Antigen

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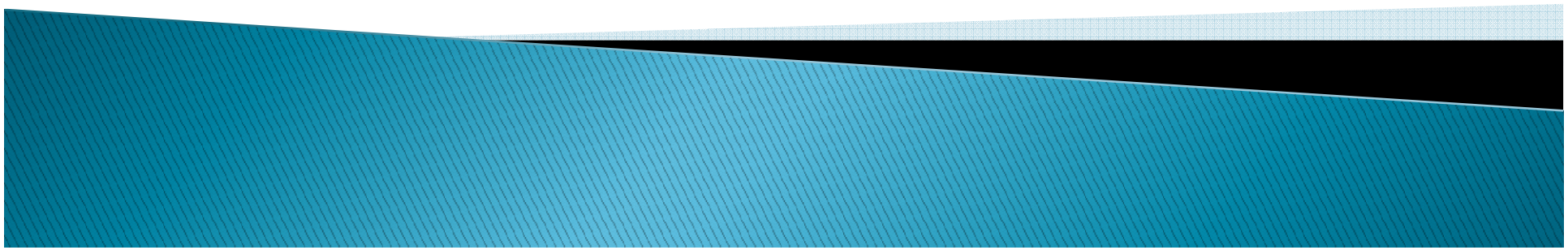


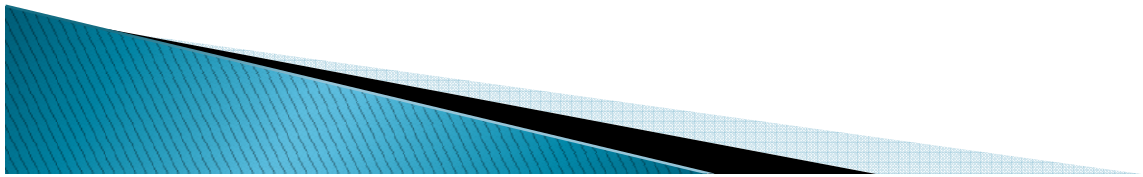
Learning objectives

By the end of this session student should be able to know

- Definition of antigen
- Factors influencing immunogenicity
- Biological classes of antigens

ANTIGEN

- Defined as any substance that satisfies two distinct immunologic properties-
  - *Immunogenicity*
  - *Antigenicity*.



Immunogenicity

- Ability of an antigen to induce immune response in the body (both humoral and/or cell mediated).
  - B cells + antigen → effector B cells (plasma cell) + memory B cells
  - T cells + antigen → effector T cells (helper T cell or cytotoxic T cell) + memory T cells
- Substance that satisfies this property i.e. immunogenicity – more appropriately called as '**immunogen**' rather than 'antigen'.

Antigenicity (immunological reactivity)

- Ability of an antigen to combine specifically with the final products antibodies and/or T cell-surface receptors.
- All molecules having immunogenicity property, also show antigenicity, but the reverse is not true
  - E.g. Haptens- which are antigenic but not immunogenic.

Epitope or *antigenic determinant*

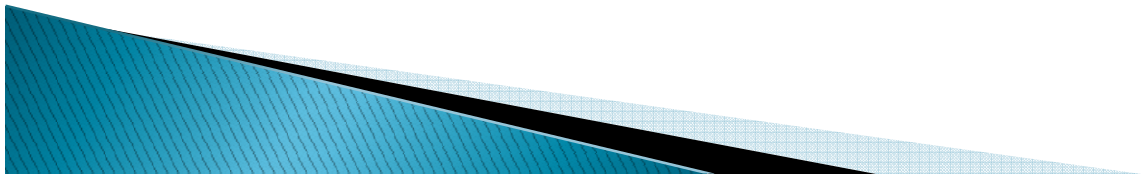
- Smallest unit of antigenicity.
- Definition – Small area present on the antigen comprising of few (four to five) amino acids or monosaccharide residues, that is capable of sensitizing T and B cells and reacting with specific site of T cell receptor or an antibody.
- Specific site of an antibody that reacts with the corresponding epitope of an antigen is called as *paratope*.

