Antibodies (Immunoglobulin)

ياسمين خليل

A Presentation by

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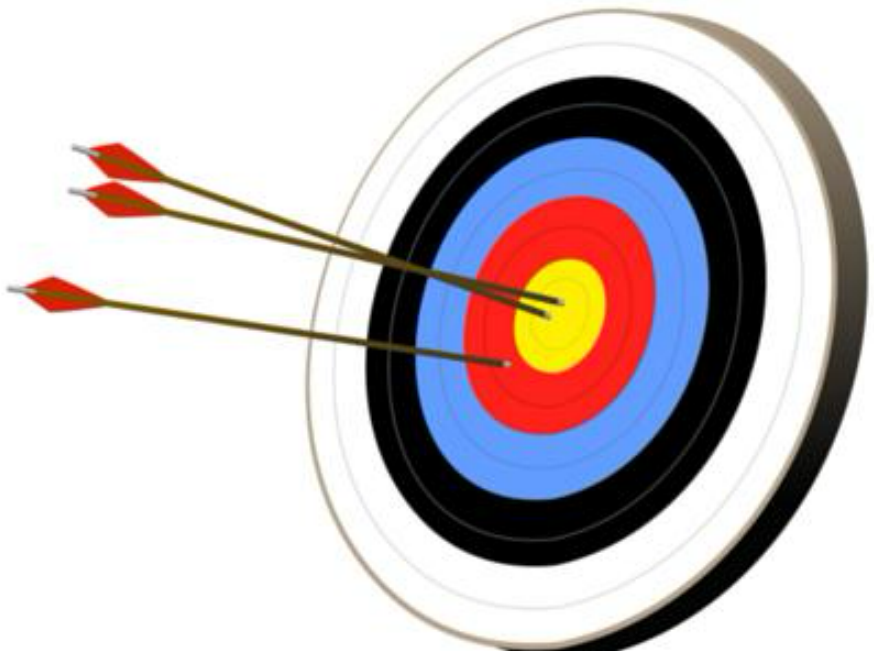
Objectives

- **Immunoglobulin structure and binding site/s.**
- **Immunoglobulin classes and their characteristics.**
- **The role of Immuoglobulines in neutralization, opsonization, antibody-dependent cellular cytotoxicity (ADCC), complement and mucosal immunity.**
- **Introduction to artificial antibodies including monoclonal and polyclonal antibodies.**

بسم الله الرحمن الرحيم



Ab: Antibody

Introduction

الأجسام المضادة ما بتشتغل بشكل عشوائي بل كل نوع antibodies بتعرف على antigen واحد بس.

- Proteins that recognize and bind to a particular antigen with very high specificity.
- Belong to a group of serum proteins called immunoglobulins (Igs).
- Ab is produced by B cells in response to a stimulation of Ag.
- Ab possesses a high degree of specificity and affinity
- Each antibody has at least two identical sites that bind antigen: Antigen binding sites.

الأجسام المضادة هي بروتينات سكرية موجودة في serum سوائل الجسم الثانية، عشان هيك بنسميها immunoglobulins.

مكنيا في تشابتر ٦ إنه الأجسام المضادة ناتجة عن B-cells عن plasma cells وهاد كلمة احتياطي للجسم الغريب إلي دخل الجسم antigen

كل جسم مضاد بكون شغال على antigen محدد

كلما كانت رابطة antibody مع antigen رح يزيد ارتباط الجسم المضاد على سطح المكروب أكثر

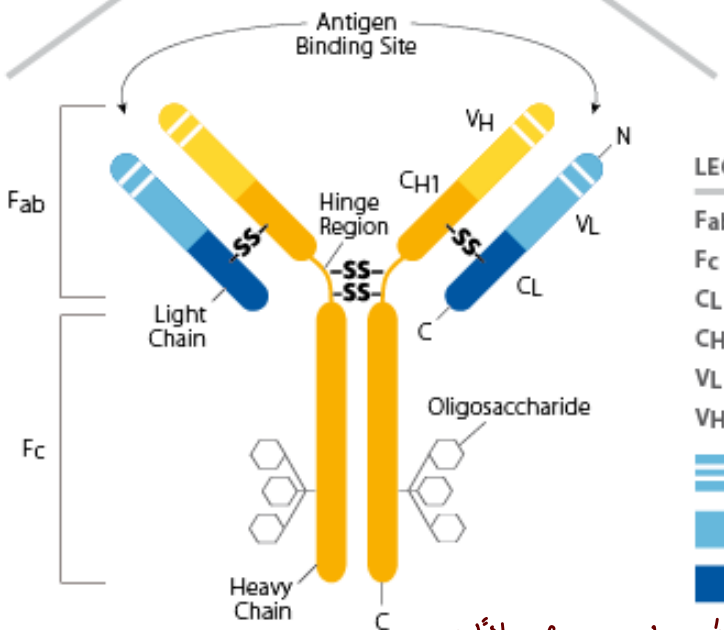
الجسم المضاد شكله زي حرف Y وأطرافه من الأعلى في ضغطتين متطابقتين

تمامًا اسمهم مواقع ارتباط بـ antigen، عشان إنهم مع قيمن ف هاد يعني إنه يكنع إمساك antigen بنفس الوقت

اللهم ارحم أيهم واغفرله
وعافه وأعف عنه واجمعه
وأهله والمسلمين في الجنة

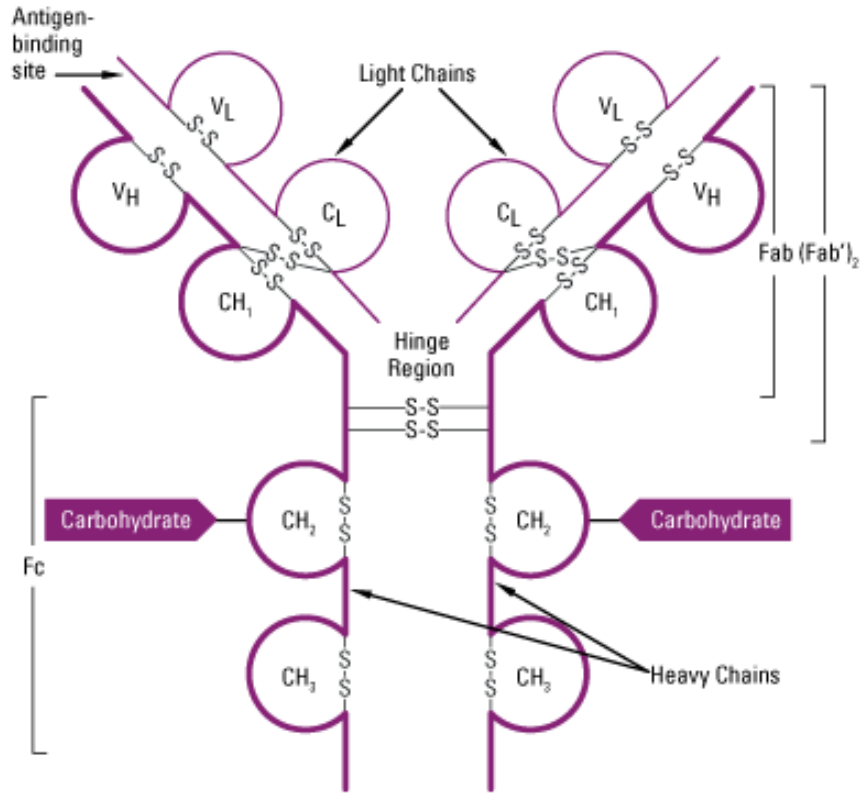
Antibodies Structure

- Immunoglobulins are glycoproteins made up of **Four polypeptide chains** (IgG):
  - Two light (L) polypeptide chains
  - Two heavy (H) polypeptide chains
- The four chains are linked by disulfide bonds



LEGEND

- **Fab** Fragment, antigen-binding
- **Fc** Fragment, crystallizable
- **CL** Constant domain, Light Chain
- **CH** Constant domain, Heavy Chain
- **VL** Variable domain, Light Chain
- **VH** Variable domain, Heavy Chain
- Hypervariable Region
- Variable Region
- Constant Region



Generalized structure of an immunoglobulin (IgG).

