Chapter 1: Generalities

Part 1: Antigen-Antibody Reaction

1- Antigens

Antigens are macromolecules of natural or synthetic origin; chemically they consist of various polymers – proteins, polypeptides, polysaccharides or nucleoproteins. Antigens display two essential properties: first, they are able **to evoke a specific immune response**, either cellular or humoral type; and, second, they **specifically interact with products of this immune response**, i.e. antibodies or immunocompetent cells.

1-A- A complete antigen – immunogen – consists of a macromolecule that bears **antigenic determinants (epitopes)** on its surface (Fig. 1). The antigenic determinant (epitope) is a certain group of atoms on the antigen surface that actually interacts with the binding site on the antibody or lymphocyte receptor for the antigen. Number of epitopes on the antigen surface determines its valency.

-Different epitopes are so organized on a single protein molecule that their spacing may affect the binding of antibody molecules in various ways.

1-B-Haptens: Low-molecular-weight compound that cannot as such elicit production of antibodies, but is able to react specifically with the products of immune response, is called **hapten (incomplete antigen)**.

1. Haptens are partial antigens. That is:

a. Haptens are **antigenic:** they can react with immune lymphocytes or antibodies.

b. However, haptens **are not immunogenic**: they can not by themselves cause the production of immune lymphocytes or antibodies.

2. Haptens are usually molecules which are too small to be immunogenic.

a. Examples of haptens are antibiotics, analgesics, and other low-molecular weight compounds.

b. Penicillin, for example, a clinically important hapten, has a molecular weight of 320 Daltons (0.3 kDa).

3. if a hapten of coupled to a larger **carrier molecule**, however, it becomes endowed with immunogenicity.

a. The carrier molecules may be albumins, globulins, or synthetic polypeptides.

b. Drugs often couple with carriers in the body and thereby acquire immunogenecity. A classic example in clinical medicine is the allergic response of some persons to penicillin.

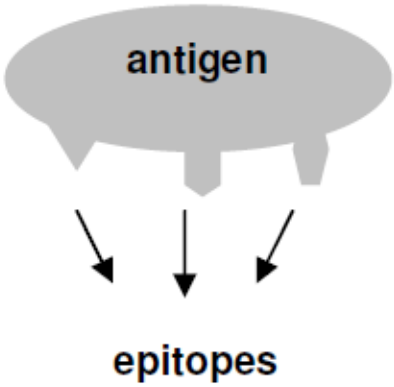


Fig. 1. Antigen and epitopes

2- Immunogenicity

Immunogenicity is the inherent ability of a substance (immunogen) to induce a specific immune response, resulting in the formation of antibodies or immune lymphocytes

The degree of immunogenicity of a molecule is influenced by several factors. The relationship can be expressed algebraically by the following formula:

Immunogenicity = (foreignness) (chemical complexity) (molecular size)

2-1- Foreignness

-An antigen must be foreign or alien to the host with which it makes contact.

-The greater the phylogenetic difference, the more foreign something becomes. The use of transplant terminology helps to clarify this concept.

a. Autologous antigens are found within the same individual; that is, they are not foreign to that individual. For example, a skin graft from an individual's thigh to his chest is an **autograft,** and is not foreign.

b. Syngeneic antigens are found in genetically identical individuals (e.g., individuals from an inbred strain of mice of identical twins). A graft between members of an inbred strain is a **syngeneic graft** or an **isograft,** and is not foreign.

c. Allogeneic antigens (alloantigens) are found in genetically dissimilar members of the same species. For example, a kidney transplant from mother to daughter is called an **allograft** or a **homograft,** and it is foreign.

D. Xenogeneic (heterogenic) antigens are found in different species. For example, a transplant of monkey kidney to humans is called a heterograft or xenografts, and it is foreign. The term heterologous is also sometimes used as a synonym for xenogeneic.

2-2- Chemical complexity

With the exception of pure lipids, most macromolecular organic chemical grouping can be immunogens.

a. Proteins. The majority of immunogens are proteins.

- Proteins are the strongest antigens, because they have the largest array of potential building blocks (amino acids).

(a) This diversity imparts epitopes of differing specificities to the molecule.

