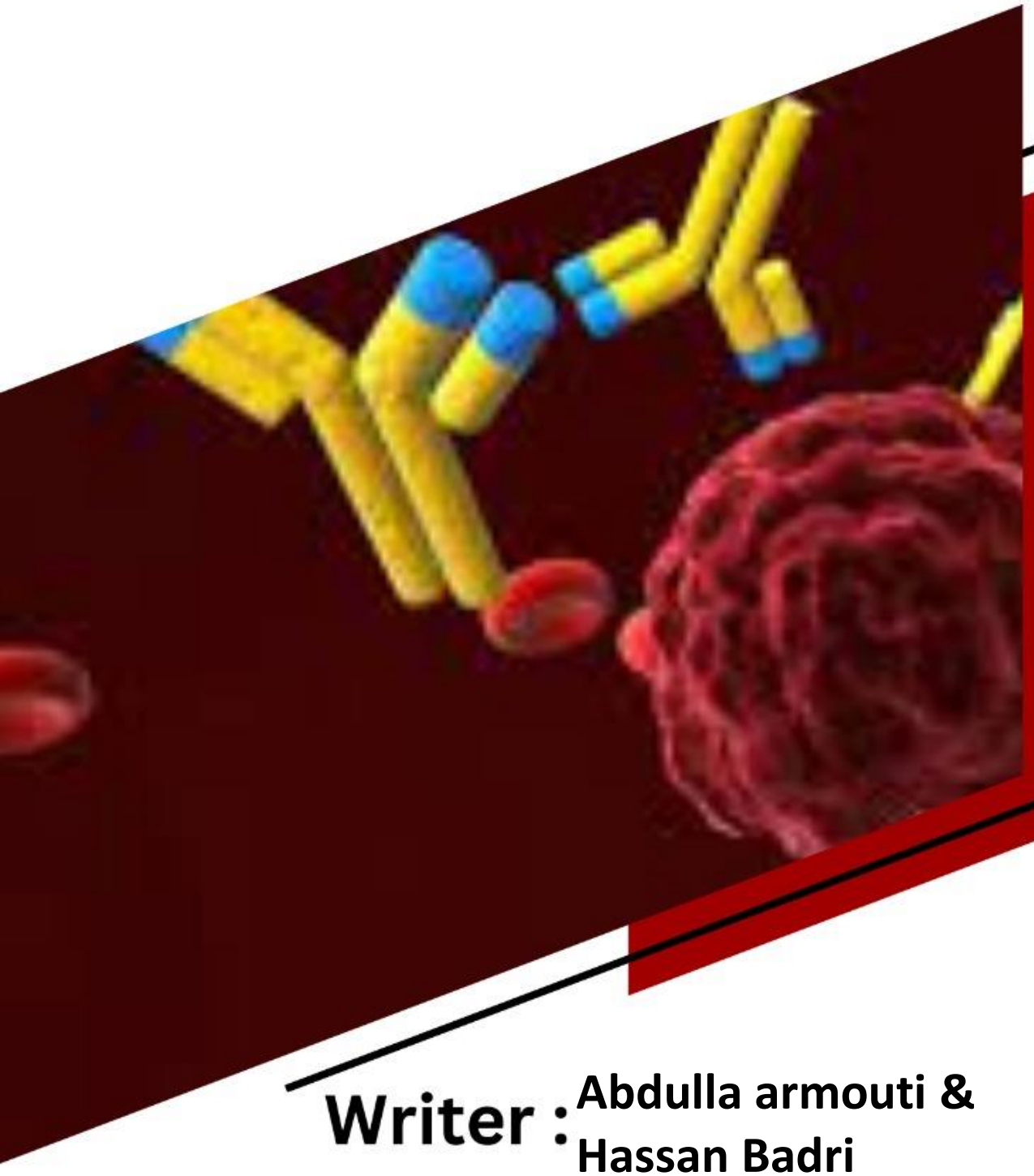
Doctor 021

IMMUNOLOGY

Sheet no. 7



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ANTIBODIES

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General revision by the doctor at the start of the lecture:

NOD like receptors (which is a cytoplasmic pattern recognition receptor) causes the formation of the inflammasome.

The inflammasome starts the cascade to cleave the protein pro IL-1 beta to Activated pro IL-1 Beta. that's why we target nod like receptors for regulation.

Some pattern recognition molecules are soluble (exp. Opsonins, neutralizing protein). which can activate complement system.

Antibodies

Antibodies were discovered when a scientist noticed that when serum is added to bacteria they died (by mac attack mostly).

Why doesn't mac attack affect our cells?

Because they have inhibitors of the complement system like cd55/factor H.

Though rarely in some pathogen the inhibitors are absent on the cell and the cells are attacked.

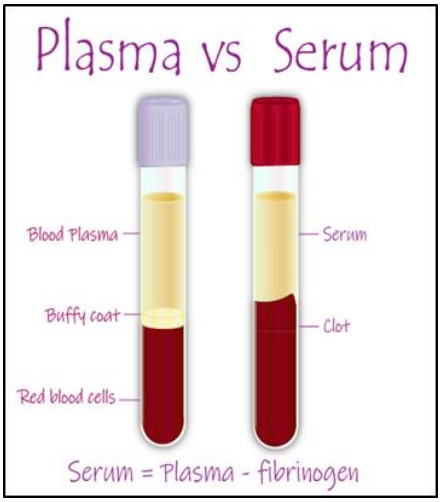
Antibodies are usually found on serum. we can diagnose certain diseases by sampling the serum (serology).