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 (V) and Constant (C) Regions

- Each H-chain and each L-chain has V-region and C-region

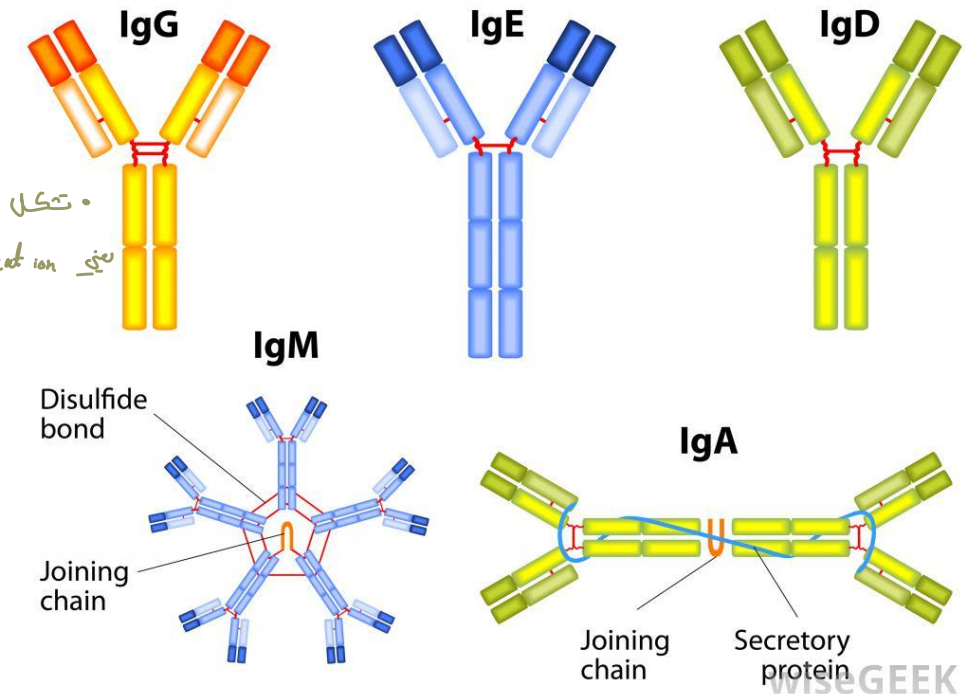
1. **V region:** Terminal portion of L-chain and terminal portion of H-chain compose antigen binding site and located within the "Fab" fragment of antibody. It shows wide variation in amino acid sequences
2. **C-region:** lies in terminal portion of molecule. C-region shows an unvarying amino acid sequence and forms Fc fragment. It is responsible for biologic functions. H-chains are distinct for each of the five Immunoglobulins

Antibodies Classes

Five classes of Antibodies:

1. IgG
2. IgM
3. IgA
4. IgD
5. IgE

GAMED



WiseGEEK

- **An antibody molecule is composed of two identical Ig heavy chains (H) and two identical light chains (L), each with a variable region (V) & constant region (C).**

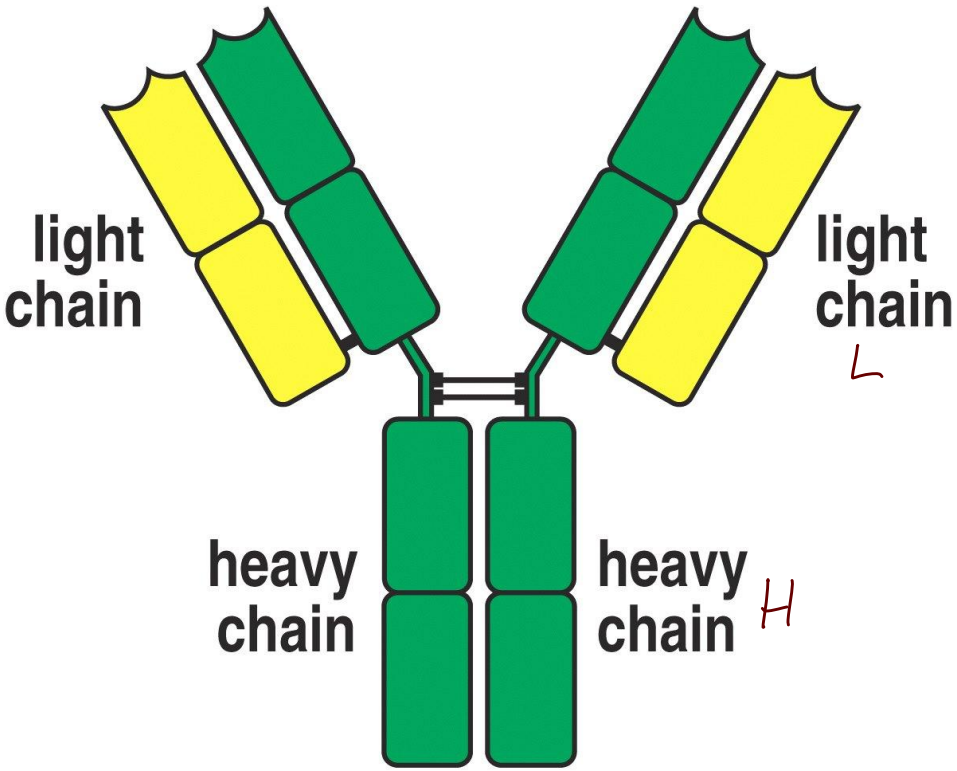
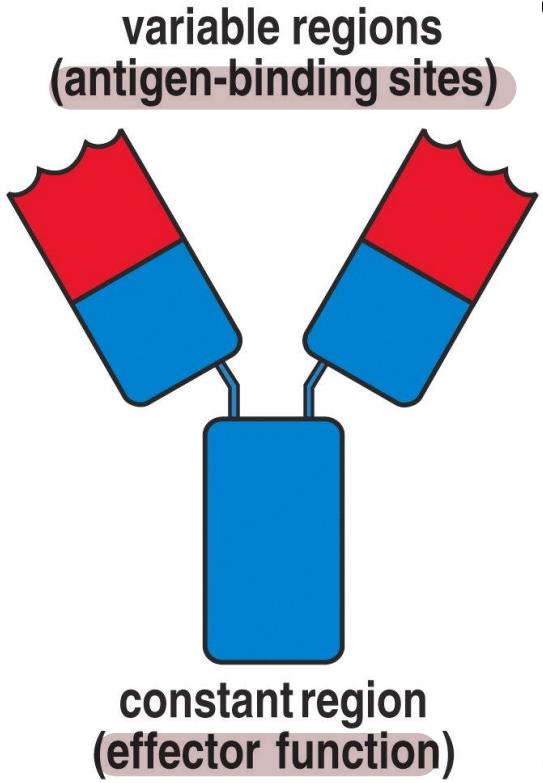


Figure 1-17 Immunobiology, 6/e. (© Garland Science 2005)



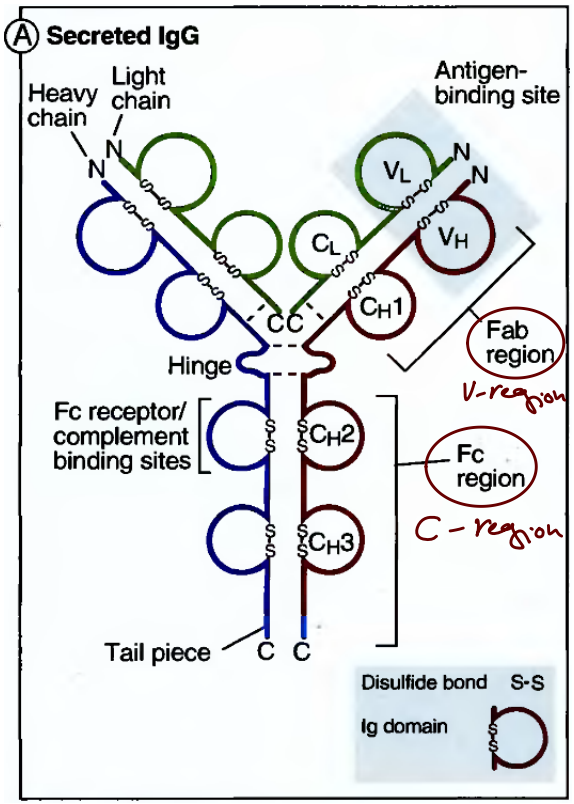


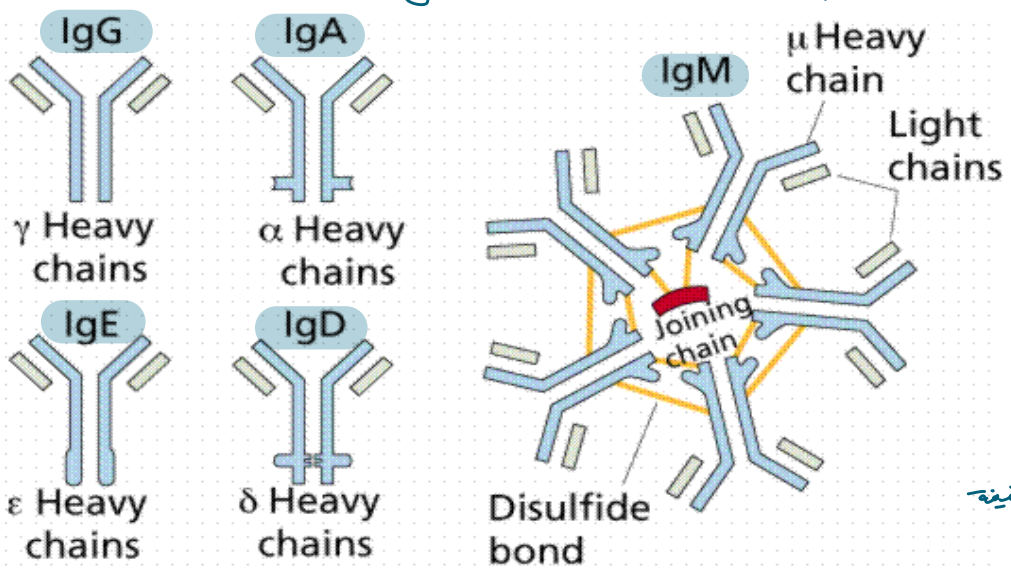
Figure 1-16 Immunobiology, 6/e. (© Garland Science 2005)

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 gamma-chains; **IgMs** have mu-chains; **IgAs** have alpha-chains; **IgEs** have epsilon-chains; and **IgDs** have delta-chains.
- **Differences** in heavy chain polypeptides **allow** these immunoglobulins 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. While there are **five** different types of heavy chains, there are only **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 10 light chains, 10 heavy chains and 10 antigen-binding.

