DR. MALABIKA BHATTACHARJEE,
PG DEPT. OF ZOOLOGY, VIVEKANANDA COLLEGE THAKURPUKUR

ANTIGENS AND IT'S PROPERTIES

1. ANITGENICITY AND IMMUNOGENICTY
2. HAPTENS AND STUDY OF ANTIGENICTY
3. THE NATURE OF THE IMMUNOGEN CONTRIBUTES TO IMMUNOGENICITY
4. CHEMICAL BONDS IN ANTIGEN-ANTIBODY REACTION
5. AFFINITY & K_d
6. AVIDITY
7. ANTIBODIES RECOGNIZE THE CONFORMATION OF ANTIGENIC DETERMINANTS
8. PROPERTIES OF B-CELL EPITOPES
9. PROPERTIES OF T-CELL EPITOPES
10. SUPERANTIGENS

1. ANTIGENICITY AND IMMUNOGENICITY

1. Substances that can be recognized by the immunoglobulin receptor of B cells, or by the T cell receptor when complexed with MHC, are called **antigens**.
2. Immunogenicity and antigenicity are related but distinct immunologic properties that sometimes are confused.

B cells + antigen → effector B cells + memory B cells
↓
(plasma cells)

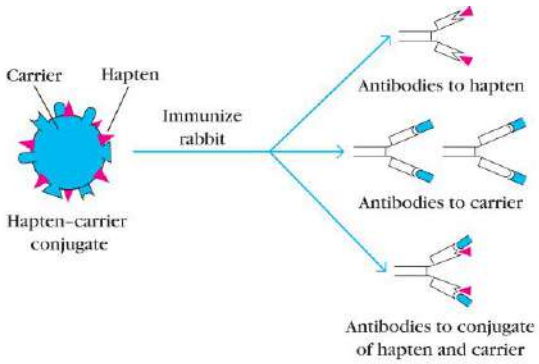
T cells + antigen → effector T cells + memory T cells
↓
(e.g., CTLs, T_Hs)

3. **Immunogenicity** is the ability to induce a humoral and/or cell mediated immune response: A substance that induces a specific immune response is more appropriately called an **immunogen.**
4. **Antigenicity** is the ability to combine specifically with the final products of the above responses (i.e., antibodies and/or cell-surface receptors).
5. Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called *haptens,* are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity.

2. HAPTENS AND THE STUDY OF ANTIGENICITY

a. The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope, on a complex protein antigen.

b. Landsteiner employed various **haptens,** small organic molecules that are antigenic but not immunogenic. Chemical coupling of a hapten to a large protein, called a **carrier,** yields an immunogenic **hapten-carrier conjugate.**



Injection with:	Antibodies formed:
Hapten (DNP)	None
Protein carrier (BSA)	Anti-BSA
Hapten–carrier conjugate (DNP-BSA)	Anti-DNP (major) Anti-BSA (minor) Anti-DNP/BSA (minor)

FIGURE 3-10 A hapten-carrier conjugate contains multiple copies of the hapten—a small nonimmunogenic organic compound such as dinitrophenol (DNP)—chemically linked to a large protein carrier such as bovine serum albumin (BSA). Immunization with DNP alone elicits no anti-DNP antibodies, but immunization with DNP-BSA elicits three types of antibodies. Of these, anti-DNP antibody is predominant, indicating that in this case the hapten is the immunodominant epitope in a hapten-carrier conjugate, as it often is in such conjugates.

c. Animals immunized with such a conjugate produce antibodies specific for
   (1) the hapten determinant,
   (2) unaltered epitopes on the carrier protein, and
   (3) new epitopes formed by combined parts of both the hapten and carrier.
d. By itself, a hapten cannot function as an ***immunogenic epitope***. But when multiple molecules of a single hapten are coupled to a carrier protein (or ***nonimmunogenic homopolymer***), the hapten becomes accessible to the immune system and can function as an immunogen.
e. The beauty of the hapten-carrier system is that it provides immunologists with a chemically defined determinant that can be subtly modified by chemical means to determine the effect of various chemical structures on immune specificity.
f. Many biologically important substances, including drugs, peptide hormones, and steroid hormones, can function as haptens. Conjugates of these haptens with large protein carriers can be used to produce hapten-specific antibodies.

