Antigens and Antibodies

Presentation · September 2022

CITATIONS
0

READS
4,790

1 author:

Irfan Ullah
Shaheed Benazir Bhutto University, Sheringal
36 PUBLICATIONS **589** CITATIONS

SEE PROFILE

ANTIGENS AND ANTIBODIES

Immunology Lecture 3

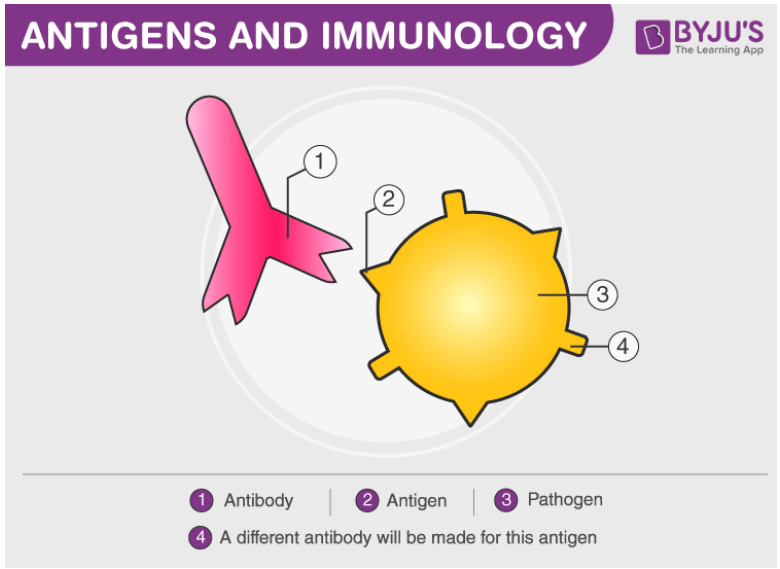
by

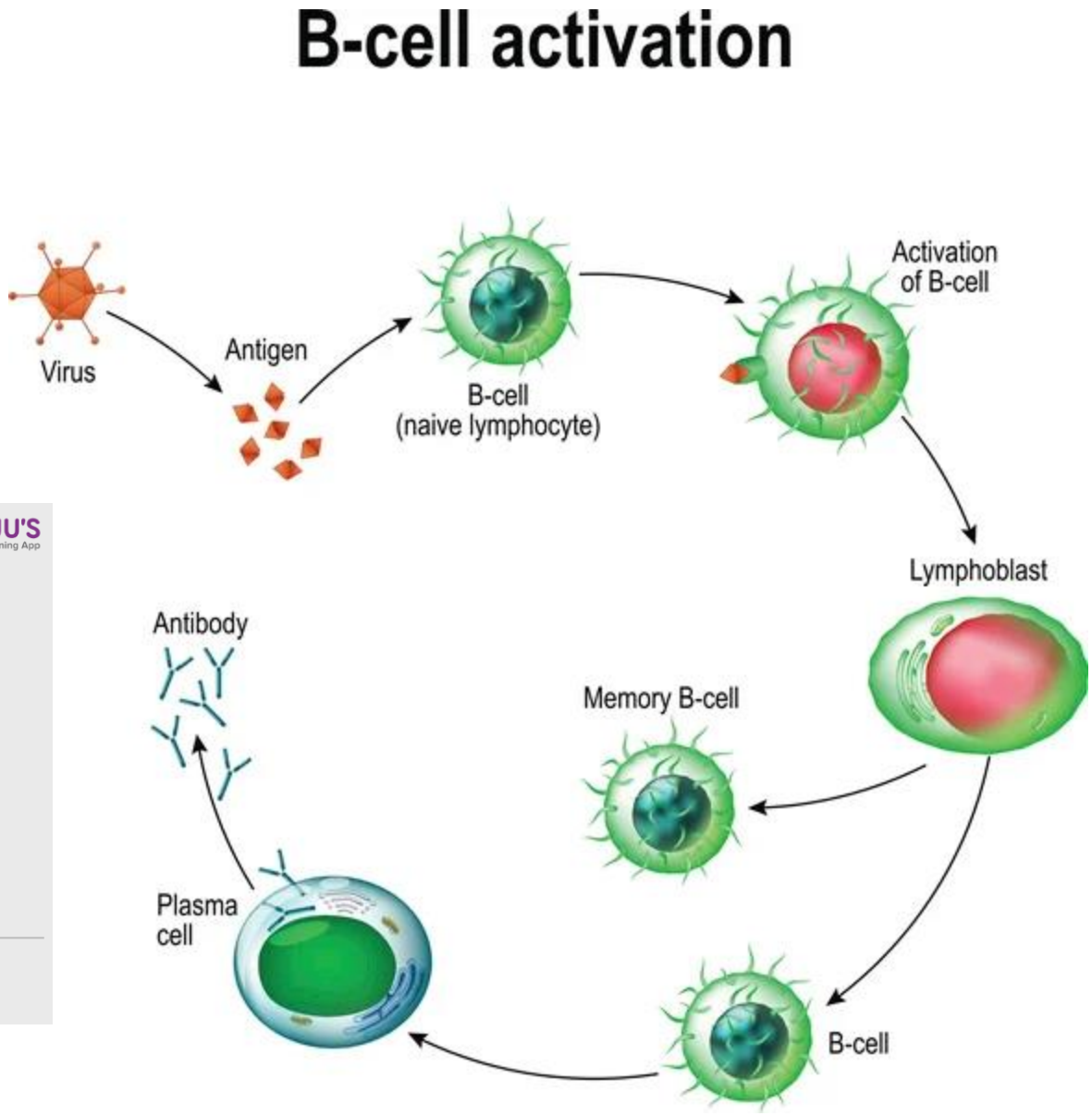
Dr. Irfanullah

Assistant Professor

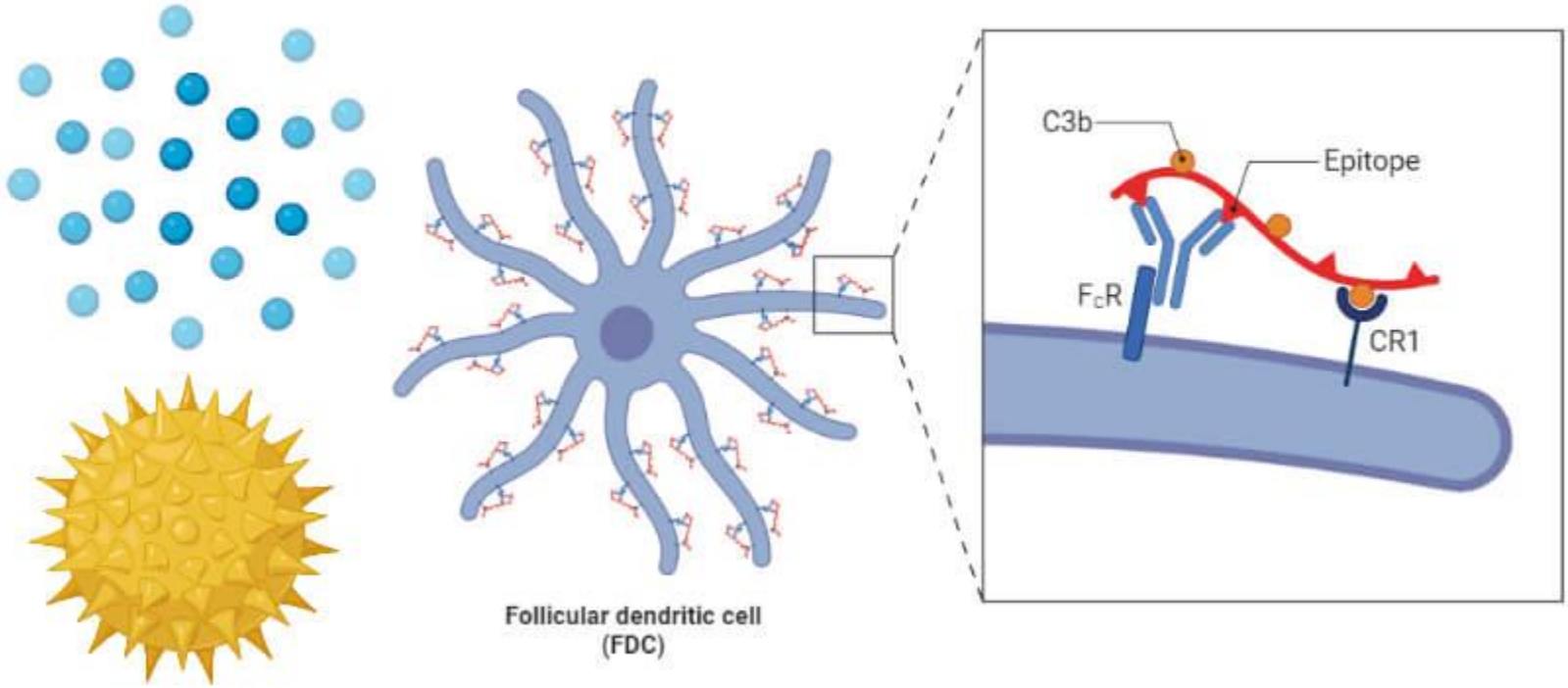
Shaheed BB University, Sheringal, Upper Dir, Pakistan

