Antigen

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APR. 28, 2020

1. Properties of Antigen (Ag)

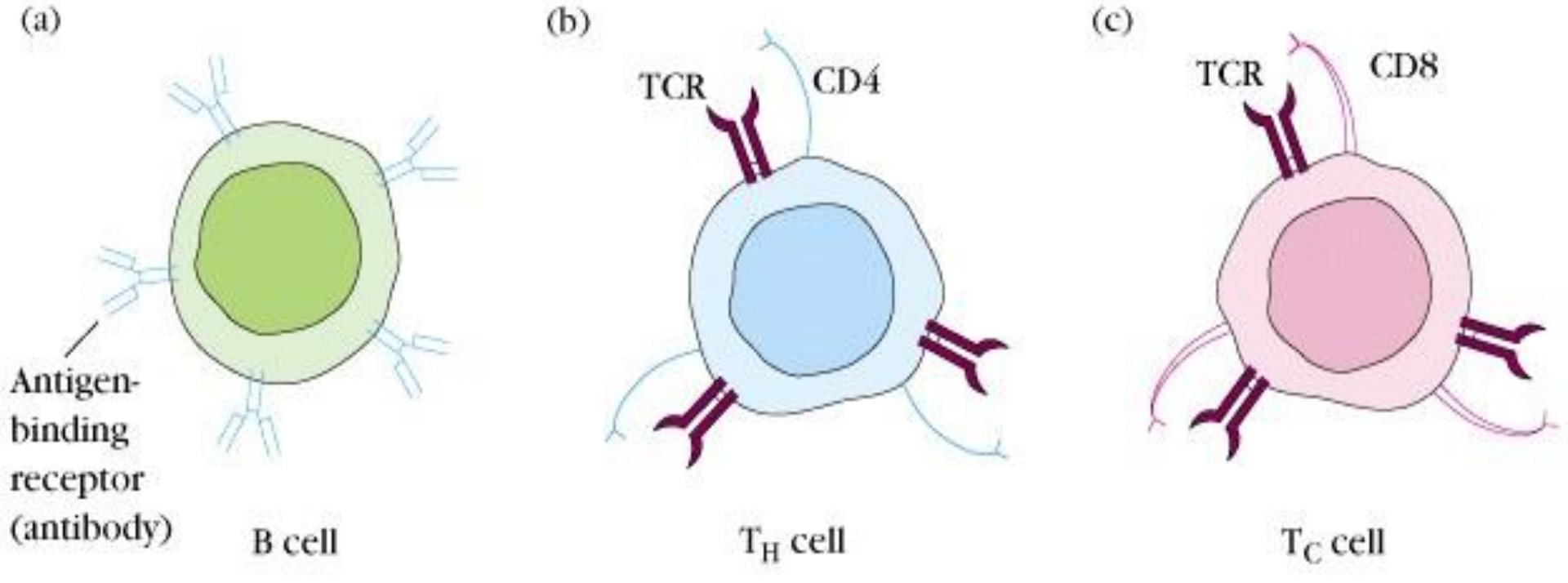
- Any substance capable of inducing an immune response is called an immunogen and is said to be **immunogenic**.

- Some immunogens activate either the humoral or the cellular responses, but most activate **both**.

- As a rule, immune responses are carried
- out only by those B and T cell clones whose surface immunoglubulin (Ig) or T cell receptor (TCR) proteins recognized the immunogen.

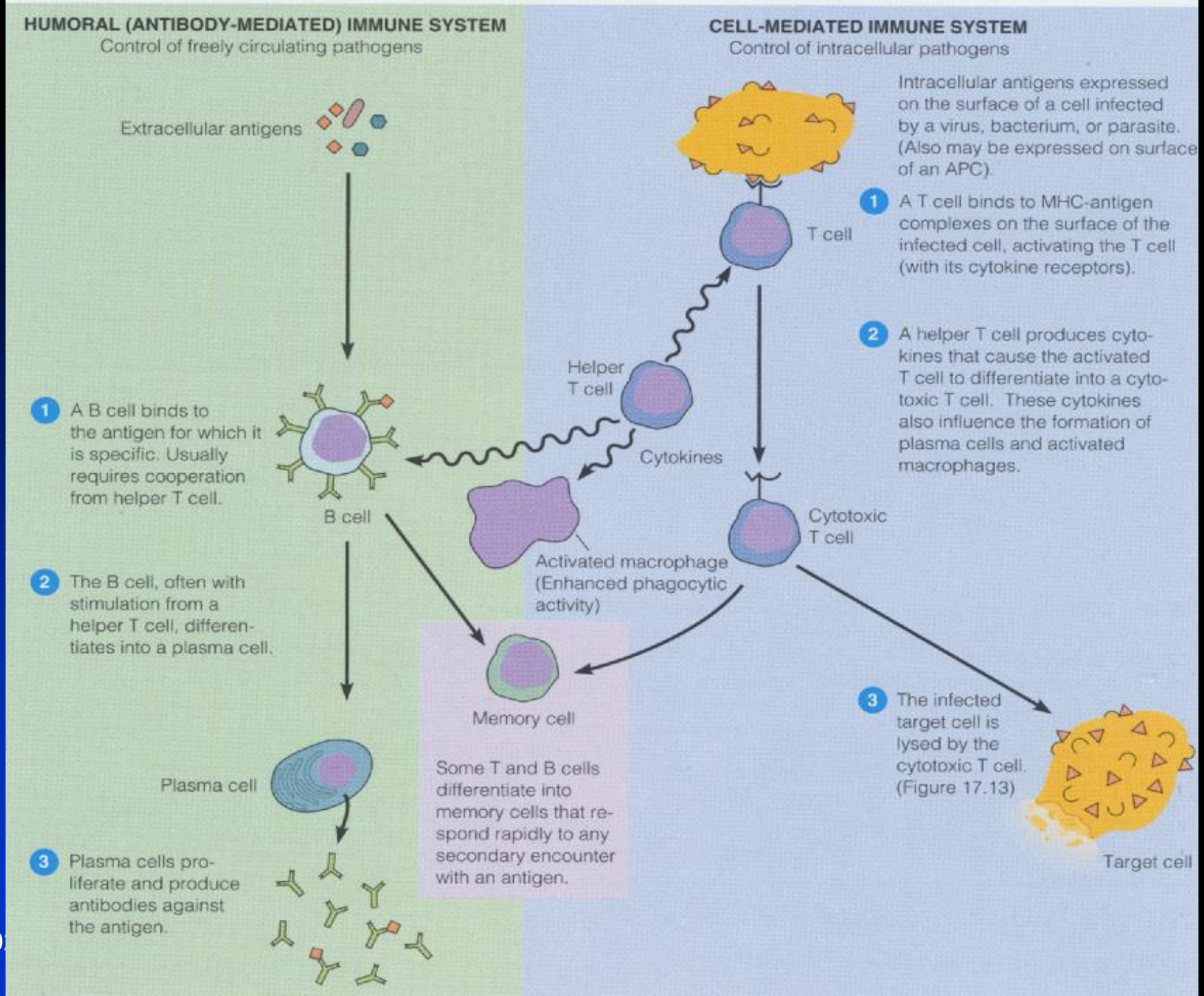
- Substances that are recognized by a particular Ig or TCR, and so can serve as
- the target of an immune response, are called antigens (Ag) and are said to be antigenic.

Recognition of Antigen by Lymphocytes (B cells and T cells)



Humoral Immunity

Cellular Immunity


HUMORAL (ANTIBODY-MEDIATED) IMMUNE SYSTEM
Control of freely circulating pathogens
CELL-MEDIATED IMMUNE SYSTEM
Control of intracellular pathogens
Extracellular antigens
Intracellular antigens expressed on the surface of a cell infected by a virus, bacterium, or parasite. (Also may be expressed on surface of an APC).
T cell
1 A T cell binds to MHC-antigen complexes on the surface of the infected cell, activating the T cell (with its cytokine receptors).
2 A helper T cell produces cytokines that cause the activated T cell to differentiate into a cytotoxic T cell. These cytokines also influence the formation of plasma cells and activated macrophages.
Helper T cell
Cytokines
1 A B cell binds to the antigen for which it is specific. Usually requires cooperation from helper T cell.
B cell
Cytotoxic T cell
Activated macrophage (Enhanced phagocytic activity)
2 The B cell, often with stimulation from a helper T cell, differentiates into a plasma cell.
Memory cell
Some T and B cells differentiate into memory cells that respond rapidly to any secondary encounter with an antigen.
3 The infected target cell is lysed by the cytotoxic T cell. (Figure 17.13)
Plasma cell
Target cell
3 Plasma cells proliferate and produce antibodies against the antigen.

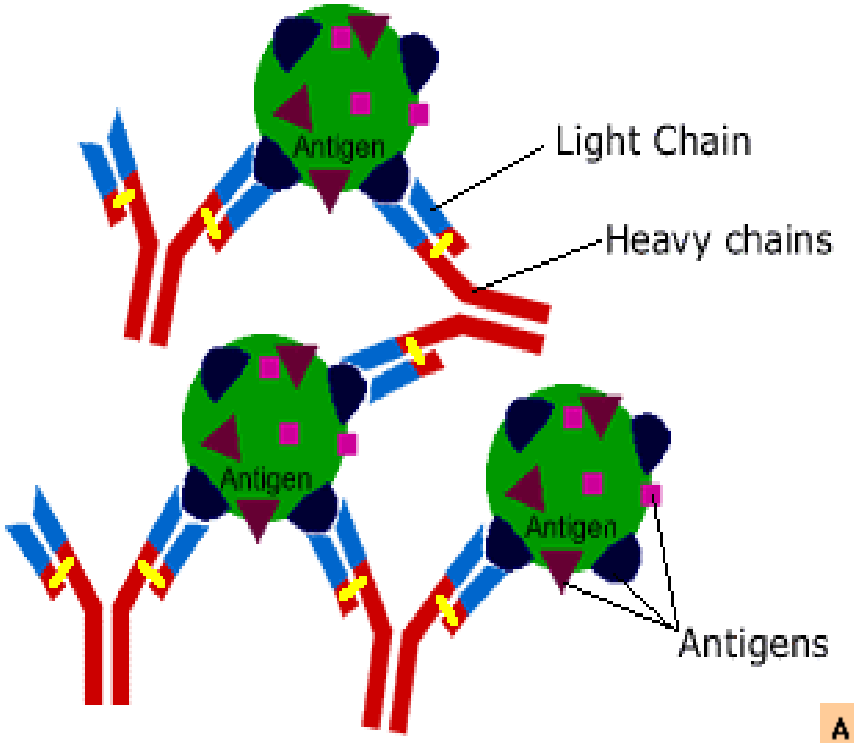
2. Characteristics of antigens

1) Specificity

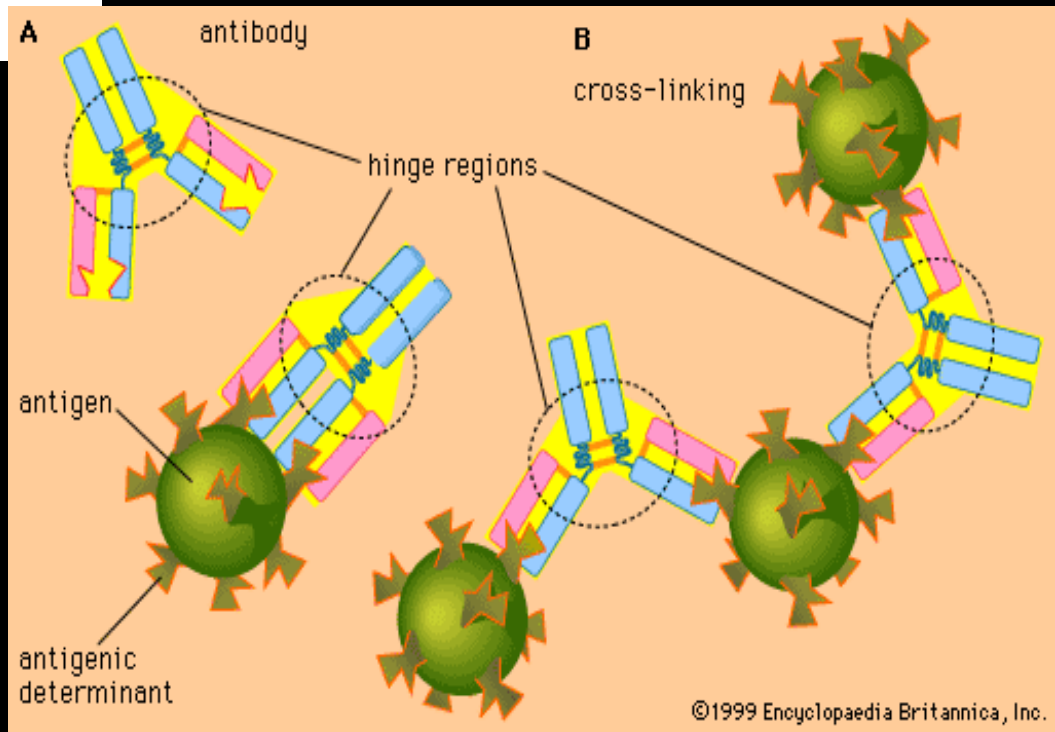
- Most of the immunogenos encountered in nature, including essentially all microbial pathogens and their metabolism substances, such as **exotoxin**, **enterotoxin** and **endotoxin** etc., are complex assemblages made up of several different types of molecules, not all of which are **antigenic.**

2)Epitopes

- An epitope is the specific site to which a particular Ig or TCR binds. It follows that every immunogen must contain one or more epitopes that enable it to serve as an Ag.
- Epitope = Antigenic Determinant = The part of an Ag molecule that interacts with the lymphocyte's receptor for Ag (B cells/ antibody (Ab); T cells /TCR).



