

M U N I
S C I

C8116 Immunochemical techniques

Immune system, part I and part II

Spring semester 2025

Hans Gorris

Department of Biochemistry

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Research and contact

Assoc. Prof. Hans H. Gorris
Department of Biochemistry
C05, office 315
Phone: 3816
E-mail: gorris@mail.muni.cz

Our research focus:

1) Analytical biochemistry:

- luminescent nanoparticles (UCNP)
- single-molecule / digital immunoassays

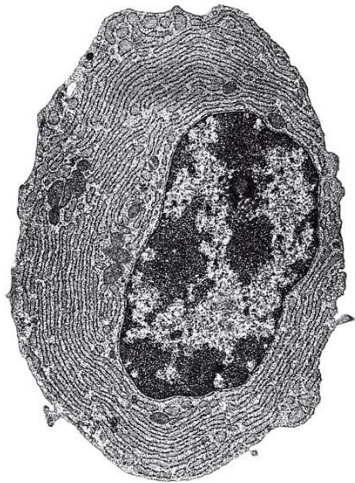
2) Single molecule studies of enzymes:

- single enzyme molecules in microchambers (50 fL)
- structure-function relationship of enzymes

=> More information provided during the lecture...

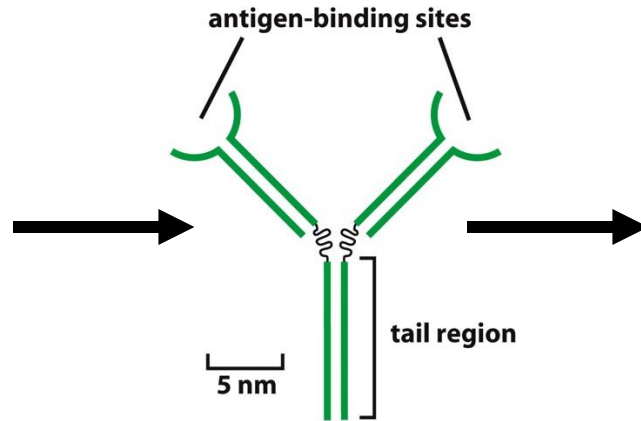
The idea behind the lecture

Immunology



effector B cell (plasma cell) 

The “tools“:
antibodies



Immunoassay



Topics of the lecture

Part A: The immune system

Part B: Antibodies as immunological tools

Part C: Immunoassays

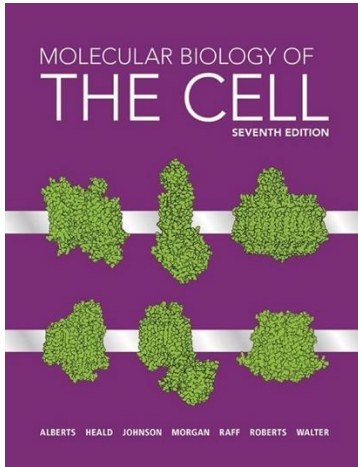
Part D: Immunoaffinity and other protein-protein affinity techniques

Part E: Advanced fluorescence microscopy for (life) cell imaging

The immune system (2 days)

- 1) General introduction to the immune system
- 2) Innate / adaptive immune system
- 3) Lymphoid organs
- 4) B cells
- 5) Progress of immune response
- 6) Structure of IgG / immunoglobulin superfamily
- 7) Binding sites of antibodies
- 8) Generation of antibody diversity / affinity maturation
- 9) Antibody affinity
- 10) Clonal selection theory / immunological tolerance
- 11) Antibody classes IgG, IgM, IgA, IgE
- 12) Complement system
- 13) B cells vs. T cells
- 14) T-cell receptor
- 15) MHC class I and II
- 16) Antigen presentation
- 17) Cytotoxic / helper T cells

Recommended reading



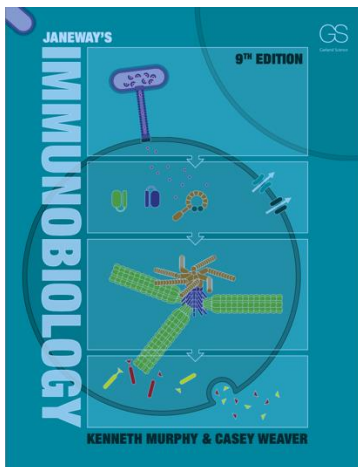
Basic text book

Molecular Biology of the Cell (7th edition)

Alberts, Heald, Johnson, Morgan, Raff, Roberts & Walter
W.W. Norton & Company, New York 2022

Chapter 24: The innate and adaptive immune system
(page 1353-1404)

<https://archive.org/details/alberts-molecular-biology-of-the-cell-7th/page/1353/mode/2up>



In depth reading

Janeway Immunobiology (9th edition)

Murphy & Weaver

Garland Science, London 2017

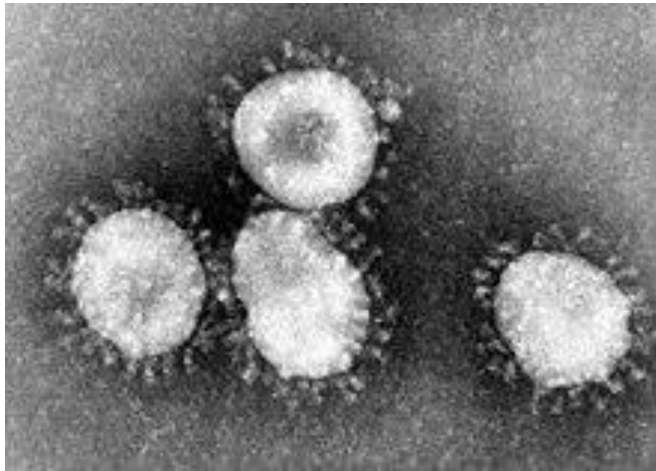
https://immunologos.wordpress.com/wp-content/uploads/2020/08/janeways-immunobiology-9th-ed_booksmedicos.org_.pdf

Slides of the lecture are available online (Learning Materials)

Overview on our body's defenses against an infection

Challenge: Great variability of infectious diseases

Coronavirus, intracellular

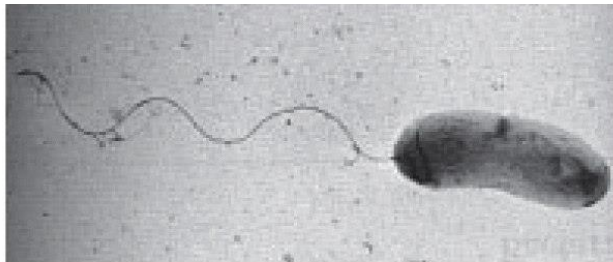


Diameter: ca. 100 nm

Toxoplasmosis:
single-celled, eukaryotic parasite, intracellular



(C)



(B)

Cholera: bacterium, in the intestine

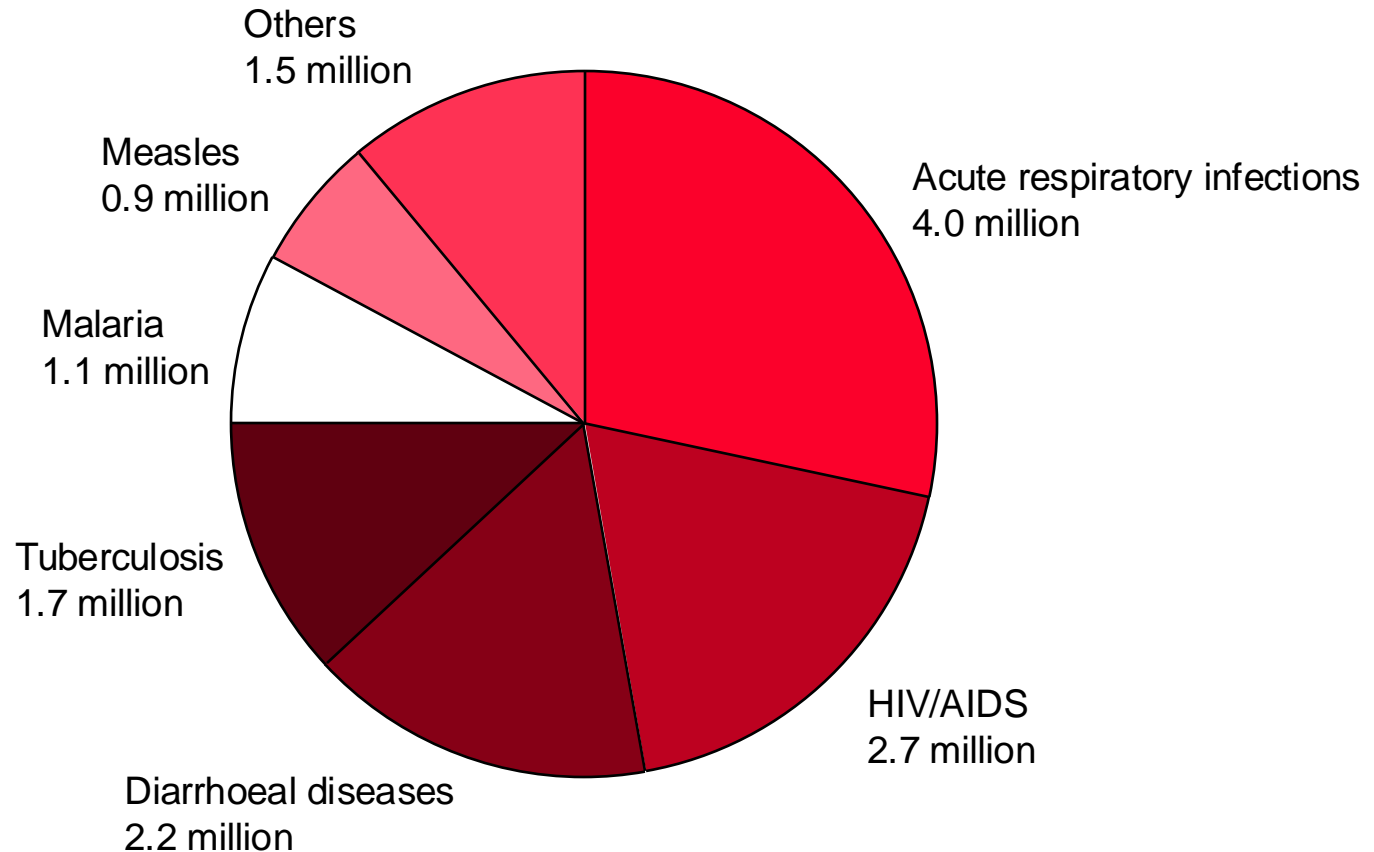


(D)

Nematodes: multicellular, eukaryotic parasite, in intestine, blood und lung

Infectious diseases

Deaths per year

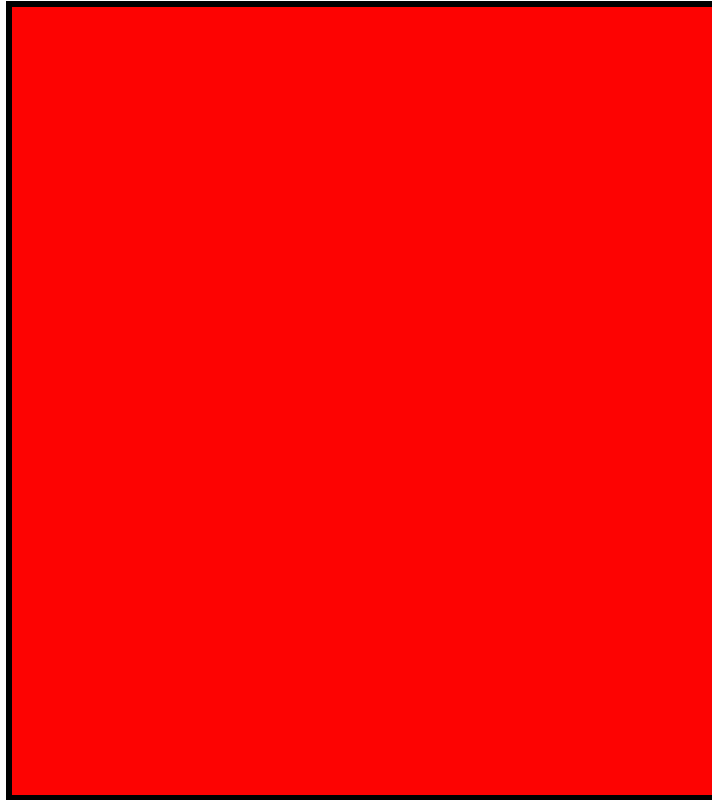


Source:
The World Health Report 2000, WHO

red colors:
Pathogens that enter our body
via mucosal surfaces

Surface areas of human body

Mucosa



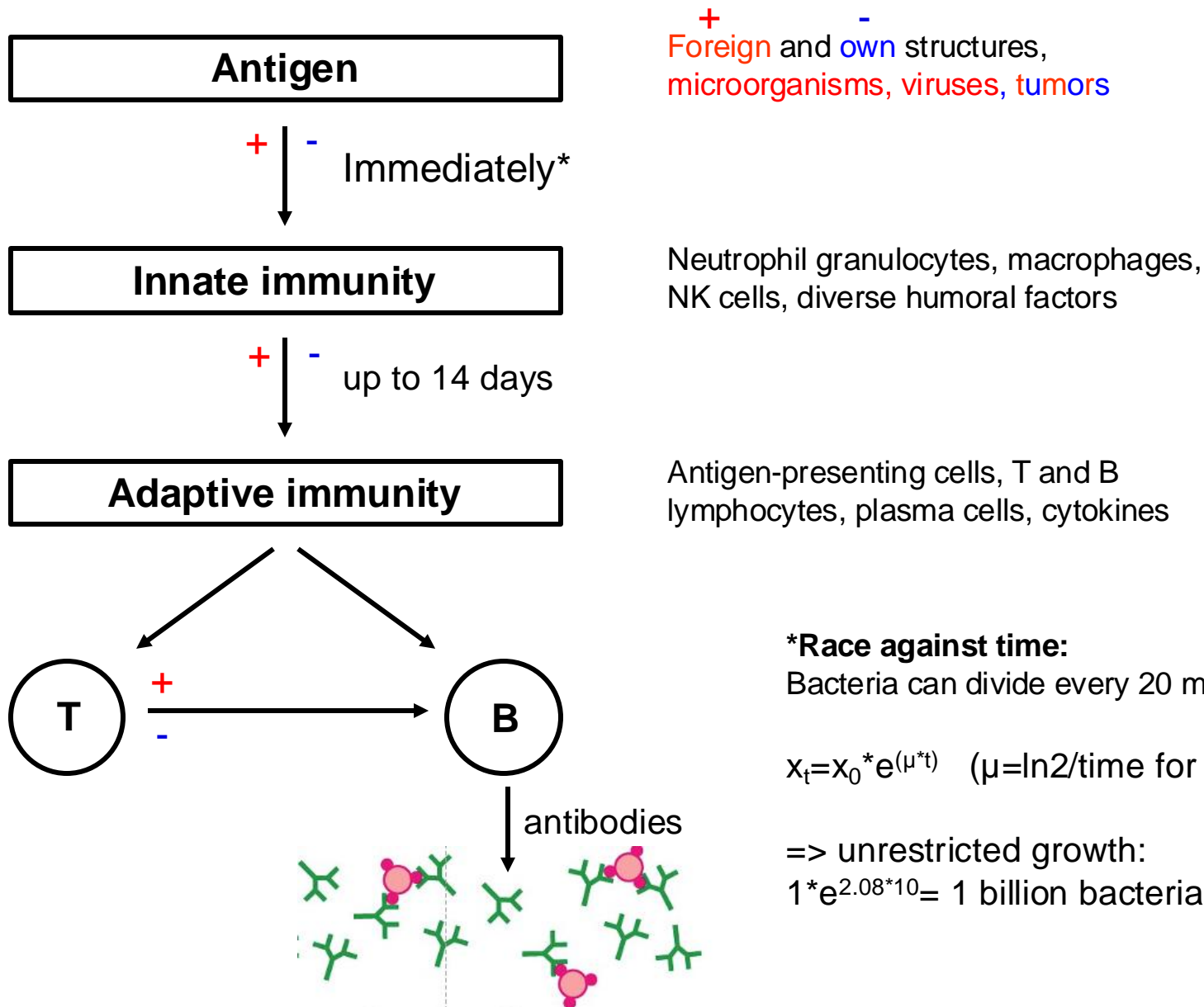
400 m²

Skin



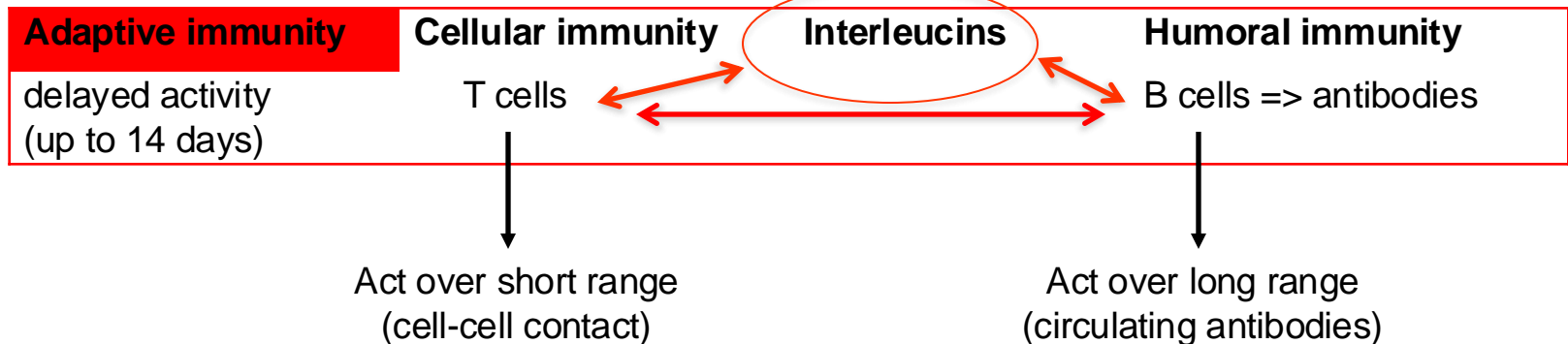
2 m²

Two lines of defence

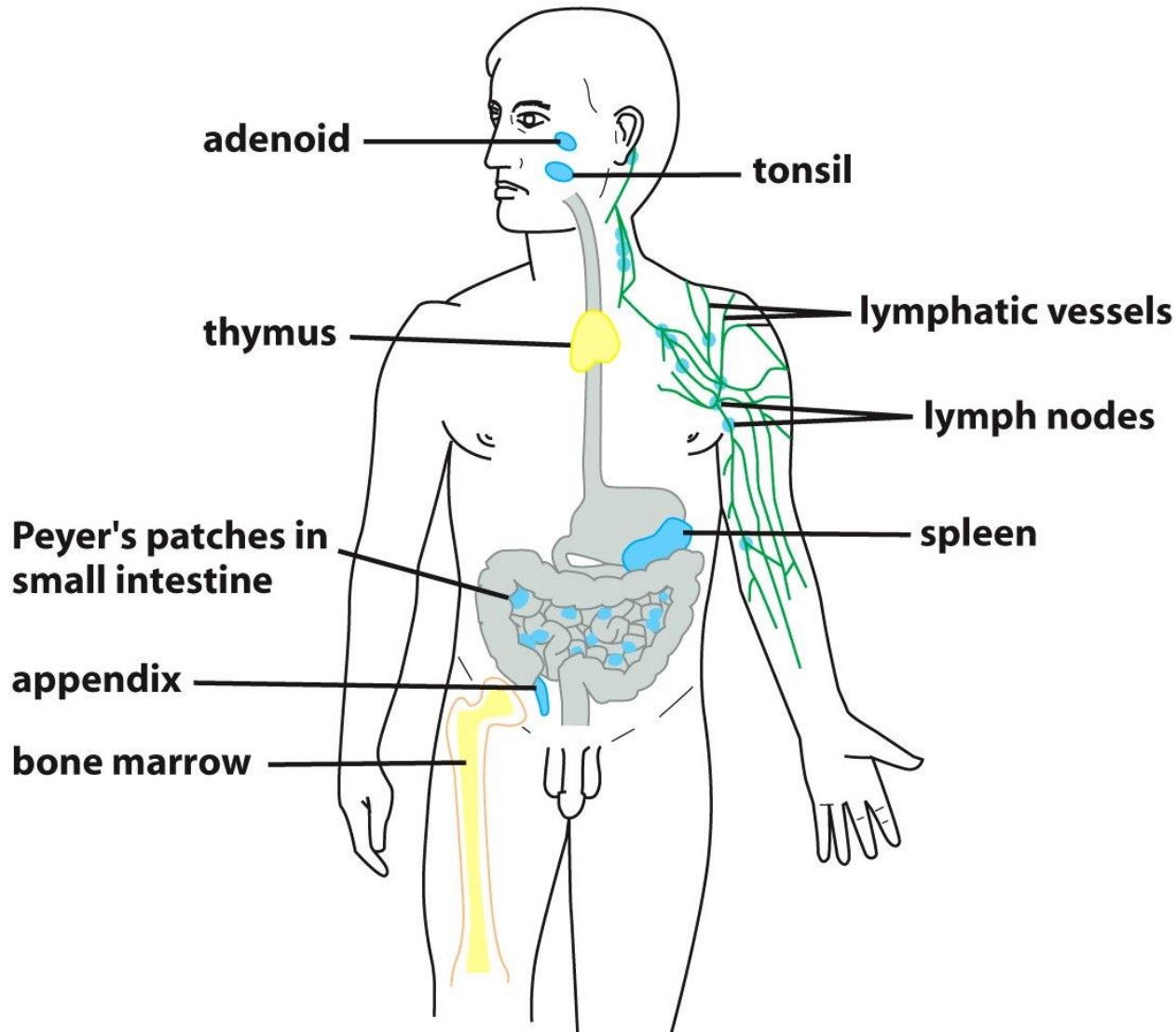


Innate / adaptive immunity

Innate immunity	Physical barriers	Cellular defence	(Bio-)chemical barriers
immediate activity	skin (2 m ²) / mucosae (400 m ²)	Macrophages Killer cells	pH (gastric acid) Lipids Enzymes (e.g. lysozyme) Complement system



Adaptive immunity: Human lymphoid organs



Primary lymphatic organs (yellow):

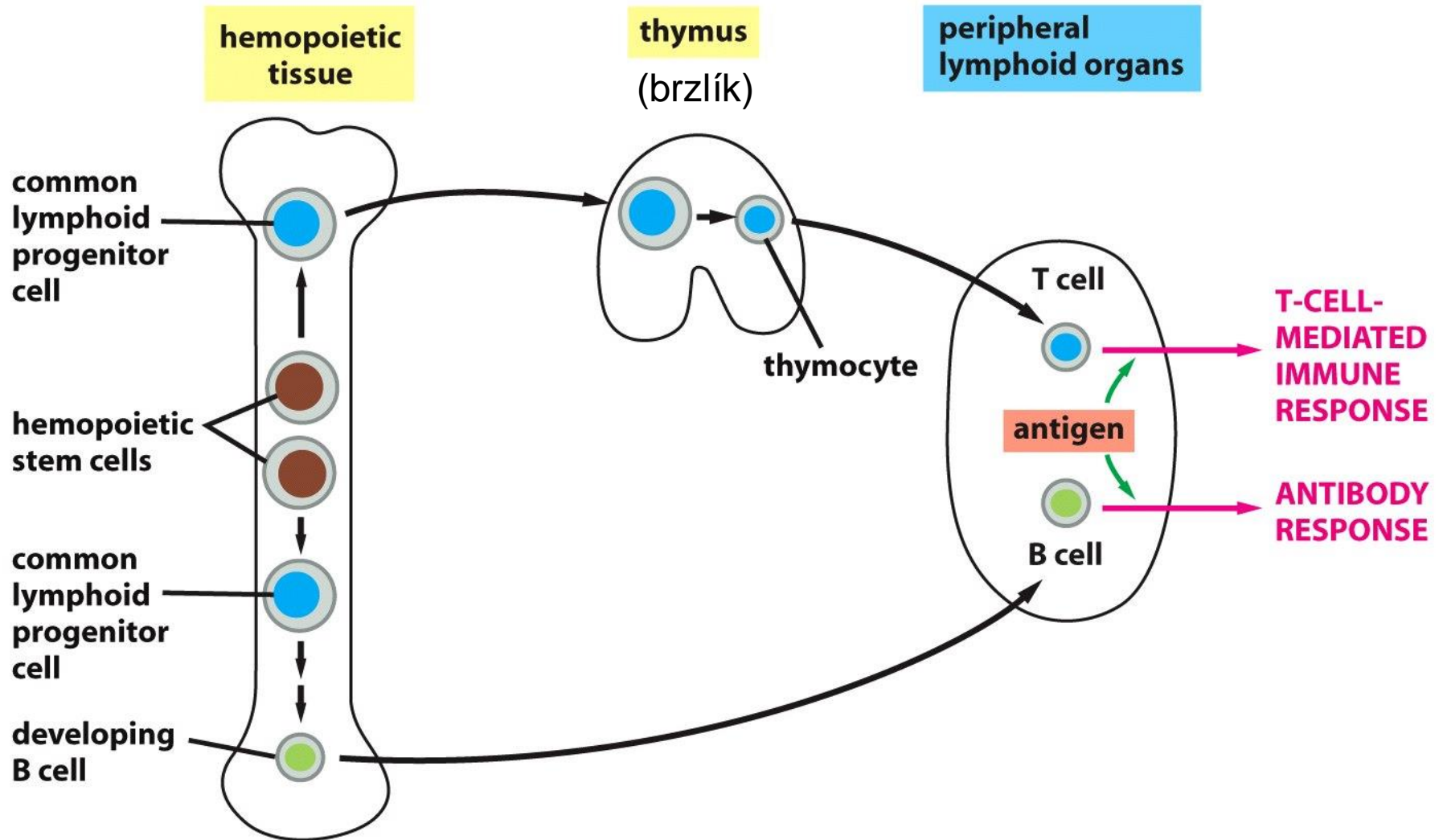
Bone marrow: B-cells
Thymus: T-cells

Secondary lymphatic organs (blue):

lymph nodes
spleen
and others

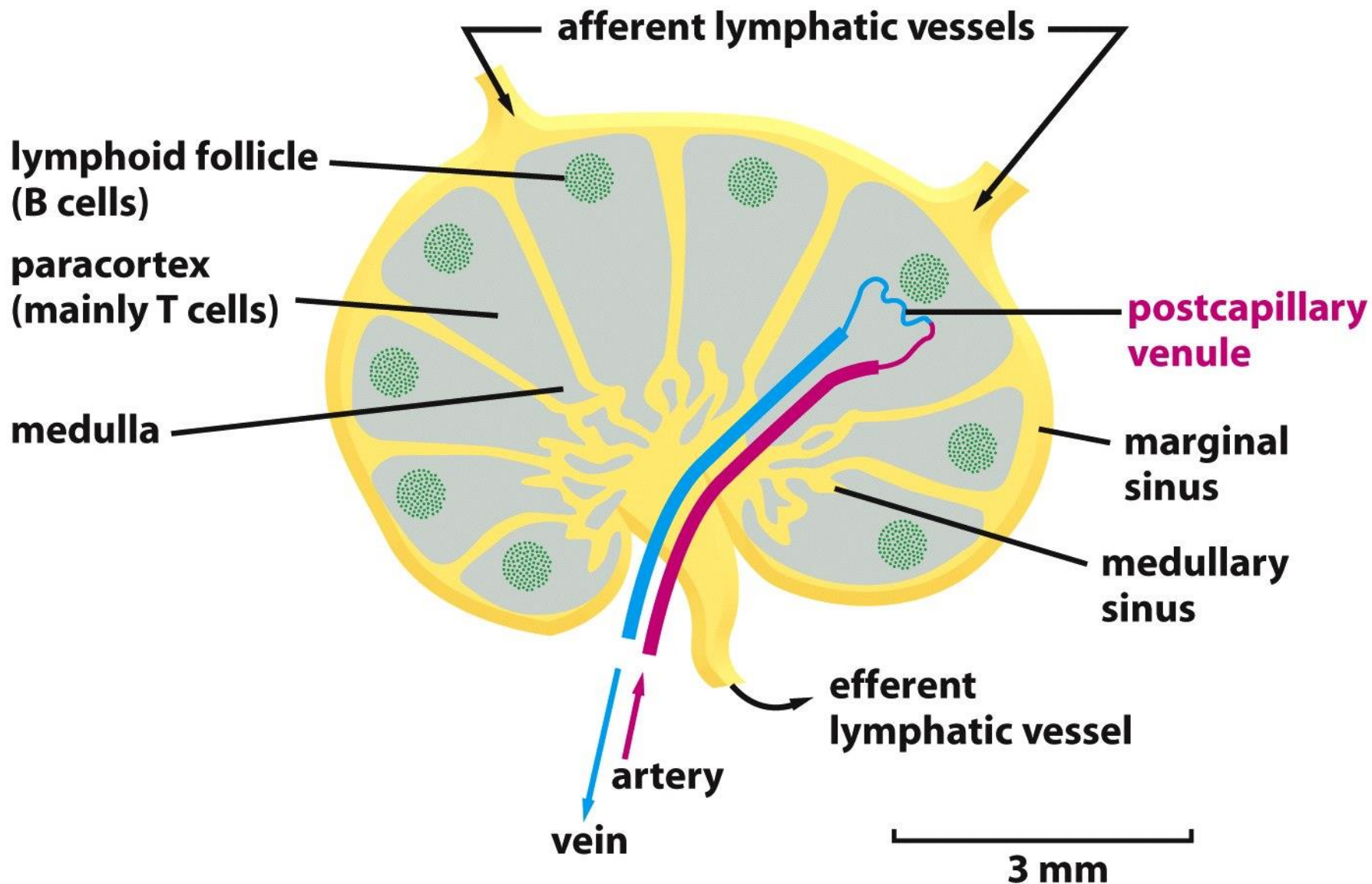
10^{12} lymphocytes (ca. 1 kg)

