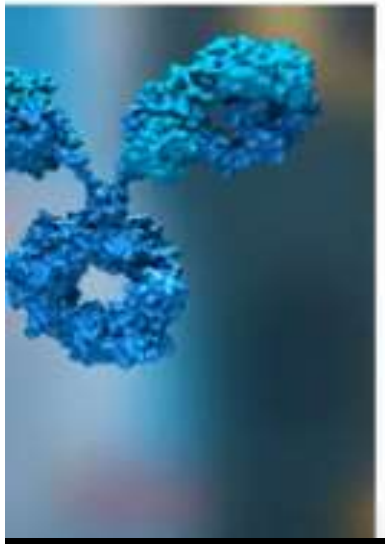
Antibody diversity (all graphics are collected from internet)

- Antibodies are Ag binding proteins present on the B-cell membrane and secreted by plasma cell.
- All antibodies share structural features, Even though they are diverse according to the antigen that caused the generation of the particular antibody.

What is antibody diversity?

- There are millions of antigens/epitope.
- Our immune system has the ability to produce specific antibody (variable region) against all antigens
- This diversification in antibody production is known as antibody diversity

Antibody Diversity-Gene Rearrangement



What is Antibody Diversity ? Source of Antibody Diversity

- Millions of antigens
- Produce specific antibody against all antigens
- Diversification

Problem?

- One gene for each protein.
- There are around 40000 genes in our genome.

But our immune system apparently produce antibody in the order of 10^{10}

Generation of Diversity

- The complete collection of antibody specificities within an individual is estimated to be 10^{11} or more
  - collectively known as our antibody repertoire
- How is this possible?

Problem…the immune system makes over one billion different antibody proteins

- In 1950's: central dogma stated DNA—to RNA—to protein
- One gene for each protein
- Required millions of genes just for the immune system
- Does not seem possible, but most scientists thought it might be
- Today we know the human genome is less than 30,000 genes
- So, what is really going on???

Two gene model hypothesis

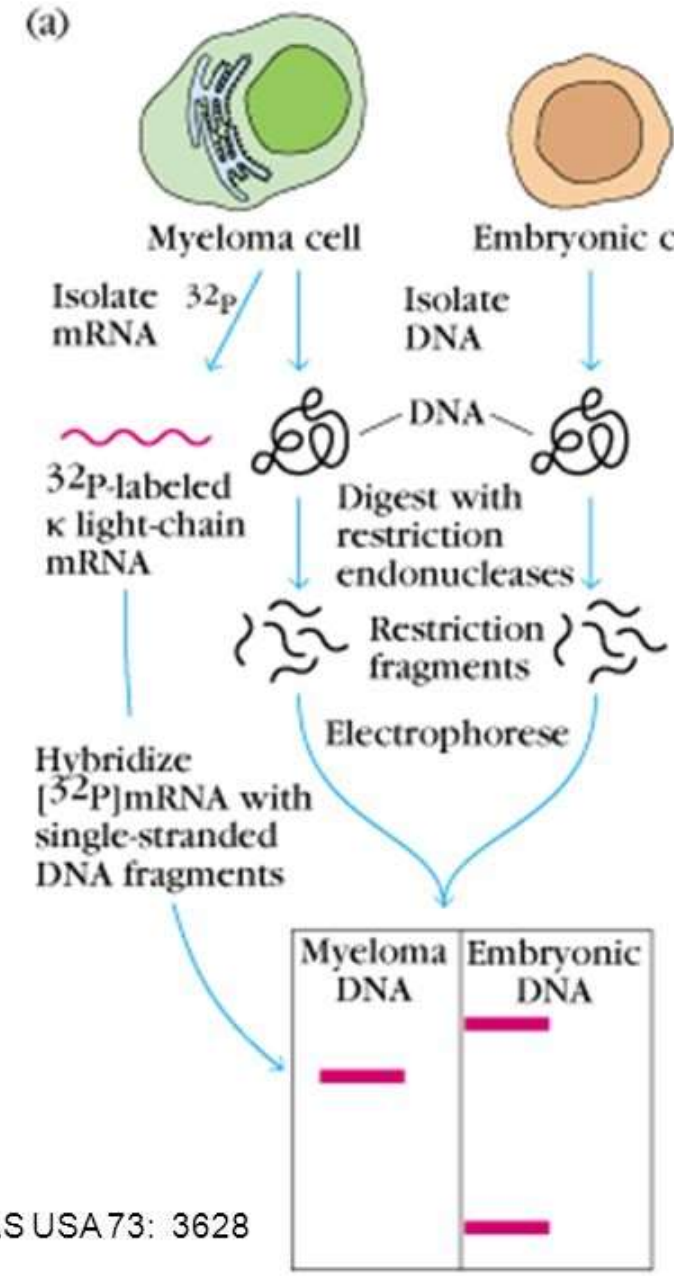
By Dreyer and Bennett, (1965)

1. **Two separate gene encode single immunoglobulin chain (H or L) one for V region and other for C region.**
2. **These two genes must somehow come together at the DNA level to form a continuous message(recombination), that can be transcribed in to a single immunoglobulin gene.**
3. **They proposed – 100 to 1000 V region genes in germ line**
4. **Only single copies of C region class and subclass genes exist.**

The hypothesis given theoretical and intellectual understanding.

The Tonegawa's Bombshell !!!!!!!

- 1976 – Direct evidence for – Separate genes encode the V and C regions of Ig
- The gene segments are rearranged in the course of B cell differentiation.
- 1986 Nobel Prize



From Hozumi and Tonegawa, 1976, PNAS USA 73: 3628

Tonegawa and Hozumi suggested that, during differentiation of lymphocytes from the embryonic state to the fully differentiated plasma-cell stage represented in their system by the myeloma cells, the V and C genes undergo rearrangement. In the embryo, the V and C genes are separated by a large DNA segment that contains a restriction-endonuclease site; during differentiation, the V and C genes are brought closer together and the intervening DNA sequence is eliminated. This experiment is also proved with the help of southern blotting in recent years.

Generation of antibody diversity

- To date : 7 means of antibody diversification have been identified in humans

Multiple germ line genes

Combinatorial V(D)J Joining

P region nucleotide addition

N region nucleotide addition

Junctional flexibility

Combinatorial association of H and L chain

Somatic hyper mutation

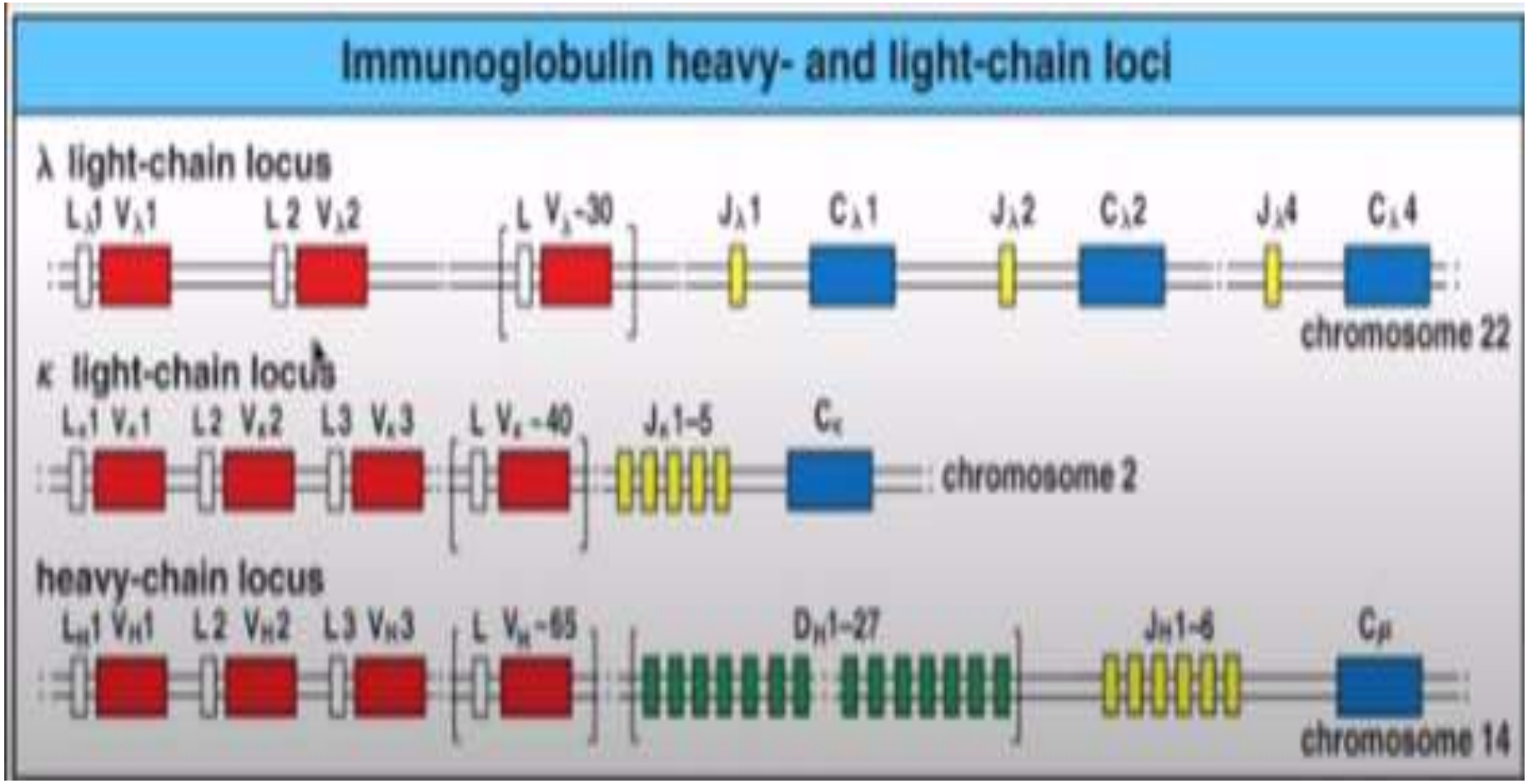
Multiple germline segment.

Multiple germ line segment generate diversity

κ and λ light chains and H chains are coded by separate multigene families situated on different chromosome.

Since Ig are product of a multigene family – different kind of Ig are produced from different genes.

	CHROMOSOME	
Gene	Human	Mouse
λ Light chain	22	16
κ Light chain	2	6
Heavy chain	14	12

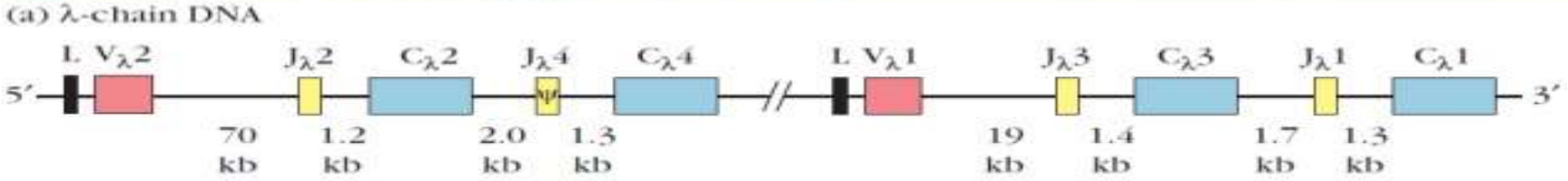

Immunoglobulin heavy- and light-chain loci
λ light-chain locus
chromosome 22
κ light-chain locus
chromosome 2
heavy-chain locus
chromosome 14

Combinatorial V-J (Light chain) and V-D-J joining (Heavy Chain)

- Ig has heavy chain and light chain
- An Ig chain has a V region and C region.
- The V region of L chain is coded by a one of the VJ recombinant.
- Similarly, V region of H chain is coded by a one of the VDJ recombinant.
- The C region of both H & L chain is coded by one of the C segment.

Lambda chain gene family

- 31 Vλ
- 4 Jλ
- 7 Cλ segments



Mouse λ chain DNA

Kappa chain gene family

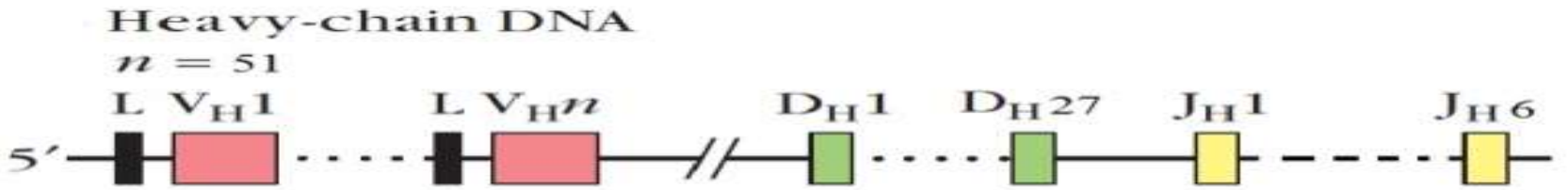
- **40 Vκ**
- **5 J κ**
- **Single Cκ**

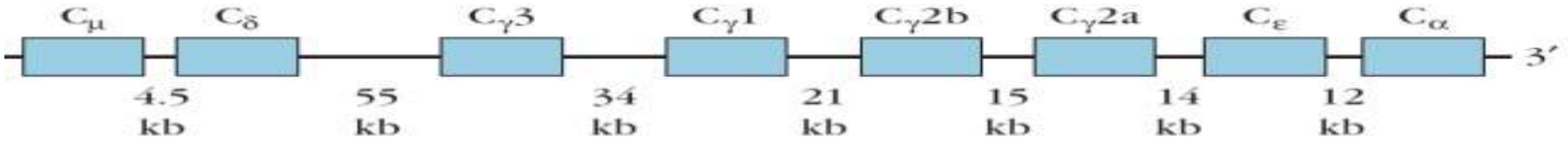
Human κ chain gene

n = 40

Heavy chain gene family

- Variable region is coded by 3 type gene segment.
- V D & J
- Constant region is coded by C region gene
- 51 VH
- 27 DH
- 6 JH
- Then C region genes in the order Cμ, Cδ, Cγ3, Cγ1, Cγ2b, C γ2a, Cε, Cα

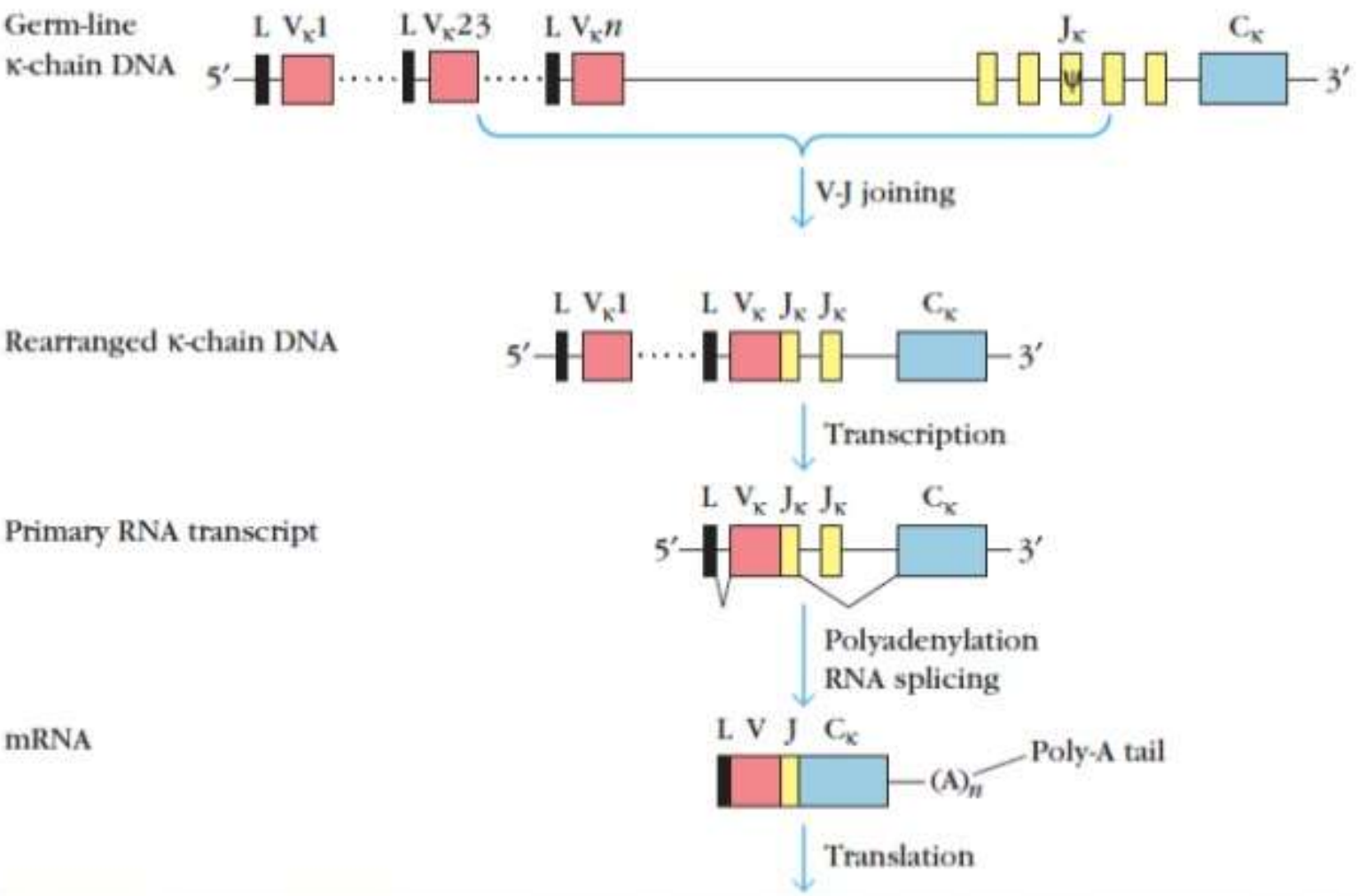




V(D)J recombination

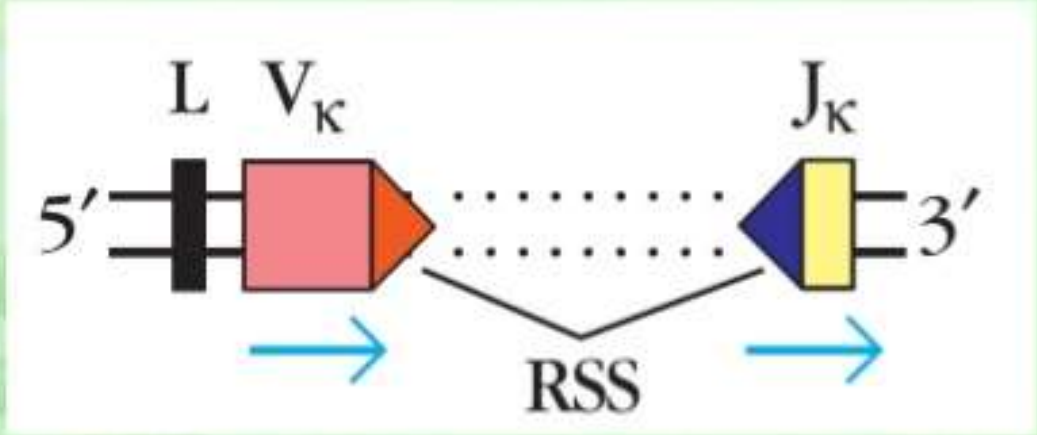
- Any of Vλ gene can combine with any of Jλ – Cλ combination (same in κ also).
- Any of V_H gene can combine with any of D_H – J_H – C_H combination.
- VDJ (first) and VJ (second) recombination – During B cell maturation in BM.
- So single antigen specific Immunocompetent cell is produced.
- RAG 1&2 and TdT (V(D)J recombinase)

VJ recombination process in κ Chain

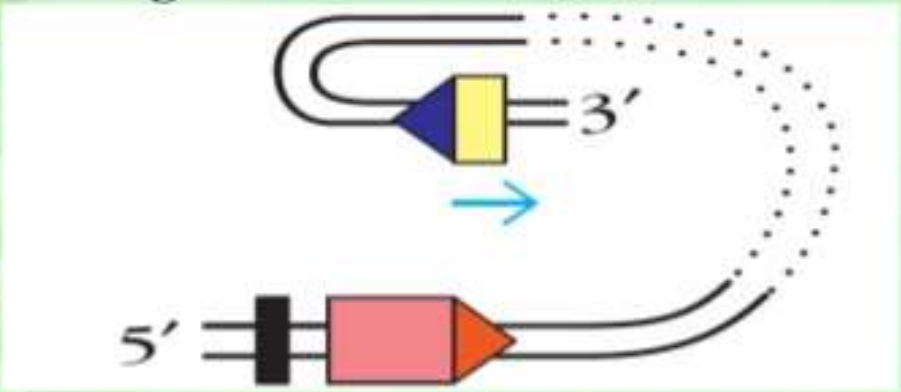


Steps in V(D)J recombination 1

- Recombinase enzyme recognise site of recombination (RSSs).

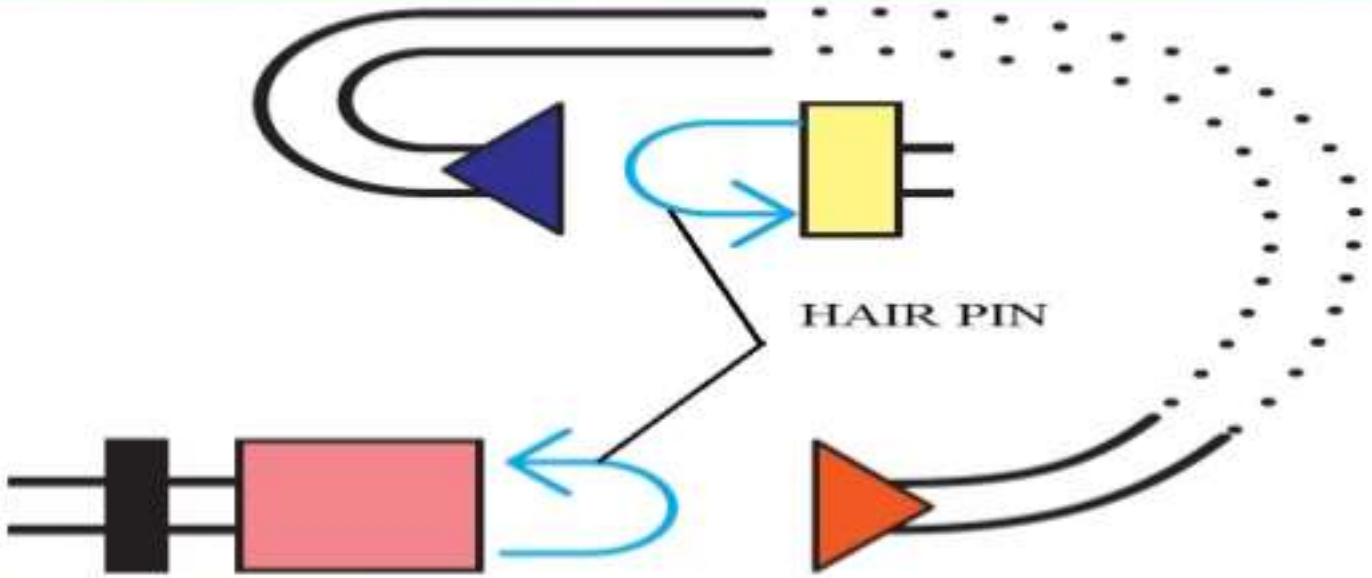


- Flanking of genes segments to be recombinated.



Steps in V(D)J recombination 2

- Cleavage of DNA by RAG 1 & RAG 2 enzymes
- 3' OH of cut DNA bind to phosphodiester linking of opposite strand **(A HAIR PIN STRUCTURE IS FORMED AT CUT END)**

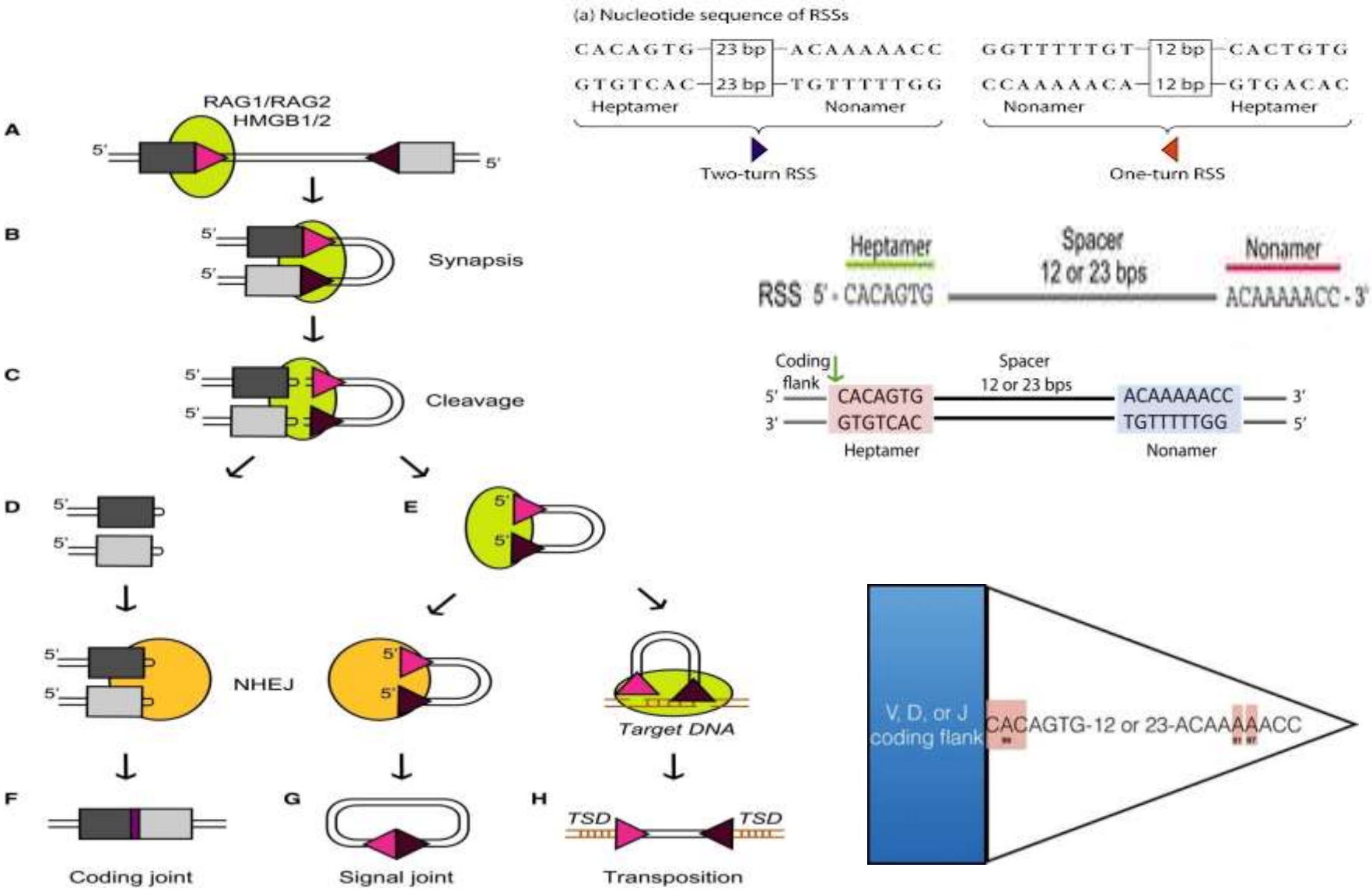


Steps in V(D)J recombination 3

- **Random cutting of hair pin and P - nucleotide addition.**
- **Trimming of few nucleotides from cut end, Catalysed by Normal ssEndonuclease.**
- **Addition of N- nucleotides at the cut end of VDJ(in case of heavy chain only)**
- **Repair and ligation of coding sequence to form coding joint by Normal DSBR enzyme.**

Result of V(D)J recombination

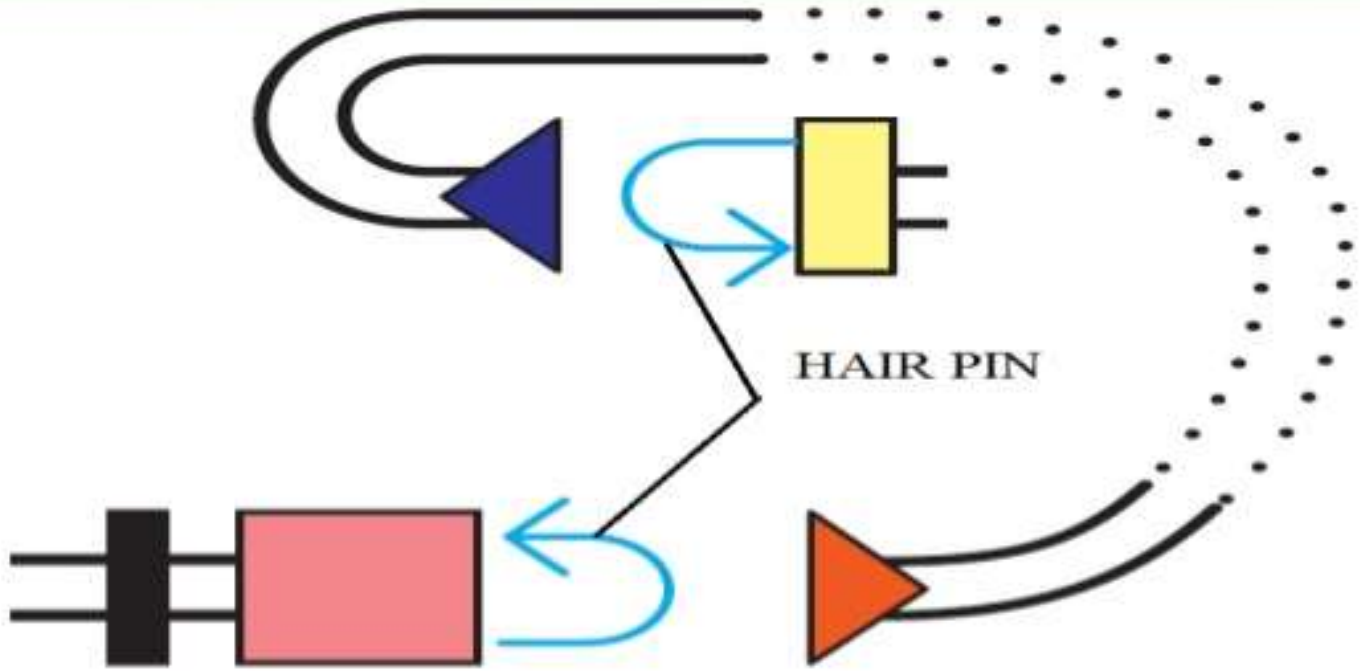
		LIGHT CHAINS	
Multiple germ-line segments	Heavy chain	κ	λ
	ESTIMATED NUMBER OF SEGMENTS IN HUMANS*		
V	51	40	30
D	27	0	0
J	6	5	4
Combinatorial V-D-J and V-J joining (possible number of combinations)	$51 \times 27 \times 6 = 8262$	$40 \times 5 = 200$	$30 \times 4 = 120$
Possible combinatorial associations of heavy and light chains†	$8262 \times (200 \times 120) = 2.64 \times 10^6$		


(a) Nucleotide sequence of RSSs
CACAGTG 23 bp ACAAAAACC
GTGTCAC 23 bp TGTTTTTGG
Heptamer
Nonamer
Two-turn RSS
GGTTTTTGT 12 bp CACTGTG
CCAAAAACA 12 bp GTGACAC
Nonamer
Heptamer
One-turn RSS
Heptamer
Spacer
12 or 23 bps
Nonamer
RSS 5' - CACAGTG
ACAAAAACC - 3'
Coding flank
Spacer
12 or 23 bps
5' CACAGTG
ACAAAAACC 3'
3' GTGTCAC
TGTTTTTGG 5'
Heptamer
Nonamer
A
RAG1/RAG2
HMGB1/2
5'
5'
B
Synapsis
C
Cleavage
D
E
NHEJ
Target DNA
F
Coding joint
G
Signal joint
H
TSD
TSD
Transposition
V, D, or J coding flank
CACAGTG-12 or 23-ACAAAAACC

P- addition generate diversity.

What is P – addition?

- During V(D)J recombination DNA cleaved by RAG 1 & RAG 2 enzymes - create a **HAIR PIN STRUCTURE AT CUT END**

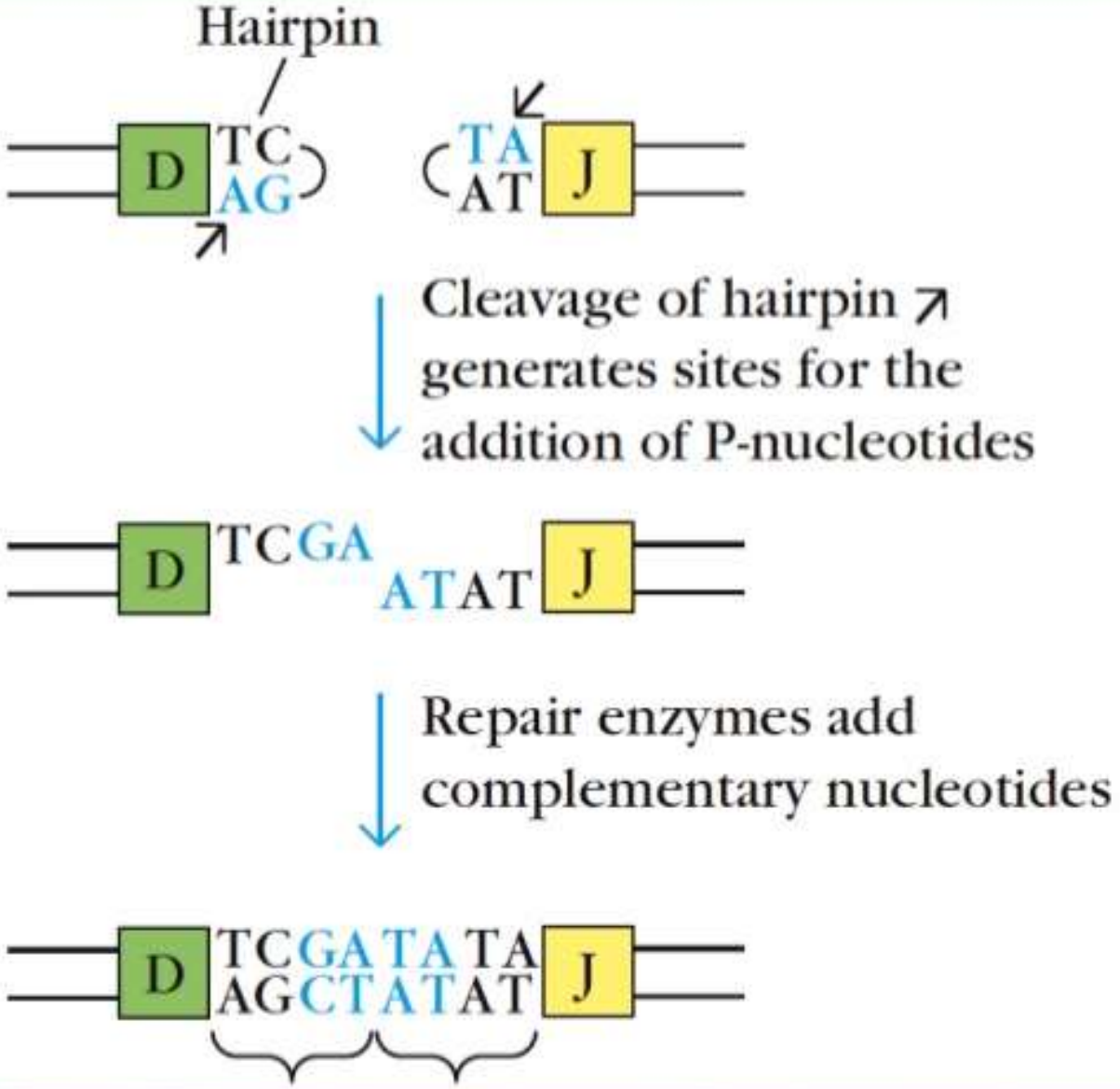


What is P – addition?

- This hair pin is a short single strand DNA.
- It undergoes random cleavage by ssEndonuclease.
- A short single strand at the cut end of coding sequence is formed.
- Subsequent repairing add complimentary nucleotide to produce palindromic sequence.
- So called P – Nucleotides.

How P – addition generate diversity?

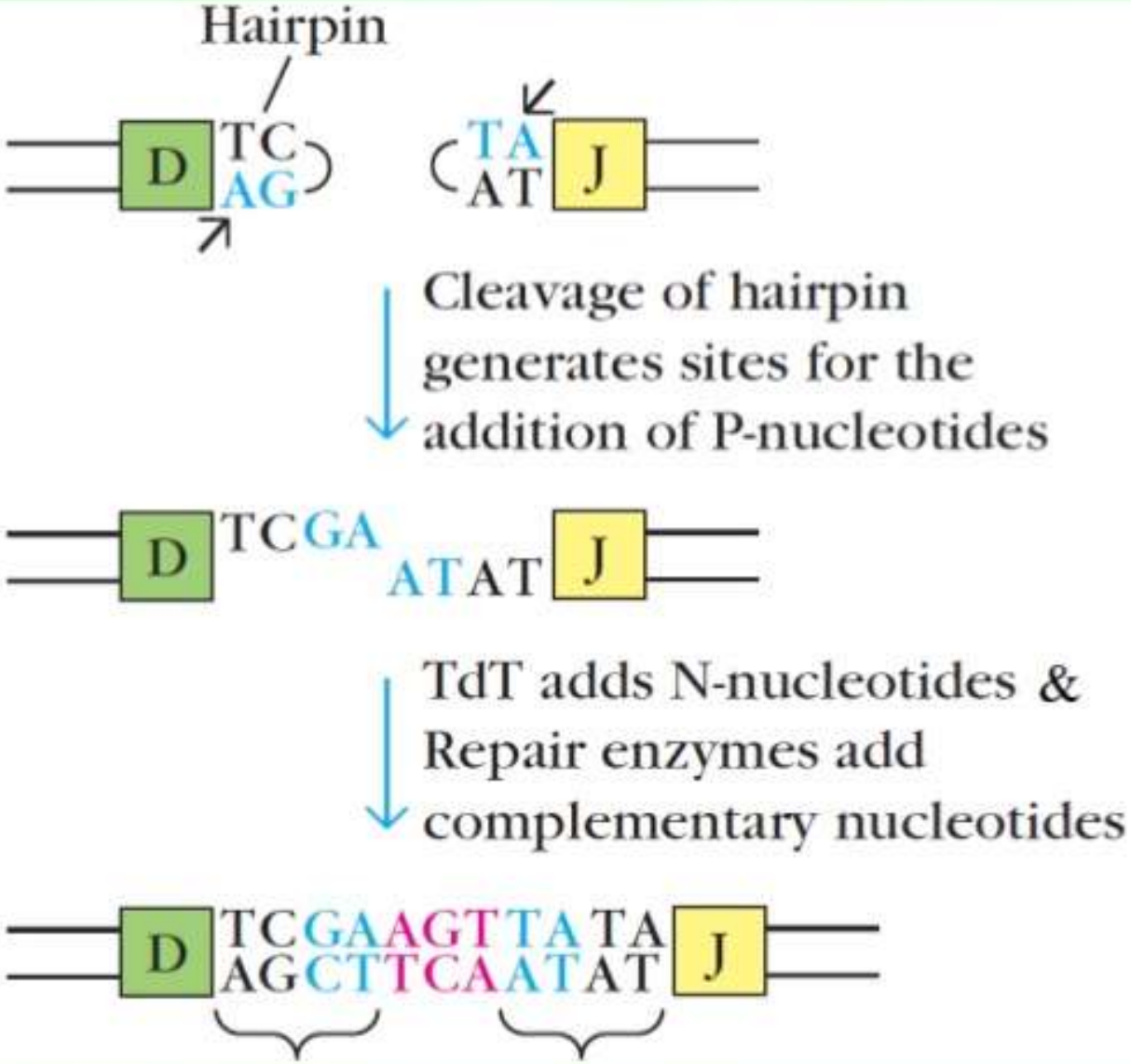
- Variation in the position of hair pin cut leads to variation in Length
- Variation in P nucleotide addition leads variations in Ab coding sequence.


Hairpin
D
TC
AG
TA
AT
J
Cleavage of hairpin ↗
generates sites for the
addition of P-nucleotides
D
TCGA
ATAT
J
Repair enzymes add
complementary nucleotides
D
TCGATATA
AGCTATAT
J

N addition generate additional diversity in Heavy chain

What is N – nucleotide addition?

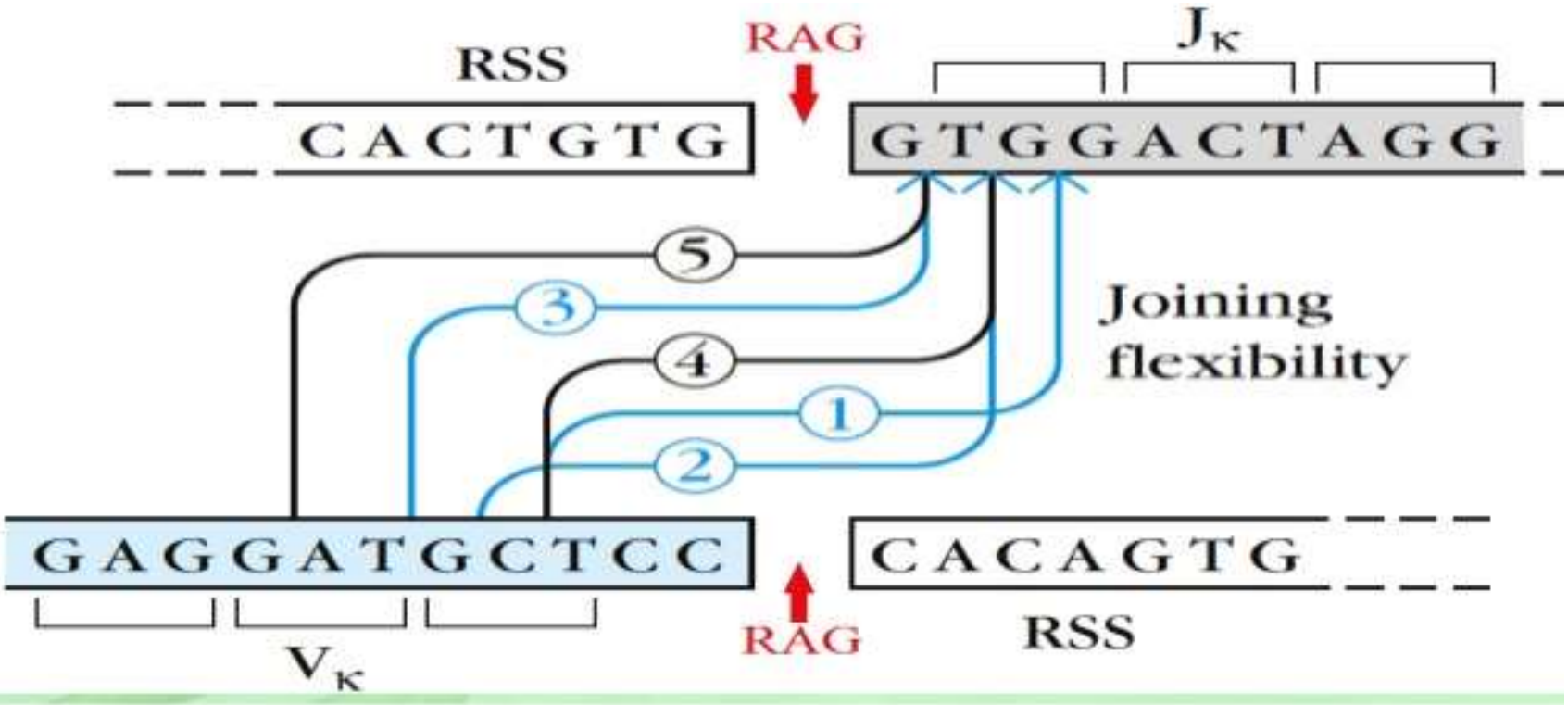
- During recombination of heavy chain an enzyme called terminal deoxynucleotidyl transferase (TdT) add some random nucleotides at cut end(in H chain only)
- N addition add up to 15 nucleotides = 5 amino acids.
- Result = $V_H N D_H N J_H$


Hairpin
D
TC
AG
TA
AT
J
Cleavage of hairpin generates sites for the addition of P-nucleotides
D
TCGA
ATAT
J
TdT adds N-nucleotides &
Repair enzymes add
complementary nucleotides
D
TCGAAGTTATA
AGCTTCAATAT
J

Junctional flexibility

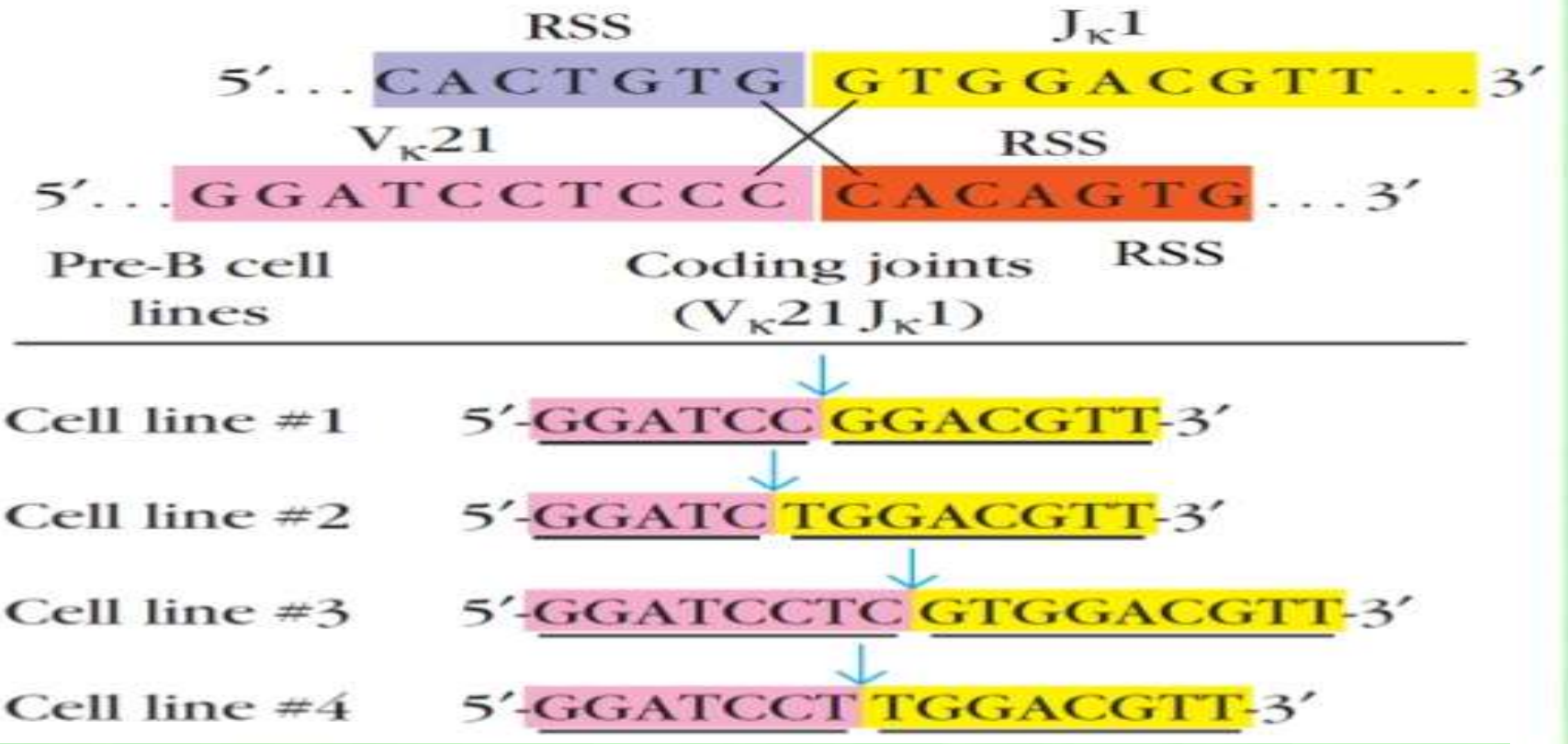
What is Junctional flexibility?

- The final Joining of coding sequences (V& J/ VD&J) segments may be imprecise.
- The variations in final trimming and ligation of coding segment / in other words formation of coding joint in a flexible fashion is called Junctional flexibility.



Productive rearrangements

	Glu	Asp	Ala	Thr	Arg
1	GAG	GAT	GCG	ACT	AGG
	Glu	Asp	Gly	Thr	Arg
2	GAG	GAT	GGG	ACT	AGG
	Glu	Asp	Trp	Thr	Arg
3	GAG	GAT	TGG	ACT	AGG



How Junctional flexibility generate diversity?

- **Junctional flexibility generate different amino acid combinations at coding joint – that generate diversity.**

Diversity by combinatorial association of H and L chain

Diversity by combinatorial association of H and L chain

- Genome has the potential to generate 8262 type H chain genes and 320 light chain gene.
- Theoretically anyone of the H chain can combine with anyone of the L chain.
- i.e.. up to 2.64×10^{10} immunonoglobulin combination.

All diversifying mechanism so far operate during maturation of B cell.

As a result of recombination and allelic exclusion B cell with single specificity come out of bone marrow.

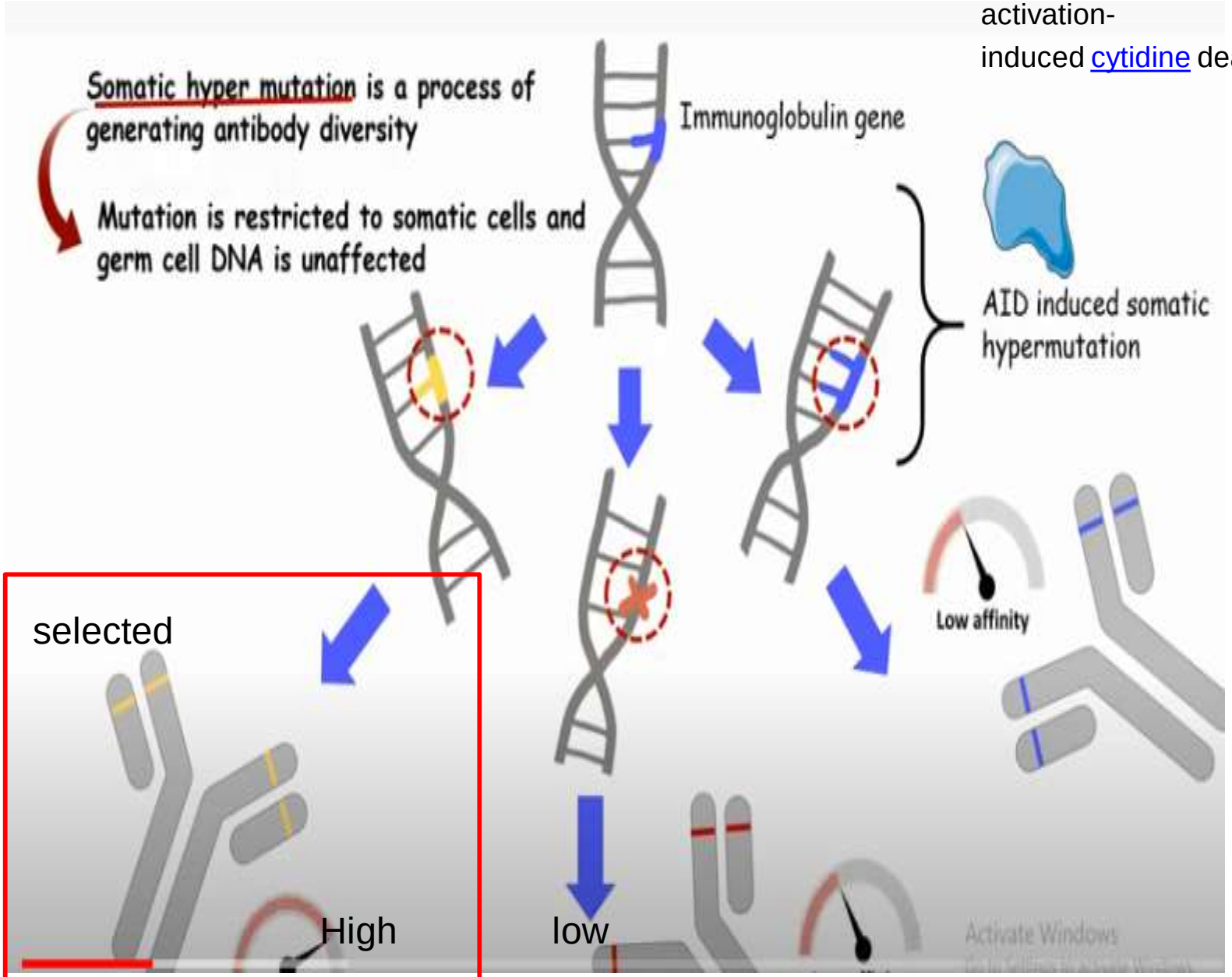
Somatic hypermutation adds diversity

- **Occur within germinal centres of secondary lymphoid organ after exposure to an antigen.(T cell dependent B cell activation)**
- **Individual nucleotides in VJ or VDJ units are replaced with alternative nucleotides.**
- **It potentially alternate the specificity of encoded Ig.**
- **The rate of this mutation is 1 Lakh times higher than spontaneous mutation.**
- **i.e. one mutation in V_H & V_L genes per every two cell division.**

Somatic hypermutation.

- Mechanism has not yet been determined.
- But most mutations are substitution rather than insertion or deletion.
- Following exposure to an Antigen, B cells with higher affinity receptors selected for survival.
- Such B cells undergo Affinity maturation takes place in germinal centres.

activation-induced cytidine deaminase



Somatic Hypermutation

Somatic hypermutation is a process in which point mutations accumulate in the antibody V-regions of both the heavy and light chains, at rates that are about 10^6-fold higher than the background mutation rates observed in other genes .

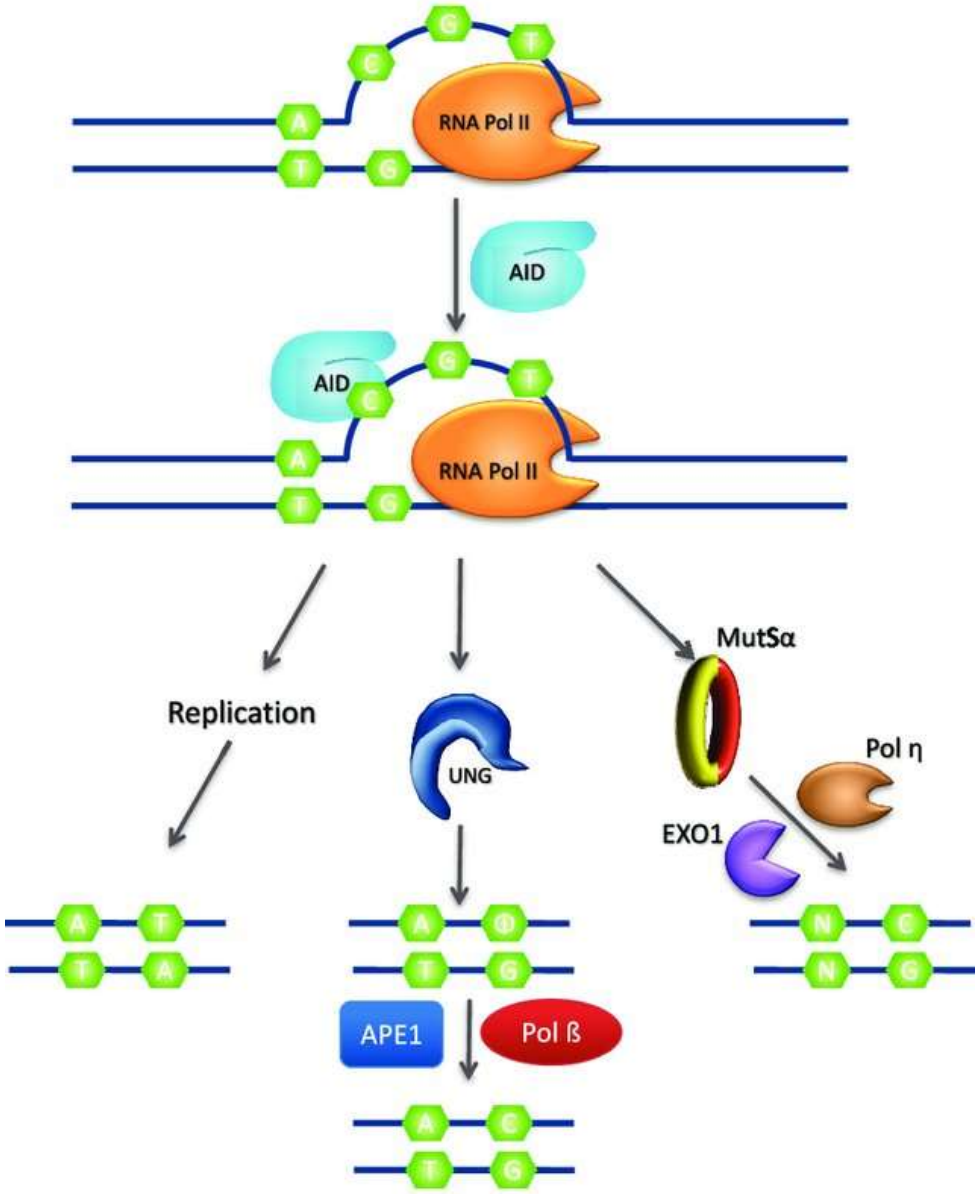
This accumulation of mutations at the V-region genes occurs at the centroblast stage of B-cell differentiation in the germinal centers of secondary lymphoid organs. Whereas the overall goal of this process is to produce high-affinity antibodies, in the absence of selection, SHM does not distinguish between favorable and unfavorable mutations and can produce antibodies with

(1) higher affinity for antigen,

(2) lower affinity for antigen, and

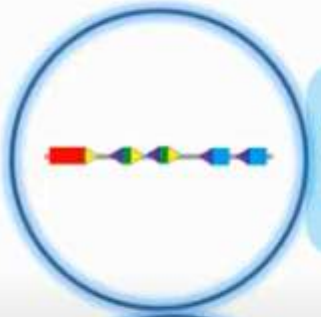
(3) no change in affinity for antigen.

Somatic hypermutation can also lead to nonfunctional antibodies, such as antibodies that cannot fold correctly, or antibody genes that harbor premature stop codons . Whereas SHM of the antibody V-region does not always produce a higher-affinity antibody, the selection process for antigen binding that occurs in the light zone of the germinal center selects for B-cells that produce the highest-affinity antibodies. Mutations tend to accumulate in the complementarity determining regions (CDRs) of the antibody V genes. Because the CDRs are the locations that directly contact the antigen, it is not surprising that these regions would have the most mutations after selection. Evolutionary selection over millions of years has facilitated this process by enriching the CDRs for codons with **activation-induced cytidine deaminase (AID)** hot spots that result in replacement mutations, whereas the codon usage in the frameworks of V genes is more likely to lead to silent or conservative mutations.

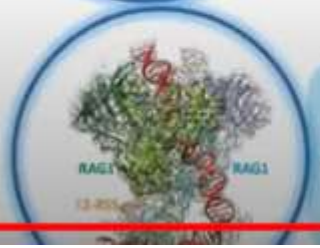


Molecular mechanism of somatic hypermutation (SHM). AID requires a single strand to initiate the SHM process. Transcription by RNA polymerase II (RNA Pol II) exposes the single-stranded DNA template for AID. AID deaminates a cytosine to create an uracil, which can then be processed by different pathways. Replication over the uracil results in C to T or G to A transition mutations. Processing by uracil DNA glycosylase (UNG) generates an abasic site (Φ) that is cleaved by the apurinic/apyrimidinic endonuclease (APE1), which removes this site and then Polβ resynthesizes the DNA. Recognition of the U-G mismatch by MutSα (represented by a torus shape) followed by the action of Exo1 and Polη spreads mutations (indicated as "N") to surrounding A-T nucleotides. UNG and Msh2/Msh6 can also act in the context of high fidelity base excision repair (BER) and mismatch repair (MMR) pathways, which results in error-free repair.

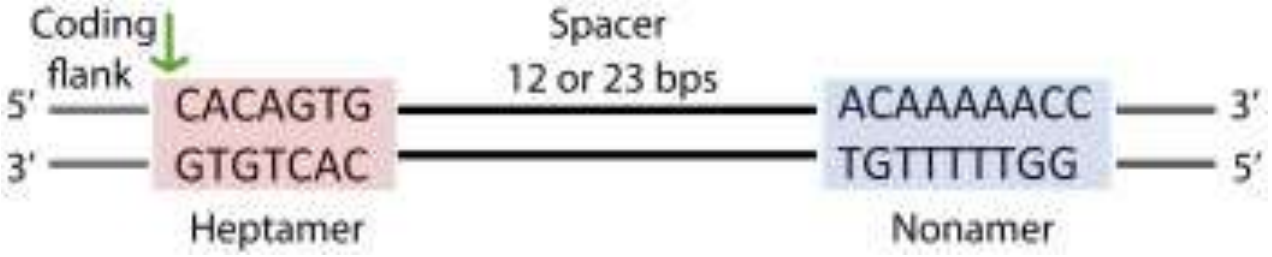
VDJ recombination is dependent on two important components



Recombination signal sequence that direct the recombination



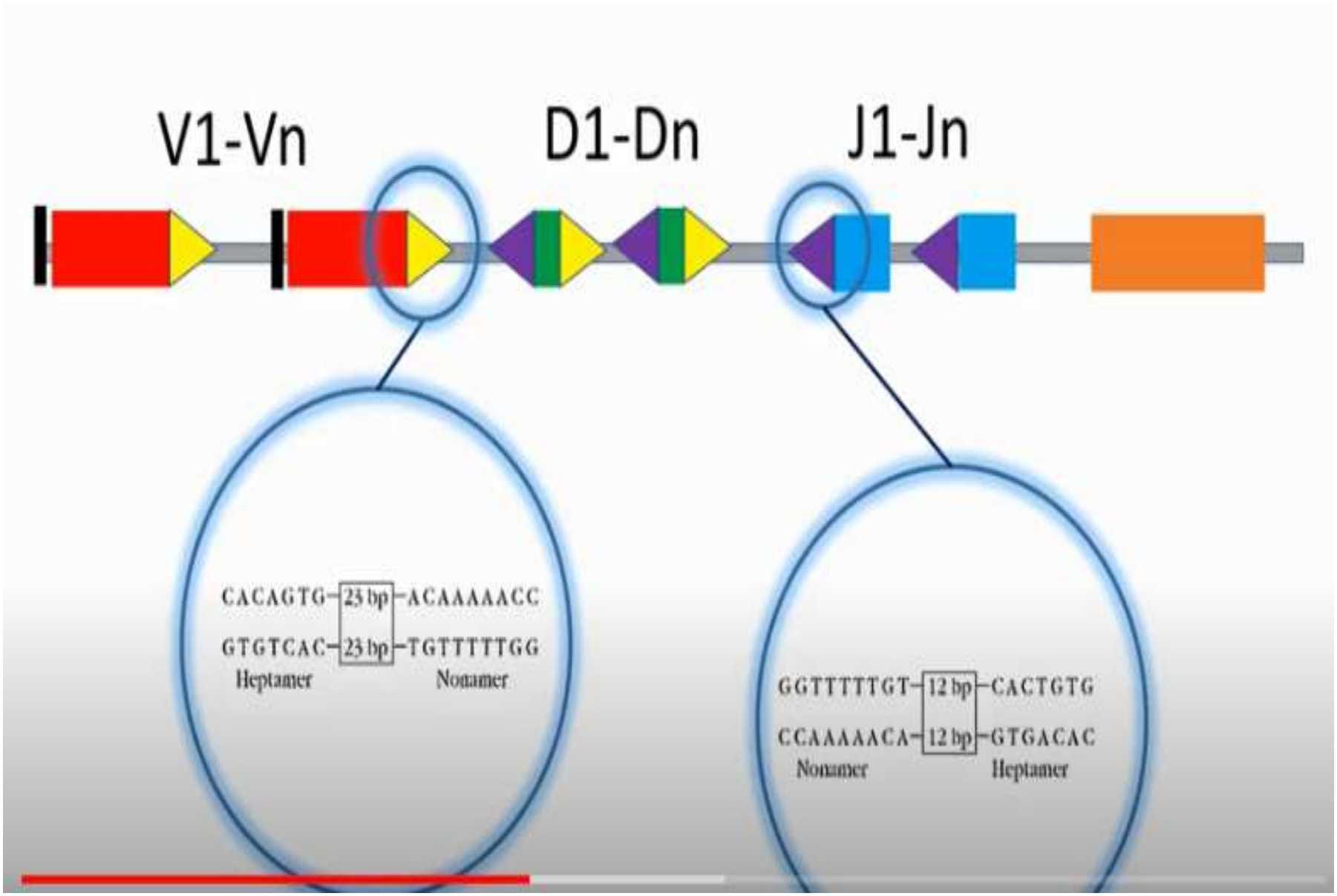
Rag1 and Rag2 recombinase

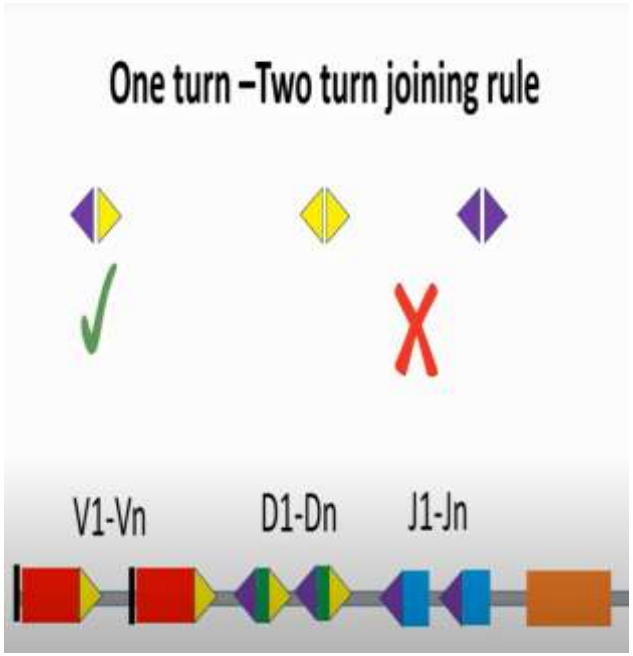


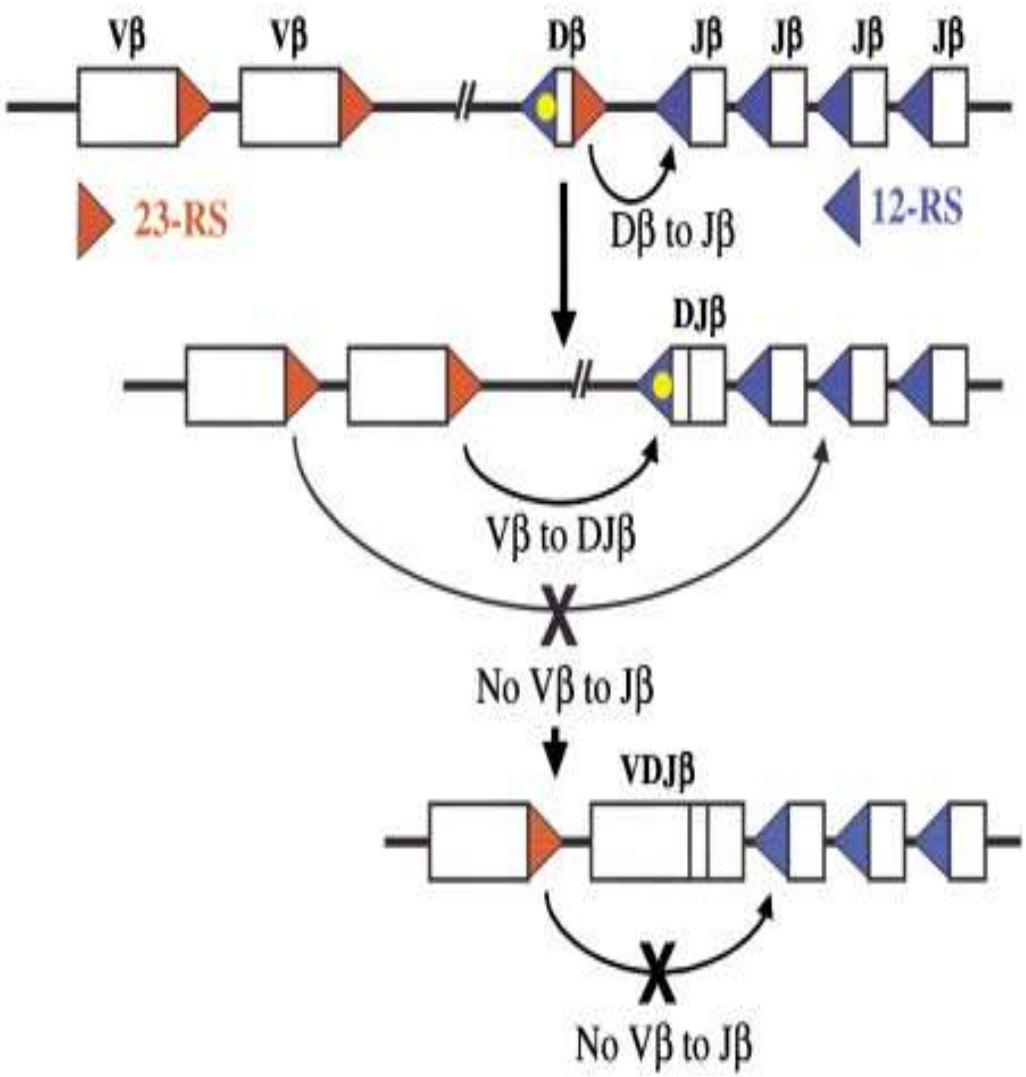
The recombination signal sequence (RSS)

Mechanism of variable region rearrangements

- Each V, D and J segments of DNA are flanked by special sequences (RSS—recombination signal sequences) of two sizes
- Single turn and double turn sequences (each turn of DNA is 10 base pairs long)
- Only single turn can combine with a double turn sequence
- Joining rule ensures that V segment joins only with a J segment in the proper order
- Recombinases join segments together


V1-Vn
D1-Dn
J1-Jn
CACAGTG
23 bp
ACAAAAACC
GTGTCAC
23 bp
TGTTTTTGG
Heptamer
Nonamer
GGTTTTTGT
12 bp
CACTGTG
CCAAAAACA
12 bp
GTGACAC
Nonamer
Heptamer


One turn –Two turn joining rule
V1-Vn
D1-Dn
J1-Jn


Vβ
Vβ
Dβ
Jβ
Jβ
Jβ
Jβ
23-RS
Dβ to Jβ
12-RS
DJβ
Vβ to DJβ
No Vβ to Jβ
VDJβ
No Vβ to Jβ

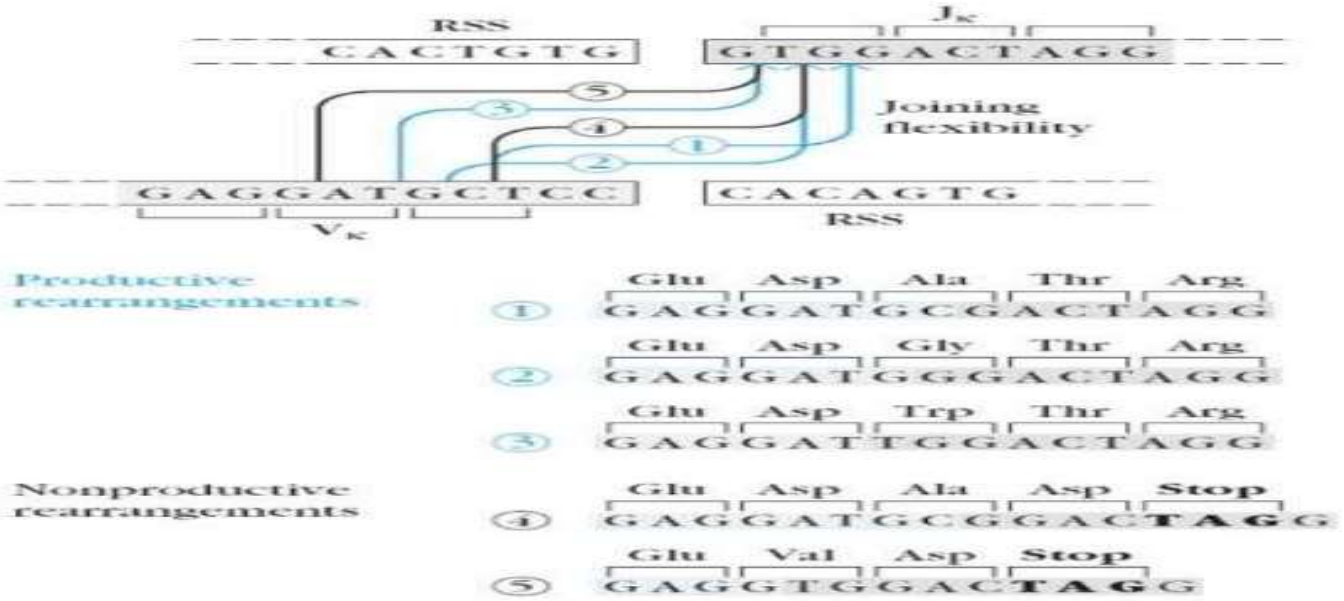
D. Productive and Nonproductive Rearrangements

- nonproductive rearrangement: imprecise joining that results in gene segments that are joined out of phase, so that the triplet reading frame for translation is not preserved.

- resulting VJ or VDJ unit will contain numerous stop codons, which interrupt translation

- Productive rearrangement: the resulting VJ or VDJ unit can be translated in its entirety, which will yield a complete antibody

- these two type of rearrangements that occur produce only about 8% of pre-B cells in the bone marrow that undergo maturation and leave as mature, immunocompetent B cells



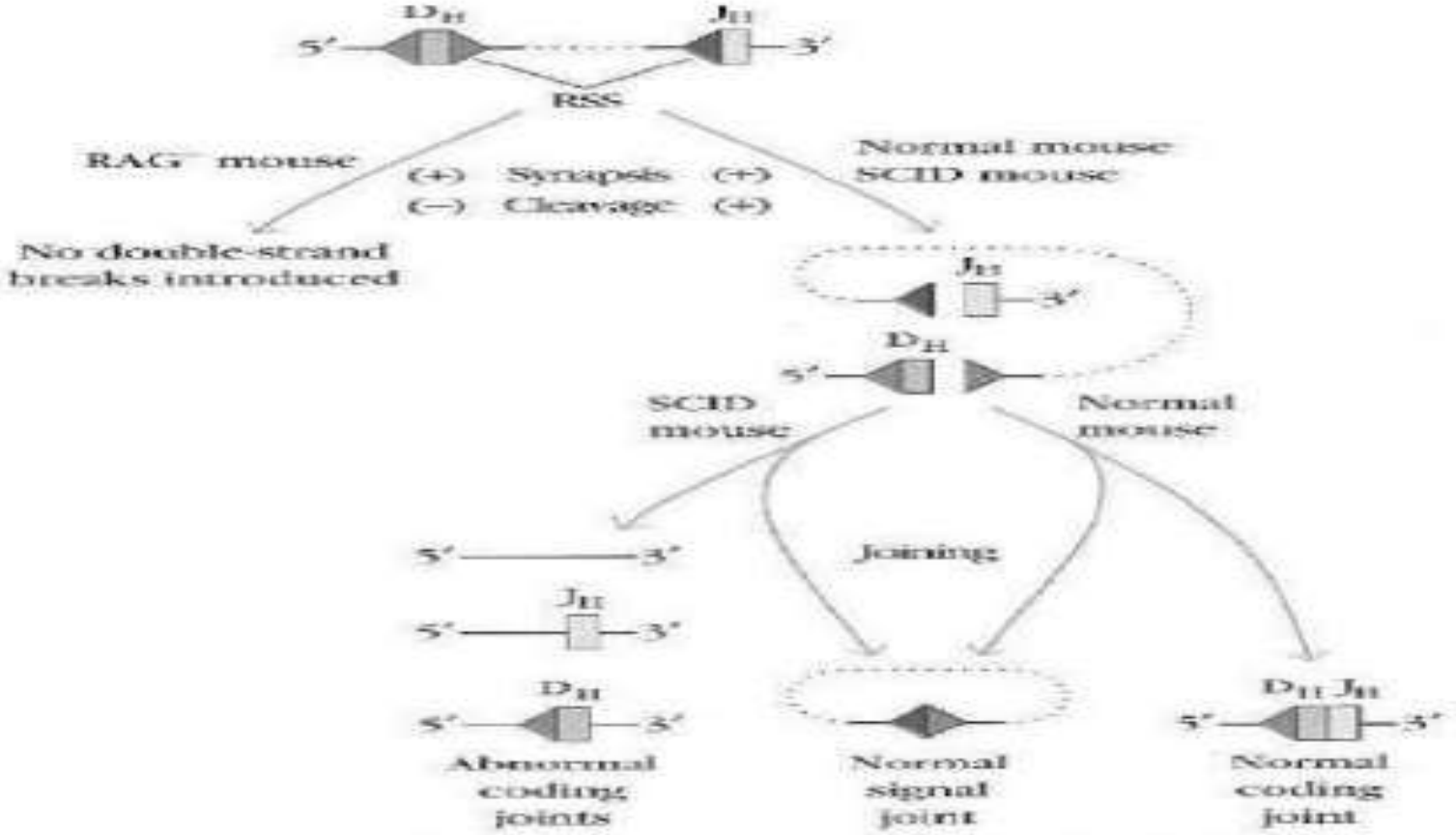


FIGURE 5-10 Recombination defects have been identified in RAG-deficient mice and SCID mice. Mice that lack a functional *RAG-1* or *RAG-2* cannot even start the recombination process. In contrast, SCID mice can carry out synapsis between D_H and J_H gene segments, introduce double-strand breaks to produce normal recombination intermediates, and form a normal signal joint. However, SCID mice cannot properly join the coding sequences. Both types of defective mice lack mature B and T cells and thus exhibit a severe combined immunodeficiency. [Adapted from FW Alt et al. 1992. *Im-*

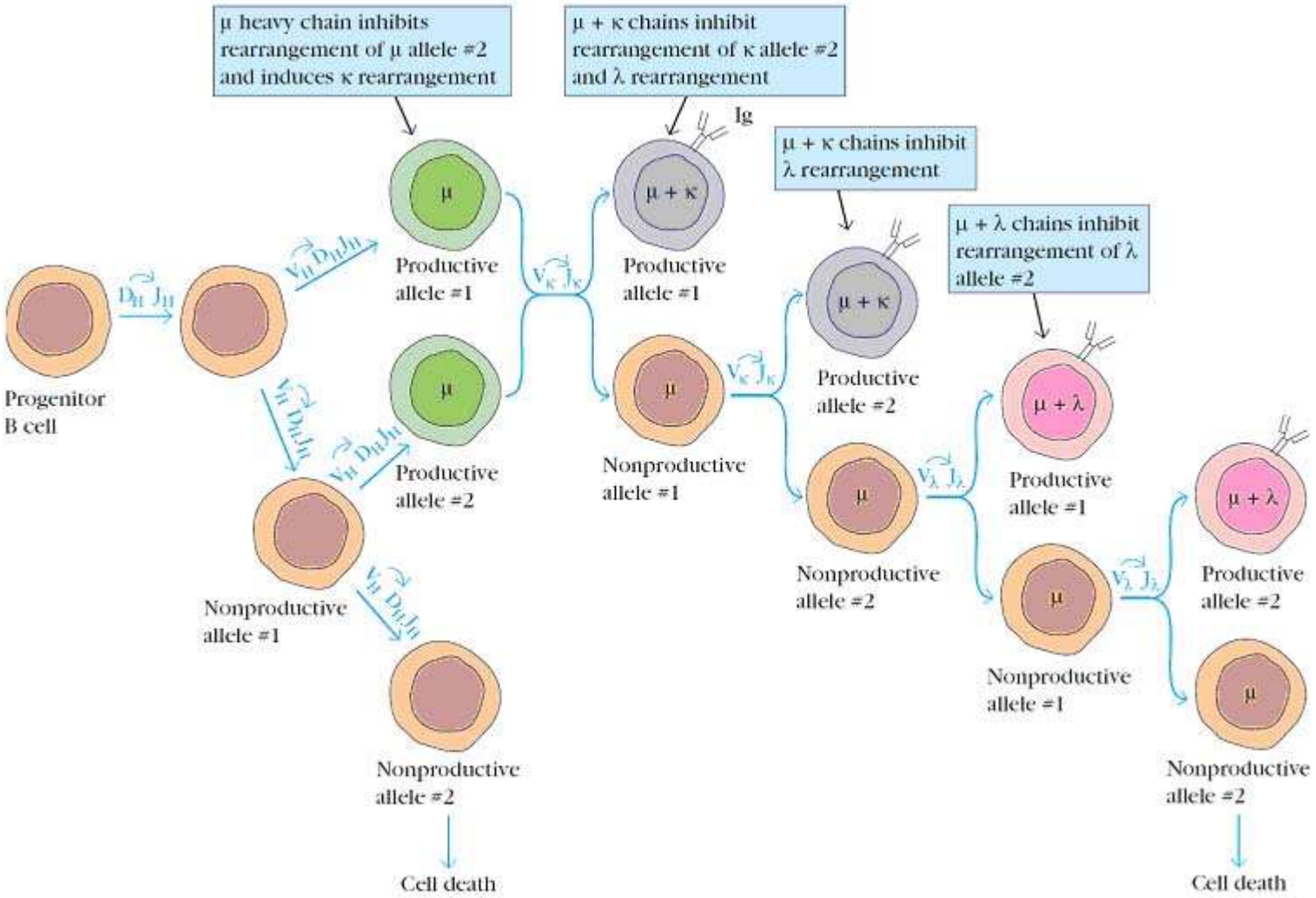
PRODUCTIVE AND NON-PRODUCTIVE REARRANGEMENTS

- Imprecise joining of rearranged segments may result in out of phase joining and the triplet reading frame for translation is not preserved.
- In such a ***non-productive rearrangement***, the resulting VJ or VDJ unit will contain numerous stop codons which interrupt translation.
- When gene segments are joined in phase, the reading frame is maintained.
- In such a ***productive rearrangement*** , the resulting VJ or VDJ unit can be translated in its entirety, yielding a complete antibody.

