Specific and Non-Specific Defense Mechanism | Immunology

Specific and Non-Specific Defense Mechanism Against Infectious Organisms within the Host !

After entering into the host tissues the infectious organisms multiply and may cause diseases.

There are some host defense mechanisms, which act against all the intruders immediately after their entry into the host; these defense mechanisms are non-specific in nature (i.e. these mechanisms can act against any infectious agent) and are usually less effective.

For any infectious agent the host raises specific immune responses (i.e. the immune responses induced against an infectious agent will act only against the agent against which the immune responses were induced) and such responses are very effective in eliminating the intruders. However, the development of specific immune responses during the first entry of

the agent takes time (about 7-10 days); and during that period the infectious agent may cause disease.

Non-Specific Defense Mechanisms:

There are many non-specific defense mechanisms (i.e. the defense mechanisms are not restricted to a particular infectious agent; and the defense mechanisms act against any infectious agent) that act immediately after the entry of microbes into the host. Many serum proteins, phagocytic cells, and inflammatory mediators released from the site of microbial infection act against the microbes.

i. From the site of bacterial infection the injured tissues release many inflammatory mediators. The inflammatory mediators as well as some of the microbial products initiate a local inflammatory response, leading to the accumulation of phagocytic cells and serum proteins at the infected site. The phagocytic cells, especially neutrophils engulf the bacteria and kill the bacteria.

ii. Acute phase proteins (such as C reactive protein) coat the surface of bacteria and lead to enhanced phagocytosis. Complement proteins initiate the

indirect pathway of complement activation and leads to bacterial lysis.

iii. Fever:

The body temperature rises during infections. It is believed that fever is a host defense mechanism against infectious agents, though it is not yet proved. On the other hand, fever itself causes many undesirable effects on the host.

Specific Defense Mechanisms against Infectious Agents:

The humoral and cell-mediated immune defense mechanisms induced against an infectious agent are specific in nature, i.e. the specific immune mechanisms act only against the microbe against which the responses were induced and not against other microbes.

Defense through Humoral Immune Responses:

Antibodies and complement components are the important mediators of humoral immune responses. Binding of the Fab regions of antibodies with specific antigen epitopes on the surface of microbes initiates the humoral defense mechanisms against the microbes.

i. Binding of Fab regions of antibody with microbe initiates the classic complement pathway activation. The complement activation leads to the formation of pores on the cell wall of the microbe and consequently the microbe dies.

ii. Antibody molecules and the complement fragment C3b (formed during complement activation) also act as opsonins. The Fc regions of antibody and C3b have receptors on the macrophage membranes. After binding with the microbe, the Fc region of antibody and C3b bind to their respective receptors on macrophage membrane; thus the microbe is bridged to the macrophage by antibody and C3b. The macrophage engulfs the microbe and kills it.

iii. The complement components formed during complement activation play many important roles (such as chemotactic activity, opsonic activity, enhancement of phagocytosis, and pore forming activity) in the inflammatory response against the microbes.

iv. Toxins produced by certain bacteria (such as tetanus toxin produced by the bacteria Clostridium

tetani) are dangerous to the host. Anti- toxin antibodies are induced against the antigen epitopes on the surface of toxins. The anti toxin antibodies bind to toxin molecules and neutralize the biological effects of toxins.

Defense through Cell-mediated Immune Responses:

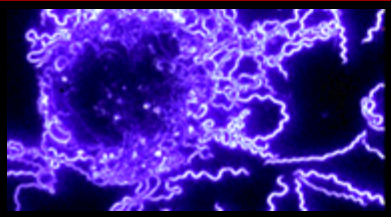
Helper T cells are central to the development of specific immune responses against microbes.

i. Helper T cells help the B lymphocytes for their activation. Thus helper T cells also play an important role in humoral responses mediated by antibodies.

ii. IFNγ secreted by helper T cells activate macrophages and augment the phagocytic and intracellular killing activity of the macrophages.

iii. Helper T cells also augment the NK cell activity and thus play important role in fight against viral infections.

iv. Helper T cells help the activation of cytotoxic T cells and thus they help in the killing of viral infected cells.



