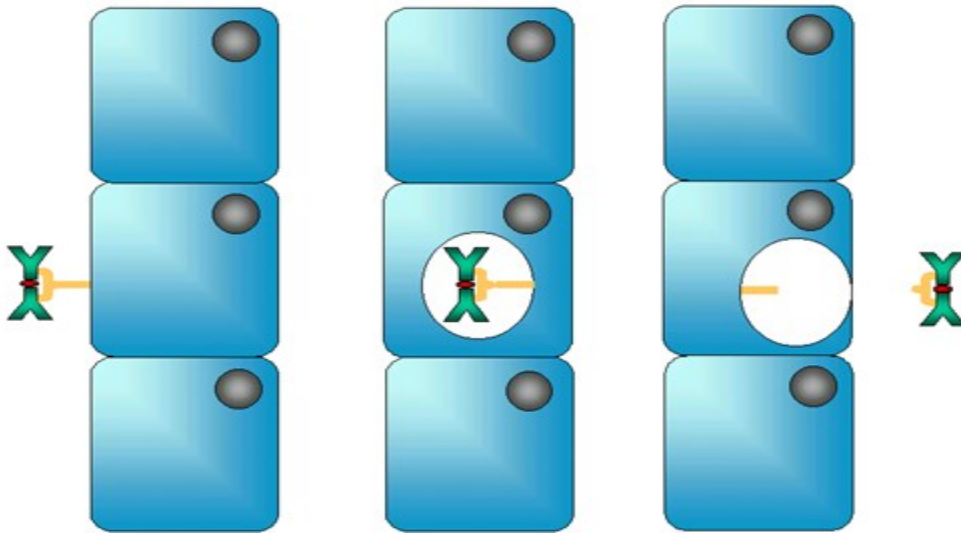
Figure 11
IgA Structure

Origin of sIgA

Figure 12
Origin of soluble IgA

IgD

Structure
The structure of IgD is presented in the Figure 13. IgD exists only as a monomer.

Properties

- IgD is found in low levels in serum; its role in serum uncertain.
- IgD is primarily found on B cell surfaces where it functions as a receptor for antigen. IgD on the surface of B cells has extra amino acids at C-terminal end for anchoring to the membrane. It also associates with the Ig-alpha and Ig-beta chains.
- IgD does not bind complement.

IgD

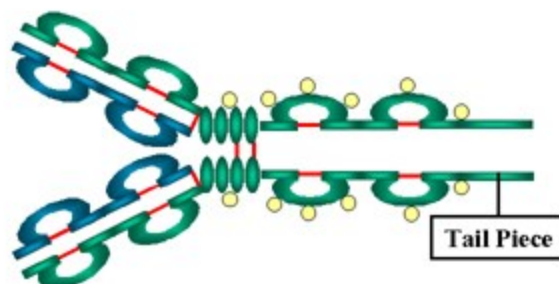- Structure
  - Monomer
  - Tail piece

Tail Piece

Figure 13
IgD Structure

IgE

Structure
The structure of IgE is presented in Figure 14. IgE exists as a monomer and has an extra domain in the constant region.

Properties

- IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.
- Involved in allergic reactions - As a consequence of its binding to basophils an mast cells, IgE is involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.
- IgE also plays a role in parasitic helminth diseases. Since serum IgE levels rise in parasitic diseases, measuring IgE levels is helpful in diagnosing parasitic infections. Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.
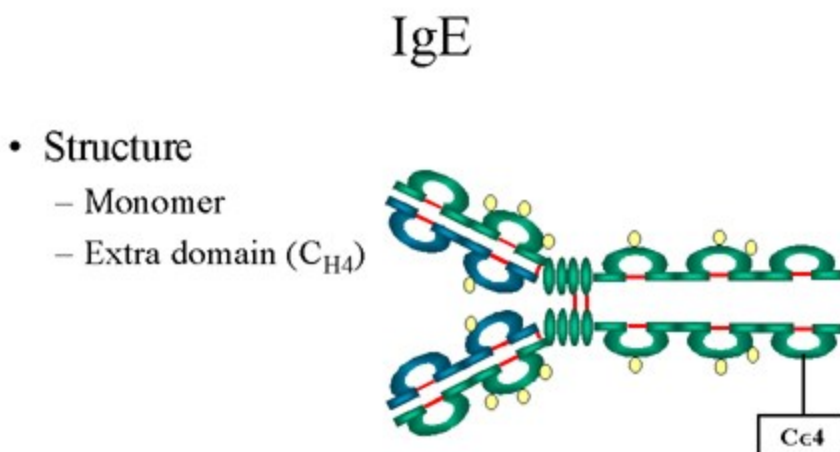- IgE does not fix complement.

IgE

- Structure
  - Monomer
  - Extra domain (C_{H4})

Figure 14
IgE Structure

CLINICAL IMPLICATIONS OF HUMAN IMMUNOGLOBULIN CLASSES

Adapted from:F.T. Fischbach in "A Manual of Laboratory Diagnostic Tests," 2nd Ed., J.B. Lippincott Co., Philadelphia, PA, 1984.

IgG

Increases in:

- Chronic granulomatous infections

- Infections of all types
- Hyperimmunization
- Liver disease
- Malnutrition (severe)
- Dysproteinemia
- Disease associated with hypersensitivity granulomas, dermatologic disorders, and IgG myeloma
- Rheumatoid arthritis

Decreases in:

- Agammaglobulinemia
- Lymphoid aplasia
- Selective IgG, IgA deficiency
- IgA myeloma
- Bence Jones proteinemia
- Chronic lymphoblastic leukemia

IgM

Increases (in adults) in:

- Waldenström's macroglobulinemia
- Trypanosomiasis
- Actinomycosis
- Carrión's disease (bartonellosis)
- Malaria
- Infectious mononucleosis
- Lupus erythematosus
- Rheumatoid arthritis
- Dysgammaglobulinemia (certain cases)

Note: In the newborn, a level of IgM above 20 ng./dl is an indication of *in utero* stimulation of the immune system and stimulation by the rubella virus, the cytomegalovirus, syphilis, or toxoplasmosis.

Decreases in:

- Agammaglobulinemia
- Lymphoproliferative disorders (certain cases)
- Lymphoid aplasia
- IgG and IgA myeloma
- Dysgammaglobulinemia
- Chronic lymphoblastic leukemia

IgA

Increases in:

- Wiskott-Aldrich syndrome
- Cirrhosis of the liver (most cases)
- Certain stages of collagen and other autoimmune disorders such as rheumatoid arthritis and lupus erythematosus
- Chronic infections not based on immunologic deficiencies
- IgA myeloma

Decreases in:

- Hereditary ataxia telangiectasia
- Immunologic deficiency states (*e.g.,* dysgammaglobulinemia, congenital and acquired agammaglobulinemia, and hypogammaglobulinemia)
- Malabsorption syndromes
- Lymphoid aplasia
- IgG myeloma
- Acute lymphoblastic leukemia
- Chronic lymphoblastic leukemia

IgD

Increases in:

- Chronic infections
- IgD myelomas

IgE

Increases in:

- Atopic skin diseases such as eczema
- Hay fever
- Asthma
- Anaphylactic shock
- IgE-myeloma

Decreases in:

- Congenital agammaglobulinemia
- Hypogammaglobulinemia due to faulty metabolism or synthesis of immunoglobulins

THE STRUCTURE AND FUNCTION OF IMMUNOGLOBULINS - ANTIBODIES

Isotypes, Allotypes and Idiotypes

ISOTYPES

Definition

Isotypes are antigenic determinants that characterize classes and subclasses of heavy chains and types and subtypes of light chains.

If human IgM is injected into a rabbit the rabbit will recognize antigenic determinants on the heavy chain and light chain and make antibodies to them. If that antiserum is absorbed with human IgG the antibodies to the light chain determinants and any determinants in common between human IgM and IgG will be removed and the resulting antiserum will be react only with human IgM. Indeed, the antibodies will only react with the constant region of the μ chain. Antibodies to the variable region are rare perhaps because only a few copies of each different variable region are represented in the IgM and thus effective immunization does not occur. The determinants that are recognized by such antibodies are called *isotypic determinants* and the antibodies to those determinants are called *anti-isotypic antibodies*. Each class, subclass, type and subtype of immunoglobulin has its unique set of isotypic determinants.

Location

Heavy chain isotypes are found on the Fc portion of the constant region of the molecule while light chain isotypes are found in the constant region. The location of isotypic determinants is illustrated in Figure 1.
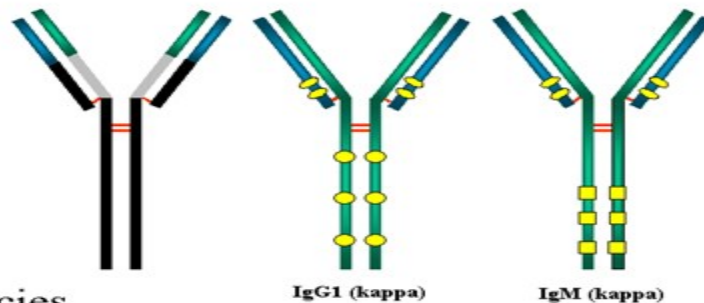

Figure 1 Location of isotype determinants

Occurrence

Isotypes are found in ALL NORMAL individuals in the species. The prefix Iso means same in all members of the species. Some individuals with immunodeficiencies may lack one or more isotypes but normal individuals have all isotypes.

Importance

Antibodies to isotypes are used for the quantitation of immunoglobulin classes and subclasses in various diseases, in the characterization of B cell leukemia and in the diagnosis of various immunodeficiency diseases.

ALLOTYPES

Definition
Allotypes are antigenic determinants specified by allelic forms of the immunoglobulin genes.

Allotypes represent slight differences in the amino acid sequences of heavy or light chains of different individuals. Even a single amino acid difference can give rise to an allotypic determinant, although in many cases there are several amino acid substitutions that have occurred.

Allotypic differences are detected by using antibodies directed against allotypic determinants. These antibodies can be prepared by injecting the immunoglobulin from one person into another. In practice, however, we obtain anti-allotype antisera from women who have had multiple pregnancies or from people who have received blood transfusions or from some patients with rheumatoid arthritis.

Location
In humans, the allotypic differences are localized to the constant region of the heavy and light chains as illustrated in the Figure 2.
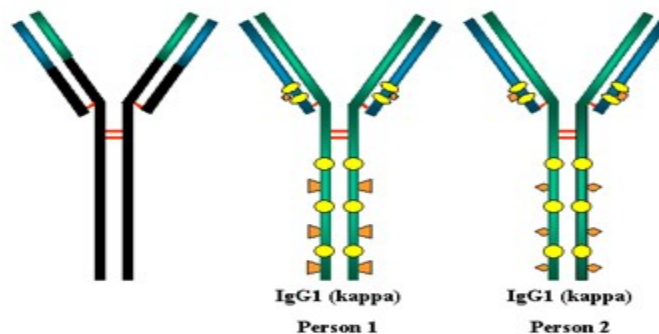

Figure 2 Immunoglobulin allotypes

Occurrence
Individual allotypes are found in individual members of a species. All allotypes are not found in all members of the species. The prefix Allo means different in individuals of a species

Human Immunoglobulin Allotypes

Nomenclature
Human immunoglobulin allotypes are named on the basis of the heavy or light chain on which it is

located. Thus, an allotype on a Gamma 1 heavy chain is given the name: G1m(3). An allotype on a Kappa light chain is given the name: Km(1). Table 1 lists some human allotypes.

Table 1 Human allotypes				
Chain	Domain	Allotype	Amino Acid	Position
IgG1	C_{H1}	G1m(f) = (3)	Arg	214
	C_{H1}	G1m(z) = (17)	Lys	
	C_{H1}	G1m(a) = (1)	Arg, Asp, Glu, Leu	355-358
κ light chain	C_L	Km(1)	Val, Leu	153, 191
	C_L	Km(3)	Ala, Val	153,191

Adapted from Stites *et al.*, Basic and Clin. Immunol., 3rd Ed., Table 7-8

Genetics

Co-dominant autosomal genes

Allotypes that represent amino acid substitutions at the same position in a heavy or light chain (eg. G1m(3) and G1m(17) or Km(1) and Km(3) are inherited as co-dominant autosomal genes. e.g.

Km(1)/Km(3) X Km(1)/Km(1)

⬇

Km(1)/Km(1) and Km(1)/Km(3)

Allelic Exclusion

Although, in a heterozygote, both alleles are expressed, any individual immunoglobulin molecule will only have one allotype. This is because an individual B cell can only express one allele. This is called allelic exclusion. Allotypes that represent amino acid substitutions at different locations in a molecule (*e.g.* G1m(1) and G1m(17)) can be found on the same molecule.

E.g. In a G1m(1,17) individual both allotypes can be on the same heavy chain

GM1(1)   G1m(17)

```
_____|_____|_____
              214                              355-358
```

Importance

- **Monitoring bone marrow grafts**
  Bone marrow grafts that produce a different allotype from the recipient can be used to monitor the graft.

- **Forensic medicine**
  Km and Gm allotypes are detectable in blood stains and semen and are useful in forensic medicine.

- **Paternity testing**
  The immunoglobulin allotypes are one of the characteristics used in legal cases involving paternity.

- **IDIOTYPES (Id)**

- **Definition**
  Unique antigenic determinants present on individual antibody molecules or on molecules of identical specificity.

- Identical specificity means that all antibodies molecules have the exact same hypervariable regions.

- Antigenic determinants created by the combining site of an antibody are called idiotypes and the antibodies elicited to the idiotypes are called anti-Id antibodies. Idiotypes are the antigenic determinants created by the hypervariable regions of an antibody and the anti-idiotypic antibodies are those directed against the hypervariable regions of an antibody.

- To understand what idiotypes are, it is helpful to understand how they are detected.

DNP-BSA ⟹ Strain A ⟹ anti-DNP Ab

⬇

| Antibody against the combining site of anti-DNP Ab | ⬅ | Strain A | ⬅ | purified anti-DNP Ab |

An antigen, in this case the hapten dinitrophenol, is injected into a mouse and antibodies (against DNP) are elicited. This antibody can be purified to homogeneity and injected into another mouse of the same strain. Most epitopes on the antibody will be seen by the second mouse's immune system as "self"; however, the epitopes that form the binding site to DNP (idiotopes - this is a term that is not often used and frequently is used interchangeably with idiotype) will be seen as foreign since the second mouse has not been injected with DNP-BSA. The second mouse will raise antibodies only against the idiotopes of the purified anti-DNP antibody. These are therefore anti-idiotypic antibodies

Antigenic determinants created by the hypervariable region of an antibody are idiotypes

Location

Idiotypes are localized on the Fab fragment of the Ig molecules as illustrated in Figure 3. Specifically, they are localized at or near the hypervariable regions of the heavy and light chains. In many instances, the actual antigenic determinant (i.e. idiotype) may include some of the framework residues near the hypervariable region. Idiotypes are usually determinants created by both heavy and light chain HVR's although sometimes isolated heavy and light chains will express the idiotype.
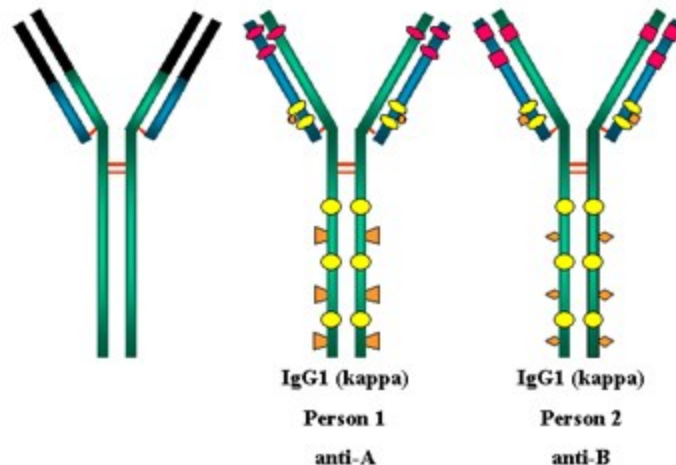

Figure 3 Immunoglobulin idiotypes

Importance

- **V region marker** -
  Idiotypes are a useful marker for a particular variable region.

- **Regulation of immune responses**
  There is evidence that immune responses may be regulated by anti-Id antibodies directed against our own Id's.

- **Vaccines**
  In some cases, anti-idiotypic antibodies actually stimulate B cells to make antibody and thus they can be used as a vaccine. This approach is being tried to immunize against highly dangerous pathogens that cannot be safely used as a vaccine.

Treatment of B cell tumors

Anti-idiotypic antibodies directed against an idiotype on malignant B cells can be used to kill the cells. Killing occurs because of complement fixation or because toxic molecules are attached to the antibodies.

GENETICS OF IMMUNOGLOBULINS

HISTORY

Amino acid sequencing data revealed that a single C region could be associated with many different V regions. Also, it was shown that a single idiotype could be associated with different C regions (eg. IgM and IgG). To explain these data it was suggested that perhaps the two regions of the immunoglobulin molecule were coded for by separate genes and that the V and C region genes were somehow joined before an immunoglobulin molecule was made (i.e. there were two genes for one polypeptide). This was a revolutionary concept but with the advent of recombinant DNA technology, it has been shown to be the correct. The immunoglobulin heavy and light chains are coded for by three separate gene families each one on a separate chromosome - one for the heavy chain and one for each of the light chain types. Each of these gene families has several V region genes and one or more C region genes. The V and C regions genes are not however immediately adjacent to each other.

LIGHT CHAIN GENE FAMILIES

Germ line gene organization
The organization of the *kappa* and *lambda* light chain genes in the germ line of undifferentiated cells is depicted in Figure 1.

- ***Lambda* light chains**
  The *lambda* gene family is composed of 4 C region genes, one for each subtype of lamda chain, and approximately 30 V region genes. Each of the V region genes is composed of two exons, one (L) that codes for a leader region and the other (V) that codes for most of the variable region. Upstream of each of the C genes there is and additional exon called J (joining). The L, V, J and C exons are separated by introns (intervening non-coding sequences).

- ***Kappa* light chains**
  The *kappa* light chain gene family contains only one C region gene, since there is only one type of *kappa* light chain. There are many V region genes (approximately 250) each of which has a leader exon and a V exon. In the κ gene family there are several J exons located between the V and C genes. All of the exons are separated by introns.
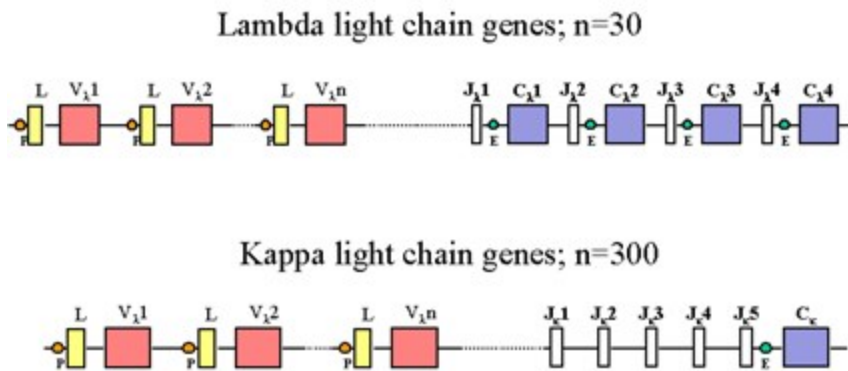

Figure 1
Organization of the *kappa* and lamda light chain genes in the germ line or undifferentiated cells

Gene rearrangement and expression

As a cell differentiates into a mature B cell that will make a light chain, there is a rearrangement of the various genes (exons) and the gene begins to be expressed as depicted in Figure 2.
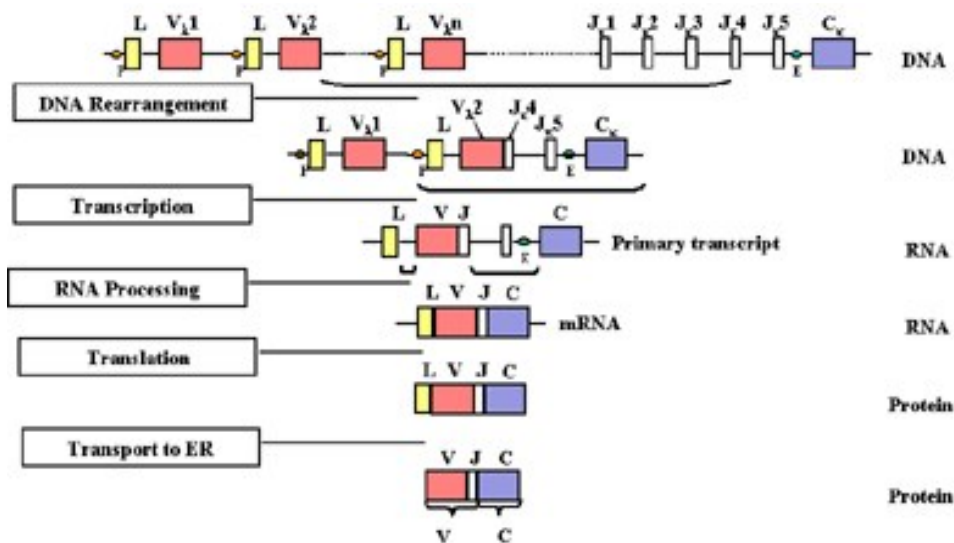

Figure 2
As a cell differentiates into a mature B cell that will make a light chain, there is rearrangement of the various genes (exons)

As a cell commits to become a B cell making a light chain, there is a rearrangement of the genes at the DNA level such that one of the V genes is brought next to one of the J regions. This occurs by a recombination event which removes the intron between the V and J regions. The selection of which V gene is used is not totally random; there is some preference for the use of V genes nearest to the J regions. However, with time all V genes can be used so that all combinations of V genes and J regions can be generated.

A consequence of this DNA rearrangement is that the gene becomes transcriptionally active because a promoter (P), which is associated with the V gene, is brought close to an enhancer (E), which is located in the intron between the J and C regions. As transcription initiates from the promoter, a pre-mRNA is made which contains sequences from the L, V J and C regions as well as sequences for the introns between L and V and between J and C (Figure 2). This pre-mRNA is processed (spliced) in the nucleus and the remaining introns are removed. The resulting mRNA has the L, V J and C exons contiguous.

The mRNA is translated in the cytoplasm and the leader is removed as the protein is transported into the lumen of the endoplasmic reticulum. The light chain is assembled with a heavy chain in the endoplasmic reticulum and the immunoglobulin is secreted via the normal route of secretory proteins. The region V region of the mature light chain is coded for by sequences in the V gene and J region and the C region by sequences in the C gene.

HEAVY CHAIN GENE FAMILY

Germ line gene organization
The organization of the heavy chain genes is depicted in Figure 3.

In the heavy chain gene family there are many C genes, one for each class and subclass of immunoglobulin. Each of the C genes is actually composed of several exons, one for each domain and another for the hinge region. In the heavy chain gene family there are many V region genes, each composed of a leader and V exon. In addition to several J exons, the heavy chain gene family also contains several additional exons called the D (diversity) exons. All of the exons are separated by introns as depicted in Figure 3.
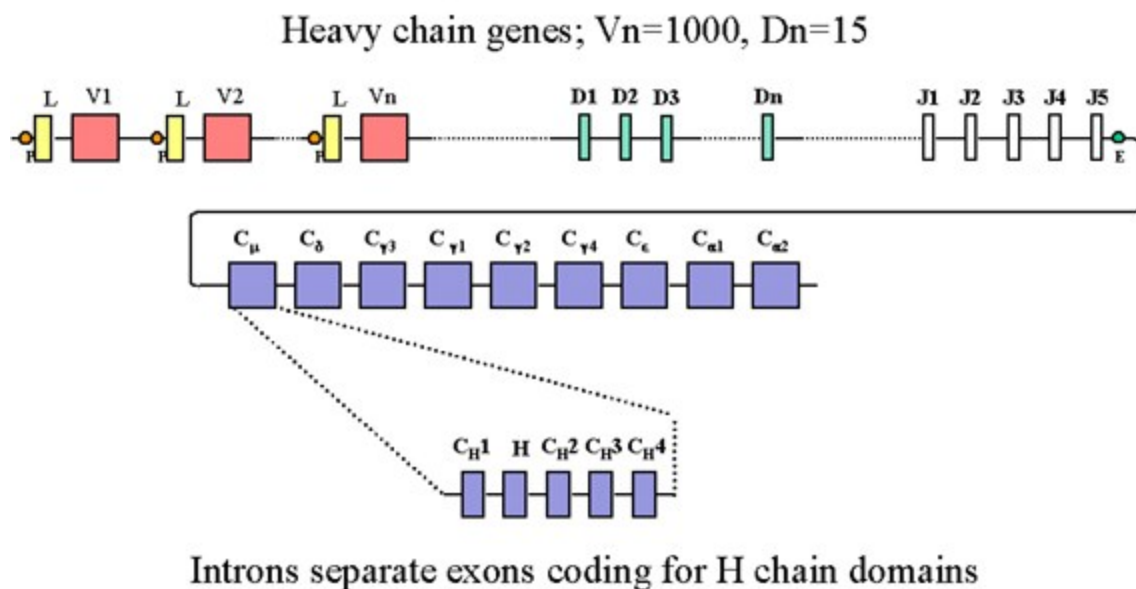

Figure 3
 In addition to several J exons, the heavy chain gene family also contains several additional exons called the D (diversity) exons. All of the exons are separated by introns

Gene rearrangements and expression
As a cell differentiates into a mature B cell that will make a heavy chain, there is a rearrangement of the various genes segments (exons) and the gene begins to be expressed as depicted in Figures 4 and 5.
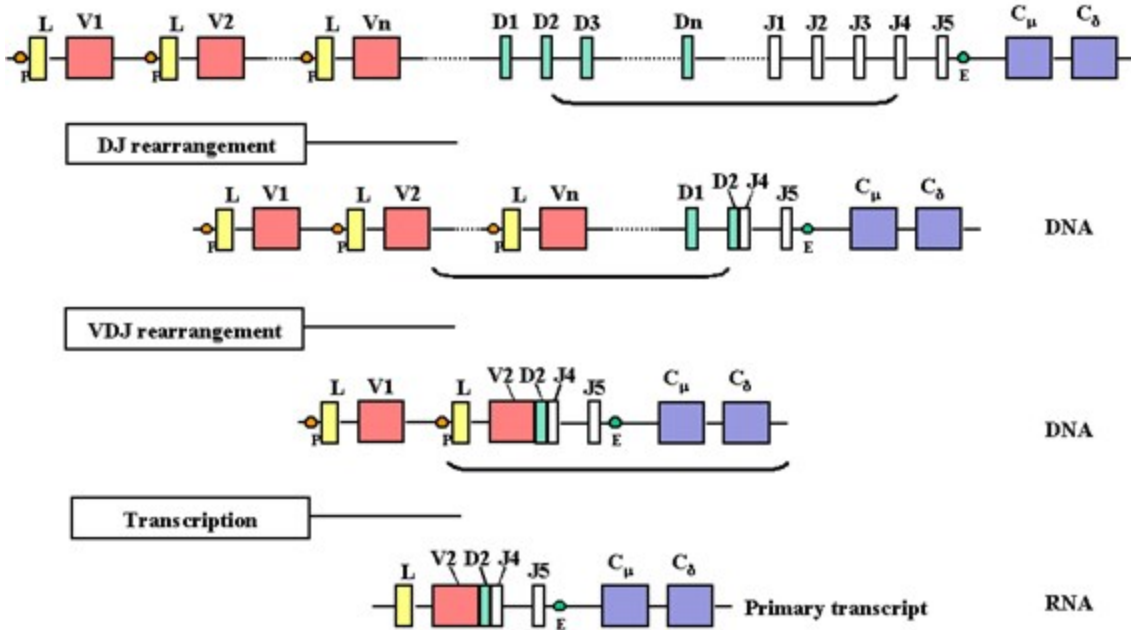
Figure 4
As transcription initiates from the promoter a pre-mRNA is made which contains sequences from the L, V, D, J Cμ and Cδ regions as well as sequences for the introns between L and V, between J and Cμ, and between Cμ and Cδ
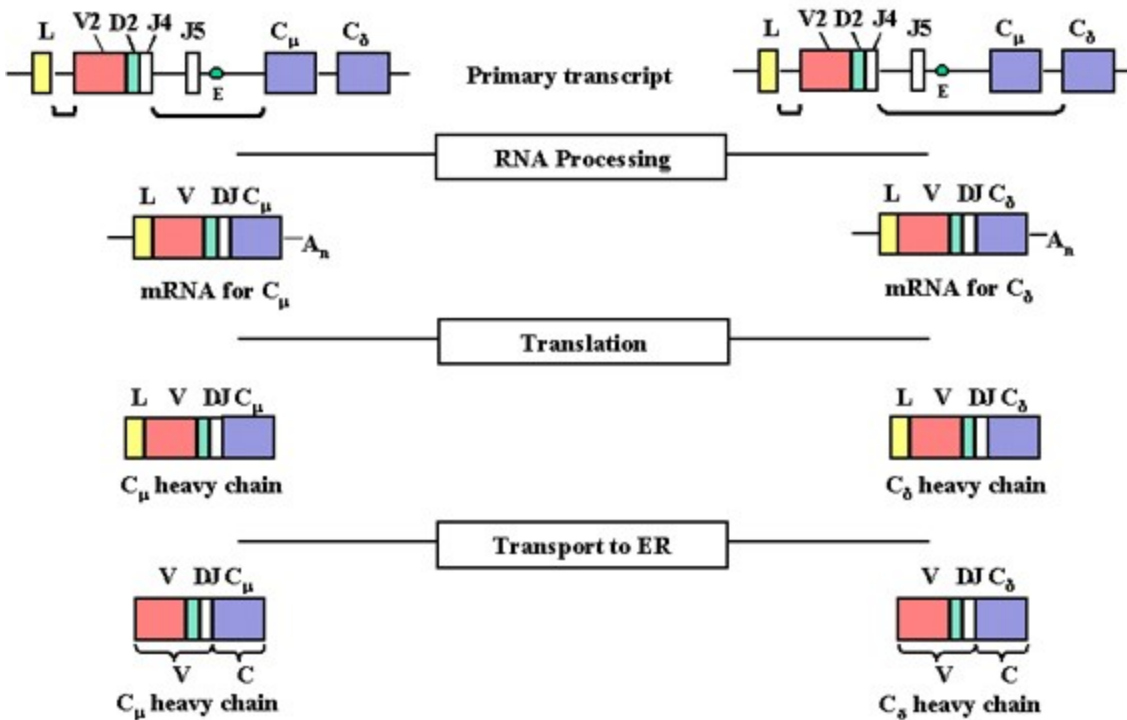

Figure 5
The pre-mRNA is processed (spliced) in the nucleus and the remaining introns, including those between the exons in the C genes, are removed

As a cell commits to become a B cell making a heavy chain, there are two rearrangements at the DNA level. First, one of the D regions is brought next to one of the J regions and then one of the V genes is brought next to the rearranged DJ region. This occurs by two recombination events which remove the

introns between the V, D and J regions. As with the light chains the selection of the heavy chain V gene is not totally random but eventually all of the V genes can be used.

A consequence of these DNA rearrangements is that the gene becomes transcriptionally active because a promoter (P), which is associated with the V gene, is brought close to an enhancer (E), which is located in the intron between the J and C_{mu} regions. As transcription initiates from the promoter a pre-mRNA is made which contains sequences from the L, V, D, J C_{mu} and C_{delta} regions as well as sequences for the introns between L and V, between J and C_{mu}, and between C_{mu} and C_{delta} (Figure 4).

The pre-mRNA is processed (spliced) in the nucleus and the remaining introns, including those between the exons in the C genes, are removed (See Figure 5). The pre-mRNA can be processed in two ways, one to bring the VDJ next to the C_{mu} gene and the other to bring the VDJ next to the C_{delta} gene. The resulting mRNAs have the L, V, D, J and C_{mu} or C_{delta} exons contiguous and will code for a *mu* and a *delta* chain, respectively.

The mRNAs are translated in the cytoplasm and the leader is removed as the protein is transported into the lumen of the endoplasmic reticulum. The heavy chain is assembled with a light chain in the endoplasmic reticulum and the immunoglobulin is secreted via the normal route of secretory proteins. The V region of the mature heavy chain is coded for by sequences in the V gene, D region and J region and the C region by sequences in the C gene.
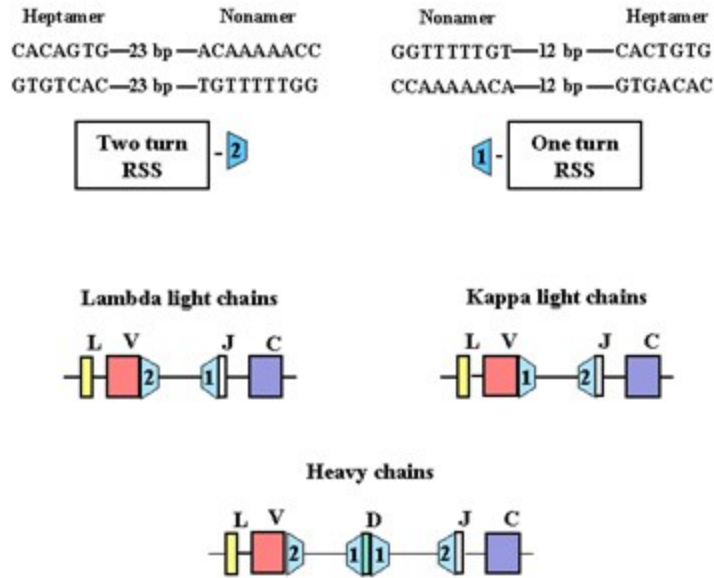
MECHANISM OF DNA REARRANGEMENTS

Flanking the V, J and D exons, there are unique sequences referred to as recombination signal sequences (RSS), which function in recombination. Each RSS consists of a conserved nonamer and a conserved heptamer that are separated by either 12 or 23 base pairs (bp) as illustrated in Figure 6. The 12bp and 23 bp spaces correspond to one or two turns of the DNA helix.

Recombination only occurs between a 1 turn and a 2 turn signal. In the case of the λ light chains there is a 1 turn signal upstream of the J exon and a 2 turn signal downstream of V_{lambda}. In the case of the κ light chains there is a 1 turn signal downstream of the V_{kappa} gene and a 2 turn signal upstream of the J exon. In the case of the heavy chains there are 1 turn signals on each side of the D exon and a 2 turn signal downstream of the V gene and a 2 turn signal upstream of the J exon. Thus, this ensures that the correct recombination events will occur.

The recombination event results in the removal of the introns between V and J in the case of the light chains or between the V, D, and J in the case of the heavy chains. The recombination event is catalyzed by two proteins, Rag-1 and Rag-2. Mutations in the genes for these proteins results in a severe combined immunodeficiency disease (both T and B cells are deficient), since these proteins and the RSS are involved in generating both the B and T cell receptors for antigen.

Mechanism of DNA Rearrangements

- **Recombination signal sequences (RSS)**
  - Nonmer
  - Heptamer
  - 1 or 2 turn signals
- **Rag-1 and Rag-2**

Heptamer		Nonamer
CACAGTG	—23 bp—	ACAAAAACC
GTGTCAC	—23 bp—	TGTTTTTGG

Nonamer		Heptamer
GGTTTTTGT	—12 bp—	CACTGTG
CCAAAAACA	—12 bp—	GTGACAC

Two turn RSS - 2

1 - One turn RSS

Lambda light chains

L V 2 1 J C

Kappa light chains

L V 1 2 J C

Heavy chains

L V 2 1 1 2 D J C

Figure 6
Flanking the V, J and D exons there are unique sequences referred to as recombination signal sequences (RSS), which function in recombination. Each RSS consists of a conserved nonamer and a conserved heptamer that are separated by either 12 or 23 base pairs

ORDER OF GENE EXPRESSION IN IMMUNOGLOBULIN GENE FAMILIES

An individual B cell only produces one type of light chain and one class of heavy chain. (*N.B.* The one exception is that a mature B cell can produce both μ and δ heavy chains but the antibody specificity is the same since the same VDJ region is found on the μ and δ chains). Since any B cell has both maternal and paternal chromosomes which code for the immunoglobulin genes there must be some orderly way in which a cell expresses its immunoglobulin genes so as to ensure that only one type of light chain and one class of heavy chain is produced.

The order in which the immunoglobulin genes are expressed in a B cell is depicted in Figure 7 and 8.

Heavy chain (Figure 7)
A cell first attempts to rearrange one of its heavy chain genes; in some cells the maternal chromosome is selected and in others the paternal chromosome is selected. If the rearrangement is successful so that a heavy chain is made, then no further rearrangements occur in the heavy chain genes. If, on the other hand, the first attempt to rearrange the heavy chain genes is unsuccessful (*i.e.* no heavy chain is made), then the cell attempts to rearrange the heavy chain genes on its other chromosome. If the cell is unsuccessful in rearranging the heavy chain genes the second time, it is destined to be eliminated.
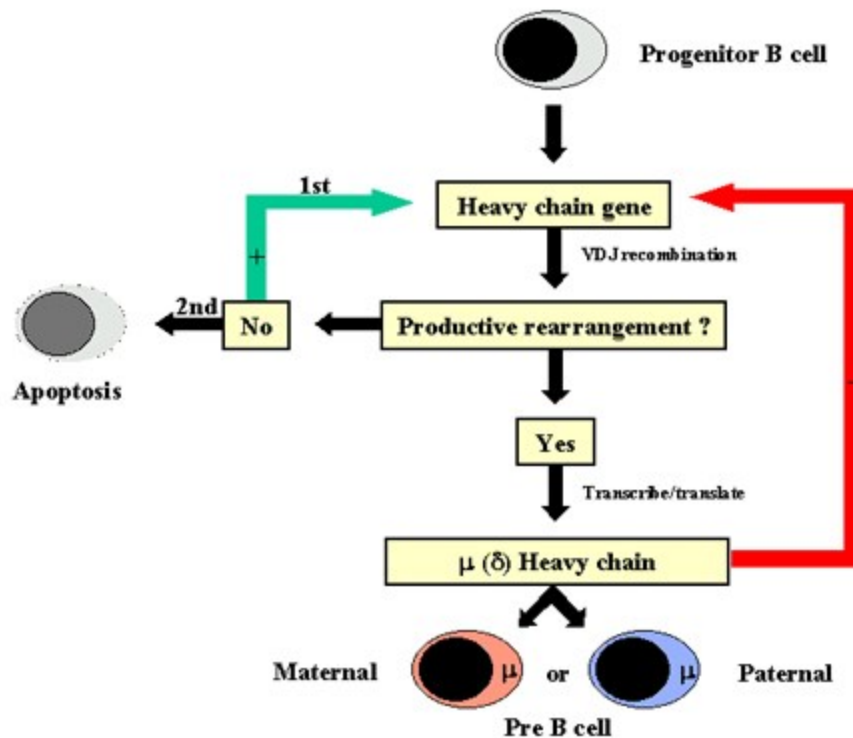
Order of Ig Gene Expression

Figure 7 Order of Ig gene expression - Heavy chain

Kappa light chain (Figure 8)

When a cell successfully rearranges a heavy chain gene, it then begins to rearrange one of its *kappa* light chain genes. It is a random event whether the maternal or paternal *kappa* light chain genes are selected. If the rearrangement is unsuccessful (*i.e.* it does not produce a functional *kappa* light chain), then it attempts to rearrange the *kappa* genes on the other chromosome. If a cell successfully rearranges a *kappa* light chain gene, it will be a B cell that makes an immunoglobulin with a *kappa* light chain.

Lambda light chain (Figure 8)

If a cell is unsuccessful in rearranging both of its *kappa* light chain genes, it then attempts to make a *lambda* light chain. It is a random event whether the maternal or paternal *lambda* light chain genes are selected. If the rearrangement is unsuccessful (*i.e.* it does not produce a functional *lambda* light chain), then it attempts to rearrange the *lambda* genes on the other chromosome. If a cell successfully rearranges a *lambda* light chain gene, it will be a B cell that makes an immunoglobulin with a *lambda* light chain.

The orderly sequence of rearrangements in the immunoglobulin gene families explains:

- Why an individual B cell can only produce one kind of immunoglobulin with one kind of heavy and one kind of light chain.

- Why a individual B cell can only make antibodies of one specificity.

- Why there is allelic exclusion in immunoglobulin allotypes at the level of an individual immunoglobulin molecule but co-dominant expression of allotypes in the organism as a whole.
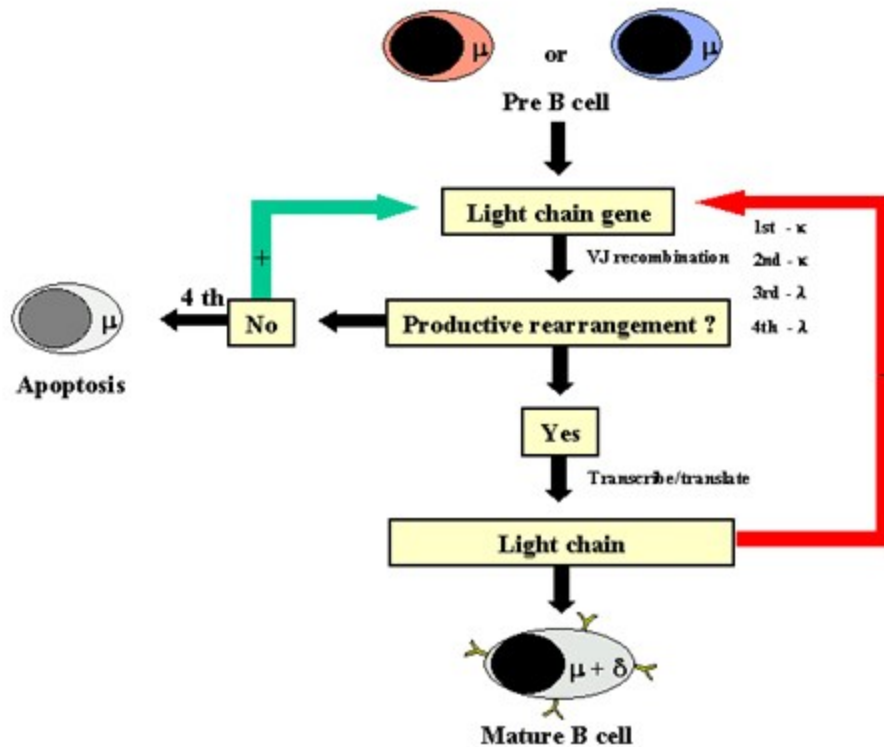
Order of Ig Gene Expression

Figure 8

Order of Ig gene expression - Light chain

ORIGIN OF ANTIBODY DIVERSITY

Background
Antibody diversity refers to the sum total of all the possible antibody specificities that an organism can make. It is estimated that we can make 10^7 - 10^8 different antibody molecules. One of the major questions in immunology has been how can we make so many different antibody molecules. Theories which have attempted to explain the origin of antibody diversity fall into two major categories.

Germ line theory
This theory states that we have a different V region gene for each possible antibody we can make.

Somatic mutation theory
This theory states that we have only one or a few V region genes and the diversity is generated by somatic mutations which occur in these genes.

Current Concepts

Our current thinking is that both the germ line and somatic mutation theories have some merit. It is thought that antibody diversity is generated by the following mechanisms.

1. A large number of V genes

There are:

a) 30 *lambda* V genes
b) 300 *kappa* V genes
c) 1000 heavy chain V genes

2. V-J and V-D-J joining

The region where the light chain V gene and J region or the heavy chain V gene and D and J regions come together is in the third hypervariable region. Since it is random which V and which J or D regions come together, there is a lot of diversity that can be generated by V-J and V-D-J joining.

3. Junctional diversity (Inaccuracies in V-J and V-D and D-J recombination) - (Figure 9)
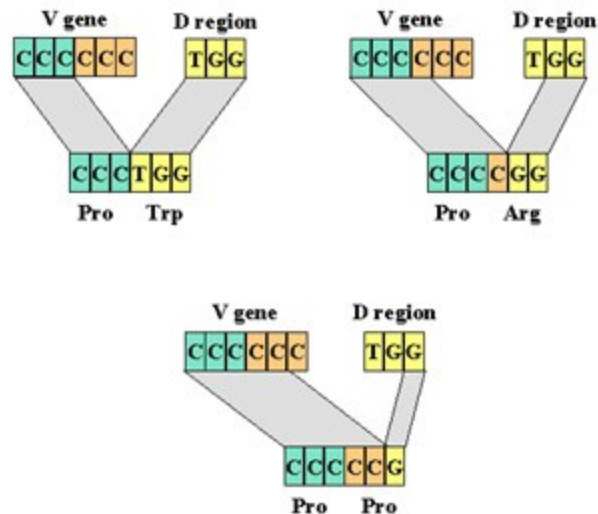

Figure 9
Origin of antibody diversity current concepts

Recombination between V-J and V-D-J is not always perfect and additional diversity can arise by errors that occur in the recombination event that brings the V region next to the J or D regions or the D region next to the J region. It is estimated that these inaccuracies can triple the diversity generated by V-J and V-D-J joining. The diversity generated by this mechanism is occurring in the third hypervariable region and thus, is directly affecting the combining site of the antibody.

4. N region insertion

At the junction between D and J segments there is often an insertion of a series of nucleotides which is catalyzed by the enzyme terminal transferase. Terminal transferase catalyzes the random polymerization of nucleotides into DNA without the need for a template. This leads to further diversity in the third hypervariable region.

5. Somatic Mutation

There is evidence that somatic mutations are occurring in the V gene, particularly in the place that codes for the second hypervariable region. Thus, somatic mutation probably contributes to antibody diversity to some extent.

6. Combinatorial Association

Any individual B cell has the potential to make any one of the possible heavy chains and any one of the possible light chains. Thus, different combinations of heavy and light chains within an individual B cell adds further diversity.

7. Multispecificity

Due to cross reactions between antigenic determinants of similar structure an antibody can often react with more than one antigenic determinant. This is termed multispecificity. Multispecificity also contributes to antibody diversity.

An example of how these mechanisms can generate a great deal of diversity is illustrated below:

	B Cell Receptor (Immunoglobulin)	
	Heavy	*Kappa*
V gene segments	1000	300
D gene segments	15	-
J gene segments	4	4
N region insertion	++	-
Junctional diversity	+++	+
Somatic mutation	+	+
Combinatorial association	V x D x J 1000 X 15 X 4	V x J 300 x 4
Total	6×10^4	1.2×10^3
Combinatorial association	7.2×10^7	

These calculations do not take into consideration the contributions of *lambda* light chains, somatic mutation junctional diversity, N region insertions or multispecificity.

The process of gene rearrangement of the heavy and light chains and the combinatorial association of these chains occurs during B cell development and is *independent of antigen*. Clones of B cells expressing all of the possible antibody specificities are produced during development and antigen simply selects those clones which have the appropriate receptor. The selected clones are then activated, proliferate and differentiate into antibody secreting plasma cells.

T CELL RECEPTOR FOR ANTIGEN

T cells also have a receptor for antigen on their surfaces. This receptor is not an immunoglobulin molecule but it is composed of two different polypeptide chains which have constant and variable regions analogous to the immunoglobulins. Diversity in the T cell receptor is also generated in the same way as described for antibody diversity (*e.g.* by VJ and VDJ joining of gene segments and combinatorial association). However, no somatic mutation has been observed in T cells.

IMMUNOGLOBULINS- ANTIGEN-ANTIBODY REACTIONS

AND SELECTED TESTS

## NATURE OF ANTIGEN-ANTIBODY REACTIONS

Lock and Key Concept

The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. X-Ray crystallography studies of antigen-antibody interactions show that the antigenic determinant nestles in a cleft formed by the combining site of the antibody as illustrated in Figure 1. Thus, our concept of antigen-antibody reactions is one of a key (*i.e.* the antigen) which fits into a lock (*i.e.* the antibody).

Non-covalent Bonds

The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds. Multiple bonding between the antigen and the antibody ensures that the antigen will be bound tightly to the antibody.

Reversibility

Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible.
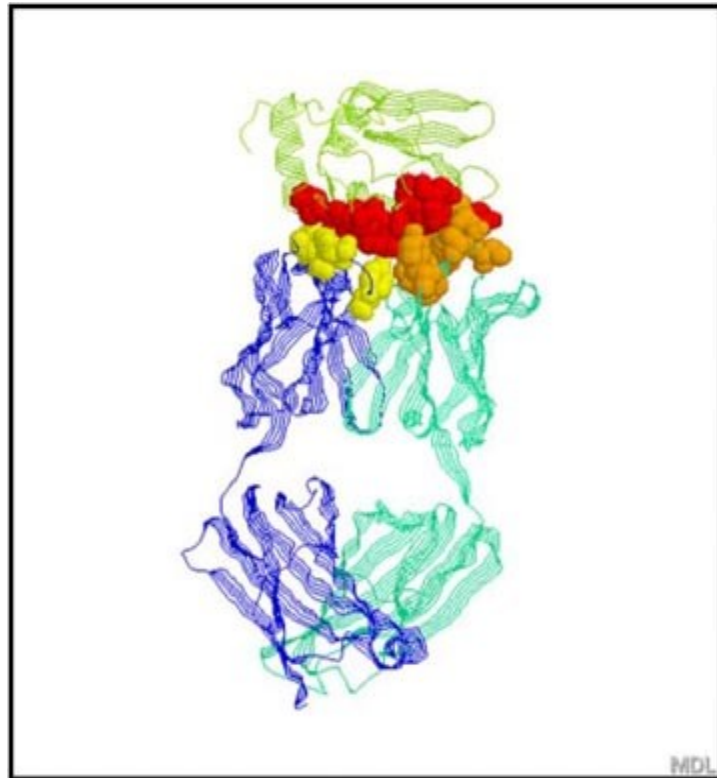
Source: Li, Y., Li, H., Smith-Gill, S. J.,
Mariuzza, R. A., Biochemistry 39, 6296, 2000

Figure 1

AFFINITY AND AVIDITY

Affinity

Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody as illustrated in Figure 2.
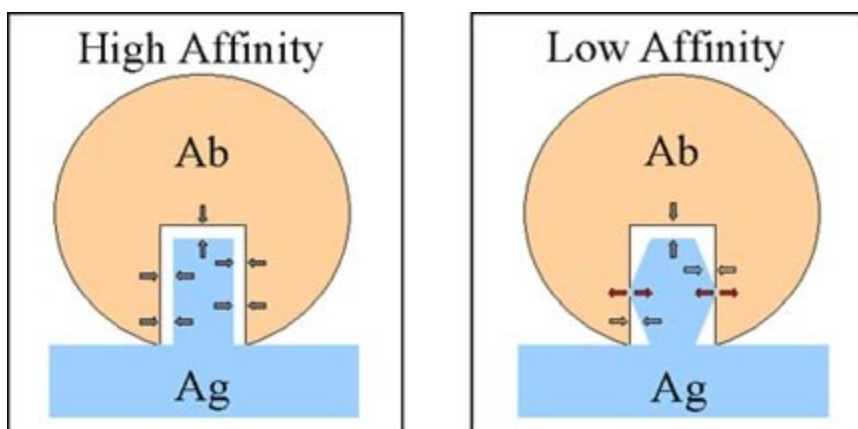

Figure 2

Affinity is the equilibrium constant that describes the antigen-antibody reaction as illustrated in Figure 3. Most antibodies have a high affinity for their antigens.
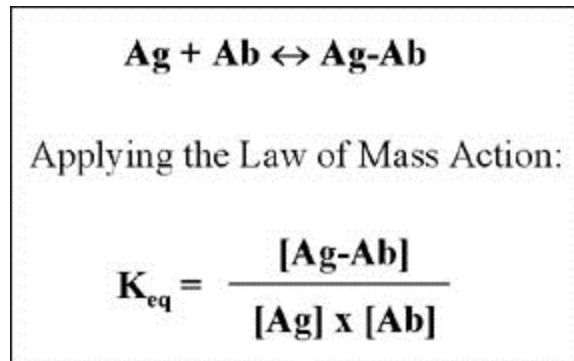
$$Ag + Ab \leftrightarrow Ag\text{-}Ab$$

Applying the Law of Mass Action:

$$K_{eq} = \frac{[Ag\text{-}Ab]}{[Ag] \times [Ab]}$$

Figure 3

Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities. This is illustrated in Figure 4.