E. Allelic Exclusion

- even though a B cell is diploid, it expresses the rearranged heavy-chain genes from only one chromosome and the rearranged light-chain genes from only one chromosome. This is known as allelic exclusion

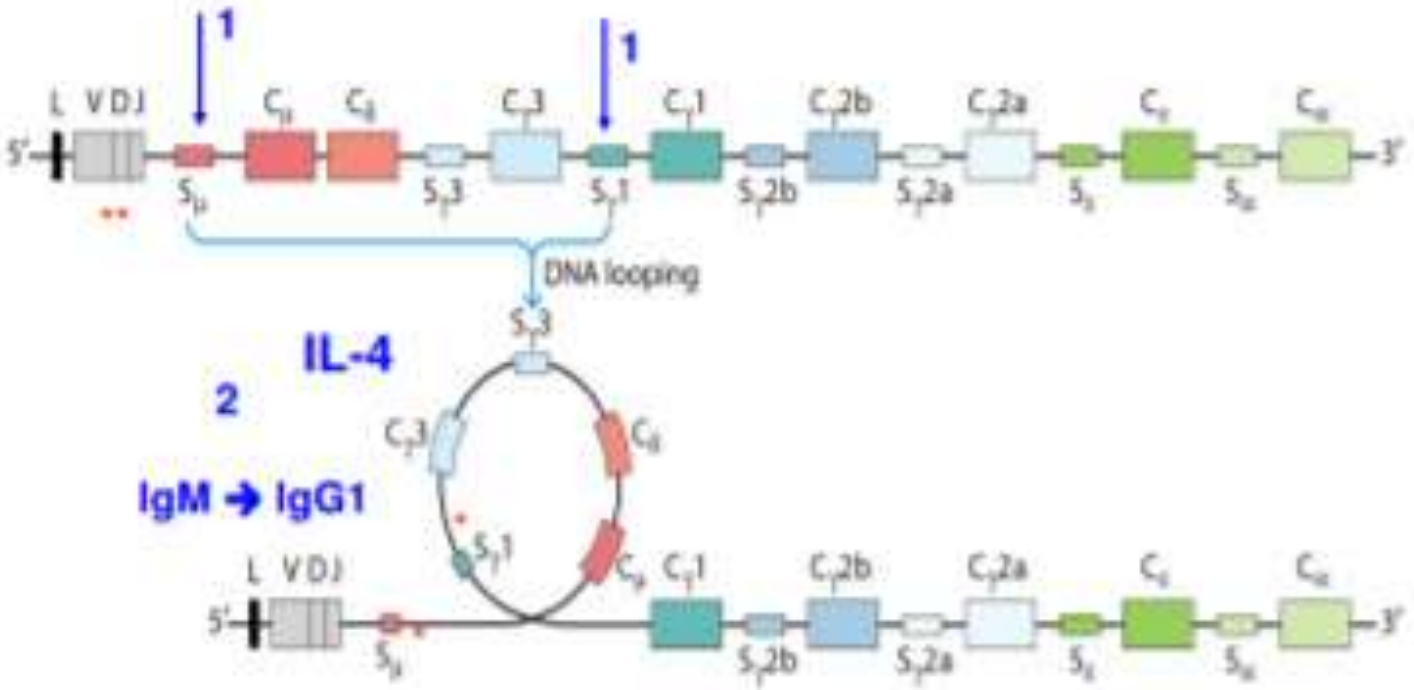
- this ensures that functional B cells never contain more than one VDJ from the heavy chain and one VJ unit from the light chain

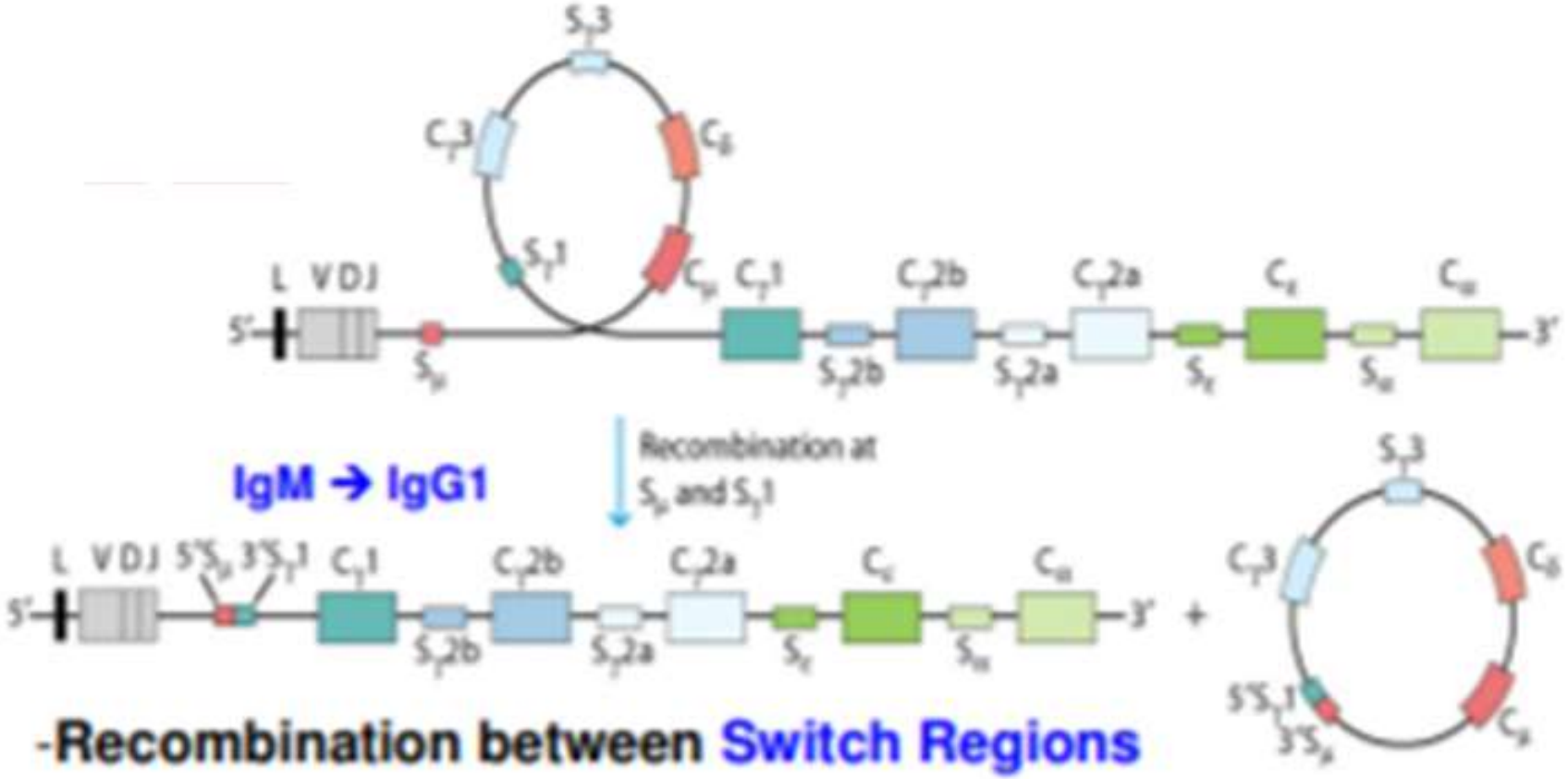

μ heavy chain inhibits rearrangement of μ allele #2 and induces κ rearrangement
μ + κ chains inhibit rearrangement of κ allele #2 and λ rearrangement
Ig
μ + κ chains inhibit λ rearrangement
μ + λ chains inhibit rearrangement of λ allele #2
Progenitor B cell
Productive allele #1
Productive allele #2
Nonproductive allele #1
Nonproductive allele #2
Cell death

Class Switching

- Antigen stimulation of a B cells → Antibodies with same variable Heavy (VDJ) with any C_H gene segment
- Process dependent on Switch Regions
- Switch Regions (2-3 kb) are located upstream from each C_H segment, except IgD (Cδ)
- Process driven by cytokines:
  - IL-4 → IgM to IgG1 or IgE
  - IFN-γ → IgM to IgG2a
- Players in regulation: 1) switch regions, 2) switch recombinases, 3) cytokine signals

Class Switching





Note: Same specificity but different H chain

Cytokine Effects on Class Switching

Certain cytokines affect class switching:

IFN-γ => IgG2a

IL-4 => IgG1, IgE

IL-5 => IgE

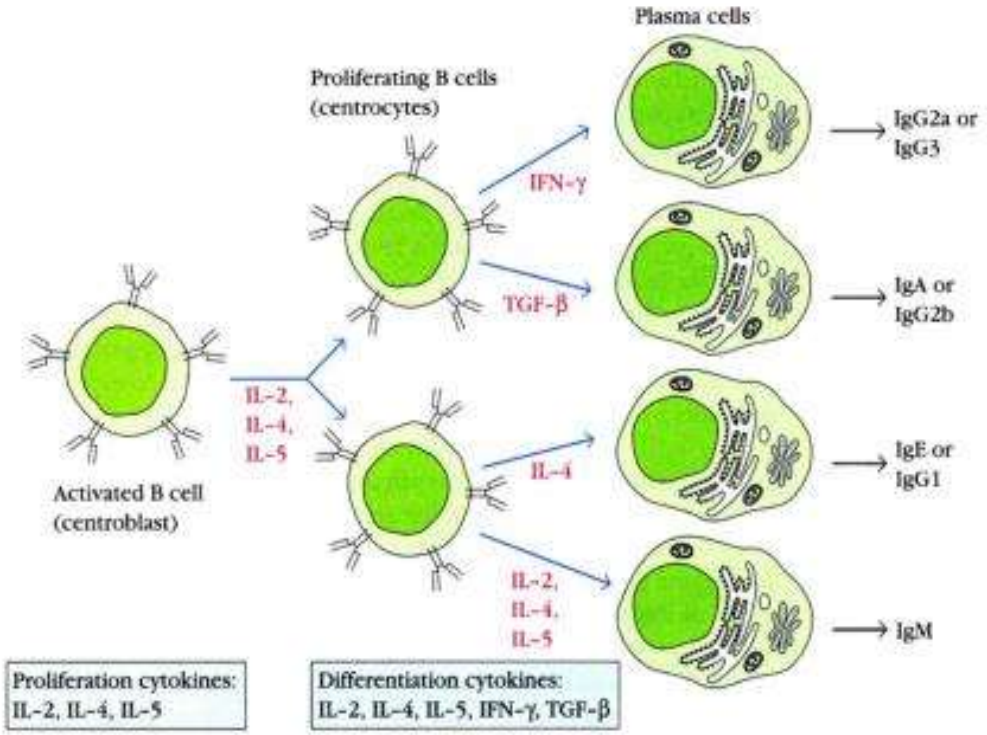
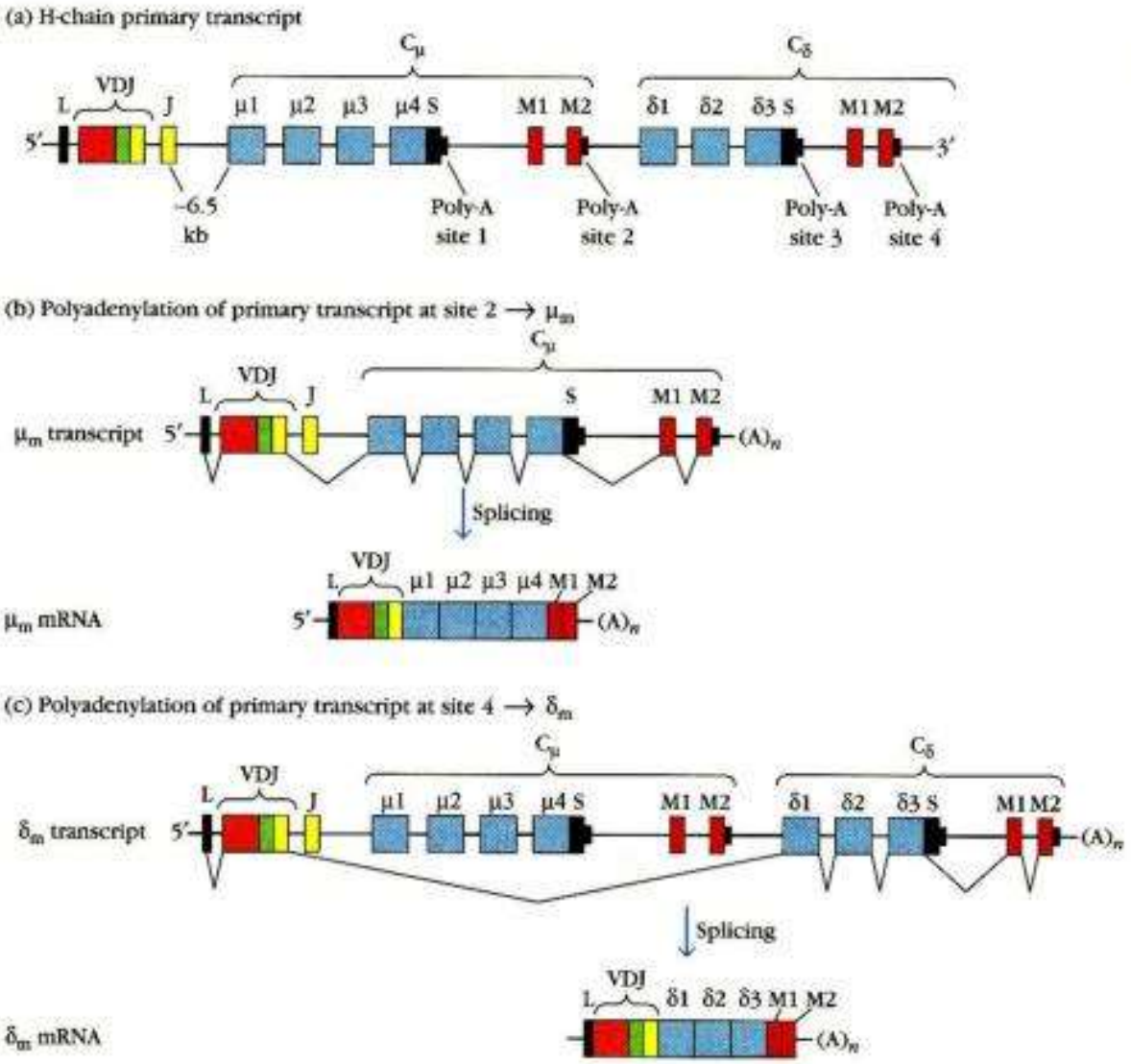


FIGURE 11-22 The interactions of numerous cytokines with B cells generate signals required for proliferation and class switching during the differentiation of B cells into plasma cells. Binding of the proliferation cytokines, which are released by activated T_H cells, provides the signal needed for proliferation of activated B cells. Similar or identical effects may be mediated by cytokines beyond the ones shown. Class switching in the response to thymus-dependent antigens also requires the CD40/CD40L interaction, which is not shown here.

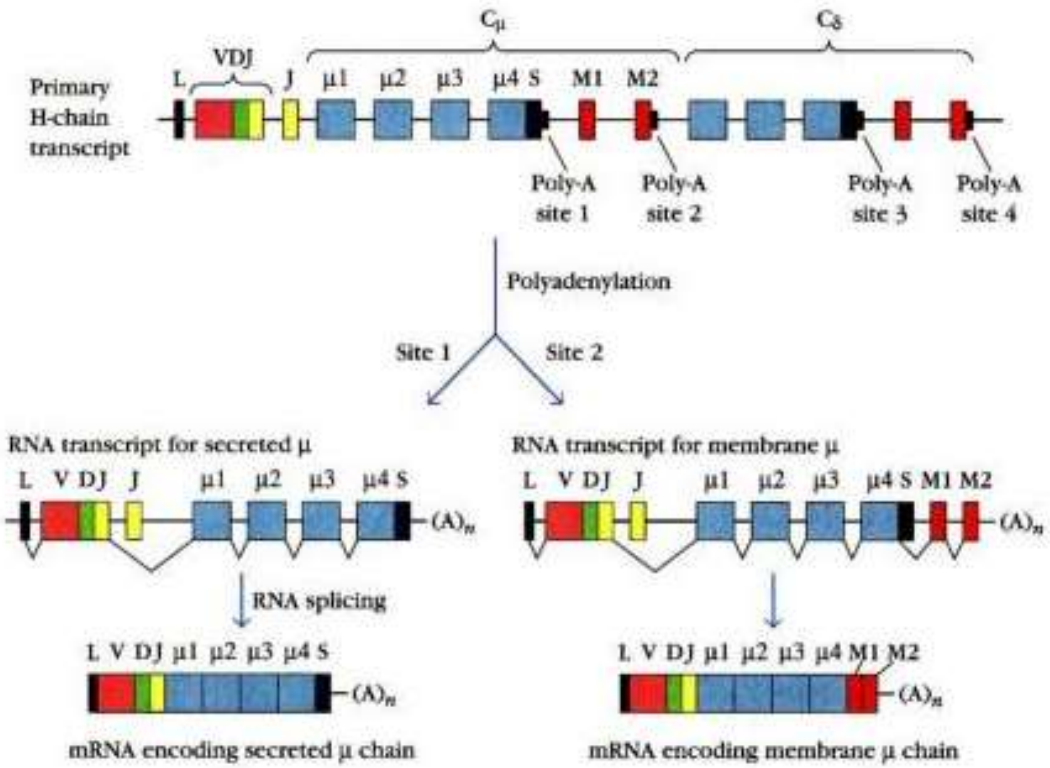
Expression of Membrane-Bound IgD and IgM

- An initial pre-mRNA transcript is produced.
- Importantly, the pre-mRNA transcript has two poly-A sites.
- If the second polyadenylation site is read, then the mRNA for membrane-bound IgM is generated by splicing.
- If the fourth polyadenylation site is read, then the mRNA for membrane-bound IgD is generated by alternate splicing.



Expression of Membrane-Bound IgM and Secreted IgM

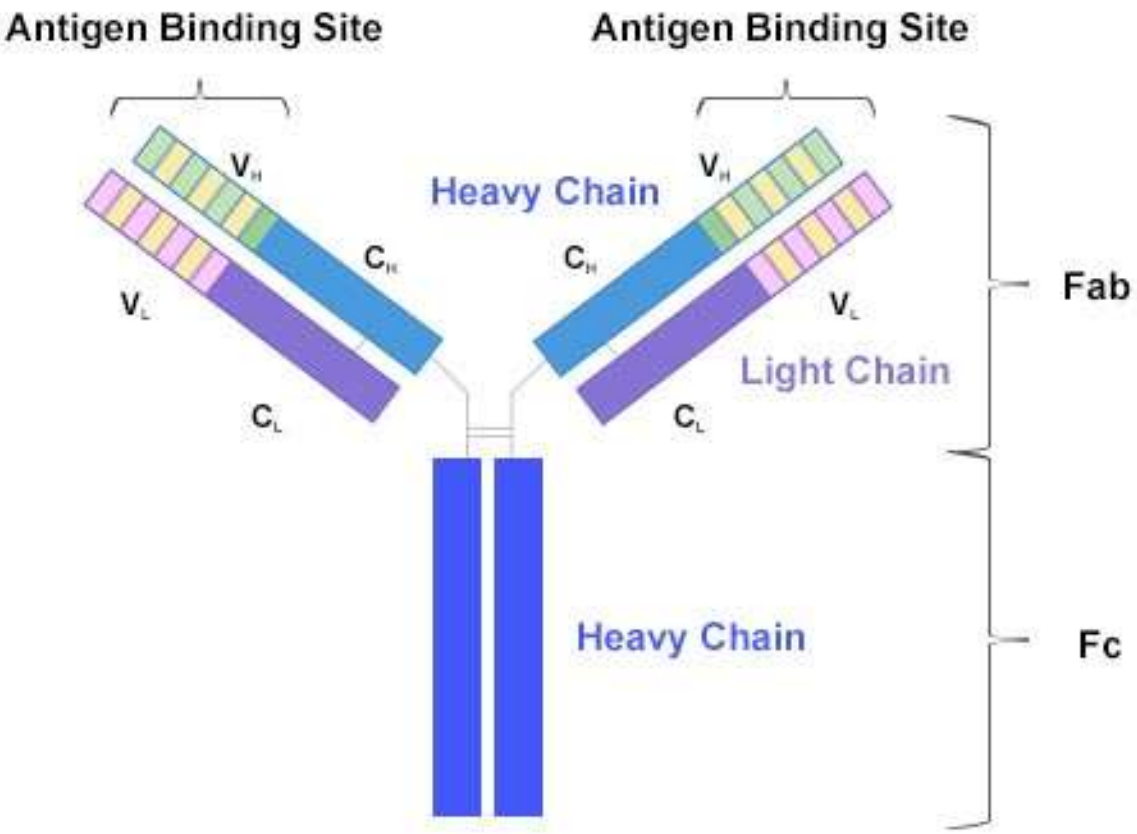
- The initial pre-mRNA transcript is synthesized.
- Importantly, the pre-mRNA transcripts have two poly-A sites within the C_μ gene segment.
- If the M1, M2 exons are spliced out, the mRNA for secreted IgM is produced.

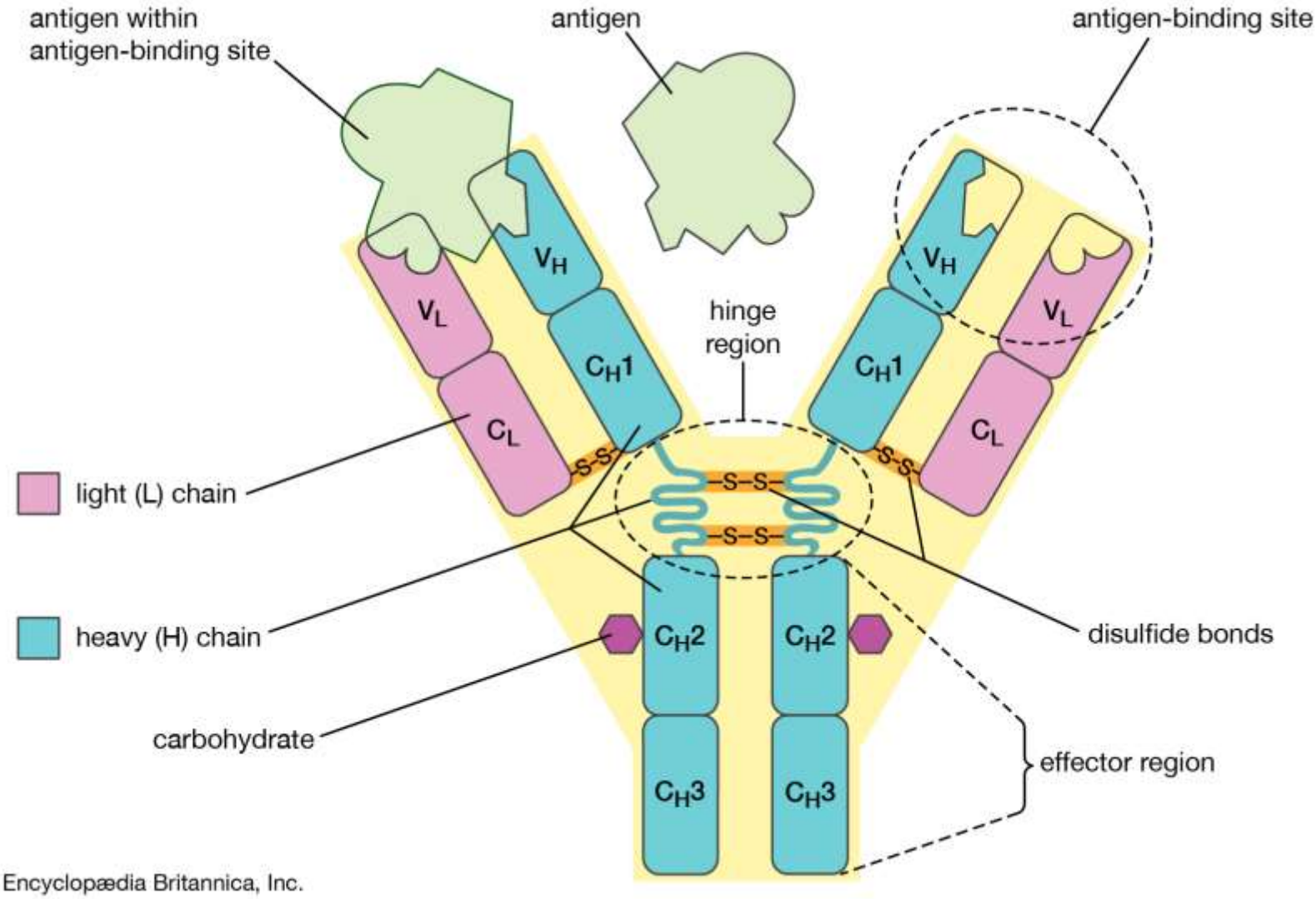


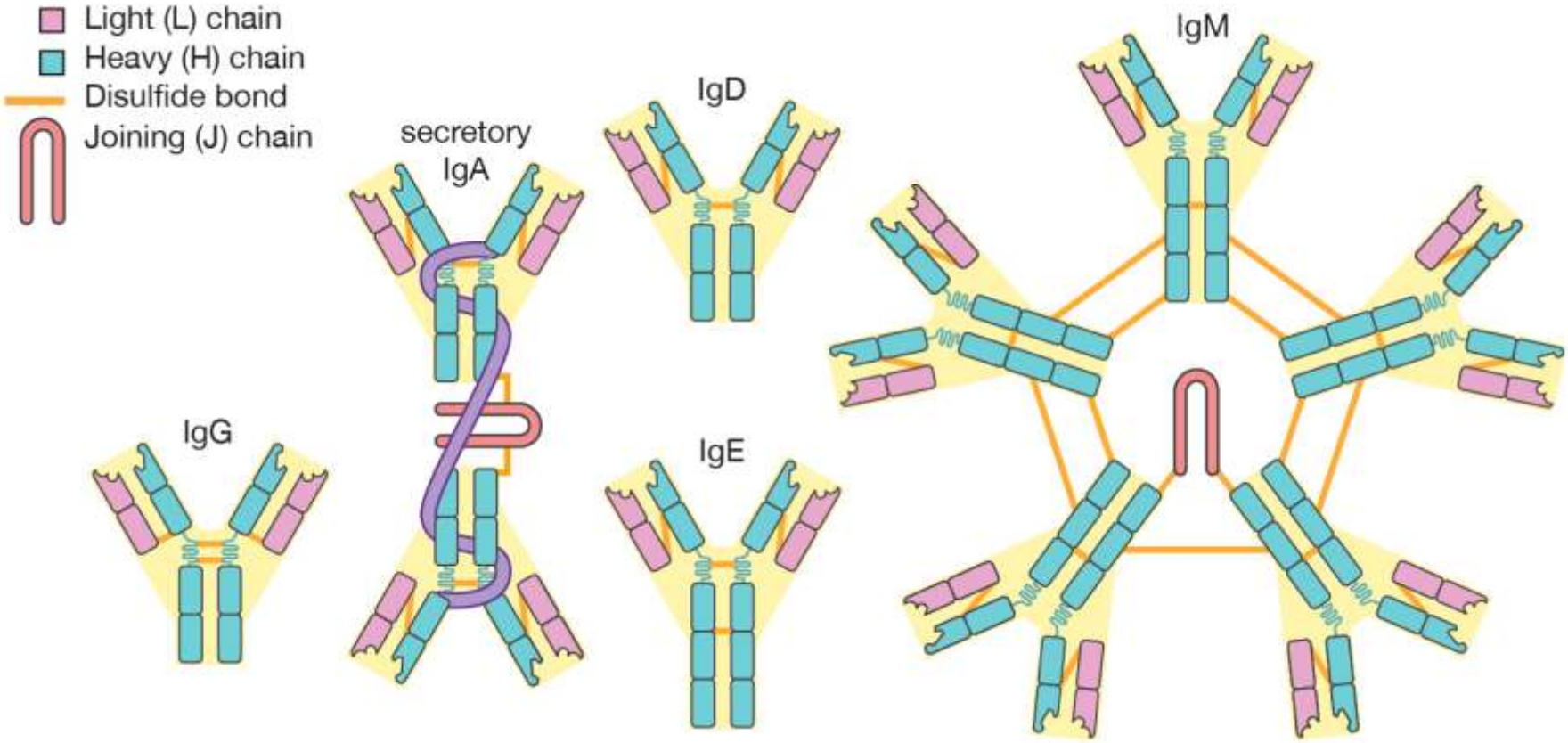
Antibody Structure and function

Grphics are collected from internet

ANTIBODY


Antigen Binding Site
Antigen Binding Site
V_H
C_H
Heavy Chain
V_H
C_H
V_L
V_L
Light Chain
C_L
C_L
Fab
Heavy Chain
Fc




Light (L) chain
Heavy (H) chain
Disulfide bond
Joining (J) chain
IgG
secretory IgA
IgD
IgE
IgM

IMMUNOGLOBULIN FRAGMENTS: STRUCTURE/FUNCTION RELATIONSHIPS

Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating structure/function relationships in immunoglobulins.

Fab

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond . This results in the formation of two identical fragments that contain the light chain and the V_H and C_{H1} domains of the heavy chain.

Antigen binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. Each Fab fragment is monovalent whereas the original molecule was divalent. The combining site of the antibody is created by both V_H and V_L. An antibody is able to bind a particular antigenic determinant because it has a particular combination of V_H and V_L. Different combinations of a V_H and V_L result in antibodies that can bind a different antigenic determinants.

Fc

Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a C_{H2} and C_{H3} domain. This fragment was called Fc because it was easily crystallized.

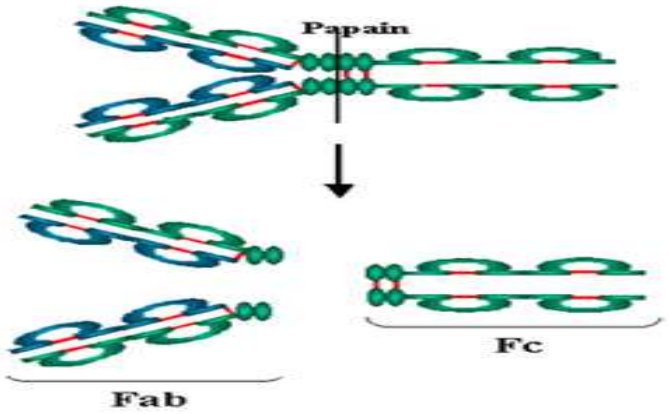
Effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule. Different functions are mediated by the different domains in this fragment . Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen; however, there are exceptions to this rule.

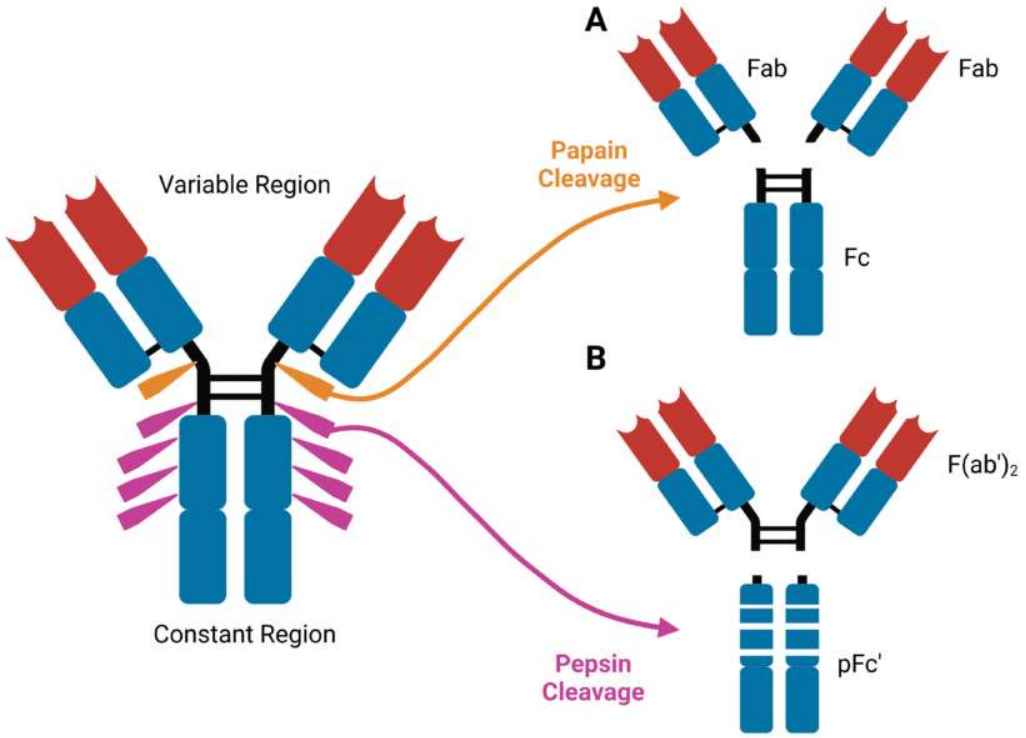
$F(ab')_2$

Treatment of immunoglobulins with pepsin results in cleavage of the heavy chain after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites . This fragment was called $F(ab')_2$ because it is divalent. The Fc region of the molecule is digested into small peptides by pepsin. The $F(ab')_2$ binds antigen but it does not mediate the effector functions of antibodies.

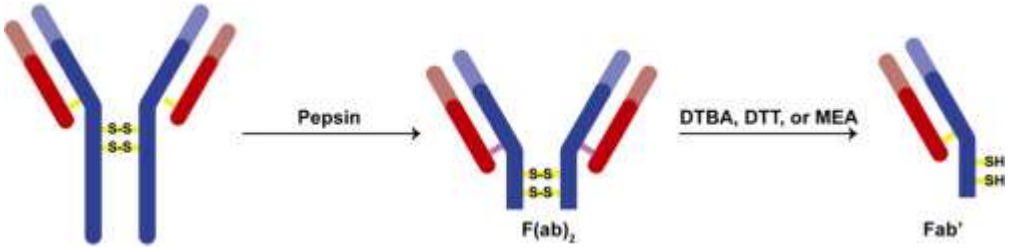
Immunoglobulin Fragments: Structure/Function Relationships

- Fab
  - Ag binding
  - Valence = 1
  - Specificty determined by V_H and V_L
- Fc
  - Effector functions





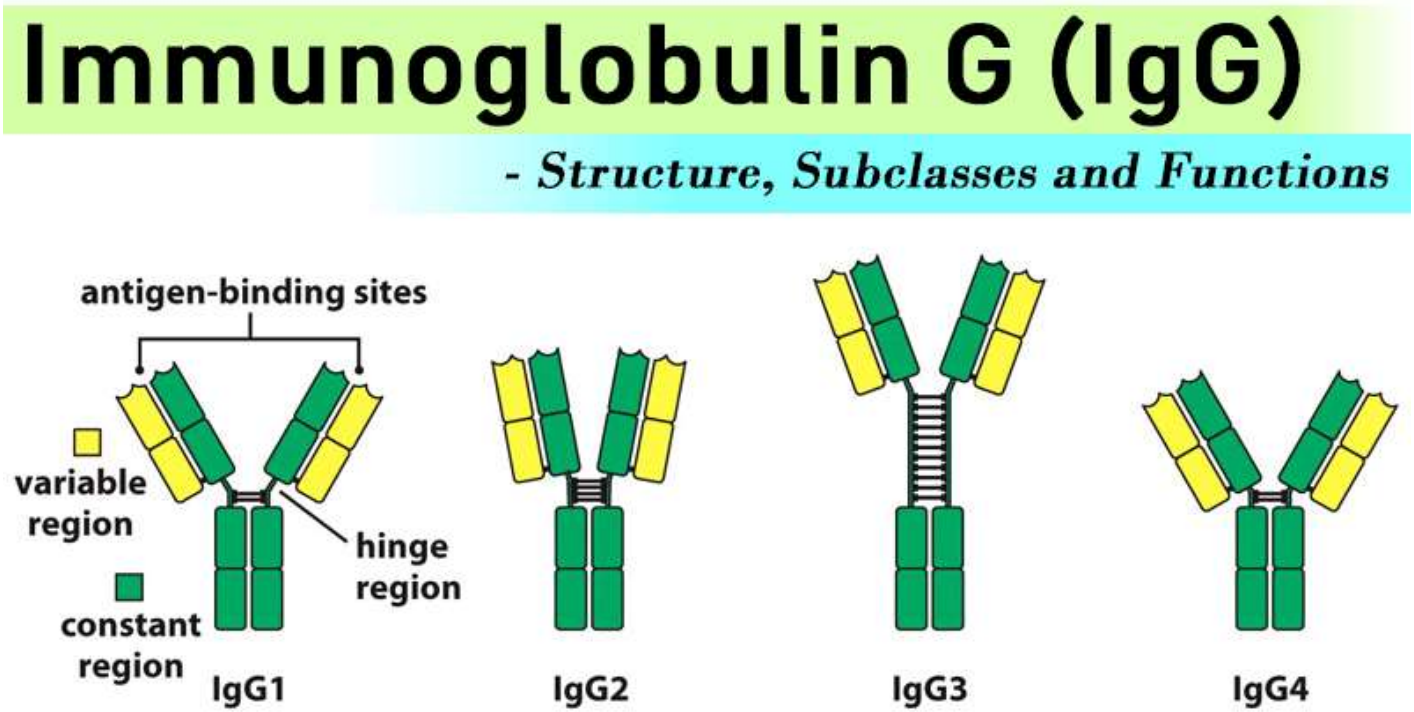
The main function of the hinge region is to provide flexibility to the Fab arm, so that the antibody can interact with different epitopes of an antigen. The region is rich in amino acids like cysteine, proline etc. IgE and IgM have no hinge region.



dithiothreitol (DTT), mercaptoethylamine (MEA), and dithiobutylamine (DTBA)

What are the functions of the antibody Immunoglobulin Ig G?
Function. Antibodies are major components of humoral immunity. IgG is the main type of antibody found in blood and extracellular fluid, allowing it to control infection of **body** tissues. By binding many kinds of pathogens such as viruses, bacteria, and fungi, IgG protects the **body** from infection.

Representing approximately 75% of serum antibodies in humans, IgG is the most common type of antibody found in blood circulation.IgG molecules are created and released by plasma B cells. Each IgG has two antigen binding sites.



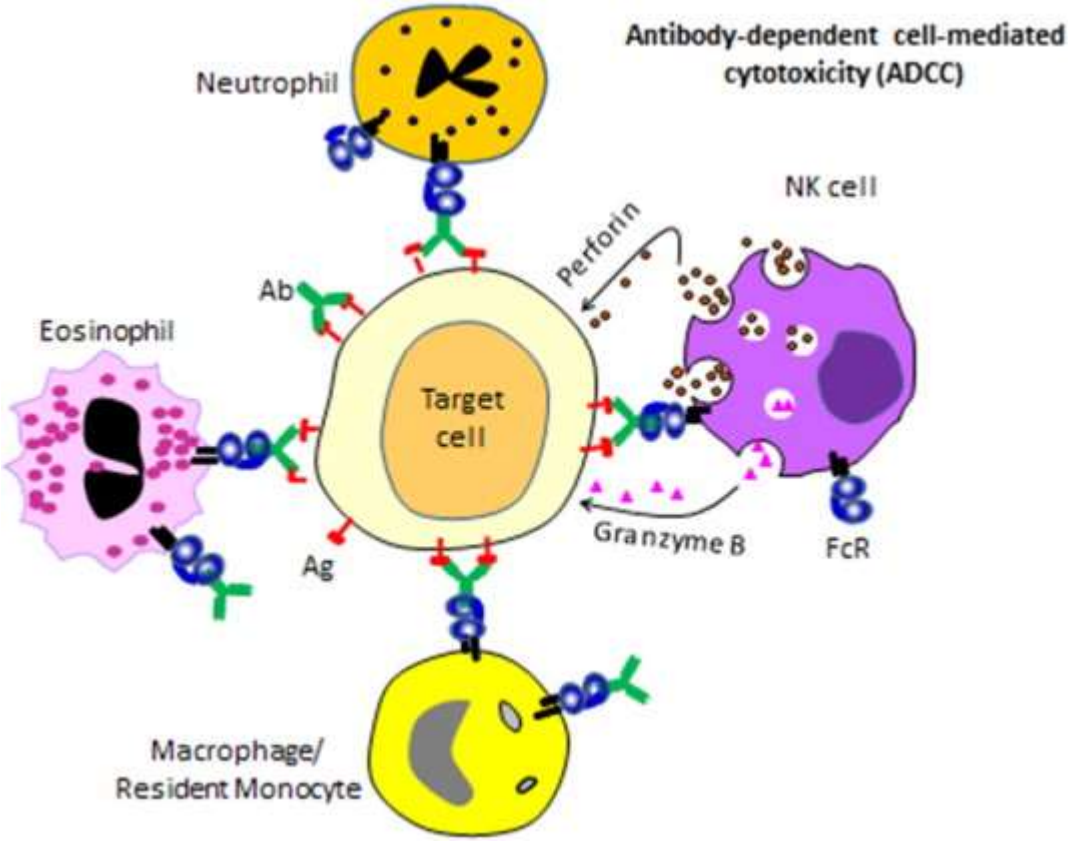
IgG-mediated binding of pathogens causes their immobilization and binding together via agglutination; IgG coating of pathogen surfaces (known as opsonization) allows their recognition and ingestion by phagocytic immune cells leading to the elimination of the pathogen itself;
IgG activates all the **classical pathway of the complement system**, a cascade of immune protein production that results in pathogen elimination;
IgG also binds and **neutralizes toxins.**
IgG also plays an important role in **antibody-dependent cell-mediated cytotoxicity (ADCC)** and **intracellular antibody-mediated proteolysis**, in which it binds to TRIM21 (the receptor with greatest affinity to IgG in humans) in order to direct marked virions to the proteasome in the cytosol;
IgG is also associated **with type II and type III hypersensitivity** reactions.

IgG antibodies are generated following class switching and maturation of the antibody response, thus **they participate predominantly in the secondary immune response.**
IgG is secreted as a monomer that is small in size allowing it to easily perfuse tissues.
It is the only antibody isotype that has receptors to facilitate passage through the **human placenta**, thereby providing protection to the fetus *in utero*.

IgG are also involved in the regulation of **allergic reactions**.
Antigens form complexes with IgG, **which then cross-link macrophage receptor FcγRIII and stimulates only platelet activating factor (PAF) release(resulting platelet aggregation and dilation of blood vessels)**

Antibody-dependent cell-mediated cytotoxicity (ADCC) by four major immune effector cells: macrophages/resident monocytes, NK cells, neutrophils, and eosinophils.

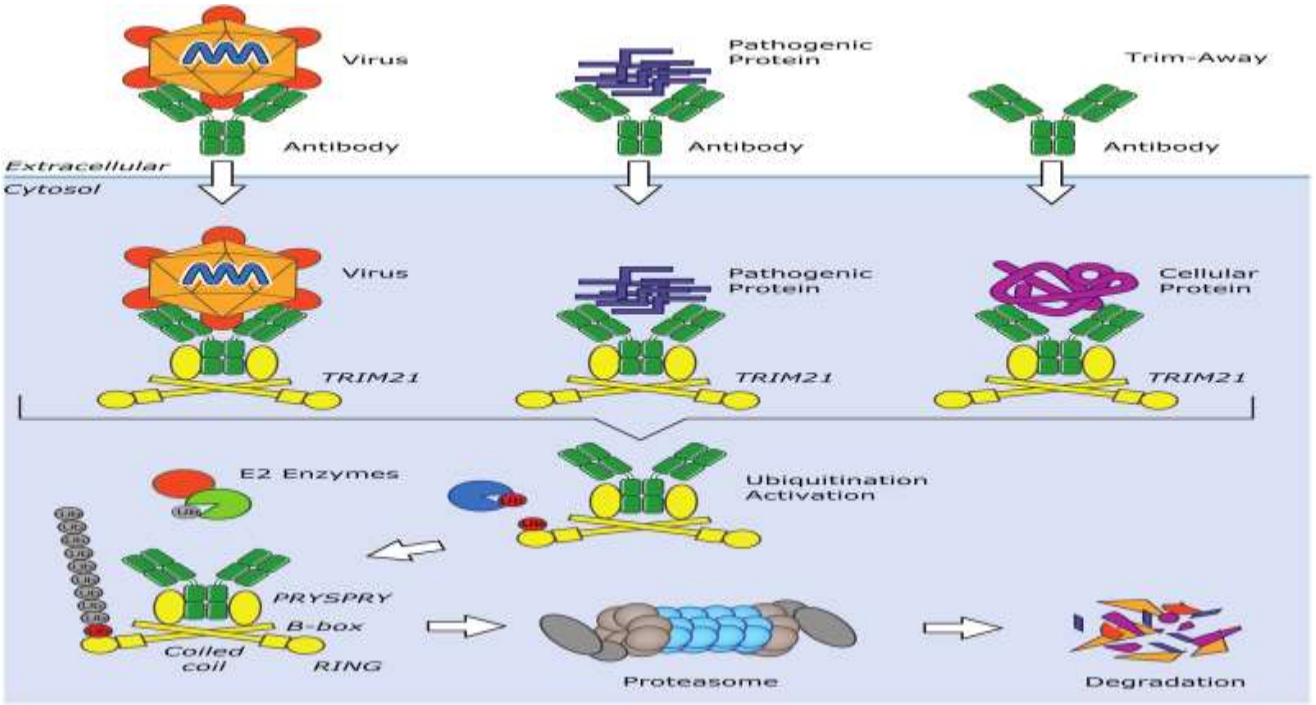
The target cell may either be microbe infected or a tumor cell that expresses the antigen recognized and bound by the antibody. Target cell killing is achieved by several cytolytic processes that also cause limitation of microbial or tumor growth.

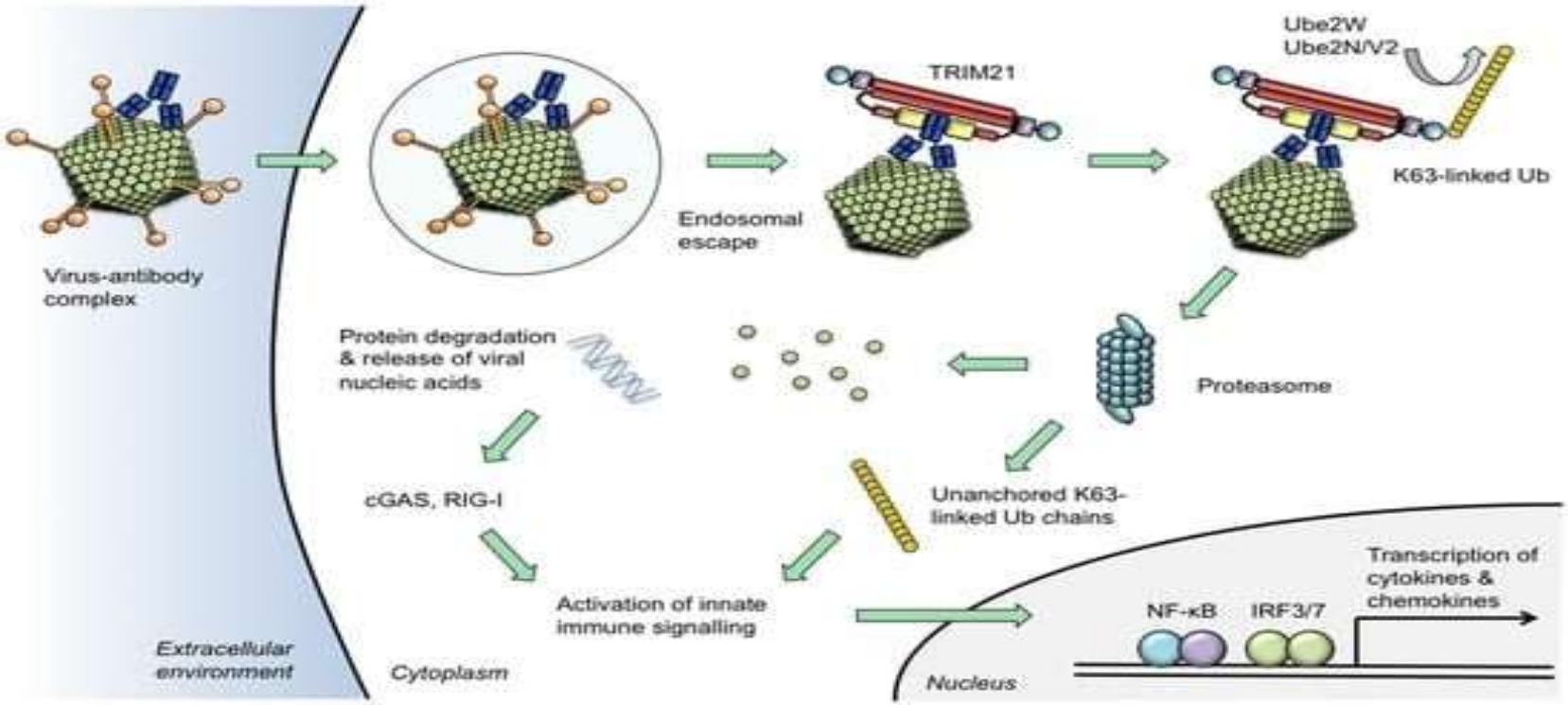


Intracellular antibody-mediated degradation (IAMD) is a neutralization mechanism of intracellular antibody-mediated immunity whereby an effector protein, TRIM21, directs antibody bound virions to the proteasome where they are degraded.

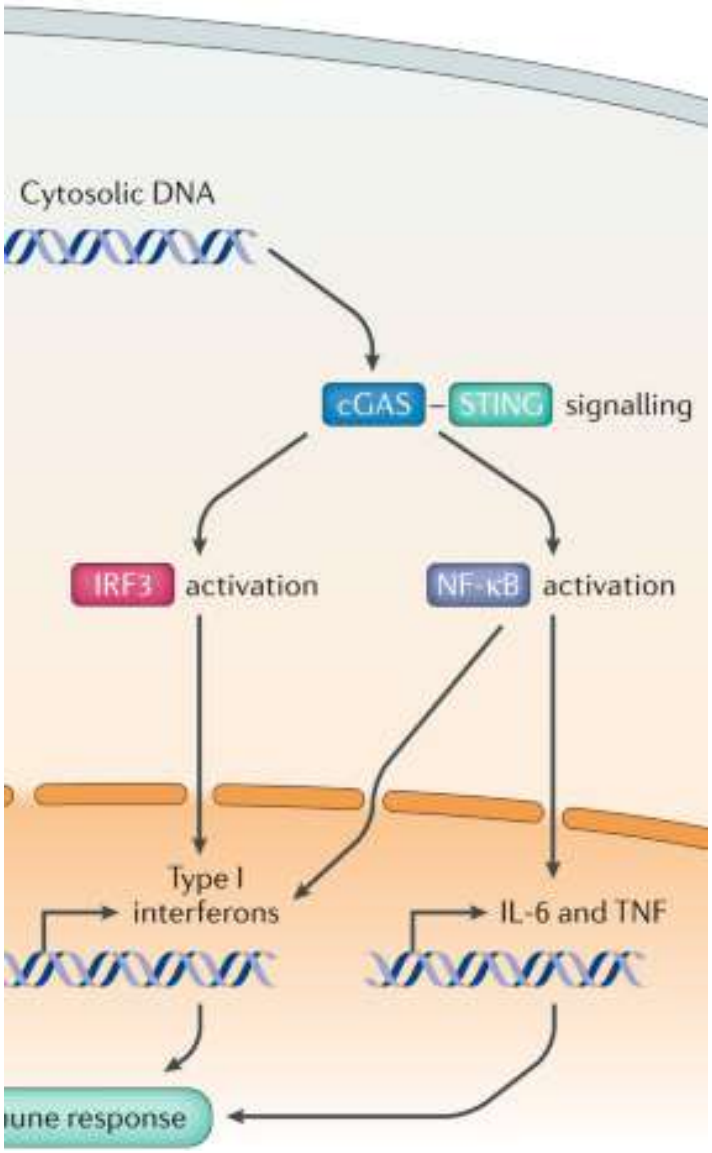
Tripartite motif containing-21 (TRIM21) is a cytosolic ubiquitin ligase and antibody receptor that **provides a last line of defense against invading viruses**. It does so by acting as a sensor that intercepts antibody-coated viruses that have evaded extracellular neutralization and breached the cell membrane.

TRIM21 binds to all 4 subclasses of IgG (IgG1, IgG2, IgG3, and IgG4) with comparable affinities, and this binding is remarkably highly conserved, meaning that human and mouse TRIM21 will bind to antibodies from other mammals

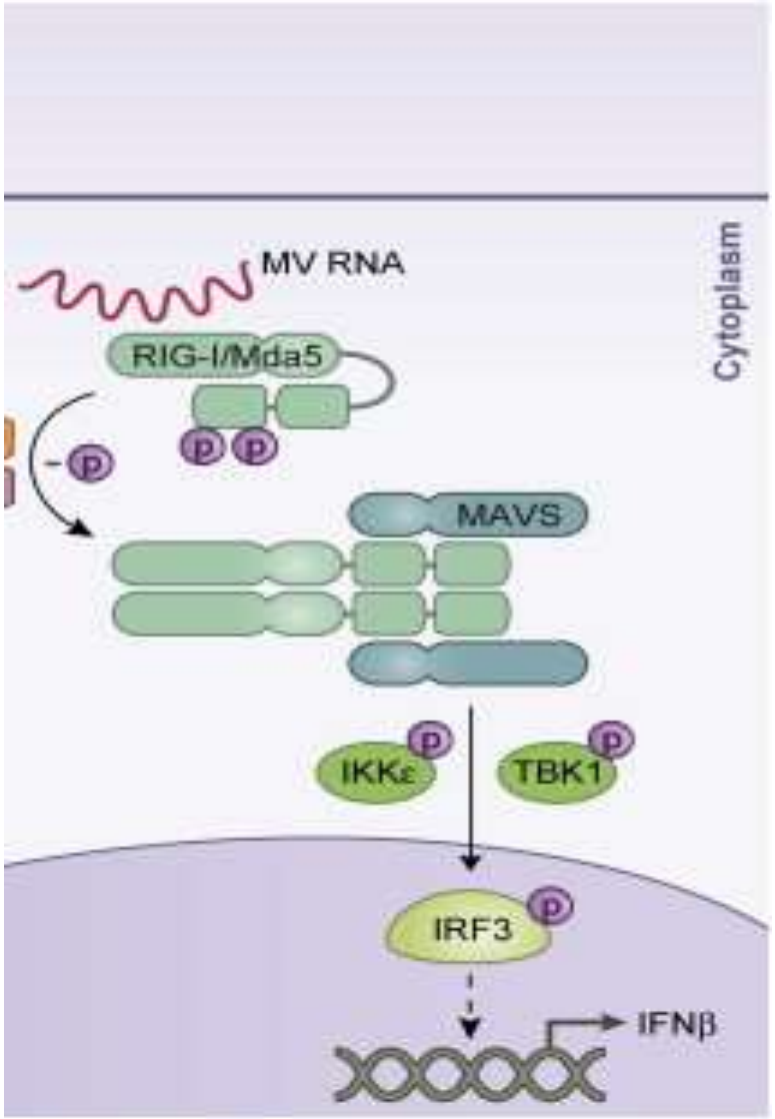




Model of TRIM21 neutralization and signalling activities. Certain viruses traffic antibodies attached to their capsids into the cytoplasm following escape from the endosome. Intracellular antibodies are bound by TRIM2. TRIM21 catalyses its auto-ubiquitination via mono-ubiquitination by Ube2W and K63-linked chain extension by Ube2n/Ube2V2. The proteasome degrades viral components causing the neutralization of infection. Concurrent with degradation, unanchored ubiquitin chains are released via the activity of proteasome-resident de-ubiquitinating enzyme POH1. Activation of NF-κB and IRF3/7 ensues which is attributable to both a direct effect of TRIM21 and through the release of viral genomes which are detected by nucleic acid sensors cGAS (for DNA) or RIG-I (for RNA). Activation of innate immune signalling stimulates production of pro-inflammatory chemokines including CCL5 and CXCL10 and cytokines such as IL-6 and interferon-β. In this way, TRIM21 uses intracellular antibody to prevent infection via its neutralization effector mechanism and to induce a transcriptional state that is refractory to viral replication in neighbouring cells.



cGAS-STING



RIG/Mda5

IgG3	IgG1	IgG2	IgG4
Ligation of all Fc receptors, including FcγRI in monomeric form (IgG3 > IgG1)		Restricted ligation of Fc receptors and only when complexed, particularly large complexes	Restricted ligation of Fc receptors and only when complexed, particularly large complexes
		Weak complement activation	No complement activation
		Most resistant of all IgG isotypes to proteolytic degradation	Produced after chronic immune stimulation, particularly parasite infections
Potent complement activation through the classical pathway (IgG3 > IgG1)		Predominant IgG subclass in plasma IgM-IgG complexes	Regulated similarly to IgE
		Only IgG subclass to undergo covalent dimerization	May form bispecific antibodies
		Predominant IgG subclass in phagocytic antibodies to polysaccharide antigens	

IgG4 is regarded as an anti-inflammatory antibody with a limited ability to elicit effective immune responses. Furthermore, IgG4 **attenuates allergic responses by inhibiting the activity of IgE**.

Immunoglobulin A (**IgA**), as the principal **antibody** class in the secretions that bathe these mucosal surfaces, acts as an important first line of defence. **IgA**, also an important serum **immunoglobulin**, mediates a variety of protective functions through interaction with specific receptors and immune mediators

Immunoglobulin A (**IgA**, also referred to as **sIgA** in its secretory form) is an antibody that plays a crucial role in the **immune function of mucous membranes**. The amount of IgA produced in association with mucosal membranes is greater than all other types of antibody combined. In absolute terms, between three and five grams are secreted into the intestinal lumen each day. This represents up to 15% of total immunoglobulins produced throughout the body.

IgA has two subclasses (IgA1 and IgA2) and can be produced as a monomeric as well as a dimeric form. **The IgA dimeric form is the most prevalent and is also called *secretory IgA* (sIgA)**. **sIgA is the main immunoglobulin found in mucous secretions**, including tears, saliva, sweat, colostrum and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium. It is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes; thus, sIgA can survive in the harsh gastrointestinal tract environment and provide protection against microbes that multiply in body secretions. sIgA can also inhibit inflammatory effects of other immunoglobulins. IgA is a **poor activator of the complement system, and opsonizes only weakly.**

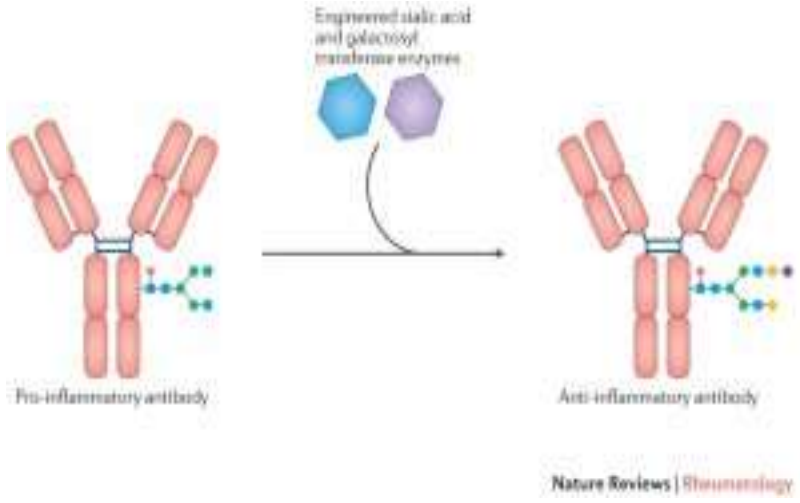
IgA1 vs. IgA2

IgA exists in two isotypes, IgA1 and IgA2. They are both heavily glycosylated proteins. While IgA1 predominates in serum (~80%), IgA2 percentages are higher in secretions than in serum (~35% in secretions); the ratio of IgA1 and IgA2 secreting cells varies in the different lymphoid tissues of the human body:

IgA1 is the predominant IgA subclass found in serum. Most lymphoid tissues have a predominance of IgA1-producing cells.

In IgA2, **the heavy and light chains are not linked with disulfide, but with non-covalent bonds**. In secretory lymphoid tissues (e.g., gut-associated lymphoid tissue, or GALT), the share of IgA2 production is larger than in the non-secretory lymphoid organs (e.g. spleen, peripheral lymph nodes).

Whereas **IgA2 acts pro-inflammatory on neutrophils and macrophages, IgA1 does not have pronounced effects**. Moreover, IgA1 and IgA2 have different glycosylation profiles, with IgA1 possessing more sialic acid than IgA2. Removal of sialic acid increases the pro-inflammatory capacity of IgA1, making it comparable to IgA2



While IgA1 predominates in the nasal and bronchial secretions of the human upper respiratory tract and in the upper GI tract, more than 60% of plasma cells in the lamina propria of the large intestine secrete IgA2

Immunoglobulin E (**IgE**) is a type of antibody (or immunoglobulin (Ig) "isotype") that has only been found in mammals.

IgE is synthesised by plasma cells.

Monomers of IgE consist of two heavy chains (ε chain) and two light chains, with the ε chain containing 4 Ig-like constant domains (Cε1-Cε4).

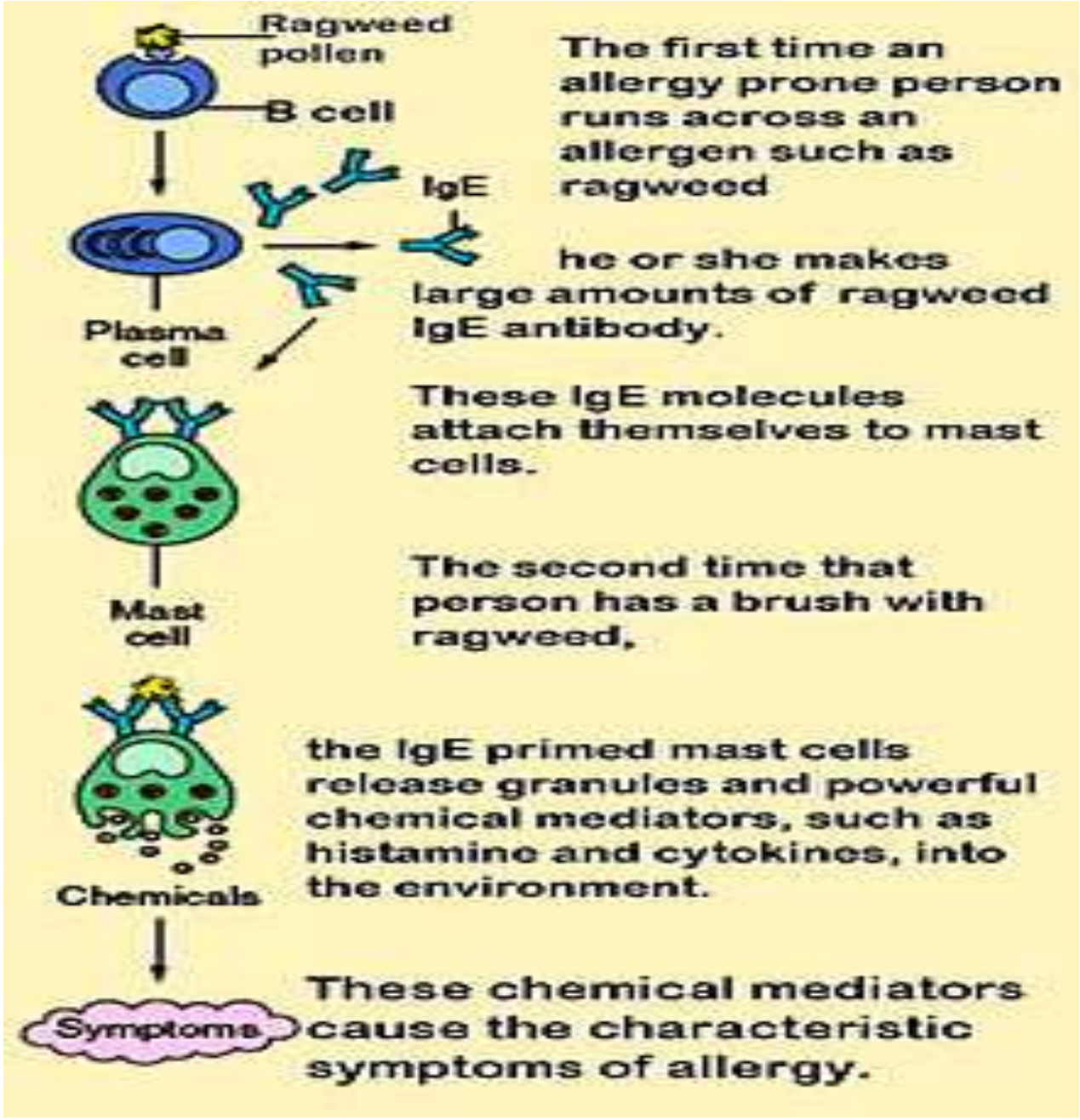
IgE's main function is immunity to **parasites such as helminths like *Schistosoma mansoni*, *Trichinella spiralis*, and *Fasciola hepatica***.

IgE is utilized during immune defense **against certain protozoan parasites such as *Plasmodium falciparum***.

IgE may have evolved as a last line of defense to protect against venoms.

IgE also has an essential role in **type I hypersensitivity,** which manifests in various allergic diseases, such as allergic asthma, most types of sinusitis, allergic rhinitis, food allergies, and specific types of chronic urticaria and atopic dermatitis.

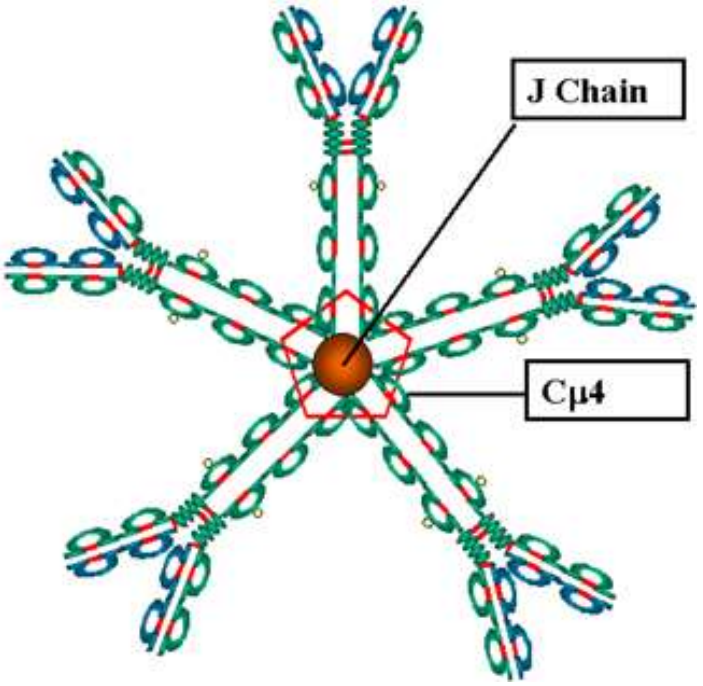
IgE also plays a pivotal role in responses to allergens, such as: **anaphylactic reactions to drugs, bee stings, and antigen preparations used in** desensitization immunotherapy.


Ragweed pollen
B cell
IgE
Plasma cell
Mast cell
Chemicals
Symptoms
The first time an allergy prone person runs across an allergen such as ragweed
he or she makes large amounts of ragweed IgE antibody.
These IgE molecules attach themselves to mast cells.
The second time that person has a brush with ragweed,
the IgE primed mast cells release granules and powerful chemical mediators, such as histamine and cytokines, into the environment.
These chemical mediators cause the characteristic symptoms of allergy.

Immunoglobulin M (**IgM**) is one of several isotypes of antibody (also known as immunoglobulin) that are produced by vertebrates. IgM is the largest antibody, and it is the first antibody to appear in the response to initial exposure to an antigen

IgM

- Structure
  - Pentamer (19S)
  - Extra domain (C_{H4})
  - J chain



Function

IgM interacts with several other physiological molecules:

IgM can bind complement component C1 and activate the **classical pathway**, leading to opsonization of antigens and cytolysis.

IgM binds to the **polyimmunoglobulin receptor (pIgR) in a process that brings IgM to mucosal surfaces, such as the gut lumen and into breast milk**. This binding depends on J chain. (The polymeric immunoglobulin receptor (**pIgR**) recognizes the J chain region of polymerized **IgA** and IgM)

Two other Fc receptors that bind **IgM—Fcα/μ-R and Fcμ-R** -- have been detected.

Fcα/μ-R, like pIgR, binds polymeric IgM and IgA.

Fcα/μ-R can mediate endocytosis, and its expression in the gut suggests a role in mucosal immunity.

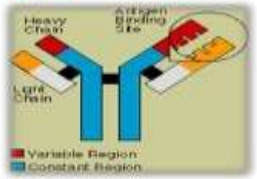
Fcμ-R (formerly known as Toso/Faim3) binds IgM exclusively and can mediate cellular uptake of IgM-conjugated antigen. Inactivation of the corresponding genes in knock-out mice produces a phenotype, but the physiological functions of these receptors are still uncertain

In B cells, **the function of IgD is to signal the B cells to be activated**. By being activated, B cells are ready to take part in the defense of the body as part of the immune system. During B cell differentiation, IgM is the exclusive isotype expressed by immature B cells.

Adaptive and innate Immune responses can be activated via membrane-anchored IgD that functions as a part of B-cell receptor (BCR) complexes or secreted-form of IgD that bounds to monocytes, mast cells, and basophil, respect

Immunoglobulin Classes

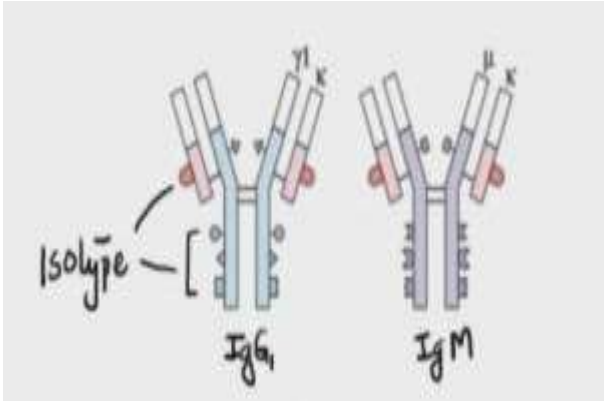
IV. IgD



- Structure: Monomer
- Percentage serum antibodies: 0.2%
- Location: B-cell surface, blood, and lymph
- Half-life in serum: 3 days
- Complement Fixation: No
- Placental Transfer: No
- Known Functions: In serum function is unknown. On B cell surface, initiate immune response.

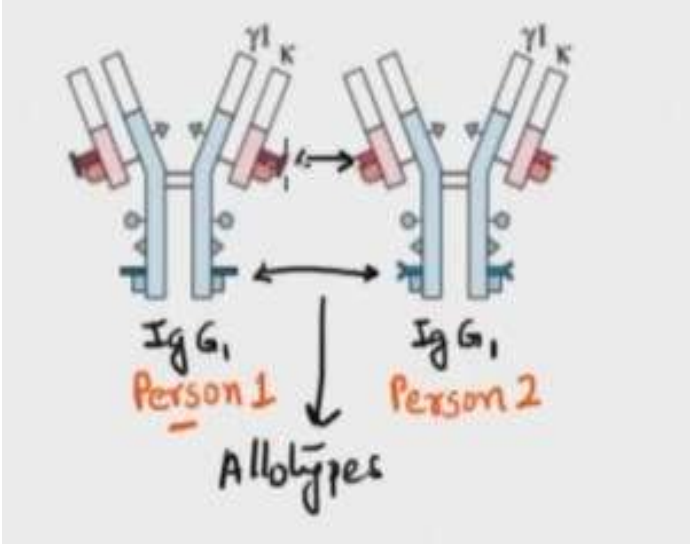
Isotypes

Each antibody has only one type of (γ, or α, or μ, or ε, or δ) heavy chain and one type of (k or λ) light chain. The structural differences in the constant region of a heavy chain or light chain determine immunoglobulin (Ig) class and sub-class, types and subtypes within a species. These constant region determinants are called isotypic determinants or isotypes.



Allotypes

Although all members of a species inherit the same set of isotype genes, **multiple alleles exist for some of the genes**. These alleles encode subtle amino acid differences. Products of allelic forms of the same gene will have slightly different amino acid sequences in the constant regions, which are known as allotypic determinants. The sum of the individual allotypic determinants displayed by an antibody determines its allotype.

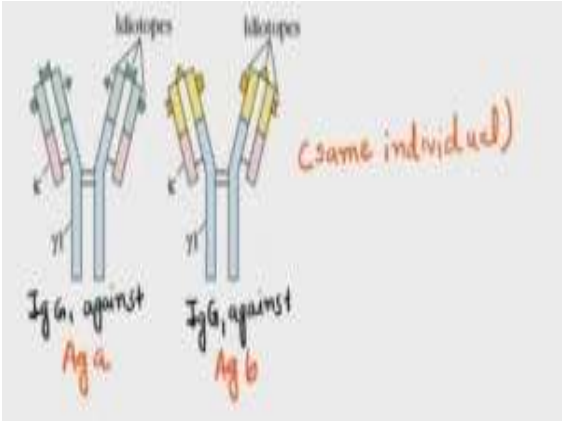


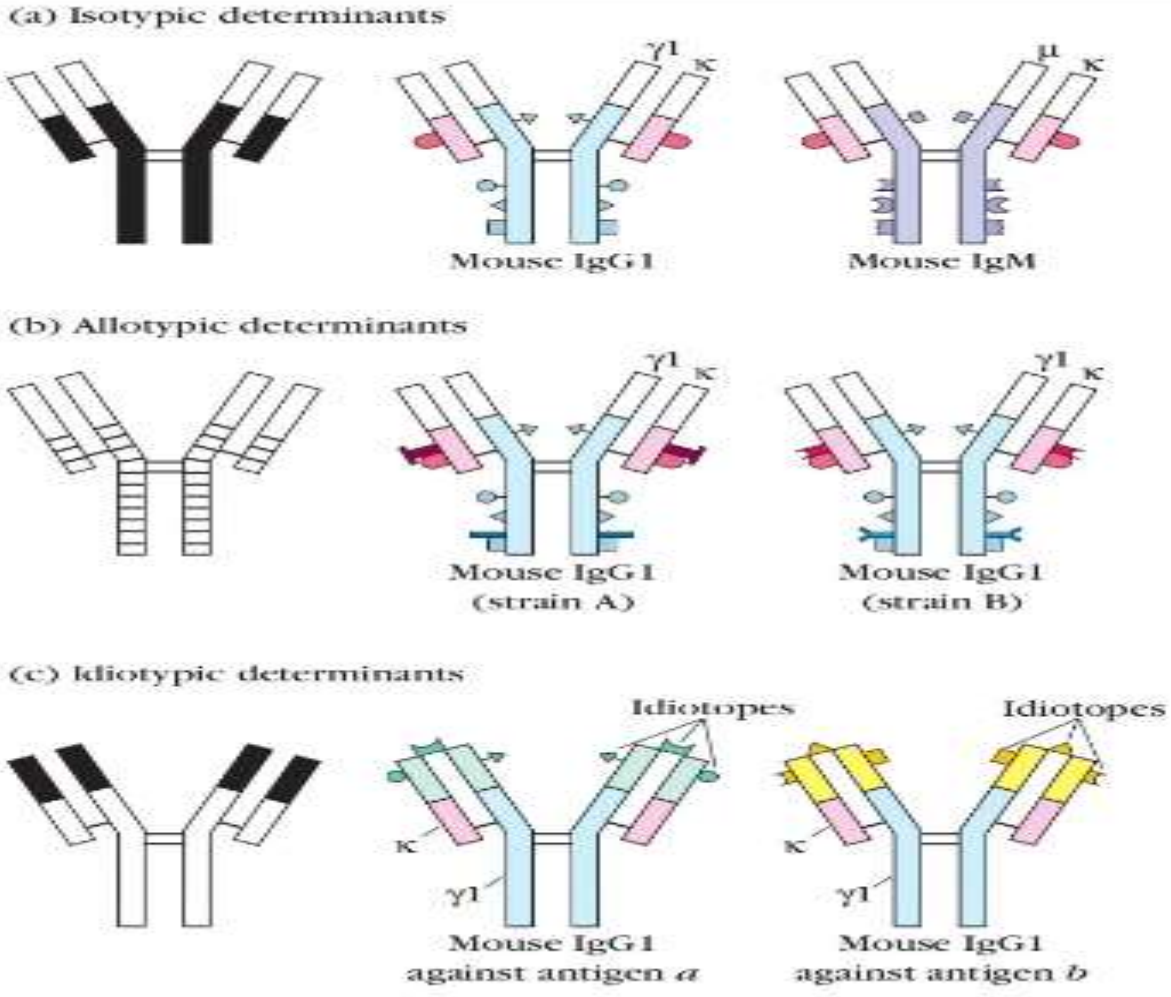
Ideotypes

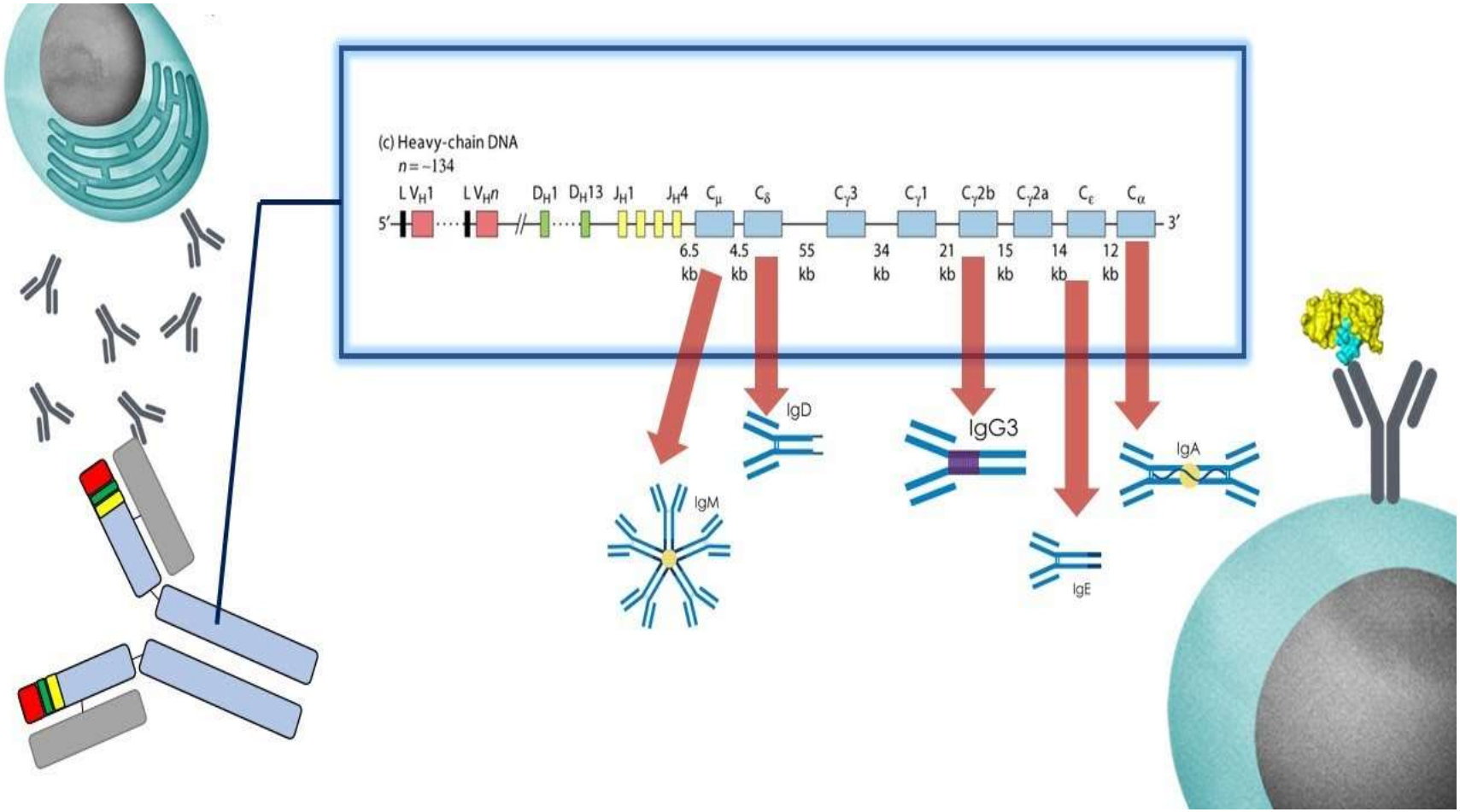
VH and VL domains of an antibody constitute an antigen-binding site. To recognize the vast array of antigens that a human can encounter in its lifetime, this variable region has different structural conformation owing to the presence of different amino acids. There are millions of such antibodies in the human body specific for each antigen.

These unique amino acid sequences present in the VH and VL domains of a given antibody also serves as a set of antigenic determinan

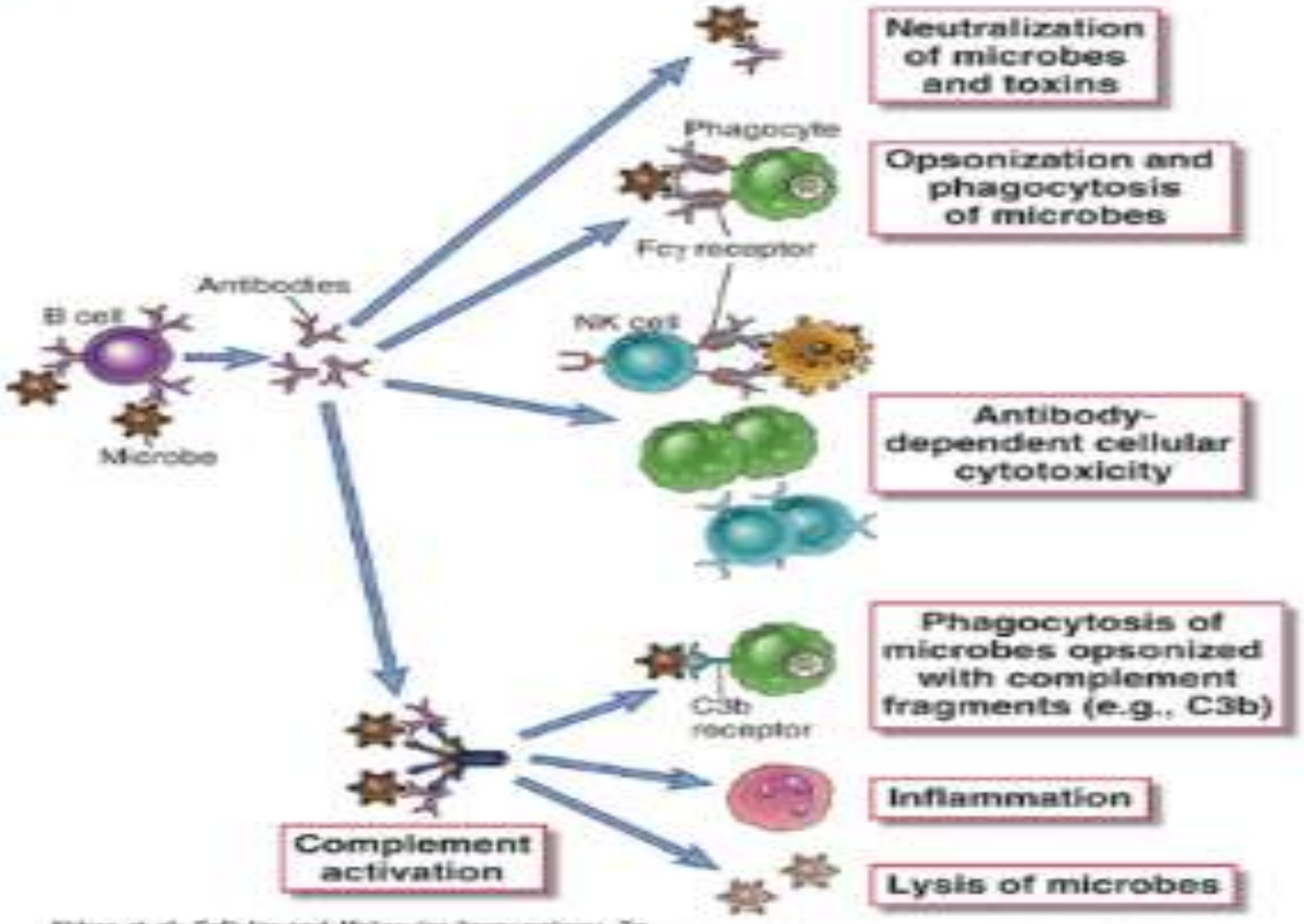
ts. Each individual **antigenic determinant of the variable region is referred to as an idiotope**. Each antibody will present multiple idiotopes; the sum of the individual idiotopes is called the idiotype of the antibody.



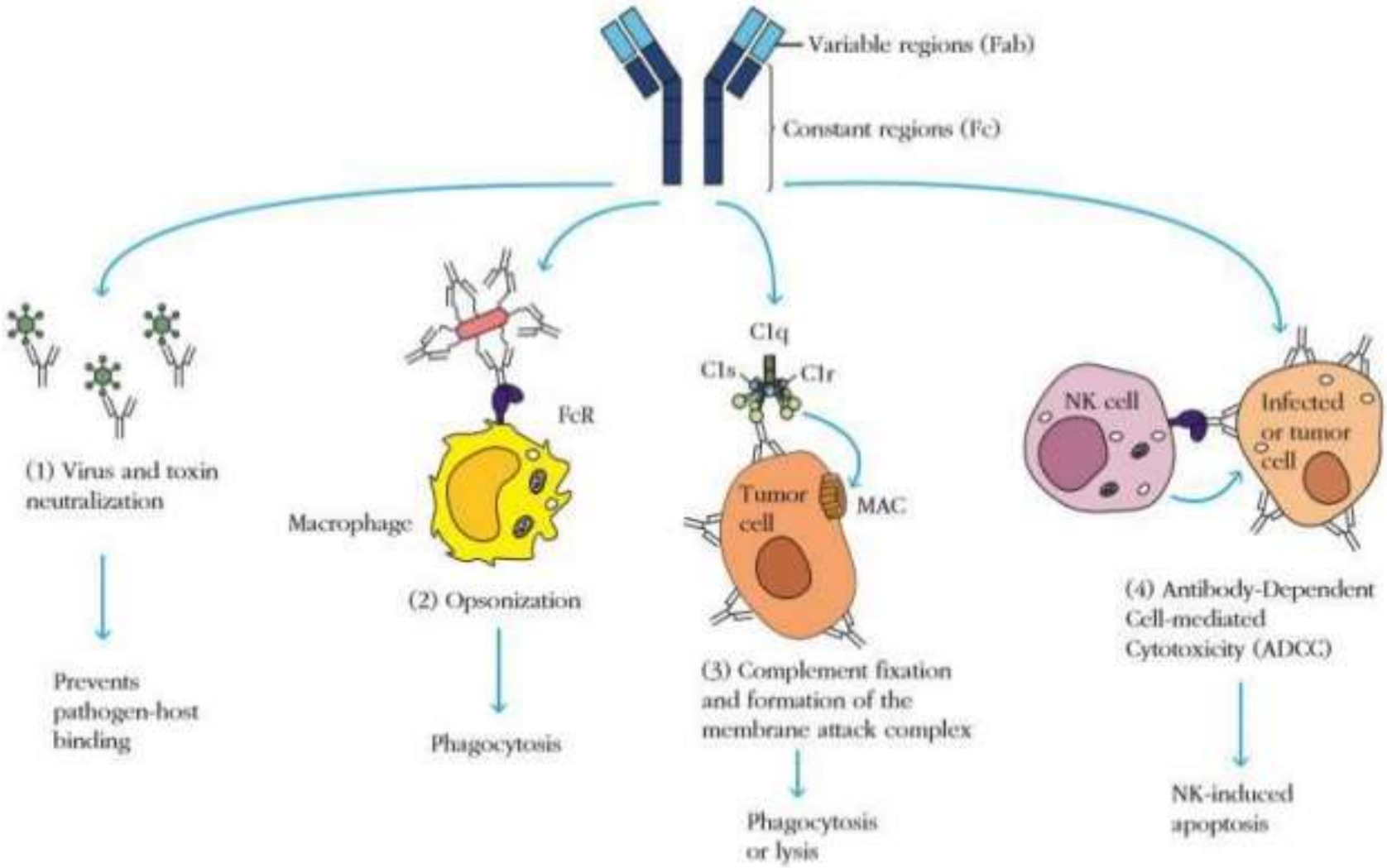

(a) Isotypic determinants
γ1
κ
μ
κ
Mouse IgG1
Mouse IgM
(b) Allotypic determinants
γ1
κ
γ1
κ
Mouse IgG1
(strain A)
Mouse IgG1
(strain B)
(c) Idiotypic determinants
Idiotopes
Idiotopes
κ
γ1
κ
γ1
Mouse IgG1
against antigen a
Mouse IgG1
against antigen b


(c) Heavy-chain DNA
n = ~134
L VH1
L VHn
DH1
DH13
JH1
JH4
Cμ
Cδ
Cγ3
Cγ1
Cγ2b
Cγ2a
Cε
Cα
5′
3′
6.5 kb
4.5 kb
55 kb
34 kb
21 kb
15 kb
14 kb
12 kb
IgM
IgD
IgG3
IgA
IgE

Major functions of the antibodies are:
Neutralization of infectivity,
Phagocytosis,
Antibody-dependent cellular cytotoxicity (ADCC),
Complement-mediated lysis of pathogens or of infected cells: Antibodies activate the complement system to destroy bacterial cells by lysis
Transcytosis, mucosal immunity & neonatal immunity
Another function is unique to Immunoglobulin E (IgE), which is **'activation of mast cells, eosinophils and basophils'.**

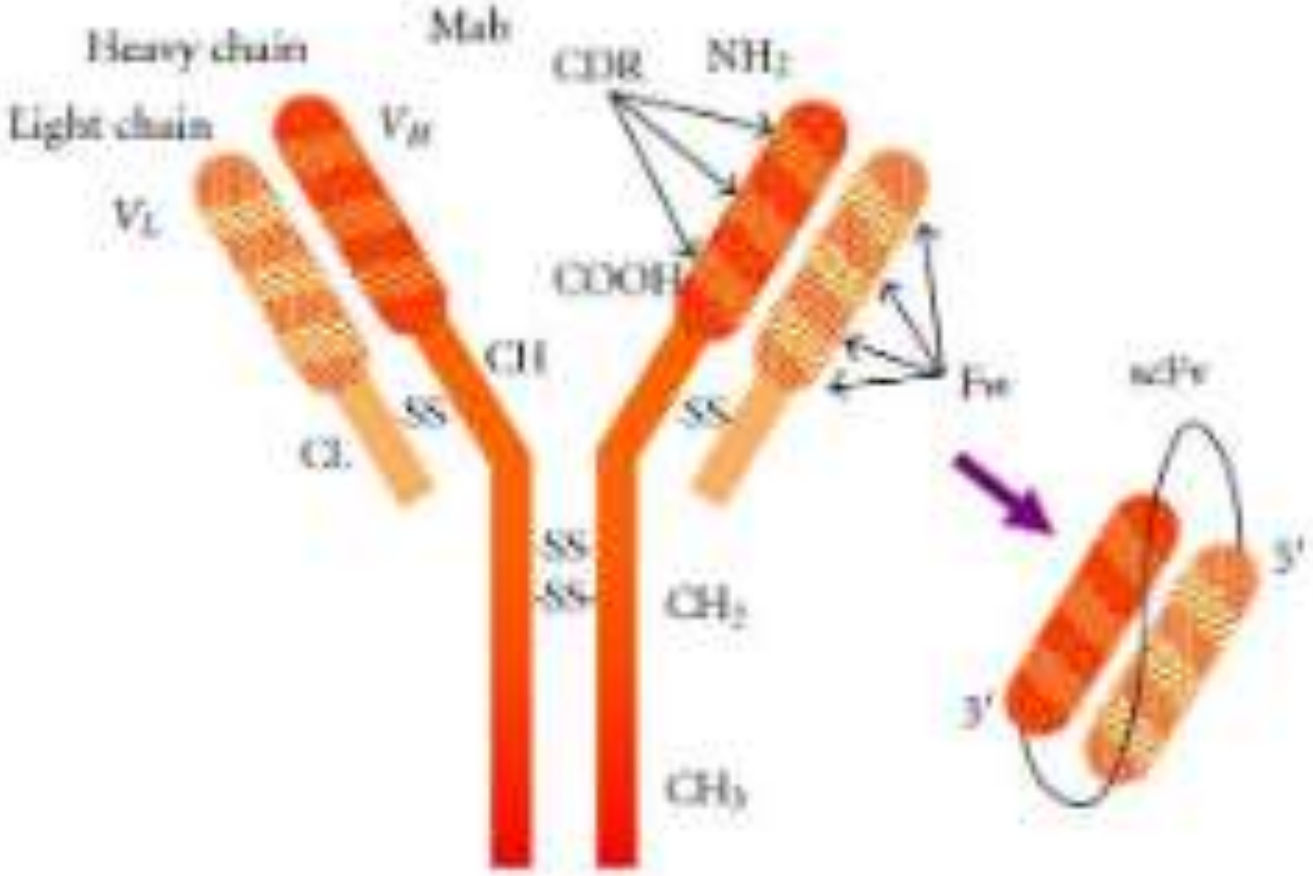


Antibody effector functions

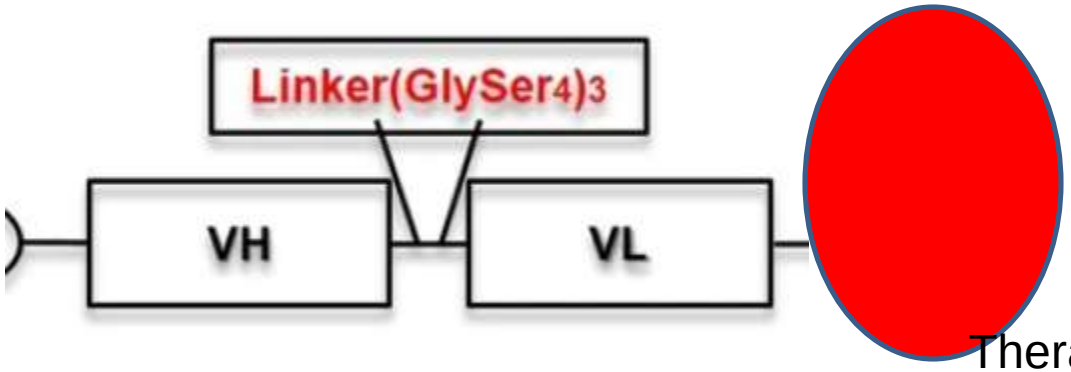


scFv Antibody

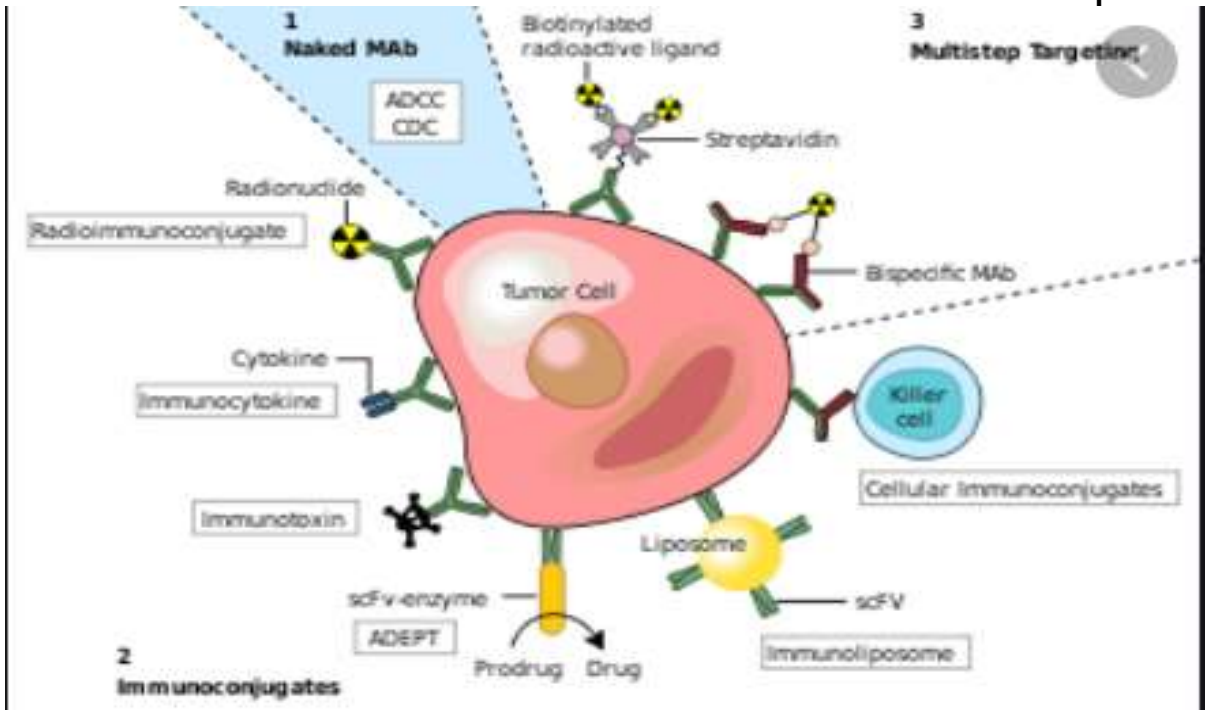
To date, **generation of single-chain fragment variable (scFv) has become an established technique used to produce a completely functional antigen-binding fragment in bacterial systems**. The advances in antibody engineering have now facilitated a more efficient and generally applicable method to produce Fv fragments. Basically, scFv antibodies produced from phage display can be genetically fused to the marker proteins, such as fluorescent proteins or alkaline phosphatase. These bifunctional proteins having both antigen-binding capacity and marker activity can be obtained from transformed bacteria and used for one-step immunodetection of biological agents. Alternatively, antibody fragments could also be applied in the construction of immunotoxins, therapeutic gene delivery, and anticancer intrabodies for therapeutic purposes.