(b) The total immune response will be the sum of all the individual antibodies that are produced.

- Immunogenicity can be enhanced by adding haptens (i.e., epitopes) to the molecule.

- Lipoproteins are a complex type of protein immunogen that exist as part of many cell membranes.

b. Polysaccharides

- Most polysaccharides are haptens or incomplete immunogens.

They do not possess sufficient chemical diversity for full immunogenicity.

In addition, they are usually rapidly degraded when they enter a host; thus they are not in contact with the immune apparatus long enough to induce a response

- How ever, polysaccharides can be immunogens, occurring in two forms:

- **Pure polysaccharide** substances (e.g., the capsular polysaccharides that are responsible for the protective immune response to the pneumococcus).

- **Lipopolysaccharides** (e.g., the endotoxins that occur within the cell membranes of gram-negative bacteria).

c. Glycoproteins

- The immunogenicity of glycoproteins is best illustrated by the A and B blood group antigens and the Rh antigens.

- The A and B substances are strong immunogens, and the immune response they induce is to the carbohydrate epitope of the molecule.

d. Polypeptides

- Polypeptide immunogens include hormones (e.g., insulin, growth hormone) and synthetic compounds (e.g., polylysine).

- Polypeptides are usually weakly immunogenic.

e. Nucleic acids and nucleoproteins

- Nucleic acids are considered to be nonimmunogenic; however, when single-stranded, they can act as immunogens.

- Nucleoproteins are stronger immunogens because the nucleic acid is coupled to protein. In patients with systemic lupus erythematosus (SLE), antibodies to autologous nucleoproteins are produced.

f. Lipids:

- These are also nonimmunogenic, although a few (e.g., cardiolipin) can function as haptens.

2-3- Molecular size:

Usually, the larger the molecule, the better the immunogen (Table-1), although there are exceptions.

Molecule	Size	Relative Immunogenicity
Hemocyanin	1000	++++
Gamma globulin	160	+++
Diphtheria toxin	58	++
Insulin	6	+
Vasopressin	1	+/-
Aspirin	0.18	-

Table-1 Relationship of Molecular Size to Immunogenicity

- As a general rule, molecules below 5 kDa will not be immunogenic. Reasonable immune responses will be induced by molecules like serum albumin (40 kDa).

- Size is important for several reasons:

a. The number of epitopes increases proportionately with the size of the protein.

b. Larger size means that the molecule will be phagocytized.

- Antibodies to most antigens are formed much more efficiently if the antigen is first "processed" by a macrophage; this involves phagocytosis of the antigen.
- Antigens that are difficult or impossible to phagocytize are not immunogenic at times.

3- Antigenicity

Antigenicity, or specific reactivity, is the property of a substance (antigen) that causes it to react specifically with the antibody o r lymphocyte that it caused to be produced.

4- Antibody

Antibodies may be defined as the proteins that recognize and neutralize any microbial toxin or foreign substance such as bacteria and viruses. The only cells that make antibodies are B lymphocytes. Mainly two forms of antibodies exist.

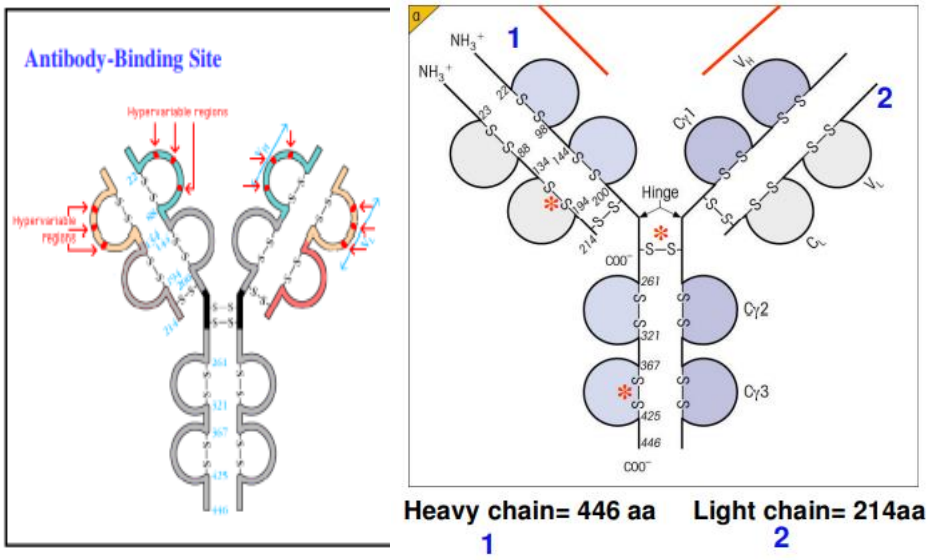
- One those that are membrane-bound and act as receptor for antigens on the surface of B lymphocytes
- The other that are involved in inhibition of entry and spread of pathogens and are found in blood circulation and connective tissues.

