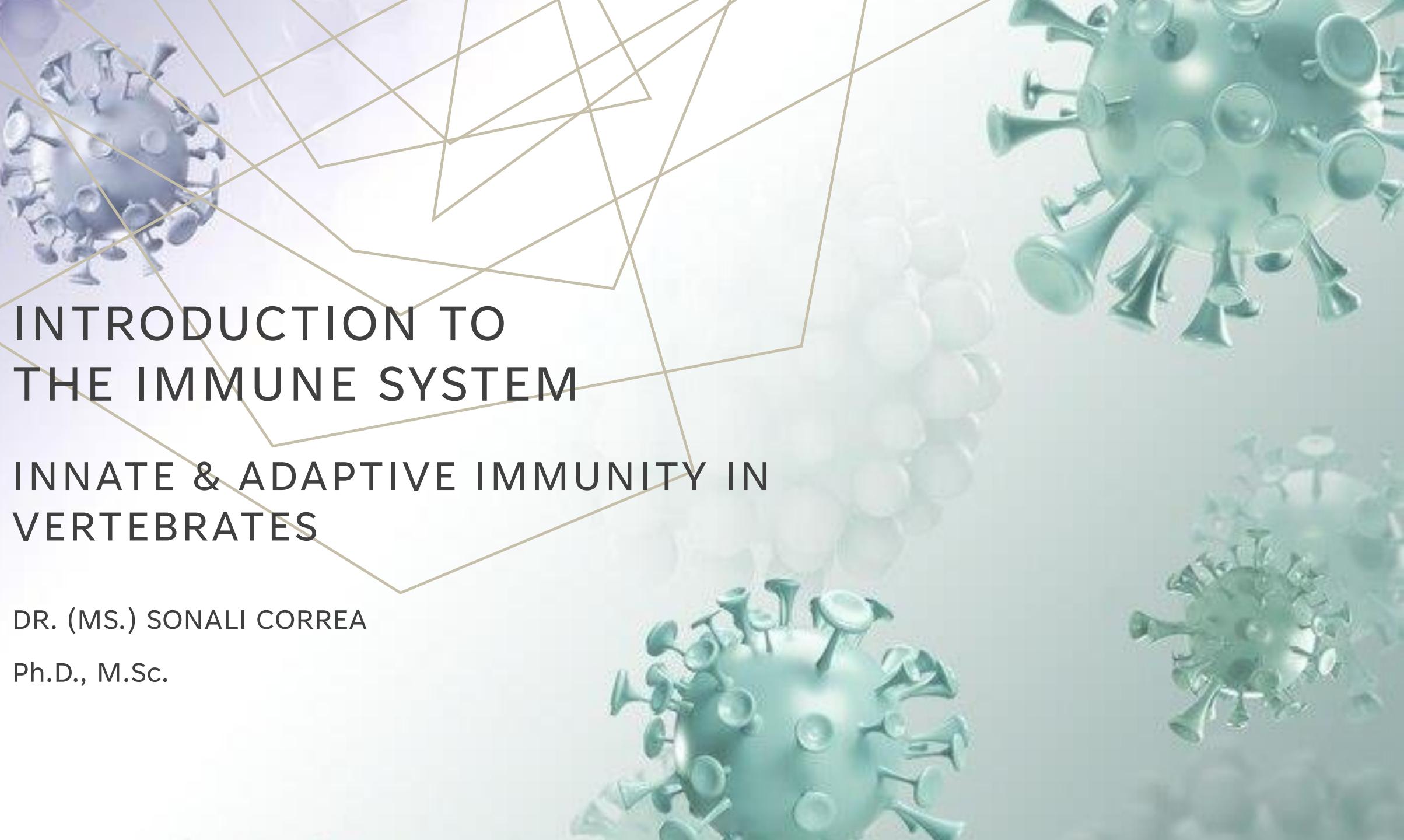


INTRODUCTION TO THE IMMUNE SYSTEM

INNATE & ADAPTIVE IMMUNITY IN VERTEBRATES

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Ph.D., M.Sc.

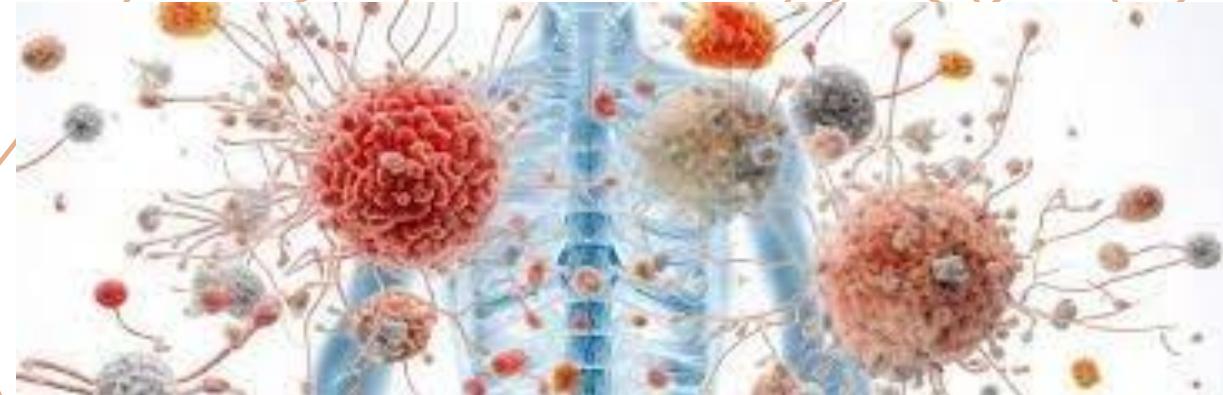


INTRODUCTION

- Immunology is a branch of biology involved with the study of the immune system, components of the immune system, its biological processes, the physiological functioning of the immune system, types, its disorders and a lot more.

History

- Edward Jenner's pioneering work in the 18th Century that would ultimately lead to **vaccination** in its modern form (an innovation that has likely saved more lives than any other medical advance), to the many scientific breakthroughs in the 19th and 20th centuries that would lead to, amongst other things, **safe organ transplantation**, the **identification of blood groups**, and the now ubiquitous use of **monoclonal antibodies** throughout science and healthcare, immunology has changed the face of modern medicine.

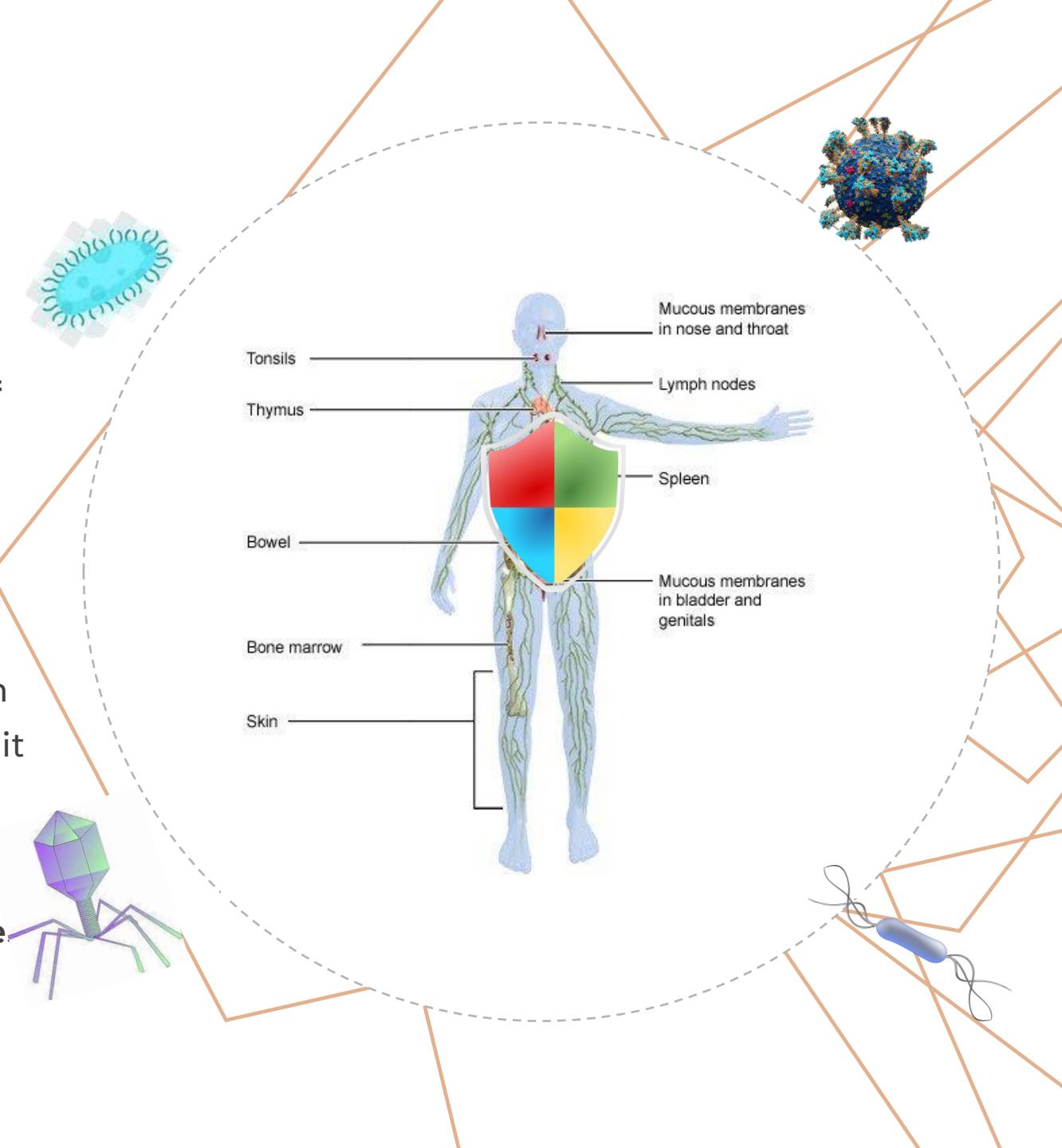
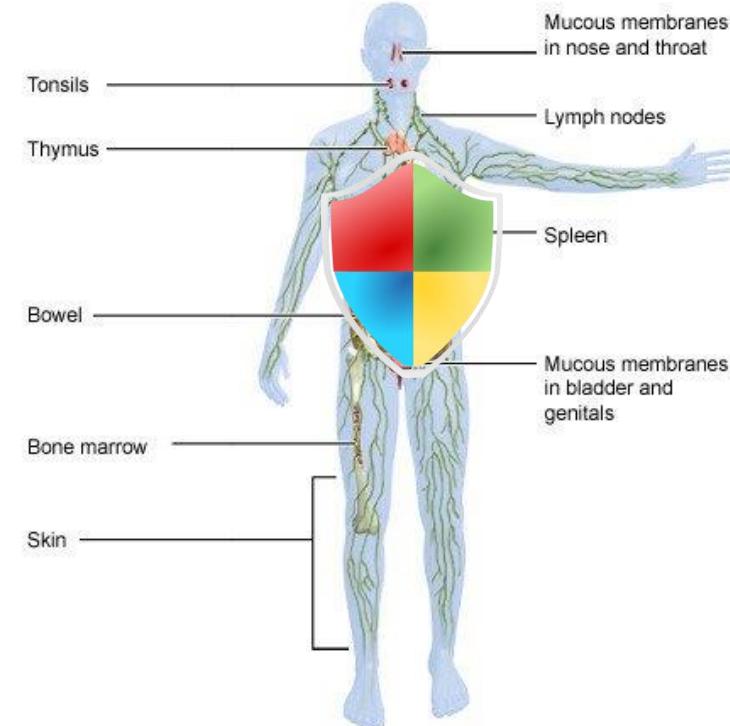


IMMUNOLOGY

"The study of the immune system, the cell-mediated and humoral aspects of immunity and immune responses."

IMMUNE SYSTEM

- The immune system is a **complex network of organs, cells and proteins** that defends the body against infection, whilst protecting the body's own cells.
- The immune system **keeps a record** of every germ (microbe) it has ever defeated so it can recognise and destroy the microbe quickly if it enters the body again.
- **Abnormalities of the immune system** can lead to allergic diseases, immunodeficiencies and autoimmune disorders.



IMMUNITY

- Immunity (immunis- Latin-exempt, state of protection from infectious diseases)
- Immunity is body's ability to **resist or eliminate** potentially harmful foreign materials or abnormal cells.

Consists of following activities:

- Defense against invading pathogens (viruses & bacteria)
- Removal of 'worn-out' cells (e.g., old RBCs) & tissue debris (e.g., from injury or disease)
- Identification & destruction of abnormal or mutant cells (primary defense against cancer)
- Rejection of 'foreign' cells (e.g., organ transplant)
- Inappropriate responses:
- Allergies - response to normally harmless substances
- Autoimmune diseases



- **A functional system – NOT an organ system**
- **Immune system** consists of different types of cells and organs which protect our body against pathogens.
- **Pathogens** are defined as microorganisms that cause infections in the body such as bacteria, fungi, viruses and protozoans.
- **Antigens** are molecules that elicit antibody generation. They can be everything that does not belong to our body, from parasites to fungi, bacteria, viruses, and haptens.
- **Haptens** are molecules that can elicit an immune response when combined with a carrier molecule.
- All the cells and molecules of the immune system are distributed in all the tissues of the body as well as lymphoid organs which eliminate microbial infectious diseases, decrease the growth of tumours and starts the repairing process of damaged tissues.
- The tissues and organs of the immune system **act as security forces** where cells act as the security guards while molecules act as the guns & bullets and use the communication system to protect you.

Types of Immune System

Innate

- First line of defence
- Physical barriers and Chemical barriers
- Function and efficiency do not change with repeated exposure to foreign pathogens.

Main elements of the innate immune system are –

- Dendritic cells.
- Phagocytic leukocytes.
- Natural killer (NK) cell.
- Physical epithelial barriers.
- Circulating plasma proteins.

Adaptive or Acquired

- Is activated when the innate system fails
- Consists lymphocytes and antibodies being the key elements
- Lymphocytes arise continuously from progenitor cells in the bone marrow & synthesize cell surface receptors or secrete proteins that specifically bind to foreign molecules.
- These secreted proteins are known as antibodies. Any molecule that can bind to an antibody is called an antigen.
- The term antibody is used interchangeably with immunoglobulin.

Passive

- Passive immunity is "borrowed" from another source and it lasts for a short time.
- For example, antibodies in a mother's breast milk give a baby temporary immunity to diseases the mother has been exposed to.

INNATE IMMUNITY

- This type of immunity is **present in an organism by birth**.
- This is **activated immediately** when the pathogen attacks. Innate immunity includes certain barriers and defence mechanisms that keep foreign particles out of the body.
- Innate immunity refers to the body's defence system.
- This immunity helps us by providing the **natural resistance** components including **salivary enzymes, natural killer cells, intact skin and neutrophils, etc.** which produce an initial response against the infections at birth prior to exposure to a pathogen or antigens.
- It is a **long-term immunity** in which our body produces the antibodies on its own. Our body has few natural barriers to prevent the entry of pathogen

TYPES OF BARRIERS

Physical barrier

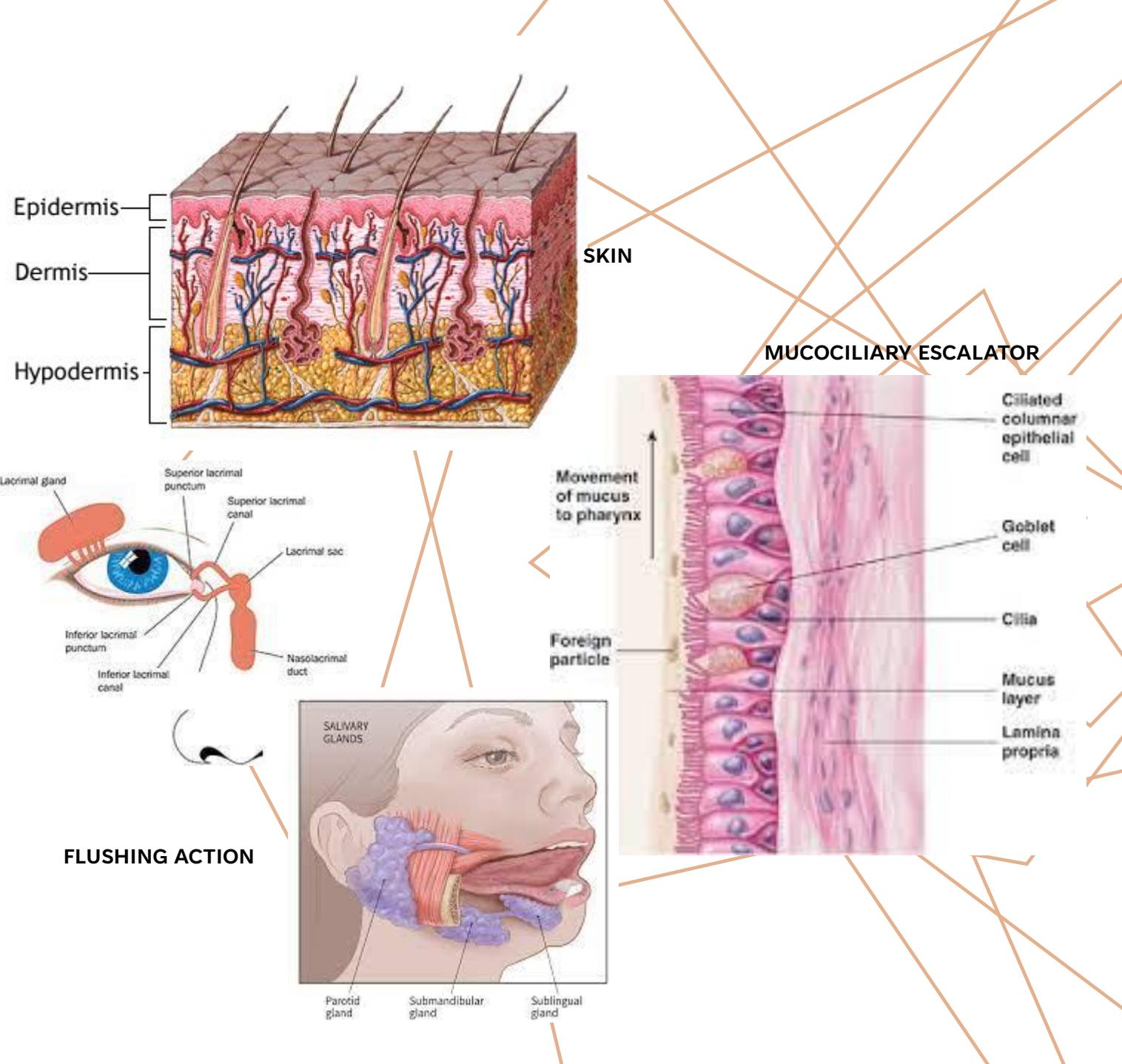
These include the **skin, body hair, cilia, eyelashes, the respiratory tract, and the gastrointestinal tract.**

These form **the first line of defence.**

The skin does more than providing us with fair or dark complexions.

Our skin acts as a physical barrier to the entry of pathogens.

The mucus coating in our nose and ear is a protective barrier which traps the pathogen before it gets inside.



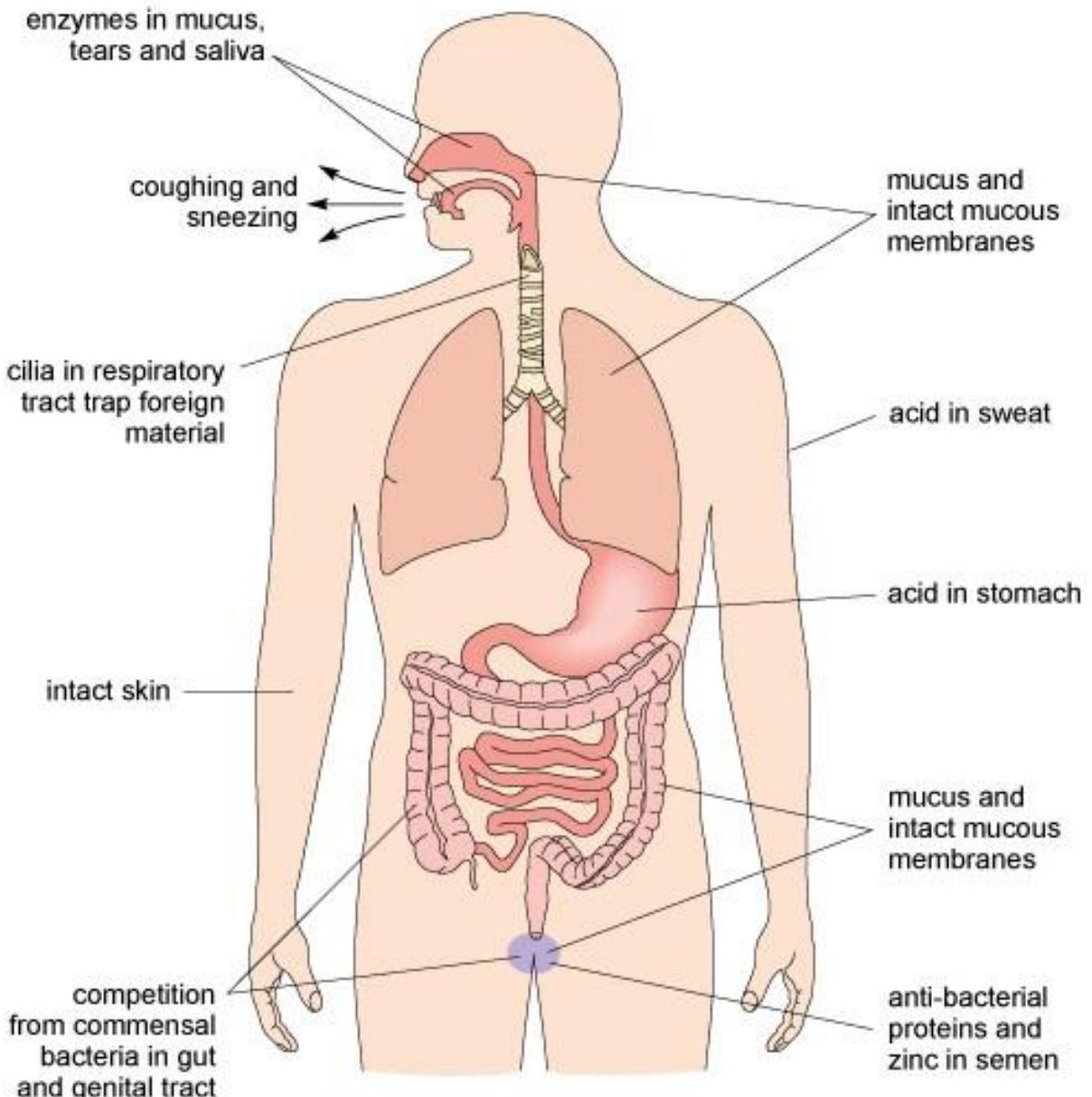
TYPES OF BARRIERS

Physiological barriers /Chemical factors

We know that our stomach uses hydrochloric acid to break down the food molecules.

Due to such a strongly acidic environment, most of the germs that enter our body along with the food are killed before the further process is carried on.

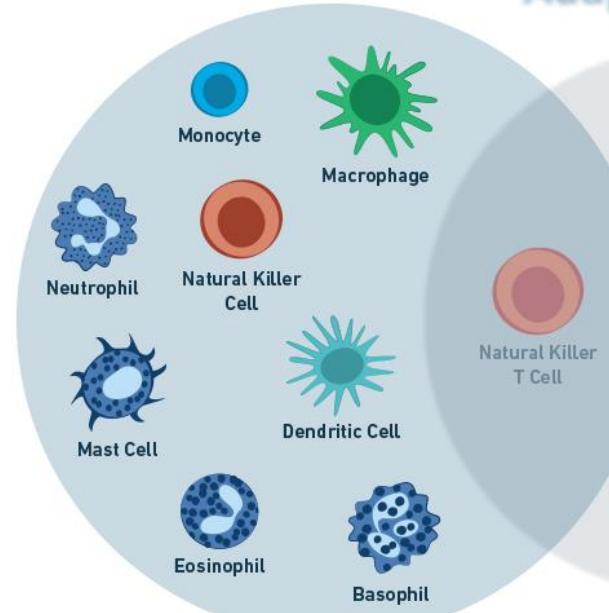
Saliva in our mouth and tears in our eyes also have the antibiotic property that does not allow the growth of pathogens even though they are exposed all day.



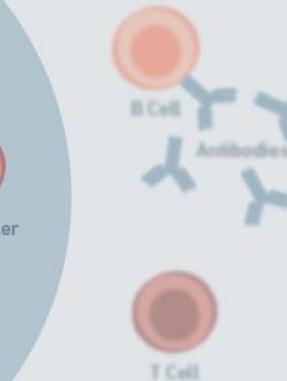
PARTS OF THE IMMUNE SYSTEM

- white blood cells
- antibodies
- complement system
- lymphatic system
- spleen
- bone marrow
- thymus.

Innate Immunity



Adaptive Immunity

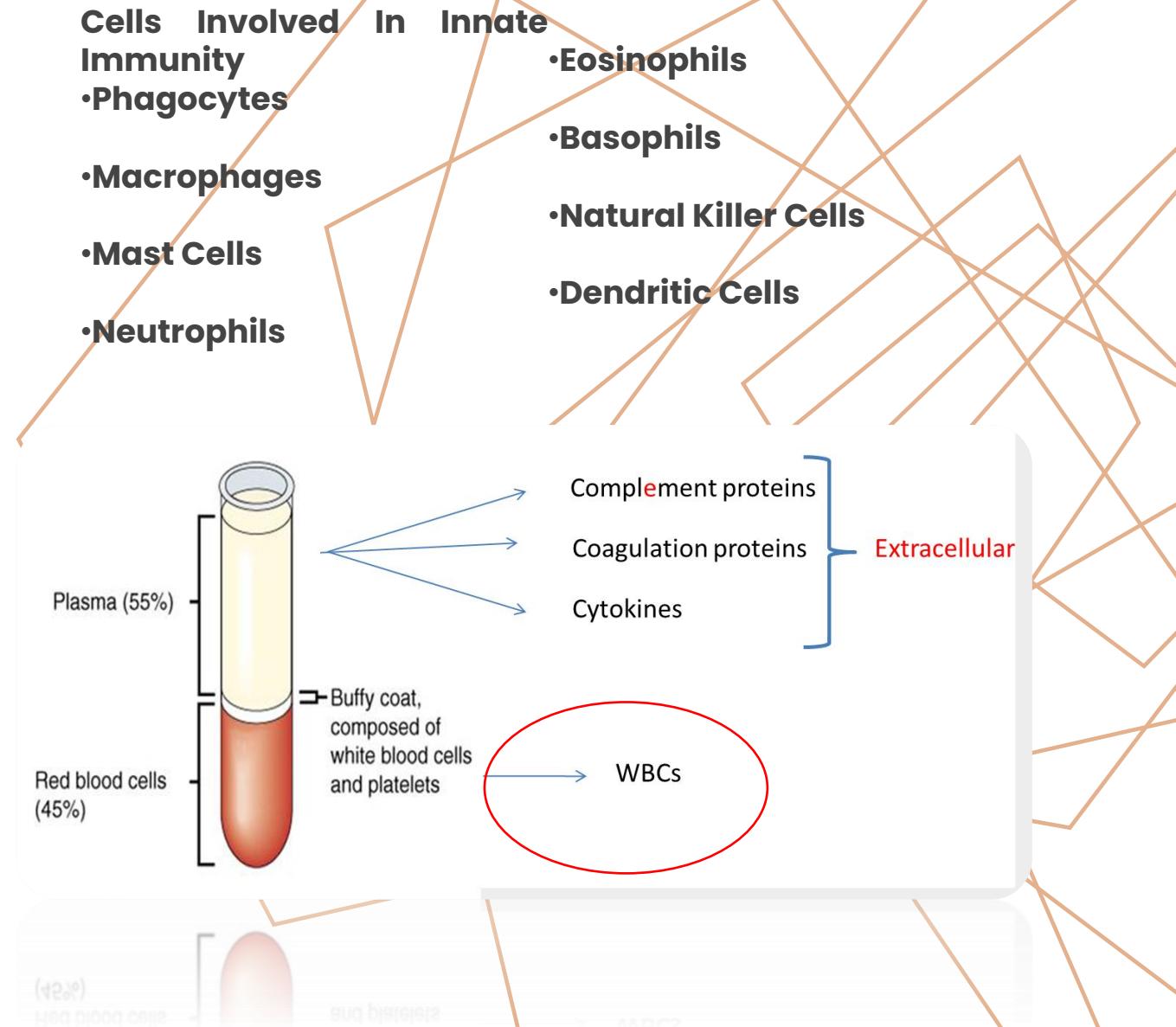


TYPES OF BARRIERS

Cellular barriers

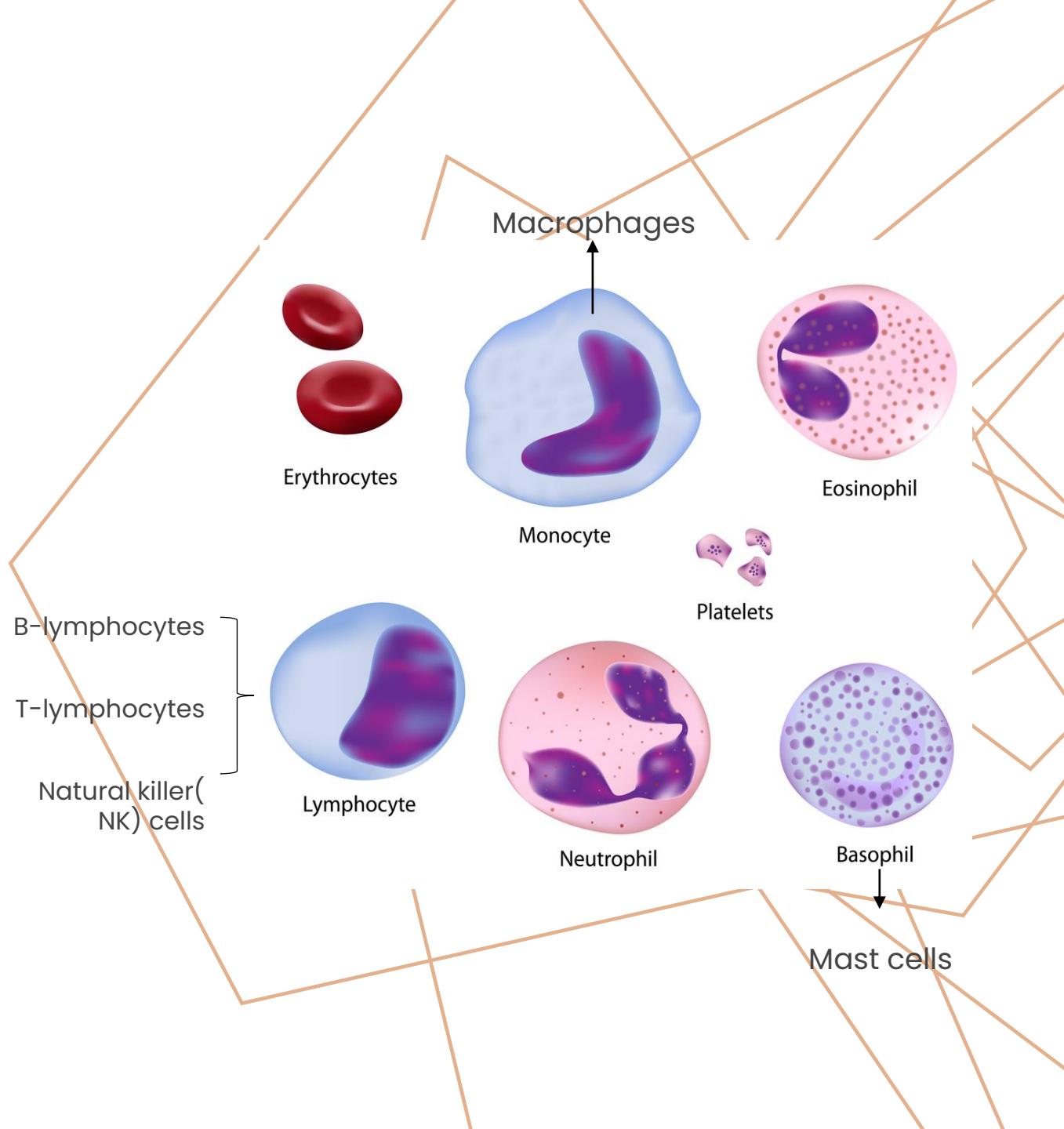
In spite of the physical and physiological barriers, certain pathogens manage to enter our body.

The cells involved in this barrier are leukocytes (WBC), neutrophils, lymphocytes, basophil, eosinophil, and monocytes. All these cells are all present in the blood and tissues.



Cells Involved In Innate Immunity

- **Phagocytes:** These circulate through the body and look for any foreign substance. They engulf and destroy it defending the body against that pathogen.
- **Macrophages:** These have the ability to move across the walls of the circulatory system. They release certain signals as cytokines to recruit other cells at the site of infections.
- **Mast Cells:** These are important for healing wounds and defence against infections.
- **Neutrophils:** These contain granules that are toxic in nature and kill any pathogen that comes in contact.
- **Eosinophils:** These contain highly toxic proteins that kill any bacteria or parasite in contact.
- **Basophils:** These attack multicellular parasites. Like the mast cells, these release histamine.
- **Natural Killer Cells:** These stop the spread of infections by destroying the infected host cells.
- **Dendritic Cells:** These are located in the tissues that are the points for initial infections. These cells sense the infection and send the message to the rest of the immune system by antigen presentation.



NEUTROPHILS IN INNATE IMMUNITY

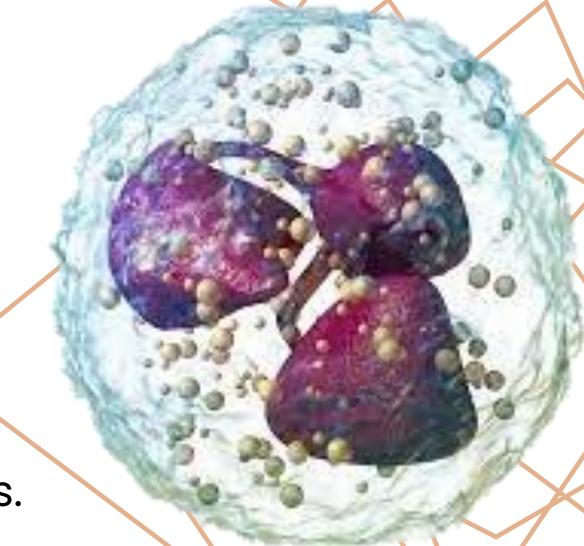
Polymorphonuclear leukocytes (PMNs or neutrophils) are the most abundant leukocyte in humans (~50-60%)

Efficient phagocytes

Most important cells of the innate immune system

the ability of neutrophils to kill microorganisms is immediate, non-specific, and not dependent on previous exposure to microorganisms.

Studies on PMN-pathogen interaction focused on the events leading to killing of microorganisms, such as recruitment/chemotaxis, transmigration, phagocytosis, and activation, whereas post-phagocytosis sequelae were infrequently considered.



PHAGOCYTOSIS

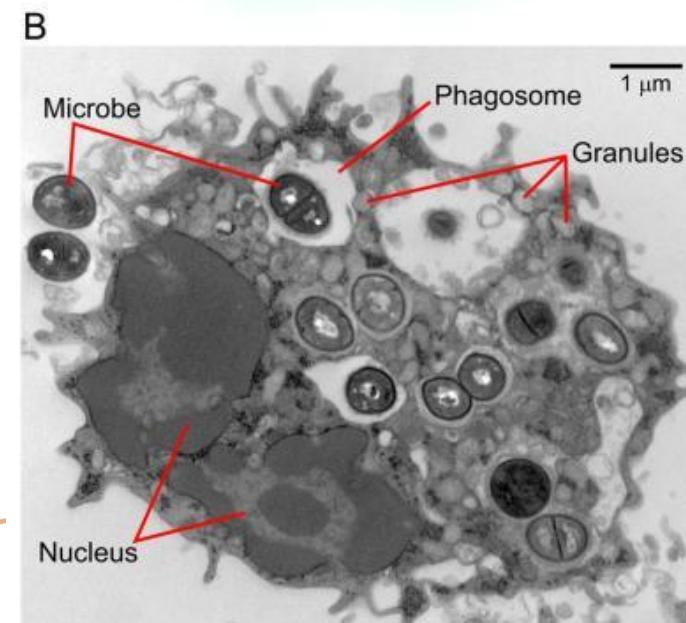
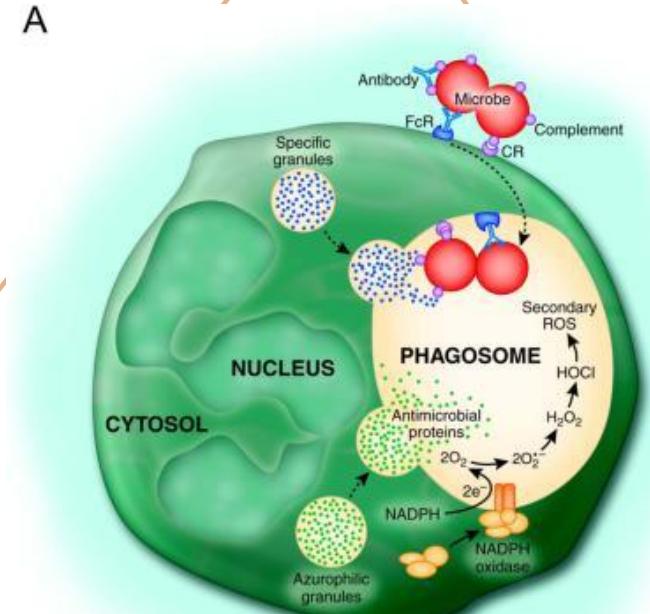
Phago = Eat Cyte = Cell

WBCs (e.g. Neutrophils) – find, eat and digest microbes

Panel A

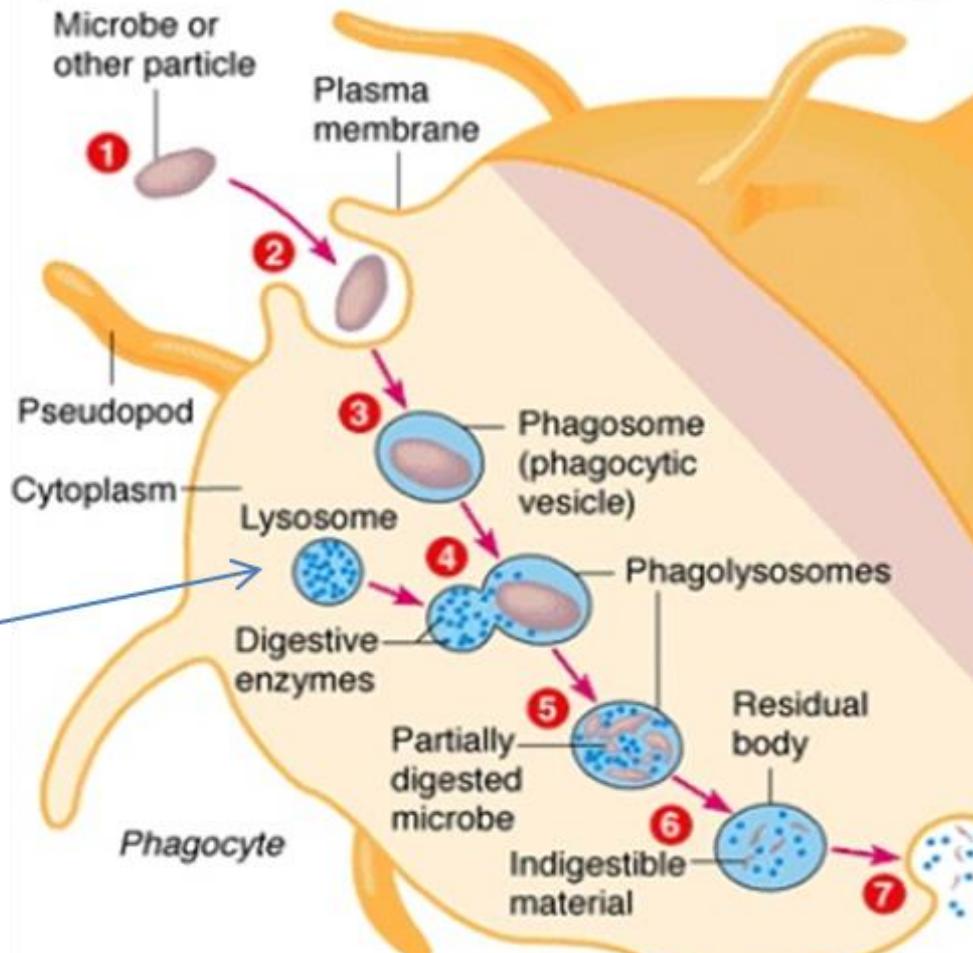
illustrates binding and phagocytosis of a microbe opsonized with antibody or serum complement. Phagocytosis triggers production of superoxide (O_2^-) from which other secondarily derived ROS are formed, including hydrogen peroxide (H_2O_2) and hypochlorous acid ($HOCl$).

Panel B is a transmission electron micrograph of a human neutrophil that has phagocytosed numerous *Staphylococcus aureus* (Microbe).



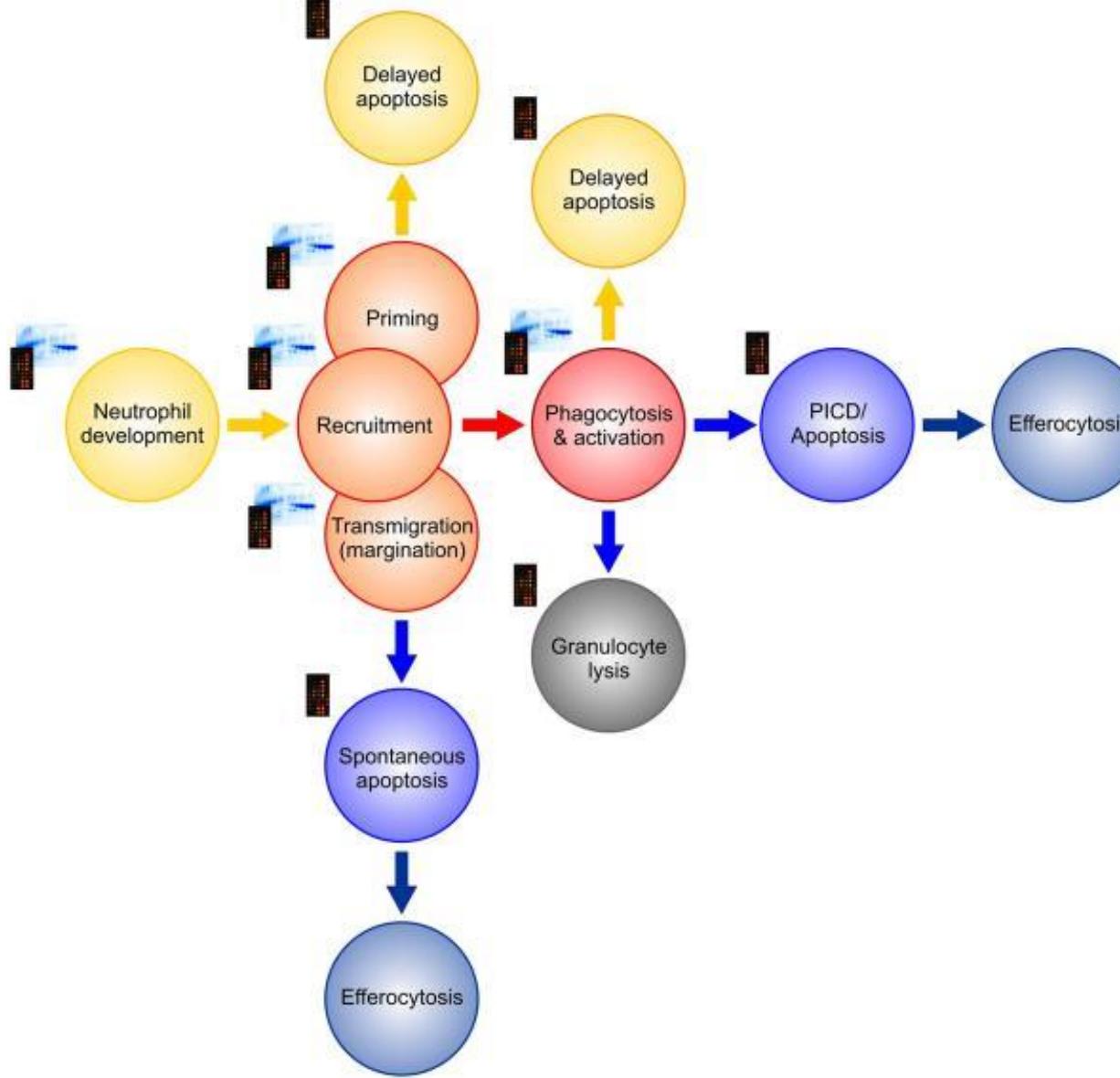
DIGESTION OF MICROBES BY NEUTROPHILS

Granules



(a) Phases of phagocytosis

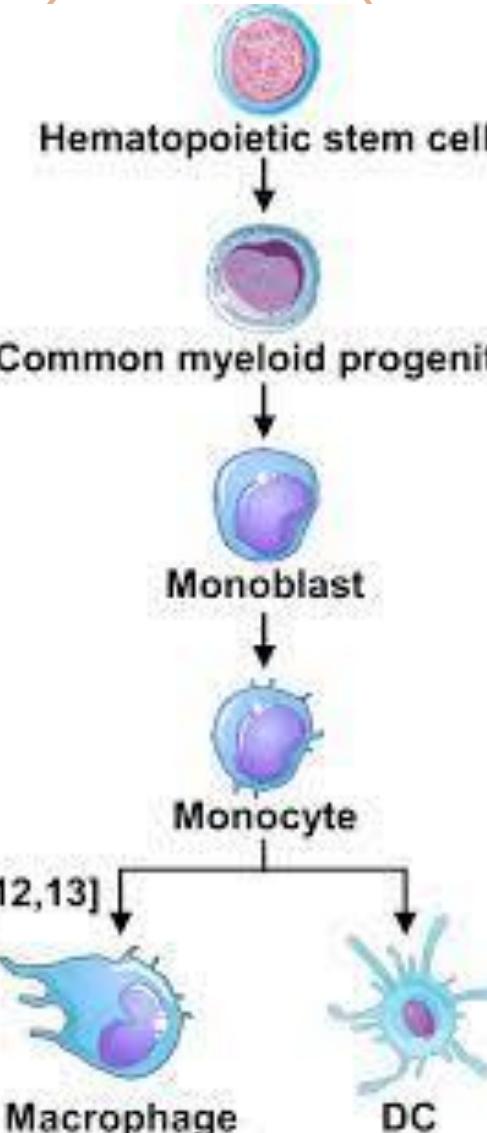
- ① Chemotaxis and adherence of microbe to phagocyte.
- ② Ingestion of microbe by phagocyte.
- ③ Formation of a phagosome.
- ④ Fusion of the phagosome with a lysosome to form a phagolysosome.
- ⑤ Digestion of ingested microbe by enzymes.
- ⑥ Formation of residual body containing indigestible material.
- ⑦ Discharge of waste materials.



MONOCYTES



- Monocytes are your cell's firefighters.
- Monocytes are bone marrow derived leukocytes that circulate in the blood and spleen.
- Monocytes are a critical component of the innate immune system.
- On maturity, they are the source of many other vital elements of the immune system, such as **macrophages and dendritic cells**.
- Monocytes play a role in both the inflammatory and anti-inflammatory processes that take place during an immune response.



DENDRITIC CELLS

- Dendritic cells (DC) are responsible for initiating all antigen-specific immune responses
- Dendritic cells are your fire department's call center. They're responsible for alerting other cells in your body to help fight infection.
- They are the master regulators of the immune response and serve this function by linking the microbial sensing features of the innate immune system to the exquisite specificity of the adaptive response
- Dendritic cells (DCs) are antigen-presenting cells that capture, process, and present antigens to lymphocytes to initiate and regulate the adaptive immune response.
- Dendritic cells reside in superficial tissues, such as just beneath your skin and in the lining of your nose, lungs, stomach and intestine.
- When a germ enters the body's tissues, dendritic cells collect the antigen of the invading germ (the molecule in the germ that produces an antibody response) and release proteins (cytokines) that notify other white blood cells to come to the site of the infection and destroy the invader.

FUNCTIONS OF DENDRITIC CELLS

Dendritic cells found in mucosal membranes where they are primed for antigen uptake but are not adept at presenting these antigens to T cells.

Dendritic cells (DCs) are professional antigen presenting cells that inform the fight against invasive pathogens while enforcing tolerance to self and harmless environmental antigens. They capture pathogens and receive signals from pathogens that influence the outcome of immune responses.

DCs activate innate lymphocytes (e.g., NK, NKT and ILCs)

On the basis of these signals, DCs orchestrate antigen specific T cell differentiation.

Alternatively they can silence self-reactive T cells by inducing deletion, anergy or regulation.

Depending on the function and development of DCs and the mechanisms by which they link innate immunity to adaptive immunity.



MACROPHAGES

- Macrophages work as innate immune cells through phagocytosis and sterilization of foreign substances such as bacteria, and play a central role in defending the host from infection.
- Macrophages are specialised cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms. In addition, they can also present antigens to T cells and initiate inflammation by releasing molecules (known as cytokines) that activate other cells.

Macrophages can be classified on basis of the fundamental function and activation.

- classically-activated macrophages(M1) macrophages,
- wound-healing macrophages (also known as alternatively-activated (M2) macrophages),
- regulatory macrophages (Mregs).

BACKGROUND

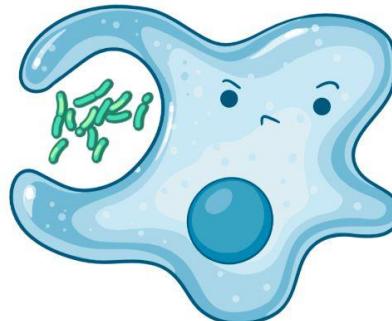
- * WHITE BLOOD CELL that PLAYS an IMPORTANT ROLE in IMMUNE SYSTEM
- * ADDITIONAL ROLES within SPECIFIC ORGAN SYSTEMS
- * ENGULF & DIGEST MICROORGANISMS
- * CLEAR OUT DEBRIS & DEAD CELLS
- * STIMULATE other CELLS INVOLVED in IMMUNE FUNCTION



FUNCTION

M1	M2
DETECT, ENGULF & DESTROY BACTERIA	REGENERATION of CONNECTIVE TISSUE
PROMOTE INFLAMMATION	PRODUCE VEGF & TGF- β 1
EXTRACELLULAR MATRIX DESTRUCTION	PHAGOCYTIZE BACTERIA & DAMAGED TISSUE
APOPTOSIS of INVADING CELLS	DEBRIDE DAMAGED TISSUE by RELEASING PROTEASES
ANTIGEN PRESENTATION	SECRETE GROWTH FACTORS

1



2



GOOD or BAD?



* OVERALL, GOOD → CRITICAL ROLE in HUMAN BODY

- ~ PROTECT BODY from BACTERIAL & VIRAL INFECTIONS
- ~ MEDIATING REPAIR
- ~ PROTECTION from NEURONAL DAMAGE in BRAIN
- ~ REGULATE IRON & BILIRUBIN LEVELS



* PATHOLOGICAL EFFECTS

- ~ TYPE of M2 MACROPHAGE PROMOTES TUMOR GROWTH
- ~ M1 & M2 PLAY ROLE in PROMOTION of ATHEROSCLEROSIS

NK CELLS

Natural killer (NK) cells play a vital role in innate immune responses to infection; they express activation receptors that recognize virus-infected cells.

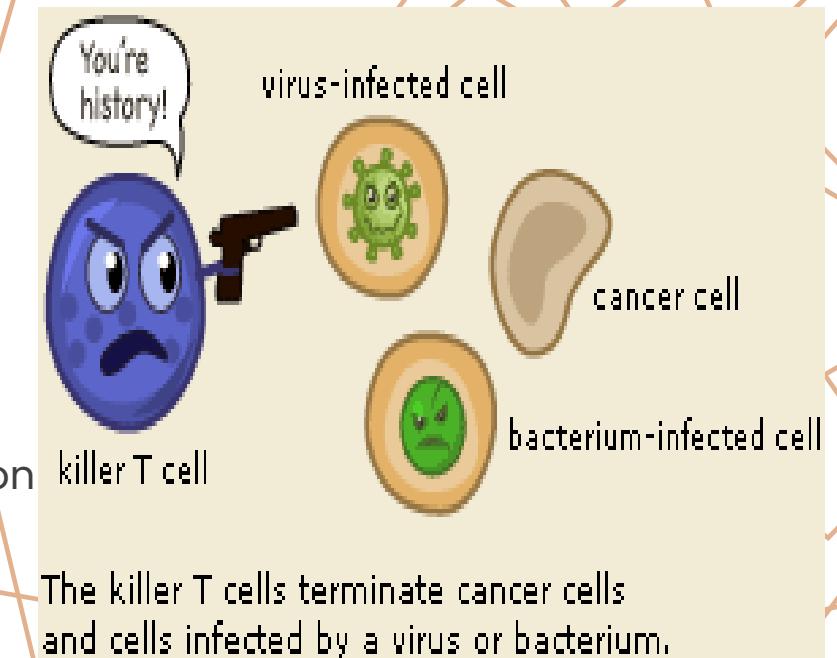
Natural killer cells are fighters in your innate immune system, which is an essential part of your immune system.

Highly related to receptors recognizing tumor cells, the activation receptors trigger cytotoxicity and cytokine production

NK cells also express inhibitory receptors for major histocompatibility complex (MHC) class I molecules that block the action of the activation receptors.

Natural killer (NK) cells target and kill aberrant cells, such as virally infected and tumorigenic cells.

Killing is mediated by cytotoxic molecules which are stored within secretory lysosomes, a specialized exocytic organelle found in NK cells.



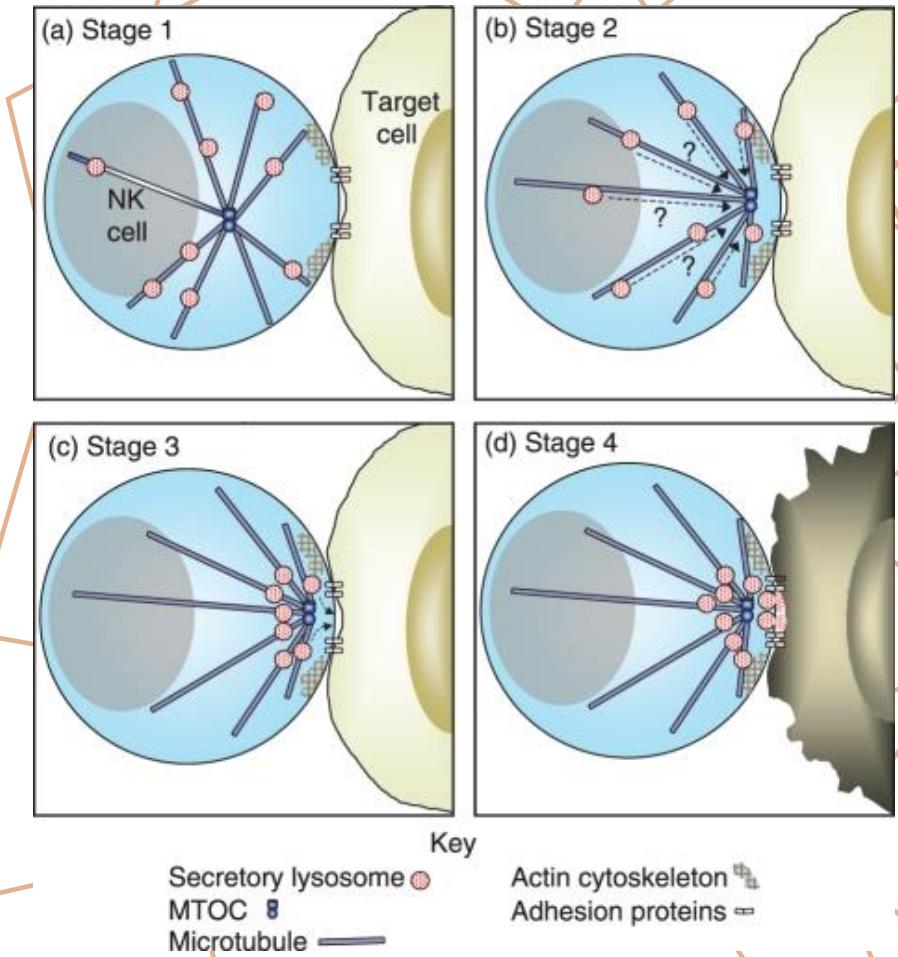
NK CELLS

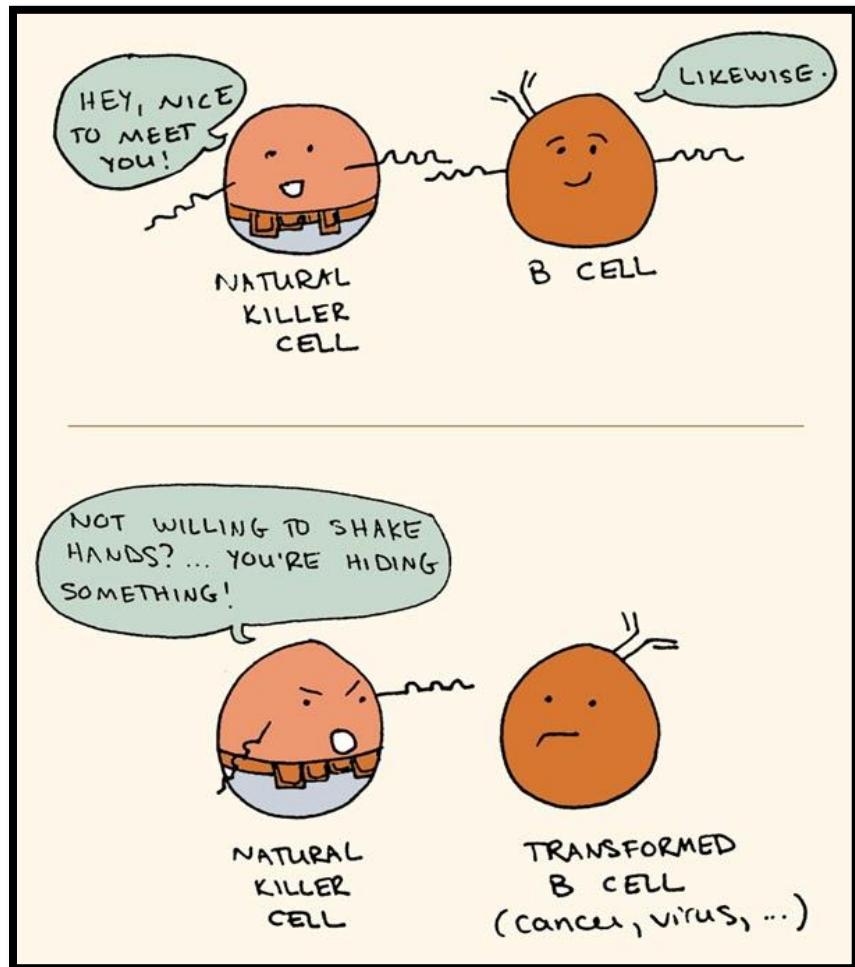
Killing is mediated by cytotoxic molecules which are stored within secretory lysosomes, a specialized exocytic organelle found in NK cells.

Target cell recognition induces the formation of a lytic immunological synapse between the NK cell and its target.

The polarized exocytosis of secretory lysosomes is then activated and these organelles release their cytotoxic contents at the lytic synapse, specifically killing the target cell.

The essential role that secretory lysosome exocytosis plays in the cytotoxic function of NK cells is highlighted by immune disorders that are caused by the mutation of critical components of the exocytic machinery



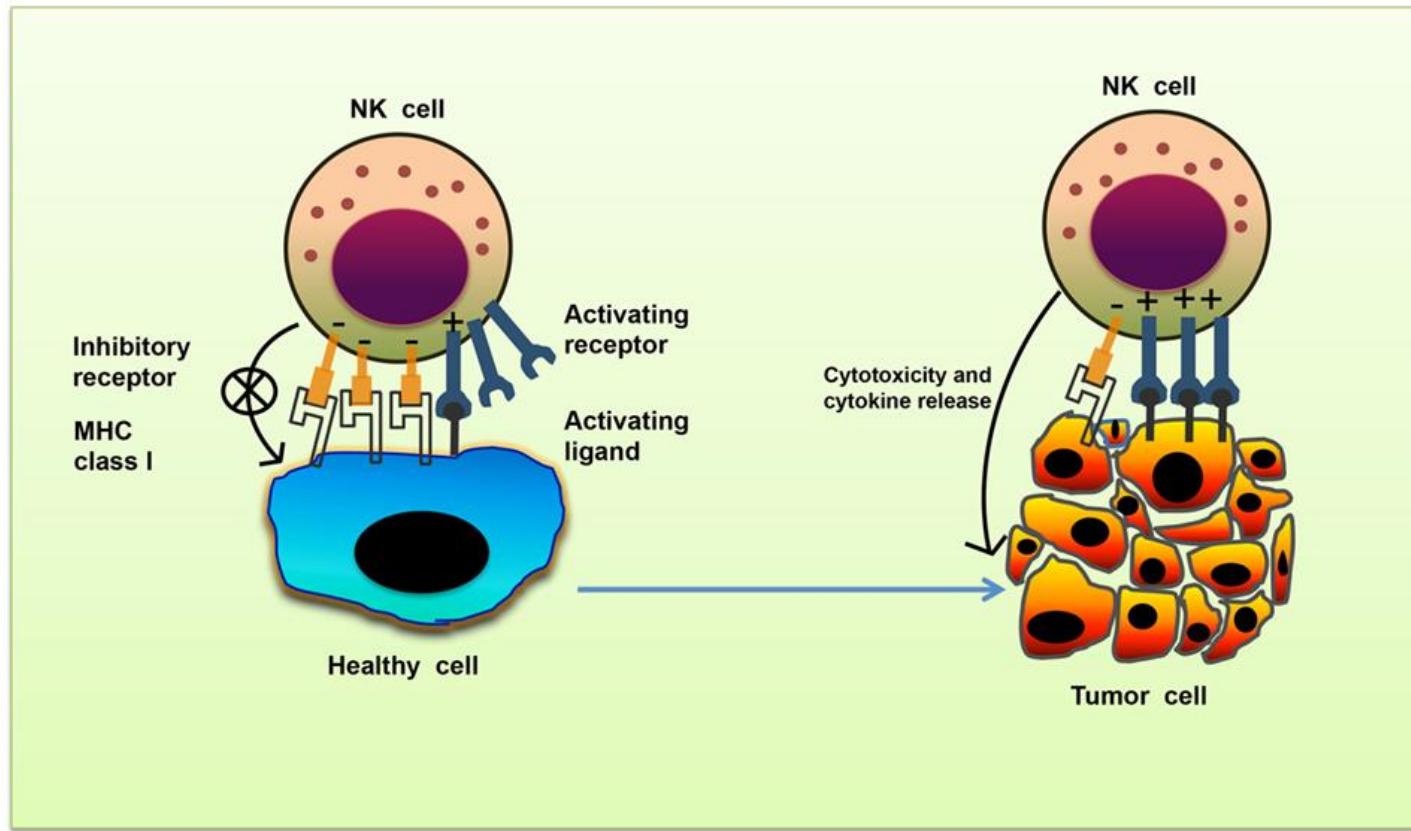


NK cells can recognize and kill cells that have down-regulated MHC class I molecules from their cell surface.

The MHC class I molecules are recognized by NK cell inhibitory receptors and the ligation of these receptors inhibits the activation of NK cells.

NK cells can kill transformed or infected cells by the release of perforin and granzymes or by using effector molecules of the tumor necrosis factor (TNF) family, such as TNF, TNF-related apoptosis inducing ligand (TRAIL), and Fas ligand, which induce apoptosis in the target cells.

HOW DO NK CELLS KILL



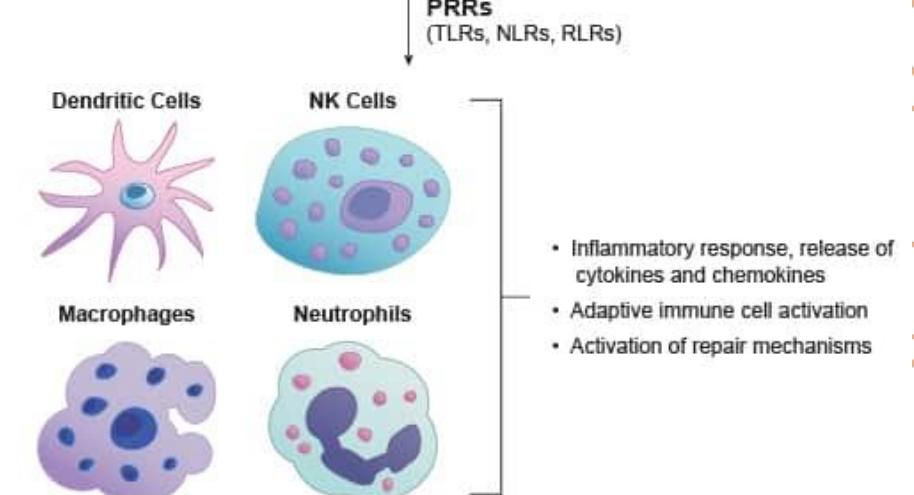
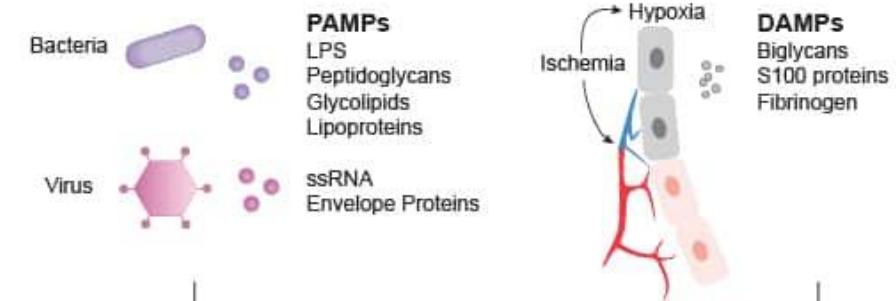
Natural killer (NK) cells target and kill aberrant cells, such as virally infected and tumorigenic cells.

NK cells release cytotoxic granules containing the pore-forming protein perforin, granulysin and serine proteases known as granzymes. Granzymes promote the cleavage and activation of a family of protease known as caspases. Caspases promote proteolytic cleavage of cellular substrates leading to apoptosis.

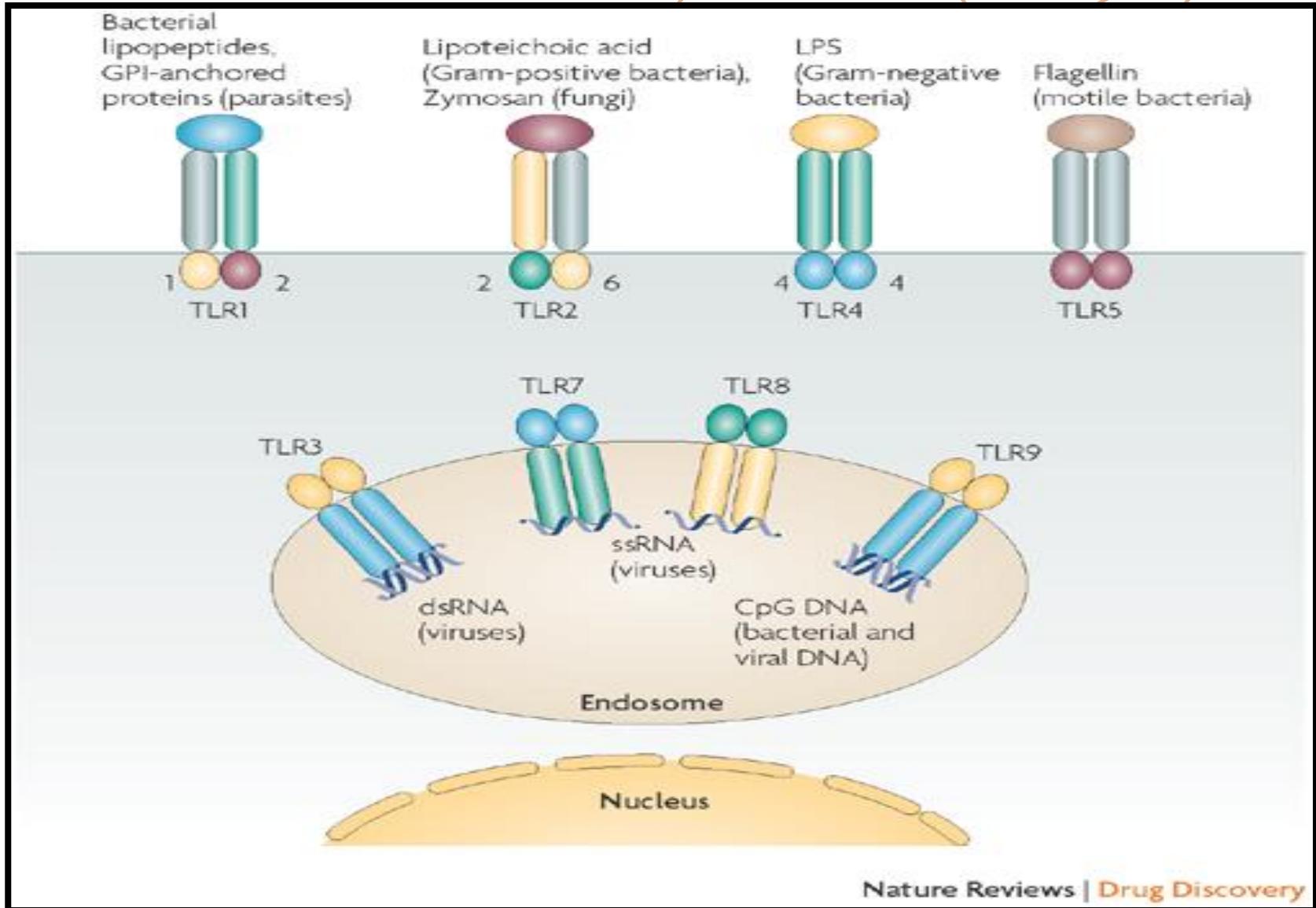
Killing is mediated by cytotoxic molecules which are stored within secretory lysosomes, a specialized exocytic organelle found in NK cells.

TLRs

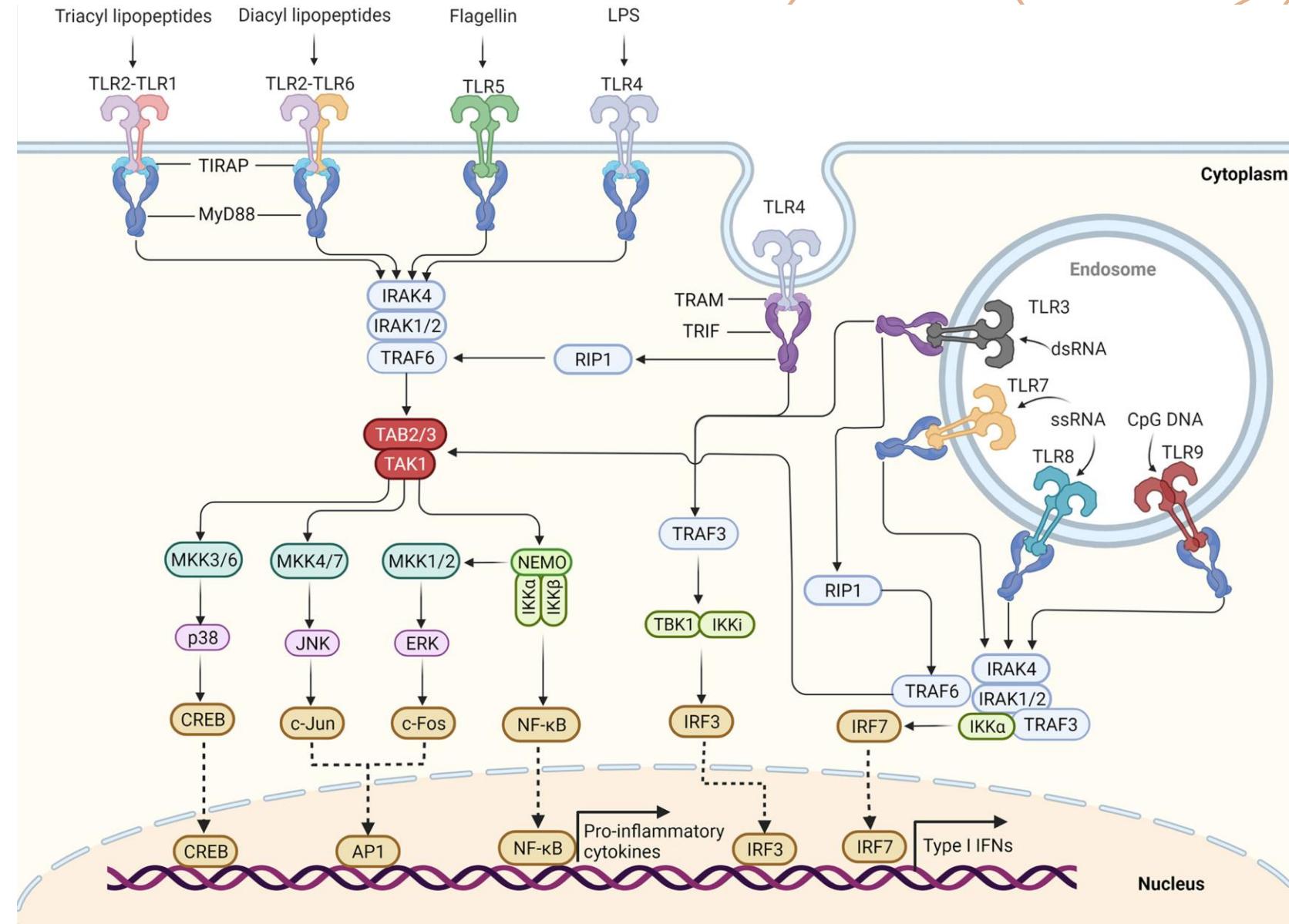
- Toll-like receptors (TLRs) has established that innate immunity is a skillful system that detects invasion of microbial pathogens.
- Recognition of microbial components by TLRs initiates signal transduction pathways, which triggers expression of genes.
- TLRs are evolutionarily conserved innate receptors expressed in various immune and non-immune cells of the mammalian host
- Toll-like receptors (TLRs) play a major role in innate immunity, since they detect conserved pathogen-associated molecular patterns (**PAMPs**) on a range of microbes, including viruses, leading to innate immune activation and orchestration of the adaptive immune response..



BINDING OF TLRS TO MICROBES & THEIR COMPONENTS



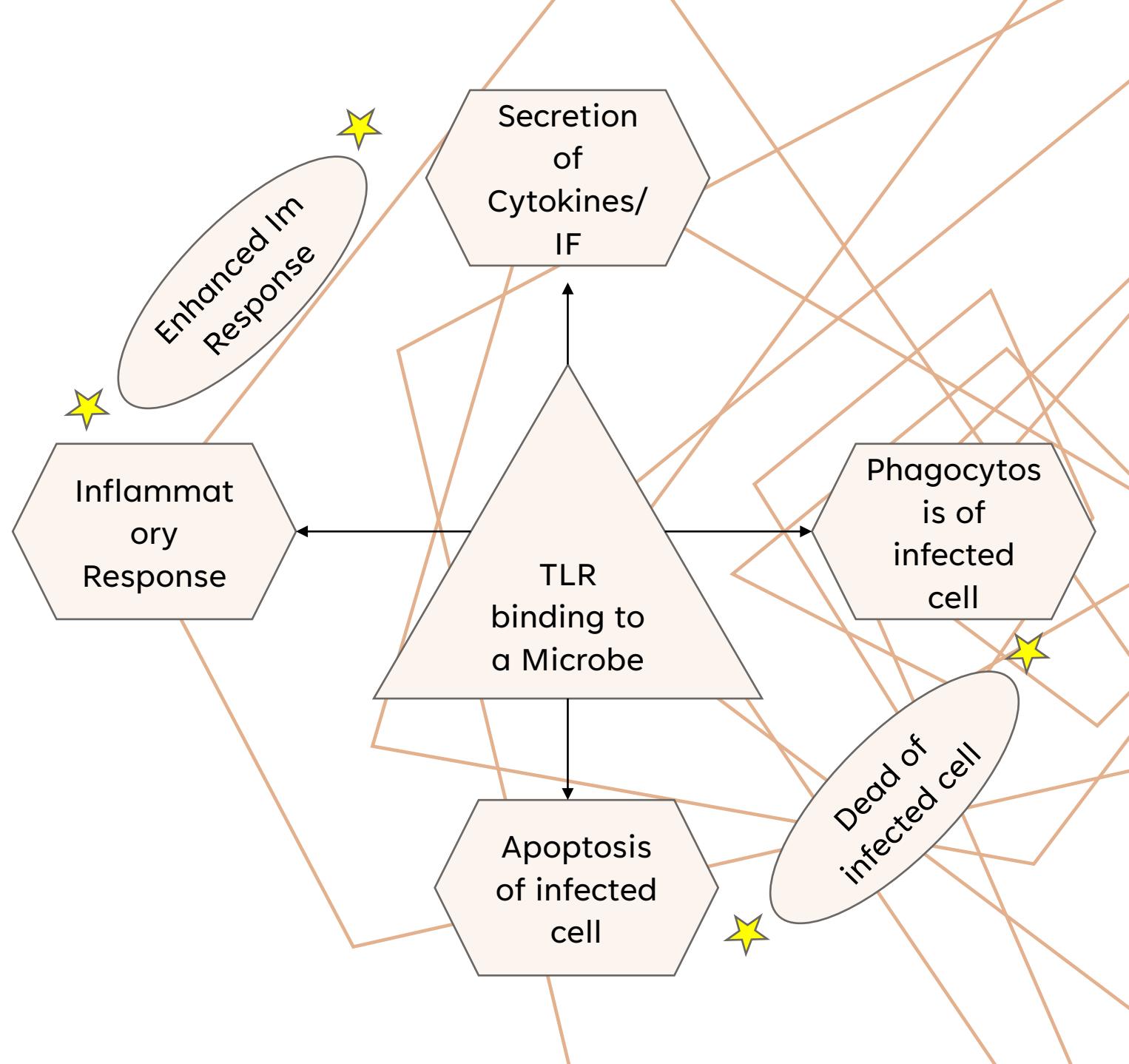
ROLES IN IMMUNE RESPONSE



WHAT HAPPENS WHEN TLRS BIND TO MICROBES

The binding of ligands to TLR activates specific intracellular signaling cascades that initiate host defense reactions.

Such binding is ligand-dependent and cell type-dependent and leads to production of pro-inflammatory cytokines and type 1 interferon.



INNATE IMMUNE RESPONSE

- Upon entering the bloodstream, a pathogen can initially be detected without the presence of a single immune cell via a mechanism referred to as the **Complement System**.
- This system works in concert with both innate and adaptive immune responses to recruit immune cells to the site of infection.
- Essentially, the Complement System consists of several types of **inert proteins** that are made by the liver and flow freely through the bloodstream.
- When they bump into a pathogen they **can bind to the surface of bacteria or parasites to flag them as foreign threats**.
- A cascade of related **complement proteins and enzymes** can then become activated which, not only continue to mark the pathogens, but may also form pores (**membrane attack complexes**) to lyse the bacteria or, alternatively, coat them in proteins to make the bacteria more enticing for immune phagocytes to engulf.
- This **process of coating a pathogen** in complement proteins is called **opsonization**.

INNATE IMMUNE RESPONSE

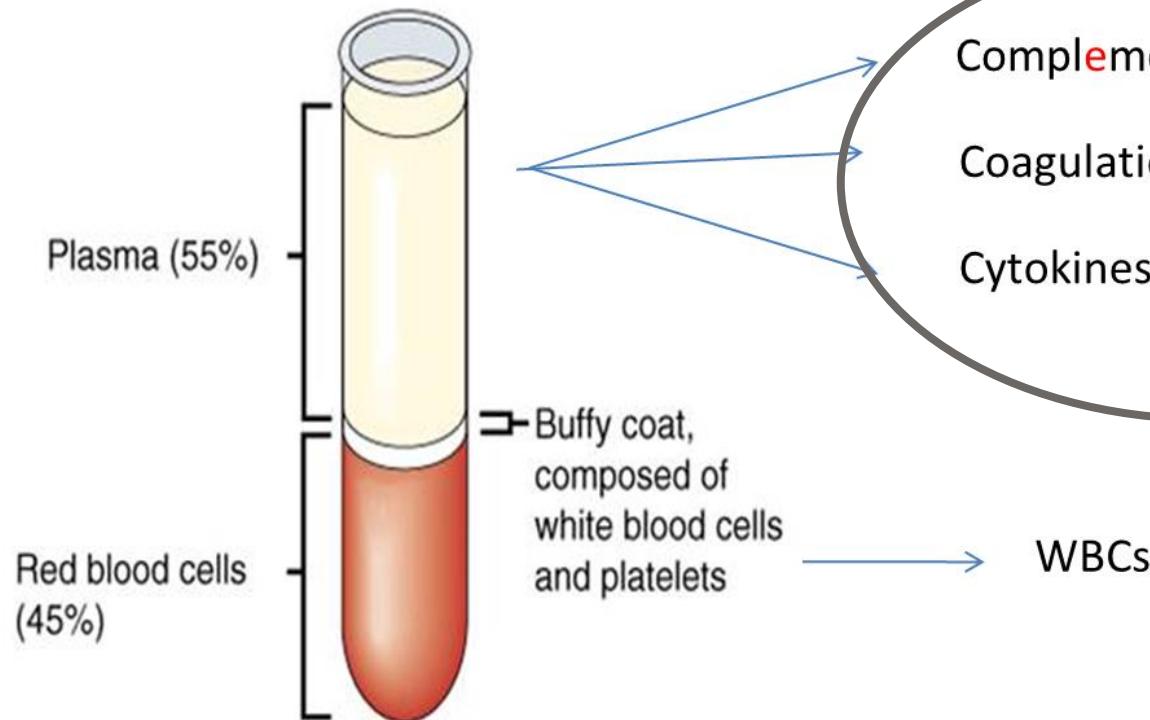
- Many of the cells actively involved in the innate immune response are **phagocytes** that patrol blood and tissue searching for potentially threatening invaders.
- Once a pathogen is found, they use **germline-encoded pattern recognition receptors (PRRs)** on their cell membrane to distinguish the invaders' molecules as foreign.
- These pattern recognition receptors are often **directed against cell surface and cell wall components of microorganisms, but bacterial DNA that contains unmethylated CpG motifs also induces an innate immune response.**
- Once activated, these cells **initiate a cascade that leads to a rapid immune response** (within minutes to hours).
- One of the first steps that certain immune cells take at the site of an infection is **to secrete cytokines into the extra-cellular fluid.**
- These cytokines, such as **interleukins (IL) or tumor necrosis factors (TNFs)**, are chemicals that attract fellow white blood cells to the site of an infiltration. Responding immune cells can also release other chemicals that initiate an inflammatory response.
- These signals lead to **vasodilation** which allows more blood to flow through the affected area and **boosts recruitment of immune cells to aid in the clearing of pathogens.**

SUMMARY INTERNAL DEFENSES – CELLULAR (WBCS)

Come into play when the external defence's are breached:

- Neutrophils
- Monocytes / macrophages
- NK cells
- TLRs

COMPONENTS OF BLOOD



(42°)

Cytokine barriers

The cells in our body are smarter than we give them credit for.

For instance, in case a cell in our body experiences a virus invasion, it automatically secretes proteins called interferons which forms a coating around the infected cell and prevents the cells around it from further infections.

Types of Cytokine Barriers

1. Innate Cytokine Barriers: These are the natural cytokine barriers that are present in the body. They provide **immediate protection** against infections. Innate cytokine barriers are activated by the presence of pathogens or foreign substances in the body. **They produce cytokines that activate the immune system and trigger an inflammatory response.**

2. Acquired Cytokine Barriers: These are the cytokine barriers that are **acquired during an infection or vaccination**. They **provide long-term protection against infections**. Acquired cytokine barriers are activated by **the recognition of specific antigens** by the immune system. They produce cytokines that **activate immune cells and trigger an inflammatory response**.

Functions of Cytokine Barriers

Cytokine barriers play a crucial role in protecting the body against infections. They have the following functions:

- 1. Activate the immune system:** Cytokines activate the immune system and stimulate the production of immune cells that can fight infections.
- 2. Trigger an inflammatory response:** Cytokines trigger an inflammatory response that helps to contain the infection and prevent its spread.
- 3. Attract immune cells:** Cytokines attract immune cells to the site of infection, where they can fight off the pathogens.
- 4. Regulate the immune response:** Cytokines regulate the immune response to ensure that it is effective but not harmful to the body.

RELEASE OF CYTOKINES

- Cells of the immune system:
- Neutrophils – when they encounter a pathogen
- Macrophages – when they encounter a pathogen
- TLRs – bind to microbe / components of a microbe
- NK cells – on encountering a microbe infected cell /tumour cell
- Lymphocytes – when they are activated
- Cytokines are IFNs, ILs TNF

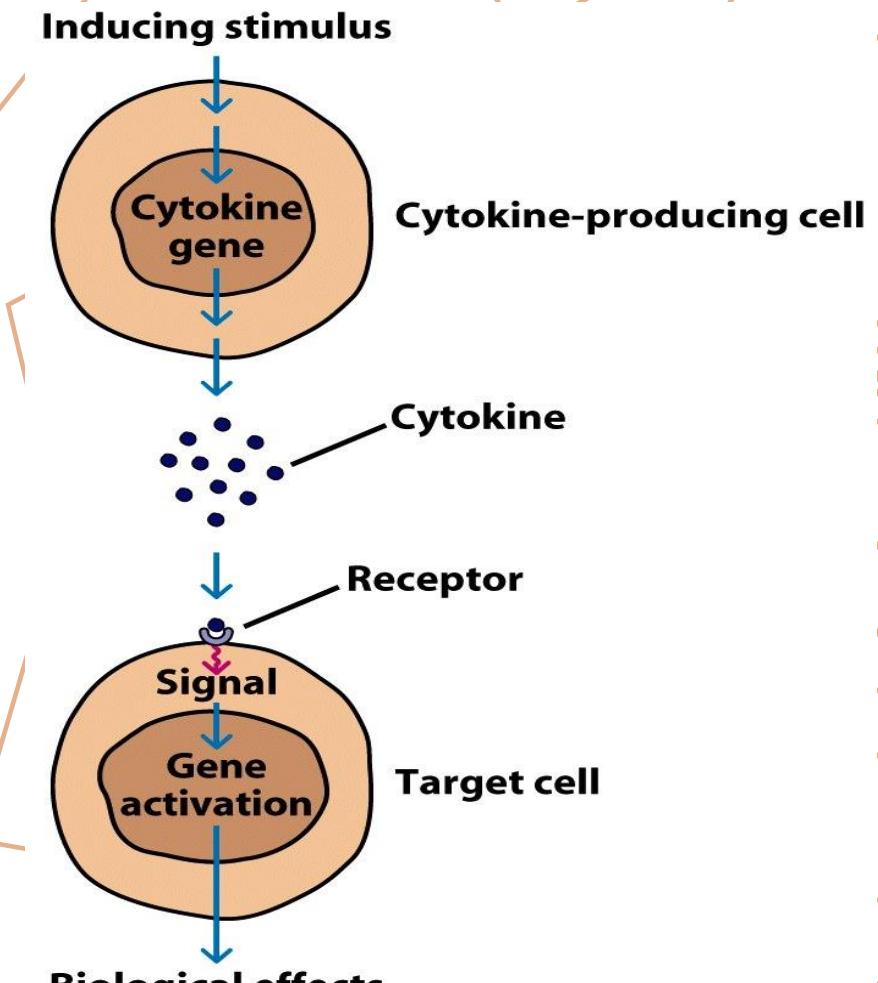
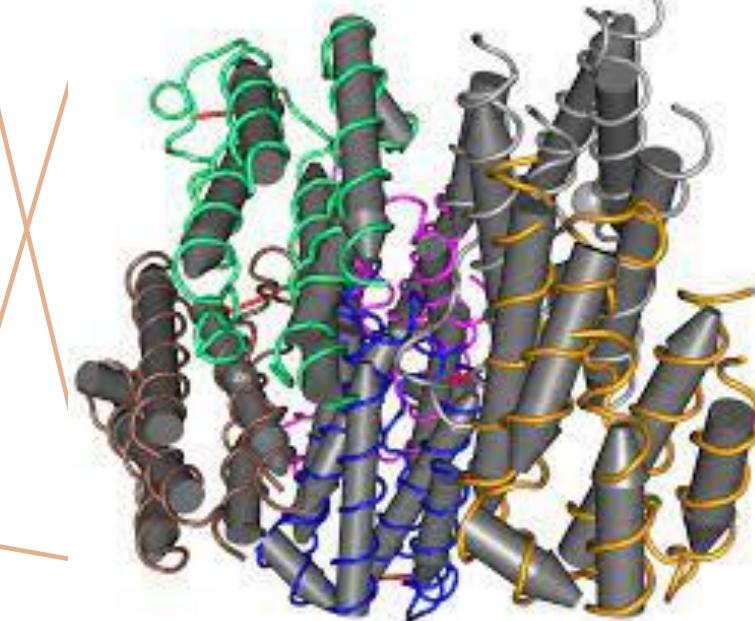


Figure 12-1a
Kuby IMMUNOLOGY, Sixth Edition
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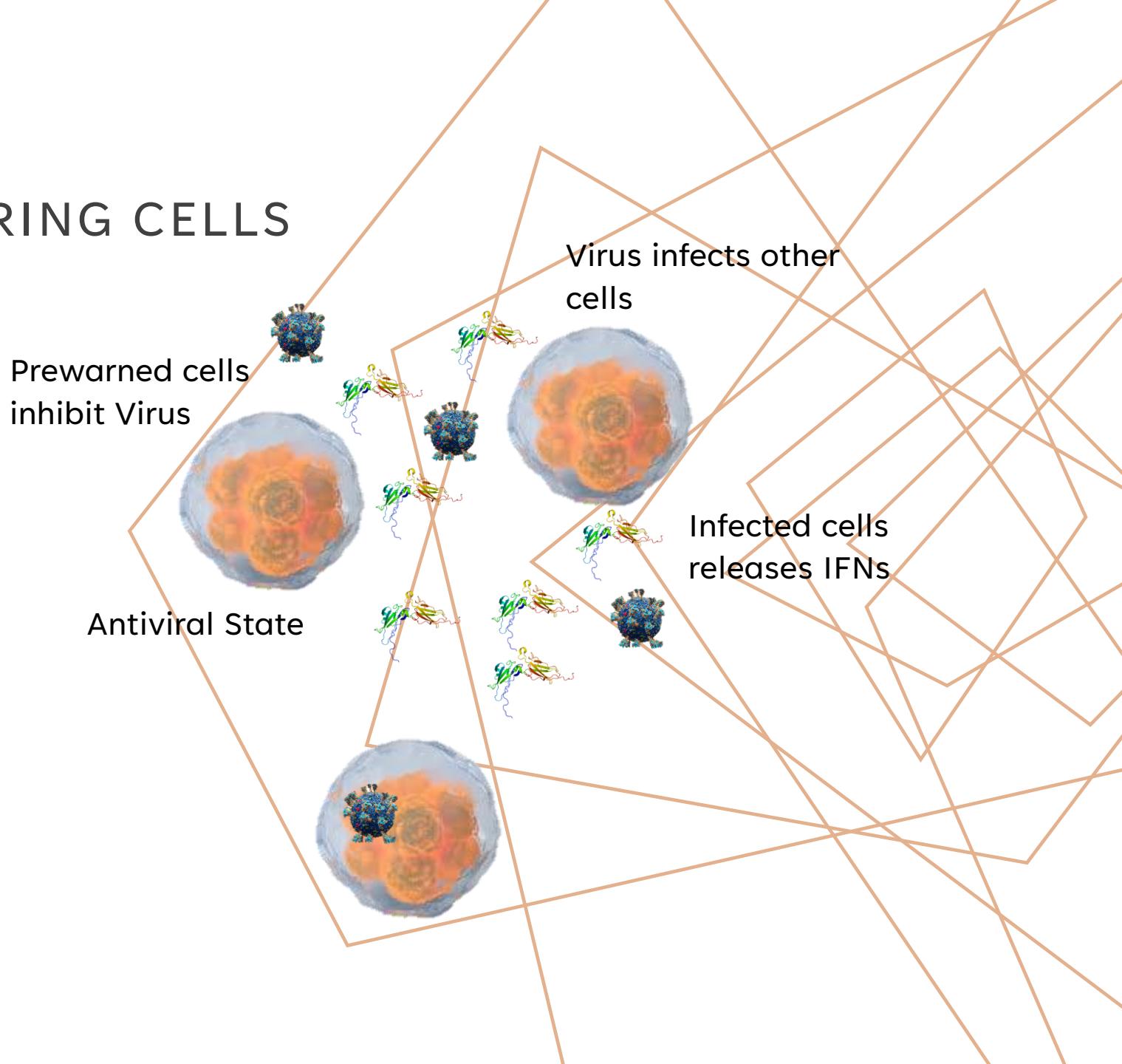
INTERFERONS (IFN)

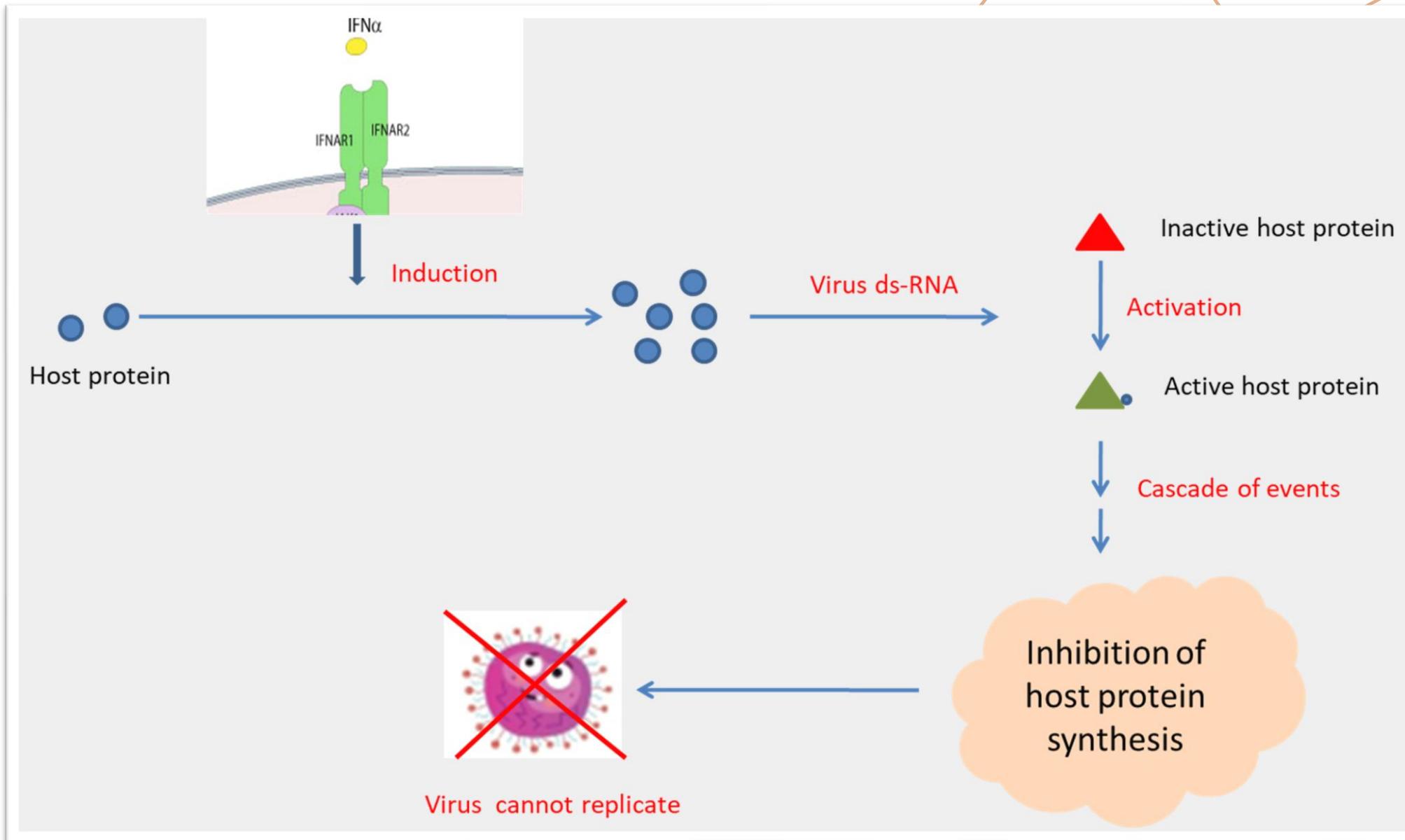
- Interferons (IFNs) are among the first vertebrate immune pathways activated upon viral infection and are crucial for control of viral replication and dissemination, especially at mucosal surfaces as key locations for host exposure to pathogens.
- Signalling proteins produced by virus infected monocytes and lymphocytes
- Secreted proteins – Key anti-viral proteins
- “Interfere” with virus replication
- Warn the neighbouring cells that a virus is around...
- If we did not have IFNs – most of us may die of influenza virus infection



IFN WARN NEIGHBORING CELLS

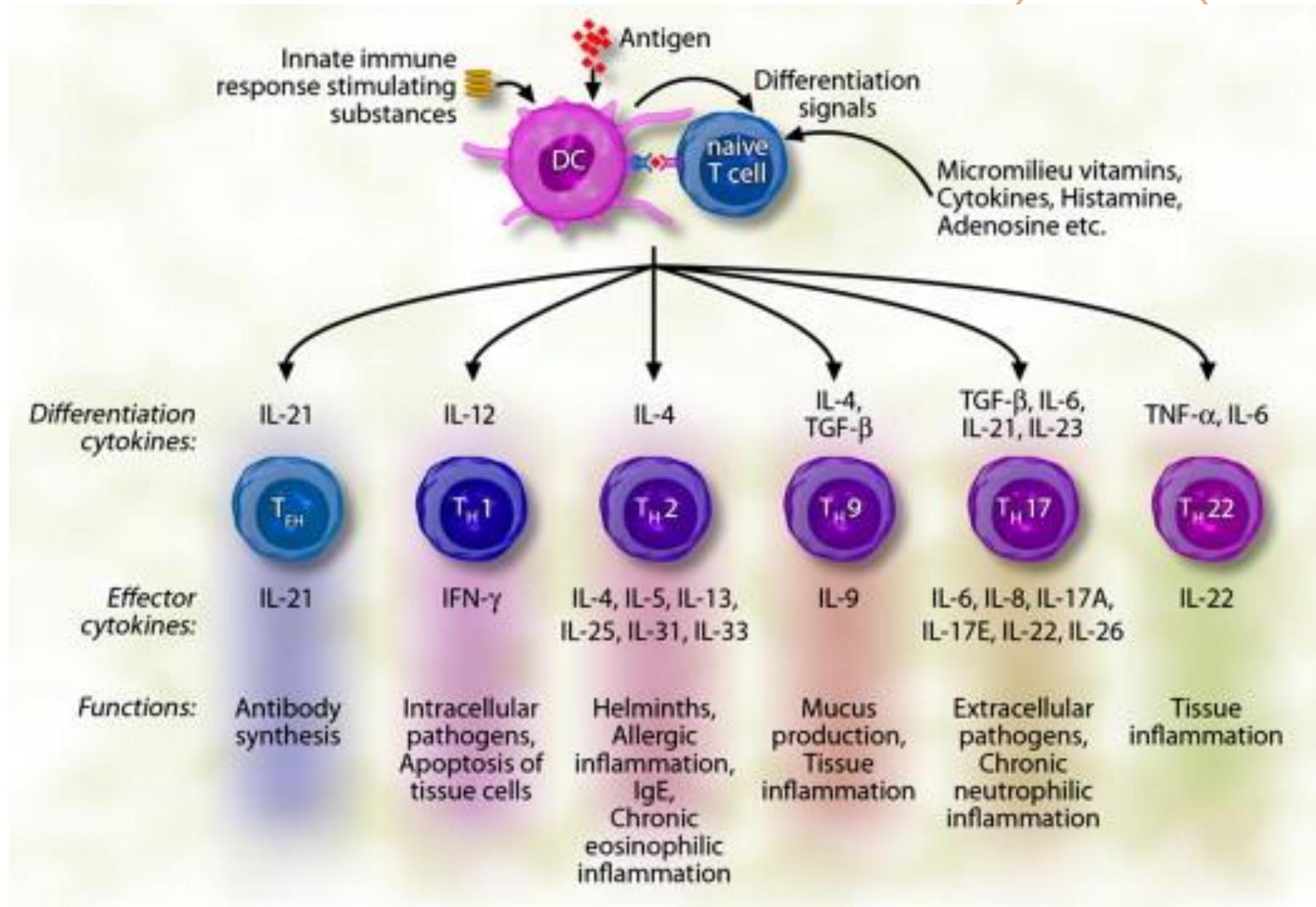
- Interferons act via
- autocrine and paracrine pathways to induce an
- antiviral state in infected cells and in neighboring cells containing interferon receptors.
- Interferons are the frontline defenders against viral infection and their primary function is to locally restrict viral propagation.





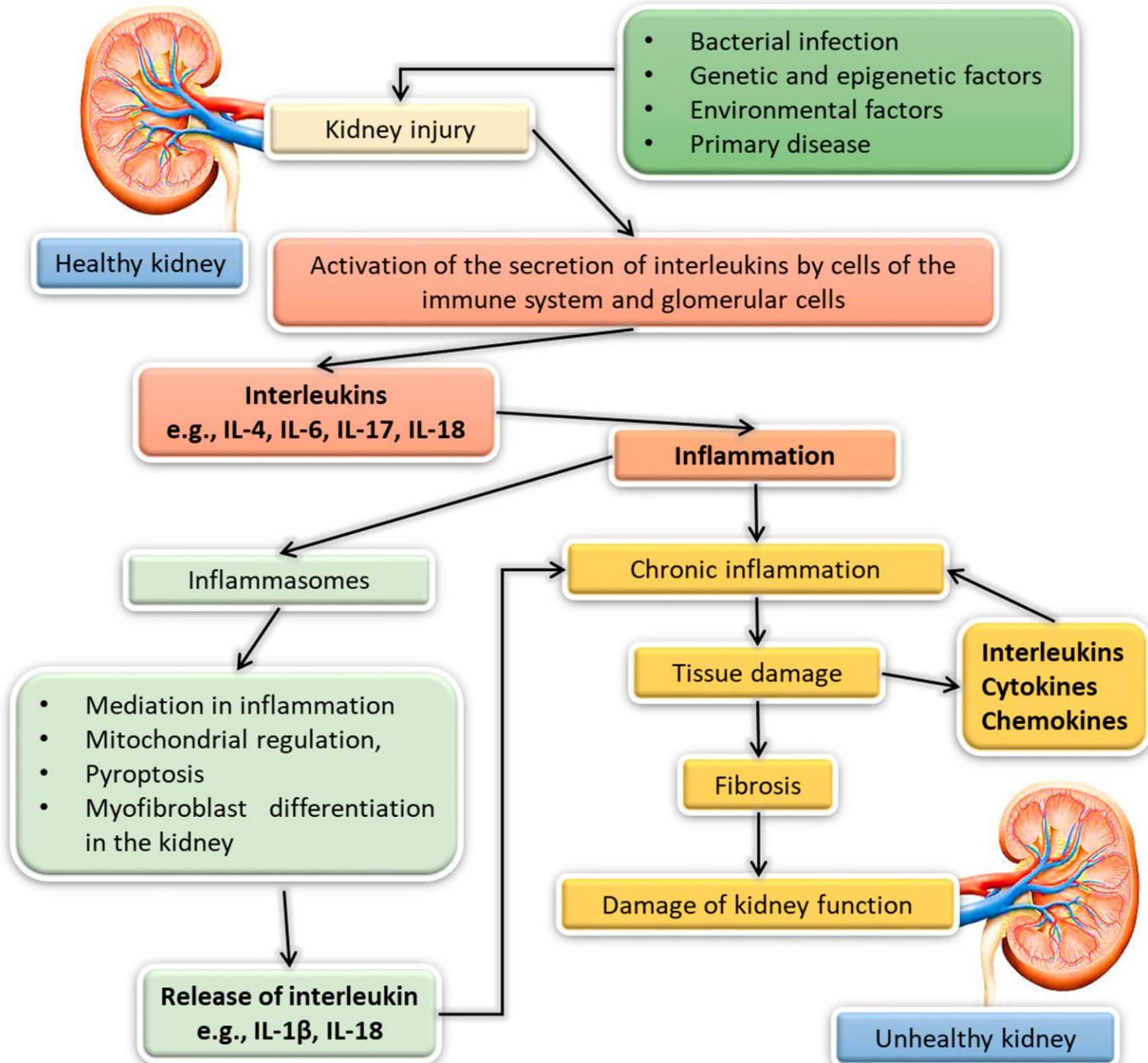
INTERLEUKINS (ILS)

- Interleukins (IL) are a type of cytokine first thought to be expressed by leukocytes alone but have later been found to be produced by many other body cells.
- They play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. Interleukins have a variety of functions,
- Interleukins – 1-37
- Not stored inside cells
- Quickly synthesized and secreted in response to infection
- Key modulators of behaviour of immune cells
- Mostly secreted by T-lymphocytes & macrophages



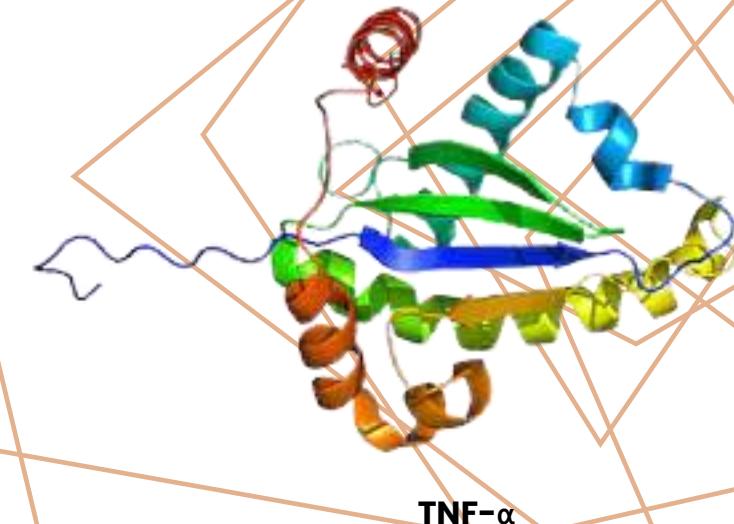
ROLE OF ILS

- Inflammatory response
- Increase production of antibodies
- proliferation of immune cells
- Activation of immune cells



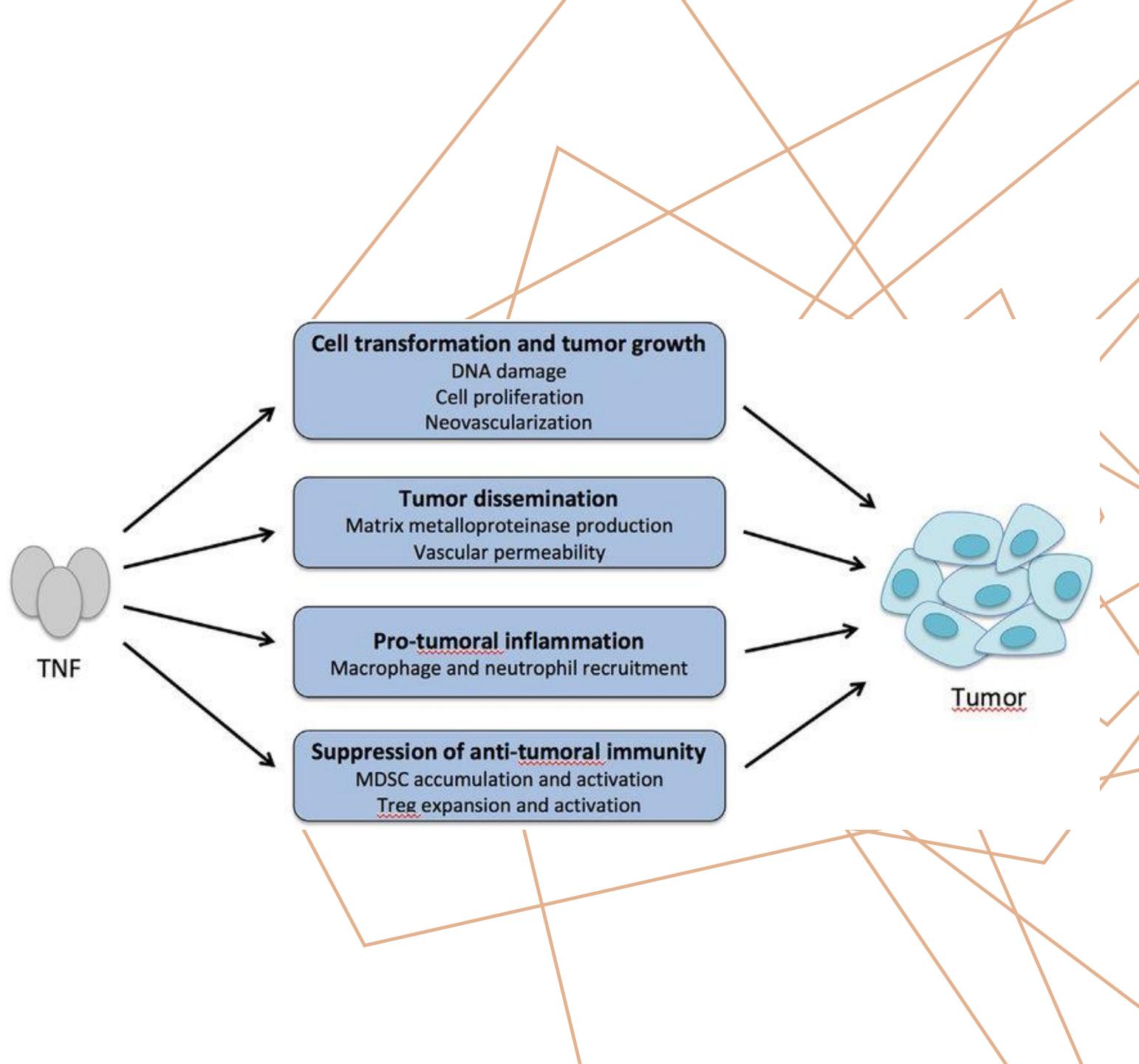
TNF

- Tumor necrosis factor (TNF) is a **multifunctional cytokine** that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death.
- Tumor necrosis factor is an **adipokine and a cytokine**.
- TNF is a **potent proinflammatory cytokine** secreted by many innate immune cells, particularly activated macrophages, but also neutrophils, mast cells, eosinophils, DCs, and NK cells
- As a pro-inflammatory cytokine, TNF is secreted by inflammatory cells, which may be involved in **inflammation-associated carcinogenesis**.



ROLES OF TNF

- TNF- α , the most widely studied cytokine within the tumor necrosis factor superfamily, is primarily released during acute phase reaction or innate immune responding.
- **TNF- α mediates fever, tumor regression, cell production and death, sepsis, cachexia, pain, and inflammation.**
- Tumour necrosis factor (TNF) regulates the switch from "antigen mode" to "inflammation mode" during terminal T cell differentiation.
- This model proposes that during the evolution of immune responses, CD4+ T cells become progressively refractory to T cell receptor (TCR) engagement.



COMPLEMENT (C')

- Complement is a major component of innate immune system involved in defending against all the foreign pathogens through **complement fragments** that participate in **opsonization, chemotaxis, and activation** of leukocytes and **through cytosis by C5b-9 membrane attack complex.**
- The complement system is made up of a large number of distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help to fight infection.
- A number of complement proteins are proteases that are themselves activated by proteolytic cleavage.

Functions of the Complement System

ROLE OF C' PROTEINS

- Facilitates Phagocytosis
- Direct Lysis of Pathogens
- Inflammation

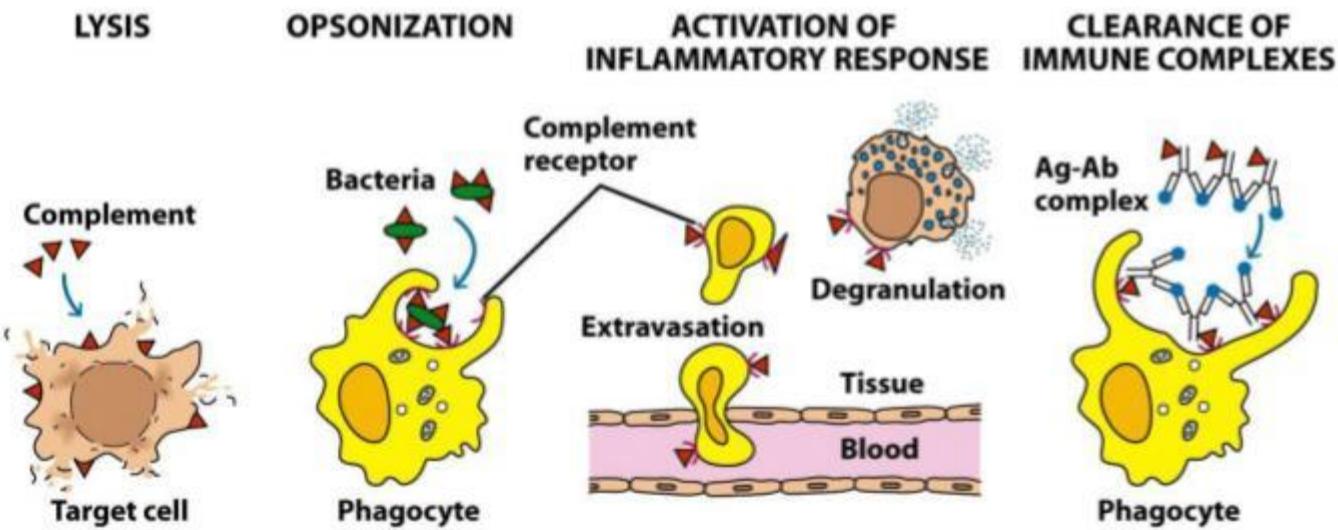


Figure 7-1
Kuby IMMUNOLOGY, Sixth Edition
© 2007 W.H. Freeman and Company

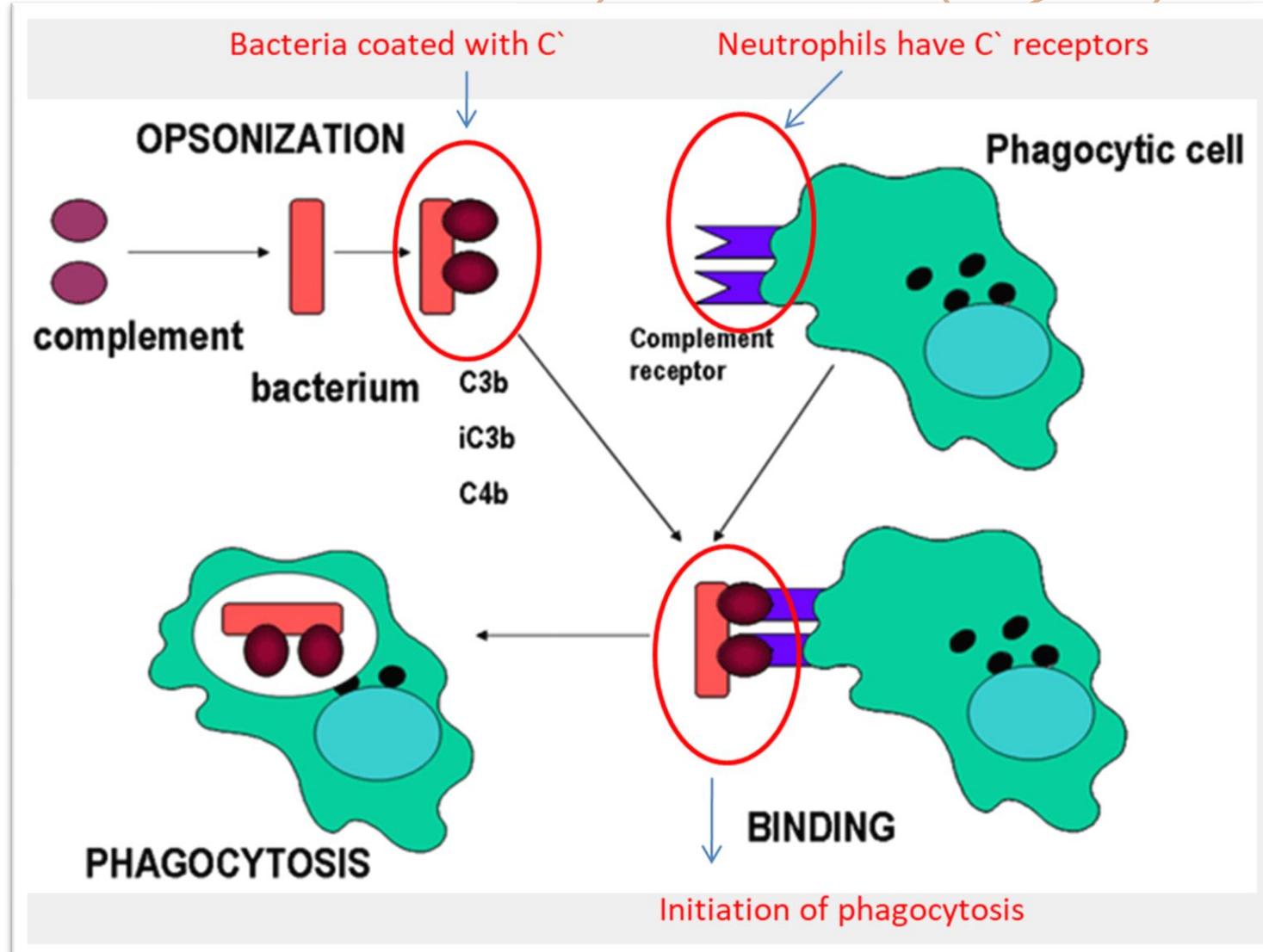
- Viral neutralization

C' PROTEINS & PHAGOCYTOSIS

A process of spirochetal opsonization begins with binding complement molecules C3b, iC3b, or C4b to the surface of the bacteria.

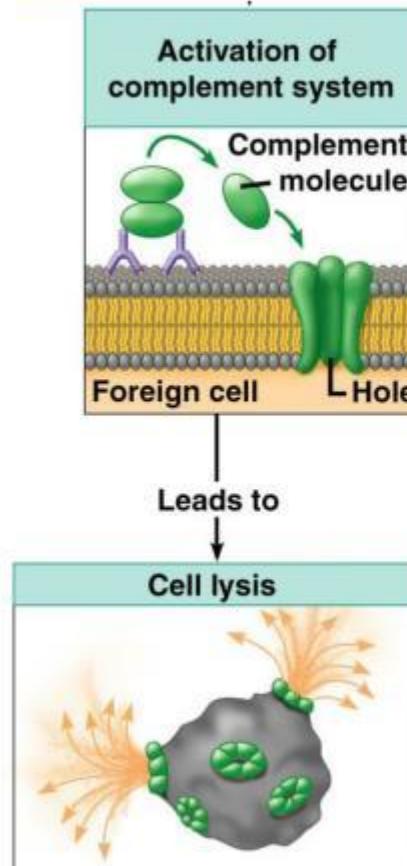
The complement molecules coat the surface of the microbe without the use of antibodies.

These molecules have a strong affinity for macrophagic CR3 or CR4. The binding of complement to these receptors allows the phagocytic cell to engulf the bacteria

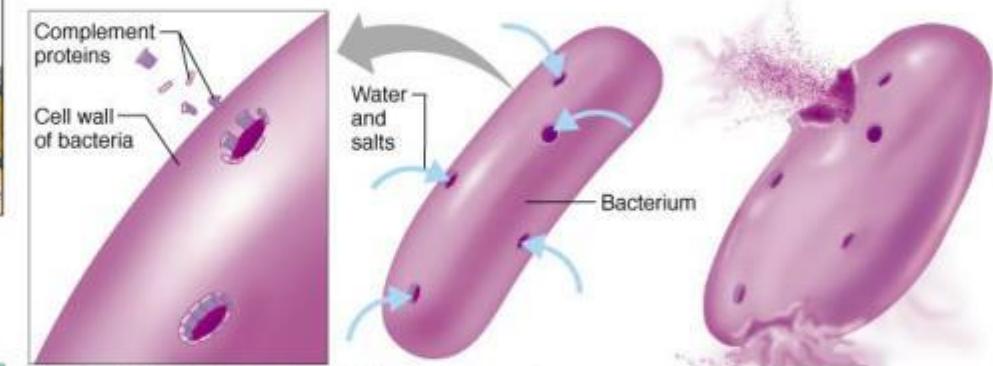


LYSIS OF PATHOGENS

Consequences of Complement Activation



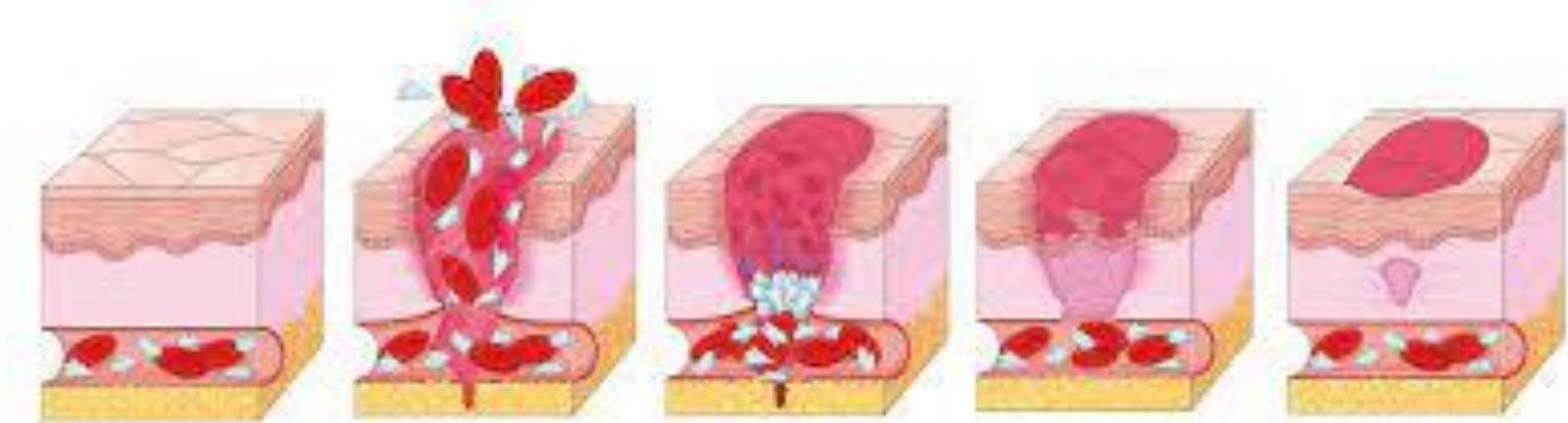
- The three complement pathways converge at the membrane-attack complex (MAC).



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COAGULATION PROTEINS



- Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot.
- It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair.
- Platelets (a type of blood cell) and proteins in your plasma (the liquid part of blood) work together to stop the bleeding by forming a clot over the injury.

COAGULATION PROTEINS

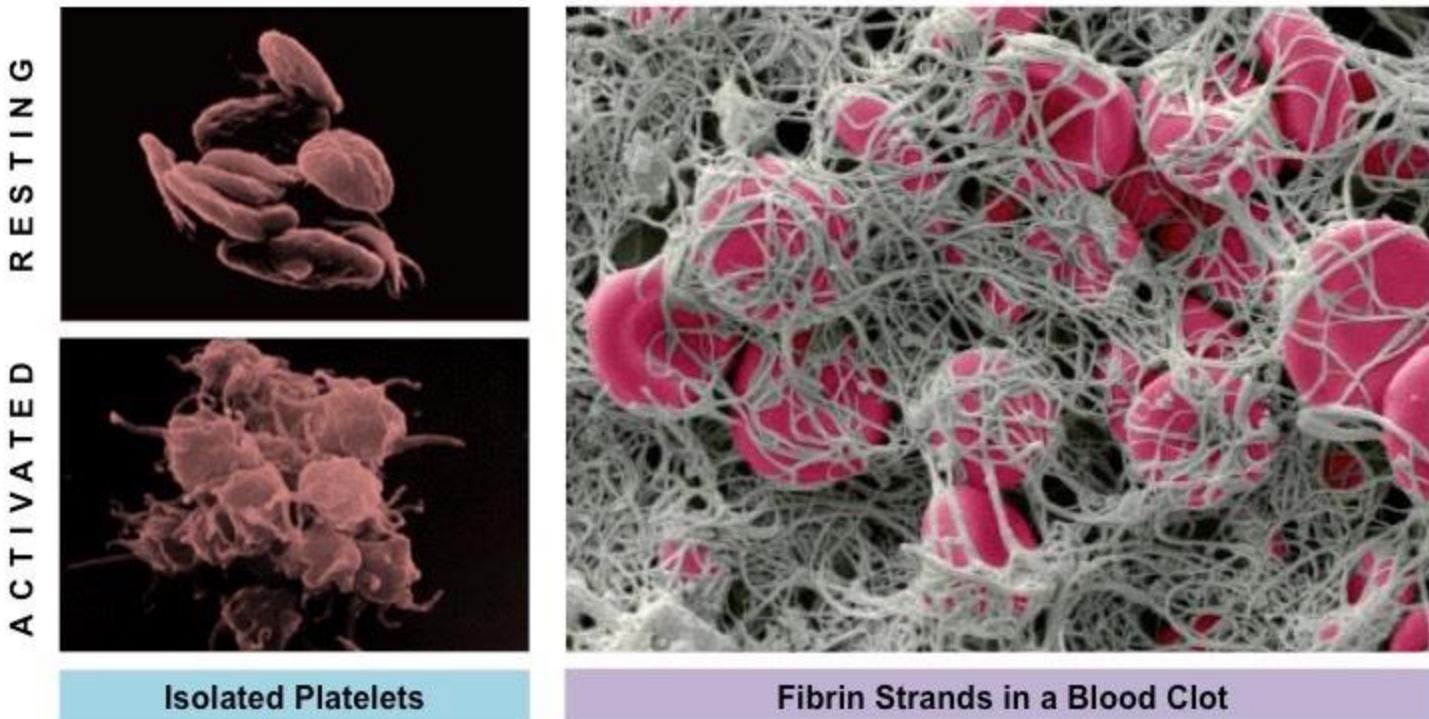
Coagulation: mechanism to stop bleeding after injury to blood vessels

Complex pathway involves:

Platelets

Coagulation factors

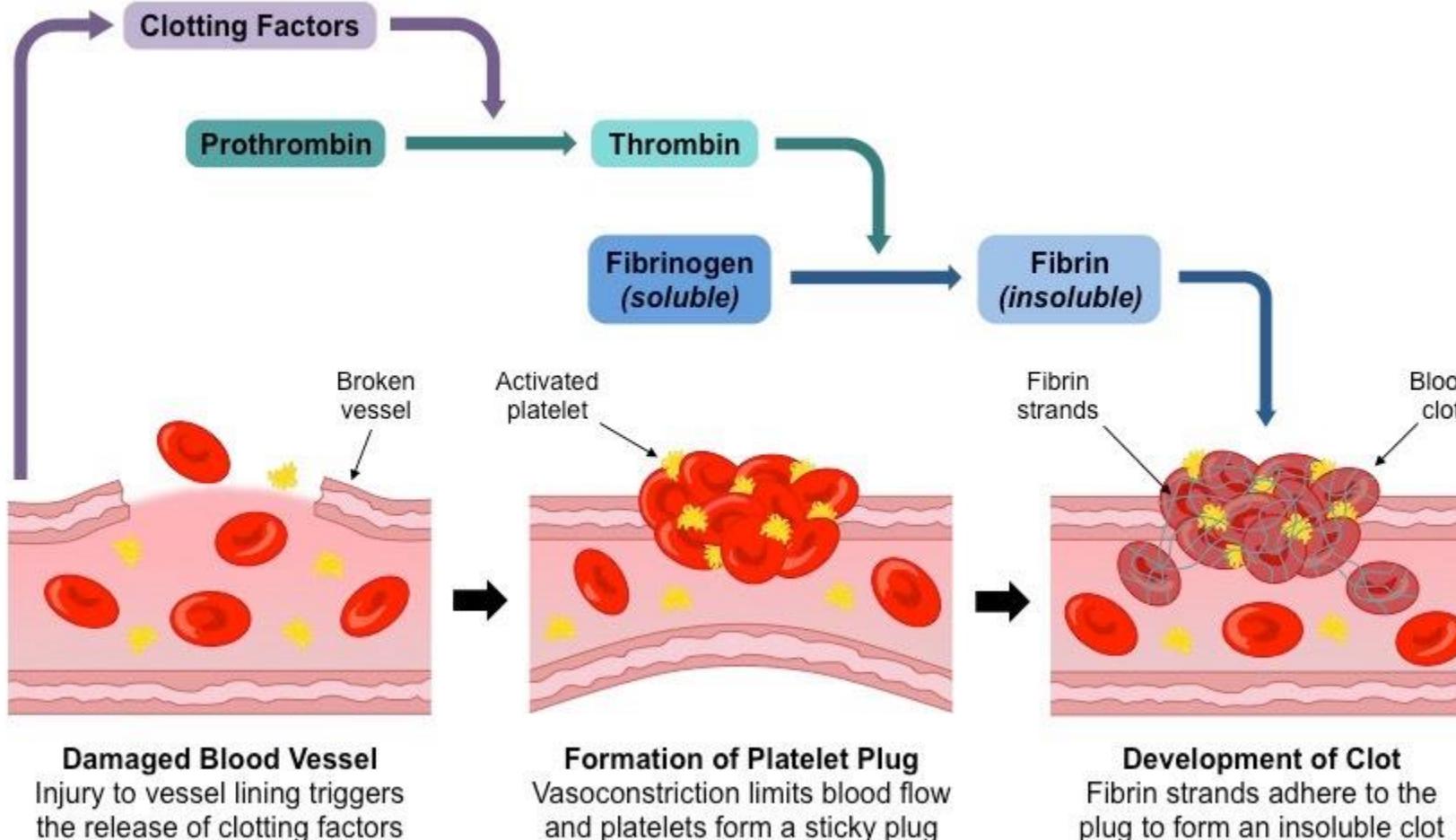
Vitamin K



The common pathway factors X, V, II, I, and XIII are also known as Stuart-Prower factor,

proaccelerin, prothrombin, fibrinogen, and fibrin-stabilizing factor, respectively.

Clotting factor IV is a calcium ion that plays an important role in all 3 pathways.



Coagulation proteins

- Blood clotting
- Inflammation
- Apoptosis (prog. Cell Death)

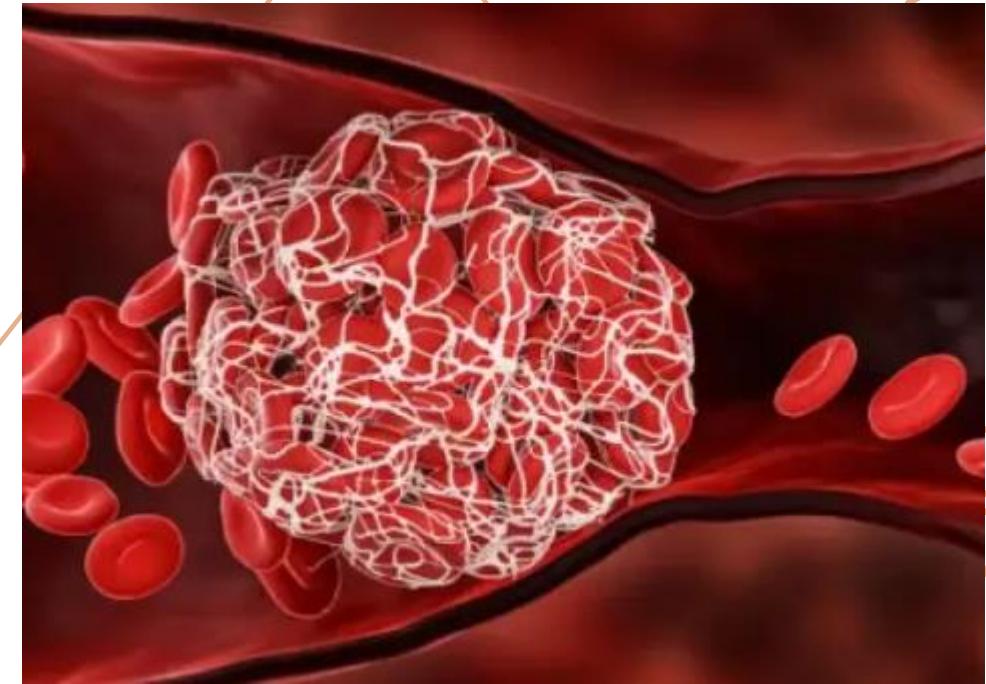
Anticoagulants

- Prevent blood clotting
- Inhibit inflammation
- Inhibit apoptosis

COAGULATION A DELICATE BALANCE



- Hemophilia
- Anemia,
- blood cancers such as leukemia, lymphoma, and myeloma.



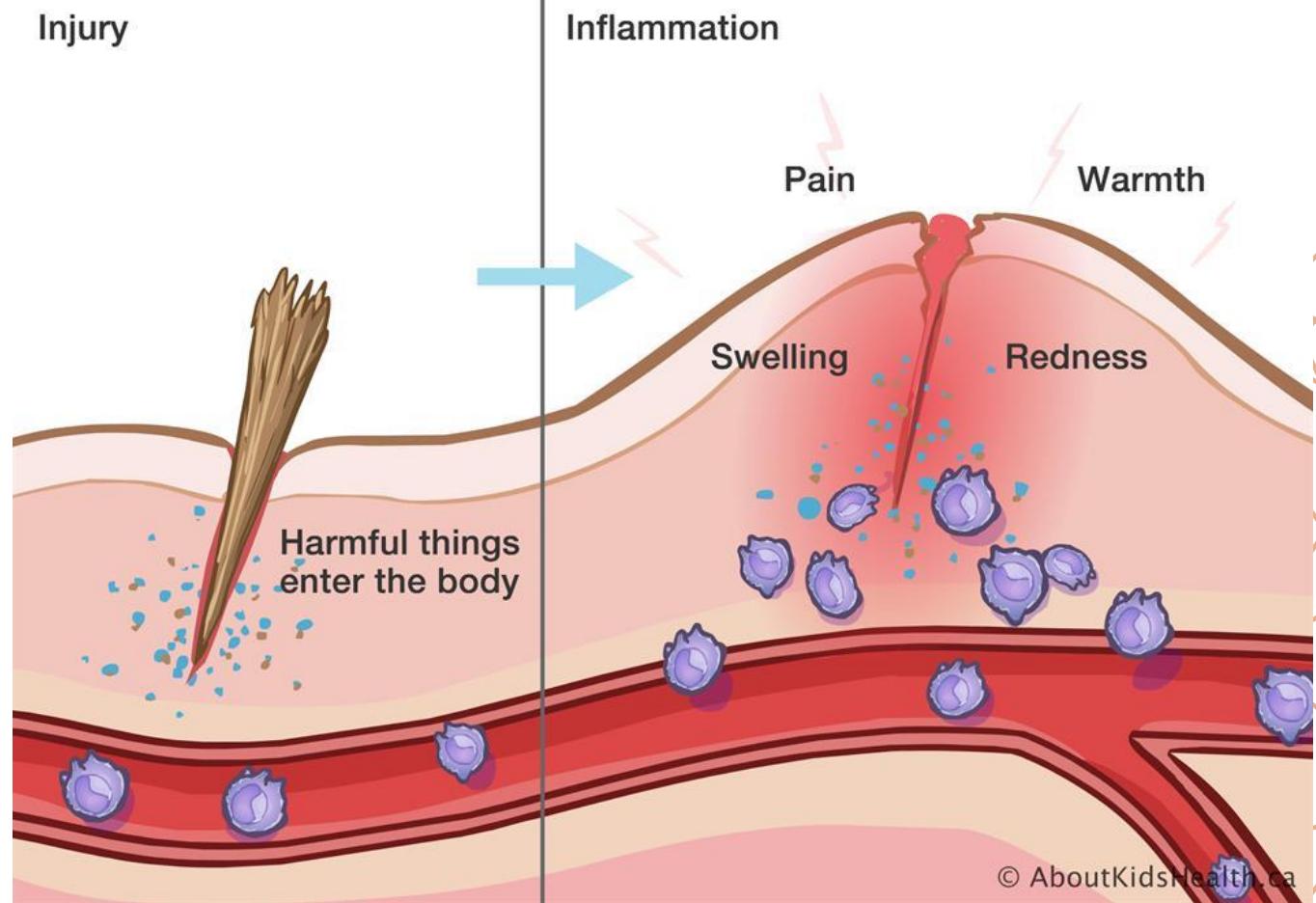
- Antiphospholipid syndrome.
- Arteriosclerosis / atherosclerosis.
- Cancer.
- Certain medications, such as oral contraceptives and hormone therapy drugs.
- Coronavirus disease 2019 (COVID-19)
- Deep vein thrombosis (DVT)
- Factor V Leiden.
- Family history of blood clots.

SUMMARY INNATE RESPONSE

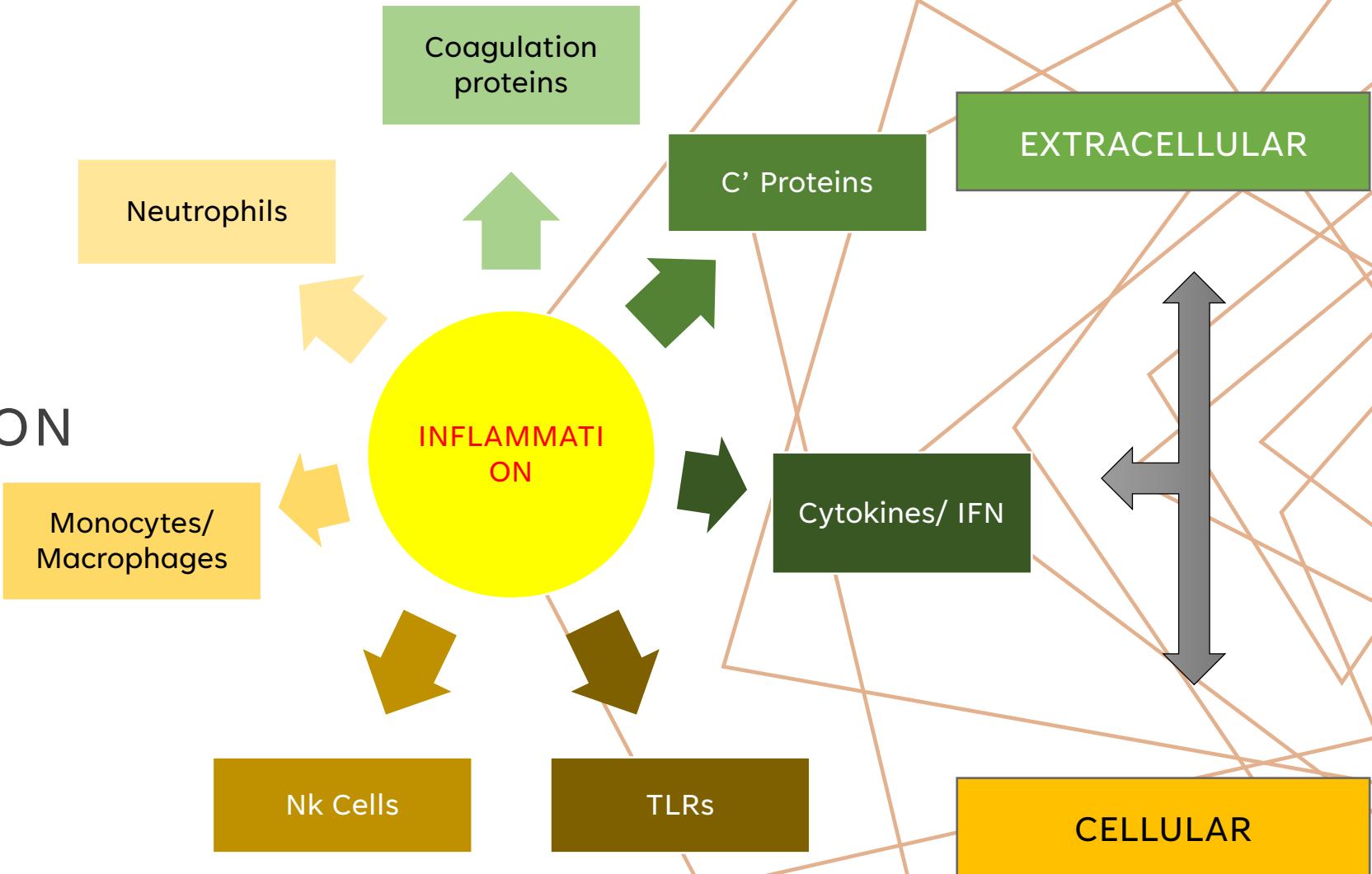
Cellular	Extracellular
Neutrophils	Cytokines
Monocytes/ Macrophages	Complement
NK cells	Coagulation
TLRs	

INFLAMMATION

- Inflammation is part of the body's defense mechanism.
- It is the process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process.
- Inflammation can be either **acute or chronic.**



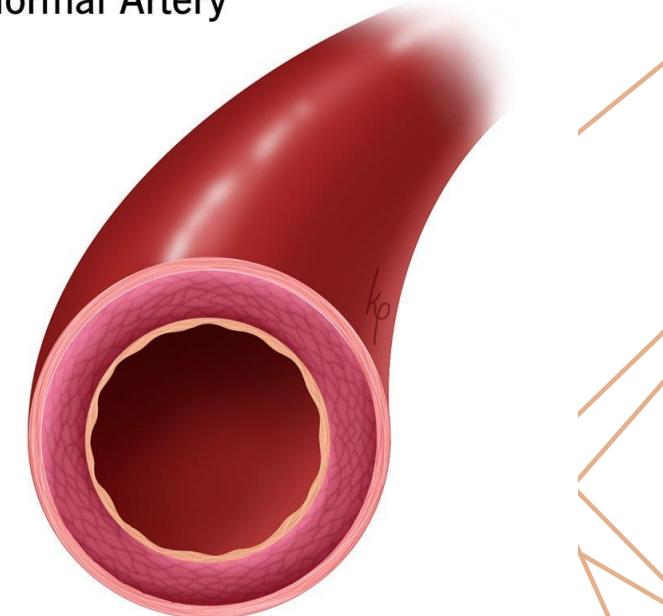
ALL ROADS LEAD TO INFLAMMATION



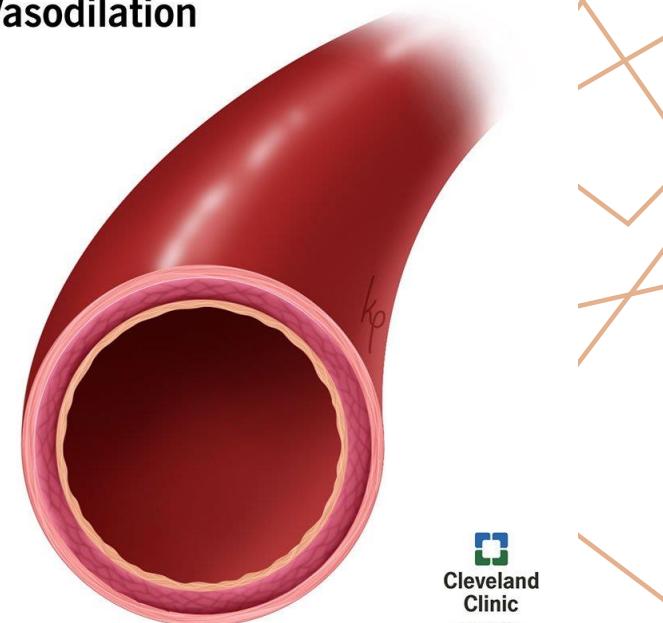
VASODILATION

- Vasodilation is the medical term for when blood vessels in your body widen, allowing more blood to flow through them and lowering your blood pressure
- This is a normal process that happens in your body without you even realizing it.
- When it happens natural
 - Blush or turn red: That's because of vasodilation of the blood vessels right under the surface of your skin (called capillaries), which increases blood flow to your face.
 - Step into a hot tub: When you enter the hot water, your body automatically tells capillaries to dilate. This is to help your body heat up more slowly.
 - Exercise: When you're physically active, your body needs more oxygen and nutrients delivered quickly to its cells (especially muscle cells). Widening your blood vessels allows more blood to flow through, meeting those needs.

Normal Artery



Vasodilation

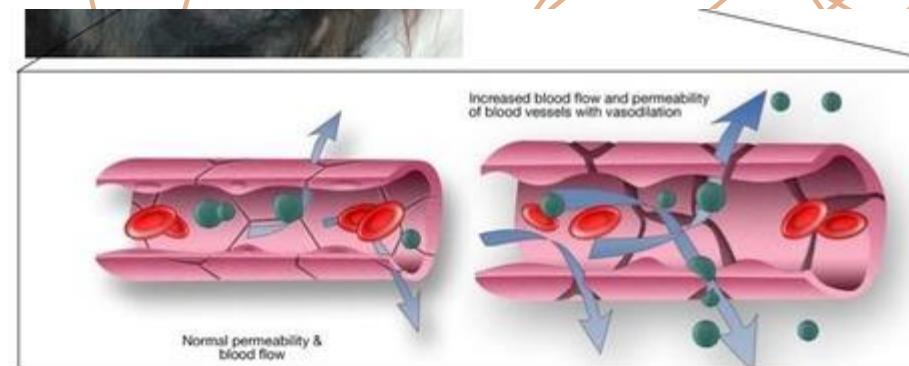


INFLAMMATION & VASODILATION

- Have an infection: Your body responds to infections by increasing blood flow to the affected area. This helps your body fight the infection and repair damage the infection caused.
- **Vasodilation assists inflammation** by increasing blood flow to damaged cells and body tissues. This enables more effective delivery of the immune cells necessary for defense and repair.
- After an inflammatory mediator is released in the bloodstream, a period of **transient vasoconstriction**, lasting only a few seconds, occurs.
- Then blood vessels **expand to undergo vasodilation from the stimulus of the vasoactive inflammatory mediator**, which increases blood flow to the area.
- This causes slowing and stasis of red blood cells, which can be involved in the clotting response needed to stop bleeding in the case of injury.
- Vasodilation is the reason for the redness, heat, and pain associated with inflammation.

INFLAMMATION & VASODILATION

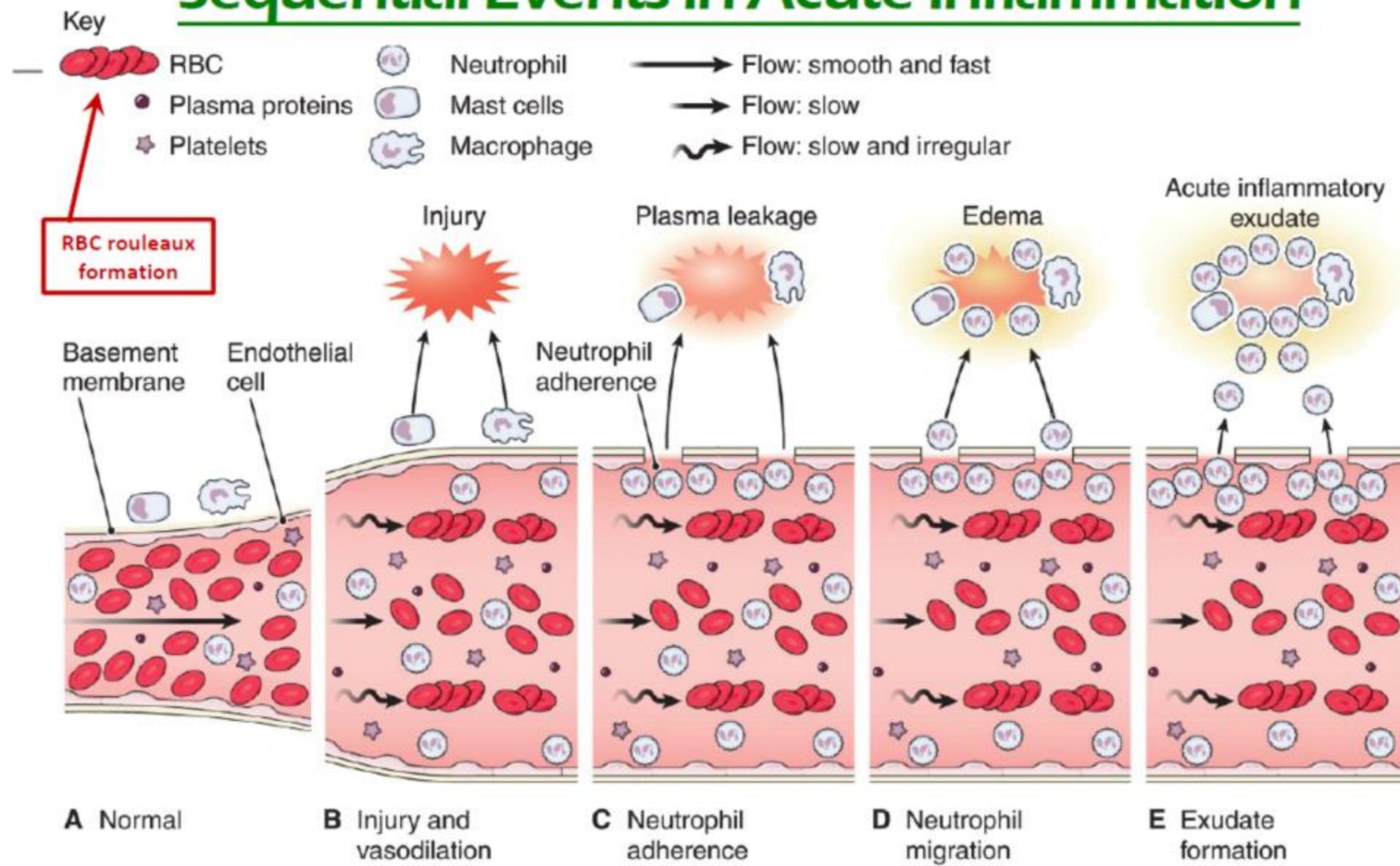
- **Increased Vascular Permeability**
- The next step of acute inflammation is an increase **in vascular permeability due to inflammatory mediator activity**, which causes the blood vessels to become more permeable.
- Normally only water and small compounds can exit the bloodstream into the tissues, but during inflammation, large proteins in the bloodstream, such as serum albumins, can leak out and into the tissues.
- Water follows these proteins due to the force of oncotic pressure that the proteins exert.
- This is called exudate, a form of edema. As exudate accumulates within the tissues, they become swollen.
- The exudate may carry antimicrobial proteins and antibodies into the tissues, and stimulates lymphatic drainage.



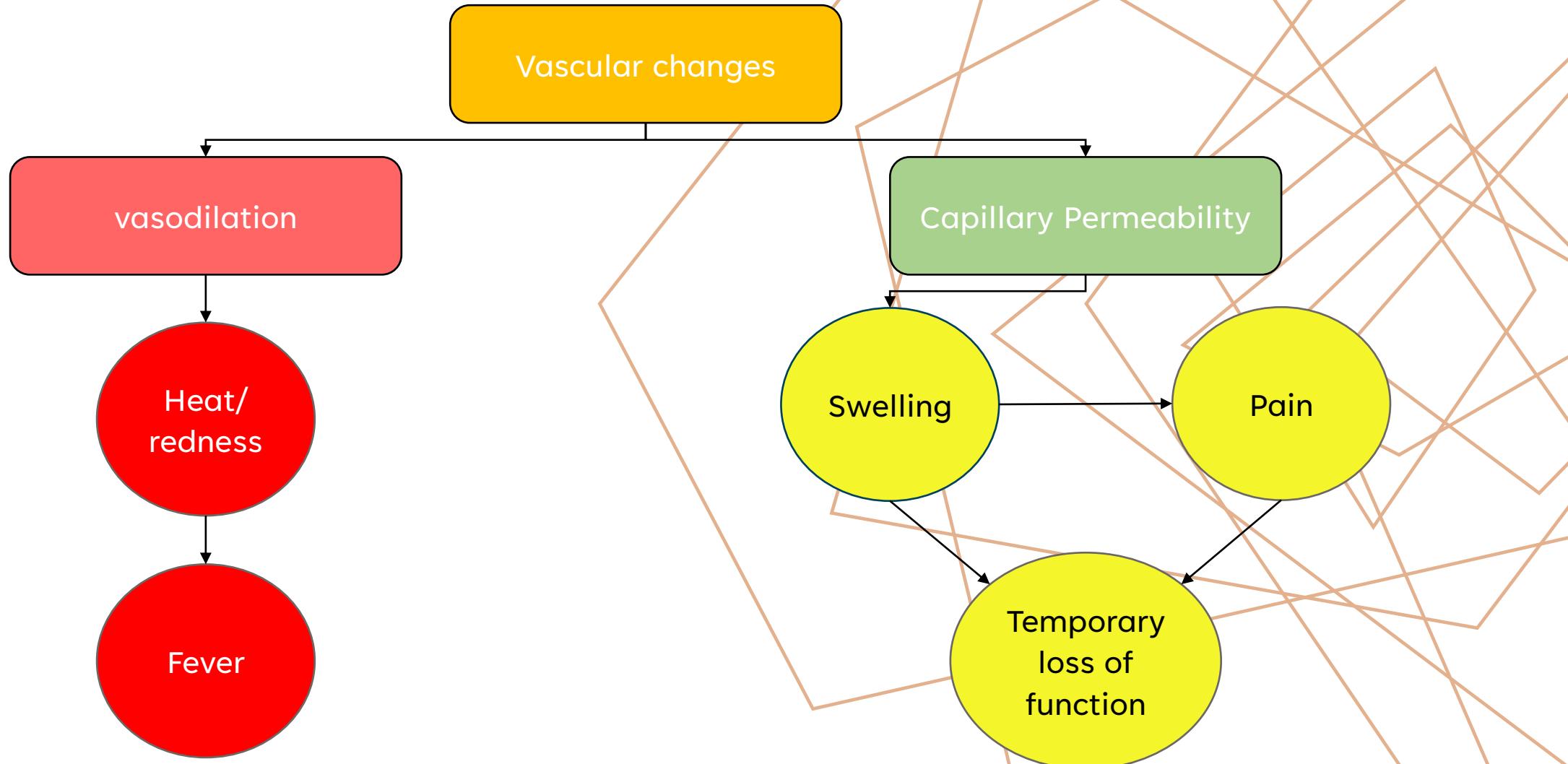
INFLAMMATION & VASODILATION

- **Leukocyte Migration to the Tissues**
- The next step of the acute inflammatory response is chemotaxis migration of neutrophils to the affected area.
- Neutrophils are recruited to the site of inflammation by various cytokines.
- Other inflammatory mediators, such as TNF-alpha and IL-1, increase the expression of adhesion molecules on vascular endothelial cells. The neutrophils loosely attach to the endothelial cells through use of selectins, a process called rolling.
- Then integrins firmly attach to the adhesion molecules on the endothelial cells, which is called adhesion.
- Together, rolling and adhesion are referred to as margination, the accumulation of leukocytes on the endothelium.
- The next step is for neutrophils to squeeze through the gaps in the endothelium into the tissues through binding with PECAM-1 expressed on the endothelium, a process called extravasation.
- Then the neutrophils follow a chemotactic gradient to the site of infection or injury in the tissues, where they will degranulate and phagocytize pathogens. Later, macrophages enter the tissues through a similar process to clean up dead neutrophils and cellular debris.