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IMMUNOLOGY - CHAPTER SEVEN

IMMUNOGLOBULINS- ANTIGEN-ANTIBOD

AND SELECTED TESTS

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TEACHING OBJECTIVES

To describe the nature of Ag-Ab reactions
To compare and contrast antibody affinity and avidity
To delineate the basis for antibody specificity and cross reactivity
To discuss the principles of commonly used tests for antigen/antibody reactions

Figure 1

KEY WORDS

Affinity
Avidity
Specificity
Cross reactivity
Agglutination
Hemagglutination
Agglutinin
Titer
Prozone
Passive hemagglutination
Direct Coomb's test
Indirect Coomb's test
Hemagglutination inhibition
Equivalence point
Antibody excess
Antigen excess
Radial immunodiffusion

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 regions of the heavy and light chains. X-Ray crystallography studies of antigen-an antigenic determinant nestles in a cleft formed by the combining site of the antibod concept of antigen-antibody reactions is one of a key (*i.e.* the antigen) which fits in

Non-covalent Bonds

The bonds that hold the antigen to the antibody combining site are all non-covalen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds. Multiple 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

AFFINITY AND AVIDITY

Affinity

Antibody affinity is the strength of the reaction between a single antigenic determin antibody. It is the sum of the attractive and repulsive forces operating between the site of the antibody as illustrated in Figure 2.

Affinity is the equilibrium constant that describes the antigen-antibody reaction as have a high affinity for their antigens.

Avidity

Avidity is a measure of the overall strength of binding of an antigen with many anti antibodies. Avidity is influenced by both the valence of the antibody and the valenc

Immunoelectrophoresis
Countercurrent immunoelectrophoresis
Radioimmunoassay
Enzyme linked immunosorbent assay
Competitive RIA/ELISA
Noncompetitive RIA/ELISA
Immunofluorescence
Flow cytometry
Complement fixation

Figure 2

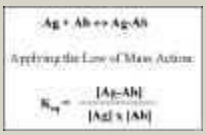

Figure 3

Figure 4

Figure 5

Figure 6

sum of the individual affinities. This is illustrated in Figure 4.

To repeat, affinity refers to the strength of binding between a single antigenic dete combining site whereas avidity refers to the overall strength of binding between m

SPECIFICITY AND CROSS REACTIVITY

Specificity

Specificity refers to the ability of an individual antibody combining site to react with ability of a population of antibody molecules to react with only one antigen. In gene 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 reac determinant or the ability of a population of antibody molecules to react with more cross reactions can arise. Cross reactions arise because the cross reacting antige immunizing antigen or because it has an epitope which is structurally similar to on (multispecificity).

TESTS FOR ANTIGEN-ANTIBODY REACTIONS

Factors affecting measurement of antigen-antibody reactions

The only way that one knows that an antigen-antibody reaction has occurred is to detecting the complexes formed between the antigen and antibody. The ease with reactions will depend on a number of factors.

Affinity

The higher the affinity of the antibody for the antigen, the more stable will be the ir can detect the interaction is enhanced.

Avidity

Reactions between multivalent antigens and multivalent antibodies are more stabl

Antigen to antibody ratio

The ratio between the antigen and antibody influences the detection of antigen-an complexes formed is related to the concentration of the antigen and antibody. This

Physical form of the antigen

The physical form of the antigen influences how one detects its reaction with an ar

generally looks for agglutination of the antigen by the antibody. If the antigen is so precipitation of the antigen after the production of large insoluble antigen-antibody

Agglutination Tests

Agglutination/Hemagglutination

When the antigen is particulate, the reaction of an antibody with the antigen can b the antigen. The general term agglutinin is used to describe antibodies that agglut antigen is an erythrocyte the term hemagglutination is used. All antibodies can the but IgM, due to its high valence, is particularly good agglutinin and one sometimes IgM class if it is a good agglutinating antibody.


Figure 7

Qualitative agglutination test

Agglutination tests can be used in a qualitative manner to assay for the presence antibody is mixed with the particulate antigen and a positive test is indicated by th (Figure 7).

For example, a patient's red blood cells can be mixed with antibody to a blood gro type. In a second example, a patient's serum is mixed with red blood cells of a kno 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 particula are made of a sample to be tested for antibody and then a fixed number of red blo particulate antigen is added. Then the maximum dilution that gives agglutination is gives visible agglutination is called the titer. The results are reported as the recipro visible agglutination. Figure 8 illustrates a quantitative hemagglutination test.


Figure 8

Prozone effect - Occasionally, it is observed that when the concentration of antibo agglutination and then, as the sample is diluted, agglutination occurs (See Patient at high concentrations of antibodies is called the prozone effect. Lack of agglutina excess resulting in very small complexes that do not clump to form visible agglutin

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 wit 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 antigen (e.g. viral antigen, a polysaccharide or a hapten) and use the coated red b antibody to the soluble antigen (Figure 9). This is called passive hemagglutination agglutination test. Applications include detection of antibodies to soluble antigens antigens.


