Immunoparasitology

Unit 2(C)

ANTIGEN - ANTIBODY INTERACTIONS

- Strength of antigen - antibody interactions
- Cross - Reactivity
- Precipitation reactions
- Agglutination reactions
- Radioimmunoassay
- ELISA
- Western Blotting
- Immunoprecipitation
- Immunofluorescence
- Flow cytometry & fluorescence
- Immunoelectron Microscopy.

ANTIGEN - ANTIBODY INTERACTIONS

- Antigen - antibody interaction is a bimolecular association similar to an enzyme - substrate interaction.
- It is reversible interaction.
- this interaction involves various noncovalent interactions -
  a) Hydrogen bonds
  b) Ionic bonds
  c) Hydrophobic interactions
  d) Van der Waals interactions
- Antigen - antibody interaction occurs between antigenic determinant or epitope, of the antigen and the variable region (V_H/V_L) domain of the antibody, particularly the hypervariable regions (CDRs)

→ CDRs - Complementarity determining regions.

- Specificity of antigen - antibody interactions led to the development of a variety of immunologic assays -
  a) Radioimmuno assay
  b) Enzyme - Linked Immunosorbent Assay ← Indirect ELISA, sandwich ELI, Competitive ELI
  c) Western Blotting
  d) Immunoprecipitation
- These assays differ in their speed & their sensitivi
- These assays can be used to (1) detect the presence of either antibody or antigen & (2) have played vital roles in diagnosing diseases.
  (3) Monitoring the level of the humoral immune response.
  (4) Identifying molecules of biological & medical interest.

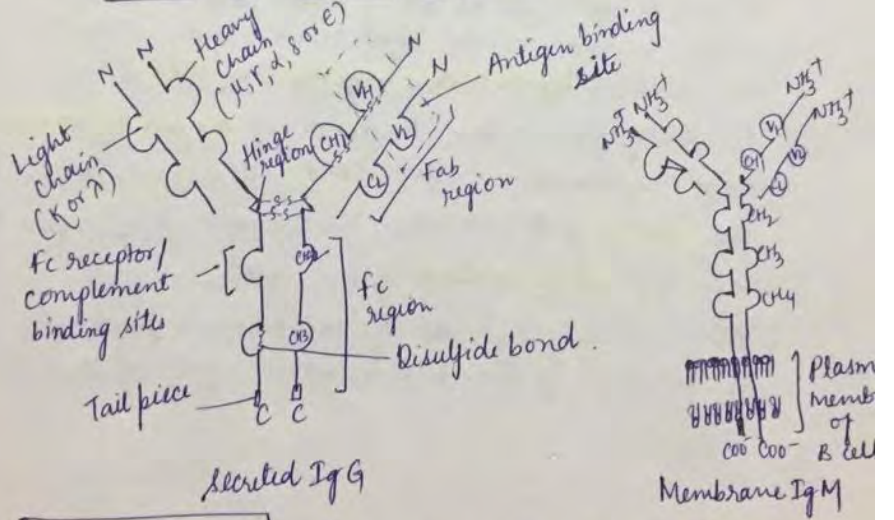
ANTIBODY

- Antibodies are circulating proteins that are produced in vertebrates in response to exposure to foreign structures or antigens.
- Antibodies, MHC molecules and T-cell antigen receptors are the three classes of molecules used by the adaptive immune system to bind antigens.
- Antibodies are synthesized only by cells of the B lymphocyte lineage & exist in two forms:-

(1) Membrane bound antibodies:- Present on the surface of B lymphocytes function as antigen receptors.

(2) Secreted antibodies:- Neutralize toxins, prevent the entry & spread of pathogens, and eliminate microbes.

STRUCTURE OF ANTIBODY MOLECULE



Ig domain

* fab → fragment, antigen binding

- Antibody molecule is composed of two identical light chains & two identical heavy chains.
- Both light & heavy chains contain a series of repeating homologous units, each about 110 amino acid residues in length that fold independently in a globular motif, called an Ig domain.
- Both light & heavy chains consist of:-
  (a) amino-terminal variable regions (V) :- Participate in antigen recognition.
  (b) carboxy-terminal constant (C) regions :- C-regions of heavy chains mediate effector functions.

Structural features of antibody variable regions:-

- Most of the sequence differences and variability among different antibodies are confined to three short stretches in the V region of the heavy chain and to three stretc in the V region of the light chain. These segments are called hypervariable regions or CDRs.
- Proceeding from either the V_L or V_H amino terminus, these regions are called CDR1, CDR2 and CDR3.
- The CDR 3s of both the V_H segment & V_L segment are the most variable of the CDRs.
- Antigen binding by antibody molecules is primarily a function of the hypervariable regions of V_H & V_L.

