Antigens & Immunogens

Professor Md. Akram Hossain

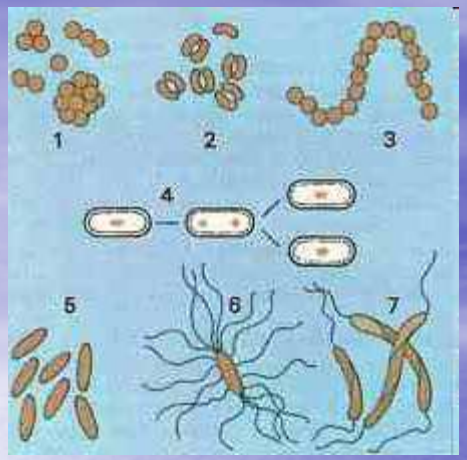
November 2010

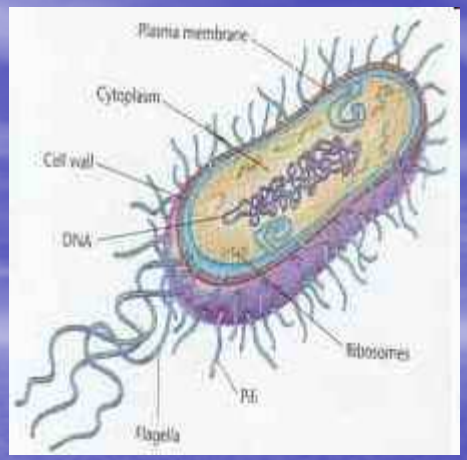
Lession plan

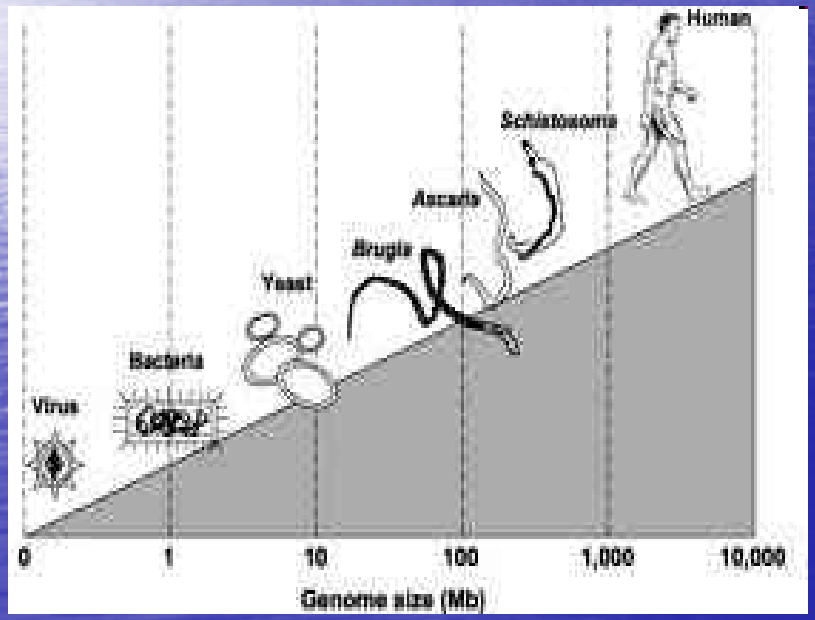
- Antigen, Immunogen, hapten
- Criteria for antigenicity
- Classification of antigens
- Antigenic determinant
- Epitope, Paratope
- superantigen

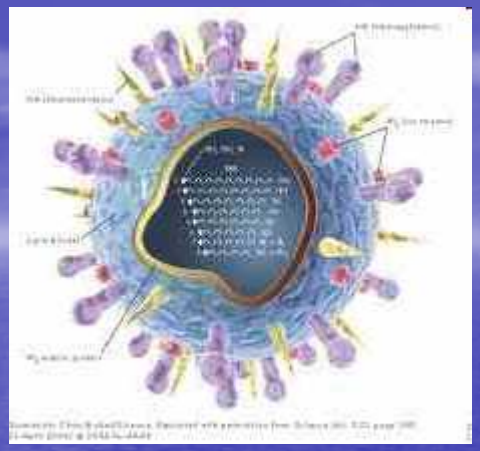
Review questions

1. What is antigen and immunogen?
2. What are the criteria for immunogenicty? Which one is most essential and why?
3. How can you classify antigens?
4. What are the differences between T-dependant and T-independent antigens? Which will give long term immunity?
5. What is super antigen
6. What is epitope and paratope? Where paratope is located?


1
2
3
4
5
6
7


Plasma membrane
Cytoplasm
Cell wall
DNA
Ribosomes
Pili
Flagella


Human
Schistosoma
Ascaris
Brugia
Yeast
Bacteria
Virus
0
1
10
100
1,000
10,000
Genome size (Mb)



Definitions

- Literally **Anti-gen** means any agent which can generate **antibody**

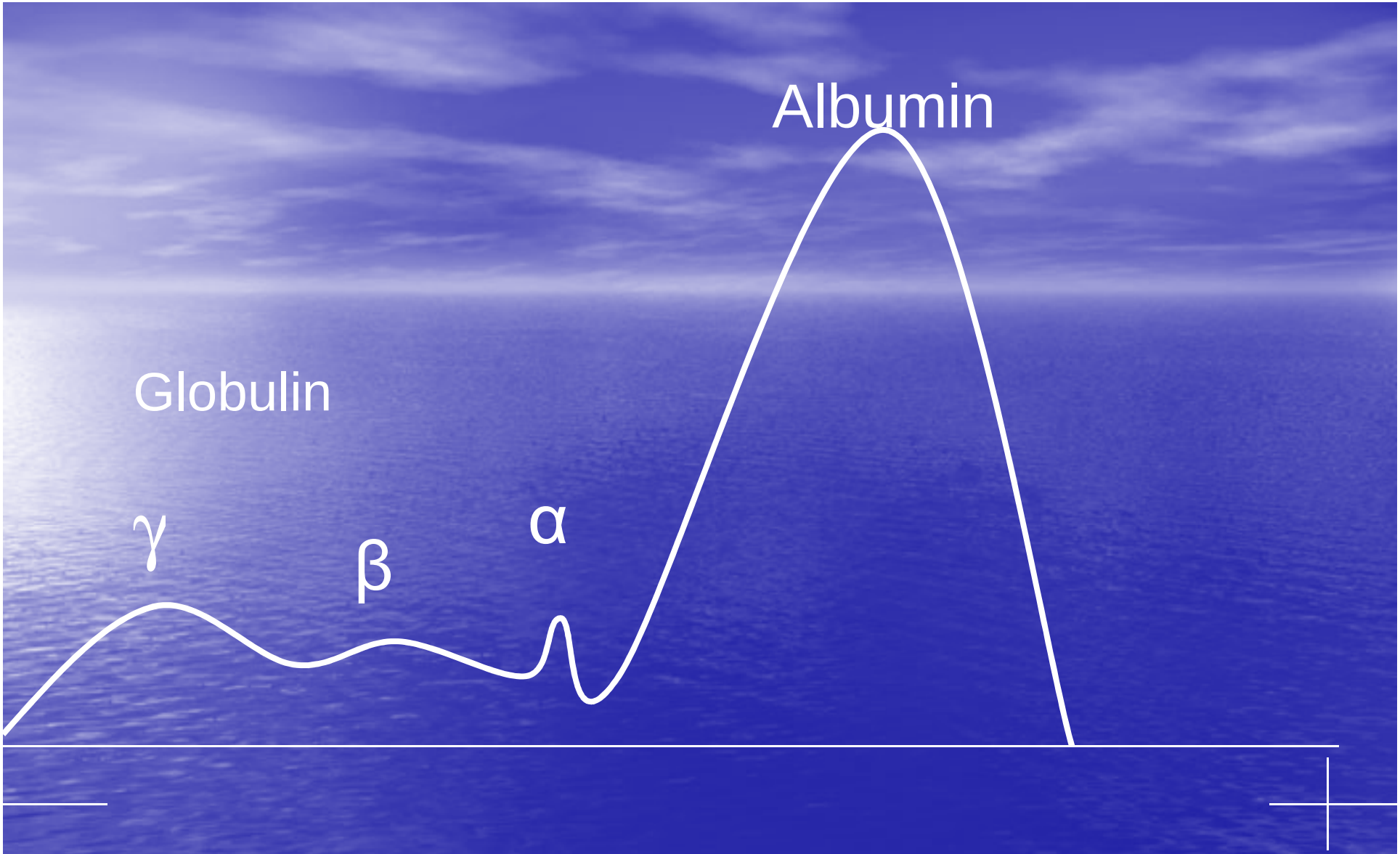
- **Immuno-gen** means any agent which generates immune response. (Antibody mediated or Cell mediated

- Immunogen: a stimulus that produces a humoral or cell-mediated immune response
  - –Humoral immune response – by antibody
  - –Cell mediated immune response – by T cells
- Antigen: any substance that binds specifically to an antibody or a T-cell receptor
- By definition all **immunogens** are antigens but all **antigens** are not immunogens.
- For simplicity, both antigens and immunogens are usually referred to as antigens.

- Antibody - a disease fighting protein developed by the body in response to the presence of an antigen

Historical and Biochemical Evidence for Immunoglobulin structure.

- Electrophoretic separation of serum proteins yields albumin, , α β γ globulin, in that order. γ globulin levels were increased in immunized animals and could be decreased by incubation with specific antigens.
- Kabat & Tiselius in 1939 showed that γ globulin fraction of serum contain antibody.
- Porter proposed of a Y-shaped structure in 1962, after discovering Fc & Fab fragment in 1959.
- Edelman discovered 4 chains of Immunoglobulin.
- Porter & Edelman Won noble prize in 1972.



