MUNI
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C8116 Immunochemical techniques

Immune system, part I

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Research and contact

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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

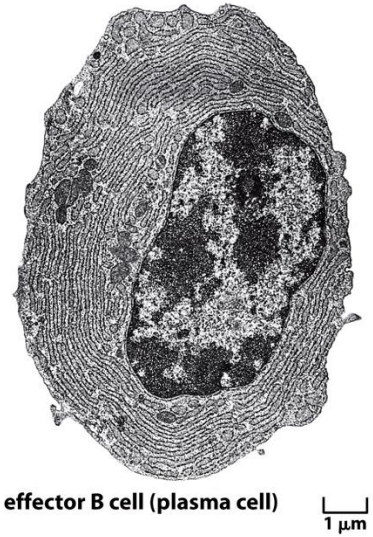
=> More information provided during the lecture…

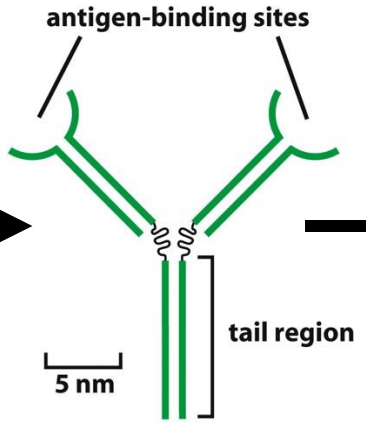
The idea behind the lecture

Immunology

The “tools“: antibodies

Immunoassay





Topics of the lecture

Part A: The immune system

Part B: Antibodies as immunological tools

Part C: Immunoassays

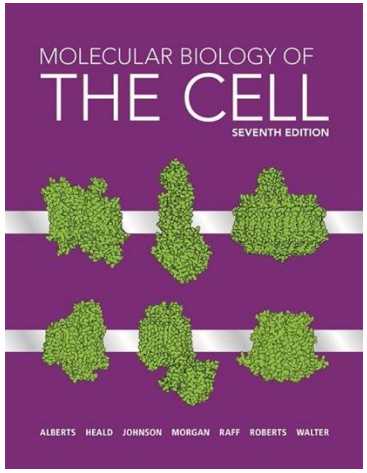
Part D: Immunoaffinity and other protein-protein affinity techniques

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

The immune system (2 days)

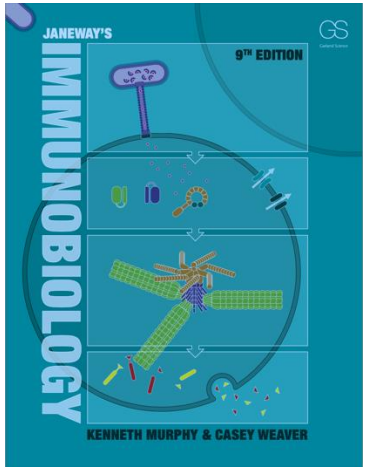
1) General introduction to the immune system
2) Innate / adaptive immune system
3) Lymphoid organs
4) B cells
5) Progress of immune response
6) Structure of IgG / immunoglobulin superfamily
7) Binding sites of antibodies
8) Generation of antibody diversity / affinity maturation
9) Antibody affinity
10) 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

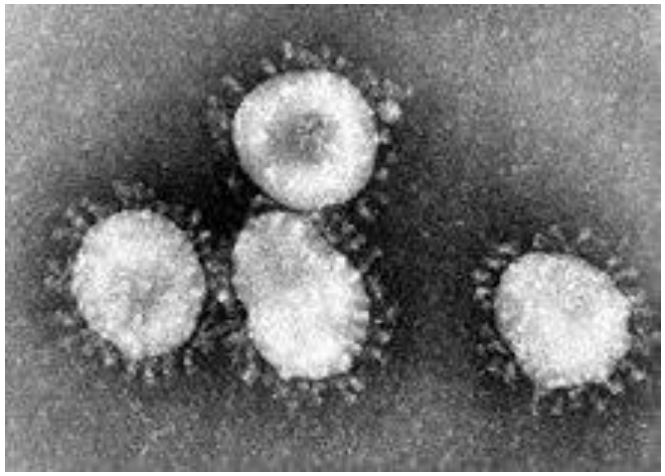
Janeway Immunobiology (9th edition)
Murphy & Weaver
Garland Science, London 2017
https://inmunologos.wordpress.com/wp-content/uploads/2020/08/janeways-immunobiology-9th-ed_booksmedicos.org_.pdf

Slides of the lecture are available online (Learning Materials)

Overview on our body‘s defenses against an infection

Challenge: Great variability of infectious diseases

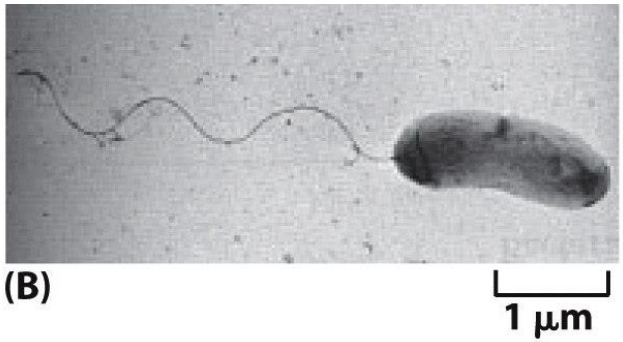
Coronavirus, intracellular



Diameter: ca. 100 nm

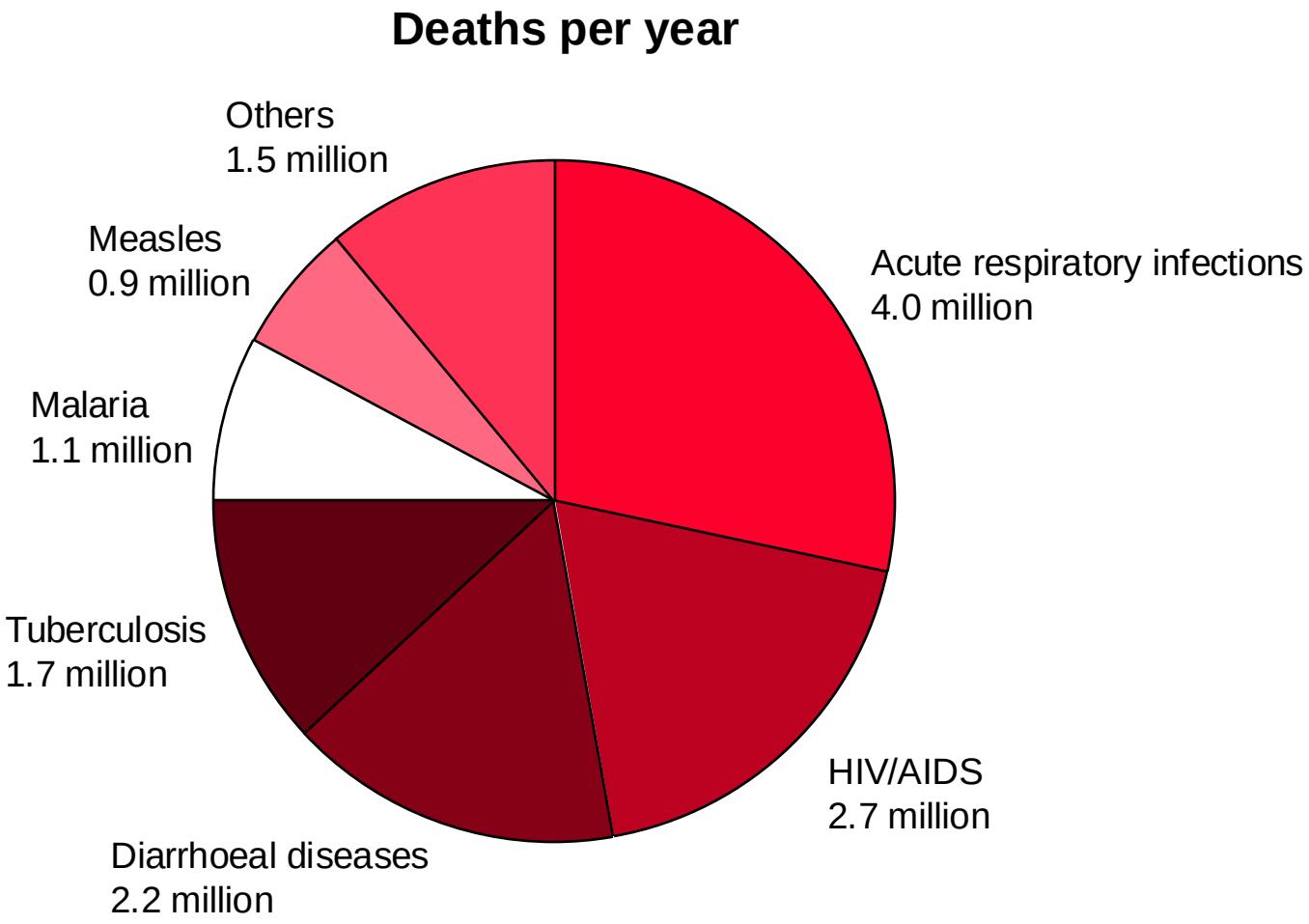
Toxoplasmosis:
single-celled, eukaryotic parasite, intracellular

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



Cholera: bacterium, in the intestine

Infectious diseases

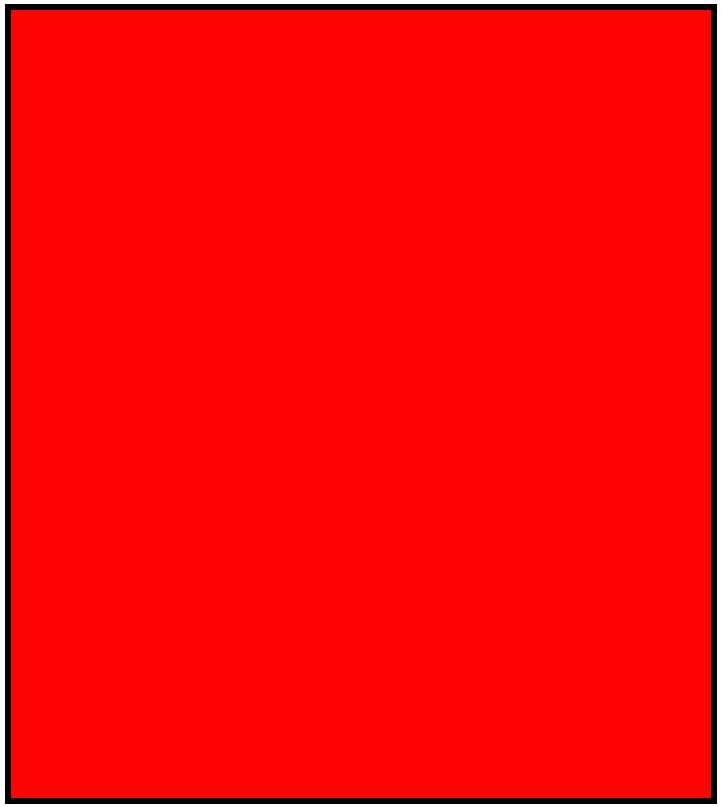


Source:
The World Health Report 2000, WHO

red colors:
Pathogens that enter our body
over mucosal surfaces

Surface areas of human body

Mucosa

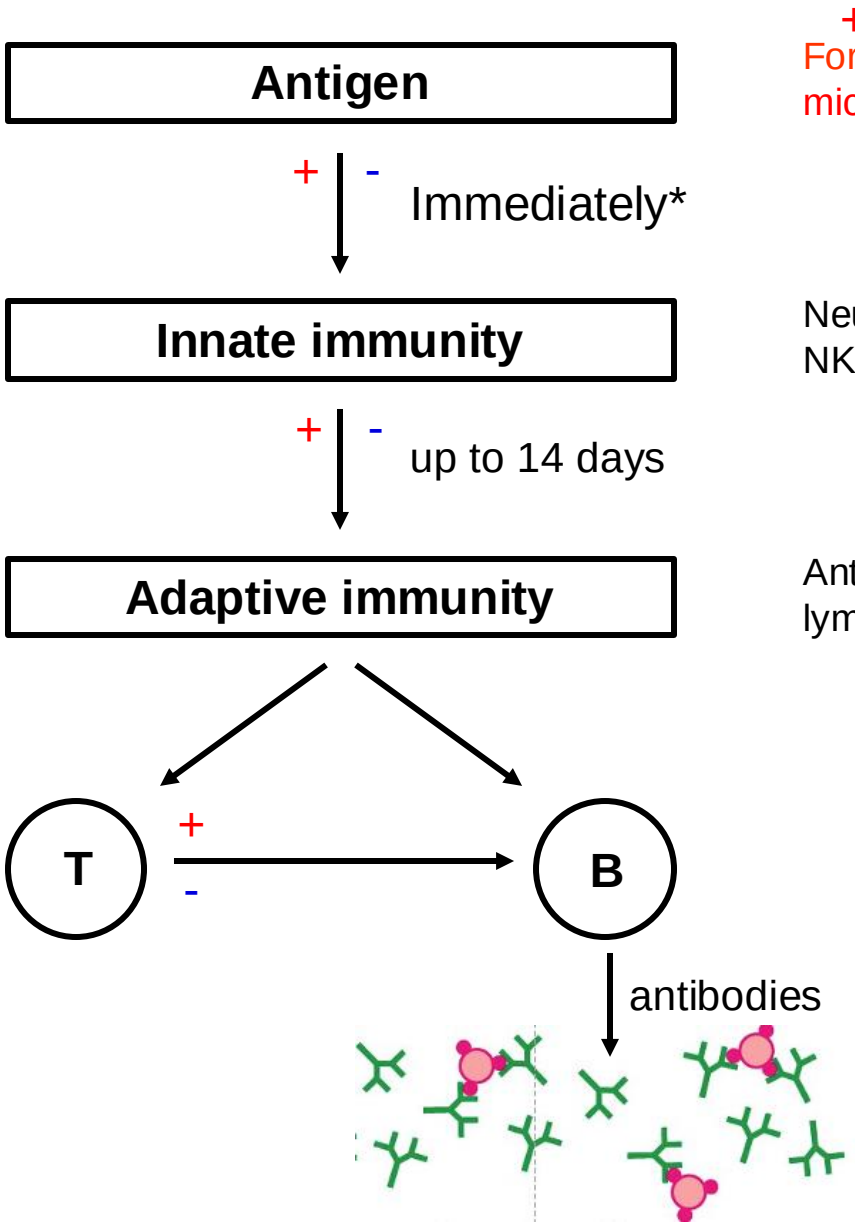


400 m^2

Skin

2 m^2

Two lines of defence



\+ Foreign and - own structures, microorganisms, viruses, tumors

Neutrophil granulocytes, macrophages, NK cells, diverse humoral factors

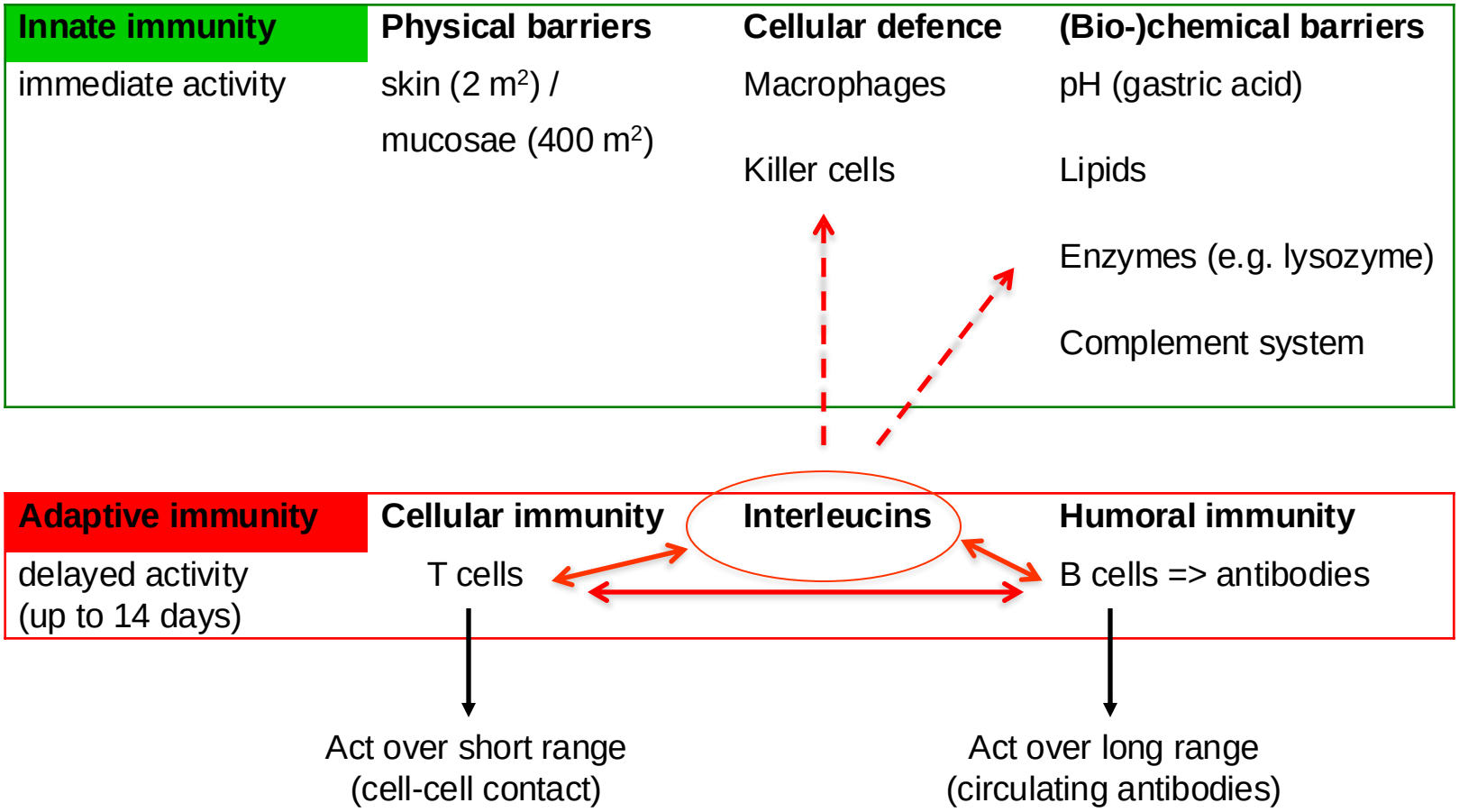
Antigen-presenting cells, T and B lymphocytes, plasma cells, cytokines

***Race against time:**
Bacteria can divide every 20 min

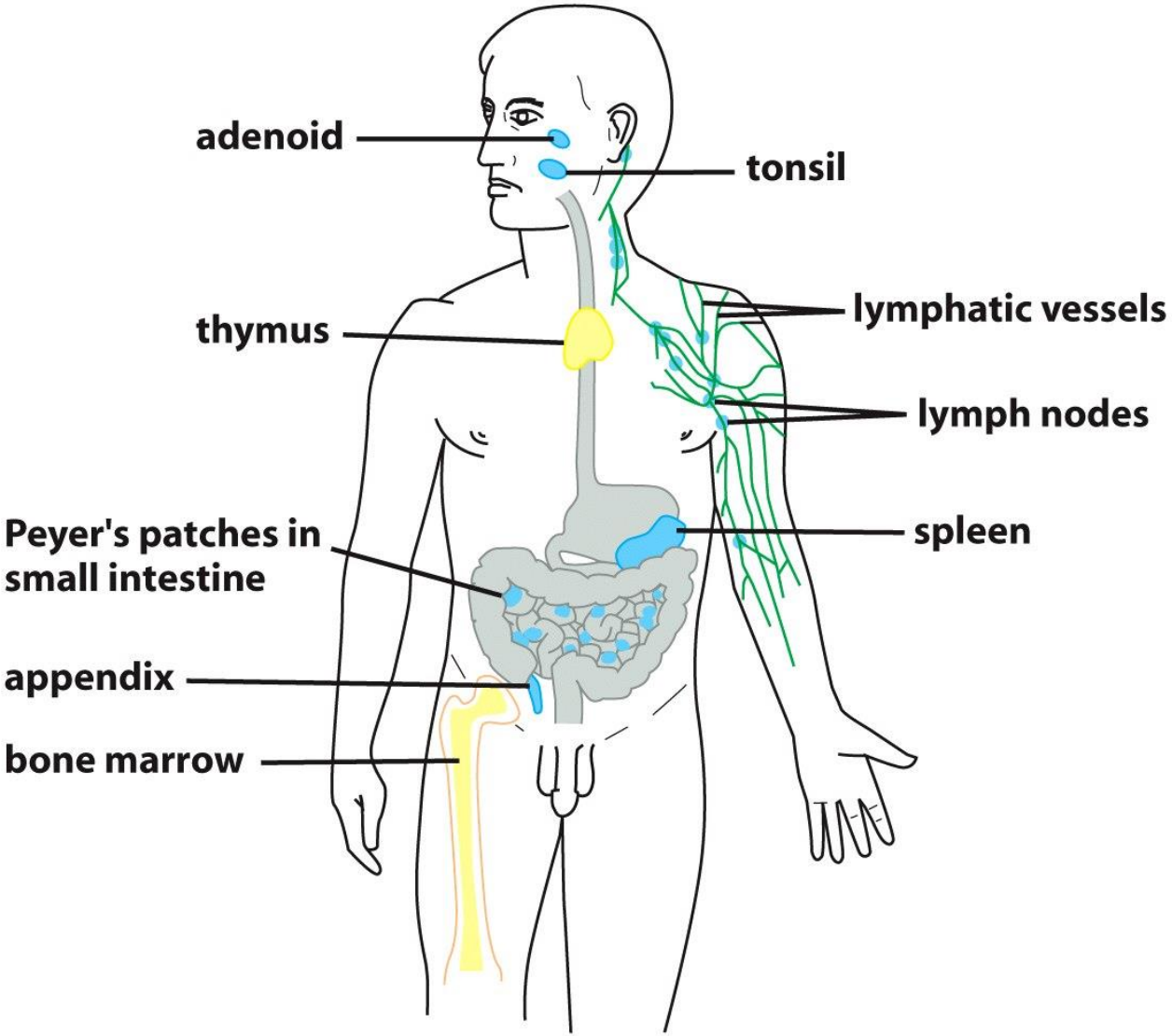
$x_t = x_0 * e^{(\mu * t)}$ (μ=ln2/time for division (hrs))

=> unrestricted growth:
$1 * e^{2.08*10}$= 1 billion bacteria after 10 hrs!

