ANTIGEN-ANTIBODY REACTIONS

SELECTED TESTS

NATURE OF ANTIGEN-ANTIBODY REACTIONS

Lock and Key Concept

The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hypervariable regions of the heavy and light chains. X-Ray crystallography studies of antigen-antibody interactions show that the antigenic determinant nestles in a cleft formed by the combining site of the antibody. Thus, our concept of antigen-antibody reactions is one of a key (*i.e.* the antigen) which fits into a lock (*i.e.* the antibody).

Non-covalent Bonds

The bonds that hold the antigen to 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 antigen and the antibody ensures that the antigen will be bound tightly to the antibody.

Reversibility

Since antigen-antibody reactions occur via non-covalent bonds, they are by their nature reversible.

AFFINITY AND AVIDITY

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

Affinity is the equilibrium constant that describes the antigen-antibody reaction .

Most antibodies have a high affinity for their antigens.

Avidity

Avidity is a measure of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. Avidity is influenced by both the valence of the antibody and the valence of the antigen. Avidity is more than the sum of the individual affinities.

To repeat, 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 antigens and antibodies.

SPECIFICITY AND CROSS REACTIVITY

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 antigen-antibody reactions. Antibodies can distinguish differences in:

- The primary structure of an antigen
- Isomeric forms of an antigen
- Secondary and tertiary structure of an antigen

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).

Agglutination Tests

Agglutination/Hemagglutination

When the antigen is particulate, the reaction of an antibody with the antigen can be detected by agglutination (clumping) of the antigen. The general term agglutinin is used to describe antibodies that agglutinate particulate antigens. When the antigen is an erythrocyte the term hemagglutination is used. All antibodies can theoretically agglutinate particulate antigens but IgM, due to its high valence, is particularly good agglutinin and one sometimes infers that an antibody may be of the IgM class if it is a good agglutinating antibody.

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. For example, a patient's red blood cells can be mixed with antibody to a blood group antigen to determine a person's blood type. In a second example, a patient's serum is mixed with red blood cells of a known blood type to assay for the presence of antibodies to that blood type in the patient's serum.

Quantitative agglutination test

Agglutination tests can also be used to measure the level of antibodies to particulate antigens. In this test, serial dilutions are made of a sample to be tested for antibody and then a fixed number of red blood cells or bacteria or other such particulate antigen is added. Then the maximum dilution that gives agglutination is determined. 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 - Occasionally, it is observed that when the concentration of antibody is high (i.e. lower dilutions), there is no agglutination and then, as the sample is diluted, agglutination 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 that do not clump to form visible agglutination.

Applications of agglutination tests

i. Determination of blood types or antibodies to blood group antigens.

ii. To assess bacterial infections

e.g. A rise in titer of an antibody to a particular bacterium indicates an infection with that bacterial type. N.B. a fourfold rise in titer is generally taken as a significant rise in antibody titer.

Practical considerations
Although the test is easy to perform, it is only semi-quantitative.

Passive hemagglutination

The agglutination test only works with particulate antigens. However, it is possible to coat erythrocytes with a soluble antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red blood cells in an agglutination test for antibody to the soluble antigen. This is called passive hemagglutination. The test is performed just like the agglutination test. Applications include detection of antibodies to soluble antigens and detection of antibodies to viral antigens.

Hemagglutination Inhibition

The agglutination test can be modified to be used for the measurement of soluble antigens. This test is called hemagglutination inhibition. It is called hemagglutination inhibition because one measures the ability of soluble antigen to inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test, a fixed amount of antibodies to the antigen in question is mixed with a fixed amount of red blood cells coated with the antigen (see passive hemagglutination above). Also included in the mixture are different amounts of the sample to be analyzed for the presence of the antigen. If the sample contains the antigen, the soluble antigen will compete with the antigen coated on the red blood cells for binding to the antibodies, thereby inhibiting the agglutination of the red blood cells.

By serially diluting the sample, you can quantitate the amount of antigen in your unknown sample by its titer. This test is generally used to quantitate soluble antigens and is subject to the same practical considerations as the agglutination test.

Precipitation tests

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

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 cure from which one can quantitate the amount of an antigen in an unknown sample. Thus, this is a quantitative test. If more than one ring appears in the test, more than one antigen/antibody reaction has occurred. This could be due to a mixture of antigens or antibodies. This test is commonly used in the clinical laboratory for the determination of immunoglobulin levels in patient samples.

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 antigen/antibody reaction occurs.

This tests is used for the qualitative analysis of complex mixtures of antigens, although a crude measure of quantity (thickness of the line) can be obtained. This test is commonly used for the analysis of components in a patient' serum. Serum is placed in the well and antibody to whole serum in the trough. By comparisons to normal serum, one can determine whether there are deficiencies on one or more serum components or whether there is an overabundance of some serum component (thickness of the line). This test can also be used to evaluate purity of isolated serum proteins.

SOURCE:

Microbiology and Immunology Online. Immunology- chapter seven. Available from: http://www.microbiologybook.org/mayer/ab-ag-rx.htm