Antigens and Epitopes

IMMUNOGENICITY V/S ANTIGENICITY

Antigen: A substance that can induce a specific immune response is usually called an antigen. The specific immune response will be generated either by B-cells or T-cells or both.

Immunogenicity: It is defined as the ability of a substance to induce a humoral and/or cell mediated immune response.

B-cells **+** antigen ⟶ effector B-cells **+** memory B-cells

↓

E.g. Plasma cells

T-cells **+** antigen ⟶ effector T-cells **+** memory T-cells

↓

E.g. T_H-cells

Therefore, a substance that induces a specific immune response is more appropriately called an immunogen.

Antigenicity: It is the ability to combine specifically with the final products of immune responses i.e. antibodies and/or cell-surface receptors.

• All molecules that have the property of immunogenicity also have the property of antigenicity. But vice versa is not true.

• Some small molecules, called hapten are antigenic but are incapable of inducing a specific immune response by themselves. Thus, they have antigenicity but lack immunogenicity.

Factors that influence immunogenicity: There are many factors that affect immunogenicity. They are grouped in 2 categories.

• **Nature of immunogen:** It includes foreignness, molecular size, chemical composition and heterogeneity, susceptibility to antigen processing and presentation.

• **Biological system:** It includes genotype of animal, immunogen dosage and route of administration, adjuvants.

Both these categories are discussed below.

1. Nature of immunogen:

A. Foreignness: It can be defined as phylogenetic distance between antigen and its host. Thus, greater the phylogenetic distance between host and antigen, stronger is the foreignness of antigen.

When an antigen is introduced into an organism, the degree of its immunogenicity depends on the degree of foreignness. So, we can say that foreignness is directly proportional to the evolutionary distance between antigen/immunogen and its host.

Example:

1. Protein BSA (Bovine serum albumin) is not immunogenic in cow (a bovine animal) but strongly immunogenic in rabbit.

2. BSA shows greater immunogenicity in chicken than in goat, because goat is more closely related to bovines.

Exception: Some macromolecules such as collagen, cytochrome C etc. have been highly conserved during evolution process. Thus, they display very little immunogenicity even at large phylogenetic distances.

B. Molecular size: There is a correlation between size of a macromolecules and its immunogenicity. Most

active immunogens have a size of 1,00,000 Da or more. Generally, substances with molecular mass of less than 5,000 – 10,000 Da are poor immunogens.

C. Chemical composition and heterogeneity: Chemical composition and heterogeneity together contribute to chemical complexity of an antigen and are important in determining its immunogenicity.

Synthetic homopolymers (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their molecule size. It is because their chemical composition is simple and homogeneous.

Co-polymers composed of different amino acids or sugars show higher immunogenicity than homopolymers of their constituents. It is because copolymers are chemically more complex and heterogeneous.