Innate / adaptive immunity



Adaptive immunity: Human lymphoid organs

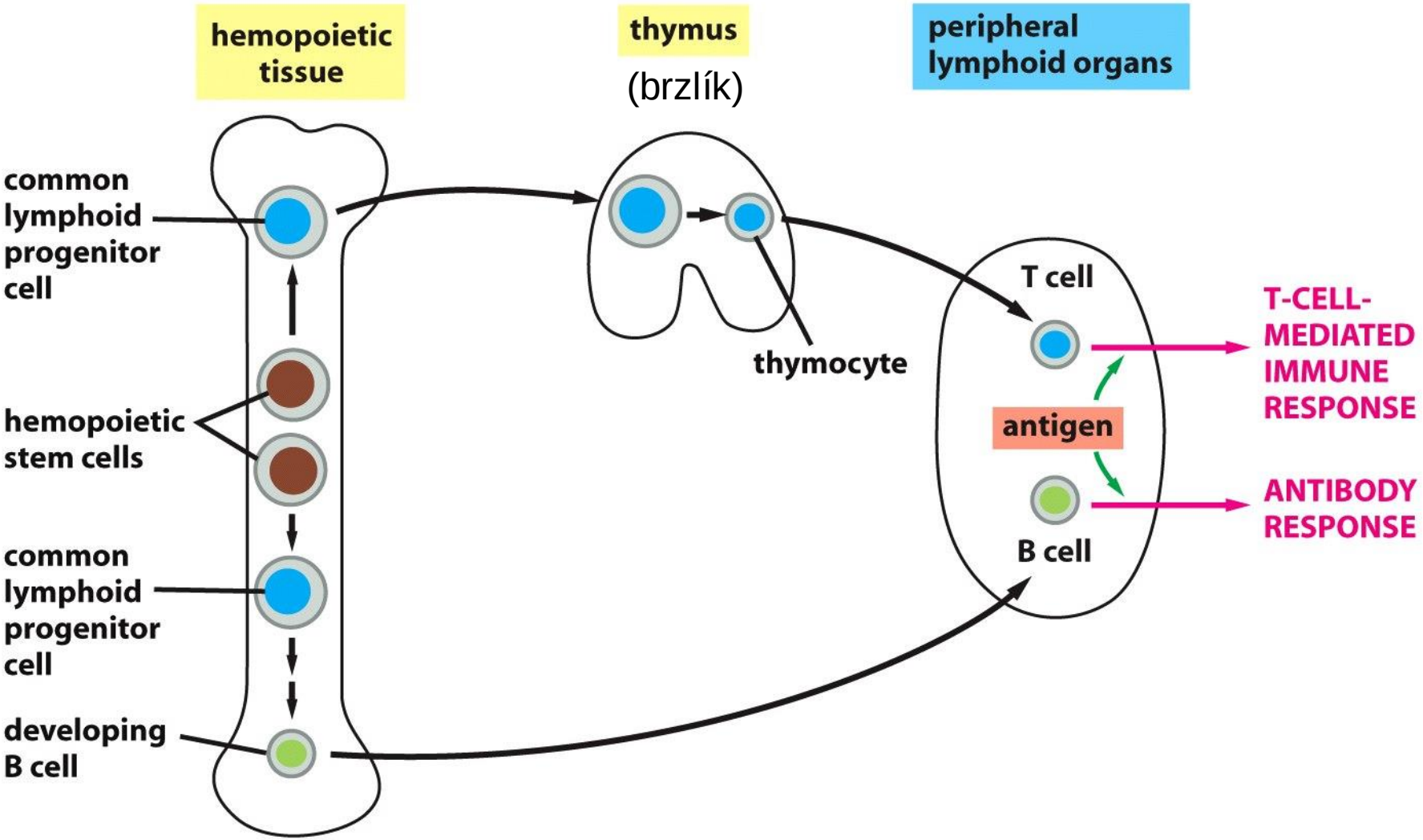


Primary lymphatic organs (yellow):
Bone marrow: B-cells
Thymus: T-cells

Secondary lymphatic organs (blue):
lymph nodes
spleen
and others

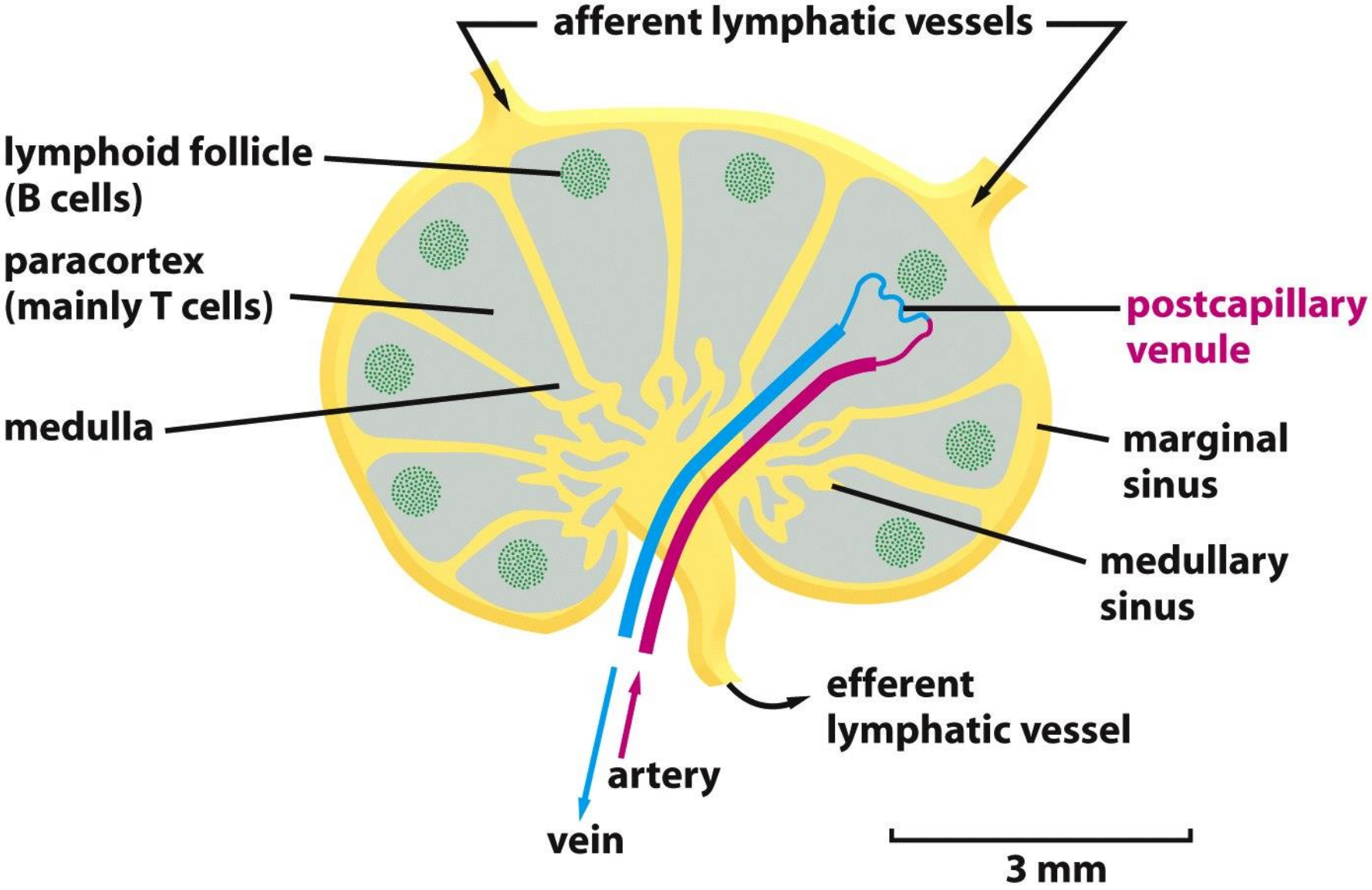
10^{12} lymphocytes (ca. 1 kg)

Development of B und T cells

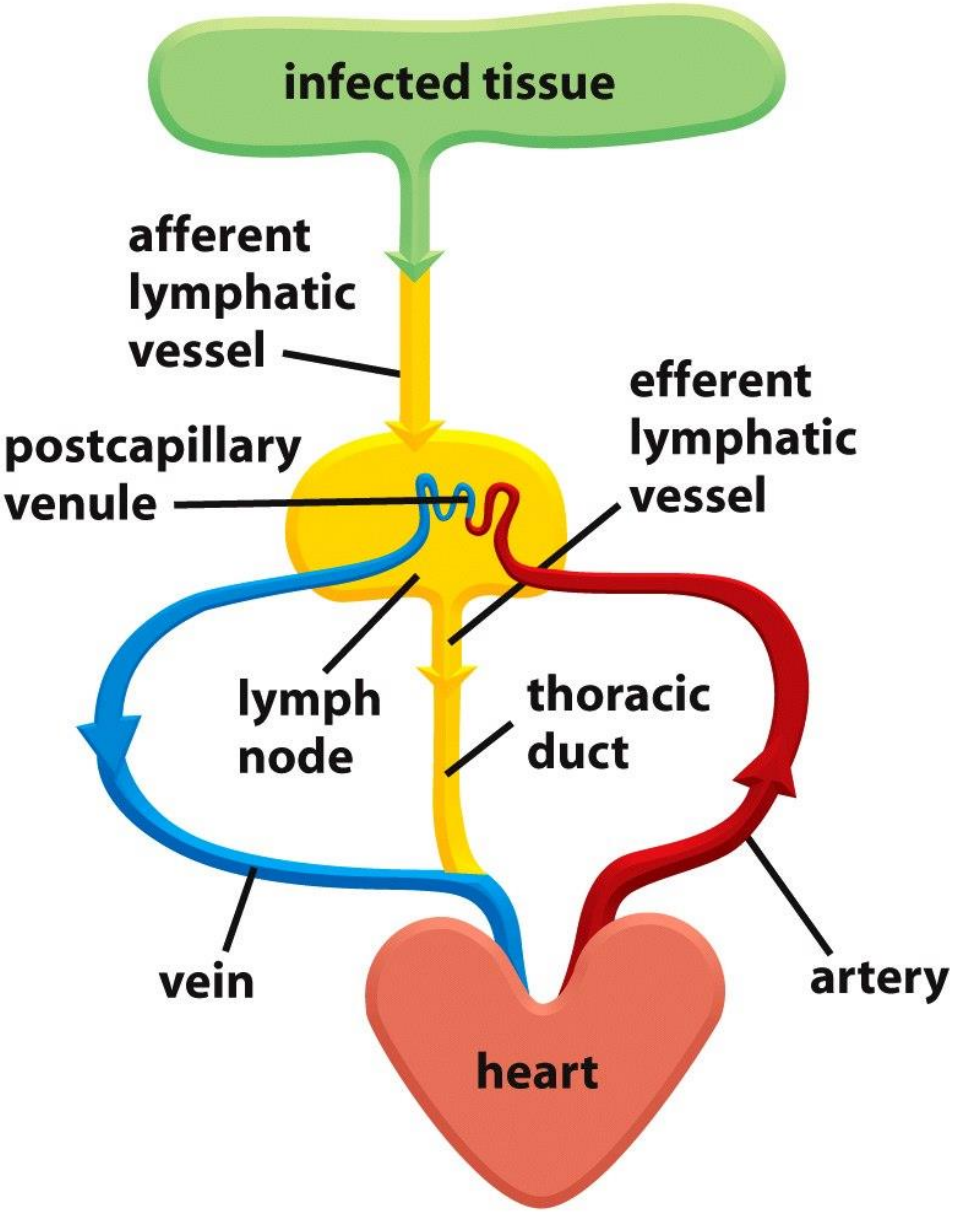


=> Bone marrow donation to reconstitute the immune system

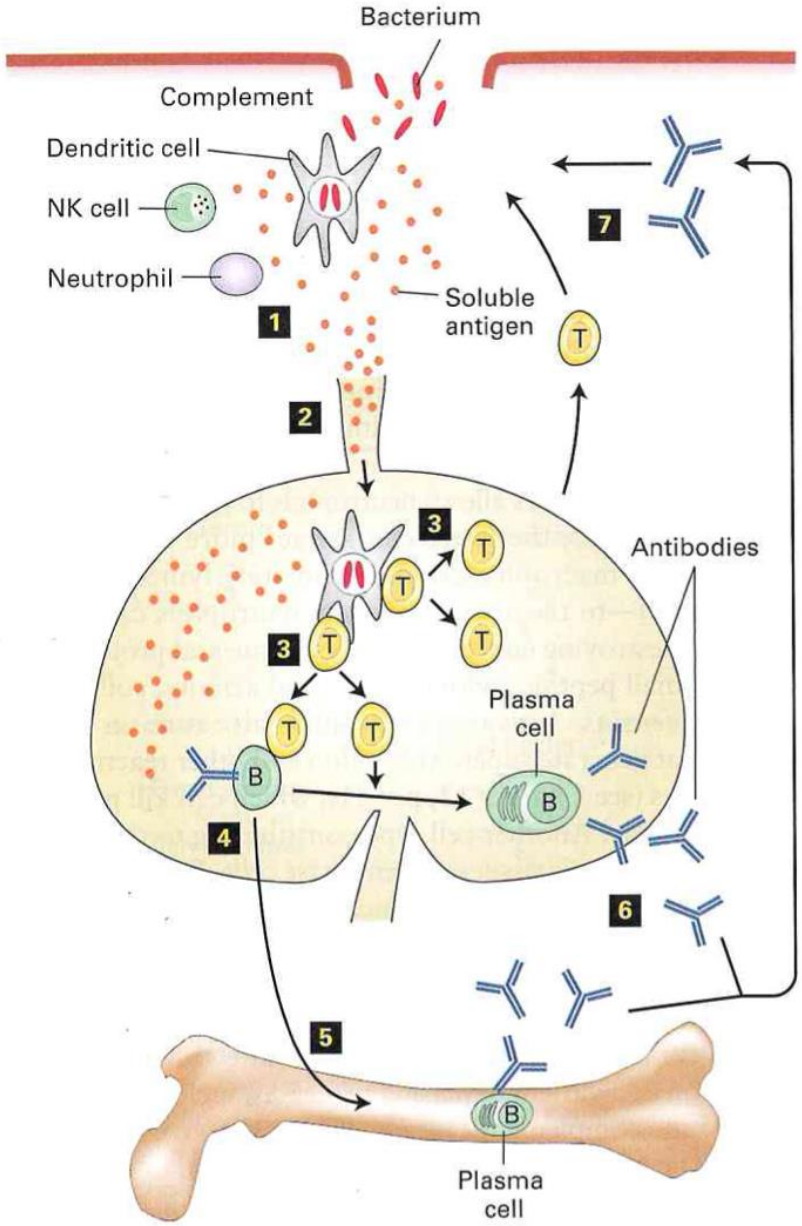
Lymph node


afferent lymphatic vessels
lymphoid follicle
(B cells)
paracortex
(mainly T cells)
medulla
postcapillary
venule
marginal
sinus
medullary
sinus
efferent
lymphatic vessel
artery
vein
3 mm

Circulation of lymphocytes



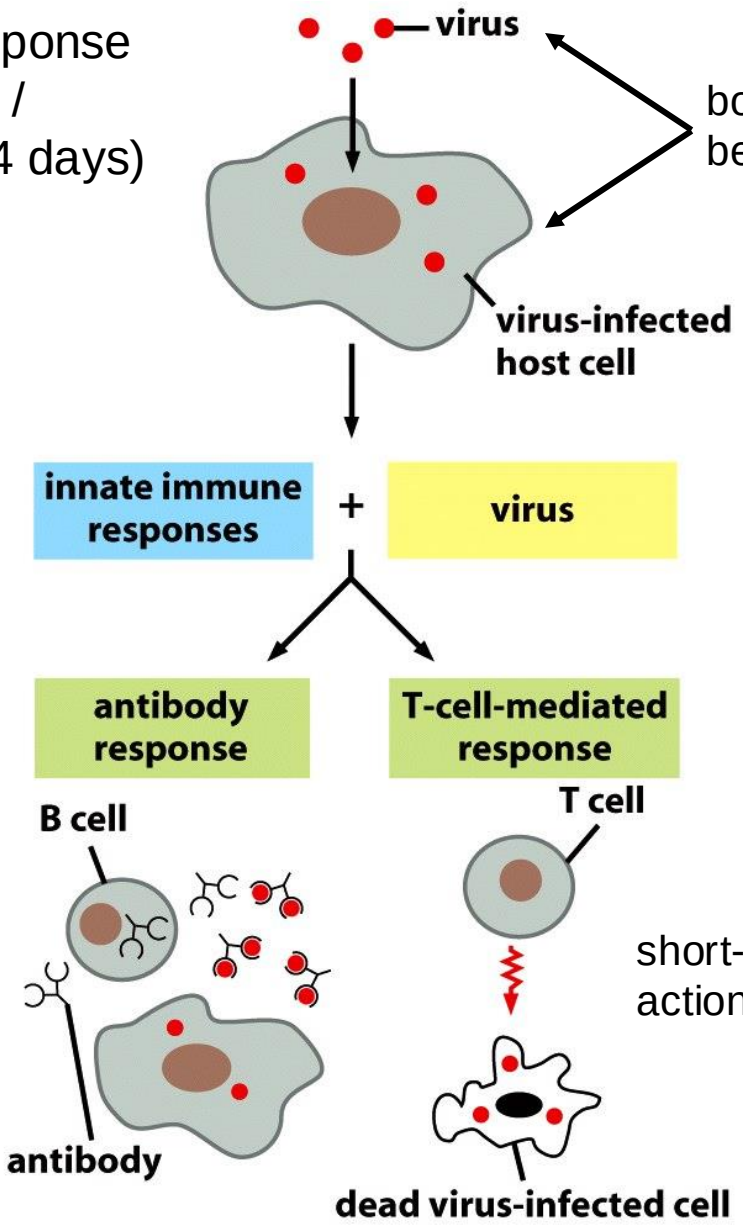
Overview of an inflammatory response



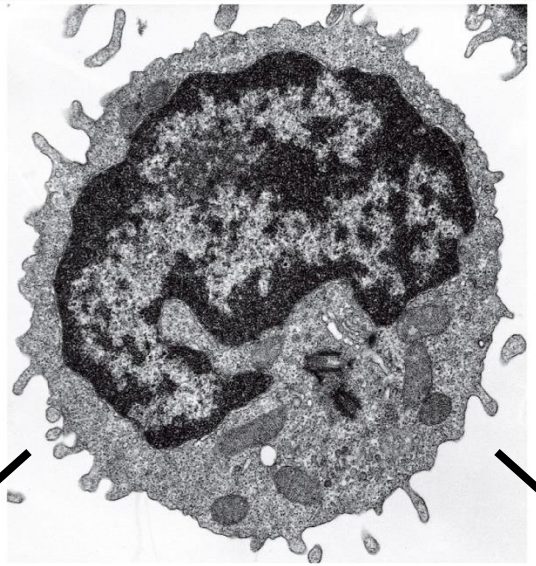
1) A bacterium encounters a first line of defense (innate immune response)
2) Breakdown of bacterium and release of antigens
3) Dendritic cells take up antigen and activate T cells
4) T cells proliferate and activate B cells
5) B cells differentiate into plasma cells
6) Plasma cells produce antibodies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

