Antigen and it's Types

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Introduction

- The term **antigen** is Greek word means *anti-against & gen- to generate*.
- The foreign materials, organisms, self and nonself substances that elicit an immune response and react with the products (immunoglobulin receptor of B cells, or by the T-cell receptor when complexed with MHC) of that immune response are called **antigens**.

Introduction

- Antigens include molecules such as proteins, nucleoproteins, polysaccharides, and some glycolipids.
- The ability of a substance to react with the specific antibodies or activated T cells that it induces is called ***antigenicity***.

Factors affecting antigenicity

- Foreignness
- Size (Molecular weight)
- Chemical complexity
- Solubility (biodegradability)
- Structural stability
- Genotype of the recipient animal
- Immunogen dosage and route of administration

ANTIGENIC DETERMINANTS (EPITOPES)

- The antigen combining sites of antibodies or T cells are smaller than whole antigen and hence it is impossible for a whole antigen to bind entirely with antibodies or T cells.
- The antibodies or T cells bind with certain surface structures found on the antigen.
- These surface structures are referred as **antigenic determinants or epitopes**

- These epitopes have different shapes and even a simple microbe may have thousands of such epitopes.
- **The immune response against all epitopes is not same.**
- **Certain epitopes elicit large amount of antibody response. Such epitopes are called as immuno-dominant epitopes.**
- Over the surface of an antigen a particular epitope may be present more than once and
- If an antigen **contain only one type of epitopes it is called as monovalent and if it contains more than one type of epitopes it is called as polyvalent.**

