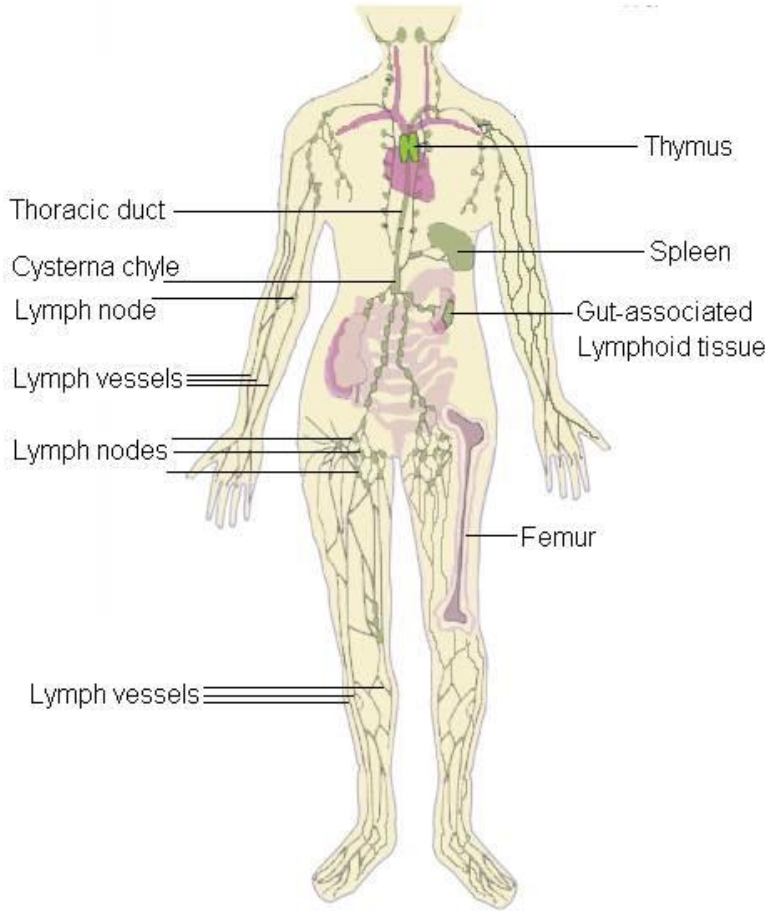
Introduction

- The **immune system** is a system of biological structures and processes within an organism that protects against disease
- To function properly, an immune system must detect a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue

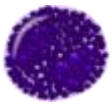
Anatomy of the immune system

- The **lymphatic system** is part of the circulatory system, comprising a network of lymphatic vessels that carry a clear fluid called lymph (from Latin *lympha* meaning *water*[1]) directionally towards the heart
- The lymph is formed when the interstitial fluid (the fluid which lies in the interstices of all body tissues) is collected through lymph capillaries. It is then transported through lymph vessels to lymph nodes before emptying ultimately into the right or the left subclavian vein, where it mixes back with blood
- One of the main functions of the lymph system is to provide an accessory return route to the blood. The other main function is that of defense in the immune system. Lymph is very similar to blood plasma but contains lymphocytes and other white blood cells
- The **central** or **primary lymphoid organs** generate lymphocytes from immature progenitor cells. This includes the bone marrow and the thymus
- **Secondary** or **peripheral lymphoid organs**, which include lymph nodes and the spleen, maintain mature naive lymphocytes and initiate an adaptive immune response. The peripheral lymphoid organs are the sites of lymphocyte activation by antigens

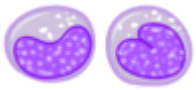


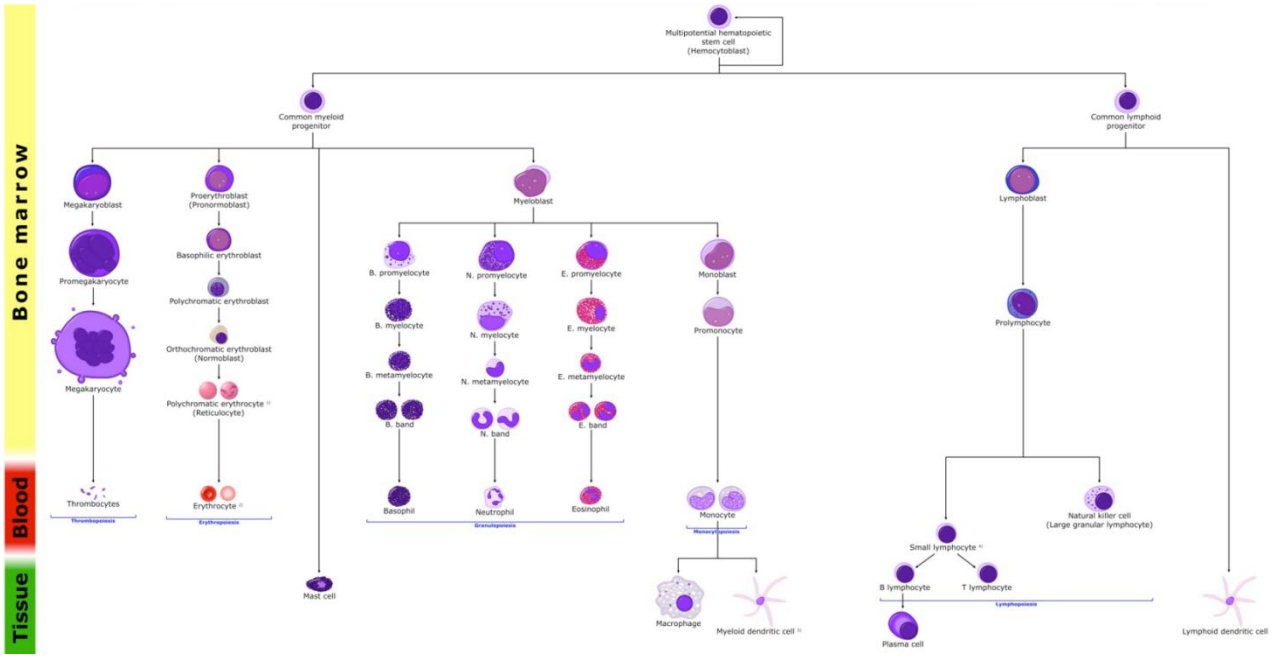
Leukocytes

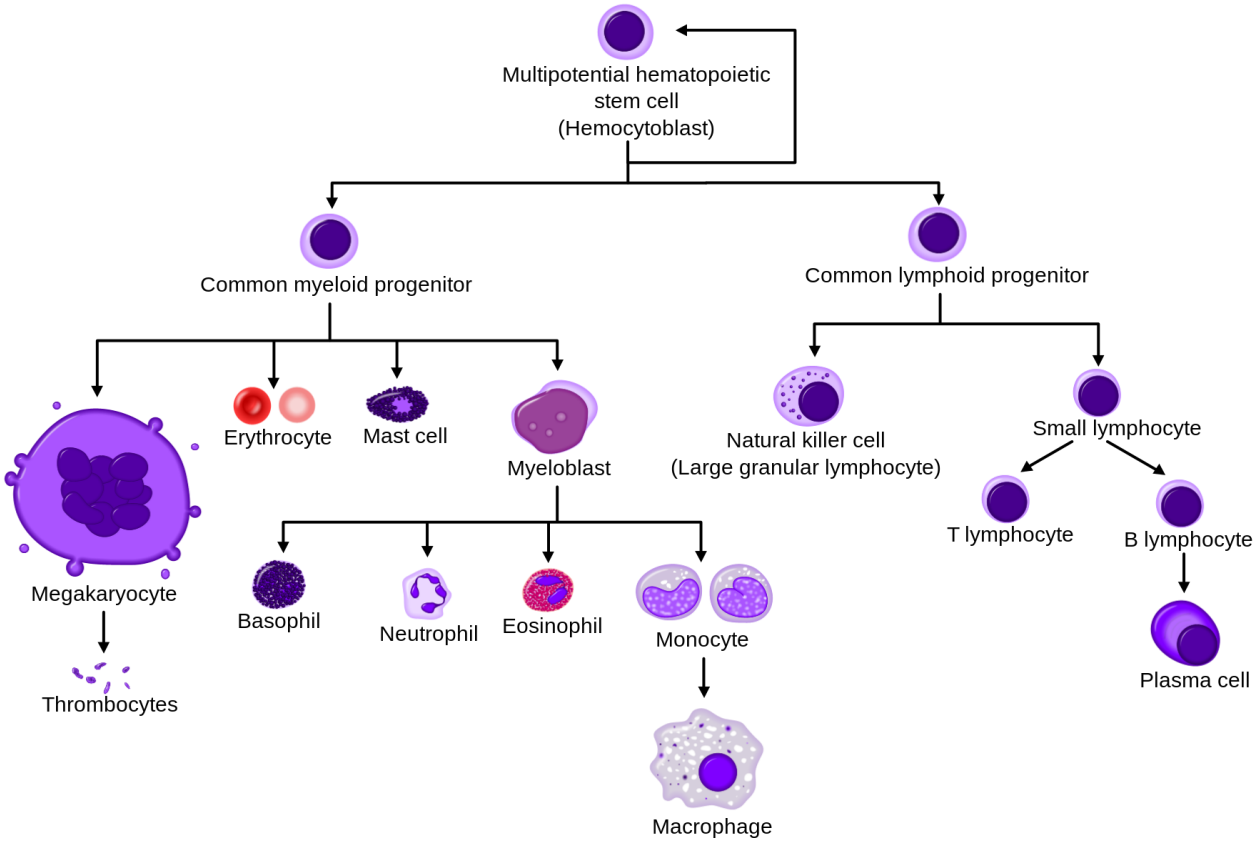
- Cells responsible for immunity are termed leukocytes (Greek leukos, white, kytos, cell). Originate from stem cells in foetal liver and bone marrow
- All leukocytes are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system