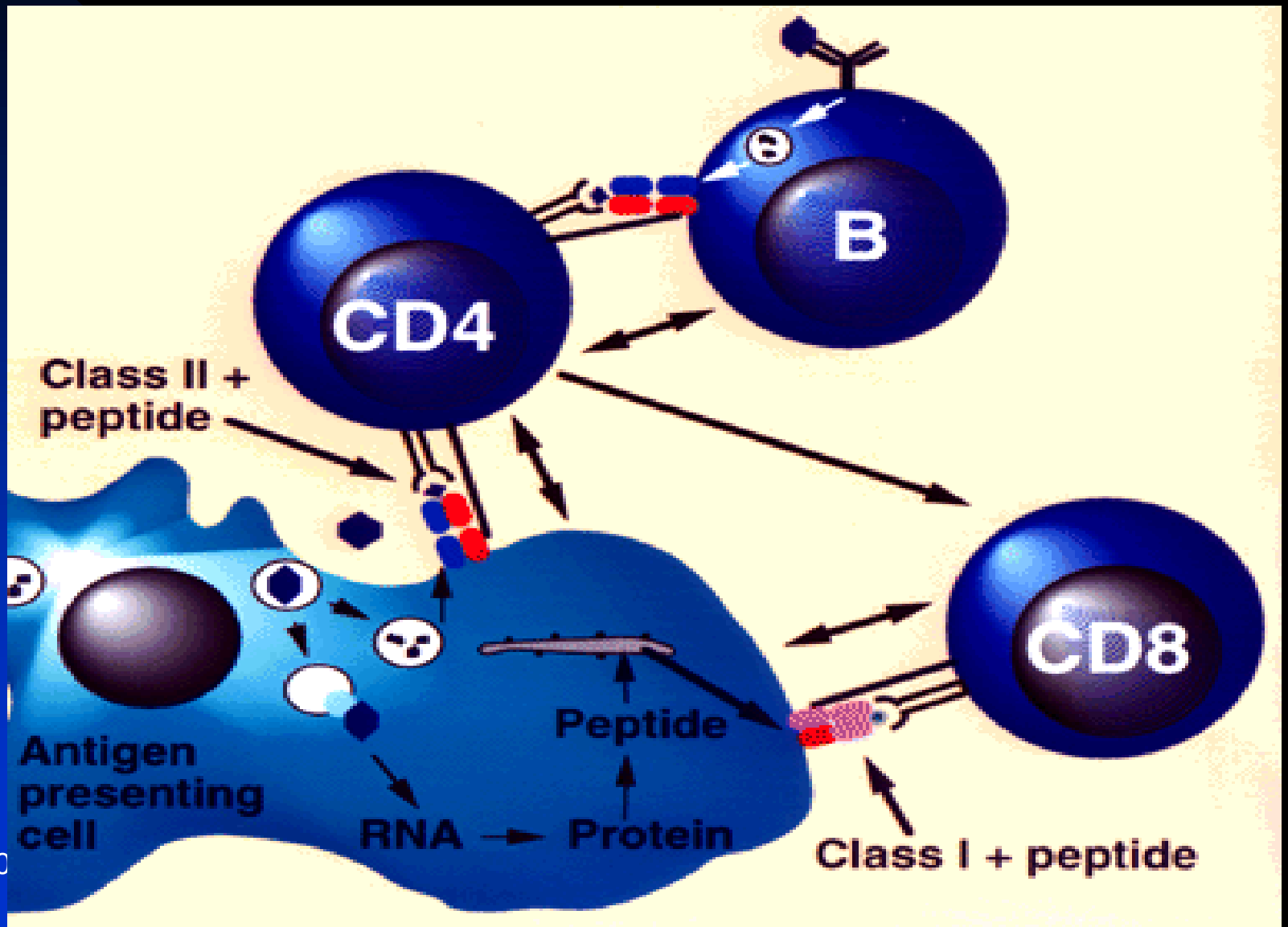
The triangles, square, and semi-circle represent a various epitopes.



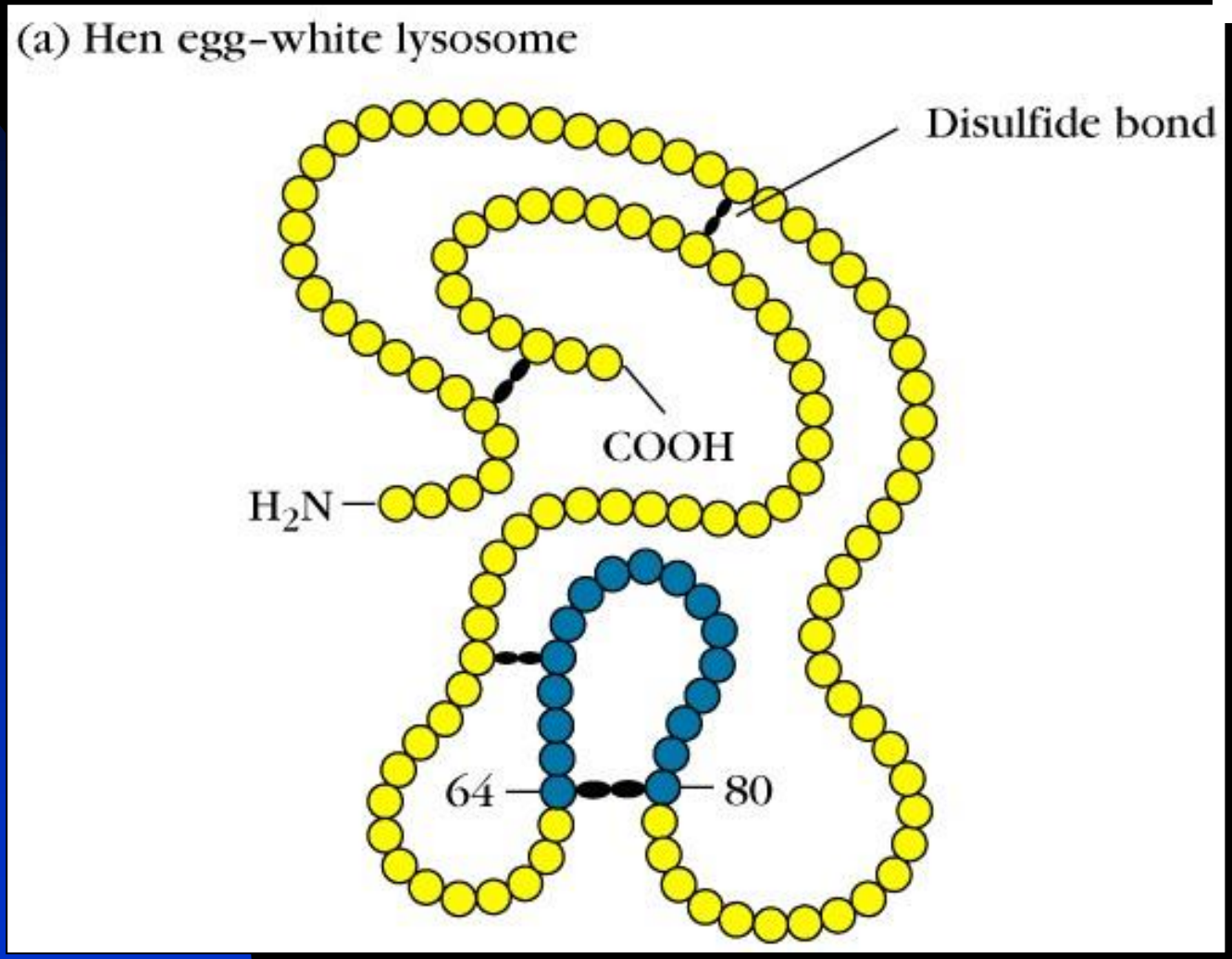
- **T and B cells Epitopes**
- **B cells and T cells "see" different parts of**
- **an Ag molecule (and in different ways).**

- **T cell Epitope as peptide presented by**
- **major histocompatibility complex (MHC) molecules.**

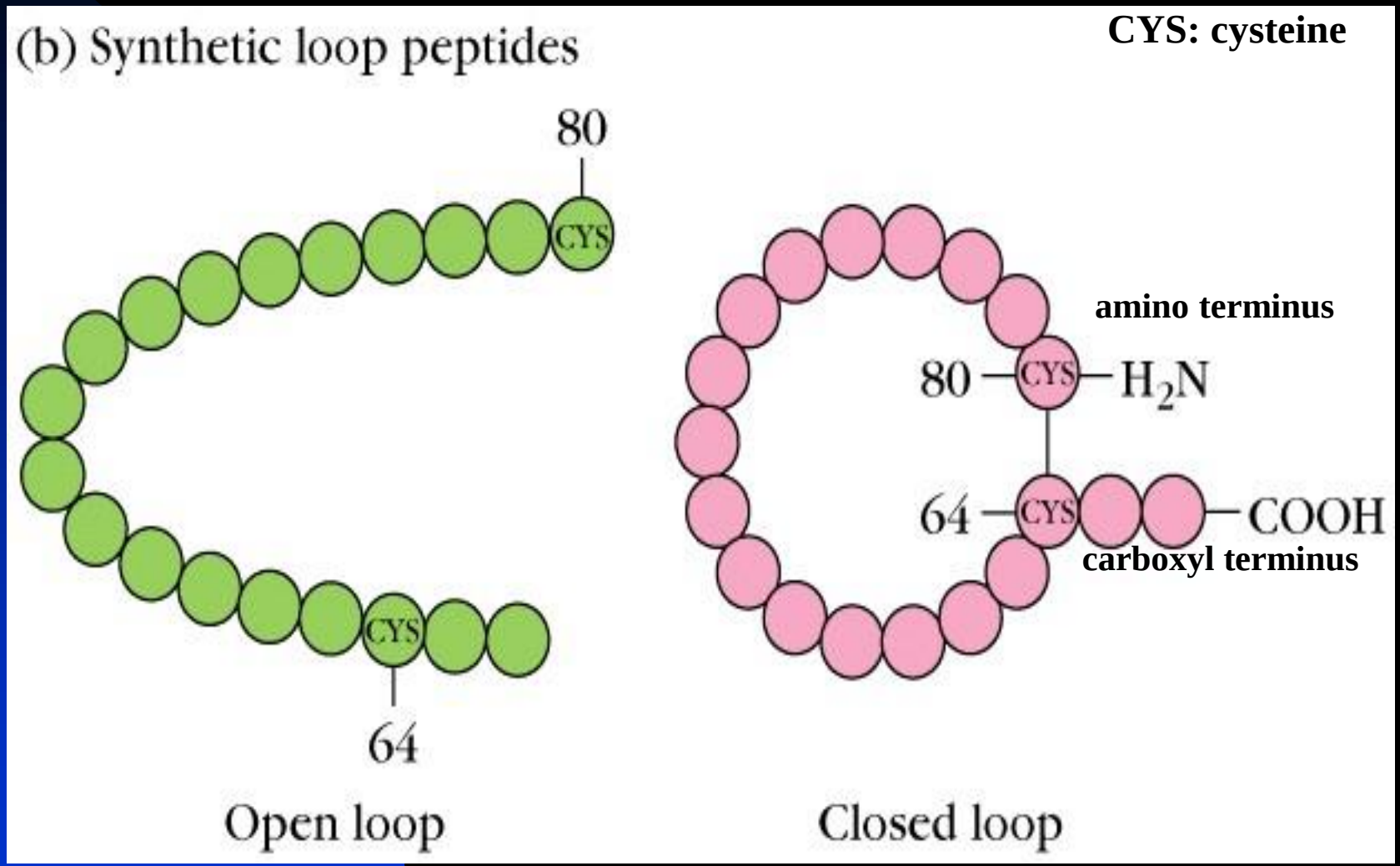
MHC mediated cell-cell interactions via Ag processing and presentation