اللهم صل وسلم

وبارك على محمد



The complement system

هو عبارة عن مجموعة من البروتينات الصغيرة (أكثر من 30 نوعًا) طافية في الدم، ومعظمها تتصنع في الكبد
في الحالة الطبيعية: تتدور في الدم وهي خاملة وطافية بتهاجم خلايا جسمك (inactive)، بتشتغل بس لما يجيلها محفز وإشارة

- The complement system consists of a number of small proteins that are synthesized by the liver, and circulate in the blood as inactive precursors. When stimulated by one of several triggers, proteases in the system cleave specific proteins to release cytokines and initiate an amplifying cascade of further cleavages.

وكلها تفعيلها إشارة ومحفز بوجود microb في أسلوب شغلة زع يكونه التتابع (cascade)
تحفيز (وجود microb) ← تكسير ← cleavage: عن طريق إنزيم protease بين تكسير البروتين الخامل لتقطع تشغلة ← تضخيم Amplification: ترجع التقطع لتشغلة لتكسر البروتينات الأخرى وبيتهاعن التفاعل إلى آلاف البروتينات في ثوان

- The end result of this complement activation or complement fixation cascade is stimulation of phagocytes to clear foreign and damaged material, inflammation to attract additional phagocytes, and activation of the cell-killing membrane attack complex.

وبالنهاية عنا 3 result من هاد الـ cascade
1 جذب phagocytes ← chemotaxis ← opsonization ← تغطية البروتينات تعمل علامات على سطح البكتيريا عشان تتسهل البروتين على phagocytes
2 inflammation: يزيد من تدفق الدم وتوسع الأوعية في منطقة الإصابة فارز يزيد عدد خلايا المناعة هناك.
3 [MAC] Membrane Attack complex: تتجمع البروتينات وتعمل شكل أنبوب أو ثقب يغرس نفسه في غشاء خلية البكتيريا فتفقد السوائل إليها وتنتفخ وتنحل lysis

- Over 30 proteins and protein fragments make up the complement system, including serum proteins, and cell membrane receptors. They account for about 10% of the globulin fraction of blood serum.

تشكل حوالي 10% من بروتينات الغلوبولين في Serum

The complement system

- The complement system, also known as complement cascade, is a part of the immune system that enhances (complements)

✓ the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism,

✓ promote inflammation,

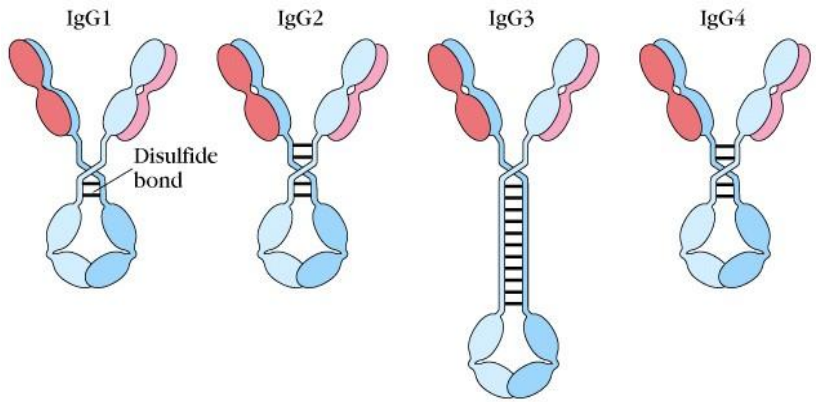
✓ and attack the pathogen's cell membrane.

- It is part of the innate immune system, which is not adaptable and does not change during an individual's lifetime.
- The complement system can, however, be recruited and brought into action by antibodies generated by the adaptive immune system.

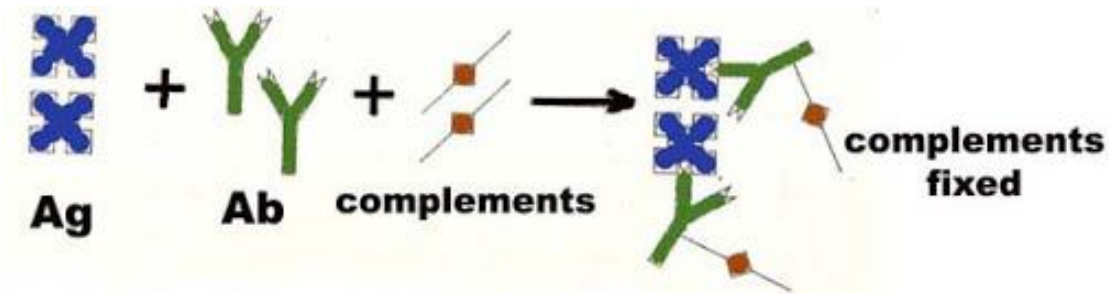
أستغفر الله العظيم وأتوب إليه

1. IgG

Four subclasses: IgG1, IgG2, IgG3, IgG4

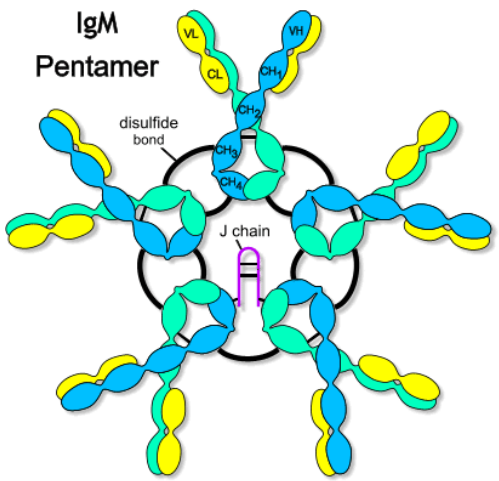


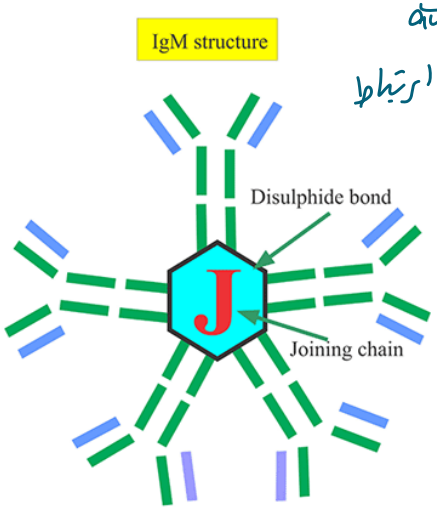
- **Structure:** Monomer
- **Percentage serum antibodies:** 80%
- **Location:** Blood, lymph, intestine
- **Half-life in serum**: 23 days
- **Complement Fixation**: Yes
- **Placental Transfer**: Yes
- **Known Functions**: Enhances phagocytosis, neutralizes toxins and viruses, protects fetus and newborn.



2. IgM

- **Structure:** Pentamer
- **Percentage serum antibodies:** 5-10%
- **Location:** Blood, lymph, B cell surface (monomer)
- **Half-life in serum:** 5 days
- **Complement Fixation:** Yes
- **Placental Transfer:** No
- **Known Functions**: First antibodies produced during an infection. Effective against microbes and agglutinating antigens.





يا حي يا قيوم

برحمتك أستغيث

أصلح لي شأني كله

ولا تكلني إلى نفسي

طرفة عين

Antibodies Fight Infections in Stages

- When an antigen is introduced into the body for the first time, large quantities of IgM are produced, while the B cells are producing the highly specific IgG more slowly.
- Once IgG is produced in quantity, the IgG takes on a greater role in the removal of antigens from the body, due to its ability to bind to the antigen molecules more tightly.
- Through the course of an infection, a rapid spike of circulating IgM can be seen in the bloodstream, followed by a decrease of IgM as the amount of IgG increases.
- Medical personnel can identify the course and duration of an infection by measuring the ratio of IgM to IgG in the bloodstream.
- A ratio high in IgM indicates that an infection is in its early stages, while a ratio high in IgG indicates that the infection is in its later stage.