$K_{eq} =$ 10^4 10^6 10^{10}
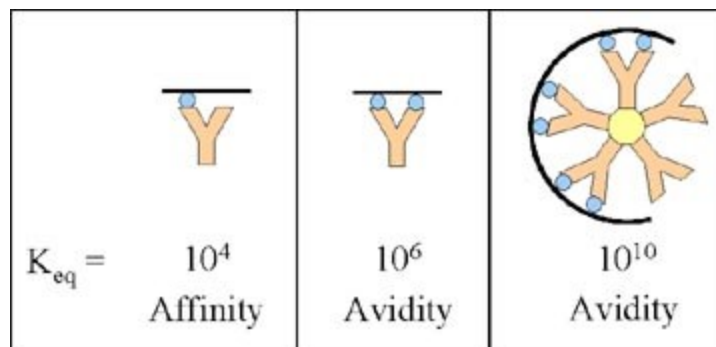
Affinity   Avidity   Avidity

Figure 4

To repeat, affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent antigens and antibodies.

SPECIFICITY AND CROSS REACTIVITY

Specificity

Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one

antigen. In general, there is a high degree of specificity in antigen-antibody reactions. Antibodies can distinguish differences in:

- The primary structure of an antigen
- Isomeric forms of an antigen
- Secondary and tertiary structure of an antigen

Cross reactivity

Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen. Figure 5 illustrates how cross reactions can arise. Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).
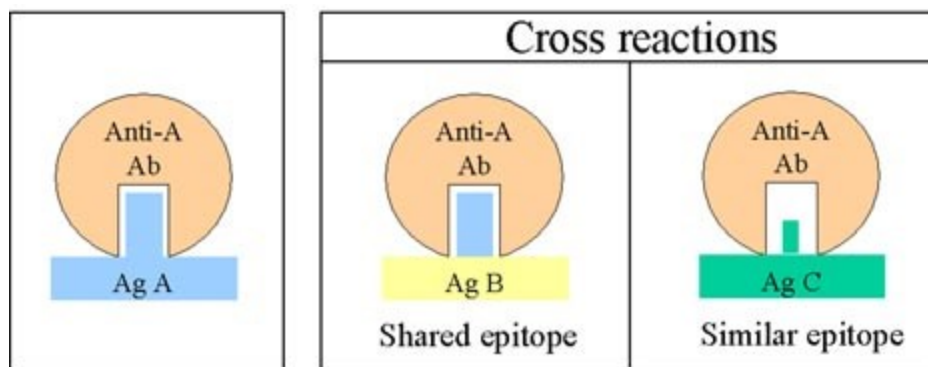

Figure 5

TESTS FOR ANTIGEN-ANTIBODY REACTIONS

Factors affecting measurement of antigen-antibody reactions

The only way that one knows that an antigen-antibody reaction has occurred is to have some means of directly or indirectly detecting the complexes formed between the antigen and antibody. The ease with which one can detect antigen-antibody reactions will depend on a number of factors.

Affinity
The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.

Avidity
Reactions between multivalent antigens and multivalent antibodies are more stable and thus easier to detect.

Antigen to antibody ratio

The ratio between the antigen and antibody influences the detection of antigen-antibody complexes because the size of the complexes formed is related to the concentration of the antigen and antibody. This is depicted in Figure 6.
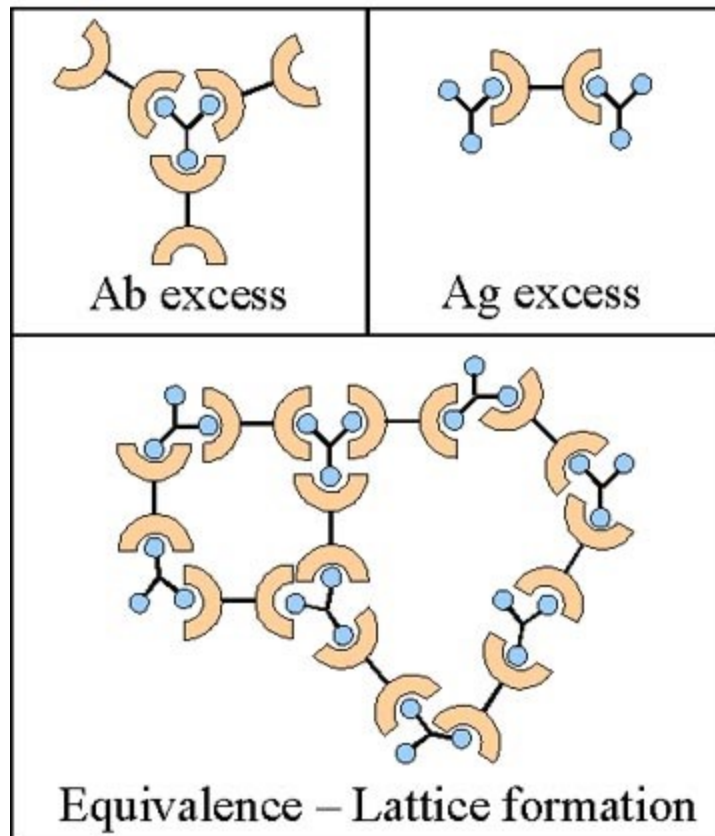

Figure 6

Physical form of the antigen

The physical form of the antigen influences how one detects its reaction with an antibody. If the antigen is a particulate, one generally looks for agglutination of the antigen by the antibody. If the antigen is soluble one generally looks for the precipitation of the antigen after the production of large insoluble antigen-antibody complexes.

Agglutination Tests

Agglutination/Hemagglutination

When the antigen is particulate, the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. The general term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term hemagglutination is used. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly good agglutinin and one sometimes infers that an antibody may be of the IgM class if it is a good agglutinating antibody.

Qualitative agglutination test

Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen. (Figure 7).

For example, a patient's red blood cells can be mixed with antibody to a blood group antigen to determine a person's blood type. In a second example, a patient's serum is mixed with red blood cells of a known blood type to assay for the presence of antibodies to that blood type in the patient's serum.
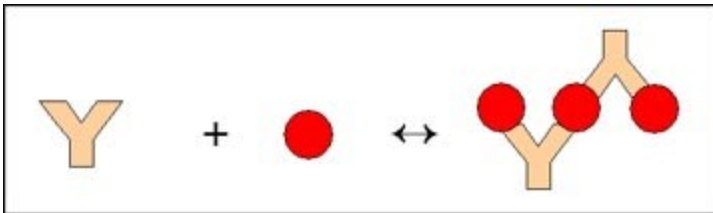

Figure 7

Quantitative agglutination test

Agglutination tests can also be used to measure the level of antibodies to particulate antigens. In this test, serial dilutions are made of a sample to be tested for antibody and then a fixed number of red blood cells or bacteria or other such particulate antigen is added. Then the maximum dilution that gives agglutination is determined. The maximum dilution that gives visible agglutination is called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination. Figure 8 illustrates a quantitative hemagglutination test.

Prozone effect - Occasionally, it is observed that when the concentration of antibody is high (i.e. lower dilutions), there is no agglutination and then, as the sample is diluted, agglutination occurs (See Patient 6 in Figure 8). The lack of agglutination at high concentrations of antibodies is called the prozone effect. Lack of agglutination in the prozone is due to antibody excess resulting in very small complexes that do not clump to form visible agglutination.

Figure 8

Applications of agglutination tests

i. Determination of blood types or antibodies to blood group antigens.

ii. To assess bacterial infections

e.g. A rise in titer of an antibody to a particular bacterium indicates an infection with that bacterial type. N.B. a fourfold rise in titer is generally taken as a significant rise in antibody titer.

Practical considerations
Although the test is easy to perform, it is only semi-quantitative.

Passive hemagglutination
The agglutination test only works with particulate antigens. However, it is possible to coat erythrocytes with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red blood cells in an agglutination test for antibody to the soluble antigen (Figure 9). This is called passive hemagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.
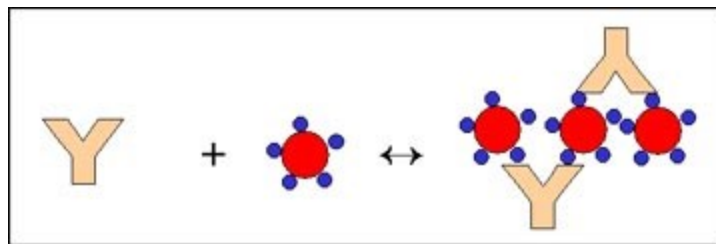

Figure 9

Coomb's Test (Antiglobulin Test)

Direct Coomb's Test
When antibodies bind to erythrocytes, they do not always result in agglutination. This can result from the antigen/antibody ratio being in antigen excess or antibody excess or in some cases electrical charges on the red blood cells preventing the effective cross linking of the cells. These antibodies that bind to but do not cause agglutination of red blood cells are sometimes referred to as incomplete antibodies. In no way is this meant to indicate that the antibodies are different in their structure, although this was once thought to be the case. Rather, it is a functional definition only. In order to detect the presence of non-agglutinating antibodies on red blood cells, one simply adds a second antibody directed against the immunoglobulin (antibody) coating the red cells. This anti-immunoglobulin can now cross link the red blood cells and result in agglutination. This test is illustrated in Figure 10 and is known as the Direct Coomb's test.
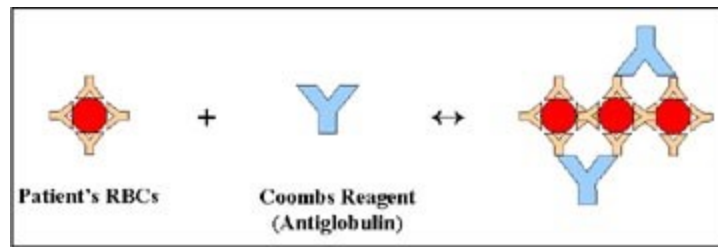
Figure 10

Indirect Coomb's Test

If it is necessary to know whether a serum sample has antibodies directed against a particular red blood cell and you want to be sure that you also detect potential non- agglutinating antibodies in the sample, an Indirect Coomb's test is performed (Figure 11). This test is done by incubating the red blood cells with the serum sample, washing out any unbound antibodies and then adding a second anti-immunoglobulin reagent to cross link the cells.
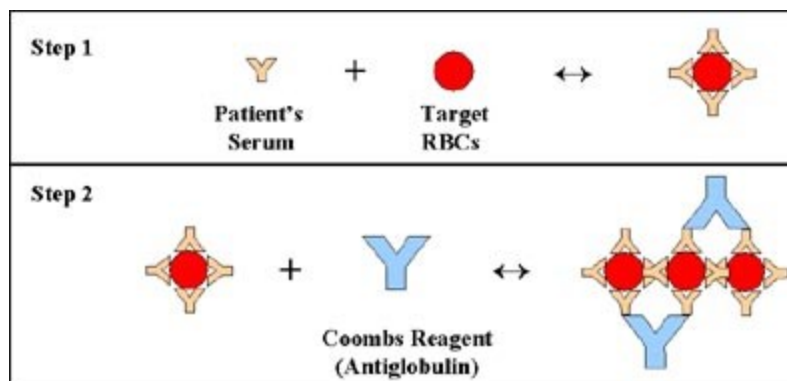

Figure 11

Applications

These include detection of anti-rhesus factor (Rh) antibodies. Antibodies to the Rh factor generally do not agglutinate red blood cells. Thus, red cells from Rh+ children born to Rh- mothers, who have anti-Rh antibodies, may be coated with these antibodies. To check for this, a direct Coombs test is performed. To see if the mother has anti-Rh antibodies in her serum an Indirect Coombs test is performed.

Hemagglutination Inhibition

The agglutination test can be modified to be used for the measurement of soluble antigens. This test is called hemagglutination inhibition. It is called hemagglutination inhibition because one measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test, a fixed amount of antibodies to the antigen in question is mixed with a fixed amount of red blood cells coated with the antigen (see passive hemagglutination above). Also included in the mixture are different amounts of the sample to be analyzed for the presence of the antigen. If the sample contains the antigen, the soluble antigen will compete with the antigen coated on the red blood cells for binding to the antibodies, thereby inhibiting the agglutination of the red blood cells. as illustrated in Figure 12.

By serially diluting the sample, you can quantitate the amount of antigen in your unknown sample by its titer. This test is generally used to quantitate soluble antigens and is subject to the same practical considerations as the agglutination test.
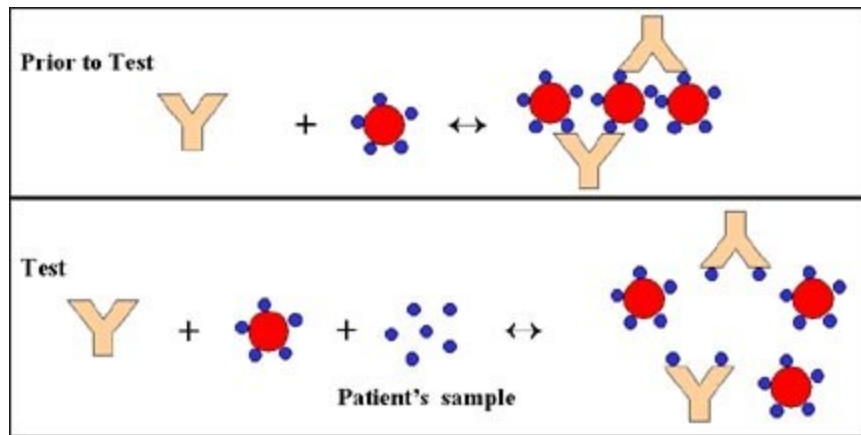

Figure 12

Precipitation tests

Radial Immunodiffusion (Mancini)
In radial immunodiffusion antibody is incorporated into the agar gel as it is poured and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel, it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed as illustrated in Figure 13.

The diameter of the ring is proportional to the log of the concentration of antigen since the amount of antibody is constant. Thus, by running different concentrations of a standard antigen one can generate a standard cure from which one can quantitate the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction has occurred. This could be due to a mixture of antigens or antibodies. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.
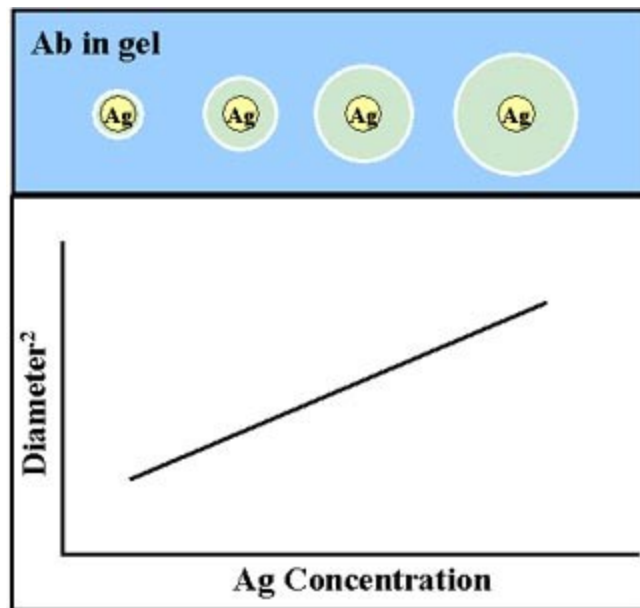
Figure 13

Immunoelectrophoresis

In immunoelectrophoresis, a complex mixture of antigens is placed in a well punched out of an agar gel and the antigens are electrophoresed so that the antigen are separated according to their charge. After electrophoresis, a trough is cut in the gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lines are produced in the equivalence zone when an antigen/antibody reaction occurs as illustrated in Figure 14.

This tests is used for the qualitative analysis of complex mixtures of antigens, although a crude measure of quantity (thickness of the line) can be obtained. This test is commonly used for the analysis of components in a patient' serum. Serum is placed in the well and antibody to whole serum in the trough. By comparisons to normal serum, one can determine whether there are deficiencies on one or more serum components or whether there is an overabundance of some serum component (thickness of the line). This test can also be used to evaluate purity of isolated serum proteins.
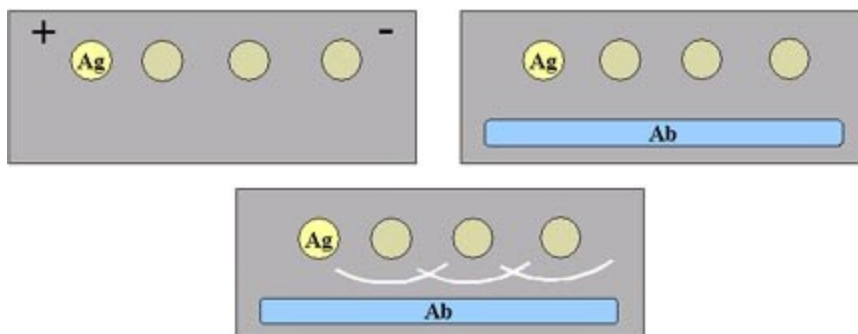

Figure 14

Countercurrent electrophoresis

In this test the antigen and antibody are placed in wells punched out of an agar gel and the antigen and antibody are electrophoresed into each other where they form a precipitation line as illustrated in Figure 15. This test only works if conditions can be found where the antigen and antibody have opposite charges. This test is primarily qualitative, although from the thickness of the band you can get some measure of quantity. Its major advantage is its speed.

Figure 15

Radioimmunoassay (RIA)/Enzyme Linked Immunosorbent Assay (ELISA)

Radioimmunoassays (RIA) are assays that are based on the measurement of radioactivity associated with immune complexes. In any particular test, the label may be on either the antigen or the antibody. Enzyme Linked Immunosorbent Assays (ELISA) are those that are based on the measurement of an enzymatic reaction associated with immune complexes. In any particular assay, the enzyme may be linked to either the antigen or the antibody.

Competitive RIA/ELISA for Ag Detection

The method and principle of RIA and ELISA for the measurement of antigen is shown in Figure 16. By using known amounts of a standard unlabeled antigen, one can generate a standard curve relating radioactivity (cpm) (Enzyme) bound versus amount of antigen. From this standard curve, one can determine the amount of an antigen in an unknown sample.

The key to the assay is the separation of the immune complexes from the remainder of the components. This has been accomplished in many different ways and serves as the basis for the names given to the assay:
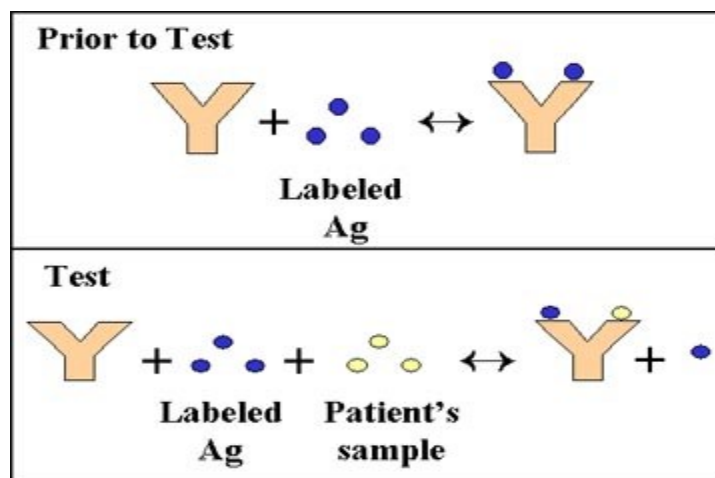

Figure 16

Precipitation with ammonium sulphate

Ammonium sulphate (33 - 50% final concentration) will precipitate immunoglobulins but not many antigens. Thus, this can be used to separate the immune complexes from free antigen. This has been called the Farr Technique

Anti-immunoglobulin antibody

The addition of a second antibody directed against the first antibody can result in the precipitation of the immune complexes and thus the separation of the complexes from free antigen.

Immobilization of the Antibody

The antibody can be immobilized onto the surface of a plastic bead or coated onto the surface of a plastic plate and thus the immune complexes can easily be separated from the other components by simply washing the beads or plate (Figure 17). This is the most common method used today and is referred to as Solid phase RIA or ELISA. In the clinical laboratory, competitive RIA and ELISA are commonly used to quantitate serum proteins, hormones, drugs metabolites.
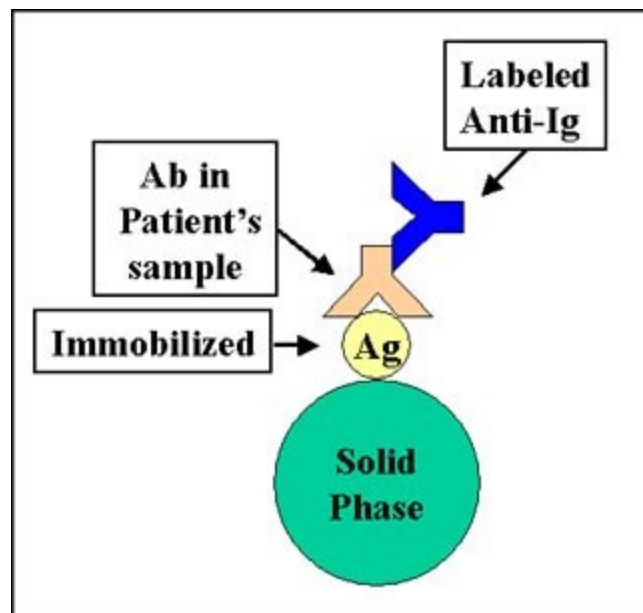

Figure 17

Non-competitive RIA/ELISA for Ag or Ab

Non-competitive RIA and ELISAs are also used for the measurement of antigens and antibodies. In Figure 18, the bead is coated with the antigen and is used for the detection of antibody in the unknown sample. The amount of labeled second antibody bound is related to the amount of antibody in the unknown sample. This assay is commonly employed for the measurement of antibodies of the IgE class directed against particular allergens by using a known allergen as antigen and anti-IgE antibodies as the labeled reagent. It is called the RAST test (radioallergosorbent test). In Figure 19, the bead is coated with antibody and is used to measure an unknown antigen. The amount of labeled second antibody that binds is proportional to the amount of antigen that bound to the first antibody.
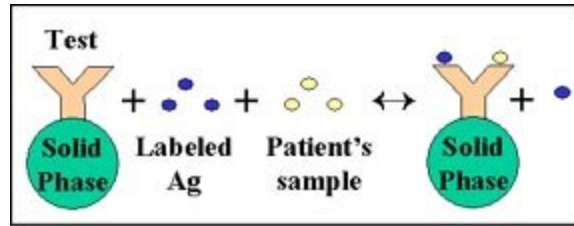
Figure 18
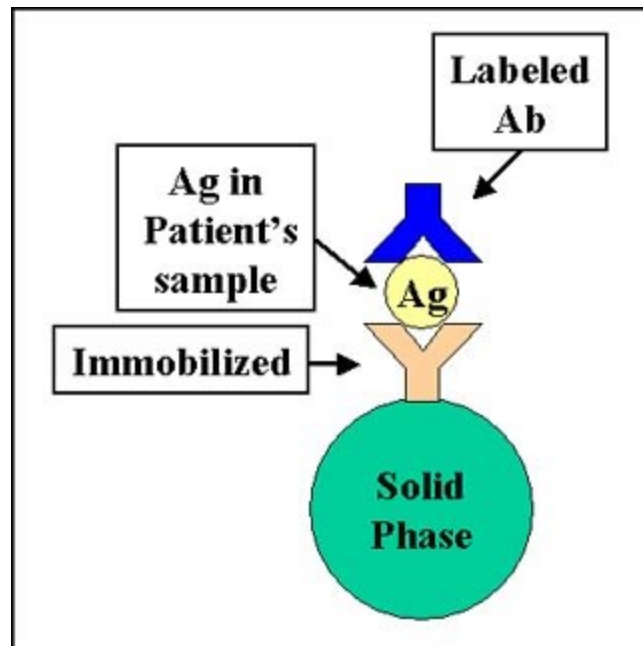
Figure 19

Tests for Cell Associated Antigens

Immunofluorescence

Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

Direct Immunofluorescence

In direct immunofluorescence, the antibody specific to the antigen is directly tagged with the fluorochrome (Figure 20).

Figure 20

Indirect Immunofluorescence

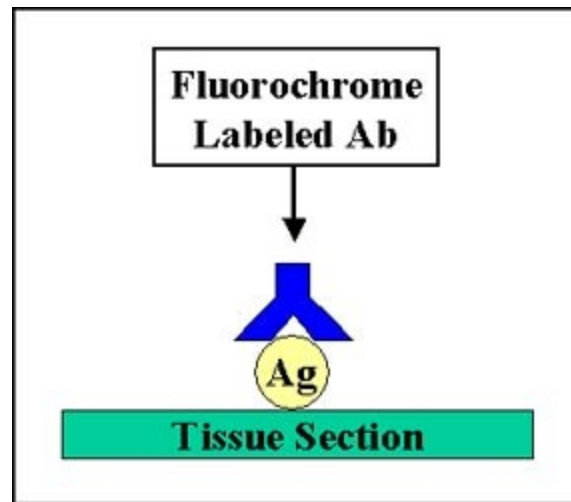In indirect immunofluorescence, the antibody specific for the antigen is unlabeled and a second anti-immunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome (Figure 21). Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.
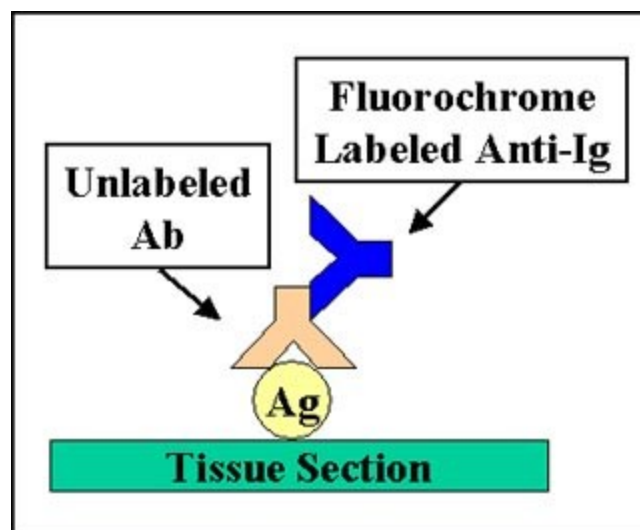
Figure 21

Flow Cytometry

Flow cytometry is commonly used in the clinical laboratory to identify and enumerate cells bearing a particular antigen. Cells in suspension are labeled with a fluorescent tag by either direct or indirect immunofluorescence. The cells are then analyzed on the flow cytometer.

Figure 22 illustrates the principle of flow cytometry. In a flow cytometer, the cells exit a flow cell and are illuminated with a laser beam. The amount of laser light that is scattered off the cells as they passes through the laser can be measured, which gives information concerning the size of the

cells. In addition, the laser can excite the fluorochrome on the cells and the fluorescent light emitted by the cells can be measured by one or more detectors.

Figure 22

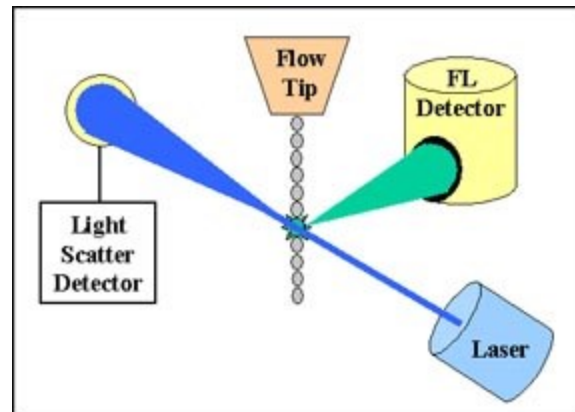The type of data that is obtained from the flow cytometer is shown in Figure 23. In a one parameter histogram, increasing amount of fluorescence (*e.g.* green fluorescence) is plotted on the x axis and the number of cells exhibiting that amount of fluorescence is plotted on the y axis. The fraction of cells that are fluorescent can be determined by integrating the area under the curve. In a two parameter histogram, the x axis is one parameter (*e.g.* red fluorescence) and the y axis is the second parameter (*e.g.* green fluorescence). The number of cells is indicated by the contour and the intensity of the color.
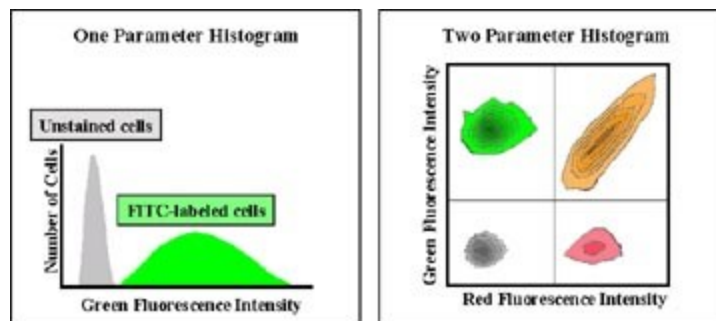
Figure 23

Complement Fixation

Antigen/antibody complexes can also be measured by their ability to fix complement because an antigen/antibody complex will "consume" complement if it is present, whereas free antigens or antibodies do not. Tests for antigen/antibody complexes that rely on the consumption of complement are termed complement fixation tests and are used to quantitate antigen/antibody reactions. This test will only work with complement fixing antibodies (IgG and IgM are best).

The principle of the complement fixation test is illustrated in Figure 24. Antigen is mixed with the test serum to be assayed for antibody and antigen/antibody complexes are allowed to form. A control tube in which no antigen is added is also prepared. If no antigen/antibody complexes are

present in the tube, none of the complement will be fixed. However, if antigen/antibody complexes are present, they will fix complement and thereby reduce the amount of complement in the tube. After allowing complement fixation by any antigen/antibody complexes, a standard amount of red blood cells, which have been pre-coated with anti-erythrocyte antibodies is added. The amount of antibody-coated red blood cells is predetermined to be just enough to completely use up all the complement initially added, if it were still there. If all the complement was still present (i.e. no antigen/antibody complexes formed between the antigen and antibody in question), all the red cells will be lysed. If antigen/antibody complexes are formed between the antigen and antibody in question, some of the complement will be consumed and, thus, when the antibody-coated red cells are added not all of them will lyse. By simply measuring the amount of red cell lysis by measuring the release of hemoglobin into the medium, one can indirectly quantitate antigen/antibody complexes in the tube. Complement fixation tests are most commonly used to assay for antibody in a test sample but they can be modified to measure antigen.
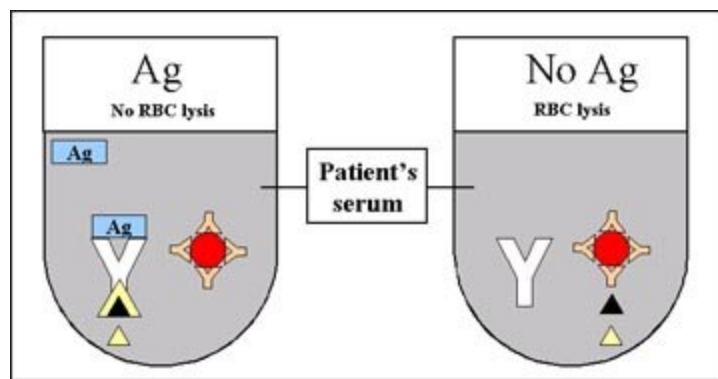
Figure 24

ANTIBODY FORMATION

GENERAL CHARACTERISTICS OF THE ANTIBODY RESPONSE

Self / non-self discrimination

One characteristic feature of the specific immune system is that it normally distinguishes between self and non-self and only reacts against non-self.

Memory

A second feature of the specific immune response is that it demonstrates memory. The immune system "remembers" if it has seen an antigen before and it reacts to secondary exposures to an antigen in a manner different than after a primary exposure. Generally only an exposure to the same antigen will illicit this memory response.

Specificity

A third characteristic feature of the specific immune system is that there is a high degree of specificity in its reactions. A response to a particular antigen is specific for that antigen or a few closely related antigens.

N.B. These are characteristic of all specific immune responses.

ANTIBODY FORMATION

Fate of the immunogen

Clearance after primary injection
The kinetics of antigen clearance from the body after a primary administration is depicted in Figure 1.

Equilibrium phase
The first phase is called the equilibrium or equilibration phase. During this time the antigen equilibrates between the vascular and extravascular compartments by diffusion. This is normally a rapid process. Since particulate antigens don't diffuse, they do not show this phase.

Catabolic decay phase
In this phase the host's cells and enzymes metabolize the antigen. Most of the antigen is taken up by macrophages and other phagocytic cells. The duration will depend upon the immunogen and the host.

Immune elimination phase
In this phase, newly synthesized antibody combines with the antigen producing antigen/antibody complexes which are phagocytosed and degraded. Antibody appears in the serum only after the immune elimination phase is over.

Clearance after secondary injection
If there is circulating antibody in the serum, injection of the antigen for a second time results in a rapid immune elimination. If the is no circulating antibody then injection of the antigen for a second time results in all three phases but the onset of the immune elimination phase is accelerated.
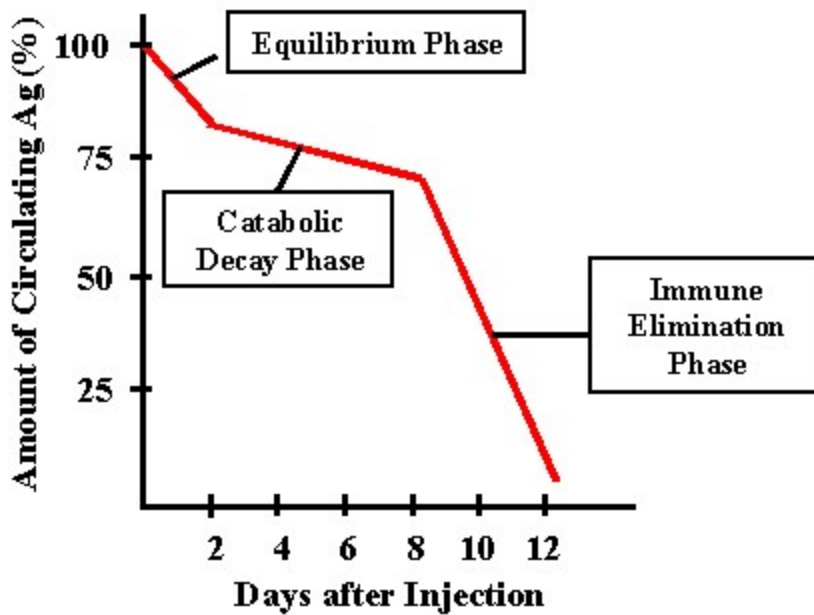
Figure 1

Kinetics of antibody responses to T-dependent Antigen

Primary (1°) Antibody response
The kinetics of a primary antibody response to an antigen is illustrated in Figure 2.

Inductive, latent or lag phase
In this phase, the antigen is recognized as foreign and the cells begin to proliferate and differentiate in response to the antigen. The duration of this phase will vary depending on the antigen but it is usually 5 to 7 days.

Log or Exponential Phase
In this phase, the antibody concentration increases exponentially as the B cells that were stimulated by the antigen differentiate into plasma cells which secrete antibody.

Plateau or steady-state phase
In this phase, antibody synthesis is balanced by antibody decay so that there in no net increase in antibody concentration.

Decline or decay phase
In this phase, the rate of antibody degradation exceeds that of antibody synthesis and the level of antibody falls. Eventually the level of antibody may reach base line levels.
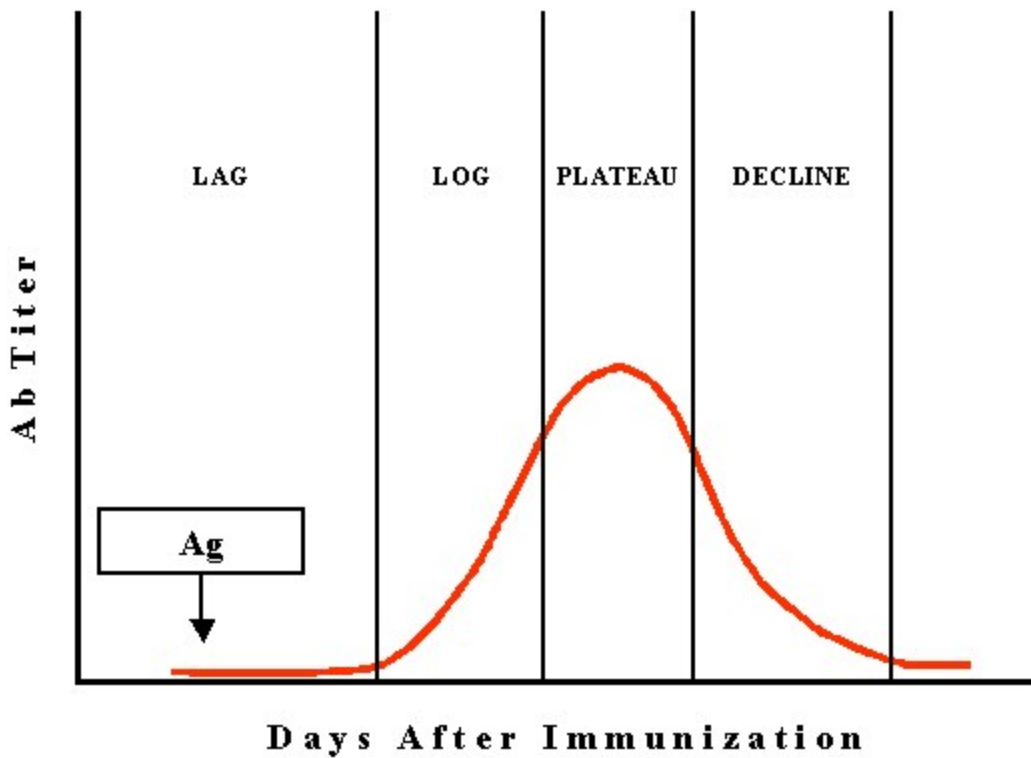
Figure 2

Secondary (2°), memory or anamnestic response (Figure 3)

Lag phase
In a secondary response, there is a lag phase by it is normally shorter than that observed in a primary response.

Log phase
The log phase in a secondary response is more rapid and higher antibody levels are achieved.

Steady state phase

Decline phase
The decline phase is not as rapid and antibody may persist for months, years or even a
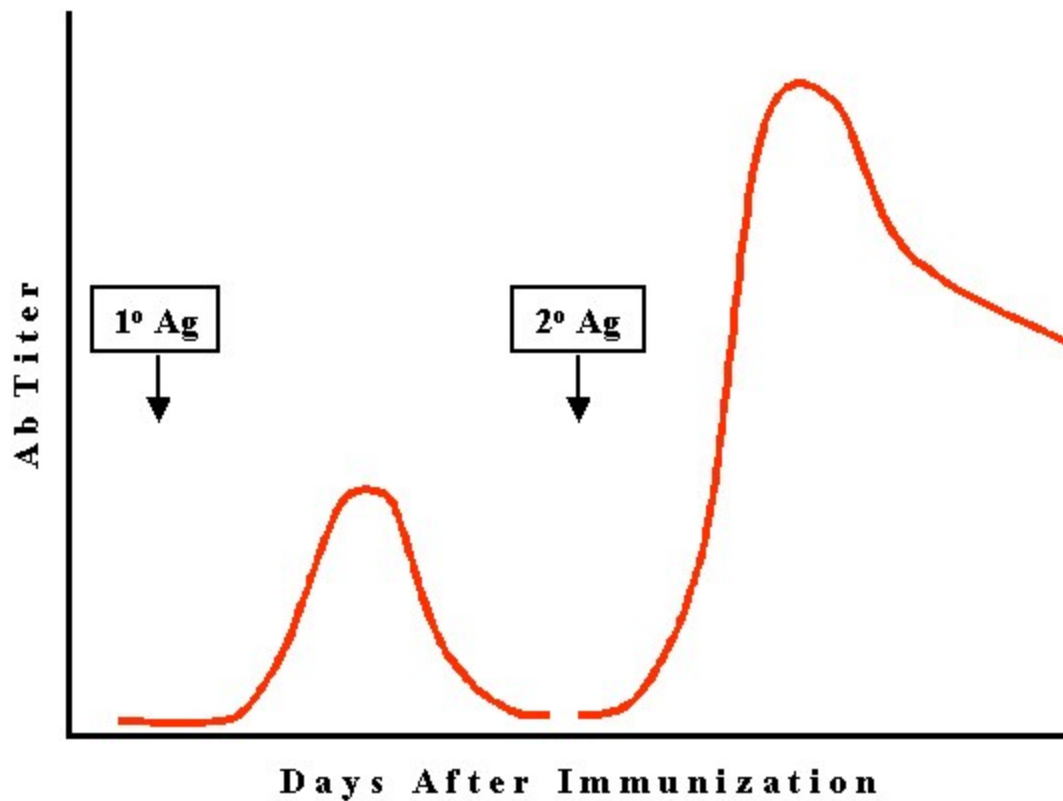
Figure 3

Specificity of primary and secondary responses

Antibody elicited in response to an antigen is specific for that antigen, although it may also cross react with other antigens which are structurally similar to the eliciting antigen. In general secondary responses are only elicited by the same antigen used in the primary response. However, in some instances a closely related antigen may produce a secondary response, but this is a rare exception.

Qualitative changes in antibody during primary and secondary responses

Immunoglobulin class variation
In the primary response, the major class of antibody produced is IgM whereas in the secondary response it is IgG (or IgA or IgE) (Figure 4). The antibodies that persist in the secondary response are the IgG antibodies.
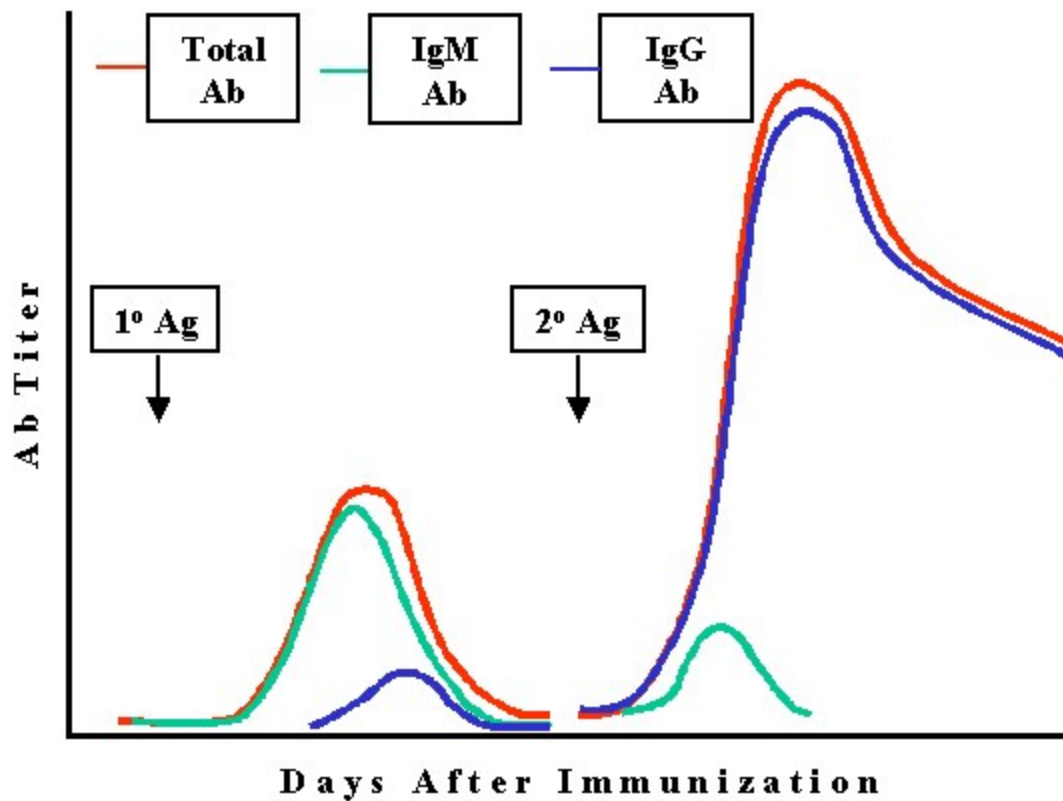
Figure 4

Affinity

The affinity of the IgG antibody produced increases progressively during the response, particularly after low doses of antigen (Figure 5). This is referred to as affinity maturation. Affinity maturation is most pronounced after secondary challenge with antigen.
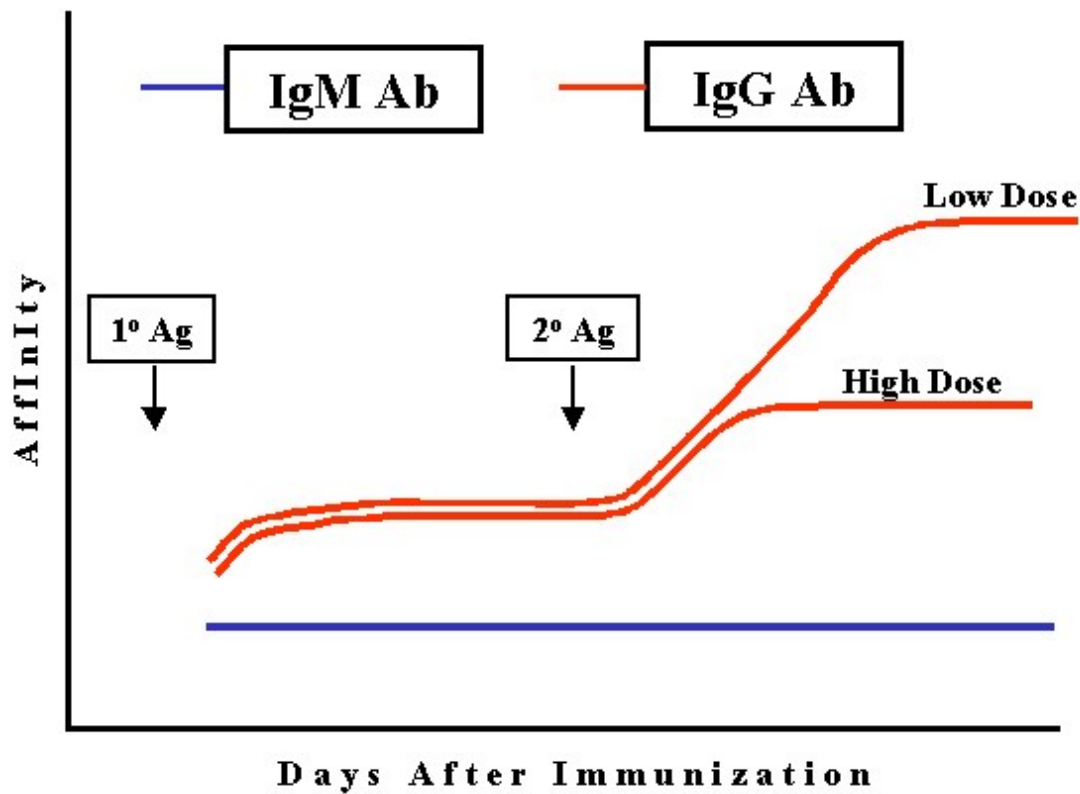
Figure 5

One explanation for affinity maturation is clonal selection as illustrated in Figure 6. A second explanation for affinity maturation is that, after a class switch has occurred in the immune response, somatic mutations occur which fine tune the antibodies to be of higher affinity. There is experimental evidence for this mechanism, although it is not known how the somatic mutation mechanism is activated after exposure to antigen.
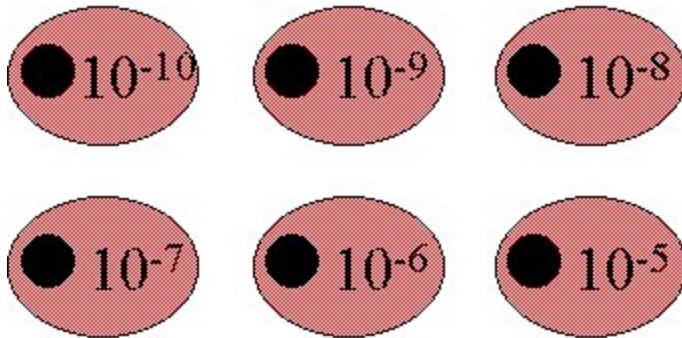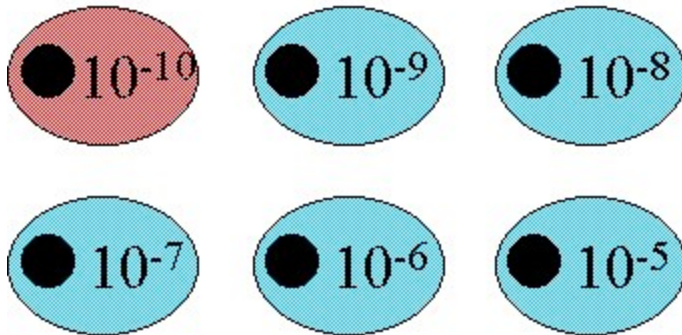
Figure 6

Avidity
As a consequence of increased affinity, the avidity of the antibodies increases during the response.

Cross-reactivity
As a result of the higher affinity later in the response, there is also an increase in detectible cross reactivity. An explanation for why increasing affinity results in an increase in detectible cross reactivity is illustrated by the following example.

Affinity of Ab for Ag

	Early	Late
Immunizing Ag	10^{-6}	10^{-9}
	+	++
Cross reacting Ag	10^{-3}	10^{-6}
	-	+

If a minimum affinity of 10^{-6} is needed to detect a reaction, early in an immune response the reaction of a cross reacting antigen with an affinity of 10^{-3} will not be detected. However, late in a response when the affinities increase 1000 fold, the reaction with both the immunizing and cross reacting antigens will be detected.

Cellular events during primary and secondary responses to T-dependent antigen

Primary response (Figure 7)

Lag phase
Clones of T and B cells with the appropriate antigen receptors bind antigen, become activated and begin to proliferate. The expanded clones of B cells differentiate into plasma cells which begin to secrete antibody.

Log phase
The plasma cells initially secrete IgM antibody since the C_μ heavy chain gene is closest to the rearranged VDJ gene. Eventually some B cells switch from making IgM to IgG, IgA or IgE. As more B cells proliferate and differentiate into antibody secreting cells the antibody concentration increases exponentially.

Stationary phase
As antigen is depleted, T and B cells are no longer activated. In addition, mechanisms which down regulate the immune response come into play. Furthermore, plasma cells begin to die. When the rate of antibody synthesis equals the rate of antibody decay the stationary phase is reached.