Other immune mediators found in the serum are **complement proteins**

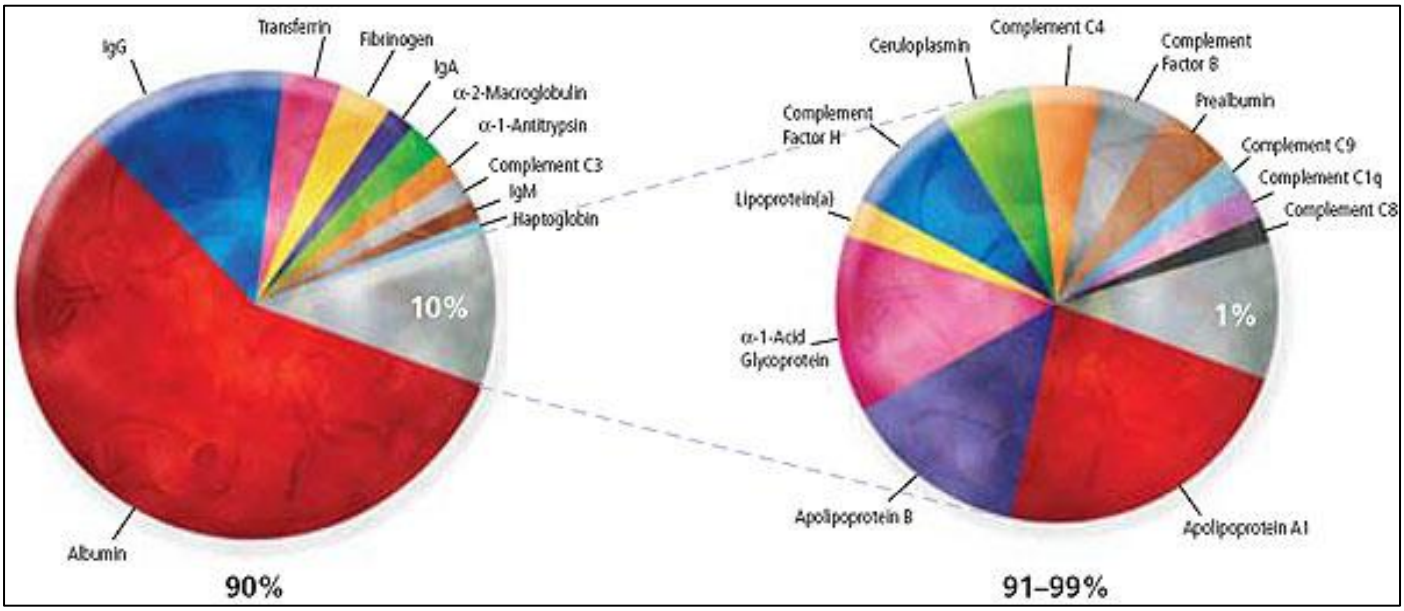
The study of antibodies and their reactions with antigens is classically called serology.

Serum lacks coagulation factors but otherwise contains all the proteins found in plasma. Any serum sample that contains detectable antibody molecules that bind to a particular antigen is commonly called an antiserum.

A healthy 70-kg adult human produces about 2 to 3 g of antibodies every day.



Plasma proteomics holds great promise for the future of biomarker discovery, as well as *in vitro* diagnostics. Although plasma is readily accessible for analysis, the study of the plasma proteome is fundamentally limited by its vast dynamic range (10 orders of magnitude).



IgG,igA.igM and complement are present in abundant quantity.

Antibodies are circulating proteins that are produced in vertebrates in response to exposure to foreign structures known as antigens

The strength of the binding between a single site of an antibody and an epitope of an antigen is called the affinity of the antibody.

Epitope:

- An epitope, also known as antigenic determinant,
- Are the binding site on the antigen which bind to an antibody
- is **the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells**.

1-Usually the higher the **affinity** the more stable is the antibody-antigen interaction thus enhancing the antibody Ability to fight pathogens. The overall affinity of all the antibodies to antigens is called the **AVIDITY.**

2-THE AVIDITY also depends on the arrangement of antibodies on the cell surface (distribution of the Antigens not just their availability). See the cartoon on the next slide for better understanding.

There are two sites to bind the antigen to the antibody forming a Y shaped region.

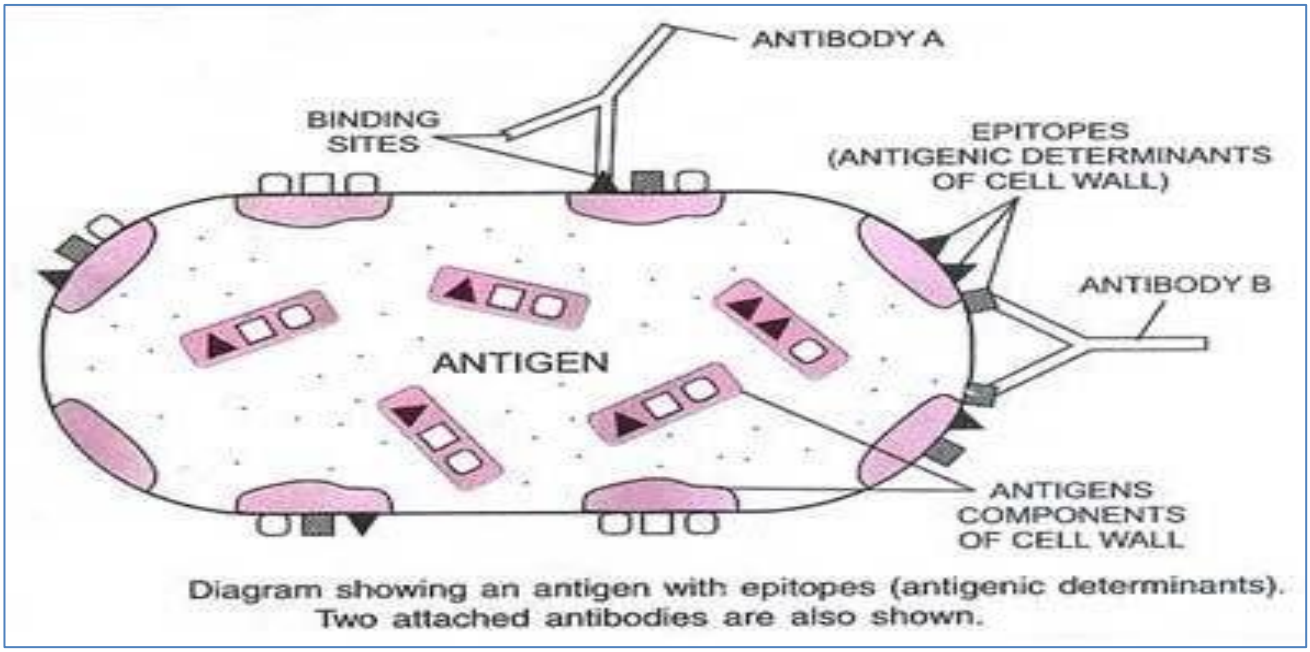


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.