Mab
Heavy chain
Light chain
V_H
V_L
CDR
NH_2
COOH
CH
SS
CL
SS
SS
SS
CH_2
CH_3
Fc
scFv
3'
3'

Targeted drug delivery

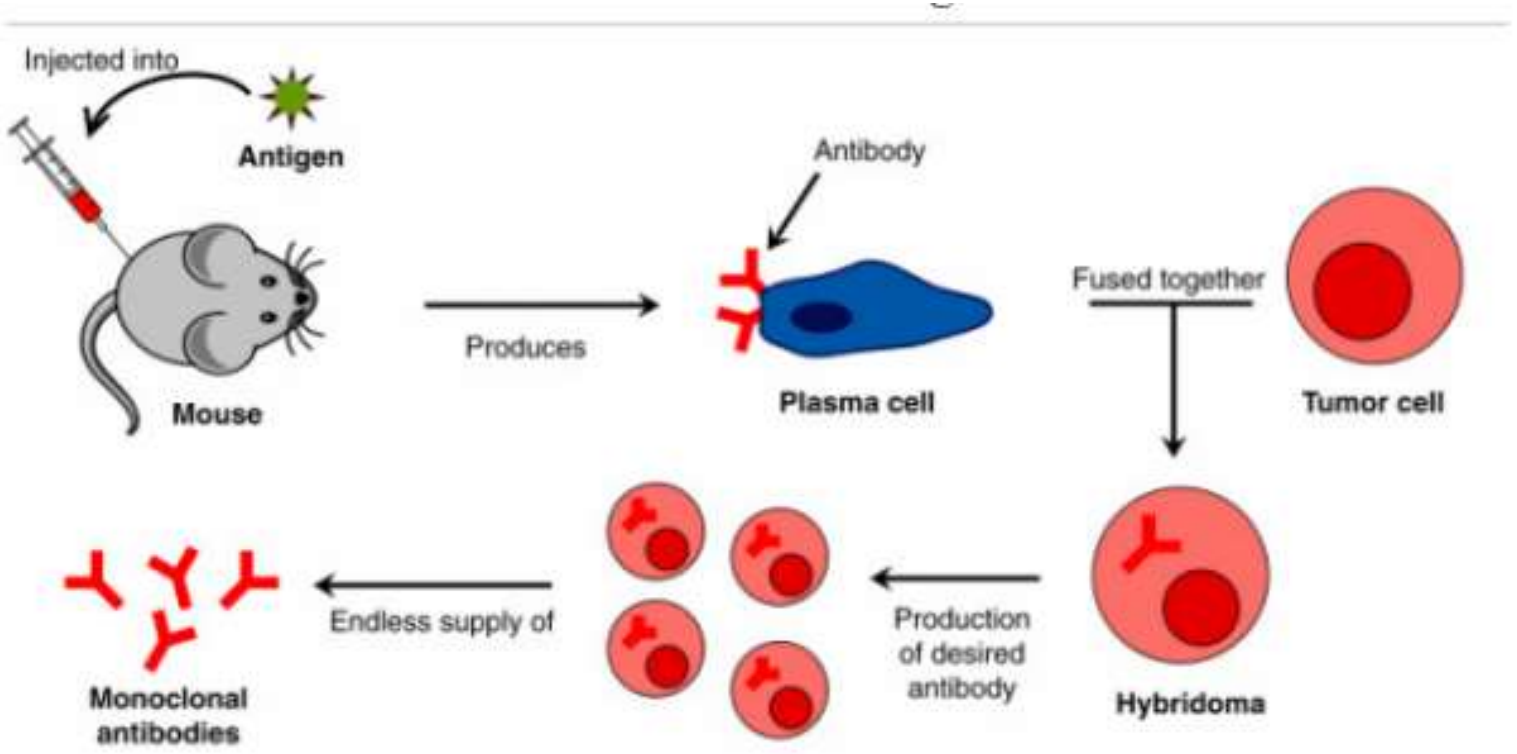


Therapeutic agents

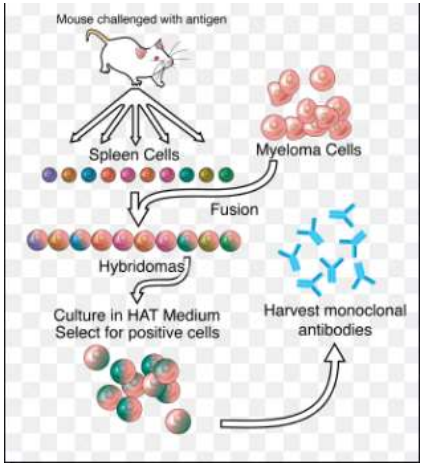


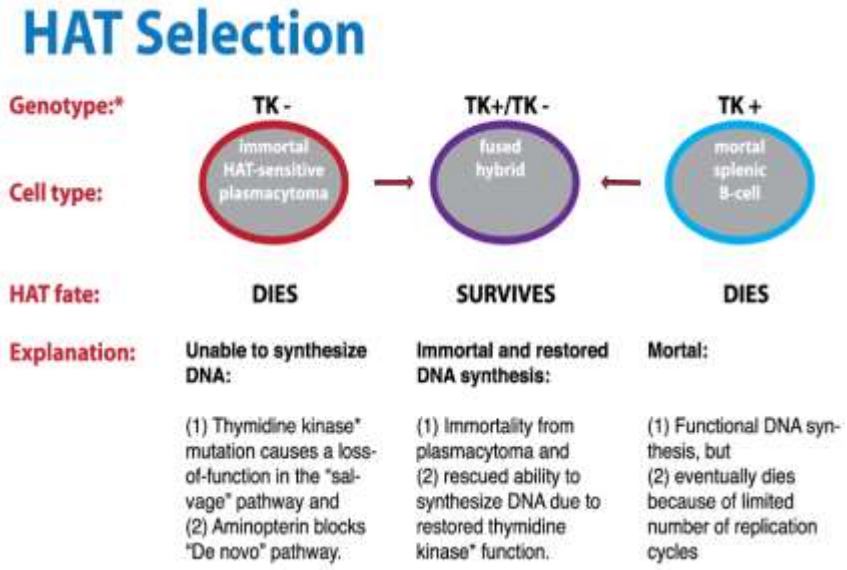
Monoclonal antibody

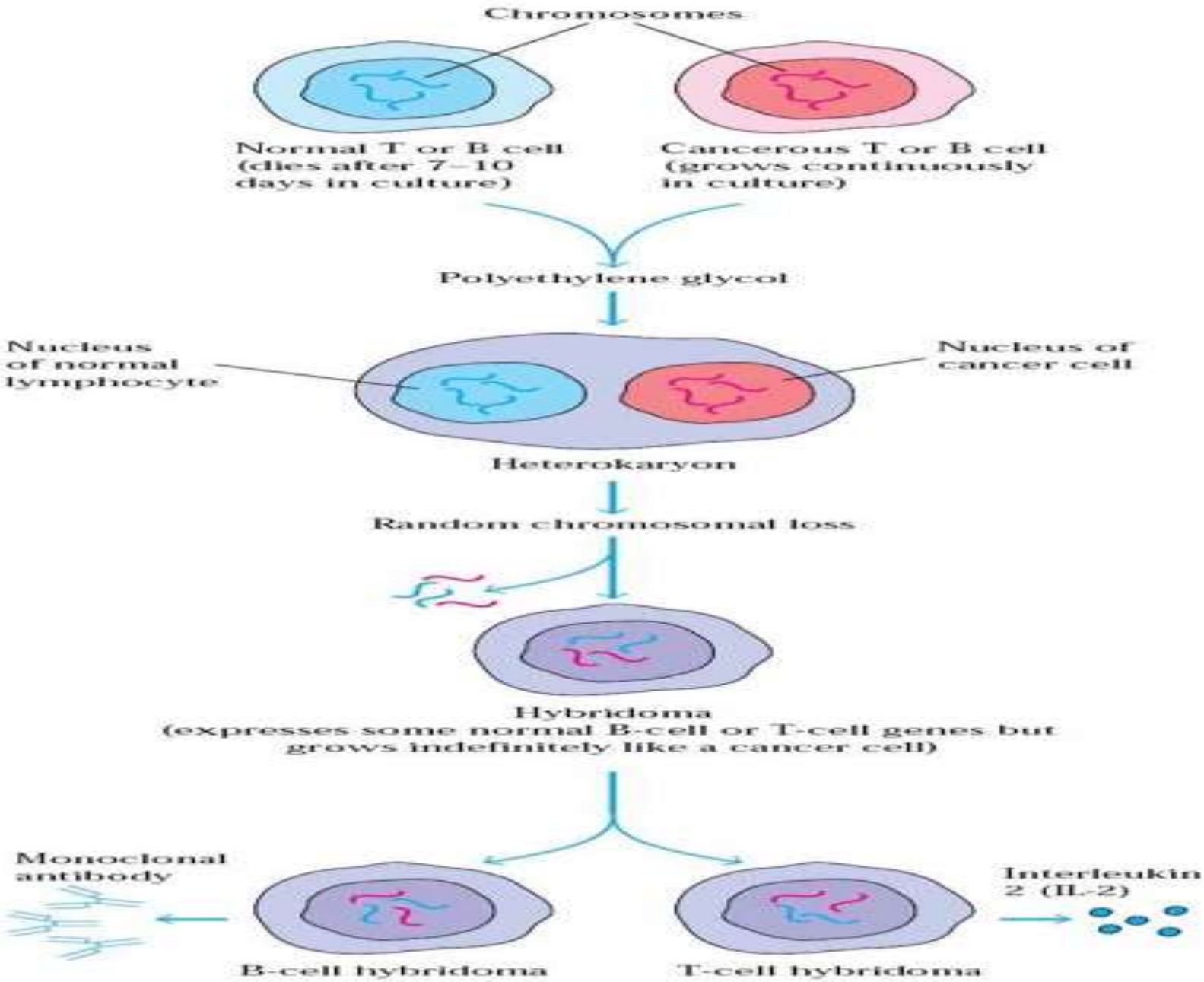
Monoclonal vs polyclonal



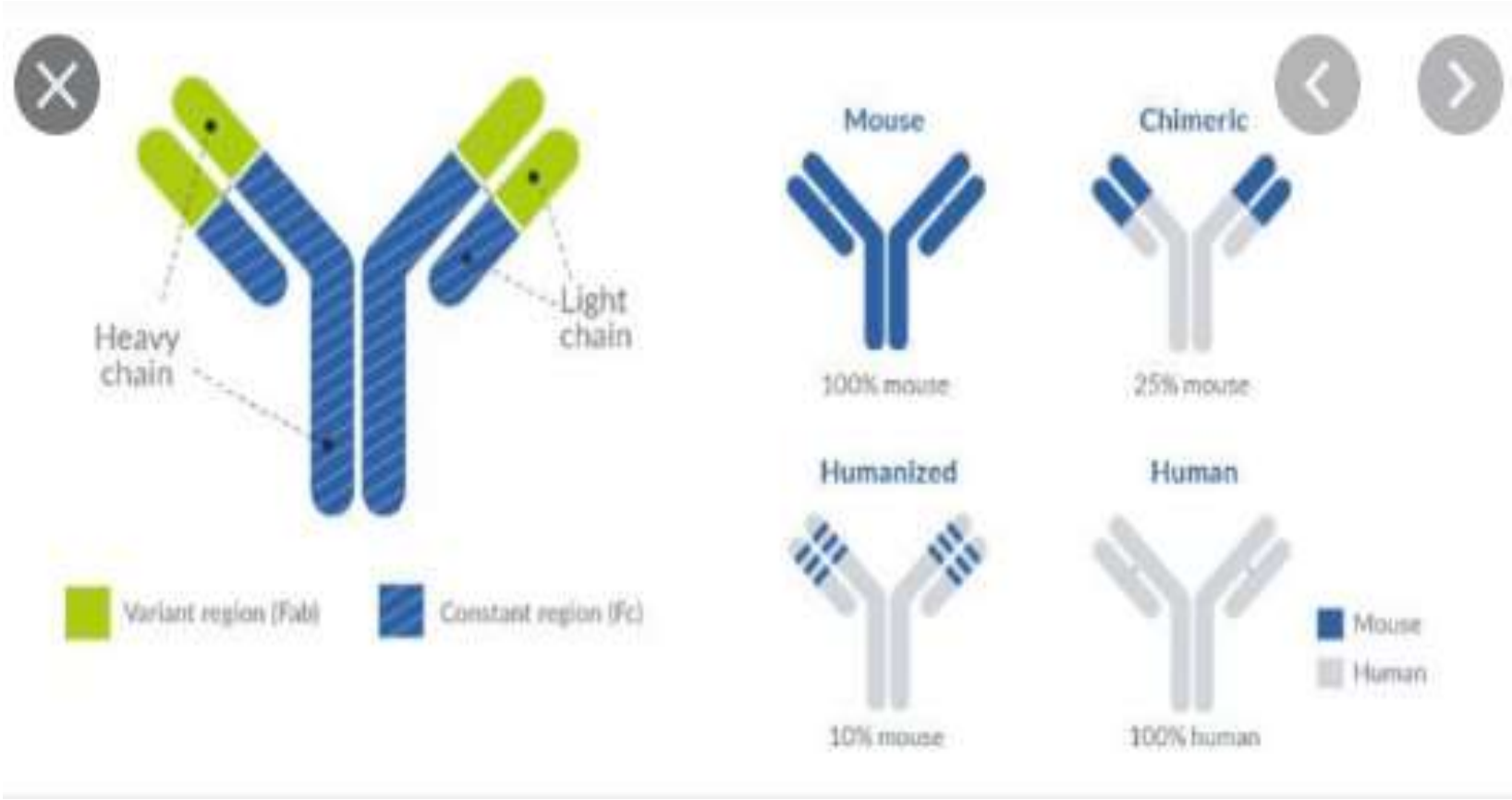
HAT Medium (**hypoxanthine-aminopterin-thymidine medium**) is a selection medium for mammalian cell culture, which relies on the combination of aminopterin, a drug that acts as a powerful folate metabolism inhibitor by inhibiting dihydrofolate reductase, with hypoxanthine (a purine derivative) and thymidine (a deoxynucleoside) which are intermediates in DNA synthesis. The trick is that aminopterin blocks DNA *de novo* synthesis, which is absolutely required for cell division to proceed, but hypoxanthine and thymidine provide cells with the raw material to evade the blockage (the "salvage pathway"), provided that they have the right enzymes, which means having functioning copies of the genes that encode them.

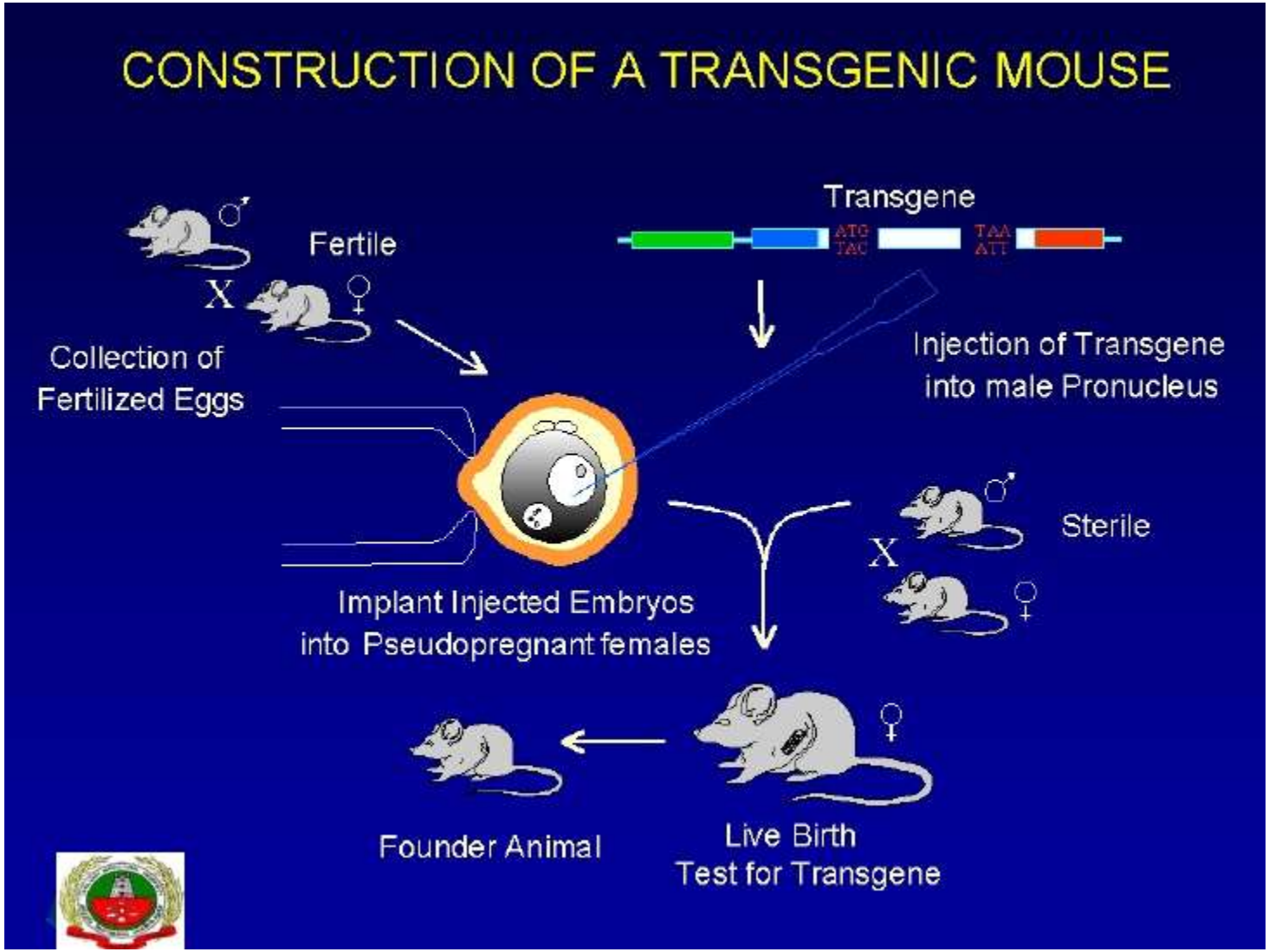





Chromosomes
Normal T or B cell (dies after 7–10 days in culture)
Cancerous T or B cell (grows continuously in culture)
Polyethylene glycol
Nucleus of normal lymphocyte
Nucleus of cancer cell
Heterokaryon
Random chromosomal loss
Hybridoma (expresses some normal B-cell or T-cell genes but grows indefinitely like a cancer cell)
Monoclonal antibody
Interleukin 2 (IL-2)
B-cell hybridoma
T-cell hybridoma

Humanized antibody




CONSTRUCTION OF A TRANSGENIC MOUSE
Transgene
ATG
TAC
TAA
ATT
Fertile
X
Collection of
Fertilized Eggs
Injection of Transgene
into male Pronucleus
Sterile
X
Implant Injected Embryos
into Pseudopregnant females
Founder Animal
Live Birth
Test for Transgene

Cell mediated effector response

(Graphics are collected from internet)

Cell mediated effector response

The effector cells of the **cell-mediated immune system** include

- cells of the **innate immune system** (**natural killer [NK] cells**)
- cells of the **adaptive immune system**
  - Helper CD4T cells (TH cells) and
  - CD8 cytotoxic T lymphocytes (CTLs or TC cells).

.

NKT cells are also participants and have features of both the **innate and adaptive immune systems**

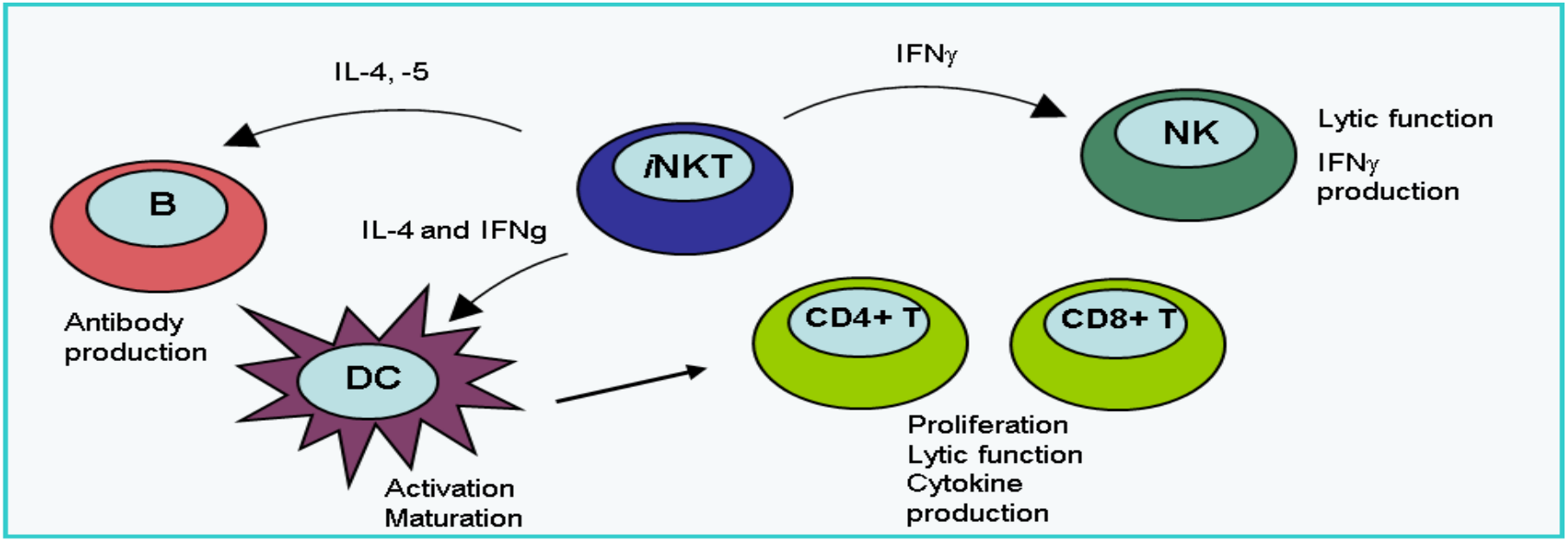
Natural killer T (**NKT**) **cells** are a heterogeneous group of T cells that share properties of both T cells and natural killer cells. Many of these cells recognize the non-polymorphic **CD1d molecule**, an antigen-presenting molecule that binds self and foreign lipids and glycolipids. They constitute only approximately 1% of all peripheral blood T cells. Natural killer T cells should neither be confused with natural killer cells nor killer T cells (cytotoxic T cells)

The best-known subset of CD1d-dependent NKT cells expresses an invariant T-cell receptor (TCR) α chain. These are referred to as type I or invariant NKT cells (iNKT) cells. They are notable for their ability to respond rapidly to danger signals and pro-inflammatory cytokines

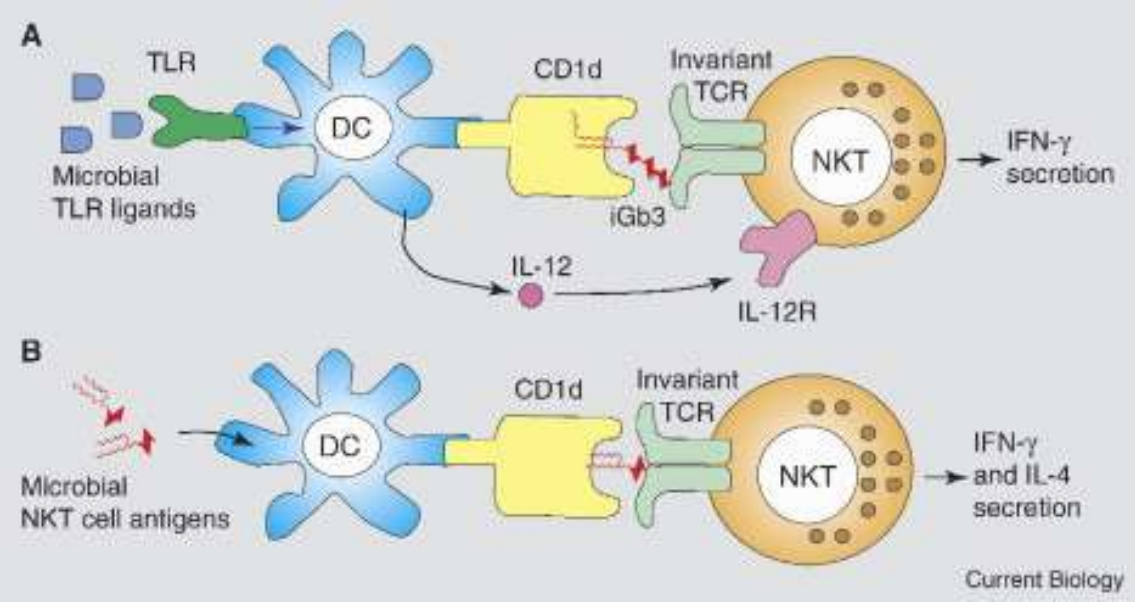
iNKT cells recognize lipid antigens presented by CD1d, a non-polymorphic major histocompatibility complex class I-like antigen presenting molecule.

Invariant natural killer T (iNKT) **cells**, also known as type I or classical **NKT cells**, are a distinct population of T **cells** that express an invariant aβ T-**cell** receptor (TCR) and a number of **cell** surface molecules in common with natural killer (NK) **cells**.

Both **NK** and **NKT cells** are cytotoxic **cells**, which induce **cell** death of pathogenic **cells** as well as tumor **cells**. The main **difference between NK cells and NKT cells** is that **NK cells** are large granular lymphocytes while **NKT cells** are a type of T **cells**.



Some examples of the interactions of iNKT cells with other cell types

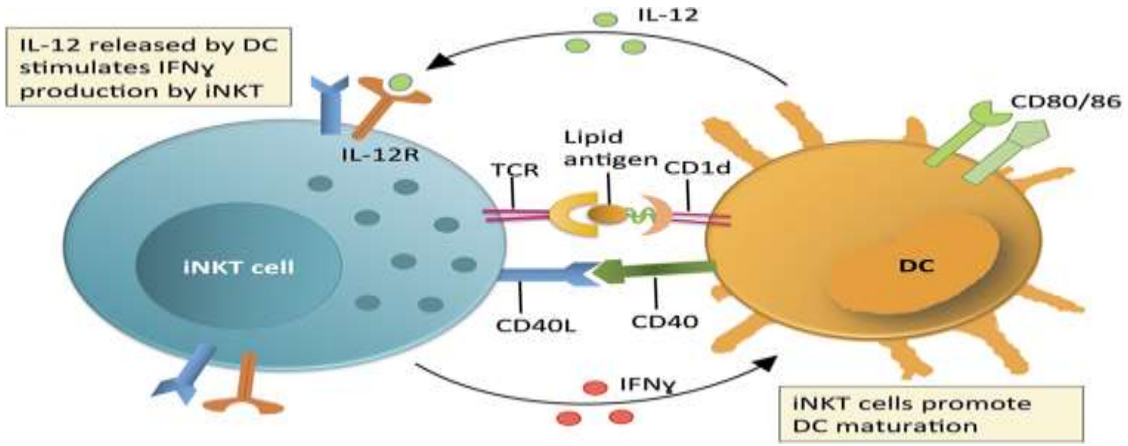


CD1D is the human gene that encodes the protein **CD1d**, a member of the CD1 (cluster of differentiation 1) family of glycoproteins expressed on the surface of various human antigen-presenting cells

A) Microbes containing TLR ligands such as LPS can activate NKT cells by inducing IL-12 production by DCs, which amplifies the weak responses of the invariant NKT cell receptor to iGb3–CD1d complexes. (B) Alternatively, microbes may activate NKT cells directly via glycosphingolipids that bind CD1d on the DCs and interact with the invariant NKT cell receptor, without the need for endogenous NKT cell antigens such as iGb3.

Isoglobotriosylceramide, Gal(α1→3)Gal(β1→4)Glcβ(1→1)Cer, abbreviated as **iGb3**, is an iso-globo-series of glycosphingolipid, which mysteriously disappeared in most mammals studied (pig, mouse, and human), except trace amount reported in the thymus.

NKT-cell development. Natural killer T (**NKT**) **cells** arise in the thymus from a common precursor pool of $CD4^+CD8^+$ double positive (DP) thymocytes that have undergone random T-**cell** receptor (TCR) gene rearrangement and expression.



Upon activation, NKT cells are able to produce large quantities of interferon gamma, IL-4, and granulocyte-macrophage colony-stimulating factor, as well as multipleother cytokines and chemokines (such as IL-2, IL-13, IL-17, IL-21, and TNF-alpha).NKT cells recognize protected microbial lipid agents which are presented by CD1d-expressing antigen presenting cells. This serves as a pathway for NKT cells to fight against infections and enhance the humoral immunity. The NKT cells provide support and help to B cells which act as a microbial defense and aid in targeting for B-cell vaccines

NKT cells seem to be essential for several aspects of immunity because their dysfunction or deficiency has been shown to lead to the development of autoimmune diseases (such as diabetes or atherosclerosis) and cancers. NKT cells have recently been implicated in the disease progression of human asthma

Effector cytotoxic cells arise from both the adaptive and innate immune systems and, therefore, include both **antigen-specific and -nonspecific cells**

Antigen non-specifi c (innate immune) cells that contribute to the clearance of infected cells include **NK cells and non lymphoid cell** types such as macrophages, neutrophils, and eosinophils

Antigen-specific cytotoxic cells include **CD8 T lymphocytes** (CTLs or TC cells), as well as **the CD4 NKT cell subpopulation**

Populations of cytotoxic CD4 TH cells may contribute to **delayed-type hypersensitivity.**

Two major categories of cell-mediated immune responses:

- Effector cells that have direct cytotoxic activity.

- Effector cells that mediate delayed-type hypersensitivity (DTH) reactions

Three types of effector T cells:

1. $CD4^+$ $T_H1$cells
2. $CD4^+$ T_H2 cells
3. $CD8^+$ CTLs

Characteristics:

- less stringent activation requirements
- increased expression of cell-adhesion molecules
- production of both membrane-bound and soluble effector molecules

TABLE 14-1 COMPARISON OF NAIVE AND EFFECTOR T CELLS

Property	Naive T cells	Effector T cells
Co-stimulatory signal (CD28-B7 interaction)	Required for activation	Not required for activation
CD45 isoform	CD45RA	CD45RO
Cell-adhesion molecules (CD2 and LFA-1)	Low	High
Trafficking patterns	HEVs* in secondary lymphoid tissue	Tertiary lymphoid tissues; inflammatory sites

*HEV = high endothelial venules, sites in blood vessel used by lymphocytes for extravasation.

- The CD45RO isoform associates with the TCR complex and CD4/CD8 much better than does the CD45RA isoform.
- CD2 ⟷ LFA-3, LFA-1 ⟷ ICAMs

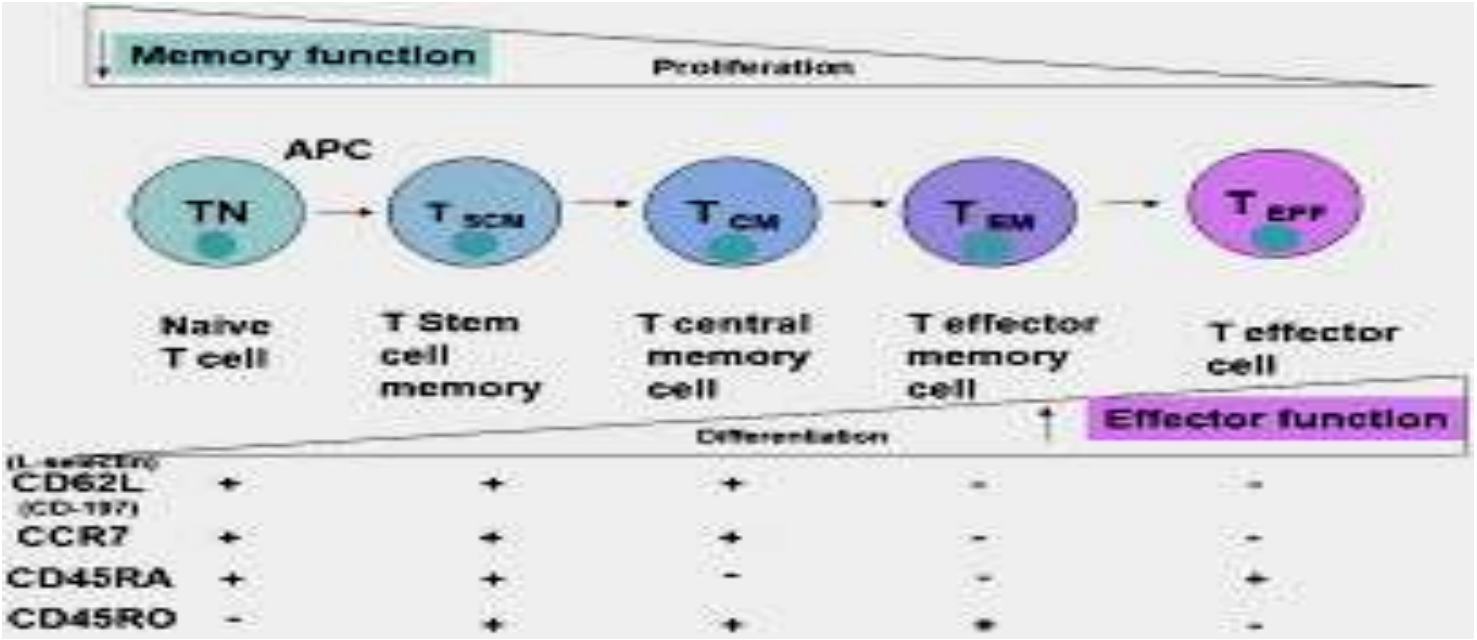
HEV: High endothelial Venules (Plump endothelial cell)

CD45 is a Ppase, has different isoform CD45RA and CD45RO : alt. Splicing

CD45RO :Expressed in effector cells
Can be associated with TCR and CD4/CD8

CD45RA : Expressed in Naïve cells
Less associated with TCR and CD4/CD8

Memory cells produce both CD45RA and CD45RO , but they predominantly express CD45RO.



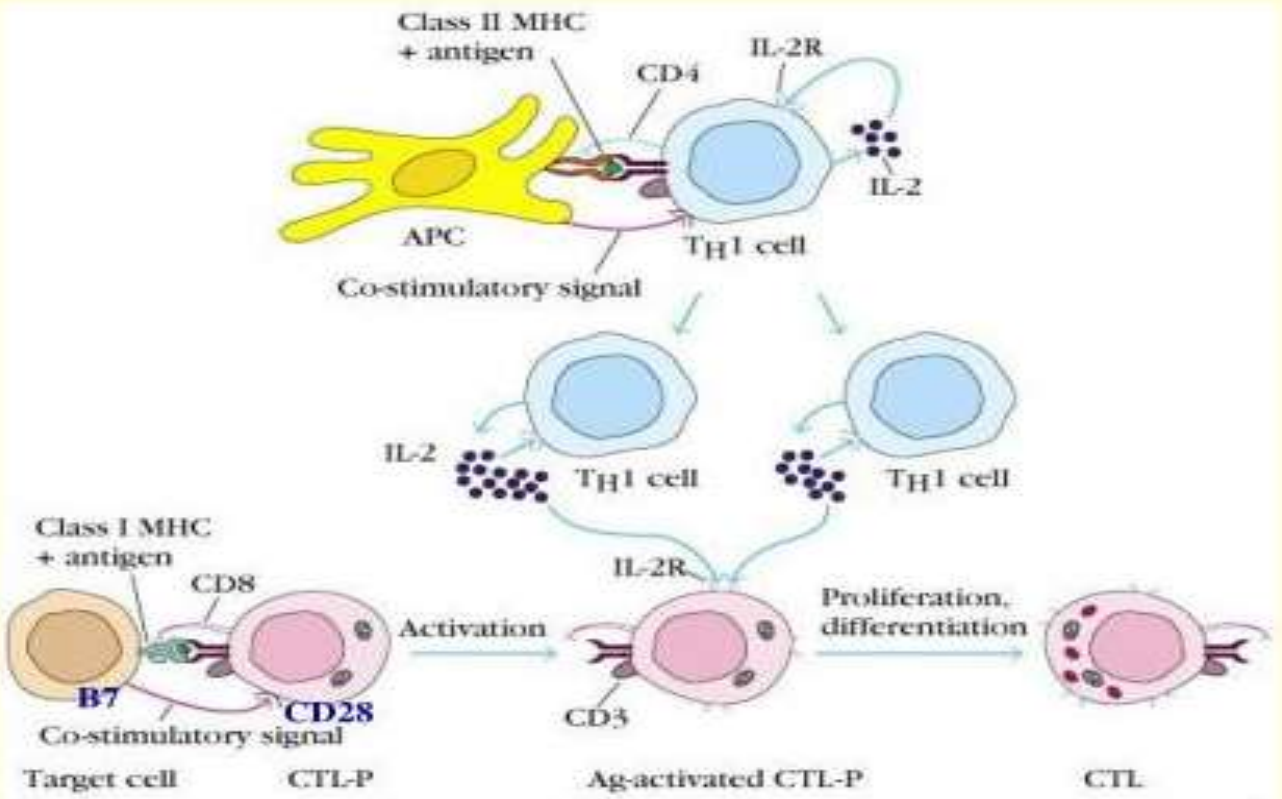
CD62L is a cell surface component that is a member of a family of adhesion/homing receptors that play important roles in lymphocyte-endothelial cell interactions.

TABLE 14-2 EFFECTOR MOLECULES PRODUCED BY EFFECTOR T CELLS

Cell type	Soluble effectors	Membrane-bound effectors
CTL	Cytotoxins (perforins and granzymes), IFN-γ, TNF-β,	Fas ligand (FAS)
T_H1	IL-2, IL-3, TNF-β, IFN-γ, GM-CSF (high)	Tumor necrosis factor β (TNF-β)
T_H2	IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, GM-CSF (low)	CD40 ligand

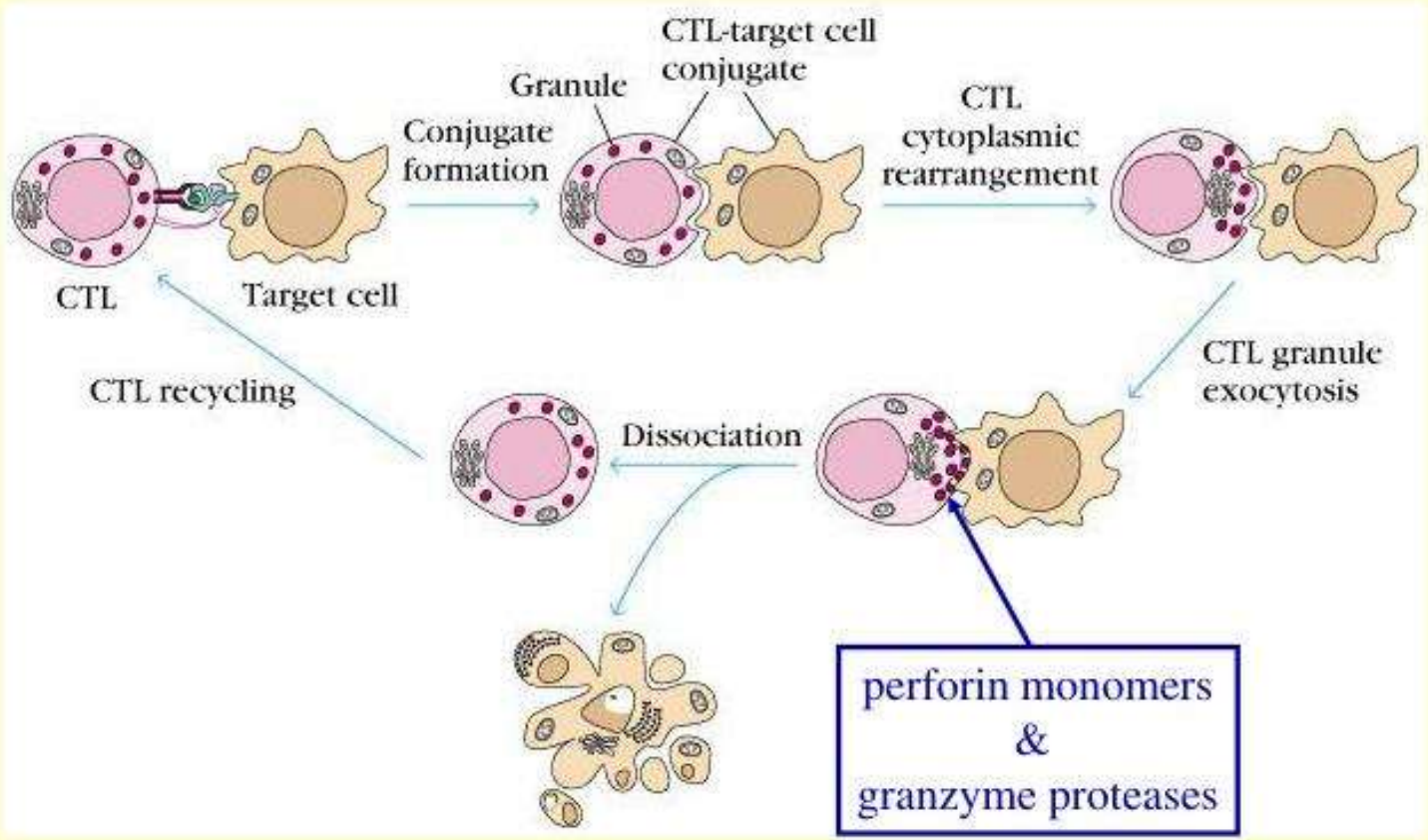
- The FasL, perforins, and granzymes mediate target cell destruction by the CTLs.
- Membrane-bound TNFβ and soluble IFNγ and GM-CSF promote macrophage activation by the T_H1 cell.
- The membrane-bound CD40L and soluble IL-4, IL-5, IL-6, and IL-10 play a role in B cell activation by the T_H2 cell.

Generation of Effector CTLs

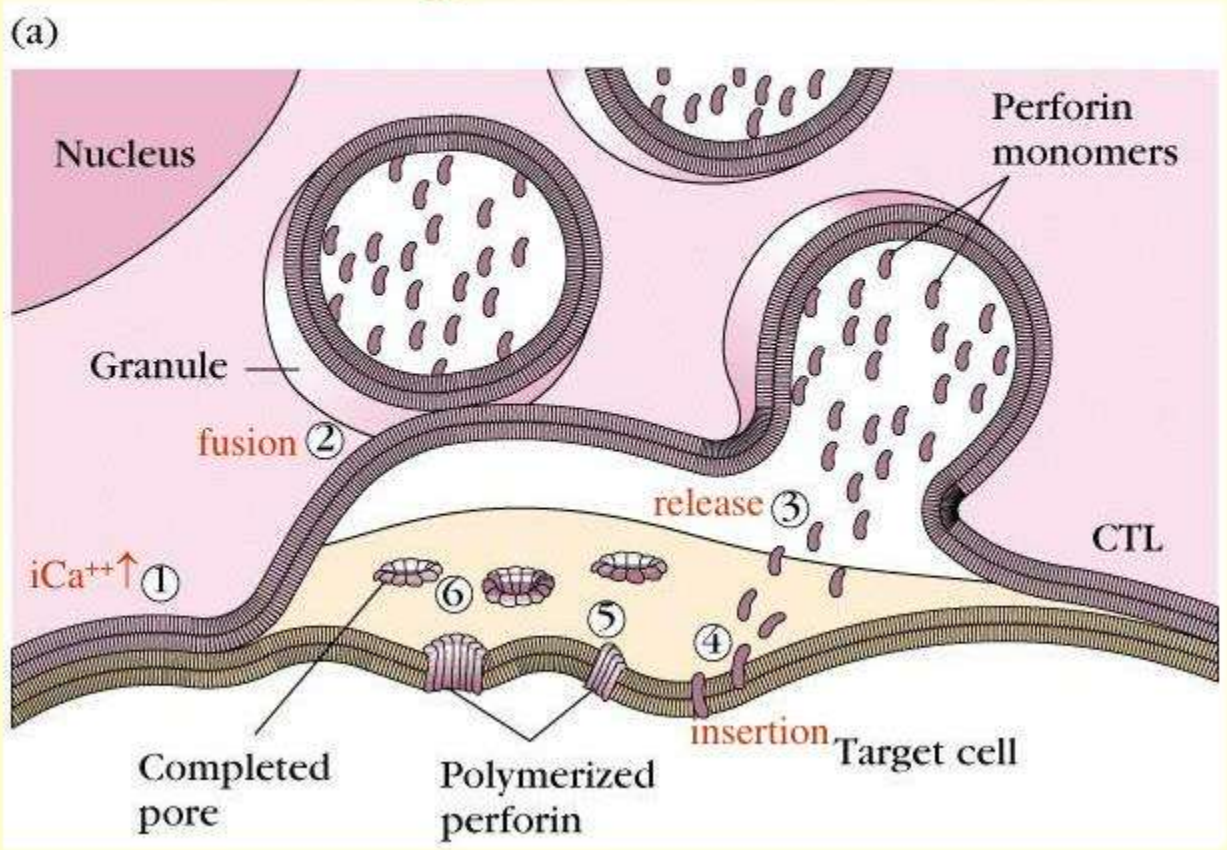


IL-2R expression	–	+	+
IL-2 expression	–	±	±
Proliferation	–	–	+
Effector cytotoxic function	–	–	+

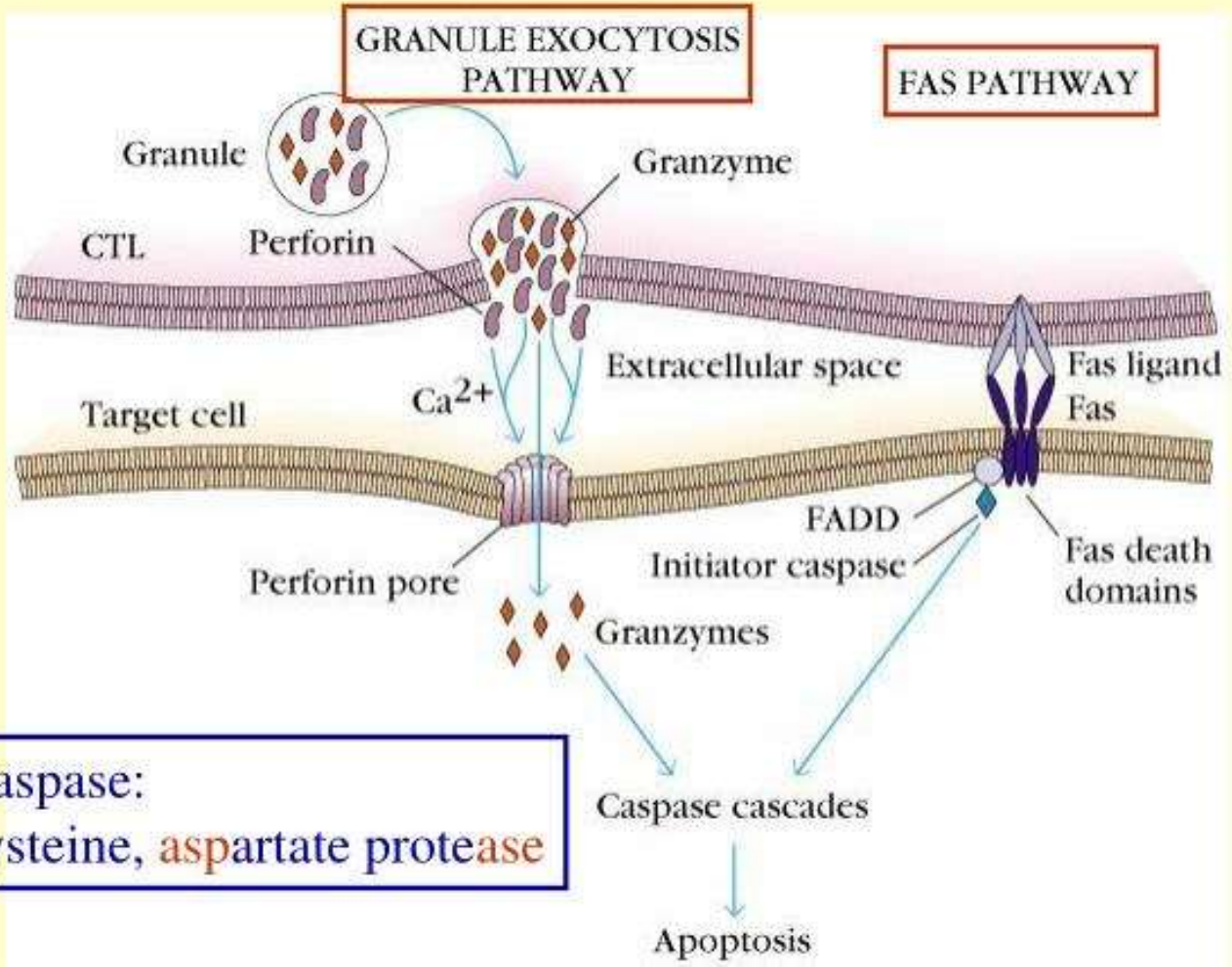
CTL-Mediated Killing of Target Cells



Cell-Mediated Pore Formation in Target-Cell Membrane



CTL-Mediated Apoptotic Pathways



Caspase:
cysteine, aspartate protease

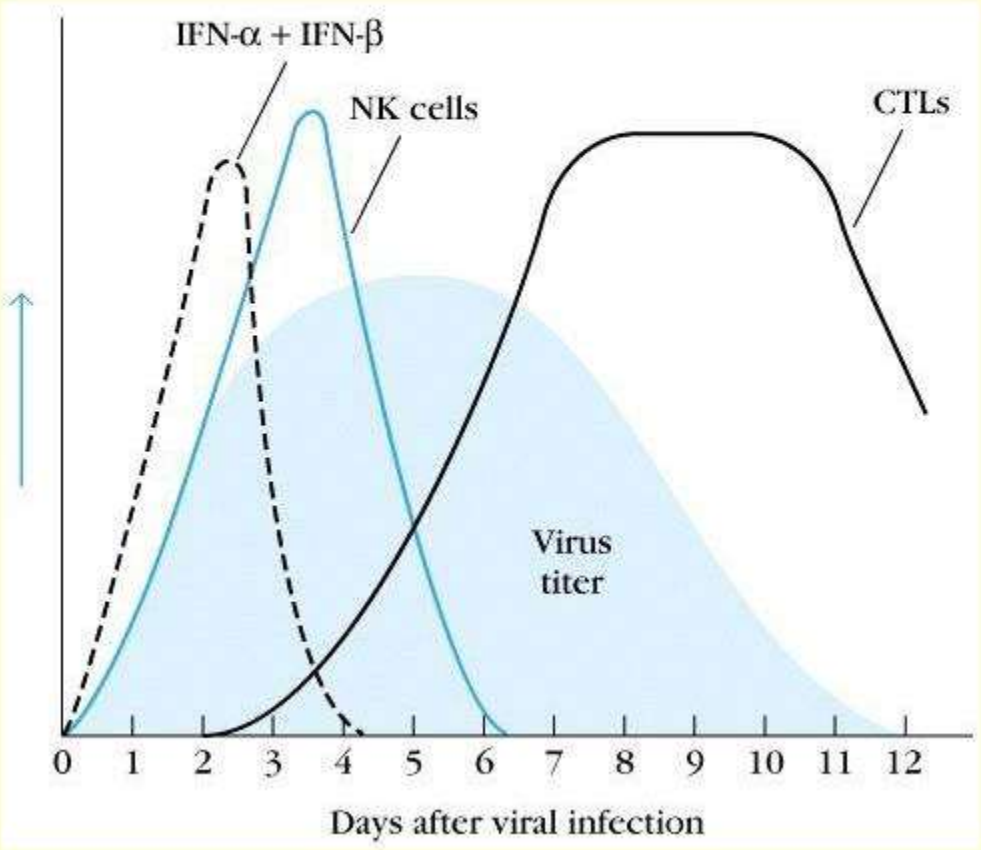
NK cells (belonging to the group of innate lymphoid cells) are one of the three kinds of cells differentiated from the common lymphoid progenitor, the other two being B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation. **NK cells differ from natural killer T cells (NKTs) phenotypically, by origin and by respective effector functions; often, NKT cell activity promotes NK cell activity by secreting interferon gamma. In contrast to NKT cells, NK cells do not express T-cell antigen receptors (TCR) or pan T marker CD3 or surface immunoglobulins (Ig) B cell receptors, but they usually express the surface markers CD16 (FcγRIII) and CD57 in humans, NK1.1 or NK1.2 in C57BL/6 mice.**

In addition to the knowledge that natural killer cells are effectors of innate immunity, recent research has uncovered information on both activating and inhibitory NK cell receptors which play important functional roles, including self tolerance and the sustaining of NK cell activity. NK cells also play a role in the adaptive immune response: numerous experiments have demonstrated their ability to readily adjust to the immediate environment and formulate antigen-specific immunological memory, fundamental for responding to secondary infections with the same antigen. **The role of NK cells in both the innate and adaptive immune responses is becoming increasingly important in research using NK cell activity as a potential cancer therapy.**

Natural Killer (NK) Cells:

- 5 - 10% of the recirculating lymphocyte population
- No immunization is required. No memory
- a population of large granular lymphocytes
- constitutively cytotoxic, always having large granules
- involved in the defense against viruses and tumors
- Activity is stimulated by IFNα, IFNβ, and IL-12.
- express CD16 (FcγRIII)
- do not express TCR/CD3
- Recognition is not MHC-restricted.
- normal in RAG-1, RAG-2, and SCID mice
- Cytotoxicity depends on perforin and granzymes.

Time Course of Viral Infection



NK-Cell Receptors

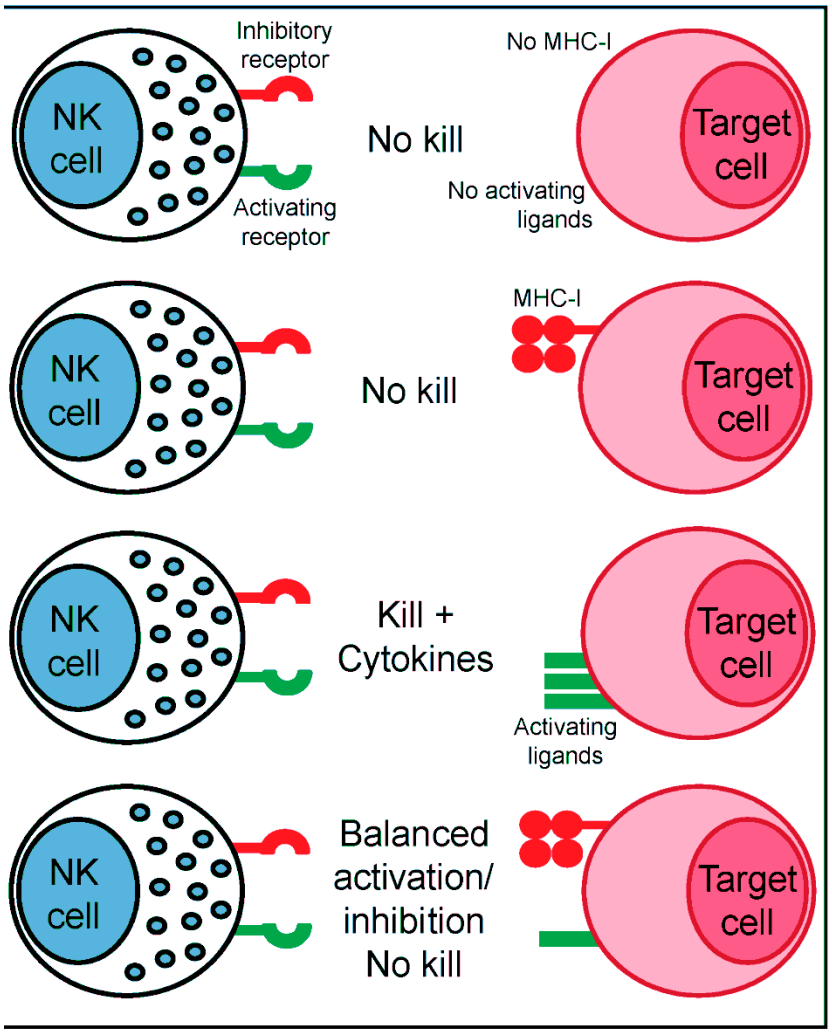
Activation Receptors:

NKR-P1 (a C-type lectin recognizing carbohydrates)

Inhibitory Receptors:

CD94/NKG2 (recognizing HLA-E with an HLA peptide)

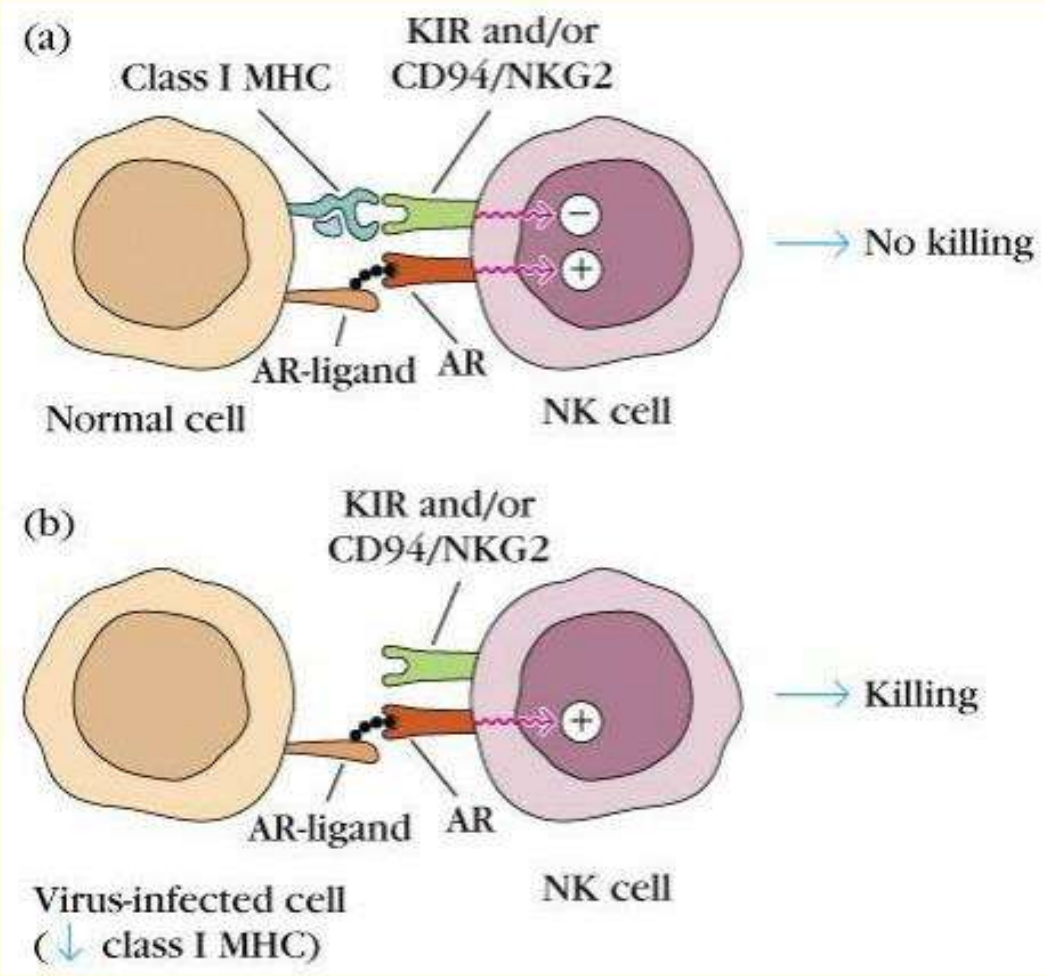
KIR (> 50 members; specific for one or a limited number of polymorphic products of particular HLA loci)



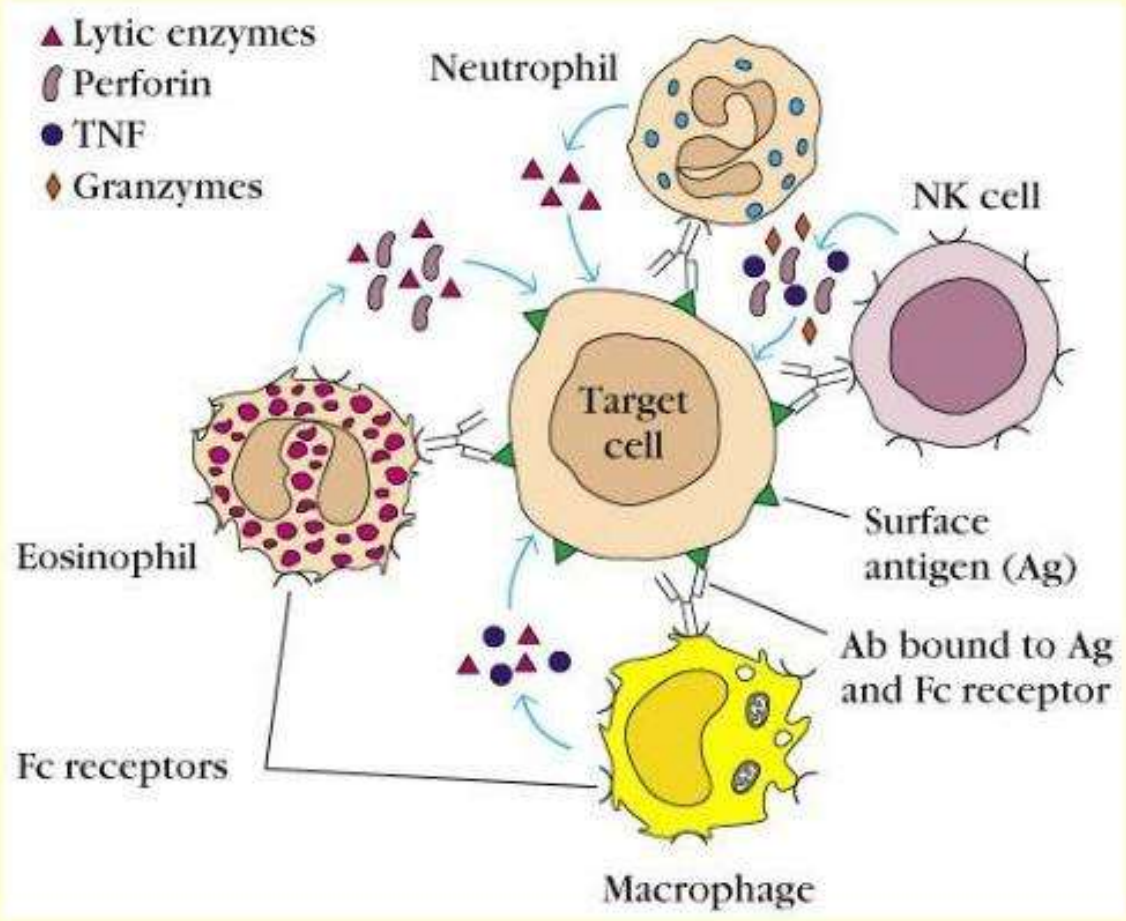
Opposing-signals Model of NK Activity

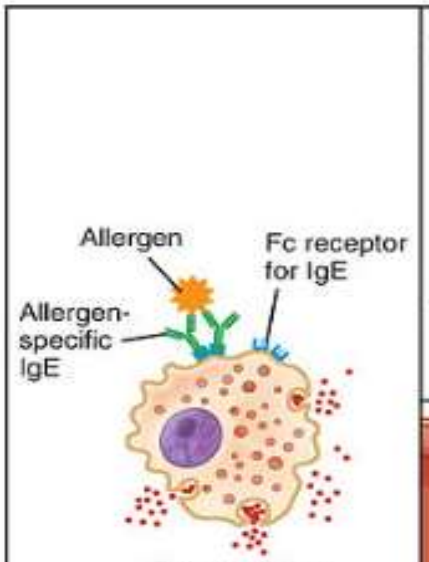
KIR:
killing inhibitory receptor

AR:
activation receptor

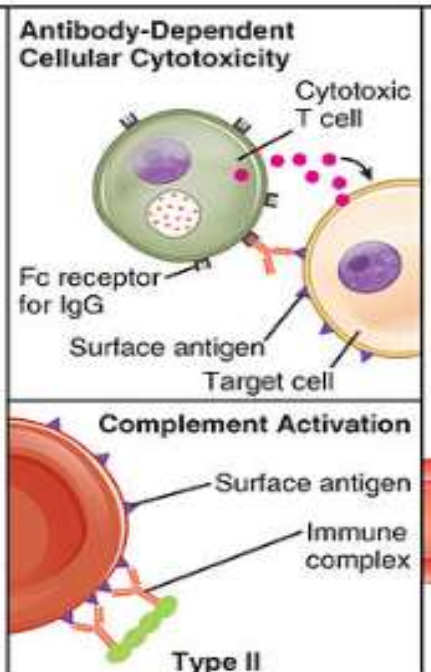


Ab-Dependent Cell-Mediated Cytotoxicity (ADCC)

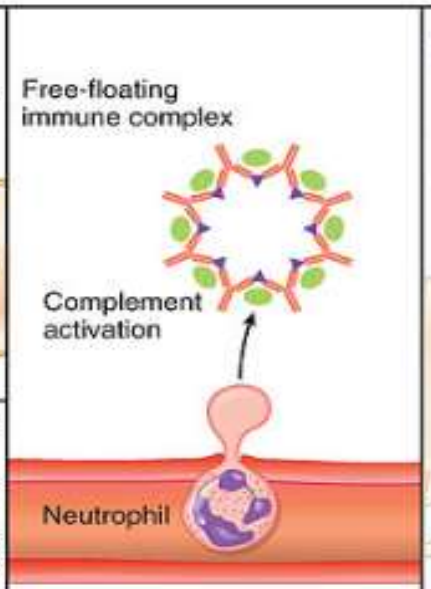




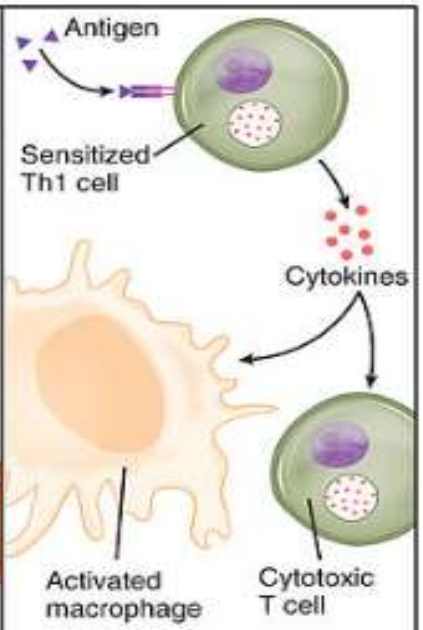
Type I



Type II



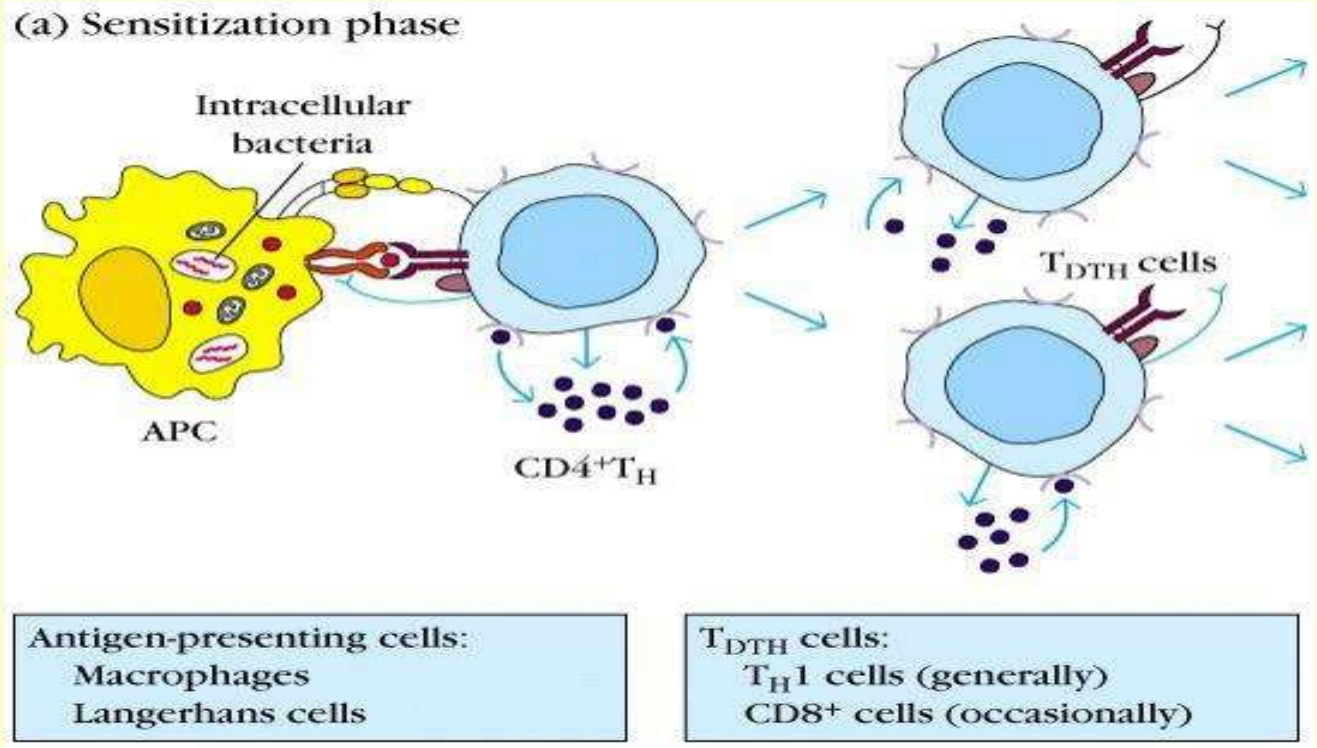
Type III



Type IV

IgE-Mediated Hypersensitivity	**IgG-Mediated Cytotoxic Hypersensitivity**	**Immune Complex-Mediated Hypersensitivity**	**Cell-Mediated Hypersensitivity**
IgE is bound to mast cells via its Fc portion. When an allergen binds to these antibodies, crosslinking of IgE induces degranulation.	Cells are destroyed by bound antibody, either by activation of complement or by a cytotoxic T cell with an Fc receptor for the antibody (ADCC)	Antigen–antibody complexes are deposited in tissues, causing activation of complement, which attracts neutrophils to the site	Th1 cells secrete cytokines, which activate macrophages and cytotoxic T cells and can cause macrophage accumulation at the site
Causes localized and systemic anaphylaxis, seasonal allergies including hay fever, food allergies such as those to shellfish and peanuts, hives, and eczema	Red blood cells destroyed by complement and antibody during a transfusion of mismatched blood type or during erythroblastosis fetalis	Most common forms of immune complex disease are seen in glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Most common forms are contact dermatitis, tuberculin reaction, autoimmune diseases such as diabetes mellitus type I, multiple sclerosis, and rheumatoid arthritis

Overview of the Delayed Type Hypersensitivity (DTH) Response



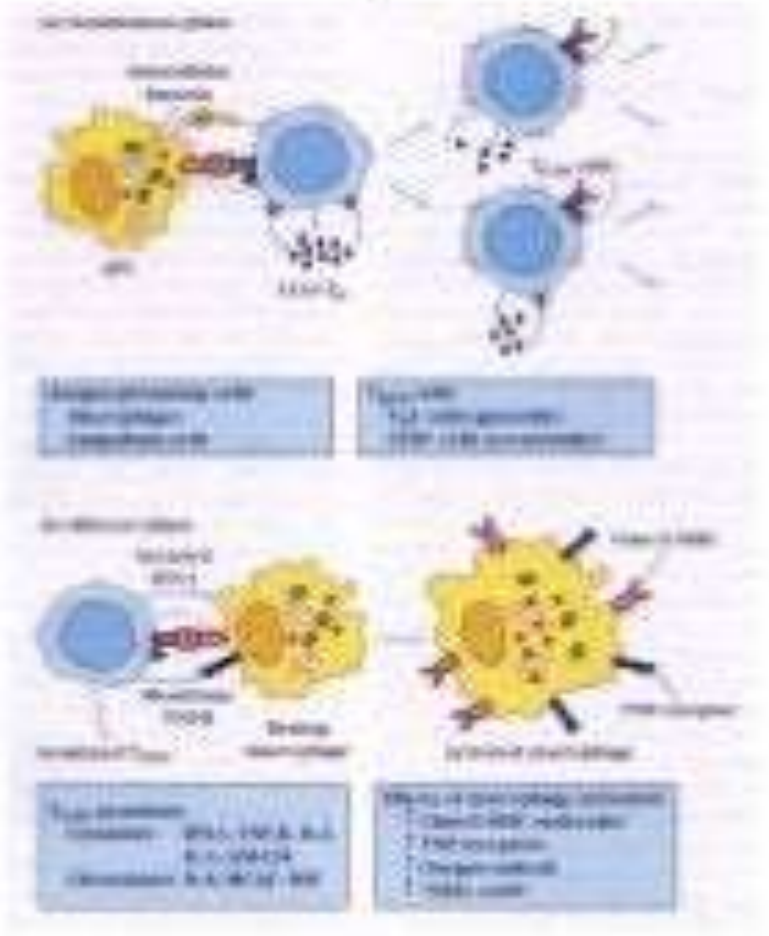
Type IV **hypersensitivity** is often called **delayed type hypersensitivity** as the reaction takes several days to develop. Unlike the other **types**, it is not antibody-mediated but rather is a **type** of cell-mediated response. This response involves the interaction of T-cells, monocytes, and macrophages**Type IV hypersensitivity** typically occurs at least 48 hours after exposure to an antigen. It involves activated T cells, which release cytokines and chemokines, and macrophages and cytotoxic $CD8^+$ T cells that are attracted by these moieties.

Phases of the DTH Response

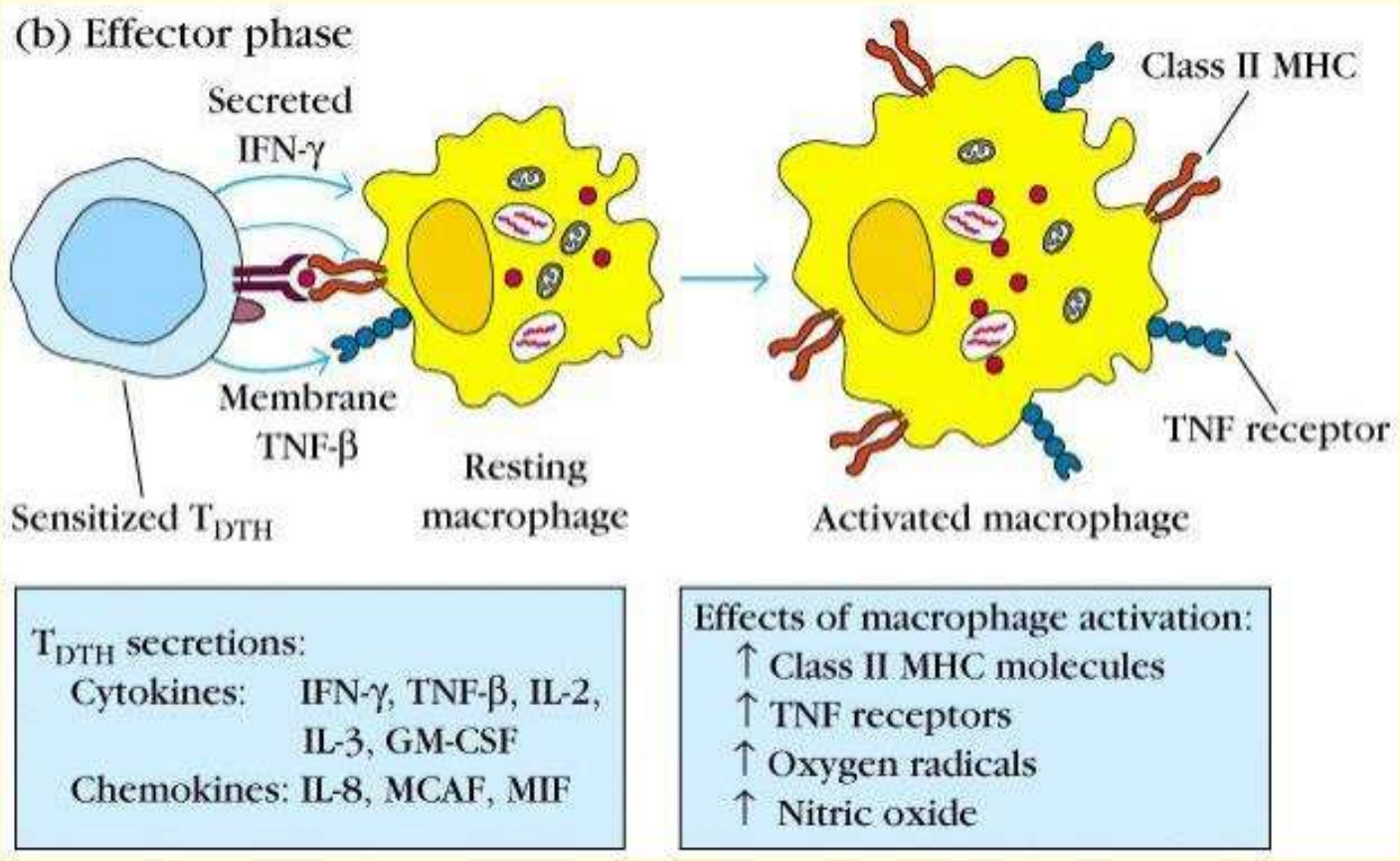
Effector phase: occurs upon subsequent exposure to the Ag

What happens during this phase?

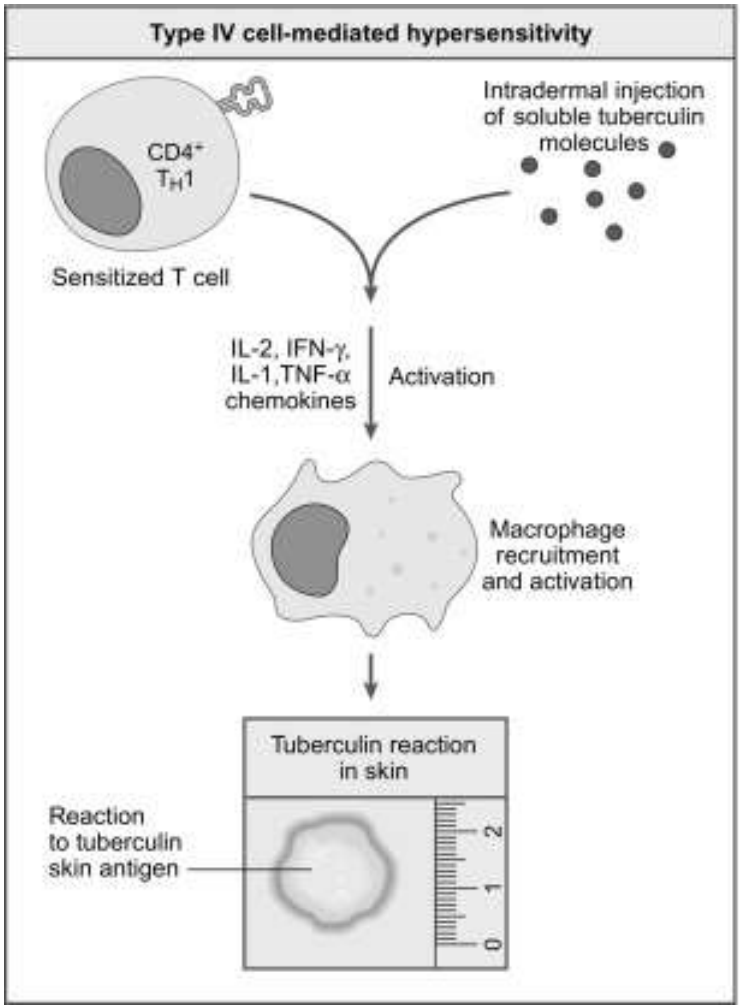
- T_{DTH} cells secrete a variety of cytokines and chemokines, which recruit and activate macrophages
- Macrophage activation promotes phagocytic activity and increased concentration of lytic enzymes for more effective killing
- Activated macrophages are also more effective in presenting Ag and function as the primary effector cell



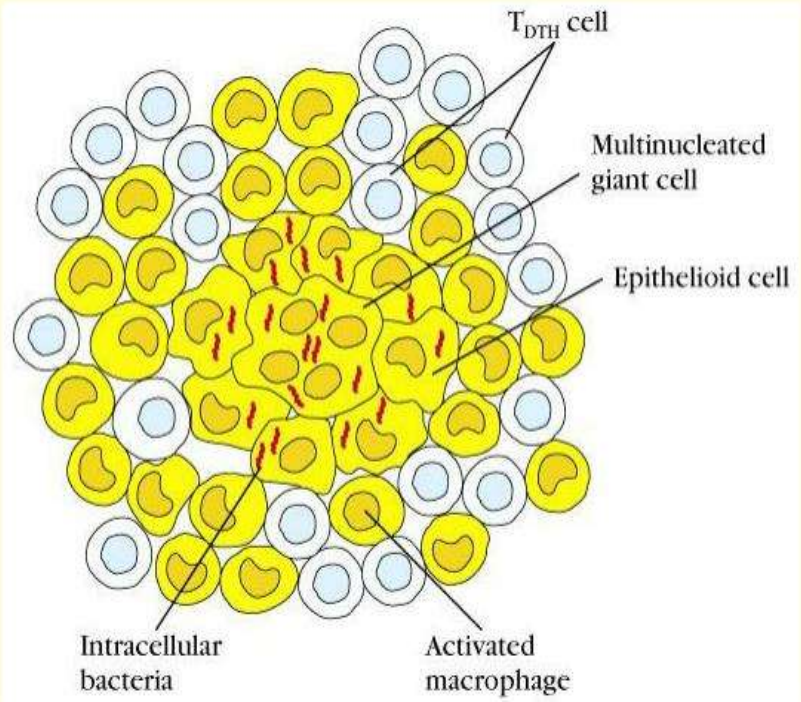
T helper cells (specifically T_h1 cells) are activated by an antigen presenting cell. When the antigen is presented again in the future, the memory Th1 cells will activate macrophages and cause an inflammatory response. This ultimately can lead to tissue damage



Delayed-type hypersensitivity also describes a positive response to the common test
for **tuberculosis** (subcutaneous injection of mycobacterial purified protein derivative, or PPD). In this case, inactivated toxoid from mycobacteria recruits pre-primed T cells and macrophages, which initiate an inflammatory response.