Development of B und T cells

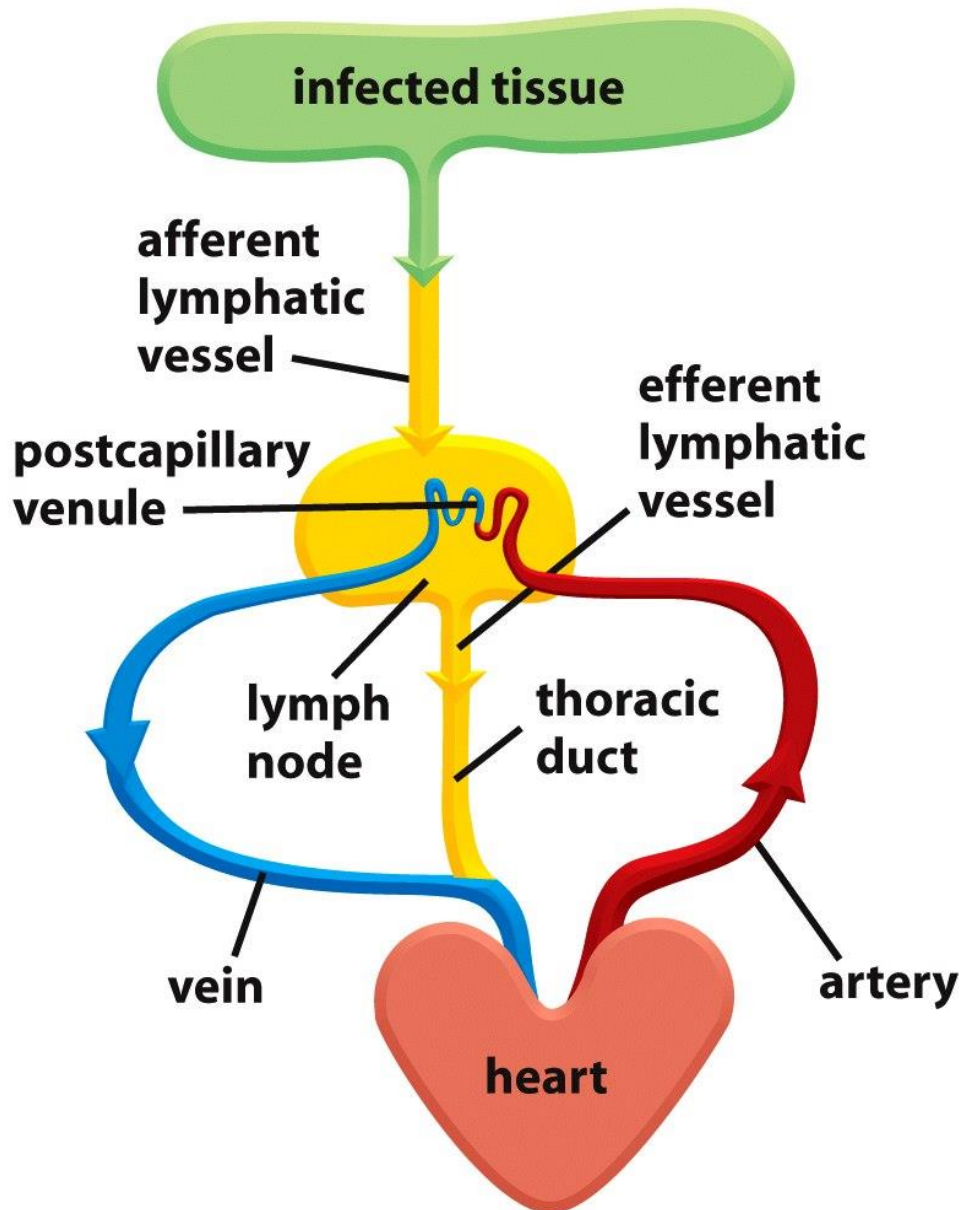


=> Bone marrow donation to reconstitute the immune system

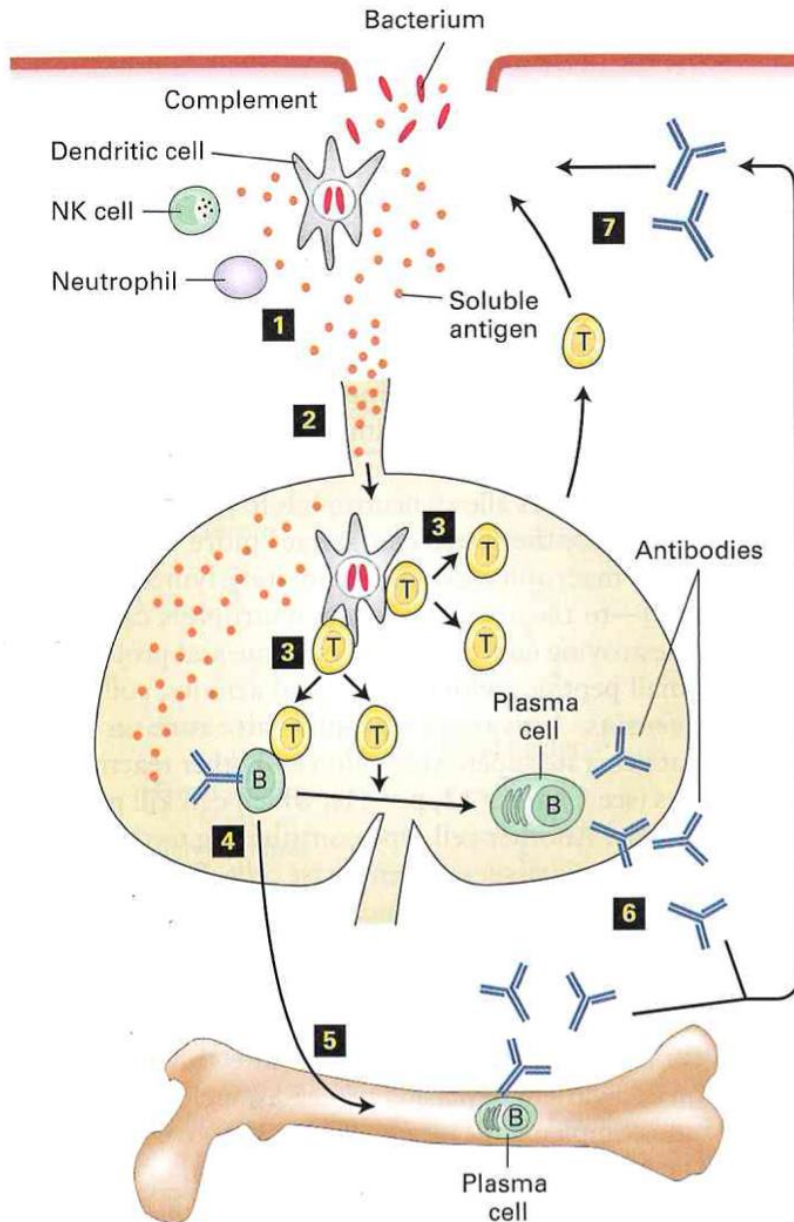
Lymph node



Circulation of lymphocytes



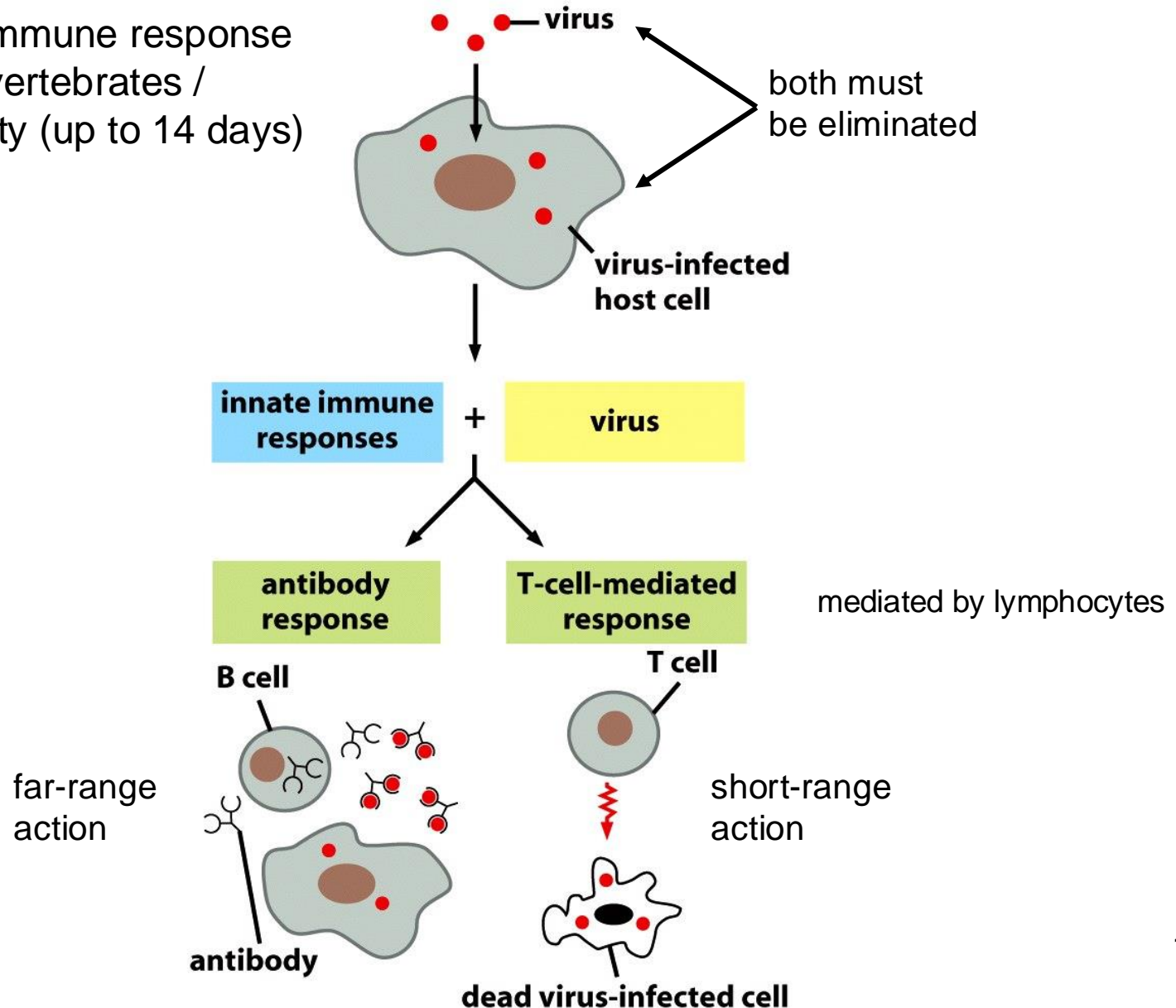
Overview of an inflammatory response



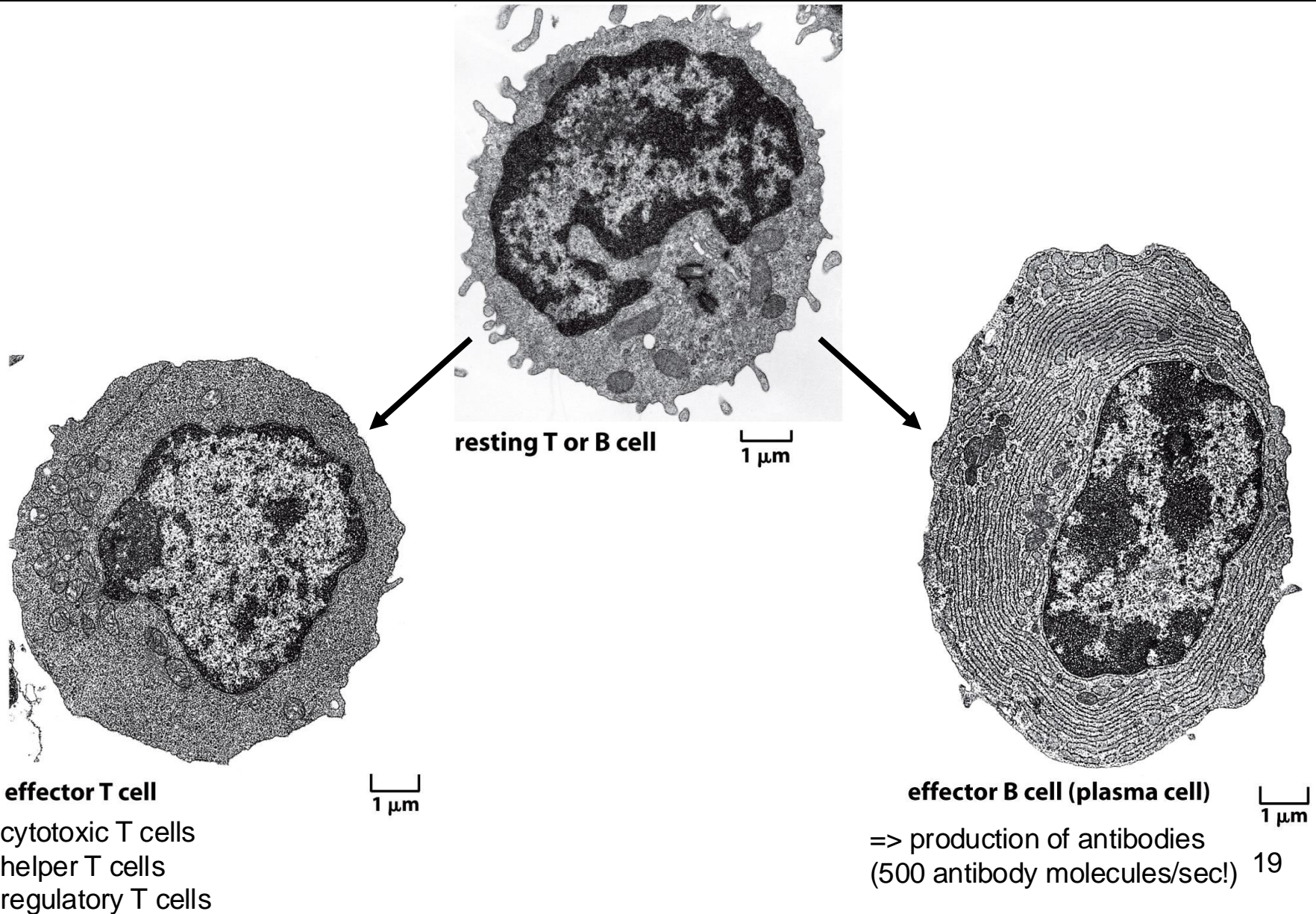
- 1) A bacterium encounters a first line of defense (innate immune response)
- 2) Breakdown of bacterium and release of antigens
- 3) Dendritic cells take up antigen and activate T cells
- 4) T cells proliferate and activate B cells
- 5) B cells differentiate into plasma cells
- 6) Plasma cells produce antibodies
- 7) Antibodies neutralize bacterium

Two classes of adaptive immune responses

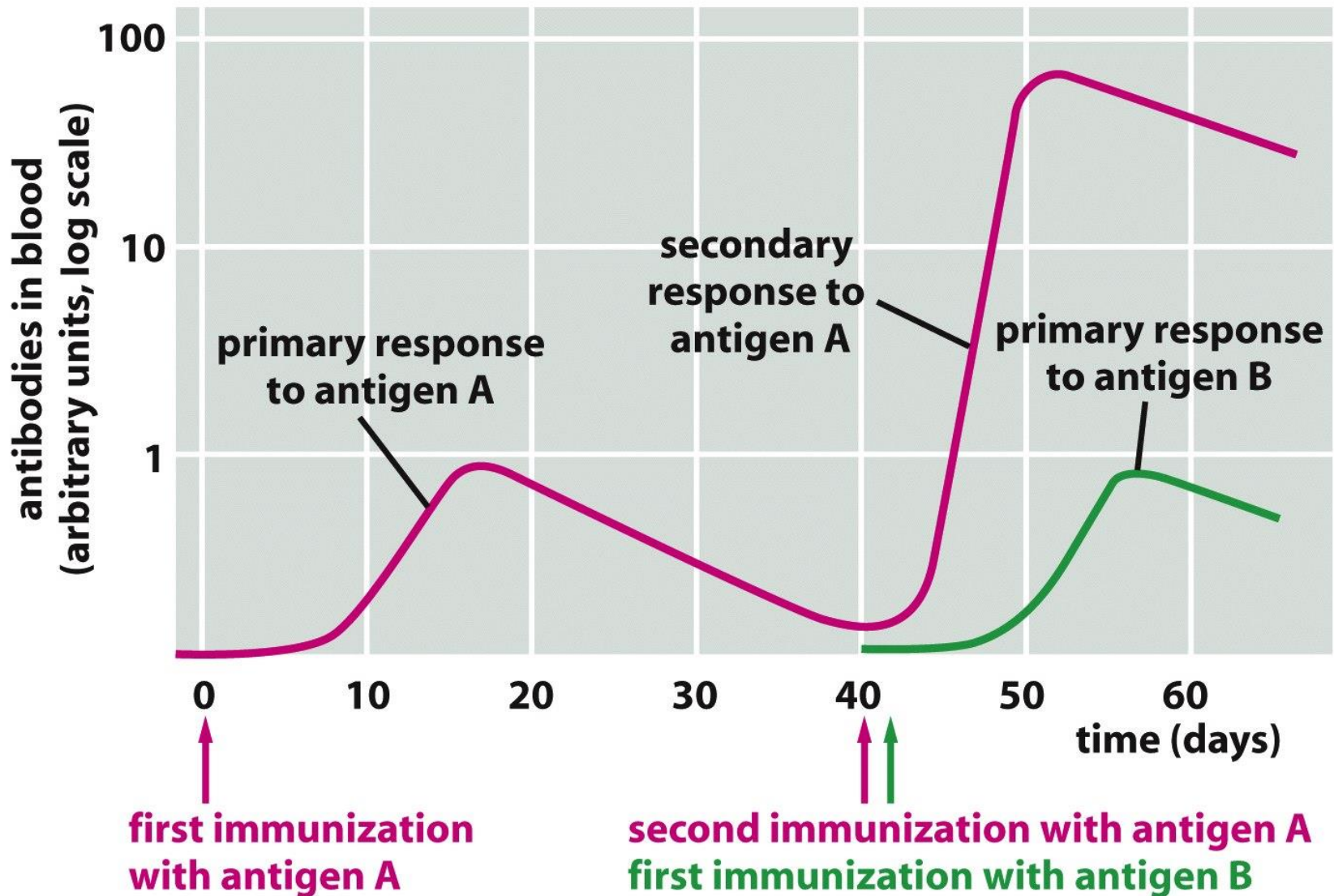
An adaptive immune response is unique for vertebrates / delayed activity (up to 14 days)



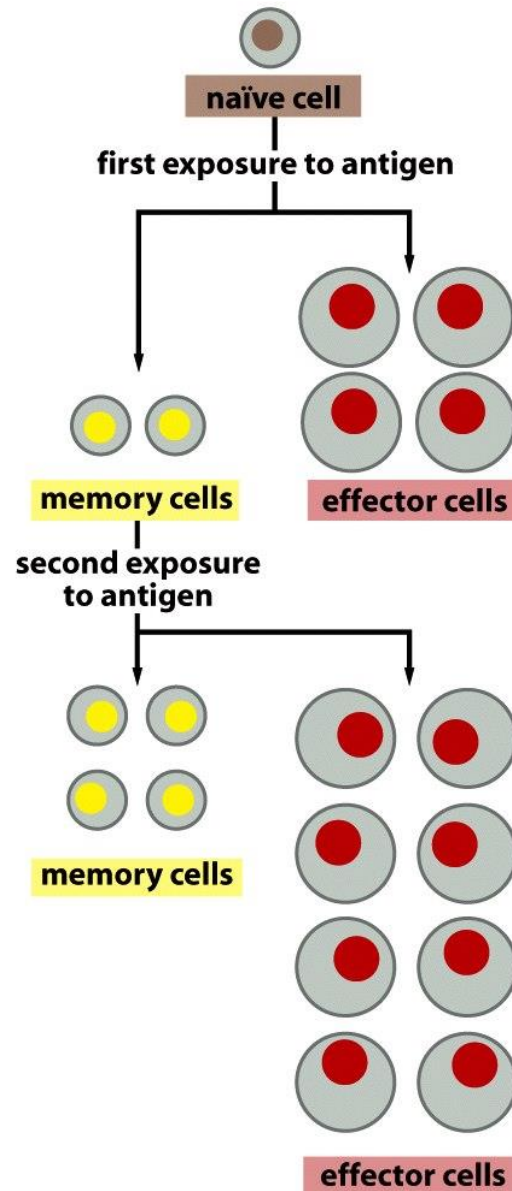
Activation of lymphocytes



Progress of immune response

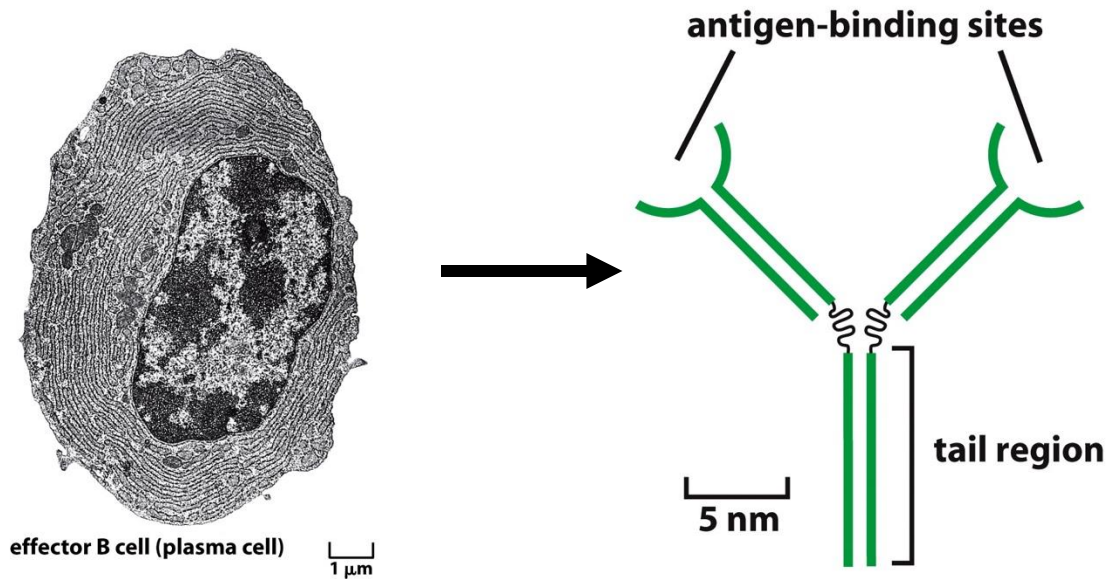


Immunological memory

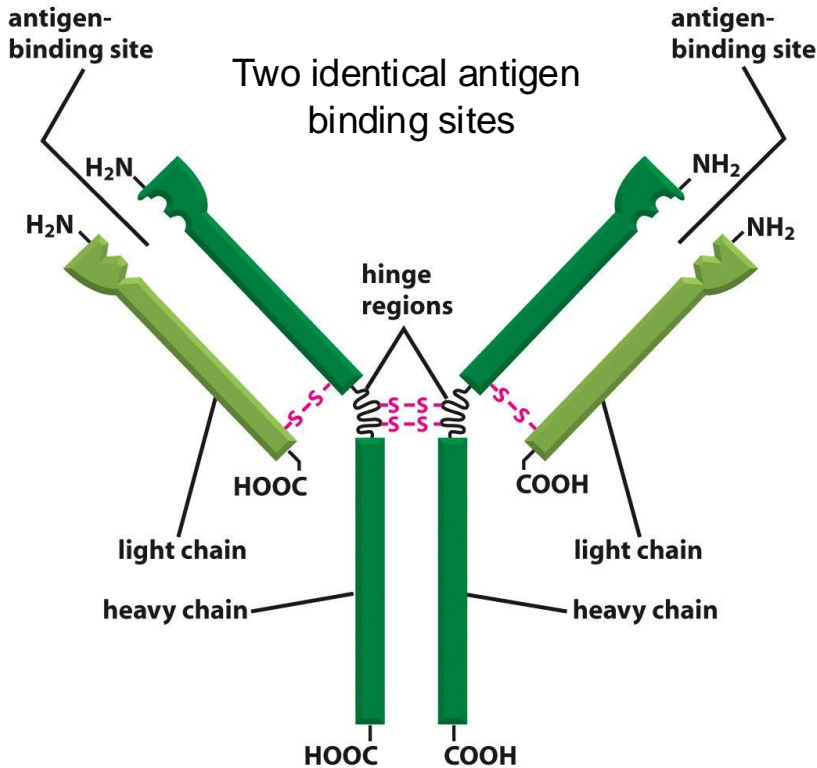


Vaccination!

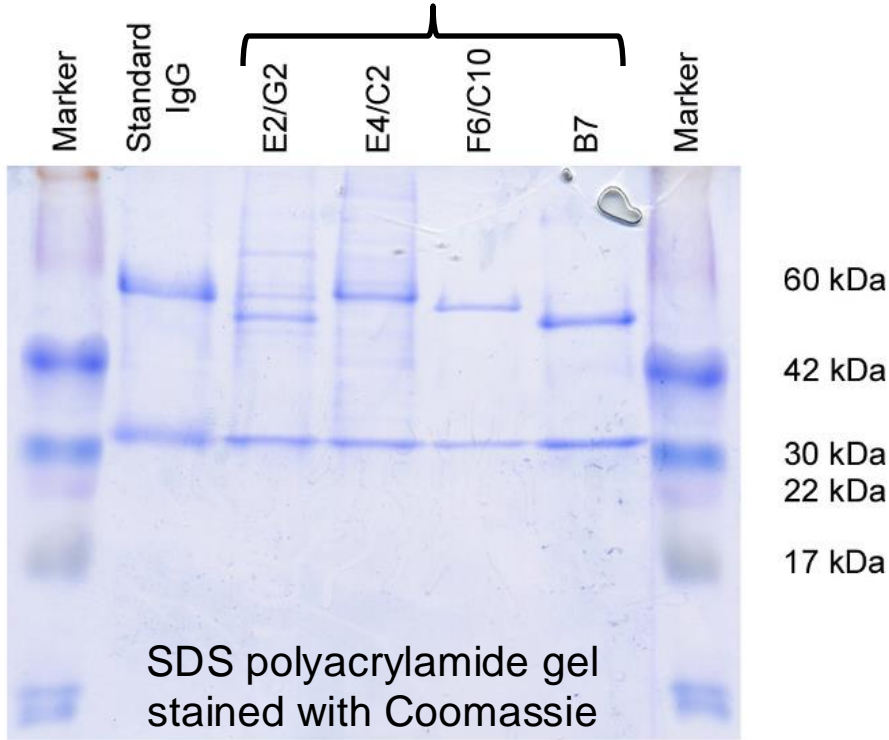
B cells and antibodies



Structure of IgG



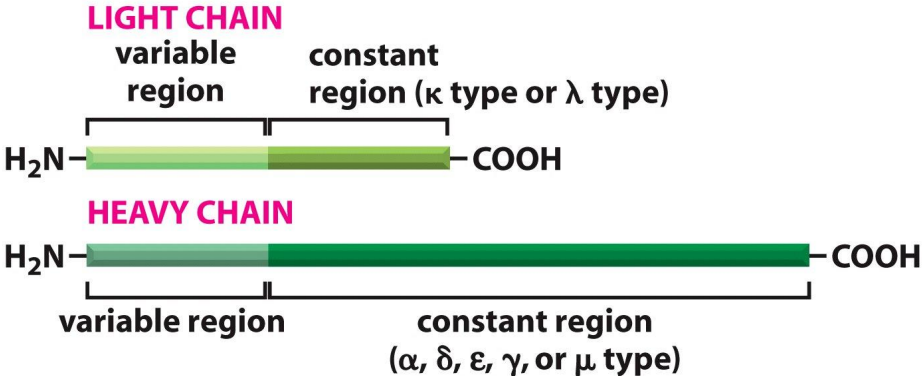
4 different IgG antibody clones against the same antigen



⇒ 2 light chains (25 kDa, light green)

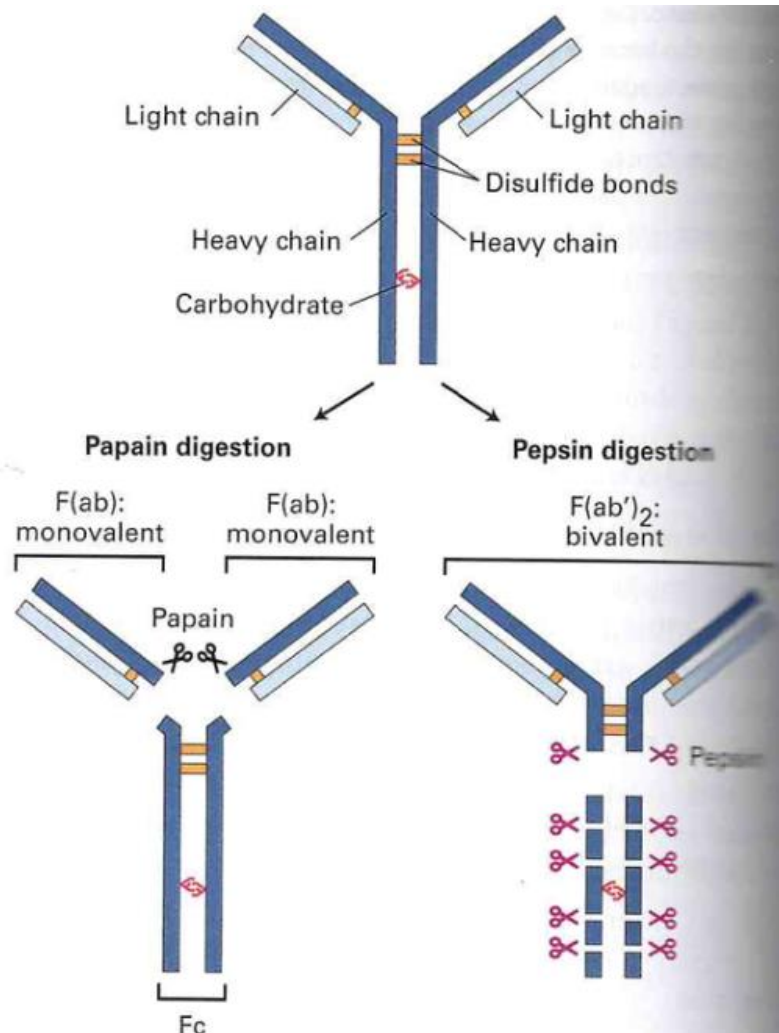
⇒ 2 heavy chains (50 kDa, dark green)

total mass: 150 kDa

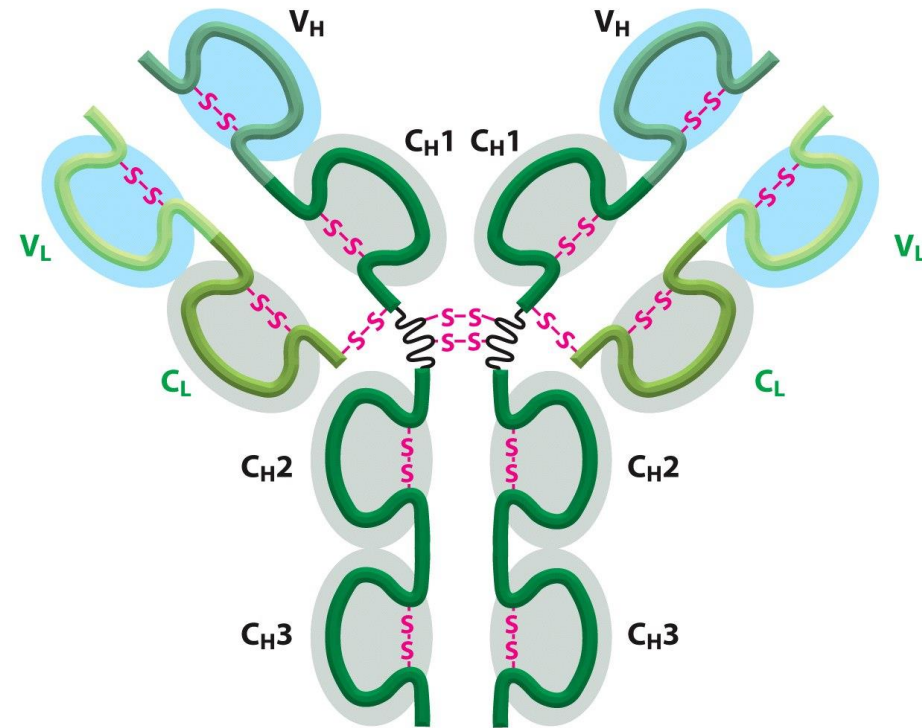


Structure of IgG

Fragmentation of antibody

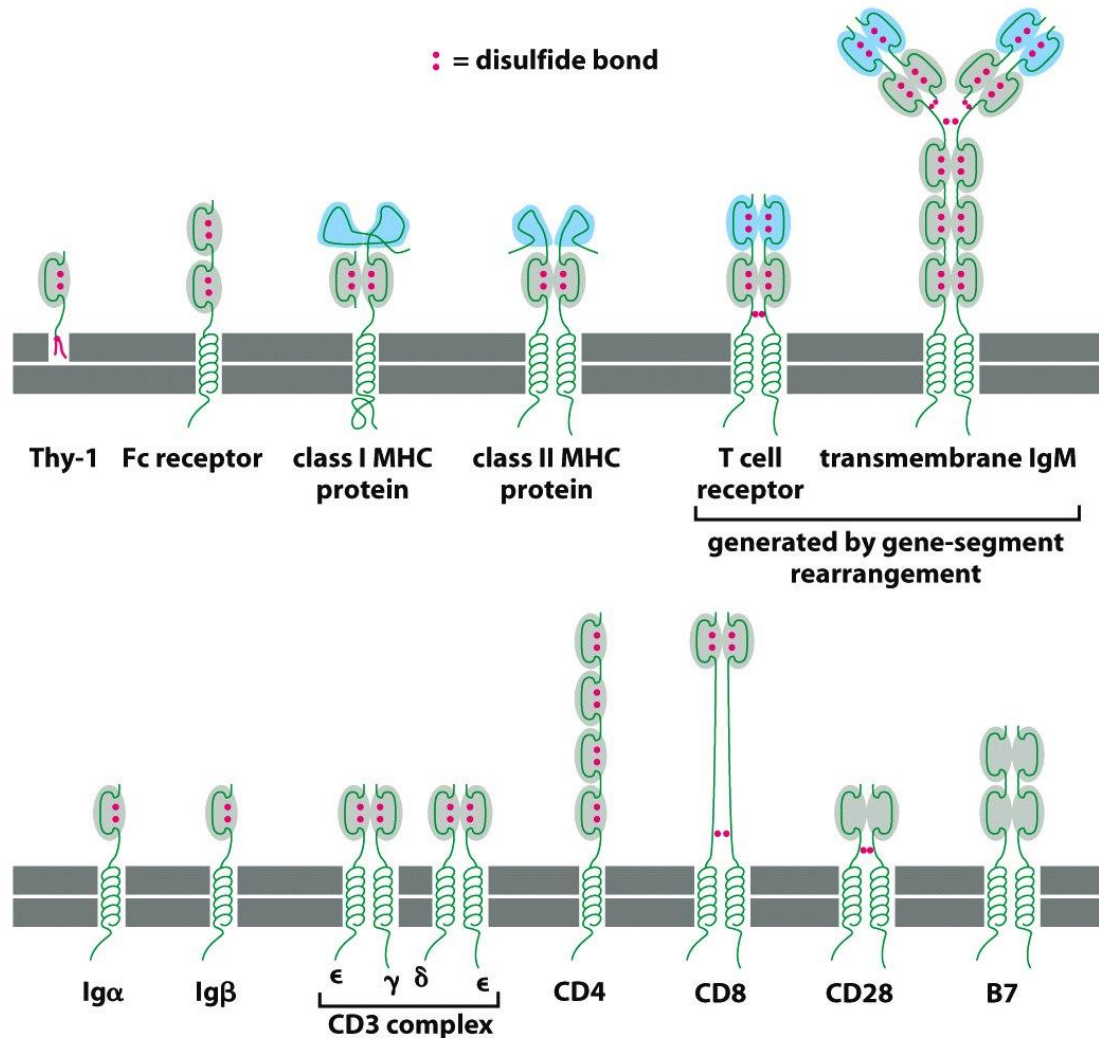


Immunoglobulin domains



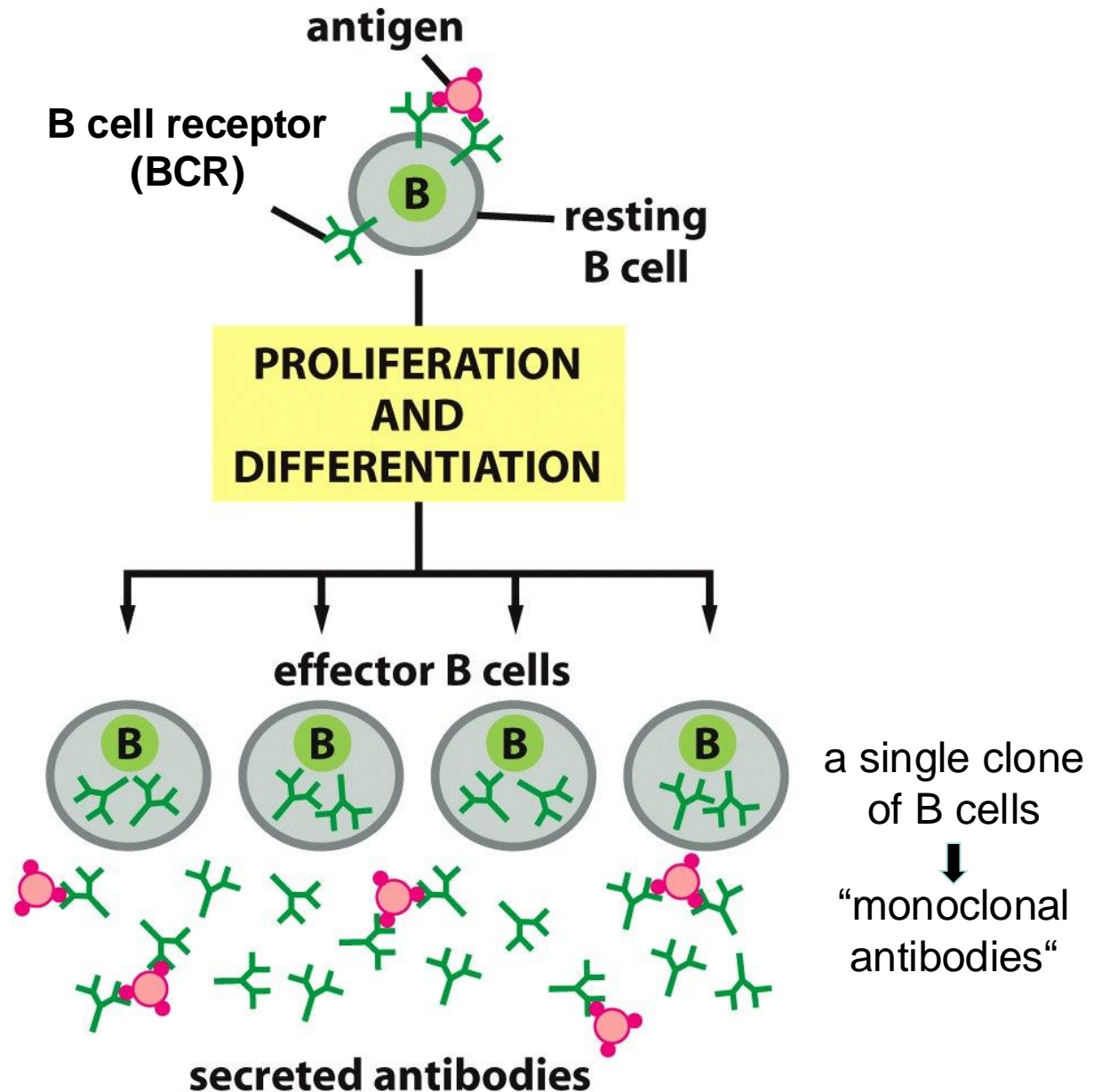
F: fragment
ab: antigen binding
c: crystallizable (constant)

