ANTIGEN – ANTIBODY REACTIONS

The interaction between antigen and antibody is called antigen-antibody reaction. It is abbreviated as ag - ab reaction.

Antigen-antibody reaction is the basis of humoral immunity or antibody mediated immune response.

Antigen-antibody reaction is characterised by the following salient features:

Immune Complex

When antigen and antibody are brought together, the antibody binds with the antigen to form a complex molecule called immune complex or antigen – antibody complex (ag – ab complex).

Specific of Ag – Ab Reaction

The reaction between antigen and antibody is highly specific. Specificity refers to the discriminate ability of a particular antibody to combine with only one type of antigen.

An antibody will combine with the antigen which is the cause for its production. For example, the antibody produced against lens – antigen will react with only lens – antigen and not with any other type of antigen. Similarly, the antibody produced against kidney – antigen will react with only kidney – antigen. Thus, each antigen has a separate antibody for antigen – antibody reaction.

The specificity of ag – ab reaction can be compared to lock and key system. A standard lock can be opened by its own key only as one antibody can react with its own antigen.

Binding Sites of Antigen and Antibody

In antigen – antibody reaction, the antibody attaches with the antigen. The part of the antigen which combines with the antibody is called epitope or antigenic determinant. An antigen may contain 10 to 50 antigenic determinants. Some times it may go up to 200.

The part of the antibody which combines with the antigen is called paratope or antigen binding site. Most of the antibodies are bivalent having two binding sites. But the antibody IgM is multivalent having 5 to 10 binding sites.

Binding Forces of Antigen and Antibody

The binding between antigen and antibody in ag – ab reaction is due to three factors namely.

a. Closeness between antigen and antibody

b. Intermolecular forces

c. Affinity of antibody

Closeness between antigen and antibody

When the antigen and antibody are closely fit, the strength of binding is great. When they are apart, the binding strength is low.

Intermolecular forces

The forces which operates between two macromolecules as for example between protein and protein, also exist between antigen and antibody in ag – ab reaction. The intermolecular forces which operate between antigen and antibody are non-covalent forces and are of 4 types.

1. Electrostatic forces
2. Hydrogen bonding
3. Hydrophobic bonding
4. Vander Walls bonding

Affinity of antibody

Affinity refers to the strength of binding between a particular molecule of antibody and a single antigenic determinant. Antibodies which bind strongly to the antigenic determinant are called high affinity antibodies. Affinity is used to denote the binding capacity of an antibody with a univalent antigen.

Avidity

Avidity refers to the capacity of an antiserum containing various antibodies to combine with the whole antigen that stimulated the production of antibodies. Avidity is used to denote the overall capacity of antibodies to combine with multivalent antigen.

Avidity is the strength of the bond after the formation of antigen – antibody complexes.

A multivalent antigen has many types of antigenic determinants. When it is injected into the blood, each antigenic determinant stimulates the production of a particular antibody. The various antibodies produced by a single antigen combine with the different antigenic determinants of the antigen.

Bonus Effect

In antigen – antibody reaction, the antibody not only binds with the antigen but also the antigens are bridged by a single antibody. In some cases two antigens may be bridged by a single antibody. Such a binding is weak and the Ag – Ab reaction may be reversible in such cases.

But when two antigens are bridged by two antibodies, the binding will be strong and the Ag – Ab complex will not dissociate.

This phenomenon of giving extra – strength to the antigen – antibody complex by the binding of two antibodies to two antigen molecules is called bonus effect.

The bonus effect is highly possible because the antigens are multivalent and there are many types of antibodies.

Bonus effect increases the binding strength of antigen and antibody molecules.

Cross Reaction

An antiserum raised against a given antigen may sometimes react with another closely related antigen. This reaction is called cross reaction and the antigen which produces the cross reaction is called cross reactive antigen. The cross reaction is due to the presence of ne or more identical antigenic determinants on the related antigen.

As far as strength of reaction, the reaction of the antiserum with its own antigen is very strong. But the reaction (cross reaction) with the related antigen is weak.

Example for Cross Reaction

1. The antiserum raised against the albumen of hen's egg cross react with the albumen of duck's egg.
2. The antiserum raised against human insulin will react with the insulin of pig, sheep whale, etc.
3. The antiserum raised against pnemococcal polysaccharide will react with E.Coli, blood group A, collagen, etc.