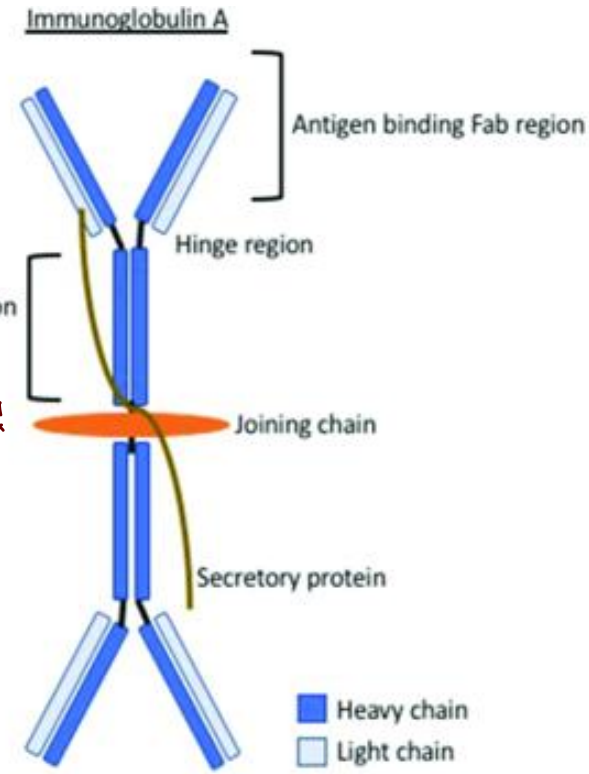
3. IgA

وحدتين Y بيعملوا ارتباط

- **Structure:** Dimer
- **Percentage serum antibodies:** 10-15%
- **Location:** Secretions (tears, saliva, intestine, milk), blood and lymph.
- **Half-life in serum:** 6 days
- **Complement Fixation:** No
- **Placental Transfer:** No → يحصل عليها الجنين من الرضاعة من أثناء الحمل
- **Two subclasses**: IgA1, IgA2
- **Known Functions**:

➢ Localized protection of mucosal surfaces.

➢ Provides immunity to infant digestive tract.



وجود مخاط، لعاب
في أفرازات الأنف، حليب الأم

لحمايته الطفل من ميكروبات الأم لأن جهازه الهضمي لسه ضعيف

ويحمي GIT في الأشهر الأولى من عمره

4. IgD

- **Structure:** Monomer
- **Percentage serum antibodies:** 0.2%
- **Location:** B-cell surface, blood, and lymph
- **Half-life in serum**: 3 days
- **Complement Fixation**: No
- **Placental Transfer**: No
- Known Functions: In serum function is unknown. On B cell surface (signal the B cells to be activated), initiate immune response.

اللهم انصر

الإسلام

والمسلمين

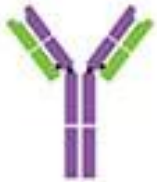
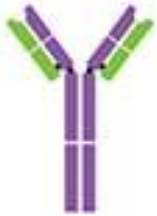
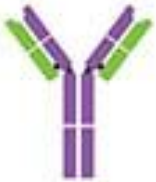
5. IgE

- **Structure:** Monomer
- **Percentage serum antibodies:** 0.002%
- **Location:** Bound to mast cells and basophils throughout body, blood.
- **Half-life in serum:** 2 days
- **Complement Fixation:** No
- **Placental Transfer:** No
- **Known Functions:**

✓Allergic reactions.

✓Possibly lysis of worms.

Eosinophils

The Five Immunoglobulin (Ig) Classes					
	IgM pentamer	IgG monomer	Secretory IgA dimer	IgE monomer	IgD monomer
					
Heavy chains	μ	γ	α	ε	δ
Number of antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000
Percentage of total antibody in serum	6%	80%	13%	0.002%	1%
Crosses placenta	no	yes	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to		phagocytes		mast cells and basophils	
Function	Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor	Main blood antibody of secondary responses, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva, colostrum	Antibody of allergy and antiparasitic activity	B cell receptor

Antibodies Functions

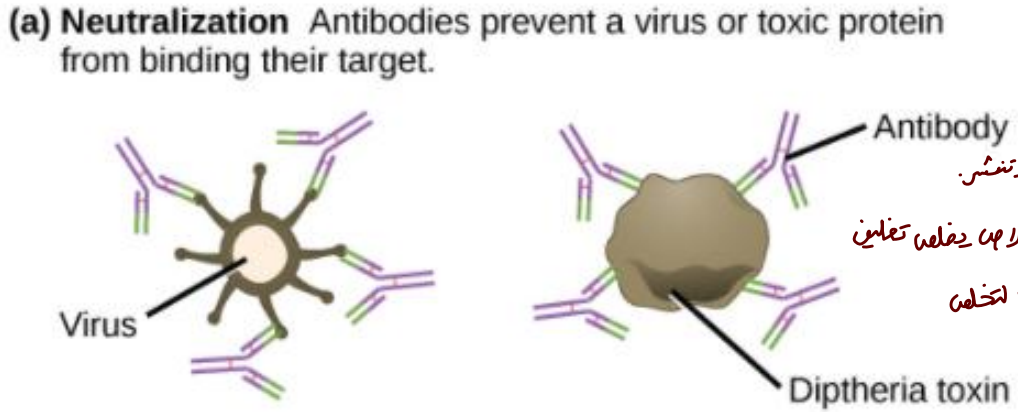
- Differentiated plasma cells are crucial players in the humoral immunity response.
- The antibodies they secrete are particularly significant against extracellular pathogens and toxins.
- Once secreted, antibodies circulate freely and act independently of plasma cells.
- Sometimes, antibodies can be transferred from one individual to another. For instance, a person who has recently produced a successful immune response against a particular disease agent can donate blood to a non-immune recipient, conferring temporary immunity through antibodies in the donor's blood serum.
- This phenomenon, called passive immunity, also occurs naturally during breastfeeding, which makes breastfed infants highly resistant to infections during the first few months of life.

❑ Antibodies Functions

- **Neutralization:** Bind antigen- neutralize toxins, virus particles
- **Opsonization**
- **Complement activation- IgG,M**
- **Antibody-Dependent Cell Mediated Cytotoxicity (ADCC)**
- **Mast cells activation**
- **Transcytosis- movement across epithelial cells**

a. Neutralization

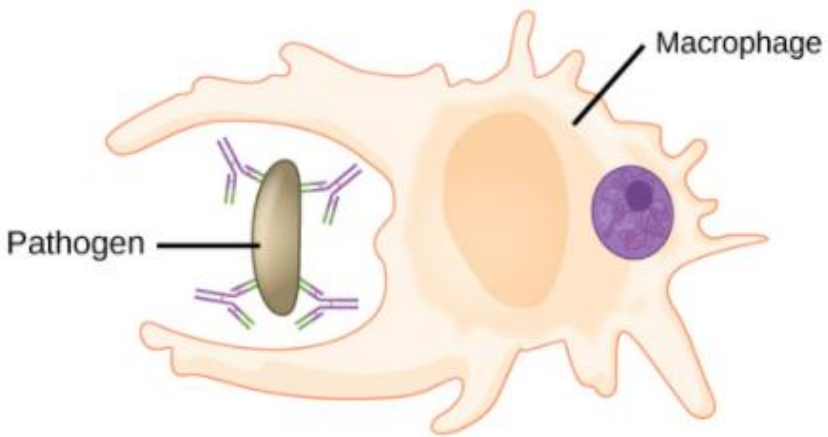
- The first step in a microbial infection involves attachment of the organism to the outside surface of the human body, either some part of the skin or the mucosal surfaces
- High-affinity antibodies that bind to the microbial ligand and prevent the microbe's attachment to human epithelium stop the infection before it starts
- Antibodies thus bind and inactivate foreign antigenic entities directly.
- Antibodies coat extracellular pathogens and neutralize them by blocking key sites on the pathogen that enhance their infectivity, such as receptors that "dock" pathogens on host cells.
- **Antibody neutralization** can prevent pathogens from entering and infecting host cells, as opposed to the cytotoxic T-cell-mediated approach of killing cells that are already infected to prevent progression of an established infection. The neutralized antibody-coated pathogens can then be filtered by the spleen and eliminated in urine or feces.