Immunoglobulin (Ig) superfamily

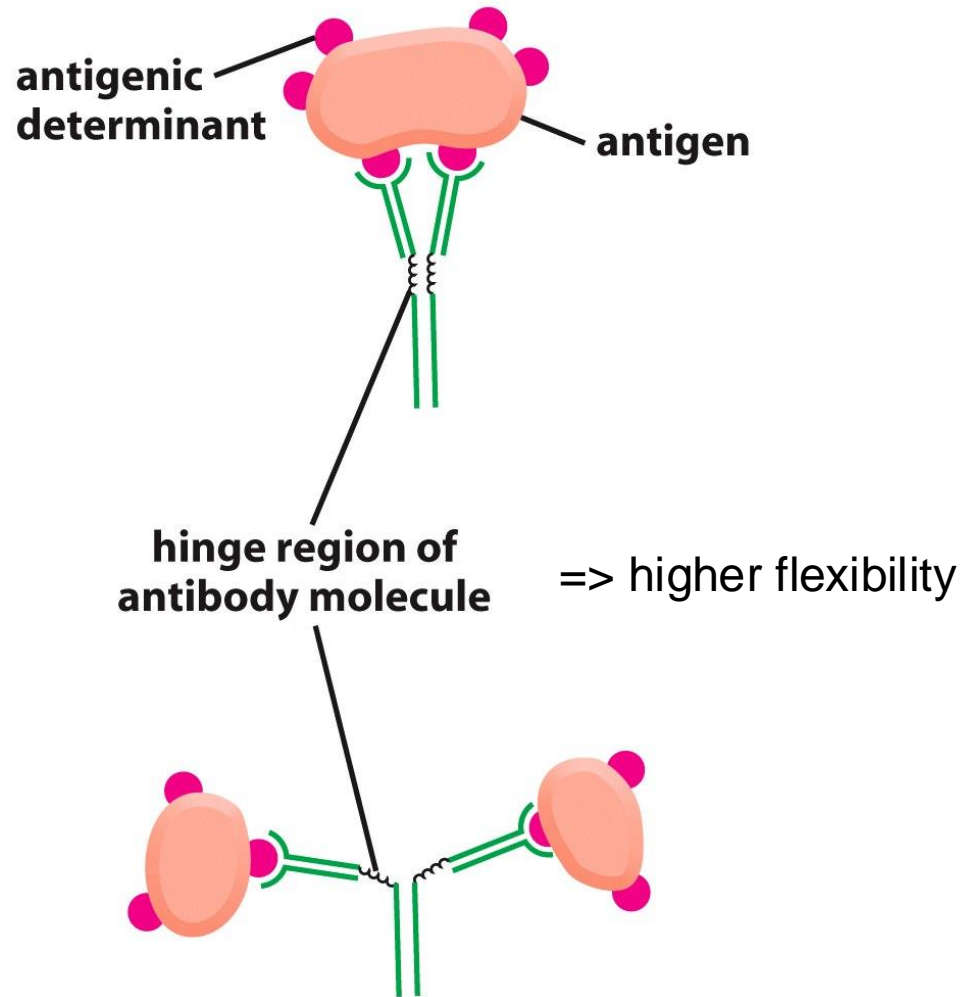


Shown: important membrane-bound molecules of the immune system
 more than 750 members in total (also cell-cell interactions); many cell surface proteins

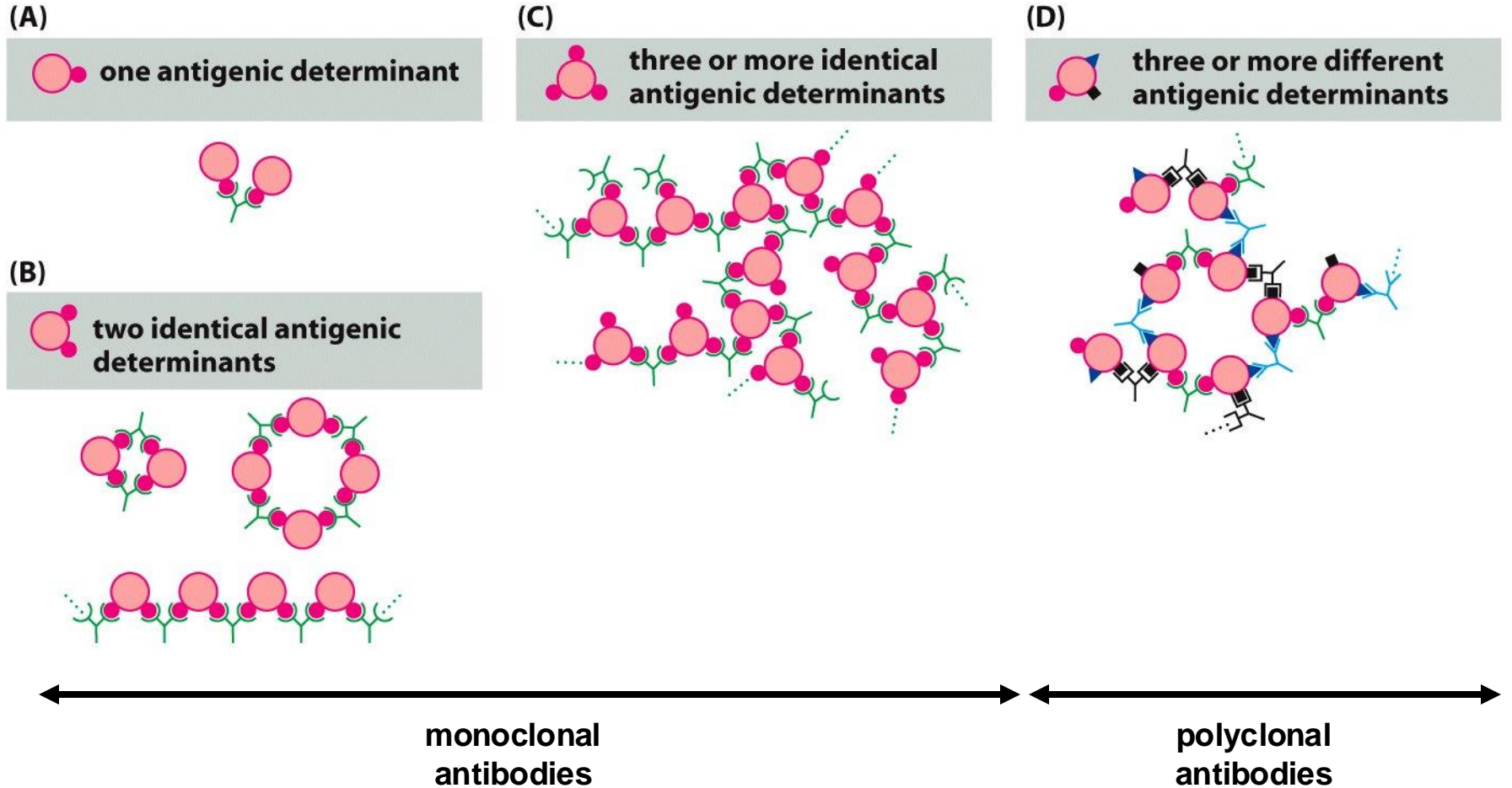
Membrane-bound BCR and secreted antibodies



The hinge region

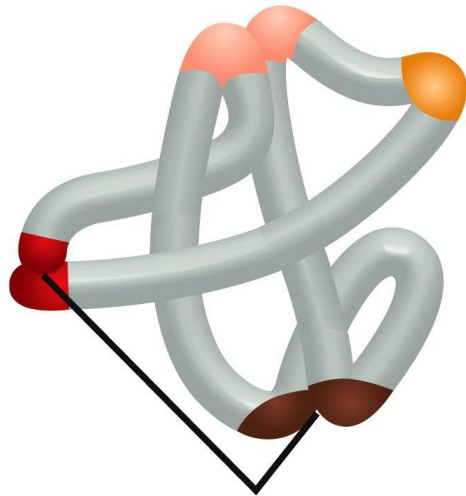


Interactions of antibody and antigen



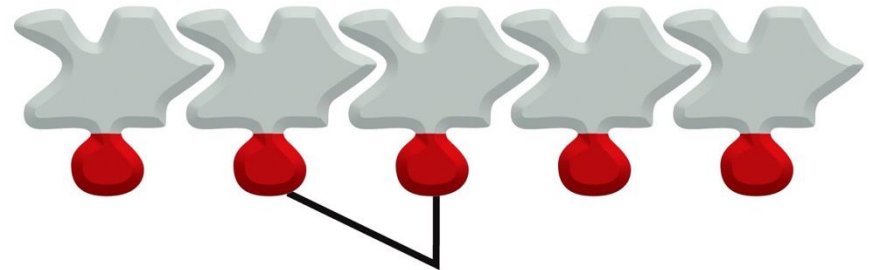
Multiple antigenic determinants: epitope

MULTIVALENT ANTIGEN



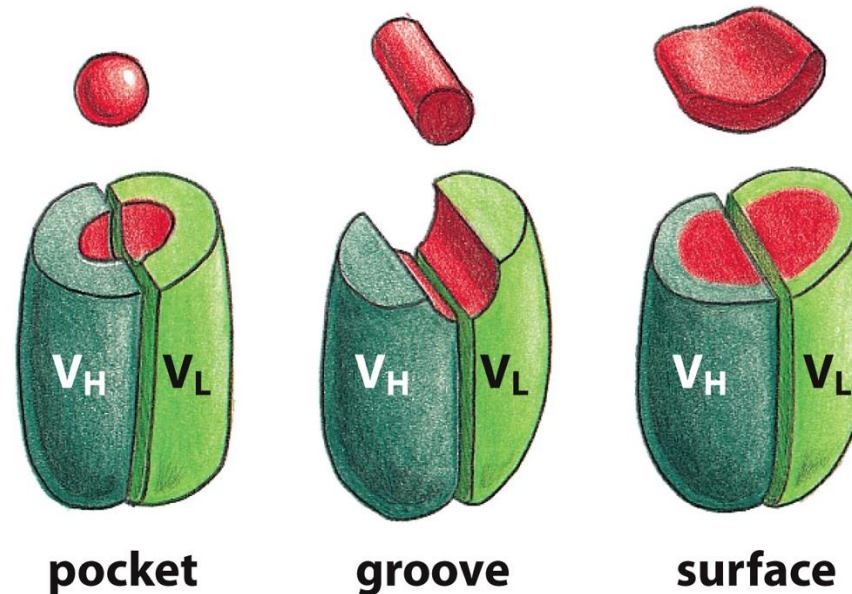
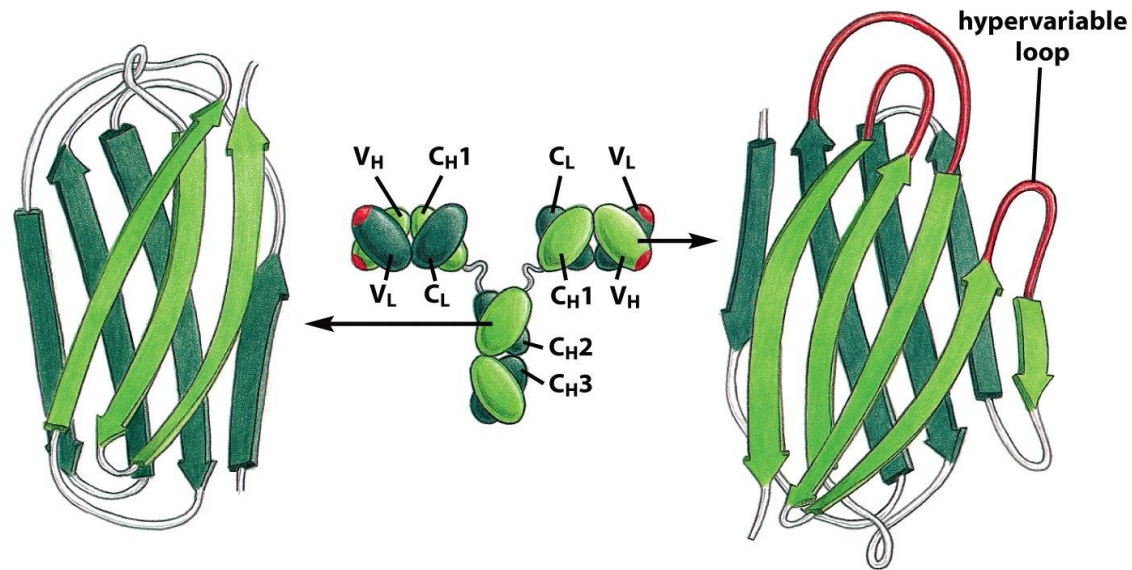
multiple **different** antigenic determinants

POLYVALENT ANTIGEN



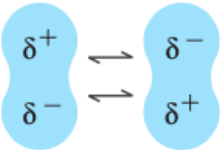
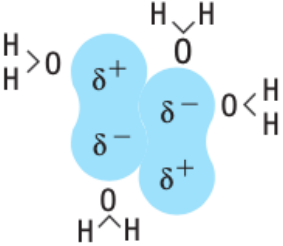
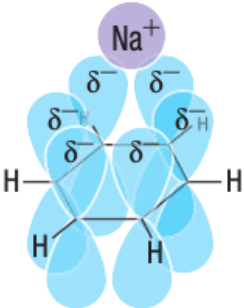
multiple **identical** antigenic determinants

Antigen-binding sites of antibodies

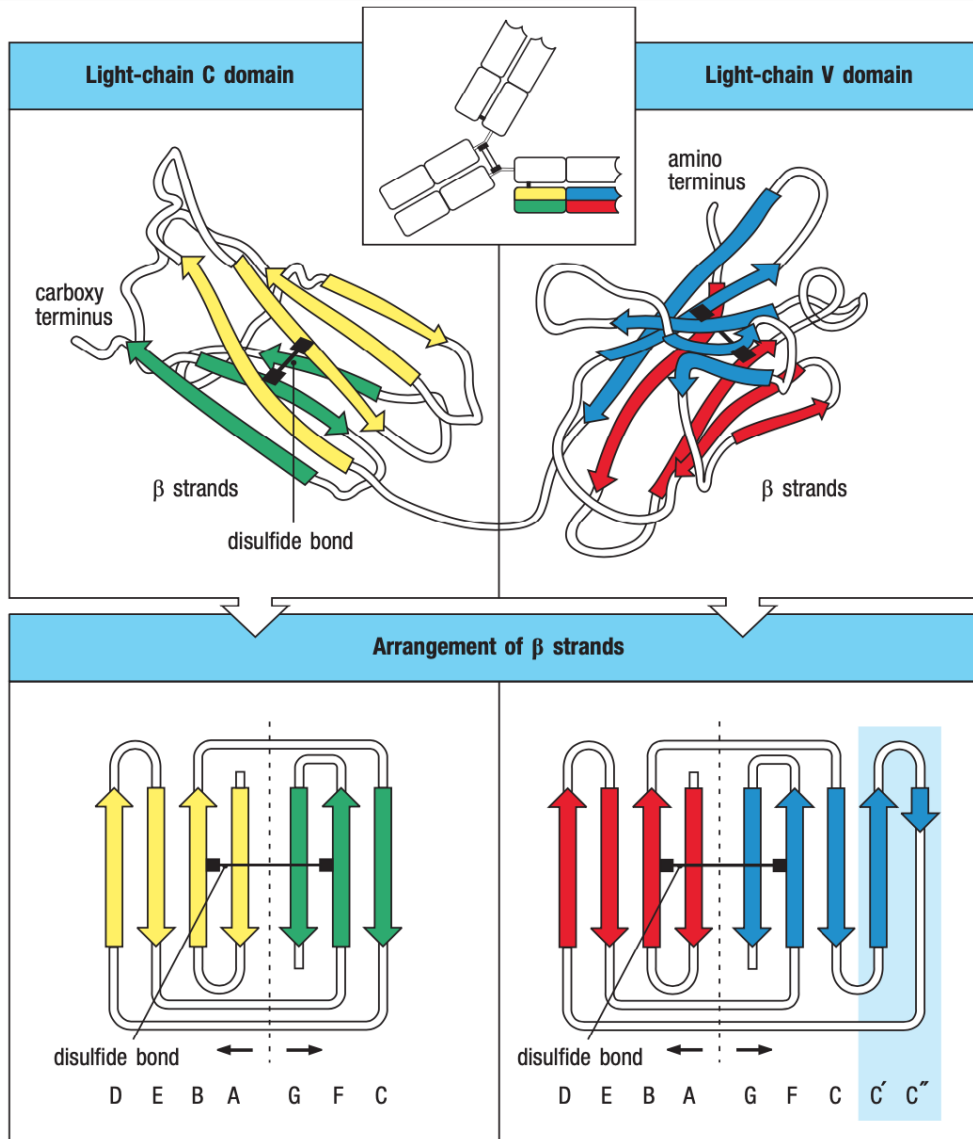


Different antigenic structures

Non-covalent binding forces [AgAb]

Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$-\overset{\oplus}{\text{N}}\text{H}_3 \quad \overset{\ominus}{\text{O}}\text{OC}-$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N, O)	$\begin{array}{c} \diagup \text{N} - \text{H} - - \text{O} = \text{C} \diagdown \\ \delta^- \quad \delta^+ \quad \delta^- \end{array}$
Van der Waals forces	Fluctuations in electron clouds around molecules polarize neighboring atoms oppositely	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	
Cation-pi interaction	Non-covalent interaction between a cation and an electron cloud of a nearby aromatic group	

Detailed structure of antibody



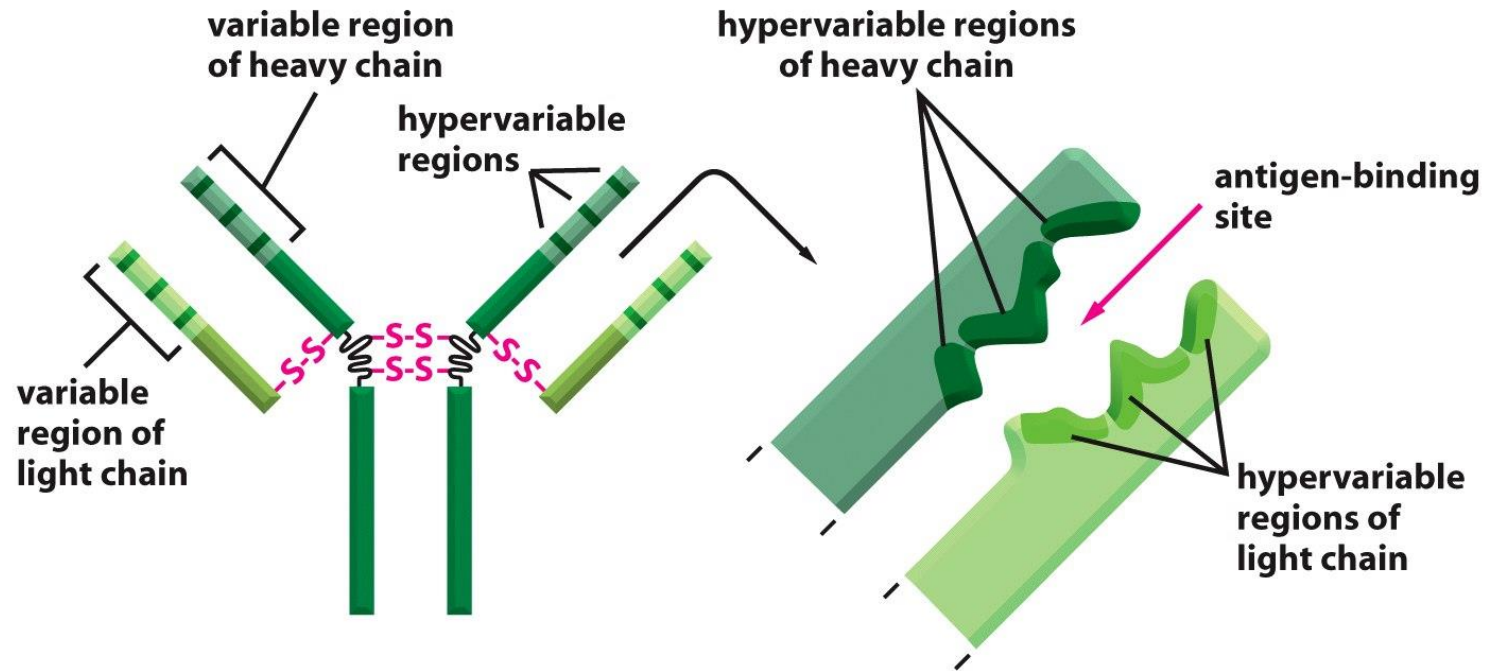
anti-parallel β sheets form a β barrel

C' and C'' are not present in the C domain

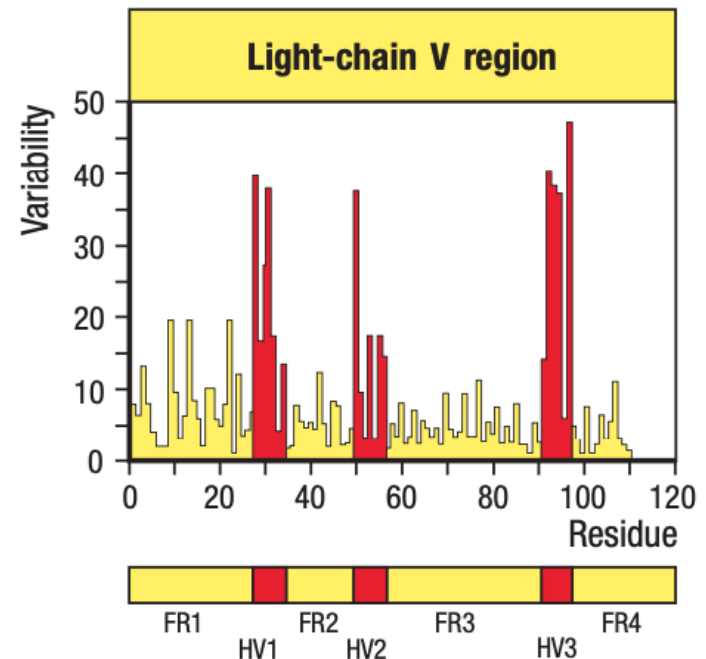
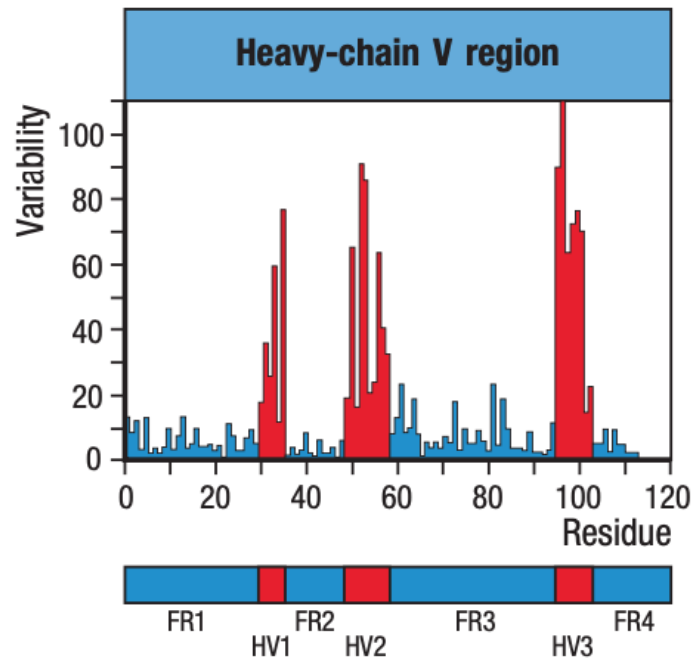
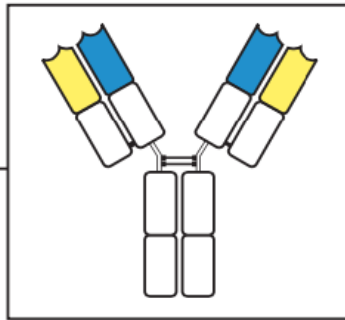
4 strand + 3 strand

4 strand + 5 strand

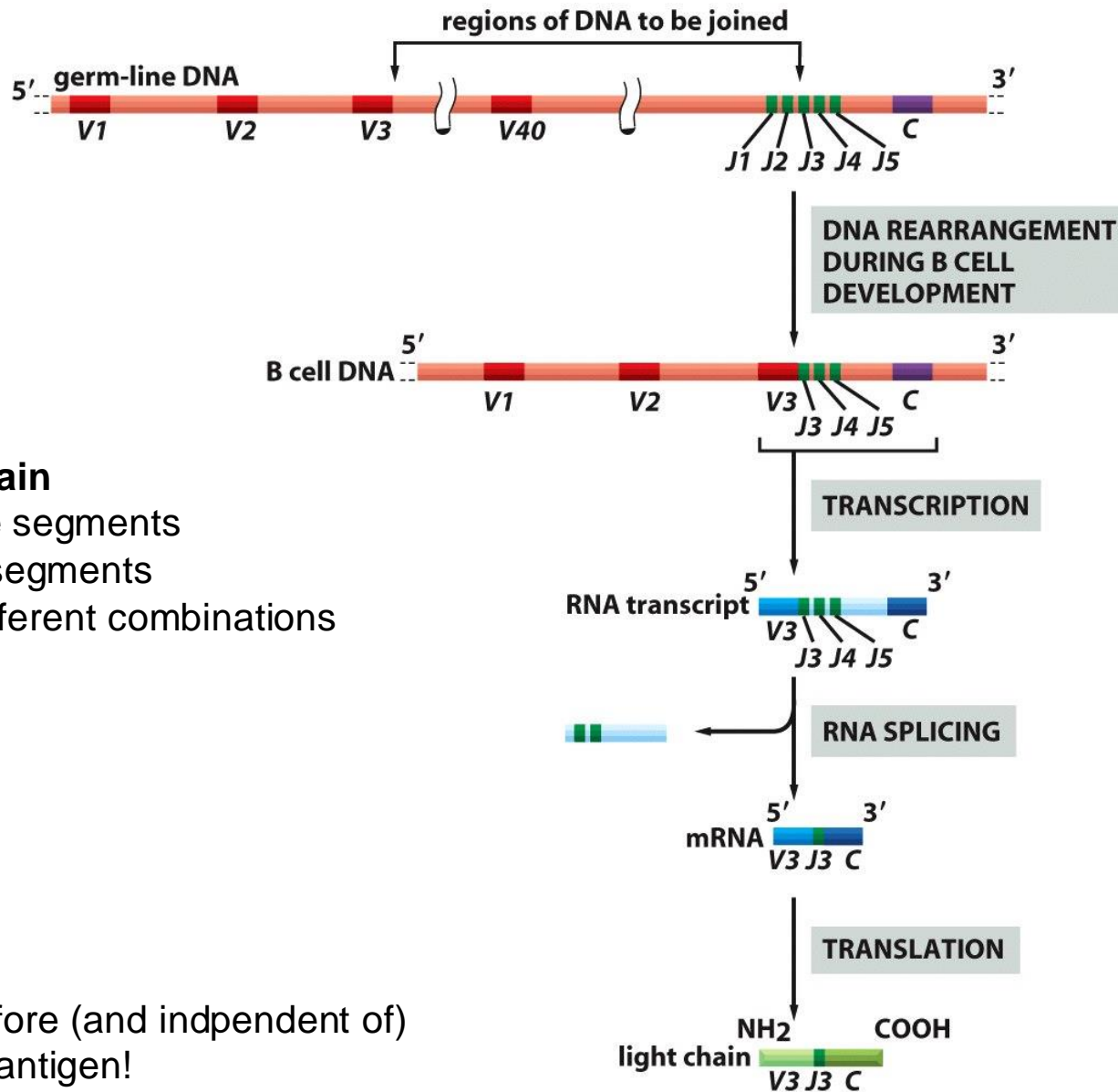
Hypervariable regions of binding sites



Hypervariable regions of binding sites



Generation of antibody diversity: light chain



κ light chain

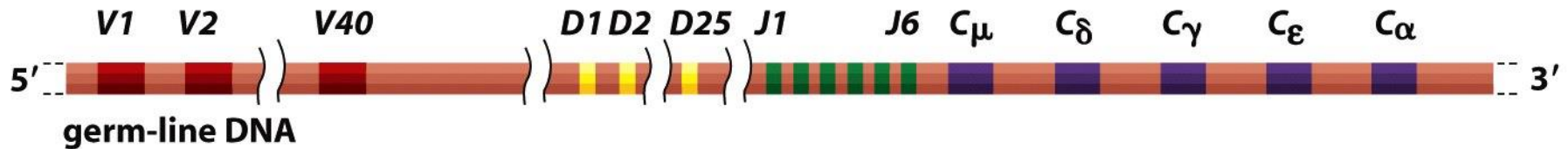
40 V gene segments

5 J gene segments

=> 200 different combinations

This happens before (and independent of) any contact with antigen!

Generation of antibody diversity: heavy chain



heavy chain

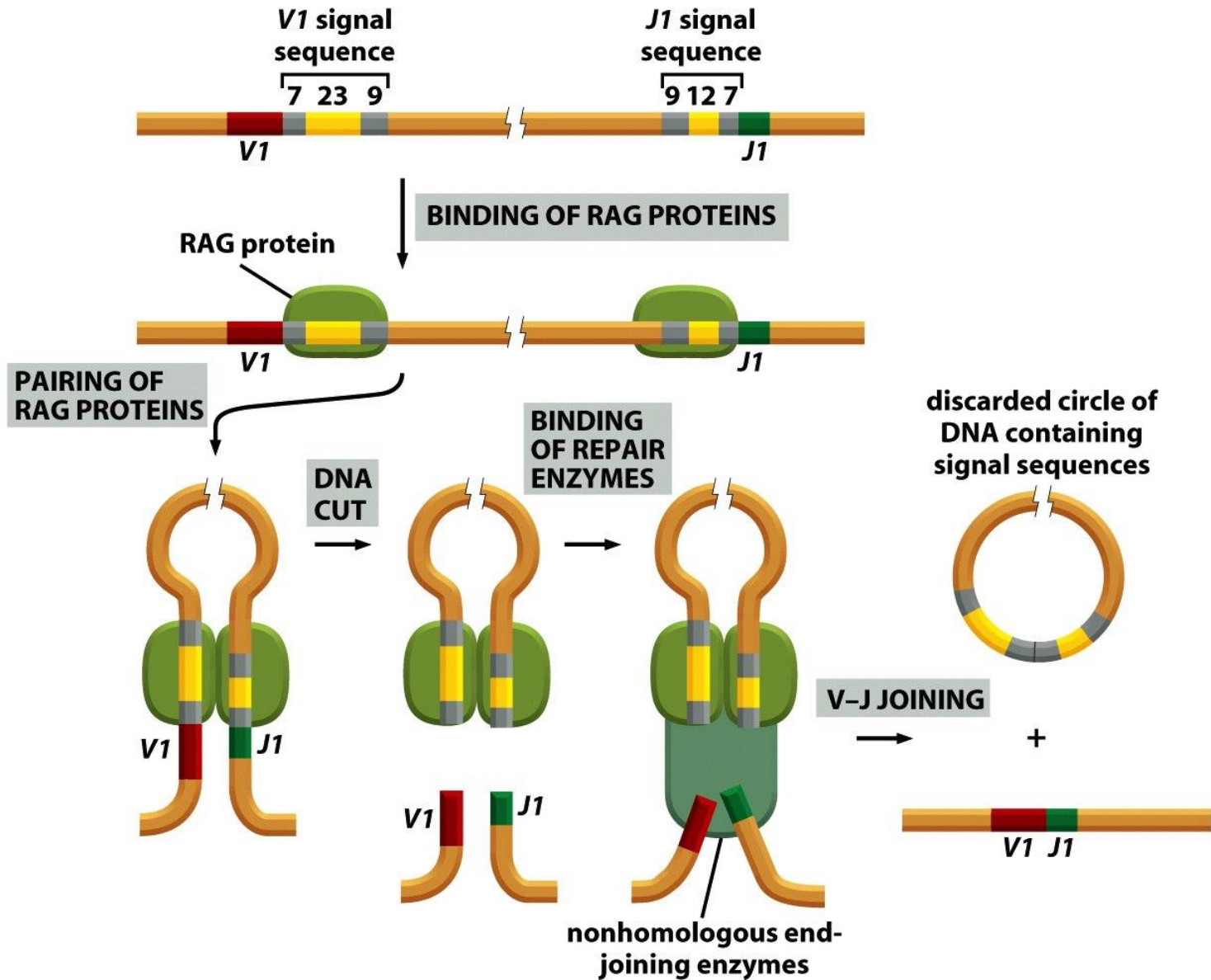
40 V gene segments

25 D gene segments

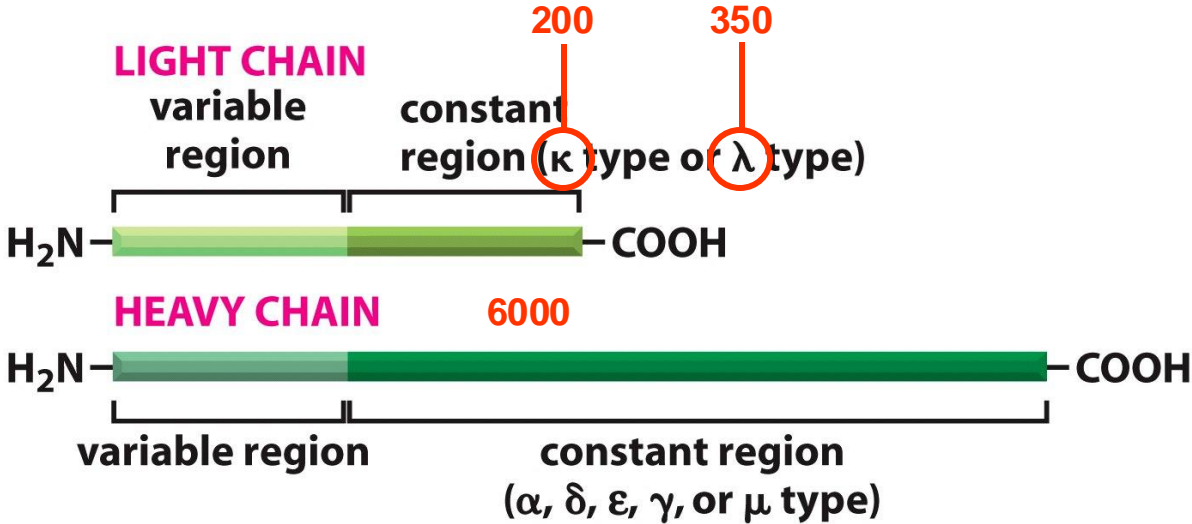
6 J gene segments

=> 6000 combinations

Gene segment joining



Generation of antibody diversity

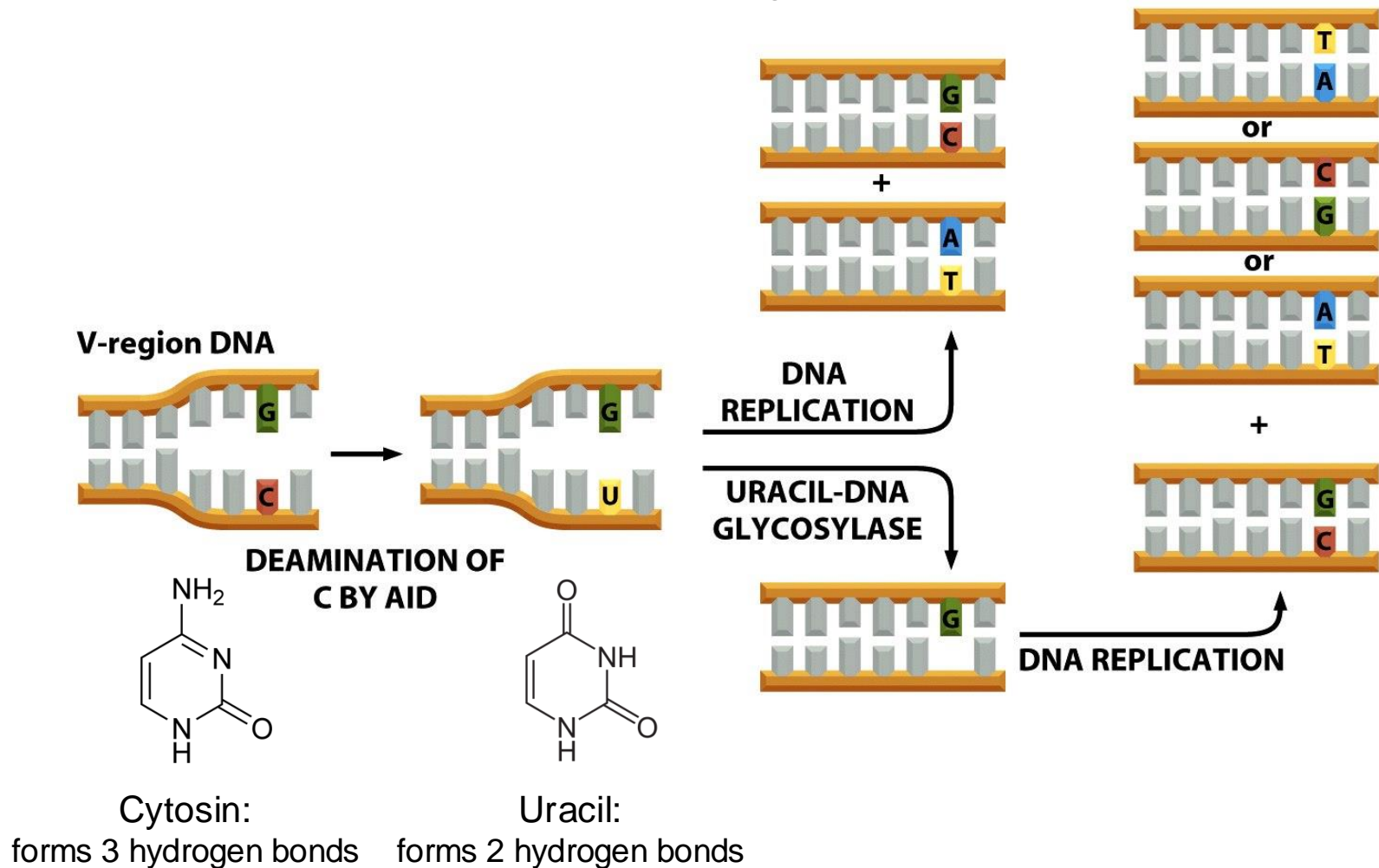


=> about 2.000.000 combinations

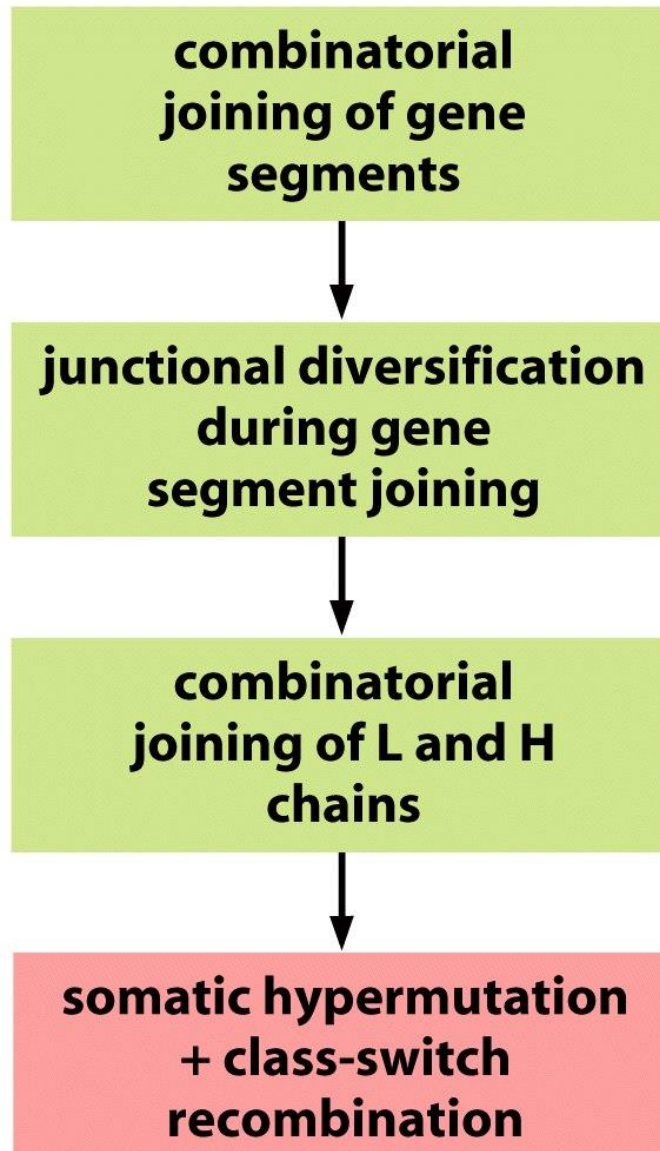
Affinity maturation of antibodies

Somatic hypermutation by activity-induced deaminase (AID)