Antigens


ANTIGENS AND IMMUNOLOGY
BYJU'S
The Learning App
1
2
3
4
1 Antibody
2 Antigen
3 Pathogen
4 A different antibody will be made for this antigen


B-cell activation
Virus
Antigen
B-cell
(naive lymphocyte)
Activation
of B-cell
Lymphoblast
Memory B-cell
B-cell
Plasma
cell
Antibody

Antigen- Definition, Properties, Structure, Types, Examples



Antigens

"An antigen is a molecule that initiates the production of an antibody and causes an immune response."

- Antigens are molecules or molecular structures that are foreign to the body and generally induce an immune reaction in the form of the production of antibodies against them.
- In simple words, antigens can be anything that doesn't belong to the body and are foreign.
- Even though antigens are usually defined by the induction of an immune response, all antigens might not induce an immune response. The antigens that induce a response are termed immunogens.
- The ability of antigens to elicit an immune response depends on the presence of specific regions on the antigens called antigenic determinants. The determinants bind to receptor molecules with the complementary structure on immune cells to elicit a response.
- Antigens are indicated by the term 'Ag', and these can occur in different forms like pollen, viruses, chemicals, or bacteria.
- The concept of antigen arose from the fact that our body can distinguish between the components of the body and foreign particles.
- In response to these antigens, the body induces the production of antibodies that act against the said antigens.
- Most antigens in humans are proteins, peptides, or polysaccharides; however, lipid and nucleic acids can also act as antigens when combined with proteins or polysaccharides.
- In addition, antigens might also be intentionally introduced into the body in the form of vaccines in order to induce the adaptive immune system of the body against the antigen.

Properties of Antigens

2. Chemical Nature

- The most potent and commonly encountered antigens are proteins followed by polysaccharides.
- However, other molecules like lipids and nucleic acids can also act as antigens when complex with proteins and polysaccharides.
- In the case of proteins, the antigen should contain immunogenic regions with at least 30% of amino acids like lysine, glutamine, arginine, glutamic acids, aspargine, and aspartic acid, along with a high number of hydrophilic or charged groups.
- The level of immunogenicity also increases with the heterogenicity of the molecules. Homopolymers are usually less immunogenic than heteropolymers.

3. Molecular Size

- The molecular size of the antigens is also crucial in the immunogenicity of the molecules.
- It has been established that antigens should have a minimum size of greater than 5000 Da before they can be considered immunogenic.
- However, low molecular weight substances can demonstrate immunogenicity when coupled with large-sized carriers.
- The low molecular weight substances are termed haptens that are considered ‘partial antigens’ with at least one antigenic determinant.

Properties of Antigens

4. Molecular Rigidity and Complexity

- The rigidity and complexity of molecules are essential factors that determine immunogenicity.
- In general, rigid molecules are good antigens as they can raise antibodies to certain structures when compared to the less rigid ones.
- The complexity of the structure is also an essential factor as a peptide antigen with a repeating unit of a single amino acid is less immunogenic than a molecule with two or more repeating amino acids units.

5. Antigenic Determinants and Cross-reactivity

- Antigenic determinants are regions in an antigen molecule that is involved in the reaction with antibodies.
- Usually, antigens with two or more antigenic determinants can induce antibody production. Thus, a smaller antigen usually doesn’t induce antibody production as it is not possible for a small molecule to have more than one antigenic determinant.
- Cross-reactivity of antigens is also an essential factor where antibodies induced by a different antigen can interact with another antigen.

Antigen Structure

- The molecular structure of an antigen is characterized by its ability to bind to the antigen-binding site of an antibody.
- Antibodies differentiate between different antigens on the basis of the specific molecular structures present on the surface of the antigen.
- Most antigens are proteins or polysaccharides. These can include coats, capsules, flagella, toxins, and fimbriae of bacteria, viruses, or other microorganisms. Besides, secretions and other chemicals of the same nature can also act as antigens.
- Lipids and nucleic acids of these microorganisms are only antigenic when these are combined with proteins or polysaccharides.
- The structure of antigens might be different depending on the nature of the antigen, their size, and immunogenicity.
- All immunogenic antigens have a specific structural component called epitope or antigenic determinant.
- The number of epitopes differs in different antigens and determines the number of antibodies a single antigen can bound to.
- The structural components of interaction in antigens are different, which determines the classes of antibodies they bound to.
- The region on antibodies that interacts with antigens is called a paratope. It has been established that the structure of epitope and paratope can be defined with a lock and key metaphor as the structures are specific and fit with one another.

Types of Antigens

Antigens can be grouped into different types based on different factors. Some of the common classifications are based on the origin of the antigen and its immunogenicity.

1. Types of antigen-based on their origin

Antigens can be classified into two groups on the basis of their origin;

a. Exogenous Antigens

- Exogenous antigens are the antigens that are originated outside the body of the host and, thus, are foreign to the host.
- These antigens might enter the body through inhalation, ingestion, or injection and then circulate throughout the body via bodily fluids.
- The uptake of exogenous antigens is primarily mediated by phagocytosis via Antigen Processing Cells (APCs) like macrophages, dendritic cells, etc.
- Many antigens like intracellular viruses might begin as exogenous antigens and later become endogenous.

Types of Antigens

b. Endogenous Antigens

- Endogenous antigens are antigens that originate within the body of the host during metabolism or as a result of intracellular viral or bacterial infection.
- Endogenous antigens are usually the cells of the body or fragments, compounds, or antigenic products of metabolism.
- These are usually processed in the macrophages and are later detected by cytotoxic T-cells of the immune system.
- Endogenous antigens include antigens that are xenogenic or heterologous, autologous, and idiotype or allogenic.
- Endogenous antigens might result in autoimmune diseases as the host immune system detects its own cells and particles as immunogenic.

Types of Antigens

Autoantigens

- Autoantigens are proteins or protein complexes of the host that are attacked by the host's immune system, resulting in autoimmune disease.
- Autoantigens can be deadly to the host as the body's own cells should not be targeted by the immune system.
- The immunological tolerance to such antigens is lost as a result of genetic and environmental factors.