Electrophoretic mobility of serum proteins

Antigens

- Most are proteins or large polysaccharides from a foreign organism.
  - **Microbes**: Capsules, cell walls, toxins, viral capsids, flagella, etc.
  - **Nonmicrobes**: Pollen, egg white , red blood cell surface molecules, serum proteins, and surface molecules from transplanted tissue.
- Lipids and nucleic acids are only antigenic when combined with proteins or polysaccharides.

- **Hapten: Small foreign molecule that is not antigenic. Must be coupled to a carrier molecule to be antigenic. Once antibodies are formed they will recognize hapten.**
- **Karl Landsteiner discovered Hapten, who also discovered blood group antigen and got noble prize**

Substances that act as antigens

Infectious materials

a. **microbial structures**

(cell walls, capsules, flagella, pili, viral capsids, envelope-associated glycoproteins, etc.

b. **microbial toxins**

Noninfectious materials

a. **allergens** (dust, pollen, hair, foods, dander, bee venom, drugs, and other agents causing allergic reactions);

b. **foreign tissues and cells** (from transplants and transfusions); and

c. **the body's own cells that the body fails to recognize as "normal self"** (cancer cells, infected cells, cells involved in autoimmune diseases).

Classification of antigens

- According to chemical nature
  1. Proteins- virtually all
  2. Polysaccharides – potentially but not always
  3. Nucleic acids – poor antigens
  4. Lipids- may act as haptens
- According to mode of action
  1. Thymus dependent – Protein antigens
  2. Thymus independent - Polysaccharides
- According to epitope
  1. Unideterminant univalent
  2. Unideterminant multivalent
  3. multdeterminant multvalent
- According to Source
  1. Exogenous
  2. Endogenous

Types of Antigens

- **T-independent antigens**
  - Complex carbohydrates
  - Do not require processing
  - Can directly interact with B cells
  - No memory
- **T-dependent antigens**
  - Require macrophages or other APC
  - Require T-helper cells
  - Require major histocompatibility antigens
  - Mostly proteins

Types of Antigens…

Exogenous:

External antigens e.g. bacterial infection

Endogenous:

Typically peptides derived from *any* protein; e.g. viral infections an infected cell

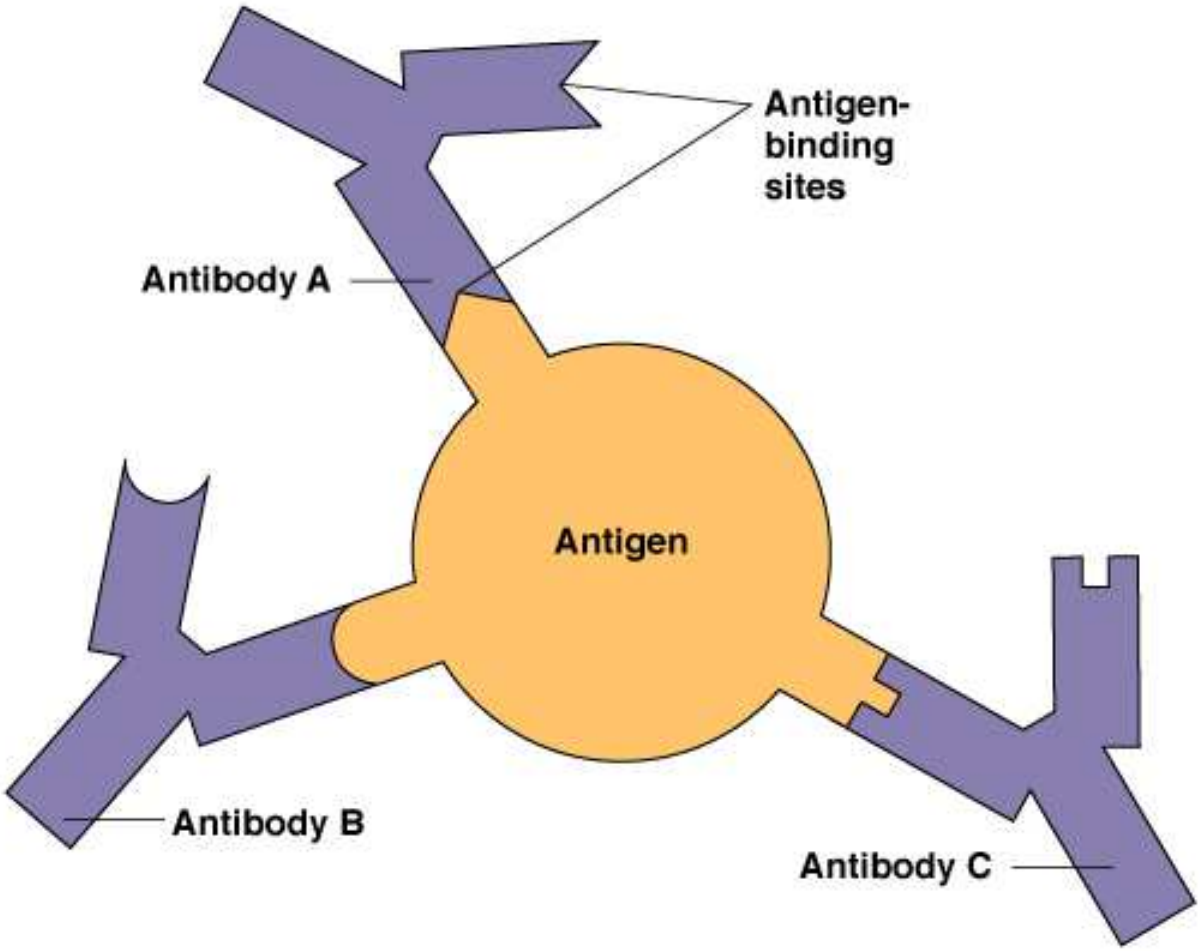
The basis of immunogenicity…

1. Foreignness – most essential
2. Molecular size
3. Chemical composition and heterogeneity
4. Degradability
5. Adequate dose & route
6. Genetic constitution of host

•Epitope: the portion of an antigen that is recognized and bound by an Ab or TCR/MHC complex (aka antigenic determinant)

•Paratope: “The site in the variable (V) domain of an antibody or T-cell receptor that binds to an epitope on an antigen

Epitopes: Antigen Regions that Interact with Antibodies



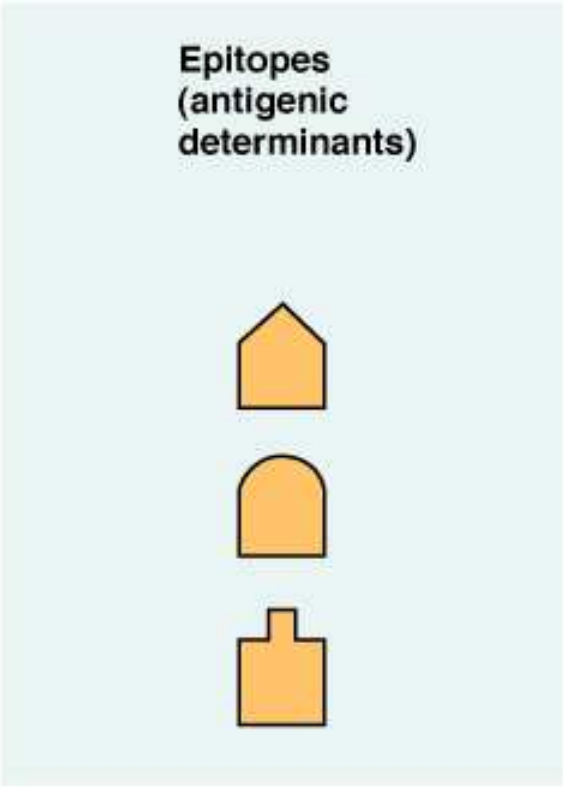
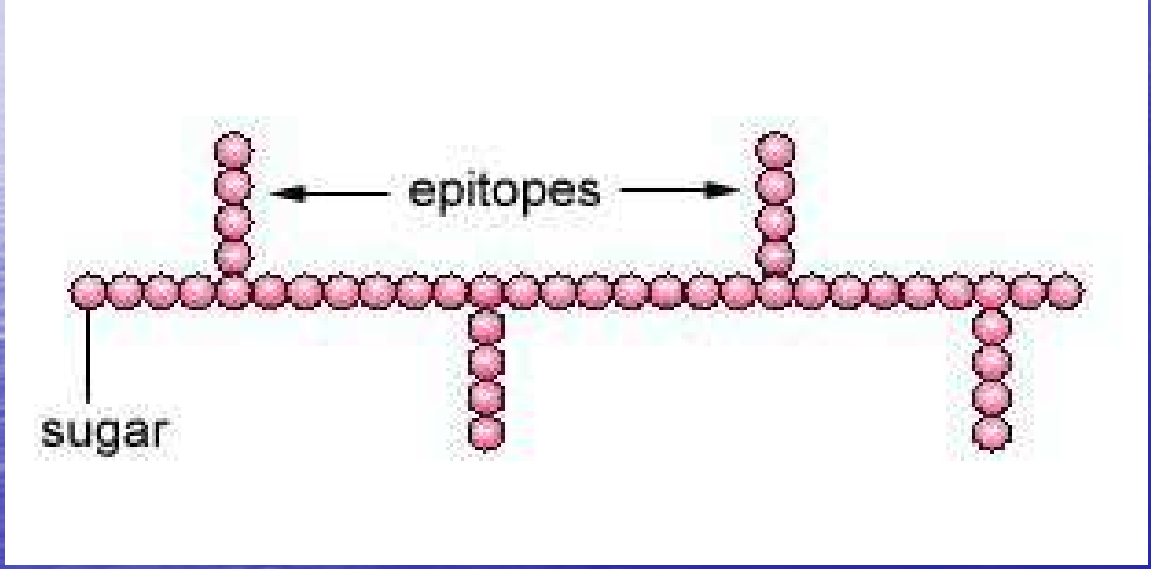
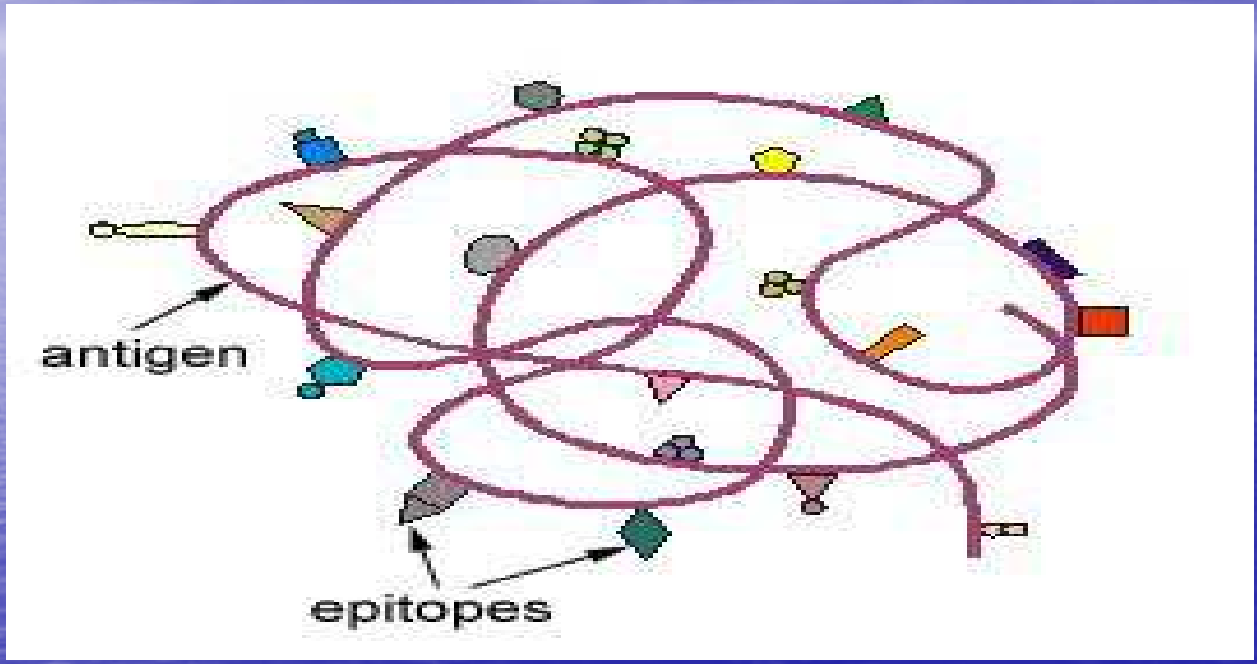