=> 1 mutation per V region per cell cycle



Main mechanisms of antibody diversity



After contact with antigen

⇒ There is an even larger repertoire of combinations than the 12^{12} existing B cells.

Antibody affinity limits during immune responses

Binding rate k_{on} : 10^5 - $10^6 \text{ M}^{-1}\text{s}^{-1}$

=> controlled by diffusion

Release rate k_{off} : 10^{-3} - 10^{-4} s^{-1}

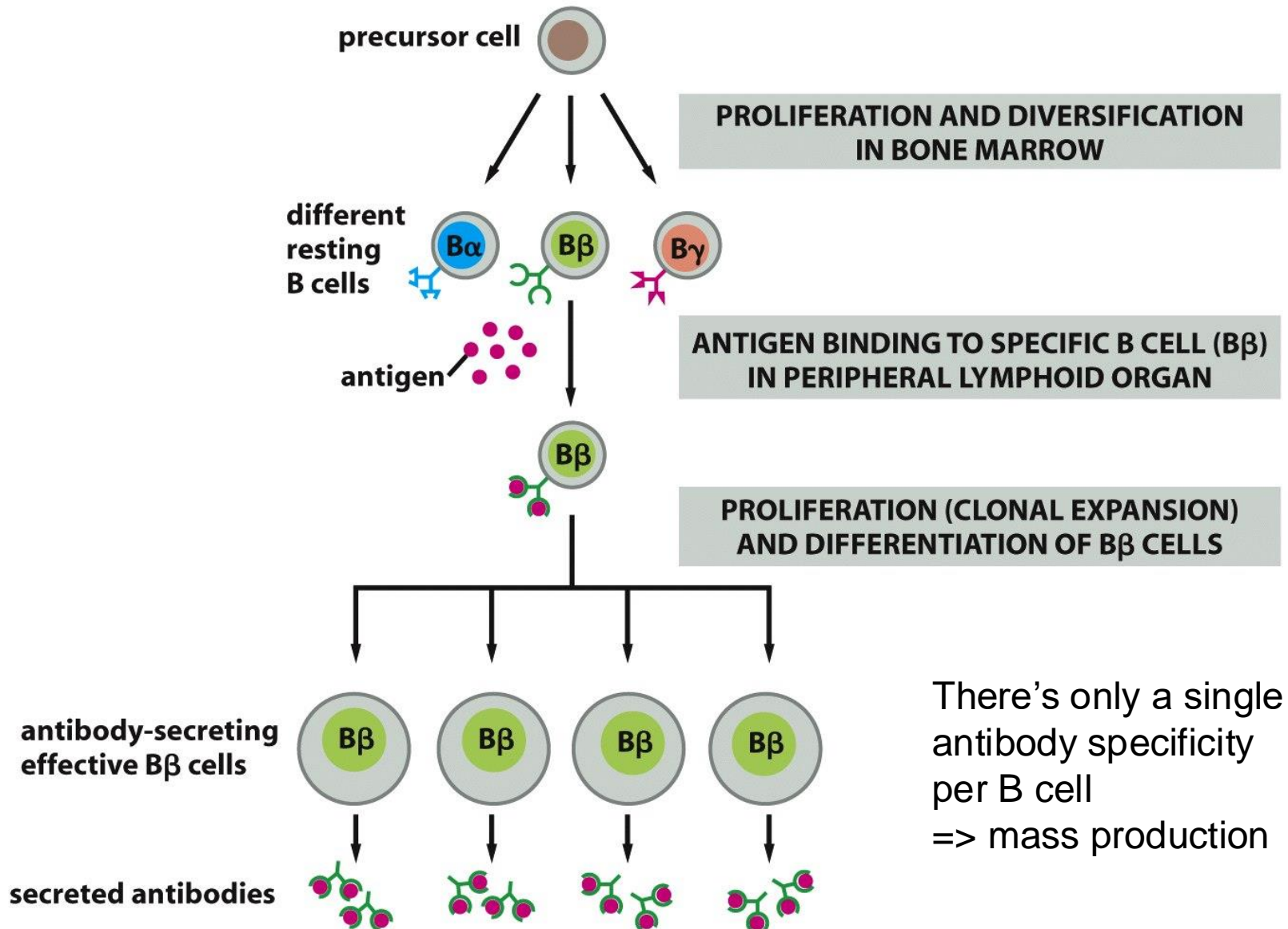
=> controlled by time for signal transduction/endocytosis
after antigen binding to cell surface receptors

Maximum affinity* of antibodies: $K_a = k_{\text{on}}/k_{\text{off}} = 10^{10} \text{ M}^{-1}$

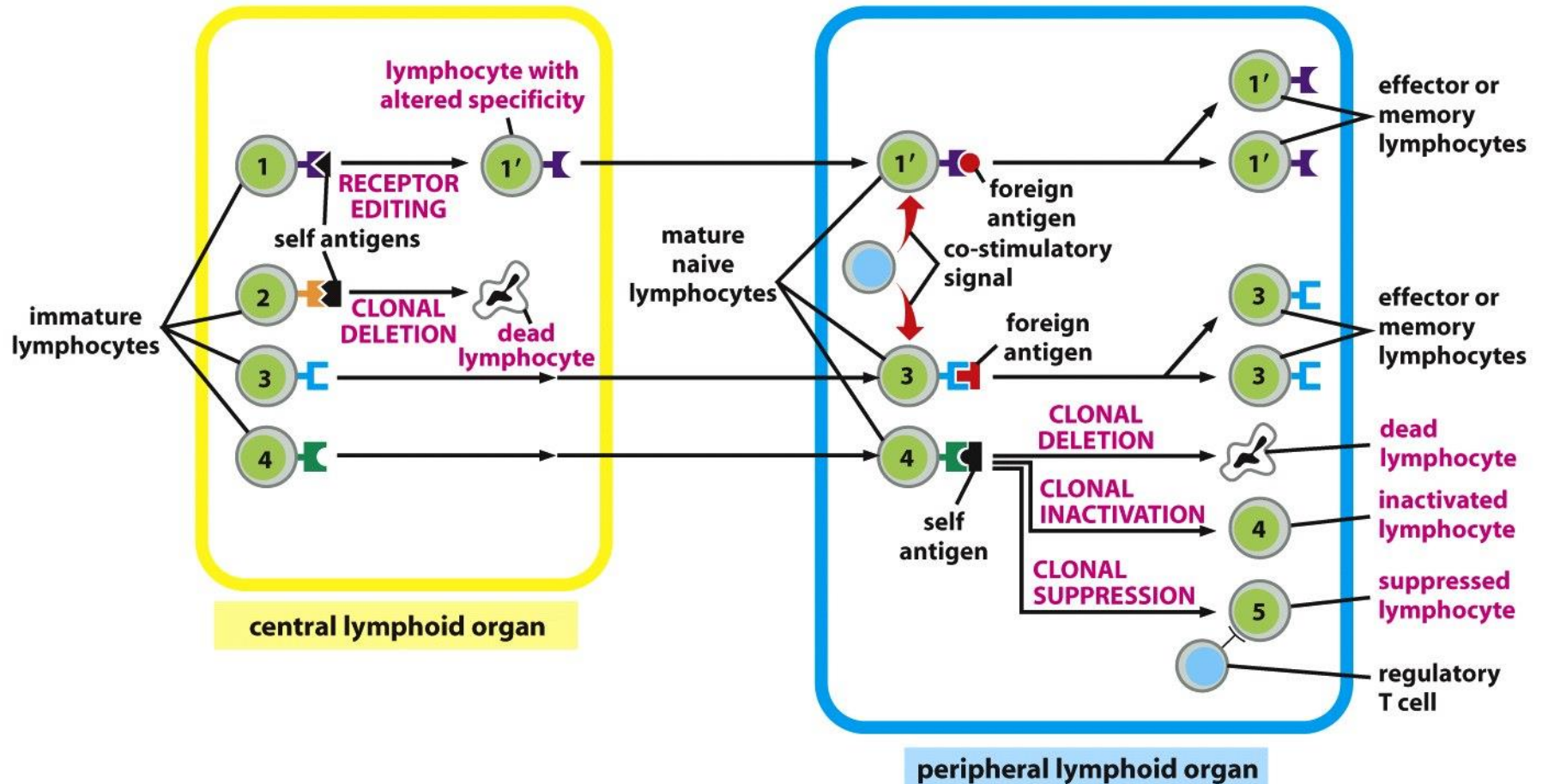
=> Higher affinity antibodies may arise but would have no selective advantage
(affinity ceiling)

*for comparison: biotin-streptavidin: $K_a = 10^{14} \text{ M}^{-1}$

Clonal selection theory



Immunological tolerance



But this system is not perfect: **autoimmune diseases**
e.g.: Epstein-Barr virus is suspected to induce multiple sclerosis

Theoretical considerations of antigen recognition

Innate immune response:

=> Elimination of everything that is recognized as foreign

Problem: through natural evolution, a pathogen can adapt to hide or change its distinct antigenic signatures

(pathogens have a big evolutionary advantage because they have a much shorter lifecycle (bacteria > 20 min) and larger populations than animals (> 1 year) in principle they can adapt 30,000x faster!)

Time to acquire 2% difference in genome sequences

Humans: 8 million years

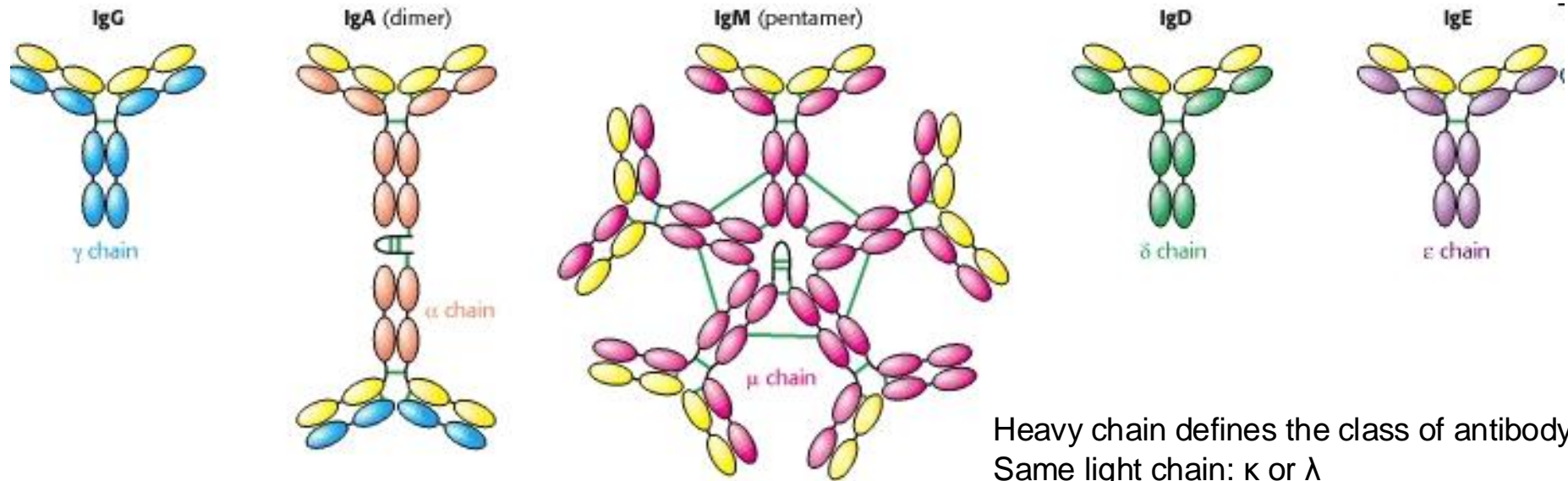
Poliovirus: 5 days

Adaptive immune response:

=> Elimination of anything that is **not** recognized as **own**

Solution: each individual person starts its mini-evolution within its leucocytes (instead of a whole life cycle, a pathogen-specific immune response is ready in less than 2 weeks)

Antibody classes



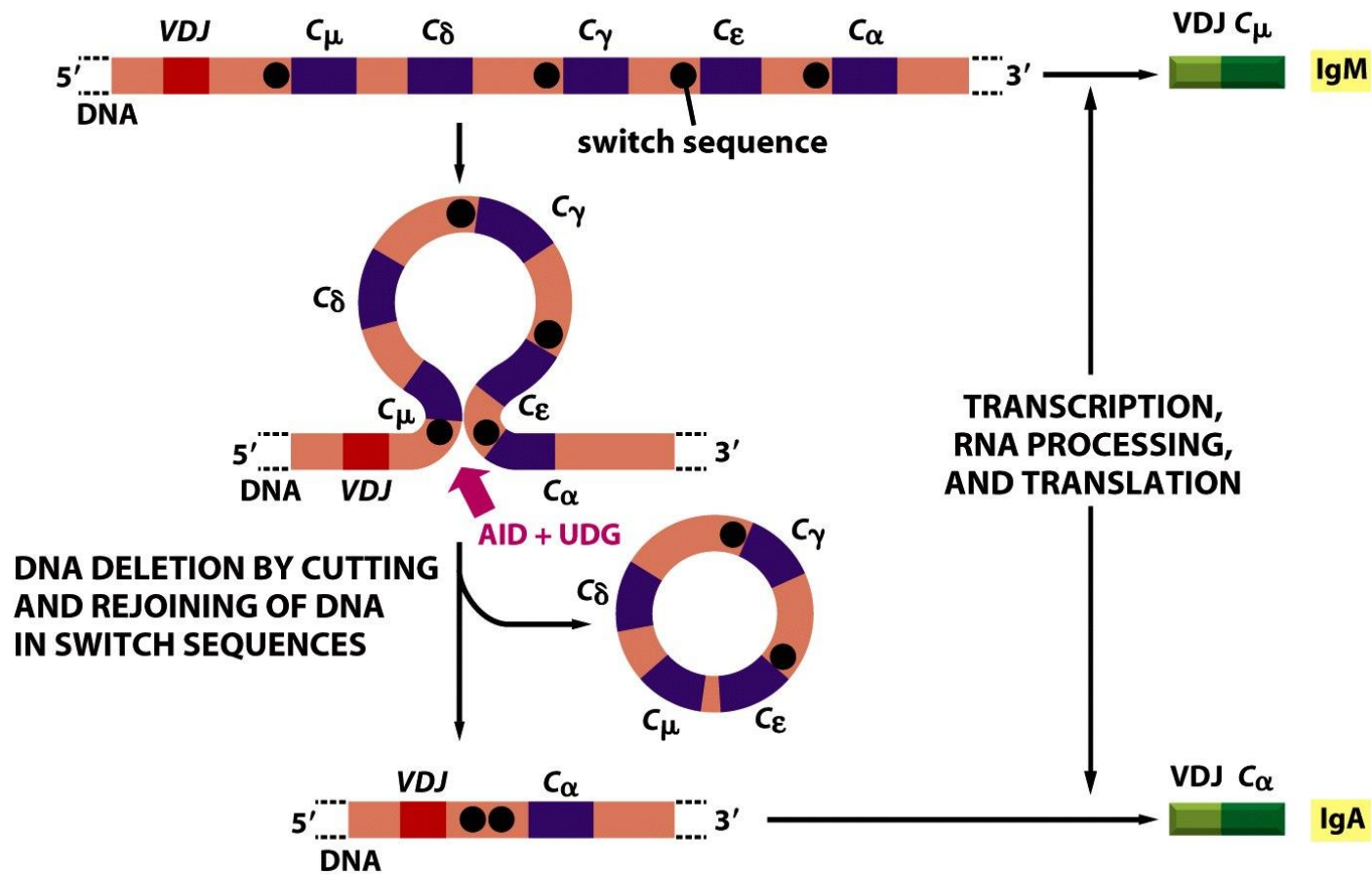
Heavy chain defines the class of antibody
Same light chain: κ or λ

PROPERTIES	CLASS OF ANTIBODY				
	IgM	IgD	IgG	IgA	IgE
Heavy chains	μ	δ	γ	α	ϵ
Light chains	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Number of four-chain units	5	1	1	1 or 2	1
Percentage of total Ig in blood	10	<1	75	15	<1
Activates complement	++++	-	++	-	-
Crosses placenta	-	-	+	-	-
Binds to macrophages and neutrophils	-	-	+	-	-
Binds to mast cells and basophils	-	-	-	-	+
	primary		secondary		

=> B cells can switch between the production of antibody classes

classes of antibody

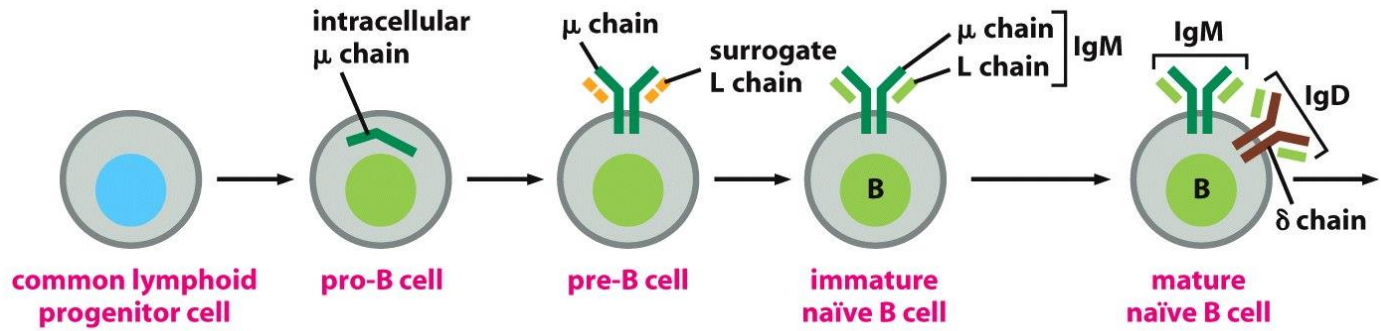
Class switch mediated by DNA rearrangement



Class switch DNA recombination (not splicing!) => irreversible
depends on switch sequences (consisting of tandem repeats) and
the enzymes activation induced deaminase (AID) + uracil-DNA glycosylase (UDG)

IgM: First antibody class

on cell surface



common lymphoid progenitor cell

pro-B cell

pre-B cell

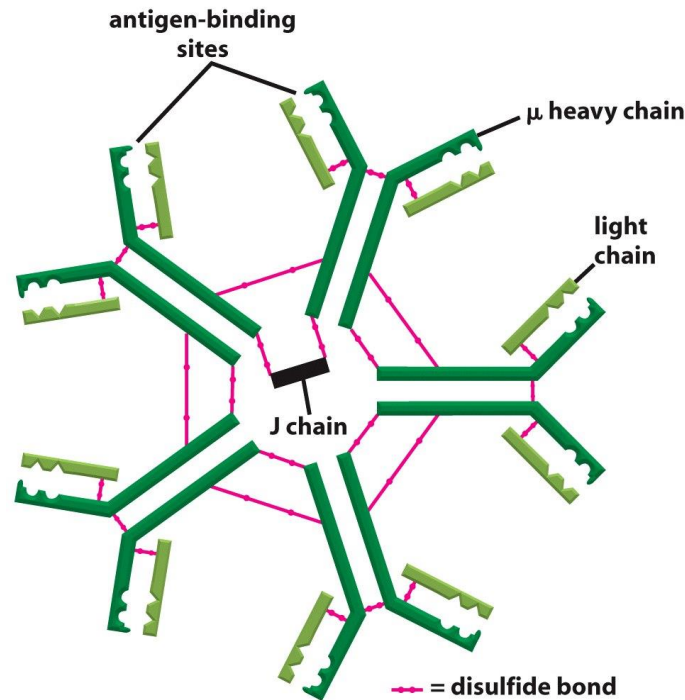
immature naïve B cell

mature naïve B cell

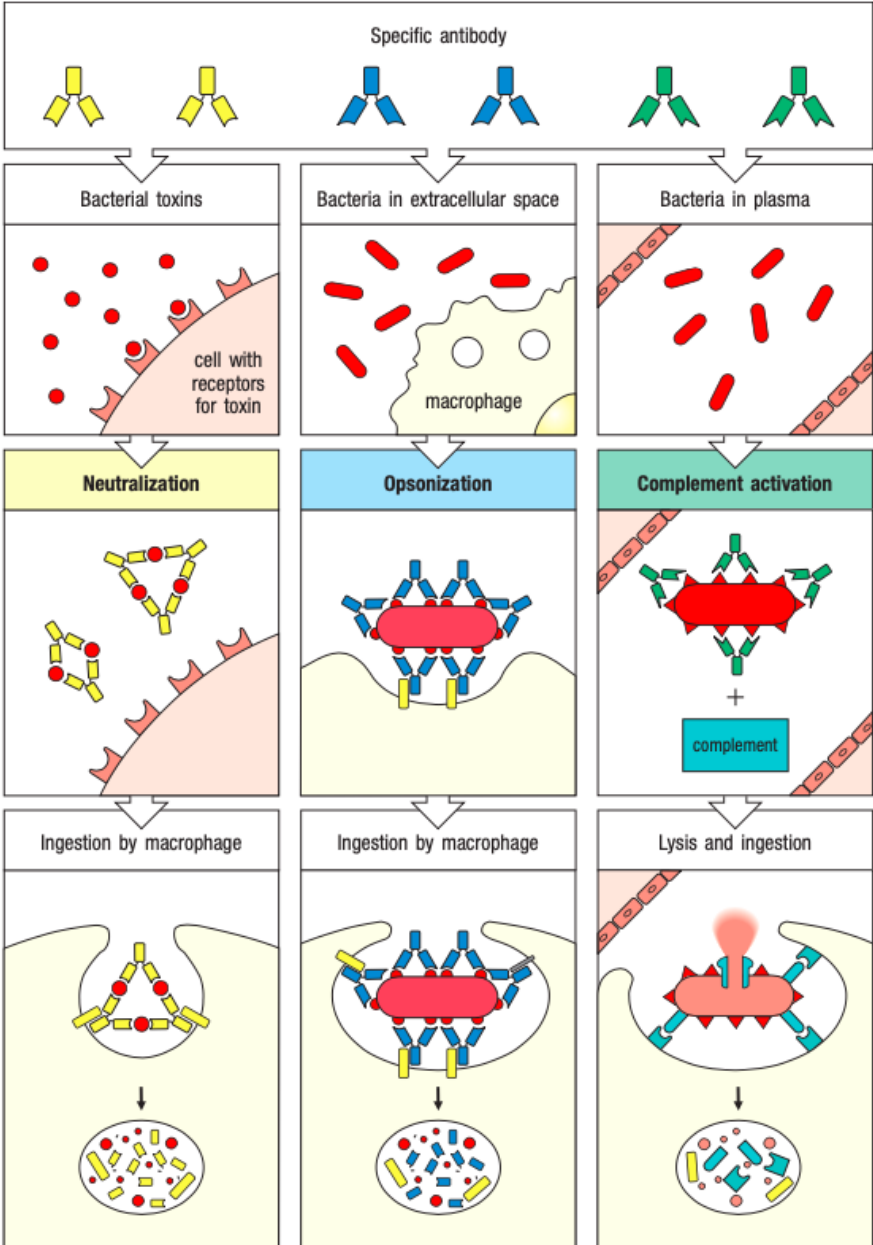
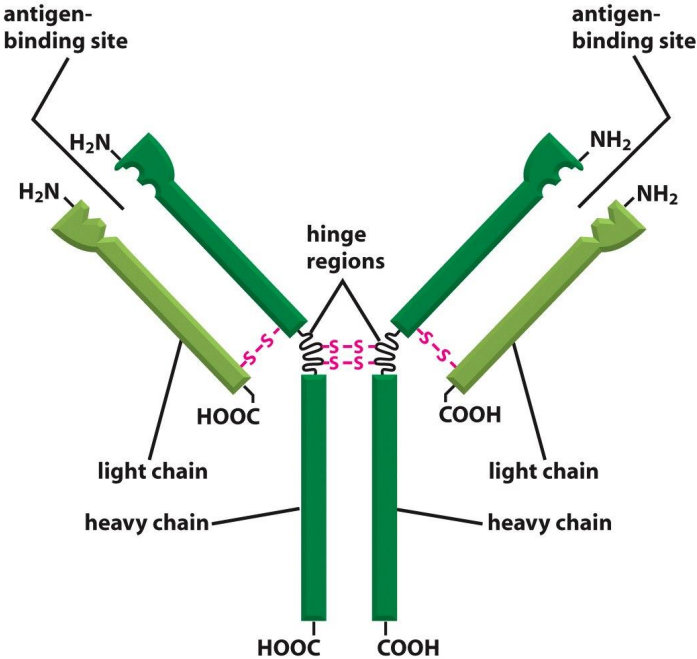
Development in bone marrow

Circulation through peripheral lymphoid organs

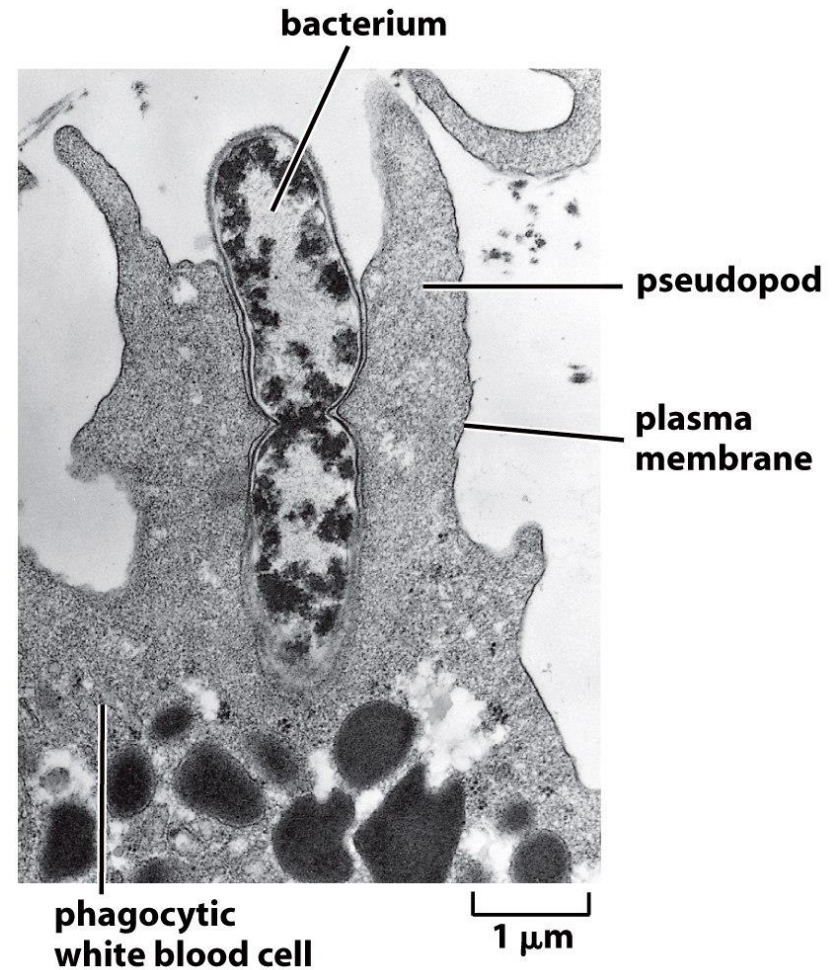
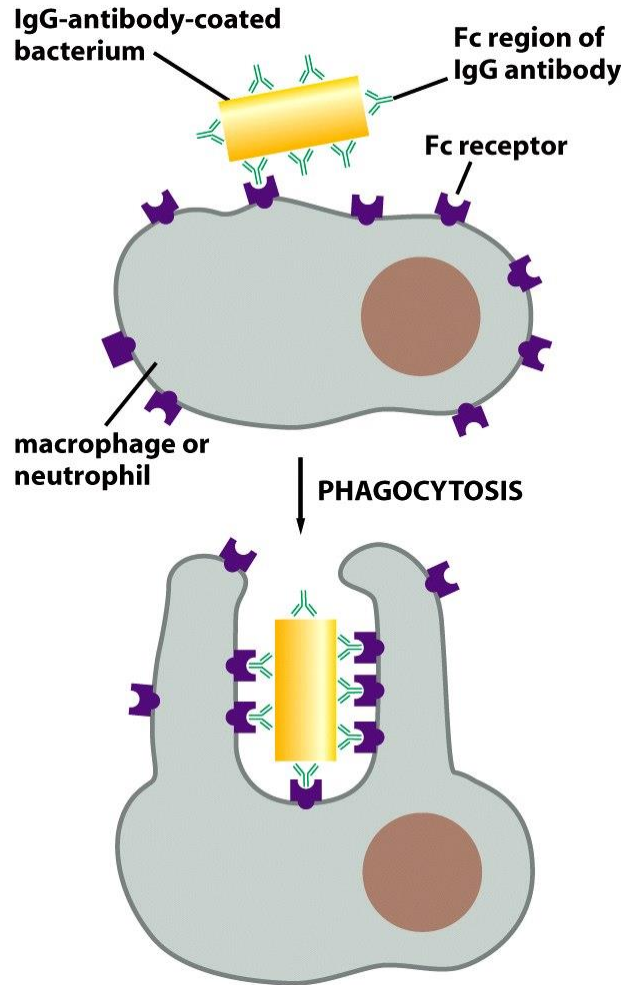
circulating in blood