Tumor Antigens (Neoantigens)

- Tumor antigens or neoantigens are presented by Major Histocompatibility Complex (MHC) I and II on the surface of tumor cells.
- The antigens are produced as a result of a tumor-specific mutation during the malignant transformation of normal cells.
- These antigens usually do not induce an immune response as the tumor cells develop ways to evade antigen presentation and immune defense.

Native Antigens

- Native antigens are antigens that are not processed by any antigen-presenting cells (APC), and thus immune cells like T-cells cannot bind to these antigens.
- However, B-cells can be activated such antigens even without any processing.

Types of Antigens

2. Types of antigens on the basis of immune response

Antigens can be classified into two distinct groups on the basis of immune response;

a. Complete antigens/ Immunogens

- Complete antigens or Immunogens are antigens that elicit a specific immune response.
- These antigens can induce an immune response by themselves without any carrier particles.
- These are usually proteins, peptides, or polysaccharides with high molecular weight (greater than 10,000 Da).

b. Incomplete antigens/ Haptens

- Incomplete antigens or haptens are antigens that cannot generate an immune response by themselves.
- These are usually non-protein substances that require a carrier molecule to form a complete antigen.
- Haptens have a low molecular weight (usually less than 10,000 Da) and fewer antigenic determinant sites.
- The carrier molecule bonded to the hapten is considered a non-antigenic component and is a protein or a polysaccharide molecule.

Antbodies

- An antigen-antibody complex or immunogenic complex is a molecule formed by binding multiple antigens to antibodies.
- The binding of antibody and antigen is determined by the epitope and paratope present in the antigen and antibody, respectively.
- The ability of antibodies to fight against multiple pathogens is due to their ability to distinguish between different antigens.
- The interaction between antigens and antibodies is highly specific, and it is determined by the amino acid sequence in the epitope and paratope of the species.
- The complex is formed by an antigen-antibody reaction which is then subject to a number of responses like complement deposition, opsonization, and phagocytosis.
- The shape and size of the immune complex are determined by the ratio of antigen to antibody. The size, in turn, determines the effect of the immune complex.
- Antigen-antibody complexes have become an important tool in understanding the antigen-antibody interaction and determining the basis of molecular recognition between an antibody and antigens.
- Immune complexes also play a role in regulating antibody production as the binding of antigen to cell receptors activates signaling cascade leading to the activation of antibodies.
- Even though immune complexes are essential for different immune functions, the deposition of the immune complex can lead to several autoimmune diseases like arthritis and scleroderma.

Structure of Antibodies

- An antibody, also known as an immunoglobulin, is a Y-shaped structure which consists of four polypeptides — two heavy chains and two light chains.
- This structure allows antibody molecules to carry out their dual functions: antigen binding and biological activity mediation.
- Each function is carried out by different parts of the antibody: fragment antigen-binding (Fab fragment) and fragment crystallizable region (Fc region).
- Fab fragment is a region on an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy and the light chain.
- These domains shape the paratope — the antigen-binding site — at the amino terminal end of the monomer.
- Fc region is the tail region of an antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system.
- This property allows antibodies to activate the immune system.
- The Fc regions of immunoglobulin Gs bear a highly conserved N-glycosylation site.

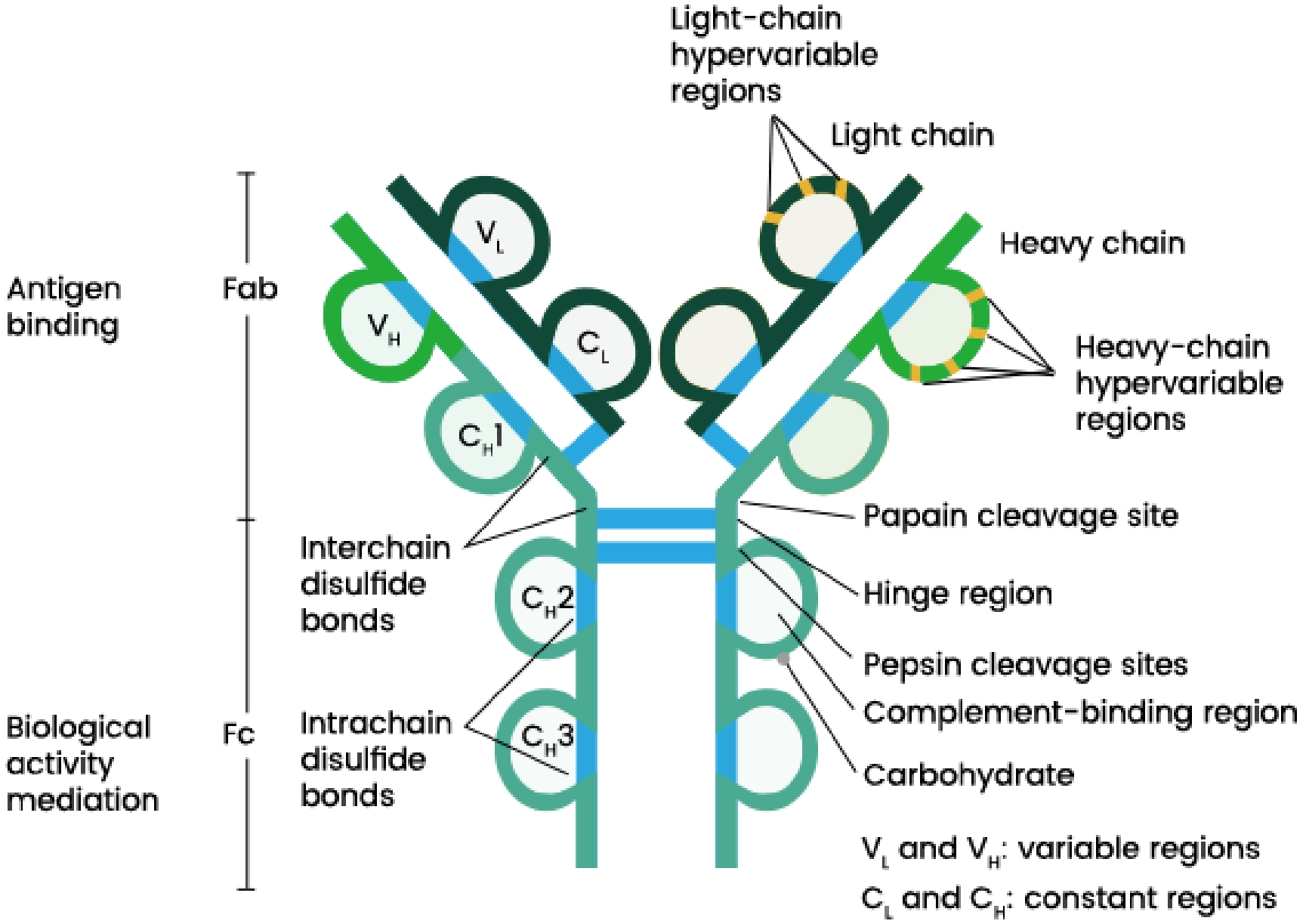
Structure of Antibodies

- Antibodies are heavy (~150 kDa) globular plasma proteins. The basic structure of all antibodies are same.
- There are four polypeptide chains: two identical heavy chains and two identical light chains connected by disulfide bonds.
- Light Chain (L) consists polypeptides of about 22,000 Da and Heavy Chain (H) consists larger polypeptides of around 50,000 Da or more.
- There are five types of Ig heavy chain (in mammal) denoted by the Greek letters: α, δ, ε, γ, and μ.
- There are two types of Ig light chain (in mammal), which are called lambda (λ) and kappa (κ).