Fig. 1: Epitopes of an Antigen (Polysaccharide)



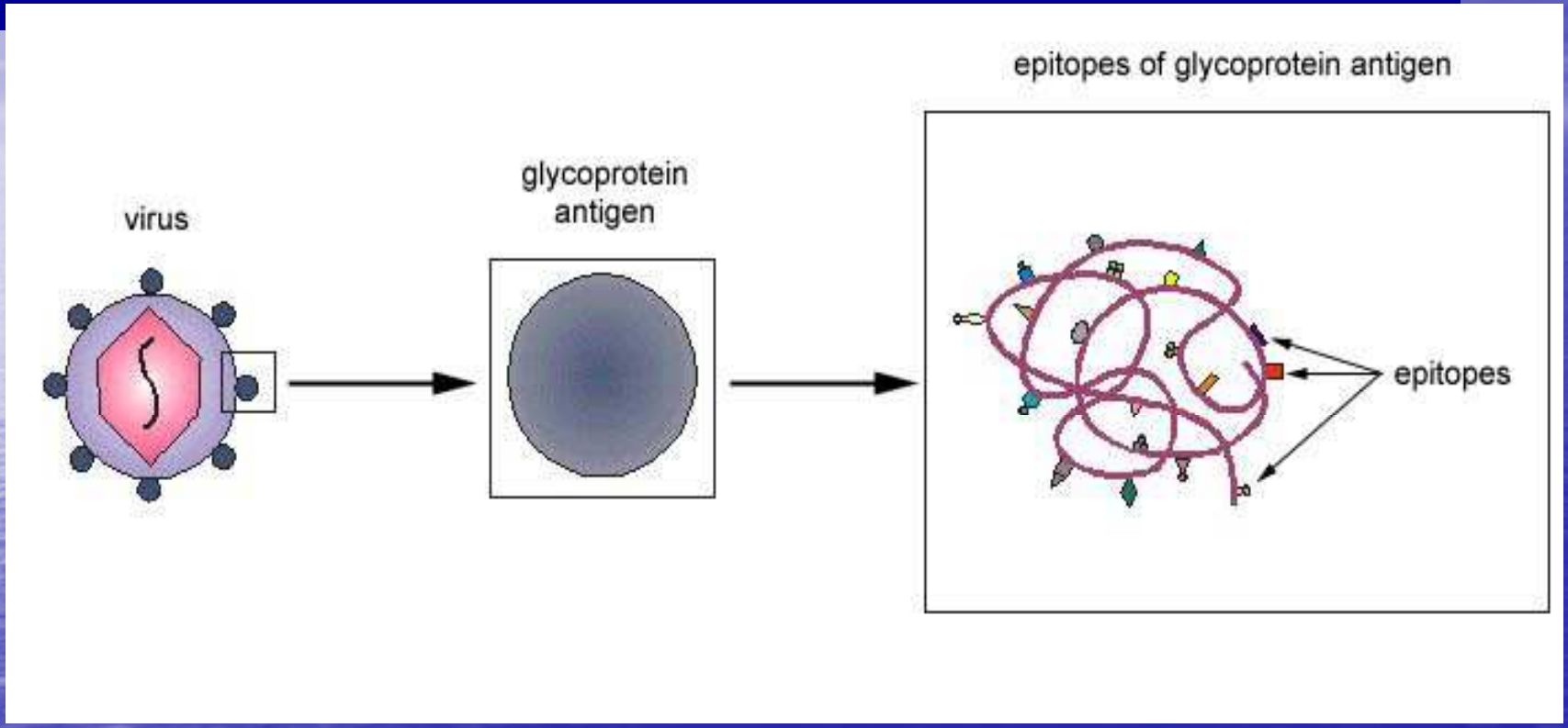
Polysaccharides have many epitopes but of similar specificities.

Fig. 2: Epitopes of an Antigen (Protein)



Proteins have many epitopes of different specificities.

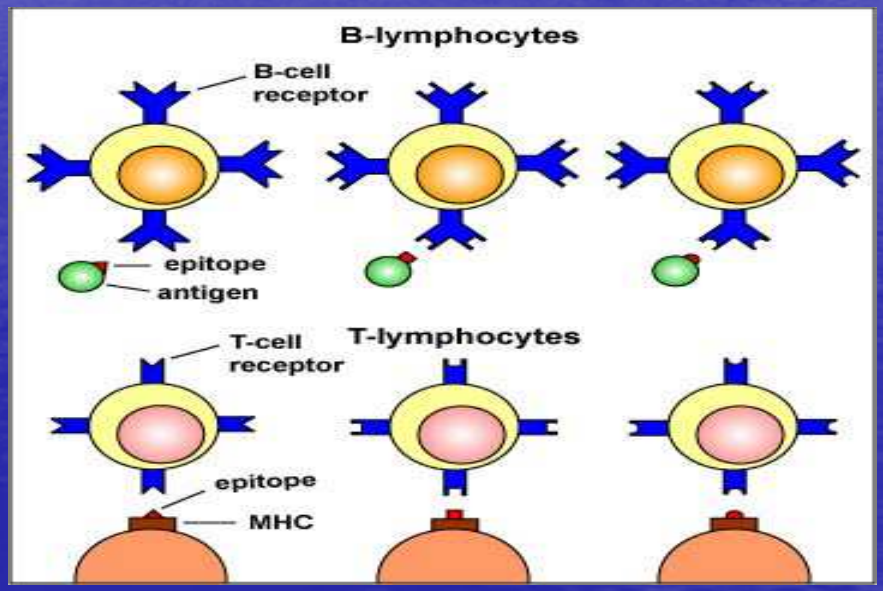
Fig. 3: Antigens and Epitopes of a Virus