IgG: Main class in blood

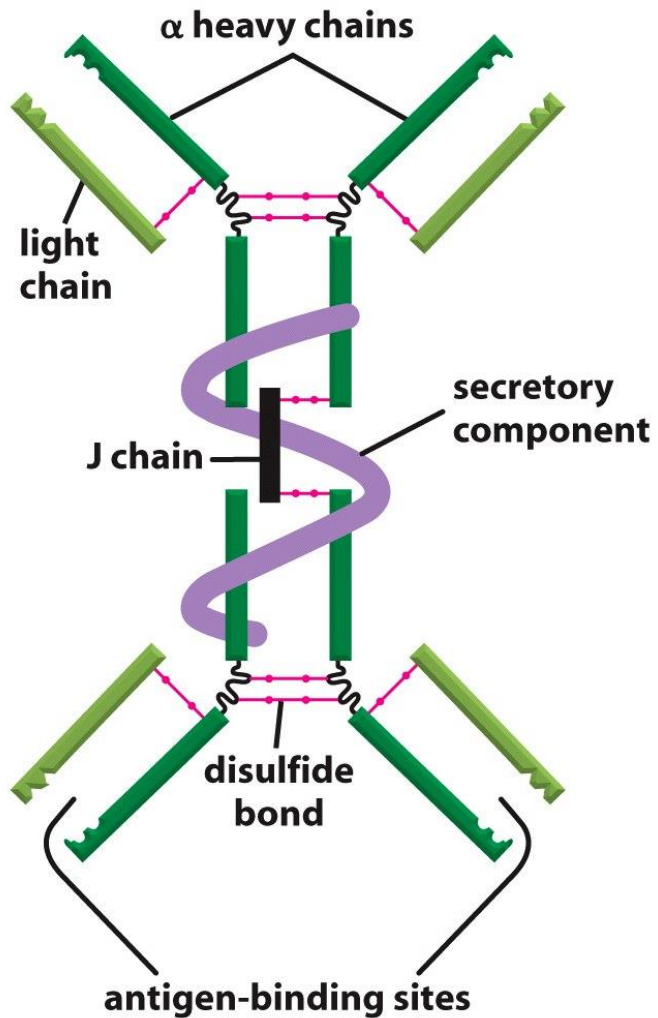


Opsonization

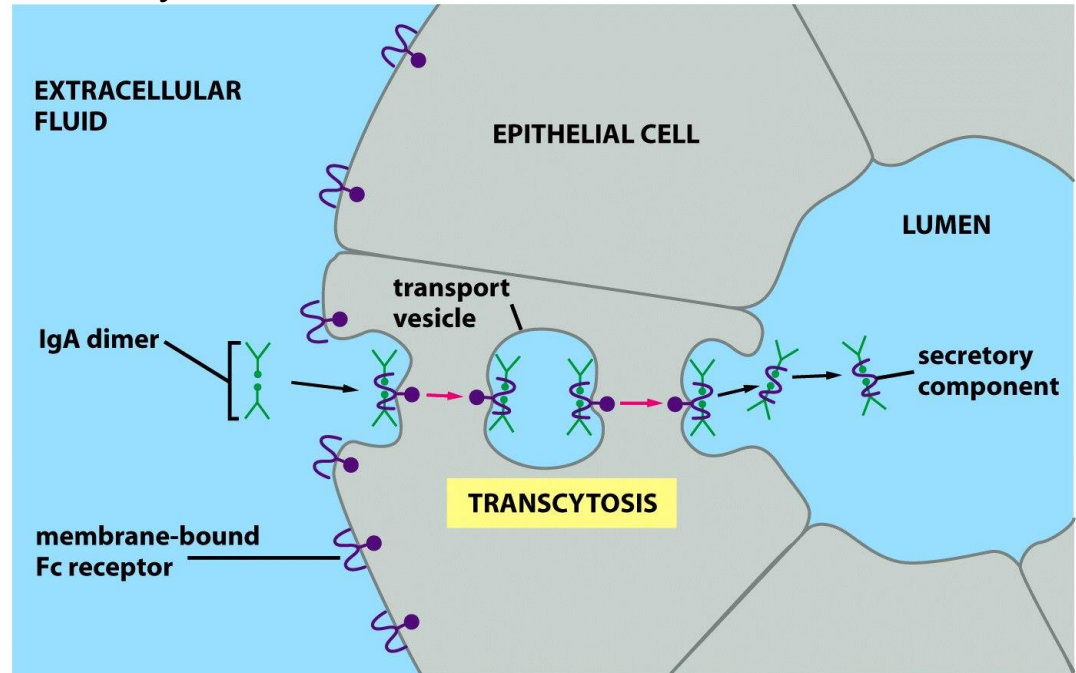


TEM image

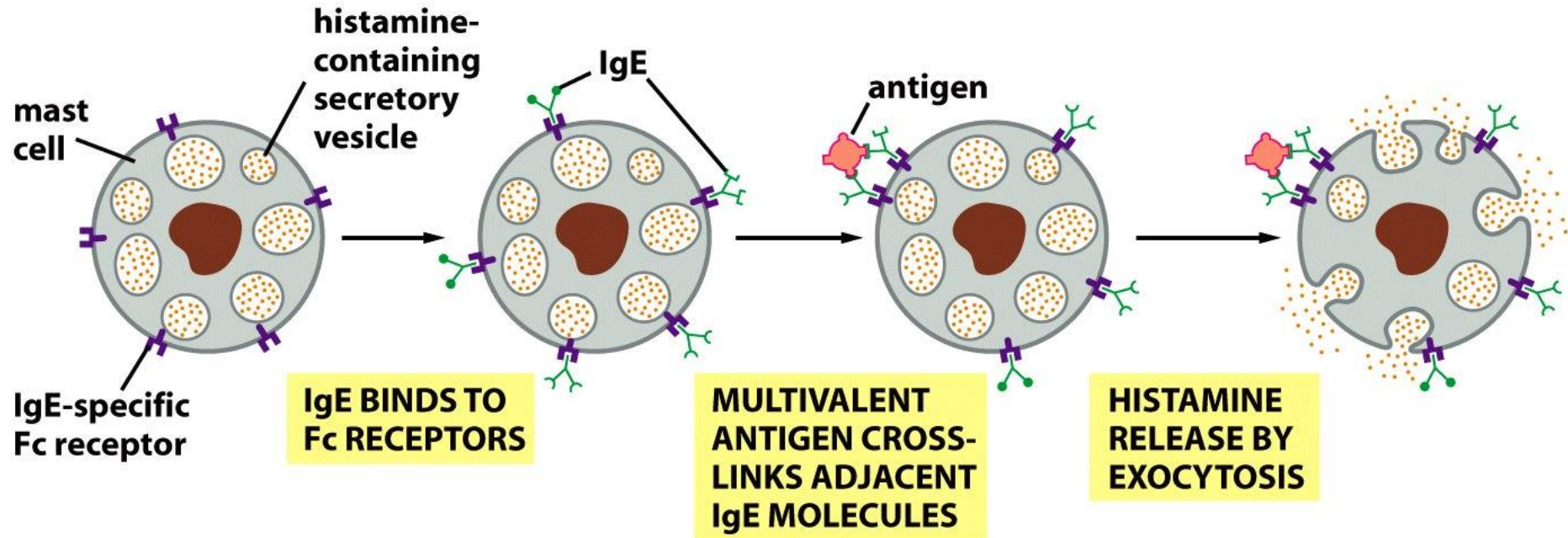
IgA: Defence of mucosal surfaces



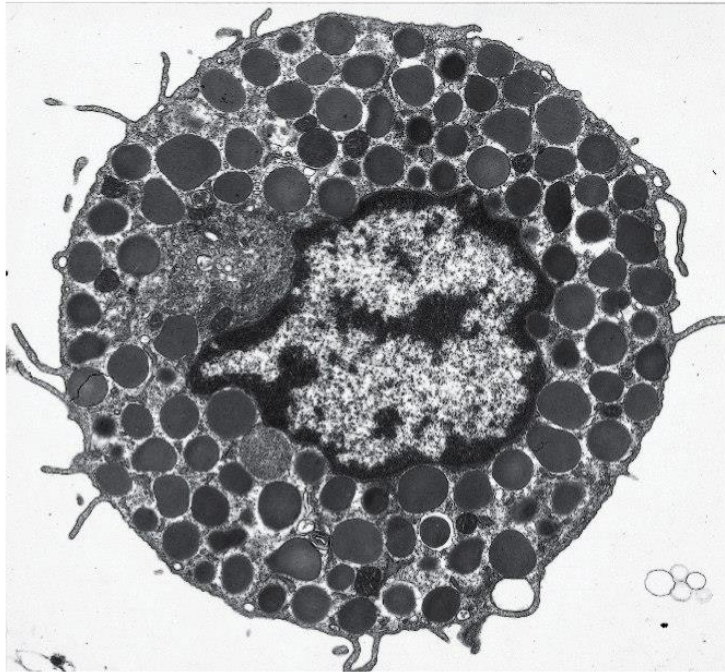
Transcytosis



IgE: Protection against large parasites

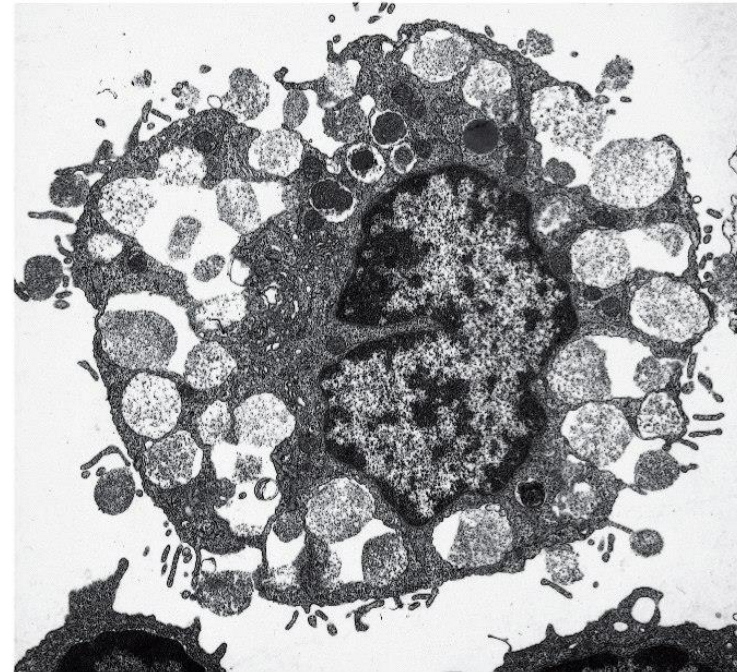


Release of histamin by mast cells

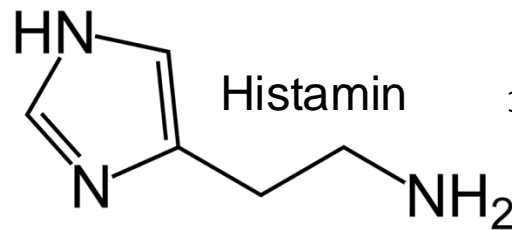


(A)

5 μm



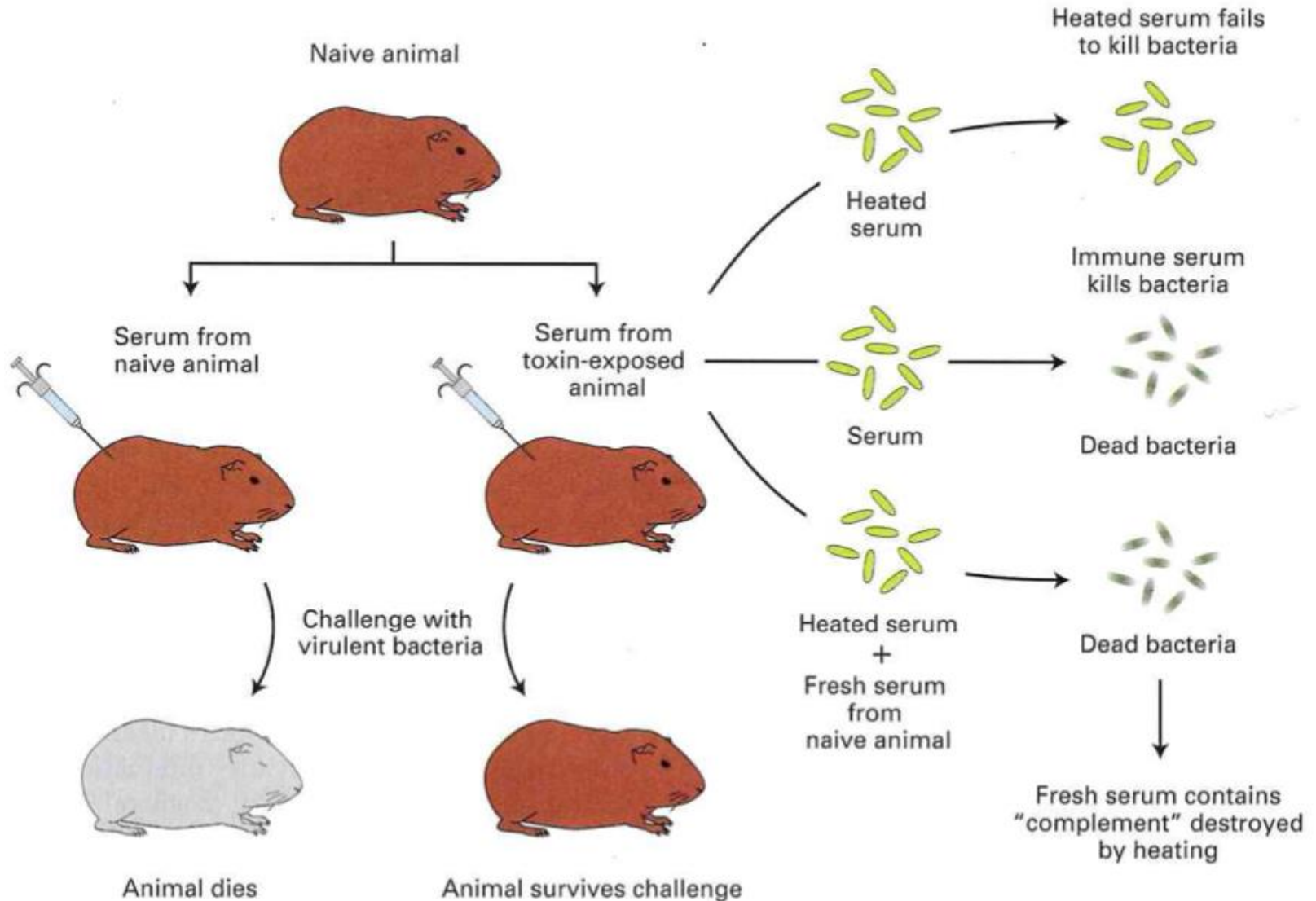
(B)



Protection against parasites

Allergies

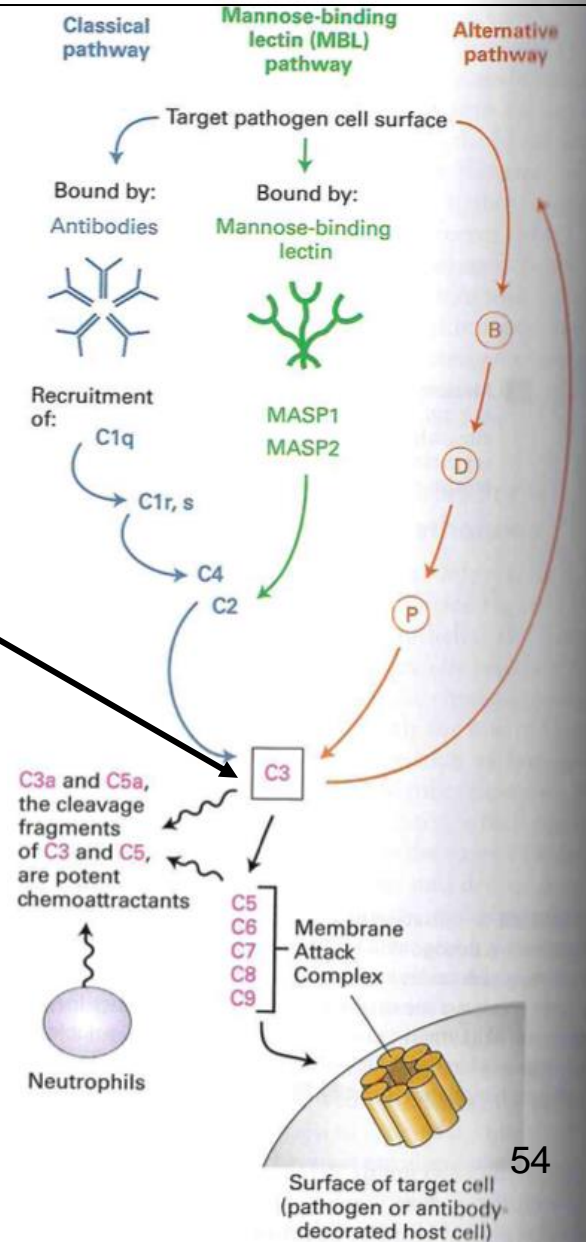
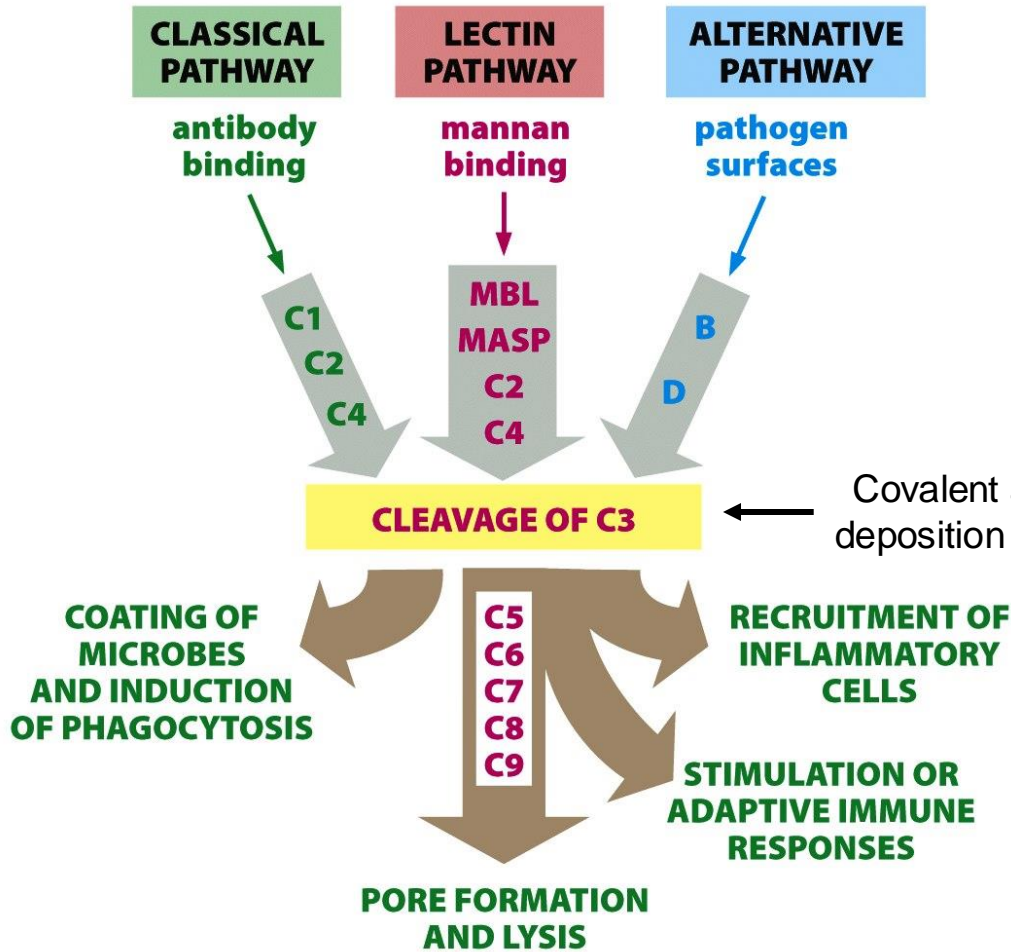
Classic experiment



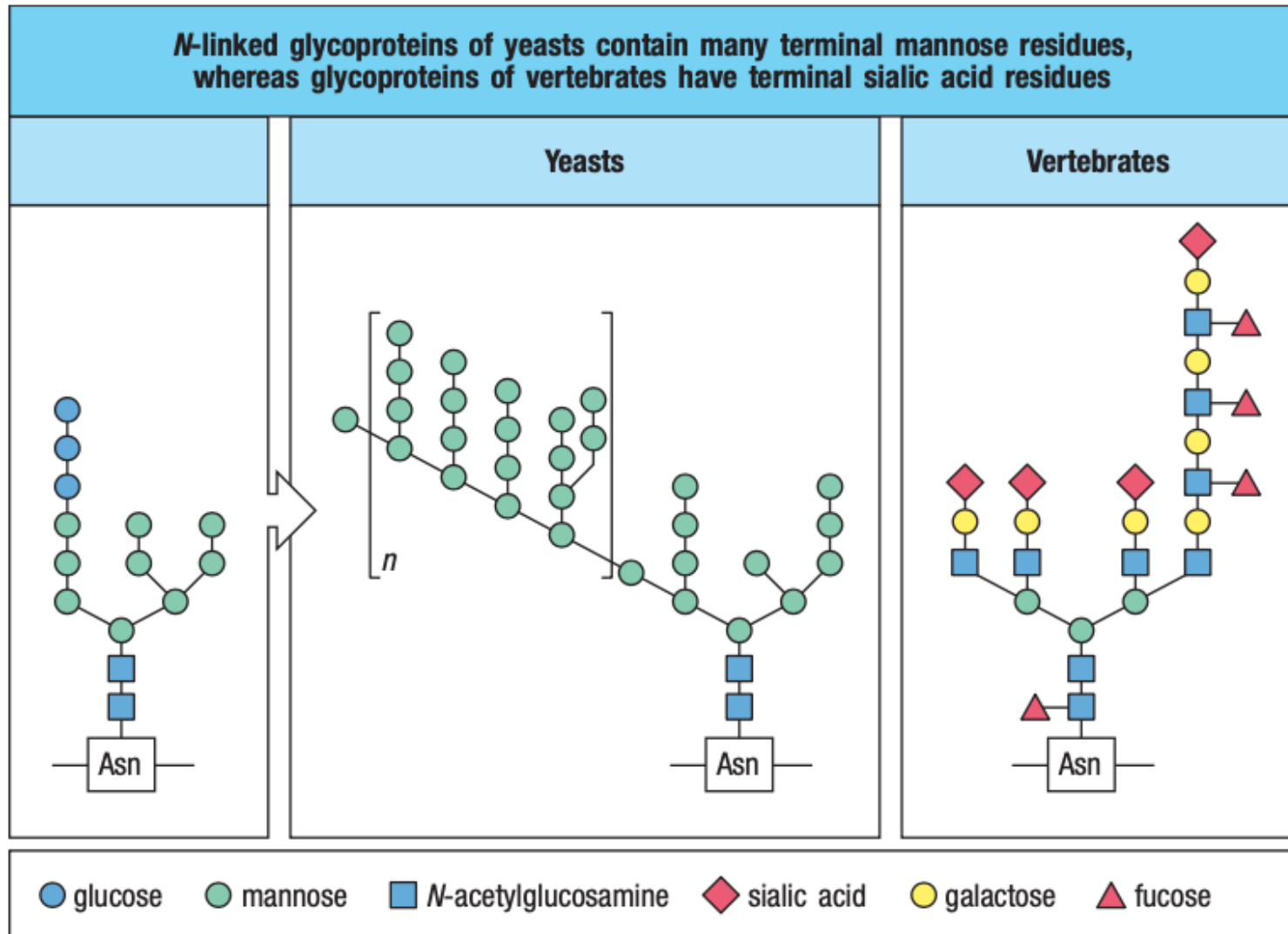
=> Behring/Kitasato (ca. 1890)

Complement system

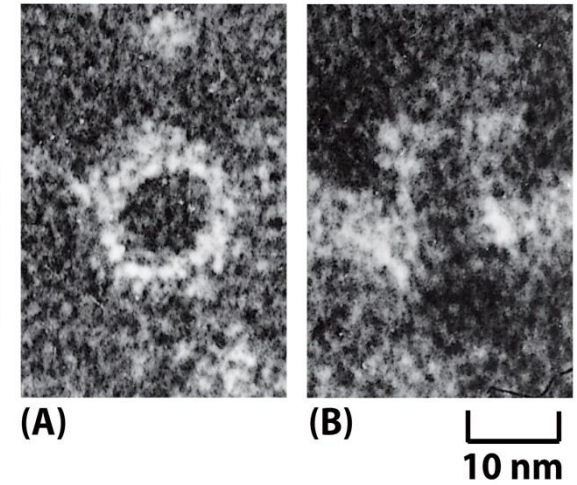
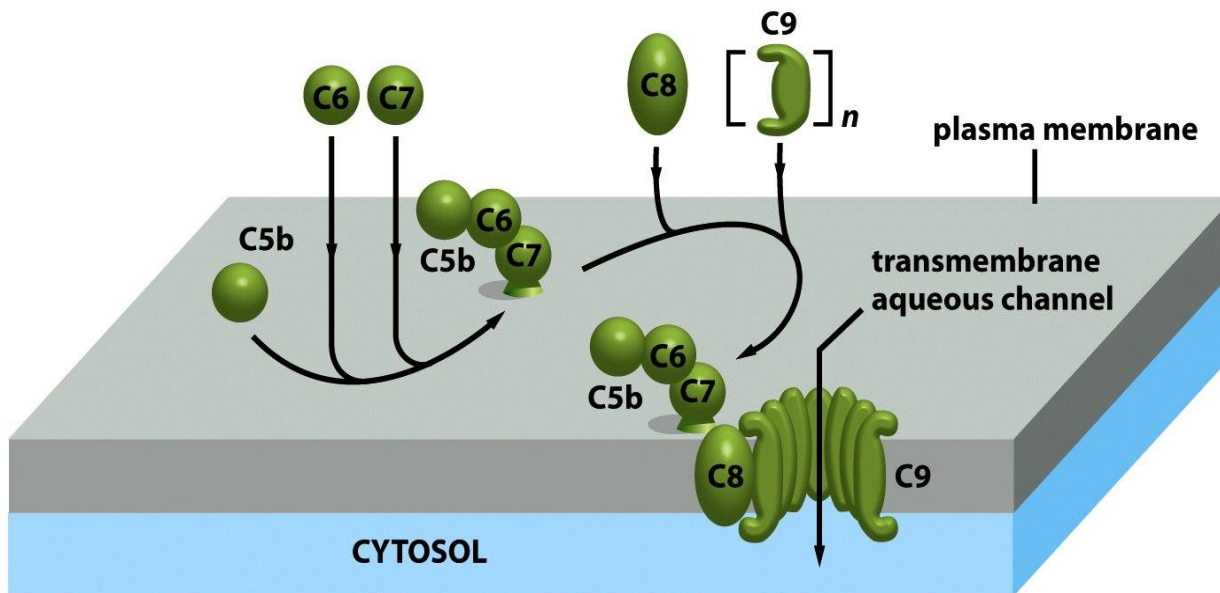
=> A protease cascade: amplification steps



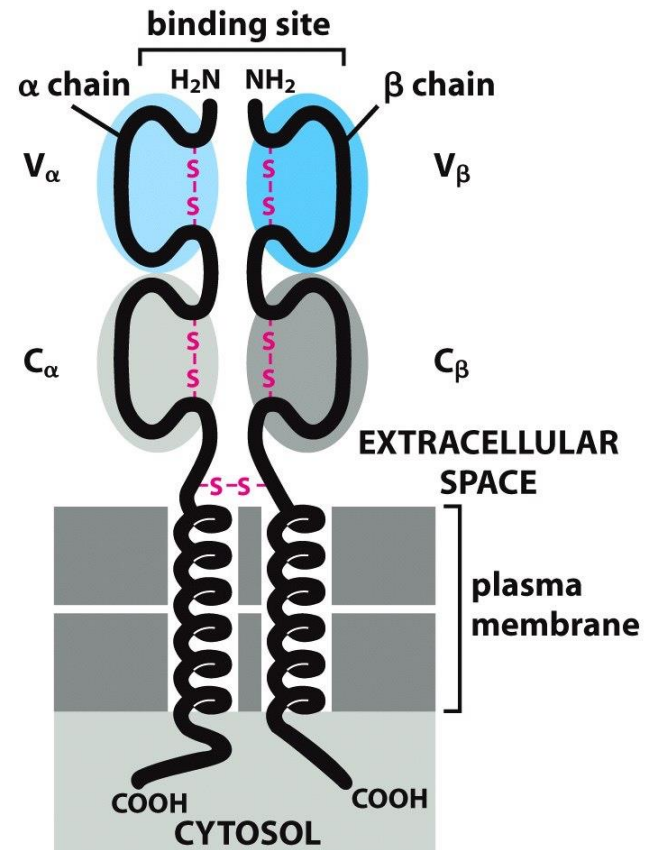
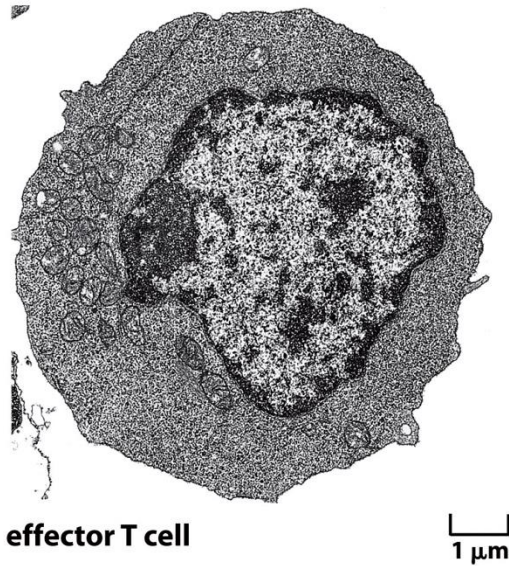
Lectin pathway



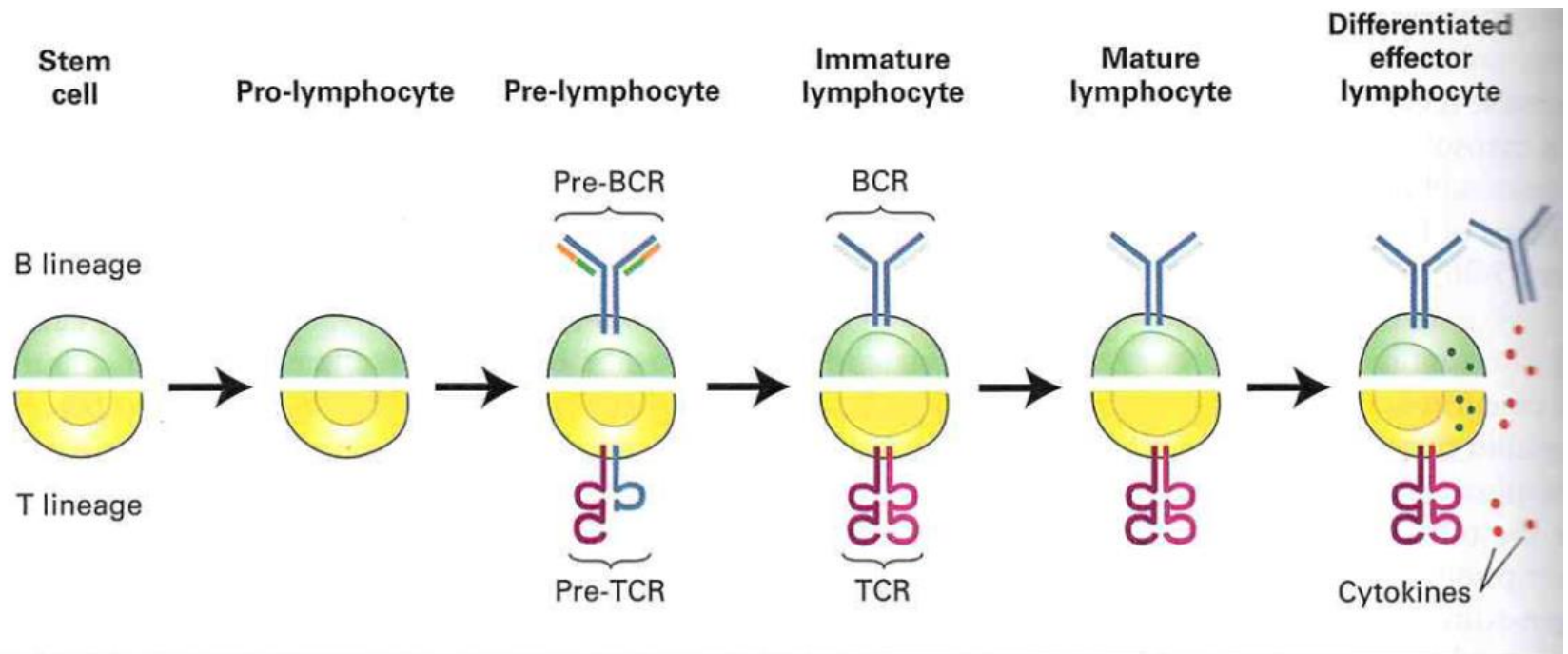
Complement system: pore formation/lysis



T cells and T cell receptor (TCR)

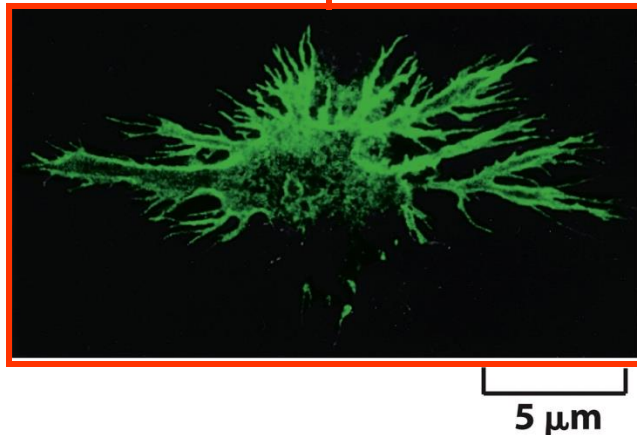
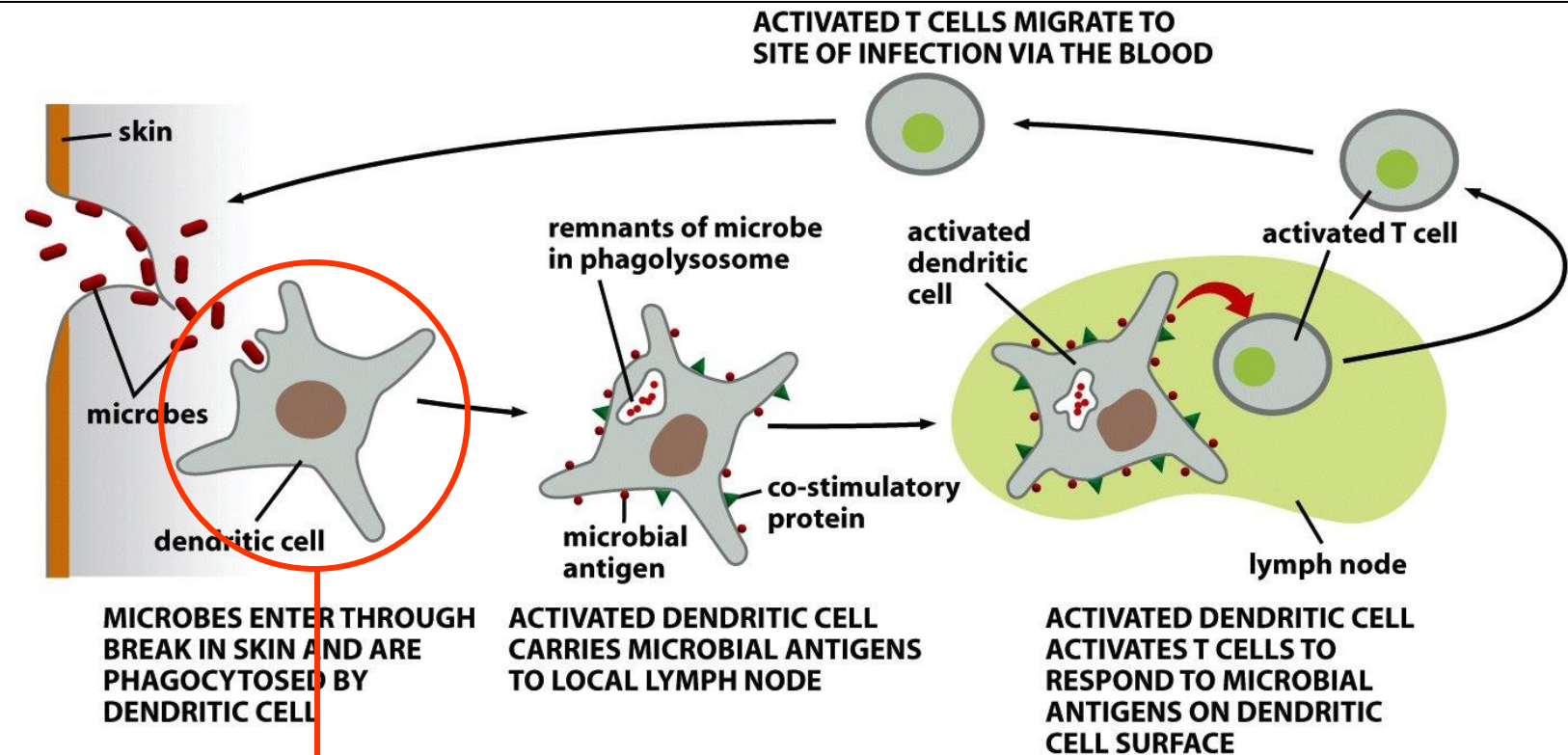


