FACULTY OF ENGINEERING AND TECHNOLOGY

Unit- 2

Antigen Antibody Reaction

Antigen

Any substance which, when introduced parentrally into the body stimulates the production of antibody specifically.

• This is a traditional definition since it had got some exception.

– Polio vaccine – oral administration

– Some antigen may not produce antibodies

• Specificity is the hallmark of all immunological reactions

Structure of Antigen:

• Smallest unit of antigenicity (antigenic determinant) – **epitope.**

• Consist of 4-5 amino residues or monosaccharide residues.

• Posses a specific chemical structure, electric charge and spatial configuration.

• Capable of sensitizing of an imunocytes.

• **Paratope: area on antibody molecule on which** epitope binds

•This produces specificity of antigenic reaction

Properties of Antigen

Size

– Antigenicity is related to molecular size

– Larger molecules are highly antigenic where as **haptens are low** molecular weight having low antigenicity

• **Chemical Nature**

– Antigenicity is directly proportional to degree of structural diversity

– Amino acid> monosachharide> lipid> nucleic acid

• **Susceptibility**

– Only substance metabolized by tissue enzyme to epitope fragments – potential to produce antigen

– **Polystyrene not antigenic**

– Also antigen rapidly broken down doesn't show Antigenicity properties

– D-amino acids are not metabolized in the body where as Lamino acids do - **prevention of autoimmunity**

Properties of Antigen

Foreignness

•Described by **Ehrlich.**

•Normal body contain numerous antigens or antigen producing substance which may not produce antibody.

•But introduction of this into other body produces immune response

•This response is directly related to degree of foreignness – antigen from same species shows less Antigenicity than others

•

Specificity

Antigen shows various types of specificity they are mainly:-

•Antigenic specificity
•Species specificity
•Iso-specificity
•Auto-specificity
•Organ specificity
•Heterogenic specificity

Function of Antigen

Immunogenicity : induction of immune response.

Immunological Reactivity: specific reaction with antibodies or sensitized cells

• Based on this function antigen is classified as
– **Complete antigen: induce antibody formation and** specific immune response
– **Partial Antigen (Hapten): a specific non-protein** substance incapable of inducing antibody formation itself but elicit immune response when couple with **carrier protein**
• **Complex hapten : polyvalent – 2 or more antibody** combining site
• **Simple Hapten: univalent**

Antigen-Antibody Reaction

Use of Antigen-Antibody Reactions

- **In Body**

– It forms the basis of antibody mediates immunity against infectious disease

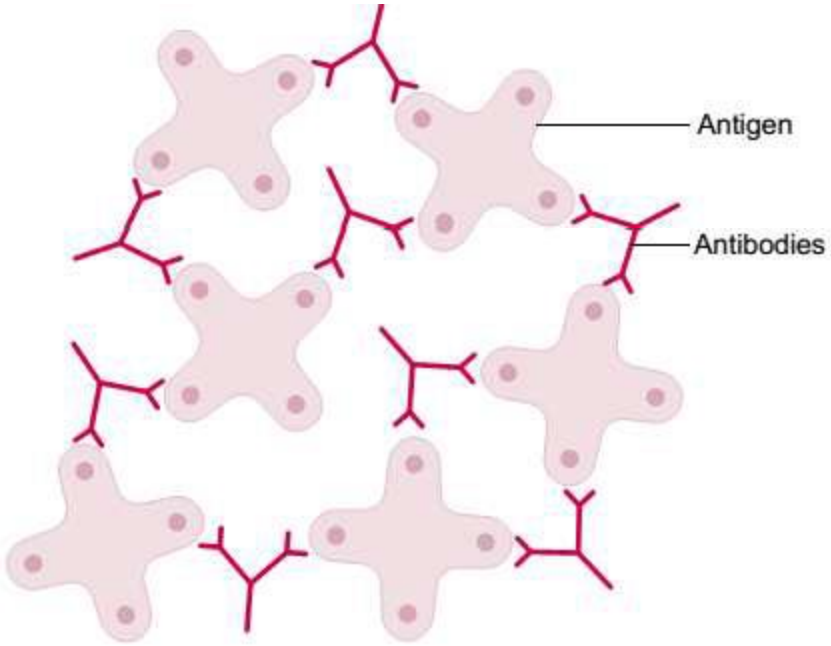
– Tissue injury like Hypesensitivity and autoimmunity

- **In the Lab – Serological Reaction**

– Diagnosis of infection

– Identification of infectious agents and difference between non infectious antigen

– Quantification of antigen or antibody



Stages of Antigen-Antibody reactions

It occurs mainly in 3 stages

– **Primary Stages**

• Initial interaction without any visible effect

• Occurs very rapidly – obeys the laws of chemistry

• Reversible

• Can be represented by estimating free and bound antibodies – physical and chemical method includind markers like radioistopte, fluorescent dyes etc

– **Secondary Stages: demonstrates the events like**

• Precipitation

• Agglutination

• Lysis of Cell

• Neutralisation of toxins and other biologically active antigen

• Complement fixation

• Immobilisation of motile organism

• Enhancement of Phagocytosis

– **Tertiary Stages : Complete destruction of injurious antigen and** tissue damage including humoral immunity

Features of Antigen-Antibodies Reaction

Specific

– antigen combines only with its homologus antibody,

– Sometimes **cross-reactivity may occur due to antigenic** similarity

• **Completeness: entire molecules reacts and not** fragments

• **No denaturation of antigen or anotibodies**

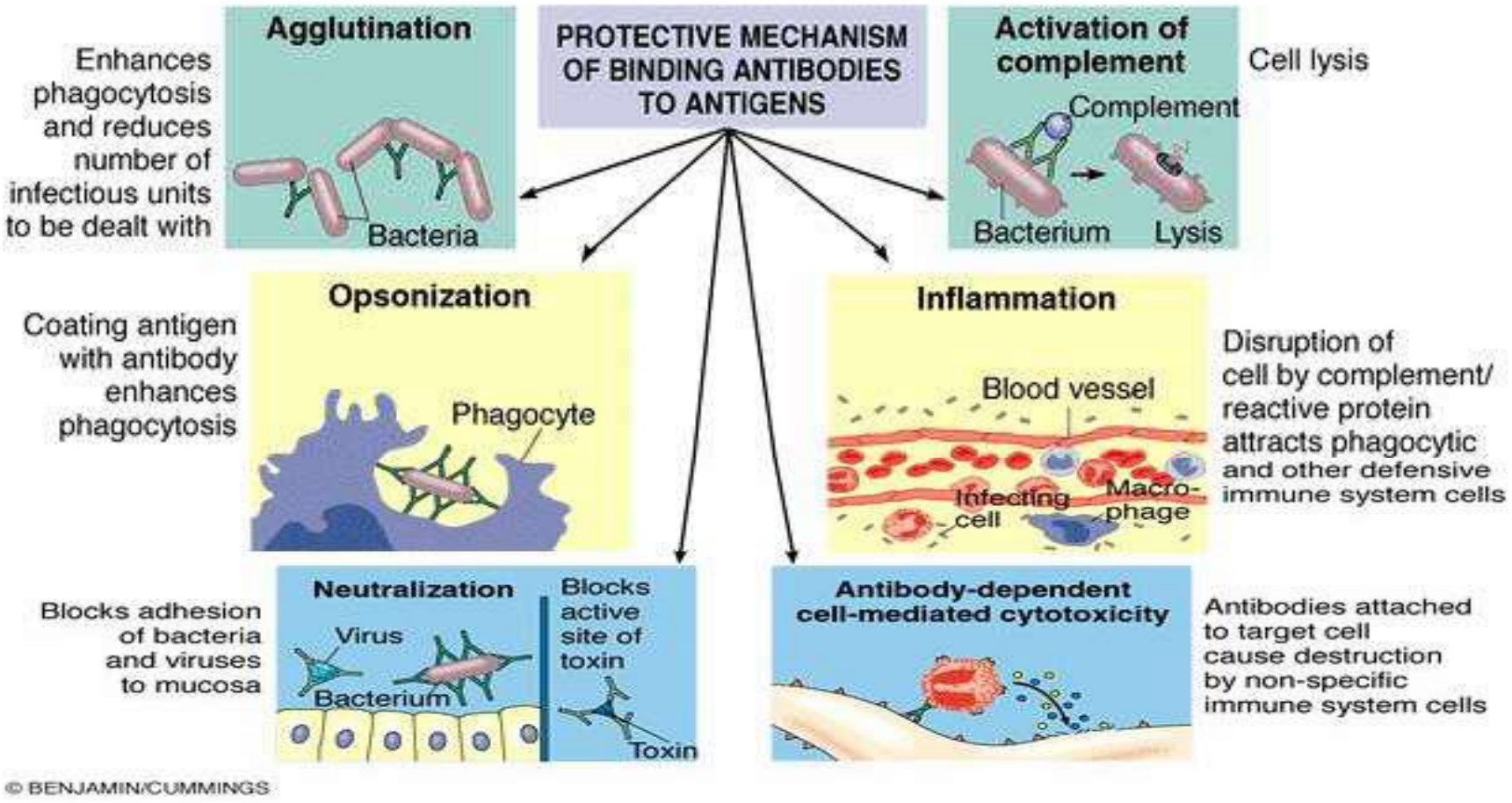
• Combination occurs on the **surface**

• The reaction is firm but **reversible**

• **Varying proportion**

• both antigen and antibodies are multivalent

• Antibody are generally bivalent – though IgM: 5-10 combining sites


PROTECTIVE MECHANISM OF BINDING ANTIBODIES TO ANTIGENS
Agglutination
Enhances phagocytosis and reduces number of infectious units to be dealt with
Bacteria
Activation of complement
Cell lysis
Complement
Bacterium
Lysis
Opsonization
Coating antigen with antibody enhances phagocytosis
Phagocyte
Inflammation
Blood vessel
Infecting cell
Macro-phage
Disruption of cell by complement/ reactive protein attracts phagocytic and other defensive immune system cells
Neutralization
Blocks adhesion of bacteria and viruses to mucosa
Virus
Bacterium
Blocks active site of toxin
Toxin
Antibody-dependent cell-mediated cytotoxicity
Antibodies attached to target cell cause destruction by non-specific immune system cells
© BENJAMIN/CUMMINGS

Precipitation Reaction

•When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at suitable temperature and pH forms **insoluble precipitate. It either**

– Settles down **– Sedimentation**

– Suspended as floccules **– Flocculation**

• It can be carried out either in **liquid or gel** media.