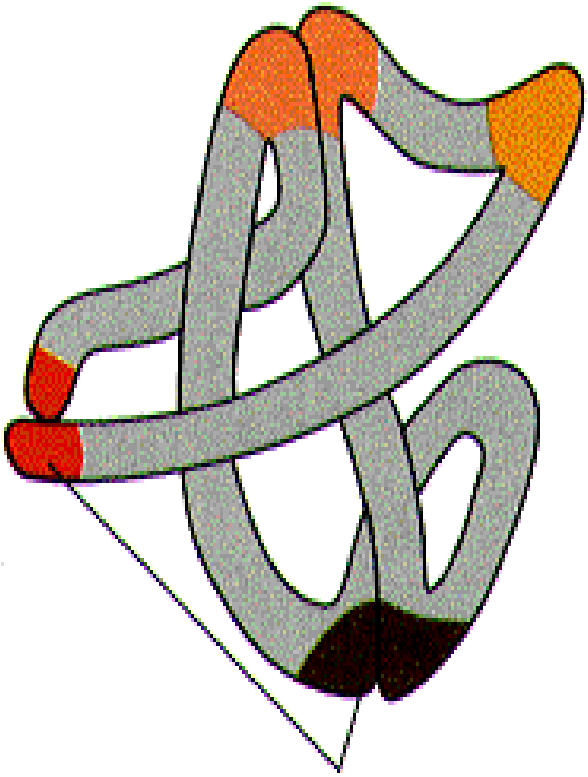
- B cell receptors see 3D or native parts of Ag.
- T cell receptors see linear peptides in the context of MHC molecules.
- B cells differentiate to plasma cells that secrete a form of the receptor for Ag.
- I.E. secreted Ab. T cells express only surface receptor for Ag, TCR.



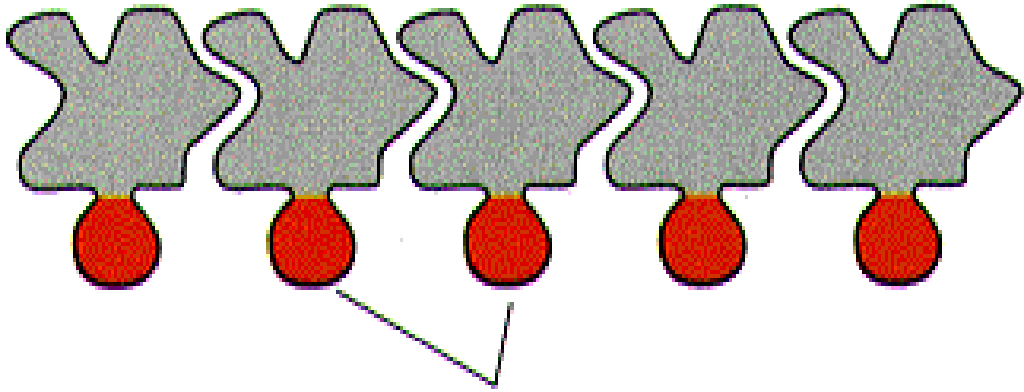
Conformational and liner epitopes in Ag.


(b) Synthetic loop peptides
CYS: cysteine
80
CYS
CYS
64
Open loop
amino terminus
80
CYS
H_2N
64
CYS
COOH
carboxyl terminus
Closed loop

Epitopes or antigenic determinants

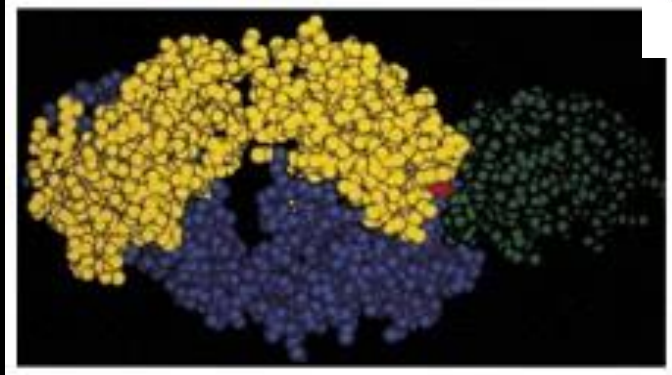


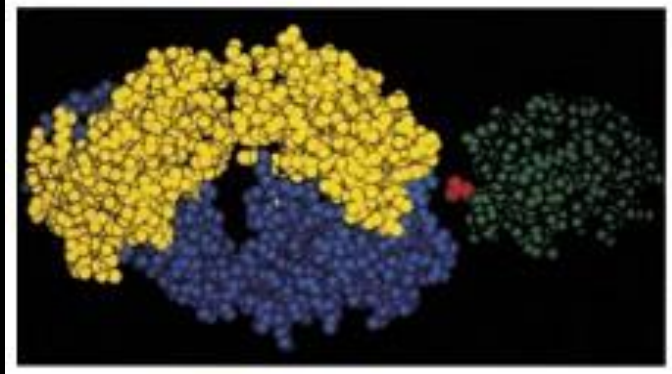
multiple different antigenic determinants

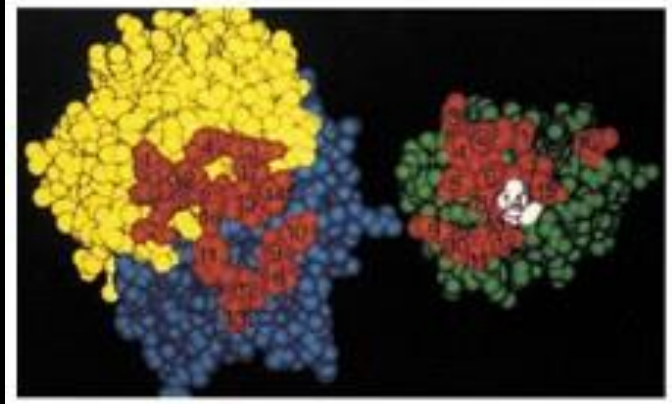


multiple identical antigenic determinants
(a multivalent antigen)

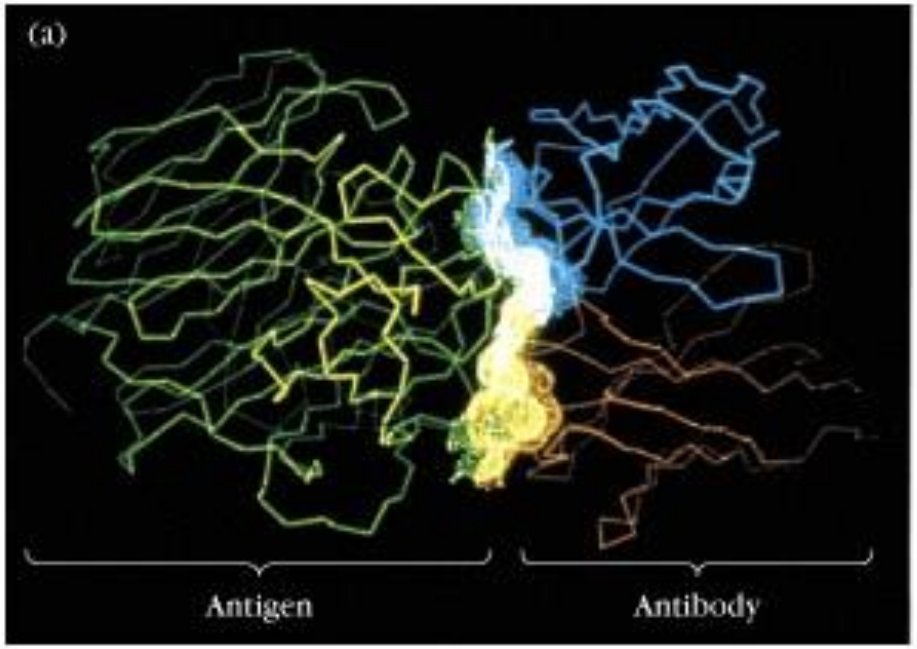
Three dimensions (3D) conformation

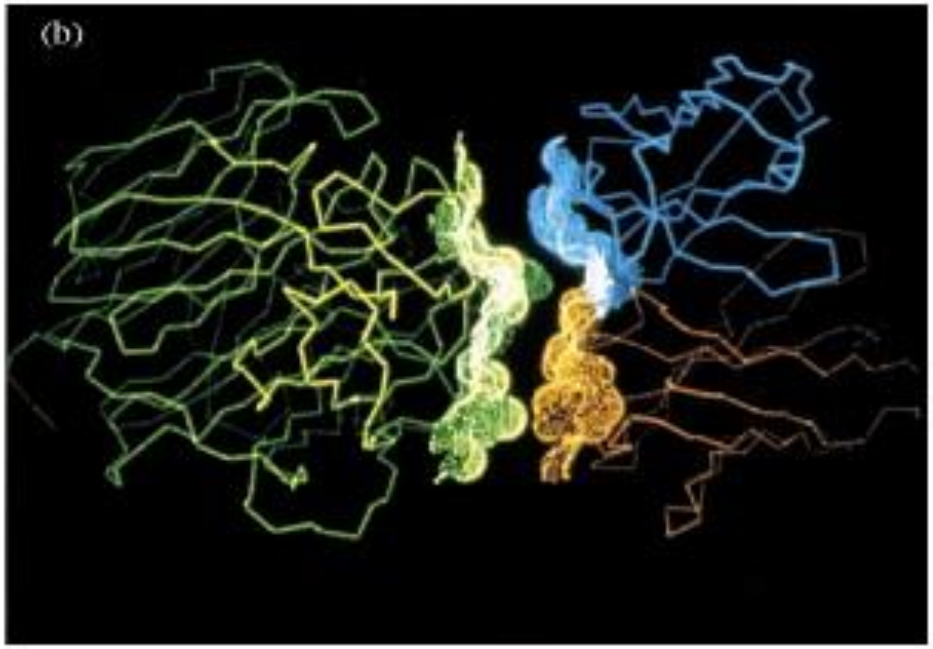






3D conformation





Ag reacts with antibody (Ab)