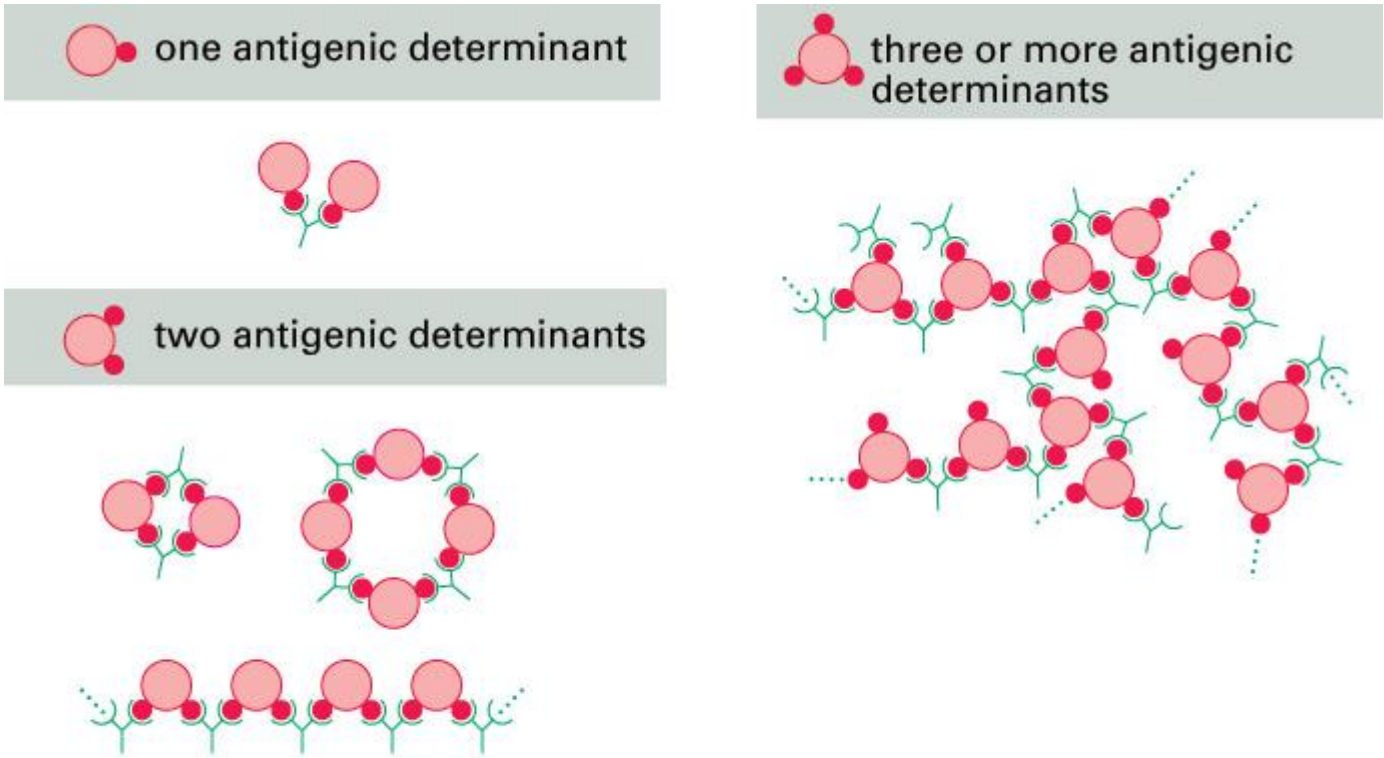


Figure 24–19. Molecular Biology of the Cell, 4th Edition.

- In certain antigens the epitopes are arranged spatially so that binding of an antibody to one may not interfere with binding of another antibody to different epitope. Such epitopes are referred as **non-overlapping epitopes**.
- Where as in some antigen one epitope will interfere with binding of antibody to another epitope and such epitopes are called as **overlapping epitopes.**
- Some times binding of an antibody to an epitope will result in conformational changes that may in turn help in binding of another antibody to different epitope. This is called **allosteric effect**.

- The epitopes can be further classified into **linear epitopes**, **conformational epitopes** and **neo antigenic epitopes** depending upon the arrangement and post synthesis modifications of the building blocks.
- Multivalent antigens generally elicit a stronger immune response than do monovalent antigens.
- Antibody **affinity** known to be strength of binding between a single binding site of a molecule(antibody) and a ligand (of antigen) at a given antigen-binding site.
- The **avidity** of an antibody relates to its overall ability to bind antigen at all antigen-binding sites.

Types of antigen

1. Functional Classification

- Complete antigen:

  Individually able to induce immune system. Produces a specific and observable immune response

  Ex. Bacterial antigens *i.e.* **somatic antigen (O)**, **Capsular antigen (K)**, **flagella antigen (H)**

- Incomplete antigen:

  Substances which individually not able to induce immune response by themselves but if joined with carrier (adjuvant) can provoke the immune system.

  Ex. Haptens

HAPTENS

- The concept of haptens emerged from the work of **Karl Landsteiner**, who pioneered to use synthetic haptens to study immunochemical phenomena.
- Origin fromG*reek word Haptein meaning to Grasp or fasten*
- Haptens are small molecules (less than 1000 daltons) that cannot elicit immune response individually but they become immunogenic when coupled with larger molecules.
- These larger molecules that make the haptens immunogenic are called as carrier molecules.
- Haptens can react with pre formed antibodies. In other words haptens are antigenic but not immunogenic.

- Haptens are classified according to the number of antigenic determinants they have. If a hapten has only epitope it is classified as simple hapten and if it has got two or more different epitopes it is classified as complex hapten.
- Example:

1. Penicillin ….. break in to penicilloyl….. when bind with serum protein such as albumin
2. Urushiol protein from Ivy plant touches with any body protein such as skin protein