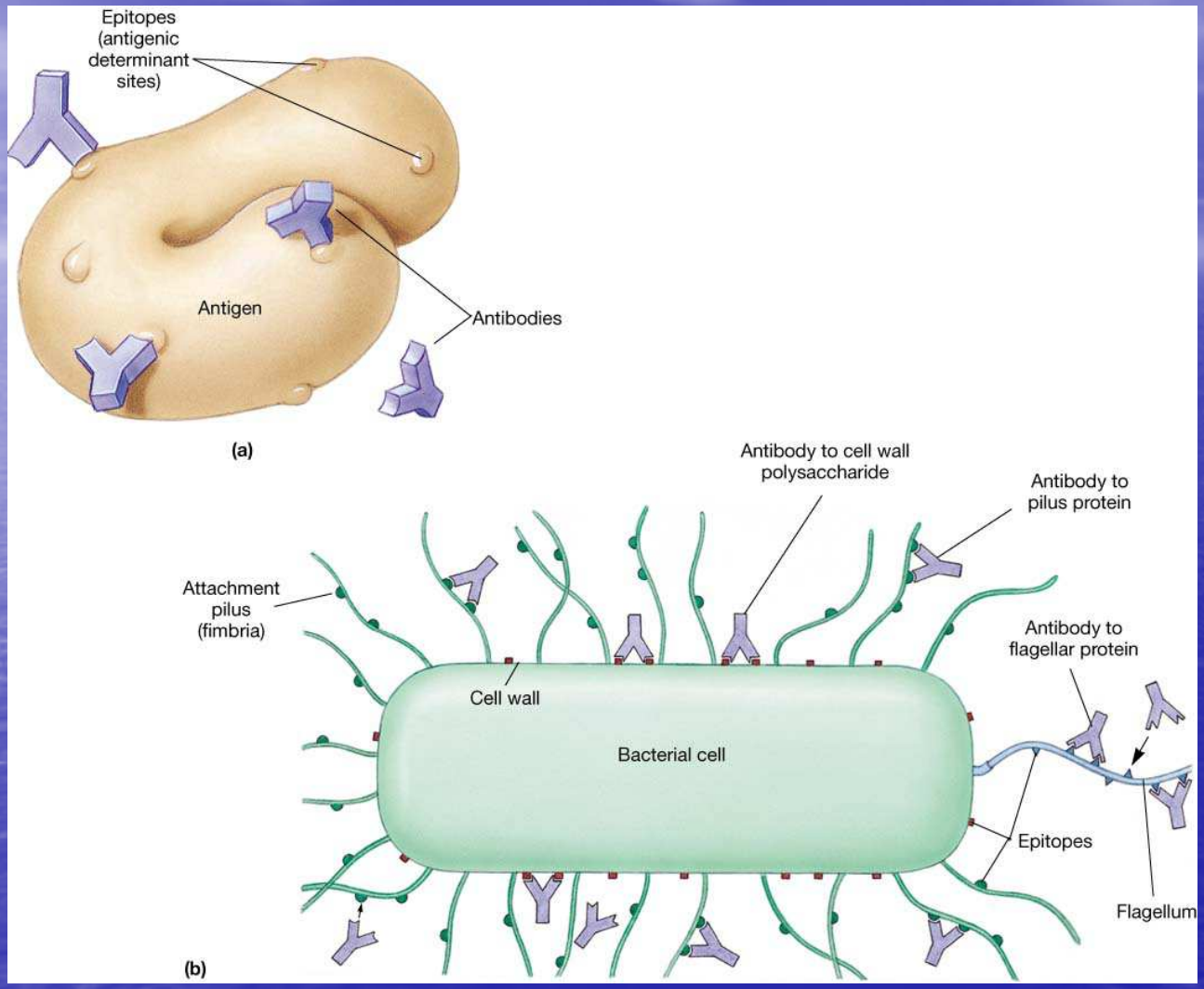


Each different protein and glycoprotein of a virus constitutes a different antigen. Each different antigen contains a number of different epitopes.

Epitope-Specific Receptors on the Surface of B- and T-Lymphocytes

B-lymphocytes have B-cell receptors (sIg) that recognize epitopes directly on antigens. T-lymphocytes have TCR molecules that recognize epitopes only after they have been placed on the body's own cells by way of MHC molecules.




Epitopes (antigenic determinant sites)
Antigen
Antibodies
(a)
Antibody to cell wall polysaccharide
Antibody to pilus protein
Attachment pilus (fimbria)
Antibody to flagellar protein
Cell wall
Bacterial cell
Epitopes
Flagellum
(b)

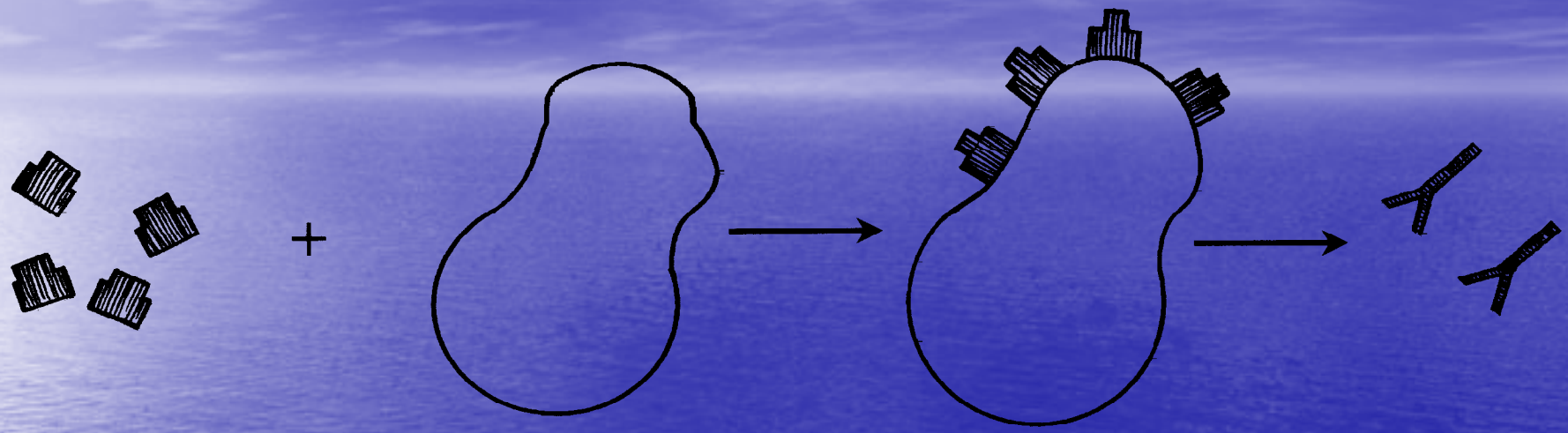
Hapten

- Hapten: A molecule too small to be immunogenic alone, but which can be immunogenic if coupled to a larger molecule referred to as a carrier.

By itself, a hapten can react with an Ab

Example: Penicillin acts as a hapten

Haptens

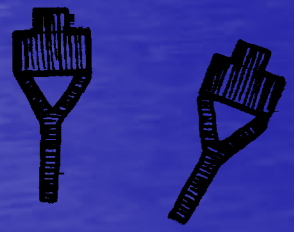


Hapten molecules

Carrier

Complete ag

Abs



Hapten molecules combine with abs

Haptens…

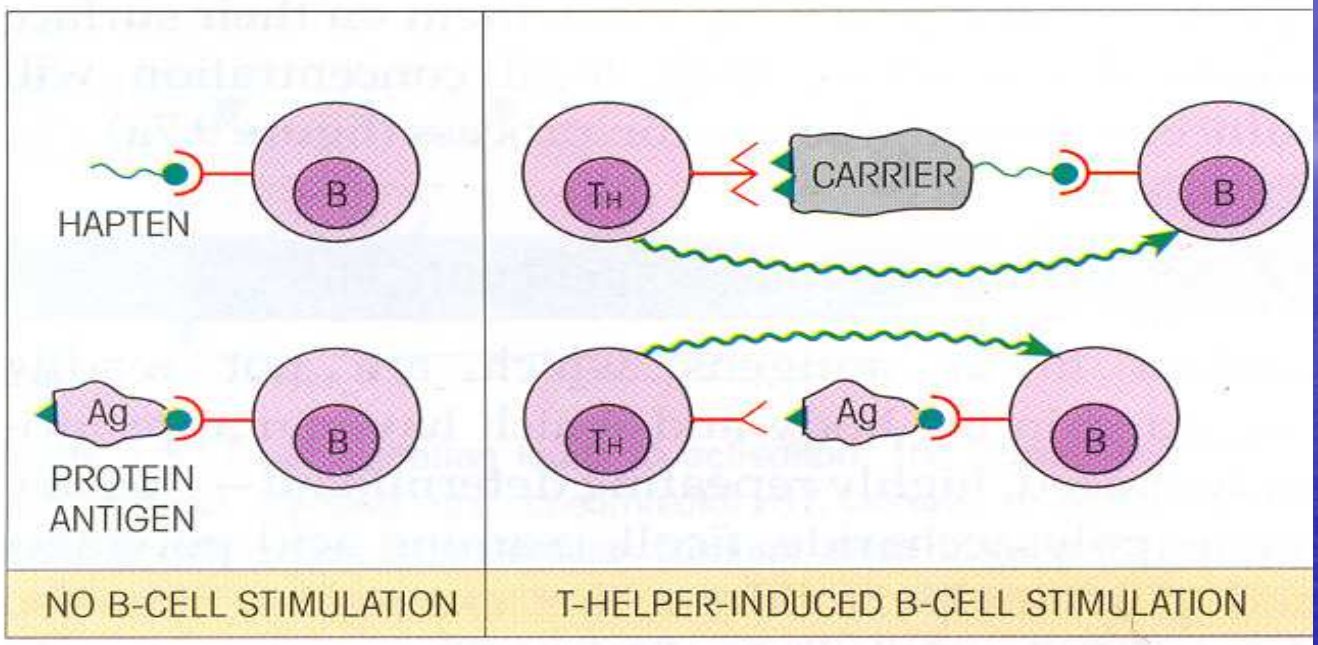


Figure 9.8. T-helper cells cooperate through protein carrier determinants to help B-cells respond to hapten or equivalent determinants on antigens by providing accessory signals. (For simplicity we are ignoring the MHC component and epitope processing in T-cell recognition, but we won't forget it.)