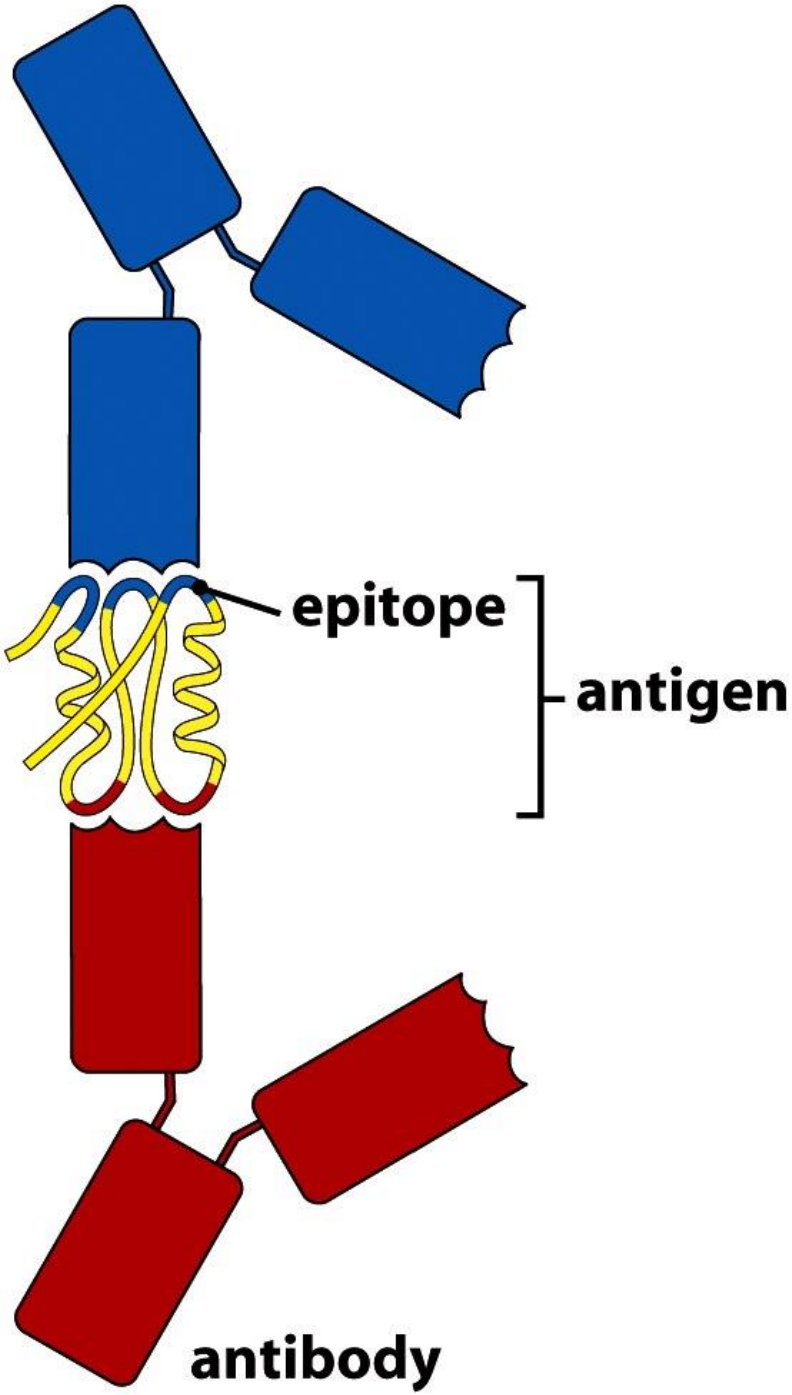


Figure 1-15 Immunobiology, 7ed. (© Garland Science 2008)

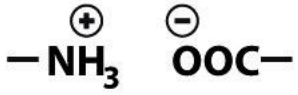
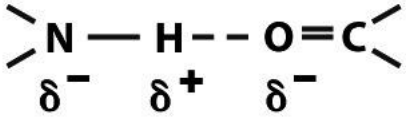
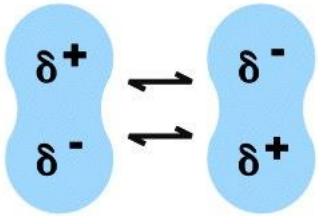
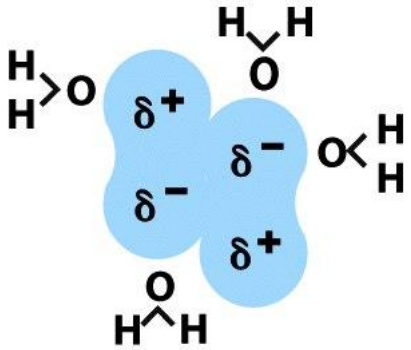
Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	
Hydrogen bonds	Hydrogen shared between electronegative atoms (N,O)	
Van der Waals forces	Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	

Figure 3-9 Immunobiology, 7ed. (© Garland Science 2008)

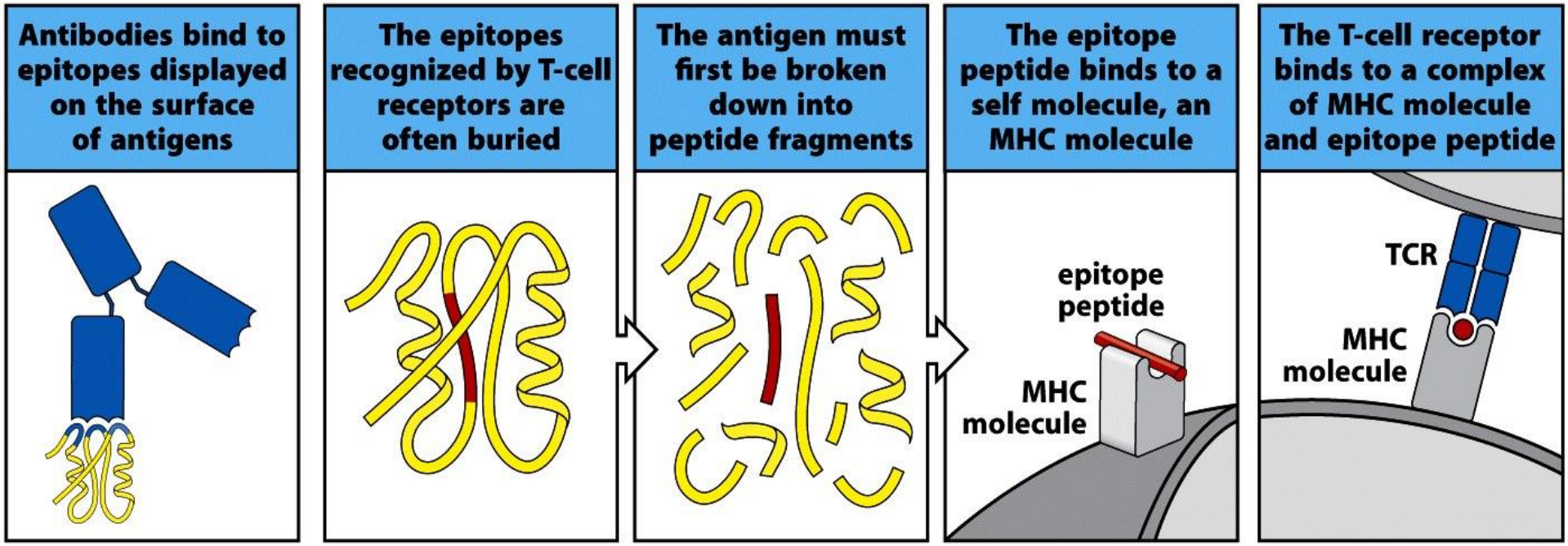


Figure 1-16 Immunobiology, 7ed. (© Garland Science 2008)

Polyclonal antibody

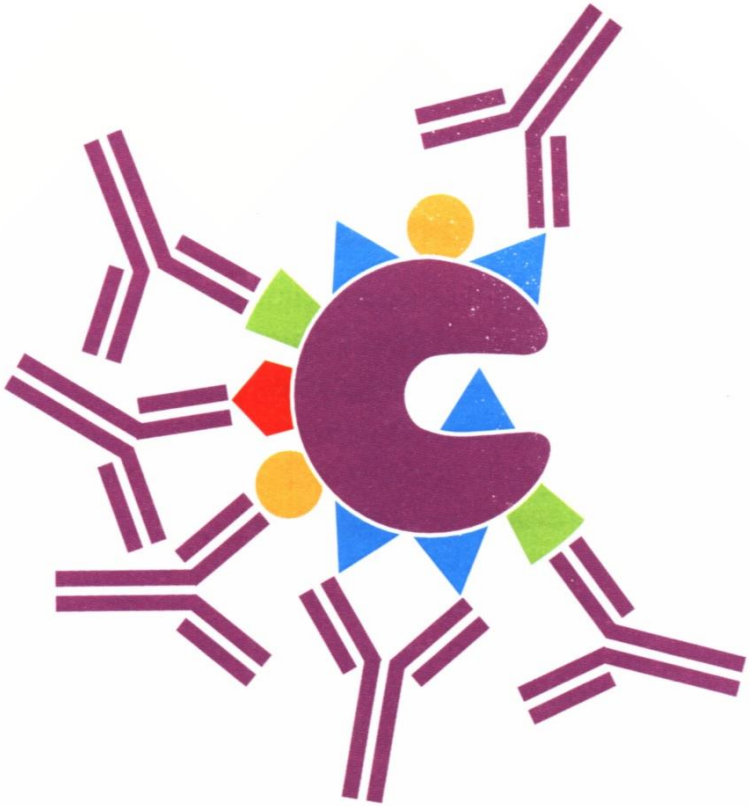


Figure 4: Schematic diagram of polyclonal antibodies binding to various epitopes on an antigen.

Monoclonal antibody (McAb)

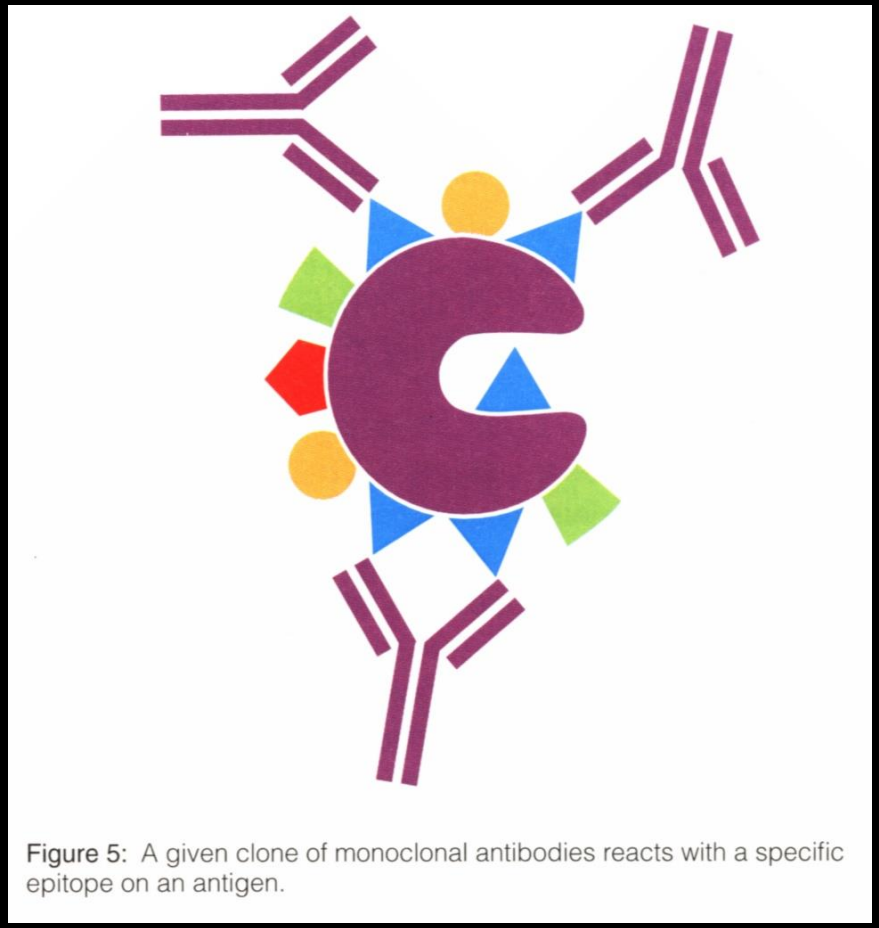


Figure 5: A given clone of monoclonal antibodies reacts with a specific epitope on an antigen.

Do T cells“care”about secondary structure of proteins?