Two classes of adaptive immune responses

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

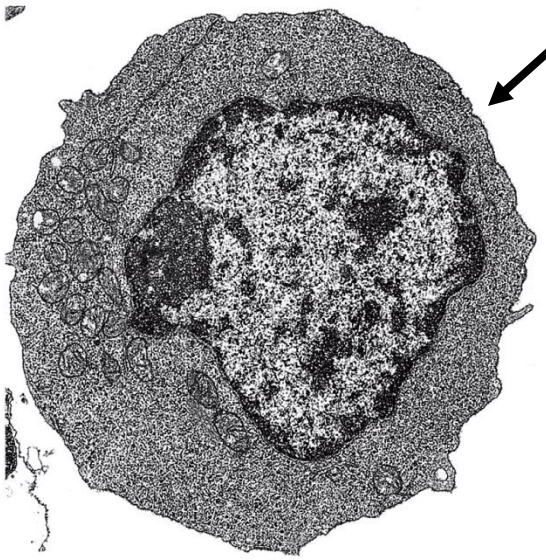


Activation of lymphocytes



resting T or B cell

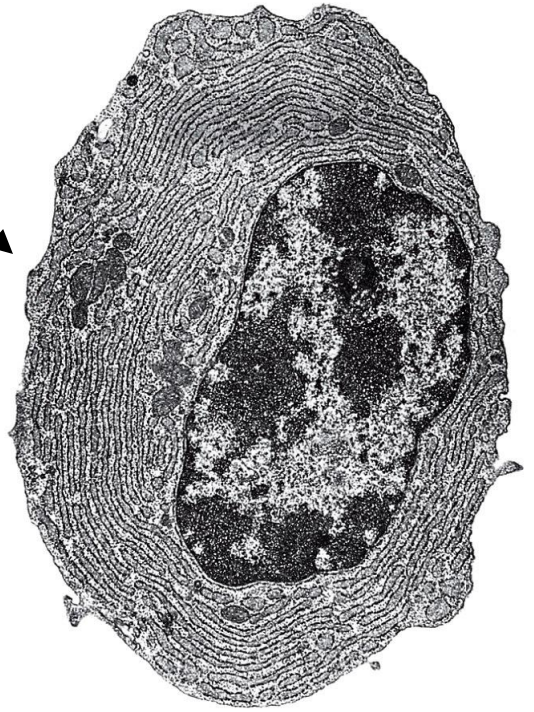
1 μm



effector T cell

1 μm

cytotoxic T cells
helper T cells
regulatory T cells

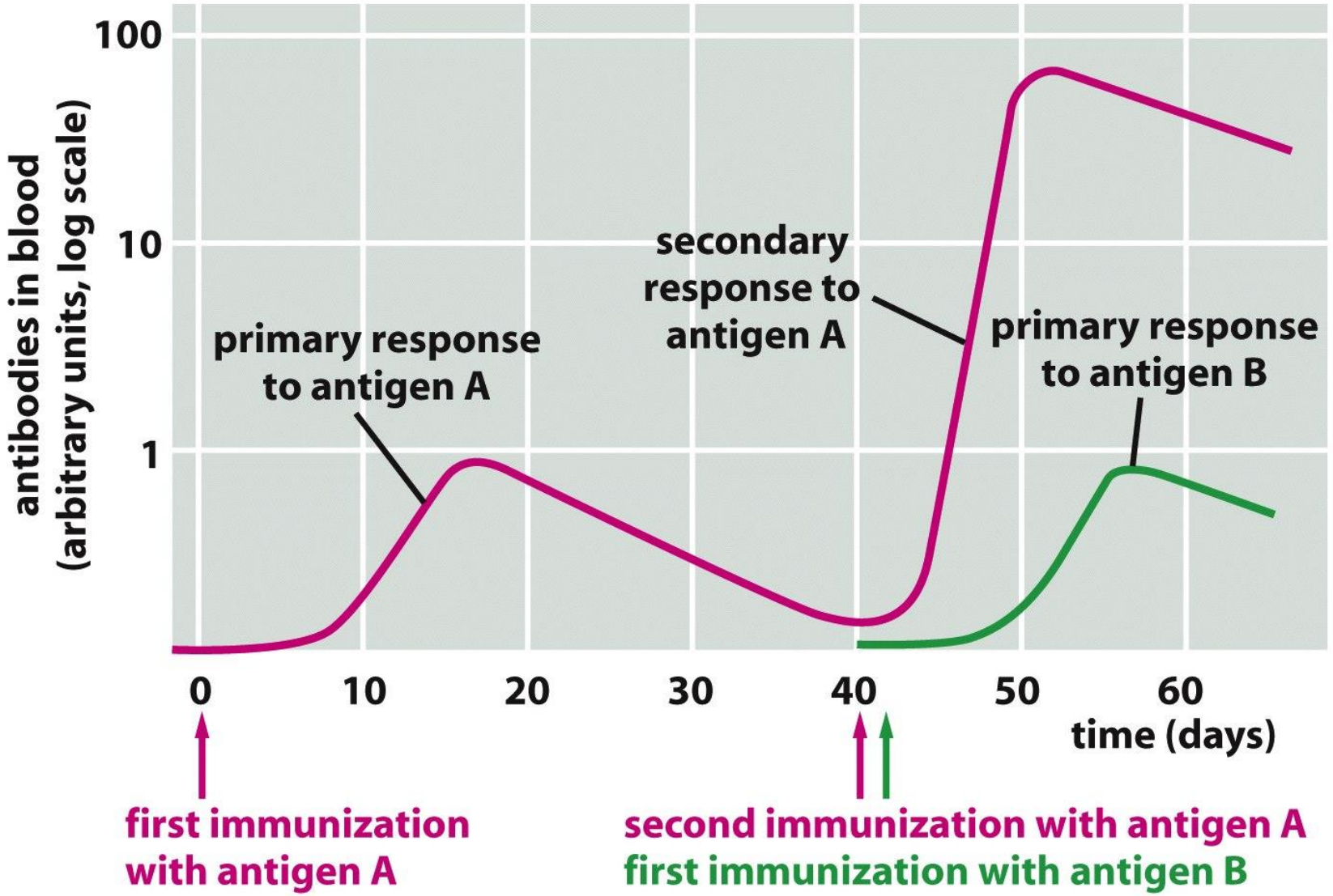


effector B cell (plasma cell)

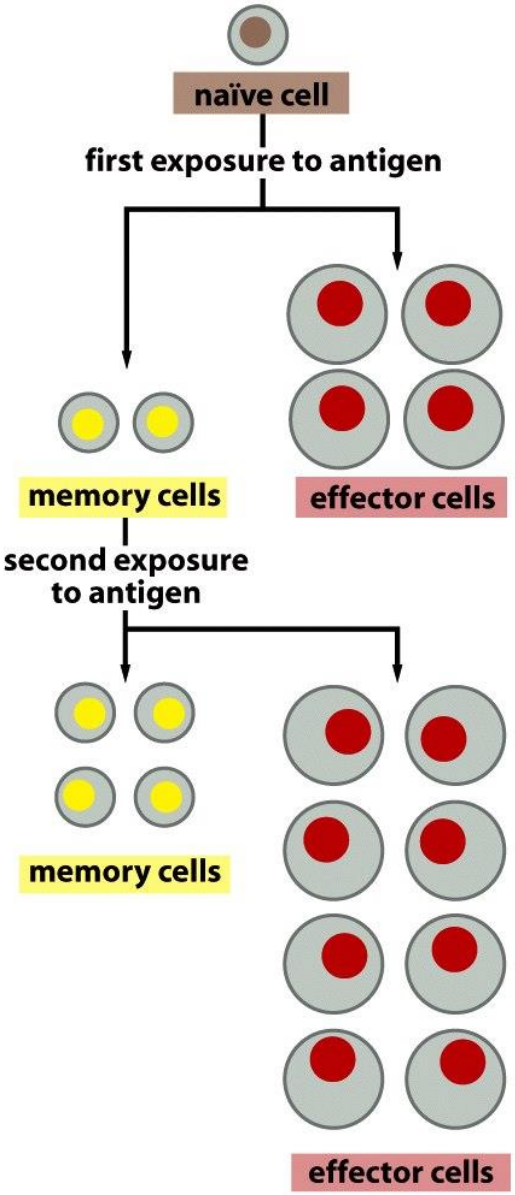
1 μm

=> production of antibodies
(500 antibody molecules/sec!)

Progress of immune response

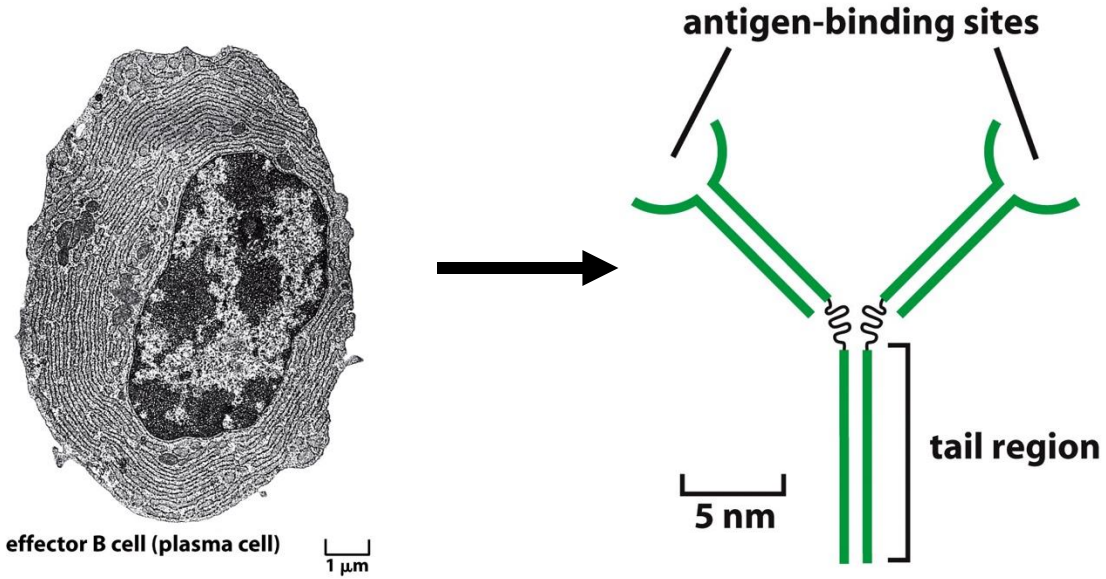


Immunological memory

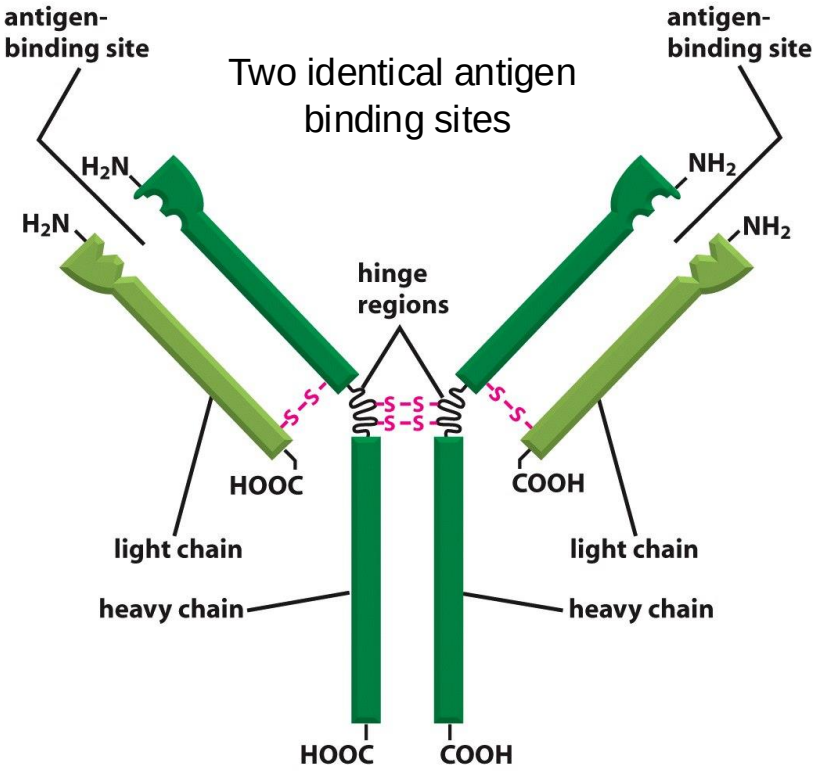


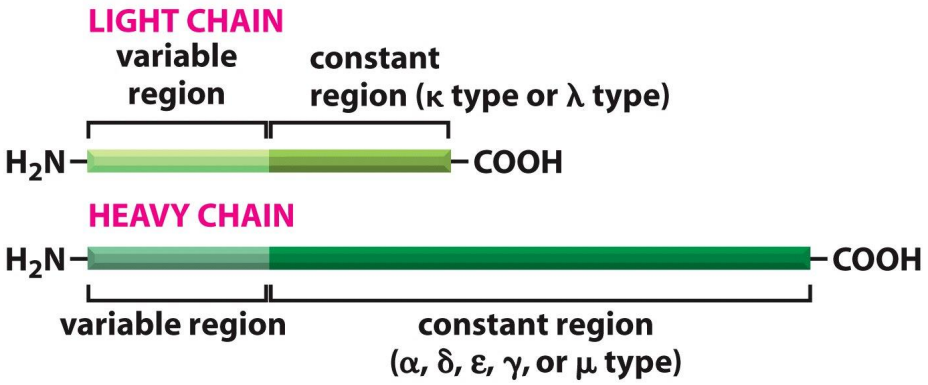
Vaccination!

B cells and antibodies

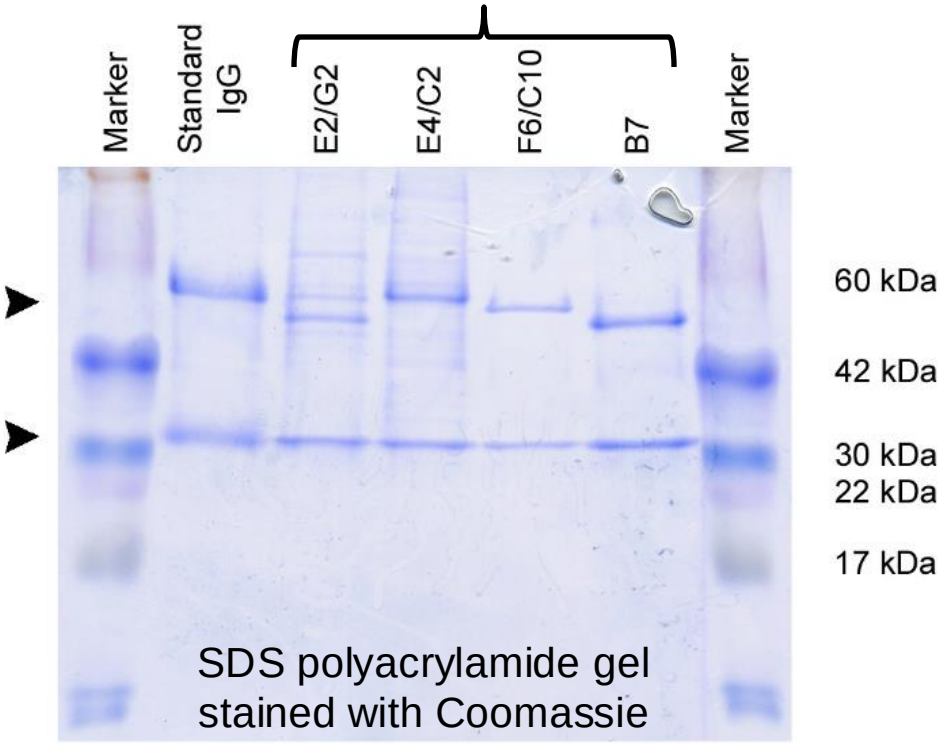


Structure of IgG





4 different IgG antibody clones against the same antigen



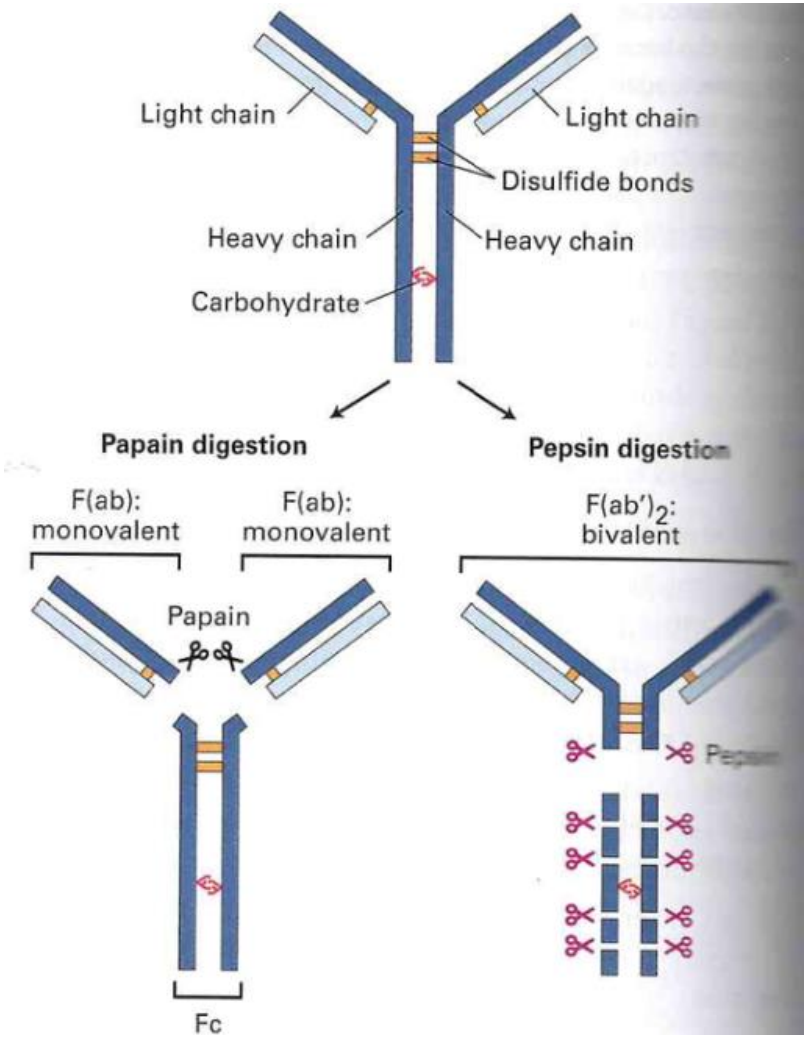
⇒ 2 light chains (25 kDa, light green)

⇒ 2 heavy chains (50 kDa, dark green)