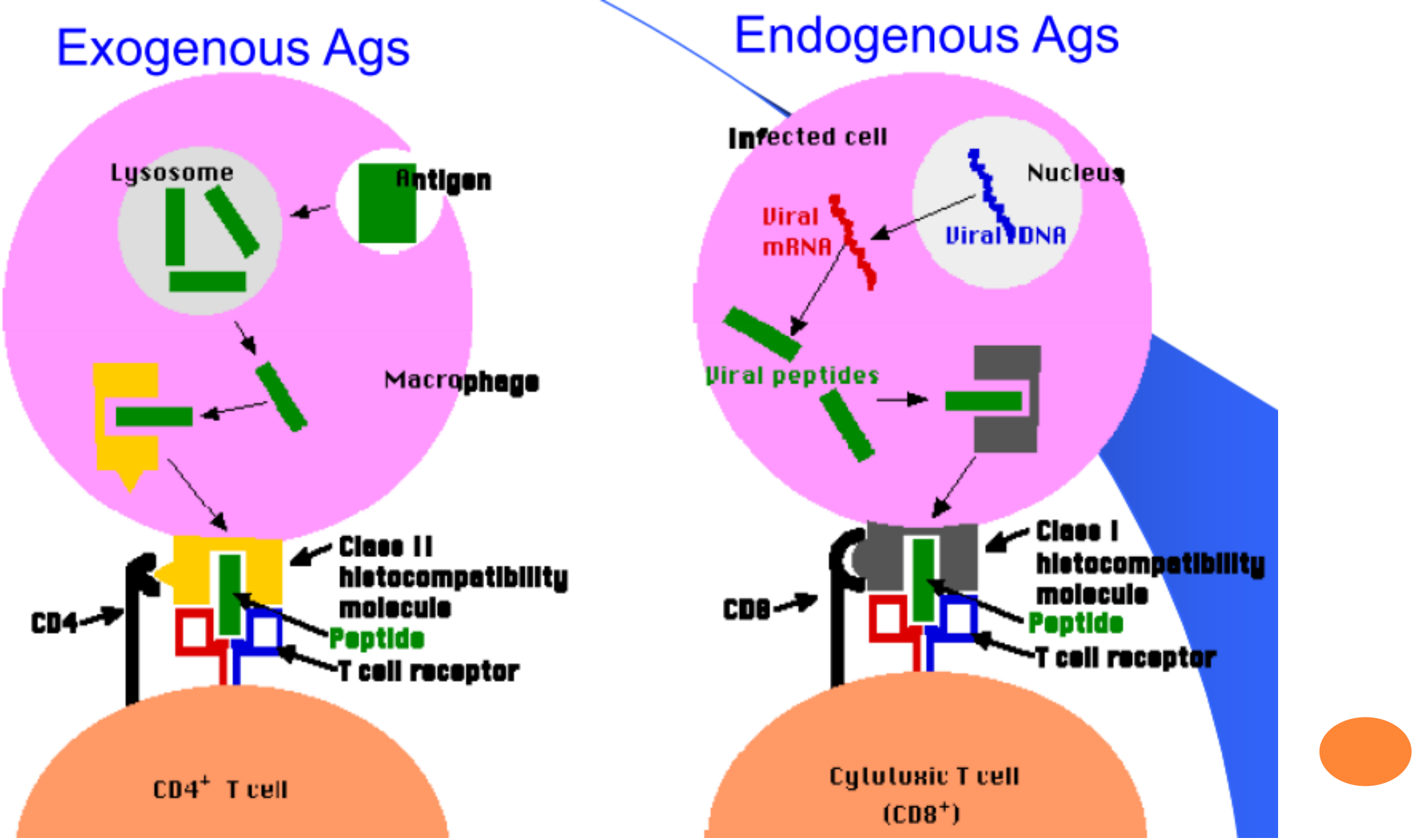
Classification based on Origin of Antigens

1. Exogenous Ags:

- Origin from outside of host
- Enter in the body by inhalation, ingestion or injection.
- Taken by the APCs and degraded into small peptides.
- APCs then present them to helper T cells by using MHC type II molecules.

2. Endogenous Ags:

- Generated within the cell as a result of normal cell metabolism, or because of viral or intracellular pathogenic infection.
- The fragments are presented along with MHC type I molecules to cytotoxic T cells.


Exogenous Ags
Lysosome
Antigen
Macrophage
Class II
histocompatibility
molecule
CD4
Peptide
T cell receptor
CD4+ T cell
Endogenous Ags
Infected cell
Nucleus
Viral
mRNA
Viral DNA
Viral peptides
Class I
histocompatibility
molecule
CD8
Peptide
T cell receptor
Cytotoxic T cell
(CD8+)

Biological Classes (Mechanisms) of Antigen

- Depending on the ability to induce antibody formation/ T cell activation, Antigens are classified as:
  1. T cell dependent (TD) Ags
  2. T cell independent (TI) Ags

1. T cell dependent (TD) Ags

- Ags that require T cells to generate an immune response
- Structurally complex e.g. RBCs, S. proteins
- Immunogenic over a wide dose range and do not cause tolerance.
- Produce immunological memory
- Requires processing by APCs.
- Rapidly metabolised