TABLE 3-4 ANTIGEN RECOGNITION BY T AND B LYMPHOCYTES REVEALS QUALITATIVE DIFFERENCES

		Secondary immune response	
Primary immunization	Secondary immunization	Antibody production	Cell-mediated T_{DTH} response*
Native protein	Native protein	+	+
Native protein	Denatured protein	−	+

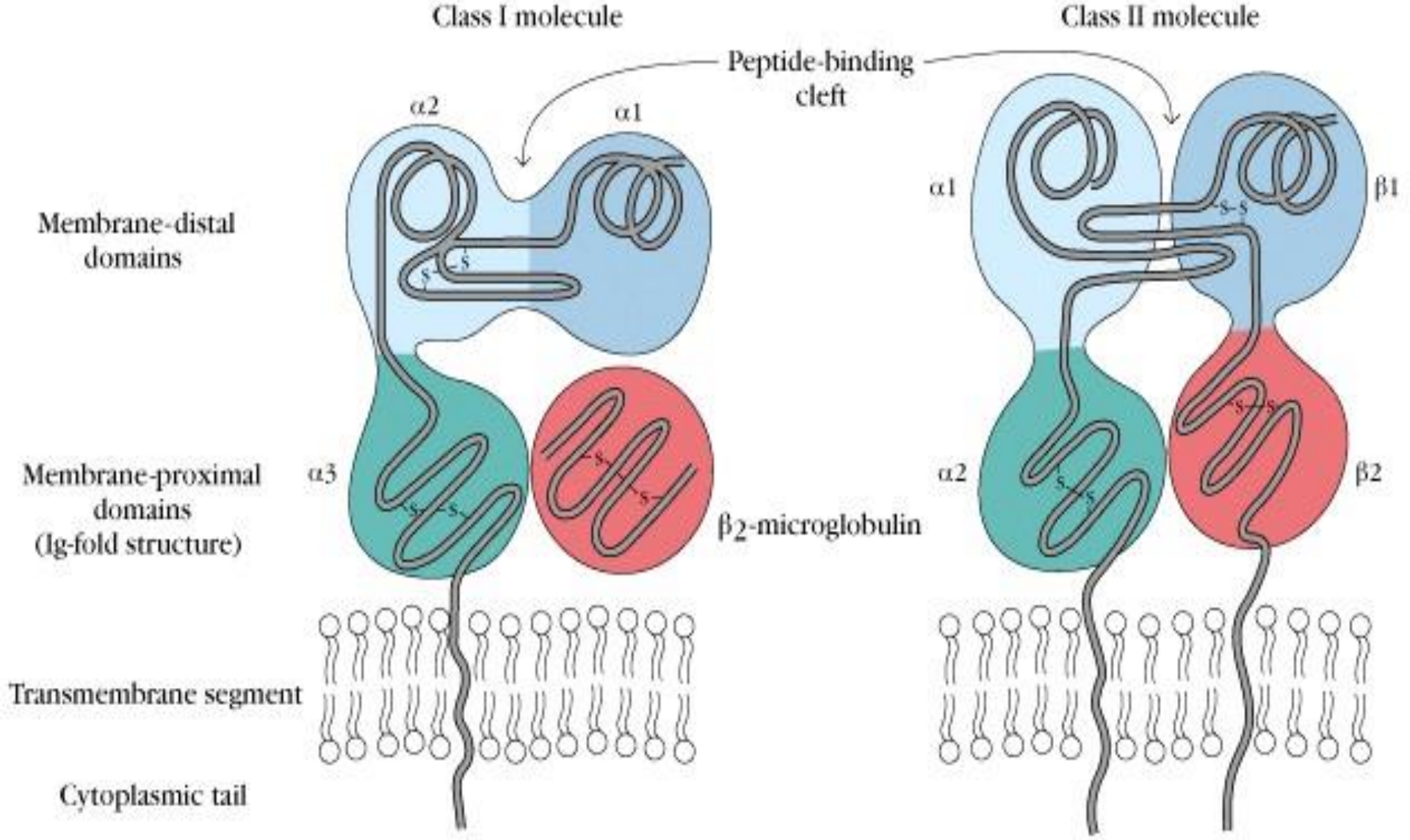
*T_{DTH} is a subset of $CD4^+$ T_H cells that mediate a cell-mediated response called delayed-type hypersensitivity (see Chapter 14).

Immunization: deliberate stimulation of the host's immune response

TABLE 3-3 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

Structure of MHC Molecules. Domains

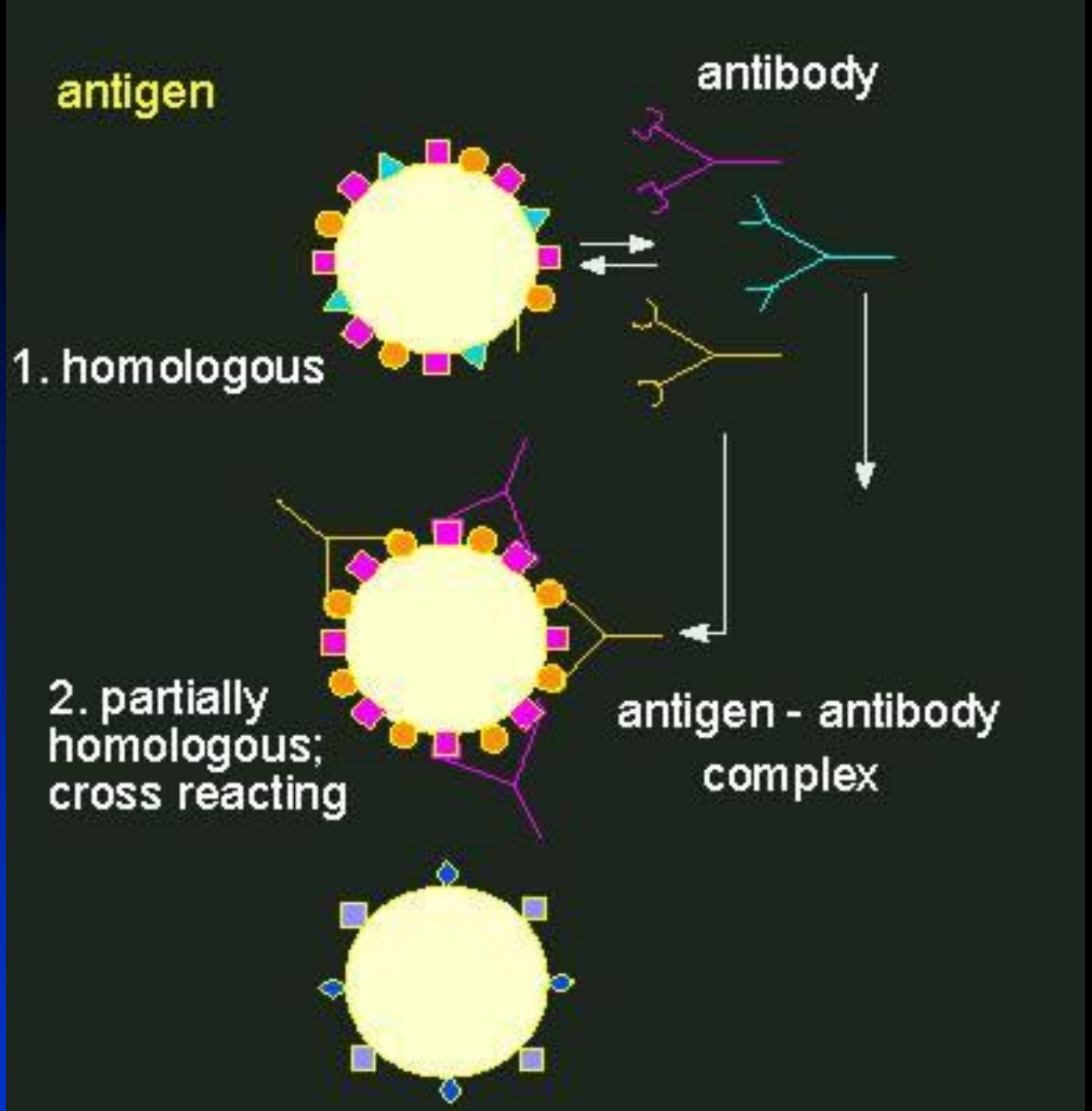


Ig family member. Ig fold in membrane proximal domains

3) Common Ag and Cross Reaction

- Some Ag can not only react with Ab or activate lymphocytes by themselves to induce an immune response, but also can react with Ab or activate lymphocytes by other inducing, there Ag frequent strip multi-Ag epitope.

- These different Ag contain sameness or similitude Ag epitope is common Ag.


antigen
antibody
1. homologous
2. partially homologous; cross reacting
antigen - antibody complex
3. heterologous ; not cross reacting

3. Factors influencing immunogenicity

1) **Physicochemical properties of Ag.**

Protein are usually the most effective immunogens.

- **Size** or weight
- **Foreignness** (donkey, horse, monkey, etc.)
- Chemical composition and heterogeneity
- Susceptibility to **Ag** processing and presentation

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

Polysaccharides, short polypeptides, and some synthetic organic polymers can also be immunogenic under certain circumstances.

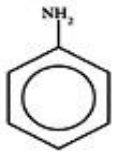
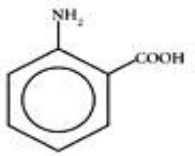
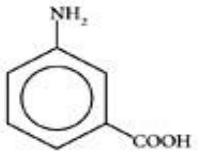
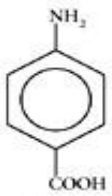
- **Nucleic acid** and **lipids** from mammalian cells are not immunogenic, but **Ab** that react with them can be elicited by immunization with **nucleoprotein** or **lipoprotein** complexes, this is probably the mechanism of origin of **anti-DNA Ab** found in the serum of many patients, such as systemic lupus erythematosus (**SLE**).

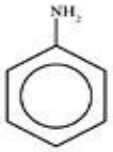
- Thus, nucleic acids and most lipids are examples of molecules that are antigenic
- but not immunogenic.

- Growing evidence has shown that DNA,
- RNA, etc. can serve as a "danger signals".

- DAMPs: danger/damage-associated
- molecular patterns /pathogens(PAMPs)
- PAMPs-PRRs

TABLE 3-5 REACTIVITY OF ANTISERA WITH VARIOUS HAPTENS

	Reactivity with			
				
Antiserum against	Aminobenzene (aniline)	*o*-Aminobenzoic acid	*m*-Aminobenzoic acid	*p*-Aminobenzoic acid
Aminobenzene	+++	0	0	0
o-Aminobenzoic acid	0	+++	0	0
m-Aminobenzoic acid	0	0	++++	0
p-Aminobenzoic acid	0	0	0	+++/+++

	Reactivity with			
				
Antiserum against	Aminobenzene (aniline)	*p*-Chloroamino benzene	*p*-Toluidine	*p*-Nitroamino benzene
Aminobenzene	+/++	+	+±	+
p-Chloroaminobenzene	+++	++	++	+/++
p-Toluidine	++/+	++	++	+
p-Nitroaminobenzene	+	++	+/++	+

KEY: 0 = no reactivity; +++ and ++++ = strong reactivity; ++ and + = lesser degrees of reactivity

SOURCE: Based on K Landsteiner, 1962, *The Specificity of Serologic Reactions*, Dover Press. Modified by J Klein, 1982, *Immunology: The Science of Self-Nonself Discrimination*, John Wiley.

2) Specificity of Ab Binding to hapten (half Ag) determinant

3) Effect of Host

The ability to respond to a particular immunogen is genetically predetermined. For example, pure polysaccharides are immunogenic when injected into mice or human adults but not when injected into guinea pigs or rabbits.

Selective responsiveness of this type reflects a number of hereditary factors.

4) Mode of Contact

Whether a substance will evoke an immune response also depends on the dosage and the route by which it enters the body.

1) Route of administration.

- Injection of intravenousiv (i.v.)
- Injection of subcutaneously (s.c.)
- Injection of intramuscularis (i.m.)
- Injection of intraperitoneal (i.p.)

An immunogen that contacts the intestinal mucosa typically evokes the production of a different type of Ab than would be produced.

If it entered though the bloodstream, and this can affect subsequent events in the immune response.