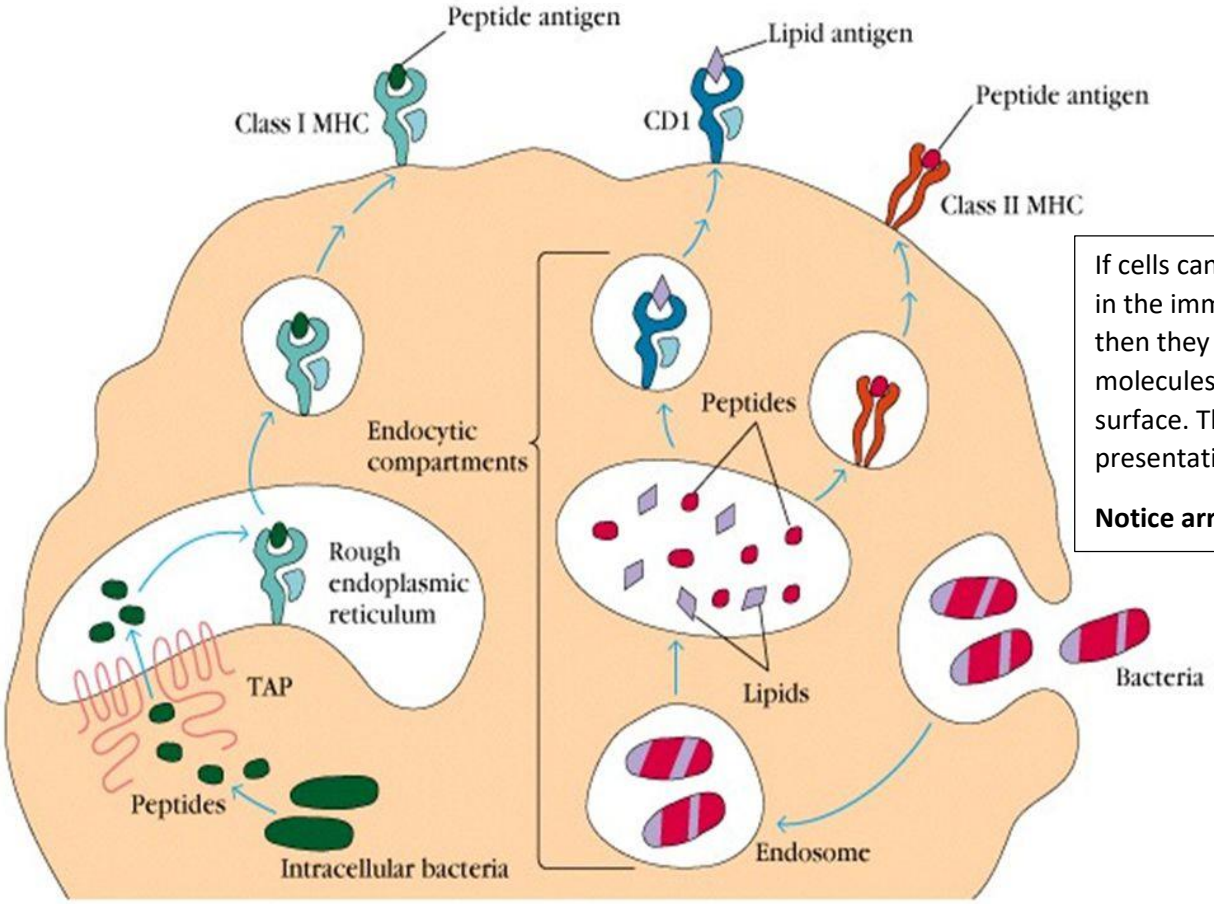
So, we can deduce that chemical complexity contributes to immunogenicity.

Note: Any protein has 4 levels of organization primary, secondary, tertiary and quaternary. All of them contribute to complexity of protein. Thus, if a protein is immunogen/antigen, all and levels will affect its immunogenicity.

D. Susceptibility to antigen processing and presentation: Development of adaptive immune response requires endocytosis of antigen, its digestion in the cell, association of digested antigen to MHC class -I or class-II, its localization on cell surface, interaction of T-cell with MHC bound antigen and development of the adaptive immune response.

Thus, we can say that larger molecules that can readily be phagocytosed and processed are more immunogenic than small molecules whose phagocytosis and processing are less probable.

Also macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens.



If cells can't digest the proteins in the immunogen into peptides, then they can't bind to MHC molecules and move to the cell surface. This blocks the antigen presentation.

Notice arrows.

Figure for Antigen Processing and Presentation

2. The biological system: For factors under this section, detailed discussion is beyond the scope. Only introductory concepts have been outlined.

A. Genotype of recipient: The genetic constitution (genotype) of an animal influences the type and degree of immune response to an antigen. This relates mainly to the MHC genes.

B. Immunogen dosage and route of administration:

Immunogen dosage: The amount of immunogen entering host's body is important. An insufficient dosage may fail to stimulate immune system. Many small/insufficient dosages can induce tolerance to antigen.

Booster dosage: In many vaccination, a single dosage may not activate the immune system strongly. Thus, repeated dosage of vaccines is needed over a period of weeks/months to strongly activate the immune system and develop long term protection.

These repeat dosages are called booster dosages.

Route of administration: There are 5 routes for entry or administration of an antigen. These are
- Intravenous (IV) - into a vein.
- Intradermal (ID) - into the skin.
- Subcutaneous (SC) - beneat the skin.
- Intramuscular (IM) - into a muscle.
- Intraperitoneal (IP) - into the peritoneal cavity.

The administration route strongly influences nature and profile of immune response. However, detailed discussion is out of scope.

C. Adjuvants: Adjuvants (Latin adjuvare - to help) are substances that when mixed with antigen and then injected with antigen, enhance the immunogenicity of the antigen.

Adjuvants are used to boost the immune response when
- the antigen has low immunogenicity.
- only small amounts of an antigen are available.

Example:
1. Freund's complete adjuvant. It is 1st deliberately formulated, highly effective adjuvant.
2. Freund's incomplete adjuvant.
3. Aluminium potassium sulphate (alum).
4. Bacterial lipopolysaccharide.
5. Synthetic polyribonucleotides such as poly IC.

Epitopes: Immune cells do not interact with or recognize an entire immunogen/antigen. These cell recognize and interact with discreet sites on antigen/immunogen.
These sites are called epitopes or antigenic determinants.