Formation of Granuloma

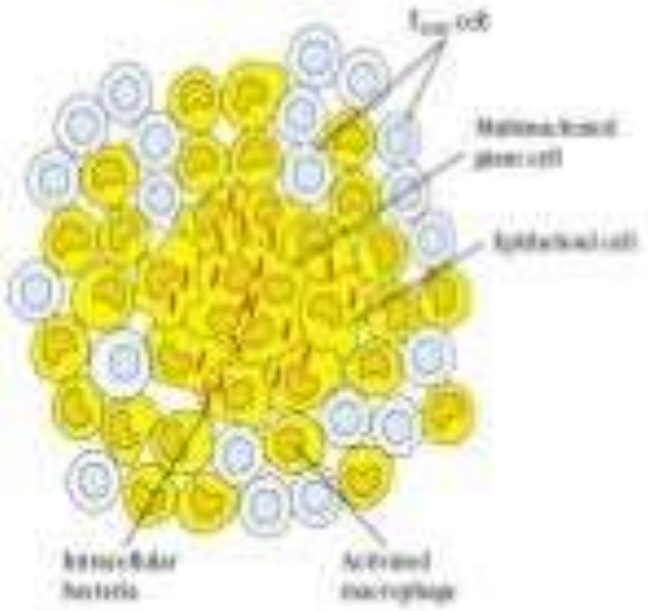


Tuberculosis is the **formation** of an organized structure called **granuloma**. It consists mainly in the recruitment at the infectious stage of macrophages, highly differentiated cells such as multinucleated giant cells, epithelioid cells and Foamy cells, all these cells being surrounded by a rim of lymphocytes.

What happens if the DTH response is prolonged?

A granuloma develops…

- Continuous activation of macrophages induces the macrophages to adhere closely to one another, assuming an epithelioid shape and sometimes fusing together to form giant, multinucleated cells.



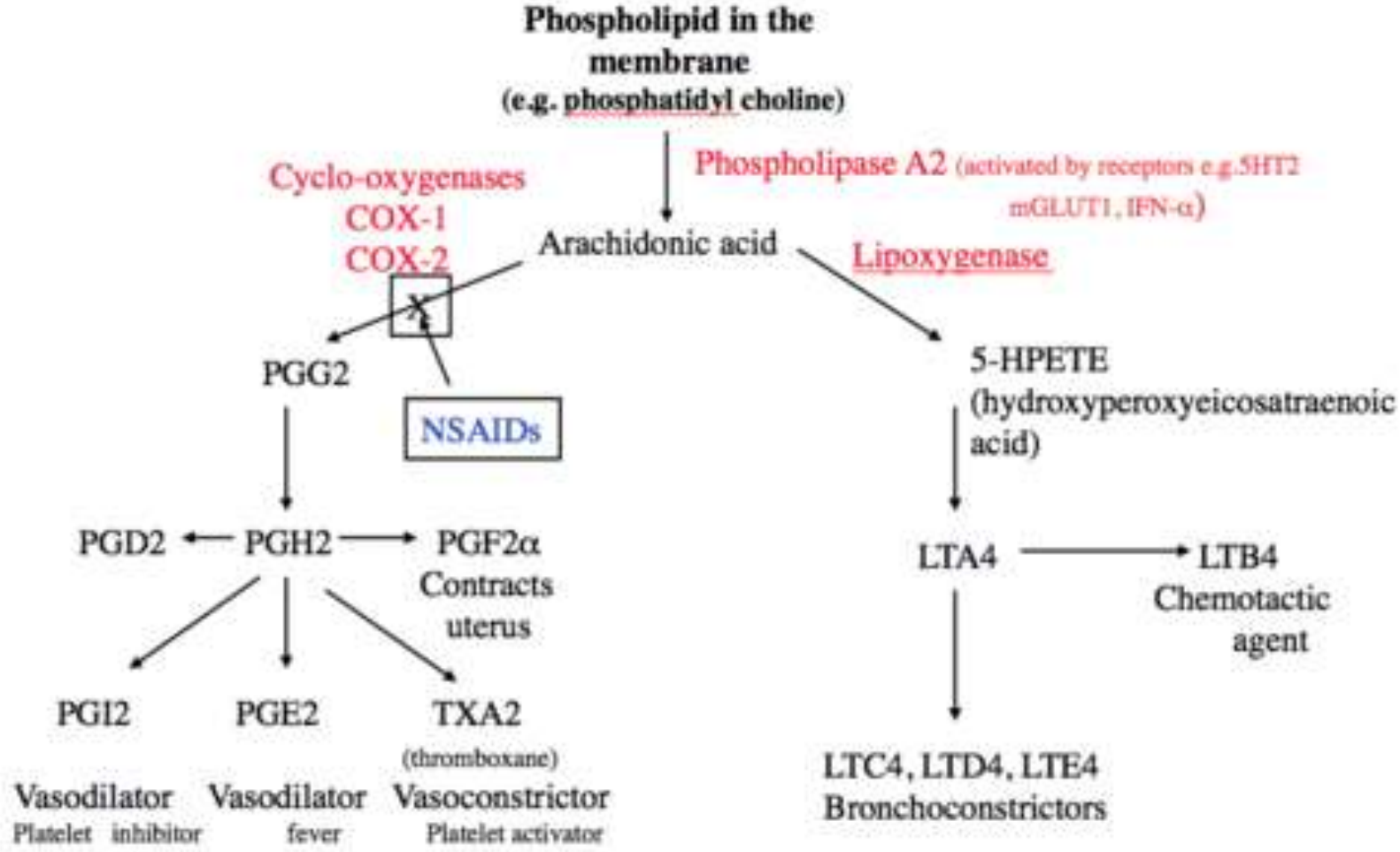
Protective Role of DTH Response

Intracellular bacteria	Intracellular viruses	Contact Antigens
Mycobacterium tuberculosis	Herpes simplex virus	Hair dyes
Mycobacterium leprae	Measles virus	Poison ivy

- A variety of intracellular pathogens and contact antigens can induce a DTH response.
- Cells harboring intracellular pathogens are rapidly destroyed by lytic enzymes released by activated macrophages

Detrimental Effects of DTH Response

- The initial response of the DTH is nonspecific and often results in significant damage to healthy tissue
- In some cases, a DTH response can cause such extensive tissue damage that the response itself is pathogenic
- Example: *Mycobacterium tuberculosis* – an accumulation of activated macrophages whose lysosomal enzymes destroy healthy lung tissue
- In this case, tissue damage far outweighs any beneficial effects.


Phospholipid in the membrane
(e.g. phosphatidyl choline)
Phospholipase A2 (activated by receptors e.g.5HT2 mGLUT1, IFN-α)
Cyclo-oxygenases
COX-1
COX-2
Arachidonic acid
Lipoxygenase
PGG2
NSAIDs
5-HPETE (hydroxyperoxyeicosatraenoic acid)
PGD2
PGH2
PGF2α
Contracts uterus
LTA4
LTB4
Chemotactic agent
PGI2
PGE2
TXA2
(thromboxane)
LTC4, LTD4, LTE4
Bronchoconstrictors
Vasodilator
Platelet inhibitor
Vasodilator
fever
Vasoconstrictor
Platelet activator

Hypersesitivity : Inappropiate immune response is termed hypersensitivity or allergy
They develop during the course of either humoral or cell medisted response

Four types of hypersensitivity reaction
Type I – IgE mediated
Type II Antibody IgG/IgM mediated
Type III Immune complex mediated
Type IV DTH

Type I hypersensitivity (or **immediate hypersensitivity**) is an allergic reaction provoked by re-exposure to a specific type of antigen referred to as an allergen. Type I is distinct from type II, type III and type IV hypersensitivities.
Exposure may be by ingestion, inhalation, injection, or direct contact.

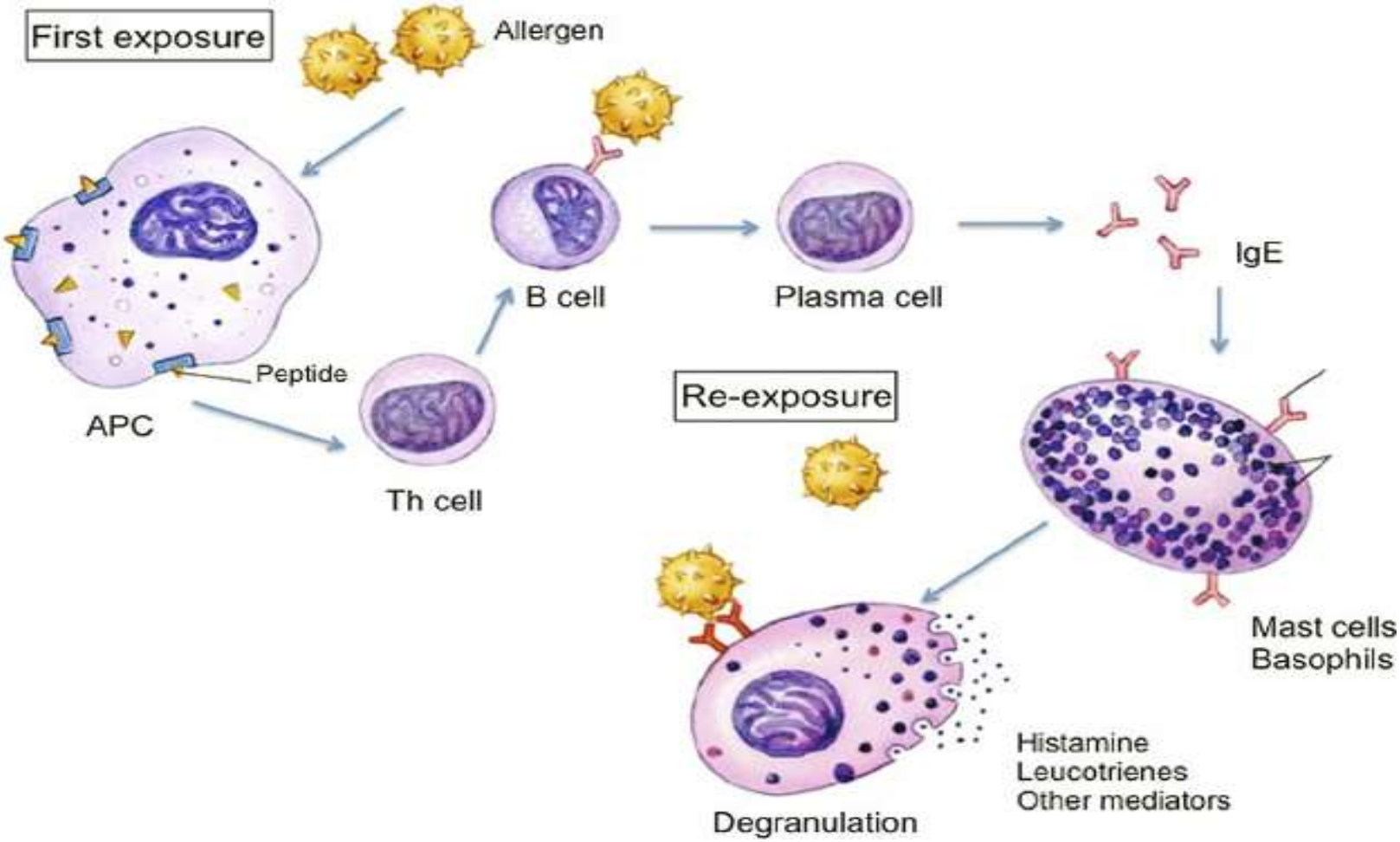
In type 1 hypersensitivity, B-cells are stimulated (by CD4+TH2 cells) to produce IgE antibodies specific to an antigen. The difference between a normal infectious immune response and a type 1 hypersensitivity response is that in type 1 hypersensitivity, the antibody is IgE instead of IgA, IgG, or IgM.

During sensitization, the IgE antibodies bind to FcεRI receptors on the surface of tissue mast cells and blood basophils.Mast cells and basophils coated by IgE antibodies are "sensitized". Later exposure to the same allergen cross-links the bound IgE on sensitized cells, resulting in anaphylactic degranulation, which is the immediate and explosive release of pharmacologically active pre-formed mediators from storage granules and concurrent synthesis of inflammatory lipid mediators from arachidonic acid some of these mediators include histamine, leukotriene (LTC4 and LTD4 and LTB4), and prostaglandin, which act on proteins (e.g., G-protein coupled receptors) located on surrounding tissues. The principal effects of these products are vasodilation and smooth-muscle contraction.

Type 1 hypersensitivity can be further classified into immediate and late-phase reactions. The immediate hypersensitivity reaction occurs minutes after exposure and includes release of vasoactive amines and lipid mediators, whereas the late-phase reaction occurs 2–4 hours after exposure and includes the release of cytokines.

List of a few mediators released by mast cells in type 1 hypersensitivity and their actions

Vasodilation and increased permeability	•Histamine •PAF •Leukotriene C4, D4, and E4 •Prostaglandin D2 •Neutral proteases
Smooth muscle spasm	•Histamine •PAF •Leukotriene C4, D4, and E4 •Prostaglandin
Leukocyte extravasation	•Cytokines (e.g. chemokines and TNF) •Leukotriene B4 •Chemotactic factors for neutrophils and eosinophils


First exposure
Allergen
B cell
Plasma cell
IgE
Peptide
APC
Th cell
Re-exposure
Mast cells
Basophils
Histamine
Leucotrienes
Other mediators
Degranulation

IMMEDIATE — *Allergy IgE/Mast Cell Activation*

1ST EXPOSURE

HELPER T CELL

B CELL

ANTIGEN + MHC II

IgE-coated cell is "sensitized"

IgE

IgE Fc RECEPTOR

MAST CELL

2ND EXPOSURE

Cross-linking receptors activates cell.

Granules:
Histamines
Proteases
Heparin

Membrane Phospholipids:

Platelet-activating factor

Arachidonic acid

Prostaglandin D2

Leukotrienes B4, C4, D4

Cytokines:
TNF, IL-1, IL-4
Chemokines

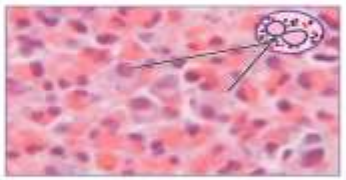
Early Phase

Vasodilation
Leakage
Gland secretion

ANTI-HISTAMINES prevent histamine receptor activation.

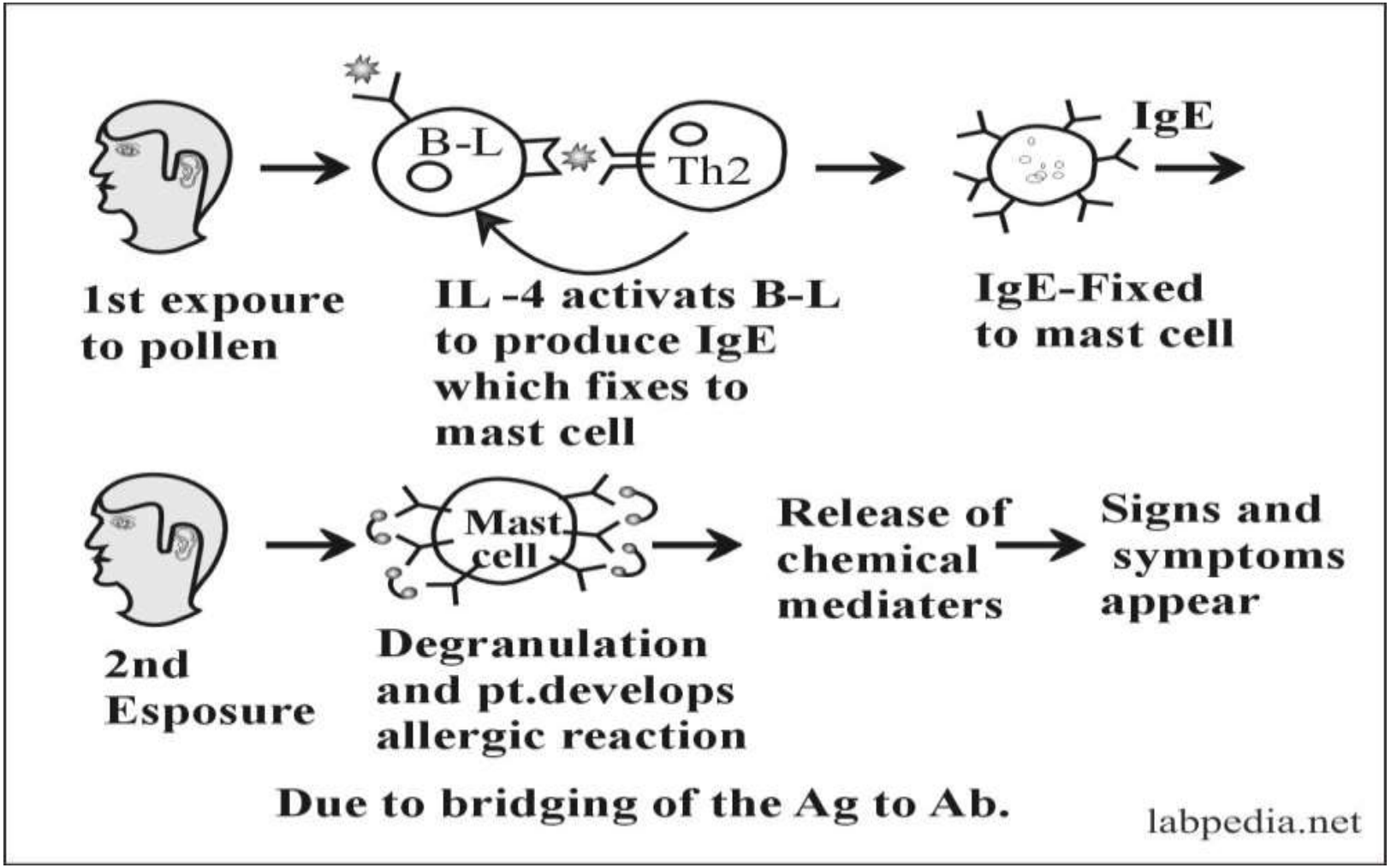
Late Phase

Eosinophils
MBP
ECP
IL-4



Broad-spectrum anti-inflammatory drugs suppress immune cell activity.

Stages of Type 1 Hypersensitivity Reactions are



This reaction takes place in two stages:

First Stage: This is the stage where there is sensitization of the host and formation of IgE Ab, which once formed attach to the receptors on mast cells or basophil.

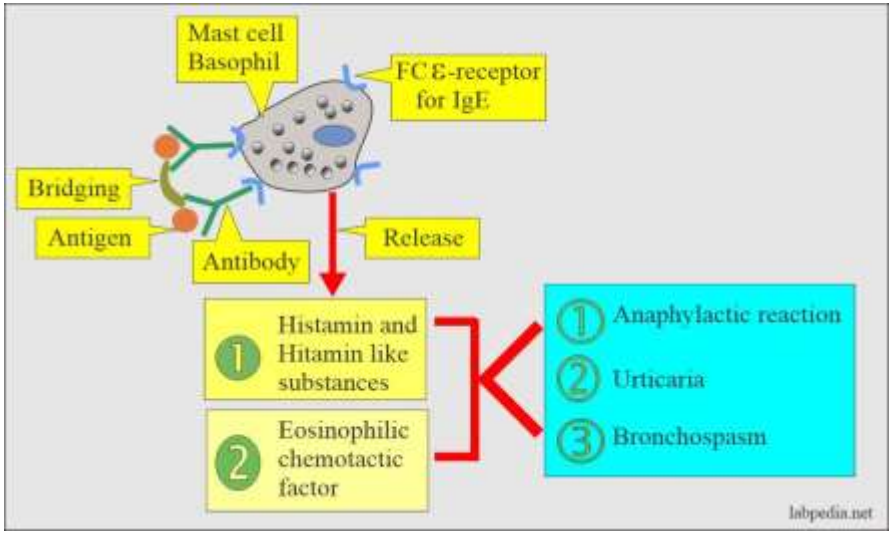
Second Stage: This is the stage of reaction or shocking dose in this stage where patients will have histamine effects and called histamine poisoning.

Basically, type 1 reaction has four stages:

- Activation of the B-lymphocytes to produce IgE.
- Antigen and IgE will sensitize mast cells.
- Mast cells release chemical mediators.
- The patient will have asthma, hay fever, or atopic eczema.
- Ag and Ab (Ag +IgE) bridging leads to degranulation, where Ag makes bridging between two IgE-Ab molecules present on the mast cell or basophils.

Degranulation is an active process, where:
There is an influx of calcium.
Initially, there is a rise in cAMP, and later on, it decreases.

Degranulation leads to the release of:
Primary mediators
Secondary mediators
Cytokines



Anaphylaxis (an-a-fi-LAK-sis) is a serious, life-threatening allergic reaction. The most common **anaphylactic** reactions are to foods, insect stings

There are two forms of anaphylaxis:

Systemic anaphylaxis: In some individuals, a severe reaction occurs within minutes, leading to symptomatology such as acute asthma, laryngeal edema, diarrhea, urticaria, and shock. Classic examples are penicillin allergy and bee sting allergy.

Local anaphylaxis (atopy): About 10% of people have "atopy" and are easily sensitized to allergens that cause a localized reaction when inhaled or ingested. This can produce hay fever, hives, asthma, etc. Classic examples are food allergies and hay fever to ragweed pollen.

Laboratory Findings

Type 1 hypersensitivity reactions may be accompanied by an increase in eosinophils, as noted with differential count of peripheral white blood cells.

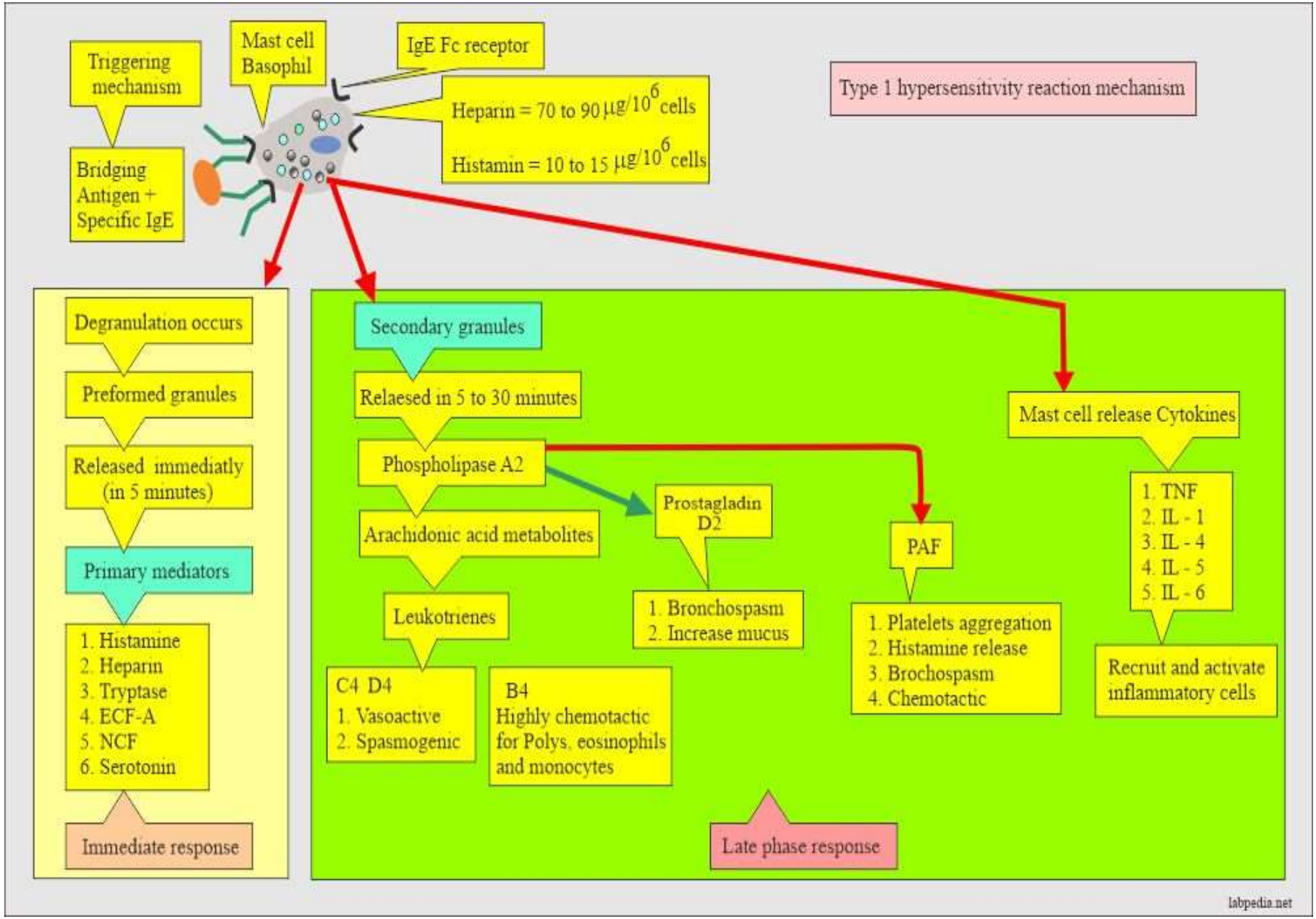
The serum tryptase (**Tryptase** is a trypsin-like proteinase that is found most abundantly in mast cells and basophils)may be increased in the hour following mast cell activation.Measurement of serum total IgE and levels of specific IgE for certain antigens may be undertaken when allergy therapies are planned. Testing for total or specific IgE should be done only when the history is consistent with allergy and specific allergens are suspected as the cause.

- **Systemic anaphylaxis**
  - May result in circulatory collapse and death
- **Localized anaphylaxis**
  - Hives, hay fever, and asthma

Chemical Mediators of type 1 Hypersensitivity Reaction are:

Primary or Preformed Mediators:
These are as follows:
Histamine
ECF-A (Eosinophilic chemotactic factor of anaphylaxis).
Neutrophil chemotactic factors (NCF).
Serotonin.
Secondary Mediators (Arachidonic Acid Metabolites):
These are:
Slow releasing substances of anaphylaxis (SRS-A) and now called leukotrienes, e.g., LTB4, LTC4, LTD4, LTE4.
Platelet-activating factor (PAF).
Prostaglandin D2 (PGD2).
Mast Cell Associated Cytokines:
These cells recruit more inflammatory cells.
TNFa, IL1, IL3, IL4, IL5, IL6, GM-CSF

Eosinophil degranulation results in the release of several cytotoxic cationic granule proteins. Furthermore, release of cytokines by eosinophils and other cells involved in inflammation **amplifies and regulates localized immune responses**


Type 1 hypersensitivity reaction mechanism
Triggering mechanism
Bridging Antigen + Specific IgE
Mast cell Basophil
IgE Fc receptor
Heparin = 70 to 90 µg/10^6 cells
Histamin = 10 to 15 µg/10^6 cells
Degranulation occurs
Preformed granules
Released immediatly (in 5 minutes)
Primary mediators
1. Histamine
2. Heparin
3. Tryptase
4. ECF-A
5. NCF
6. Serotonin
Immediate response
Secondary granules
Relaesed in 5 to 30 minutes
Phospholipase A2
Arachidonic acid metabolites
Leukotrienes
C4 D4
1. Vasoactive
2. Spasmogenic
B4
Highly chemotactic for Polys, eosinophils and monocytes
Prostagladin D2
1. Bronchospasm
2. Increase mucus
PAF
1. Platelets aggregation
2. Histamine release
3. Brochospasm
4. Chemotactic
Mast cell release Cytokines
1. TNF
2. IL - 1
3. IL - 4
4. IL - 5
5. IL - 6
Recruit and activate inflammatory cells
Late phase response
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Histamine

It is 10% of the weight of cells. It has the highest level in the morning and the lowest level in the late afternoon.

Histamine acts through separate receptors, and these are named as:-

H1 – present on smooth muscles of bronchi and action stops by the antihistamine.

H2 – Present in the stomach and action stops by cimetidine.

H3 – Present in CNS. It is under research.

Histamine clinically leads to:-

Wheal and flare.

Broncho-constriction.

Increase mucous secretions.

Hypotension due to vasodilatation and increased vascular permeability.

Cardiac arrhythmia.

Histamine target areas show:-

Skin – edema and hives.

Trachea and bronchi due to broncho-constriction lead to asthma and increased mucous secretions.

Eyes and nose – increase secretion and red eyes.

Uterus – smooth muscle contraction leads to abortion and pain.

Gastrointestinal tract. There is nausea, vomiting, abdominal pain, and diarrhea.

Secondary Mediators

Leukotrienes:

These are the metabolites of arachidonic acid metabolism. These have the same action as histamine but more potent and strong, almost several thousands time more active than histamine.

These are vasoactive and spasmogenic leads to contraction of smooth muscles and increase vascular permeability.

LTB4 – It is chemotactic for eosinophils, ploys, and monocytes.

Another leukotriene LTC4 (Previously) is called SRS-A.

Platelet Activating Factors (PAF)

It is generated from complex -lipids stored in the cell membrane. It leads to:-

Platelet aggregation and their lysis leading to histamine release.

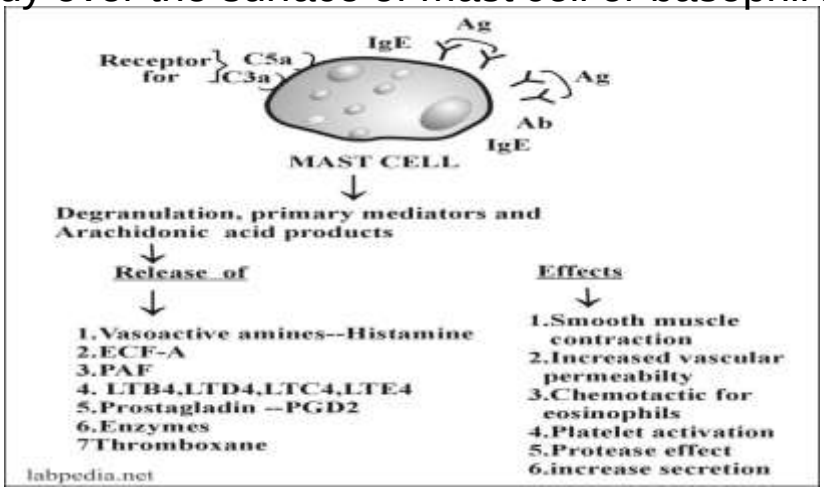
It activates neutrophils and eosinophils.

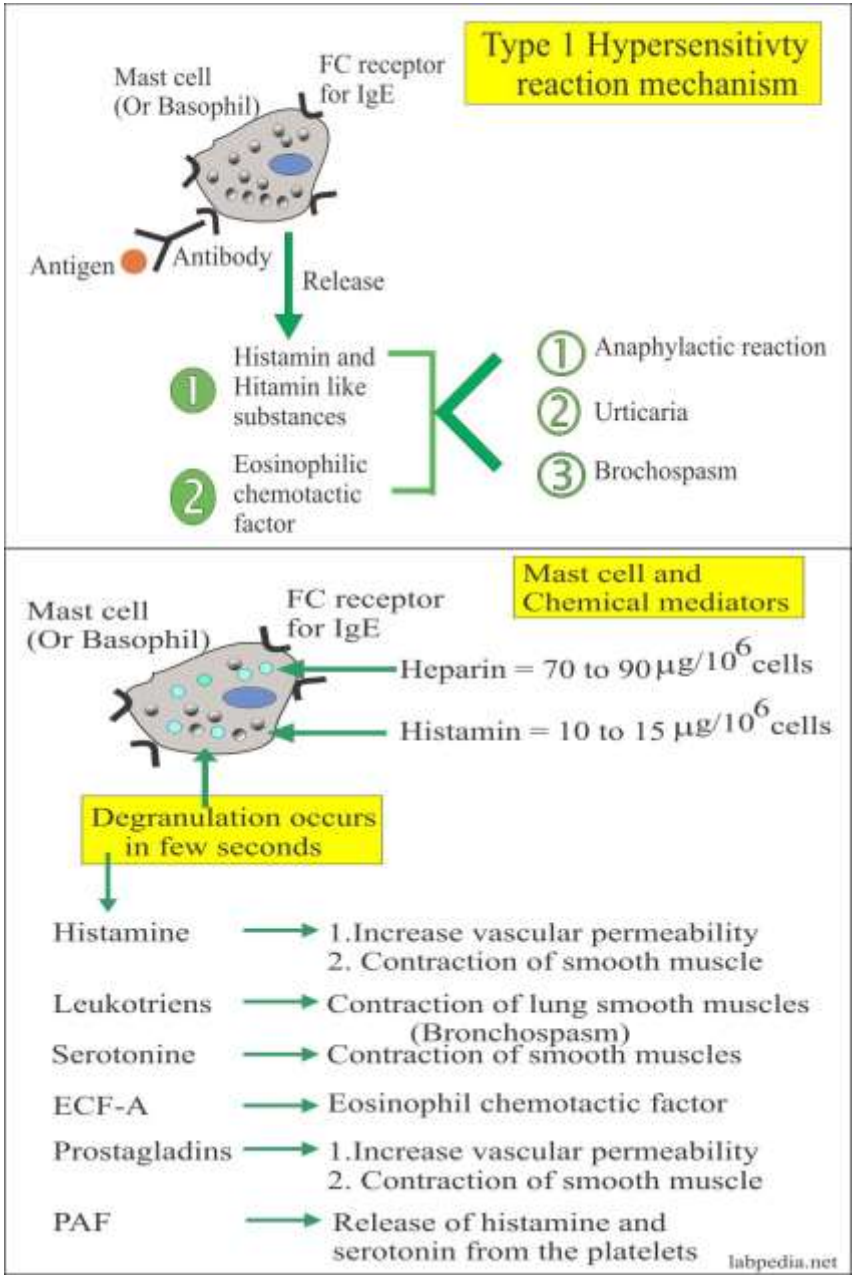
It is the most potent eosinophil chemotactic factor.

Prostaglandin (PGD2)

It is produced by human mast cells and leads to increased secretion, edema, and smooth muscle contraction.

Bridging of antigen and antibody over the surface of mast cell or basophil leads to the release of mediators




Type 1 Hypersensitivty reaction mechanism
Mast cell (Or Basophil)
FC receptor for IgE
Antigen
Antibody
Release
1 Histamin and Hitamin like substances
2 Eosinophilic chemotactic factor
1 Anaphylactic reaction
2 Urticaria
3 Brochospasm
Mast cell and Chemical mediators
Mast cell (Or Basophil)
FC receptor for IgE
Heparin = 70 to 90 µg/10^6 cells
Histamin = 10 to 15 µg/10^6 cells
Degranulation occurs in few seconds
Histamine → 1.Increase vascular permeability 2. Contraction of smooth muscle
Leukotriens → Contraction of lung smooth muscles (Bronchospasm)
Serotonine → Contraction of smooth muscles
ECF-A → Eosinophil chemotactic factor
Prostagladins → 1.Increase vascular permeability 2. Contraction of smooth muscle
PAF → Release of histamine and serotonin from the platelets
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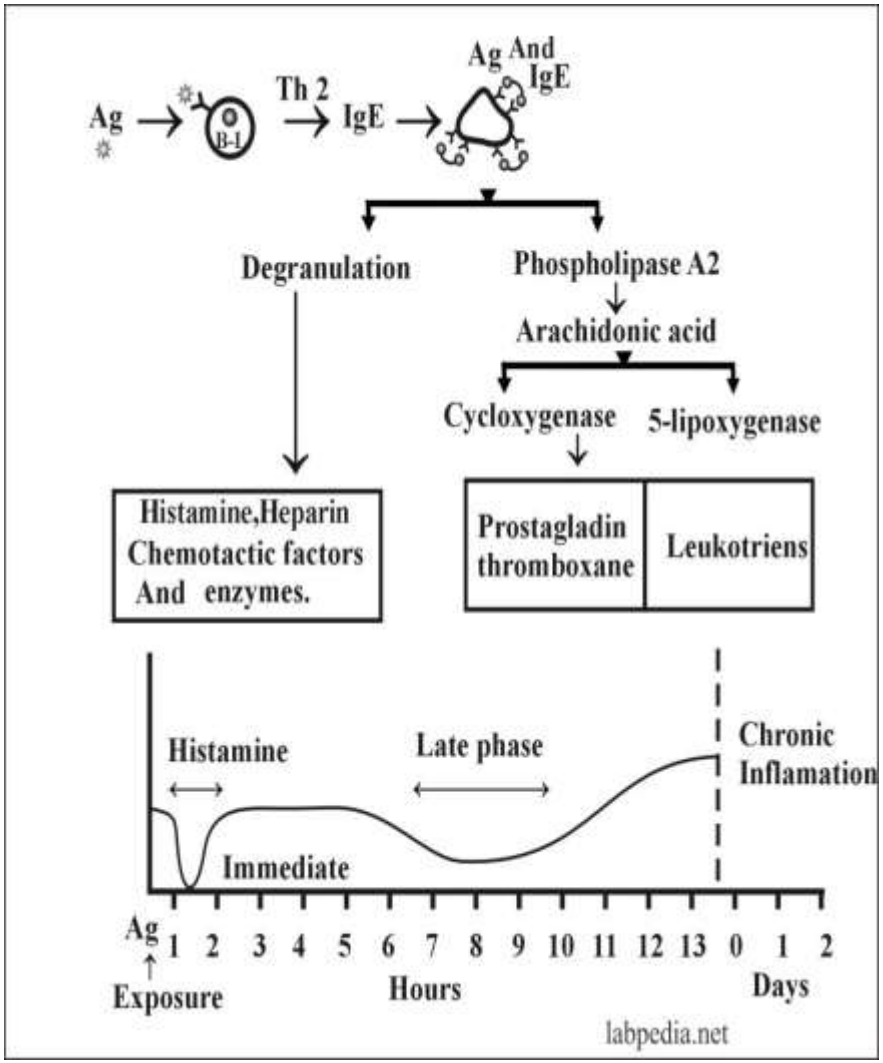
Ag
B-L
Th 2
IgE
Ag And IgE
Degranulation
Phospholipase A2
Arachidonic acid
Cycloxygenase
5-lipoxygenase
Histamine,Heparin Chemotactic factors And enzymes.
Prostagladin thromboxane
Leukotriens
Histamine
Late phase
Chronic Inflamation
Immediate
Ag
1 2 3 4 5 6 7 8 9 10 11 12 13 0 1 2
Exposure
Hours
Days
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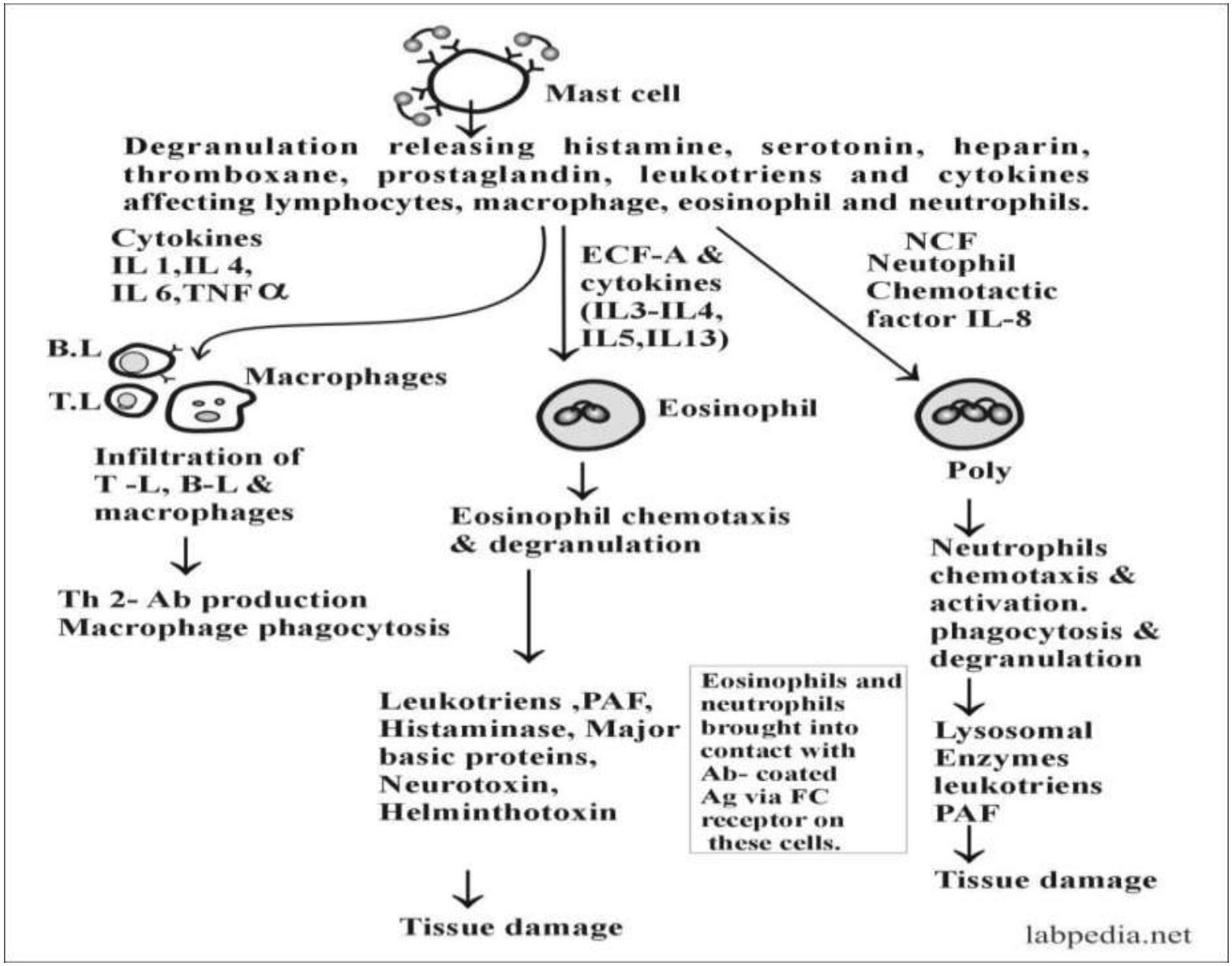
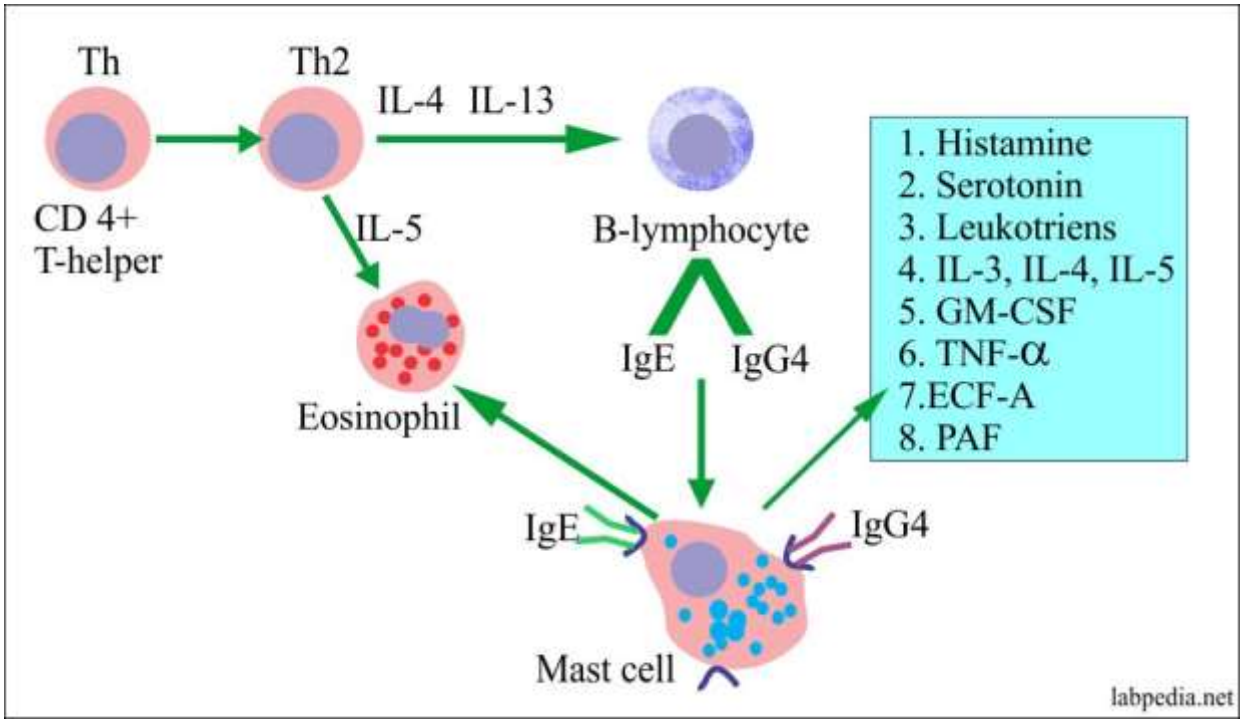


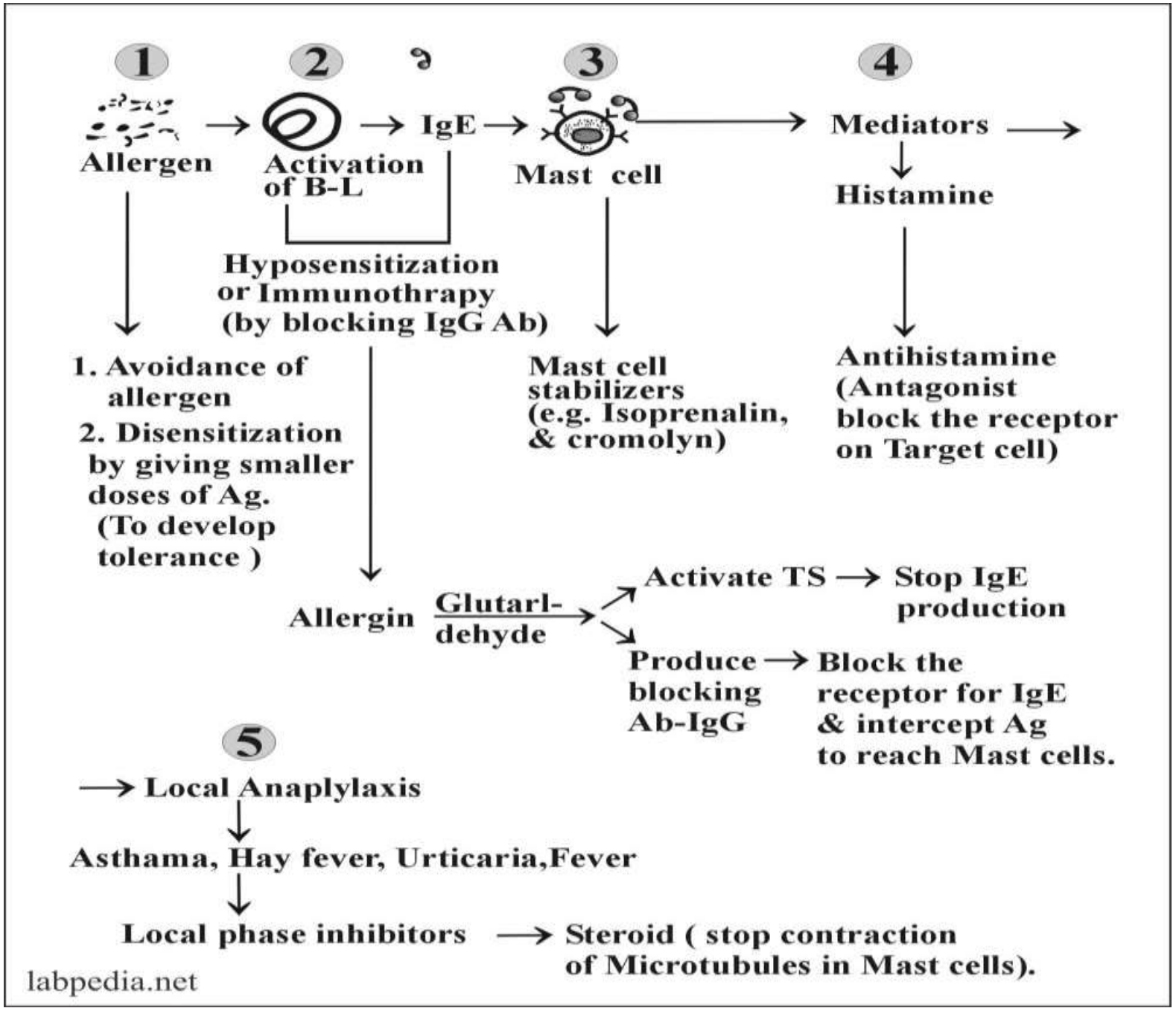
In late-phase activation of Mast Cells and its mediators ultimately leading to tissue damage

Type 1 hypersensitivity reaction and the Role of T-cells



Role of T-lymphocytes in the activation of B-lymphocyte and formation of IgE and IgG4:

- IgE production depends on the T-lymphocytes, which activate the B-lymphocytes and produce IgE and IgG4.
- The T-cells can suppress the formation of IgE by the production of interferon-gamma (IFN-γ).
- The production of IgE is dependent upon the Th-2 helper cells. At the same time, Th-1 cells will suppress IgE production


1
2
3
4
Allergen
Activation of B-L
IgE
Mast cell
Mediators
Histamine
Hyposensitization or Immunothrapy (by blocking IgG Ab)
1. Avoidance of allergen
2. Disensitization by giving smaller doses of Ag. (To develop tolerance)
Mast cell stabilizers (e.g. Isoprenalin, & cromolyn)
Antihistamine (Antagonist block the receptor on Target cell)
Allergin
Glutarl-dehyde
Activate TS → Stop IgE production
Produce blocking Ab-IgG → Block the receptor for IgE & intercept Ag to reach Mast cells.
5
→ Local Anaplylaxis
Asthama, Hay fever, Urticaria,Fever
Local phase inhibitors → Steroid (stop contraction of Microtubules in Mast cells).
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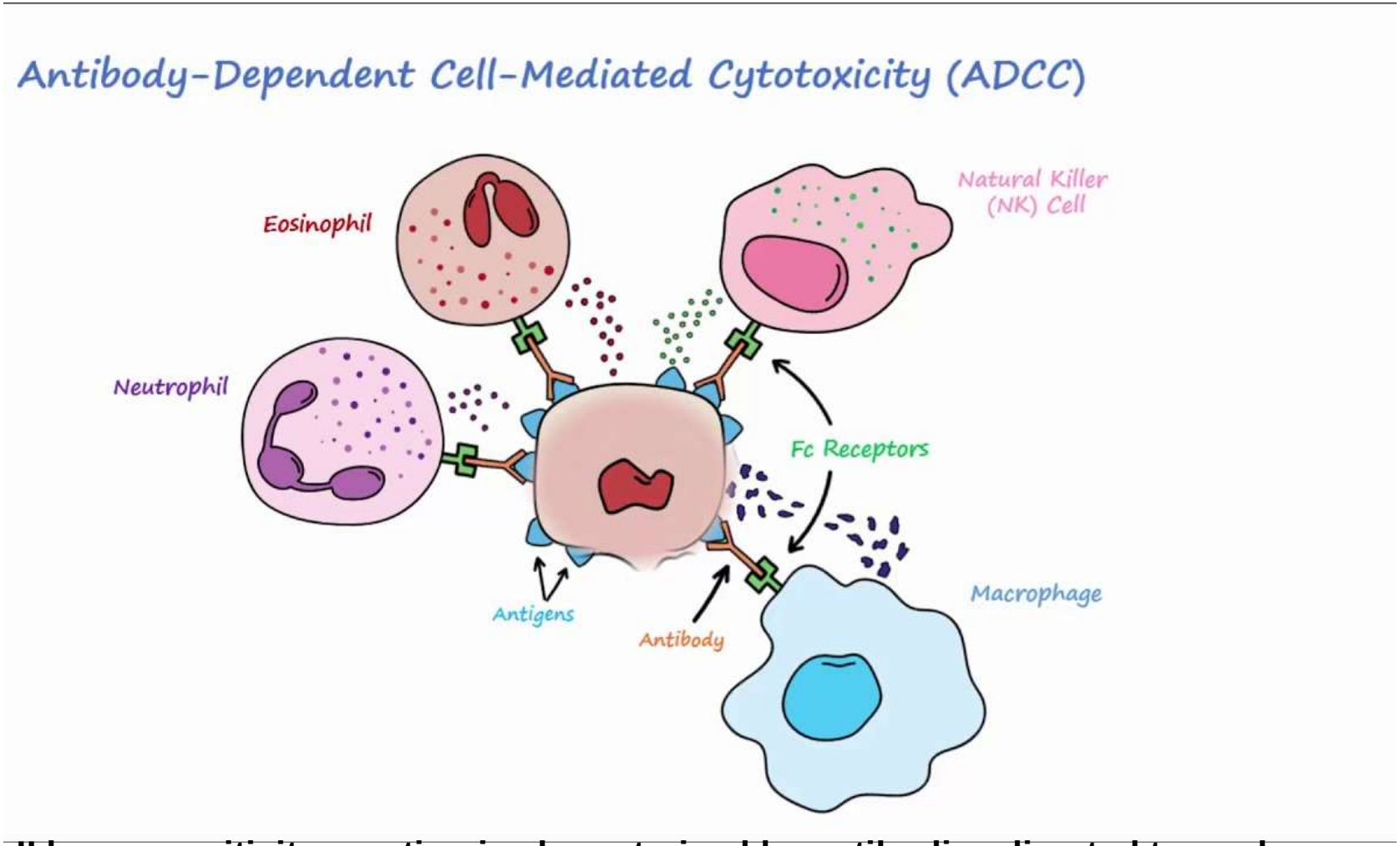
Type II Hypersensitivity is one of the basic mechanisms by which immune-mediated injury to host tissues can occur. The reaction occurs due to direct binding of antibody to host tissues resulting in either functional derangement of the tissue or inflammatory damage.

Examples of type II HS include **some forms of anemia, blood transfusion reactions, certain platelet disorders, and some types of tissue transplant rejection, erythroblastosis fetalis, and autoimmune hemolytic anemia.**

.

Type II hypersensitivity reactions are mediated by antibodies, such as IgG and IgM, directed against antigens, which cause cell destruction by complement activation or antibody-dependent cell-mediated cytotoxicity..

How is Hypersensitivity reaction – Type II Treated?
intragam infusion: this is infusing the body with antibodies. ...
plasmaphoresis: this is removing the blood autoantibodies.
other drugs: interferon, cyclophosphamide, cyclosporin.



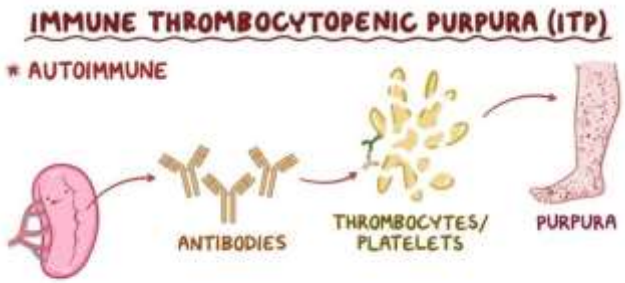
Type II hypersensitivity reaction is characterised by antibodies directed toward antigens (substance that attracts the antibody to bind with) that are present on cell surfaces outside the cells.

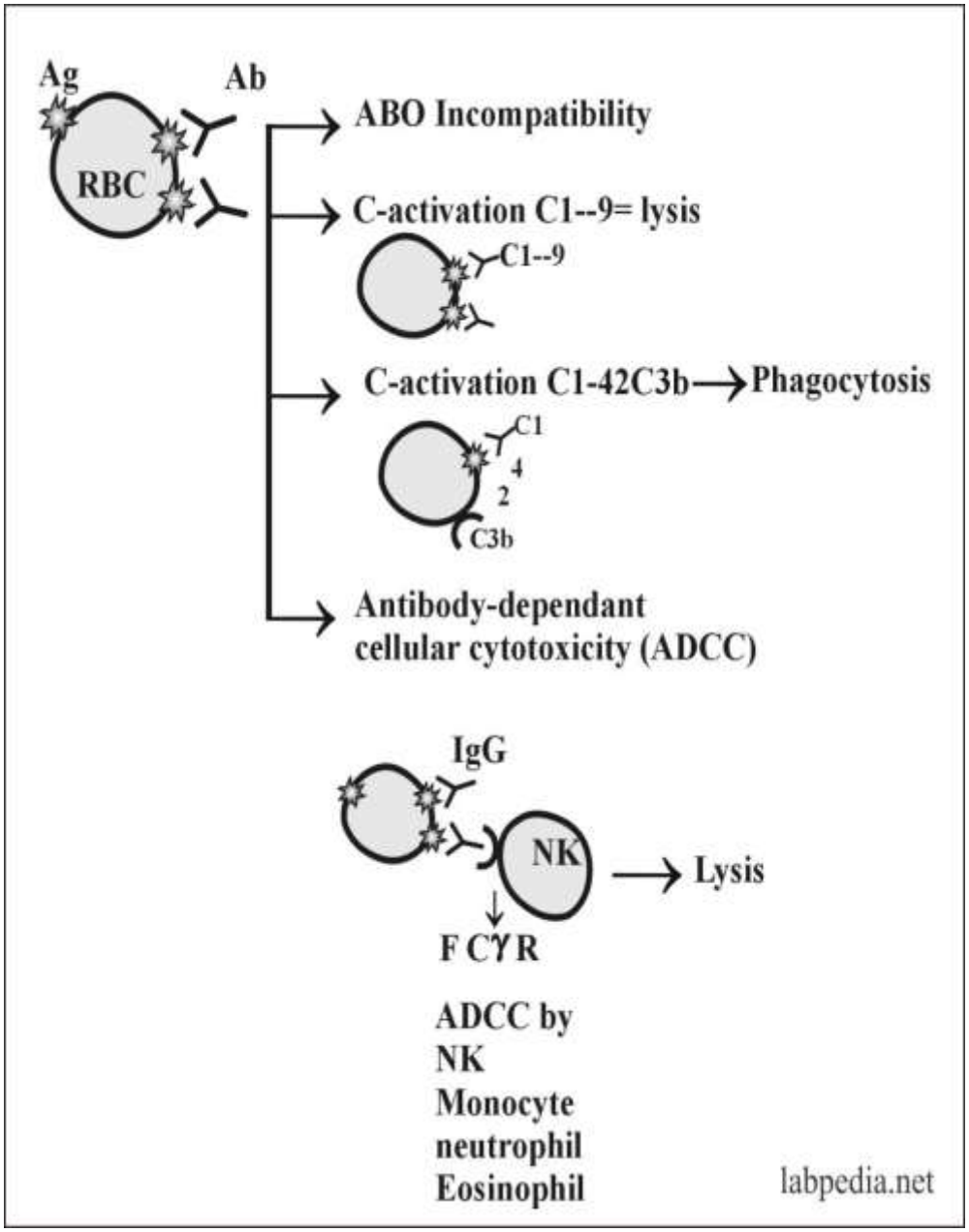
The antigens can either be from the body itself or from outside the body (for example, bacteria or microorganisms that infect the body).

By the various biochemical mechanisms, the end result are tissue damage to the body.

Type II hypersensitivity diseases can be widely different from one to another. It can be life-threatening such as in blood transfusion reaction or severe immune thrombocytopaenic purpura (ITP, platlet reduce) – they need medical attention immediately if the diseases are severe. Some conditions such as pernicious anaemia have good prognosis as treatment can usually be administered to control the disease.

Immune thrombocytopenic purpura (ITP) is **a blood disorder characterized by a decrease in the number of platelets in the blood**. Immune thrombocytopenia usually happens when **immune system mistakenly attacks and destroys platelets**. In adults, this may be triggered by infection with HIV , hepatitis or H. pylori — the type of bacteria that causes stomach ulcers.





Complement activation:

The antibody attaches to Antigen on the surface of cells and activates the complement system, which leads to lysis.

Antibody-dependent cellular toxicity (ADCC):

Sometimes Antibody is attached to Antigen on the cell surface will bring this complex near to NK cells or other phagocytic cells possessing the Fc-Receptor and leads to antibody dependant cellular cytotoxicity (ADCC).

Opsonization and phagocytosis:

The antibody binds to an antigen and makes it a target for phagocytosis, and this process is called opsonization.

Type III hypersensitivity occurs when there is accumulation of immune complexes (antigen-antibody complexes) that have not been adequately cleared by innate immune cells, giving rise to an inflammatory response and attraction of leukocytes. Such reactions may progress to immune complex diseases.

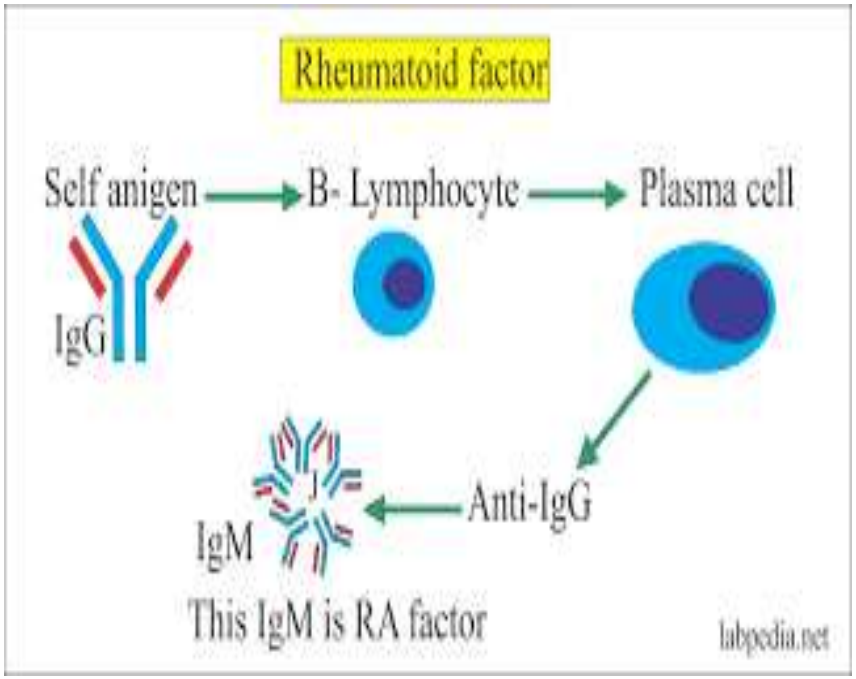
Examples of type III hypersensitivity reactions include drug-induced serum sickness, farmer's lung and systemic lupus erythematosus, **rheumatoid arthritis etc**

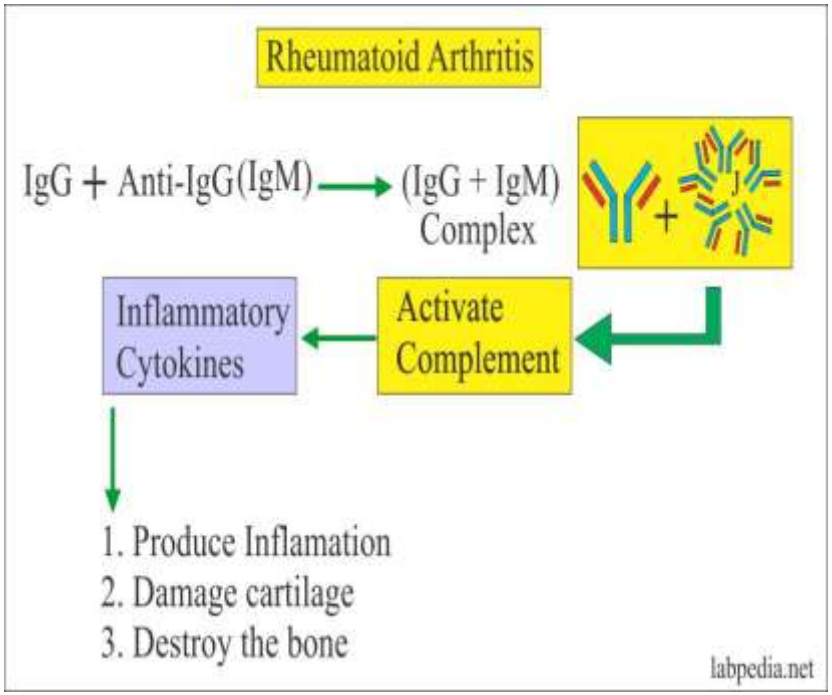
Type III Hypersensitivity

Antigens Associated with Immune Complex Disorders

ANTIGEN	CLINICAL MANIFESTATION
EXOGENOUS	
Infectious agents:	
Bacteria: *Y. enterocolitica*	Arthritis
Streptococci	GN, Infective endocarditis
T. pallidum	Glomerulonephritis
Viruses: Hep. B, CMV	Polyarteritis nodosa
Parasites: *Plasmodium*	Glomerulonephritis
Schistosoma	
Fungi: *Actinomycetes*	Farmer's lung
ENDOGENOUS	
Nuclear antigens	SLE
Immunoglobulins	Rheumatoid arthritis
Tumor antigens	Glomerulonephritis

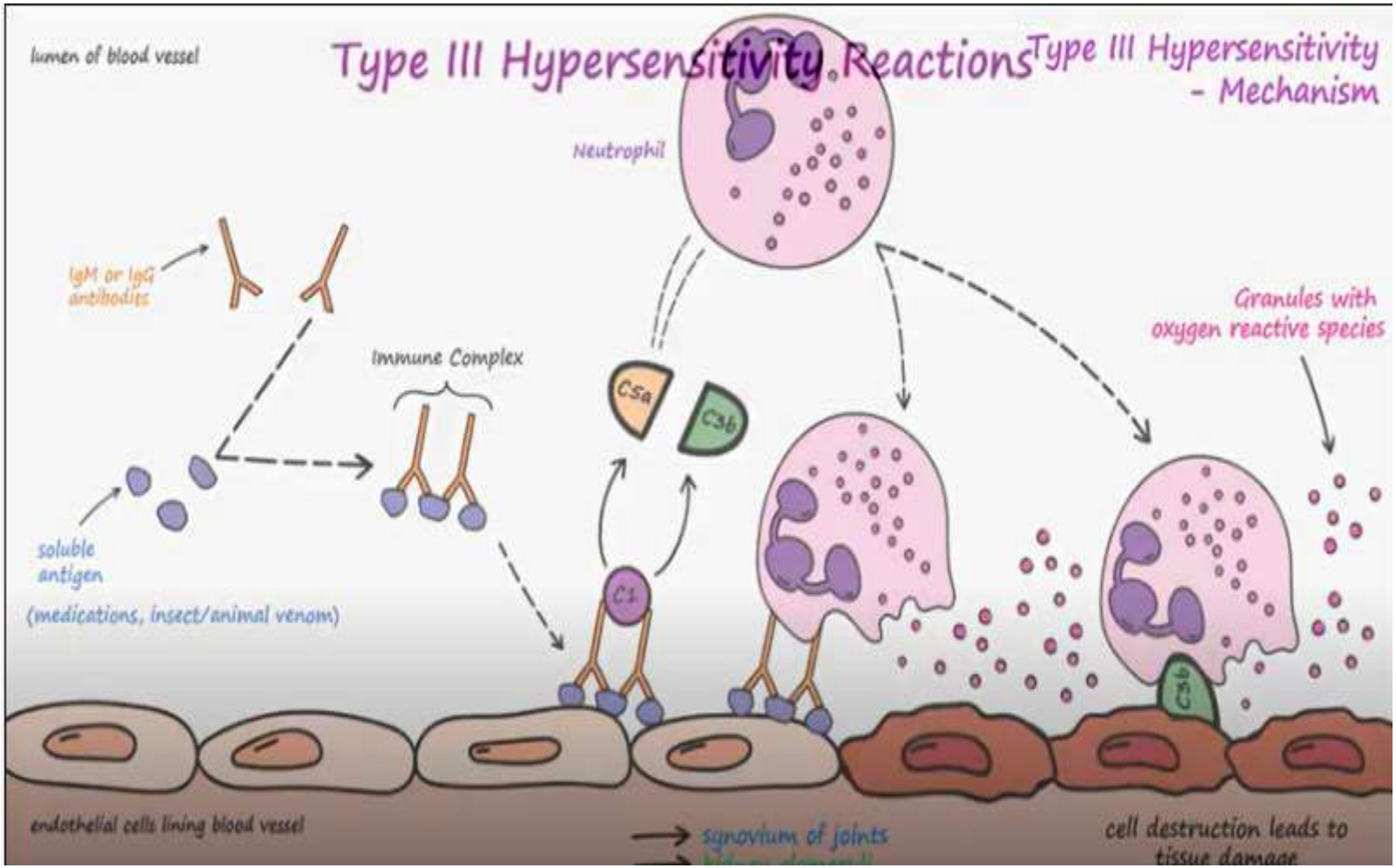
Possible antigens are:Self- IgG.
Viral-like EBV may play a role that may suppress T cells or give rise to polyclonal activation of B-Lymphocytes. Other viruses may be blamed like Herpes, Rubella, and Mycoplasma.
Synovial lymphocytes produce IgG which is recognized as foreign and give rise immune complex (IgG + Anti-IgG).





The damaging inflammatory reaction is triggered by a soluble antigen capable of forming large insoluble immune complexes (IC) with IgM or IgG antibodies in the circulation. These complexes are too large to phagocytose, so, rather than being cleared, the ICs are deposited either at a single site or in various locations in the body (often in the walls of vessels). The presence of the ICs in the tissues initiates immune responses that damage surrounding cells, leading to localized pain, edema, and inflammation. Histologically, type III HS is distinguished by an accumulation of neutrophils at the site of tissue injury at about 4–6 hours after exposure to the antigen.

Arthus reaction, is typically seen with repeated insect stings, where a red swollen lesion develops after a sting. The tissue damaging mechanisms are similar to those described for the antigen-antibody complexes that form in type II responses. The response times of types II and III hypersensitivity reactions are slower than that of type I reactions; they typically develop 3–6 h after exposure to antigen. The response can also become chronic, particularly in autoimmune reactions, where antigen persists.


Type III Hypersensitivity Reactions
Type III Hypersensitivity - Mechanism
lumen of blood vessel
Neutrophil
IgM or IgG antibodies
Immune Complex
C5a
C3b
Granules with oxygen reactive species
soluble antigen
(medications, insect/animal venom)
C1
C3b
endothelial cells lining blood vessel
synovium of joints
cell destruction leads to tissue damage

Serum Sickness

an allergic reaction to an injection of serum, typically mild and characterized by skin rashes, joint stiffness, and fever

Many nonprotein **drugs**, including beta-lactam antibiotics, ciprofloxacin, sulfonamides, bupropion, streptokinase, metronidazole, carbamazepine, insulin detemir, and others, have been reported to **cause serum sickness**–like reactions

- **Serum sickness**: originally has been observed as reaction against treatment with horse serum as passive immunization
  - Type III systemic inflammatory response to the presence of immune complexes .
    - not like arthus reaction which is localized)
  - Symptoms appear few days after exposure to antigen and include
    - Fever, urticaria (hives), arthralgia, lymphadenopathy, spleenomegaly
  - Symptoms disappear after few days when antibody level increases and antigen level falls down

Urticaria:pale red bumps

Arthralgia: pain in joint

Lymphaldenopathy: swelling in lymp gland

Farmer,s Lung: a type of pneumonitis caused by an allergic reaction to spores in mouldy hay.

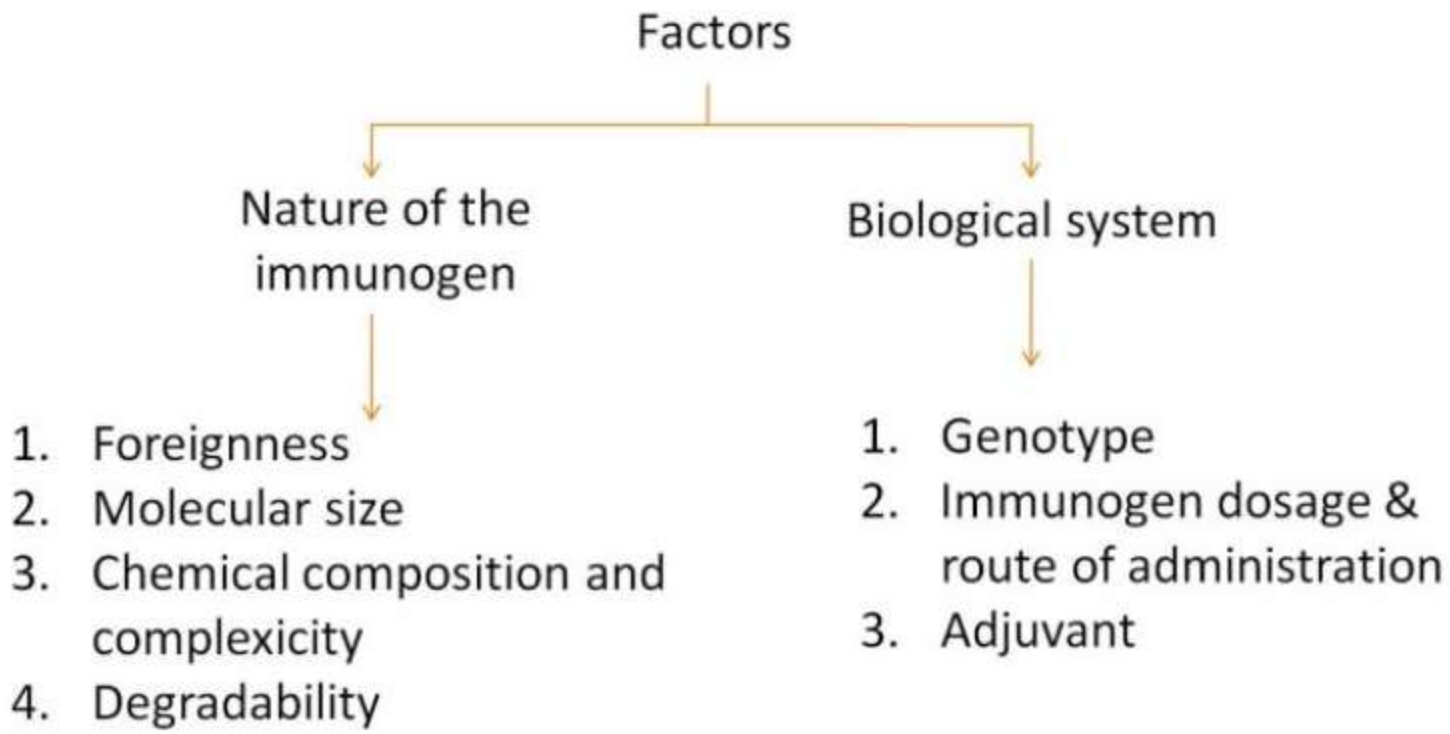
is a hypersensitivity pneumonitis induced by the inhalation of biologic dusts coming from hay dust or mold spores or any other agricultural products. It results in a type III hypersensitivity inflammatory response and can progress to become a chronic condition which is considered potentially dangerous.

Immunogen and Antigen (collected graphics from internet)

Immunogens

- Immune response generator—can bind *and* induce an immune response
- The immunogen is the target of the response it induces
- Subsequent exposures result in increased responsiveness
- Proteins tend to be more immunogenic than lipids, carbohydrates, and nucleic acids
- Important for vaccines

Few factors affect the immunogenicity:



What factors influence Immunogenecity?

Nature of the Immunogen

Foreignness
- Non-self , phylogenetic distance , sequestered antigens

Molecular Size
- > 100kDa or more are active immunogens.
- With exceptions: dextran
- Low m.w. chemicals : formaldehyde

Chemical composition & Heterogeneity
- Synthetic homopolymers lack immunogenecity.
- Copolymers composed of different amino acids / sugars immunogenic than homopolymers.
- Complexicity imp factor
- Structural stability important. Ex: gelatin , proteins with aromatic amino acids

Susceptibility to Ag processing & Presentation
- Large insoluble macromolecules> immunogenic soluble ones
- Macromolecules not degraded – poor immunogens
- D-amino acids - poor immunogens
- L-amino acids - good immunogens.

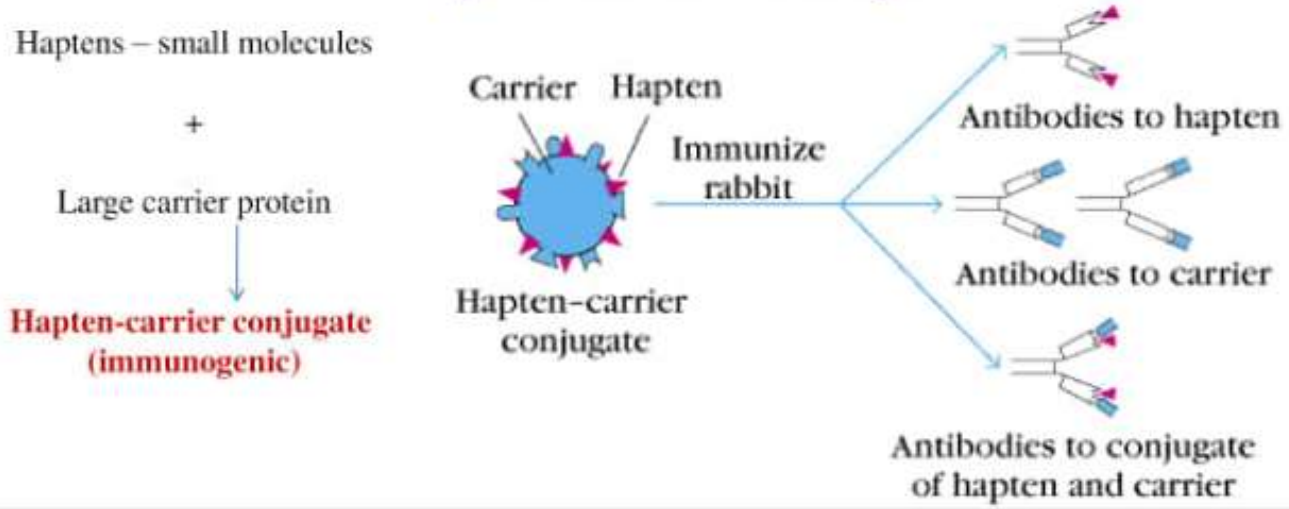
Lipids
- Lipid-proteins as hapten-carrier conjugates.
- Recognition of lipids by T cells through CD1

Immunogen, antigen, epitope, hapten

- Immunogen: a stimulus that produces a humoral or cell-mediated immune response
- Antigen: any substance that binds specifically to an antibody or a T-cell receptor
- Epitope: the portion of an antigen that is recognized and bound by an Ab or TCR/MHC

HAPTENS

Antigenic but not immunogenic



Response of the Host (biological system) receiving the Antigen is important

Genotype of recipient and Age

- Different genotype respond differently to immunogens.

  ex: same Ag ----different Ab response.
- The kind of immune responsiveness depends upon MHC genes of the host.
- Impaired at very young and old age.

Dosage & route of administration

- Each immunogen has a dose-response curve. High or low dose can induce tolerance. Ex. Pnemococcal capsular polysaccharide.
- Booster immunizations.
- **iv , id , sc , im , ip**

Adjuvants

- Adjuvants enhance immunogenecity of the Ag.
- Augment immune response by :

  1. prolong Ag persistence
  2. enhance co-stimulatory signals
  3. increase local inflammation
  4. stimulate non-specific proliferation of lymphocytes.

Freund's adjuvant is a solution of antigen emulsified in mineral oil and used as an immunopotentiator (booster).
The **complete form**, Freund's Complete Adjuvant (FCA or CFA) is composed of inactivated and dried mycobacteria (usually *M. tuberculosis*), whereas the **incomplete form** (FIA or IFA) lacks the mycobacterial components (hence just the water in oil emulsion). It is named after Jules T. Freund

Incomplete Freund's Adjuvant (IFA) = strong Th2 immune response + low Th1 response

Complete Freund's Adjuvant (CFA) = Strong Th1 immune response

IFAVax Incomplete Freund's Adjuvant (IFA) and CFAVax Complete Freund's Adjuvant (CFA) are water-in-oil emulsions consisting in mixtures of mineral oil and an emulsifier in a ratio of 85% v/v oil and 15% v/v emulsifier.

Contrary to the Complete Freund's Adjuvant (CFA), IFA does not contain heat-killed **mycobacteria (*Mycobacterum tuberculosis*).**

IFA and CFA have been used extensively in experimental immunology and IFA has been used for decades in practical veterinary vaccination.

Importantly, Freund's adjuvants are not pre-formed emulsions and thus they must be mixed with an equal volume of aqueous solution of antigens and subsequently emulsified prior to use.

Even if the mechanisms of action of oil emulsions are still poorly understood, some evidences suggest a partial requirement for NOD2 (inflamasome). Moreover, these emulsions are prone to cause cellular damage upon injection and thus, endogenous signals released during necrotic cell death may also contribute to their adjuvant activity.

Immune response directed by CFA is dramatically enhanced by the presence of the mycobacterial component that attracts macrophages and immune cells at the site of injection.

CFA induces principally a Th1 response and can cause granulomas and an intense inflammatory reaction at the inoculation site. Indeed, the use of CFA should be used responsibly and with care in order to avoid or minimize the adverse effects of excessive inflammation.

As opposed to CFA that induces principally a Th1 response, IFA that lacks mycobacterial components primes a Th2 response .

For most applications, CFA is usually only necessary for the initial immunization, while IFA is the adjuvant of choice for subsequent immunizations. IFAVax and CFAVax adjuvants are designed to provide continuous release of antigens necessary for stimulating strong persistent immune response

ALUMINIUM ADJUVANT (FOR TH2 IMMUNE RESPONSE)

Alum is the most common adjuvant used in approved prophylactic vaccines because of its excellent safety profile and ability to enhance protective humoral immune response (Th2). It consists of precipitates of **aluminum phosphate and / or aluminum hydroxide** to which antigens are adsorbed through hydrophobic and electrostatic interactions or entrapped.

AlumVax hydroxide is positively charged at a physiological pH of 7.4 and **binds acidic proteins**. **AlumVax phosphate** on the other hand is negatively charged and therefore **binds basic proteins**.

The association of antigen with Alum favors a high local antigen concentration and an improved uptake by APC. Thus early observations described that Alum enhances the response to antigens by extending the time during which the antigen is available.

Furthermore, it is now admitted that Alum, like many other adjuvants, acts by **direct activation of the immune cells**. In mice, Alum induces a profoundly polarized Th2 immune response which is characterized by the production of IL-4 and IL-5 and the strong induction of antibodies production as immunoglobulin IgE and IgG1. Consequently, Alum is very effective against pathogens that require Th2 humoral-mediated immunity. In parallel, Alum alone can activate the NLRP3 inflammasome to produce mature IL-1 creating a favorable environment for the immune response . Nonetheless; **Alum fails to induce Th1 responses associated with the induction of INF-γ and cytotoxic T lymphocytes which are required to clear the body of intracellular infections.**

Th1 cells play important roles in the **identification and eradication of intracellular pathogens such as viruses and bacteria**, including Mycobacterium tuberculosis, Mycobacterium leprae, and Leishmania.

Th2 cells **mediate the activation and maintenance of the humoral, or antibody-mediated, immune response against extracellular parasites, bacteria, allergens, and toxins**. Th2 cells mediate these functions by producing various cytokines such as IL-4, IL-5, IL-6, IL-9, IL-13, and IL-17E (IL-25)

Squalene is widely used for numerous vaccine and drug delivery **emulsions** due to its stability-enhancing effects and biocompatibility.

Squalane is **derived** by hydrogenation of squalene. It is naturally present in the skin lipid barrier of plants, animals and humans, preventing moisture loss while restoring skin's suppleness and flexibility. Due to the complete saturation of **squalane**, it is not subject to auto-oxidation

Oil content and particle size of droplets control the quantity and the quality of the immune response generated.

an oily liquid hydrocarbon which occurs in shark liver oil and human sebum, and is a metabolic precursor of sterols.

SqualVax is an oil-in-water emulsion made of squalene droplets in a continuous aqueous phase. **Squalene droplets are stabilized by the addition of two non-ionic surfactants that are widely used as emulsifiers in food, cosmetics and pharmaceuticals** . It is fully biodegradable, which is an important advantage over alternative oils used in emulsion adjuvants, like Freund's adjuvant that contains mineral oil (paraffin oil) and thus has long term persistence in the organisms.