The substance or molecule identified by antibodies or that can evoke antibody response is called **an antigen**.

4-1-Antibody structure

Antibodies are also called as immunoglobulins and are Y- shaped protein structures. Antibodies consist of two identical light and heavy chains.

- Amino terminal variable (V) regions are found in both heavy and light chains and they take part in antigen recognition.
- Effector functions are directed by carboxy – terminal constant (C) regions of the heavy chains but C regions are also found in both the chains.
- Both the heavy and light chains are composed of Immunoglobulin (Ig) domain.
- Light chains consist of 2 domains (C and V).
- Heavy chains have 4-5 domains (depending on the class of antibody)
- Each domain is about 110 amino acids in length and contains an intrachain disulfide bond between two cysteines about 60 amino acids apart
- **Constant region** – amino acid sequence in the C terminal regions of the H and L chains **is the same.**
- **Variable region** – amino acid sequence in the N terminal regions of the H and L chains **is different**. This region provides antibodies with **unique specificity.**
- **Hyper-variable regions** are regions within the variable regions (**greater specificities).**
- Within the variable domains are three regions of extreme variability. These are referred to as the hypervariable regions. These regions of the variable domains actually contact the antigen.
- They therefore make up the antigen-binding site. These regions are also called the complementarity determining regions, or CDRs



4-2-Monoclonal antibodies The concept of monoclonal antibodies was given for the first time by **Georges Kohler** and **Cesar Milstein** in the year 1975. Monoclonal antibodies are the antibodies that are specific to one particular antigen as they are made by identical immune cells that are several copies of a same parent cell e.g. any tumor cell of a specific region say plasma cells, are monoclonal and thus have the ability to produce antibodies of same specificity. The basic technique involved in making of monoclonal antibodies relies on fusion of B cells from an immunized mouse with a myeloma (tumor cell line) cell line and let the cells grow in a condition where unfused normal and tumor cells cannot survive. The cells that are fused and able to grow through this procedure are called as **hybridomas**.

4-2-1 Uses of monoclonal antibodies

1) Monoclonal antibodies help in immunodiagnosis by detection of a particular antigen or antibody.

2) Many tumor-specific antibodies help in tumor detection.

3) Some of the monoclonal antibodies have therapeutic uses. E.g. cytokine tumor necrosis factor (TNF) is used to treat many inflammatory conditions.

4) Monoclonal antibodies help in identification of individual cell population's e.g lymphocyte and leukocyte differentiation has become possible now.

5) They help in the purification of cells in order to generate the info about their features and functions.

4-2-Polyclonal antibodies (conventional antibodies) are prepared by immunisation of animals (rabbits, goats, sheep) with the antigen. Blood serum of the immunised animal that contains antibodies against the antigen used, is called an antiserum. If one antigen (e.g. one protein) is used for immunisation, monospecific antibodies (antiserum) result. However, as every epitope stimulates

different clone of B cells, and complex antigens bear several epitopes, the antiserum contains mixture of monoclonal antibodies, differing in their affinity and specificity towards particular epitopes on the antigen used for immunisation.

Immunisation of an animal with mixture of antigens results in production of polyspecific antibodies1, containing immunoglobulins against many antigens (e.g. antiserum against human serum proteins used in immunoelectrophoresis).