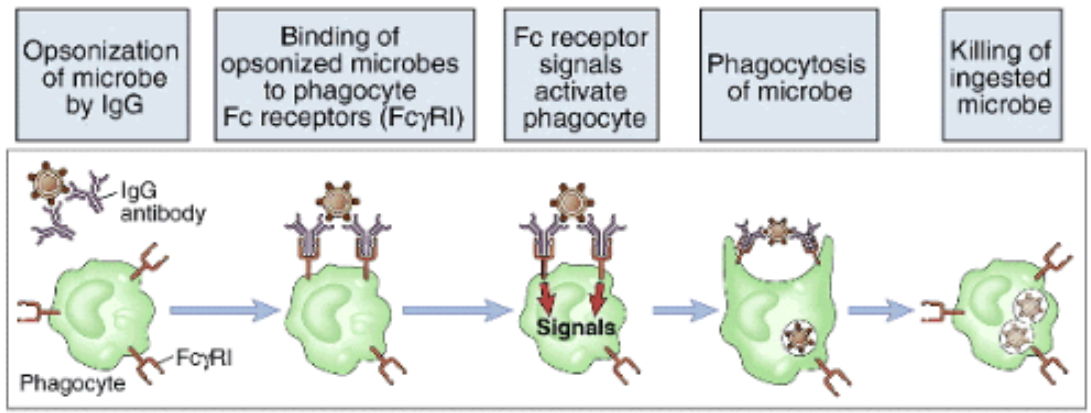


b. Opsonization

- Antibodies also mark pathogens for destruction by phagocytic cells, such as macrophages or neutrophils, because they are highly attracted to macromolecules complexed with antibodies. Phagocytic enhancement by antibodies is called opsonization.
- Many bacteria are coated with polysaccharide → slippery and hard to endocytose
- But IgG can bind polysaccharide
- Macrophage can specifically bind IgG via FC-γ receptors

(b) Opsonization A pathogen tagged by antibodies is consumed by a macrophage or neutrophil.





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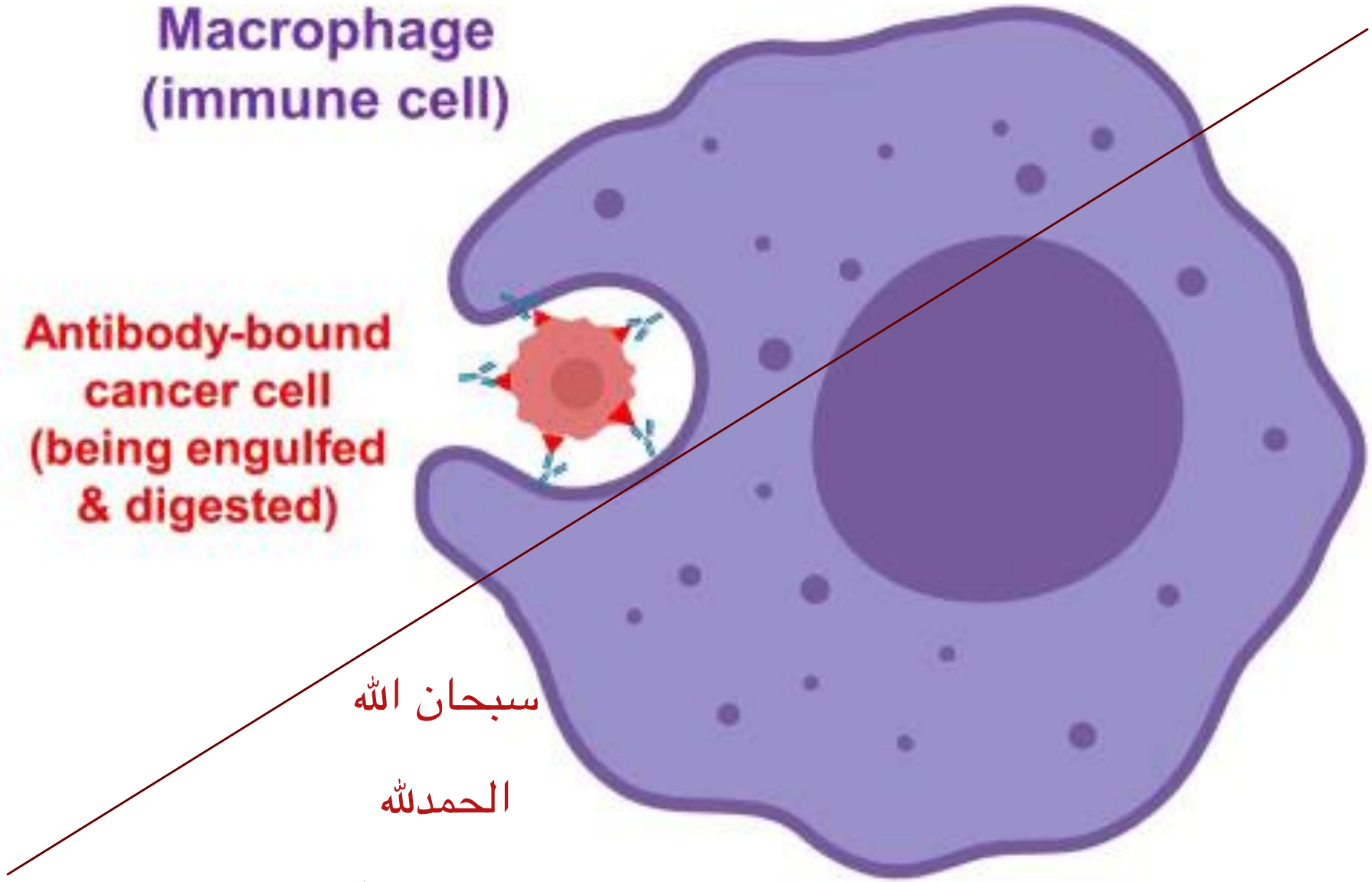


سبحان الله

الحمدلله

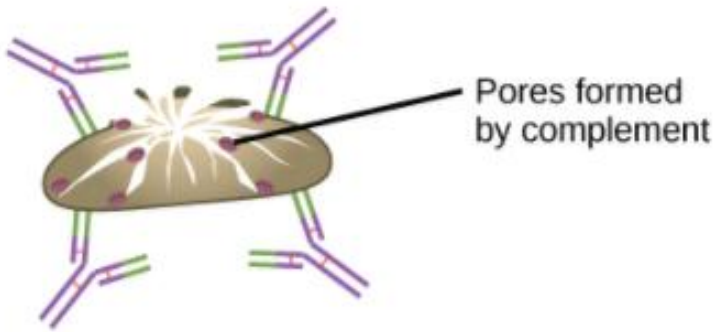
لا إله إلا الله

الله أكبر

c. Complement Activation

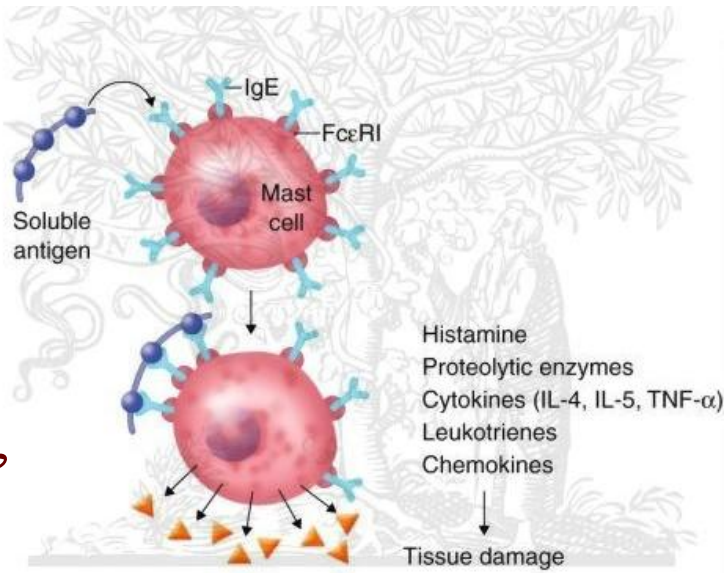
- In another process, complement fixation, IgM and IgG in serum bind to antigens, providing docking sites onto which sequential complement proteins can bind.
- The combination of antibodies and complement enhances opsonization even further, promoting rapid clearing of pathogens.
1. Classical: IgM or 2 adjacent IgG's binds to C1Q on bacterial surface results in cascade that can cause bacterial lysis
2. Alternative: antibody binding attracts C3B → phagocytosis and opsonization

(c) Complement activation Antibodies attached to the surface of a pathogen cell activate the complement system.



d. Mast Cell Activation

- IgE exists in serum at very low concentration (ng/ml)
- IgE binds to FC-ε recptors on Mast, Basophil, and Langerhan cells
- Antigen cross links bound antibodies → degranualtion and release of histamine, heparin, proteases, chemotaxins which attracts WBC's
- This induce Phospholipase activity → mucus production, sneezing and other allergic symptoms