Because the hinge region of antibodies gives them flexibility, a single antibody may attach to a single multivalent antigen by more than one binding site. the strength of attachment of the antibody to the antigen must take into account binding of all the sites to all the available epitopes. This overall strength of attachment is called the avidity.

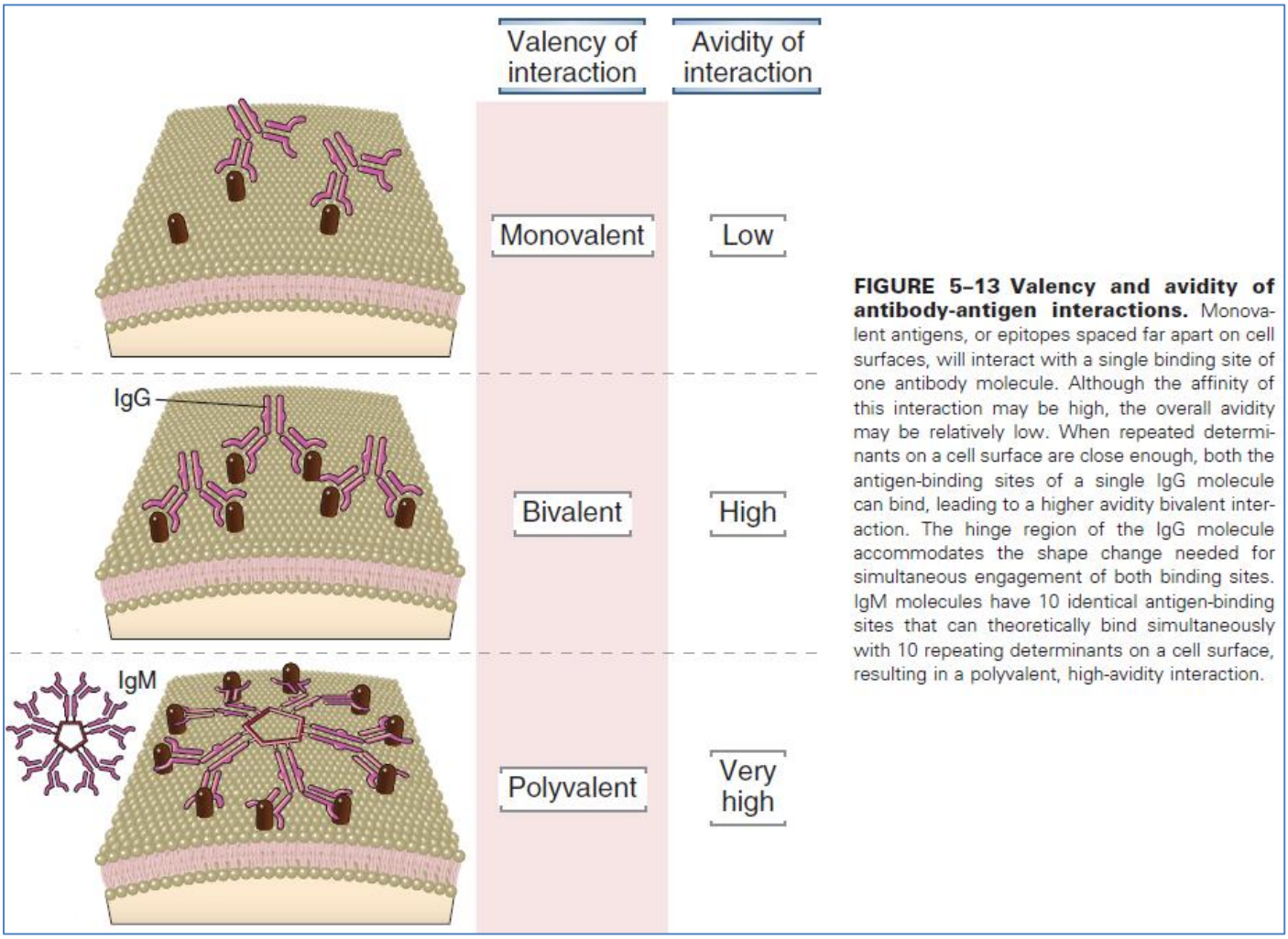


FIGURE 5–13 Valency and avidity of antibody-antigen interactions. Monovalent antigens, or epitopes spaced far apart on cell surfaces, will interact with a single binding site of one antibody molecule. Although the affinity of this interaction may be high, the overall avidity may be relatively low. When repeated determinants on a cell surface are close enough, both the antigen-binding sites of a single IgG molecule can bind, leading to a higher avidity bivalent interaction. The hinge region of the IgG molecule accommodates the shape change needed for simultaneous engagement of both binding sites. IgM molecules have 10 identical antigen-binding sites that can theoretically bind simultaneously with 10 repeating determinants on a cell surface, resulting in a polyvalent, high-avidity interaction.

Most of the time a polyvalent interaction is of a much higher avidity than a monovalent one **however** there could be some exceptions for example:

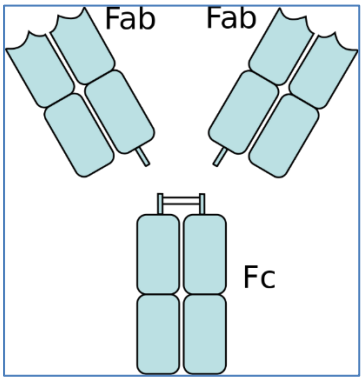
The igM doesn't necessarily have higher avidity than igG because igG could have higher affinity for the antigen in a <u>certain</u> case.

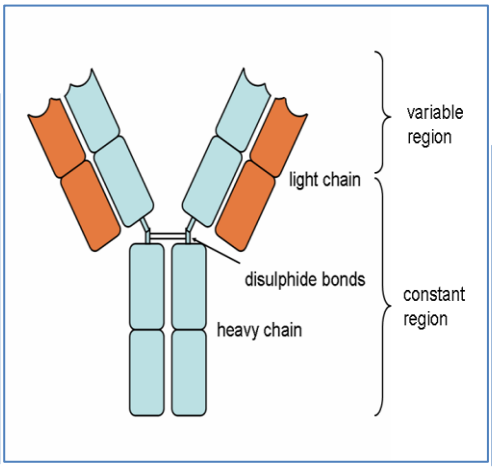
Antibodies (immunoglobulins) are made of 2 heavy chains and 2 light chains, those chains combined give us an antibody binding region (Fab) and the fragment crystallizable region (Fc region) which is the tail region of an antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system.