Type	Diagram	Approx. % in adults	Main targets	Granules
Neutrophil		72%	• Bacteria • Fungi	Fine, faintly pink (H&E stain)
Eosinophil		2.3%	• Larger parasites • Modulate allergic inflammatory responses	Full of pink-orange (H&E stain)
Basophil		0.4%	• Release histamine for inflammatory responses	Large blue

Lymphocyte		30%	• B cells: releases antibodies and assists activation of T cells
			• T cells:
			  ○ CD4+ Th (T helper) cells: activate and regulate T and B cells
			  ○ CD8+ cytotoxic T cells: virus-infected and tumor cells.
			  ○ γδ T cells: bridge between innate and adaptive immune responses; phagocytosis
			  ○ Regulatory (suppressor) T cells: prevents autoimmunity
			• Natural killer cells: virus-infected and tumor cells.
				NK-cells and cytotoxic T-cells

Monocyte		5.3%	• Differentiate into tissue resident macrophages • Become Kupffer cells in the liver	None





Innate vs Adaptive

These two "arms" are inextricably linked- the nature of the acquired response depends on the innate responses that are triggered.

Innate immune system	Adaptive immune system
Response is non-specific	Pathogen and antigen specific response
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Surface Barriers

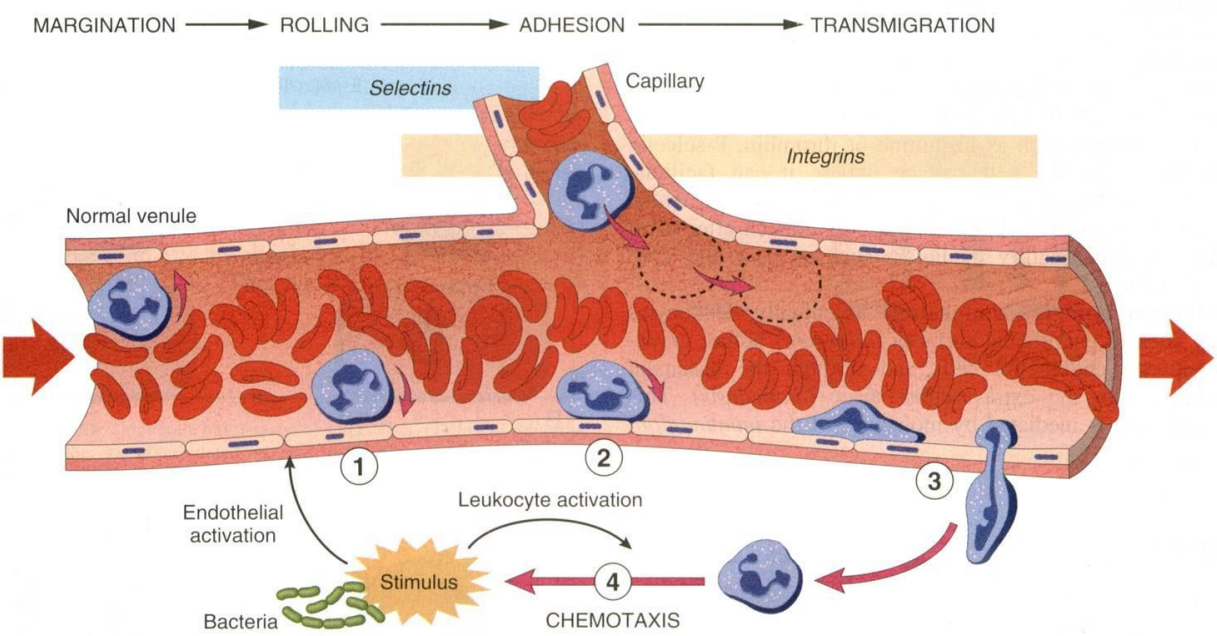
- Several barriers protect organisms from infection, including mechanical, chemical, and biological barriers
- Skin forms an effective barrier against infection, as it consists of a thick outer layer of keratinocytes which are regularly shed, are dry, mildly acidic, and already colonised by normal microbiota which compete against pathogens
- However, as organisms cannot be completely sealed from their environments, other systems act to protect body openings such as the lungs, intestines, and the genitourinary tract
- Lysozymes are enzymes that damage bacterial cell walls. It is abundant in a number of secretions, such as tears, saliva, human milk, and mucus

Anatomical barrier	Additional defense mechanisms
Skin	Sweat, desquamation, flushing, organic acids
Gastrointestinal tract	Peristalsis, gastric acid, bile acids, digestive enzyme, flushing, thiocyanate, defensins, gut flora
Respiratory airways and lungs	Mucociliary elevator, surfactant,[4] defensins[4]
Nasopharynx	Mucus, saliva, lysozyme
Eyes	Tears

- **Immunoglobulin A** (**IgA**, also referred to as **sIgA**) is an antibody that plays a critical role in mucosal immunity. IgA is the main immunoglobulin found in mucous secretions, including tears, saliva, sweat, colostrum and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium

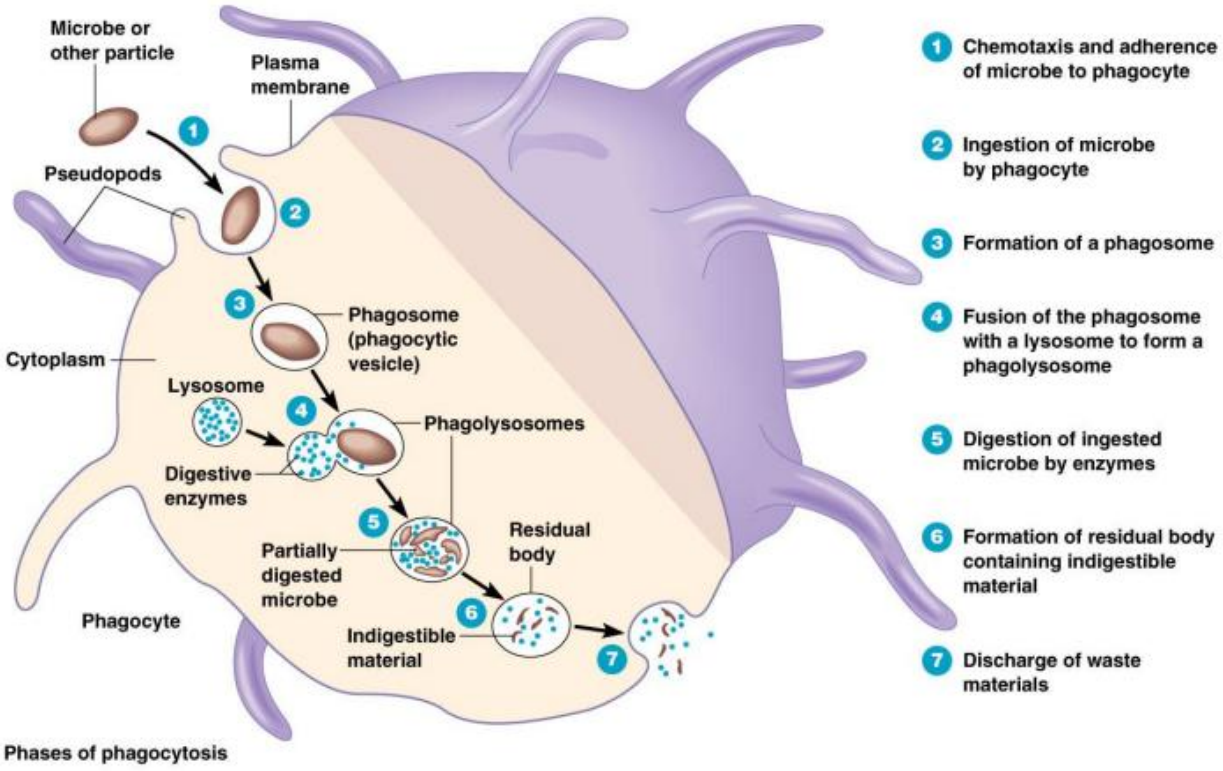
Inflammation

- Inflammation is a protective immunovascular response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair
- The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity
- Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process
- Damaged cells release chemicals including histamine, bradykinin, and prostaglandins. These chemicals cause blood vessels to leak fluid into the tissues (extravasation), causing swelling. This helps isolate the foreign substance from further contact with body tissues. The chemicals also attract white blood cells called phagocytes that "eat" germs and dead or damaged cells
- Inflammatory mediators released by injured cells decrease pH. This activates Bradykinin, a chemical which activates Mast cells, that then release histamine, which increases the permeability of the capillaries to white blood cells and some proteins



Phagocytotic Cells

- The process by which a cell—often a phagocyte or a protist—engulfs a solid particle to form an internal vesicle known as a phagosome. The phagosome of ingested material is then fused with the lysosome, forming a phagolysosome and leading to degradation by various enzymes
- **Toll-like receptors** (**TLRs**) are a class of proteins that play a key role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors usually expressed in sentinel cells such as macrophages and dendritic cells. TLR recognise specific, pathogen associated molecules
- Activation of TLRs is how the "innate" immune system differentiates self and non-self, as self cells do not contain PAMPS
- Monocytes and macrophages: a type of white blood cell that engulfs and digests cellular debris, foreign substances, microbes, and cancer cells in a process called phagocytosis. They also help initiate specific defense mechanisms (adaptive immunity) by recruiting other immune cells such as lymphocytes
- Neutrophils: the most abundant (40% to 75%) type of white blood cells. Neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation
- Dendritic cells: phagocytes in tissues that are in contact with the external environment; therefore, they are located mainly in the skin, nose, lungs, stomach, and intestines
- Recognition can be opsonin-dependent or opsonin-independent



Non-Phagocytotic Cells

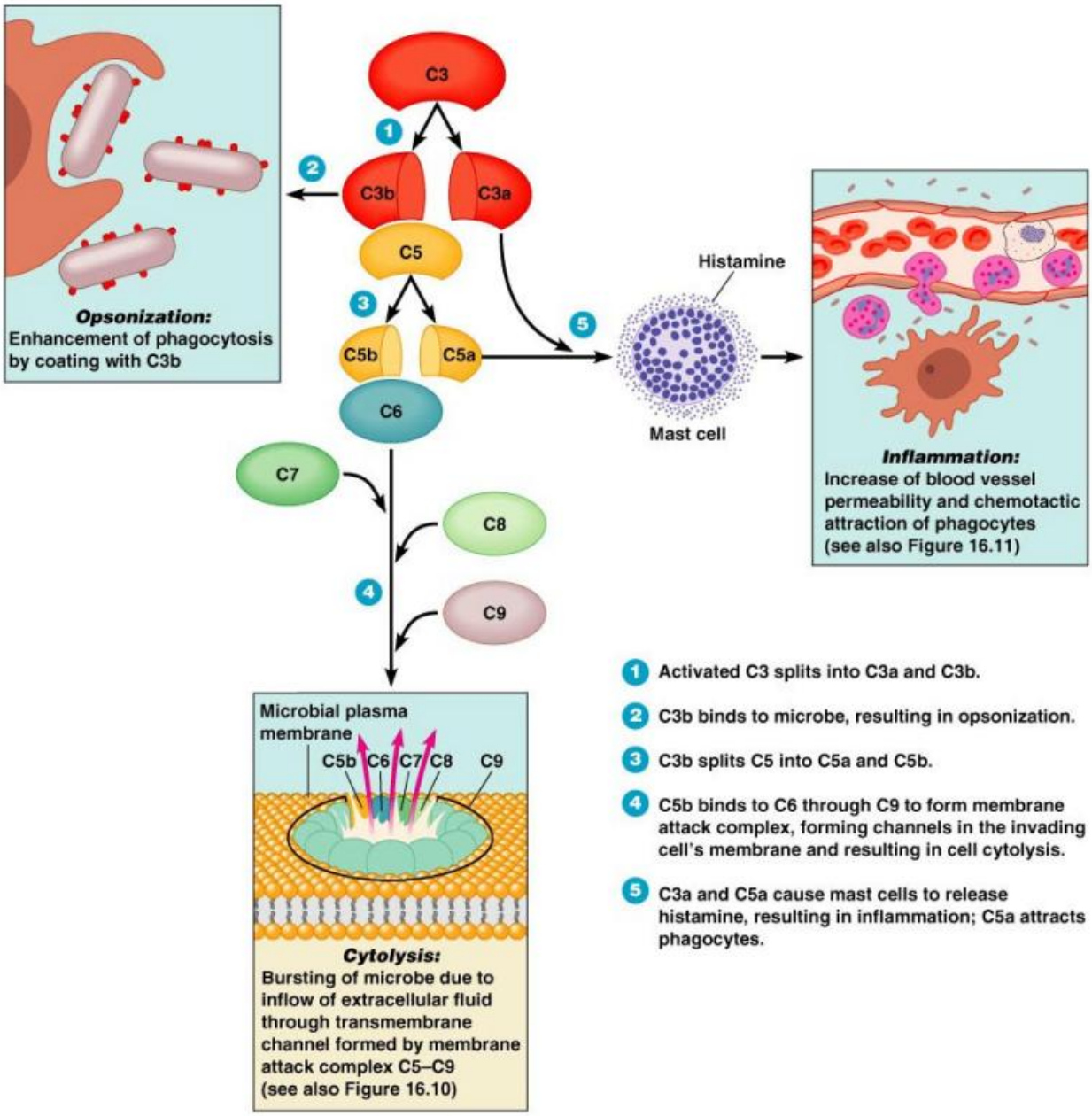
- Natural Killer cells: a component of the innate immune system which does not directly attack invading microbes. Rather, NK cells destroy compromised host cells, such as tumor cells or virus-infected cells, recognizing such cells by a condition known as "missing self." This term describes cells with low levels of a cell-surface marker called MHC I (major histocompatibility complex) – a situation that can arise in viral infections of host cells. They kill cells by releasing toxins which punch holes in the membrane, and also triggering apotosis
- Basophils: appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues
- Eosinophils: one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. Along with mast cells, they also control mechanisms associated with allergy and asthma

Complement System

- The complement system consists of a number of small proteins found in the blood, in general synthesized by the liver, and normally circulating as inactive precursors (pro-proteins)
- Activation of complement is by a sequential cascade involving at least 30 proteins
- Three pathways of activation
    - Classical: requires antibody
    - Alternative: stimulated by endotoxins, polysaccharides etc.
    - Lectin: stimulated by mannose-binding protein released by macrophages after phagocytosis
- Four main functions
    - Opsonization (C3b): enhancing phagocytosis of antigens
    - Chemotaxis (C3a, C5a): attracting macrophages and neutrophils

- Cell Lysis (C5a, C6-9): rupturing membranes of foreign cells
- Agglutination: clustering and binding of pathogens together (sticking)

- C5b initiates the membrane attack pathway, which results in the **membrane attack complex** (MAC), consisting of C5b, C6, C7, C8, and C9. MAC is the cytolytic endproduct of the complement cascade; it forms a transmembrane channel, which causes osmotic lysis of the target cell