Detection of Antigen-antibody Reaction

(Types of antigen-antibody Reaction)

Antigen – antibody reaction is brought about by the contact of antigen and antibody. This process of contact cannot be seen by the naked eye. But once the contact is made, the Ag – Ab reaction leads to visible manifestations such as precipitation, agglutination, cytolysis, etc.

The antigen – antibody reaction can be detected by the following techniques:

1. Preciption
2. Agglutination
3. Cytolysis
4. Complement fixation
5. Flocculation
6. Opsonization

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Preciption

Preciption refers to an antigen – antibody reaction between a soluble antigen and its antibody resulting in the formation of insoluble precipitate. The antibody causing precipitation is called precipitin.

Mechanism of Precipitation

Precipitation is due to the formation of antigen – antibody complex

The antigen is multivalent and the antibody is bivalent. As each antibody is a bivalent molecule, it can bridge two multivalent antigen molecule. This bridging leads to the formation of a lattice which forms the precipitate.

When antigen and antibody are in optimal concentra tion, the precipitation is complete and a large lattice is formed.

Precipitin Test

Precipitin test is a test is a test of antigen - antibody reaction. Precipitin reaction can be carried out by a classical experiment. A set of 5 or more reaction tubes are arranged serially and are marked as A, B, C, D and E. A constant volume of antiserum is added to each tube. The antigen is added in increasing volume from tube A to E.

Antigen and antibody react together resulting in precipitation. The amount of precipitate formed is determined by the proportion of antigen and antibody.

Maximum amount of precipitate is formed when the antigen and antibody are in optimal proportion. This occurs in the central tube. When the antibody is in excess or the antigen is in excess, the amount of precipitate formed will be less. This occurs in the side tubes.

When the amount of precipitate formed in different tubes is plotted on a graph paper a curve is obtained. This curve is called precipitin curve.

The curve shows a peak where maximum precipitate is formed. This occurs when the proportion of antigen and antibody is optimum. The amount of precipitate formed on the side tubes is low and hence the curve descends on the sides.

The precipitin curve shows 3 zones, namely

a. Zone of antibody excess

b. Zone of equivalence

c. Zone of antigen excess

The zone of equivalence lies in the peak of the curve (tube C). Here the proportion of antigen and antibody is optimum. Here all the antigen and antibody are completely precipitated into a large lattice which is insoluble.

The zone of antibody excess (tubes A and B) lies in the ascending part of the curve. Here uncombined antibody is present.

All the available antigentic determinants of antigen are occupied by the binding sites of antibody. There is no antigenic determinant left out. Hence the ag – ab complex is insoluable.

The zone of antigen excess (tubes D and E) lies in the descending part of the curve. Here uncombined antigenic determinants are present. All the antibodies are combined. In antigen excess, the binding between antigen and antibody is weak and hence the ag – ab complexes are soluble because large lattice formation is inhibited.

Precipitin test is used to find out the amount of antibody present in the serum of an immunised animal.

Application of Precipitin Reaction

Precipitin reaction is the principle of the following techniques

1. *Single immunodiffusion*
2. *Double immunodiffusion*
3. *Radial immunodiffusion*
4. *Immuno electrophoresis*
5. *Rocket immunodiffusion*

Agglutination

Agglutination is an antigen – antibody reaction where the antibody of serum cause the cellular antigens to adhere to one another to form clumps. It is the clumping of a particular antigen and its antibody. The antibodies that cause agglutination are called agglutinins and the particulate antigens aggregated are called agglutinogens.

The particulate antigens include bacteria, viruses, RBC, platelets, lymphocytes, etc.

When red blood cells are agglutinated, the reaction is called haemagglutination. When bacterial cells are agglutinated, the agglutination is called bacterial agglutination.

Mechanism of Agglutination

Agglutination is brought about by the linking of antigens and antibodies. As most of the antibodies are bivalent, an antibody can link two adjacent antigens. The IgM is multivalent and it contains 5 or 10 combining sites. Hence it can link more number of antigens. Hence IgM antibody has the capacity to make clumps more effectively with a lesser number of molecules than that of IgG antibody molecule.

The univalent antibodies (antibodies with a single combining site) cannot form clumps or lattice and hence agglutination will not occur.

Agglutination Test

Agglutination test refers to the examination of clump formation when particulate antigen and its antibodies are combined.