Squalene emulsion induces local stimulation and recruitment of DCs and granulocytes, differentiation of monocytes into DCs and increased uptake of antigen by APC

Compared to aluminium salts, a stronger immune response is elicited (e.g higher antibody and T-cell response) with a mixed and more balanced Th1/Th2 cell phenotype

EPITOPE

- **Discrete sites on an immunogenic macromolecule.**
- **Immunologically active regions of an immunogen.**
- **T cells and B cells recognize different epitopes on the same antigenic molecule.**
- **Lymphocytes interact with a complex antigen on several levels of antigen structure.**

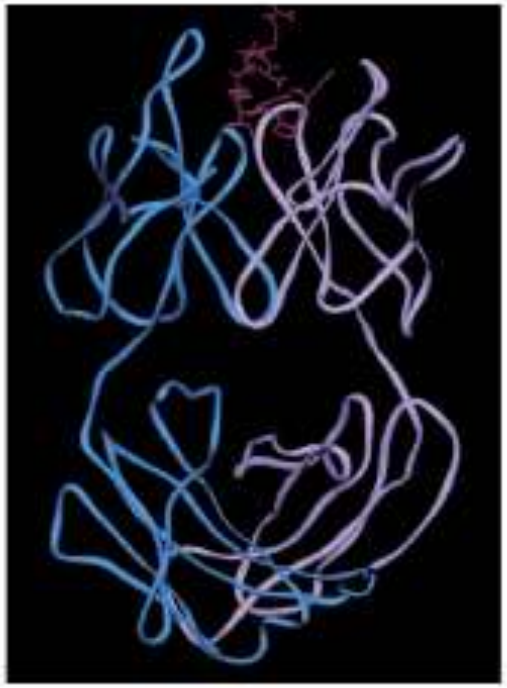
TABLE 3-3 COMPARISON OF ANTIGEN RECOGNITION BY T CELLS AND B CELLS

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

Properties of B cell epitopes

The ability to function as a B cell epitope is determined by the nature of the antigen-binding site of the antibody molecules displayed by B cells.

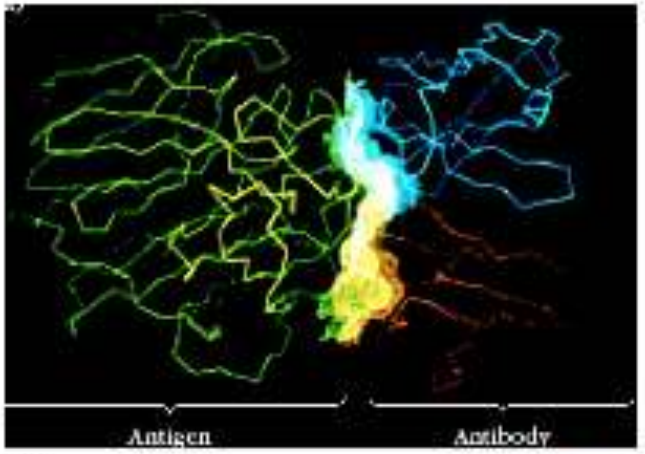
- **Complementary shapes** of Ag-binding site and the epitope
  - Strong bond
- **Size of the epitope** not larger than the size of the Ag-binding site of the Ab.
- **Shape of the epitope --------- Shape** assumed by the sequence of amino acids in the binding site.
- **Larger areas of the globular protein** Ag are engaged by the binding site. Shape of epitope : tertiary conformation of native protein.
- In contrast, **small peptides fold into compact structures** that occupy less space and fit into cleft of the binding site.

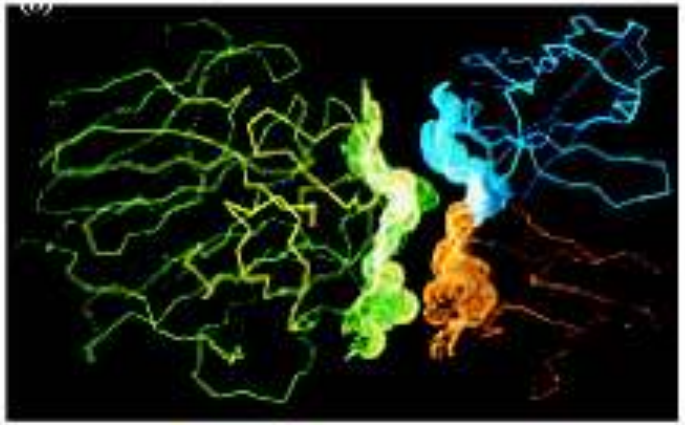


Properties of B cell epitopes

The B cell epitopes on native proteins generally are composed of hydrophilic amino acids on the protein surface that are topographically accessible to the membrane-bound or free antibody.

- Must be **accessible**.
- **Protruding regions** most likely to be recognized as epitopes predominantly **hydrophilic amino acids.**
- Amino acid sequences in the **interior of the protein-hydrophobic-** not function as B cell epitopes.
- **Complementary protrusions and depressions**.
- **Bonds:** Hydrogen bonds, ionic and hydrophobic interactions.





Properties of B cell epitopes

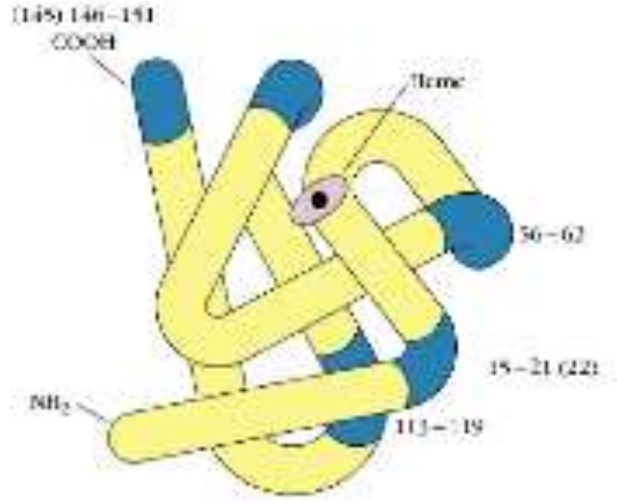
B cell epitopes can contain sequential or nonsequential amino acids.

- Sequential **contiguous residues.**

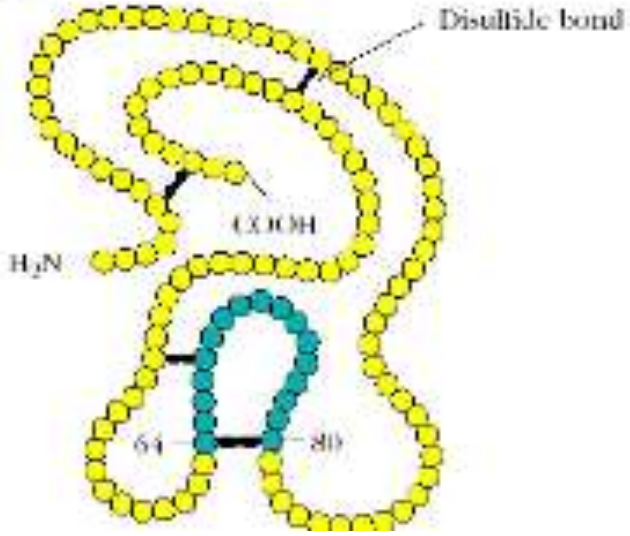
- **Or non sequential residues** from the segments of the chain brought together by the **folded conformation of an antigen.**

- **Antibodies** to native protein **do not bind to the denatured protein.**

- Binding of Ab to Ag depends on maintenance of the **tertiary structure** of the epitopes by **intrachain disulphide bonds.**



(a) Hen egg-white lysozyme



Properties of B cell epitopes

B cell epitopes tend to be located in the flexible regions of an immunogen and display site mobility.

- **Major Antigenic determinants** in proteins generally located in the most **mobile regions**.
- **Site mobility** of epitopes **maximizes complementarity** with the Ag binding site.
- **But is of lower affinity due to loss of entropy.**

Complex proteins contain multiple overlapping B cell epitopes, some of which are immunodominant

- **Most of the surface** of a protein is potentially **antigenic**.
- **Subset of antigenic sites** on a given protein recognized by the immune system is much **smaller** than the **potential antigenic repertoire.**
- **Immunodominant epitopes** induce a more **pronounced immune response** than other epitopes of the same protein.

Properties of T cell epitopes

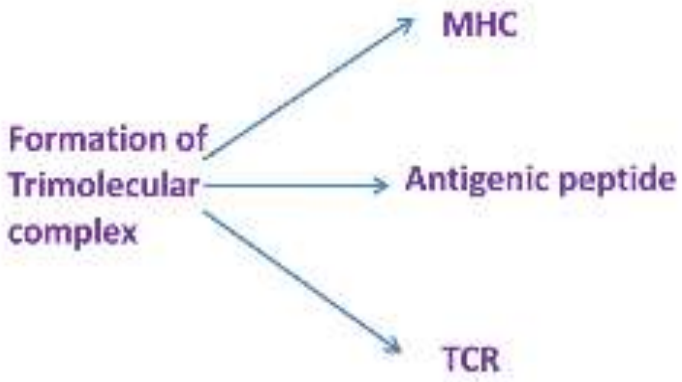
Antigen-derived peptides

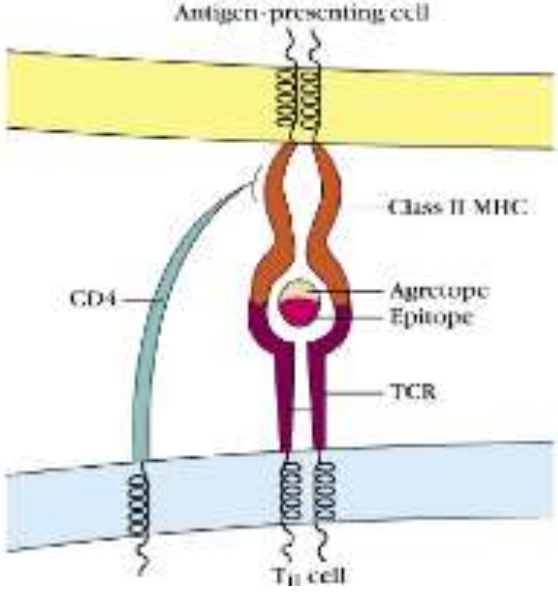
TABLE 3-4 ANTIGEN RECOGNITION BY T AND B LYMPHOCYTES REVEALS QUALITATIVE DIFFERENCES

		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).

Presentation of peptides in context of MHC molecule





Antigen processing is required to generate peptides that interact specifically with MHC molecules

- Endogenous antigens processed into peptides within the cytoplasm
- Exogenous antigen processed by the endocytic pathway.

Binding of MHC to Antigenic peptide does not have the fine specificity of the epitope-Ab interaction.

- A MHC molecule can bind to a wide variety of peptides.
- Antibody can bind to only that epitope for which it is specific.

Epitopes recognized by T cells are often internal

- T cells recognize internal peptides that are exposed by processing within Ag-presenting cells or altered self-cells.

Vaccines;

1. Killed
2. Subunit
3. Peptide
4. DNA
5. Vaccinia virus
6. Live attenuated

MHC and antigen presentation

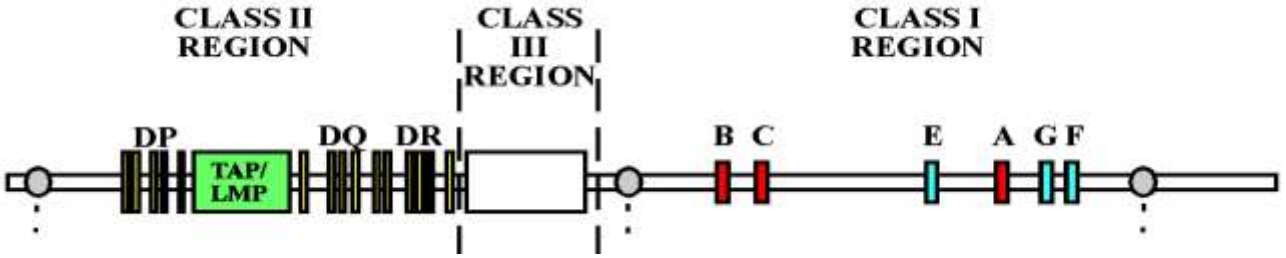
(Graphics from internet)

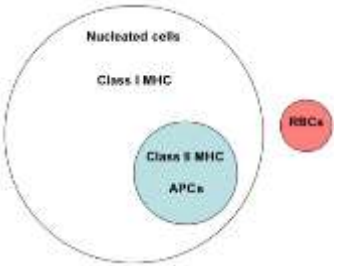
Major Histocompatibility Complex (MHC)

- Group of antigens first identified in graft patients
- Important in determining the compatibility of tissues in successful grafting
- Major histocompatibility antigens are glycoproteins found in the membranes of most cells of vertebrate animals
- Function to hold and position antigenic determinants for presentation to T cells

- Genes in the MHC were first identified as being important genes in rejection of transplanted tissues
- Genes within the MHC were highly polymorphic
- Studies with inbred strains of mice showed that genes within the MHC were also involved in controlling both humoral and cell-mediated immune responses
  - Responder/Non-responder strains

- There were three kinds of molecules encoded by the MHC
  - Class I
  - Class II
  - Class III
- Class I MHC molecules are found on <u>all nucleated</u> cells (not RBCs)
- Class II MHC molecules are found on APC
  - Dendritic cells, Macrophages, B cells, other cells





In humans:	In the Mouse:
Class I = A, B and C (also called HLA-A, HLA-B and HLA-C) - Ag (peptide) presentation to CD8+ cells	Class I = K, D and L molecules (also called H-2D, H-2K and H-2L)
Class II = DP, DQ and DR (also called HLA-DP, HLA-DQ and HLA-DR) - Ag (peptide) presentation to CD4+ cells	Class II = A and E (also called I-A and I-E)
Class III = Complement proteins, Tumor	Class III = Complement proteins, Tumor necrosis factor (TNFs)-α, β

MHC polymorphism The loci that encode class I and class II MHC molecules are the most polymorphic known in higher vertebrates. Within any species, there are many different alleles for each class I and class II MHC molecule.

Humans: HLA Class-I genes: A (240), B (470), C (110) alleles (1.2 x 107)

HLA Class-II genes: DP= DPB1 (96) alleles DQ= DQA1 (22), DQB1 (49) alleles DR= DRB1 (304), DRB1 (1), DRB1 (35), DRB1 (11), DRB1 (15) alleles 1.8 x 1011 different

Class II combinations, and (1.2 x 107) x (1.8 x 1011) = 2.25 x 1018 different combinations of Class I and Class II possible combinations

Terminology:

Haplotype: set of alleles present in each parental chromosome (two sets).

• Inbred mouse strains: same set of alleles (homozygous) at each locus (K, IA, IE, S, D).

• Inbred strains are SYNGENIC = identical at all genetic loci

• Inbred strains have been bred by brother-sister mating for > 20 generations

• Outbred mouse strains: different set of alleles at each locus ~ like humans.

• Congenic strains = genetically identical except at a single loci

Mouse Strains

• Thus, the strain C57BL/6 was designated H-2b haplotype and said to possess the ‘b’ allele at each MHC locus.

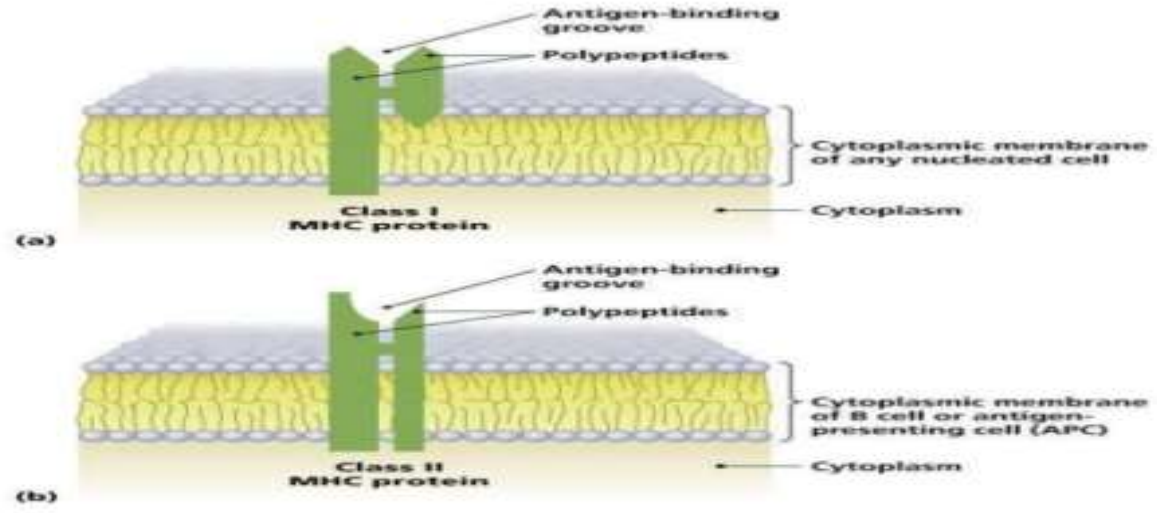
Thus, it is: H-2b = Kb , Db , Lb , I-Ab , I-Eb

• Another strain, CBA/2 was found to possess different alleles than C57BL/10 and was arbitrarily designated as having the k haplotype (I.e. H-2k).

• Thus, it is: H-2k = Kk , Dk , Lk , I-Ak , I-Ek

MHC Molecules

- Major histocompatibility antigen
  - Body cell surface proteins coded by a family of highly polymorphic genes
  - MHC class I: found on all nucleated cells
  - MHC class II: found only on APCs
- T cell receptors recognize antigenic peptide/MHC complexes
  - CD4+ T cells: restricted by class II
  - CD8+ T cells: restricted by class I



Antigen Processing

- T-independent antigen
  - Large antigen molecules with readily accessible, repeating antigenic determinants
  - B cells can bind these directly without being processed
    - Stimulates B cells to differentiate into a plasma cell and produce antibodies

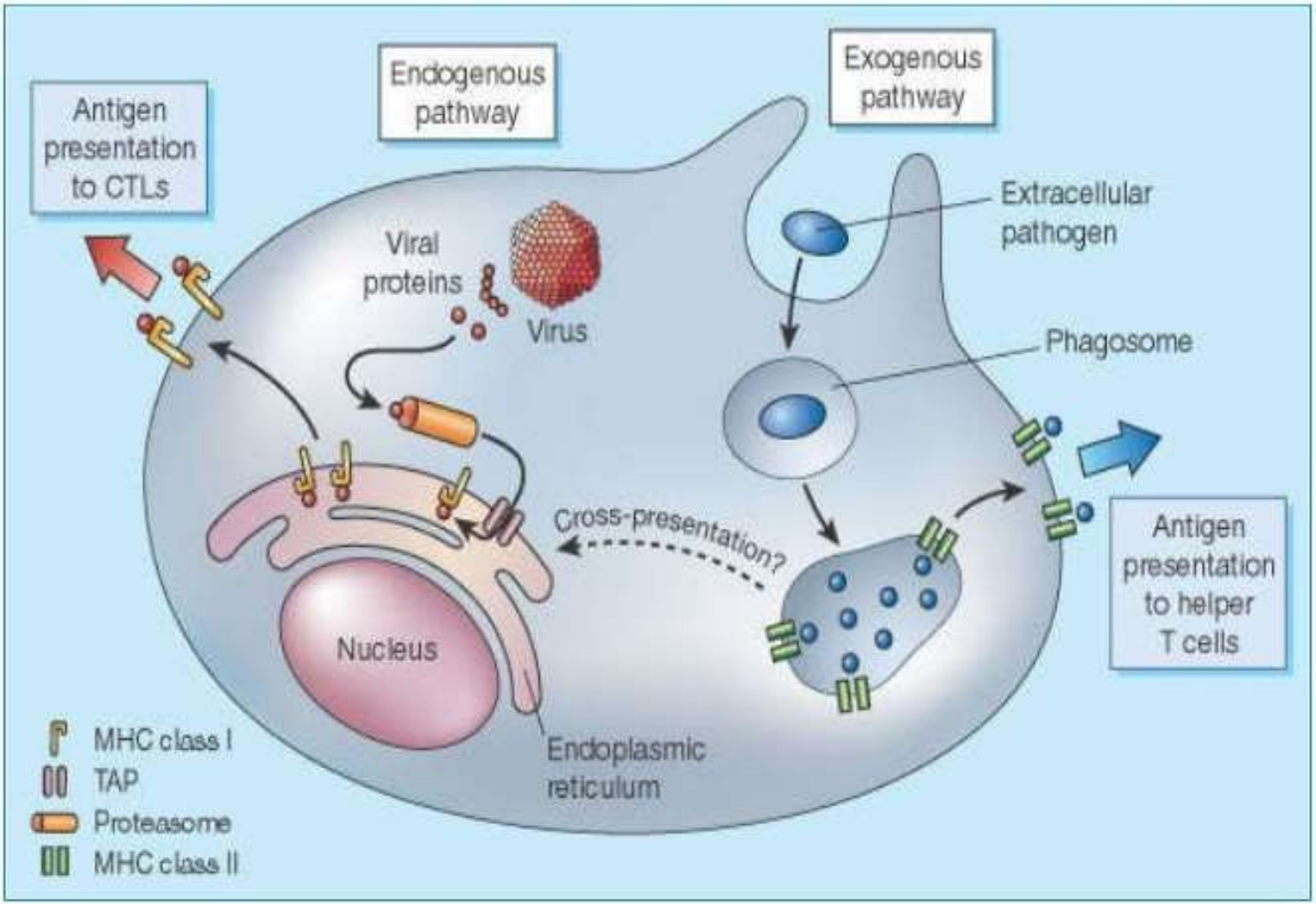
Processing of Exogenous Antigens

- APC internalizes the invading pathogen and enzymatically digests it into smaller antigenic fragments which are contained within a phagolysosome
- Phagolysosome fuses with a vesicle containing MHCII molecules
- Each fragment binds to the antigen-binding groove of a complementary MHCII molecule
- The fused vesicle then inserts the MHCII-antigen complex into the cytoplasmic membrane so the antigen is presented on the outside of the cell

Processing of Endogenous Antigens

- The intracellular pathogens are also digested into smaller antigenic determinants
- Each fragment binds to a MHCI molecule located in the endoplasmic reticulum membrane
- The membrane is packaged into a vesicle by a Golgi body which is inserted into the cytoplasmic membrane so the antigen is displayed on the cell's surface

Endogenous and Exogenous Antigen Presenting Pathways



Endogenous and Exogenous Antigen Presenting Pathways

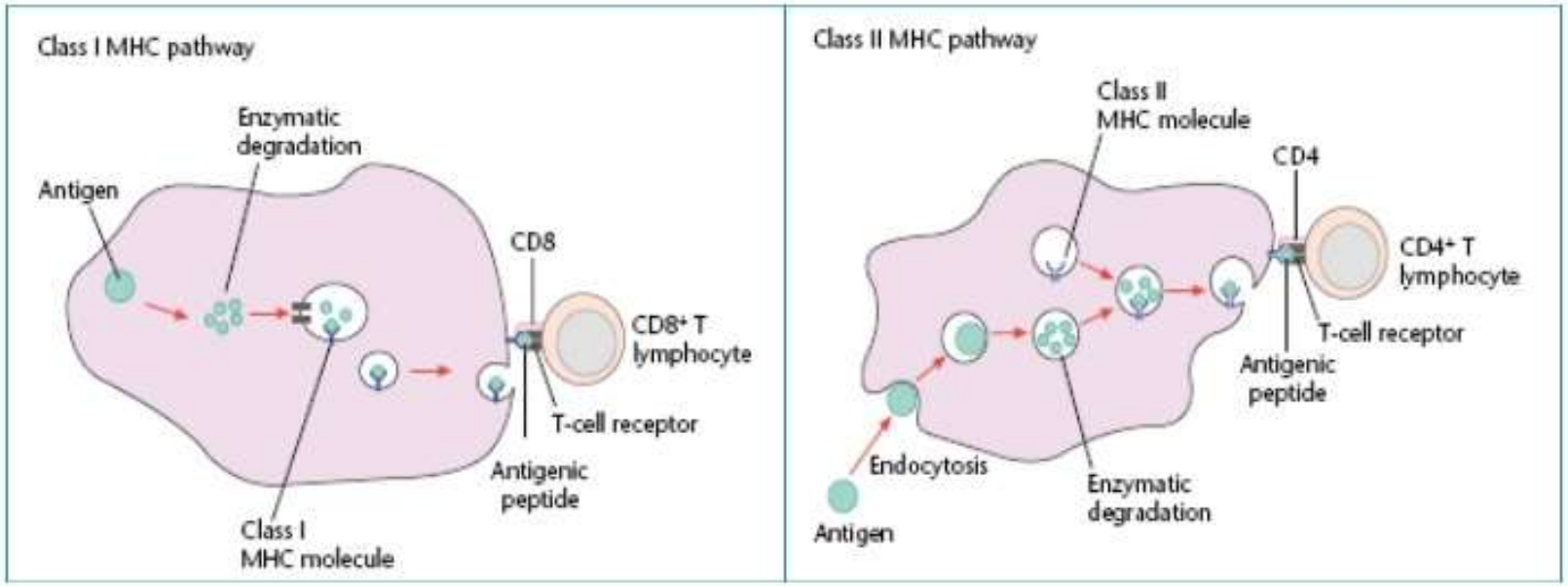
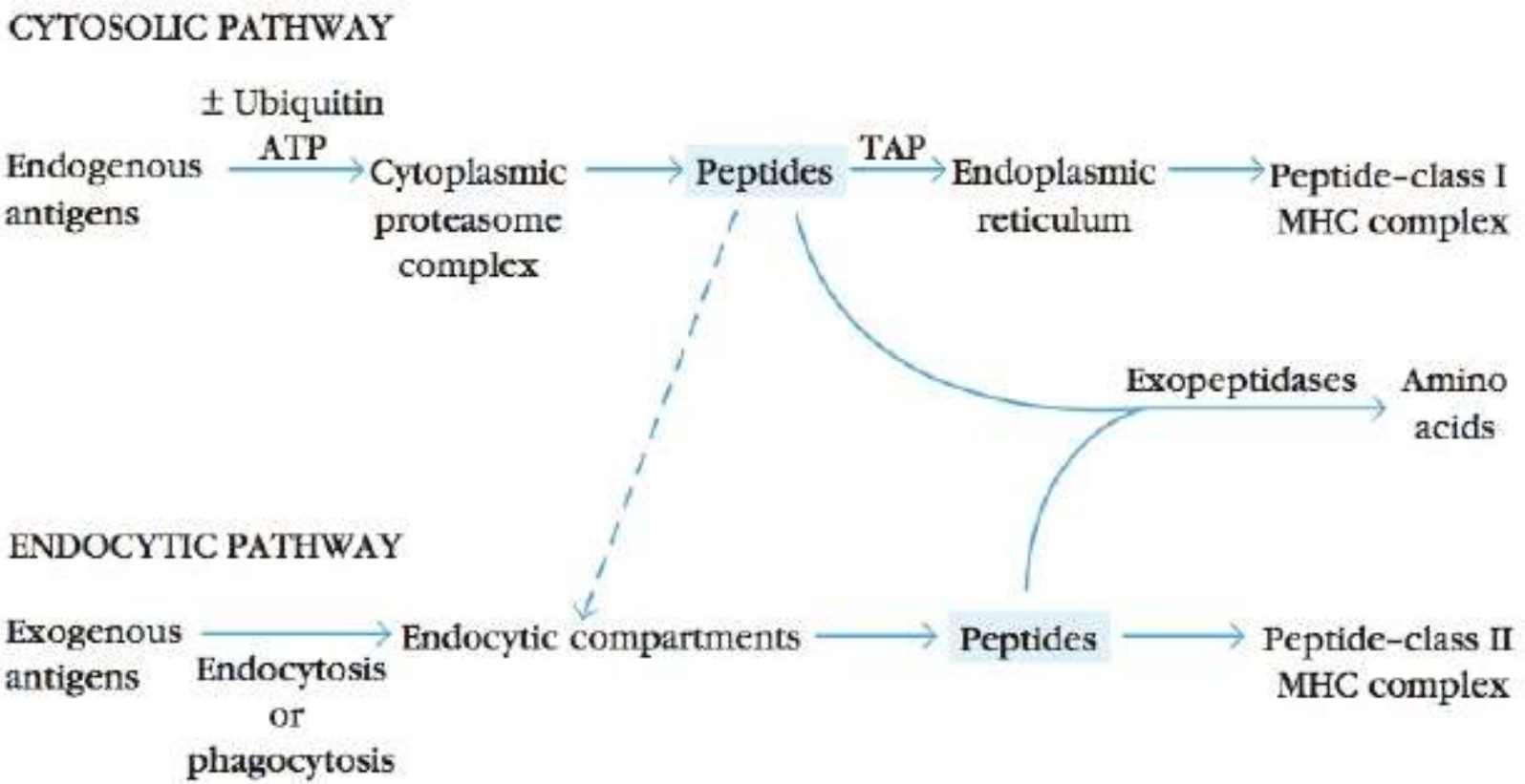


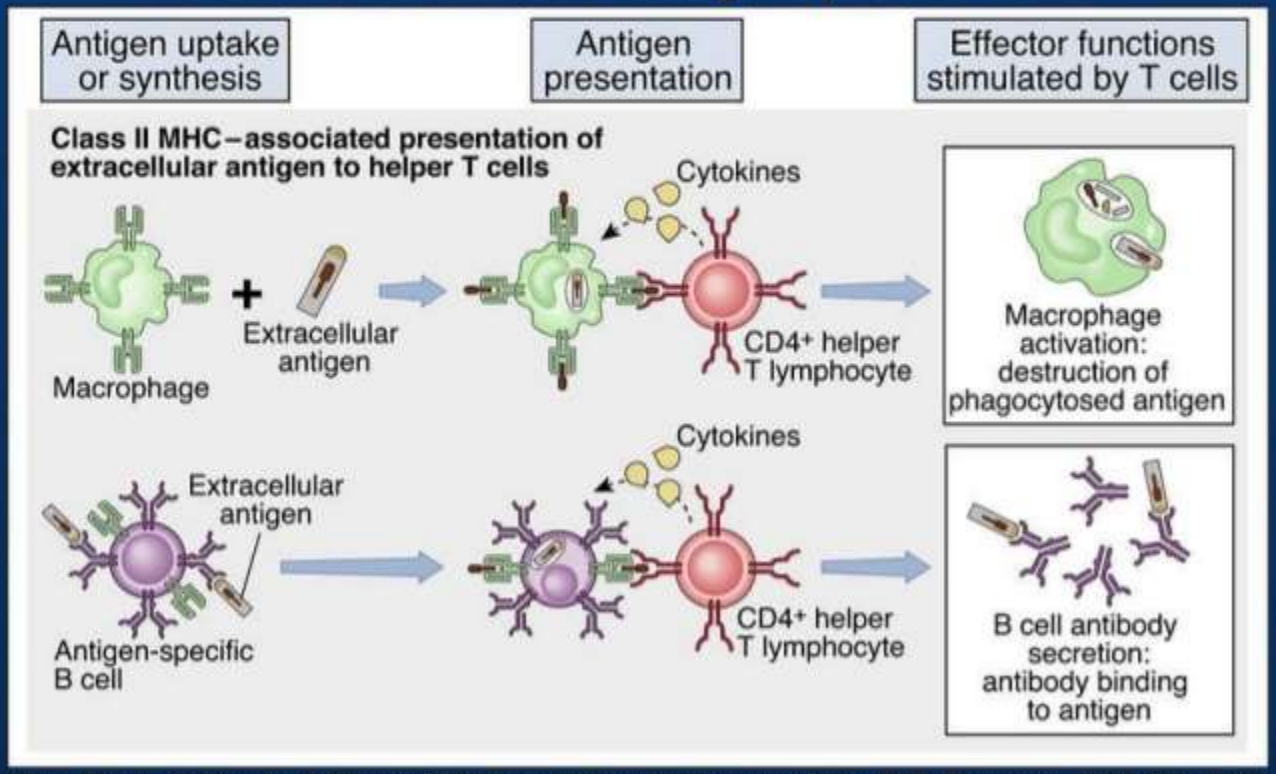
Figure 3 Antigen processing and presentation by antigen-presenting cells (APCs). Class II major histocompatibility complex (MHC) molecules are found only on all professional APCs (dendritic cells, macrophages and B lymphocytes). As a general rule, antigens ingested from outside the APC enter this pathway and are processed into peptide fragments by enzymatic degradation in endosomal compartments. These peptides are picked up by class II MHC molecules and presented to CD4 ('helper') T lymphocytes. In contrast, class I MHC molecules are found on all nucleated cells in the body (including APCs). These molecules present processed peptides to CD8 (cytotoxic) T lymphocytes (CTLs), and antigens presented by this pathway usually originate from within the cell (as is the case in viral infection) and are processed in the cytosol. By this means, all cells have the capacity to be recognized and killed by CTLs should they become infected. There is evidence that dendritic cells can also load class I from antigen derived from outside the cell.

EVIDENCE FOR TWO PROCESSING AND PRESENTATION PATHWAYS



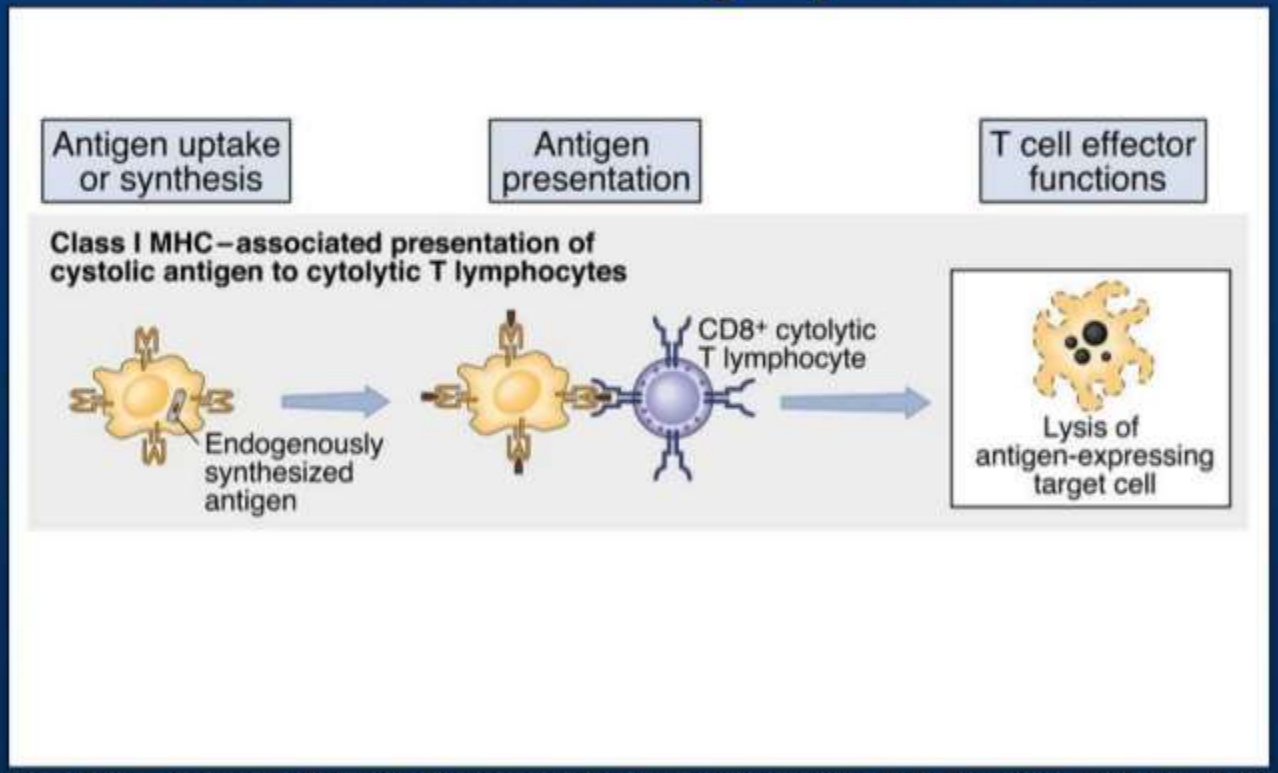
Slide 5-17

Physiologic significance of class II-associated antigen presentation

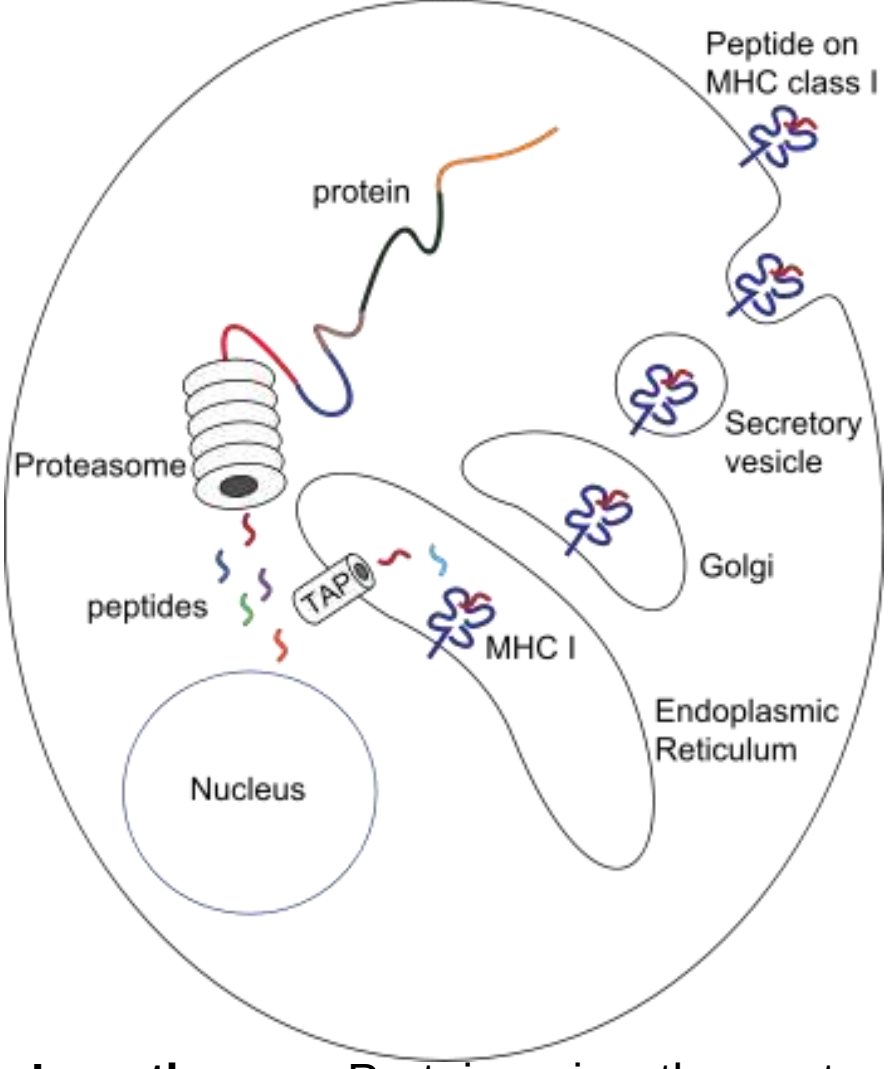


From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 5-14a

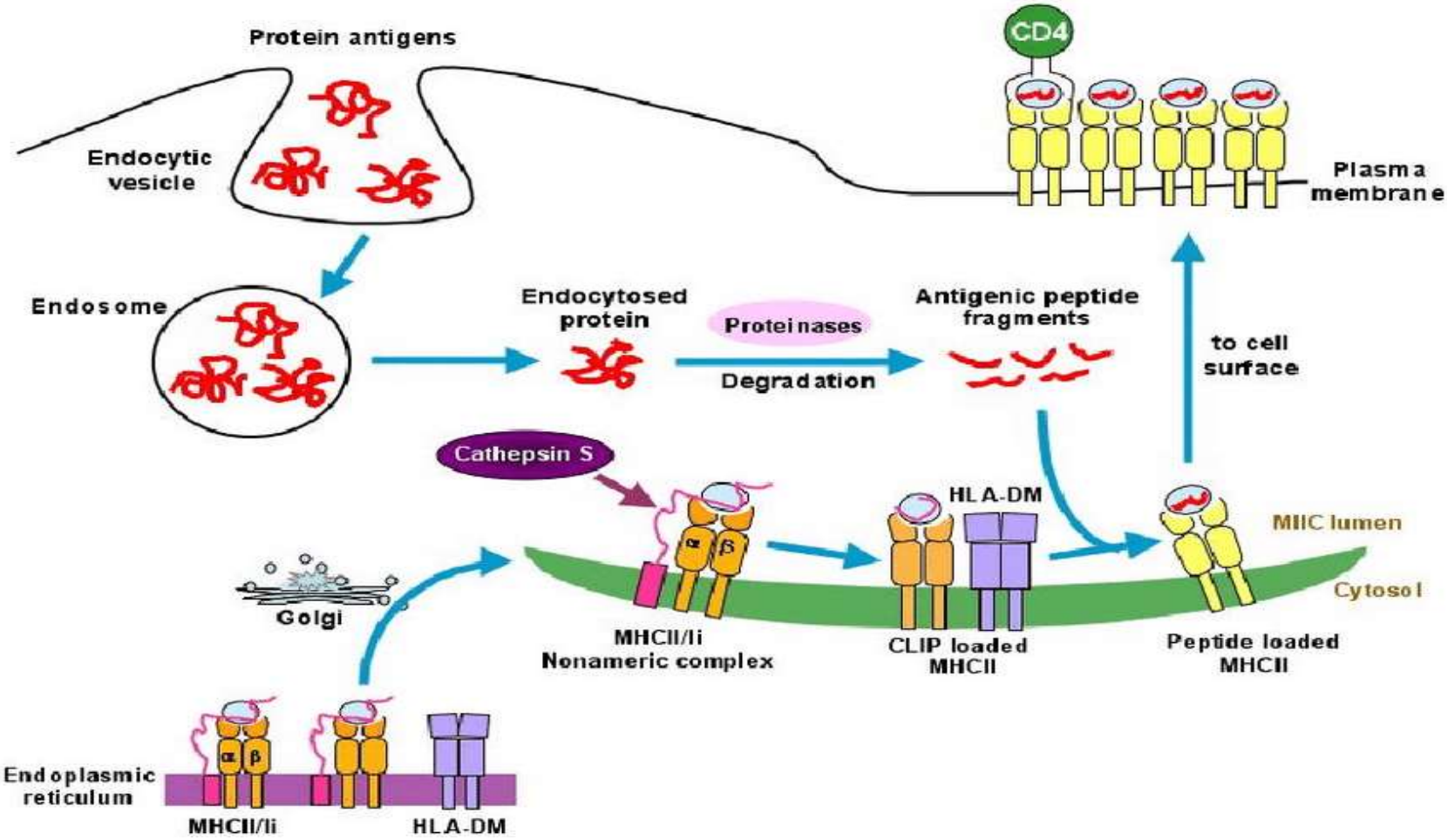
Physiologic significance of class I-associated antigen presentation



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 5-14b



MHC class I pathway: Proteins in the cytosol are degraded by the proteasome, liberating peptides internalized by TAP channel in the endoplasmic reticulum, there associating with MHC-I molecules freshly synthesized. MHC-I/peptide complexes enter Golgi apparatus, are glycosylated, enter secretory vesicles, fuse with the cell membrane, and externalize on the cell membrane interacting with T lymphocytes.



MHCII are assembled as dimmers in the endoplasmic reticulum with help of the specialized chaperone invariant chain (Ii), which in addition occupies the peptide-binding groove. The MHCII/Ii complexes form a nanomeric complex that is transported to the MHCII containing compartments (MIIC). Here the invariant chain is degraded by cathepsins such as cathepsin S until only the part occupying the peptide-binding grove is left, which is called CLIP. In these compartments, MHCII also encounters antigenic peptide fragments derived from proteins degraded in the endocytic track. CLIP is then exchanged for one of these fragments with help of chaperone HLA-DM, and the peptide-loaded MHCII is transported to plasma membrane for presentation to the immune system.

Privileged Sites

- Sites at which grafts are not likely to be rejected
- Different sites are privileged for different reasons
  - The brain lacks lymphatic vessels, and its blood vessel walls are impermeable to lymphocytes such as T cells
  - Cornea lacks extensive blood vessels
  - Eyes and testes contain naturally high levels of immunosuppressive molecules
  - Other sites either lack dendritic cells or express low levels of MHC molecules, so antigen processing does not occur

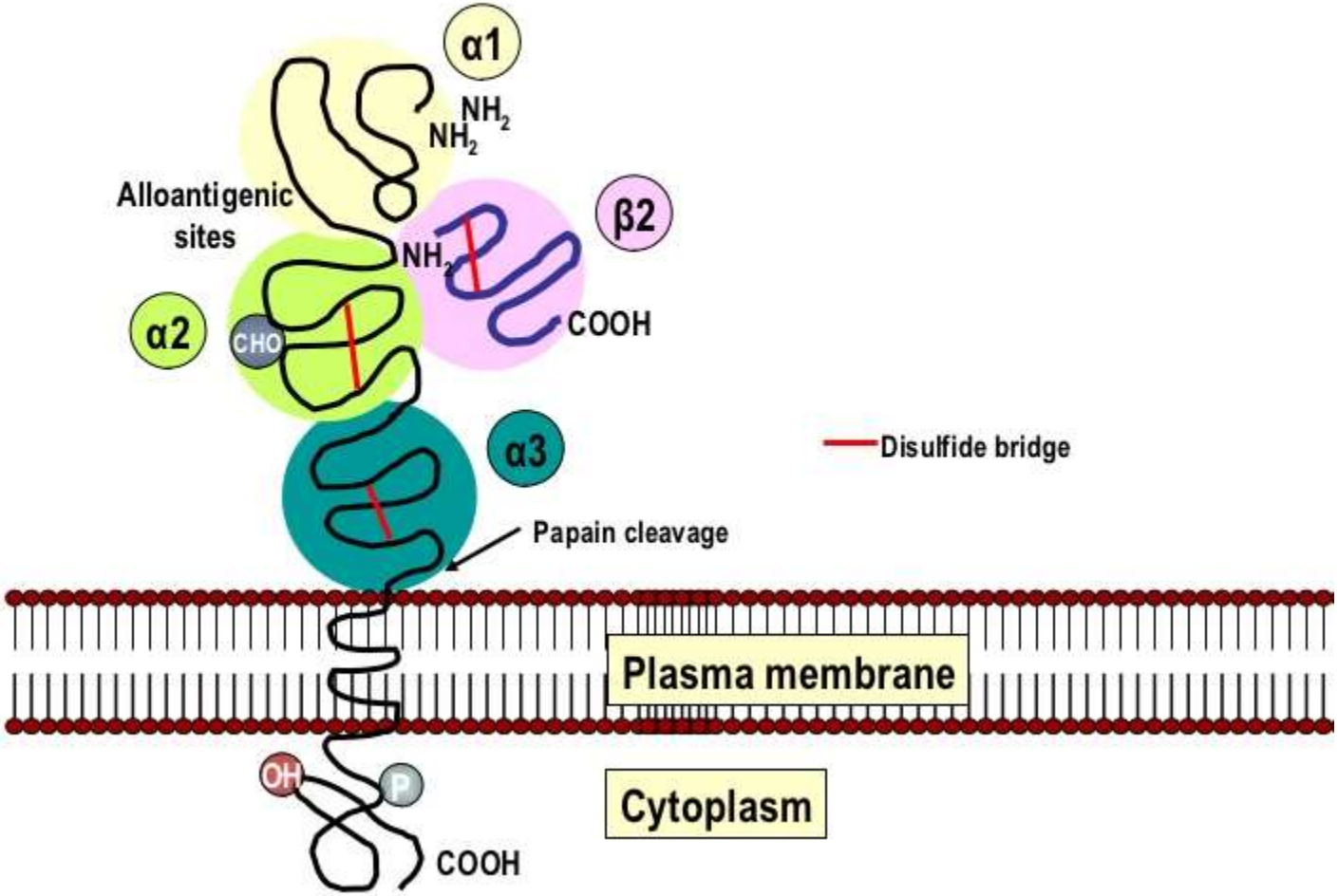
Why Fetuses are Not Rejected

- The fetus is not a privileged site but is not rejected
- Rejection is prevented by the many different immunosuppressive mechanisms
  - Early embryos do not express MHC class I and II molecules on the placental layer that is in contact with maternal tissues
  - Cytokines that enhance MHC expression have no effect on placental cells
  - T cells are prevented from functioning in the placenta to reject the fetus

Immune Evasion Examples

Mycobacteria	: Inhibits phagolysosome fusion so that it survives within the phagosome
Herpes simplex virus	: Interferes with TAP transporter (inhibits antigen presentation)
Cytomegalovirus	: Inhibits proteasome activity and removal of MHC I from ER
Epstein-Barr virus	: Inhibits proteasome activity; produces IL-10 to inhibit macrophage activation
Pox virus	: Produces soluble cytokine receptors to inhibit activation of effector cells

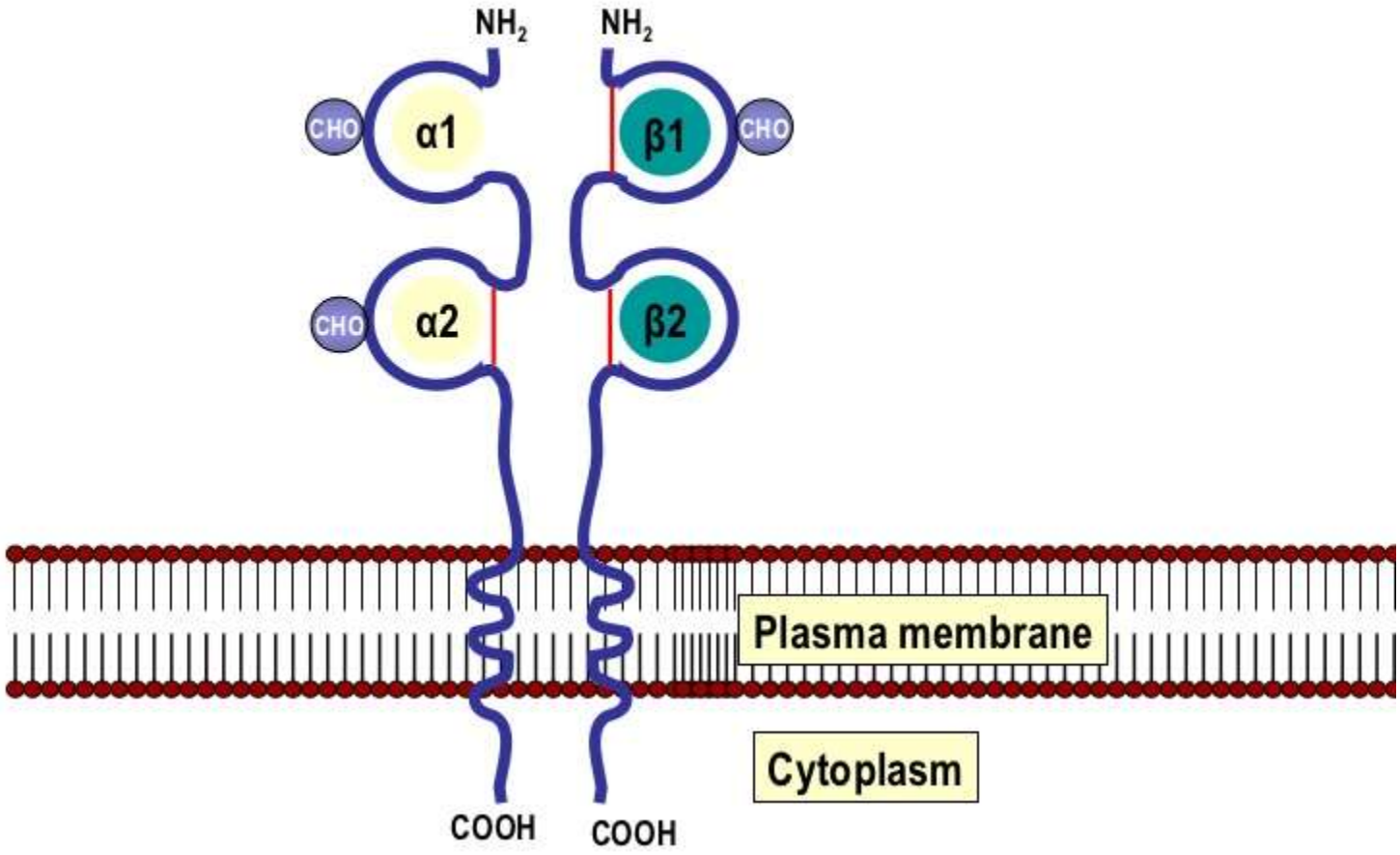
Structure of Class I MHC



Structure of Class I MHC Peptide-binding Region

- a “groove” composed of an α-helix on two opposite walls and eight β-pleated sheets forming the floor
- residues lining groove most polymorphic
- peptide in groove 8-10 amino acids long
- specific amino acid on peptide required for “anchor site” in groove

Structure of Class II MHC



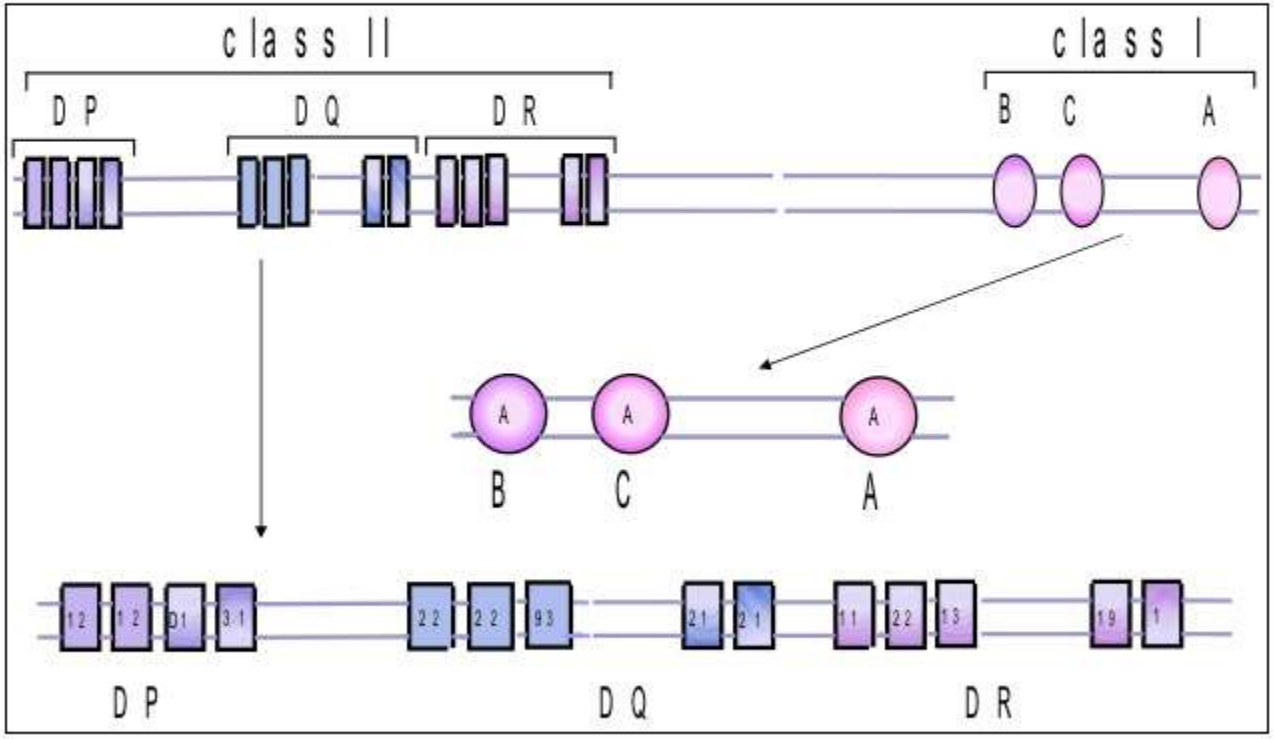
Structure of Class II MHC

- Two polypeptide chains, α and β, of roughly equal length.
2. Peptide-binding region – a groove formed from the α1 and β1 domains of the α and β chains – site of polymorphism
3. Immunoglobulin-like region – conserved α2 and β2 domains – β2 is site to which CD4 on T cell binds

Peptide-binding grooves for class I and class II MHC are structurally similar

- Both have a peptide-binding groove with a wall of two α helices and a floor of eight β-pleated sheets
- Close-ended groove for class I MHC requires an 8-10 amino acid-length peptide to bind; open-ended groove for Class II MHC lets it bind a peptide 13-25 amino acids long, not all of which lie in the groove
- Anchor site rules apply to both classes

The human MHC genes



Class I polymorphism

Locus	Number of alleles (allotypes)
HLA - A	218
HLA - B	439
HLA - C	96
There are also HLA - E, HLA - F and HLA - G	Relatively few alleles

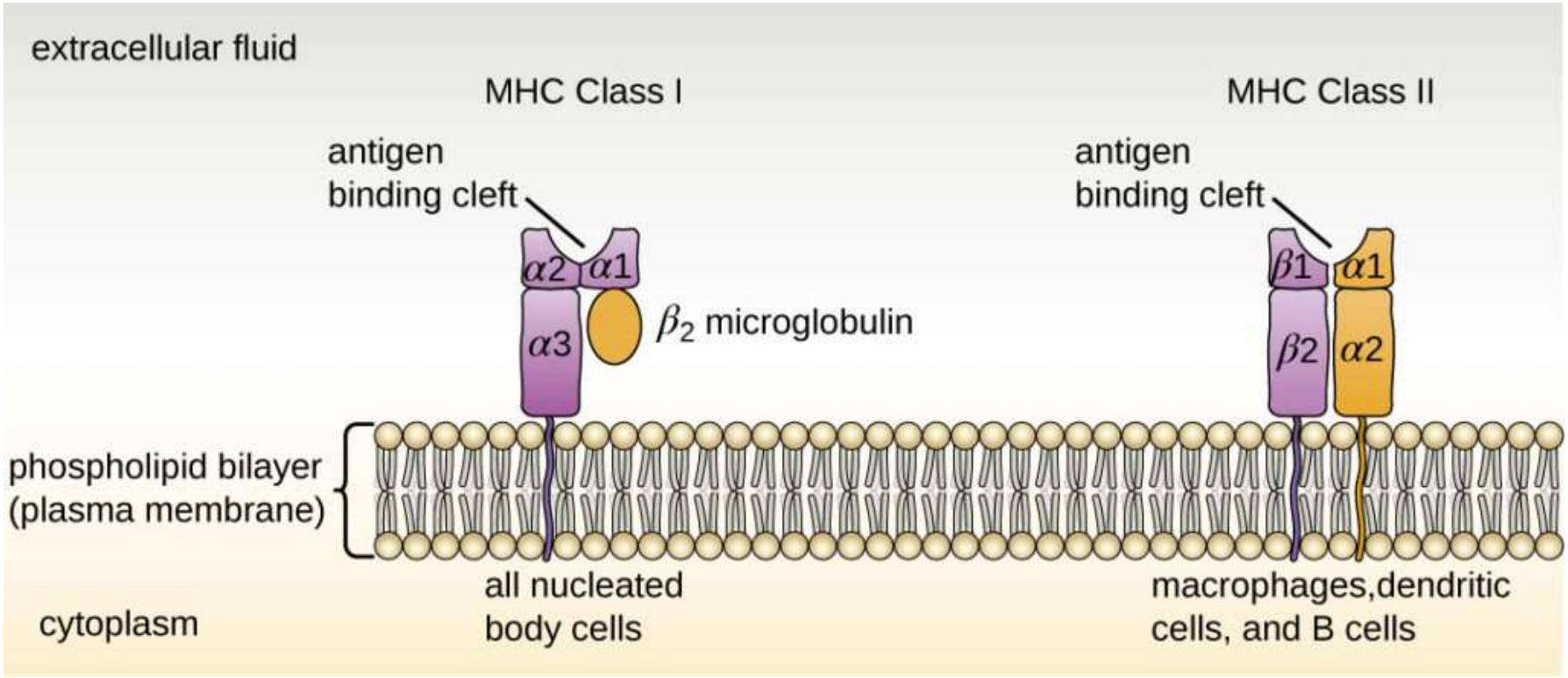
Class II polymorphism

Locus	Number of alleles (allotypes)
HLA - DP_A	12
HLA - DP_B	88
HLA - DQ_A	17
HLA - DQ_B	42
HLA - DR_A	2
HLA - DR_{B1}	269
HLA – DR_{B3}	30
HLA – DR_{B4}	7
HLA – DR_{B5}	12
There are also HLA - DM and HLA - DO	Relatively few alleles

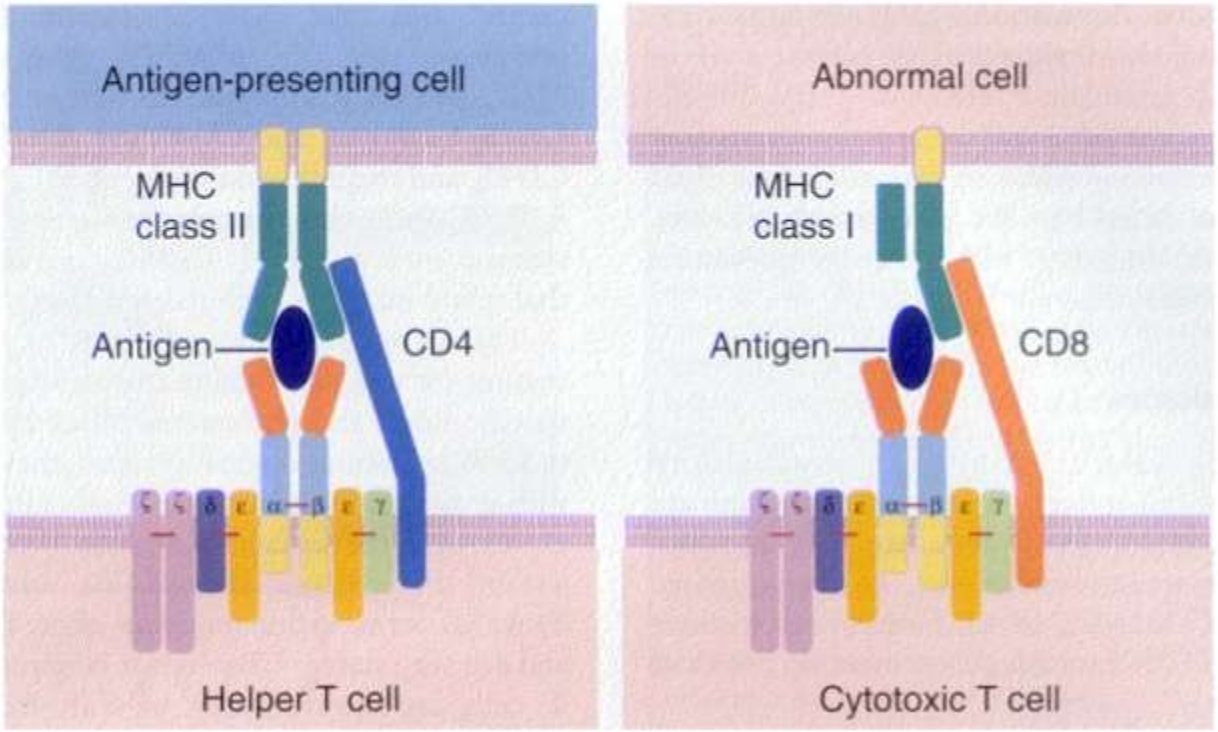
Comparison: MHC Class I and II Structure

TABLE 7-1
A Comparison of MHC Class I and Class II Structure

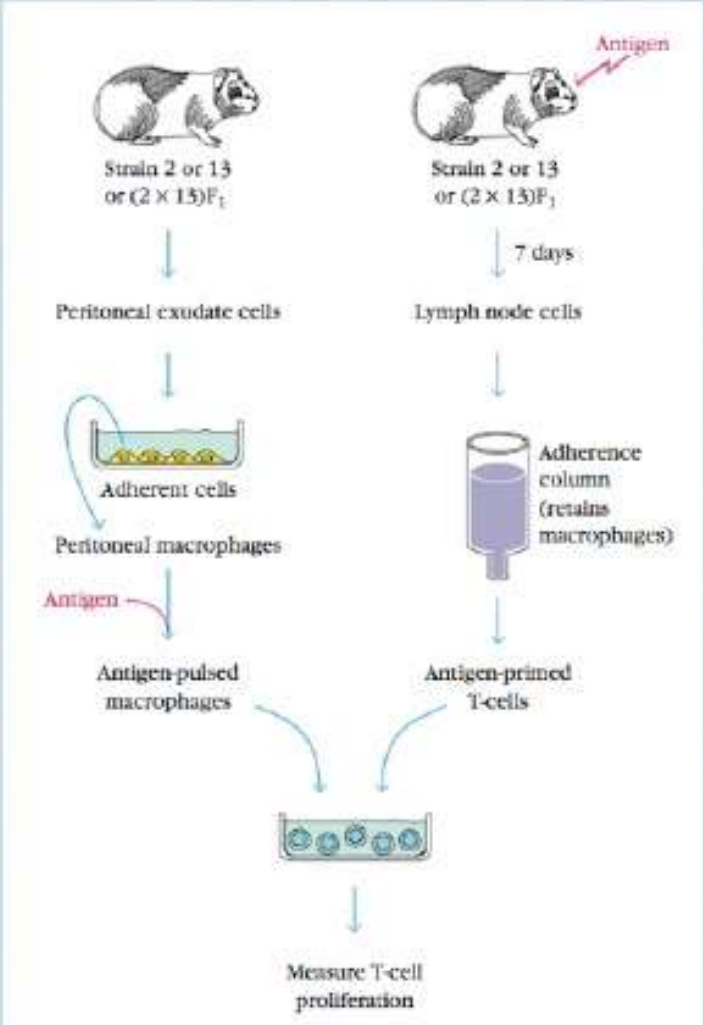
	Class I	Class II
Loci include	Typically A, B, and C	DP, DQ, and DR
Distribution	Most nucleated cells	B cells, macrophages, and dendritic cells
Function	Present antigen to cytotoxic T cells	Present antigen to T helper cells
Result	T-cell-mediated toxicity	T-cell-mediated help


extracellular fluid
MHC Class I
MHC Class II
antigen
binding cleft
antigen
binding cleft
α2
α1
α3
β2 microglobulin
β1
α1
β2
α2
phospholipid bilayer
(plasma membrane)
cytoplasm
all nucleated
body cells
macrophages,dendritic
cells, and B cells

Role of CD4 and CD8 in promoting T-cell responses



Experiment 1: Rosenthal and Shevach



- Showed that ***"antigen-specific proliferation of TH cells occurred only in response to antigen presented by macrophages of the same MHC haplotype as the T cells"***

- Guinea pig macrophages from strain 2 were initially incubated with an antigen

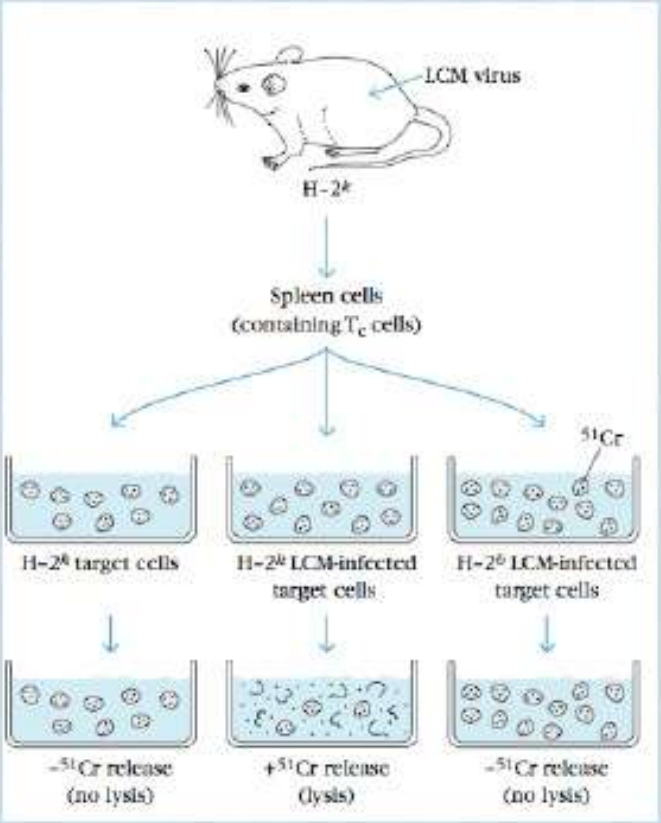
- After the "antigen-pulsed" macrophages had processed the antigen and presented it on their surface, they were mixed with T cells from the same strain (strain 2), a different strain (strain 13), or (2 x 13) F1 animals, and the magnitude of T-cell proliferation in response to the antigen-pulsed macrophages was measured

Antigen-primed T cell	Antigen-pulsed macrophages		
	Strain 2	Strain 13	$(2 \times 13)F_1$
Strain 2	+	−	+
Strain 13	−	+	+
$(2 \times 13)F_1$	+	+	+

- Results showed that strain-2 antigen-pulsed macrophages activated strain-2 and F1 T-cells but not strain-13 T-cells
- Similarly, strain-13 antigen-pulsed macrophages activated strain-13 and F1 T-cells but not strain-2 T-cells
- Subsequently, congenic and recombinant congenic strains of mice, which ***differed from each other only in selected regions of the H-2 complex***, were used as the source of macrophages and T cells

- These experiments ***confirmed that the CD4+TH cell is activated and proliferates*** only in the presence of antigen-pulsed macrophages that share ***class II MHC alleles***
- ***THUS: antigen recognition by the CD4+Tcell is class II MHC restricted***

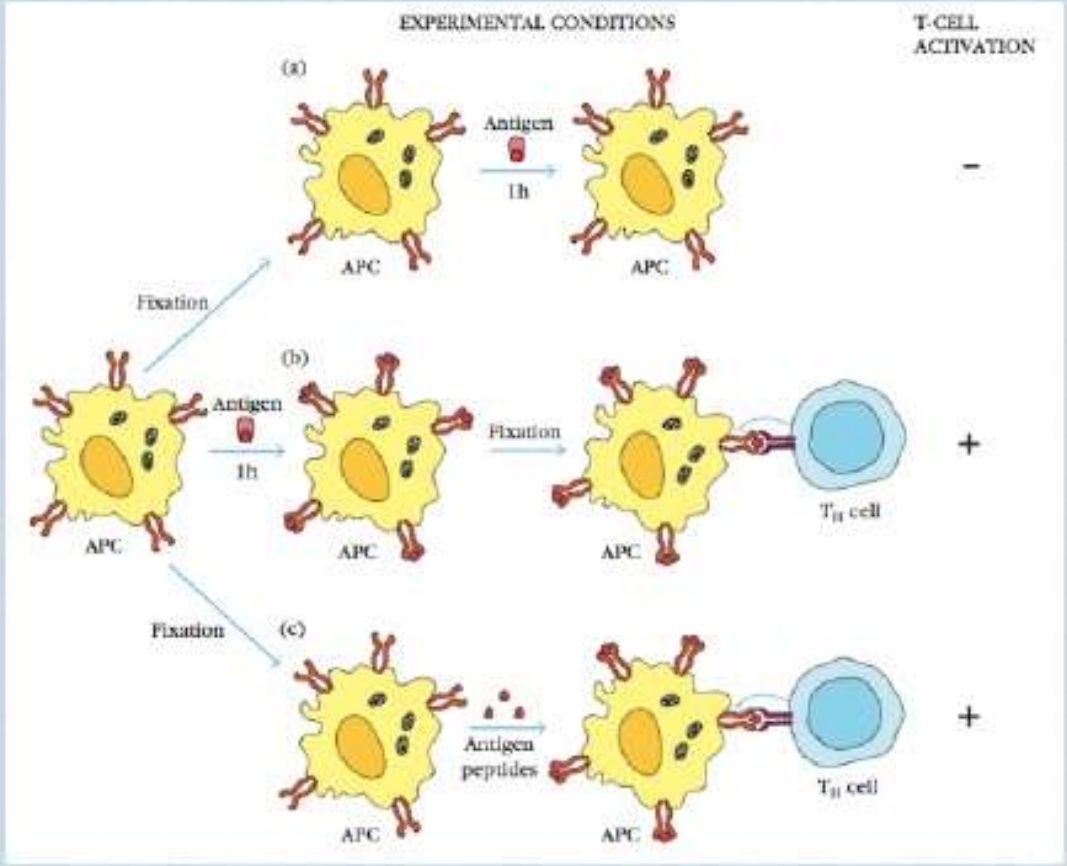
Experiment 2: Zinkernagel & Doherty



- ***In 1996, Doherty and Zinkernagel were awarded the Nobel prize for their major contribution to the understanding of cell-mediated immunity***

- ***demonstrated the self-MHC restriction of CD8+ T-cells***
- Mice were immunized with lymphocytic choriomeningitis (LCM) virus
- Several days later, the animals' spleen cells, which included TC-cells specific for the virus, were isolated and incubated with LCM-infected target cells of the same or different haplotype
- Found that the TC-cells killed only syngeneic virus-infected target cells
- Later studies with congenic and recombinant congenic strains showed that the TC-cell and the virus-infected target cell must share class I molecules encoded by the K or D regions of the MHC
- ***THUS : antigen recognition by CD8+TC cells is class I MHC restricted demonstrated the self-MHC restriction of CD8+ T-cells***

Experiment 3: Ziegler and Unanue



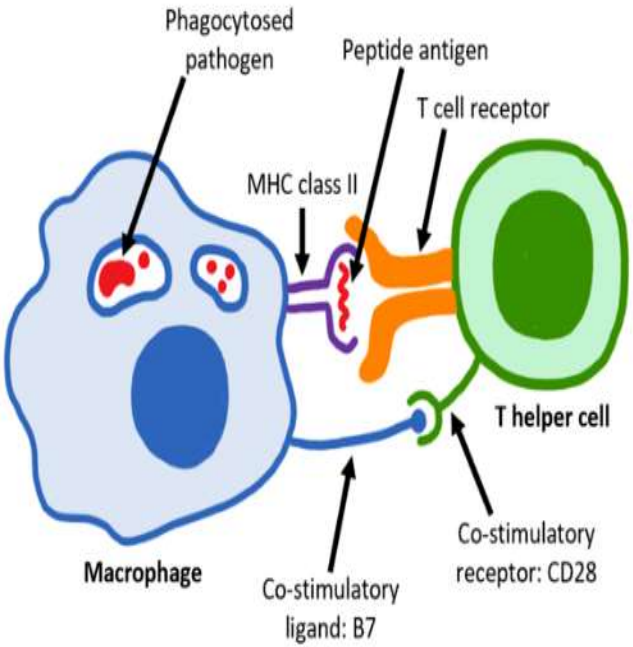
- ***During that 1-3 h interval, the APC had processed the antigen and had displayed it on the membrane in a form able to activate T cells (figure a and b)***

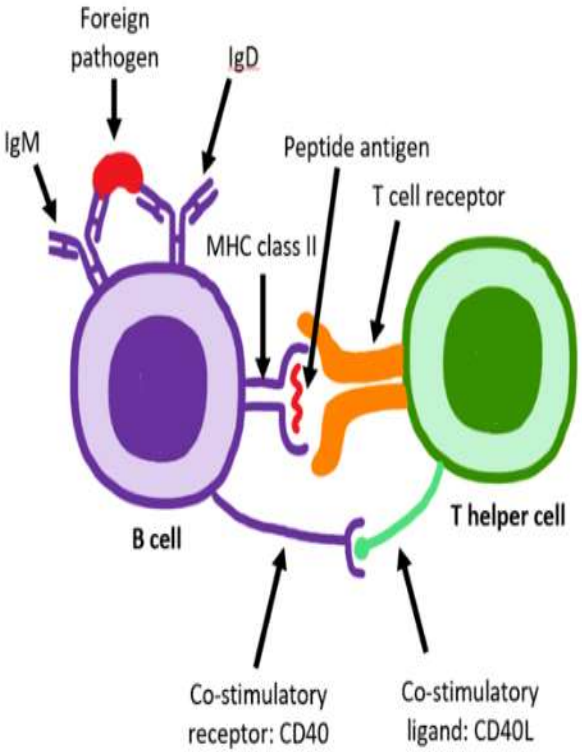
- ***CONTRADICTED*** that antigen recognition by B cells and T cells was ***similar***

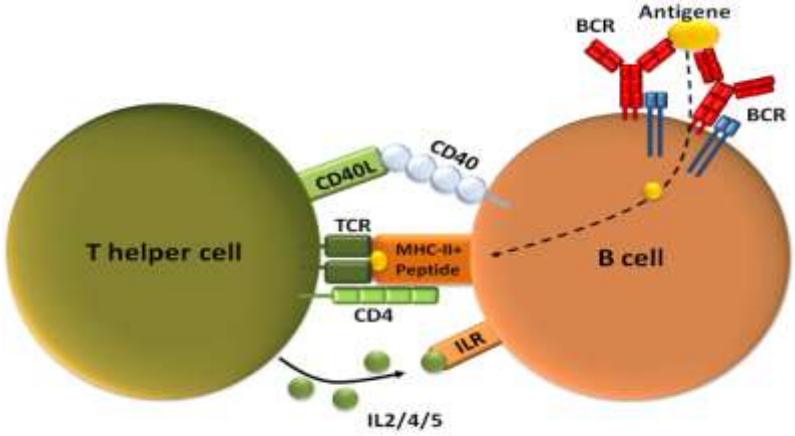
- observed that Th cell activation by bacterial protein antigens was prevented by treating the antigen presenting cells with paraformaldehyde prior to antigen exposure

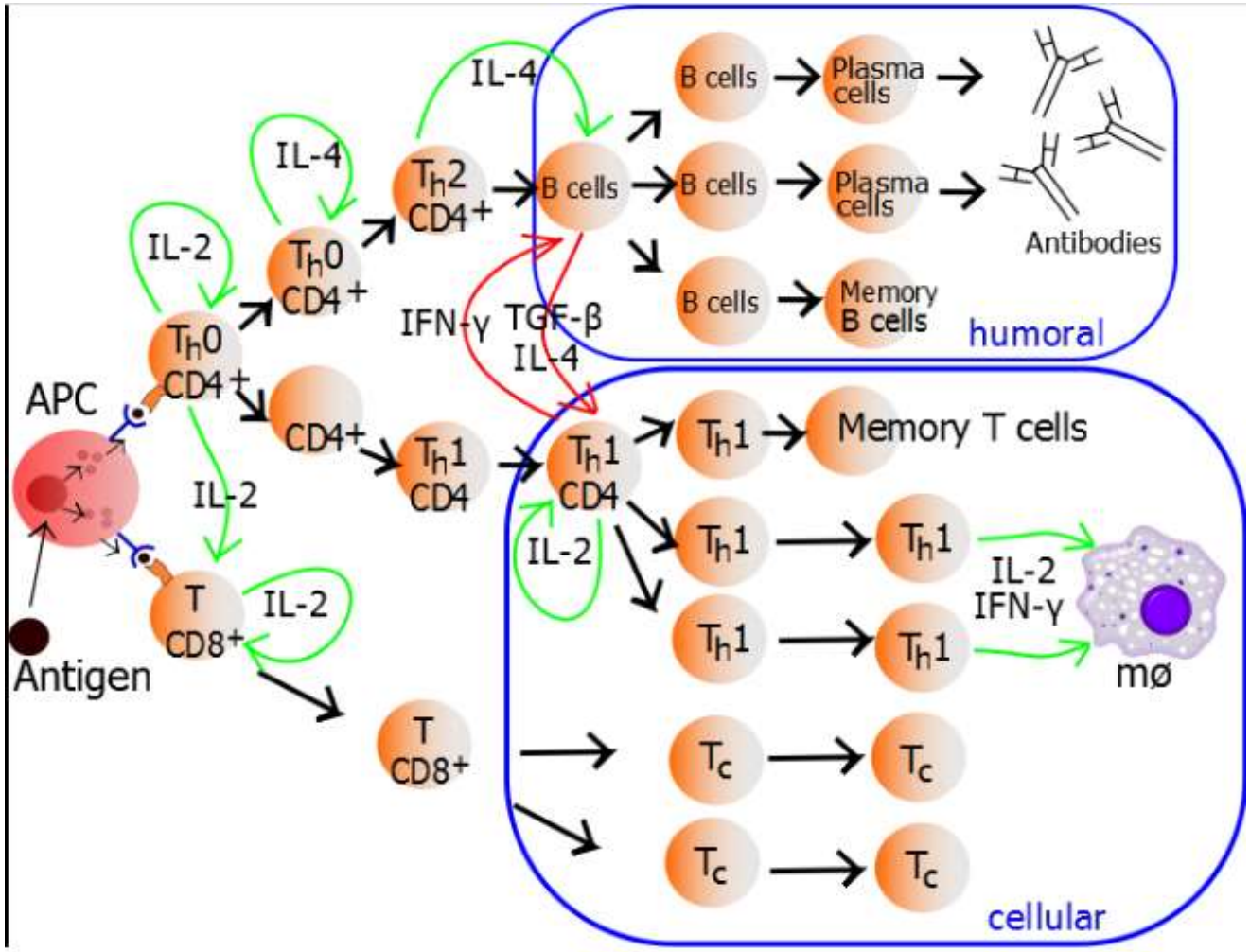
- however if the APC were first allowed to ingest the antigen and were fixed with paraformaldehyde 1-3 hours later, Th cell activation still occurred

- APCs were treated with glutaraldehyde and then incubated with native ovalbumin or with ovalbumin that had been subjected to partial enzymatic digestion
  - ***NOTE: fixation***
- the digested ovalbumin was able to interact with the treated APCs thereby activating ovalbumin-specific Th cells
  - whereas the native ovalbumin failed to do so
- ***suggests that antigen processing involved digestion of the protein into peptides that are recognized by the ovalbumin-specific Th cells***


Phagocytosed pathogen
Peptide antigen
T cell receptor
MHC class II
T helper cell
Macrophage
Co-stimulatory ligand: B7
Co-stimulatory receptor: CD28


Foreign pathogen
IgD
IgM
Peptide antigen
T cell receptor
MHC class II
B cell
T helper cell
Co-stimulatory receptor: CD40
Co-stimulatory ligand: CD40L


BCR
Antigene
BCR
CD40L
CD40
TCR
MHC-II+ Peptide
T helper cell
B cell
CD4
ILR
IL2/4/5

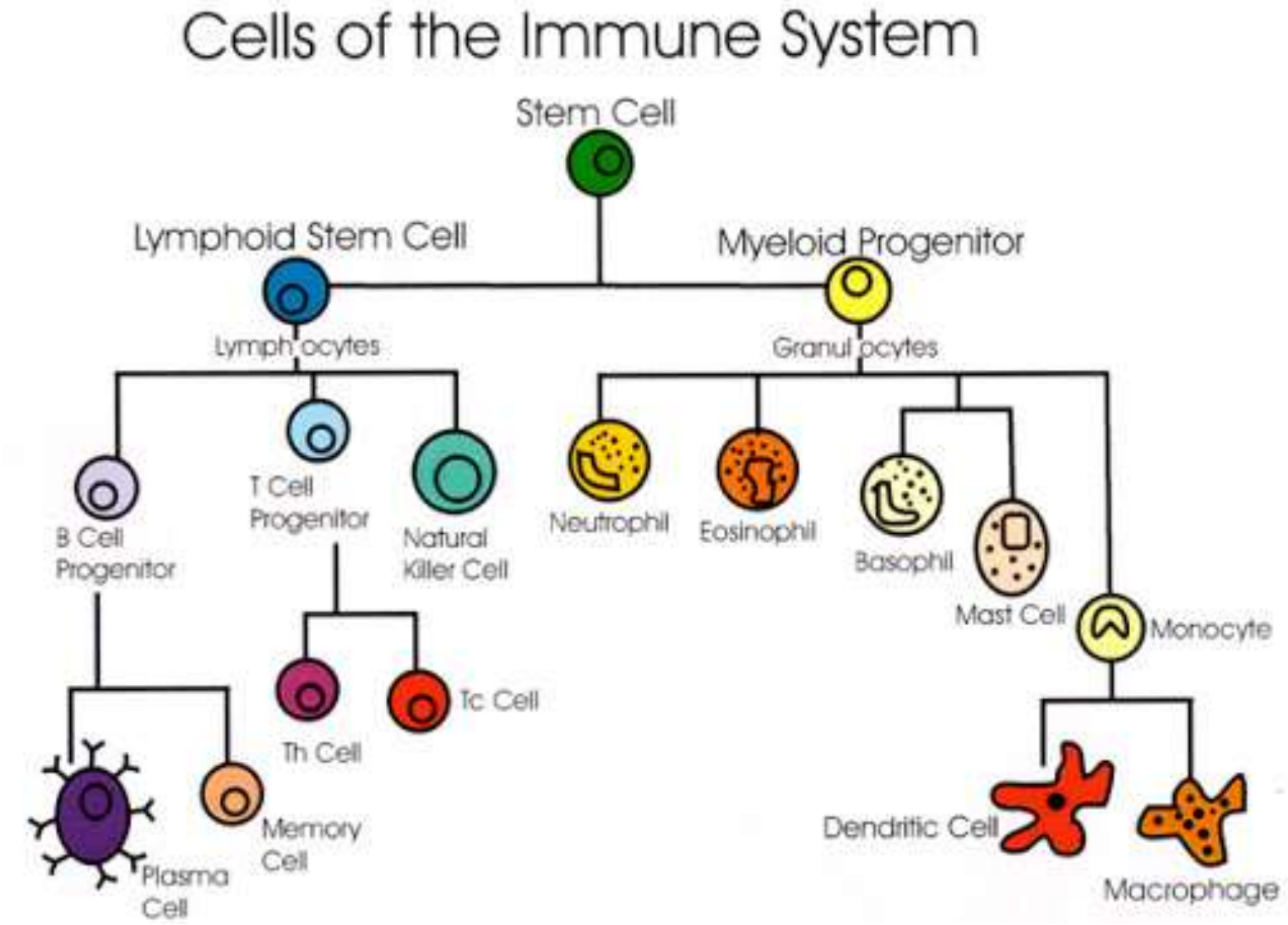


T_h1/T_h2 Model for helper T cells. An antigen is ingested and processed by an APC. It presents fragments from it to T cells. The upper, T_h0, is a T helper cell. The fragment is presented to it by MHC2. IFN-γ, interferon γ; TGF-β, transforming growth factor β; mø, macrophage; IL-2, interleukin 2; IL-4, interleukin 4

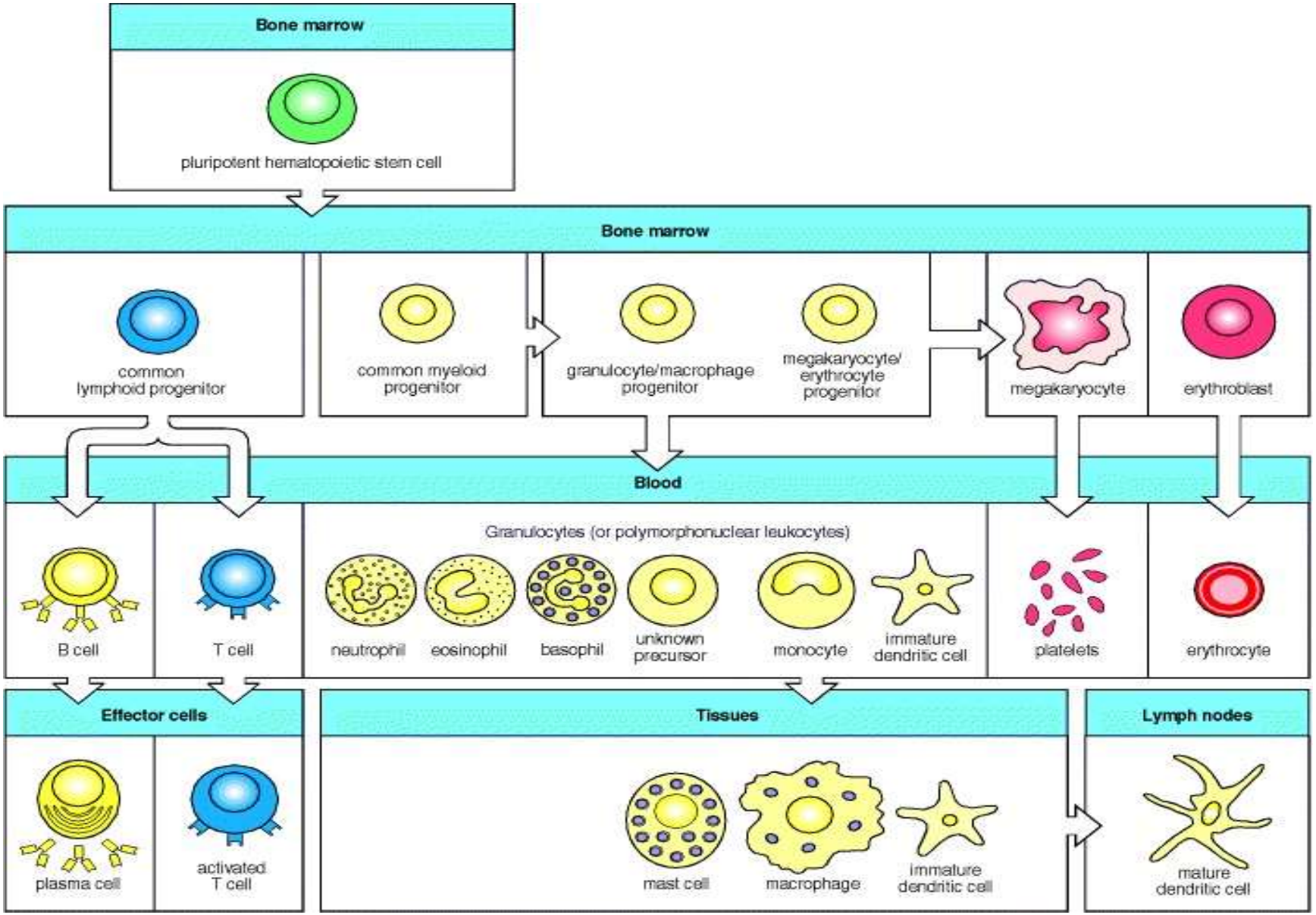
Immune cells : origin and functions

(All Graphics are collected from Internet)

The **cells** of the **immune system** originate in the bone marrow, where many of them also mature. They then migrate to guard the peripheral tissues, circulating in the blood and in a specialized **system** of vessels called the lymphatic **system**.