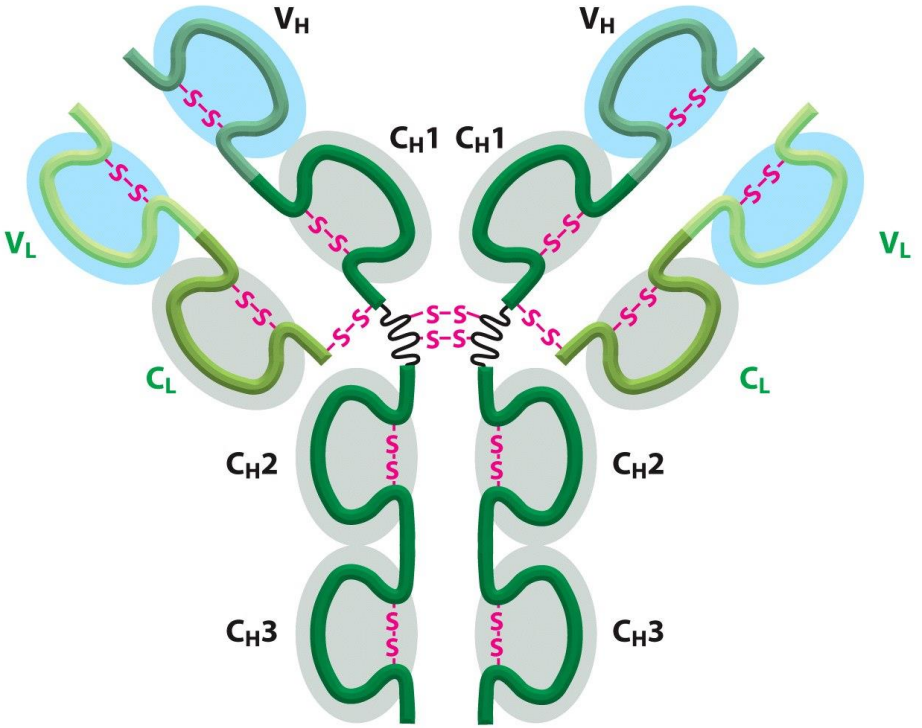
total mass: 150 kDa

Structure of IgG

Fragmentation of antibody

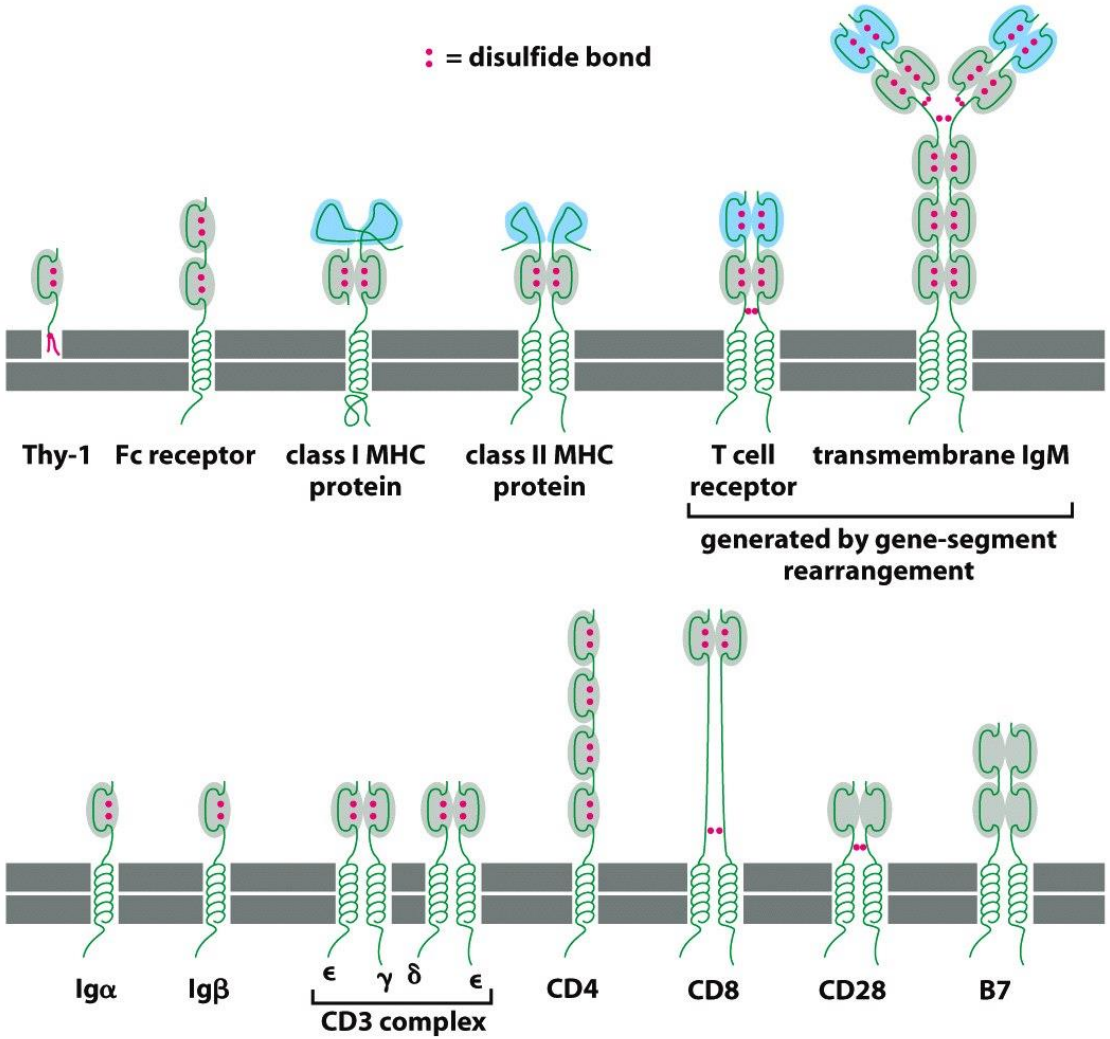


Immunoglobulin domains



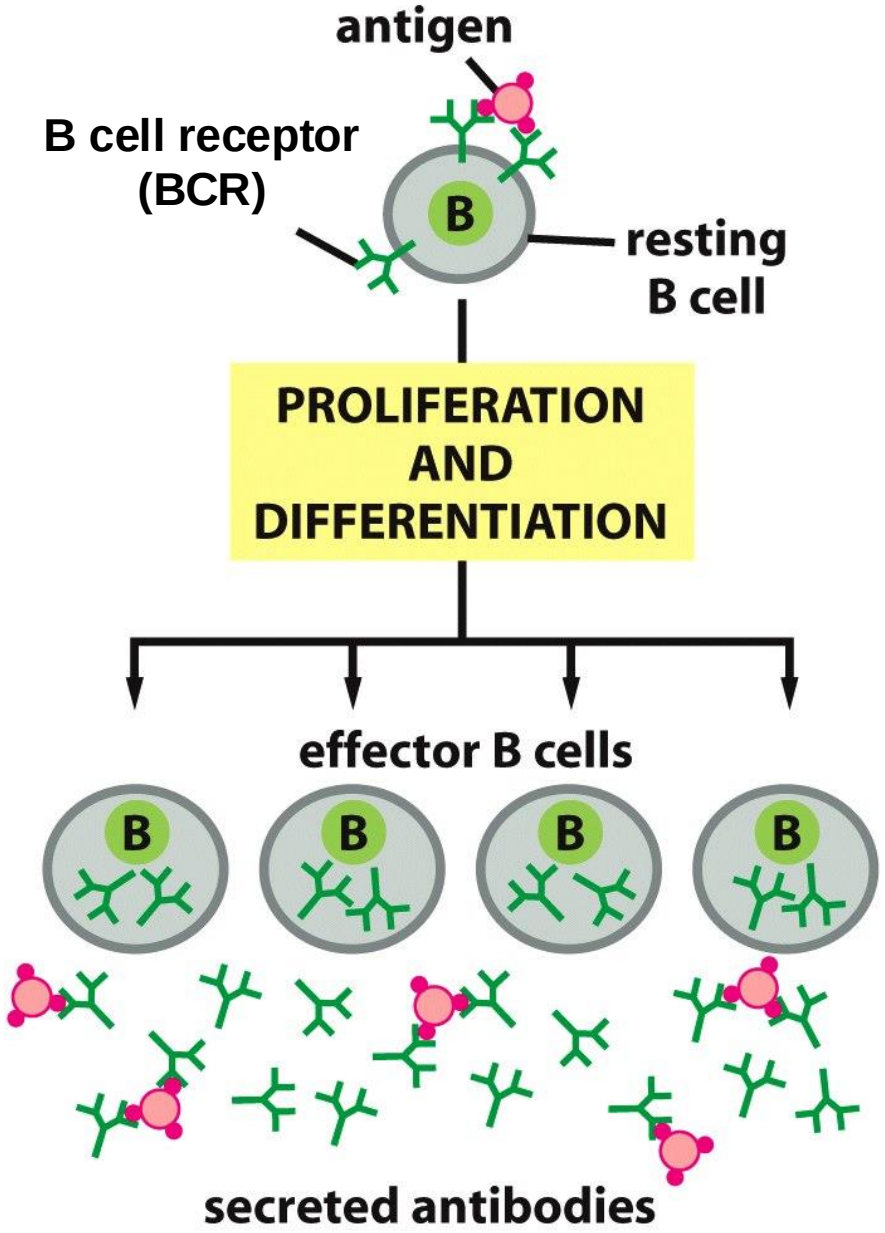
F: fragment
ab: antigen binding
c: crystallizable (constant)

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

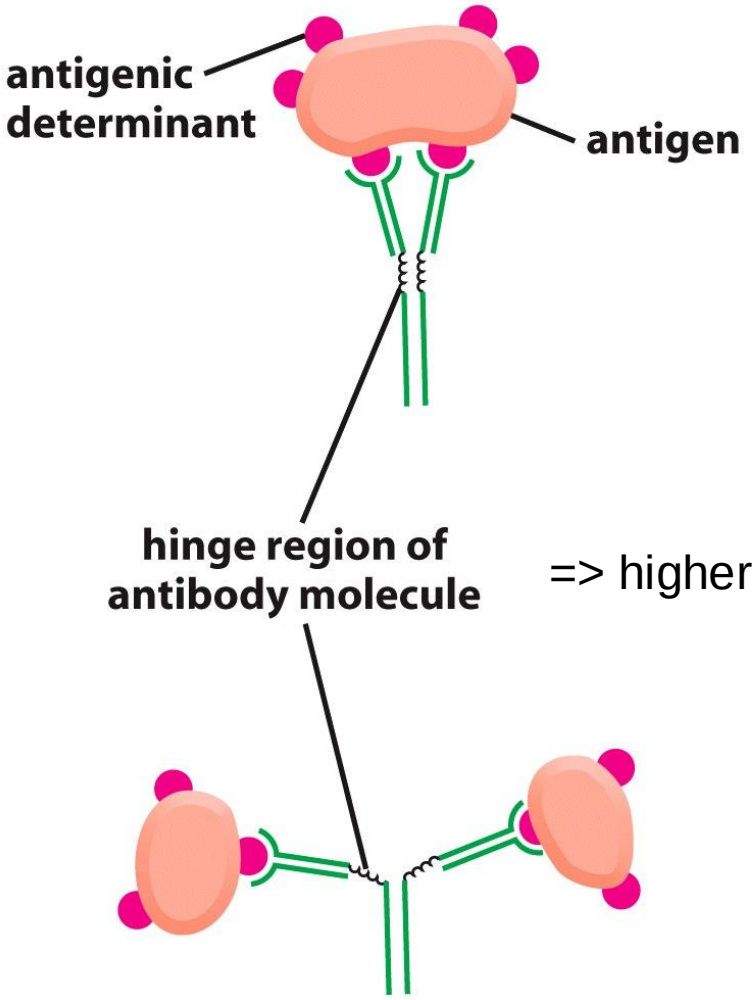


a single clone
of B cells

⬇

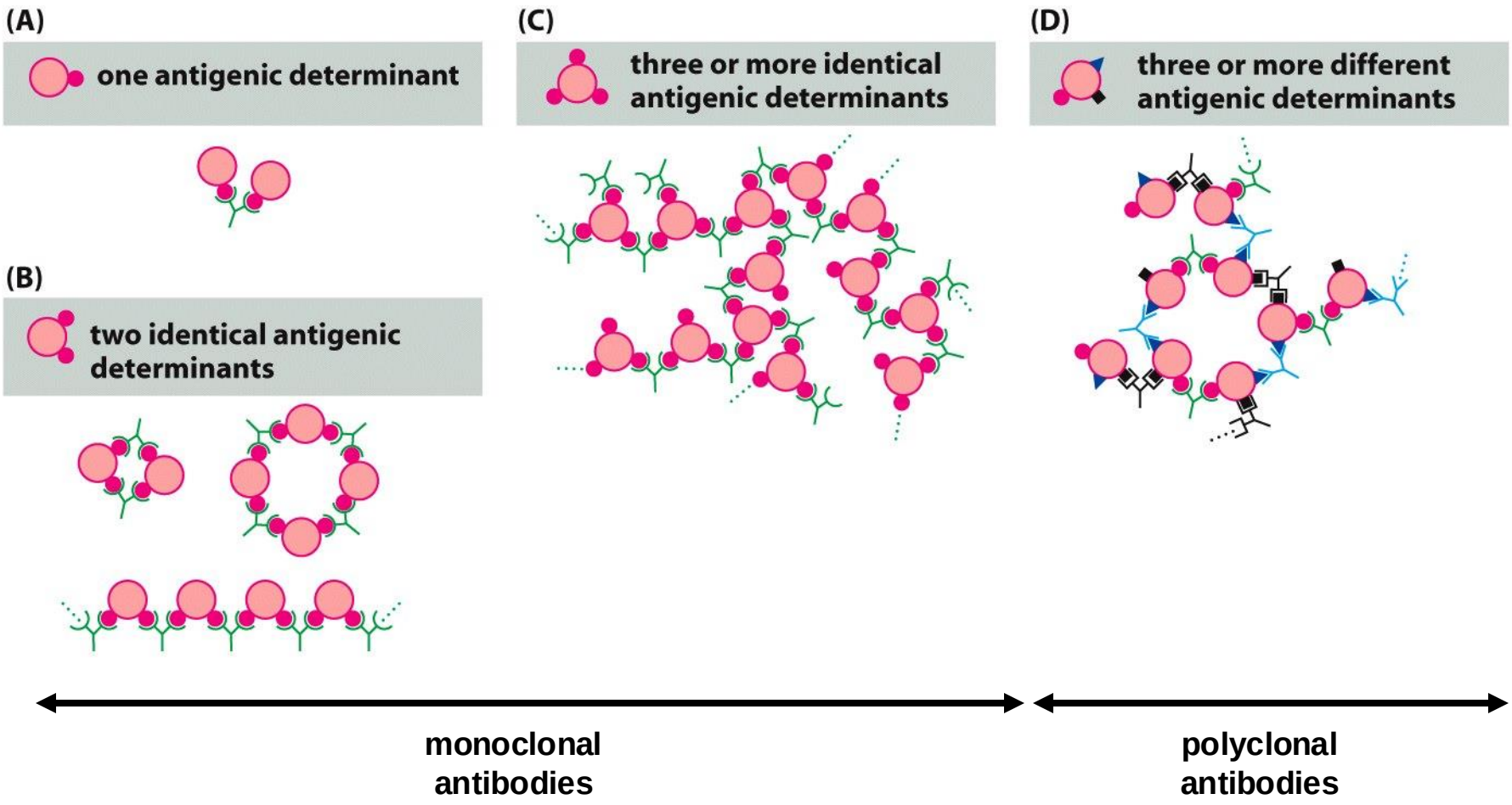
“monoclonal
antibodies“

The hinge region



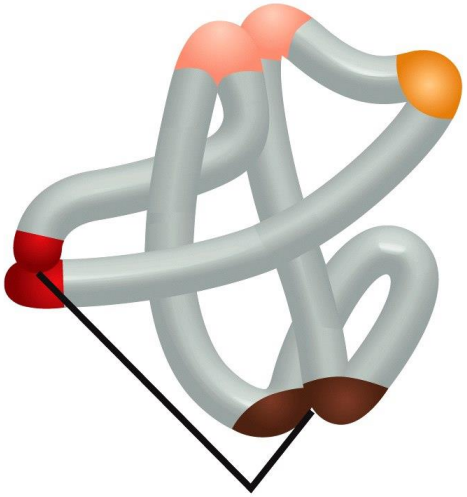
=> higher flexibility

Interactions of antibody and antigen



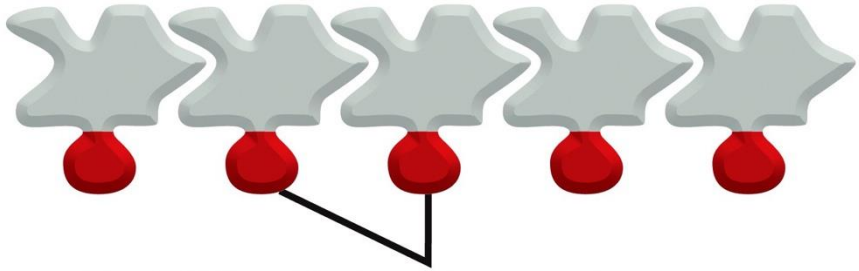
Multiple antigenic determinants: epitope

MULTIVALENT ANTIGEN



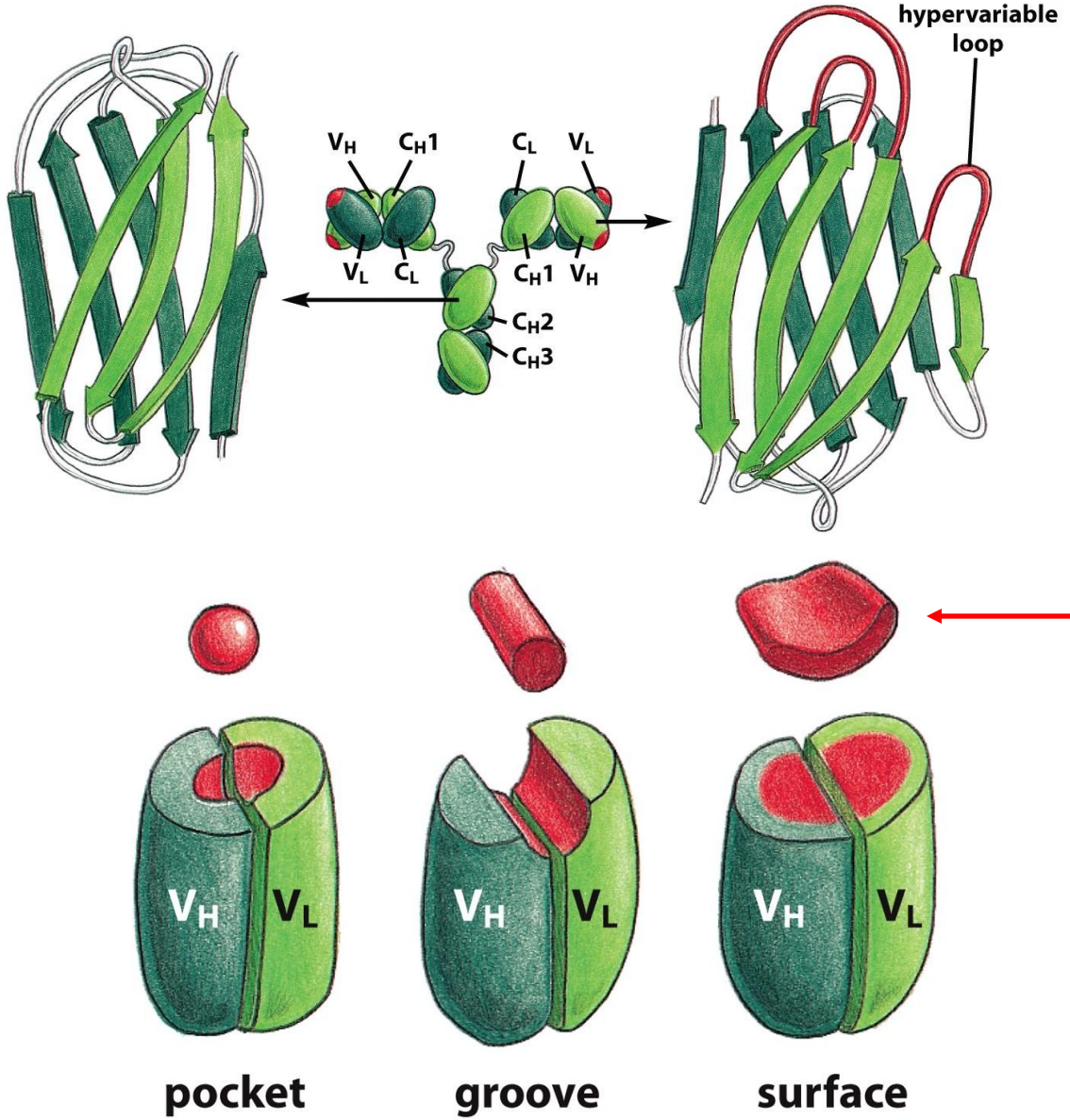
multiple **different** antigenic determinants

POLYVALENT ANTIGEN

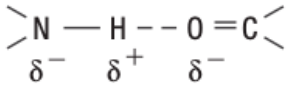
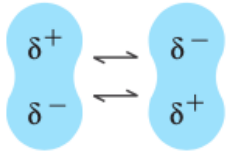
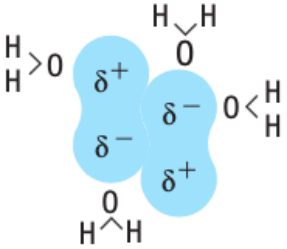
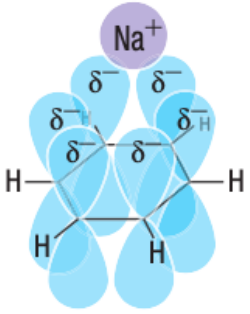


multiple **identical** antigenic determinants

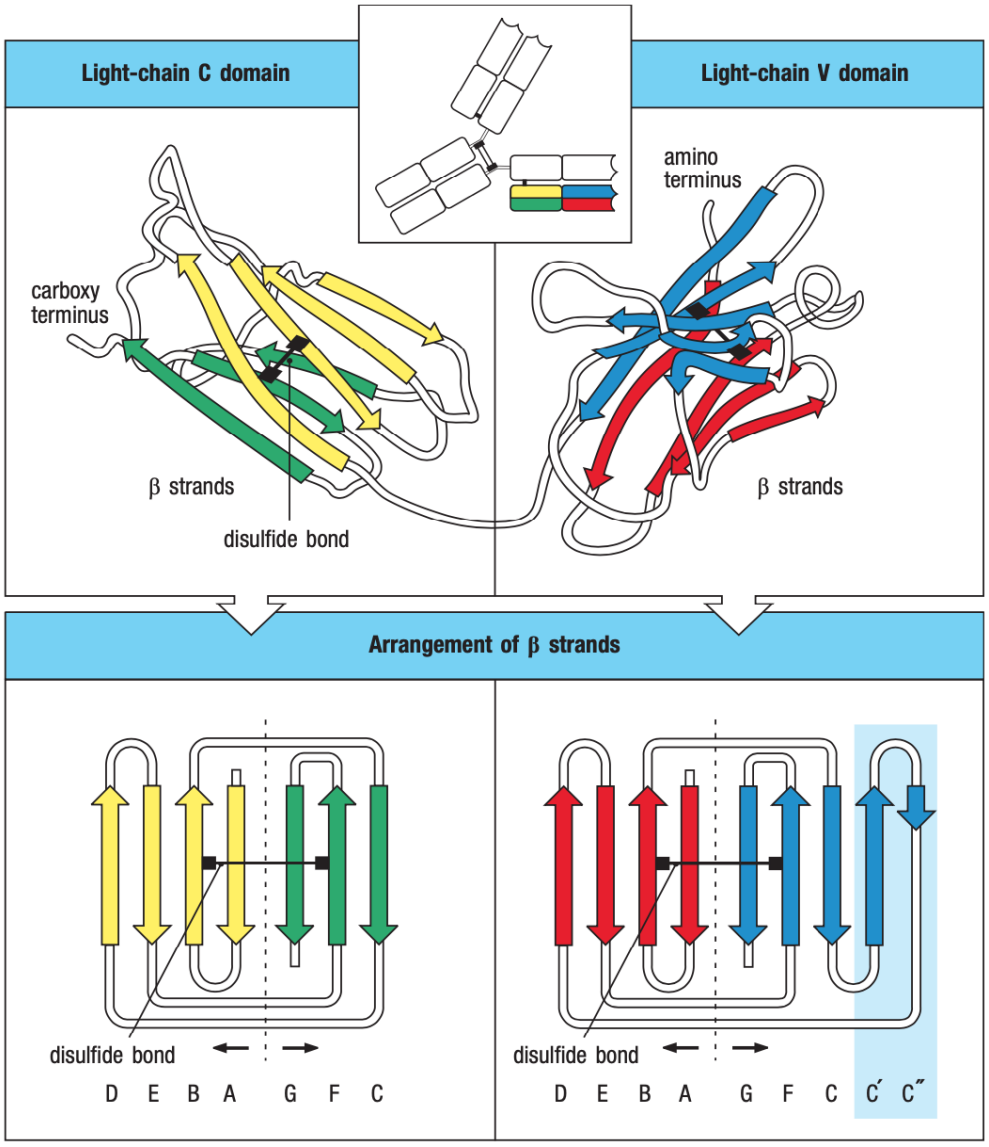
Antigen-binding sites of antibodies



Non-covalent binding forces [AgAb]

Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	
Hydrogen bonds	Hydrogen shared between electronegative atoms (N, O)	
Van der Waals forces	Fluctuations in electron clouds around molecules 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



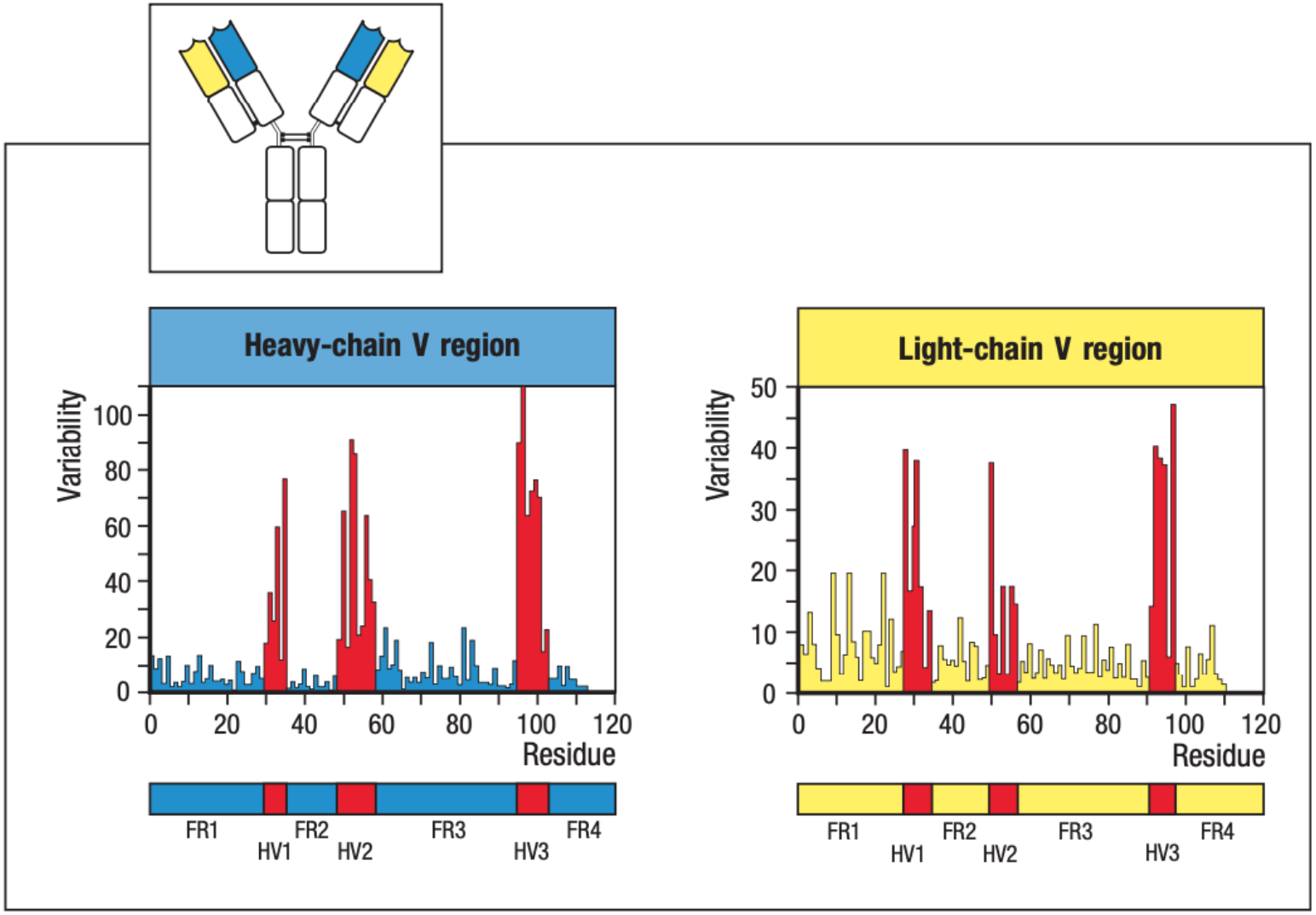
4 strand + 3 strand

4 strand + 5 strand

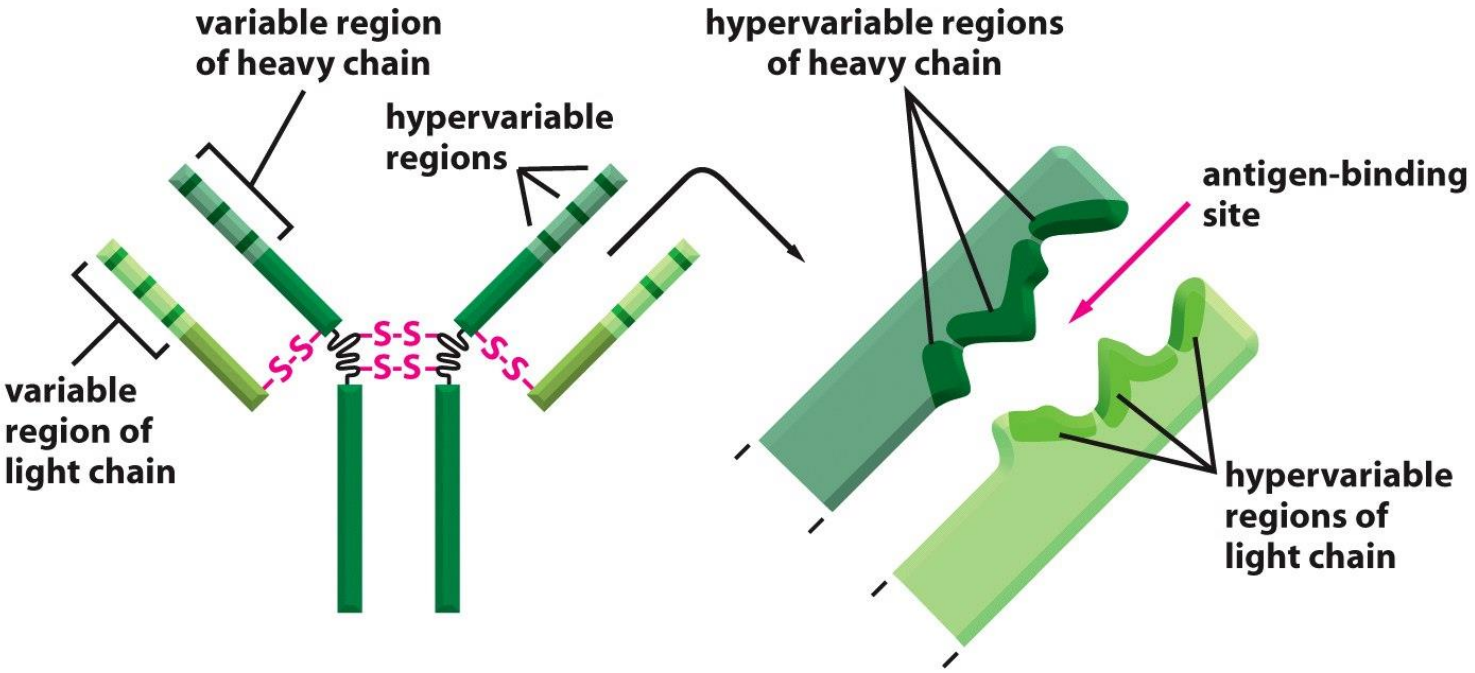
anti-parallel β sheets form a β barrel

C‘ and C“ are not present in the C region

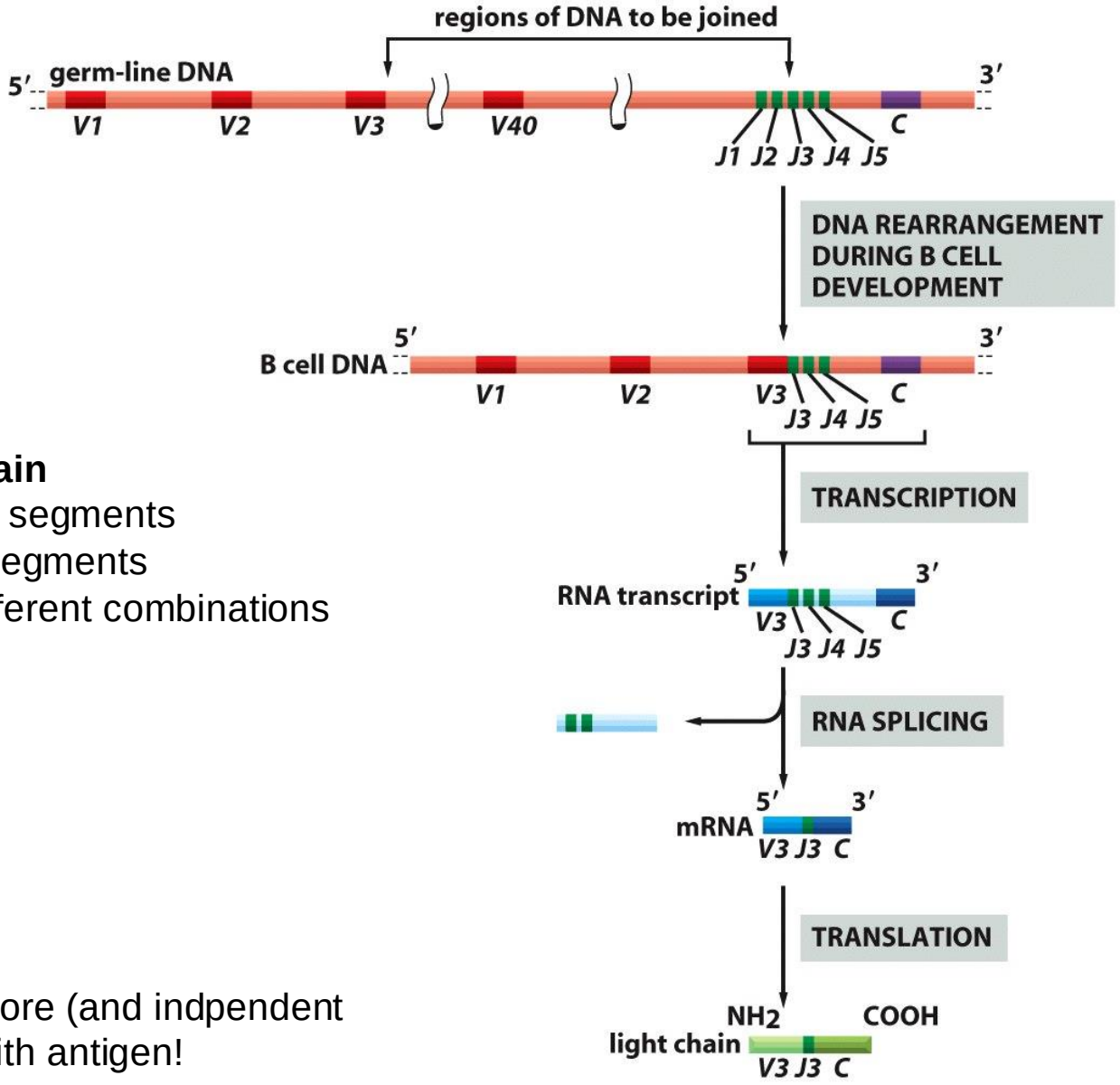
Hypervariable regions of binding sites



Hypervariable regions of binding sites



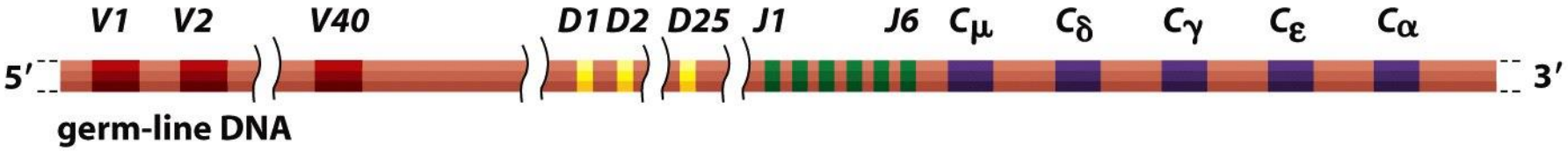
Generation of antibody diversity: light chain



κ light chain
40 V gene segments
5 J gene segments
=> 200 different combinations

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

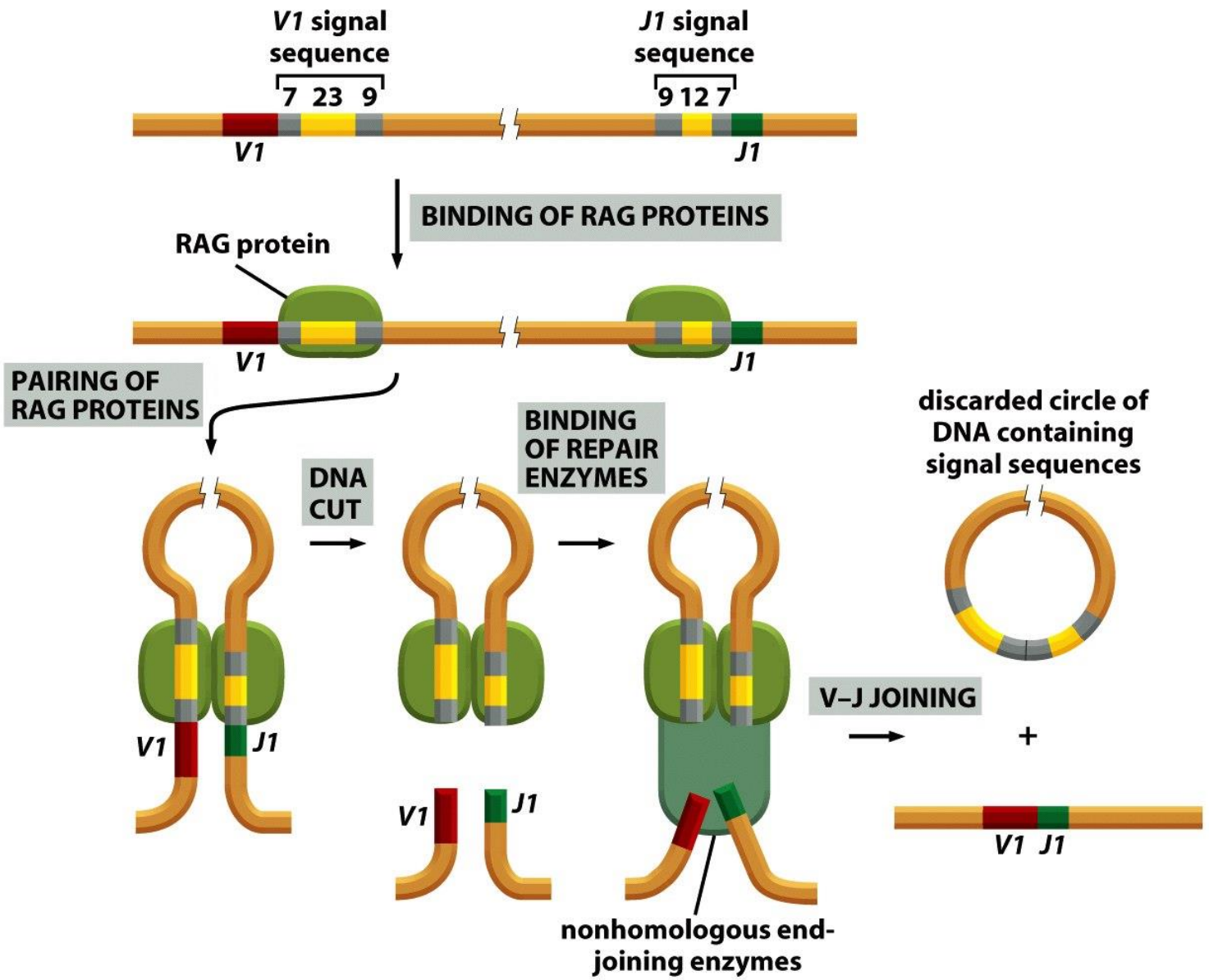
Generation of antibody diversity: heavy chain



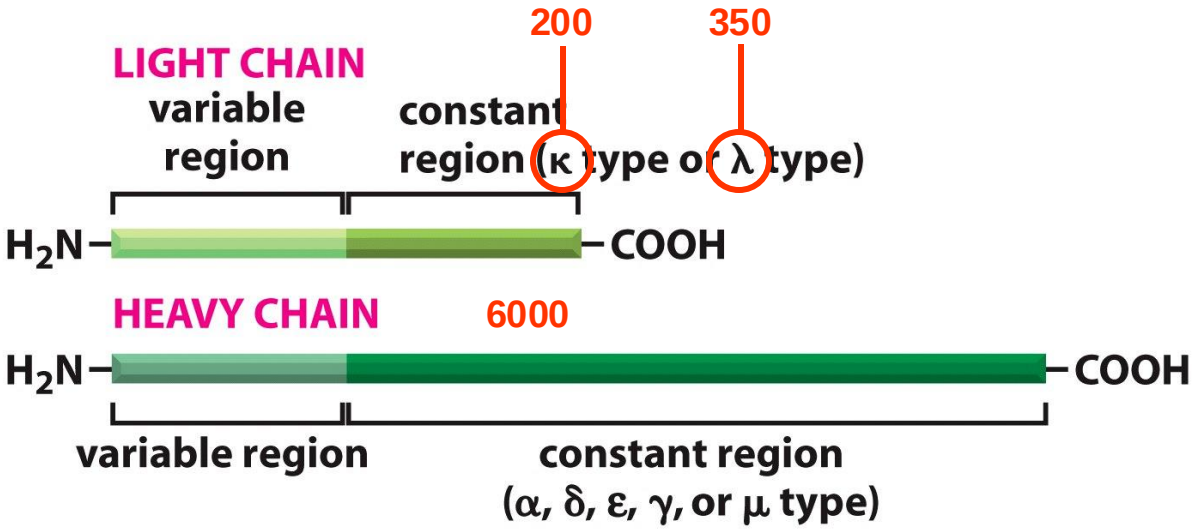
heavy chain
40 V gene segments
25 D gene segments
6 J gene segments