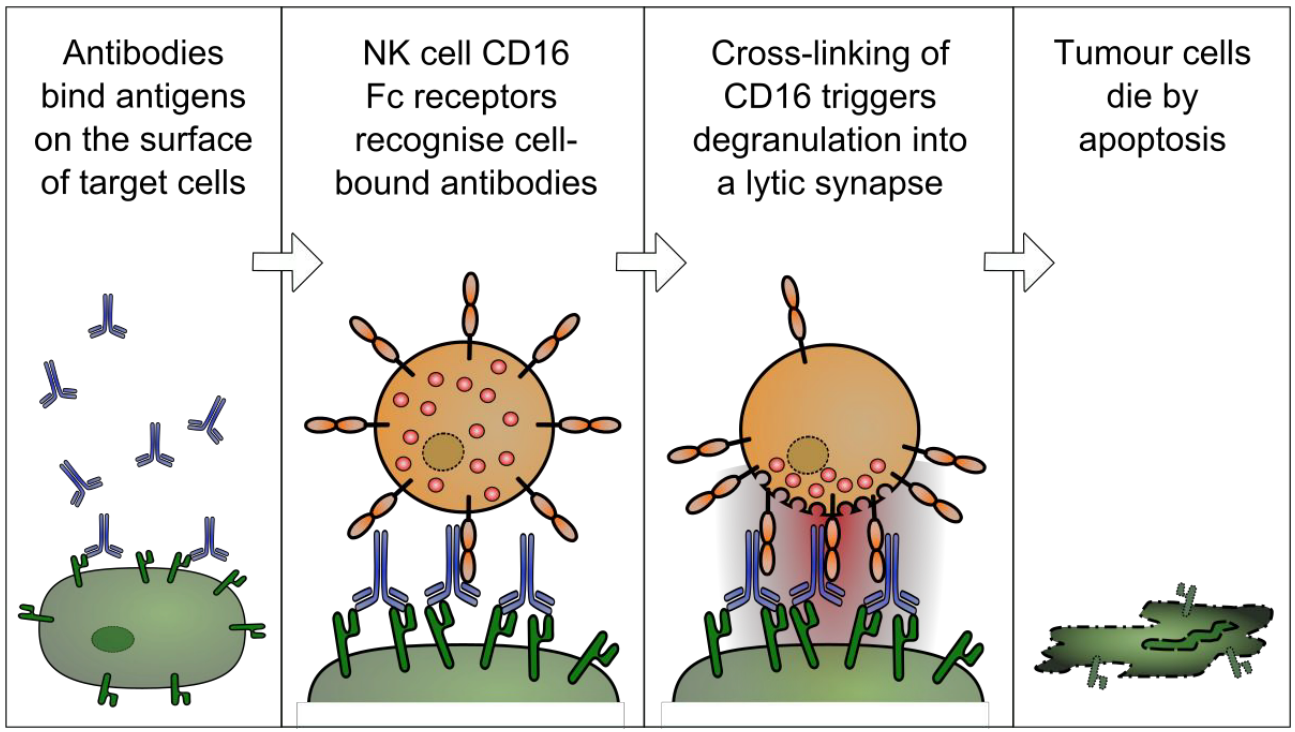
هاي ممكن نتجاهلها من قبل إنها رابطة بين المناعة الفطرية والمكتسبة.

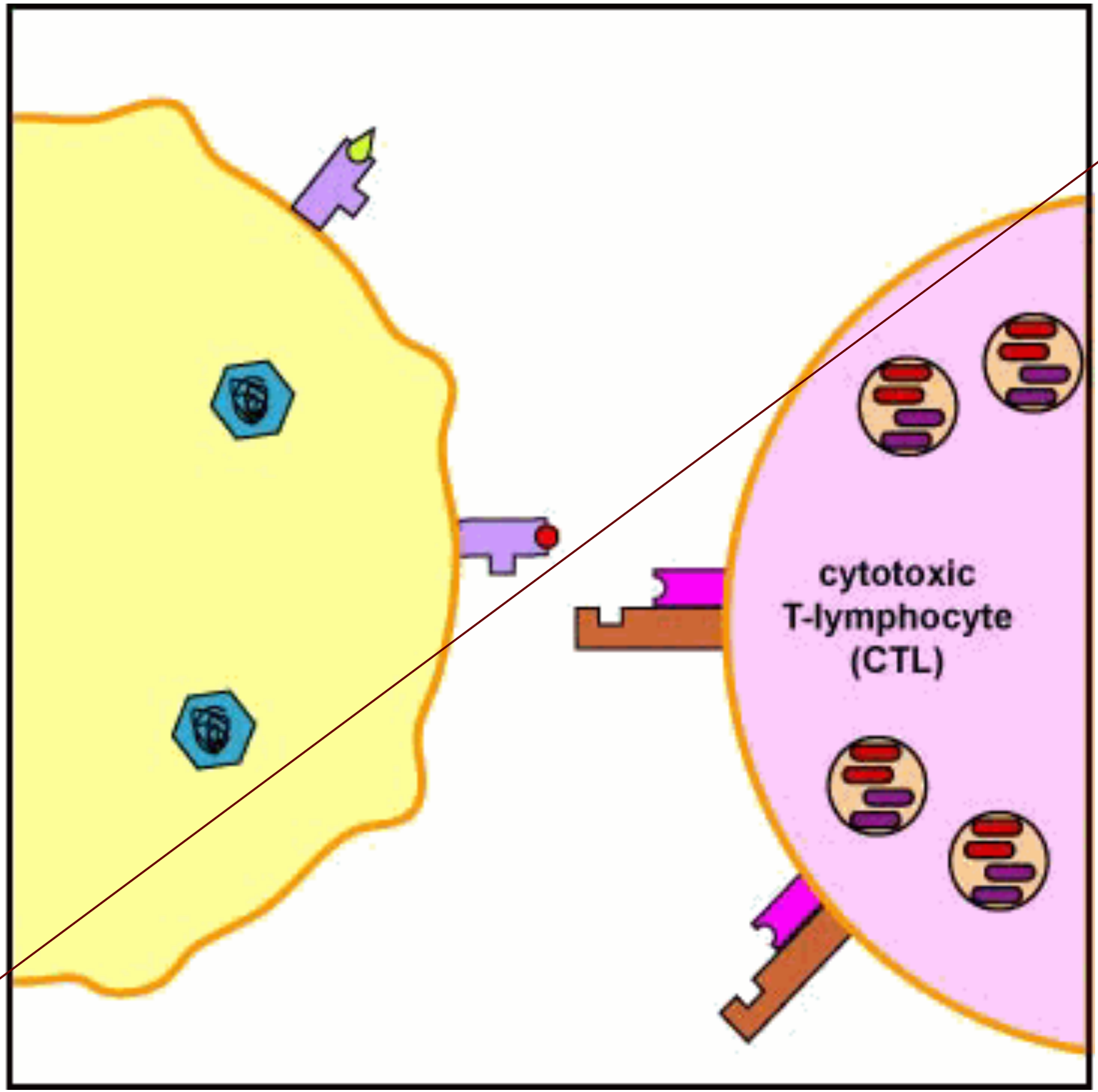
e. ADCC: Antibody-Dependent Cell Mediated Cytotoxicity

تهاجم الأهداف الكبيرة جدًا مثل الطفيليات، قبل الخلية بالمكتسبة IgG الـ antigens على سطح الخلية
الهدف وترتبط بها، الجزء الأساسي من IgG يسمى بالخلية المناعية، أما الخلفي F_c يبرز للخارج كإشارة استفزاز قوية.

- ADCC is an adaptive immune response largely mediated by NK cells through FC-γR receptor that binds the Fc portion of IgG antibodies triggering the lysis of targeted cells.
- IgG binds target cell (virally infected or tumorigenic)
- Crosslinking of receptors → perforin/protease release by NK

$Fc-\gamma R$ في NK عندها مستقبلات متخصصة
في بعض الخلايا
بعدما تفرز NK محتوياتها
من Perforin (بروتين)
بعمل ثقوب في غشاء الخلية المصابة)
و إنزيمات تدخل هاي الثقوب وتحبر
الخلية كلها Apoptosis





اللهم

اهدني

لأحسن

الأخلاق

f. Transport/trancytosis

النقل عبر الخلايا

- Transcytosis (also known as cytopempsis)
- It is a type of transcellular transport in which various macromolecules are transported across the interior of a cell.

وسيلة يستخدمها الجسم لنقل الجزيئات الضخمة

① يتم احتجاز الجزيئات داخل حويصلات على جانب واحد من الخلية

- Macromolecules are captured in vesicles on one side of the cell, drawn across the cell, and ejected on the other side.