Biological Classes (Mechanisms) of Antigen

2. T cell independent (TI) Ags

- Directly stimulate Ab production by B cells, without the participation of T cells.
- Structurally simple, being composed of a limited number of repeating epitopes. e.g. Pneumococcal capsular polysaccharide, bacterial LPS, flagellar protein
- Immune response is dose dependent.
- Too little - non immunogenic
- Too much – tolerance
- Do not produce immunological memory.
- Do not require processing by APCs.
- Remain in the body for long periods

Based on Source of an Antigen

1. **Microbial antigens/ Infectious:** The microbial antigens could be classified into bacterial, viral and other microbial antigens.

a. ***Bacterial antigens:***

- Bacteria possess different types of antigen mainly based on its morphological features. Some of the common bacterial antigens are **somatic antigen (O)**, **Capsular antigen (K)**, **flagella antigen (H)** and **fimbrial or pili antigen**.
- Exotoxins are highly immunogenic and stimulate the production of antibodies. **The antibodies against exotoxins are called antitoxins.**
- When these exotoxins are precipitated by mild protein denaturing agents such as formaldehyde, the exotoxin loses its pathogenicity but retains its immunogenicity. Such **precipitated toxins are called as toxoids.**

Based on Source of an Antigen

b. **Viral *antigens:***

- The proteins of the outer coat (capsid) of virus are good antigens. These capsid proteins elicit good antibody response when they are present in the circulation.
- Types of virus proteins that are produced inside the cell are called **endogenous antigens**.

c. **Other microbial antigens:**

- The other micro and macro parasites like fungi, protozoa and worms also are composed of different types of antigens composed of major building blocks like lipids, carbohydrates and proteins.
- They also elicit immune response in the body.

Based on Source of an Antigen

2. **Non-microbial Antigens/ Non infectious:** The non-microbial antigens could be classified into cell surface antigens, auto (self) antigens, Non-self antigens and miscellaneous.

a. **Cell surface antigens**:

- The surface of most of the cells is covered with different antigens. When these antigens are given to heterogonous host an immune response is mounted.
- Some of the important cell surface antigens are blood group antigens, and CD receptors of the leukocytes (The abbreviation CD stands for cluster of differentiation. There are more than 130 CD receptors.)

b. Autoantigens:

- Belong to the host itself - not immunogenic.
- Hosts do not react to their own antigens by exhibiting a mechanism called immunological tolerance.
- Such substances are called as **autoantigens** and such immune response is called as **autoimmunity.**
- Some of the normal components of body against which immune response is elicited are basement membrane, myelin, mitochondrial proteins, nuclear proteins, hormones etc.
- Sometimes, the self-antigens are biologically altered (e.g. lens protein, tumor cells) and can become immunogenic.

Based on Source of an Antigen

c. Non-self or foreign antigens

- These are immunogenic
- Three different types based on their phylogenetic distance to host.

i. Xenoantigen – foreign Ag, from different species e.g. bacteria, viruses

ii. Alloantigen – different individual from same species e.g. blood group Ag

iii. Heterophile antigen – Common/ related Ags shared by different species e.g. M protein of *Streptococus* spp. bears common antigen determinant with basement membrane of kidney. They share epitopes with each other.

d. **Miscellaneous antigens:**

- Non-microbial antigens like dust particle, certain type of food particle, pollen grains, venom etc. can also elicit immune response in certain individuals.
- These substances produce antibodies on entering the body.

Example

- Forssmann antigen
- Superantigens

Forssmann antigen

- Forssman antigen was first described by J. Forssman in 1911 as a universal heterophile antigen.
- He immunized rabbits with homogenates of guinea pig organs and detected an antibody that hemolyzed sheep erythrocytes.
- Several groups proposed that Forssman antigen is not protein, but lipid
- It is a lipid carbohydrate complex present in all animals (except rat, rabbits, cattle and pig), plants and bacteria. Hence, anti-Forssmann antibody can be prepared in rabbits.

Diagnostic Applications of Heterophilic Antigen

- **Weil-Felix test:**

  Patients suffering from certain rickettsial diseases will produce antibodies that will react and agglutinate certain non-motile strains of Proteus (OXK, OX2, and OX19).

- **Paul-Bunnel test:**

  Patients suffering from infectious mononucleosis due to Epstein-Barr virus (EBV) will produce antibodies that will react and agglutinate sheep erythrocytes.

- **Streptococcus MG agglutination test:**

  Patients suffering from *Mycoplasma pneumoniae* develop heterophile antibodies to Streptococcus MG, which are titrated in a tube agglutination test.