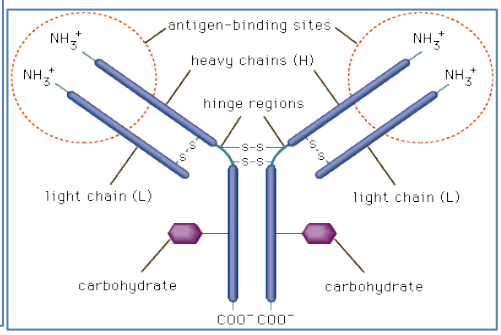
Immunoglobulins are divided into different classes (isotypes).

Fab region is variable and Fc region is constant. The Fc is constant because it deals with Fc receptors on body cells like macrophages and mast cells and it also interacts with complement system (C1q). in contrast to the Fab variable region because it deals with a very wide range of antigens.

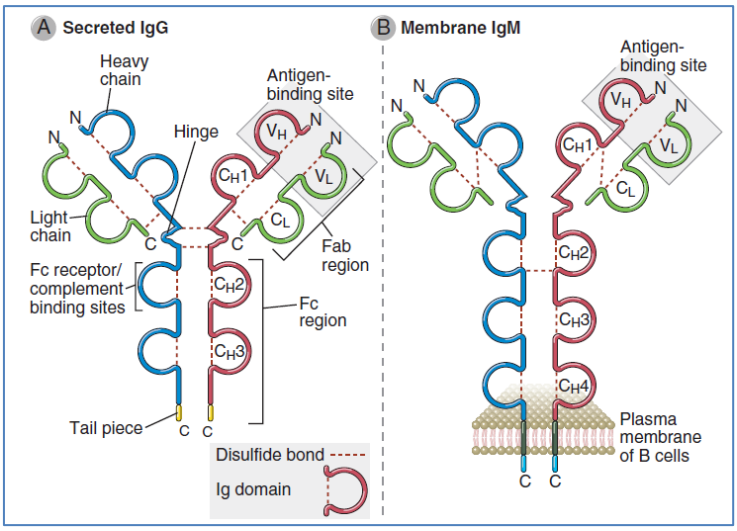
- Changes in the Fab makes changes in specificity
- Changes in the heavy chains of the Fc makes different classes of isotypes.







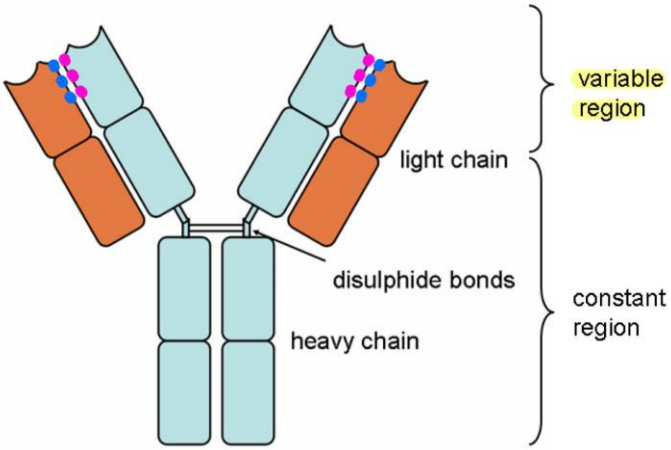
- **Antibodies can exist in two forms: membrane-bound antibodies on the surface of B lymphocytes function as receptors for antigen, and secreted antibodies that reside in the circulation, tissues, and mucosal sites**
- **Both heavy chains and light chains consist of amino terminal variable (V) regions that participate in antigen recognition and carboxyl-terminal constant (C) regions; the C regions of the heavy chains mediate effector functions.**

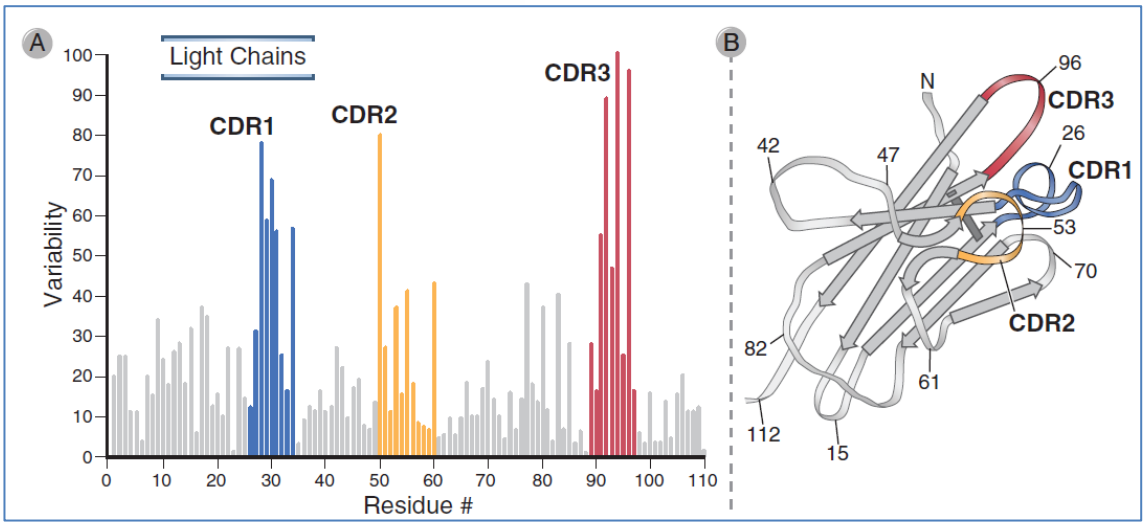


When an antibody is a membrane immunoglobulin it's considered a B cell receptor

When the cell encounters its antigen and starts producing antibodies the receptor is secreted into the circulation

Most of the sequence differences and variability among different antibodies are confined to (three short stretches in the V region of the heavy chain) in blue below and to (three stretches in the V region of the light chain) in pink. These diverse stretches are known as hypervariable segments, Because these sequences form a surface that is complementary to the three-dimensional structure of the bound antigen, the hypervariable regions are also called complementarity-determining regions (CDRs).