② تتحرك الحويصلات عبر cytoplasm

③ وتندمج الحويصلة مع الغشاء في الجانب الآخر وتطرد وتقذف محتواها للخارج

- Examples of macromolecules transported include IgA
- While transcytosis is most commonly observed in epithelial cells,
- Submucosal lymphoid follicles secretes **IgA** (trachea, for example).
- Epithelial M cells phagocytose/pinocytose foreign particles in lumen transport; digested antigens into follicle and stimulate antibody production

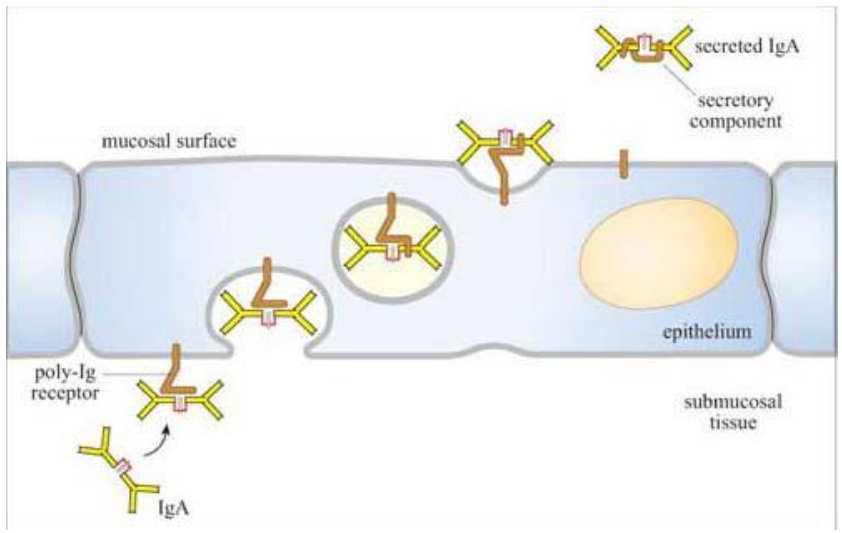
تتم تقديم أجزاء البروتين الخاصة للجهاز المناعي لتحفيز إنتاج antibody

- Placental transport: active transport of IgG across placenta give protection for baby up to six months

الوصيد

IgA : by milk

IgG: by placental



Affinity, Avidity, and Cross reactivity

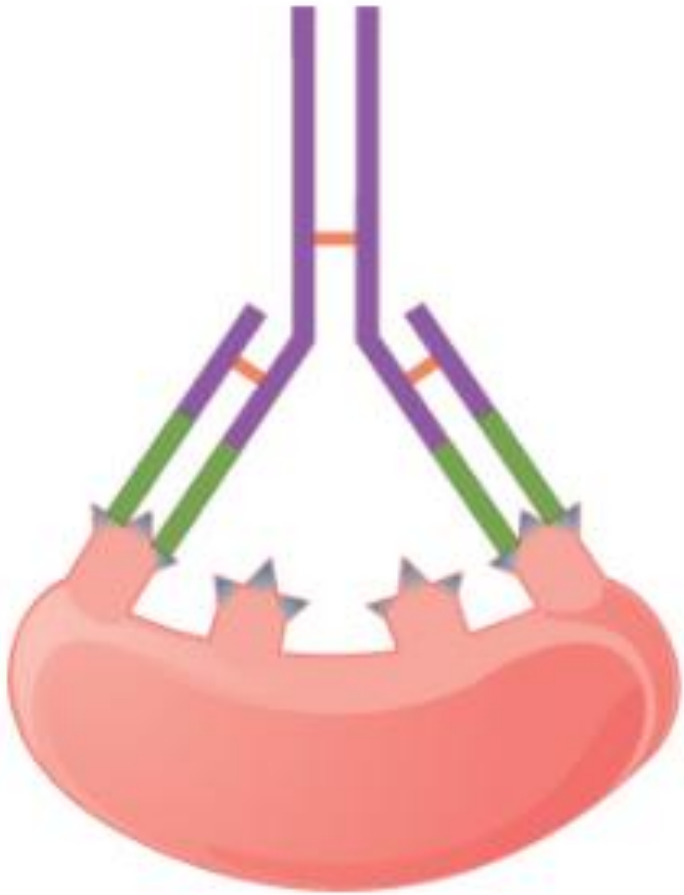
a. Affinity versus Avidity

- Not all antibodies bind with the same strength, specificity, and stability.
- In fact, antibodies exhibit different affinities (attraction) depending on the molecular complementarity between antigen and antibody molecules.
- An antibody with a higher affinity for a particular antigen would bind more strongly and stably.
- It would be expected to present a more challenging defense against the pathogen corresponding to the specific antigen.
- The avidity depends on the **number** of identical binding sites on the antigen being detected, as well as other physical and chemical factors.
- Typically, multimeric antibodies, such as pentameric IgM, are classified as having lower affinity than monomeric antibodies, but high avidity.
- Essentially, the fact that multimeric antibodies can bind many antigens simultaneously balances their slightly-lower-binding strength for each antibody/antigen interaction.

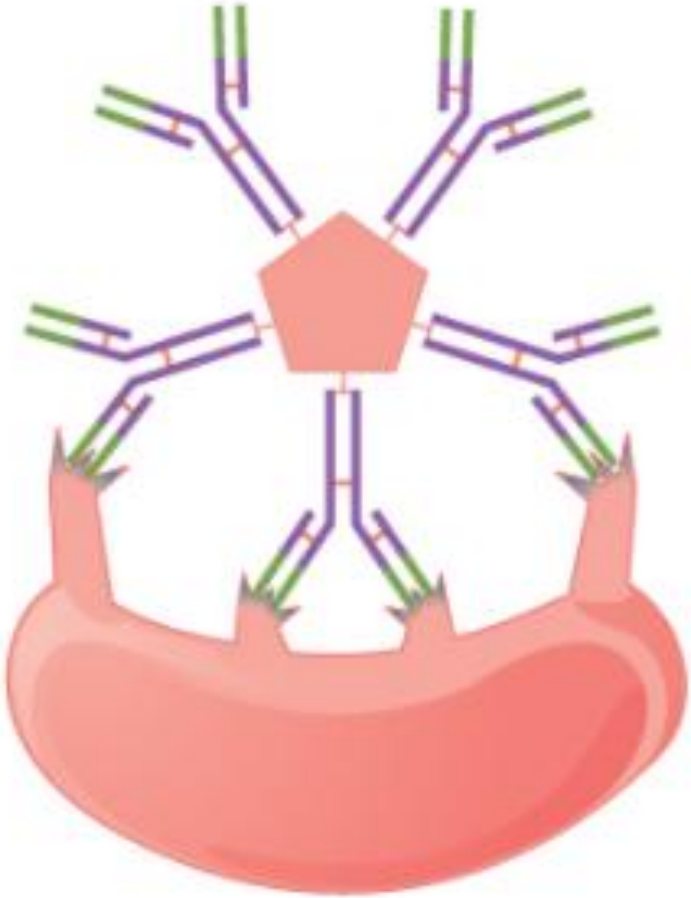
Affinity = قوة الرابط الواحد (Quality).

- Avidity = قوة الارتباط الكلي (Quantity + Quality).

(a) Affinity versus avidity



Affinity refers to the strength of a single antibody–antigen interaction. Each IgG antigen binding site typically has high affinity for its target.



Avidity refers to the strength of all interactions combined. IgM typically has low affinity antigen binding sites, but there are ten of them, so avidity is high.

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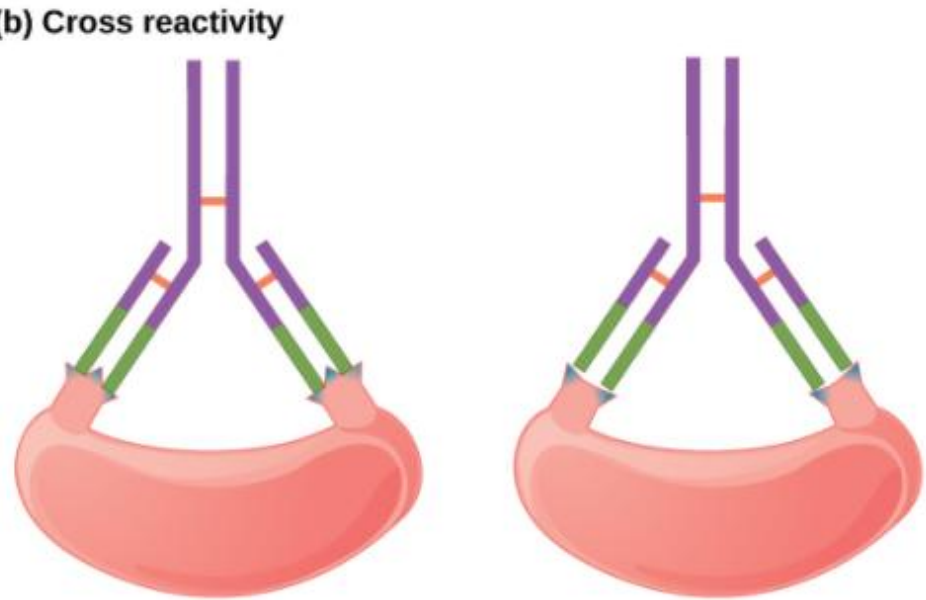
Affinity, Avidity, and Cross reactivity

b. Cross reactivity

- Cross reactivity occurs when an antibody binds not to the antigen that elicited its synthesis and secretion, but to a different antigen.
- Because an epitope corresponds to such a small region (the surface area of about four to six amino acids), it is possible for different macromolecules to exhibit the same molecular identities and orientations over short regions.

