Lecture 5: Dr. Nihad A Jafar

AG-AB REACTIONS

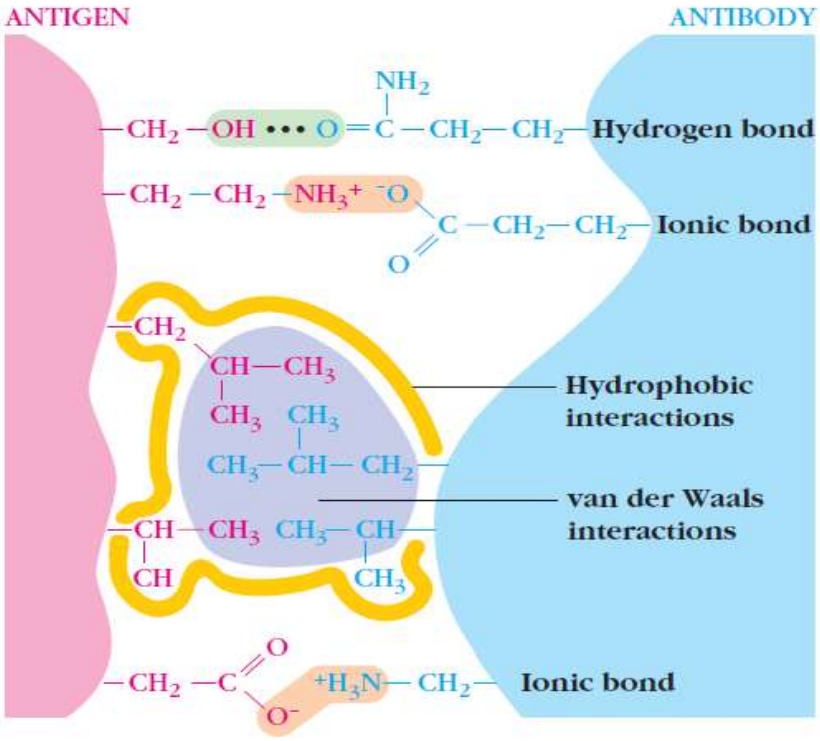
The interactions between antigens and antibodies are known as ***antigen–antibody reactions***. The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens.

I. NATURE OF AG-AB REACTIONS

A. Lock and Key Concept - The combining site of an antibody is located in the Fab portion of the molecule (hypervariable regions of the heavy and light chains). Studies of antigens and antibodies interacting shows that the antigenic determinant nestles in a cleft formed by the combining site of the antibody. Thus, our concept of Ag-Ab reactions is one of a key (i.e. the Ag) which fits into a lock (i.e. the Ab).

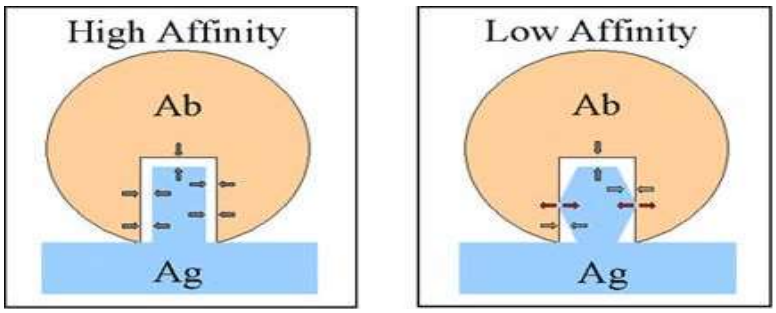
B. Non-covalent Bonds - The bonds that hold the Ag in the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds. Multiple bonding between the Ag and the Ab ensures that the Ag will be bound tightly to the Ab.

C. Reversible - Since Ag-Ab reactions occur via non-covalent bonds they are reversible.



II. AFFINITY AND AVIDITY

A. Affinity - Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody. It is the sum of the attractive and repulsive forces operating between the antigenic determinant and the combining site of the antibody.



Most antibodies have a high affinity for their antigens.

B. Avidity - Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Affinity refers to the strength of binding between a single antigenic determinant and an individual antibody combining site whereas avidity refers to the overall strength of binding between multivalent Ag's and Ab's.

Avidity is influenced by both the valence of the antibody and the valence of the antigen.

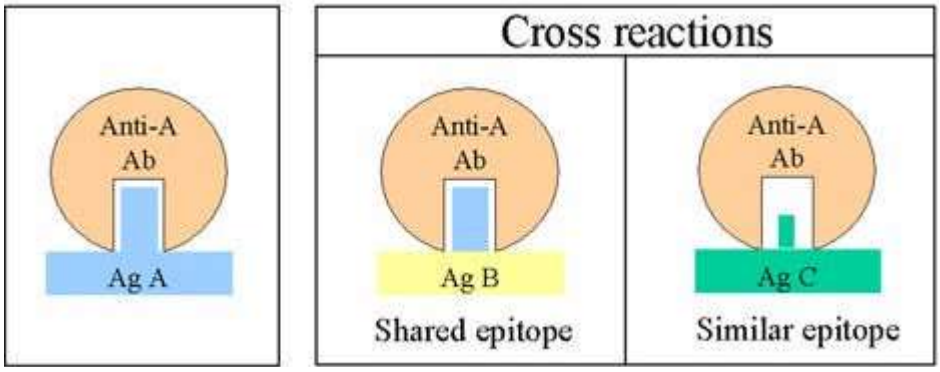
III. SPECIFICITY AND CROSS REACTIVITY

A. Specificity - Specificity refers to the ability of an individual antibody combining site to react with only one antigenic determinant or the ability of a population of antibody molecules to react with only one antigen. In general, there is a high degree of specificity in Ag-Ab reactions. Antibodies can distinguish differences in