=> 6000 combinations

Gene segment joining



Generation of antibody diversity

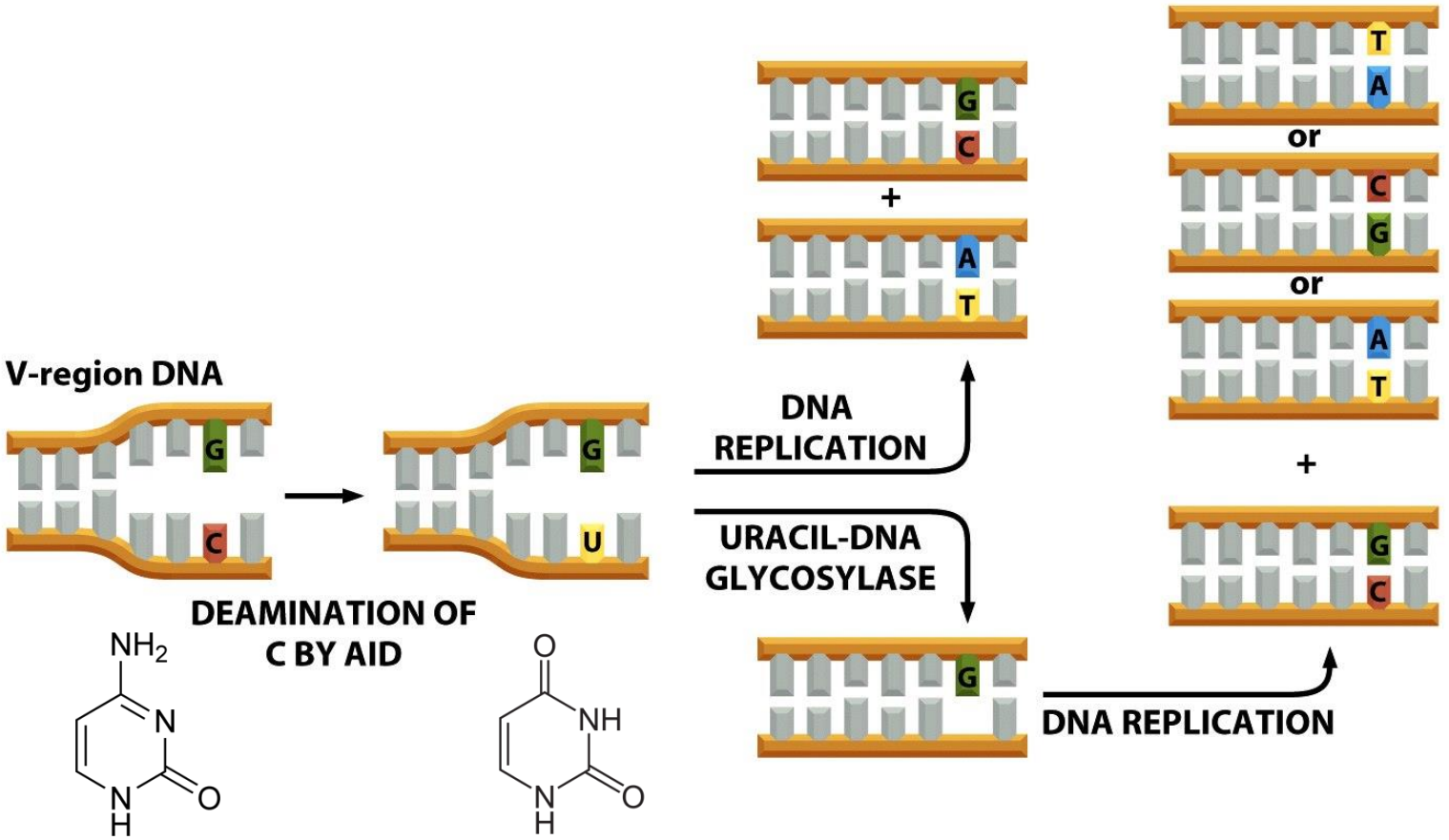


=> about 2.000.000 combinations

Affinity maturation of antibodies

Somatic hypermutation by activity-induced deaminase (AID)

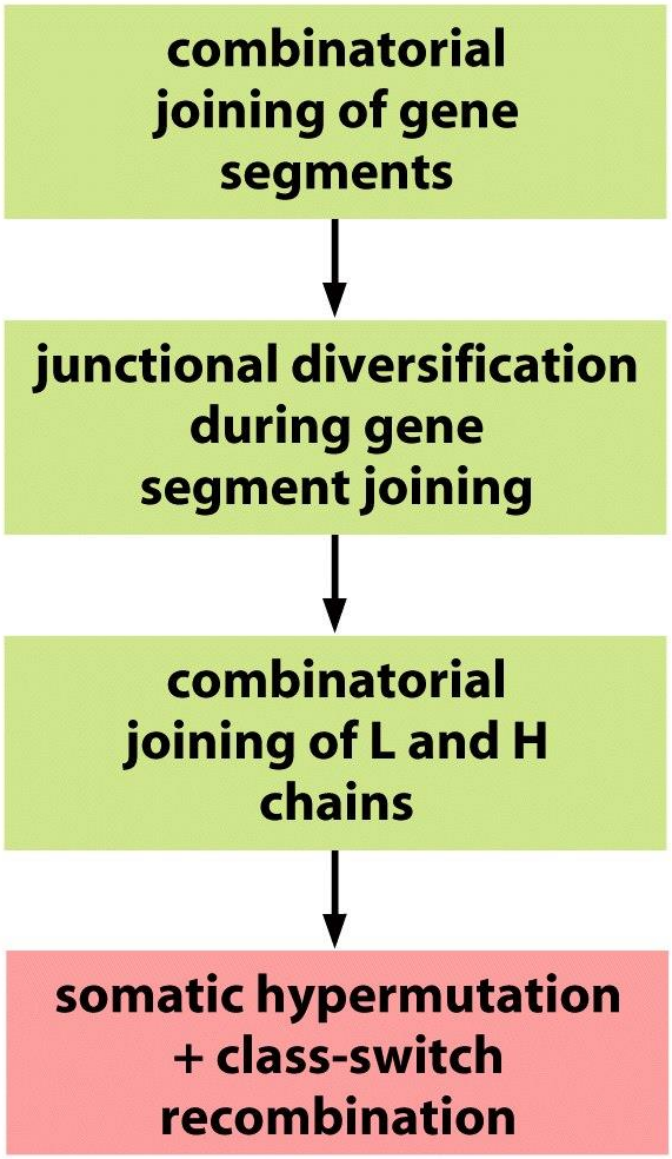
=> 1 mutation per V region per cell cycle



Cytosin:
forms 3 hydrogen bonds

Uracil:
forms 2 hydrogen bonds

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 $M^{-1}s^{-1}$
=> controlled by diffusion

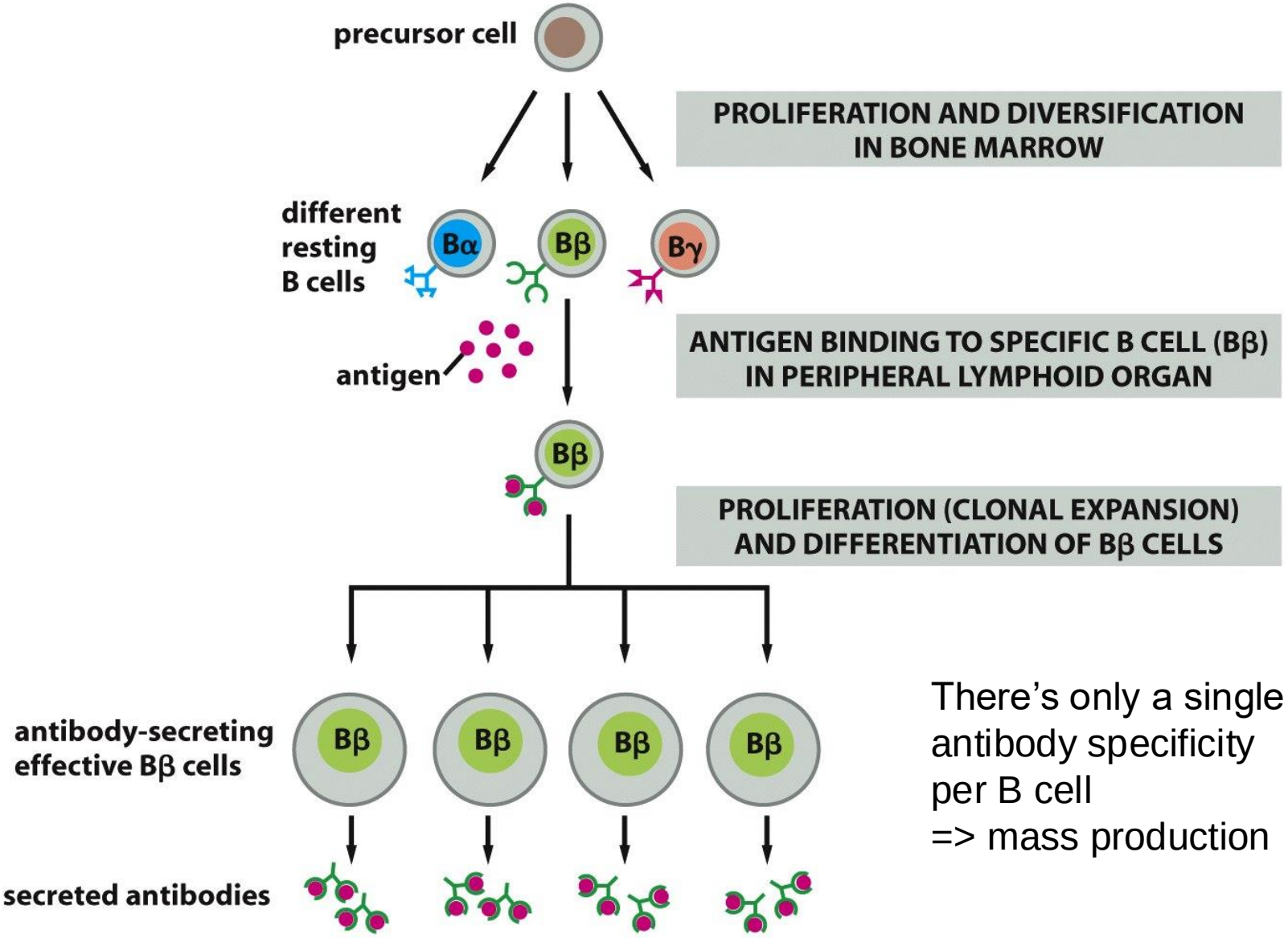
Release rate k_{off}: 10^{-3}-10^{-4} s^{-1}
=> controlled by time for signal transduction/endocytosis
after antigen binding to cell surface receptors

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

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

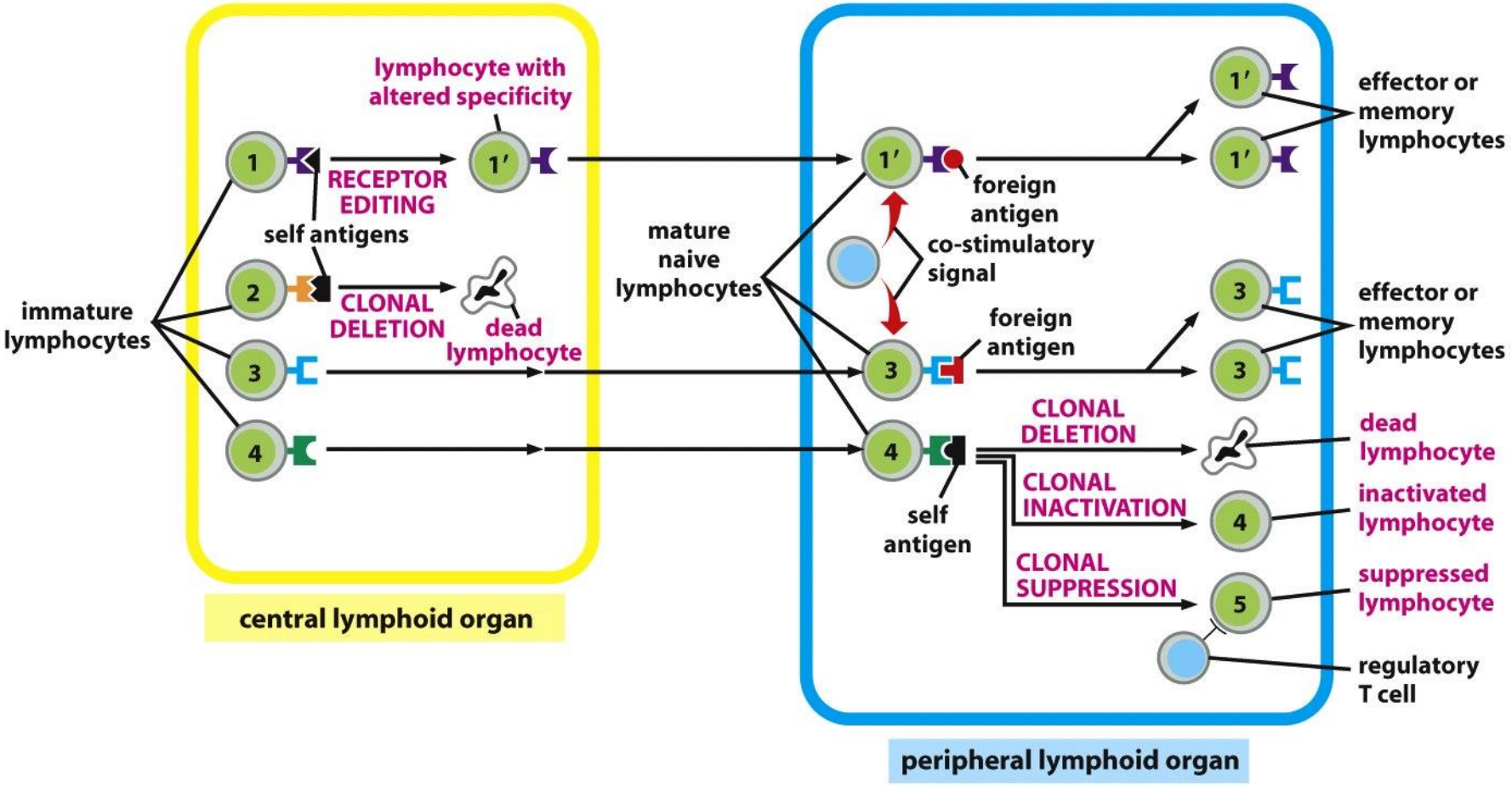
*for comparison: biotin-strepatividin: $K_a = 10^{14}$ M^{-1}

Clonal selection theory



There's only a single antibody specificity per B cell
=> mass production

Immunological tolerance



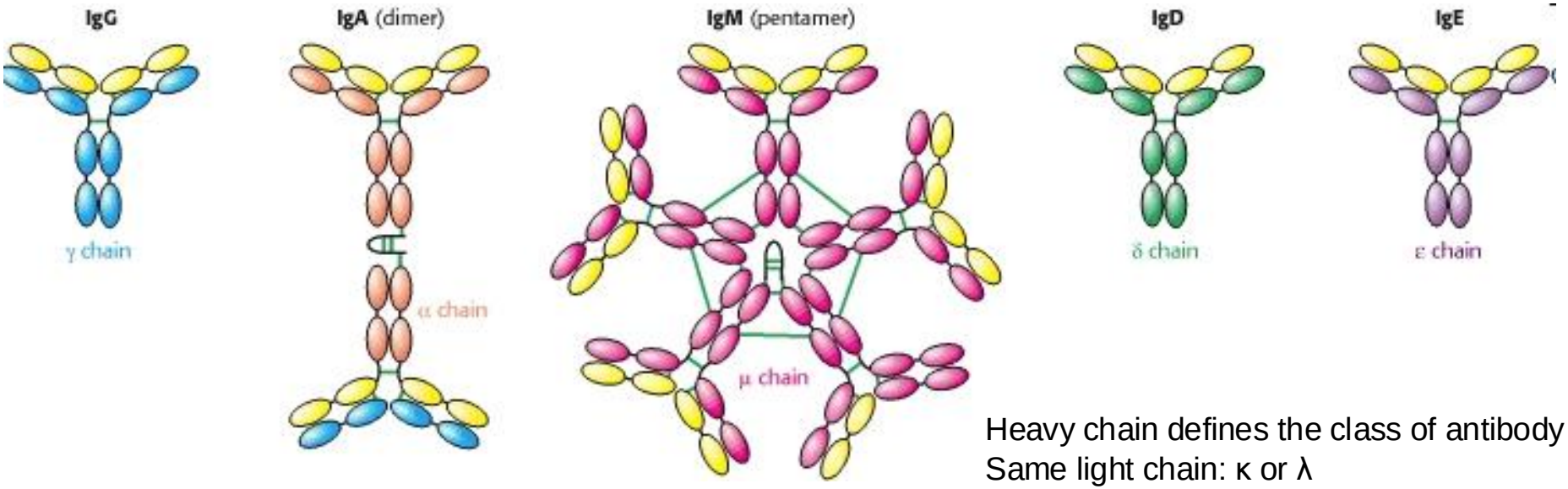
But this system is not perfect: **autoimmune diseases**
e.g.: Eppstein-Barr virus is suspected to induce multiple sclerosis

Theoretical considerations of antigen recognition

Innate immune response:
=> Elimination of everything
that is recognized as foreign

Adaptive immune response:
=> Elimination of anything
that is ***not*** recognized as ***own***

Antibody classes



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

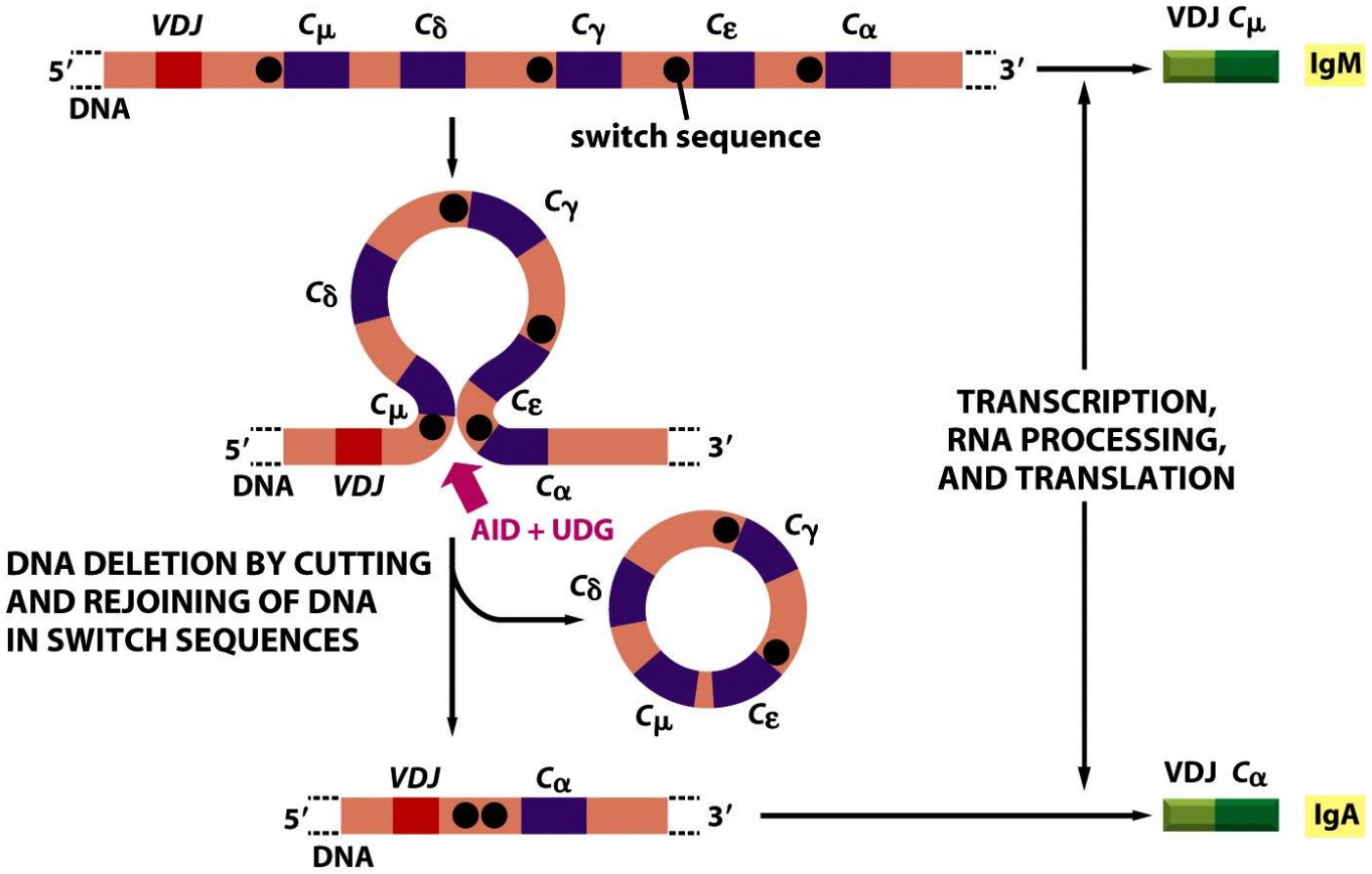
PROPERTIES	CLASS OF ANTIBODY				
	IgM	IgD	IgG	IgA	IgE
Heavy chains	μ	δ	γ	α	ε
Light chains	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Number of four-chain units	5	1	1	1 or 2	1
Percentage of total Ig in blood	10	<1	75	15	<1
Activates complement	++++	–	++	–	–
Crosses placenta	–	–	+	–	–
Binds to macrophages and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

primary | secondary

classes of antibody

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

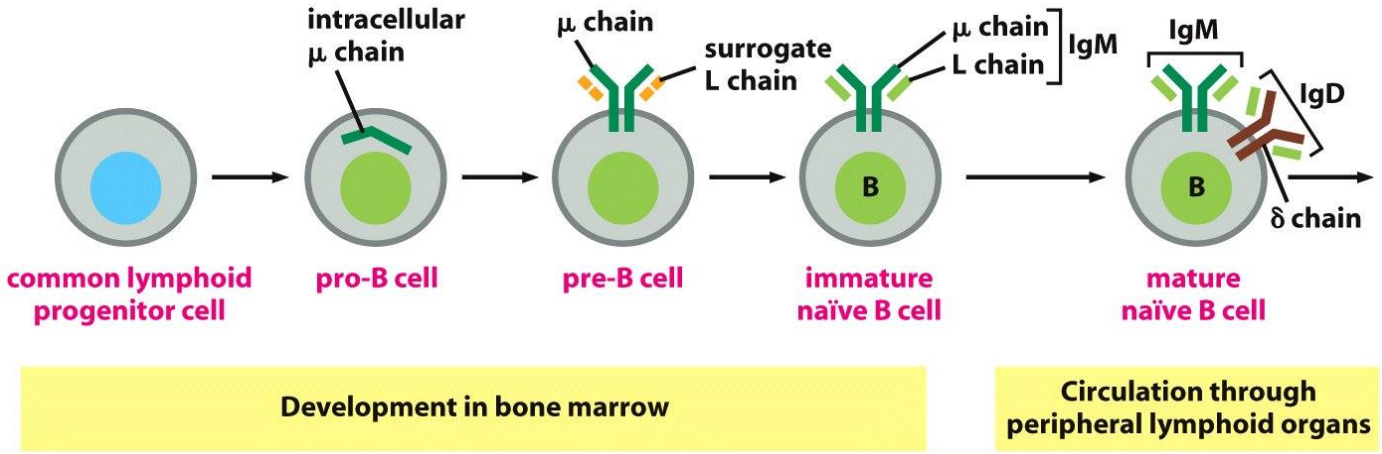
Class switch mediated by DNA rearrangement