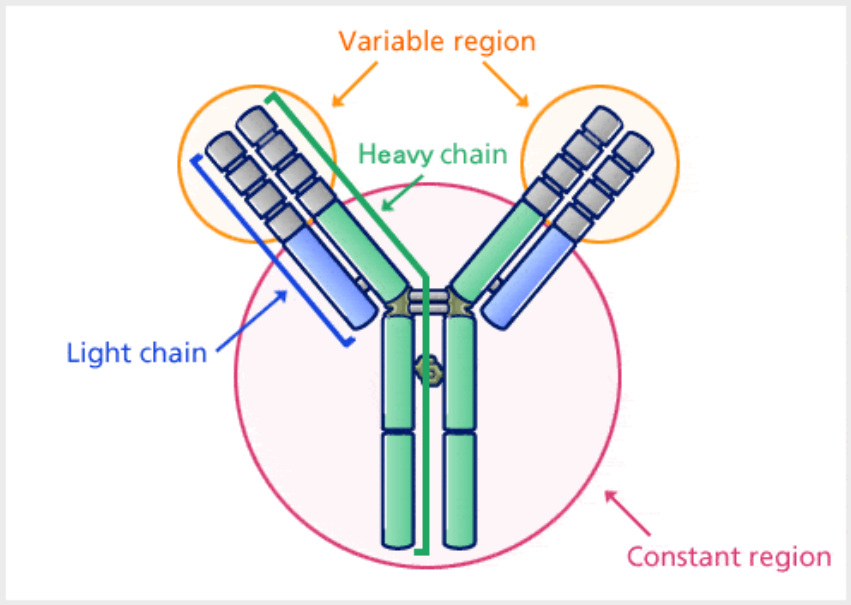
- An antibody is made up of a variable region and a constant region, and the region that changes to various structures depending on differences in antigens is called the variable region, and the region that has a constant structure is called the constant region.

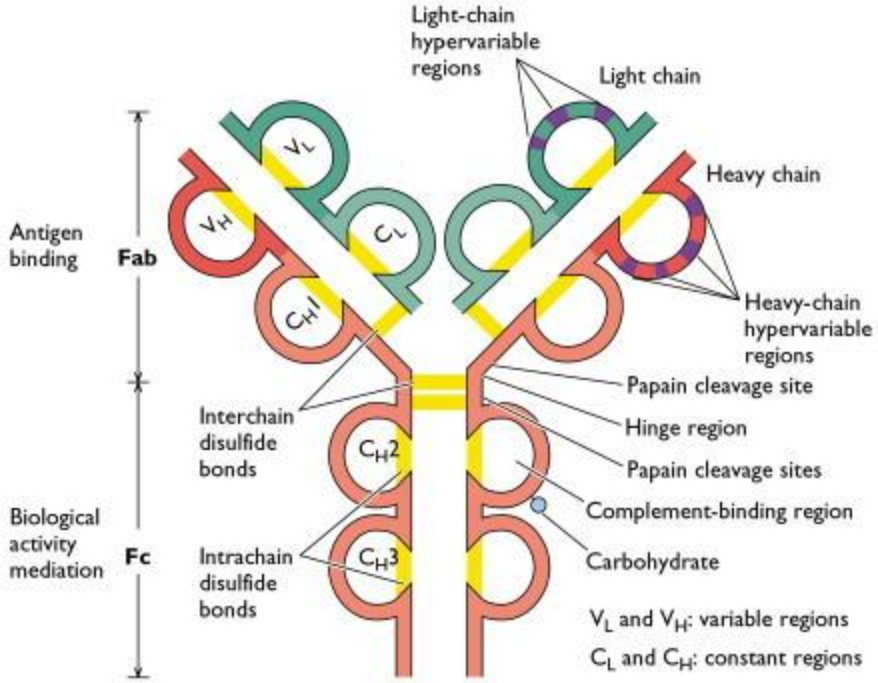
Structure of Antibodies

- Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region that consists of 100 to 110 amino acids and differ from one antibody to another.
- The remainder of each chain in the molecule – the constant (C) region exhibits limited variation that defines the two light chain subtypes and the five heavy chains subclasses.
- Some heavy chains (α, δ, γ) also contain a proline-rich hinge region. The amino terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the carboxy-terminal domains.
- The ε and μ heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule. CHO denotes a carbohydrate group linked to the heavy chain.


Light-chain hypervariable regions
Light chain
Heavy chain
Heavy-chain hypervariable regions
Antigen binding
Fab
V_L
V_H
C_L
C_H1
Papain cleavage site
Interchain disulfide bonds
Hinge region
C_H2
Pepsin cleavage sites
Complement-binding region
Biological activity mediation
Fc
Intrachain disulfide bonds
C_H3
Carbohydrate
V_L and V_H: variable regions
C_L and C_H: constant regions

Structure of Antibodies





Functions of Antibodies

- IgG provides long term protection because it persists for months and years after the prescence of the antigen that has triggered their production.
- IgG protect against bacteris, viruses, neutralise bacterial toxins, trigger compliment protein systems and bind antigens to enhance the effectiveness of phagocytosis.
- Main function of IgA is to bind antigens on microbes before they invade tissues. It aggregates the antigens and keeps them in the secretions so when the secretion is expelled, so is the antigen.
- IgA are also first defense for mucosal surfaces such as the intestines, nose, and lungs.
- IgM is involved in the ABO blood group antigens on the surface of RBCs.
- IgM enhance ingestions of cells by phagocytosis.
- IgE bind to mast cells and basophils wich participate in the immune response.
- Some scientists think that IgE’s purpose is to stop parasites.
- IgD is present on the surface of B cells and plays a role in the induction of antibody production.

Classes of Antibodies

There are five immunoglobulin classes of antibody molecules found in serum: IgG, IgM, IgA, IgE and IgD. They are distinguished by the type of heavy chain they contain.

- IgG antibody structure and function
- Immunoglobulin G (IgG) antibodies are large globular proteins with a molecular weight of about 150 kDa made of four peptide chains. It contains two identical γ (gamma) heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure.

- IgG provides long term protection because it persists for months and years after the presence of the antigen that has triggered their production. IgG protects against bacteria, viruses, neutralises bacterial toxins, triggers complement protein systems and binds antigens to enhance the effectiveness of phagocytosis.

- IgM antibody structure and function
- Immunoglobulin M (IgM) antibodies are constructed of five or six units (i.e. mostly as pentamers but also hexamers occur) which are each comprised of two heavy-chains (μ-chains) and two light chains, bound together by disulfide bonds and a so-called J-chain.

- IgM is involved in the ABO blood group antigens on the surface of RBCs. IgM enhances ingestions of cells by phagocytosis.

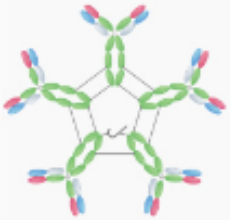
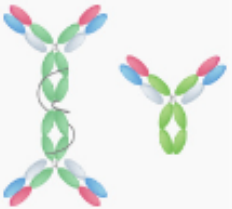
- IgA antibody structure and function
- Immunoglobulin A (IgA) antibodies consist of heavy (H) and light (L) chains. Each H chain is comprised of the constant region (Cα1, Cα2, Cα3), hinge region and the Variable (V) region. Light chains consist of the CL and Vκ or Vλ elements.

- The main function of IgA is to bind antigens on microbes before they invade tissues. It aggregates the antigens and keeps them in the secretions so when the secretion is expelled, so is the antigen. IgA is also first defense for mucosal surfaces such as the intestines, nose, and lungs.

- IgE antibody structure and function
- Immunoglobulin E (IgE) antibodies have only been found in mammals. IgE is synthesised by plasma cells. Monomers of IgE consist of two heavy chains (ε chain) and two light chains, with the ε chain containing 4 Ig-like constant domains (Cε1-Cε4).