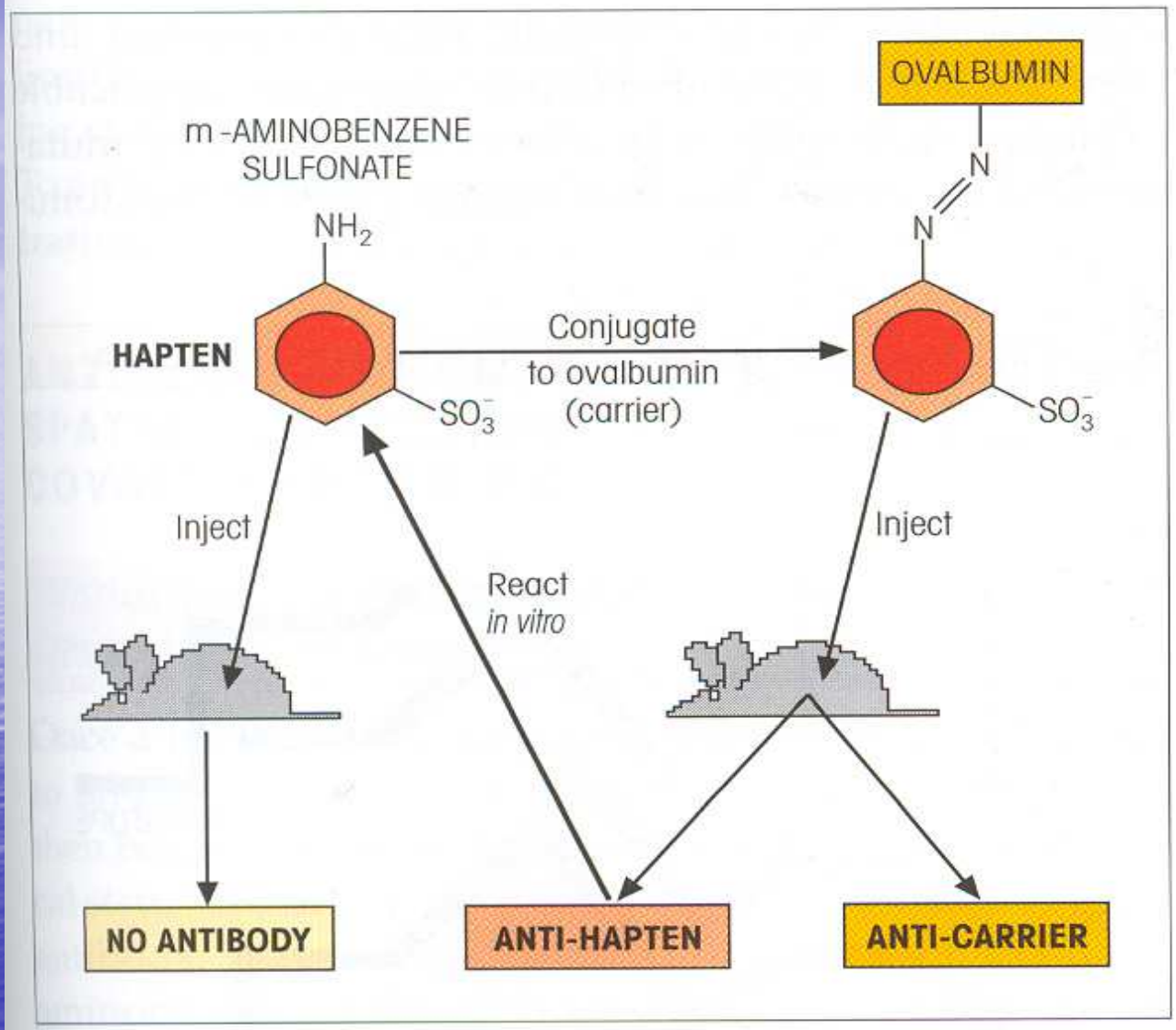
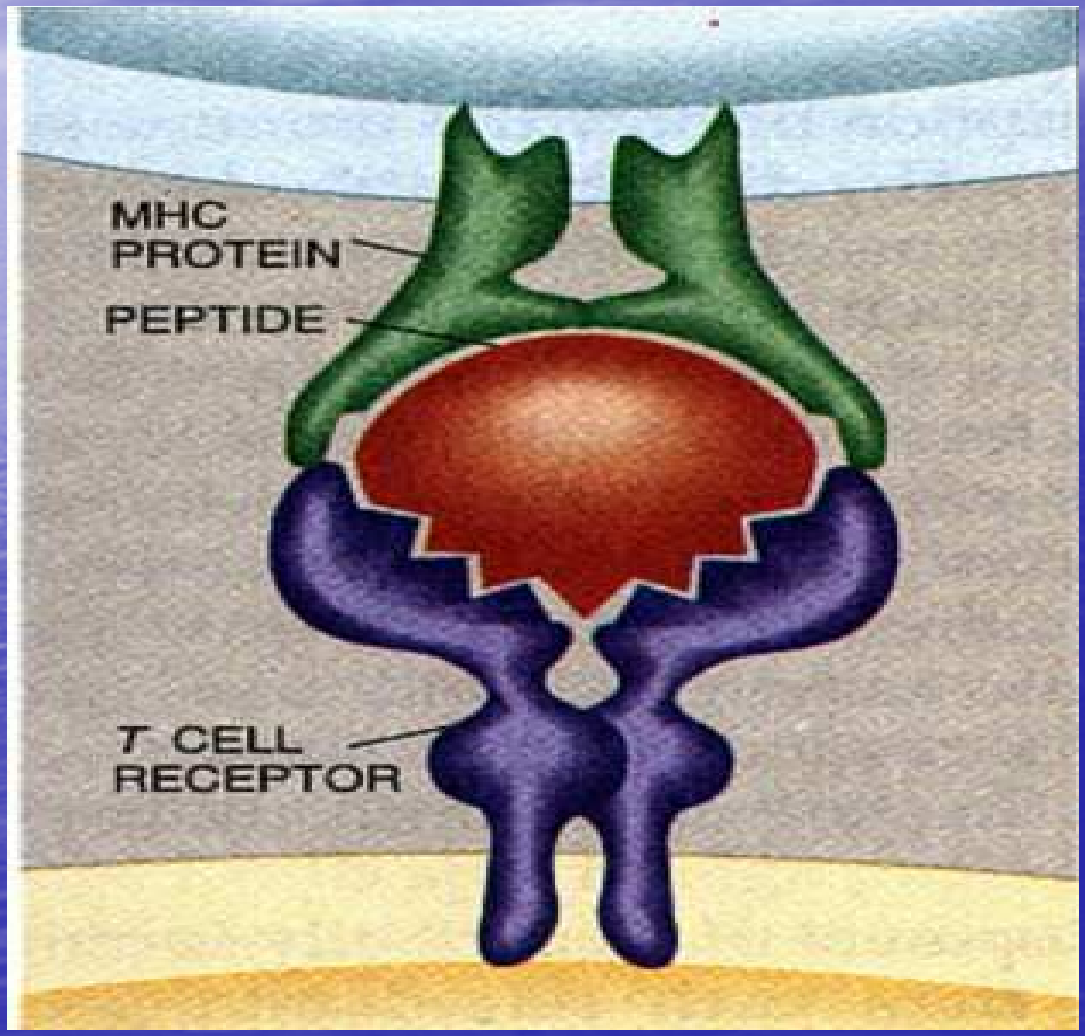


Figure 5.1. **A hapten on its own will not induce antibodies.** However, it will react *in vitro* with antibodies formed to a conjugate with an immunogenic carrier.

Antibody-Antigen Interactions

- Binding of antigen to antibody
- Occurs in variable region of antibody molecule
- Instantaneous
- Exothermic
- May form complexes
- Cytotoxicity mediated by complement (lysis)

The key event…



The key event…

A processed antigen in an MHC is seen by a TCR.

The TCR asks the MHC, "Are you me?" and receives an affirmative answer, "Yes."

The TCR asks the processed antigen, "Are you me?" and receives the negative answer, "No!"

Thus, the processed antigen is seen as "not-self," *i. e.,* "foreign."

The key event…

A processed antigen in an MHC is seen by a TCR. This "viewing" occurs in the ***ternary complex.***

The TCR asks the MHC, "Are you me?" and receives an affirmative answer, "Yes." Here the TCR looks at the MHC ***histotope.***

The TCR asks the processed antigen, "Are you me?" and receives the negative answer, "No!" Here the TCR uses its ***paratope*** and looks at the ***epitope.***

The key event…

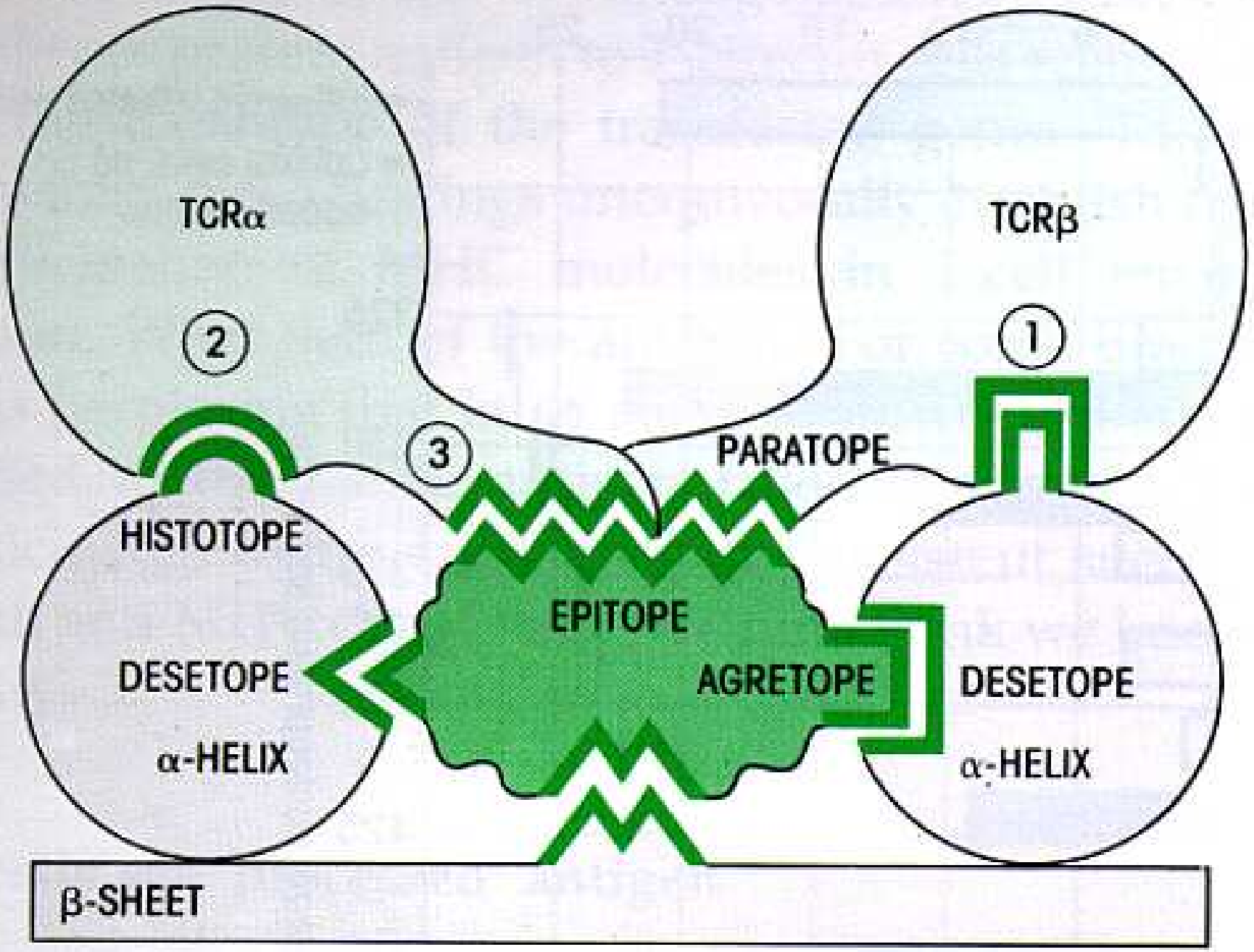
A processed antigen in an MHC is seen by a TCR.