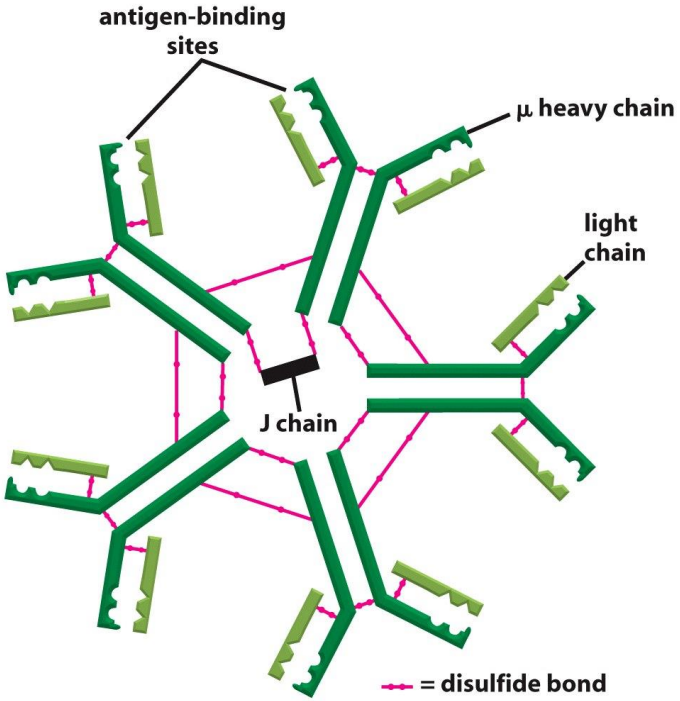
Class switch DNA recombination (not splicing!) => irreversible
depends on switch sequences (consisting of tandem repeats) and
the enyzmes activiation indcued deaminase (AID) + uracil-DNA glycosylase (UDG)