B and T cell maturation follow a similar course

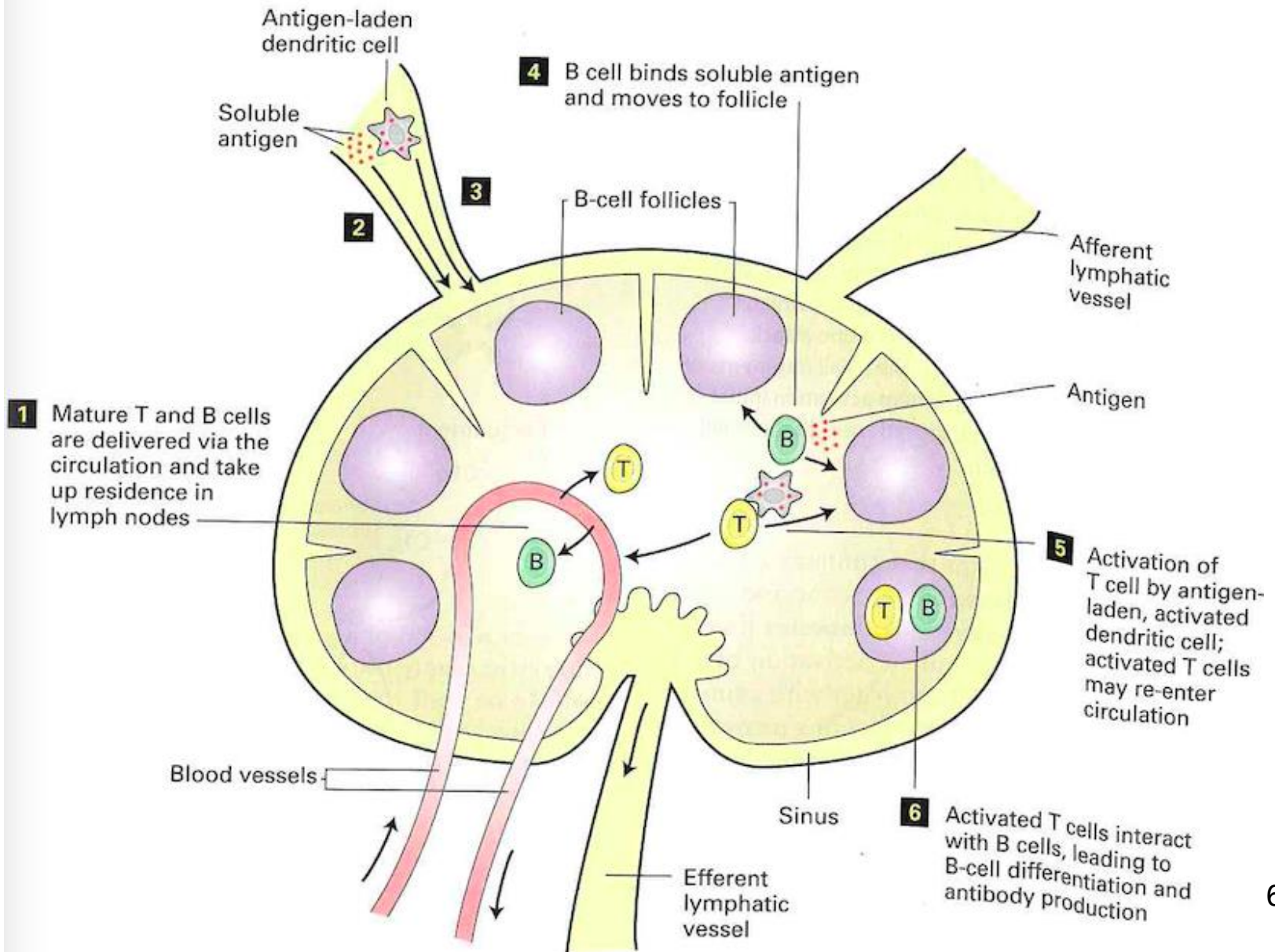


Bone marrow or thymus			Periphery
No		Self antigen	Foreign antigen
Early maturation and expansion	Pre-antigen receptor expression	Completion of antigen receptor; selection of receptor repertoire; differentiation	Performance of effector functions

We take a larger picture: Antigen presentation

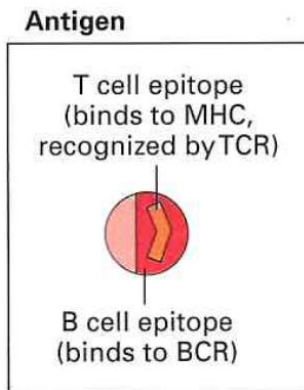


Larger picture: initiation of immune response



Better double check!

B cell epitope \longleftrightarrow recognition \longleftrightarrow BCR

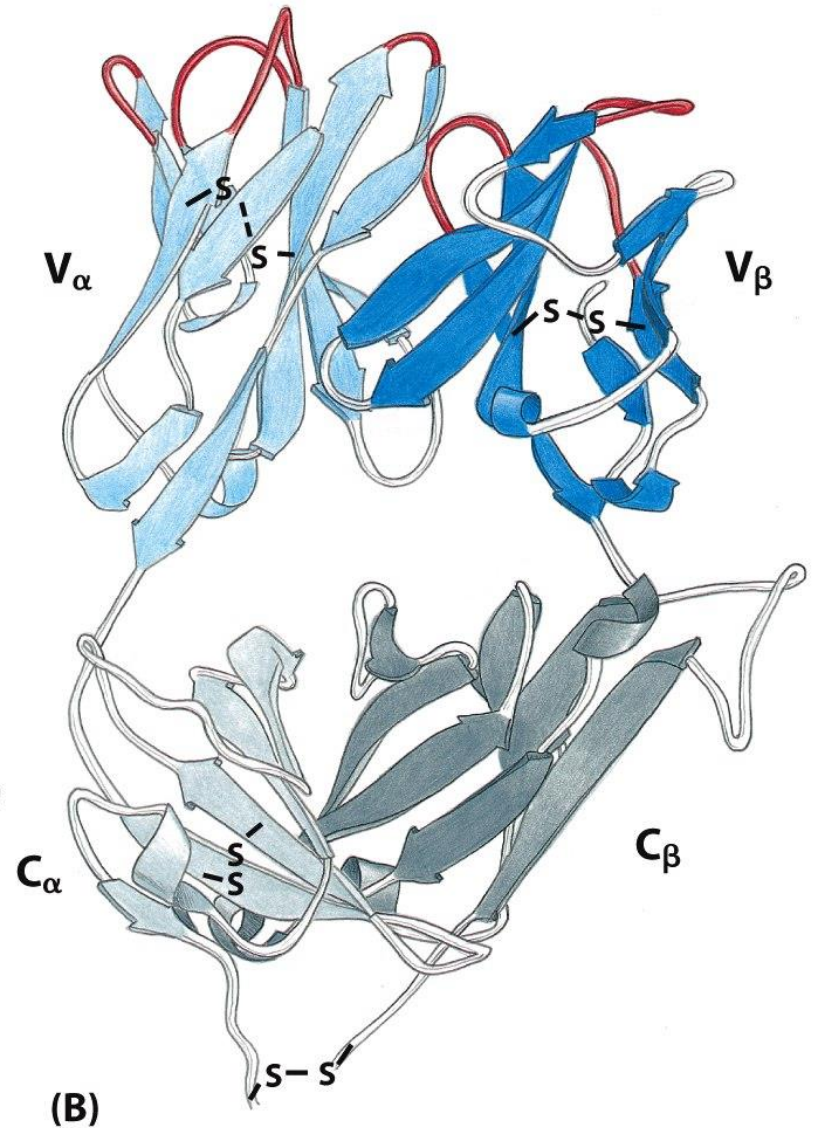
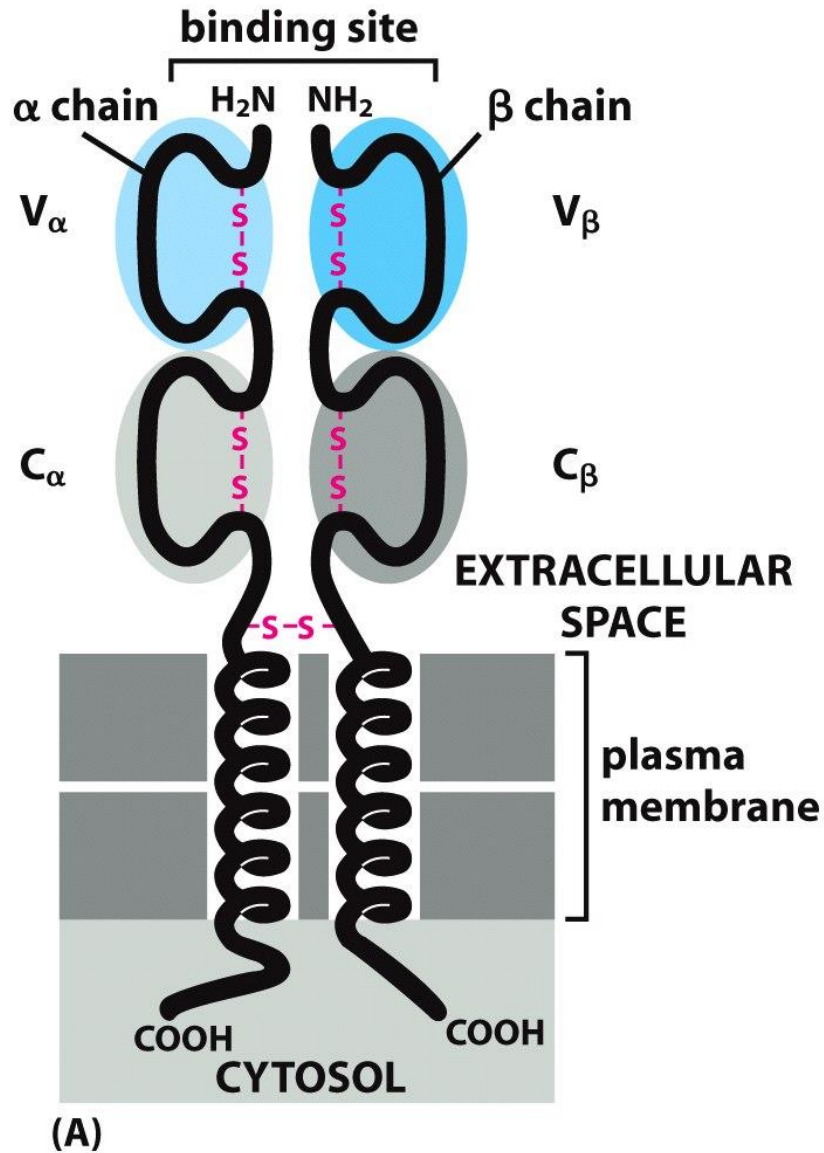


T cell epitope \longleftrightarrow recognition \longleftrightarrow TCR



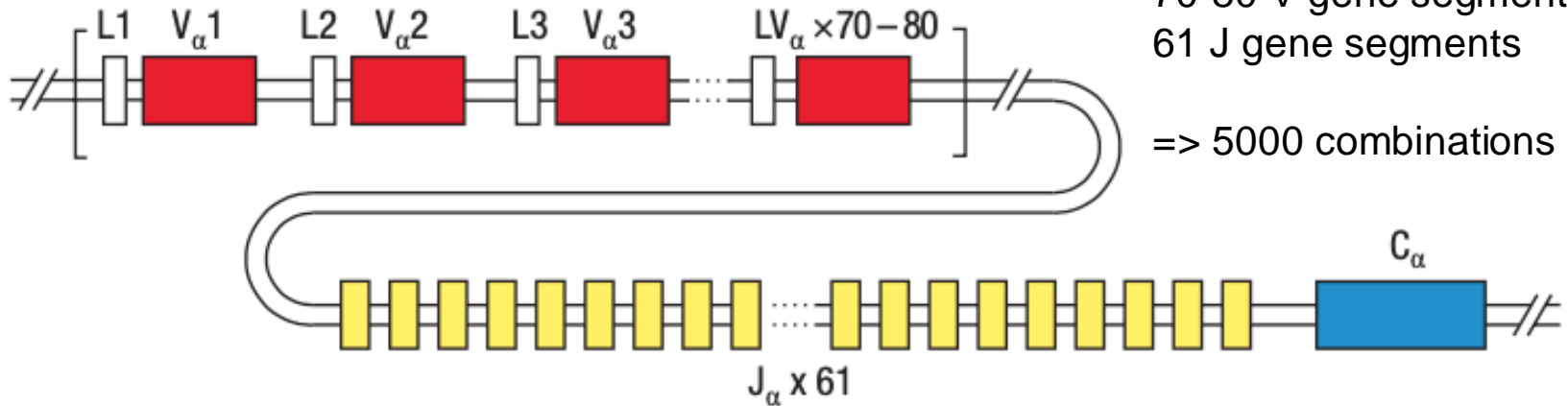
⇒ Minimizing the risk of wrong classification (friend/foe)
to prevent e.g. autoimmune diseases, allergies

T cell receptor (TCR)



Generation of TCR diversity

α -chain locus

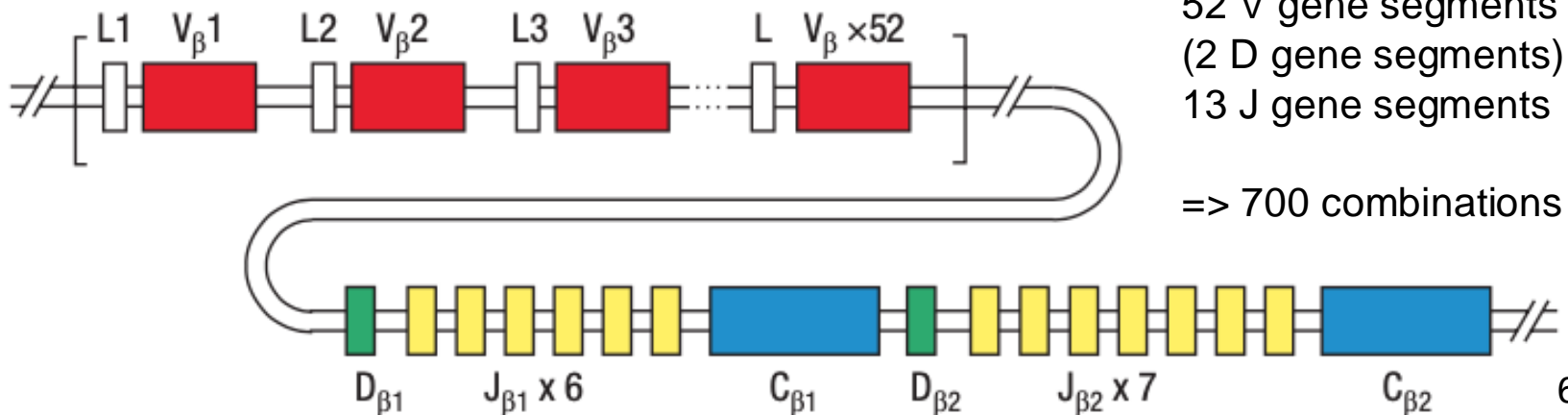


α -chain

70-80 V gene segments
61 J gene segments

=> 5000 combinations

β -chain locus

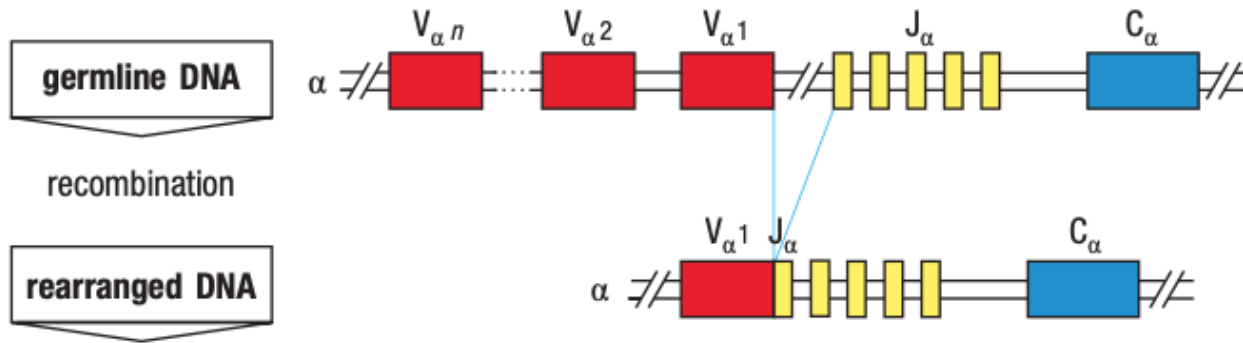


β -chain

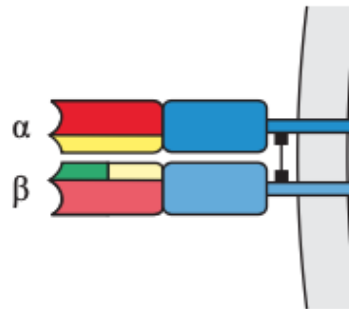
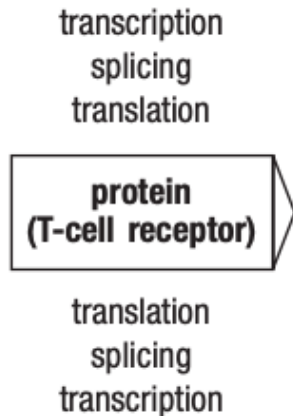
52 V gene segments
(2 D gene segments)
13 J gene segments

=> 700 combinations

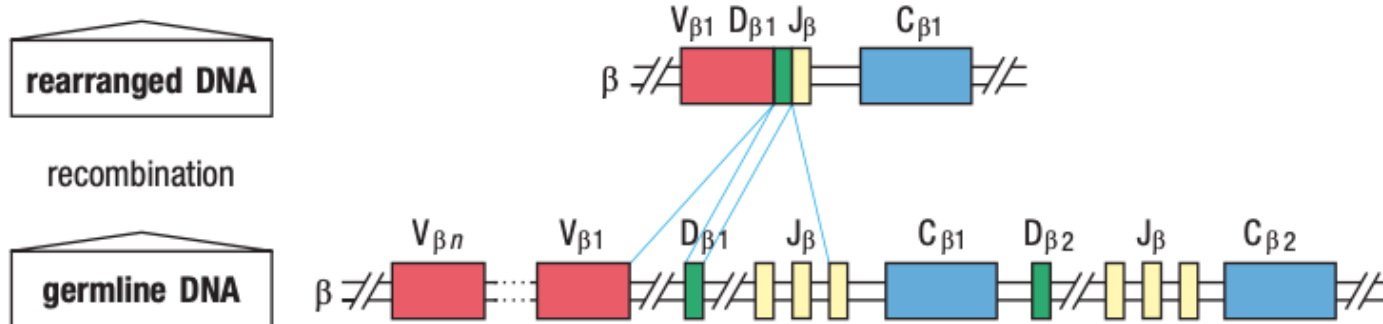
Generation of TCR diversity



=> Same enzymes are used for the gene rearrangement as in B cells

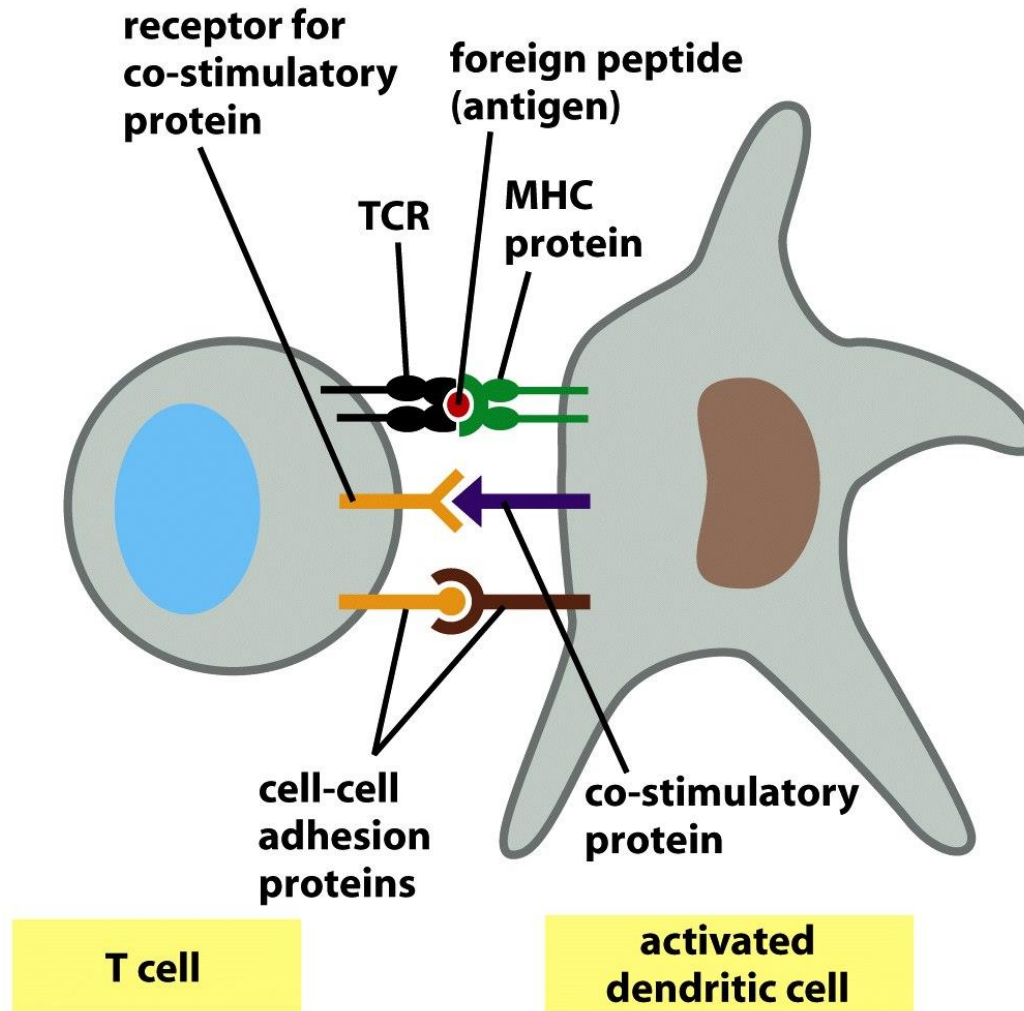


=> about 3.500.000 combinations



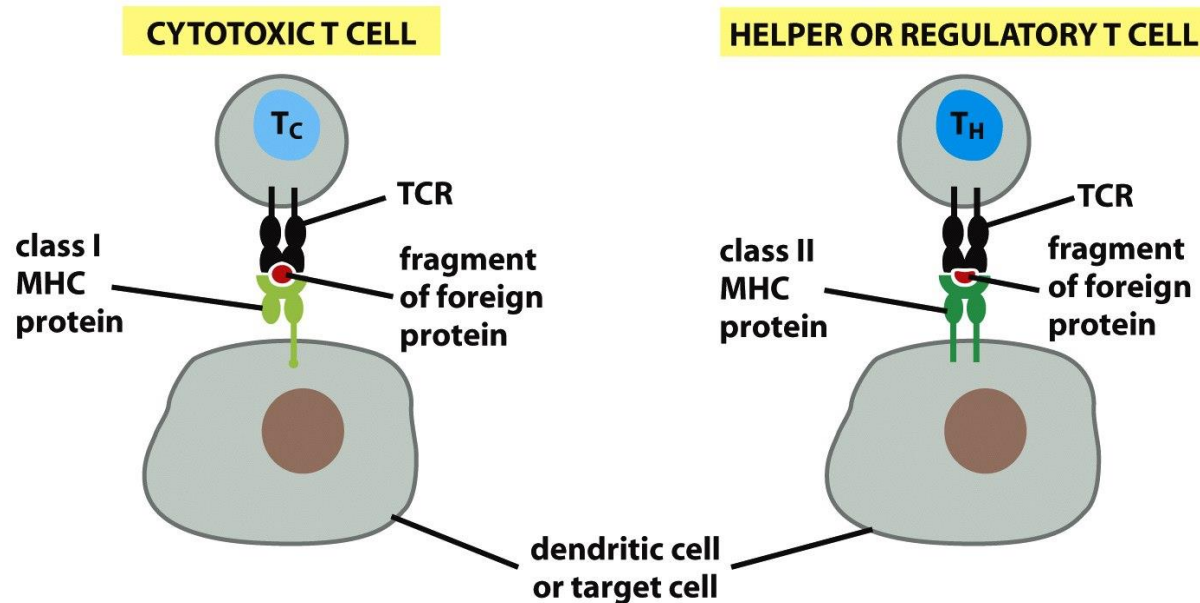
Unlike BCR no somatic hypermutation => only lower affinity ($K_a = 10^5 - 10^7 \text{ M}^{-1}$)

T cell activation



A **TCR** recognizes the antigen only in context of an **MHC**

Major histocompatibility complex (MHC)



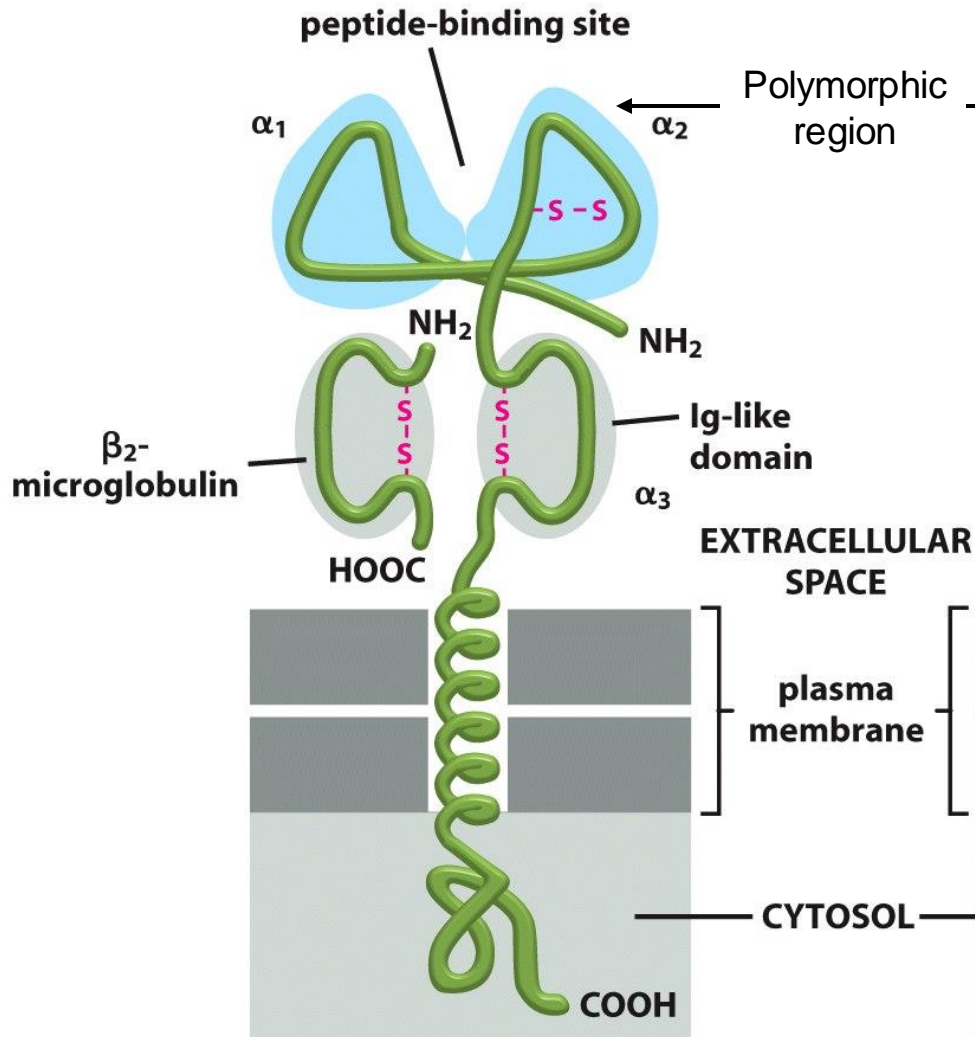
Properties of Human Class I and Class II MHC Proteins

	CLASS I	CLASS II
Genetic loci	<i>HLA-A, HLA-B, HLA-C</i>	<i>DP, DQ, DR</i>
Chain structure	α chain + β_2 -microglobulin	α chain + β chain
Cell distribution	most nucleated cells	dendritic cells, B cells, macrophages, thymus epithelial cells, some others
Presents antigen to	cytotoxic T cells	helper T cells, regulatory T cells
Source of peptide fragments	mainly proteins made in cytoplasm	mainly endocytosed plasma membrane and extracellular proteins
Polymorphic domains	$\alpha_1 + \alpha_2$	$\alpha_1 + \beta_1$
Recognition by co-receptor	CD8	CD4

Major histocompatibility complex (MHC)

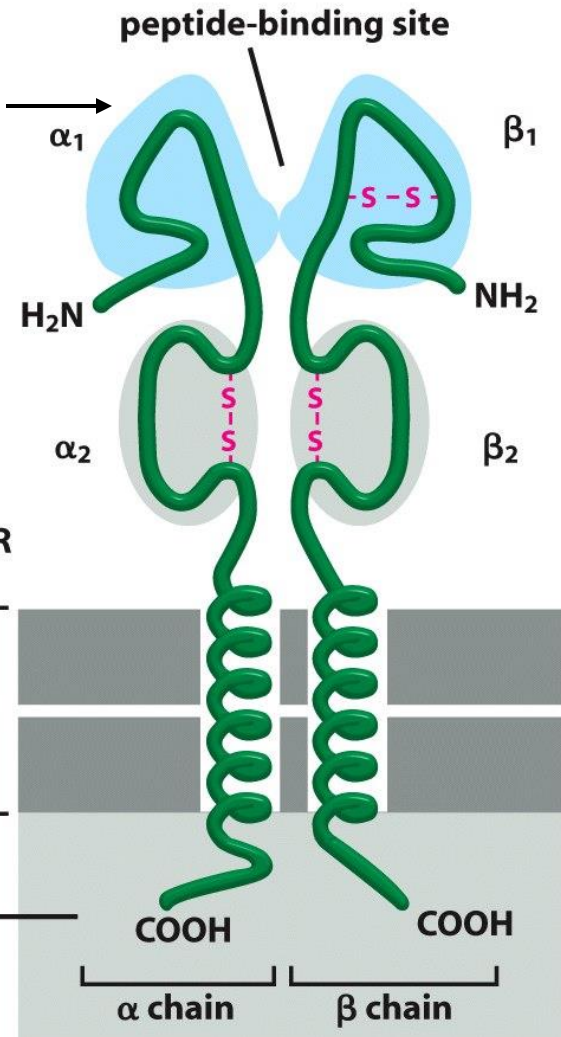
Class I MHC protein

=> on (almost) all cells

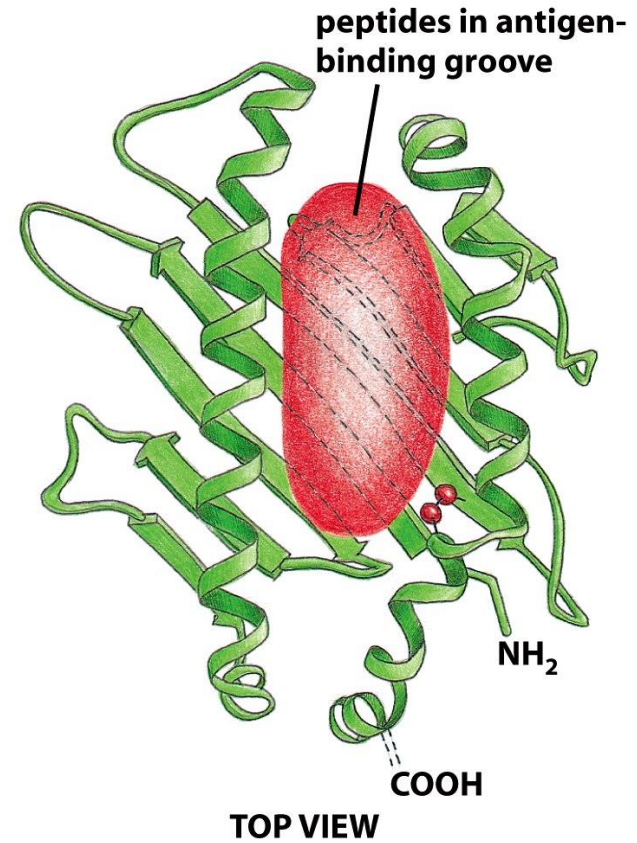
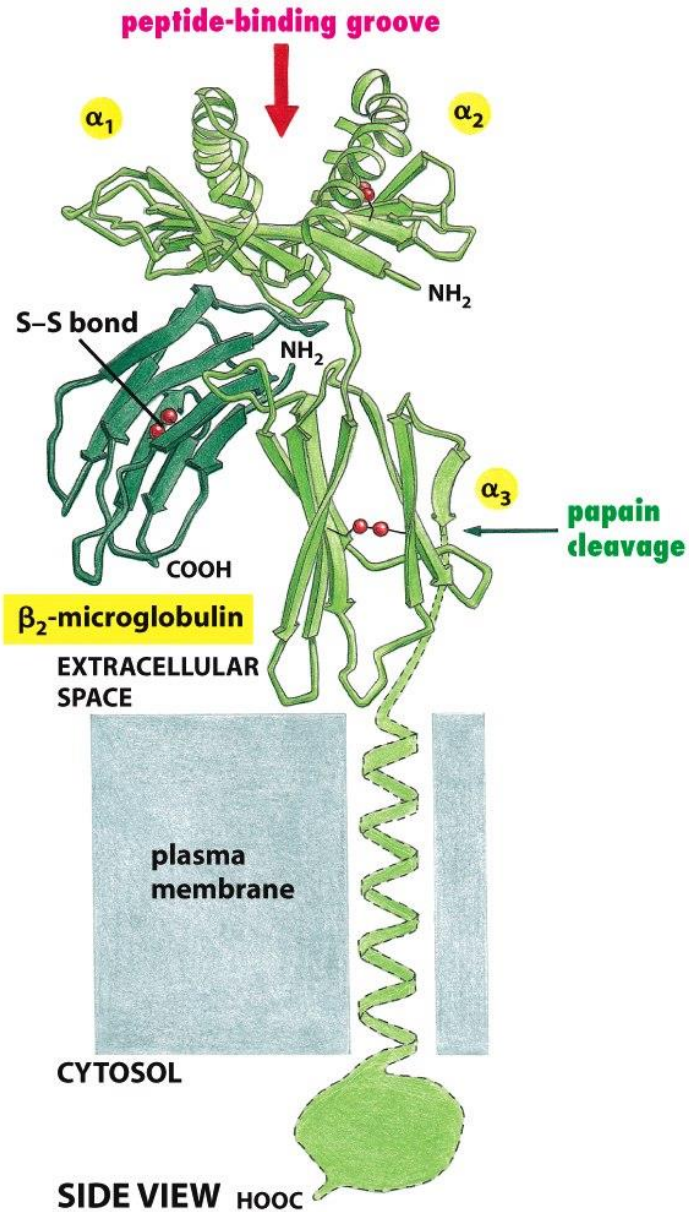