Figure 9

Coomb's Test (Antiglobulin Test)

Direct Coomb's Test

Figure 10

When antibodies bind to erythrocytes, they do not always result in agglutination. T ratio being in antigen excess or antibody excess or in some cases electrical charg effective cross linking of the cells. These antibodies that bind to but do not cause a sometimes referred to as incomplete antibodies. In no way is this meant to indicat structure, although this was once thought to be the case. Rather, it is a functional presence of non-agglutinating antibodies on red blood cells, one simply adds a se immunoglobulin (antibody) coating the red cells. This anti-immunoglobulin can nov in agglutination. This test is illustrated in Figure 10 and is known as the Direct Coo

Indirect Coomb's Test

Figure 11

If it is necessary to know whether a serum sample has antibodies directed against to be sure that you also detect potential non- agglutinating antibodies in the sampl (Figure 11). This test is done by incubating the red blood cells with the serum sam and then adding a second anti-immunoglobulin reagent to cross link the cells.

Applications

These include detection of anti-rhesus factor (Rh) antibodies. Antibodies to the Rh blood cells. Thus, red cells from Rh^+ children born to Rh^- mothers, who have anti-I antibodies. To check for this, a direct Coombs test is performed. To see if the mot an Indirect Coombs test is performed.

Hemagglutination Inhibition

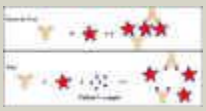

Figure 12

The agglutination test can be modified to be used for the measurement of soluble hemagglutination inhibition. It is called hemagglutination inhibition because one m inhibit the agglutination of antigen-coated red blood cells by antibodies. In this test antigen in question is mixed with a fixed amount of red blood cells coated with the above). Also included in the mixture are different amounts of the sample to be ana the sample contains the antigen, the soluble antigen will compete with the antigen to the antibodies, thereby inhibiting the agglutination of the red blood cells. as illus

By serially diluting the sample, you can quantitate the amount of antigen in your u generally used to quantitate soluble antigens and is subject to the same practical

Precipitation tests

Radial Immunodiffusion (Mancini)

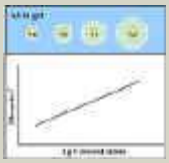

Figure 13

In radial immunodiffusion antibody is incorporated into the agar gel as it is poured placed in holes punched into the agar. As the antigen diffuses into the gel, it react equivalence point is reached a ring of precipitation is formed as illustrated in Figur

The diameter of the ring is proportional to the log of the concentration of antigen s Thus, by running different concentrations of a standard antigen one can generate quantitate the amount of an antigen in an unknown sample. Thus, this is a quantit in the test, more than one antigen/antibody reaction has occurred. This could be d This test is commonly used in the clinical laboratory for the determination of immu

Figure 14

Figure 15

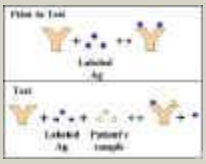

Figure 16

Figure 17

TUTORIAL
ELIZA ASSAY
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Immunoelectrophoresis

In immunoelectrophoresis, a complex mixture of antigens is placed in a well punch are electrophoresed so that the antigen are separated according to their charge. A gel and antibodies are added. As the antibodies diffuse into the agar, precipitin lin when an antigen/antibody reaction occurs as illustrated in Figure 14.

This tests is used for the qualitative analysis of complex mixtures of antigens, alth (thickness of the line) can be obtained. This test is commonly used for the analysis Serum is placed in the well and antibody to whole serum in the trough. By compar determine whether there are deficiencies on one or more serum components or w some serum component (thickness of the line). This test can also be used to evalu

Countercurrent electrophoresis

In this test the antigen and antibody are placed in wells punched out of an agar ge electrophoresed into each other where they form a precipitation line as illustrated i conditions can be found where the antigen and antibody have opposite charges. T from the thickness of the band you can get some measure of quantity. Its major ad

Radioimmunoassay (RIA)/Enzyme Linked Immunosorbent Ass

Radioimmunoassays (RIA) are assays that are based on the measurement of radi complexes. In any particular test, the label may be on either the antigen or the ant Assays (ELISA) are those that are based on the measurement of an enzymatic rea complexes. In any particular assay, the enzyme may be linked to either the antige

Competitive RIA/ELISA for Ag Detection

The method and principle of RIA and ELISA for the measurement of antigen is sho amounts of a standard unlabeled antigen, one can generate a standard curve rela versus amount of antigen. From this standard curve, one can determine the amou

The key to the assay is the separation of the immune complexes from the remainc accomplished in many different ways and serves as the basis for the names given

Precipitation with ammonium sulphate

Ammonium sulphate (33 - 50% final concentration) will precipitate immunoglobulin be used to separate the immune complexes from free antigen. This has been calle

Anti-immunoglobulin antibody

The addition of a second antibody directed against the first antibody can result in t and thus the separation of the complexes from free antigen.