2) Dose

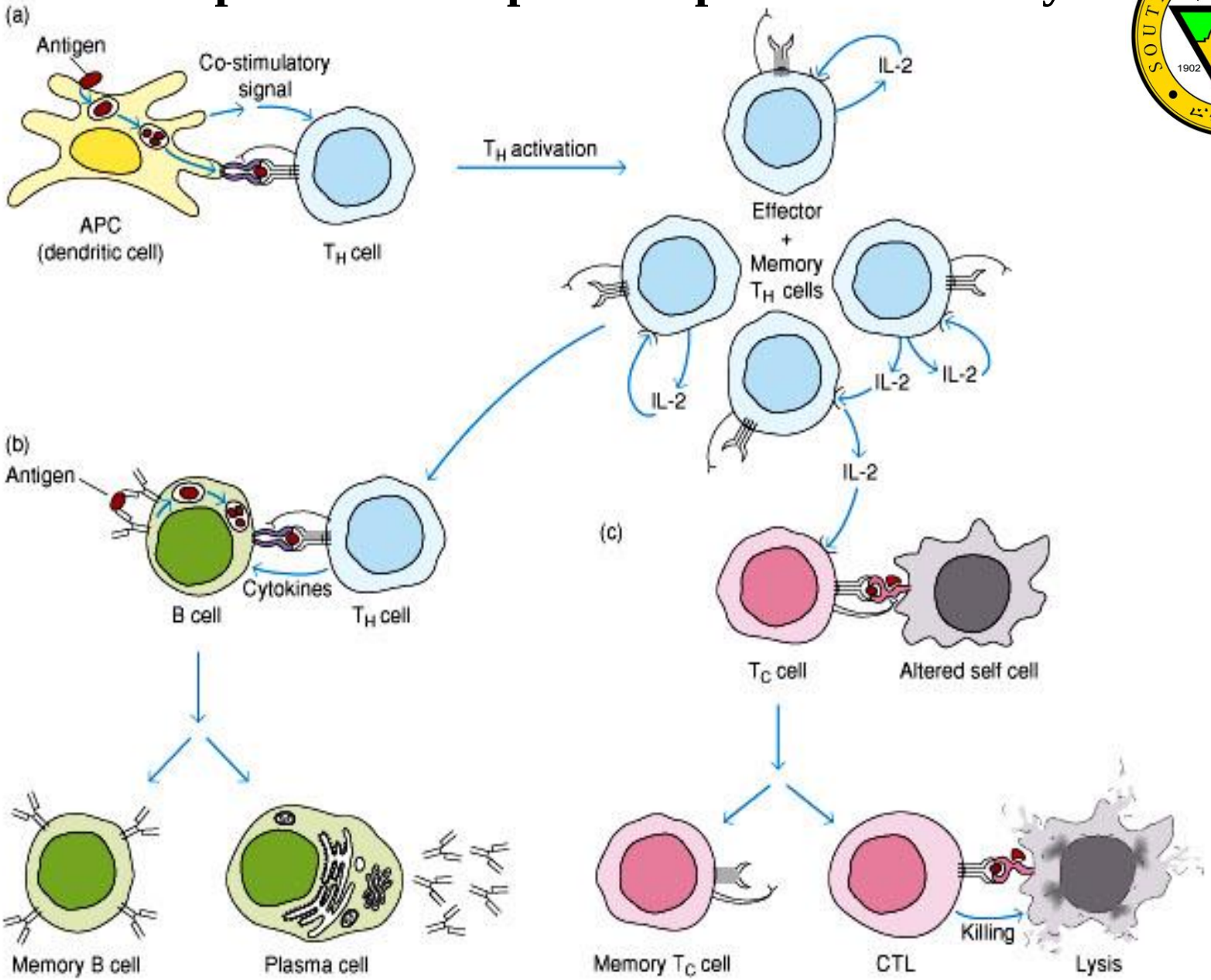
The threshold dose required for a response under particular conditions varies among immunogens. In general, once the threshold dose is exceeded, increasing dose leads to increasing response, though less than proportionate response. Excessive doses, however, may not only fail to induce a response but may instate establish a state of specific unresponsiveness or tolerance.

4. Classification of antigens

1) TD-Ag

Generally, Ag contact alone is insufficient to activate B cells because most protein Ag depends on both T cells and B cells recognizing the Ag in a linked fashion. This type of Ag is called T-dependent Ag (TD-Ag).

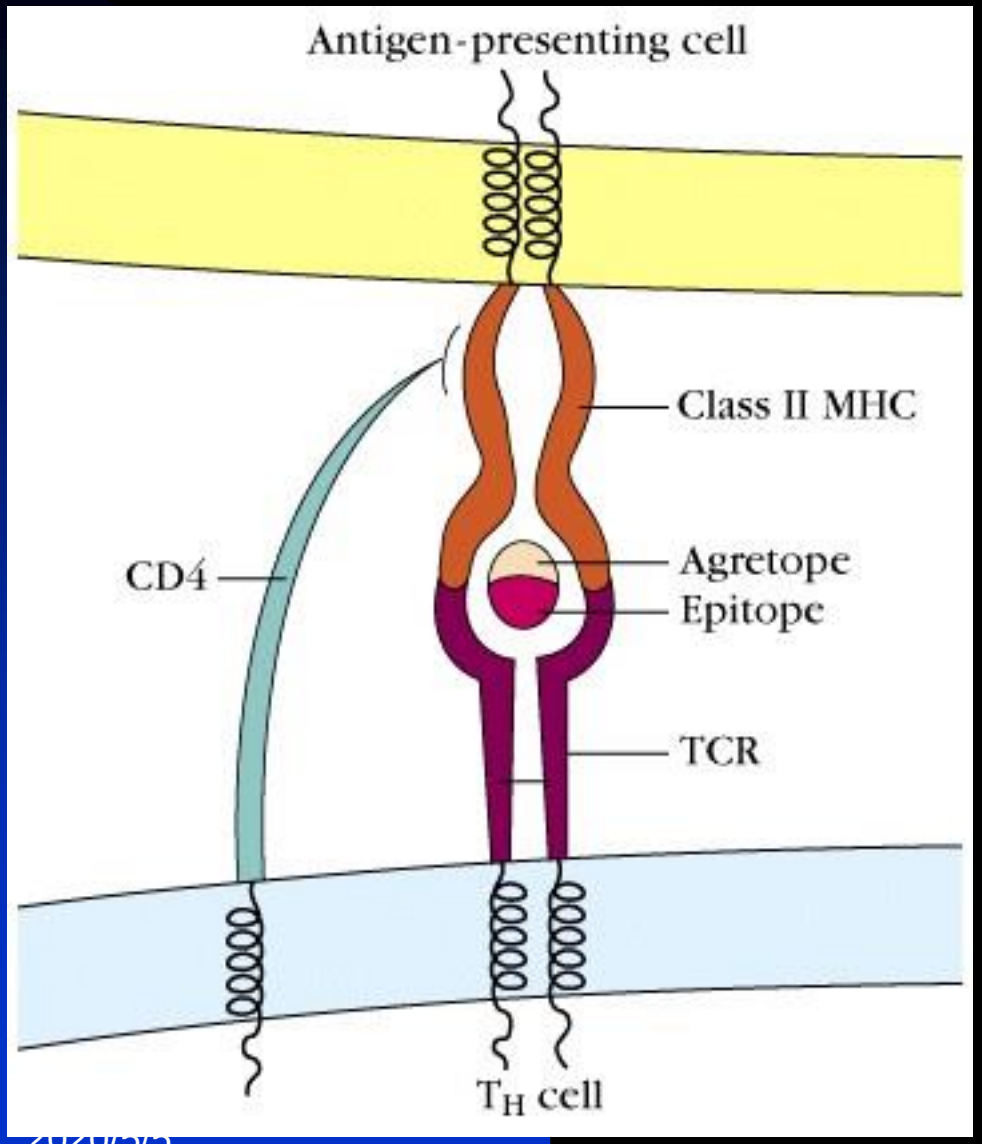
Helper T cells help B cells produce antibody

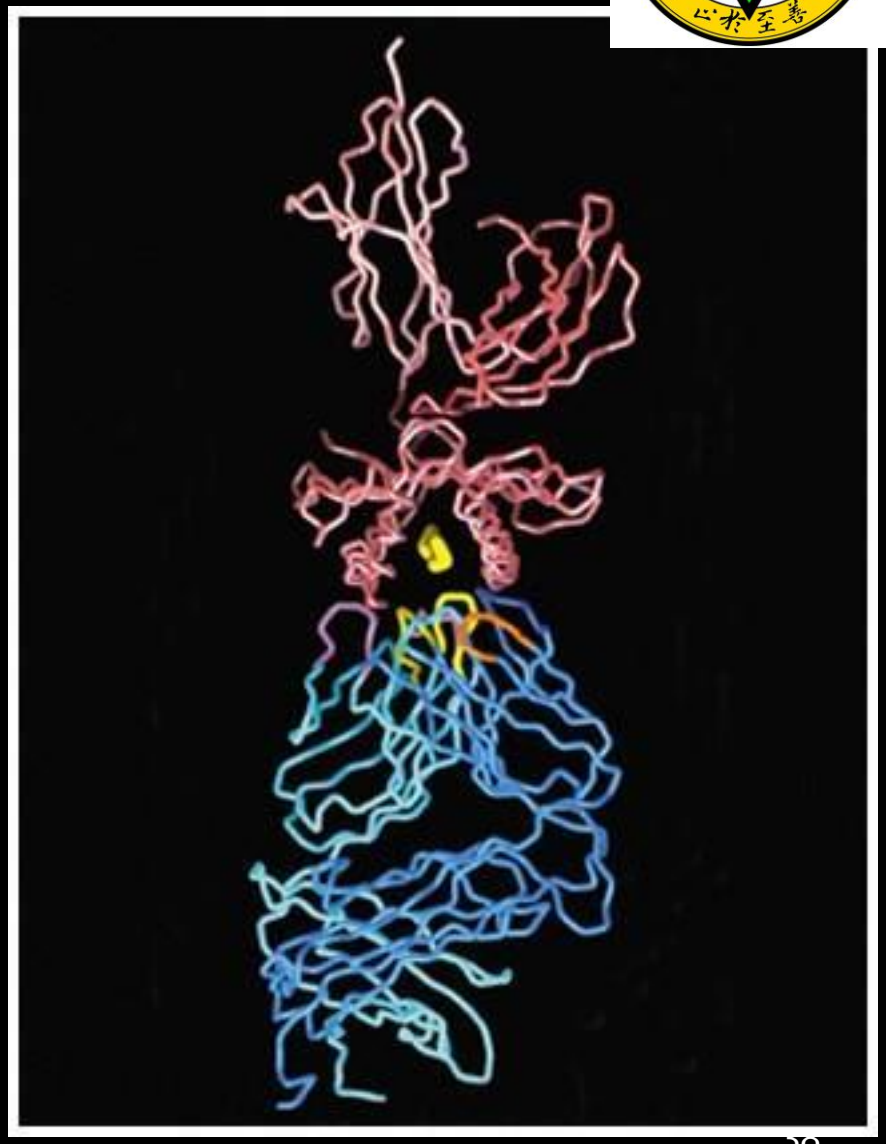


- By manipulating the cell populations in these experiments, it is shown that **Th cells** are responsible for recognizing the carrier, whereas the **B cells** recognize **hapten** (incomplete **Ag**).

- The secondary responses to **TD-Ag** is far stronger and has a large **IgG** component.

Recognition of Antigen by T cells via MHC





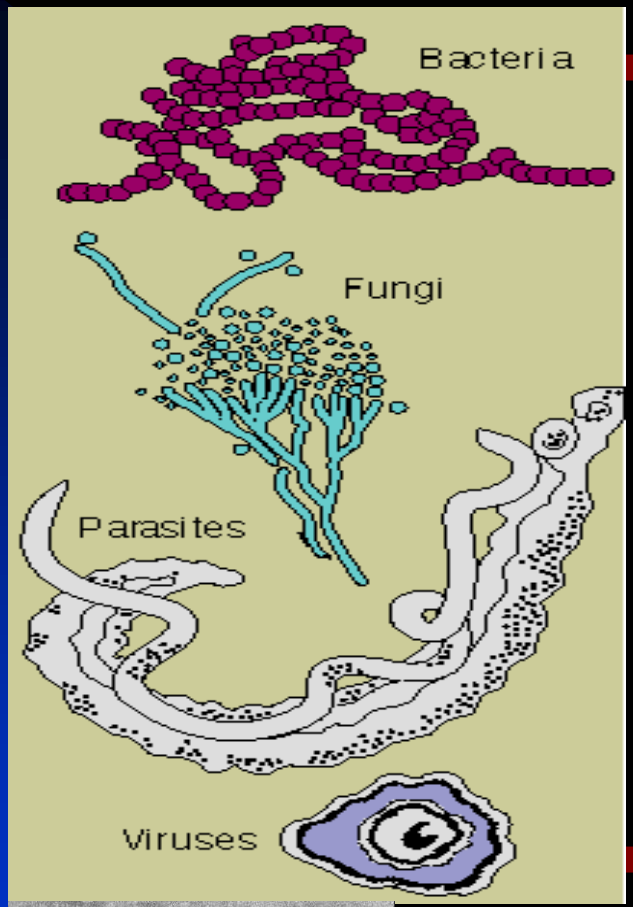
- **2) TI-Ag**
- **Some Ag do not require the presence of**
- **helper T cells, and these Ag are called T-independent Ag (TI-Ag), which typically fall into either of two categories, with different mechanistic properties.**

- **The first group, called TI-1 Ag, such as**
- **lipopolysaccharide (LPS), from gram negative bacterial cell walls , can induce**

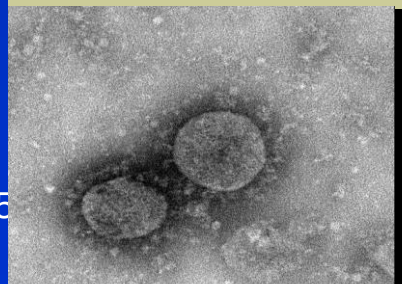
immunological defense reactions in a number of invertebrate as well as vertebrate organisms. Mammalian cells recognize LPS with Toll-like receptor (TLR4) and several other bacterial cell wall components with the closely related TLR2.