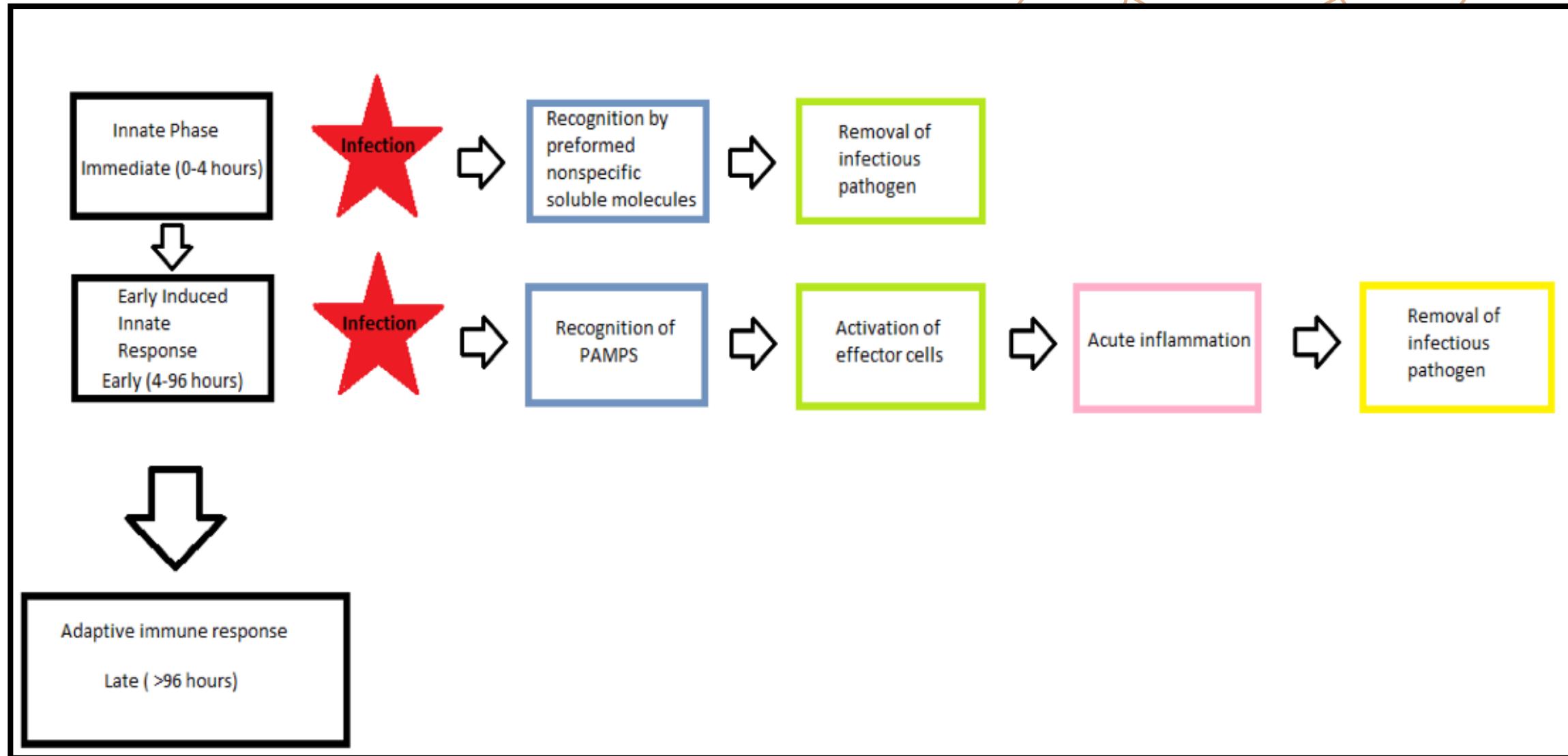
Sequential Events in Acute Inflammation



SIGNS OF INFLAMMATION



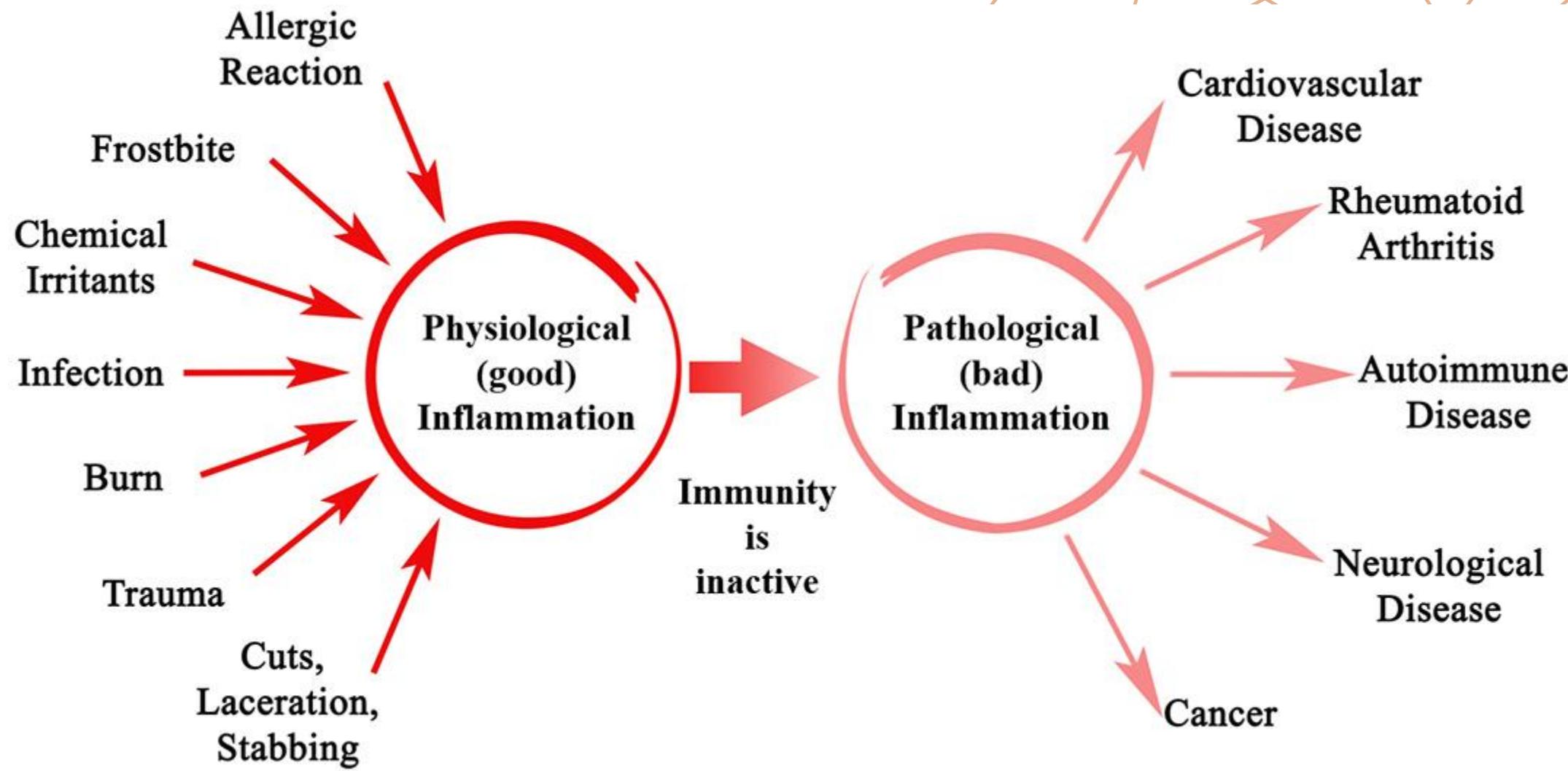
INFLAMMATION IN INNATE IMMUNITY



ROLE OF INFLAMMATION IN INNATE IMMUNITY

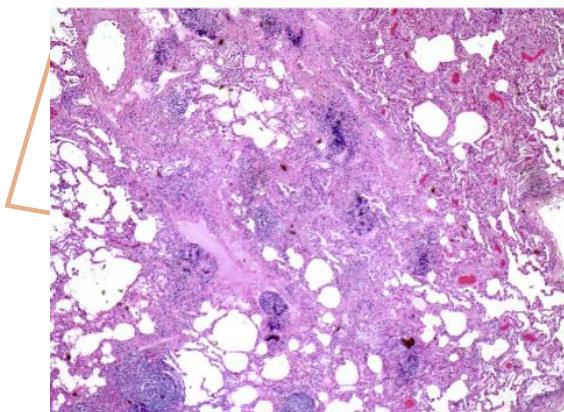
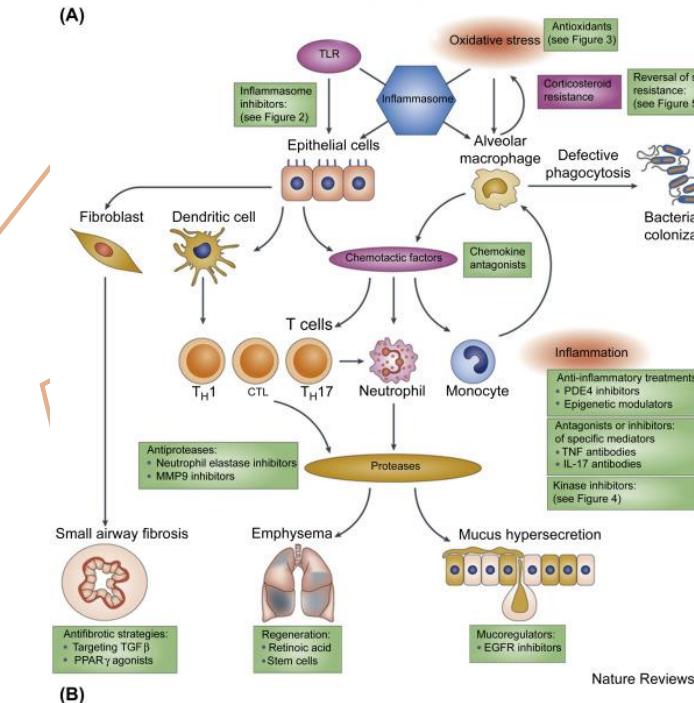
- Initiation of phagocytosis – killing of pathogen
- Limiting the spread of infection
- Stimulate adaptive immune response
- Initiate tissue repair

GOOD, BAD & UGLY ABOUT INFLAMMATION



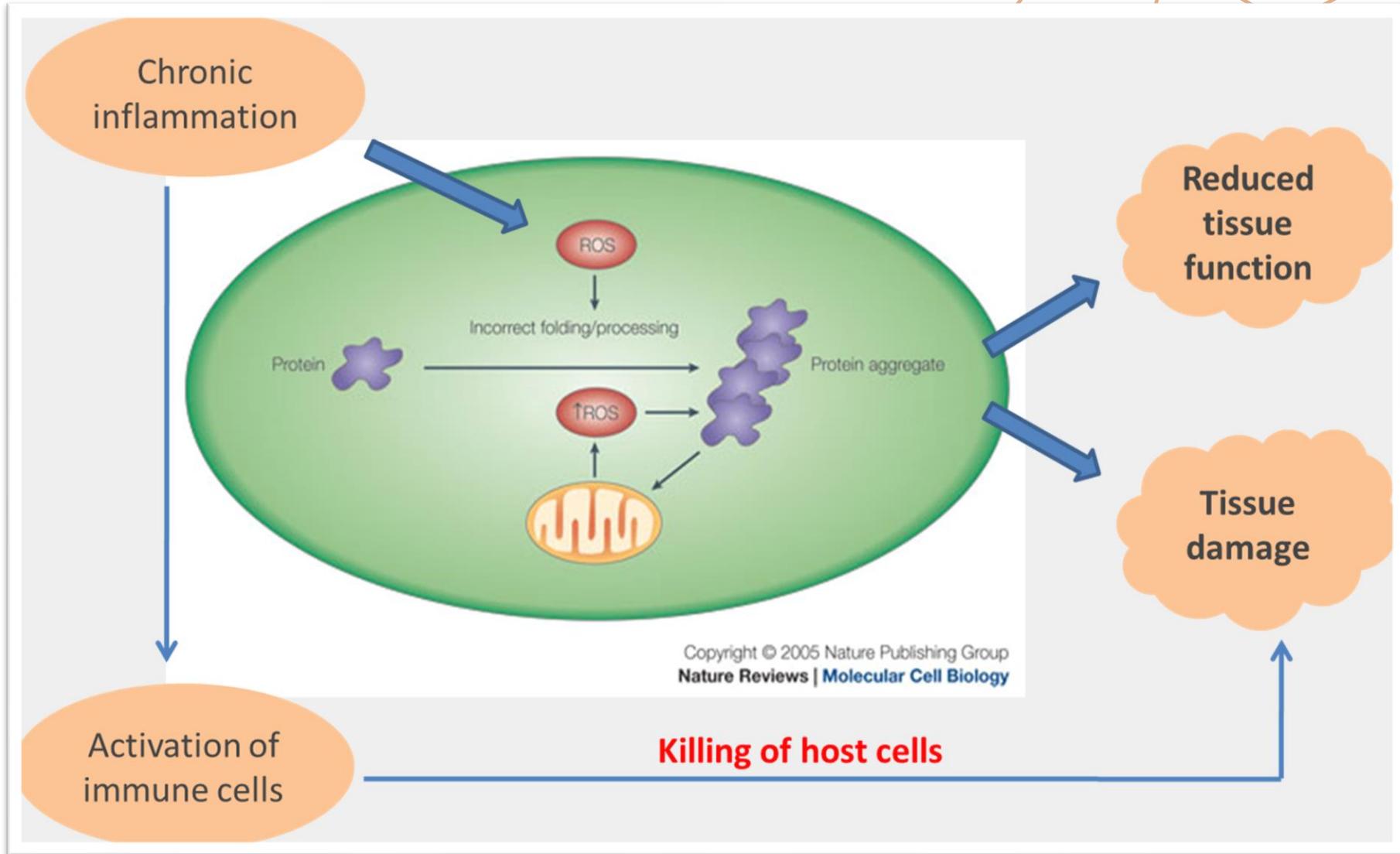
CHRONIC INFLAMMATION

- Chronic inflammation - macrophages in the injured tissue.
- Macrophages release toxins (including reactive oxygen species or ROS) that injure tissues
- Chronic inflammation is almost always accompanied by tissue destruction.

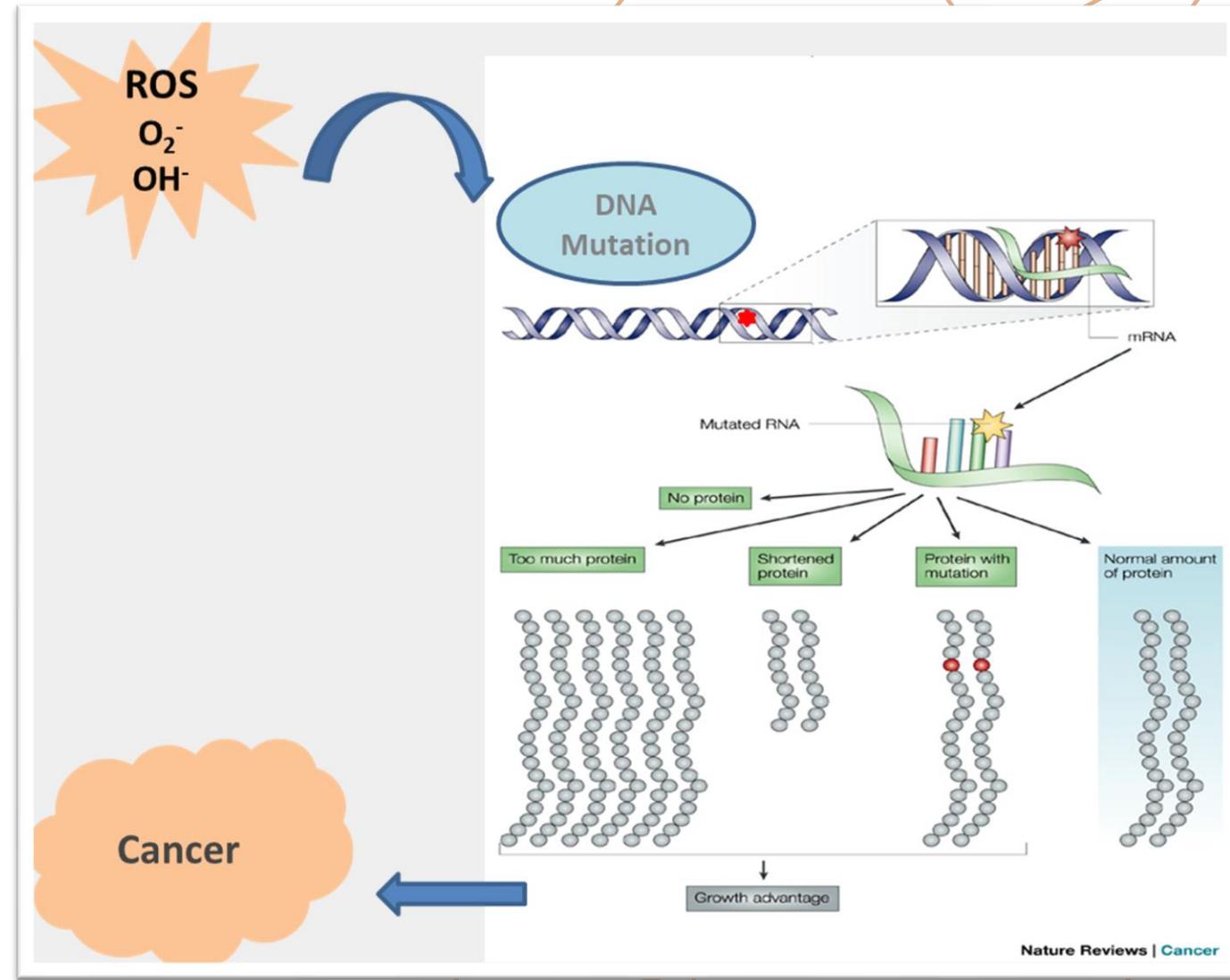


(A) Pathways of chronic pulmonary inflammation, (B) lung with chronic inflammation showing organized B-cell follicles.

CHRONIC INFLAMMATION AND TISSUE DAMAGE:

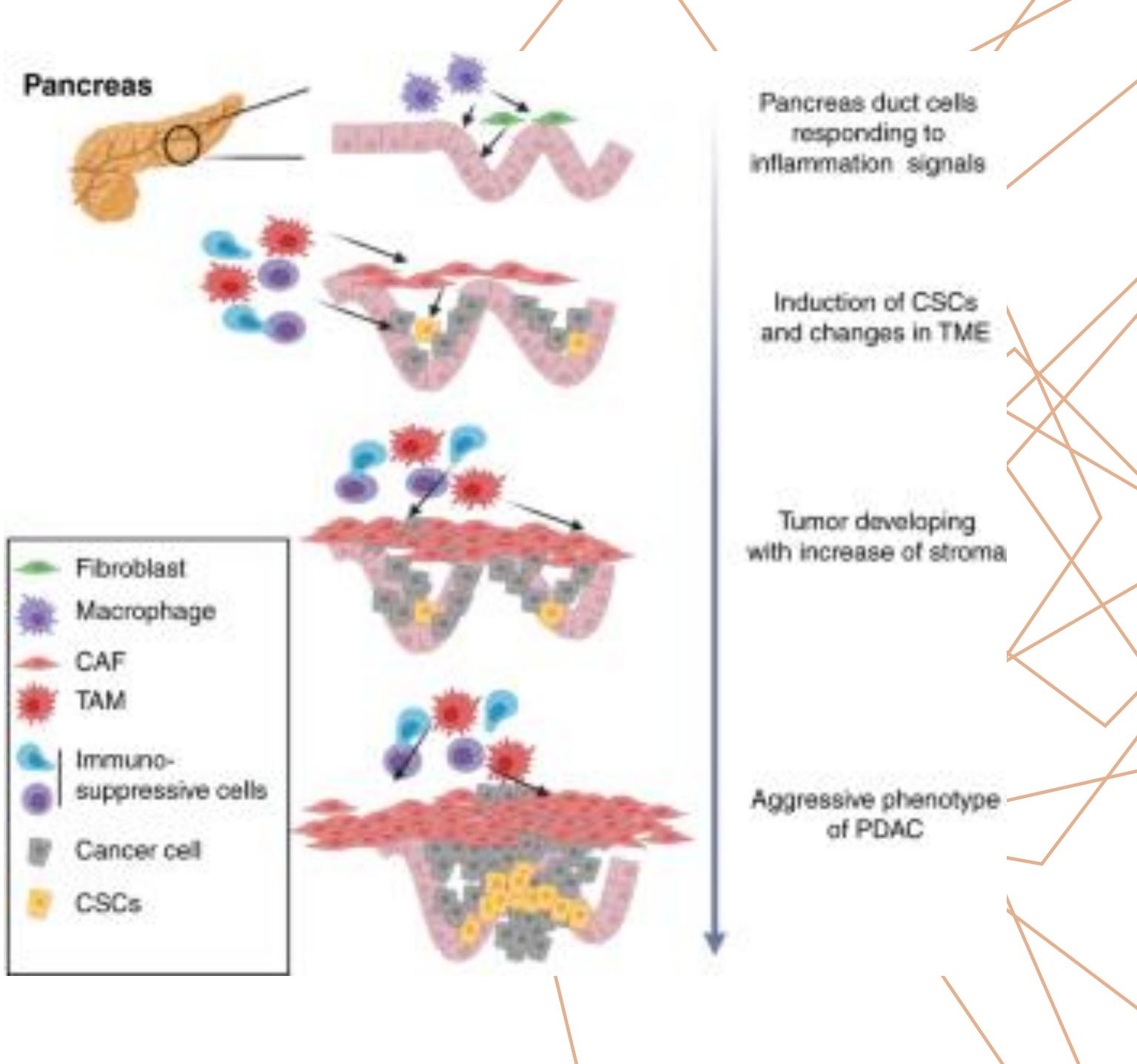


INFLAMMATION AND CANCER



PANCREATIC CANCER

Afify, S.M., Hassan, G., Seno, A. et al. Cancer-inducing niche: the force of chronic inflammation. *Br J Cancer* **127**, 193–201 (2022).
<https://doi.org/10.1038/s41416-022-01775-w>



IMMUNOGEN/ ANTIGENS

- An immunogen refers to a molecule that is capable of eliciting an immune response by an organism's immune system,
- whereas an antigen refers to a molecule that is capable of binding to the product of that immune response.
- So, an immunogen is necessarily an antigen, but an antigen may not necessarily be an immunogen.

ANTIGEN VERSUS IMMUNOGEN

ANTIGEN

A substance specifically bind to antibodies or a cell surface receptors of B cells and T cells

Can be either immunogenic or non-immunogenic

Not all are immunogens

Can be either proteins, polysaccharides, lipids or nucleic acids

Haptens are low-molecular-weight molecules, which bind to antibodies

IMMUNOGEN

An antigen capable of inducing an immune response

Immunogenic

All are antigens

Normally proteins and large polysaccharides

Haptens become immunogenic when binding to larger carrier molecules

IMMUNOGENICITY

- Immunogenicity is defined as the **ability of cells/tissues to provoke an immune response** and is generally considered to be an undesirable physiological response.
- . Immunogenicity is the ability to induce a humoral (antibody) and/or cell-mediated immune response
- the immunogenicity of an antigen using the following three aspects: (
 - the ability to defend the immune system (immunological defense), which is the ability to repel an exogenous antigen and to fight against infection;
 - the ability to keep the immune system stable (immunological homeostasis), which is the ability of the body to recognize and eliminate damaged tissue, inflammation and/or senescent cells, and
 - the ability to kill and to remove abnormally mutated cells so as to monitor and inhibit the growth of malignancies in the body (immunological surveillance).
- Thus, immunogenicity reflects the strength of these three functions.

WHAT DETERMINES IMMUNOGENICITY

- Foreignness: essential for immunogenicity (self-responsive immune cells are eliminated during lymphocyte development), an antigen must be perceived as foreign by the biological system or recognized as non-self
- Size: Bigger > Smaller The most potent immunogens typically fall within a molecular mass range of 14,000 to 600,000 Daltons (Da), with a majority exceeding 100,000 Da
- Chemical composition: Proteins > nucleic acids / polysaccharides / lipids substances that are chemically more complex tend to exhibit higher immunogenicity. This complexity can arise from the presence of multiple epitopes within the antigen, allowing for a greater variety of interactions with the immune system.
- Structure: Primary /secondary /tertiary structures play a role
- Physical form: Particulate > Soluble particulate antigens are more immunogenic compared to soluble antigens
- Susceptibility to antigen processing and presentation Antigens that can be efficiently processed and presented by MHC molecules are more likely to elicit a strong immune response.

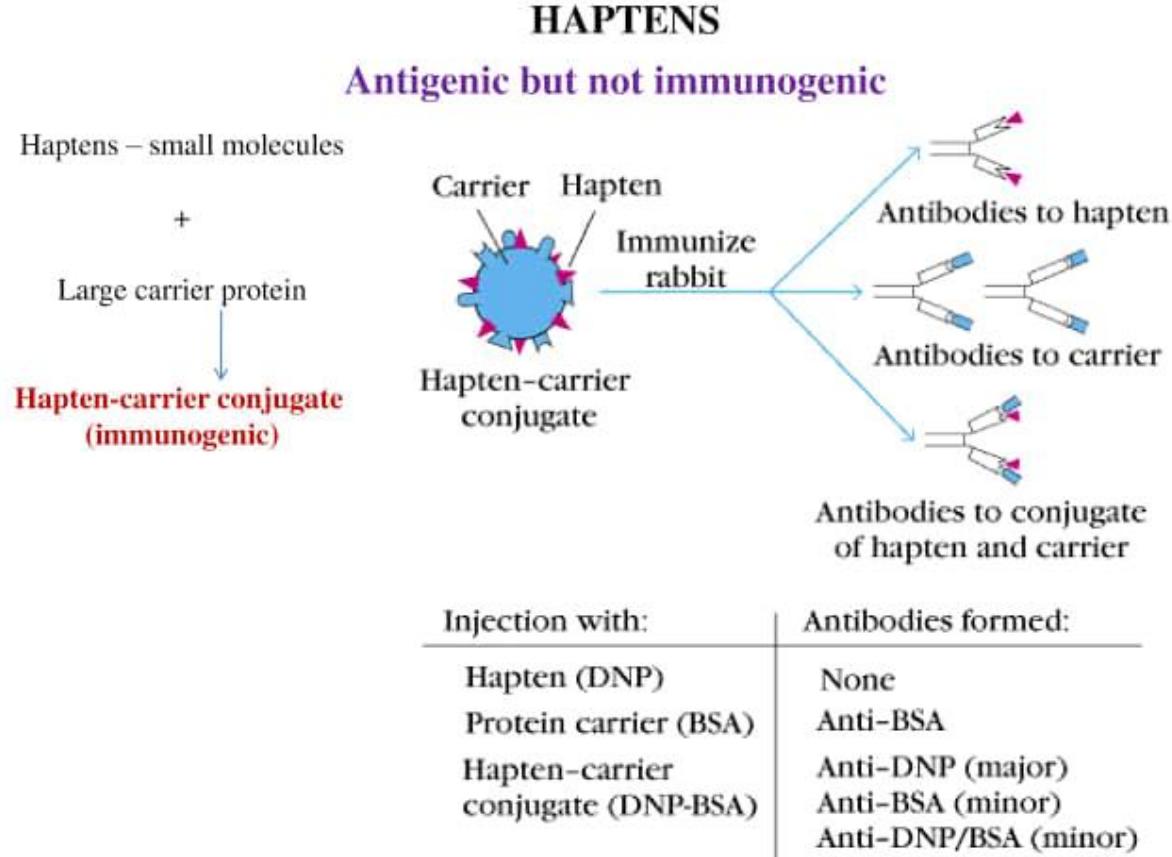
<https://microbiologynote.com/which-factors-affects-immunogenicity-factors-affecting-immunogenicity/#:~:text=Several%20factors%20influence%20immunogenicity%2C%20including,genetic%20factors%2C%20and%20the%20presence>

HOST FACTORS AFFECTING IMMUNOGENICITY

- **The genotype of the recipient animal:** Different species or individuals within a species may exhibit varying levels of immunogenicity towards specific substances
- **The dosage and route of administration of an immunogen:** Insufficient dosage may fail to elicit a response, state of tolerance: high dosage unresponsiveness or tolerance
- **Adjuvants** are substances that, when mixed and injected with an antigen, enhance its immunogenicity. Adjuvants work through various mechanisms to enhance the immune response: Adjuvants can provide co-stimulatory signals, prolong the presence of antigens, and enhance non-specific proliferation of lymphocytes, all of which contribute to improved immunogenicity.

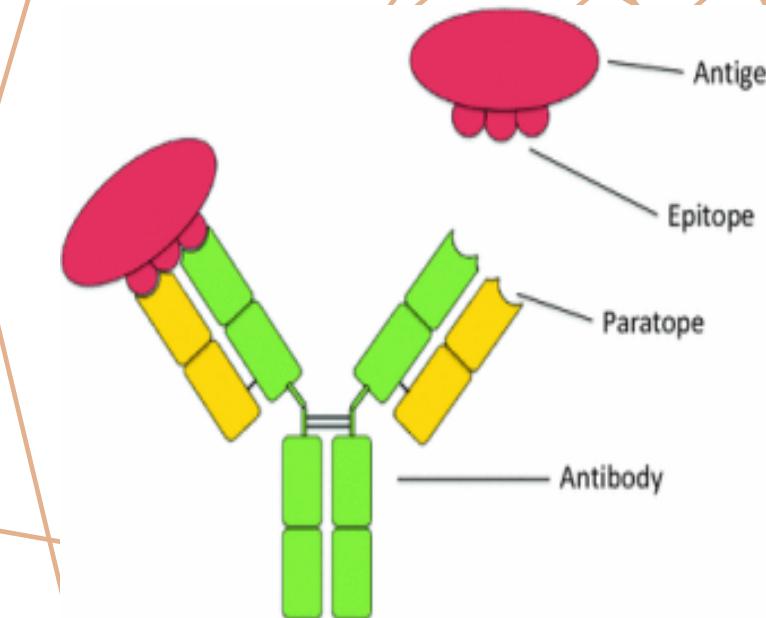
HAPTENS

- A haptens are small molecules which are non-immunogenic but can combine with a specific antibody but lacks antigenicity of its own.
- small molecules of $M_r < 1000$ such as toxins, drugs and hormones are not capable of invoking immune response when injected directly into animals.
- These small-molecular-weight compounds need to be linked to a large molecule, such as a protein like bovine serum albumin to make them immunogenic before injecting into an animal to get the desired antibody.
- The reactive carboxylic or amino group in a haptens could be used to conjugate it to the protein molecule.

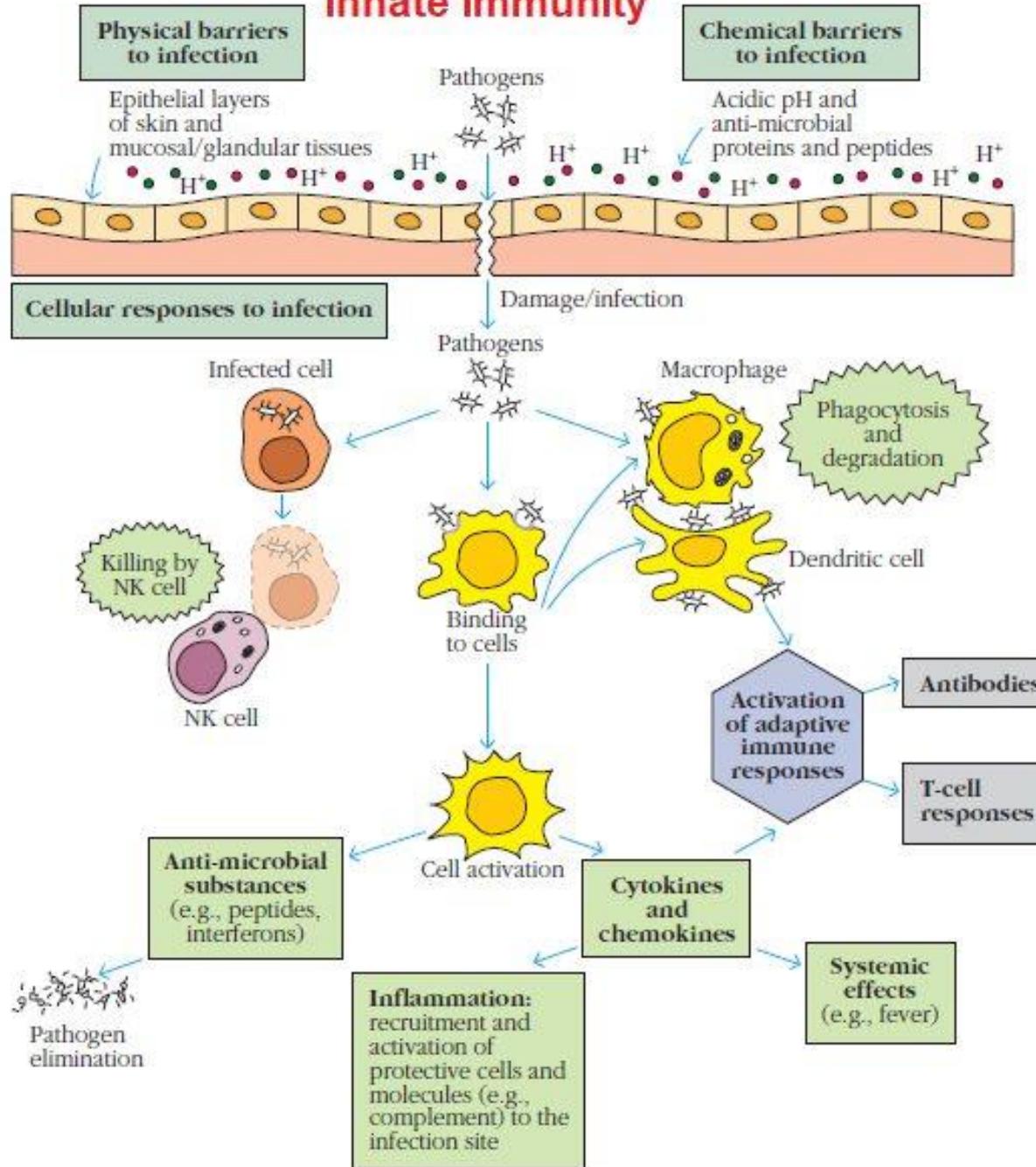


- Epitopes, also known as **antigenic determinants**, are the immunologically active discrete sites on the antigen molecule that physically bind to antibodies, B-cell receptors, or T-cell receptors.
- Is a **specific piece of the antigen to which an antibody binds**
- The **part of an antibody that binds to the epitope** is called a **paratope**
- This paratope is only capable of binding with one unique epitope.
- B cells can recognize an **epitope alone** but T cells can recognize an **epitope** only when it is associated with an MHC molecule on the surface of a self-cell (either an antigen-presenting cell or an altered self-cell).

EPITOPE



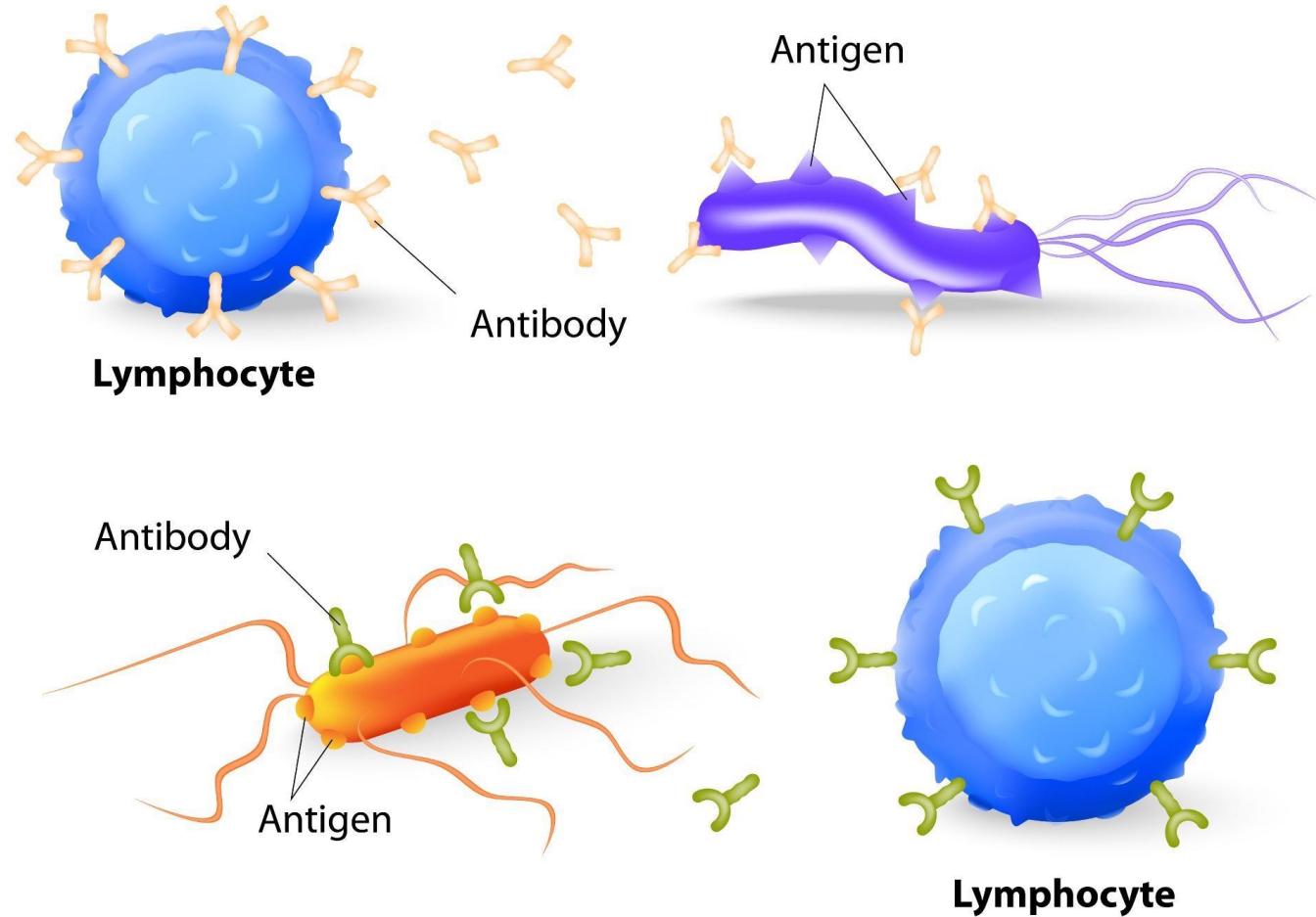
Innate Immunity



HUMORAL IMMUNITY

- Results in production of proteins called "Immunoglobulin's" or "antibodies"
- Humoral immunity is the process of adaptive immunity manifested by the production of antibodies by B lymphocytes.
- It develops in bone marrow.
- B cells may be triggered to proliferate into plasma cells.
- Plasma cells produce antibodies.
- Antibodies are produced when the antigen bonds the B cell receptor (BCR).

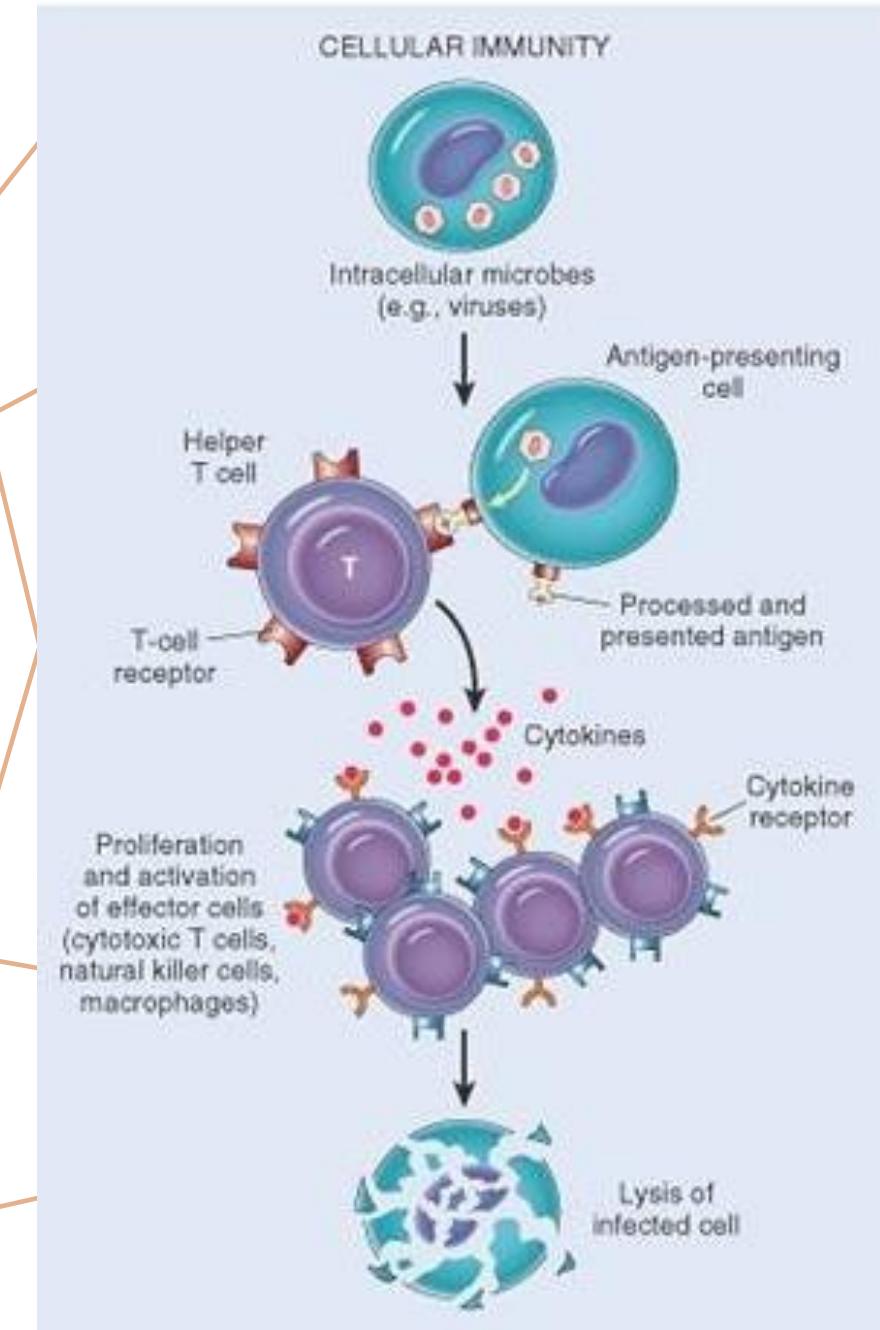
HUMORAL IMMUNITY



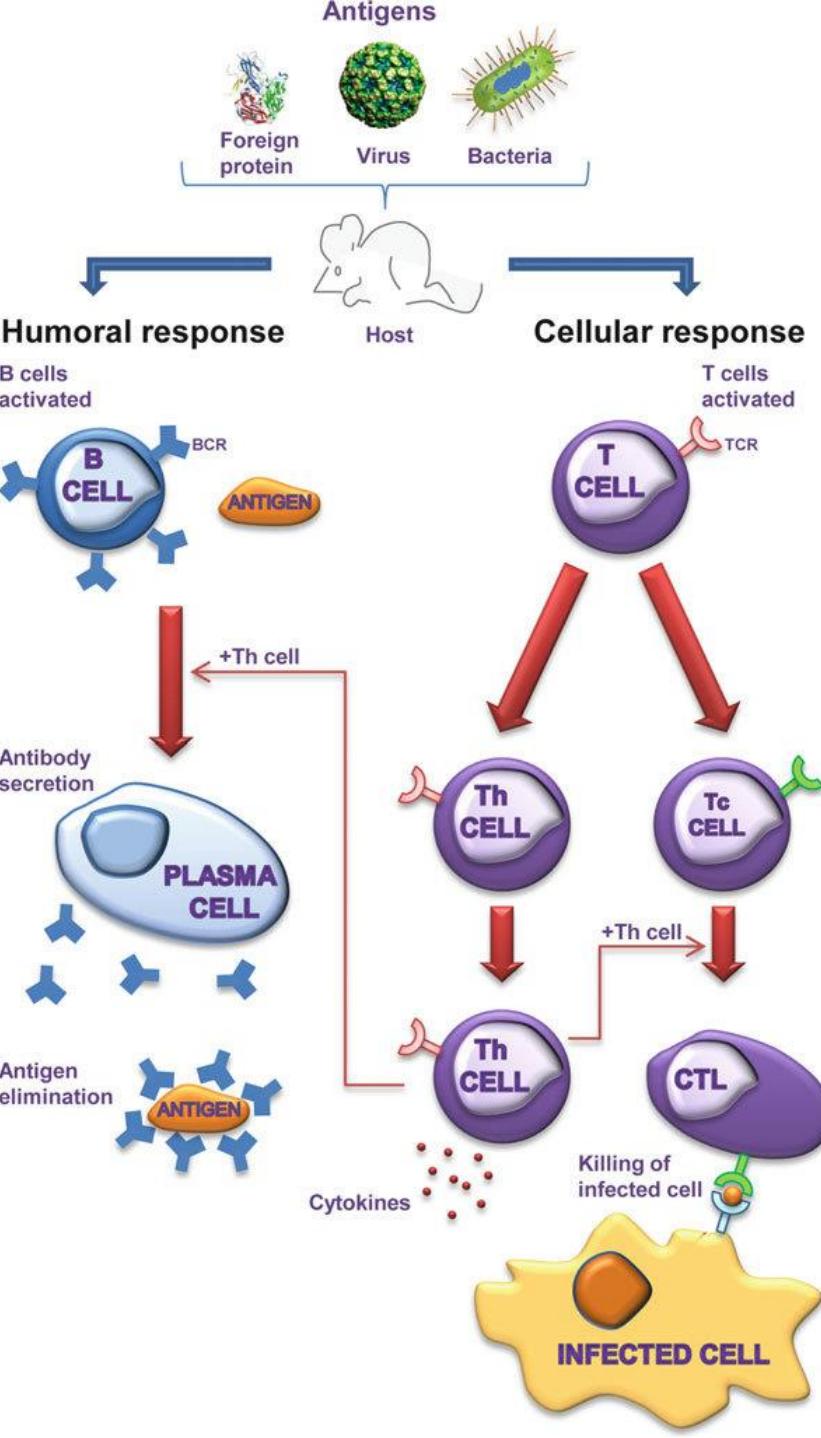
- When foreign material - antigens - is recognized in the body, the body responds with an antibody-mediated reaction.
- Extracellular intruders, such as bacteria, are commonly found in this foreign material.
- B cell lymphocytes, a type of immune cell that makes antibodies after detecting a specific antigen, are principally responsible for this method.
- Lymphocytes known as naive B cells circulate throughout the body via the lymphatic system.
- These cells produce antigen-specific molecules that are necessary for detecting infectious pathogens in the human body.
- When naive B cells in the lymphatic system come into contact with an antigen, they begin the differentiation process that results in the formation of memory B cells and effector B cells.
- Memory B cells and effector B cells produce the same antigen-specific molecules as their parent naive B cell during this development.
- The activated memory B cells express these antigen-specific molecules on their surface with the help of T cell lymphocytes, which are activated by MHC class II receptors that recognize microbial-associated antigens.
- The effector B cells secrete these molecules in the blood to bind the antigen of interest.

CELL-MEDIATED IMMUNITY

- CMI is produced by specific cells of our bodies which is different from the immunity provided by antibodies.
- CMI is the third line of defense mechanism and it gets initiated when the first line of defense (innate immunity) and the second line of defense (nonspecific resistance) fails to protect the body.
- CMI comes under the acquired or adaptive type of immune response which means that it is produced in the body only upon exposure to pathogens and then the memory cells are developed.
- Cell-mediated immunity, unlike humoral immunity, does not rely on antibodies to perform adaptive immunological activities..

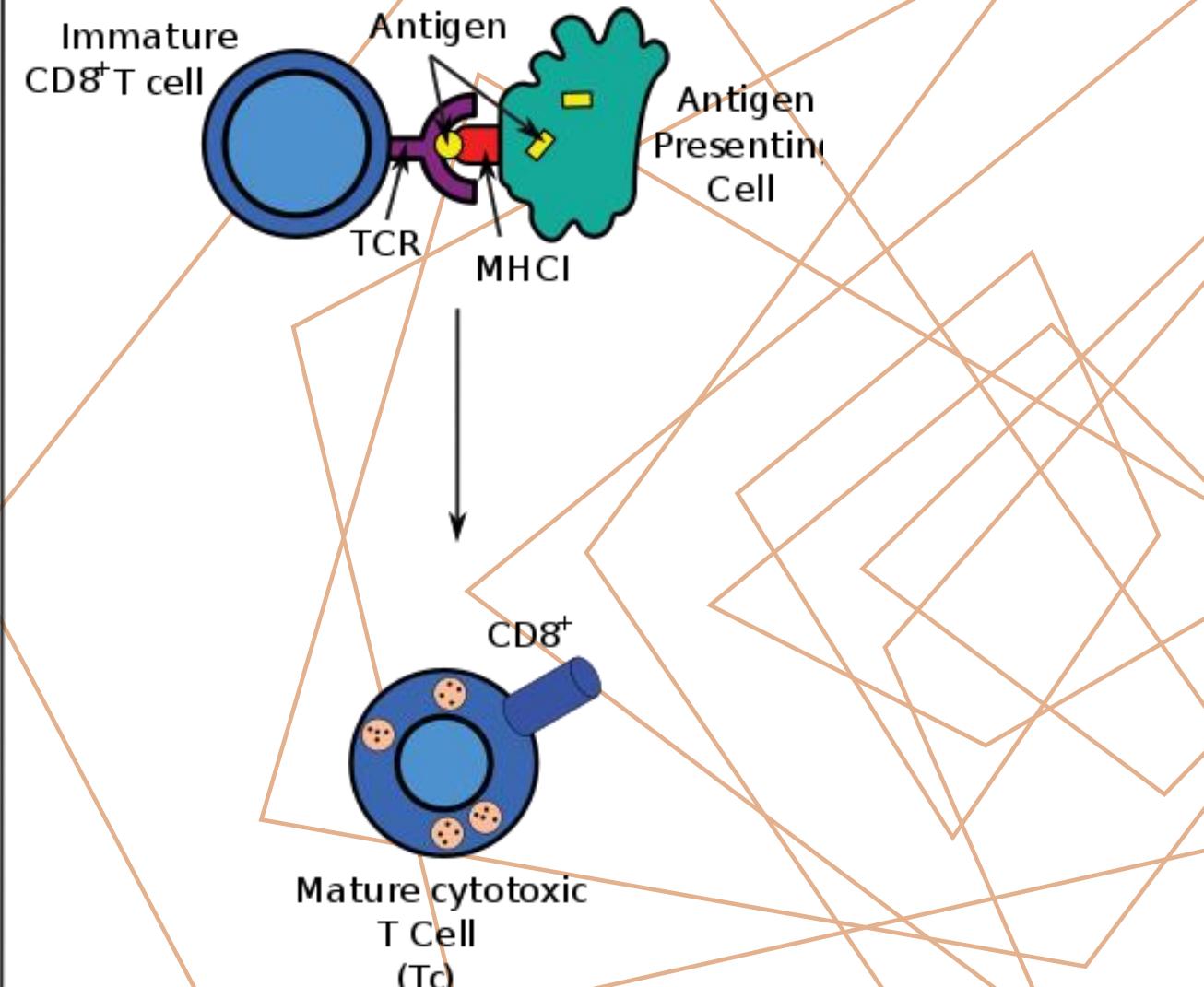
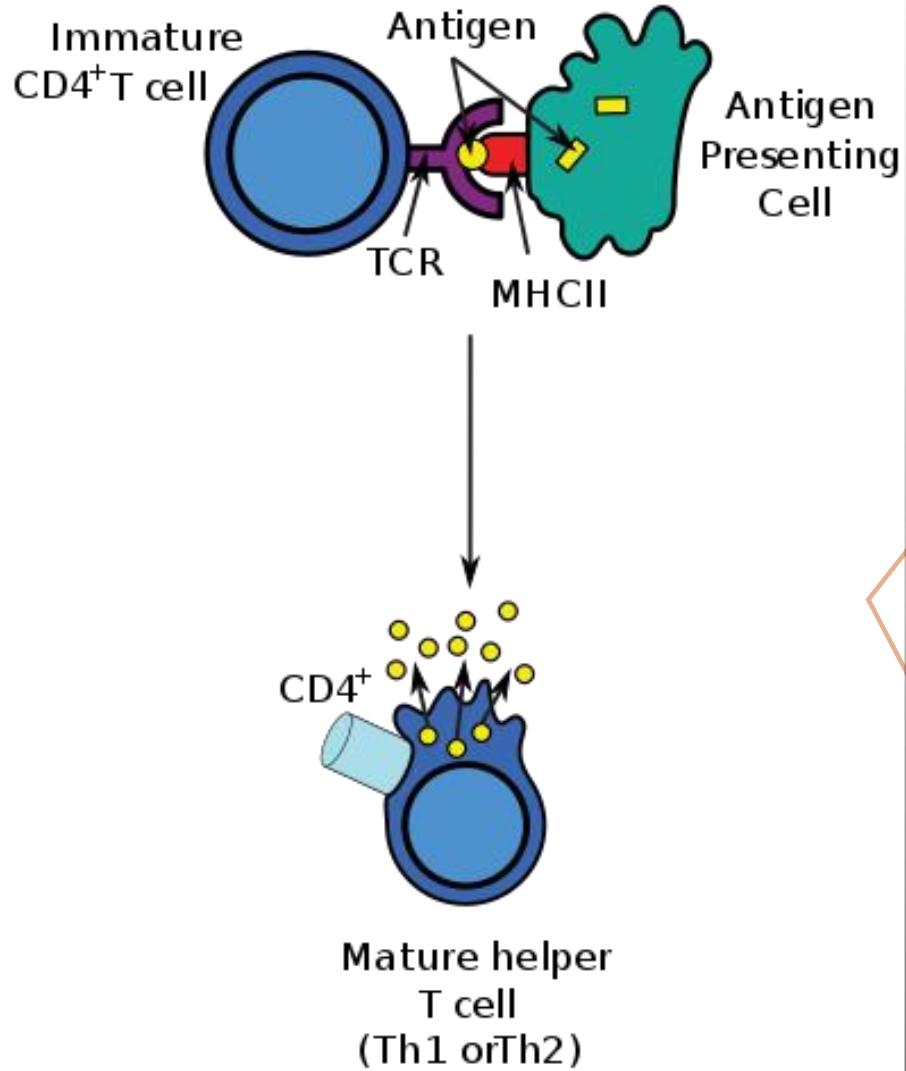


- Mature T cells, macrophages, and the production of cytokines in response to an antigen are the main drivers of cell-mediated immunity.
- To recognize intracellular target antigens, T cells that participate in cell-mediated immunity rely on antigen-presenting cells that have membrane-bound MHC class I proteins.
- The maturation and differentiation of naive T cells into **helper or killer T cells** are dependent on the binding specificity of MHC proteins to external antigens.
- Cell-mediated immunity is activated when cells in the body are infected by a virus, bacterium, or fungus (intracellular invaders).
- T lymphocytes can detect malignant cells with the help of MHC class I proteins.
- Helper T cells, killer T cells, and macrophages are the three main kinds of lymphocytes involved in cell-mediated immunity.
- When a "helper" T cell encounters an antigen-presenting cell in the body, it releases cytokines, which are signaling proteins. These cytokines cause "killer" T lymphocytes and macrophages to flock to the antigen-presenting cell in an attempt to eliminate it.



ANTIGEN PRESENTING CELLS

- Antigen-presenting cells (APCs) are a heterogeneous group of immune cells that mediate the cellular immune response by processing and presenting antigens for recognition by certain lymphocytes such as T cells.
- Classical APCs include dendritic cells, macrophages, Langerhans cells (unique population of tissue-resident macrophages that form a network of cells across the epidermis of the skin) and B cells
- Invading foreign organisms are either engulfed by macrophages through phagocytosis or trapped by dendritic cells.
- Later, the antigen from these organisms is digested into small peptide products.
- These antigenic peptide products move towards the surface of the antigen presenting cells and bind with human leukocyte antigen (HLA).
- HLA is a genetic matter present in the molecule of class II major histocompatibility complex (MHC), which is situated on the surface of the antigen presenting cells.
- B-cells ingest the foreign bodies by means of pinocytosis. Role of B cells as antigen-presenting cells in the body is not fully understood..

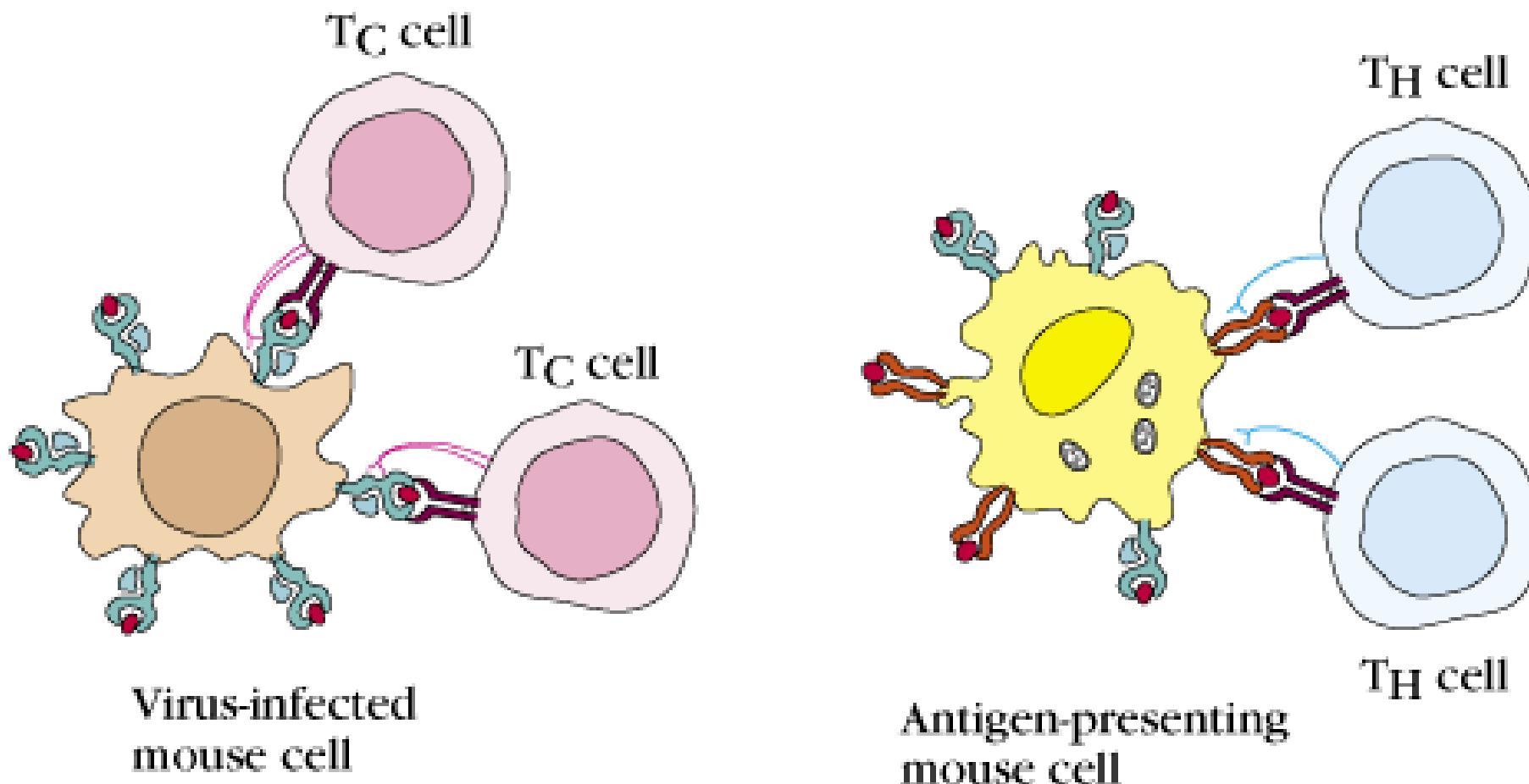
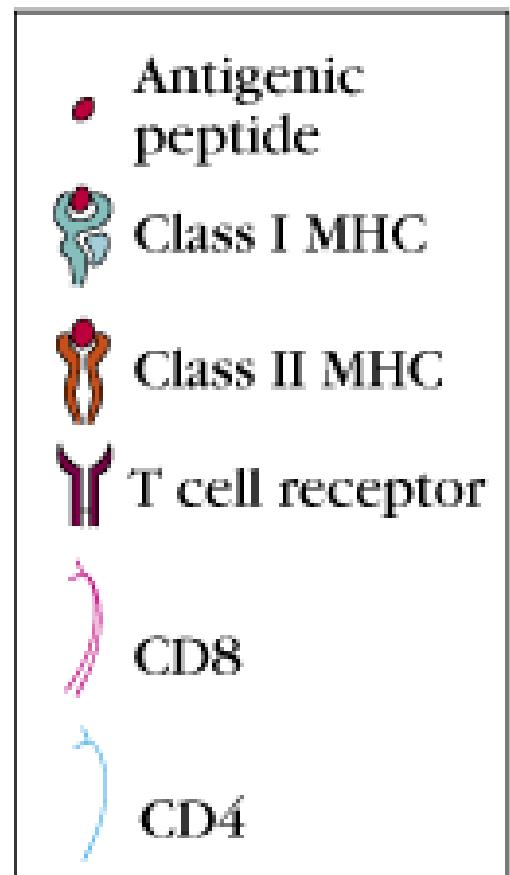


MAJOR HISTO-COMPATIBILITY COMPLEX (MHC)

- Is a set of surface proteins located in the cell membrane of nucleated cells.
- It plays an important role in identification of self and non self antigen body, intracellular recognition and responsible for antigen presentation
- This locus got its name because it was discovered via the study of transplanted tissue compatibility.
- Histo referred to tissue and compatibility referred to as harmonious living
- Is a large locus on vertebrate DNA containing a set of closely linked polymorphic genes that code for cell surface proteins essential for the adaptive immune system.
- These cell surface proteins are called MHC molecules.
- The function of MHC molecules is to bind peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T cells.
- In humans the MHC is found on Chromosome 6 referred to as the HLA complex

MAJOR HISTO-COMPATIBILITY COMPLEX (MHC)

- The first descriptions of the MHC were made by British immunologist Peter Gorer in 1936. MHC genes were first identified in inbred mice strains.
- He identified the four group of MHC molecules in the blood sample of mice when he identified the blood group antigen and was designated as class I to IV
- MHC molecules in human beings are divided into two types:
- 1) **Class I MHC molecule:** It is found on every cell in human body. It is specifically responsible for presentation of endogenous antigens (antigens produced intra-cellularly such as viral proteins and tumor antigens) to cytotoxic T cells.
- 2) **Class II MHC molecule:** It is found on B cells, macrophages and other antigen-presenting cells. It is responsible for presenting the exogenous antigens (antigens of bacteria or viruses which are engulfed by antigen-presenting cells) to helper T cells.
- Antigen-presenting cells present their class II MHC molecules together with antigen-bound HLA to the helper T cells. This activates the helper T cells through series of events

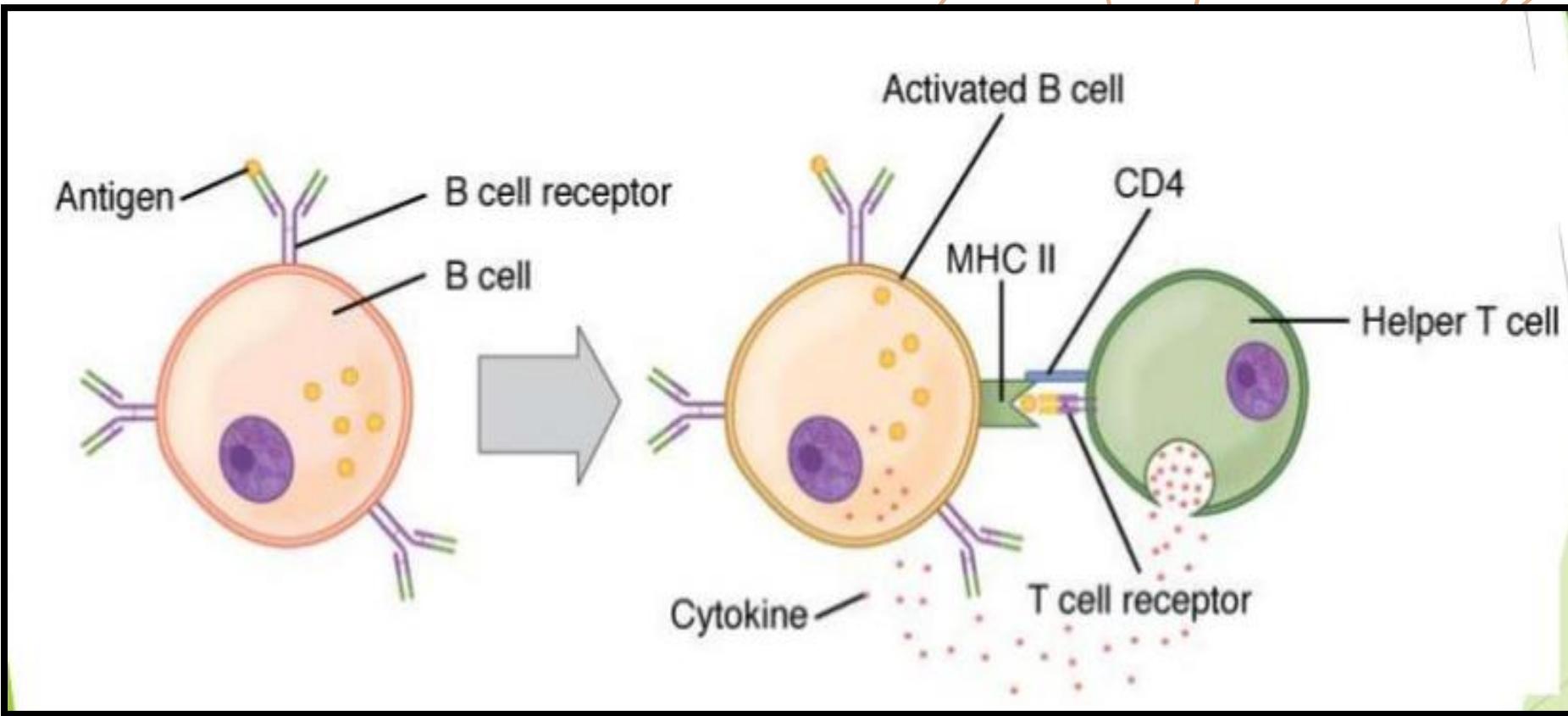


ANTIGEN PRESENTATION & PROCESSING

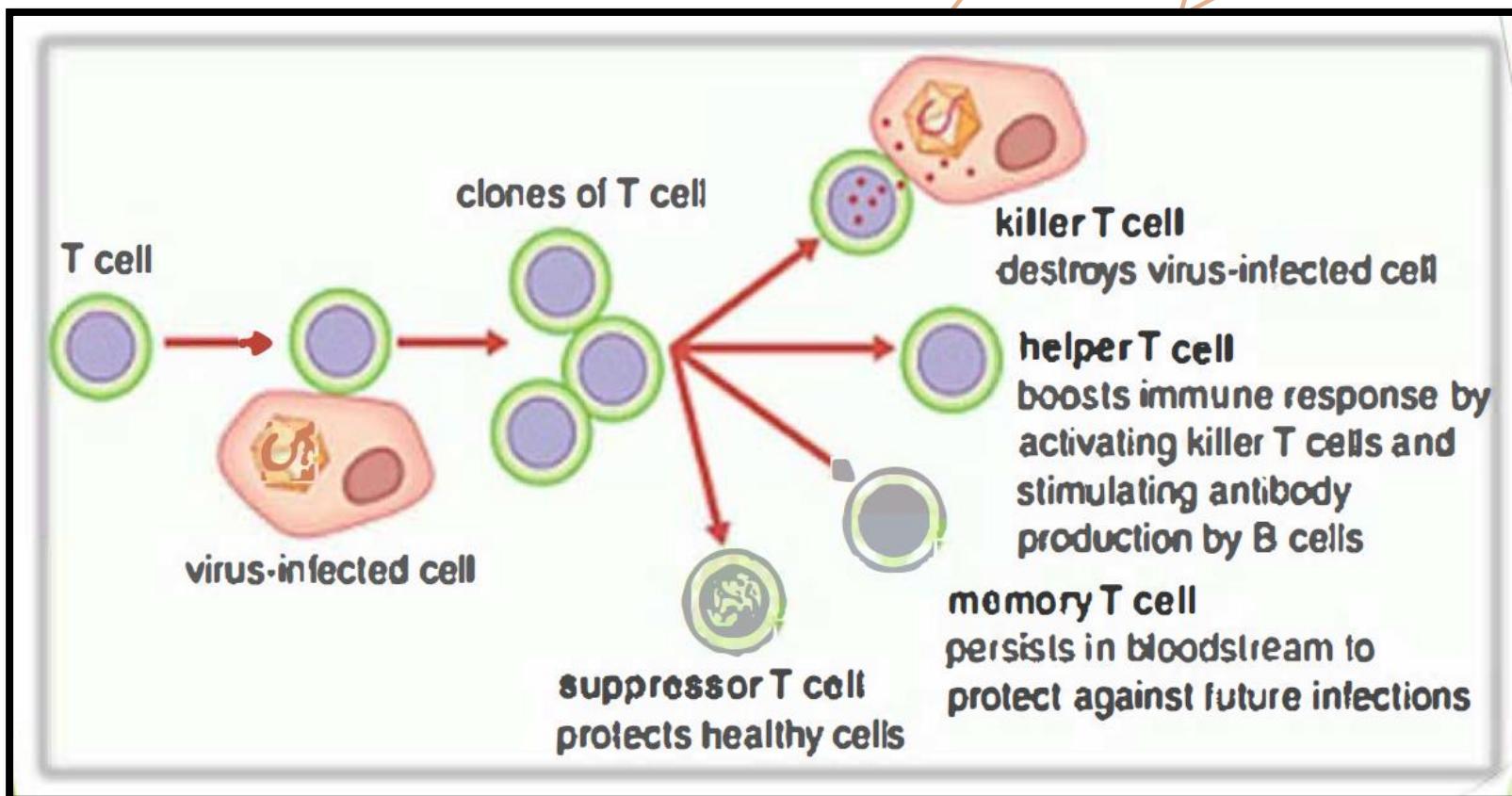
- The recognition of protein antigens by T-lymphocytes required that the antigens be processed by Antigen-presenting Cells, then displayed within the cleft of the MHC molecules on the membrane of the cell.
- This involves the degradation of the protein antigens into peptides, a process known as antigen processing.
- When the antigen has been processed and degraded into peptides, it then associates with MHC molecules within the cell cytoplasm forming a peptide-MHC complex. This complex is then transported to the membrane, where it is displayed by a process of antigen presentation.
- The MHC Class I and class II MHC molecules associated with peptides that have been processed in different intracellular compartments.
- The Class I MHC molecules bind peptides derived from endogenous antigens that have been processed within the cytoplasm of the cell such as tumor proteins, bacterial proteins, or viral proteins, or cellular proteins, and processed within the cytosolic pathway.
- Class II MHC molecules bind peptides derived from exogenous antigens that are internalized by phagocytosis or endocytosis and processed within the endocytic pathway.

SEQUENTIAL ACTIVATION OF T HELPER (T_H) CELLS

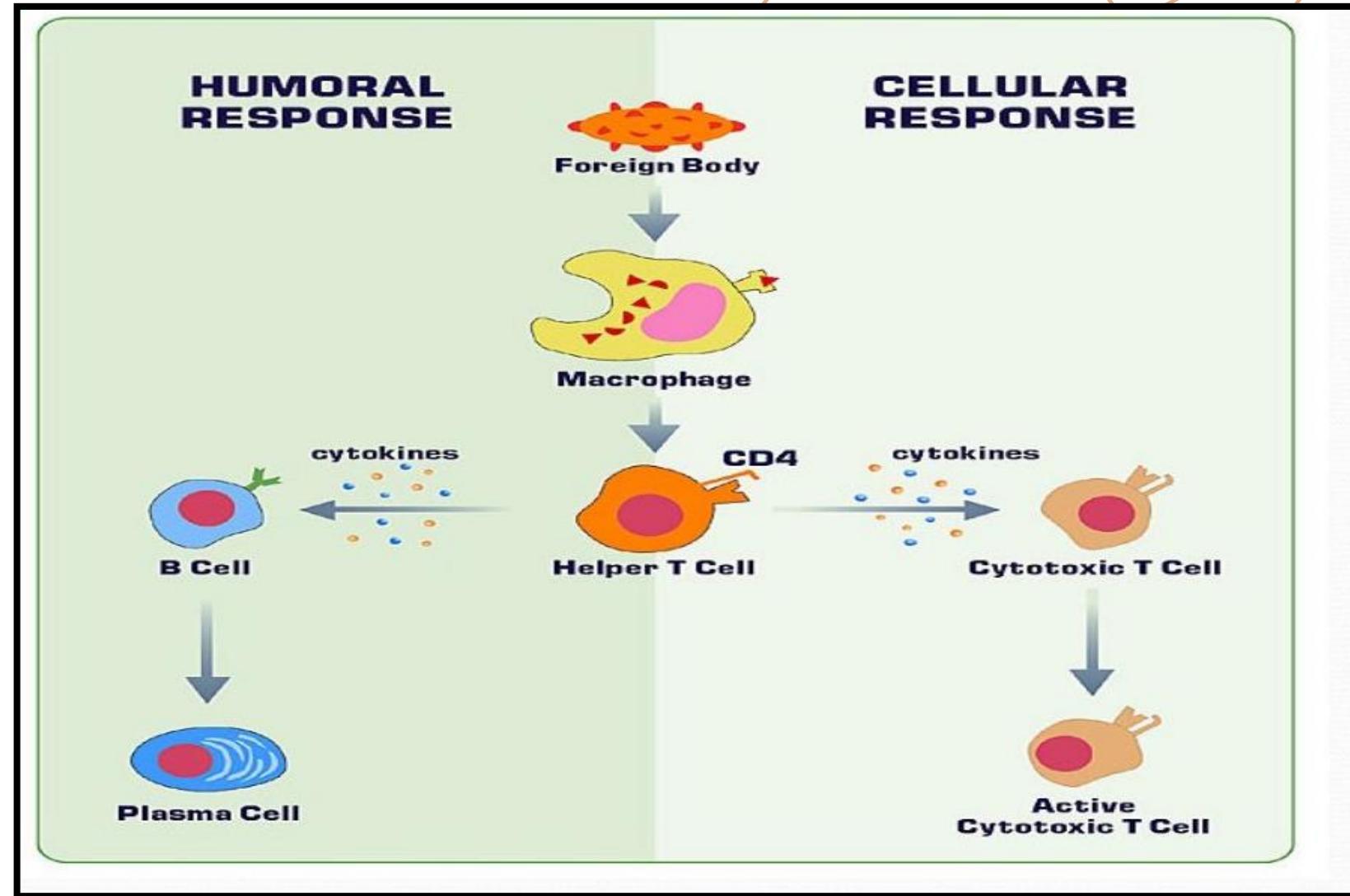
- Helper T cell recognizes the antigen displayed on the surface of the antigen presenting cell with the help of its own surface receptor protein called T cell receptor.
- Recognition of the antigen by the helper T cell initiates a complex interaction between the helper T cell receptor and the antigen. This reaction activates helper T cells.
- At the same time, macrophages (the antigen-presenting cells) release interleukin-1, which facilitates the activation and proliferation of helper T cells.
- Activated helper T cells proliferate and the proliferated cells enter the circulation for further actions.
- Simultaneously, the antigen which is bound to class II MHC molecules activates the B cells also, resulting in the development of humoral immunity



ROLES OF T CELLS



ANTIBODY

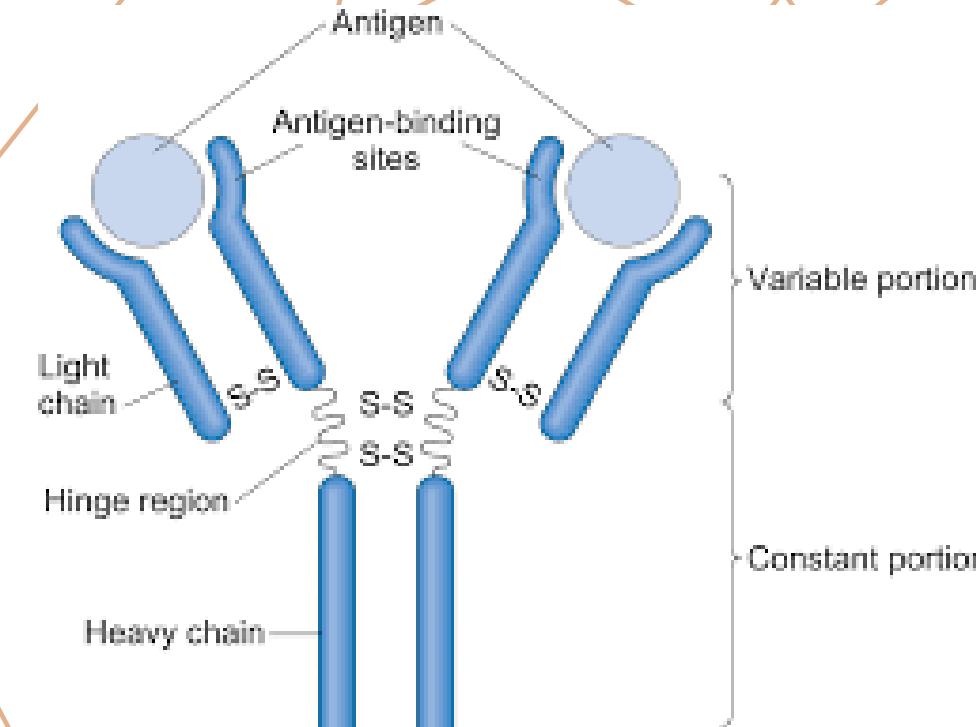


ANTIBODY

- Also called immunoglobulin (Ig)
- Immunoglobulin is a glycoprotein that is made in response to an antigen and can recognize and bind to the antigen that caused its production.
- Protects us from microbial infection. They're gamma globulins.
- Synthesized by plasma cells and constitute 25-30 % of total serum proteins
- Antibodies are present in serum, tissue fluids and mucosal surfaces and on surface of B-cells where they acts as antigen receptor.

ANTIBODY

- Composed of 4 polypeptide chains.
- 2 identical light chains (25 kDa each) and 2 identical heavy chains (50-73 kDa each)
- Linked by di-sulphide bonds
- Light chains similar in all immunoglobulins
- Light chains occur in 2 varieties :-I kappa(κ) and lambda(λ)
- Heavy chains:- gamma, alpha, mu, delta and epsilon.
- Kappa chains are more frequently found.



L CHAIN

- L- chain of antibody is composed of about 220 amino acids.
- Around 100-110 amino acids are located at N-terminal (amino-terminal) and the amino acids sequences varies among antibodies. This region of L-chain is known as variable (V) region.
- Remaining 110 amino acids located at C-terminal (carboxyl-terminal) of L-chain are almost constant among antibodies. This region of L-chain is known as constant (C) region. Two types of constant region sequences are found ie. Lambda (λ) and Kappa (κ). In a particular antibody either – 2 lambda or 2 kappa chains are present but not 1 lambda and kappa.
- In human 60% light chain are kappa and 40% are lambda whereas in mice 95% of light chain are kappa and 5% are lambda.

H CHAIN

- In H-chain about 110 amino acids are located at N-terminal which shows great variation among antibody. This region is known as Variable (V) region.
- Remaining amino acid sequences of H-chain is somewhat constant but reveals five different types of constant (C) heavy chain region ie. μ , α , δ , ϵ and γ .
- The length of constant region of H-chain is 330 aminoacids for α , γ and δ and 440 amino acids for μ and ϵ .
- Antibodies molecules are classified into five class on the basis of constant region of H-chain.
- There are five different types of heavy chains in mammals that are designated by letters: α , δ , γ , ϵ and μ .

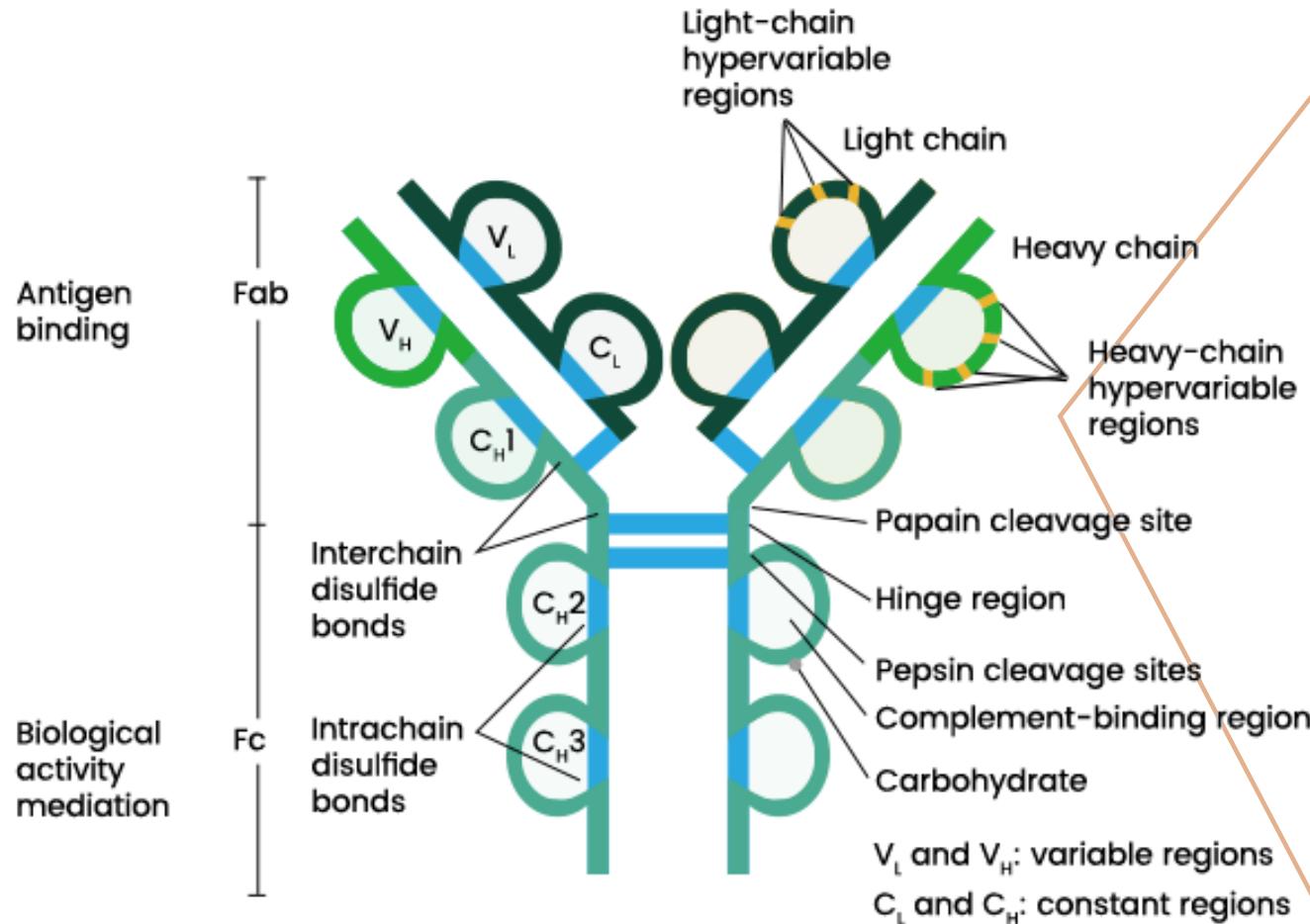
VARIABLE & CONSTANT REGION

- Light and Heavy chains are subdivided into variable and constant region.
- Each heavy and light chain contains amino terminal in variable region and carboxy terminal in constant region
- Variable region extends from N-terminal about 100 -n- amino acids and the amino acid sequences in these region's are highly variable.
- Constant region extends from end of variable region to C-terminal and here the amino acid sequence is relatively constant.

VARIABLE & CONSTANT REGION

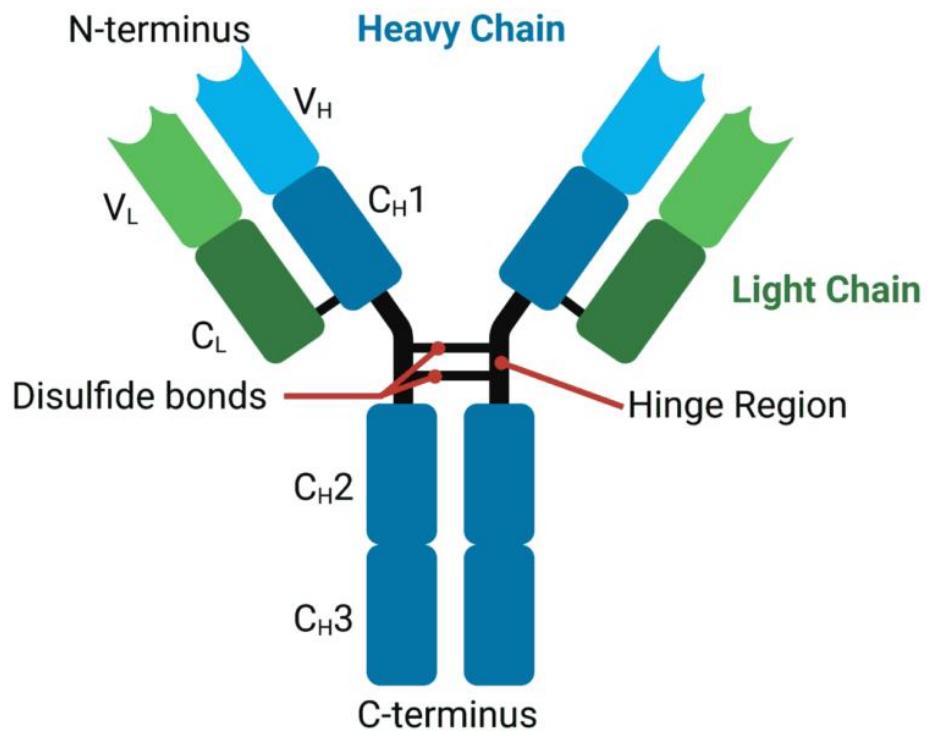
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- Constant region extends from end of variable region to C-terminal and here the amino acid sequence is relatively constant.

STRUCTURE



- Antibodies or immunoglobulins (Ig) maintain a common **quaternary structure** consisting of two identical **heavy chains** (HCs) and two identical **light chains** (LCs).
- The structure is heterotetrameric, with **disulfide bridges** linking HCs and LCs together to form the canonical immunoglobulin '**Y**' shape.
- **Variable domains** (V) of heavy and light chains (V_H and V_L , respectively) give rise to antigen specificity through highly variable amino acid sequences.
- While the V domain interacts with antigens, the **constant domains** (C) on heavy and light chains interact with effector proteins and molecules. HCs have three constant regions, in contrast to the one on LCs and as such, are numbered from the N-terminus to the C-terminus (CH_1 , CH_2 and CH_3)

HINGE REGION

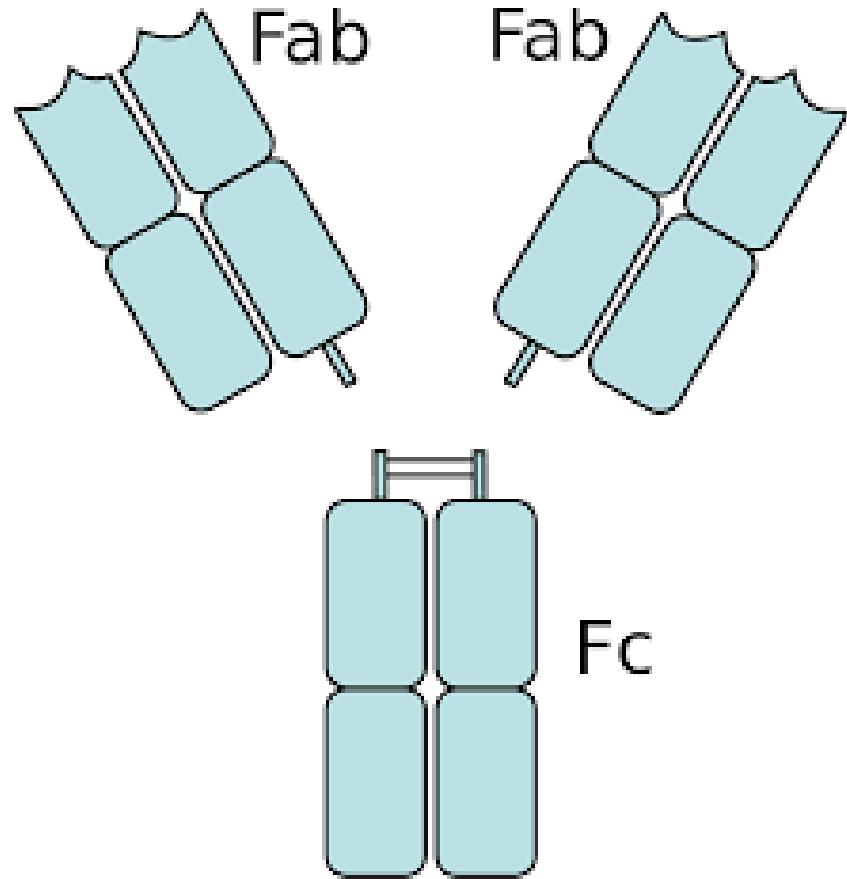


The γ , δ and α heavy chain contains an extended peptide sequence between CH1 and CH2 domain that has no homology with other domain, this region is known as hinge region.

Hinge region is rich in proline residue and is flexible. Therefore IgG, IgD and IgA are flexible.

The flexibility given by hinge region enable Fab region to assume various angle to bind antigen.

FAB 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.

Antigen binding is accomplished by amino-terminal (N-terminal) region and effector functions by carboxyl terminal (C-terminal) region of antibody.

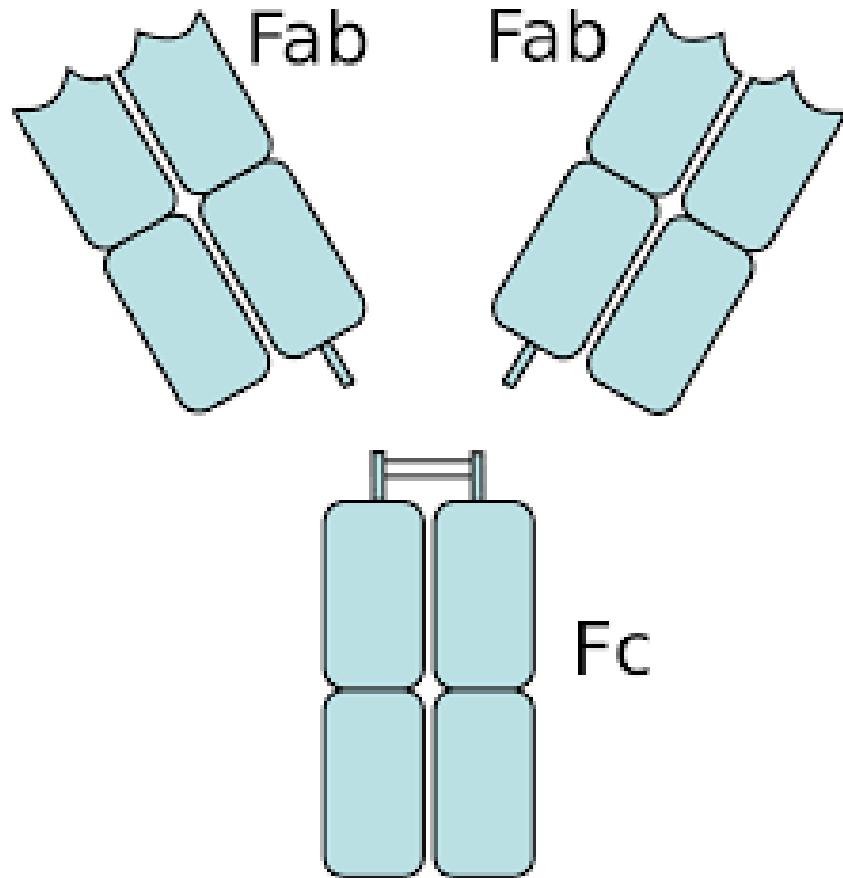
In an antibody molecule two Fab regions are found and they binds antigens.

Hypervariable region on L-chain (VL domain) and H-chain (VH domain) form antigen binding site.

The variability plot of VH and VL domains shows maximum variation in certain region which is known as hypervariable region and this forms antigen binding site.

Antigen binding site is complementary to epitope of antigen, so it is also known as complementary determining regions (CDRs).

FC REGION



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.

Fc region of immunoglobulin allows for interaction of immune complex with other phagocytic cells and complement.

Take parts in various biological functions that are determined by amino acid sequences of each domains of constant region.

Many different form of Fc receptors exists.

ANTIGEN PROCESSING & PRESENTATION

In order to be capable of engaging the key elements of adaptive immunity (specificity, memory, diversity, self/non-self discrimination), antigens have to be processed and presented to immune cells.

Antigen presentation is mediated by MHC class I molecules, and the class II molecules found on the surface of antigen-presenting cells (APCs) and certain other cells.

MHC class I and class II molecules are similar in function: they deliver short peptides to the cell surface allowing these peptides to be recognized by CD8+ (cytotoxic) and CD4+ (helper) T cells, respectively.

ANTIGEN PROCESSING & PRESENTATION

Antigen processing is a metabolic process that digests the proteins into peptides which can be displayed on the cell membrane together with a class-I or class-II MHC molecules and recognized by T-cells.

Antigen presentation is the process by which certain cell in the body especially antigen presenting cells (APCs) express processed antigen on their cell surface along with MHC molecules in the form recognizable to T cell.

If antigen is presented along with class-I MHC molecule, it is recognized by CD8+ Tc-cell and if presented along with class-II MHC molecule, it is recognized by CD4+ TH cells.

ENDOGENOUS ANTIGENS

Endogenous antigens are derived from proteins produced **inside the cell**.

These includes altered self-protein antigens (e.g. tumor antigens) and non-self protein antigens (e.g. viral antigens).

Endogenous antigens associate with Class I MHC molecules that activate cytotoxic CD8+ T cells for killing infected cells and tumor cells (target or effector cells).

Endogenous antigens can be processed and presented by any nucleated cell.

EXOGENOUS ANTIGENS

Exogenous antigens are derived from proteins produced outside the cell.

These includes various bacterial, viral, protozoal, fungal and parasitic antigens which are derived from outside the body.

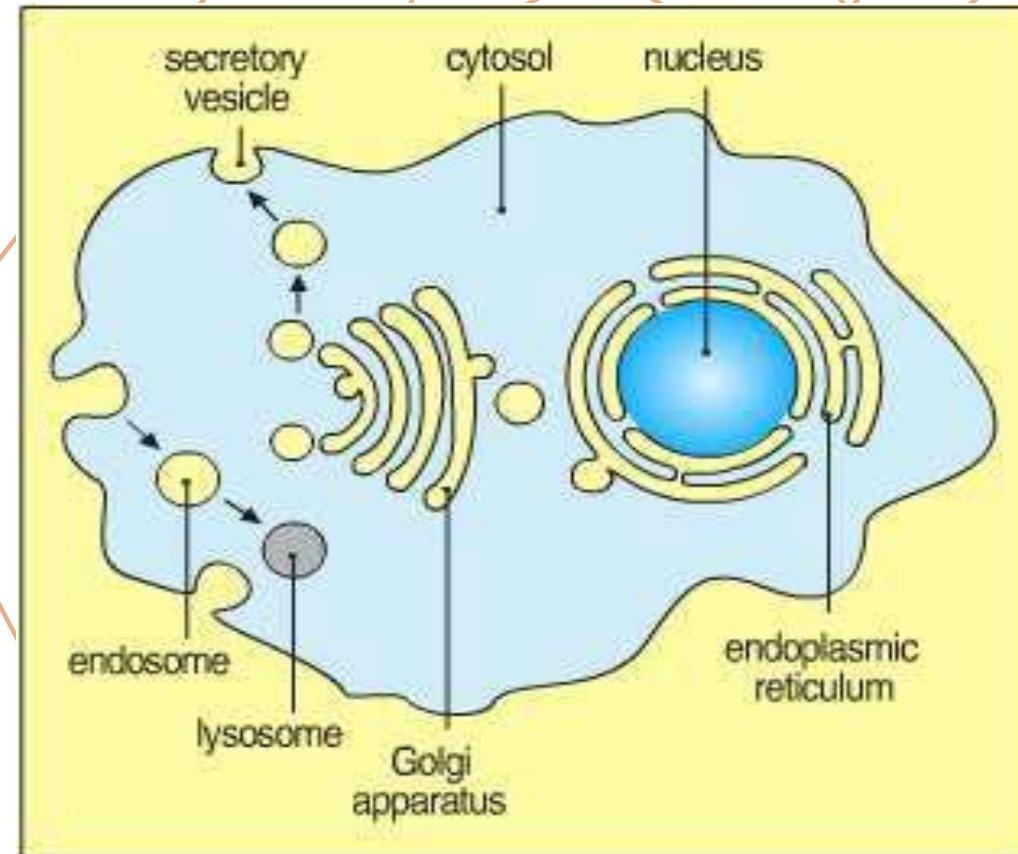
Exogenous antigens associate with Class II MHC molecules that activate helper CD4+ T cells for providing help to B and Tc cells.

Exogenous antigens are processed and presented by APCs

EXO AND ENDOGENOUS PROTEINS:

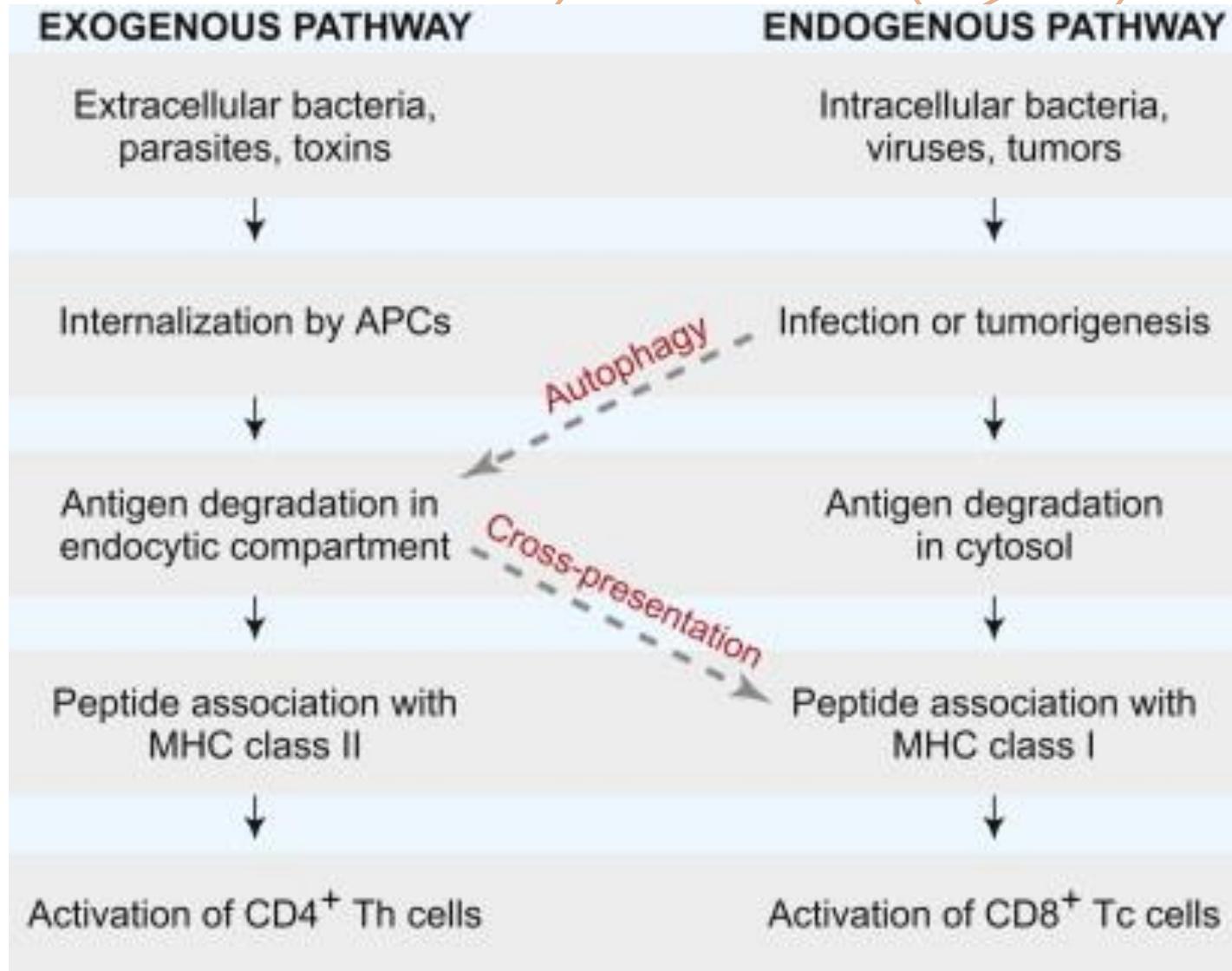
Endogenous proteins are processed in cytosol or in secretory vesicles and presented on class I MHC molecules to CD8+ T cells.

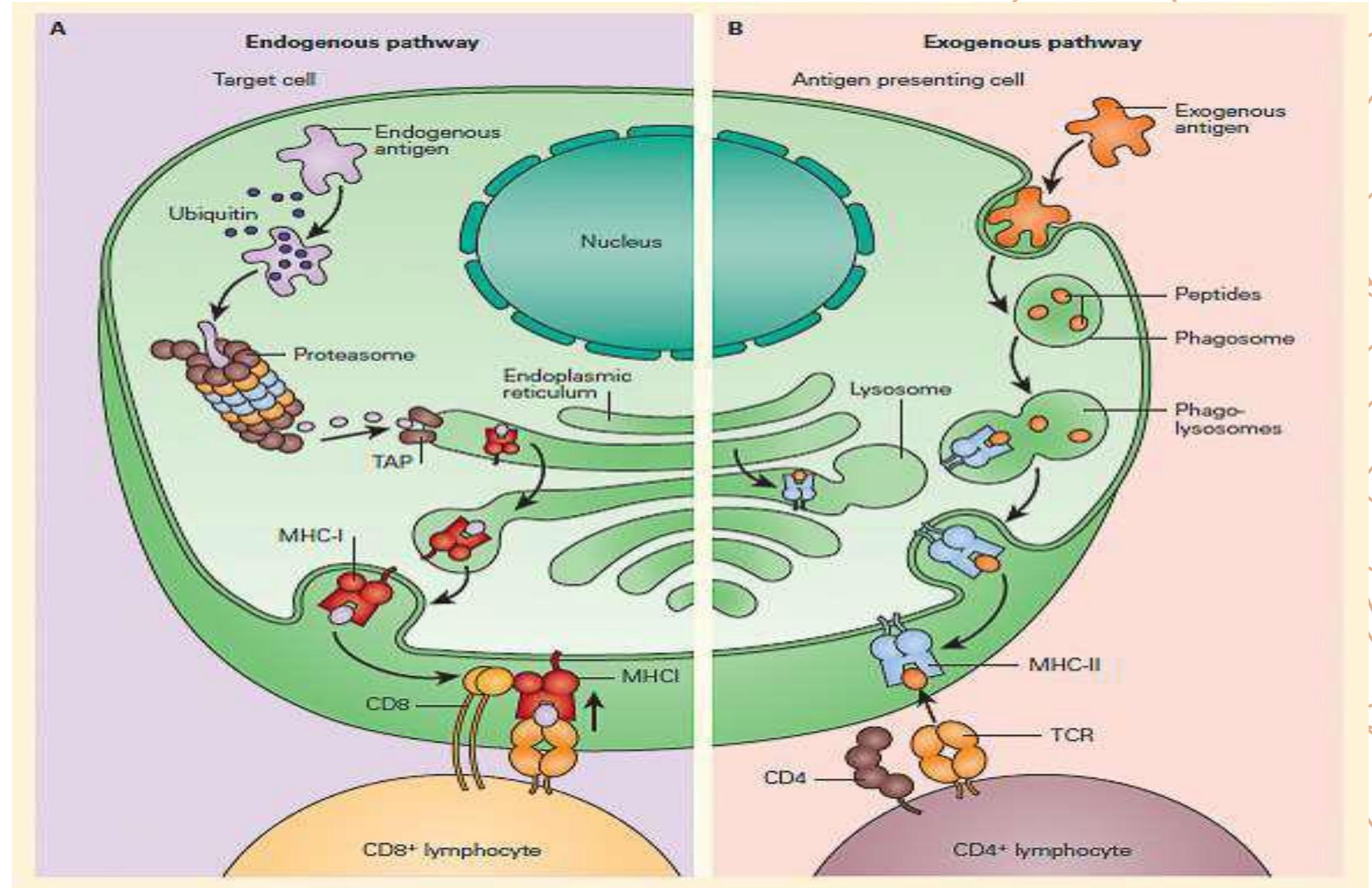
Exogenous proteins are processed in endosomes and presented on class II MHC molecules to CD4+ T cells



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PATHWAYS





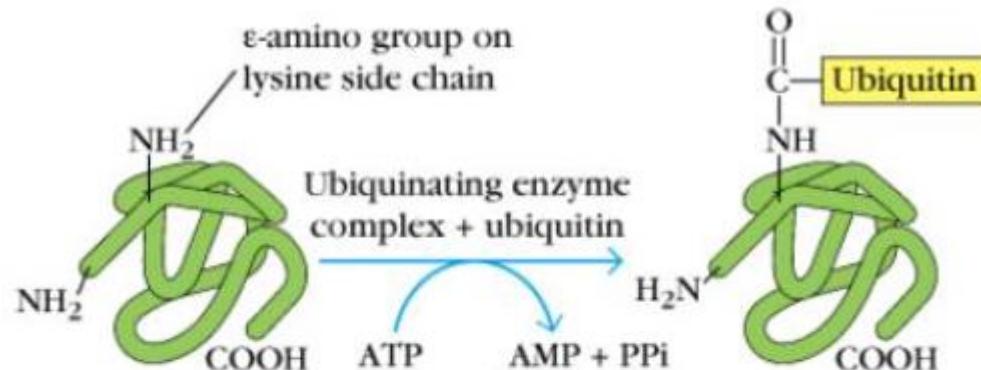
ENDOGENOUS ANTIGENS PROCESSING PATHWAY (CYTOSOLIC PATHWAY)

Endogenous (MHC class I) pathway

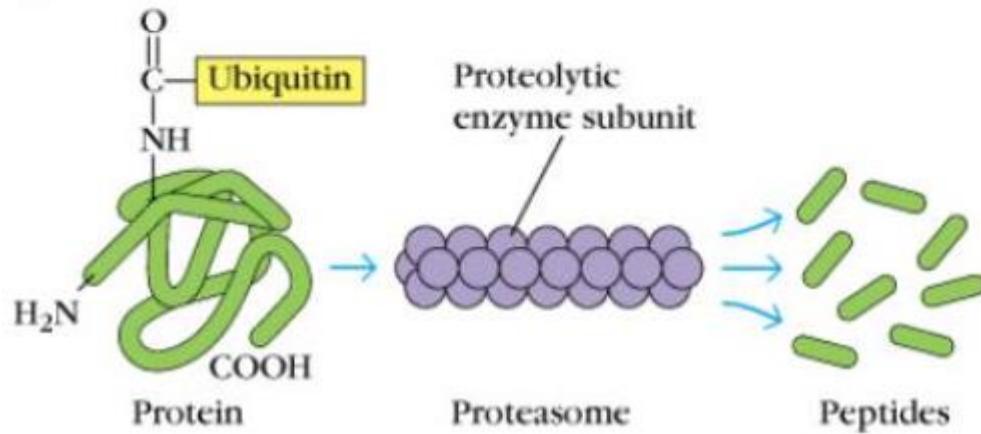
1. Processing of antigens into peptides
2. Assembly of MHC and peptide loading complex
3. Peptide loading and MHC-peptide transport

STEP 1A UBIQUITINATION

(a)

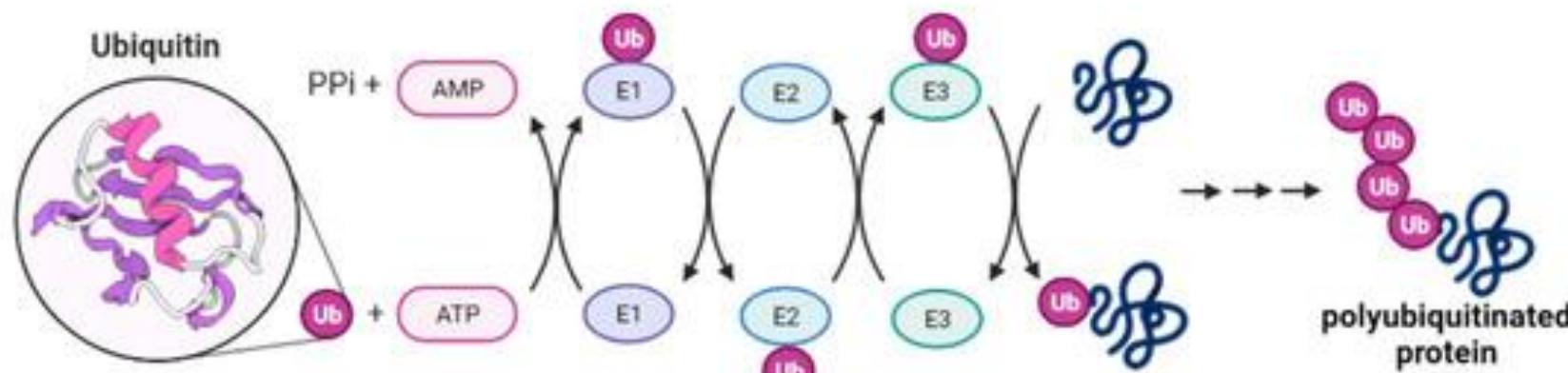


(b)

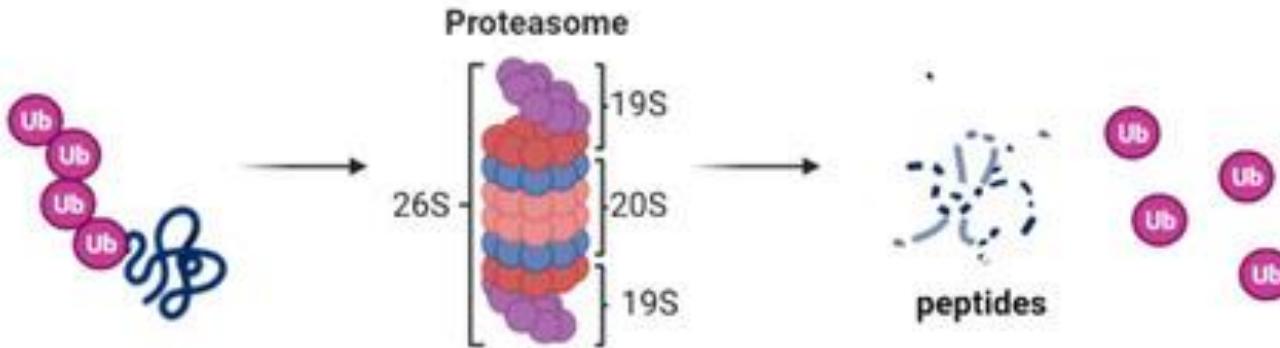


- The ubiquitin-proteasome pathway (UPP) is one of the major destruction ways to control the activities of different proteins.
- The function of UPP is to eliminate dysfunctional/misfolded proteins via the proteasome, and these specific functions enable the UPP to regulate protein quality in cells.
- defects in UPP are expected to disturb cellular homeostasis and are detrimental to cell survival.
- step A is a specific recognition process, employing the Ub conjugation cascade that uses a highly modular approach in different combinations for different purposes.
- step B, degradation of the tagged substrate by the 26S proteasome is an indiscriminate destruction process, mediated by the proteolytic proteasome core.
- This indiscriminate proteolytic step provides directionality for a signaling pathway, i.e., once a protein is committed for degradation, there is no return, ensuring that partially degraded proteins do not interfere with biological processes.

a Protein Ubiquitination Pathway



b Polyubiquitinated Protein Degradation



Ubiquitin-proteasome system **(a)** Protein polyubiquitination process using the ubiquitin-activating enzyme E1, conjugating enzymes E2 and the E3 ligase; **(b)** Polyubiquitinated proteins are degraded by 26S proteasome into small peptides following its deubiquitination.

STEP 1B PROTEASOME-MEDIATED PROCESSING

The **proteasome** is a cylindrical shaped catalytic protease complex of 28 subunits for cytosolic protein degradation.



The proteasome unfolds proteins and then **cleaves proteins** into peptides and amino acids by proteases

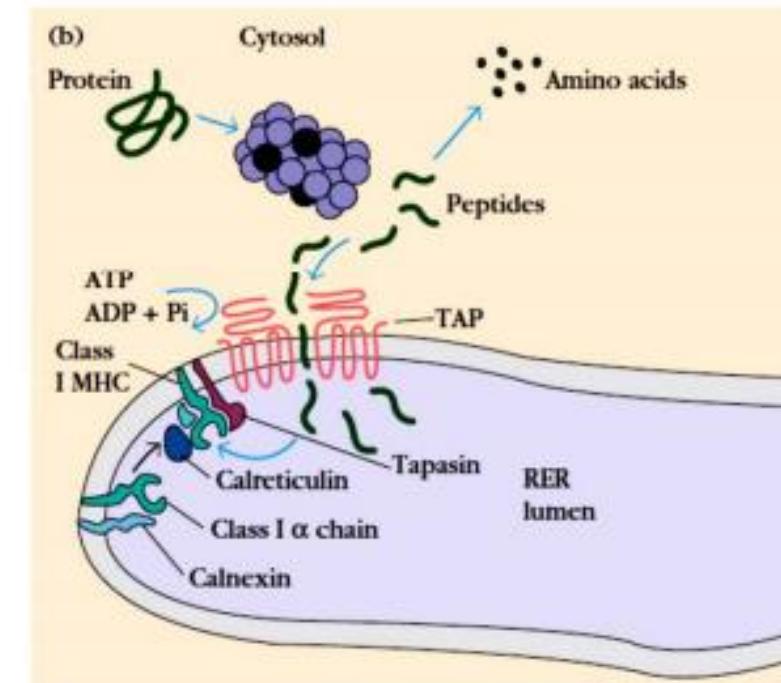
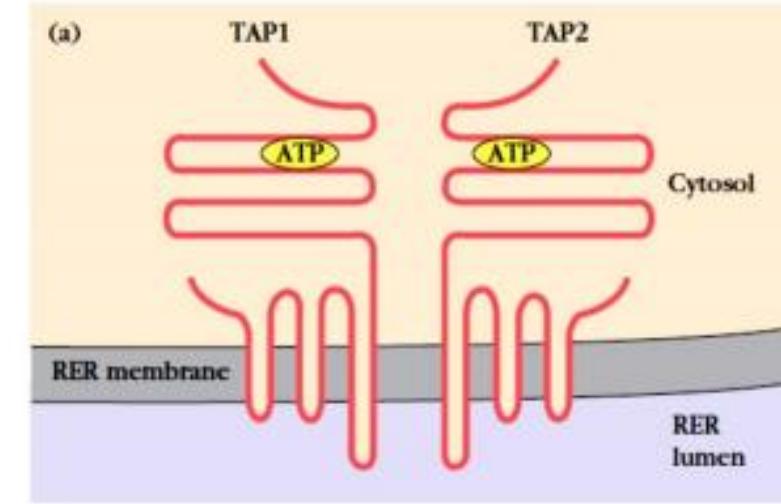
Conserved throughout the eukaryotes and the archaeabacteria

ENDOGENOUS ANTIGENS PROCESSING PATHWAY (CYTOSOLIC PATHWAY)

- After monoubiquitination of the targeted protein, the C-terminus of each ubiquitin molecule can be linked to any of the other seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) on the previous ubiquitin to extend the ubiquitin chain and form the polyubiquitinated tagged protein
- the signal for protein degradation by the proteasome usually involves the linking of Ub to the K48 of the previous Ub on the protein
- K11, K29, and K63 linked chains have also been shown to play a role in proteasomal degradation
- Proteasome inhibition is a therapeutic approach for the treatment of cancer.
- Proteasome activation by small molecules is a proposed strategy for the treatment of age-related diseases and several neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's Disease (AD), Huntington's Disease (HD), and Amyotrophic Lateral Sclerosis
- Increasing the proteolytic activity of proteasome enhances the degradation of specific intrinsically disordered proteins (IDPs) such as α -synuclein, β -amyloid, and tau, to mention a few, which are associated with the pathogenesis of these neurodegenerative diseases.

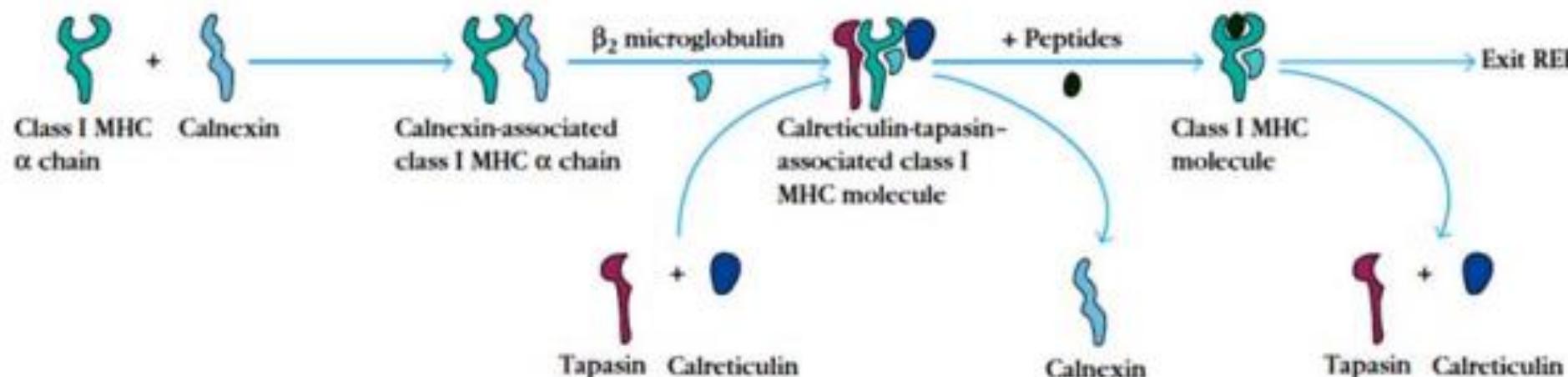
STEP-II: TRANSFER OF PEPTIDES BY TAP PROTEINS

- TAP proteins (Transporters associated with Antigen Processing)
- TAP 1 and TAP 2 form heterodimer in membrane of ER to facilitate selective transport of peptides from cytoplasm into lumen of ER.
- TAP pump preferentially transport peptides with a length of 8–15 amino acids



Peptides Assemble with Class I MHC Aided by Chaperone Molecules

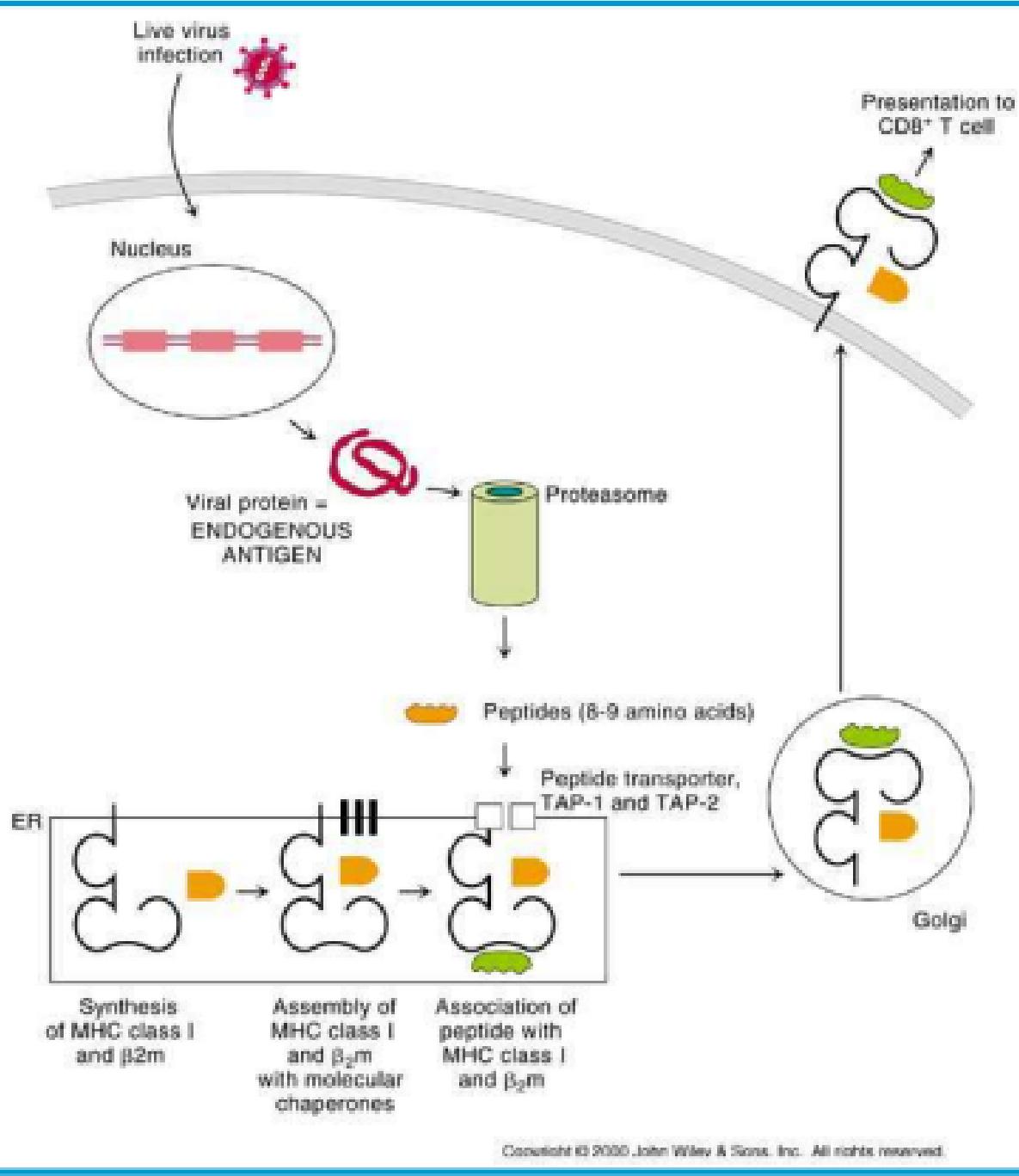
STEP-III: GENERATION OF CLASS I MHC PEPTIDES



- the α- chain and β₂-microglobulin components of the class I MHC molecule are synthesized
- The assembly process involves several steps and includes the participation of molecular chaperones, which facilitate the folding of polypeptides
- Calnexin, membrane protein of the ER, associates with the free class I α-chain and promotes its folding
- β₂-microglobulin binds to the αchain, calnexin is released, class I molecule associates with the chaperone calreticulin and with tapasin
- TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide.

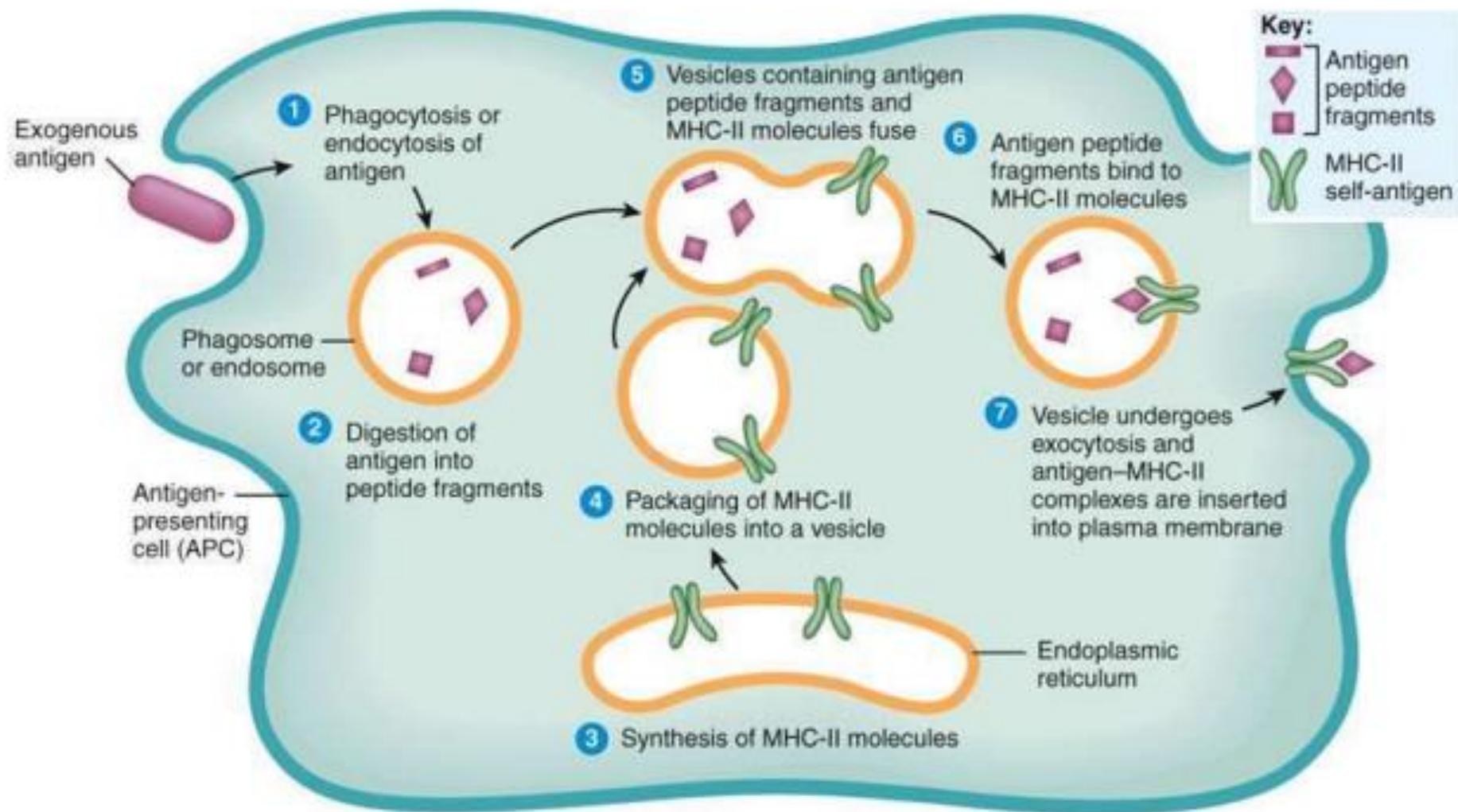
STEP-IV AND V:TRANSPORT OF PMHC TO CELL SURFACE AND PRESENTATION:

- peptide binding, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER, and proceed to the cell surface via the Golgi.
- The pMHC-I complex is transported from ER via Golgi bodies in a membrane bound vesicle to the cell surface.
- The membrane of transport vesicle fuse with the cell membrane and pMHC complex bind to membrane presenting peptide lodged towards the exterior to be recognised by Tc cell



EXOGENOUS ANTIGENS PROCESSING PATHWAY (ENDOCYTIC PATHWAY):

- Exogenous (MHC class II) pathway:
- 1. Uptake and processing of exogenous antigen
- 2. MHC assembly and transport to peptide loading compartment
- 3. Peptide loading (CLIP exchange) and MHC peptide transport



APCs present exogenous antigens in association with MHC-II molecules

STEP-I: HOW ARE PEPTIDES GENERATED?

Once an antigen is internalized, it is degraded into peptides within compartments of endocytic processing pathway.

The endocytic pathway appears to involve three increasingly acidic compartments, early endosomes (pH 6-6.5), late endosomes or endo-lysosome (pH 5-6) and lysosomes (pH 4.5-5).

The internalized antigens move from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a lower pH in each compartment.

Within the compartment, antigen is degraded into oligopeptides of about 13-18 residues.

The mechanism by which internalized Ag moves from one endocytic compartment to next has not been clearly demonstrated.

It has been suggested that early endosome move from periphery to inward to become late endosome and finally lysosomes.

Alternatively, small transport vesicles may carry Ag from one compartment to next.

STEP-I: HOW ARE PEPTIDES GENERATED?

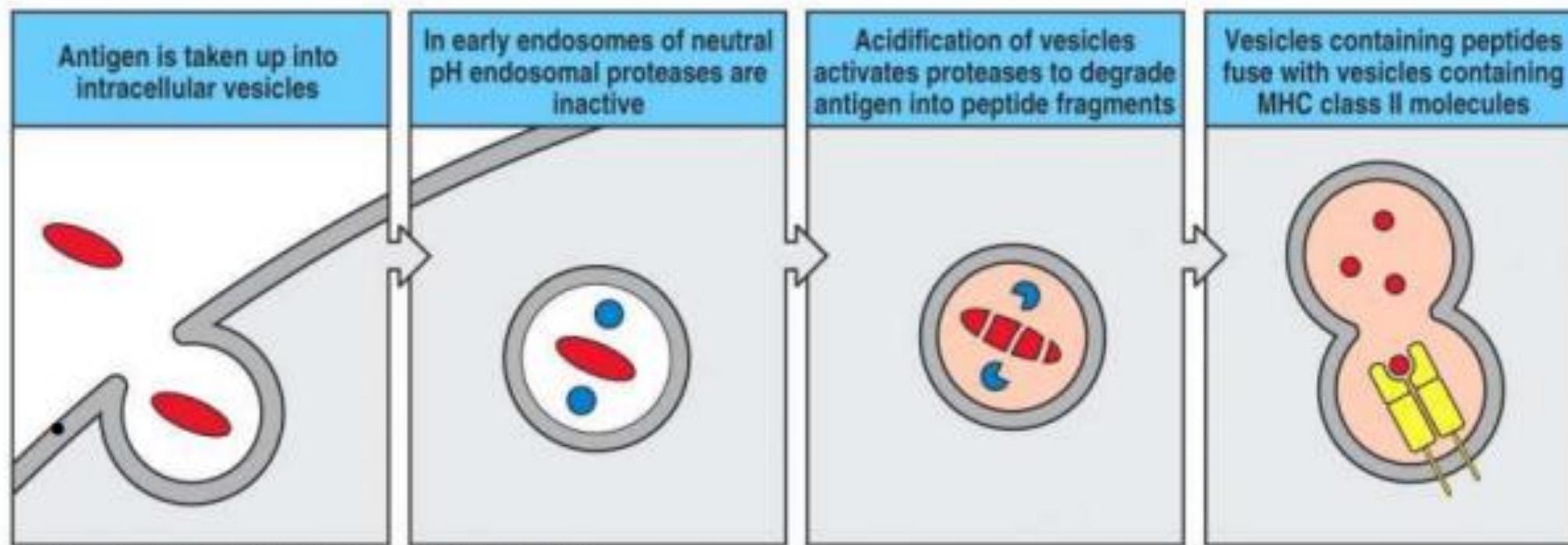


Figure 5-7 Immunobiology, 6/e. (© Garland Science 2005)

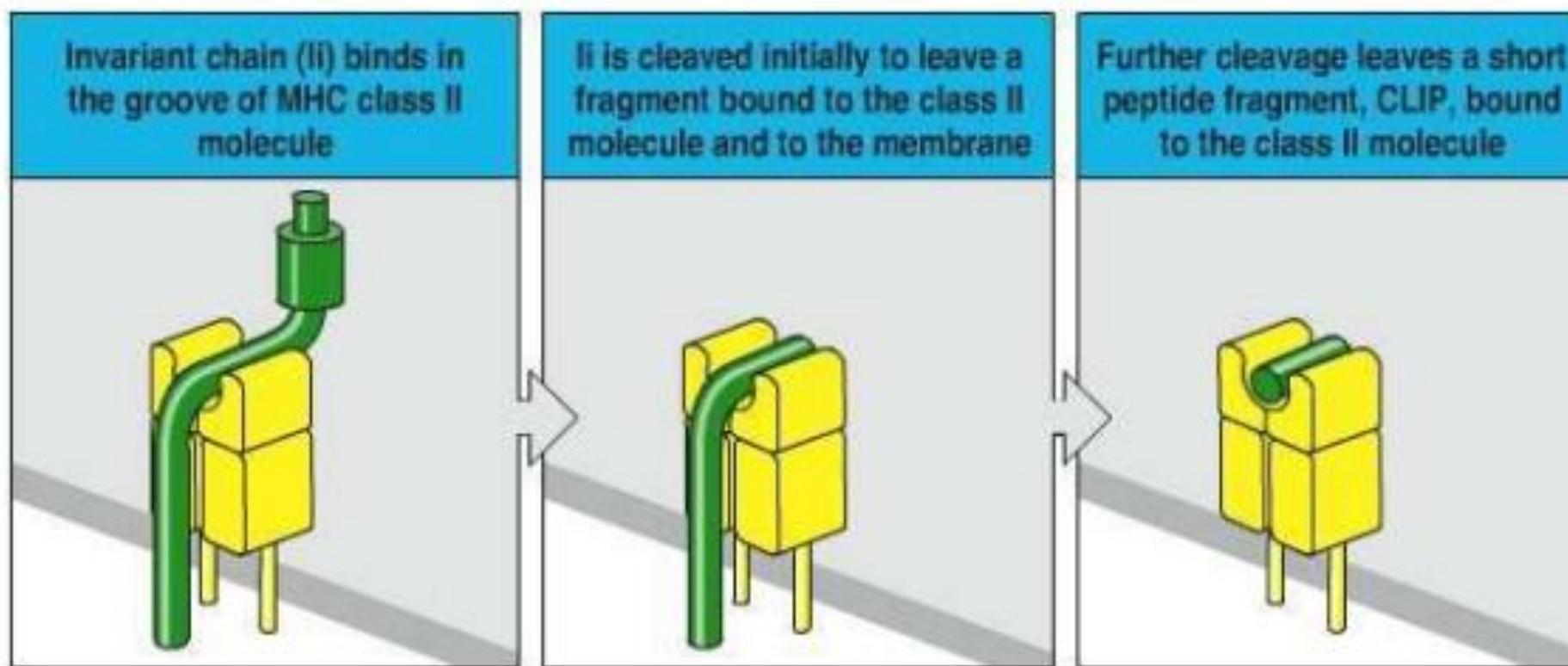
STEP-II: GENERATION OF MHC CLASS II MOLECULES

- Class-II MHC molecules consist of two trans-membrane polypeptides (α and β) and a third molecule nestled in the groove they form.
- All three components of this complex must be present in the ER for proper assembly.
- A protein called the invariant chain ("li") temporarily occupies the groove till the antigenic peptides are not transported.
- The steps:
 - The two chains α and β of the class II molecule associate into the membrane of the ER.
 - They bind one molecule of li in groove.
 - This trimolecular complex is transported through the Golgi apparatus and the trans golgi network into specialised vesicles.
 - These specialised vesicles deliver MHC class II to specialized compartments where peptide loading occurs

STEP-II: GENERATION OF MHC CLASS II MOLECULES

- Invariant chain (li) binds to Class II MHC molecules in ER to prevent endogenous peptide binding.
- Also, the invariant chain transports the MHC class II molecule from the Golgi apparatus to the endocytic compartments.
- Signals in the cytoplasmic tail of li lead to proper sorting of MHC class II.
- In the endocytic compartments li is cleaved to leave a peptide fragment (CLIP) in the binding groove.

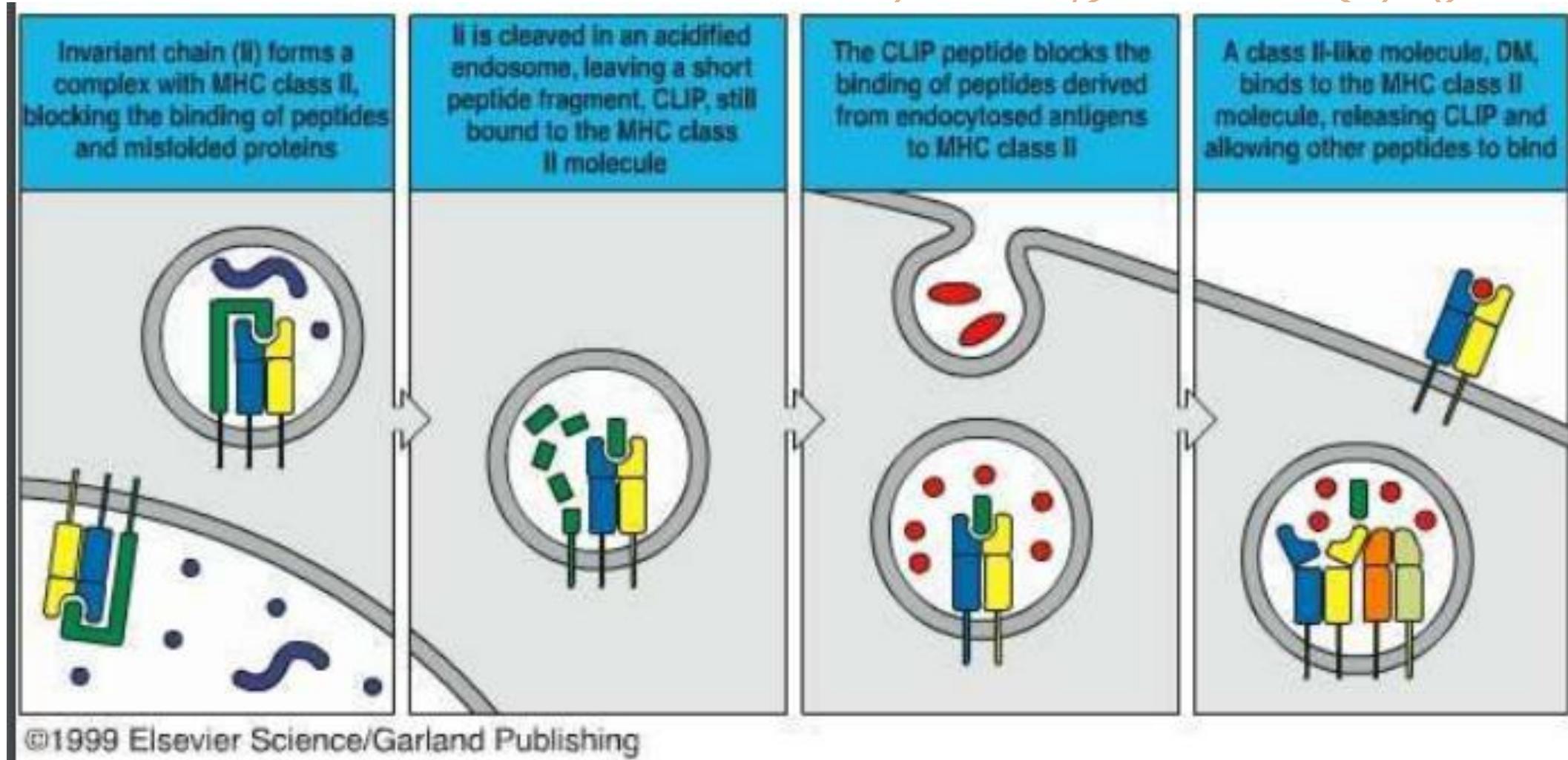
II IS CLEAVED TO LEAVE CLIP PEPTIDE IN CLASS II MHC GROOVE



STEP-III: CLASS II MHC PEPTIDE LOADING

- Class II MHC molecule with Ii is transported to endosomes where processed peptides are present for loading into its groove .
- In the endocytic compartment Ii is cleaved by proteases into a small fragment called as CLIP.
- CLIP prevents premature binding of peptides to MHC class II molecules.
- A non-classical MHC class II molecule, called MHC-DM, removes CLIP from the peptide-binding cleft and helps to load the antigenic peptide into the groove (gerotope) of nascent MHC class II molecule to form pMHC
- Acidic pH is required for exchange of peptides.

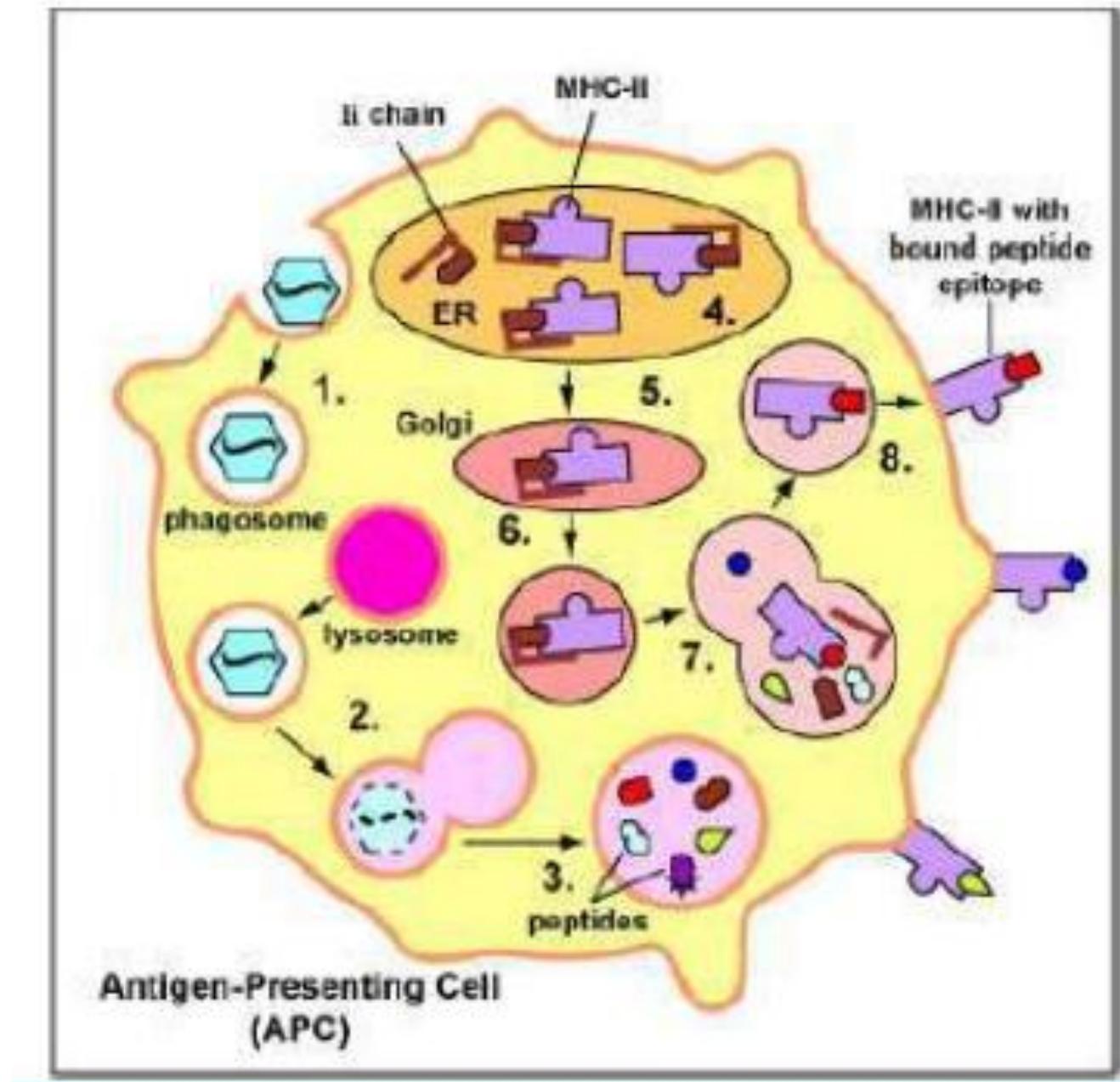
I.I CHAIN PREVENTS NEWLY SYNTHESIZED SELF PROTEINS FROM BINDING CLASS II MHC GROOVE UNTIL CLASS II MHC IS IN ENDOSOMES



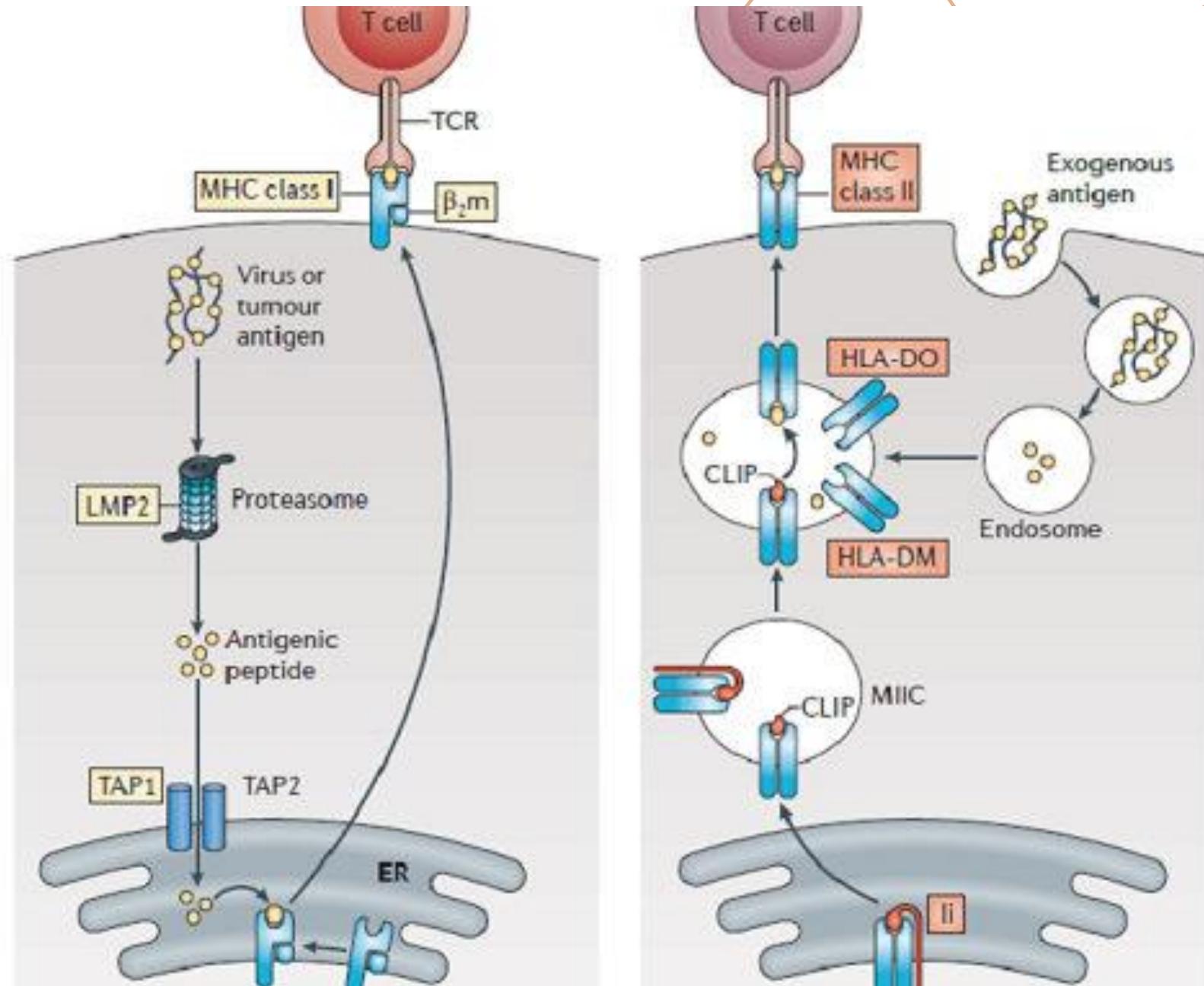
STEP-IV: MHC-PEPTIDE TRANSPORT

- The peptide loaded Class II MHC molecule – pMHC is transported into a membrane bound vesicle to the plasma membrane.
- The membrane of transport vesicle fuse with the cell membrane and pMHC complex bind to membrane and displayed at the cell surface
- It is presented to Th cells with appropriate TCR and CD4 molecules

SUMMARY



SUMMARY



Exogenous versus Endogenous pathways of Ag processing

Feature	Exogenous Pathway	Endogenous Pathway
Type of MHC	Class II	Class I
Source of Ag	Exogenous	Endogenous
Types of APC	DC, MO, B cells	All nucleated
Responsive T cell	CD4 T cells	CD8 T cells
Cellular compartment	Endosome	Cytosol
Enzymes responsible For peptide degradation	Endosomal and lysosomal proteases	Cytosolic proteasome
Molecules involved in Transport of peptides and Loading of MHC molecules	Invariant chain (Ii), HLA-DM	TAP

CHALLENGES TO THE IMMUNE SYSTEM

Sometimes a person may have an immune response even though there is no real threat.

This can lead to problems such as allergies, asthma, and autoimmune diseases. If you have an autoimmune disease, your immune system attacks healthy cells in your body by mistake.

Other immune system problems happen when your immune system does not work correctly.

These problems include immunodeficiency diseases. If you have an immunodeficiency disease, you get sick more often. Your infections may last longer and can be more serious and harder to treat. They are often genetic disorders.

There are other diseases that can affect your immune system. For example, HIV is a virus that harms your immune system by destroying your white blood cells. If HIV is not treated, it can lead to AIDS (acquired immunodeficiency syndrome). People with AIDS have badly damaged immune systems. They get an increasing number of severe illnesses.

FACTORS THAT AFFECT THE IMMUNE SYSTEM

Multiple factors like stress, age, body composition or our lifestyle can affect the performance of our immune system and any deviation from the normal pattern can affect our immunity.

Stress

When we're stressed, the body produces stress hormone corticosteroid or cortisol which decreases the body's ability to fight against infections making you more susceptible. Too much of stress can also lead to binge-eating on unhealthy snacks or consumption of alcohol which can lead to nutritional deficiencies and weaken your immunity.

Age

Our immune system's capacity declines as we get older, especially above the age of 70 years due to decrease in functioning of T-cells as a result of the degeneration of the thymus gland in the body which is the main site for T-cell production

FACTORS THAT AFFECT THE IMMUNE SYSTEM

Body Composition

Too much or too little body fat can lead to suppression of the immune system. Excess weight gain can put you at the risk of developing co-morbid conditions like type 2 diabetes, hypertension & heart disease. This can lead to a decrease in the body's ability to fight against infections due to a weak immune response.

Lifestyle Factors

A healthy diet and lifestyle results in a better immune function. Eating a balanced diet on a regular basis provides proper nourishment to the body and also prevents any vitamin & mineral deficiencies which may hinder the immune response. Some sort of regular physical activity also supports the immune system by increasing the number of fighter cells in the body. One must try to obtain adequate amounts of rest everyday in order to minimize the stress levels which can in turn affect our immunity.

FACTORS THAT AFFECT THE IMMUNE SYSTEM

Gut Flora

It is surprising to note that 70% of our immune system is dependent on our gut microbiome. Healthy gut bacteria prevent crowding of harmful bacteria in the intestine, produce lactic acid to stop their growth & work integrally with our immune system. Including fermented foods like curd, buttermilk, kefir, kombucha, kimchi etc in your diet will help support the growth of good gut bacteria.