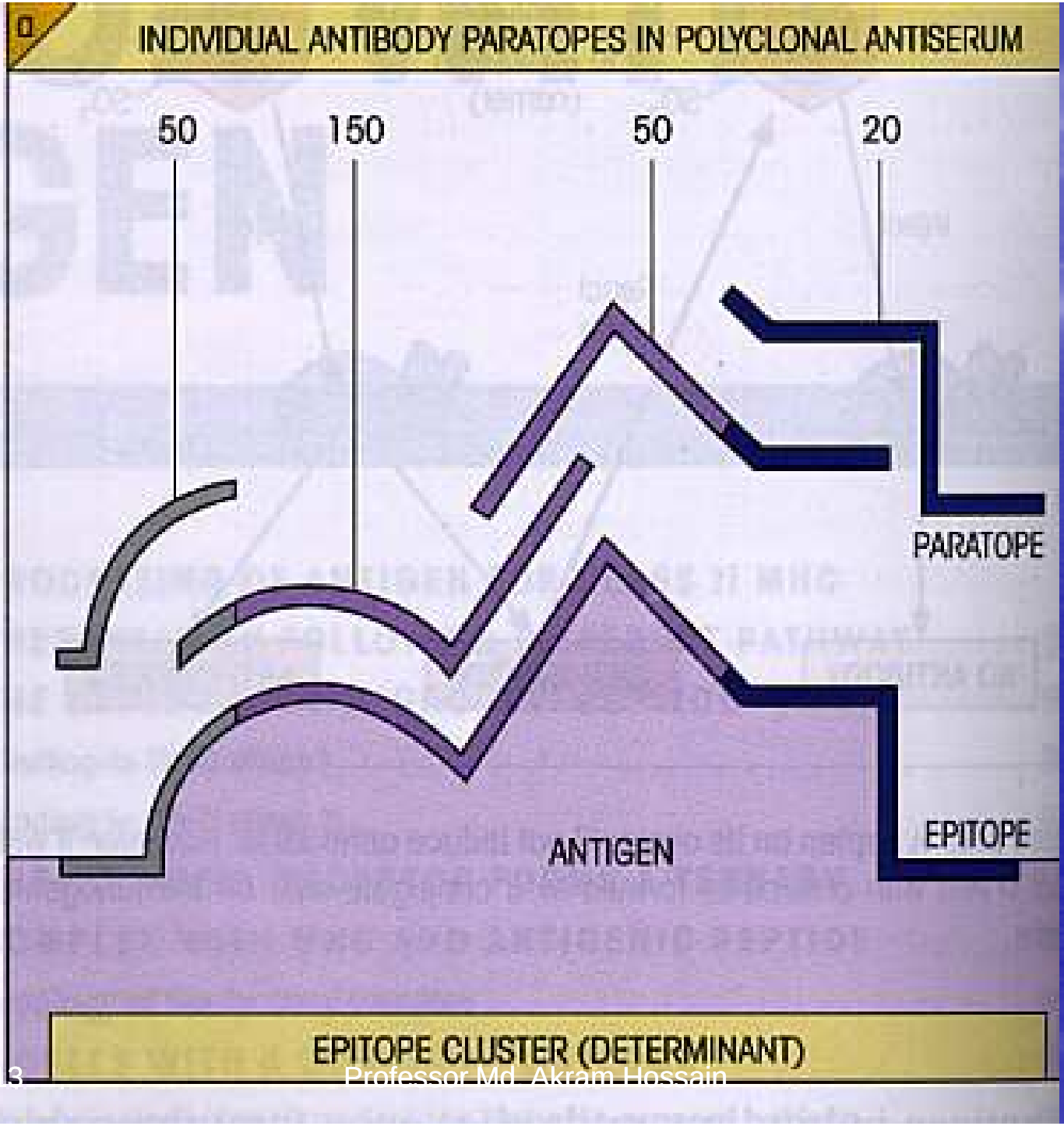
The TCR asks the MHC, "Are you me?" and receives an affirmative answer, "Yes."

The TCR asks the processed antigen, "Are you me?" and receives the negative answer, "No!"

But what if the TCR asks the processed antigen, "Are you me?" and receives the answer, "Yes." TCR's which can see "self" are *eliminated* in a process called clonal deletion.

Clonal deletion assures that TCR's don't see "self."


TCRα
TCRβ
2
1
3
PARATOPE
HISTOTOPE
EPITOPE
DESETOPE
AGRETOPE
DESETOPE
α-HELIX
α-HELIX
β-SHEET


a
INDIVIDUAL ANTIBODY PARATOPES IN POLYCLONAL ANTISERUM
50
150
50
20
PARATOPE
ANTIGEN
EPITOPE
EPITOPE CLUSTER (DETERMINANT)