Types of epitope

- **Sequential** or **linear** epitope- Present as a single linear sequence of few amino acid residues.
- **Conformational** or **non sequential** epitopes
  - Found on the flexible region of complex antigens having tertiary structures.
  - Formed by bringing together the surface residues from different sites of the peptide chain during its folding into tertiary structure.
- T cells recognize sequential epitopes, while B cells bind to the conformational epitopes.

HAPTENS

- Low molecular weight molecules that *lack immunogenicity* (cannot induce immune response) but *retain antigenicity* or immunological reactivity (i.e. can bind to their specific antibody or T cell receptor).
- Haptens can become immunogenic when combined with a larger protein molecule called ‘carrier’.
- The hapten-carrier complex is capable of inducing immune response in the body.

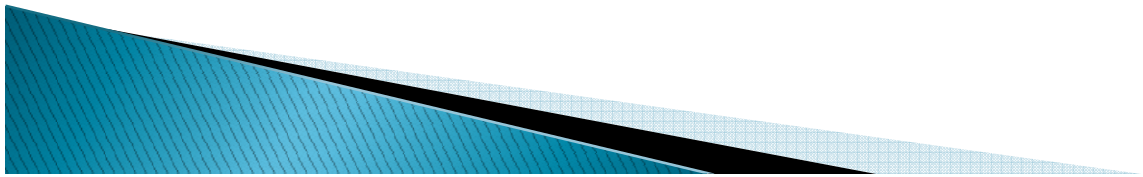


HAPTENS

- It is observed that animals immunized with such a hapten-carrier conjugate produce antibodies specific for-
  - Epitopes of hapten
  - Unaltered epitopes on the carrier protein &
  - New epitopes formed by combined parts of both the hapten and carrier.

Haptens – Classification

- Complex haptens:
  - Contain two or more epitopes.
- Simple haptens:
  - Contain only one epitope (univalent).



ANTIGEN AND HOST RELATIONSHIP

- Based on the antigen-host relationship, antigens can be grouped into two groups:
  - *Self* or *auto antigens*
  - *Non-self* or *foreign antigens*

Self or *auto antigens*

- Belong to the host itself – not immunogenic.
- Hosts do not react to their own antigens by exhibiting a mechanism called *immunological tolerance*.
- Sometimes, the self-antigens are biologically altered (e.g. as in cancer cells) and can become immunogenic.

Non-self or *foreign antigens*-

- Immunogenic and are of three types based on their phylogenetic distance to the host.
  - *Alloantigens* are species specific. Tissues of all individuals in a species contain species-specific antigens.
  - *Isoantigens* are type of antigens which are present only in subsets of a species, e.g. blood group antigens and histocompatibility antigens. The histocompatibility antigens are highly specific as they are unique to every individual of a species.
  - *Heteroantigens* – Antigens belonging to two different species are called as heteroantigens, e.g. antigens of plant or animal or microorganisms etc. A *heterophile antigen* is a type of hetero antigen that exists in unrelated species.

Heterophile antigens

- **Heterophile antigens** – are a type of heteroantigens that are present in two different species; but they share epitopes with each other.
- **Forssmann antigen** is universal heterophile antigen. It is a lipid carbohydrate complex present in all animals, plants and bacteria, but absent in rabbits. Hence, anti-Forssmann antibody can be prepared in rabbits.

Diagnostic application – Heterophile antigens

- *Weil- Felix reaction*
- *Paul-Bunnell test*
- *Cold agglutination test* and *Streptococcus MG* test