Medications

Medications for autoimmune disorders, cancer, HIV or disorders with chronic inflammation like asthma, Crohn's disease¹, rheumatoid arthritis² etc can also limit the immune response and weaken the body's ability to fight against infections.

SIX TIPS TO ENHANCE IMMUNITY

Eat Well

Eating well means emphasizing plenty of fruits and vegetables, lean protein, whole grains, and fat-free or low-fat milk and milk products. Eating well also means limiting saturated fats, cholesterol, salt, and added sugars. Eating well provides multiple nutrients that support optimal immune function.

Be Physically Active

Regular physical activity helps you feel better, sleep better, and reduce anxiety. Combined with eating well, physical activity can help a person maintain a healthy weight.

Get Enough Sleep

Scientific evidence is building that sleep loss can negatively affect different parts of the immune system. This can lead to the development of a wide variety of disorders.

SIX TIPS TO ENHANCE IMMUNITY

Quit Smoking

Smoking can make the body less successful at fighting disease. Smoking increases the risk for immune system problems, including rheumatoid arthritis.

Avoid Too Much Alcohol

Over time, excessive alcohol use can weaken the immune system.

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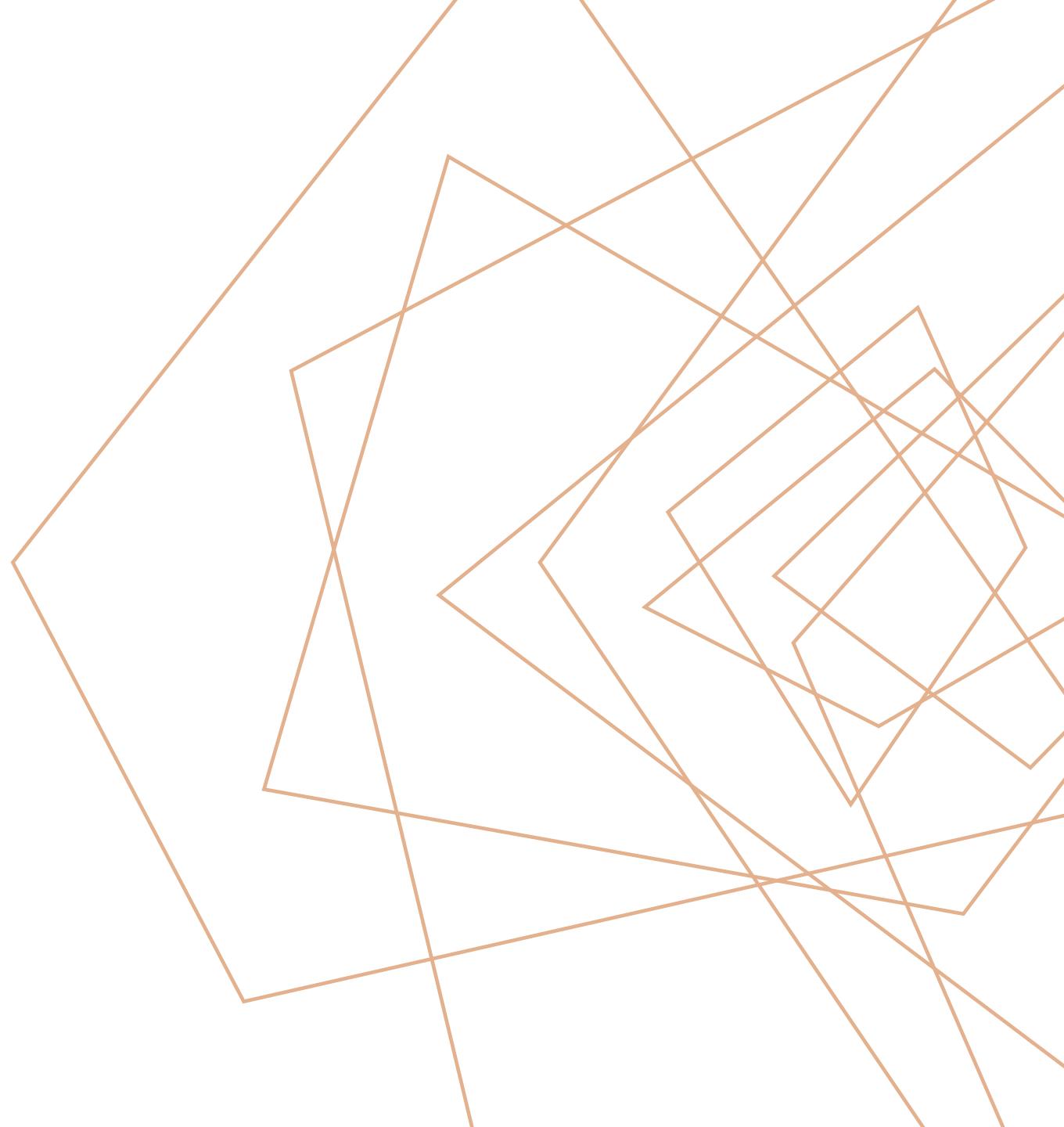
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2. <https://www.biology-pages.info/A/AntigenPresentation.html>
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THANK YOU





MISCELLANEOUS MATERIAL

Antigen processing and Antigen presentation

- **Antigen processing** is a metabolic process that digests the proteins into peptides which can be displayed on the cell membrane together with a class-I or class-II MHC molecules and recognized by T-cells.
 - **Antigen presentation** is the process by which certain cell in the body especially antigen presenting cells (APCs) express processed antigen on their cell surface along with MHC molecules in the form recognizable to T cell.
 - If antigen is presented along with class-I MHC molecule, it is recognized by CD8⁺ Tc-cell and if presented along with class-II MHC molecule, it is recognized by CD4⁺ TH cells.
- On the basis of types of antigen to be processed and presented, antigen processing and presenting pathway are of two types:

Cytosolic pathway of antigen processing and presentation

- Cytosolic pathway processed and presented the endogenous antigens i.e. those generated within cell eg. Viral infected cells, tumor cells and intracellular pathogens (*M. tuberculosis*, *Histoplasma capsulatum*).
- The processed antigen is presented on the cell membrane with MHC-class I molecule which is recognized by CD8⁺ Tc-cell for degradation.

Steps involved in cytosolic pathways are:

- Proteolytic degradation of Ag (protein) into peptides
- Transportation of peptides from cytosol to RER
- Assembly of peptides with class I MHC molecules

- **Proteolytic degradation of proteins into peptides:**

- Intracellular proteineous antigen are larger in size to be bound to MHC molecule.
- So, it is degraded into short peptides of about 8-10 amino acids.
- These proteins are degraded by cytosolic proteolytic system present in cell called proteasome.
- The large (20S) proteasome is composed of 14 sub-units arranged in barrel-like structure of symmetrical rings.
- Some, but not all the sub-units have protease activity.
- Proteins enter the proteasome through narrow channel at each end.
- Many proteins targeted for proteolysis have a small protein called ubiquitin attached to them.
- Ubiquitin attached to them ubiquitin-protein complex consisting of 20S proteasome and 19S regulatory component added to it.
- The resulting 26S proteasome cleaves peptide bonds which is ATP-dependent process.
- Degradation of ubiquitin protein complex is thought to occur within the central hollow of the proteasome to release peptides.

- **Transportation of peptides from cytosol to Rough Endoplasmic Reticulum (RER):**

- Peptides generated in cytosol by proteasome are transported by TAP (transporter associated with antigen processing) into RER (Rough endoplasmic reticulum) by a process which require hydrolysis of ATP.
- TAP is membrane spanning heterodimer consisting of two proteins, TAP1 and TAP2.
- TAP has affinity for peptides having 8-16 amino acids.

- The optimal peptide length required by class-I MHC for binding is nine, which is achieved by trimming the peptides with the help of amino-peptidase present in RER. Eg. ERAP.
- In addition to it, TAP favor peptides with hydrophobic or basic carboxyl terminal amino acids, that preferred anchor residues for class-I MHC molecules.
- TAP deficiency can lead to a disease syndrome that has both immune-deficiency and auto-immunity aspects.

• Assembly of peptides with class-I MHC molecule:

- Like other proteins, the α -chain and $\beta 2$ microglobulin components of the class-I MHC molecule are synthesized on polysome along the rough endoplasmic reticulum.
- Assembly of these components into stable class-I MHC molecule that can exit the RER require binding of peptides into peptide binding groove of class-I MHC molecules.
- The assembly process involves several steps and needs help of molecular chaperone.
- The first molecular chaperone involved in assembly of class-I MHC is calnexin.
- It is a resident membrane protein of RER.
- Calnexin associated with free class-I α -chain and promotes its folding.
- When $\beta 2$ -microglobulin binds class-I α -chain, calnexin is released and class-I MHC associates with another chaperone calreticulin and tapasin (TAP-associated protein).

- Tapasin brings TAP transporter carrying peptides to the proximity with class-I MHC molecule and allows to acquire the antigenic peptides.
- An additional protein with enzymatic activity, ERp57, form disulfide bond to tapasin and non-covalently associates with calreticulin to stabilize the interaction and allows release of MHC-I-class after acquiring antigenic peptides.
- As a consequence, the productive peptide binding with MHC of class-I releases from the complex of calreticulin, tapasin and ERp57, exit from RER and displays on the cell surface via golgi complex.

Endocytic pathway of antigen processing and presentation:

- The endocytic pathway processes and presents the exogenous Ag. i.e. antigens generated outside the cells. E.g. Bacteria.
- At first APC phagocytosed, endocytosed or both, the antigen.
- Macrophage and dendritic cells internalize the antigen by both the process.
- While other APCs are non-phagocytic or poorly phagocytic. E.g. B cell internalizes the antigen by receptor-mediated endocytosis.
- Then antigen is processed and presented on the cell surface along with class-II MHC molecules which are recognized by CD4⁺ TH cell.

• Steps involved in endocytic pathway:

- Peptide generation from internalized molecules (Ag) in endocytic vesicles.
 - Transport of class-II MHC molecule to endocytic vesicles.
 - Assembly of peptides with Class-II MHC molecules.
-
- i. Peptide generation from internalized molecules (Ag) in endocytic vesicles:
 - Once an antigen is internalized, it is degraded into peptides within compartments of endocytic processing pathway.
 - The endocytic pathway appears to involve three increasingly acidic compartments, early endosomes (pH 6-6.5), late endosomes or endo-lysosome (pH 5-6) and lysosomes (pH 4.5-5).

- The internalized antigens move from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a lower pH in each compartment.
- Within the compartment, antigen is degraded into oligopeptides of about 13-18 residues.
- The mechanism by which internalized Ag moves from one endocytic compartment to next has not been clearly demonstrated.
- It has been suggested that early endosome move from periphery to inward to become late endosome and finally lysosomes.
- Alternatively, small transport vesicles may carry Ag from one compartment to next.

• Transport of class-II MHC molecule to endocytic vesicles:

- When class-II MHC molecules are synthesized within RER, three pairs of class-II $\alpha\beta$ -chains associated with a pre-assembled trimer of a protein called invariant chain (Li, CD74).
- This trimeric protein prevents any endogenously antigen to bind to the cleft.
- The invariant chain consists of sorting signals in its cytoplasmic tail.
- It directs the transport of class-II MHC molecule to endocytic compartments from the trans-golgi network.

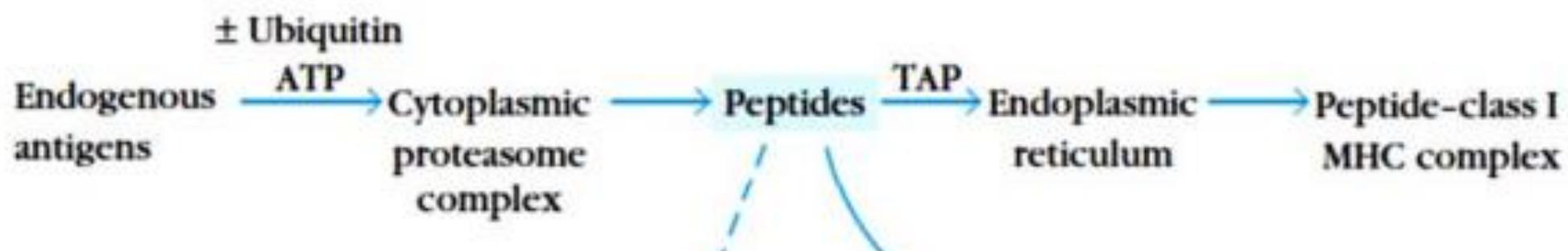
- **Assembly of peptides with class-II MHC molecules:**

- Class-II MHC-invariant chain complexes are transported from RER through golgi complex and golgi-network and through endocytic compartment, moving from early endosome to late endosome and finally to lysosome.
- The proteolytic activities increase in each compartment, so the invariant is slowly degraded.
- However, a short fragment of invariant chain remained termed as CLIP (Class-II associated invariant chain).
- CLIP physically occupies the peptide binding, cleft of class-II MHC molecule, presumably preventing any premature binding of antigenic peptides.
- A non-classical class-II MHC molecule known as HLA-DM is required to catalyze the exchange of CLIP with antigenic peptides.
- The reaction between HLA-DO, which binds to HLA-DM and lessens the efficiency of the exchange reactions.
- Conditions of higher acidity in endocytic compartment weakens the association of DM/DO and increase the possibility of antigenic peptide binding despite of DO.
- As with class-I MHC molecule, peptide binding is required to maintain the structure and stability of class-II MHC molecules.
- Once a peptide has bound the peptide-class II MHC complex is transported to the plasma membrane where neutral pH enables the complex to assume the compact and stable form.

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CYTOSOLIC PATHWAY



ENDOCYTIC PATHWAY



1. Endogenous Antigens: The Cytosolic Pathway

DIFFERENCE BETWEEN

INNATE IMMUNITY

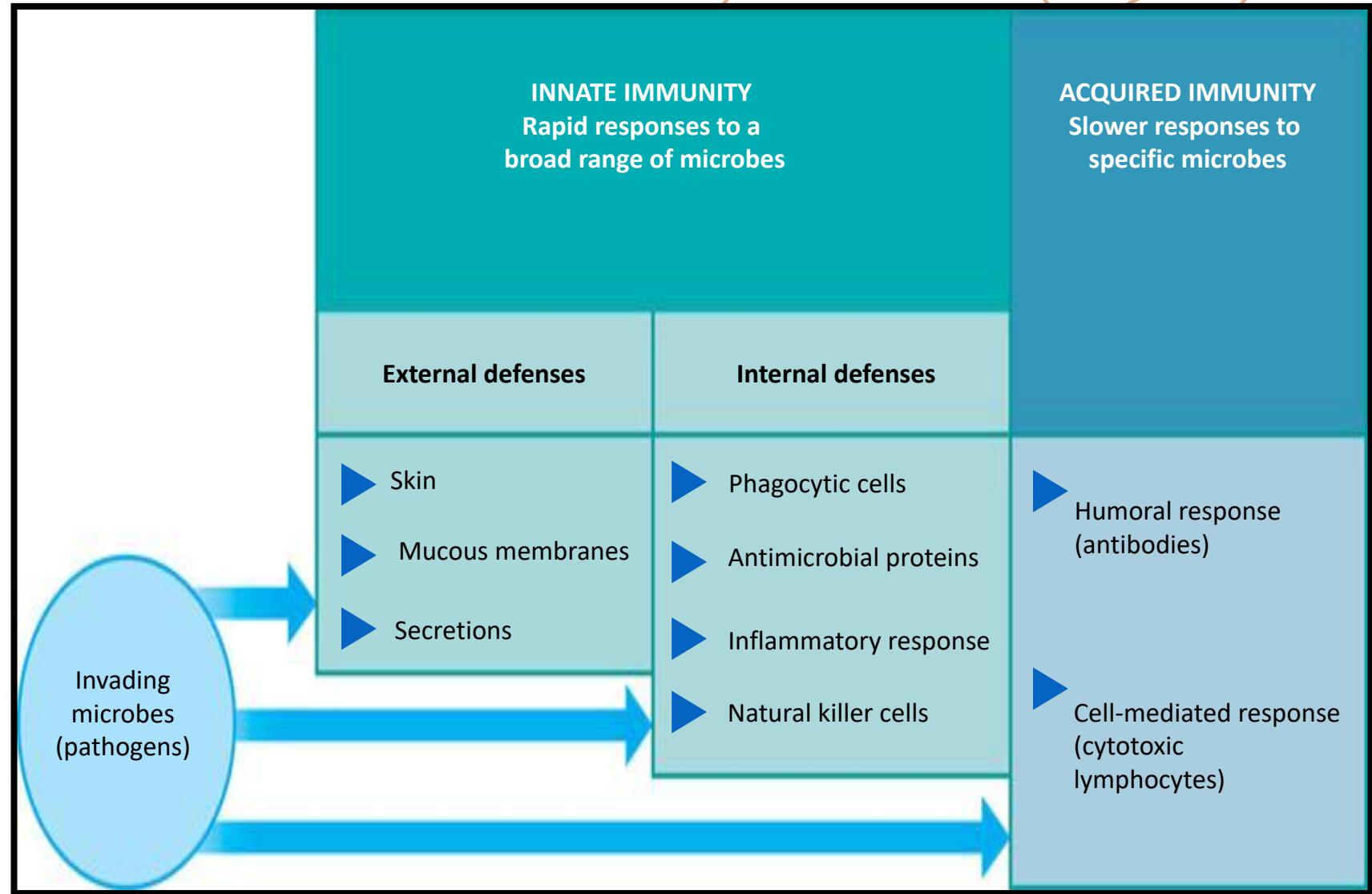
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ADAPTIVE IMMUNITY

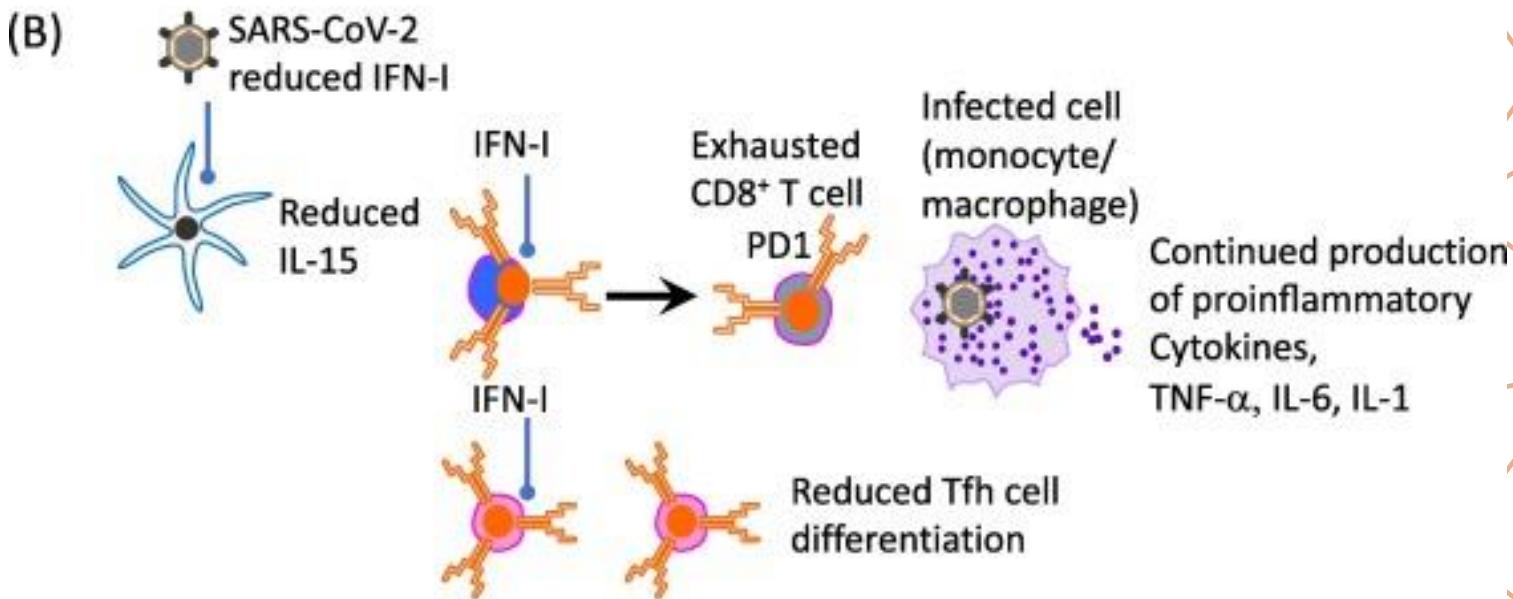
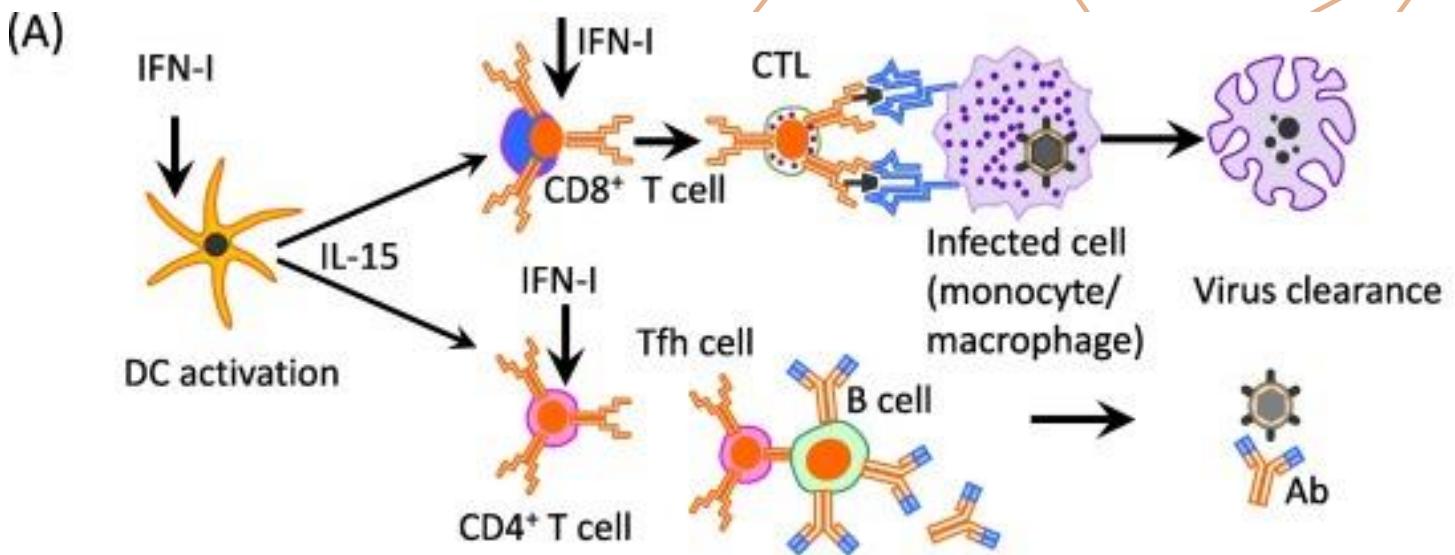
Innate immunity refers to a naturally occurring immunity by the genetic constituents and the physiology of a person	Adaptive immunity refers to an acquired immunity, mediated by T cells and B cells and characterized by an immunological memory	Temperature, pH, skin, and mucous membranes are the barriers	Lymph nodes, spleen, and lymphoid tissues are the barriers
Known as natural immunity	Known as acquired immunity	Does not develop memory cells	Develops memory cells
Generates a non-specific immune response	Generates a specific immune response	Possesses a less diversity	Possesses a higher diversity
Always present in the body	Generated in response to exposure to an external factor	Less potent	Exhibits a higher potency
Generates a rapid response	Delayed 5-6 days	Does not produce allergic reactions	Develops allergic reactions; immediate and delayed hypersensitivity
Plasma proteins, phagocytes, physical and chemical barriers are the components	Humoral and cell-mediated immunity are the components	Ex: Redness and swelling caused by the white blood cells around a wound	Ex: Vaccination against a virus

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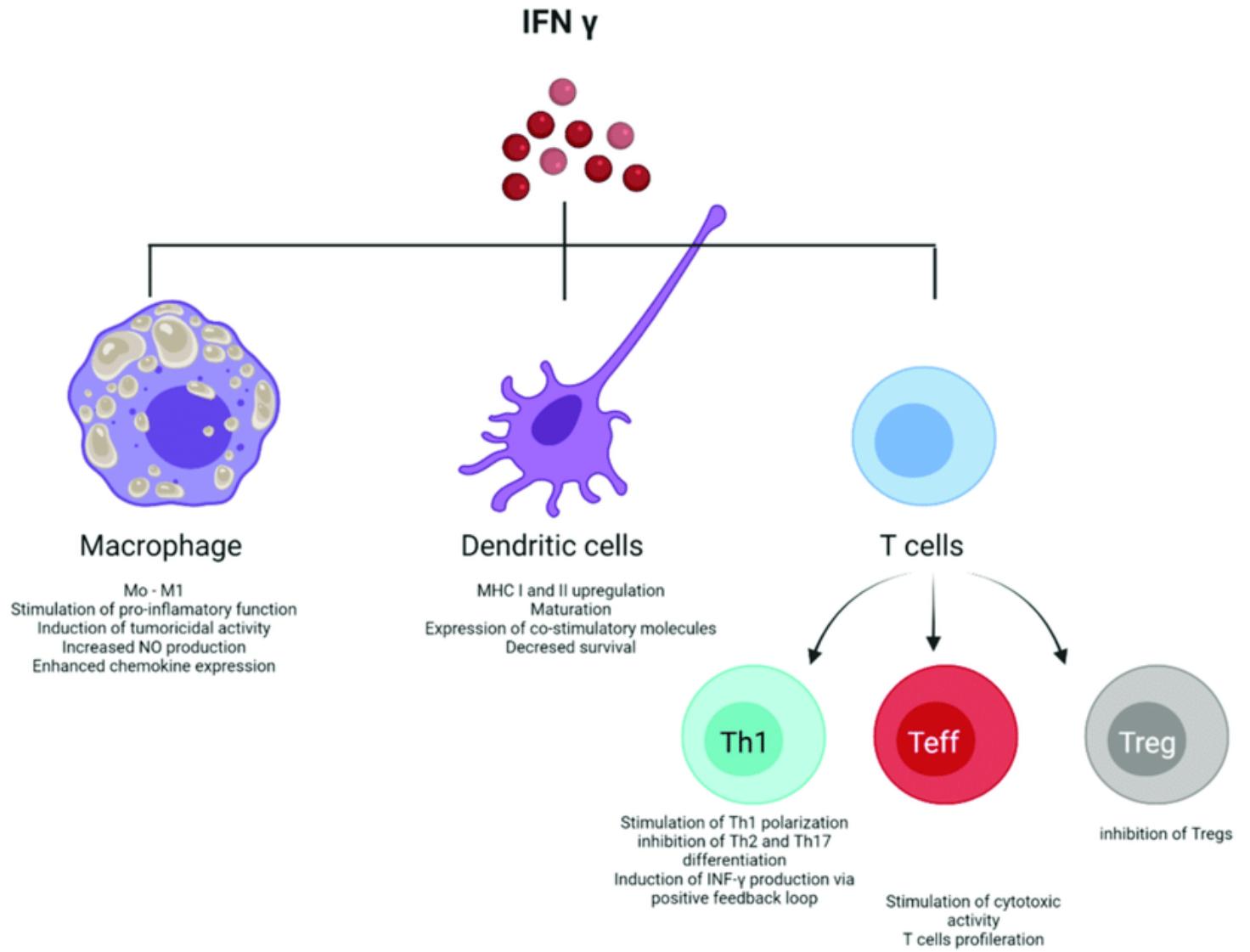
IMMUNE RESPONSE



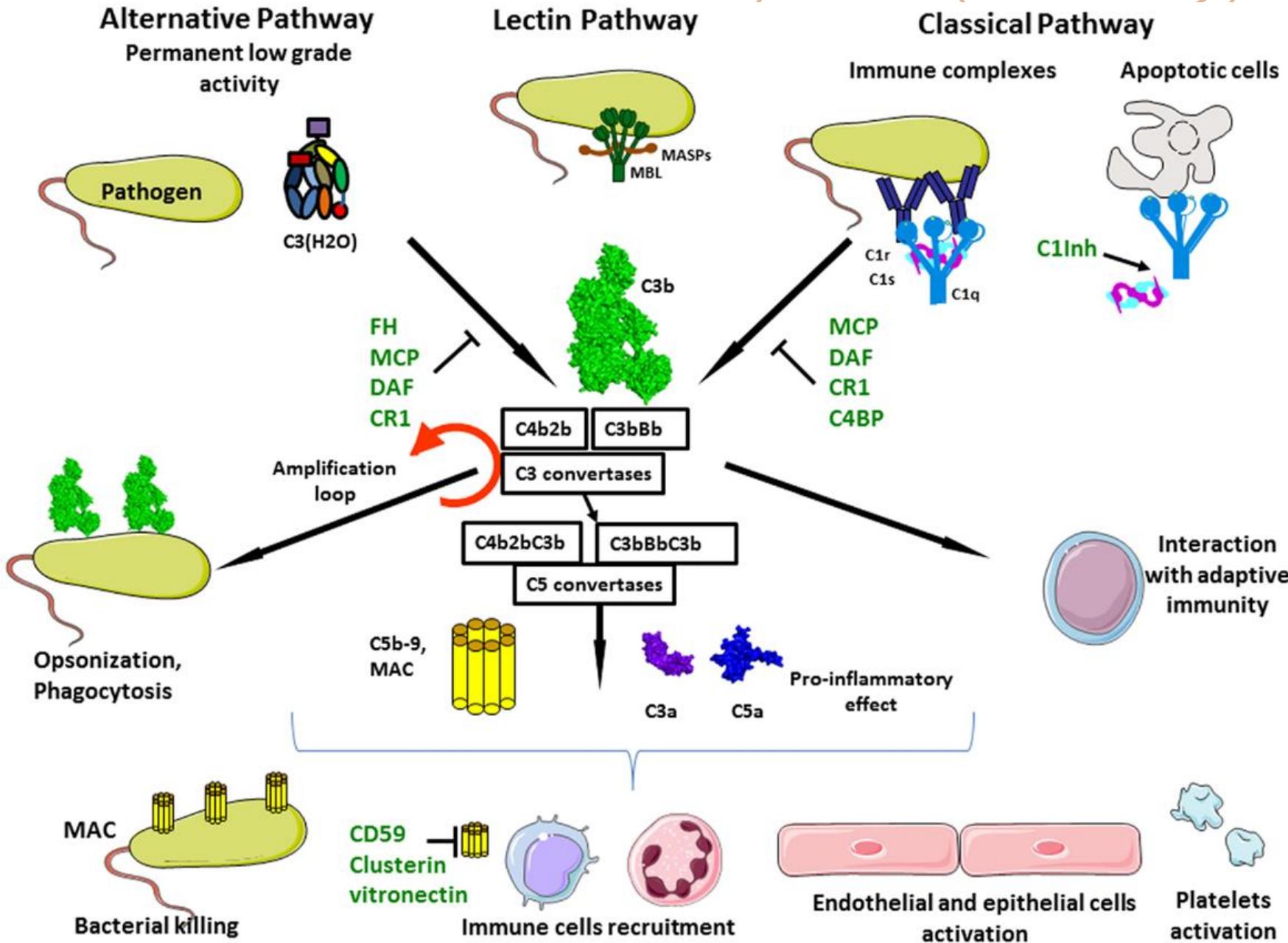
ROLE OF IFNS IN SARS



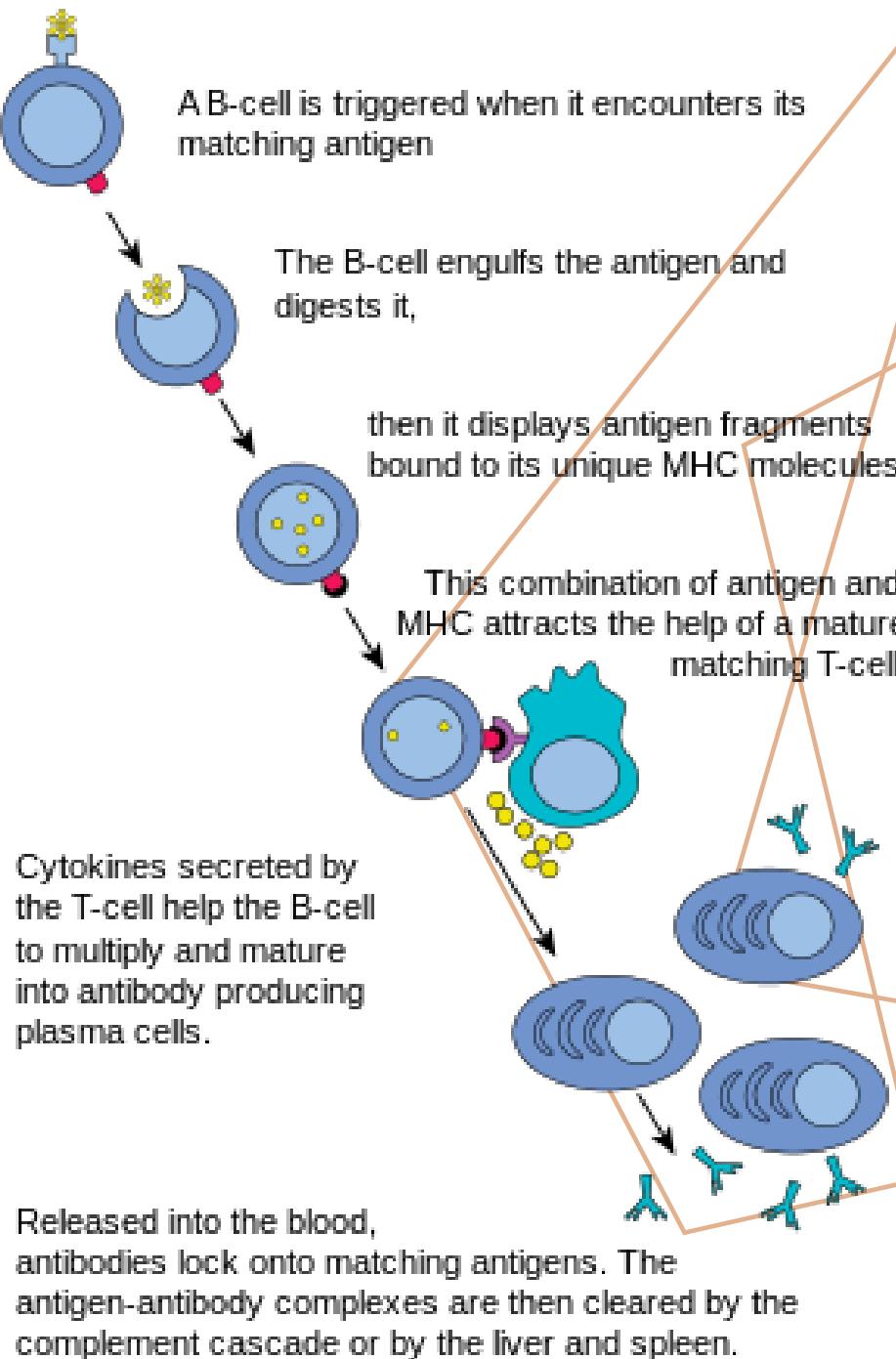
IFN

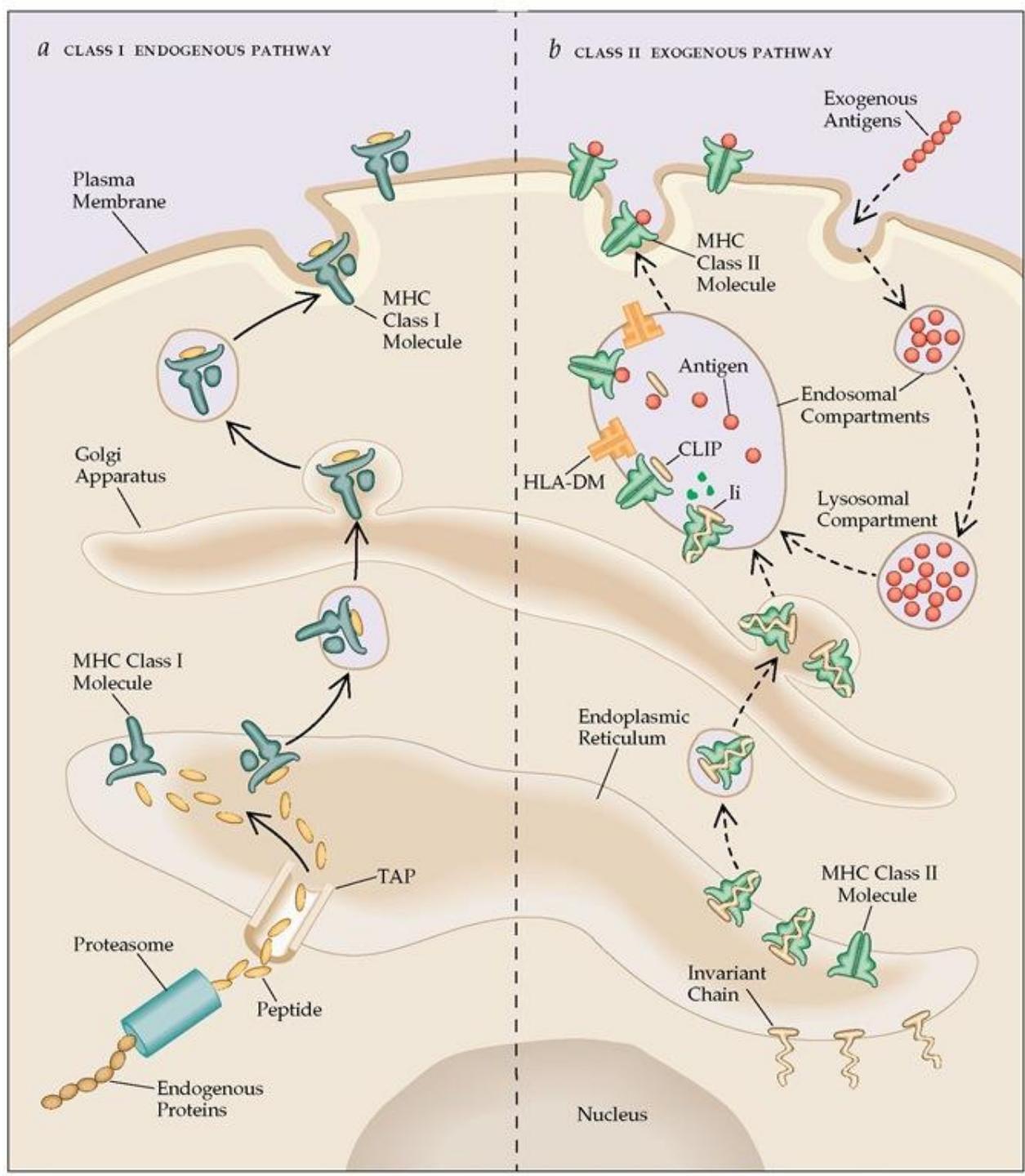


COMPLEMENT PATHWAYS



HUMORAL RESPONSE

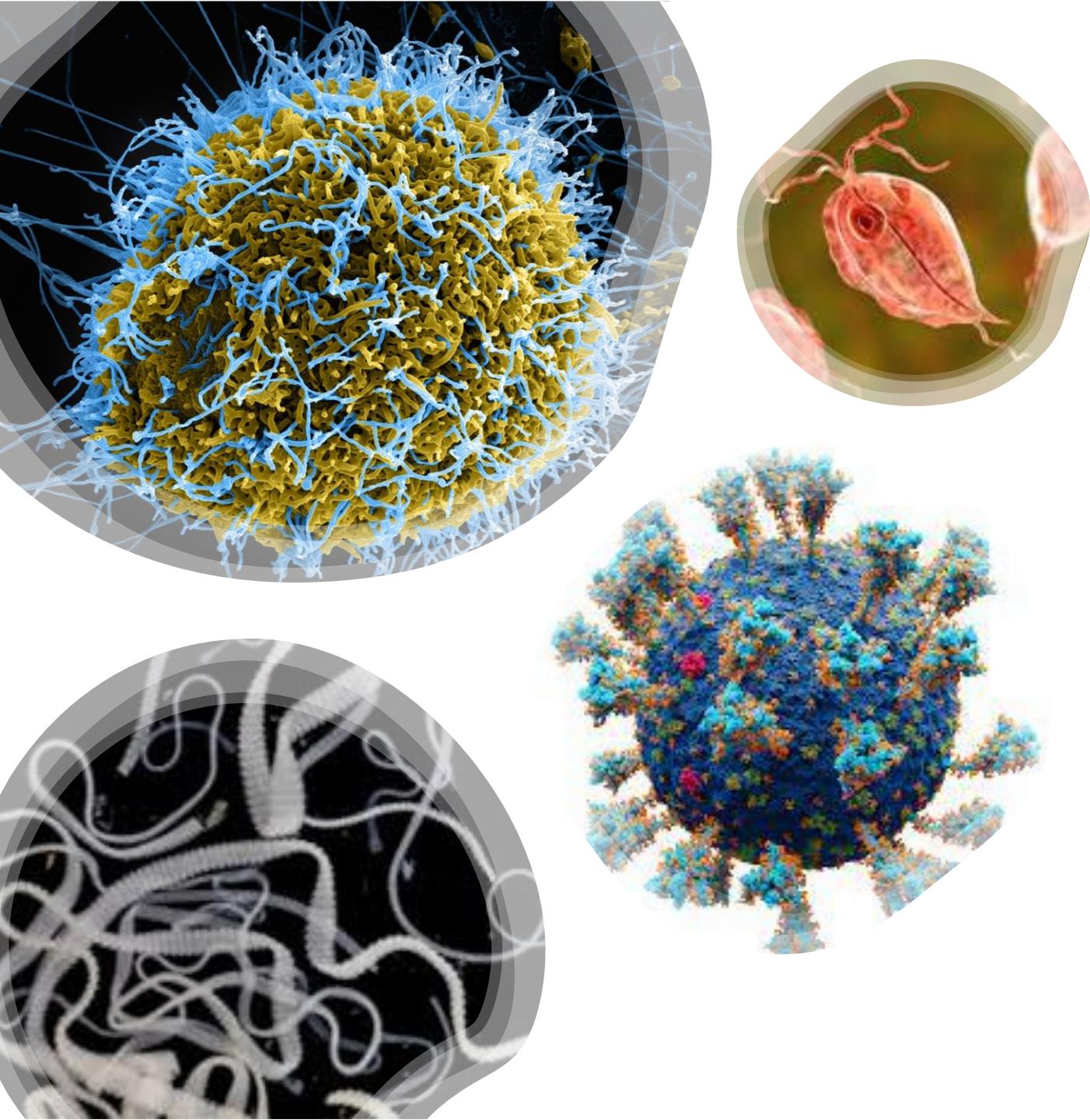




Infectious Diseases

Dr. (Ms.) Sonali Correa
M.Sc., Ph.D.



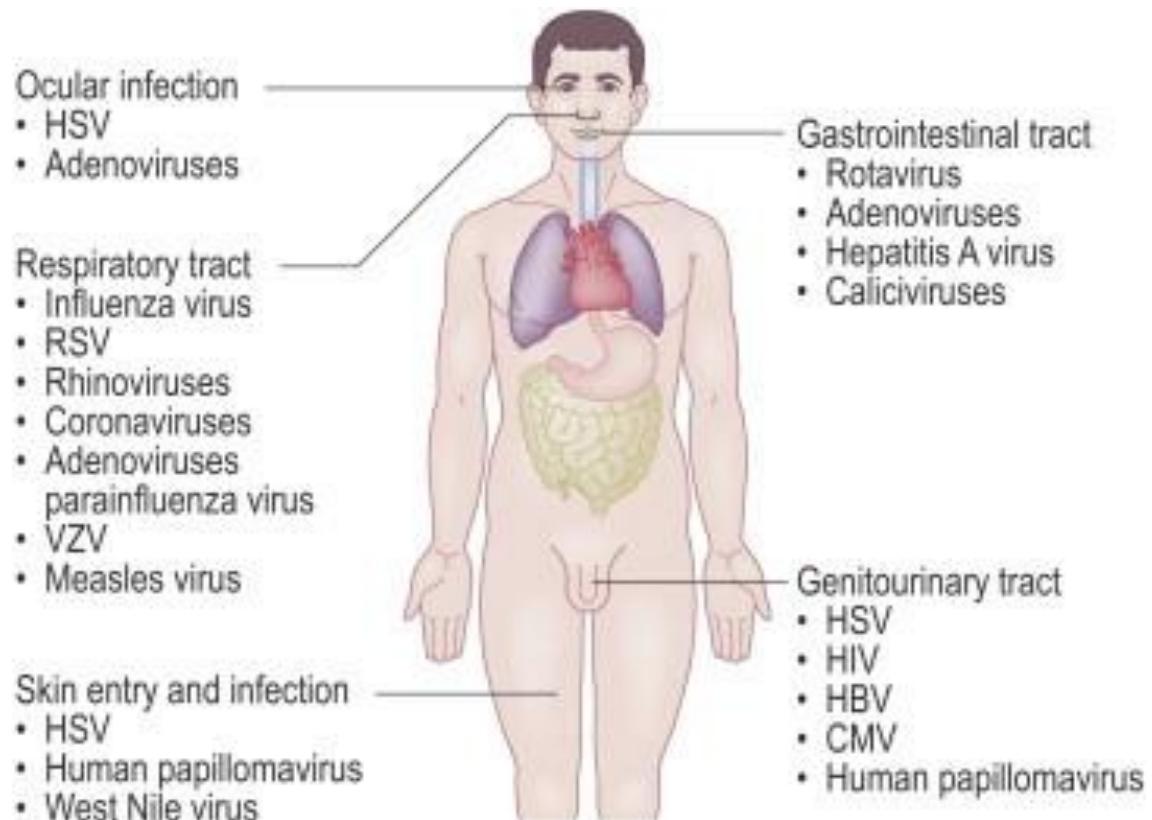


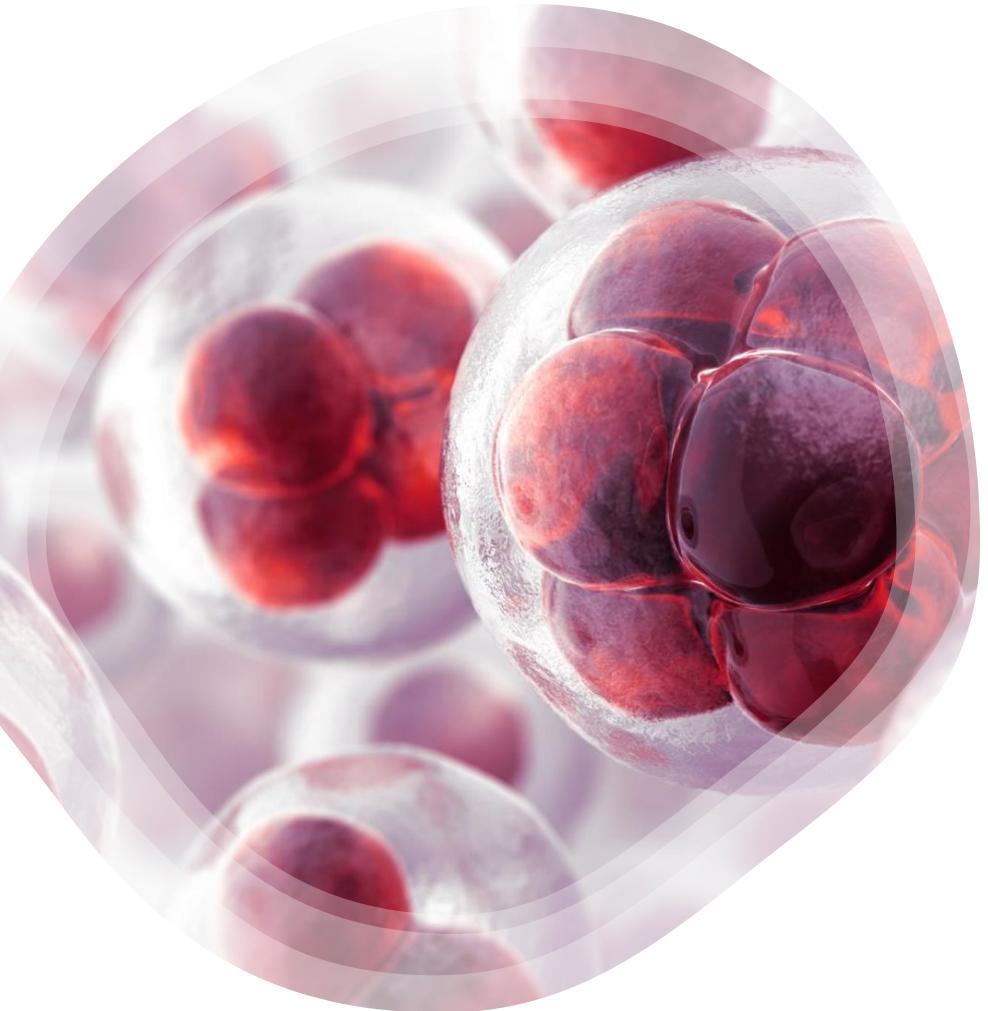
Introduction

- Infectious diseases are illnesses caused by harmful agents (pathogens) that get into your body.
- The most common causes are
 - viruses,
 - bacteria,
 - protozoa
 - Helminths
- Infectious diseases usually spread from person to person, through contaminated food or water and through bug bites. Some infectious diseases are minor and some are very serious.

Viral infection

- Access to target tissues presents numerous obstacles for entry and infection by most human viruses.
- Most effective of these are the mechanical barriers provided by the skin and mucosal surfaces, as well as the chemically hostile environment of the gut
- What are the types of viral infections?
- Some viruses, like herpes viruses and adenoviruses, can cause many different types of illness.
- Types of viral infections include:
- Respiratory infections.
- Digestive system infections.
- Viral hemorrhagic fevers.
- Sexually transmitted infections (STIs).
- Exanthematous (rash-causing) infections.
- Neurological infections.
- Congenital infections.





INNATE IMMUNITY TO VIRUSES

- Viral infection induces an extensive array of defense mechanisms in the host.
- Innate defenses come into play to block or inhibit initial infection, to protect cells from infection, or to eliminate virus-infected cells, and occur well before the onset of adaptive immunity
- The innate immune defenses are initiated via pathogen recognition receptors of the Toll-like receptor (TLR) family or a family of DExD/H box RNA helicases
- These cellular sensors promote the expression of type I (α/β) interferons (IFN) and a variety of IFN-stimulated genes and inflammatory cytokines.
- TLRs are cell surface or endosomal membrane-bound proteins expressed by numerous cells including dendritic cells (DC), macrophages, lymphocytes, and parenchymal cells
- Expression of TLRs is largely inducible in most cell types, though some (TLR7/8/9) are constitutively expressed at high levels by specialized plasmacytoid DC for rapid IFN production.

Key Concepts: Major Antiviral Innate Defense Mechanisms

- **Acting to block infection:**
 - Natural antibodies
 - Complement components
 - Some chemokines
- **Acting to protect cells from infection:**
 - Interferon- α/β
 - Interferon- γ
- **Acting to destroy or inhibit virus-infected cells:**
 - Natural killer cells
 - NKT cells
 - Macrophages
 - Neutrophils
 - $\gamma\delta$ T cells
 - Nitric oxide
- **Involved in regulating antiviral inflammatory response:**
 - Interleukins-1, 6, 10, 12, 18, 23
 - Transforming growth factor- β
 - Chemokines

- Different TLR molecules recognize specific viral products such as single- and double-stranded RNA (TLR 3 and TLR7/8, respectively) or double-stranded DNA (TLR9). The more recently described non-TLR RNA helicases, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene (MDA-5), mediate cytoplasmic recognition of viruses.
- It is thought that other cytoplasmic sensors of viruses are also likely to exist such as the recently discovered cytosolic dsDNA sensor DAI (DNA-dependent activator of IFN).
- The innate defense system consists of multiple cellular components and many specialized proteins.
- The longest-known and best-studied antiviral proteins are the α/β IFNs, which act by binding to the type I IFN receptor and result in the transcription of more than 100 IFN-stimulated genes. One consequence of this ‘antiviral state’ is the inhibition of cell protein synthesis and the prevention of viral replication.
- Type I IFNs also activate natural killer (NK) cells and induce other cytokines such as interleukin (IL)-12 that promote NK responses.
- NK cells produce proinflammatory cytokines, they can kill infected cells and interact with DC, and are an important component of innate defense against viruses

- NK cells are regulated by an array of activating and inhibitory receptors whose expression and function are just beginning to be understood.
- Uninfected cells are usually protected from NK cell cytolysis as they deliver negative signals such as high expression of MHC molecules.
- In contrast, virus-infected cells are killed either because they deliver positive signals or because they lack adequate MHC-negative signals.
- The NK defense system appears important against some herpes viruses, which downregulate MHC expression in the cells they infect.
- NK cells are also important in resistance to mouse and human cytomegalovirus, and possibly to HIV, influenza virus, and Ebola viruses.
- A distinct NK cell population, NKT cells, may provide some antigen-specific innate immune protection against certain viruses.
- Many other leukocytes are involved in innate defense, including macrophages, DC, neutrophils and perhaps T cells expressing $\gamma\delta$ T-cell receptors for antigen
- In addition to IFN- α/β , several other host proteins function in antiviral defense.
- These include natural antibody, which may play a role in defense against some virus infections, as well as the complement proteins

- Some viruses may be directly inactivated by complement activation or be destroyed by phagocytic cells that bind and ingest complement-bound virions.
- Several cytokines and chemokines induced by virus infection also play a role in defense. These include the cytokines TNF- α , IFN- γ , IL-12, IL-6, and chemokines such as MIP-1 α . In particular, IL-12 is a potent inducer of IFN- γ from NK cells
- Inflammatory chemokines may also play an important role in innate antiviral defense by orchestrating macrophage, neutrophil, DC, and NK responses at the site of infection
- Not only are these components of innate immunity involved in mediating initial protection against viruses, several components (such as the TLRs and type I IFN and IL-12) serve to shape the nature and effectiveness of the subsequent adaptive response to viral antigens.

Key Concepts: Principles of Antiviral Immunity

- Many human viral infections are successfully controlled by the immune system
- Certain emerging viruses may overwhelm the immune system and cause severe morbidity and mortality
- Other viruses have developed mechanisms to overwhelm or evade the immune system and persist
- Individuals with defects in innate or adaptive immunity demonstrate more severe viral infections
- T-cell immunity is more important for control than antibody with many viral infections
- Antibody is important to minimize reinfection, particularly at mucosal sites
- Immune memory is often sufficient to prevent secondary disease, though not in all viral infections

ADAPTIVE IMMUNITY TO VIRUSES

- The two major divisions of adaptive immunity, antibody and T-cell-mediated, are mainly directed at different targets.
- Antibodies usually function by binding to free viral particles, and in so doing block infection of the host cell.
- In contrast, T cells act principally by recognizing and destroying virus-infected cells
- As all viruses replicate within cells and many of them spread directly between cells without re-entering the extracellular environment, resolution of infection is reliant more on T-cell function than on antibody.
- Antiviral antibody, however, does assume considerably more importance as an additional immunoprotective barrier against reinfection.
- It is the presence of antibody at portals of entry – most often mucosal surfaces – that is of particular relevance to influenza and HIV infections. Accordingly, vaccinologists try to design vaccines that optimally induce mucosal antibody.

ADAPTIVE IMMUNITY TO VIRUSES

- Initiation of adaptive immunity is closely dependent upon early innate mechanisms that activate antigen-presenting cells (APC).
- APC and lymphocytes are drawn into lymphoid tissues by chemokine and cytokine signals and retained there for a few days in order to facilitate effective interactions between these cells.
- The architecture of the secondary lymphoid tissues supports the coordinated interactions between cells of the adaptive immune system through a network of supportive stromal cells and local chemokine gradients.
- The **induction events occur** in lymph nodes draining the infection site, or in the spleen if virus enters the bloodstream. The passage of viral antigens to lymph nodes usually occurs in DCs.
- Some viruses are able to compromise the function of APC, such as HSV and measles virus, which can inhibit DC maturation.
- B-cell activation occurs following antigen encounter in the B-cell follicles, and possibly the T-cell zones, in the spleen or lymph nodes
- Some activated B cells become short-lived plasma cells while others move the edges of the B-cell follicles and interact with antigen-specific helper CD4 T cells via presentation of antigenic peptides on B-cell MHC class II molecules

ADAPTIVE IMMUNITY TO VIRUSES

- These activated B cells initiate germinal center (GC) reactions, which ensure somatic **hypermutation and affinity maturation for the selection of high-affinity, antibody-producing long-lived plasma cells as well as memory B cells**
- Antibody binding to epitopes expressed by native proteins at the surface of free virions usually blocks viral attachment or penetration of target cells.
- Sometimes the consequence is viral lysis (with complement proteins also involved), opsonization, or sensitization for destruction by Fc receptor-bearing cells that mediate antibody-dependent cellular cytotoxicity (ADCC).
- Fc receptor binding of antibody-bound virus may facilitate infection and result in more severe tissue damage. This occurs in dengue fever and may happen in some instances in HIV infection
- Like B-cell responses, T-cell responses to viral infections also begin within the lymphoid tissues. Specific CD8 cytotoxic T lymphocyte (CTL) precursors recognize antigen in the context of MHC class I-peptide antigen complexes on professional APC, such as DC.
- The CD8 T cells become activated, proliferate, and differentiate into effectors.

ADAPTIVE IMMUNITY TO VIRUSES

- Expansion of these naïve antigen-specific precursors is considerable, often exceeding 10 000-fold, and results in an effector population that can account for 40% or more of a host's total CD8 T-cell population
- Various factors, including antigen and APC, co-stimulatory molecules (such as CD28 and 4-1BB) and inflammatory cytokines (such as IFN- α/β and IL-12) are required to program the development of functional effector lymphocytes.
- The CTL effectors enter the efferent lymph and bloodstream and access almost all body locations, including both primary and subsequent sites of infection.
- Effectors do not stay activated for long once the virus is cleared, and approximately 95% die by a process termed activation-induced cell death.
- Following this contraction phase, the remaining cells differentiate into memory cells, which remain as a more or less stable population in the host for many years.
- They represent an expanded pool of CTL precursors that can be activated upon secondary encounter with antigen, and provide enhanced protection upon reinfection with the same virus.

ADAPTIVE IMMUNITY TO VIRUSES

- T-cell immunity against a particular virus commonly involves both CD4 and CD8 T-cell subsets. Both CD4 and CD8 T cells recognize peptides derived from viral antigens bound to surface MHC proteins (class II and class I, respectively)
- Complexes of viral peptides bound to MHC class II proteins are generated by APC from scavenged and processed virus-infected cells or viral particles.
- Antigen–MHC class I complexes are expressed on the surface of infected cells, and antigen can also be transferred to APC from infected cells by a process known as cross-priming.
- Recent experiments in mice have also demonstrated a role for transfer of antigen between DC18 as they migrate from infected tissues to the lymphoid tissues.
- Curiously, although many peptides derived from viral proteins have an appropriate motif that permits MHC binding, the majority of CD8 T cells, and possibly CD4 T cells, are often specific for a few immunodominant epitopes
- CTL function by recognizing virus-infected cells and killing them
- This often involves perforins and cytotoxic granules containing granzymes.

ADAPTIVE IMMUNITY TO VIRUSES

- Effector CTL can also induce death in target cells following engagement of Fas ligand on the CTL with Fas on target cells.
- Both pathways lead to apoptosis of the target cell, involving the degradation of nucleic acids, including those of the virus.
- CD8 T cells also mediate defense through the release of various cytokines following antigen recognition. Some of the cytokines and chemokines most highly produced by CTL include IFN- γ , TNF- α , lymphotxin- α , and RANTES(regulated upon activation, normal T cell expressed and secreted) (is a prototypical T-cell-derived chemokine and potent inflammatory mediator that activates basophils and mast cells and attracts T cells)
- These cytokines can have multiple antiviral effects on infected cells and the cells around them, including purging of virus from infected cells without killing the cell. This is particularly important for viruses like HSV which infects nonrejuvenating cells such as nerve cells.

ADAPTIVE IMMUNITY TO VIRUSES

- CD4 T cells are also involved in antiviral defense. They are important, though not always essential, for controlling infections such as HSV, influenza virus, HIV, and many others. CD4 T cells participate in antiviral immunity in several ways.
- the subset acts as helper cells for the induction of both antiviral antibodies and CD8 T-cell responses to most virus antigens
- CD4 T cells also function as antiviral effector cells, and generate stable memory cell populations similar to those of CD8 T cells.
- The differentiation of CD4 T cells into effectors occurs in a manner very similar to that with CD8 T cells.
- CD4 T cells are activated by recognizing viral peptides.
- These are larger than those involved in CD8 T-cell recognition and are associated with class II MHC molecules present on more specialized cells such as APC
- CD4 T cells rarely recognize viral epitopes present on cells as a consequence of viral gene expression within that cell, dictating their function as helper cells for B cells and CD8 T cells, and as producers of cytokines for help and viral clearance.

Effector systems	Recognized molecules	Control mechanisms
Antibody	Surface proteins or virions	Neutralization of virus, opsonization, or destruction of infected cells by ADCC
Antibody + complement	Surface proteins expressed on infected cells	Infected cell destruction by ADCC or complement-mediated lysis
Mucosal antibody (IgA)	Surface proteins or virions	Viral neutralization, opsonization, and transcytosis
CD4 T cells	Viral peptides (10–20 mers) presented on MHC class II – surface, internal or nonstructural proteins presented by APC	Antiviral cytokine and chemokine production; help for CD8 T-cell and B-cell responses; killing infected cells; regulatory functions to reduce immunopathology
CD8 T cells	Viral peptides (8–10 mers) presented on MHC class I – surface, internal or nonstructural proteins presented on infected cells or by cross-presentation	Killing infected cells or purging virus without cell death; antiviral cytokine and chemokine production

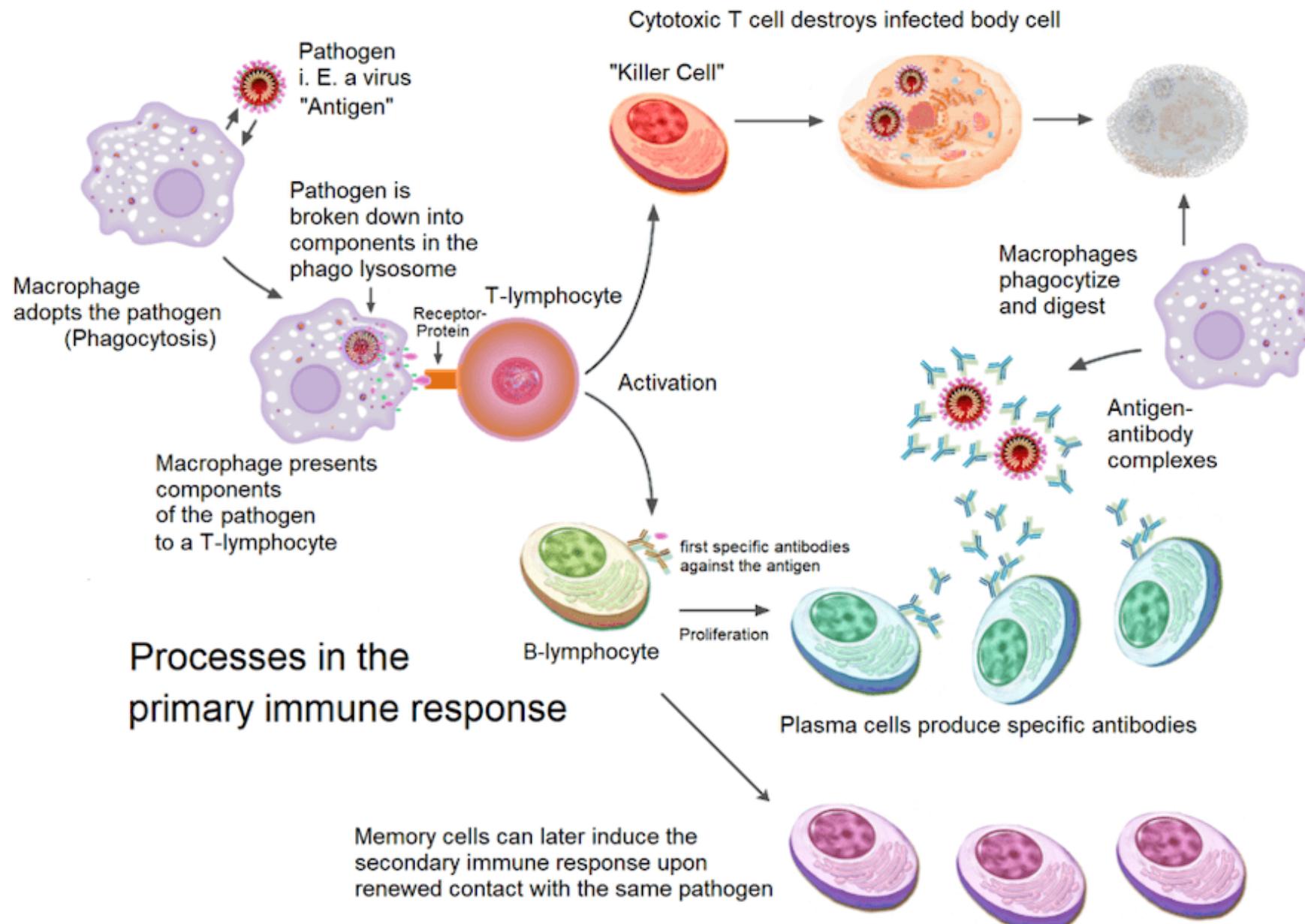
ADCC, antibody-dependent cellular cytotoxicity; APC, antigen-presenting cell; IgA, immunoglobulin A; MHC, major histocompatibility complex.

IMMUNOLOGICAL MEMORY

- Immunological memory is a cardinal feature of adaptive immunity.
- Immunological memory is defined by a pool of antigen-specific cells whose increased frequency enables rapid control of viral reinfection
- Recent studies identified a population of IL-7 receptor-alpha-expressing effector cells as the precursors of this memory pool.
- This population of cells, which constitutes ~5–10% of the effector pool, preferentially survives the contraction phase, and gradually differentiates into a stable memory population.
- Upon reinfection, these memory cells can be rapidly activated, and by virtue of their increased frequency mediate more rapid clearance of the viral pathogen.
- Moreover, repeated stimulation of memory cells via multiple infections with the same virus, or prime-boost vaccine regimes, further increases the size of the antigen-specific memory T-cell pool.
- Re-stimulation also affects the activation status and tissue distribution of memory T cells, which may enhance protection from viral infection in mucosal, and other, tissues.

IMMUNOLOGICAL MEMORY

- Experiments in humans and mice have demonstrated that memory T cells are heterogeneous. Memory T cells have been divided into effector memory (TEM) and central memory (TCM) subsets, defined by expression of two surface molecules involved in T-cell migration: CD62L and CCR7.
- The CD62L^{lo}CCR7^{lo} TEM subset is found primarily in nonlymphoid tissues and spleen, whereas the CD62L^{hi}CCR7^{hi} TCM subset are largely present in the lymph nodes and spleen.
- The current model predicts that effector T cells form the TEM subset; these cells gradually convert to a TCM phenotype over time.



IMMUNE EVASION AND IMMUNITY TO CHRONIC VIRAL INFECTIONS

- Many, if not all, viruses employ evasion strategies to circumvent aspects of the immune system, allowing them time to replicate further or escape detection
- One such mechanism may involve killing or infecting APC.
- Viruses may also delay or prevent apoptosis induced by CTL within infected cells. Other viral evasion measures aimed at the CD8 T-cell-mediated antiviral defense system serve to inhibit antigen processing, thereby minimizing effector CTL induction.
- Many viruses also downregulate MHC molecules on the surface of infected cells to escape CTL killing.
- In addition, viruses may produce various mimics or modulators/inhibitors of cytokines, chemokines, or other components of the immune system or their receptors.
- Viruses also resort to antigenic hypervariability to escape antibody or T-cell recognition.
- This can occur during transmission from host to host (i.e., influenza virus), or within hosts during chronic infection through the generation of viral escape mutants (i.e., HIV).

Mechanism	Example
Interference with viral antigen processing and presentation	HSV (ICP47), EBV (EBNA-1), HIV (Nef, Tat), HPV (E5), CMV (UL6)
Evasion of NK cell function	HIV (Nef), EBV (EBNA-1), CMV (UL40, UL18)
Inhibition of cell apoptosis	Adenovirus (RID complex and E1B), HIV (Nef), EBV (BHRF-1)
Destruction of T cells	HIV
Interference with antiviral cytokines and chemokines	EBV (IL-10 homologue), CMV (US28 chemokine receptor homologue), vaccinia virus (IL-18-binding protein), HIV (Tat chemokine activity)
Inhibition of complement action	HSV, pox viruses
Inhibition of DC maturation	HSV, vaccinia virus
Frequent antigenic variation	Influenza virus, HIV
Infection of immune privileged site	Measles virus, VZV and HSV (neurons)
Immune exhaustion	HIV, HCV, HBV

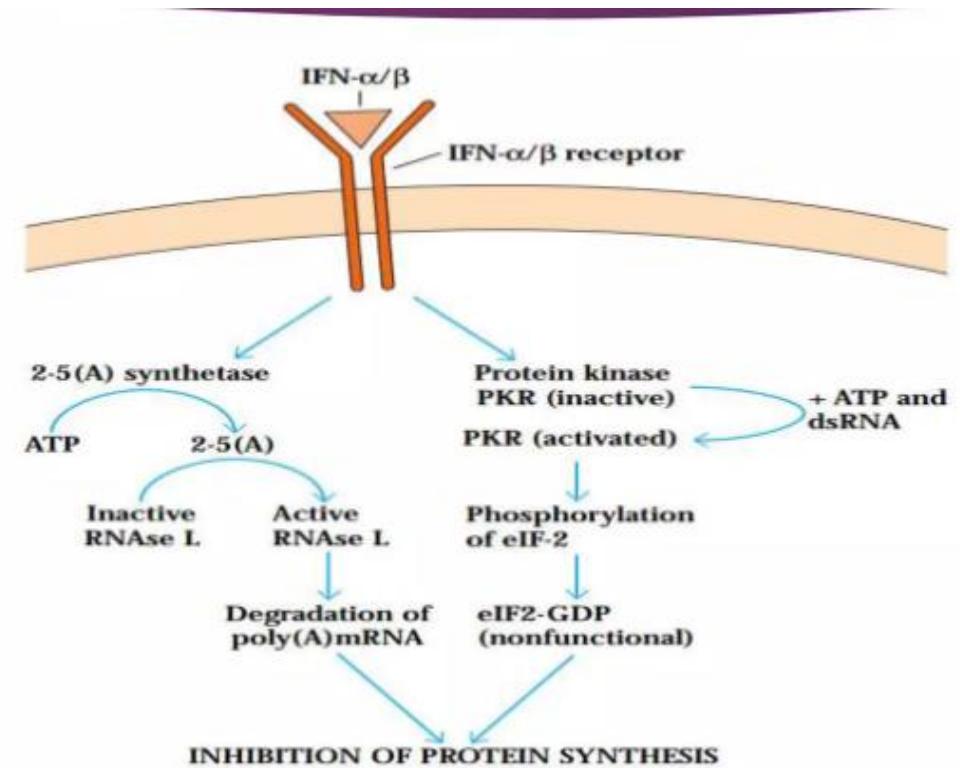
CMV, cytomegalovirus; DC, dendritic cell; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HSV, herpes simplex virus; IL-18, interleukin-18; NK, natural killer; RID, receptor internalization and degradation; VZV, varicella-zoster virus.

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- Viruses also resort to antigenic hypervariability to escape antibody or T-cell recognition.
- This can occur during transmission from host to host (i.e., influenza virus), or within hosts during chronic infection through the generation of viral escape mutants (i.e., HIV).
- The success of many viral pathogens rests in their ability to subvert the host immune response. The most successful human viruses can escape the immune system and persist for the life of the host

Induction of antiviral activity

- Induction of antiviral activity by IFN α and INF β
- These interferons bind to the IFN receptor which in turn induces the syntheses of both 2-5(A) synthetase and protein kinase (PK)
- The action of 2-5(A) synthetase results in the activation of RNase L, which can degrade messenger RNA
- PK inactivates the translation initiation factor eIF-2 by phosphorylating it
- Both pathways thus result in the inhibition of protein synthesis and thereby effectively block viral replication.

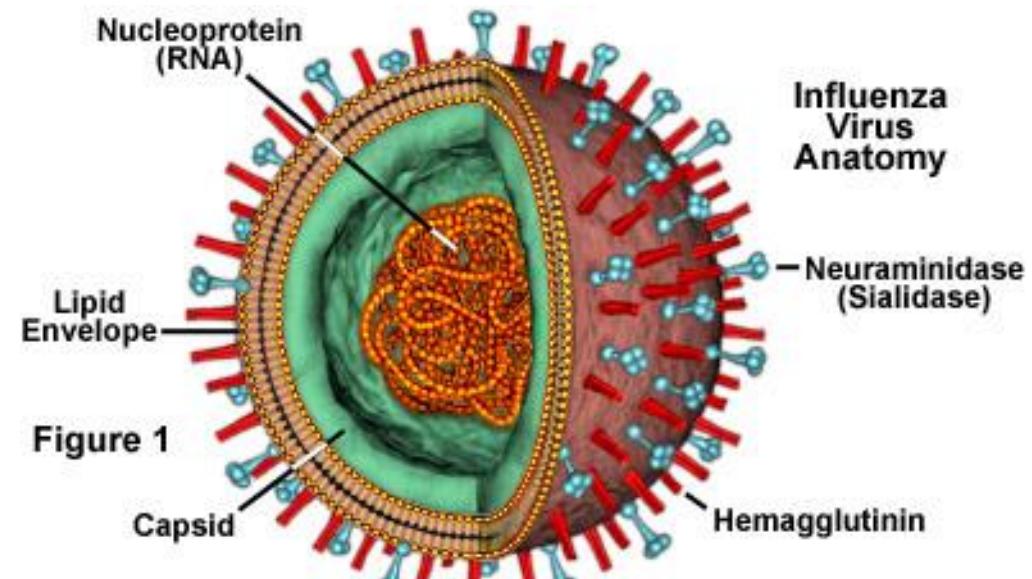


Influenza virus

- The influenza virus attacks the upper respiratory tract ie. lungs, nose and throat.
- Young children, older adults, pregnant women and people with chronic disease or weak immune systems are at high risk.
- Symptoms include fever, chills, muscle aches, cough, congestion, runny nose, headaches and fatigue.
- Influenza is highly contagious and is more common during the colder months of the year.
- The influenza virus is chiefly transmitted through airborne respiratory secretions released when an infected individual coughs or sneezes. Incubation typically is from one to two days from the time of infection, and most people begin to naturally recover from symptoms within a week.

Structure

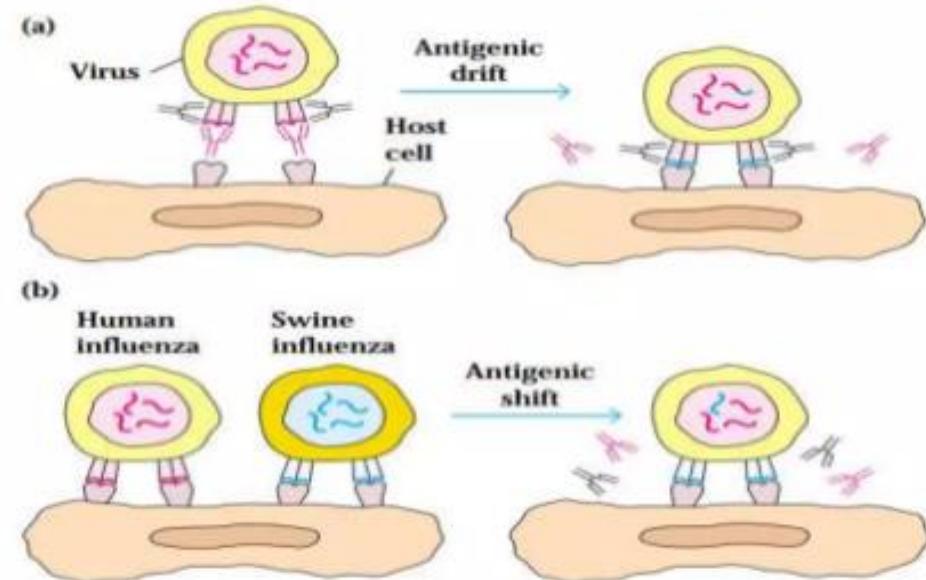
- variable, but the virion particles are usually spherical or ovoid in shape and 80 to 120 nanometers in diameter.
- The influenza virion is an enveloped virus that derives its lipid bilayer from the plasma membrane of a host cell.
- Two different varieties of glycoprotein spike are embedded in the envelope.
- 80 percent of the spikes are hemagglutinin
- 20 percent or so of the glycoprotein spikes consist of neuraminidase
- On the inner side of the envelope that surrounds an influenza virion is an antigenic matrix protein lining.
- influenza genome, which is organized into eight pieces of single-stranded RNA (A and B forms only; influenza C has 7 RNA segments).
- The RNA is packaged with nucleoprotein into a helical ribonucleoprotein form, with three polymerase peptides for each RNA segment.



Host response to influenza action

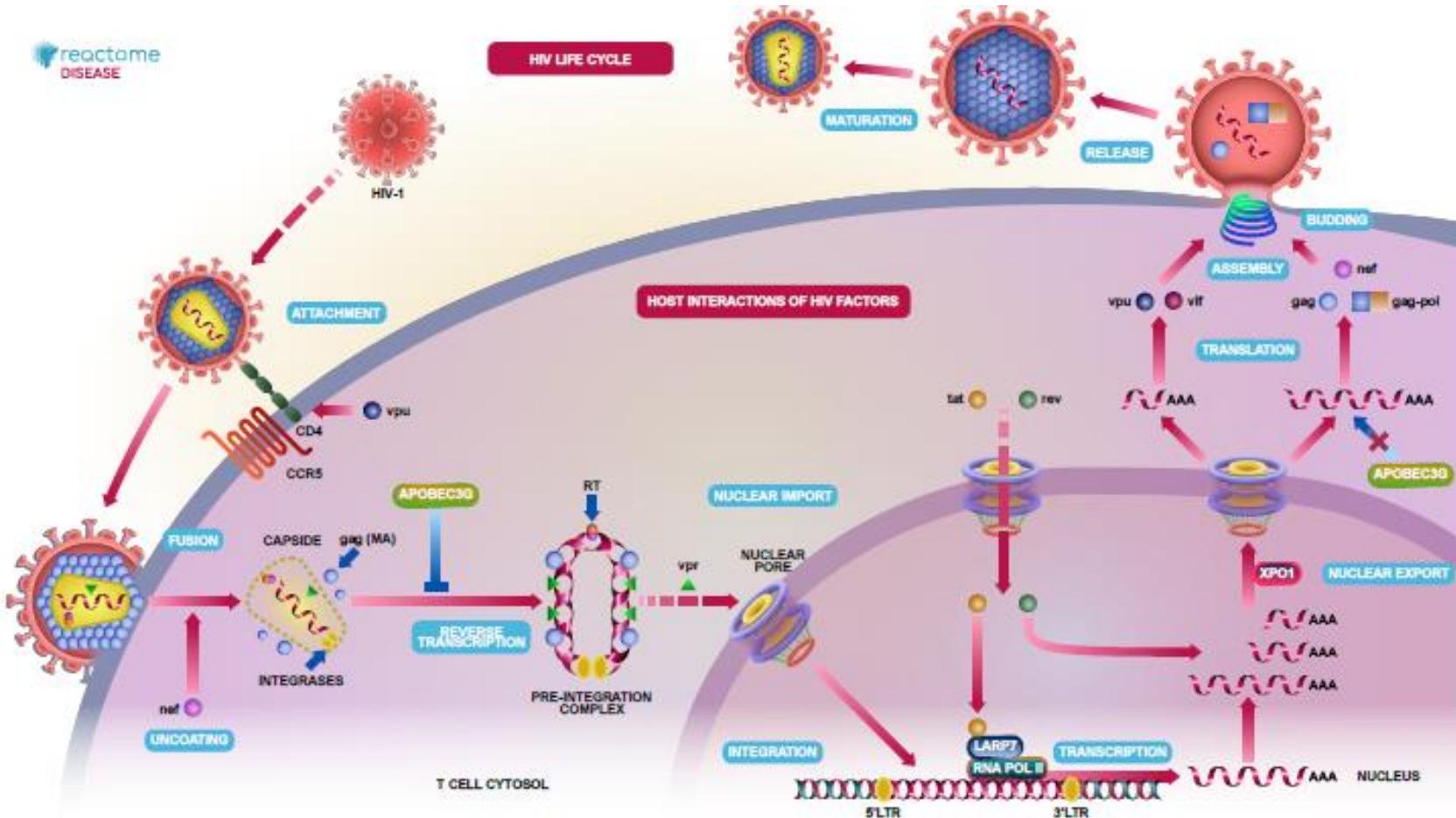
Two mechanism generate variations in influenza surface antigens.

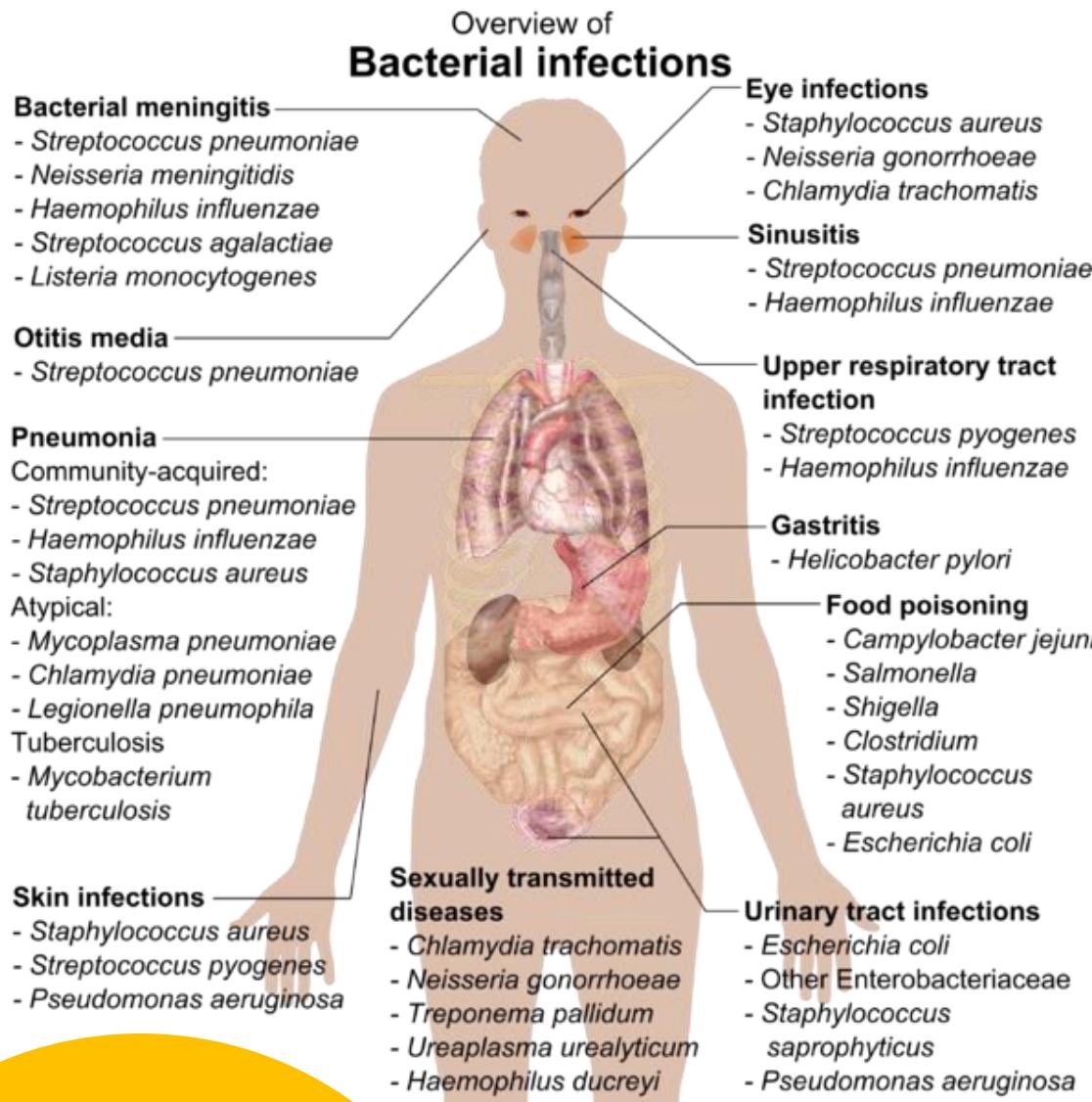
- In antigenic drift, the accumulation of point mutations eventually yield a variant protein that is no longer recognized by antibody to the original antigen.
- Antigenic shift may occur by reassortment of an entire ssRNA between human and animal virions infecting the same cell. Only four of the eight RNA strand are depicted.



Infection by HIV

<https://reactome.org/PathwayBrowser/#/R-HSA-162906>



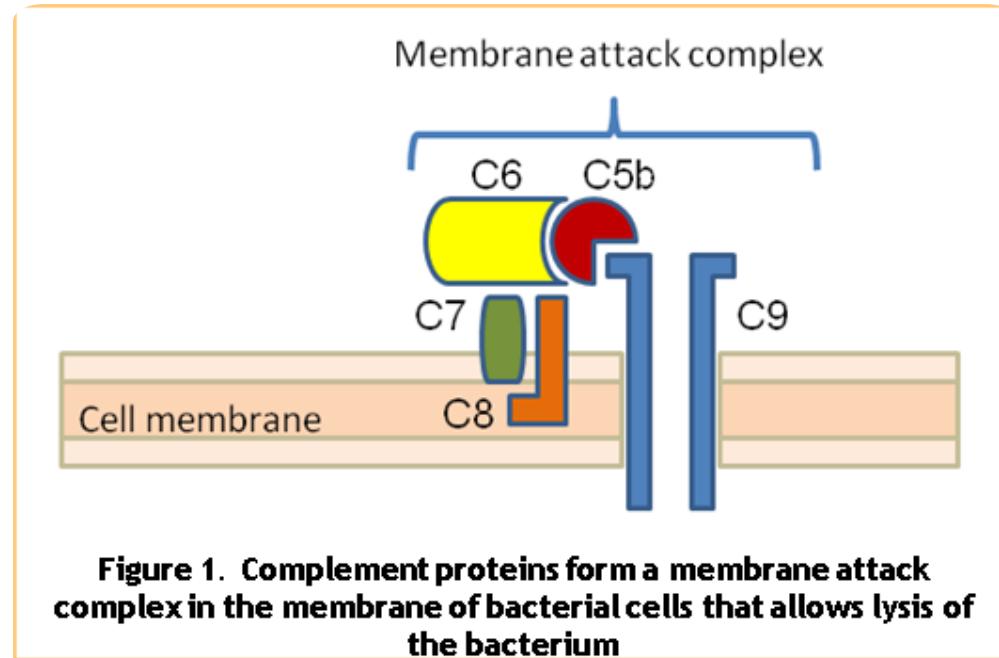


Bacterial infections

- Bacterial infections have a large impact on public health.
- Disease can occur at any body site and can be caused by the organism itself or by the body's response to its presence.
- Bacteria are transmitted to humans through air, water, food, or living vectors.
- Some examples of bacterial infections are:
 - Legionnaires' disease.
 - meningococcal disease.
 - Q fever.
 - strep throat.
 - tuberculosis (TB)
 - whooping cough (pertussis)

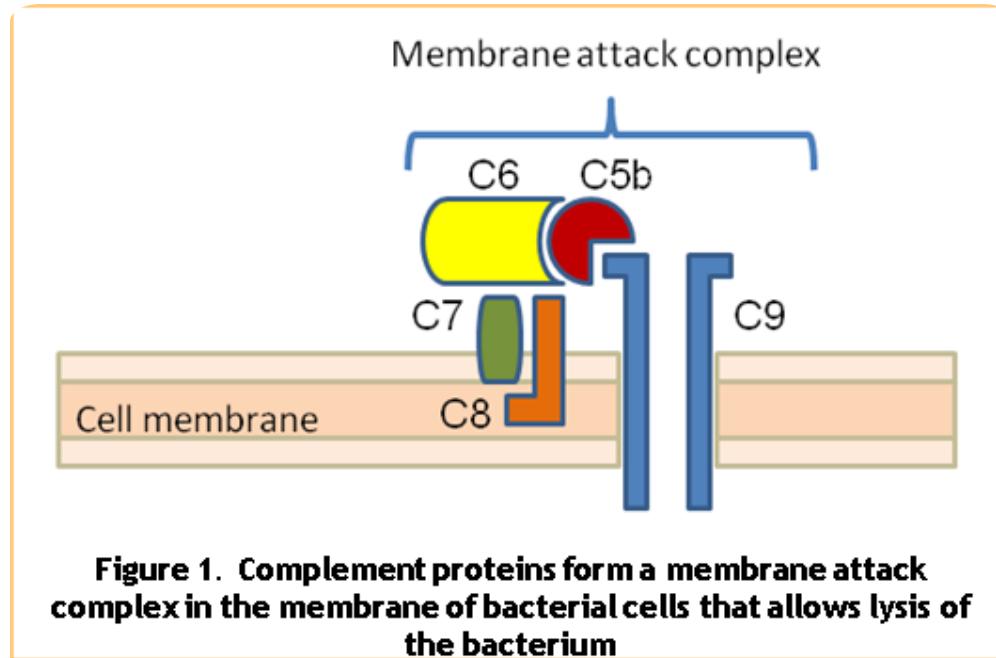
- The principal modes of transmission of bacterial infection are contact, airborne, droplet, vectors, and vehicular.
- Steps in bacterial infection:
- Attachment to host cell
- Proliferation
- Invasion of host tissue
- Toxin induced damage to host cells

Via complement-mediated lysis



- When bacteria, such as *Neisseria meningitidis*, invade the body, they are attacked by immune proteins called complement proteins.
- Complement proteins assist in bacterial killing via three pathways, the classical complement pathway, the alternative complement pathway or the lectin pathway.
- The first steps of the classical complement pathway require the binding of antibodies to the surface of the target bacterium.
- The antibodies then become targets for one particular complement protein complex, known as C1 – C1 binds to the tail (known as Fc region) of the antibody.

Via complement-mediated lysis



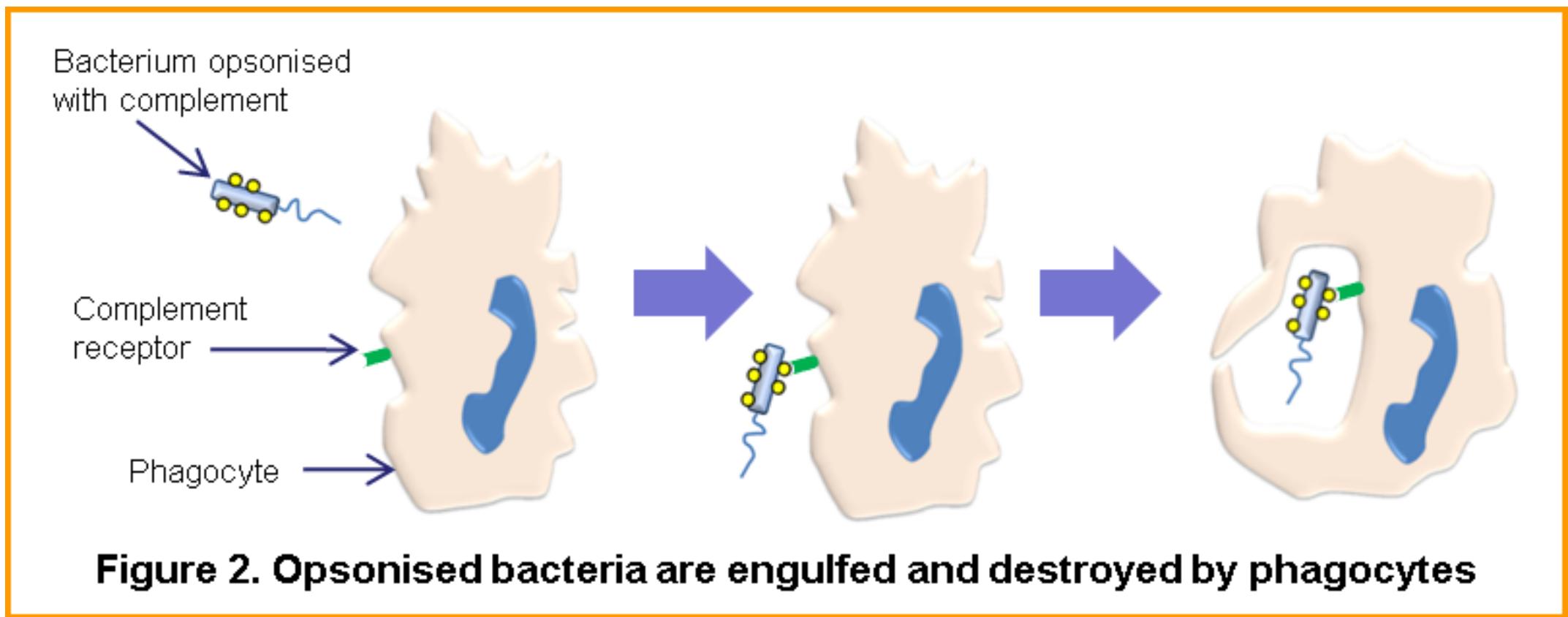
- Once bound, C1 initiates a cascade of cleavage and reforming of complement complexes that ends in the binding of several complement proteins to the surface of the bacterium in the form of a membrane attack complex (MAC) or can generate opsonins that label a bacterium for destruction.
- MAC can insert into the cell membrane of Gram-negative, but not Gram-positive, bacteria.
- There, it produces pores that allow the entry of membrane damaging molecules, such as lysozyme, and makes the bacterium susceptible to osmotic lysis.

Lectin pathway

- The alternative complement pathway does not require antibody to initiate the lysis of bacteria.
- In this pathway, complement proteins from a complex known as C3 directly bind to bacteria and activate downstream components in the complement cascade, once again ending in formation of MAC that causes lysis of the bacterium.
- During the lectin pathway, mannan-binding lectin (MBL) binds to proteins containing mannose residues that are found in some types of bacteria (such as *Salmonella* spp.).
- Once bound, MBL forms a complex with an enzyme called MBL-activated serine protease (MASP).
- In this form, this enzyme activates C3 convertase (by cleaving C2 and C4 complement components) that participates in forming MAC.

Via phagocytosis

- Bacteria may also be killed by phagocytes.
- Immune proteins like acute phase proteins (like complement) and antibodies bind to the surface of bacteria by a process called opsonisation.
- Opsonised bacteria are, therefore, coated with molecules that phagocytic cells recognise and respond to.
- Activated phagocytes engulf and destroy opsonised bacteria by a process called phagocytosis. Complement C3b is a particularly important opsonisation protein for controlling bacterial infections by this mechanism.
- Opsonisation allows killing of Gram-positive bacteria (e.g. *Staphylococcus* spp.) that are resistant to killing by MAC.
- After bacteria are ingested by phagocytosis, they are killed by various processes that occur inside the cell, and broken into small fragments by enzymes.
- Phagocytes present the fragments on their surface via class II major histocompatibility (MHC class II) molecules.

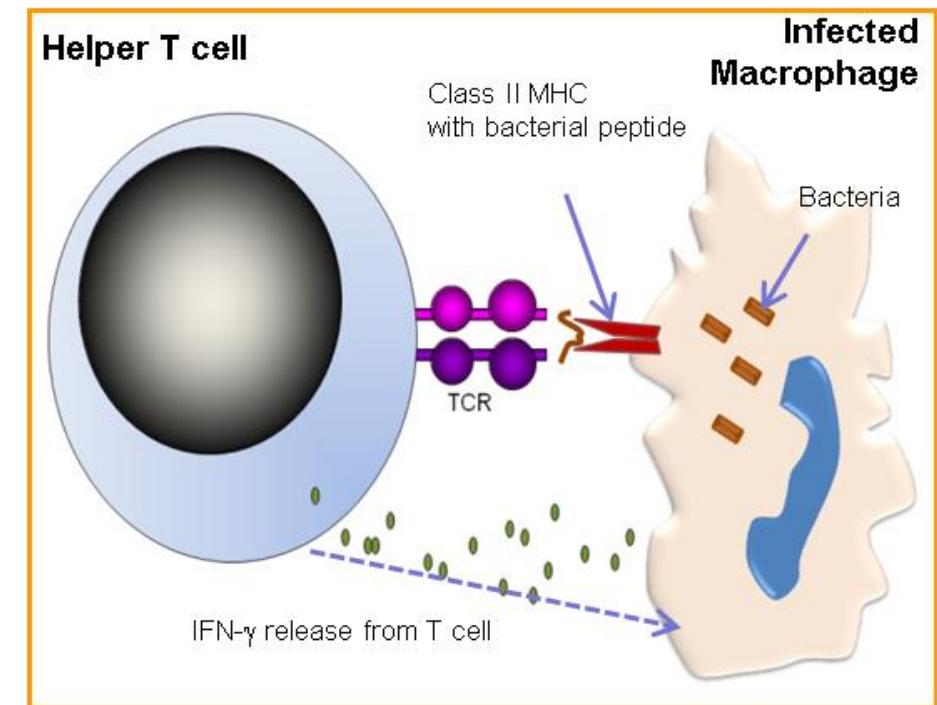


Via phagocytosis

- Circulating helper T cells recognise these bacterial fragments and begin to produce proteins called cytokines.
- Two major groups of helper T cells are known as Th1 and Th2 cells.
- These cell types differ in the types of cytokine they secrete.
- Th1 cells predominantly produce interferon-g (IFN-g), which promotes cell-mediated immune mechanisms.
- Th2 cells produce mostly interleukin-4 (IL-4), which promotes humoral immunity by activating B cells. B cells make antibodies that stick to extracellular bacteria and prevent their growth and survival.

Via cell-mediated immunity

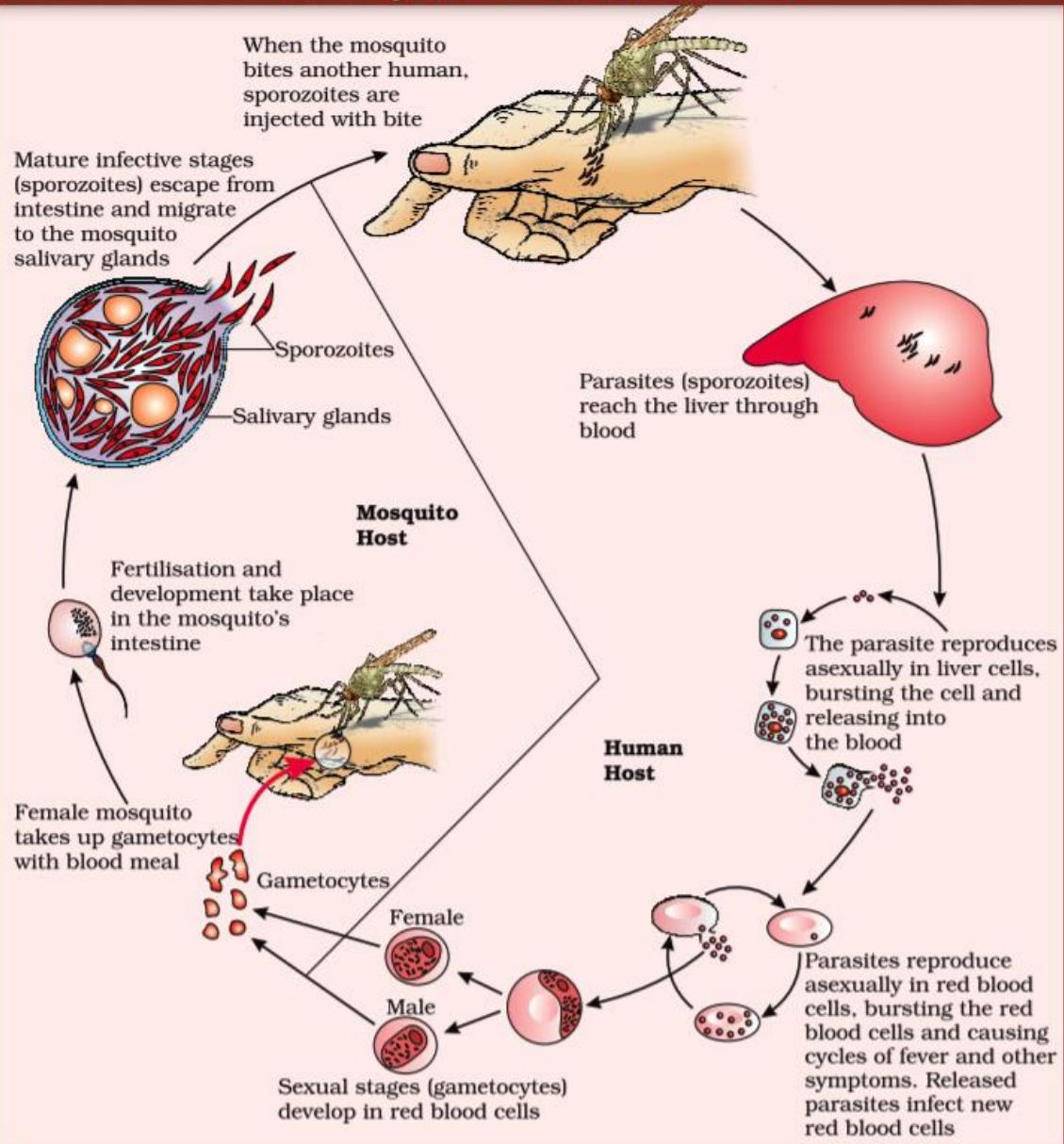
- Some bacteria engulfed during phagocytosis avoid the killing mechanisms of the phagocyte to survive inside cells.
- Macrophages are a common targets for intracellular bacteria (e.g. *Salmonella* spp.) that live inside cell compartments.
- These bacteria cannot be detected by complement or antibody but, instead, are eliminated using a cell-mediated response. Infected macrophages present bacterial peptides on their cell surface using MHC class II molecules.
- This mechanism is called antigen presentation.
- A helper T cell surveys MHC class II molecules with its T-cell receptor (TCR) to observe the peptides they hold.
- If a bacterial peptide is presented, the Th1 cell releases IFN- γ .
- This cytokine stimulates killing mechanisms, (such as production of lysozyme) inside the infected macrophage to digest and destroy the invading bacterium. IFN- γ also increases antigen presentation by cells, making the bacterium more visible to the immune system and more prone to attack



Protozoan diseases

- Protozoans are single-celled organisms that have membrane-bound organelles and nuclei. These eukaryotes can live independently or in symbiosis with other organisms
- These microorganisms mostly reside in resting cysts, which let them survive in dry conditions.
- *Toxoplasma Gondii*, *Isospora Belli*, *Blastocystis hominis*, *Balantidium coli*, *Naegleria spp* and *Acanthamoeba spp* are the protozoa variants that spread diseases through water.

Life Cycle of Plasmodium



Life cycle of plasmodium

- Sporozoites enter the Bloodstream when an infected mosquito takes a blood meal.
- The Sporozoites migrate to the liver, where they multiply, transforming liver hepatocytes into giant multinucleate schizonts, which release thousands of merozoites into the bloodstream.
- The merozoites infect red blood cells, which eventually rupture, releasing more merozoites.
- Eventually some of the merozoites differentiate into male and female gametocytes, which are ingested by a mosquito and differentiate into gametes.
- The gametes fuse to form a zygote that differentiates to the sporozoite stage within the salivary gland of the mosquito.

Natural and specific immune response to protozoa

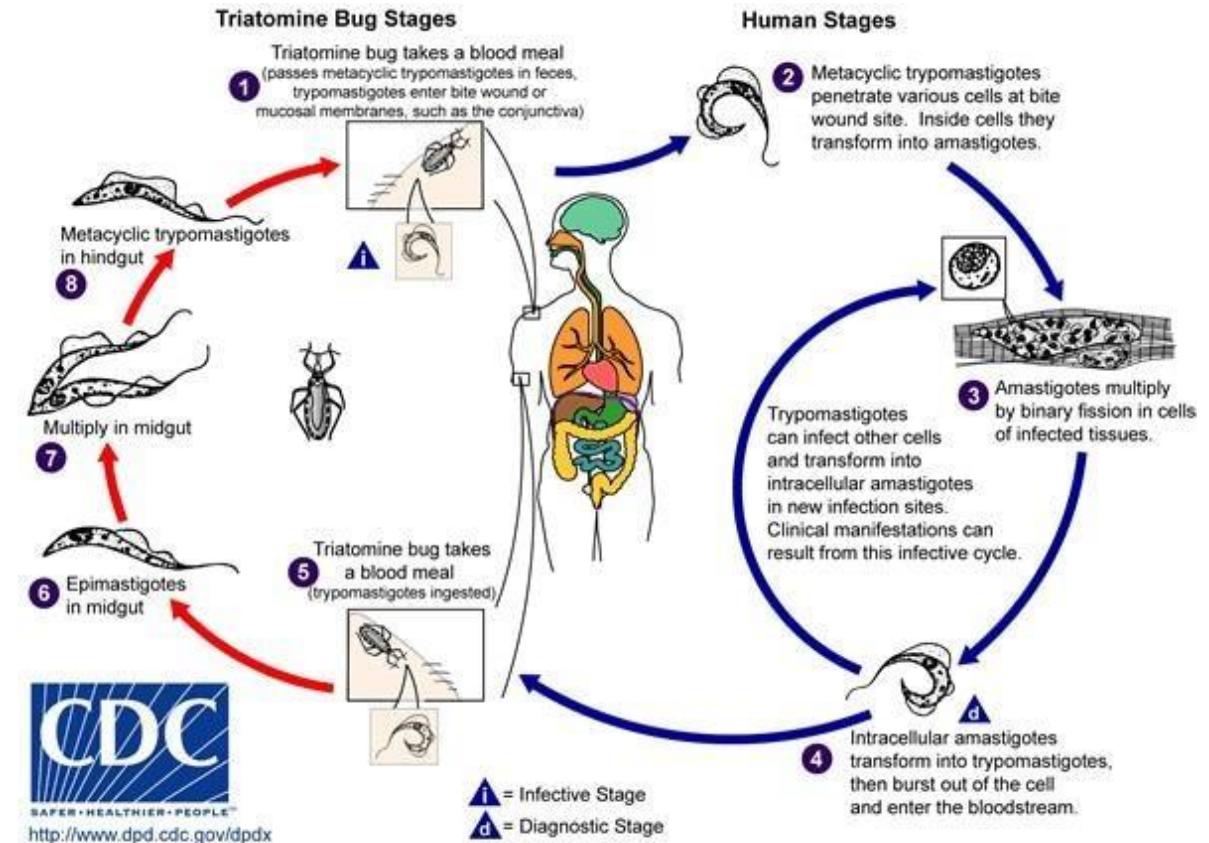
- Protozoa activate quite distinct specific immune responses, which are different from the responses to fungi, bacteria and viruses.
- Protozoa may be phagocytozed by macrophages, but many are resistant to phagocytic killing and may even replicate within macrophages.
- *T. brucei gambiense* is the best example of protozoa which can induce humoral immune response because of its extra-cellular location.
- In *Leishmania* sp. infections, cellular defense mechanisms depend upon CD4+ T-lymphocytes and activate macrophages as effector cells that are regulated by cytokines of Th1 subset.
- *Plasmodium* sp. is a protozoa which show the diversity of defence mechanisms which can be cellular or humoral, depending on Ag and protozoa's location.

Immune evasion mechanisms of protozoa

- Different protozoa have developed remarkably effective ways of resisting specific immunity:
 - a) anatomic sequestration is commonly observed with protozoa Plasmodium and *T. gondii*;
 - b) some protozoa can become resistant to immune effector mechanisms: *Trypanosoma*, *Leishmania* and *T. gondii*;
 - c) some protozoa have developed effective mechanisms for varying their surface antigens: *Plasmodium* and *Trypanosoma*;
 - d) some protozoa shed their antigen coats, either spontaneously or after binding with specific antibodies: *E. histolytica*;
 - e) some protozoa alter host immune response by nonspecific and generalized immunosuppression (abnormalities in cytokine production, deficient T cell activation): *Trypanosoma*, *Leishmania*, *Toxoplasma*, *Entamoeba*.

Protozoan trypanosoma (African sleeping disease)

- The *T cruzi* life cycle consists of 3 main developmental forms.
- Epimastigotes are an extracellular and noninfective form of the parasite found in the midgut of insect vectors, where they multiply by binary fission.
- As epimastigotes move to the hindgut, they differentiate into metacyclic trypomastigotes, which are nondividing forms resistant to mammalian complement that have the capacity to infect mammalian cells.
- They enter local cells through breaks in the skin, mucous membranes, or the conjunctivas and transform into the third morphologic form, amastigotes.
- Amastigotes multiply intracellularly until the host cell is overwhelmed, at which point they transform into bloodstream trypomastigotes



- The modes of transmission of *T cruzi* to humans
- Historically, most transmission of *T cruzi* to humans has resulted from the contamination of vulnerable surfaces (eg, breaks in the skin, mucosae, and the conjunctivas) with the feces of infected vectors.
- vector-borne transmission has been reduced markedly in many endemic countries.
- Transfusion transmission was a major public health problem in endemic countries for decades, but, as accurate serologic assays for *T cruzi* infection were developed and screening of blood donors became mandatory and were implemented throughout the endemic range, this problem essentially has been eliminated
- *T cruzi* can be transmitted via transplantation of organs obtained from persons with chronic infection, and occasional reports of this in Latin America [30] and in the United States
- As transmission of congenital (transplacental) transmission from mothers with chronic *T cruzi* infection to their newborns by vectors and through transfusion of contaminated blood have been reduced, the proportion of new *T cruzi* infections that result from congenital transmission has increased.

Disease caused by Parasitic worms Helminths

Helminths are large, multicellular organisms that reside in humans but do not ordinarily multiply there and are not intracellular pathogens.

Although helminths are more accessible to the immune system than protozoans.

The immune system is not strongly engaged and the level of immunity generated to helminths is often very poor.

Several helminthes are important pathogens of domestic animals and invade humans who ingest contaminated food.

Taenia, a tapeworm of cattle

Trichinella, the roundworm of pigs

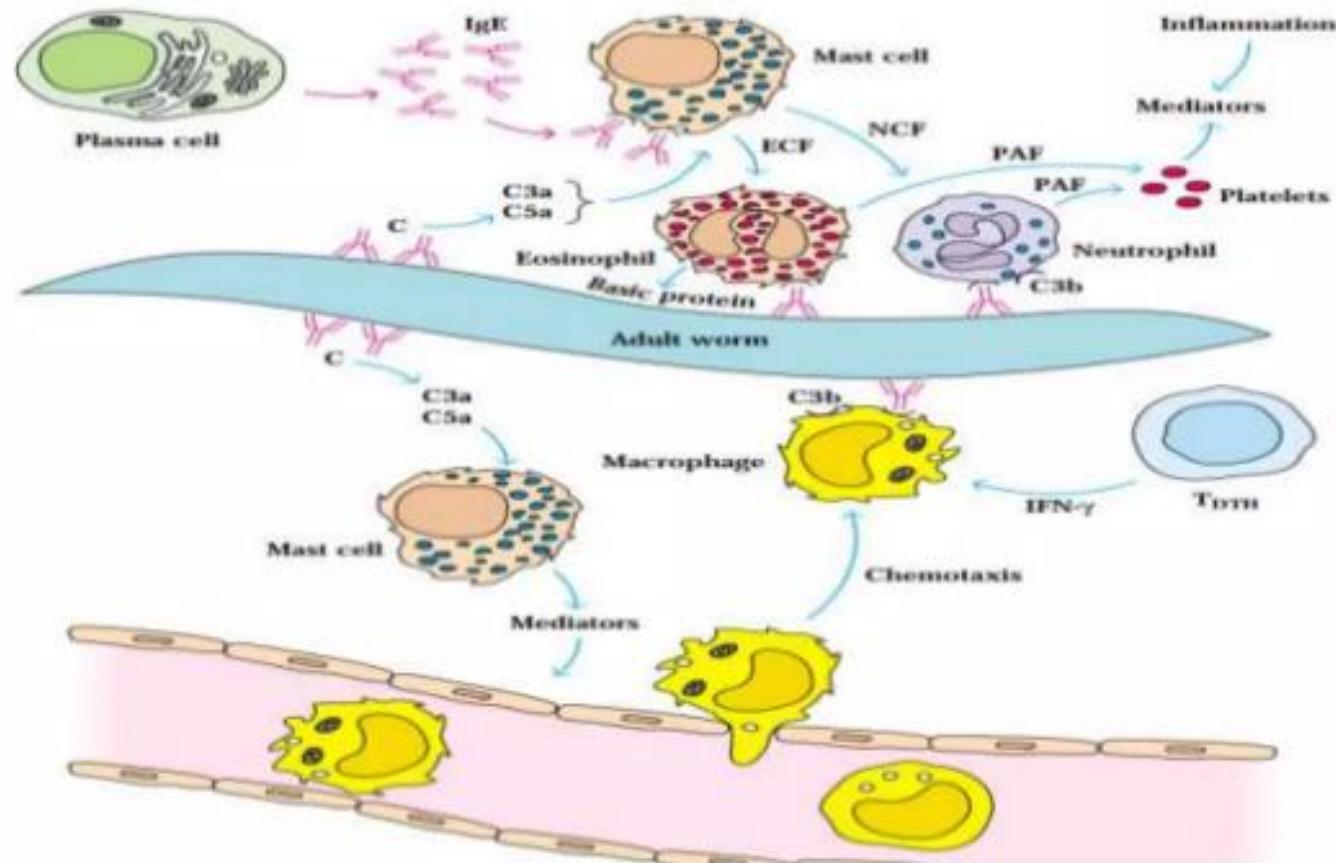
Immune response

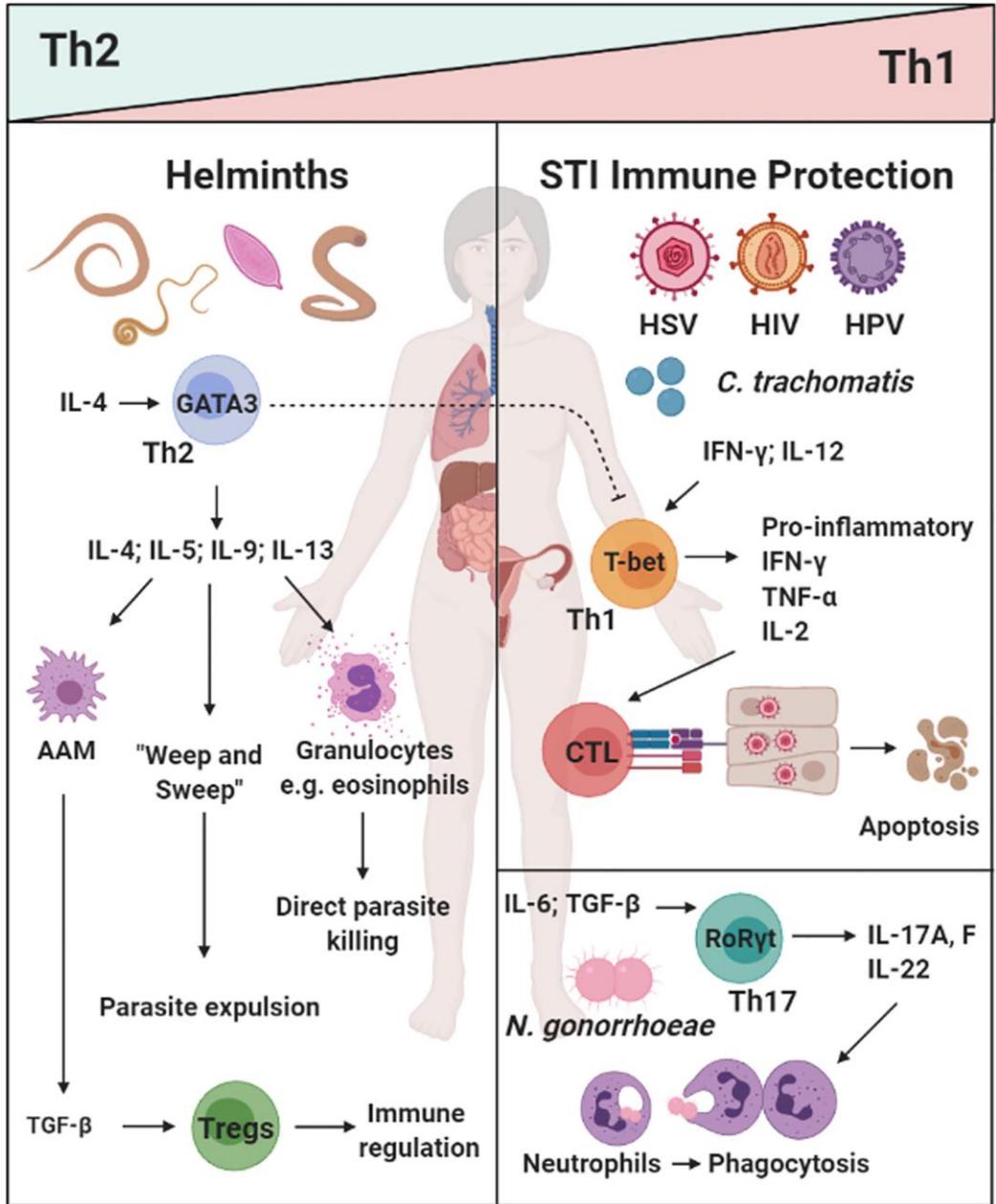
The response includes an IgE humoral component and a cell-mediated component involving CD4 T cells.

C = complement

ECF = eosinophil chemotactic factor

PAF = platelet-activating factor





- 1) The dichotomy of helminth-induced Th2/type 2 and regulatory immunity, and protective responses against sexually transmitted infections (STIs) in the female reproductive tract (FRT): Helminth infections (e.g. *A. lumbricoides*, *T. trichiura*, *Schistome* eggs) commonly induce a potent Th2/type 2 immune response characterized by type 2 cytokines IL-4, IL-9, and IL-13, which induce a potent type 2 effector cells and functions (e.g. eosinophils, alternatively activated macrophages(AAMs), “weep and sweep” responses)). Prevalent viral [Herpes Simplex Virus type II (HSV-2), Human Immunodeficiency Virus (HIV), and Human Papillomavirus (HPV)] and bacterial (*C. trachomatis* and *N. gonorrhoeae*) vaginal infection are a serious health concern for women in low- and middle-income countries (LMICs). Protective immunity against these pathogens can be classified a Th1/type 1 and Th17 responses i.e. cytotoxic killing of infected cells or phagocytosis of extracellular pathogens).
- How helminth exposure and immune modulation may influence susceptibility and control of STIs, is not fully

Reference

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VACCINES

DR. (MS.) SONALI CORREA, M.SC.,
PH.D.



AGENDA

- DEFINITION
 - INTRODUCTION
 - HISTORY
 - IMMUNITY
 - ACQUIRED IMMUNITY VS
ADAPTIVE IMMUNITY
 - VACCINE?
- VACCINATION VS
IMMUNIZATION
 - VACCINE TERMINOLOGIES
 - TYPES OF VACCINE'S



DEFINITION



- A vaccine is a biological preparation that improves immunity to a particular disease by stimulating the body's immune response.
- It contains certain agents that not only resembles a disease-causing microorganism, but it also stimulates body's immune system recognize the foreign agents.
- A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe.
- Vaccines are usually administered through needle injections, but some can be administered by mouth or sprayed into the nose.

INTRODUCTION

- Vaccination is a simple, safe, and effective way of protecting people against harmful diseases, before they come into contact with them.
- Vaccines train your immune system to create antibodies, just as it does when it's exposed to a disease.
- However, because vaccines contain only killed or weakened forms of germs like viruses or bacteria, they do not cause the disease or put you at risk of its complications.
- Vaccines reduce risks of getting a disease by working with your body's natural defences to build protection.
- When you get a vaccine, your immune system responds. We now have vaccines to prevent more than 20 life-threatening diseases, helping people of all ages live longer, healthier lives.

HISTORICAL BACKGROUND

- In May 1796, English physician Edward Jenner expands on this discovery and inoculates 8-year-old James Phipps with matter collected from a cowpox sore on the hand of a milkmaid. Despite suffering a local reaction and feeling unwell for several days, Phipps made a full recovery.
- Two months later, in July 1796, Jenner inoculates Phipps with matter from a human smallpox sore in order to test Phipps' resistance.
- Phipps remains in perfect health, and becomes the first human to be vaccinated against smallpox.
- The term '**vaccine**' is later coined, taken from the Latin word for cow, vacca.



1806

French Emperor Napoleon Bonaparte and American President Thomas Jefferson acknowledge Dr Edward Jenner's work and endorse the smallpox vaccine.



1885

Louis Pasteur successfully prevents rabies through post-exposure vaccination.



1967

Mass vaccination begins with the World Health Organization announcing the Intensified Smallpox Eradication Programme.



1971

The measles vaccine (1963) is combined with recently developed vaccines against mumps (1967) and rubella (1969) into a single vaccination (MMR).

Read more about the [HISTORY OF THE MEASLES VACCINE](#) →



2016

The success of the Meningitis Vaccine Project highlights the key role public-private partnerships can play in helping to develop vaccines.



2019

WHO prequalifies an Ebola vaccine for use in countries at high risk.

Read more about the [HISTORY OF THE EBOLA VACCINE](#) →



1918–19

The "Spanish Flu" pandemic kills 1 in 67 United States soldiers, making an influenza vaccine a US military priority.

Read more about the [HISTORY OF THE INFLUENZA VACCINE](#) →

1988

WHO launches the Global Polio Eradication Initiative.

Read more about the [HISTORY OF THE POLIO VACCINE](#) →



2021

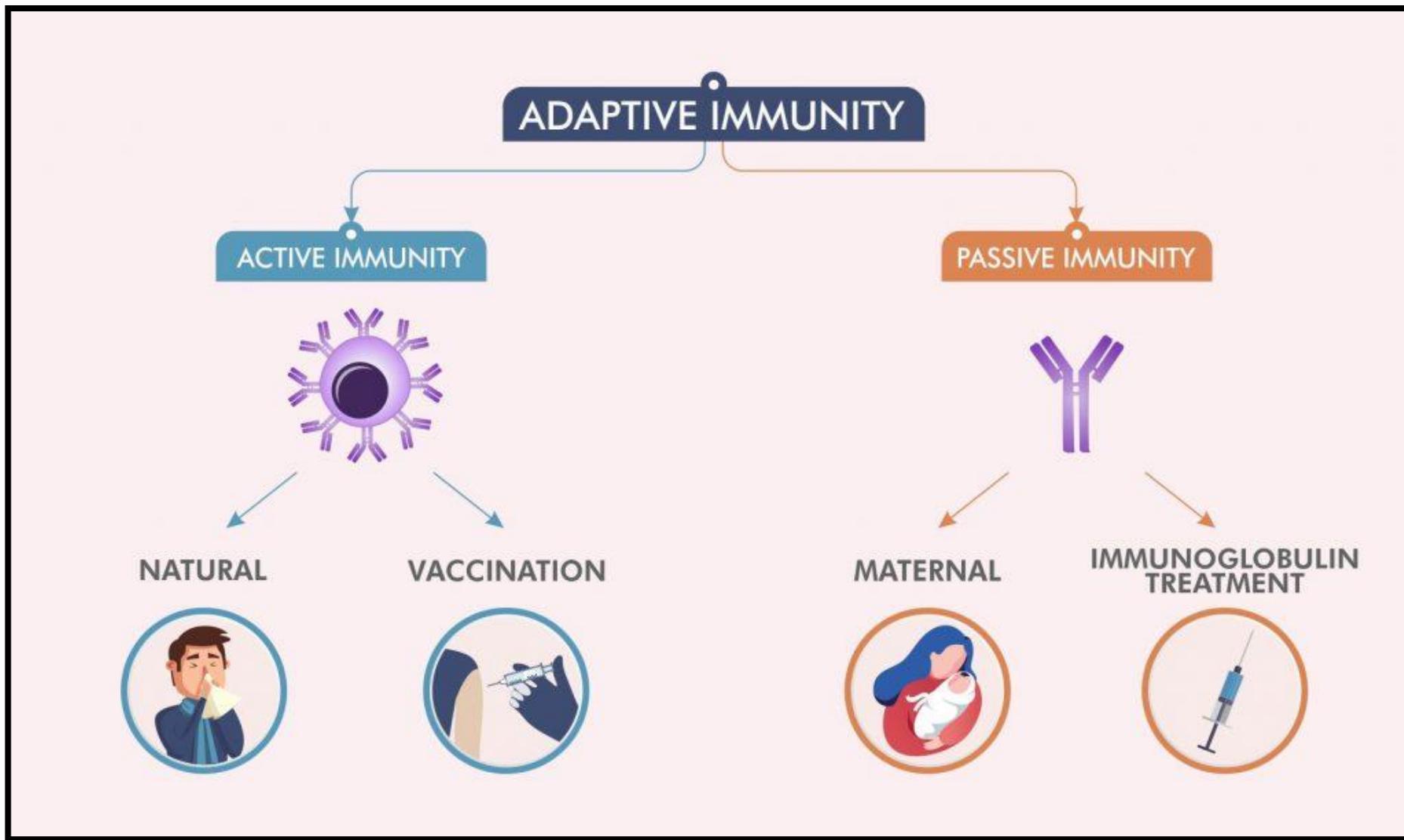
WHO calls on Member States to prioritize vaccination against COVID-19 of health workers and at-risk groups in all countries.



VACCINES & IMMUNITY

- Immunity is the ability of the body to defend itself against disease-causing organisms.
- Everyday our body comes in contact with several pathogens, but only a few result in diseases.
- The reason is, our body has the ability to release antibodies against these pathogens and protects the body against diseases.
- **This defense mechanism is called immunity.**
- There are two major types of immunity:
 - Innate Immunity or Natural or Non-specific Immunity.
 - Acquired Immunity or Adaptive Immunity.
- Vaccines help your immune system fight infections faster and more effectively.
- When you get a vaccine, it sparks your immune response, helping your body fight off and remember the germ so it can attack it if the germ ever invades again.
- Vaccines thus play a role in ACQUIRED/ADAPTIVE immunity

ACQUIRED / ADAPTIVE IMMUNITY



Acquired Immunity

Immunity you develop during your life

Active Immunity

Immunity you develop after being exposed to an infection or from getting a vaccine

Passive Immunity

Immunity you acquire from someone or something else

Natural

Antibodies made after exposure to an infection

Artificial

Antibodies made after getting a vaccination

Natural

Antibodies transmitted from mother to baby (e.g., via mother's milk)

Artificial

Antibodies acquired from an immune serum medicine

ACTIVE IMMUNITY VS PASSIVE IMMUNITY

Active immunity refers to immunity, which results from the production of antibodies by the person's own immune system in response to a direct contact of an antigen	Passive immunity refers to a short-term immunity which results from the introduction of antibodies from the outside
Mediated by the antibodies produced by the person's own cells	Mediated by the antibodies produced outside the body
The pathogen has direct contact with the body	The pathogen doesn't have direct contact with the body
Does not generate a rapid response	Generates a rapid response
May last for a long time	May not last for a long time
Generates an immunological memory	Does not generate an immunological memory
Side effects are very low	The body may react to antisera
Does not work in immunodeficient hosts	Works in immunodeficient hosts

VACCINE

- A vaccine typically contains an agent that resembles a disease causing agent.
- It is often made up from weakened or killed forms of the microbe, its toxins or one of its surface proteins.
- The vaccine stimulates the body's immune system to recognize the agent **as a threat, destroy it, and keep a record of it**, so that the immune system can more easily recognize and destroy any of these pathogens that it later encounters.

PURPOSE OF A VACCINE

- By priming the immune system with vaccination, when the vaccinated individual is later exposed to the live pathogens in the environment, the immune system can destroy them before they can cause fatality.
- Vaccination: The act of introducing a vaccine to give you immunity to a specific disease
- Immunization: The process by which vaccination protects you from a disease

Vaccination	Immunization
The process involves introducing a weakened / deactivated disease causing microbes into a person	The process starts after the person is exposed to the vaccine and the body starts building resistance to that disease
It is usually injected or administered orally	It is not administered in any way. The body develops resistance from vaccines.
Imovax Rabies is the trade name for rabies vaccine	The body builds up immunity through this vaccine for the disease rabies.
Vaccination does not guarantee complete resistance to a disease	Complete immunity occurs when the person fully recovers from the disease.
Usually, if mutation happens to microbes, it might render the vaccine ineffective (this is the reason why common cold has no vaccine)	Similarly, variations of a disease impact the body's ability to generate an immune response.

VACCINE TERMINOLOGY

- Active immunity: The production of antibodies against a specific disease by the immune system. Active immunity can be acquired in two ways, either by contracting the disease or through vaccination. Active immunity is usually permanent, meaning an individual is protected from the disease for the duration of their lives.
- Adjuvant: A vaccine component distinct from the antigen that enhances the immune response to the antigen.
- Adverse events: An “adverse event” is any health problem that happens after a shot or other vaccine. An adverse event might be truly caused by a vaccine, or it might be pure coincidence.
- Allergy: A condition in which the body has an exaggerated response to a substance (e.g. food or drug). Also known as hypersensitivity.
- Antibody: A protein found in the blood that is produced in response to foreign substances (e.g. bacteria or viruses) invading the body. Antibodies protect the body from disease by binding to these organisms and destroying them.

VACCINE TERMINOLOGY

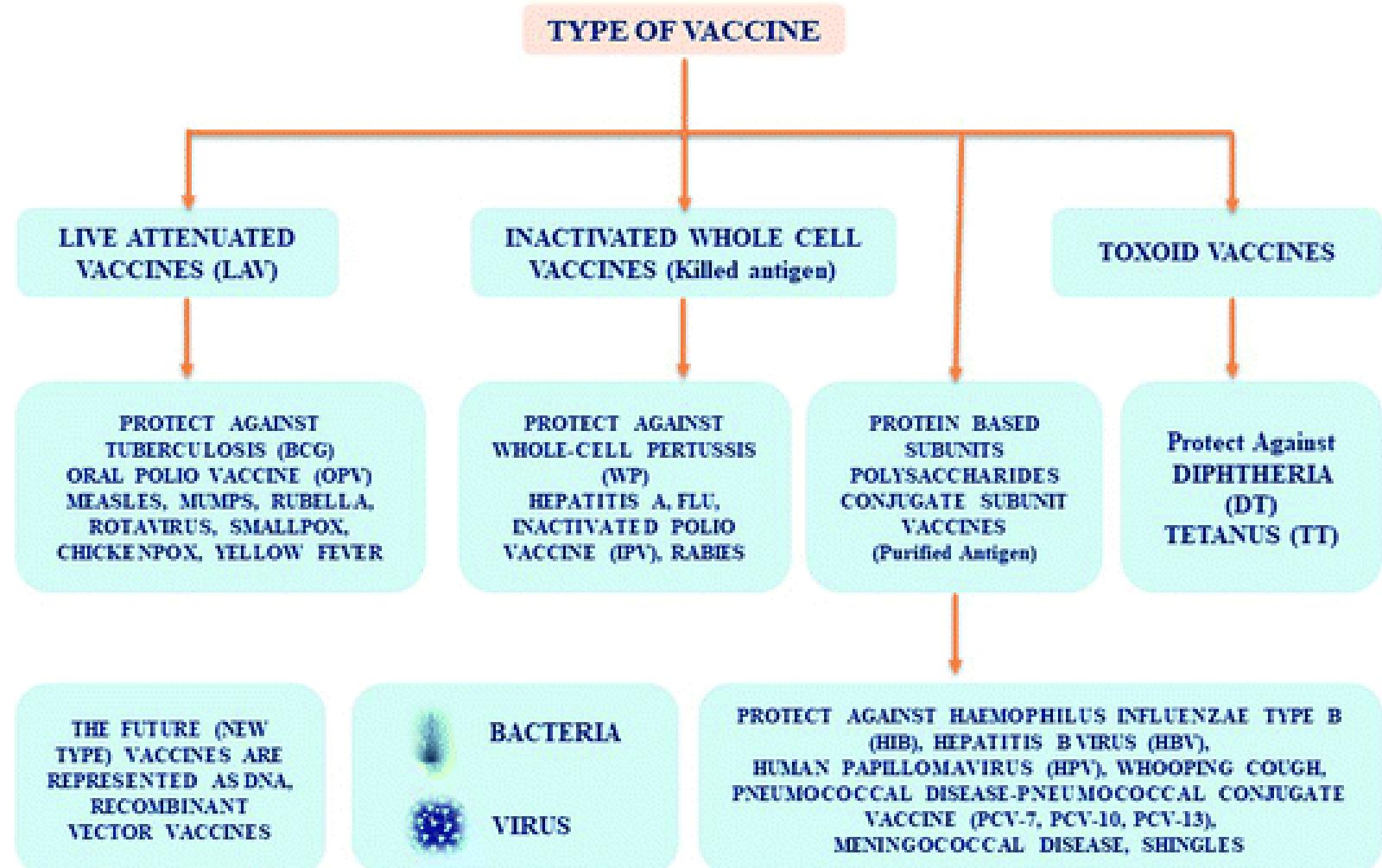
- Antigens: Foreign substances (e.g. bacteria or viruses) in the body that are capable of causing disease. The presence of antigens in the body triggers an immune response, usually the production of antibodies.
- Antitoxin: A solution of antibodies against a toxin. Antitoxin can be derived from either human (e.g., tetanus immune globulin) or animal (usually equine) sources (e.g., diphtheria and botulism antitoxin). Antitoxins are used to confer passive immunity and for treatment.
- Antiviral: Literally “against-virus” — any medicine capable of destroying or weakening a virus.
- Efficacy rate: A measure used to describe how good a vaccine is at preventing disease.
- Epidemic: The occurrence of disease within a specific geographical area or population that is in excess of what is normally expected.
- Endemic: Present in a given area, though usually at low or baseline levels.
- Hypersensitivity: A condition in which the body has an exaggerated response to a substance (e.g. food or drug). Also known as an allergy.

VACCINE TERMINOLOGY

- Hypersensitivity: A condition in which the body has a weakened or delayed reaction to a substance.
- Immune globulin: A protein found in the blood that fights infection. Also known as gamma globulin.
- Memory Cell: A group of cells that help the body defend itself against disease by remembering prior exposure to specific organisms (e.g. viruses or bacteria). Therefore these cells are able to respond quickly when these organisms repeatedly threaten the body.
- Pathogens: Organisms (e.g. bacteria, viruses, parasites and fungi) that cause disease in human beings.
- Recombinant: Of or resulting from new combinations of genetic material or cells; the genetic material produced when segments of DNA from different sources are joined to produce recombinant DNA.
- Side Effect: Undesirable reaction resulting from immunization.
- Strain: A specific version of an organism. Many diseases, including HIV/AIDS and hepatitis, have multiple strains.
- Viremia: The presence of a virus in the blood.
- Virulence: The relative capacity of a pathogen to overcome body defenses.

TYPES OF VACCINES

- Live, attenuated vaccines
- Inactivated vaccines
- Subunit vaccines
- Toxoid vaccines
- Conjugate vaccines
- DNA vaccines
- Recombinant vector vaccines



LIVE, ATTENUATED VACCINE

- Microorganisms can be attenuated or disabled so that they lose their ability to cause significant disease (pathogenicity) but retain their capacity for transient growth within an inoculated host.
- The first vaccine used by Jenner is of this type. Inoculation of humans with vaccinia (cowpox) virus confers immunity to smallpox (without causing smallpox).
- Attenuation can often be achieved by growing a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions.
- Live-attenuated vaccines differ from traditional inactivated vaccines where the pathogen is “killed”, and as the name suggests the pathogen (typically a virus) remains active in live vaccines, however, is attenuated or modified in a way that the pathogen is not able to cause disease itself but can produce a robust immune response.
- This helps to select mutants that are better suited for growth in the abnormal culture conditions than in the natural host.

LIVE, ATTENUATED VACCINE

- For example, an attenuated strain of *Mycobacterium bovis* called *Bacillus Calmette- Guerin (BCG)* was developed by growing *M. bovis* on a medium containing increasing concentrations of bile.
- After 13 years, this strain had adapted to growth in strong bile and became sufficiently attenuated that it was suitable as a vaccine for tuberculosis.
- The attenuated vaccines can replicate within host cells and particularly suitable for inducing cell-mediated responses. It requires only a single immunization.
- LAV vaccines are more similar to the actual infection.
- Albeit, it is efficient, but not everyone can be administered these vaccines, including children and patients undergoing chemotherapy as their immune system is too weak.

LIVE, ATTENUATED VACCINE

- Live-attenuated vaccines can be produced by reverse genetics including RNAi. Briefly, reverse genetic tools are used to create live-attenuated vaccines.
- Genes from current (novel) viruses are combined with previously altered (attenuated) viruses belonging to the same generic strain.
- The immunological mechanism of live-attenuated vaccines usually involves a broad immune response including the involvement of CD4+ & CD8+ T lymphocytes (T-cells) as well as antibodies against the pathogen (produced by B-cells). Live-attenuated vaccines usually result in long-term (potentially life-long) protection without requiring additional doses in adulthood.

Advantages	Disadvantages
live-attenuated vaccines over some other forms of vaccines (such as inactivated) include the production of a robust, strong antibody and cell-mediated immune response, long-lasting immunity, with a relatively quick onset of action.	may include production, maintenance & transport considerations due to the use of live pathogens and the fact that because they are live pathogens it could lead to issues in immunocompromised individuals

EXAMPLES OF LAV

- Live-attenuated Influenza (flu) vaccines (LAIV)
- Measles, Mumps & Rubella (MMR)
- Polio – nearing global eradication due to mass vaccination
- Smallpox – officially eradicated due to mass vaccination
- Whilst the majority of these vaccines are for viruses, some do exist for specific bacterial infections. These include cholera, TB and oral typhoid vaccines.
- Chickenpox
- Yellow Fever
- Japanese encephalitis
- Shingles
- Rotavirus.

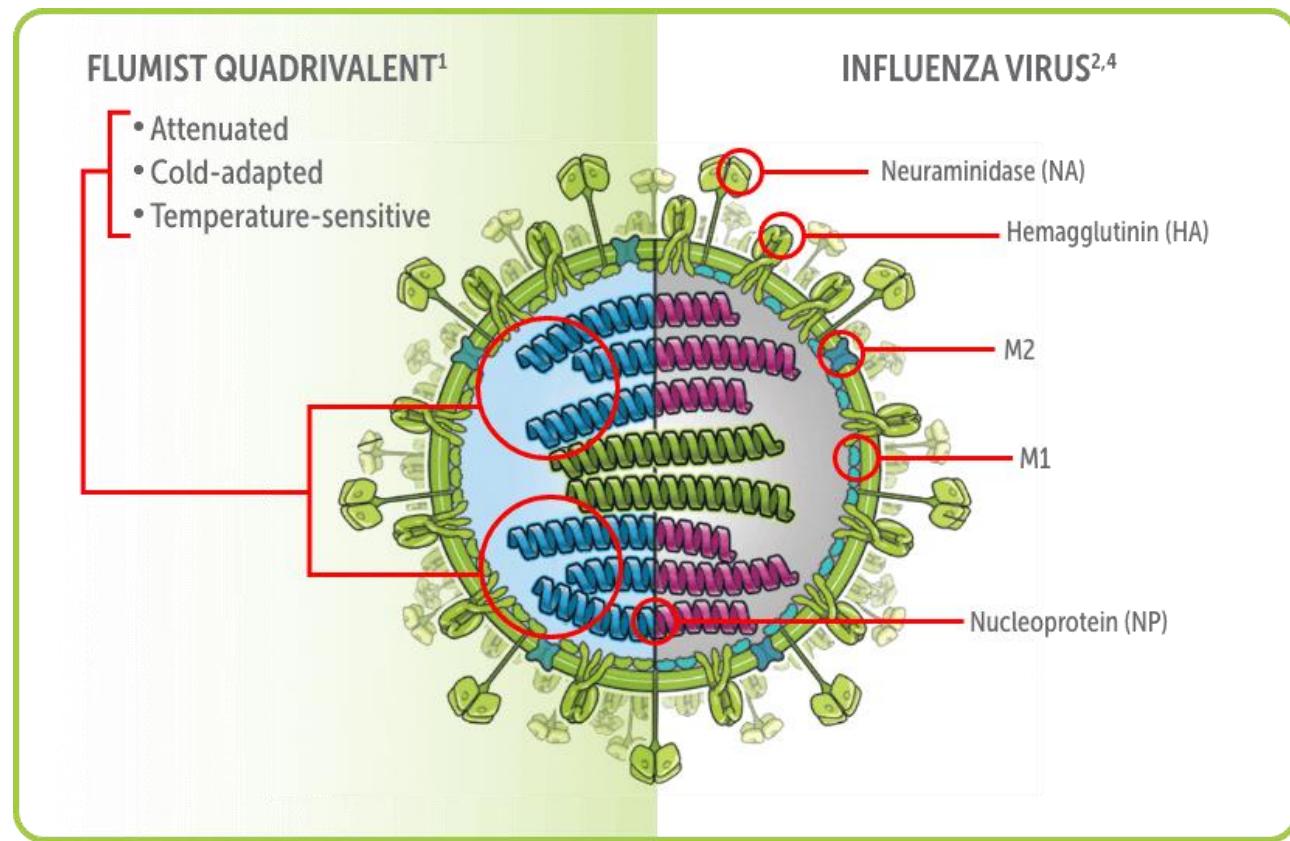
LIVE-ATTENUATED INFLUENZA VACCINES (LAIV)

Influenza causes worldwide seasonal epidemics each year

To combat influenza, a variety of different vaccines are available to use including inactivated vaccines and live-attenuated.

The LAIV can provide up to 90% protection in adults under 65 years of age, and up to 40% of adults over 65 (though inactivated vaccines are preferred for older individuals).

LAIVs are usually administered via the nose – mimicking the natural infection route of the influenza virus.



<https://www.flumistquadrivalenthcp.com/laiv-composition-and-replication.html>

LIVE-ATTENUATED INFLUENZA VACCINES (LAIV)

- The influenza A virus has 8 RNA segments.
- In the live attenuated vaccine, a combination of 6 attenuated segments combined with 2 normal (WT) segments – engineered into plasmids – creating a 6:2 reassortment LAIV.
- LAIVs are typically produced in 10–11-day old chicken embryonated eggs using a WHO-approved virus variant and a master donor virus (MDV).
- The 6:2 reassorted virus is passaged in the presence of neutralizing antibodies against the MDV at low temperatures so that it becomes cold-adapted & temperature-sensitive.
- This means it no longer replicates in the lower-respiratory tract, which is warmer and where influenza causes disease.
- Instead, it allows it to replicate in the slightly colder upper respiratory tract to elicit an immune response without causing the disease.

MUMPS, MEASLES & RUBELLA (MMR) VACCINE

- The MMR vaccine offers a high level of protection (over 90% effective overall) against mumps, measles and rubella and is usually given as part of a 2-dose regimen in toddlers.
- Due to the MMR vaccine, the levels of mumps, measles and rubella infections and fatalities are incredibly low, though cases have increased in unvaccinated populations.
- The MMR vaccine usually contains an attenuated rubella virus grown in human cell strains, whereas the attenuated measles and mumps viruses are grown using chicken embryo cells (not chicken embryonated eggs).
- The MMR vaccine may also incorporate attenuated varicella virus; chickenpox, in the MMRV vaccine.
- Whilst these vaccines are incredibly safe, they do come with some side effects, and the MMRV vaccine has a higher rate of side effects compared to the MMR vaccine.



BCG VACCINE

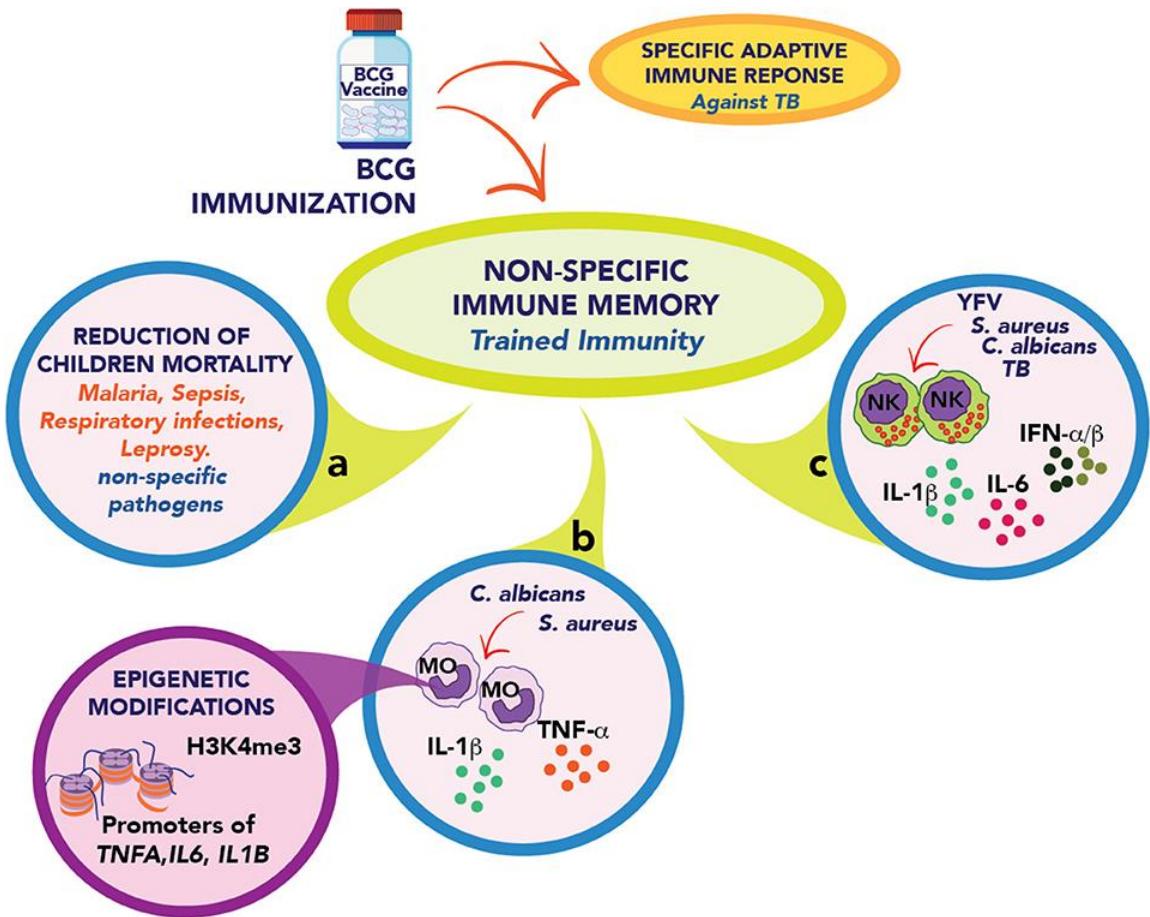
- BCG, or bacille Calmette-Guerin, is a vaccine for tuberculosis (TB) disease.
- Bacillus Calmette-Guerin (BCG) is the live attenuated vaccine form of *Mycobacterium bovis* used to prevent tuberculosis and other mycobacterial infections.
- The vaccine was developed by Calmette and Guerin and was first administered to human beings in 1921.
- BCG is the only vaccine against tuberculosis.
- It is the most widely administered vaccine and usually a part of the routine newborn immunization schedule.
- BCG vaccine also offers protection against non-tuberculous mycobacterial infections like leprosy and Buruli ulcer.
- It is also used in the treatment of superficial carcinoma of the bladder.

BCG VACCINE

BCG can induce the expansion of T cells that recognize epitopes against other bacteria (different than *M. tb*) and viruses [e.g., severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)].

Although the underlying mechanism for cross-protection is not yet fully understood, it is thought to rely on epigenetic changes and metabolic modifications on immune cells.

New data have suggested an association between decreased mortality rates due to SARS-CoV-2 infection with a high rate of BCG vaccination, suggesting that this vaccine might potentially generate cross-protective immunity against this virus, which remains to be robustly tested.



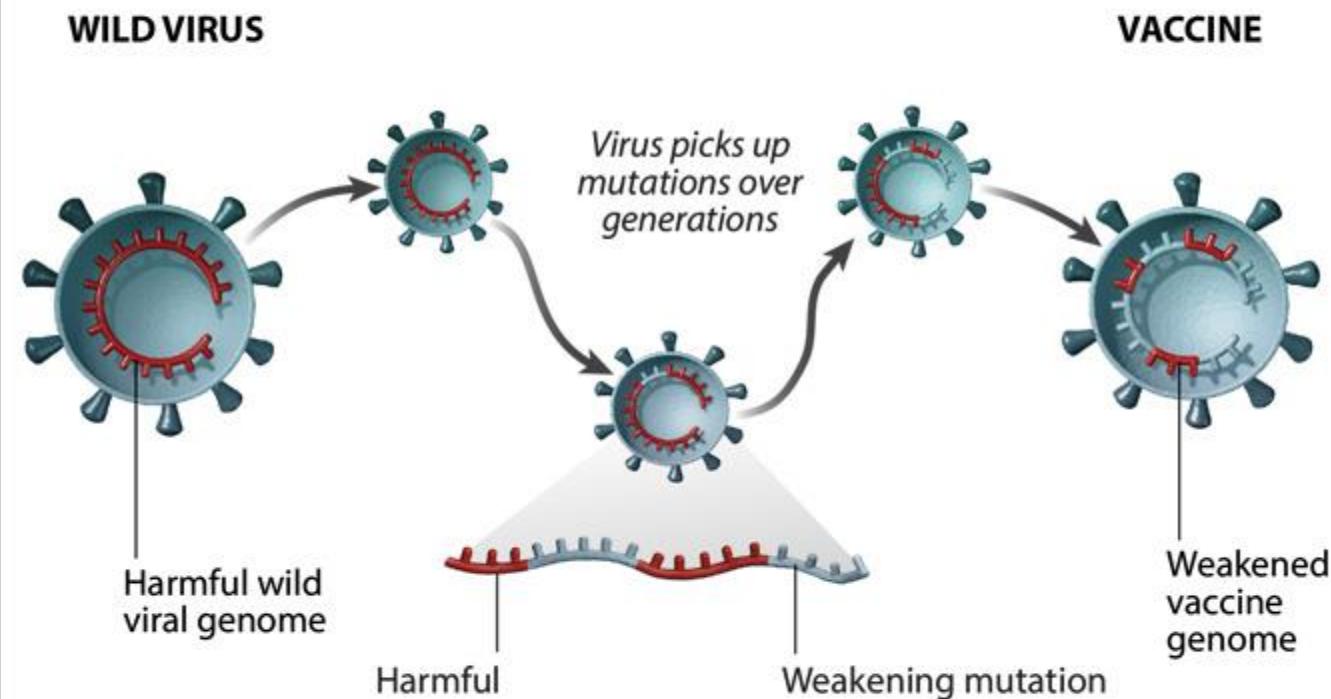
Schematic representation of trained immunity elicited by BCG immunization. (A) The BCG vaccine develops a specific adaptive and protective immune response against *M. tuberculosis*. It also promotes a non-specific immune memory called Trained immunity. The BCG vaccine contributes in many countries to reducing the infection rate of children against other unrelated pathogens such as malaria, respiratory infections, and leprosy. (B) BCG vaccination in adults leads to a trained phenotype in circulating monocytes (MO) that quickly respond, secreting IL-1 β , TNF- α , and IL-6 after stimulation with unrelated pathogens such as *S. aureus* and *C. albicans*. This response is explained by epigenetic modifications in regulatory elements of *tnfa*, *il6*, and *il1b* genes. (C) In healthy human volunteers, the vaccination enhanced the capacity of NK cells to secrete proinflammatory cytokines and type I interferons after stimulation with *M. tuberculosis*, *S. aureus*, *C. albicans*, and Yellow fever virus (YFV).

CONSIDERATIONS OF LAV

- Whilst live-attenuated vaccines lead to strong, robust and long-term immunity to viruses (and some bacteria) in most healthy individuals, there may be cases where such vaccines may not be appropriate. Specifically, individuals with compromised immune systems – either due to chemotherapy, HIV infection, or primary disorders of immunodeficiency should not be given live-attenuated vaccines.
- Compared to inactivated vaccines, live-attenuated vaccines also need to be carefully prepared, stored, transported and administered. These normally need to be kept uninterruptedly at cold temperatures, and this may present a challenge to more remote areas of the world or where such facilities do not exist.
- In summary, live-attenuated vaccines are highly effective and safe vaccines used in the prevention of a variety of viral diseases (including influenza, measles, mumps, rubella & chickenpox) as well as some bacterial diseases (such as cholera and TB). In these vaccines, the pathogen remains viable, but altered in a way that it can cause an immune response, but not lead to infection. Generally, these vaccines produce more robust and broader immune responses compared to inactivated (killed pathogen) vaccines.

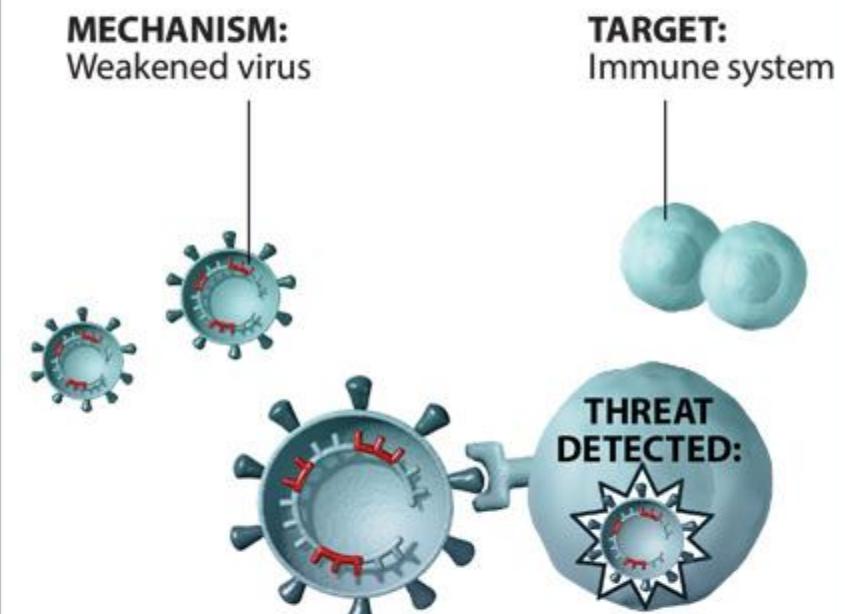
What are Live, Attenuated Vaccines?