SUPERANTIGENS

- Superantigens are unconventional antigens which recognise immune receptors outside their usual recognition sites.
- These are unusual microbial toxins that can interact with a large number of different CD4+ T cells (up to 20% of T cells can be activated by a single type of superantigen).
- Most known superantigens are peptides of between 22 and 29kD that are resistant to proteases and heat inactivation and share common structural features.
- Superantigens can bind directly to MHC class II molecules and T-cell receptors on CD4+ T cells to cause T-cell activation without prior processing.

SUPERANTIGENS

- Superantigen binding is predominantly controlled by the shape of the TCR-ß variable region, and superantigens typically bind to all T-cell receptors that derive from a single family of TCR-ß variable region gene segments (e.g., the Vß8).

Example:

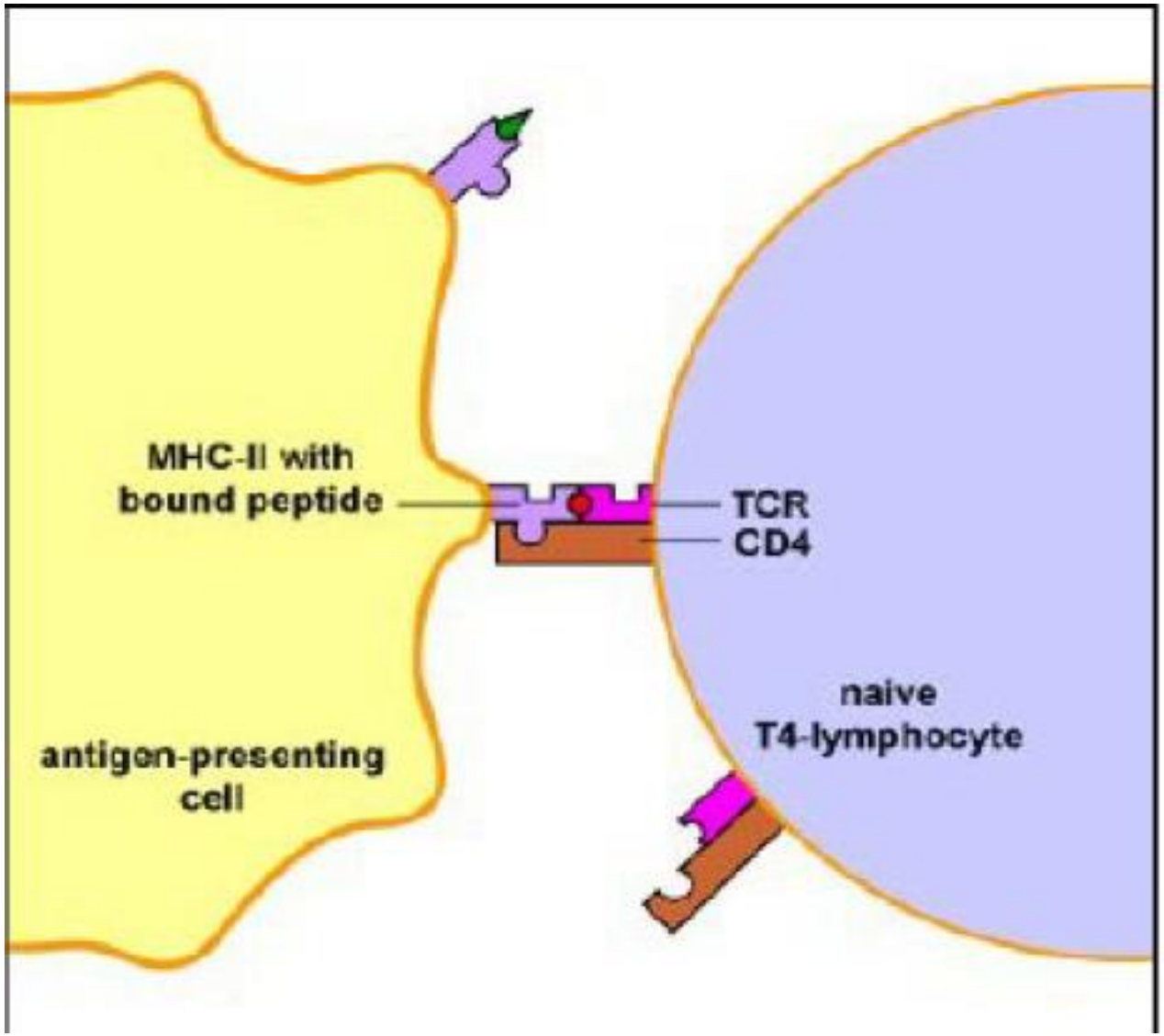
Bacterial superantigen:

- Staphylococcal toxin- Toxic shock syndrome toxin-1(TSST-1); Exfoliative toxin; Enterotoxins
- Streptococcal toxin- Strptococcal pyrogenic exotoxin (SPE)-A and C
- Mycoplasma arthritidis mitogen-I

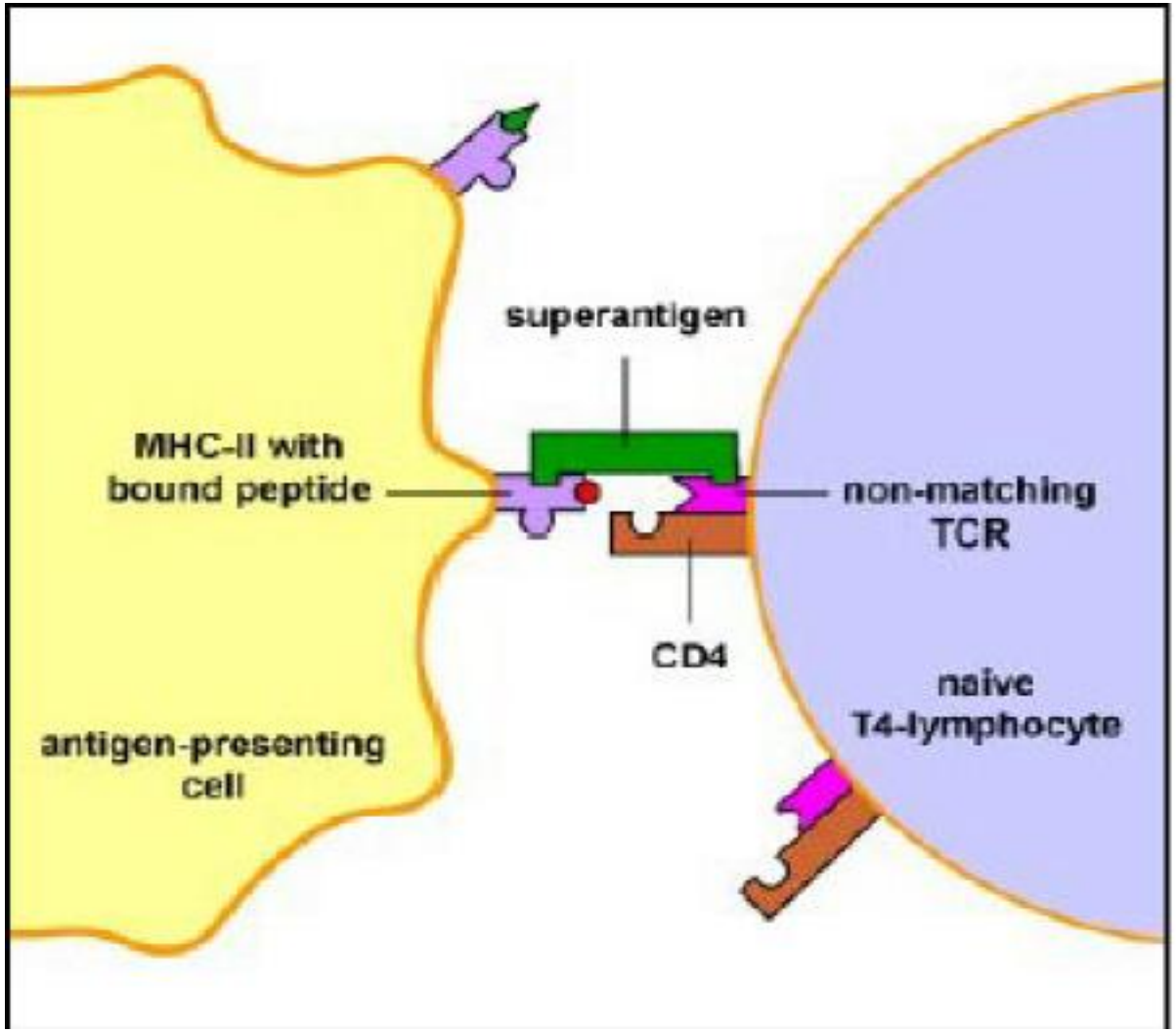
Fungal superantigen: *Malassezia furfur*