FACTORS INFLUENCING IMMUNOGENICITY

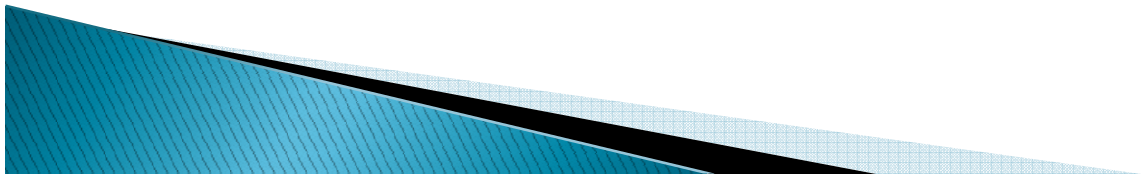
- Size of the antigen
- Chemical nature of the antigen
- Susceptibility of antigen to tissue enzymes
- Structural complexity
- Foreignness to the host
- Genetic factor
- Optimal dose of antigen
- Route of antigen administration:
- Repeated Number of doses of antigens
- Multiple antigens:
- Effect of prior administration of antibody:

Size of the antigen

- Larger is the size; more potent is the molecule as an immunogen.
- Molecules of > 10,000 Dalton molecular weight only can induce immune response (e.g. hemoglobin).

Chemical nature of the antigen

- Proteins are stronger immunogens than carbohydrates followed by lipid and nucleic acids.

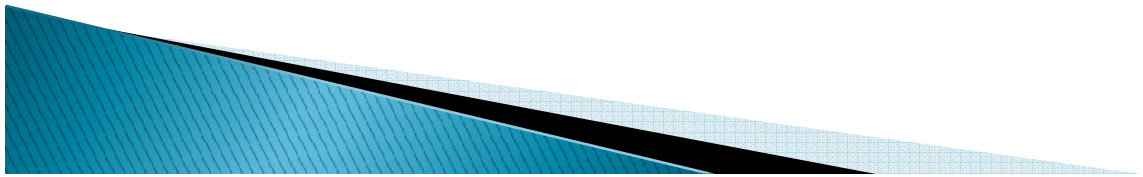


Susceptibility of antigen to tissue enzymes

- Only substances that are susceptible to the action of tissue enzymes are immunogenic.
- Degradation of the antigen by the tissue enzymes produces several immunogenic fragments having more number of epitopes exposed.

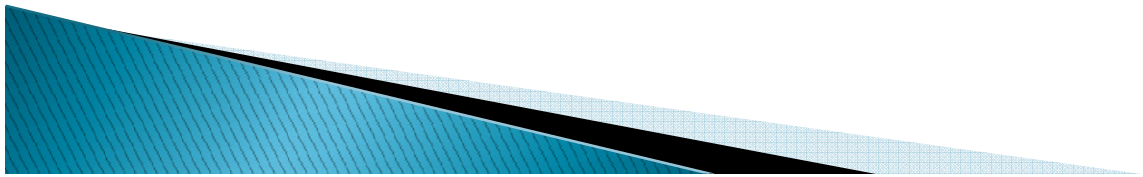
Structural complexity

- Simple homopolymers made up of single amino acid lack immunogenicity.
- Polymers made up of two or more amino acids are immunogenic.
- Addition of aromatic amino acids increases immunogenicity.



Foreignness to the host

- Key factor which determines immunogenicity.
- Higher is the phylogenetic distance between the antigen and the host; more is the immunogenicity.

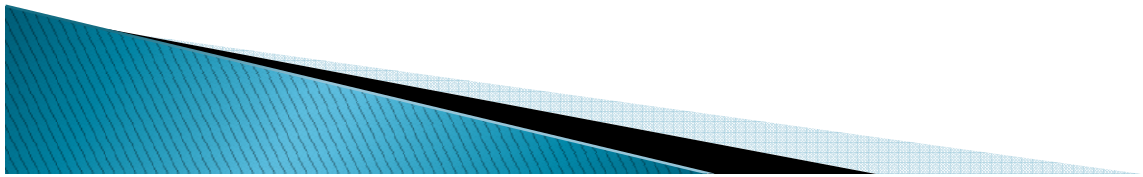


Genetic factor

- Different individuals of a given species show different types of immune responses towards the same antigen.
  - *Responders*- are the individuals who produce antibody faster
  - *Slow responders*- are the individuals who produce antibody slowly and may need repeated antigenic exposures
  - *Non-responders* - are the individuals who do not produce antibody in spite of repeated antigenic exposures.