Live vaccines are “wild” viruses or bacteria that have been weakened.* In the lab, generally the virus is passed through many generations of cells to pick up genetic mutations which weaken it - so much it won’t cause disease in your body.



Vaccine Target

Live, attenuated vaccines target your body’s immune system directly. They are strong enough to trigger the immune response, but too weak to cause disease.



*Did You Know?: “Attenuated” means weakened.

INACTIVATED OR "KILLED" VACCINE

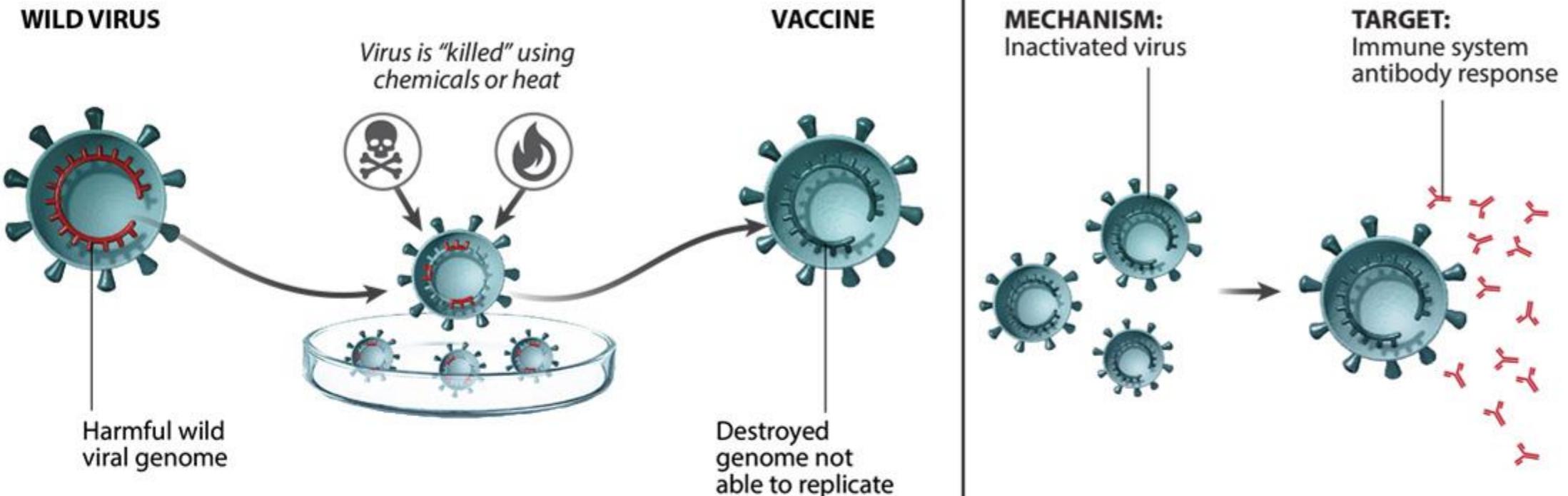
- The pathogen is treated with heat or chemicals, killed making it incapable of replication, but allows it to induce an immune response to at least some of the antigens contained within the organism.
- It is important to maintain the structure of epitopes on the surface antigens during inactivation. Heat inactivation is often unsatisfactory because it causes extensive denaturation of proteins.
- Chemical inactivation with formaldehyde or various alkylating agents has been successful.
- Killed vaccines often require repeated boosters to achieve a protective immune status as they do not replicate in the host.
- Killed vaccines typically induce a predominantly humeral antibody response and are less effective than attenuated vaccines in inducing cell-mediated immunity.
- The safety of inactivated vaccines is greater than that of live attenuated vaccines.
- Inactivated vaccines usually don't provide immunity (protection) that's as strong as live vaccines. So you may need several doses over time (booster shots) in order to get ongoing immunity against diseases.

ADVANTAGES AND DISADVANTAGES OF INACTIVATED VIRUS VACCINES

- Well-established technology
- Suitable for people with compromised immune systems
- No live components, so no risk of the vaccine triggering disease
- Relatively simple to manufacture
- Relatively stable
- Booster shots may be required

What are Inactivated Vaccines?

Live vaccines are “wild” viruses or bacteria that have been inactivated.* In the lab, a wild virus is “killed” with heat or chemicals so it cannot replicate or cause disease in your body, and is safe for immunodeficient people.



INACTIVATED OR "KILLED" VACCINE

Inactivated vaccines include whole-cell inactivated vaccines (e.g., polio, hepatitis A, and rabies vaccines),

subunit vaccines (e.g., influenza and pneumococcal vaccines),

toxoids (e.g., diphtheria and tetanus toxoid),

recombinant vaccines (e.g., hepatitis B, human papillomavirus [HPV], and influenza

[Flublok brand]).

WHOLE-CELL INACTIVATED VACCINES

- contain bacteria or viruses that have been killed through a physical or chemical process.
- Whole virus vaccines use a weakened (attenuated) or deactivated form of the pathogen that causes a disease to trigger protective immunity to it.
- Inactivated virus vaccines also contain the disease-causing virus, or parts of it, but their genetic material has been destroyed.
- For this reason, they are considered safer and more stable than live attenuated vaccines, and they can be given to people with compromised immune systems.
- Even though their genetic material has been destroyed, inactivated viruses usually contain many proteins which the immune system can react to.
- But because they cannot infect cells, inactivated vaccines only stimulate antibody-mediated responses, and this response may be weaker and less long-lived.
- To overcome this problem, inactivated vaccines are often given alongside adjuvants (agents that stimulate the immune system) and booster doses may be required.

SUBUNIT VACCINES

- Subunit vaccines are made from a piece of a pathogen, not the whole organism, so they do not contain any live pathogens. Some important subunit vaccines are polysaccharide vaccines, conjugate vaccines, and protein-based vaccines.
- Polysaccharide vaccines target an immune response to pathogenic bacteria that are encased in a layer of sugar, this means they help you make protective responses against the surface of the bacteria, allowing your body to kill the bacteria. These do not work, and therefore are not used, in children under 2.
- Conjugate vaccines are the same in that they have a polysaccharide component, but that sugar is stuck to a protein so your immune system will respond to the sugar on the bacteria better, They also help your body remember the bacteria better, so if you get infected in the future, the immune response will be better. Importantly, these vaccines do work in children under 2.

SUBUNIT VACCINES

- Protein-based vaccines allow you to make a protective response against a protein on the surface of a virus, against a protein on the surface of a bacteria, or against a secreted toxin. In this case, the immune response is against the protein components of the bacteria or virus, not the sugar coat. Certain proteins on the surface of bacteria or viruses help the pathogen cause disease, so inducing an immune response against them can help the body fight against the infection or the toxic effects of the toxin.
- Subunit vaccines can be made one of two ways: from the original pathogen or recombinantly. Recombinant vaccines use another organism to make the vaccine antigen.
- Examples: Haemophilus influenzae type B (Hib) vaccine (conjugate), pneumococcal vaccine (polysaccharide or conjugate), shingles vaccine (recombinant protein), hepatitis B (recombinant protein), acellular pertussis, MenACWY (conjugate).

Advantages and disadvantages of protein subunit vaccines

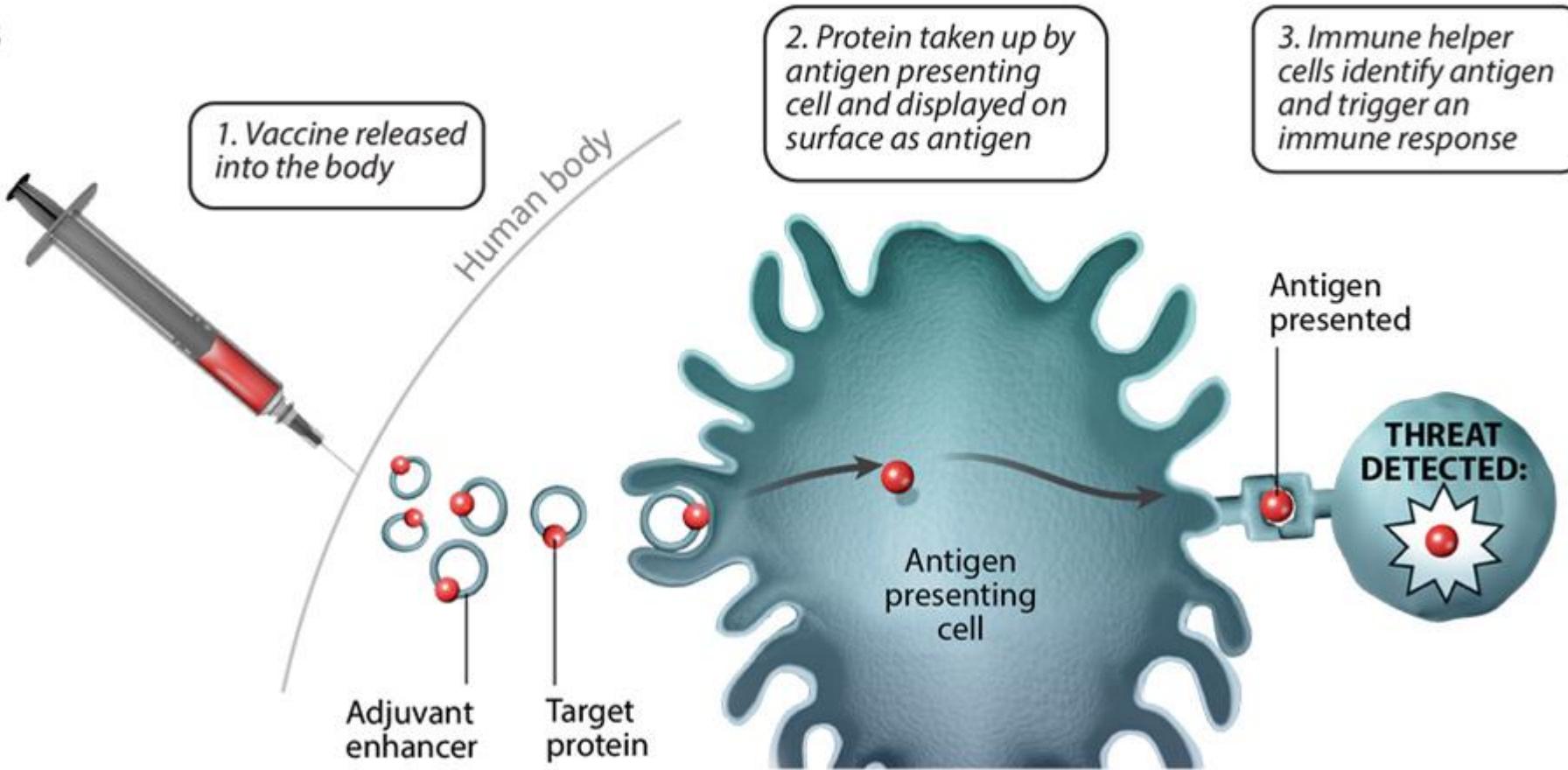
- | | |
|--|---|
| <ul style="list-style-type: none">• Well-established technology• Suitable for people with compromised immune systems• No live components, so no risk of the vaccine triggering disease | <ul style="list-style-type: none">• Relatively stable• Relatively complex to manufacture• Adjuvants and booster shots may be required• Determining the best antigen combination takes time |
|--|---|

What are Subunit (recombinant, polysaccharide, and conjugate) vaccines?

Subunit vaccines use a portion of a bacteria or virus to cause an immune response independent of its virus or bacteria of origin. Elements of subunit vaccines can be proteins, polysaccharide chains, or a combination of these.

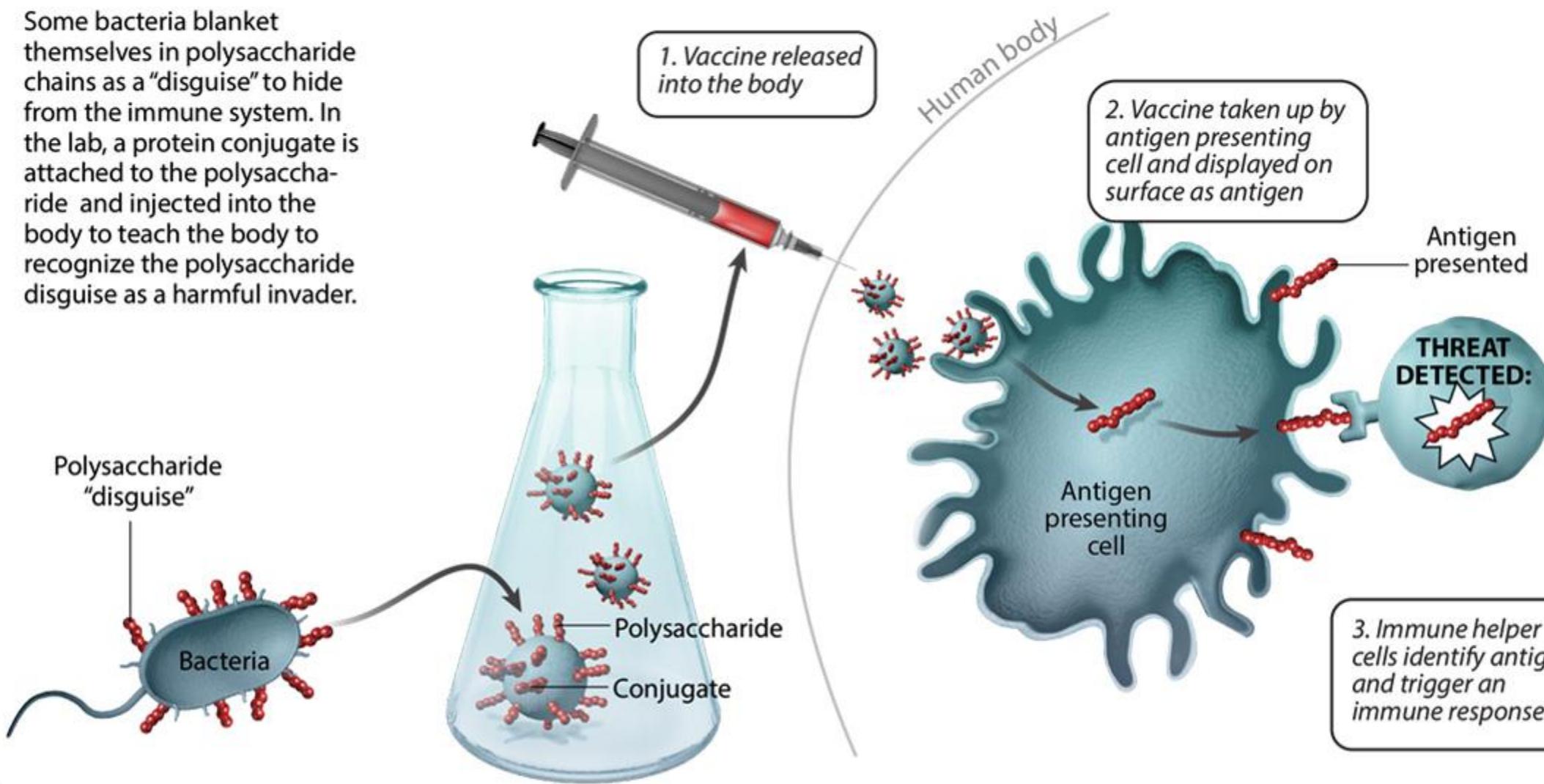
PROTEIN VACCINES

Viral proteins are isolated in a lab, mixed with an adjuvant immune-system stimulator, and injected into the body to cause an immune response without the virus that makes you sick.



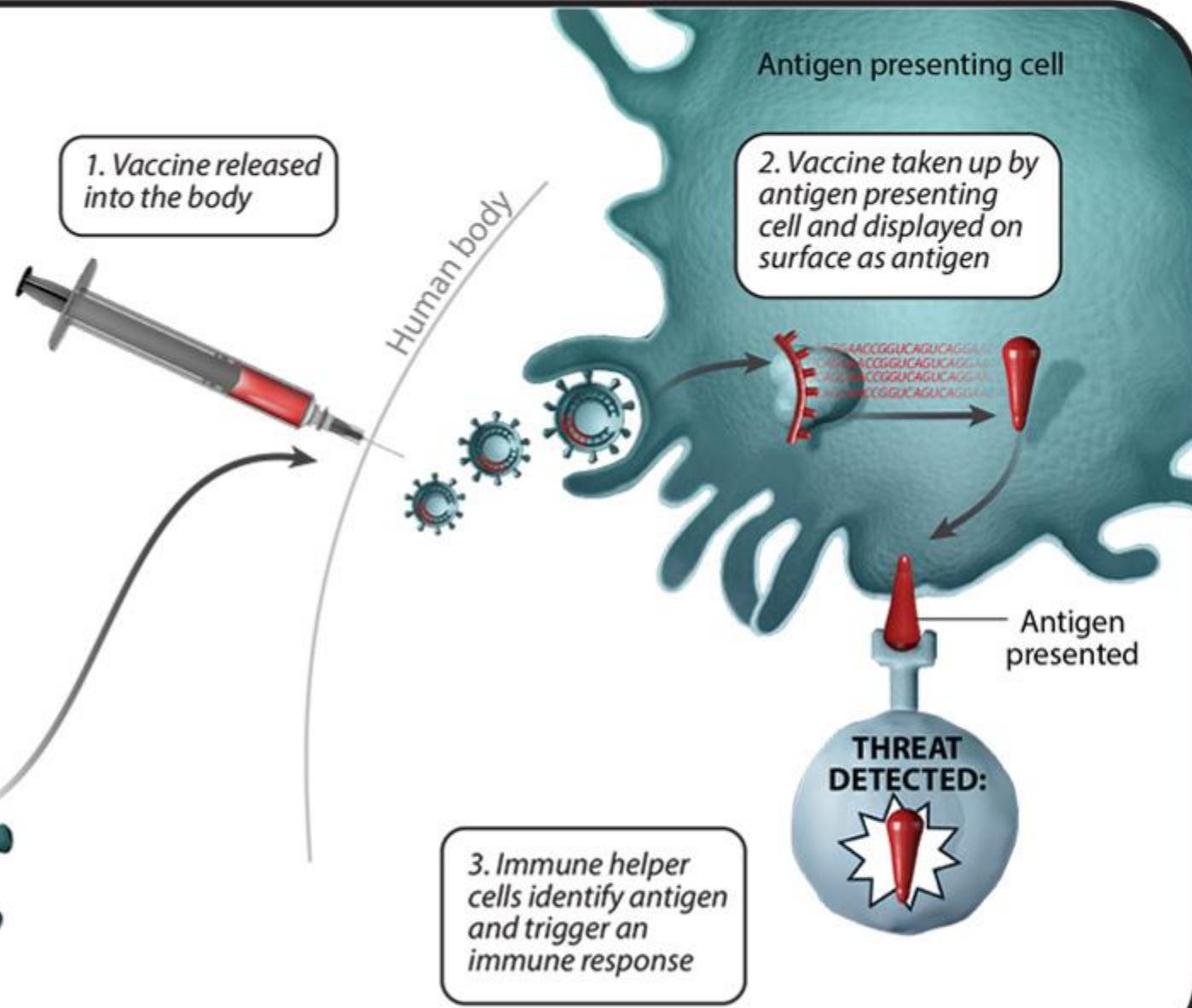
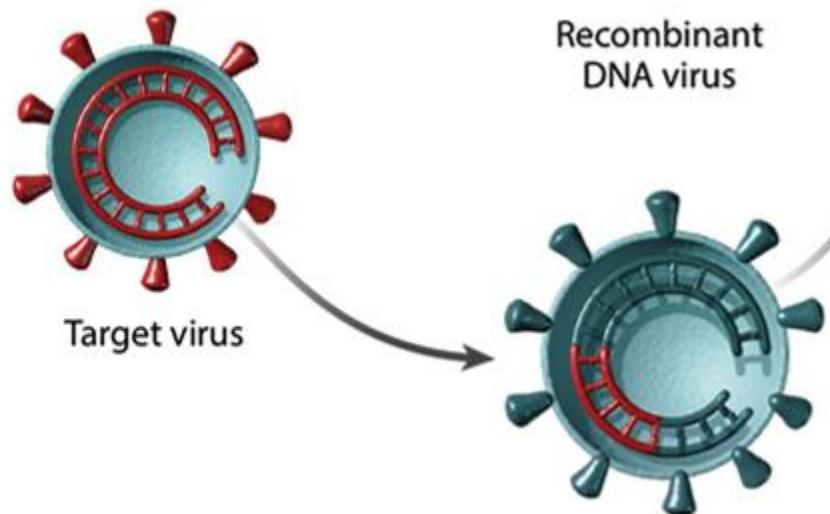
POLYSACCHARIDE AND CONJUGATE VACCINES

Some bacteria blanket themselves in polysaccharide chains as a "disguise" to hide from the immune system. In the lab, a protein conjugate is attached to the polysaccharide and injected into the body to teach the body to recognize the polysaccharide disguise as a harmful invader.



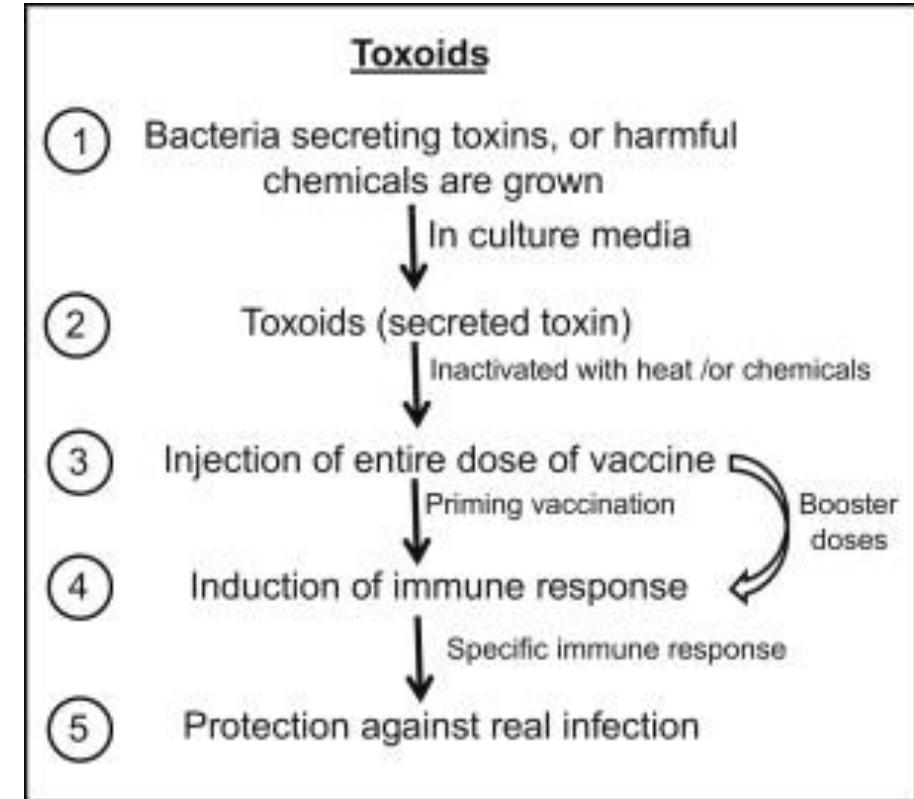
RECOMBINANT VACCINES

Viruses, bacteria, or cells can be created in the lab which carry DNA coding for surface proteins from a virus or bacteria. These harmless hybrids can be injected into the body to cause an immune response to the viral surface proteins without making you sick.



TOXOID VACCINE

- Some bacteria produce disease in their host by producing exotoxins.
- Toxoid Vaccines are inactivated exotoxins. The exotoxins are treated with heat/ chemicals to inactivate it.
- This makes them unable to cause the disease but can stimulate the body to produce antitoxoid antibodies which are capable of binding to the toxins and neutralizing their effects.
- Long-lasting immunity against bacterial diseases such as tetanus and diphtheria is induced by a course of toxoid vaccines which cause an immune response against weakened versions of specific bacterial toxins called toxoids.
- Example: Tetanus, Diphtheria bacterial vaccines



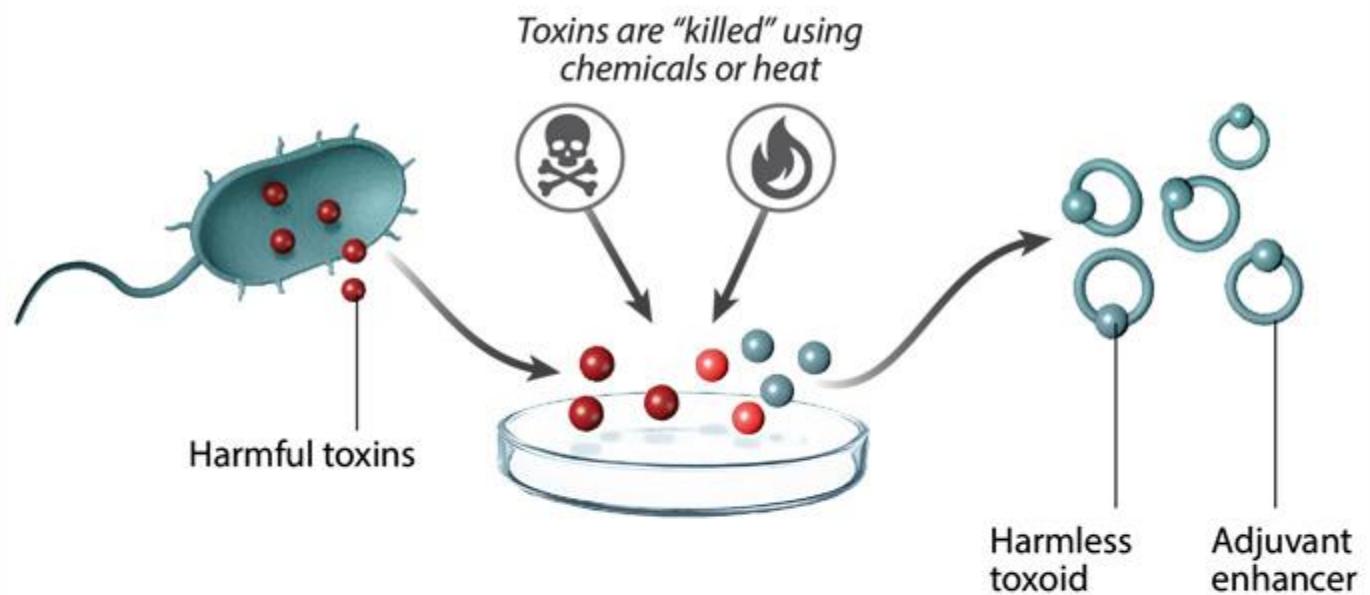
TOXOID VACCINE

- Toxoid vaccines use toxoids (as antigens) to induce an immune response in protecting against diseases caused by toxins secreted by specific bacteria.
- By using toxoids, the body is able to form an immune response to the original toxin (maintained immunogenicity), but since the toxoid is a weakened form of the toxin, it cannot lead to any toxicity or toxin-induced disease.
- Compared to other vaccines, toxoid vaccines are more stable and less susceptible to damage caused by temperature, humidity, or light.
- During the immune response mounted by the body in response to exposure to toxoids, Th2 (CD4+) and B-cells become activated which produce immunoglobulins against the immunogenetic part of the toxoid (which is the same as it is in the toxin), allowing for protection against the actual toxin should exposure ever occur.
- toxoid vaccines are given as part of a course of multiple doses throughout childhood and adulthood for maximum protection, and booster shots can be given if you are traveling to a high-risk country for example.

What are Toxoid Vaccines?

Toxoid* vaccines neutralize the toxic activity created by bacteria, instead of the harmful bacteria itself, neutralizing activity which normally makes you sick.

WILD BACTERIA PRODUCING TOXINS

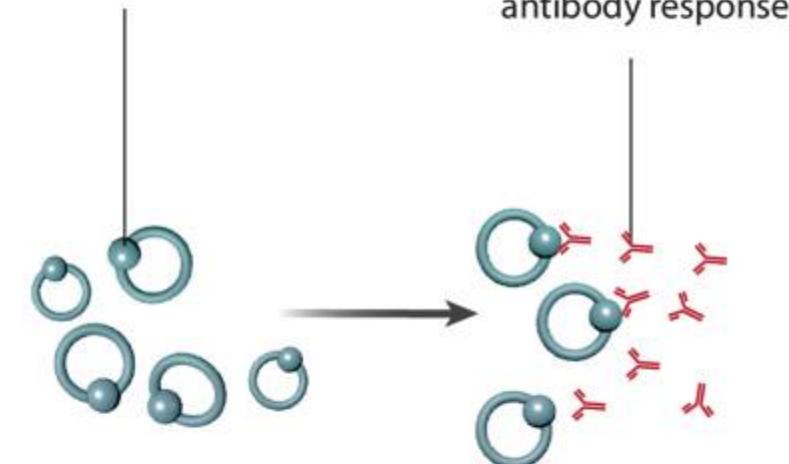


Vaccine Target

The vaccine targets toxic activity, creating an antibody response to that toxin.

MECHANISM:

Inactivated toxins



TARGET:

Immune system antibody response

*Did You Know?: A toxoid is an inactivated, harmless form of a toxin.

TETANUS

- Common diseases caused by bacterial toxins are typically immunized against using toxoid vaccines. Specific examples include vaccinations against tetanus (*Clostridium tetani*), diphtheria (*Corynebacterium diphtheriae*), botulism (*Clostridium botulinum*) and whooping cough; pertussis (*Bordetella pertussis* – though this tends to be bacterial components rather than toxoids per se, but components are incorporated alongside toxoids of other bacteria).
- Tetanus (caused by *Clostridium tetani*) leads to muscular spasms occurring across the body which last for a few minutes at a time.
- Whilst recovery can occur naturally in most people over the course of many months, it is also fatal in approximately 10% of all non-vaccinated cases. Vaccinations against tetanus have dramatically cut the number of cases worldwide (up to 95%) as well as the fatality rate.
- Almost all cases of tetanus that occur nowadays happen in non-immunized and inadequately immunized individuals.
Unlike a majority of toxins and pathogens, exposure and recovery to natural tetanus toxins do not result in natural immunity due to the potency of the tetanus toxin.
- However, exposure to the tetanus toxoid (TT) vaccine results in robust immunological memory. It is a safe and effective vaccine, but it can result in localized inflammation (redness and pain) at the site of injection in as many as 25-85% of all people, though severe allergic reactions are extremely rare.

DIPHTHERIA

- Diphtheria (caused by *Corynebacterium diphtheriae*) leads to symptoms of fever, swollen glands, and a thick grey-white coating at the back of the throat. If it infects the skin (such as through wounds or cuts), it can cause large pus-filled blisters or ulcers.
- The Diphtheria vaccine has resulted in a 90% reduction in cases worldwide and tends to have very few side effects apart from a raised bump at the site of injection which may last for several weeks. Alongside tetanus, diphtheria is immunized against using a combination vaccine.

DPT VACCINE

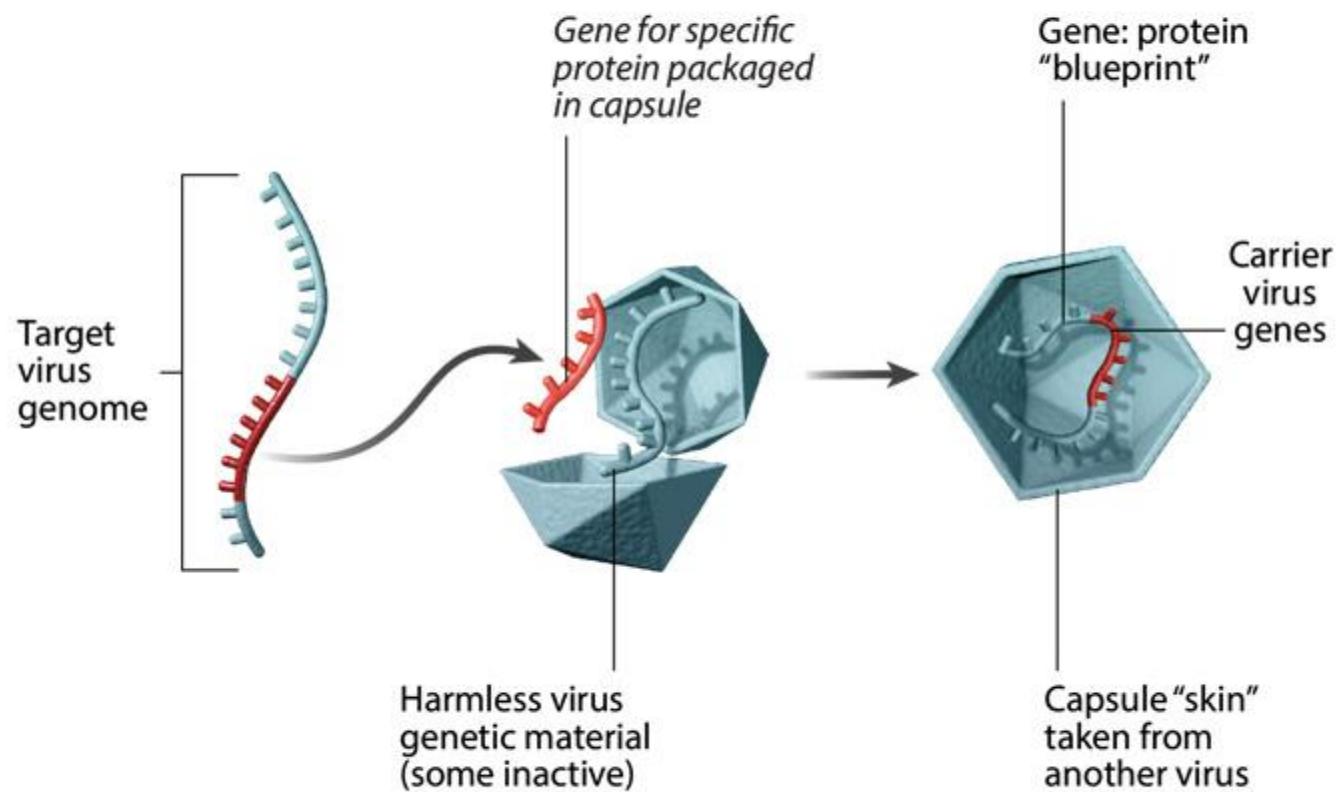
- The Diphtheria, Pertussis & Tetanus (DPT) vaccine is a trivalent vaccine that incorporates toxoids and killed bacteria from the 3 different bacteria which cause the 3 diseases. Typically, the DPT vaccine contained toxoids of *Corynebacterium diphtheriae* & *Clostridium tetani*, whilst containing killed cells from *Bordetella pertussis*.
- The DTP vaccine is typically given as part of a course of immunization starting in young children (part of a hexavalent 6-in-1 vaccine) and as boosters (DTP only) in teenagers (as part of 5 doses in the UK for example). Specific administration of doses varies in different countries. The DTP vaccine should not be given to those that suffer from severe anaphylaxis to a previous toxoid vaccine, or to one of the ingredients in the vaccine.
- There are variations of the DTP vaccine including the DTaP and Tdap vaccines. The DTaP vaccine (DTPa/TDaP) also protects against the 3 diseases, but the pertussis component is acellular rather than the whole-cell components used in the DTP vaccine. The TDaP vaccine is a tetanus toxoid with reduced diphtheria toxoid and reduced acellular pertussis components usually given in adults or as boosters.

RECOMBINANT VECTOR VACCINES

- Live attenuated vaccines prolong antigen delivery and encourage cell-mediated responses, but have the disadvantage of reverting to pathogenic forms rarely.
- Recombinant vectors maintain the advantages of live attenuated vaccines while avoiding this major disadvantage.
- Individual genes that encode key antigens of especially virulent pathogens can be introduced into attenuated viruses or bacteria.
- The attenuated organism serves as a vector, replicating within the vaccinated host and expressing the gene product of the pathogen.
- Since most of the genome of the pathogen is missing, reversion potential is virtually eliminated.
- Recombinant vector vaccines have been prepared utilizing existing licensed live, attenuated vaccines and adding to them genes encoding antigens present on newly emerging pathogens.
- Such chimeric virus vaccines can be more quickly tested and approved than an entirely new product.

What is a Viral Vector Vaccine?

Made of a small section of a virus' genetic material - the instructions or 'blueprint' for a specific protein. The viral capsule or shell from another virus carries the gene safely to your cells.

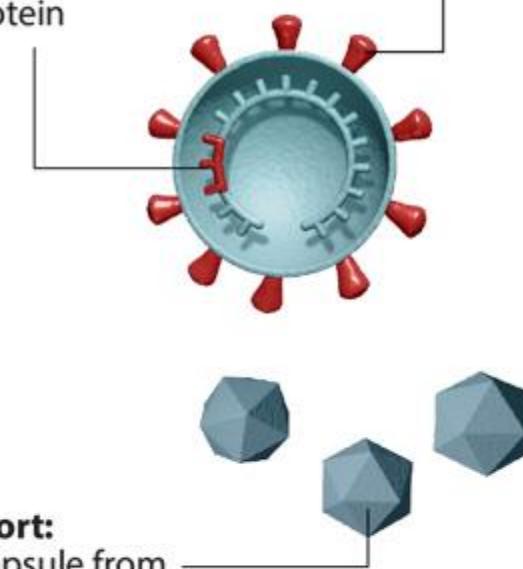


Vaccine Target

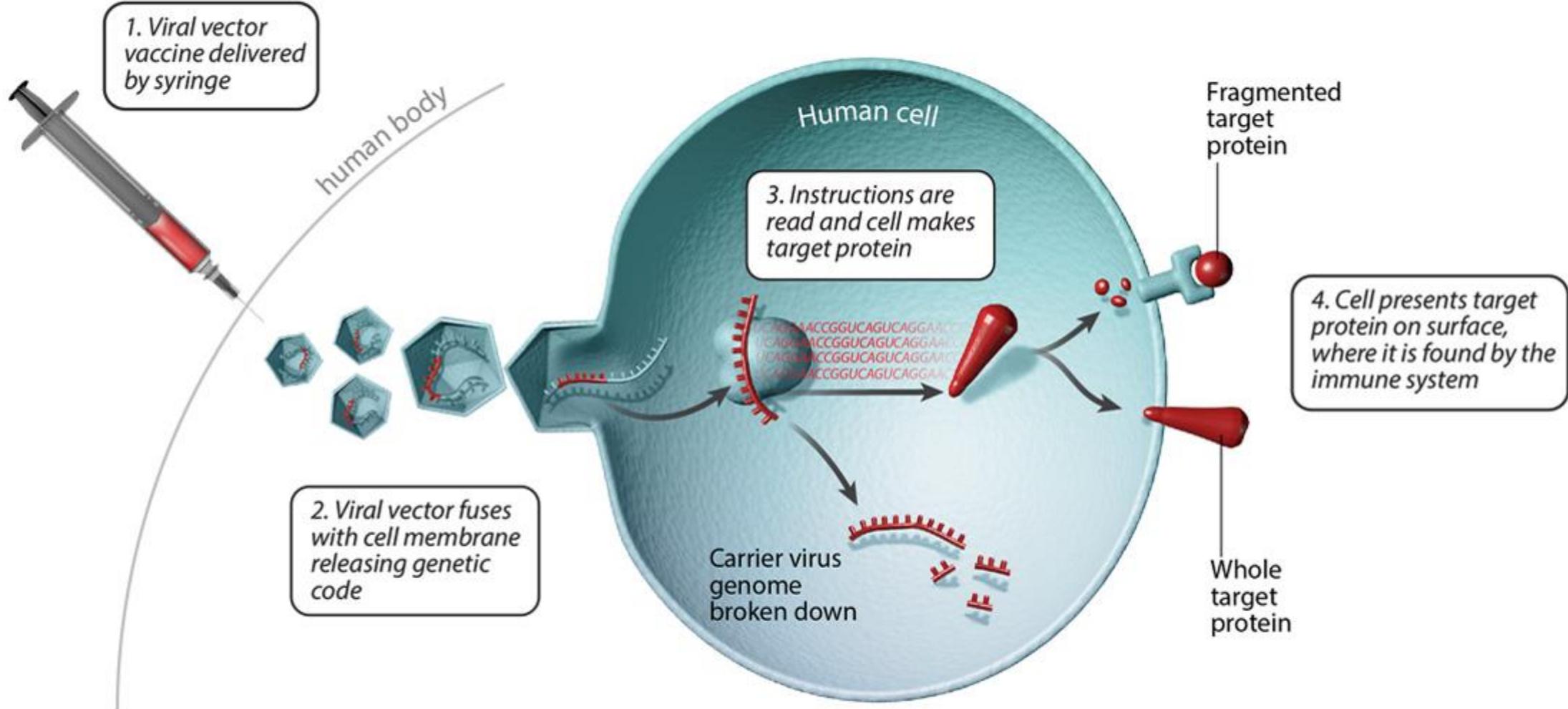
The AstraZeneca and Johnson & Johnson COVID viral vector vaccines carry genetic code for the spike protein, and build immunity against invaders carrying it on their surface.

MECHANISM:
Genetic
"blueprint" for
spike protein

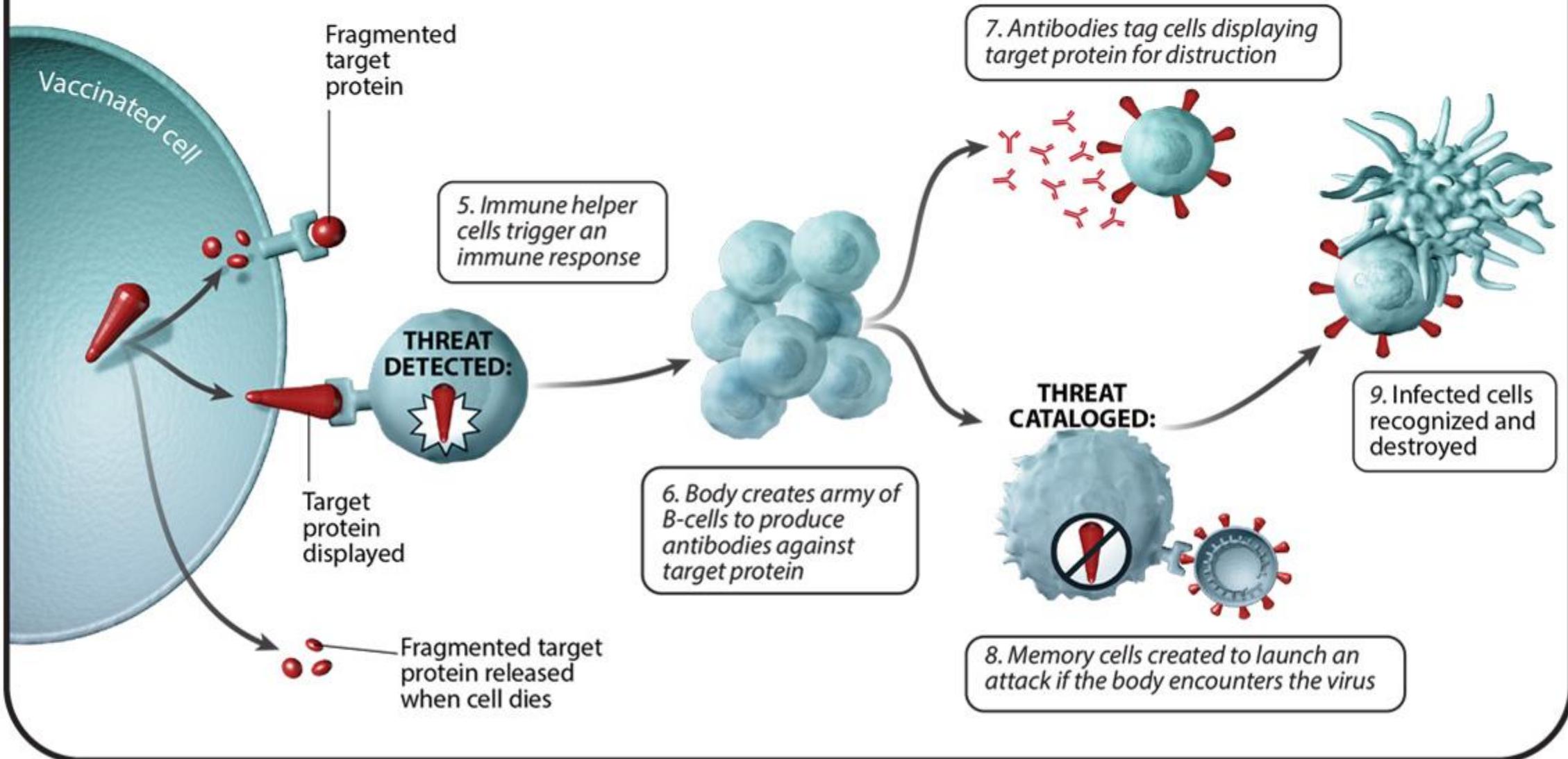
TARGET:
Spike protein



How does a Viral Vector Vaccine work?



How does a Viral Vector Vaccine create immunity?



HPV

- Human papillomavirus (HPV) quadrivalent recombinant vaccine is a mixture of virus-like particles derived from the L1 capsid proteins of HPV types 6, 11, 16 and 18.
- It is administered intramuscularly in a three-dose regimen, with the initial injection followed by subsequent doses at months 2 and 6.
- The vaccine is indicated for use in the prevention of cervical cancer, vulvar and vaginal precancer and cancers, precancerous lesions and genital warts associated with HPV types 6, 11, 16 or 18 infection in adolescents and young women.
- Vaccine efficacy and antibody responses for 9vHPV are found to persist for at least five years while longer-term observational studies are ongoing to monitor long-term vaccine effectiveness.

CHARACTERISTICS OF HPV VLP VACCINES

Manufacturer	Merck™ (Gardasil®)	GlaxoSmithKline™ (Cervarix®)	Merck™ (Gardasil® 9)
L1 VLP types	6, 11, 16, and 18	16 and 18	6, 11, 16, 18, 31, 33, 45, 52, and 58
Dose	20/40/40/20 µg	20/20 µg	30/40/60/40/20/20/20/20 µg
Producer cells	<i>Saccharomyces cerevisiae</i> (baker's yeast) expressing L1	<i>Trichoplusia ni</i> (Hi 5) insect cell line infected with L1 recombinant baculovirus	<i>Saccharomyces cerevisiae</i> (baker's yeast) expressing L1
Adjuvant	225 µg aluminium hydroxyphosphate sulfate	500 µg aluminium hydroxide, 50 µg 3-O-deacylated-4'-monophosphoryl lipid A	500 µg aluminium hydroxyphosphate sulfate
Vaccination schedule	0, 2, and 6 months	0, 1, and 6 months	0, 2, and 6 months

DNA VACCINE

- DNA vaccination is developing rapidly.
- Vaccines currently being developed use not only DNA, but also include adjuncts that assist DNA to enter cells, target it towards specific cells, or that may act as adjuvants in stimulating or directing the immune response.
- The first such vaccines licensed for marketing are likely to use plasmid DNA derived from bacterial cells.
- In future, others may use RNA or may use complexes of nucleic acid molecules and other entities. These guidelines address the production and control of vaccines based on plasmid DNA intended for use in humans. The purpose of these guidelines is to indicate:
 - appropriate methods for the production and control of plasmid DNA vaccines; and
 - specific information that should be included in submissions by manufacturers to national control authorities in support of applications for the authorization of clinical trials and marketing.

DNA VACCINE

- A DNA vaccine, utilizes plasmid DNA encoding antigenic proteins that are injected directly into the muscle of the recipient.
- This strategy relies on the host cells to take up the DNA and produce the immunogenic protein *in vivo*, thus directing the antigen through endogenous MHC class I presentation pathways, helping to activate better CTL responses.
- The DNA appears either to integrate into the chromosomal DNA or to be maintained for long periods in an episomal form, and is often taken up by dendritic cells or muscle cells in the injection area.
- Tests in animal models have shown that DNA vaccines are able to induce protective immunity against a number of pathogens, including influenza and rabies viruses.
- The addition of a follow-up booster shot with protein antigen or inclusion of supplementary DNA sequences in the vector, may enhance the immune response.
- These vaccines dispense with both the whole organism and its parts and get right down to the essentials: the microbe's genetic material.

DIFFERENT TYPES OF COVID-19 VACCINES: HOW THEY WORK

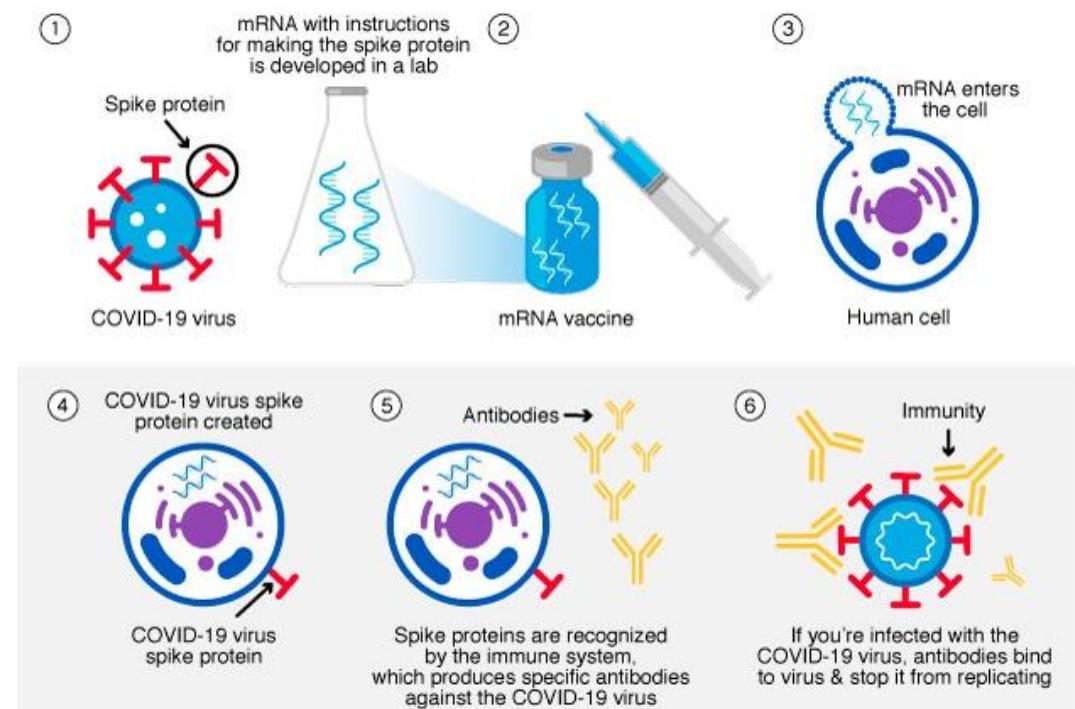
Messenger RNA (mRNA) vaccine.

This type of vaccine gives your cells instructions for how to make the S protein found on the surface of the COVID-19 virus. After vaccination, your muscle cells begin making the S protein pieces and displaying them on cell surfaces. This causes your body to create antibodies. If you later become infected with the COVID-19 virus, these antibodies will fight the virus.

Once the protein pieces are made, the cells break down the instructions and get rid of them.

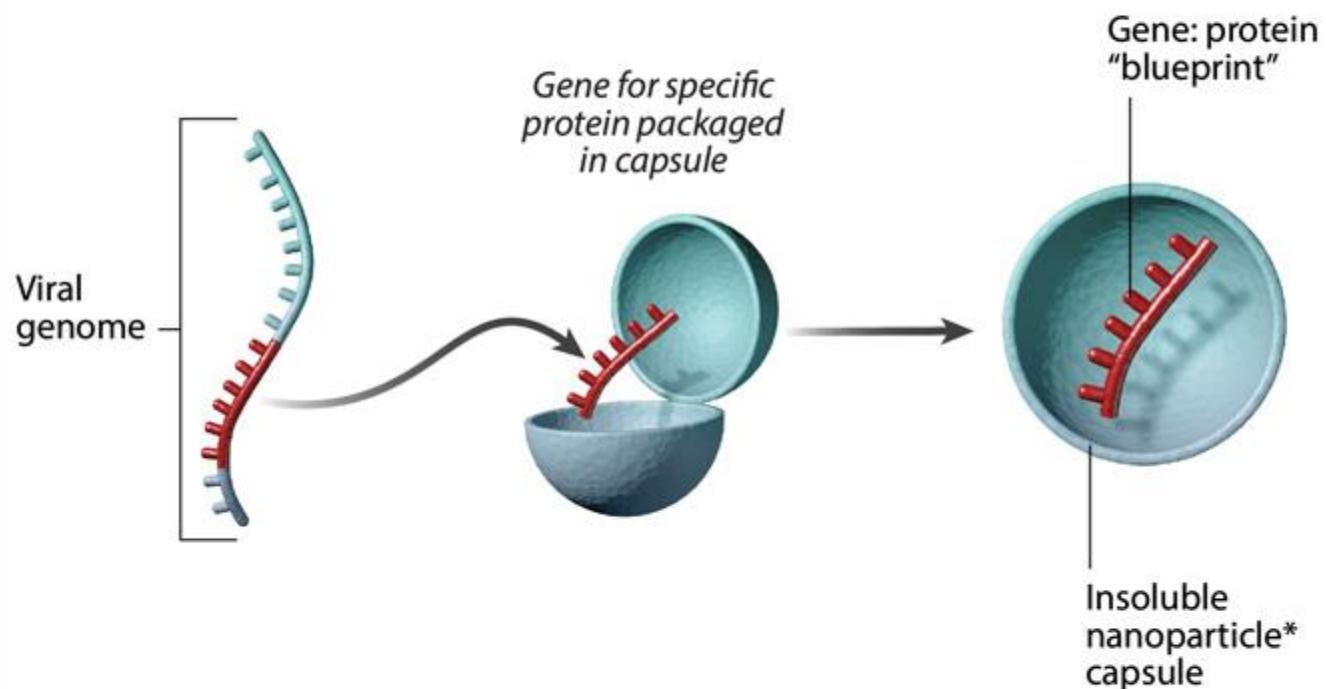
The mRNA in the vaccine doesn't enter the nucleus of the cell, where DNA is kept.

Both the Pfizer-BioNTech and the Moderna COVID-19 vaccines use mRNA.



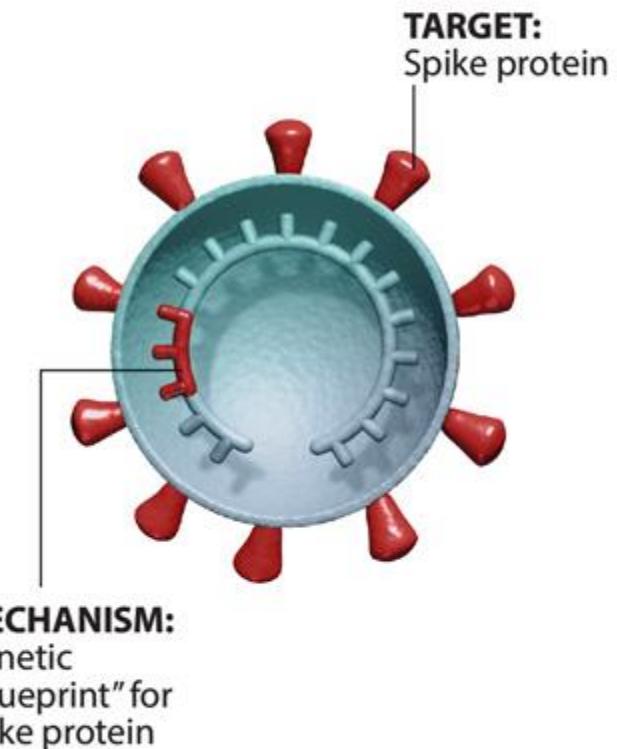
What is the Messenger RNA (mRNA) vaccine?

Made of a small section of a virus' genetic material - the instructions or 'blueprint' for a specific protein. A insoluble nanoparticle* capsule carries the gene safely to your cells.



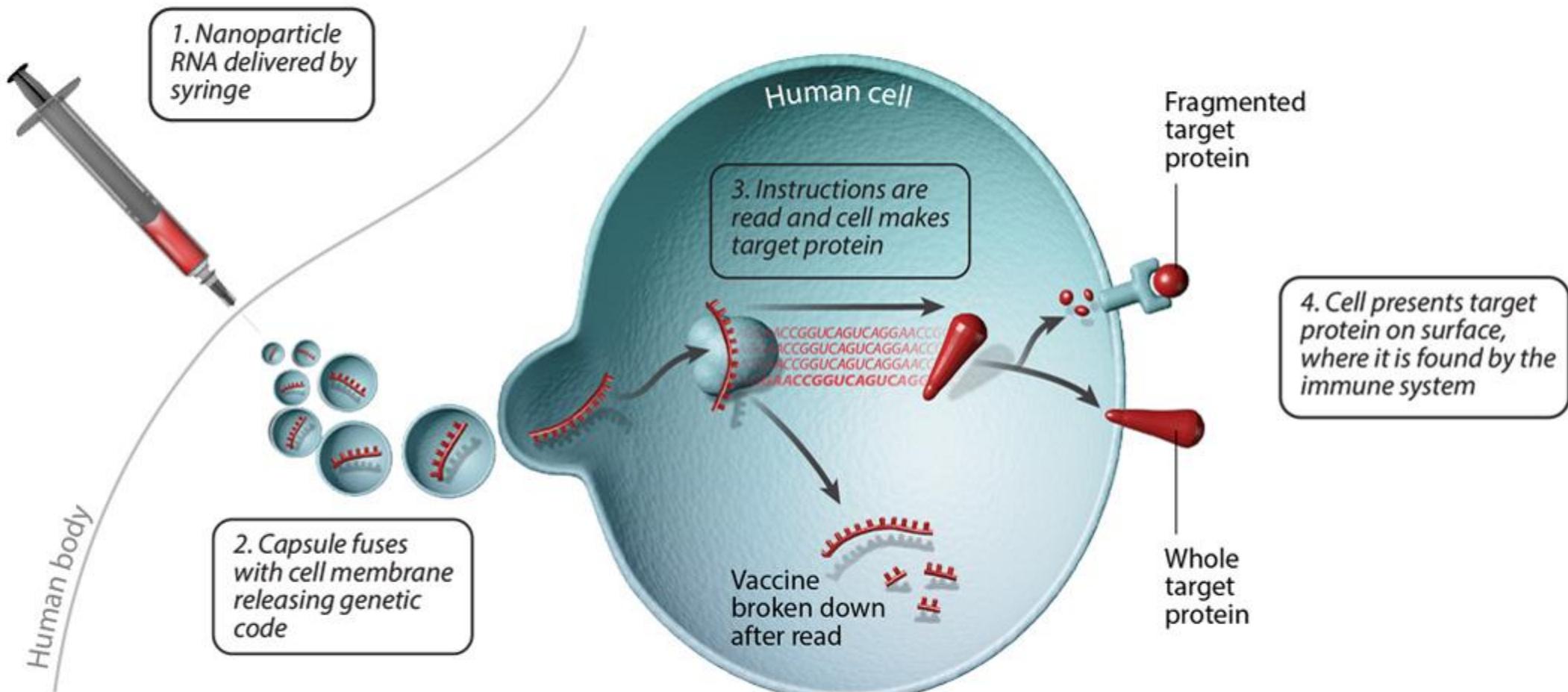
Vaccine Target

Pfizer's mRNA COVID vaccine carries the genetic blueprint for the spike protein. Your body will make this protein and build immunity against any invaders carrying it on their surface.

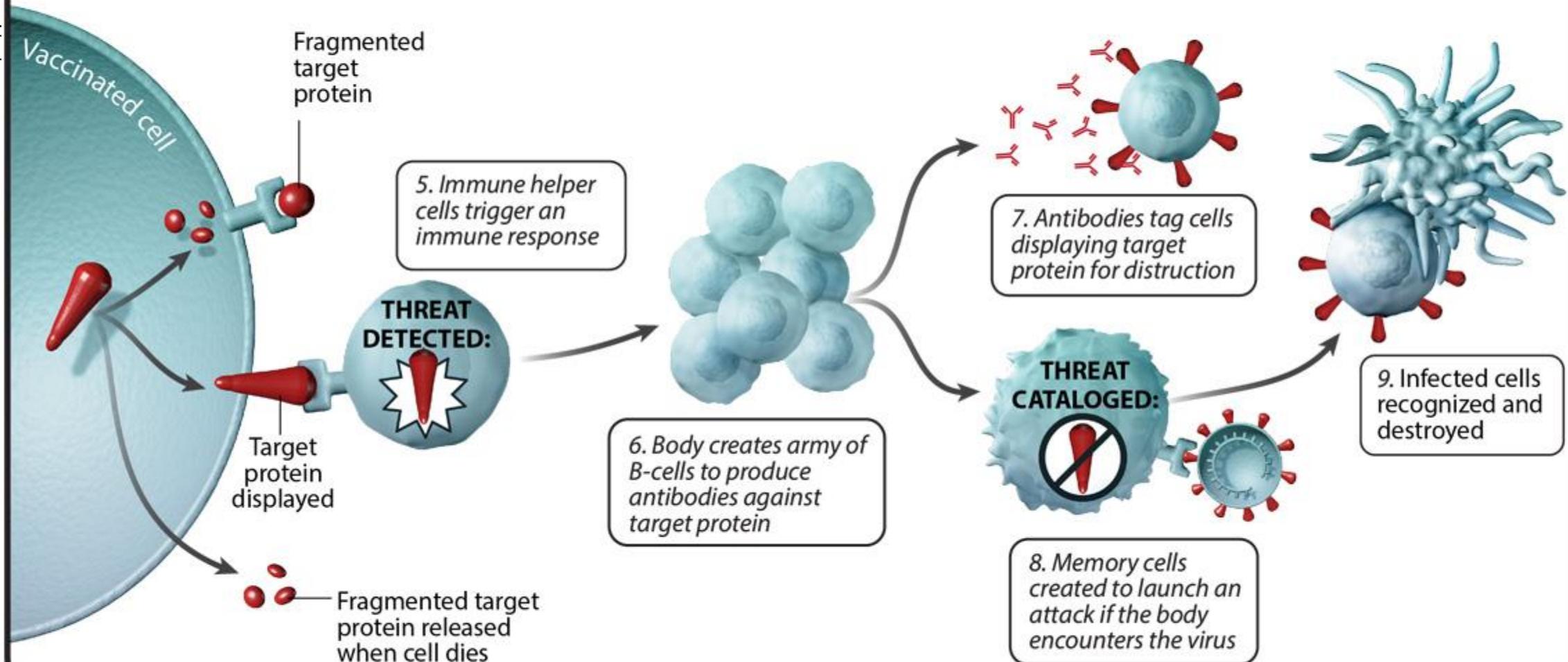


*Did You Know?: "Nano" means small.

How does an mRNA vaccine work?

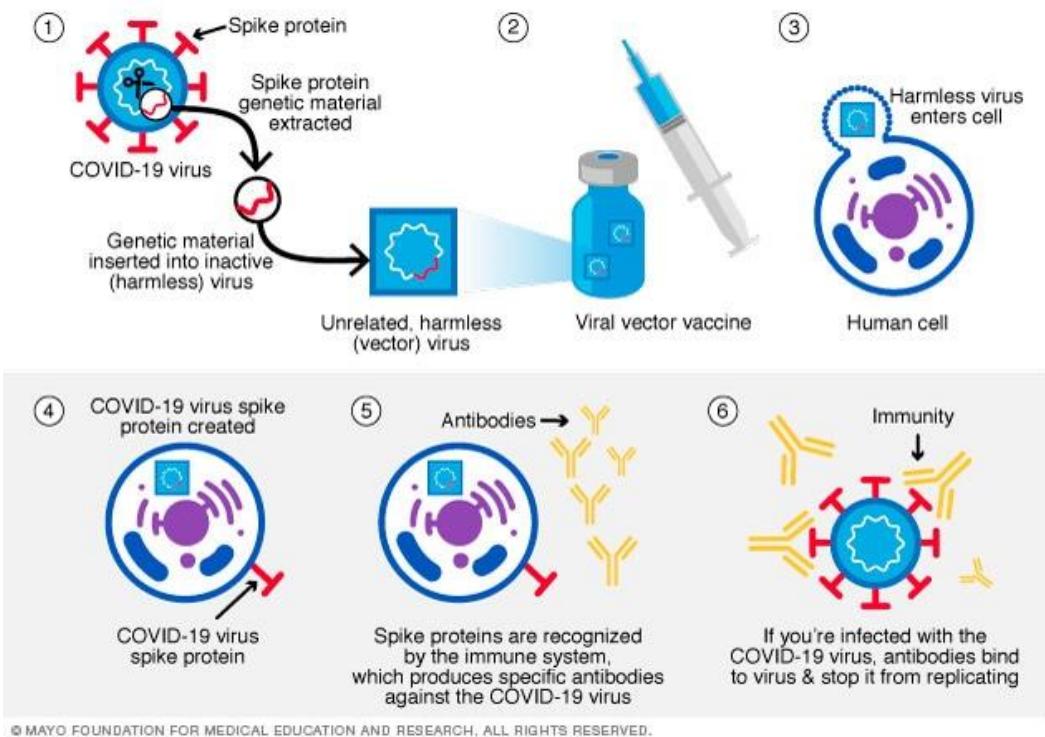


How does an mRNA vaccine create immunity?



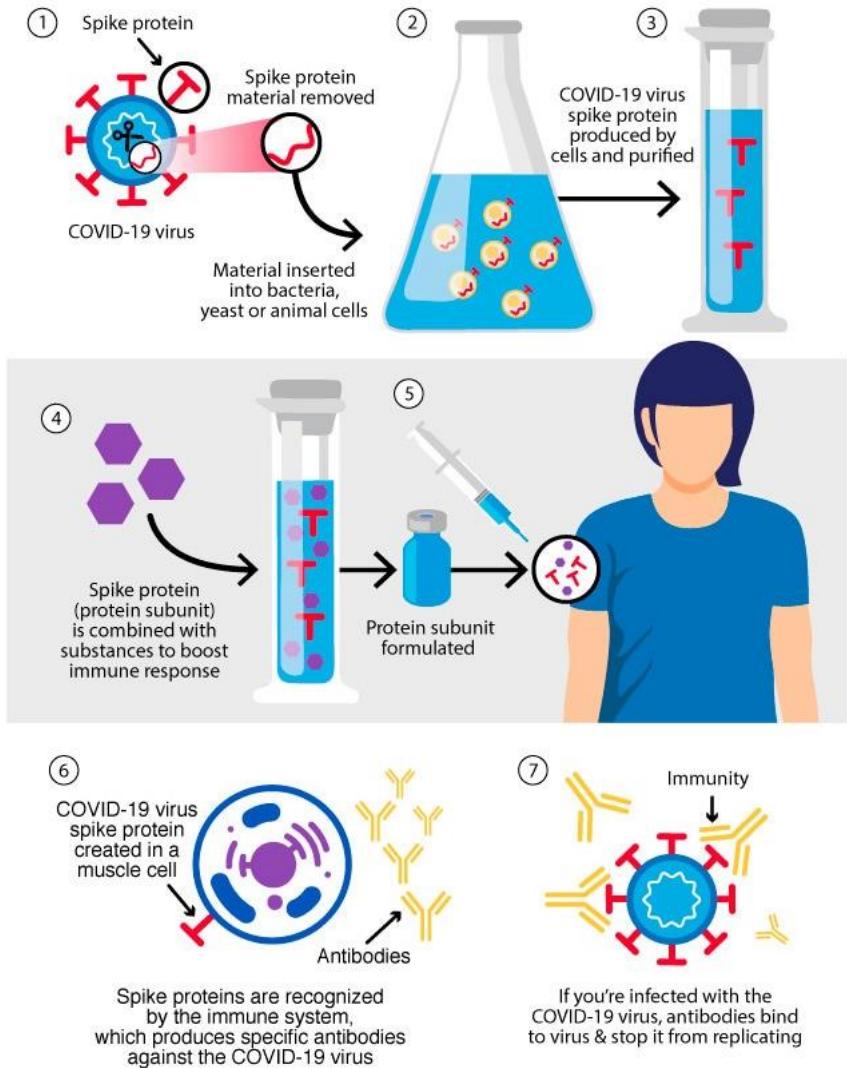
DIFFERENT TYPES OF COVID-19 VACCINES: HOW THEY WORK

- Vector vaccine.
- In this type of vaccine, material from the COVID-19 virus is placed in a modified version of a different virus (viral vector). The viral vector gives your cells instructions to make copies of the COVID-19 S protein. Once your cells display the S proteins on their surfaces, your immune system responds by creating antibodies and defensive white blood cells. If you later become infected with the COVID-19 virus, the antibodies will fight the virus.
- Viral vector vaccines can't cause you to become infected with the COVID-19 virus or the viral vector virus. The Janssen/Johnson & Johnson COVID-19 vaccine is a vector vaccine. AstraZeneca and the University of Oxford also have a vector COVID-19 vaccine.



DIFFERENT TYPES OF COVID-19 VACCINES: HOW THEY WORK

- Protein subunit vaccine.
- Subunit vaccines include only the parts of a virus that best stimulate your immune system. This type of COVID-19 vaccine contains harmless S proteins. Once your immune system recognizes the S proteins, it creates antibodies and defensive white blood cells. If you later become infected with the COVID-19 virus, the antibodies will fight the virus.
- The Novavax COVID-19 vaccine is a protein subunit vaccine.



“TO VACCINATE OR NOT TO VACCINATE?” THAT IS THE QUESTION

Evaluating the benefits and risks
of a medicine or vaccine

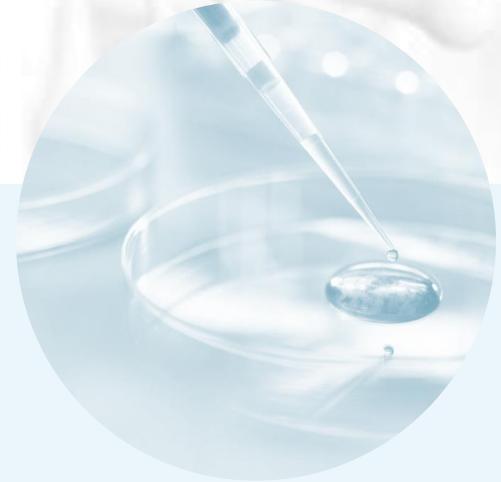


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THANK YOU

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AUTOIMMUNITY

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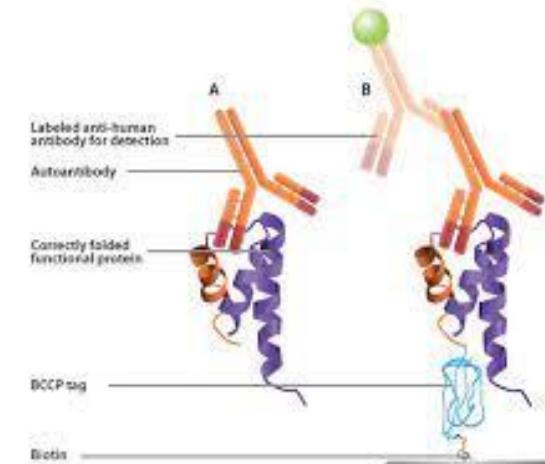
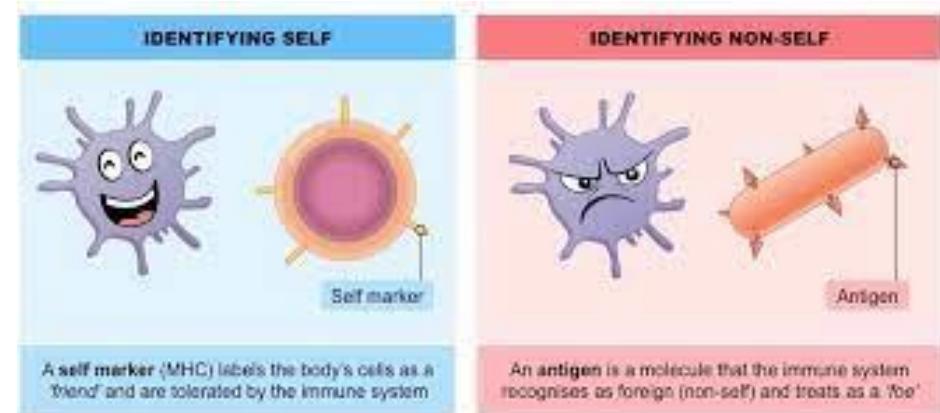
Introduction

- **Auto- antigen**

- Self-antigens are receptors on the surface of cells.
- The immune system uses self-antigens as identifiers to recognize a cell that belongs to the body it is protecting.
- Self-antigens also inform immune cells that antigens from the cell should be immunologically tolerated.

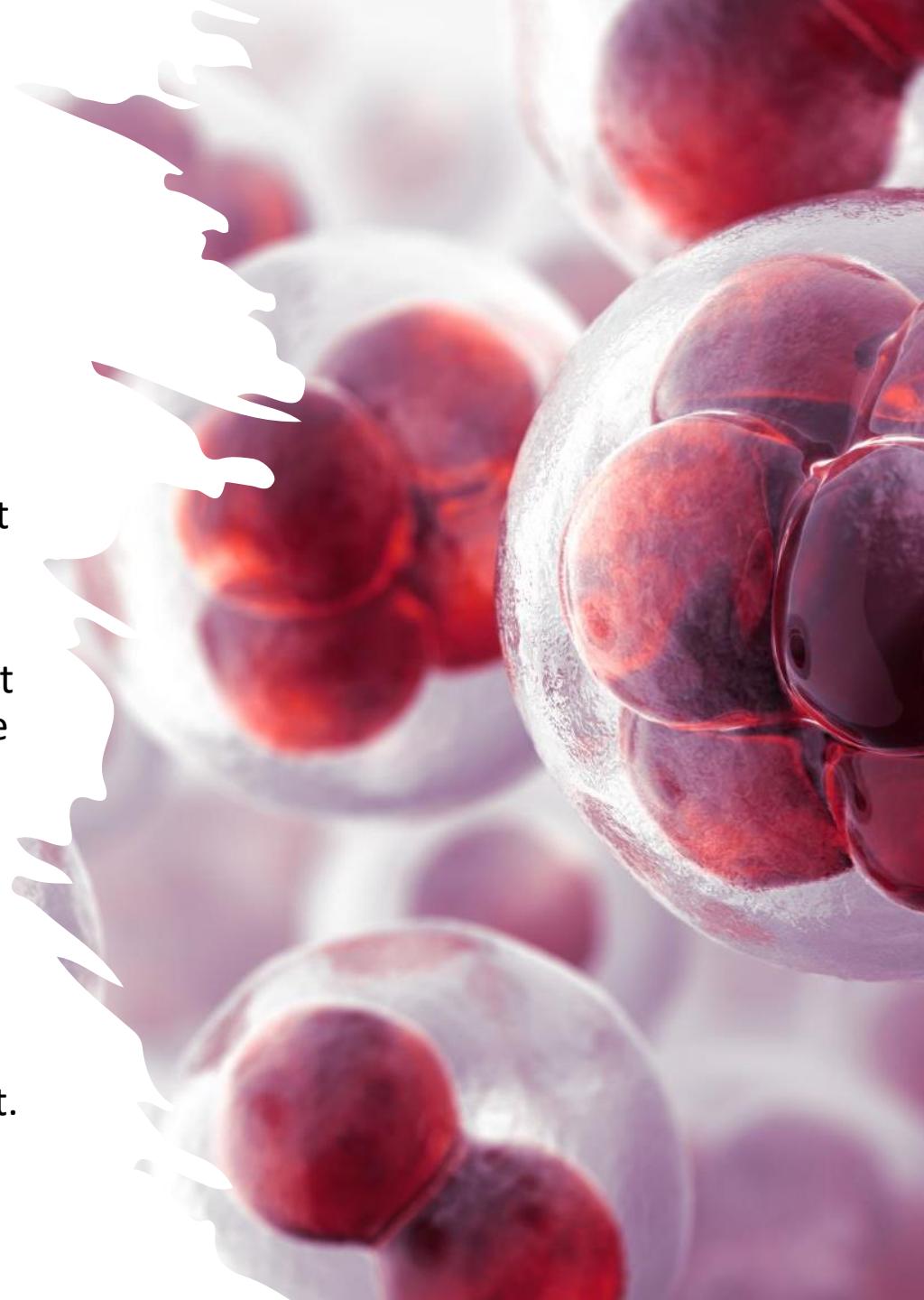
- **Autoantibodies**

- They are antibodies that react with self-antigens. These antigens may be found in all cell types (e.g. chromatin, centromeres) or be highly specific for a specific cell type in one organ of the body (e.g. thyroglobulin in cells of the thyroid gland).

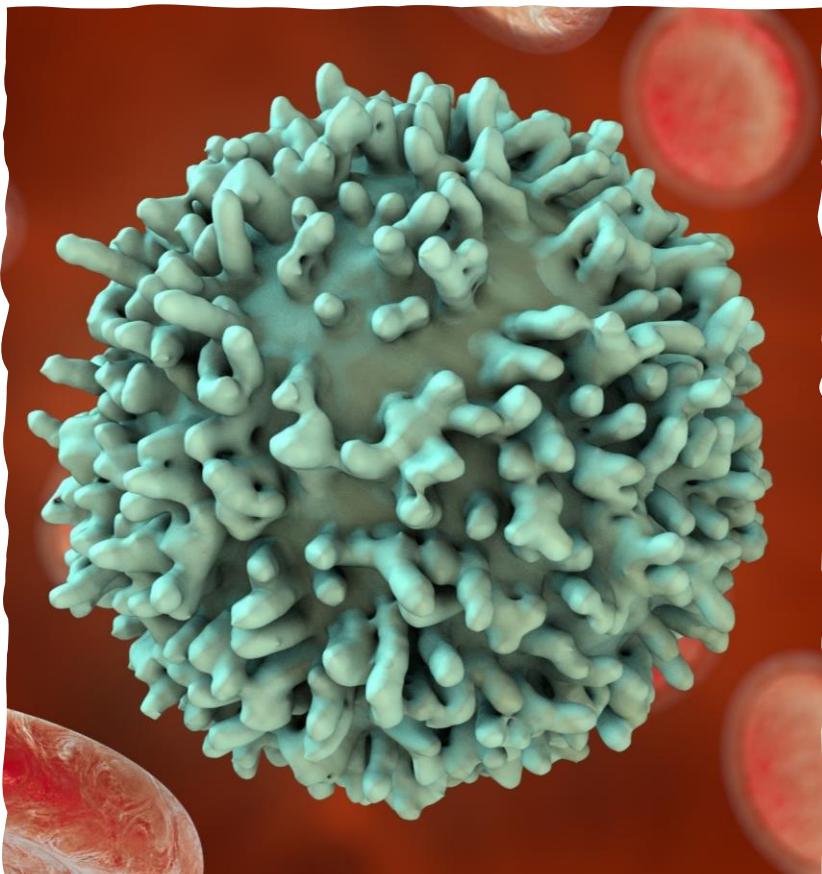


AUTOIMMUNITY

- The term autoimmunity refers to the failure of the human body's immune system to recognize its cells and tissues as "self."
- In contrast, humoral and cell-mediated immune responses by B lymphocytes and T lymphocytes, respectively, are launched against the normal components of an individual (autoantigens) as if they were foreign or invading bodies.
- Autoimmunity refers to an immune reaction that is directed against an individual's own tissue that may cause tissue damage and cause disease.
- Autoimmune diseases develop when the auto-reactive B lymphocytes (autoantibodies) and T lymphocytes described above cause pathological and/or functional damage to the organ/tissue containing the target autoantigen(s).
- In autoimmune diseases, the auto-reactive lymphocytes are the actual cause of the disease rather than a harmless accompaniment.



Pathophysiology of autoimmune disorders



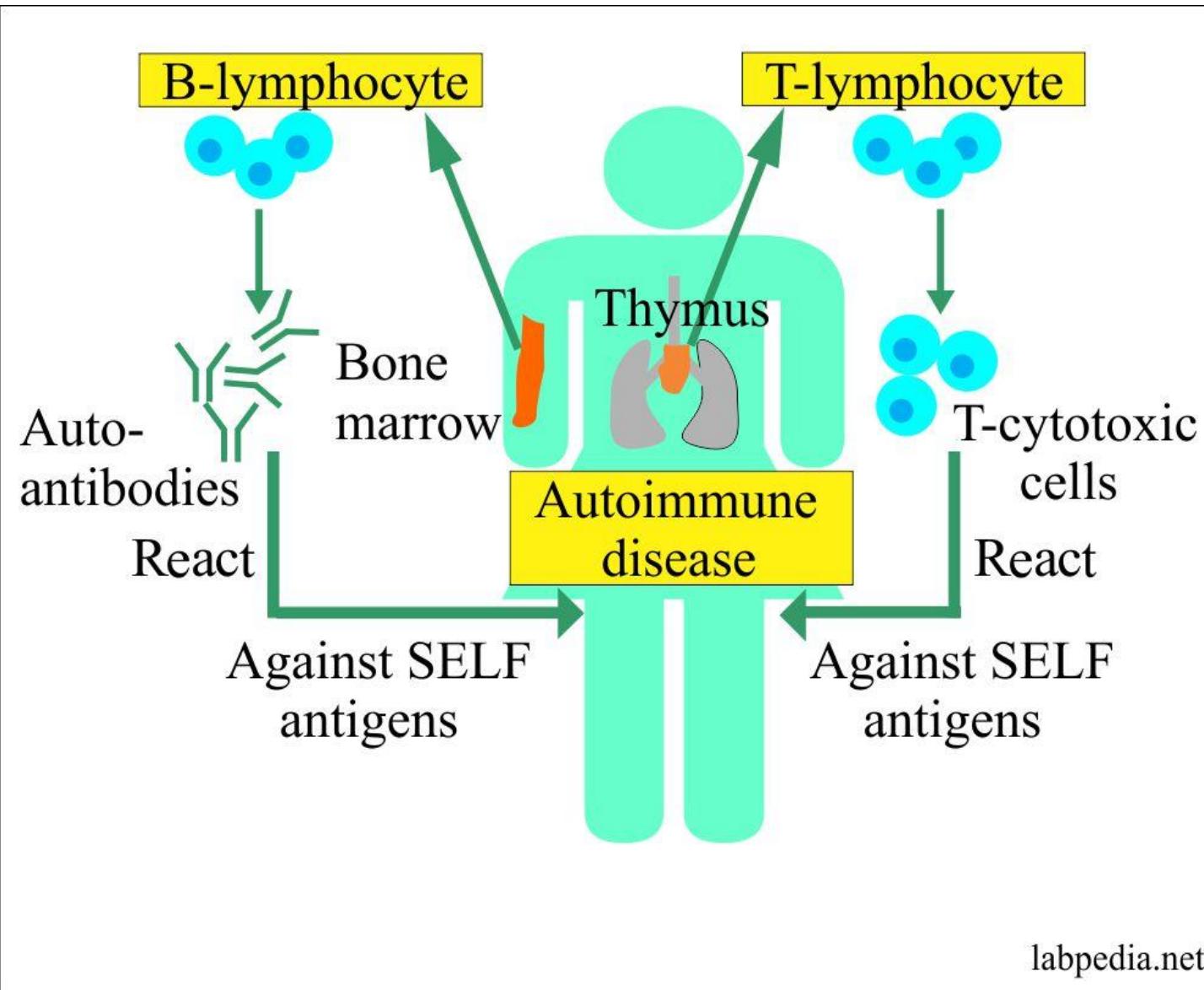
- B lymphocytes develop into plasma cells and produce antibodies to combat the infectious invaders or antigens that trigger immune responses.
- For the immune system to function appropriately, it must be able to distinguish cells that are "self" from substances that are non-self or foreign, and when the immune system fails to do this, it instead produces antibodies directed toward the body's own tissues, auto-antibodies.
- B lymphocytes are produced and mature in the bone marrow as part of the normal process of B cell maturation, and a few B lymphocytes may develop immunoglobulin receptors that are reactive to self-antigens.
- Therefore, they can release antibodies that react with self-antigens.
- To prevent the development of autoimmune diseases, autoreactive B lymphocytes must be eliminated before they can produce harmful reactions.

Pathophysiology of autoimmune disorders

- The process of removal of autoreactive lymphocytes is called tolerance and involves the actual death or functional inactivation of autoreactive lymphocytes.
- Likewise, T lymphocytes arise in the bone marrow but mature in and undergo tolerization in the thymus gland.
- The presence of autoimmune diseases indicates inefficient tolerization avoidance of apoptotic elimination, immune escape of autoreactive lymphocytes, and reversal of an anergic state.
- **In autoimmune diseases, autoreactive T and B lymphocytes emerge from primary lymphoid tissues, escape the regulatory mechanisms that normally delete them or keep them in check, and make their way to secondary lymphoid tissues where activation of autoreactive cells occurs.**
- Besides these mechanisms, infectious agents with antigenic sites similar to self-antigens may cause autoimmune diseases with the breaking of tolerance for those self-antigens. Rheumatic fever is caused by this immune mechanism.
- In the spleen, lymph nodes, and blood, many immune reactions are initiated that regulate immune cell function and the population of the lymphoid system. Altered function and imbalance of immune regulatory cells may predispose to the development of autoimmune disorders.

Pathophysiology of autoimmune disorders

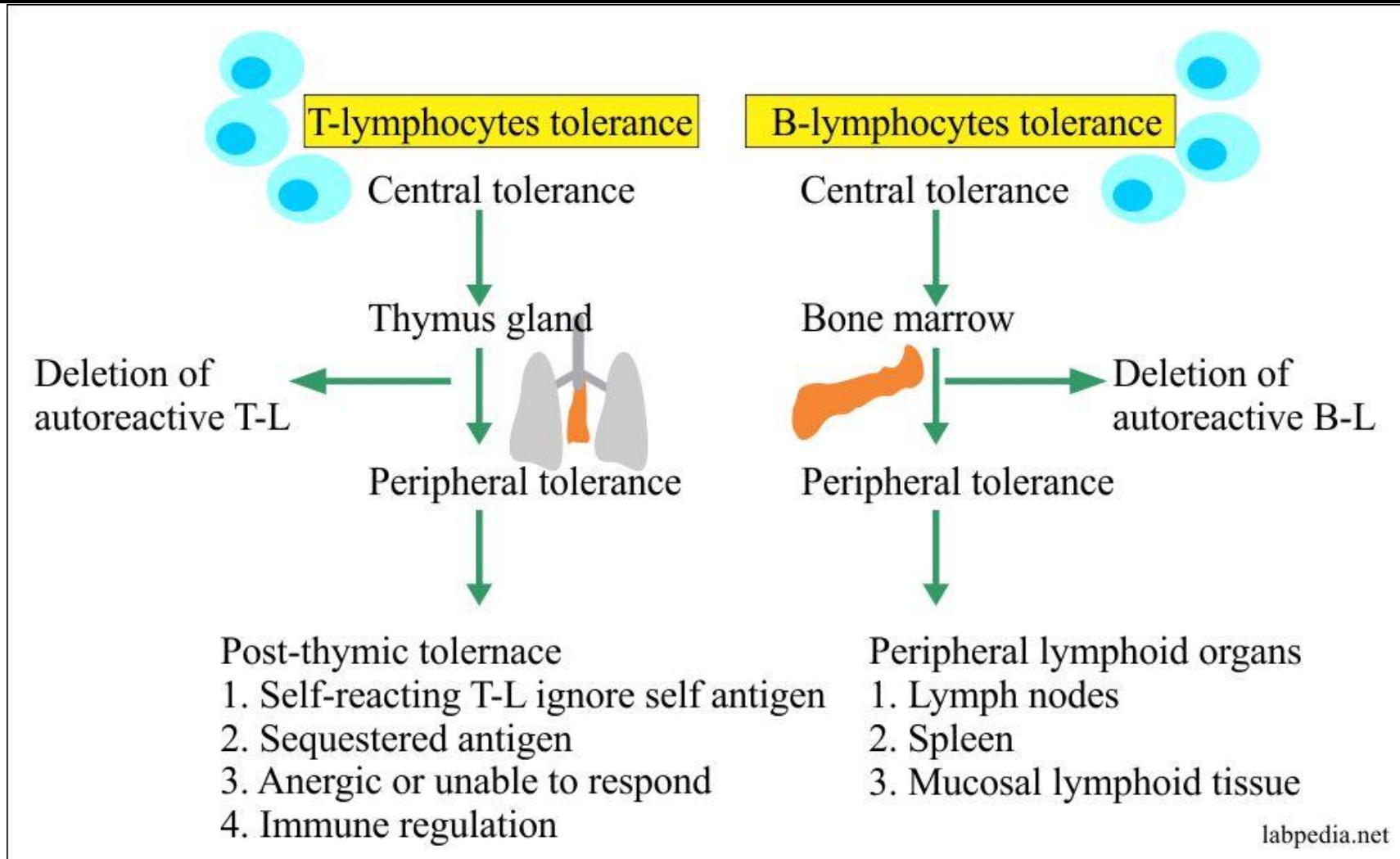
- For example, reduced levels of transforming growth factor-beta (TGF- β) due to macrophage inhibition and elevated levels of interferon-gamma production by CD4 Th1 lymphocytes may activate autoreactive B or T lymphocytes, respectively.
- The effector mechanisms appear to be no different from those used to combat exogenous agents (invading viruses, bacteria, or other pathogens) and include soluble products such as antibodies (humoral immunity), as well as direct cell-to-cell interaction leading to specific cell-lysis (cell-mediated immunity).
- Taken together, autoimmune diseases can be considered a manifestation of **immune dysregulation**.
- In normal circumstances, a healthy immune system is tolerant of the tissue constituents of the host but intolerant of nonhosts (or "non-self") matter, whereas, in autoimmunity, the immune system mounts responses against tissue constituents of the host organism (auto-antigens).
- The autoimmune disease could be considered one end of a spectrum of autoimmunity stretching from physiology to pathology. However, the fact that the vast majority suggests that regulatory mechanisms are critical. It is a state of balanced, physiological autoimmunity that may be described as **Tolerance**.
- **autoimmune diseases as a pathological process leading to the breakdown of Self-Tolerance.**



IMMUNOLOGIC TOLERANCE

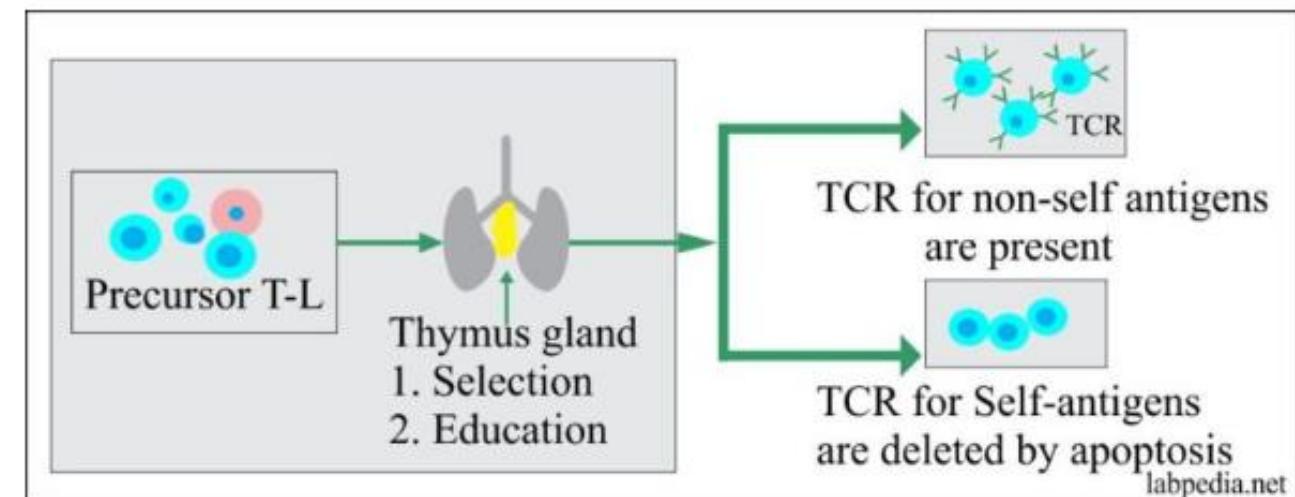
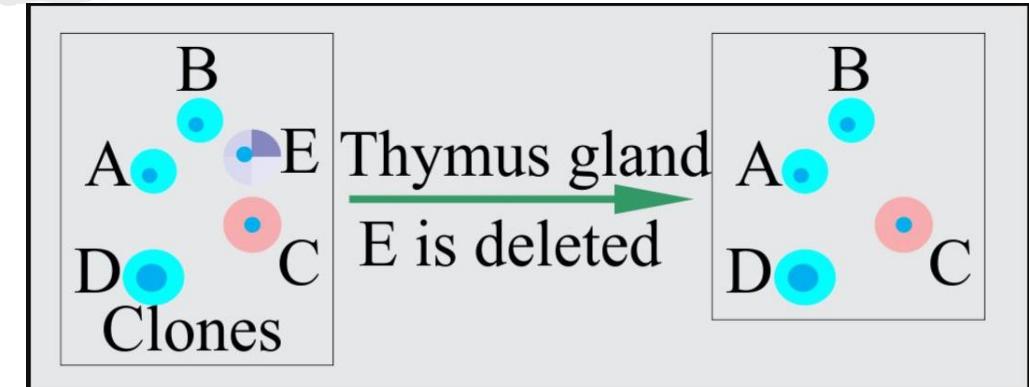
- Immunologic Tolerance is a state in which an individual is incapable of developing an immune response to a specific antigen.
 - Immunologic tolerance prevents harmful reactivity against the body's own tissue.
-
- Immunologic Tolerance may be:
 - Present at birth—prenatal tolerance.
 - It is acquired or present in adult age.
 - Immunologic Tolerance may be:
-
- Central—induced centrally in the primary lymphoid organs, e.g., thymus for T-L tolerance and bone marrow for B-L tolerance. This is the first stage of tolerance.
 - Peripheral—Tolerance in mature B and T-cells. This is acquired in the Lymph nodes, spleen, and mucosal lymphoid tissues. This is the second stage of tolerance.

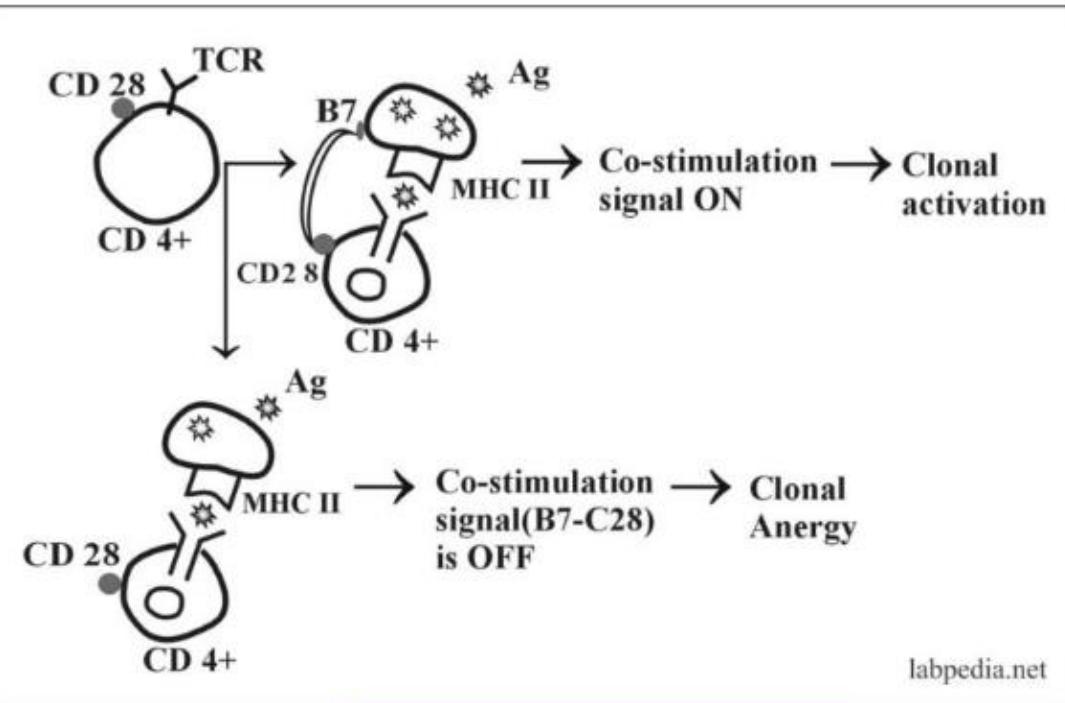
Mechanism of Autoimmunity immunologic tolerance



Self Immunologic Tolerance

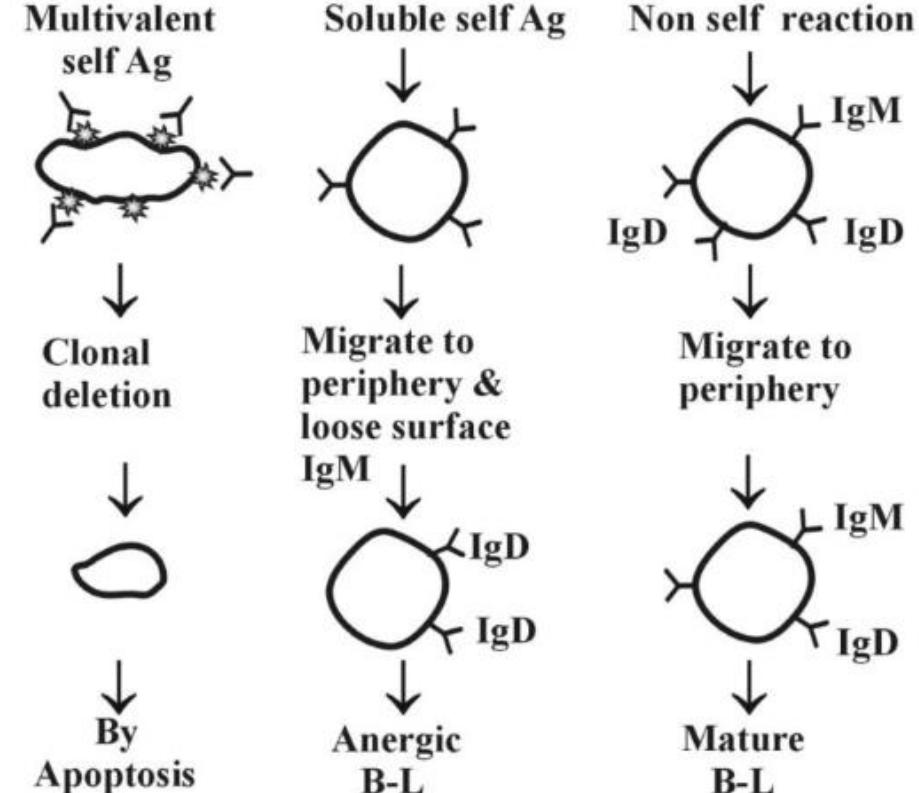
- The following are the different explanations and theories for the development of self Immunologic Tolerance.
- 1. Burnet Clonal Deletion Theory: It is believed that self-antigen reacting B and T-L clones are deleted from the circulation, e.g., during the development stages of lymphoid tissue.
- This process takes place in the thymus gland.
- The same process takes place for B –Lymphocytes. That self Antigen bearing B-cells are deleted by apoptosis in the bone marrow.
- There was an objection to clonal deletion theory because, later on, self-antigen reacting clones of lymphocytes are present in our circulation.
- This theory was modified that there is clonal anergy.





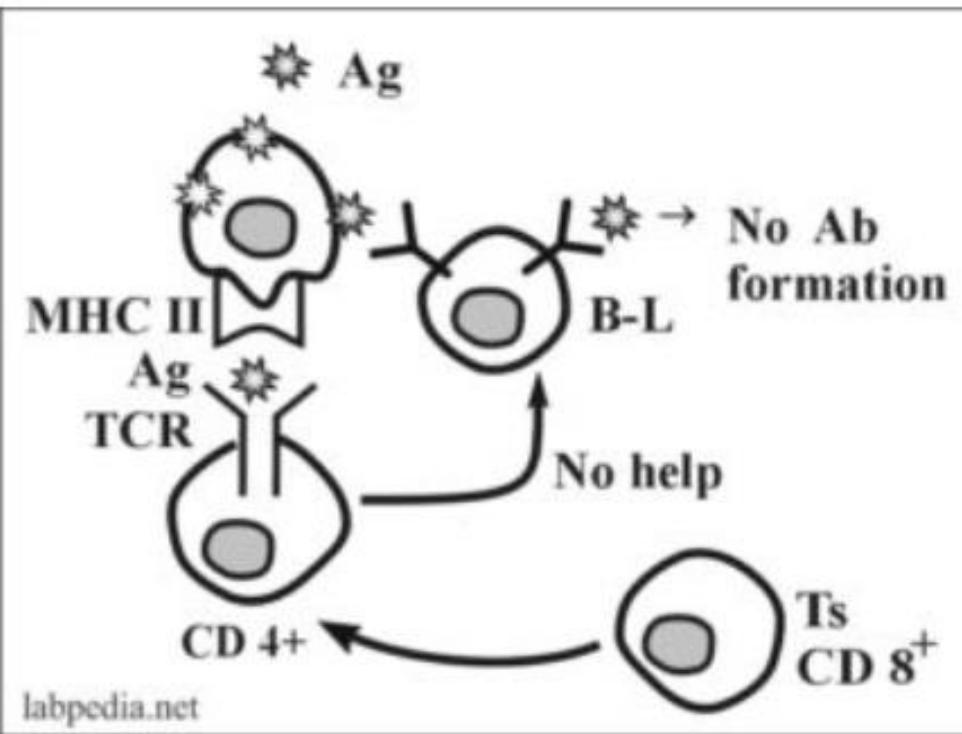
CD 28 and B7 role in Clonal Anergy

- 2. Clonal Anergy:- This is prolonged irreversible functional inactivation of lymphocytes.
- This is also called clonal purging or clonal silence, clonal abortion, or obligatory paralysis.
- Ag-Specific T – L (CD4+) requires two signals:
- Recognition of Ag-peptide with MHC-II.
- The second co-stimulating signal provided by APC-T – L associated molecule, CD28, must bind to its ligand B7 on APC.



- 3. B-L also has clonal anergy (B-lymphocyte Tolerance):-
- B-lymphocytes during maturation, Ag-receptor (IgM) after self-Ag recognition are endocytosed, and Ag-receptor (IgM) is not expressed.

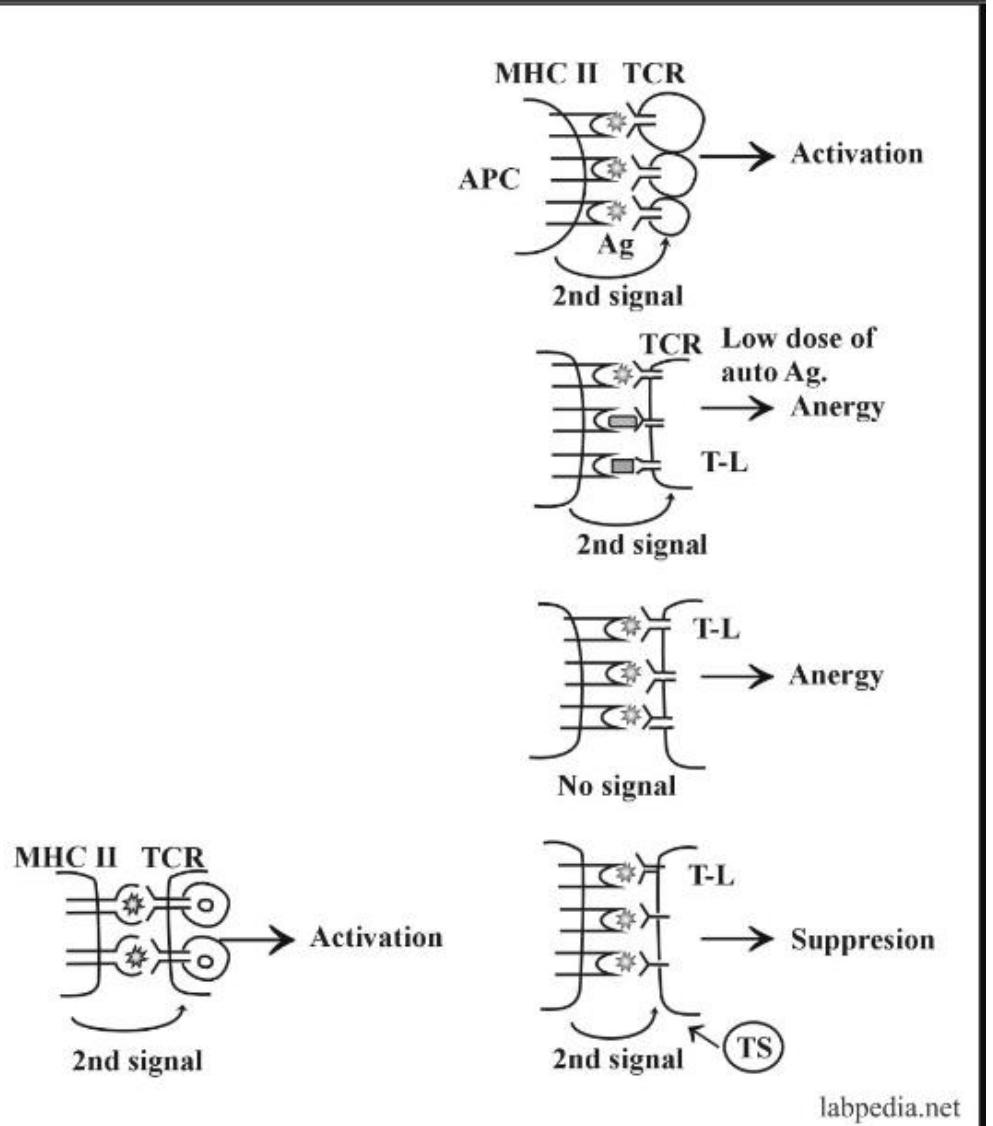
Immunologic Tolerance for Self-Reacting B-Lymphocytes



Ts (CD8+) suppress the B-lymphocytes

- 4. Peripheral Suppression by Ts (CD8+) Cells

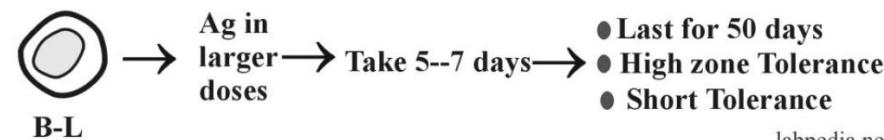
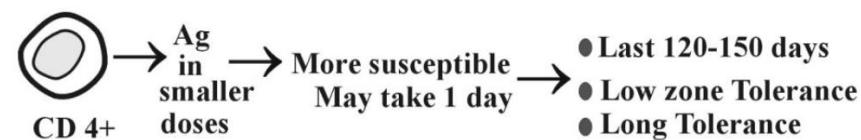
- Ts(CD8+) suppress the immune response by producing cytokines. These are:
 - TGF β 1 – It down-regulates many immune responses.
 - Directly suppress:
 - Th-cells.
 - B-lymphocyte.
 - Prevents autoimmune response



Peripheral Suppression at T Lymphocytes

- 5. Tolerance at Adult Age (Acquired or Peripheral Tolerance):-

- B and T lymphocytes can be made tolerant by giving antigens in various doses.
- The (CD4+) cells are made tolerant by giving antigens in smaller doses, while B-L is made tolerant by giving antigens in larger doses.
- So peripheral T-L suppression may be due to:
- Low antigen concentration.
- APC, the presenting cell, is incapable of a second co-stimulatory signal.
- Regulatory Ts cell suppresses the autoreactive T-L.



Tolerance at Adult Age

Diagnosis of autoimmune disorders

- In autoimmune diseases, auto-reactive lymphocytes expand polyclonally (forming multiple types of autoreactive lymphocytes) since the mechanisms that normally keep them at bay fail.
- The lymphocytes that expand have different antigen receptors on their surface, recognizing different targets (called epitopes) within a single protein or a group of proteins.
- Immunization studies have indicated that some autoantibody responses may arise sequentially, a process termed epitope spreading in mice; however, whether this mechanism applies to humans needs further investigation.
- Autoantibodies have been the tool used by clinical laboratories to diagnose and monitor autoimmunity.
- The molecular spectrum of autoantigenic targets, together with their exquisite antigenic specificity, has made autoantibodies valuable reagents in molecular and cellular biology.
- In both organ-specific and systemic autoimmune diseases, *in vivo* deposition of autoantibody in tissues and organs has clinical significance, as it indicates sites of inflammation and possible pathological lesions.

AUTOIMMUNE DISEASES AND THEIR MECHANISM

- A variety of mechanisms have been proposed to account for the T-cell mediated generation of autoimmune disease. And it is likely that autoimmune disease does not develop from a single event rather from a number of different events.
- Several other pathological processes could break tolerance and lead to autoimmunity.
 - Forbidden clone
 - Altered antigen
 - Sequestered antigen
 - Immunological deficiency theory
 - Genetic influence

- **Tolerance to self-antigen may be broken by:**
 - Defect in immune regulatory pathways.
 - The presence of Ag similarities between pathogenic organisms and self-proteins.
 - The provision of new T-cell epitopes to bypass tolerant T-cells.
 - The release of “hidden” self-antigen.
 - The so-called “aberrant” expression of the MHC class II molecule.
 - The influence of cytokines.
 - Circumstantial evidence of familial tendency.
 - Lymphocytic infiltrate.
 - MHC–association.
 - Clinical improvement with immunosuppressive therapy.

- **Criteria for Autoimmune Diseases:**

- Presence of autoimmune disease.
- Clinical or experimental evidence of the disease is not due to tissue damage, e.g., when administering thyroglobulin will produce thyroiditis.
- Absence of any other evidence of disease.

Forbidden clone

- Mutation in the lymphocytes may result in the formation of changed or altered clone. These altered clone may recognize host as foreign and lead to development of autoimmunity.

Altered antigen

- Some of the antigen on the host cell get altered by chemical, biological or physical means. Thus formed new antigenic determinants which may be recognized as foreign by the host.

Immunological deficiency theory

- According to this theory, mutation or loss of immune regulatory power. i.e. deficiency in immune system results in a condition in which self-antigen behaves as foreign.

Molecular Mimicry

- antigenic determinants are identical to host cell

Genetic influence

- This was determined by family studies. It is well recognized that certain immune disorder predominate in females and families.
- This has strongly supported the role of genetic influence in autoimmunity.
- Genetic links have occurred between disease and HLA antigens.

Neo antigens

- Altered or modified antigens- by physical (irradiation), chemical (drug induced) or microbial agents (intracellular virus)

Cessation tolerance

- It may result when tolerance to self- Ag is evaded

Cross Reacting antigens

- Cross-reactivity measures the extent to which different antigens appear similar to the immune system.
- The molecular determinants of specificity and cross-reactivity define the nature of antigenic variation and the selective processes that shape the distribution of variants in populations.
- Foreign Ag which resembles self
- Many species share organ specific Ag.
- E.g. human and sheep brain Ag

Cytokines

- Cytokines are soluble factors that affect both locally and systemically cells of the immune system. These cytokines also play some role in the development of autoimmune disease e.g.
- **IL-2** is **the T-lymphocyte growth and differentiation factor** used therapeutically in some solid organs. Tumors to enhance immune-mediated anti-tumor responses. During such IL-2 treatment, an inflammatory lesion with lymphocytic infiltration has been seen in several organs, including myocardium, skin, liver, and thyroiditis, like Hashimoto's, which may progress hypothyroidism in 10% of the cases.
- **IFN- α therapy:** This is used to treat and clear hepatitis C virus infection. These patients may develop type-I diabetes with islets antibody and insulin autoantibody as evidence of autoimmune disease.

Idiotype-Bypass Mechanism

- It is postulated that idiotype and anti-idiotype network regulate immune response, inhibiting or activating the response.
- One of the examples is Grave's disease, where auto-antibody against TSH-receptor stimulates the thyroid gland.

Bypass Of Th (T-Helper) Tolerance:

- This can take place by modification of the molecular structure of antigen or carrier e.g.
- 1. Modification of the molecular structure of potential autoantigen (Hapten) complexes with new carriers, e.g., α -methyldopa complex with e-antigen on RBC, and lead to autoimmune hemolytic anemia.
- Partial degradation of auto-antigen is the exposure of new antigens like degraded collagen, thyroglobulin, and γ -globulin.

Imbalance of Ts-Th Function

- Imbalance of Ts – Th cells is seen with increasing age. In SLE, it is found that there is an overactivity of the Helper cells.

The emergence of Sequestered Antigens

- There are some autoantigens, which are not exposed to the immune system during developmental stages. These are exposed; autoantibodies form, e.g., spermatozoa, thyroglobulin, and eye lens.
- In 1956–Dressler described autoantibodies formation after myocardial infarction where autoantibody forms against cardiac myocytes because of injury to these muscles. Here the function of the autoantibody is to clear the damaged tissue and recycle the affected protein.
- Another mechanism is that **virus** may damage the tissue and expose the intracellular proteins. These autoantigens may incite an immune response.
- The last possibility is that appearance of a small number of **hidden autoantigens** to which peripheral tolerance exists may be sufficient to break it since tolerance is dependent on the concentration of the antigens.

Thymic Defects

- The intact thymus gland plays a vital role in immunologic stability because a thymectomy patient is more prone to develop autoimmune diseases.

Defects in Macrophages

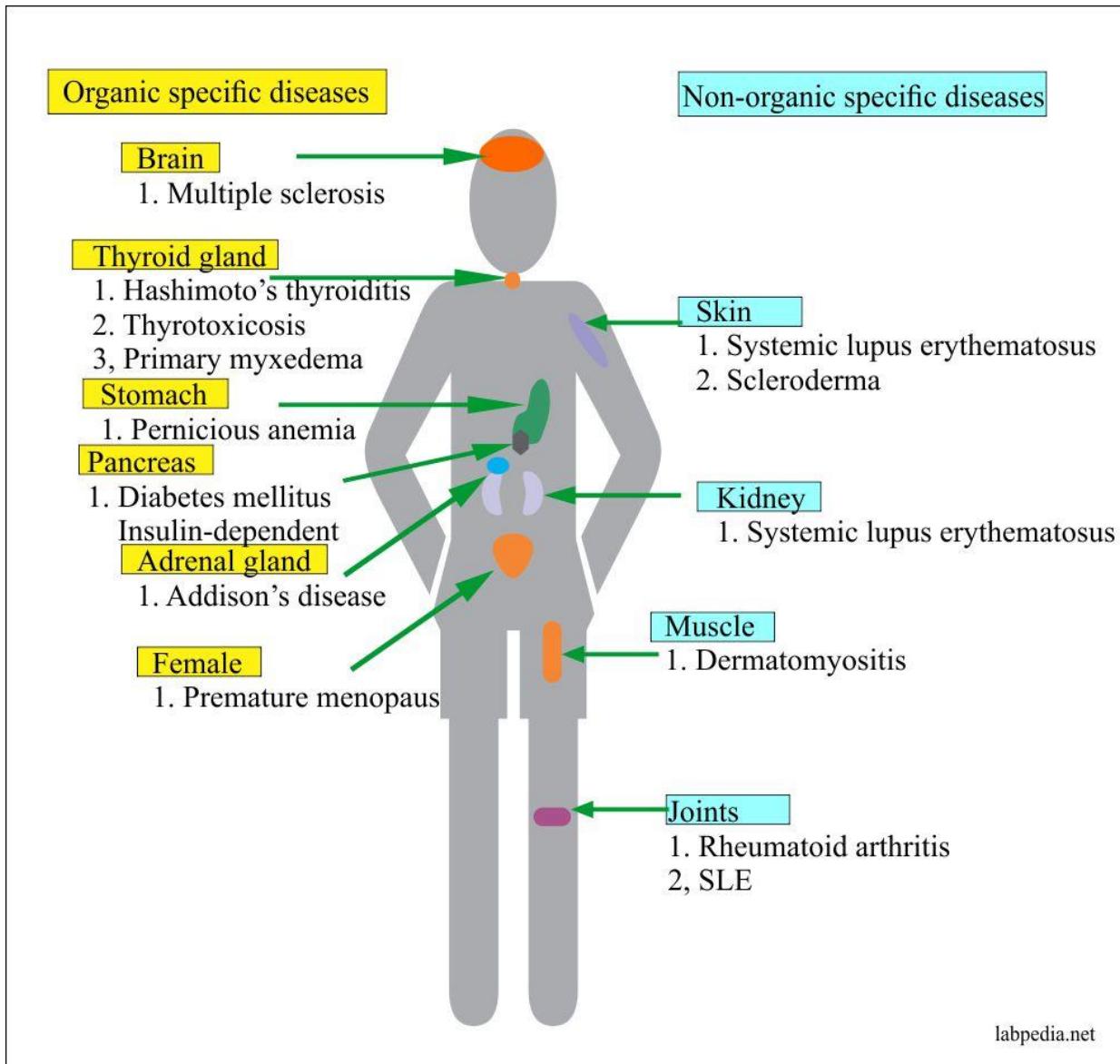
- As one knows, macrophagic cells are needed to process antigens and their presentation to immune cells. So any defect or abnormality of macrophagic cells may lead to autoimmune diseases.

Polyclonal Activation of B-Lymphocytes

- There may be direct activation of B-L, and Th-cells may be bypassed.
- This may take place by:
 - 1.Exogenous substances—lipopolysaccharides, Nystatin, PPD, and Amphotericin B.
 - 2.Endogenous or intrinsic substances.
 - 3.Microbes, e.g., viruses, bacteria, and parasites.
 - 4.Proteolytic enzyme-like trypsin.
 - 5.Viruses are a significant cause of B-lymphocytes' polyclonal activation; they can lead to a Bypass of Th-cell.
 - 6.Direct stimulation of B-L.
 - 7.Loss of Ts.
- **The B-lymphocytes activation may be due to:**
 - 1.Genetic abnormality.
 - 2.Intrinsic B-cell abnormality.
 - 3.Ts-function loss.

Classification of Autoimmune Diseases

- Autoimmune diseases are divided into two classes:
 - organ-specific
 - systemic.
-
- An organ-specific disease is one in which an immune response is directed against antigens in a single organ; examples include Addison disease, in which autoantibodies attack the adrenal cortex, and myasthenia gravis, in which they attack neuromuscular cells.
 - In systemic diseases the immune system attacks self antigens in several organs. Systemic lupus erythematosus, for example, is characterized by inflammation of the skin, joints, and kidneys, among other organs.



Organ specific autoimmune disease:

- This autoimmune disease is directed against a component of one particular type of organ.
- The organ specific autoimmune disease can further be divided into two groups:
- **i. Autoimmune disease mediated by direct cellular damage:**
- This type of damage occur when lymphocytes or antibodies bind to cell membrane antigens, causing cellular lysis or inflammatory response in affected organ.
- The damaged cellular structure is then replaced by connective tissue (fibrous) & it losses its function.
- Examples: Hasimoto's thyroiditis, autoimmune anaemia, Good pasteur's syndrome, Insulin dependent diabetes mellitus.

- **Autoimmune disease mediated by stimulating or blocking auto antibodies:**
- In some cases, antibodies act as antagonist & bind to hormone receptor stimulating inappropriate activity. This usually leads to overproduction of mediators or increase cell growth.
- They also bind to hormone receptor function and thereby block receptor function. This causes impaired secretion of mediators and gradual atrophy of the affected organ.
- Examples: Grave's disease, Myasthenia gravis.

Systematic autoimmune disease :

- It is the type of autoimmune disease which is directed against an antigen that is present in many different sites and can include involvement of several organs and tissues.
- These disease reflect a general defect in immune regulation that result in hyperactive T-cells and B-cells.
- Examples: Rheumatoid arthritis, Systematic lupus erythematosus (SLE), multiple sclerosis.

Autoimmune Diseases

- **Grave's disease:**
 - The production of thyroid hormones is carefully regulated by thyroid stimulating hormone (TSH) produced by pituitary gland. The binding of TSH to receptor on thyroid cell activates adenylate cyclase enzyme stimulating synthesis of thyroxine and tri-iodo thyroxine.
 - A patient with Graves' disease produces autoantibody (LATS) that bind to receptor of TSH & mimic the normal action of TSH, activating adenylate cyclase & resulting in production of thyroid hormones. Unlike TSH, however autoantibody are not regulated and consequently they overstimulate the thyroid gland.
- **Myasthenia gravis:**
 - It is an autoimmune disease mediated by blocking antibodies.
 - A patient with this disease produces auto antibodies that bind the acetylcholine receptor on motor end plates of muscles, blocking the normal binding of acetyl choline. The result is progressive weakening the skeletal muscles.
 - It also inducing complement mediated lysis of cells and the antibodies cause the destruction of the cells bearing receptors.

- **Hashimoto's Thyroiditis:**
- In Hashimoto thyroiditis, an individual produces antibodies & sensitized TH1 Cell specific for thyroid antigens.
- An attending delayed type hypersensitivity (DTH) response is characterized by an intense infiltration of the thyroid gland by lymphocytes, macrophages and plasma cells which form lymphocytic follicles and germinal centers.
- The ensuing inflammatory response cause goiter or visible enlargement of the thyroid gland, a physiological response to hypothyroidism.
- Hypothyroidism is caused when antibodies are formed to a number of thyroid proteins including thyroglobulin and thyroid peroxidase, both of which are involved in the uptake of iodine.
- Binding of auto-antibodies to these protein interfere with thyroid gland functioning.

- **Goodpasture's Syndrome:**
- In Goodpasture's syndrome, auto-antibodies specific for certain basement membrane antigen bind to basement membrane of kidney glomeruli and alveoli of lungs.
- Subsequent complement activation leads to direct cellular damage and an ensuing inflammatory response mediated by a buildup of complement split products.
- Damage to the glomerulus and alveolar basement membrane leads to progressive kidney damage and pulmonary hemorrhage.
- Death may ensue within several months of the onset of symptoms.
- **Autoimmune anemias:**
- Autoimmune anemias include pernicious anemia, autoimmune hemolytic anemia (AIHA) and drug induced hemolytic anemia.
- Pernicious anemia is caused by auto- antibodies to intrinsic factor, a membrane bound intestinal protein on gastric parietal cells which facilitate uptake of vitamin B12 from small intestine. Binding of auto-antibody to intrinsic factor blocks absorption of vitamin B12. In absence of sufficient vitamin B12, which is necessary for proper hematopoiesis, the number of functional mature RBC decrease below normal.

- **Autoimmune hemolytic anemia:** An individual with autoimmune hemolytic anemia makes auto-antibody to Red- blood cell antigen, triggering complement mediated lysis or antibody-mediated opsonization & phagocytosis of RBC.
- **In drug induced hemolytic anemia,** certain drugs such as penicillin or anti-hypertensive agents like methyldopa interact with RBC, the cells become antigenic.
- **Insulin Dependent Diabetes mellitus (IDDM):**
- IDDM is caused by an autoimmune attack on pancreas.
- The attack is directed against specialized insulin producing beta-cell that are located in spherical cluster islets of Langerhans, scattered throughout the pancreas.
- The autoimmune attack destroys beta cell resulting in decreased production of insulin and consequently increased level of blood glucose.
- Several factors are important in destruction of beta cells, first activated CTLs migrate into an islet and begin to attack the insulin producing cells.
- The CTL infiltration & activation of macrophages, frequently referred to as insulitis which is followed by cytokine release and presence of auto antibodies which leads to a cell mediated DTH.
- The auto-antibodies to beta cells may contribute to cell distribution by facilitating either antibody-mediated complement lysis or antibody-dependent cell-mediated cytotoxicity (ADCC).

- **Systemic Lupus erythematosus:**
- One of the best example of a systemic autoimmune disease is systemic lupus erythematosus (SLE).
- The individual affected by SLE may produce auto-antibodies to a vast array of tissues, antigens, such as DNA, histones, RBCs, platelets, leukocytes, and clotting factors.
- Interaction of these auto-antibodies with their specific antigens produces various symptoms.
- Auto antibody specific for RBC and platelets for examples, can lead to complement mediated lysis resulting in hemolytic anemia and thrombocytopenia, respectively.
- When immune complex of auto antibodies with various nuclear antigens are deposited along the walls of small blood vessels, a type III hypersensitivity reaction develops.
- The complexes activates the complement system and generate membrane- attack complexes and complement split products that damage the wall of the blood vessel, resulting in vasculitis and glomerulonephritis.

- **Multiple sclerosis (MS):**
- Multiple sclerosis (MS) is the most common cause of neurologic disability associated with autoimmune disease in western country.
- With this disease production of auto-reactive T-cell that participate in the formation of inflammatory lesions along the myelin sheath of nerve fibers.
- The cerebrospinal fluid of patient with active MS contains activated T lymphocytes, which infiltrate the brain tissue and cause characteristic inflammatory lesions, destroying the myelin.
- Since myelin function to insulate the nerve fibers, a breakdown in the myelin sheath leads to numerous neurologic dysfunctions.
- **Rheumatoid arthritis:**
- Rheumatoid arthritis is common autoimmune disorder.
- Many individuals with rheumatoid arthritis produce a group of auto-antibodies called rheumatoid factors that are reactive with determinants of Fc region of IgG antibody.
- The classic rheumatoid factor is an IgM antibody with that reactivity. Such auto-antibodies bind to normal circulating IgG, forming IgM –IgG complexes that are deposited in the joints.
- The immune complexes can activate the complement cascade, resulting in type III hypersensitive reaction which leads to chronic inflammation of the joints

Autoimmune diseases and their possible source of antigens:

- Overlap Of Auto-Antibodies:
- There is also an overlap of auto-antibodies in the same group; e.g., in patients with thyroid diseases, 30% may show at the same time antibodies against parietal cells while thyroid antibodies are found in 50% of pernicious anemia cases.
- Similarly, Rheumatoid arthritis patients are clinically associated with a clinical picture of SLE.

Disease	Antigen
1. Hashimoto's thyroiditis	1.Thyroglobulin 2.The second colloidal antigen
2. Primary Myxedema	1.Cytoplasmic microsome cell-surface
3. Thyrotoxicosis	1.Cell surface TSH receptor
4. Pernicious anemia	1.Intrinsic factor 2.Parietal cell microsome
5. Addison's disease	1.The cytoplasm of adrenal cells
6. Premature menopause	1.The cytoplasm of steroid producing cells
7. Juvenile diabetes	1.Islet cell cytoplasm 2.Insulin
8. Good Pasteur Syndrome	1.The basement membrane (BM) of glomeruli 2.BM of lung
9. Pemphigus Vulgaris	1.Desmosomes of the Prickle cell
10. Myasthenia gravis	1.Acetylcholine receptor of skeletal muscles 2.And heart muscles.
11. Autoimmune Hemolytic Anaemia	1.RBC
12. Idiopathic thrombocytopenic purpura (ITP)	1.Platelets
13. Primary biliary cirrhosis	1.Mitochondrial pyruvate dehydrogenase
14. Chronic active hepatitis	1.Smooth muscle nuclei 2.Cell surface Lipoprotein
15. Ulcerative colitis	1.Colon lipopolysaccharides
16. Sjogren syndrome	1.Duct 2.Mitochondria 3.IgG 4.Nuclei 5.Thyroid Ag
17. Rheumatoid arthritis	1.IgG 2.Collagen
18. Scleroderma	1.IgG
19. Dermatomyositis	1.Nucleus 2.IgG
20. Systemic lupus erythematosus	1.DNA 2.Nuclear proteins 3.IgG 4.Cytoplasmic proteins, 5.Formed elements of blood 6.Clotting factors

Classification of Autoimmune Diseases

Organ specific autoimmune diseases		
Disease	Self-antigen	Immune response
Addison's disease	Adrenal cells	Autoantibodies
Autoimmune haemolytic anaemia	RBC membrane proteins	Autoantibodies
Goodpasture's syndrome	Renal and lung basement membrane	Autoantibodies
Grave's disease	TSH receptor	Autoantibody (stimulating)
Idiopathic thrombocytopenic purpura	Platelet membrane proteins	Autoantibodies
Hashimoto's thyroiditis	Thyroid proteins and cells	TDTH cells, autoantibodies
Myasthenia gravis	Acetylcholine receptors	Autoantibody (blocking)
Myocardial infarction	Heart	Autoantibodies
Pernicious anaemia	Gastric parietal cell, intrinsic factor	Autoantibody
Poststreptococcal glomerulonephritis	Kidney	Antigen-antibody complex
Spontaneous infertility	Sperm	autoantibodies

Systemic autoimmune diseases		
Disease	Self-antigen	Immune response
Ankylosing spondylitis	Vertebrae	Immune complexes
Multiple sclerosis	Brain or white matter	TDTH and Tc cells, autoantibodies
Rheumatoid arthritis	Connective tissue, IgG	Autoantibodies, immune complexes
Scleroderma	Nuclei, heart, lungs, GIT, kidney	Autoantibodies
Sjogren's syndrome	Salivary gland, liver, kidney, thyroid	Autoantibodies
Systemic Lupus Erythematosus (SLE)	DNA, nuclear protein, RBC and platelet membrane	Autoantibodies, immune complexes

Diagnosis of Autoimmune diseases

- **Initial laboratory evaluation**
- Inflammatory diseases will cause abnormalities in routine laboratory studies. Characteristic findings can include a normochromic, normocytic anemia indicating the chronicity or severity of disease. Common hematologic parameters also include an elevated or decreased platelet count and/or white blood cell count. Leukopenia and thrombocytopenia are common in patients with systemic lupus erythematosus (SLE).
- **Testing will find aberrations in serum levels** of specific organ enzymes or abnormalities in metabolic processes that are reflected in the comprehensive metabolic panel.
- **Coagulation studies** such as a prolongation of the activated partial thromboplastin time (aPTT) and/or the prothrombin time (PT) that does not correct with mixing studies suggests an inhibitor of the clotting process is present as seen in the antiphospholipid syndrome.



Diagnosis of Autoimmune diseases

- **The urinalysis** is commonly used to assess renal injury (e.g. glomerulonephritis, interstitial nephritis) and will show proteinuria, hematuria or active sediment (white blood cell casts or red blood cell casts).
- **Inflammatory markers**
- Serum proteins that are produced in response to inflammation can be referred to as inflammatory markers. These proteins are mainly produced by the liver in response to stress and can also be called acute phase reactants. Pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha induce synthesis of some acute phase reactants that include CRP, fibrinogen and haptoglobin.
- **Erythrocyte sedimentation rate (ESR)**
- The ESR is the measure of the quantity of red blood cells (RBC) that precipitate in a tube in a defined time and is based upon serum protein concentrations and RBC interactions with these proteins. Inflammation causes an increase in the ESR. Multiple factors influence the ESR and include patient's age, gender, RBC morphology, hemoglobin concentration, and serum levels of immunoglobulin.



Diagnosis of Autoimmune diseases

- **C-reactive protein (CRP)**
- C-reactive protein (CRP/CRP-high sensitivity) was discovered and named for its reactivity to the C polysaccharide in the cell wall of *S. pneumoniae*. CRP, an innate immune protein, helps opsonize pathogens for phagocytosis and activates the complement system. CRP production is under the control of IL-1, IL-6, and TNF-alpha. Changes in serum CRP concentration change more quickly than ESR and therefore CRP may be a better reflection of current inflammation. Unlike the ESR, CRP is a fairly stable serum protein whose measurement is not time-sensitive and is not affected by other serum components. The magnitude of inflammation directly relates to the concentration of CRP.
- **Ferritin**
- Serum ferritin is a storage protein for iron and its synthesis is regulated by intracellular iron, cytokines (TNF-alpha, IL-1, and IL-6), products of oxidative stress, and growth factors. Elevated levels can indicate acute or chronic sepsis, inflammation or malignancy.



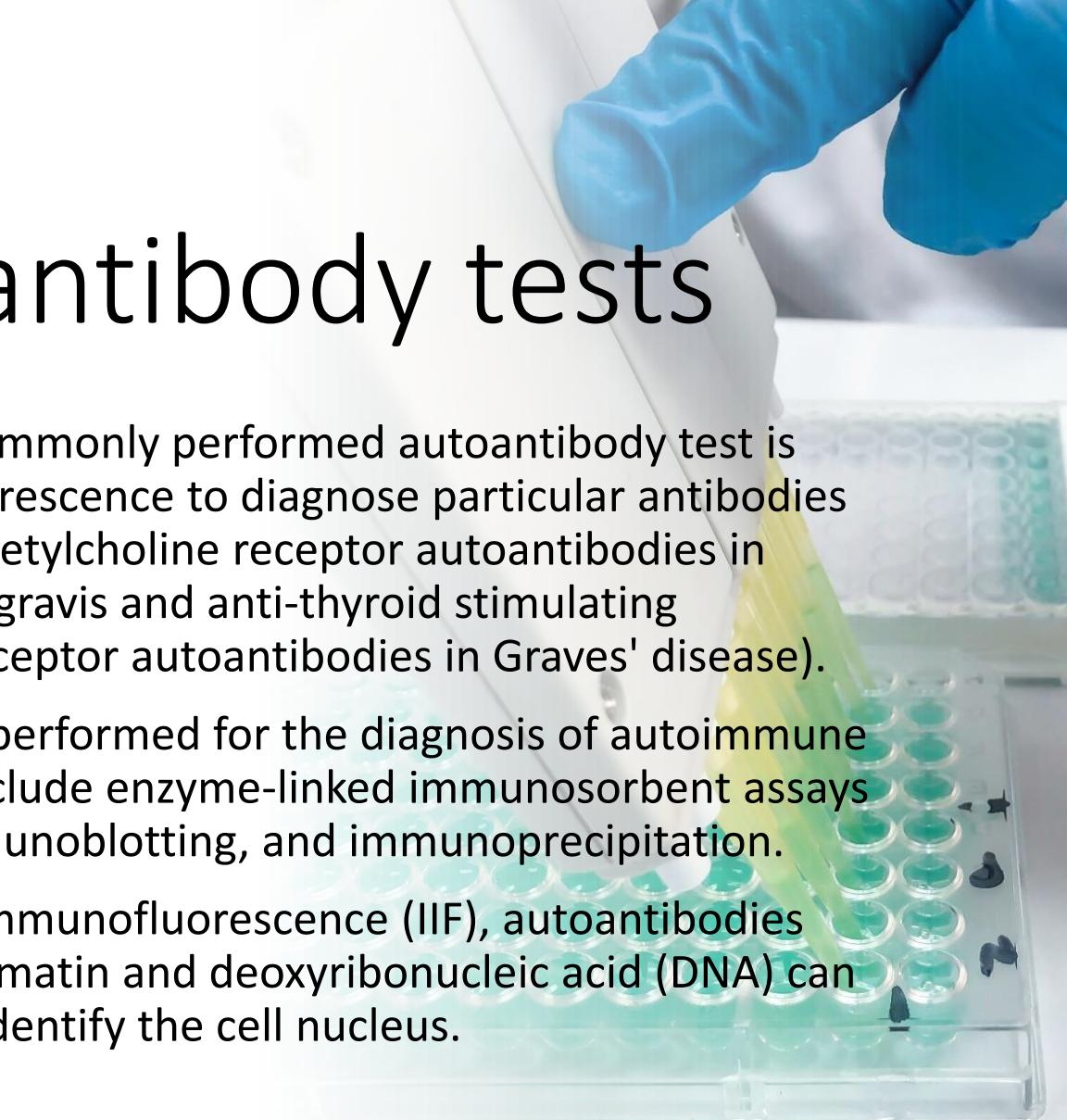
Diagnosis of Autoimmune diseases

- **Ceruloplasmin** the major copper containing protein in blood that plays a role in iron metabolism and is increased in acute and chronic inflammatory states, pregnancy, lymphoma, rheumatoid arthritis and Alzheimer's disease
- **Fibrinogen** a hemostatic coagulation factor produced in response to tissue injury. Fibrinogen synthesis is controlled at the transcription level and is increased in the presence of inflammation and stress that is mediated by IL-6.
- **Haptoglobin** is produced in response to tissue injury. Increased levels of haptoglobin can be seen during inflammation, malignancy, surgery, trauma, peptic ulcer disease and ulcerative colitis. Decreased levels may indicate chronic liver disease or anemia.
- **Albumin** a serum protein synthesized by the liver that aids body tissues in maintaining oncotic pressure necessary for proper body fluid distribution. The average amount of albumin in the plasma is approximately 300 to 400 grams, and about 15 grams is produced by the liver per day. While the rate of synthesis can double in situations of rapid albumin loss as seen in glomerulonephritis or inflammatory bowel disease, serum levels will decline.



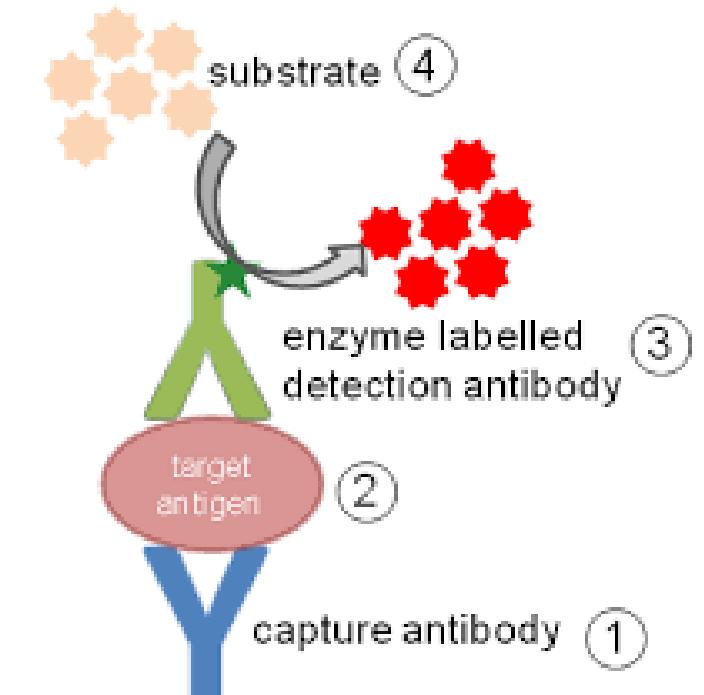
Autoantibody tests

- The most commonly performed autoantibody test is immunofluorescence to diagnose particular antibodies (e.g., anti-acetylcholine receptor autoantibodies in myasthenia gravis and anti-thyroid stimulating hormone receptor autoantibodies in Graves' disease).
- Other tests performed for the diagnosis of autoimmune disorders include enzyme-linked immunosorbent assays (ELISA), immunoblotting, and immunoprecipitation.
- In indirect immunofluorescence (IIF), autoantibodies against chromatin and deoxyribonucleic acid (DNA) can be used to identify the cell nucleus.



Autoantibodies and Immunologic Studies

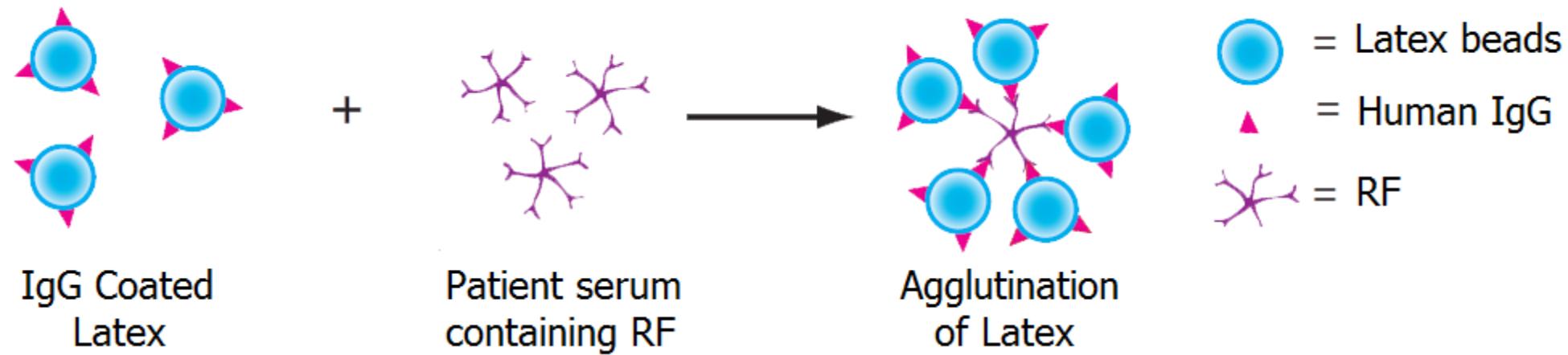
- **Enzyme-linked immunosorbent assay (ELISA)**
- ELISA is an immunometric method for detecting and measuring specific antibodies. The basic components of this laboratory method include a substrate where an antigen is fixed (typically a 96 well micro-well plate), patient's sera, washing solutions and a detection method where an enzyme is linked to an antibody that detects the antigen.
- In a typical double-antibody sandwich ELISA, an antibody that is attached to the bottom of a well provides both antigen capture and immune specificity, while another antibody linked to an enzyme provides detection and acts as an amplification factor.
- This allows for accurate and sensitive detection of the antigen of interest. However, performance is largely dependent on antibody quantity, kit manufacturer, and operator skill and experience.
- ELISA permits measurement of only one antigen at a time for a given aliquot of sample. Furthermore, ELISA has a limited dynamic range (that is, the range over which there is a linear relationship between antigen concentration and absorbance reading) – the range is narrow relative to the range for other technologies such as multiplex assays



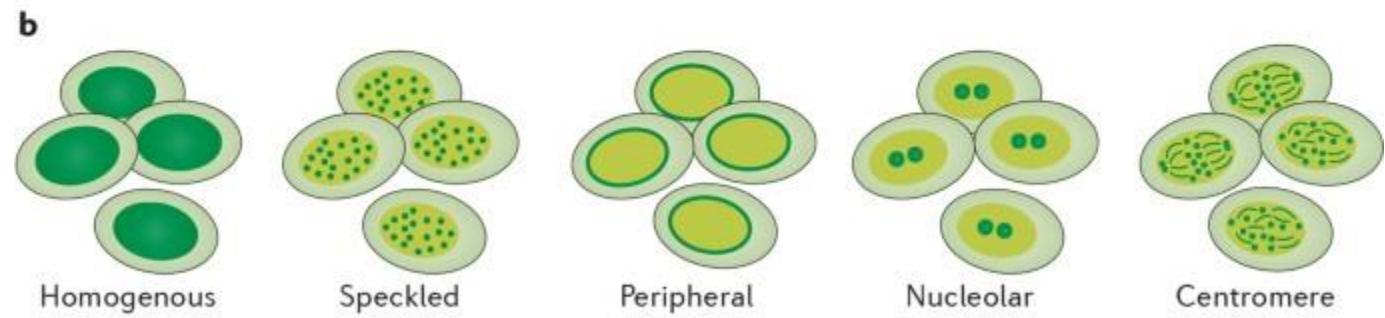
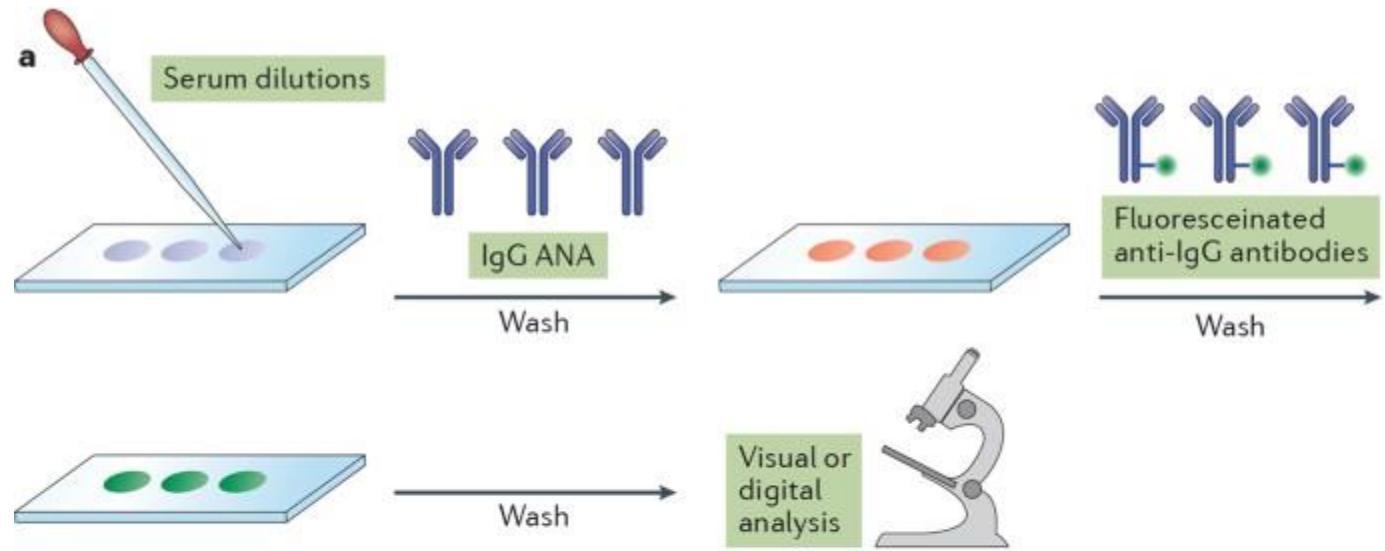
Autoantibodies and Immunologic Studies

- **Rheumatoid factor (RF) and Anti-cyclic citrullinated peptide antibody (CCP)**
- RF is an autoantibody that reacts to the Fc portion of polyclonal IgG; but they can be any class of immunoglobulin. Most assays detect the IgM rheumatoid factor. RF is helpful when evaluating patients who may have rheumatoid arthritis as the sensitivity is ~70% with a specificity of ~70%. Rheumatoid factor is absent in ~15% of patients with rheumatoid arthritis. However, ~15% of the healthy population may have a low titer RF.
- Rheumatoid factor positive patients are more likely to have progressive, erosive arthritis with loss of joint mobility and also have extraarticular manifestations including rheumatoid nodules, vasculitis, Felty's syndrome and secondary Sjögren's syndrome. In addition, the presence of RF is seen in other autoimmune disorders including Sjogren's syndrome, SLE, cryoglobulinemia, in pulmonary diseases such as interstitial fibrosis and silicosis and various infectious diseases.

Rheumatoid Factor



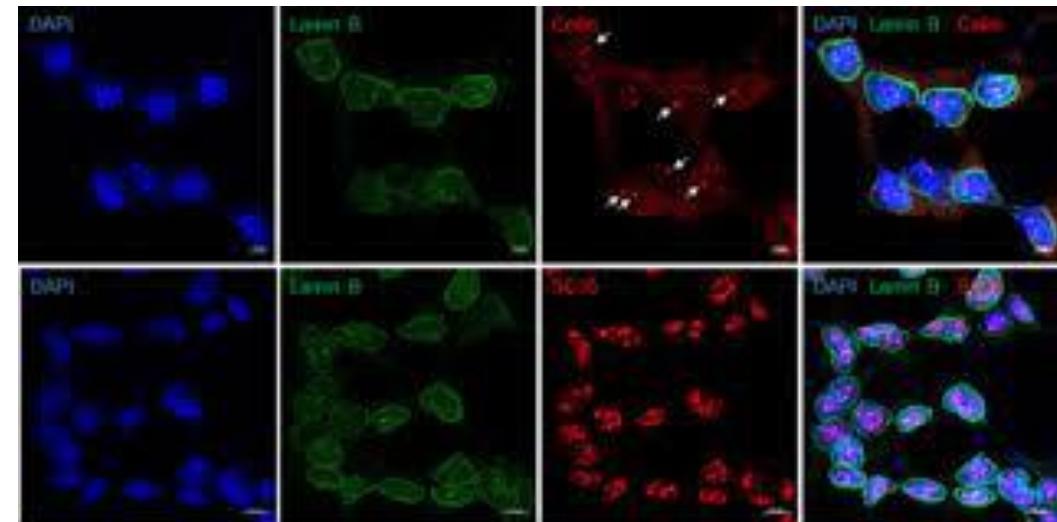
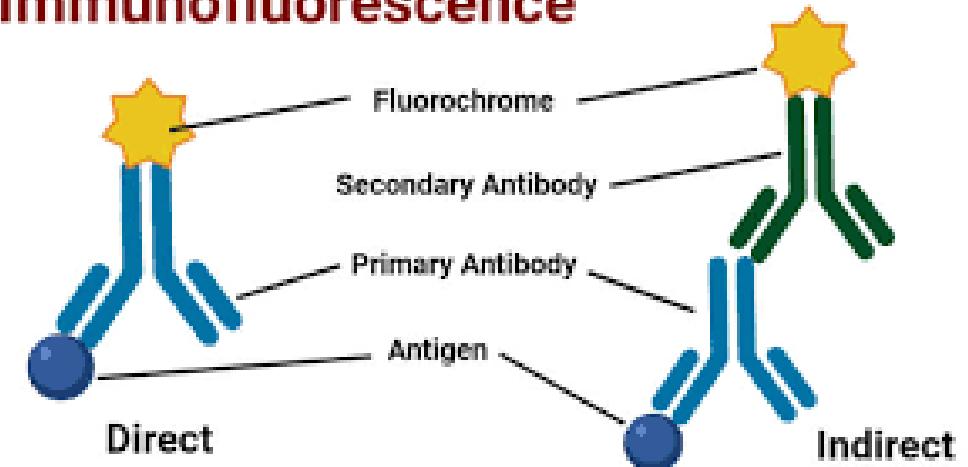
- **Anti-nuclear antibody (ANA)**
- Autoantibodies to nuclear antigens are a diverse group of antibodies that react against nuclear, nucleolar, or perinuclear antigens.
- These antigens represent cellular components such as nucleic acid, histone, chromatin, nuclear and ribonuclear proteins.
- Classically, the ANA hallmarks the serologic diagnosis of SLE, but finding an ANA is common to most other autoimmune diseases. Methods used for detection utilize immunofluorescence testing of the patient's serum, at various dilutions, using a cell substrate.
- Typically, screening patient's serum for the detection of an ANA with ELISA provides high sensitivity but lacks specificity.
- Results are reported as either the dilution of serum that tests positive or the degree of positivity measured by the testing procedure.
- Historically, HEp2 cells (a human laryngeal epithelioma cancer cell line) have been used as the cell substrate because the result offers the advantage of detecting a nuclear fluorescent pattern. The fluorescent patterns (homogenous, diffuse, speckled, peripheral and rim) suggest clinical associations with certain autoimmune diseases. However, because of the time and expense for testing with HEp2 cells, the assay procedures are largely done by ELISA methods.



- **Immunofluorescence**

- is particularly useful as an initial screening test for those individuals suspected of having an autoimmune disease – SLE, Sjögren's syndrome, RA, mixed connective tissue disease (MCTD), scleroderma, polymyositis/dermatomyositis (PM/DM). However, one must use caution when interpreting ANA as this autoantibody is found in nonrheumatic diseases such as Hashimoto's thyroiditis, Graves' disease, autoimmune hepatitis, primary autoimmune cholangitis, primary pulmonary hypertension, and in various infections and malignancies. Furthermore, the presence of low titer ANA occurs more frequently in elderly populations.

Immunofluorescence



- **Anti-double stranded DNA (anti-dsDNA)**
- Autoantibodies to double stranded DNA are an important marker used in the diagnosis and monitoring of SLE. Antibodies to dsDNA are highly specific for SLE.
- However, some patients with other rheumatic diseases or chronic active hepatitis may have mildly or moderately elevated serum titers.
- Previously, anti-dsDNA was typically measured using radioimmunoassay (particularly the Farr assay).
- The more common current tests employ an immunofluorescence assay (IFA) or ELISA.
- **Anti-extractable nuclear antigen (anti-ENA)**
- The extractable nuclear antigens consist of the Smith (Sm) antigen, ribonuclear protein (RNP) or U1RNP, anti-SSA (Ro) and anti-SSB (La).
- They are called extractable because they are readily soluble or extractable in neutral buffers.
- The Sm antigen is highly specific for SLE, but it is found only in ~25% of SLE patients. The U1RNP antigen is seen in patients with SLE plus systemic sclerosis and in patients with mixed connective tissue disease. The SSA (Ro) and SSB (La) nuclear antigens are often found together in those patients with Sjögren's syndrome. Anti-SSA and anti-SSB are also seen in some subsets of SLE patients.

- **Anti-signal recognition particle (anti-SRP), anti-JO-1, anti-Mi2, anti-PM/Scl**
- Anti-SRP, anti-JO-1, anti-Mi2 and anti-PM/Scl
- are termed myositis specific antibodies because of the high specificity to the autoimmune inflammatory myopathies (IIM). Anti-SRP antibodies are directed toward an RNA-protein complex consisting of 6 proteins and a 300-nucleotide RNA molecule (7SL RNA).
- Patients with this antibody have a distinct type of IIM that is characterized by acute onset of muscle weakness, a muscle biopsy that lacks inflammation and the patient shows a poor response to therapy. Anti-JO-1 autoantibodies are the most common autoantibody found in the group of inflammatory myopathies called the anti-synthetase syndrome.
- **Antineutrophil cytoplasmic antibody (ANCA) [myeloperoxidase (MPO), proteinase-3 (PR3)]**
- Antineutrophil cytoplasmic antibodies (ANCA) react with cytoplasmic granules of neutrophils. Initial ANCA testing screens sera for the presence of ANCA and two general immunofluorescent staining patterns are observed – cytoplasmic (cANCA) and perinuclear (pANCA). The immunofluorescence pattern is helpful to distinguish various ANCA associated vasculitis syndromes.