Decline phase
When no new antibody is produced because the antigen is no longer present to activate T and B cells and the residual antibody slowly is degraded, the decay phase is reached.
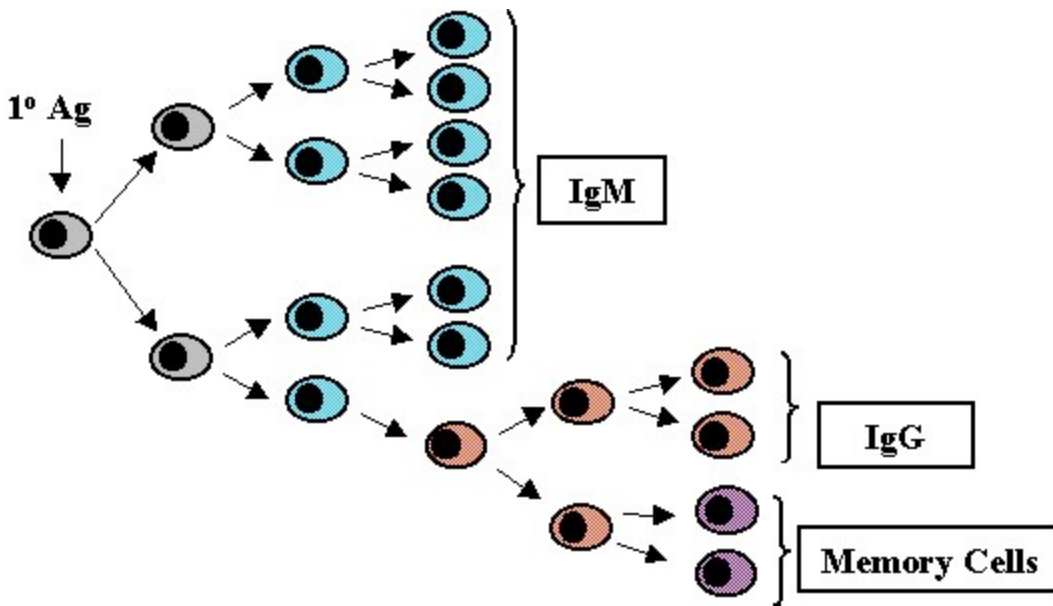
Figure 7

Secondary response (Figure 8)

Not all of the T and B cells that are stimulated by antigen during primary challenge with antigen die. Some of them are long lived cells and constitute what is refer to as the memory cell pool. Both memory T cells and memory B cells are produced and memory T cells survive longer than memory B cells. Upon secondary challenge with antigen not only are virgin T and B cells activated, the memory cells are also activated and thus there is a shorter lag time in the secondary response. Since there is an expanded clone of cells being stimulated the rate of antibody production is also increased during the log phase of antibody production and higher levels are achieved. Also, since many if not all of the memory B cells will have switched to IgG (IgA or IgE) production, IgG is produced earlier in a secondary response. Furthermore since there is an expanded clone of memory T cells which can help B cells to switch to IgG (IgA or IgE) production, the predominant class of Ig produced after secondary challenge is IgG (IgA or IgE).
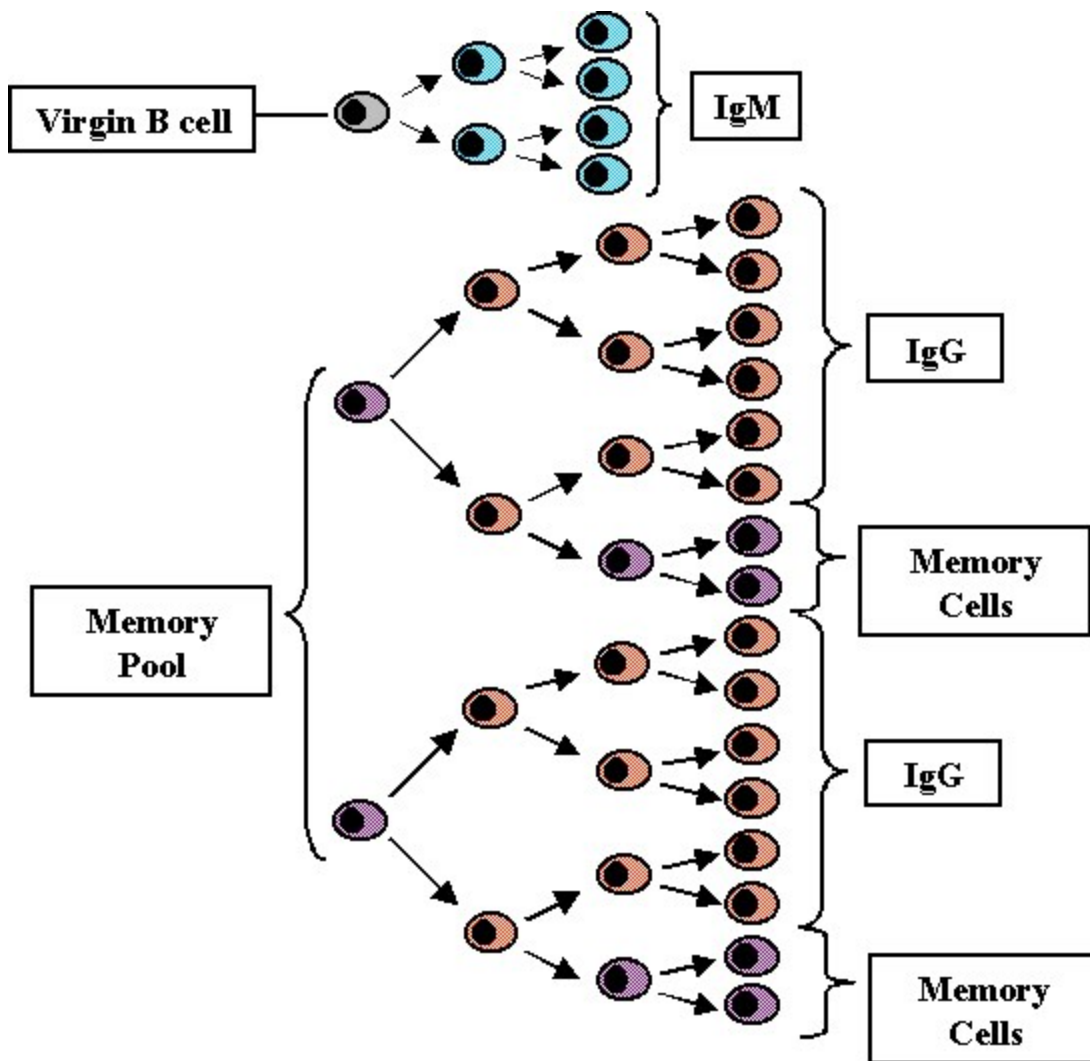
Figure 8

Ab response to T-independent antigen

Responses to T-independent antigen are characterized by the production of almost exclusively IgM antibody and no secondary response. Secondary exposure to the antigen results in another primary response to the antigen as illustrated in Figure 9.
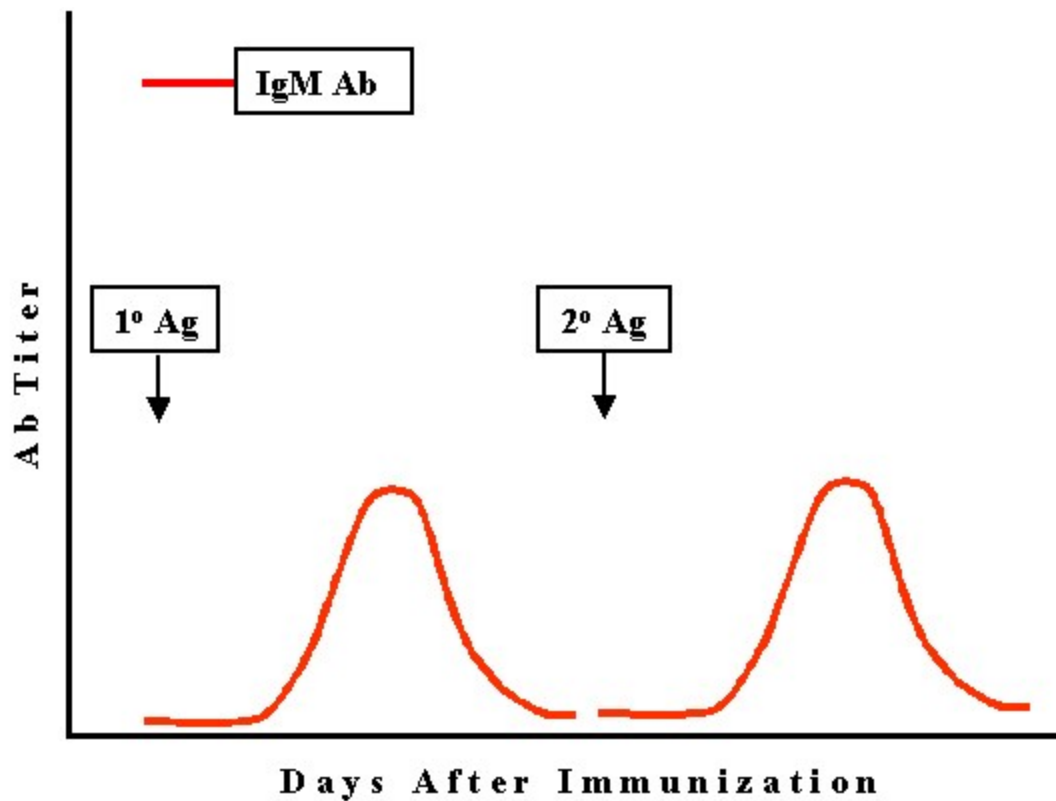
Figure 9

Class switching

During an antibody response to a T-dependent antigen a switch occurs in the class of Ig produced from IgM to some other class (except IgD). Our understanding of the structure of the immunoglobulin genes, helps explain how class switching occurs (Figure 10).

During class switching another DNA rearrangement occurs between a switch site (S_μ) in the intron between the rearranged VDJ regions and the C_μ gene and another switch site before one of the other heavy chain constant region genes. The result of this recombination event is to bring the VDJ region close to one of the other constant region genes, thereby allowing expression of a new class of heavy chain. Since the same VDJ gene is brought near to a different C gene and since the antibody specificity is determined by the hypervariable regions within the V region, the antibody produced after the switch occurs will have the same specificity as before.

Cytokines secreted by T helper cells can cause the switch to certain isotypes.
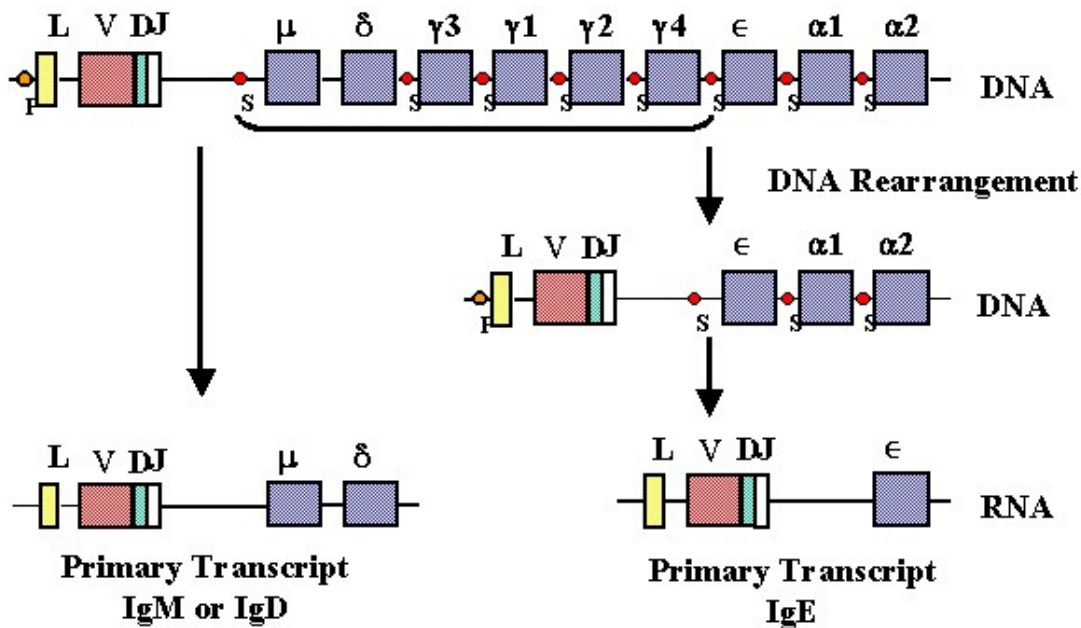
Figure 10

Membrane and secreted immunoglobulin

The specificity of membrane immunoglobulin on a B cell and the Ig secreted by the plasma cell progeny of a B cell is the same. An understanding of how the specificity of membrane and secreted Ig from an individual B cell can be the same comes from an understanding of immunoglobulin genes (Figure 11).

There are two potential polyA sites in the immunoglobulin gene. One after the exon for the last heavy chain domain and the other after the exons that code for the trans- membrane domains. If the first polyA site is used, the pre-mRNA is processed to produce a secreted protein. If the second polyA site is used, the pre-mRNA is processed to produce a membrane form of the immunoglobulin. However, in all cases the same VDJ region is used and thus the specificity of the antibody remains the same. All C regions genes have these additional membrane pieces associated with them and thus after class switching other classes of immunoglobulins can be secreted or expressed on the surface of B cells.
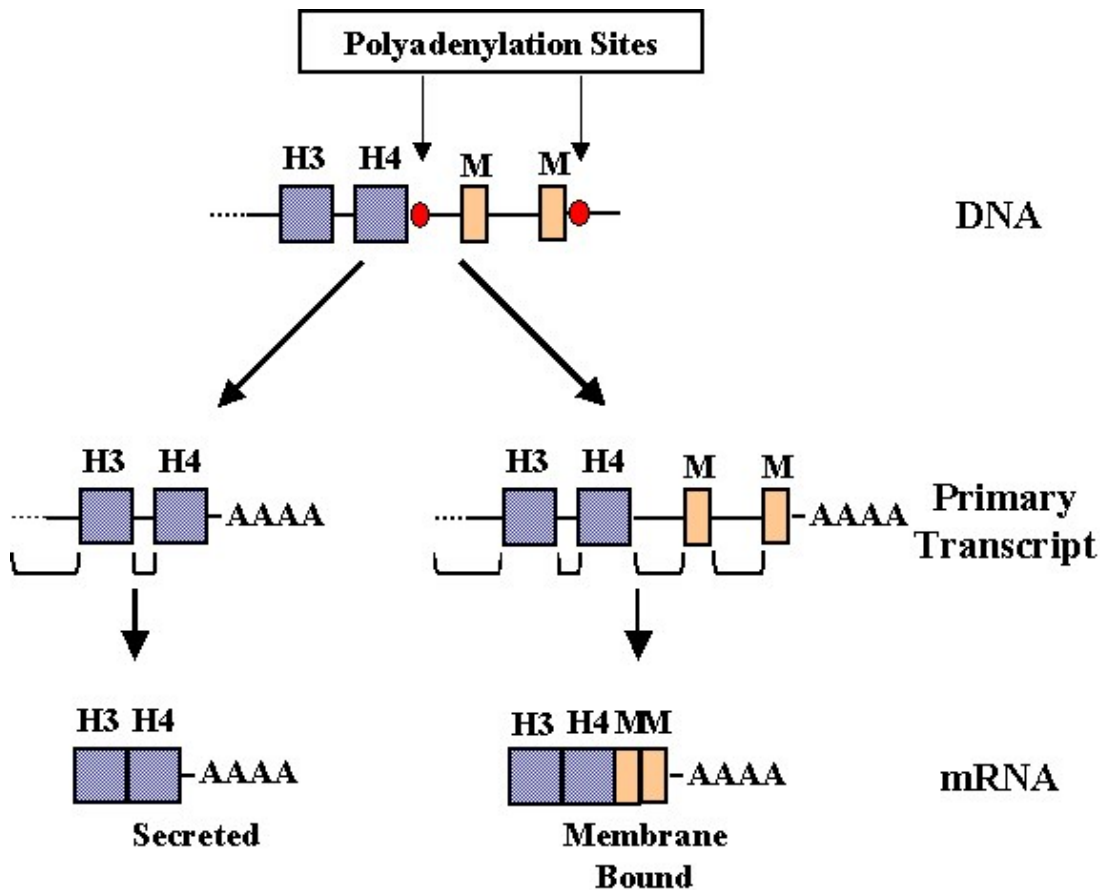
Figure 11

CELLS INVOLVED IN IMMUNE RESPONSES AND ANTIGEN RECOGNITION

OVERVIEW

The immune system has developed to protect the host from pathogens and other foreign substances. Self/non-self discrimination is one of the hallmarks of the immune system. There are two main sites where pathogens may reside: extracellularly in tissue spaces or intracellularly within a host cell, and the immune system has different ways of dealing with pathogens at these sites. Although immune responses are tailored to the pathogen and to where the pathogen resides, most pathogens can elicit both an antibody and a cell-mediated response, both of which may contribute to ridding the host of the pathogen. However, for any particular pathogen an antibody or a cell-mediated response may be more important for defense against the pathogen.

Extracellular pathogens

Antibodies are the primary defense against extracellular pathogens and they function in three major ways:

- Neutralization (Figure 1a)
  By binding to the pathogen or foreign substance antibodies, can block the association of the pathogen with their targets. For example, antibodies to bacterial toxins can prevent the binding of the toxin to host cells thereby rendering the toxin ineffective. Similarly, antibody binding to a virus or bacterial pathogen can block the attachment of the pathogen to its target cell thereby preventing infection or colonization.
- Opsonization (Figure 1b)
  Antibody binding to a pathogen or foreign substance can opsonize the material and facilitate its uptake and destruction by phagocytic cells. The Fc region of the antibody interacts with Fc receptors on phagocytic cells rendering the pathogen more readily phagocytosed.
- Complement activation (Figure 1c)
  Activation of the complement cascade by antibody can result in lysis of certain bacteria and viruses. In addition, some components of the complement cascade (*e.g.* C3b) opsonize pathogens and facilitate their uptake via complement receptors on phagocytic cells.
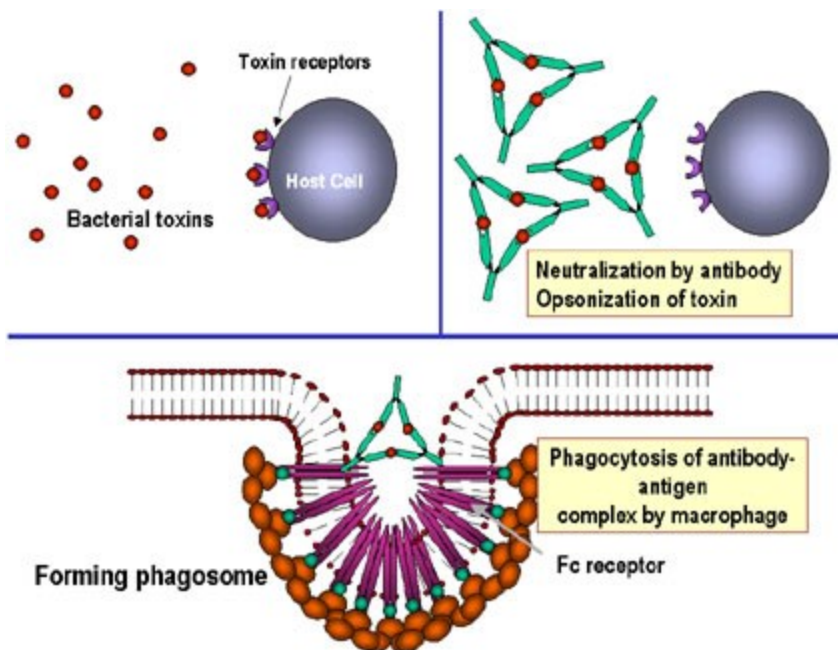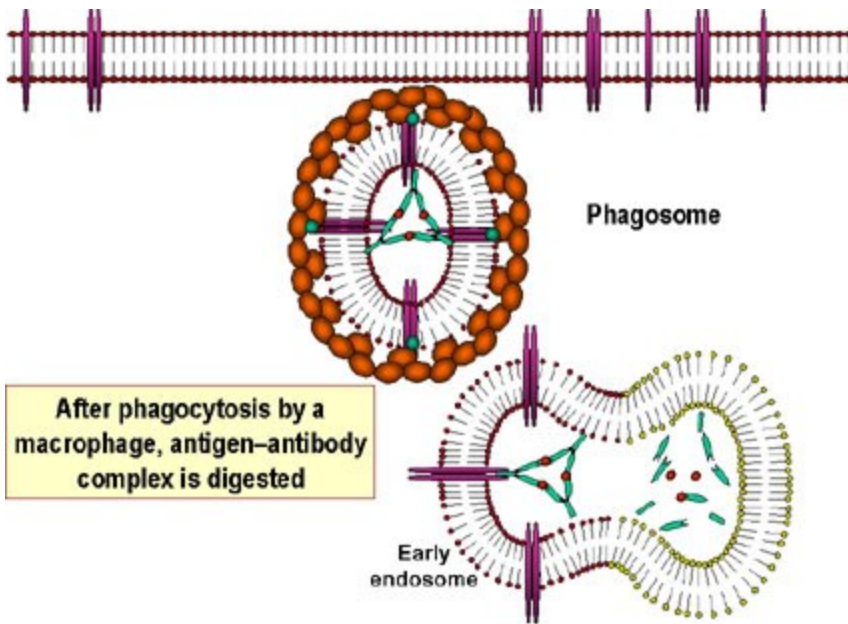
Figure 1 A   Antibodies binding to and neutralizing a bacterial toxin, preventing it from interacting with host cells and causing pathology. Unbound toxin can react with receptors on the host cell, whereas the toxin:antibody complex cannot. Antibodies also neutralize complete virus particles and bacterial cells by binding to them and inactivating them. The antigen: antibody complex is eventually scavenged and degraded by macrophages. Antibodies coating an antigen render it recognizable as foreign by phagocytes (macrophages and polymorphonuclear leukocytes), which then ingest and destroy it; this is called opsonization
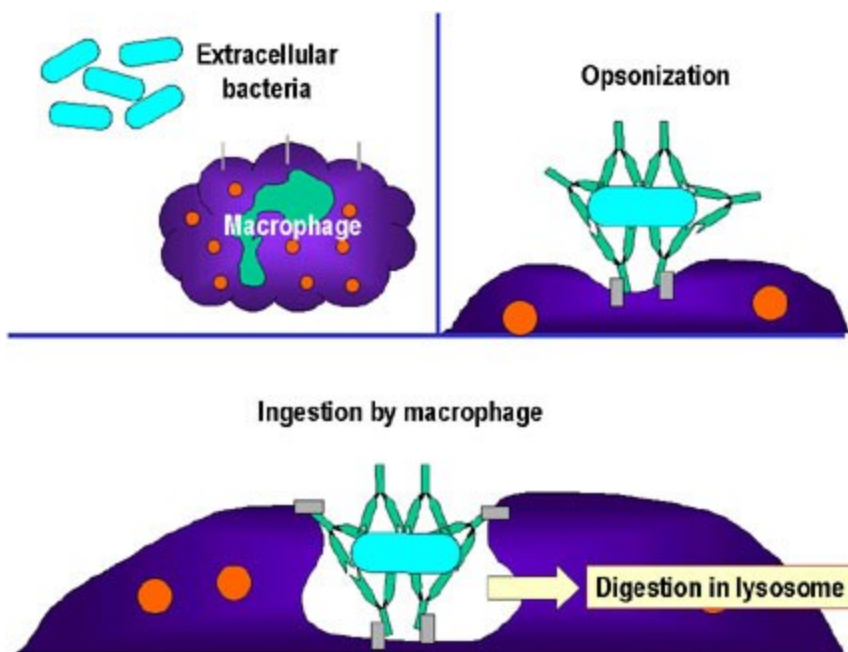
Figure 1 B   Opsonization and phagocytosis of a bacterial cell
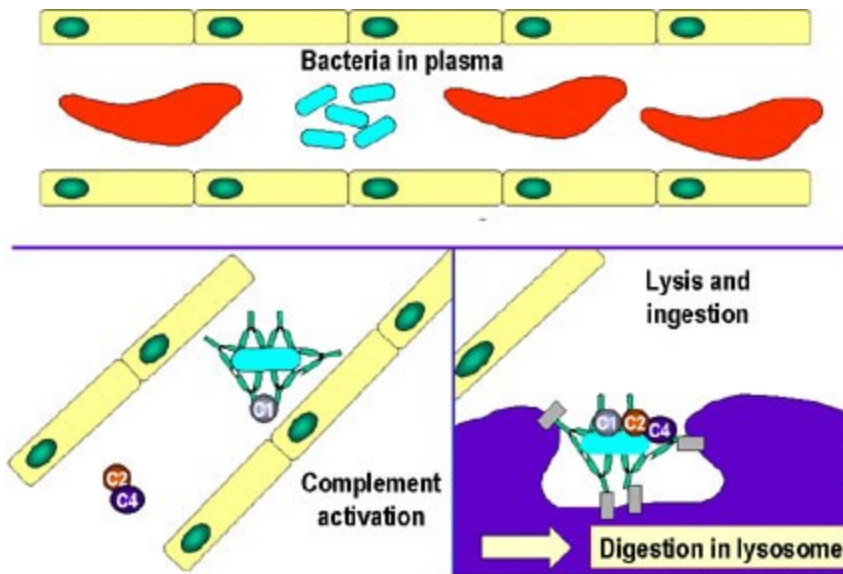
Figure 1 C   Activation of the complement system by antibodies coating a bacterial cell. Bound antibodies form a receptor for the first protein of the complement system, which eventually forms a protein complex on the surface of the bacterium that in some cases, can kill the bacterium directly but more generally favors its uptake and destruction by phagocytes. Thus, antibodies target pathogens and their products for disposal by phagocytes

Intracellular pathogens

Because antibodies do not get into host cells, they are ineffective against intracellular pathogens. The immune system uses a different approach to deal with these kinds of pathogens. Cell-mediated responses are the primary defense against intracellular pathogens and the approach is different depending upon where the pathogen resides in the host cell (*i.e.,* in the cytosol or within vesicles). For example, most viruses and some bacteria reside in the cytoplasm of the host cell, however, some bacteria and parasites actually live within endosomes in the infected host cell. The primary defense against pathogens in the cytosol is the cytotoxic T lymphocyte (Tc or CTL). In contrast, the primary defense against a pathogen within vesicles is a subset of helper T lymphocytes (Th1).

--Cytotoxic T lymphocytes (Figure 2)
CTLs are a subset of T lymphocytes that express a unique antigen on their surface called CD8. These cells recognize antigens from the pathogen that are displayed on the surface of the infected cell and kill the cell thereby preventing the spread of the infection to neighboring cells. CTLs kill by inducing apoptosis in the infected cell.
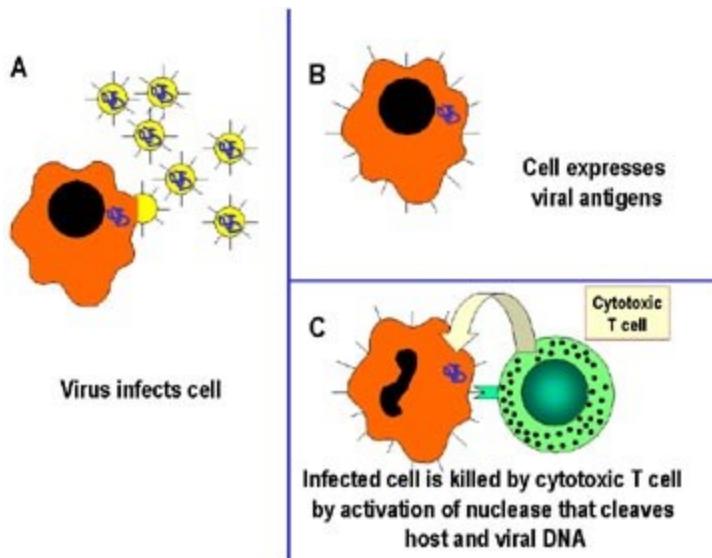
Figure 2

Mechanism of host defense against intracellular infection by viruses. Cells infected by viruses are recognized by specialized T cells called cytotoxic T lymphocytes (CTLs), which kill the infected cells directly. The killing mechanism involves the activation of nucleases in the infected cell, which cleave host and viral DNA.

--Th1 Helper T cells (Figure 3)

Th cells are a subset of T cells that express a unique antigen on their surface called CD4. A subpopulation of Th cells, Th1 cells, is the primary defense against intracellular pathogens that live within vesicles. Th1 cells recognize antigen from the pathogen that are expressed on the surface of infected cells and release cytokines that activate the infected cell. Once activated, the infected cell can then kill the pathogen. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis, infects macrophages but is not killed because it blocks the fusion of lysosomes with the endosomes in which it resides. Th1 cells that recognize *M. tuberculosis* antigens on the surface of an infected macrophage can secrete cytokines that activate macrophages. Once activated the lysosomes fuse with endosomes and the *M. tuberculosis* bacteria are killed.
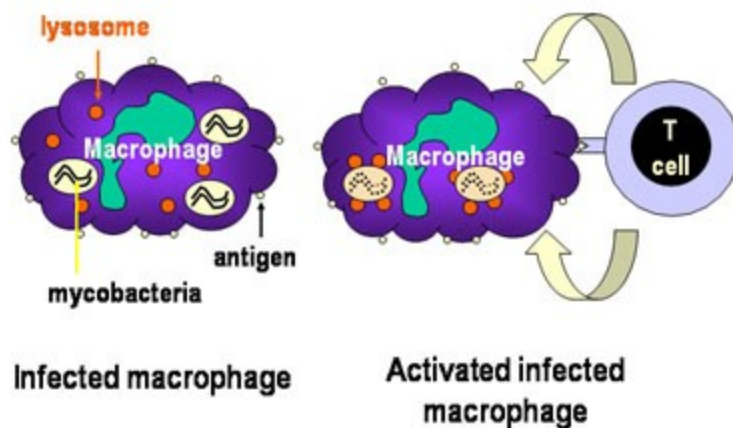
Figure 3

Mechanism of host defense against intracellular infection by mycobacteria. Mycobacteria infecting macrophages live in cytoplasmic vesicles that resist fusion with lysosomes and consequent destruction of the bacteria by macrophage bacteriocidal activity. However, when the appropriate T cell recognizes an infected macrophage it releases macrophage-activating molecules that induce lysosomal fusion and the activation of macrophage bactericidal activities

Although immune responses are tailored to the pathogen and to where the pathogen resides, most pathogens can elicit both an antibody and a cell-mediated response, both of which may contribute to ridding the host of the pathogen. However, for any particular pathogen an antibody or a cell-mediated response may be more important for defense against the pathogen.

CELLS OF THE IMMUNE SYSTEM

All cells of the immune system originate from a hematopoietic stem cell in the bone marrow, which gives rise to two major lineages, a myeloid progenitor cell and a lymphoid progenitor cell (Figure 4). These two progenitors give rise to the myeloid cells (monocytes, macrophages, dendritic cells, meagakaryocytes and granulocytes) and lymphoid cells (T cells, B cells and natural killer (NK) cells), respectively. Theses cells make up the cellular components of the innate (non-specific) and adaptive (specific) immune systems.
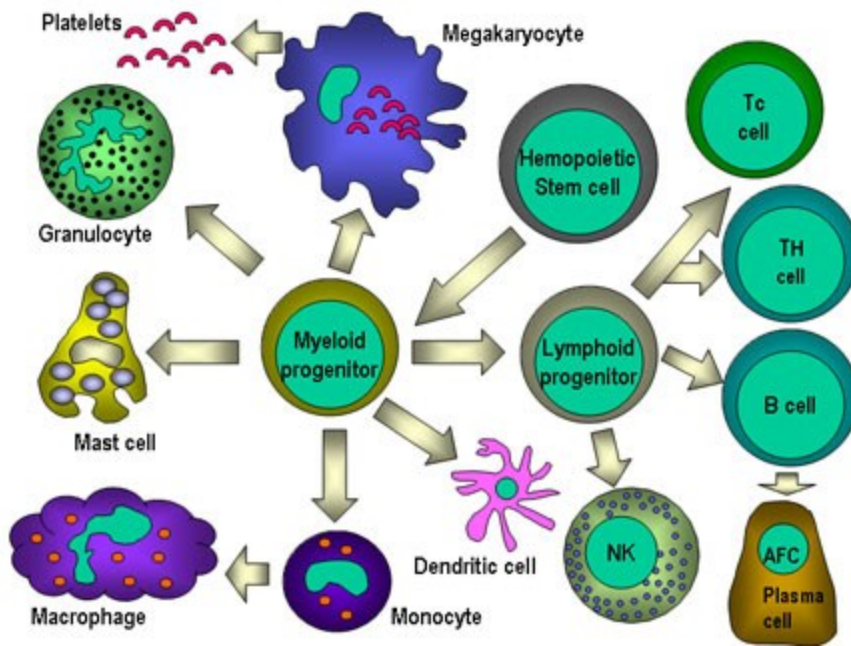
Figure 4

All hematopoietic cells are derived from pluripotent stem cells which give rise to two main lineages: one for lymphoid cells and one for myeloid cells. The common lymphoid progenitor has the capacity to differentiate into either T cells or B cells depending on the microenvironment to which it homes. In mammals, T cells develop in the thymus while B cells develop in the fetal liver and bone marrow. An AFC is an antibody-forming cell, the plasma cell being the most differentiated AFC. NK cells also derive from the common lymphoid progenitor cell. The myeloid cells differentiate into the committed cells on the left. The collective name "granulocyte" is used for eosinophils, neutrophils and basophils

Cells of the innate immune system

Cells of the innate immune system include phagocytic cells (monocyte/macrophages and PMNs), NK cells, basophils, mast cells, eosinophiles and platelets. The roles of these cells have been discussed previously (see non-specific immunity). The receptors of these cells are pattern recognition receptors (PRRs) that recognize broad molecular patterns found on pathogens (pathogen associated molecular patterns, PAMPS).

Cells that link the innate and adaptive immune systems

A specialized subset of cells called antigen presenting cells (APCs) are a heterogenous population of leukocytes that play an important role in innate immunity and also act as a link to the adaptive immune system by participating in the activation of helper T cells (Th cells). These cells include dendritic cells and macrophages. A characteristic feature of APCs is the expression of a cell surface molecule encoded by genes in the major histocompatibility complex, referred to as class II MHC molecules. B lymphocytes also express class II MHC molecules and they also function as APCs, although they are not considered as part of the innate immune system. In addition, certain other cells (e.g., thymic epithelial cells) can express class II MHC molecules and can function as APCs.

Cells of the adaptive immune system

Cells that make up the adaptive (specific) immune system include the B and T lymphocytes. After exposure to antigen, B cells differentiate into plasma cells whose primary function is the production of antibodies. Similarly, T cells can differentiate into either T cytotoxic (Tc) or T helper (Th) cells of which there are two types Th1 and Th2 cells.

There are a number of cell surface markers that are used in clinical laboratories to distinguish B cells, T cells and their subpopulations. These are summarized in Table 1.

Table 1. Main distinguishing markers of T and B cells			
Marker	B cells	Tc	Th
CD3	-	+	+
CD4	-	-	+
CD8	-	+	-
CD19 and/or CD20	+	-	-
CD40	+	-	-
Ag receptor	BCR (surface Ig)	TCR	TCR

SPECIFICITY OF THE ADAPTIVE IMMUNE RESPONSE

Specificity on the adaptive immune response resides in the antigen receptors on T and B cells, the TCR and BCR, respectively. The TCR and BCR are similar in that each receptor is specific for one antigenic determinant but they differ in that BCRs are divalent while TCRs are monovalent (Figure 5). A consequence of this difference is that while B cells can have their antigen receptors cross-linked by antigen, TCRs cannot. This has implications as to how B and T cells can become activated.

B cell antigen receptors:
Surface Immunoglobulins
Two identical antigen recognition sites

T cell antigen receptors:
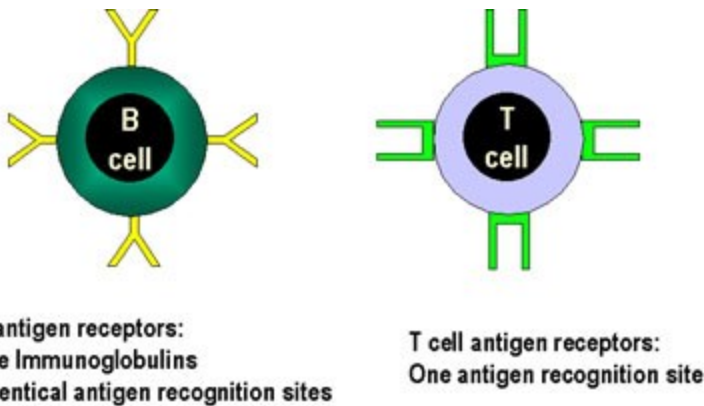One antigen recognition site

Figure 5
The antigen receptors of B cells have two antigen-recognition sites whereas those of T cells have only one

Each B and T cells has a receptor that is unique for a particular antigenic determinant and there are a vast array of different antigen receptors on both B and T cells. The question of how these receptors are generated was the major focus of immunologists for many years. Two basic hypotheses were proposed to explain the generation of the receptors: the instructionist (template) hypothesis and the clonal selection hypothesis.

Instructionist hypothesis

The instructionist hypothesis states that there is only one common receptor encoded in the germline and that different receptors are generated using the antigen as a template. Each antigen would cause the one common receptor to be folded to fit the antigen. While this hypothesis was simple and very appealing, it was not consistent with what was known about protein folding (*i.e.* protein folding is dictated by the sequence of amino acids in the protein). In addition this hypothesis did not account for self/non-self discrimination in the immune system. It could not explain why the one common receptor did not fold around self antigens.

Clonal selection hypothesis

The clonal selection hypothesis states that the germline encodes many different antigen receptors - one for each antigenic determinant to which an individual will be capable of mounting an immune response. Antigen selects those clones of cells that have the appropriate receptor. The four basic principles of the clonal selection hypothesis are:

- Each lymphocyte bears a *single* type of receptor with a unique specificity.
- Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with a high affinity leads to lymphocyte activation.
- The differentiated effector cells derived from an activated lymphocyte will bear receptors of an identical specificity to those of the parental cell from which that lymphocyte was derived.
- Lymphocytes bearing receptors for self molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes.

The clonal selection hypothesis is now generally accepted as the correct hypothesis to explain how the adaptive immune system operates. It explains many of the features of the immune response: 1) the specificity of the response; 2) the signal required for activation of the response (*i.e.* antigen); 3) the lag in the adaptive immune response (time is required to activate cells and to expand the clones of cells); and 4) self/non-self discrimination.

LYMPHOCYTE RECIRCULATION

Since there are relatively few T or B lymphocytes with a receptor for any particular antigen (1/10,000 – 1/100,000), the chances for a successful encounter between an antigen and the appropriate lymphocyte are slim. However, the chances for a successful encounter are greatly enhanced by the recirculation of lymphocytes through the secondary lymphoid

organs. Lymphocytes in the blood enter the lymph nodes and percolate through the lymph nodes (Figure 6). If they do not encounter an antigen in the lymph node, they leave via the lymphatics and return to the blood via the thoracic duct. It is estimated that 1-2% of lymphocytes recirculate every hour. If the lymphocytes in the lymph nodes encounter an antigen, which has been transported to the lymph node via the lymphatics, the cells become activated, divide and differentiate to become a plasma cell, Th or Tc cell. After several days the effector cells can leave the lymph nodes via the lymphatics and return to the blood via the thoracic duct and then make their way to the infected tissue site.
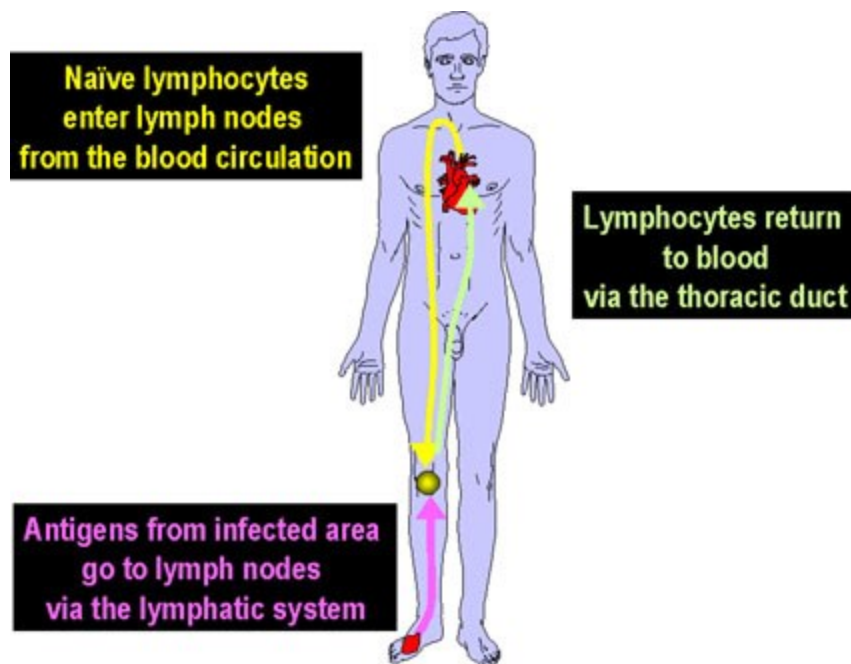
Figure 6
Circulating lymphocytes encounter antigen in peripheral lymphoid tissues

 Naive (virgin) lymphocytes enter the lymph nodes from the blood via High Endothelial Venules (HEVs) Homing receptors on the lymphocytes direct the cells to the HEVs. In the lymph nodes, lymphocytes with the appropriate antigen receptor encounter antigen, which has been transported to the lymph nodes by dendritic cells or macrophages. After activation the lymphocytes express new receptors that allow the cells to leave the lymph node and reenter the circulation. Receptors on the activated lymphocytes recognize cell adhesion molecules expressed on endothelial cells near the site of an infection and chemokines produced at the infection site help attract the activated cells (Figure 7).
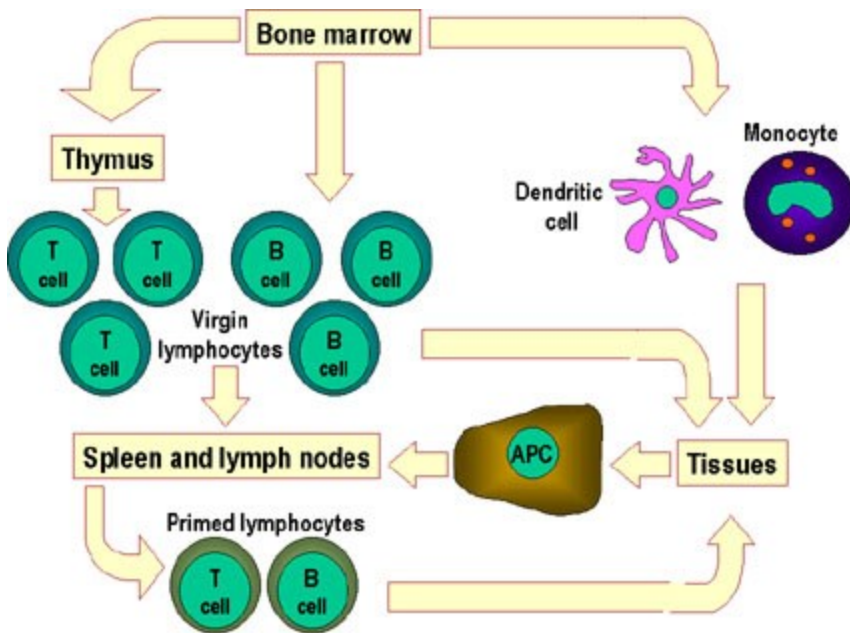
Figure 7
Virgin lymphocytes from the primary lymphoid tissues such as bone marrow migrate to secondary lymphoid tissues, i.e. the spleen and lymph nodes. Antigen-presenting cells (APCs), including dendritic cells and mononuclear phagocytes (monocytes), also derive from bone marrow stem cells. These APCs enter tissues, take up antigen and transport it to the lymphoid tissues to be presented to T cells and B cells. Primed lymphocytes then migrate from the lymphoid tissues and accumulate preferentially at sites of infection and inflammation

IMMUNITY: CONTRASTS BETWEEN NON-SPECIFIC AND SPECIFIC

Non-specific (natural, native, innate)

- System in place prior to exposure to antigen
- Lacks discrimination among antigens
- Can be enhanced after exposure to antigen through effects of cytokines

Specific (acquired, adaptive)

- Induced by antigen
- Enhanced by antigen
- Shows fine discrimination

The hallmarks of the specific immune system are memory and specificity.

- The specific immune system "remembers" each encounter with a microbe or foreign antigen, so that subsequent encounters stimulate increasingly effective defense mechanisms.
- The specific immune response amplifies the protective mechanisms of non-specific immunity, directs or focuses these mechanisms to the site of antigen entry, and thus makes them better able to eliminate foreign antigens.
-

CELLS OF THE IMMUNE SYSTEM

All cell types in the immune system originate from the bone marrow

Figure 8

Human T-lymphocyte Attacking Fibroblast Tumor / Cancer Cells (SEM x4,000)