Agglutination test has a wide application in the clinical field. It is used to test blood groups and infectious diseases. The following are the applications of agglutination test:

1. ABO blood grouping
2. Rh blood grouping
3. Widal test for typhoid
4. Well felix test for typhus

5. Coomb's test for the identification of anti-Rh antibodies.

6. Brucella agglutination test for brucellosis.

7. Leptospira agglutination test for leptospirosis.

8. Cold agglutination test for pneumonia, malaria, trypanosomiasis.

9. Haemagglutination inhibition test for the diagnosis of certain viral and parasitic diseases.

ABO Blood Typing

the typing of blood, for ABO groups or Rh groups, involves agglutination reaction. For typing blood,a drop of the blood sample is mixed with a drop of antiserum A and another drop of the blood sample is mixed with a drop of antiserum B on a glass slide.

If the blood sample is clumped with antiserum A, the sample belongs to group A; if the sample is clumped with antiserum B, the sample belongs to B, if the sample is clumped with both antiserum A and antiserum b, the blood sample belongs to group AB. If there is no agglutination, the blood sample belongs to group O.

Cytolysis

Cytolysis is the dissolution of a cell. When an RBC is lysed the cytolysis is called haemolysis. When a bacterial cell is lyssed, the lysis is called bacteriolysis. It is caused by complement – fixing union between antibody and cell bound antigen.

Mechanism of Cytolysis

The antigen – antibody complex activates the complement. The activated complement binds to the surface antigen of microbe or cell.

The complement fixed on the surface of the cell, cause the disruption of the lipid bilayer of the membrane of the microbe. As a result, a hole is made on the microbe. Through this hole the contents of the cell are released and the cell is lysed.

Complement Fixation

The binding of complement to antigen – antibody complex is called complement fixation.

When complement is added to a serum containing an antigen and its antibody, the complement is activated and immediately it binds to the antigen – antibody complex and the complement is said to be fixed. In this system, the complement does not remain unbound in the serum.

Flocculation

Flocculation is an antigen – antibody reaction brought about by exotoxins* and antitoxins*. This reaction produces floccules which do not sediment but remain dispersed in the medium. Flocculation is somewhat like precipitation, but the precipitate will not sediment.

Opsonization

Opsonization is the process by which a particulate antigen becomes more susceptible to phagocytosis by combination which an opsonin.

Opsonin is an antibody, when combines with a particulate antigen, increases the susceptibility of the antigen to phagocytosis. This antigen is also called opsonizing antibody.

In Opsonization, the antibody combines with the surface antigen of bacteria. This antigen – antibody complex activates complement system. The activated complement C3 is attached to the antigen – antibody complex to from an antigen – antibody complement complex.

Exotosins

Extracellular protein or proteinaceous toxins produced by microorganisms.

Antitoxzin

An immune serume that neutralises toxins such as exotoxin. It develops in the body as a result of repeated infection.

The phagocytic cell have surface receptors for C3 and the Fc fragment of the antibody. As a result the antigen – antibody – complement is adhered to the phagocytic cells. The microbe (antigen) fixed on the phagocytic cell is killed by phagocytosis or lysis.

Immunofluorescence

When antibodies are mixed with fluorescent dyes such as flurescein or rhodamine, they emit radiation. This phenomenon of emiting rediation by antibodies labelled with fluorescent dyes is called immunoflurescence. The immunofluorescence can be observed by a fluorescent microscope.

Application of Immunofluorescence

The immunofluorescence test can be applied in three methods, namely direct method, indirect method and sandwich method.

Direct Method

In this method the antibody labelled with fluorescent dye is applied on the tissue sections. The labelled antibody binds with specific antigen. This can be observed under the fluorescent microscope.

Indirect Method

In indirect method unlabelled fluorescent dyes, is applied on the tissues. The labelled anti- immunogloulin antibody is highly specific for unlabelled antibody and hence it binds with the already linked unlabelled antibody. In this method cell or tissue antigens as well as specific antibodies can be detected.

Sandwich Method

This is an immunofluorescence method used to test the number of cells producing antibodies for a specific antigen.

The cells producing antibodies, for example, lymphocytes are fixed in ethanol. The fixed cells are treated with the polysaccharide antigen of Pneumococcus. This antigen will specifically combine with those lymphocytes which have the capacity to produce antibody against pneumococcus polysaccharide antigen.

In this test the antigen is sandwiched between the antibody present in the lymphocytes and the fluorescent labelled antibody. Hence, this test is called sandwich test.