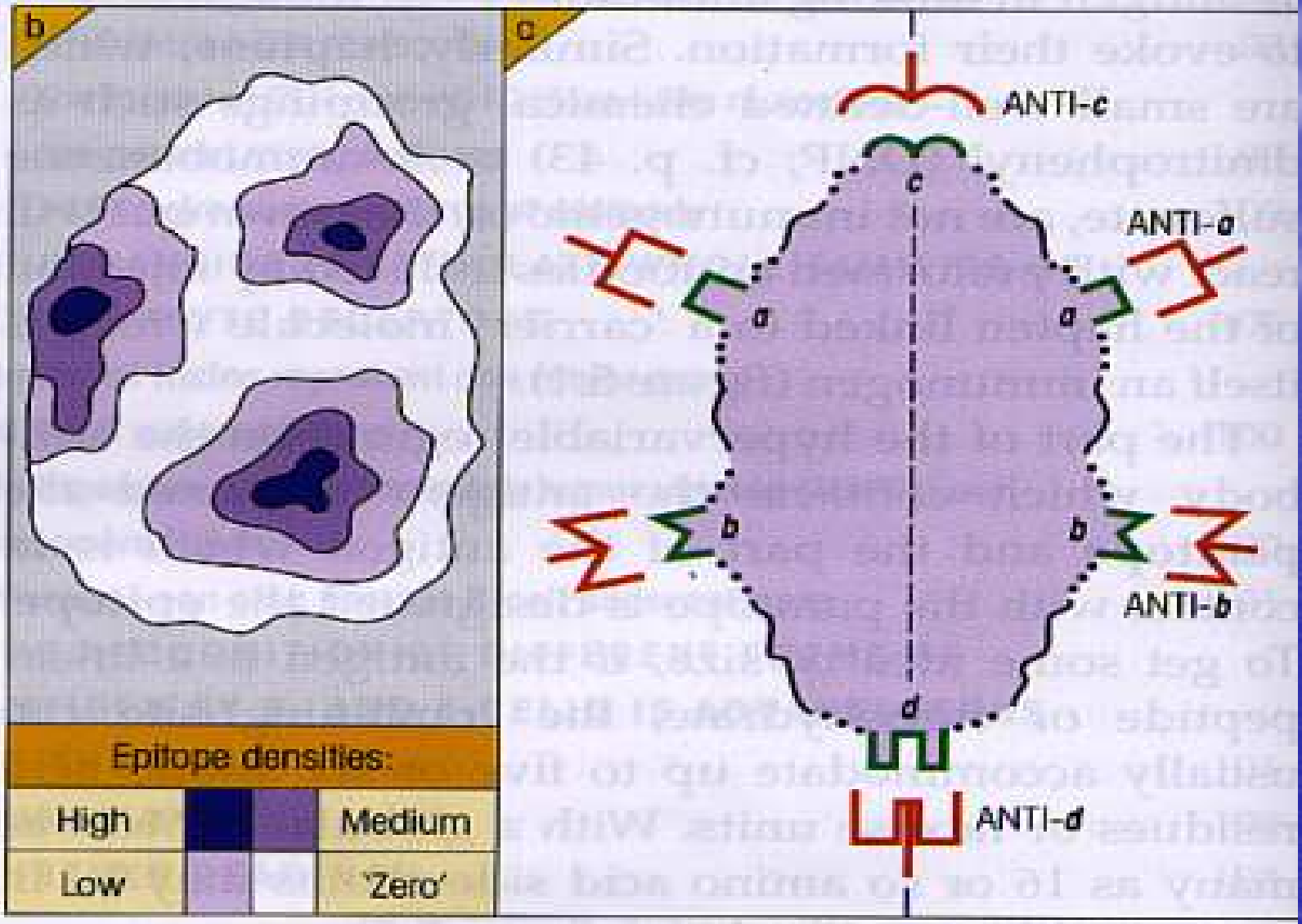


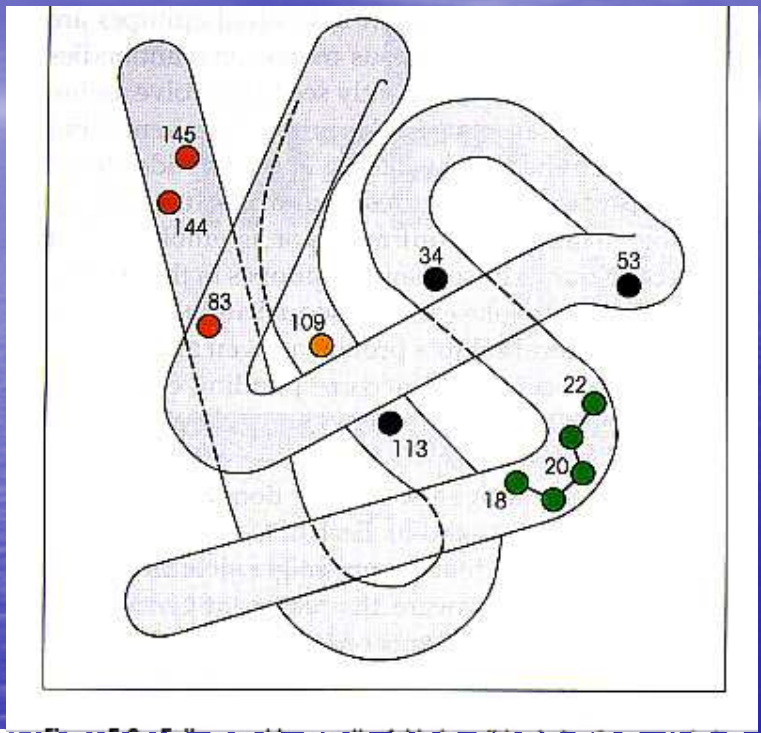
Figure 5.4. A globular protein antigen usually bears a mosaic of determi-
nants (dominant epitope clusters) on its surface, defined by the hetero-

Experimental systems…

TABLE 3-1 MOLECULAR WEIGHT OF SOME COMMON EXPERIMENTAL ANTIGENS USED IN IMMUNOLOGY

Antigen	Approximate molecular mass (Da)
Bovine gamma globulin (BGG)	150,000
Bovine serum albumin (BSA)	69,000
Flagellin (monomer)	40,000
Hen egg-white lysozyme (HEL)	15,000
Keyhole limpet hemocyanin (KLH)	>2,000,000
Ovalbumin (OVA)	44,000
Sperm whale myoglobin (SWM)	17,000
Tetanus toxoid (TT)	150,000

Epitopes for B-cells *versus* T-cells



By examining myoglobin one can see that the Ag's seen by B-cells and T-cells are different. B-cells see a continuous or discontinuous series of amino acids; by some circumstance, amino acid residue 109 has *never* been a part of an epitope for any monoclonal antibody; yet residue 109 is *always* part of the processed antigen seen by a TCR.

Presentation of processed antigen...

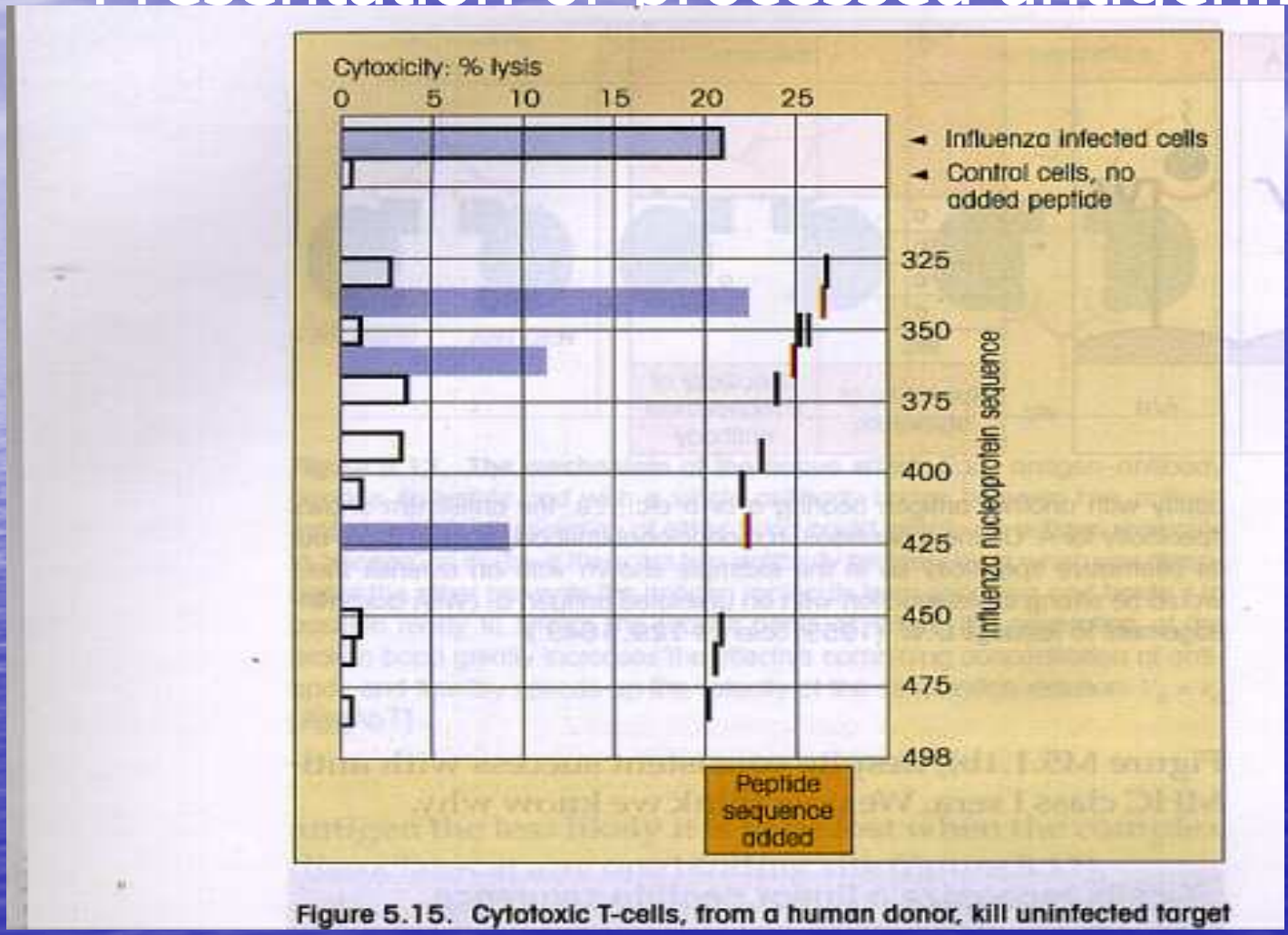
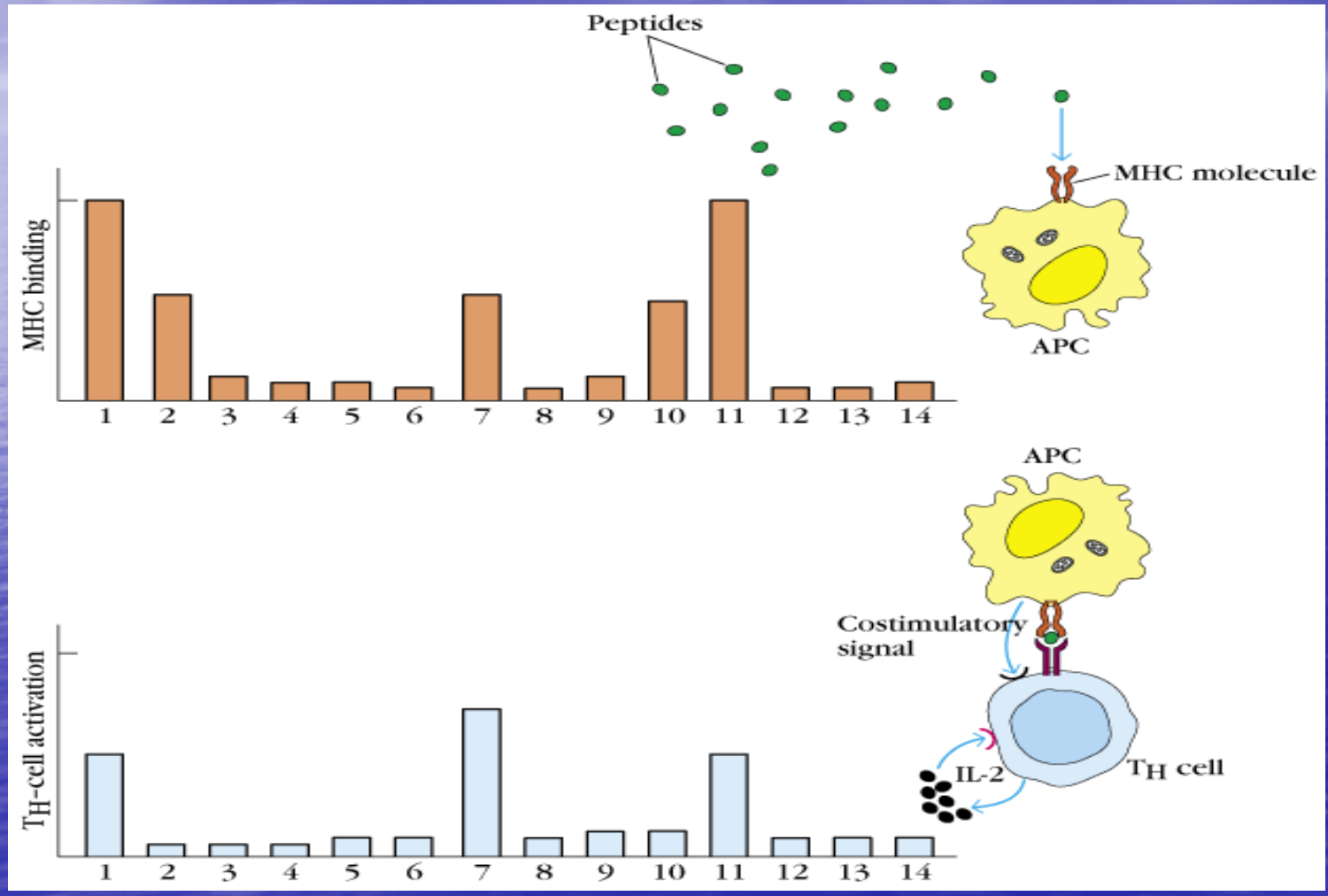


Figure 5.15. Cytotoxic T-cells, from a human donor, kill uninfected target

Presentation of processed antigen…



There are two classes of T-cells

T_H have **CD4** which interacts with MHC-II; thus, $CD4^+$ T-cells are "MHC-II restricted."