- **The secondary group, called TI-2 Ag.**
- **In contrast, TI-2 Ag, do not have polyclonal B cell activator properties, nor do they activate macrophage. These Ag are generally highly repetitive polymeric Ag such as polysaccharides from bacterial cell wells, or polymeric protein structures such as bacterial flagella.**

Pathogens (Ag)
4 main groups of infectious organisms



- *Bacteria*
  - prokaryotic
  - Gram^{+} & Gram^{-}
- *Fungi*
  - Eukaryotic: single-celled & multi-cellular
  - yeasts, molds
- *Parasites*
  - host dependent
  - worms & protozoa
- *Viruses*
  - replicate only in living cells
  - RNA & DNA viruses



2019-nCoV (RNA virus)

- **5. Classification based on relationship**
- **with host**
- **1) Heterophilic Ag is a kind common Ag, exists in human, animals and microorganism, as well as Forssman Ag. For example, there are common Ag in a strain hemolytic streptococcus surface component and cardiac muscle self-tissue, therefore, hemolytic streptococcus-induce Ab may crossly react with heart, kidney tissue, and results in nephritis and cardiac muscle inflammation.**

- **2) Xenogenic Ag come from different**
- **genus and generic. An important xenogenic Ag are pathogenic microorganism, plant, protein, animal serum and heterogeneity organ implant, and so on.**

- **3) Allogenic Ag, the specific Ag exists in**
- **different individual of same genus. The human allogenic Ag include: blood type Ag, included ABO red cell blood type Ag, Rh Ag system, and**

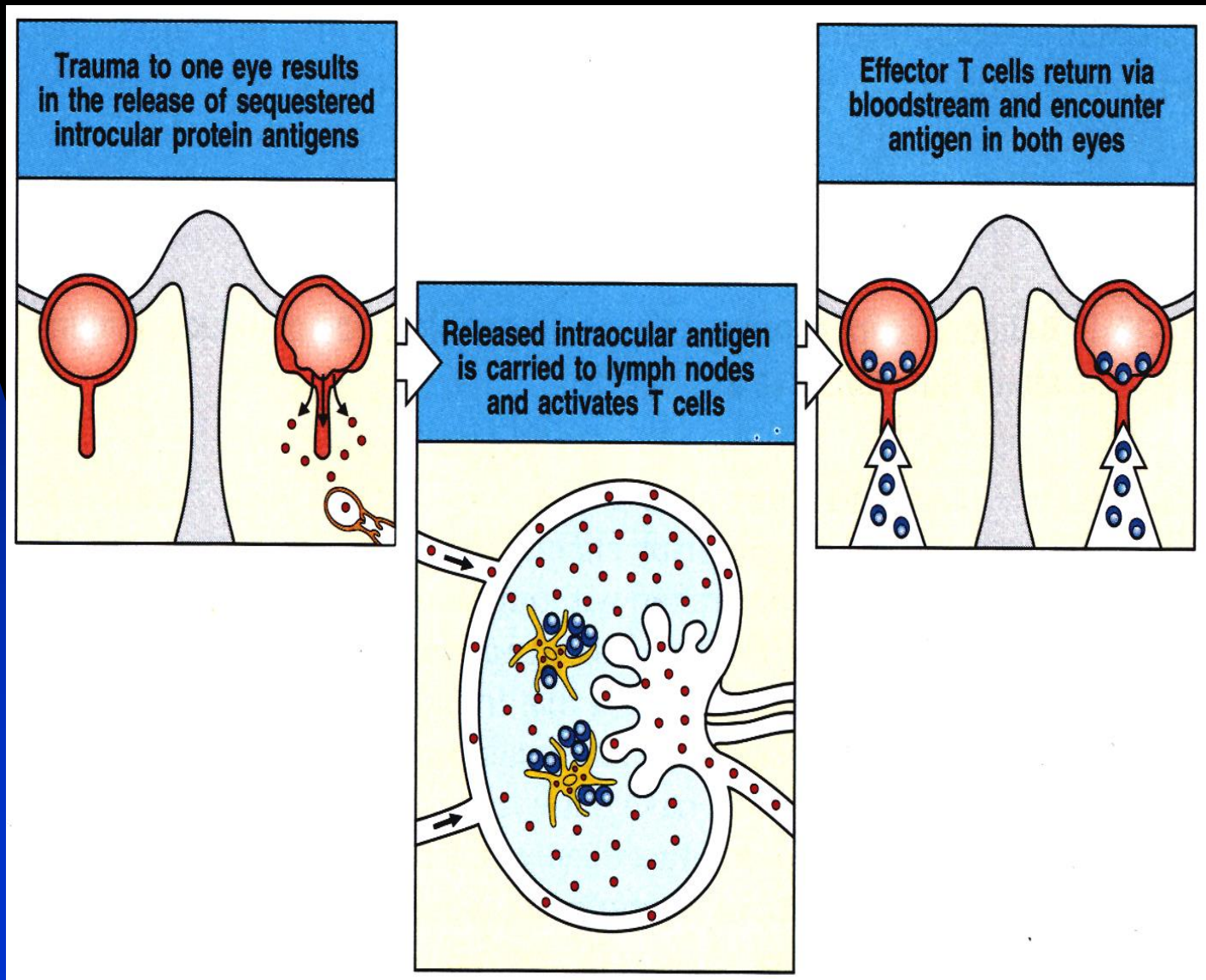
- human leukocyte Ag (HLA) etc.

- 4) Autoantigen.
- In common, immunity system does not response against self-tissues or cells, in other words, it gets tolerance to its own body.

- In some pathology (such as enshrouded or isolated Ag; self-Ag occur changed or decorated), oneself component can induce body to self immune response.

Release of Sequestered Antigen from Immunoprivileged Site

- The eye is not normally "sampled" by T cells
- Trauma to the eye can release antigens unique to the eye (not presented in the thymus)
- These antigens can be brought to lymph nodes where they activate T cells.
- Primed T cells can traffic through privileged sites and cause tissue damage if they recognize antigen



5) Idiotypic antigen Ab's idiotype

Endogenous Ag

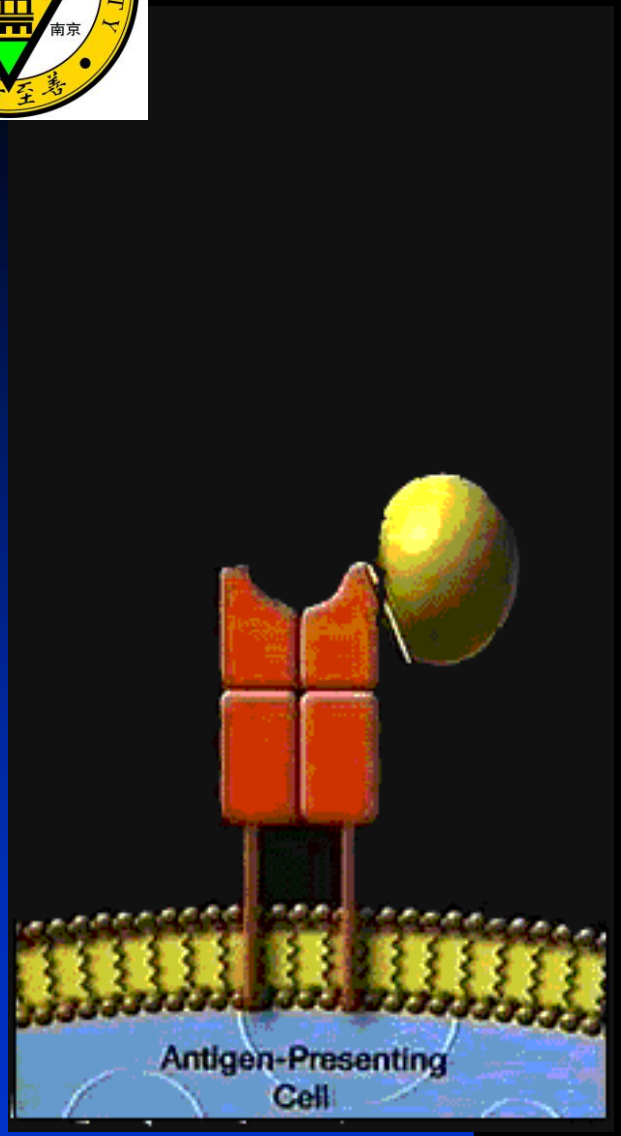
Exogenous Ag

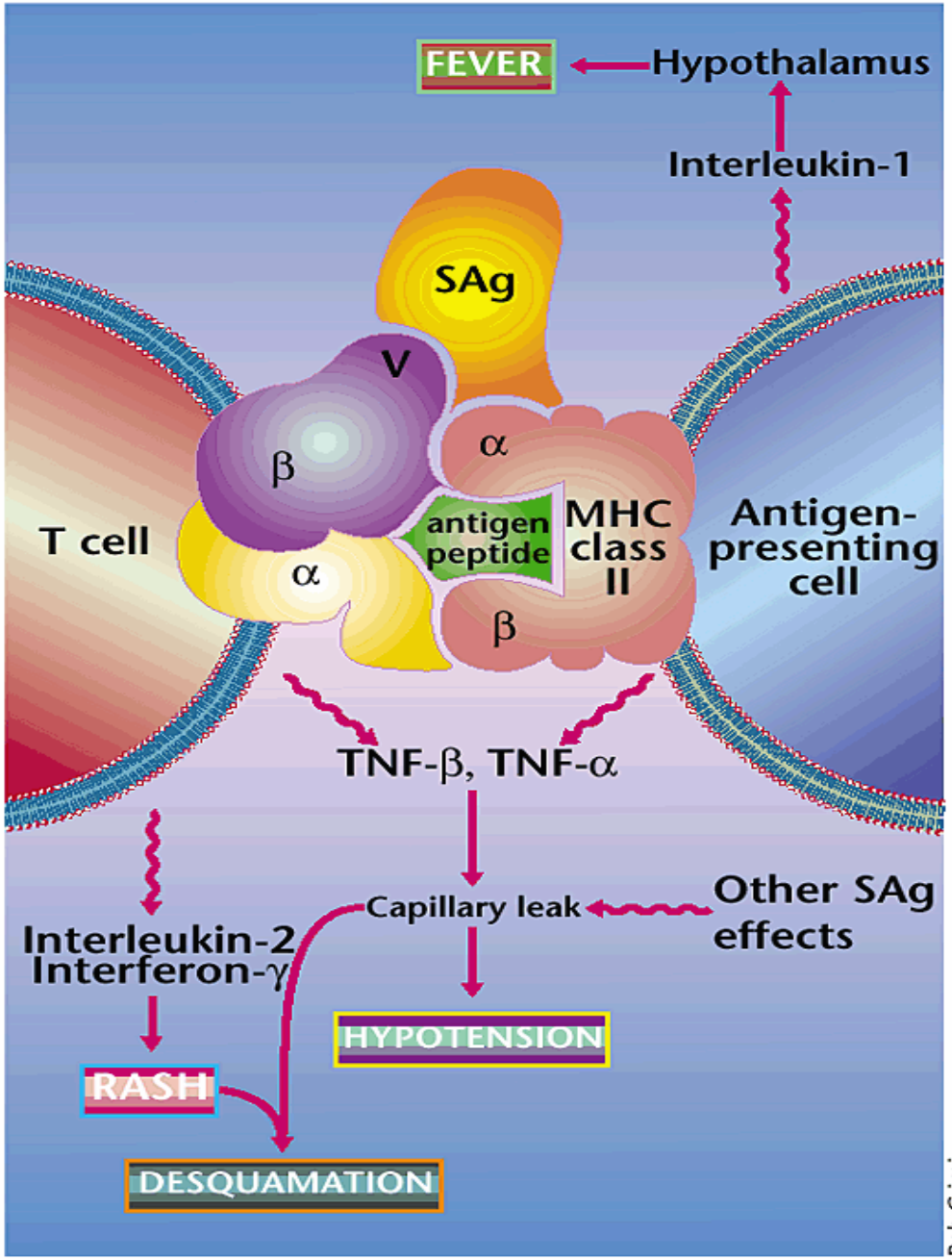
TSA, TAA

Artificial Ag, Synthetic Ag, etc.