Surface of any antigen presents a large number of epitopes (say 100). The subset of epitopes recognized by the immune system is much less (say 50) than total number of epitope (100). The recognized epitopes are called immunogenic.

Of these recognized epitopes (50), some induce (say 10) a very strong immune response than other epitopes (40). These strong response generating epitopes are called immunodominant epitopes.

- Nature of epitopes recognized by B-cells and T-cells is very different. However, their discussion is beyond the scope. But some important points w.r.t. epitopes in general are given below.

• An antibody or TCR binds to an epitope by weak non covalent interaction. These interactions/forces/bonds are van der Waal's bonds, hydrogen bonds, ionic bonds, hydrophobic interaction.

• If an antibody is made against a protein antigen in its native conformation, the antibody will not bind to denatured protein antigen. This is because denaturation will change the epitopes in native protein. Antibody will not find its epitopes in denatured protein and thus, will not bind to it.

However, it will bind perfectly to the protein antigen in its native conformation.

• Surface amino acids in 3-D/native conformation of a proteins is hydrophilic, thus, they make up for epitope in a native protein. Generally, these make up B-cell epitopes.

• Hydrophobic amino acids are buried deep in 3-D/native conformation of a protein and thus, act as epitopes, only when protein has been denatured i.e. its 3-D/native structure destroyed. Generally, these make up T-cell epitopes as the structure of protein is destroyed during antigen processing and presentation.

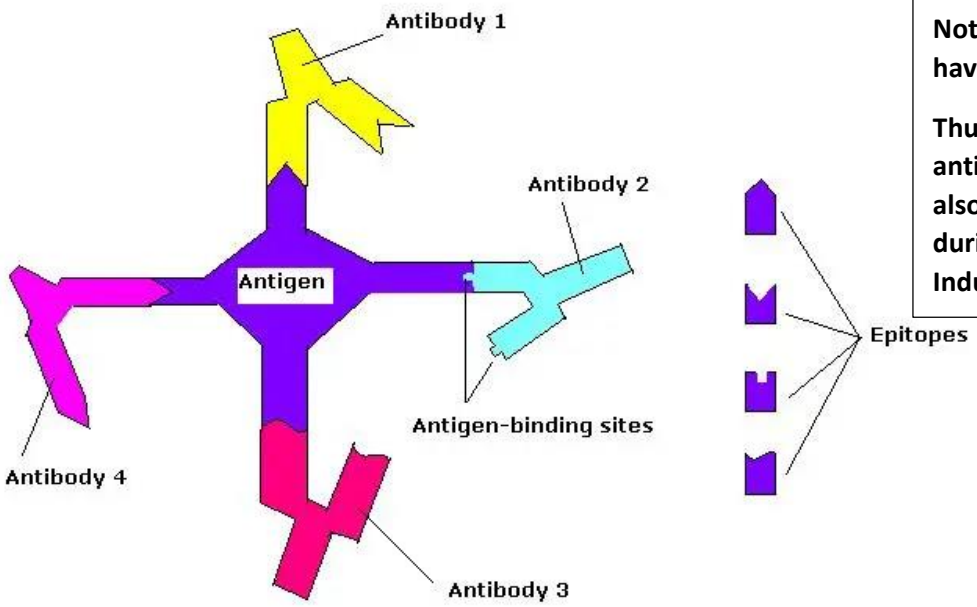
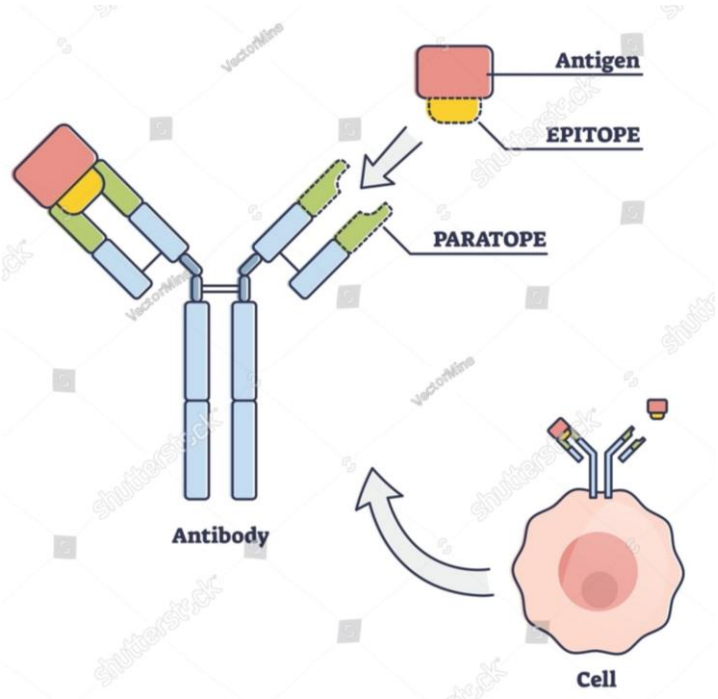


Figure showing different B – cell epitopes.