Class II MHC protein

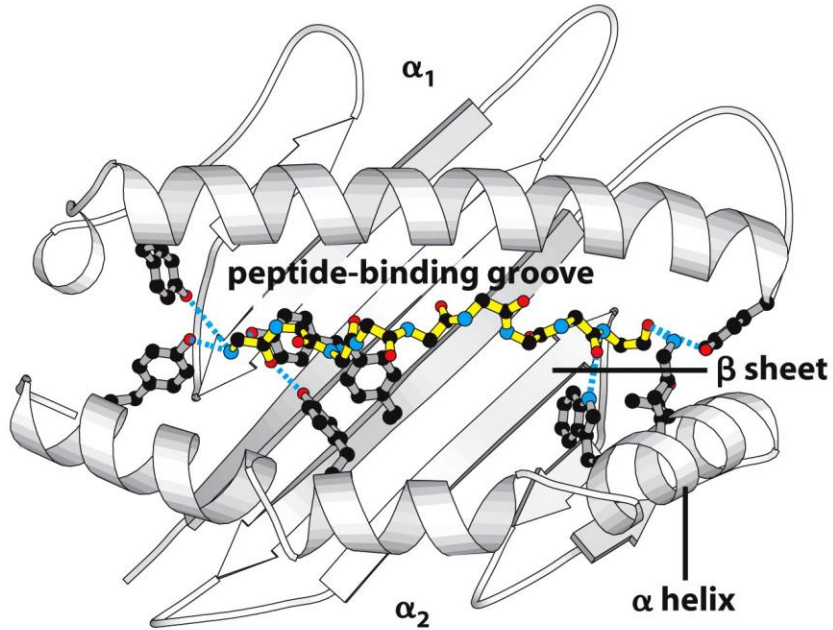
=> Only on professional antigen-presenting cells (e.g. dendritic cells)



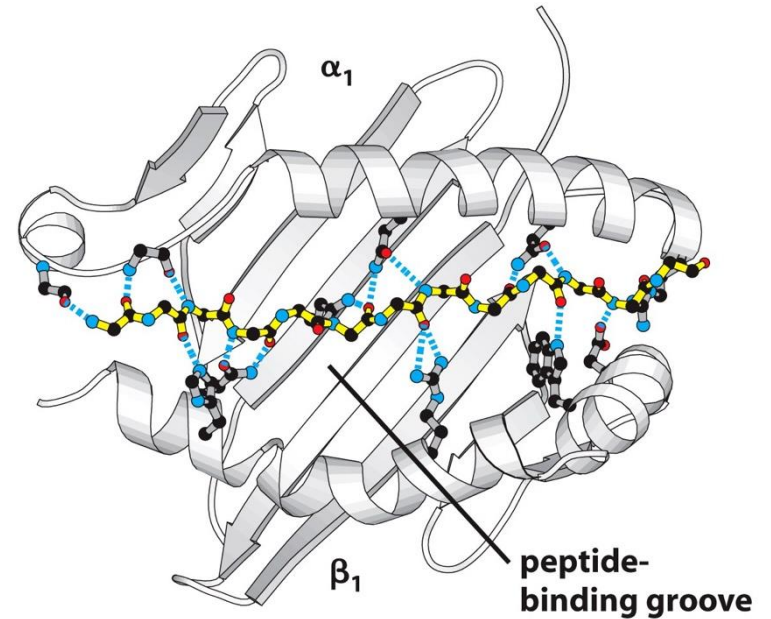
Peptide bound to MHC



Peptide bound in the groove of MHC

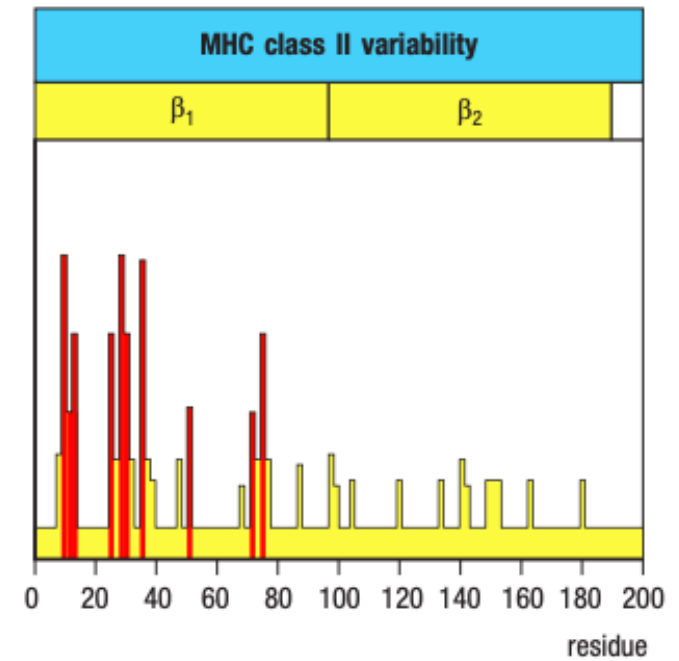
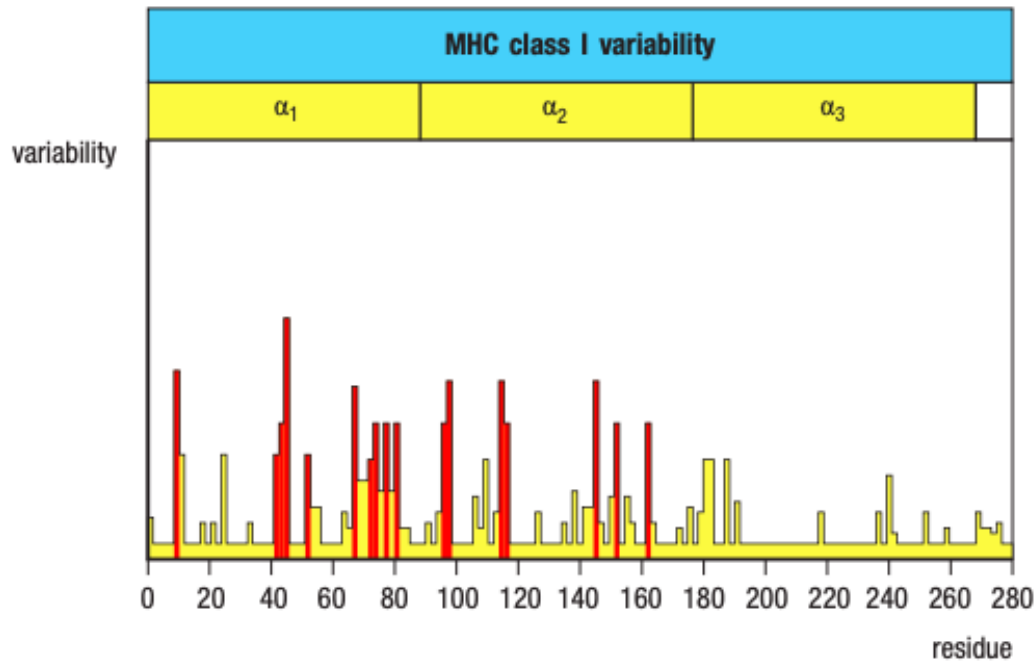
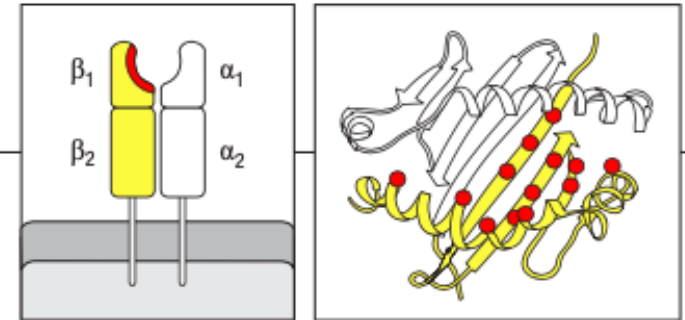
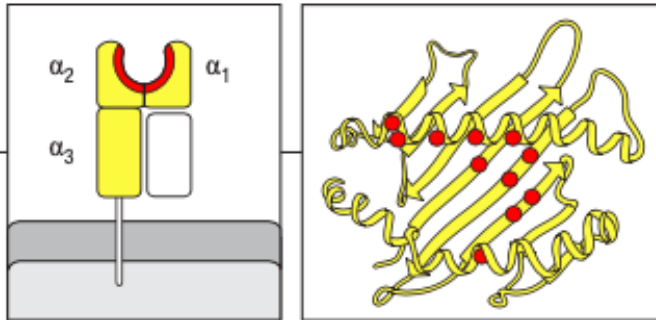


Peptides bound to **MHC-I**:
8-10 amino acids long



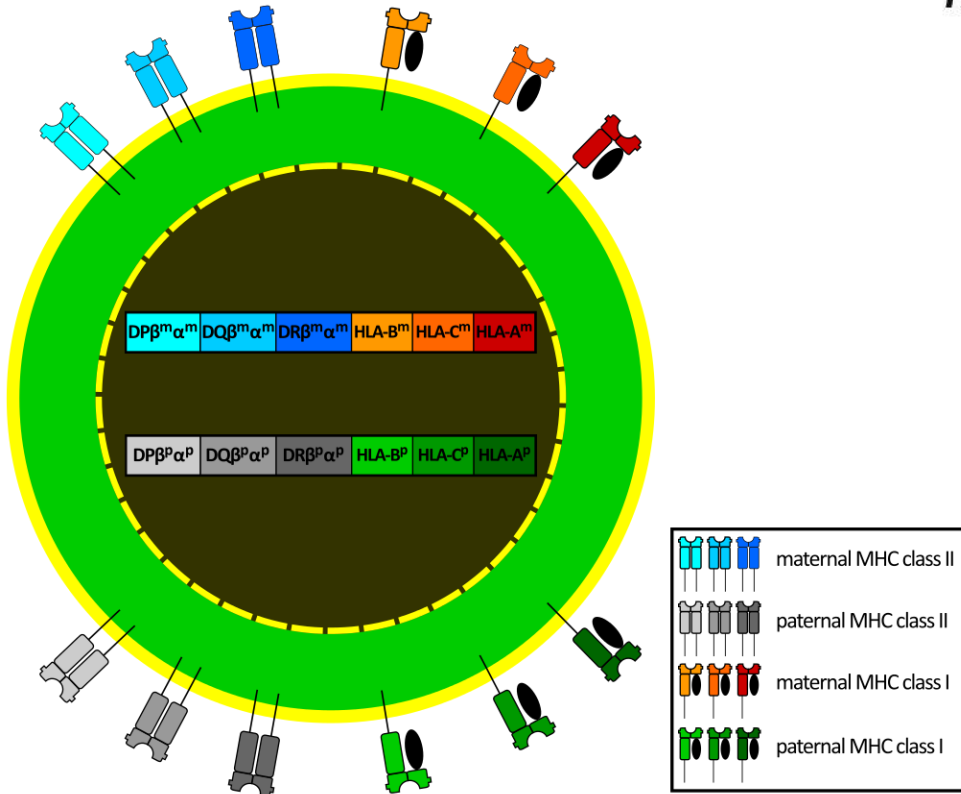
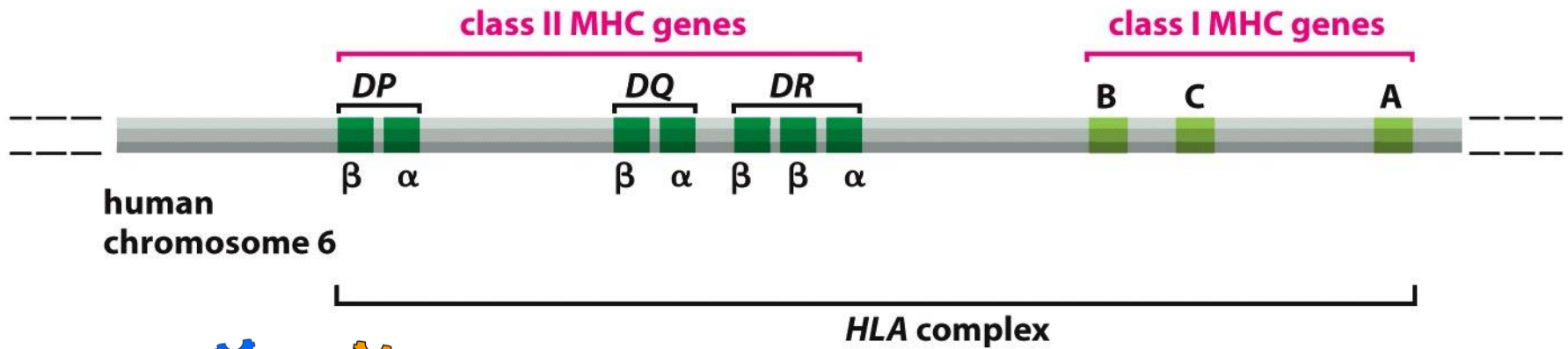
Peptides bound to **MHC-II**:
10-12 amino acids long

Allelic variation in MHC genes



Red: peptide binding regions

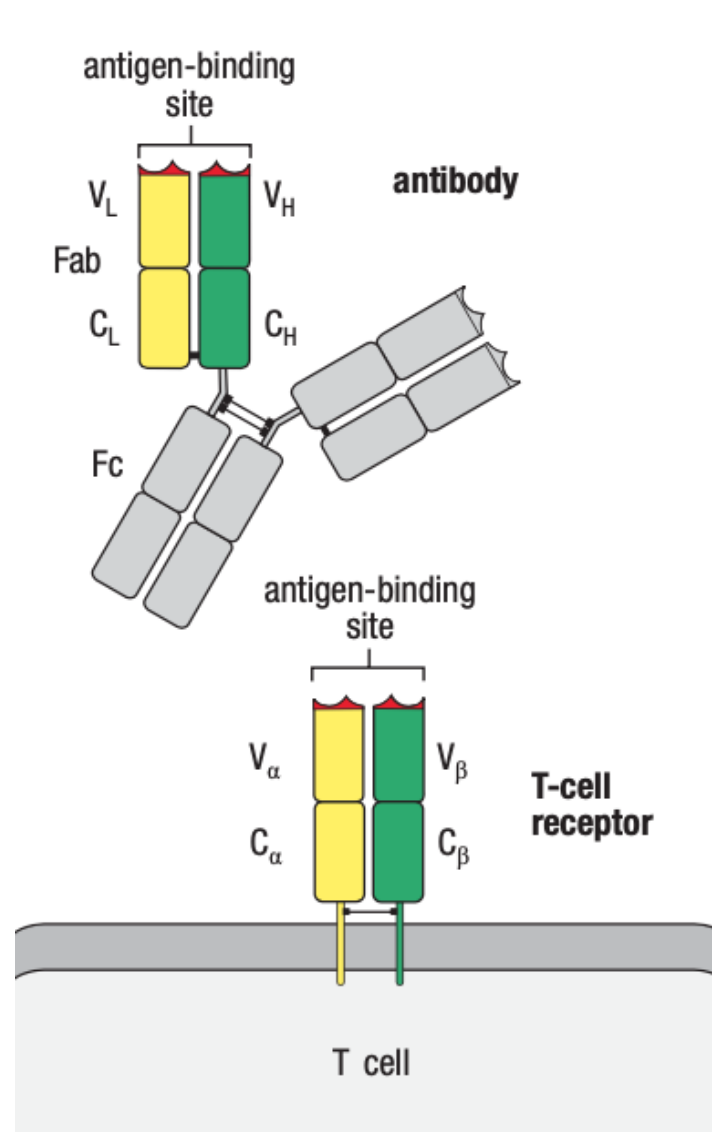
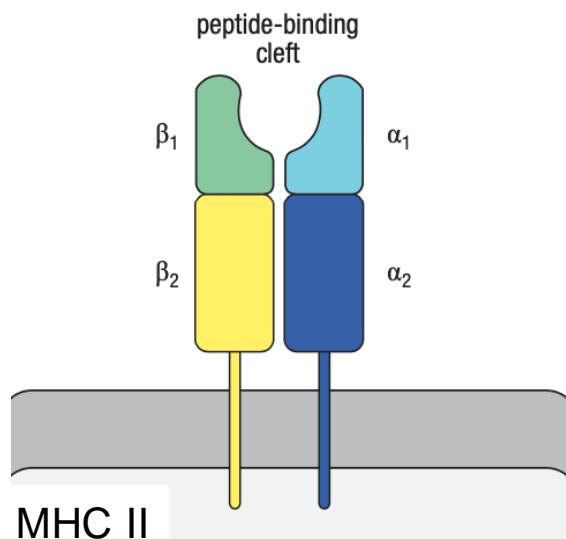
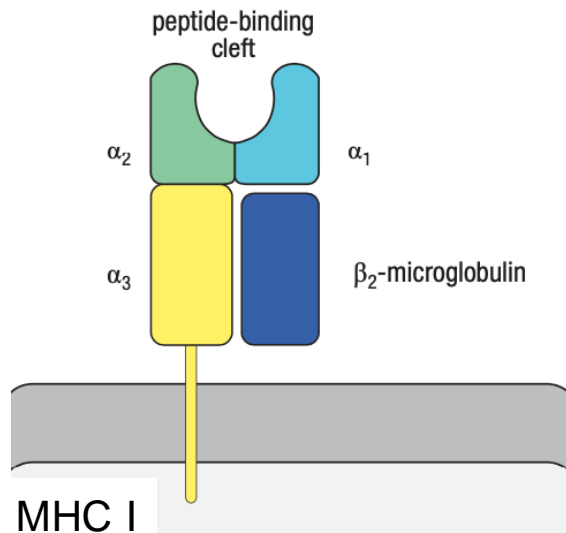
Human MHC genes



There is a large variability of MHC molecules in a population, but in each individual:

MHC-I	MHC-II
2x HLA-A	2x HLA-DP
2x HLA-B	2x HLA-DQ
2x HLA-C	2x HLA-DR
=> 6	=> 6-8

Structural comparison: antibody, MHC and TCR



Large diversity in the recognition of antigens

BCR and antibodies: gene rearrangement + somatic hypermutation

=> Each individual can recognize any hapten/epitop
(linear *and* conformational epitopes)

TCR: gene rearrangement

=> Each individual can recognize any linear peptide in context with MHC molecule

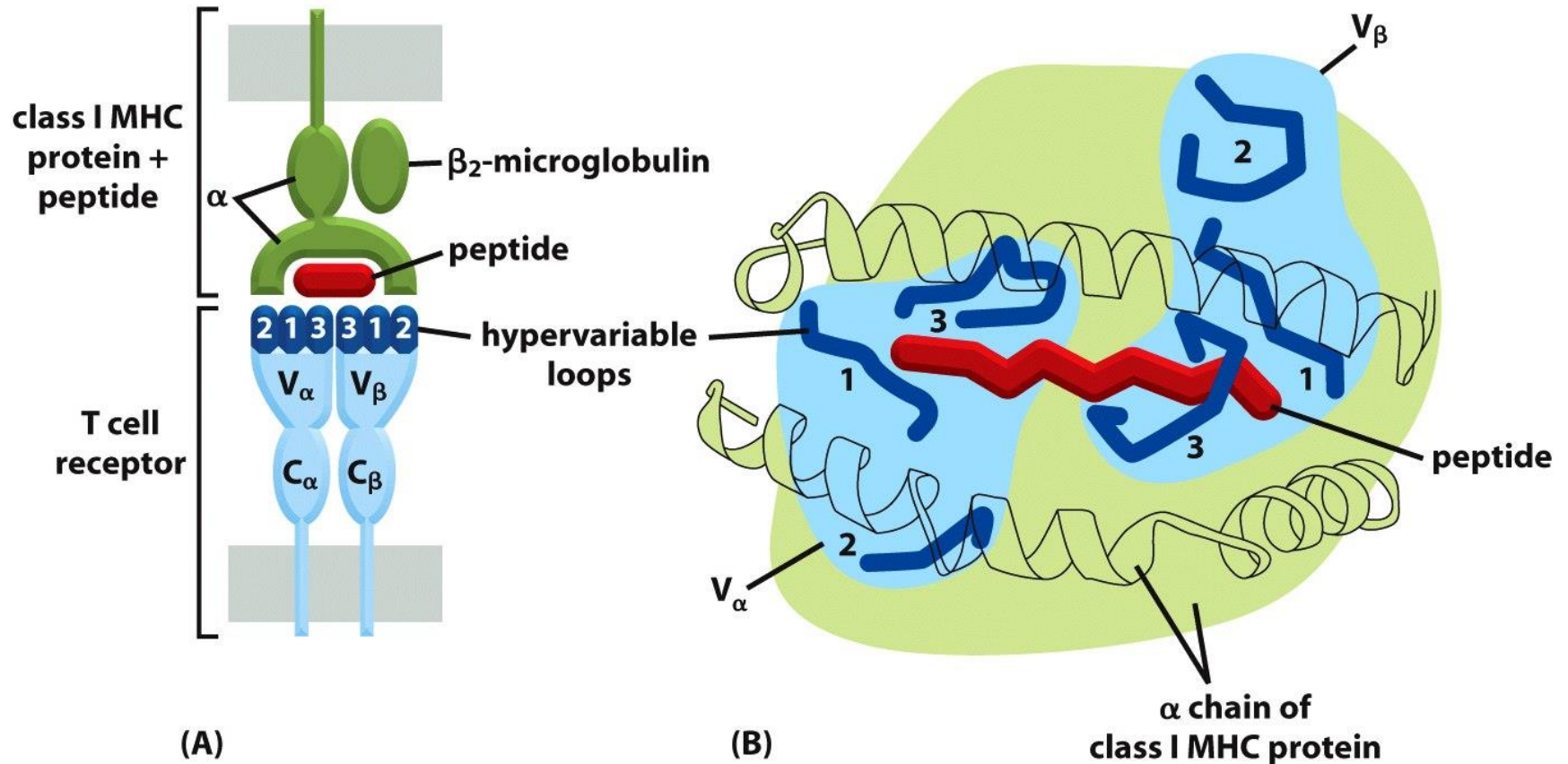
MHC: no gene rearrangement but 3 genes and several thousand alleles in a population

=> Can bind a large variety of peptides (but not all)

=> a whole population is well protected but there is an individual risk of missing some pathogenic peptides

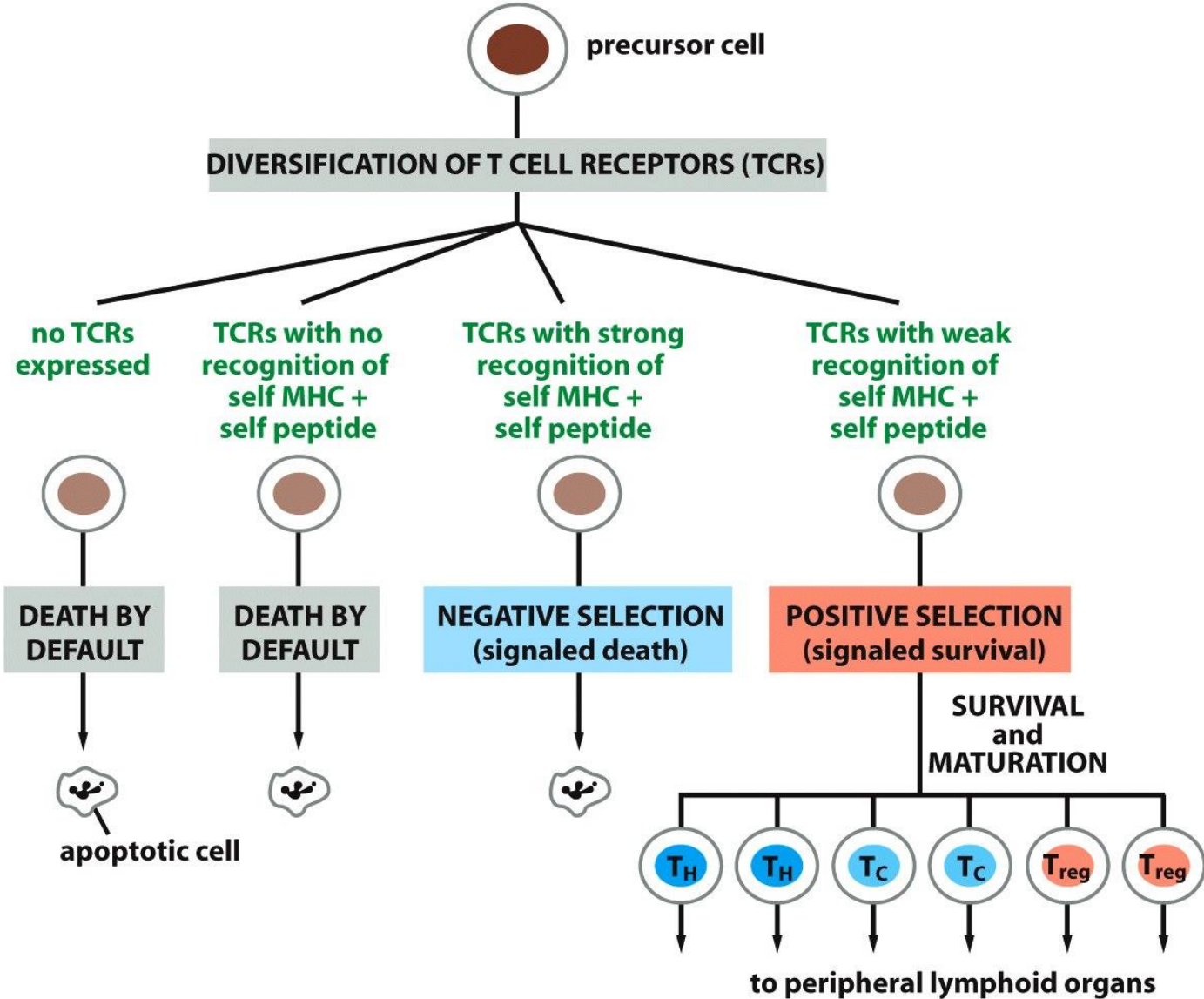
=> populations with a large gene pool are more resistant to an epidemic

Interaction of TCR with a peptide on MHC class I

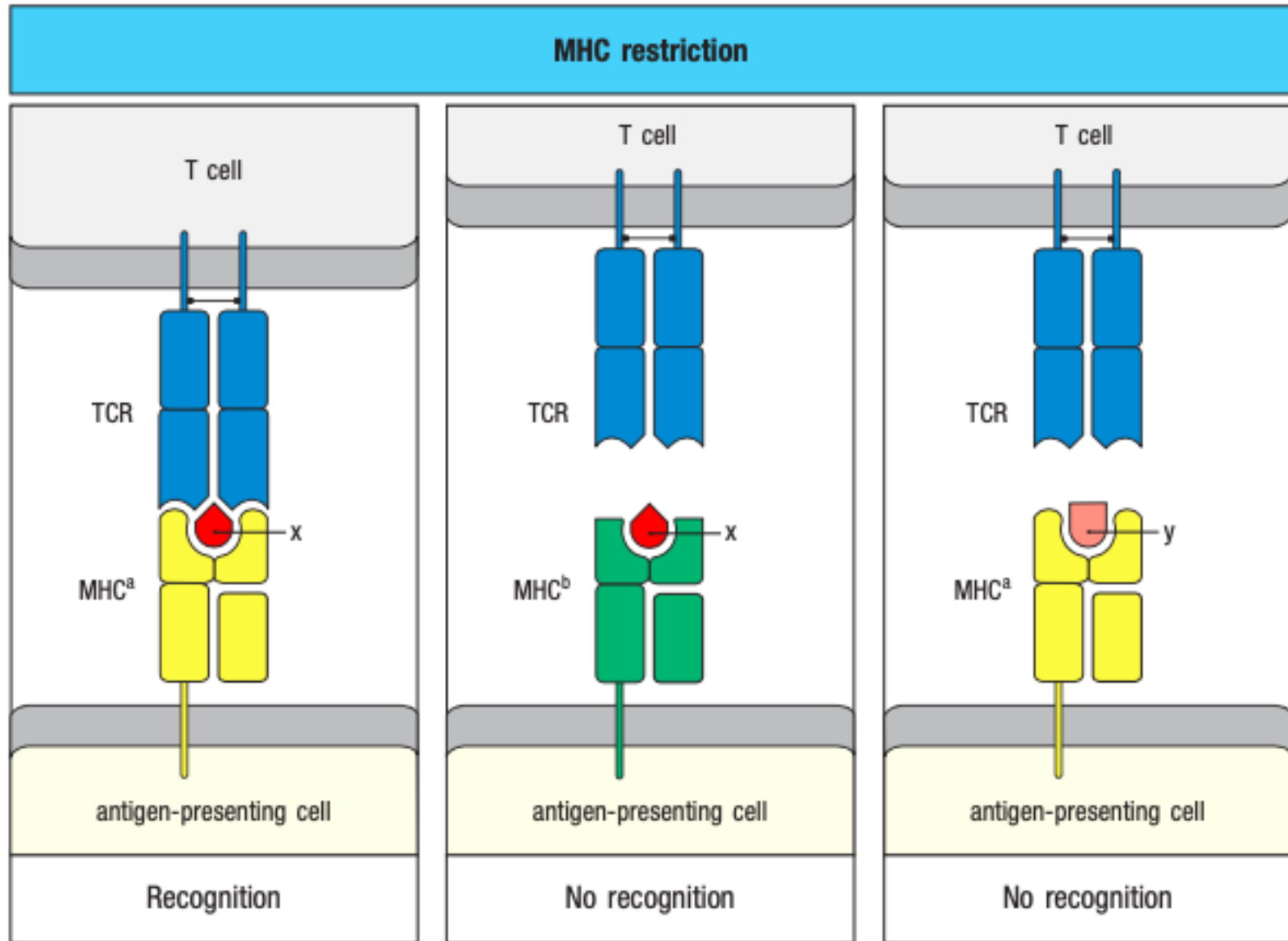


=> Only linear peptide epitopes

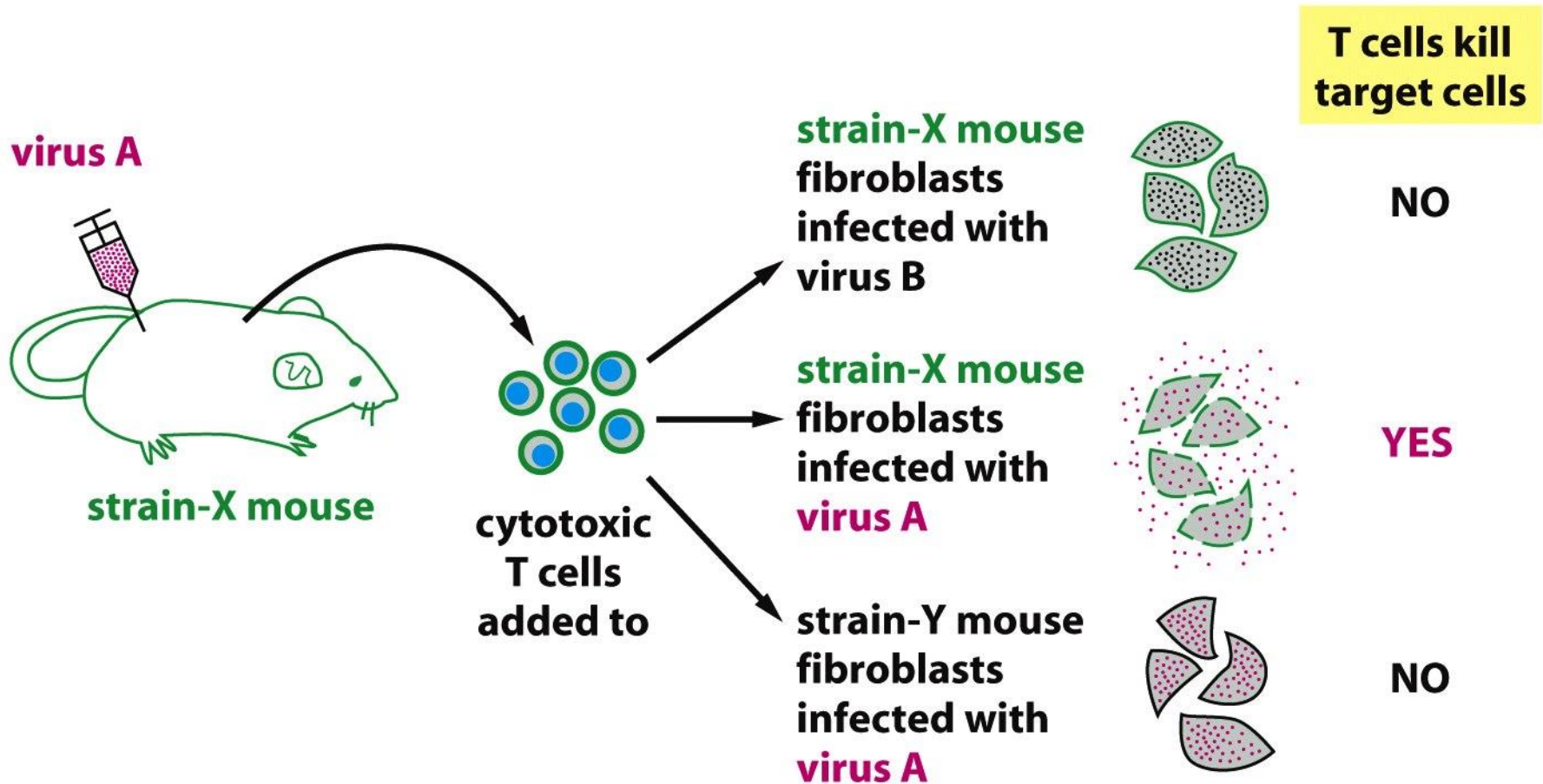
Friend / foe recognition by T cells



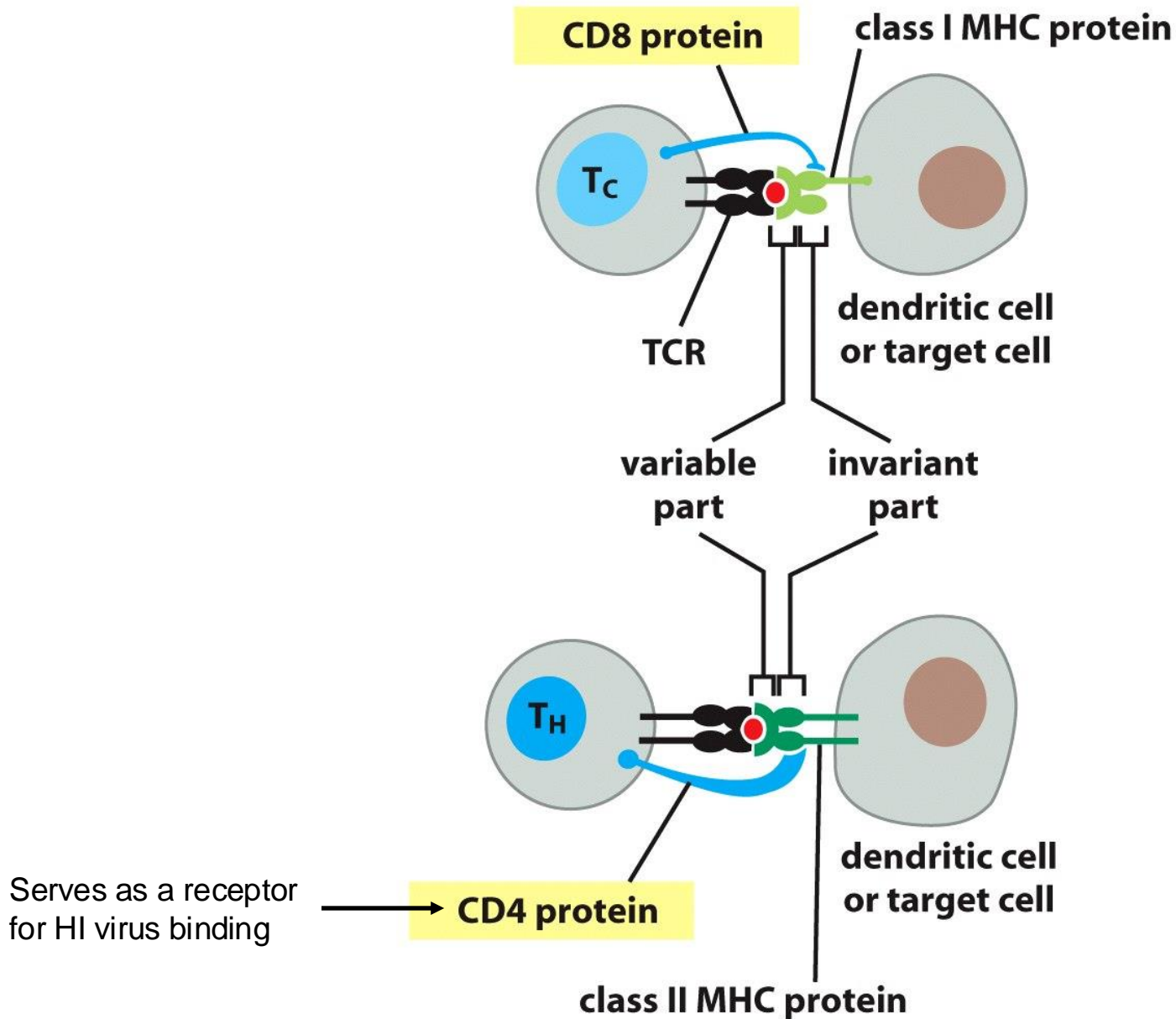
T cell recognition of antigens is MHC restricted