5- Isotopes:

The isotype or class of a given antibody molecule is determined based on the constant region sequence: IgM(m), IgG(g), IgA(a), IgD(d), or IgE(e). Each isotype can have either lambda or kappa light chains. Depending on the minor differences in the amino acid sequences of the γ, α heavy chains, they are further classified into subisotypes that determine the subclass of antibody molecules they constitute. In humans, there are two subisotypes of A heavy chains and thus two subclasses, IgA1 and IgA2 and four subisotypes of g heavy chains and therefore four subclasses, IgG1, IgG2, IgG3, and IgG4.

*** lgG**

exists as both surface and secreted monomeric molecules(80% - 85%). four human lgG subclasses, lgG1 , lgG2, lgG3, and lgG4. Collectively, lgG subclasses make up the greatest amount of immunoglobulin in the serum . Many lgG antibodies are effective in activating complement, opsonizing and neutralizing microorganisms and viruses, and initiating antibody-dependent cell-mediated cytotoxicity, and they function in a wide

***lgM**

is found either as a cell surface-bound monomer or as a secreted pentamer (5% - 10%) Most unstimulated B cells display lgM on their cell surfaces. In general , lgM is the first immunoglobulin to be formed following antigenic stimulation. lgM is effective both at immobilizing antigen (agglutination) and in activating the classical pathway of complement. IgM is actually more than five times larger than the IgG

- IgM pentamer contain five unites monomer with J chain

- H chain are longer than IgG

The multiple antigen-binding sites of IgM make it very efficient in many functions like phagocytosis

***IgA**

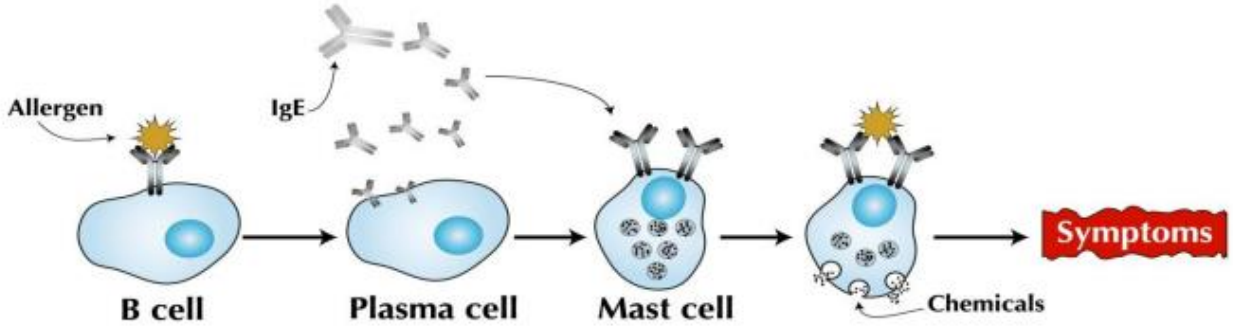
IgA constitutes only 10%–15% of the total immunoglobulin in serum and the predominant secretary immunoglobulin found in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. IgA is found in monomeric as well as polymeric forms dimers, trimers, and sometimes tetramers all containing a J-chain polypeptide.

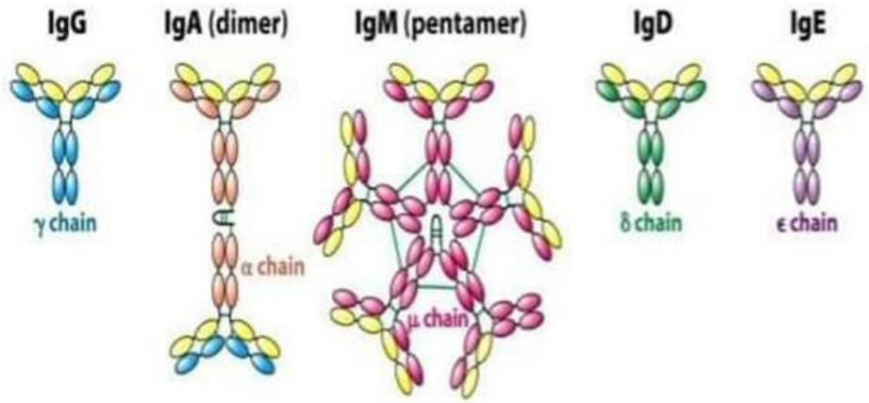
***IgD**

(1%) has a monomeric structure and is almost exclusively displayed on B-cell surfaces. Little is known of its function.