Notice that for each different epitope we have a different antibody.

Thus, we see that antibody binds to the antigen using complementary fit. Since it also involves changes in conformation during binding, it is called as binding by Induced Fit Hypothesis.



The part of antigen against which there is immune response is called as epitope.

Epitope is also defined as part of the antigen where antibody binds.

The site on an antibody which actually binds the epitope on the antigen is called as paratope.

So, epitope is always on the antigen and paratope is always on the antibody.

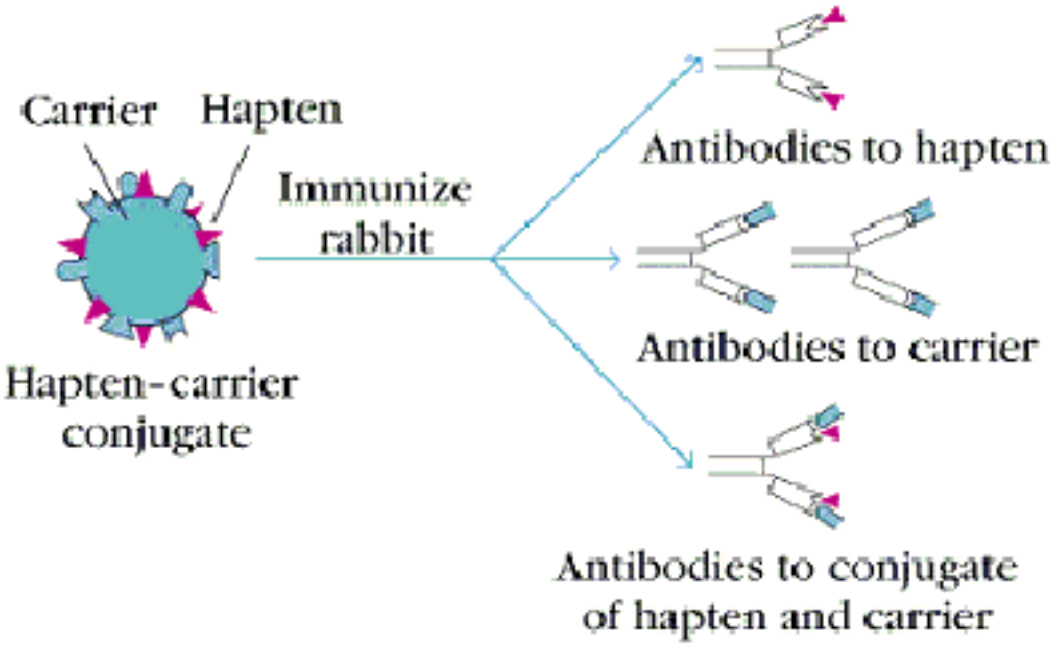
Haptens: Hapten is any small, organic molecule that is antigenic but not immunogenic.

• Hapten, when covalently bound to a large carrier molecule becomes immunogenic. It is called hapten – carrier conjugate and it has many hapten molecules covalently bound to a single, large carrier molecule.

• By conjugation, hapten becomes visible and accessible to immune system due to increase in its molecular size. Thus, it activates the immune system and functions as an immunogen.

- Since it can activate the immune system when it is made visible to the immune system, the haptens do have epitopes but they can't activate the immune system alone due to their small size.

• This system of hapten-carrier conjugate was developed by Karl Landsteiner.



When we use hapten – carrier conjugate for immunization then we get different antibodies as shown in the figure. But major response is always against the haptens.