Human T-lymphocyte   (SEM x12,080
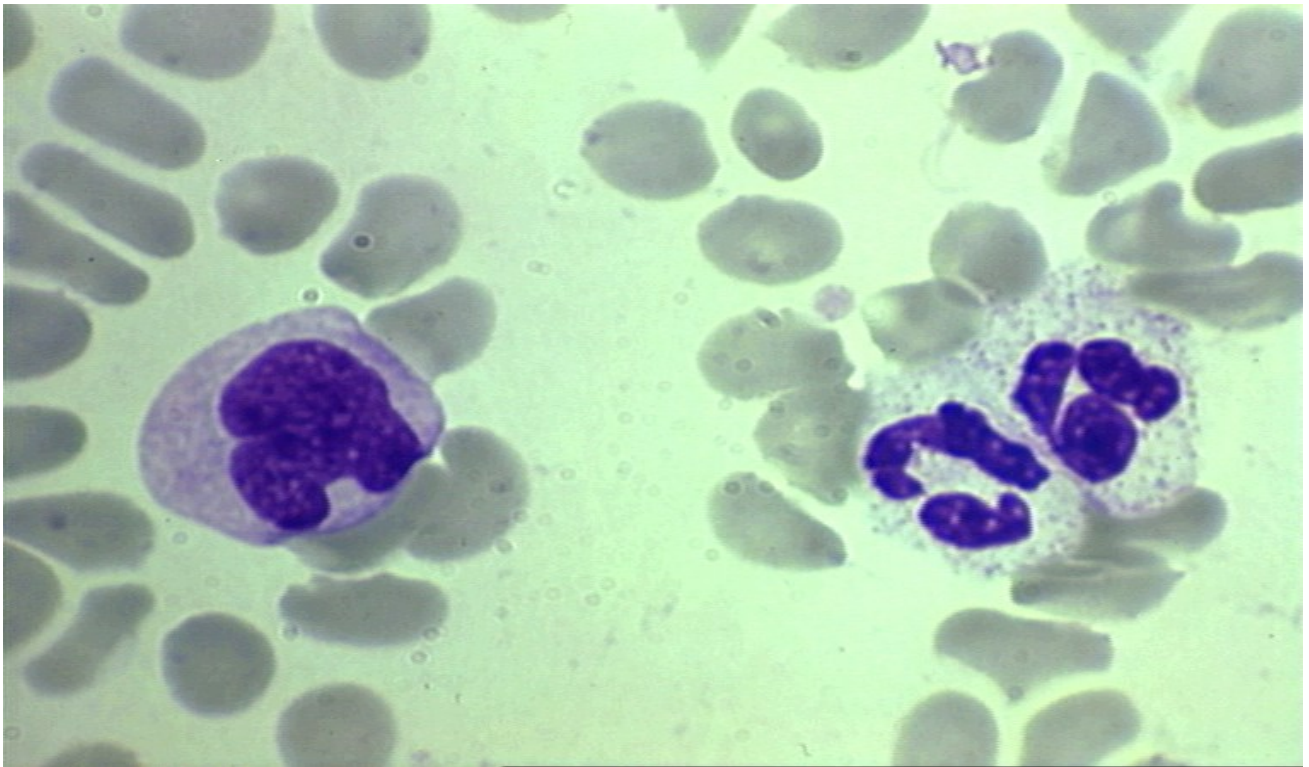
Blood film showing a monocyte (left) and two neutrophils
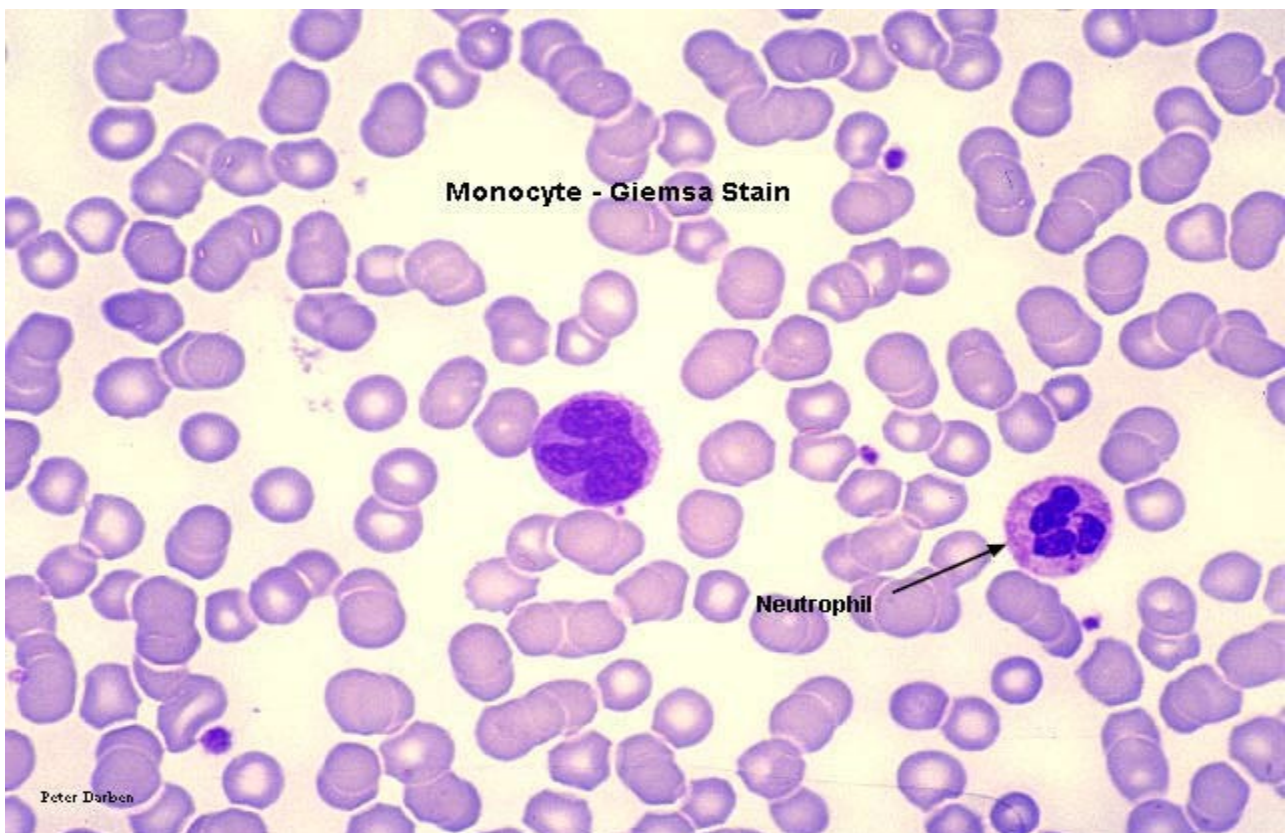
Monocyte, giemsa stained peripheral blood film
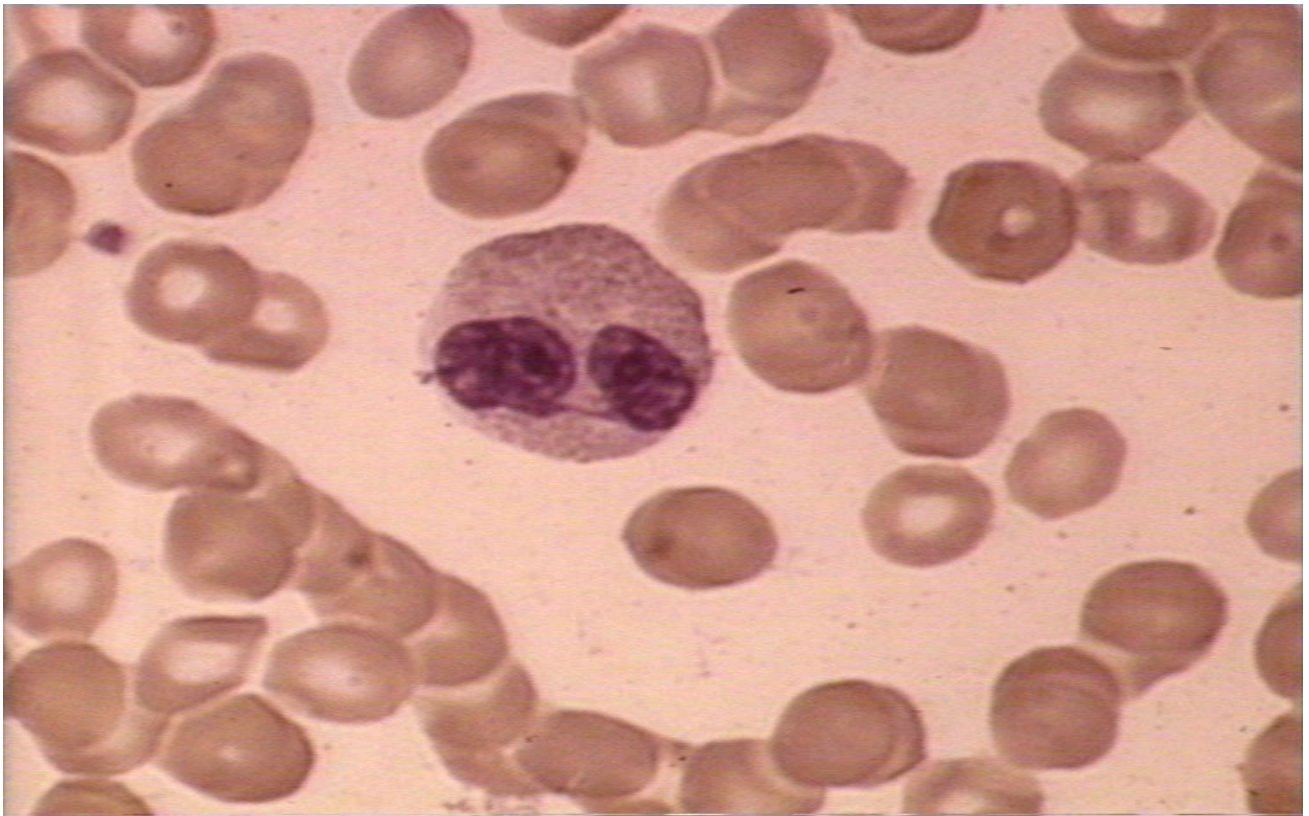
Eosinophil in blood film
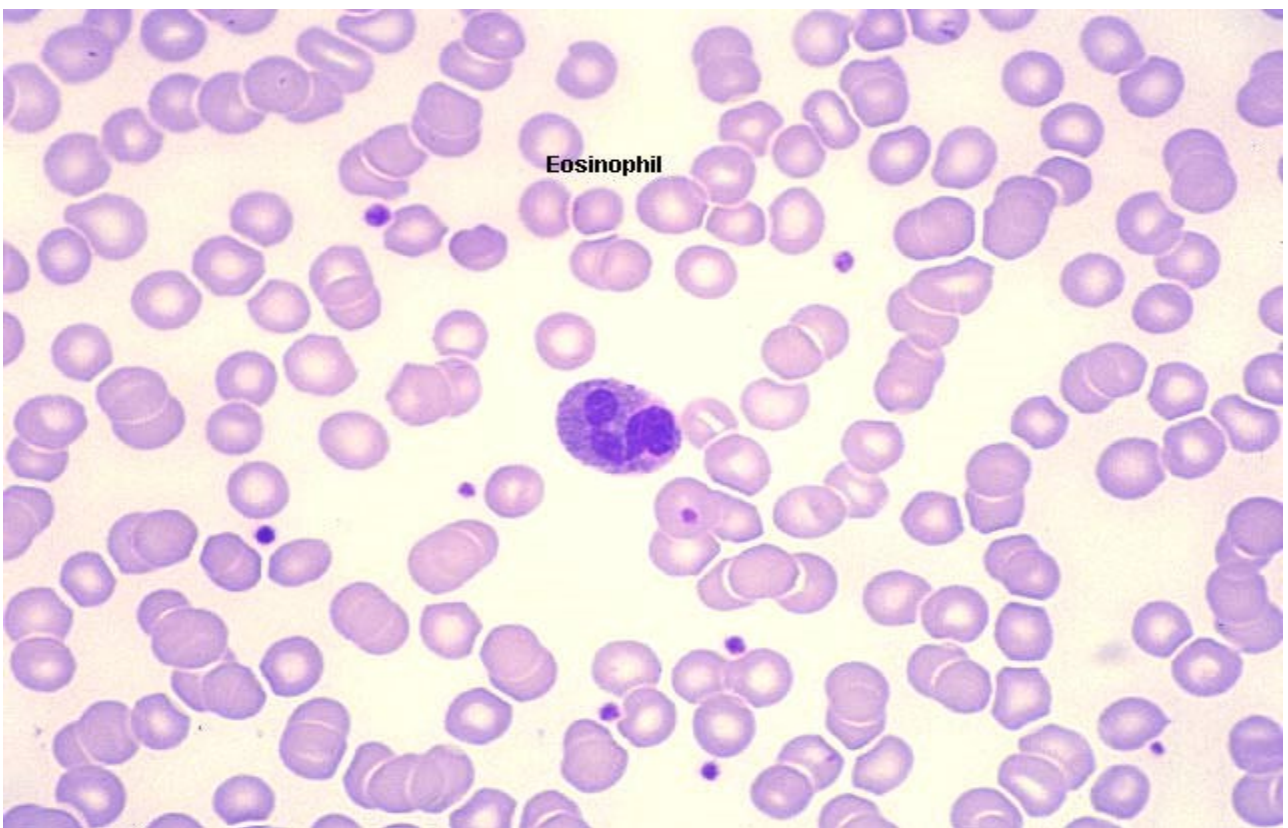
Eosinophil, giemsa stained peripheral blood film
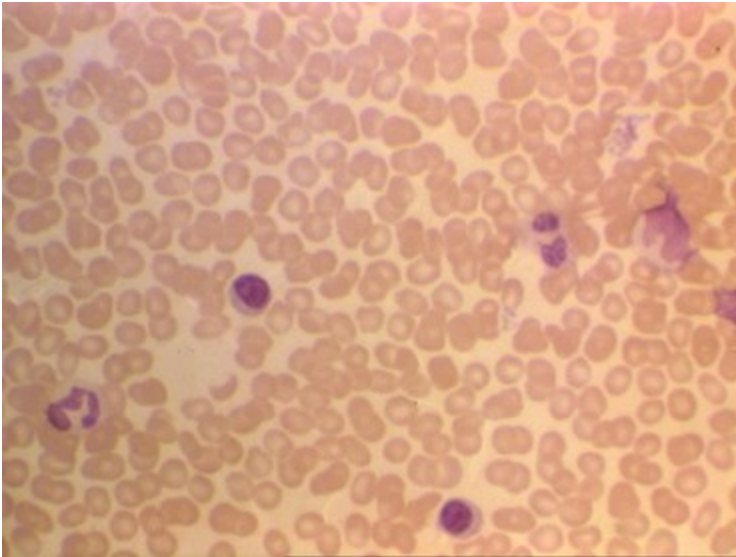
Blood film showing small lymphocytes
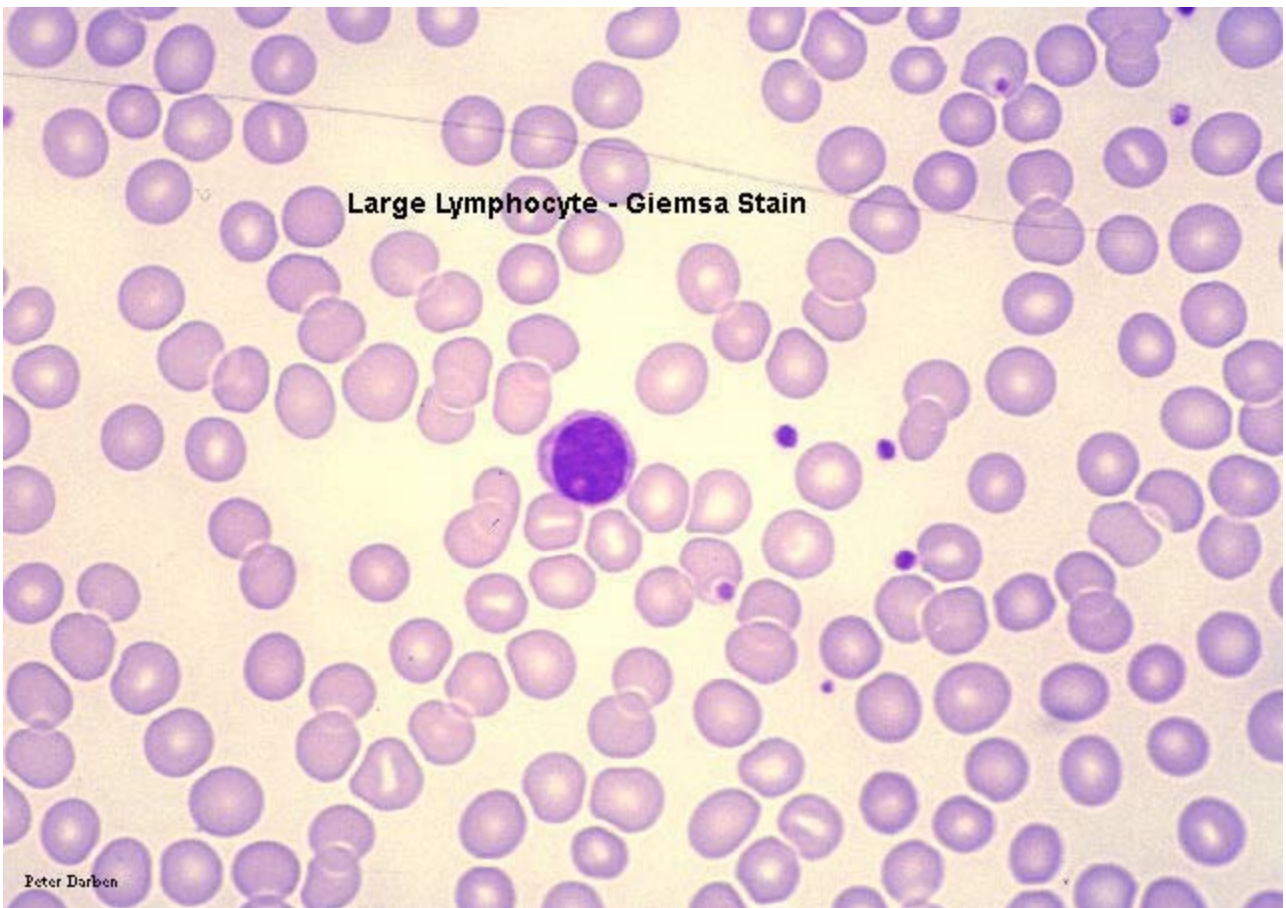
Large Lymphocyte, giemsa stained peripheral blood film
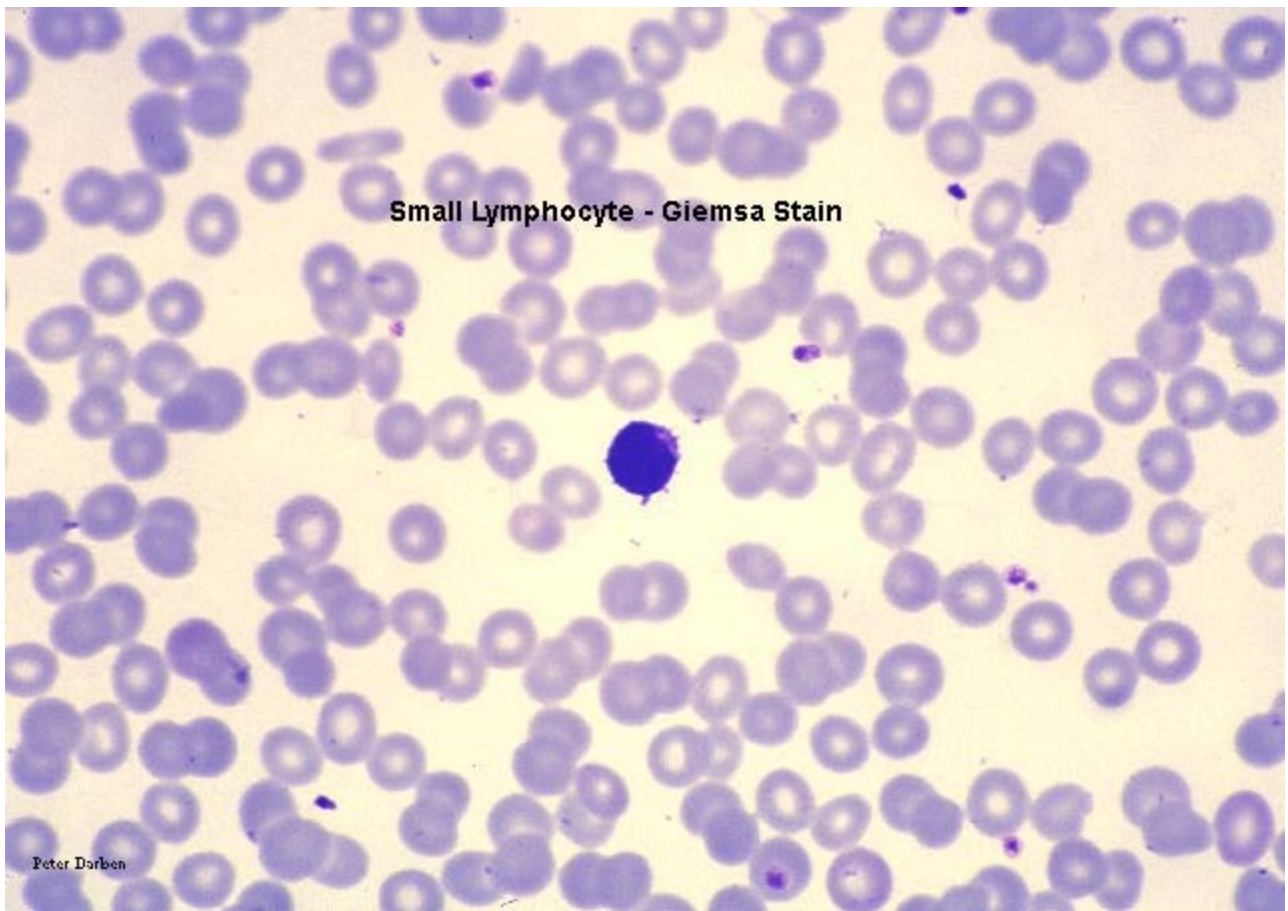
Small Lymphocyte, giemsa stained peripheral blood film
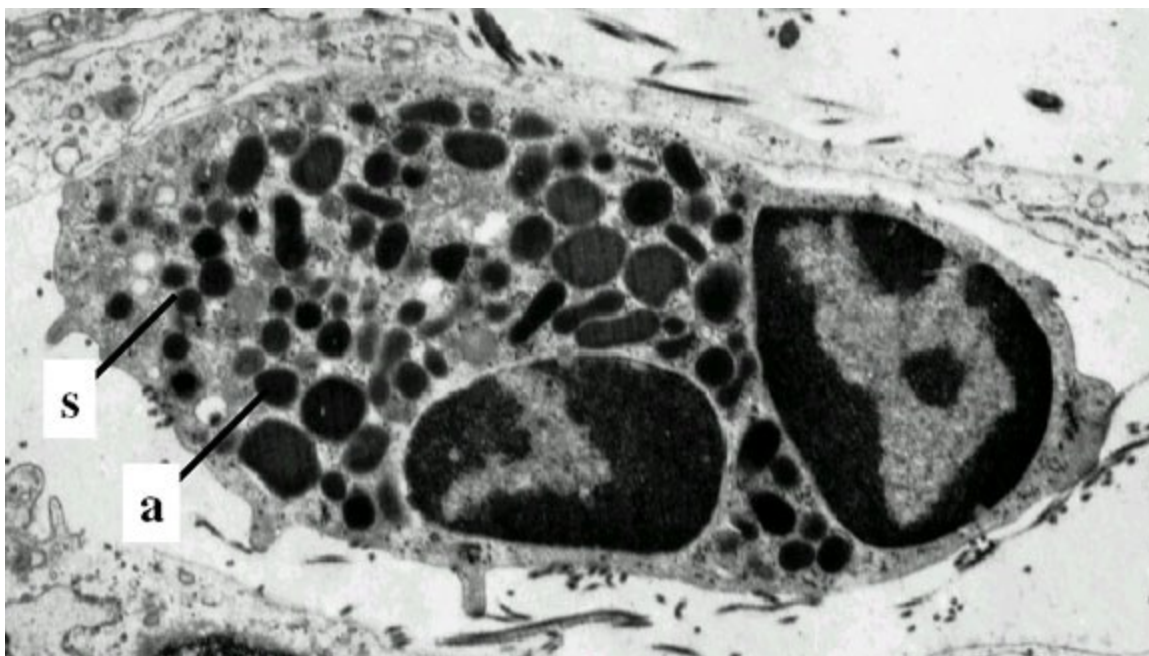
Neutrophil - electron micrograph. Note the two nuclear lobes and the azurophilic granules

Neutrophil, giemsa stained peripheral blood film

Copyright © 2004 Dennis Kunkel Microscopy, Inc.
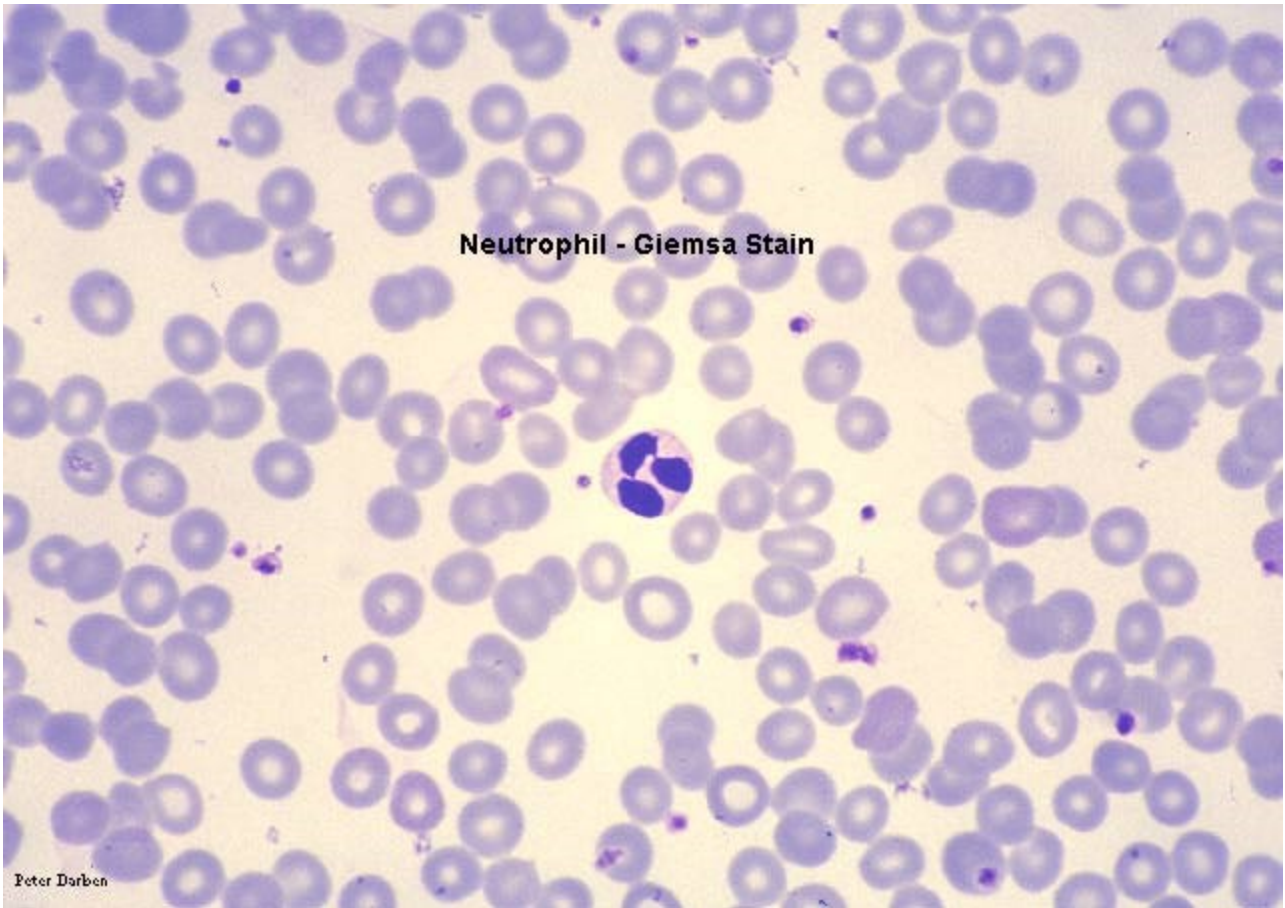
T lymphocytes (pre-T cells) and granulocyte (neutrophil)

There are two main lineages that derive from the hemopoietic stem cell:

- The lymphoid lineage

T lymphocytes (T cells)
B lymphocytes (B cells)
Natural killer cells (NK cells)

- The myeloid lineage

Monocytes, macrophages
Langerhans cells, dendritic cells
Megakaryocytes
Granulocytes (eosinophils, neutrophils, basophils)

Clonal selection
The four basic principles of the clonal selection hypothesis
Each lymphocyte bears a single type of receptor of a unique specificity
Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with high affinity leads to lymphocyte activation
The differentiated effector cells derived from an activated lymphocyte will bear receptors of an identical specificity to those of the parental cell from which that lymphocyte was derived
Lymphocytes bearing receptors specific for self molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) AND T-CELL RECEPTORS - ROLE IN IMMUNE RESPONSES

HISTORICAL OVERVIEW

Cell-cell interactions of the adaptive immune response are critically important in protection from pathogens. These interactions are orchestrated by the immunological synapse whose primary components are the T cell antigen receptor (TCR) and the Major histocompatibility complex (MHC) molecule. The major function of the TCR is to recognize antigen in the correct context of MHC and to transmit an excitatory signal to the interior of the cell. Since binding of peptide within the MHC is not covalent, there are many factors while help stabilize the immunological synapse.

Gene products encoded in the MHC were first identified as being important in rejection of transplanted tissues. Furthermore, genes in the MHC were found to be highly polymorphic (i.e. in the population there were many different allelic forms of the genes). Studies with inbred strains of mice showed that genes in the MHC were also involved in controlling both humoral and cell-mediated

immune responses. For example, some strains of mice could respond to a particular antigen but other strains could not and these strains differed only in one or more of the genes in the MHC. Subsequent studies showed that there were two kinds of molecules encoded by the MHC – Class I molecules and class II molecules which are recognized by different classes of T cells. Class I molecules were found on all nucleated cells (not red blood cells) whereas class II molecules were found only on antigen presenting cells, (APCs) which included dendritic cells, macrophages, B cells and a few other types (Figure 1).

It was not until the discovery of how the TCR recognizes antigen that the role of MHC genes in immune responses was understood. The TCR was shown to recognize antigenic peptides in association with MHC molecules. T cells recognize portions of protein antigens that are bound non-covalently to MHC gene products. Cytotoxic T cells (Tc) recognize peptides bound to class I MHC molecules and helper T cells (Th) recognize peptides bound to class II MHC molecules. The three dimensional structures of MHC molecules and the TCR have been determined by X-ray crystallography so that a clear picture of how the TCR, MHC gene products and antigen interact has emerged.
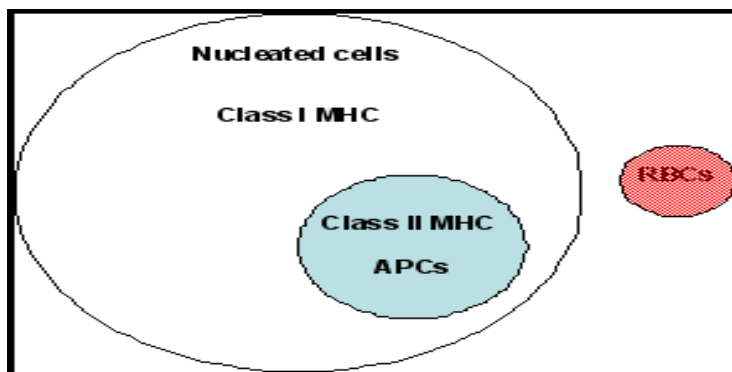
Figure 1
Distribution of class I and class II MHC molecules on human cells

STRUCTURE OF CLASS I MHC MOLECULES

The molecule

Class I MHC molecules are composed of two polypeptide chains, a long α chain and a short β chain called β2-microglobulin (figure 2). The α chain has four regions.

- A cytoplasmic region, containing sites for phosphoylation and binding to cytoskeletal elements.
- A transmembrane region containing hydrophic amino acids by which the molecule is anchored in the cell membrane.
- A highly conserved α3 immunoglubilin-like domain to which CD8 binds.
- A highly polymorphic peptide binding region formed from the α1 and α2 domains. The β2- microglobulin associates with the α chain and helps maintain the proper conformation of the molecule.
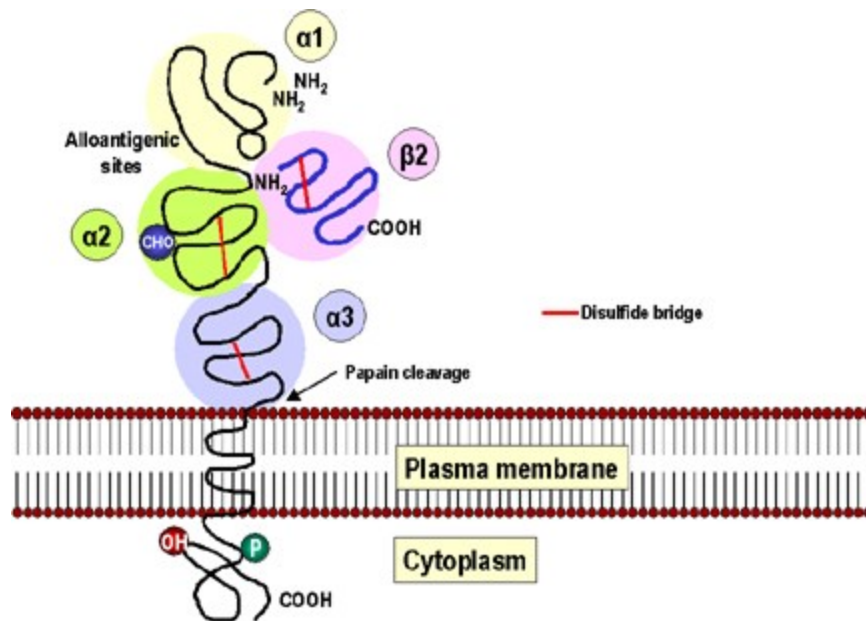
Figure 2
The MHC class 1 molecule has three globular domains alpha 1 (yellow), alpha 2 (green) and alpha 3 (blue). The alpha 3 domain is closely associated with the non-MHC -encoded beta 2 microglobulin (pink). The latter is stabilized by a disulfide bridge (red) and is similar to an immunoglobulin domain in three-dimensional structure. The alloantigenic sites which carry determinants specific to each individual are found in the alpha 1 and 2 domains. The latter also has a carbohydrate chain (blue, CHO). There is a phosphate in the cytoplasmic domain. Papain cleaves near the outer surface of the plasma membrane

The antigen-binding groove

An analysis of which part of the class I MHC molecules is most variable demonstrates that variability is most pronounced in the α1 and α2 domains, which comprise the peptide binding region (Figure 3). The structure of the peptide binding groove, revealed by X-ray crystallography, shows that the groove is composed of two α helices forming a wall on each side and eight β-pleated sheets forming a floor. The peptide is bound in the groove and the residues that line the groove make contact with the peptide (Figure 4). These are the residues that are the most polymorphic. The groove will accommodate peptides of approximately 8-10 amino acids long. Whether a particular peptide will bind to the groove will depend on the amino acids that line the groove. Because class I molecules are polymorphic, different class I molecules will bind different peptides. Each class I molecule will bind only certain peptides and will have a set of criteria that a peptide must have in order to bind to the groove. For example, Figure 5 shows that one class I molecule will bind peptides that have a leucine (L) as the carboxy-terminal amino acid and either tyrosine (Y) or phenylalanine (F) as the 4th amino acid from the carboxy-terminal end. As long as these two conditions are met a peptide will

bind, regardless of what the other amino acids are. Similarly a different class I molecule will bind any peptide that has a tyrosine (Y) as the second amino acid from the amino-terminal end and either a valine (V), isoleucine (I) or leucine (L) at the carboxy-terminal end (Figure 5). Thus, for every class I molecule, there are certain amino acids that must be a particular location in the peptide before it will bind to the MHC molecule. These sites in the peptide are referred to as the "anchor sites". The ends of the peptide are buried within the closed ends of the class I binding groove while the center bulges out for presentation to the TCR.

Within the MHC there are 6 genes that encode class I molecules HLA-A, HLA –B, HLA-C, HLA-E, HLA-F and HLA-G. Among these HLA-A, HLA –B, and HLA-C are the most important and are most polymorphic. Table 1 shows the degree of polymorphism at each of these loci.

Figure 3 Most variability in amino acids at different positions along the alpha chain of class I MHC molecules occurs in the alpha 1 and alpha 2 regions. The greatest polymorphism is found for amino acids that line the wall and floor of the groove that binds the peptides

Figure 4

a. Peptide binding groove of class I MHC molecules.

b. Groove with highlighted highly variable residues. The variable residues are clustered around the peptide-binding pocket

Figure 5

Anchor sites in peptides that bind to class I MHC molecules (adapted from Janeway et al. Immunobiology 6th Edition

Table 1. Polymorphism of class I MHC genes	
Locus	**Number of alleles (allotypes)**
HLA-A	218
HLA-B	439
HLA-C	96
HLA-E, HLA-F and HLA-G	Relatively few alleles

STRUCTURE OF CLASS II MHC MOLECULES

The molecule

Class II MHC molecules are composed of two polypeptide chains an α and a β chain of approximately equal length (Figure 6). Both chains have four regions:

- A cytoplasmic region containing sites for phosphoylation and binding to cytoskeletal elements
- A transmembrane region containing hydrophic amino acids by which the molecule is anchored in the cell membrane
- A highly conserved α2 domain and a highly conserved β2 domain to which CD4 binds
- A highly polymorphic peptide binding region formed from the α1 and β1 domains
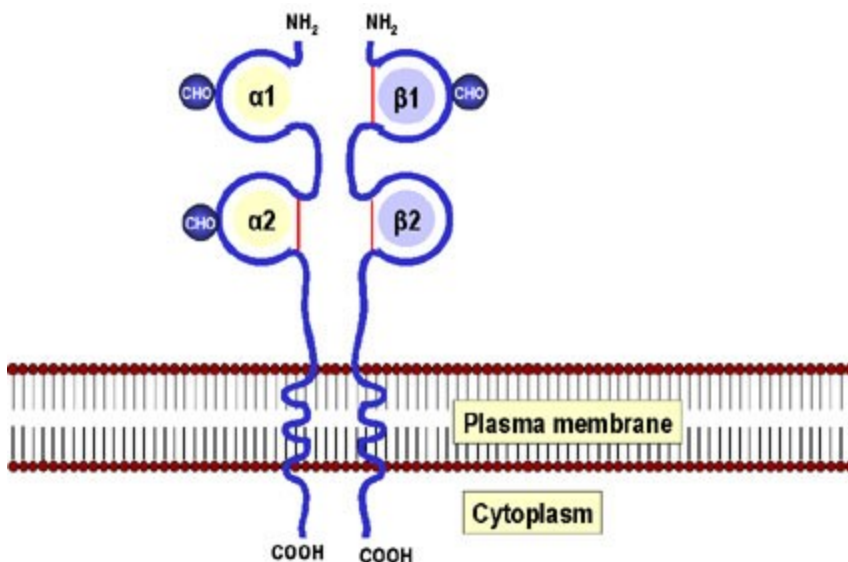
Figure 6 MHC class II molecules comprise two non-identical peptides (alpha and beta) which are non-covalently associated and traverse the plasma membrane with the N terminus to the outside of the cell. The domains closest to the membrane in each chain are structurally related to immunoglobulins. With the exception of the alpha 1 domain, all domains are stabilized by disulfide bridges (red). Both the alpha and beta chains are glycosylated. The beta chain is shorter than the alpha chain (beta mol. wt = 28,000) and contains the alloantigenic sites. There is some polymorphism in the alpha chain of some MHC II molecules

The antigen-binding groove

As with Class I MHC molecules, an analysis of which part of the class II MHC molecule is most variable demonstrates that variability is most pronounced in the α1 and β1 domains, which comprise the peptide binding region (Figure 7). The structure of the peptide binding groove, revealed by X-ray crystallography, shows that, like class I MHC molecules, the groove is composed of two α helices forming a wall on each side and eight β-pleated sheets forming a floor. Both the α1 and β1 chain contribute to the peptide binding groove. The peptide is bound in the groove and the residues that line the groove make contact with the peptide. These are the residues that are the most polymorphic. The groove of Class II molecules is open at one end so that the groove can accommodate longer peptides of approximately 13-25 amino acids long with some of the amino acids located outside of the groove. Whether a particular peptide will bind to the groove will depend on the amino acids that line the groove. Because class II molecules are polymorphic, different class II molecules will bind different peptides. Like class I molecules, each class II molecule will bind only certain peptides and will have a set of criteria that a peptide must have in order to bind to the groove (i.e. "anchor sites").

Within the MHC there are 5 loci that encode class II molecules, each of which contains a gene for an α chain and at least one gene for a β chain. The loci are designated as HLA-DP, HLA –DQ, HLA-DR, HLA-DM, and HLA-DO. Among these, HLA-DP, HLA –DQ, and HLA-DR are the most important and are most polymorphic. Table 2 shows the degree of polymorphism at each of these loci.
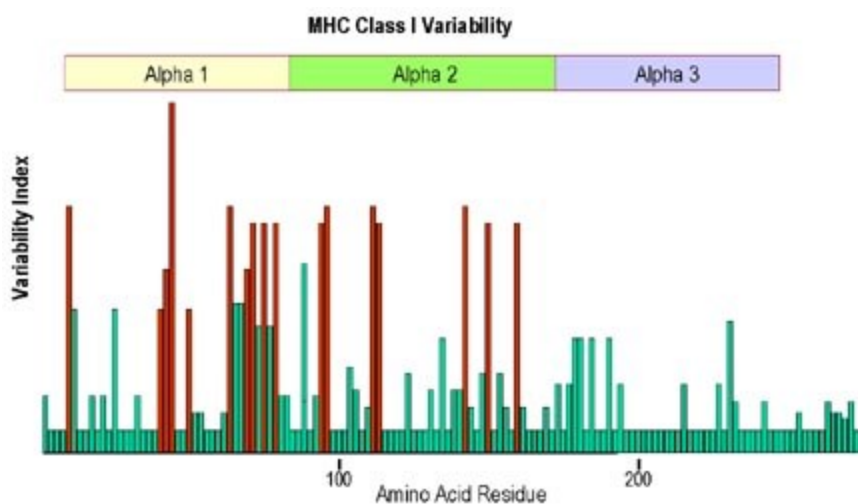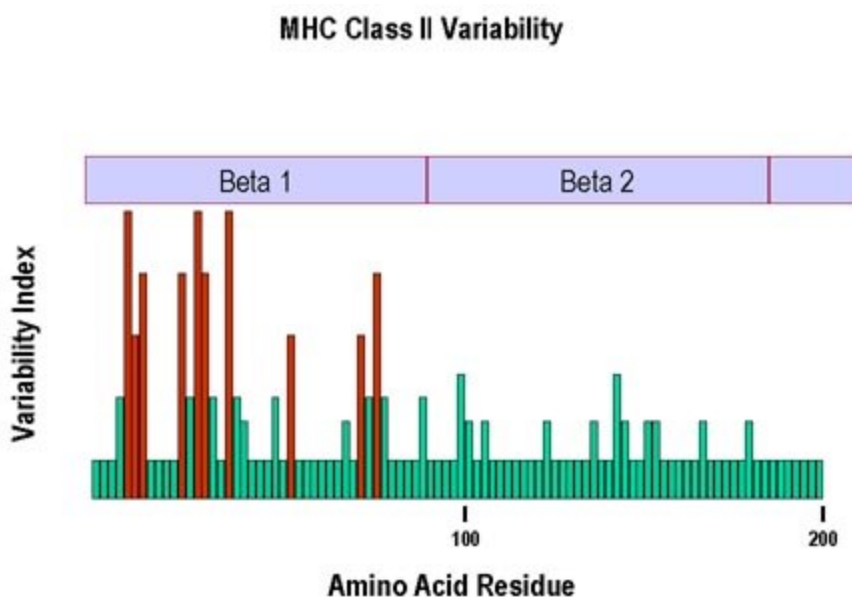

Figure 7
The greatest polymorphism for the beta chain of class II MHC molecules is found for those amino acids in the beta I region that line the wall and floor of the groove that binds the peptide

IMPORTANT ASPECTS OF MHC

- Although there is a high degree of polymorphism for a species, an individual has maximum of six different class I MHC products and only slightly more class II MHC products (considering only the major loci).
- Each MHC molecule has only one binding site. The different peptides a given MHC molecule can bind all bind to the same site, but only one at a time.
- Because each MHC molecule can bind many different peptides, binding is termed degenerate.
- MHC polymorphism is determined only in the germline. There are no recombinational mechanisms for generating diversity.
- MHC molecules are membrane-bound; recognition by T cells requires cell-cell contact.
- Alleles for MHC genes are co-dominant. Each MHC gene product is expressed on the cell surface of an individual nucleated cell.
- A peptide must associate with a given MHC of that individual, otherwise no immune response can occur. That is one level of control.
- Mature T cells must have a T cell receptor that recognizes the peptide associated with MHC. This is the second level of control.
- Cytokines (especially interferon-γ) increase level of expression of MHC.
- Peptides from the cytosol associate with class I MHC and are recognized by Tc cells. Peptides from within vesicles associate with class II MHC and are recognized by Th cells.
- Polymorphism in MHC is important for survival of the species.

Table 2. Polymorphism of class II MHC genes	
Locus	Number of alleles (allotypes)
HLA-DPA	12
HLA-DPB	88
HLA-DQA	17
HLA-DQB	42
HLA-DRA	2
HLA-DRB1	269
HLA-DRB3	30
HLA-DRB4	7
HLA-DRB5	12
HLA-DM and HLA-DO	Relatively few alleles

HOW DO PEPTIDES GET INTO THE MHC GROOVE?

Peptides from the cytosol associate with class I MHC and are recognized by CTL cells. The peptides enter the endoplasmic reticulum and bind in the MHC class I groove. This complex is then exported to the cell surface through the Golgi. MHC class II molecules are formed with an invariant (Ii) chain as a place holder while in the ER and Golgi. The Ii chain is cleaved and removed once the complex

is in a vesicle. Peptides from within the vesicle associate with class ll MHC and are then exported to the cell surface where they are recognized by Th cells.

THE ROLE OF TCR IN THE IMMUNE RESPONSE

The TCR is a surface molecule found on T cells that recognizes antigen presented in the correct MHC context. The TCR is similar to immunoglobulin and is part of the immunoglobulin superfamily. There are two types of TCRs, the predominant αβ which is commonly found in lymphoid tissues, and the γδ which is found at mucosal surfaces.

STRUCTURE OF THE T CELL RECEPTOR (TCR)

The TCR is a heterodimer composed of one α and one β chain of approximately equal length (Figure 8). Each chain has a short cytoplasmic tail but it is to small to be able to transduce an activation signal to the cell. Both chains have a transmembrane region comprised of hydrophobic amino acids by which the molecule is anchored in the cell membrane. Both chains have a constant region and a variable region similar to the immunoglobulin chains. The variable region of both chains contains hypervariable regions that determine the specificity for antigen. Each T cell bears a TCR of only one specificity (*i.e.* there is allelic exclusion).
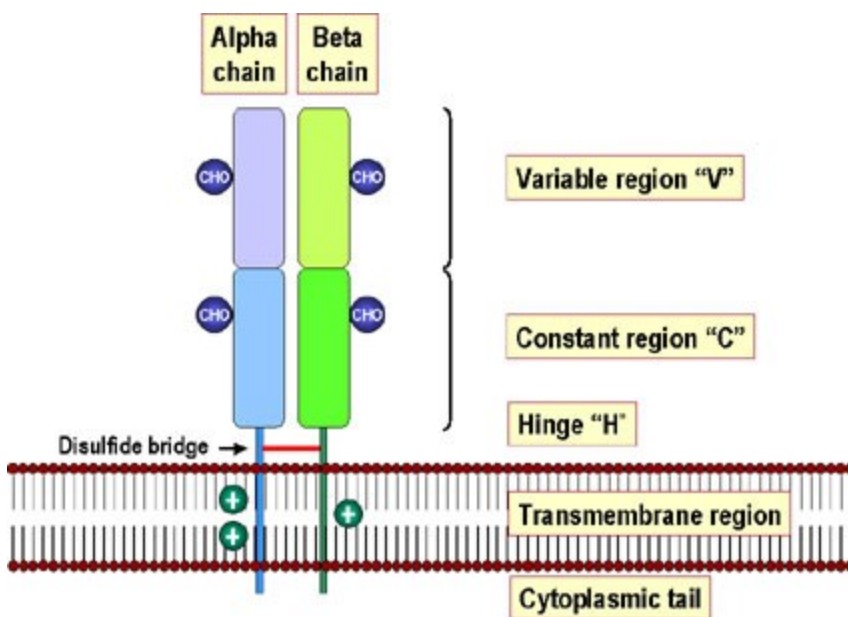
Figure 8

The T cell receptor heterodimer comprises two transmembrane glycoproteins, the alpha and beta chains. There are two domains in the external part of each chain and these resemble immunoglobulin variable and constant regions. There are sugar chains on each domain. There is a short sequence similar to the immunoglobulin hinge region that connects the immunoglobulin-like domains to the transmembrane sequence. This contains cysteines that form a disulfide bridge. The hydrophobic transmembrane helical structures are unusual in that they contain positively charged amino acids (basic amino acids). The alpha chain has two positively charged residues while the beta chain has one.

THE GENETIC BASIS FOR RECEPTOR GENERATION

The genetic basis for the generation of the vast array of antigen receptors on B cells has been discussed previously (see lecture on Ig genetics). The generation of a vast array of TCRs is accomplished by similar mechanism. The germline genes for the TCR β genes are composed of V, D and J gene segments that rearrange during T cell development to produce many different TCR β chains (Figure 9). The germline genes for the TCR α genes are composed of V and J gene segments which rearrange to produce α chains. The specificity of the TCR is determined by the combination of α and β chains.

There is a small population of T cells that express TCRs that have γ and δ chains instead of α and β chains. These gamma/delta T cells predominate in the mucosal epithelium and have a repertoire biased toward certain bacterial and viral antigens. The genes for the δ chains have V, D and J gene segments whereas the genes for the γ chains have only V and J gene segments but the repertoire is considerably smaller that than that of the alpha/beta T cells. The gamma/delta T cells recognize antigen in an MHC-independent manner unlike the alpha/beta T cells.
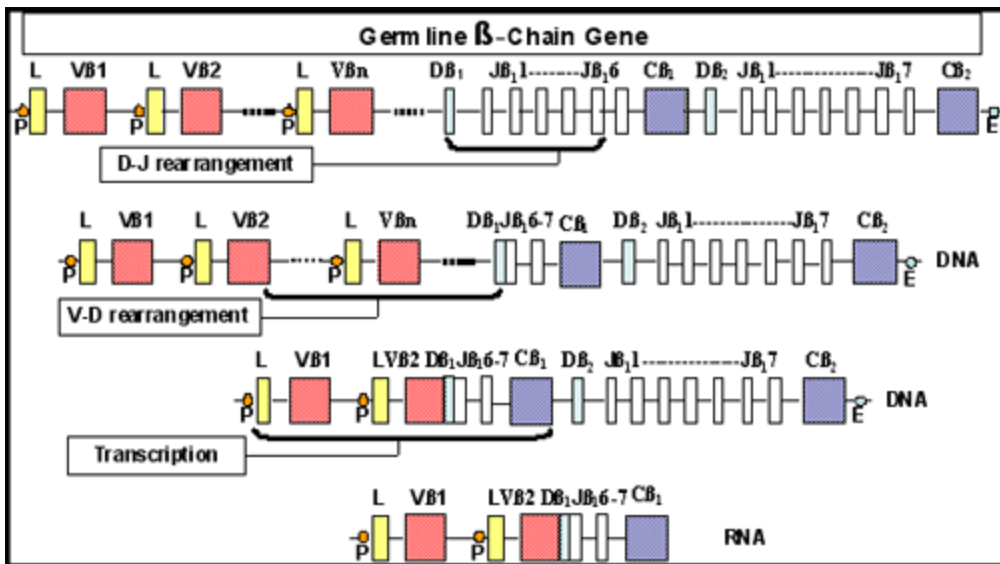
Figure 9
Rearrangement of the TCR beta chain genes

TABLE 3 COMPARISON OF THE MAJOR PROPERTIES OF IMMUNOGLOBULIN (Ig) AND T-CELL RECEPTOR (TCR) GENES AND PROTEINS		
GENES		
Properties	Ig	TCR
Many VDJs, Few C's	Yes	Yes
VDJ Rearrangement	Yes	Yes
V pairs form antigen-recognition site	Yes	Yes
Somatic hypermutation	Yes	No
PROTEINS		
Transmembrane forms	Yes	Yes
Secreted forms	Yes	No
Isotypes with distinct functions	Yes	No
Valency	2	1
Adapted from Janeway and Travers, Immunobiology		
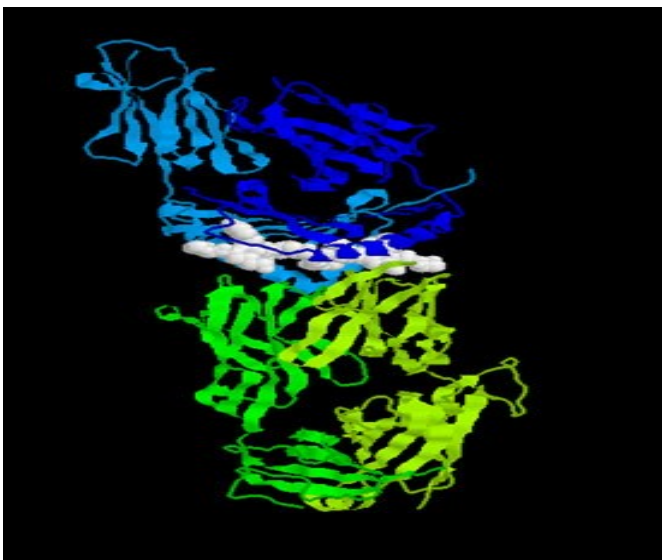
Structure of a crystal structure of a complex of a human T cell receptor, influenza Ha Antigen Peptide and an MHC Class II Molecule. The alpha and beta chains of the MHC II molecules are in dark and light blue. The T cell receptor is in yellow and green. The influenza peptide is in gray

TCR AND CD3 COMPLEX

The TCR is closely associated with a group of 5 proteins collectively called the CD3 complex (Figure 10). The CD3 complex is composed of one γ, one δ, two ε and 2 ξ chains. All of the proteins of the CD3 complex are invariant and they do not contribute to the specificity in any way. The CD 3 complex is necessary for cell surface expression of the TCR during T cell development. In addition, the CD3 complex transduces activation signals to the cell following antigen interaction with the TCR.
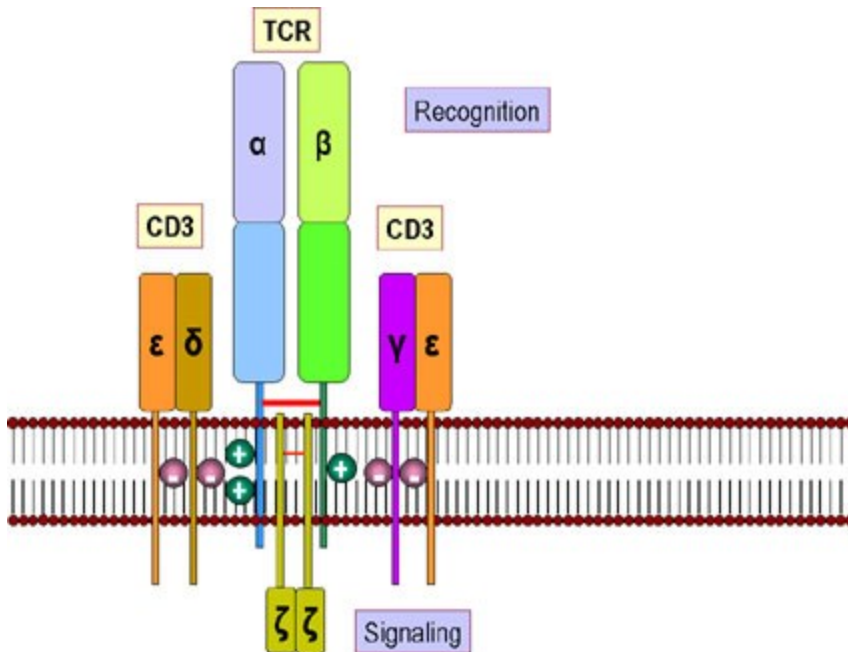
Figure 10
The receptor for antigens on the T cell surface comprises eight proteins.
(a) Two disulfide-bonded chains of the T cell receptor which form a heterodimer. These recognize peptides associated with MHC molecules.
(b) Four chains, collectively termed CD3, that associate with the T cell receptor dimer and participate in its transport to the surface of the cell. The CD3 complex together with the zeta chains, which form a homodimer, transduce the signal after antigen has bound

THE "IMMUNOLOGICAL SYNAPSE"

The interaction between the TCR and MHC molecules are not very strong. Accessory molecules are necessary to help stabilize the interaction (Figure 11a,b). These include:

- CD4 binding to Class II MCH, which ensures that Th cells only interact with APCs
- CD8 binding to class I MHC, which ensures that Tc cells can interact with target cells
- CD2 binding to LFA-3
- LFA-1 binding to ICAM-1
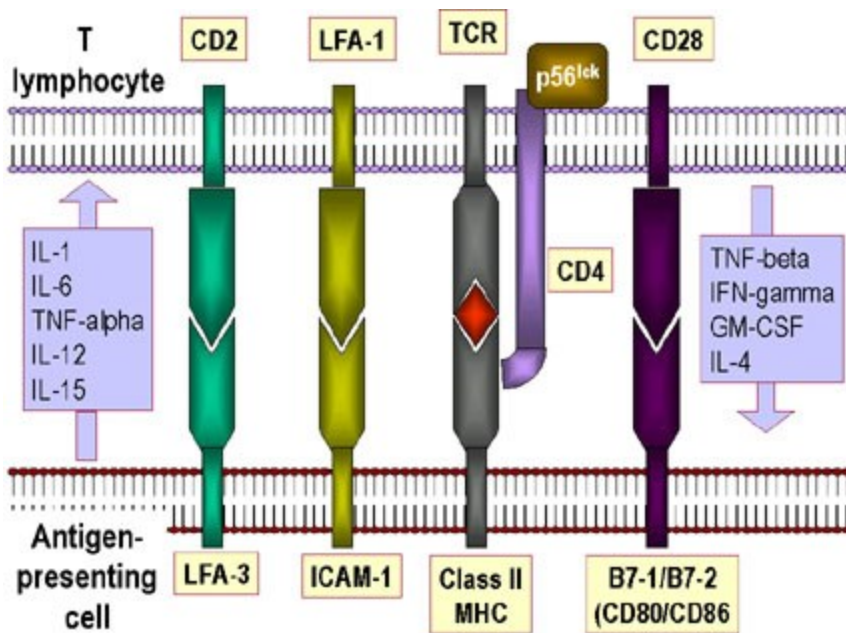
Figure 11 A. Molecules involved in the interaction between T cells and antigen-presenting cells. Some cytokines produced by each cell type are shown
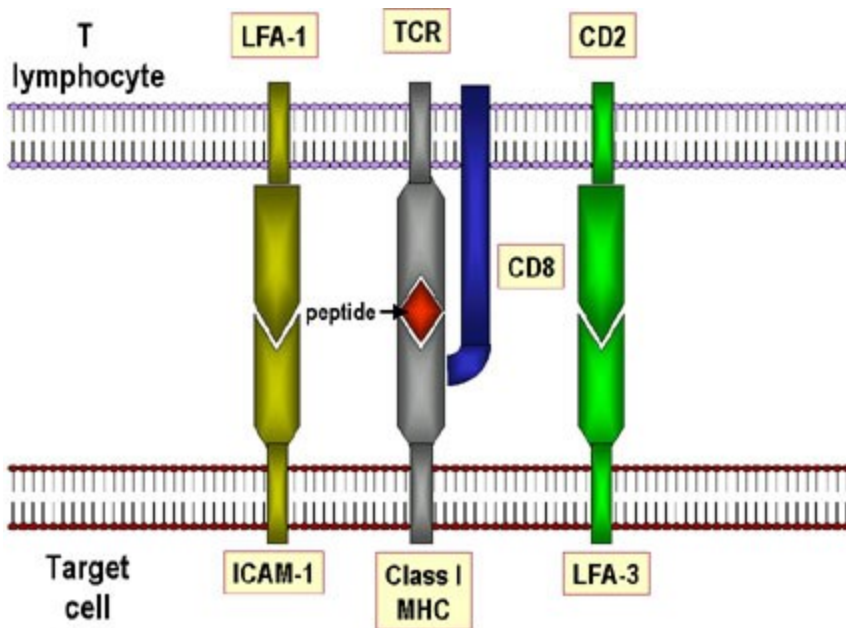
B. Ligands involved in the interaction of cytotoxic T cells and their target cells

The accessory molecules are invariant and do not contribute to the specificity of the interaction, which is solely determined by the TCR. The expression of accessory molecules can be increased in response to cytokine, which is one way that cytokines can modulate immune responses.