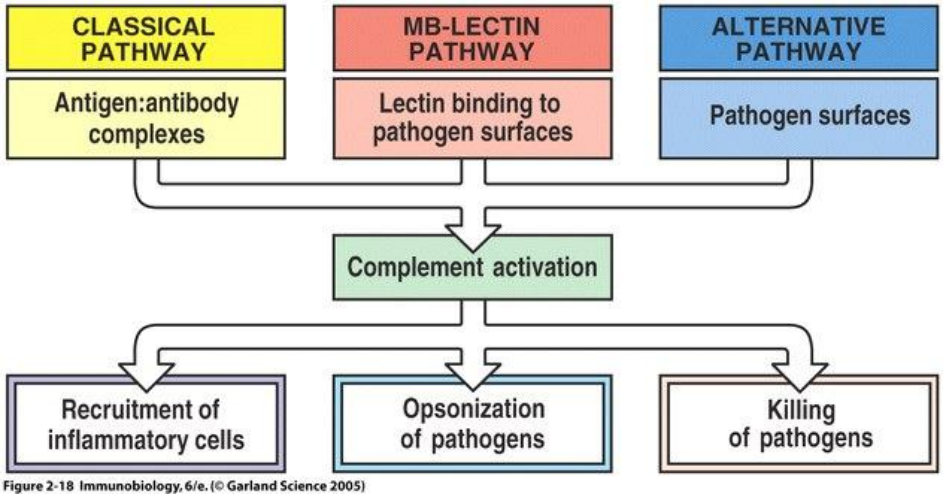


Figure 2-18 Immunobiology, 6/e. (© Garland Science 2005)

Cytokines

- A broad and loose category of small proteins that are important in cell signaling
- Cytokines are involved in the differentiation of stem cells into immune system cells
- Can effect the cell from which they were secreted (autocrine function), nearby cells (paracrinefunction), or distributed into the circulation (endocrine function)
- Cytokines bind to specific cellular receptors to switch on genes, proteins produced
- Common cytokines include interleukins that are responsible for communication between white blood cells; chemokines that promote chemotaxis; and interferons that have anti-viral effects, such as shutting down protein synthesis in the host cell
- Certain cytokines cause fever after an infection, which restricts the pathogens survival

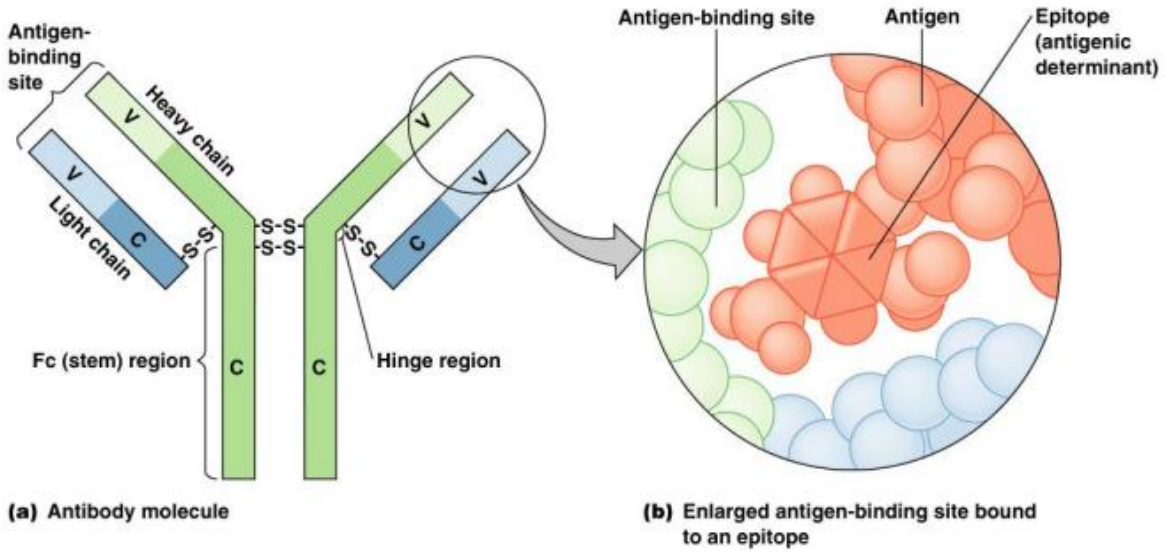
TABLE 17.3 A Summary of Some Important Cytokines

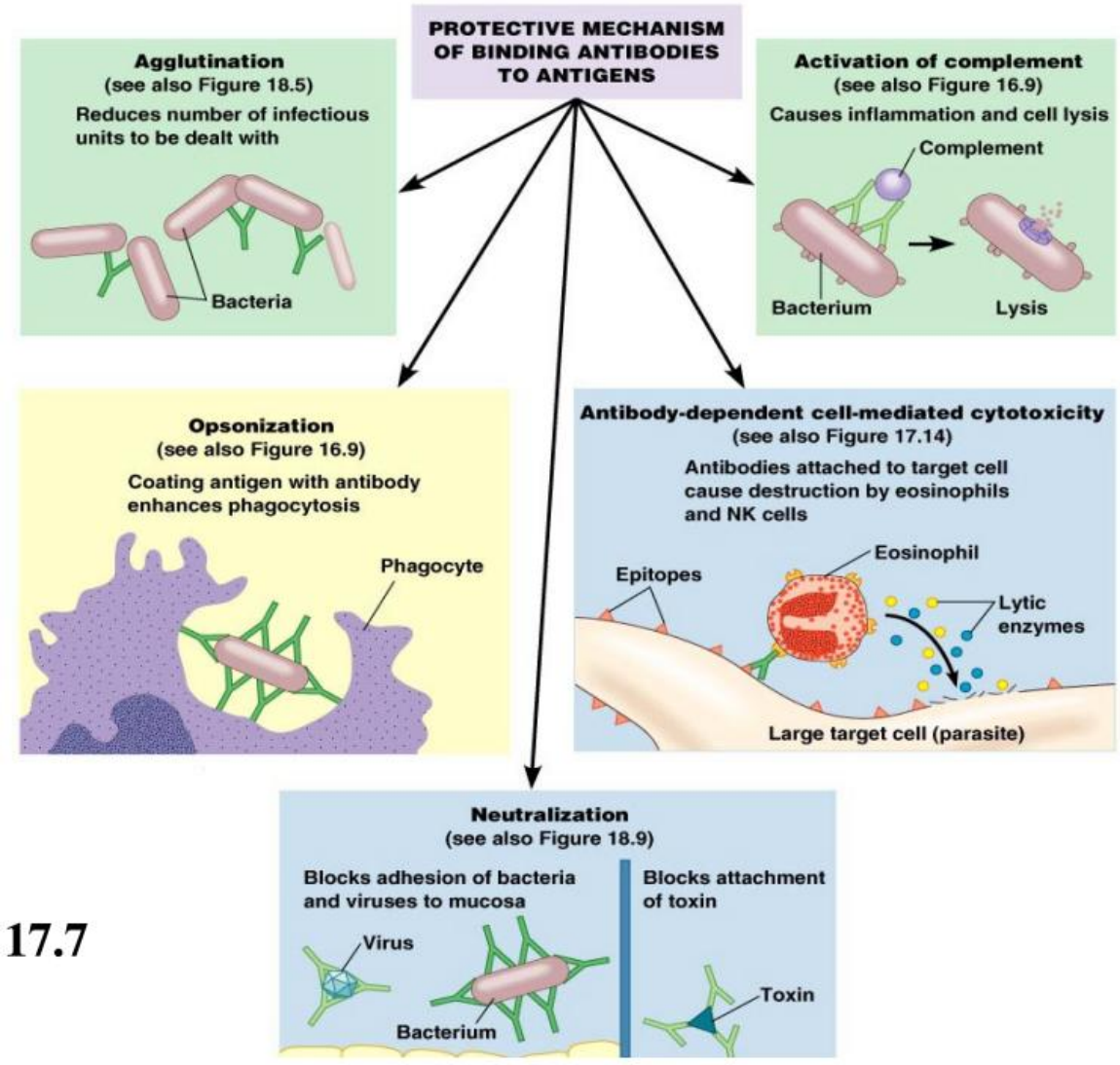
Cytokine	Representative Activity
Interleukin-1 (IL-1)	Stimulates T_H cells in presence of antigens; chemically attracts phagocytes in inflammatory response
Interleukin-2 (IL-2)	Involved in proliferation of antigen-stimulated T_H cells, proliferation and differentiation of B cells, and activation of T_C cells and NK cells
Interleukin-8 (IL-8)	Chemoattractant for immune system cells and phagocytes to site of inflammation
Interleukin-10 (IL-10)	Secreted by T_H2 cells and T_R cells; interferes with activation of T_H1 cells
Interleukin-12 (IL-12)	Mainly involved in differentiation of CD4 T cells
Interferons (IFNs)	
α-IFN and β-IFN	Induces antiviral activity in nucleated cells (interferes with protein synthesis)
γ-IFN	Activates macrophages; improves antigen presentation
Alpha-tumor necrosis factor (α-TNF)	Cytotoxic to tumor cells: enhances activity of phagocytic cells