Structural features of Antibody constant regions:-

- Antibody molecules can be divided into classes & subclasses on the basis of differences in the structure of their heavy chain C regions. The classes of antibody molecules are also called isotypes.
- Isotypes are:- IgA, IgD, IgE, IgG and IgM.
- In humans,
  - 2 subclasses or subtypes of IgA Isotype:- IgA1 and IgA2
  - " " " IgG " :- IgG1, IgG2, IgG3 & IgG4
- The heavy chain C regions of all antibody molecules of one isotype or subtype have essentially the same amino acid sequence.
- In human, IgM, IgE] C region contain 4 tandem Ig domains (CH1, CH2, CH3 and CH4)

  IgG, IgA, IgD] C region contain 3 Ig domains. (CH1, CH2 and CH3)

Note: Ig domains are designated as CH domains.

- There are two classes, or isotypes, of light chains called κ (kappa) and λ, that are distinguished by their carboxyl-terminal constant (C) regions.

Imp: Each antibody molecule has either two identical κ light chains or two identical λ light chains.

FEATURES OF BIOLOGIC ANTIGENS

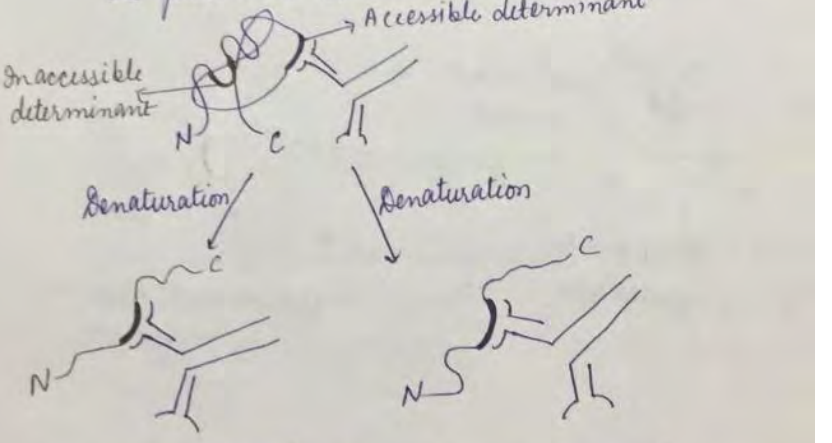
- An **antigen** is any substance that may be specifically bound by an antibody molecule or T cell receptor.
- Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes.
- Molecules that stimulate immune responses are called **immunogens**.
- Antibody binds to only a portion of the macromolecule, which is called a **determinant** or **an epitope**.
- The presence of multiple identical determinants in an antigen is referred to as **polyvalency** or **multivalency**.
- The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways:-
  1. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other, such determinants are said to be **non-overlapping determinants**.
  2. When two determinants are close to one another, the binding of antibody to the first determinant may cause steric hindrance with the binding of antibody to the second, such determinants are said to be **overlapping determinants**.
  3. In rarer cases, binding of one antibody may cause a conformational change in the structure of the antigen, positively or negatively influencing the

binding of a second antibody at another site on the protein by means other than steric hindrance. Such interactions are called allosteric effects.

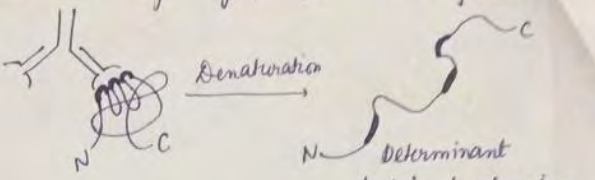
Nature of the antigenic determinants

(1) Linear determinant: - Epitopes formed by several adjacent amino acid residues are called linear determinants.

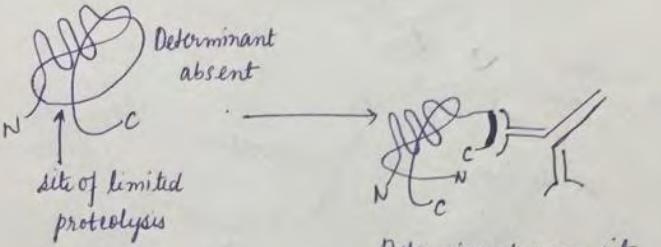
- The antigen-binding site of an antibody can usually accomodate a linear determinant made up of about six amino acids.
- If linear determinants appear on the external surface or in a region of extended conformation in the native folded protein, they may be accessible to antibodies.
- In other cases, linear determinants may be inaccessible in the native confirmation and appear only when the protein is denatured.