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Immunoglobulin	Increased	Decreased
Ig G	Infection, inflammation, hyperimmunization, IgG multiple myeloma, liver disease, rheumatic fever, systemic rheumatic disease	Agammaglobulinemia, amyloidosis, leukemia, myeloma, preeclampsia
IgM	Early HIV infection, infectious mononucleosis, lymphoma, macroglobulinemia, myeloma, rheumatoid arthritis	Rarely agammaglobulinemia, amyloidosis, leukemia, myeloma
IgA	Chronic infections (especially of gastrointestinal tract), inflammatory bowel disease, myeloma, rheumatic fever	Agammaglobulinemia, hereditary IgA deficiency, myeloma or protein-losing enteropathy

- **Flow cytometry**
- Flow cytometry is a technique where particles or tagged cells flow through laser light so that populations of particle/cells can be counted and phenotyped using cell characteristics and surface proteins. Initial applications of flow cytometry pertained to the interest in certain cell populations, for example the numbers of lymphocytes in patients infected with human immunodeficiency virus (HIV).
- **Cytokine studies**
- Cytokines are molecules secreted by a variety of cells that function in cellular communication. Immunologists are keenly interested in cytokines, particularly those that influence immune function and inflammation. Commercial testing laboratories do not routinely assay most serum cytokine levels, as this testing is largely done in research laboratories. Testing is laborious because of the labile nature of these small molecules.
- Cytokines that influence inflammation include IL-1, IL-6 and TNF-alpha.
- **Major Histocompatibility Complex (MHC) (human leukocyte antigen (HLA))**
- Human leukocyte antigen (HLA) is synonymous with the major histocompatibility complex (MHC). MHC class I and II genes are the major genetic determinants of susceptibility to many autoimmune diseases.

- MHC class I molecules include HLA-A, -B, and –C. MHC class II molecules include HLA-DR, HLA-DQ, and HLA-DP.
- Detection of HLA type can be done routinely and can be assayed using several methods that include gel electrophoresis, polymerase chair reaction (PCR), ELISA, and newer methods employing high-throughput detection of nucleic acid.

Treatment

- Autoimmune disorders in general cannot be cured, but the condition can be controlled in many cases.
- Historically, treatments include:
 - anti-inflammatory drugs – to reduce inflammation and pain
 - corticosteroids – to reduce inflammation. They are sometimes used to treat an acute flare of symptoms
 - pain-killing medication – such as paracetamol and codeine
 - immunosuppressant drugs – to inhibit the activity of the immune system
 - physical therapy – to encourage mobility
 - treatment for the deficiency – for example, insulin injections in the case of diabetes
 - surgery – for example, to treat bowel blockage in the case of Crohn's disease
 - high dose immunosuppression – the use of immune system suppressing drugs (in the doses needed to treat cancer or to prevent the rejection of transplanted organs) have been tried recently, with promising results. Particularly when intervention is early, the chance of a cure with some of these conditions seems possible.

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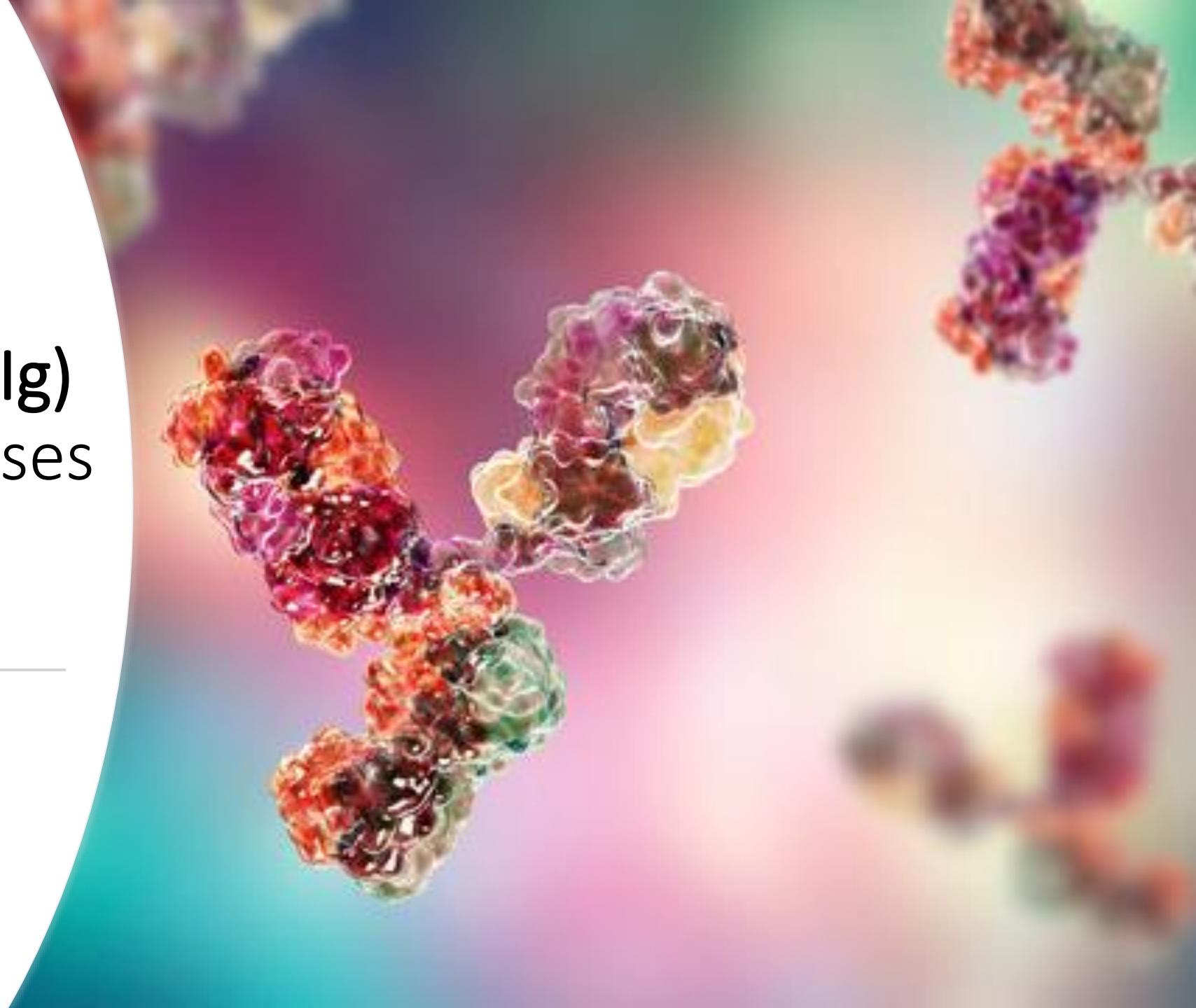
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Unit 2: Module 2.1

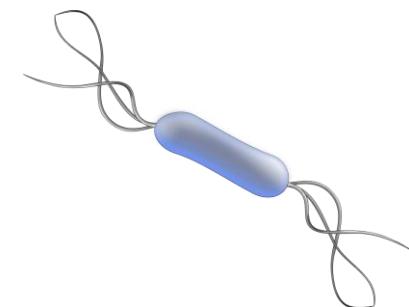
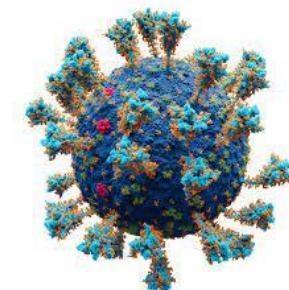
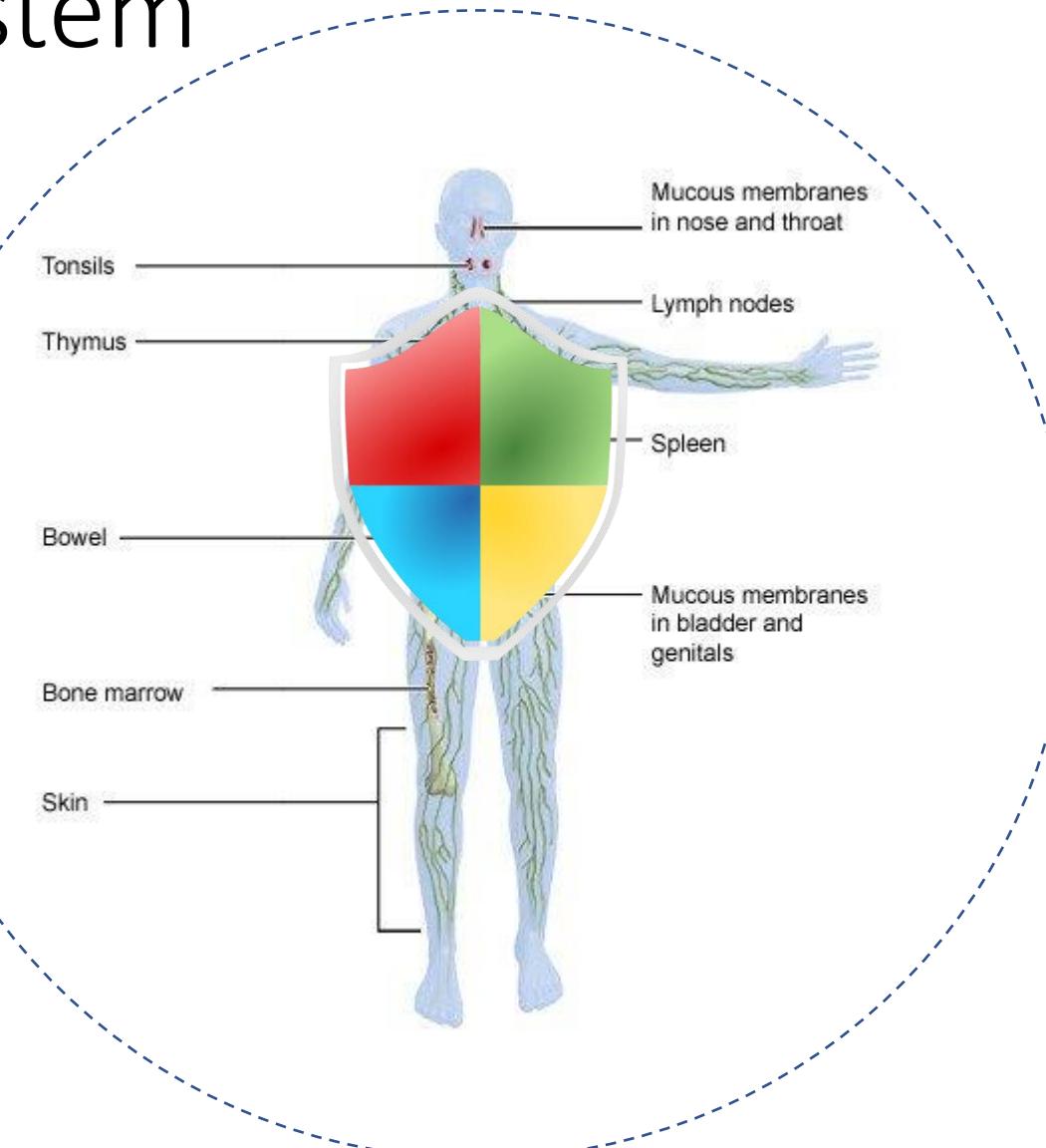
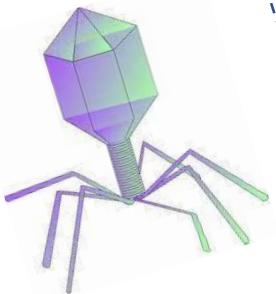
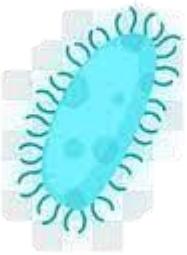
Immunoglobulin (Ig) Classes & Subclasses

Antibodies, Receptors & MHC

Dr. (Ms.) Sonali Correa
MSc., PhD.



Immune system



Immune System

Innate

- **First line of defence**
- **Physical barriers and Chemical barriers**
- **Function and efficiency do not change** with repeated exposure to foreign pathogens.

Adaptive or Acquired

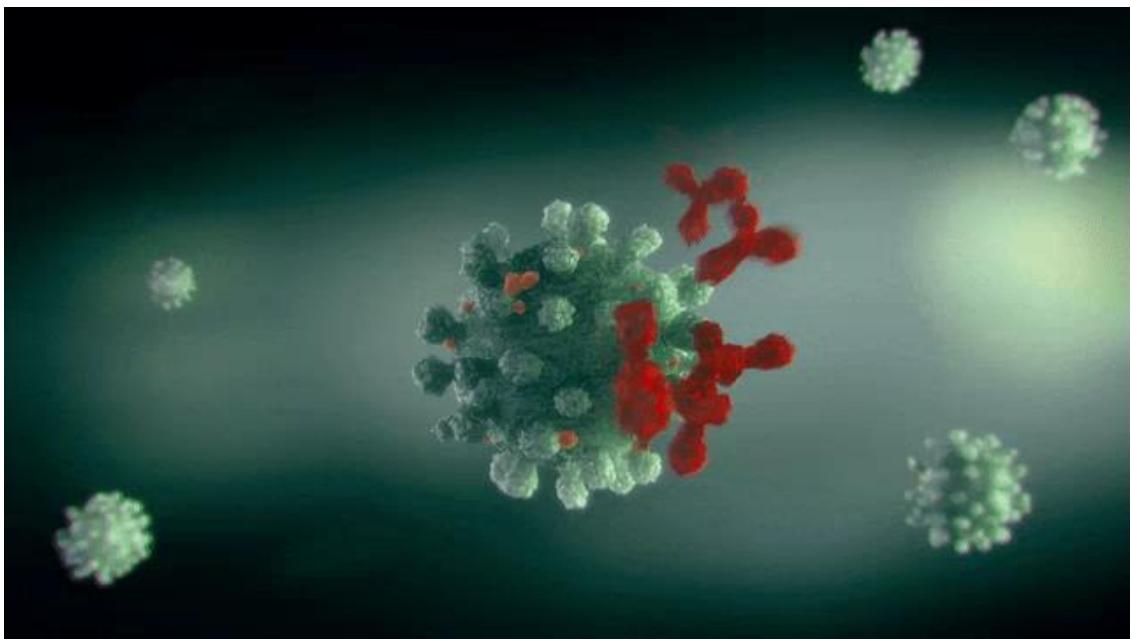
- Is activated when the innate system fails
- Consists **lymphocytes and antibodies** being the key elements
- Lymphocytes arise continuously from **progenitor cells** in the bone marrow & **synthesize cell surface receptors** or **secrete proteins** that specifically bind to foreign molecules.
- These secreted proteins are known as **antibodies**. Any molecule that can bind to an antibody is called an antigen.
- The term **antibody** is used interchangeably with **immunoglobulin**.

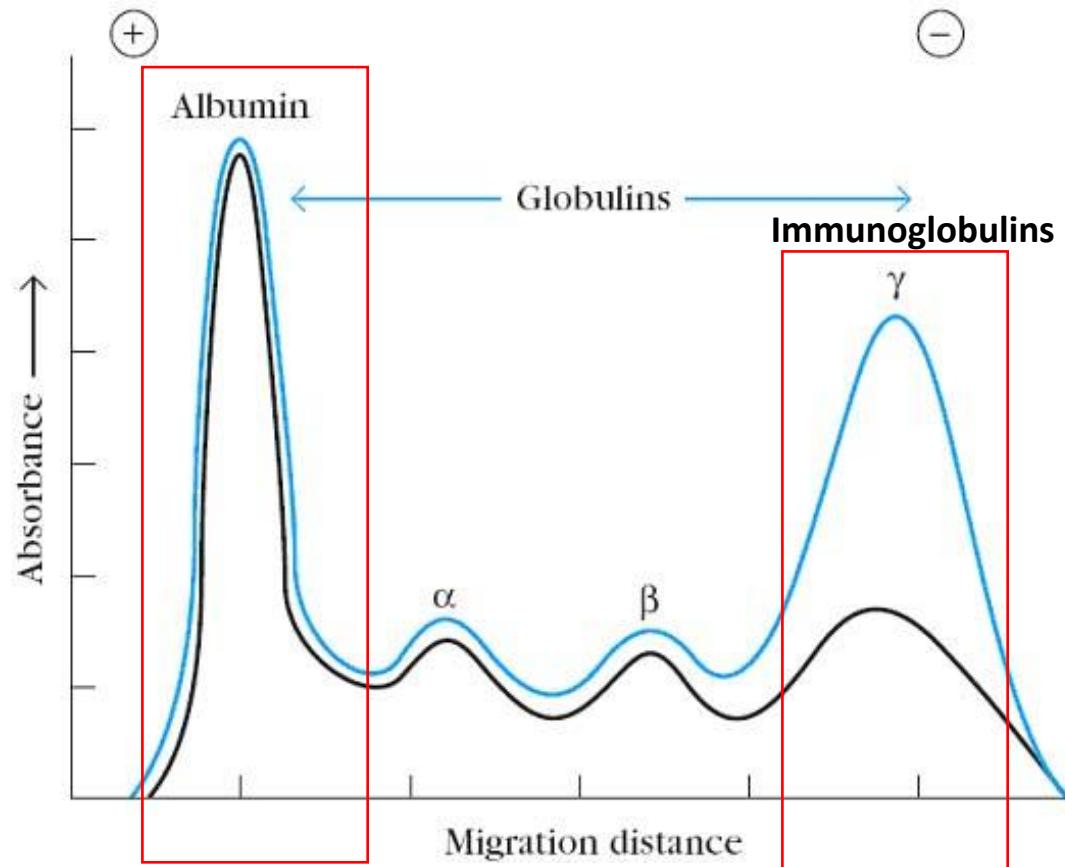
Passive

- Passive immunity is "**borrowed**" from another source and it lasts for a short time.
- For example, antibodies in a mother's breast milk give a baby temporary immunity to diseases the mother has been exposed to.

Immunoglobulin

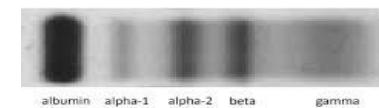
- Immunoglobulins (**Ig**), also known as antibodies (**Ab**), are **glycoprotein molecules** produced by plasma cells or white blood cells
- They **recognize and bind to specific epitopes** (the part of an **antigen molecule** to which an antibody attaches itself) on antigens
- Present on B cell membrane & secreted by plasma cells, and they circulate in blood, where they eliminate/neutralize an antigen





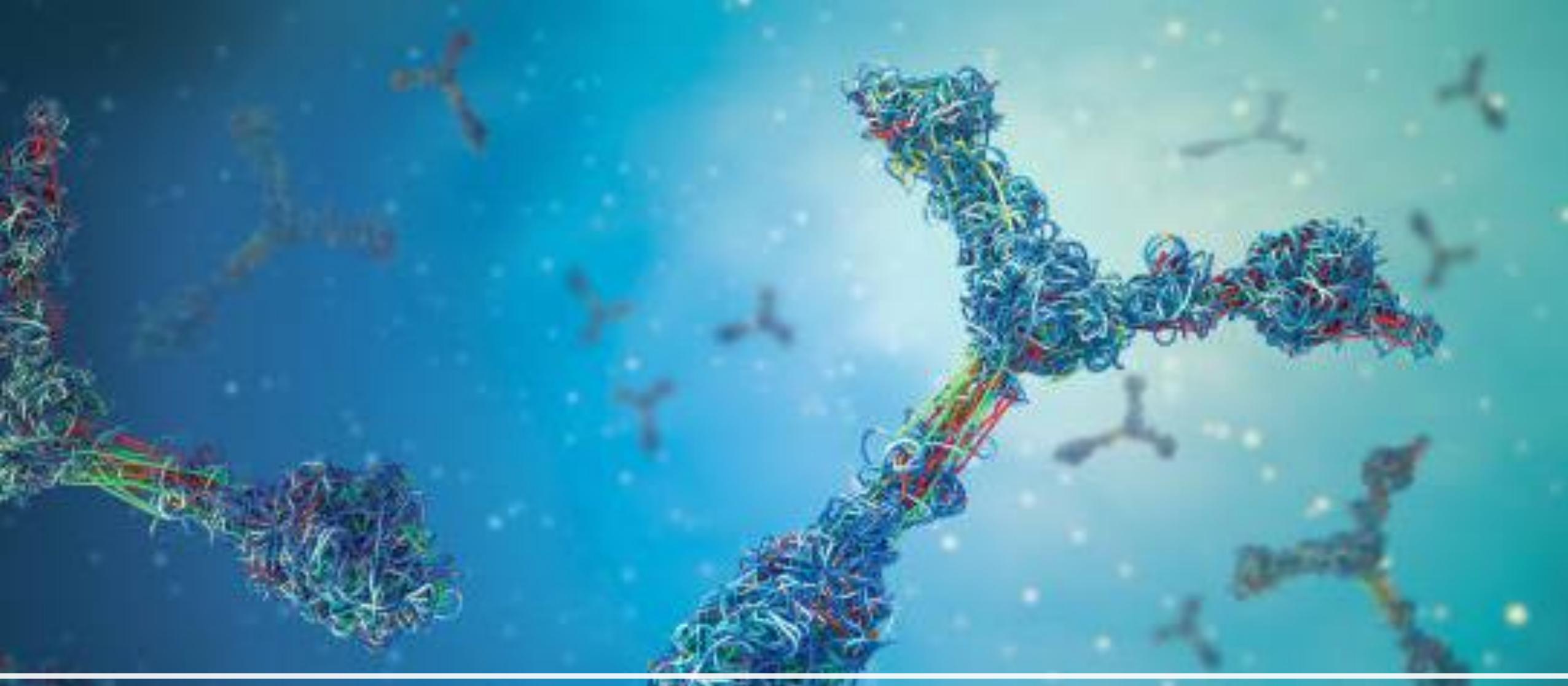
Immunoglobulins constitute of 20-25% of serum proteins

- Tiselius (1937), Swedish biochemist carried out the serum electrophoresis (immune sera) which separated in 4 fractions (*albumin, alpha-, beta- & gamma globulins*)
- Most antibody activity was found in Gamma globulin & thus antibody molecule is considered as “Gamma-globulin fraction”
- As they immunologically react with the antigen, they were given the name **Immunoglobulin**

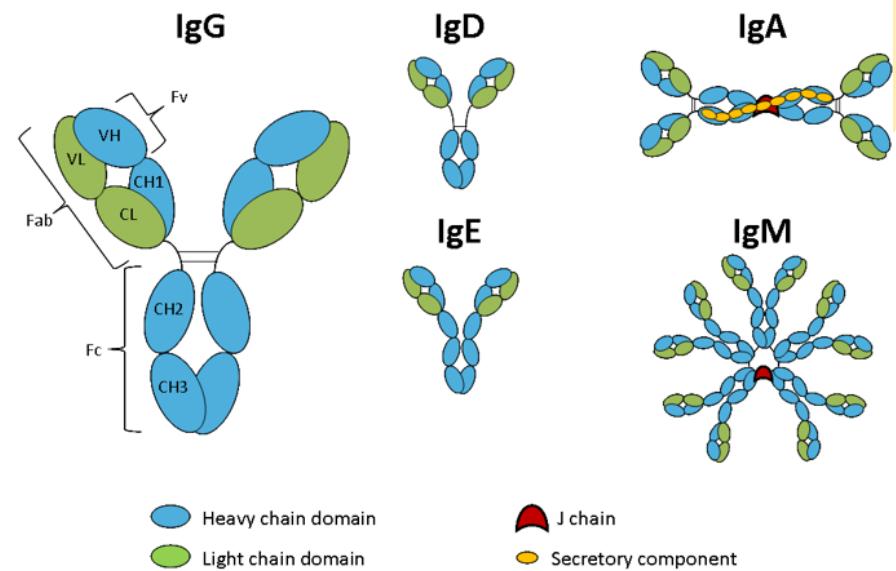
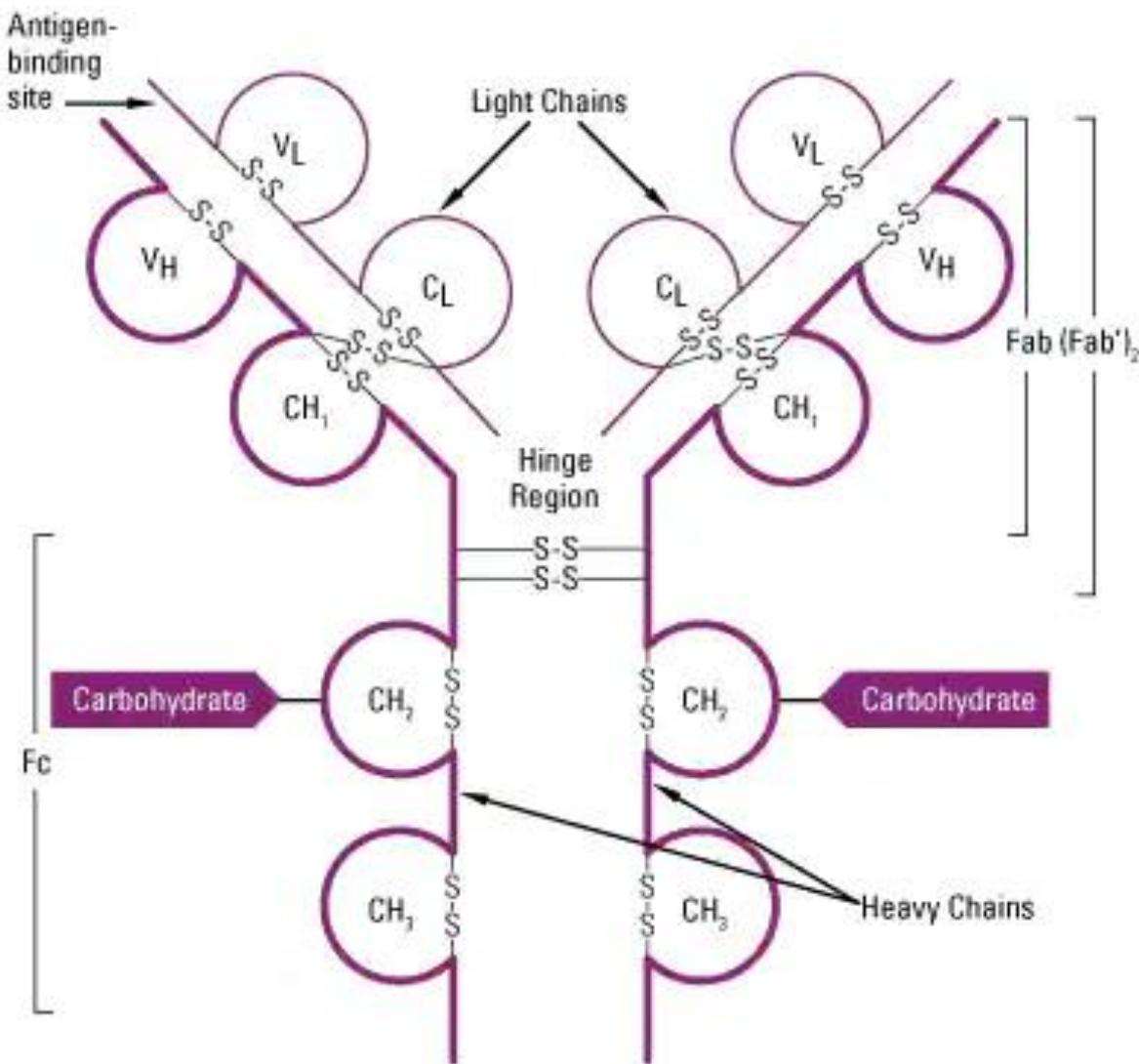


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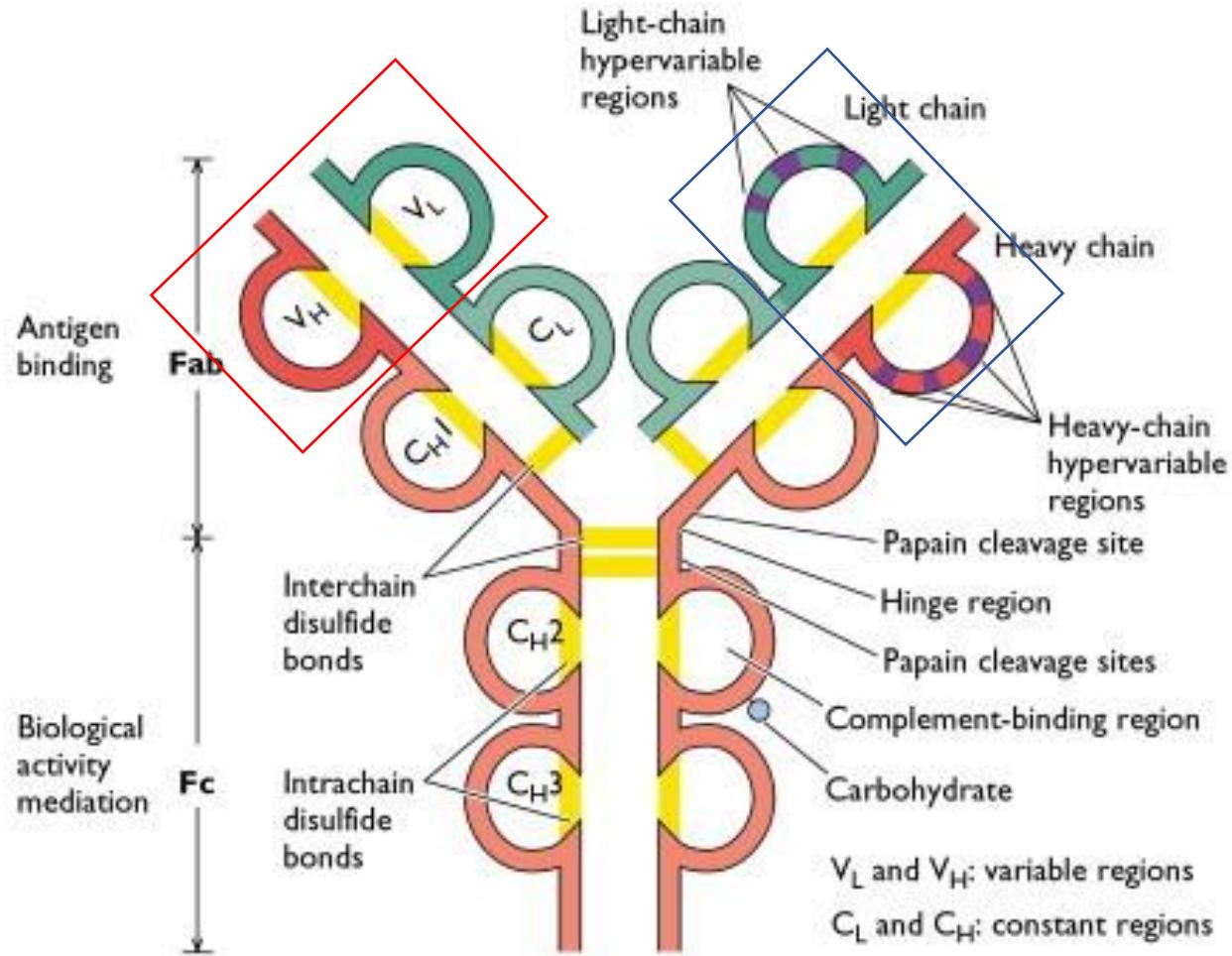
STRUCTURE OF IG



Ig	Heavy Chain
IgG	Gamma γ
IgA	Alpha α
IgM	Mu μ
IgD	Delta δ
IgE	Epsilon ϵ

- Made of 4 polypeptide chains: **2 heavy chains (H) & 2 light chains (L)**
- Structure is divided into 2 regions: **Constant & variable**
- 2 fragment: **Fc fragment (Fragment crystallizable) & Fab (antigen-binding) fragment**
- The amino terminal ends of the polypeptide chains show considerable variation in amino acid composition and are referred to as the variable (V) regions to distinguish them from the relatively constant (C) regions.
- Each L chain consists of **one variable domain**, VL, and **one constant domain**, CL.
- The H chains consist of a **variable domain**, VH, and **three constant domains** CH1, CH2 and CH3.
- Each heavy chain has about **twice the number of amino acids** and molecular weight (~50,000) as each light chain (~25,000), resulting in a total immunoglobulin monomer molecular weight of approximately 150,000.
- Heavy and light chains are held together by a combination of non-covalent interactions and covalent interchain **disulfide bonds**, forming a bilaterally symmetric structure.
- The V regions of H and L chains comprise the **antigen-binding sites** of the immunoglobulin (Ig) molecules. Each **Ig monomer contains two antigen-binding sites** and is said to be bivalent.
- The hinge region is the area of the H chains between the first and second C region domains and is held together by disulfide bonds. This flexible hinge (found in IgG, IgA, and IgD, but not IgM or IgE) region allows the distance between the two antigen-binding sites to vary.

Variable & Constant Regions



Fab fragments: represent **the antigen binding fragment** of an intact antibody containing both the variable and constant regions of both heavy and light chains

Fc fragments: The fragment crystallizable region (Fc region) is the tail region, that interacts with **cell surface receptors** called Fc receptors and some proteins of the complement system. Allows antibodies to activate the immune system.

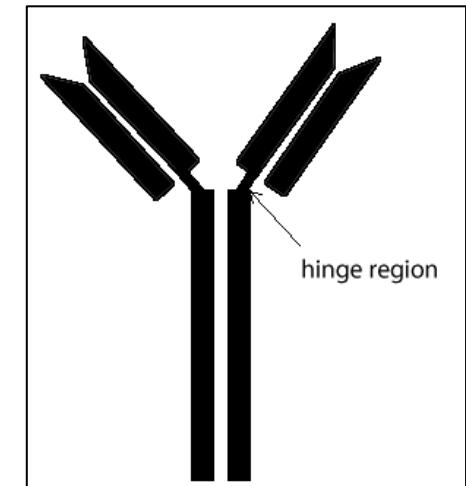
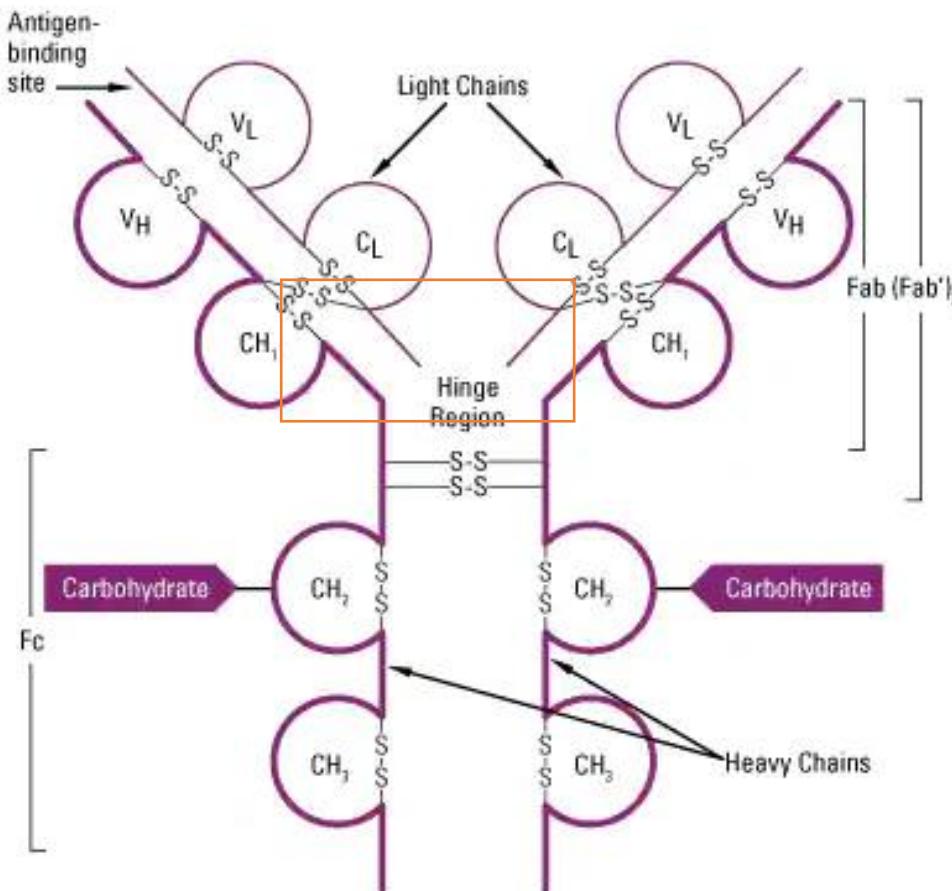
Variable Region: Antigen Binding site

Hyper-variable region: Within the V region there are hot spots that show higher variability in the amino acid sequences. Also known Complementarity determining regions

The site on the HVR that make actual contact with the epitope of the antigen is known as paratope

Hinge Region

- Permits flexibility between the two arms of the Y-shaped antibody molecule.
- This allows them to open and close to accommodate binding to two identical antigens separated by a fixed distance
- Rich in proline & cysteine
- Highly sensitive to various **enzymatic digestions**

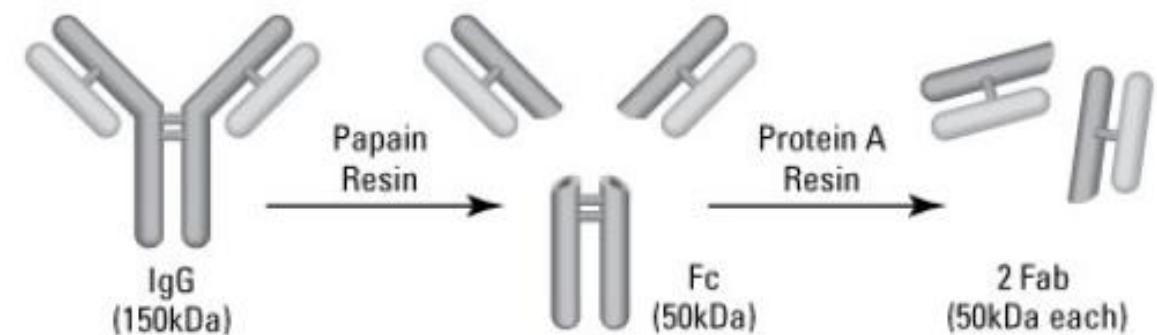


IgG—Preparing Fab, F(ab')2 and Fc fragments

- The hinge region of an immunoglobulin monomer (IgG) is readily accessible to proteolytic attack by enzymes.
- Cleavage at this point produces F(ab')2 or Fab fragments and the Fc fragment.
- The Fc fragment may remain intact or become further degraded, depending upon the enzyme and conditions used.
- Proteolytic IgG fragmentation using three different enzymes is discussed below.
- Traditionally, proteolysis was accomplished in solution using free enzyme. We have developed immobilized enzyme products that enable better control of digestion and efficient separation of reaction-products from the protease. Thus, the diagrams featured below refer to enzyme "resins."
- Most procedures also include Protein A resin antibody purification steps to separate Fab and Fc fragments.

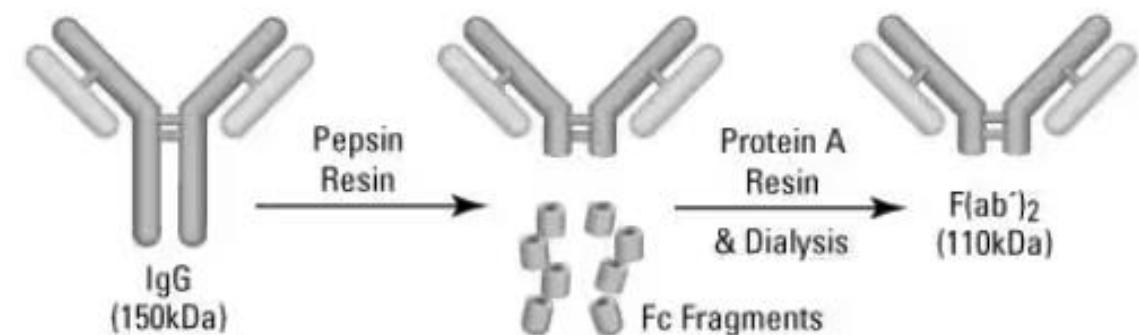
Papain digestion: Fab from IgG

- Papain is a nonspecific, thiol-endopeptidase that has a sulfhydryl group in the active site, which must be in the reduced form for activity.
- When IgG molecules are incubated with papain in the presence of a reducing agent, one or more peptide bonds in the hinge region are split, producing three fragments of similar size: two Fab fragment and one Fc fragment
- When Fc fragments are of interest, papain is the enzyme of choice because it yields an intact 50,000-dalton Fc fragment.



Pepsin digestion: F(ab')₂ from IgG

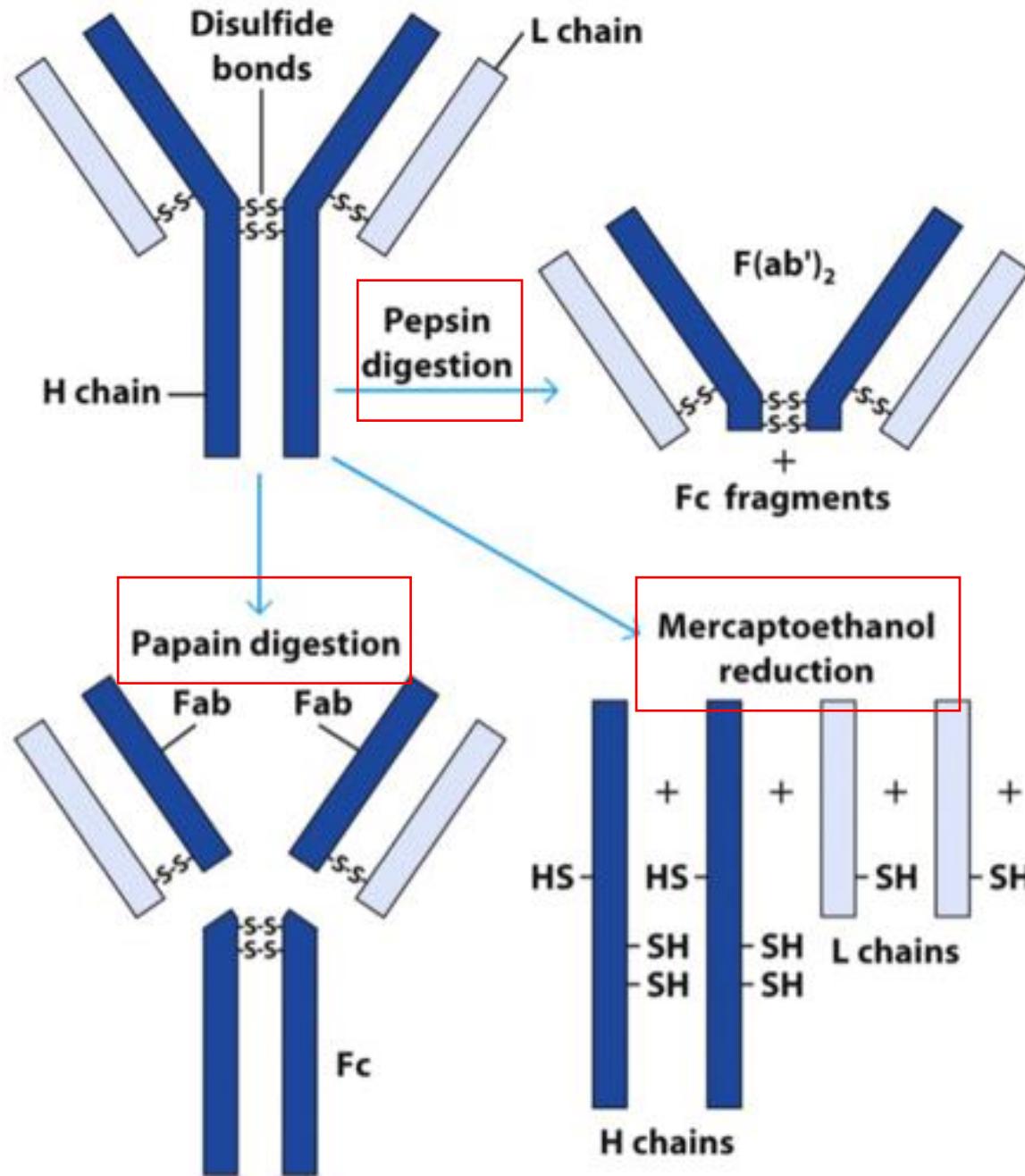
- Pepsin is a nonspecific endopeptidase that is active only at acid pH.
- It is irreversibly denatured at neutral or alkaline pH.
- Digestion by the enzyme pepsin normally produces one F(ab')₂ fragment and numerous small peptides of the Fc portion.
- The resulting F(ab')₂ fragment is composed of two disulfide-connected Fab units.
- The Fc fragment is extensively degraded, and its small fragments can be separated from F(ab')₂ by dialysis, gel filtration or ion exchange chromatography.



Mercaptoethanol reduction

- F(ab')₂ can be separated by mild reduction into two sulfhydryl-containing, univalent Fab' fragments.
- The advantage of Fab' fragments is that they can be conjugated to detectable labels directly through their sulfhydryl groups, ensuring that the active binding site remains unhindered and active.
- Use 2-Mercaptoethylamine•HCl (2-MEA) for mild reduction of F(ab')₂ fragments.
- The free sulfhydryls of each Fab' can be targeted for conjugation, or they can be blocked with an alkylating reagent, such as N-Ethylmaleimide (NEM) to prevent re-formation of the F(ab')₂.

Enzymatic digestion



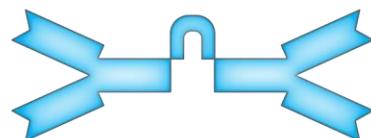
Valency

The valency of antibody refers to the **number of Fab regions it possesses thus how many binding sites an antibody may have**



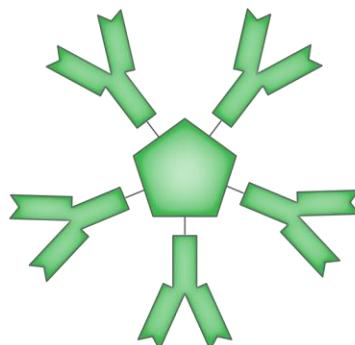
Monomer
IgD, IgE, IgG

Valency 2



Dimer
IgA

Valency 4



Pentamer
IgM

Valency 10 (however
not more than 5)



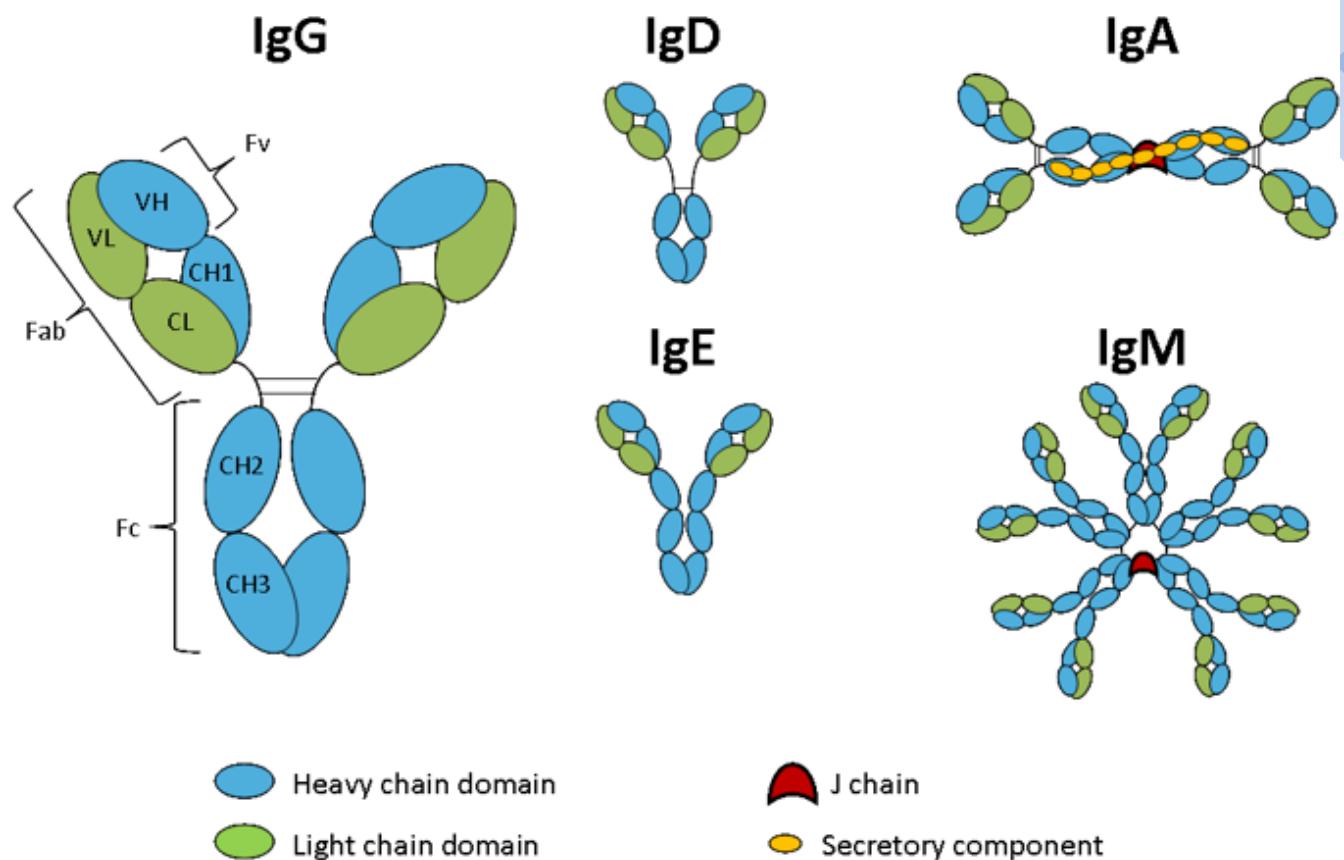
CLASSES OF IMMUNOGLOBULINS

- The five primary classes of immunoglobulins are IgG, IgM, IgA, IgD, and IgE.

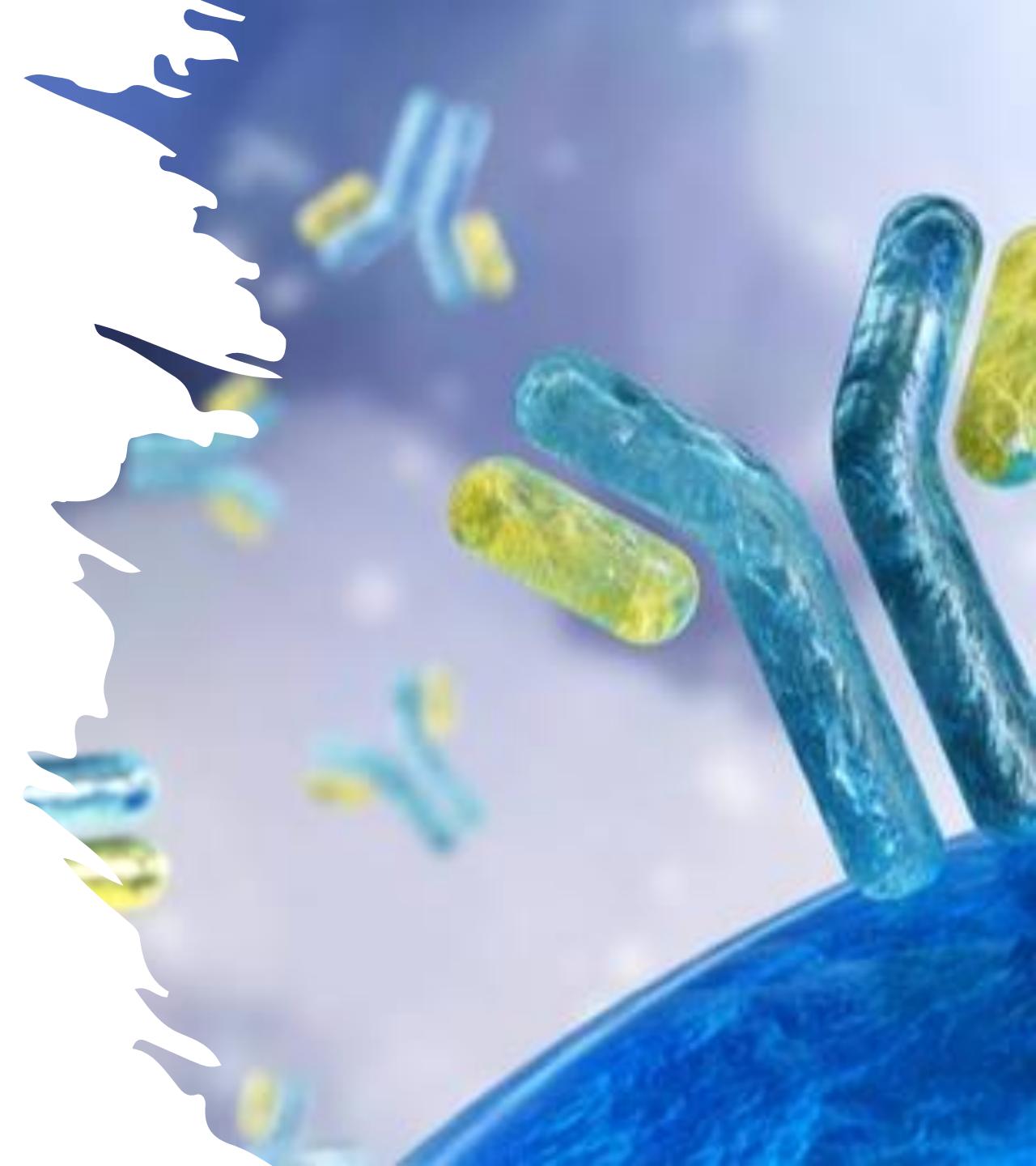
- These are distinguished by the type of heavy chain found in the molecule.

- IgG molecules have heavy chains known as γ gamma-chains
- IgMs have μ mu-chains;
- IgAs have α alpha-chains
- IgEs have ϵ epsilon-chains
- IgDs have δ delta-chains.

GAMED

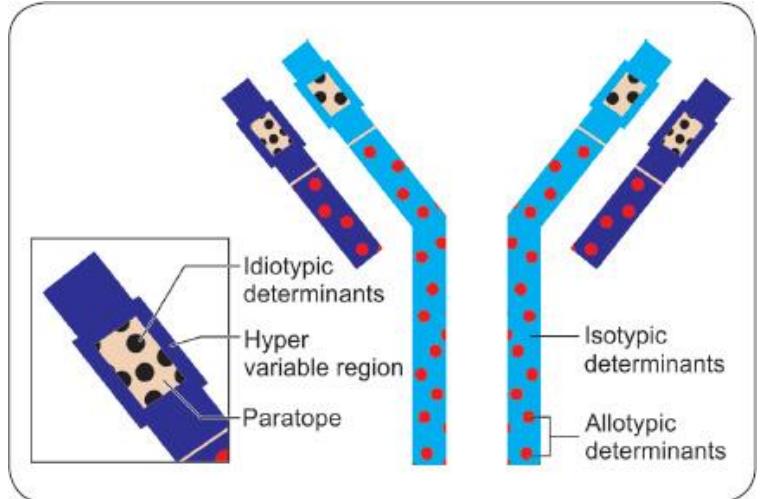


- Differences allow to function in different types of immune responses and at particular stages of the immune response.
- The polypeptide protein sequences responsible for these differences are found primarily in the Fc fragment.
- There are two main types of light chains: kappa (κ) and lambda (λ).
- Antibody classes differ in valency as a result of different numbers of Y-like units (monomers) that join to form the complete protein.
 - For example, in humans, functioning IgM antibodies have five Y-shaped units (pentamer) containing a total of ten light chains, ten heavy chains, and ten antigen-binding

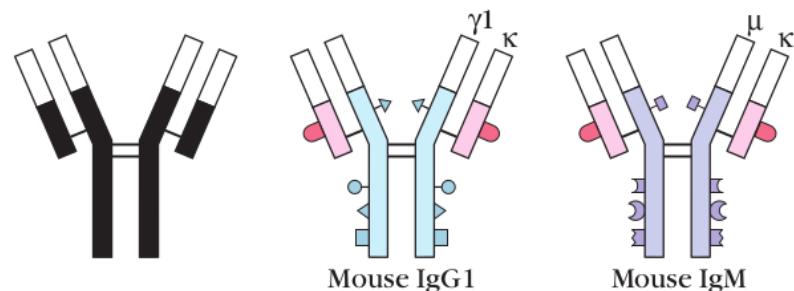


Properties	IgG	IgA	IgM	IgD	IgE
Form	Monomer	Mono-, Dimer	Mono-, Pentamer	Monomer	Monomer
MW (Da)	150,000	320,000	900,000	180,000	200,000
Valency	2	2 or 4	2 or 10	2	2
Chains	-	J chain secretory component	J chain	-	-
Sub Classes	G1, G2, G3, G4	A1, A2	-	-	-
Half-life days	23*	6	5	3	2.5
Intravascular dist (%)	45	42	80	75	50
Classical Complement Act	++ (IgG3>2>1)-	-	+++	-	-
Alternate	-	+	-	-	-
Placental Trnfer	+ (Except G2)	-	-	-	-
Mediates coagglutination	+ (Except G3)	-	-	-	-
Muscosal trnspt	-	+	-	-	-
Mast Cell	-	-	-	-	+

Isotypes



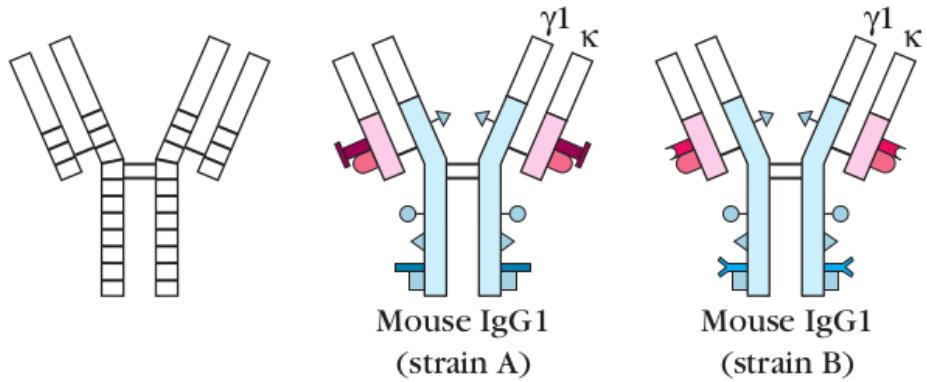
(a) Isotypic determinants



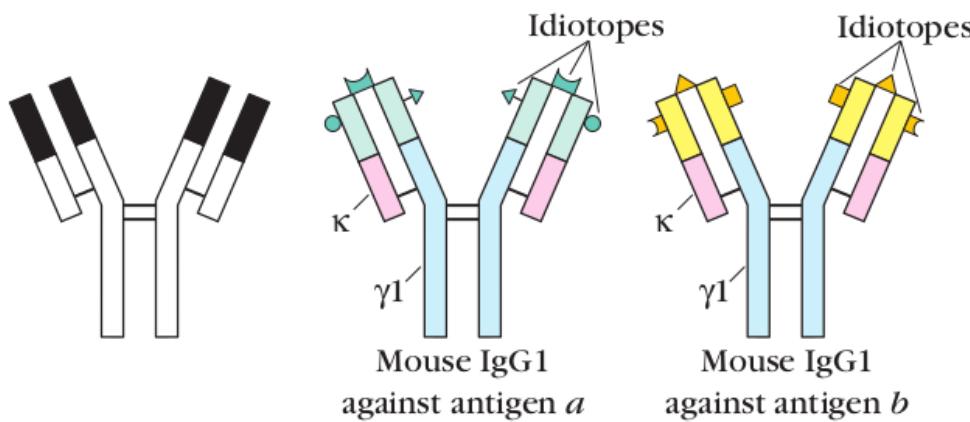
- The five classes of IgGs and their subclasses are called “isotypes”
- Vary from each other in the amino acid sequences in the constant region of their heavy chains

- Isotypes have different heavy chains.
- They represent classes of Abs

(b) Allotypic determinants



(c) Idiotypic determinants



Idiotype & allotype

- **Allotypes** have same constant region with minor but immunologic differences
- Different individuals have different allotypes

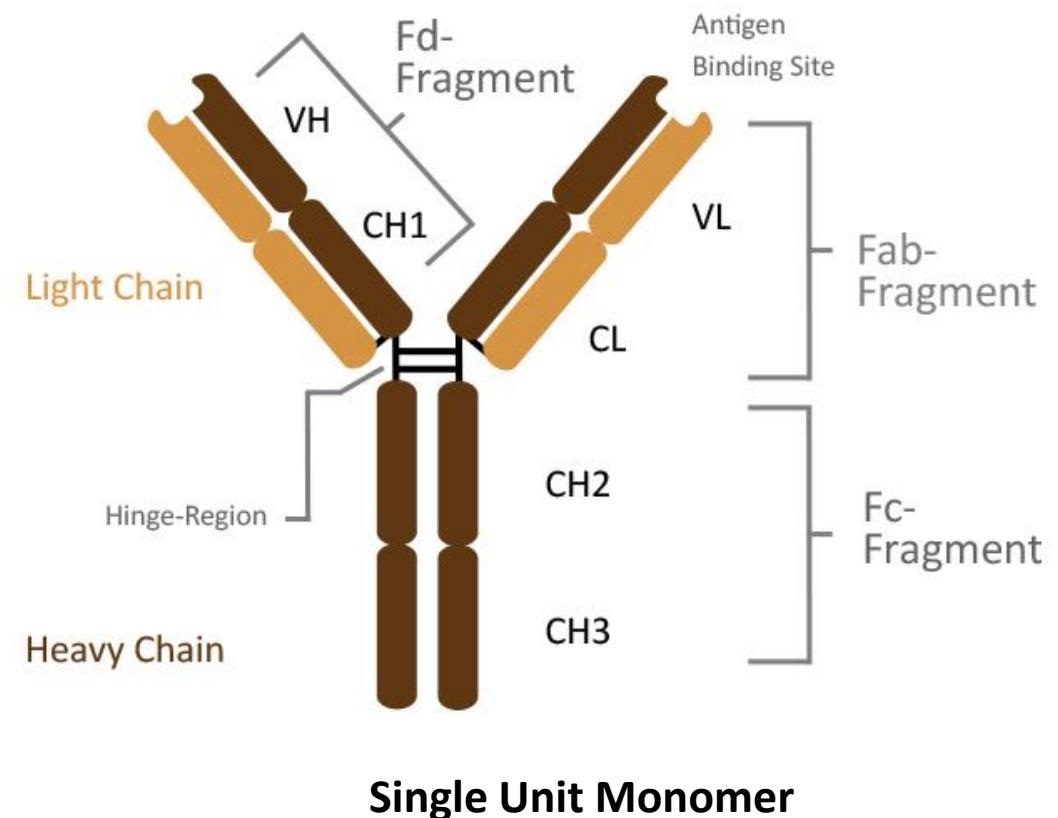
- **Idiotypes** are Abs that recognize different specific epitopes (on the paratope)
- Each idiotype is composed of several idiotypes or combining sites

IgG class

- IgG - Immunoglobulin G is the **main immunoglobulin**
- present in the **blood and represents 70% to 75%**
- Easily crosses blood vessels & the placental barrier and are responsible for defense against infection in the first few months of a baby's life.
- Provides long term Immunity

Properties of IgG:

- Molecular weight: 150,000 Da
- H-chain type (MW): gamma (53,000 Da)
- Serum concentration: 10 to 16 mg/mL
- Half-Life: 23 days (time required for $\frac{1}{2}$ Abs to disappear)
- Percent of total immunoglobulin: 75%
- Glycosylation (by weight): 3%
- Distribution: intra- and extravascular
- Function: secondary response

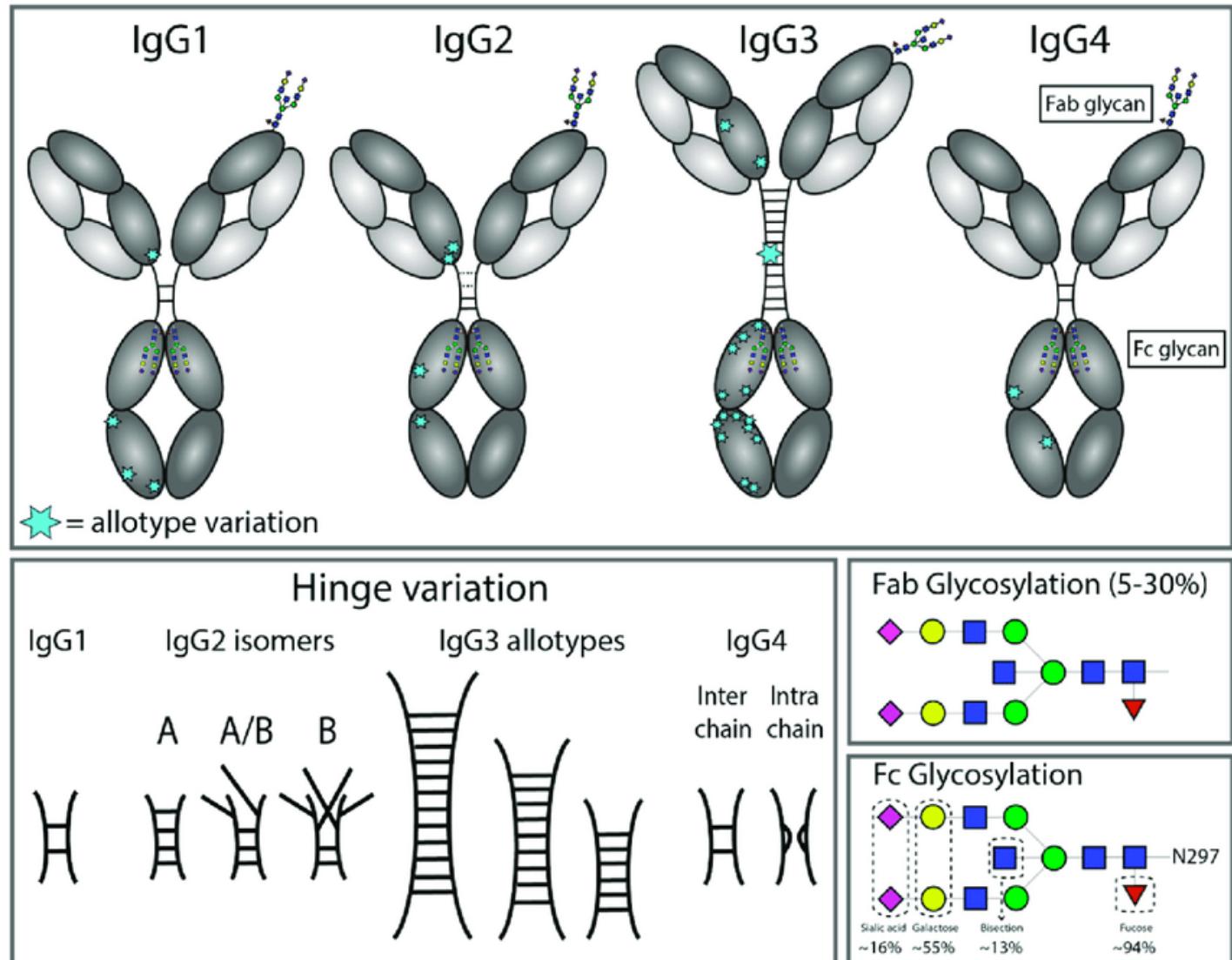


Role of IgG

- IgG is the major immunoglobulin in blood, lymph fluid, cerebrospinal fluid and peritoneal fluid and a key player in the **humoral immune response**.
- Serum IgG in healthy humans presents approximately **15% of total protein** beside albumins, enzymes, other globulins, and many more.
- The **Fc portion of IgG**, but not F(ab')2 or Fab fragments, can cross the placenta of a mother and enter fetal circulation, providing the fetus with postpartum protection.
- IgG molecules are able to react with Fc γ receptors (Fc γ Rs) that are present on the surface of macrophages, neutrophils and natural killer cells, and can activate the complement system.
- The binding of the Fc portion of IgG to the receptor present on a phagocyte is a critical step in the opsonization. Phagocytosis of particles coated with IgG antibodies is a vital mechanism that cells use to cope with microorganisms.
- IgG is produced in a delayed response to an infection and can be retained in the body for a long time. The longevity in serum makes IgG most useful for passive immunization by transfer of this antibody. Detection of IgG usually indicates a prior infection or vaccination.

IgG SubClasses

- IgG subclasses are more than **90% identical** on the amino acid level
- They have a unique profile with respect to **structure, antigen binding, immune complex formation, complement activation, triggering of Fc γ R, half-life, and placental transport**
- They differ in the **number of disulfide bonds** and the **length and flexibility** of the hinge region.



-
- Except for their variable regions, all immunoglobulins within one class share about 90% homology, but only 60% among classes.
 - Determination of IgG subclasses can be a valuable tool in indicating a potential antibody deficiency.
 - Selective IgG subclass deficiencies are associated with disease with prolonged or severe infections, determination of IgG levels can provide additional insight into the manifestation of disease. It is important to interpret IgG subclass concentrations in correlation to the donor's age since the immune system matures during childhood.
 - Because of its relative abundance and excellent specificity toward antigens, IgG is the principal antibody used in immunological research and clinical diagnostics

Definitions & terms

Immune complex formation: An immune complex, sometimes called an antigen-antibody complex or antigen-bound antibody, is a molecule formed from the binding of multiple antigens to antibodies.

Fc-gamma receptors (FcγRs) recognize IgG-coated targets, such as opsonized pathogens or immune complexes (ICs). Cross-linking leads to internalization of the cargo with associated activation of down-stream signaling cascades.

Activating Fc γ receptors (FcγRs) stimulate immune cell effector mechanisms, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis (ADCP), which combine to facilitate antibody-mediated tumor cell killing

Antibody effector functions are an important part of the humoral immune response and form an essential link between innate and adaptive immunity. Most of these effector functions are induced via the constant (Fc) region of the antibody, which can interact with complement proteins and specialized Fc-receptors.

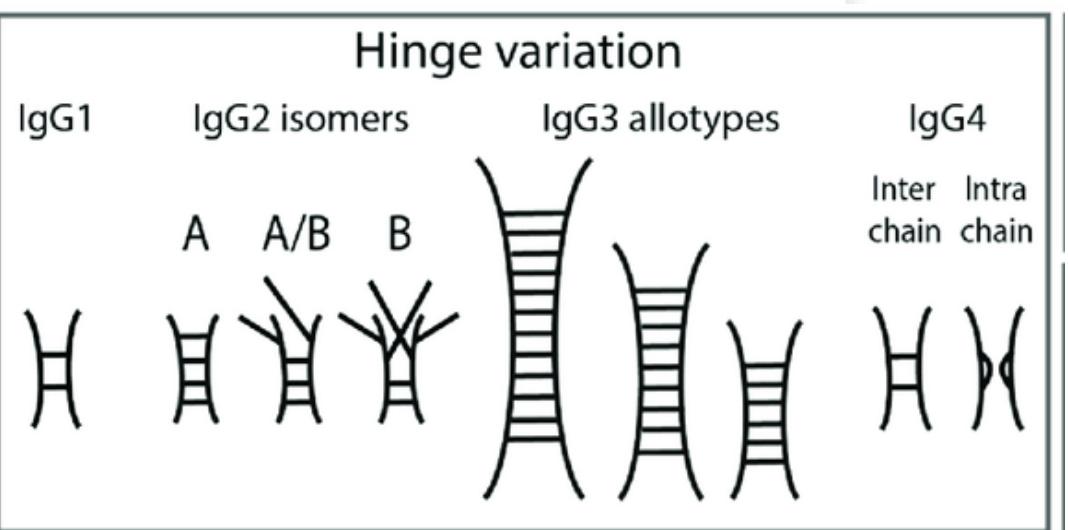
Antibody Effector Functions

- Neutralization occurs when antibodies bind to pathogens or toxins in order to prevent the pathogens from infecting cells.
- Opsonization is an immune process which uses opsonins to tag foreign pathogens for elimination by phagocytes.
- Complement Activation Complement is a system of plasma proteins that can be activated directly by pathogens or indirectly by pathogen-bound antibody, leading to a cascade of reactions that occurs on the surface of pathogens and generates active components with various effector functions.
- **Antibody-Dependent Cellular Cytotoxicity (ADCC)** also referred to as antibody-dependent cell-mediated cytotoxicity, is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies

IgG1

- Most abundant 60–65%
- Mediates antibody responses against viral pathogens
- It does so by binding to soluble proteins and membrane protein antigens via its variable domain and activating effector mechanisms of the innate immune system.
- IgG1 can effectively bind to C1q causing complement-dependent cytotoxicity (CDC) and can bind to each of the different Fc receptors resulting in antibody-dependent cell-mediated cytotoxicity (ADCC)
- relatively high thermostability, monomeric nature, and average flexible hinge region containing only two disulfide bonds.
- goal of the therapeutic requires ADCC or CDC (e.g., elimination of cancer cells), IgG1 is preferred.
- IgG1 molecules can be engineered with reduced binding to C1q or Fc γ Rs

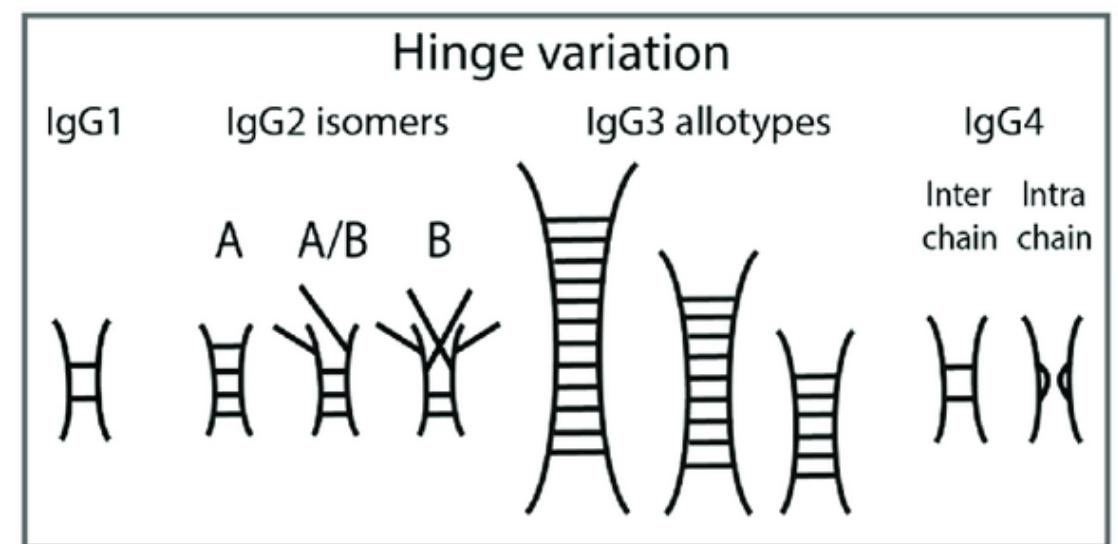
IgG2



- IgG2 plays a role in protection against protein antigens but is predominantly responsible for **anticarbohydrate IgG responses against bacterial capsular polysaccharides**.
- IgG2 is **limited** in its ability to **engage effector functions**
- relatively **short hinge** region (12 amino acids) containing four disulfide bonds within the hinge short hinge, combined with unique disulfide bond isoforms, can restrict the conformational flexibility of the Fab arms relative to the Fc portion of the IgG2 molecule
- IgG2 can exist in three dominant forms based on its disulfide configuration: IgG2A, IgG2B, and IgG2A/B
- IgG2A is a representative of the **canonical Y-shaped IgG molecule** with **the disulfide bonds of the Fab portion being independent of those in the hinge**.
- IgG2B is more **constrained** due to the Fab arms being covalently attached to the hinge via disulfide bonds and can be depicted as a **T-shaped molecule**
- IgG2A/B is a **combination of both the A and B forms** with one Fab arm being independent of the Fc and the other being covalently attached.

IgG3

- comprises around 5–10% of total IgG
- IgG3 is very **effective at engaging effector mechanisms** but represents a relatively small percentage of circulating IgG in human serum
- dominant feature of IgG3 is the **long hinge connecting the Fab domains to the Fc portion of the molecule.**
- The hinge of IgG3 is **62 amino acids long**, more than four times that of IgG1 and containing 11 disulfide bonds.
- They attribute this heightened potency to the long hinge region of IgG3, **facilitating the cross-linking of the spike protein on the viral surface**
- long hinge of IgG3 affords it **greater flexibility**, but also **increased susceptibility to degradation when purified** and as a result has not extensively been examined for use as a therapeutic molecule
- Depending on the allotype of IgG3, its half-life is considerably less than that of the other subclasses



IgG4

- Comprising usually less than 4% of total IgG
- IgG4 are believed to constitute a veritable antigen “garbage disposal” system, which can attenuate inflammation or protect against type I hypersensitivity by inhibiting IgE activity, as well as prevent type II and III hypersensitivity by blocking immune complex formation
- IgG4 does not bind to polysaccharides

Table 1 | Properties of human IgG subclasses.

	IgG1	IgG2	IgG3	IgG4	
General					
Molecular mass (kD)	146	146	170	146	
Amino acids in hinge region	15	12	62 ^a	12	
Inter-heavy chain disulfide bonds	2	4 ^b	11 ^a	2	
Mean adult serum level (g/l)	6.98	3.8	0.51	0.56	
Relative abundance (%)	60	32	4	4	
Half-life (days)	21	21	7/~21 ^a	21	
Placental transfer	++++	++	++/++++ ^a	+++	
Antibody response to:					
Proteins	++	+/-	++	++ ^e	
Polysaccharides	+	+++	+/-	+/-	
Allergens	+	(-)	(-)	++	
Complement activation					
C1q binding	++	+	+++	-	
Fc receptors					
FcyRI	+++ ^c	65 ^d	-	++	34
FcyRIIa _{H131}	+++	5.2	++	++	0.17
FcyRIIa _{R131}	+++	3.5	+	++	0.21
FcyRIIb/c	+	0.12	-	+	0.20
FcyRIIIa _{F158}	++	1.2	-	-	0.20
FcyRIIIa _{V158}	+++	2.0	+	++	0.25
FcyRIIIb	+++	0.2	-	-	-
FcRn (at pH < 6.5)	+++	+++	++/+++ ^a	+++	

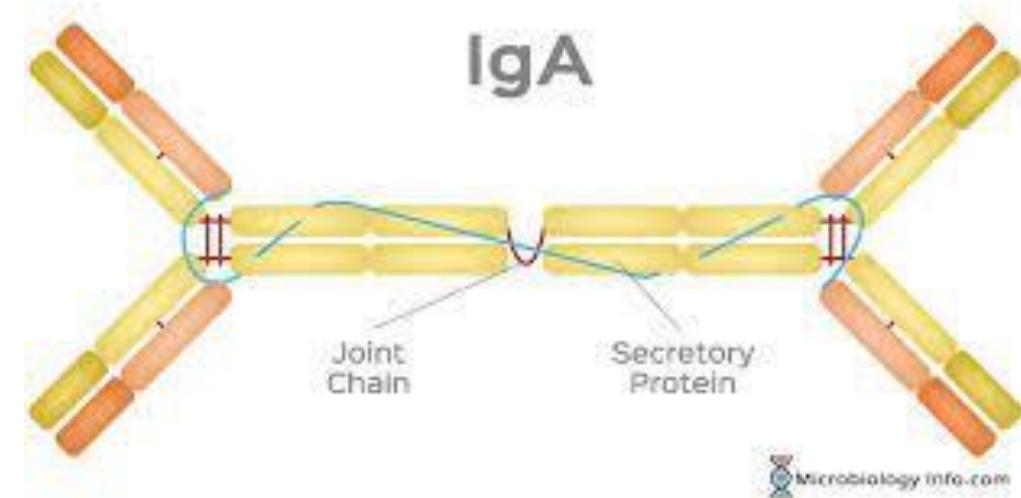
^aDepends on allotype.^bFor A/A isomer.^cMultivalent binding to transfected cells. Adapted from Bruhns et al. (2).^dAssociation constant ($\times 10^6 \text{ M}^{-1}$) for monovalent binding (2).^eAfter repeated encounters with protein antigens, often allergens.

IgA class

- IgA exists in serum in both monomeric and dimeric forms, comprising approximately 15% of the total serum Ig.
- Secretory IgA, a dimer, provides the primary defense mechanism against some local infections because of its abundance in mucosal secretions (e.g., saliva and tears). The principal function of secretory IgA may be not to destroy antigens but to prevent passage of foreign substances into the circulatory system.

Properties of IgA:

- Molecular weight: 320,000 Da (secretory)
- H-chain type (MW): alpha (55,000 Da)
- Serum concentration: 1 to 4 mg/mL
- Percent of total immunoglobulin: 15%
- Glycosylation (by weight): 10%
- Distribution: intravascular and secretions
- Function: protect mucus membranes



Role of IgA

- IgA in serum is mainly monomeric, but in secretions, such as saliva, tears, colostrums, mucus, sweat, and gastric fluid. Most present in secreted form.
- IgA is found as a dimer connected by a joining peptide.
- This is believed to be due to its properties in preventing invading pathogens by attaching and penetrating epithelial surfaces. IgA is a very weak complement-activating antibody; hence, it does not induce bacterial cell lysis via the complement system.
- However, secretory IgA works together with lysozymes (also present in many secreted fluids), which can hydrolyze carbohydrates in bacterial cell walls thereby enabling the immune system to clear the infection.
- IgA is predominantly found on epithelial cell surfaces where it acts as a neutralizing antibody.

IgA subclasses

IgA1

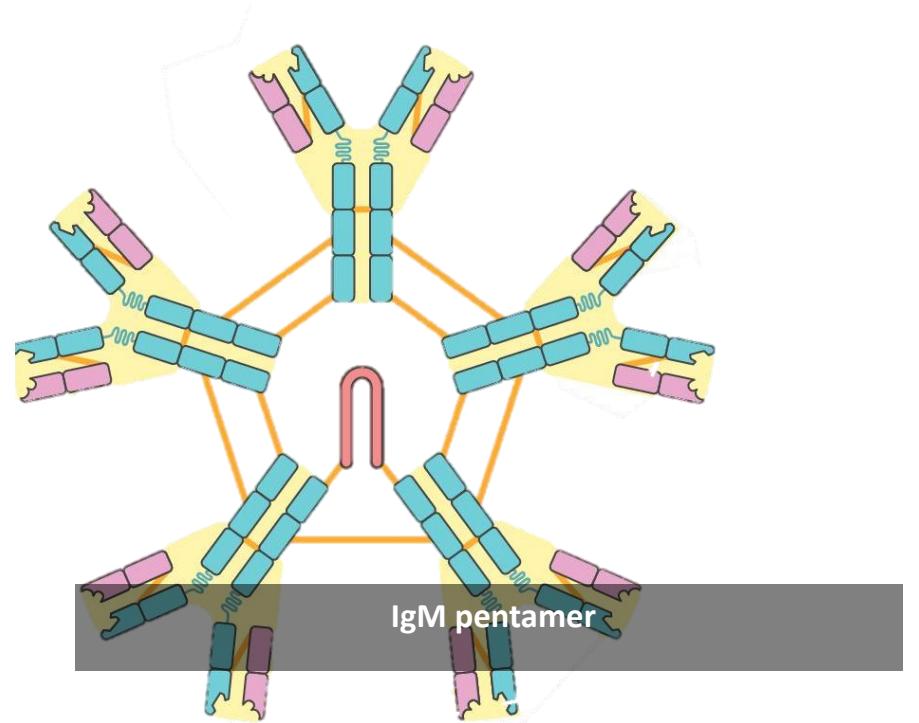
- Comprises approximately 85%
- Although IgA1 shows a broad resistance against several proteases, there are some that can affect/splice on the hinge region.
- IgA1 shows a good immune response to protein antigens and, to a lesser degree, polysaccharides and lipopolysaccharides.

IgA2

- Comprises approximately 15%
- Crucial role in the mucosa of the airways, eyes and the gastrointestinal tract to fight against polysaccharide and lipopolysaccharide antigens.
- It also shows good resistance to proteolysis and many bacterial proteases, supporting the importance of IgA2 in fighting bacterial infections.

IgM class

- IgM is the first class of immunoglobulin made by B cells as they mature
 - It is the form most commonly present as the antigen receptor on the B-cell surface.
 - Five of the basic Y-shaped units become joined together to make a large pentamer molecule with 10 antigen-binding sites
-
- Properties of IgM:
 - Molecular weight: 900,000 Da
 - H-chain type (MW): mu (65,000 Da)
 - Serum concentration: 0.5 to 2 mg/mL
 - Percent of total immunoglobulin: 10%
 - Glycosylation (by weight): 12%
 - Distribution: mostly intravascular
 - Function: primary response

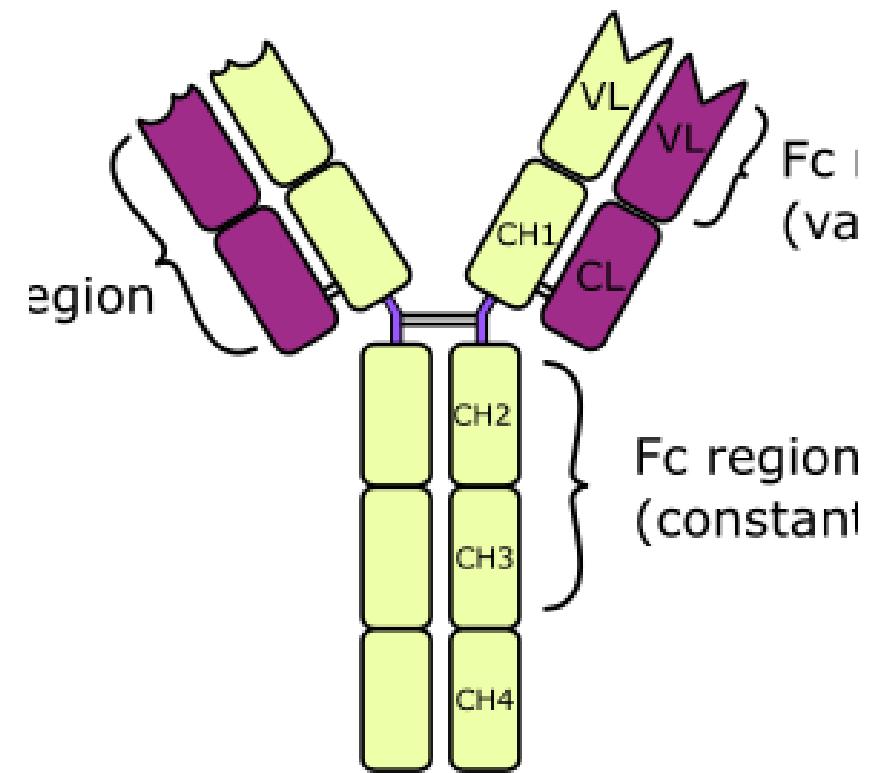


Role IgM

- Immunoglobulin M is the **third most common** serum Ig and takes one of two forms:
- a **pentamer** where all **heavy and light chains are identical**
- a monomer (e.g., found on B lymphocytes as B cell receptors)
- Allows **building of bridges** between **encountered epitopes** on molecules that are **too distant** as to be connected by smaller IgG antibodies.
- **First antibody** built during an immune response.
- It is responsible for **agglutination and cytolytic** reactions
- Due to conformational constraints among the 10 Fab portions, **IgM only has a valence of 5**.
- Vital importance in **complement activation** and **agglutination**.
- IgM is predominantly found in the **lymph fluid and blood** and is a very effective neutralizing agent in the early stages of disease.
- Elevated levels can be a sign of **recent infection** or **exposure to antigen**.
- IgM is not as versatile as IgG.

IgE

- IgE primarily defends against parasitic invasion and is responsible for allergic reactions.
- Properties of IgE:
- Molecular weight: 200,000
- H-chain type (MW): epsilon (73,000)
- Serum concentration: 10 to 400 ng/mL
- Percent of total immunoglobulin: 0.002%
- Glycosylation (by weight): 12%
- Distribution: basophils and mast cells in saliva and nasal secretions
- Function: protect against parasites



Role of IgE

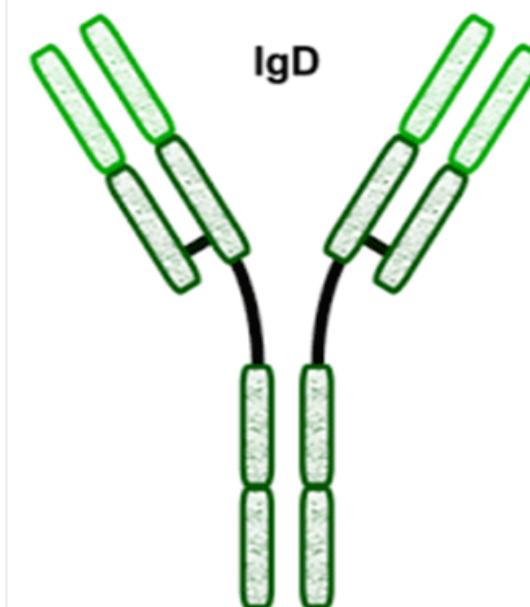
- The heavy chain of IgE contains an extra domain, by which it attaches with high affinity to Fc epsilon Receptor I (Fc ϵ RI) found primarily on eosinophils, mast cells and basophils.
- When antigens such as pollen, venoms, fungus, spores, dust mites or pet dander bind with the Fab portion of the IgE attached to the cells, the cells degranulate and release factors like heparin, histamine, proteolytic enzymes, leukotrienes and cytokines.
- vasodilatation and increased small vessel permeability causes fluid to escape from capillaries into the tissues, leading to the characteristic symptoms of an allergic reaction.
- typical allergic reactions like mucus secretion, sneezing, coughing or tear production are considered beneficial to expel remaining allergens from the body.
- Studies have shown that conditions such as asthma, rhinitis, eczema, urticaria, dermatitis and some parasitic infections (e.g., helminths and tapeworms) lead to increased IgE levels
- Low levels of IgE can occur in a rare inherited disease that affects muscle coordination (ataxia-telangiectasia).

IgD

- IgE and IgD are found in serum in much smaller quantities than other Igs.
- IgD emerged soon after IgM at the time of inception of the adaptive immune system.
- Membrane IgD is a receptor for antigen found mostly on mature B-lymphocytes.

- **Properties of IgD:**

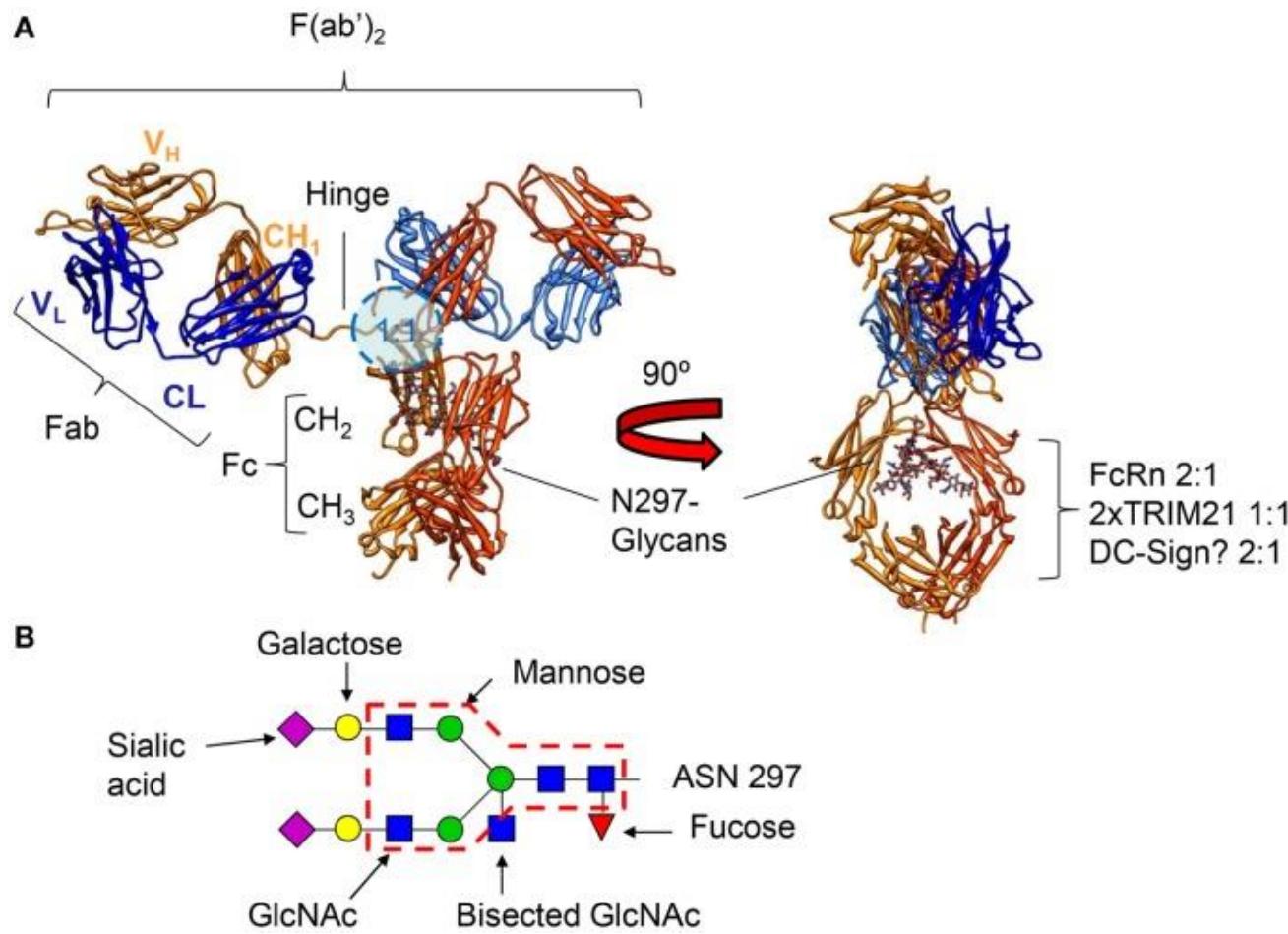
- Molecular weight: 180,000
- H-chain type (MW): delta (70,000)
- Serum concentration: 0 to 0.4 mg/mL
- Percent of total immunoglobulin: 0.2%
- Glycosylation (by weight): 13%
- Distribution: lymphocyte surface



Role of IgD

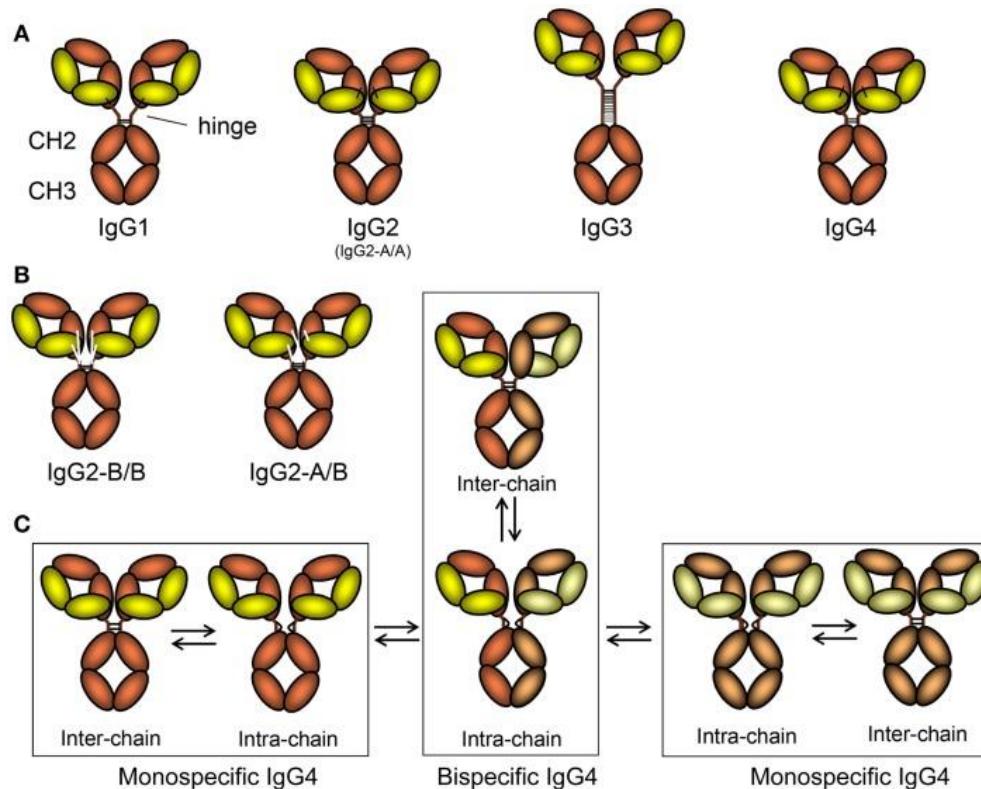
- Secreted IgD appears to **enhance mucosal homeostasis** and **immune surveillance** by "**arming**" **myeloid effector cells** such as basophils and mast cells with IgD antibodies reactive against **mucosal antigens**, including **commensal and pathogenic microbes**.
- **Mature B cells** undergo **alternative mRNA splicing** to express IgD and IgM receptors with identical antigenic specificity.
- The enigma of dual IgD and IgM expression has been tackled by several recent studies showing that **IgD helps peripheral accumulation of physiologically autoreactive B cells through its functional unresponsiveness to self-antigens but prompt readiness against foreign antigens**.
- IgD achieves this balance by **attenuating IgM-mediated anergy** (absence of the normal immune response to a particular antigen or allergen) while promoting specific responses to **multimeric non-self-antigens**.

Structure of IgG



(A) Crystal structure of an human IgG1 molecule (1HZH) viewed from two different angles, demonstrating the flexibility of the two Fab fragments with respect to each other and the Fc tail. The binding location for FcγR, binding IgG asymmetrically in a 1:1 configuration (46–49), is indicated by the blue circle (lower hinge, upper CH2) on the left, and the location of the binding motifs for FcRn, TRIM21, and the potential site for binding of DC-SIGN on the right (intersection of CH2 and CH3). FcRn, and the potential binding site of DC-SIGN bind IgG in a 2:1 configuration (50–52), respectively, while a dimer of TRIM21 binds IgG in a 1:1 configuration (53). The N-linked glycan at position 297 attached to each of the heavy chains is shown on the right. **(B)** The N-linked glycan found at position 297 can be found as a core structure, common to all IgG found in human beings and rodents (core structure indicated with a red dashed line), but can be found with either an addition of fucose, bisecting N-acetylglucosamine (GlcNAc), one or two galactose, and one or two sialic acid residues.

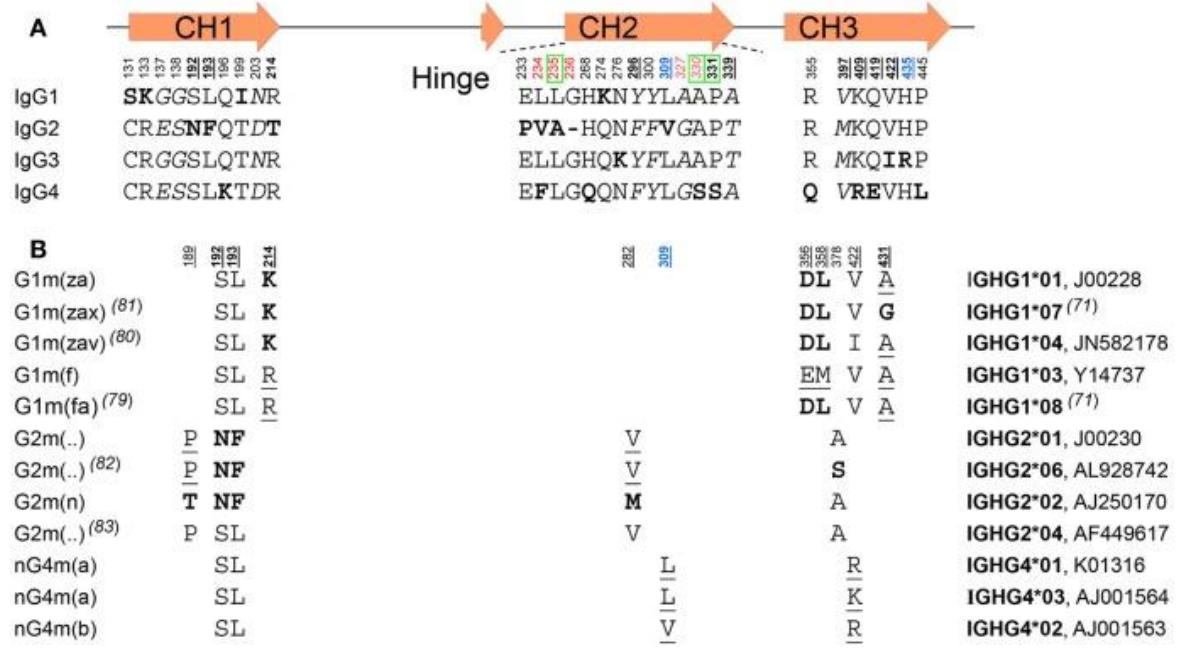
- Consists of **four polypeptide chains**, composed of two identical 50 kDa heavy (H) chains and two identical 25 kDa kappa or lambda (L) light chains, linked together by inter-chain disulfide bonds
- heavy chain consists of an N-terminal variable domain (VH) and three constant domains (CH1, CH2, CH3), with an additional “hinge region” between CH1 and CH2
- light chains consist of an N-terminal variable domain (VL) and a constant domain (CL).
- The light chain associates with the VH and CH1 domains to form a Fab arm
- the V regions interact to form the antigen-binding region – acquired through differential assembly of Variable, Diversity (VH only), and Joining gene segments and inclusion of somatic mutations
- Two heavy chain–light chain heterodimers (HL) combine into a single antibody molecule(H2L2) via disulfide bonds in the hinge region and non-covalent interactions between the CH3 domains
- The part of the antibody formed by the lower hinge region and the CH2/CH3 domains is called “Fc”



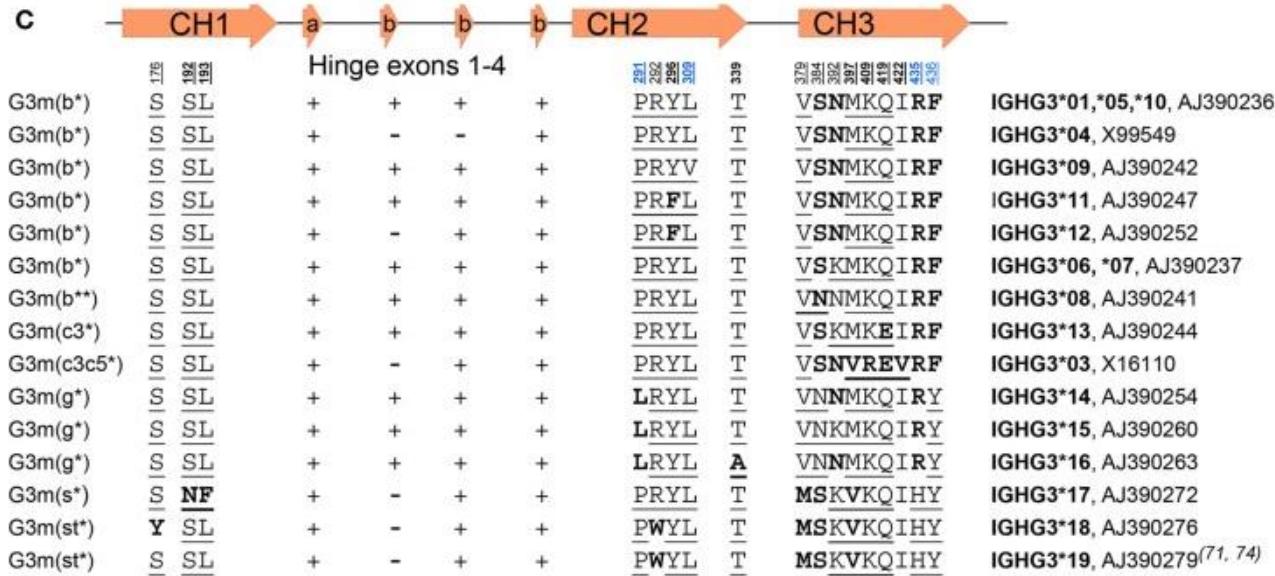
- four human IgG subclasses are very similar
- 90% homology in amino acid sequence
- Much variation is found in the hinge region and N-terminal CH2 domain
- residues most proximal to the hinge region in the CH2 domain of the Fc part are responsible for effector functions of antibodies as it contains a largely overlapping binding site for C1q (complement) and IgG-Fc receptors (Fc γ R) on effector cells of the innate immune system
- highly conserved N-linked glycosylation site at position 297 is located at the interface between the two CH2/CH3 forming the Fc, it is responsible for important changes in the quaternary structure allowing exposed docking site for Fc γ R
- these glycans also directly participate in the Fc γ R binding, but can also modulate these interactions through highly specific modifications of the N297 glycan
- The interface between the CH2–CH3 domains also contains the **binding site for the neonatal Fc receptor (FcRn)**, responsible for the prolonged half-life of IgG, placental passage, and transport of IgG to and from mucosal surfaces. Little variation exists in this region, with FcRn binding only minimally affected, except perhaps for IgG3 as discussed further below.

- The schematic layout of the IgG subclasses and isomers thereof.
- (A) The IgG subclasses, indicating how the different heavy and light chains are linked, the length of the hinge, and the number of disulfide bridges connecting the two heavy chains. For orientation, and comparison with Figure 1, the location of the hinge, CH2, and CH3 domains are shown. The classical A/A isoform of IgG2 with four different disulfide bridges between the two heavy chains is depicted here, but in
- (B) the B/B form, with only two disulfide bridges and alternative linkages of the light chain to the heavy chain form is shown, together with the intermediate A/B form.
- (C) Isomers of IgG4 resulting in half-molecule exchange. On the far left and far right, two classically depicted IgG4 clones in slightly different colors are shown just after secretion from B-cells. These are connected with two inter-chain disulfide bridges. However, these are in fact in equilibrium where these are reduced creating forms without covalent linkages between the symmetric molecules. This form can either revert back to covalently linked form or swap heavy chains in a stochastic process with that of neighboring IgG4 molecule creating an asymmetric bispecific IgG4 (bottom middle) that is also in flux, reverting into covalently linked IgG4 (top, middle). By this process, most IgG4 found in human beings (expressing the IgG4-R409 allotype, see text for more details and Figure 3) are monovalent-bispecific molecules.

Structural Variation in the Hinge Region



- The hinge exon of **IgG1** encompasses **15 amino acids** and is very flexible.
- IgG2 has a shorter hinge than IgG1, with **12 amino acid** residues. The lower hinge region of IgG2 (actually encoded by the CH2 region) also has a **one amino acid deletion** (lacking one of the double Glycines found at position 235-6), resulting in IgG2 having the shortest hinge of all the IgG subclasses.
- The hinges of **IgG2** are even **more rigid** due to a **poly-proline helix**, stabilized by up to four extra inter-heavy chain disulfide bridges
- the hinge region of IgG4 also contains **12 amino acids, shorter** than that of IgG1.
- Its **flexibility is intermediate** between that of IgG1 and IgG2
- It does **encode for the CH2-encoded glycines 235-6** in the lower hinge



IgG3 has a **much longer hinge** region than any other IgG subclasses or Ig human isotypes

four times as long as the IgG1 hinge, 62 amino acids (including 21 prolines and 11 cysteines), forming a poly-proline helix with **limited flexibility**

The exact length of the hinge varies between allotypes of IgG3

- The Fab fragments are relatively far away from the Fc fragment, providing a greater flexibility.
- This long hinge of IgG3 is a result of duplications of a hinge exon, encoded by one exon in IgG1, IgG2, and IgG4, but up to four exons in IgG3.
- One of those exons is common to all IgG3 allotypes, but it also has 1–3 copies of a homologous second type of IgG3-hinge exon, also responsible for its higher molecular weight compared to the other subclasses
- The difference in hinge flexibility influences the relative orientation and movement of the Fab arms and Fc tail of the IgG antibody.
- The relative flexibility of the Fab arms with respect to the Fc differs between subclasses as follows: IgG3 > IgG1 > IgG4 > IgG2 , which also reflects the relative binding of these subclasses to Fc γ R and C1q, although this only partially explains the respective activities of the IgG subclass

Effector Mechanisms & Receptors

Definitions

Effector

- In biology, an effector is a general term that can refer to several types of molecules or cells depending on the context:

Small molecule effectors:

- A small molecule that selectively binds to a protein to regulate its biological activity can be called an effector. An example of such an effector is oxygen, which is an allosteric effector of hemoglobin - oxygen binding to one of the four hemoglobin subunits greatly increases the affinity of the rest of the subunits to oxygen.

Protein effectors

- An effector can also be used to refer to a protein that is involved in cellular signal transduction cascades.

Antibody Effectors

are effectors involved with the production and secretion of molecules involved in pathogen defense, such as Immunoglobulin. Many antibodies then act as effector molecules for the immune system of the organism, typically as enzyme activators.

Definitions

RNA effectors

- Certain plant pathogens, such as *Botrytis cinerea*, secrete small RNAs (sRNAs) into the host cells and downregulate plant proteins involved in the immune response by RNA interference

Effector cells

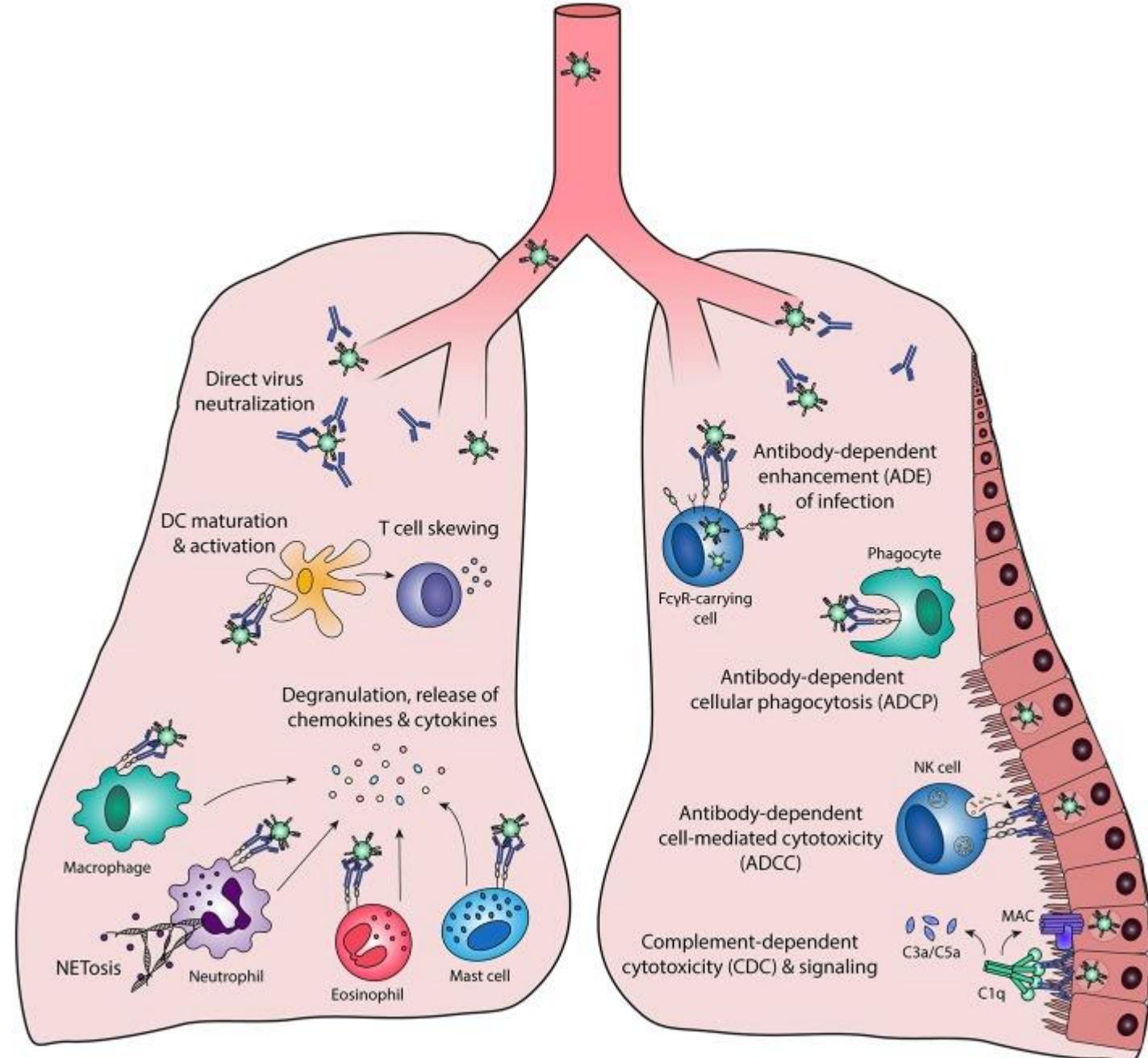
- In immunology, effector cells are cells of either the innate or the adaptive immune system that mediate the immune response.
- **Effector Mechanisms**
- Effector mechanisms seen in vitro include: (a) lysis of infected target cells expressing recognizable surface antigenic determinants; (b) release of lymphokines acting on macrophages and other leukocytes; (c) immune interferon acting on both leukocytes and tissue parenchymal cells.

Fc-Mediated Antibody Effector Functions

- Antibody effector functions are an important part of the humoral immune response and form an essential link between innate and adaptive immunity.
- Most of these effector functions are induced via the constant (Fc) region of the antibody, which can interact with complement proteins and specialized Fc-receptors.
- The latter can induce activating or inhibitory pathways, depending on the type of receptor, and are found on B cells and most innate immune cells in various combinations.
- **The most well-known Fc-mediated antibody effector functions are antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC).**
- In addition, antibodies have been found to mediate inflammation and immunomodulation through the induction of cellular differentiation and activation.

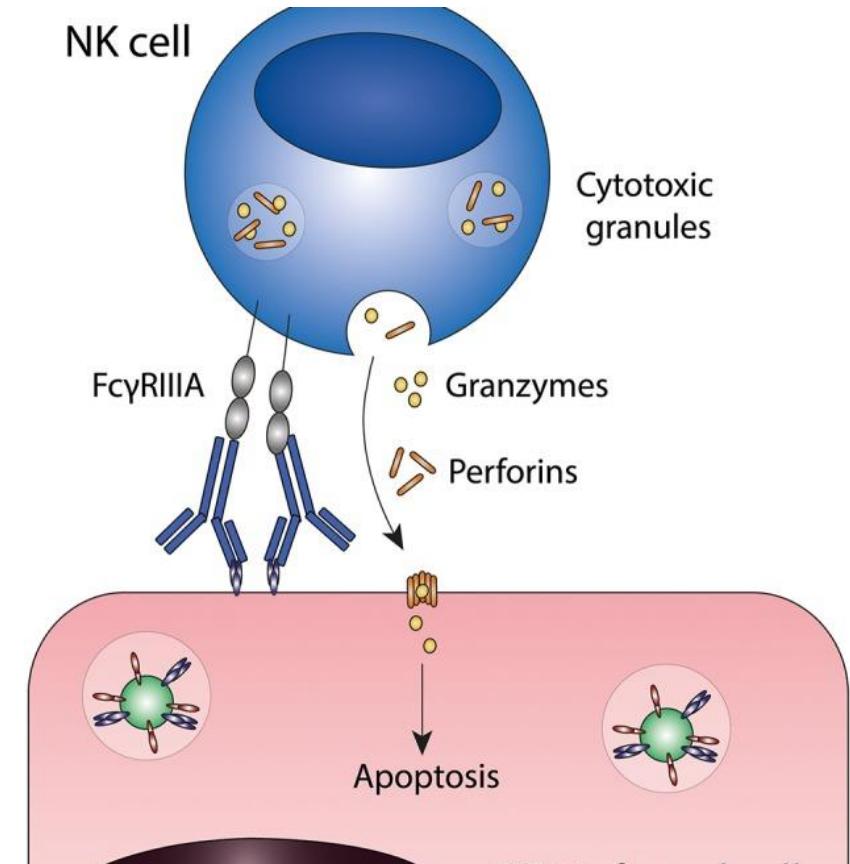
Fc-mediated antibody effector functions.

- Antibodies elicit a wide range of effector functions during viral infections.
- These include but are not necessarily limited to the functions depicted in this figure.
- **DC, dendritic cell; Fc γ R, Fc gamma receptor; MAC, membrane attack complex; NK cell, natural killer cell.**



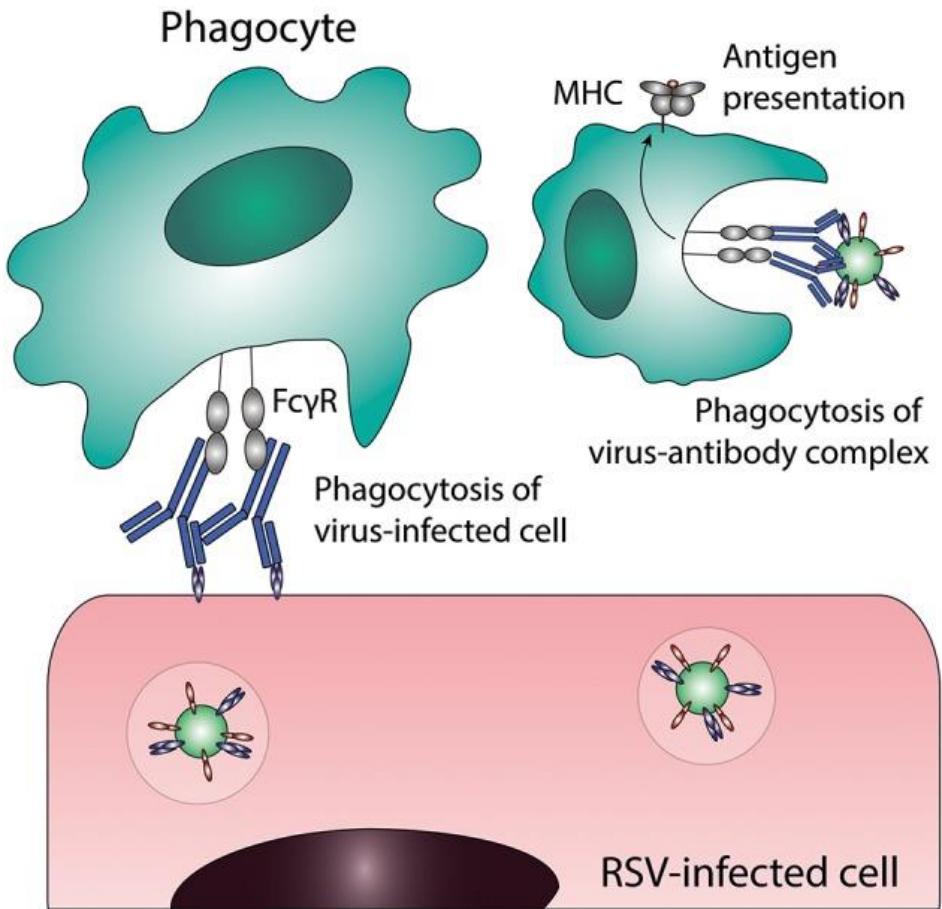
Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

- ADCC is induced when Fc gamma receptors (Fc γ Rs) on innate effector cells are engaged by the Fc domain of antibodies that are bound to viral proteins on the surface of virus-infected cells.
- This interaction induces the release of cytotoxic granules (containing perforins and granzymes) resulting in killing of infected cells.
- Multiple innate effector cells, including natural killer (NK) cells, neutrophils, monocytes, and macrophages, are capable of ADCC in vitro. However, the most important contributors to ADCC in vivo are thought to be NK cells, which express only Fc γ RIIIA.



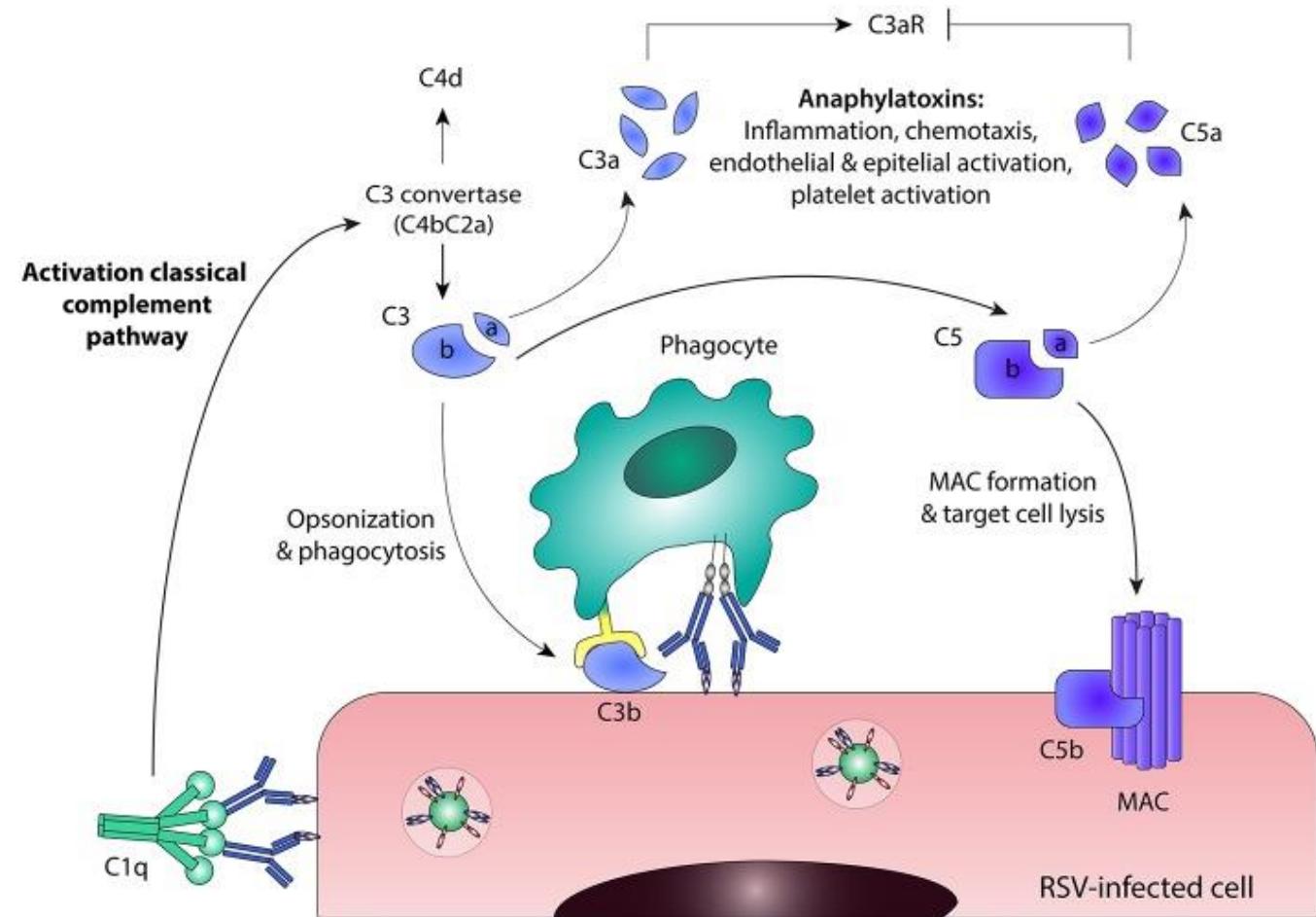
Antibody-Dependent Cellular Phagocytosis (ADCP)

- ADCP or opsonophagocytosis is the uptake of virus-antibody complexes or antibody-coated virus-infected cells by phagocytic cells
- Phagocytic cells, including monocytes, macrophages, neutrophils, eosinophils and dendritic cells (DCs), express Fc γ RI, Fc γ RII, and Fc α RI, which can all mediate immune complex uptake.
- The exact phagocytic capacity of effector leukocytes is dependent upon the cell type, differentiation stage, and level of Fc γ R expression.
- ADCP results in the clearance of immune complexes from the infected host, by trafficking of the complexes to lysosomes for degradation and antigen processing for presentation on Major Histocompatibility Complex (MHC)-molecules on the cell surface.



Antibody-Mediated Complement Activation

- Besides ADCC and ADCP, antibodies can also induce complement activation.
- The complement cascade contributes to pathogen elimination either directly, by means of complement-dependent cytotoxicity (CDC), or indirectly, through phagocytic clearance of complement-coated targets and the induction of an inflammatory response.
- Activation of the classical complement pathway results from binding of the recognition molecule C1q to the Fc domain of antibodies bound to virus-infected cells
- Upon binding of C1q, the proteases of the classical pathway are activated, leading to cleavage of C2 and C4.

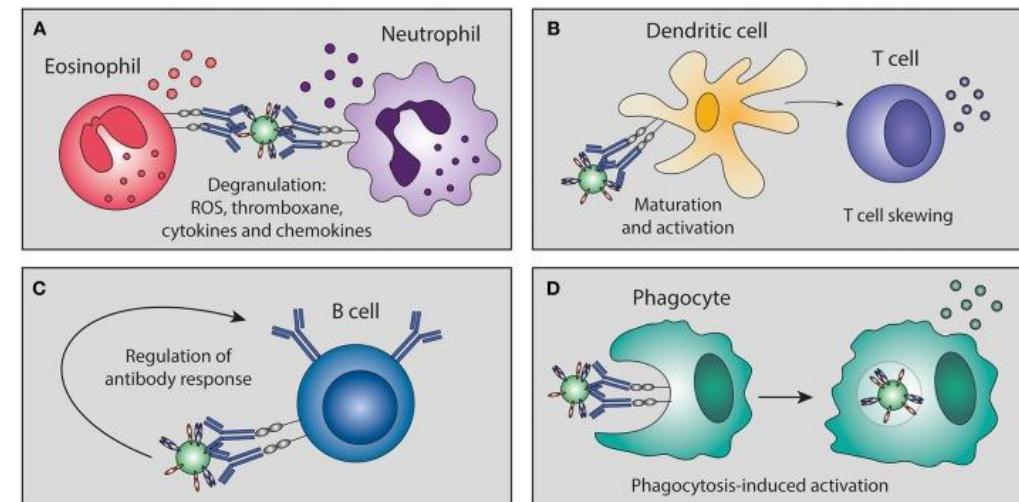


Antibody-Mediated Complement Activation

- Together, the resulting cleavage products form the C3 convertase ($C4bC2a$) that cleaves C3 into C3a and C3b. One of the mechanisms by which the complement cascade is regulated, is cleavage of active C4b, which serves as a marker for complement activation.
- The release of anaphylatoxins C3a and C5a stimulates a pro-inflammatory environment by inducing the recruitment of immune effector cells and the activation of leukocytes, endothelial cells, epithelial cells, and platelets.
- The highly reactive C3b binds to pathogens and infected cells, leading to immune complex clearance and phagocytosis through complement receptors found on immune cells.
- The terminal complement components will assemble into the membrane attack complex (MAC), resulting in lysis of the infected cell. Besides direct antiviral activity, the complement system can also regulate B cell responses.
- The binding of complement-coated immune complexes to complement receptor 2 on B cells is reported to lower the B cell activation threshold, thereby promoting long-lived adaptive immunity and higher antibody levels

Antibody-Mediated Immunomodulation

- Besides the well-defined classical Fc-mediated effector functions (ADCC, ADCP, CDC), immune complexes can also promote immune cell maturation and activation, leading to a wide range of effector activities and production of pro-inflammatory and immunomodulatory mediators
- Some of these pro-inflammatory cytokine responses correlate with protection as has been shown for influenza and HIV
- The importance of Fc γ Rs in this process has been shown by the use of Fc γ R-deficient mice.
- In contrast to the pro-inflammatory responses caused by immune complexes, injection with intravenous immunoglobulin (IVIg) can induce an anti-inflammatory state.

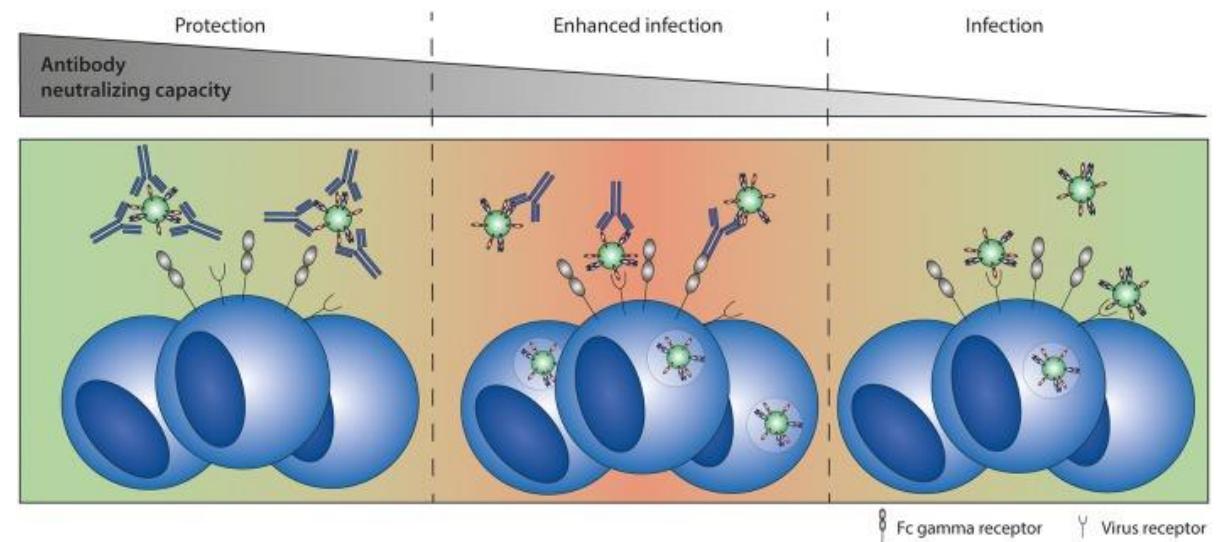


Antibody-Mediated Immunomodulation

- Immune complexes can also regulate cellular maturation and activation.
- The balance between inhibitory and activating Fc γ R interactions is crucial in regulating B cell IgG responses, and skewing APC maturation and antigen presentation, which can modulate T cell activation. Immune complexes have also been shown to bias the macrophage immune response toward a Th2-like phenotype
- It is proposed that this anti-inflammatory effect is partly due to the presence of sialylated antibodies in IVIg, which induce expression of Fc γ RIIB (the only inhibitory Fc γ R) and thereby dampen the inflammatory response

Antibody-Dependent Enhancement (ADE) of Infection

- ADE refers to a phenomenon in which virus-specific antibodies promote, rather than inhibit, infection and/or disease.
- In ADE of infection, also known as extrinsic ADE, the number of virus-infected cells is increased in the presence of (natural or monoclonal) antibodies that are non-neutralizing or present in sub-neutralizing concentrations. ADE of infection requires the presence of Fc_YRs on target cells and is an efficient *in vitro* tool to assess Fc-Fc_YR interactions.
- However, while ADE of infection has been observed for many viruses *in vitro* [as extensively reviewed in, its significance *in vivo* remains uncertain.



Binding Effector Receptors

Binding capacity and functionality of IgG subclasses.

Subclass	Serum abundance (%)	Fc γ RI	Fc γ RIIa	Fc γ RIIb	Fc γ RIIIa	Fc γ RIIIb	C1q	Effector functions
IgG1	60	+++	+++	+	++	+++	++	ADCC, ADCP, CDC
IgG2	32	-	++	-	-	-	+	
IgG3	4	++++	++++	++	++++	++++	+++	ADCC, ADCP, CDC
IgG4	4	++	++	+	-	-	-	

Binding Effector molecules

- IgG1 and IgG3 have the highest affinity for Fc γ Rs and are potent activators of complement, ADCC and phagocytosis
- IgG3 is the subclass with the highest potential to activate both Fc γ Rs and complement, but due to its short half-life the preferred subclass for therapeutic cytotoxic activity is IgG1
- In contrast, receptor-blocking antibodies are often of the IgG2 or IgG4 subclass to avoid Fc-mediated cytotoxic side effects
Induction of specific subclasses can have major effects on the outcome of vaccine trials as has been shown for the HIV RV144 and VAX003 vaccines.
- RV144 recipients produced highly functional IgG3 antibodies that provided 31.2% efficacy, whereas VAX003 recipients elicited a monofunctional IgG4 antibody response that was not protective at all

Binging Effector molecules

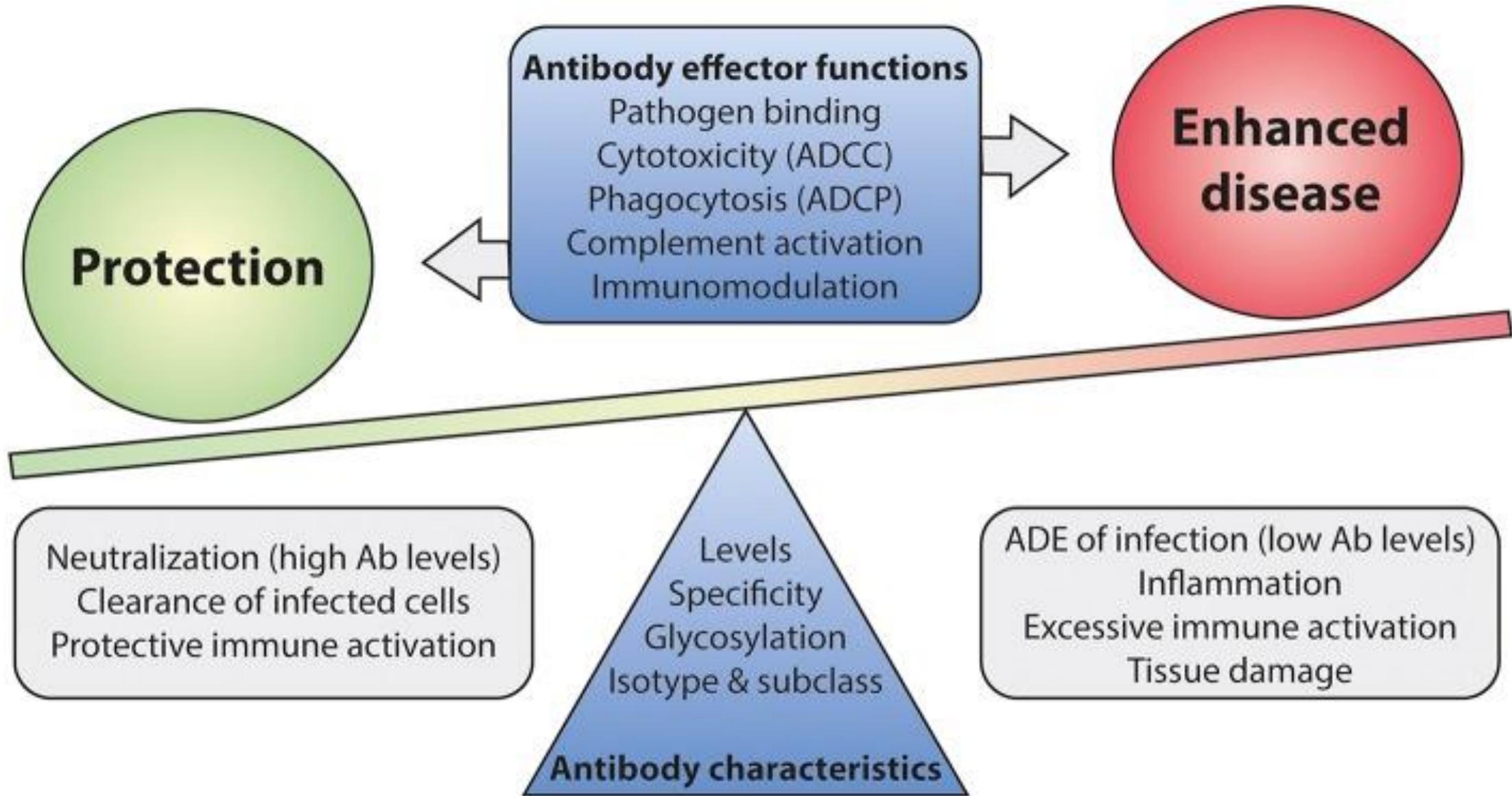
- **IgG1 and IgG3** are potent triggers of effector mechanisms
- **IgG2 and IgG4** will induce more subtle responses, and only in certain cases
- All remain capable of neutralizing virus particles and toxins
- **C1q:** Complement activation is initiated through binding and subsequent activation of C1q, leading to deposition of C3b to further **opsonize** the target, but also to the **formation of the membrane attack complex**, C5–C9, causing disruption of the targeted bilipid membrane
- **IgG1 and IgG3** can efficiently trigger this classical route of complement
- **IgG2 and IgG4** does so much less efficiently due reduced binding to C1q
- Residues in the CH2 region important for C1q binding include L235, D270, K322, P329, and P331
- gG2, reduced C1q binding appears to be largely caused by residue A235
- IgG4, P331 is – at least in part – responsible for the reduced or absent binding of C1q
- Structural determinants in the middle or “core” hinge region (residues 226–230) can influence the binding of C1q
- rigidity in this region contributes favorably to C1q binding, whereas removal of cysteine bonds negatively affects binding thus long hinge of IgG3 makes the C1q binding site more accessible resulting in more efficient complement activation

Fc-gamma receptors

- All of the Fc γ receptors (Fc γ R) belong to the immunoglobulin superfamily and are the most important Fc receptors for inducing phagocytosis of opsonized (marked) microbes.
- This family includes several members, Fc γ RI (CD64), Fc γ RIIA (CD32), Fc γ RIIB (CD32), Fc γ RIIIA (CD16a), Fc γ RIIIB (CD16b), which differ in their antibody affinities due to their different molecular structure.
- For instance, Fc γ RI binds to IgG more strongly than Fc γ RII or Fc γ RIII does.
- Fc γ RI also has an extracellular portion composed of three immunoglobulin (Ig)-like domains, one more domain than Fc γ RII or Fc γ RIII has.
- This property allows Fc γ RI to bind a sole IgG molecule (or monomer), but all Fc γ receptors must bind multiple IgG molecules within an immune complex to be activated.
- The Fc-gamma receptors differ in their affinity for IgG and likewise the different IgG subclasses have unique affinities for each of the Fc gamma receptors.
- These interactions are further tuned by the glycan (oligosaccharide) at position CH2-84.4 of IgG. For example, by creating steric hindrance, fucose containing CH2-84.4 glycans reduce IgG affinity for Fc γ RIIIA.
- In contrast, G0 glycans, which lack galactose and terminate instead with GlcNAc moieties, have increased affinity for Fc γ RIIIA

FcRn: Neonatal crystallizable fragment receptor

- the existence of a receptor responsible for the unusually long half-life of IgG (3 weeks) and efficient transport from mother to young was first proposed by Brambell.
- FcRn is strikingly similar to MHC-class I molecules
- MHC-class I and other MCH-class I-like molecules, FcRn is co-expressed with the non-glycosylated 12 kD β 2-microglobulin, encoded on chromosome 15.
- α -chain of human FcRn, a 45 kD polypeptide chain, is encoded on chromosome 19 at a locus harboring various other immune receptors
- FcRn starts its function early in life by transport of IgG – and thereby humoral immunity – across the placenta from mother to young
- In adult life, FcRn is expressed on many epithelial cells, and continues to function in IgG transport across FcRn expression epithelial barriers
- FcRn is able (in all species) to bi-directionally transcytose cargo across polarized (both epithelial and endothelial) cells, but the net transport direction depends on the tissue





Complementarity Determining Regions (CDR)

Definition

- Complementarity-determining regions (CDRs) are immunoglobulin (Ig) **hypervariable domains** that determine **specific antibody (Ab) binding**.
- a **variable sequence of amino acids** that **folds into loops capable** of binding to an **antigenic amino acid sequence**, also known as an epitope
- Are part of the **variable chains** in immunoglobulins and T cell receptors, **generated by B-cells and T-cells** respectively, where these molecules bind to their **specific antigen**.
- A set of CDRs constitutes a **paratope**.
- CDRs are crucial to the **diversity of antigen specificities** generated by lymphocytes.

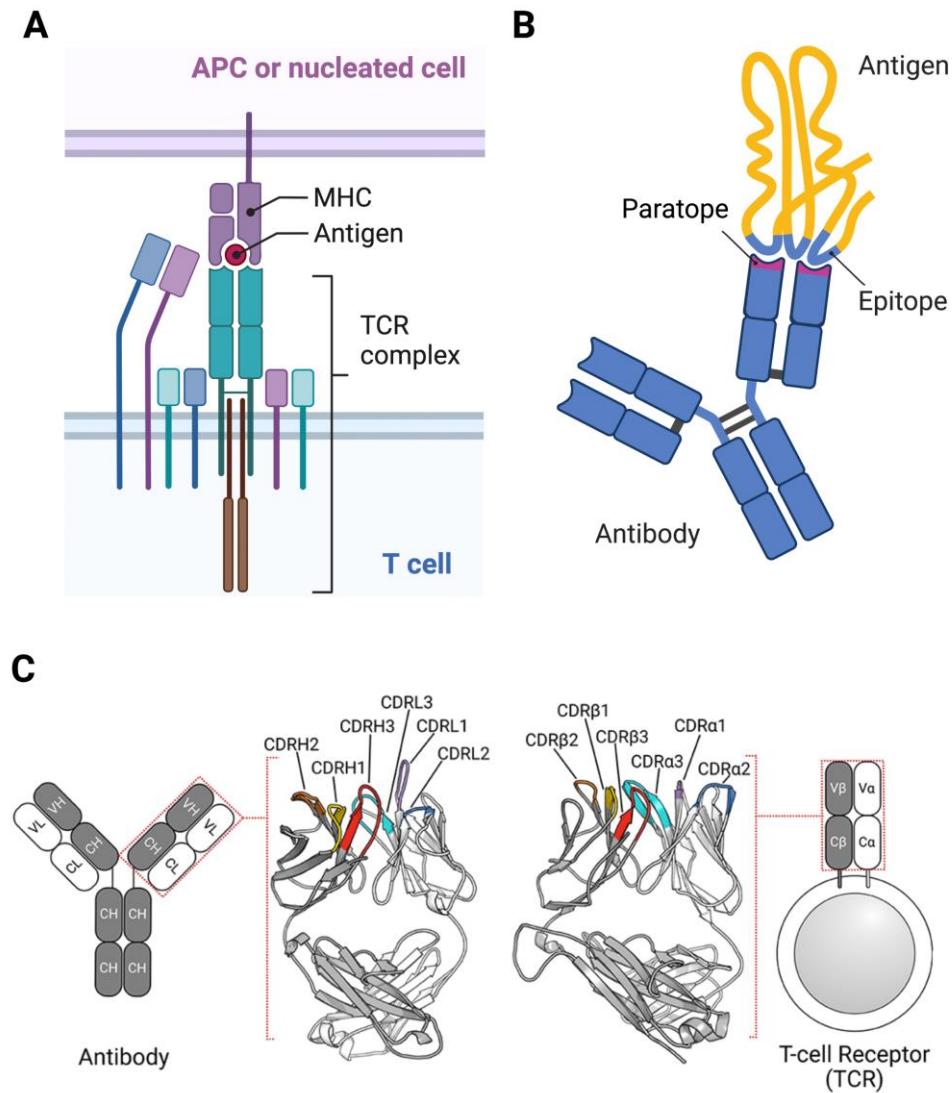
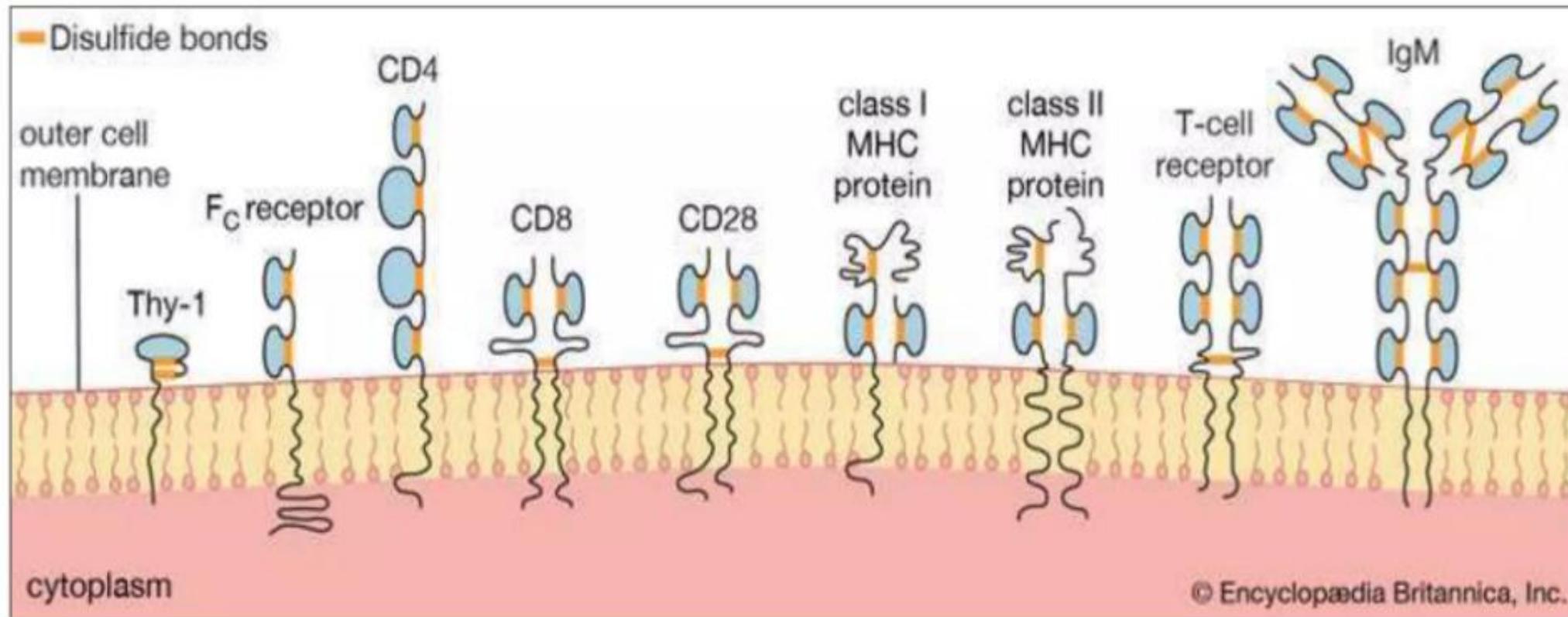


Figure 1. Complementarity Determining Regions (CDRs) are responsible for mediating the interactions between antigens presented by immune cells known as antigen-presenting cells (APCs) or other nucleated cells and the T cell receptor (TCR) part of the TCR complex of T cells (A), and between antigens and antibodies (B). The regions containing the CDRs are known as antigen-binding sites or paratopes (B). The antigenic area to which an antibody or TCR paratope binds is called the epitope (B). Both light and heavy chains of an antibody and the two extracellular chains of TCRs contain three CDRs each (C).

Immunoglobulin SF

- The immunoglobulin superfamily is a group of cell surface proteins characterized by the presence of a variable number of related 70–110 amino acid Ig-like domains originally described in the Ig variable and constant regions.
- Included are CD2, CD3, CD4, CD7, CD8, CD28, T cell receptor (TCR), MHC class I and MHC class II molecules, leukocyte function-associated antigen 3 (LFA-3), the IgG receptor, and a dozen other proteins
- These molecules share in common with each other an immunoglobulin-like domain, with a length of approximately 100 amino acid residues and a central disulfide bond that anchors and stabilizes antiparallel β strands into a folded structure resembling immunoglobulin.
- 30 years ago Kabat and Wu identified sub regions within the variable region called Complementarity Determining Regions



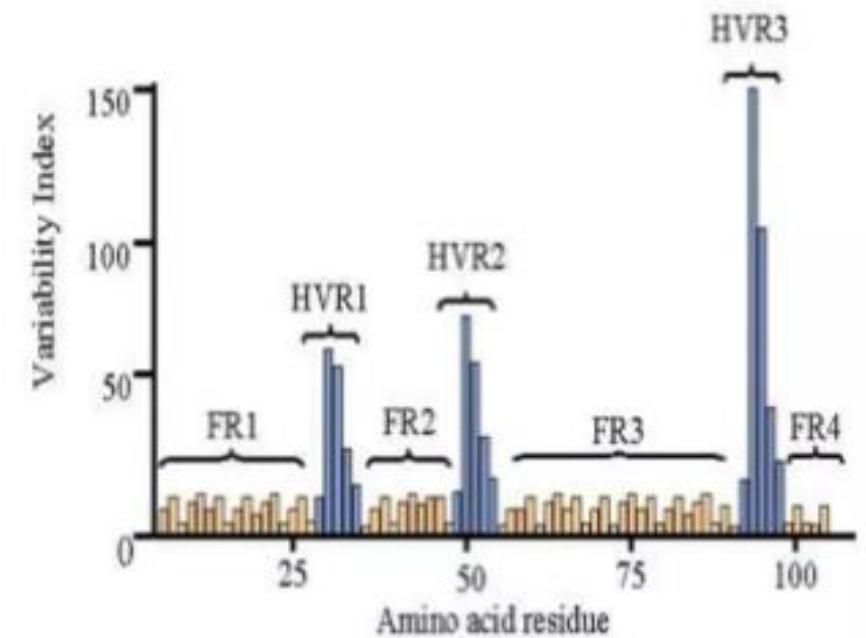
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Fig 1. Schematic representation of some proteins of Ig superfamily.

All members of the superfamily are involved in recognizing other cells/foreign particles. They share basic structural similarity in the Ig-domain(shades in blue),indicating that the genes encoding these proteins evolved from a common ancestral gene involved in cell-to-cell recognition.