Antibody

- An antibody (AB), also known as an immunoglobulin (Ig), is a large Y-shape protein produced by plasma cells that is used by the immune system to identify and neutralize foreign objects such as bacteria and viruses
- Each tip of the "Y" of an antibody contains a paratope (a structure analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Each antigen can contain several epitopes, called multivalent antigens. Small molecules (haptens) can become immunogenic if coupled to a carrier
- Antibodies can occur in two physical forms, a soluble form that is secreted from the cell, and a membrane-bound form that is attached to the surface of a B cell and is referred to as the B cell receptor (BCR)
- The BCR is found only on the surface of B cells and facilitates the activation of these cells and their subsequent differentiation into either antibody factories called plasma cells or memory B cells that will survive in the body and remember that same antigen so the B cells can respond faster upon future exposure
- In most cases, interaction of the B cell with a T helper cell is necessary to produce full activation of the B cell and, therefore, antibody generation following antigen binding
- Antibodies that bind to surface antigens (for example, on bacteria) will attract the first component of the complement cascade with their Fc region and initiate activation of the "classical" complement system

- Opsonization involves the binding of an opsonin, e.g., antibody, to an epitope on an antigen. After opsonin binds to the membrane, phagocytes are attracted to the pathogen

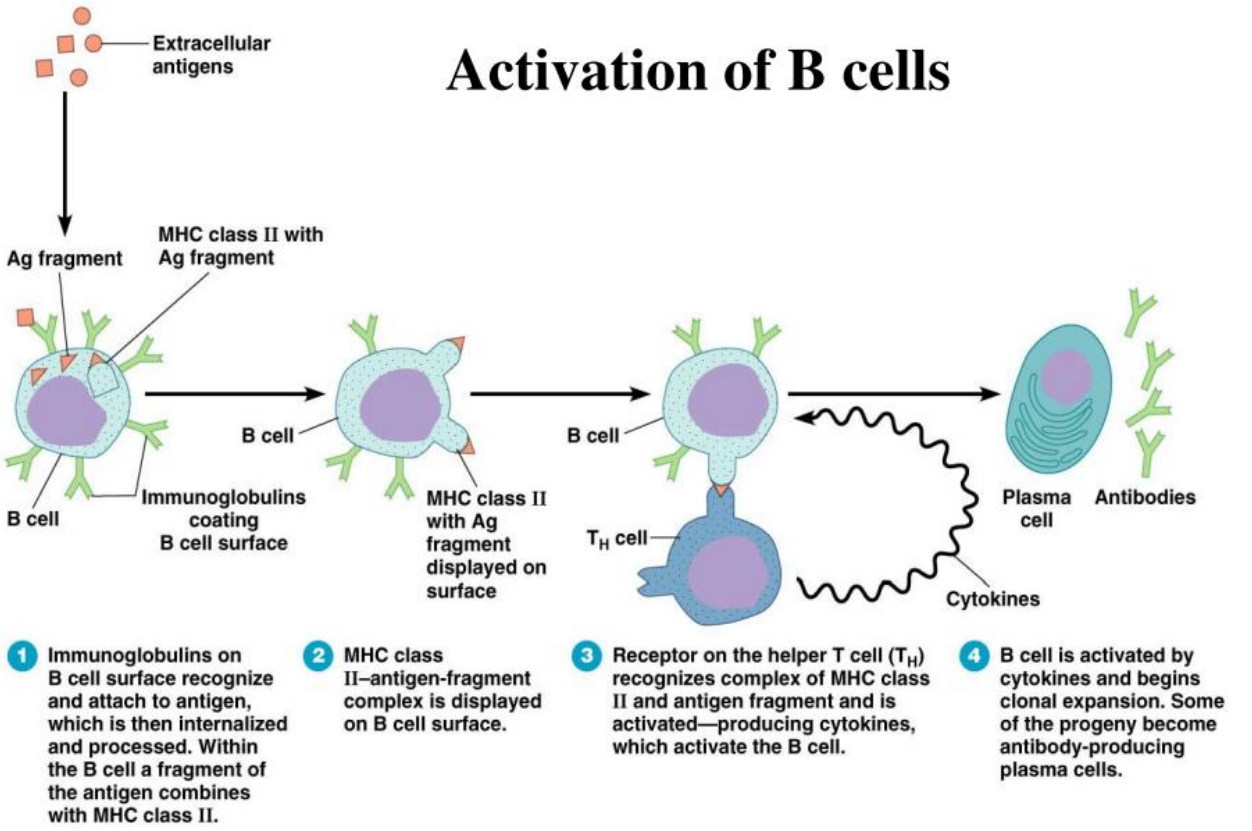




17.7

B Cells (Humoral)

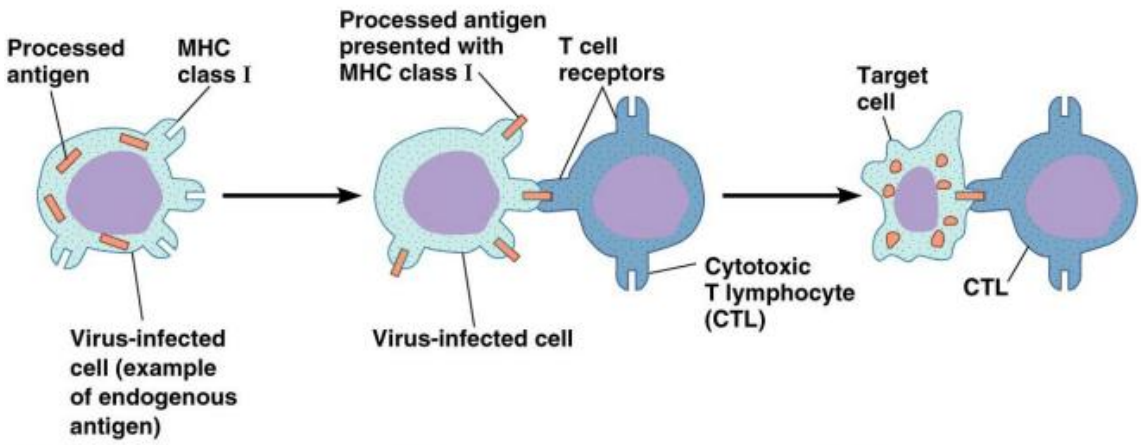
- B cells or B lymphocytes are a type of lymphocyte in the humoral immunity of the adaptive immune system. B cells can be distinguished from other lymphocytes, such as T cells and natural killer cells (NK cells), by the presence of a protein on the B cell's outer surface known as a B cell receptor (BCR). This specialized receptor protein allows a B cell to bind to a specific antigen
- In mammals, immature B cells are formed in the bone marrow
- The principal functions of B cells are to make antibodies against antigens, to perform the role of antigen-presenting cells (APCs), and to develop into memory B cells after activation by antigen interaction. B cells also release cytokines (proteins), which are used for signaling immune regulatory functions
- Each B cell has a unique receptor protein (referred to as the B cell receptor (BCR)) on its surface that will bind to one particular antigen. The BCR is a membrane-bound immunoglobulin, and it is this molecule that allows the distinction of B cells from other types of lymphocyte, as well as being the main protein involved in B cell activation
- Once a B cell encounters its cognate antigen and receives an additional signal from a T helper cell, it can further differentiate into one of the two types of B cells: plasma B cells and memory B cells
- **Memory B cells** differentiate from activated B cells predominantly from a germinal center and are specific to the antigen encountered during the primary immune response. These cells are able to live for a long time, and can respond quickly following a second exposure to the same antigen.
- **Plasma B cells** are large B cells that have been exposed to antigen and produce and secrete large amounts of antibodies, which assist in the destruction of microbes by binding to them and making them easier targets for phagocytes and activation of the complement system. They are sometimes referred to as *antibody factories.* An electron micrograph of these cells reveals large amounts of rough endoplasmic reticulum, responsible for synthesizing the antibody, in the cell's cytoplasm.