All the cellular elements of blood, including the lymphocytes of the adaptive immune system, arise from hematopoietic stem cells in the bone marrow



there are two major subsystems of the immune system: **the innate immune system and the adaptive immune system**. Both subsystems use humoral immunity and cell-mediated immunity to perform their functions.

Immune System

Innate (inborn or non-specific) Immune System

First line of defense

Prevent microbial attachment, colonization and entry

Disposes pathogens rapidly and nonspecifically if they can e

Adaptive (acquired or specific) Immune System

Second line of defense when the innate system fails

Devotes specialized sets of cells for each pathogen

to identify, mark for disposal and retaining memory for future

Adaptive Immunity

Adaptive immune system has two arms

Adaptive Immunity

Humoral Immunity

- Provided by B lymphocytes
- Can recognize protein, polysaccharide, phospholipid and nucleic acid antigens
- Can act against soluble or free antigens
- Provides immunity to extracellular bacteria, viruses and toxins
- Causes Type I, II & III hypersensitivity

Cell mediated Immunity

- Provided by T lymphocytes
- Can recognize only protein antigens
- Recognizes antigens presented by APCs with Class I or Class II MHC molecule
- Provides immunity to intracellular bacteria, viruses, fungi and protozoa
- Causes Type IV hypersensitivity
- Causes acute graft rejection

Cooperation between Innate and Adaptive Immunity

- Adaptive immunity is not independent of innate immunity
- The phagocytic cells crucial to nonspecific immune responses are intimately involved in activating the specific immune response
- Various soluble factors produced by a specific immune response have been shown to augment the activity of these phagocytic cells
- Through the carefully regulated interplay of adaptive and innate immunity, the two systems work together to eliminate a foreign invader

Components of the immune system	
Innate immune system	Adaptive immune system
Response is non-specific	Pathogen and antigen specific response
Composed of leukocytes	Composed of antigens, B cells, T cells
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

Overview of Innate Immunity

- First line of defense against microbes
- Exist before exposure to microbes
- Found in Plants, Insects, Vertebrates
- *3 Essential function*
  1.) Initial response to microbes
  2.) Eliminate damaged cells and initiate the process of tissue repair
  3.) Stimulates adaptive immune response
- *2 Major type of responses*
  1.) Inflammation
  2.) Antiviral defense

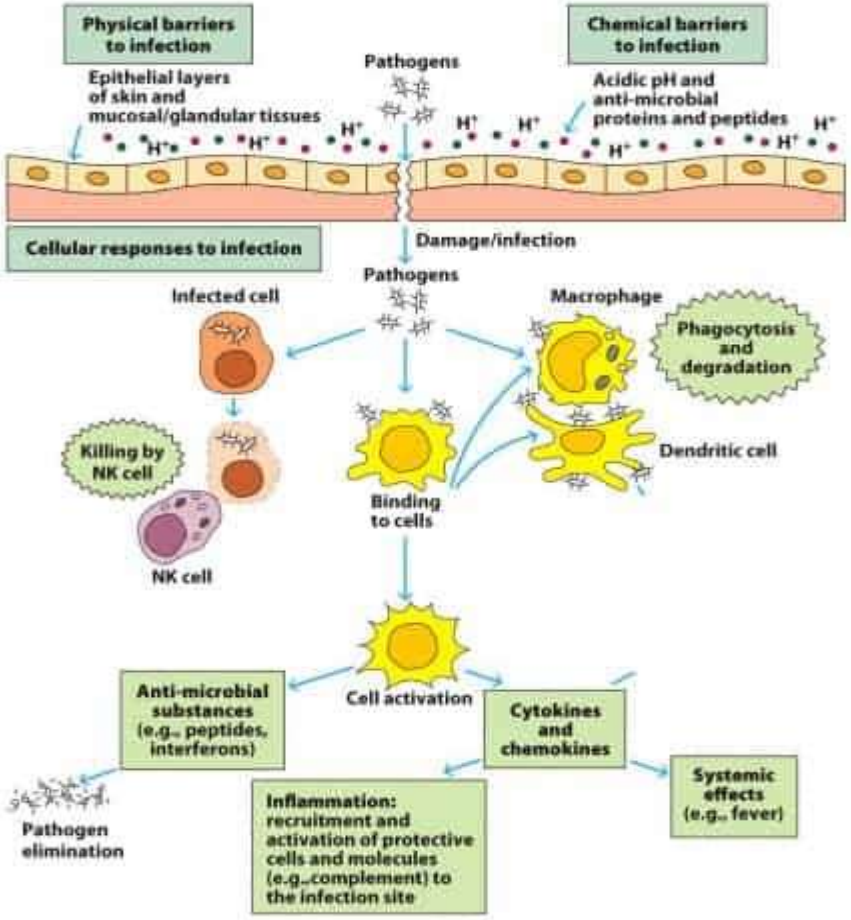
Kuby Immunology 7th edition.

Innate VS Adaptive

TABLE 5-1 Innate and adaptive immunity

Attribute	Innate immunity	Adaptive immunity
Response time	Minutes/hours	Days
Specificity	Specific for molecules and molecular patterns associated with pathogens and molecules produced by dead/damaged cells	Highly specific; discriminates between even minor differences in molecular structure of microbial or nonmicrobial molecules
Diversity	A limited number of conserved, germ line–encoded receptors	Highly diverse; a very large number of receptors arising from genetic recombination of receptor genes in each individual
Memory responses	Some (observed in invertebrate innate responses and mouse/human NK cells)	Persistent memory, with faster response of greater magnitude on subsequent exposure
Self/nonself discrimination	Perfect; no microbe-specific self/nonself patterns in host	Very good; occasional failures of discrimination result in autoimmune disease
Soluble components of blood	Many antimicrobial peptides, proteins, and other mediators	Antibodies and cytokines
Major cell types	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, other leukocytes, epithelial and endothelial cells	T cells, B cells, antigen-presenting cells

Component of innate immunity

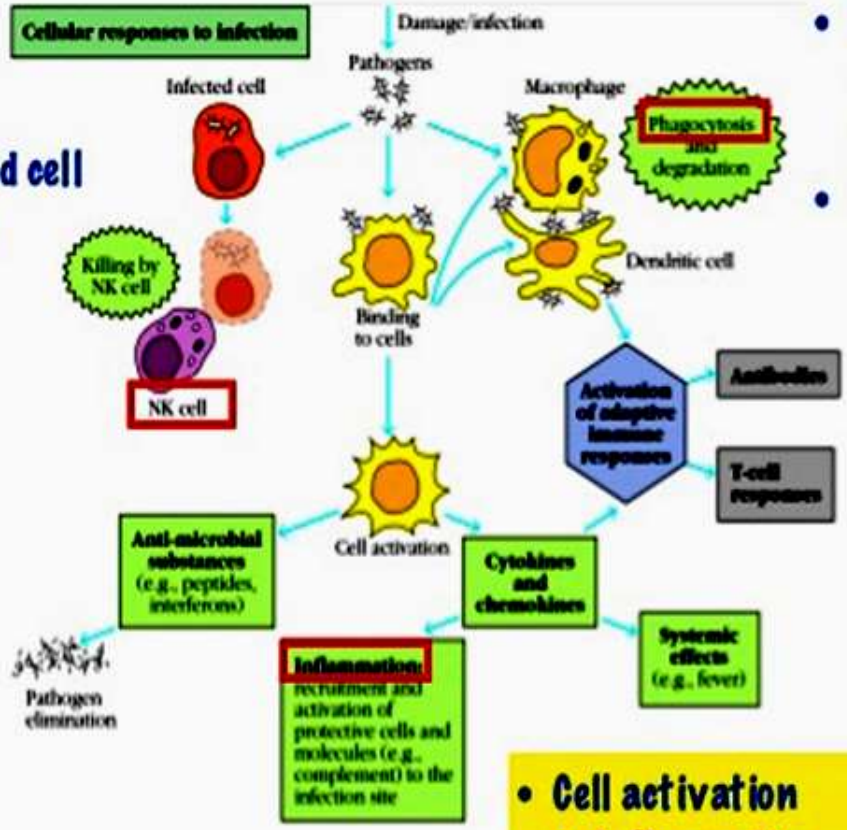


1. Anatomical barrier
   - Physical barriers
   - Chemical barriers
2. Cell
   - Phagocytic cells
   - Dendritic cell
   - NK cells, ILC
3. Soluble proteins
   - Complement
   - Cytokines, Chemokines
   - Anti-microbial substances

Cellular Response (Recognized pathogen by receptors : PRRs)

Soluble proteins

- NK cell
  - Viral infected cell
  - Malignancy



- Phagocytic cells
  - -Macrophage, Neutrophil
  - "Phagocytosis"
- Dendritic cell
  - >> Activated adaptive immune response

- Cell activation
  - >> Inflammation
  - >> Antiviral defense

Cellular response

- Innate immune system recognizes
  - PAMPs (Pathogen-associated molecular pattern)
    : molecular structures of microbial pathogen
    that required for survival
  - DAMPs (Damage-associated molecular pattern)
    : result of cell damage by infections

- Cellular receptors : PRRs (Pattern recognition receptors)

One well-known **PAMP** is lipopolysaccharide (LPS), which is found on the outer cell wall of gram-negative bacteria.

DAMPs are derived from host cells including tumor cells, dead or dying cells, or products released from cells in response to signals such as hypoxia.

Protein **DAMPs** include intracellular proteins, such **as heat-shock proteins or HMGB1,** and materials derived from the extracellular matrix that are generated following tissue injury, such as haluronan fragments.

Non-protein **DAMPs** include ATP, uric acid, heparin sulfate and DNA.

PAMPs

1.) Produced only by microbes, not by their hosts

2.) PAMP structures are usually fundamental to the integrity, survival, and pathogenicity of the microorganisms

3.) Shared by entire classes of pathogens

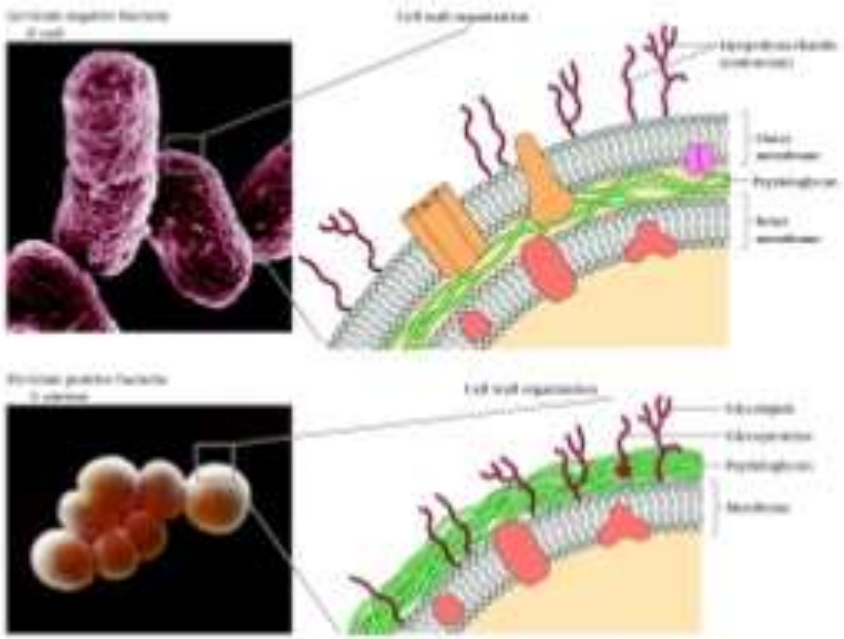


TABLE 4-2 Examples of PAMPs and DAMPs

Pathogen-Associated Molecular Patterns		Microbe Type
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	
Crystals	Monosodium urate	
Nuclear proteins	HMGB1	

Uric acid crystals [**monosodium urate** (MSU)] have emerged as an important factor for both gouty arthritis and **immune** regulation

This simple crystalline structure appears to activate innate host defense mechanisms in multiple ways and triggers robust inflammation and immune activation.

- *Structure:* Several families of protein

- *Location:* Expressed on many effector cells
  esp. most importantly on the professional APC
  (Macrophages, Dendritic cells, and B cells)
  1.) Transmembrane PRRs
  2.) Cytosolic PRRs

- *Functions:* 3 classes
  - Secreted PRRs
  - Endocytic PRRs
  - Signaling PRRs

Principle functions of PRRs

1.) Opsonization
2.) Activation of complement
3.) Phagocytosis
4.) Activation of proinflammatory signaling pathways
5.) Induction of apoptosis

Endocytic pattern-recognition receptors are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction. They include mannose receptors, scavenger receptors, and opsonin receptors

Janeway C.et al. NEMJ 2000. 343(5), 338-44.
Liu A.et al. Middleton's 8th Edition, 1-19.

1.) Secreted PRRs

- **Secreted pattern-recognition receptors**. These bind to microbial cell walls and enable them to be **recognized by the complement pathways and phagocytes**.
- E.g. mannose-binding lectin is synthesized by the liver and released into the bloodstream. MBL recognizes carbohydrate patterns, found on the surface of a large number of pathogenic micro-organisms, including bacteria, viruses, protozoa and fungi.

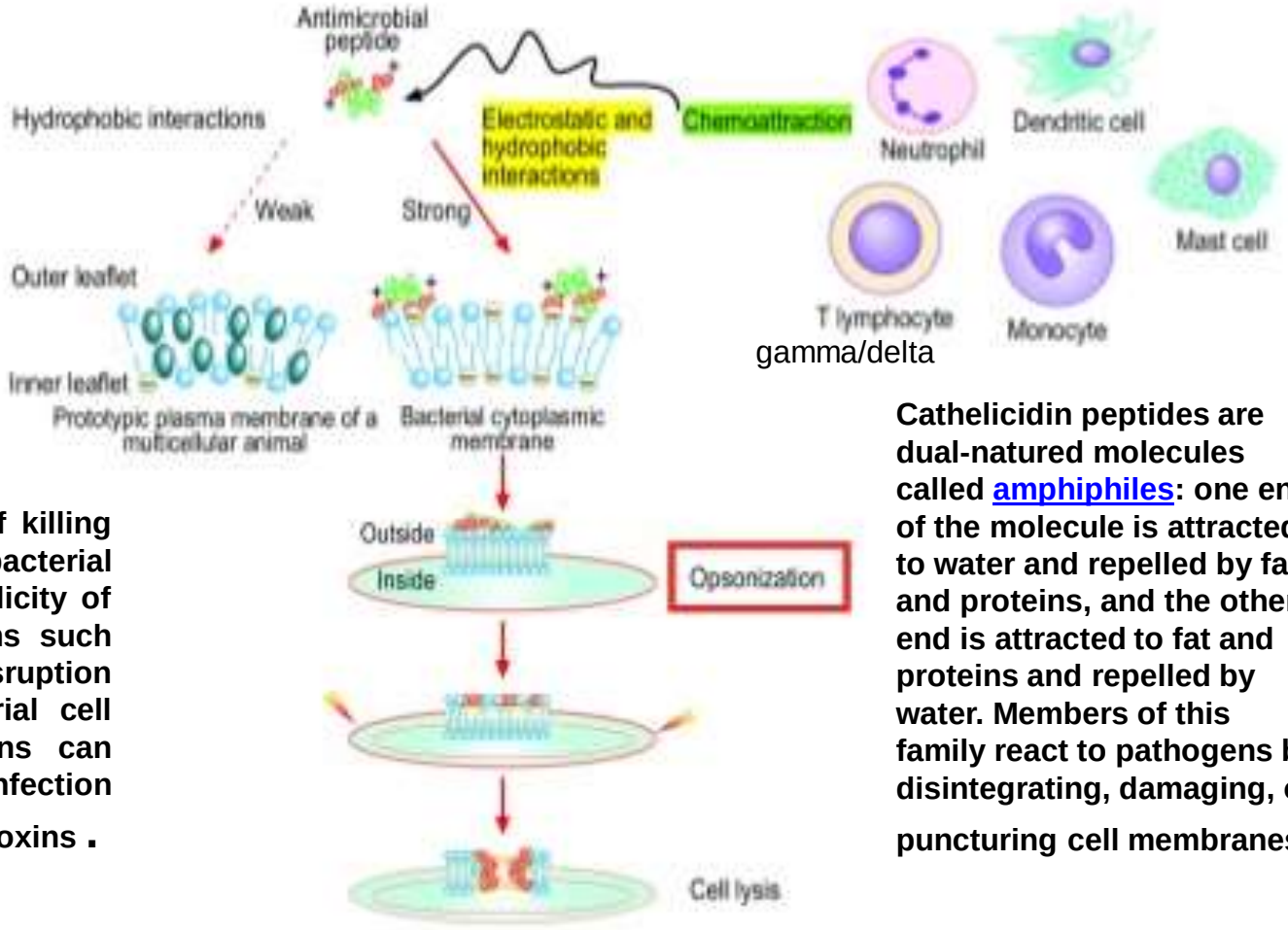
A number of PRRs can be **secreted** by cells, and bind directly to invading micro-organisms. Some **examples** of these proteins are collectins, pentraxins, ficolins, lipid transferases, peptidoglycan recognition proteins (PGRs) and the leucine-rich repeat receptor (LRR).

AMPs

Antimicrobial peptides

Defensins & Cathelicidins

(LL-37 peptide in the Cathelicidin family found only in the human body)



Defensins are capable of killing bacteria or inhibiting bacterial growth through a multiplicity of antimicrobial mechanisms such as direct membrane disruption and inhibition of bacterial cell wall synthesis. Defensins can also reduce bacterial infection by neutralizing secreted toxins .

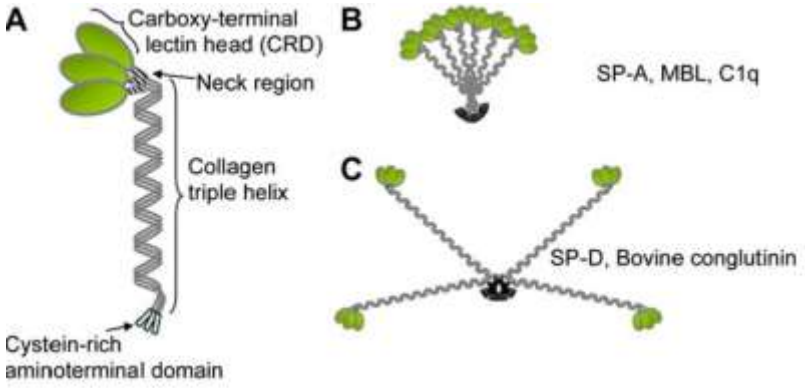
Cathelicidin peptides are dual-natured molecules called amphiphiles: one end of the molecule is attracted to water and repelled by fats and proteins, and the other end is attracted to fat and proteins and repelled by water. Members of this family react to pathogens by disintegrating, damaging, or puncturing cell membranes.

Collectins (collagen-containing C-type lectins) are a part of the innate immune system. They form a family of collagenous Ca^{2+}-dependent defense lectins, which are found in animals. **Collectins** are soluble pattern recognition receptors (PRRs).

The **lectin pathway** is initiated by binding of collectins
Exp: **SP-A, MBL, C1q,SP-D and conglutinin**

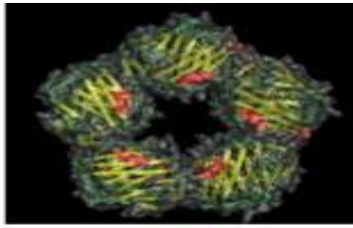
Binding of collectins to microorganisms may trigger elimination of microorganisms by aggregation, complement activation, opsonization, activation of phagocytosis, or inhibition of microbial growth. Other functions of collectins are modulation of inflammatory, allergic responses, adaptive immune system and clearance of apoptotic cells.

Collectins can bind to the surface of microorganisms and between carbohydrate ligands. Due to these properties, the interaction can result in aggregation.



Pentraxins (PTX), also known as pentaxins, are an evolutionary conserved family of proteins characterised by containing a **pentraxin** protein domain. Proteins of the **pentraxin** family are involved in acute immunological responses. They are a class of pattern recognition receptors (PRRs).

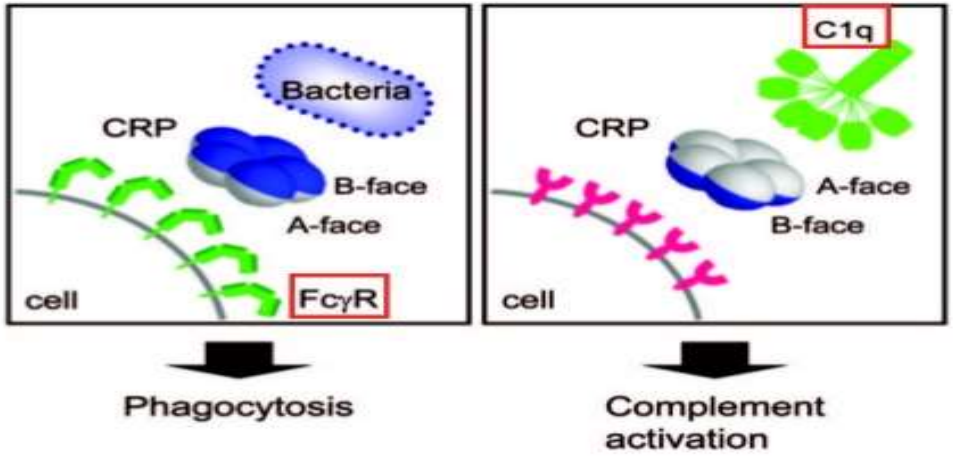
Three of the principal members of the pentraxin family are serum proteins: namely**, C-reactive protein (CRP), serum amyloid P component protein (SAP), and female protein (FP)**



Pentraxins

C-reactive protein (CRP)

pneumonia ,**influenza virus and adenovirus.**



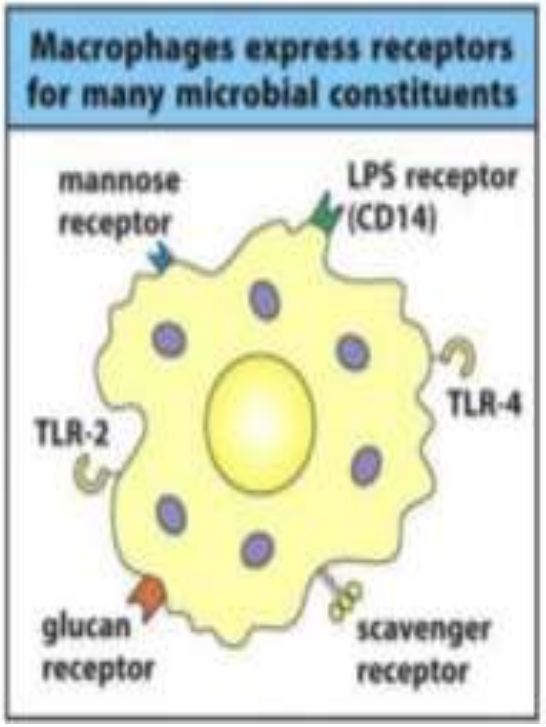
C-reactive protein (**CRP**) is an annular (ring-shaped) pentameric protein found in blood plasma, whose circulating concentrations rise in response to inflammation. It is an acute-phase protein of hepatic origin that ***increases following interleukin-6 secretion by macrophages and T cells***. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system

2.) Endocytic PRRs

Endocytic pattern-recognition receptors, also called phagocytic **pattern-recognition receptors**, are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction

Mannose receptors (CD206)

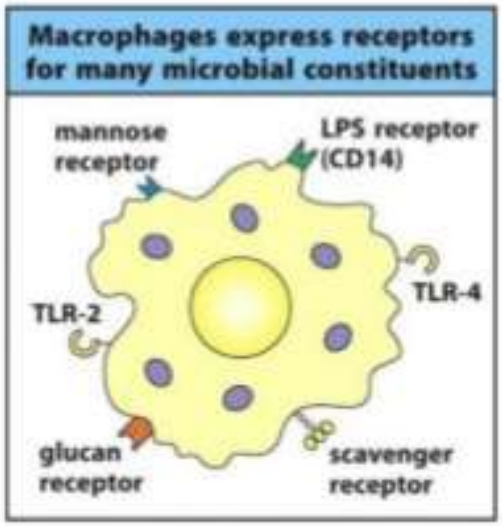
- C - Type Lectin Receptors
- Recognized carbohydrates (Mannose)
  - Bacterial, Fungus, Parasite
- Expressed on Macrophage
- Phagocytosis, NF-kB activation



Scavenger receptors

The receptors **mediate the uptake of oxidized lipoproteins into cells**. Scavenger receptors also mediate the uptake of microbes and contribute to the response of macrophages to mycobacteria.

- Recognize Lipoproteins on gram + and gram - bacteria
- Eliminated old & apoptotic cells
- Expressed on Macrophage
- Phagocytosis, NF-kB activation



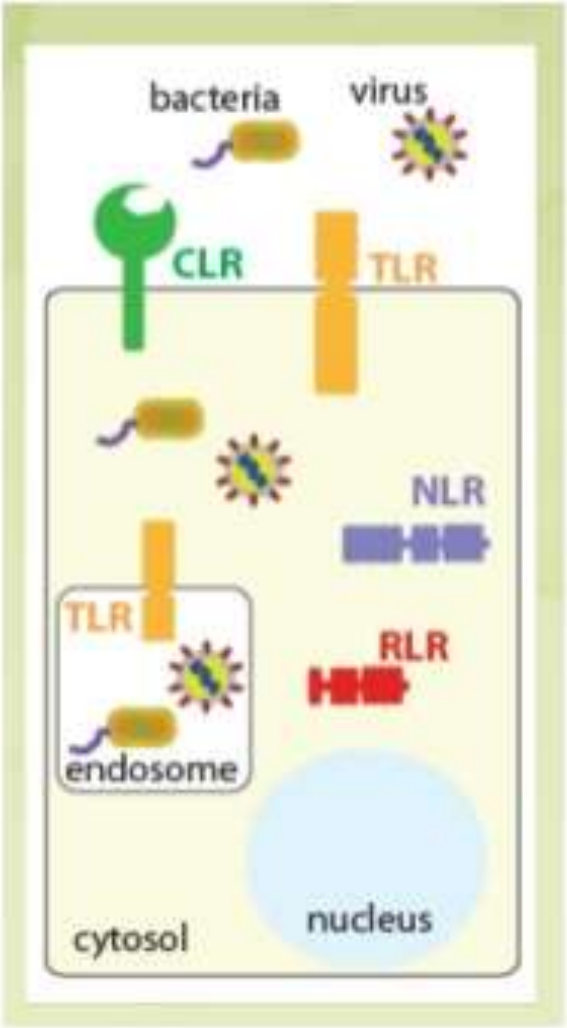
ability to bind modified low-density lipoproteins (LDLs), such as LDL molecules that have been oxidized or acetylated during cell injury or apoptosis.

Atherosclerosis arises from the deposition and retention of serum lipoproteins in the artery wall. Macrophage scavenger receptors **bind modified lipoproteins and promote cellular cholesterol accumulation in the artery wall**

3.) Signaling PRRs

4 Major classes of Signaling PRRs

1.) Toll-like receptors (TLRs) : Transmembrane PRRs
2.) C-Type Lectin Receptors (CLRs): Transmembrane PRRs
3.) NOD-like receptors (NLRs) : Cytosolic PRRs
4.) Retinoid acid–inducible gene (RIG)-like receptors (RLRs) : Cytosolic PRRs



NLR=nucleotide-binding oligomerization domain and leucine-rich repeat receptors

Cellular location of TLRs

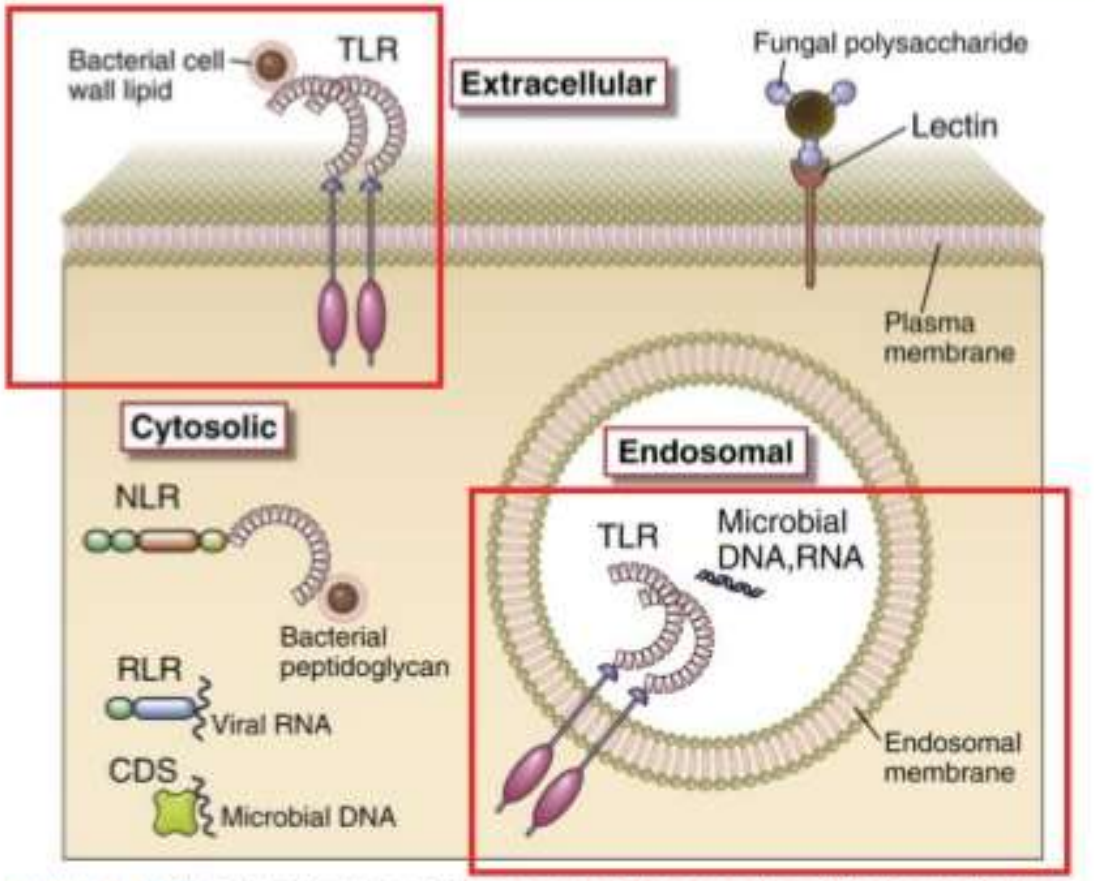
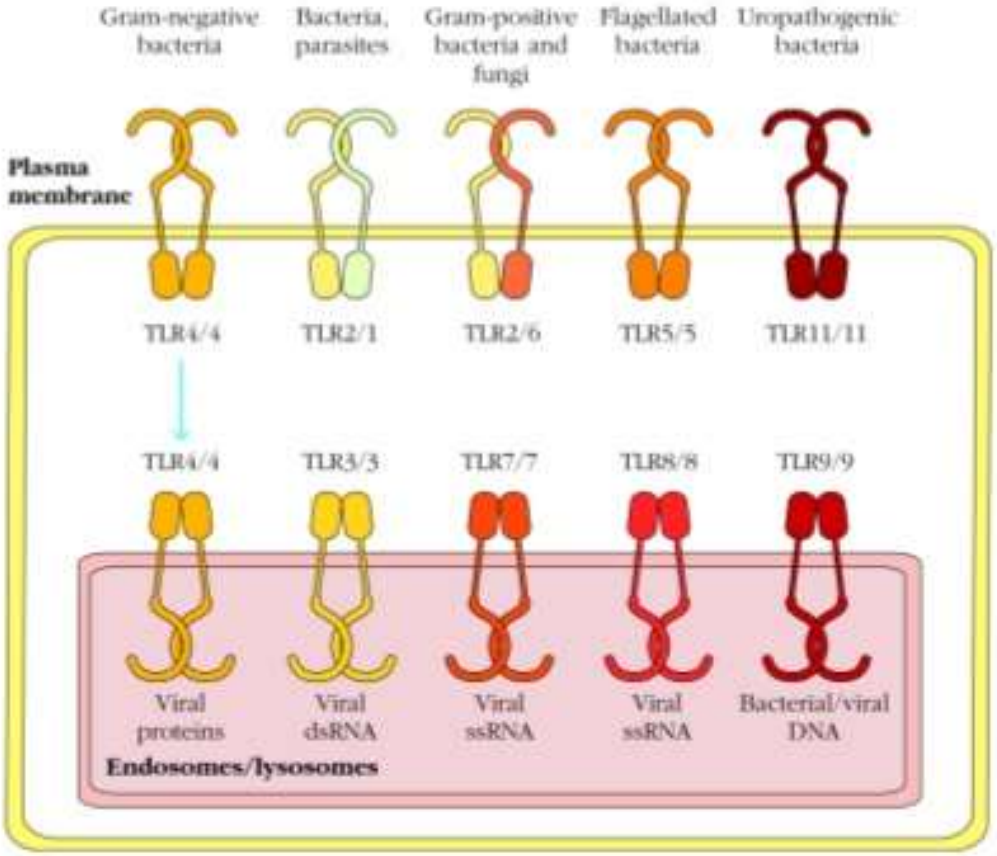


FIGURE 4-1 Cellular locations of pattern recognition receptors of the innate immune...

cytosolic dsDNA sensors (CDSs)



Plasma membrane

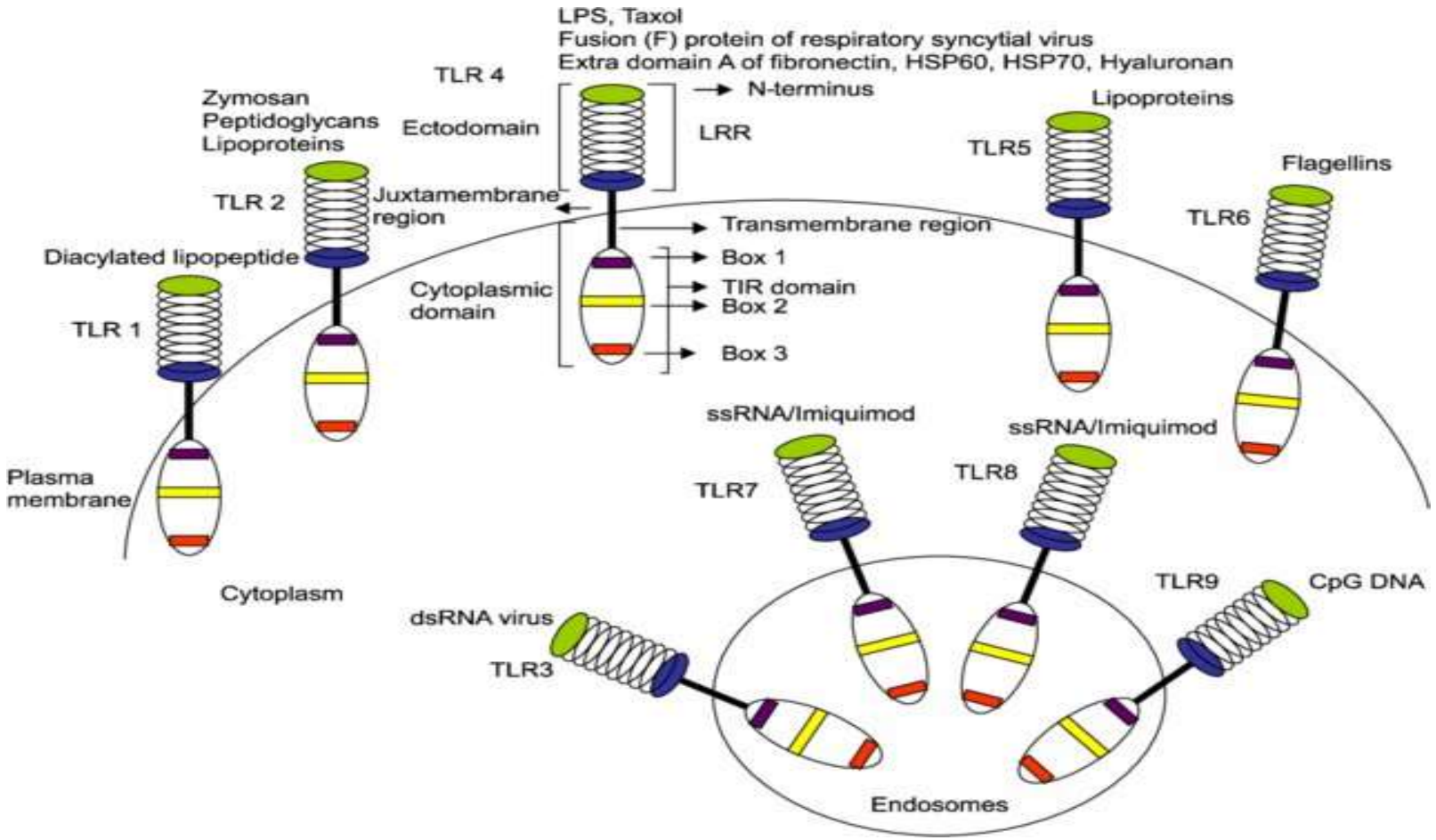
1/2, 2/6, 4, 5, 11

Endosomes/ Lysosomes membrane

3, 7, 8, 9, 13

Kuby Immunology 7th edition.

Toll-like receptors (TLRs) are expressed on innate immune cells, like macrophages and dendritic cells. They are located on the cell surface or in intracellular compartments because microbes may be found in the body or inside infected cells. TLRs recognize general microbial patterns, and they are essential for innate immune-cell activation and inflammatory responses.



Nucleotide-binding Oligomerization Domain (NOD)

NOD-like receptors (NLR)s respond to various microbial products and stress, thereby mediating the formation of inflammasomes

NOD-Like Receptors (NLRs)

- Cytosolic PRRs
- Recognized - PAMPs & DAMPs
  - Stress/ Damage signal
- Similar to the TLRs : linked to signal transduction
- NLR family : 23 Members

NOD Response to bacterial cell wall peptidoglycans

- NOD 1 : iE-DAP
- NOD 2 : MDP

NOD-like receptor family, detects conserved motifs in bacterial peptidoglycan and promotes their clearance through activation of a proinflammatory transcriptional program and other innate immune pathways, including autophagy and endoplasmic reticulum stress. An inactive form due to mutations or a constitutive high expression of NOD2 is associated with several inflammatory diseases

NOD 1

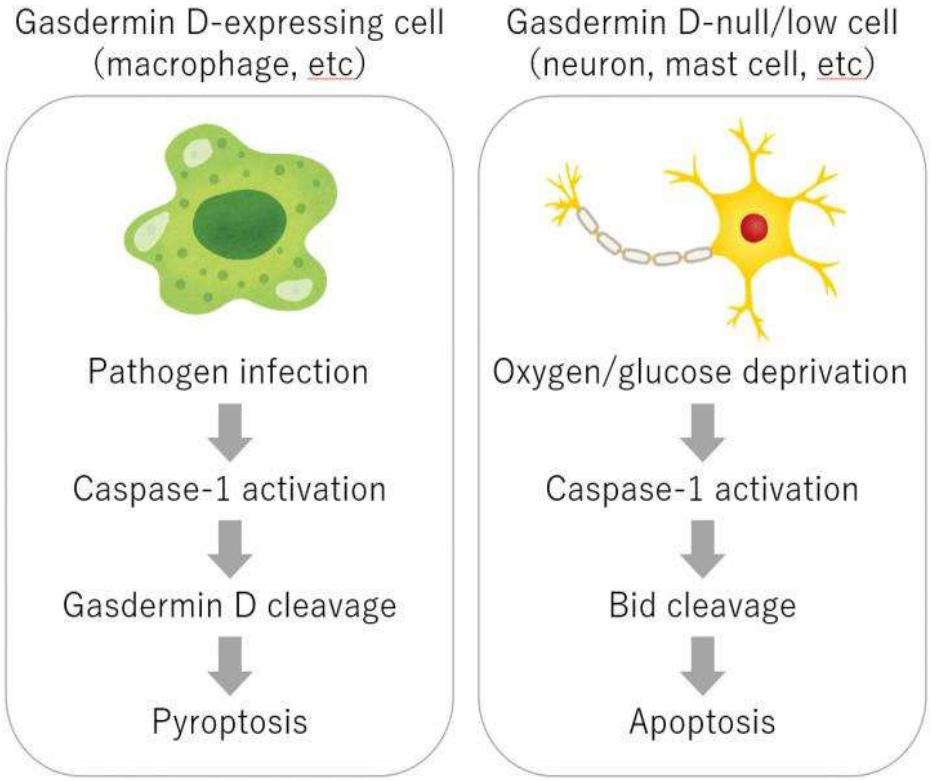
- Recognizes Diaminopimelic acid (DAP) derived mainly from gram-negative bac. peptidoglycans

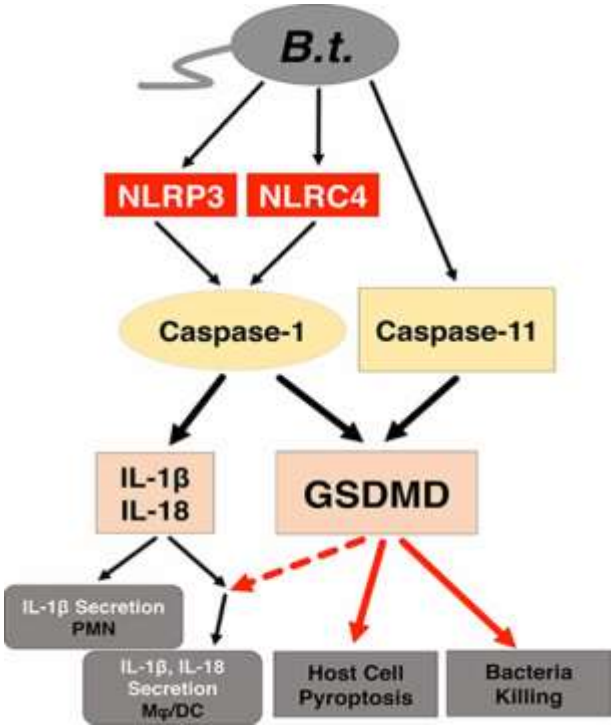
NOD 2

- Recognizes Muramyl dipeptide (MDP) derived both gram-negative & gram-positive bac. peptidoglycans
- Highly expressed in intestinal Peneth cells
- Mutations : Crohn disease
  (Bac. overgrowth –> chronic inflammation)
- Gain-of-function : Blau's syndrome (systemic inflammatory disease)

Nucleotide-binding domain and leucine-rich repeat containing (NLR) proteins

Inflammasomes are cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses.Activation and assembly of the inflammasome promotes proteolytic cleavage, maturation and secretion of pro-inflammatory cytokines interleukin 1β (IL-1β) and interleukin 18 (IL-18), as well as cleavage of Gasdermin-D





Pyroptosis is a form of lytic programmed cell death initiated by inflammasomes, which detect cytosolic contamination or perturbation. This drives activation of caspase-1 or caspase-11/4/5, which cleave **gasdermin D**,separating its N- terminal pore-forming domain (PFD) from the C-terminal repressor domain (RD)

The **inflammasome** is a multiprotein intracellular complex that detects pathogenic microorganisms and sterile stressors, and that activates the highly pro-inflammatory cytokines interleukin-1b (IL-1b) and IL-18. **Inflammasomes** also induce a form of cell death termed pyroptosis.

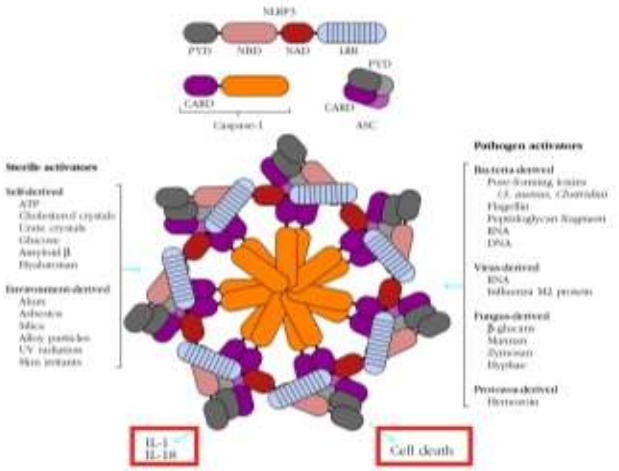
Inflammasome

- Large protein complex that activates caspase-1 to generate IL-1
- Containing

1.) NLR subfamily
- NLRP 1
- NLRP 3
- NLRC 4/ IPAF

2.) Non-NLR/ Adaptor protein
Ex. ASC, AIM2

3.) Caspase-1 (inactivate)



Inflammasomes are molecular complexes that are comprised of three basic protein units:

➢A sensor molecule, which may include one of these; NLRP1, NLRP2, **NLRP3**, NLRC4, AIM2, or pyrin.

➢The adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain or CARD). .

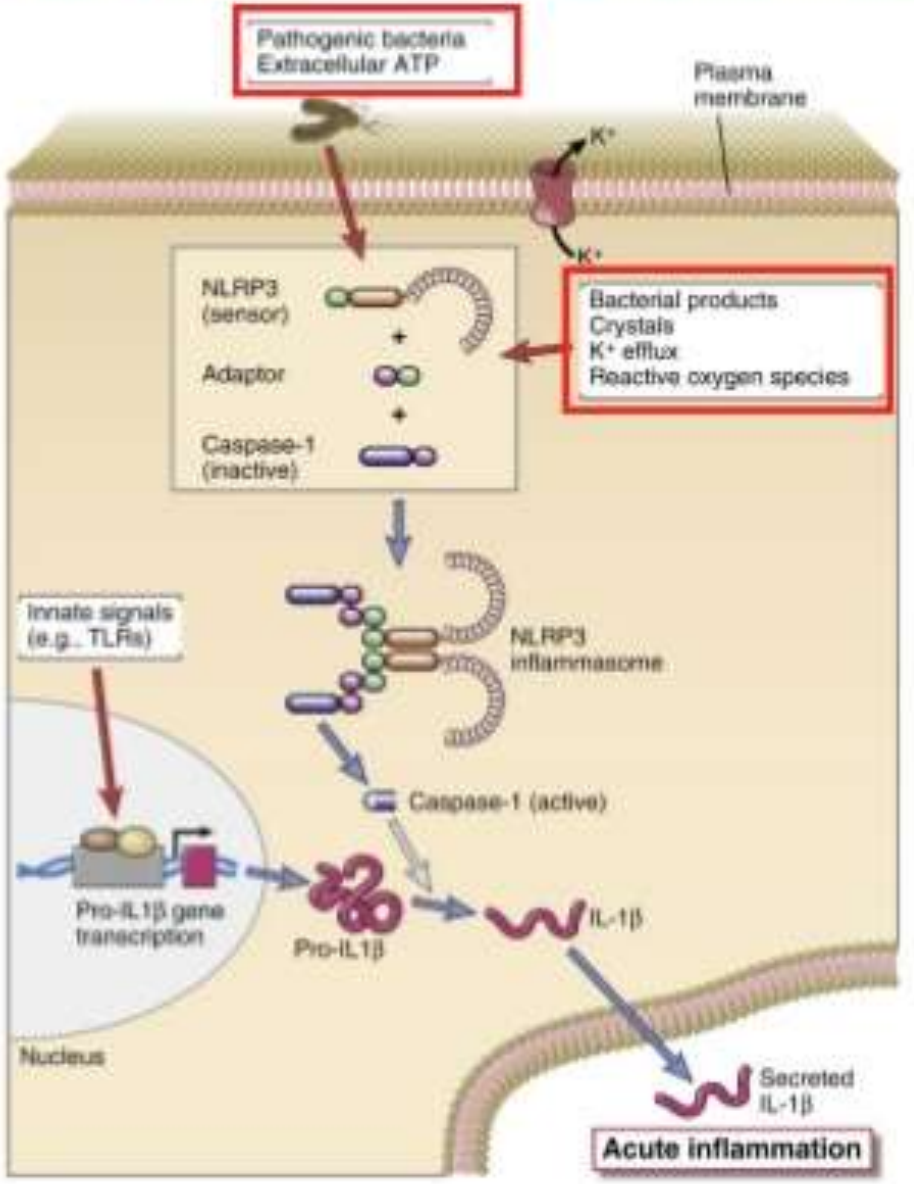
➢Pro-caspase 1

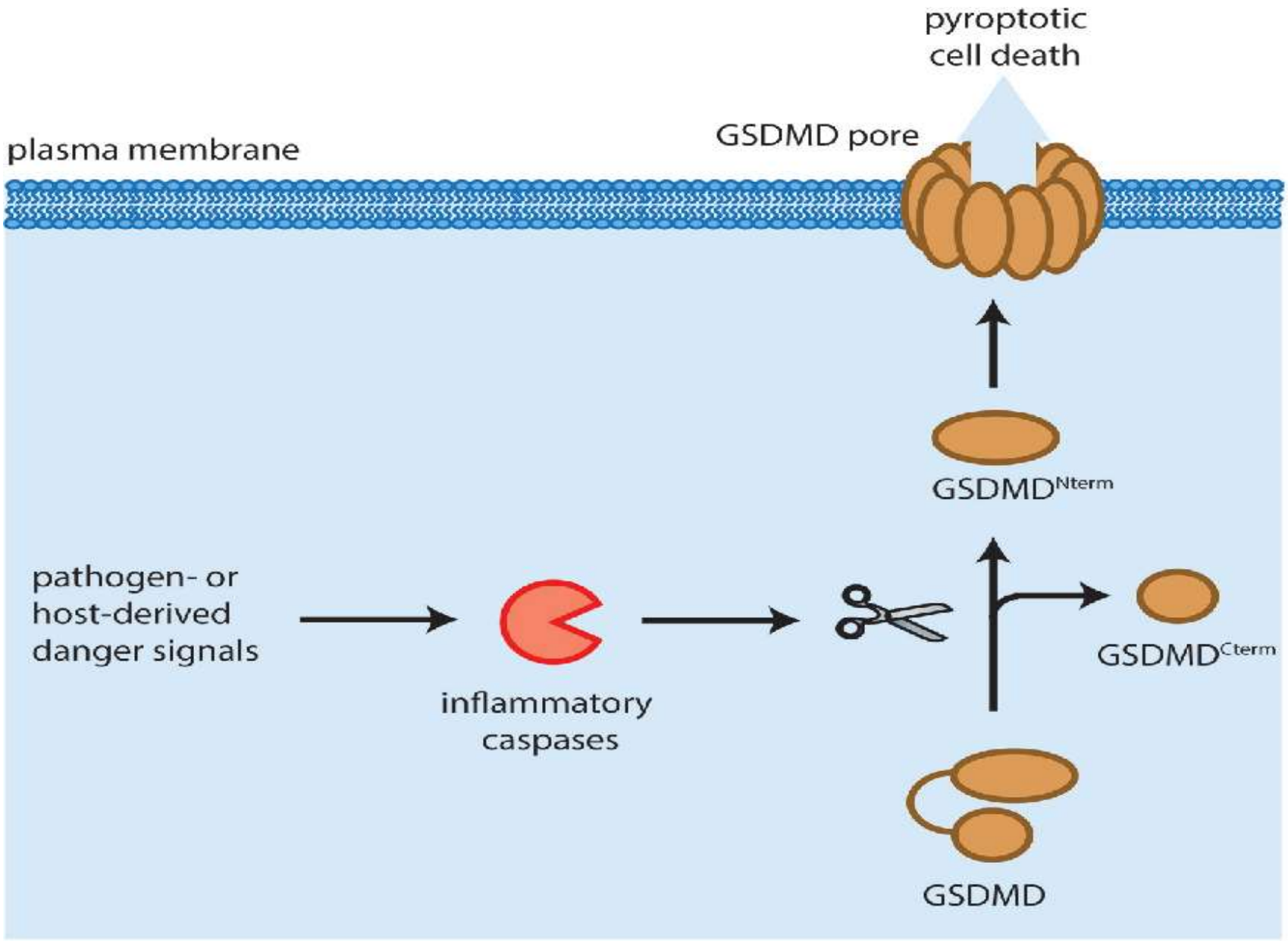
The **inflammasome** plays an **important** role in the innate immune pathway and regulates at least two protective responses of the host: the secretion of proinflammatory cytokines (IL-1β and IL-18) and the induction of pyroptosis, which is a form of cell death

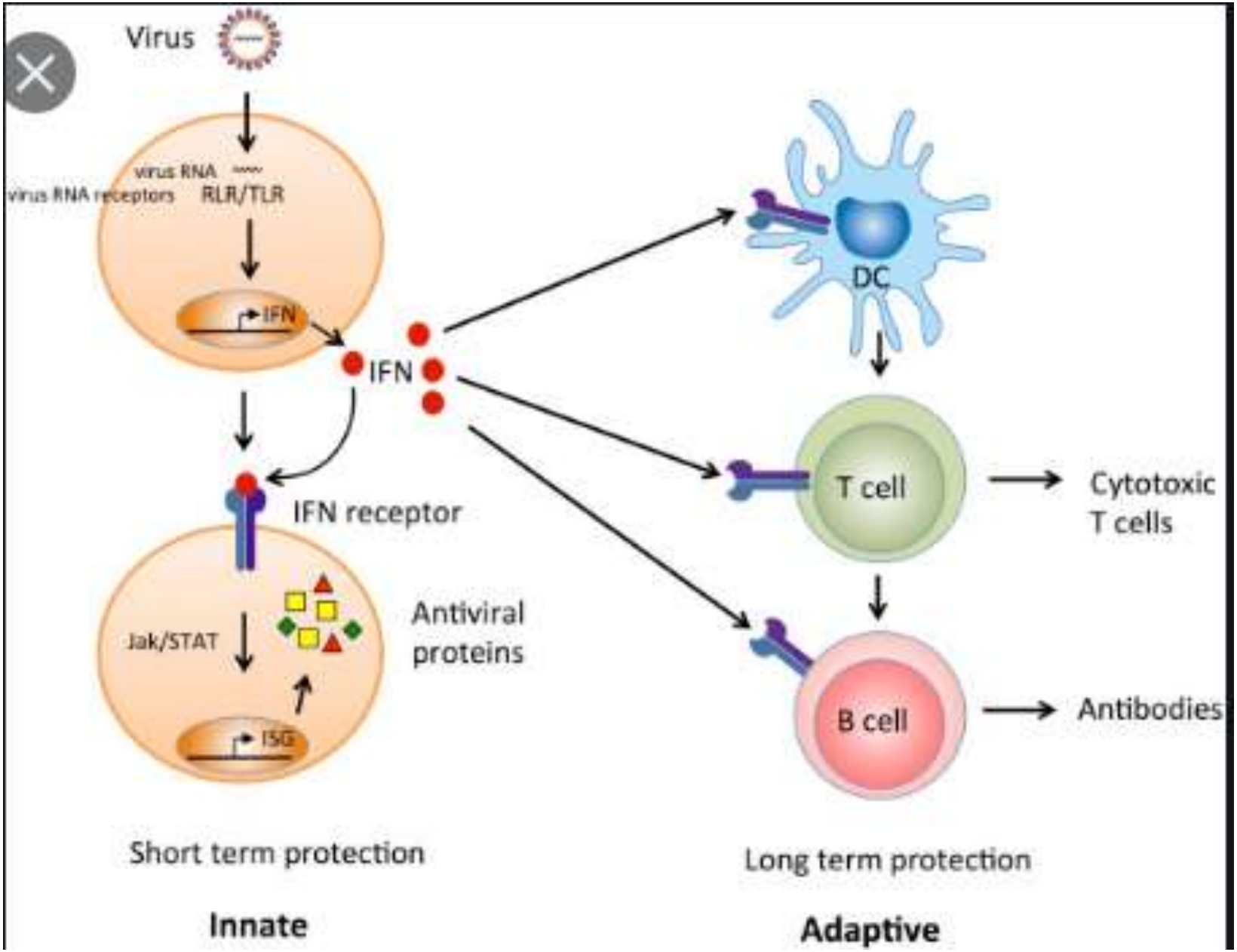
NLRP3 Inflammasome

- Monocyte
- Macrophage
- Neutrophils
- DC
- Lymphocyte

Variety stimuli activated Inflammasome




pyroptotic
cell death
GSDMD pore
plasma membrane
GSDMD^Nterm
pathogen- or
host-derived
danger signals
inflammatory
caspases
GSDMD^Cterm
GSDMD


Virus
virus RNA
virus RNA receptors
RLR/TLR
IFN
IFN
DC
T cell
Cytotoxic
T cells
IFN receptor
Jak/STAT
Antiviral
proteins
ISG
B cell
Antibodies
Short term protection
Long term protection
Innate
Adaptive

C-Type Lectin Receptors (CLRs)

- Plasma membrane receptors
  - monocytes, macrophages, dendritic cells, neutrophils, B cells, and T-cell subsets

- Recognize carbohydrate components of fungi, mycobacteria, viruses, parasites, and some allergens (peanut and dust mite proteins)

- Human : 15 CLRs that function as PRRs
  - Most recognize sugar moieties
    - mannose –> mannose receptor, DC-SIGN
    - fucose –> Dectin-2, DC-SIGN
    - glucans –> Dectin-1

Dectin-1 is a pattern recognition receptor expressed by myeloid phagocytes (macrophages, dendritic cells and neutrophils) that detects **β-glucans in fungal cell walls** and triggers direct cellular anti-microbial activity, including phagocytosis and production of reactive oxygen species

RIG-Like Receptors (RLRs)

Retinoic acid-inducible gene

- Soluble PRRs
- Sensors of viral infection ex. Influenza, Measles
- Recognize the RNA viruses in the cytoplasm of infected cells

  –> induce inflammatory cytokines and type I interferons
- 3 Members : CARD-containing RNA helicase
  1.) RIG-I
  2.) MDA5
  3.) LGP2

Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are key sensors of virus infection, mediating the transcriptional induction of type I interferons and other genes that collectively establish an antiviral host response

Granulocytes include basophils, eosinophils, and neutrophils. **Basophils and eosinophils are important for host defense against parasites.** They also are involved in **allergic reactions**. Neutrophils, the most numerous innate immune cell, patrol for problems by circulating in the bloodstream. They can phagocytose, or ingest, bacteria, degrading them inside special compartments called vesicles

Mast cells also are important for defense against parasites. Mast cells are found in tissues and can mediate allergic reactions by releasing inflammatory chemicals like histamine.

Monocytes, which develop into *macrophages*, also patrol and respond to problems. They are found in the bloodstream and in tissues. Macrophages, "big eater" in Greek, are named for their ability to ingest and degrade bacteria. Upon activation, monocytes and macrophages coordinate an immune response by notifying other immune cells of the problem. Macrophages also have important non-immune functions, such as recycling dead cells, **like red blood cells, and clearing away cellular debris. These "housekeeping" functions occur without activation of an immune response.**

Dendritic cells **(DC)** are an important antigen-presenting cell (APC), and they also can develop from monocytes. Antigens are molecules from pathogens, host cells, and allergens that may be recognized by adaptive immune cells. APCs like DCs are responsible for processing large molecules into "**readable"** fragments (antigens) recognized by adaptive B or T cells. However, antigens alone cannot activate T cells. They must be presented with the appropriate major histocompatiblity complex (MHC) expressed on the APC. MHC provides a checkpoint and helps immune cells distinguish between host and foreign cells.

Natural killer **(NK)** cells have features of both innate and adaptive immunity. They are important for recognizing and killing virus-infected cells or tumor cells. They contain intracellular compartments called granules, which are filled with proteins that can form holes in the target cell and also cause apoptosis, the process for programmed cell death. It is important to distinguish between apoptosis and other forms of cell death like necrosis. Apoptosis, unlike necrosis, does not release danger signals that can lead to greater immune activation and inflammation. Through apoptosis, immune cells can discreetly remove infected cells and limit bystander damage. Recently, researchers have shown in mouse models that NK cells, like adaptive cells, can be retained as memory cells and respond to subsequent infections by the same pathogen.

Adaptive Cells

B cells have two major functions: **They present antigens to T cells, and more importantly, they produce antibodies to neutralize infectious microbes.**

Antibodies coat the surface of a pathogen and serve three major roles: neutralization, opsonization, and complement activation.

Neutralization occurs when the pathogen, because it is covered in antibodies, is unable to bind and infect host cells. In opsonization, an antibody-bound pathogen serves as a red flag to alert immune cells like neutrophils and macrophages, to engulf and digest the pathogen. Complement is a process for directly destroying, or lysing, bacteria.

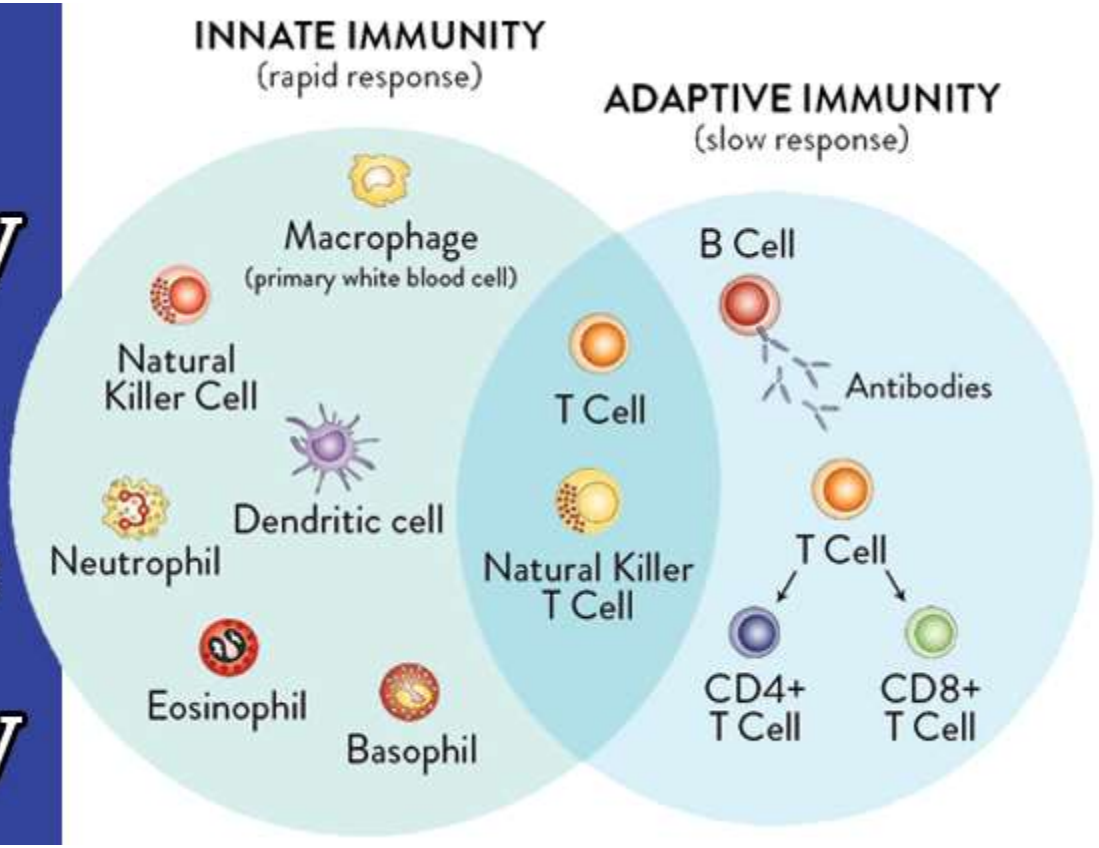
T cells have a variety of roles and are classified by subsets. T cells are divided into two broad categories: **CD8+ T cells or CD4+ T cells**, based on which protein is present on the cell's surface. T cells carry out multiple functions, including killing infected cells and activating or recruiting other immune cells.

CD8+ T cells also are called cytotoxic T cells or cytotoxic lymphocytes (CTLs). They are crucial for recognizing and removing virus-infected cells and cancer cells. CTLs have specialized compartments, or granules, containing cytotoxins that cause apoptosis, i.e., programmed cell death. Because of its potency, the release of granules is tightly regulated by the immune system.

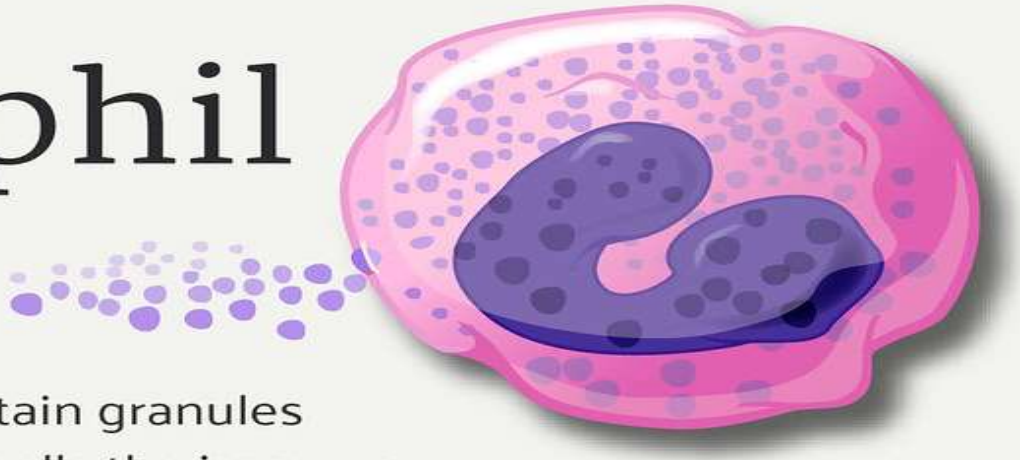
The four major CD4+ T-cell subsets are TH1, TH2, TH17, and Treg, with "TH" referring to "T helper cell." TH1 cells are critical for coordinating immune responses against intracellular microbes, especially bacteria. They produce and secrete molecules that alert and activate other immune cells, like bacteria-ingesting macrophages. TH2 cells are important for coordinating immune responses against extracellular pathogens, like helminths (parasitic worms), by alerting B cells, granulocytes, and mast cells. TH17 cells are named for their ability to produce interleukin 17 (IL-17), a signaling molecule that activates immune and non-immune cells. TH17 cells are important for recruiting neutrophils.

Regulatory T cells (Tregs), as the name suggests, monitor and inhibit the activity of other T cells. They prevent adverse immune activation and maintain tolerance, or the prevention of immune responses against the body's own cells and antigens.

Innate Immunity VS Adaptive Immunity



Eosinophil



White Blood Cell

Function: These cells contain granules full of molecules that kill cells the immune system has marked for destruction. They help clear parasitic infections and mediate inflammation.

Disease: Several immune disorders involve the presence of too many or overactive eosinophils. These include an increasingly diagnosed disease called eosinophilic esophagitis, which causes difficulty swallowing, nausea, and vomiting. Eosinophils are also believed to contribute to asthma.

Location: Eosinophils circulate in the blood and then migrate to tissues that interact with the outside environment, like the lungs and the lining of the gastrointestinal tract.

Basophil

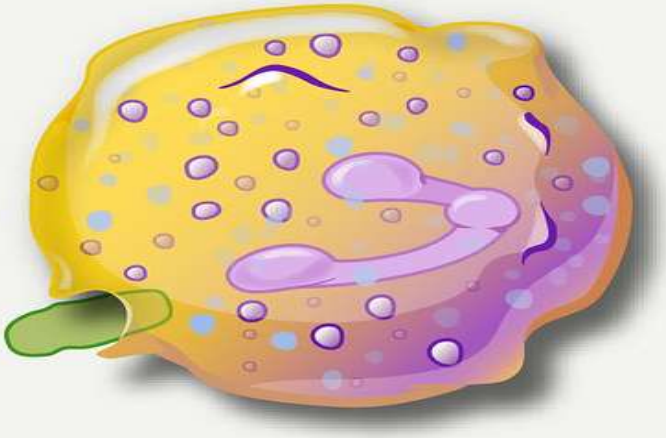
White Blood Cell

Function: These cells are loaded with granules that can be released by various inflammatory, infectious and allergic triggers. The granules are filled with various proteins and chemicals. One of these chemicals, histamine, is especially important to increase blood flow during an inflammatory response. The beneficial functions of basophils are not clear yet. They may play a role in control of parasites and insect vectors of disease.

Disease: In some people, an otherwise harmless substance, like tree pollen or peanut, can make basophils release chemicals and proteins, causing allergy symptoms. When allergies are severe, basophils contribute to inflammation that can cause airway tightening and low blood pressure, a life-threatening condition known as anaphylaxis.

Location: In most people, basophils make up less than 1 percent of the immune cells in the blood. Mast cells, another immune cell type that plays a large role in allergic responses, are located in the body's tissues.

Neutrophil



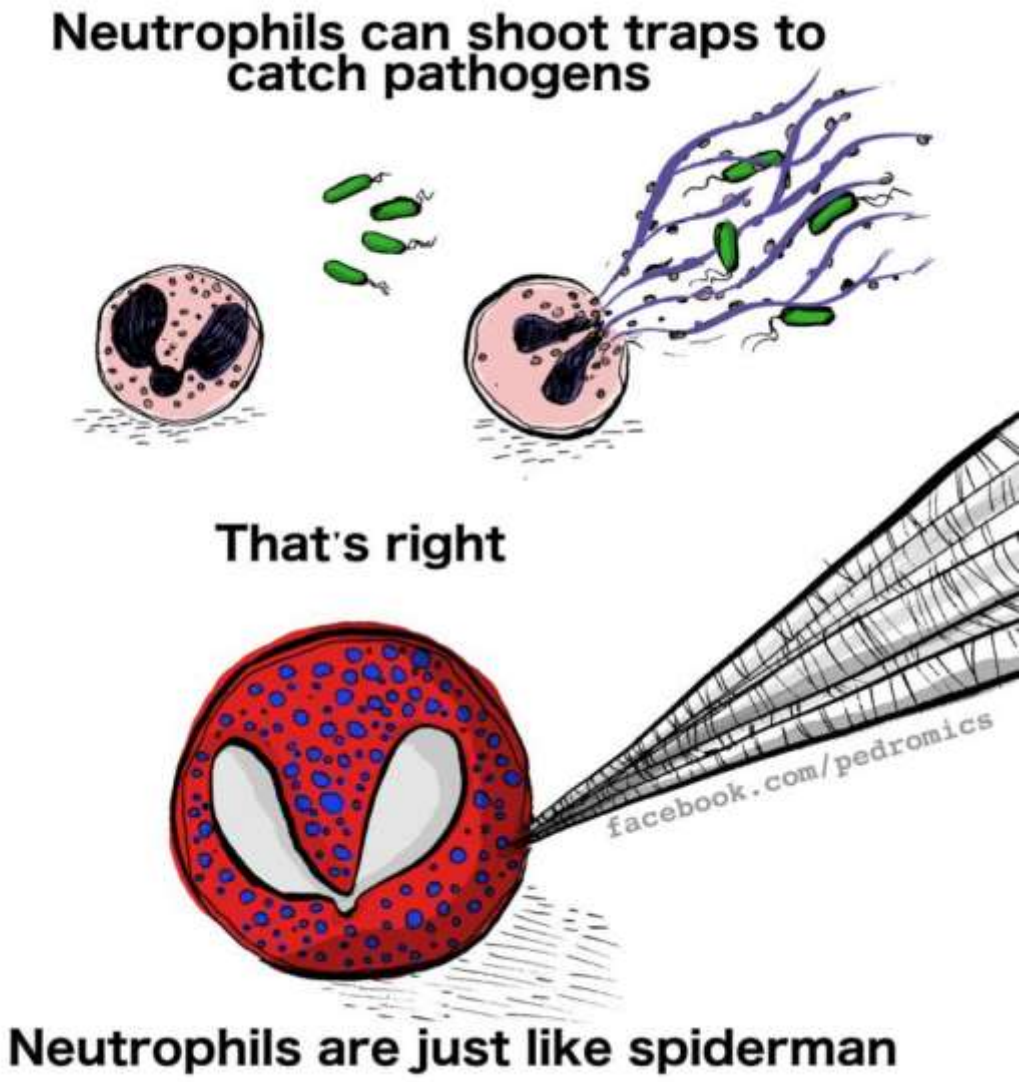
White Blood Cell

Function: Neutrophils engulf and destroy bacteria and other pathogens. They are the most abundant type of white blood cell in most people's bloodstreams and play a large role in fighting many types of infection.

Disease: Because of genetic anomalies, some people are born with too few neutrophils, a condition known as neutropenia, or with neutrophils that do not function properly. This causes people to be more prone to infections.

Location: Neutrophils circulate in the blood and quickly move to sites of infection or injury to fight off pathogens.

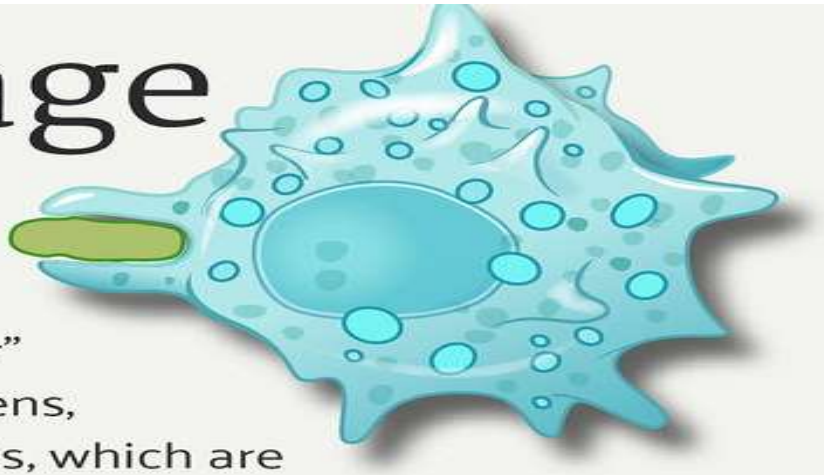
Neutrophil Extracellular Traps (NETs)



Neutrophil extracellular traps (NETs) are **made** of a network of **extracellular** strings of DNA that bind pathogenic microbes. Histones and several **neutrophil** granule proteins associated with the DNA framework damage entrapped microorganisms.

NETs contain antigens, some of which are modified histones that act as neoantigens to induce formation of autoantibodies that induce or accelerate vasculitis, rheumatoid arthritis, and thrombus formation.

Macrophage



White Blood Cell

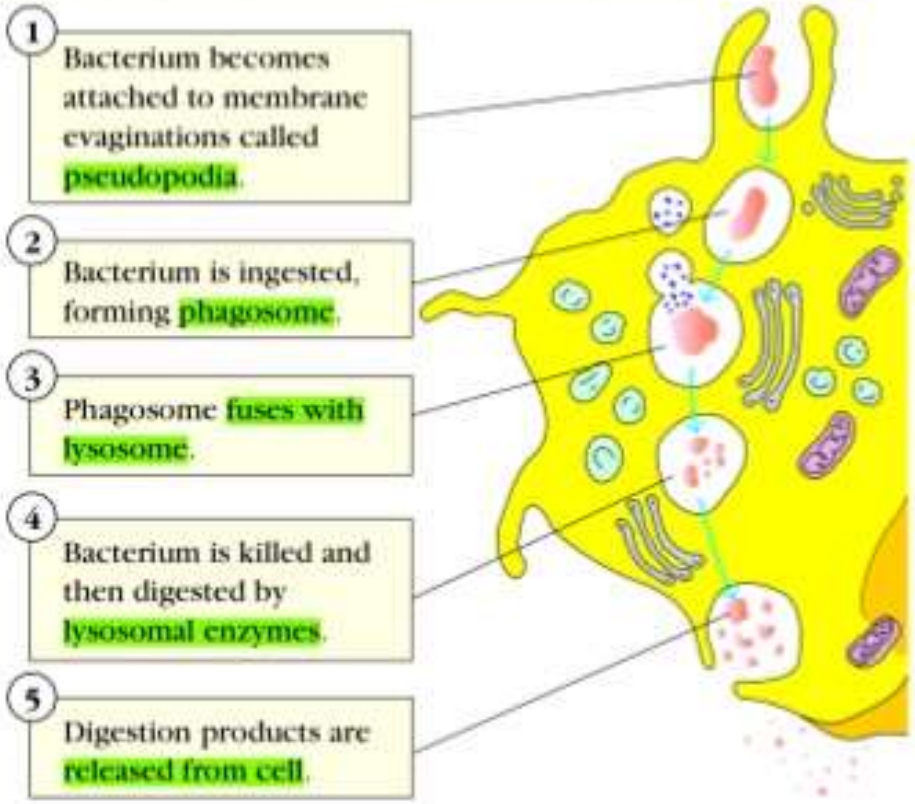
Function: Also known as "big eater" cells, macrophages ingest pathogens, cancer cells and microscopic debris, which are then destroyed, degraded and recycled with harsh chemicals and enzymes.

Disease: Unfortunately, macrophages sometimes fail to destroy ingested pathogens, which then may replicate inside the macrophage and hide from other immune responses. Macrophages also play a large roll in controlling inflammation and their malfunction can impact inflammatory diseases.

Location: Macrophages develop in the bone marrow as "monocytes," which circulate in the blood stream and then settle down in other body tissues. Macrophages are given different names depending on the type of tissue they are found in. For example, they are called microglia in the central nervous system and osteoclasts in bone.

- Macrophage, Neutrophil, DC : Tissue
- Monocyte : Blood

1.) Direct : PRRs rec.



Kuby Immunology 7th edition.

2.) Indirect : Opsonin rec.

" Opsonization " (to make it tasty)

- recognition of soluble proteins that have bound to microbial surfaces
- Soluble prot. = Opsonin

Opsonin receptors		Microbe-binding opsonins (soluble; bind to microbes)
Collagen-domain receptor	CD91/calreticulin	Collectins SP-A, SP-D, MBL; L-ficolin; C1q
Complement receptors	CR1, CR3, CR4, CRIg, C1qRp	Complement components and fragments*
Immunoglobulin Fc receptors	FcαR	Specific IgA antibodies bound to antigen[‡]
	FcγRs	Specific IgG antibodies bound to antigen;[#] C-reactive protein

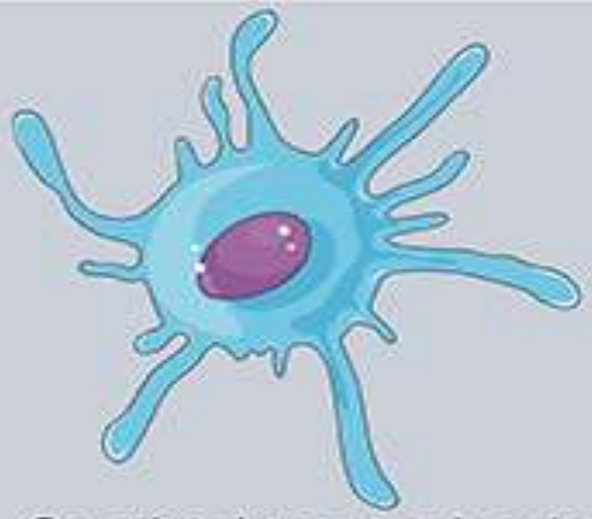
Major opsonin 1.) Lectin
2.) C3b
3.) IgG

Immature Dendritic Cells

- ↓ Co-stimulatory molecules
- ↓ MHC II Expression
- ↓ Secretion of pro-inflammatory cytokines
- ↑ Phagocytic capacity
- ↓ CCR7 expression
- ↓ Glycolysis

Pathogens
Cytokines
PAMPs
DAMPs

Mature Dendritic Cells

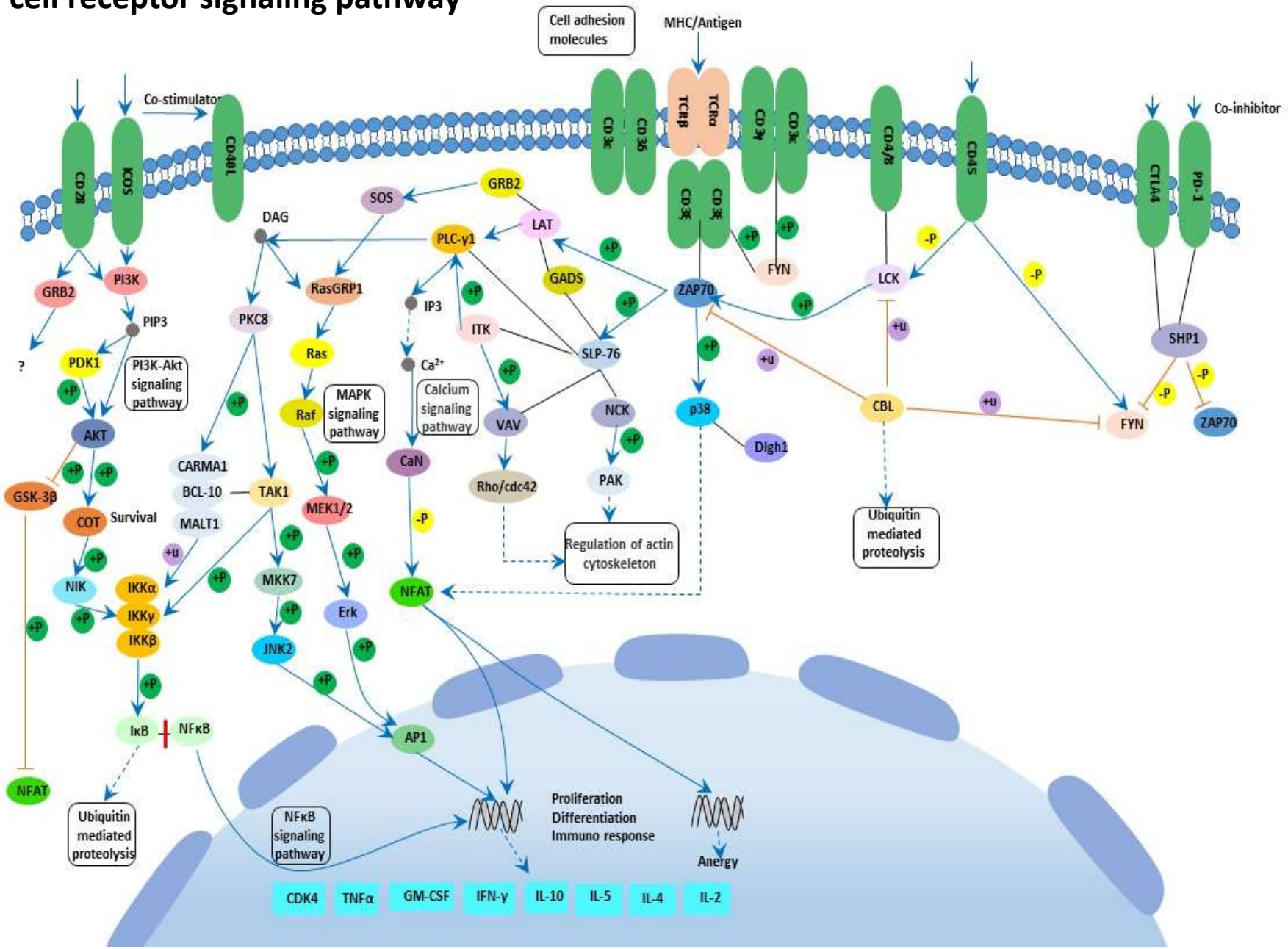


- ↑ Co-stimulatory molecules
- ↑ MHC II Expression
- ↑ Secretion of pro-inflammatory cytokines
- ↓ Phagocytic capacity
- ↑ CCR7 expression
- ↑ Glycolysis

T cell receptor signaling pathway

(All graphics collected from internet)

T cell receptor signaling pathway



Activation of T Cells

T cell activation is a complex process, including receiving signal stimulation, signal transduction, intracellular enzyme activation, gene transcription expression and cell amplification.

T cell activation requires dual signal stimulation.

First signal: antigen peptide -MHC molecular complex on antigen presenting cells binds to TCR specifically.

Second signal: interaction between T cells and costimulatory molecules present on the surface of antigen presenting cells produces costimulatory signals, of which CD28 and B7(CD80/CD86) are relatively important.

The integration of the two signals was the most effective way to induce T cell activation, while the lack of costimulation signals resulted in decreased T cell response, and in some cases can induce tolerance or T cell anergy. Blocking the co-stimulus signal of T cell activation can negatively regulate T cell activity and induce T cell immune tolerance.

The Signals Transduction Molecules of TCR Signaling Pathway

Intracellular signaling is accomplished by the following **kinases and phosphatases**. The molecules involved in TCR signal transduction mainly include upstream kinases (**Lck, Fyn, ZAP-70, Itk),** scaffold proteins (LAT, Gads, SLP-76, Grb2), phospholipases, phosphatases, etc. Activation of TCR signals not only induces T cell proliferation and cytokine production, but also promotes T cell differentiation and T cell function.