CDRs

- HVR are domains on Ig heavy and light chain V regions that are in contact with antigen and are frequently mutated to allow diver specific antigenic specificities to be recognised
- HVR form the antigen-binding site of the antibody molecule
- Three areas in the V region of the heavy and light chain are highly variable and form the distinct loops in the Ig protein structure and termed as CDR1, CDR2, CDR3
- CDRs have different orientation in different Abs



- ✓ The division of labor is:
- ✓ V regions are responsible for epitope recognition.
- ✓ C regions are responsible for triggering a useful response

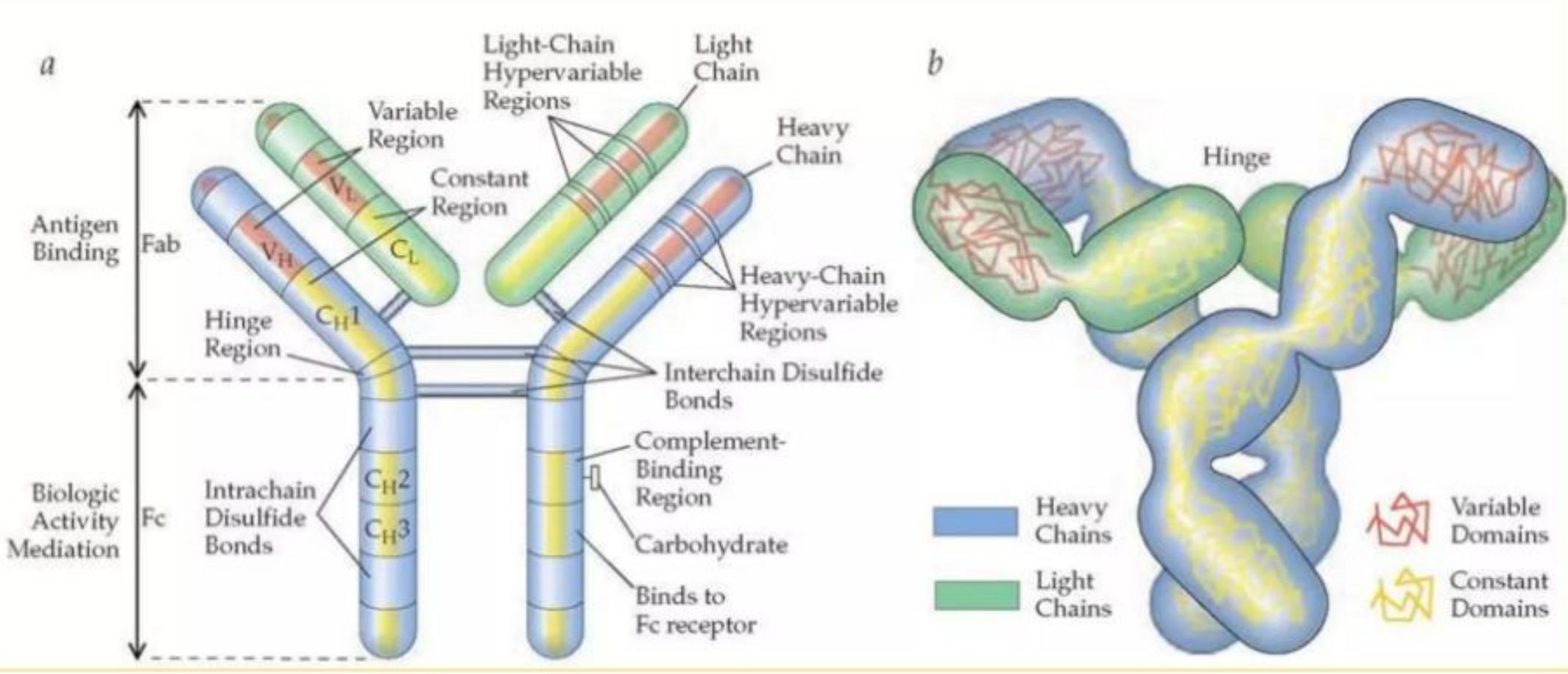
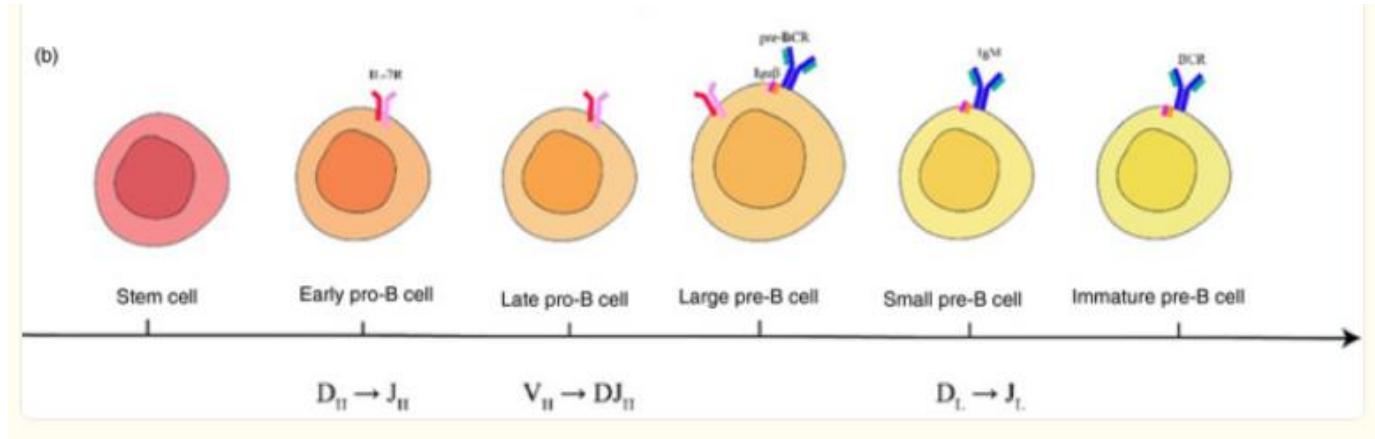


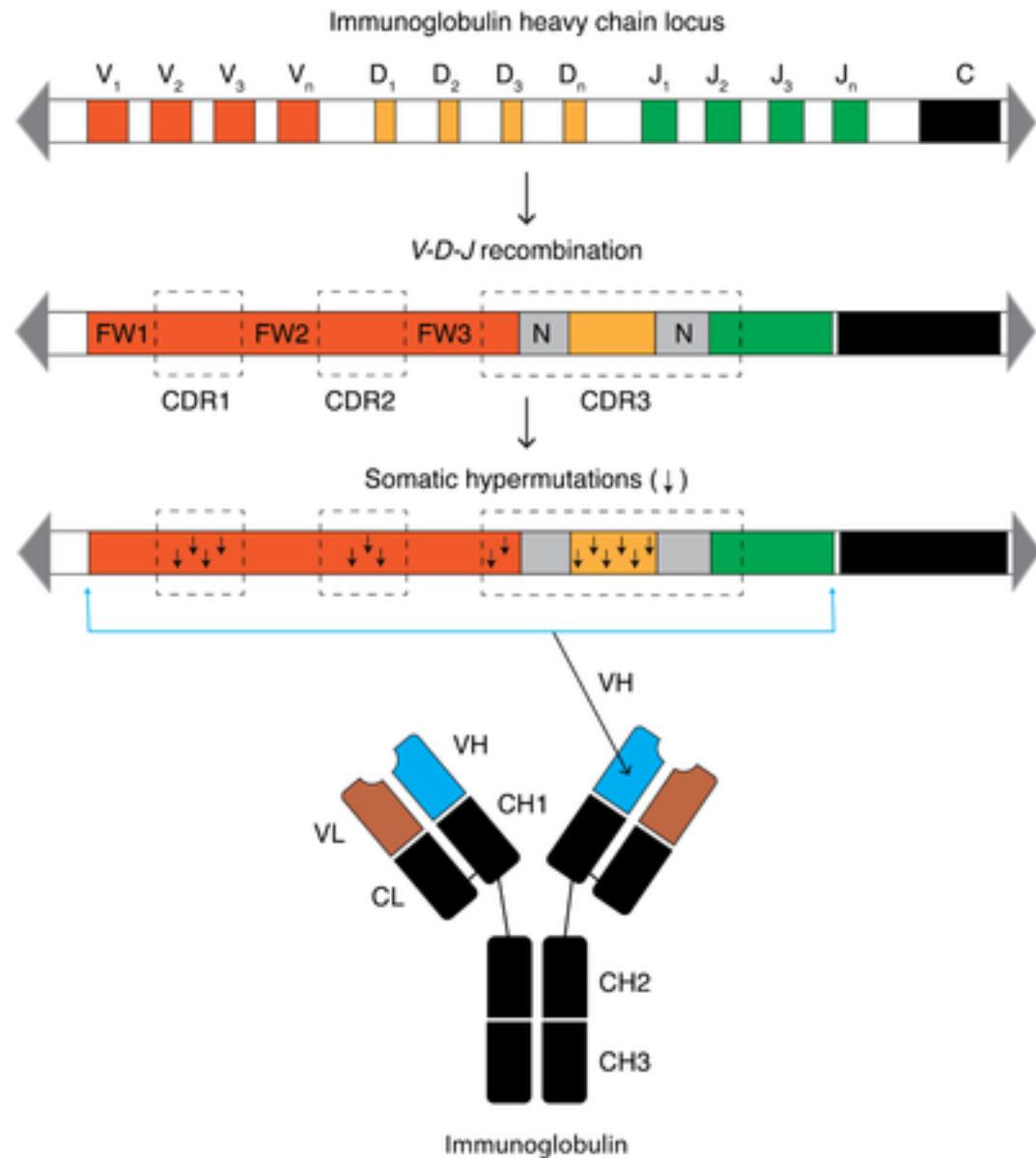
Fig 2(a) Each polypeptide has regions whose amino acid sequences are constant (white and yellow) and variable (red). The variable regions also contain hypervariable regions. **(b)** Schematic model of the domain structure of an antibody molecule.

Diversity

- The specificity of a particular antibody, i.e. what the antibody recognises, is determined by the shape of its variable region, a particular antibody will bind to a protein that has a region with a complementary structure to the antibody's own variable region.
- Diversity in the specificity of antibodies is initially generated at the earliest stages of B-cell development.
- While still at the B-cell progenitor stage in the bone marrow, B cells randomly rearrange their variable (V), diversity (D), and joining (J) genes to form the blueprint for the variable regions of their antibodies.
- Diversity comes from the fact that there are multiple copies of the V, D and J genes that can be joined together in different combinations

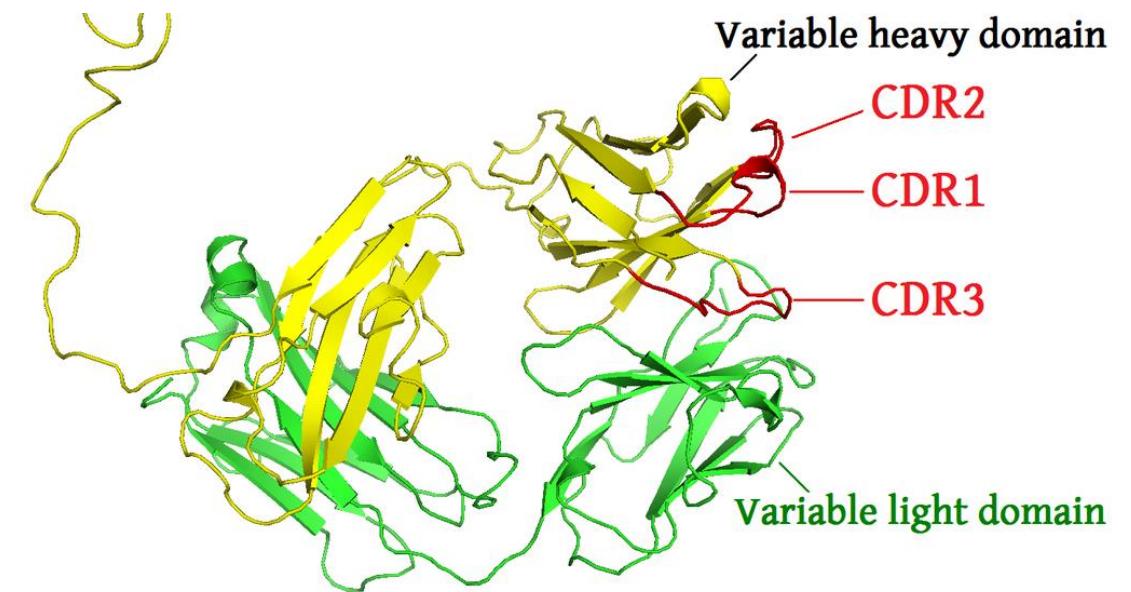


- During the course of an infection, B cells can further alter the specificity of the antibody they produce.
- The recombination of the V(D)J gene is the key mechanism to produce antibody diversity.
- The recombinational process, including randomly choosing a pair of V, D, J segments, introducing double-strand breaks adjacent to each segment, deleting (or inverting in some cases) the intervening DNA and ligating the segments together, is defined as V(D)J recombination, which contributes to surprising immunoglobulin diversity in vertebrate immune systems.
- When a mature B cell meets an antigen that its B-cell receptor recognises (the B-cell receptor comprises the antibody the cell produces anchored on the cell surface) then the B cell can undergo a process called **somatic hypermutation**.
- Here an enzyme called activation-induced cytidine deaminase (AID) makes random mutations in the antibody variable region genes.
- If the mutations result in an antibody that more strongly binds to their targets then these B cells will survive and may differentiate into antibody-producing plasma cells with the new specificity.



Structure

- There are **three CDRs (CDR1, CDR2 and CDR3)** arranged non-consecutively on the amino acid sequence of a variable domain of an antigen receptor.
- There are **six CDRs for each antigen receptor** (3 CDR loops per variable domain in antibodies 3 on heavy chain and 3 on light chain (LDR)) that can **collectively come into contact** with the antigen
- A single antibody molecule has **two antigen receptors** and therefore **contains twelve CDRs total**.
- Since most sequence variation associated with immunoglobulins and T cell receptors are found in the CDRs, these regions to as Hypervariable regions



- Within the variable domain, CDR1 and CDR2 are found in the variable (V) region of a polypeptide chain, and CDR3 includes some of V, all of diversity (D, heavy chains only) and joining (J) regions. CDR3 is the most variable.
- The CDRs are separated by structurally conserved regions called framework regions (**FR-1,-2,-3, and -4**) **that form a “core” β-sheet structure displaying these loops on the surface of the variable domain.**
- The length and composition of the CDR sequences are highly variable, especially in the CDR3.
- the origin of this diversity lies in the complexity of the genetic mechanisms that generate **the highly variable pool of antibodies** from a relatively small number of antibody genes.
- Variable regions are assembled from two genes (V and J, for λ and κ light chains) or three genes (V, D and J for heavy chains), following the V(D)J recombination mechanism.
- Variability in CDR3 length and sequence is introduced by the mechanisms that permit addition or deletion of nucleotides in those junctions and by somatic hypermutations in the recombined genes.

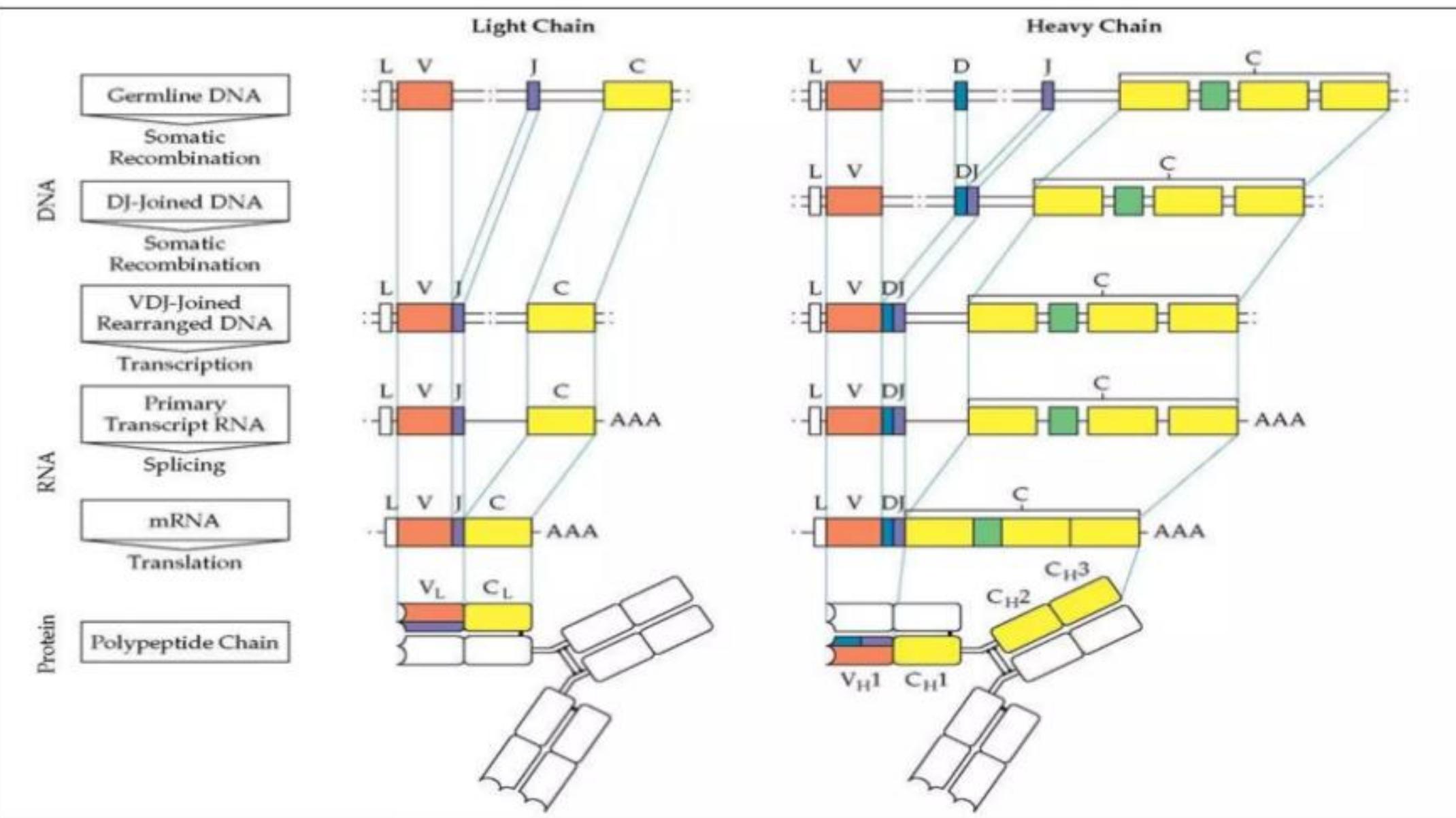


Fig 4. construction of heavy and light chains

- **Somatic hypermutation** involves introduction of point mutations into V regions of rapidly proliferating B-cells in the germinal centers of Lymphoid follicles.
- Antigen-driven somatic hypermutation of variable immunoglobulin genes can result in an increase in binding affinity of the B-cell receptor for its cognate ligand.
- **affinity maturation** is the process by which B cells produce antibodies with increased **affinity** for antigen during the course of an immune response.
- With repeated exposures to the same antigen, a host will produce antibodies of successively greater **affinities**.
- Somatic hypermutation occurs at a high rate, thought to be on the order of about 1×10^{-3} mutations per base-pair per generation, which is approximately 106 times higher than the mutation rate of cellular housekeeping genes

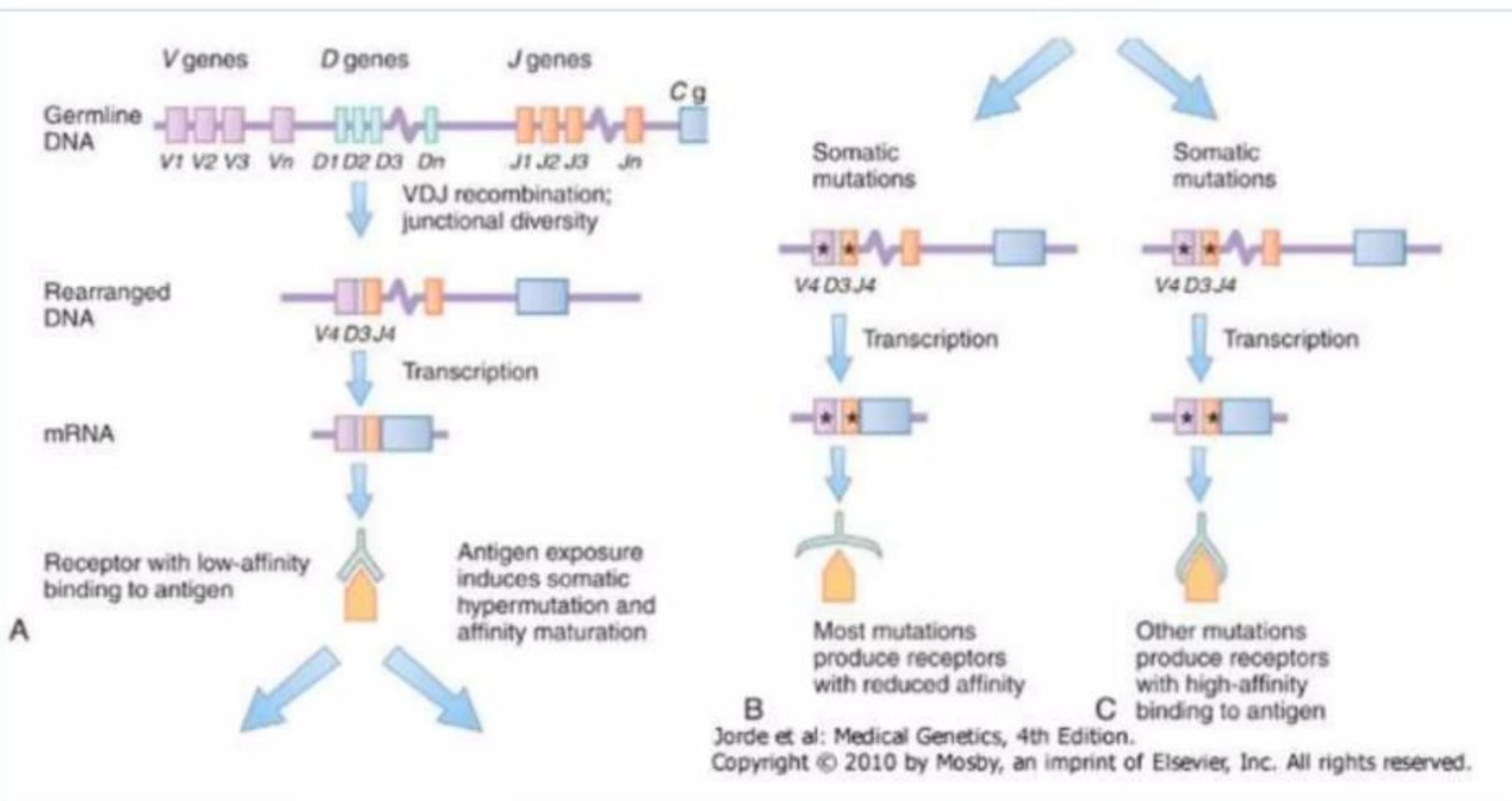


Fig 5. VDJ recombination, affinity maturation and somatic hypermutations

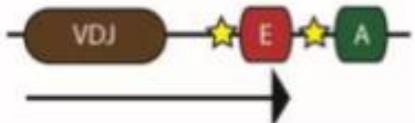
Mature B cells express IgM and IgD as they are the first heavy chain segments in the Ig locus.



After a B cell is activated segments of the locus are cut out and the locus is joined back together.



Now another heavy chain segment, IgE, is first on the Ig locus and it is expressed.



There are five different types of Immunoglobulin antibodies.

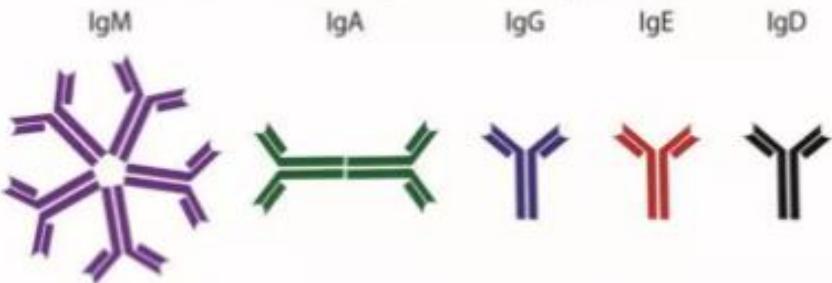


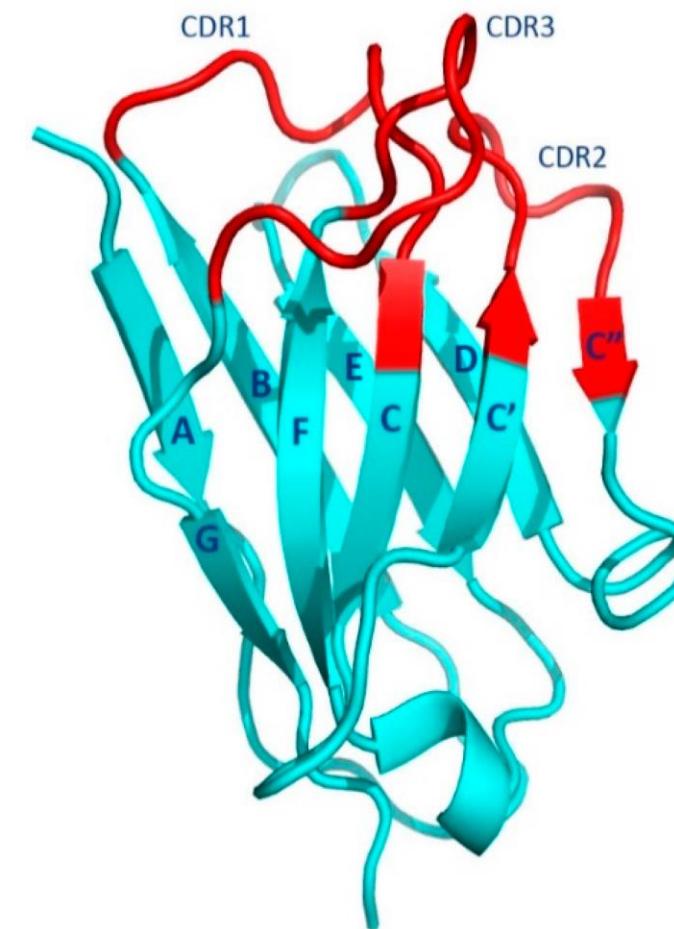
Fig 6. Class switch recombination. After VDJ recombination, class switch recombination may occur. Here unwanted Ig genes are excised so that the desired gene can be expressed. In this depiction excision occurs and IgE is expressed.

- Once activated, B cells may undergo class switch recombination
- In their inactivated state B cells express IgM/IgD but once activated they may express IgA, IgE, IgG or retain IgM expression.
- they do this by excision of the unwanted isotypes
- A master gene, activation-induced deaminase(AID), is essential for both somatic mutation of variable-region genes and the switch of the immunoglobulin isotype from IgM to IgG, IgA, or IgE during the immune response
- Cytokines produced by T cells and other cells are important in determining what isotype the B cells express.

Antigen combining (binding) site and CDRs

- The antigen combining site (also called the antigen binding site), or paratope, is defined by the set of amino acid residues that make contact with the antigen
- To determine the exact paratope, the experimental structure of the antibody in complex with its corresponding antigen needs to be elucidated by co-crystallography and structural elucidation
- VH and VL combine by non-covalent association to form the FV region, which contains the antigen binding or combining site.
- Each domain contributes three hypervariable loops (HVLs) or CDRs, with **CDR-L1, CDR-L2, and CDR-L3 (LDR regions)** formed by VL and **CDR-H1, CDR-H2, and CDR-H3** by VH. In the FV, the two β-sheets and the non-hypervariable loops are referred to as Framework Regions (FRs).

- **CDR-L1 and CDR-H1 HVLs** correspond to the residues within the loops connecting β -strands **B** and **C**
- **CDR-L2 and CDR-H2**, the HVLs are formed by the loops connecting β -strands **C'** and **C''**, and for **CDR-L3 and CDR-H3**, the HVLs are formed by the loops connecting β -strands **F** and **G**
- Due to the large number of different V-regions that can comprise the Fv, both amino acid sequence and length can vary significantly for the HVLs



Variability in Antibody CDR Sequences

Because antibodies' CDRs are highly variable, it can prove difficult to identify their exact amino acid sequence.

Essentially, CDRs are novel protein sequences.

Routine identification of proteins is often executed via genomics and proteomics methodologies that require database searches.

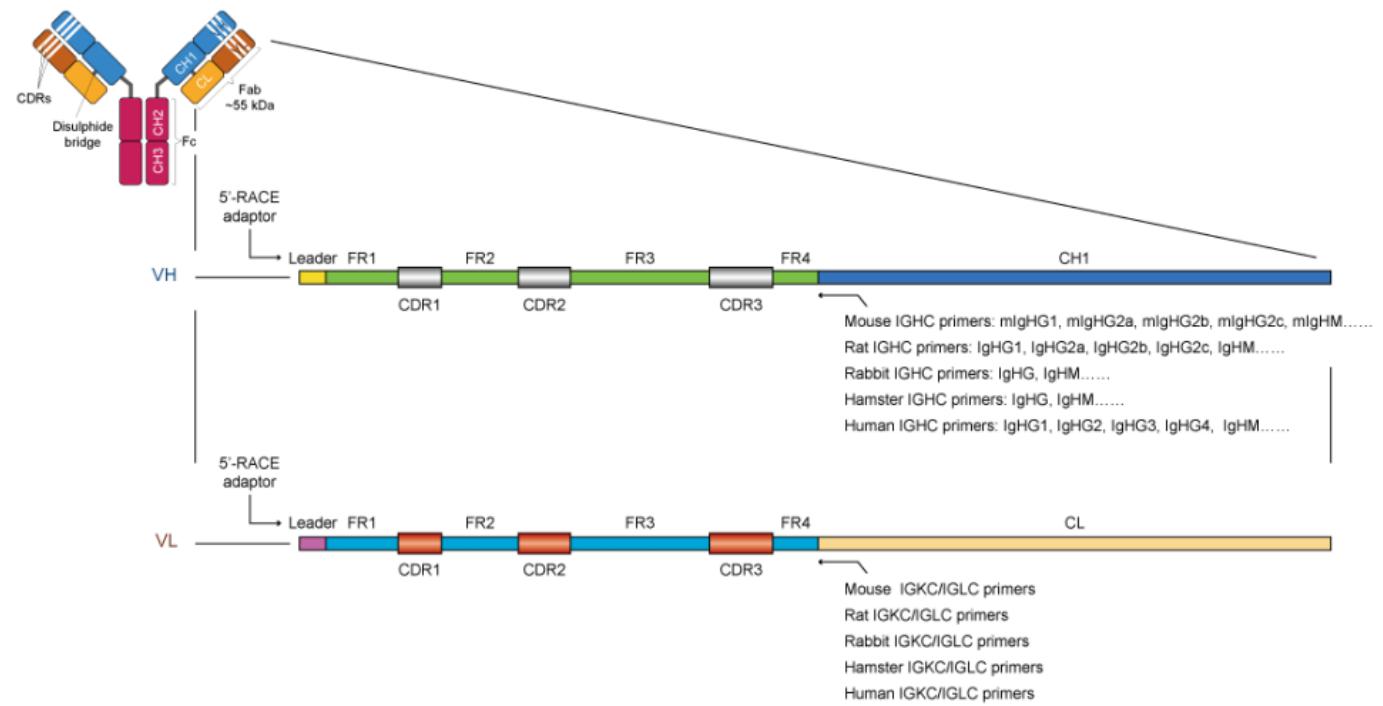
Since antibody sequences are rarely found in databases, more advanced techniques are required.

Antibody Sequencing

- The antibody sequencing protocol involves
 - isolating the mRNA from hybridoma cells
 - followed by cDNA synthesis
 - PCR amplification of heavy- and light-chain variable region genes.
 - The genes are then cloned into a vector and sequenced.
- sequencing report states
 - the nucleotide and amino acid sequences of both the heavy-chain variable (VH), and light-chain variable (VL), regions of the monoclonal antibody.
 - This will also include annotated VH and VL amino acid sequences highlighting the complementary determining, and framework regions of the antibody, and the similarity of the sequences to unarranged germline mouse or rat antibody sequences

Why?

- Antibody numbering, which standardizes a residue index at each position of an antibody variable domain, is an important step in immunoinformatic analysis.
- It provides an equivalent index for the comparison of sequences or structures, which is particularly valuable for antibody modelling and engineering.



Antigen- antibody interaction database

- Antigen–Antibody Interaction Database (AgAbDb) is an immunoinformatics resource developed at the Bioinformatics Centre, University of Pune, and is available online at <http://bioinfo.net.in/AgAbDb.htm>
- AgAbDb lists not only the residues of binding sites of antigens and antibodies, but also interacting residue pairs.
- The Antigen–Antibody Interaction Finder (AAIF), a program developed in-house, is used to compile the molecular interactions, viz. van der Waals interactions, salt bridges, and hydrogen bonds. As well as curating water- mediated interactions has also been developed.
- various residue level features, viz. accessible surface area, data on epitope segment, and secondary structural state of binding site residues, are also compiled
- AgAbDb can be used as a benchmark dataset to validate algorithms for prediction of B-cell epitopes

- The AgAbDb is implemented as a relational database using MySQL Server 5. T
- The database comprises of 12 tables to compile, curate, and archive data on antigens, antibodies, and interactions and is normalized up to Third Normal Form (3NF)
- AgAbDb archives data of antigens, antibodies, and molecular interactions under eight categories, viz. Summary, IR: Epitope-Paratope, IR: Epitope Segments, Binding Site: IR + BR, Atomic Level Interactions, Water-Mediated Interactions, View Interactions, and Statistics.

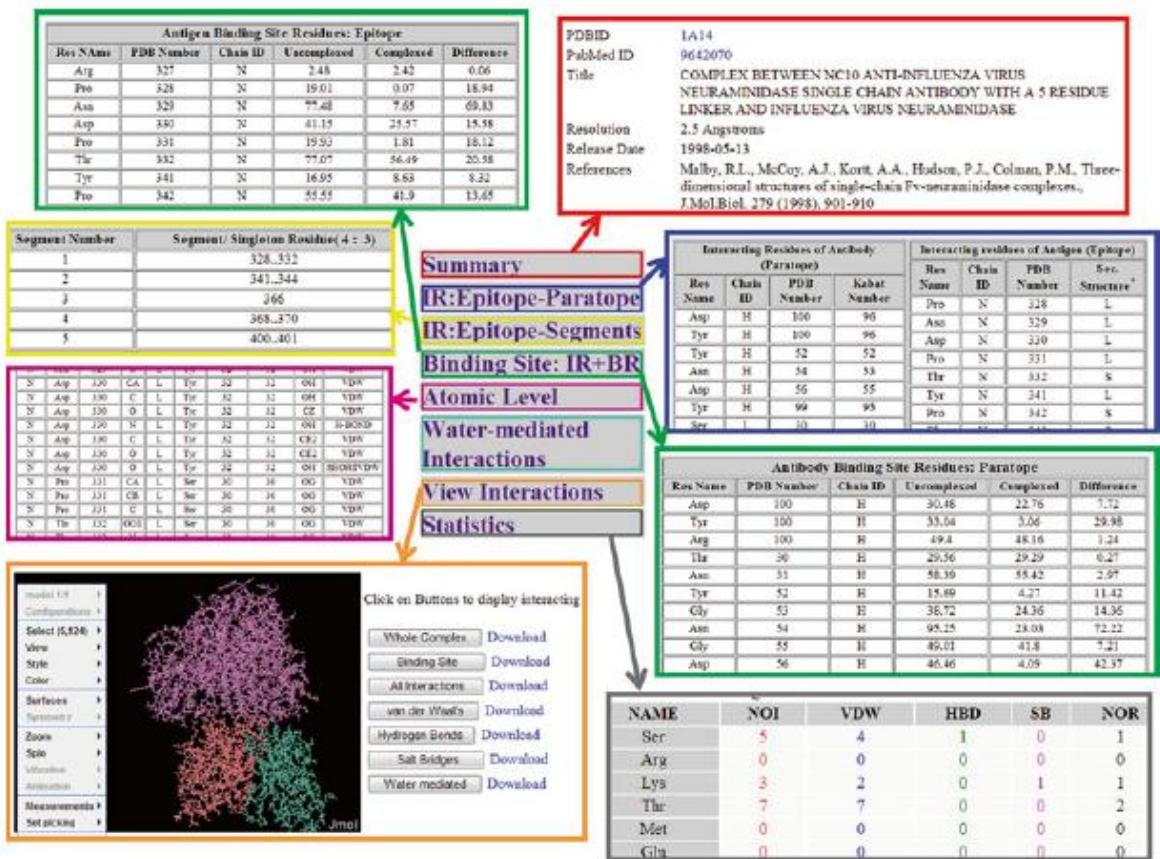


Fig. 5 Snapshots of various data archived in AgAbDb. The PDB ID: 1A14 (complex of neuraminidase and anti-body NC10) is used as a case study

Sequence numbering

- The recently generated **deep-sequencing data** and the increasing number of solved three-dimensional structures of antibodies from human and non-human origins have led to the emergence of numerous databases, different databases **use different numbering conventions and CDR definitions**.
- **Antibody engineering methods** require **precise identification** of the residues that have **an impact on the interaction and/or affinity** of the antibody for its target antigen

Kabat Numbering Scheme

- Is a scheme for the numbering of amino acid residues in antibodies based upon variable regions. The scheme is useful when comparing these variable regions between antibodies
- aligned 77 Bence-Jones protein and immunoglobulin light chain sequences in order to study the statistical variability in amino acid composition at the sequential positions of the variable antibody regions.
- They defined the “variability parameter” as
- $$\text{Variability parameter} = \frac{\text{no.of diff amino acids at a given position}}{\text{freq of most occurring amino acid at that position}}$$
- This analysis revealed three hypervariable regions in the variable region of the light chains
- These hypervariable regions would cluster at one side of the folded domain to form a surface responsible for specific antigen recognition and referred to these hypervariable Regions as CDR-1-2-3
- proposed a **standardized numbering scheme** for the variable regions of immunoglobulins

- In their compilation of “Sequences of Proteins of Immunological Interest”, the amino acid sequences of the variable region of the light (l, k) and heavy chain of antibodies as well as the variable region of T cell receptors ($\alpha, \beta, \gamma, \delta$) were aligned and numbered.
- observed that the analysed sequences exhibited variable lengths and that gaps and insertions could only be included at precise positions.
- Interestingly, the points of insertion were located inside the CDRs, except for CDRL2, but also at some positions inside the framework regions.
- **Limitations:**
 - this scheme was built on the alignments of a limited number of sequences from antibodies with the most common sequence lengths
 - sequences with unconventional insertions or deletions in the CDRs or in the framework regions were not included
 - it doesn't match very well with the 3D structure of antibodies.
- useful numbering tool named ABnum2 that numbers the amino acid sequences of variable domains according to a much larger and regularly updated database (Abysis3), takes into account insertions of variable lengths, particularly in CDR2 by adding an insertion point at position L54.

Chothia Numbering Scheme

- a structure-based numbering scheme for antibody variable regions
- They aligned crystal structures of antibody variable regions, defined the loop structures that form the CDRs and corrected the position numbers of the insertion points inside CDRL1 and CDRH1 so that they better fit their topological positions
- they classified the CDR loops of heavy and light chains in a small number of conserved structures, called “canonical” classes
- Chothia CDR definition ensures a better correspondence to the structural loops
- The loop structure of CDRH3 identified by Chothia matches well the Kabat HV region. In contrast, the other loops are shorter than the hypervariable sequences defined by Kabat, except for CDRH1 which extends from H26 to H32
- The main advantage that topologically aligned residues from different antibodies are localized at the same position number and that the Chothia CDR definition corresponds in most antibody sequences to the structural antigen-binding loops
- **Limitations:**
 - ‘limited use of this numbering scheme compared to the Kabat or the IMGT numbering schemes
 - ignores sequences with unconventional length

- CDR-H1 as defined by Kabat starts nine residues after Cys22 and is followed by Trp36. CDR-H1, as defined by Chothia, generally starts at about residue 26 of the V_H chain, four residues after Cys22, and is typically $\sim 8\text{--}10$ residues in length.
- Thus the Kabat CDR-H1 definition is offset from the Chothia definition by about five residues.

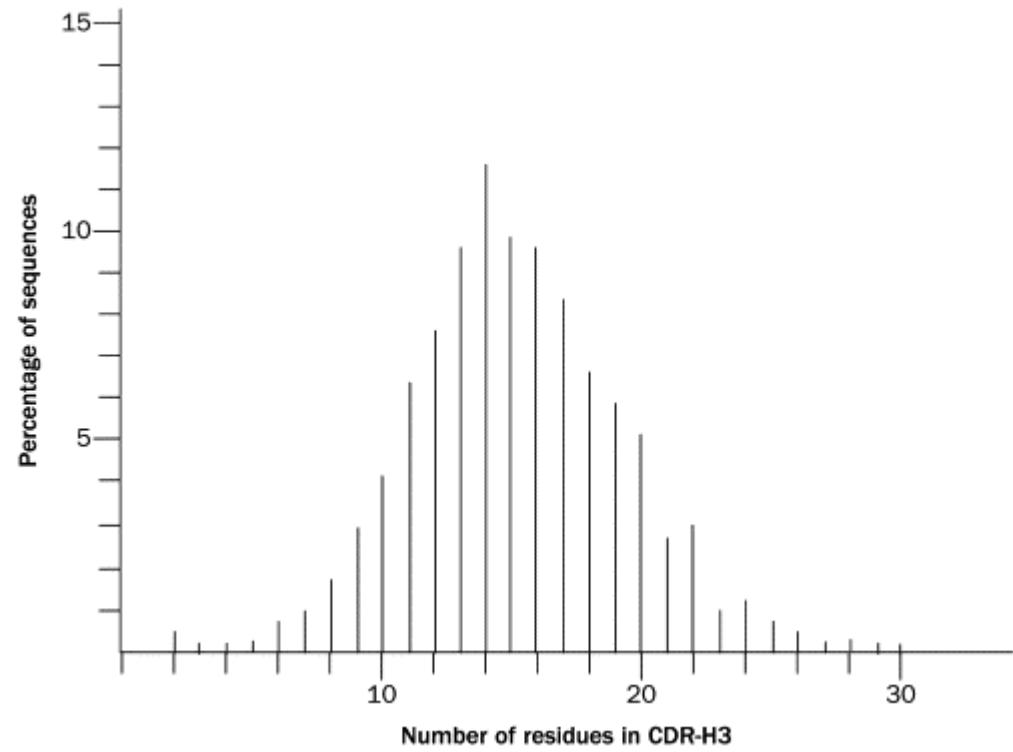
CDR	Kabat CDR*	Chothia CDR*	Contact range*
CDR-H1	V_H 31–35	V_H 26–32	V_H 30–35
CDR-H2	V_H 50–65	V_H 52–56	V_H 46–55
CDR-H3	V_H 95–102	V_H 95–102	V_H 89–96
CDR-L1	V_L 24–34	V_L 24–34	V_L 24–34
CDR-L2	V_L 50–56	V_L 50–56	V_L 50–56
CDR-L3	V_L 89–97	V_L 89–97	V_L 89–97

* Using Chothia numbering system (modified from MacCallum et al., 1996)

Example – CDR-H1:

Chothia number:	22-23-24-25-26-27-28-29-30-31-31a-31b-32-33-34-35-	36....
Kabat number:	22-23-24-25-26-27-28-29-30-31-	32-33-34-35-35a-35b-36....
Example sequence:	C K A S G Y T F T G	Y Y M H W
Kabat CDR-H1:	<hr/>	
Chothia CDR-H1:	<hr/>	
CDR-H1 contact range:	<hr/>	

- CDR-H3 occurs 33 residues after the end of the Kabat-defined CDR-H2 and always starts at the third residue after a Cys residue (often Cys-Ala-Arg). CDR-H3 is nearly always followed with a Trp-Gly-xxx-Gly sequence and can be as short as three residues in length or as long as 25 or more residues in length
- CDR-L1 generally starts around residue 24, is typically 10–17 residues in length, and is always flanked by a Cys residue on the N-terminal side and a Trp residue on the C-terminal side.
- CDR-L2 is typically found 16 residues after the end of CDR-L1, is preceded by hydrophobic residues such as Ile-Tyr, Val-Tyr, Ile-Lys, or Ile-Phe, and is seven residues in length.
- CDR-L3 starts 33 residues after the end of CDR-L2, is always preceded by a Cys residue, and is followed by a Phe-Gly-xxx-Gly sequence. CDR-L3s are generally 7–11 residues in length, although some CDR-L3s with as few as three residues have been found.



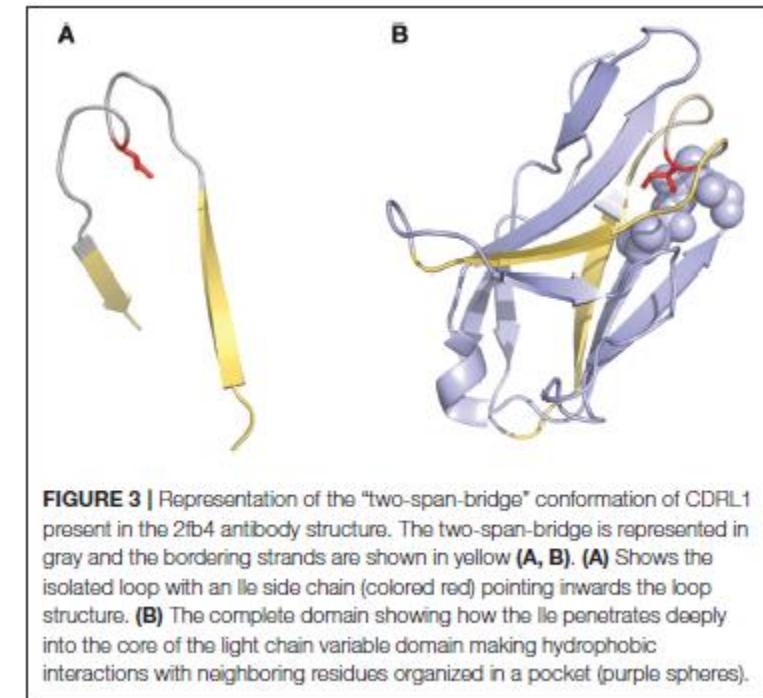
Kabat and Chothia definitions for CDR-H1 and CDR-H2 are different, but the definitions for CDR-H3, CDR-L1, CDR-L2, and CDR-L3 are essentially identical.

Martin Numbering Scheme

- focussed on the structural alignment of different framework regions of unconventional lengths
- They highlighted residues that are absent in most sequences and structures and therefore the authors defined these as deletion positions
- By analyzing sequences and structures, they also proposed a correction of the insertion point within the framework region 3 of the heavy chain domain from position H82 to H72.
- In addition and by analogy with CDRH2, they amended the position of the insertion point for the CDRL2 that locates now at position L52
- They used the numbering software, Abnum, recommended a new numbering scheme that consists of the Chothia numbering system corrected by the ABnum software
- this software uses the much larger Abysis database, which integrates sequences from Kabat, IMGT, and the PDB databases.
- the ABnum program defines a novel insertion/deletion position at position H6 in the Chothia and Kabat numbering schemes ,and corrects this point of insertion and shifts it toward position H8
- The Martin numbering scheme should be considered as the most recent version of the Chothia numbering as it analyses sequences and structures of larger databases correctly inserting positions, defines new ones & highlights the location of deletion in order to fit the topological positions of residues

Gelfand Numbering Scheme

- This numbering system results in a relatively complex nomenclature
- The variable chain sequences are divided into 21 fragments termed “words,” each of these “words” matches with a secondary structure element (a strand or a loop).
- The strands are defined by a letter in alphabetical order (e.g., A, B, C) and the loops by two letters that corresponds to the neighboring strands (e.g., AB, BC, . . .)
- there are two exceptions in this terminology: the three N-terminal residues of the variable chain (named OA since they are not part of the first b-strand) and the loop connecting the B and C strands, which has a ‘two span bridge’ conformation with one residue deeply inserted into the structure
- This loop is divided into two words named BC and CB.
- Limitation:
 - does not include gaps or deletion points but permits a precise comparison of secondary structures (loops and strands) between aligned sequences.
 - noticeable that the Gelfand definition of several loops does not exactly correspond to Chothia’s definition of loops.



IMGT Numbering Scheme

<http://www.imgt.org/IMGTindex/CDR.html>

- introduced a new and standardized numbering system for all the protein sequences of the immunoglobulin superfamily, including variable domains from antibody light and heavy chains as well as T cell receptor chains from different species
- based on amino acid sequence alignment of the germ-line V genes.
- Extension of numbering scheme to the entire variable domains and developed various tools to analyze the full-length sequences
- possesses its own definitions of the framework regions (named FR-IMGT) and CDR (named CDR-IMGT)
- numbering method counts residues continuously from 1 to 128 based on the germ-line V sequence alignment.
- It avoids the use of insertion codes, except between position 111 and 112 for CDR3-IMGT with more than 13 amino acids.
- no number is attributed when a residue is missing in a particular sequence
- It is the primary reference in immunogenetics and immuno-informatics
- Main advantage that it is based on alignments of sequences from a complete reference gene database (44, 45) including the whole immunoglobulin superfamily.

- This has led to the development of highly useful tools.
- amino acid alignment and numbering can be performed by the IMGT/DomainGapAlign This tool also enables to analyse sequence domain polymorphisms by identifying the corresponding VDJ genes coding for the variable region. It is coupled with another interesting application known as IMGT- “Collier de Perles”
- Limitations:
 - due to the continuous numbering of the amino acids along the sequence, the IMGT numbering scheme does not allow an intuitive visualization of insertion positions, even for the most common ones
 - is less flexible.
 - it is more difficult to adapt the IMGT scheme for potential sequences with new amino acid insertions

	27	28	29	30	31	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	62	63	64	65	66	
IGHV1-8*01	G	Y	T	F	-	-	T	S	Y	D	I	N	W	V	R	Q	A	T	G	Q	G	L	E	W	M	G	W	M	N	P	N	-	S	G	N	T	G	
IGHV1-18*01	G	Y	T	F	-	-	T	S	Y	G	I	S	W	V	R	Q	A	P	G	Q	G	L	E	W	M	G	W	I	S	A	Y	-	N	G	N	T	N	
IGHV2-5*02	G	F	S	L	S	T	S	G	V	G	V	G	W	I	R	Q	P	P	G	K	A	L	E	W	L	A	L	I	Y	W	D	-	-	D	D	K	R	
IGHV3-7*01/02	G	F	T	F	-	-	S	S	Y	W	M	S	S	W	V	R	Q	A	P	G	K	G	L	E	W	V	A	N	I	K	Q	D	-	G	S	E	K	Y
IGHV3-11*01	G	F	T	F	-	-	S	D	Y	Y	M	S	S	W	I	R	Q	A	P	G	K	G	L	E	W	V	S	Y	I	S	S	-	G	S	T	I	Y	
IGHV3-21*01	G	F	T	F	-	-	S	S	Y	S	M	N	W	V	R	Q	A	P	G	K	G	L	E	W	V	S	S	I	S	S	S	-	S	S	Y	I	Y	
IGHV3-23*01	G	F	T	F	-	-	S	S	Y	A	M	S	S	W	V	R	Q	A	P	G	K	G	L	E	W	V	S	A	I	S	G	S	-	G	G	S	T	Y
IGHV4-39*01/07	G	G	S	I	S	S	S	S	Y	Y	W	G	W	I	R	Q	P	P	G	K	G	L	E	W	I	G	S	I	Y	Y	S	-	-	G	S	T	Y	
IGHV4-59*01/08	G	G	S	I	-	-	S	S	Y	Y	W	S	S	W	I	R	Q	P	P	G	K	G	L	E	W	I	G	Y	I	Y	Y	S	-	-	G	S	T	N
IGHV5-51*01	G	Y	S	F	-	-	T	S	Y	W	I	G	W	V	R	Q	M	P	G	K	G	L	E	W	M	G	I	I	Y	P	G	-	D	S	D	T	R	
IGHV6-1*01	G	D	S	V	S	S	N	S	A	A	W	N	W	I	R	Q	S	P	S	R	G	L	E	W	L	G	R	T	Y	Y	R	S	K	W	Y	N	D	

CDRH1

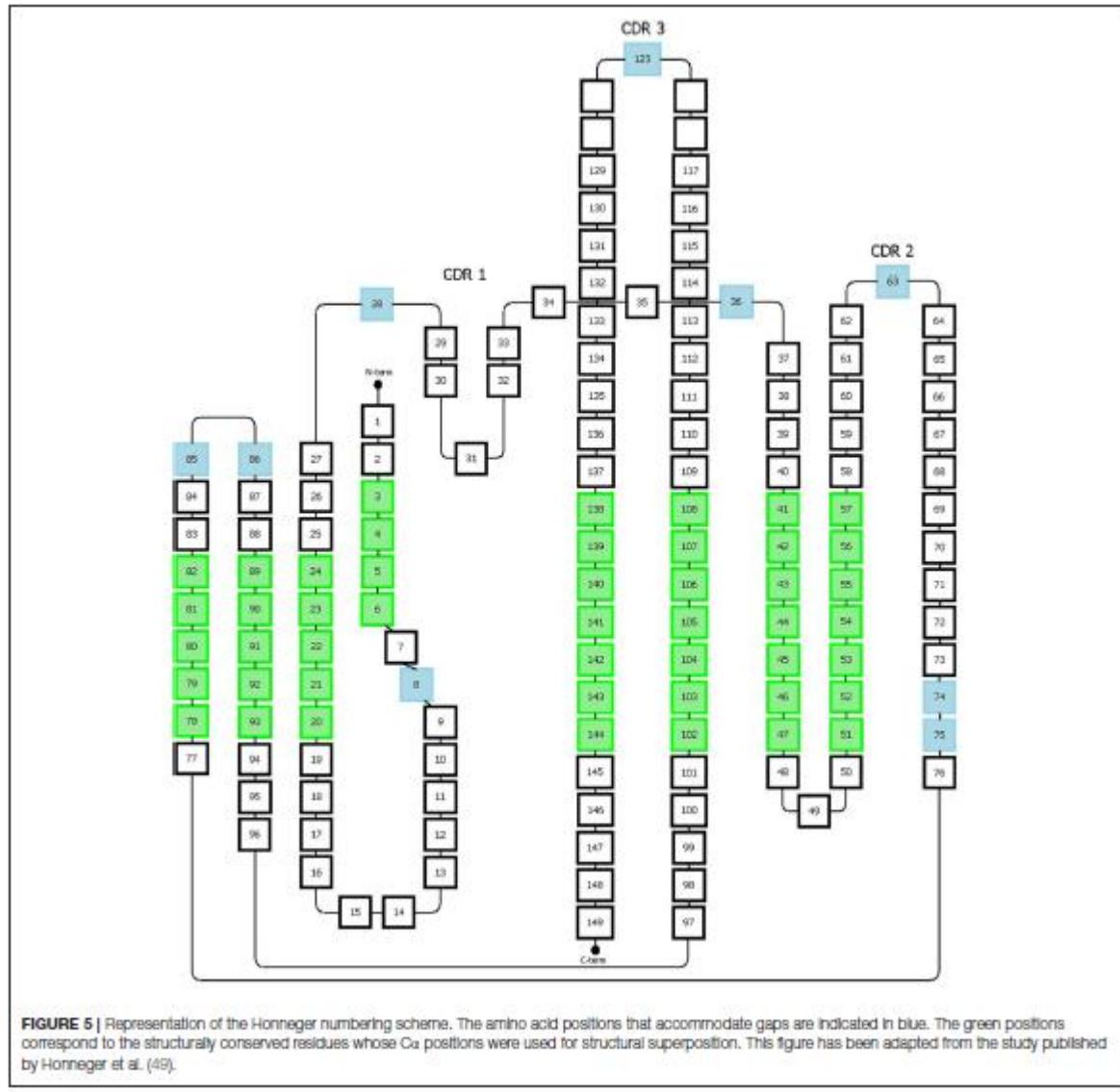
CDRH2

	67	68	69	70	71	72	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104
IGHV1-8*01	Y	A	Q	K	F	Q	G	R	V	T	M	T	R	N	T	S	I	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V	Y	Y	C
IGHV1-18*01	Y	A	Q	K	L	Q	G	R	V	T	M	T	T	D	T	S	T	S	T	A	Y	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	Y	C
IGHV2-5*02	Y	S	P	S	L	K	S	R	L	T	I	T	K	D	T	S	K	N	Q	V	V	L	T	M	T	N	M	D	P	V	D	T	A	T	Y	Y	C
IGHV3-7*01/02	Y	V	D	S	V	K	G	R	F	T	I	S	R	D	N	A	K	N	S	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C
IGHV3-11*01	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	K	N	S	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C
IGHV3-21*01	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	A	K	N	S	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C
IGHV3-23*01	Y	A	D	S	V	K	G	R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L	R	A	E	D	T	A	V	Y	Y	C
IGHV4-39*01/07	Y	N	P	S	L	K	S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C
IGHV4-59*01/08	Y	N	P	S	L	K	S	R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C
IGHV5-51*01	Y	S	P	S	F	Q	G	Q	V	T	I	S	A	D	K	S	I	S	T	A	Y	L	Q	W	S	S	L	K	A	S	D	T	A	M	Y	Y	C
IGHV6-1*01	Y	A	V	S	V	K	S	R	I	T	I	N	P	D	T	S	K	N	Q	F	S	L	Q	N	S	V	T	P	E	D	T	A	V	Y	Y	C	

Honneger's Numbering Scheme (AHo's)

- numbers the variable domains of the immunoglobulin superfamily in a homogenized format
- This system is based on structural alignments of the 3D structures of the immunoglobulin variable regions covering the observed length variation.
- It allows to define structurally conserved Ca positions and therefore deduces appropriate framework regions and CDR lengths
- also defines conserved residues (C23, W43, C106, G140) and gaps on specific positions (#27-28, #36, #63, #123)
- The CDR1 has a “two span bridge” conformation created by a conserved hydrophobic residue at position #31 which is deeply inserted into the structure and therefore divides the loop into two distinct parts, it describes two gap regions located onto these two parts, one located in the first part (#27 and 28) and the other one located in the second part (#36)
- main advantage of the Honneger numbering system is that it is based on structural alignments and therefore it matches better to antibody 3D structures features, in a similar manner to the Chothia numbering scheme

- Limitations:
 - Similarly to the IMGT scheme, the AHo's can skip some numbers in the sequential residue numbering which can be puzzling when analyzing the sequence numbering
 - Less flexible and adaptable to include immunoglobulins with new or larger insertions.





Structural Classification of the CDRs

- Chothia's group classified the CDR loops of heavy and light chains in a small number of conserved structures, called "canonical" classes
- This classification system indicates that the CDRs of the light chain (CDR L1, L2, L3) and the first two CDRs of the heavy chain (CDR H1,H2) adopt only a few different structures
- The authors identified that only very few conserved residues (13 and 7 in the light and heavy chains, respectively) found within the CDR and FR regions are responsible for the conformations of the CDRs
- studies suggest that the structural classification of the CDRs based on structure prediction from the CDR sequences can be a very useful tool for antibody engineering.
- it is important to keep in mind that residues from the framework regions can also influence the CDR conformation

	C																		W																		F/W G																																																			
VL Kabat	23	24	25	26	27	27A	27B	27C	27D	27E	27F	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99					
VL Chothia	23	24	25	26	27	28	29	(30)	30A	30B	30C	30D	30E	30F	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99					
VL Martin	23	24	25	26	27	28	29	(30)	30A	30B	30C	30D	30E	30F	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	(52)	52A	52B	52C	52D	52E	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
IMGT	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99											
Aho	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99											
VH Kabat	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99										
VH Chothia	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99										
VH Martin	22	23	24	25	26	27	28	29	30	(31)	31A	31B	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99								
VL Kabat	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																																	
VL Chothia	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																																	
VL Martin	45	46	47	48	49	50	51	(52)	52A	52B	52C	52D	52E	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																												
IMGT	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																																							
Aho	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																																									
VH Kabat	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																																		
VH Chothia	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																																		
VH Martin	46	47	48	49	50	51	(52)	52A	52B	52C	52D	52E	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99																													

FIGURE 6 | Alignment of residue positions of the CDRs within the light and heavy chain variable domains showing the disparity in the CDR definition according to the different numbering schemes. The CDRs are highlighted in pale green. Amino acid insertion positions are indicated in red. In the Aho numbering system the structurally conserved positions are colored in purple. For Martin's CDR definition, the CDRs correspond to the antigen contact residues. Conserved residues surrounding CDR 1 and 3 are indicated in green.

- Chothia – a structure-based numbering scheme used by Rapid Novor in annotation of REmAb antibody sequencing reports
- Kabat – the most widely adopted sequence-based scheme for numbering antibody residues
- Martin – the most recent version of the Chothia numbering with correction on insertion sites in framework regions
- Glefand – a numbering method that provides comparison of secondary structures between aligned sequences
- IMGT – a standardized numbering system based on alignments of sequences from a complete reference gene database
- Honnegers – the “Aho” numbering scheme based on known three-dimensional structures of immunoglobulin domain

Software

- Software options, such as AbNum¹⁷ ANARCI,²² PyIgClassify,²³ ProABC,²⁴ and DIGIT,²⁵ pre-annotated a large set of antibody sequences, and made sequence alignment to build hidden Markov models (HMMs)

Conclusion

- highlights the importance of standardized numbering system for antibody engineering strategies, especially for antibody humanization tasks
- an effective amino acid numbering system should be able to assign the same number of residues to structurally aligned positions in antibodies from different species
- it is recommended to compare the different numbering systems as inaccuracies are still possible, especially for variable antibody domains with unconventional lengths
- a precise identification of the paratope established using an appropriate amino acid numbering scheme is necessary to engineer humanized antibodies with high affinity, stability and low immunogenicity\
- Designing a fully functional paratope on another framework should not only be restricted to grafting the antigen contacting and interacting residues, but should also include the amino acids that assist in fixating the antigen-binding loops and the residues at positions that affect the relative orientation of the paired VL/VH. All these residues have to occupy identical positions in the 3D structure.

References

- Van Erp EA, Luytjes W, Ferwerda G, van Kasteren PB. Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. *Front Immunol.* 2019 Mar 22;10:548. doi: 10.3389/fimmu.2019.00548. PMID: 30967872; PMCID: PMC6438959.



Immunogenetics & Immunogenomics, Humanization of Abs

Dr. (Ms.) Sonali Correa

Definition

- Immunogenetics is a field of biology that incorporates immunology, molecular biology, and genetics to examine inherited factors that influence immunity, intraspecific heterogeneity, tissue receptor inheritance, genetic, and population dimensions of host-microbe interactions, and tissue incompatibility.
- Immunogenetics is the study of the **genetic basis of the immune response**.
- It includes the study of **normal immunological pathways** and the **identification of genetic variations** that result in immune defects, which may result in the **identification of new therapeutic targets for immune diseases**.
- A branch of immunology concerned with the interrelations of heredity, disease, and the immune system and its components

Introduction

- How can the body identify and eliminate foreign invaders. especially since microorganisms are constantly evolving ways to avoid detection?
- The trick is to be able to distinguish between “self” and “non-self”: to recognize molecules in the body that don’t belong there.
- Foreign molecules (often on the surface of foreign organisms) raise an immune response in the body.
- The primary defence is a set of antibody molecules (also called _____, Ig).
- The human body produces over 1,000,000 different antibodies for this purpose.
- Antibody molecules bind to antigens, which are molecules that are non-self. Each antibody is specific for a particular_____.
- Some soluble antibodies mark foreign cells for attack by the complement system, a series of proteins that punches holes in the cell membranes.
- Other antibodies are on the surface of immune system cells (lymphocytes), which are stimulated to engulf and digest foreign cells by phagocytosis.

The Immune System

- The lymphocytes, or white blood cells, mostly travel through the body in the lymph vessels, a separate circulatory system that is connected to the blood system. The cells collect in lymph nodes, where large numbers of lymphocytes can attack foreign invaders.
- There are two main branches of immune system: __cells and __cells. Both originate in the _____, from the same stem cells as the red blood cells.
- T cells then move to the thymus gland to mature.
- B cells were originally named for their site of maturation in birds: the Bursa of Fabricius. This organ doesn't exist in humans. B cells mature in the bone marrow.
- B cells secrete _____ antibodies: _____ immunity.
- T cells interact directly with their targets: _____ immunity

Antibody Molecules

- Basic structure: 2 heavy chains plus 2 light chains, joined together by _____ bridges between cysteine amino acids.
- The molecule has a "Y" shape, with the two ends of the fork being composed of both heavy and light chain regions.
- These ends are the regions that bind the antigens (Ag). Each Ab molecule has two identical Ag binding regions, and thus the Ab molecules can bind together large groups of Ag's.
- This makes an insoluble complex that is easy for other cells in the immune system to find and eat.
- Each light (L) chain has 2 domains, a _____(V) region and a _____ (C) region. There are only a small number of C regions in each person, but there are very many different V regions. Note that the V and C regions are together on the same _____ chain!
- Each heavy (H) chain has 4 domains, __V domain followed by __C domains. The C domains determine the class (IgG, IgM, etc) of the antibody. • Ig's come in 5 classes: Ig_ (early response), Ig_ (main blood Ig), Ig_ (in body secretions), Ig_ (allergic response), and Ig_ (mostly a cell surface molecule in the early response).
- IgM comes in both membrane-bound and soluble forms. The soluble form is 5 Ig's connected together in a star shape.
- IgA is two Ig's connected together tail to tail.
- In many cases, the constant class-specific regions of the H chains bind to receptors on the surface of specific cells. For instance, IgA binds to secretory cells so it gets secreted into tears, mucus, etc. Also, IgE binds to mast cells that trigger histamine release and other rapid responses to invasion.

Splicing

• Light chain

- V-JC joining by DNA splice (lambda)
- V-J joining by DNA splice (kappa)
- VJ-C intron removal (RNA splice)

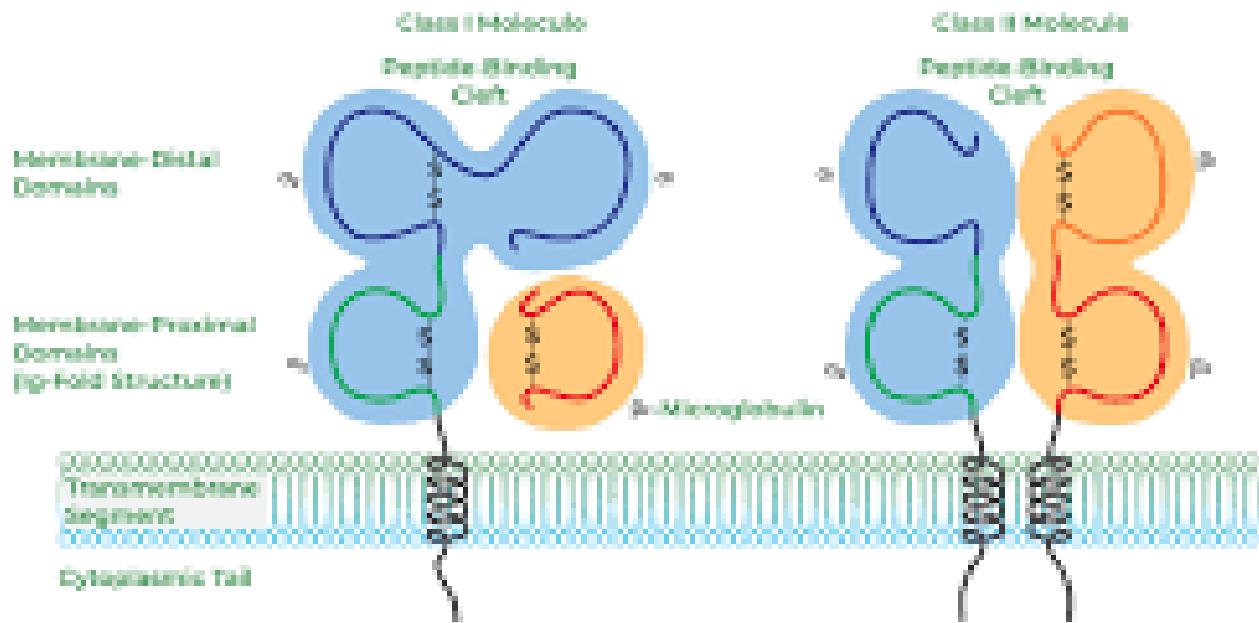
Heavy chain

- D-J joining by DNA splice
- V-DJ joining by DNA splice
- VDJ-C intron removal by RNA splice
- IgM membrane bound 3rd C domain to soluble 3rd C domain by RNA splice
- Class switching by DNA splice

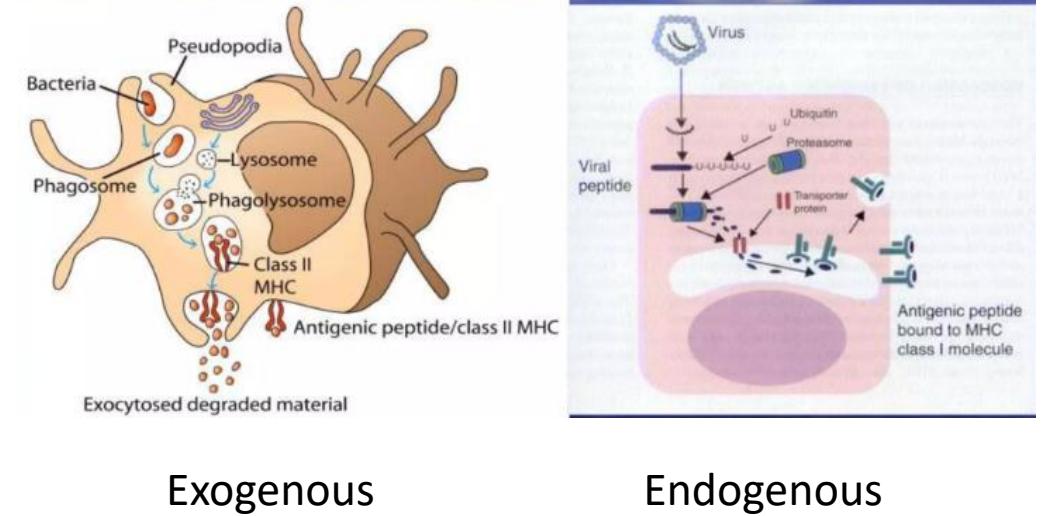
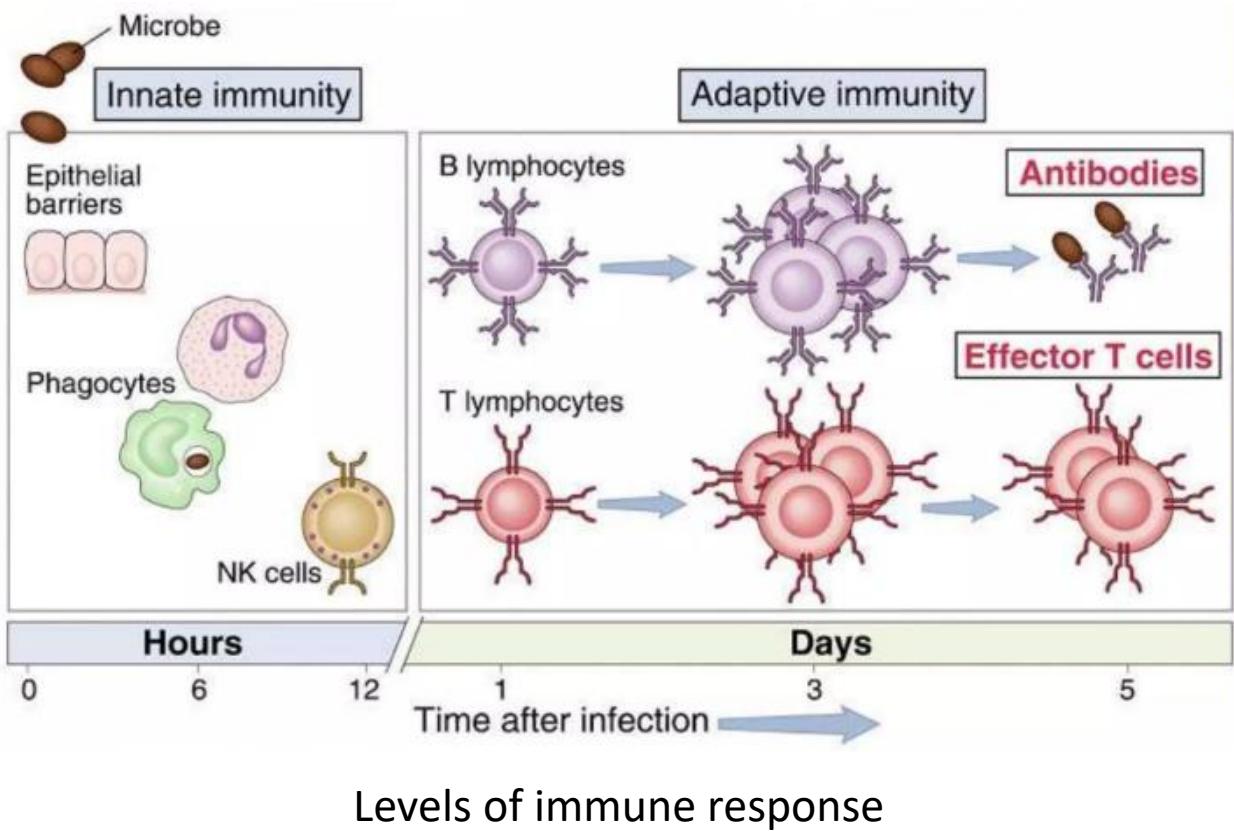
MHC

- The major histocompatibility complex (MHC) of genes consists of a linked set of genetic loci encoding many of the proteins involved in antigen presentation to T cells, most notably the MHC class I and class II glycoproteins (the MHC molecules) that present peptides to the T-cell receptor.

MHC Class I vs MHC Class II

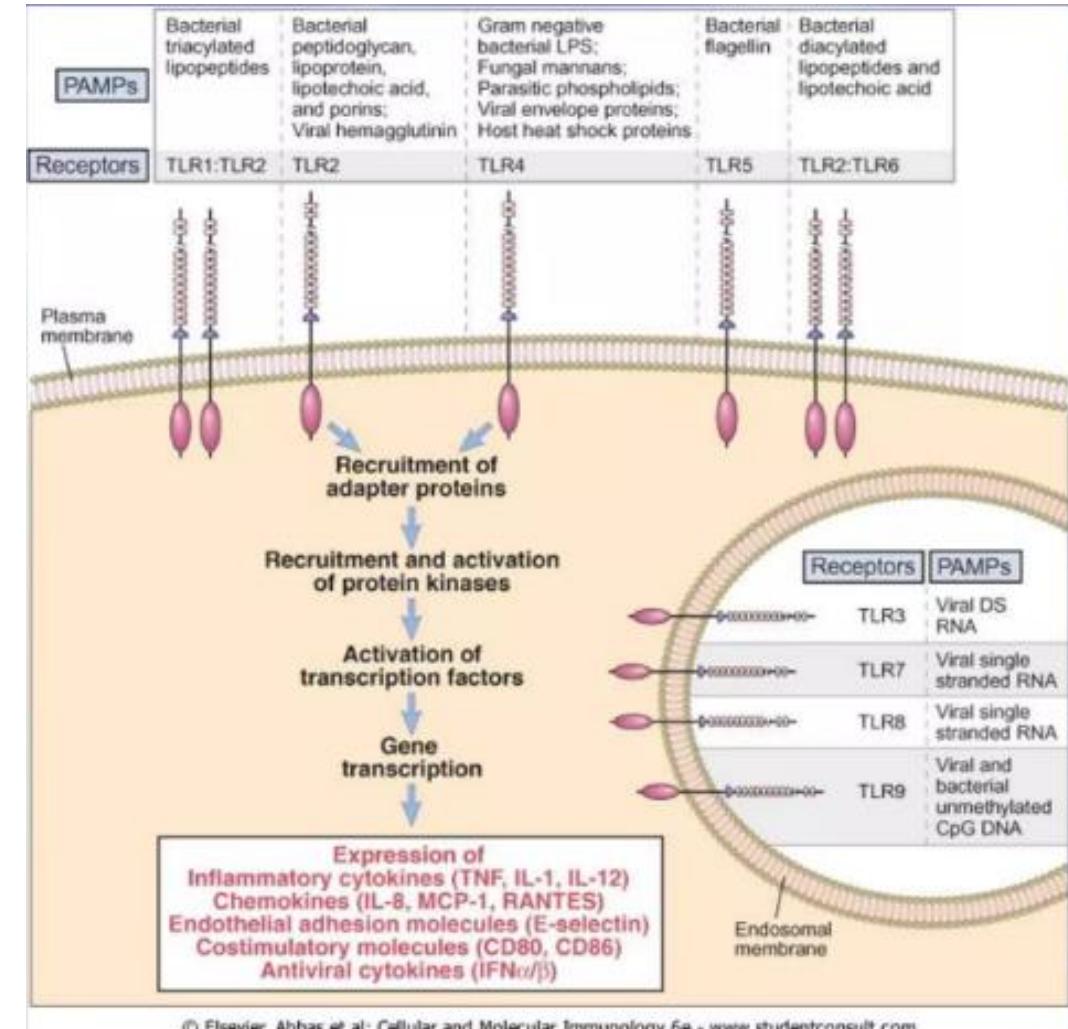
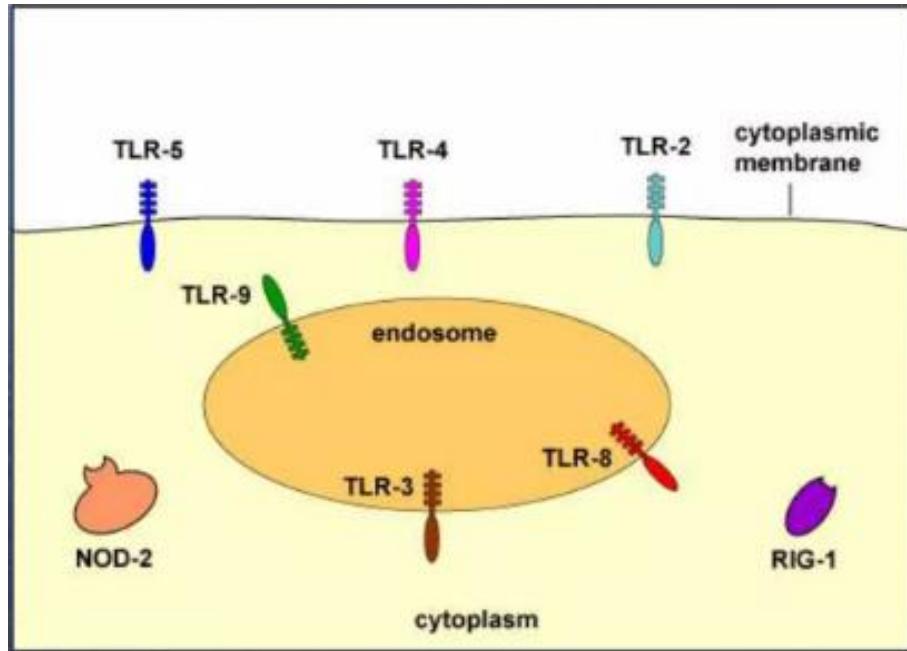


Antigen recognition



Pattern Recognition Receptors

TLRs



TCR & BCR

TCR

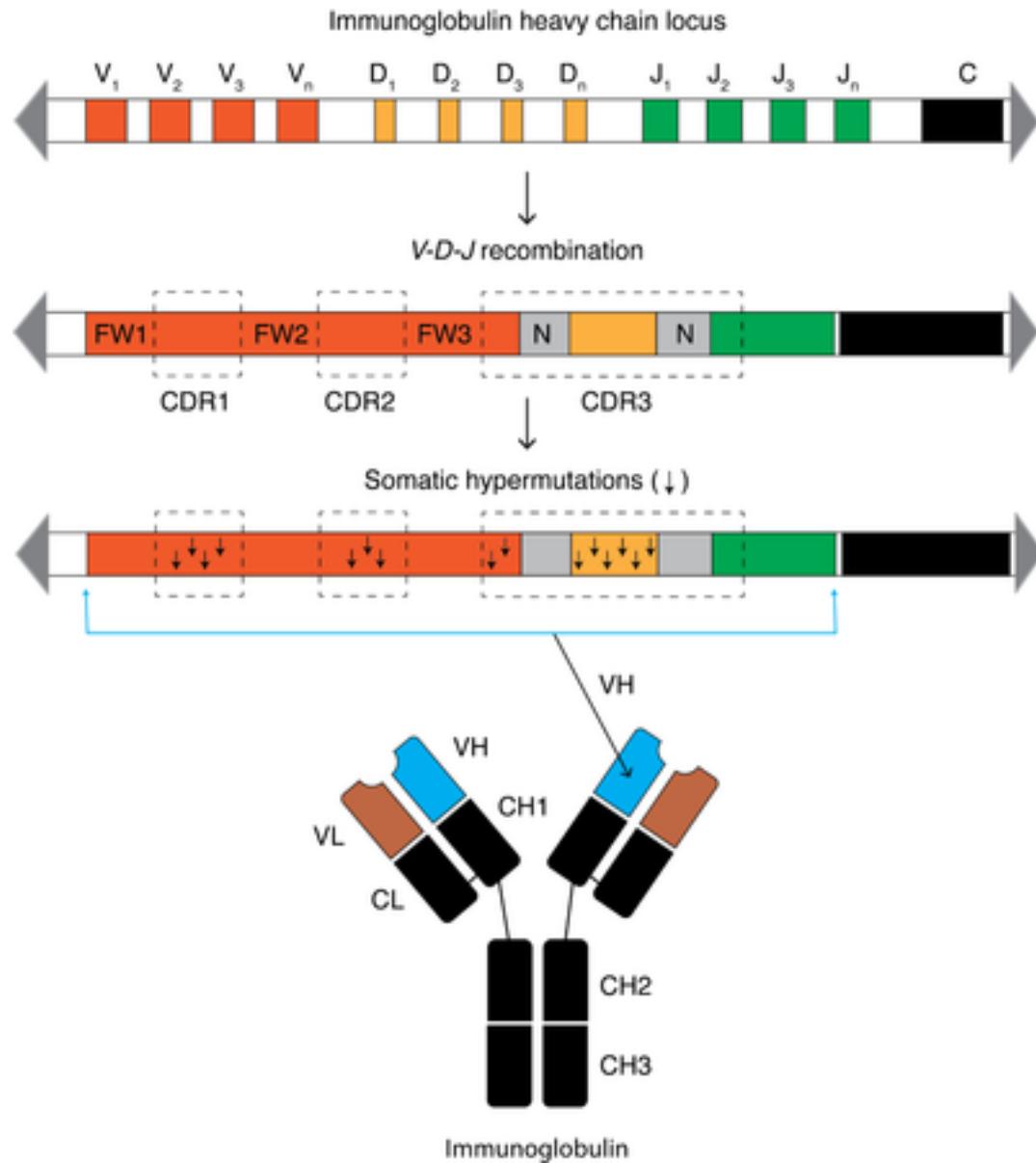
- The TCR protein has 2 subunits and one antigen binding site.
- The alpha subunit has V and J segments (similar to Ig light chains)
- The beta subunit has V, D and J regions, like the Ig heavy chain.
- Both segments undergo DNA splicing rearrangements like the Ig genes. The joining is not precise and short additions or deletions of bases can occur, as in the Ig genes. However, affinity maturation and somatic hypermutation do not occur.
- The TCR protein is membrane bound. It is only found on T cells.
- TCR only recognize antigens when displayed on MHC molecules
- Appearance of half an Ig molecule

BCR

- BCR heavy chains are encoded by different combinations of V, D, and J genes, and BCR light chain loci have only V and J genes.
- BCRs detect and bind to soluble antigens that are present freely
- affinity maturation and somatic hypermutation occur
- Appearance of a Ig molecule

VDJ Recombination & SHM

- V(D)J recombination is the mechanism of somatic recombination that occurs only in developing lymphocytes during the early stages of T and B cell maturation
- Somatic hypermutation is a cellular mechanism by which the immune system adapts to the new foreign elements that confront it (e.g. microbes), whereas affinity maturation refers to the process of increasing the specificity and strength of the interaction between an antibody and its target antigen to increase the effectiveness of the immune response.



MHC Proteins

- Class 1 MHC molecules are found on the surface of all _____ cells. They are involved in cellular immunity.
- Class 2 MHC molecules are only found on the surface of cells that display _____: macrophages and B cells. They are involved in humoral immunity.
- Structure: Both class 1 and class 2 molecules have ___ domains, but they are divided differently. MHC1 has 3 domains on the alpha subunit and one domain on the beta subunit. MHC2 has two domains on both alpha and beta subunits.
- The peptide-binding groove is between the alpha-1 and alpha-2 domains on MHC1, and between alpha-1 and beta-1 in MHC2. Most of the differences between the many different **MHC alleles** lie in this region, which allows binding of different peptides.
- The MHC proteins are encoded by a series of genes at the MHC locus on chromosome ___. MHC genes are well known to be very polymorphic. This polymorphism is the major cause of tissue graft rejection: the immune system almost never recognizes MHC genes from another individual as self.
- There are three main MHC class 1 genes: A, B, and C, which encode the alpha subunit. The beta subunit (beta2 microglobulin), is not very polymorphic, and it is encoded elsewhere. Also, there are about 25 “non-classical” class 1b genes and pseudogenes clustered nearby. The class 1b genes are mostly monomorphic and their function is not well understood.
- The class 2 genes are encoded by 6 regions: DM, DN, DO, DP, DQ, and DR. Each region has at least one alpha and one beta gene. There are also some other genes and pseudogenes in the region.

Activation of B Cells

In the embryo, each B cell undergoes the **DNA splicing** necessary to produce a single type of antibody. This antibody is an IgM bound to the surface of the cell. No antibody is secreted at this time.

Foreign invaders are swallowed (phagocytosis) by macrophages, which are lymphocytes that non-selectively eat particles found in the body

The macrophage partially digests the invader, converting it into peptides and other small molecules.

The macrophage then combines the foreign peptides with an MHC class 2 molecule that gets “displayed” on the surface of the macrophage cell.

Helper T cells that have a T cell receptor proteins that match (bind to) the displayed antigen get activated.

Helper T cells are the central regulatory element in humoral immunity. They are also the primary target of the AIDS virus.

Activation of B Cells

- The activated helper T cells then divide (proliferate). They also activate B cells.
- The inactive B cells start the activation process by binding to an antigen with their IgM molecules, engulfing it and processing it.
- The antigen peptides are displayed on MHC 2 molecules on the cell surface, just like the macrophages do.
- Activated helper T cells interact with B cells that have the same antigen displayed on their surface: the TCR on the helper T cell binds to the antigen displayed on MHC2 on the surface of the B cell.
- the CD4 protein on the surface of the helper T cell is also necessary for this interaction. • The helper T cells secrete cytokines, which stimulate the B cells to proliferate and differentiate into plasma cells, which secrete antibodies.
- this is clonal selection: only those B cells with usable antibodies are selected to proliferate.
- Some descendants of the activated B cell become **plasma cells**, while others become **memory cells**.
- Memory cells stay present in the blood for long periods of time. The next time their specific antigen appears, the memory cells quickly start proliferating and differentiating into plasma cells. This provides a rapid response to a second appearance of the antigen.

Cellular Immunity

- Cytotoxic (or killer) T cells are the main actor in cellular immunity. Cytotoxic cells (also called CD8 cells) kill cells that are infected with viruses or otherwise contain foreign proteins.
- Cytotoxic T cells are activated in the same way that helper T cells are.
- Macrophages eat things in the blood, digest them to peptide fragments, and display the fragments on their surfaces bound to MHC class 1 molecules (as opposed to class 2 for helper T cells).
- Inactive cytotoxic T cells have a specific T cell receptor protein on their surface. If their TCR binds to the peptide displayed on a macrophage, the cytotoxic T cell is activated.
- the CD8 protein on the T cell surface facilitates this interaction.
- All cells digest proteins in their lysosomes. Some of the resulting peptide fragments are displayed in MHC 1 proteins on the surface of the cell. Thus, every cell displays a summary of the proteins inside it on its surface. Mostly these are acceptable proteins, but if the cell has been infected by a virus, foreign viral protein fragments will be displayed.
- If the TCR on an activated cytotoxic T cell binds to a peptide fragment displayed on the surface of a cell, the T cell kills it by secreting perforin proteins that punch holes in the cell's membrane.

Immunogenomics

- **an information science**
- Adding, for each of us, the millions of uniquely randomized T- and B-cell receptor genes that encode our immune repertoires
- This is what make us most unique

- Immunogenomics describes molecular features and changes in immune cells associated with disease using functional genomics technologies
- Oncology is seeing an explosion in immunogenomics due to the recent clinical successes of immunotherapy, and this chapter is focused on cancer immunogenomics
- brings together experts in genomics, immunology, computational biology, and clinical research to tackle the complexity of tumor progression and treatment outcomes
- This field takes advantage of transcriptome analysis , whole-genome sequencing, and targeted sequencing of both host T cells and immune cells as well as epigenetics studies.
- Advancements in next-generation sequencing are helping us understand the clonal heterogeneity intrinsic to tumor progression
- Immune activation and exhaustion

Chimeric antigen receptor T cell therapy

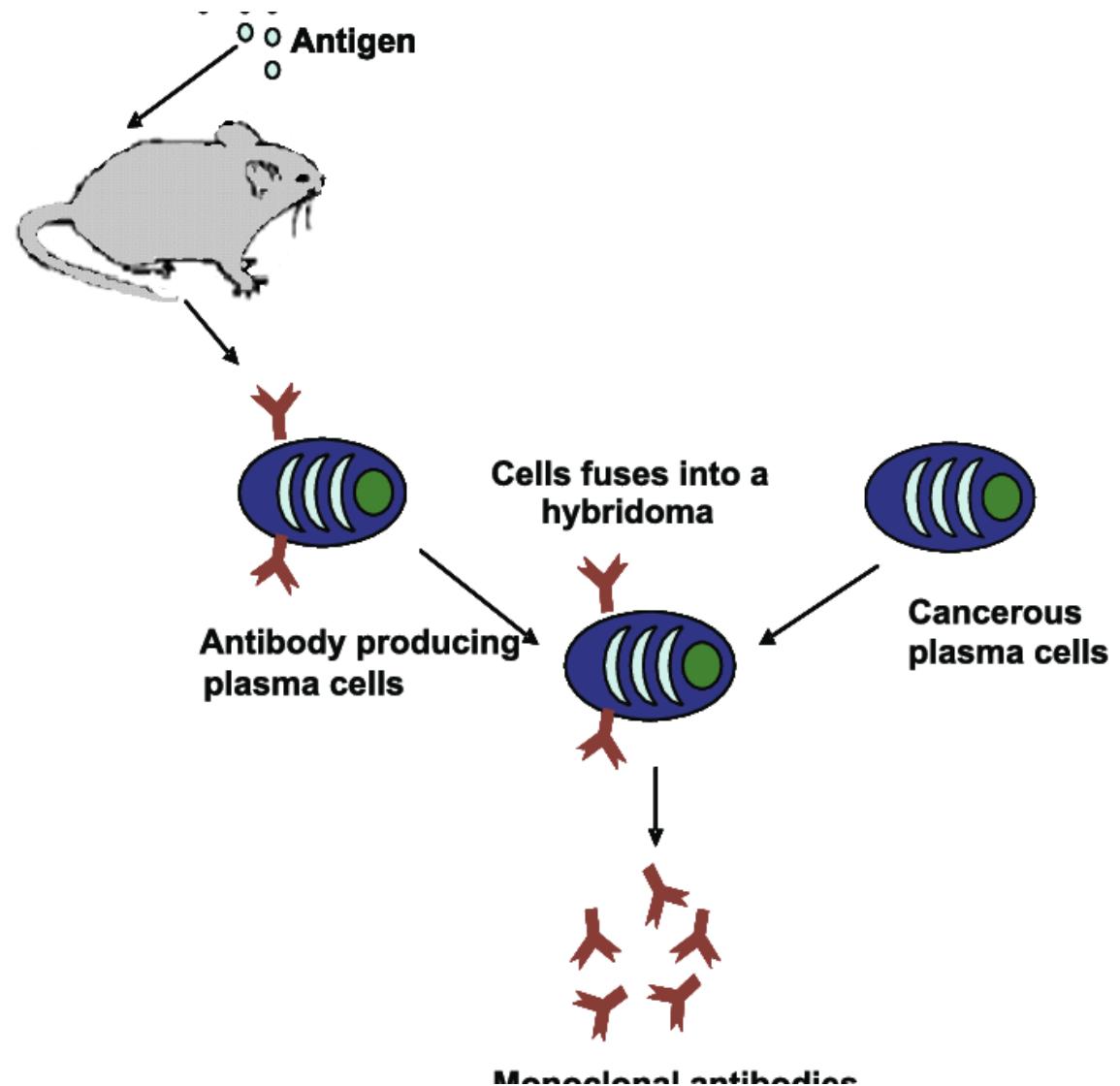
- **CAR T cell therapy, in essence, is a “living drug” derived from the patient’s own blood.**
- In the CAR T cell manufacturing process, T cells are purified from the blood of the patients before being genetically engineered to express a CAR.
- This CAR molecule is armored with an extracellular antibody-like domain fused to an intracellular signaling domain, which enables T cells to simultaneously detect and kill cancer cells and while boosting T cell activity.
- These engineered T cells are then expanded in sufficient number ex vivo before being infused back into the patient.
- A successful treatment is supposed to see CAR T cell further expand in the body while homing in on and killing target cancer cells.
- CART cells do have potentially serious adverse effects including cytokine release syndrome (CRS). development efforts are underway for new generations of safer CAR-T cells that include sophisticated genetic circuitry to control activation
- They are ideally specific for tumor neoantigens and capable of recognizing intact membrane antigens in an MHC unrestricted fashion. Bypassing the steps of antigen processing and presentation by MHC helps alleviate the risk of immune escape due to loss of MHC expression.

Benefits

- The widespread availability and decreasing cost of NGS-based genomic technologies have generated novel mechanistic insights into the genetic control of immune responses, the genomic landscape and clonal architecture of human tumors, and the complexity of tumor-immune system interactions.
- These insights are rapidly being translated to the clinic, with new generations of genetically engineered immunotherapeutics and companion diagnostics based on genomic platforms.
- We can now envision a future when the patient's germline genome and tumor mutational and gene expression profiles are used in routine clinical practice to guide immunotherapeutic decisions.

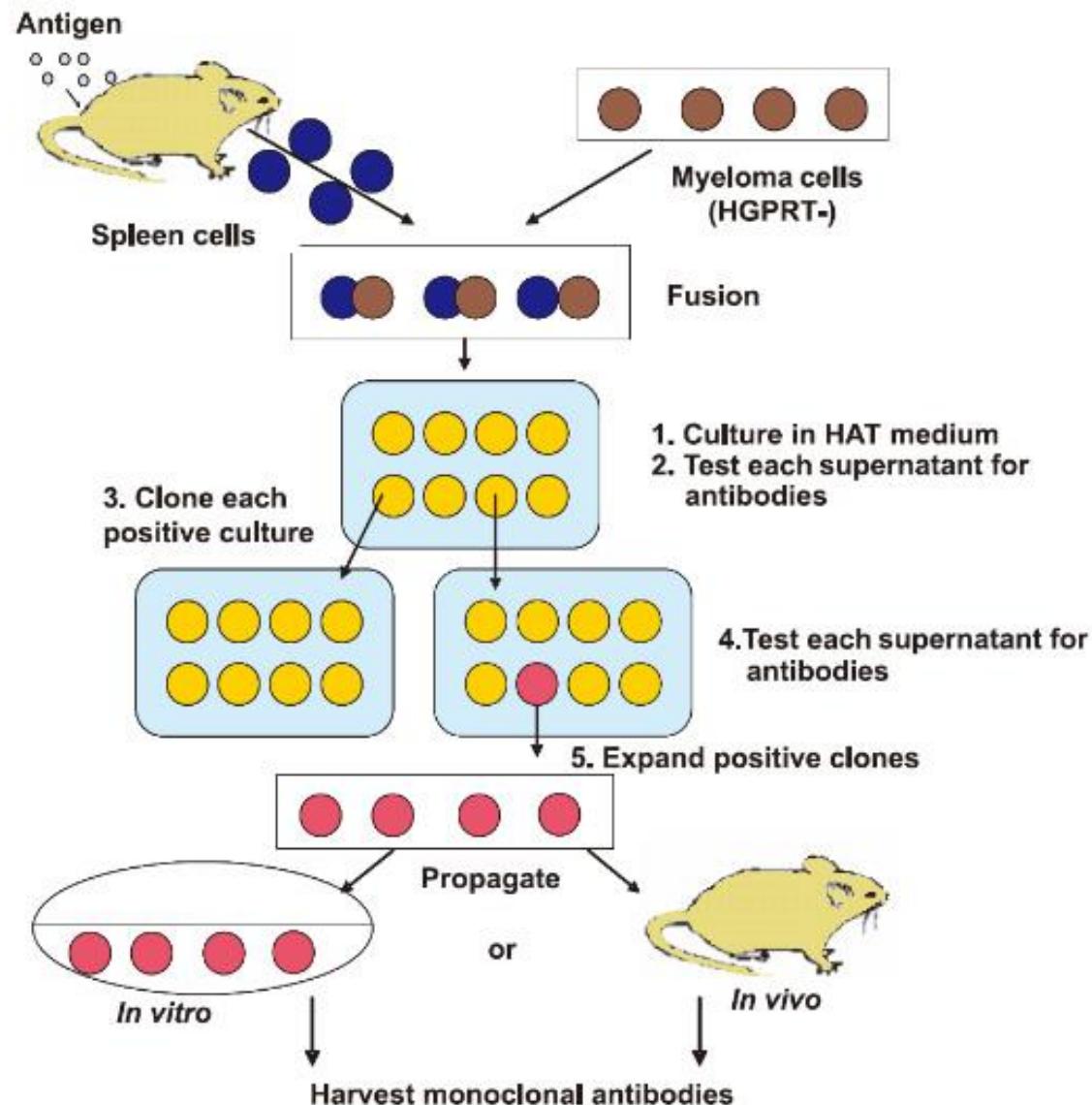
Hybridoma Technology

- In 1975, Kohler and Milstein discovered a technique called hybridoma technology for the production of monoclonal antibodies
- Hybridoma technology is one of the most common methods used to produce monoclonal antibodies mAbs.
- **antibody-producing B lymphocytes** are isolated from mice **after immunizing the mice with specific antigen** and are fused with **immortal myeloma cell lines** to form hybrid cells, called **hybridoma cell lines**



Steps Involved in Hybridoma Technology

- Hybridoma technology is composed of several technical procedures, including **antigen preparation**, **animal immunization**, **cell fusion**, **hybridoma screening** and **subcloning**, as well as characterization and production of specific antibodies.
- mAb generation by the hybridoma approach requires knowledge of multiple disciplines and practice of versatile technical skills, ranging from animal handling, immunology to cellular and molecular biology.
- Generation and identification of high-quality hybridoma clones is a comprehensive and labor-intensive process and requires months of work during the time frame from immunization to specific hybridoma identification.



Cell fusion

- Polyethylene glycol (PEG) and electrofusion are commonly used to induce cell fusion in hybridoma production. PEG fuses the plasma membranes of adjacent myeloma and/or antibody-secreting cells, forming a single cell with two or more nuclei. This heterokaryon retains these nuclei until the nuclear membranes dissolve before mitosis. Electrofusion joins the membranes of neighboring cells by the application of a pulsed electrical field. Electrofusion is more efficient than PEG and the results are reproducible.

Hybridoma screening

- Even in the most efficient hybridoma fusions, only about 1% of the starting cells are fused, and only about 1 in 10^5 form viable hybrids. This leaves a large number of unfused cells still in culture. The cells from the immunized animal (antibody secreting cell) do not continue to grow in tissue culture and so do not confuse further work. However, the myeloma cells are well adapted to tissue culture and must be killed, which can be achieved by drug selection.
- Commonly, the myeloma cells have a defective HGPRT enzyme (hypoxanthine-guanine phosphoribosyl transferase), blocking their ability to use the salvage pathway. These cells containing a non-functional HGPRT protein will die in HAT medium. Only the hybridoma cells have got the ability to divide and proliferate on the HAT medium because genome from the B-lymphocyte makes them HGPRT positive and genome from the myeloma cells they can divide indefinitely.

mAb production

- Hybridoma antibodies can be produced *in vitro* and *in vivo*.
- For production of monoclonal antibodies ***in vitro***, hybridomas are expanded by transfer to 24 well tissue culture plates followed by 25 cm² flask and a 75 cm² flask containing suitable medium. The cell density is maintained between 10⁵ and 10⁶ cells/ml. Typical culture supernatants yield up to 100µg/ml of antibody, the exact amount depending upon the cell density and rate of growth. Culture *in vitro* provides a more pure preparation of antibody.
- For producing monoclonal antibodies ***in vivo***, mice are primed by intraperitoneal injection with 10⁵ - 10⁷ hybridoma cells. The rate of growth of the resulting ascites tumour is in general very variable and can be from less than two or more than five weeks. The ascites fluid can be collected from an anaesthetized mouse. It is possible to obtain 10 ml of ascites fluid or more from a mouse by regular tapping. Ascites fluid will be contaminated with mouse immunoglobulins to a small extent and if a very pure antibody is required this may prove inconvenient.

Clinical significance of hybridoma technology



- **mAb therapeutics**
- Compared with other biologics, mAbs are able to maintain an extremely high affinity towards their target.
- Due to this high affinity and specificity, researchers began investigating the therapeutic potential of mAbs as metabolic activators, inhibitors and immuno-modulators.
- While the first few US FDA-approved mAb therapeutics, such as muromonab-CD3, were generated solely in mice, it became evident that in order to avoid immune rejection, future mAb-based therapeutics needed to undergo humanization.
- Since the approval of muromonab-CD3 in 1986, the FDA has approved approximately 80 more mAb therapeutics for diseases ranging from autoimmune disorders, to inflammatory diseases, HIV and cancer.
- Interestingly, despite the discovery of combinatorial display libraries in 1984 as an alternative mAb discovery platform, the majority of these mAb therapeutics were originally discovered using hybridoma technology in either fully murine or humanized mice.
- The reason for this preference is likely attributed to the natural ability of the murine immune system to generate highly specific mAbs that elicit strong constant domain functionality with limited immunoreactivity after humanization.

A list of FDA-approved therapeutic mAbs by hybridoma technology

Murine mAb (- omab) 3	ORTHOCLONE OKT3® <i>muromonab-CD3</i> (1986)	ZEVALIN® <i>ibritumomab tiuxetan</i> (2002)	BEXXAR® <i>tositumomab</i> (2003)		
Chimeric mAb (- ximab) 5	REOPRO® <i>abciximab</i> (1994)	RITUXAN®/ MABTHERA® <i>rituximab</i> (1997)	SIMULECT® <i>basiliximab</i> (1998)	REMICADE® <i>infliximab</i> (1998)	ERBITUX® <i>cetuximab</i> (2004)
Hybridoma Origin (26 mAbs)	ZENAPAX® <i>dacizumab</i> (1997)	SYNAGIS® <i>palivizumab</i> (1998)	HERCEPTIN® <i>trastuzumab</i> (1998)	CAMPATH® MABCAMPAT® <i>alemtuzumab</i> (2001)	XOLAIR® <i>omalizumab</i> (2003)
Humanized mAb (- zumab) 11	AVASTIN® <i>bevacizumab</i> (2004)	TYSABRI®/ ANTEGREN® <i>natalizumab</i> (2004)	LUCENTIS® <i>ranibizumab</i> (2006)	SOLIRIS® <i>eculizumab</i> (2007)	CIMZIA® <i>certolizumab pegol</i> (2008)
	Actemra® <i>Tocilizumab</i> (2010)				
Human mAb (- umab) 7	VECTIBIX® <i>panitumumab</i> (2006)	SIMPONI® <i>golimumab</i> (2009)	STELARA® <i>ustekinumab</i> (2009)	ARZERRA® <i>ofatumumab</i> (2009)	ILARIS® <i>canakinumab</i> (2009)
	PROLIA®/ XGEVA® <i>Denosumab</i> (2010)	YERVOY® <i>ipilimumab</i> (2011)			

FDA-approved monoclonal antibodies

Margetuximab, a drug that is used in conjunction with chemotherapy to treat patients with HER2-positive metastatic breast cancer, was approved in the year 2020

Evinacumab is used in conjunction with other medications to decrease cholesterol in adults and children aged 12 and above, who have a hereditary form of high cholesterol

Dostarlimab is used to treat individuals with endometrial cancer that has progressed or relapsed following treatment with previous chemotherapeutic drugs

Satralizumab is used in the treatment of neuromyelitis optica spectrum disease (NMOSD) which is a rare, chronic autoimmune illness that affects the central nervous system and causes inflammation of the optic nerves, spinal cord, or brain

Isatuximab is used in the treatment of multiple myeloma

Crizanlizumab is used to ease severe pain caused in patients with sickle cell anemia

Ibalizumab helps manage HIV infection; this medicine is used with other HIV drugs. It aids in the reduction of HIV in the body, allowing the immune system to function more effectively

Benralizumab is used in adults and children (12 years of age and above?) in combination with other medicines to prevent chest tightness, wheezing, trouble breathing, and cough caused by asthma

Atoltivimab, maftivimab, and odesivimab-ebgn are FDA-approved mAbs used for the treatment of Zaire ebolavirus. It comprises a fixed-dose combination of three monoclonal antibodies

Aducanumab is a drug used in the treatment of Alzheimer's disease (AD). It is an amyloid beta-directed monoclonal antibody that works to decrease the development of aggregated forms of Amyloid-beta (A) in the brains of patients suffering from Alzheimer's disease

Eptinezumab is a medicine intended to assist people with migraines. It is a monoclonal antibody that targets the alpha and beta forms of calcitonin gene-related peptides (CGRP) in the brain

Brolucizumab is used to treat severe eye disease (wet age-related macular degeneration). This medicine can aid in the preservation of eyesight and the prevention of blindness

Risankizumab is used in the treatment of plaque psoriasis

Romosozumab is used to treat bone density loss (osteoporosis) in post-menopausal women who are at a high risk of bone fractures. It works by boosting bone strength and density

Diagnostic testing

- Monoclonal antibodies are commonly employed in the diagnosis of a variety of disorders. It is used to check the presence of any foreign antigen such as toxins, drugs, hormones, or internal and surface proteins of bacteria or viruses

Testing of pregnancy

- Monoclonal antibodies are used to identify the presence of human chorionic gonadotropin [hCG] as a mark for recognition of pregnancy

Radioimmunodetection (RID) of cancer

- Monoclonal antibodies are also used to detect the presence of specific tumor-type in the body. In this technology, antibodies are labeled with radioactive tags to check the presence of any type of carcinomas or cancer-specific cells in the body

Malaria herpes virus testing

- mAbs are used in the diagnosis of various diseases caused by viruses such as malaria herpes viruses

Identification of different strains of pathogens

- Monoclonal antibodies can be used to differentiate between different strains of a single pathogen, for example, *Neisseria gonorrhoeae*

Serological identification of ABO blood groups

- Monoclonal antibodies can also be used in the serological identification of blood groups. The antibodies can be isolated from the sera of a person stimulated by the A or B blood group

Radioimmunotherapy (RIT) of cancer

- This technique is similar to RID; in RIT, monoclonal antibodies are used to target tumor cells; following this, the targeted cells are then killed using a lethal dose of radiation; the advantage of this technique is that it minimizes the radiation exposure by other normal body cells

Cancer treatments through drugs

- Monoclonal antibodies are also used in targeted chemotherapy. A drug named rituximab, sold under the brand name of Rituxan, was approved by FDA for commercial use, for the targeted treatment of cancers mainly lymphomas

Viral disease treatment

- Monoclonal antibodies are also being tested in treatments of previously incurable diseases such as AIDS

Specific cell identification and their functions

- Monoclonal antibodies can be used to identify and monitor certain cell populations or even molecules in a living system.

Organ transplantation

- Monoclonal antibodies are used for the inactivation of T-lymphocytes that play a role in the rejection of transplanted organs. Monoclonal antibodies such as OKT3 play an important role by interfering with T-cell function in graft rejection. It is a monoclonal antibody that targets the CD3 receptor, a membrane protein found on T cell surfaces

Rhesus disease immunization

- The UK Blood Products laboratory has been working on research to develop a possibility of substituting mAb rhesus immunization and replacing it with serum

Immunopurification

- This technique has been used in the purification of individual interferons and can be used in the purification of proteins and enzymes.

Advantages of hybridoma technology

- Produces **highly pure and specific antibodies** (monoclonal antibodies).
- Highly **reproducible and scalable**
- Provides an **unlimited production** of monoclonal antibodies.
- Used to perform **highly sensitive and specific** assays.
- There is no need to maintain the animal in the laboratory for the production of antibodies [in vitro method].
- The **purity of antigen or immunogen is not a prerequisite**.
- The selection method is useful in the identification of the right clones against a specific antigen.
- In vivo production of antibodies ensures the formation of a mixture of variable and constant domains. The generated antibodies have a high affinity towards the epitope of a targeted substance.
- This method is not labor-intensive; in vitro antibody generation techniques require the use of immune libraries
- Antibody reliability is vital to each analysis and assay development and is one of the key features of hybridoma technology. Once hybridoma cells become stable, these offer limitless production of cost-effective, homogenized antibodies.
- The perfect tool for research, in various fields such as toxicology, animal biotechnology, medicine, pharmacology, etc.
- Monoclonal antibodies are **widely employed** in diagnostic and therapeutic procedures.
- It is **used** in various chemotherapeutic regimens to treat various cancer types.
- Used **widely in research** for the production of vaccines.

Challenges of hybridoma technology

- This is a time-consuming method, requiring 6 to 9 months.
- The method is quite expensive and requires considerable effort in production.
- This method is not suitable for producing antibodies against small peptides and fragment antigens.
- Hybridoma culture suffers from a high risk of contamination.
- This system of antibody production is now developed for only mice and rats and researchers are working continuously to develop antibodies of human origin [19].
- The viable efficiency of cells is quite low. More than 99% of the cells die during the process of cell fusion reducing the efficiency of the method and also reducing the variety of helpful antibodies which will be made against a specific substance.
- If monoclonal antibodies are generated against a single antigenic determinant, they do not show cross-reactivity with other antigenic determinants. Retroviruses are a common incidence within mammalian chromosomes. Generally, animals like mice that are used in the production of monoclonal antibodies could carry many viruses such as viscus virus, retrovirus, reovirus, herpes virus, and thymic virus, leading to cross-contamination or infection in humans [18]. This poses a major threat for cross disease transfer from mice or rats to the human.
- Despite purification, there is no guarantee that monoclonal antibodies made using the hybridoma technique are virus-free.
- The fusion of human lymphocyte and mouse myeloma cells may result in the production of unstable fused cells.
- In humans, there are no stable myeloma cells, suitable for the process of antibody production that can be used to substitute mouse myeloma cells.

Advancement in the field of hybridoma technology

Transgenic mice (first generation, the 1970s): The first generation of transgenic mice protein features two identical heavyweight chains and two identical lightweight chains (CH, CL), as well as variable domains (VH, VL). This protein also contains associated antigen-binding sites (CDRs), and immunogenicity is induced by the constant (Fc) region of the associate protein

Chimeric antibody (second generation, the 1980s): Chimeric monoclonal antibodies are created by substituting a consistent segment of human IgG protein for the constant segment of mouse protein.

Humanized antibody: More than 90% of human sequences are present in the humanized protein. Human immunoglobulin G antibodies are completely humanized by fusing the DNA of three CDRs from the mouse variable regions into human immunoglobulin G antibodies. These totally humanized antibodies are made with the help of transgenic mice that have human immunoglobulin; therefore, they include 100% human sequences and are needed in fewer quantities than the antibodies of mice origin

A bispecific antibody (third generation, the 1990s, and 2000s) aids in the prolongation of the half-life of monoclonal antibodies and the enhancement of their therapeutic activities. Bispecific antibodies are created from 2 distinct antigen-binding regions, and thus, they can bind to two totally different antigens .

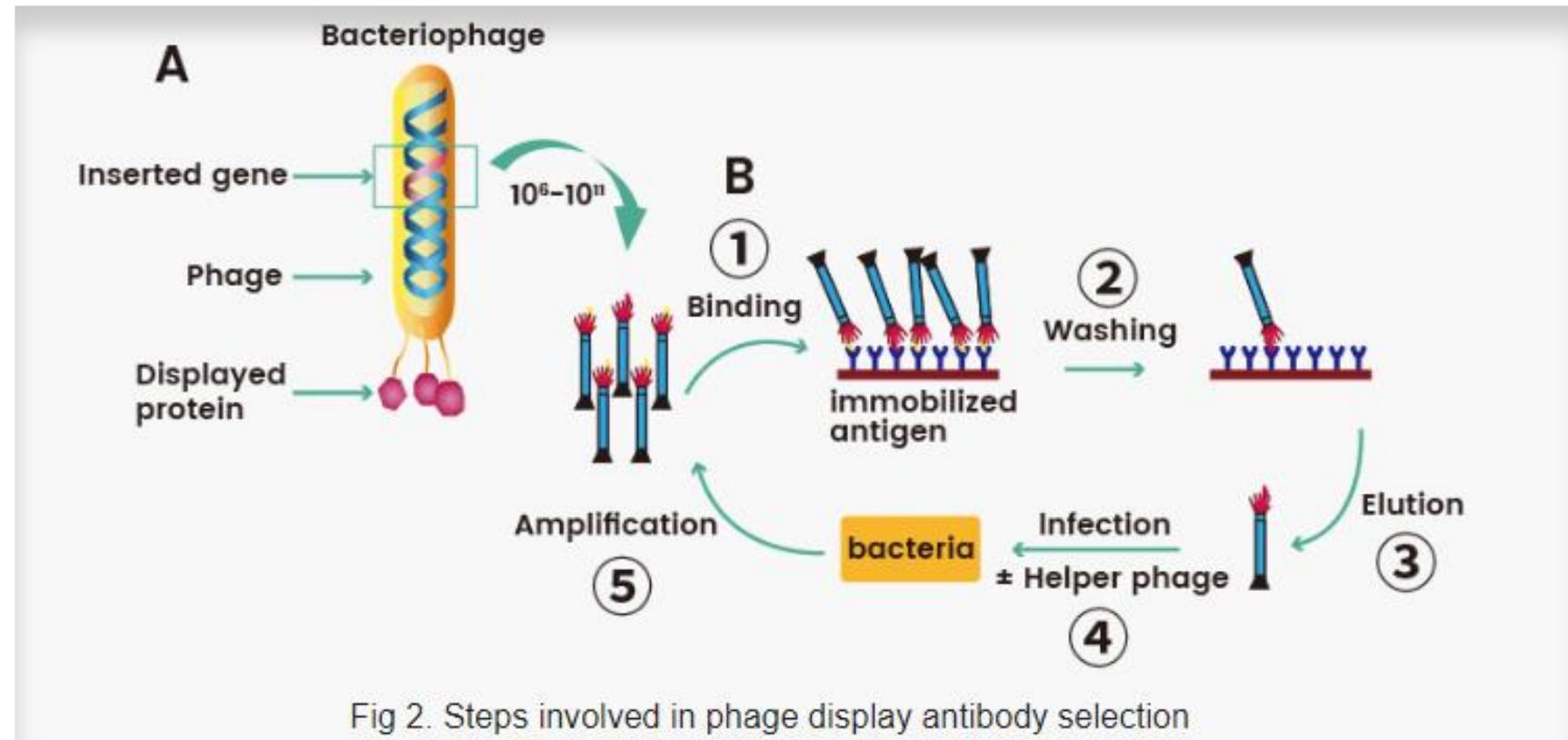
Humanization of Abs by Design

- The process of "humanization" is usually applied to monoclonal antibodies developed for administration to humans (for example, antibodies developed as anti-cancer drugs).
- Humanization can be necessary when the process of developing a specific antibody involves generation in a non-human immune system (such as that in mice).
- The protein sequences of antibodies produced in this way are partially distinct from homologous antibodies occurring naturally in humans, and are therefore potentially immunogenic when administered to human patients (see also Human anti-mouse antibody).
- Humanized antibodies are genetically engineered by grafting each of the complementarity determining regions (CDRs) from an antibody with the desired antigen binding specificity into the corresponding CDRs of another antibody, for example, grafting the CDRs from a mouse monoclonal antibody into a human IgG1 antibody.

Use of recombinant DNA in humanization process

- The humanization process takes advantage of the fact that production of monoclonal antibodies can be accomplished using recombinant DNA to create constructs capable of expression in mammalian cell culture.
- That is, gene segments capable of producing antibodies are isolated and cloned into cells that can be grown in a bioreactor such that antibody proteins produced from the DNA of the cloned genes can be harvested en masse.
- The step involving recombinant DNA provides an intervention point that can be readily exploited to alter the protein sequence of the expressed antibody. The alterations to antibody structure that are achieved in the humanization process are therefore all effectuated through techniques at the DNA level.
- Not all methods for deriving antibodies intended for human therapy require a humanization step (e.g. phage display) but essentially all are dependent on techniques that similarly allow the "insertion" or "swapping-out" of portions of the antibody molecule.

Phage Display

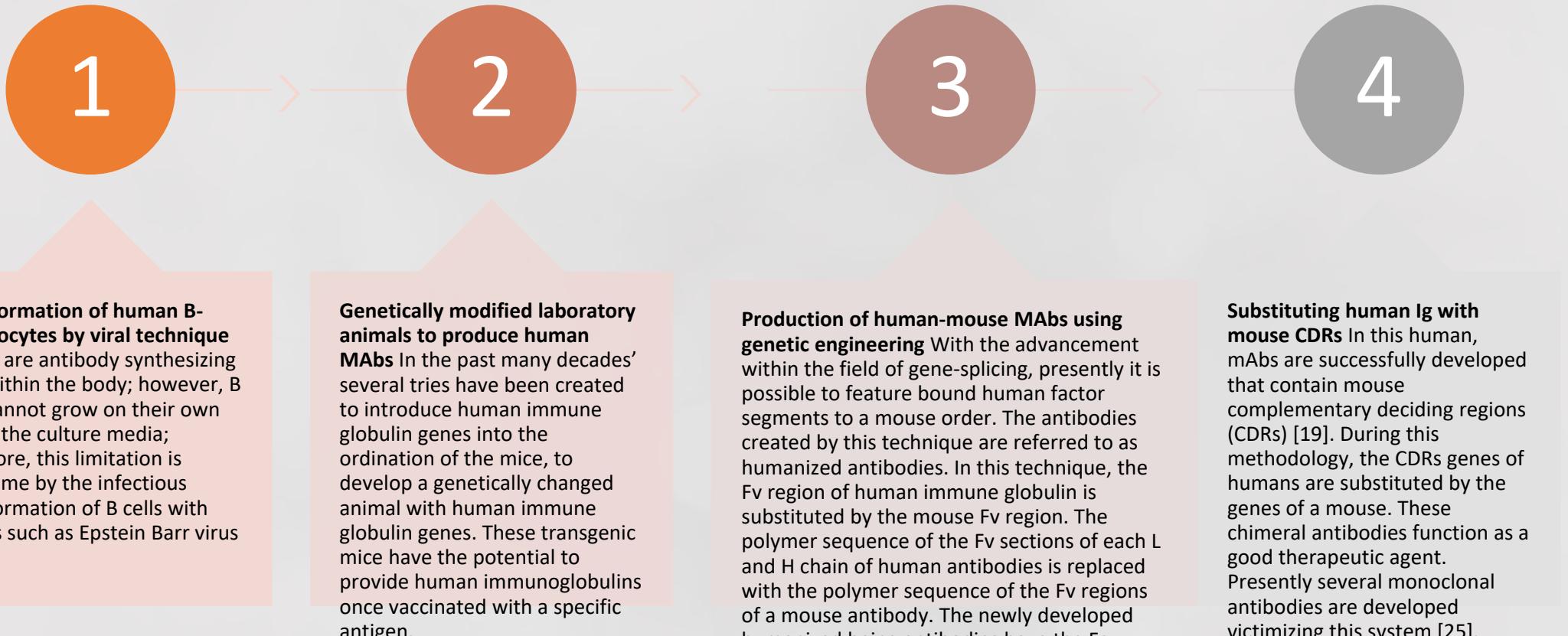


- Phage display is a selection technique based on genetic engineering of bacteriophages and repeated rounds of antigen-guided selection and phage propagation. It allows *in vitro* selection and production of recombinant monoclonal antibodies of high specificity and affinity.
- In 1985, Smith was first to discover that foreign DNA fragments can be fused to the gene encoded for pIII coat protein of a nonlytic filamentous phage and expressed as a fusion protein on the virion surface without disturbing the infectivity of the phage. Winter flipped the process of phage display by using the phage to display antibodies (rather than proteins) then fishing out the desired antibodies that bind to molecules or even cells. The invention of antibody phage display has revolutionised monoclonal antibody drug discovery.
- For monoclonal antibody production, both hybridoma and phage display are very important technologies.

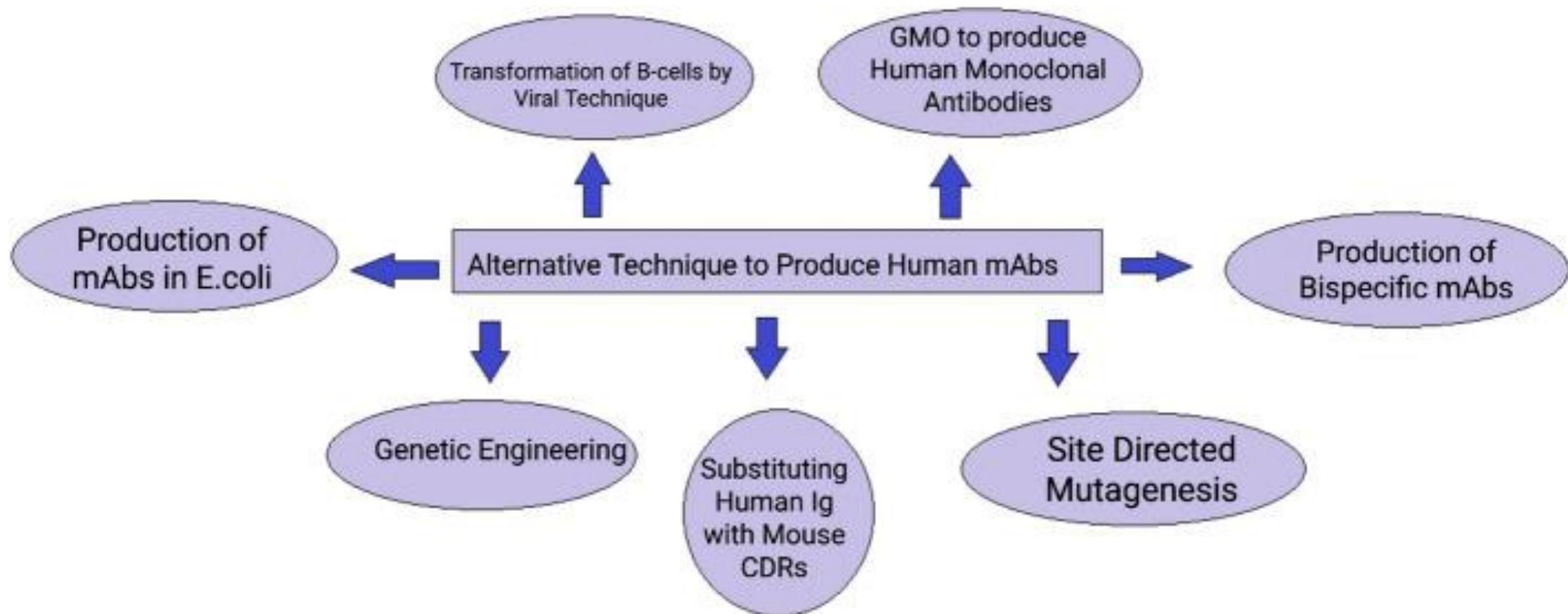
	Phage Display	Hybridoma technology
Advantages	<ul style="list-style-type: none"> • Large scale production • Fast process • Great control over the selection process • Easy to screen a large diversity of clones • Possible to directly screen human libraries • Possible to screen toxic antigens • No immunogenicity issue (for naïve libraries) • No clone viability issues • Direct access to sequence • No animal use (for naïve libraries) 	<ul style="list-style-type: none"> • Large scale production • High antibody yield • High specificity • High antibody sensitivity • Lower cost
Disadvantages	<ul style="list-style-type: none"> • More expensive • Binders may have lower affinity • Technically more difficult 	<ul style="list-style-type: none"> • Long generation time • Incomplete epitope identification • Often requires humanization

- Humanized antibodies contain murine-sequence derived CDR regions that have been engrafted, along with any necessary framework back-mutations, into human sequence-derived V regions.
- Fully human sequence derived antibodies have no murine sequence, and are largely produced via two sources: phage display technologies and transgenic mice.
- The first fully human sequence-derived antibody to be approved for therapeutic use was adalimumab (Humira), a fully human IgG1 antibody specific for TNF α that was selected via phage display of human VH and VL sequences
- fully human sequence antibodies isolated from mice carrying genetic modifications such that the murine immunoglobulin genes were disabled and replaced with functional human immunoglobulin loci have been approved for therapeutic use

Advance techniques to produce humanized monoclonal antibodies



- **Production of Bi-specific monoclonal antibodies** In this technique, the arms of fab (antigen-binding arms) have 2 completely different specificities for 2 different epitopes of two different antigens; these forms of antibodies are referred to as Bi-specific monoclonal antibodies. These are generated either by fusing 2 completely different hybridomas or by recombinant DNA technology. These antibodies are helpful in the treatment of various diseases
- **Production of monoclonal antibodies using *E. coli*** as a host Hybridoma technology is a kind of backbreaking, expensive, and long method. To beat these disadvantages, scientists attempt to create genetically changed microorganisms like bacteria to produce monoclonal antibodies. The aim is to develop bioreactors for the large-scale production of monoclonal antibodies in *E. coli* or in little microorganisms. The antigen-binding sites on the antibodies have a vital function within the binding of antibodies with the antigen; the Fv and Fab fragment play a major role. On the opposite hand, the Fc portion can be variable
- **Site-directed Mutagenesis to produce monoclonal antibodies** The site-directed mutagenesis could be a technique utilized in biotechnology; this method has created its potential to introduce amino acid residues at a very important position on the antibody. These amino acid residues can increase the potency of atom labeling and are established to be helpful in diagnostic imaging and radioimmunotherapy
- Anti-SARS-CoV-2 monoclonal antibodies **The spike (S), envelope (E), membrane (M), and nucleocapsid (N) structural proteins, as well as non-structural and auxiliary proteins, are all encoded by the SARS-CoV-2 genome.** S1 and S2, two components of the spike protein, mediate host cell adhesion and invasion. In the treatment of SARS-CoV-2 infection, monoclonal antibodies that target the spike protein have been found to be effective. The Food and Drug Administration (FDA) has issued Emergency Use Authorizations (EUAs) for three anti-SARS-CoV-2 monoclonal antibody products for the treatment of mild to moderate COVID-19 infection among non-hospitalized individuals with SARS-CoV-2 infection verified in the lab , such as:
 - Bamlanivimab plus etesevimab: These are neutralizing monoclonal antibodies that bind to distinct but overlapping epitopes in the SARS-CoV-2 spike protein RBD
 - Casirivimab plus imdevimab: It is human monoclonal antibodies that bind to non-overlapping epitopes of the SARS-CoV-2 spike protein, RBD. It is also used as a post-exposure prophylactic for those who are at high risk of contracting SARS-CoV-2
 - Sotrovimab: This monoclonal antibody was discovered in a SARS-CoV survivor in 2003. It binds to an epitope in the spike protein's RBD that is shared by SARS-CoV and SARS-CoV-2



Conclusion

- Presently, the monoclonal antibodies used are either raised in mice or rats; this poses a risk of disease transfer from mice to humans.
- There is no guarantee that antibodies thus created are entirely virus-free, despite the purification process.
- Also, there are some immunogenic responses observed against the antibodies of mice origin.
- Technologically advanced techniques such as genetic engineering helped in reducing some of these limitations.
- Advanced methods are under development to make lab-produced monoclonal antibodies as human as possible

Thank you

**THANK YOU FOR BEING
AWESOME STUDENTS!!!**

The background of the slide features a close-up photograph of several red blood cells, each containing a central nucleus. A large, semi-transparent diagonal graphic element runs from the top-left towards the bottom-right. This graphic consists of three parallel bands: a dark grey band closest to the top, a light blue band in the middle, and a darker blue band closest to the bottom.

Membrane receptors for antigen

Dr. (Ms) Sonali Correa

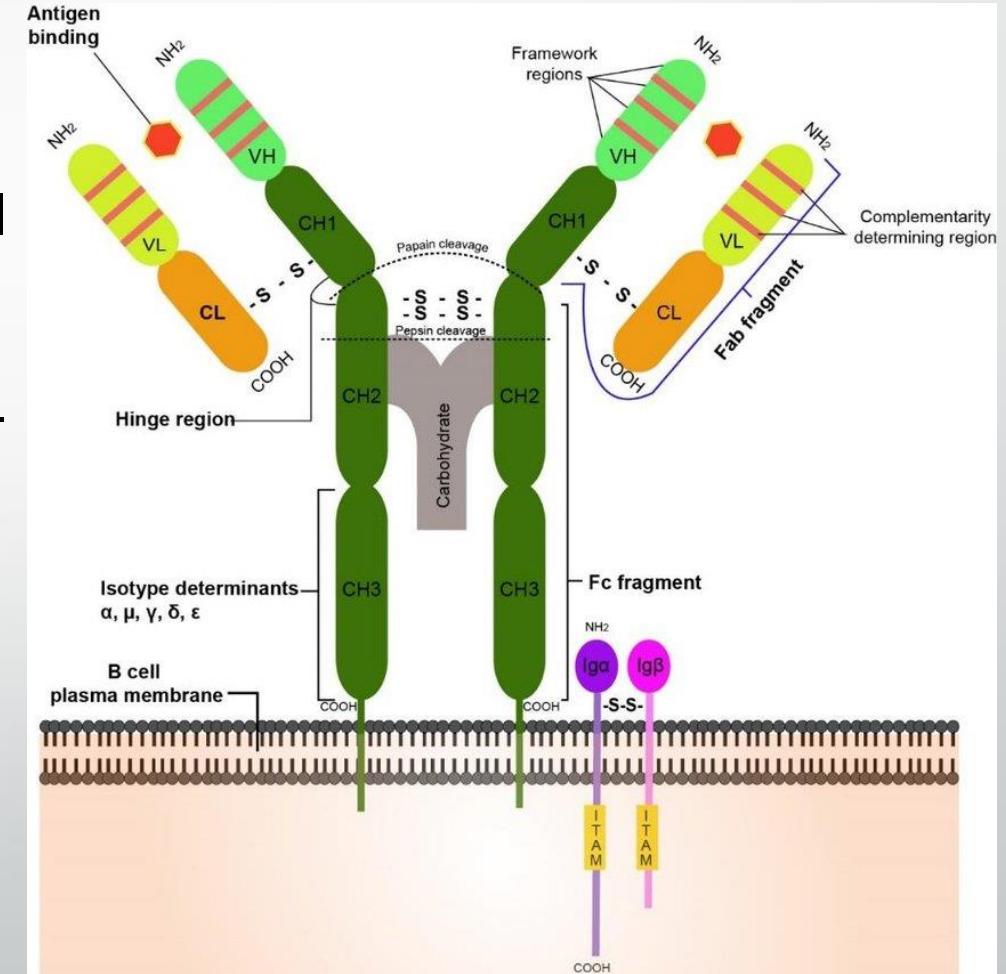
INTRODUCTION

- the body is defended by innate immune responses, but these will only work to control pathogens that have certain molecular patterns or that induce interferons and other secreted yet non-specific defences
- Most crucially, they do not allow memory to form as they operate by receptors that are coded in the genome.
- innate immunity is good for preventing pathogens from growing freely in the body, but it does not lead to the most important feature of adaptive immunity, which is long-lasting memory of specific pathogen.
- To recognize and fight the wide range of pathogens an individual will encounter, the lymphocytes of the adaptive immune system have evolved to recognize a great variety of different antigens from bacteria, viruses, and other disease-causing organisms

- A receptor is a general term used for a molecule that receives signals from its ligands i.e. molecules it binds to
- Receptors expressed on membranes have specific domains
 - Extracellular domain- specifically binds to ligands
 - Transmembrane – spans the plasma membrane
 - Cytoplasmic- participates in signal transduction
- The receptors themselves or molecules associated with them link the exterior of the cell to the interior
- In the duration of 60s-70s of last decade, the discovery of B cells and its participations on immune responses drew attention to the origin and functions of lymphocytes

B cell antigen receptor

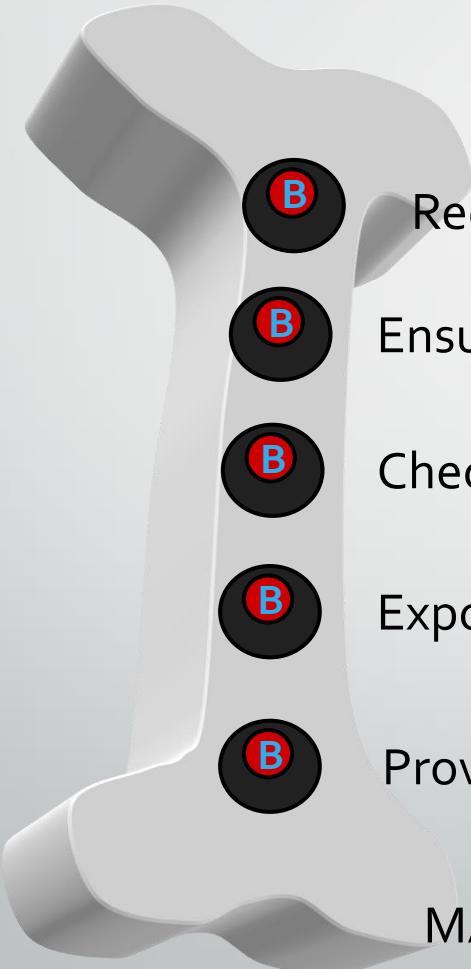
- The antigen-recognition molecules of B cells are the immunoglobulins, or Ig.
- These proteins are produced by B cells in a vast range of antigen specificities, each B cell producing immunoglobulin of a single specificity
- Membrane-bound immunoglobulin on the B-cell surface serves as the cell's receptor for antigen, and is known as the **BCR**.
- BCR consists of an antigen-binding transmembrane Ig (mlg) in complex with 2 transmembrane polypeptides viz. Ig α , Ig β containing tyrosine activation motifs (ITAMs) which enable transmission of intracellular signalling



Structure

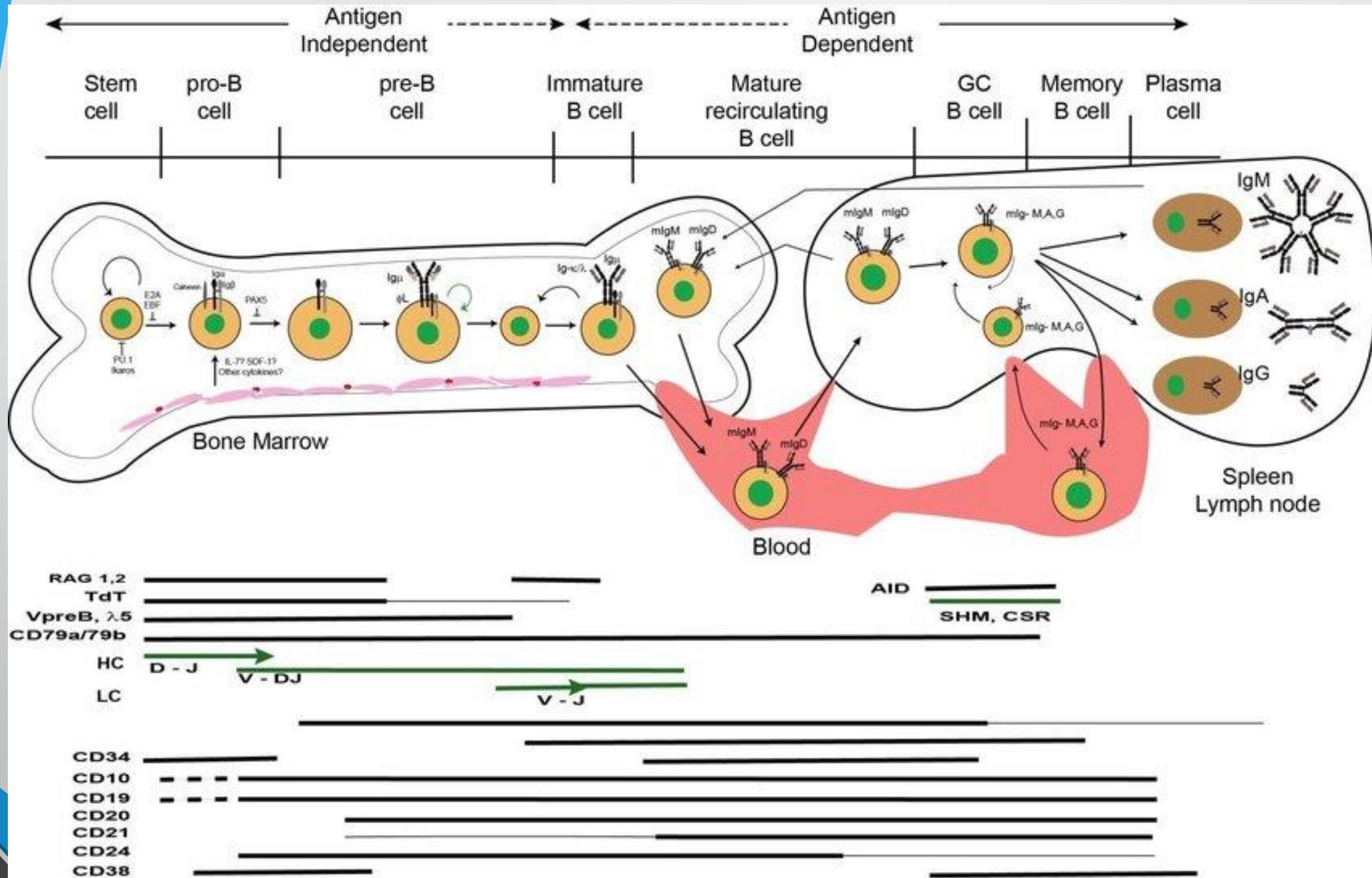
- The BCR is a transmembrane protein complex comprising multiple subunits including polypeptide chains of two identical Ig heavy chains (IgHC) having disulfide (-s-s-) bonds in between
- B cell receptors are made up of four peptides – two light chains and two heavy chains – that comprise two antigen-binding regions.
- Light chains are classified as either kappa or lambda, while the heavy chains can be IgG, IgA, IgM, IgD, or IgE isotypes.