T Cells (Cellular)

- T cells or T lymphocytes are a type of lymphocyte that plays a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface
- T cells mature in the thymus. They do not recognise native antigen, but recognise antigen "presented" by another cell (antigen presenting cell, APC). The antigen is presented as short peptides bound to a Major Histocompatibility Complex (MHC) protein
- The binding between TCR and antigen is of relatively low affinity and is degenerate: that is, many TCR recognize the same antigen and many antigens are recognized by the same TCR
- T cells recognize a "non-self" target, such as a pathogen, only after antigens (small fragments of the pathogen) have been processed and presented in combination with a "self" receptor called a major histocompatibility complex (MHC) molecule
- Macrophages, dendritic cells, B cells etc are professional antigen presenting cells. They present small peptides with (MHC molecules) on their surface. This includes self peptides. Antigen presentation occurs in the lymphatic tissue (spleen, lymph nodes, respiratory, gut etc associated Lymphoid tissue)
- The **major histocompatibility complex** (**MHC**) is a set of cell surface molecules encoded by a large gene family which controls a major part of the immune system. The major function of MHCs is to bind to peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T-cell
- MHC Class I, are present on surface of all nucleated cells and presents peptides derived from internally synthesized antigens, while MHC Class II, only on APCs (e.g. macrophage, dendritic cell, B cell) and presents peptides derived from external antigens
- **Helper T cells** (CD4) express T cell receptors (TCR) that recognize antigen bound to Class II MHC molecules (macrophage, dendritic, B cell). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response
- **Killer T cells** (CD8) are a sub-group of T cells that kill cells that are infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional. As with B cells, each type of T cell recognizes a different antigen. Killer T cells are activated when their T cell receptor (TCR) binds to this specific antigen in a complex with the MHC Class I receptor of another cell. The T cell then travels throughout the body in search of cells where the MHC I receptors bear this antigen. When an activated T cell contacts such cells, it releases cytotoxins, such as perforin, which form pores in the target cell's plasma membrane
- **Memory T cells** are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections
- **Regulatory T cells**, formerly known as **suppressor T cells**, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus.

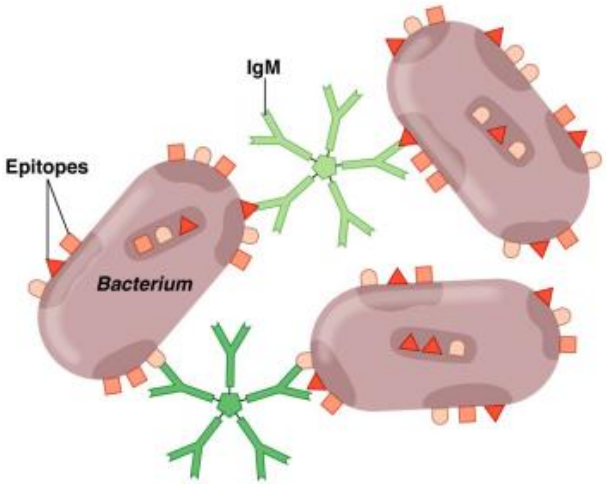
Cytotoxic (CD8) T cell killing



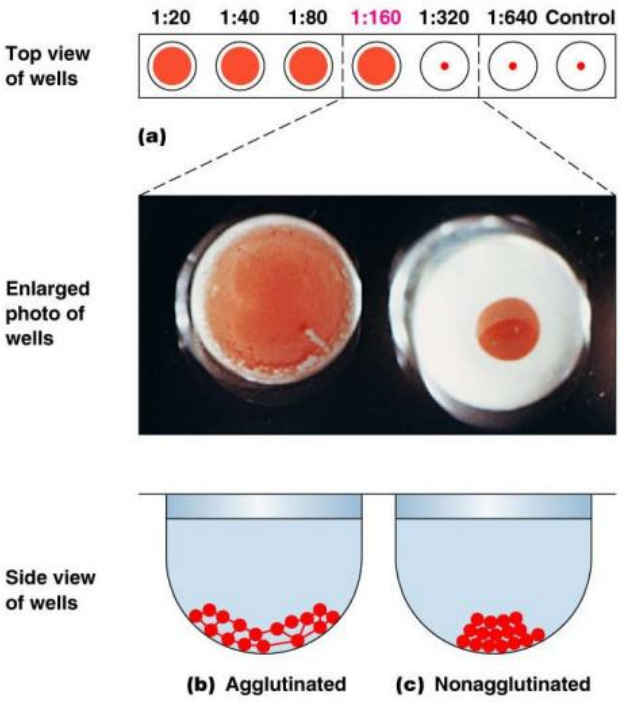
1. A normal cell will produce endogenous antigens that will not trigger a response by a cytotoxic T lymphocyte (CTL), but a virus-infected cell or a cancer cell produces abnormal endogenous antigens.
2. The abnormal antigen is presented on the cell surface in association with MHC class I molecules. CD8 cells with receptors for the antigen are transformed into CTLs.
3. The CTL induces destruction of the virus-infected cell by apoptosis.

Agglutination

- **Agglutination** is the clumping of particles, and occurs if an antigen is mixed with its corresponding antibody. **Haemagglutination**, is a specific form of agglutination that involves red blood cells



- **Titer hemagglutination assay:** determined by the last viable "lattice" structure found. This is because it is at the point where, if diluted any more, the amount of virus particles will be less than that of the RBCs and thus not be able to agglutinate them together. The HA titer is the reciprocal of the dilution in this well.

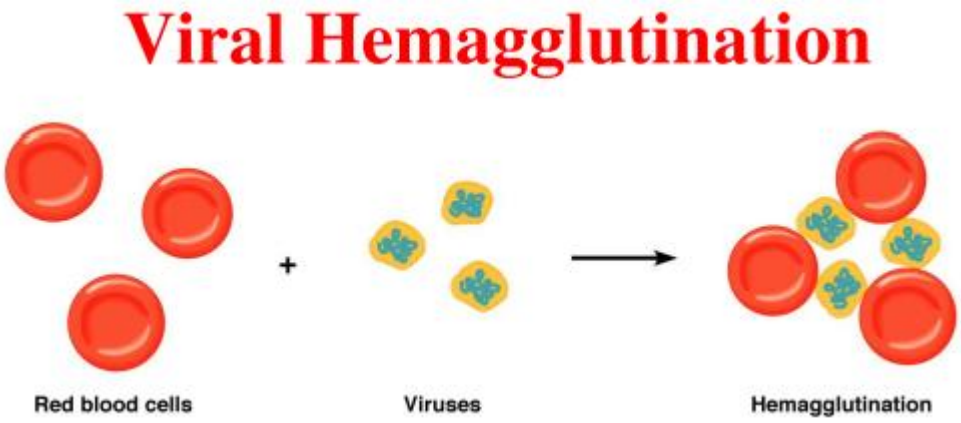


(a) Each well in this microtiter plate contains, from left to right, only half the concentration of serum that is contained in the preceding well. Each well contains the same concentration of particulate antigens, in this instance red blood cells.

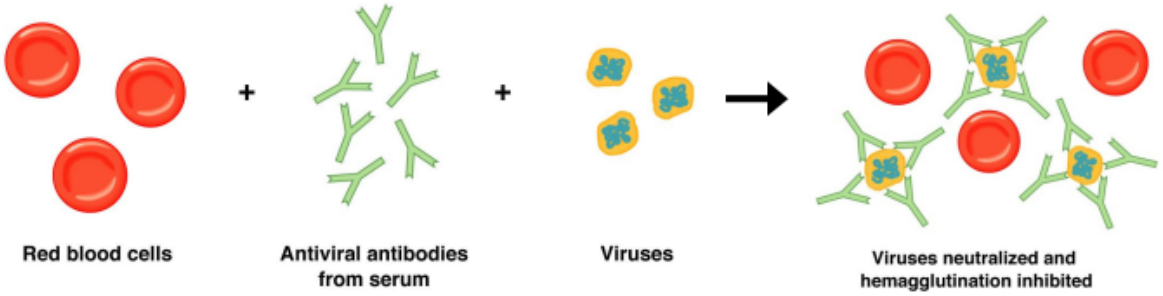
(b) In a positive (agglutinated) reaction, sufficient antibodies are present in the serum to link the antigens together, forming a mat of antigen–antibody complexes on the bottom of the well.

(c) In a negative (nonagglutinated) reaction, not enough antibodies are present to cause the linking of antigens. The particulate antigens roll down the sloping sides of the well, forming a pellet at the bottom. In this example, the antibody titer is 160 because the well with a 1:160 concentration is the most dilute concentration that produces a positive reaction.