3. THE NATURE OF THE IMMUNOGEN CONTRIBUTES TO IMMUNOGENICITY

Immunogenicity is determined, in part, by four properties of the immunogen: its foreignness, molecular size, chemical composition and complexity, and ability to be processed and presented with an MHC molecule on the surface of an antigen- presenting cell or altered self-cell.

A. FOREIGNNESS

1. In order to elicit an immune response, a molecule must be recognized as **nonself** by the biological system. When an antigen is introduced into an organism, the **degree of its immunogenicity depends on the degree of its foreignness.**
2. Generally, the greater the phylogenetic distance between two species, the greater the structural (and therefore the antigenic) disparity between them.
3. For example, the common experimental antigen bovine serum albumin (BSA) is not immunogenic when injected into a cow but is strongly immunogenic when injected into a rabbit.
4. There are some exceptions to this rule.
   a. Some macromolecules (e.g., **collagen and cytochrome c**) have been highly conserved throughout evolution and therefore display very ***little immunogenicity across*** diverse species lines.
   b. Conversely, some self-components (e.g., **corneal tissue and sperm**) are effectively sequestered from the immune system, so that if these tissues are injected even into the animal ***from which they originated, they will function as immunogens.***

B. MOLECULAR SIZE

1. The most active immunogens tend to have a molecular mass of ***100,000 daltons (Da).***
2. Generally, substances with a molecular mass less than ***5000–10,000 Da*** are poor immunogens, although a few substances with a molecular mass less than ***1000 Da*** have proven to be immunogenic.

C. CHEMICAL COMPOSITION AND HETEROGENEITY

1. Size and foreignness are not, by themselves, sufficient to make a molecule immunogenic; other properties are needed as well. For example, ***synthetic homopolymers (polymers composed of a single amino acid or sugar) tend to lack immunogenicity regardless of their size***.
2. Studies have shown that copolymers ***composed of different amino acids or sugars are usually more immunogenic*** than homopolymers of their constituents.
3. These studies show that ***chemical complexity contributes to immunogenicity.*** In this regard it is notable that all four levels of protein organization—primary, secondary, tertiary, and quaternary—contribute to the structural complexity of a protein and hence affect its immunogenicity

D. LIPIDS AS ANTIGENS

1. Appropriately presented lipoidal antigens can induce B- and T-cell responses. For the stimulation of B-cell responses, lipids are used as haptens and attached to suitable carrier molecules such as the proteins keyhole **limpet hemocyanin (KLH) or bovine serum albumin (BSA)**. Using this approach, antibodies have been raised against a wide variety of lipid molecules including steroids, complex fatty-acid derivatives,and fat-soluble vitamins such as vitamin E.
2. T cells recognize peptides derived from protein antigens when they are presented as peptide-MHC complexes. **Lipoidal** compounds such as **glycolipids** and some **phospholipids** can be recognized by T-cell receptors when presented as complexes with molecules that are very much like MHC molecules. These lipid-presenting molecules are members of the CD1 family and are close structural relatives of class I MHC molecules.
3. Recognition of lipids is a part of the immune response to some pathogens, and T cells that recognize lipids arising from ***Mycobacterium tuberculosis*** and ***Mycobacterium leprae***, which respectively cause tuberculosis and leprosy, have been isolated from humans infected by these mycobacteria.

E. SUSCEPTIBILITY TO ANTIGEN PROCESSING AND PRESENTATION

1. ***Large, insoluble macromolecules generally are more immunogenic*** than small, soluble ones because the larger molecules are more readily phagocytosed and processed. Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens.
2. This can be illustrated with ***polymers of D-amino acids***, which are ***stereoisomers of the naturally occurring L-amino acids***. Because the ***degradative enzymes within antigen-presenting cells can degrade only proteins containing L-amino acids***, polymers of D-amino acids cannot be processed and thus are poor immunogens.

F. THE BIOLOGICAL SYSTEM CONTRIBUTES TO IMMUNOGENICITY

These properties include the ***genotype of the recipient***, ***the dose and route of antigen administration***, and the administration of substances, ***called adjuvants***, that increase immune responses.