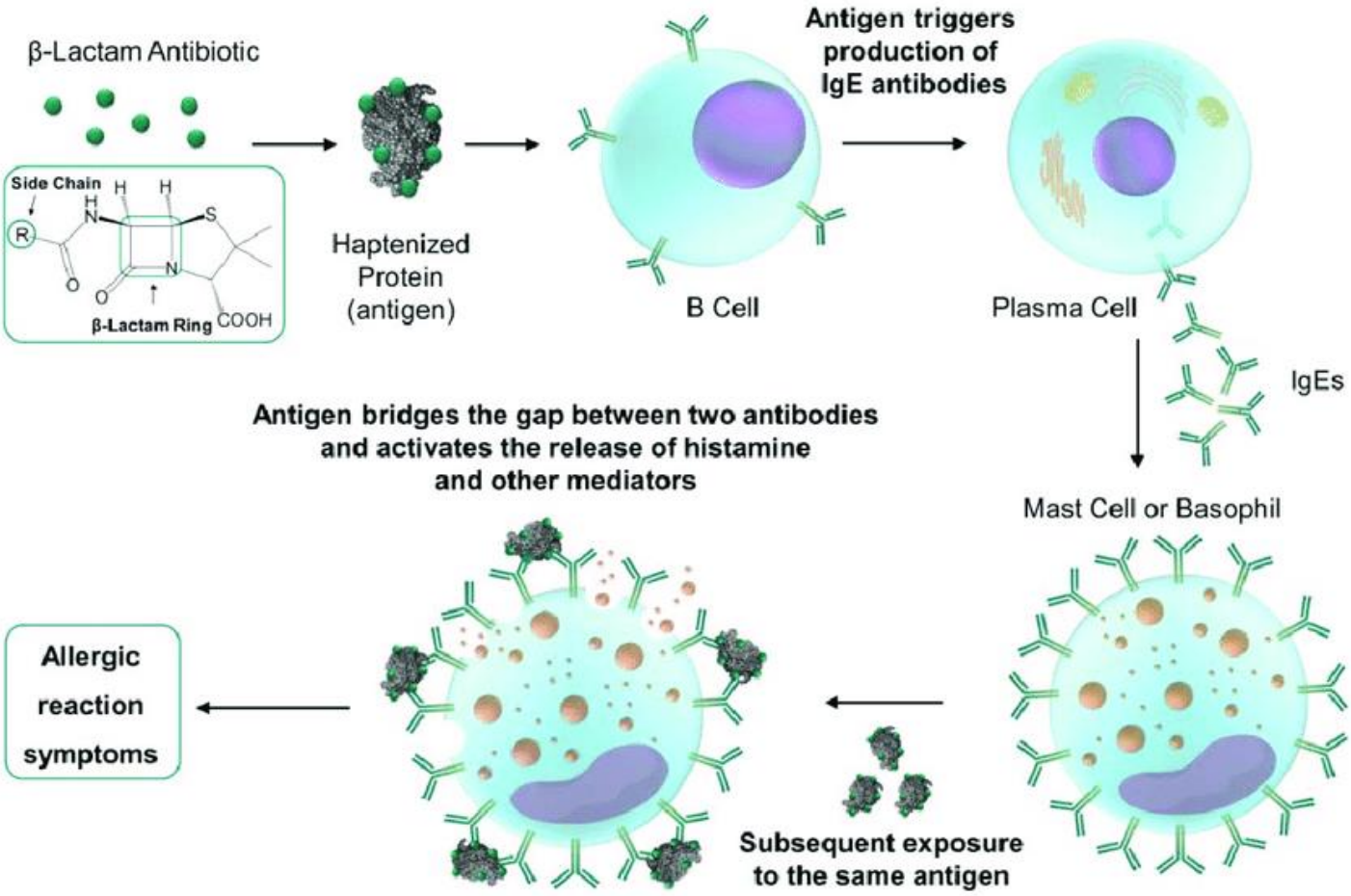
• Many biological molecules can act as hapten. These include drugs, peptide hormones and steroid hormones.

• Formation of hapten-carrier conjugate system is responsible for penicillin and many other drug allergies.
• It develops as under:

1. Penicillin + cellular protein = adduct
2. Adduct is hapten-carrier conjugate and is recognized by immune system as an immunogen.
3. IgE antibody is produced as a response by immune system.
4. IgE binds to its receptors on surface of mast cells and basophils.
5. When penicillin is administered again, it binds to membrane bound IgE antibody.
6. This initiates and leads to allergic reaction.
7. Streptomycin and aspirin also become allergic by same mechanism.

When allergy is developing for the first time then symptoms are minor such as rashes, hives, arthritis like pain and get cured when medicine is discontinued. But if the same drug enters again the reactions are very severe and can proceed to anaphylaxis which if not treated in time could be fatal.

Drugs used in such situation of anaphylaxis are epinephrine, pheniramine and hydrocortisone. They are given via injection.


β-Lactam Antibiotic
Side Chain
β-Lactam Ring
Haptenized Protein (antigen)
B Cell
Antigen triggers production of IgE antibodies
Plasma Cell
IgEs
Mast Cell or Basophil
Subsequent exposure to the same antigen
Antigen bridges the gap between two antibodies and activates the release of histamine and other mediators
Allergic reaction symptoms