Very minimal change in the amino acids of these CDRs can have a huge effect on the specificity of the antibody

(Remember that the specificity of the antibody is a result of random genetic recombination during the maturation of the antibody. However, this may not be the final shape of the antibody as **Affinity maturation** can take place.

Affinity maturation: the development of the antibody to become more affinitative. This is due to simple changes in the CDRs (the **specificity** is still the same)

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

Antibodies of different classes differ in their location around the body, appear at different stages of an adaptive immune response.

The heavy chain C regions of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence. This sequence is different in antibodies of other isotypes or subtypes.

The different isotypes (classes) of immunoglobulins are formed by changing the constant region of the heavy chains
Different antibody classes have different functions, are found in different regions of the body and are secreted differently

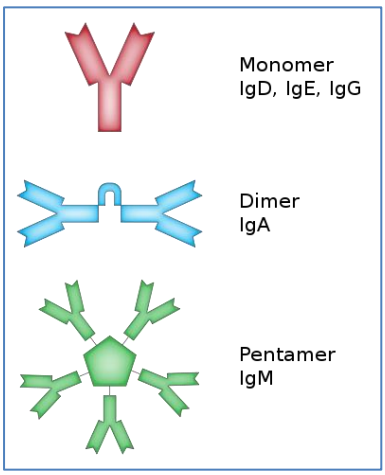


TABLE 5–2 Human Antibody Isotypes

Isotope of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	IgA (dimer) Monomer, dimer, trimer	Mucosal immunity
IgD	None (δ)	Trace	3	None	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	IgE Monomer	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	IgG1 Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	IgM Pentamer	Naive B cell antigen receptor, complement activation

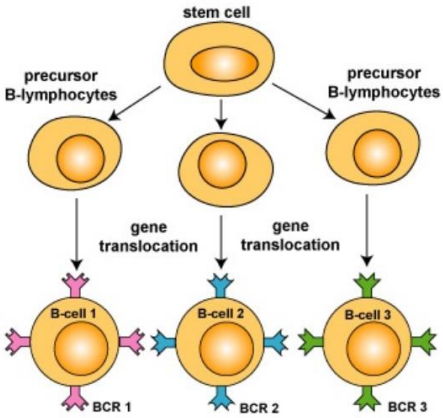
IgG: The most abundant in the serum and has the LONGEST half life

IgG, IgA & IgM: Make up the majority (98%) of antibodies in the serum

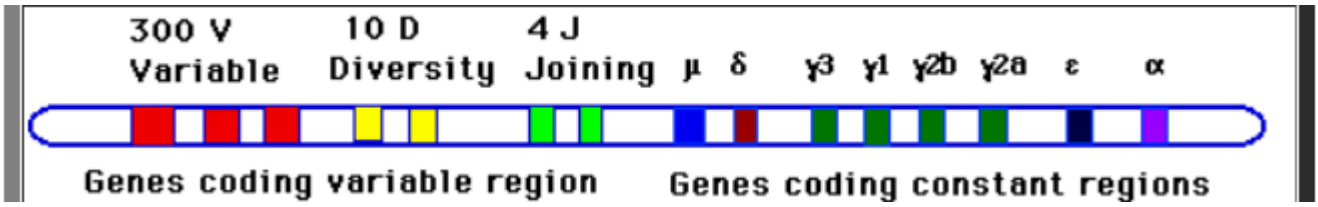
B lymphocytes are the cells responsible for antibody responses.

During its development, each B-lymphocyte becomes genetically programmed through a series of gene-splicing reactions to produce an antibody molecule with a unique specificity

It is estimated that the human body has the ability to recognize 10^7 or more different epitopes, due to the wide range of possible combinations during gene splicing.



Naive B-lymphocytes with unique B-cell receptors.



ANTIBODY PRODUCTION

Initial contact with a new antigen evokes the primary response, which is characterized by a lag phase of approximately 1 week between the challenge and the detection of circulating antibodies.

Once antibody is detected in serum, the levels rise exponentially to attain a maximal steady state in approximately 3 weeks, then decline gradually with time.

The first antibodies synthesized in the primary immune response are IgM and, then IgG antibodies arise and eventually predominate.

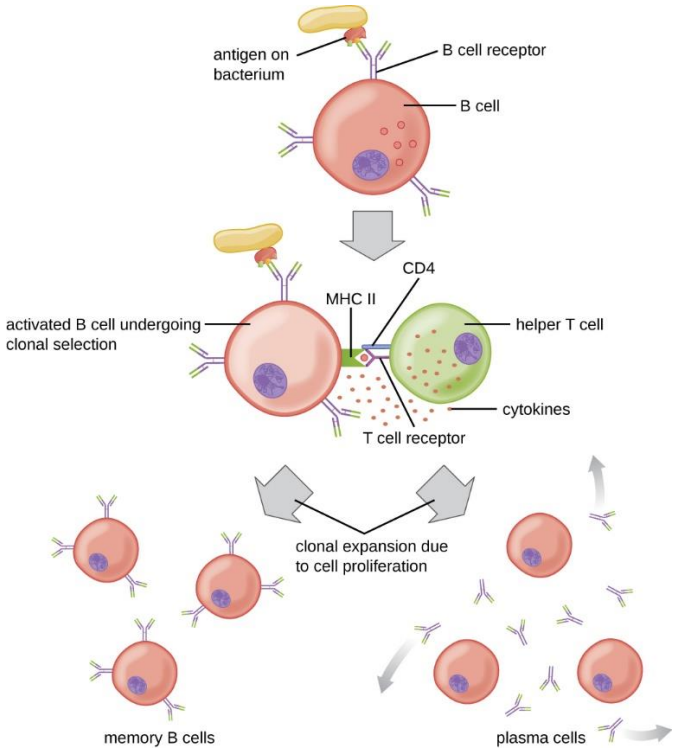
Resting B cells become activated by antigen via binding to the BCR and internalization.