- IgE bind to mast cells and basophils which participate in the immune response. Some scientists think that IgE's purpose is to stop parasites.

- IgD antibody structure and function
- Immunoglobulin D (IgD) antibodies are expressed in the plasma membranes of immature B-lymphocytes. IgD is also produced in a secreted form that is found in small amounts in blood serum. IgD plays a role in the induction of antibody production.

IgG		IgG is the main antibody in blood and it has a powerful ability to bind to bacteria and toxins, and thus it takes on an important role in the biological defense system. It is the only isotype that can pass through the placenta, and IgG transferred from the mother's body protects a newborn.
IgM		IgM is constructed of five units of basic Y-shaped structures and is mainly distributed to the blood. Produced first upon pathogen invasion by B cells, IgM has a key role in the initial immune system defense for protecting the body.
IgA		While in blood, IgA is mainly present as monomers (the shape of a single Y), but it forms dimers (a combination of 2 Ys) in secretions such as bowel fluid, nasal discharge, and saliva, to prevent bacterial invasion from a mucous membrane. It is also present in breast milk and protects the gastrointestinal tract of newborns from bacterial and viral infection.
IgD		IgD is present on the surface of B cells and it is reported to play a role in the induction of antibody production and the prevention of respiratory tract infections.
IgE		It is believed that IgE was originally related to immunity reactions to parasites. By binding to mast cells, IgE is believed to be involved in allergies such as pollinosis.

Major Histocompatibility Complex

- The major histocompatibility complex, or simply MHC, is a complex of proteins found on the membrane of our cells.
- They have two very important functions.
- Firstly, they allow our immune cells of the body to recognize our healthy cells and distinguish them from infected cells and pathogens.
- The special name of this particular type of complex is the major histocompatibility complex class I (MHC class I).
- Secondly, they also function in helping our immune cells to actually interact and communicate with one another and this class of complexes is called the major histocompatibility complex class II (MHC class II).
- The major histocompatibility complex contains a cleft that can attach either its own proteins (self-antigens) or pathogenic proteins (antigens).
- When leukocytes approach these complexes, they will recognize their own healthy cells and attack the cells that contain the pathogenic antigens.

Major Histocompatibility Complex

1. The Major Histocompatibility complex is a genetic locus that encodes the glycoprotein molecules (transplantation antigens) which are responsible for tissue rejection of grafts between genetically unidentical individuals.
2. It is also the molecule that binds the peptide antigens processed by Antigen-presenting Cells and presents them to T-cells, hence they are responsible for antigen recognition by the T-cell receptors.
3. Unlike the B-cell receptors that directly interact with the antigens, the T-cell receptors have an intertwined relationship with the MHC molecule, in that T-cell receptors can only receive and bind processed antigens in form of peptides that are bound to the MHC molecule, and therefore, T-cell receptors are specific for MHC molecules.
4. In humans, the Major Histocompatibility complex is known as Human Leukocyte Antigen (HLA). There are three common MHC molecules i.e class I, class II, and class III MHC proteins.
5. The genes of the MHC exhibit genetic variability; and the MHC has several genes for each class hence it is polygenic.
6. The MHC is also polymorphic, meaning a large number of alleles exist in the population for each of the genes.
7. Therefore, a large number of alleles exist in the population for each of the genes. Each individual inherits a restricted set of alleles from his or her parent. Sets of MHC genes tend to be inherited as a block or haplotype. There are relatively infrequent cross-over events at this locus.
8. The structure of the MHC class I have two domains that are distant from each other, made up of two parallel α helices on top of a platform that is created by a β-pleated sheet. The general structure looks like a cleft whose sides are formed by the α helices and the floor is β-sheet.
9. Generally, the MHC molecules have a broad specificity for peptide antigens and many different peptides can be presented by any given MHC allele binding a single peptide at a time.
10. The α helices forming the binding clefts are the site of the amino acid residues that are polymorphic (varying allelic forms) in MHC proteins, meaning that different alleles can bind and present different peptide antigens. For all these reasons, MHC polymorphism has a major effect on antigen recognition.
11. The function of T-cells on interaction with the MHC molecules reveals that the peptide antigens associated with class I MHC molecules are recognized by CD8+ cytotoxic T-lymphocytes (Tc cells) and MHC class-II associated with peptide antigens that are recognized by CD4+ Helper T-cells (Th cells).
12. The MHC in humans is known as human leukocyte antigens (HLA) complex.

Major Histocompatibility Complex

HLA complex

- In humans, the HLA complex of genes is located on short arm of chromosome 6 containing several genes that are critical to immune function. The HLA complex of genes is classified into three classes as follows:
- Class I: HLA-A, HLA-B, and HLA-C.
- Class II: HLA-DR, HLA-DQ, and HLA-DP. All of these are present within HLA-D region of HLA complex.
- Class III: Complement loci that encode for C2, C4, and factor B of complement system and TNFs alpha and beta.

Gene Products of HLA complex

- Class I MHC genes encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of endogenous peptide antigens to CD8+ T cells.
- Class II MHC genes encode glycoproteins expressed predominantly on APCs (macrophages, dendritic cells, and B cells), where they primarily present exogenous antigenic peptides to CD4+ T cells.
- Class III MHC genes encode several different proteins, some with immune functions, including components of the complement system and molecules involved in inflammation.

Major Histocompatibility Complex (MHC) Types

- In humans, the MHC molecules are divided into three types, Class I, Class II and Class III.
- Class I MHC molecules are coded from three different locations called A, B and C and these molecules are expressed in all nucleated cells.
- Class II MHC genes are located in the D region and there are several loci such as DR, DQ and DP and these molecules are expressed only in antigen-presenting cells.
- Class III MHC genes are coded in the region between Class I and Class II genes.
- Class III MHC genes codes for cytokines and complement proteins which play an important role during the immune response.

Major Histocompatibility Complex (MHC) Types

Class I MHC Molecule

- The structure of Class I MHC molecule consists of two polypeptide chains α and β. These two chains are connected together by non-covalent bonds. The α chain is characterized as an internal membrane glycoprotein with a molecular weight of 45000 Da (in humans). B chain, on the other hand, is an extracellular microglobulin with a molecular mass of 12kDa.
- The α chain is made up of approximately 350 amino acids and also divided into three globular domains α1, α2 and α3. Each of these domains contains roughly 90 amino acids. The N terminal of α chain is the place of α1 domain, while α2 and α3 are present after α1 The α2 domain is characterized by the formation of a loop of 63 amino acids; the loop is formed due to intrachain disulfide bond. α3 also contains a disulfide bond enclosing 86 amino acids. The α1 and α2 domains interact to form peptide-binding units of class I MHC molecule.
- Moreover, α chain also consists of a stretch of 26 hydrophobic amino acids which holds the α chain on the plasma membrane. This transmembrane segment is present as a form of α helix at the hydrophobic region of the plasma membrane. An intracellular domain or the carboxyl-terminal of α chain is located inside the cell and it contains around 30-40 amino acids.
- In humans the β chain is non-polymorphic and it is dimorphic in mice. α3 and β chain are structurally similar to the immunoglobulin C domain and also characterized as a disulfide loop. A peptide binding platform is formed by β plated sheets of α1 and α2
- Tcyt Cell (cytotoxic T cell) has specificity towards cells containing peptides associated with Class I MHC due to the presence of CD8 antigen on the surface of Tcyt Cell. CD8 antigen has an affinity towards the α3 domain of Class I MHC molecules.