***IgE**

is present in relatively low serum concentration ; most is adsorbed on the surfaces of mast cells and eosinophils. Mast cells and basophils have isotype-specific receptors for the Fc portion of free lgE molecules. Cross-linking of lgE on mast cell surfaces by antigen triggers the release of histamine and other inflammatory mediators, leading to immediate hypersensitivity (allergic) responses.





1- What is the Complement System?

The complement system plays a major role in the host defense system to combat microorganisms and other infectious agents, including the inflammatory response. The complement system comprises of a number of plasma proteins which function together to initiate a cascade which helps the host immune response. The complement system also includes those specific receptors for these complement plasma protein which are present on cells of the immune system and the inflammatory cells. The complement system is activated by the classical pathway which is antibody dependent or the alternative pathway which is antibody independent.

2- The basic functions of Complement system:

After initial activation, the various complement component interact in highly regulated cascade to carry out a number of basic functions including:

1. Cell lysis: rupturing membranes of foreign cells.

2. Opsonization: enhancing phagocytosis of antigens.

3. Chemotaxis: attracting macrophages and neutrophils.

4. Agglutination: clustering and binding of pathogens together (sticking).

3- The components of Complement :

Complement is not a single substance but consisted of multiple proteins. Separated serum components, water-soluble pseudoglobulin and insoluble euglobulin, had no bactericidal activity.

But when mixed together, complement activity was restored. The sequential actions of the serum fractions demonstrated that the lytic activity requires at least two factors one present in insoluble fraction which was termed as midpiece, which reacted with antibody and the other present in soluble fraction named endpiece, which reacted after midpiece to complete the lytic reaction.

The complement components are designated by numerals (C1…..C9), by letter symbols (Factor D, B, H and I) or by trivial names (homologous restriction factor). In most cases the smaller fragment resulting from cleavage of component is designated "a" and the largest fragment "b" (e.g. C3a, C3b) exception in C2 cleavage C2a is the largest fragment designated.

The complement fragments interact with one another to form functional complexes which have enzymatic activity and designated by a bar over the number or symbol (e.g.C4b2a).

5- Biochemical pathways activate the complement system:

Sequential activation of complement components occurs via three main pathways:

- Classical complement pathway
- Alternative complement pathway
- Manose lectin pathway (MLP).

5-1- classical pathway

Antibody that combined with antigen interacts with the inactive enzyme C1making it active, this enzyme then splits C4 and C3 resulting in an enzyme called C3 which cleaves C3 into C3a and C3b (specific).