Immobilization of the Antibody

The antibody can be immobilized onto the surface of a plastic bead or coated onto the immune complexes can easily be separated from the other components by sim 17). This is the most common method used today and is referred to as Solid phas competitive RIA and ELISA are commonly used to quantitate serum proteins, horm

Figure 18

Figure 19

Figure 20

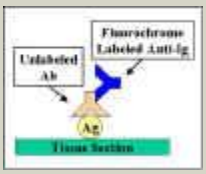

Figure 21

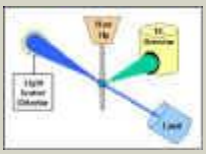

Figure 22

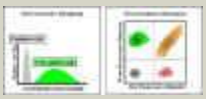

Figure 23

Figure 24

PowerPoint animation of figure

Non-competitive RIA/ELISA for Ag or Ab

Non-competitive RIA and ELISAs are also used for the measurement of antigens a coated with the antigen and is used for the detection of antibody in the unknown s antibody bound is related to the amount of antibody in the unknown sample. This a measurement of antibodies of the IgE class directed against particular allergens b anti-IgE antibodies as the labeled reagent. It is called the RAST test (radioallergos coated with antibody and is used to measure an unknown antigen. The amount of proportional to the amount of antigen that bound to the first antibody.

Tests for Cell Associated Antigens

Immunofluorescence

Immunofluorescence is a technique whereby an antibody labeled with a fluoresce one of many other fluorescent dyes) is used to detect the presence of an antigen i fluorescence emitted by the bound antibody.

Direct Immunofluorescence

In direct immunofluorescence, the antibody specific to the antigen is directly tagge

Indirect Immunofluorescence

In indirect immunofluorescence, the antibody specific for the antigen is unlabeled a antibody directed toward the first antibody is tagged with the fluorochrome (Figure sensitive than direct immunofluorescence since there is amplification of the signal.

Flow Cytometry

Flow cytometry is commonly used in the clinical laboratory to identify and enumera Cells in suspension are labeled with a fluorescent tag by either direct or indirect im analyzed on the flow cytometer.

Figure 22 illustrates the principle of flow cytometry. In a flow cytometer, the cells e laser beam. The amount of laser light that is scattered off the cells as they passes which gives information concerning the size of the cells. In addition, the laser can the fluorescent light emitted by the cells can be measured by one or more detecto

The type of data that is obtained from the flow cytometer is shown in Figure 23. In amount of fluorescence (*e.g.* green fluorescence) is plotted on the x axis and the r fluorescence is plotted on the y axis. The fraction of cells that are fluorescent can under the curve. In a two parameter histogram, the x axis is one parameter (*e.g.* r second parameter (*e.g.* green fluorescence). The number of cells is indicated by th

Complement Fixation

Antigen/antibody complexes can also be measured by their ability to fix compleme will "consume" complement if it is present, whereas free antigens or antibodies do complexes that rely on the consumption of complement are termed complement fi

24 of this figure

antigen/antibody reactions. This test will only work with complement fixing antibod

The principle of the complement fixation test is illustrated in Figure 24. Antigen is r
for antibody and antigen/antibody complexes are allowed to form. A control tube ir
prepared. If no antigen/antibody complexes are present in the tube, none of the co
antigen/antibody complexes are present, they will fix complement and thereby red
tube. After allowing complement fixation by any antigen/antibody complexes, a sta
have been pre-coated with anti-erythrocyte antibodies is added. The amount of an
predetermined to be just enough to completely use up all the complement initially
complement was still present (i.e. no antigen/antibody complexes formed between
the red cells will be lysed. If antigen/antibody complexes are formed between the a
the complement will be consumed and, thus, when the antibody-coated red cells a
simply measuring the amount of red cell lysis by measuring the release of hemogl
quantitate antigen/antibody complexes in the tube. Complement fixation tests are
antibody in a test sample but they can be modified to measure antigen.

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