In addition to accessory molecules which help stabilize the interaction between the TCR and antigen in association with MHC molecules, other molecules are also needed for T cell activation. Two signals are required for T cell activation – one is the engagement of the TCR with Ag/MHC and the other signal comes from the engagement of co-stimulatory molecules with their ligands. One of the most important (but not the only) co-stimulatory molecule is CD28 on T cells which must interact with B7-

1 (CD80) or B7-2 (CD81) on APCs . Like accessory molecules the co-stimulatory molecules are invariant and do not contribute to the specificity of the interaction. The multiple interactions of TCR with Ag/MHC and the accessory and co-stimulatory molecules with their ligands have been termed the "immunological synapse.

Not only is co-stimulation necessary for T cell activation, a lack of co-stimulation may result in anergy (i.e., inability to respond to antigen) or down-regulation of the response. Figure 12 shows the possible outcomes of a T cell receiving one or both of the signals necessary for activation. Engagement of the TCR with Ag/MHC but no co-simulation results in anergy. Engagement of only the co-stimulatory molecule has no effect. Engagement of TCR with Ag/MHC and co-stimulatory molecules with their ligand results in activation. Engagement of the TCR with Ag/MHC and engagement of B7 ligand with CTLA-4, molecules similar to CD28, results in down-regulation of the response. CTLA-4/B7 interaction sends an inhibitory signal to the T cell rather than an activating signal. This is one of the ways that immune responses are regulated. CTLA-4 is expressed on T cells later in an immune response and this helps to turn off the response.
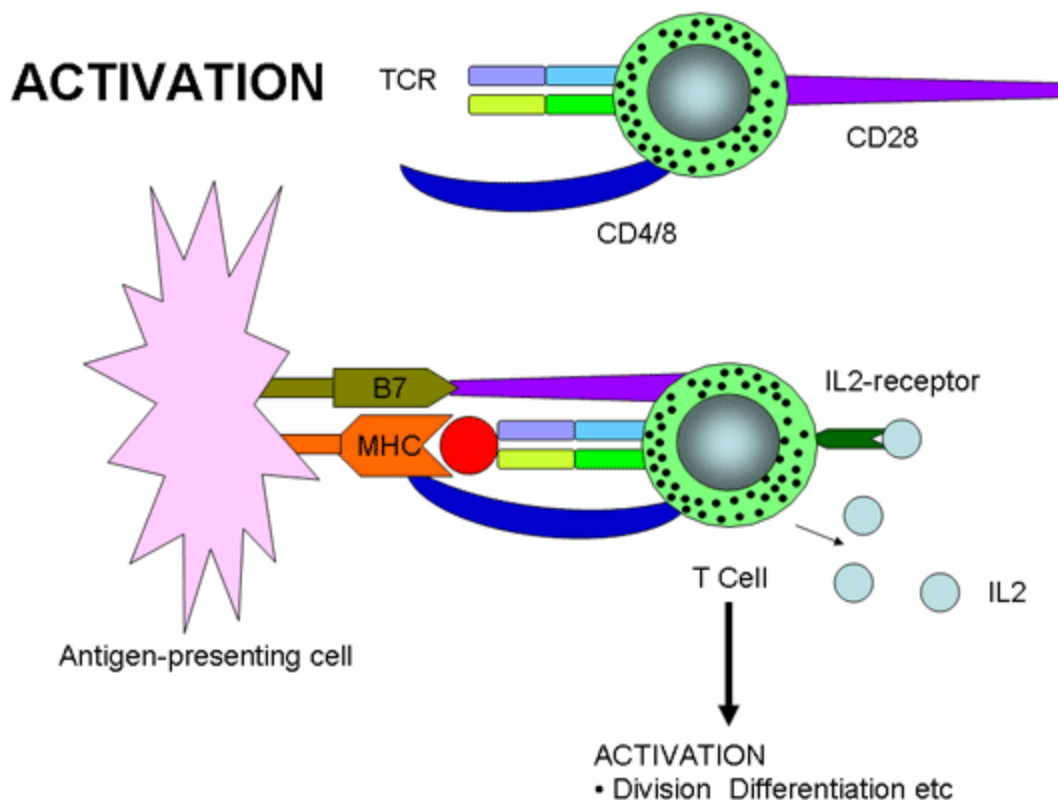
Figure 12a Activation of T cells only occurs when both TCR and co-stimulatory molecules are engaged with their respective ligands

Figure 12 b Down regulation occurs if CTLA-4 interacts with B7: CTLA-4 sends an inhibitory signal

Figure 12c Engagement of TCR and antigen/MHC in the absence of co-stimulation may lead to anergy

ENGAGEMENT OF CO-STIMULATORY MOLECULES IN THE ABSENCE OF TCR: NO RESPONSE
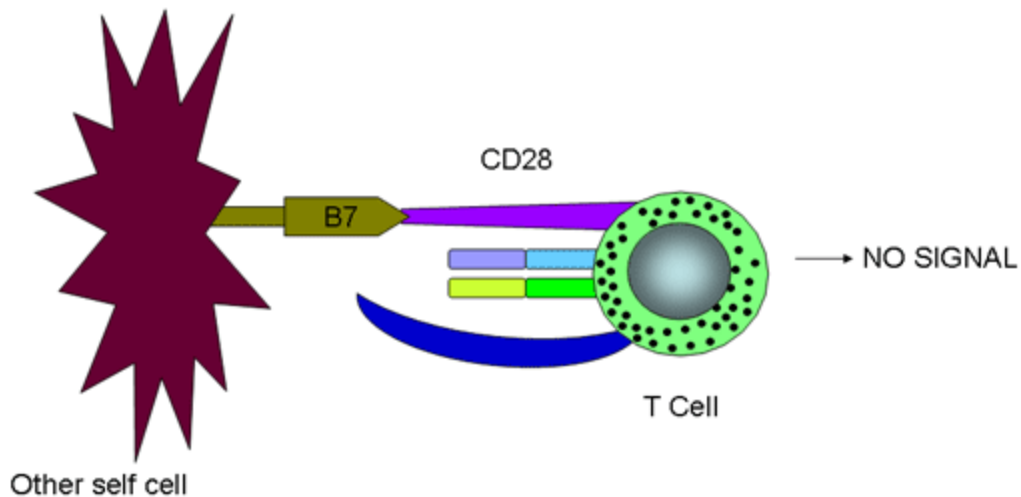
Figure 12d Engagement of co-stimulatory molcules in the absence of TCR engagement results in no response

TABLE 4 IMPORTANT ACCESSORY MOLECULES	
T cell molecule	**Ligand on second cell**
CD4 on helper T cells	class II MHC molecules
CD8 on cytotoxic T cells	class I MHC molecules
LFA-2 (CD2)	LFA-3
LFA-1	ICAM-1, ICAM-2
LFA = Leukocyte Function-associated Antigen	
ICAM = Intercellular Adhesion Molecule	

RESPONSE TO ANTIGEN: PROCESSING AND PRESENTATION

MHC RESTRICTION AND ROLE OF THE THYMUS

COMPARISON OF BCR AND TCR

B cells and T cells recognize different substances as antigens and in a different form. The B cell uses cell surface-bound immunoglobulin as a receptor and the specificity of that receptor is the same as the immunoglobulin that it is able to secrete after activation. B cells recognize the following antigens in soluble form:

- Proteins (both conformational determinants and determinants exposed by denaturation or proteolysis)
- Nucleic acids
- Polysaccharides
- Some lipids
- Small chemicals (haptens)

In contrast, the overwhelming majority of antigens for T cells are proteins, and these must be fragmented and recognized in association with MHC products expressed on the surface of nucleated cells, not in a soluble form. T cells are grouped functionally according to the class of MHC molecules that associate with the peptide fragments of the protein: helper T cells recognize only those peptides associated with class II MHC molecules, and cytotoxic T cells recognize only those peptides associated with class I MHC molecules.

ANTIGEN PROCESSING AND PRESENTATION

Antigen processing and presentation are processes that occur within a cell that result in fragmentation (proteolysis) of proteins, association of the fragments with MHC molecules, and expression of the peptide-MHC molecules at the cell surface where they can be recognized by the T cell receptor on a T cell. However, the path leading to the association of protein fragments with MHC molecules differs for class I and class II MHC. MHC class I molecules present degradation products derived from intracellular (endogenous) proteins in the cytosol. MHC class II molecules present fragments derived from extracellular (exogenous) proteins that are located in an intracellular compartment.

Antigen processing and presentation in cells expressing class I MHC

All nucleated cells express class I MHC. As shown in Figure 1, proteins are fragmented in the cytosol by proteosomes (a complex of proteins having proteolytic activity) or by other proteases. The fragments are then transported across the membrane of the endoplasmic reticulum by transporter proteins. (The transporter proteins and some components of the proteosome have their genes in the MHC complex). Synthesis and assembly of class I heavy chain and beta$_2$ microglobulin occurs in the endoplasmic reticulum. Within the endoplasmic reticulum, the MHC class I heavy chain, beta$_2$microglobulin and peptide form a stable complex that is transported to the cell surface.
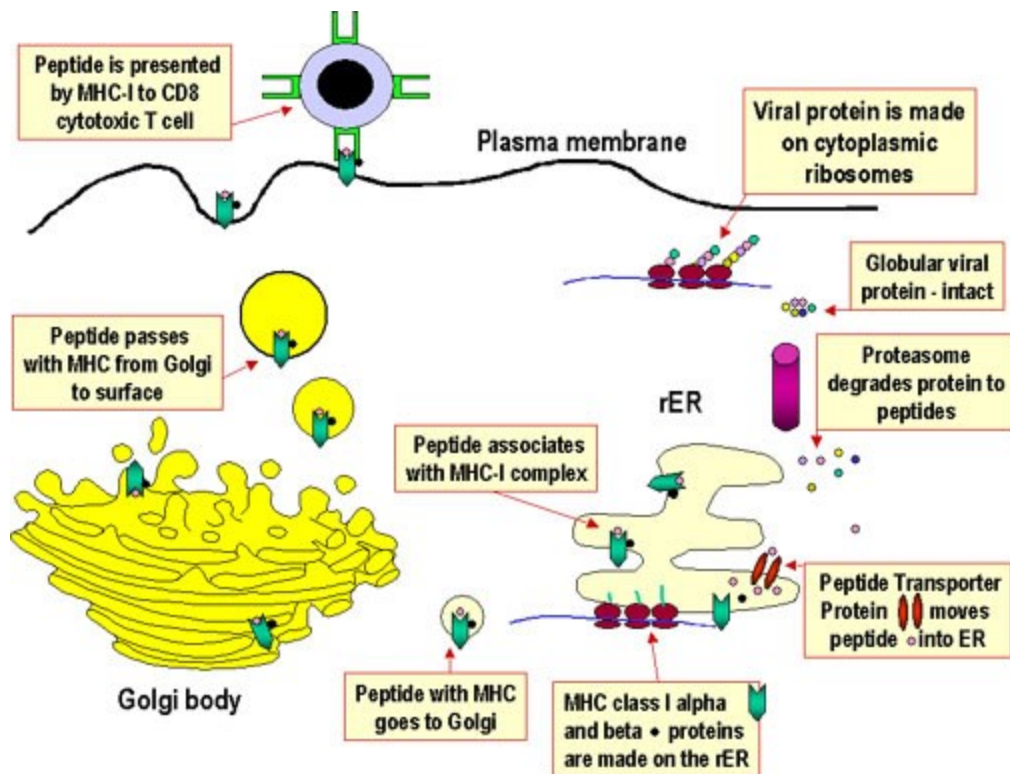
Figure 1 Pathway of class I MHC restricted presentation of an endogenously synthesized antigen. An example of such an antigen would be a viral protein made in the cell as a result of infection

Antigen processing and presentation in cells expressing class II MHC

Whereas all nucleated cells express class I MHC, only a limited group of cells express class II MHC, which includes the antigen presenting cells (APC). The principal APC are macrophages, dendritic cells (Langerhans cells), and B cells, and the expression of class II MHC molecules is either constitutive or inducible, especially by interferon-gamma in the case of macrophages.

As shown in Figure 2, exogenous proteins taken in by endocytosis are fragmented by proteases in an endosome. The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized, assembled in the endoplasmic reticulum, and transported through the Golgi and trans-Golgi apparatus to reach the endosome, where the invariant chain is digested, and the peptide fragments from the exogenous protein are able to associate with the class II MHC molecules, which are finally transported to the cell surface.
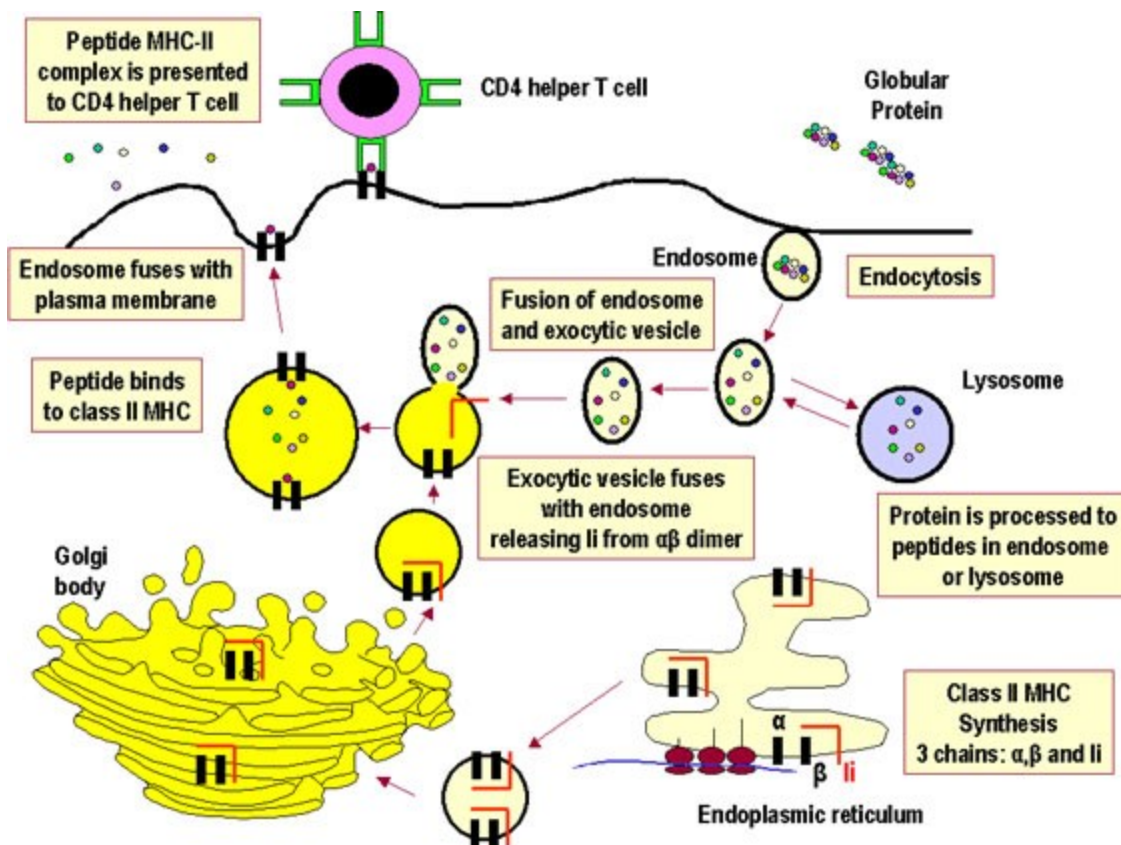
Figure 2 Pathway of class II MHC-restricted presentation of an exogenous antigen

Important aspects of antigen processing and presentation

- One way of rationalizing the development of two different pathways is that each ultimately stimulates the population of T cells that is most effective in eliminating that type of antigen.

Viruses replicate within nucleated cells in the cytosol and produce endogenous antigens that can associate with class I MHC. By killing these infected cells, cytolytic T cells help to control the spread of the virus.

Bacteria mainly reside and replicate extracellularly. By being taken up and fragmented inside cells as exogenous antigens that can associate with class II MHC molecules, helper Th2 T cells can be activated to assist B cells to make antibody against bacteria, which limits the growth of these organisms.

Some bacteria grow intracellularly inside the vesicles of cells like macrophages. Inflammatory Th1 T cells help to activate macrophages to kill the intracellular bacteria.

- Fragments of self, as well as non-self, proteins associate with MHC molecules of both classes and are expressed at the cell surface.

- Which protein fragments bind is a function of the chemical nature of the groove for that specific MHC molecule.

SELF MHC RESTRICTION

In order for a T cell to recognize and respond to a foreign protein antigen, it must recognize the MHC on the presenting cell as self MHC. This is termed self MHC restriction. Helper T cells recognize antigen in context of

class II self MHC. Cytolytic T cells recognize antigen in context of class I self MHC. The process whereby T cells become restricted to recognizing self MHC molecules occurs in the thymus.

The experimental systems demonstrating self MHC restriction for APC-helper T cell interactions and for class I MHC-cytotoxic T cell interactions are shown in Figures 3 and 4, respectively.
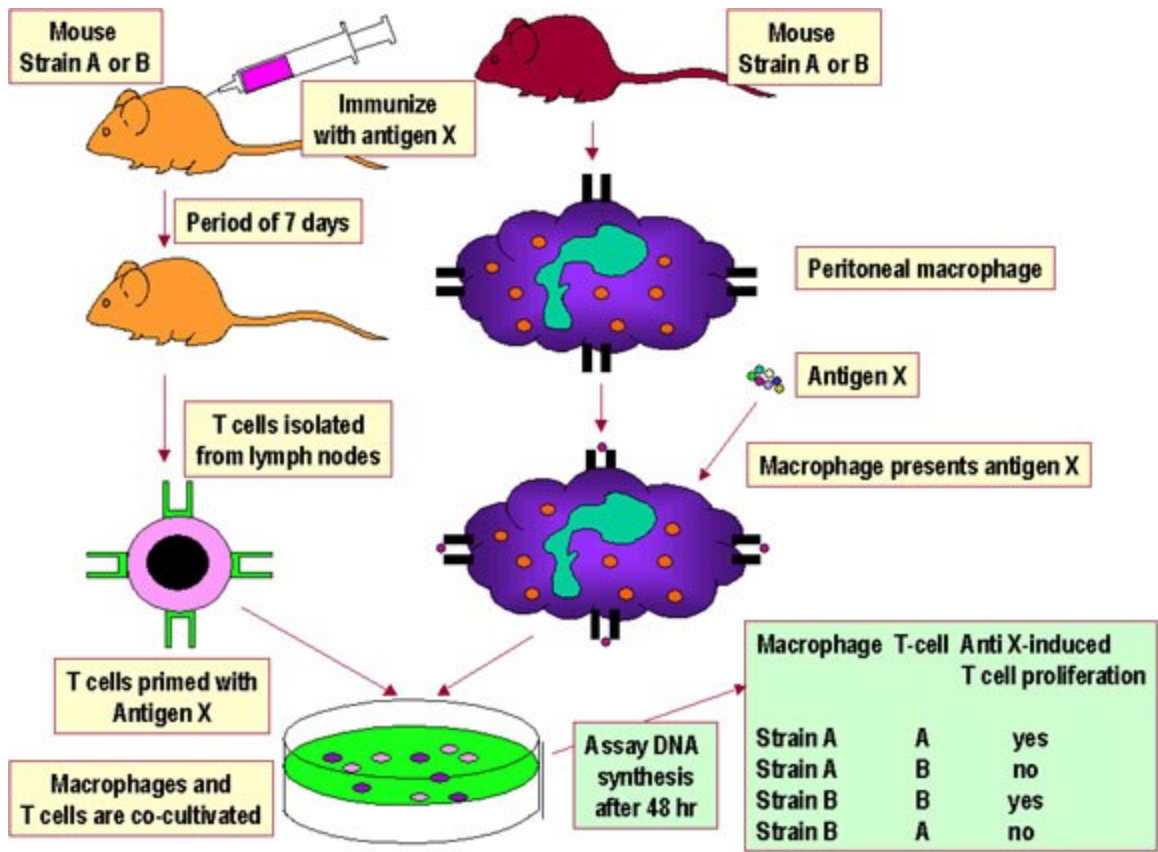
Figure 3 Self MHC Restriction of Th/APC Interactions

Mouse Strain A or B

Immunize with virus

Period of 7 days

Isolate spleen

Virus-specific cytotoxic T cells (CTLs) isolated from spleen

Purify Fibroblasts

Mouse Strain A or B

Infect fibroblasts with virus and radiolabel with 51Cr

51Cr labeled fibroblast presents antigen

Virus-specific CTLs

CTLs and fibroblasts are co-cultivated

Assay 51Cr release

Spleen CTLs immunized with	Fibroblast (target cells) infected with	51Cr release from fibroblasts (lysis)
Strain A	A	yes
Strain A	B	no
Strain B	B	yes
Strain B	A	no

Figure 4 Virus-specific CTLs from a strain A or strain B mouse lyse only syngeneic target cells infected with a specific virus. The CTLs do not lyse uninfected target cells and are not alloreactive. Further analysis has shown that the CTLs and target cells must come from animals that share class I MHC alleles in order for the target to present viral antigens to the CTLs.

ANTIGEN PRESENTING CELLS

The three main types of antigen presenting cells are dendritic cells, macrophages and B cells, although other cells, that express class II MHC molecules, (*e.g.,* thymic epithelial cells) can act as antigen presenting cells in some cases. Dendritic cells, which are found in skin and other tissues, ingest antigens by pinocytosis and transport antigens to the lymph nodes and spleen. In the lymph nodes and spleen they are found predominantly in the T cells areas. Dendritic cells are the most effective antigen presenting cells and can present antigens to naïve (virgin) T cells. Furthermore, they can present internalized antigens in association with either class I or class II MHC molecules (cross presentation), although the predominant pathway for internalized antigen is the class II pathway. The second type of antigen presenting cell is the macrophage. These cells ingest antigen by phagocytosis or pinocytosis. Macrophages are not as effective in presenting antigen to naïve T cells but they are very good in activating memory T cells. The third type of antigen presenting cell is the B cell. These cells bind antigen via their surface immunoglobulin and ingest antigens by pinocytosis. Like macrophages these cells are not as effective as dendritic cells in presenting antigen to naïve T cells. B cells are very effective in presenting antigen to memory T cells, especially when

the antigen concentration is low because surface immunoglobulin on the B cells binds antigen with a high affinity.

PRESENTATION OF SUPERANTIGENS

Superantigens are antigens that can polyclonally activate T cells (see antigens) to produce large quantities of cytokines that can have pathological effects. These antigens must be presented to T cells in association with class II MHC molecules but the antigen does not need to be processed. Figure 5 compares how conventional antigens and superantigens are presented to T cells. In the case of a superantigen, the intact protein binds to class II MHC molecules and to one or more V_β regions of the TCR. The antigen is not bound to the peptide binding groove of the MHC molecule or to the antigen binding site of the TCR. Thus, any T cell that uses a particular V_β in its TCR will be activated by a superantigen, resulting in the activation of a large numbers of T cells. Each superantigen will bind to a different set of V_β regions.
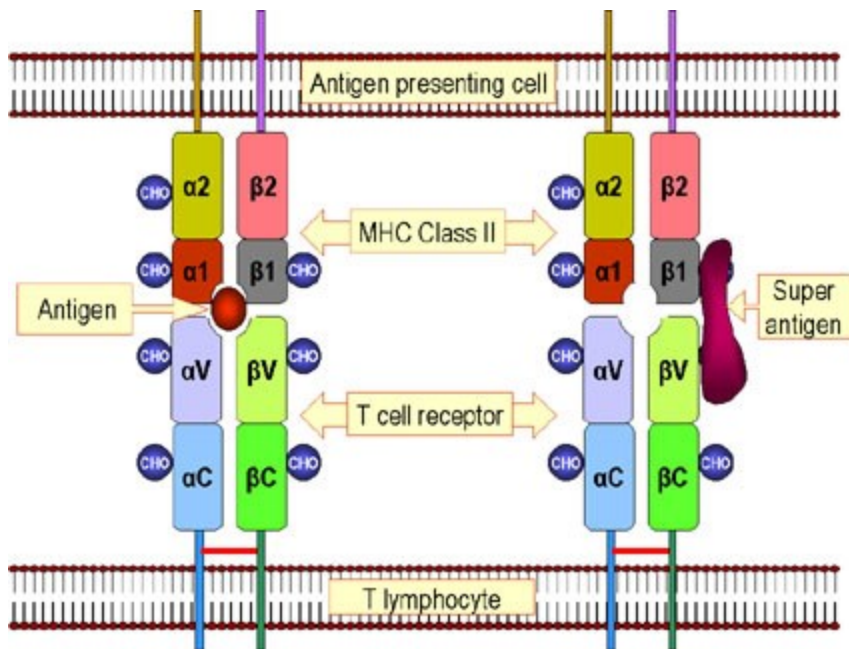
Figure 5 Differences between antigen and super antigen. Antigenic peptides are processed within the cell and presented on the cell surface in association with class II MHC molecules. They then trigger the T-cell receptor on a T lymphocyte. Superantigens are not processed but bind to the class II MHC protein and to the V beta chain of the T cell receptor. A given superantigen activates a distinct class of T cells that express a certain V beta chain.

Note: In the case of MHC II-TCR interaction with a normally processed peptide, recognition of the peptide on the MHC molecule requires V alpha, J alpha, V beta, D beta and J beta segments of the TCR. Such an interaction occurs at low frequency. In the case of MHC II-TCR interaction with an unprocessed superantigen, only a given V beta region is recognized. This clearly would occur at a much higher frequency

THYMIC EDUCATION

Both Th and Tc cells are self-MHC restricted. In addition, T cells do not normally recognize self antigens. How are self MHC restricted T cells generated and why are self reacting T cells not produced? Random VDJ rearrangements in T cells would be expected to generate some T cells that can recognize non-self MHC and some T cells that can recognize self antigens. It is the role of the thymus to ensure that the only T cells that get to the periphery are self-MHC restricted and unable to react with self antigen. Functional T cells in the periphery have to recognize foreign antigens associated with self MHC, because APC or target cells present foreign antigen associated with self MHC. However, an individual does not need functional T cells in the periphery that recognize antigen (self or foreign) associated with foreign MHC. An individual especially does not want functional T cells in the periphery that can recognize self antigens associated with self MHC because they could lead to damage of healthy, normal tissues.

As a result of random VDJ recombination events occurring in immature T cells within the thymus, TCRs of all specificities are produced. Processes in the thymus determine which TCR specificities are retained. There are two sequential steps shown in Figure 6. First, T cells with the ability to bind to self MHC molecules expressed by cortical thymic epithelial cells are retained. This is known as positive selection. Those that do not bind, undergo apoptosis. Thus, T cells having a TCR that recognizes self MHC survive. Next, T cells with the ability to bind to self MHC molecules associated with self molecules expressed by thymic epithelial cells, dendritic cells and macrophages are killed. This is known as negative selection. Those that do not bind are retained. As a result of these two steps, T cells having a TCR that recognizes self MHC and foreign antigen survive. Each T cell that survives positive and negative selection in the thymus and is released into the periphery retains its specific T cell receptor.
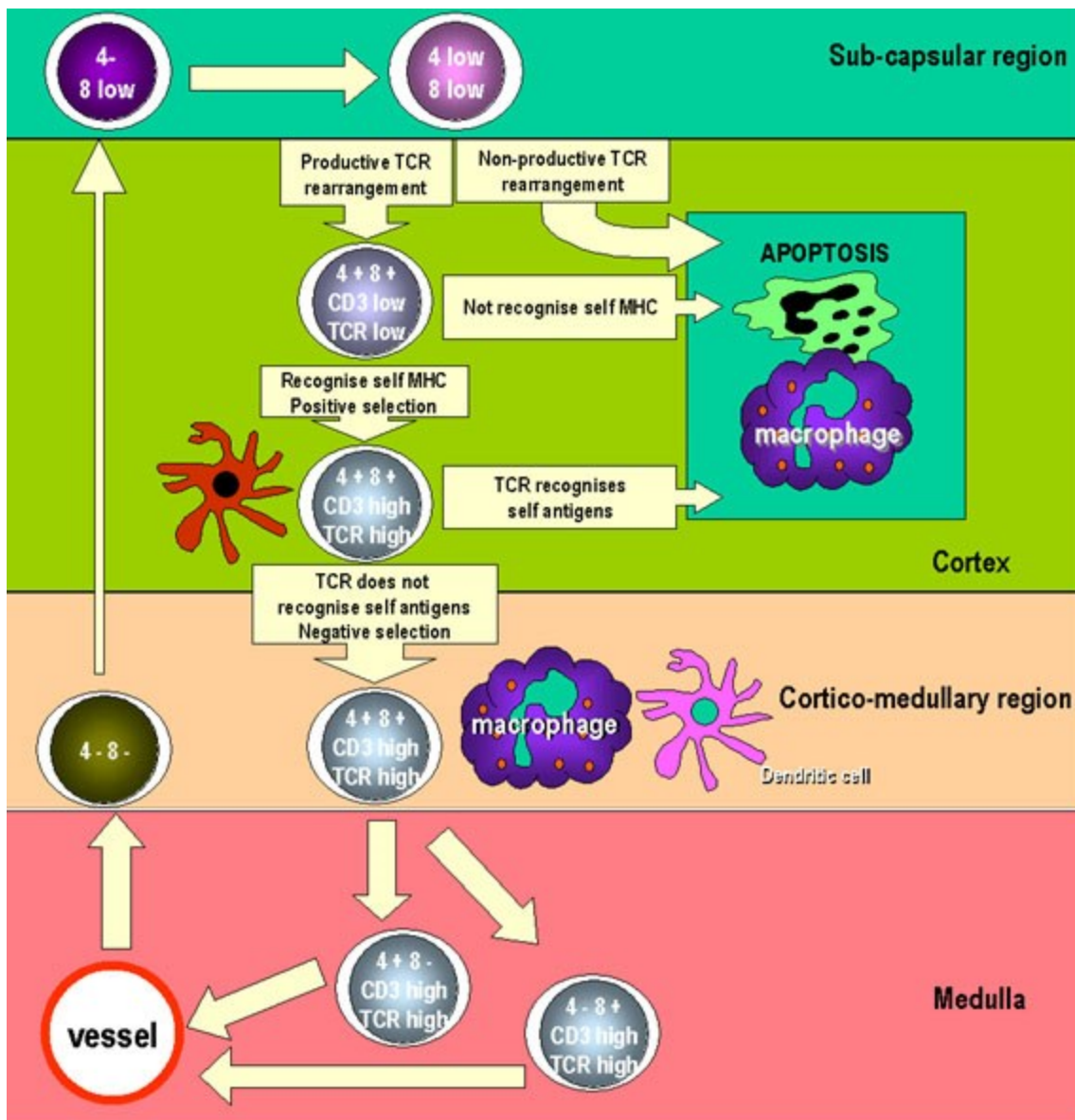
Figure 6 Prethymic T cells enter the thymus rudiment and proliferate as large lymphoblasts in the sub-capsular region of the thymus. The lymphoblasts replicate resulting in a pool of cells that differentiate. Here the cells become CD8 and CD4 positive but expression is low. TCR genes are also rearranged in these cells and the products may also be expressed on the cell surface at low levels. As the cells mature, they move into the cortex where they adhere to cortical epithelial cells which are long and branched, providing a large surface area to interact with other cells. TCRs on the surfaces of thymocytes interact with the MHC molecules on the epithelial cells leading to positive selection. The cells that are not selected are subject to apoptosis and are phagocytosed by macrophages. As the thymocytes migrate further into the cortex of the thymus, the expression of CD3, CD4, CD8 and TCR increases. TCRs with self-reactivity are deleted because of contact with autoantigens presented by dendritic cells and macrophages. This leads to negative selection. Cells that express CD4 *or* CD8 appear and migrate to the periphery by specialized vessels in the cortico-medullar region.

While positive and negative selection is occurring in the thymus the immature T cells are also expressing CD4 or CD8 antigens on their surface. Initially the pre-T cell that enters the thymus is CD4-CD8-. In the thymus it becomes CD4+CD8+ and as positive and negative selection proceeds a cell becomes either a CD4+ or CD8+ cell. The commitment to become either a CD4+ or CD8+ cells depends on which class of MHC molecule the cell encounters. If a CD4+CD8+ cell is presented with a class I molecule it will down regulate CD4 and become a CD8+ cell. If a cell is presented with a class II MHC molecule it will down regulate CD8 and become a CD4+ cell (Figure 7).
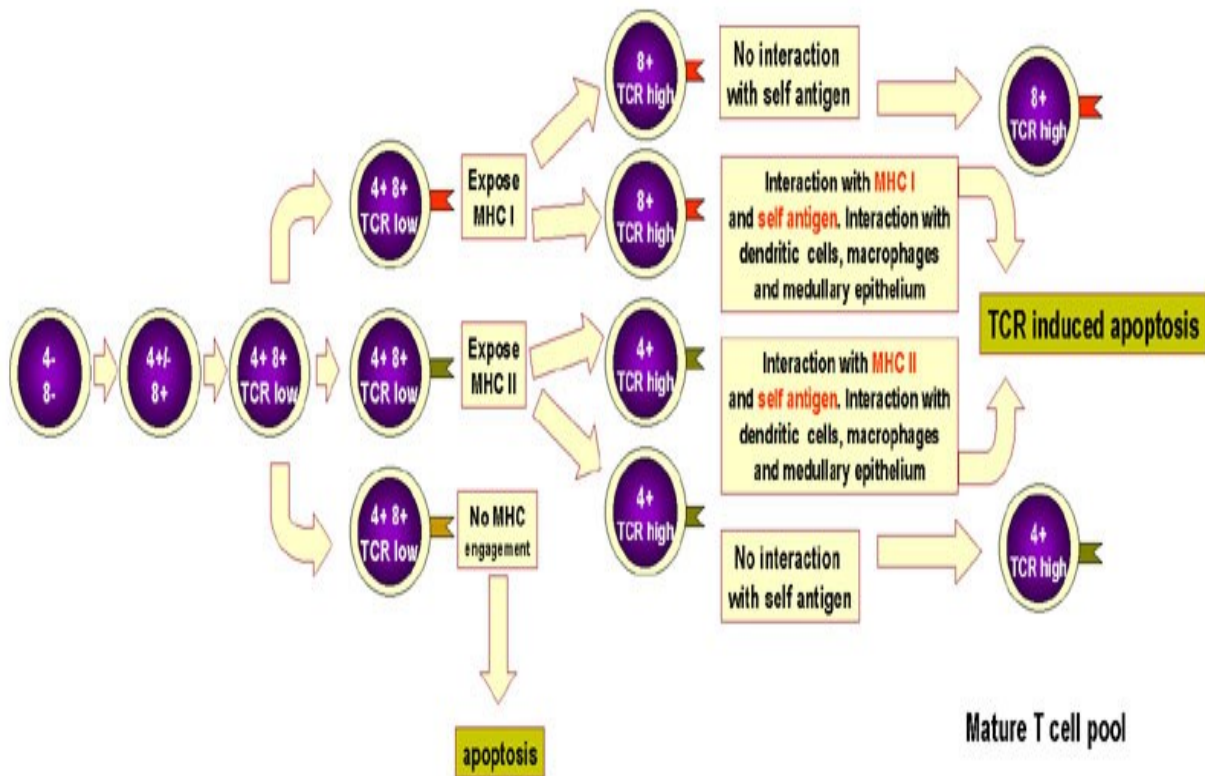
Figure 7 CD4- CD8- precursor thymocytes become double positive, CD4+CD8+ cells expressing low levels of the alpha and beta chains of the T cell receptor (TCR). Positive selection for interaction with self MHC-I or MHC-II molecules occurs in the cortical epithelium. The majority of the cells are unselected and undergo apoptosis. The cells that remain either interact with MHC-I and lose their CD4 antigen or interact with MHC-II and lose their CD8 antigen. Autoreactive cells are then removed as a result of their interaction with self antigen peptides that are presented by cells in the corticomedullary junction and the medulla of the thymus

NEGATIVE SELECTION IN THE PERIPHERY

Positive and negative selection in the thymus is not a 100% efficient process. In addition, not all self antigens may be expressed in the thymus. Thus some self reactive T cells may get to the periphery. Thus, there are additional mechanisms that are designed to eliminate self reactive T cells in the periphery. These will be discussed in the tolerance chapter.

B CELL SELECTION

Since B cells are not MHC-restricted there is no need for positive selection of B cells. However, negative selection (i.e., elimination of self-reactive clones) of B cells is required. This occurs during B cell development in the bone marrow. However, negative selection of B cells is not a critical as for T cells since, in most instances, B cells require T cell help in order to become activated. Thus, if a self reactive B cell does get to the periphery it will not be activated due to lack of T cell help.

CELL-MEDIATED IMMUNITY:
Cell-cell interactions in specific immune responses

CENTRAL ROLE OF TH CELLS IN IMMUNE RESPONSES

As depicted in Figure 1, after Th cells recognize specific antigen presented by an antigen-presenting cell (APC), they can initiate several key immune processes. These include:

- Selection of appropriate effector mechanisms (e.g., B cell activation or Tc generation);

- Induction of proliferation of appropriate effector cells

- Enhancement of the functional activities of other cells (e.g., granulocytes, macrophages, NK cells).

There are four subpopulations of Th cells: Th0, Th1, Th2 and Th17 cells. When naïve Th0 cells encounter antigen in secondary lymphoid tissues, they are capable of differentiating into inflammatory Th1 cells, helper Th2 cells or pathogenic T17 cells, which are distinguished by the cytokines they produce (Figure 2). Whether a Th0 cells becomes a Th1, a Th2 or a T17 cell depends upon the cytokines in the environment, which is influenced by antigen. For example some antigens stimulate IL-4 production which favors the generation of Th2 cells while other antigens stimulate IL-12 production, which favors the generation of Th1 cells. Th1, Th2 and Th17 cells affect different cells and influence the type of an immune response, as shown in Figure 3 for Th1 and Th2 cells.

Cytokines produced by Th1 cells activate macrophages and participate in the generation of cytoxic lymphocytes (CTL), resulting in a cell-mediated immune response. In contrast cytokines produced by Th2 cells help to activate B cells, resulting in antibody production.

In a relatively recent discovery, Th17 cells (designated as such by their production of IL-17) differentiate (in humans) in response to IL-1, IL-6, and IL-23. TGF-β is important for Th17 differentiation in mice, but not in humans. IL-17 enhances the severity of some autoimmune diseases including multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis. Equally important, each subpopulation can exert inhibitory influences on the other. IFN-γ produced by Th1 cells inhibits proliferation of Th2 cells and differentiation of Th17 cells and IL-10 produced by Th2 cells inhibits production of IFN-γ by Th1 cells. In addition, although not shown, IL-4 inhibits production of Th1 cells and differentiation of Th17 cells. Thus, the immune response is directed to the type of response that is required to deal with the pathogen encountered – cell-mediated responses for intracellular pathogens or antibody responses for extracellular pathogens.
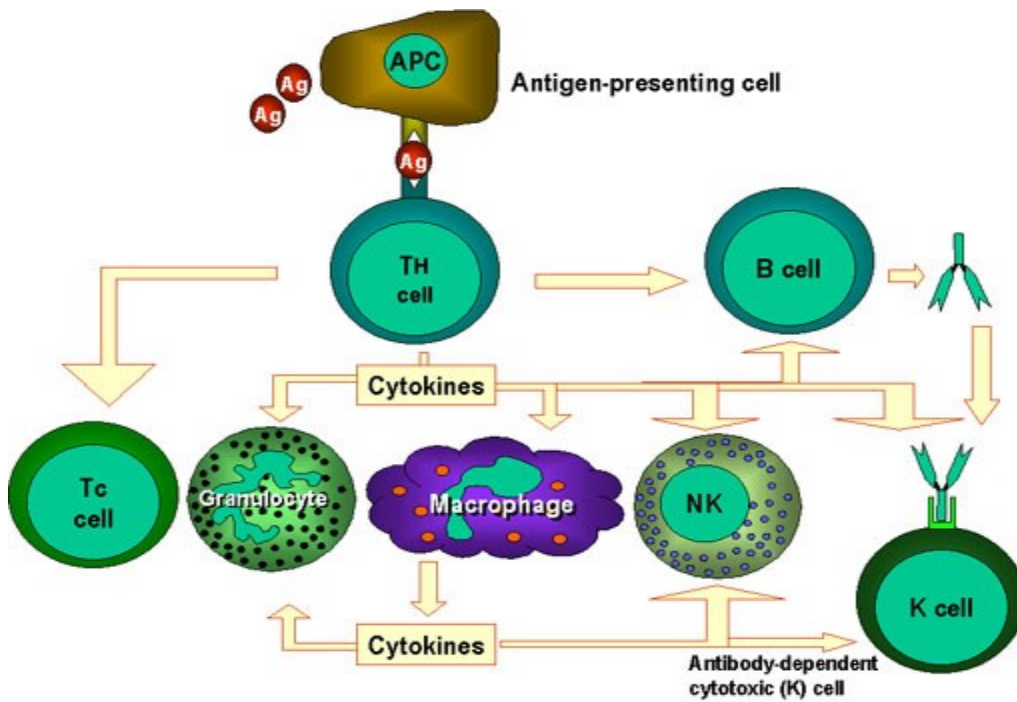
 Figure 1 Th cells are at the center of cell-mediated immunity. The antigen-presenting cells present antigen to the T helper (Th) cell. The Th cell recognises specific epitopes which are selected as target epitopes. Appropriate effector mechanisms are now determined. For example, Th cells help the B cells to make antibody and also activate other cells. The activation signals produced by Th cells are cytokines (lymphokines) but similar cytokines made by macrophages and other cells also participate in this process
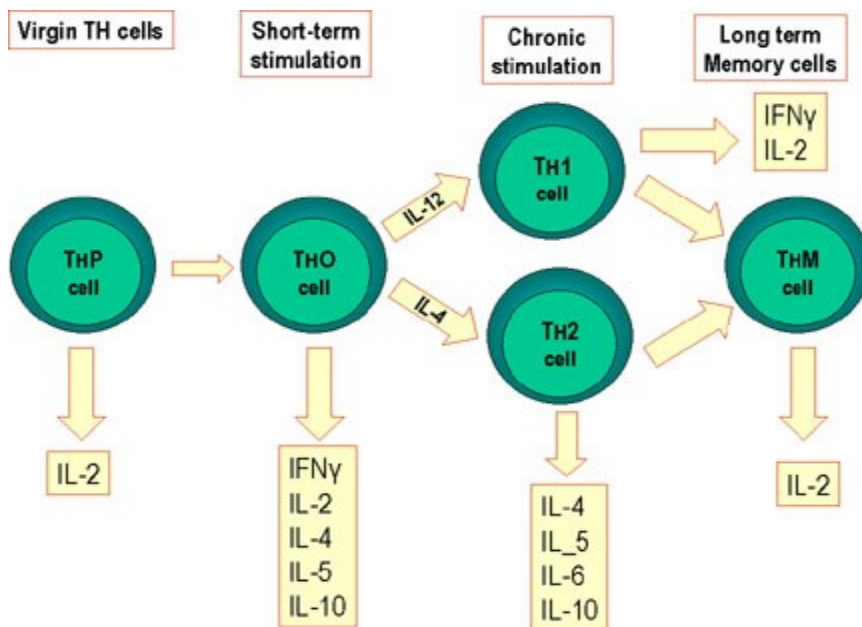
Figure 2 Differentiation of murine Th cells.   Mouse Th cells differentiate into subsets that synthesize different patterns of lymphokines. This also occurs in humans
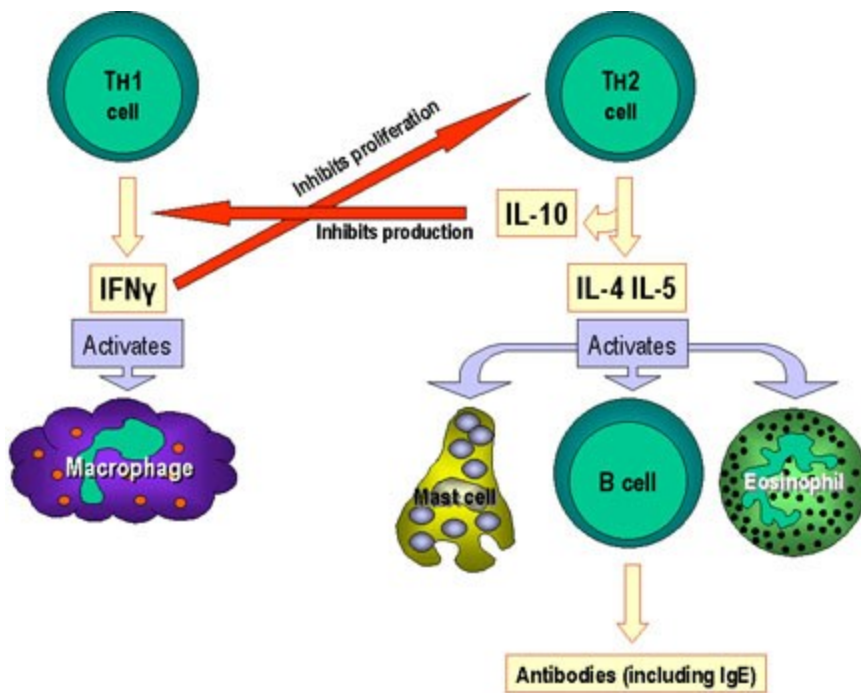
 Figure 3 Selection of effector mechanisms by Th1 and Th2 cells. In addition to determining various effector pathways by virtue of their lymphokine production, Th1 cells switch off Th2 cells and vice versa

CELL-CELL INTERACTIONS IN ANTIBODY RESPONSES TO EXOGENOUS T-DEPENDENT ANTIGENS

Hapten-carrier model

Historically one of the major findings in immunology was that both T cells and B cells were required for antibody production to a complex protein. A major contribution to our understanding of this process came from studies on the formation of anti-hapten antibodies. Studies with hapten-carrier conjugates established that:

- Th2 cells recognized the carrier determinants and B cells recognized haptenic determinants
- Interactions between hapten-specific B cells and carrier-specific Th cells was self MHC restricted
- B cells can function both in antigen recognition and in antigen presentation

B cells occupy a unique position in immune responses because they express immunoglobulin and class II MHC molecules on their cell surface. They therefore are capable of producing antibody having the same specificity as that expressed by their immunoglobulin receptor; in addition they can function as an antigen presenting cell. In terms of the hapten-carrier conjugate model, the mechanism is thought to be the following: The hapten is recognized by the immunoglobulin receptor, the hapten-carrier is brought into the B cell, processed, and peptide fragments of the carrier protein are presented to a helper T cell. Activation of the T cell results in the production of cytokines that enable the hapten-specific B cell to become activated to produce soluble anti-hapten antibodies. Figure 4

summarizes the B cell-T cell interactions that occur.
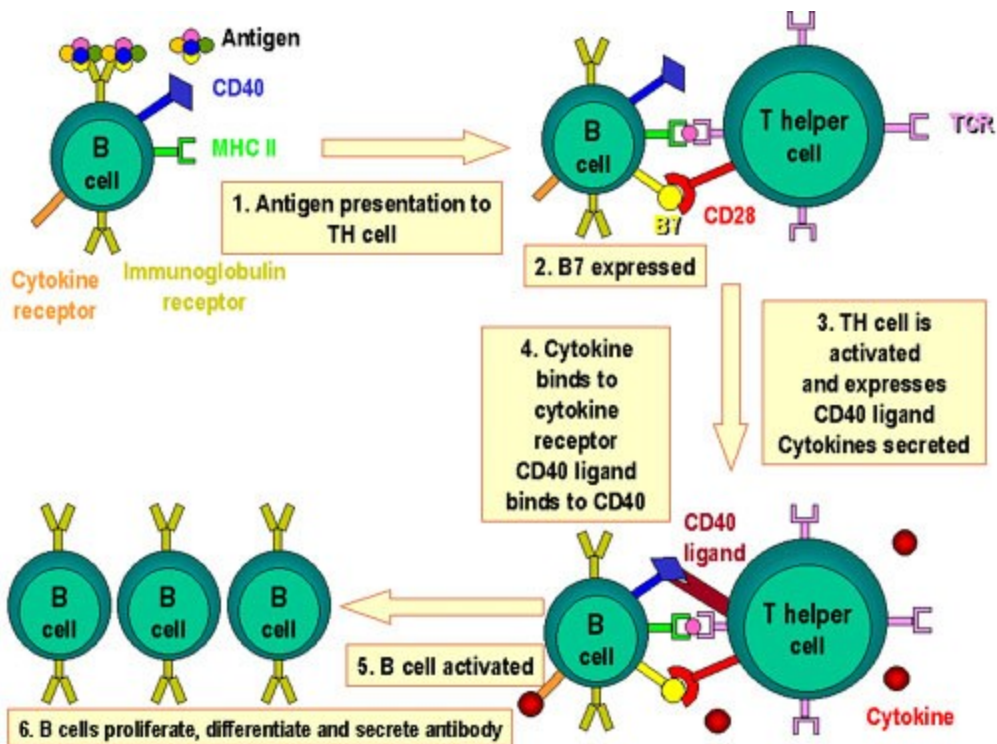
Figure 4

Molecules   involved   in   the   interactions   of   B   and   TH   cells

Antigen 🔵 is processed by B cell. Co-stimulators are expressed. The processed antigen peptide🔴 is presented in association with MHC class II antigens. The T cell recognizes the peptide along with the MHC antigen and the co-stimulators. The T cell expresses CD40 ligand. The latter binds to CD40 antigen on the B cell and the B cells divide and differentiate. Antibodies are produced by the B cell

Note that there are multiple signals delivered to the B cells in this model of Th2 cell-B cell interaction. As was the case for activation of T cells where the signal derived from the TCR recognition of a peptide-MHC molecule was by itself insufficient for T cell activation, so too for the B cell. Binding of an antigen to the immunoglobulin receptor delivers one signal to the B cell, but that is insufficient. Second signals delivered by co-stimulatory molecules are required; the most important of these is CD40L on the T cell that binds to CD40 on the B cell to initiate delivery of   a   second   signal.