B cell development in the bone marrow

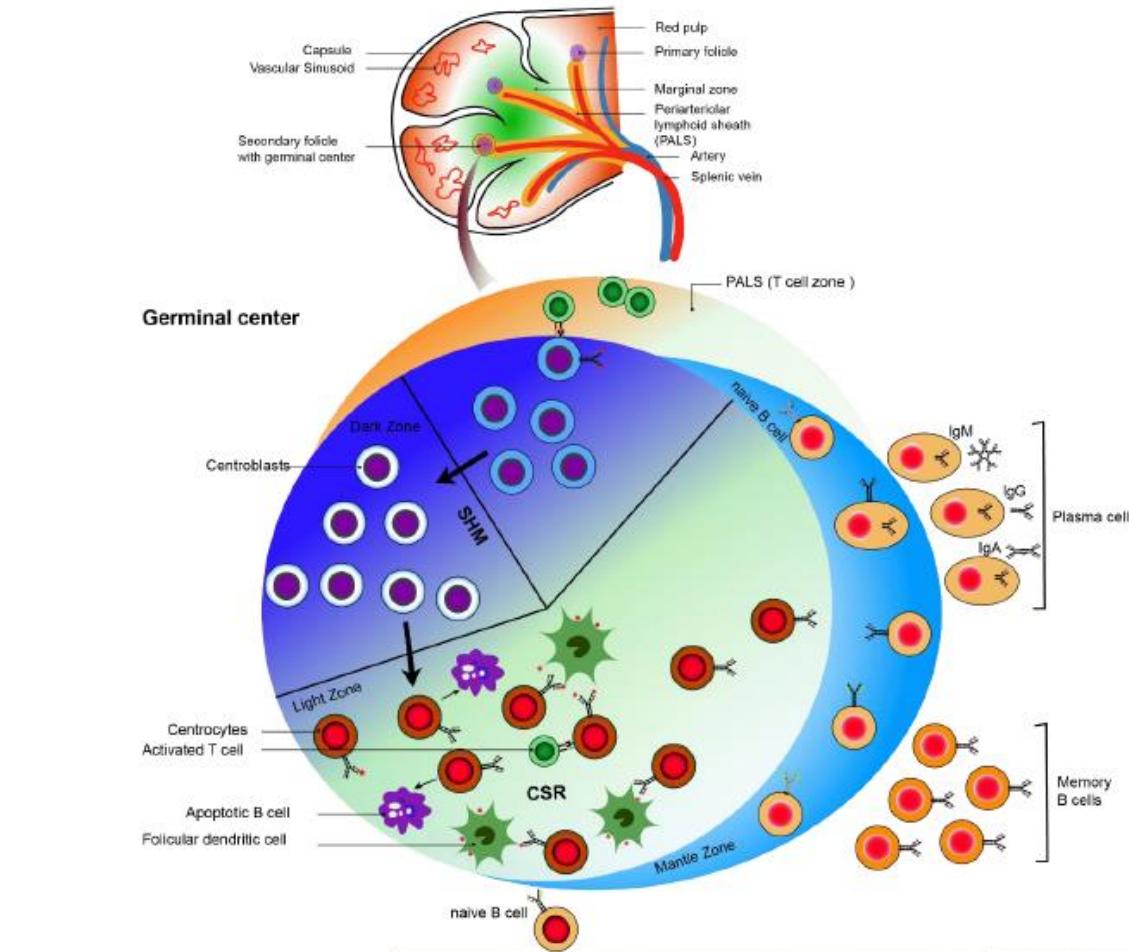


- Regulates construction of an antigen receptor
- Ensures each cell has only one specificity
- Checks and disposes of self-reactive B cells
- Exports useful cells to the periphery
- Provides a site for antibody production

Bone Marrow provides a
MATURATION & DIFFERENTIATION MICROENVIRONMENT
for B cell development



Spleen



Stages in B Cell Development

	stem cell	early pro-B cell	late pro-B cell	large pre-B cell	small pre-B cell	immature B cell	mature B cell
H chain genes	germline	D-J joining	V-DJ joining	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged
L chain genes	germline	germline	germline	germline	V-J joining	VJ rearranged	VJ rearranged
Surface Ig	none	none	none	μ chain in pre-B receptor	μ chain in cytoplasm and on surface	membrane IgM	membrane IgM and IgD
RAG, TdT expression	no	yes	yes	no	yes	yes	no
Surrogate L chain expression	no	yes	yes	yes	no	no	no
Ig $\alpha\beta$ expression	no	yes	yes	yes	yes	yes	yes
btk*	no	little	yes	yes	yes	yes	yes
Membrane markers	CD34	CD34 CD45 (B220) Class II	CD45R Class II CD19 CD40	CD45R Class II pre-B-R CD19 CD40	CD45R Class II pre-B-R CD19 CD40	CD45R Class II IgM CD19 CD40	CD45R Class II IgM IgD CD19 CD21 CD40

B CELL STAGE

Stem cell

Early pro-B

Late pro-B

Large pre-B

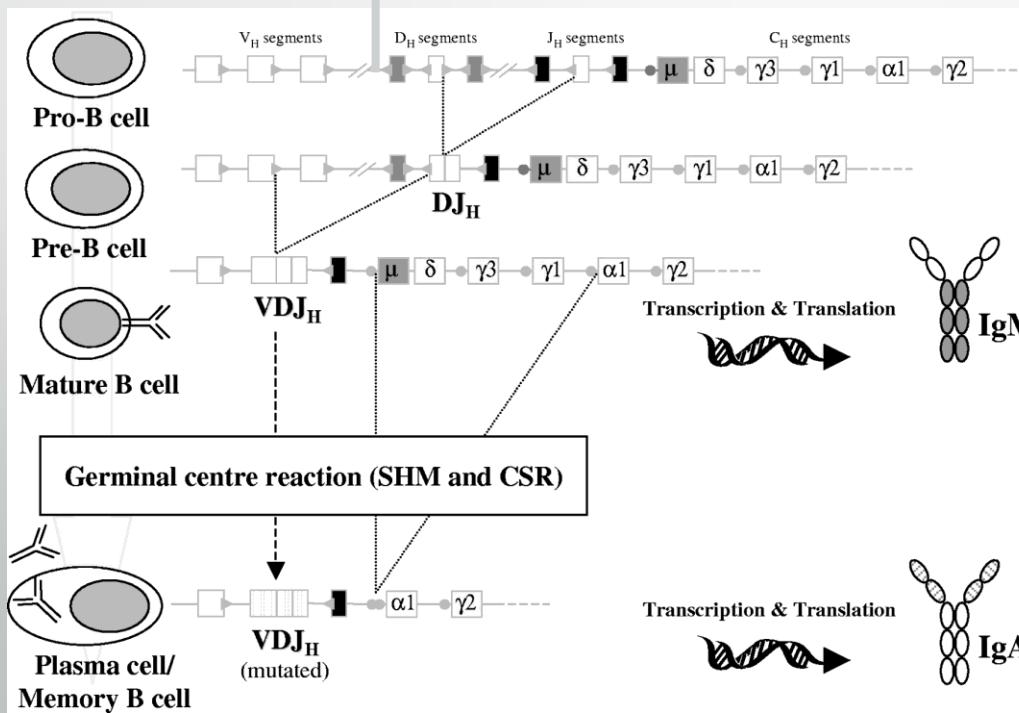
*IgH GENE
CONFIGURATION*

Germline

D_H to J_H

V_H to D_HJ_H

V_HD_HJ_H



Pre-B cell
receptor
expressed

Heavy and light chain rearrangement is potentially wasteful



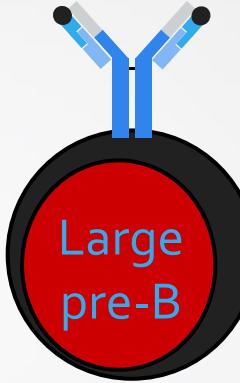
Germline



D_H-J_H joining



V_H-D_HJ_H joining



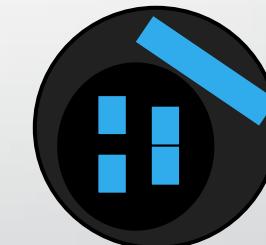
With two “random” joins to generate a heavy chain
there is a 1:9 chance of a rearrangement being in frame



Germline



V_L-J_L joining



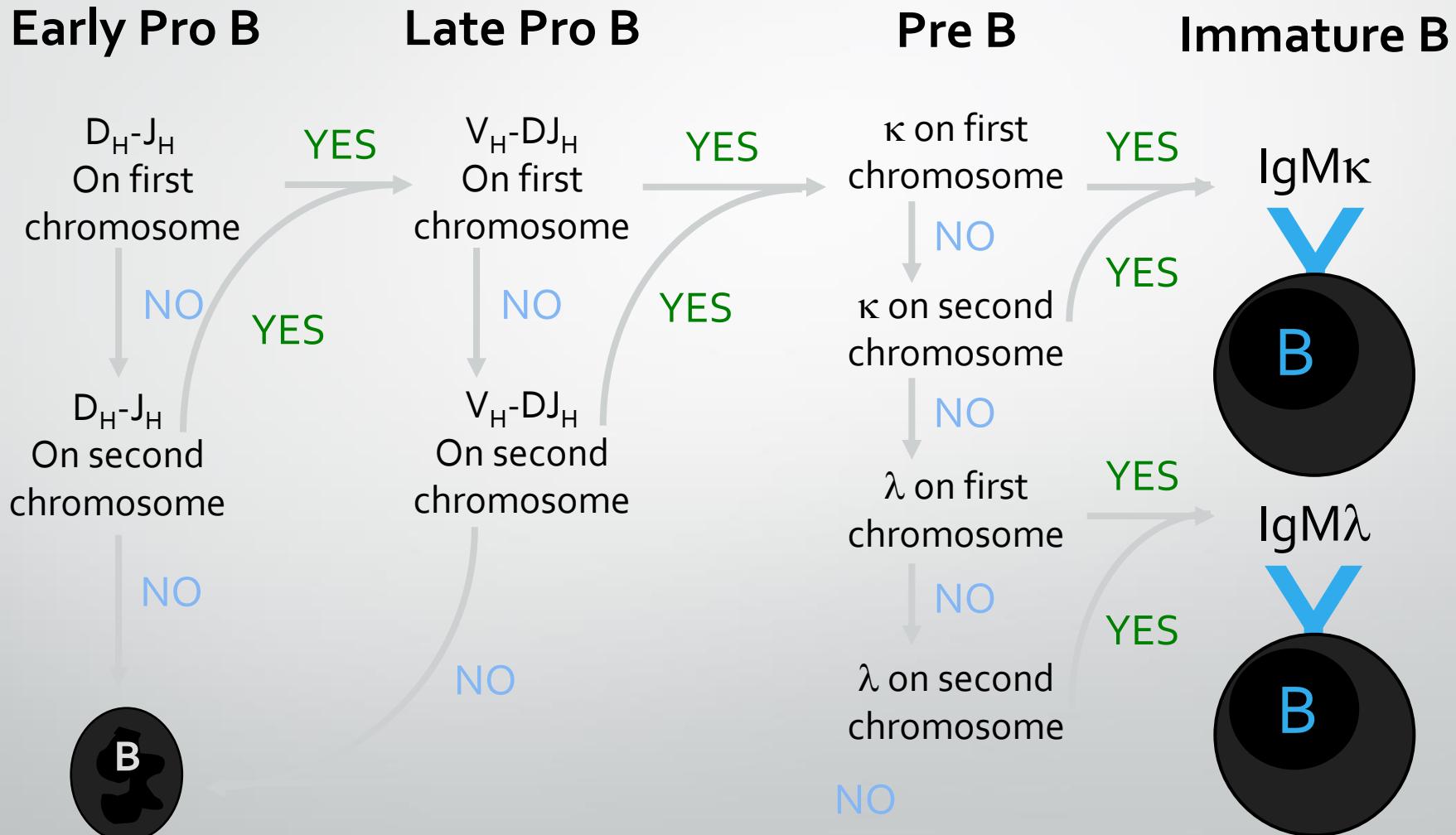
With one “random” join to generate a light chain
there is a 1:3 chance of a rearrangement being of frame

Small
pre-B

There is, therefore, only a 1:27 chance of an in frame rearrangement

Out of frame rearrangements arrest further B cell maturation

B cells have several chances to successfully rearrange Ig genes



Ig α and Ig β in B cell

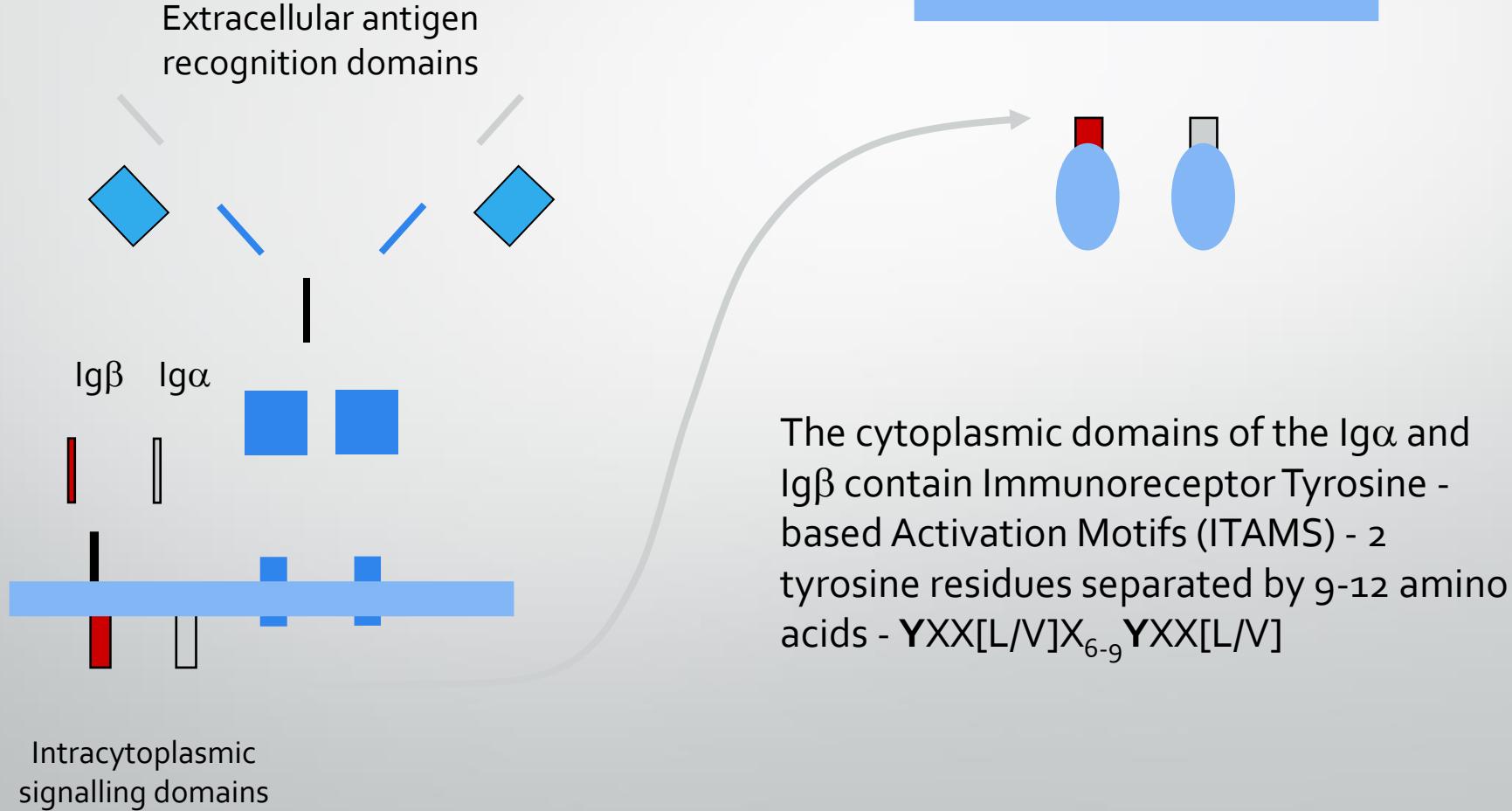
- For signal initiation, the BCR complex requires Ig α also naming CD79a (or MB-1 membrane glycoprotein) and Ig β also naming CD79b (or B-cell-specific glycoprotein B29)
- The Ig α /Ig β heterodimer contains a conserved aa sequence called immunoreceptor tyrosine-based activation motif (ITAM), in which any two aa separate a tyrosine from a leucine or isoleucine giving the signature YxxL/I
- Two of this signature are repeated in the Ig α /Ig β cytoplasmic domain separated by between 6 and 8 aa, giving the signature D/E(X)₇-D/E(X)₂Y(X)₂L/I(X)₆₋₈Y(X)₂L/I
- Here X represents any aa, D represents aspartic acid, E represents glutamic acid, Y represent tyrosine residues, L and I represent leucine and isoleucine residues

- After BCR stimulation, the tyrosine residues of the ITAM become phosphorylated, which results in recruitment and activation of protein tyrosine kinases (PTKs) like Lyn, Syk, Btk. The cooperative action of these PTKs lead to activation of a plethora of signaling pathways such as phospholipase C γ 2 (PLC γ 2) or phosphoinositide 3-kinase (PI3K)
- the Ig α ITAM tyrosines and Y204 non-ITAM tyrosine are required for efficient pre-B cell differentiation. Ig α is differentially N-glycosylated in IgM- and IgD- expressing cells, whereas membrane-proximal extracellular part of μ HC and δ HC determine the unique glycosylation pattern of the associated Ig α molecule

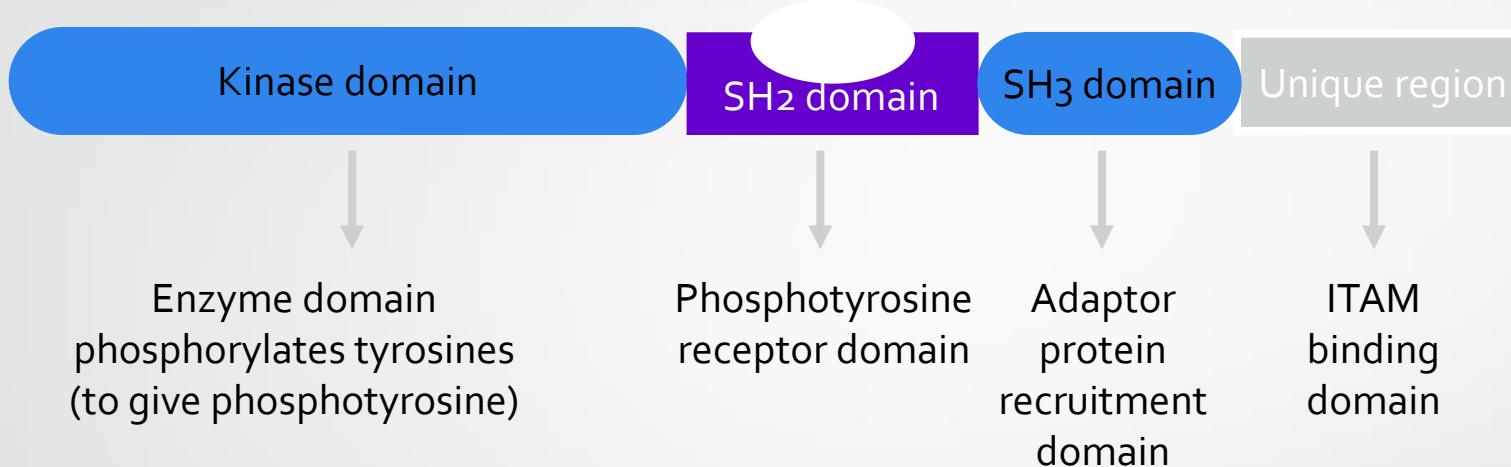
Signal transduction from B-cell receptors

- The resting pre-BCRs or BCRs transduce signals through a well-organized signaling complex consisting of several molecules
- B cells generate antigen-specific effector cells, recognize antigen and give response by engaging cell surface immunoreceptors
- BCRs can give either antigen-induced responses or ligand-independent (autonomous) responses and thereby influence fate decisions of B cells leading to
 - cell proliferation, differentiation, apoptosis and anergy
- BCRs also process antigen by internalization leading to the presentation of antigen derived peptides to T-helper cells

Transduction of signals by the B cell receptor

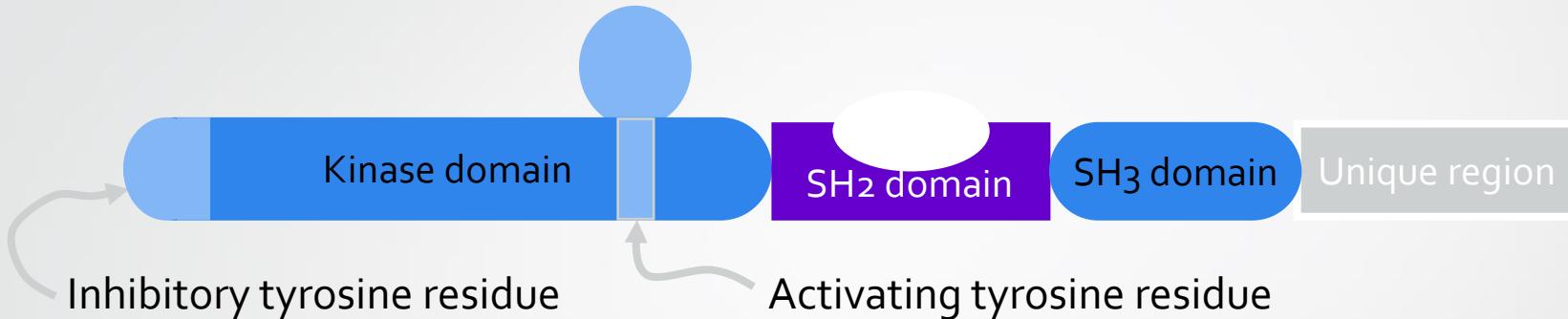


Phosphorylation by Src kinases

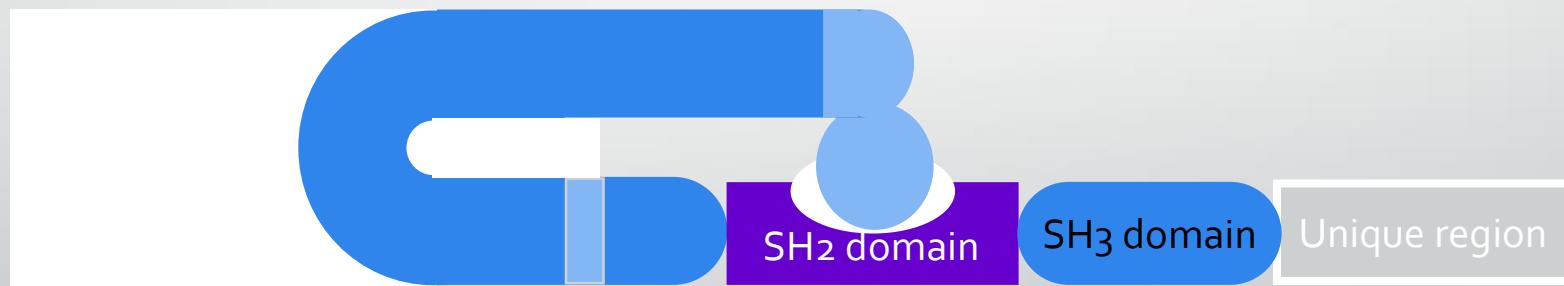


- Phosphorylation changes the properties of a protein by changing its conformation
- Changes in conformation may activate or inhibit a biochemical activity or create a binding site for other proteins
- Phosphorylation is rapid, requires no protein synthesis or degradation to change the biochemical activity of a target protein
- It is reversible via the action of phosphatases that remove phosphate

Regulation of Src kinases

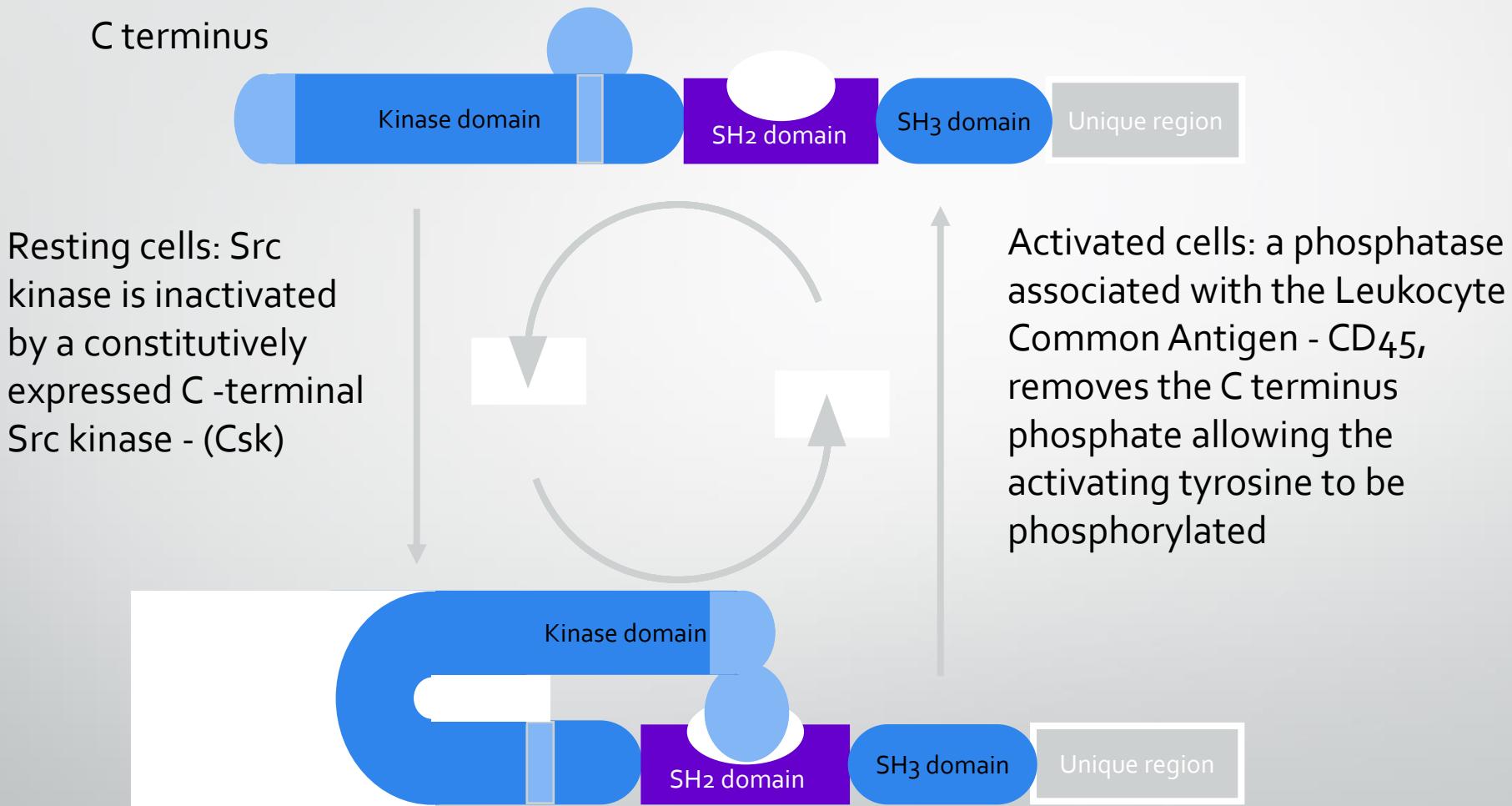


Phosphorylation of 'Activating Tyrosine' stimulates kinase activity



Phosphorylation of 'Inhibitory Tyrosine' inhibits kinase activity
by blocking access to the Activating Tyrosine Residue

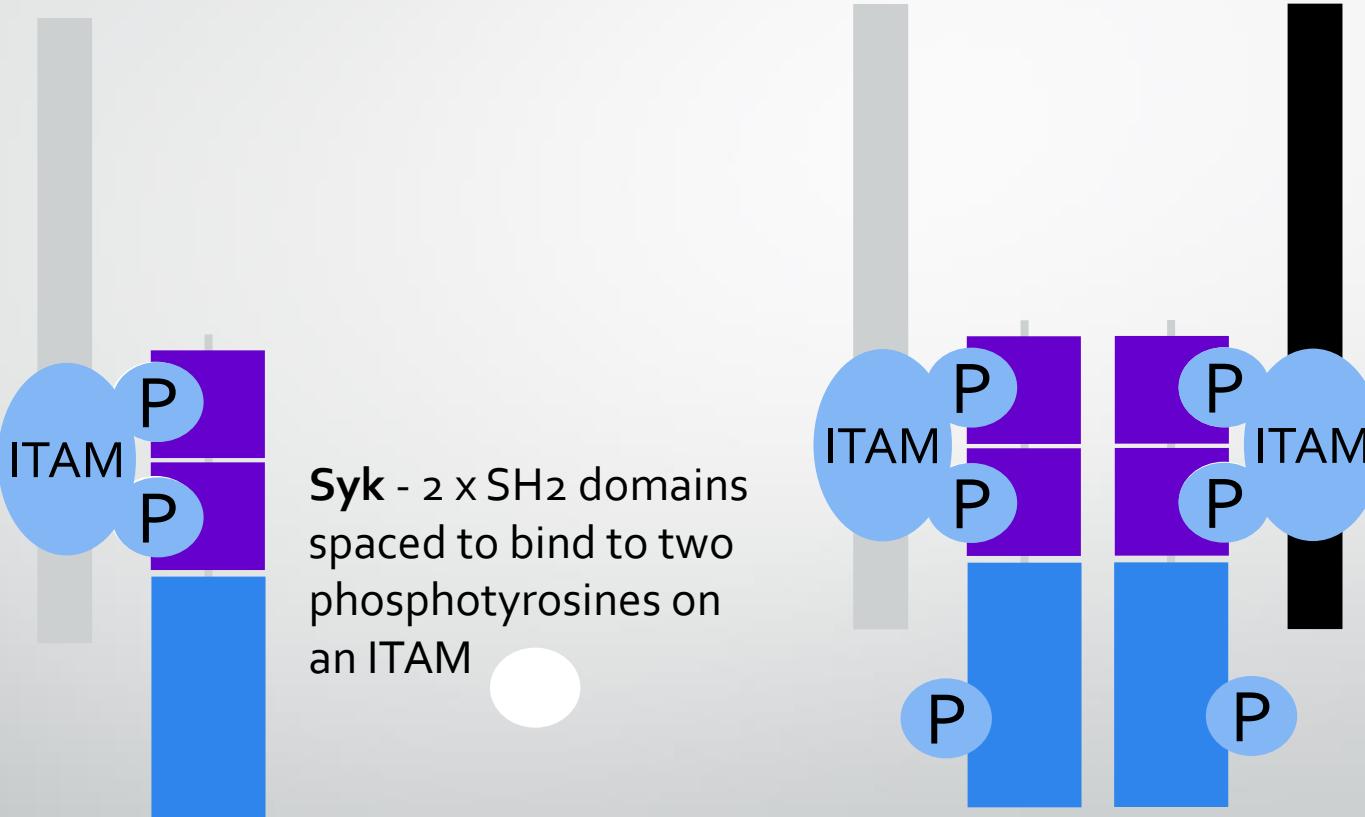
Regulation of Src kinases by Csk and CD45



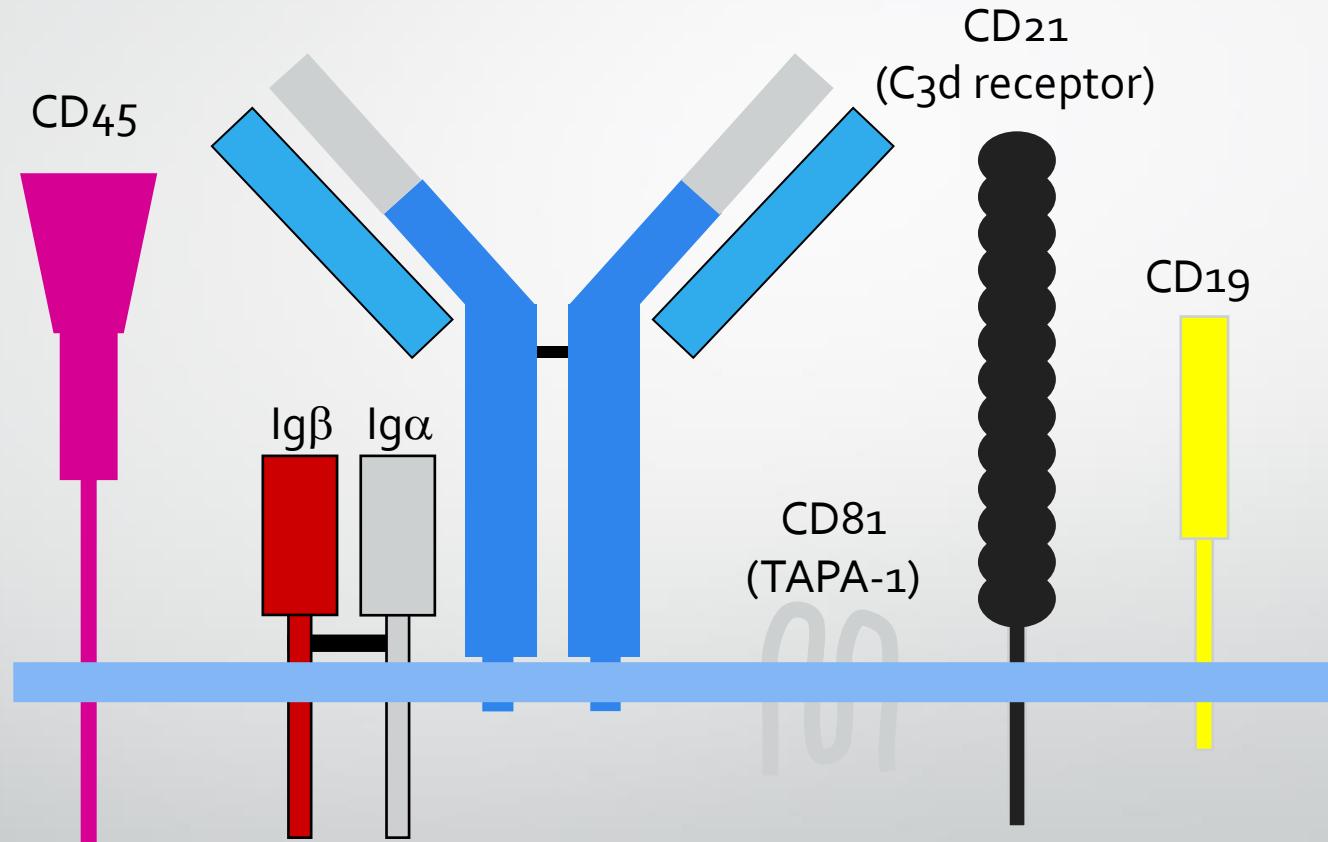
The balance between Csk and CD45 phosphatase activity sets the threshold for initiating receptor signalling

Syk protein Tyrosine kinases

- CD45 phosphatase allows activation of Src family kinases Blk, Fyn & Lyn
- Receptor cross-linking activates Src kinases that phosphorylate ITAMs in the Ig α and Ig β

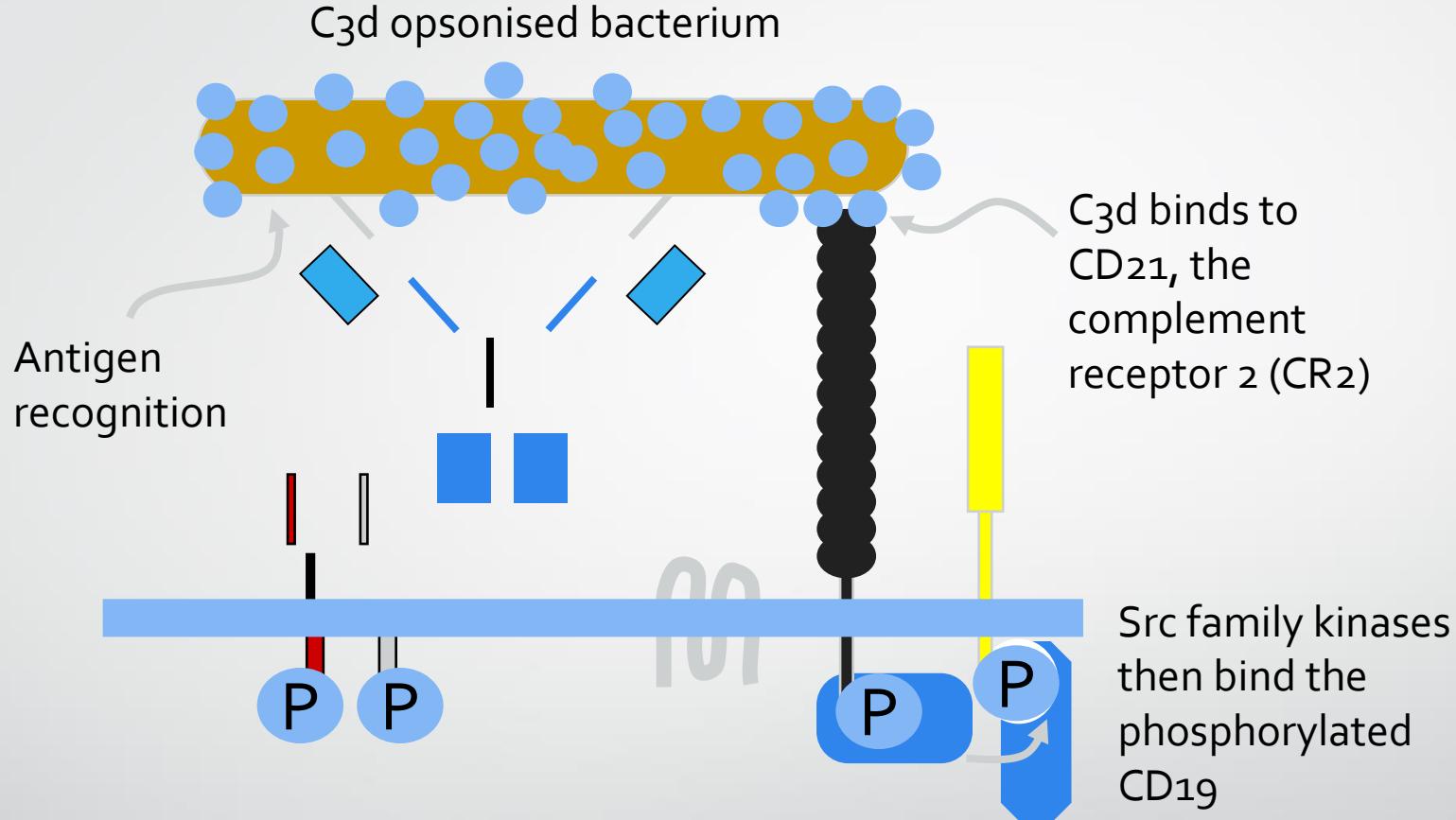


The B cell co-receptor



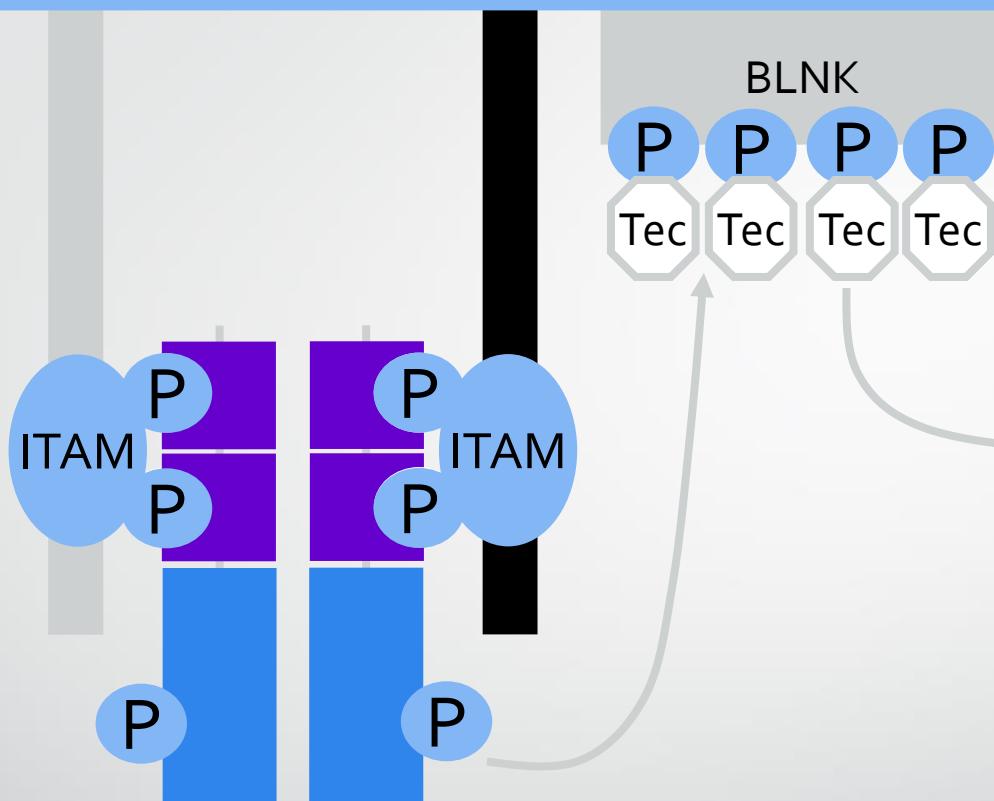
The B cell co-receptor

Co-receptor phosphorylation



- mlg and CD21 are cross-linked by antigen that has activated complement
- CD21 is phosphorylated and receptor-associated kinases phosphorylate CD19
- Phosphorylated CD19 activates more Src family kinases
- Ligation of the co-receptor increases B cell receptor signalling 1000 -10,000 fold

Activation of signals that affect gene transcription



Activated Syk phosphorylates BLNK

Activated Syk phosphorylates
Guanine-nucleotide exchange factors (GEFS)
that in turn activate small GTP binding
proteins Ras and Rac

Cell membrane-associated B cell
Linker protein - BLNK - contains
many Tyrosine residues

BLNK binds Tec kinases

Tec kinases activate Brutons tyrosine kinase (BTK)
phospholipase C- γ (PLC- γ)

PLC- γ cleaves
phosphatidylinositol
bisphosphate (PIP₂) to yield
diacylglycerol (**DAG**) and inositol
trisphosphate (**IP₃**)

Ras and Rac activate the **MAP
kinase** cascade

Transmission of signals from the cell surface to the nucleus

- B cell-specific parts of the signalling cascade are associated with receptors unique to B cells - mlg, CD19 etc.
- Subsequent signals that transmit signals to the nucleus are common to many different types of cell.
- The ultimate goal is to activate the transcription of genes, the products of which mediate host defence, proliferation, differentiation etc.

Once the B cell-specific parts of the cascade are complete, signalling to the nucleus continues via three common signalling pathways via:

1. *The mitogen-activated protein kinase (MAP kinase) pathway*
2. *Increased in intracellular Ca²⁺ mediated by IP₃*
3. *The activation of Protein Kinase C mediated by DAG*

Simplified scheme linking antigen recognition with transcription of B cell-specific genes

- **MAP Kinase cascade**

Small G-protein-activated MAP kinases found in all multicellular animals - activation of MAP kinases ultimately leads to phosphorylation of transcription factors from the AP-1 family such as **Fos** and **Jun**.

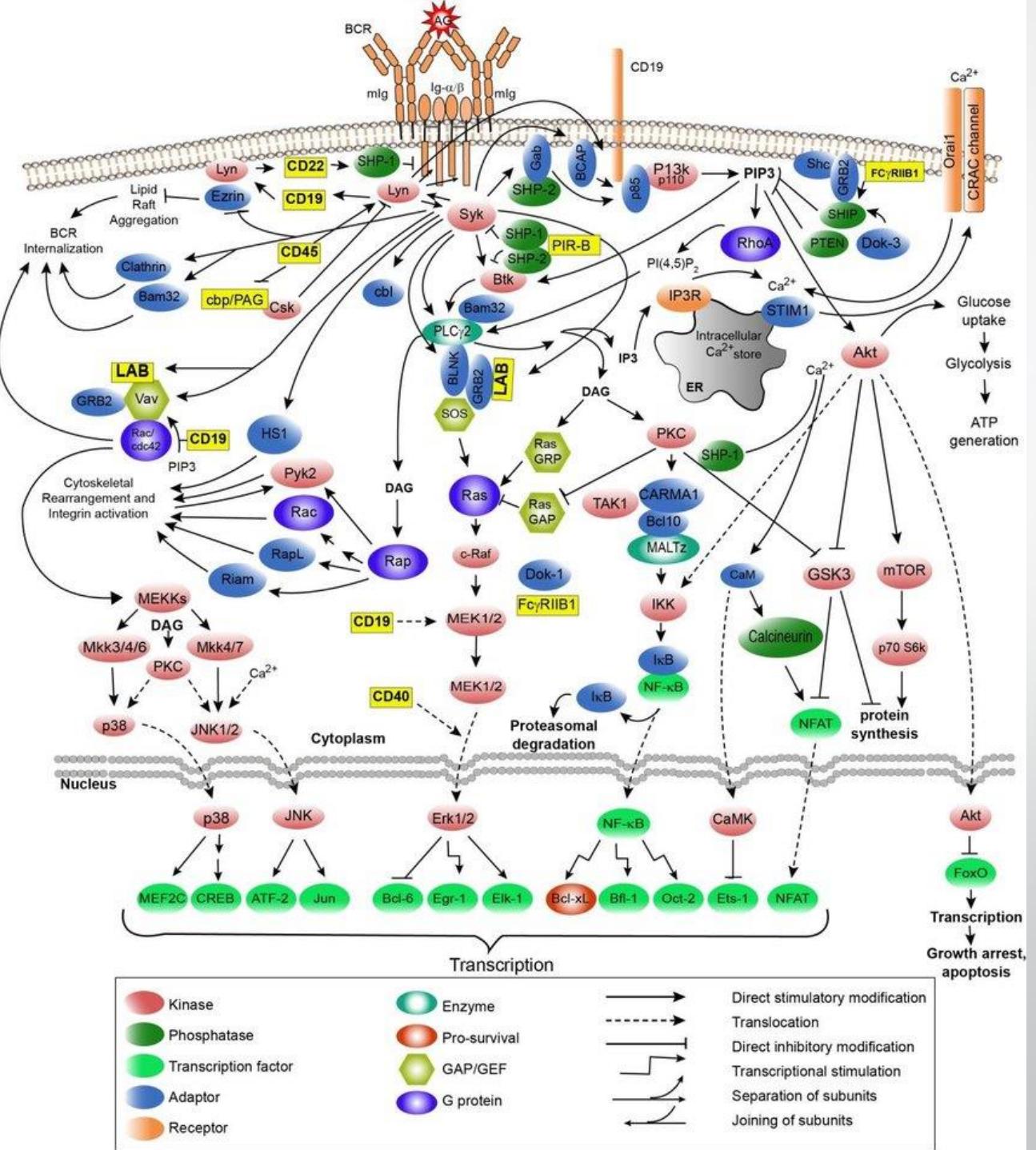
- **Increases in intracellular calcium via IP₃**

IP₃, produced by PLC- γ , binds to calcium channels in the ER and releases intracellular stores of Ca⁺⁺ into the cytosol. Increased intracellular [Ca⁺⁺] activate a phosphatase, calcineurin, which in turn activates the transcription factor **NFAT**.

- **Activation of Protein Kinase C family members via DAG**

DAG stays associated with the membrane and recruits protein kinase C family members. The PKC, serine/threonine protein kinases, ultimately activate the transcription factor **NF κ B**

The activated transcription factors AP-1, NFAT and NF κ B induce B cell proliferation, differentiation and effector mechanisms



Summary

- You should know:
- Where B cells come from
- What happens to B cells in the bone marrow
- How B cell differentiation is linked with Ig gene rearrangement
- The B cell developmental ‘check points’ that ensure each cell produces a single specificity of antibody that does not react with self
- How B cells transmit information from the shape and charge of an antigen through the cell membrane to allow the expression of genes in the nucleus

Membrane antigen receptors- TcR

Dr. (Ms.) Sonali Correa

Introduction

T cell is a type of lymphocyte

T cells are one of the important white blood cells

T cells are born from hematopoietic stem cells, found in the bone marrow

Developing T cells then migrate to the thymus gland to develop (or mature).

Precursor cells mature into several distinct types of T cells.

T cell differentiation also continues after they have left the thymus.

Groups of specific, differentiated T cell subtypes have a variety of important functions in controlling and shaping the immune response.

The T-cell receptor (TCR) is a protein complex found on the surface of T cells, or T lymphocytes, that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules.

T lymphocytes use co-receptors to bind to the MHC molecules. Co-receptors can be either CD4 or CD8.

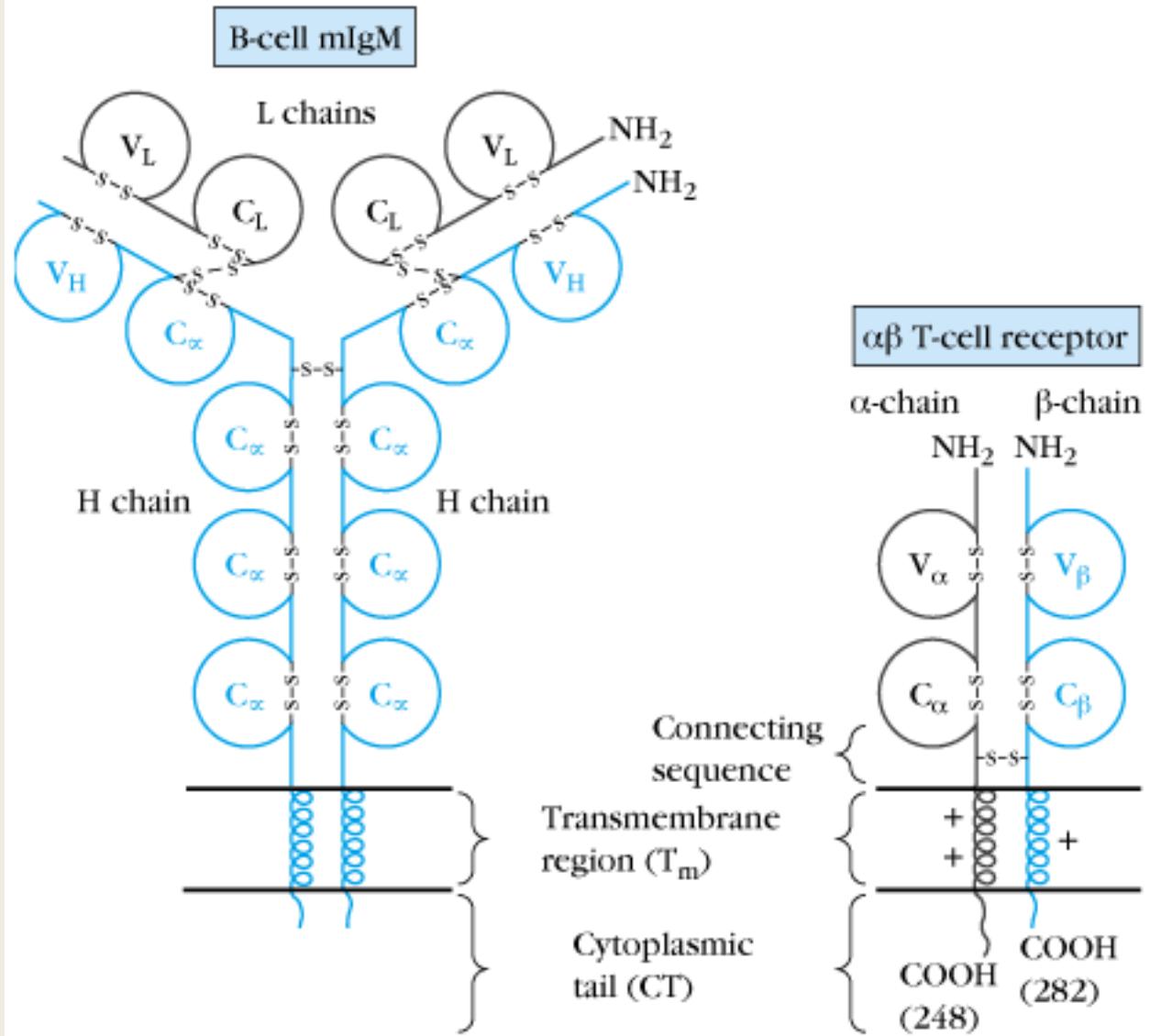
CD proteins help to differentiate major groups of effector T lymphocytes.

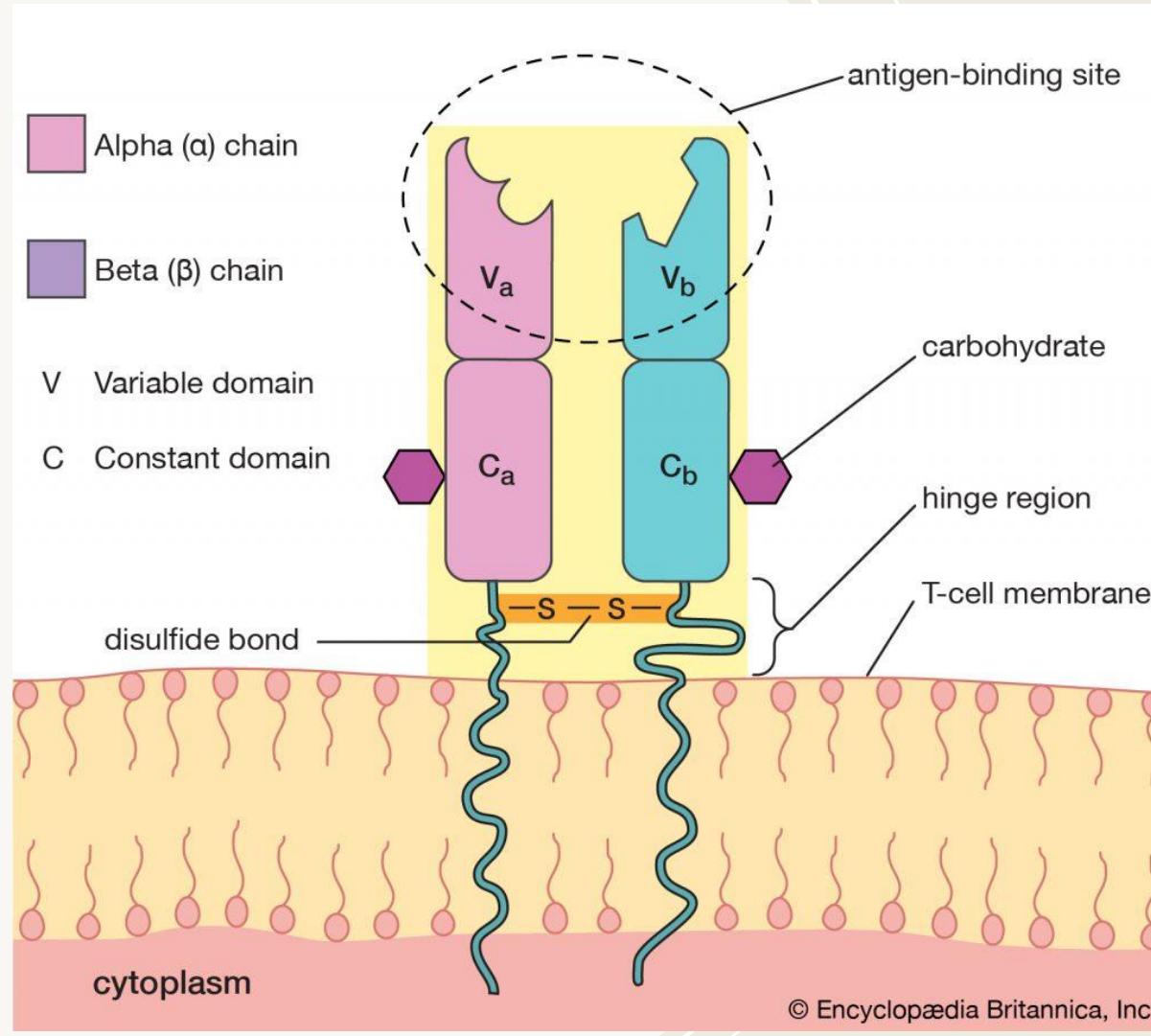
Naïve CD8+ T lymphocytes will become cytotoxic T lymphocytes

CD4+ T lymphocytes will become T helper lymphocytes

The binding between TCR and antigen peptides is of relatively low affinity and is degenerate: that is, many TCRs recognize the same antigen peptide and many antigen peptides are recognized by the same TCR

Structure of T-cell receptor (TCR)





The TCR is composed of two different protein chains (it is a heterodimer).

The domain structure of $\alpha\beta$ and $\gamma\delta$ TCR heterodimers are similar to those of Igs; thus they are classified as members of **immunoglobulin superfamily**.

It is a **disulfide-linked membrane-anchored** heterodimeric protein

In humans, in 95% of T cells the TCR consists of the **highly variable alpha (α) and beta (β) chains** expressed as part of a complex with the invariant CD3 chain molecules and are referred to as $\alpha:\beta$ (or $\alpha\beta$) T cells,

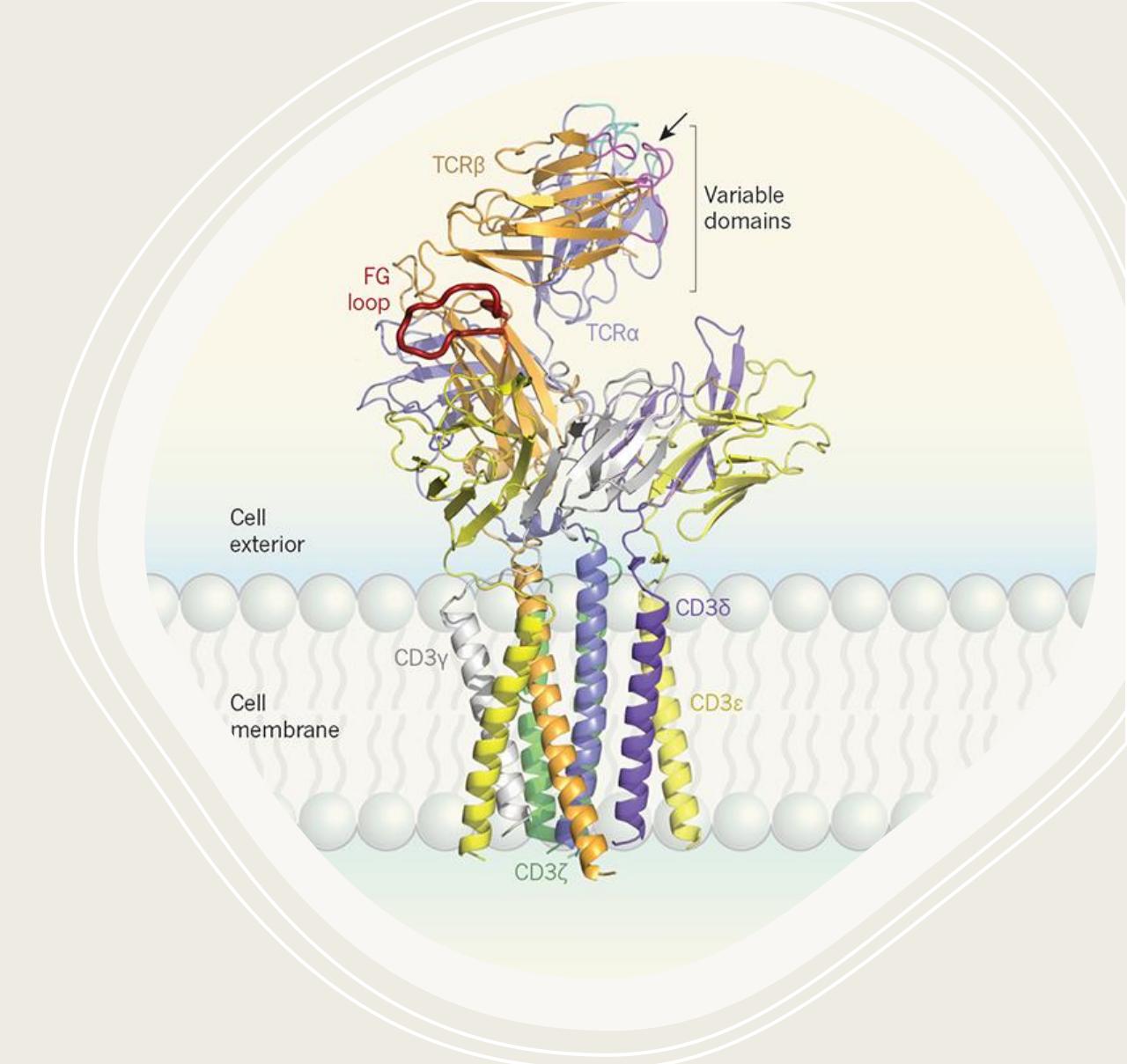
whereas in 5% of T cells, the TCR consists of **gamma and delta (γ/δ) chains** (encoded by TRG and TRD, respectively)

Variable (V) region and a Constant (C) region, both of Immunoglobulin superfamily (IgSF) domain **forming antiparallel β -sheets**.

The Constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the Variable region binds to the peptide/MHC complex.

The variable domain of both the TCR α -chain and β -chain each **have three hypervariable or CDRs**.

There is also an additional area of hypervariability on the β -chain (HV4) that does not normally contact antigen and, therefore, is not considered a CDR



Residues in these variable domains are located in two regions of the TCR, at the interface of the α - and β -chains and in the β -chain framework region that is thought to be in proximity to the CD3 signal-transduction complex.

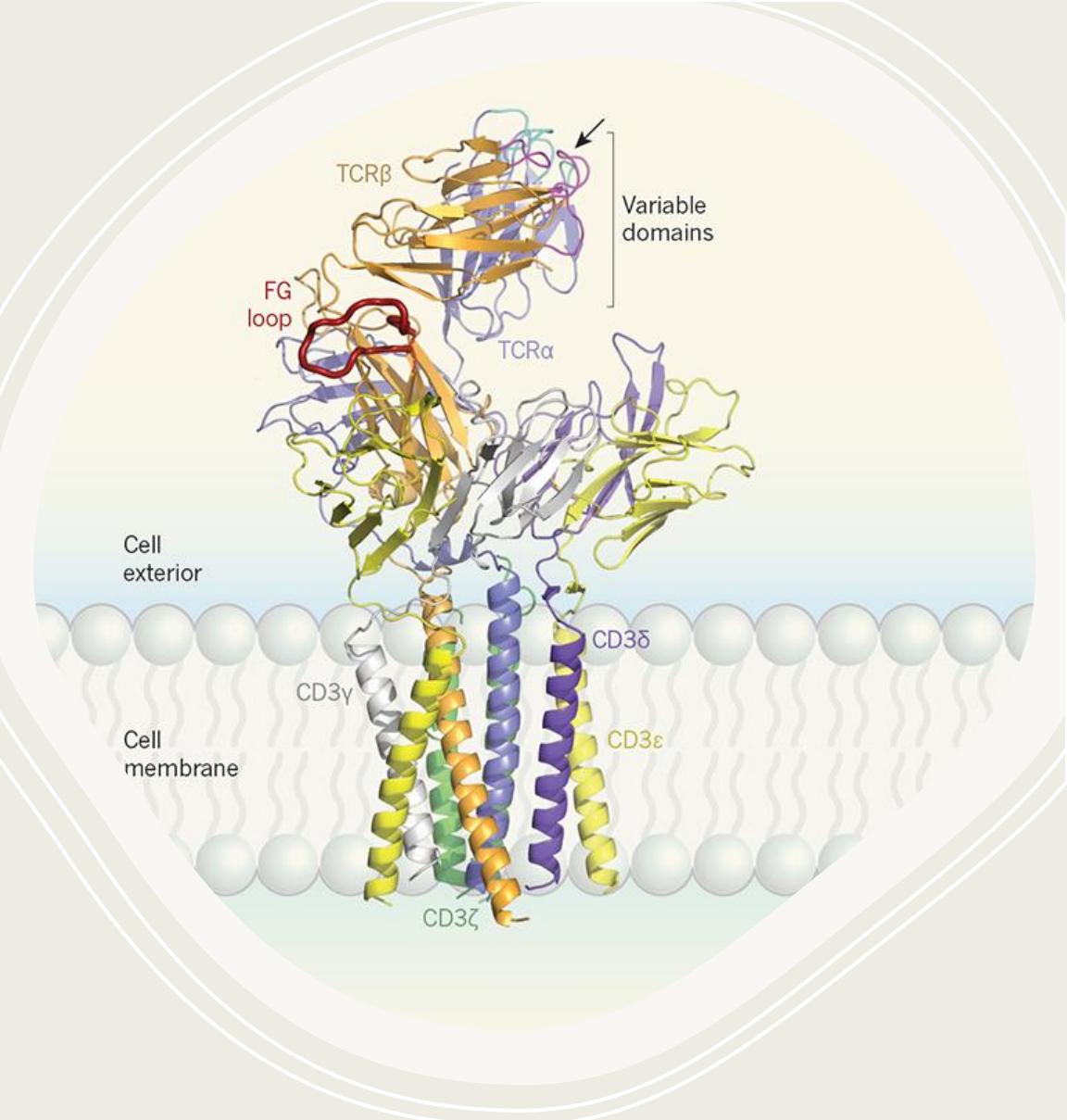
CDR3 is the main CDR responsible for recognizing processed antigen,

CDR1 of the alpha chain has also been shown to interact with the **N-terminal part of the antigenic peptide**

CDR1 of the β -chain interacts with the **C-terminal part of the peptide**

CDR2 is thought to **recognize the MHC**

CDR4 of the β -chain is not thought to participate in antigen recognition, but has been shown to **interact with superantigens**



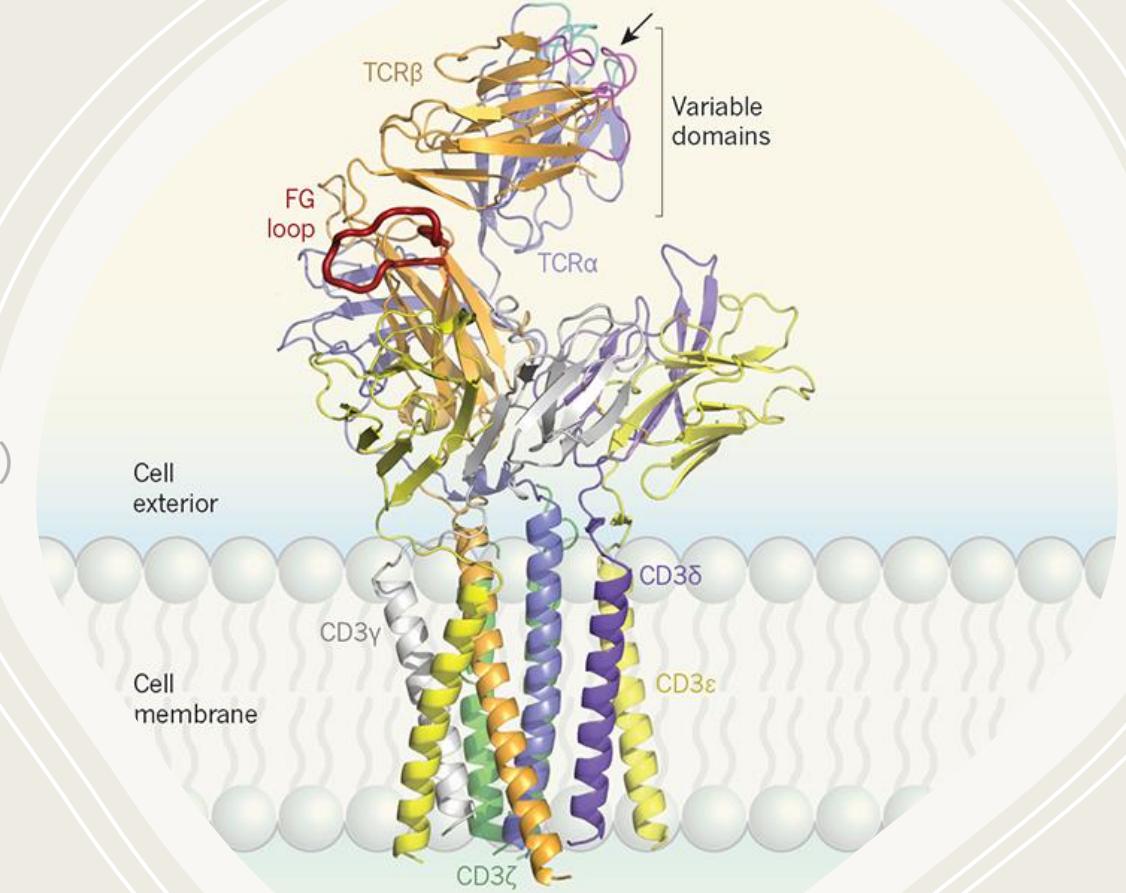
The constant domain of the TCR consists of short connecting sequences in which a cysteine residue forms disulfide bonds, which form a link between the two chains

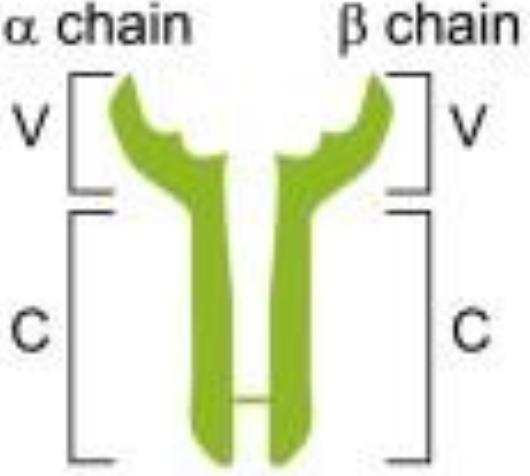
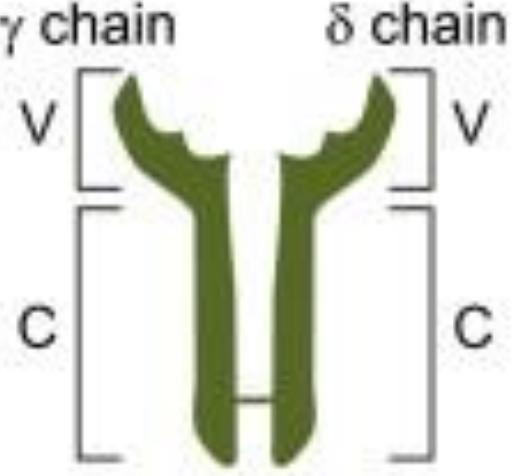
The TCR is similar to a half-antibody consisting of a single heavy and single light chain, except the heavy chain is without its crystallisable fraction (Fc) and the subunits are twisted together

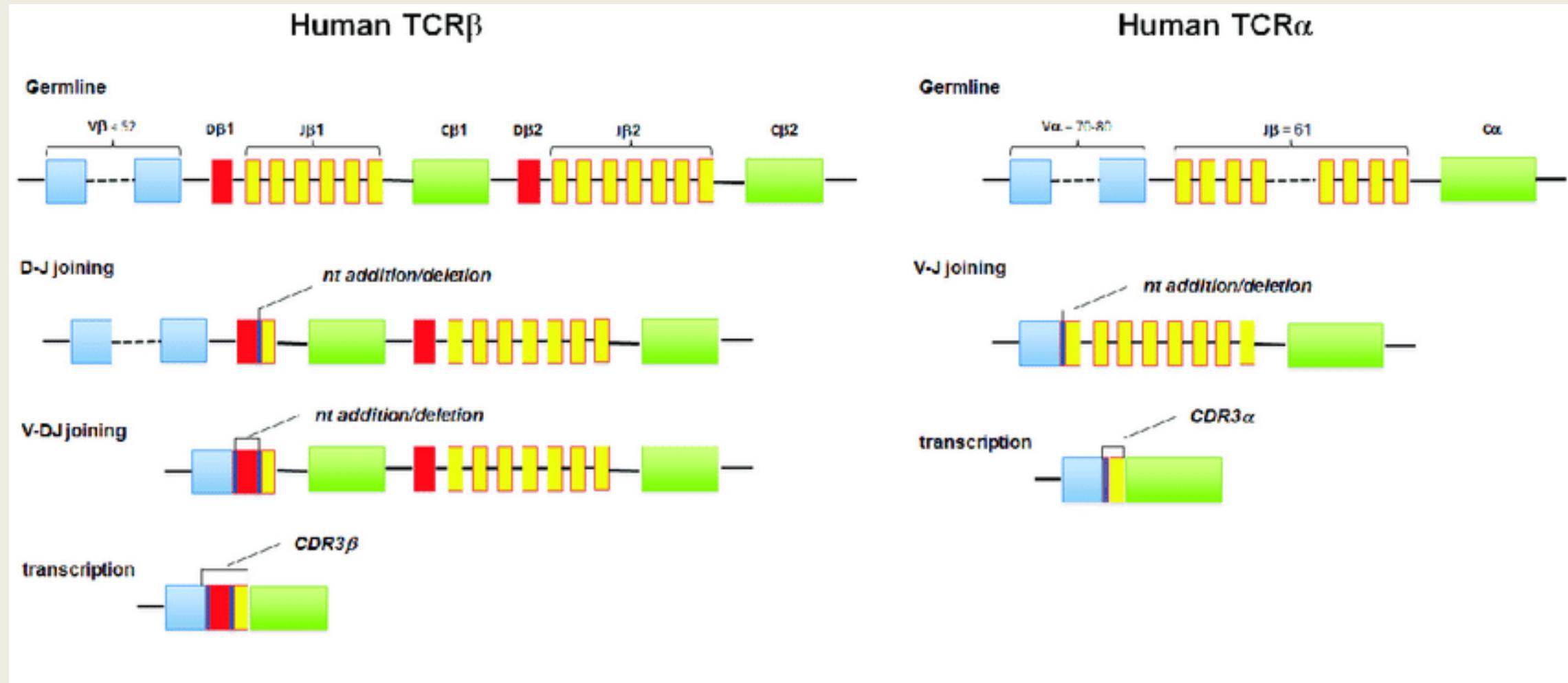
the antibody uses its Fc region to bind to Fc Receptors on leukocytes, TCR is already docked onto the cell membrane

it is not able to mediate signal transduction itself due to its short cytoplasmic tail, so TCR still requires CD3 and zeta to carry out the signal transduction in its place

MHC-TCR-CD3 interaction for T cells is functionally similar to the antigen(Ag)-immunoglobulin(Ig)-FcR interaction for myeloid leukocytes, and Ag-Ig-CD79 interaction for B cells.



	TCR$\alpha\beta$	TCR$\gamma\delta$
TCR icon		
% Mature T Cells in Humans	>90%	<10%
Tissue Distribution	Secondary lymphoid tissues	Intraepithelial tissues
Nature of Ligand	Peptide–MHC	Processed or unprocessed ligand



(a,b) The diversity of T-cell receptor (TCR) $\alpha\beta$ is a result of genetic recombination and diversification mechanisms occurring at the α and β TCR chain loci. Diversity is first created in the germline via recombination of variable V, diversity D (for β chain), and joining J segments. Further diversification occurs through imprecise junctions of these gene segments (addition of P-and N-nucleotides adjacent to the D segment), and the combination of α and β chains.

The generation of TCR diversity is similar to that for antibodies and B-cell antigen receptors.

It arises mainly from genetic recombination of the DNA-encoded segments in individual somatic T cells by somatic V(D)J recombination using RAG1 and RAG2 recombinases.

TCR genes do not undergo somatic hypermutation, and T cells do not express activation-induced cytidine deaminase(AID).

The recombination process that creates diversity in BCR (antibodies) and TCR is unique to lymphocytes (T and B cells) during the early stages of their development in primary lymphoid organs (thymus for T cells, bone marrow for B cells).

Each recombined TCR possess unique antigen specificity, determined by the structure of the antigen-binding site formed by the α and β chains in case of $\alpha\beta$ T cells or γ and δ chains on case of $\gamma\delta$ T cells

The TCR alpha chain is generated by VJ recombination, whereas the beta chain is generated by VDJ recombination (both involving a random joining of gene segments to generate the complete TCR chain

generation of the TCR gamma chain involves VJ recombination, whereas generation of the TCR delta chain occurs by VDJ recombination.

Intersection of these specific regions (V and J for the alpha or gamma chain; V, D, and J for the beta or delta chain) corresponds to the CDR3 region that is important for peptide/MHC recognition

It is the unique combination of the segments at this region, along with palindromic and random nucleotide additions (respectively termed "P-" and "N-"), which accounts for the even greater diversity of T-cell receptor specificity for processed antigenic peptides.

individual CDR loops of TCR can be re-edited in the periphery outside thymus by reactivation of recombinases using a process termed TCR revision (editing) and change its antigenic specificity.

TCR complex

In the plasma membrane the TCR receptor chains α and β associate with six additional **adaptor proteins to form an octameric complex**

The complex contains

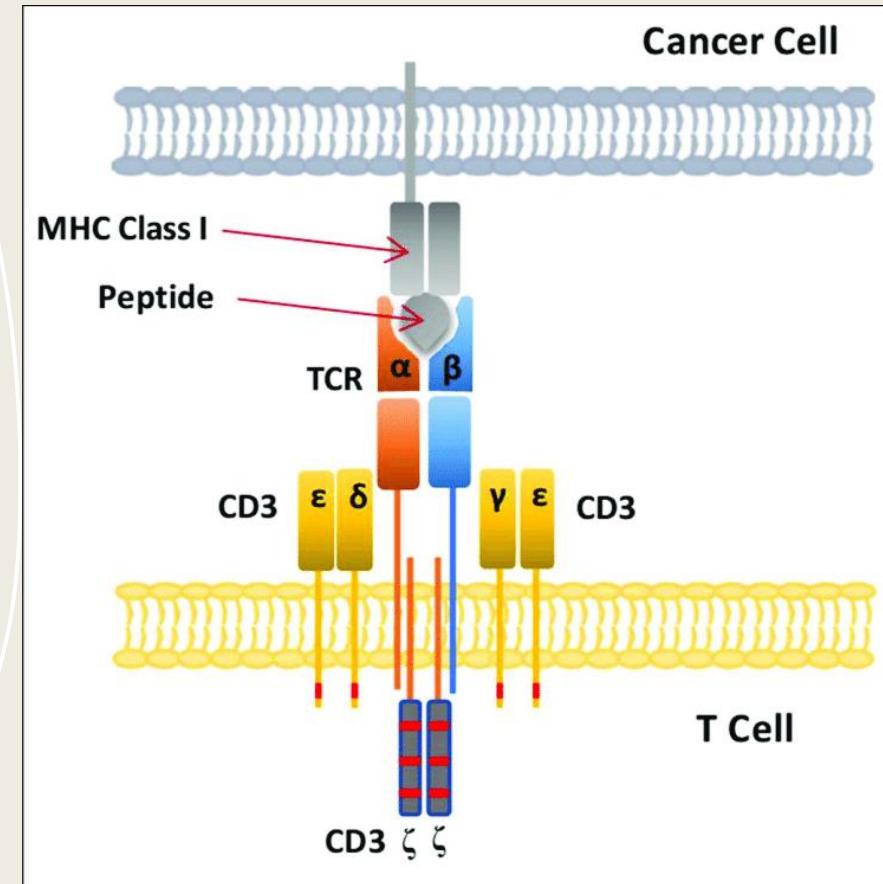
both **α and β chains**

forming the ligand-binding site

the signalling modules CD3 δ , CD3 γ , CD3 ϵ and CD3 ζ in the stoichiometry TCR α β - CD3 $\epsilon\gamma$ - CD3 $\epsilon\delta$ - CD3 $\zeta\zeta$

Charged residues in the transmembrane domain of each subunit form polar interactions allowing a **correct and stable assembly of the complex**

The cytoplasmic tail of the TCR is **extremely short**, hence the CD3 adaptor proteins contain the signalling motifs needed for propagating the signal from the triggered TCR into the cell



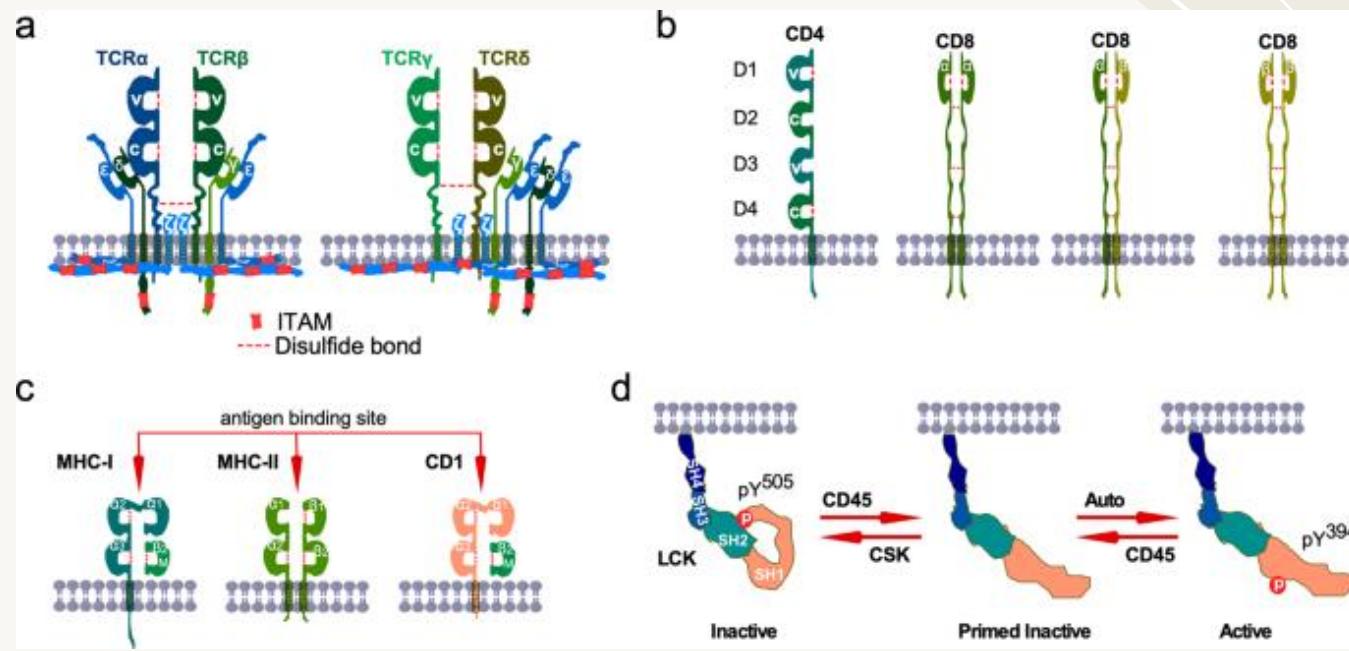
The signalling motifs involved in TCR signalling are tyrosine residues in the cytoplasmic tail of these adaptor proteins that can be phosphorylated in the event of **TCR-pMHC binding**

The tyrosine residues reside in a specific amino acid sequence of the signature $Y_{xx}(L/I)_{x6-8}Y_{xx}(L/I)$, where Y, L, I indicate tyrosine, leucine and isoleucine residues, x denotes any amino acids, the subscript 6-8 indicates a sequence of 6 to 8 amino acids in length

This motif is very common in **activator receptors of the non-catalytic tyrosine-phosphorylated receptor (NTR) family and is referred to as immunoreceptor tyrosine-based activation motif (ITAM)**

CD3 δ , CD3 γ and CD3 ϵ each contain a single ITAM, while CD3 ζ contains three ITAMs.
In total the TCR complex contains 10 ITAMs

Phosphorylated ITAMs act as binding site for **SH2-domains** of additionally recruited proteins.



TCR components

a TCR α /TCR β and TCR γ /TCR δ heterodimers form complexes with the CD3 molecules. Heterodimers of CD3 ϵ /CD3 δ and CD3 γ /CD3 ϵ , and a homodimer of CD3 ζ /CD3 ζ form complexes with TCR dimers. TCR heterodimers contain intramolecular and intermolecular disulfide bonds. CD3 chains contain 10 ITAMs distributed in different CD3 molecules. The variable region (V) of TCR heterodimers recognize the antigen peptide-loaded on MHC (pMHC). In the absence of pMHC, the intracellular part of the CD3 molecules forms a close conformation in which ITAMs are inaccessible to the kinases for phosphorylation.

b Coreceptor CD4 acts as a single molecule while CD8 α and CD8 β can form homodimers or heterodimers.

c MHC-I consists of an α -chain containing three immunoglobulin domains ($\alpha_1, \alpha_2, \alpha_3$) and $\beta 2$ -microglobulin ($\beta 2m$). MHC-II is the heterodimer of an α chain and a β chain containing two immunoglobulin domains (α_1, α_2 , and β_1, β_2) in each chain.

d LCK-loaded CD4 molecules bind to the MHC-II bound TCR (TCR α /TCR β) complex. This allows LCK to phosphorylate two distinct sites on ITAMs. Then ZAP-70 interacts with the phosphotyrosine sites and mediates more tyrosine phosphorylation. CD4 and MHC-II interaction is mediated through the membrane-proximal α_2 and $\beta 2$ domains of MHC-II and the membrane-distal D1 domain of CD4.

Role of TCR-CD3 complex in antigen recognition

TCR-CD3 complex majorly recognizes the Ag-MHC molecules and various other membrane molecule play important role in Ag recognition and T-cell activation.

Some of these membrane molecules strengthen interaction between T cell and APC or target cells and some act in signal transduction and some do both.

CD4 and CD8 co-receptors binds to conserved region of MHC-II (α_2 , β_2) or MHC-I (α_3 , β_2 micro-globulin) molecules.

CD4 is a 55KDa monomeric membrane glycoprotein that contains 4 extracellular immunoglobulin like domains (D1-D4), a hydrophobic trans-membrane region and a long cytoplasmic tail containing three serine residue that can be phosphorylated.

CD8 generally takes the form of a disulfide linked $\alpha\beta$ heterodimer or $\alpha\alpha$ homodimer. Both α and β chain of CD8 are small glycoprotein of approximately 30 to 38 KDa. Each chain consists of a single extracellular immunoglobulin like domains a stalk region, a hydrophobic transmembrane are region and a cytoplasmic tail containing 25-27 residues several of which can be phosphorylated.

Role in Antigen presentation

These TCR receptors encoded by $\alpha\beta$ genes interact with peptide Ag processed and presented on antigen presenting cells (APCs).

The remaining TCR were found to be $\gamma\delta$ which interacts with non-peptide antigen presented by the products of CD1 family of genes.

Role of TCR in signal transduction however TCR itself cannot transduce the signal. For signal transduction TCR associates with CD3 molecule forming TCR-CD3 complex.

The CD3 molecules participate in signal transduction after interaction of T-cell (TCR) with antigens. However CD3 does not influence interaction of antigen.

CD3 is a complex of five invariant polypeptide chains that associate to form three dimers; a heterodimer of gamma and epsilon ($\gamma\epsilon$), a heterodimer of delta and epsilon chain ($\delta\epsilon$) and a homodimer of two zeta chains ($\zeta\zeta$) or heterodimer of zeta and eta chain ($\zeta\eta$).

The ζ and η chains are encoded by same gene but differ in their carboxyl terminal ends because of difference in RNA splicing of the primary transcript.

About 90% of the CD3 complexes examined to date incorporate the ($\zeta\zeta$) homodimer; the remainder have the ($\zeta\eta$) heterodimer.¹³

Antigen discrimination

Each T cell expresses clonal TCRs which recognize a specific peptide loaded on a MHC molecule (pMHC), either on MHC class II on the surface of antigen-presenting cells or MHC class I on any other cell type

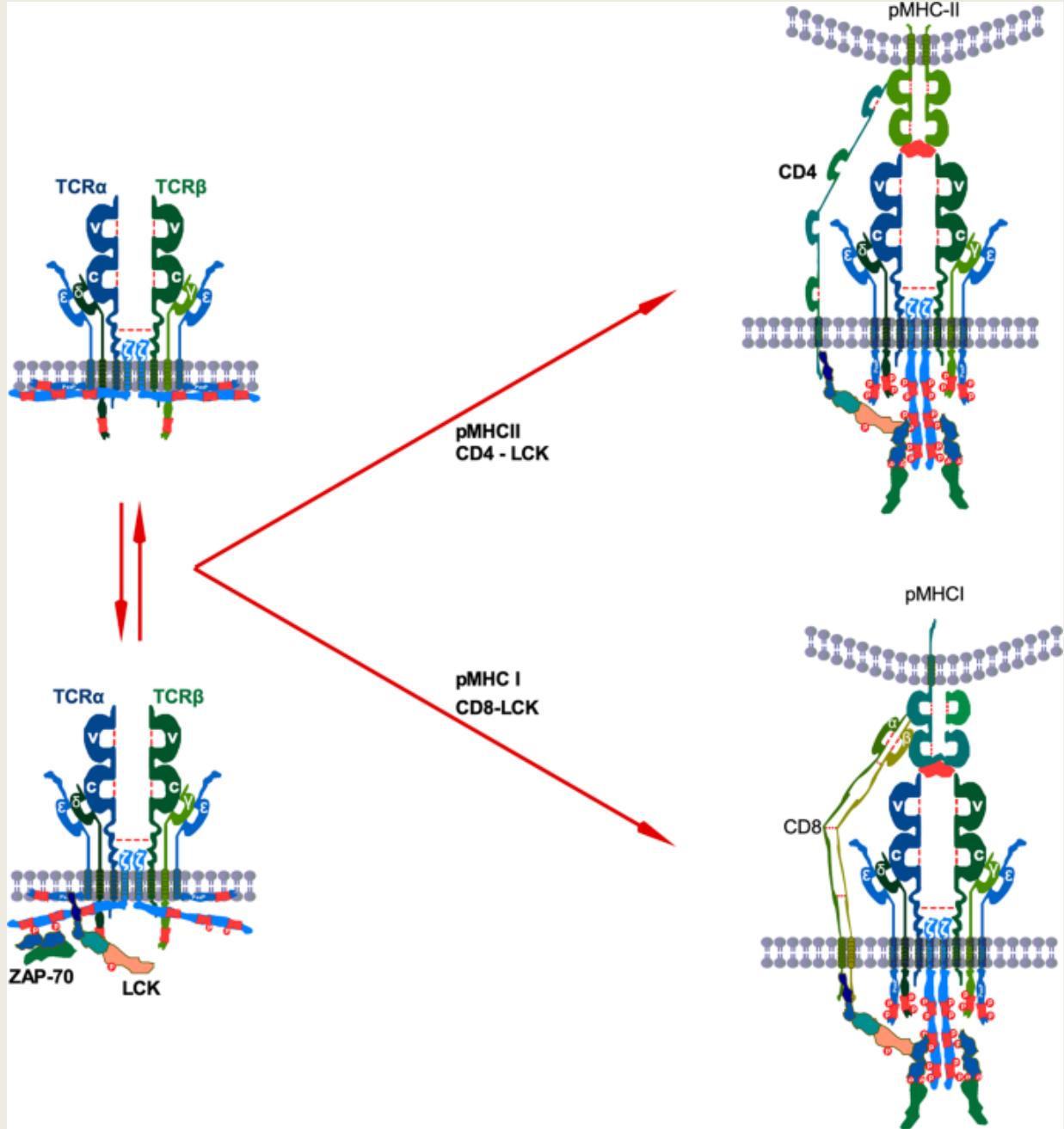
A unique feature of T cells is their ability to discriminate between peptides derived from **healthy, endogenous cells and peptides from foreign or abnormal** (e.g. infected or cancerous) cells in the body

the T-cell receptor signalling should not be activated by self-pMHC so that endogenous, healthy cells are ignored by T cells

The ability of T cells to ignore healthy cells but respond when these same cells express a small number of foreign pMHCs is known **as antigen discrimination**

T cells have a very high degree of antigen specificity, despite the fact that the affinity to the peptide/MHC ligand is rather low in comparison to other receptor types

TCR Activation & Signalling



TCR activation & Signaling pathway

The essential function of the TCR complex is to identify specific bound antigen derived from a potentially harmful pathogen and elicit a distinct and critical response.

The signal transduction mechanism by which a T cell elicits this response upon contact with its unique antigen is termed T-cell activation

Upon binding to pMHC, the TCR initiates a signalling cascade, involving transcription factor activation and cytoskeletal remodelling resulting in T cell activation

Active T cells secrete cytokines, undergo rapid proliferation, have cytotoxic activity and differentiate into **effector and memory cells**.

When the TCR is triggered, T cells form an immunological synapse allowing them to stay in contact with the antigen presenting cell for several hours

T cell activation depends on the strength of **TCR stimulation, the dose-response curve of ligand to cytokine production is sigmoidal**

Signal 1

- is provided by the T-cell receptor when recognising a specific antigen on a MHC molecule.

Signal 2

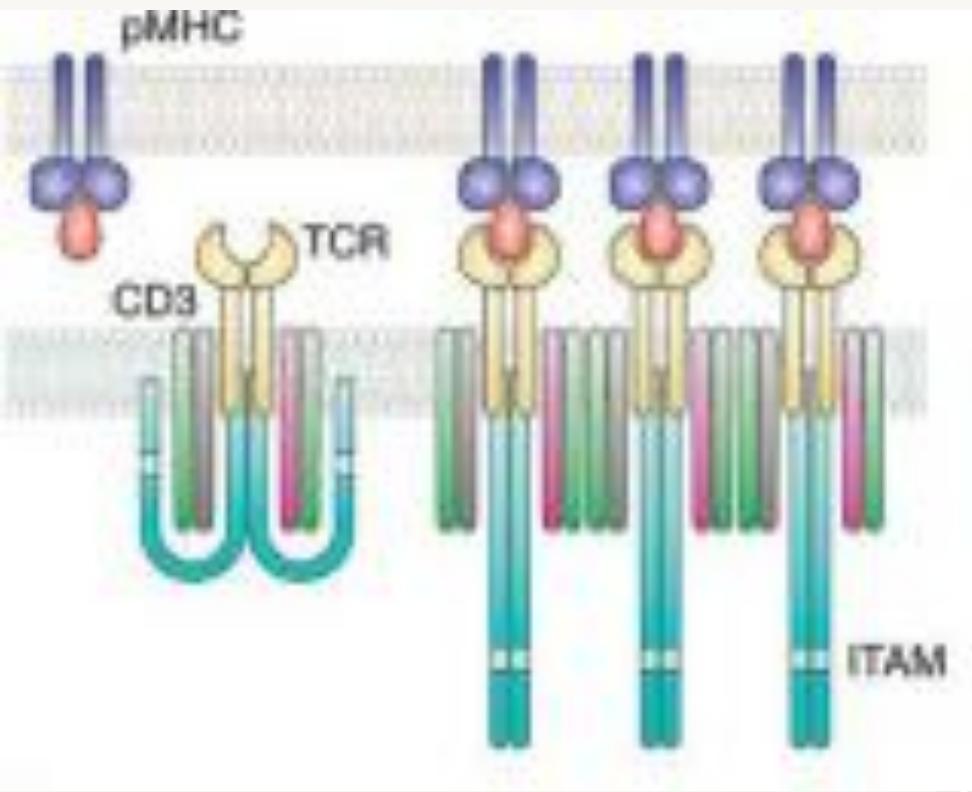
- comes from co-stimulatory receptors such as CD28, presented on the surface of other immune cells. It is expressed only when an infection was detected by the innate immune system, it is a "Danger indicating signal". This two-signal system makes sure that T cells only respond to harmful pathogens and not to self-antigens.

Signal 3

- is provided by cytokines, which regulate the differentiation of T cells into different subsets of effector T cells.

- There are myriad molecules involved in the complex biochemical process (called trans-membrane signaling) by which T-cell activation occurs.

Receptor activation



In the aggregation model, pMHC binding induces oligomerization of TCR-CD3 complexes. This clustering could increase the proximity of associated Lck molecules, resulting in activation of receptors in the aggregate by *trans-autophosphorylation*.

The initial triggering follows the mechanism common for all NTR receptor family members.

Once the TCR binds a specific pMHC, the tyrosine residues of the ITAMs in its CD3 adaptor proteins are phosphorylated.

The residues serve as docking sites for downstream signalling molecules, which can propagate the signal

Phosphorylation of ITAMs is mediated by the **Src kinase Lck**.

Lck is anchored to the plasma membrane by associating with the co-receptor CD4 or CD8, depending on the T cell subtype

CD4 is expressed on helper T cells and regulatory T cells, and is specific for MHC class II. CD8, on the other hand, specific for MHC class I, is expressed on cytotoxic T cells.

Binding of the co-receptor to the MHC brings Lck in close proximity to the CD3 ITAMs

It has been shown that 40% of Lck is active even before the TCR binds pMHC and therefore has the ability to constantly phosphorylate the TCR.

Tonic TCR signalling is avoided by the presence of phosphatase CD45 that removes phosphorylation from tyrosine residues and inhibits signal initiation

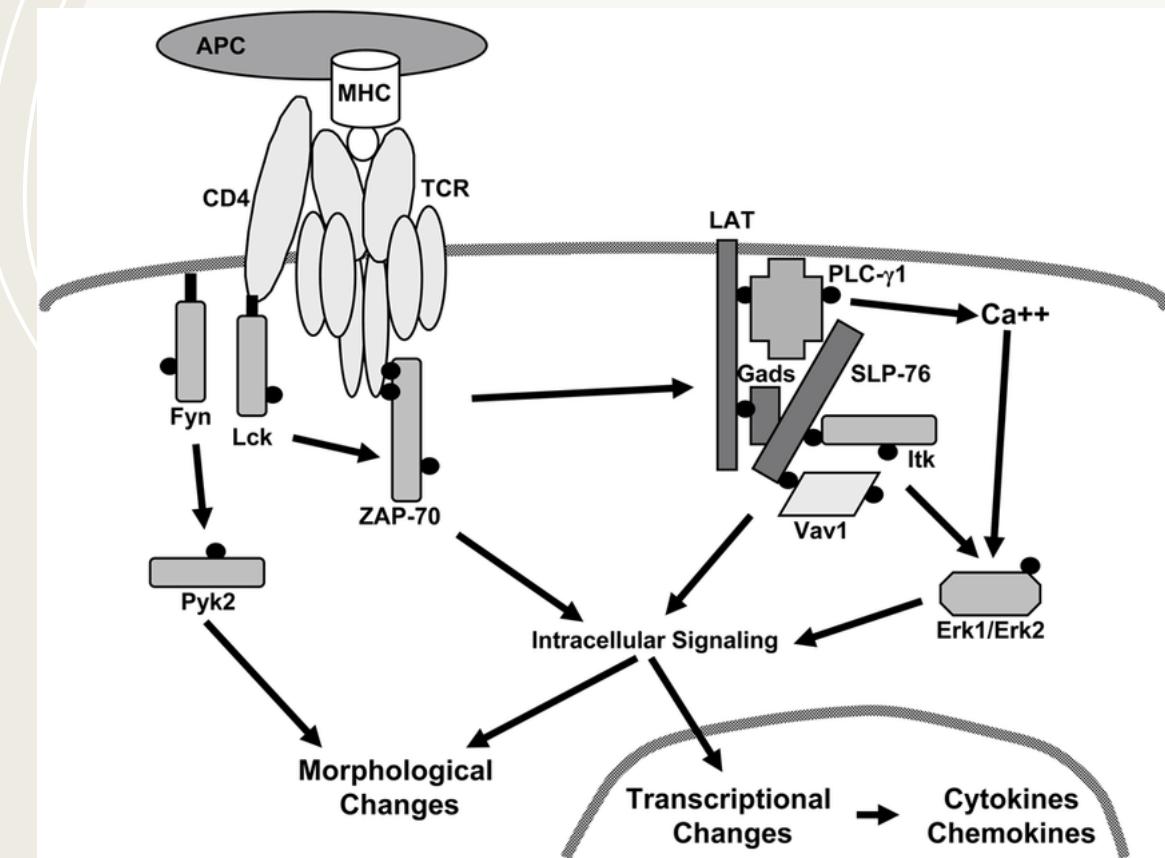
Proximal TCR signaling

Phosphorylated ITAMs in the cytoplasmic tails of CD3 recruit protein tyrosine kinase Zap70 that can bind to the phosphorylated tyrosine residues with its SH2 domain.

This brings Zap70 into close proximity to Lck which results to its phosphorylation and activation by Lck.

Lck phosphorylates a number of different proteins in the TCR pathway.

Zap70 is able to phosphorylate multiple tyrosine residues of the transmembrane protein LAT (Linker for activation of T cells). LAT is a scaffold protein associated with the membrane



LAT associates with another scaffolding protein Slp-76 via the Grap2 adaptor protein, which provides additional binding sites

LAT and Slp-76 provide a platform for the recruitment of many downstream signalling molecules

LAT/Slp76 complex act as a highly cooperative signalosome

Molecules that bind the LAT/Slp76 complex include: Phospholipase Cy1 (PLCy1), SOS via a Grb2 adaptor, Itk, Vav, Nck1 and Fyb

Signal transduction to the nucleus

PLC γ is a very important enzyme in the pathway as it generates second messenger molecules

Direct activation of the human phospholipase C- γ isozymes (PLC- γ 1, - γ 2) by tyrosine phosphorylation is fundamental to the control of diverse biological processes, including chemotaxis, platelet aggregation, and adaptive immunity

It is activated by the tyrosine kinase Itk which is recruited to the cell membrane by binding to Phosphatidylinositol (3,4,5)-trisphosphate (PIP3)

PIP3 is produced by the action of Phosphoinositide 3-kinase(PI-3K), which phosphorylates Phosphatidylinositol 4,5-bisphosphate (PIP2) to produce PIP3.

The interaction between PLC γ , Itk and PI-3K could be the point in the pathway where the first and the second signal are integrated

Only if both signals are present, PLC γ is activated.

Once PLC γ is activated by phosphorylation, it hydrolyses PIP2 into two secondary messenger molecules, namely the membrane-bound diacyl glycerol(DAG) and the soluble inositol 1,4,5-trisphosphate (IP3).

These second messenger molecules amplify the TCR signal and distribute the prior localised activation to the entire cell and activate protein cascades that finally lead to the activation of transcription factors(regulation of genes in order to express them)

Transcription factors involved : are the NFAT, NF- κ B and AP1, a heterodimer of proteins Fos and Jun.

All three transcription factors are needed to activate the transcription of interleukin-2(IL2) gene.

NFAT

NFAT activation depends on calcium signaling

IP₃ produced by PLC- γ is no longer bound to the membrane and diffuses rapidly in the cell

Binding of IP₃ to calcium channel receptors on the endoplasmic reticulum (ER) induces the release of calcium (Ca²⁺) into the cytosol

The resulting low Ca²⁺ concentration in the ER causes STIM1 clustering on the ER membrane, which in turn leads to activation of cell membrane CRAC channels that allows additional calcium to flow into the cytosol from the extracellular space

This cytosolic calcium binds calmodulin, inducing a conformational change of the protein such that it can then bind and activate calcineurin

Calcineurin, in turn, dephosphorylates NFAT. In its deactivated state, NFAT cannot enter the nucleus as its nuclear localisation sequence (NLS) cannot be recognised by nuclear transporters due to phosphorylation by GSK-3

When dephosphorylated by Calcineurin translocation of NFAT into the nucleus is possible

PI-3K via signal molecules recruits the protein kinase AKT to the cell membrane.

AKT is able to deactivate GSK3 and thereby inhibiting the phosphorylation of NFAT, which could contribute to NFAT activation

NF-κB

Nuclear factor kappa-light-chain-enhancer plays a critical role in regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells

NF-κB activation is initiated by DAG

DAG binds and recruits Protein kinase C θ (PKCθ) to the membrane where it can activated the membrane bound scaffold protein CARMA1 Capsase recruitment domain containing protein aka CARD

CARMA1 then undergoes a conformational change which allow it to oligomerise and bind the adapter proteins BCL10, CARD domain and MALT1 Mucosa-associated lymphoid tissue lymphoma translocation protein 1

This multi-subunit complex binds the Ubiquitin ligase TRAF6

Ubiquitination of TRAF6 serves as scaffold to recruit NEMO, I κ B kinase (IKK) and TAK1

AK 1 phosphorylates IKK, which in turn phosphorylates the NF- κ B inhibitor I- κ B, leading to the ubiquitination and subsequent degradation of I- κ B. I- κ B blocks the NLS of NF- κ B therefore preventing its translocation to the nucleus

I- κ B is degraded, it cannot bind to NF- κ B and the NLS of NF- κ B becomes accessible for nuclear translocation

AP1

Activation of AP1 involves three MAPK signalling pathways which use a phosphorylation cascade of three successive acting protein kinases to transmit a signal.

The three MAPK pathways in T cells involve kinases of different specificities belonging to each of the MAP3K, MAP2K, MAPK families

Initial activation is done by the GTPase Ras or Rac which phosphorylate the MAP3K

A cascade involving the enzymes Raf, MEK1, ERK results in the phosphorylation of Jun, conformational change allows Jun to bind to Fos and hence AP-1 to form

AP-1 then acts as transcription factor.

Raf is activated via the second messenger DAG, SOS, and Ras.

DAG recruits among other proteins the RAS guanyl nucleotide-releasing protein (RasGRP), a guanine nucleotide exchange factor (GEF), to the membrane.

RasGRP activates the small GTPase Ras by exchanging Guanosine diphosphate (GDP) bound to Ras against Guanosine triphosphate (GTP).

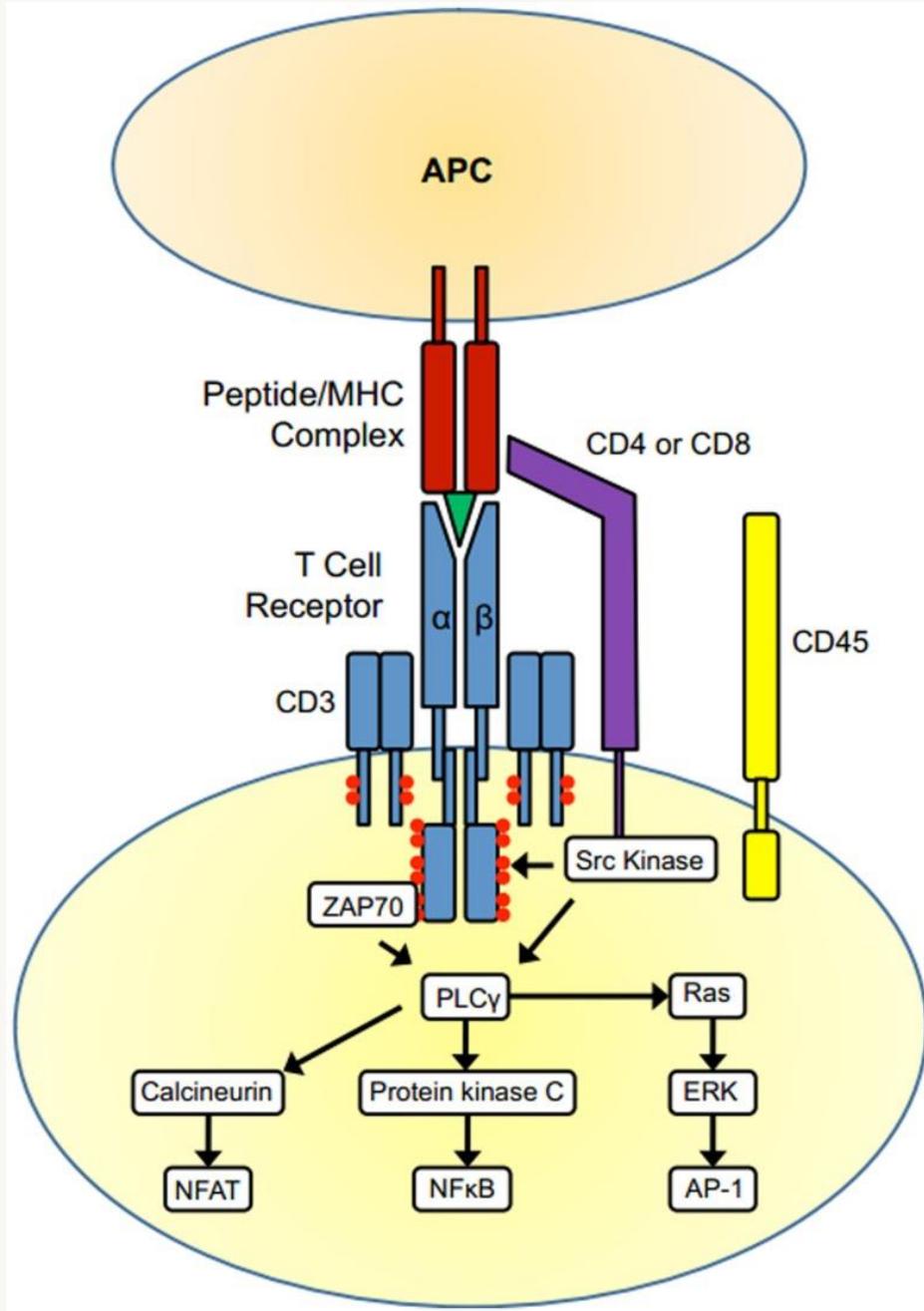
Ras can also be activated by the guanine nucleotide exchange factor SOS which binds to the LAT signalosome.

Ras then initiates the MAPK cascade.

The second MAPK cascade with MEKK1, JNKK, JNK induces protein expression of Jun.

Another cascade, also involving MEKK1 as MAPK3, but then activating MKK3 /6 and p38 induces Fos transcription.

Activation of MEKK1, additionally to being activated by Ras, involves Slp-76 recruiting the GEF Vav to the LAT signalosome, which then activates the GTPase Rac. Rac and Ras activate MEKK1 and thereby initiate the MAPK cascade



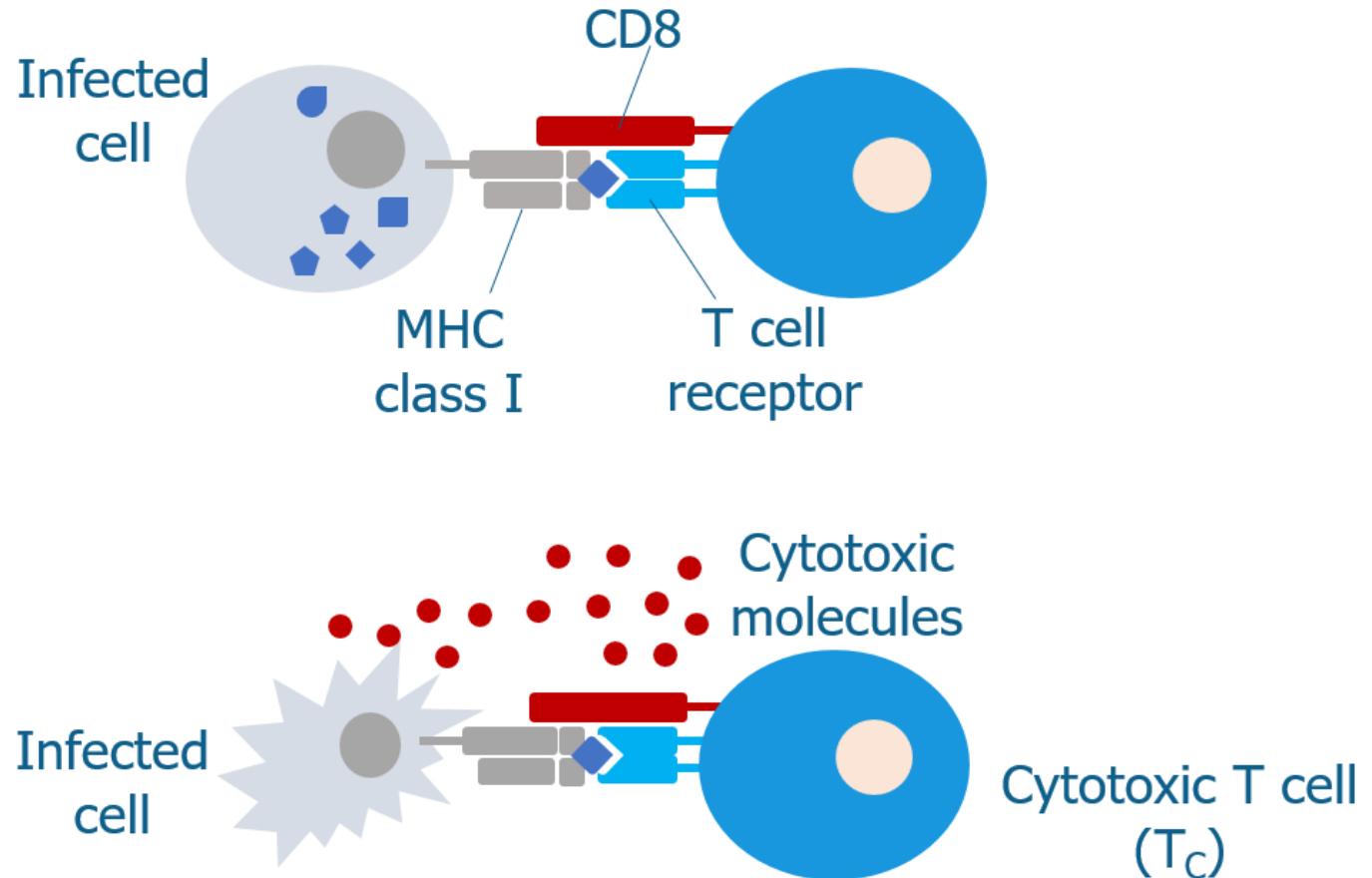
CD8 Co-Receptor

The CD8 co-receptor is predominantly expressed on the surface of cytotoxic T cells, but can also be found on natural killer cells, cortical thymocytes, and dendritic cells.

The CD8 molecule is a marker for cytotoxic T cell population.

It is expressed in T cell lymphoblastic lymphoma and hypopigmented mycosis fungoides

1. The T cell receptor on a CD8+ T cell recognizes the pathogen peptide displayed on the surface of an infected cell
2. The T cell releases cytotoxic molecules that kill the infected cell and stop the pathogen from spreading



Structure

To function, CD8 forms a dimer, consisting of a pair of CD8 chains.

The most common form of CD8 is composed of a CD8- α and CD8- β chain, both members of the immunoglobulin superfamily with an immunoglobulin variable (IgV)-like extracellular domain connected to the membrane by a thin stalk, and an intracellular tail.

The molecular weight of each CD8 chain is about 34 kDa

Function

The extracellular IgV-like domain of CD8- α interacts with the α_3 portion of the Class I MHC molecule

This affinity keeps the T cell receptor of the cytotoxic T cell and the target cell bound closely together during antigen-specific activation. Cytotoxic T cells with CD8 surface protein are called CD8+ T cells.

The main recognition site is a flexible loop at the α_3 domain of an MHC molecule

The flexible α_3 domain is located between residues 223 and 229 in the genome

In addition to aiding with cytotoxic T cell antigen interactions the CD8 co-receptor also plays a role in T cell signaling

The cytoplasmic tails of the CD8 co-receptor interact with Lck (lymphocyte-specific protein tyrosine kinase).

Once the T cell receptor binds its specific antigen Lck phosphorylates the cytoplasmic CD3 and ζ -chains of the TCR complex which initiates a cascade of phosphorylation eventually leading to activation of transcription factors like NFAT, NF- κ B, and AP-1 which affect the expression of certain genes.

CD4

In molecular biology, CD4 (cluster of differentiation 4) is a glycoprotein that serves as a co-receptor for the T-cell receptor (TCR).

CD4 is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells.

originally known as leu-3 and T4 (after the OKT4 monoclonal antibody that reacted with it) before being named CD4 in 1984

CD4 protein is encoded by the CD4 gene

CD4+ T helper cells are white blood cells that are an essential part of the human immune system.

They are often referred to as CD4 cells, T-helper cells or T4 cells.

They are called helper cells because one of their main roles is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious particle.

If CD4 cells become depleted, for example in untreated HIV infection, or following immune suppression prior to a transplant, the body is left vulnerable to a wide range of infections that it would otherwise have been able to fight.

Structure

CD4 is a member of the immunoglobulin superfamily.

It has four immunoglobulin domains (D1 to D4) that are exposed on the extracellular surface of the cell:

D1 and D3 resemble immunoglobulin variable (IgV) domains.

D2 and D4 resemble immunoglobulin constant (IgC) domains.

The immunoglobulin variable (IgV) domain of D1 adopts an immunoglobulin-like β -sandwich fold with seven β -strands in 2 β -sheets

CD4 interacts with the β 2-domain of MHC class II molecules through its D1 domain.

T cells displaying CD4 molecules (and not CD8) on their surface, therefore, are specific for antigens presented by MHC II and not by MHC class I (they are MHC class II-restricted). MHC class I contains Beta-2 microglobulin.

The short cytoplasmic/intracellular tail (C) of CD4 contains a special sequence of amino acids that allow it to recruit and interact with the tyrosine kinase Lck.

Function

CD4 is a co-receptor of the T cell receptor (TCR) and assists the latter in communicating with antigen-presenting cells.

The TCR complex and CD4 bind to distinct regions of the antigen-presenting MHC class II molecule.

The extracellular D1 domain of CD4 binds to the $\beta 2$ region of MHC class II. The resulting close proximity between the TCR complex and CD4 allows the tyrosine kinase Lck bound to the cytoplasmic tail of CD4 to phosphorylate tyrosine residues of immunoreceptor tyrosine activation motifs (ITAMs) on the cytoplasmic domains of CD3 to amplify the signal generated by the TCR

. Phosphorylated ITAMs on CD3 recruit and activate SH2 domain-containing protein tyrosine kinases (PTK), such as ZAP70, to further mediate downstream signalling through tyrosine phosphorylation.

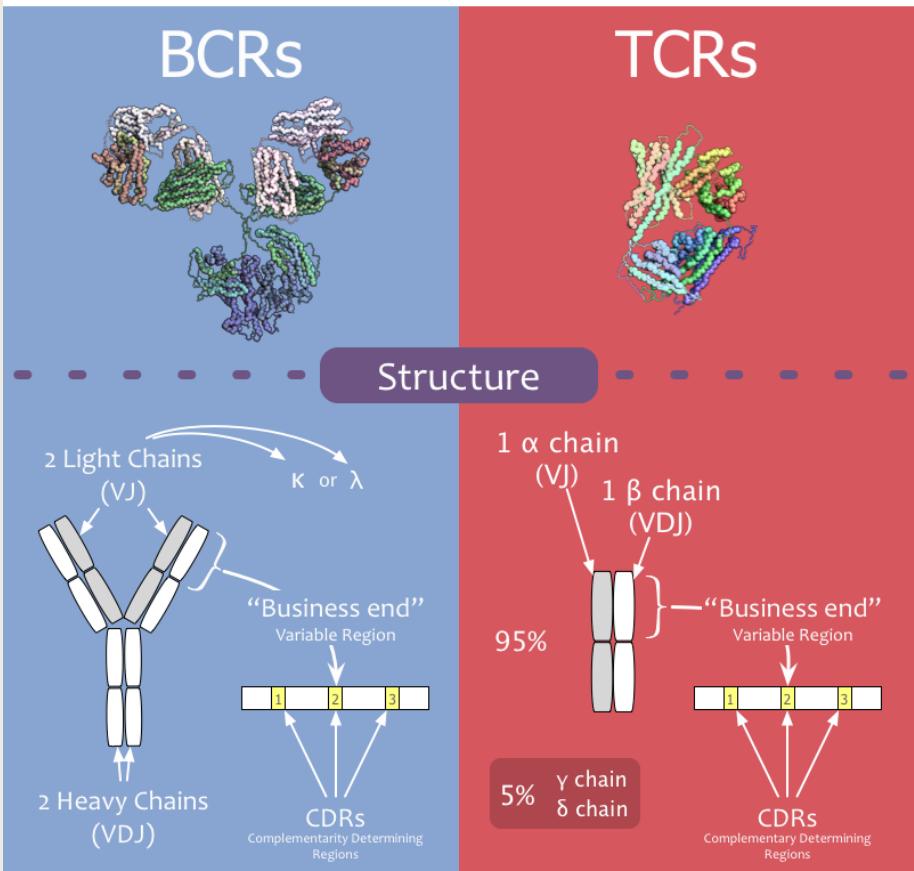
These signals lead to the activation of transcription factors, including NF- κ B, NFAT, AP-1, to promote T cell activation.

	Type	Stimulus cytokines	Transcription factor	Function	Effector molecules	Target organisms
CD8	Cytotoxic T cells	IL-12IL-18	T-betBlimp1	Kills virus-infected cells.	GranzymePerforin TNF- α Fas ligand	Viruses
CD4	T _H 1 cells	IL-12IFN- γ IL-2	T-bet	Activates macrophages. Helps cytotoxic T-cells. Provides B-cell help for antibody production	IL-12IFN- γ IL-2	Intracellular (mycobacteria, listeria, leishmania) and extracellular bacteria.Fungi.
	T _H 2 cells	IL-4	GATA3STAT6	Provide help to B cells for antibody production- especially IgE antibodies. Activates eosinophils and mast cells.	IL-4IL-5 IL-13	HelminthExtracellular Parasites

	Type	Stimulus cytokines	Transcription factor	Function	Effector molecules	Target organisms
CD4	T _H 17 cells	IL-6IL-21 TGF-β	ROR-γTSTAT3	Enhance neutrophil response. Improve epithelial barrier function.	IL-17IL-21 IL-22 IL-26 IL-6	Extracellular bacteria (e.g. <i>Salmonella enterica</i>)
	TFH cells	IL-6IL-21	Bcl6	Germinal centre formation. B cell antibody isotype switching. Antibody affinity maturation. Enables B cells to develop into plasma cells for Long term humoral immunity	IL-10IL-21 IL-4	
	T regulatory cells	TGF-β	Foxp3	Suppresses other immune cells, particularly CD4+ and CD8+ responses.	TGF-βIL-10 CTLA-4	T regulatory cells

Antibodies (BCRs) vs T Cell Receptors (TCRs)

Similarities and Differences



Structure

Diversity

V(D)J recombination



Junctional Diversity
Insertions / Deletions

Constant Regions
($\alpha, \delta, \epsilon, \gamma, \mu$)
Class Switching

Somatic Hyper Mutation (SHM)

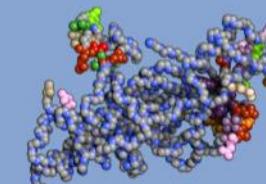
V(D)J recombination



Junctional Diversity
Insertions / Deletions

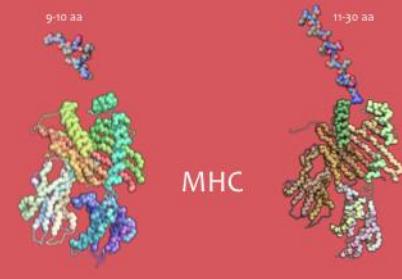
Antigens

Proteins - 3D structure
Also peptides, chemicals



Discontinuous or continuous epitopes

Linear Peptides
Processed, then presented by MHC proteins



B CELL RECEPTOR VERSUS ANTIBODY

B CELL RECEPTOR

An immunoglobulin molecule which serves as a type of transmembrane protein on the surface of B cells

A type of membrane-bound immunoglobulin

Two types of B cell receptors: IgD and IgM

Has a C-terminal, hydrophobic region in the heavy chains and another transmembrane domain for signal transduction

Bind with a specific antigen to activate the B cell

ANTIBODY

A blood protein produced in response to and counteracting a specific antigen

An antibody is a type of a secreted immunoglobulin

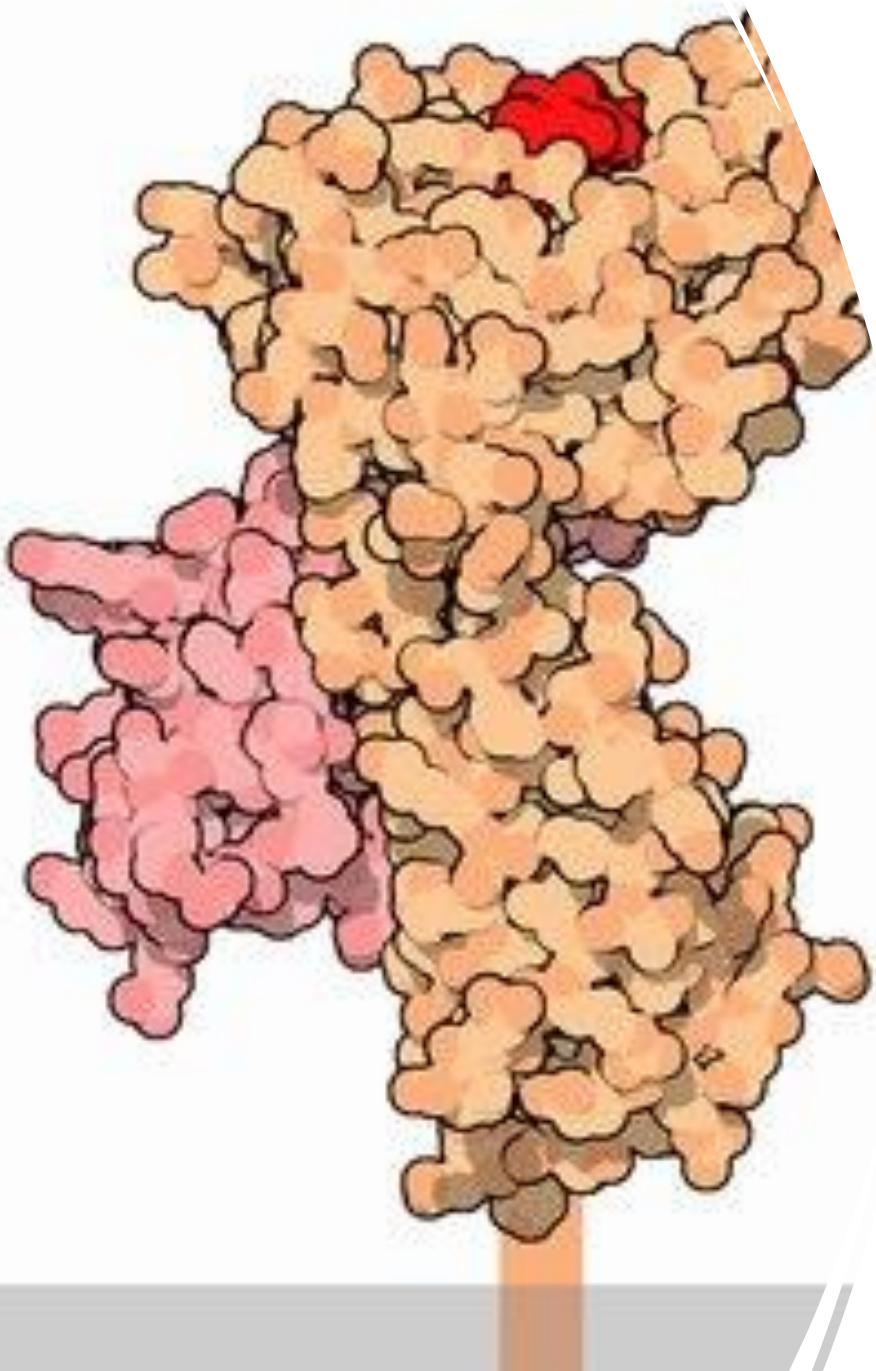
Five classes of antibodies: IgA, IgD, IgE, IgG, and IgM

Do not contain such transmembrane domains

Can bind to the antigen and elicit immune responses through the complement pathway and recruit other immune cells to destroy the pathogen

References

1. <https://www.nature.com/articles/s41392-021-00823-w>



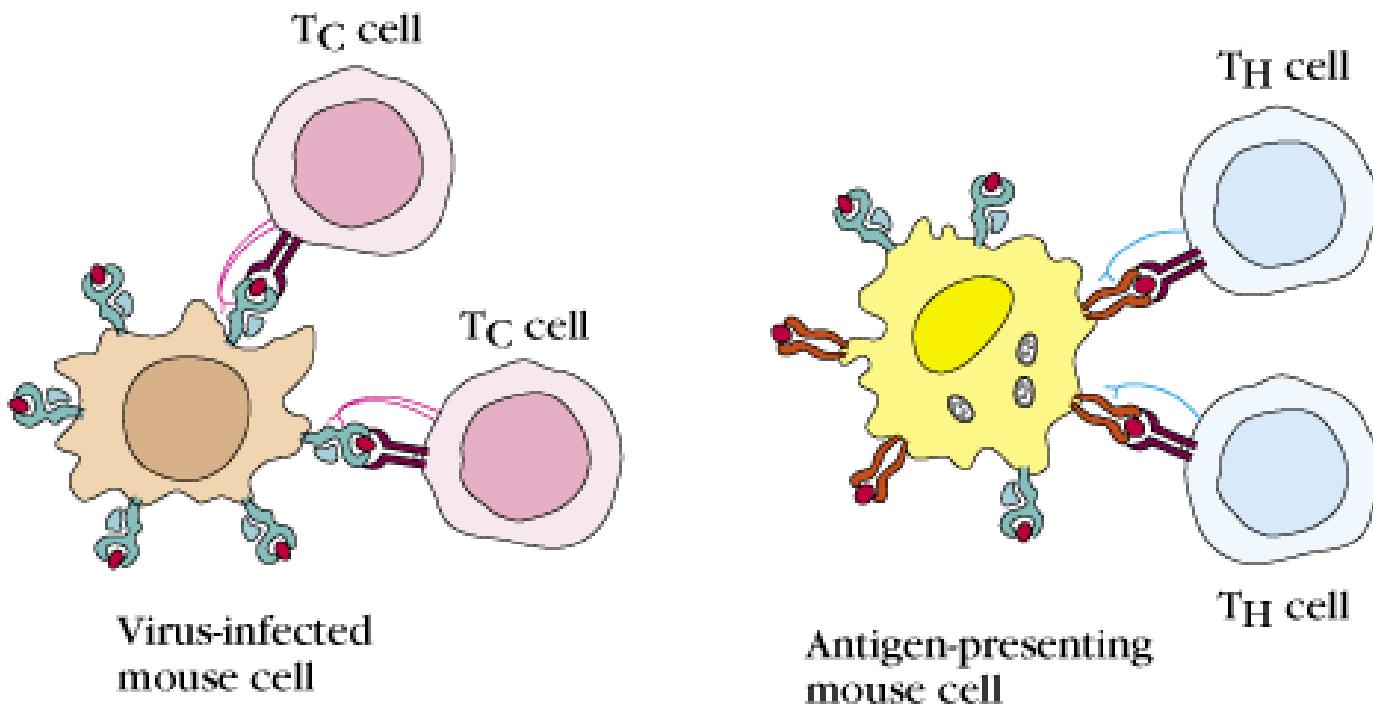
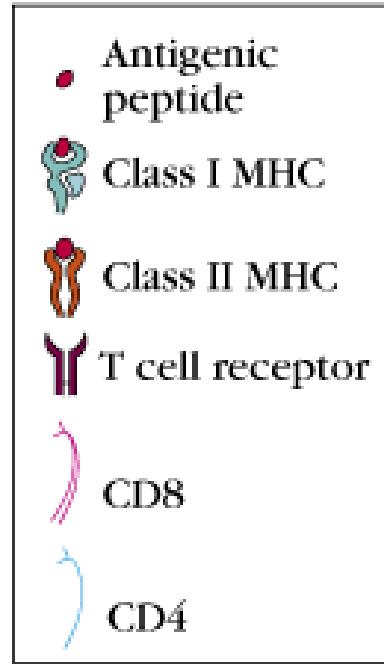
Major Histocompatibility Complex

Dr. (Ms.) Sonali Correa

INTRODUCTION

- Is a set of surface proteins located in the cell membrane of nucleated cells.
- It plays an important role in identification of self and non self antigen body, intracellular recognition and responsible for antigen presentation
- This locus got its name because it was discovered via the study of **transplanted tissue compatibility**.
- Histo referred to tissue and compatibility referred to as harmonious living
- **Is a large locus** on vertebrate DNA containing a set of **closely linked polymorphic genes** that **code** for cell surface proteins essential for the **adaptive immune system**.
- These cell surface proteins are **called MHC molecules**.
- The function of MHC molecules is to **bind peptide fragments** derived from pathogens and display them on the **cell surface for recognition by the appropriate T cells**.
- In humans the MHC is found on **Chromosome 6** referred to as the **Human Leukocyte Antigen (HLA) complex** and contains more than 200 genes
- The major histocompatibility complex is located on **chromosome 17** in the mouse and extends over some 4 centimorgans of DNA, about 4×10^6 base pairs and are known as **the H-2 genes**.





The major histocompatibility complex (MHC) of genes consists of a linked set of genetic loci encoding many of the proteins involved in antigen presentation to T cells, most notably the MHC class I and class II glycoproteins (the MHC molecules) that present peptides to the T-cell receptor.



HISTORICAL BACKGROUND

- The first descriptions of the MHC were made by British immunologist Peter Gorer in 1936. MHC genes were first identified in inbred mice strains.
- He identified the four group of MHC molecules in the blood sample of mice when he identified the blood group antigen and was designated as class I to IV



STRUCTURE AND FUNCTIONS OF MHC



MHC CLASS-I

- Class-I MHC **gene encodes glycoprotein molecule** which **expressed on the surface of all nucleated cells and platelets** i.e. by all body cells except red blood cells aka HLA-A, -B, and -C genes.
- It present antigens **Tc cells**
- Present intracellular antigens to CD8+ T-cells
- When a cell becomes cancerous or is invaded by a virus, unfamiliar proteins are synthesized in the cell. These proteins are endogenous antigens—that is, **antigens produced inside the cell**. Portions of these antigens are combined with MHC-I glycoproteins and, when displayed on the plasma membrane, indicate a nonself cell.
- MHC-I molecule contains a two polypeptide chains
 - 45KDa α -chain associated non-covalently with a
 - 12KDa $\beta 2$ microglobulin molecule.
- Association of α -chain and $\beta 2$ microglobulin is required for expression of class-I MHC molecule on cell membrane.
- There are three class I α -chain genes in humans, called HLA-A, -B, and -C



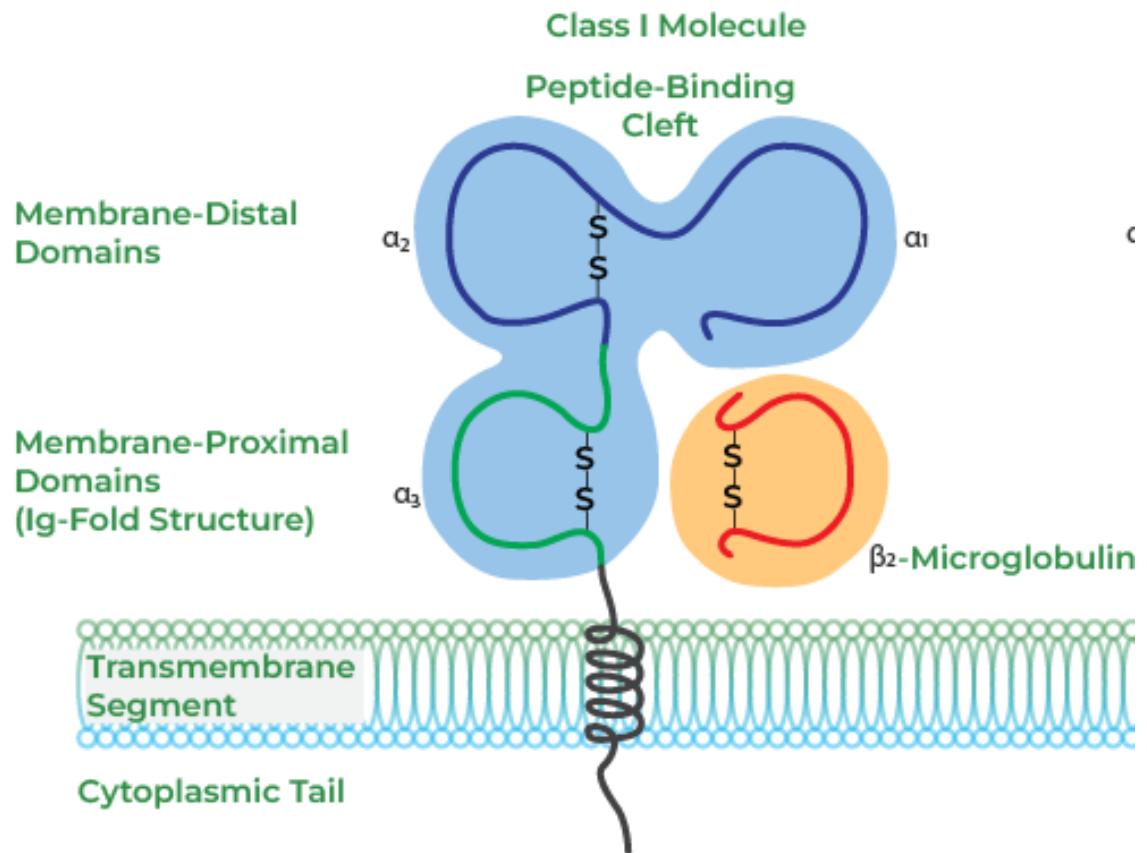
CLASS I MHC MOLECULE

- **α -chain**

- 3 external domains- each approximately 90 amino acids long
- A transmembrane domain – 25 hydrophobic amino acids & a short chain of charged hydrophilic amino acids
- A cytoplasmic anchor segment- 30 amino acids

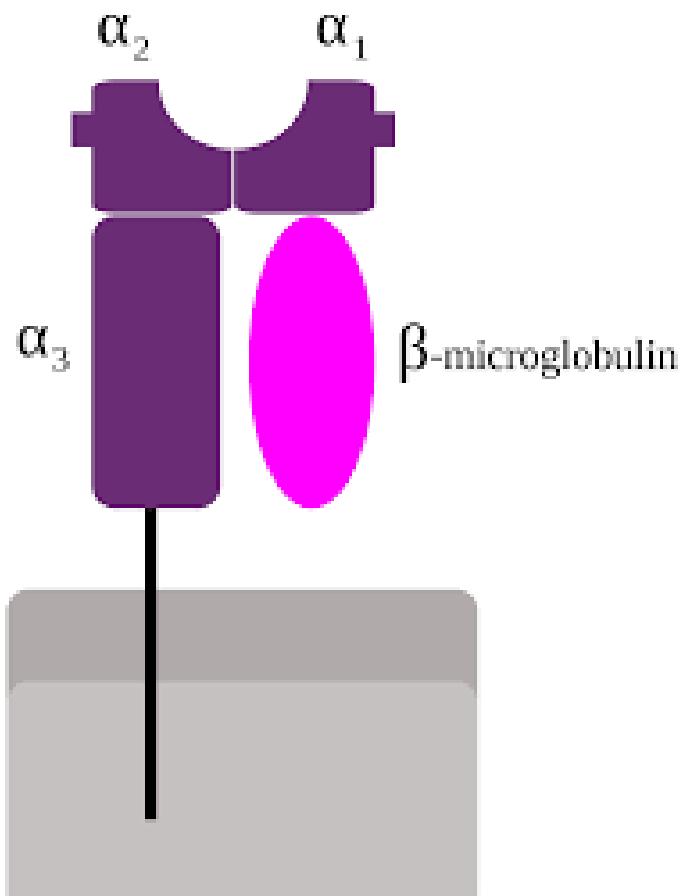
- **$\beta 2$ microglobulin**

- Similar in size & organization to $\alpha 3$ domain
- No transmembrane region
- Bound non covalently to the α domain



STRUCTURE OF MHC CLASS I

- MHC Class I molecules in both human and mouse consist of two polypeptide chains that dramatically differ in size.
- The larger (α) chain has a molecular weight of 44 kDa in humans and 47 kDa in the mouse, and is encoded by an MHC Class I gene.
- The smaller chain, called β -2 microglobulin, has a molecular weight of 12 kDa in both species, and is encoded by a nonpolymorphic gene that is mapped outside of the MHC complex.
- There are no known differences in the structure of the human MHC Class I antigen α chains encoded by the HLA-A locus compared to those encoded by the HLA-B or the HLA-C loci, or in the structure of the murine MHC Class I antigen α chains encoded by the H-2K locus compared to those encoded by the H-2D or H-2L loci.

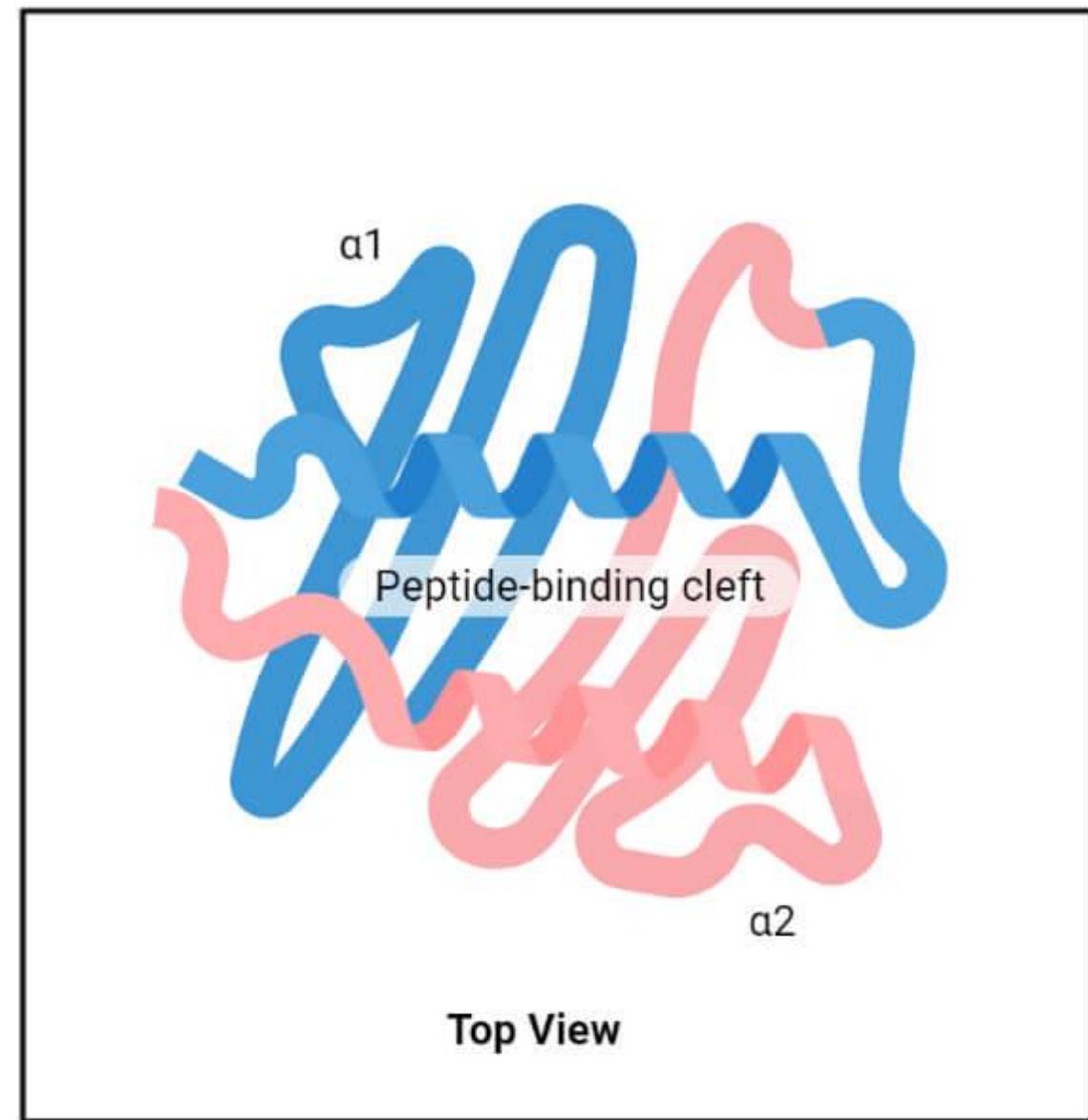
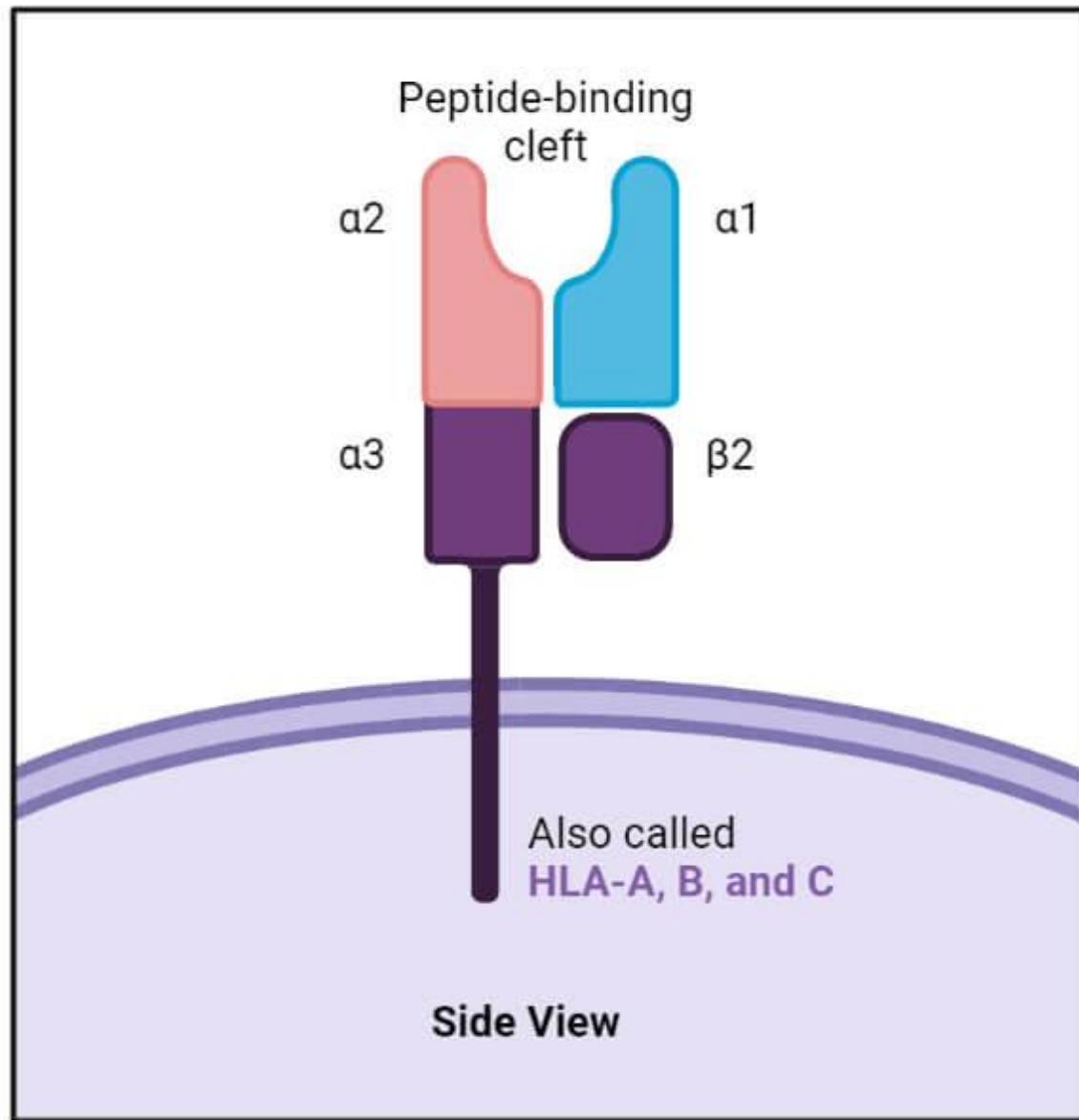


ALPHA CHAIN + BETA2-MICROGLOBULIN

- The α chain is **highly polymorphic**, meaning it exists in various allelic forms leading to a diverse array of MHC Class I molecules in the population.
- The α chain consists of three domains: $\alpha 1$, $\alpha 2$, and $\alpha 3$.
- The $\alpha 1$ and $\alpha 2$ domains form a peptide-binding cleft,
- while the $\alpha 3$ domain anchors the complex to the cell membrane.
- The $\beta 2$ -microglobulin which is the non-polymorphic is essential for the proper folding and stability of the MHC Class I molecule.



MHC Class I



- Regardless of which of these loci codes it, the α chain can be subdivided into the following regions or domains:
 1. the peptide-binding domain;
 2. the immunoglobulin-like domain;
 3. the transmembrane domain; and
 4. the cytoplasmic domain.
- The peptide-binding domain is the most N-terminal; it is the only region of the molecule where allelic differences in the amino acid sequence can be localized.
- As seen from its name, the peptide-binding domain of the molecule includes the site to which antigenic peptides bind.
- It makes much sense to have this site exactly where the allelic differences are, because different MHC alleles accommodate peptides better or worse, thus influencing on the magnitude of the T-cell response.
- X-ray crystallography showed that the peptide-binding site in the MHC Class I molecules looks like a cleft that has a “floor” and two “walls” formed by spiral shaped portions of the alpha chain, called alpha 1 and alpha 2.
- Since the “floor” of the peptide-accommodating cleft is closed, only relatively small peptides, consisting of 9 to 11 amino acid residues, can be “stuffed” there.
- The immunoglobulin-like domain is structurally conserved, and resembles a domain of an antibody C-region.
- It contains the binding site for the T-cell accessory molecule CD8.
- The transmembrane and the cytoplasmic domains ensure that the alpha chain spans the membrane and is properly expressed by the cell.
- The β -2-microglobulin chain is also vitally important for the proper expression of the alpha chain.
- There are some mutant lymphoid cell lines (notably Daudi) that do not express MHC Class I molecules because of the defect in the β -2-microglobulin gene.

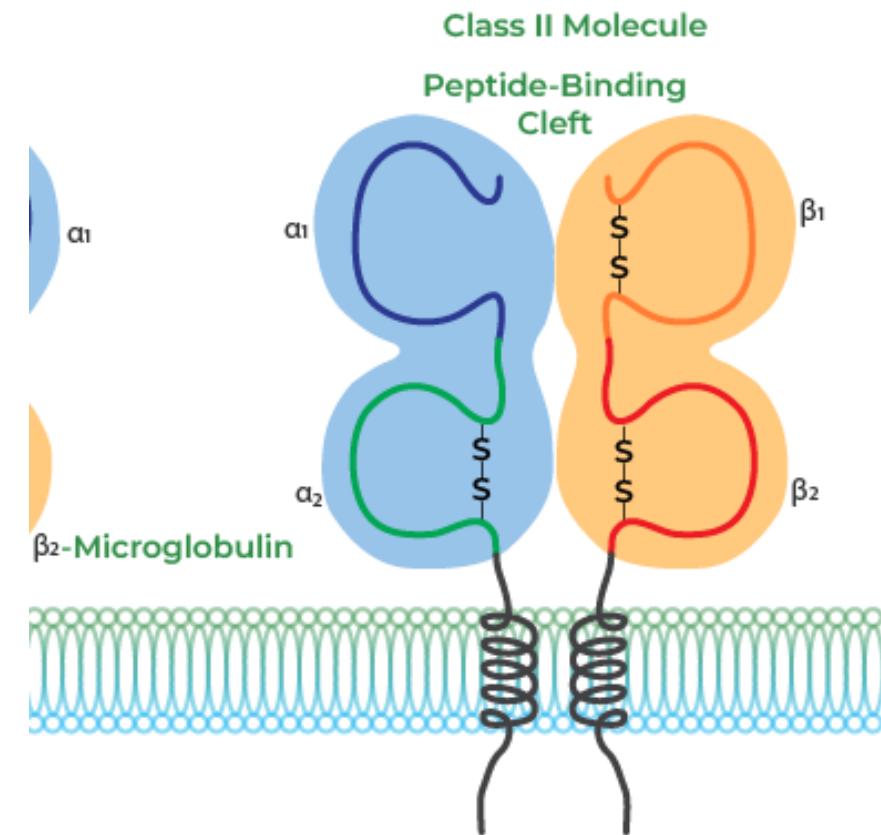
CLASS II MHC MOLECULE

- The MHC Class II proteins are essential components of the immune system involved in antigen presentation and immune regulation.
- They play a crucial role in recognizing and presenting antigens derived from the extracellular pathogens to CD4+ T cells, also known as helper T cells.
- The antigens presented by class II peptides are derived from extracellular proteins (not cytosolic as in MHC class I)
- Consists of two different polypeptide chains
 - α -chain- 33 kDa
 - β -chain- 28 kDa



CLASS II MHC MOLECULE

- α -chain
 - Contains 2 extracellular domains – α_1 & α_2
 - A transmembrane segment
 - A cytoplasmic anchor segment
- β -chain
 - Similar to the α chain
 - Contains 2 extracellular domains - β_1 and β_2
- The peptide biding cleft is a open ended groove formed between α -chain and β -chain at proximal end.
- The cleft can bind antigenic peptide of 13-18 aminoacids long.

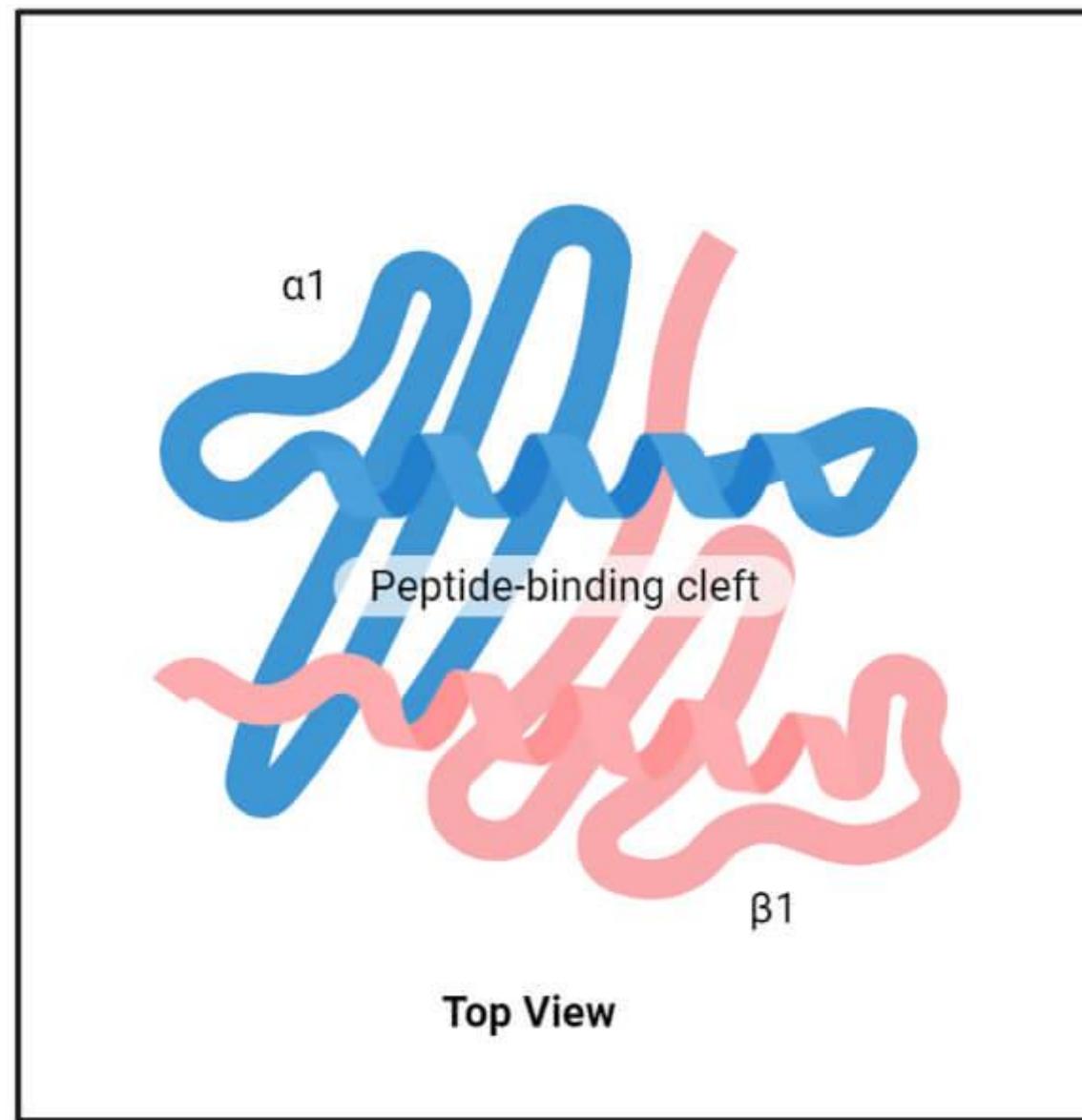
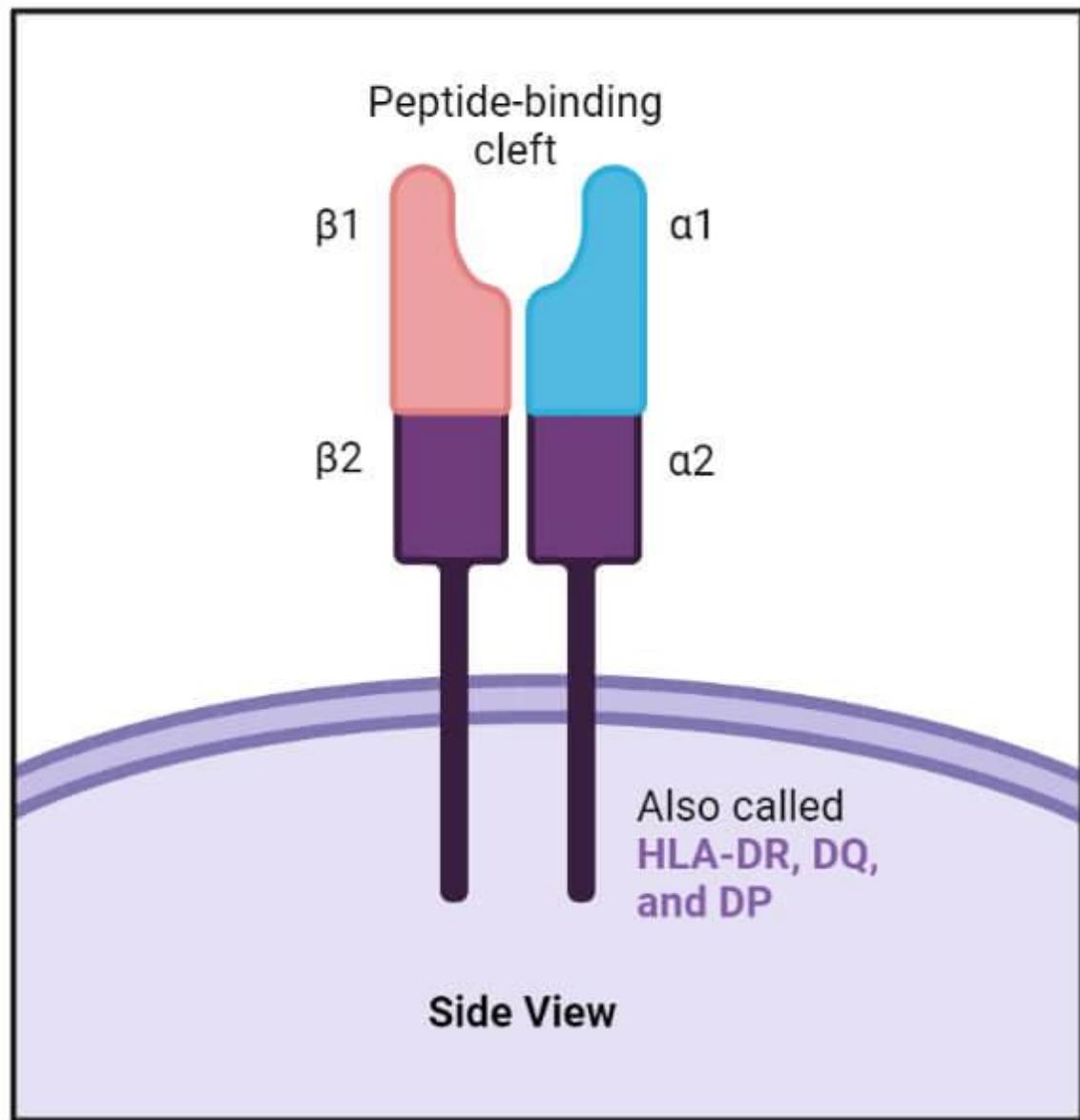


ALPHA AND BETA CHAIN

- The MHC Class II complex is expressed primarily on the surface of antigen-presenting cells, including dendritic cells, macrophages, and B cells.



MHC Class II



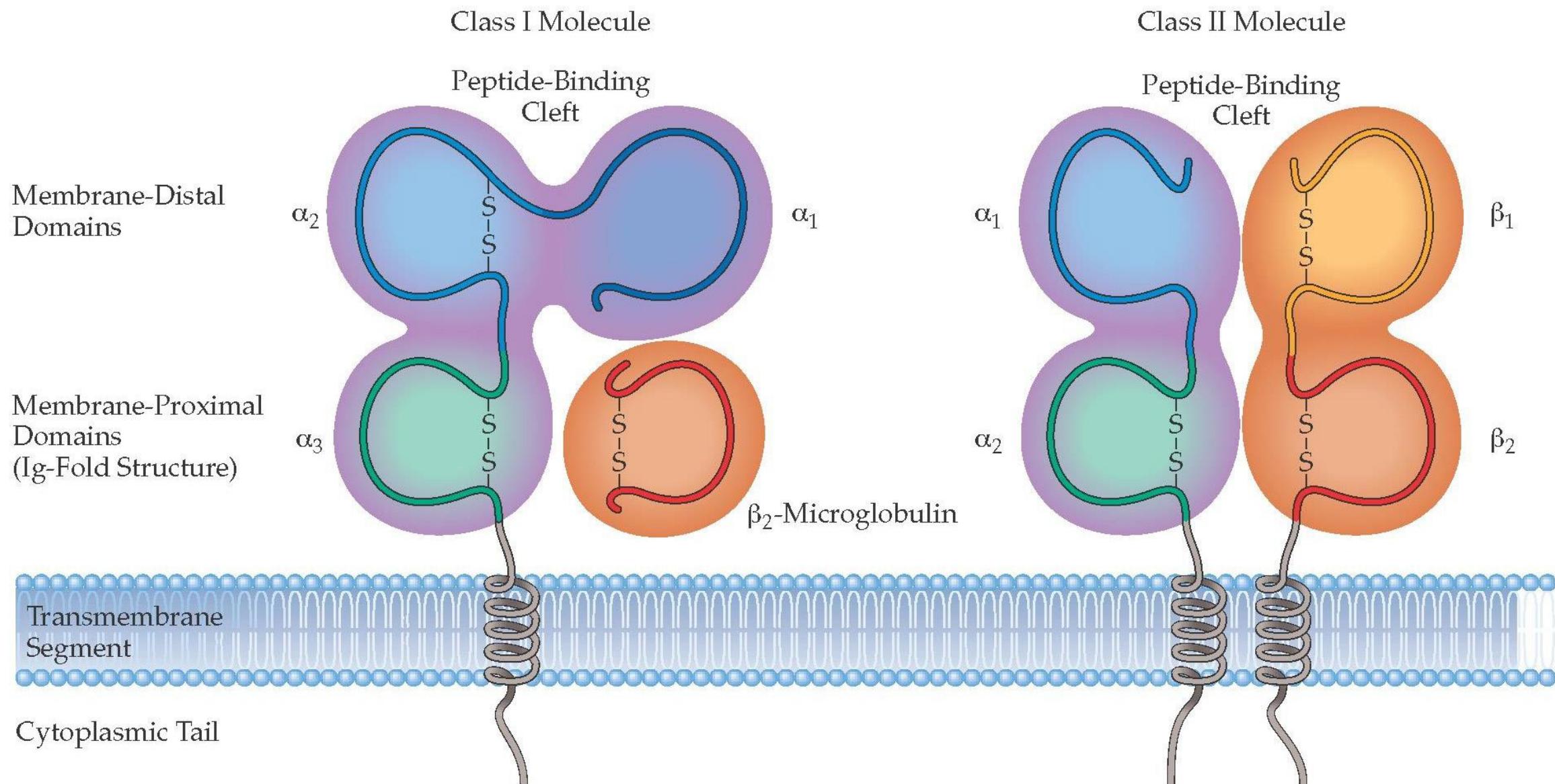
- The structure of the α and the β chains of the MHC Class II molecules resembles that of the alpha chain of the MHC Class I molecules in that the former can be also divided into the peptide-binding, the immunoglobulin-like, the transmembrane, and the cytoplasmic domains.
- One important difference, however, is that the peptide-binding cleft in Class II molecules is formed by both alpha and beta chains.
- Although positioned close to each other in space, the spirals of the alpha and the beta chains that form the cleft are not physically bound to each other.
- Because of that, the “floor” of the peptide-accommodating cleft in Class II MHC molecules is “open,” or “has a hole” in it.
- That allows MHC Class II molecules to accommodate peptides that are larger than those that fit MHC Class I molecules.
- The immunoglobulin-like domain of the MHC Class II molecules contains the binding site for a T-cell accessory molecule, CD4.
- This site cannot bind the above-mentioned CD8 molecule.

CLASS III

- Class III MHC genes encode for various secreted proteins that have immune functions, including the component of the complement system and molecules that are involved in inflammation such as cytokines.



Differences between MHC Class I and MHC Class II



Characteristics	MHC Class I Proteins	MHC Class II Proteins
Structure	The α chain + $\beta 2$ -microglobulin	the α chain + β chain
Cell Surface Expression	The Ubiquitous (expressed on most nucleated cells)	The Restricted to the antigen-presenting cells (dendritic cells, macrophages, B cells)
Antigen Source	The Intracellular pathogens (viruses, intracellular bacteria)	The Extracellular pathogens (bacteria, parasites)
Antigen Processing	The Endogenous pathway: antigens derived from inside the cell are processed and presented on MHC Class I molecules	The Exogenous pathway: antigens internalized from the extracellular environment are processed and presented on MHC Class II molecules
Peptide Size	The Small peptides (8-10 amino acids)	The Larger peptides (13-25 amino acids)
T Cell Interaction	The CD8+ T cells (cytotoxic T cells)	The CD4+ T cells (helper T cells)
Co-receptor	The CD8 molecule	The CD4 molecule
Immune Function	Cytotoxicity (killing infected or abnormal cells)	The Immune regulation, coordination, and activation of other immune cells
Location	The Present of all nucleated cells	The Present primarily on antigen-presenting cells

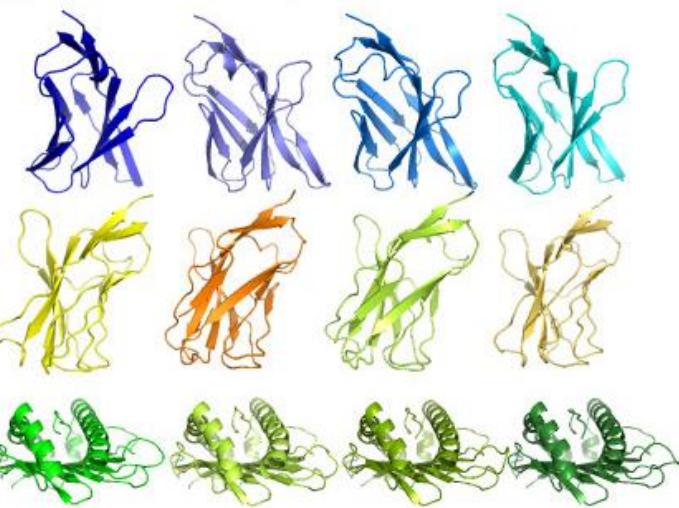


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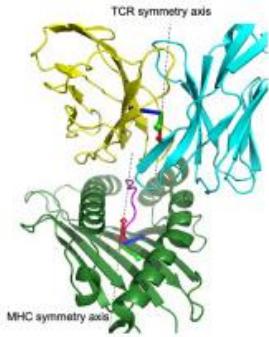
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TRAJ42*01
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TCR beta
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TRBJ2-7*01
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peptide:MHC
HLA-A*02:01
GILGFVFTL

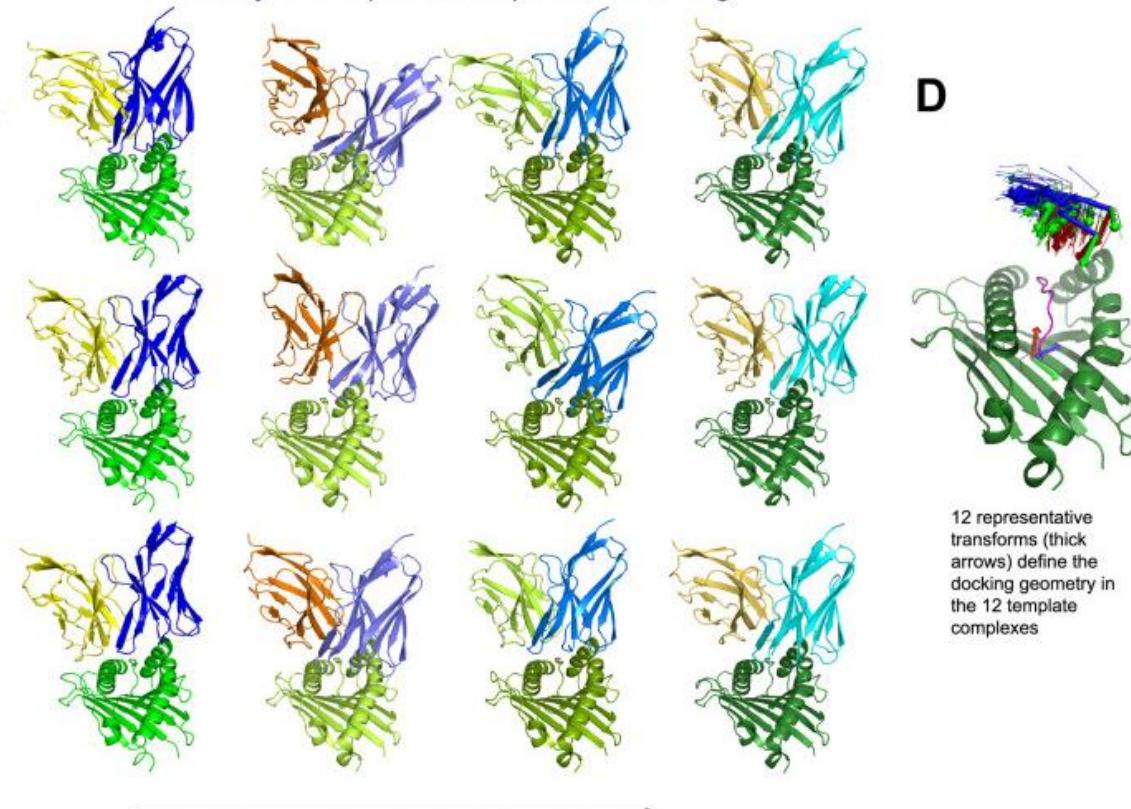


reference frames

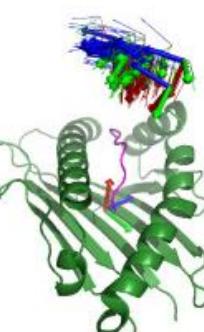


C

Twelve hybrid templates for AlphaFold modeling



D



12 representative
transforms (thick
arrows) define the
docking geometry in
the 12 template
complexes

Four template complexes per AlphaFold run

Three AlphaFold runs per target

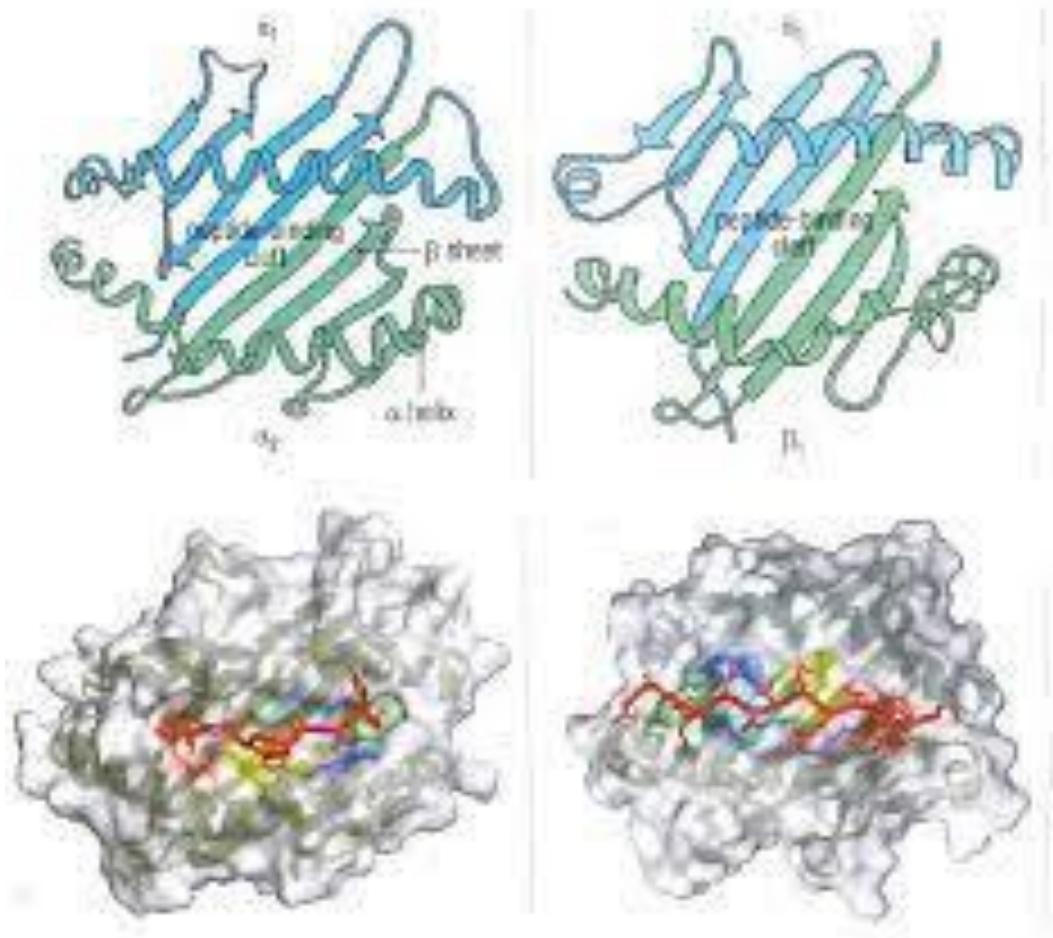
PEPTIDE-MHC INTERACTIONS

- The regulatory and effector functions of T cells are initiated by the binding of their cell-surface T cell receptor (TCR) to peptides presented by major histocompatibility complex (MHC) proteins on other cells.
- The specificity of TCR:peptide-MHC interactions, thus, underlies nearly all adaptive immune responses

Figure: Constructing diverse hybrid templates for AlphaFold modeling.