(2) Conformational determinants: The formation of ... residues that are not in a sequence but become spatially juxtaposed in the folded protein.



(3) Neo antigenic determinant :- Proteins may be subjected to modifications such as glycosylation, phosphorylation, ubiquitination, acetylation and proteolysis. These modifications, by altering the structure of the protein, can produce new epitopes. Such epitopes are called neoantigenic determinants.



STRENGTH OF ANTIGEN-ANTIBODY INTERACTIONS

- Basis for strength of antigen-antibody interactions are non-covalent interactions.
- Non-covalent interactions are:- a) Hydrogen bonds
  b) Ionic bonds
  c) Hydrophobic interactions
  d) Van der Waals interactions
- Large number of such interactions are required to form a strong Ag-Ab interaction because strength of each of these interactions is weak (as compared to covalent bond)
- Each of these noncovalent interactions operates over a very small distance, generally about 1×10^{-7} mm (1 Å).
- A strong Ag-Ab interaction depends on a very close fit between the antigen and antibody.

ANTIBODY AFFINITY

- The strength of the total noncovalent interactions between a single antigen-binding site on an antibody and a single epitope is the affinity of the antibody for that epitope.
- Low affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high affinity antibodies bind antigen more tightly and remain bound longer.

• The association between a binding site on an Ab with a monovalent Ag can be described by the equation:-

$$Ag + Ab \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} Ag\text{-}Ab$$

Where, K_1 = forward (association) rate constant

K_{-1} = Reverse (dissociation) " "

Ag = Unbound Antigen

Ab = " Antibody

Ag-Ab = Antigen-antibody complex.

from above eqⁿ,

$$\boxed{K_a = \frac{K_1}{K_{-1}}} \quad \text{(or)}$$

$$K_a = \frac{[Ag\text{-}Ab]}{[Ag]\,[Ab]} = \frac{\text{Conc of Product}}{\text{Conc. of substrates}}$$

where, K_a = equilibrium constant for above eqⁿ or association constant (a measure of affinity)

Note: (i) Value of K_a varies for different Ag-Ab complexes & dependson both K_1 and K_{-1}

(ii) Unit of K_1 = L/mol/sec. (2^{nd} order reaction)

(iii) Unit of K_{-1} = sec^{-1} (1^{st} order reaction)

(iv) for small heptens, the forward rate constant can be extremely high ($\approx 4 \times 10^8$ L/mol/s)

(v) for larger antigens, K_1 is smaller (range $\times 10^5$ L/mol/s)

Note: (a) If $[Ag] > [Ab]$ = weak complex of Ag-Ab

(b) $[Ag] < [Ab]$ = " " "

(c) $[Ag] \approx [Ab]$ = Strong complex (either both Ag and Ab have equal high conc or equal low conc)

for some purposes, the dissociation of the antigen-antibody complex,

$$Ag\text{-}Ab \rightleftharpoons Ab + Ag$$

$$K_d = \frac{[Ab][Ag]}{[Ag\text{-}Ab]} = \frac{1}{K_a}$$

Where, K_d = equilibrium constant

- K_d is a quantitative indicator of the stability of an Ag-Ab complex.
- High Kd value - Less stable complexes
- Low kd value - more stable complexes

Methods to determine the affinity of Ag-Ab interactions:-

1. Equilibrium dialysis – Older method
2. Surface plasmon resonance (SPR) - Modern method

1. Equilibrium dialysis:- Association constant (K_a) can be determined by this method.

→ This procedure uses a dialysis chamber containing two equal compartments separated by a semipermeable membrane.

→ Antibody is placed in one compartment and a radioactively labeled ligand that is small enough to pass through semipermeable membrane is placed in the other compartment.

→ In the absence of antibody, ligand added to compartment will equilibrate on both sides of the membrane.

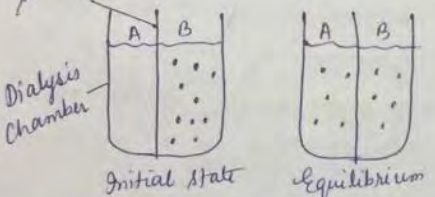
→ In the presence of antibody, part of the labelled ligand will be bound to the antibody at equilibrium and the unbound ligand will be equally distributed in both compartments. Thus the total concentration of ligand will be greater in the compartment containing antibody.

→ The difference in the ligand concentration in the two compartments represents the concentration of ligand bound to the antibody.