Cell-cell interactions in the primary antibody response

B cells are not the best antigen presenting cell in a primary antibody response; dendritic cells or macrophages are more efficient. Nevertheless, with some minor modifications the hapten-carrier model of cell-cell interactions described above also applies to interactions in a primary antibody response (Figure 5). In a primary response the Th2 cell first encounters antigen presented by dendritic cells or macrophages. The "primed" Th2 cell can then interact with B cells that have encountered antigen and are presenting antigenic peptides in association with class II MHC molecules. The B cells still require two signals for activation – one signal is the binding of antigen to the surface immunoglobulin and the second signal comes from CD40/CD40 ligand engagement during Th2/B cell-

cell interaction. In addition, cytokines produced by the Th2 cells help B cells proliferate and differentiate into antibody secreting plasma cells.
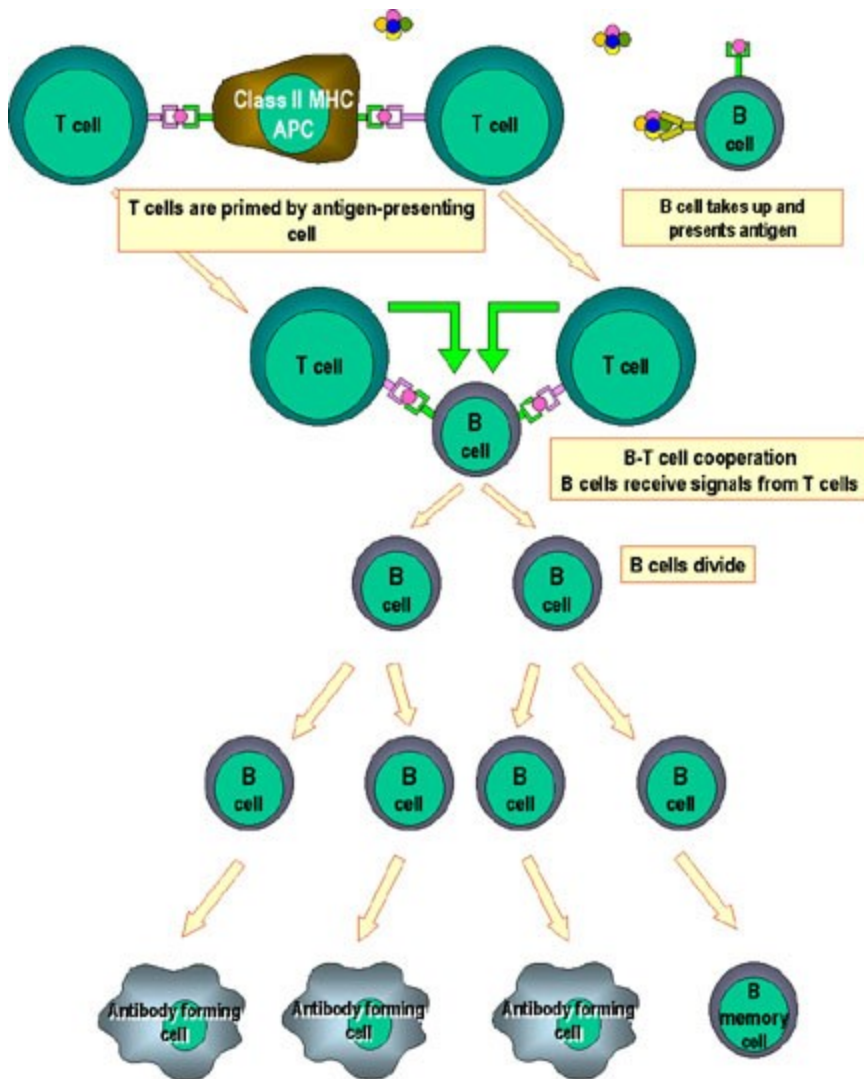
Figure 5 Cooperation of cells in the immune response Antigen-presenting cells (e.g. dendritic cells) present processed antigen to virgin T cells, thereby priming them. B cells also process the antigen and present it to the T cells. They then receive signals from the T cells that cause them to divide and differentiate. Some B cells form antibody-forming cells while a few form B memory cells

Cell-cell interactions in the secondary antibody responses

As a consequence of a primary response, many memory T and B cells are produced. Memory B cells have a high affinity immunoglobulin receptor (due to affinity maturation), which allows them to bind and present antigen at much lower concentrations than that required for macrophages or dendritic cells. In addition, memory T cells are more easily activated than naïve T cells. Thus, B/Th cell interactions are sufficient to generate secondary antibody responses. It is not necessary (although it can occur) to "prime" memory Th cells with antigen presented by dendritic cells or macrophages.

Cytokines and class switching

Cytokines produced by activated Th2 cells not only stimulate proliferation and differentiation of B cells, they also help regulate the class of antibody produced. Different cytokines influence the switch to different classes of antibodies with different effort functions. In this way the antibody response is tailored to suit the pathogen encountered (e.g. IgE antibodies for parasitic worm infections). Table 1 shows the effects of different cytokines on the class of antibody produced.

Cytokine	IgG1	IgG2a	IgG2b	IgG3	IgA	IgE	IgM
IL-4	Induce	Inhibit		Inhibit		Induce	Inhibit
IL-5					Augment production		
IFN-gamma	Inhibit	Induce		Induce		Inhibit	Inhibit
TGF-beta			Induce	Inhibit	Induce		Inhibit

Isotype regulation by murine T cell cytokines. Certain cytokines either induce (green) or inhibit (pink) the production of certain antibody isotypes. Inhibition mostly results from switch to the different isotype

Table 1

CELL-CELL INTERACTIONS IN ANTIBODY RESPONSES TO EXOGENOUS T-INDEPENDENT ANTIGENS

Antibody responses to T-independent antigens do not require cell-cell interactions. The polymeric nature of these antigens allows cross-linking of antigen receptors on B cells resulting in activation. No secondary responses, affinity maturation or class switching occurs. Responses to T-independent antigens are due to the activation of a subpopulation of B cells called CD5+ B cells (also called B1 cells), which distinguishes them from conventional B cells that are CD5- (also called B2 cells).

CD5+ (B1) cells

CD5+ cells are the first B cells to appear in ontogeny. They express surface IgM but little or no IgD and they produce primarily IgM antibodies from minimally somatically mutated germ line genes. Antibodies produced by these cells are of low affinity and are often polyreactive (bind multiple antigens). Most of the IgM in serum is derived from CD5+ B cells. CD5+ B cells do not give rise to memory cells. An important characteristic of these cells is that they are self-renewing, unlike conventional B cells which must be replaced from the bone marrow. CD5+ B cells are found in peripheral tissues and are the predominant B cell in the peritoneal cavity. B1 cells are a major defense against many bacterial pathogens that characteristically have polysaccharides in their cell walls. The importance of these cells in immunity is illustrated by the fact that many individuals with T cell defects are still able to resist many bacterial pathogens.

CELL-CELL INTERACTIONS IN CELL-MEDIATED IMMUNITY - GENERATION OF TC CELLS IN RESPONSE TO ENDOGENOUS ANTIGENS IN THE CYTOSOL

Cytotoxic T lymphocytes are not fully mature when they exit the thymus. They have a functional TCR that recognizes antigen, but they cannot lyse a target cell. They must differentiate into fully functional effector Tc cells. Cytotoxic cells differentiate from a "pre-CTL" in response to two signals:

- Specific antigen in the context of class I MHC, on a stimulator cell
- Cytokines produced by Th1 cells, especially IL-2, and IFN-gamma. This is shown in Figure 6
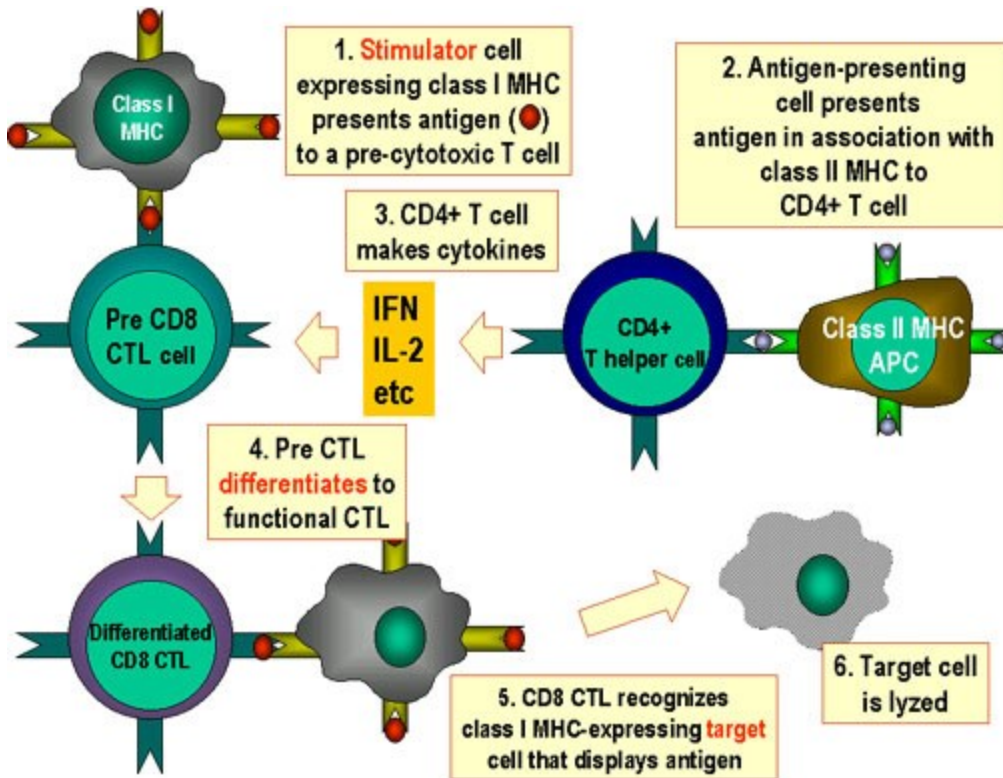
Figure 6 CTL cells must differentiate in response to antigen. In order to differentiate into functional cytotoxic T lymphocytes, pre-CD8+ CTLs must receive two different signals. First, they must recognize antigen presented by MHC-I expressing cells (the stimulator cells) and, second, they must be stimulated by cytokines. IL-2, interferon-gamma and others are made by CD4+ helper T cells as a result of their interaction with class II MHC-expressing antigen presenting cells. As a result of these two signals, the pre-CTL differentiates into an active CTL that can then lyse target cells that bear the same antigen. Adapted from Abbas, et. al. *Cellular and Molecular Immunology*. 3rd Ed., p. 292.

Features of CTL-mediated lysis

- CTL killing is antigen-specific. To be killed by a CTL, the target cell must bear the same class I MHC-associated antigen that triggered pre-CTL differentiation.
- CTL killing requires cell contact. CTL are triggered to kill when they recognize the target antigen associated with a cell surface MHC molecule. Adjacent cells lacking the appropriate target MHC-antigen are not affected.
- CTLs are not injured when they lyse target cells. Each CTL is capable of killing sequentially numerous target cells.

Mechanisms of CTL-mediated killing

CTLs utilize several mechanisms to kill target cells, some of which require direct cell-cell contact and others that result from the production of certain cytokines. In all cases death of the target cells is a result of apoptosis.

- Fas- and TNF-mediated killing (Figure 7) Once generated CTLs express Fas ligand on their surface, which binds to Fas receptors on target cells. In addition, TNF-α secreted by CTLs can bind to TNF receptors on target cells. The Fas and TNF receptors are a closely related family of receptors, which when they encounter their ligands, for trimers of the receptors. These receptors also contain death domains in the cytoplasmic portion of the receptor, which after trimerization can activate caspases that induce apoptosis in the target cell.
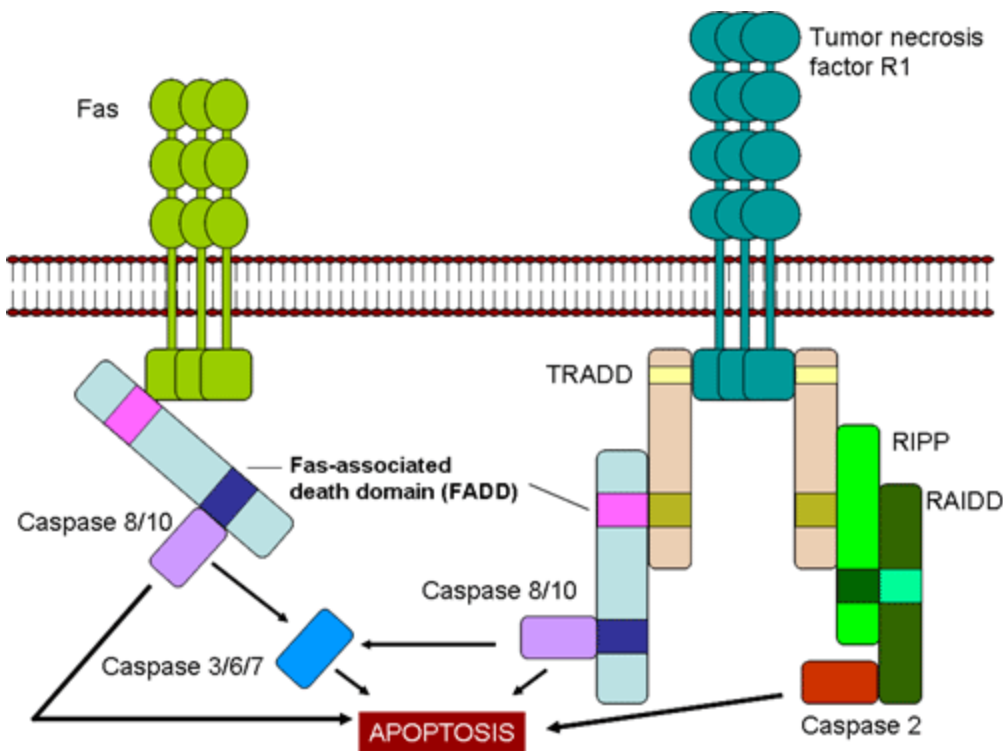
Figure 7 Fas- and TNF-mediated killing of target cells by CTLs

- Granule-mediated killing (Figure 8) Fully differentiated CTLs have numerous granules that contain perforin and granzymes. Upon contact with target cells, perforin is released and it polymerizes to form channels in the target cell membrane. Granzymes, which are serine proteases, enter the target cell through the channels and activate caspases and nucleases in the target cell resulting in apoptosis.

Figure 8 Mechanisms for the CTL destruction of target cells
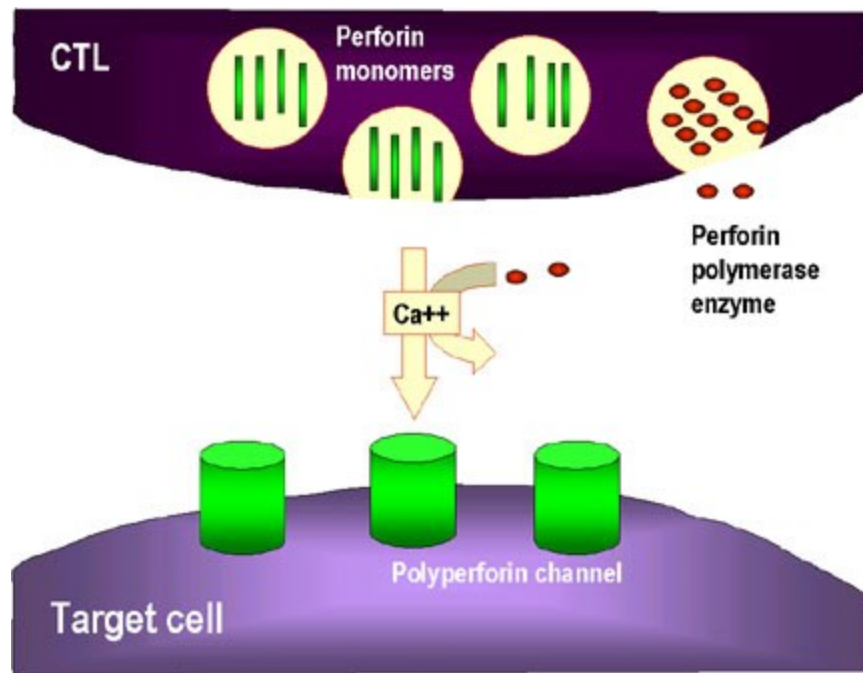
1. CTL degranulates and releases perforin monomers into the surroundings. Enzymes that polymerize perforin to form polyperforin channels are also released and these along with Ca++ catalyze channel formation in the membrane of the target cell
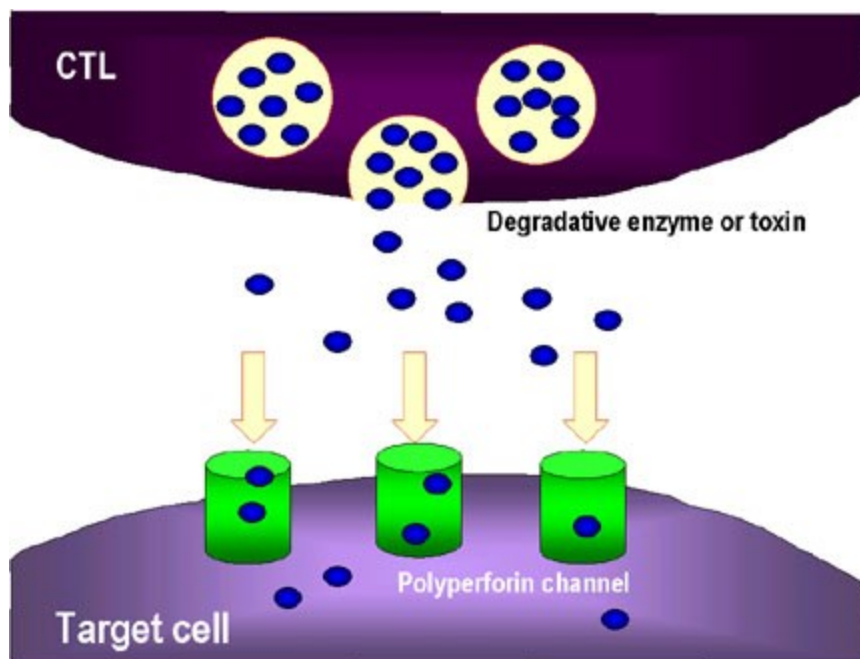
2. The CTL may also release degradative enzymes and toxins which travel through the perforin chanels and damage the target cell
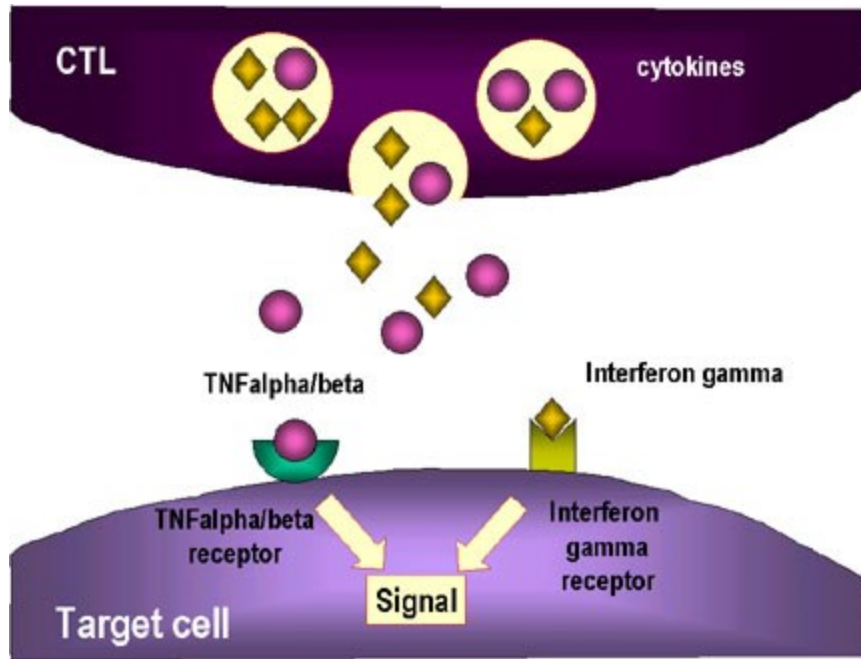
3. Cytokines such as TNF alpha and TNF beta are released from the CTL or nearby macrophages. Interferon gamma may also be released from the CTLs or from other nearby lymphoid cells. These bind to receptors on the target cell and trigger apoptosis

CELL-CELL INTERACTIONS IN CELL-MEDIATED IMMUNITY - ACTIVATION OF MACROPHAGES IN RESPONSE TO ENDOGENOUS ANTIGENS IN VESICLES

Macrophages play a central role in the immune system. As shown in Figure 9, macrophages are involved in:

- Initial defense as part of the innate immune system
- Antigen presentation to Th cells
- Various effector functions (*e.g.,* cytokine production, bactericidal and tumoricidal activities).

Indeed macrophages play an important role not only in immunity but also in reorganization of tissues. However, because of their potent activities, macrophage can also do damage to tissues. Table 2 summarizes the many functions of macrophages in immunity and inflammation.
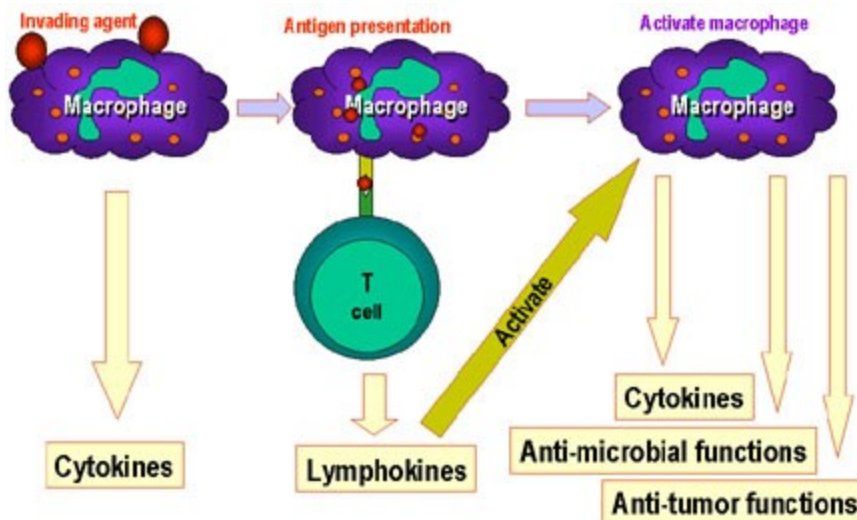
Figure 9 Macrophages play a central role in the immune system. before T and B-cell immunity starts. Macrophages process antigens and present them to T cells which then release lymphokines which activate the macrophages to perform various other functions including the production of more cytokines

Inflammation - Fever	Damage to tissues
Production of: IL-6, TNF alpha, IL-1 – act as pyrogen	Hydrolases Hydrogen peroxide production Complement C3a TNF alpha production

Immunity
 	
Selection of lymphocytes to be activated:
IL-12 results in Th1 activation
IL-10 results in Th2 activation
 	
Activation of lymphocytes:
Production of IL-1
Processing and presentation of antigen
	Antimicrobial action
	
	Oxygen –dependent production of:
	   hydrogen peroxide
	    superoxide
	   hydroxyl radical
	   hypochlorous acid
	
	Oxygen-independent production of:
	  acid hydrolases
	   cationic proteins
	   lysozyme

Reorganization of tissues **Secretion of a variety of factors:** Degradative enzymes (elastase, hyaluronidase,collagenase) Fibroblast stimulation factors Stimulation of angiogenesis	**Anti-tumor activity** Toxic factors Hydrogen peroxide Complement C3a Proteases Arginase Nitric oxide TNF alpha

Table 2

Many of these macrophage functions can only be performed by activated macrophages. Macrophage activation can be defined as quantitative alterations in the expression of various gene products that enable the activated macrophage to perform some function that cannot be performed by the resting macrophage.

Macrophage activation is an important function of Th1 cells. When Th1 cells get activated by an APC such as a macrophage, they releases IFN-γ, which is one of two signals required to activate a macrophage. Lipopolysaccharide (LPS) from bacteria or TNF-α produced by macrophages exposed to bacterial products deliver the second signal (Figure 10).

Effector mechanisms employed by macrophages include production of:

- TNF-α, which can induce apoptosis
- Nitric oxide and other reactive nitrogen intermediates
- Reactive oxygen intermediates
- Cationic proteins and hydrolytic enzymes

Macrophage activation by Th1 cells is very important in protection against many different pathogens For example, *Pneumocystis carinii*, an extracellular pathogen, is controlled in normal individuals by activated macrophages; it is, however, a common cause of death in AIDS patients because they are deficient in Th1 cells. Similarly, *Mycobacterium tuberculosis*, an intracellular pathogen that resides in vesicles, is not efficiently killed by macrophages unless they are activated; hence this infection is a problem in AIDS patients.
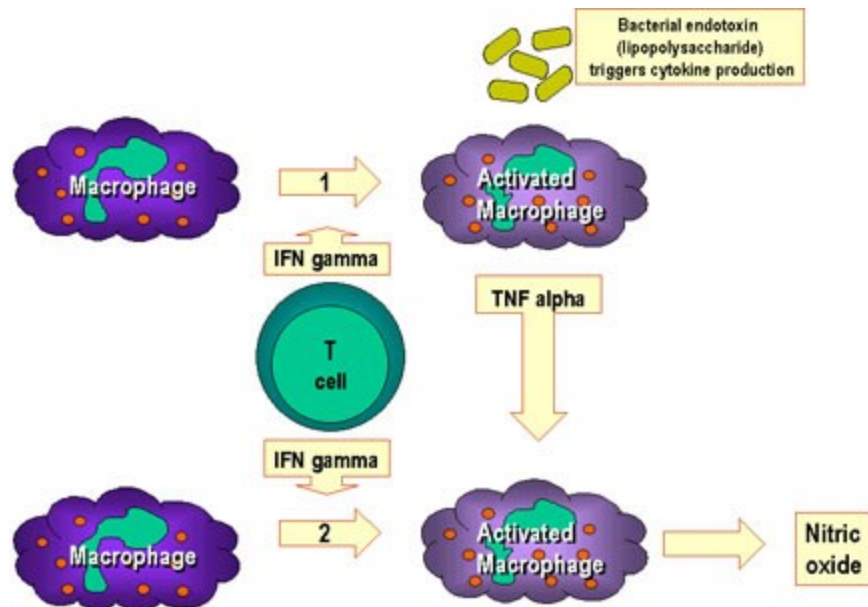
Figure 10 Macrophage activation results from the interaction of multiple cytokines and other factors.
In pathway 1, TNF-alpha is released from macrophages as a result of activation by interferon gamma and interaction with bacterial components that trigger cytokine production. An example of such a triggering component is bacterial lipopolysaccharide. The TNF-alpha from pathway 1 leads to the production of nitric oxide by the interferon-activated macrophage in pathway 2.

CELL-CELL INTERACTIONS IN CELL-MEDIATED IMMUNITY - ACTIVATION OF NK CELLS

Cytokines produced by activated Th1 cells, particularly Il-2 and IFN-γ, also activate NK cells to become lymphokine activated killer cells (LAK cells). LAK cells are able to kill virus infected and tumor cells in a non-MHC-restricted manner. Indeed, susceptibility of target cells to killing by NK and LAK cells is inversely proportional to the expression of MHC class I molecules (see lecture on innate immunity). The effector mechanisms used by NK and LAK cells to kill target cells is similar to those used by CTLs (*e.g.*, perforin and granzymes). NK and LAK cells are also able to kill antibody coated target cells by ADCC.

CYTOKINES AND IMMUNOREGULATION

OVERVIEW

Cytokines are a diverse group of non-antibody proteins that act as mediators between cells. They were initially identified as products of immune cells that act as mediators and regulators of immune processes but many cytokines are now known to be produced by cells other than immune cells and they can have effects on non-immune cells as well. Cytokines are currently being used clinically as biological response modifiers for the treatment of various disorders. The term cytokine is a general term used to describe a large group of proteins but there are other terms that are commonly used to describe particular kinds of cytokines. These include:

- Monokines, cytokines produced by mononuclear phagocytic cells
- Lymphokines, cytokines produced by activated lymphocytes, especially Th cells
- Interleukins, cytokines that act as mediators between leukocytes
- Chemokines, small cytokines primarily responsible for leucocyte migration

Cytokines function as part of a larger inter-related system of proteins and signaling cascades, the cytokine network. These are complex interactions in which different cells can respond differently to the same cytokine depending upon other signals received by the cell. Cytokine signaling is very flexible and can induce both protective and damaging responses. One cytokine often influences the synthesis of other cytokines. They can produce cascades, or enhance or suppress production of other cytokines. In addition, they can often influence the action of other cytokines. The effects can be: antagonistic, additive, or synergistic.

Cytokines are not typically stored as preformed proteins. Rather their synthesis is initiated by gene transcription and their mRNAs are short lived. They are produced as needed in immune responses. Genes encoding cytokines can produce variants through alternative splicing to yield proteins with slightly different but biologically significant bioactivities.

Many individual cytokines are produced by many cell types involved in both the innate and adaptive immune response. Individual cytokines also act on many cell types (*i.e.,* they are pleotropic) and in many cases cytokines have similar actions (*i.e.,* they are redundant). Redundancy is due to the nature of the cytokine receptors.

Receptors for cytokines are heterodimers (sometimes heterotrimers) that can be grouped into families based on common structural features; one subunit is common to all members of a given family. Some examples are shown in Figure 1.
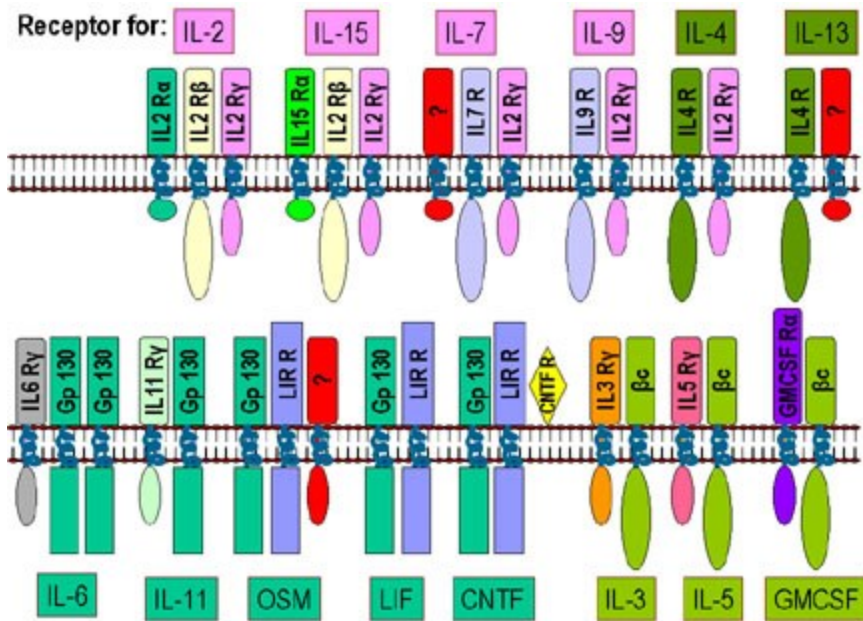
Figure 1A Receptors for various cytokines showing common subunits

- Type 1 cytokine receptors (IL-2R family) are the largest family of cytokine receptors. This family is divided into three subsets based on common components: IL2Rγ, common β, and gp130 (Figure 1A). These receptors lack intrinsic protein tyrosine kinase activity. Ligand (cytokine) binding leads to receptor dimerization and initiation of intracellular signaling.
- Type 2 cytokine receptors (IFNR family) have conserved cysteines in the extracellular domains of the subunits. The extracellular domains also have tandem immunoglobulin-like domains characteristic of this cytokine receptor family. These receptor subunits also have intrinsic tyrosine kinase activity (denoted by the * in Figure 1B).
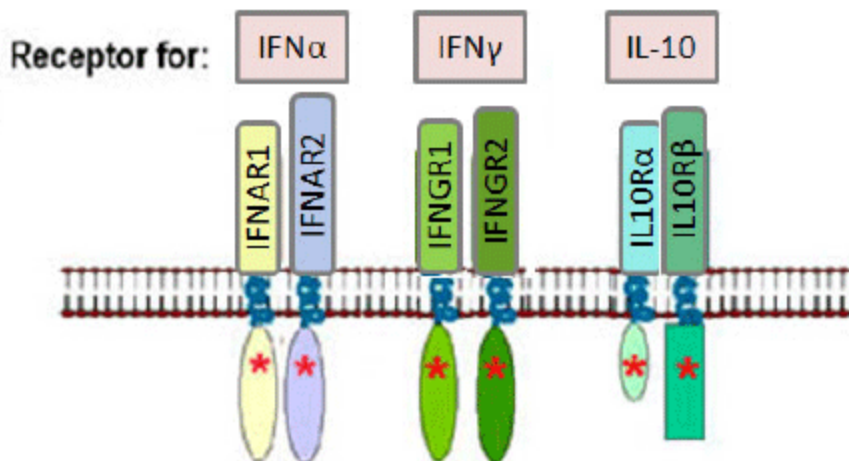
Figure 1B Interferon receptor family

Chemokine receptors all have seven transmembrane segments linked to GTP-binding proteins. They are selectively expressed on particular lymphocyte populations and are named based on the family

of chemokines to which they bind; CCR (the CC receptor) binds CC chemokines as its ligand while the CXCR binds CXC chemokines as its ligand (chemokines naming convention will be discussed below).

Since the subunit common to all members of the family functions in binding cytokine and in signal transduction, a receptor for one cytokine can often respond to another cytokine in the same family. Thus, an individual lacking IL-2, for example, is not adversely affected because other cytokines (IL-15, IL-7, IL-9, etc.) assume its function. Similarly, a mutation in a cytokine receptor subunit other than the one in common often has little effect. On the other hand, a mutation in the common subunit has profound effects. For example, a mutation in the gene for the IL-2R gamma subunit causes human X-linked severe combined immunodeficiency (XSCID) characterized by a complete or nearly complete T and B cell defects.

Cytokines bind to specific receptors on target cells with high affinity and the cells that respond to a cytokine are either:

- The same cell that secreted cytokine (autocrine)
- A nearby cell (paracrine)
- A distant cell reached through the circulation (endocrine). Cellular responses to cytokines are generally slow (hours) because they require new mRNA and protein synthesis.
- **CATEGORIES OF CYTOKINES**

  Cytokines can be grouped into different categories based on their functions or their source but it is important to remember that because they can be produced by many different cells and act on many different cells, any attempt to categorize them will be subject to limitations.

- **Mediators of natural immunity (innate immune response)**
  Cytokines that play a major role in the innate immune system include: TNF-α, IL-1, IL-10, IL-12, type I interferons (IFN-α and IFN-β), IFN-γ, and chemokines.

- **TNF-α**
  Tumor necrosis factor alpha is produced by activated macrophages is response to microbes, especially the lipopolysaccharide (LPS) of Gram negative bacteria. It is an important mediator of acute inflammation. It mediates the recruitment of neutrophils and macrophages to sites of infection by stimulating endothelial cells to produce adhesion molecules and by producing chemokines which are chemotactic cytokines. TNF- α also acts on the hypothalamus to produce fever and it promotes the production of acute phase proteins.

  IL-1
  Interleukin 1 is another inflammatory cytokine produced by activated macrophages. Its effects are similar to that of TNF-α and it also helps to activate T cells.

  IL-10
  Interleukin 10 is produced by activated macrophages and Th2 cells. It is predominantly an

inhibitory cytokine. It inhibits production of IFN-γ by Th1 cells, which shifts immune responses toward a Th2 type. It also inhibits cytokine production by activated macrophages and the expression of class II MHC and co-stimulatory molecules on macrophages, resulting in a dampening of immune responses.

IL-12
Interleukin 12 is produced by activated macrophages and dendritic cells. It stimulates the production of IFN-γ and induces the differentiation of Th cells to become Th1 cells. In addition, it enhances the cytolytic functions of Tc and NK cells.

Type I interferons

Type I interferons (IFN-α and IFN-β) are produced by many cell types and they function to inhibit viral replication in cells. They also increase expression of class I MHC molecules on cells making them more susceptible to killing by CTLs. Type I interferons also activate NK cells.

INF-γ
Interferon gamma is an important cytokine produced by primarily by Th1 cells, although it can also be produced by Tc and NK cells to a lesser extent. It has numerous functions in both the innate and adaptive immune systems as depicted in Figure 2.
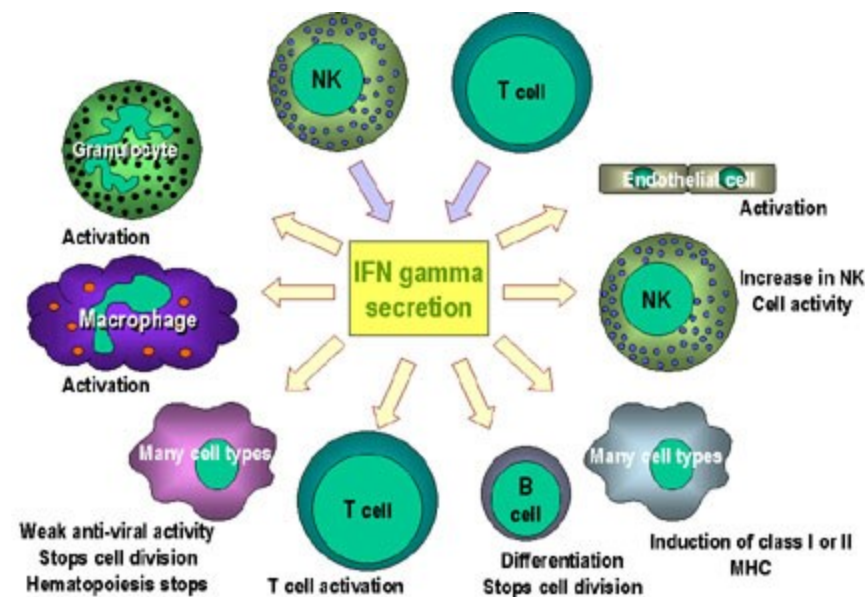
Figure 2 Immunoregulatory actions of interferon gamma on the immune system. Note the anti-proliferation and anti-ral activities are weaker than those of IFN alpha and IFN beta. IFN gamma is the most potent of the three at macrophage activation and in inducing class II MHC expression

Chemokines
Chemokines are chemotactic cytokines produced by many kinds of leukocytes and other cell types. They represent a large family of molecules that function to recruit leukocytes to sites of infection and play a role in lymphocyte trafficking by determining which cells will cross the

epithelium and where they are directed to go. There are four families of chemokines based on spacing of conserved cysteine. Two examples are the α-chemokines which have a CXC structure (two cysteines with a different amino acid in between) and the β-chemokines which have a CC structure (two neighboring cysteines). Individual chemokines (within the same family) often bind more than one receptor.

- ## Mediators of adaptive immunity

  Cytokines that play a major role in the adaptive immune system include: IL-2, IL-4, IL-5, TGF-β, IL-10 and IFN-γ.

- ## IL-2
  Interleukin 2 is produced by Th cells, although it can also be produced by Tc cells to a lesser extent. It is the major growth factor for T cells. It also promotes the growth of B cells and can activate NK cells and monocytes as depicted in Figure 3.
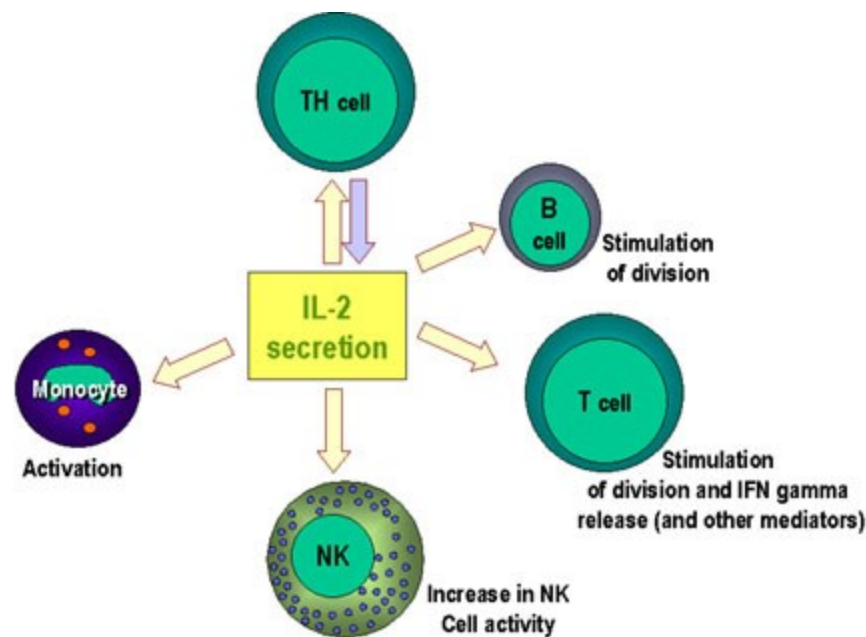
Figure   3
Immuno-regulatory actions of interleukin-2

- IL-2 acts on T cells in an autocrine fashion. Activation of T cells results in expression of IL-2R and the production of IL-2. The IL-2 binds to the IL-R and promotes cell division. When the T cells are no longer being stimulated by antigen, the IL-2R will eventually decay and the proliferative phase ends Figure 4.
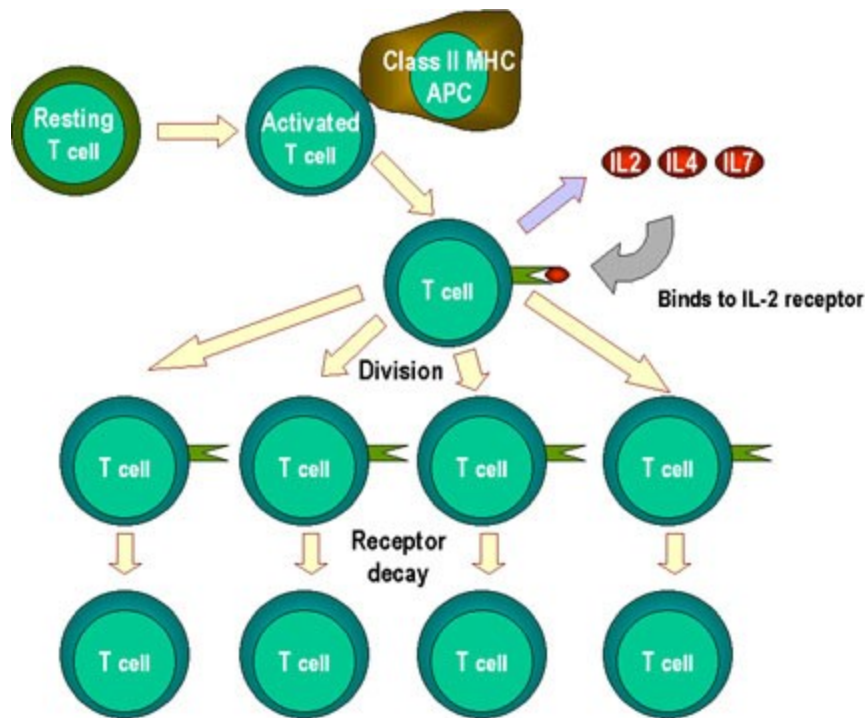
Figure 4 T cell proliferation and cytokines. When T cells are resting, they do not make cytokines such as interleukins 2, 4 or 7. Nor do they express large amounts of their receptors. There are no IL-2 receptors. Activation of T cells results in the formation of high affinity IL-2 receptors and induction of the synthesis and secretion of IL-2 and Il-4. These bind to their receptors and the cells proliferate. When stimulation by interleukins declines (e.g. when antigen stimulation declines), receptors decay and the proliferative phase is at an end. Note: stimulation by the cytokines can be paracrine or autocrine

- **IL-4**
  Interleukin 4 is produced by macrophages and Th2 cells. It stimulates the development of Th2 cells from naïve Th cells and it promotes the growth of differentiated Th2 cells resulting in the production of an antibody response. It also stimulates Ig class switching to the IgE isotype.

  IL-5
  Interleukin 5 is produced by Th2 cells and it functions to promote the growth and differentiation of B cells and eosinophiles. It also activates mature eosinophiles.

  TGF-β
  Transforming growth factor beta is produced by T cells and many other cell types. It is primarily an inhibitory cytokine. It inhibits the proliferation of T cells and the activation of macrophages. It also acts on PMNs and endothelial cells to block the effects of pro-inflammatory cytokines.
- **Stimulators of hematopoesis**
- Some cytokines stimulate the differentiation of hematopoetic cells. These include GM-CSF which promotes the differentiation of bone marrow progenitors, M-CSF, which promotes growth and differentiation of progenitors into monocytes and macrophages and G-CSF, which promotes production of PMNs.
- **Interleukin 17**

IL-17 is proinflammatory cytokine approximately 150 amino acids long. The IL-17 family includes six members which share sequence homology but differential tissue expression. IL-17 is produced by Th17 cells and its over expression has been associated with autoimmune disease including multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease.

CYTOKINE NETWORKS

Although the focus of most research has been on the production and action of cytokines on cells of the immune system important to remember that many of them have effects on other cells and organ systems. In fact, the cytokine network is complex and represents a series of overlapping and inter-related connections amongst cytokines. Within this network, one cytokine may induce or suppress its own synthesis, induce or suppress the synthesis of other cytokines, induce or suppress synthesis cytokine receptors (both its own and other cytokine receptors), and antagonize or synergize with other cytokines.

A diagram showing some of the interactions in the cytokine network is presented in Figure 5a, b and c.
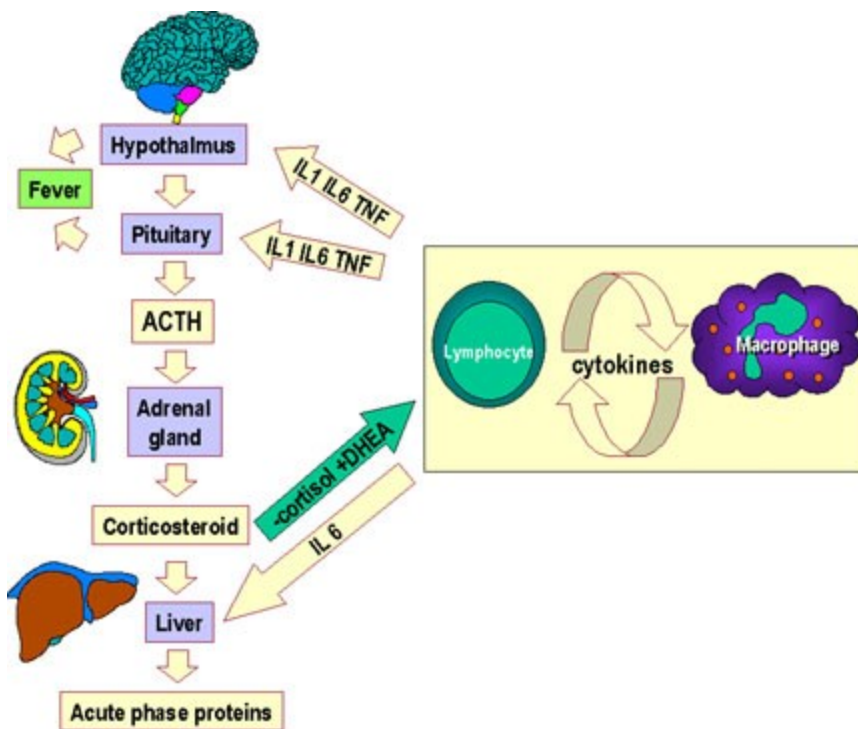
Figure 5a Cytokine network. Communication between lymphocytes and macrophages and the hypothalamus, adrenals and the liver
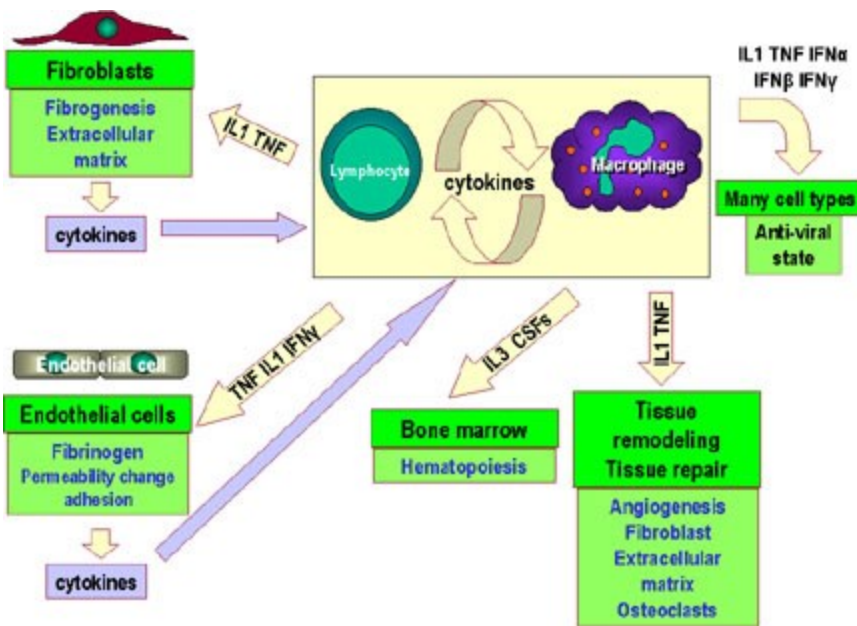
Figure 5b Cytokine network. Communication between lymphocytes and macrophages and other cells and tissues
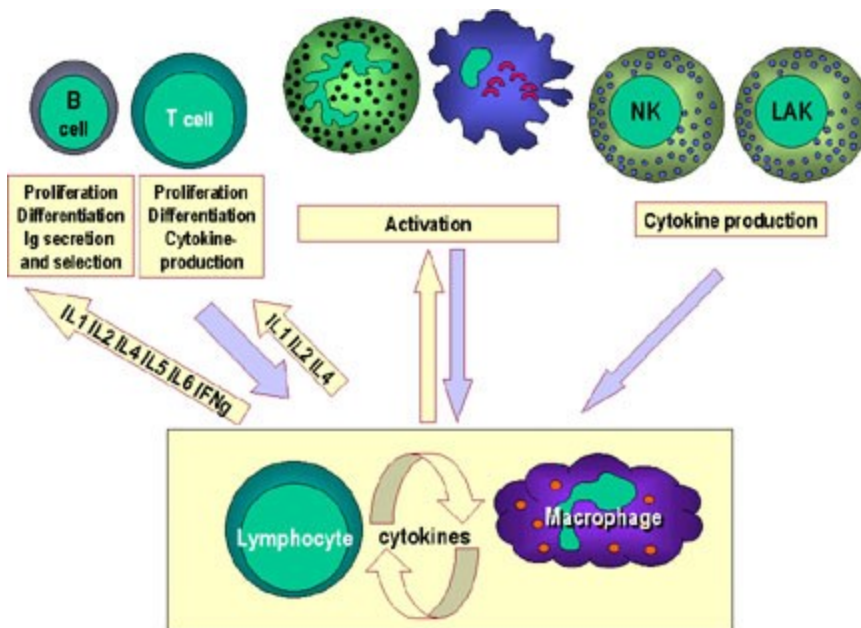
Figure 5c Cytokine network. Communication between lymphocytes and macrophages and other components of the immune system

IMMUNOREGULATION

The magnitude of an immune response is determined by the balance between antigen-driven activation of lymphocytes and negative regulatory influences that prevent or dampen the response. Regulatory mechanisms can act at the recognition, activation or effector phases of an immune response. Examples of regulation that have already been discussed include:

- Recognition of antigen in the absence of co-stimulation resulting in anergy
- Recognition of antigen with CTLA-4 engagement of B7 resulting in down regulation of T cell activation
- Cytokines with stimulatory or inhibitory activities on immune cells
- Idiotype/anti-idiotype interactions leading to stimulation or inhibition of immune responses
- Dose and route of antigen exposure can induce differential Th responses which in one case can protect and in another can tolerize.

In addition to these there are other ways in which immune responses can be regulated.

Regulation by antibody (Figure 6)

Soluble antibody can compete with antigen receptors on B cells and block or prevent B cell activation. In addition antigen antibody complexes can bind to Fc receptors on B cells, sending an inhibitory signal to B cells. In this case the regulation is occurring at the recognition level.
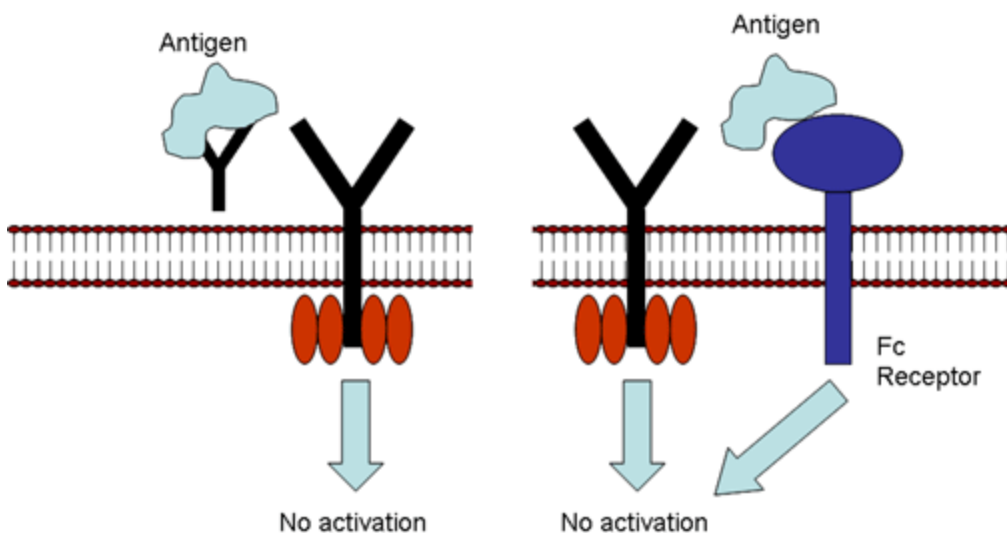
Figure 6 Regulation by antibody. Soluble antibody competes with cell surface Ig for binding to antigen (left) or soluble antibody binds to Fc receptor resulting in an inhibitory signal (right).

In addition, antigen-antibody complexes can bind to Fc receptors on B cells, sending an inhibitory signal to B cells. Here regulation occurs at the activation level.

Antibody can also regulate activation (enhance) by maintaining a source of antigen for APC. In this case, antibody binds antigen forming an immune complex which binds and activated the complement system. Complement activation allows for ligation to the complement receptor on the APC.

Regulation by cytokines

Cytokines are positive or negative regulators. They act at many stages of the immune response, but their activity is dependent upon the other cytokines present in the microenvironment as well as

receptor expression on effector cells. Cytokines regulate the type and extent of the immune response generated.

Regulation by regulatory T cells (Tregs)

Regulatory T cells (Tregs) are a recently described populations of cells that can regulate immune responses. They do not prevent initial T cell activation; rather, they inhibit a sustained response and prevent chronic and potentially damaging responses. They do not have characteristics of Th1, Th2 or TH17 cells but they can suppress both Th1 and Th2 responses.

Naturally occurring Tregs

The thymus gives rise to CD4+/CD25+/Foxp3+ cells that functions as Tregs. These Tregs suppress immune responses in a cell contact dependent manner but the mechanism of suppression has not been established.

Induced Tregs

In the periphery some T cells are induced to become Tregs by antigen and either IL-10 or TGF-β. Tregs induced by IL-10 are CD4+/CD25+/Foxp3- and are referred to as Tr1 cells. These cells suppress immune responses by secretion of IL10. Tregs induced by TGF-β are CD4+/CD25+/Foxp3+ and are referred to as induced Tregs. These cells suppress by secretion of TGF-β

CD8+ Tregs

Some CD8+ cells can also be induced by antigen and IL-10 to become a Treg cell. These cells are CD8+/Foxp3+ and they suppress by a cell contact dependent mechanism or by secretion of cytokines. These cells have been demonstrated *in vitro* but it is not known whether they exist *in vivo*.

Genetic factors influencing immunoregulation

MHC-linked genes help control response to infection. Certain HLA haplotypes are associated with individuals who are responders or nonresponder, those who are susceptible or resistant.

Non-MHC genes are also involved in immunoregulation. An example is a gene related to macrophage activity encoding a transporter protein involved in transport of nitrite (NO2-) into the phagolysosome, natural resistance-associated macrophage protein-1 (Nramp1). Polymorphisms in this gene could change the activity of macrophages.

Cytokine, chemokine, and their receptors are involved in immunoregulation as discussed above. Polymorphisms in the genes encoding these, in particular the receptors, have been shown to correlate to susceptibility to infection or generation of autoimmune disease.

Table 1 - FEATURES OF CYTOKINES

Cytokine	Cell Source	Cell Target	Primary Effects
IL-1	Monocytes Macrophages Fibroblasts Epithelial cells Endothelial cells Astrocytes	T cells; B cells Endothelial cells Hypothalamus Liver	Costimulatory molecule Activation (inflammation) Fever Acute phase reactants
IL-2	T cells NK cells	T cells B cells Monocytes	Growth Growth Activation
IL-3	T cells	Bone marrow progenitors	Growth and differentiation
IL-4	T cells	Naive T cells T cells B cells	Differentiation into a T_H2 cell Growth Activation and growth; Isotype switching to IgE
IL-5	T cells	B cells Eosinophils	Growth and activation
IL-6	T cells Macrophages Fibroblasts	T cells B cells, Mature B cells Liver	Costimulatory molecule Growth (in humans) Acute phase reactants
IL-8 family	Macrophages Epithelial cells Platelets	Neutrophils	Activation and chemotaxis
IL-10	T cells (T_H2)	Macrophages T cells	Inhibits APC activity Inhibits cytokine production
IL-12	Macrophages NK cells	Naive T cells	Differentiation into a T_H1 cell
IFN-gamma	T cells NK cells	Monocytes Endothelial cells Many tissue cells - especially macrophages	Activation Activation Increased class I and II MHC
TGF-beta	T cells Macrophages	T cells Macrophages	Inhibits activation and growth Inhibits activation
GM-CSF	T cells Macrophages	Bone marrow progenitors	Growth and differentiation

	Endothelial cells Fibroblasts		
TNF-alpha	Macrophages T cells	Similar to IL-1	Similar to IL-1

IL = interleukin GM-CSF = granulocyte-macrophage colony stimulating factor
IFN = interferon TNF = tumor necrosis factor
TGF = transforming growth factor

IMMUNIZATION

Immunization is a means of providing specific protection against many common and damaging pathogens by stimulating an organism's immune system to either produce humoral antibodies against the pathogen (or toxins produced by the pathogen) or T cells that can provide cell-mediated immunity.

The type of immunity that is needed to neutralize a specific pathogen depends on the site of the pathogen and the mechanism of its pathogenesis. For example, some pathogens produce disease by secreting exotoxins. If this is the case, the only immune mechanism effective against the organism would be neutralizing antibodies that prevent exotoxin binding to the appropriate receptor on its target cell and promoting its clearance and degradation by phagocytes.

If the pathogen produces disease by other means, an antibody will have to react with the pathogen itself and eliminate it either by complement-mediated lysis or phagocytosis and intracellular killing. However, if the pathogenic organism is localized intracellularly, it will not be accessible to antibodies and the cell harboring it will have to be destroyed instead; only then could antibody have any effect on the pathogen. Most viruses, together with intracellular bacteria and protozoa, are examples of such pathogens. In this case, the harboring cells can be destroyed by elements of cell-mediated immunity or, if they cause the infected cell to express unique antigens recognizable by antibody, antibody-dependent and complement-mediated killing of the infected cell can expose the pathogen to elements of humoral immunity. It is also possible for cells harboring intracellular pathogen to be activated to kill the pathogen. Such is clearly not the case with pathogens that have the capability of surviving within phagocytic cells.

Specific immunity can result from either passive or active immunization and both modes of immunization can occur by natural or artificial processes (Figure 1C).

PASSIVE IMMUNITY

Immunity can be acquired, without the immune system being challenged with an antigen. This is done by transfer of serum or gamma-globulins from an immune donor to a non-immune individual.

Alternatively, immune cells from an immunized individual may be used to transfer immunity. Passive immunity may be acquired naturally or artificially.

Naturally acquired passive immunity

Immunity is transferred from mother to fetus through placental transfer of IgG or colostral transfer of IgA.

Artificially acquired passive immunity

Immunity is often artificially transferred by injection with gamma-globulins from other individuals or gamma-globulin from an immune animal. Passive transfer of immunity with immune globulins or gamma-globulins is used in numerous acute situations of infection (diphtheria, tetanus, measles, rabies, etc.), poisoning (insects, reptiles, botulism), and as a prophylactic measure (hypogammaglobulinemia). In these situations, gamma-globulins of human origin are preferable, although specific antibodies raised in other species are effective and used in some cases (poisoning, diphtheria, tetanus, gas gangrene, botulism). While this form of immunization has the advantage of providing immediate protection, heterologous gamma-globulins are effective for only a short duration and often result in pathological complications (serum sickness) and anaphylaxis. Homologous immunoglobulins also carry the risk of transmitting hepatitis and HIV.

Passive transfer of cell-mediated immunity can also be accomplished in certain diseases (cancer, immunodeficiency). However, it is difficult to find histocompatible (matched) donors and there is severe risk of graft versus host disease.

Figure 1A. Edward Jenner carries out a vaccination
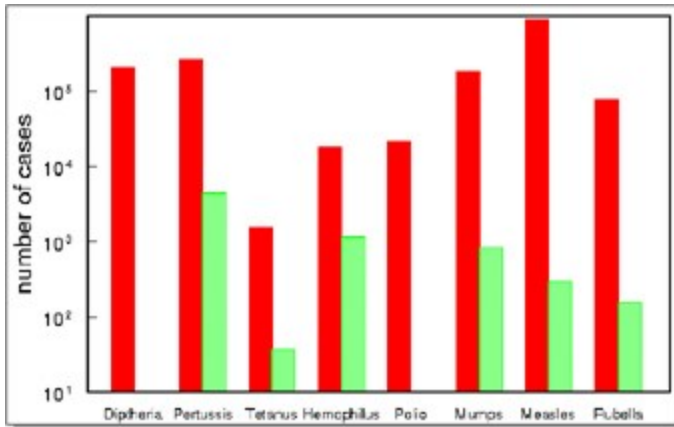
B. Pre and post vaccine incidence of common infectious diseases
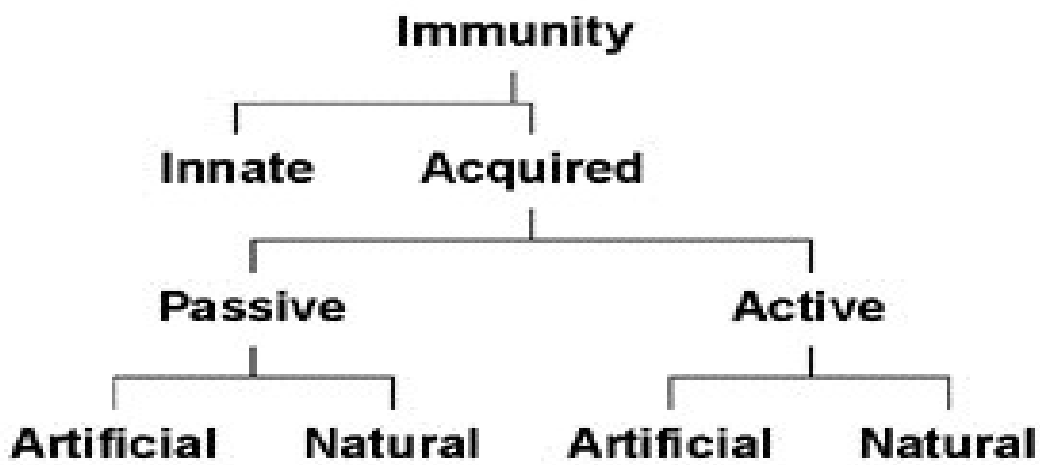
C. Modes of immunization

Milestones in immunization

3000BC
- Evidence of sniffing powdered small pox crust in Egypt

2000BC
- Sniffing of small pox crust in China

1500BC
- Turks introduce variolation

1700AD
- Introduction of variolation in England and later in the US

1780AD
- Edward Jenner discovers small pox vaccine

1885AD
- Pasteur discovers rabies attenuated vaccine

D. Milestones of immunization

ACTIVE IMMUNITY

This refers to immunity produced by the body following exposure to antigens.

Naturally acquired active immunity

Exposure to various pathogens leads to sub-clinical or clinical infections which result in a protective immune response against these pathogens.

Artificially acquired active immunity

Immunization may be achieved by administering live or dead pathogens or their components. Vaccines used for active immunization consist of live (attenuated) organisms, killed whole organisms, microbial components or secreted toxins (which have been detoxified).

Live vaccines

The first live vaccine was cowpox virus introduced by Edward Jenner as a vaccine for smallpox (see vaccine section); however, variolation (innoculation using pus from a patient with a mild case of smallpox) has been in use for over a thousand years (figure 2)

Introduction of variolation

The wife of the British Ambassador in Turkey, in March 1717 wrote, following the variolation of her son, to a friend in England: "The small pox, so fatal, so general amongst us, is entirely harmless here by the invention of ingrafting….I am patriot enough to bring this invention into fashion in England.

Figure 2 Introduction of variolation

Live vaccines are used against a number of viral infections (polio (Sabin vaccine), measles, mumps, rubella, chicken pox, hepatitis A, yellow fever, *etc*.) (figure 3). The only example of live bacterial vaccine is one against tuberculosis (*Mycobacterium bovis*: Bacille Calmette-Guerin vaccine: BCG). This is is used in many African, European and Asian countries but not in the United States. Whereas many studies have shown the efficacy of BCG vaccine, a number of studies also cast doubt on its benefits.

Live Attenuated Vaccines

➔polio*
→ not used in std. schedule

➔measles, mumps & rubella

➔Varicella zoster
→ children with no history of chicken pox

➔hepatitis A
→ not required in SC

➔yellow fever
→ Military and travelers

➔tuberculosis
→ not used in this country

Figure 3 Live attenuated vaccines

Live vaccines normally produce self-limiting non-clinical infections and lead to subsequent immunity, both humoral and cell-mediated, the latter being essential for intracellular pathogens. However, they carry a serious risk of causing overt disease in immunocompromised individuals. Furthermore, since live vaccines are often attenuated (made less pathogenic) by passage in animals or thermal mutation, they can revert to their pathogenic form and cause serious illness. It is for this reason that live polio (Sabin) vaccine, which was used for many years, has been replaced in many countries by the inactivated (Salk) vaccine.

Killed vaccines

Killed (heat, chemical or UV irradiation) viral vaccines include those for polio (Salk vaccine), influenza, rabies, influenza, rabies, etc. Most bacterial vaccines are killed organisms (typhoid, cholera, plague, pertussis, *etc.*) (figure 4).

Killed Whole-Organism Vaccines

- polio
- influenza
  - elderly and at risk
- rabies
  - post exposure
- Q fever
  - population at risk
- typhoid, cholera, plague
  - epidemics and travelers
- pertussis
  - replaced by the acellular vaccine

Figure 4 Killed whole organism vaccines

Sub-unit vaccines

Some anti-bacterial vaccines utilize purified cell wall components (haemophilus, pertussis, meningococcus, pneumococcus, etc.) (figure 5).

Microbial Fragment Vaccines

- *Bordetella. Pertussis*
  - virulence factor protein
- *Haemophilus influenzae B*
  - protein conjugated polysaccharide
- *Streptococcus pneumoniae*
  - Polysaccharide mixture
- *Neisseria meningitidis*
  - polysaccharide

Microbial Fragment Vaccines

- *Clostridium tetani (tetanus)*
  - inactivated toxin (toxoid)
- *Corynebacterium diphtheriae*
  - inactivated toxin (toxoid)
- *Vibrio cholerae*
  - toxin subunits
- Hepatitis B virus
  - cloned in yeast

Figure 5 Microbial fragment vaccines

Some viral vaccines (hepatitis-B, *etc.*) consist of purified antigenic proteins manufactured after expression from a gene cloned into a suitable vector (*e.g.*, yeast). When the pathogenic mechanism of an agent involves a toxin, a modified form of the toxin (toxoid, which has lost its toxicity while remaining immunogenic) is used as a vaccine (*e.g.*, diphtheria, tetanus, cholera) (figure 6).

Figure 6 Modification of toxin to toxoid

These subunit vaccines are designed to reduce the toxicity problems. Each type of vaccine has its own advantages and disadvantages (figure 7).

Advantages and Disadvantages of Passive Immunization

Advantages	Disadvantages
immediate protection	no long term protection
	serum sickness
	risk of hepatitis and Aids
	graft vs. host disease (**cell graft only**)

Figure 7 Advantages and disadvantages of passive immunization

Subunit vaccines may consist of proteins or polysaccharides. Since polysaccharides are relatively weak T-independent antigens, and produce only IgM responses without immunologic memory, they are made more immunogenic and T-dependent by conjugation with proteins (e.g., haemophilus, meningococcus, pneumococcus, etc.).

Other novel vaccines

A number of novel approaches to active immunization are in the investigative stage and are used only experimentally. These include anti-idiotype antibodies, DNA vaccines and immunodominant peptides (recognized by the MHC molecules) and may be available in the future.

- Anti-idiotype antibodies against polysaccharide antibodies produce long lasting immune responses with immunologic memory.
- DNA vaccines (viral peptide genes cloned into vectors) must be injected. They transfect host cells and consequently produce a response similar to that produced against live-attenuated viruses (both cell-mediated and humoral). Several anti-HIV DNA vaccines have been developed but none has so far shown much efficacy.

- Immunodominant peptides are simple and easy to prepare and, when incorporated into MHC polymers, can provoke both humoral and cell mediated responses.

Table 1. Selected adjuvants in clinical or experimental use		
Adjuvant type	**Human use**	**Experimental only**
Salts: aluminum hydroxide, aluminum phosphate-calcium phosphate	Yes Yes	Slow release of antigen, TLR interaction and cytokine induction
Beryllium hydroxide	No	
Synthetic particles: Liposomes, ISCOMs, polylactates	No No	Slow release of antigen
Polynucleotides: CpG and others	No*	TLR interaction and cytokine induction
Bacterial products: *B.pertussis*	Yes	TLR interaction and cytokine induction
M. bovis (BCG and others)	No	
Mineral oils	No	Antigen depot
Cytokines: IL-1, IL-2, IL12, IFN-γ, *etc*.	No*	Activation and differentiation of T - and B cells and APC
*Experimental use in human malignancies		

The protective immunity conferred by a vaccine may be life-long (measles, mumps, rubella, small pox, tuberculosis, yellow fever, etc.) or may last as little as a few months (cholera). The primary immunization may be given at the age of 2 to 3 months (diphtheria, pertussis, tetanus, polio), or 13 to 15 months (mumps, measles, rubella). The currently recommended schedule for routine immunization in the United States (recommended by CDC and AIP) is summarized in Table 2. This schedule is revised on a yearly basis or as need by the CDC Advisory Committee on Immunization Practice (AICP).

Table 2   Schedule for Active Immunization of Normal Children*

Age / Vaccine	Birth	Months								Years	
		1	2	4	6	12	15	18	19-23	2-3	4-6
Hepatitis-B [1]	HeB	HeB		1	HeB						HeB
Rotavirus [2]			Rota	Rota	Rota						
Diphtheria, Tetanus, Pertussis [3]			DTaP	DTaP	DTaP	3	DTaP				DTaP
Hemophilus influenzae-b (CV) [4]			Hib	Hib	Hib4	Hib					
Pneumococcal [5]			PCV	PCV	PCV	PCV				PPV	
Inactivated Poliovirus			IPV	IPV	IPV						IPV
Influenza [6]					Influenza (yearly)						
Measles, Mumps, Rubella [7]						MMR				MMR	MMR
Varicella [8]						Var					
Hepatitis A [9]						Hep A (2 doses)				HepA series	
Meningococcal [10]											MCV4

*Recommended by Advisory Committee on Immunization , American academy of Pediatrics

NOTES

Range of recommended ages	Certain high risk groups

CDC Immunization schedules

Prophylactic versus therapeutic immunization
Most vaccines are given prophylactically, i.e. prior to exposure to the pathogen. However, some vaccines can be administered therapeutically, i.e. post exposure (e.g., rabies virus). The

effectiveness of this mode of immunization depends on the rate of replication of the pathogen, incubation period and the pathogenic mechanism. For this reason, only a booster shot with tetanus is sufficient if the exposure to the pathogen is within less than 10 years and if the exposure is minimal (wounds are relatively superficial). In a situation where the pathogen has a short incubation period, only a small amount of pathogenic molecules could be fatal (e.g., tetanus and diphtheria); therefore both passive and active post exposure immunization are essential. This is also the case when a bolus of infection is relatively large

Passive prophylactic immunization is also normal in cases of defects in the immune system, such as hypogammaglobulinemias.

Adverse effects of immunization

Active immunization may cause fever, malaise and discomfort. Some vaccine may also cause joint pains or arthritis (rubella), convulsions, that may sometimes be fatal (pertussis), or neurological disorders (influenza). Allergies to eggs may develop as a consequence of viral vaccines produced in eggs (measles, mumps, influenza, yellow fever). Booster shots result in more pronounced inflammatory effects than the primary immunization. The serious side effects have been documented after use of the DTP vaccine (Table 3). Most of these were attributable to the whole pertussis component of the vaccine and have been eliminated by the use of an acellular pertussis preparation.

Adverse event occurring within 48 hours DTP vaccination

Event	Frequency
Local: redness, swelling, pain	1 in 2-3 doses

Mild/moderate systemic:
    fever, drowsiness, fretfulness vomiting,
    anorexia
	1 in 2-3 doses
	1 in 5-15 doses

More serious systemic:
    persistent crying, fever
    collapse, convulsions
    acute encephalopathy permanent
    neurological deficit
	1 in 100-300 doses
	1 in 1750 doses
	1 in 100,000 doses
	1 in 300,000 doses

Adverse events occurring with 48 hours of DPT vaccination

Table 3. Approximate rates of adverse event occurring within 48 hours DTP vaccination	
Event	**Frequency**
Local	
Redness, swelling, pain	1 in 2-3 doses
Mild/moderate systemic	
Fever, drowsiness, fretfulness	1 in 2-3 doses
Vomiting, anorexia	1 in 5-15 doses
More serious systemic	
Persistent crying, fever	1 in 100-300 doses
Collapse, convulsions	1 in 1750 doses
Acute encephalopathy	1 in 100,000 doses
Permanent neurological deficit	1 in 300,000 doses

You have learned:

Different modes of acquiring immunity

Which mode is used or applicable in what situation

Advantages and disadvantages of different modes of immunization

Rationale for vaccine design

Risk and benefits of vaccination

TOLERANCE AND AUTOIMMUNITY

TOLERANCE

INTRODUCTION

Tolerance refers to the *specific* immunological non-reactivity to an antigen resulting from a previous exposure to the same antigen. While the most important form of tolerance is non-reactivity to self antigens, it is possible to induce tolerance to non-self antigens. When an antigen induces tolerance, it is termed tolerogen.

TOLERANCE TO SELF ANTIGENS

We normally do not mount a strong immune response against our own (self) antigens, a phenomenon called self-tolerance. When the immune system recognizes a self antigen and mounts a strong response against it, autoimmune disease develops. Nonetheless, the immune system has to recognize self-MHC to mount a response against a foreign antigen. Thus, the immune system is constantly challenged to discriminate self vs non-self and mediate the right response.

INDUCTION OF TOLERANCE TO NON-SELF

Tolerance can also be induced to non-self (foreign) antigens by modifying the antigen, by injecting the antigen through specific routes such as oral, administering the antigen when the immune system is developing, etc. Certain bacteria and viruses have devised clever ways to induce tolerance so that the host does not kill these microbes. Ex: Patients with lepromatous type of leprosy do not mount an immune response against *Mycobacterium leprae*.

TOLERANCE TO TISSUES AND CELLS

Tolerance to tissue and cell antigens can be induced by injection of hemopoietic (stem) cells in neonatal or severely immunocompromised (by lethal irradiation or drug treatment) animals. Also, grafting of allogeneic bone marrow or thymus in early life results in tolerance to the donor type cells and tissues. Such animals are known as chimeras. These findings are of significant practical application in bone marrow grafting.

TOLERANCE TO SOLUBLE ANTIGENS

A state of tolerance to a variety of T-dependent and T-independent antigens has been achieved in various experimental models. Based on these observations it is clear that a number of factors determine whether an antigen will stimulate an immune response or tolerance (Table 1).

Table 1

Factors that determine induction of immune response or tolerance following challenge with antigen

Factors that affect response to Ag	Favor immune response	Favor tolerance
Physical form of antigen	Large, aggregated, complex molecules;	Soluble, aggregate-free, relatively smaller, less complex molecules, Ag not processed by APC or processed by cell without class II MHC
Route of Ag administration	Sub-cutaneous or intramuscular	Oral or sometimes intravenous
Dose of antigen	Optimal dose	Very large (or sometime very small) dose
Age of responding animal	Older and immunologically mature	Newborn (mice), immunologically immature
Differentiation state of cells	Fully differentiated cells; memory T and memory B cells	Relatively undifferentiated: B cells with only IgM (no IgD), T cells (*e.g.* cells in thymic cortex)

IMMUNOLOGIC FEATURES OF TOLERANCE

Tolerance is different from non-specific immunosuppression and immunodeficiency. It is an active antigen-dependent process in response to the antigen. Like immune response, tolerance is specific and like immunological memory, it can exist in T-cells, B cells or both and like immunological memory, tolerance at the T cell level is longer lasting than tolerance at the B cell level.

Induction of tolerance in T cells is easier and requires relatively smaller amounts of tolerogen than tolerance in B cells. Maintenance of immunological tolerance requires persistence of antigen. Tolerance can be broken naturally (as in autoimmune diseases) or artificially (as shown in experimental animals, by x-irradiation, certain drug treatments and by exposure to cross reactive antigens).

Tolerance may be induced to all epitopes or only some epitopes on an antigen and tolerance to a single antigen may exist at the B cell level or T cell level or at both levels.

MECHANISM OF TOLERANCE INDUCTION

The exact mechanism of induction and maintenance of tolerance is not fully understood. Experimental data, however, point to several possibilities.

Clonal deletion

T and B lymphocytes during development come across self antigens and such cells undergo clonal deletion through a process known as apoptosis or programmed cell death. For example, T cells that develop in the thymus first express neither CD4 nor CD8. Such cells next acquire both CD4 and CD8 called double-positive cells and express low levels of αβ TCR. Such cells undergo positive selection after interacting with class I or class II MHC molecules expressed on cortical epithelium. During this process, cells with low affinity for MHC are positively selected. Unselected cells die by apoptosis, a process called "death by neglect". Next, the cells loose either CD4 or CD8. Such T cells then encounter self-peptides presented by self MHC molecules expressed on dendritic cells. Those T cells with high affinity receptors for MHC + self-peptide undergo clonal deletion also called negative selection through induction of apoptosis. Any disturbance in this process can lead to escape of auto-reactive T-cells that can trigger autoimmune disease. Likewise, differentiating early B cells when they encounter self-antigen, cell associated or soluble, undergo deletion. Thus, clonal deletion plays a key role in ensuring tolerance to self antigen.

Peripheral tolerance

The clonal deletion is not a fool proof system and often T and B cells fail to undergo deletion and therefore such cells can potentially cause autoimmune disease once they reach the peripheral lymphoid organs. Thus, the immune system has devised several additional check points so that tolerance can be maintained.

Activation-induced cell death

T cells upon activation not only produce cytokines or carryout their effector functions but also die through programmed cell death or apoptosis. In this process, the death receptor (Fas) and its ligand (FasL) play a crucial role. Thus, normal T cells express Fas but not FasL. Upon activation, T cells express FasL which binds to Fas and triggers apoptosis by activation of caspase-8. The importance of Fas and FasL is clearly demonstrated by the observation that mice with mutations in Fas (lpr mutation) or FasL (gld mutation) develop severe lymphoproliferative and autoimmune disease and die within 6 months while normal mice live up to 2 years. Similar mutations in these apoptotic genes in humans leads to a lymphoproliferative disease called autoimmune lymphoproliferative syndrome (ALPS).

Clonal anergy

Auto-reactive T cells when exposed to antigenic peptides on antigen presenting cells (APC) that do not possess the co-stimulatory molecules CD80 (B7-1) or CD86 (B7-2) become anergic (nonresponsive) to the antigen. Also, while activation of T cells through CD28 triggers IL-2 production, activation of CTLA4 leads to inhibition of IL-2 production and anergy. Also, B cells when exposed to large amounts of soluble antigen down-regulate their surface IgM and become anergic. These cells also up-regulate the Fas molecules on their surface. An interaction of these B cells with Fas-ligand bearing T cells results in their death via apoptosis.

Clonal ignorance

T cells reactive to self-antigen not represented in the thymus will mature and migrate to the periphery, but they may never encounter the appropriate antigen because it is sequestered in inaccessible tissues. Such cells may die out for lack of stimulus. Auto-reactive B cells, that escape deletion, may not find the antigen or the specific T-cell help and thus not be activated and die out.

Anti-idiotype antibody

These are antibodies that are produced against the specific idiotypes of other antibodies. Anti-idiotypic antibodies are produced during the process of tolerization and have been demonstrated in tolerant animals. These antibodies may prevent the B cell receptor from interacting with the antigen.

Regulatory T cells (Formerly called suppressor cells)

Recently, a distinct population of T cells has been discovered called regulatory T cells. Regulatory T cells come in many flavors, but the most well characterized include those that express CD4+ and CD25+. Because activated normal CD4 T cells also express CD25, it was difficult to distinguish regulatory T cells and activated T cells. The latest research suggests that regulatory T cells are defined by expression of the forkhead family transcription factor Foxp3. Expression of Foxp3 is required for regulatory T cell development and function. The precise mechanism/s through which regulatory T cells suppress other T cell function is not clear. One of the mechanisms include the production of immunosuppressive cytokines such as TGF-β and IL-10. Genetic mutations in Foxp3 in humans leads to development of a severe and rapidly fatal autoimmune disorder known as Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome. This disease provides the most striking evidence that regulatory T cells play a critical role in preventing autoimmune disease.

Termination of tolerance

Experimentally induced tolerance can be terminated by prolonged absence of exposure to the tolerogen, by treatments which severely damage the immune system (x-irradiation) or by immunization with cross reactive antigens. These observations are of significance in the conceptualization of autoimmune diseases.

AUTOIMMUNITY

DEFINITION

Autoimmunity can be defined as breakdown of mechanisms responsible for self tolerance and induction of an immune response against components of the self. Such an immune response may not always be harmful (e.g., anti-idiotype antibodies). However, in numerous autoimmune diseases it is well recognized that products of the immune system cause damage to the self.

EFFECTOR MECHANISMS IN AUTOIMMUNE DISEASES

Both antibodies and effector T cells can be involved in the damage in autoimmune diseases.

GENERAL CLASSIFICATION

Autoimmune diseases are generally classified on the basis of the organ or tissue involved. These diseases may fall in an organ-specific category in which the immune response is directed against antigen(s) associated with the target organ being damaged or a non-organ-specific category in which the antibody is directed against an antigen not associated with the target organ (Table 2). The antigen involved in most autoimmune diseases is evident from the name of the disease (Table 2).

GENETIC PREDISPOSITION FOR AUTOIMMUNITY

Studies in mice and observations in humans suggest a genetic predisposition for autoimmune diseases. Association between certain HLA types and autoimmune diseases has been noted (HLA: B8, B27, DR2, DR3, DR4, DR5 *etc*.).

ETIOLOGY OF AUTOIMMUNITY DISEASE

The exact etiology of autoimmune diseases is not known. However, various theories have been offered. These include sequestered antigen, escape of auto-reactive clones, loss of suppressor cells, cross reactive antigens including exogenous antigens (pathogens) and altered self antigens (chemical and viral infections).

Sequestered antigen

Lymphoid cells may not be exposed to some self antigens during their differentiation, because they may be late-developing antigens or may be confined to specialized organs (*e.g.*, testes, brain, eye, *etc.*). A release of antigens from these organs resulting from accidental traumatic injury or surgery can result in the stimulation of an immune response and initiation of an autoimmune disease.

Escape of auto-reactive clones

The negative selection in the thymus may not be fully functional to eliminate self reactive cells. Not all self antigens may be represented in the thymus or certain antigens may not be properly processed and presented.

Lack of regulatory T cells

There are fewer regulatory T-cells in many autoimmune diseases.

Table 2 Spectrum of autoimmune diseases, target organs and diagnostic tests				
	Disease	**Organ**	**Antibody to**	**Diagnostic Test**
Organ-Specific	Hashimoto's thyroiditis	Thyroid	Thyroglobulin, thyroid peroxidase (microsomal)	RIA, CF, hemagglutination
	Primary Myxedema	Thyroid	Cytoplasmic TSH receptor	Immunofluorescence (IF)
	Graves' disease	Thyroid		Bioassay, Competition for TSH receptor

Disease	Organ/Tissue	Antigen	Test
Pernicious anemia	Red cells	Intrinsic factor (IF), Gastric parietal cell	B-12 binding to IF immunofluorescence
Addison's disease (Fig 1)	Adrenal	Adrenal cells	Immunofluorescence
Premature onset menopause	Ovary	Steroid producing cells	Immunofluorescence
Male infertility	Sperm	Spermatozoa	Agglutination, Immunofluorescence
Insulin dependent juvenile diabetes	Pancreas	Pancreatic islet beta cells	
Insulin resistant diabetic	Systemic	Insulin receptor	Competition for receptor
Atopic allergy	Systemic	beta-adrenergic receptor	Competition for receptor
Myasthenia graves	Muscle	Muscle, acetyl choline receptor	Immunofluorescence, competition for receptor
Goodpasture's syndrome	Kidney, lung	Renal and lung basement membrane	Immunofluorescence (line staining) (Fig. 2)
Pemphigus	Skin	Desmosomes	Immunofluorescence (Fig
Pemphigoid	Skin	Skin basement membrane	Immunofluorescence (Fig
Phacogenic uveitis	Lens	Lens protein	
AI hemolytic anemia	Erythrocytes Platelet	Erythrocytes	Passive hemagglutination Direct Coomb's test
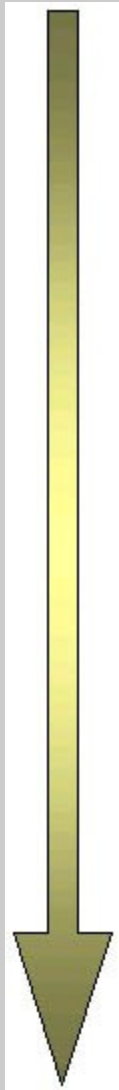
Disease	Tissue	Antigen	Detection method
Idiopathic thrombocytopenia		Platelet	Immunofluorescence
Primary biliary cirrhosis	Liver	Mitochondria	Immunofluorescence
Idiopathic neutropenia	Neutrophils	Neutrophils	Immunofluorescence
Ulcerative colitis	Colon	Colon lipopolysaccharide	Immunofluorescence
Sjogren's syndrome	Secretory glands (Fig 5)	Duct mitochondria	Immunofluorescence
Vitiligo	Skin Joints	Melanocytes (fig 6)	Immunofluorescence
Rheumatoid arthritis	Skin, kidney, joints etc	IgG	IgG-latex agglutination
Systemic lupus erythematosus	joints, etc.	DNA, RNA, nucleoproteins	RNA-, DNA-latex agglutination, IF (granular kidney)

Non-organ Specific

Diseases are listed from the most organ-specific (top) to the least specific (bottom)
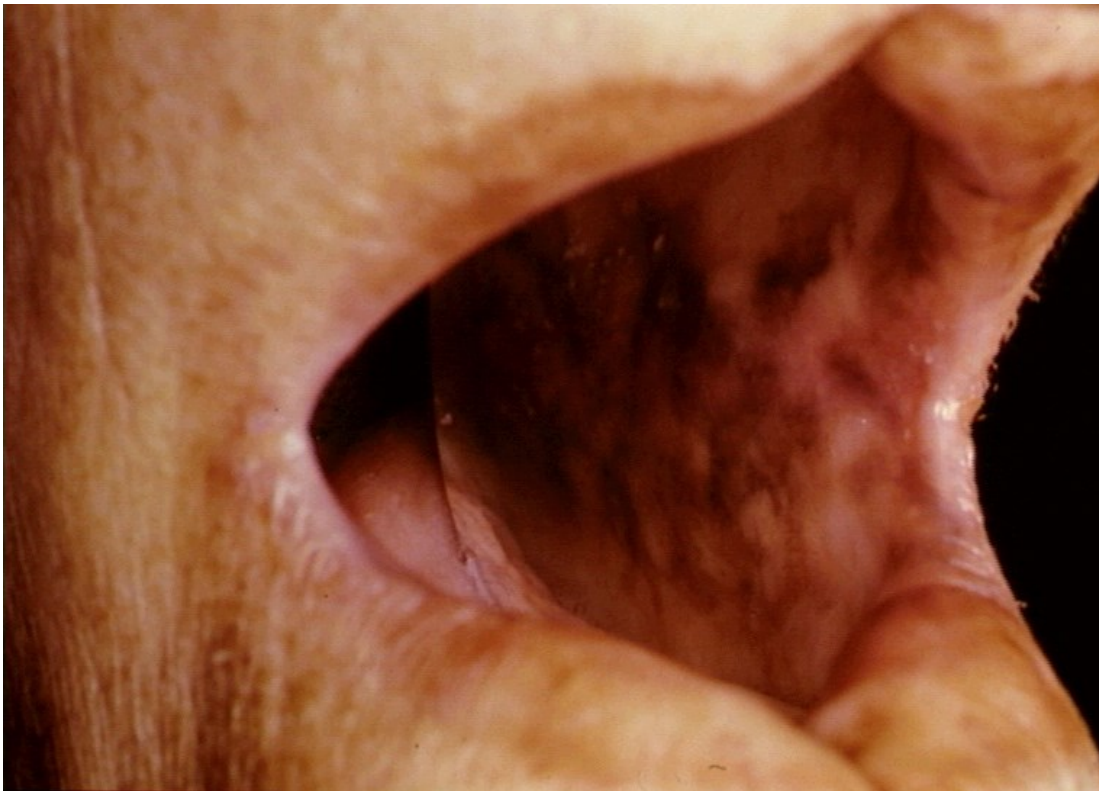
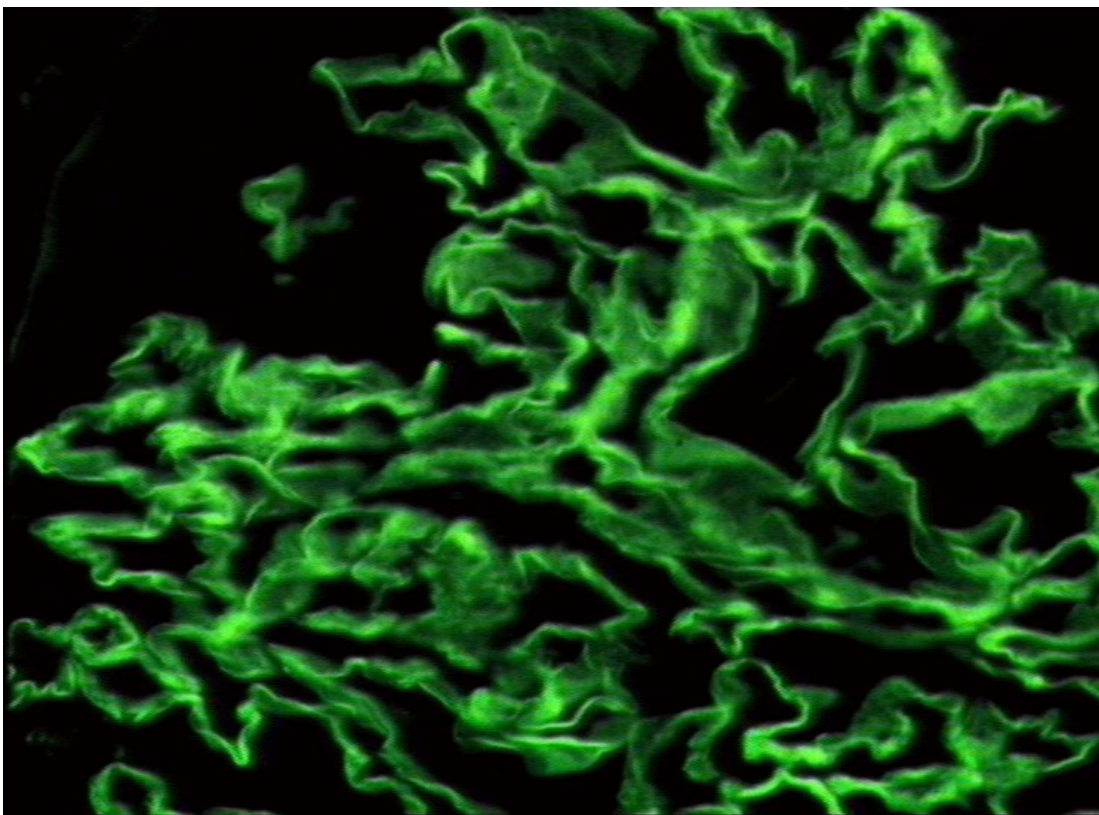Figure 1 Hyperpigmentation of buccal mucosa in Addison's disease

Figure 2 Immunofluorescent stain of immunoglobulin G (IgG) showing linear pattern in Goodpasture's syndrome
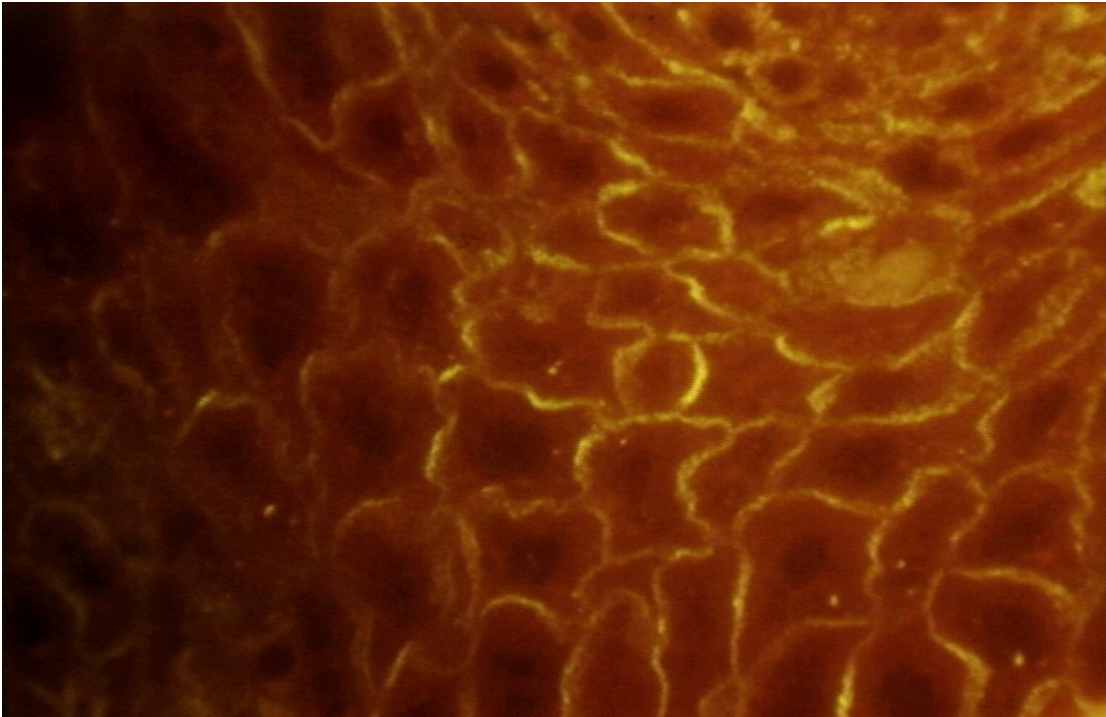
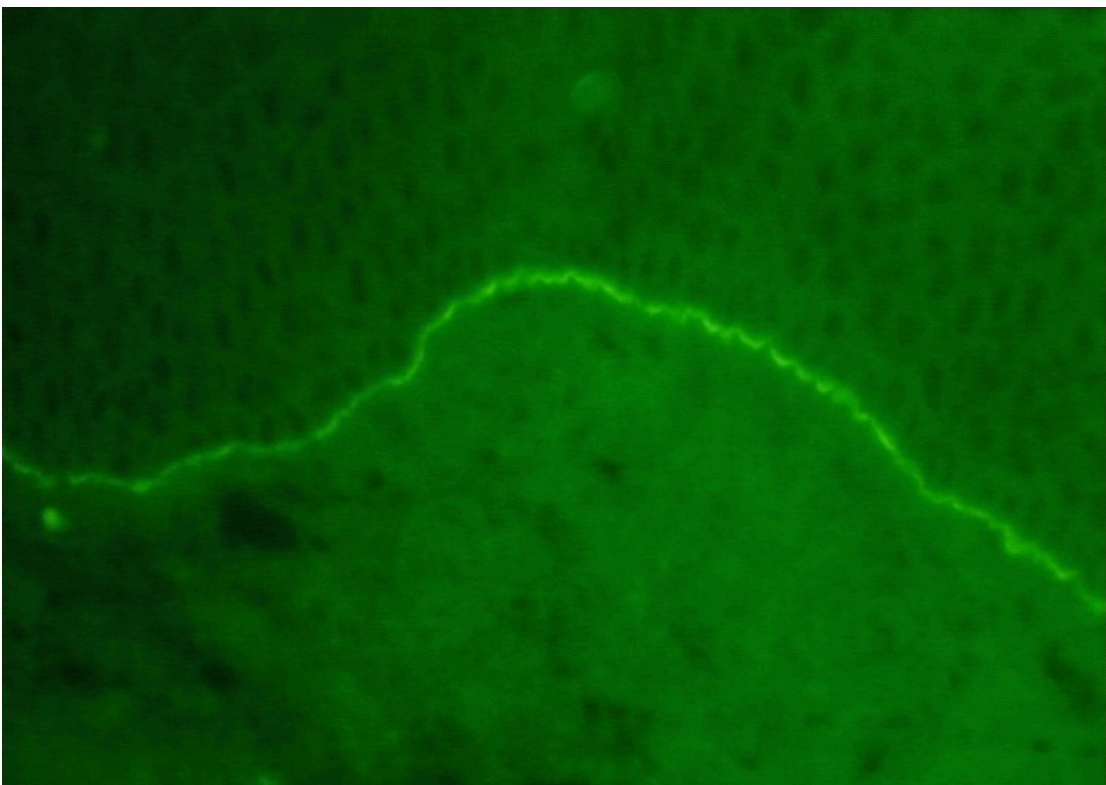Figure 3 Pemphigus vulgaris - immunofluorescence

Figure 4 Mucous membrane pemphigoid - immunofluorescence

Figure 5 Parotid enlargement in Sjogren's syndrome

Figure 6 Depigmentation in vitiligo

HYPERSENSITIVITY REACTIONS

Hypersensitivity refers to excessive, undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the normal immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Hypersensitivity reactions can be divided into four types: type I, type II, type III and type IV, based on the mechanisms involved and time taken for the reaction. Frequently, a particular clinical condition (disease) may involve more than one type of reaction.

TYPE I HYPERSENSITIVITY

Type I hypersensitivity is also known as immediate or anaphylactic hypersensitivity. The reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis). The reaction may cause a range of symptoms from minor inconvenience to death. The reaction usually takes 15 - 30 minutes from the time of exposure to the antigen, although sometimes it may have a delayed onset (10 - 12 hours).

Immediate hypersensitivity is mediated by IgE. The primary cellular component in this hypersensitivity is the mast cell or basophil. The reaction is amplified and/or modified by platelets, neutrophils and eosinophils. A biopsy of the reaction site demonstrates mainly mast cells and eosinophils.

The mechanism of reaction involves preferential production of IgE, in response to certain antigens (often called allergens). The precise mechanism as to why some individuals are more prone to type-I hypersensitivity is not clear. However, it has been shown that such individuals preferentially produce more of TH2 cells that secrete IL-4, IL-5 and IL-13 which in turn favor IgE class switch. IgE has very high affinity for its receptor (Fcε; CD23) on mast cells and basophils.

A subsequent exposure to the same allergen cross links the cell-bound IgE and triggers the release of various pharmacologically active substances (figure 1). Cross-linking of IgE Fc-receptor is important in mast cell triggering. Mast cell degranulation is preceded by increased Ca++ influx, which is a crucial process; ionophores which increase cytoplasmic Ca^{++} also promote degranulation, whereas, agents which deplete cytoplasmic Ca^{++} suppress degranulation.

The agents released from mast cells and their effects are listed in Table 1. Mast cells may be triggered by other stimuli such as exercise, emotional stress, chemicals (*e.g.*, photographic developing medium, calcium ionophores, codeine, *etc.*), anaphylotoxins (*e.g.*, C4a, C3a, C5a, *etc.*). These reactions, mediated by agents without IgE-allergen interaction, are not hypersensitivity reactions, although they produce the same symptoms.

Table 1. Pharmacologic Mediators of Immediate Hypersensitivity
MEDIATOR

Preformed mediators in granules

Histamine	Bronchoconstriction, mucus secretion, vasodilatation, vascular permeability
Tryptase	Proteolysis
Kininogenase	Kinins and vasodilatation, vascular permeability, edema
ECF-A (tetrapeptides)	Attract eosinophil and neutrophils

Newly formed mediators

Leukotriene B_4	Basophil attractant
Leukotriene C_4, D_4	Same as histamine but 1000x more potent
Prostaglandins D_2	Edema and pain
PAF	platelet aggregation and heparin release: microthrombi

The reaction is amplified by PAF (platelet activation factor) which causes platelet aggregation and release of histamine, heparin and vasoactive amines. Eosinophil chemotactic factor of anaphylaxis (ECF-A) and neutrophil chemotactic factors attract eosinophils and neutrophils, respectively, which release various hydrolytic enzymes that cause necrosis. Eosinophils may also control the local reaction by releasing arylsulphatase, histaminase, phospholipase-D and prostaglandin-E, although this role of eosinophils is now in question.