F.1. GENOTYPE OF THE RECIPIENT ANIMAL

1. The genetic constitution **(genotype)** of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response.
2. Numerous experiments with simple defined immunogens have demonstrated ***genetic control of immune responsiveness, largely confined to genes within the MHC.***
3. ***MHC gene products***, which function to present processed antigen to T cells, ***play a central role in determining the degree to which an animal responds to an immunogen.***
4. The response of an animal to an antigen is ***also influenced by the genes that encode B-cell and T-cell receptors and by genes that encode various proteins involved in immune regulatory mechanisms***. Genetic variability in all of these genes affects the immunogenicity of a given macromolecule in different animals. These genetic contributions to immunogenicity

F.2. IMMUNOGEN DOSAGE AND ROUTE OF ADMINISTRATION

1. Each experimental ***immunogen exhibits a particular dose-response curve***, which is determined by measuring the immune response to different doses and different administration routes. An antibody response is measured by determining the level of antibody present in the serum of immunized animals.

2. A single dose of most experimental immunogens will not induce a strong response; rather, repeated administration over a period of weeks is usually required.Such repeated administrations, or **boosters,** increase ***the clonal proliferation of antigen-specific T cells or B cells and thus increase the lymphocyte populations specific for the immunogen***.
3. Experimental immunogens are generally administered parenterally (*para,* around; *enteric,* gut)—that is, by routes other than the digestive tract. The following administration routes are common.
    a. _ Intravenous (iv): into a vein
    b. _ Intradermal (id): into the skin
    c. _ Subcutaneous (sc): beneath the skin
    d. _ Intramuscular (im): into a muscle
    e. _ Intraperitoneal (ip): into the peritoneal cavity
4. The administration route strongly influences which immune organs and cell populations will be involved in the response. ***Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes***. Differences in the lymphoid cells that populate these organs may be reflected in the subsequent immune response.

G. ADJUVANTS

1. **Adjuvants** (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.
2. Precisely how adjuvants augment the immune response is not entirely known, but they appear to exert one or more of the following effects:
    a. _ Antigen persistence is prolonged.
    b. _ Co-stimulatory signals are enhanced.
    c. _ Local inflammation is increased.
    d. _ The nonspecific proliferation of lymphocytes is stimulated.

Example.1

a. ***Aluminum potassium sulfate (alum)*** prolongs the persistence of antigen.
b. When an antigen is mixed with alum, the salt precipitates the antigen.
c. 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.

Example.2

a. 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.

Example.3

b. **Freund's complete adjuvant,** the first deliberately formulated highly effective adjuvant, developed by ***Jules Freund*** many years ago and containing ***heat-killed Mycobacteria as an additional ingredient***.
c. ***Muramyl dipeptide***, a component of the mycobacterial cell wall, ***activates macrophages,*** making Freund's complete adjuvant far more potent than the incomplete form.
d. ***Activated macrophages*** are more phagocytic expressing higher levels of class II MHC molecules and the membrane molecules of the B7 family. The increased expression of ***class II MHC increases the ability of the antigen-presenting cell to present antigen to T_H cells***.
e. B7 molecules on the antigen presenting cell bind to CD28, a cell-surface protein on T_H cells, triggering co-stimulation, an enhancement of the T cell immune response. Thus***, antigen presentation and the requisite co-stimulatory signal usually are increased in the presence of adjuvant.***

NOTE:

a. ***Alum and Freund's adjuvants*** also stimulate a local, chronic inflammatory response that attracts both phagocytes and lymphocytes. This infiltration of cells at the site of the adjuvant injection often results in formation of a ***dense, macrophage-rich mass of cells called a granuloma.*** Because the macrophages in a granuloma are activated, this mechanism also enhances the activation of T_H cells.

b. Other adjuvants (e.g., ***synthetic polyribonucleotides*** and ***bacterial lipopolysaccharides***) stimulate the nonspecific proliferation of lymphocytes and thus increase the likelihood of antigen-induced clonal selection of lymphocytes.

Based on the above discussion, it may be said that the use of adjuvants is beneficial in improving antigen delivery to antigen-presenting cells as well as processing and presentation by antigen-presenting cells.