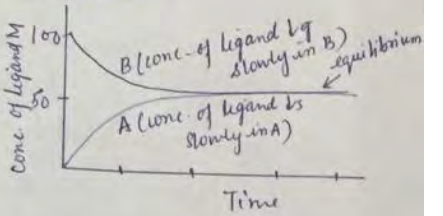
→ The higher the affinity of the antibody, the more ligand is bound.

Determination of ab-ag by equilibrium dialysis:-

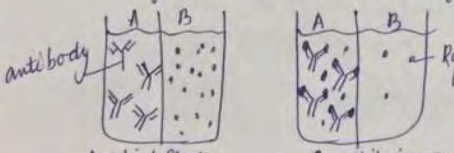
(a) Control: No antibody present (ligand equilibrates on both sides equally)



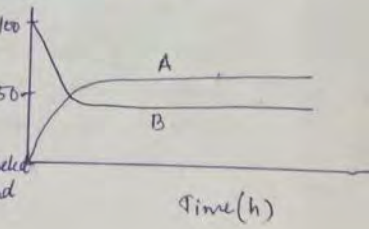
(b) Control:



Experimental: Antibody in A (at equilibrium more ligand in A due to Ab binding)



Experimental:



Equilibrium eqn can be rewritten as,

$$K_a = \frac{[Ab\text{-}Ag]}{[Ab][Ag]} = \frac{r}{n - r(c)}$$

where, r = ratio of the conc. of bound ligand to total antibody conc.

c = conc. of free ligand

n = number of binding sites per antibody molecule

By arranging above eqn. we get **Scatchard equation**:

$$\frac{r}{c} = K_a n - K_a r$$

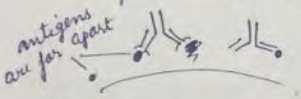
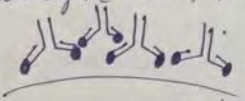
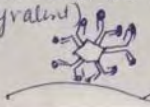
→ Values for r and c can be obtained by repeating the equilibrium dialysis with the same conc. of antibody with different concentrations of ligand.

ANTIBODY AVIDITY

- The affinity of one binding site does not always reflect the true strength of the antibody-antigen interaction.
- When complex antigens containing multiple, repeating antigen determinants are mixed with antibodies containing multiple binding sites, the interaction of an antibody molecule with an antigen molecule at one site will increase the probability of reaction between those two molecules at a second site.
- The strength of such multiple interactions between a multivalent antibody and antigen is called the **avidity**.

- The avidity of an antibody is a better measure of its binding capacity within biological systems (eg: the reaction of an antibody with antigenic determinants on a virus or bacterial cell) than the affinity of its individual binding sites.
- High avidity can compensate for low affinity.
- eg:- Secreted pentameric IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.

Valency & avidity of antibody-antigen interactions:-

	Valency of interaction	Avidity of interaction
(1) Monovalent antigens or epitopes spaced far apart on cell surfaces. 	Monovalent	Low (Affinity high)
(2) When repeated determinants on a cell surface are close enough (In case of IgG). 	Bivalent	High
(3) IgM molecules have 10 identical antigen binding sites that can bind with 10 repeating determinants on cell surface (polyvalent) 	Polyvalent	Very high

Cross-Reactivity

- Cross reactivity occurs if two different antigens share an identical epitope or if antibodies specific for one epitope also bind to an unrelated epitope possessing similar chemical properties.
- In the latter case, the antibody's affinity for the cross-reacting epitope is usually less than that for the original epitope.
- Cross-reactivity is often observed among polysaccharide antigens that contain similar oligosaccharide residues.

  eg: ABO blood group antigens - These are glycoproteins expressed on RBCs. Subtle differences in the terminal sugar residues distinguish the A and B blood group antigens.

Blood type	Antigens on RBCs	Serum antibodies
A	A	Anti-B
B	B	Anti-A
AB	A and B	Neither
O	Neither	Anti-A and Anti-B

- Cross-reactivity is the basis for the presence of these blood-group antibodies, which are induced in an individual not by exposure to red blood cell antigens but by exposure to cross-reacting microbial antigens present on common intestinal bacteria. These cross-reacting microbial antigens induce the formation of antibodies in individuals lacking these antigens on their red blood cells.