Optimal dose of antigen

- An antigen is immunologically active only in the optimal dose range.



Route of antigen administration

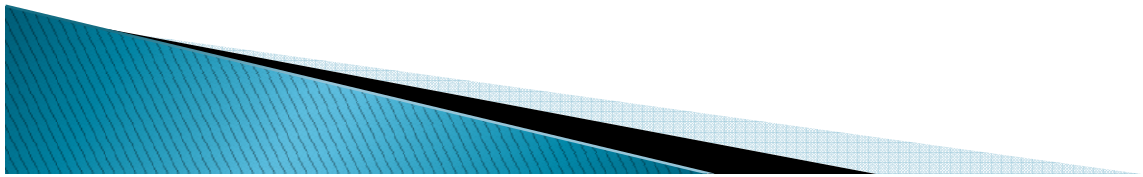
- Immune response is better induced following parenteral administration of an antigen.
- Depends on the type of antibody produced.
- *Site of injection* may influence immunogenicity:

Repeated doses of antigens

- Repeated doses of antigens over a period of time are needed to generate an adequate immune response.
- This is due to the role of memory cells in secondary immune response.
- However, after a certain doses of antigens, no further increase in antibody response is seen.

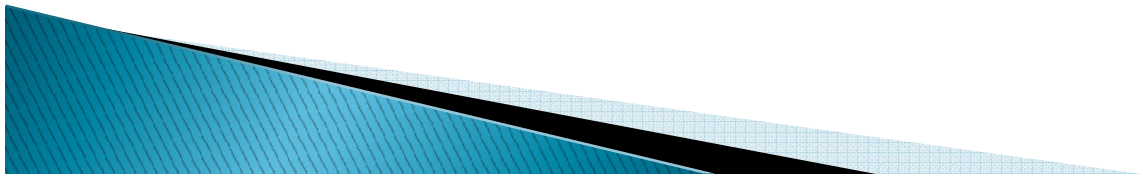
Multiple antigens

- When two or more antigens are administered simultaneously, the effects may vary.
- Antibody response to one or the other antigen may be equal or diminished (due to antigenic competition) or enhanced (due to adjuvant like action).



Adjuvant

- Any substance that enhances the immunogenicity of an antigen.
- Added to vaccines to increase the immunogenicity of the vaccine antigen.

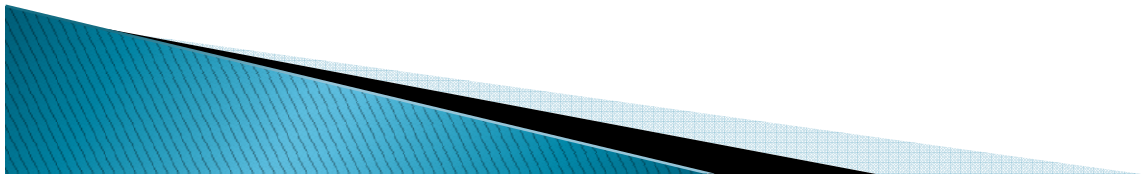


Adjuvants

- *Alum* (aluminium hydroxide or phosphate)
- *Mineral oil* (liquid paraffin)
- *Freund's incomplete adjuvant*- It is a water-in-oil emulsion containing a protein antigen in the aqueous phase.
- *Freund's complete adjuvant* is the mixture of Freund's incomplete adjuvant & suspension of killed tubercle bacilli in the oil phase.
- *Lipopolysaccharide* (LPS) fraction of Gram-negative bacilli
- Other bacteria or their products-
  - *Mycobacterium bovis*
  - Toxoid (diphtheria toxoid and tetanus toxoid act as adjuvant for *Haemophilus influenzae*-b vaccine)
- Nonbacterial products: Silica particles, beryllium sulfate, squalene, and thimerosal.

Mechanism of adjuvant action

1. *Delaying the release of antigen*
2. *By activating phagocytosis*
3. *By activating T_H cells*
4. B*y granuloma formation*



Effect of prior administration of antibody

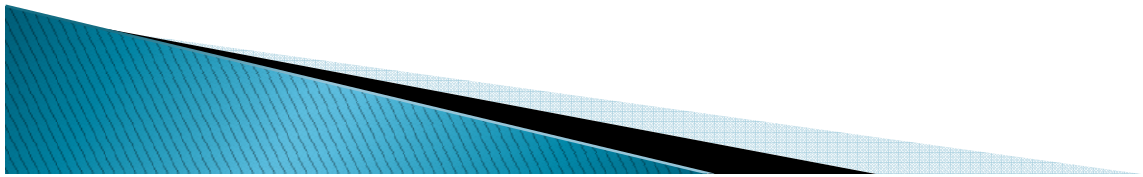
- Primary immune response is more susceptible to get suppressed than the secondary immune response.
- Therapeutic application
  - In Rh negative women carrying an Rh positive fetus, the anti-Rh globulin is administrated immediately following delivery (within 72 hours) which prevents the Rh sensitization in Rh negative women by a negative feedback mechanism.

BIOLOGICAL CLASSES OF ANTIGENS

- Depending on the mechanisms of inducing antibody formation, antigens are classified as:
  - T cell dependent (TD) antigens.
  - T cell independent (TI) antigens.

T-dependent (TD) Antigens

- Most of the normal antigens are T cell dependent, they are processed and presented by antigen-presenting cells (APCs) to T cells which leads to T cell activation.
- Activated T cells secrete cytokines that in turn stimulate the B cells to produce antibodies.



T-independent (TI) Antigens

- Antigens such as *bacterial capsule, flagella and LPS* (lipopolysaccharide) that do not need the help of T cells and APCs.
- Directly bind to immunoglobulin receptors present on B cells and stimulate B cells polyclonally.
- Leads to increased secretion of non- specific antibodies (hypergamma-globulinemia).
- TI antigens can activate both mature and immature B cells. B cells can only differentiate into activated cells.
- No memory cells formation.
- Activated B cells do not undergo affinity maturation and class switch over (both properties are unique to TD antigen stimulated B cells) ; thus such an activated B cell can produce only limited classes of antibodies such as IgM and IgG3.

Differences between T cell dependent and T cell independent antigens

T independent Antigen	T dependent Antigen
Structurally simple- LPS, capsular polysaccharide, flagella	Structurally complex- protein in nature
Dose dependent Immunogenicity	Immunogenic over wide range of dose
No memory	Memory present
No antigen processing	Antigen processing step is needed
Slowly metabolized	Rapidly metabolized

Differences between T cell dependent and T cell independent antigens

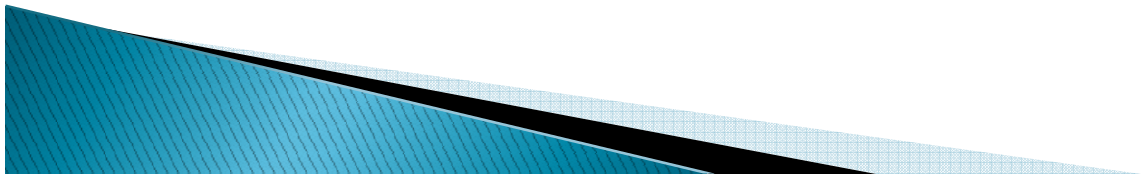
T Independent Antigen	T dependent Antigen
Activate B cells polyclonally	Activate B cells monoclonally
Activate both mature and immature B cells	Activate mature B cells only
B cells stimulated against T independent antigen do not undergo- • Affinity maturation • Class switch over	B cells stimulated against T dependent antigen undergo • Affinity maturation • Class switch over
Antibody response is restricted to IgM and IgG3	Antibodies of all classes can be produced

Superantigens

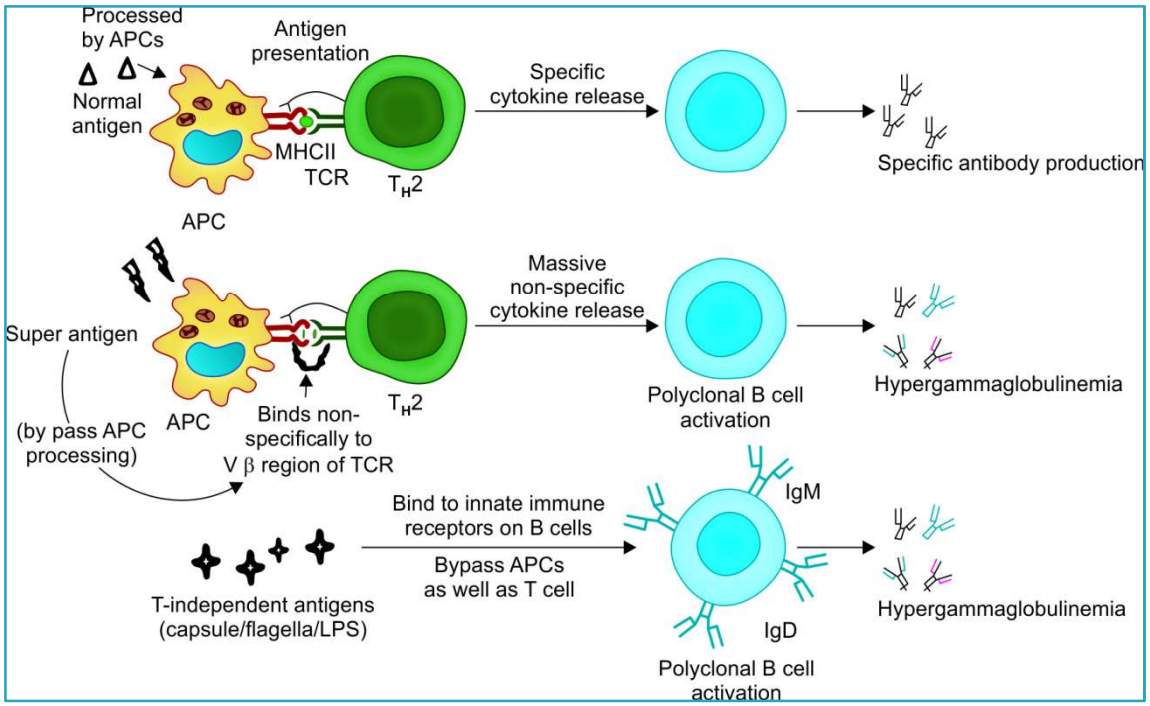
- Superantigens are the third variety of biological class of antigens, recently described in the last decade.
- Unique feature of superantigens is, they can activate T cells directly without being processed by antigen presenting cells (APCs).
- The variable β region of T cell receptor (vβ of TCR) appears to be the receptor for superantigens.

Superantigens

- Directly bridge non-specifically between major histocompatibility complex (MHC)-II of APCs and T cells.
- Non-specific activation of T cells leads to massive release of cytokines which can activate B cell polyclonally, which leads to increased secretion of non- specific antibodies (hypergammaglobulinemia)



Superantigens



Superantigen

Bacterial superantigen	
	Staphylococcal toxin- • Toxic shock syndrome toxin-1(TSST-1); Exfoliative toxin;Enterotoxins
	Streptococcal toxin- Streptococcal pyrogenic exotoxin (SPE)-A and C
	Mycoplasma arthritidis mitogen-I
	Yersinia enterocolitica Yersinia pseudotuberculosis
Viral superantigen	
	Epstein-Barr virus associated superantigen
	Cytomegalovirus associated superantigen
	Rabies nucleocapsid
	HIV encoded superantigen (nef- negative regulatory factor)
Fungal superantigen	
	Malassezia furfur

Disease associated with superantigens

- Conditions associated with staphylococcal toxins are as follows-
  - Toxic shock syndrome
  - Food poisoning
  - Scalded skin syndrome
  - Rare conditions such as- Atopic dermatitis, Kawasaki syndrome, psoriasis, acute disseminated encephalomyelitis.