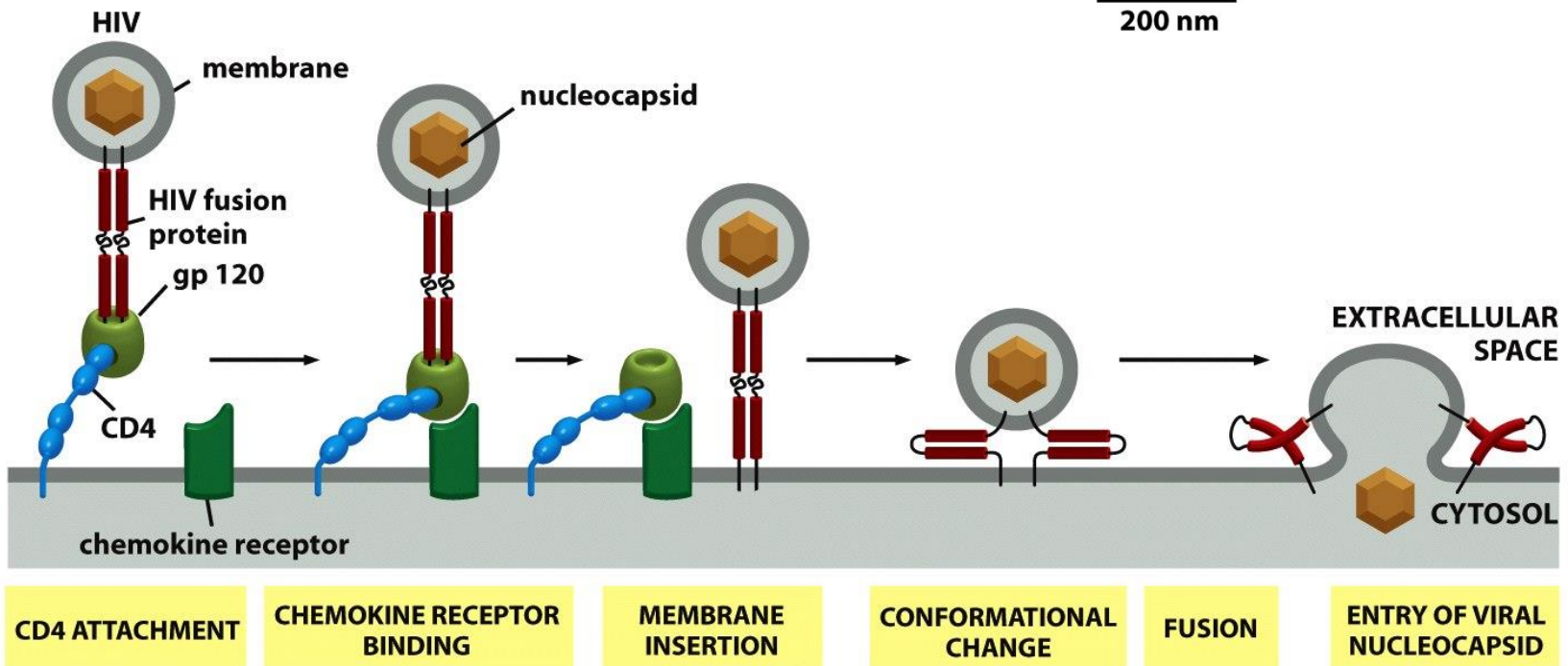
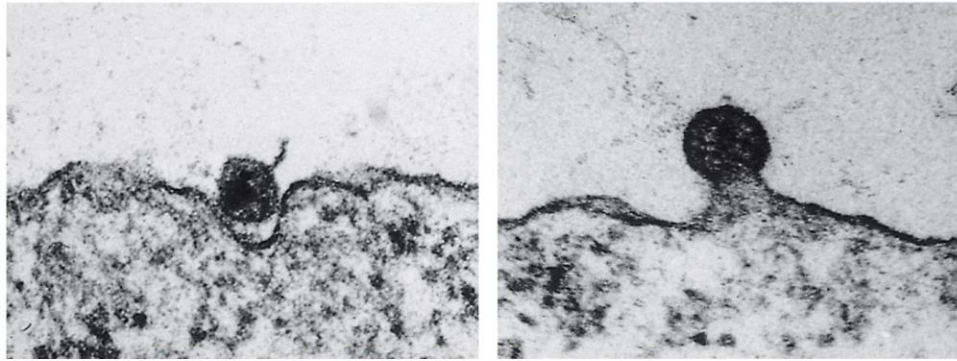
T cell recognizes viral antigen *and* host target cell



Co-receptors on T cells

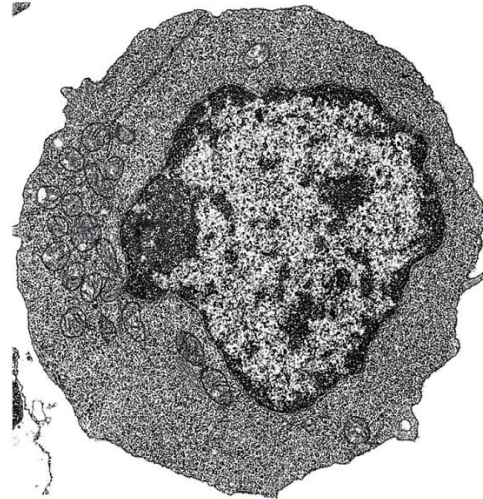


Excursion: HI virus infecting T cell



=> depletion of T_H cells: **AIDS** (Aquired Immunodeficiency Syndrome)

Classification of T cells



effector T cell

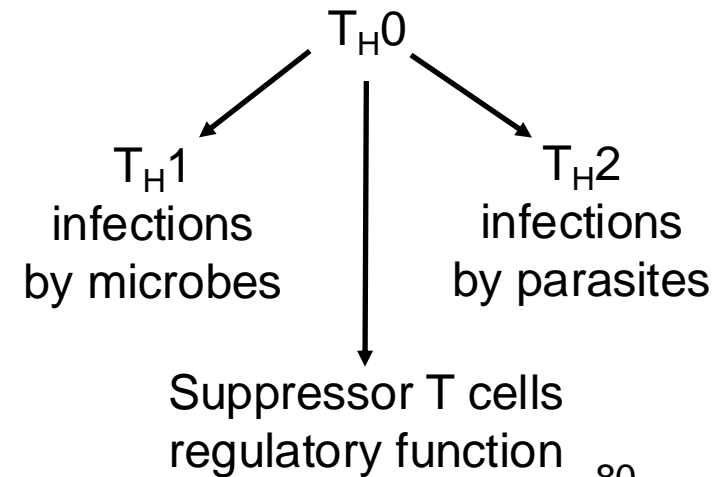
1 μm

Cytotoxic T cells (T_C)
($CD8^+$ cells)

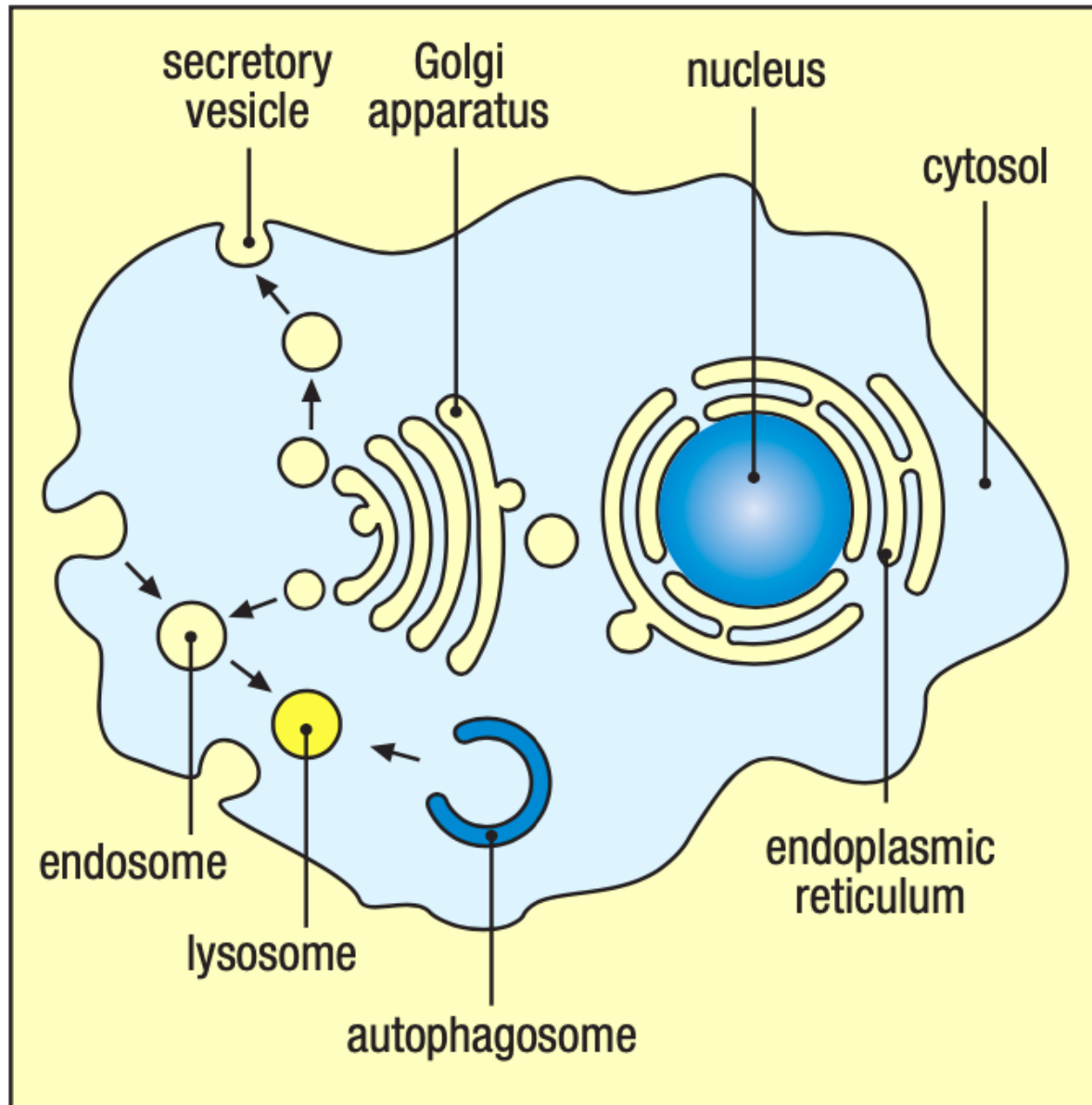
=> recognize peptides on MHC I

T helper cells (T_H)
($CD4^+$ cells)

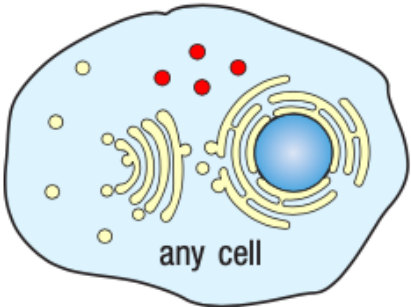
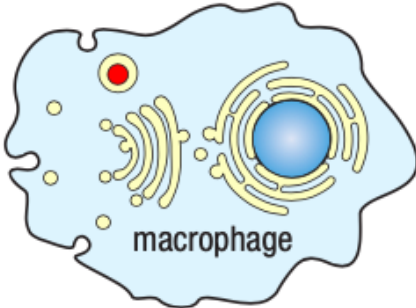
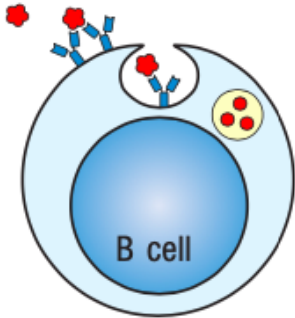
=> recognize peptides on MHC II



Topologically equivalent compartments



Antigen acquisition sites

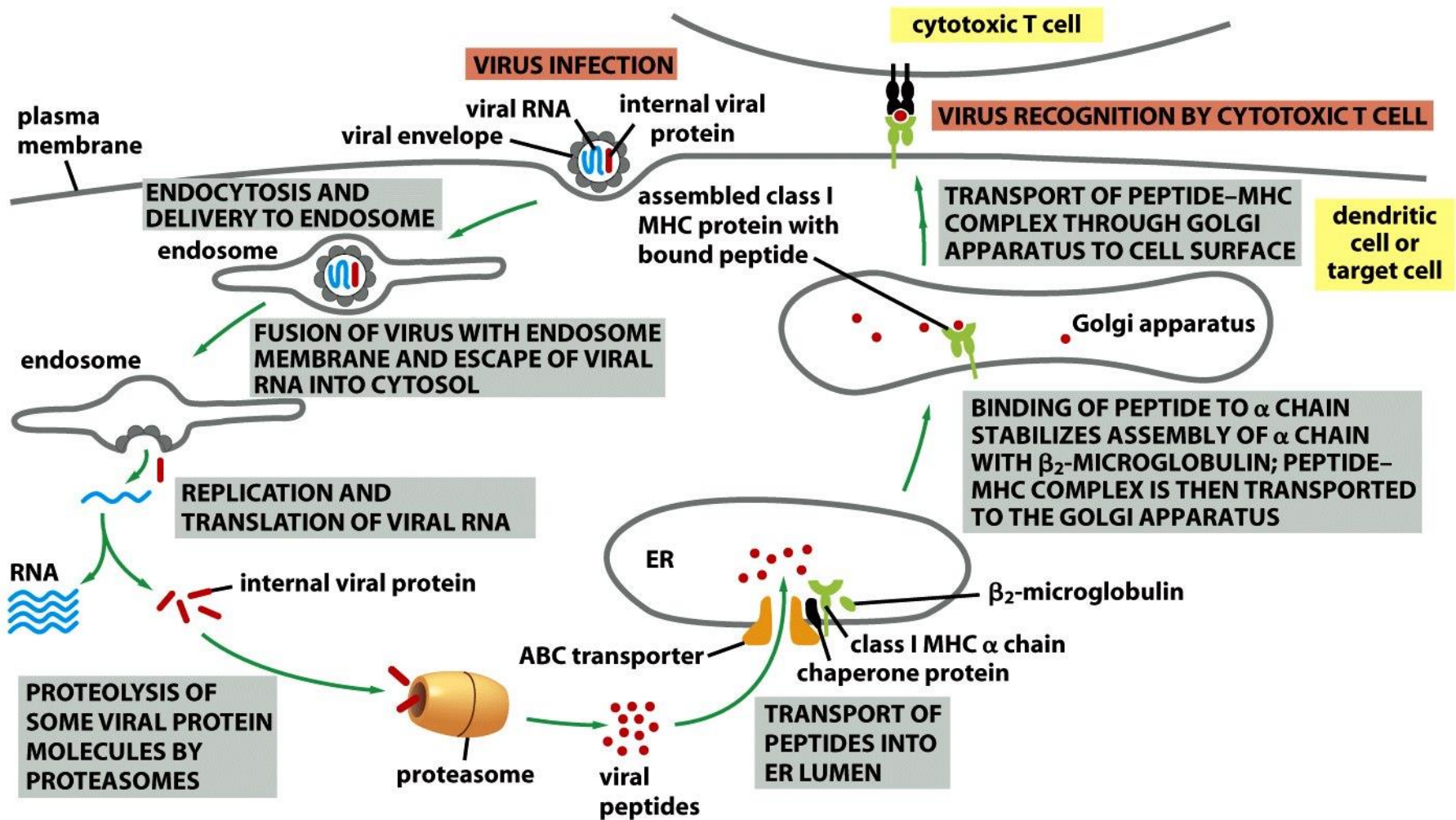
	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
	 <p>any cell</p>	 <p>macrophage</p>	 <p>B cell</p>
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	Effector CD8 T cells	Effector CD4 T cells	Effector CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins

=> T_C

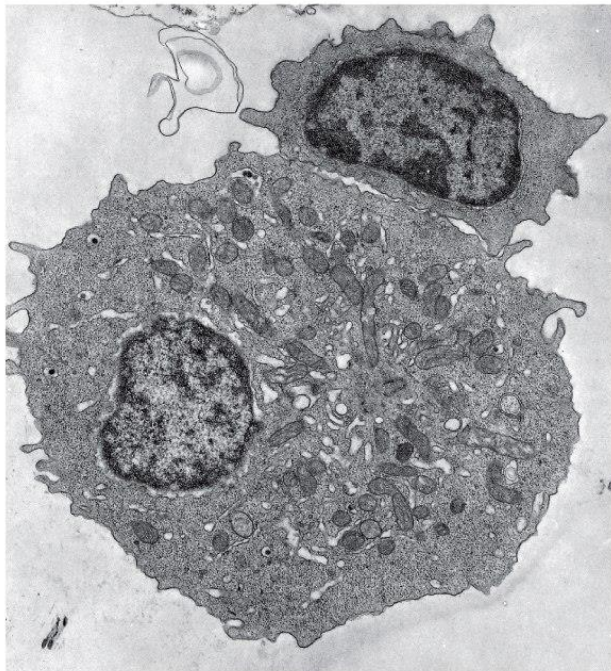
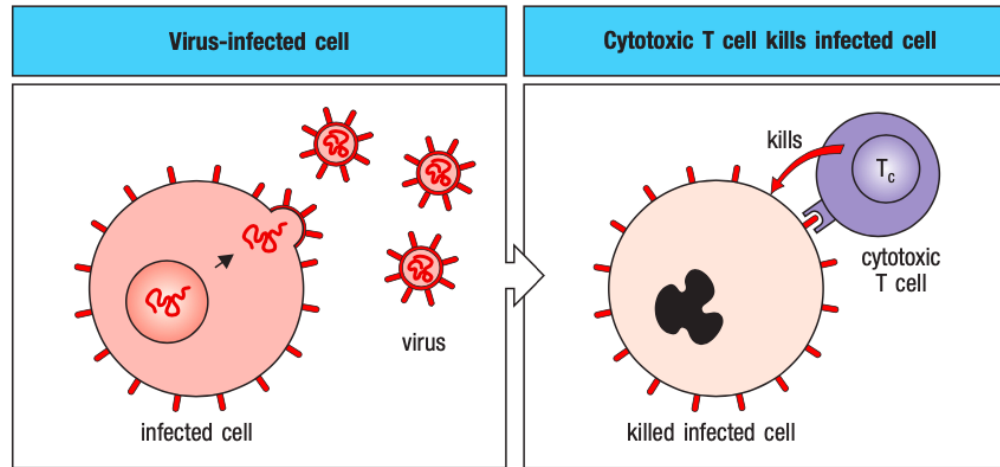
=> T_{H1}

=> T_{H2}

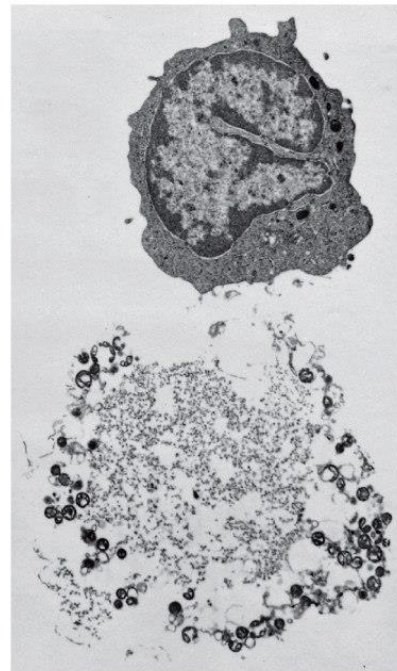
Antigen presentation by MHC-I



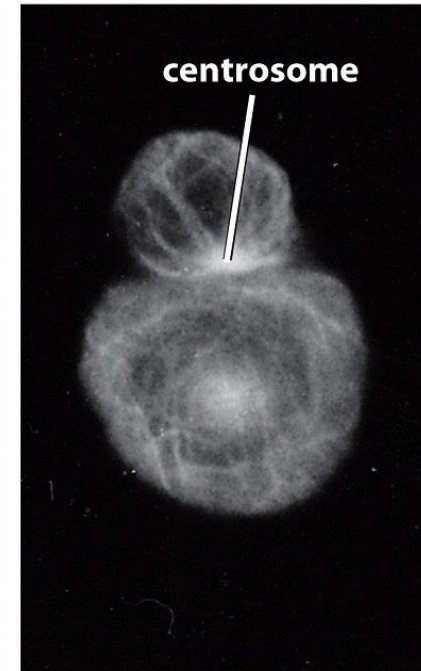
Activation of cytotoxic T cells



(A)



(B)

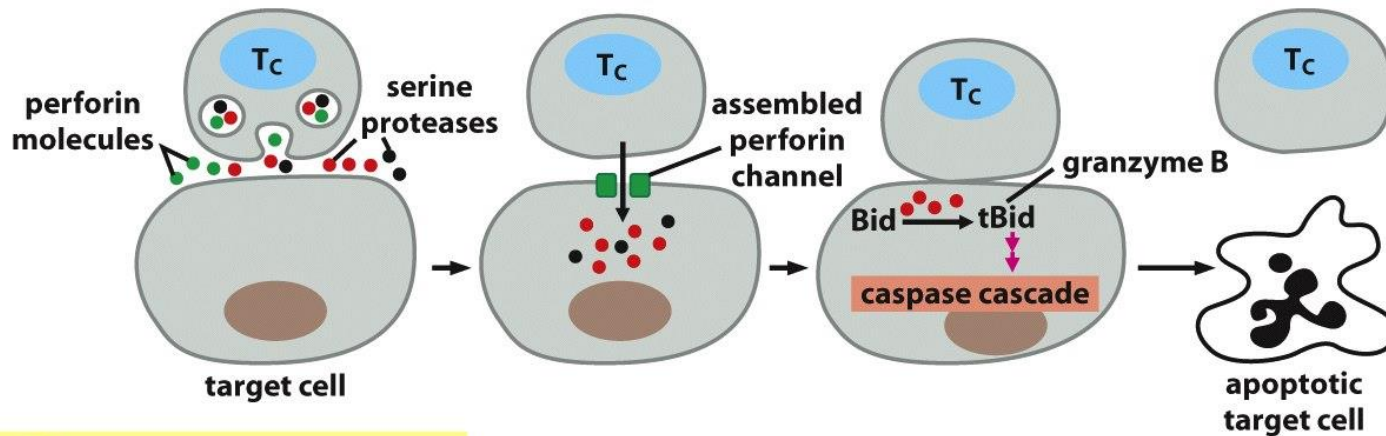


(C)

Cytotoxic T cells induce apoptosis

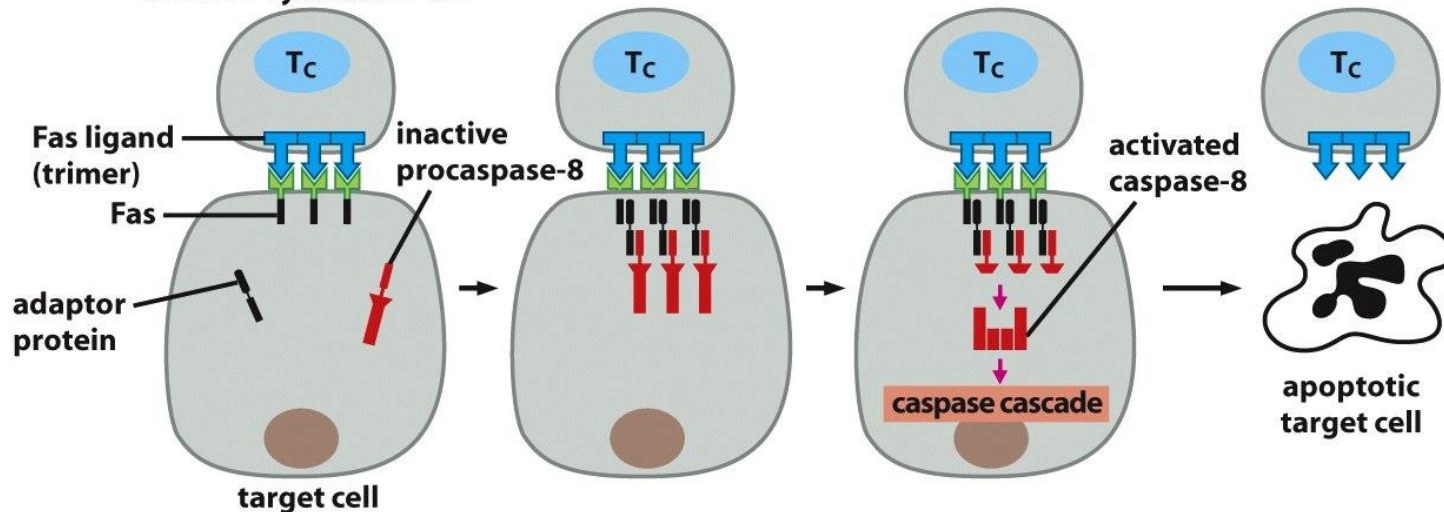
A Perforin-dependent killing

effector cytotoxic T cell

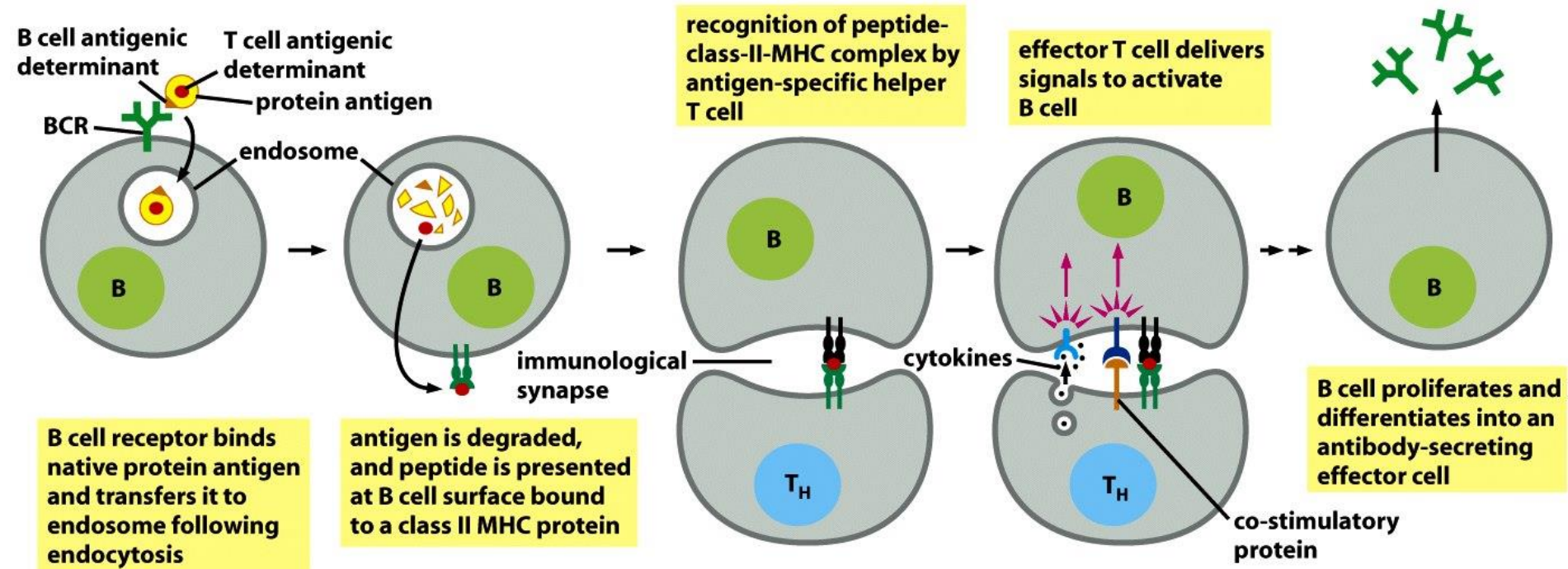


B Fas-dependent killing

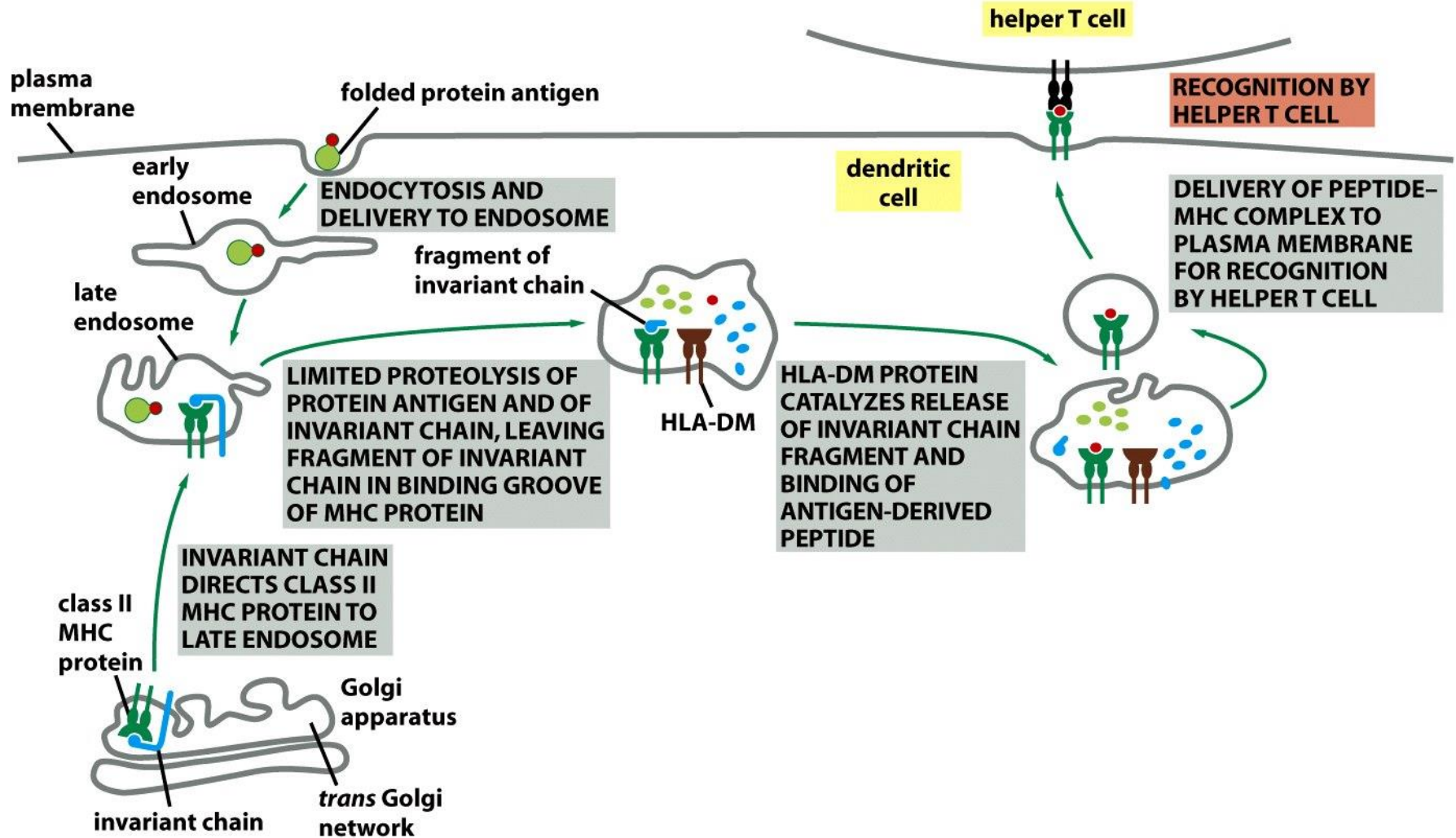
effector cytotoxic T cell



Activation of a B cell by an antigen *and* T_H cell



Antigen presentation by MHC-II



Summary of interplay between T_H and B cells

Antigen

T cell epitope
(binds to MHC,
recognized by TCR)



B cell epitope
(binds to BCR)