The mechanism by which adjuvants exert their biological eff ect appears to be as follows:

a. Antigen mixed with oil/water emulsion is slowly released, prolonging the time of exposure to the immunogen from days to a few weeks.
b. Adjuvants that have gel (for example, alum) or emulsion (for example, Freund's) associate with the antigen and facilitate the transport of the antigen to the draining lymph node where the immune response occurs.
c. Adjuvants (such as alum) bind antigens and increase antigen size, thereby increasing the chances of phagocytosis.
d. Adjuvants may increase the non-specific proliferation of committed lymphocytes thereby increasing the chances of antigen-specific clonal proliferation.
e. Adjuvants increase the efficiency of macrophage-processing of antigen by inducing a local inflammatory response, which attracts macrophages. This is especially important in Freund's adjuvant.

Adjuvant Type	Composition	Mode of Action
Alum	Aluminium phosphate gel Aluminium hydroxide gel	Slow release of antigen; increased uptake of antigen by APC
Freund's complete adjuvant	Oil-in-water emulsion with killed mycobateria	Slow release of antigen; stimulates macrophages; increased uptake of antigen by APC
Freund's incomplete adjuvant	Oil-in-water emulsion	Slow release of antigen
Alum		
+BCG	Aluminium phosphate + BCG	Slow release of antigen; stimulates APC
+Muramyl dipeptide	Aluminium phosphate + muramyl dipeptide	Slow release of antigen; stimulates APC
+Lipopolysaccharide	Aluminium phosphate + bacterial lipopolysaccharide	Slow release of antigen; stimulates APC
+Diphtheria and tetanus toxoid	Aluminium phosphate/ hydroxide + toxoid	Slow release of antigen; stimulates APC
Glucans/Dextrans	Glucans and dextrans	Non-specific stimulator of APC

4. CHEMICAL BONDS IN ANTIGEN-ANTIBODY REACTION

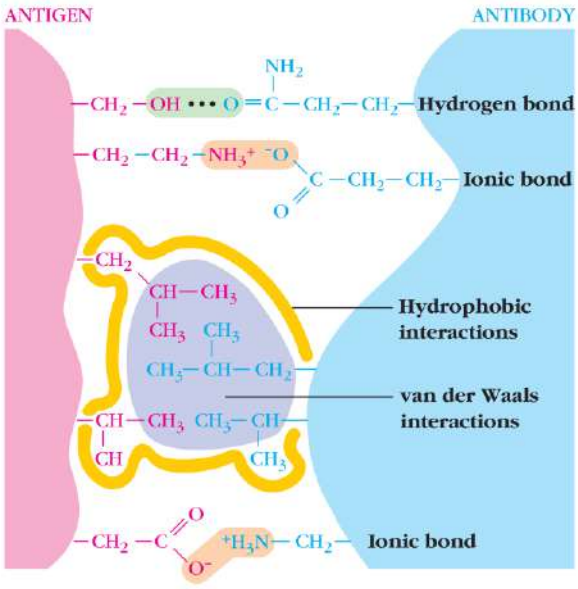


FIGURE 6-1 The interaction between an antibody and an antigen depends on four types of noncovalent forces: (1) hydrogen bonds, in which a hydrogen atom is shared between two electronegative atoms; (2) ionic bonds between oppositely charged residues; (3) hydrophobic interactions, in which water forces hydrophobic groups together; and (4) van der Waals interactions between the outer electron clouds of two or more atoms. In an aqueous environment, noncovalent interactions are extremely weak and depend upon close complementarity of the shapes of antibody and antigen.

5. AFFINITY & K_d

1. The combined strength of the noncovalent interactions between a *single* antigen-binding site on an antibody and a *single* epitope is the **affinity** of the antibody for that epitope.
2. Low-affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer. The association between a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation

$$\mathrm{Ag} + \mathrm{Ab} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{Ag\text{-}Ab}$$

where k_1 is the forward (association) rate constant and k_{-1} is the reverse (dissociation) rate constant. The ratio k_1/k_{-1} is the association constant K_a (i.e., $k_1/k_{-1} = K_a$), a measure of affinity. Because K_a is the equilibrium constant for the above reaction, it can be calculated from the ratio of the molar concentration of bound Ag-Ab complex to the molar concentrations of unbound antigen and antibody at equilibrium as follows:

$$K_a = \frac{[\mathrm{Ag\text{-}Ab}]}{[\mathrm{Ab}][\mathrm{Ag}]}$$

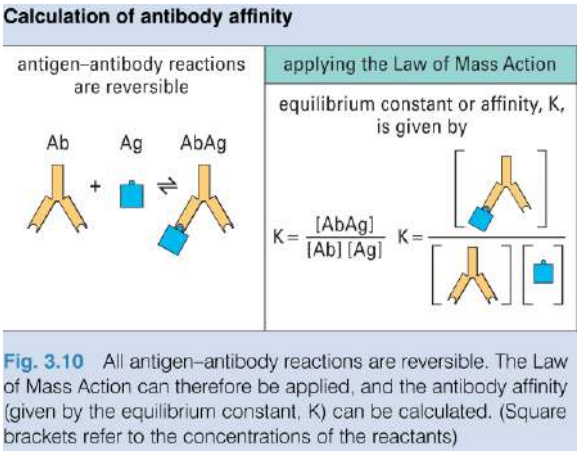


Fig. 3.10 All antigen–antibody reactions are reversible. The Law of Mass Action can therefore be applied, and the antibody affinity (given by the equilibrium constant, K) can be calculated. (Square brackets refer to the concentrations of the reactants)

3. The value of K_a varies for different Ag-Ab complexes and depends upon both k_1, which is expressed in units of ***liters/mole/second (L/mol/s)***, and k_{-1}, which is expressed in units of 1/second. For small haptens, the forward rate constant can be extremely high; in some cases, k_1 can be as high as 4×10^8 L/mol/s, approaching

the theoretical upper limit of diffusion-limited reactions (10^9 L/mol/s). For larger protein antigens, however, k_1 is smaller, with values in the range of 10^5 L/mol/s.

4. For some purposes, the dissociation of the antigen-antibody complex is of interest:

$$\text{Ag-Ab} \rightleftharpoons \text{Ab} + \text{Ag}$$

5. The equilibrium constant for that reaction is K_d, the reciprocal of K_a

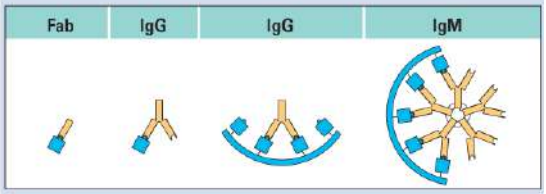
$$K_d = [\text{Ab}][\text{Ag}]/[\text{Ab-Ag}] = 1/K_a$$

and is a quantitative indicator of the stability of an Ag-Ab complex; very stable complexes have very low values of K_d, and less stable ones have higher values.

6. AVIDITY

1. When complex antigens containing multiple, repeating antigenic determinants are mixed with antibodies containing multiple binding sites, the interaction of an antibody molecule with an antigen molecule at one site will increase the probability of reaction between those two molecules at a second site. The strength of such multiple interactions between a multivalent antibody and antigen is called the **avidity.**
2. The avidity of an antibody is a better measure of its binding capacity within biological systems (e.g., the reaction of an antibody with antigenic determinants on a virus or bacterial cell) than the affinity of its individual binding sites.
3. High avidity can compensate for low affinity. For example, secreted pentameric IgM often has a lower affinity than IgG, but the high avidity of IgM, resulting from its higher valence, enables it to bind antigen effectively.

Affinity and avidity



antibody	Fab	IgG	IgG	IgM
effective antibody valence	1	1	2	up to 10
antigen valence	1	1	n	n
equilibrium constant (L/mol)	10^4	10^4	10^7	10^{11}
advantage of multivalence	–	–	10^3-fold	10^7-fold
definition of binding	affinity	affinity	avidity	avidity
	intrinsic affinity		functional affinity	

Fig. 3.11 Multivalent binding between antibody and antigen (avidity or functional affinity) results in a considerable increase in stability as measured by the equilibrium constant, compared with simple monovalent binding (affinity or intrinsic affinity, here arbitrarily assigned a value of 10^4 L/mol). This is sometimes referred to as the 'bonus effect' of multivalency. Thus there may be a 10^3-fold increase in the binding energy of IgG when both valencies (combining sites) are used and a 10^7-fold increase when IgM binds antigen in a multivalent manner.