Major Histocompatibility Complex (MHC) Types

Class II MHC Molecule

- Class II MHC molecules are heterodimers and characterized by two non-covalently connected polypeptide chains. The chains are termed a heavy chain (α, 30kDa) and light chain (β, 26kDa).
- Similar to class I MHC molecules, class II MHC molecules are also characterized by an extracellular amino-terminal domain, a transmembrane domain, and an intracellular carboxy-terminal tail.
- The class II MHC molecules are expressed on the surface of the antigen-presenting cells such as B cells, dendritic cells, and macrophages.
- The α chain is divided into two domains α1 and α2, while the β chain is also divided into two groups β1 and β2. The β2 domain is responsible for the binding of T cell co-receptor CD4. The α1 and β1 domains, on the other hand, are involved in the formation of the antigen-binding sites. Peptides containing 13-20 amino acids can bind at the antigen-binding site of class II MHC.
- The presence of disulfide bonds in α2, β1, and β2 domains is also an important structural feature of the class II MHC molecules.

Major Histocompatibility Complex (MHC) Types

Class III MHC Molecule

- There are several serum proteases which involve in the complement system that come under the group of class III MHC molecules.
- Class III MHC molecules do not have any involvement in antigen presentation.
- The complement components such as asC2, C4A, and C4B, and factor B are the most important compounds involved as class III MHC molecules. Apart from these tumor necrosis factors α and β and some heat shock proteins also come under this category.

Major Histocompatibility Complex

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

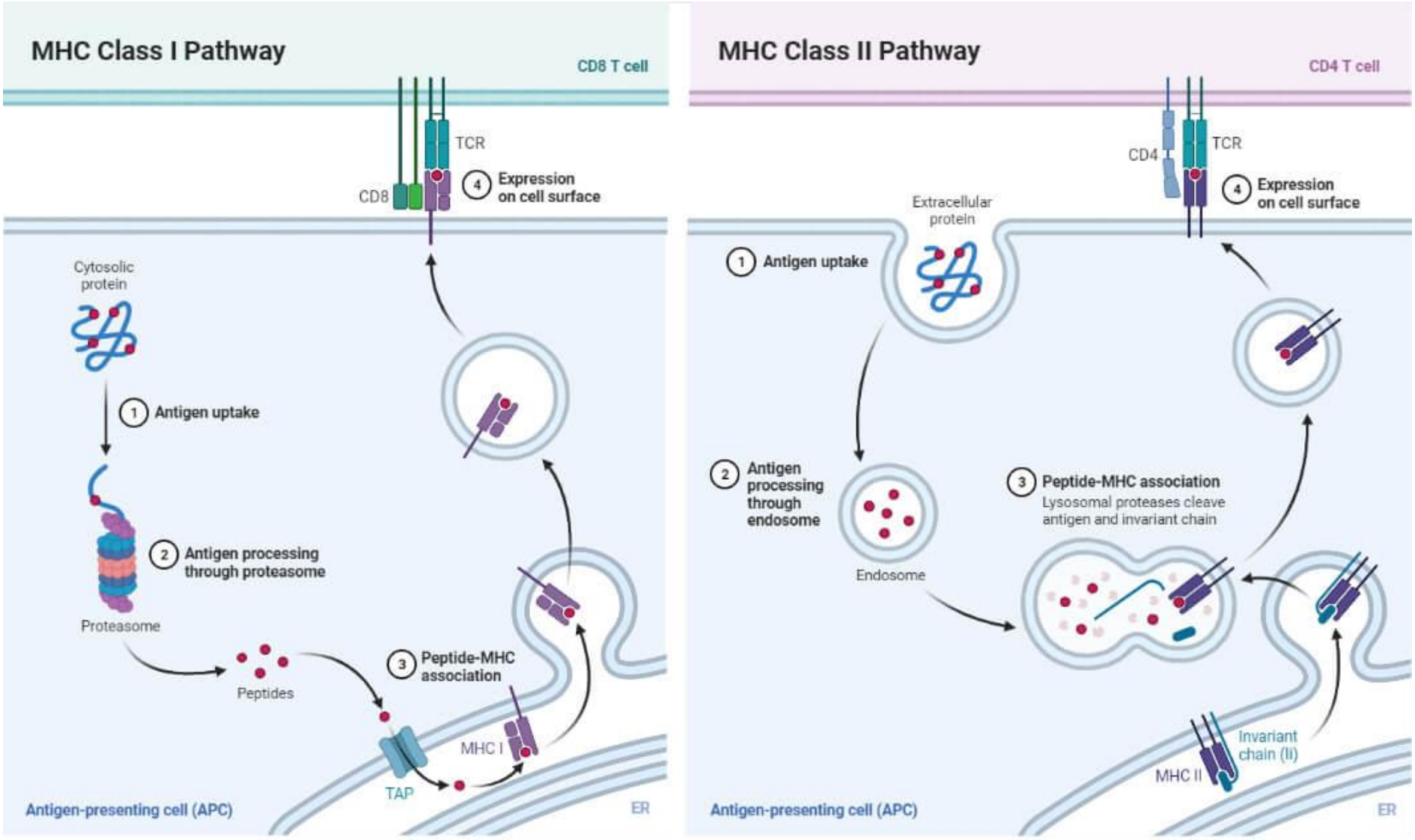
Antigen Processing and Presentation

Antigen processing and presentation is the process of digestion of antigens into smaller peptide fragments by an antigen-presenting cell (APCs) that are then displayed on the surface of the cells via antigen-presenting molecules like MHC class I and II for recognition by lymphocytes.

Antigen processing and presentation can occur via three different pathways;

1. Endogenous Pathway or Classical MHC class I Presentation

- The endogenous pathway of antigen processing and presentation utilizes mechanisms similar to those involved in the normal turnover of intracellular proteins.
- The antigen-presenting cells degrade the protein antigen into short peptides by a specified cytosolic proteolytic system called the proteasome.
- The proteasome involved in the immune system is called the immunoproteasome, which has components induced by exposure to interferon-γ or TNF-α.
- The next step of the proteolytic mechanisms is the peptide trimming by aminopeptidases in the ER lumen.
- The peptides formed after proteolysis are transported to the ER lumen by the Transporter associated with Antigen Processing (TAP).
- In addition to TAP, tapasin and calnexin-calreticulin system mediates the trimming and loading of peptides on MHC class I molecules.
- Depending on the affinity of the loaded peptides, MHC class I molecules will either be transported to the cell membrane or recycled by a mechanism dependent on UDP-glucose glycoprotein transferase-1.
- In the case of high-affinity MHC class-1 complexes, the peptides are transported through the Golgi apparatus to the cell membrane in order to elicit antigen-specific CD8+ T cell responses.



Antigen Processing and Presentation

- **2. Exogenous Pathway/ Classical MHC class II Presentation**
- In the case of the exogenous pathway, APCs internalize the antigen by simple phagocytosis, where the material first binds to specific surface receptors.
- The degradation of proteins into peptides occurs within the compartments of the cell by the endocytic processing pathway.
- The internalized antigen progresses through different acidic compartments encountering hydrolytic enzymes and low pH in each compartment.
- The APCs have a unique endosome in the MHC class II-containing compartment (MIIC), where the final protein degradation and peptide loading take place.
- Since APCs express both classes of MHC, there is a distinct mechanism to prevent the interaction of antigenic peptides for the class I molecules.
- When class II MHC molecules are synthesized, the class II αβ chains associate with a protein called the invariant chain. This protein interacts with the class II peptide-binding groove preventing the binding of endogenously derived peptides to the class II molecule.
- As the invariant chain moves through different compartments, it is degraded until it forms a short fragment termed CLIP (class II-associated invariant chain peptide).
- Later, a class II MHC molecule catalyzes the exchange of CLIP with antigenic peptide.
- The peptide binding is required to maintain the structure and stability of class II MHC molecules.
- After the binding of the peptide, the class II MHC –peptide complex is transported to the plasma membrane, where the neutral pH causes the complex to form a compact, stable form.