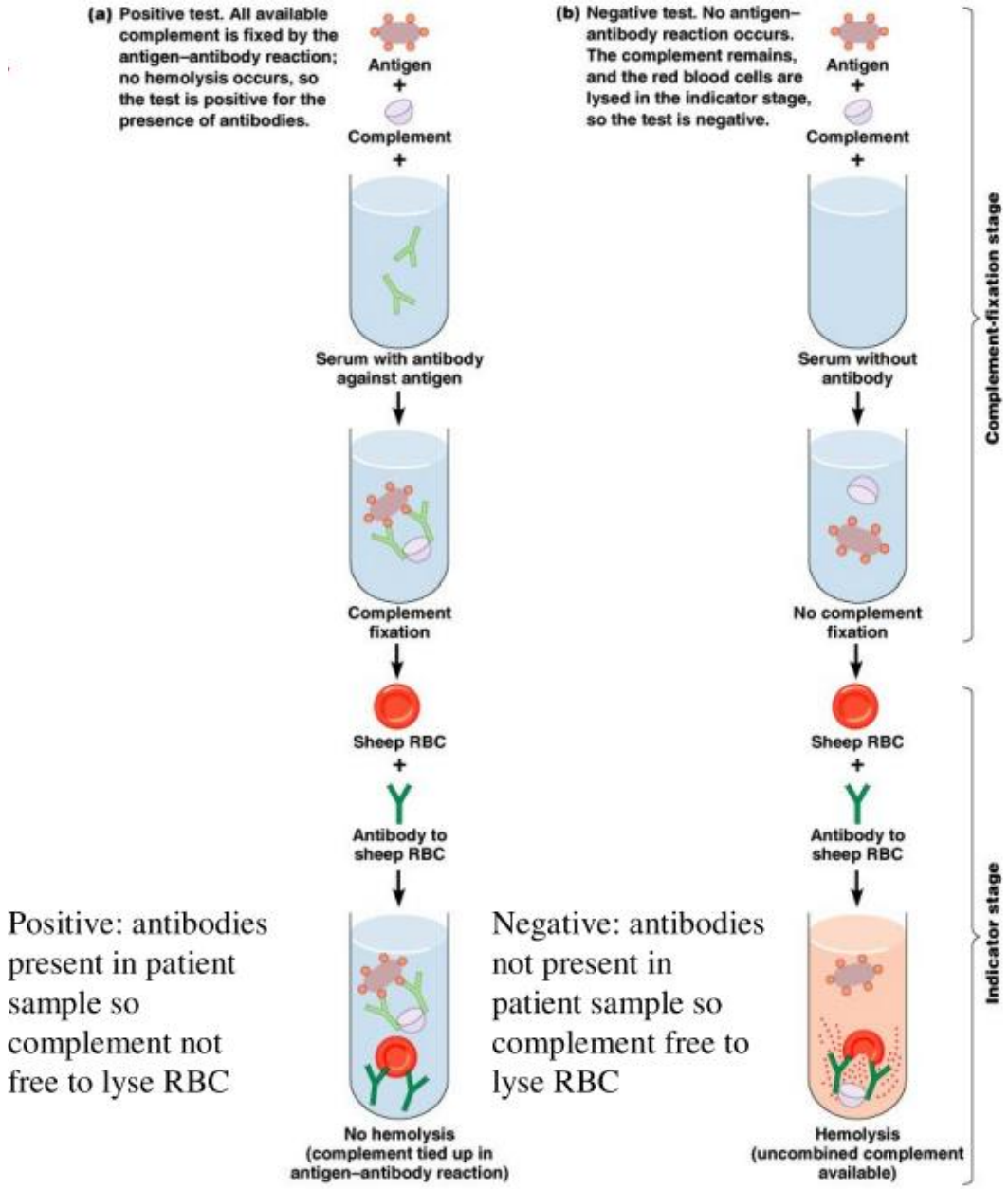
- **The hemagglutination inhibition assay**: a common variation of the HA assay used to measure flu-specific antibody levels in blood serum. In this variation, serum antibodies to the hemmagglutinin (HA) surface glycoprotein will interfere with the virus attachment to red blood cells. Therefore, hemagglutination is inhibited when antibodies are present at a sufficient concentration



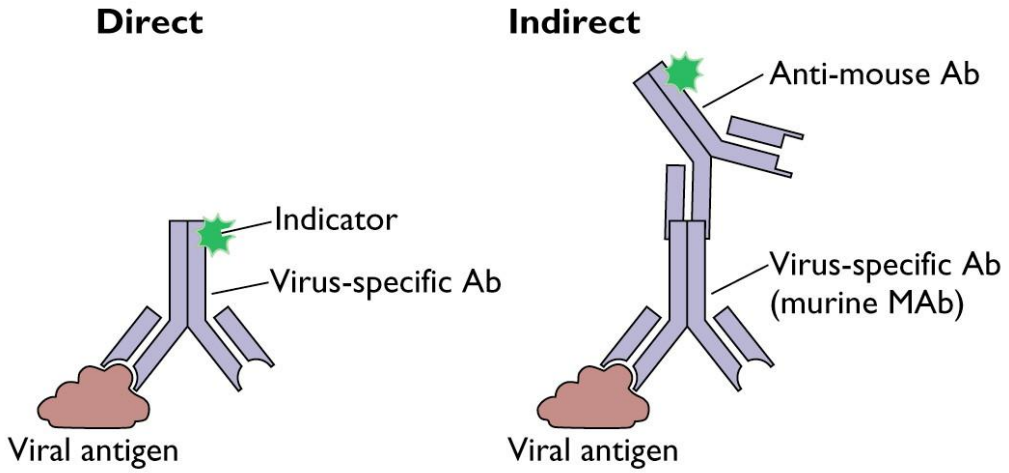
Haemagglutination inhibition



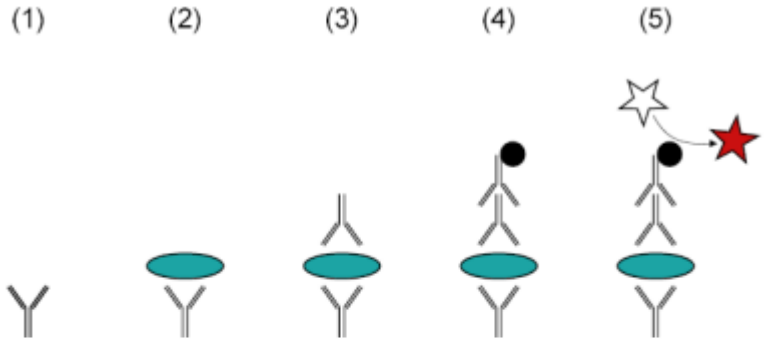
- **Complement fixation test**: blood serum is heated in such a way that all of the complement proteins but none of the antibodies within it are destroyed. Then a known amount of standard complement proteins are added to the serum, and then the antigen itself. Sheep red blood cells are then added. If the solution turns red, it means that complement proteins remained to lyse the sheep cells. Thus a pink colour change is a negative result for the presence of antibodies



- Direct ELISA: detects antigen

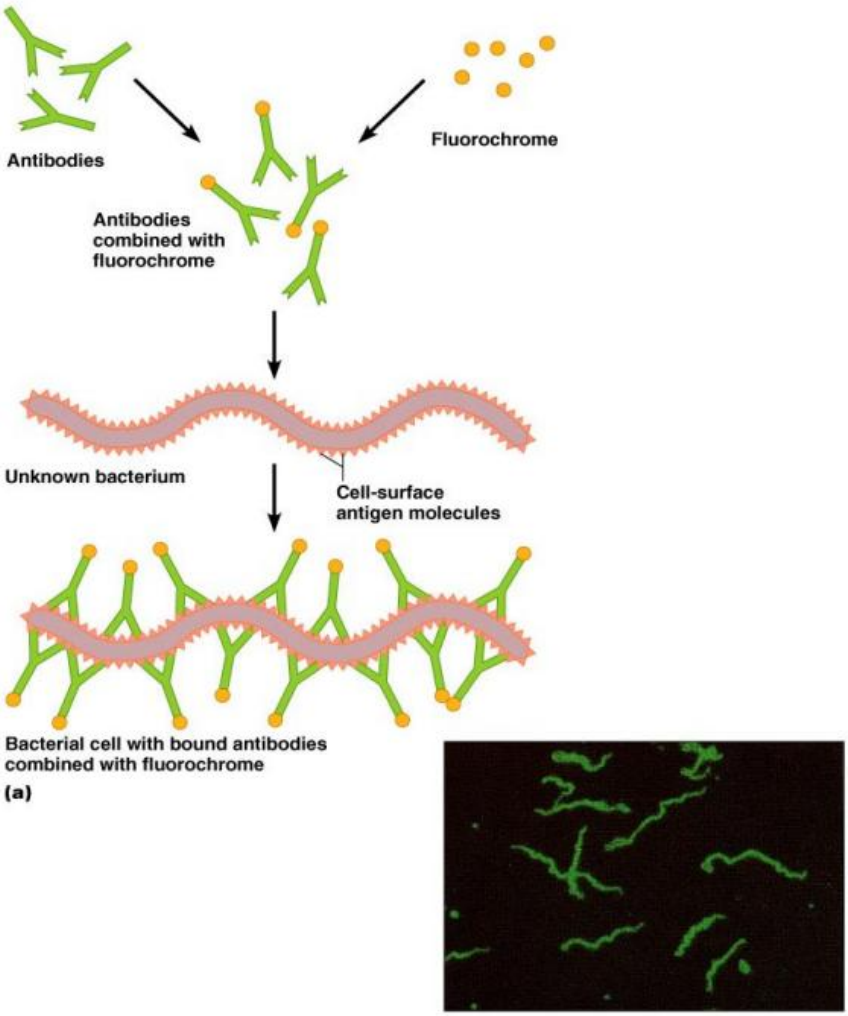


- Indirect ELISA: detects the antibody, so can't directly differentiate between current and prior infection
- Sandwich ELISA:



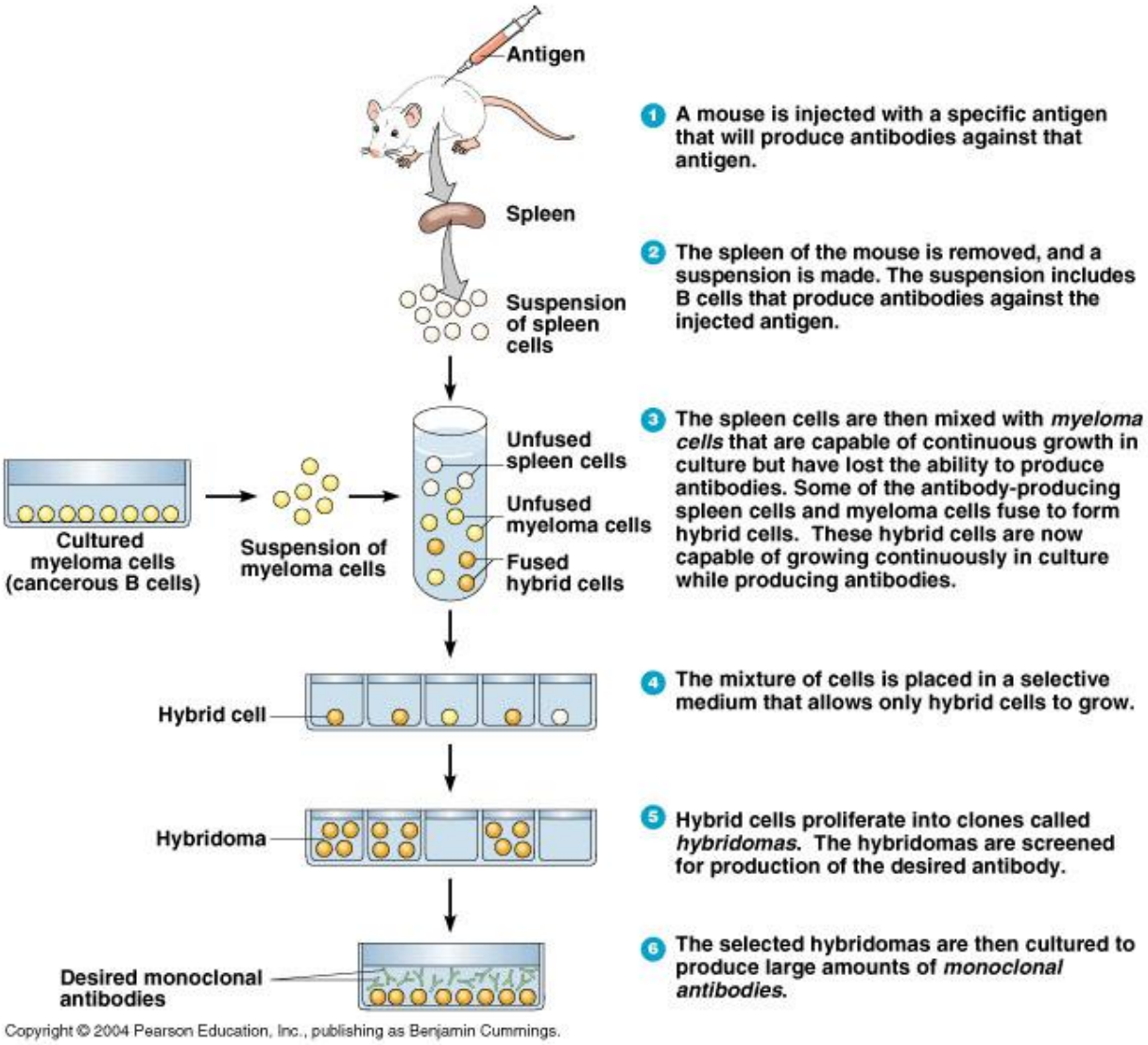
(1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form. (Source Wikipedia ELISA)

- Fluorescent Antibodies: Fluorescent microscopes can be used to image antibody interactions when antibodies are tagged with fluorescent dyes



Monoclonal antibodies

- **Monoclonal antibodies** (**mAb** or **moAb**) are monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope
- Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine.



Immunity to Infectious Diseases

- Animals or humans may acquire immunity to a disease in several ways
- By acquiring (and surviving!) the infection. This is termed natural active immunity
- By injection of components of the infective agent. This is termed artificial active immunity (or vaccination)
- By injection of antiserum from an immune individual. This is termed artificial passive immunity
- By transfer of maternal IgG to the fetus. This is natural passive immunity

16 First exposure response vs secondary response

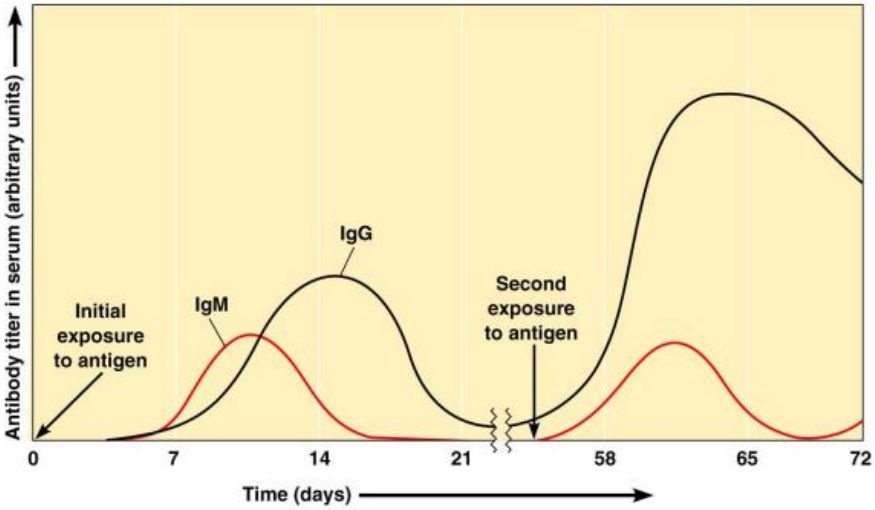
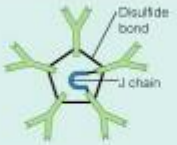
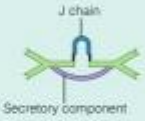


TABLE 17.1 A Summary of Immunoglobulin Classes

Characteristics	IgG	IgM	IgA	IgD	IgE
					
Structure	Monomer	Pentamer	Dimer (with secretory component)	Monomer	Monomer
Percentage of total serum antibody	80%	5–10%	10–15%*	0.2%	0.002%
Location	Blood, lymph, intestine	Blood, lymph, B cell surface (as monomer)	Secretions (tears, saliva, mucus, intestine, milk), blood, lymph	B cell surface, blood, lymph	Bound to mast and basophil cells throughout body, blood
Molecular weight	150,000	970,000	405,000	175,000	190,000
Half-life in serum	23 days	5 days	6 days	3 days	2 days
Complement fixation	Yes	Yes	No†	No	No
Placental transfer	Yes	No	No	No	No
Known functions	Enhances phagocytosis; neutralizes toxins and viruses; protects fetus and newborn	Especially effective against microorganisms and agglutinating antigens; first antibodies produced in response to initial infection	Localized protection on mucosal surfaces	Serum function not known; presence on B cells functions in initiation of immune response	Allergic reactions; possibly lysis of parasitic worms

*Percentage in serum only; if mucous membranes and body secretions are included, percentage is much higher.
† May be yes via alternate pathway.