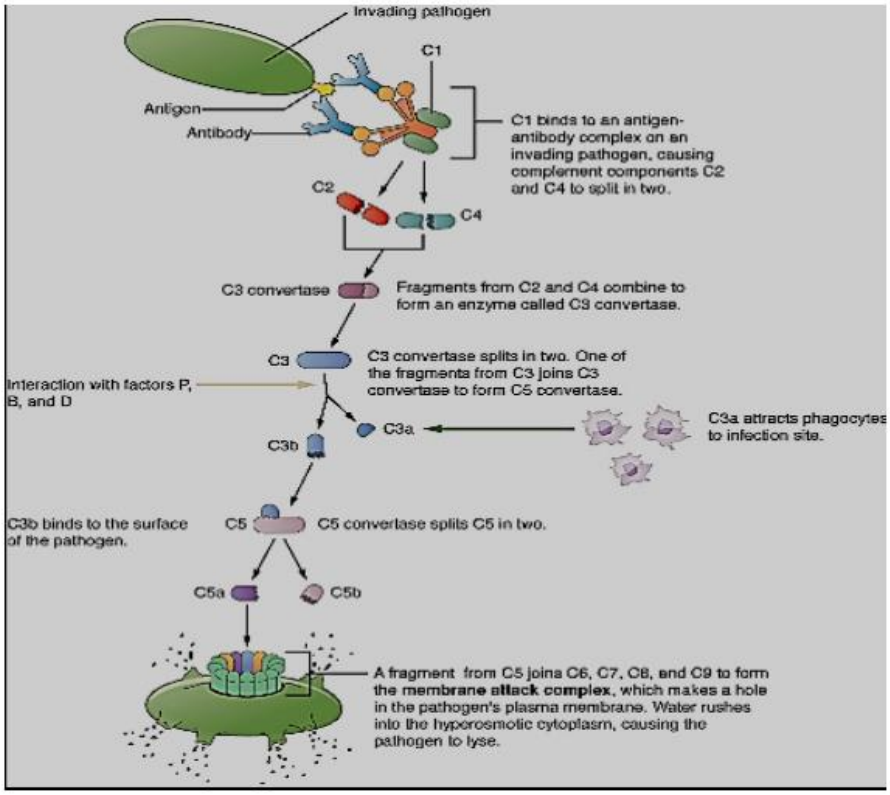
 Ag – Ab complex induce conformational changes in IgM that expose a binding site for the C1 component of the complement system. C1 in serum is a macromolecular complex consist of C1q and two molecules of C1r and C1s held together to form complex (C1qr2S2) which stabilized by Ca+2 ions.

 C1qr2S2 cleave C4 into C4a and C4b, and cleave C2 into C2a and C2b then form (C4b2a) complex which is called C3 convertase.

 C3 convertase cleave C3 into C3a and C3b leading to the formation of (C4b2a3b) complex which is called C5 convertase.

 C5 will cleave into C5a and C5b.

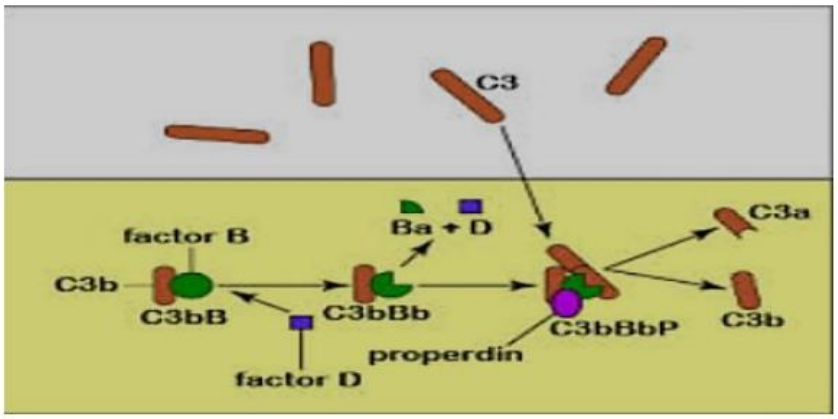
 C5b will bind to C6 to form C5b6789 and initiate the formation of MAC (membrane attack complex) which form a large pore in the membrane of microbial cell.



5-2- alternative pathway

Is triggered when some complement proteins are activated on microbial surfaces (cell walls, capsules, polysaccharides) the cascade begins with C3. A small amount of C3b is always found in circulation as a result of spontaneous cleavage of C3.so the microbial surfaces react with a tiny amount of C3b. properdin and factors B and D lead production of C3 convertase which cleaves C3 to C3a and C3b (nonspecific).

- Spontaneously cleavage of C3 into C3a and C3b, C3b will bind to the surface of cell wall of microbial cell.
- C3b will bind with B factor in the presence of D factor cleavage into Ba and Bb to form C3bBb which has C3 convertase activity.
- C3 will cleave into C3a and C3b to form C3bBb3b binding with properdin stabilizes.
- C3bBb3bp has C5 convertase activity cleave C5 and form C5b6789 which leading to form MAC.