The Function T Cell Receptor Signaling

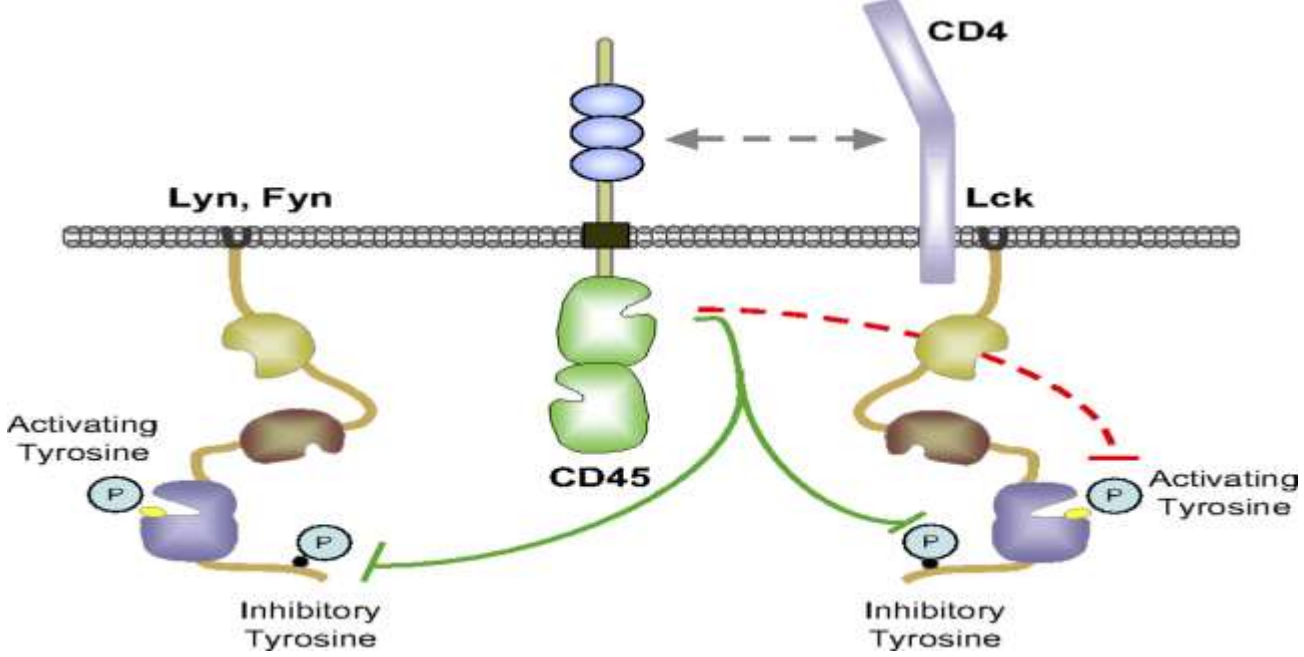
The T cell receptor is located on the surface of T cells, which specifically recognizes antigenic peptides presented by MHC on the surface of antigen-presenting cells, and activates signal pathways such as **ERK, JNK, and NF-κB in T cells**. Through several different signaling pathways, many transcription factors associated with cell division and differentiation are activated to regulate cell functions such as T cell proliferation, differentiation, death, and cytokine release. Typical intracellular signals activated by TCR also include: **MAPK (Mitogen-activated protein kinase), PKC (Protein Kinase C), calcium signal pathways.**

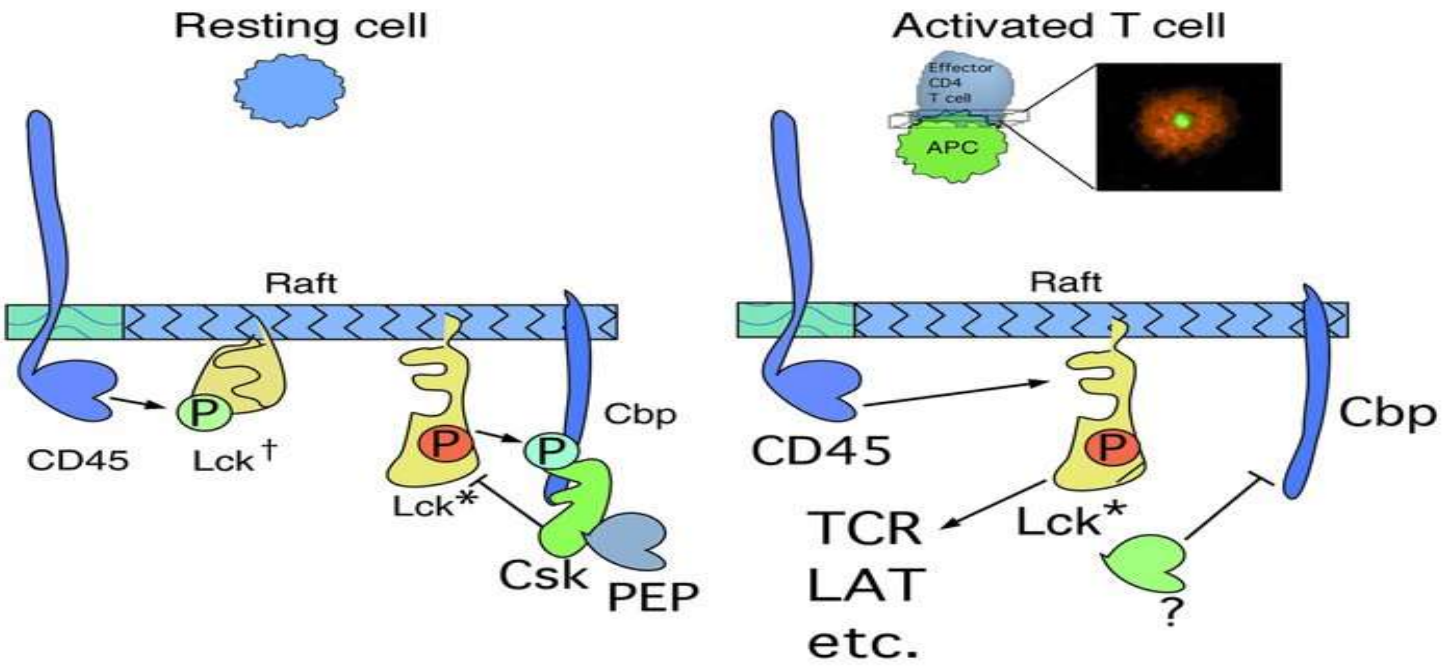
Proximal T-cell signaling cascade.

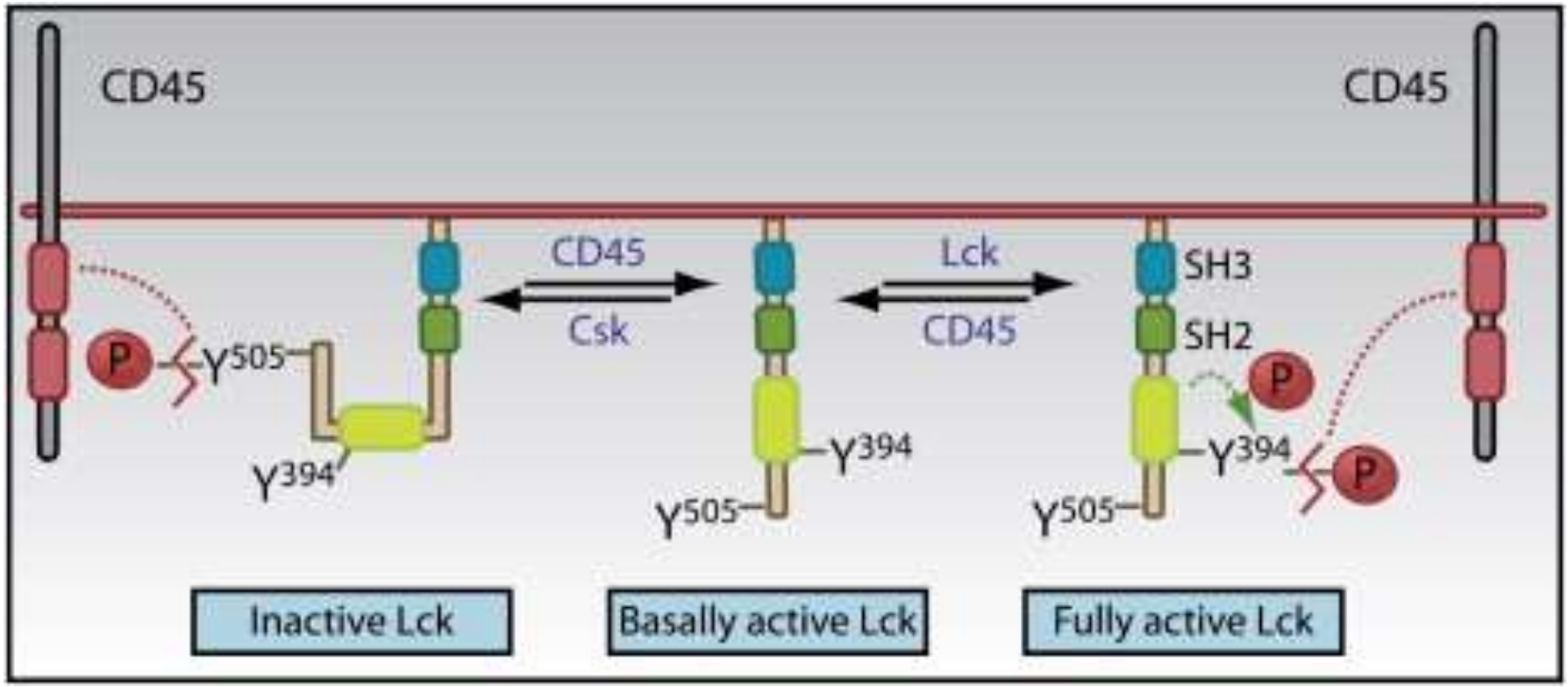
Proximal signaling pathways downstream of the T-cell receptor (TCR)-antigen presenting cell (APC) signaling complex are responsible for the cascade of events leading to metabolic reprogramming including the transcription of amino acid transporter and enzymes involved in metabolism of nutrients and biosynthesis of polyamines .

Phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytoplasmic side of the TCR/CD3 complex engage numerous cascading interactions largely mediated by phosphorylation, dephosphorylation or ubiquitinylation resulting in cellular activation

The initiating signal is generated by lymphocyte protein tyrosine kinase (Lck) and other proto-oncogene tyrosine-protein kinase (Src) family tyrosine kinases including the zeta-chain associated protein kinase (Zap-70) that is recruited to the TCR/CD3 complex.


CD4
Lyn, Fyn
Lck
Activating
Tyrosine
CD45
Activating
Tyrosine
Inhibitory
Tyrosine
Inhibitory
Tyrosine


Resting cell
Activated T cell
Effector
CD4
T cell
APC
Raft
Raft
CD45
Lck†
Lck*
Cbp
Csk
PEP
CD45
Lck*
Cbp
TCR
LAT
etc.
?

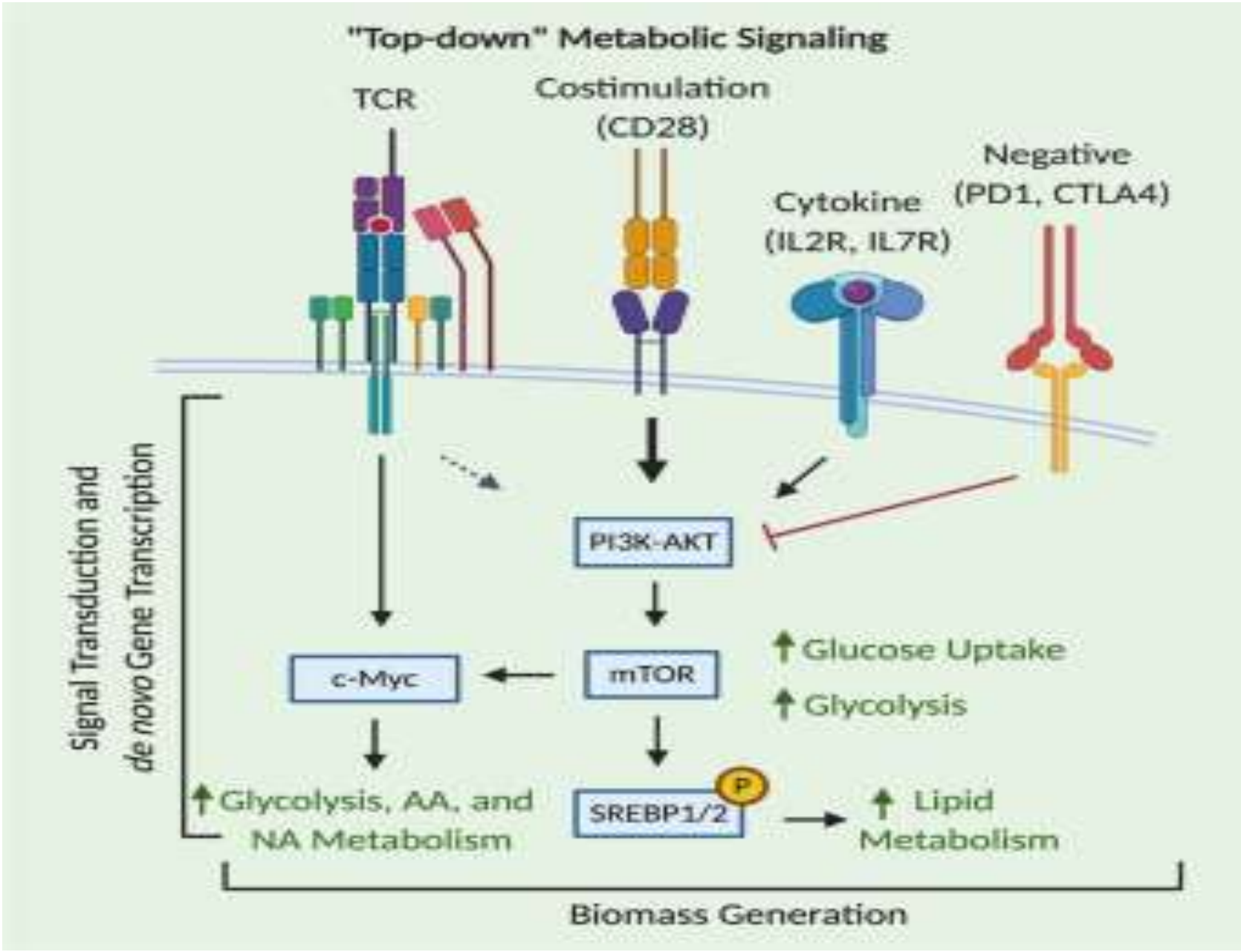

CD45
CD45
CD45
Csk
Lck
CD45
SH3
SH2
P
Y505
Y394
Y394
Y505
Y394
Y505
P
P
Inactive Lck
Basally active Lck
Fully active Lck

Costimulation through leukocyte-associated antigen-1 (LFA1) which is an integrin involved in T-cell migration

or CD28 interaction with CD80 (B7-1) or CD86 (B7-2) activates the phosphorylation of the YXXM or YNPP signaling motifs which regulates glucose metabolism.

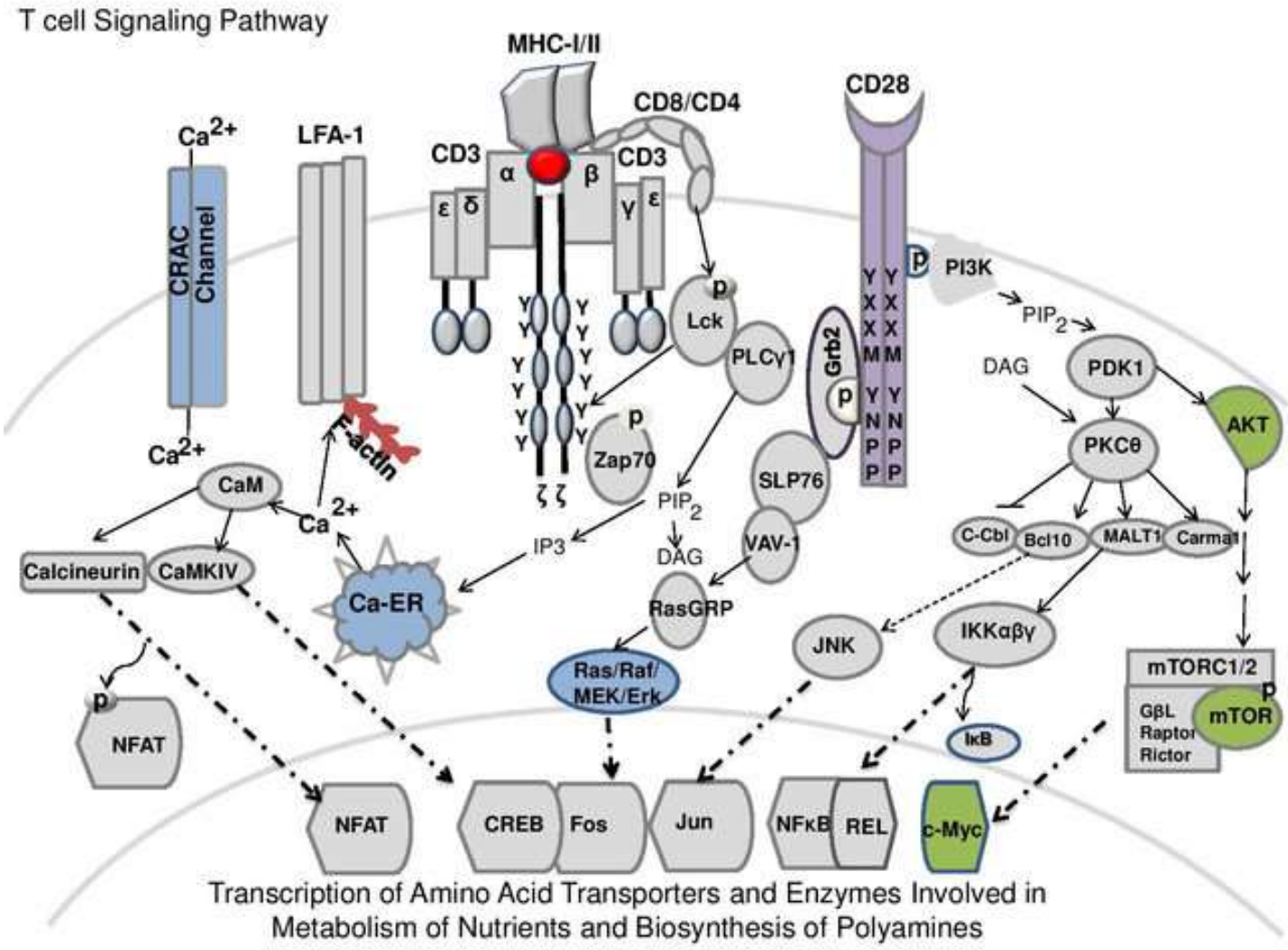
CD28 leads to stable recruitment of the adaptor protein Grb2/GADS along with interleukin-2-indicible T-cell kinase (Itk), Lck, and phosphatidylinositide 3 kinase (PI3K) heterodimer p85/p110 and SLP76.

<u>These interactions promote the activation of VAV-1, RasGRP, and the Ras/Raf/MEK/Erk pathway downstream of phosphorylated SLP-76 and Zap-70 modulating the TCR signal strength .</u>


"Top-down" Metabolic Signaling
TCR
Costimulation
(CD28)
Negative
(PD1, CTLA4)
Cytokine
(IL2R, IL7R)
PI3K-AKT
c-Myc
mTOR
Glucose Uptake
Glycolysis
Signal Transduction and
de novo Gene Transcription
Glycolysis, AA, and
NA Metabolism
SREBP1/2
P
Lipid
Metabolism
Biomass Generation

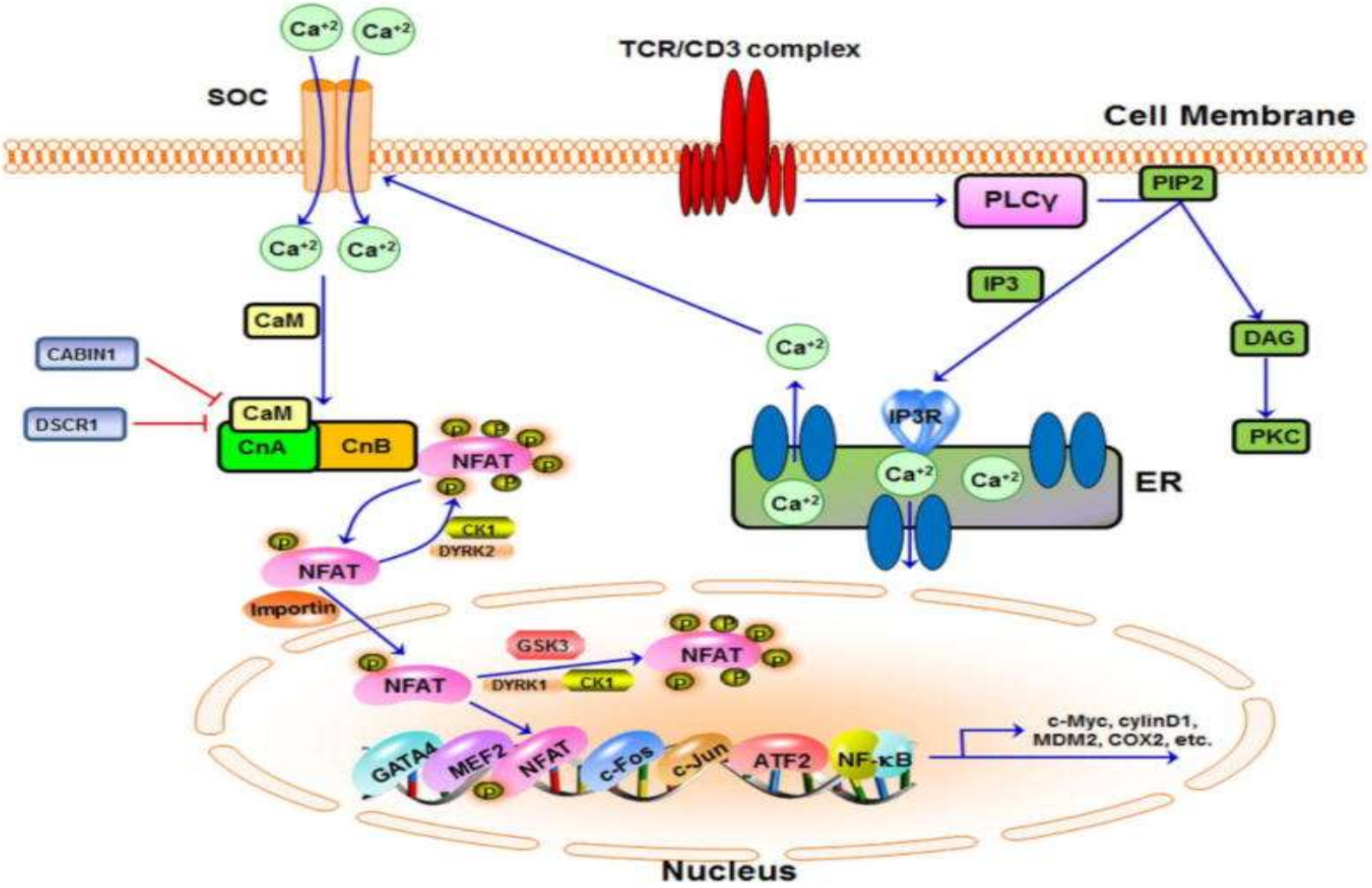
A complement of transcription factors nuclear factor of **activated T-cells (NFAT),** cAMP response element-binding protein (CREB), Fox family transcription factor c-Fos, Jun (when in combination with c-Fos forms the AP-1 early response transcription factor complex, nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), an NFκB family member c-Rel, and c-Myc which coordinately regulate gene expression. Activation of CD28 leads to the phosphorylation of PI3K, phosphatidylinositol-3,4 bisphosphate (PIP2) and phosphoinositide-dependent kinase 1 (PDK1) which integrates the TCR and CD28 signaling to induce the NFκB pathway including protein kinase C-theta (PKC-θ), and inhibits the ubiquitin ligase c-Cbl leading to activation of Bcl10, Malt1, Carma1 (CBM) complex leading to IKKαβγ activation of NFκB and REL .

In addition to PKC-θ, phosphorylation of Akt is critical for the regulation of mTORC1 and mTORC2 complexes of mTOR that bind GβL and raptor or rictor, respectively. This is a critical step in c-Myc-dependent transcriptional regulation that stimulates dramatic changes in metabolism including glucose, amino acid, nucleotide and polyamine biosynthesis . Divalent cations such as calcium (Ca 2+) are induce downstream of phospholipase C γ1, PIP2, and indo inositol-1,4,5 triphosphate (IP3) which mobilizes the release of intracellular Ca 2+ stores from the endoplasmic reticulum (Ca 2+-ER) a potential metabolic switch that suppresses intratumoral T-cell function. Sustained signaling then promotes the influx of extracellular Ca 2+ into the cells through calcium release-activated Ca 2+ (CRAC) channels. Calcium-calmodulin interactions (Ca 2+ /CaM) then activates the phosphatase calcineurin and calcium/calmodulin-dependent protein kinase type IV calmodulin (CaMKIV), which dephosphorylates the cytoplasmic subunits of nuclear factor of activated T-cells (NFAT) exposing a nuclear localization signal resulting in nuclear transport and phosphorylates CREB, respectively



tyrosines in the activation loop of the ZAP-70 kinase domain are phosphorylated by Lck or by ZAP-70 itself in *trans* to further promote its catalytic activity. A number of signaling proteins, **including the linker for the activation of T cells (LAT) and the SH2-domain-containing leukocyte protein of 76 kDa (SLP-76)**, are subsequently phosphorylated by active ZAP-70. The phosphorylated LAT and SLP-76 proteins function as scaffolds to recruit many other signaling molecules . The consequences of these early signaling events eventually lead to T-cell activation, proliferation, and differentiation.

Nuclear factor of **activated T cells** (**NFAT**) is a transcription factor regulated by calcium influx. When phosphorylated, it is confined to the **cell** cytoplasm where it is inactive. After **T-cell activation**, the ensuing calcium influx **activates** the phosphatase calcineurin that **activates NFAT** by dephosphorylating it.



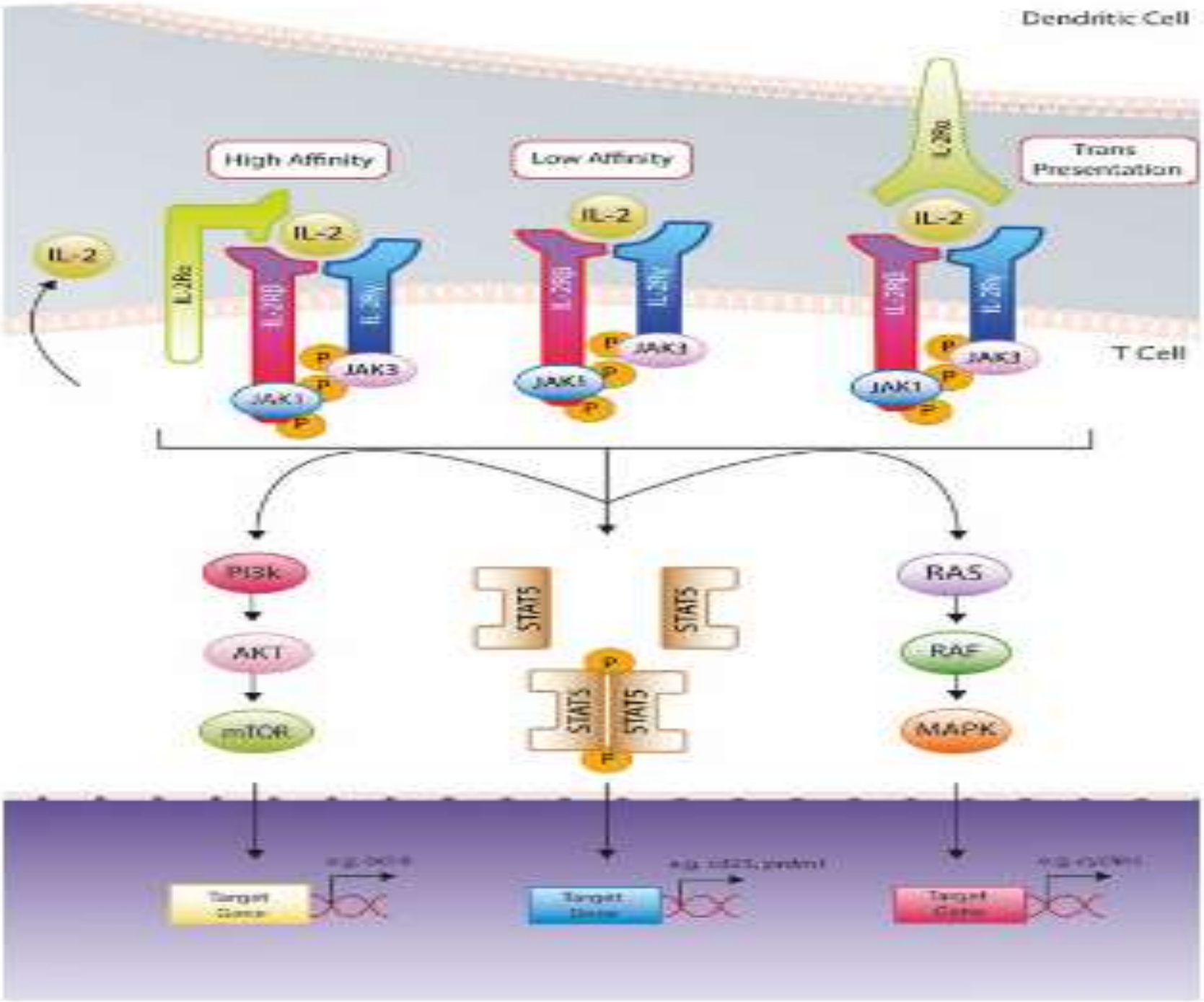
(NFAT)p stay in cytoplasm

Calcineurin dephosphorylate NFAT------ (NFAT)N nuclear localization

Actually NFAT phosphorylation mask the NLS of NFAT, dephopshorylation Unmask NLS.

The **interleukin-2 receptor** (**IL-2R**) is a heterotrimeric protein expressed on the surface of certain immune cells, such as lymphocytes, that binds and responds to a cytokine called IL-2.

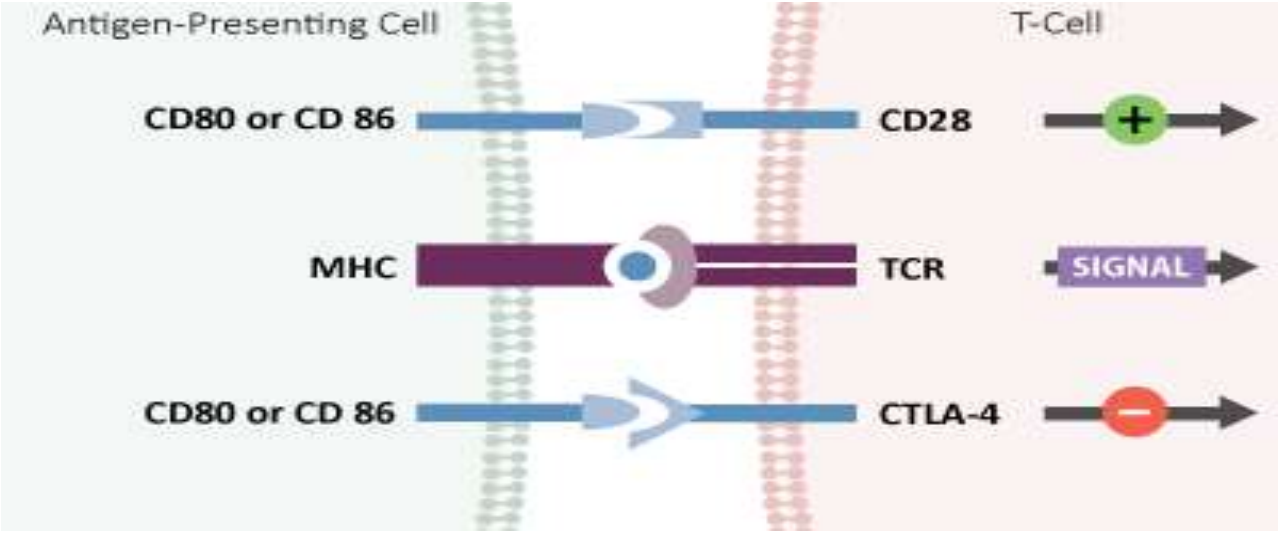
The three receptor chains are expressed separately and differently on various cell types and can assemble in different combinations and orders to generate low, intermediate, and high affinity IL-2 receptors.
The α chain binds IL-2 with low affinity, the combination of β and γ together form a complex that binds IL-2 with intermediate affinity, primarily on memory T cells and NK cells; and all three receptor chains form a complex that binds IL-2 with high affinity (Kd ~ 10^{-11} M) on activated T cells and regulatory T cells. The intermediate and high affinity receptor forms are functional and cause changes in the cell when IL-2 binds to them

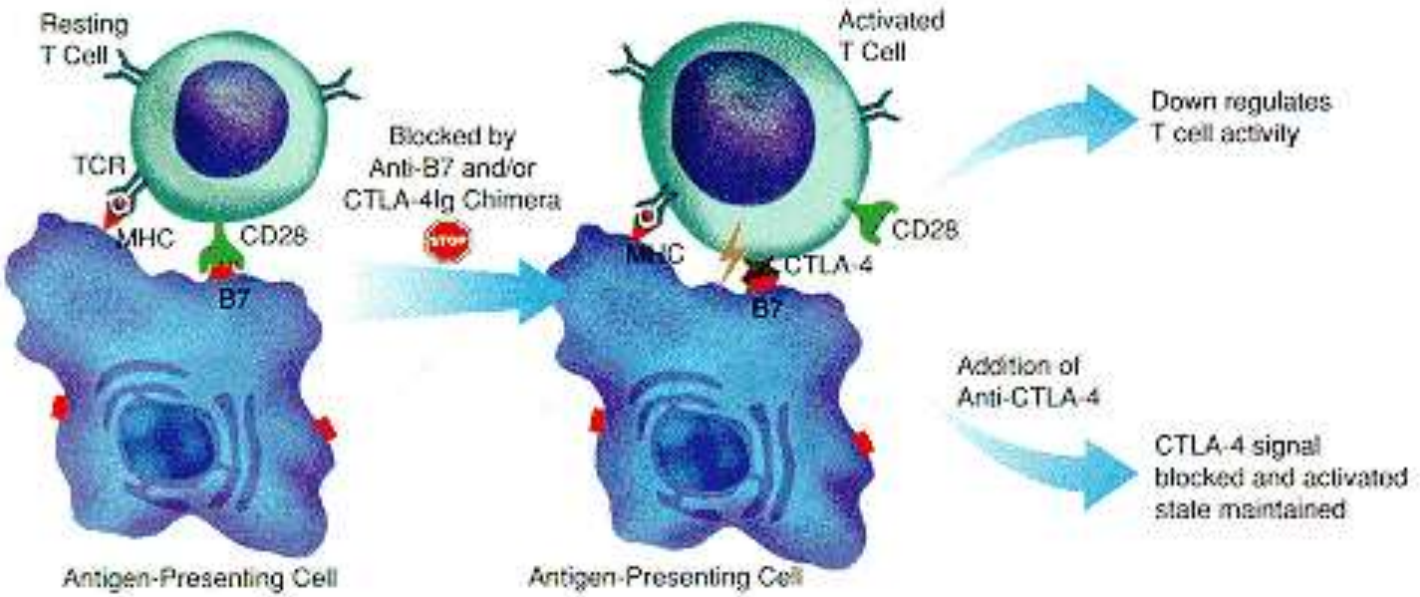

Dendritic Cell
High Affinity
Low Affinity
Trans Presentation
IL-2
IL-2Rα
IL-2Rβ
IL-2Rγ
JAK3
JAK1
P
T Cell
PI3K
AKT
mTOR
STAT5
RAS
RAF
MAPK
Target Gene

CD28 can be considered a highly expressed but low-affinity receptor, whereas **CTLA-4** is a low abundance but higher-affinity receptor where both receptors interact with CD80 and CD86. ... On T cells **CD28** expression is constitutive whereas **CTLA-4** is not expressed by resting T cells.

CTLA4 or **CTLA-4** (**cytotoxic T-lymphocyte-associated protein 4**), also known as **CD152** (cluster of differentiation 152), is a protein receptor that functions as an immune checkpoint and downregulates immune responses. CTLA4 is constitutively expressed in regulatory T cells but only upregulated in conventional T cells after activation – a phenomenon which is particularly notable in cancers. It acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells.

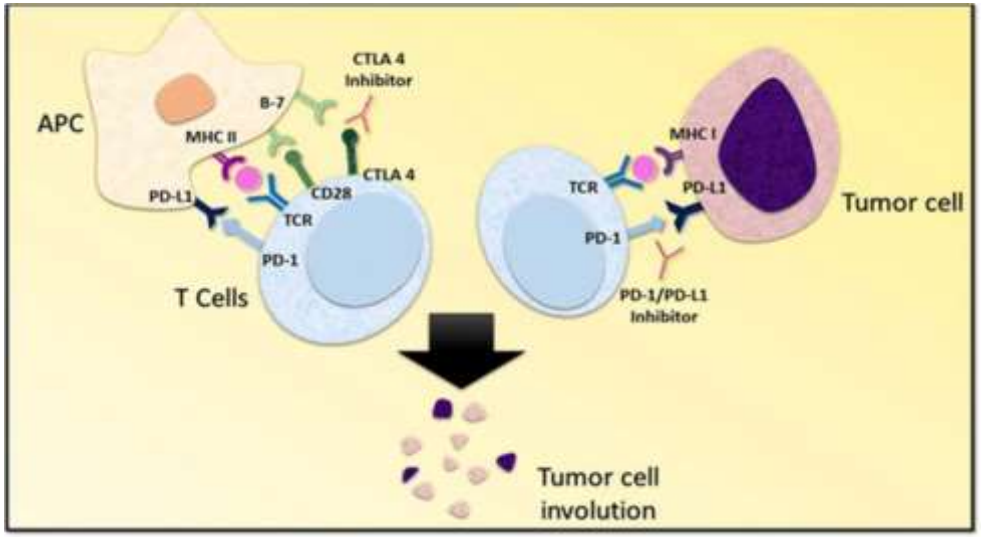
The mechanism by which CTLA-4 acts in T cells remains somewhat controversial. Biochemical evidence suggested that CTLA-4 recruits a phosphatase to the T cell receptor (TCR), thus attenuating the signal. This work remains unconfirmed in the literature since its first publication. More recent work has suggested that CTLA-4 may function in vivo by capturing and removing B7-1 and B7-2 from the membranes of antigen-presenting cells, thus making these unavailable for triggering of CD28


Antigen-Presenting Cell
T-Cell
CD80 or CD 86
CD28
+
MHC
TCR
SIGNAL
CD80 or CD 86
CTLA-4
−


Resting
T Cell
TCR
MHC
CD28
B7
Antigen-Presenting Cell
Blocked by
Anti-B7 and/or
CTLA-4Ig Chimera
STOP
Activated
T Cell
MHC
CTLA-4
CD28
B7
Antigen-Presenting Cell
Down regulates
T cell activity
Addition of
Anti-CTLA-4
CTLA-4 signal
blocked and activated
state maintained

Programmed **cell** death **protein** 1 (PD1) is an inhibitory receptor that is expressed by all T cells during activation. It regulates T **cell** effector functions during various physiological responses, including acute and chronic infection, cancer and autoimmunity, and in immune homeostasis

Programmed death-1 (PD-1), also known as CD279, is another important negative costimulatory molecule following the discovery of CTLA4. Its ligand is PD-L1, also known as CD274, which, like PD-1, belongs to the CD28/B7 family. PD-1 binds to its ligand and transmits a negative costimulatory signal, inhibits T lymphocyte proliferation, and plays a key role in regulating T cell activation and immune tolerance.



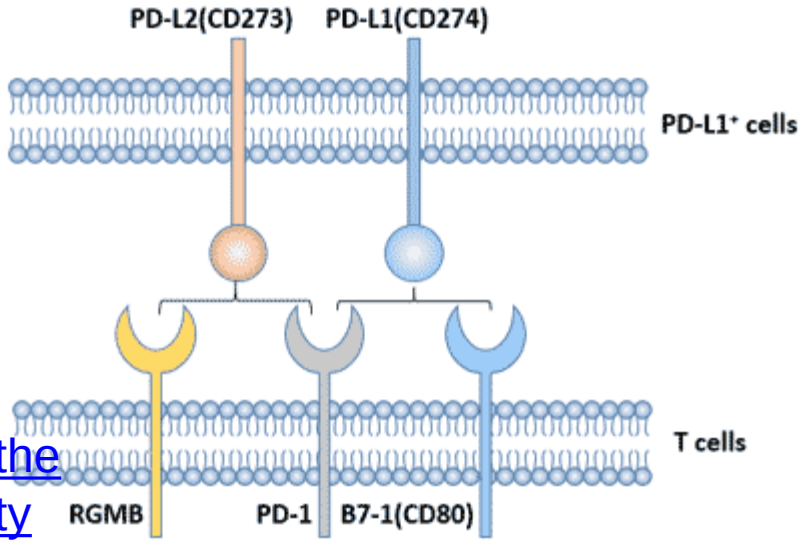
Programmed cell death protein 1, also known as PD-1 and CD279, is a cell surface receptor that plays an important role in down-regulating the immune system and promoting self-tolerance by suppressing T cell inflammatory activity. In humans, it is encoded by the PDCD1 gene .

PD-1 is mainly expressed in activated CD4 + and CD8 + T cells, activated B cells, natural killer (NK) cells, natural killer T cells, dendritic cells (DC) and activated monocytes, and is closely related to their differentiation and apoptosis.

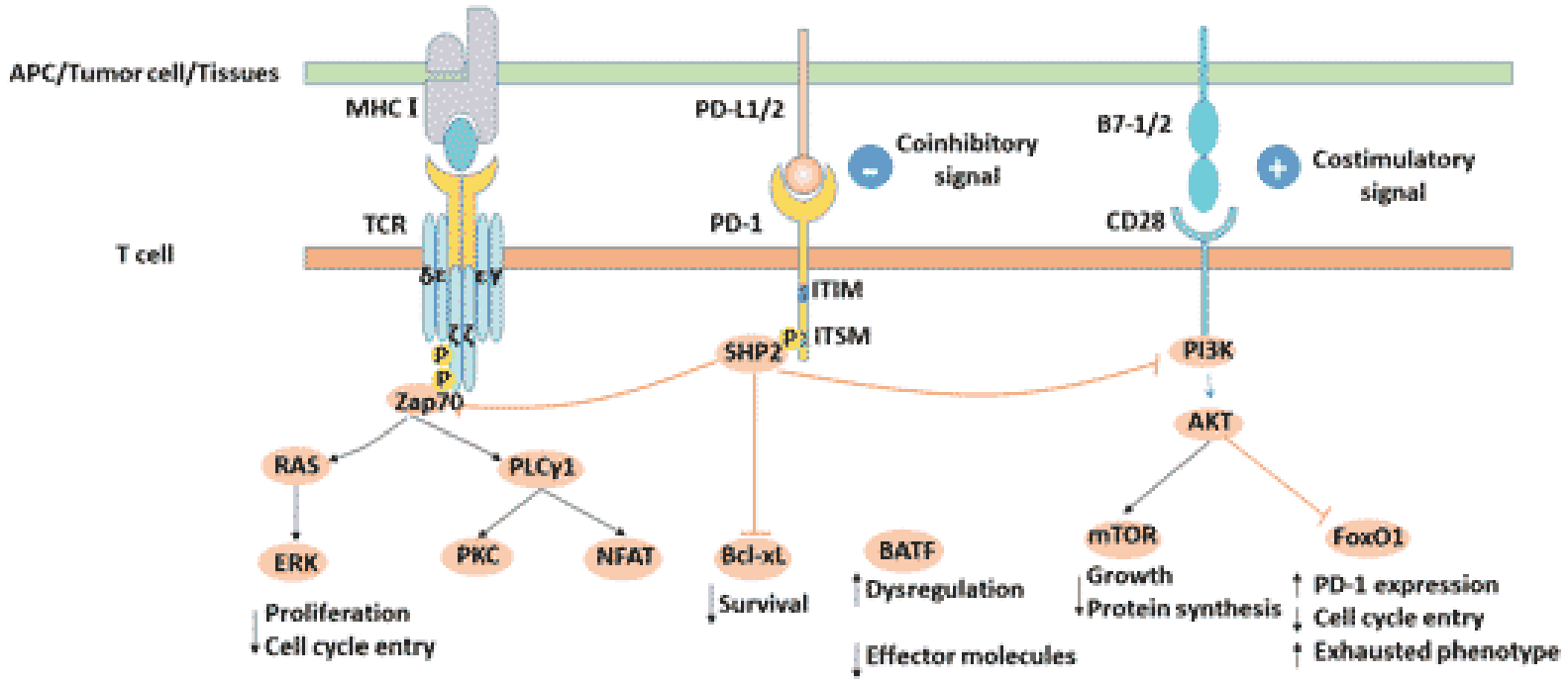
The expression of PD-1 can be induced by TCR or B cell receptors, and tumor necrosis factor can enhance the expression of PD-1.

Extensive expression of PD-1 suggests that it has an essential role in maintaining a negative immune response in the body.

The ligands of PD-1 are mainly PD-L1 and PD-L2, and PD-L1 is the main ligand , which is highly expressed in various malignant tumors.



RGMB enhances the suppressive activity



PD-L1/PD-1 Signaling Pathway

After PD-1 binds to its ligand **PD-L1, tyrosine in the ITSM domain of PD-1 cytoplasmic region is phosphorylated, and SHP2 phosphatase molecules are recruited to cell membrane , and further inhibition of PI3K/PKB mediated Zap-70/CD3 zeta and PKC theta phosphorylation, inhibiting T cell proliferation, differentiation and cytokine production**

Function of PD-1 / PD-L1 Signal Pathway

IFN-γ up-regulates PD-L1 on the surface of normal tissue cells and different tumor tissues, and down-regulates the immune response of the tumor microenvironment by inducing PD-L1 expression.

After T cells are stimulated by inflammatory signals, PD-1 is induced to produce and limit the function of T cells in a variety of peripheral tissues dominated by infections and tumors, thereby limiting the amplification and duration of the immune response, thereby avoiding damage to normal tissue.

Although the physiological functions of PD-L1 and PD-1 are up-regulated to avoid the expansion of inflammation and reduce tissue damage, the induced production of PD-L1 in the tumor microenvironment will promote the apoptosis of activated T cells and stimulate the production of IL-10 in human peripheral blood T cells to regulate the immunosuppression.

The interaction of PD-1/PD-L1 plays an important role in inhibiting T cells in vivo. PD-L1 binds to receptor PD-1 on T cell membrane to produce an inhibitory signal, which can prevent proliferation and activation of CD4+ T cells and CD8+ T cells, down-regulate the expression of some anti-apoptotic molecules and pro-inflammatory factors, and change the tumor microenvironment.

This weakens the ability of the body to monitor and clear tumor cells, resulting in immune escape, allowing tumor cells to proliferate indefinitely in the body [10]. Therefore, blocking the PD-1/PD-L1 pathway restores the killing effect of the immune system on tumor cells.

PD-1/PD-L1 Signaling Pathway and Chronic Viral Infection

Virus infection can lead to increased expression of PD-1 and **PD-L1 on the surface of antigen-specific T cells, inhibit proliferation of CD4 + and CD8 + T cells, reduce secretion or spread of cytokines interleukin-2 and interferon gamma , and lead to decreased immune function or even failure of specific T lymphocytes.**

This weakens the host's anti-infective immune response, leading to damage to the target organ, which ultimately leads to the development of related diseases such as persistent viral infection. **This suggests that the PD-1/PD-L1 pathway may be one of the leading causes of chronic viral infection and chronic disease.**

The PD-1/PD-L pathway has been shown to be associated with chronic viral infections such as HIV, HBV, HCV .

In chronic viral infection, CD8+T cells can be reactivated, proliferated and differentiated by blocking PD-1 with antibodies, and their virus-killing ability can be restored, resulting in decreased viral titer

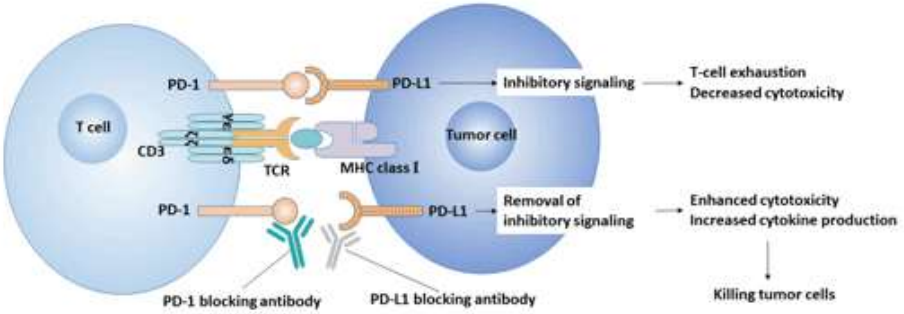
PD-1/PD-L and Tumor

In humans, antigens expressed in tumor tissue are recognized by host T cells but not necessarily eliminated.

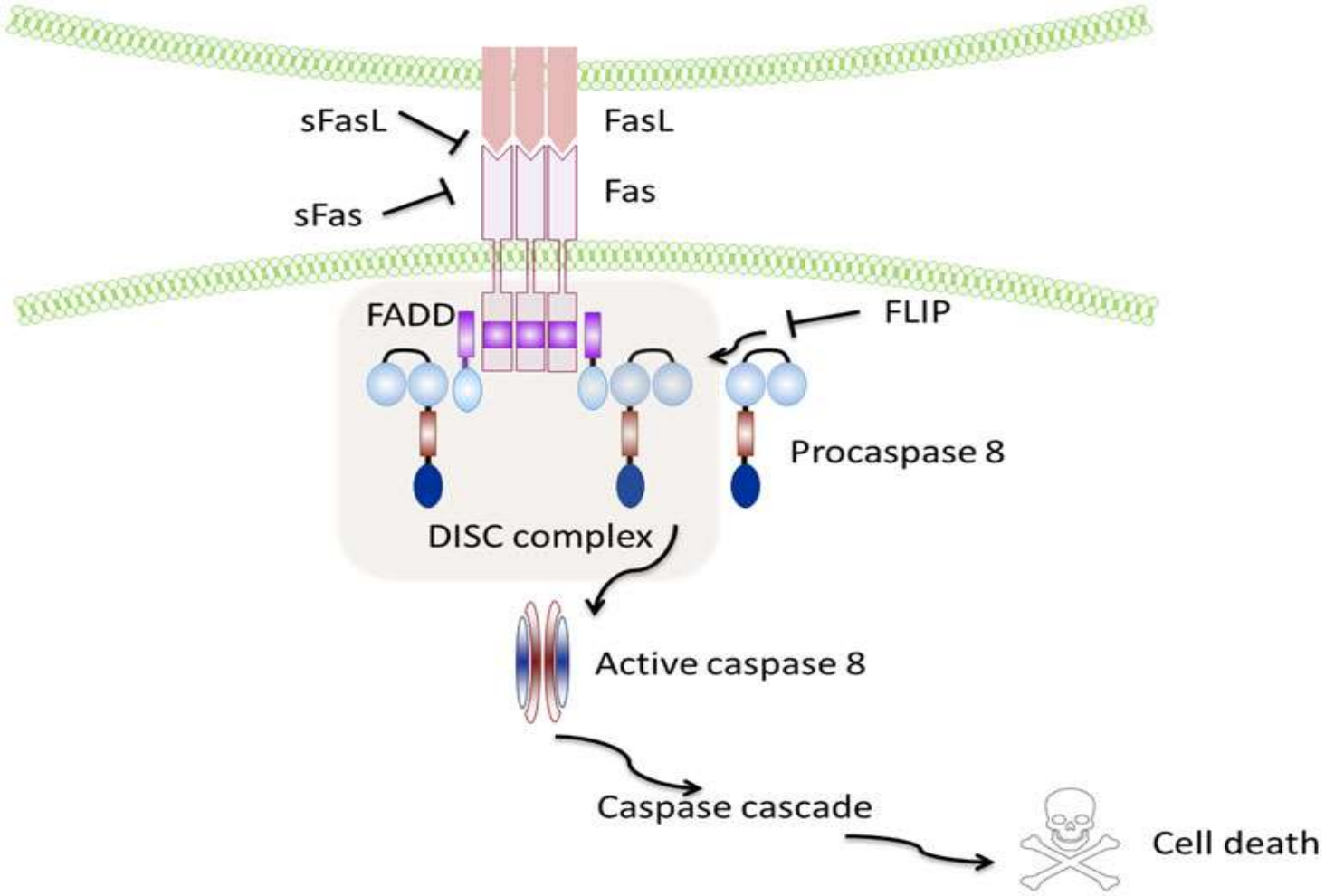
One of the reasons is the **up-regulated expression of PD-L1 and PD-1 in the tumor microenvironment. The expression of PD-L1 in tumor tissues (such as lymphoma, choriocarcinoma, melanoma, esophageal cancer) and up-regulation of PD-1 expression on tumor-infiltrating lymphocytes are involved in related immunosuppressive signaling.**

Activation of PD-1/PD-L1 signaling pathway inhibits proliferation and activation of CD4+ T cells and CD8+ T cells, inhibits the expression of cytokines, changes the tumor microenvironment. This promotes tumor cells to evade immune surveillance and killing . Therefore, the PD-1/PD-L pathway has become a new target for the treatment of tumors.

At present, the main anti-PD-1/PD-1 antibodies are nivolumab, Pembrolizumab, ipidilizumab, avelumab, atezolizumab and durvalumab. Among them, nivolumab and pembrolizumab are PD-1 antibodies, and avelumab, atezolizumab and durvalumab are PD-L1 antibodies.



T cell death


sFasL
FasL
sFas
Fas
FADD
FLIP
Procaspase 8
DISC complex
Active caspase 8
Caspase cascade
Cell death

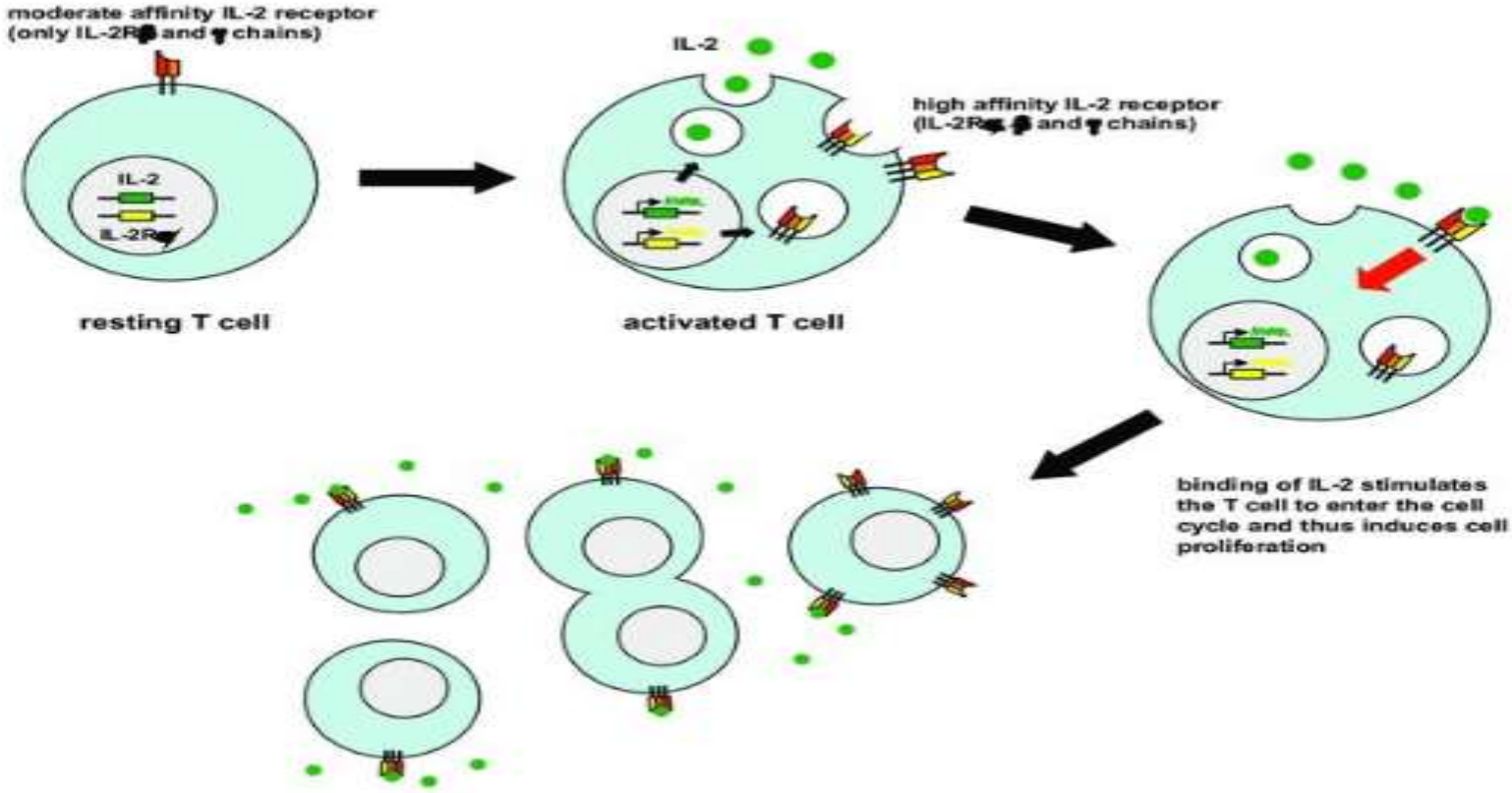
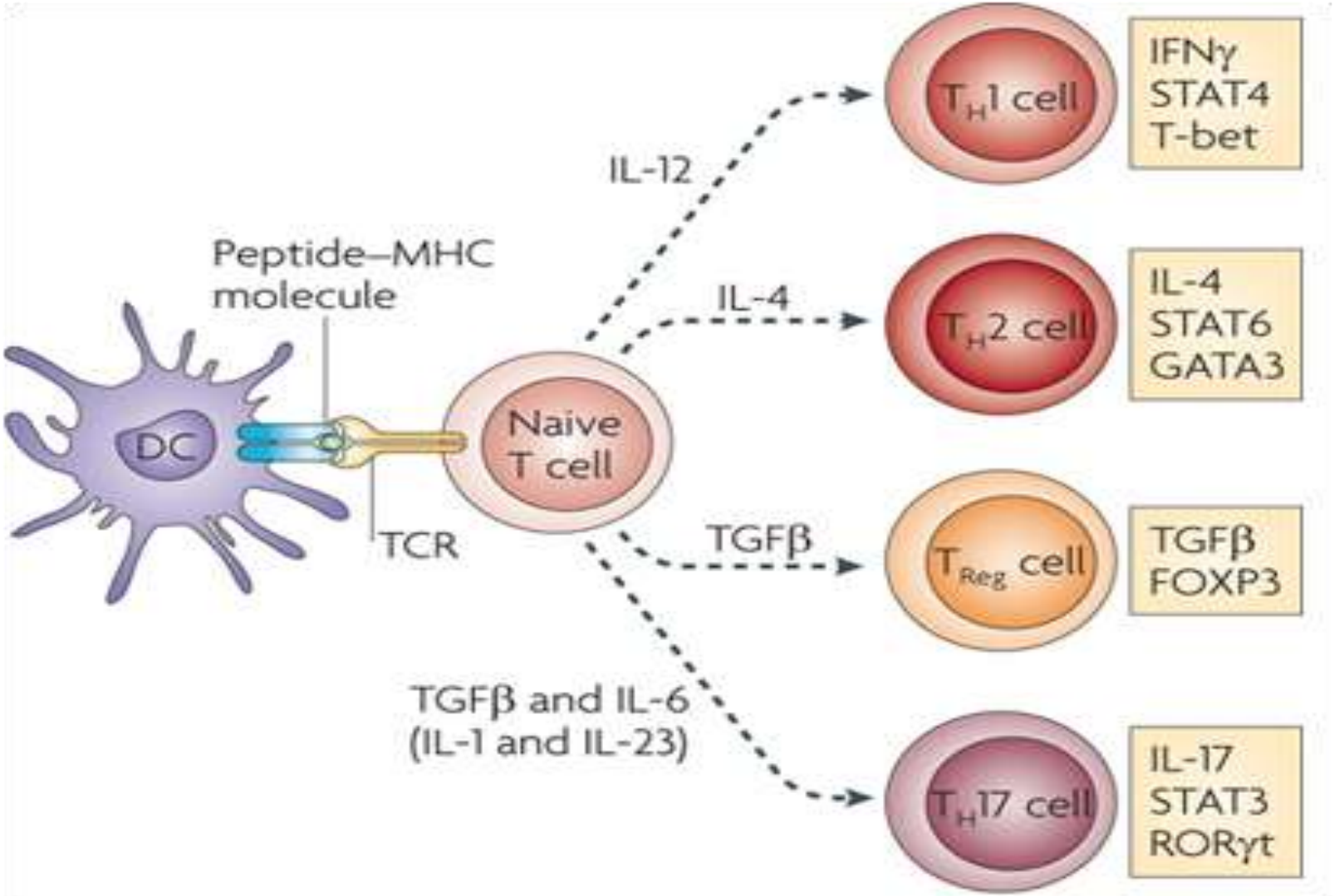
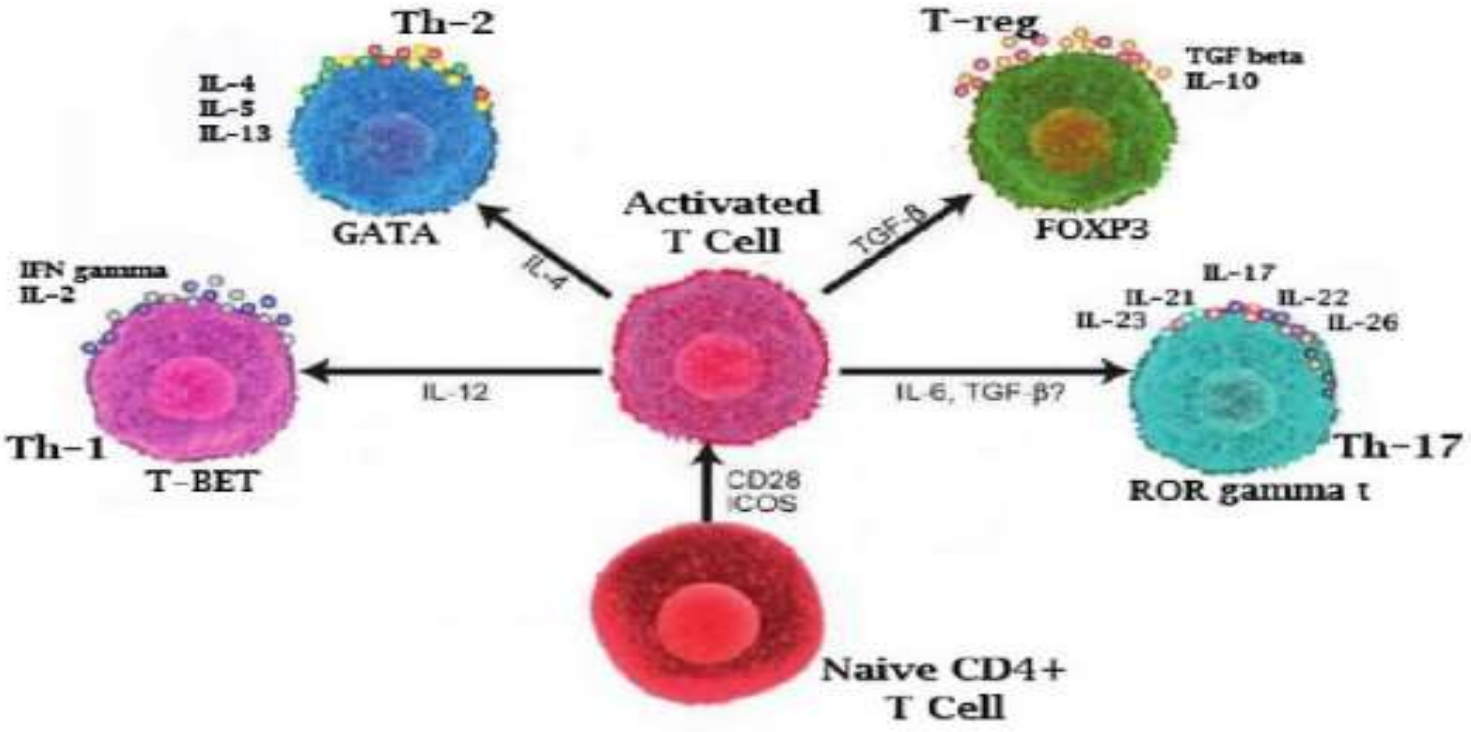


Figure 8. IL-2 secretion. IL-2 secreted by activated T cells induces T cell proliferation. Resting T cells express moderate affinity IL-2 receptor consisting of only the β and γ chains. Once T cells become activated they start to express IL-2 and the IL-2Rα chain. IL-2 binds then to this high affinity IL-2 receptor consisting of an α, a β and a γ chain, and thus promotes T cell proliferation in an autocrine fashion.

Differentiation of helper $CD4^+$ T cell subsets. After activation by antigen-presenting cells, $CD4^+$ T cells can differentiate into several subsets: T helper 1 (T_H1), T_H2, T_H17 and regulatory T cells. The differentiation of each T cell subset is regulated by different transcription factors





Pathways of differentiation, transcription factors expression and cytokine secretion profile of various subpopulations of CD4 T lymphocytes: Th1 cells are generated under the influence of IL-12, by the T-BET transcription factor expression, and secrete IL-2 and IFN- γ ; Th2 cells appear under the influence of IL-4 , by the GATA transcription

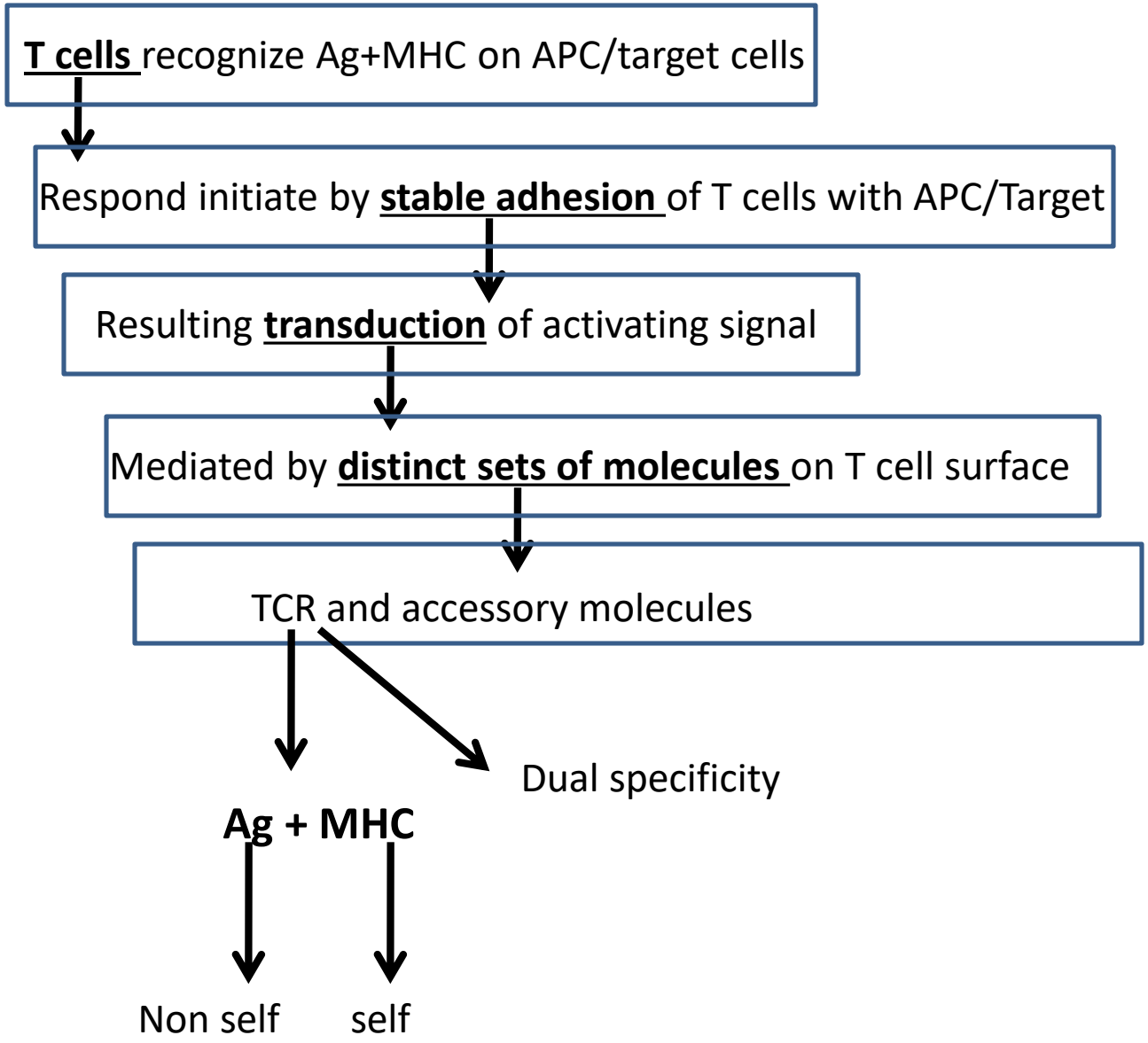
one of the best characterized of the costimulatory molecules is the receptor CD40. This receptor, a member of the tumor necrosis factor receptor family, is expressed by B cells, professional antigen-presenting cells, as well as non-immune cells and tumors. CD40 binds its ligand CD40L, which is transiently expressed on T cells and other non-immune cells under inflammatory conditions. A wide spectrum of molecular and cellular processes is regulated by CD40 engagement including the initiation and progression of cellular and humoral adaptive immunity

CD40L/CD40 interactions exert profound effects on DCs, B cells, and endothelial cells, among many cells of the hematopoietic and non-hematopoietic compartments. It has been demonstrated that CD40 engagement on the surface of DCs promotes their cytokine production, the induction of costimulatory molecules on their surface, and facilitates the cross-presentation of antigen

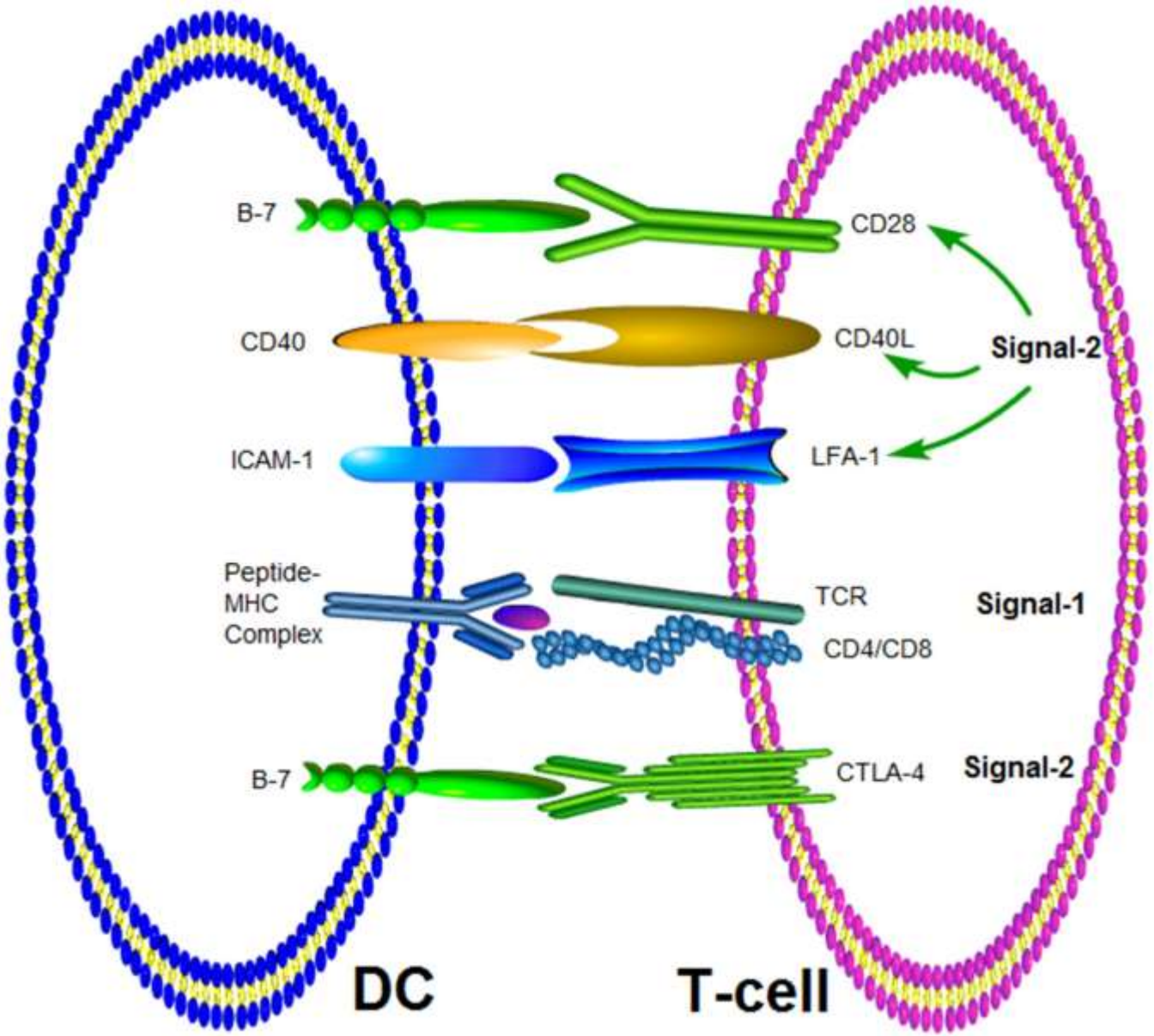
Overall, the impact of CD40 **signaling 'licenses' DCs** to mature and achieve all of the necessary characteristics to effectively trigger T-cell activation and differentiation. **CD40 signaling of B cells promotes germinal center (GC) formation, immunoglobulin (Ig) isotype switching, somatic hypermutation (SHM) of the Ig to enhance affinity for antigen, and finally the formation of long-lived plasma cells and memory B cells**

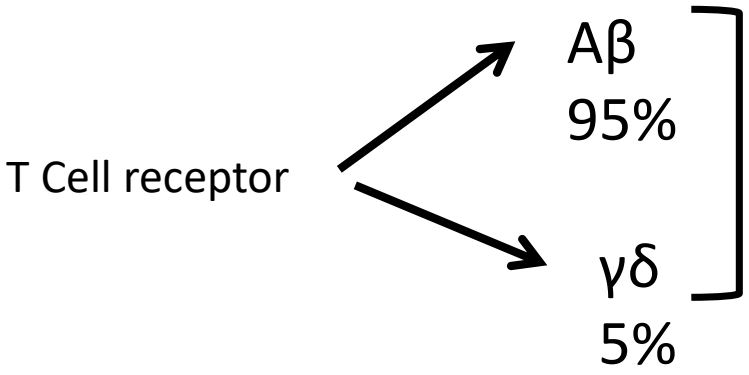
T cell receptor

(All graphics from internet)


T cells recognize Ag+MHC on APC/target cells
Respond initiate by stable adhesion of T cells with APC/Target
Resulting transduction of activating signal
Mediated by distinct sets of molecules on T cell surface
TCR and accessory molecules
Dual specificity
Ag + MHC
Non self
self

Basic Interaction of T cells and APC





Transmembrane heterodimer

Disulphide bond

Each α and β chain -----N terminal variable region, one Ig like constant domain
A hydrophobic transmembrane domian
Short cytoplasmic trail

N terminal variable region and CDR

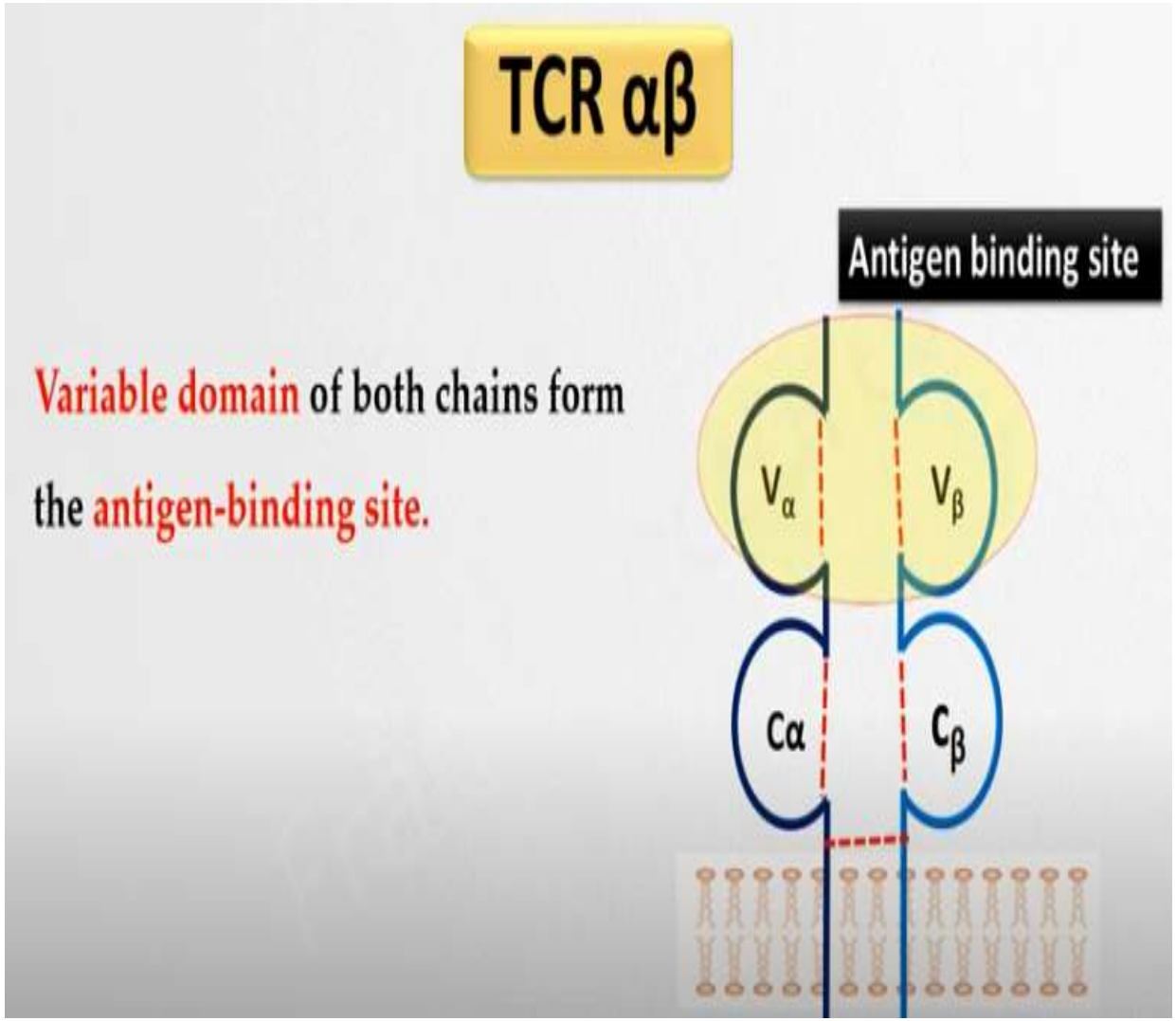
Key Concepts in T-cell Receptor (TCR)

1. T-cell antigen receptor (TCR) is similar to the F(ab) of Ab but only located on the surface of T cells
   => No secreted form
   => Two major types: αβ TCR and γδ TCR

2. TCR functions to recognize Ag peptide and then to activate T cells => Adaptive immunity

3. Ag recognition by αβ TCR requires Ag presented by Major Histocompatibility Complex (MHC).
   => consider both Ag peptide & MHC
   => Cell-Cell interaction

4. The Ag-binding site region of the TCR is formed by the Vα and Vβ regions.



α and γ VJ

β and δ VDJ

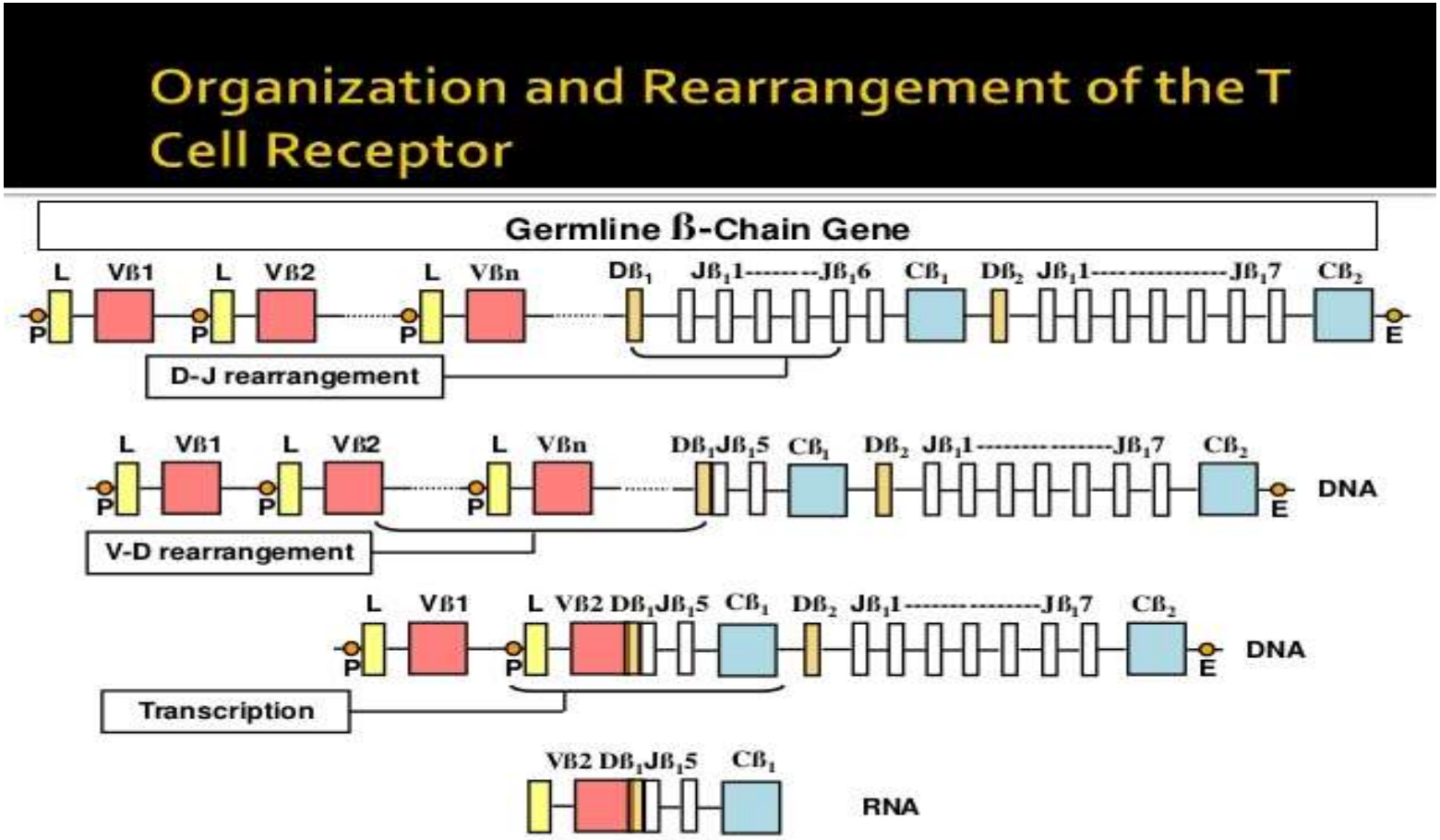
δ chain within V α and J α

Thus productive Rearrangement of α delete δ chain
Aβ and γδ cannot be Coexpressed in same cells

Multiple germ line gene segment, mechnisim is same as B cells , RAG1/2, TDT, RSS etc
Antibody and TCR cannot express in same cells ----------

Difference between TCR and antibody

TCR is not secreted no effector function,no isotype switching, no affinity maturation

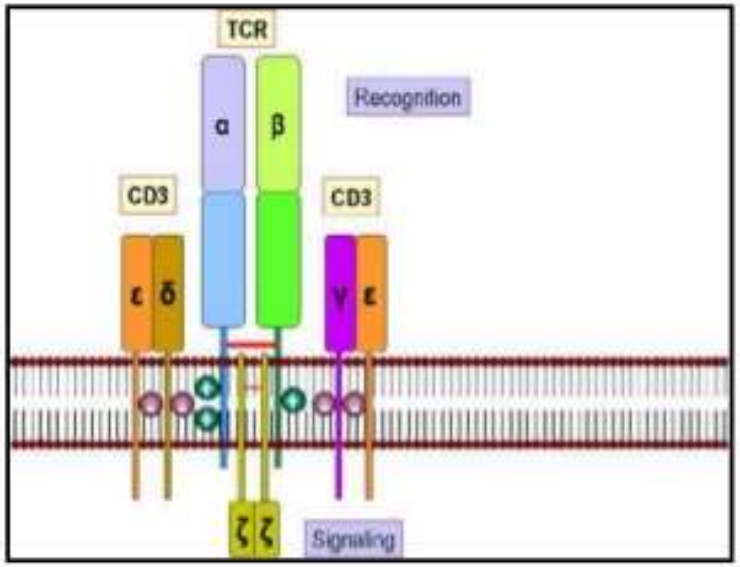


γδ TCR

- Gamma/Delta T cells can recognize antigen in an MHC-independent manner
- Gamma/Delta T cells play a role in responses to certain viral and bacerial pathogens