- Cross reactivity can be **beneficial** if an individual develops immunity to several related pathogens despite having been exposed to or vaccinated against only one of them.
- Conversely, antibodies raised against pathogenic molecular components that resemble self molecules may incorrectly mark host cells for destruction, causing autoimmune damage.



An antibody may react with two different epitopes.

Artificial Antibodies

- Antibodies made artificially
- Two types:

1. Polyclonal Ab:
   - A mixture Ab with different specificities and affinities
   - Generate in a natural response or artificial immunization
2. Monoclonal Ab:
   - Ab produced by single clone (or one hybridomas clone) and having a single specificity

اللهم اغفر لي ولوالدي وللمسلمين

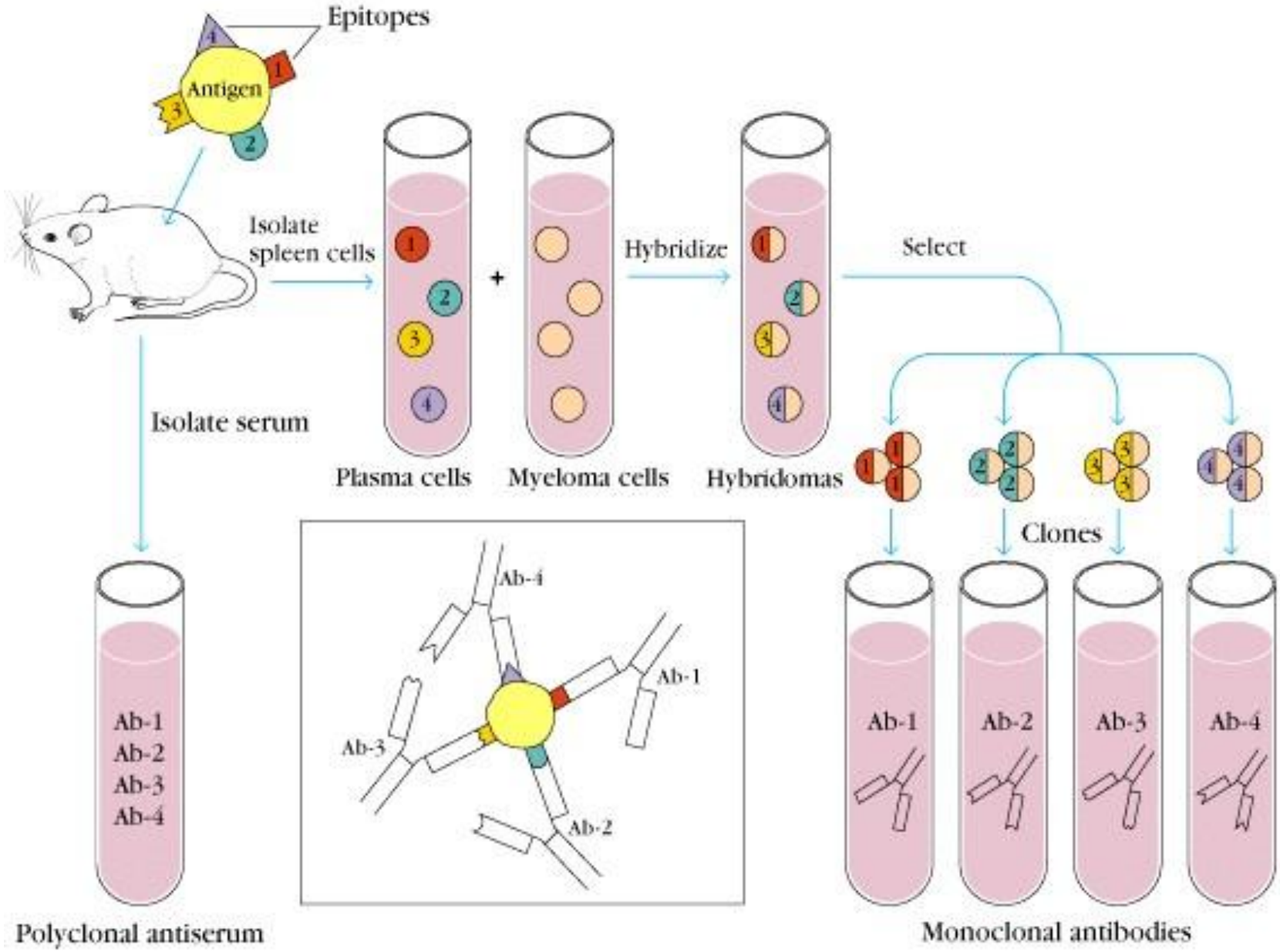
والمسلمات والمؤمنين والمؤمنات الأحياء

والأموات

MAbs

Monoclonal Ab Applications

- Diagnostic Tests
  - mAbs are capable to detect tiny amounts (pg/mL) of molecules
  - Ex. Pregnancy hormones
- Diagnostic Imaging
  - mAbs that recognize tumor antigens are radiolabeled with iodine I-131
- Immunotoxins
  - mAbs conjugated with toxins
- mAbs to Clear Pathogens
- mAbs for treatment (thrombotic diseases, cancer..)


Epitopes
Antigen
1
2
3
4
Isolate spleen cells
Plasma cells
Myeloma cells
Hybridize
Hybridomas
Select
Clones
Isolate serum
Ab-1
Ab-2
Ab-3
Ab-4
Polyclonal antiserum
Monoclonal antibodies

Artificial antibodies

POLYCLONAL.	MONOCLONAL.
Derived from different B Lymphocytes cell lines	Derived from a single B cell clone
Batch to Batch variation affecting Ab reactivity & titre	mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity
NOT Powerful tools for clinical diagnostic tests	Enable the development of secure immunoassay systems.

KEY TAKEAWAYS

- **Key Points**

- Antibodies are produced by plasma cells, but, once secreted, can act independently against extracellular pathogen and toxins.
- Antibodies bind to specific antigens on pathogens; this binding can inhibit pathogen infectivity by blocking key extracellular sites, such as receptors involved in host cell entry.
- Antibodies can also induce the innate immune response to destroy a pathogen, by activating phagocytes such as macrophages or neutrophils, which are attracted to antibody-bound cells.
- Affinity describes how strongly a single antibody binds a given antigen, while avidity describes the binding of a multimeric antibody to multiple antigens.
- A multimeric antibody may have individual arms with low affinity, but have high overall avidity due to synergistic effects between binding sites.
- Cross reactivity occurs when an antibody binds to a different-but-similar antigen than the one for which it was raised; this can increase pathogen resistance or result in an autoimmune reaction.

- **Key Terms**

- **Avidity**: the measure of the synergism of the strength individual interactions between proteins
- **Affinity**: the attraction between an antibody and an antigen

The measurement of antibodies specific to COVID-19

- The body responds to a viral infection immediately with a non-specific innate response in which macrophages, neutrophils, and dendritic cells slow the progress of virus and may even prevent it from causing symptoms.
- This non-specific response is followed by an adaptive response where the body makes antibodies that specifically bind to the virus. These antibodies are proteins called immunoglobulins.
- The body also makes T-cells that recognize and eliminate other cells infected with the virus. This is called cellular immunity.
- This combined adaptive response may clear the virus from the body, and if the response is strong enough, may prevent progression to severe illness or re-infection by the same virus (This process is often measured by the presence of antibodies in blood).

The measurement of antibodies specific to COVID-19

- WHO continues to review the evidence on antibody responses to SARS-CoV-2 infection. Most of these studies show that people who have recovered from infection have antibodies to the virus.
- However, some of these people have very low levels of neutralizing antibodies in their blood, suggesting that cellular immunity may also be critical for recovery.
- Laboratory tests that detect antibodies to SARS-CoV-2 in people, including rapid immunodiagnostic tests, need further validation to determine their accuracy and reliability.
- Inaccurate immunodiagnostic tests may falsely categorize people in two ways:

➢The first is that they may falsely label people who have been infected as negative.

➢The second is that people who have not been infected are falsely labelled as positive.

➢These tests also need to accurately distinguish between past infections from SARS-CoV-2 and those caused by the known set of six human coronaviruses. Four of these viruses cause the common cold and circulate widely. The remaining two are the viruses that cause Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome.

➢People infected by any one of these viruses may produce antibodies that cross-react with antibodies produced in response to infection with SARS-CoV-2.

Successful transfer of anti–SARS-CoV-2 immunity using convalescent plasma in an MM patient with hypogammaglobulinemia and COVID-19

- EXCEPTIONAL CASE REPORT/ OCTOBER 8, 2020
- **Key Points**
- A severely immunocompromised patient with MM and COVID19 who received a convalescent plasma product showed SARS-CoV-2 clearance.
- The convalescent plasma showed humoral immunity against all structural SARS-CoV-2 proteins, which was successfully transferred to the patient.

- https://ashpublications.org/bloodadvances/article/4/19/4864/464130/Successful-transfer-of-anti-SARS-CoV-2-immunity