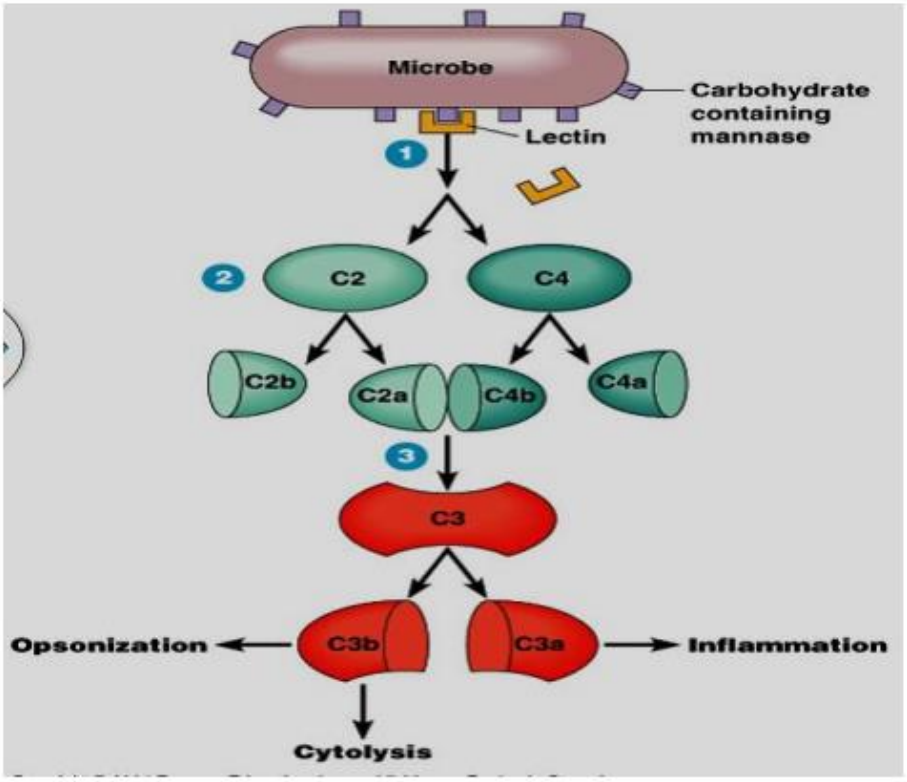


" Alternative Complement Pathway"

5-3-lectin pathway

Mannose in the pathogen's surface (mannose is present in many bacterial cell walls) binds to the lectin, a protein that binds carbohydrates. This substance called mannanbinding lectin (MBL) is found normally in the blood. The combination of MBL is similar to part of the C1component ,so they activates complement enzymes. (nonspecific)

- Activation of C1 which cleave C4 into C4a andC4b also C2 into C2a and C2b and form C4b2a complex.
- C4b2a complex has C3 convertase activity to cleavage C3 into C3a and C3b and form C4b2a3b complex.
- C4b2a3b complex has C5 convertase activity to cleave C5 into C5a and C5b, C5b bind and form C5b6789 and form MAC.



" Lectin Complement Pathway"

6-Regulation of complement system:

The complement system has the potential to be extremely damaging to host tissues, meaning its activation must be tightly regulated. The complement system is regulated by complement control proteins, which are present at a higher concentration in the blood plasma than the complement proteins themselves. A number of regulatory mechanisms have evolved to restrict complement activity to designated targets, it is required passive and active regulatory mechanism.

- **Regulation before assembly of convertase activity:**

  - C1 inhibitor bind to C1r2s2 causing dissociation from C1q.

  - Factor H prevent binding of C3b and factor B.

- **Regulation after assembly of convertase:**

  - C3 convertase dissociated by complement receptor 1 CR1, factor H and decay – accelerating factor (DAF) (CD55) which is glycoproteins.

- **Regulation at assembly of MAC:**

  - S protein prevent insertion of C5b67 MAC component into the membrane.

- Homologous restriction factor (HRF) (CD59) bind to C5b678 preventing assembly with C9 and block the formation of MAC.

7- Complement deficiencies:

Genetic deficiencies have been described for each of the complement components, homozygous deficiencies in any of the classical pathway like C1q, C1r, C1s result immune- complex disease such as systemic lupus erythromatous. Individuals with C3 deficiency suffer from recurrent Neisseria infection and Individual with MAC deficiency developed meningococcal and gonococcal infection.