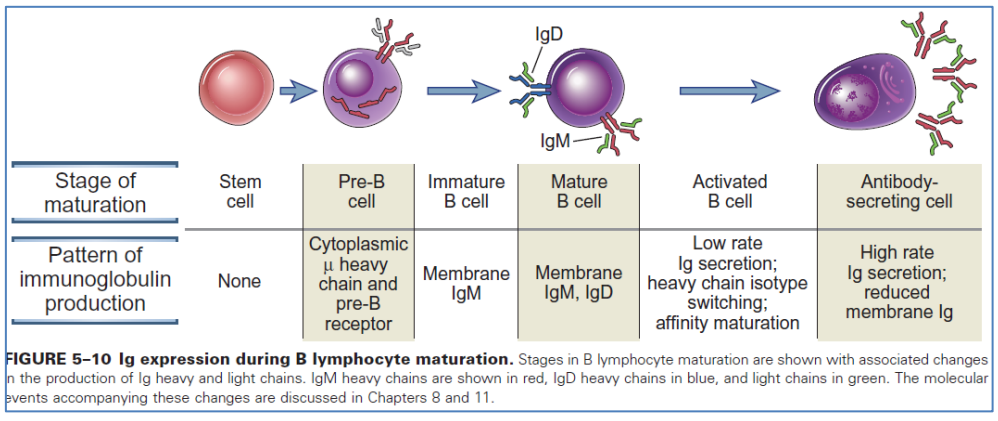
Once internalized inside the B cell, the protein antigen is processed and presented with MHC II. The presented antigen is then recognized by helper T (aka CD-4) cells specific to the same antigen.

Once activated by linked recognition, T-cells produce and secrete cytokines that activate the B cell and cause proliferation into clonal daughter cells.

Clonal expansion: leads to two types of b cells which are memory B cell and the remaining will go on to create plasma cells.

After several rounds of proliferation, additional cytokines provided by the T-cells stimulate the differentiation of activated B cell clones into memory B cells, which will quickly respond to subsequent exposures to the same antigen, and plasma cells that lose their membrane BCRs and initially secrete pentameric IgM





Stage of maturation	Stem cell	Pre-B cell	Immature B cell	Mature B cell	Activated B cell	Antibody-secreting cell
Pattern of immunoglobulin production	None	Cytoplasmic μ heavy chain and pre-B receptor	Membrane IgM	Membrane IgM, IgD	Low rate Ig secretion; heavy chain isotype switching; affinity maturation	High rate Ig secretion; reduced membrane Ig

FIGURE 5–10 Ig expression during B lymphocyte maturation. Stages in B lymphocyte maturation are shown with associated changes in the production of Ig heavy and light chains. IgM heavy chains are shown in red, IgD heavy chains in blue, and light chains in green. The molecular events accompanying these changes are discussed in Chapters 8 and 11.

After a subsequent exposure or booster injection of the same antigen, a different sequence called the secondary response ensues.

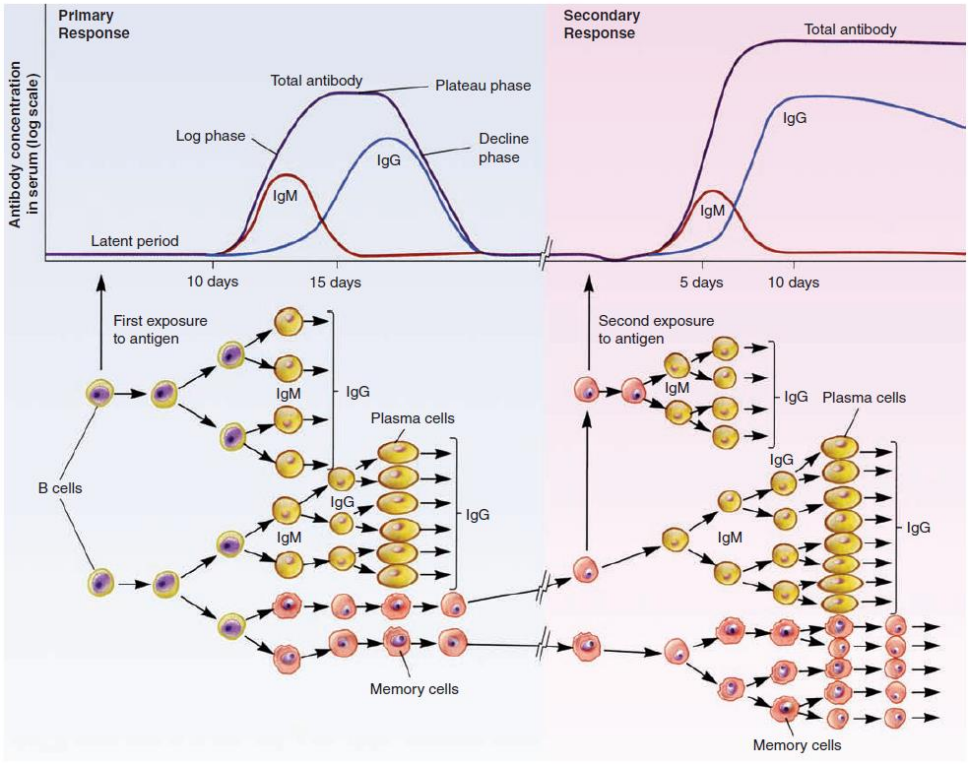
In the secondary response;

the lag time between the immunization and the appearance of antibody is shortened,

the rate of exponential increase to the maximum steady-state level is more rapid,

and the steady-state level itself is higher, representing a larger amount of antibody.

Another key factor of the secondary response is that the antibodies formed are predominantly of the IgG class.