7. ANTIBODIES RECOGNIZE THE CONFORMATION OF ANTIGENIC DETERMINANTS

1. Analysis of antibodies to protein antigens reveals that specificity may be for epitopes:
   a. consisting of a ***single contiguous stretch of amino acids (a continuous epitope);***
   b. dependent on the ***native conformation of the antigen and formed from two or more stretches of sequence separated in the primary structure (discontinuous or conformational epitopes).***
2. Continuous epitopes are unique three dimensional structures whilst discontinuous epitopes may be formed of a flexible peptide that assumes a unique conformation when bound to a paratope. i.e., the paratope may influence the conformation of the epitope by an induced fit mechanism.

8. PROPERTIES OF B-CELL EPITOPES

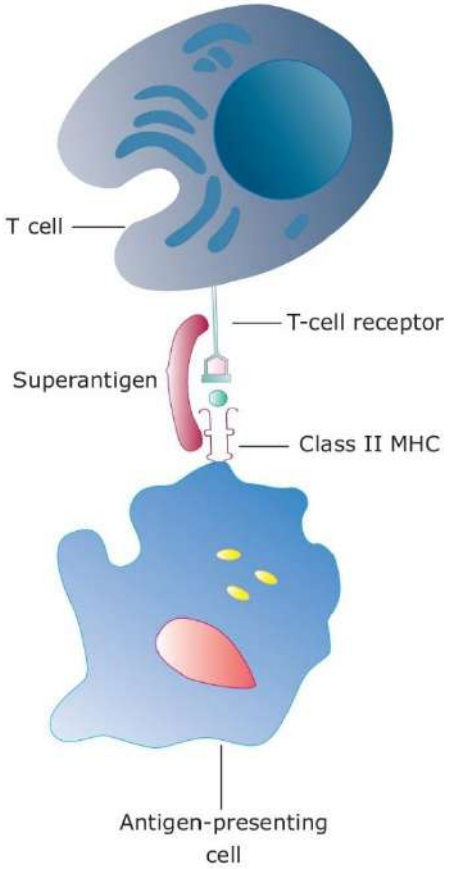
1. The properties of B-cell epitopes are as under:
   a. generally composed of hydrophilic amino acids;
   b. located on the surface of the native protein so that it is topographically accessible to the antibody or antibody-like B-cell receptor;
   c. can contain linear or conformational epitopes;
   d. tend to be located on the flexible region of the immunogen, thus maximizing its easy binding with the antibody that might not otherwise react with it, if it was rigid; and
   e. may contain overlapping and non-overlapping determinants.

2. Bovine serum antigen (BSA) has 25 overlapping antigens on its surface. Some determinants induce a more pronounced immune response than other epitopes in a given animal. Such epitopes are called immunodominant. These are usually those epitopes that project distally from the central mass of the immunogen.

9. PROPERTIES OF T-CELL EPITOPES

1. Th e properties of T-cell epitopes are as follows:
    a. Processing of an antigen is required before it can be presented to a T cell. Th is processing yields peptides which bind to class I or II MHC molecules and this complex is then presented to T cells.
    b. T-cell epitopes are always presented together with MHC. T-cell receptors do not recognize any epitopes that are presented alone.
    c. Antigens recognized by T cells have two regions—epitope, that interacts with T-cell receptor, and agretope, that interacts with the MHC molecule. Interactions between the epitope and T-cell receptor, and the agretope and MHC are purely non-covalent.
    d. Epitopes recognized by T cells are often internal and usually sequential.

10. SUPERANTIGENS



1. Recently, a class of protein antigens with "super" antigenic properties has been characterized and named superantigens. Superantigens bind simultaneously to T-cell receptors and class II MHC molecules.
2. This non-specific binding activates into the lymphoproliferative phase in T cells and induces pronounced cytokine production by T cells in vivo, thereby causing a variety of pathological consequences such as fever, malaise, diarrhoea, etc.
3. Unlike conventional antigens, superantigens are not internalized and degraded by antigen-presenting cells. Instead, they bind directly to class II MHC molecules outside of the antigen-binding cleft. Superantigens bind on the side of the T-cell receptor, far from the normal antigen-binding site on the receptor.
4. Most superantigens activate an impressive 5–25 per cent of T cells although most benign protein antigens can activate less than 0.01 per cent of T cells.
5. To date, the most extensively studied superantigen is ***Staphylococcal enterotoxin B (SEB)***.
6. Among other important bacterial superantigens are ***pyrogenic exotoxins*** from ***Streptococcus pyrogens, Mycoplasma arthritidis (MAS)*** and ***Staphylococcal exfoliative toxin***.