Antigen Processing and Presentation

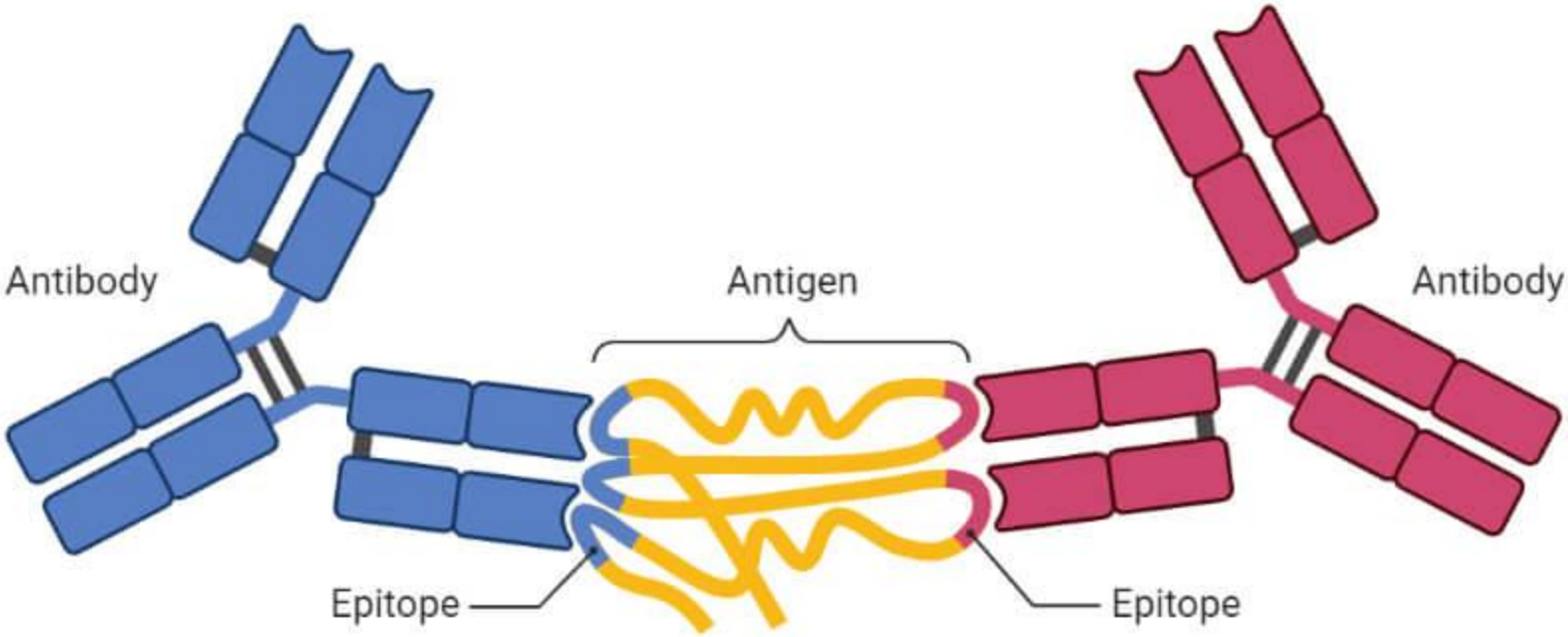
3. Cross-presentation

- Cross-presentation is the process where the APCs will divert antigen obtained by endocytosis (exogenous pathway) to a class I MHC loading and peptide presentation.
- The phenomenon of cross-presentation, however, requires the internalized antigens to be handled by the exogenous pathway leading to class II MHC presentation somehow are redirected to a class I loading pathway.
- Cross-presentation is primarily observed in the case of dendritic cells that accomplish cross-presentation by one of the two possible means.
- The first mechanism assumes that cross-presenting cells contain special antigen-processing machinery that enables the loading of exogenously derived peptides onto class I MHC molecules.
- The second mechanism hypothesizes that specialized endocytosis machinery is found in the cells that send internalized antigen directly to an organelle, where the peptides are then loaded onto class I MHC molecules.
- Cross-presentation of antigens has an advantage as APCs can capture viruses from extracellular environments, process them and activate cytotoxic T-cell lymphocytes that can attack virus-infected cells, which inhibit the further spread of the virus.

Antigen-Antibody Complex

- An antigen-antibody complex or immunogenic complex is a molecule formed by binding multiple antigens to antibodies.
- The binding of antibody and antigen is determined by the epitope and paratope present in the antigen and antibody, respectively.
- The ability of antibodies to fight against multiple pathogens is due to their ability to distinguish between different antigens.
- The interaction between antigens and antibodies is highly specific, and it is determined by the amino acid sequence in the epitope and paratope of the species.
- The complex is formed by an antigen-antibody reaction which is then subject to a number of responses like complement deposition, opsonization, and phagocytosis.
- The shape and size of the immune complex are determined by the ratio of antigen to antibody. The size, in turn, determines the effect of the immune complex.
- Antigen-antibody complexes have become an important tool in understanding the antigen-antibody interaction and determining the basis of molecular recognition between an antibody and antigens.
- Immune complexes also play a role in regulating antibody production as the binding of antigen to cell receptors activates signaling cascade leading to the activation of antibodies.
- Even though immune complexes are essential for different immune functions, the deposition of the immune complex can lead to several autoimmune diseases like arthritis and scleroderma.

Antigen Recognition by Antibodies



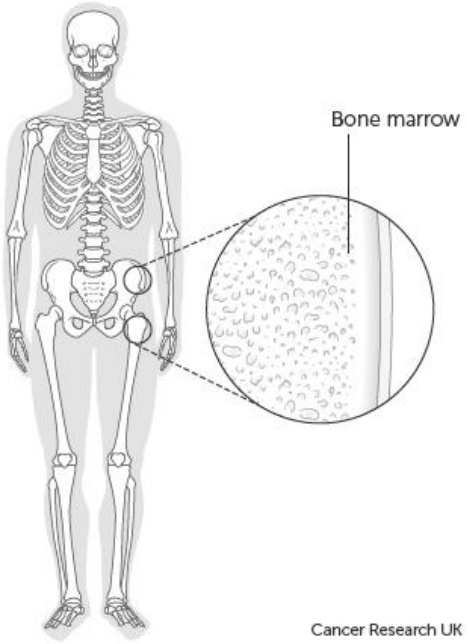
Myeloma and Hybridoma Immunoglobulins

What is myeloma?

Myeloma is a type of blood cancer that develops from cells in the bone marrow called plasma cells.

Bone marrow is the spongy tissue found inside the inner part of some of our large bones.

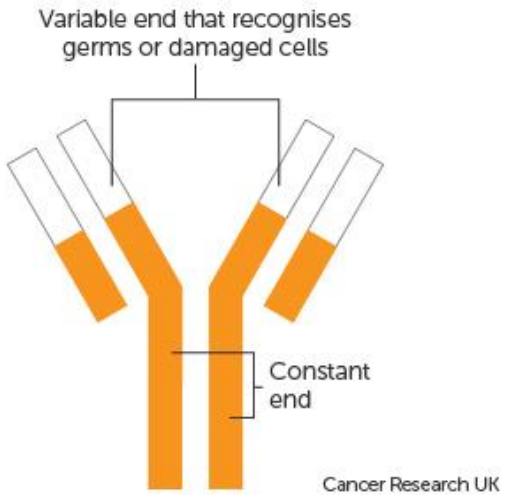
The bone marrow produces different types of blood cells.



Myeloma and Hybridoma Immunoglobulins

What are plasma cells?