Cyclic nucleotides appear to play a significant role in the modulation of immediate hypersensitivity reaction, although their exact function is ill understood. Substances which alter cAMP and cGMP levels significantly alter the allergic symptoms. Thus, substances that increase intracellular cAMP seem to relieve allergic symptoms, particularly broncho-pulmonary ones, and are used therapeutically (Table 2). Conversely, agents which decrease cAMP or stimulate cGMP aggravate these allergic conditions.

Table 2 - Relationship between allergic symptoms and cyclic-nucleotides	
Lowering of cyclic-AMP	**Elevation of cyclic-AMP**
Stimulation of α-adrenergic receptor (nor-epinephrin, phenyl-epinephrin) or Blocking of β-adrenergic receptor (propanolol)	Stimulation of β-adrenergic receptor (epinephrine, isoproterenol) Blocking of α-adrenergic receptor (phenoxybenzamine) Inhibition of phosphodiesterase (theophylline) Binding of histamine-2 or PGE to their receptors
Elevation of cyclic-GMP	
Stimulation of γ-cholinergic receptor (acetyl choline, carbacol)	
WORSENING OF SYMPTOMS	**IMPROVEMENT OF SYMPTOMS**

Diagnostic tests for immediate hypersensitivity include skin (prick and intradermal) tests (fig. 1A), measurement of total IgE and specific IgE antibodies against the suspected allergens. Total IgE and specific IgE antibodies are measured by a modification of enzyme immunoassay (ELISA). Increased IgE levels are indicative of an atopic condition, although IgE may be elevated in some non-atopic diseases (*e.g.*, myelomas, helminthic infection, *etc.*).
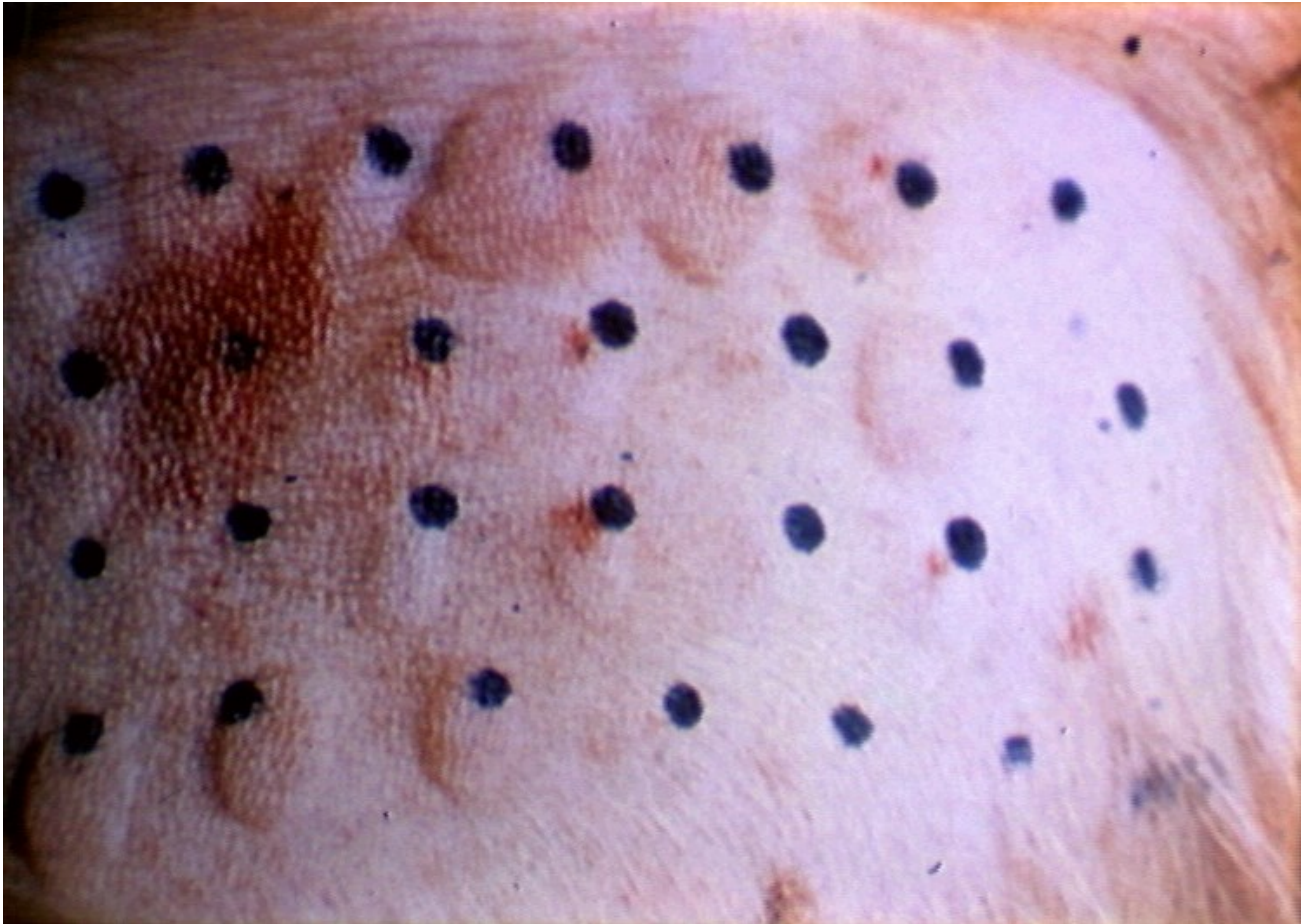
Figure 1A close-up view of intradermal skin test with multiple positive allergen responses

There appears to be a genetic predisposition for atopic diseases and there is evidence for HLA (A2) association.

Symptomatic treatment is achieved with anti-histamines which block histamine receptors. Chromolyn sodium inhibits mast cell degranulation, probably, by inhibiting Ca^{++} influx. Late onset allergic symptoms, particularly bronchoconstriction which is mediated by leukotrienes, are treated with leukotriene receptor blockers (Singulair, Accolate) or inhibitors of the cyclooxygenase pathway (Zileutoin). Symptomatic, although short term, relief from bronchoconstriction is provided by bronchodilators (inhalants) such as isoproterenol derivatives (Terbutaline, Albuterol). Thophylline elevates cAMP by inhibiting cAMP-phosphodiesterase and inhibits intracellular Ca^{++} release is also used to relieve bronchopulmonary symptoms.

The use of IgG antibodies against the Fc portions of IgE that binds to mast cells has been approved for treatment of certain allergies, as it can block mast cell sensitization.

Hyposensitization (immunotherapy or desensitization) is another treatment modality which is successful in a number of allergies, particularly to insect venoms and, to some extent, pollens. The mechanism is not clear, but there is a correlation between appearance of IgG (blocking) antibodies and relief from symptoms. Suppressor T cells that specifically inhibit IgE antibodies may play a role.

TYPE II HYPERSENSITIVITY

Type II hypersensitivity is also known as cytotoxic hypersensitivity and may affect a variety of organs and tissues. The antigens are normally endogenous, although exogenous chemicals (haptens) which can attach to cell membranes can also lead to type II hypersensitivity. Drug-induced hemolytic anemia, granulocytopenia and thrombocytopenia are such examples. The reaction time is minutes to hours. Type II hypersensitivity is primarily mediated by antibodies of the IgM or IgG classes and complement (Figure 2). Phagocytes and K cells may also play a role.
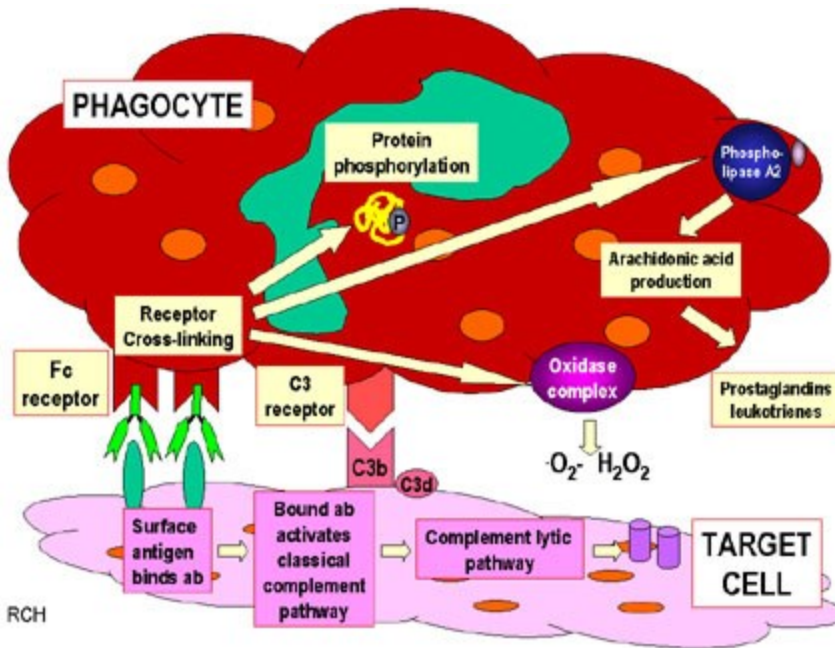
Figure 2. Type II cytotoxicity mechanism

The lesion contains antibody, complement and neutrophils. Diagnostic tests include detection of circulating antibody against the tissues involved and the presence of antibody and complement in the lesion (biopsy) by immunofluorescence. The staining pattern is normally smooth and linear, such as that seen in Goodpasture's nephritis (renal and lung basement membrane) (figure 3A) and pemphigus (skin intercellular protein, desmosome) (figure 3B).
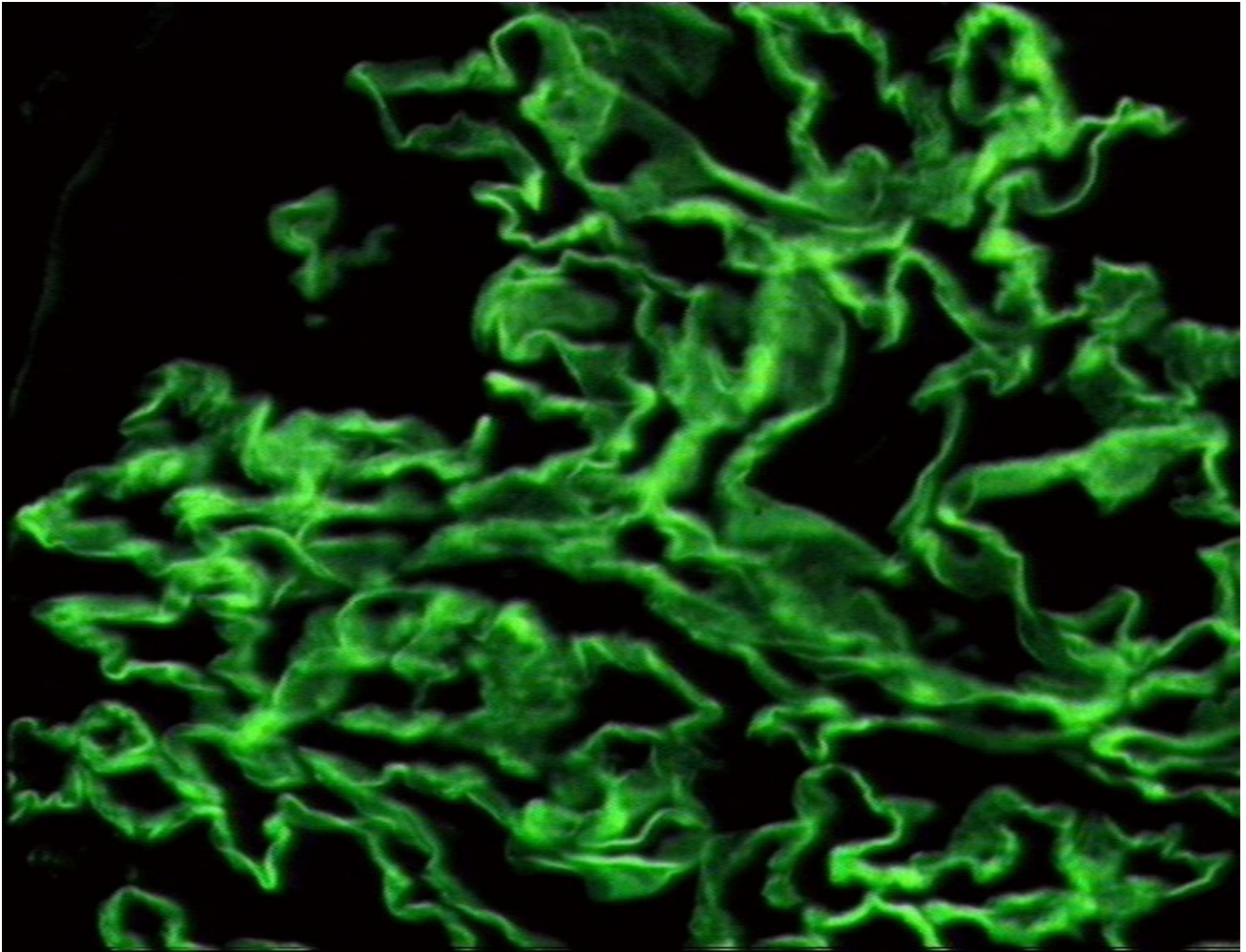
Figure 3A   Immunofluorescent stain of immunoglobulin G (IgG) showing linear pattern in Goodpasture's syndrome
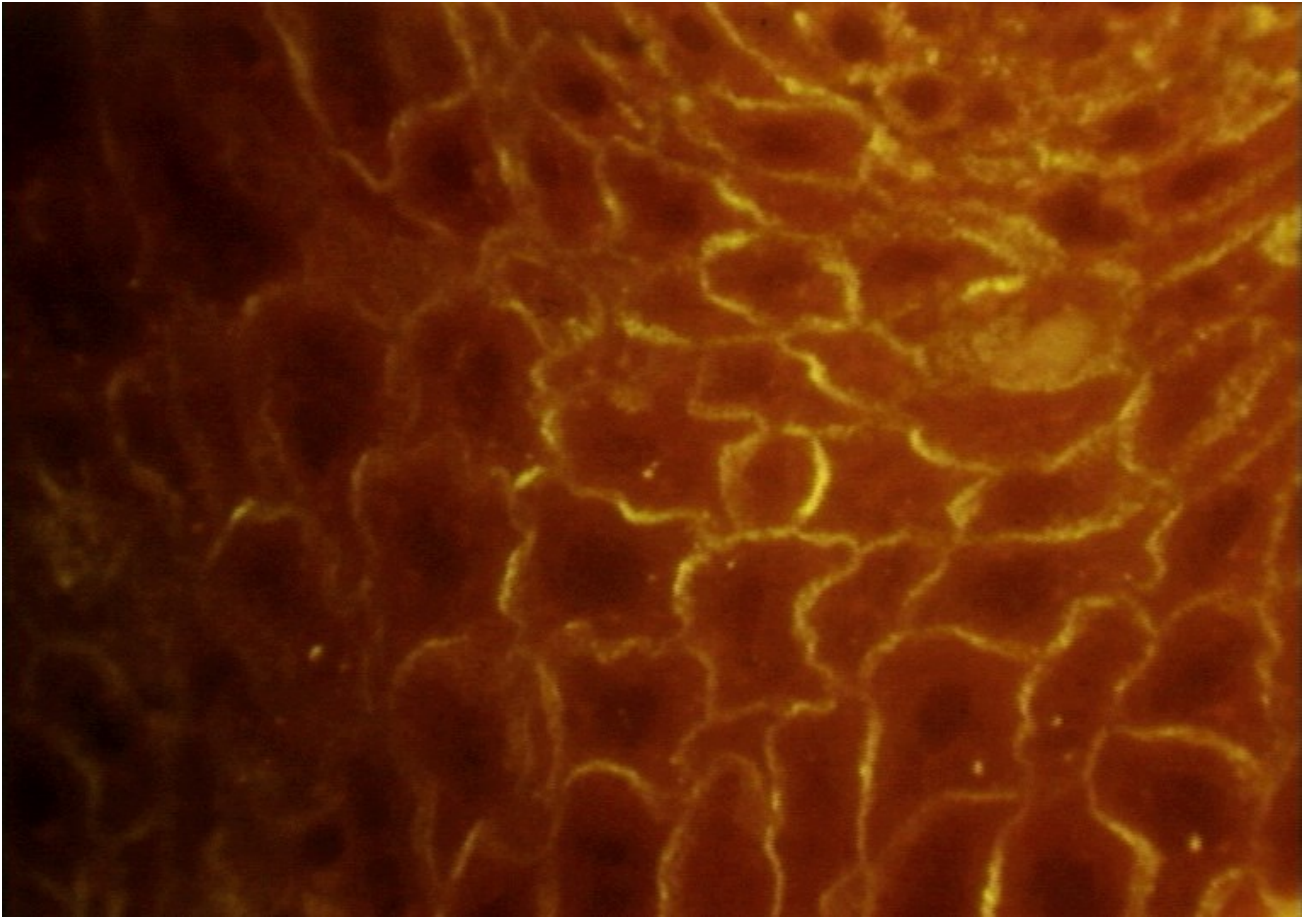
Figure 3B Pemphigus vulgaris - immunofluorescence

Treatment involves anti-inflammatory and immunosuppressive agents.

TYPE III HYPERSENSITIVITY

Type III hypersensitivity is also known as immune complex hypersensitivity. The reaction may be general (*e.g.*, serum sickness) or may involve individual organs including skin (*e.g.*, systemic lupus erythematosus, Arthus reaction), kidneys (*e.g.*, lupus nephritis), lungs (*e.g.*, aspergillosis), blood vessels (*e.g.*, polyarteritis), joints (*e.g.*, rheumatoid arthritis) or other organs. This reaction may be the pathogenic mechanism of diseases caused by many microorganisms.

The reaction may take 3 - 10 hours after exposure to the antigen (as in Arthus reaction). It is mediated by soluble immune complexes. They are mostly of the IgG class, although IgM may also be involved. The antigen may be exogenous (chronic bacterial, viral or parasitic infections), or endogenous (non-organ specific autoimmunity: *e.g.*, systemic lupus erythematosus, SLE). The antigen is soluble and not attached to the organ involved. Primary components are soluble immune complexes and complement (C3a, 4a and 5a). The damage is caused by platelets and neutrophils (Figure 4). The lesion contains primarily neutrophils and deposits of immune complexes and complement. Macrophages infiltrating in later stages may be involved in the healing process.

The affinity of antibody and size of immune complexes are important in production of disease and determining the tissue involved. Diagnosis involves examination of tissue biopsies for deposits of immunoglobulin and complement by immunofluorescence microscopy. The immunofluorescent staining in type III hypersensitivity is granular (as opposed to linear in type II such as seen in Goodpasture's syndrome). The presence of immune complexes in serum and depletion in the level of complement are also diagnostic. Polyethylene glycol-mediated turbidity (nephelometry) binding of C1q and Raji cell test are utilized to detect immune complexes. Treatment includes anti-inflammatory agents.

TYPE IV HYPERSENSITIVITY

Type IV hypersensitivity is also known as cell mediated or delayed type hypersensitivity. The classical example of this hypersensitivity is tuberculin (Montoux) reaction (figure 5) which peaks 48 hours after the injection of antigen (PPD or old tuberculin). The lesion is characterized by induration and erythema.

Table 3 - Delayed hypersensitivity reactions				
Type	**Reaction time**	**Clinical appearance**	**Histology**	**Antigen and site**
Contact	48-72 hr	Eczema	Lymphocytes, followed by macrophages; edema of epidermis	Epidermal (organic chemicals, poison ivy, heavy metals, *etc.*)
Tuberculin	48-72 hr	Local induration	Lymphocytes, monocytes, macrophages	Intradermal (tuberculin, lepromin, *etc.*)
Granuloma	21-28 days	Hardening	Macrophages, epitheloid and giant cells, fibrosis	Persistent antigen or foreign body presence (tuberculosis, leprosy, *etc.*)

Type IV hypersensitivity is involved in the pathogenesis of many autoimmune and infectious diseases (tuberculosis, leprosy, blastomycosis, histoplasmosis, toxoplasmosis, leishmaniasis, *etc.*)

and granulomas due to infections and foreign antigens. Another form of delayed hypersensitivity is contact dermatitis (poison ivy (figure 6), chemicals, heavy metals, *etc.*) in which the lesions are more papular. Type IV hypersensitivity can be classified into three categories depending on the time of onset and clinical and histological presentation (Table 3).

Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages. Cytotoxic T cells (Tc) cause direct damage whereas helper T (TH1) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage (figure 4). The delayed hypersensitivity lesions mainly contain monocytes and a few T cells.
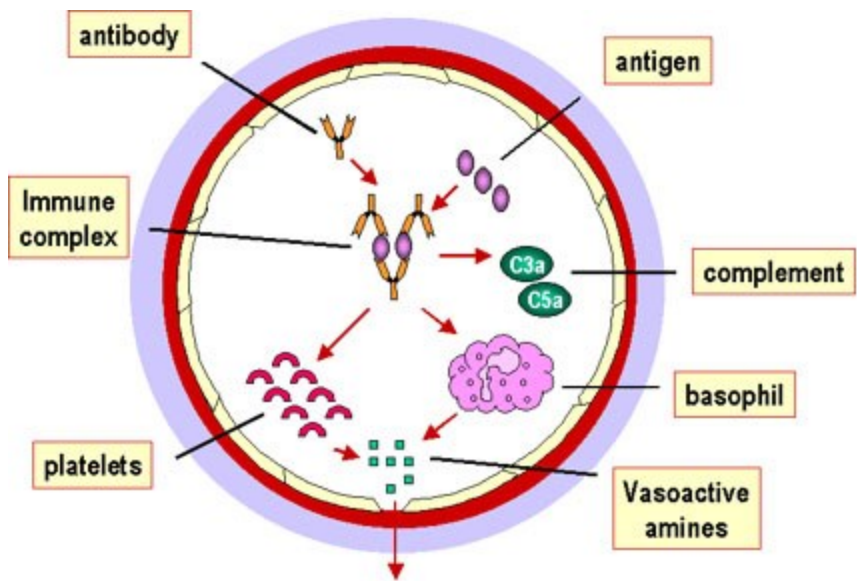
RCH
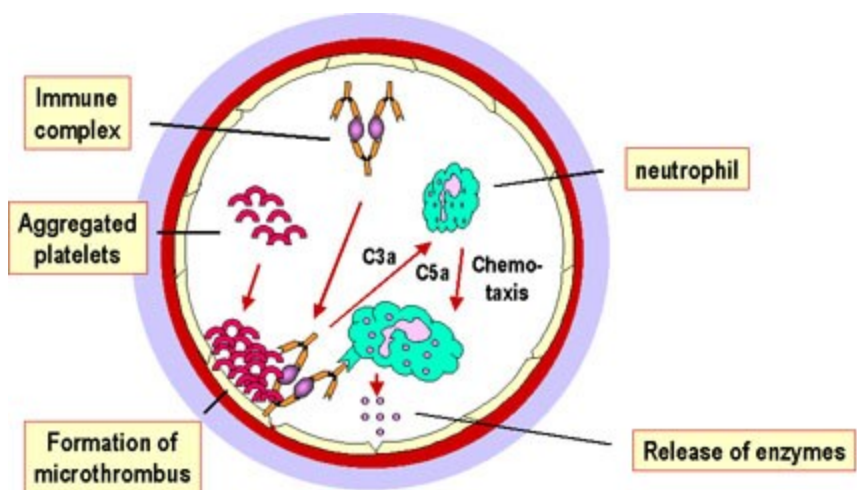
RCH

Figure 4. Mechanism of damage in immune complex hypersensitivity

Major lymphokines involved in delayed hypersensitivity reaction include monocyte chemotactic factor, interleukin-2, interferon-gamma, TNF alpha/beta, *etc.*

Diagnostic tests *in vivo* include delayed cutaneous reaction (*e.g.* Montoux test (figure 5)) and patch test (for contact dermatitis). In vitro tests for delayed hypersensitivity include mitogenic response, lympho-cytotoxicity and IL-2 production.

Figure 5 Mantoux intradermal tuberculin skin test for tuberculosis

Corticosteroids and other immunosuppressive agents are used in treatment.

Table 5 - Comparison of Different Types of hypersensitivity				
Characteristics	Type-I (anaphylactic)	Type-II (cytotoxic)	Type-III (immune complex)	Type-IV (delayed type)
Antibody	IgE	IgG, IgM	IgG, IgM	None

Antigen	Exogenous	Cell surface	Soluble	Tissues and organs
Response time	15-30 minutes	Minutes-hours	3-8 hours	48-72 hours
Appearance	Weal and flare	Lysis and necrosis	Erythema and edema, necrosis	Erythema and induration
Histology	Basophils and eosinophil	Antibody and complement	Complement and neutrophils	Monocytes and lymphocytes
Transferred with	Antibody	Antibody	Antibody	T-cells
Examples	Allergic asthma, hay fever	Erythroblastosis fetalis Goodpasture's nephritis	SLE, farmer's lung disease	Tuberculin test, poison ivy, granuloma

Figure 6 Poison Ivy CDC

IMMUNODEFICIENCY

IMMUNODEFICIENCY

Immunodeficiency is the failure of the immune system to protect against disease or malignancy.

Primary Immunodeficiency is caused by genetic or developmental defects in the immune system. These defects are present at birth but may show up later on in life.

Secondary or acquired immunodeficiency is the loss of immune function as a result of exposure to disease agents, environmental factors, immunosuppression, or aging.

PRIMARY IMMUNODEFICIENCIES

Primary immunodeficiencies are inherited defects of the immune system (figure 1).
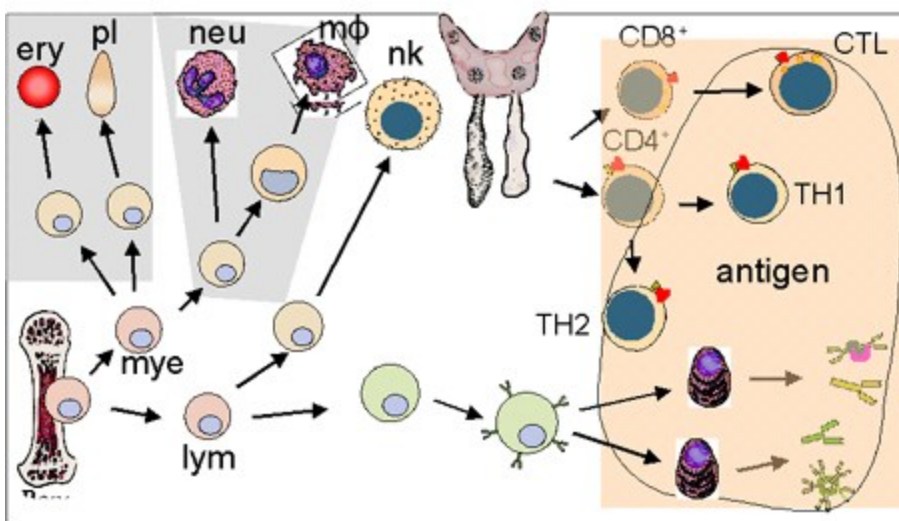
Figure 1 Developmental defects in primary immunodeficiencies

These defects may be in the specific or non-specific immune mechanisms. They are classified on the basis of the site of lesion in the developmental or differentiation pathway of the immune system.

Individuals with immunodeficiencies are susceptible to a variety of infections and the type of infection depends on the nature of immunodeficiency (Table 1).

Table 1. Characteristic infections of the primary immunodeficiencies

Component	Primary pathogen	Primary site	Clinical example
T-cells	Intracellular, bacteria viruses, protozoa, fungi,	Non-specific	SCID, DiGeorge
B-cells	*Pneumococcus*, *Streptococcus*, *Haemophilus*	Lung, skin, CNS	IgG, IgM deficiency
	Enteric bacteria and viruses	GI, nasal, eye	IgA deficiency
Phagocytes	*Staphylococcus*, *Klebsiella Pseudomonas*	Lung, skin, regional lymph node	chronic granulomatous disease (CGD)
Complement	*Neisseria*, *Haemophilus*, *Pneumococcus*, *Streptococcus*	CNS, lung, skin	C3, Factors I and H, late C components

SPECIFIC IMMUNE SYSTEM

There are variety of immunodeficiencies which result from defects in stem cell differentiation and may involve T-cells, B-cells, and/or immunoglobulins of different classes and subclasses (Table 2).

A defect in the early hematopoiesis which involves stem cells results in reticular dysgenesis that leads to general immune defects and subsequent susceptibility to infections. This condition is often fatal but very rare. It can be treated successfully by bone marrow transplantation.

Lymphoid lineage immunodeficiency

If the lymphoid progenitor cells are defective, then both the T and B cell lineages are affected and result in the severe combined immunodeficiency (SCID). Infants suffer from recurrent infections especially by opportunistic microrganisms (bacterial, viral, mycotic and protozoan infections).

In about 50% of SCID patients, the immunodeficiency is x-linked whereas in the other half the deficiency is autosomal. Both are characterized by an absence of T cell and B cell immunity and absence (or very low numbers) of circulating T and B lymphocytes. Thymic shadows are absent on X-rays.

The *x*-linked severe SCID is due to a defect in the gamma-chain of IL-2 also shared by IL-4, -7, -11 and 15, all of which are involved in lymphocyte proliferation and/or differentiation. The autosomal

SCIDs arise primarily from defects in adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) genes which results is accumulation of dATP or dGTP, respectively, and cause toxicity to lymphoid stem cells.

Other genetic defects leading to SCID include those for RAG1, RAG2 and IL-7-alpha. If suspected of SCID, the patient must not receive live vaccine, as it will result in progressing disease.

Diagnosis is based on enumeration of T and B cells and immunoglobulin measurement. Severe combined immunodeficiency can be treated with a bone marrow transplant (see MHC and transplantation). Recently, autosomal SCID patients with ADA deficiency have been treated with a retroviral vector transfected with the gene with some success.

SCID includes several disorders

Recombinase activating genes

Patients having both T and B cell deficiency lack recombinase activating genes (RAG1 and 2) that are responsible for the T cell receptor and immunoglobulin gene rearrangements. These patients are athymic and are diagnosed by examining the T cell receptor (TCR) gene rearrangement. Defects in B cells are not observed in early infant life because of passive antibodies obtained from the mother. NK cells are normal in these patients. This is an autosomal recessive trait.

CD3 chain

In some SCID patients, T cells may be present but functionally defective because of deficiency in signaling mediated by the CD3 chain that is associated with the TCR.

Interleukin-2 receptor

Interleukin-2 receptor common gamma chain (IL-2Rγc) may be lacking in patients thereby preventing signaling by IL-2 and other cytokines which act as growth factors. This leads to a defect in the proliferation of T cells, B cells and NK cells. This is an autosomal recessive trait.

Adenosine deaminase

Adenosine deaminase (ADA) is an enzyme responsible for converting adenosine to inosine. ADA deficiency leads to accumulation of adenosine which results in the production of toxic metabolites that interfere with DNA synthesis. The patients have defects in T, B and NK cells.

SCIDs are autosomal recessive traits and can be treated by gene therapy or stem cell transplantation.

Table 2. Summary of T cell and B cell immunodeficiency diseases (ID)

Disease	T-cells		B-cells No	Immunoglobulins			Inheritance
	No.	Fx		IgM	IgG	IgA	
Reticular dysgenesis	A	A	A	A	A	A	u
CID (autosomal)	A/L	A/L	A/L	A/L	A/L	A/L	a
SCID (x-linked)	A/L	A/L	A/L	A/L	A/L	A/L	x
DiGeorge's syndrome	A/L	A/L	N/V	N/V	N/V	N/V	a/x
Ataxia telangiectasia	L	L	L	N/V	L/V	L	a
Wiskott-Aldrich	?V	L	L/V	L also high IgE	N	H	x
X-linked hypo-gamma- globulinemia	N	N	L	L	L	L	x
Selective IgA immunodeficiency	N	N	N	N	L/V	L	a/x
Hyper-IgM hypo-gamma- globulinemia	N	N	N	H	L	L	x
Transient hypo-gamma- globulinemia	N	N	N	N	L	L	a?
Common variable hypo-gamma-globulinemia (teens-adult)	N	N	N	N	L	L	none

**A: absent; a: autosomal; H: high; L: low; N: normal; U; unknown; V: variable; x: x-linked**

Disorders of T cells

T cell disorders affect both cell-mediated and humoral immunity making the patient susceptible to viral, protozoal and fungal infections. Viral infections such as those by cytomegalovirus and attenuated measles in the vaccine can be fatal in these patients.
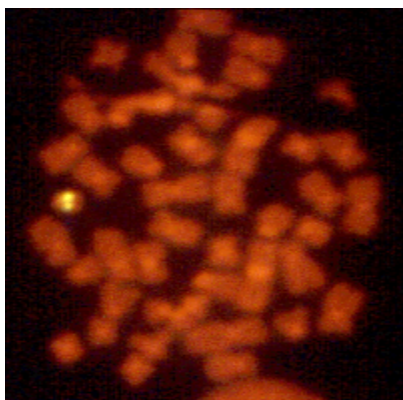
DiGeorge's Syndrome (Deletion 22 Syndrome)

This the most clearly defined T-cell immunodeficiency and is also known as congenital thymic aplasia/hypoplasia, or immunodeficiency with hypoparathyroidism. The syndrome is associated with hypoparathyroidism, congenital heart disease, low set notched ears and fish shaped mouth. These defects results from abnormal development of the fetus (3rd and 4th pharyngeal pouch) during the 6th to 10th week of gestation when parathyroid, thymus, lips, ears and aortic arch are being formed. No genetic predisposition is clear and not all DiGeorge syndrome babies have thymic aplasia. A thymic graft taken from an early fetus (13 - 14 weeks of gestation) can be used for treatment. Older grafts may result in GVH reaction. In severely immunodeficient DiGeorge patients, live vaccines may cause progressive infections.

DiGeorge syndrome is autosomal dominant (figure 2)
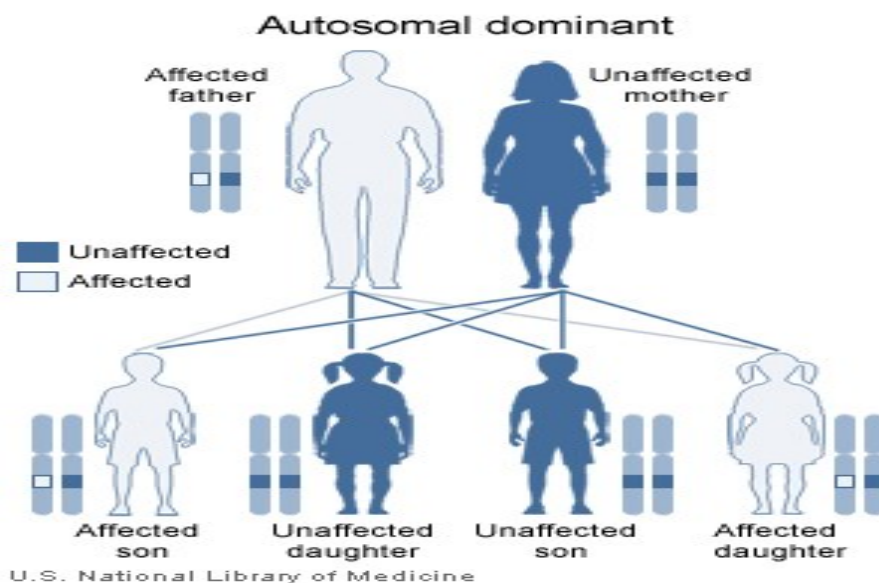
Figure 2 In DiGeorge's syndrome, 22q11.2 deletion is inherited in an autosomal dominant pattern. National Library of Medicine - NIH

and is caused by a deletion in chromosome 22 (figure 3).

Figure 3 Deletion of genes in DiGeorge syndrome can be visualized by a fluorescent signal on only one of the two copies of chromosome 22 David Ian Wilson, University of Newcastle on Tyne - NIH

The deletions are of variable size but size does not correlate with severity of disease. In about 6% of cases, the chromosome 22 microdeletion is inherited but most cases result from *de novo* deletion which may be caused by environmental factors. Patients may be treated with a thymic graft.

T cell deficiencies with variable degrees of B cell deficiency

Ataxia-telangiectasia

Ataxia-telangiectasia is a deficiency of T cells associated with a lack of coordination of movement (ataxis) and dilation of small blood vessels of the facial area (telangiectasis). T-cells and their functions are reduced to various degrees. B cell numbers and IgM concentrations are normal to low. IgG is often reduced and IgA is considerably reduced (in 70% of the cases). There is a high incidence of malignancy, particularly leukemias, in these patients. The defects arise from a breakage in chromosome 14 at the site of TCR and immuinoglobulin heavy chain genes.

Wiskott-Aldrich syndrome

Wiskott-Aldrich syndrome syndrome is associated with normal T cell numbers with reduced functions, which get progressively worse. IgM concentrations are reduced but IgG levels are normal. Both IgA and IgE levels are elevated. Boys with this syndrome develop severe eczema, petechia (due to platelet defect and thrombocytopenia). They respond poorly to polysaccharide antigens and are prone to pyogenic infection. Wiskott-Aldrich syndrome is an X-linked disorder (figure 4) due to defect in a cytoskeletal glycoprotein, CD43.
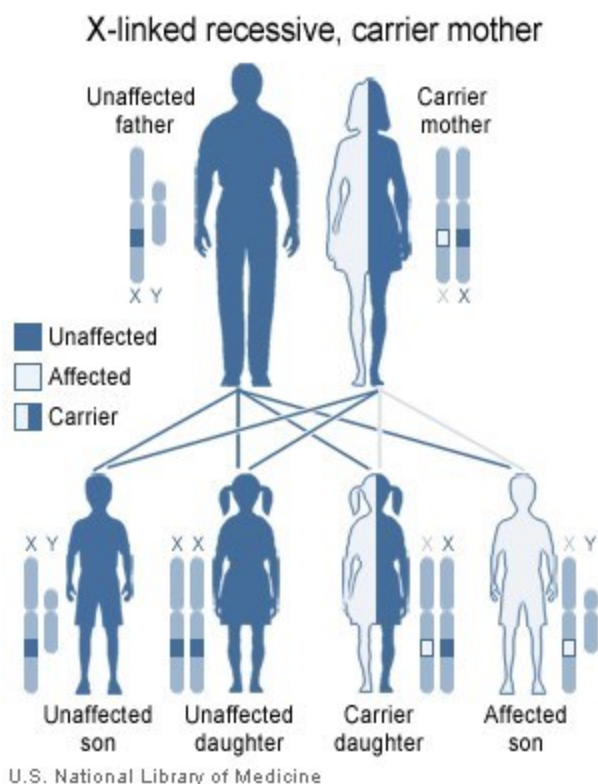

Figure 4 Wiskott-Aldrich syndrome is an X-linked disorder National Library of Medicine - NIH

MHC deficiency (Bare leukocyte syndrome)

A number of cases of immunodeficiency have been described in which there is a defect in the MHC class II transactivator (CIITA) protein gene, which results in a lack of class II MHC molecules on their APC. Since the positive selection of CD4 cells in the thymus depends on the presence of these MHC molecules, these patients have fewer CD4 cells and are infection prone. There are also individuals who have a defect in their transport associated protein (TAP) gene and hence do not express the class I MHC molecules and consequently are deficient in CD8$^+$ T cells.

Disorders of B lymphocytes

There are a number of diseases in which T cell numbers and functions are normal: B cell numbers may be low or normal but immunoglobulin levels are low.

X-linked infantile hypogammaglobulinemia

X-linked hypogammaglobulinemia, also referred to as Bruton's hypoglobulinemia or agammaglobulinemia, is the most severe hypogammaglobulinemia in which B cell numbers and all immunoglobulin levels are very low. The patients have failure of B-cell maturation associated with a defective B cell tyrosine kinase (*btk*) gene. Thus, B cells exist as pre-B cells with H chains but not L chains rearranged. Diagnosis is based on enumeration of B cells and immunoglobulin measurement. Patients have no immunoglobulins and suffer from recurrent bacterial infections.

Transient hypogammaglobulinemia

Children, at birth, have IgG levels comparable to that of the mother. Because the half life of IgG is about 30 days, its level gradually declines, but by three months of age normal infants begin to synthesize their own IgG. In some infants, however, IgG synthesis may not begin until they are 2 to 3 years old. This delay has been attributed to poor T cell help. This results in a transient deficiency of IgG which can be treated with gamma-globulin.

Common variable hypogammaglobulinemia (Late onset hypogammaglobulinemia)
These individuals have deficiencies of IgG and IgA in the 2nd or 3rd decade of their life because B cells fail to differentiate into plasma cells. These patients are susceptible to a variety of pyogenic bacteria and intestinal protozoa. They should be treated with specially prepared gamma-globulin for intravenous use.

IgA deficiency

IgA deficiency is the commonest of all immunodeficiencies (1/700 of all Caucasians) and results from a defect in class switching. About 20% of individuals with IgA deficiency also have low IgG. IgA-deficient patients are very susceptible to gastrointestinal, eye and nasopharyngeal infections. Patients with IgA deficiency have a high incidence of autoimmune diseases (particularly immune complex type) and lymphoid malignancies. Anti-IgA antibodies (IgG) are detected in 30 to 40 percent of patients who should not be treated with γ-globulins. Laboratory diagnosis is based on IgA measurement.

Selective IgG deficiency

Deficiencies of different IgG subclasses have been found. These patients are susceptible to pyogenic infections.

X-linked Hyper-IgM immunodeficiency

Individuals with this type of immunodeficiency have low IgA and IgG concentrations with abnormally high levels of IgM. These patients cannot make a switch from IgM to other classes which is attributed to a defect in CD40L on their CD4 cells. They are very susceptible to pyogenic infection and should be treated with intravenous gamma-globulins.

NON-SPECIFIC IMMUNE SYSTEM - DEFECTS IN THE MYELOID LINEAGE

Primary immunodeficiencies of the non-specific immune system include defects in phagocytic and NK cells and the complement system.

Congenital Agranulomatosis

Patients have a decrease in the neutrophil count. This is due to a defect in the myeloid progenitor cell differentiation into neutrophils. These patients are treated with granulocyte-macrophage colony stimulating factor (GM-CSF) or G-CSF.

Defects of the phagocytic system

Defects of phagocytic cells (numbers and/or functions) can lead to increased susceptibility to a variety of infections.

Cyclic neutropenia

This is marked by low numbers of circulating neutrophil approximately every three weeks. The neutropenia lasts about a week during which the patients are susceptible to infection. The defect appears to be due to poor regulation of neutrophil production.

Chronic granulomatous disease (CGD)

CGD is characterized by marked lymphadenopathy, hepato- splenomegaly and chronic draining lymph nodes. Leukocytes have poor intracellular killing (figure 5) and low respiratory burst. In majority of these patients, the deficiency is due to a defect in NADPH oxidase (cytochrome b_{558} : gp91^{phox}, or rarely gp22^{phox}) or other cofactor proteins (gp47^{phox}, gp67^{phox}) that participate in phagocytic respiratory burst. These patients can be diagnosed on the basis of poor Nitroblue tetrazolium (NBT) reduction which is a measure of respiratory burst. Interferon-gamma therapy has been successful.

Leukocyte Adhesion Deficiency

In this disease, T cells and macrophages lack the complement receptor CR3 due to a defect in CD11 or CD18 peptides and consequently they cannot respond to C3b opsonin. Alternatively there may a defect in integrin molecules, LFA-1 or mac-1 arising from defective CD11a or CD11b peptides, respectively. These molecules are involved in diapedesis and hence defective neutrophils cannot respond effectively to chemotactic signals. Treatment is with bone marrow (devoid of T cells and MHC-matched) transplantation or gene therapy.

Chediak-Higashi syndrome

Chediak-Higashi syndrome is marked by reduced (slower rate) intracellular killing and chemotactic movement accompanied by inability of phagosome and lysosome fusion and proteinase deficiency. Giant lysosomes (intracellular granules) are often seen (figure 6). The respiratory burst is normal. Accompanying NK cell defects and platelet and neurological disorders are noted.
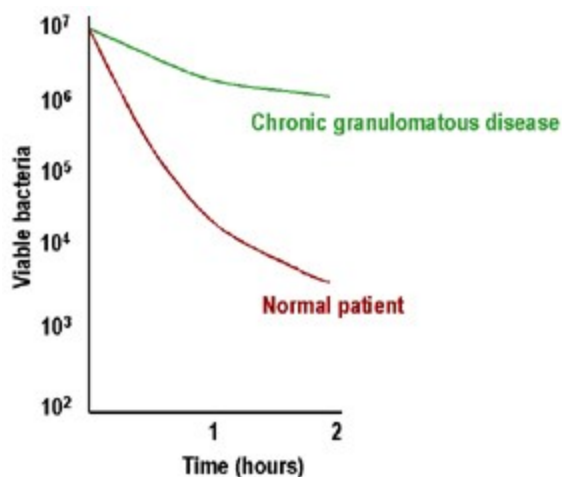

Figure 5 Poor intracellular killing of bacteria in chronic granulomatous disease
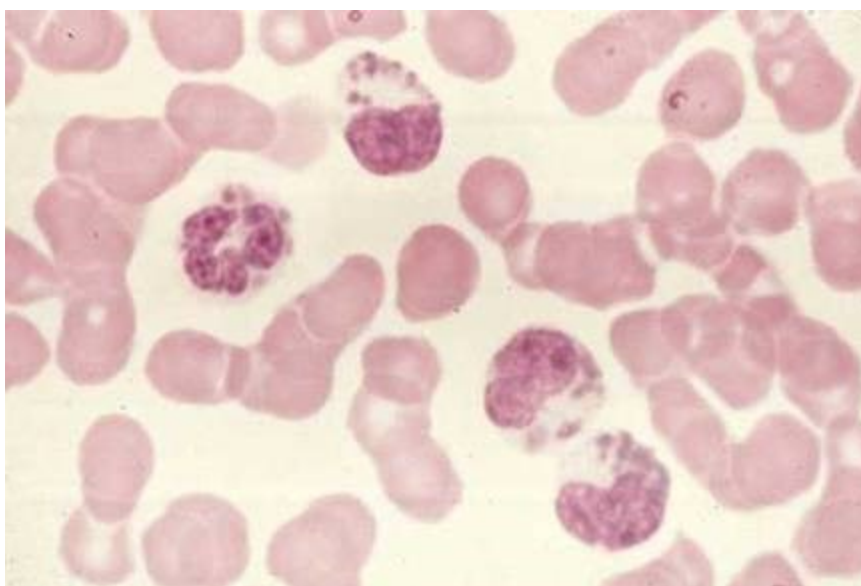
Figure 6 This slide is from a patient with Chediak-Higashi Syndrome. Extremely large granules are seen in the cytoplasm of granulocytes. They result from abnormal fusion of granules during their formation. The abnormal granules are found in many other cell types throughout the body National Cancer Inst

DISORDERS OF COMPLEMENT SYSTEM

Complement abnormalities also lead to increased susceptibility to infections. There are genetic deficiencies of various components of complement system, the most serious of which is the C3 deficiency which may arise from low C3 synthesis or deficiency in factor I or factor H.

SECONDARY (ACQUIRED) IMMUNODEFICIENCIES

Immunodeficiencies associated with infections

Bacterial, viral, protozoan, helminthic and fungal infections may lead to B cell, T cell, PMN and macrophage deficiencies. Most prominent among these is acquired immunodeficiency syndrome (AIDS). Secondary immunodeficiencies are also seen in malignancies.

Immunologic abnormalities in the AIDS

All acquired immunodeficiencies have been outdone by AIDS that is caused by Human Immunodeficiency Virus (HIV)-1. This virus was first discovered in 1981 and the patients exhibited fungal infections with opportunistic organisms such as *Pneumocystis carinii* and in other cases, with a skin tumor known as Kaposi's sarcoma. There are two major types of HIV: HIV-1 and 2, the former being the strain frequently found in North America. HIV is spread through sexual intercourse, infected blood and body fluids as well as from mother to offspring. HIV is a retrovirus with RNA that is reverse transcribed to DNA by reverse transciptase (RT) following entry into the cell. The DNA is integrated into the cell genome as a provirus that is replicated along with the cell. HIV-1 does not replicate in most other animals but infects chimpanzees although it does not induce AIDS in them. Severe combined immunodeficient mice (SCID) reconstituted with human lymphocytes can be infected with HIV-1. The HIV-1 virion consists of a viral envelope made up of the outer lipid bilayer of the host cell in which are embedded glycoproteins composed of the transmembrane gp41 along with the associated gp120. The gp120 binds the CD4 expressed on host cells. Within the viral envelope is the viral core or nucleocapsid consisting of a layer of matrix protein composed of p17 and an inner capsid made up of p24. The viral genome consists of two single stranded RNA molecules associated with two RT molecules as well as other enzymes including a protease and an integrase.

Replication cycle and targets of therapy

The virus attaches to the CD4 molecule on Th cells, monocytes and dendritic cells through the gp120 of HIV. For HIV infection, a co-receptor is required. The co-receptor is a chemokine receptor such as CXCR4 or CCR5. CCR5, expressed predominantly on macrophages, and CXCR4 on CD4+ T cells serve as coreceptors for HIV infection. After the fusion of HIV envelope and the host membrane, the nucleocapsid enters the cell. The RT synthesizes viral DNA which is transported to the nucleus where

it integrates with the cell DNA in the form of a [provirus](). The provirus can remain latent until the cell is activated when the provirus also undergoes transcription. Virions, consisting of the transcribed viral RNA and proteins, are produced. These bud out of the host cell membrane from where they acquire the envelope. Thus, therapeutic agents have been developed that target viral entry and fusion, as well as serve as RT, protease and integrase inhibitors. [Highly active anti-retroviral therapy]() is a cocktail of 3 or more such agents.

Immunological Changes

The virus replicates rapidly and within about two weeks the patient may develop fever. The viral load in the blood increases significantly and peaks in two months, after which there is a sudden decline because of the latent virus found in germinal centers of the lymph nodes. CTL develop very early whereas antibodies can be detected between 3 - 8 weeks. The CTL killing of Th cells around 4 - 8 weeks leads to a decrease in CD4+ T cells. When the CD4+ T cell count decreases below 200 per cubic mm, full blown AIDS develops.

Immunotherapy
There are several barriers to development of an effective HIV vaccine.

- Attenuated vaccine may induce the disease
- CD4+ T cells may be destroyed by the vaccine
- Antigenic variation of HIV
- Low immunogenicity of the virus by downregulation of MHC molecules
- Lack of animal models
- Lack of in vitro tests

The following reagents have been considered in developing vaccines

- Immunization with deletion mutants to reduce pathogenicity
- Vaccination with recombinant proteins
- Gene encoding proteins introduced into virus vectors may be used for vaccination
- Chemokines that compete for the co-receptors
- IL-2 to boost the Th cells.

For more on HIV and AIDS go [here]()

Immunodeficiencies associated with aging

These include a progressive decrease in thymic cortex, hypo-cellularity of and reduction in the size of thymus, a decrease in suppressor cell function and hence an increase in auto-reactivity, a decrease in CD4 cells functions. By contrast B cells functions may be somewhat elevated.

Immunodeficiencies associated with malignancies and other diseases

B cell deficiencies have been noted in multiple myeloma, Waldenstrom's macroglobulinemia, chronic lymphocytic leukemia and well differentiated lymphomas. Hodgkin's disease and advanced solid tumors are associated with impaired T-cell functions. Most chemotherapeutic agents used for treatment of malignancies are also immunosuppressive.

Other conditions in which secondary immunodeficiencies occur are sickle cell anemia, diabetes mellitus, protein calorie malnutrition, burns, alcoholic cirrhosis, rheumatoid arthritis, renal malfunction, *etc*.