Quick summary of this graph:

- Primary response is the 1st time of meeting the antigen
- Secondary response is the 2nd time of meeting the antigen
- Adaptive immunity takes around 10 days to develop
- The first type of Ig to be produced is IgM. This may be because of their pentameric structure can bind more pathogens (remember: at this period, you need to contain as much as possible of the pathogen) this way IgM worked as a glue which glues pathogens to prevent them from replicating and reaching host cells
- After a period, IgG (a better activator of the immune system) becomes more prominent than IgM
- This difference in concentrations can help us determine the stage of an infection when testing
- The switching from IgM to IgG is by altering the constant region of the heavy chain (this does not mean that an actual IgM converts to IgG NO!! This means that the plasma cell producing IgM converts its production to IgG)

In secondary responses

1. More IgG is produced
2. IgM duration is shorter
3. The production of antibodies starts earlier (Has shorter latency period (it's faster)) and is in higher concentrations
4. The plateau phase persists for a longer time

CLASS SWITCHING

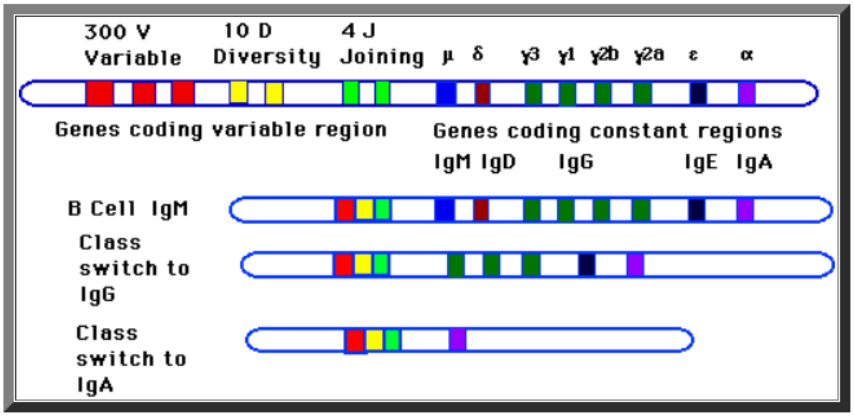
After initial secretion of IgM, cytokines secreted by T- cells stimulate the plasma cells to switch from IgM production to production of IgG, IgA, or IgE.

This process, called class switching or isotype switching, allows plasma cells cloned from the same activated B cell to produce a variety of antibody classes with the same antigen specificity.

Class switching is accomplished by genetic rearrangement of gene segments encoding the constant region, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original antigen specificity.

Class switch

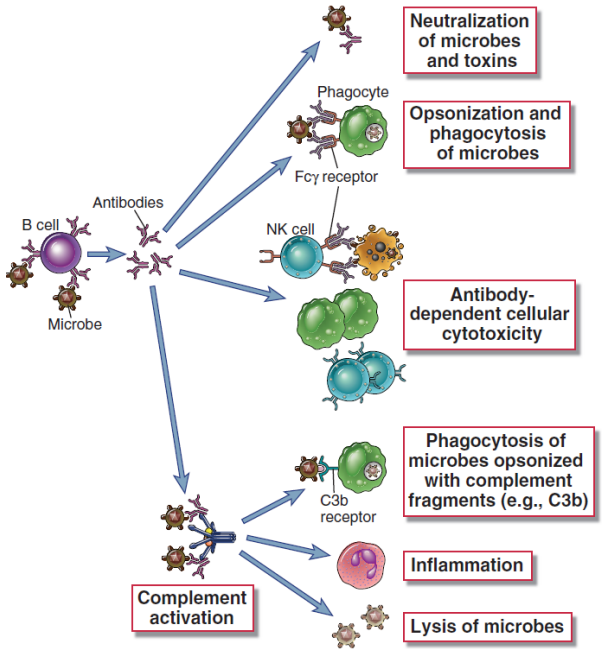
- DNA rearrangement changing the heavy chain constant gene in memory cells.



The ability of antibodies in any individual to specifically bind a large number of different antigens is a reflection of antibody diversity, and the total collection of antibodies with different specificities represents the antibody repertoire. The genetic mechanisms that generate such a large antibody repertoire occur exclusively in lymphocytes. This diversity is generated by random recombination of a limited set of inherited germline DNA sequences to form functional genes that encode the V regions of heavy and light chains as well as by the addition of nucleotide sequences during the recombination process.

Somatic mutation in antigen-stimulated B lymphocytes that generates new V domain structures, some of which bind the antigen with greater affinity than did the original V domains. Those B cells producing higher affinity antibodies preferentially bind to the antigen and, as a result of selection, become the dominant B cells with each subsequent exposure to the antigen. This process is called affinity maturation.

EFFECTOR MECHANISMS OF HUMORAL IMMUNITY



Neutralization: Prevents the microbe from 1. binding to host cells 2. releasing toxins

Opsonization: By the interaction between Fc region of the anti-body and fc receptor on the phagocyte (phagocytosis is much more effective with opsonins)

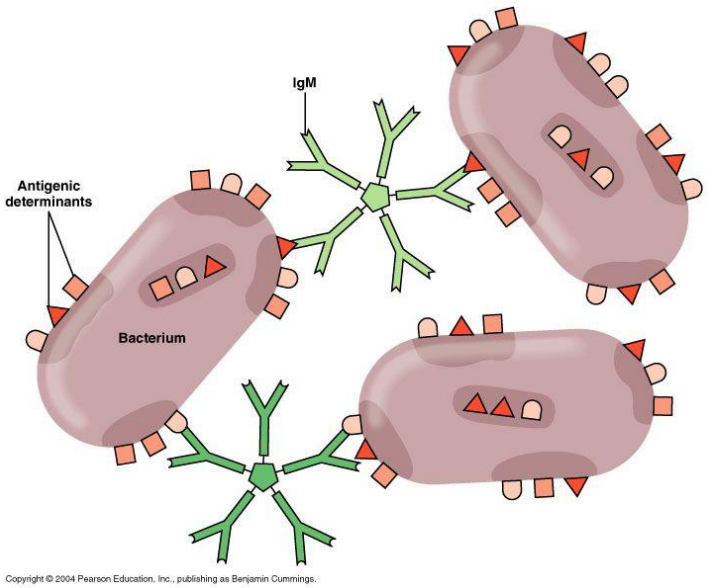
Antibody dependent cellular cytotoxicity: when antibodies bind to a cell it's a "kill me" Marker or signal

Complement: C1q bind to the Fc region. This activates the classical pathway

IGM

Because of its many specific binding sites, IgM is particularly effective in agglutinating particles carrying antigens against which it is directed.

IgM is particularly active in bringing about complement-mediated cytolytic damage to foreign antigen-bearing cells. → IgG are more efficient in activating complement



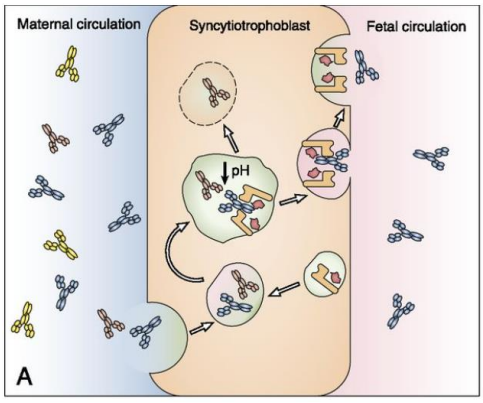
IGG

Immunoglobulin G (IgG) is the most abundant immunoglobulin in health and provides the most extensive and long-lived antibody response to the various microbial and other antigens.

IgG antibody is characteristically formed in large amounts during the secondary response to an antigenic stimulus, and usually follows production of IgM in the course of a viral or bacterial infection.

IgG is the only immunoglobulin class able to cross the placental barrier and, thus, provides <u>passive immune protection</u> to the newborn in the form of maternal antibody.

Infants are most susceptible to pathogen when maternal antibodies are lost until they develop their own

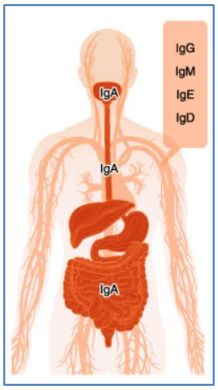


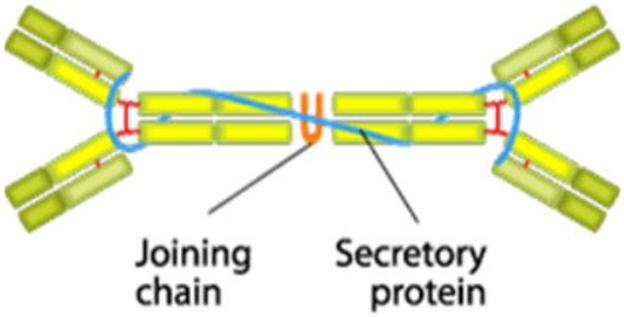
IGA

Immunoglobulin A (IgA) has a special role as a major determinant of so-called local immunity in protecting epithelial surfaces from colonization and infection.
→**(Mucosal immunity)** very important because of the abundance of pathogens in mucosal surfaces

At the epithelia, two IgA molecules combine with another protein, termed the secretory piece, which is present on the surface of local epithelial cells. The complex, then termed secretory IgA (sIgA),

The major role of sIgA is to prevent attachment of antigen-carrying particles to receptors on mucous membrane epithelia



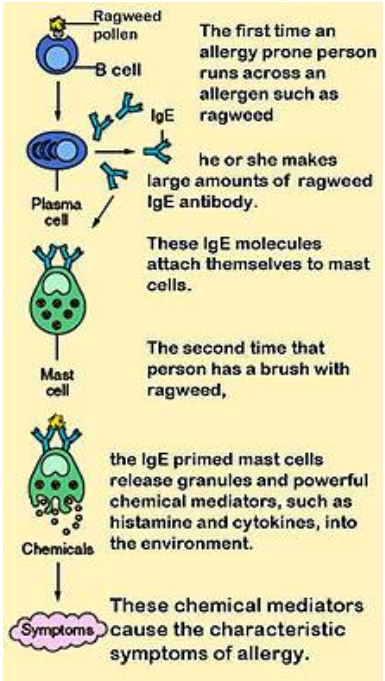


IGE

IgE not only provides protective immunity against helminth parasites but can also mediate the type I hypersensitivity reactions that contribute to the pathogenesis of allergic diseases such as asthma, allergic rhinitis and atopic dermatitis.

Atopic dermatitis (eczema) is a condition that causes dry, itchy and inflamed skin.

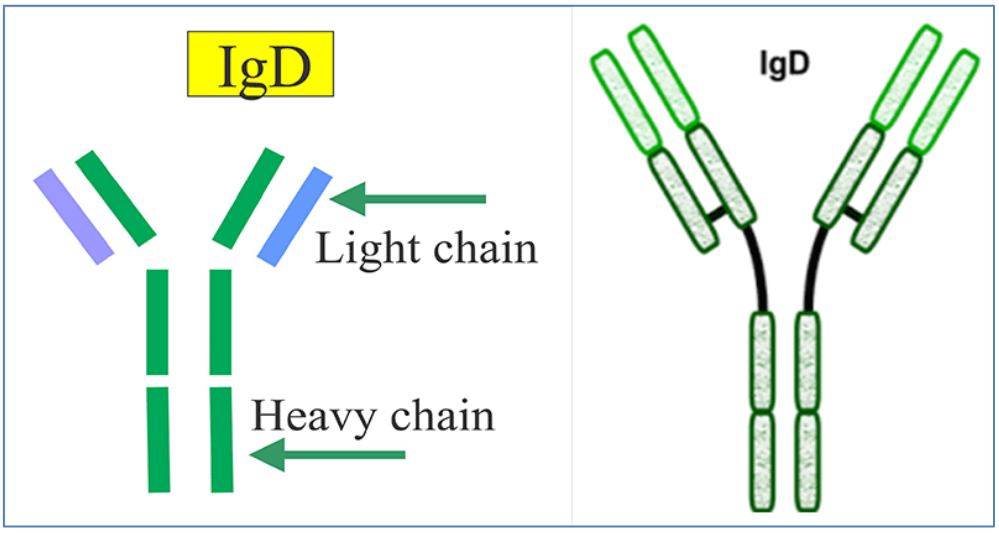
Allergic rhinitis is where your nose gets irritated by something you're allergic to,



IGD

IgD (IgD) is a monomeric antibody isotype that is expressed in the plasma membranes of immature B-lymphocytes. IgD is also produced in a secreted form that is found in small amounts in blood serum.

the function of IgD is to signal the B cells to be activated.



16) Natural antibodies found in circulation are mainly secreted by which of the following cells?

A) Plasmacytoid dendritic cell
B) Follicular B-cells
C) CD4+ T-cells
D) BI B-cells
E) Macrophages

Answer: D

6) Which of the following complement components/complexes inhibits complement activation on a surface?

A) C5b-9
B) Factor B
C) Cir
D) Factor H
E) c3b

Answer: D

19) How many complementarity determining regions (CDR) in one Fc portion of an antibody?

A) 0
B) 6
C) 4
D) 3
E) 2

Answer: A

*0 because CDRs are on the fab region