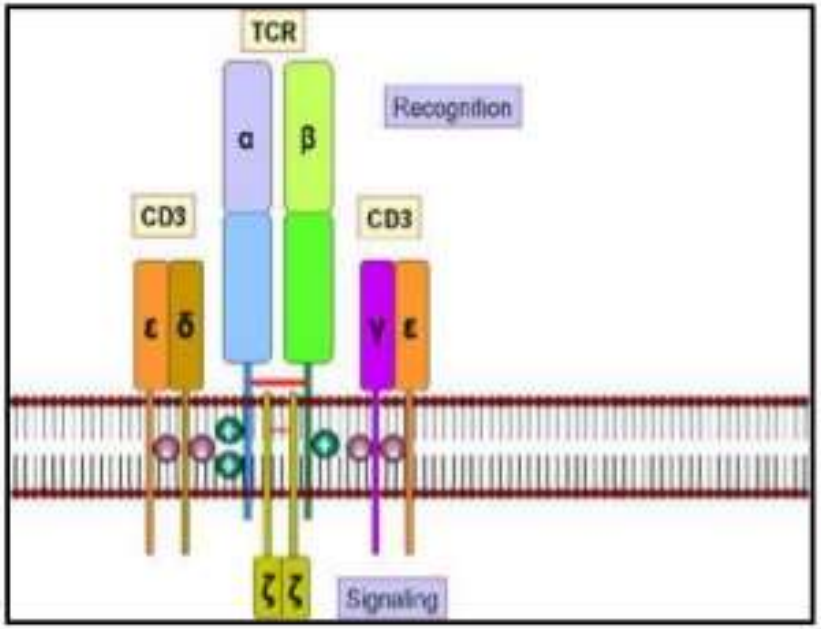
TCR and CD3 Complex

- TCR is closely associated with a group of 5 proteins collectively called the CD3 complex
  - γ chain
  - δ chain
  - 2 ε chains
  - 2 ξ chains
- CD3 proteins are invariant

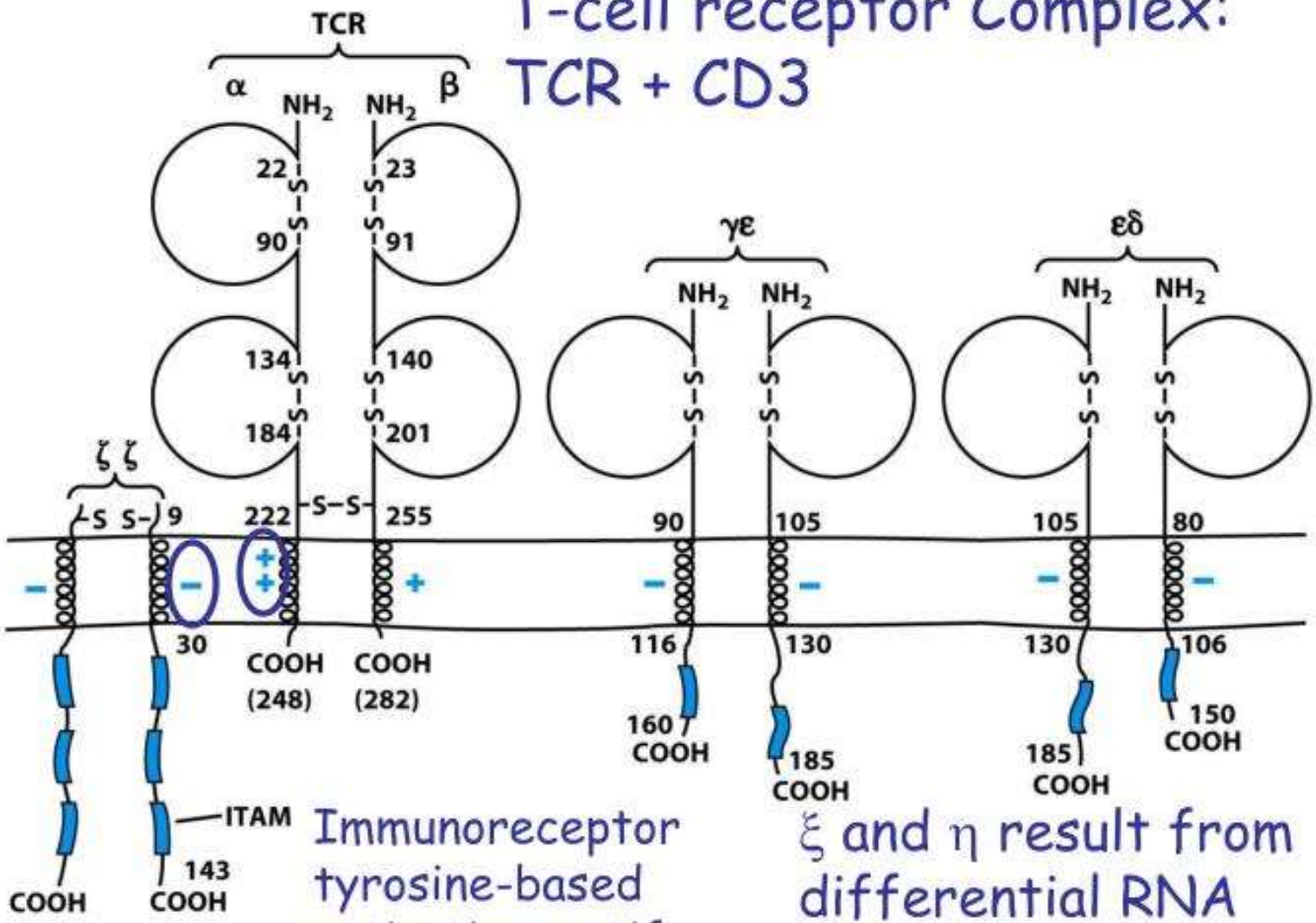


Role of CD3 Complex

- CD3 complex necessary for cell surface expression of TCR during T cell development
- CD3 complex transduces signals to the interior of the cells following interaction of Ag with the TCR



T-cell receptor Complex: TCR + CD3



Immunoreceptor tyrosine-based activation motif

ξ and η result from differential RNA splicing

Figure 9-9a
Kuby IMMUNOLOGY, Sixth Edition

TCR accessory proteins

Ig domains

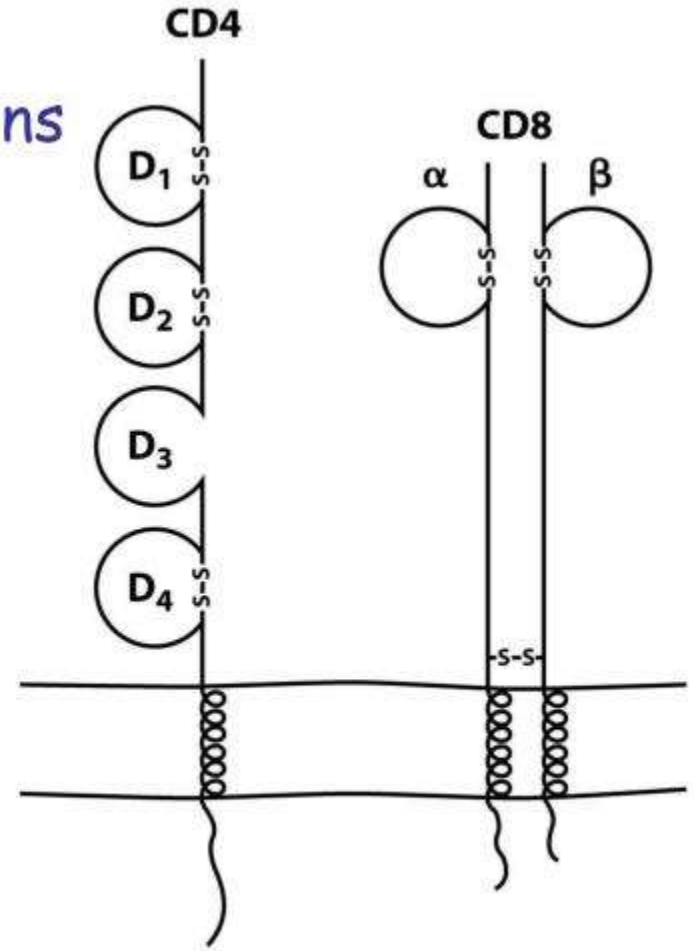


Figure 9-10
Kuby IMMUNOLOGY, Sixth Edition

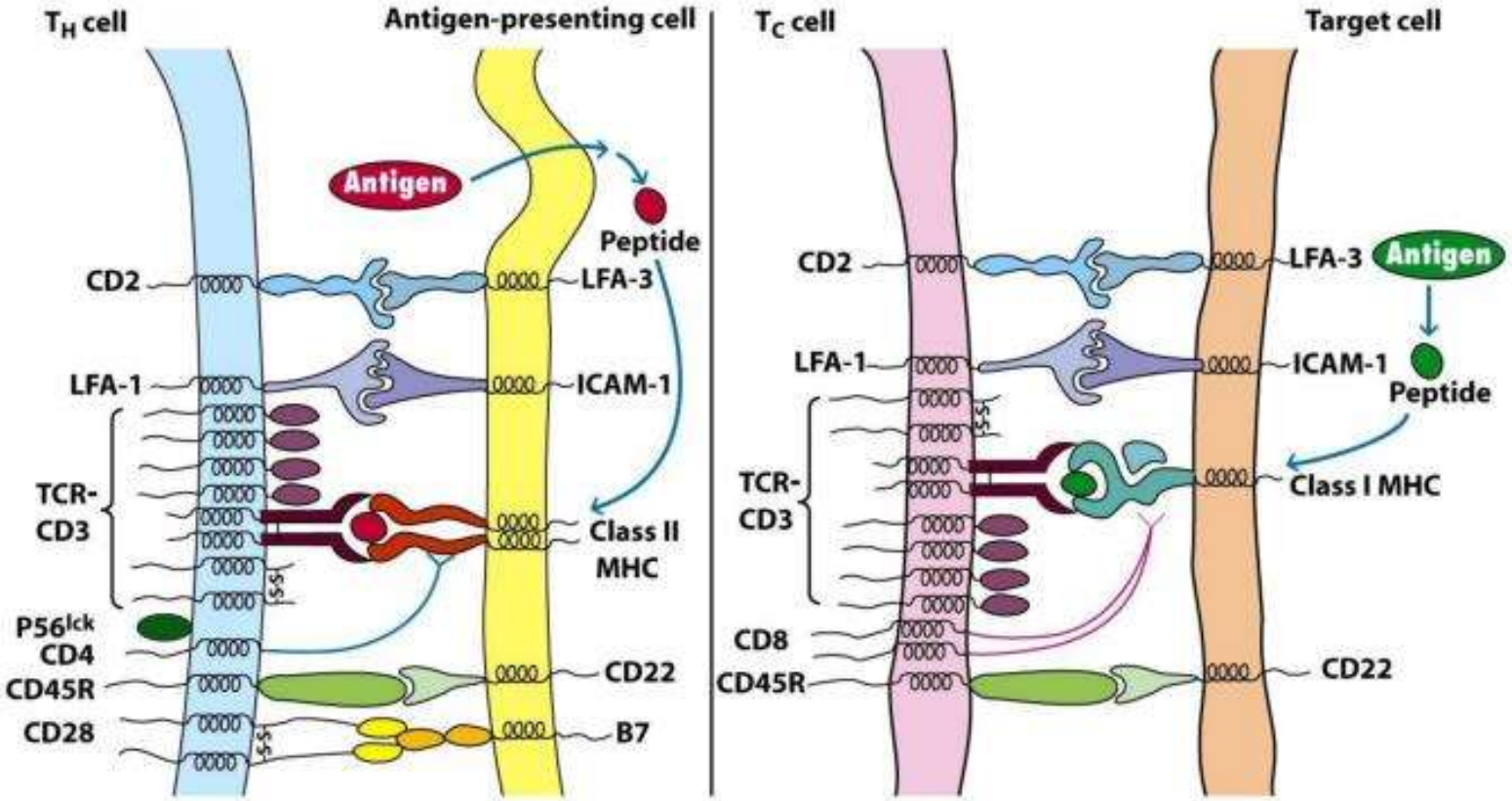
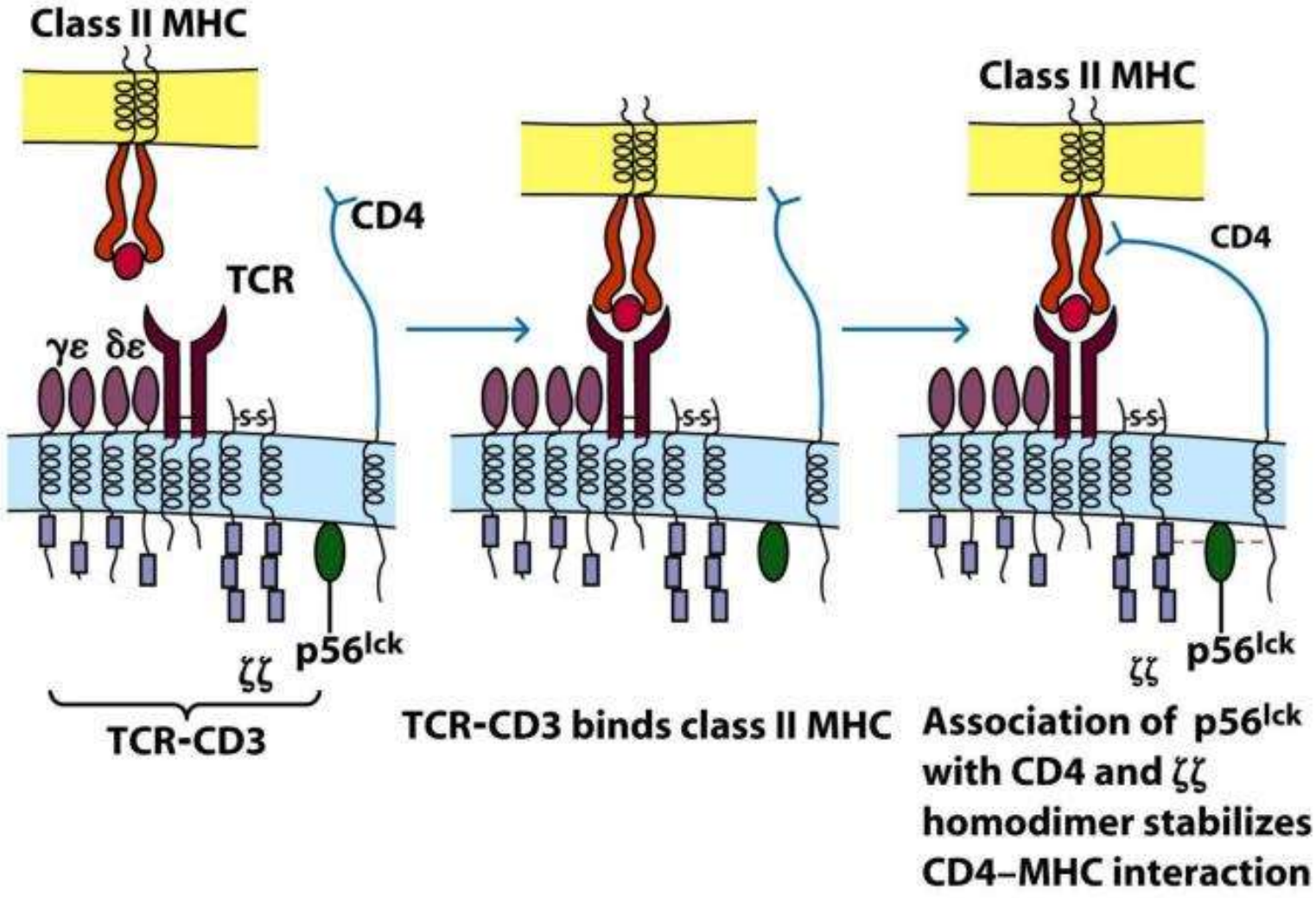
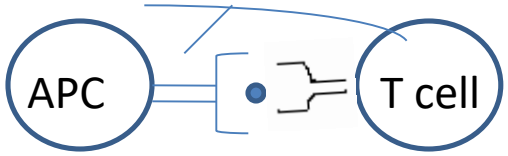


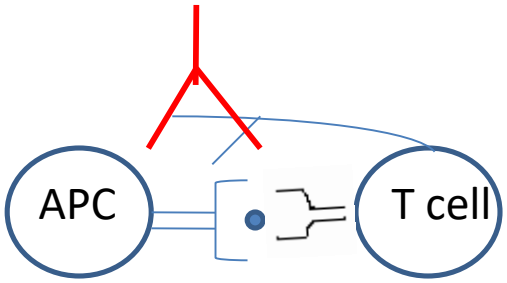
Figure 9-12b
Kuby IMMUNOLOGY, Sixth Edition


Class II MHC
CD4
TCR
γε δε
s-s
p56lck
ζζ
TCR-CD3
TCR-CD3 binds class II MHC
Class II MHC
CD4
s-s
ζζ
p56lck
Association of p56lck with CD4 and ζζ homodimer stabilizes CD4–MHC interaction

Experiment : role of Co-receptor



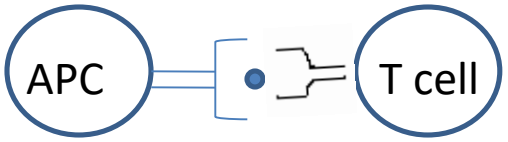
Anti CD4



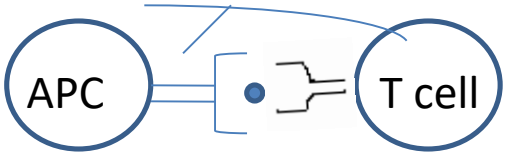
T cell response

no response

CD4 negative



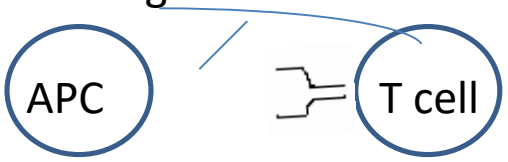
Transfect with CD4



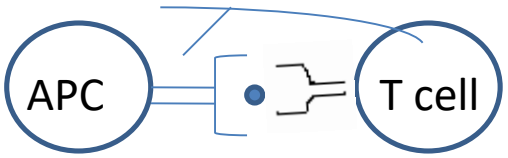
no response

T cell response

MHC negative



Transfect with MHC



no response

T cell response

If transfect with mutation of MHC where CD4 binds -----no T cells response

T cell maturation

T cells with αβ T-cell receptors (TCRs) respond to foreign antigen in the form of short peptides that are bound to MHC class I or class II proteins. These TCRs are generated by somatic DNA rearrangements and random chain pairing, and hence, lack predictable specificity for their ligands.

Optimal signalling in response to MHC ligands requires co-engagement of the TCR and either a CD4 (MHC-class-II-binding) or CD8 (MHC-class-I-binding) co-receptor. Immature thymocytes express both CD4 and CD8, but mature functional T cells express the co-receptor molecule that has an MHC-class specificity that matches that of the cell's TCR.

During T-cell differentiation in the thymus, $CD4^+CD8^+$ double-positive (DP) precursors must make a lineage decision to become $CD4^+$ (helper) or $CD8^+$ (cytotoxic) T cells.

Two models have been proposed to explain how a T cell that expresses a TCR with an unpredictable specificity emerges from the thymus with the required match between the MHC-binding preferences of TCR and co-receptor.

The instruction model postulates that TCR–CD4 binding of a self-peptide–MHC-class-II ligand generates a signal that is distinct from that produced on co-binding of a TCR and CD8 molecule to a self-peptide–MHC-class-I ligand. These unique signals 'instruct' the precursor DP T cell to choose the correct lineage fate and develop into a $CD4^+$ or $CD8^+$ T cell.

The selection model postulates that precursor DP T cells randomly choose a fate and lose expression of either CD4 or CD8. Further differentiation and survival depends on the cell having chosen correctly, so that it receives a signal from coordinate binding of the TCR and co-receptor to a self-peptide–MHC ligand. For TCRs that are specific for MHC class I, CD8 must be retained, whereas for TCRs with MHC class-II specificity, CD4 must be retained; the wrong choice dooms the cell at this checkpoint.

Recent data indicate that a combination of these two models is a more accurate description of reality.

The current 'strength of signal' model proposes that the intensity/duration of initial signalling dictates lineage choice; strong/long signalling leads to the CD4 pathway, whereas weaker/shorter signalling prompts the CD8 choice. This is generally correlated with MHC class-II versus class-I binding, respectively, because of differential association of LCK with the two co-receptors in DP thymocytes.

A combination of negative selection and cell loss due to a failure to sustain signalling removes most of the cells that make 'incorrect' choices (overly strong MHC class-I reactivity that promotes CD4 lineage choice or very weak MHC class-II reactivity that leads to the CD8 lineage).

Changes in cell surface molecules throughout T-cell maturation in the Thymus

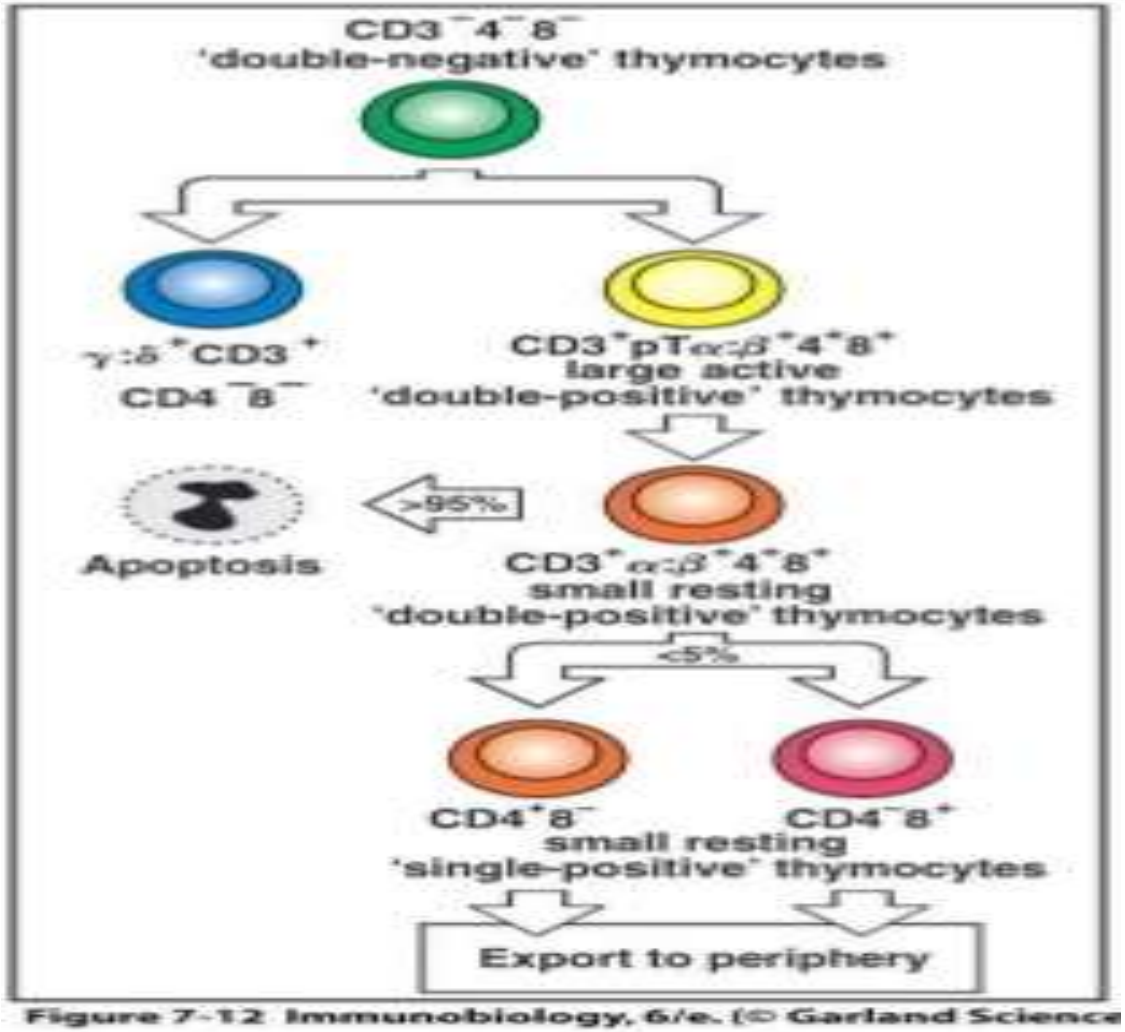
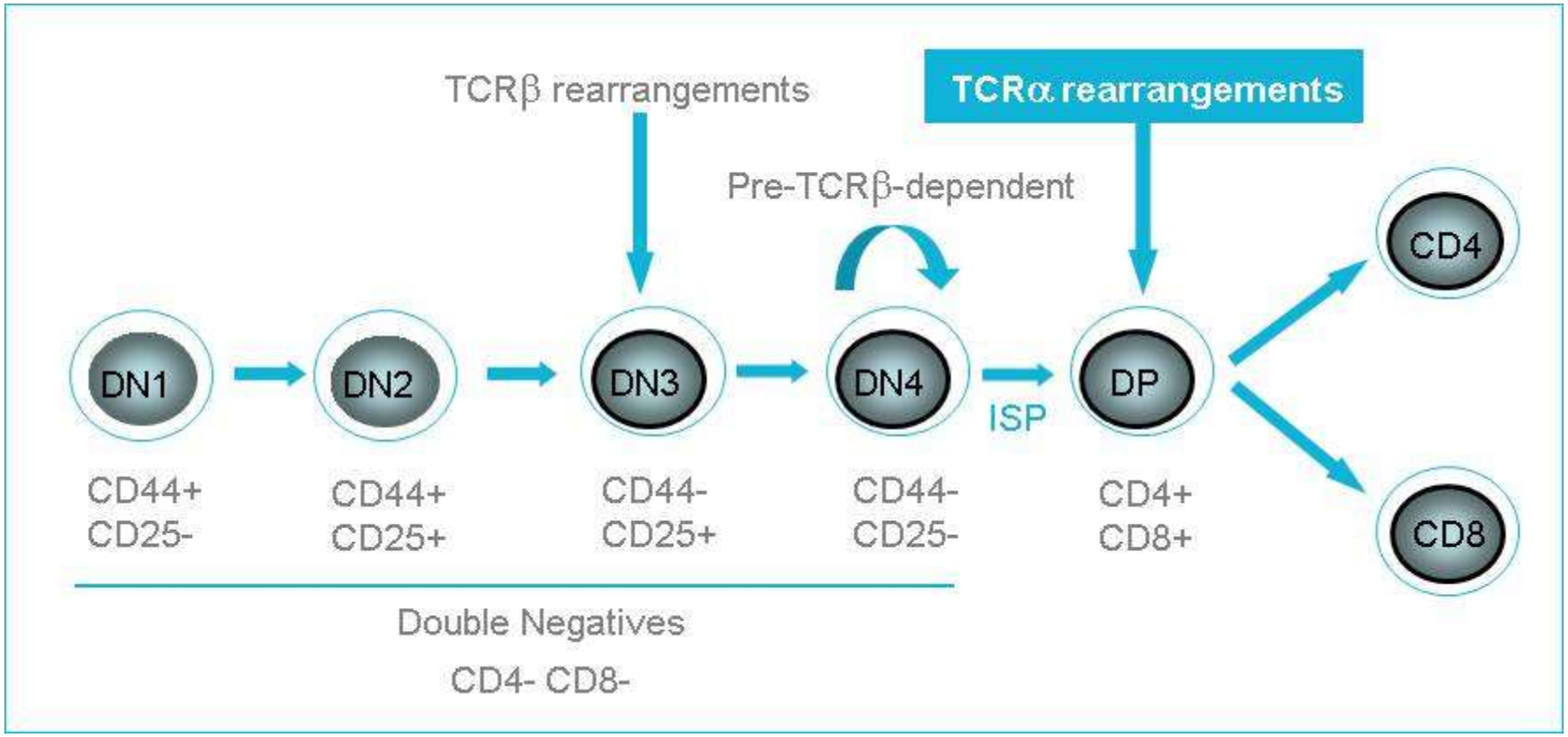


Figure 7-12 Immunobiology, 6/e. (© Garland Science

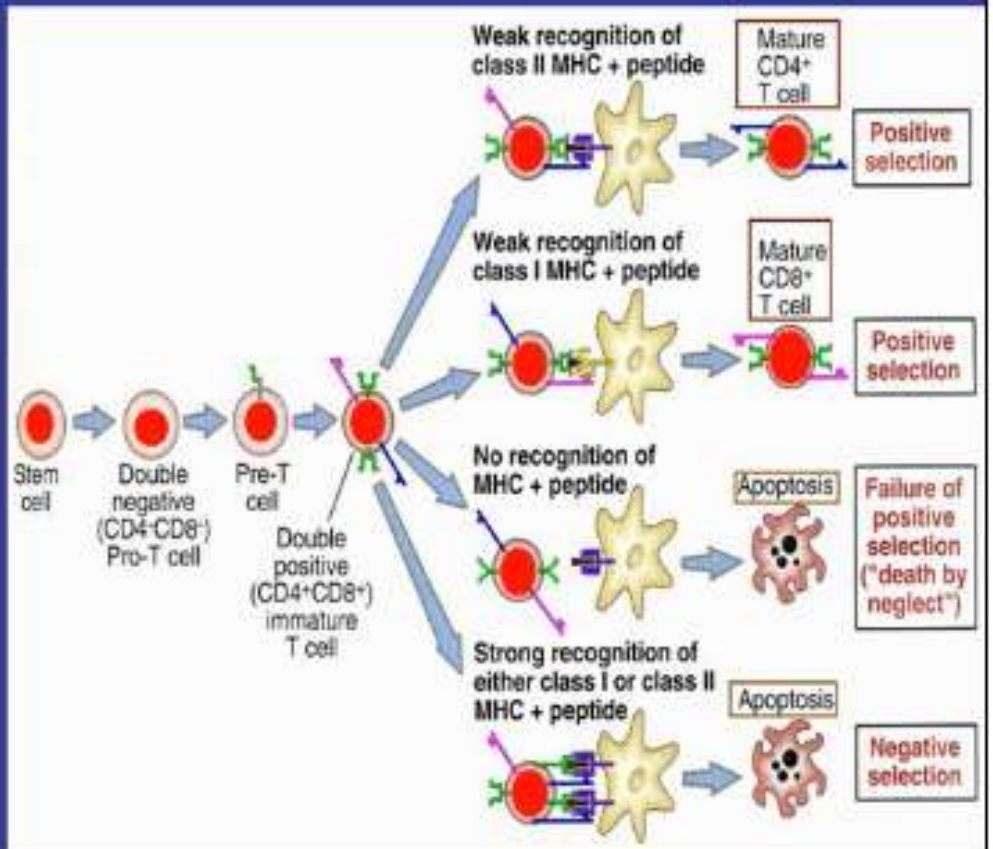
T-cell development in thymus



αβ T cell development, showing the different cell surface markers expressed at the different stages of T cell development in the mouse.

T Cell Maturation and Selection

- Occurs in the thymus.
- Positive selection
  - T cells must recognize a MHC molecule in order to survive.
  - Makes sure TCR molecule can recognize MHC + peptide.
- Failure of positive selection
  - No recognition of MHC+ peptide.
- Negative selection
  - T cells who strongly recognize a MHC molecule undergo apoptosis.
  - Eliminates self protein reacting T cells.

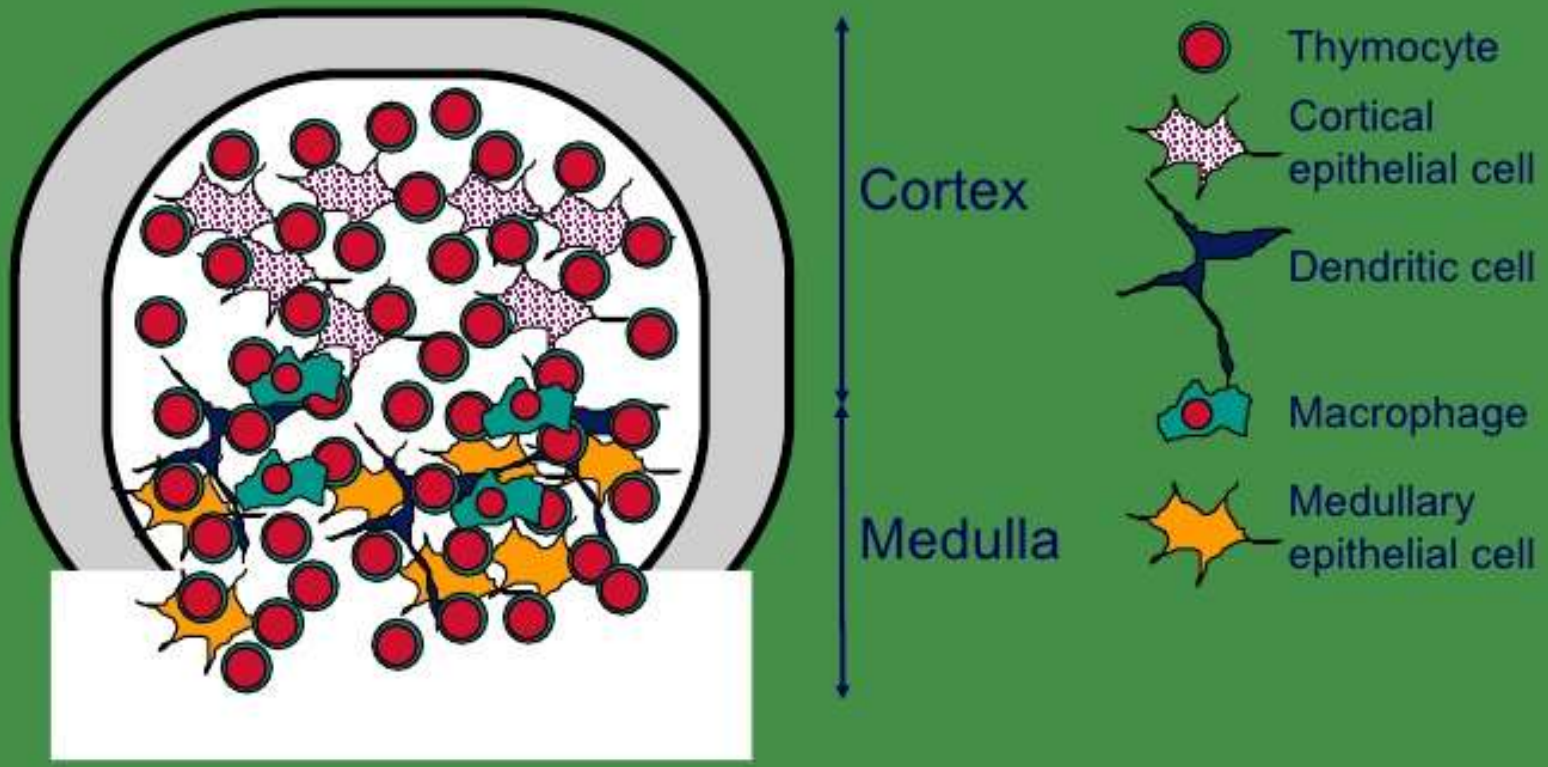


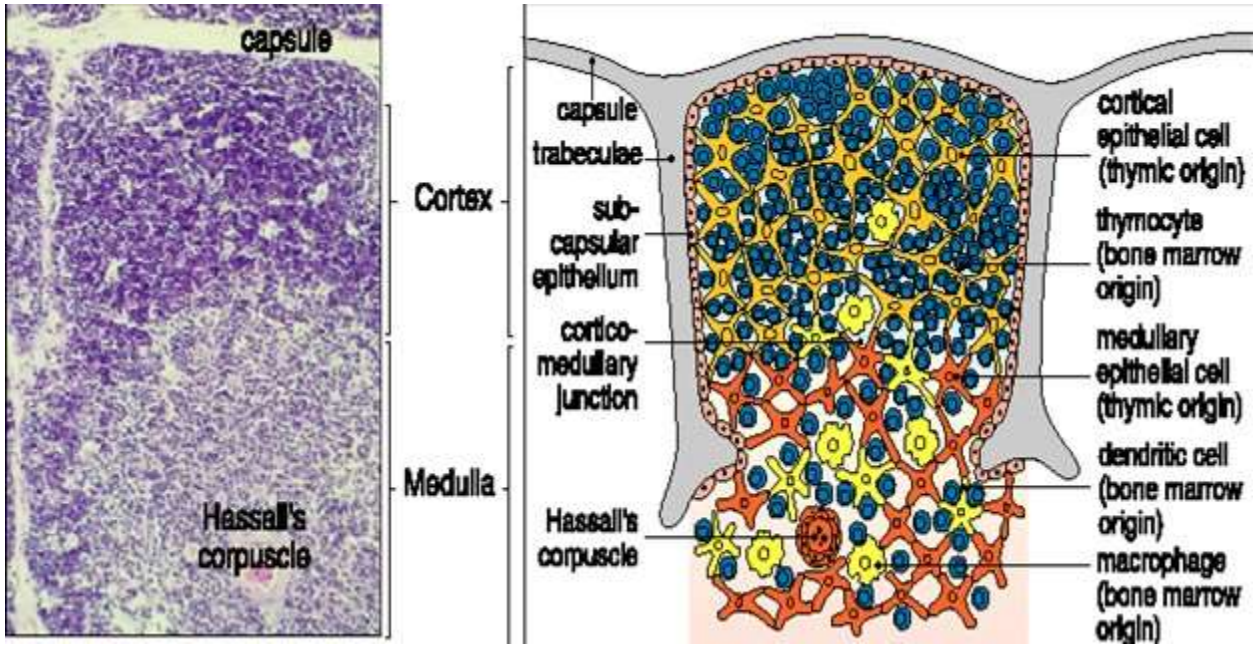
The thymus

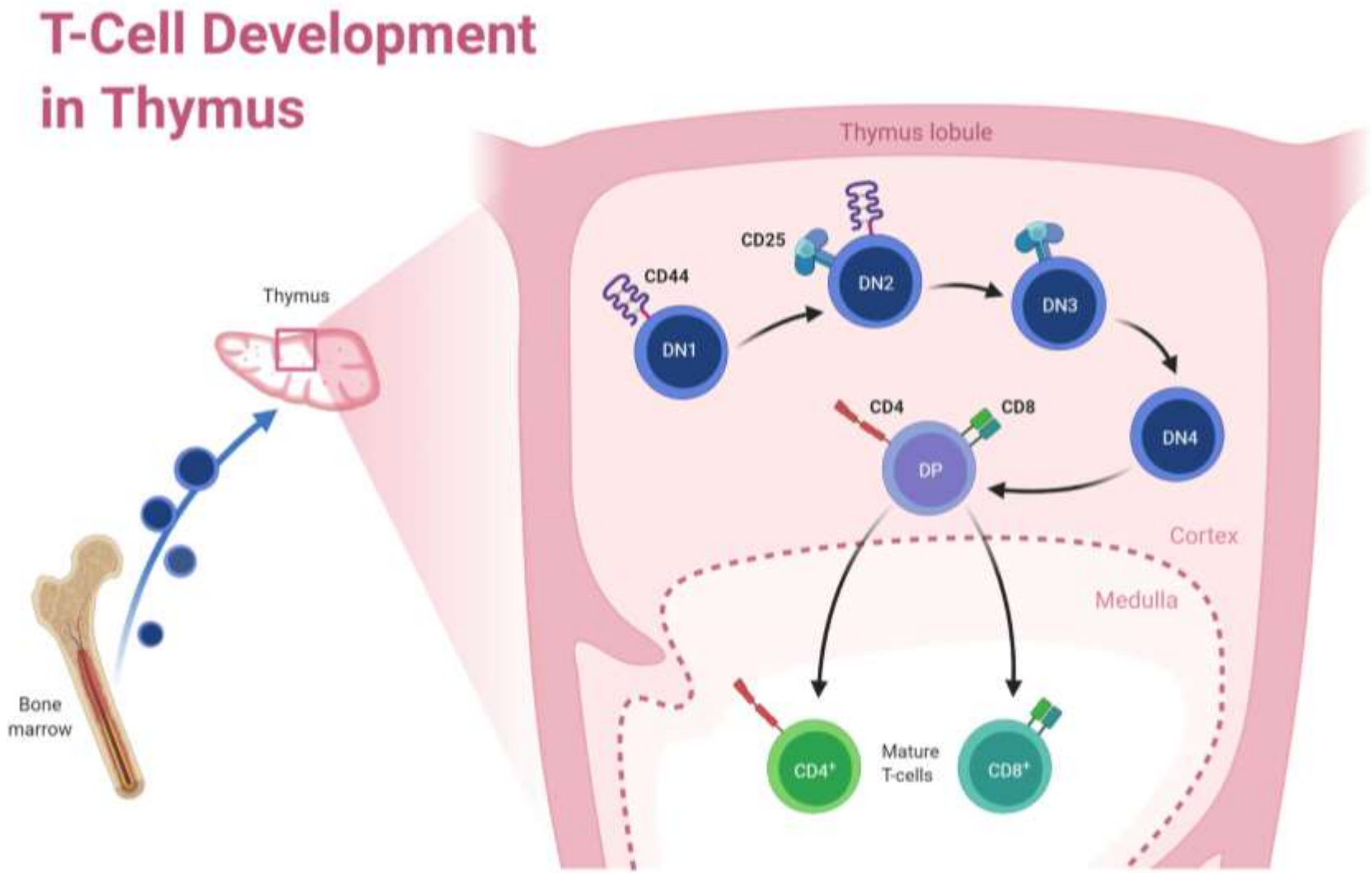
Lobulated structure with a STROMA of epithelial cells & connective tissue

Stroma provides a microenvironment for T cell development & selection

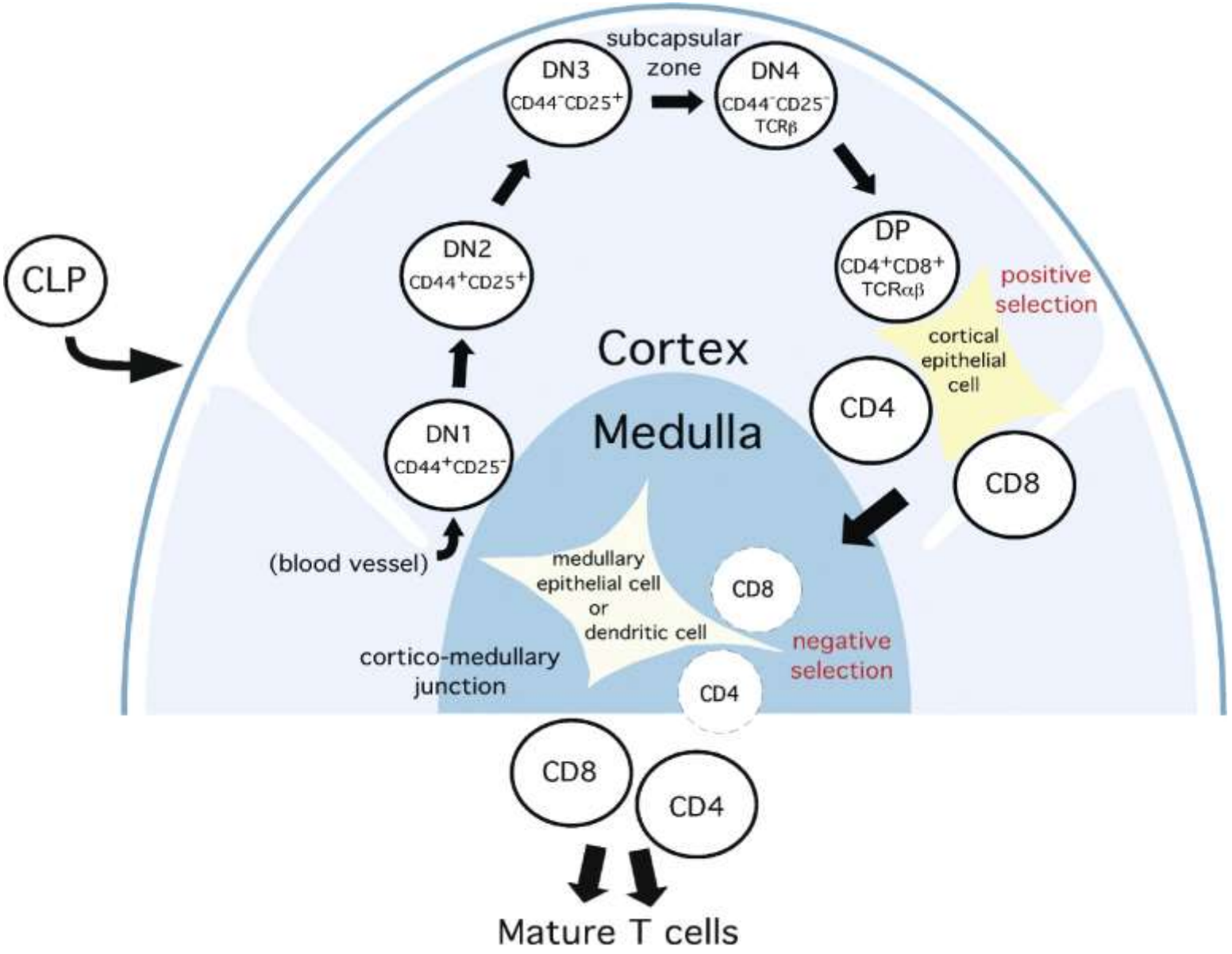
Lobules differentiated into an outer CORTEX & inner MEDULLA, both filled with bone-marrow-derived THYMOCYTES



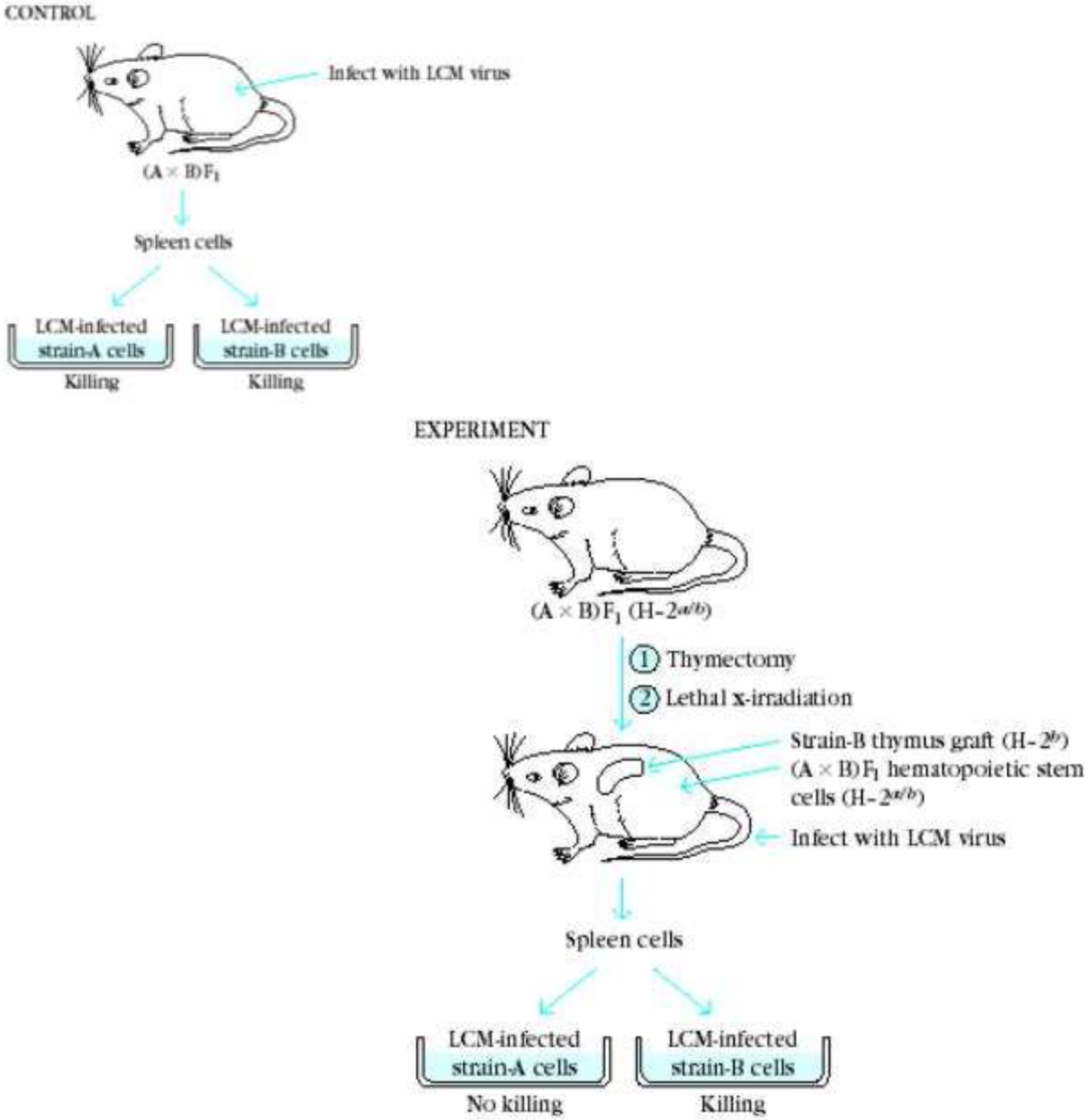

capsule
Cortex
Medulla
Hassall's corpuscle
capsule
trabeculae
sub-capsular epithelium
cortico-medullary junction
Hassall's corpuscle
cortical epithelial cell (thymic origin)
thymocyte (bone marrow origin)
medullary epithelial cell (thymic origin)
dendritic cell (bone marrow origin)
macrophage (bone marrow origin)



CD44 (an adhesion molecule) and CD25 (Interleukin-2 receptor α chain)


subcapsular
zone
DN3
CD44⁻CD25⁺
DN4
CD44⁻CD25⁻
TCRβ
DP
CD4⁺CD8⁺
TCRαβ
positive
selection
cortical
epithelial
cell
CD4
CD8
CLP
DN2
CD44⁺CD25⁺
Cortex
Medulla
DN1
CD44⁺CD25⁻
(blood vessel)
medullary
epithelial cell
or
dendritic cell
CD8
negative
selection
cortico-medullary
junction
CD4
CD8
CD4
Mature T cells

Role of thymus



Only strain-B target cells were lysed, suggesting that the H-2*b* grafted thymus had selected for maturation only those T cells that could recognize antigen combined with H-2*b* MHC molecules.

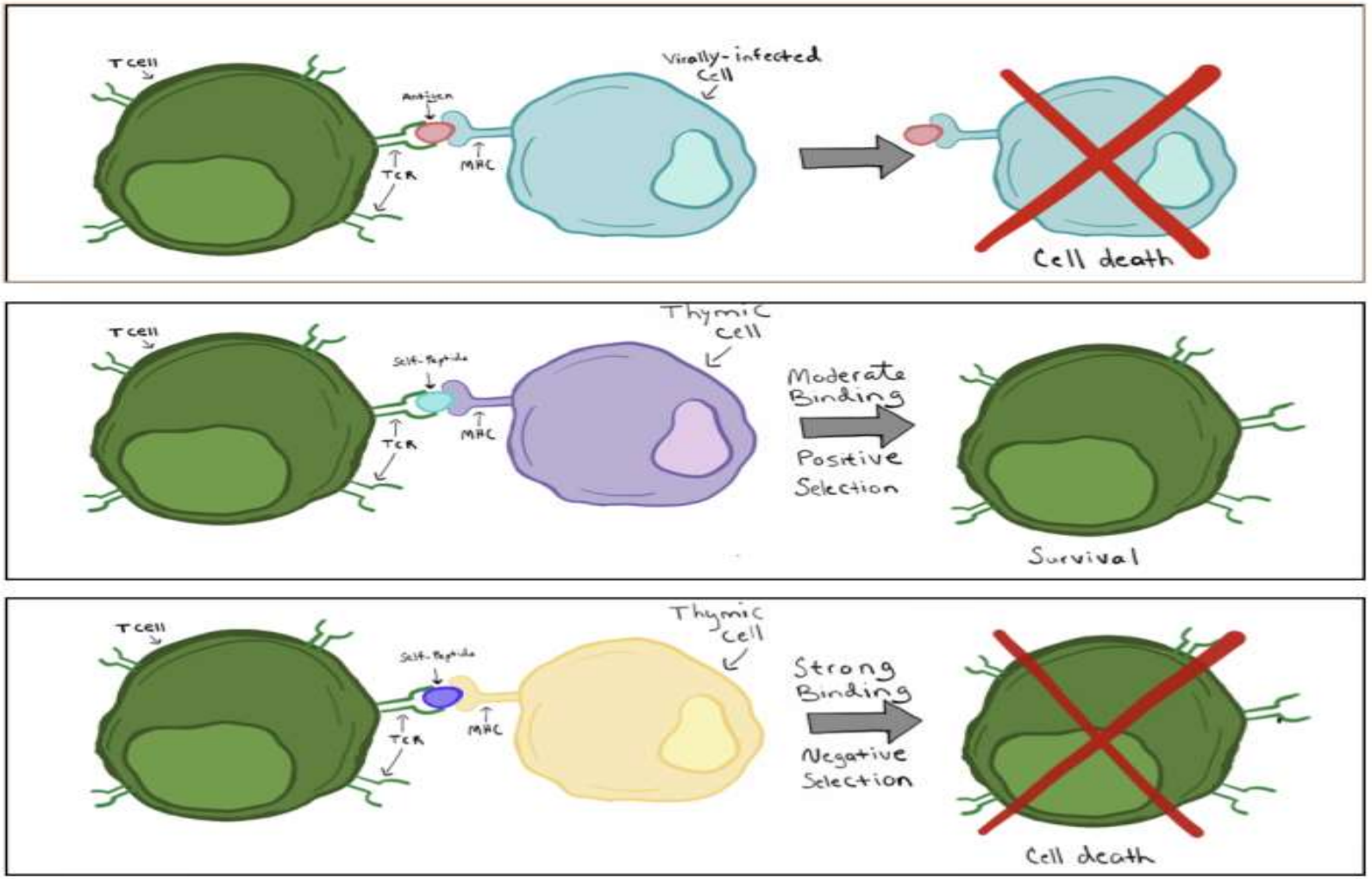
Positive Selection

- Results in MHC restriction
- Mechanism:
  - Immature thymocytes cluster with MHC molecules on the cortical cells of the thymus
    - If TCR interacts with MHC → *protective signal* results that prevents apoptosis.
    - If TCR does not interact with MHC → *no protective signal* and apoptosis occurs.
- Result? Only reactive thymocytes survive.

Negative Selection

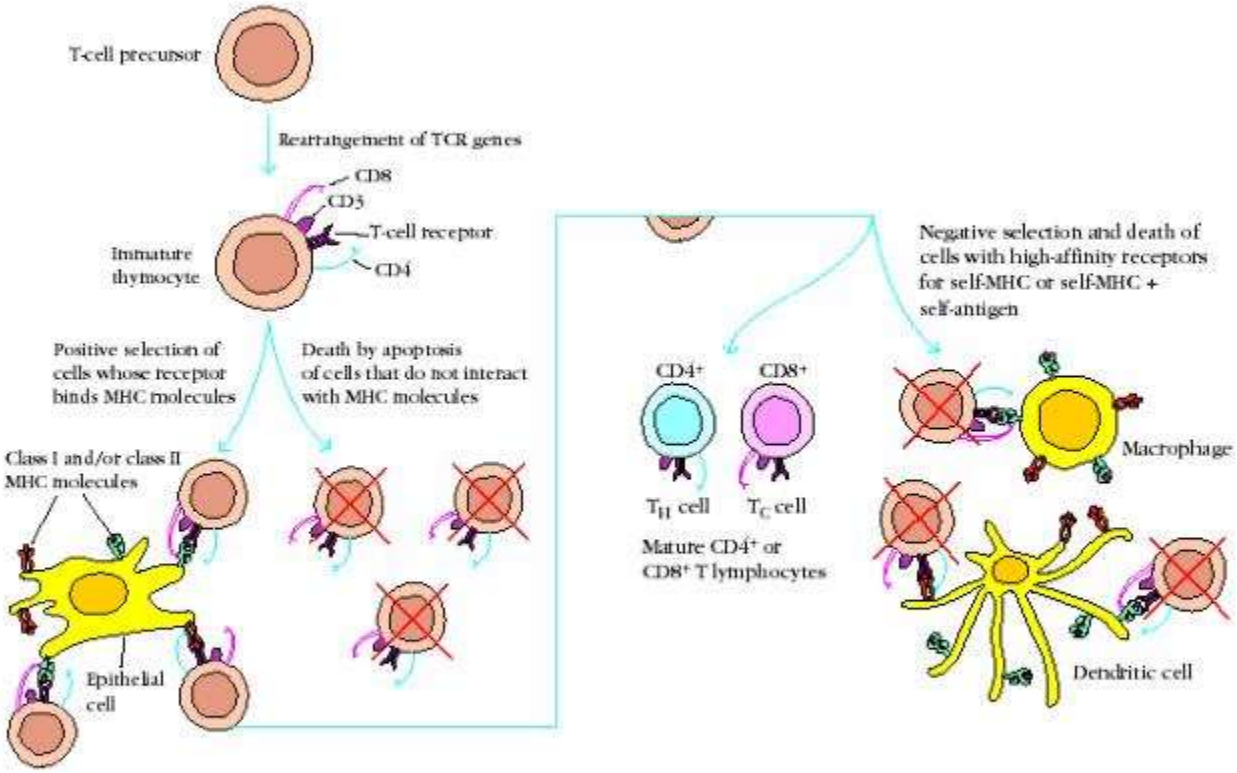
- Ensures ***self-tolerance***
- Weeds out High affinity thymocytes
- Mechanism:
  - APC's bearing MHC's interact with thymocytes
    - If avidity is too strong → thymocyte undergoes apoptosis.
    - Details unknown…
- Result? Only self-tolerant thymocytes survive.

Avidity (functional affinity) is the accumulated strength of multiple affinities.

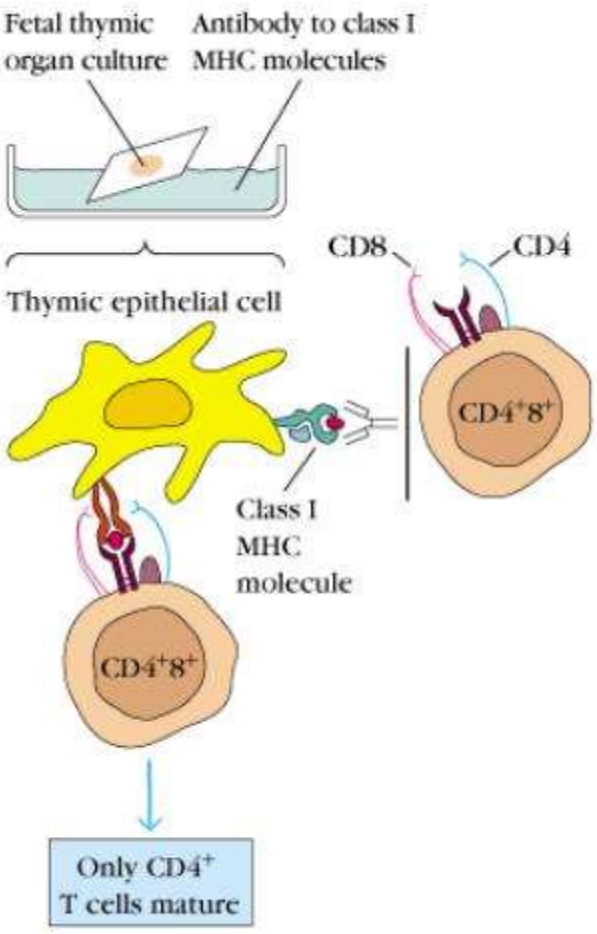


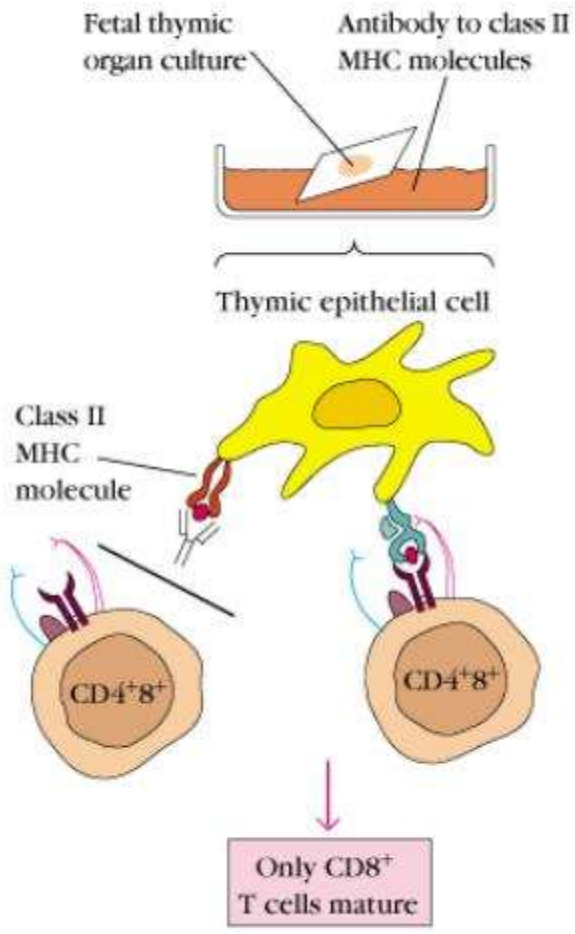
. *When a TCR binds an antigen-MHC complex displayed by a sick or infected cell, the T cell can induce cell death called apoptosis (top). In order for mature, antigen-recognizing T cells to develop without being self-reactive and causing autoimmunity, T cells must go through both positive and negative selection. In positive selection, T cells in the thymus that bind moderately to MHC complexes receive survival signals (middle). However, T cells whose TCRs bind too strongly to MHC complexes, and will likely be self-reactive, are killed in the process of negative selection (bottom*

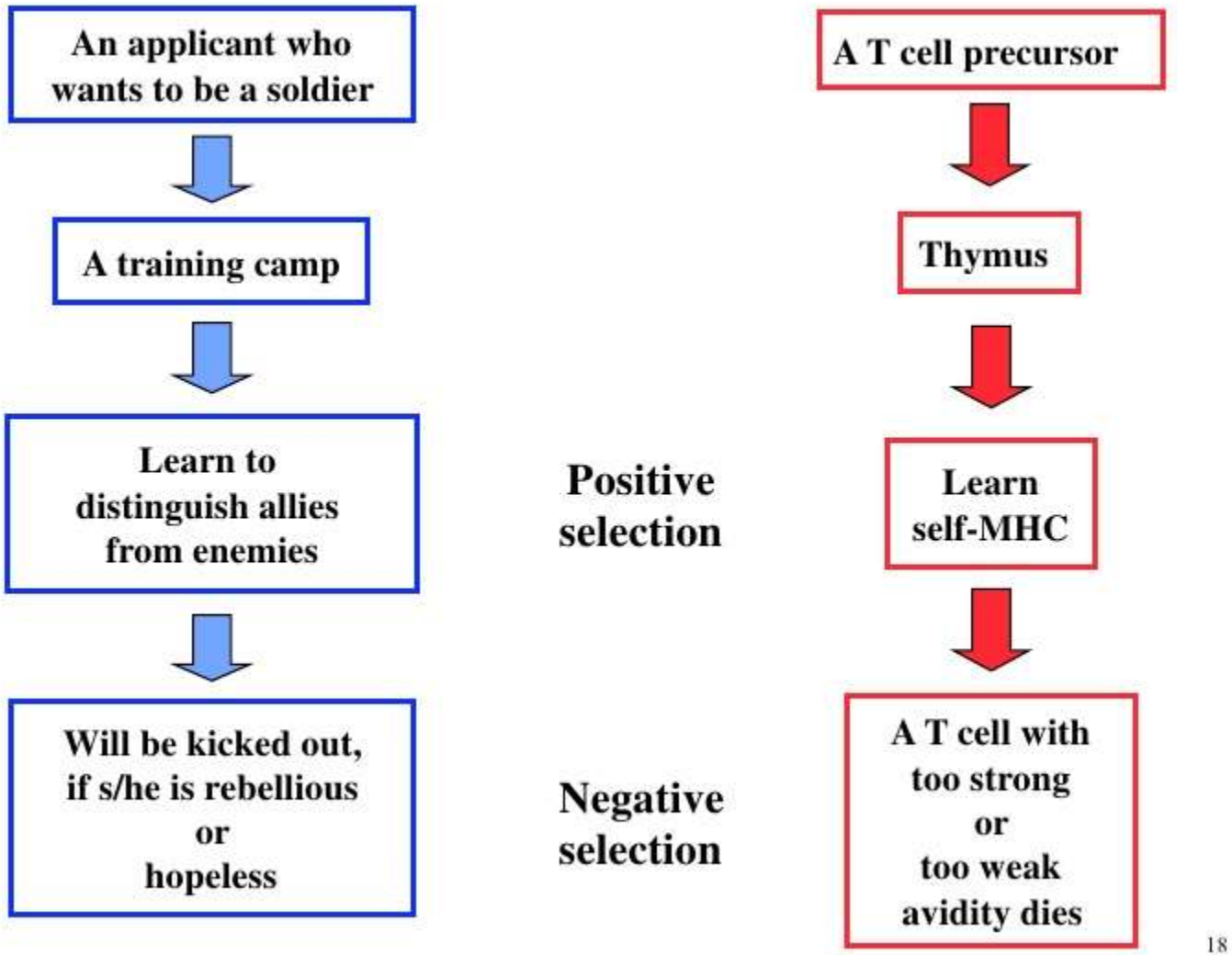
1) Positive Selection Ensures MHC Restriction



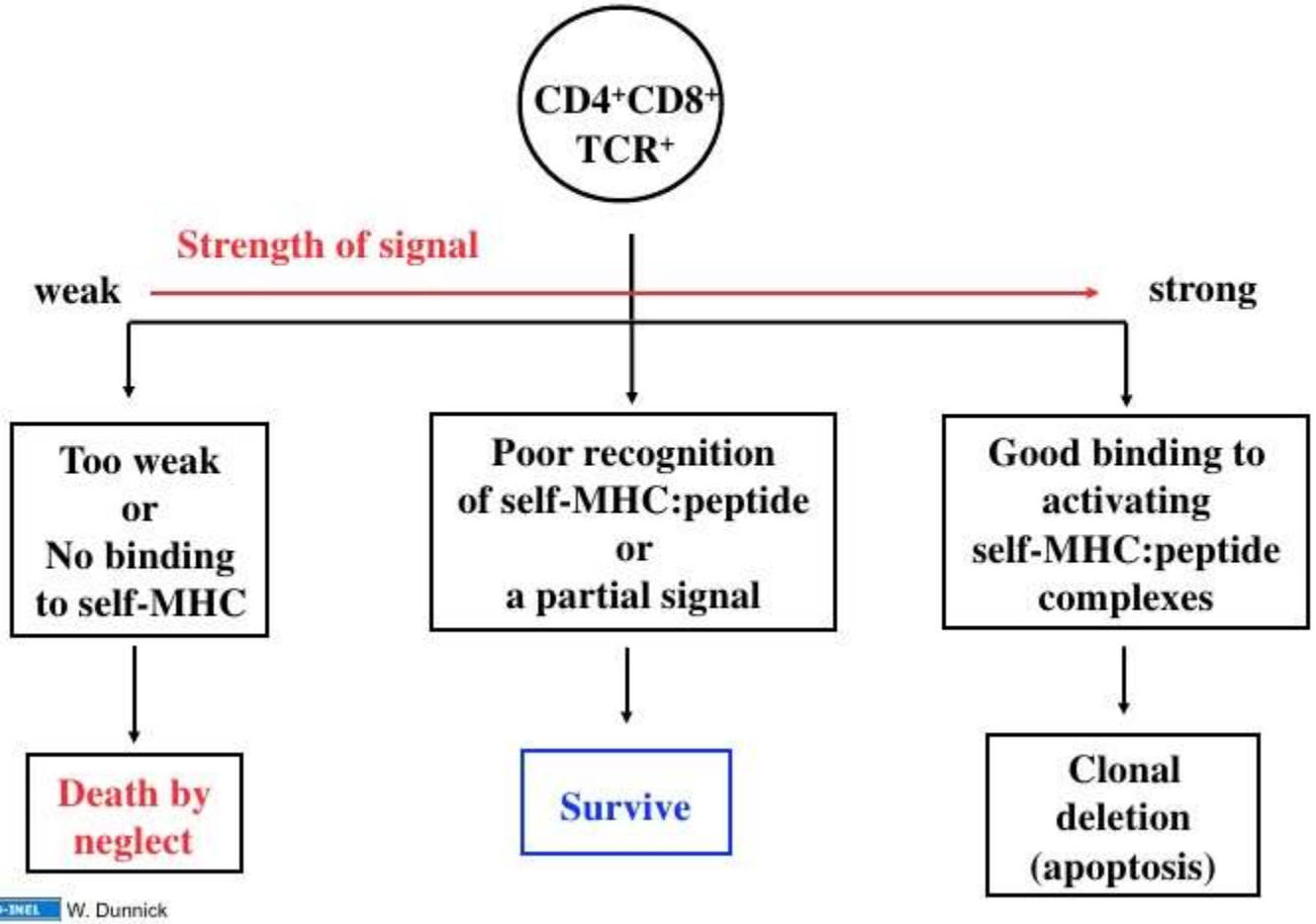
2) Negative Selection Ensures Self-Tolerance


Fetal thymic organ culture
Antibody to class I MHC molecules
Thymic epithelial cell
CD8
CD4
CD4+8+
Class I MHC molecule
CD4+8+
Only CD4+ T cells mature


Fetal thymic organ culture
Antibody to class II MHC molecules
Thymic epithelial cell
Class II MHC molecule
CD4+8+
CD4+8+
Only CD8+ T cells mature


An applicant who wants to be a soldier
A training camp
Learn to distinguish allies from enemies
Will be kicked out, if s/he is rebellious or hopeless
Positive selection
Negative selection
A T cell precursor
Thymus
Learn self-MHC
A T cell with too strong or too weak avidity dies

A simple view of the thymic selection



If T cells escape from selection processes in the thymus, what would be the consequences?

1. Failure of positive selection;

   Lack of functional T cells

2. Failure of negative selection;

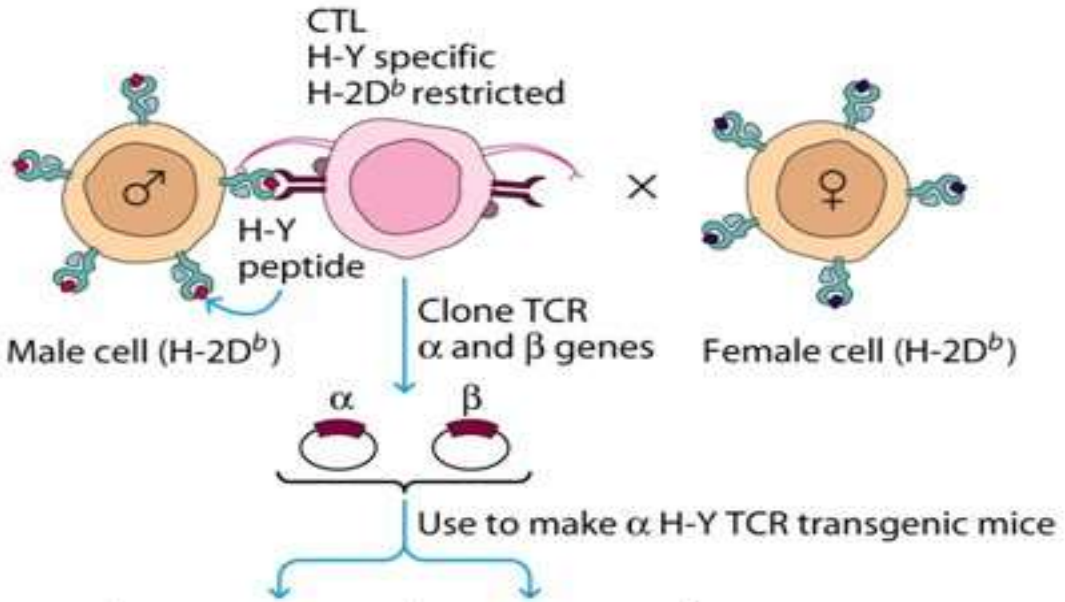
   Self-reactive T cells in the periphery resulting in autoimmunity

The ultimate goal of selection is to produce T cells that are

- **Self-MHC restricted to recognize foreign antigenic peptides with self-MHC**

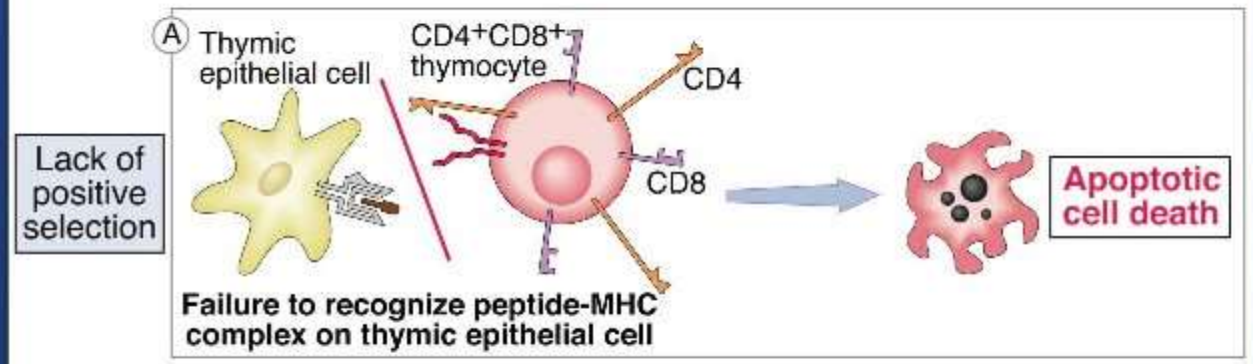
AND

- **Self tolerant not to respond to self-peptides**



	Male H-2D^b	Female H-2D^b
H-Y expression	+	–
Thymocytes		
CD4$^-$8$^-$	++	+
CD4$^+$8$^+$	+	++
CD4$^+$	+	+
CD8$^+$	–	++

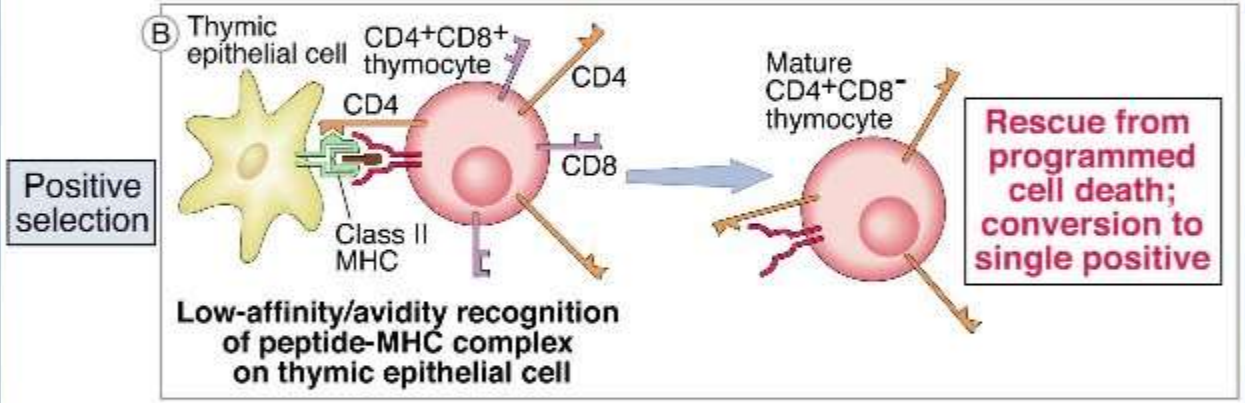
Selection processes in the thymus (a)



Death by Neglect

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-20a

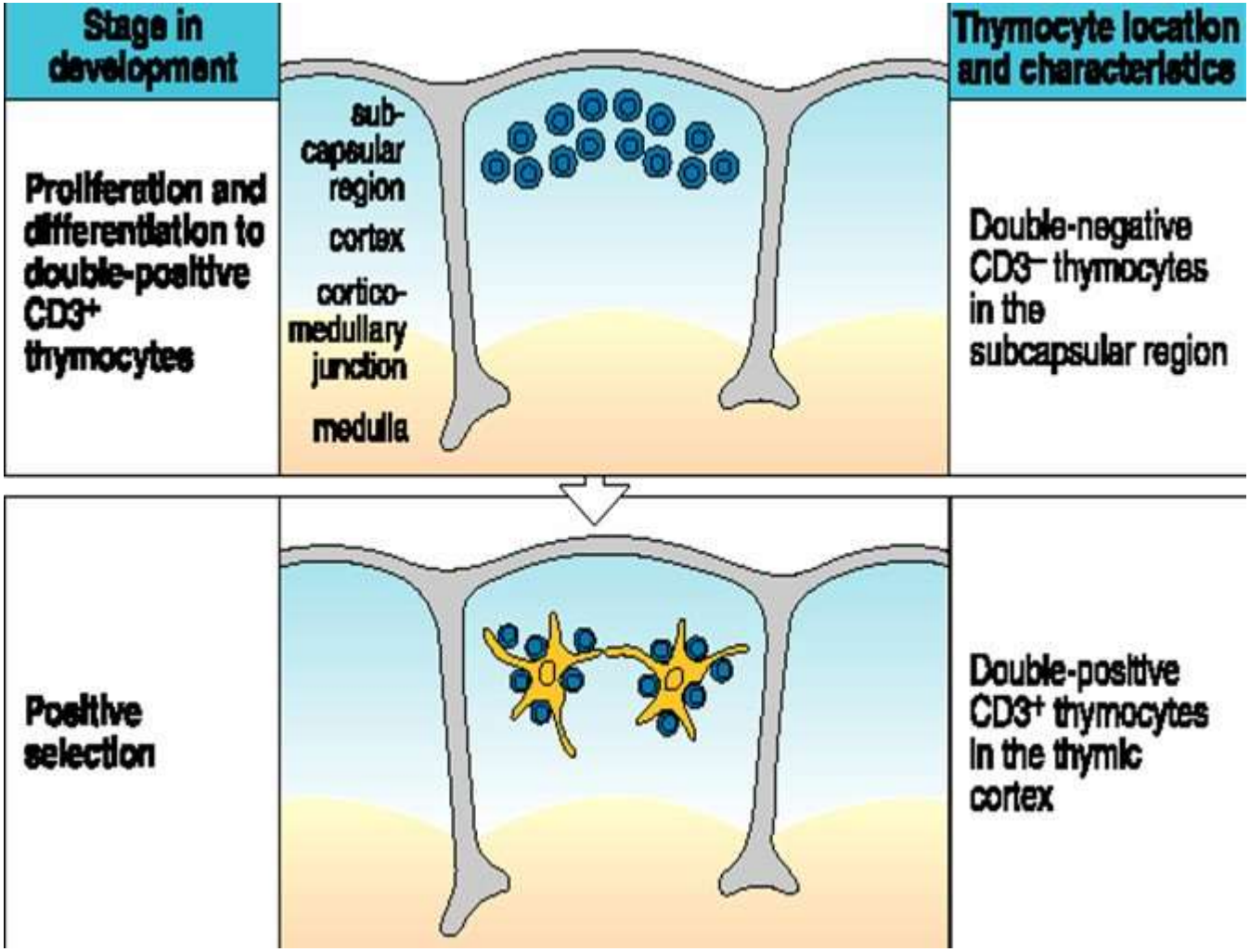
Selection processes in the thymus (b)

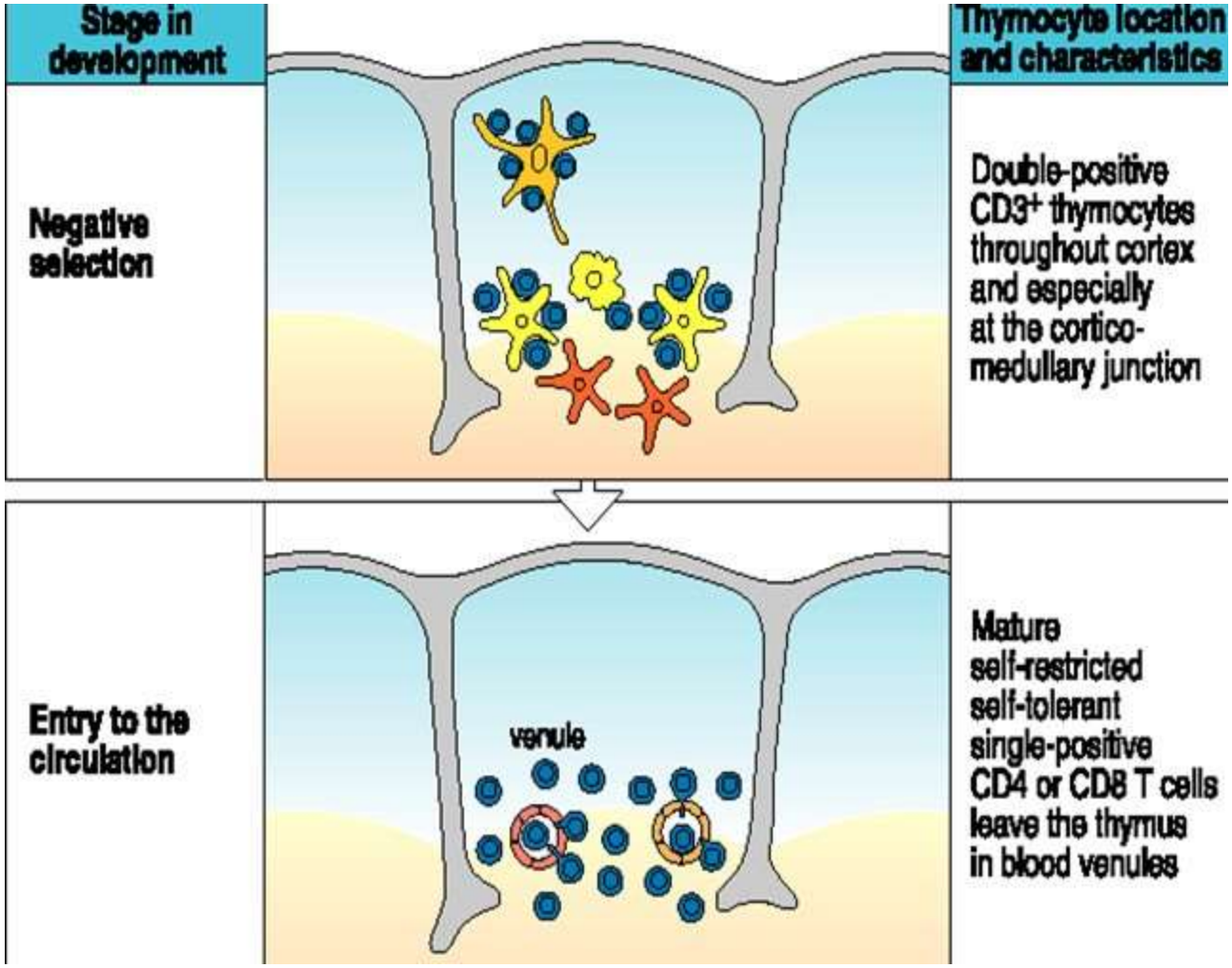


From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-20b

TABLE 10-1 CHARACTERISTICS OF T-CELL SELECTION IN THE THYMUS

Property	Positive selection	Negative selection
Site	Cortex	Medulla
Stromal cells involved	Epithelial cells	Macrophages and dendritic cells
Selection mechanism	Survival of thymocytes bearing receptors for self-MHC	Elimination of thymocytes bearing high-affinity receptors for self-MHC alone or self-antigen + self-MHC
Immune consequence	Self-MHC restriction	Self-tolerance


Stage in development
Thymocyte location and characteristics
Proliferation and differentiation to double-positive CD3+ thymocytes
sub-capsular region
cortex
cortico-medullary junction
medulla
Double-negative CD3− thymocytes in the subcapsular region
Positive selection
Double-positive CD3+ thymocytes in the thymic cortex


Stage in development
Thymocyte location and characteristics
Negative selection
Double-positive CD3+ thymocytes throughout cortex and especially at the cortico-medullary junction
Entry to the circulation
venule
Mature self-restricted self-tolerant single-positive CD4 or CD8 T cells leave the thymus in blood venules

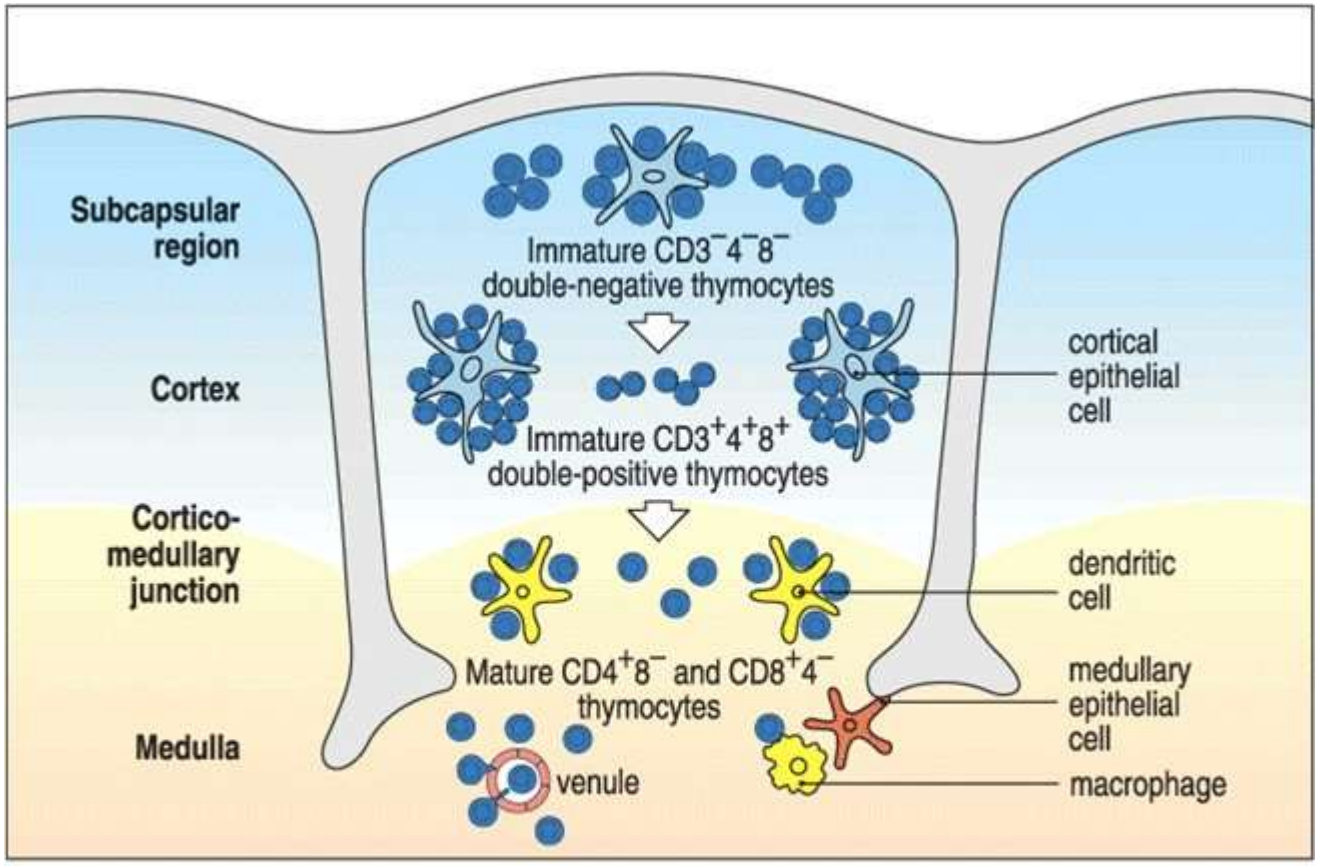


Fig 7.13 © 2001 Garland Science