- A number of viruses and bacteria have antigenic determin identical to normal host cell components.
- eg: The bacterium Streptococcus pyogenes expresses cell wall proteins called M antigens. Antibodies produced to streptococcal M antigens have been shown to cross-react with several myocardial and skeletal muscle proteins and have been implicated in heart and kidney damage following streptococcal infections.
- Some vaccines also exhibit cross-reactivity.

  eg: Vaccinia virus, which causes cowpox, expresses cross reacting epitopes with variola virus, the causative agent of small pox. This cross-reactivity was the basis of Jenner's method of using vaccinia virus to induce immunity to smallpox.

Precipitation Reactions

- The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops into a visible precipitate.
- Antibodies that thus aggregate soluble antigens are called precipitins.
- Although formation of the soluble Ag-Ab complex occurs within minutes, formation of the visible precipitate occurs more slowly and often takes a day or two to reach completion.
- Formation of an Ag-Ab lattice depends on the valency of both the antibody and antigen :-

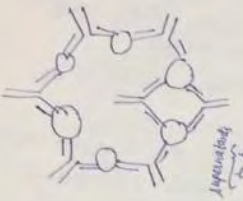
(a) The antibody must be bivalent, a precipitate will not form with monovalent Fab fragments.

(b) The antigen must be either bivalent or polyvalent, i.e., it must have at least two copies of the same epitope or have different epitopes that react with different antibodies present in polyclonal antisera.

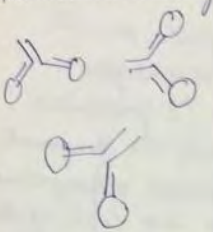
eg: Myoglobin precipitates well with specific ~~mono~~ polyclonal antisera but fails to precipitate with a specific monoclonal antibody because it contains multiple, distinct epitopes but only a single copy of each epitope.

*(a)

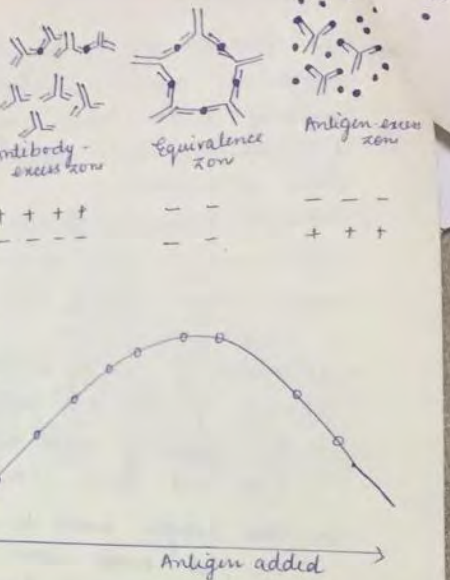
Polyclonal antiserum



Monoclonal antibody



*(b)



Precipitation reactions:-

*(a) Polyclonal antibodies can form lattice or large aggregates, that precipitate out of solution.
Monoclonal antibody can link with two molecules of antigen and no precipitate is formed.

*(b) A precipitation curve for a system of one antigen and its antibodies.

(i) Zone of antibody excess - precipitation is inhibited and antibody not bound to antigen can be detected in the supernatent.

(ii) Equivalence zone - maximum precipitation, neither antigen nor antibody can be detected in the supernatent.

(iii) Zone of antigen excess - precipitation is inhibited and antigen not bound to antibody can be detected in the supernatent.

Agglutination Reactions

- The interaction between antibody and a particulate antigen results in visible clumping called agglutination.
- Antibodies that produce such reactions are called agglutinins.
- Agglutination reactions are similar in principle to precipitation reactions, they depend on the crosslinking of polyvalent antigens.
- Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions, this inhibition is called the prozone effect.
- Several mechanisms can cause the prozone effect:- First, at high antibody concentrations, the number of antibody binding sites may greatly exceed the number of epitopes. As a result, antibodies can bind antigen only univalently instead of multivalently. Antibodies that bind univalently can not crosslink one antigen to another. Prozone effects are readily diagnosed by performing the assay at a variety of antibody (or antigen) concentrations. When one is using polyclonal antibodies, the prozone effect can also occur for another reason. The antiserum may contain high concentrations of antibodies that bind to the antigen but do not induce agglutination; these antibodies are called incomplete antibodies (of the Ig G class). At high concentration of Ig G, incomplete antibodies may occupy all of the antigenic sites, thus blocking access by Ig M, which is good agglutinin. This effect is not seen with agglutinating monoclonal antibodies

- The lack of agglutinating activity of an incomplete antibody may be due to restricted flexibility in the hinge region, making it difficult for the antibody to assume the required angle for optimal cross-linking of epitopes on two or more particulate antigens.

Hemagglutination

→ Agglutination reactions are routinely performed to type RBCs. In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide.

→ Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions.