ANTIGENS

Antigen may be defined as a substance which when introduced into the tissue of an animal stimulates the production of an Ab with which it reacts specifically or reacts with sensitized cells of the immune system. The sensitization of specific lymphocytes lead to cell mediated immunity. An Ag introduced into the body reacts only with those particular immunocytes (B or T-cells) which carry specific marker for that Ag.

The two attributes of antigenicity are-

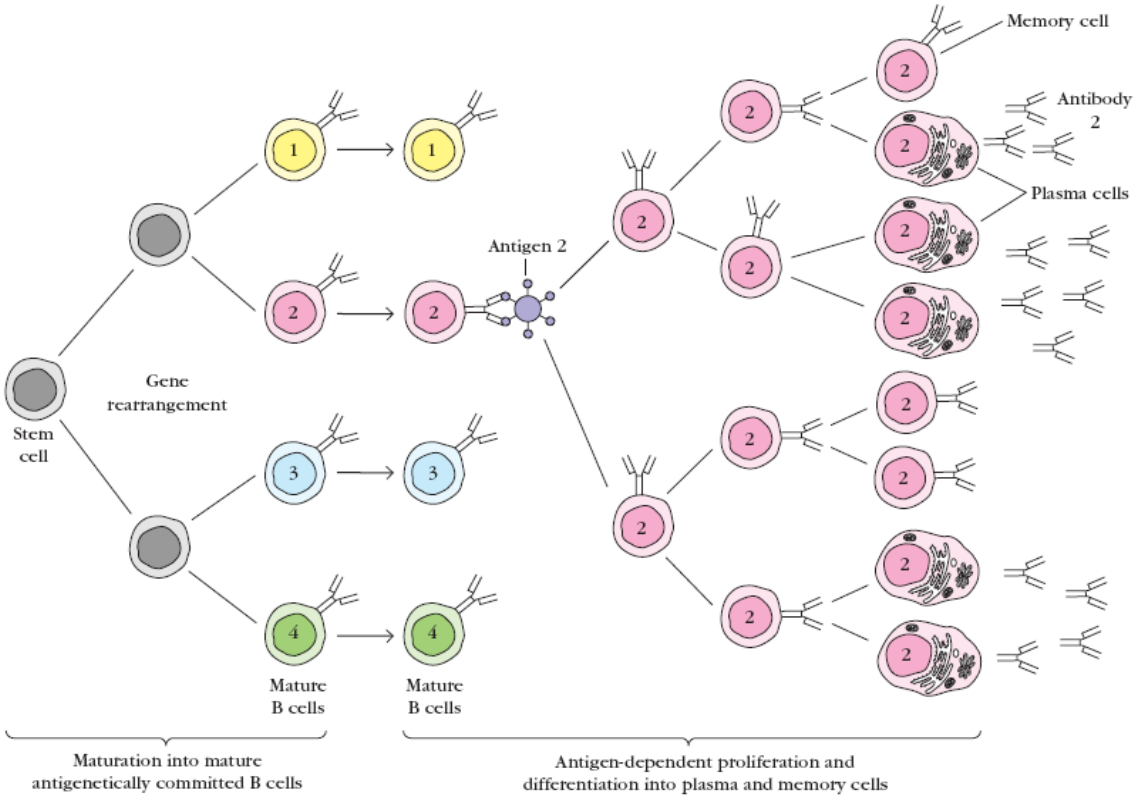
1) Induction of an immune response (**Immunogenicity)**
2) Specific reaction with Ab's or sensitized cells (**Immunological reactivity)**

The most remarkable feature of immune system is its ability to respond to millions of different foreign antigens in a highly specific way. The **clonal selection theory** explains that during development each lymphocyte becomes committed to react to a particular Ab, even before being exposed to it.

The lymphocytes express cell surface receptor proteins which bind to the specific Antigen. This binding leads to proliferation and maturation of specific lymphocytes (antigen specific lymphocytes).

Clonal selection theory: The immune system contains millions of different lymphocyte clones and hundreds of which may be activated by a particular Ag, leading to proliferation of specific lymphocyte clones. B-cells can directly take up the soluble Ag's, where as T cells take up Ag in association with a MHC molecule present on Antigen Presenting cells.

Clonal selection theory:



Classsification of Antigens: Based on the ability to bind to Ag to induce Immune response, they are classified into different types.

1) Complete antigens: These Ag's induces specific Ab's production and also combines with the Ab's so produced.

2) Haptens: The molecules which by themselves cannot induce immune response but can bind to the specific Ab's are called Haptens. These can become immunogenic (Capable of inducing immune response) when coupled with larger molecules called **carriers**.

Epitope: The part of the Ag that can combine with Ag-binding site on an Ab molecule or a lymphocyte receptor are called **Antigenic determinants or epitopes.** The epitope consists of four or five aminoacids or monosaccharide residues, possessing a specific chemical structure, electrical charge and spatial configuration.

The epitopes may be present as a linear segment of a primary sequence of an Ag (**Sequential or linear epitope**s) or it may be formed by aminoacid residues form different sites of a peptide chain folded into a tertiary structure (**Conformational**

epitopes). T-cell recognizes linear epitopes while B-cell recognizes conformational epitopes.

The area of the Ab that combines with Ag is called **Paratope.** An Ag can have many numbers of epitopes and also many types of epitopes. A virus or a bacterium carries many different epitopes. For a highly folded protein molecule only the surface epitopes are functional. Denaturizing or unfolding of the protein leads to exposure of the hidden epitopes.

Valency: The number of epitopes per molecule of an Ag.

Eg: Bovine serum Albumin (BSA)- Mol.wt- 70,000da, has 6 functional epitopes. Therefore its valency is 6. Denaturation of the proteins revealed that it valency is 18. The rest of the 12 epitopes are hidden epitopes.

Presence of different types of epitopes leads to induction of specific Ab's for each epitope. Some epitopes are more immunogenic than others, so that the reaction to that epitope may be dominant during the overall immune response. Such antigenic determinants are said to be **Immunodominant.**

The smallest Ag is called Hapten. Example: Dinitrophenyl (DNP), which when coupled to a carrier induces production of anti-DNP Ab's, which are polyclonal in origin. This means that different clones of Bcells respond to the Ag and leads to formatin of Ab's with different Ag-binding sites which have their own characteristic affinity for the Ag.

When only few clones respond the response is said to be **Oligoclonal**, and if only a single clone is responding it is called **monoclonal.** The response to most Ag's is polyclonal as they contain different types of epitopes.

Biological classes of Ag's: Depending on their ability to induce Ab formation, Ag's are classified into two types, a) **T dependent (TD) antigens**

b) **T-independent(TI) antigens**

Antibody production is the property of B-lymphocytes. Some Ag's can directly stimulate the production of Ab's by the B-cells and these are called T independent antigens (TI Ag's). The others which require the participation of T cells are called T-dependent or TD Ag's.

TI Ag's: These are structurally simple and are composed of few repeating epitopes.

Eg: Pneumococcla capsular polysaccharides, Bacterial lipopolysaccharides and Flagellar protein –flagellin.

They induce formation of IGM and IgG_3 Ab's.

No immunological memory is produced.

They so not require preliminary processing by the macrophages and they remain in the body for long time as they are processed very slowly.

TD Ag's: These are structurally complex.

Exampe: Erythrocytes, Serum proteins, variety of protein –hapten complexes.

They induce formation of all the types of Ab's (GAMED).

They require preliminary processing by macrophages and are rapidly metabolized in the body.

T-cells recognize the Ag presented to it by the Macrophages on their surface MHC molecules. The T-cells then induce the B-cell proliferation and maturation. The plasma cell formed as a result produce antibodies.

Comparison of Antigen recognition by T-cells and B-cells

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complex of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, and MHC molecule
Binding of soluble antigen	Yes	No
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC-like molecules
Epitope properties	Accessible, hydrophilic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules

Major classes of Antigens: The following are the classes of Ag's.

1) **Carbohydrates:** Not all polysaccharides are immunogenic. Normally polysaccharides which form part of a cell surface (glycoproteins) of a bacteria or virus, elicit immune response. Eg: ABO blood group antigens are glycoproteins on the surface of RBC.

2) **Lipids:** These are rarely immunogenic, but in combination with a protein induce immune response. Immune response to glycolipids and sphingolipids are also determined.
3) **Nucleic acids:** These form poor Antigens. DNA in its pure double helical form is non-immunogenic. But sometimes immune responses to nucleic acids have been reported as in case of **Systemic lupus erythematosus.** In this antibodies are produced against single stranded as well as double stranded DNA molecules against the same individuals DNA (autoimmune disease).
4) **Proteins:** The most common immune responses are those of proteins. It was observed that the greater the degree of complexity of a protein, the more vigorous will be the immune response to that protein. In general proteins are multi determinant (have many epitopes) Ag's. Some of the most common Ag's used in Immunological studies are,
a) Bovine gamma globulin(BGG) mol.wt-150,00Da.
b) Bovine Serum Albumin (BSA) mol.wt-69,000Da.
c) Hen Egg white lysozyme (HEL) mol.wt- 15,000Da
d) Keyhole Limpet hemocyanin (KHL)-20,00,000 Da.

Immunogen dosage: Each experimental immunogen exhibits a specific dose –response curve. An insufficient dosage fails to activate enough lymphocytes which lead to immunogenic unresponsiveness or tolerance. The same way a very high dose also fails to induce immune response.
Eg: In mice 0.5mg dose fails to induce immune response, where as 5×10^{-4} mg of the same Ag was shown to induce humoral immune response.
A single dose of an Ag may not be enough
to get a strong immune response,
where as repeated administration
(booster dose), increases the clonal
proliferation of Ag specific Tcells
or B cells resulting in an increase
in lymphocyte population for that Ag.

Routes of administration of Ag:

1. Intravenous (iv): administration into vein
2. Intradermal(id): into the skin
3. Subcutaneous (sc): beneath the skin
4. Intramuscular(im): Into the muscle
5. Intraperitoneal(ip): into the peritoneal cavity

Adjuvants: (in Latin Adjuvare- to help)

These are substances which enhance the immunogenicity of an Ag when mixed with it. These are used to enhance the immune response of an Ag if it has either low immunogenicity or if it is available in small amounts.

Example: If BSA is given with an adjuvant the immune response increases by 5 fold.

The adjuvants exert one of the following effects during the immune response:

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

Examples of adjuvants and their postulated mode of action.

	POSTULATED MODE OF ACTION			
Adjuvant	Prolongs antigen persistence	Enhances co-stimulatory signal	Induces granuloma formation	Stimulates lymphocytes nonspecifically
Freund's incomplete adjuvant	+	+	+	−
Freund's complete adjuvant	+	++	++	−
Aluminum potassium sulfate (alum)	+	?	+	−
Mycobacterium tuberculosis	−	?	+	−
Bordetella pertussis	−	?	−	+
Bacterial lipopolysaccharide (LPS)	−	+	−	+
Synthetic polynucleotides (poly IC/poly AU)	−	?	−	+

When an Ag is given with alum(Aluminum Potassium Sulfate), the salt first precipitates the Ag. Injection of this alum precipitate when given, causes slow release of the precipitated Ag. So the effective time of exposure of the Ag increases from few days to several weeks. Alum also increases the size of Ag, thus promoting phagocytosis.

Freunds Incomplete Adjuvant: It contains Ag in aqueous solution, mineral oil and an emulsifying agent such as Mannide monooleate. This causes dispersion of the oil into small droplets which surround Ag and this causes slow release of Ag.

Freunds complete adjuvant: It is the firstly formulated highly effective adjuvant developed by Jules Freund. It contains heat killed Mycobacteria as an additional ingredient. Muramyl peptide, a component of the bacterial cell wall, activates macrophages making it a far more potent form than the previous one. Macrophages express high levels of Class II MHC molecules which present the Ag to T_H.

Alum and Freunds Adjuvants stimulate infiltration of macrophages at the site of adjuvant injection. A dense mass of macrophages called **Granuloma** which in turn enhances the activity of T_H cells.

Factors that effect induction of Immune Response

1) Size: Antigenicity is related to Molecular weight. Large molecules like Haemocyanin (Mol.Wt. 6.75 x 10^6Da) is highly antigenic when compared to a Insulin having a mol wt of 5000Da. If mol.wt is less than 5000Da the molecules are less antigenic or non-antigenic.

Insulin can be made antigenic by adsorbing it on to large inert particle such as Bentonite or Koalin. Chloride which have low mol.wt can be made antigenic by applying on skin. These bind to tissue proteins and become immunogenic.

Hapten can induce immune response when combined with carriers like Gelatin. The resulting anti-hapten antibodies cannot bind to free carrier but bind to a hapten-carrier complex.

Eg of Hapten- DNP(Dinitro phenol), TNP(Trinitro Phenol), TABA(Tryosine Azobenzinoic acid).

2) Chemical Nature: Proteins are highly antigenic when compared to lipids and polysaccharides. Their structural diversity also effects antigenicity. A protein having 20 different amino acids is more antigenic than a polysaccharides having just 4 or 5 monosaccharide. Not all proteins are antigenic, structural stability of the molecule also effects antigenicity.

Eg: gelatin is non-immunogenic because of its unstable structure.

3) Susceptibility to tissue enzymes: The Ag when enters into the body is fragmented by the intracellular enzymes of phagocytic cells. The Ag is broken down into immunogenic fragments which induce immune response when presented to T cells.

Substances which are very rapidly broken down by the tissue enzymes as well as some substances which are not at all susceptible to tissue enzymes (Like the polystyrene, latex) both are not antigenic. Some polypeptides having D-aminoacids instead of L-aminoacids are also not antigenic.

4) Foreignness: The antigens which are non-self (foreign) induce immune response. Immune system does not normally react with its own antigens. This was termed by Ehrlich as the concept of " Horror autotoxicus", which is fear of self poisoning. The tolerance for self Ag's is broken down. It leads to autoimmunization resulting in autoimmune diseases.

The degree of foreignness also influences antigenicity. Ag's from individuals of the same species are less antigenic than those from individuals of other species.

5) Antigenic Specificity: The basis of antigenic specificity is the spatial arrangement of the molecule (Stereochemical). This was first demonstrated by Obermayer and Pick and was confirmed by Landsteiner.

The importance of the position of the antigenic determinant group in the Ag molecule was evidenced by the differences in specificity in compounds with the group attached at the ortho-, meta- or parapositions. The influence of spatial configuration of the determinant group was shown by the differences in antigenic specificity of the dextro, levo and meso isomers of substances such as tartaric acid.

When Ag's exhibit stereochemical similarities, they lead to cross reactivity. This is due to sharing of similar antigenic determinants (epitopes) within the two Ag's.

The specificity of natural tissue Ag's of animals maybe studied under the following categories.

a) Species specificity: Tissues of all individual species contain species specific Ag's. They exhibit some degree of cross reaction.

Eg: An individual sensitized with horse serum can react with serum from horses or animals of the same species, but will not exhibit cross reactivity to BSA.

b) Isospecificity: A species may be grouped depending on the presence of different isoantigens in its members.

Eg: Human blood groups are genetically determined. They are clinically important in blood transfusions and isoimmunisation during pregnancy. Parental disputes were ones settled by blood groups, but now by DNA finger printing.

Histocompatibility Ag's are the cellular Ag's specific to each individual of a species. They are recognized when attempts are made to transfer or transplant cellular material from one individual to another.

c) Autospecificity: Self Ag's are usually not antigenic. But some proteins like the lens proteins are normally not found in the circulation and this is identified as self –Ag. Also some Ag's that are absent during embryonic life and attained at adult stages are also not identified as self Ag's.

d) Organ specificity: Some organs such as brain, kidney and lens protein of different species, share the same Ag. Such Ag's are called organ specific Ag's. Eg: During antirabic vaccination, the vaccine is developed using sheep brain. The sheep brain Ag's present the vaccine induce immunological reactions damaging the human nervous system.

6) Heterogenetic (heterophile) specificity: Some times closely related Ag's may exist in different biological species, classes and kingdoms. These are named heterophile Ag's.

Eg: Frossman Ag- it is a lipid carbohydrate complex(Hapten), distributed in many animals, plants, birds and even in bacteria. It is absent in rabbits and hence anti-frossman Ab's can be raised in rabbits. In us it is located in the gastrointestinal tract.

Important Questions:

1) Define the following terms.(each definition carries two marks)

a) Antigen

b) Hapten

c) Epitope or antigenic determinant

d) Adjuvant

e) Conformational epitopes

2) Write a brief note on T-dependent and T-independent Antigens. -4

3) Explain the role of adjuvant in inducing immune response with examples. -7

4) What are the important features of an Antigen. -3