1) the primary structure of an antigen
2) isomeric forms of an antigen
3) secondary and tertiary structure of an antigen.

B. Cross reactivity - Cross reactivity refers to the ability of an individual antibody combining site to react with more than one antigenic determinant or the ability of a population of antibody molecules to react with more than one antigen.

Cross reactions arise because the cross reacting antigen shares an epitope in common with the immunizing antigen or because it has an epitope which is structurally similar to one on the immunizing antigen (multispecificity).



IV. TESTS FOR ANTIGEN-ANTIBODY REACTIONS

A. Factors affecting measurement of Ag/Ab reactions - The only way that one knows that an antigen-antibody reaction has occurred is to have some means of directly or indirectly detecting the complexes formed between the antigen and antibody.

Detection of antigen-antibody reactions will depend on a number of factors.

1. Affinity - The higher the affinity of the antibody for the antigen, the more stable will be the interaction. Thus, the ease with which one can detect the interaction is enhanced.
2. Avidity - Reactions between multivalent antigens and multivalent antibodies are more stable and thus easier to detect.
3. Ag:Ab ratio - The ratio between the antigen and antibody influences the detection of Ag/Ab complexes because the sizes of the complexes formed is related to the concentration of the antigen and antibody.
4. Physical form of the antigen - The physical form of the antigen influences how one detects its reaction with an antibody. If the antigen is a particulate, one generally looks for agglutination of the antigen by the antibody. If the antigen is soluble one generally looks for the precipitation of the antigen after the production of large insoluble Ag/Ab complexes.

Agglutination Tests

1. Agglutination/Hemagglutination - When the antigen is particulate the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. When the antigen is an erythrocyte the term hemagglutination is used. The term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term hemagglutinin is often used. All antibodies can theoretically agglutinate particulate antigens but IgM due to its high valence is particularly good agglutinin.

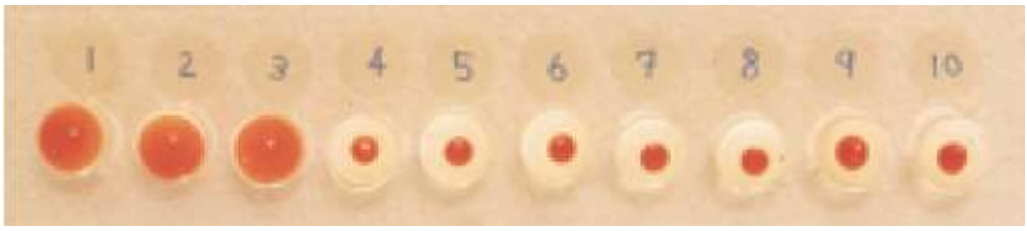
a) **Qualitative agglutination test** - Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. The antibody is mixed with the particulate antigen and a positive test is indicated by the agglutination of the particulate antigen.

e.g. A patients red blood cells mixed with antibody to a blood group antigen to determine a persons blood type.

e.g. A patients serum mixed with red blood cells of known blood type to assay for the presence of antibodies to that blood type in the patient's serum.

b) **Quantitative agglutination test** - Agglutination tests can also be used to quantitate the level of antibodies to particulate antigens. In this test one makes serial dilutions of a sample to be tested for antibody and then adds a fixed number of red blood cells or bacteria or other such particulate antigen and determines the maximum dilution which gives agglutination. The

maximum dilution that gives visible agglutination is called the titer. The results are reported as the reciprocal of the maximal dilution that gives visible agglutination.



Prozone effect - On occasion one observes that when the concentration of antibody is high (i.e. lower dilutions) there is no agglutination and then as the sample is diluted agglutination occurs. The lack of agglutination at high concentrations of antibodies is called the prozone effect.

Lack of agglutination in the prozone is due to antibody excess resulting in very small complexes which do not clump to form visible agglutination.