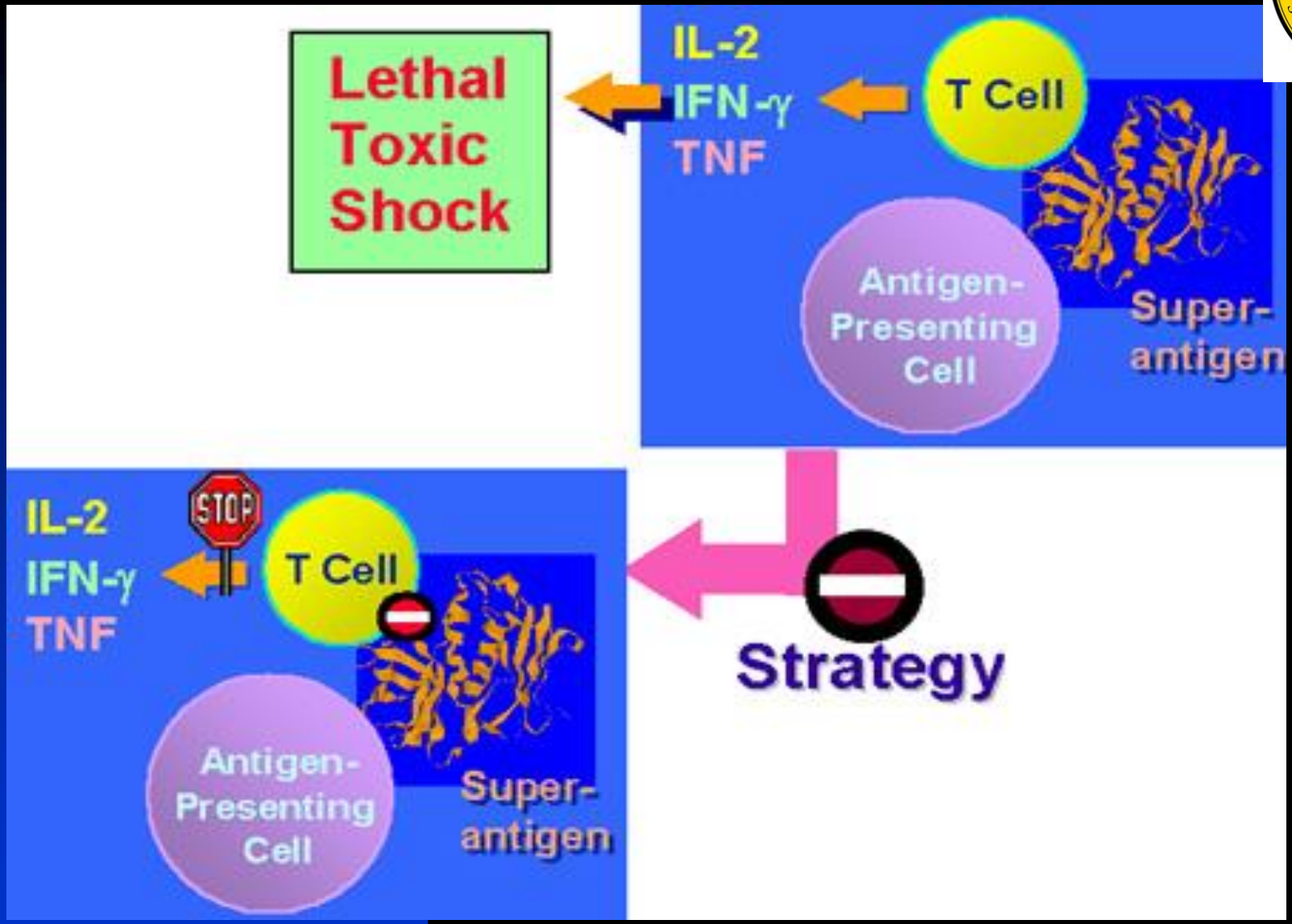
6. Non-Specific Stimulators

- **Super Ag.**
- **Superantigens (SAg) are a class of bacterial toxins and retroviral proteins that have the ability to bind MHC class II molecules and the TCR β chain. In so doing, they act as a "clamp" between the TCR and class II molecule, providing signals to the T cells.**





Moving figure


Lethal
Toxic
Shock
IL-2
IFN-γ
TNF
T Cell
Antigen-
Presenting
Cell
Super-
antigen
Strategy
STOP
IL-2
IFN-γ
TNF
T Cell
Antigen-
Presenting
Cell
Super-
antigen

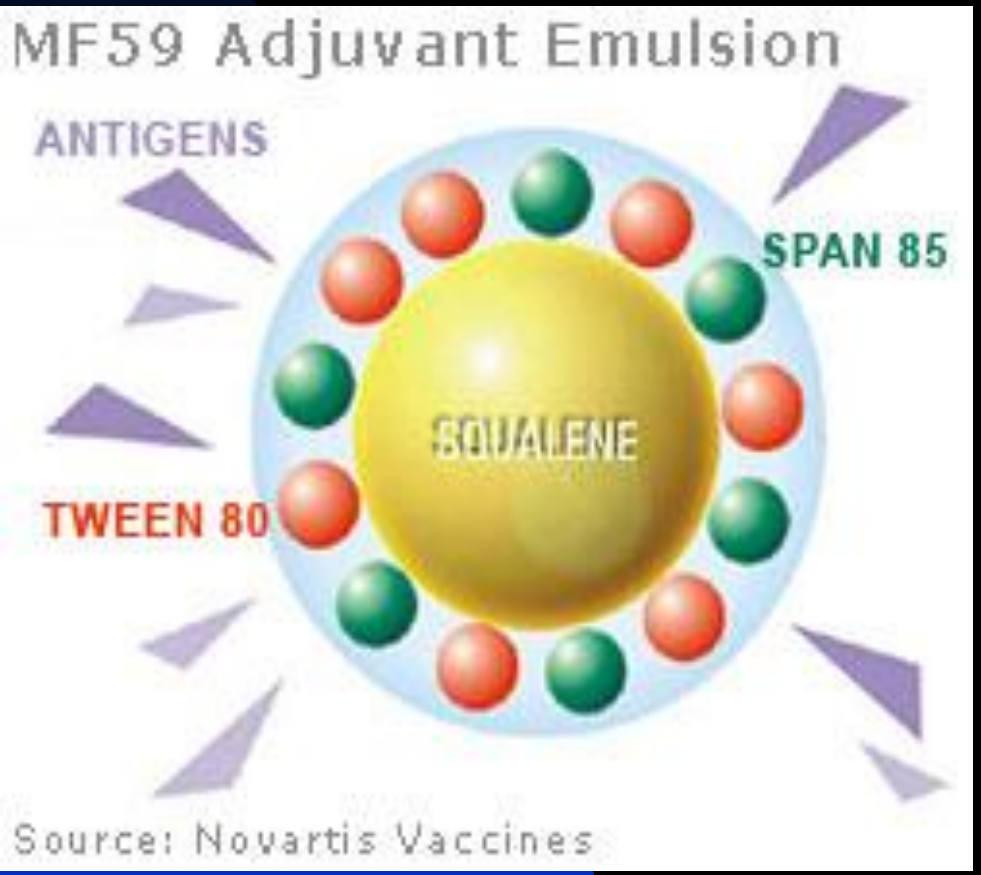
- **Adjuvants**
  - Antigen persistence
  - Co-stimulatory signals
  - Granuloma formation.
  - Activate macrophages /dendritic cells
  - Activate polyclonally or nonspecifically.
  - Cytokines.

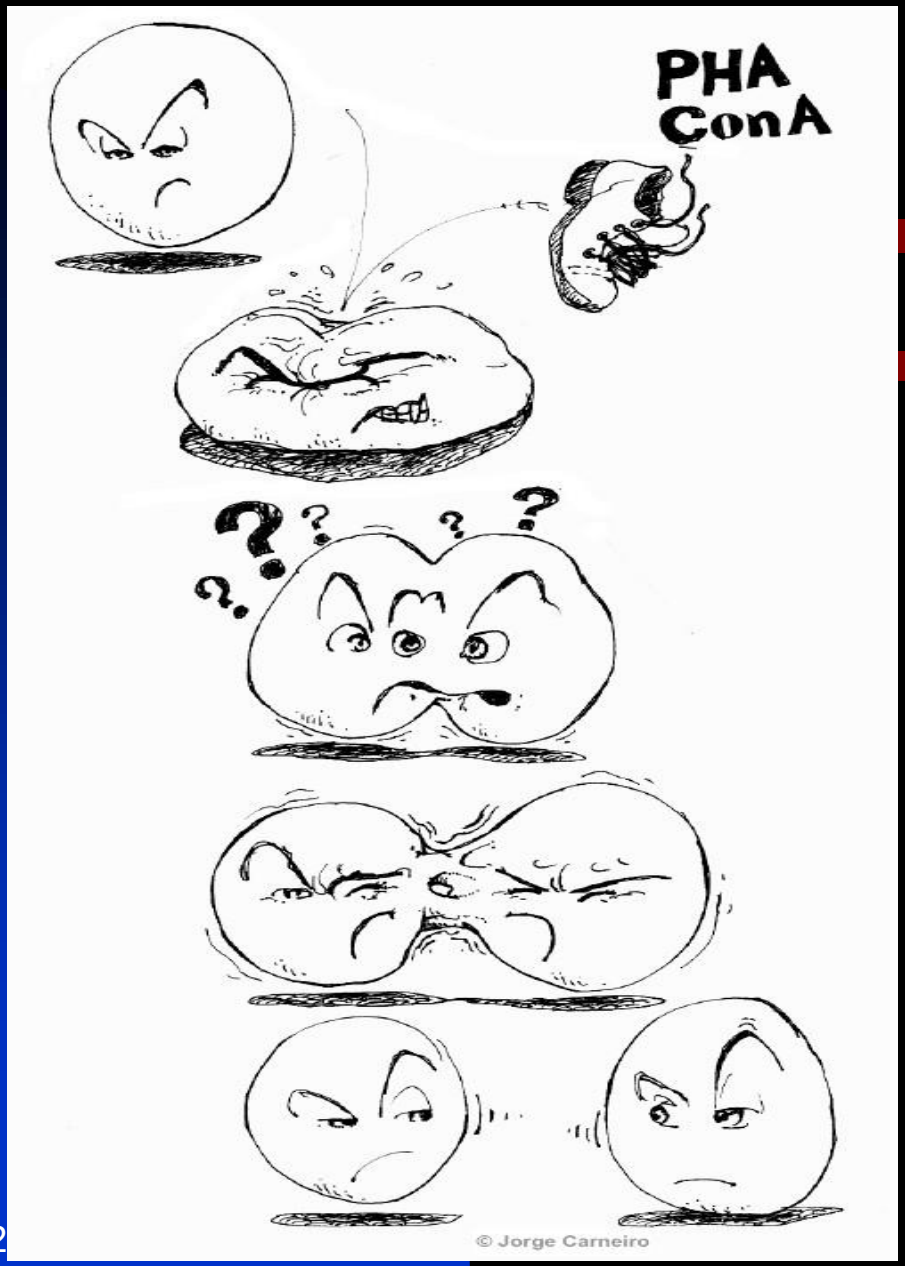
Adjuvants

TABLE 3-2 POSTULATED MODE OF ACTION OF SOME COMMONLY USED ADJUVANTS

Adjuvant	Postulated mode of action			
	Prolongs antigen persistence	Enhances costimulatory 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)	−	?	−	+

Adjuvants


MF59 Adjuvant Emulsion
ANTIGENS
SPAN 85
SQUALENE
TWEEN 80
Source: Novartis Vaccines



Mitogen

Activation can also be induced under artificial conditions by **cross-linking** other types of surface molecules.

Reference books:

Medical Immunology, by Yunqing An and Zhi ao. 2017-2. ISBN: 978-7-5659-0750-0.

Primer to the immune response ---Tak Mak et al
Immunology ------ Roitt et al
Kuby Immunology--- Goldsby et al
Immunobiology ----- Janeway et al

Short notes:

1. Epitopes or antigenic determinants
2. T and B cells Epitopes
3. Common Ag and Cross Reaction
4. Heterophilic Ag and Autoantigen
5. Hapten

Questions:

1. Comparison of TD-Ag Versus TI-Ag, please!
2. How to understand the Super Ag?
3. How to understand the enshrouded Ag?
4. How to understand the antigenic specificity?