- Plasma cells are part of the immune system. Normal plasma cells make proteins called antibodies. These antibodies are also called immunoglobulins.
- The plasma cells make antibodies when the body responds to infections. They make different antibodies for different infections. Antibodies attack and help to kill bacteria and viruses and so protect us from infections.
- There are 5 main types of antibody (immunoglobulin) – A, G, M, D and E.



Myeloma and Hybridoma Immunoglobulins

How does myeloma develop?

- Myeloma develops when there is a change in the DNA of the plasma cells.
- DNA is the instructions for the cell so it knows what to do and when.
- The change happens to the DNA when the bone marrow is making new plasma cells.
- The abnormal plasma cell then produces more abnormal cells. These are myeloma cells.
- The myeloma cells produce an abnormal form of one of the types of antibody.
- They aren't able to work normally and so can't help fight infections.

Myeloma and Hybridoma Immunoglobulins

Blood cells and myeloma

- To understand why myeloma affects you the way it does, it helps to understand how blood cells are normally produced and what they do.

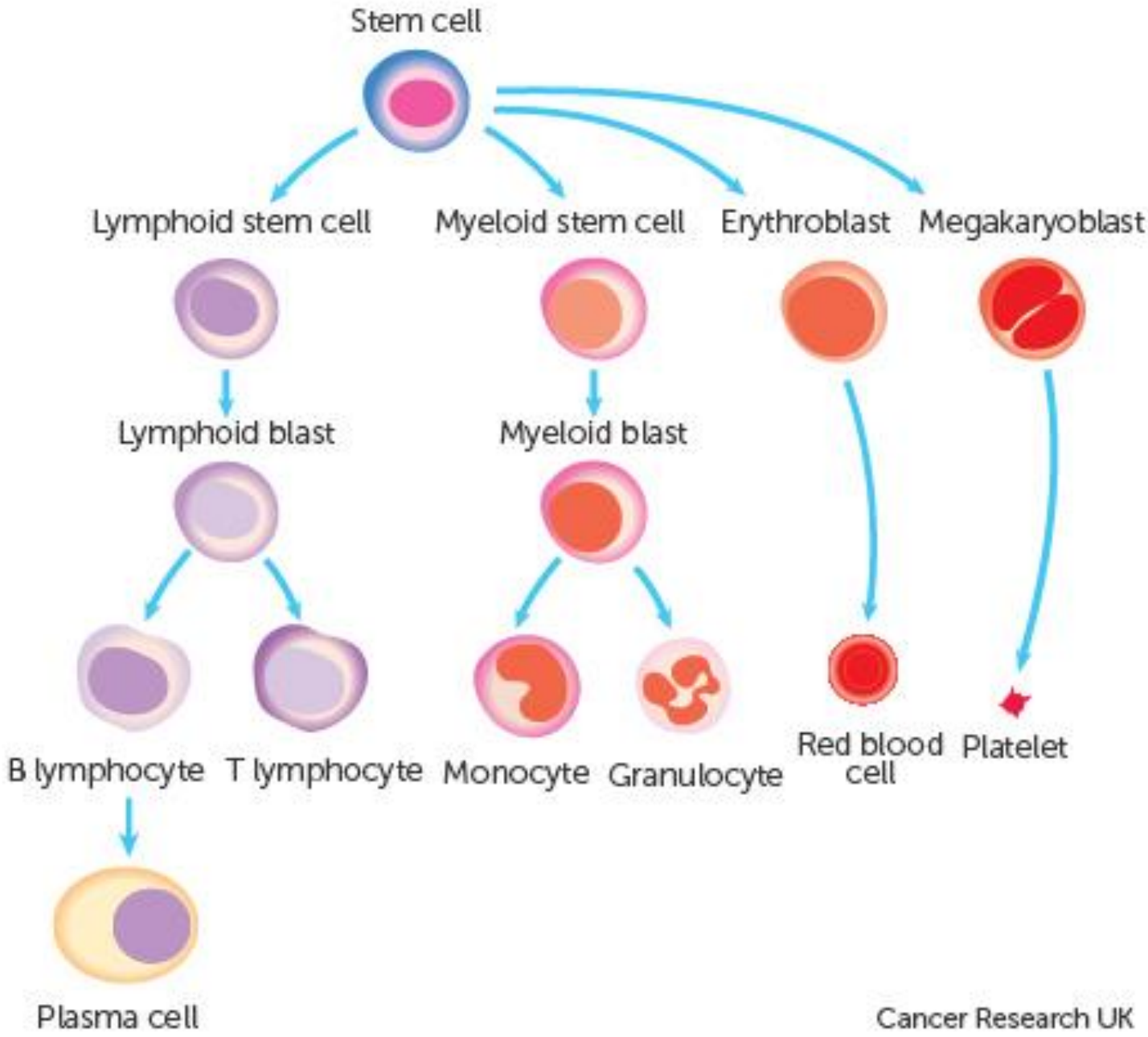
- Usually bone marrow makes blood cells in a controlled way, when your body needs them. All blood cells start as the same type of cell, called a stem cell. As they develop (mature), they turn into one of three types of blood cell:

❖ white blood cells (leucocytes)

❖ red blood cells (erythrocytes)

❖ platelets (thrombocytes)

- Plasma cells develop from a type of white blood cell called B lymphocytes.
- In myeloma, too many plasma cells are made and they are all of the same type.
- They crowd the bone marrow.
- This means that there is not enough space for making normal white cells, red cells and platelets.


Stem cell
Lymphoid stem cell
Myeloid stem cell
Erythroblast
Megakaryoblast
Lymphoid blast
Myeloid blast
B lymphocyte
T lymphocyte
Monocyte
Granulocyte
Red blood cell
Platelet
Plasma cell
Cancer Research UK

Myeloma and Hybridoma Immunoglobulins

Polyclonal antibodies (pAbs) are antibodies that are secreted by different B cell lineages within the body.

They are a collection of immunoglobulin molecules that react against a specific antigen, each identifying a different epitope.

A monoclonal antibody (mAb, more rarely called moAb) is an antibody produced from a cell line made by cloning a unique white blood cell.

All subsequent antibodies derived this way trace back to a unique parent cell.

Monoclonal antibodies can have monovalent affinity, binding only to the same epitope (the part of an antigen that is recognized by the antibody).

Applications of Monoclonal Antibodies

Diagnostic tests

- Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance.
- Proteins can be detected using the Western blot and immuno dot blot tests.
- In immunohistochemistry, monoclonal antibodies can be used to detect antigens in fixed tissue sections, and similarly, immunofluorescence can be used to detect a substance in either frozen tissue section or live cells.

Analytic and chemical uses

Antibodies can also be used to purify their target compounds from mixtures, using the method of immunoprecipitation.

Therapeutic uses

Therapeutic monoclonal antibodies act through multiple mechanisms, such as blocking of targeted molecule functions, inducing apoptosis in cells which express the target, or by modulating signalling pathways.

Cancer treatment

One possible treatment for cancer involves monoclonal antibodies that bind only to cancer-cell-specific antigens and induce an immune response against the target cancer cell.

Such mAbs can be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate or to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell.

Every intact antibody can bind to cell receptors or other proteins with its Fc region.

Hybridoma

- Hybridoma technology is a method for producing large numbers of identical antibodies (also called monoclonal antibodies).
- This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response.
- A type of white blood cell, the B cell, produces antibodies that bind to the injected antigen.
- These antibody producing B-cells are then harvested from the mouse and, in turn, fused with immortal B cell cancer cells, a myeloma to produce a hybrid cell line called a hybridoma, which has both the antibody-producing ability of the B-cell and the longevity and reproductivity of the myeloma.
- The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal).
- The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis.
- In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.