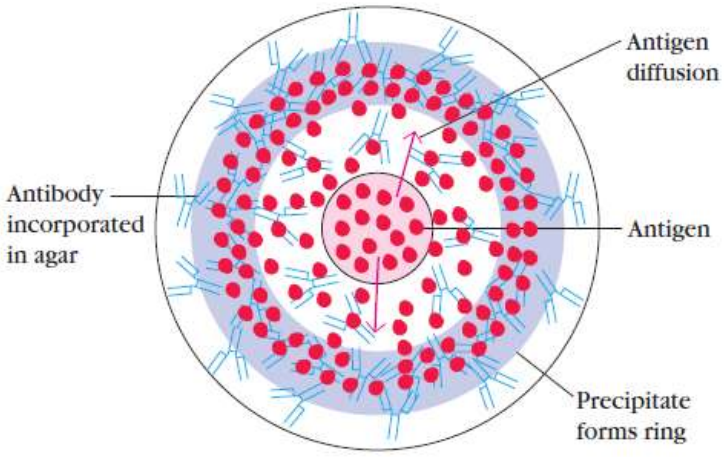
c) Applications of agglutination tests

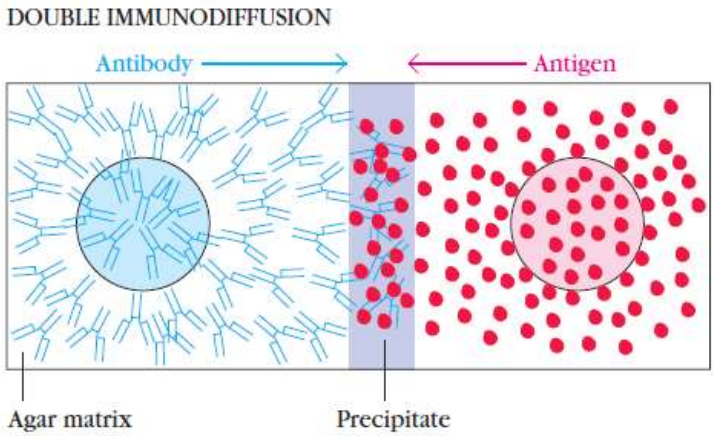
1) Determination of blood types or antibodies to blood group antigens.

2) To assess bacterial infections

Precipitation tests

1. **Radial Immunodiffusion (Mancini)** - In radial immunodiffusion antibody is incorporated into the agar gel as it is poured and different dilutions of the antigen are placed in holes punched into the agar. As the antigen diffuses into the gel it reacts with the antibody and when the equivalence point is reached a ring of precipitation is formed as illustrated in the figure:





The diameter of the ring is proportional to the log of the concentration of antigen since the amount of antibody is constant. Thus, by running different concentrations of a standard antigen one can generate a standard curve from which one can quantitate the amount of an antigen in an unknown sample. Thus, this is a quantitative test. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

2. Immunoelectrophoresis - In immunoelectrophoresis a complex mixture of antigens is placed in a well punched out of an agar gel and the antigens are electrophoresed so that the antigen are separated according to their charge. After electrophoresis a trough is cut in the gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lines are produced in the equivalence zone when an Ag/Ab reaction occurs.

Radioimmunoassay (RIA)/Enzyme Linked Immunosorbent Assay (ELISA)

Radioimmunoassays (RIA) are assays which are based on the measurement of radioactivity associated with immune complexes. In any particular test, the label may be on either the antigen or the antibody. Enzyme Linked Immunosorbent assays (ELISA) are those that are based on the measurement of an enzymatic reaction associated with immune complexes. In any particular assay the enzyme may be linked to either the antigen or the antibody.

Immunofluorescence

Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.

a) Direct Immunofluorescence - In direct immunofluorescence the antibody specific to the antigen is directly tagged with the fluorochrome.

b) Indirect Immunofluorescence - In indirect immunofluorescence the antibody specific for the antigen is unlabeled and a second antiimmunoglobulin antibody directed toward the first antibody is tagged with the fluorochrome.

Note: Indirect fluorescence is more sensitive than direct immunofluorescence since there is amplification of the signal.

Complement Fixation

Antigen/Antibody complexes can also be measured by their ability to fix complement because an Ag/Ab complex will "consume" complement if it is present whereas free Ag's or Ab's do not. Tests for Ag/Ab complexes that rely on the consumption of complement are termed complement fixation tests and are used to quantitate Ag/Ab reactions. This test will only work with complement fixing antibodies (IgG, IgM best).

Antigen is mixed with the test serum to be assayed for antibody and Ag/Ab complexes are allowed to form. A control tube in which no Ag is added is also prepared. If no Ag/Ab complexes are present in the tube, none of the complement will be fixed. However, if Ag/Ab complexes are present, they will fix complement and thereby reduce the amount of complement in the

tube. After allowing for complement fixation by any Ag/Ab complexes, a standard amount of red blood cells, which have been pre-coated with anti-erythrocyte antibodies is added. The amount of antibodycoated RBC is predetermine to be just enough to completely use up all the complement initially added if it were still there. If all the complement was still present (i.e. no Ag/Ab complexes formed between the Ag and Ab in question), all the RBC will be lysed. If Ag/Ab complexes are formed between that Ag and Ab in question, some of the complement will be consumed and thus when the antibodycoated RBC's are added not all of them will lyse. By simply measuring the amount of RBC lysis by measuring the release of hemoglobin into the medium, one can indirectly quantitate Ag/Ab complexes in the tube. Complement fixation tests are most commonly used to assay for antibody in a test sample but they can be modified to measure antigen.