• Amount of precipitate is greatly influenced by antigen/antibody ratio.

•Based on the antigen/antibody ratio, the precipitation reactions can be classified in 3 zones i.e **Zone phenomenon**

– **Prozone : Antibody is in excess. False negative** precipitation may occur.

– **Zone of equivalence : equal proportion of antigen** and antibody i.e. optimal proportion, most rapid and abundant reaction

– **Postzone: Antigen is in excess. Precipitation is** again weak or even absent

Agglutination Reaction

•When a particulate antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.

• More sensitive for antibodies detection.

• Same **Zonal principle applies for agglutination as** that of precipitation.

• Incomplete or monovalent antibodies doesn't cause agglutination – combine with antigen : **Blocking antibodies**

•Positive reaction is flowed by clumping together of particles

• There are various application for agglutination reactions

– **Slide Agglutination**

– **Tube agglutination: Widal test for Typhoid, Paul** Bunnel Test – mononucleosis, Cold agglutination test - mycoplasma pneumonia

– **Antiglobulin (Coombs) test – Rh antibody**

– **Passive agglutination test – Rose Waaler test for** Rheumatoid arthritis

Complement Fixation Test (CFT)

Complement takes part in many immunological reactions.

- In the presence of antibodies complement
  – Lyses and kills erythrocytes and bacteria
  – Immobilizes motile organism
  – Promotes phagocytosis and immune adherence
  – Hypersensitivity
- The ability of antigen-antibody complexes to fix complement is made use of the **Complement Fixation Test.**

- Very versatile and sensitive test – detecting as little as **0.04 mg of antibody and 0.1 mg of antigen.**
- Gold standard test for detection of *Treponema pallidum and Vibrio cholerae.*
- Its carried out in 2 steps and uses five reagents:-
  – Antigen
  – Antibody
  – Complement
  – Sheep Erythrocytes
  – Amboceptor – rabbit antibody to sheep erythrocytes

Immunofluorescence

•Fluorescence is the property of absorbing light rays of one particular wavelength and emitting rays with a different wavelength.

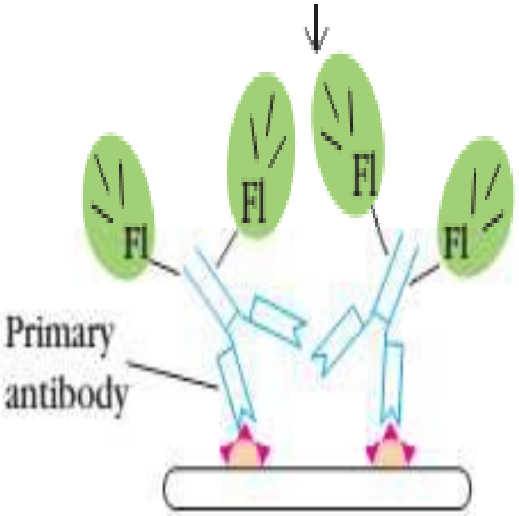
• Coons et al – demonstrated that antibody labelled with fluorescent dyes can be used to locate and identify antigen

• Commonly used dyes – **Fluorescent isothiocyante (blue** green) and **lissamine rhodamine (orange red).**

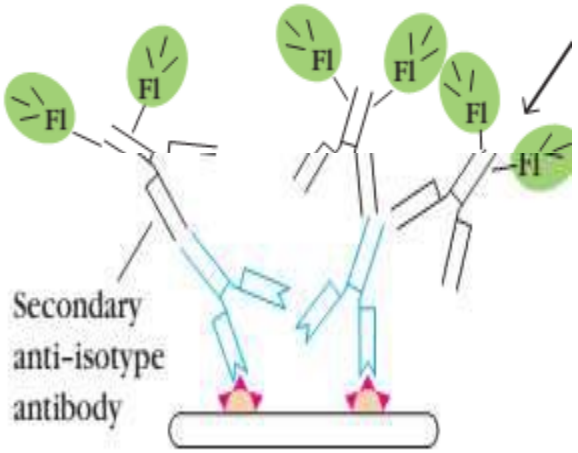
• It can be of two types:

– Direct Immunofluorescence test

– Indirect Immunofluorescence test



Direct Immunofluorescence test



Indirect Immunofluorescence test