T_H cells are "helper cells" that send signals (via cytokines and surface proteins) to other cells of the immune system. The T_H cells function as the "brain" of the immune system.

There are two classes of T-cells…

T_C have **CD8** which interacts with MHC-I; thus, $CD8^+$ T-cells are "MHC-I restricted."

T_C cells become *cytotoxic T lymphocytes* (CTL's) which attack "altered self-cells (*e. g.,* infected cells.) "Altered self-cells" are also called "target cells." They are the targets for the CTL's cytotoxicity.

"adjuvants"

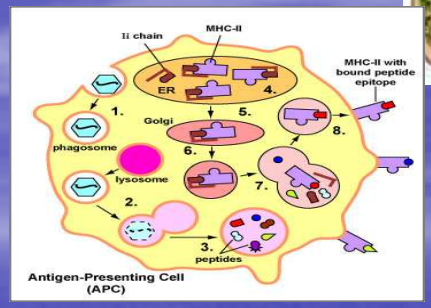
Adjuvants:

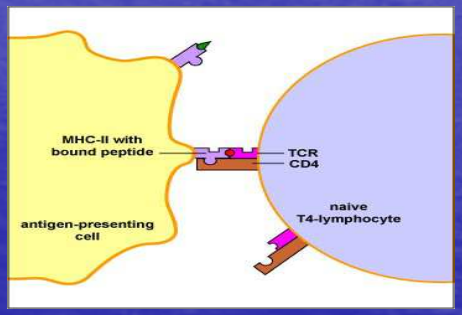
A substance that non-specifically enhances the immune response to an antigen

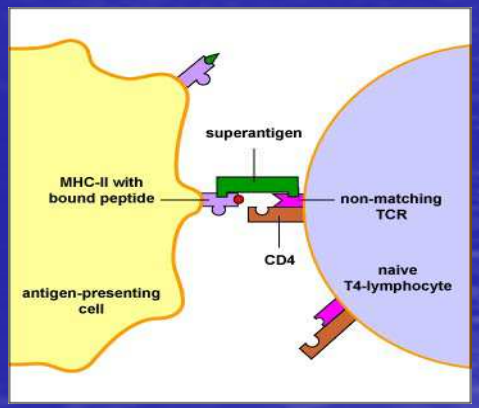
- Prolong the presence of the antigen
- Enhance production of "co-stimulatory" signals
- Induce granuloma formation (*i.e.,* an accumulation of macrophages)
- Non-specifically stimulate lymphocytes

Superantigens

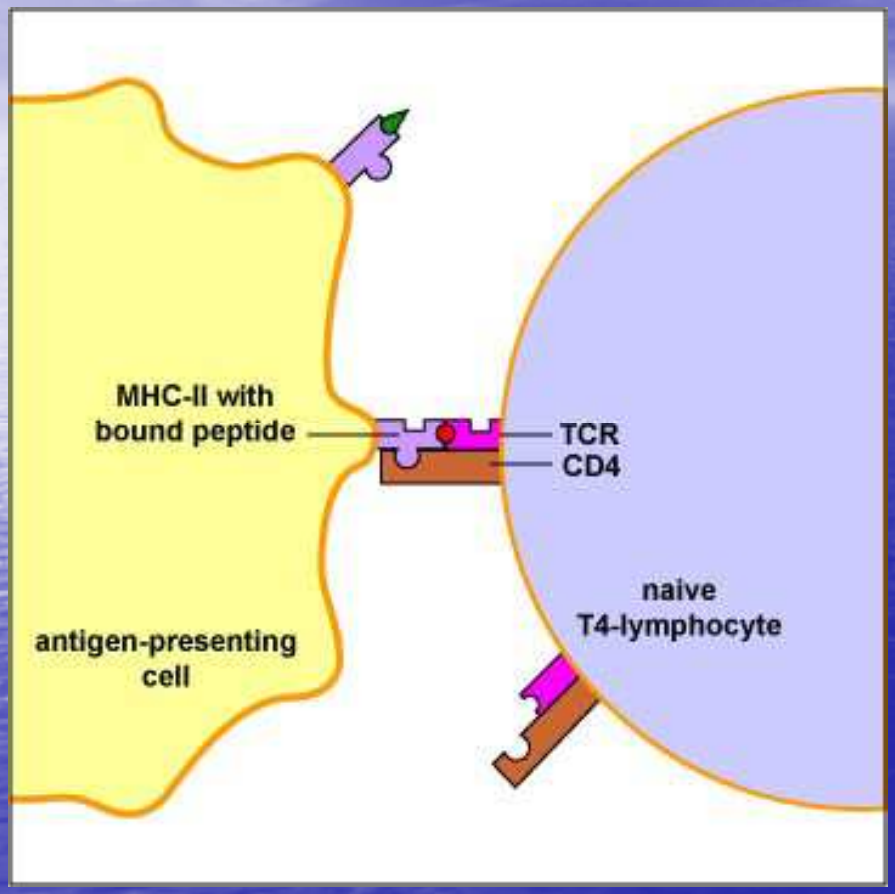
Superantigens are unusual bacterial toxins that **interact with exceedingly large numbers of T4-lymphocytes . Conventional antigens** are engulfed by antigen presenting cells (APCs), degraded into epitopes., bind to the peptide groove of MHC-II molecules, and are put on the surface of the APC (see Fig. 1). Here they are **recognized by specific T4-lymphocytes having a TCR with a corresponding shape** (see Fig. 2). **Superantigens**, however, **bind directly to the outside of MHC-II molecules and activate large numbers of T4-**

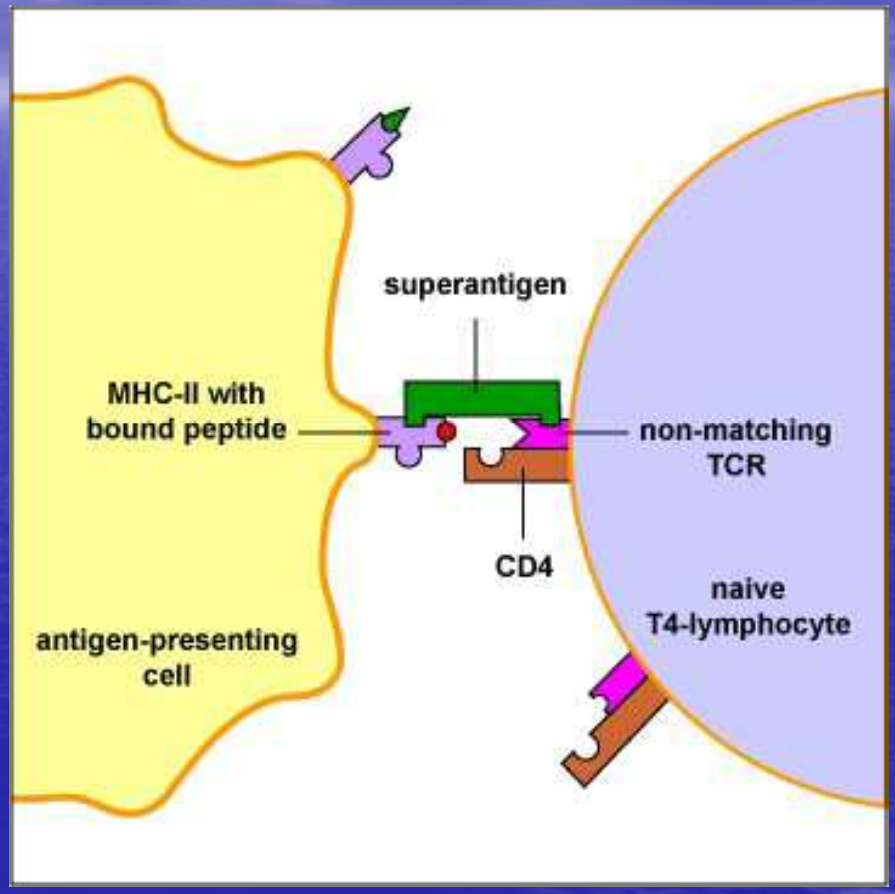






Superantigens





Regular antigen

Super antigen

Actions of superantigens

1. This activation of very large numbers of T4-lymphocytes results in the **secretion of excessive amounts of a cytokine called interleukin-2 (IL-2)** as well as the activation of self-reactive T-lymphocytes.
2. Production of high levels of IL-2 can result in circulation of IL-2 in the blood leading to symptoms such as fever, nausea, vomiting, diarrhea, and malaise.

excess stimulation of IL-2 secretion can also lead to production of other cytokines such as

- tumor necrosis factor-alpha (TNF-alpha),
- interleukin-1 (IL-1),
- inflammatory chemokines such as IL-8,
- platelet-activating factor (PAF),

can lead to the same endothelial damage, acute respiratory distress syndrome, disseminated intravascular coagulation, shock, and multiple organ system failure seen with **Endotoxin.**

3. Activation of self-reactive T-lymphocytes can also lead to autoimmune attack.

Examples of superantigens

1. **Toxic shock syndrome toxin-1 (TSST-1)**,
2. **Streptococcal pyrogenic exotoxin (Spe)**,

   produced by rare invasive strains and scarlet fever strains of *Streptococcus pyogenes* (group A beta streptococci).
3. **Staphylococcal enterotoxins (SE)**,

4. **Superantigens associated with *Streptococcus pyogenes*** are also thought to be responsible for **psoriasis**.
5. Antigens associated with ***Mycobacterium tuberculosis***, the rabies virus, and possibly HIV may also function as superantigens