Viral superantigen:

- Epstein-Barr virus associated superantigen
- Cytomegalovirus associated superantigen
- Rabies nucleocapsid
- HIV encoded superantigen (nef- negative regulatory factor)



- Conventional antigens are only recognized by specific T4-lymphocytes having a specific TCR with a shape that corresponds to a peptide of that antigen processed and presented by an antigen presenting cell and bound to MHC-II molecules



- Super antigens bind directly to the outside of MHC-II molecules and the TCRs and activate many T4-lymphocytes.

A specific TCR is not required for activation.

Disease associated with superantigens

- Toxic shock syndrome
- Food poisoning
- Scalded skin syndrome
- Rare conditions such as- Atopic dermatitis, Kawasaki syndrome, psoriasis, acute disseminated encephalomyelitis.

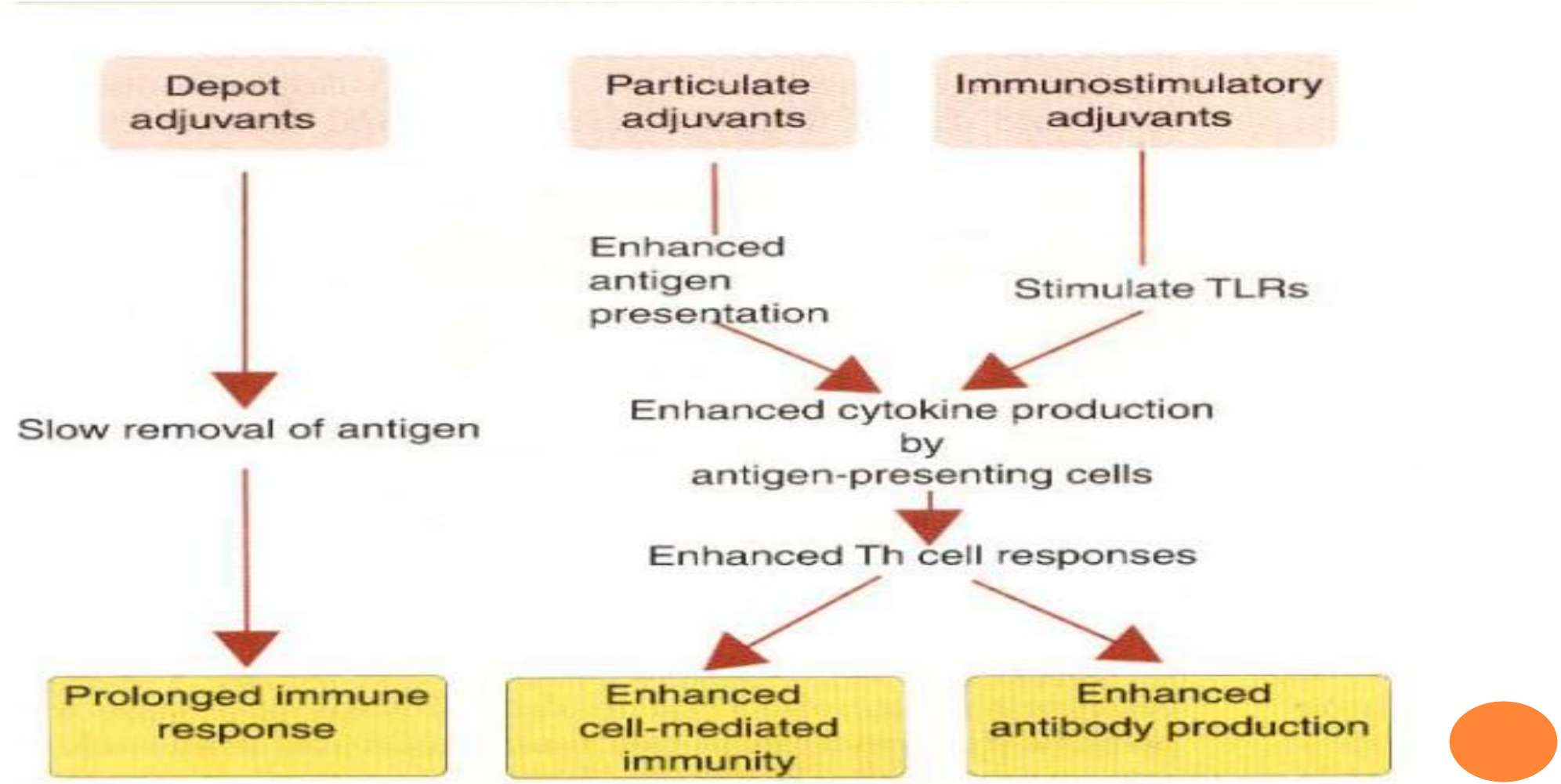
CARRIER MOLECULES (ADJUVANTS)