- Four structural templates for each TCR chain and for the peptide:MHC are identified in the Protein Databank (Berman et al., 2000) by sequence similarity search.
- TCR:pMHC docking geometry is defined by computing the rigid-body transformation between TCR and pMHC coordinate frames. Coordinate frames are oriented based on internal pseudo symmetry as described in the Methods.
- Three independent AlphaFold simulations are performed, each with four hybrid templates built from the four sets of single-chain templates oriented relative to one another using one of twelve representative docking geometries chosen to cover a wide range of experimentally determined ternary complexes.
- TCR coordinate frames from class I pMHC ternary structures and the 12 representative transforms (thicker arrows) are shown in a common coordinate system defined by their corresponding pMHC coordinate frames.

PEPTIDE-MHC INTERACTIONS



peptide binding cleft commonly accommodates peptides 8 to 11 amino acids long and the classical function of HLA-B27 is to present peptides to a variety of ligands, in particular T-cell receptors on the surface of CD8+ cytotoxic T lymphocytes (CTLs).

MHC Class I

- $\alpha 1$ and $\alpha 2$ domains form peptide-binding domains • Antiparallel eight stranded β -pleated sheet form the floor • Two long α helices, oriented adjacent and roughly parallel to each other form sides of the deep cleft • Closed ends, accommodate about 8-11 aa in a flexible, extended conformation • Solvent Accessibility • About 80% Buried.

MHC Class II •

- Two long α helices as sides and the β sheet as bottom • The ends of class II peptide-binding cleft are more open, bind longer and irregular peptides (12-16 aa be optimal)
- Longer peptides. Ends can hang out. • Central core of about 13 amino acids. • Allele-specific binding motifs. • No bulge.

Comparison of Binding Clefs

	Class I Molecules	Class II Molecules
Nature of peptide-binding cleft	Closed at both ends	Open at both ends
General size of bound peptides	8-10 amino acids	13-18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide	Anchor residues distributed along the length of the peptide
Nature of bound peptide	Extended structure with both ends interacting and middle arching away from MHC cleft	Extended structure that is held at a constant elevation above the floor of the MHC cleft



- There are about a dozen of types of classical HLA molecules on the cell surface for one individual
- • There are much, much, much more kinds of antigen peptides would be presented in one individual
- Some amino acid residues of peptides anchor the peptide into the pockets within the groove of the MHC molecule, called anchor residue.
- a given MHC molecule binds a group peptides with same anchor residues
- The different MHC molecules bind different groups of peptide



SYFPEITHI-PEPTIDE DATABASE

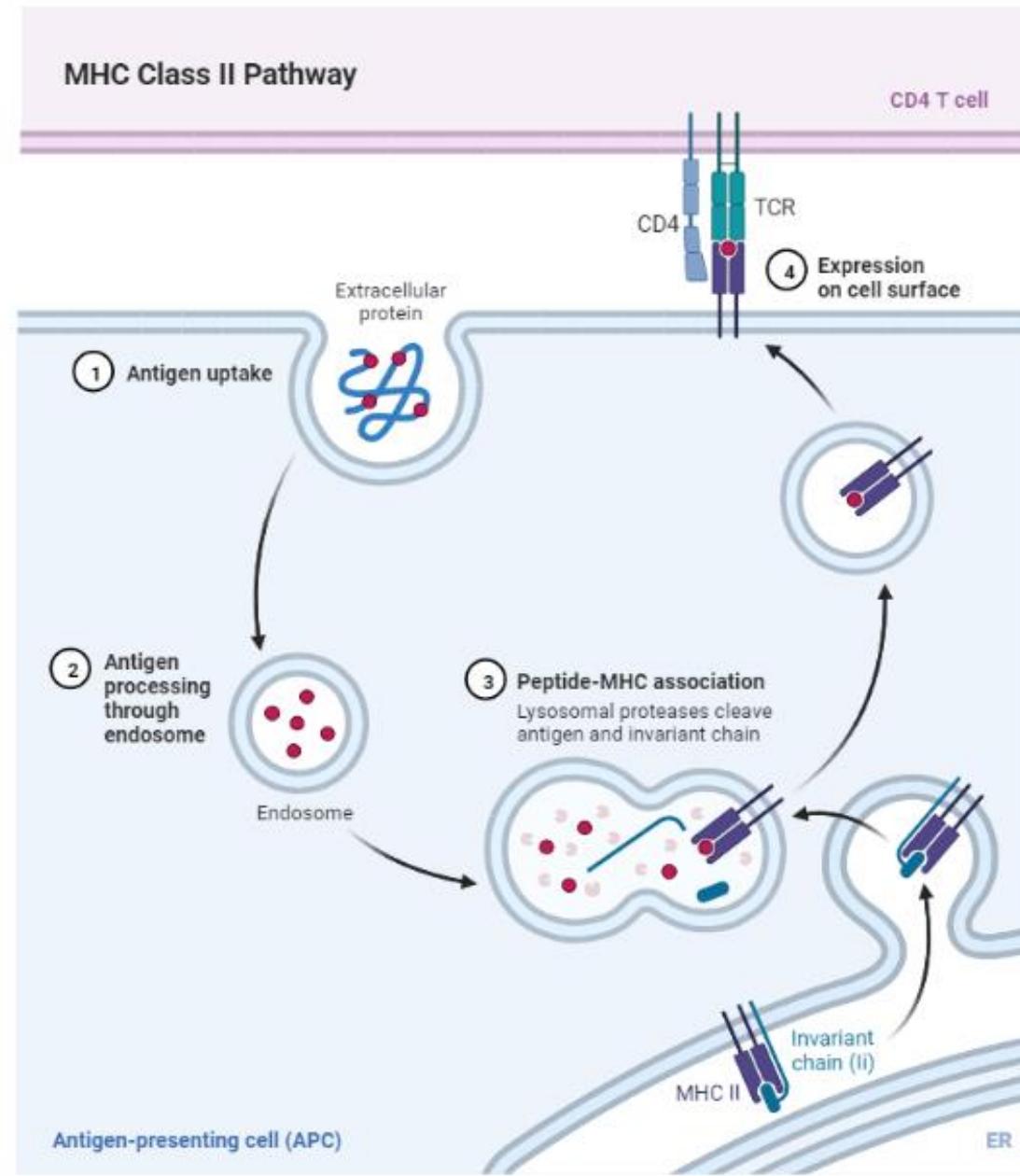
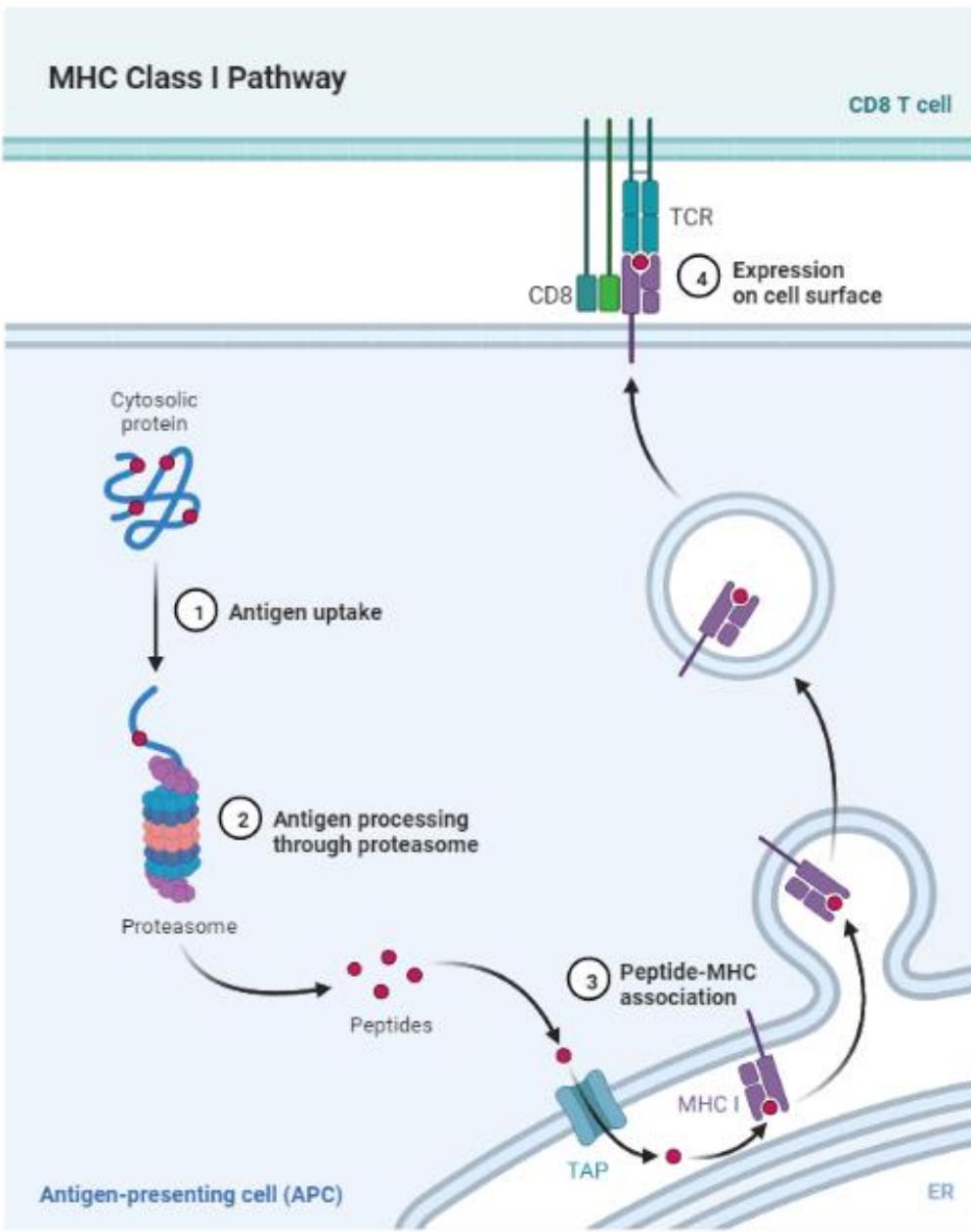
- SYFPEITHI, a database of interactions between proteins of the major histocompatibility complex (MHC) and antigenic peptides, contains information on MHC-associated peptide sequences, anchor positions, MHC molecule specificities and references to the published literature.
- It contains a collection of MHC class I and class II ligands and peptide motifs of humans and other species, such as apes, cattle, chicken, and mouse, for example, and is continuously updated. All motifs currently available are accessible as individual entries.
- Searches for MHC alleles, MHC motifs, natural ligands, T-cell epitopes, source proteins/organisms and references are possible.
- SYFPEITHI, a database of interactions between proteins of the major histocompatibility complex (MHC) and antigenic peptides, contains information on MHC-associated peptide sequences, anchor positions, MHC molecule specificities and references to the published literature. It is named after one of the first defined MHC-bound epitopes, a peptide that bound to the MHC molecule H₂-K^d.
- The database 'search' functions include epitope prediction and the retrieval of sequences on the basis of mass spectrometry data.
- The site also permits searching for epitopes associated with MHC molecules of known peptide-binding motif and allows for the definition of such motifs from sequence or mass spectrometry data.



ANTIGEN PRESENTATION & PROCESSING

- The recognition of protein antigens by T-lymphocytes required that the antigens be processed by Antigen-presenting Cells, then displayed within the cleft of the MHC molecules on the membrane of the cell.
- This involves the degradation of the protein antigens into peptides, a process known as antigen processing.
- When the antigen has been processed and degraded into peptides, it then associates with MHC molecules within the cell cytoplasm forming a peptide-MHC complex. This complex is then transported to the membrane, where it is displayed by a process of antigen presentation.
- The MHC Class I and class II MHC molecules associated with peptides that have been processed in different intracellular compartments.
- The Class I MHC molecules bind peptides derived from endogenous antigens that have been processed within the cytoplasm of the cell such as tumor proteins, bacterial proteins, or viral proteins, or cellular proteins, and processed within the cytosolic pathway.
- Class II MHC molecules bind peptides derived from exogenous antigens that are internalized by phagocytosis or endocytosis and processed within the endocytic pathway.





CYTOSOLIC PATHWAY: ENDOGENOUS ANTIGEN

- This is the pathway that processes and presents the endogenous antigen using the Class I MHC molecules.
- The antigen proteins are degraded intracellularly to short peptides by a cytosolic proteolytic system that is present in all cells. These proteins targeted for proteolysis have a small protein known as ubiquitin attached to them.
- The ubiquitin-protein conjugate then gets degraded by a multifunctional protease complex known as a proteasome.
- Each proteasome is a large (26S), cylindrical particle that consists of four rings of protein subunits and a central channel of 10–50 Å diameter.
- The proteasome can cleave peptide bonds between 2-3 different amino acid combinations in an ATP-dependent process.
- Degradation of the ubiquitin-protein complex takes place in the central hollow of the proteasome.
- The peptides are then transported from the cytosol to the rough endoplasmic reticulum. This is enabled by the transporter protein, designated TAP (transporter associated with antigen processing) is a membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2.
- The TAP1 and TAP2 proteins each have a domain projecting into the lumen of the Rough endoplasmic reticulum (RER), and an ATP-binding domain that extends into the cytosol.
- Both TAP1 and TAP2 belong to the family of ATP-binding cassette proteins found in the membranes of many cells, including bacteria.
- They mediate ATP-dependent transport of amino acids, sugars, ions, and peptides.

- the peptides that are generated in the cytosol by the proteasome, are translocated into the Rough Endoplasmic Reticulum (RER) by TAP proteins by a process that utilizes hydrolyzed ATP.
- TAP proteins have a high affinity for peptides sizes of 8-10 amino acids, the optimum length for class I MHC binding.
- Additionally, TAP proteins favor peptides with hydrophobic or basic carboxyl-terminal amino acids, which is the preferred anchor residue for class I MHC molecule, and therefore, TAP is optimized to transport peptides that will interact with class I MHC molecules.
- Next, the peptides that are assembled with class I MHC are aided by chaperone molecules that facilitate the folding of polypeptides.
- The alpha and beta-2-microglobulin components of the class I MHC molecules are synthesized on the polysomes along the rough endoplasmic reticulum.
- These components are assembled into a stable class I MHC molecules complex that can exit the RER requiring the presence of a peptide in the binding groove of the class molecule.

- The first chaperone involved is known as calnexin, which is a resident membrane protein of the endoplasmic reticulum.
- Calnexin associates with the class I α chain and promotes its folding. When the Beta-2-microglobulin binds to the α chain, the calnexin is released, and the class I molecule associates with the chaperone calreticulin and with tapasin.
- Tapasin is a TAP-associated protein that brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide. The physical association of the α chain-beta-2-microglobulin heterodimer with the TAP protein promotes peptide capture by the class I molecule before the peptides are exposed to the RER.
- The peptides not bound by class I molecules are rapidly degraded.
- After binding, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER, and proceed to the cell surface via the Golgi.
- An additional chaperone protein, ERp57, associates with calnexin and calreticulin complexes.
- The precise role of this resident endoplasmic reticulum protein in the class I peptide assembly and loading process has not yet been defined, but it is thought to contribute to the formation of disulfide bonds during the maturation of class I chains.

ENDOCYTIC PATHWAY: EXOGENOUS ANTIGEN

- Antigen-presenting cells can internalize antigen by phagocytosis, endocytosis, or both.
- Macrophages internalize antigen by both phagocytosis and endocytosis.
- Most of the other APCs are poorly phagocytic and can only internalize the antigen by pinocytosis or endocytosis, whereas most other APCs are not phagocytic and therefore they internalize the exogenous antigen only by endocytosis or by pinocytosis. B-cells which are also APCs internalizes the antigen effectively by receptor-mediated endocytosis using antigen-specific membrane antibody receptors.
- When the exogenous antigen is internalized, it is degraded into peptides in the compartments of the endocytic processing pathway.
- The breaking down of antigens into peptides takes 1-3 hours to transverse the endocytic pathway and appear at the cell surface in the form of a peptide-class II MHC complex.
- In this pathway, three acidic compartments: early endosome (pH 6.0-6.5), late endosome or endolysosomes (pH 5.0-6.0); and lysosomes (pH 4.5-5.0).
- The internalized antigen moves from the early to late endosomes and later to the lysosomes where they encounter the hydrolytic enzyme, with a decreasing pH in each compartment.



- the lysosomes have a unique collection of 40 acid-dependent hydrolases including proteases, nucleases, glycosidases, lipases, phospholipases, and phosphatases. Within the compartments of the endocytic pathway, the antigen is degraded into oligopeptide made up of 13-18 residues, that bind to class II MHC molecule.
- The hydrolytic enzymes are active in low pH, they inhibit antigen processing chemical agents that may increase the compartment pH and that of protease inhibitors.
- Movement of the peptides from one compartment to the next has been associated with small transport vesicles.
- After getting to the final compartments, they return to the cell periphery fusing with the plasma membrane, enabling the recycling of surface receptors.
- The antigen-presenting cells express both MHC I and MHC II molecules, therefore to prevent binding of MHC II to the same set of antigenic peptides as those of class I MHC, some mechanisms must exist to prevent this.
- When the MHC II has been synthesized within the RER, three pairs of class II chains associate with a preassembled trimer of a protein known as an invariant chain (Ii, CD74). The trimeric protein interacts with the peptide-binding cleft of the class II MHC molecules, preventing any endogenously derived peptides from binding to the cleft while the MHC class II remains within the RER.
- The invariant chain is also involved in the folding of class II MHC and its chains, the exit from the RER, and routing it to the endocytic processing pathway from the trans-Golgi network into the endocytic vesicles.

- Secondly, the peptides assemble with class II MHC molecule by displacing CLIP (Class-II associated invariant chain peptide).
- Most of class II MHC-invariant chain complexes are transported from the RER where they are formed through the Golgi complex and trans-Golgi network, and then through the endocytic pathway, moving from early endosomes to late endosomes then finally to the lysosomes.
- This increases the proteolytic activity from each compartment to the next.
- This causes the degradation of the invariant chain gradually, leaving a short fragment of the invariant chain known as the CLIP (Class II-associated invariant chain peptide) that remains bound to the class II molecule after the invariant chain has been cleaved with the endosomal compartment.
- CLIP occupies the peptide-binding groove of the class II MHC molecule, preventing premature binding of the antigenic peptide. HLA-DM molecule catalyzes the exchange of CLIP with the antigenic peptides.
- It is found in mammalian cells, mice, and rabbits. HLA-DM is neoclassical and nonpolymorphic.
- When the HLA-DM and class II CLIP complex react, it facilitates the exchange of CLIP for another peptide but in the presence of HLA-DO, it can bind to HLA-DM reducing the efficiency of the exchange reaction.
- The HLA-DO which has a similar structure as that of HLA-DM helps to modulate the function of HLA-DM, however, the function is obscure.

PRES^ENTATION OF NON-PEPTIDE ANTIGENS

- Nonpeptide antigens are also recognized by the immune system, these are antigens that are derived from infectious agents such as *Mycobacterium tuberculosis*.
- These antigens are recognized by T-cell Receptors known as $\delta\gamma$ -TCR (T-cell receptor are dimers of $\alpha\beta$ and $\delta\gamma$) which are derived from glycolipid of bacterial pathogens such as *Mycobacterium tuberculosis*.
- These nonprotein antigens are presented by members of the CD1 family of nonclassical class I molecules.
- The CD1 family of molecules associates with $\beta 2$ -microglobulin and it has its structure similar to that of MHC I molecules. It has 5 genes that encode for human CD1 molecules (CD1A-E, encoding the gene products CD1a-d, no E has been identified yet. These genes are located on the chromosomes and not on MHC I).
- They are classified into two groups based on sequence homology. Group 1 includes CD1A, B, C, and E; CD1D is in group 2. All mammalian species have CD1 genes, although the number varies. Rodents have only group 2 CD1 genes, whereas rabbits, like humans, have five genes, including both group 1 and 2 types.
- The sequence identity of CD1 with classical class I molecules is considerably lower than the identity of the class I molecules with each other. CD1D1 as compared to class I MHC shows that the antigen-binding groove of Cd1d1 is deeper and more voluminous than that of class I MHC molecule.



FUNCTION OF HLA MOLECULES

- • Antigen presentation
- – process : endogenous antigen and exogenous
- antigen
- – presentation recognize MHC: peptide complex
- (double recognition)
- – MHC restriction of T cell: Any individual's T cells
- respond to a specific MHC allele expressed by that
- individual, that is to “self” MHC
- • others
- – Genetically regulator of immune response, so to
- predispose individuals to particular susceptibility or
- disorders
- – Immune regulation



MHC CLASS 1 VERUS MHC CLASS 2

MHC class 1 are a class of major histocompatibility complex molecules found on the surface of all nucleated cells in mammals

Expressed on all types of nucleated cells in the body

Composed of three alpha domains and a single beta domain

Composed of a single, membrane-spanning, alpha domain

Three types are MHC-A, MHC-B, and MHC-C

MHC class 2 are a class of major histocompatibility complex molecules mainly found on antigen presenting cells such as macrophages, dendritic cells, and B cells

Expressed on antigen presenting cells such as B cells, macrophages, and dendritic cells

Composed of two alpha and beta domains

Composed of two membrane-spanning alpha and beta domains

MHC-D is the main type

Alpha domains are encoded on the MHC locus of chromosome 6; beta chains are encoded on chromosome 15

Present endogenous antigens originated from the cytoplasm

Alpha 1 and alpha 2 domains are involved in the presentation of antigens

Present antigens to cytotoxic T cells

Bind to the CD8+ receptors on the cytotoxic T cells

Responsible for the clearance of endogenous antigens

Encoded on the chromosome 6

Present exogenous antigens originated extracellularly from foreign bodies such as pathogens

Alpha 1 and beta 2 domains are involved in the antigen presentation

Present antigens to helper T cells

Bind to the CD4+ receptors on the helper T cells

Responsible for the clearance of exogenous antigens

3 MAIN TYPES OF GENETIC MECHANISMS

- The types are:
- 1. Immuno-Genetics
- 2. The HLA System
- 3. The Rh Factor.



IMMUNO-GENETICS

- Genetic mechanisms which control immune responses have opened up an entire new field of immuno-genetics.
- Basically it is the study of antigens, antibodies and their reactions.
- An antigen is a substance present in the body or introduced from outside which can initiate an immune reaction.
- The antigens present on the surface of red and white blood cells are important in immuno-genetics.
- The immune reactions in response to the antigen take place in the lymphoid organs (spleen, lymph nodes and tonsils) and result in the production of antibodies or sensitized lymphocytes which are effective in eliminating the antigen from the body.
- As there are millions of potential antigens, there are many millions of species of antibody molecules that are synthesised by the immune system.



THE RH FACTOR

- In 1940, Wiener and Landsteiner discovered that an antigenic factor Rh, which is found on the surface of red blood cells of the Indian brown monkey (*Macacus rhesus*), is also present in man.
- They injected the blood of rhesus monkey into rabbits and guinea pigs.
- Antibodies produced in the blood serum of these animals were found to agglutinate the red cells of the rhesus monkey.



THE HLA SYSTEM

- The genetic mechanisms controlling transplantation antigens play a significant role in the organ transplant technique.
- The treatment of certain human diseases requires transfer of grafts from a host to a recipient individual of a different genetic make-up (allograft).
- The existence of transplantation antigens first became known from skin grafting experiments in mice.
- Whether a graft would be rejected or accepted by the recipient mouse depended upon the presence of histocompatibility antigens present on the skin and other tissue cells.
- From the results of genetic experiments it was concluded that the histocompatibility antigens were controlled by several different genes, each gene having multiple alleles.



- That the red blood cells in humans contain blood group antigens on their surface.
- These antigens are important in blood transfusions.
- The white blood cells also carry several antigens which are important in organ transplantation.
- These are called histocompatibility antigens and are found to be controlled by four gene loci A, B, C and D on chromosome 6.
- The histocompatibility (histo meaning tissue) antigens and their corresponding genes constitute the HLA (human leukocyte antigen) system.
- When a graft is transplanted, the lymph nodes of the recipient individual respond to the histocompatibility antigens of the genetically different tissue by producing lymphocytes which are of two types, the T lymphocytes (thymus dependent) and the B lymphocytes (cells equivalent to the bursa in birds).
- The T cells are important in graft rejection, and are involved in the cell mediated immune response (CMI).
- The B cells are involved in the humoral responses (HR) which produce antibodies against viruses, bacteria and such invasions, as well as against graft cells of the host.
- It is mainly the T cells which respond to the histocompatibility antigens present on the grafted tissue.

- The genetics of HLA has been studied by performing breeding experiments in mice.
- When two mice homozygous for the histocompatibility antigens are crossed, the F1 mice are able to accept grafts from both parents.
- When F1 mice are inbred, three of the progeny mice accept grafts from either parent, while one mouse shows rejection.
- Applying the principles of Mendelian inheritance one can conclude that the parental mice differ at a single gene locus. From similar experiments a number of loci controlling histocompatibility antigens could be determined. Each locus has several alleles.
- One complex locus H-Z controls the strong transplantation antigens in mouse.
- In humans, the strongest transplantation antigens are controlled by four distinct loci A, B, C and D on chromosome 6
- Additional genes related to histocompatibility also lie in the adjacent regions.
- Studies on different individuals in the population have revealed that the A gene has more than 12 alleles, and B gene at least 20 alleles controlling more than 32 histocompatibility antigens.
- As the A and B genes are only one map unit apart, there is very little chance of crossing over between them; they are thus transmitted together in most cases.
- Since any allele at A can be associated with any allele at B, the number of possible combinations of the alleles of A and B genes in the population is about 240 (12×20).
- A combination of the alleles of the A and B genes is called the haplotype. Designating the alleles of the A locus as A₁, A₂, ..., A₁₂, and at B as B₁, B₂, ..., B₂₀, suppose one chromosome 6 of a particular individual is carrying the alleles A₅ and B₉.
- The haplotype in this case would be written as 5 9 (A allele is written to the left and B allele to the right). As there are two number 6 chromosomes in a cell, the HLA genotype of a person consists of two haplotypes.

- The presence of a particular allele in the individual is determined serologically from the existence of a specific antigen on the leukocyte surface by the technique of leukocyte typing, also called HLA typing.
- Thus individuals may be homozygous or heterozygous for any pair of alleles.
- Due to the highly polymorphic and complex nature of the HLA locus, it is very unlikely that any two persons picked at random should have the same set of leukocyte antigens.
- These genetic differences lead to rejection of allografts in organ transplantation. Due to this reason the host and recipient HLA genotypes have to be closely matched.

- **Allele**
- "Allele" is the word that we use to describe the alternative form or versions of a gene. People inherit one allele for each autosomal gene from each parent, and we tend to lump the alleles into categories
- MHC alleles are expressed in codominant fashion. This means the alleles (variants) inherited from both parents are expressed equally: Each person carries 2 alleles of each of the 3 class-I genes, (HLA-A, HLA-B and HLA-C), and so can express six different types of MHC-I

- **Allograft**

- Allograft (allo- from the Greek meaning 'other') is an organ removed from a genetically nonidentical donor and transplanted to a recipient of the same species.
- Direct allore cognition is the process by which donor-derived major histocompatibility complex (MHC)-peptide complexes, typically presented by donor-derived 'passenger' dendritic cells, are recognised directly by recipient T cells.

- **Haplotype**

- A haplotype refers to a set of DNA variants along a single chromosome that tend to be inherited together. They tend to be inherited together because they are close to each other on the chromosome, and recombinations between these variants are rare
- Extended major histocompatibility complex (MHC) haplotypes are fixed conserved regions of the short arm of the sixth human chromosome defined by their HLA-B, complotype (BF, C2, C4A, C4B), HLA-DR alleles.



WHAT ARE THE GENETIC MECHANISMS?

- Polygenicity

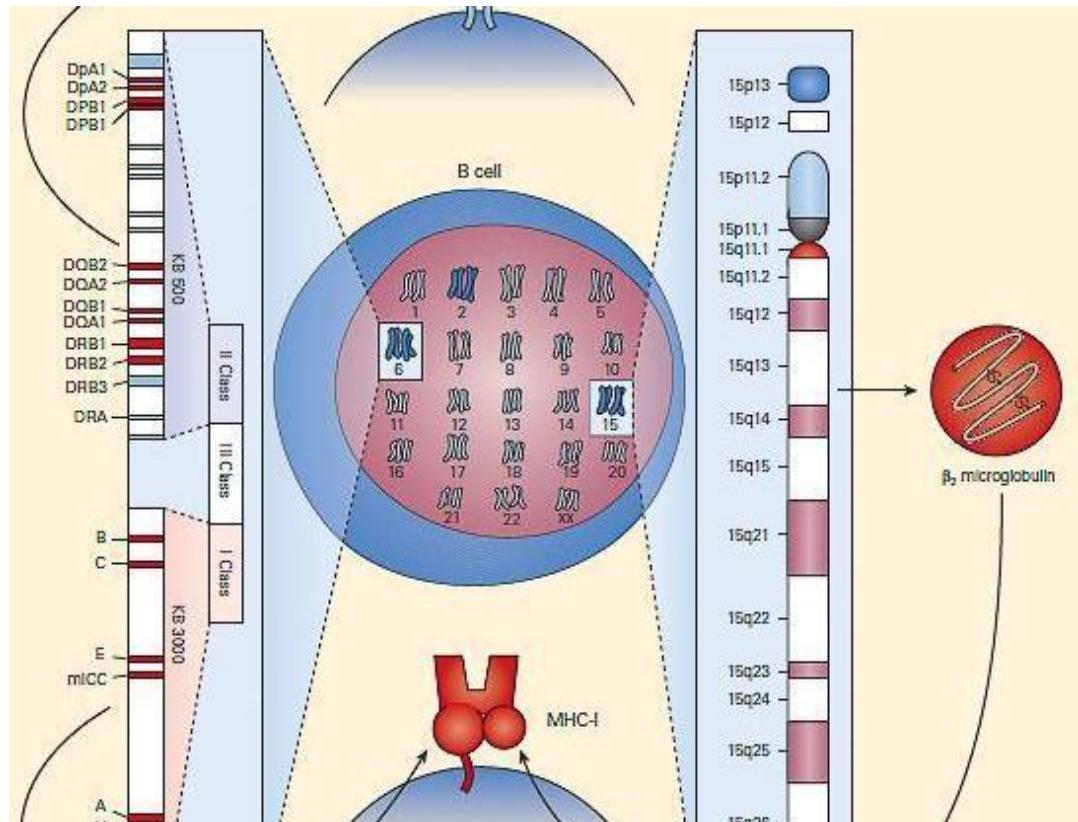
- A polygenic trait is a characteristic, such as height or skin color, that is influenced by two or more genes. Because multiple genes are involved, polygenic traits do not follow the patterns of Mendelian inheritance. Many polygenic traits are also influenced by the environment and are called multifactorial.
- it contains several different MHC class I and MHC class II genes, so that every individual possesses a set of MHC molecules with different ranges of peptide-binding specificities.

- Polymorphism

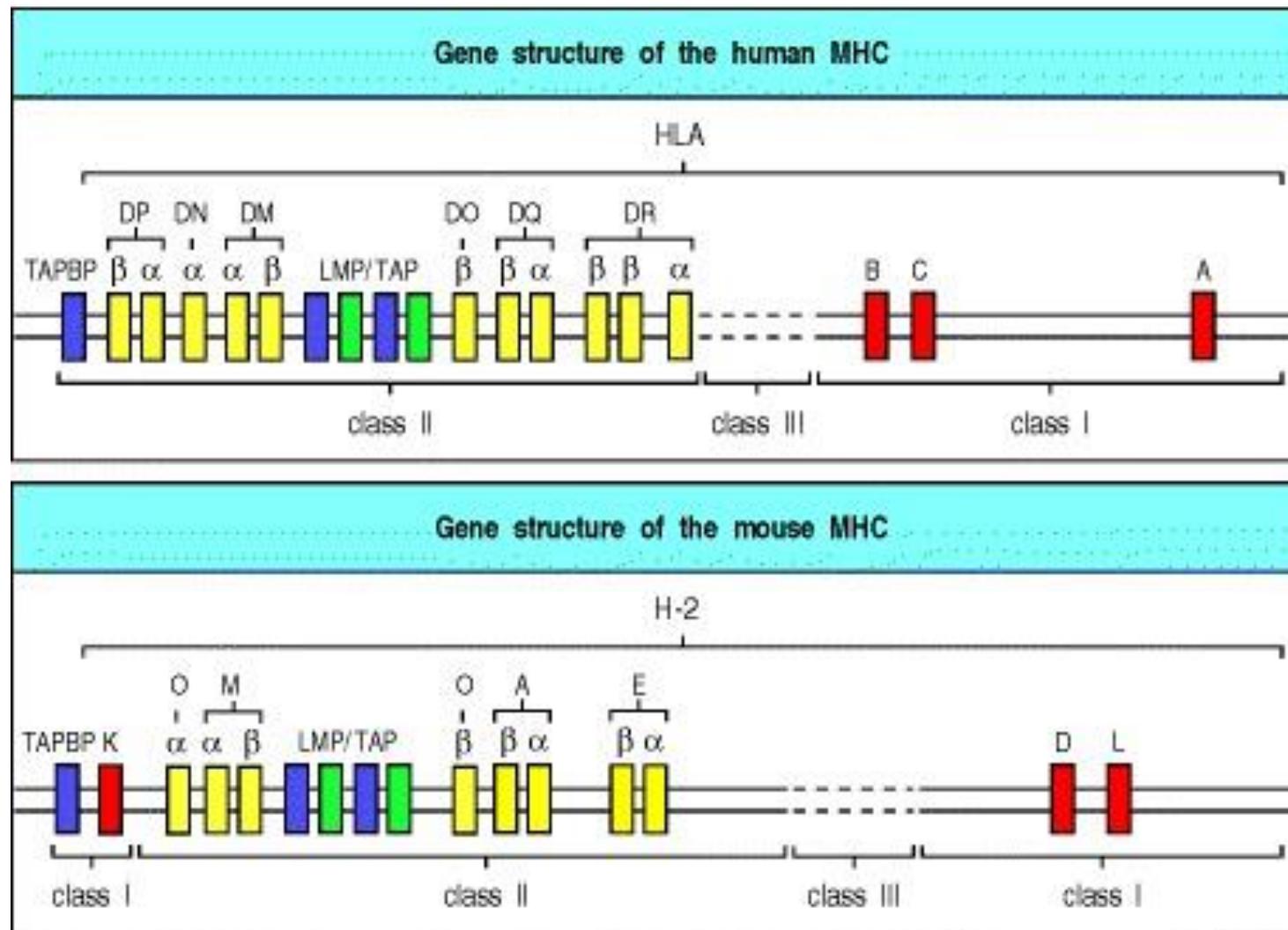
- The word “polymorphism” means having many forms. In simple words, we can define polymorphism as the ability of a message to be displayed in more than one form. A real-life example of polymorphism is a person who at the same time can have different characteristics.
- MHC class I and class II genes are highly polymorphic in the regions that encode the peptide binding groove. These polymorphisms help ensure survival of the population by increasing the variety of peptides that can be presented to T cells.



WHAT IS THE DIFFERENCE BETWEEN POLYGENIC AND POLYMORPHIC MHC?



- First, the MHC is polygenic. It contains several different MHC-I and MHC-II genes so that every individual possesses a set of MHC molecules with different ranges of peptide-binding specificities.
- Second, the MHC is extremely polymorphic. The MHC genes display the greatest degree of polymorphism in the human genome.



- The genes encoding the α chains of MHC class I molecules and the α and β chains of MHC class II molecules are linked within the complex
 - The genes for $\beta 2$ -microglobulin and the invariant chain are on different chromosomes (chromosomes 15 and 5, respectively, in humans and chromosomes 2 and 18 in the mouse).
 - In humans these genes are called **Human Leukocyte Antigen** or HLA genes

CLASSIFICATION

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C' proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-A

POLYGENECITY

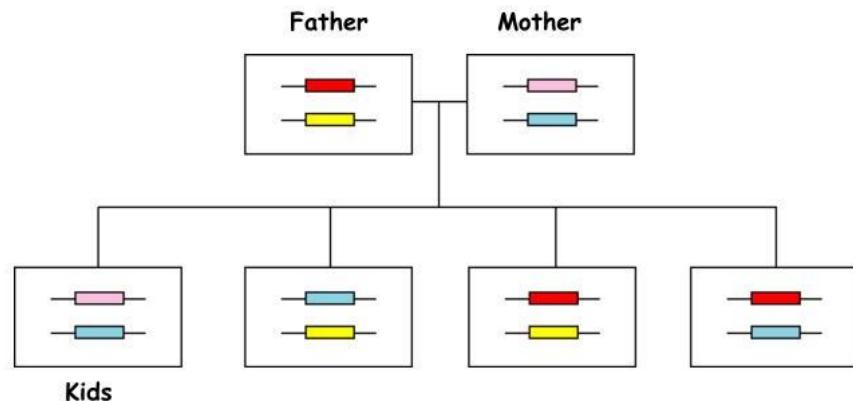
POLYMORPHISM

CO-DOMINANCE

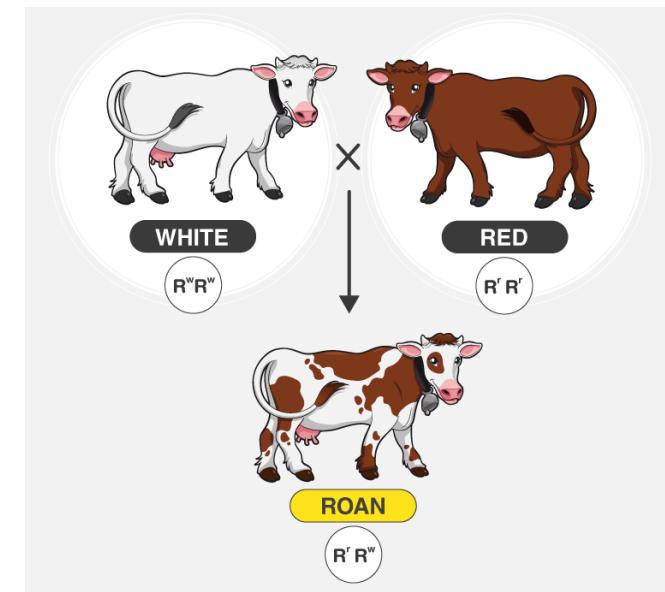
LINKAGE
DISEQUILIBRIUM



- The most intensely-studied HLA genes are the nine so-called classical MHC genes:
- HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, and HLA-DRB1.
- The A, B, C, E, F, and G genes belong to MHC class I
- whereas the six D genes belong to class II.
- MHC genes are expressed in **co-dominant** fashion. This means that the alleles inherited from both progenitors are expressed in an equivalent way.
- As there are 3 Class-I genes, named in humans **HLA-A, HLA-B and HLA-C**, and as each person inherits a set of genes from each progenitor, that means that any cell in an individual can express 6 different types of MHC-I molecules.

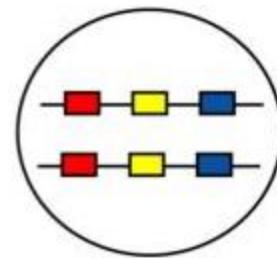
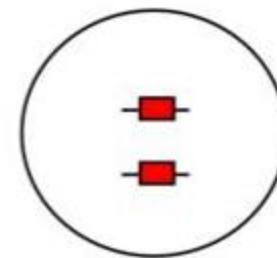


Co-dominant Expression

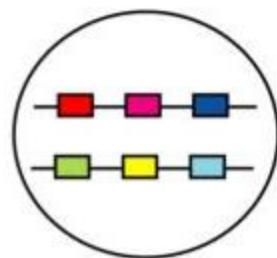
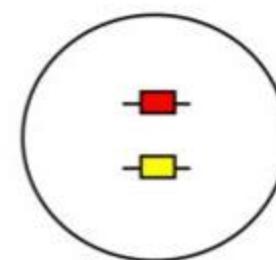


Polygeny

- In the Class-II locus, each person inherits \
- couple of genes HLA-DP (DPA1 and DPA2, which encode α and β chains),
- a couple of genes HLA-DQ (DQA1 and DQA2, for α and β chains),
- one gene HLA-DR α (DRA1)
- one or two genes HLA-DR β (DRB1 and DRB3, -4 o -5).
- That means that one heterozygous individual can inherit 6 or 8 Class-II alleles, three or four from each progenitor.
- The set of alleles that is present in each chromosome is called MHC haplotype.
- In humans, each HLA allele is named with a number.
- Each heterozygous individual will have two MHC haplotypes, one in each chromosome (one of paternal origin and the other of maternal origin).
- The MHC genes are **highly polymorphic**; this means that there are many different alleles in the different individuals inside a population.
- The polymorphism is so high that in a mixed population (non-endogamic) **there are not two individuals with exactly the same set of MHC genes and molecules, with the exception of identical twins.**



Polymorphic



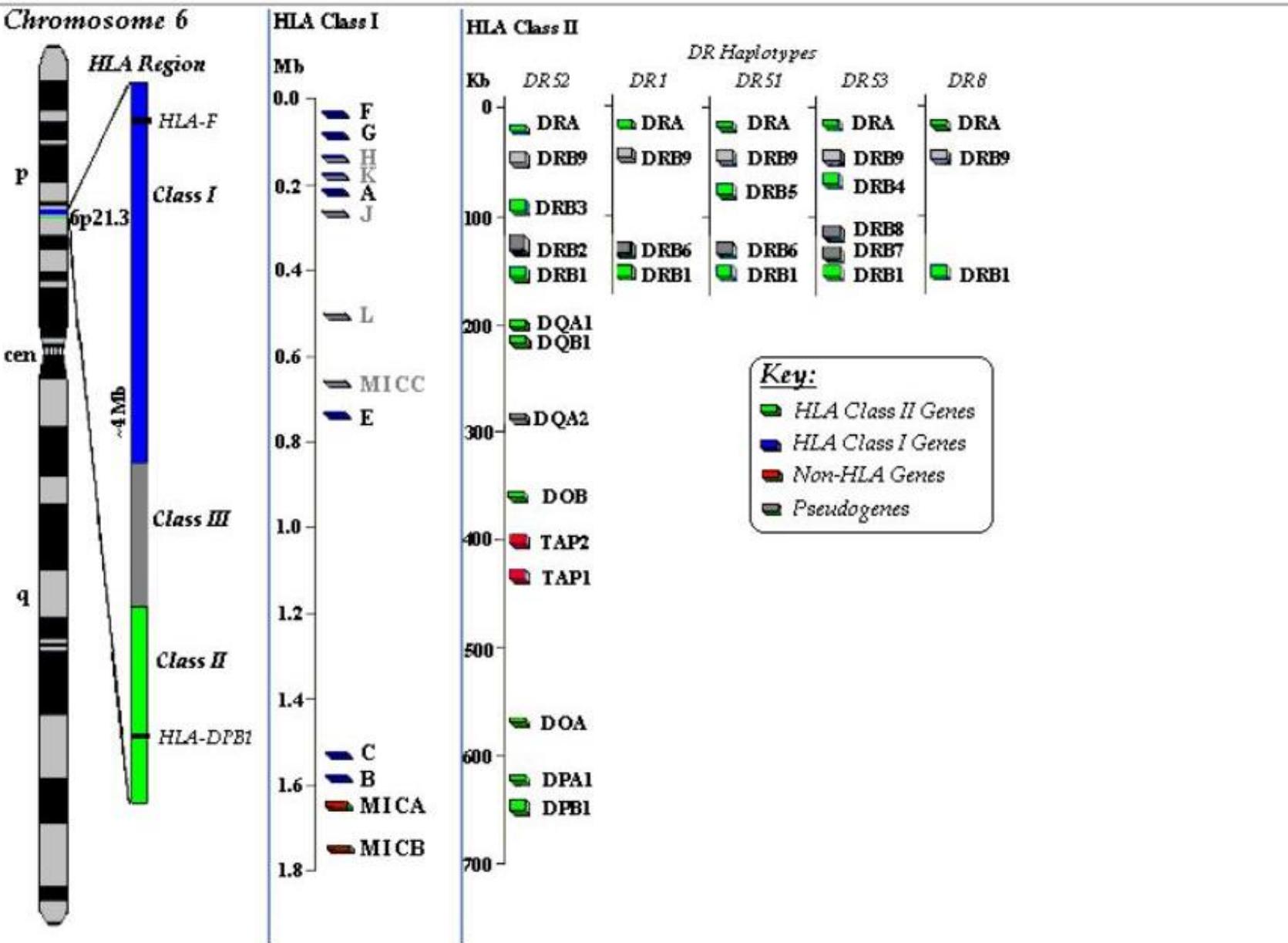
- MHC proteins (from the “haplotype”) constitute a life-long cell surface character for any vertebrate.
 - This circumstance is very different from Ig's which are constantly being generated in response to new foreign proteins and carbohydrates in the environment.
-
- The loci which specify MHC's are polymorphic.
 - Many alleles may exist at a locus:
 - HLA A locus ~ 60 alleles
 - HLA B locus ~ 110 alleles
 - HLA C locus ~ 40 alleles
 - The high level of allelism creates diversity within a species (thus restricting allografting) but does not produce diversity within an individual.



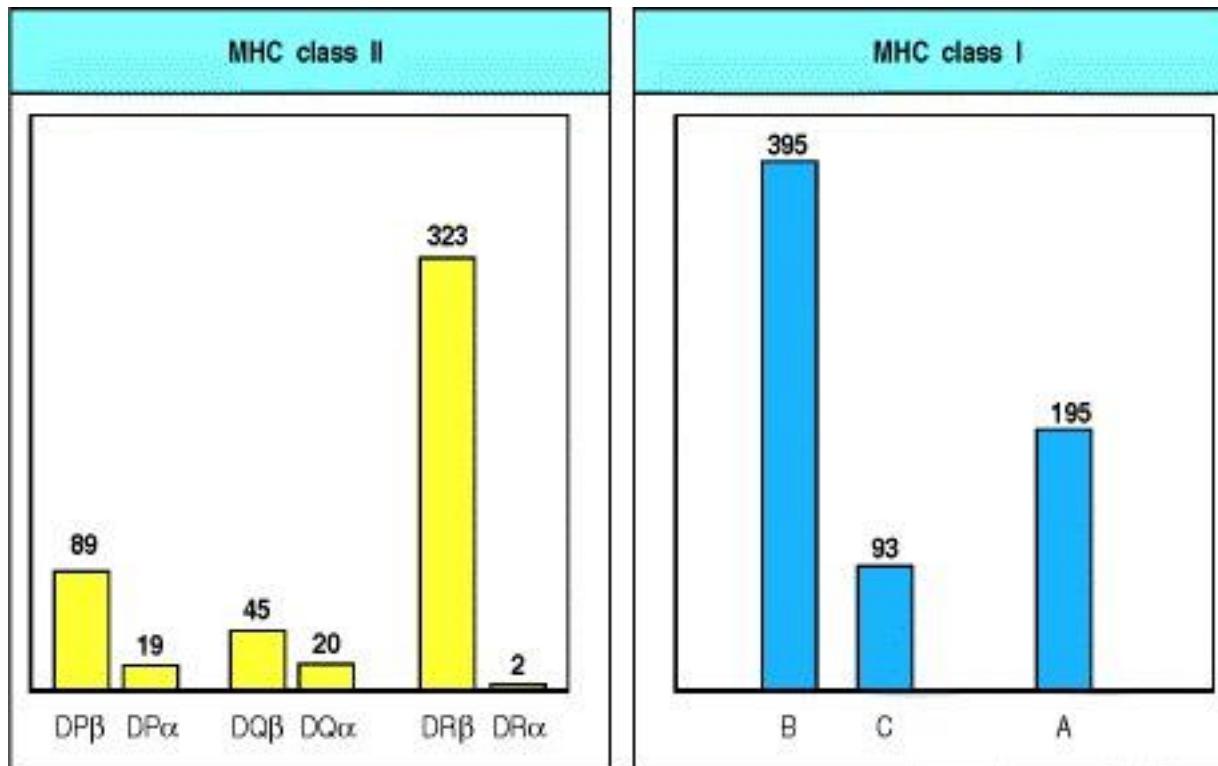
MHC GENE REGION

- The gene map for the extended major histocompatibility complex (xMHC) comprises 421 loci (excluding RNA genes) in a sequence length of 7.6 Mb — extending the previous gene map of the classical MHC, which was 3.6 Mb long and contained 224 loci.
- All 421 xMHC loci have been assigned definitive and approved gene symbols.
- About 50% of the xMHC gene loci are present in clusters or superclusters that are not restricted only to immune genes. The two largest clusters, comprising histone and tRNA genes are the largest of their type in the genome.
- Transcription hotspot analysis indicates that it is just as likely that the classical MHC is hitch-hiking with gene clusters of the xMHC as the reverse.
- About 22% of the expressed xMHC genes show a higher than average number of non-synonymous coding polymorphisms.
- About 28% of the xMHC genes can be associated with immune system function.
- About 10% of the xMHC genes are currently known to be disease-causing or disease-associated.
- About 20% of the xMHC genes have putative paralogues elsewhere in the genome, indicating considerable potential for functional redundancy.
- The gene map of the xMHC provides an invaluable resource for the study of the most important genetic region of the human genome in relation to infectious, inflammatory and autoimmune diseases.



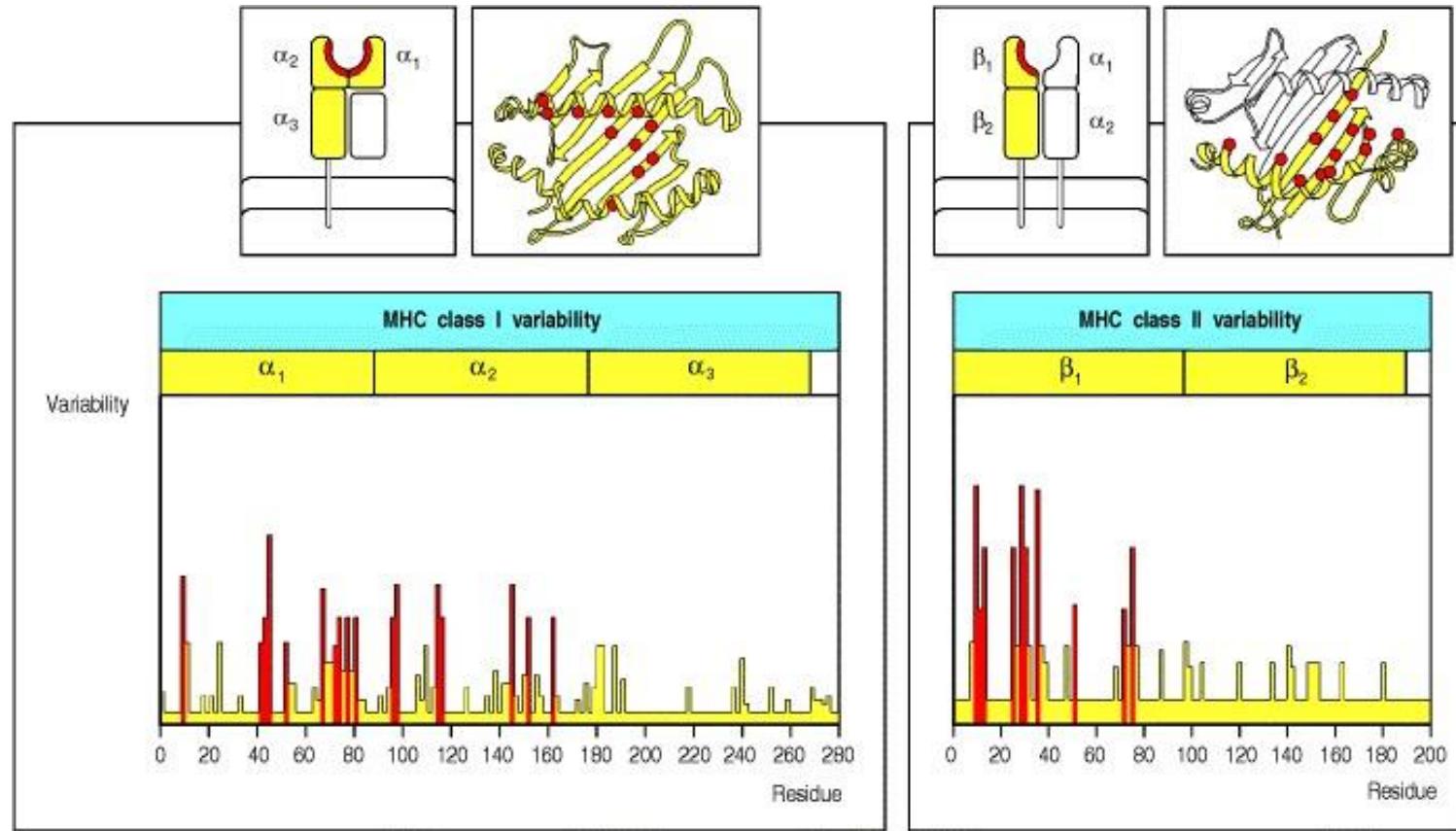


POLYMORPHISM



- The term polymorphism comes from the Greek poly, meaning many, and morphe, meaning shape or structure
- within-species variation at a gene locus, and thus in its protein product; the variant genes that can occupy the locus are termed alleles
- more than 200 alleles of some human MHC class I and class II genes
- With the notable exception of the DR α locus, which is functionally monomorphic, each locus has many alleles.

MHC POLYMORPHISM AFFECTS ANTIGEN RECOGNITION BY T CELLS



- The products of individual MHC alleles can differ from one another by up to 20 amino acids, making each variant protein quite distinct.
- Most of the differences are localized to exposed surfaces of the outer domain of the molecule, and to the peptide-binding groove in particular
- The polymorphic residues that line the peptide-binding groove determine the peptide-binding properties of the different MHC molecules.
- Polymorphism in MHC class I molecules affects which amino acids line these pockets and thus their binding specificity.
- Different allelic variants of MHC class II molecules also bind different peptides, but the more open structure of the MHC class II peptide-binding groove and the greater length of the peptides bound in it allow greater flexibility in peptide binding

MHC POLYMORPHISM & VACCINE DESIGN

- Few human will share the same set of alleles
- different people will react to a pathogen infection in a non similar manner
- A CTL based vaccine must include epitopes specific for each HLA allele in a population
- It must consist of ~800 MHC class I epitopes and ~400 class II epitopes



CLUSTERING MHC

- The MHCcluster method estimates the functional relationship between two molecules from the overlap in prediction binding specificity, and returns a heat map and graphical tree-based visualizations of the functional relationship between MHC variants
- The identification of peptides binding to major histocompatibility complexes (MHC) is a critical step in the understanding of T cell immune responses.
- The human MHC genomic region (HLA) is extremely polymorphic comprising several thousand alleles, many encoding a distinct molecule.
- The potentially unique specificities remain experimentally uncharacterized for the vast majority of HLA molecules. Likewise, for nonhuman species, only a minor fraction of the known MHC molecules have been characterized.
- Here, we describe a tool, MHCcluster, to functionally cluster MHC molecules based on their predicted binding specificity.
- The method has a flexible web interface that allows the user to include any MHC of interest in the analysis.



CLUSTERING MHC

- The output consists of a static heat map and graphical tree-based visualizations of the functional relationship between MHC variants and a dynamic TreeViewer interface where both the functional relationship and the individual binding specificities of MHC molecules are visualized.
- We demonstrate that conventional sequence-based clustering will fail to identify the functional relationship between molecules, when applied to MHC system, and only through the use of the predicted binding specificity can a correct clustering be found.
- Clustering of prevalent HLA-A and HLA-B alleles using MHCcluster confirms the presence of 12 major specificity groups (supertypes) some however with highly divergent specificities.
- Importantly, some HLA molecules are shown not to fit any supertype classification. Also, we use MHCcluster to show that chimpanzee MHC class I molecules have a reduced functional diversity compared to that of HLA class I molecules.
- MHCcluster is available at www.cbs.dtu.dk/services/MHCcluster-2.0



MHC SUPERTYPES

- The definition of an HLA supertype is that HLA molecules with similar peptide binding features are grouped into one supertype; this means that if a peptide is able to bind to one allele within a supertype, it can also bind to all other alleles in this supertype.
- Many MHC molecules have similar specificities
- MHC molecules with similar specificities can be grouped together
- Methods to define Supertypes
 - Primary (Sequence)
 - Tertiary (Structure)
- Shared peptide binding motifs
- Identification of cross reacting peptides
- Ability to generate methods that can predict cross binding peptides



HLA-I SUPERTYPES

- HLA-I has been defined **by structural similarities, shared peptide-binding motifs, and identification of cross-reacting peptides**
- Sette and Sidney: Based on motifs derived from binding data or sequencing of endogenously bound peptides, along with simple structural analyses: HLA-A1, -A2, -A3, -A24, -B7, -B27, -B44, -B58, -B62
- Ole Lund's group: using this similarity to cluster alleles into supertypes: constructed hidden Markov models (HMMs): Gibbs sampling procedure and defined a similarity measure between these sequence motifs: three new HLA-I supertypes HLA-A26, -B8, and -B39



HLA-II SUPERTYPES

- peptide binding data for HLA-II molecules is less available than those for HLA-I molecules due to the complexity of HLA-II structure
- Ou et al.: grouped HLA-DR molecules into seven different functional supertypes on the basis of their ability to bind and present antigenic peptides to T cells and their association with susceptibility or resistance to disease
- Castelli et al: defined an HLA-DP4 supertype and supported the existence of three main binding supertypes among HLA-DP molecules.
- Doytchinova et al. : applied a combined bioinformatics approach using both protein sequence and structural data, to 2,225 HLA-II molecules, to detect similarities in their peptide-binding sites for definition of HLA-II supertypes: They defined 12 HLA-II supertypes, including five DRs (DR1, DR3, DR4, DR5, and DR9), three DQs (DQ1, DQ2, and DQ3), and four DPs (DPw1, DPw2, DPw4, and DPw6).



- Greenbaum et al.: determined the binding capacity of a large panel of non-redundant peptides for a set of 27 common HLA DR, DQ, and DP molecules: measured binding data were then used to define class II supertypes on the basis of shared binding repertoires: Seven different supertypes (main DR, DR4, DRB3, main DQ, DQ7, main DP, and DP2) were defined
- according to motif-based supertype classification: seven different supertypes were defined after the analysis of 27 HLA II proteins
- All the molecules belonging to the DP genetic locus (DPB1*0101, DPB1*0201, DPB1*0401, DPB1*0402, DPB1*0501, and DPB1*1401) were grouped into a single supertype; DQ proteins were grouped into two different supertypes, each containing three HLAs: (DQB1*0301, DQB1*0302, DQB1*0401) and (DQB1*0201, DQB1*0501, DQB1*0602).
- The motif-based classification of the DR proteins is less defined compared with the other loci. The HLA-DR can be grouped into four supertypes: (DRB1*0401, DRB1*0405, DRB1*0802, DRB1*1101), (DRB3*0101, DRB3*0202), (DRB1*0301, DRB1*1302), and the fourth containing the remaining DR proteins.
- Functional and motif-based clustering of 27 defined HLA-II molecules revealed the presence of proteins sharing both functional and structural properties, thus supporting the concept of HLA-II supertypes.

HLA SUPERTYPES AND VACCINES

- One of the major drawbacks of a peptide-based vaccine strategy is that the restricting HLA genes are extremely polymorphic resulting in a vast diversity of peptide-binding HLA specificities and a low population coverage for any given peptide–HLA specificity.
- To increase population coverage, one might include defined epitopes for each HLA-I allele; however, this would lead to a vaccine comprising hundreds of peptides.
- group HLA molecules into HLA supertypes; a classification that as mentioned above refers to a group of HLA alleles with largely overlapping peptide binding specificities
- this means that a peptide, which binds to one allele within a supertype, has a high probability of binding to other allelic members of the same supertype.
- The concept of HLA supertypes has been successfully applied to characterize and identify T cell epitopes from a variety of different pathogens, including measles-mumps-rubella, SARS, EBV, HIV, HCV, HBV, HPV, influenza, LCMV, Lassa virus, *F. tularensis*, vaccinia, and cancer antigens as well



HLA SUPERTYPES AND VACCINES

- HLA supertypes have been utilized as a component in several approaches and algorithms designed for predicting peptide candidates
- The technology behind “reverse immunology” is developing rapidly in order to identify T cell epitopes from tumor antigens and infectious microorganisms
- “reverse immunology” as a powerful tool to identify T cell epitopes has now reached the stage where genome-, pathogen-, and HLA-wide scanning for HLA-binding antigenic epitopes become feasible at a scale and speed that makes it possible to exploit the genome information as fast as it can be generated
- a large-scale dataset of measured HLA-II-binding affinities covering 26 allelic variants, including a total of 44541 affinity measurements for HLA-DR alleles as well as 11 HLA-DP and DQ molecules
- classification of HLA supertypes reduces complexity of HLA polymorphisms and has a significant impact on the development of peptide-based vaccines with maximum population coverage. Since CD4+ T cells are required for priming of naïve CD8+ T cells as well as expansion of CD8+ memory T cells
- it is of critical importance to incorporate both HLA-I and -II supertype-restricted epitopes in peptide-based vaccines with maximum population coverage to obtain participation of both CD4+ and CD8+ T cells for generation of strong and long-lasting immunity



NOVEL SUPERTYPES

- Splitting the A1 supertype alleles into a new A26 supertype
- Splitting the A1 supertype alleles into a new B27 supertype
- B8 alleles may define their own supertype



MAIN FEATURES OF MHC

- Providing the strongest barrier to transplantation
- Play a central role in generation and execution of immune response – Presentation of peptide antigens to T cells
- Susceptibility to infectious diseases and development of autoimmune diseases



HLA AND CLINICAL MEDICINE

- 1. HLA and transplantation rejection
- 2. HLA related-Disease
- 3. HLA abnormal expression and disease
- 4. HLA and human ID for crime medical detection and identification of progeny
- 5. Mating preference and olfactory sense ?
- 6. CNS development and repair ?



QUESTIONS

- All are features of cell-surface HLA-B molecules, EXCEPT:
- A. They are associated with $\beta 2$ microglobulin.
- B. They bind exogenous peptides.
- C. They are polymorphic.
- D. They are expressed on B lymphocytes.
- E. They can be bound by CD8 molecules.



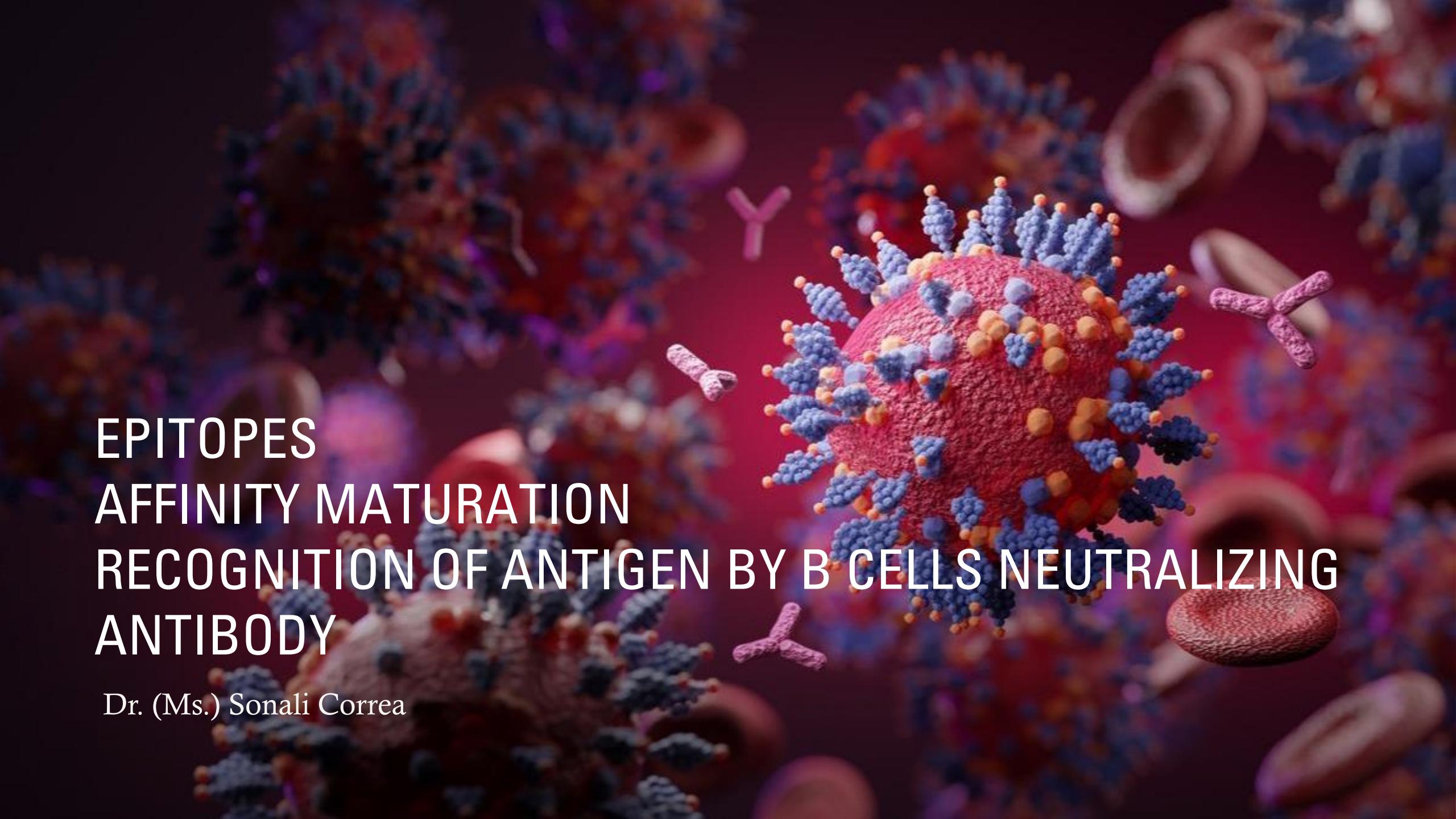
- Which one of the following is TRUE about class II MHC molecules?
- A. They consist of an alpha chain of three domains and a β 2microglobulin.
- B. They are found in all nucleated cells of our body.
- C. They are involved in antigen presentation to CD8+ cytotoxic lymphocytes.
- D. They consist of DR, DQ, and DP molecules.
- E. They are located on the X chromosomes

- Q1: What is the role of MHC proteins in the immune system?
 - Answer: The MHC proteins play a vital role in the immune system by presenting antigens to the immune cells, which triggers an immune response against foreign substances.
-
- Q2: Can MHC Class I and MHC Class II proteins present the same antigen?
 - Answer: No, MHC Class I and MHC Class II proteins present antigens derived from different sources and interact with different types of T cells.
-
- Q3: Are MHC Class I and MHC Class II genes polymorphic?
 - Answer: Yes, MHC genes are highly polymorphic, meaning they exist in different variants or alleles within a population, contributing to the diversity of antigen presentation.
-
- Q4: What happens if there is a mismatch between MHC proteins during organ transplantation?
 - Answer: The Mismatched MHC proteins can lead to the immune response, as the recipient's immune system may recognize the transplanted organ as foreign and mount an immune attack, resulting in organ rejection.



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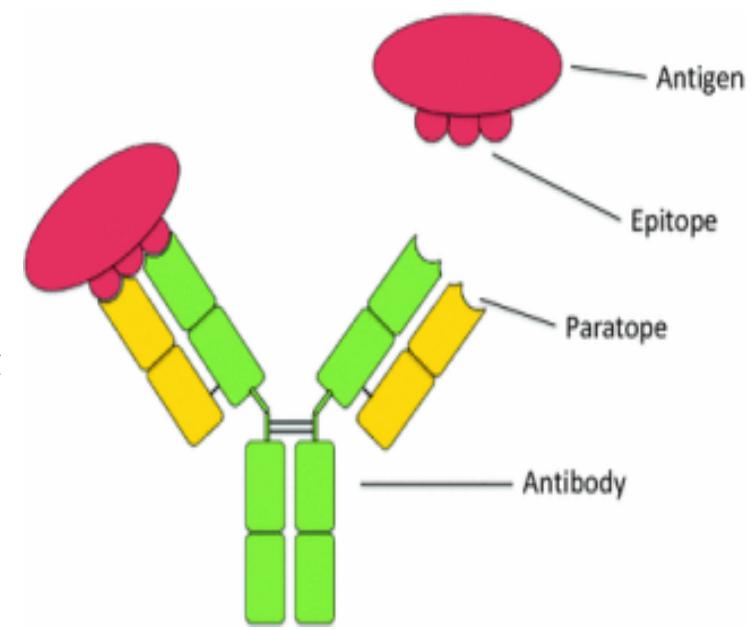


EPITOPES AFFINITY MATURATION RECOGNITION OF ANTIGEN BY B CELLS NEUTRALIZING ANTIBODY

Dr. (Ms.) Sonali Correa

EPITOPES

- Epitopes, also known as **antigenic determinants**, are the immunologically active discrete sites on the antigen molecule that physically bind to antibodies, B-cell receptors, or T-cell receptors.
- Is a specific piece of the antigen to which an antibody binds
- The part of an antibody that binds to the epitope is called a **paratope**
- Are usually **non-self proteins**, however, sequences derived from the host that can be recognized (as in the case of autoimmune diseases) are also epitopes.
- This paratope is only capable of binding with **one unique epitope**.
- B cells can recognize an **epitope alone** but T cells can recognize an epitope only when it is associated with an MHC molecule on the surface of a self-cell (either an antigen-presenting cell or an altered self-cell).



-
- The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the paratope.
 - **Conformational epitope** is a **discontinuous sequence of residues amino acids sub-units** composing an antigen that come in direct contact with a receptor of the immune system. **Can be recognized solely by B cells**
 - **Linear or a sequential epitope** is an epitope that is **recognized by antibodies** by its linear sequence of amino acids, or **primary structure**. **Can be recognized by both T and B cells**
 - In contrast, **most antibodies** recognize a conformational epitope that has a specific three-dimensional shape and its protein structure.
 - Conformational and linear epitopes **interact with the paratope** based on the **3-D conformation adopted by the epitope**, which is **determined by the surface features of the involved epitope residues and the shape or tertiary structure of other segments of the antigen**.
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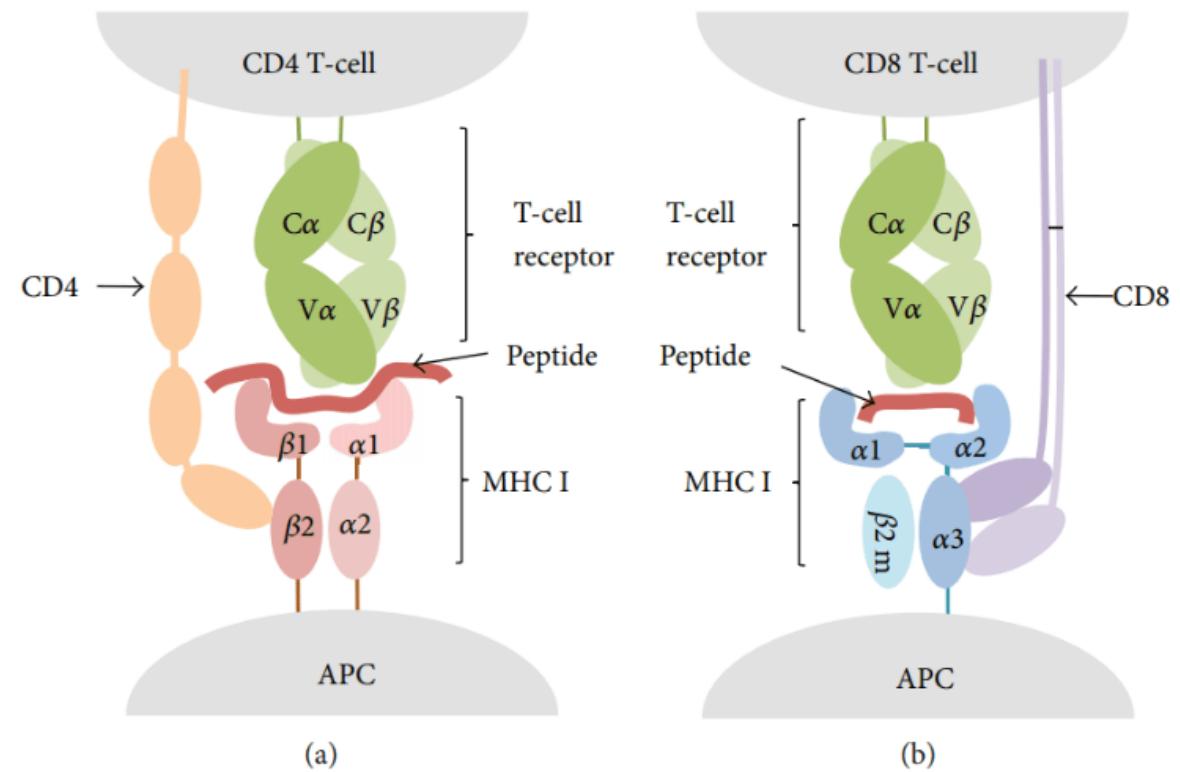
Properties	Linear epitopes	Conformational epitopes
Location	Most polysaccharides, fibrillar proteins, and single-stranded nucleic acids.	Most globular proteins and native nucleic acids
Composition	Adjacent amino acid residues in the covalent sequence	Amino acid residues brought into proximity to one another by folding
Antigen-antibody reactions	Dependent on linear structure of 6 amino acids	Dependent on the 3-dimensional structure
Availability for antibody interaction	Become available upon denaturation of proteins	Usually associated with native proteins

FUNCTIONS OF EPITOPES

- B cells recognizes an enormous variety of epitopes (also referred to as **B-cell epitopes**): those displayed on the exposed regions of bacteria or viral particles, as well as those displayed on soluble proteins, glycoproteins, polysaccharides, or lipopolysaccharides that have been released from invading pathogens.
 - When B cells are exposed to T-dependent antigens, they get activated and undergo class switching, affinity maturation, and differentiate into plasma cells.
 - **Plasma cells produce large amounts of antibody specific for the epitope recognized by their immunoglobulin receptor.**
 - T cells recognizes protein epitopes (also referred to as **T-cell epitopes**) displayed together with MHC molecules on self-cells, including altered self-cells such as virus-infected self-cells and cancerous cells.
 - As T cells **recognize only the processed peptides**, those epitopes may be located on those regions (e.g., internal proteins) which are inaccessible to B-cells.
 - Each branch of the immune system uniquely suited to recognize antigen in a different environment.
-

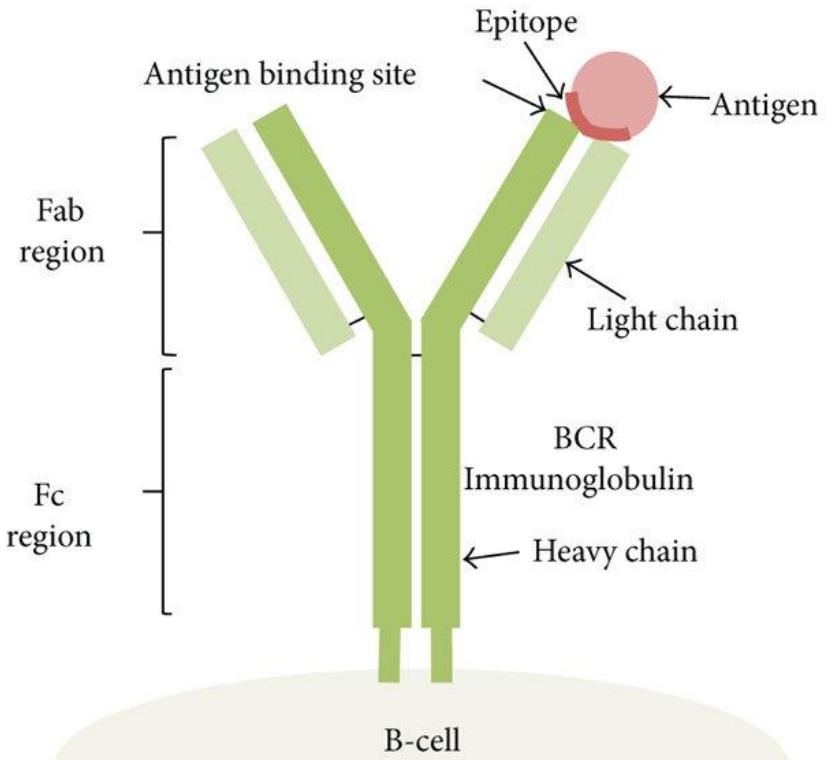
T-CELL EPITOPES

- T cell epitopes are presented on the surface of an APC
- T-cell epitopes are presented by class I (MHC I) and II (MHC II) MHC molecules that are recognized by two distinct subsets of **T-cells, CD8, and CD4 T-cells**, respectively.
- There are **CD8 and CD4 T-cell epitopes**.
- T-cells become cytotoxic T lymphocytes (CTL) following T CD8 epitope recognition. Meanwhile, primed CD4 T-cells become helper (Th) or regulatory (Treg) T-cells.
- T cell epitopes presented by MHC class I molecules are typically peptides between **8 and 11 amino acids in length**, whereas MHC class II molecules present longer peptides, **13–17 amino acids in length**, and non-classical MHC molecules also present non-peptidic epitopes such as glycolipids.

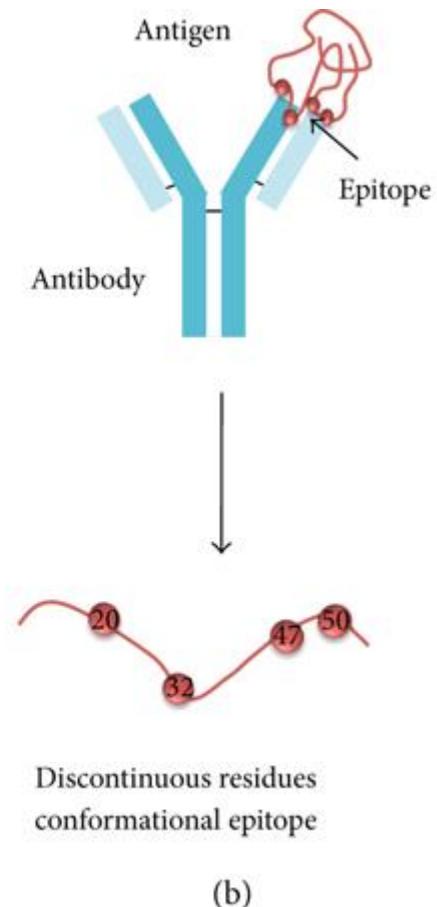
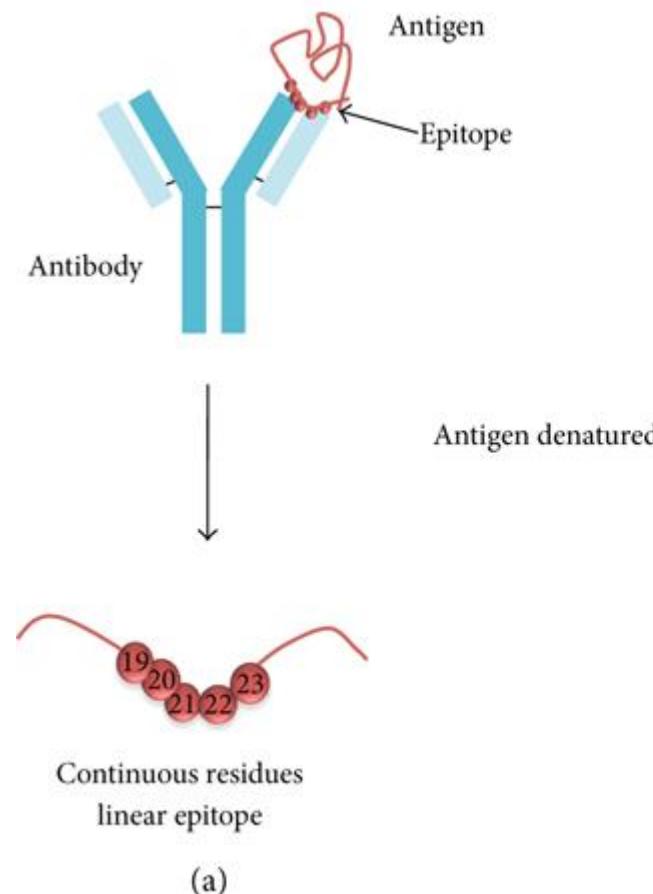


B-CELL EPITOPES

- The part of the antigen that immunoglobulin or antibodies bind to is called a B-cell epitope.
- B cell epitopes can be divided into two groups: conformational or linear.
- B cell epitopes are mainly conformational.
- There are additional epitope types when the quaternary structure is considered.
- Epitopes that are masked when protein subunits aggregate are called cryptotopes.
- Neotopes are epitopes that are only recognized while in a specific quaternary structure and the residues of the epitope can span multiple protein subunits and are not recognized once the subunits dissociate



- B-cell epitopes are categorized into linear and conformational epitopes.
- A linear b-cell epitope is a **contiguous amino acid segment in an antigen**.
- A conformational b-cell epitope is located in close proximity in the protein 3-dimensional structure **but discontinuous in the protein sequence**.
- The features of B cell epitope are **hydrophilicity, surface accessibility, beta turns, exposed surface, polarity and antigenic properties of amino acids**.
- These properties of polypeptides chains have been correlated with the location of the continuous and discontinuous conformational epitopes



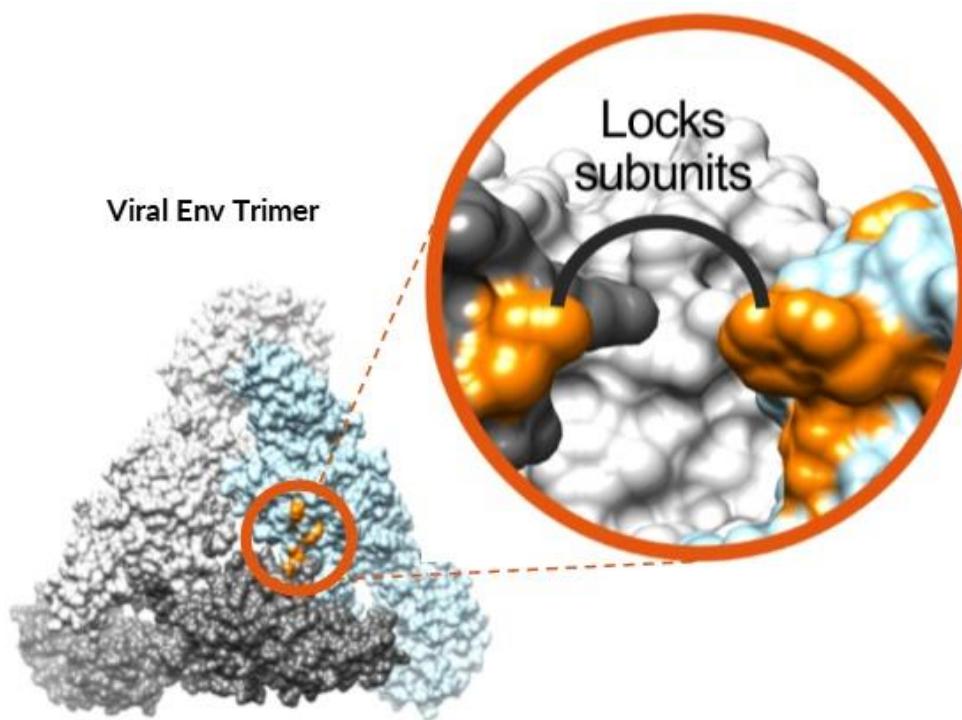
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- Most B-cell epitopes are **discontinuous in sequence**, meaning that they are composed of **residues that might be far apart in sequence and are brought together in spatial proximity by the protein folding**
 - Data describing such conformational epitopes are mainly obtained from **experimentally resolved 3D structures of antibodies** co-crystallized with their target antigen, which allows a very precise identification of the epitope residues.
 - **Methods include** protein crystallography, ELISA and peptide-chip, but in general they are expensive, time consuming, low-throughput, or have low accuracy. **Computational methods include** BepiPred, DiscoTope, CBtope, and ABCpred
 - B-cell epitope prediction tools can broadly be categorized in two groups: **sequence- and structure-based methods**
-

EPITOPE MAPPING

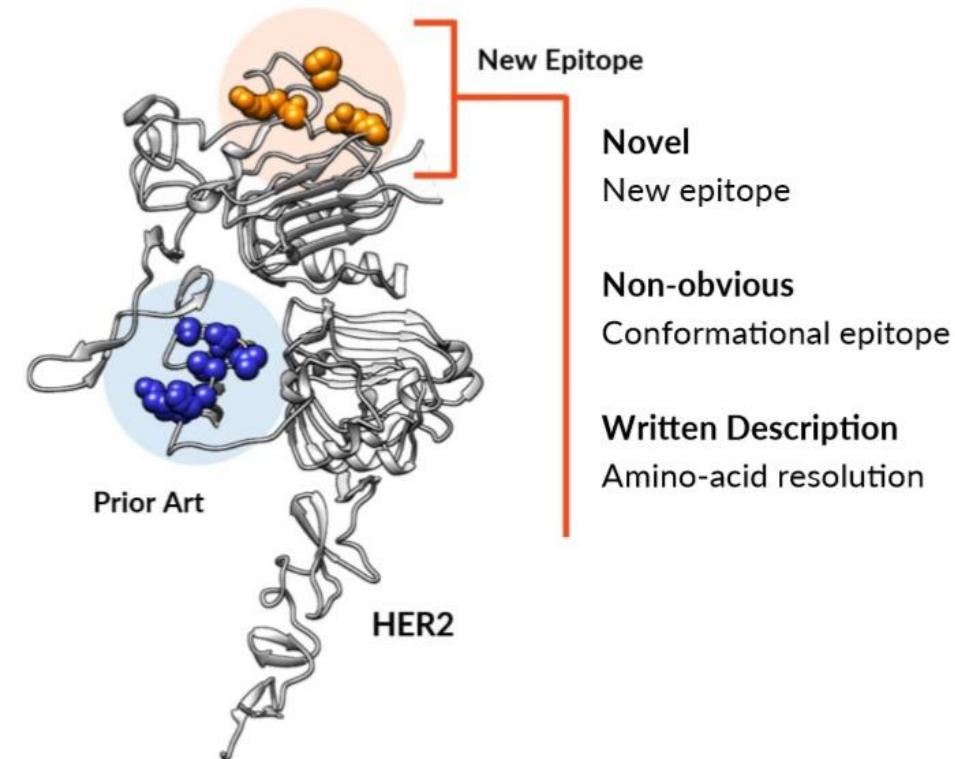
- Epitope mapping is the process of experimentally identifying the binding site, or "epitope", of an antibody on its target antigen (usually, on a protein).
 - Identification and characterization of antibody binding sites aid in the discovery and development of new therapeutics, vaccines, and diagnostics
 - epitope characterization can also help elucidate the mechanism of binding for an antibody and can strengthen intellectual property (patent) protection
 - Experimental epitope mapping data can be incorporated into robust algorithms to facilitate **in silico prediction of B-cell epitopes based on sequence and/or structural data**
-

EPITOPE DATA BENEFITS

Epitopes Differentiate Mechanism of Action



Epitope Maps Strengthen Intellectual Property



NEED FOR EPITOPE MAPPING

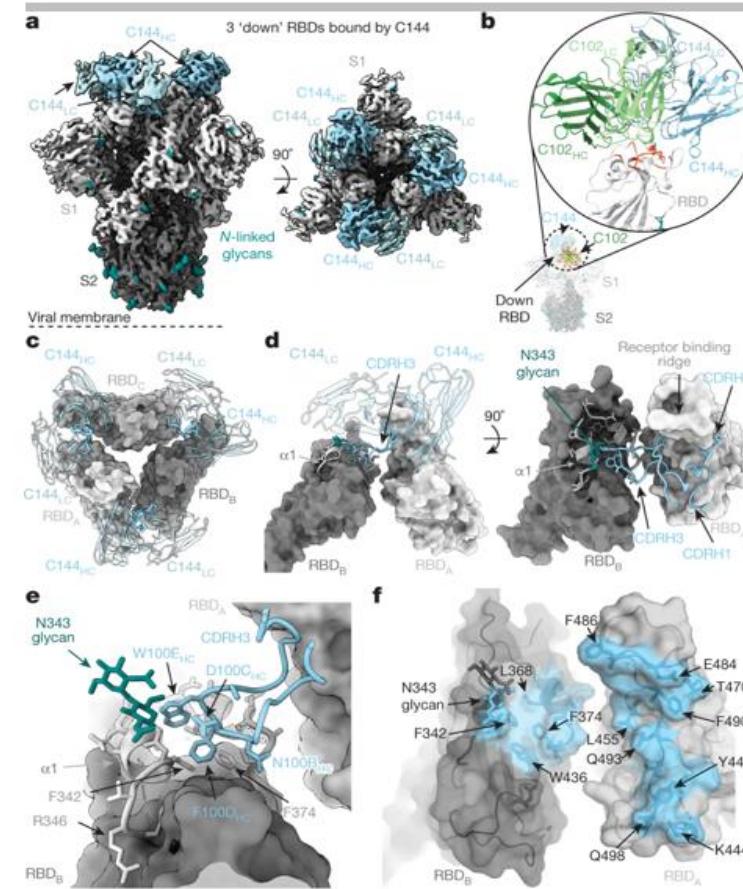
- Used for vaccine development
 - mAB and polyclonal antibodies production
 - Ab-Ag interaction
-

WHY EPITOPE MAPPING?

- Epitope mapping has been crucial to the development of vaccines against prevalent or deadly viral pathogens, such as chikungunya, dengue, Ebola, and Zika viruses, by determining the antigenic elements (epitopes) that confer long-lasting immunization effects.
 - Information about the binding epitope of an antibody can provide important mechanistic insights and indicate for what applications an antibody might be useful.
 - understanding disease etiology, immune monitoring, developing diagnosis assays, and designing epitope-based vaccines
-

METHODS

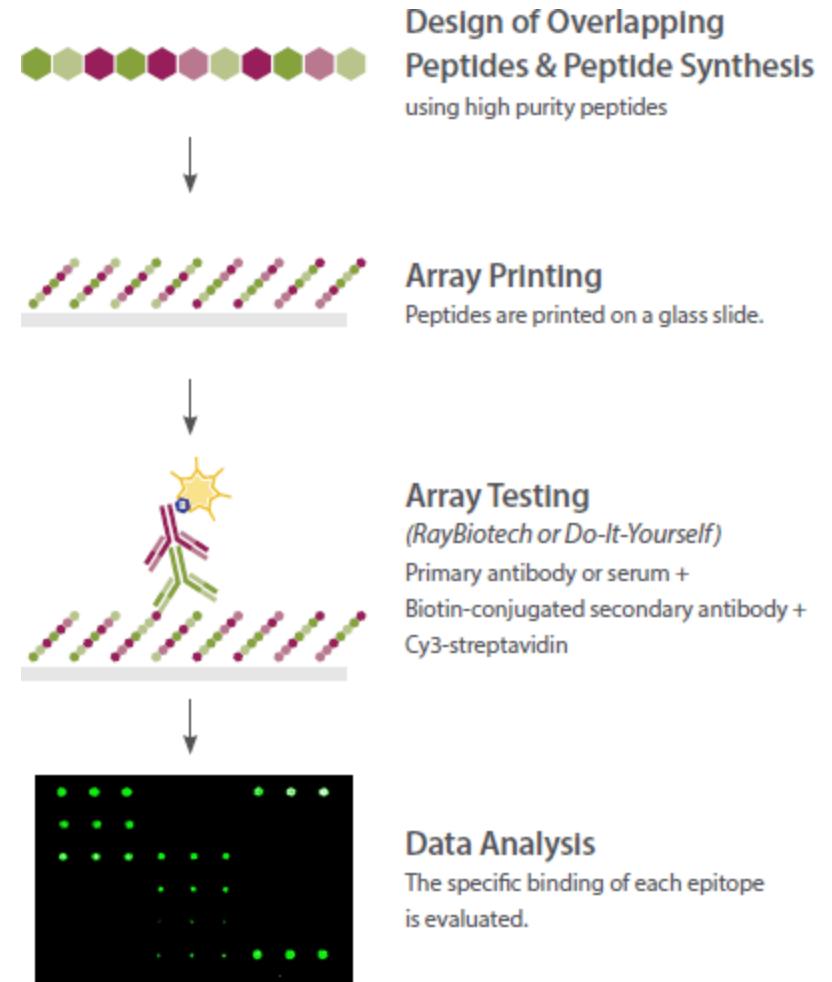
- **X-ray co-crystallography and cryogenic electron microscopy (cryo-EM)**
 - X-ray co-crystallography has historically been regarded as the gold-standard approach for epitope mapping because it allows direct visualization of the interaction between the antigen and antibody. Cryo-EM can similarly provide high-resolution maps of antibody-antigen interactions



Cryo-EM structure of the C144-S complex illustrates a distinct VH3-53 NAb binding mode. (Barnes C O, et al. 2020)

METHODS

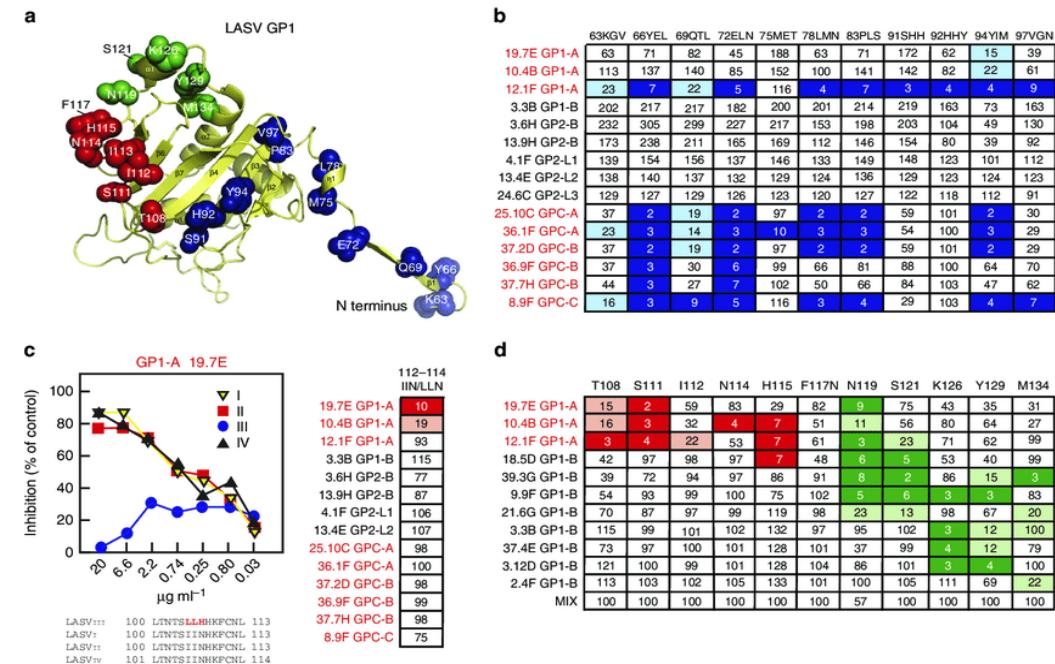
- **Array-based oligo-peptide scanning**
 - Also known as overlapping peptide scan or pepscan analysis, this technique uses a library of oligo-peptide sequences from overlapping and non-overlapping segments of a target protein, and tests for their ability to bind the antibody of interest.



METHODS

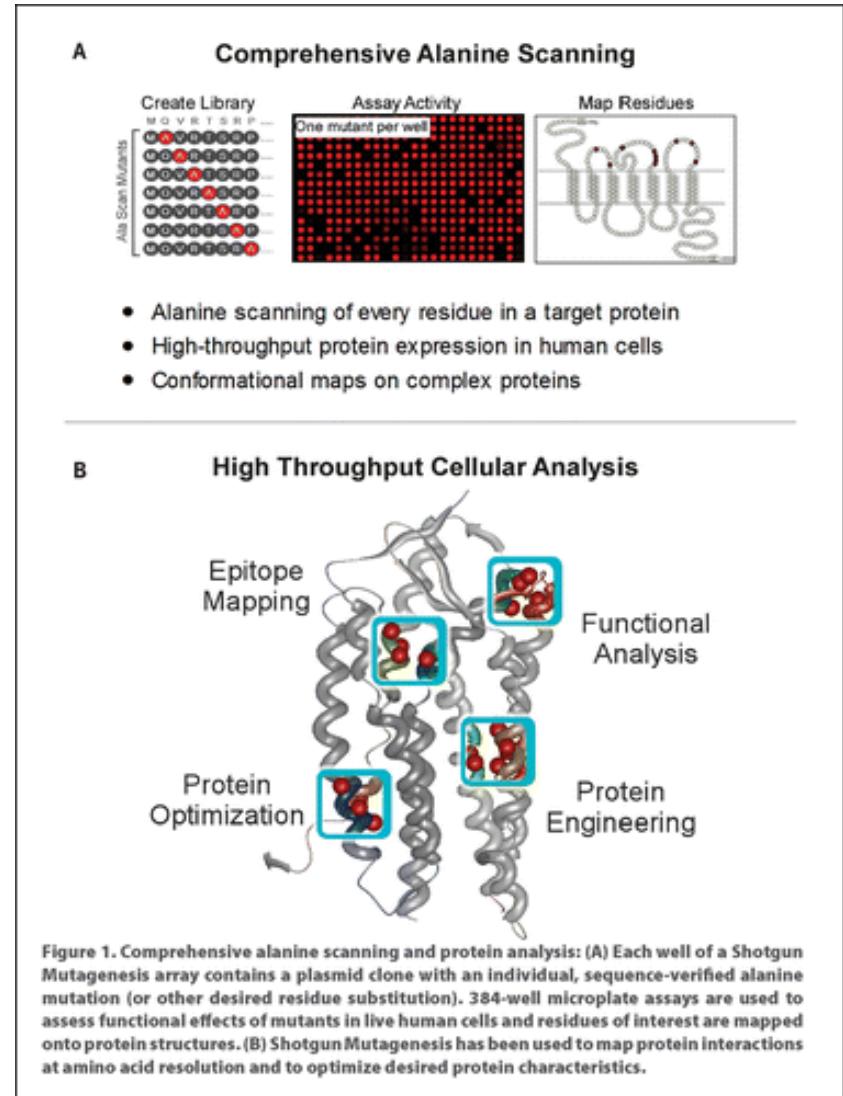
- **Site-directed mutagenesis mapping**

- The molecular biological technique of site-directed mutagenesis (SDM) can be used to enable epitope mapping. In SDM, systematic mutations of amino acids are introduced into the sequence of the target protein.

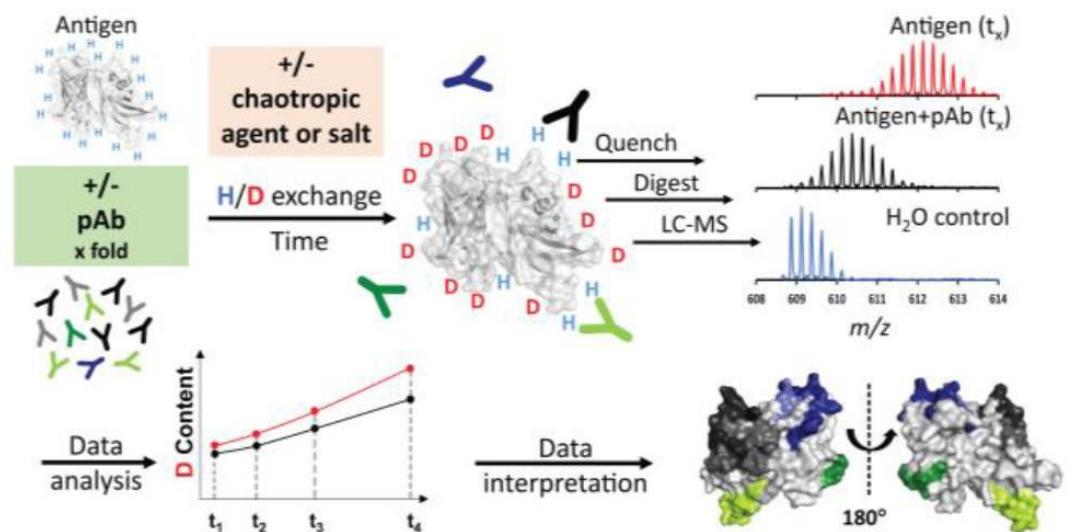


Mapping of putative epitopes recognized by LASV mAbs by site-directed mutagenesis. Wild-type recombinant LASV GPC was engineered with altered amino-acid sequences to map putative B-cell epitopes on LASV glycoproteins.

- **High-throughput shotgun mutagenesis epitope mapping**
- Shotgun mutagenesis is a high-throughput approach for mapping the epitopes of mAbs. The shotgun mutagenesis technique begins with the creation of a mutation library of the entire target antigen, with each clone containing a unique amino acid mutation (typically an alanine substitution) Hundreds of plasmid clones from the library are individually arrayed in 384-well microplates, expressed in human cells, and tested for antibody binding. Amino acids of the target required for antibody binding are identified by a loss of immunoreactivity. These residues are mapped onto structures of the target protein to visualize the epitope.



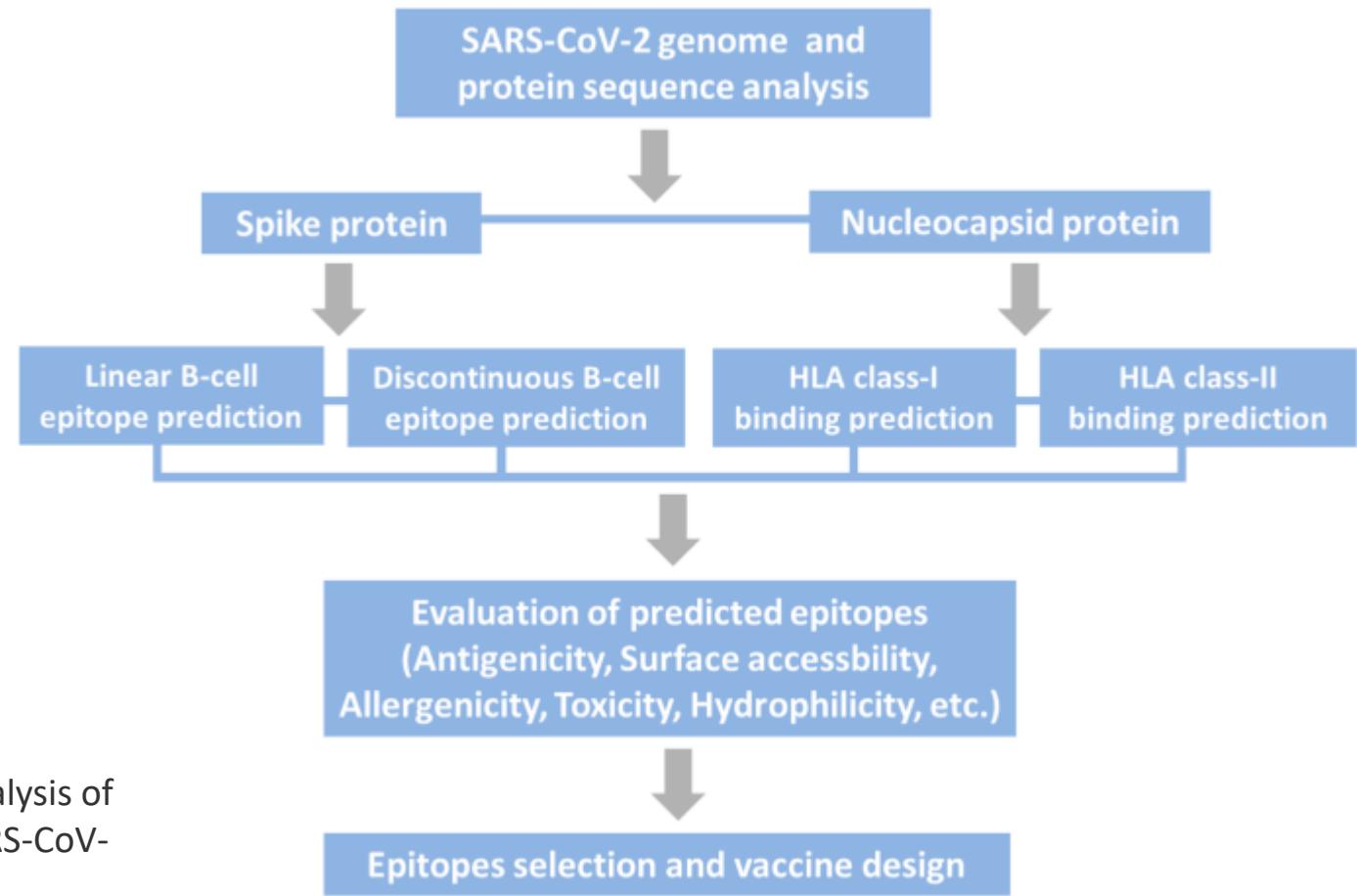
- **Hydrogen–deuterium exchange (HDX).**
- This method gives information about the solvent accessibility of various parts of the antigen and antibody, demonstrating reduced solvent accessibility in regions of protein-protein interactions. One of its advantages is that it determines the interaction site of the antigen-antibody complex in its native solution, and does not introduce any modifications (e.g. mutation) to either the antigen or the antibody.



EPITOPE BASED VACCINES

- Epitope vaccines provide maximal therapeutic efficacy with minimal side effects.
 - Reverse vaccinology can be used for designing epitope vaccines.
 - Computational immunoinformatics is the basis of epitope vaccine development.
 - Various in silico computational approaches are used in reverse vaccinology
-

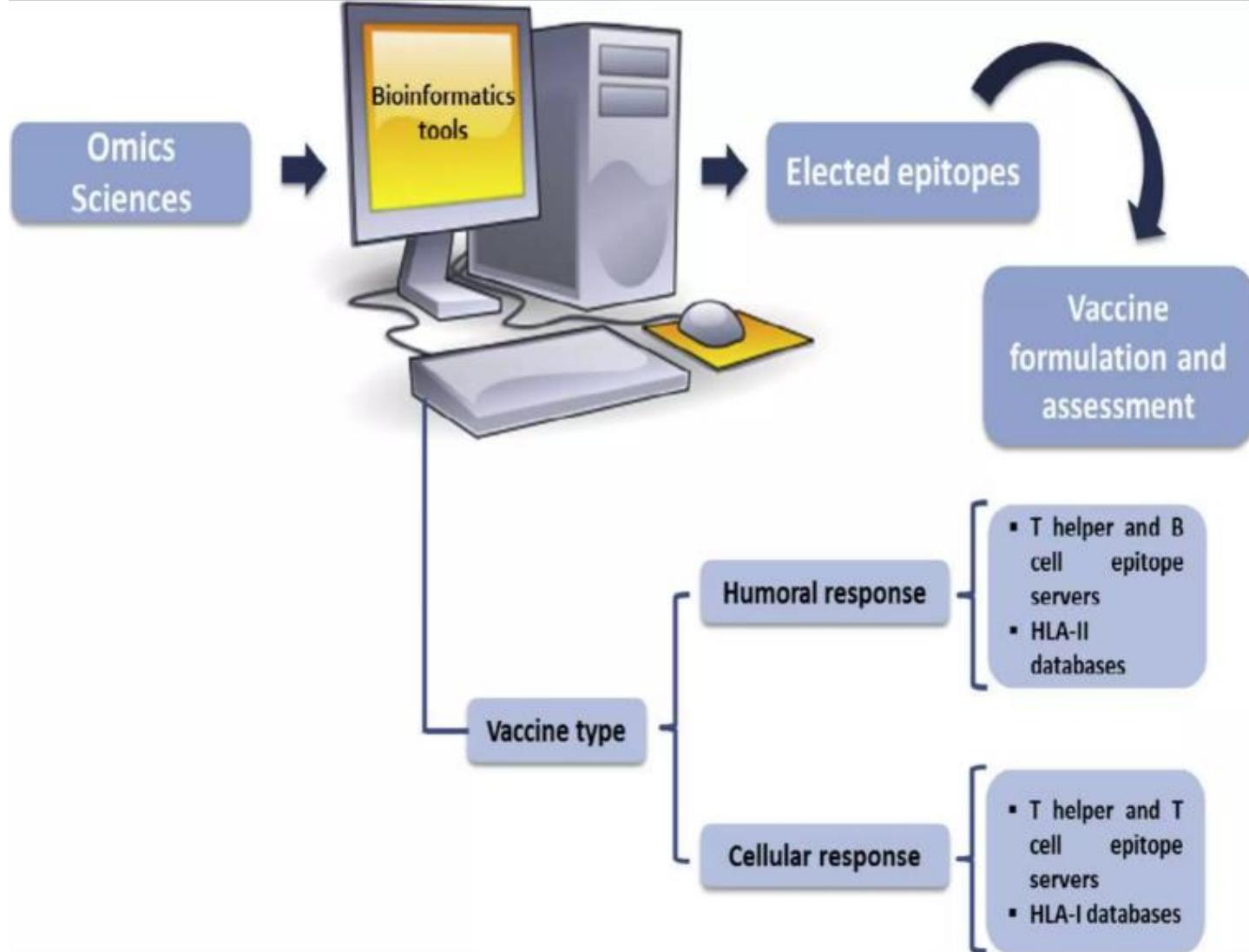
- Suitable proteins of SARS-CoV-2 were selected at the first step for epitope prediction.
- The second step comprised of B- and T-cell epitope analysis with bioinformatics approaches.
- Epitope evaluation was followed and appropriate ones were chosen for vaccine design



Chen, HZ., Tang, LL., Yu, XL. et al. Bioinformatics analysis of epitope-based vaccine design against the novel SARS-CoV-2. *Infect Dis Poverty* 9, 88 (2020).

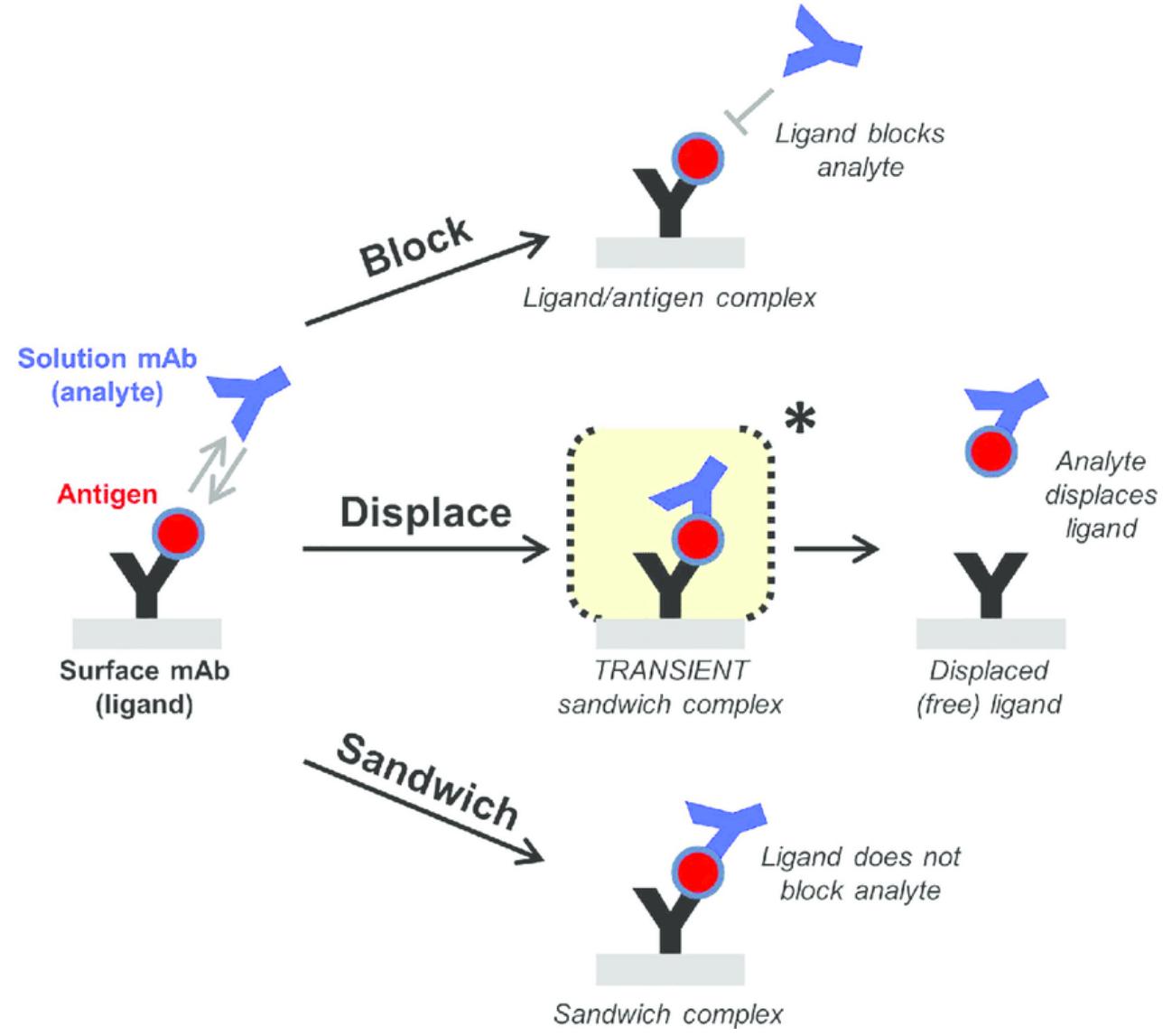
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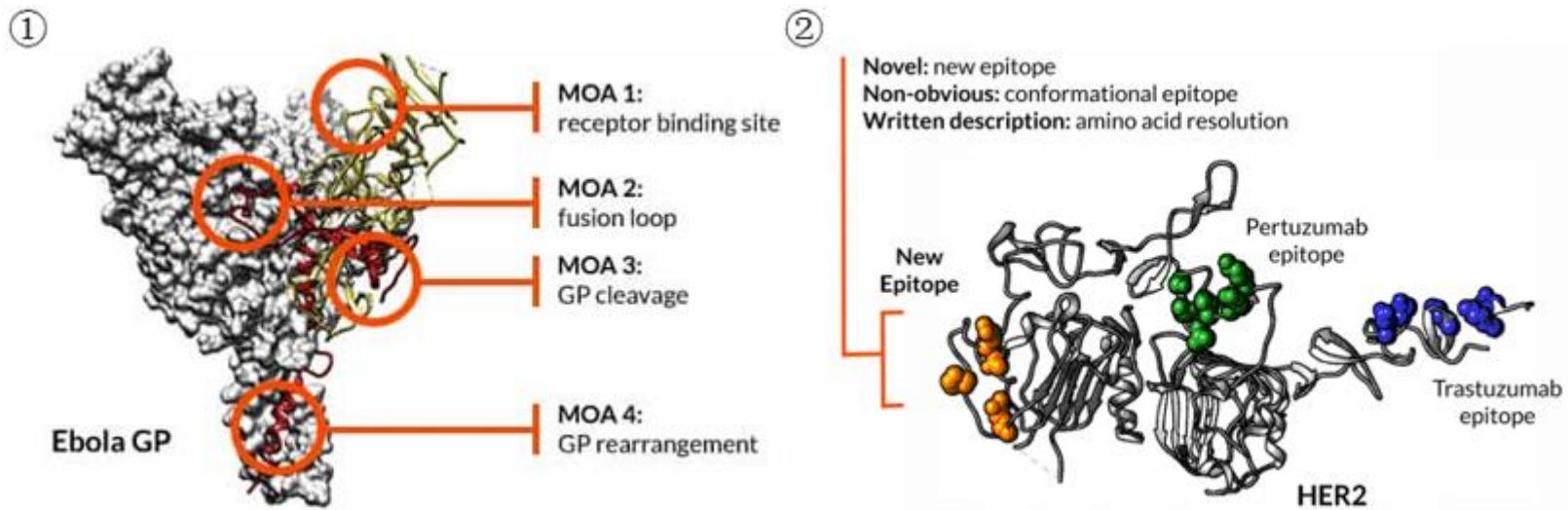
[Download citation](#)



EPITOPE BINNING

- Epitope binning is a technique used to cluster different monoclonal antibodies by the specific region on the antigen that is recognized by the antibody.
- Epitope binning is generally referenced as epitope mapping or epitope characterization in the literature.
- In epitope binning, you determine which of your **different antibodies can bind to your antigens** simultaneously, therefore recognise non-overlapping epitopes
- Epitope binning immunoassays using surface plasmon resonance (SPR) can and should be deployed in the development of new potential drug candidates.





① Assay setup according to the specific program	② Mutagenesis of the target protein	③ Antibody screening with the designed mutation array	④ Deliver final report including graphical representation of the epitope data, identification of critical residues, and mapped epitope.
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- State-of-the-art array-based SPR for high throughput epitope binning;
- BLI platform-based Octet system;
- Biacore system;
- IBIS MX96&CFM platform;
- ProteOn platform.

Epitope binning and mapping service will greatly support the mechanism of action understanding and the intellectual property claims of clients' antibodies which can further facilitate your biologics drug discovery and development.

B-CELL EPITOPE PREDICTION (IN SILICO)

- methods for continuous epitope prediction combine two or more residue properties with machine learning approaches
 - prediction methods can be divided based on the level of input information to methods based on antigen sequence and methods based on 3D structure of antigen. Structure-based methods significantly outperform sequence-based methods
 - existing prediction methods are not accurate enough and annotate general immunogenic/epitope-like regions on the antigen
 - consensus of various B-cell epitope prediction methods ensures greater accuracy of the results
-

PREDICTION OF CONTINUOUS B-CELL EPITOPES

Tool	Source (URL)	Input data
ABCpred	http://www.imtech.res.in/raghava/abcpred/	FASTA
APCPred	http://ccb.bmi.ac.cn/APCpred/	FASTA
BCPREDS	http://ailab.ist.psu.edu/bcpred/	FASTA
BepiPred	http://www.cbs.dtu.dk/services/BepiPred	FASTA or FASTA file
LBtope	http://crdd.osdd.net/raghava/lbtope/	FASTA or FASTA file
Bcepred	http://www.imtech.res.in/raghava/bcepred/	FASTA or FASTA file
SVMTriP	http://sysbio.unl.edu/SVMTriP/	FASTA

PREDICTION OF DISCONTINUOUS B-CELL EPITOPES

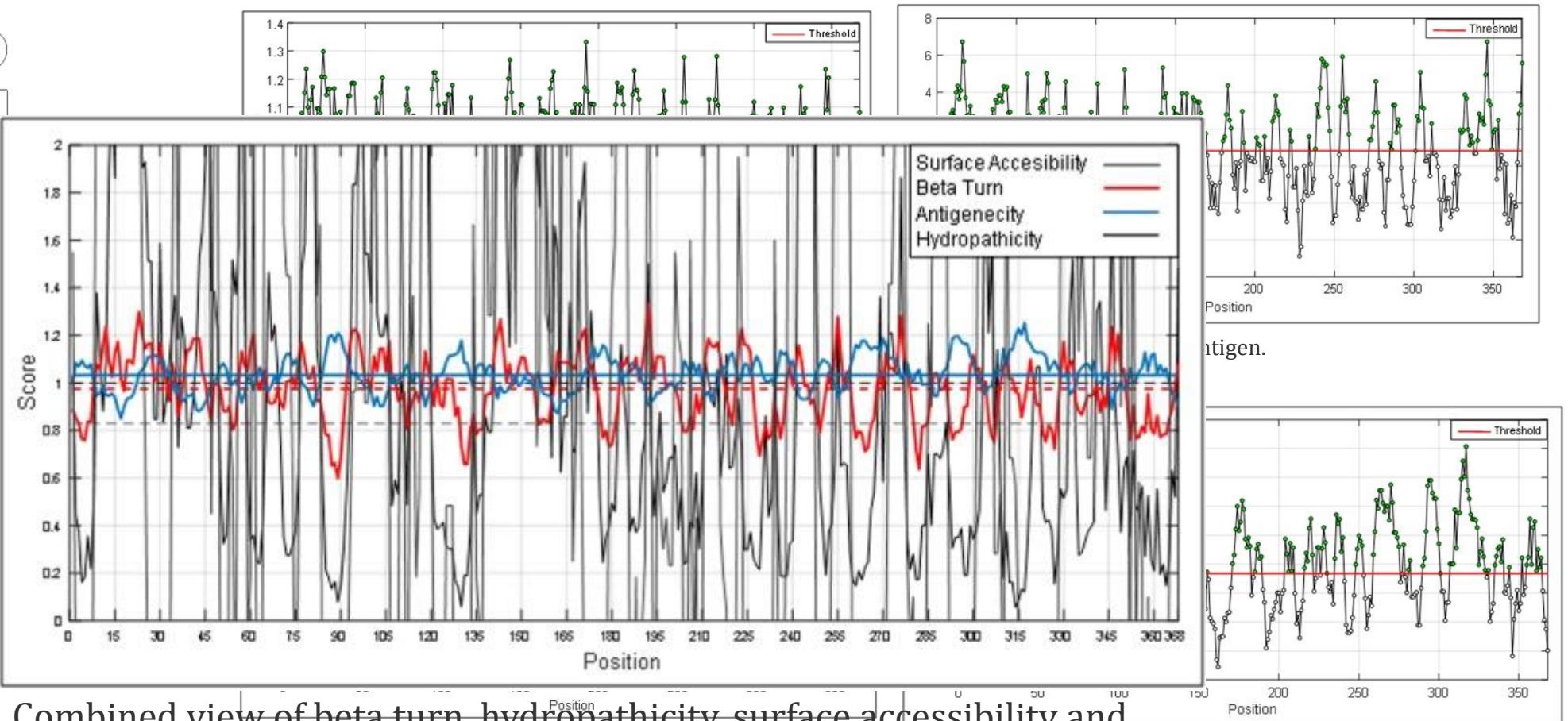
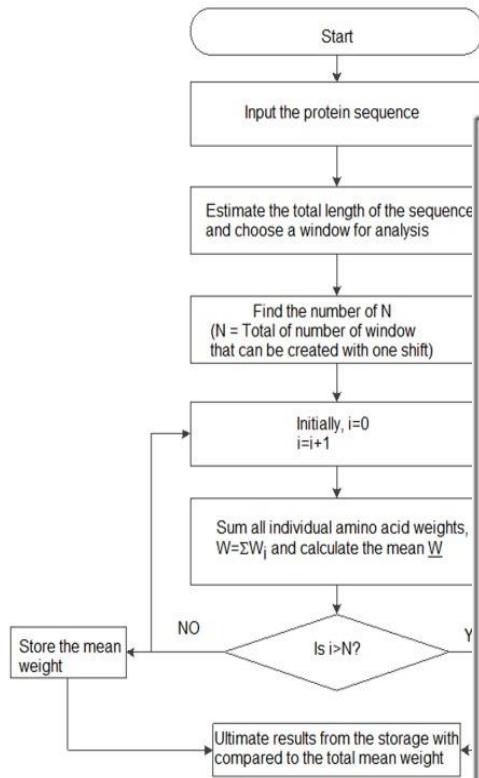
Tool	Source (URL)	Input data
DiscoTope	http://www.cbs.dtu.dk/services/DiscoTope-2.0/	PDB ID or PDB file
BePro (PEPITO)	http://pepito.proteomics.ics.uci.edu/	PDB ID or PDB file
ElliPro	http://tools.immuneepitope.org/ellipro/	FASTA or Swiss-Prot ID
SEPPA	http://badd.tongji.edu.cn/seppa/	PDB ID or PDB file
EPITOPIA	http://epitopia.tau.ac.il/	FASTA/PDB ID or PDB file
CBTOPE	http://www.imtech.res.in/raghava/cbtope/	FASTA or FASTA file
EPCES	http://sysbio.unl.edu/EPCES/	PDB ID or PDB file
EPSVR	http://sysbio.unl.edu/EPSVR/	PDB ID or PDB file
PEASE	http://www.ofranlab.org/PEASE	Ag PDB ID or PDB file Ab FASTA or FASTA file
EpiPred	http://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/EpiPred.php	PDB ID or PDB file

B-CELL EPITOPE DATABASE

Database	Source (URL)
IEDB IEDB-3D	http://www.iedb.org/
AntiJen	http://www.ddg-pharmfac.net/antijen/AntiJen/antijenhomepage.htm
CED	http://immunet.cn/ced/
Epitome	http://www.rostlab.org/services/epitome/
BciPep	http://www.imtech.res.in/raghava/bcippep/info.html
SEDB	http://sedb.bicpu.edu.in
SDAP	https://fermi.utmb.edu/
HIV Molecular Immunology Database	http://www.hiv.lanl.gov/content/immunology/index.html
FLAVIdB	http://cvc.dfci.harvard.edu/flavi/

B-CELL EPITOPE FEATURES IN ANTIGENS

- Using MATLAB programming to classify the different features of a protein sequence to help predict a potential B cell epitope from a protein or a group of protein sequences. A protein sequence (FASTA format) with the accession number AAY57281.1 from the UniProtKB database was used as a test sequence in this study.
- Beta-turn regions: Secondary structure elements in a protein are usually alpha helix, beta turn regions, and coil-coil regions. Beta turn region is relevant to epitope design.
- Hydropathicity: This scale for amino acids was used to identify potential hydrophilic regions in the query protein for generating a plot with window size from $i = 0$ to $i > N$.
- Surface accessibility: The empirical amino acid accessible surface probabilities which are fractional probabilities (0.26 to 0.97) determined for an amino acid found on the surface of a protein is used. The most surface accessible area in a protein was determined with these fractional surface probabilities for amino acids, which a surface probability after calculating normalized surface accessible values for amino acids and a plot was generated
- Antigenicity prediction: a semi-empirical method which utilizes physicochemical properties of amino acid residues and their probabilities or frequencies of occurrence in experimentally known segmental epitopes to predict antigenic determinants on proteins



Combined view of beta turn, hydropathicity, surface accessibility and antigenicity in a protein antigen to define a potential B cell epitope.

Graphical presentation of surface accessibility in a protein antigen.

Graphical presentation of antigenicity in a protein antigen.

DIFFERENCE BWTN T CELL & B CELL EPITOPES

Properties	Recognized by B cells and Antibodies	Recognized by T cells
Composition	Proteins, glycoproteins, polysaccharides, nucleic acids	Proteins
Configuration	Linear/conformational determinants	Linear determinants
Size	4-8 residues	8-15 residues
Number	Limited, located on the exposed surface of the antigen	Limited to those proteins that can be processed and bind to MHC

EPITOPE SPREADING

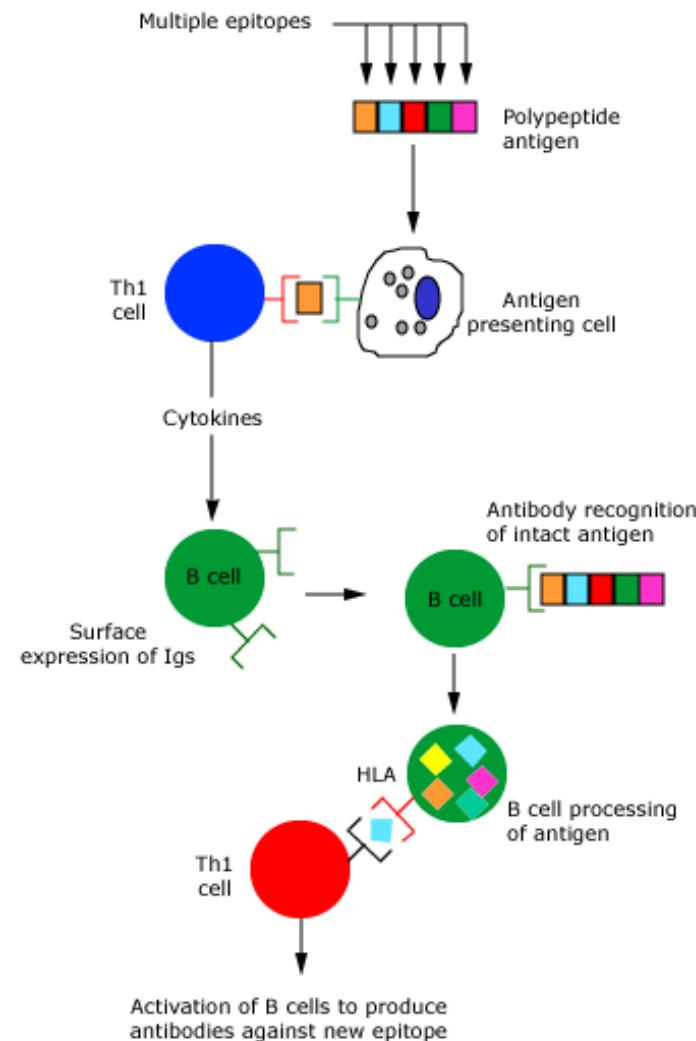
- Epitope spreading or ‘determinant spreading’ denotes ‘development of immune responses to endogenous epitopes secondary to the release of self-antigens during a viral infection or a chronic autoimmune or inflammatory response’.
 - In such conditions, sequestered autoantigens are exposed to autoreactive T cells causing autoimmune disease.
-

MECHANISM OF EPITOPE SPREADING

Multiple factors are involved in the induction of epitope spreading, such as:

- enhanced display of previously hidden antigenic determinants under the local inflammatory/cytokine environment
- Release of self-antigens following tissue damage
- Role played by B cells as antigen-presenting cells.

Mechanism of epitope spreading



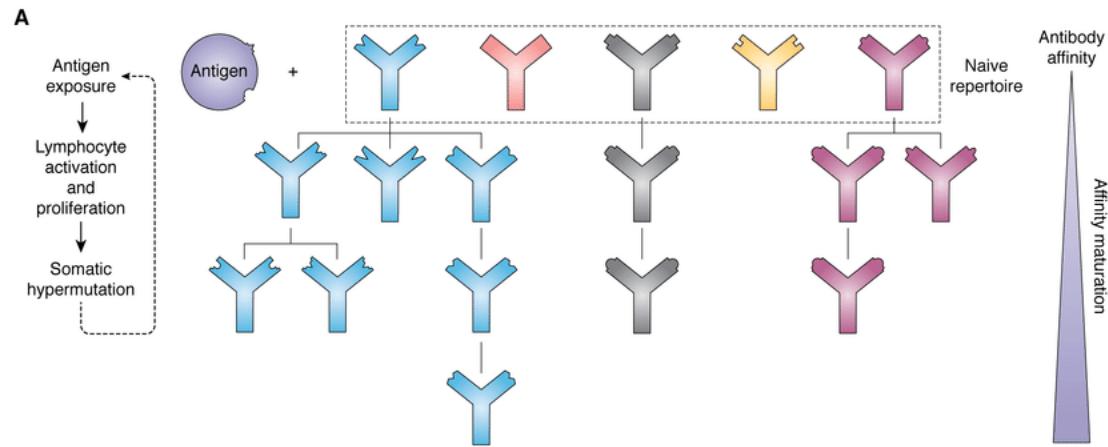
AFFINITY MATURATION

- Targeting effectual epitopes is essential for therapeutic antibodies to accomplish their desired biological functions.
 - As modulators of molecular interactions with high affinity and high specificity, monoclonal antibodies have emerged as important therapeutics targeting cancers, immune diseases and infections
 - Affinity and specificity, along with the therapeutic efficacy of a given monoclonal antibody often depends on the specific epitope recognized. i.e. exactly where on the antigen binding occurs
 - **Generation of potent antibodies by a mutation-selection process called affinity maturation is a key component of effective immune responses**
 - Affinity maturation is an important strategy in antibody optimization to generate safe and efficacious second-generation therapeutics
 - Affinity maturation is the process by which **B cells increase their affinity for a particular antigen**
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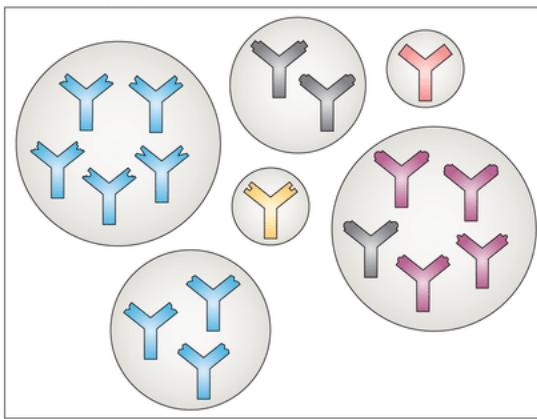
HOW DOES AFFINITY MATURATION OCCUR

- Affinity maturation primarily occurs on membrane immunoglobulin of germinal center (GC) B cells and as a direct result of somatic hypermutation (SHM) and selection by TFH cells.
 - Affinity maturation occurs within the GC, where somatically mutated BCRs undergo selection on antigen retained on follicular dendritic cells
 - Antigen is retained in the form of Immune Complexes (ICs) and involves the interaction of both complement receptors and Fc γ RIIB with these ICs on FDCs. B cells also express both complement and Fc γ RIIB.
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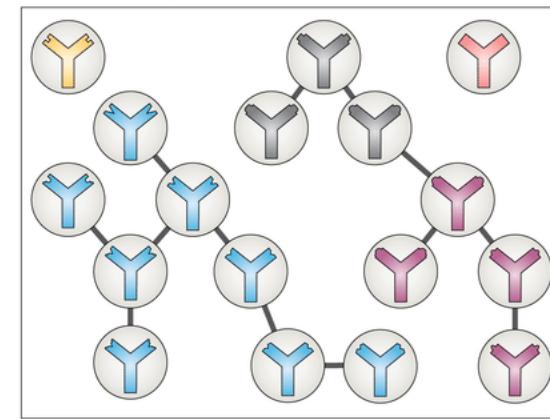
Figure 3



B Clonotyping



C Network analys

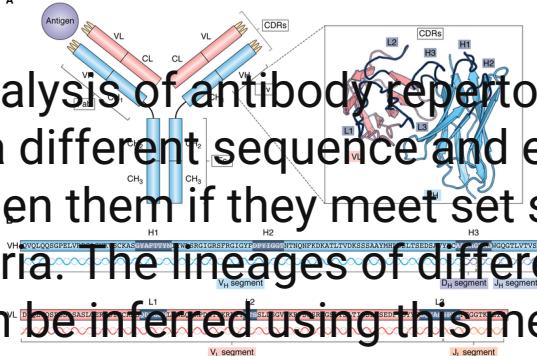


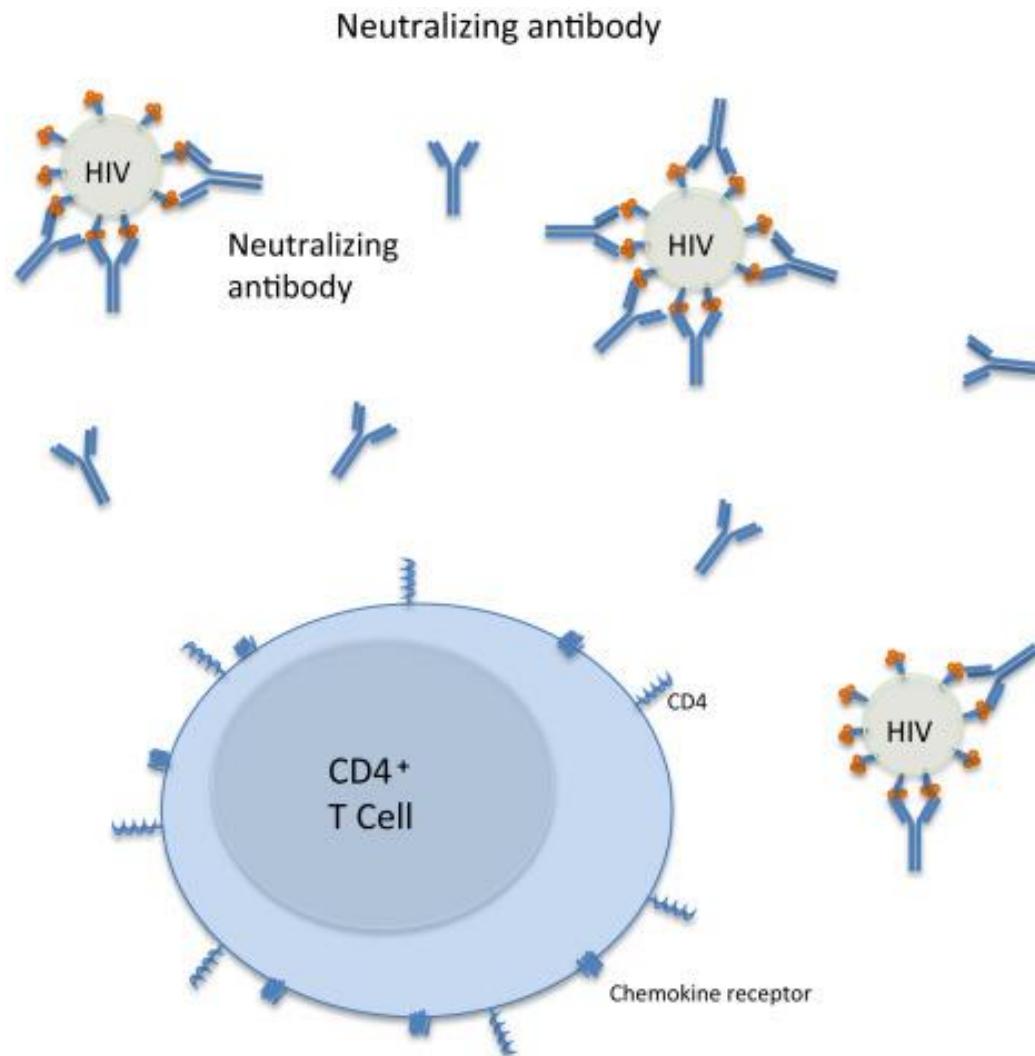
The process of affinity maturation and methods of analysing the resulting antibody repertoires.

A -Upon exposure to an antigen, those antibodies present in the naive repertoire that are able to bind to it proliferate, undergoing somatic hypermutation to produce variations upon the initial binder. Successive rounds of this process produce antibodies with high affinity.

B -Clonotyping groups antibodies in the repertoire based on sequence similarity; normally they must originate from the same V and J genes and have an H3 sequence identity of 80-100%. Antibodies of the same clonotype are predicted to bind to the same epitope.

C -Network analysis of antibody repertoires, where each node is a different sequence and edges are present between them if they meet set sequence similarity criteria. The lineages of different antibodies can be inferred using this method.





NEUTRALIZING ANTIBODY (NAB)

- A neutralizing antibody (NAb) is an antibody that defends a cell from a pathogen or infectious particle by neutralizing any effect it has biologically.
- Neutralization renders the particle no longer infectious or pathogenic
- Neutralizing antibodies are part of the humoral response of the adaptive immune system against viruses, intracellular bacteria and microbial toxin.
- By binding specifically to surface structures (antigen) on an infectious particle, neutralizing antibodies prevent the particle from interacting with its host cells it might infect and destroy.

MECHANISM

- Neutralizing antibodies can inhibit the infectivity by binding to the pathogen and block the molecules needed for cell entry.
 - This can be due to the antibodies statically interfering with the **pathogens or toxins** attaching to host cell receptors.
 - In case of a **virus infection**, NAbs can bind to glycoproteins of enveloped viruses or capsid proteins of non-enveloped viruses.
 - Can act by preventing particles from undergoing structural changes often needed for successful cell entry
 - Neutralizing antibodies are also important in neutralizing the toxic effects of bacterial toxins.
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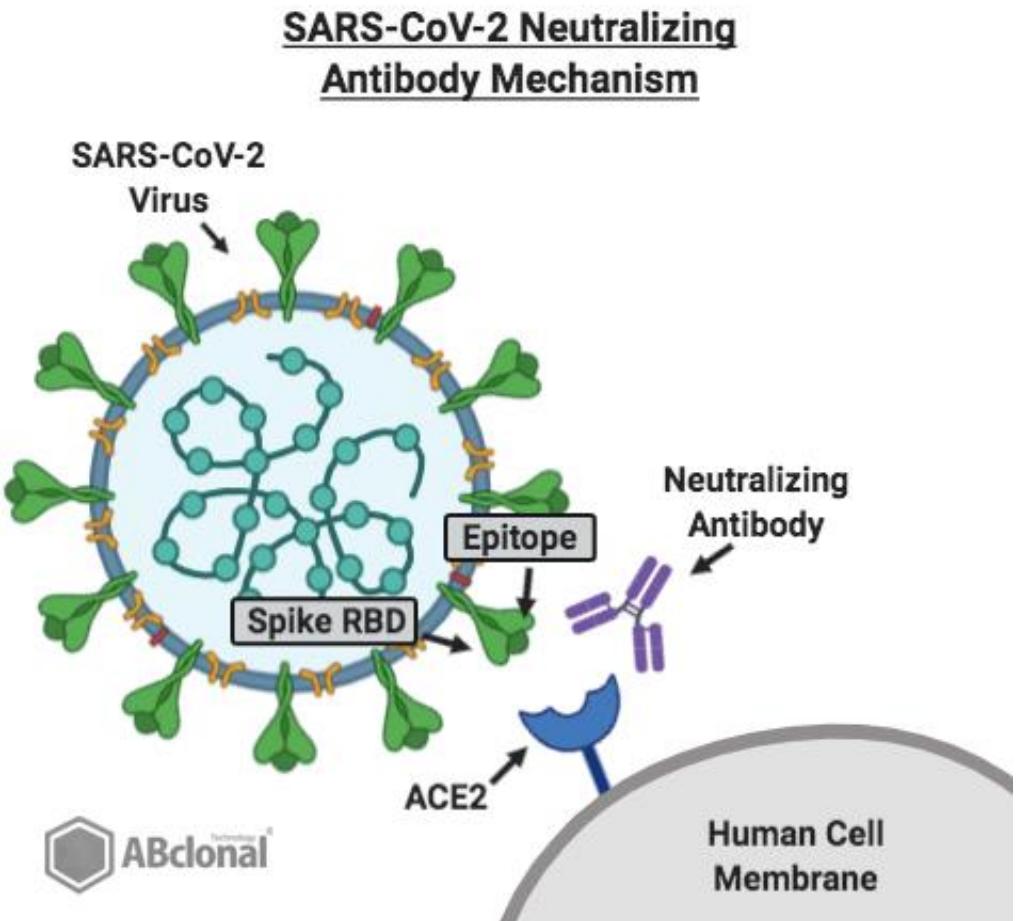
NAB IN VIRUS INFECTION

- NAbs could be defined as antibodies that bind to the free virus and prevent it from infecting cells
 - Antibody neutralization is defined as the “**abrogation of virus infectivity in vitro by the binding of antibodies to the virion**” and that binding may affect the virus at one or more stages in the viral infection cycle.
 - The role of antibodies in host protection against viral infections has been amply demonstrated.
 - Antibodies neutralize viral infection or replication by targeting **viral glycoproteins** of enveloped viruses (such as the SARS-CoV-2 Spike (S) protein) or the **protein shell of nonenveloped viruses**.
 - These proteins bind to cellular receptors and cellular membranes and mediate the viral fusion and penetration into the cytosol, respectively
-

MECHANISMS

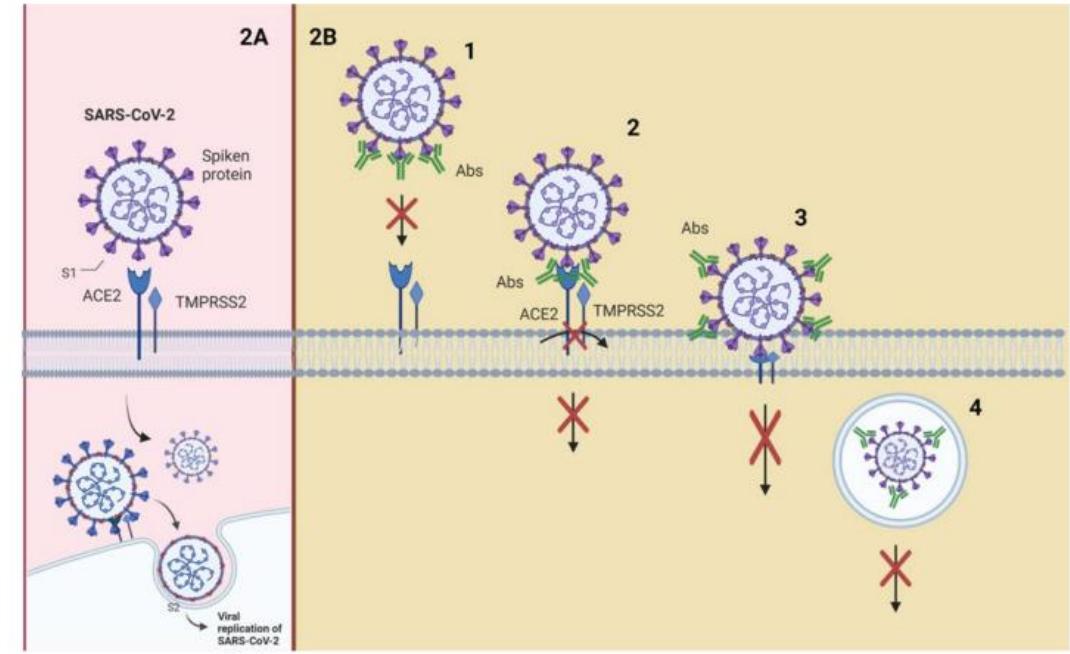
- Neutralization can be achieved through four main mechanisms
 - NAbs binding to viral surface proteins and blocking their interaction with the host cell receptor and infection
 - NAbs binding to viral protein epitopes that interact with host cell coreceptors that are key for viral infection
 - NAbs binding to viral epitopes that are not essential for host cell receptor binding but are necessary for conformational changes needed for membrane fusion
 - A variant of this mechanism would be the capability of NAbs to bind to proteins essential for host cell receptor binding, but NAbs are bound to distal epitopes of fusogenic proteins (internalized), preventing complete fusion

- NAbs generally **block the binding of the virus to cellular receptors**; however, in some cases, they may **prevent conformational changes** necessary for fusion of the virus with the cell membrane or proteolytic cleavage
- For enveloped viruses, the latest step blocked seems to be membrane fusion, i.e., entry into the cytoplasm.
- the function of NAb is mediated by a region called fragment antigen-binding (Fab), and non-neutralizing antibodies exert their effect near the crystallizable region (Fc)



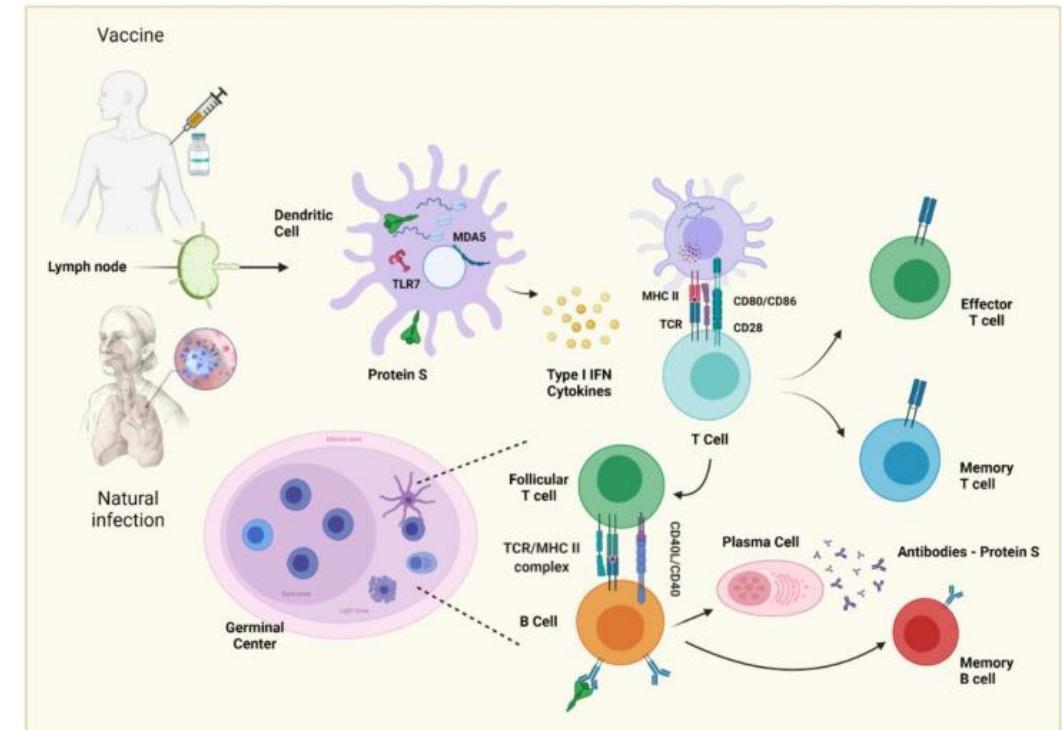
The neutralizing action is carried out through the variable fraction (Fab) of the antibody, whose primary limitation is viral resistance. Here, effector mechanisms enter to avoid viral replication

- SARS-CoV-2 attaches to the host cell with the aid of the spike (S) glycoprotein present on its envelope
- S glycoprotein is composed of two subunits (S1 and S2) that have to be cleaved to allow for viral fusion with the cell membrane, entry into the cell, and initiation of the replication process
- Transmembrane serine protease 2 (TMPRSS2) or endosomal cysteine proteases cathepsins B (CTSL) and L (CTSB) perform this excision
- Protease cleavage at the S2' site frees the fusion peptide from the new S2 N-terminal region
- This fusion peptide is inserted into the host membrane and facilitates the pulling of the viral and host cell membrane into close proximity, leading to membrane fusion
- Angiotensin-converting enzyme 2 (ACE2), an enzyme located on the outer surface of a wide variety of cells, is the primary host cell target of the receptor-binding domain (RBD) of the S1 subunit
- This suggests that disruption of the RBD–ACE2 interaction would block SARS-CoV-2 cell entry; therefore, RBD has been suggested as the main target of NAbs against SARS-CoV-2



GENERATION AND CHARACTERISTICS OF A NEUTRALIZING ANTIBODY

- Affinity maturation is based on the somatic mutation of the germline genes of immunoglobulin, a process called somatic hypermutation (SHM)
- specialized microstructures formed in secondary lymphoid tissues upon infection or immunization, producing long-lived plasma cells and memory B cells, which protect against reinfection
- GCs are organized into two regions, the dark zone (DZ) and the light zone (LZ)



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- In the DZ, there are B cells with a high proliferation rate (called centroblasts) and SHM
 - The centroblasts then enter the LZ (now are called centrocytes), where they capture and process antigens present on follicular dendritic cells (FDC)
 - they subsequently present antigenic peptides to T follicular helper (Tfh) cells to in order receive critical survival signals and undergo selection
 - FDCs can have the ability to retain the native antigen to carry out the previous process, in addition to producing cytokines, such as BAFF, that help in the survival of the B cell
 - Z class-switch recombination (CSR) also occurs, where the constant region of the heavy chain of the antibody is changed, allowing B cells to produce IgG antibodies, IgA or IgE.
 - This process diversifies the effector functions of antibodies, e.g., IgG can activate NK cells and phagocytes to eliminate cells infected by pathogens
 - final stage of the germinal center process, the centrocytes exit the GC as memory B cells or high-affinity antibody-secreting plasma cells
 - In the case of a plasma cell, the key is the activation of the *Blimp1* master regulator (Prdm1), which, among many other functions, helps to stop the expression of transcription factors
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- For memory B cells, there is no established master regulator
 - Plasma cells reside in the bone marrow and constitutively secrete antibodies do not possess antibodies
 - memory B cells express BCR but do not secrete antibodies constitutively. When they meet again with the antigen, they can reactivate and form GCs to produce antibodies with a greater affinity; moreover, these cells can give rise to plasma cells and reside in circulation or peripheral lymphoid tissue
 - NAbs can come from both populations of B cells, but it is most probably that bNAbs come from evolved memory B cells recruited into the plasma cell compartment.
 - NAbs could have increased affinity for antigen compared to the corresponding naive B-cell receptors but a higher affinity does not always define a higher neutralizing capacity.
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- The required levels of **SHM and affinity maturation** may vary from target to target; for example, while chronic infection may result in mutation levels from their germinal genes upwards of 30%, as seen in HIV-1 broadly neutralizing antibodies (bNAbs),
 - mutations of 5–20% may provide sufficient maturation for effective neutralization, which could be more readily achieved by vaccination
 - mutation levels over 20% may be difficult to achieve by vaccination; thus, they consider goal mutation levels closer to 5–15% for those NAbs targeting specific and multiple sites of vulnerability
 - The complementarity determining regions (CDRs) with more mutations are also variables for each type of antibody depending on the virus; as an example, for HIV-1, it has been seen to have a greater effect on CDR H3
 - Regarding SARS-CoV-2, Graham et al. reported a low percentage of SHM in VH and VL genes (mean of 1.9% and 1.4%, respectively) following an acute infection
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