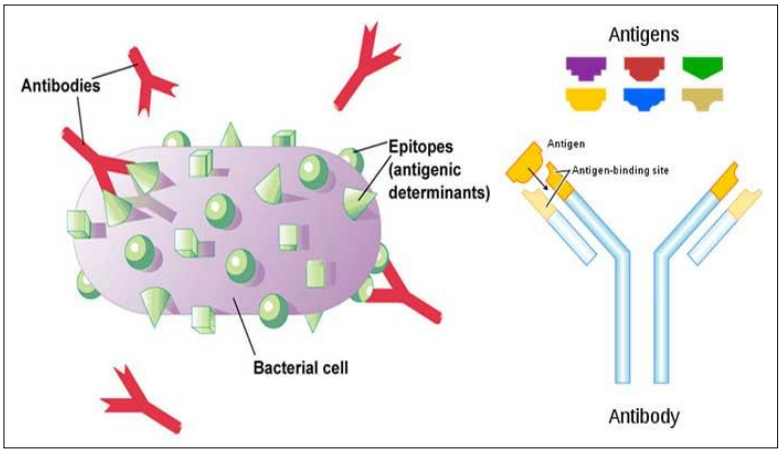
In 1899 Ladislas Deutsch (Detre) (1874–1939) named the hypothetical substances halfway between bacterial constituents and antibodies "substances immunogenes or antigenes". He originally believed those substances to be precursors of antibodies, just like zymogen is a precursor of zymase. But by 1903 he understood that an antigen induces the production of immune bodies (antibodies) and wrote that the word antigen was a contraction of "Antisomatogen

DEFINITION OF ANTIGEN

Antigen is substance which when introduced parentally into the body stimulates the production of an antibody with which it reacts specifically and in an observable manner.

Antigens are specifically defined as molecules that interact specifically with **immunoglobulin receptor of B-cell (or T-cell when complexed with MHC)** or antigen is any substance that may be specifically bound by an antibody molecule or T cell receptor. Antigen is a substance which when introduced into living animal evokes specific immune response either by producing specific antibody or by sensitized T-cell. Antigen may be soluble substance, toxin or substance present in bacteria, virus, RBC and other types of cell. It is a substance usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both. Antigens are “targeted” by antibodies.



An antigen may be a substance from the environment, such as chemicals, bacteria, viruses, or pollen or may also be from inside the body. In general, two main divisions of antigens are recognized: **foreign antigens (or hetero-antigens)** and **autoantigens (or self-antigens)**.

CLASSIFICATION OF ANTIGENS:

- **BASED ON IMMUNOGENICITY**

Complete antigen: Substances which can induce antibody formation by themselves and can react specifically with these antibodies

Incomplete antigen (haptens): substances unable to induce antibody formation on its own but can become immunogenic when covalently linked to proteins, called carrier proteins .they are of two types: Complex and Simple

- **BASED ON ORIGIN**:

Exogenous antigens: Exogenous antigens are antigens that have entered the body from the outside, for example by inhalation, ingestion, or injection. The immune system's response to exogenous antigens is often subclinical.

Endogenous antigens: Endogenous antigens are antigens that have been generated within previously normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection.

Foreign antigens
It originates from outside the body. Examples include parts of or substances produced by viruses or microorganisms (such as bacteria and protozoa), as well as substances in snake venom, certain proteins in foods, and components of serum and red blood cells from other individuals.

Auto antigens
It originates within the body. Normally, the body is able to distinguish self from non-self, but in persons with autoimmune disorders, normal bodily substances provoke an immune response, leading to the generation of autoantibodies. Ribonucleoprotein antigens in lupus-related diseases and mitochondrial antigens in primary biliary cirrhosis (PBC) etc. are examples of autoantigens.

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Antigens that stimulate immune responses are called **immunogens.** An antigen that induces an immune response i.e., stimulates the lymphocytes to produce antibody or to attack the antigen directly is called an immunogen. A substance that induces specific immune response can be called as immunogen.

Antibody binds to only a portion of the antigen, which is called a **determinant or an epitope**. **Epitope** is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants**. The small area of chemical grouping on antigen molecule which determines specific immune response and reacts specifically with antibody is known as epitope.

Antigens typically contain multiple determinants, some of which may be repeated and each of which can be bound by an antibody. The presence of multiple identical determinants in an antigen is referred to as **polyvalency or multivalency**.

The spatial arrangement of different epitopes on a single protein molecule may influence the binding of antibodies in several ways. When determinants are well separated, two or more antibody molecules can be bound to the same protein antigen without influencing each other; such determinants are said to be **non-overlapping**. When two determinants are close to one another, the binding of antibody to the first determinant may cause steric interference with the binding of antibody to the second; such determinants are said to be **overlapping**.

Immunogenicity

It is defined as the property of a substance (immunogen) that endows it with the capacity to provoke a specific immune response. Immunogenecity is the ability to induce a humoral/ cell-mediated immune response.

Antigenicity

It is defined as the property of a substance (antigen) that allows it to react with the products of a specific immune response (antibody or Tcell receptor). Antigenecity is the ability to combine specifically with the final products (Antibodies or receptors in T-Cell) of humoral /cell mediated immune response.

Thus, **all immunogens are antigens; but all antigens are not immunogens.**

Antigen and Factors Affecting Immunogenicity

The substances that induce a specific immune response and subsequently **react** with the products of a specific immune response is called antigen *(it is more appropriately called as immunogen).*

In the case of infectious diseases, the antigens are components of invading microorganism's structure that are usually composed of proteins or polysaccharides.

Ag has to be recognized by the

- Immunoglobulin receptor of B Cells or
- By the T cell receptor when complexed with MHC

Among the biological macromolecules, protein is the most potent immunogen followed by the polysaccharide. Other macromolecules such as lipids and nucleic acids do not serve as immunogen.

***F**or Cell-Mediated immunity only proteins and some lipids/glycolipids serve as immunogen.*

Properties of an Immunogen

- Immunogenicity
- Antigenicity

Immunogenicity: Ability **to induce** a humoral and/or cell-mediated immune response.

- B cells + Ag = effector B cells (plasma cells) + memory B cells
- T cells + Ag = effector T cells (e.g., CTLs, THs) + memory T cells

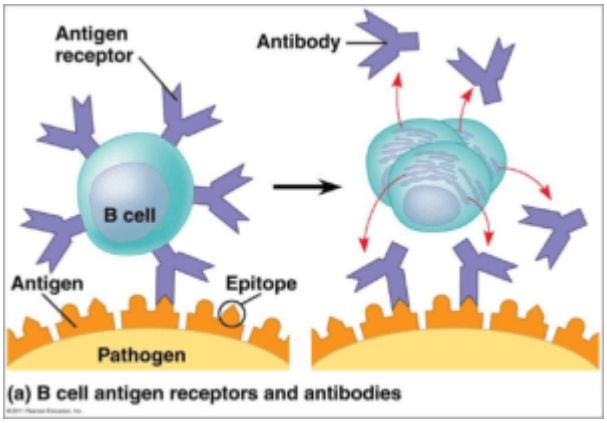
Antigenicity: The ability **to combine/react** specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors).

*All molecules that have the property of immunogenicity also have the property of antigenicity but **Reverse not true. Remember:** All Immunogen are Antigen but all Antigen are not Immunogen e.g. Hapten.*

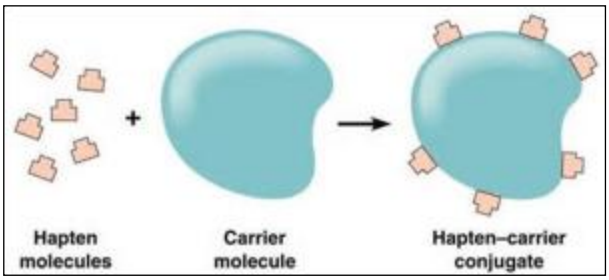
Hapten are antigenic but incapable by themselves of inducing a specific immune response, i.e., they lack immunogenicity

Epitopes

- Immune cells do not interact with or recognize an entire immunogen instead they recognize **discrete sites on the macromolecule which is called epitopes.**
- Epitopes are immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies.
- B cells and T cells recognize different epitopes on the same antigenic molecule.
- B cell bind epitopes that are present in **highly accessible sites** on the exposed surface of the immunogen.
- T cells recognize only peptides combined with MHC molecules on the surface of APCs or altered self cells.



Hapten



- A substance that is non-immunogenic but **which can react** with the products of a specific immune response.
- Haptens are small molecules which could never induce an immune response when administered by themselves but **which can** when coupled to a carrier molecule.
- Haptens have the property of antigenicity but not immunogenicity.

FACTORS INFLUENCING IMMUNOGENICITY

Nature of the Immunogen

1. Foreignness
2. Molecular size
3. Chemical composition and heterogeneity
4. Ability to be processed and presented with an MHC molecule on the surface of Antigen Presenting Cells (APCs) or altered self-cell

Biological system that the antigen encounters

1. Genotype of the recipient animal
2. Dosage and route of administration

Factors influencing Immunogenicity

Ⅰ. Foreignness

Antigens must be recognized as **non-self** by the biological system. Degree of immunogenicity depends on the degree of foreignness **i.e.** The greater the phylogenetic distances between two species, the greater the structural (and therefore the antigenic) disparity between them. e.g. If Bovine serum albumin (BSA) is injected in Cow, Rabbit and Chicken, the order of Immunogenicity will be:

Cow < Rabbit < Chicken (least for cow and most for chicken)

This property is govern by: **Tolerance to self** (specific unresponsiveness to self antigens)

Ⅱ. Molecular Size

Correlation exists between size of the macromolecule and its immunogenicity

1.Molecular Mass ≥ 1,00,000 Da: Active Immunogens
2. Molecular Mass 5000-10,000 Da: Poor immunogen

Exceptions: Few substances with molecular mass less than 1000 Da have proven to be immunogenic.

III. Chemical Composition and Heterogeneity

- Chemical complexity contributes to immunogenicity
- Copolymers composed of different amino acids or sugars are usually more immunogenic than homopolymer of their constituents.
- All four levels of protein organization contribute to the structural complexity of a protein and hence affect its immunogenicity.

IV.Susceptibility to Ag Processing and Presentation

The development of both humoral and cell-mediated immune response requires interaction of T cells **with Ag that has been processed and presented** together with MHC molecules.

- Large, insoluble macromolecules > Small, soluble macromolecules
  - *Ease of phagocytosis and processing*
- Can not degraded & presented: Poor immunogen e.g. Polymers of D-amino acids

V.Contribution of the Biological System

1. **Age:** Usually the very young and the very old have a diminished ability to mount an immune response in response to an immunogen.

2. **Genotype of the recipient animal**:

- Genetic constitution of an immunized animal influences the type of immune response the animal manifests, as well as the degree of response.
- Genetic control of immune responsiveness is largely confined to **genes within MHC.**
- MHC gene products plays central role in determining the degree to which an animal responds to an immunogen.
- Some substances are immunogenic in one individual but not in others (i.e.responders and non-responders).

VI. **SUSCEPTIBILITY TO TISSUE ENZYMES** Substances which can be metabolised and′ are able to the action of tissue enzyme behave as antigen.

VI.**Antigenic specificity** It depends upon epitope′ Position of epitope in the antigen molecule′ is important for specificity

VII. **Species specificity** Tissue of all individual in species possesses species′ specific antigens.

VIII.Isospecificity It depends on isoantigens which may be found in some′ but not all members of species.

I**X.Autospecificity** Self antigens are generally non-antigenic but in some′ case such as lens protein and sperm these are not recognised as self antigen because they are absent during the embryonic life and develop later.

X.Organspecificity Some organs such as the brain, kidney and lens protein′ of different species share the same antigens. such antigens are the characteristics of an organ or tissue found in different species and they are known as organ specific antigen.

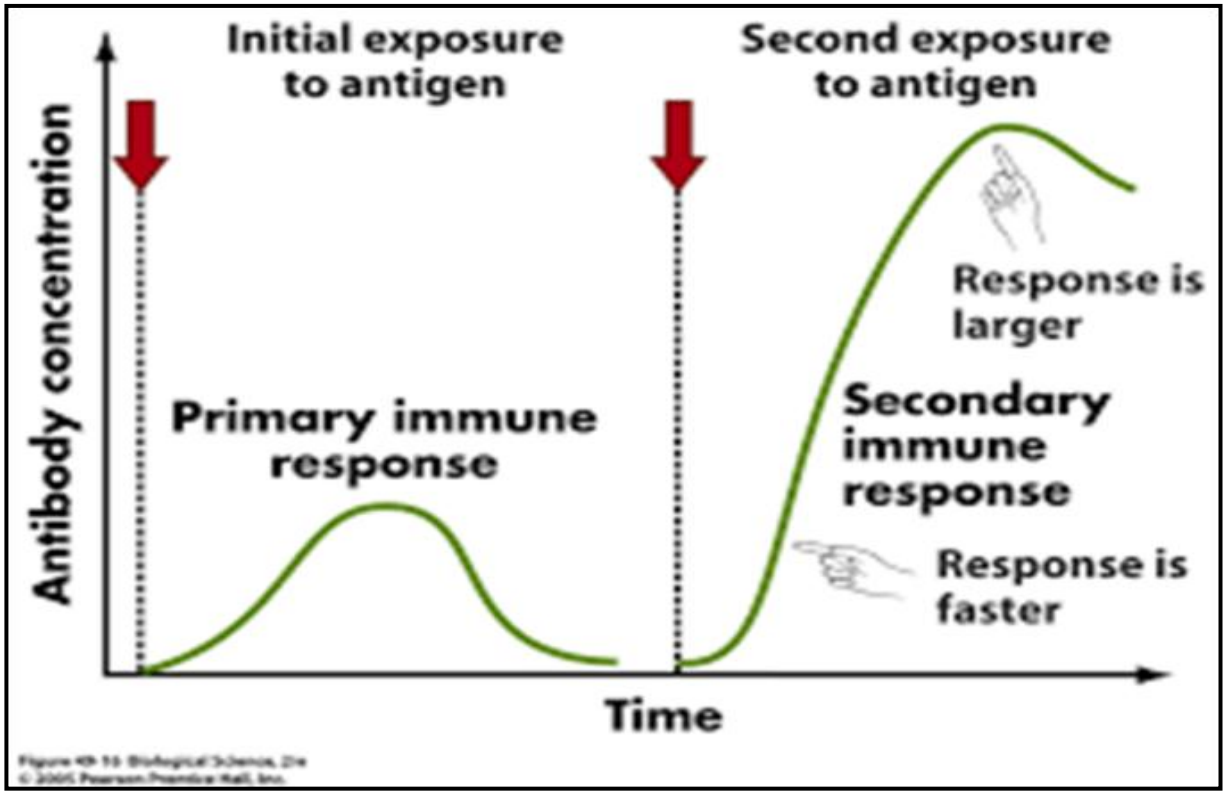
Immunogen Dosage and Route of Administration

A: Amount of Immunogen

- Optimum dose is necessary to mount good immune response.
- **Insufficient dose**: will not stimulate an immune response (fails to activate enough lymphocyte or can induce a state of immunologic unresponsiveness, or tolerance).
- **Excessively high dose**: Induces tolerance

B: Times

- **Single dose:** will not induce strong response
- **Repeated administration (boosters):** Increases clonal proliferation of antigen specific T cells or B cells and thus increase the lymphocyte populations specific for the immunogen.



PRIMARY VS. SECONDARY IMMUNE RESPONSE

C. Routes of Administration:

- Generally the subcutaneous route is better than the intravenous or intragastric routes
- Administration route strongly influences which immune organs and cell populations will be involved in the response. e.g. intravenous (spleen); subcutaneous (local lymph nodes)

Use of Antigen with or without Adjuvants

Adjuvants

- Substances that when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen.
- Used to boost the immune response when an Ag has low immunogenicity or when small amount of Ag are available
- Adjuvants appear to exert one or more of the following effects;
    - Antigen persistence is prolonged (e.g. alum, water in oil adjuvants)
    - Co-stimulatory signals are enhanced
    - Local inflammation is increased
    - Nonspecific proliferation of lymphocytes is stimulated

Freund's Adjuvant

- one of the most commonly used adjuvants
- designed to provide continuous release of antigens necessary for stimulating a strong, persistent immune response.
- It is used as a water-in-oil emulsion.
- It is prepared from non-metabolizable oils (paraffin oil and mannide monooleate).

Main disadvantage: it can cause granulomas, inflammation at the inoculation site and lesions.

Types-

Freund's incomplete adjuvant

- Ag in aqueous solution, mineral oil, mannide monooleate (emulsifying agent)
- Small droplets of oil surrounding Ag formed
- Ag is released slowly from the site of injection

Freund's complete adjuvant

- Highly effective adjuvant
- Incomplete adjuvant+ heat-killed *Mycobacterium tuberculosis*
- Muramyl dipeptide (a component of mycobacterial cell wall), **activates macrophages**, making Freund's complete adjuvant far more potent than incomplete form.

SUPERANTIGENS: When the immune system encounters a′ conventional T-dependent antigen, only a small fraction (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response). However, there are some antigens which′ polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called superantigens

ANTIGEN BINDING BY B CELLS AND T CELLS HUMAN IMMUNOLOGY

Antigen binding by B Cells and T Cells

Repeated folding of the amino acid chain (like you make a ball of thread to fly a kite) forms a protein molecule. Such a molecule will have epitopes inside the molecule as well as on the surface of the molecule (Fig.).

The B cells and T cells recognize the epitopes by different ways (Fig.).

i. B cell directly binds to the epitope of an immunogen in the body fluids. The surface immunoglobulin's (sigs) of the B cell bind directly to the epitope on the surface of an immunogen. The binding of surface epitope with sig leads to B cell activation. But B cell cannot bind to an epitope in the inner aspect of the immunogen.

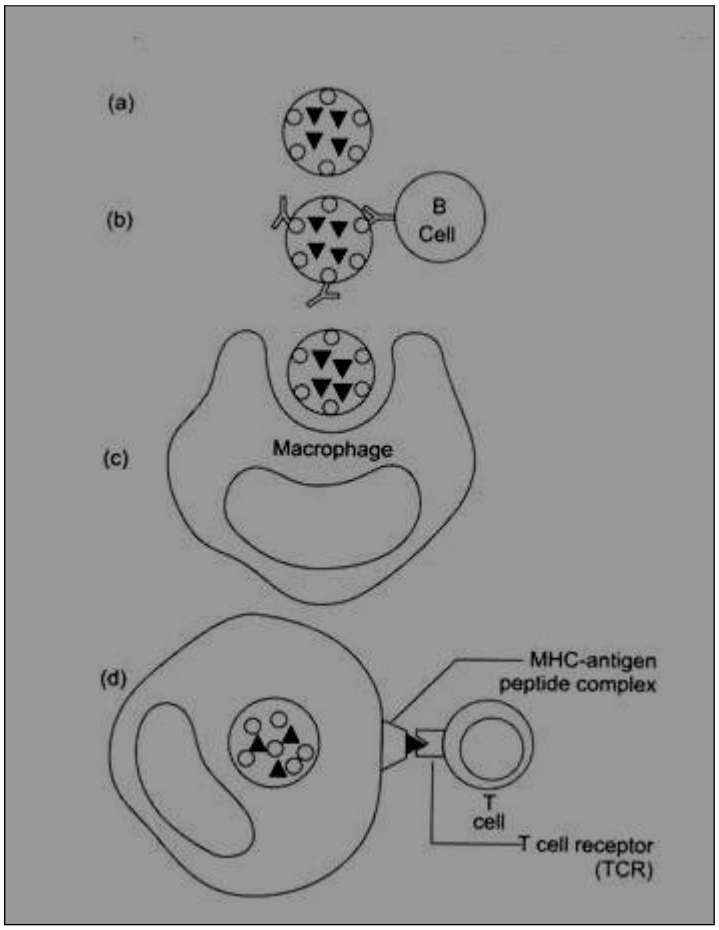
ii. Binding of a T cell to an epitope differs from the binding of the B cell to its epitope (Table 1). T cell doesn't bind directly to the epitope of an immunogen in the body fluid. T cell need the help of another cell called "antigen presenting cell (APC)" to recognize and bind to the epitope. APC phagocytose and degrade the immunogenic molecule into short peptide fragments.

Table 1: Antigen binding by B cells and T cells

	B cells	**T cells**
Antigen receptor on B or T cell	Surface immunoglobulin (sig)	T cell receptor (TCR)
Binding of soluble antigen in body fluids	Yes	No
Chemical nature of antigen	Proteins	Mostly protein.
-	Polysaccharides	Some lipids and
-	Lipids	polysaccharides are also recognized
Requirement of antigen presenting cells (APCs)	Not required	Required

Binding of epitopes on the surface or inner	Bind only to surface epitopes	Bind to epitopes on the surface
aspect of the antigen	-	As well as the inner aspect of the antigen.

During degradation, peptide fragments from the surface as well as inner aspect of the molecule are formed. These fragments are then presented as epitopes by APC to T cell. Because of the degradation of the immunogen inside the APC, epitopes from both surface and inner aspects of the immunogenic molecule are formed and presented by APC. Consequently, T cells can recognize epitopes from the surface as well as inner aspect of the immunogen.



Figs A to D: Schematic diagram showing the difference in the recognition of epitopes in an antigen by T cell and B cell.

(A) An antigen molecule has epitopes on its surface (circular) as well as in its interior aspect (triangle). (B) The surface Immunoglobulin (slg; B cell receptor) on the B cell membrane directly binds to the epitope (circular) on the surface of the antigen molecule, resulting in the B cell activation. (C and D) The macrophage engulfs the antigen; the engulfed antigen is cleared by the macrophage enzymes into small antigen peptides; the small peptides are complexed to MHC class II molecules of the macrophage; the complex is expressed on the surface of the macrophage.

The MHC-antigen peptide complex binds to the T cell receptor of T cell, resulting in T cell activation. (Details are in Epitopes from the surface as well as the Interior aspects of antigen are recognized by T cells. However, epitopes from the interior aspect of antigen are usually recognized by the T cells.

The B cell binds to unaltered immunogen (i.e. immunogen not split into peptide fragments by APCs) in the body fluids. So B cell has no access to the epitopes inside the immunogen. Consequently, the epitopes recognized by B cells are usually conformational and occur on the surface of the immunogen. On the other hand, (because of the degradation of the immunogen by APC) the epitopes recognized by T cells are denatured, sequential, and are usually derived from within the immunogenic molecule (Fig.).

Usually, T cells and B cells mount immune responses against different epitopes on the same immunogen. (For example, when mice are immunized with glucogon, antibodies are formed against epitopes in the aminoterminal position, whereas T cell responses are induced against the epitopes in the carboxy-terminal position). However, T cell and B cell responses against the same epitope in an immunogen can also occur.

An immunogen may have one or many different epitopes. Usually, B cells target only one or few of these epitopes on the surface of an immunogen. Among the few epitopes recognized by B cells, one epitope may induce larger quantity of antibodies with higher binding affinities than the other epitopes in the same immunogen. Such an epitope is said to be an immunodominant epitope.