- Adjuvants (origin from Latin adjuvare, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen.
- Adjuvants are often used to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen are available.
- For example, the antibody response of mice to immunization with BSA can be increased fivefold or more if the BSA is administered with an adjuvant.

- Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects.

1. Antigen persistence is prolonged.
2. Co-stimulatory signals are enhanced.
3. Local inflammation is increased.
4. The nonspecific proliferation of lymphocytes is stimulated.

TYPES OF ADJUVANTS



TYPES OF ADJUVANTS

Type	Adjuvant	Mode of Action
Depot adjuvants	Aluminum phosphate	Slow-release antigen depot
	Aluminum hydroxide	Slow-release antigen depot
	Alum	
	Freund's incomplete adjuvant	
Immunostimulatory adjuvants	Anaerobic corynebacteria	Macrophage stimulator
	BCG	Macrophage stimulator
	Muramyl dipeptide	Macrophage stimulator
	Bordetella pertussis	Lymphocyte stimulator
	Lipopolysaccharide	Macrophage stimulator
	Saponin	Stimulates antigen processing
	Lysolecithin	Stimulates antigen processing
	Pluronic detergents	Stimulates antigen processing
	Acemannan	Macrophage stimulator
	Glucans	Macrophage stimulator
	Dextran sulfate	Macrophage stimulator
Particulate adjuvants	Liposomes	Stimulates antigen processing
	ISCOMS	Stimulates antigen processing
	Microparticles	Stimulates antigen processing
Mixed adjuvants	Freund's complete adjuvant	Water-in-oil emulsion plus *Mycobacterium*

Other examples

- **Aluminum potassium sulfate (alum)** prolongs the persistence of antigen. When an antigen is mixed with alum, the salt precipitates the antigen.
- Injection of this alum precipitate results in a **slower release** of antigen from the injection site, so that the **effective time of exposure** to the antigen **increases** from a few days without adjuvant to several weeks with the adjuvant.
- The alum precipitate **also increases the size of the antigen**, thus increasing the likelihood of phagocytosis.

FREUND'S ADJUVANT

- Water-in-oil adjuvants also prolong the persistence of antigen.
- A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as mannide monooleate, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection.
- This preparation is based on **Freund's complete adjuvant**, the first deliberately formulated highly effective adjuvant, developed by **Jules T. Freund** many years ago and containing heat-killed **Mycobacteria** as an additional ingredient.

- The immune response mediated by T- cytotoxic cells is called ……..
- Write four intrinsic factors of antigen affecting immunogencity
- Define the term active immunity
- Write a brief note on auto- immunity
- a. Define term immunity, b. Classification of immunity, c. briefly describe the mechanism of innate immunity
- ……. laboratory animal is considering as queen for production of hyper immune serum
- Which among the following is most antigenic

1. Exotoxin 2. Endotoxin 3. viruses 4. none

- Heterophile antigen
- Adjuvants using in veterinary vaccines
- The smallest unit of an antigen molecule that is capable of binding with antibody or T- cell receptor is called ……
- Generation of immune response towards self- antigen lead to……
- The antigen associated with bacterial flagella referred as
- 1. K ag. 2. H ag. 3. F ag 4. All
- Father of Immunology ???

- Define Immunogen and Haptens
- The antigenic determinant site of an antigen is called as……..
- Mode of action of adjuvants

Thank You

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