IgM: First antibody class

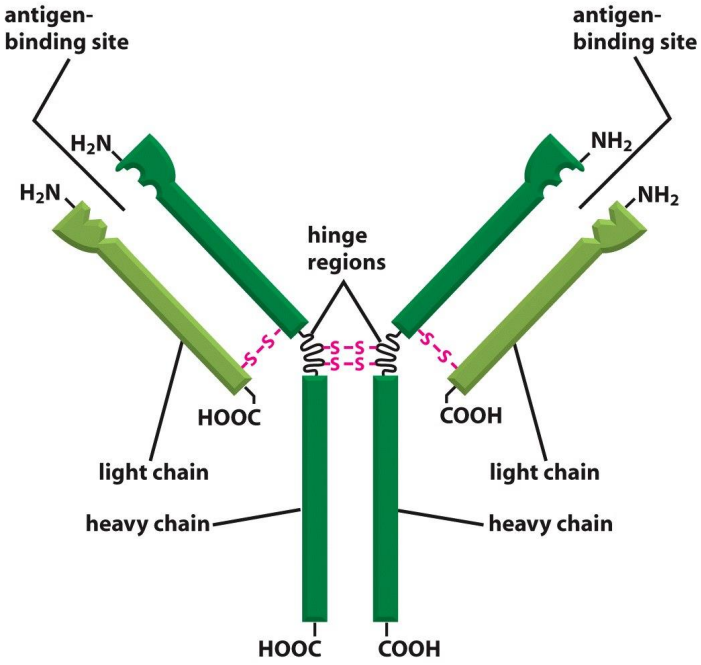
on cell surface

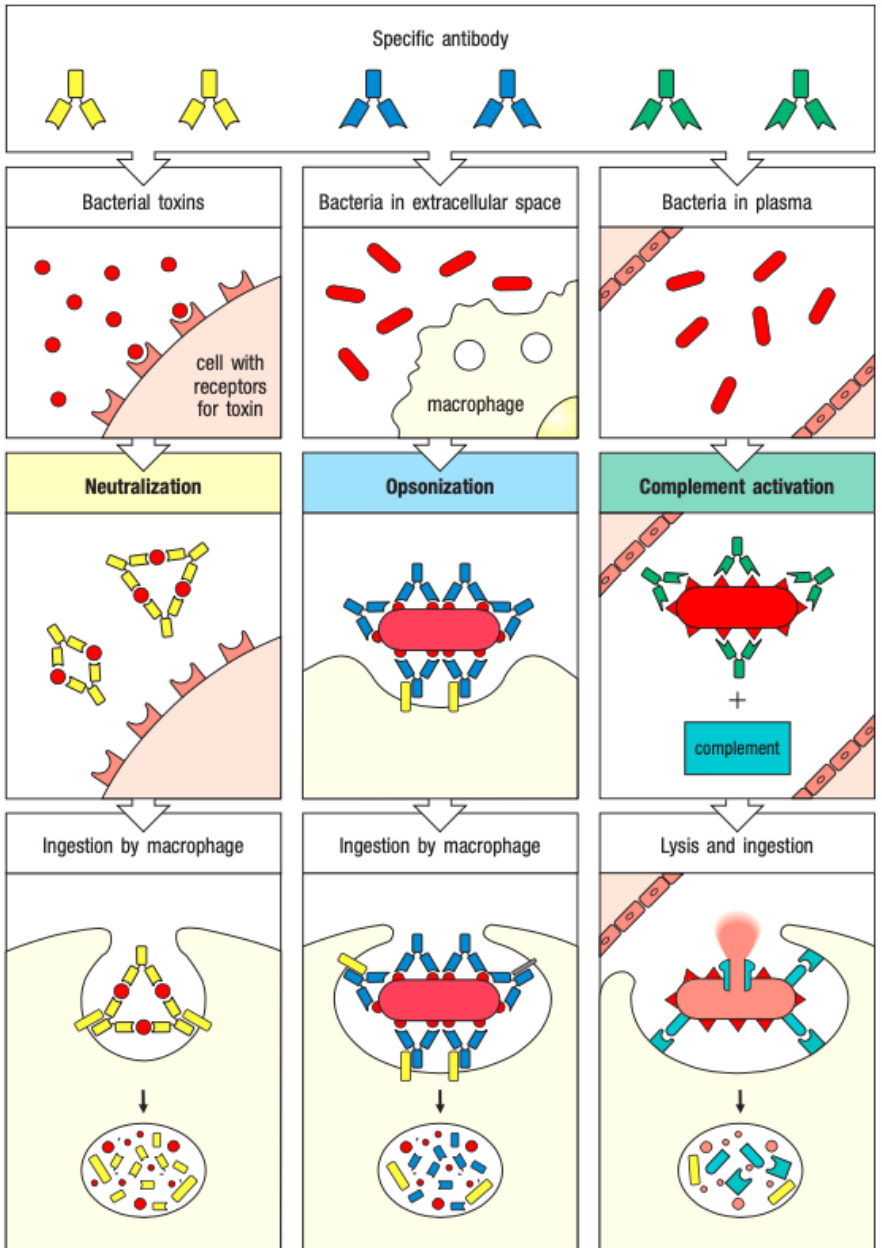


circulating in blood

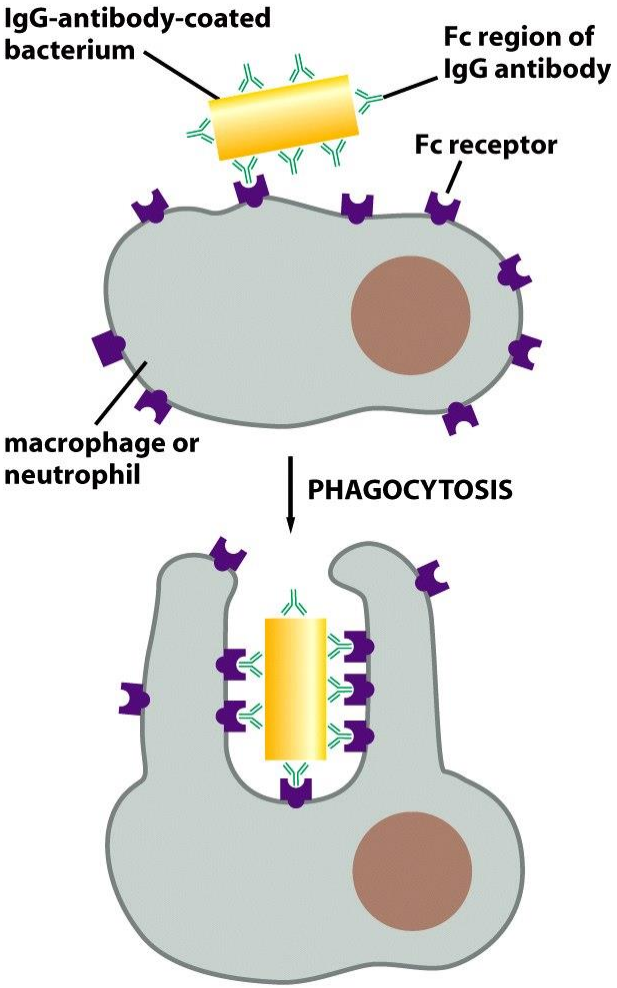


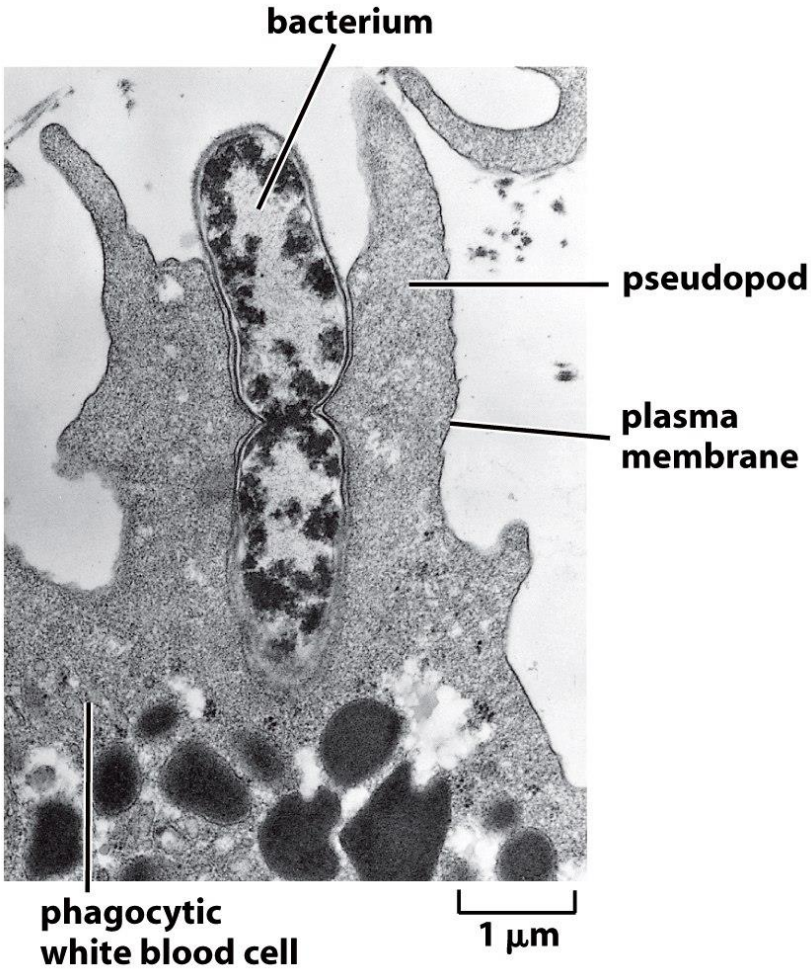
IgG: Main class in blood





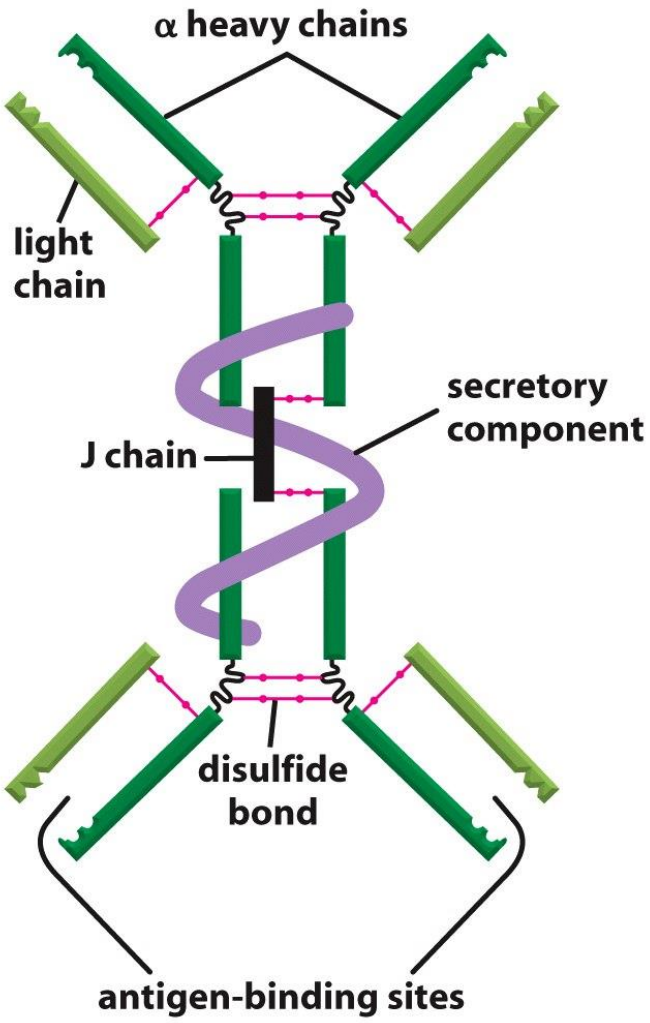
Opsonization



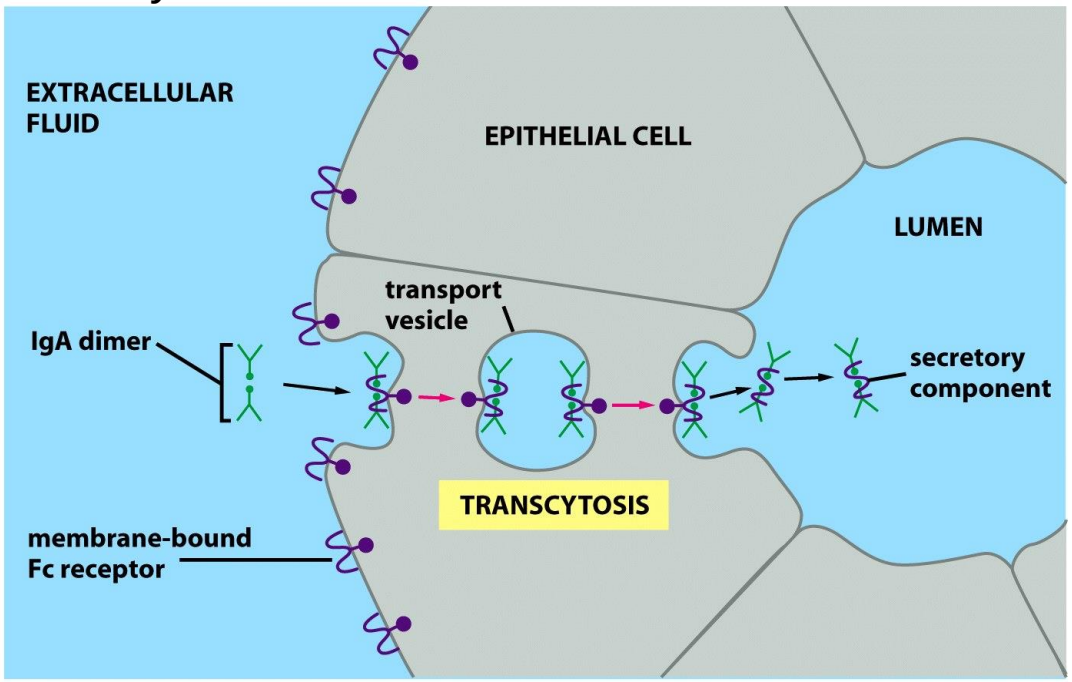


TEM image

IgA: Defence of mucosal surfaces

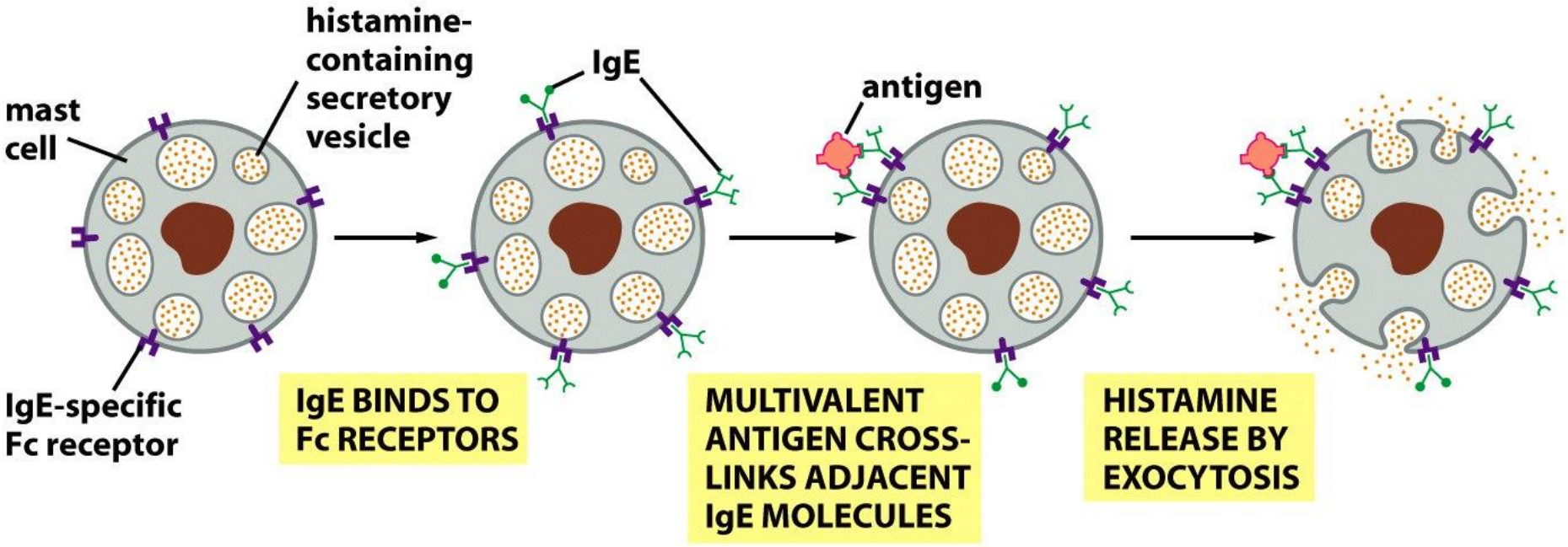


Transcytosis

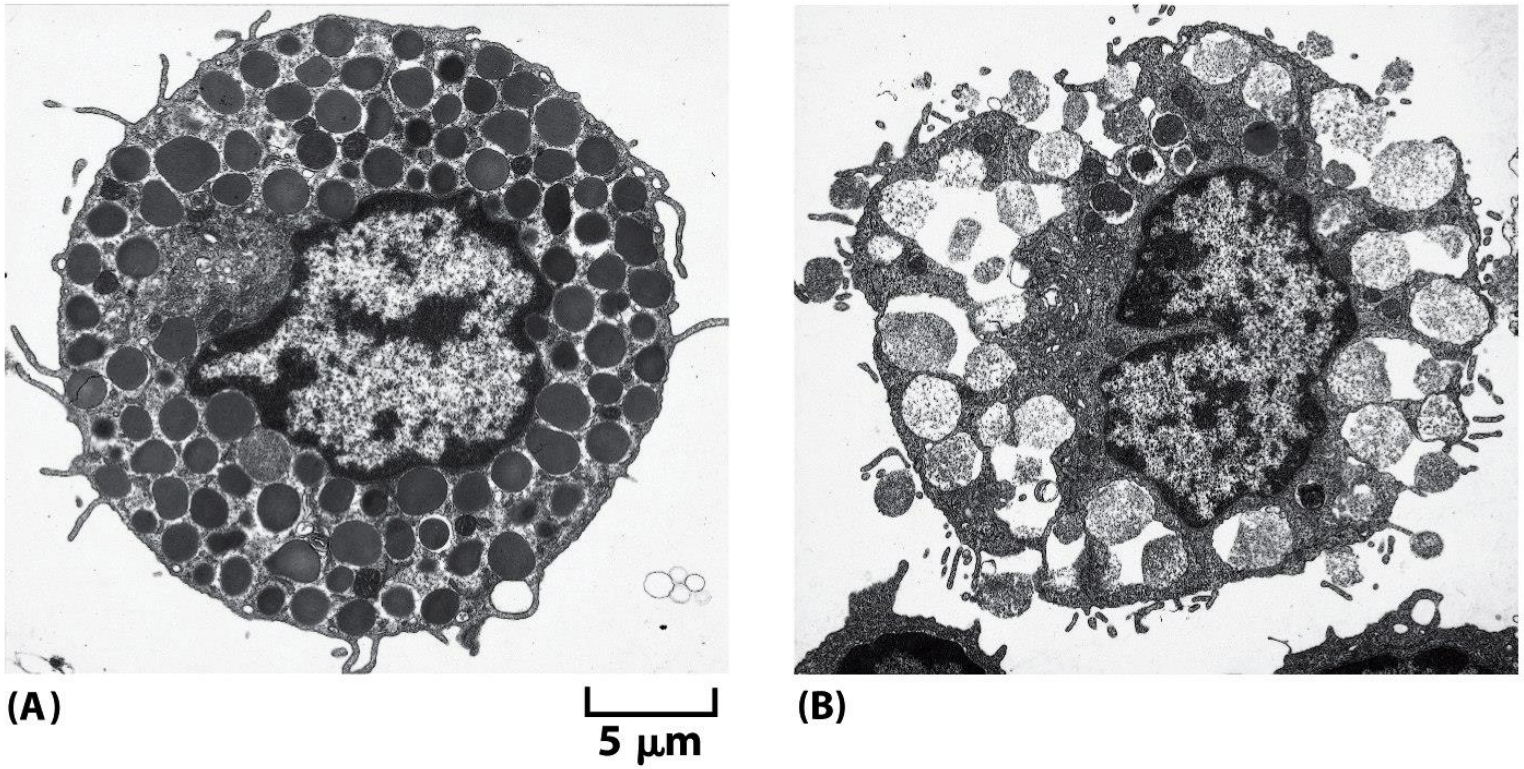


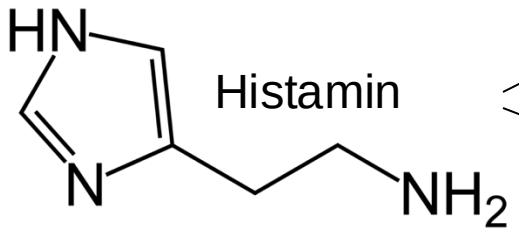
⇒ Similar mechanism of *IgG* transcytosis across the placenta to protect the fetus

IgE: Protection against large parasites



Release of histamin by mast cells



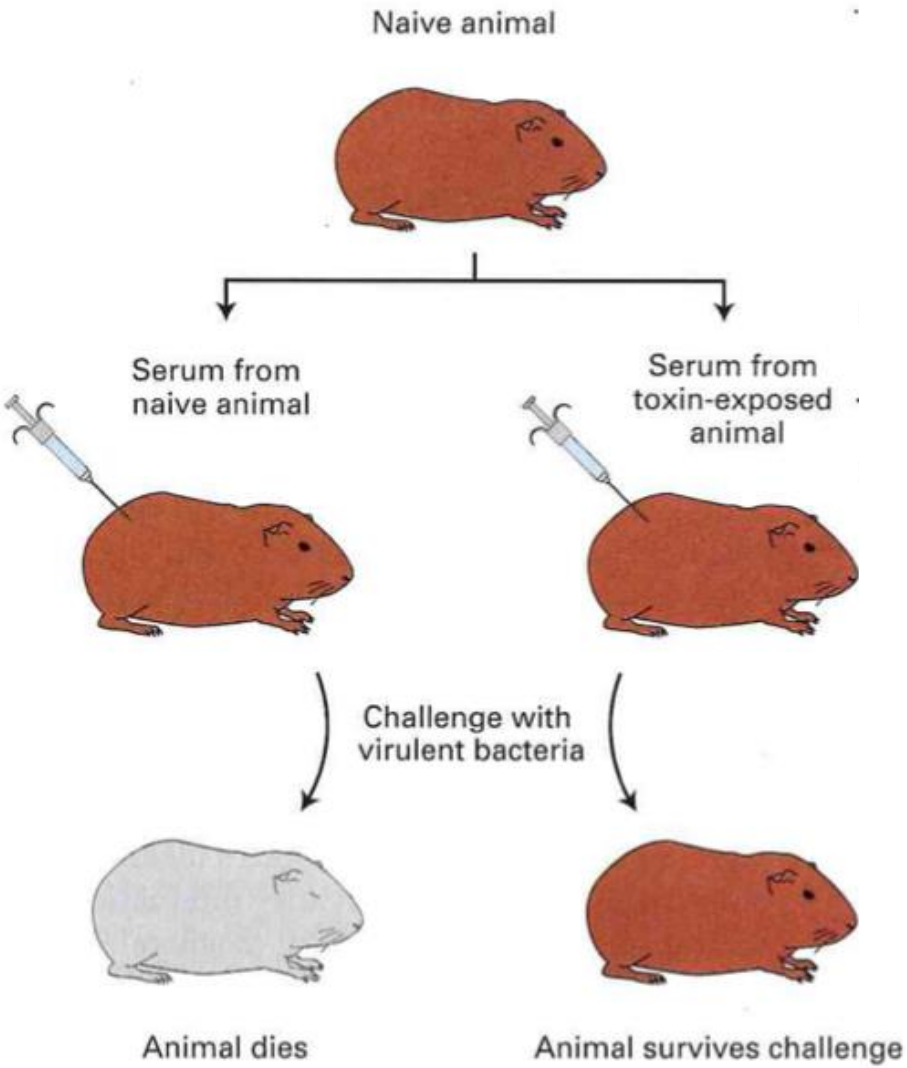


Histamin

→ Protection against parasites

→ Allergies

Classic experiment

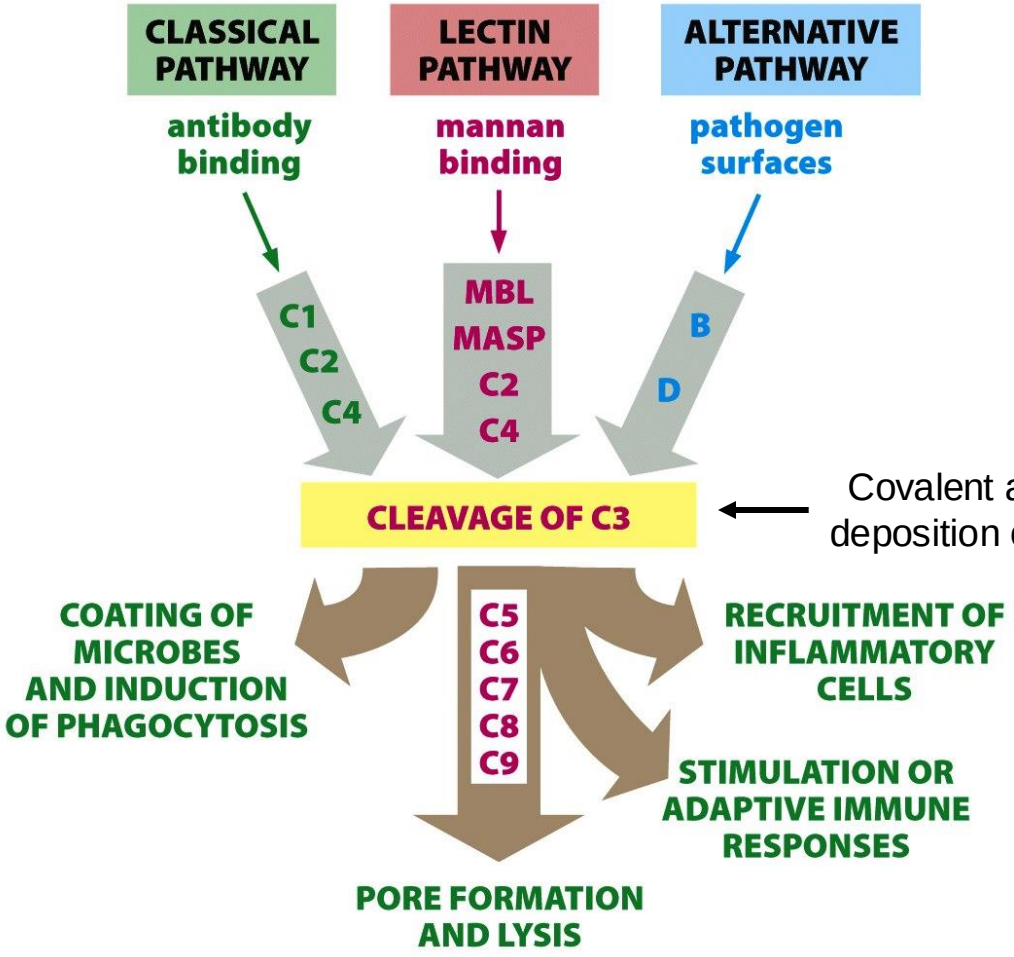


How can this be explained?

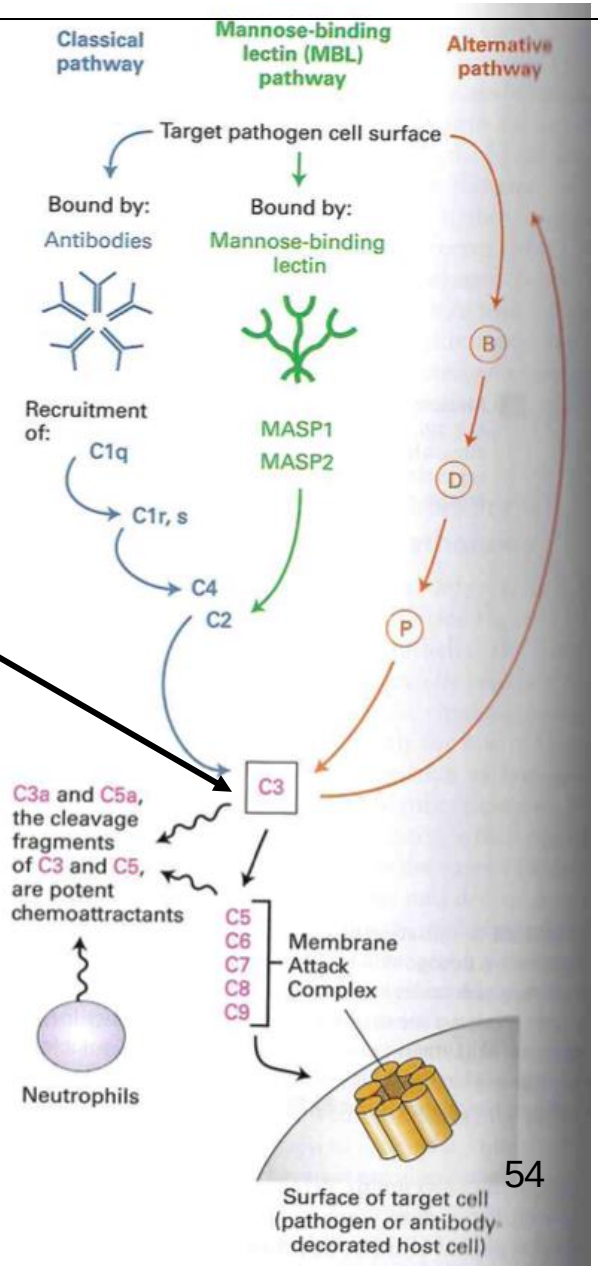
=> Behring/Kitasato (ca. 1890)

Complement system

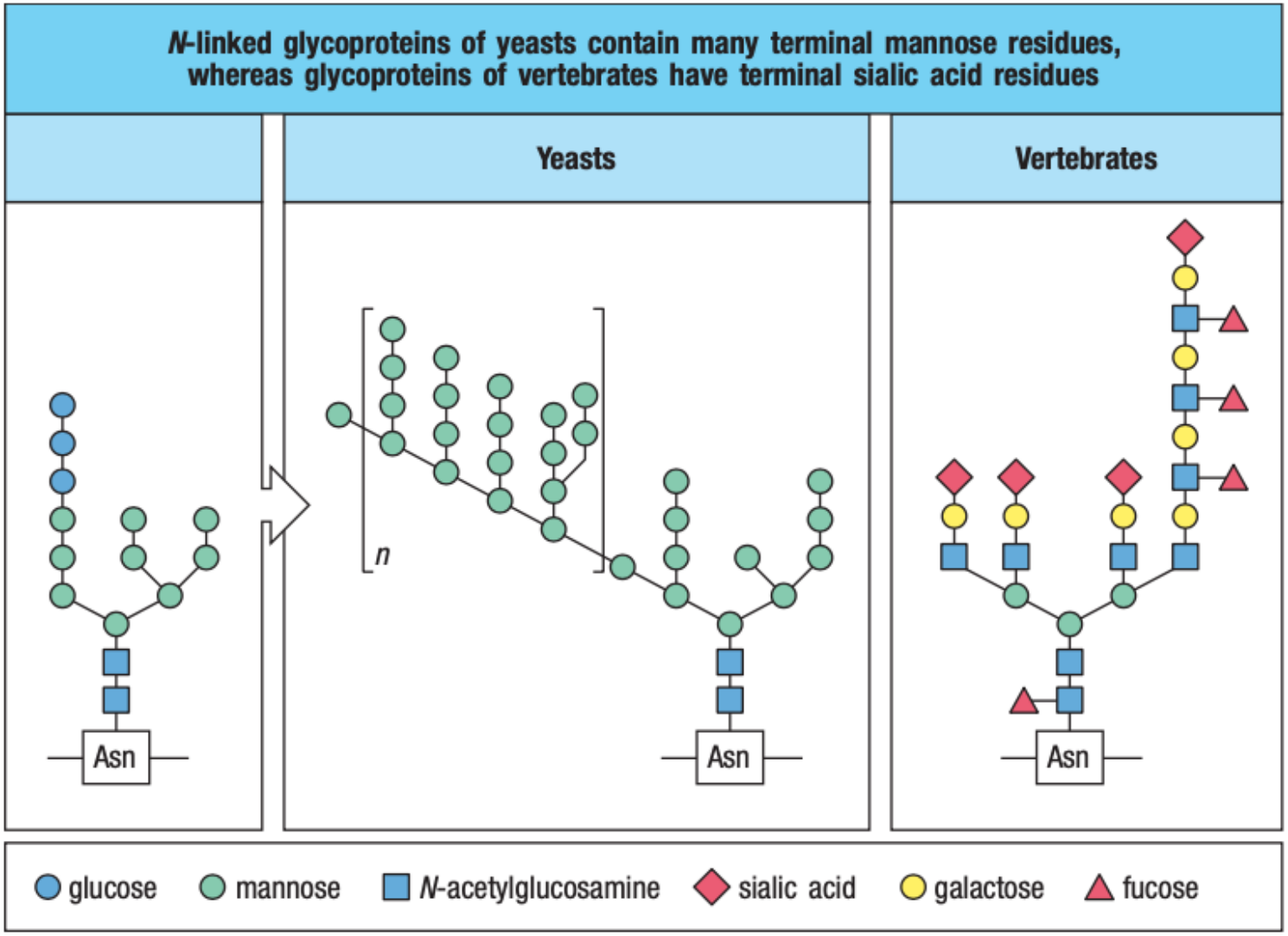
=> A protease cascade: amplification steps



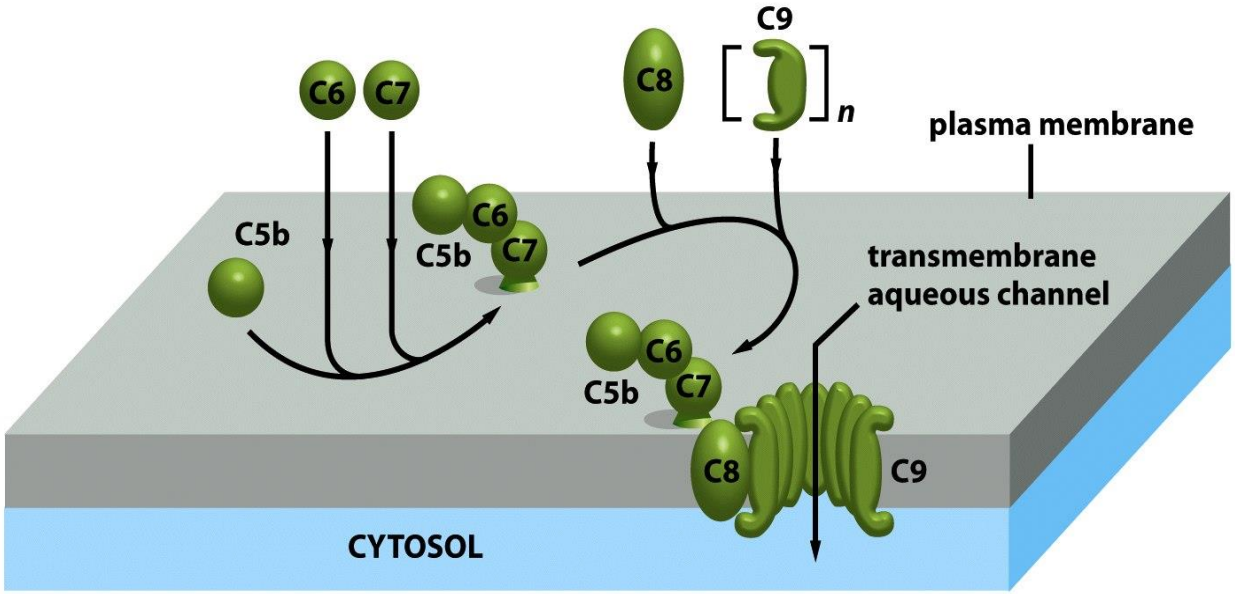
Covalent attachment / deposition on target cell

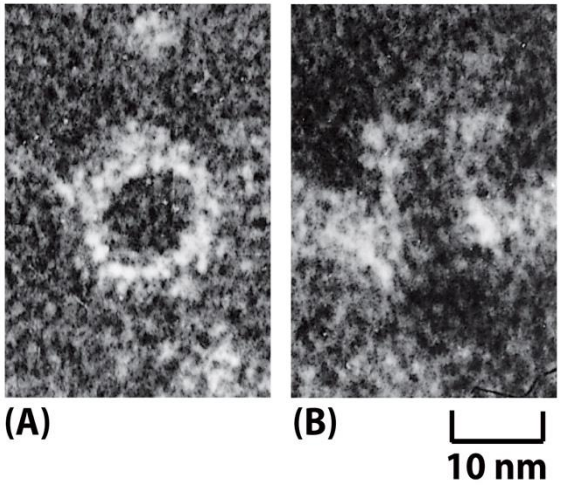


Lectin pathway

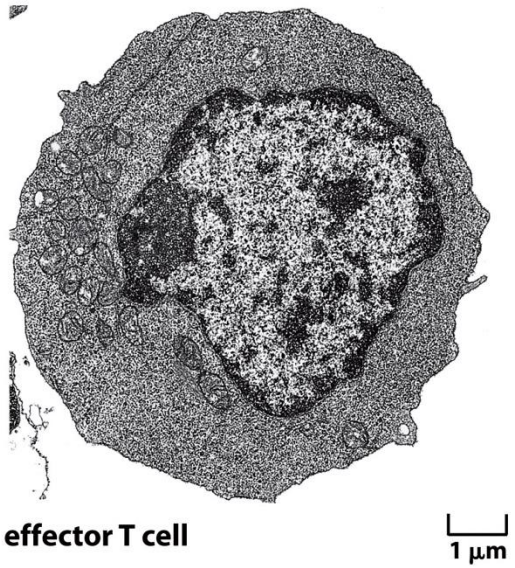


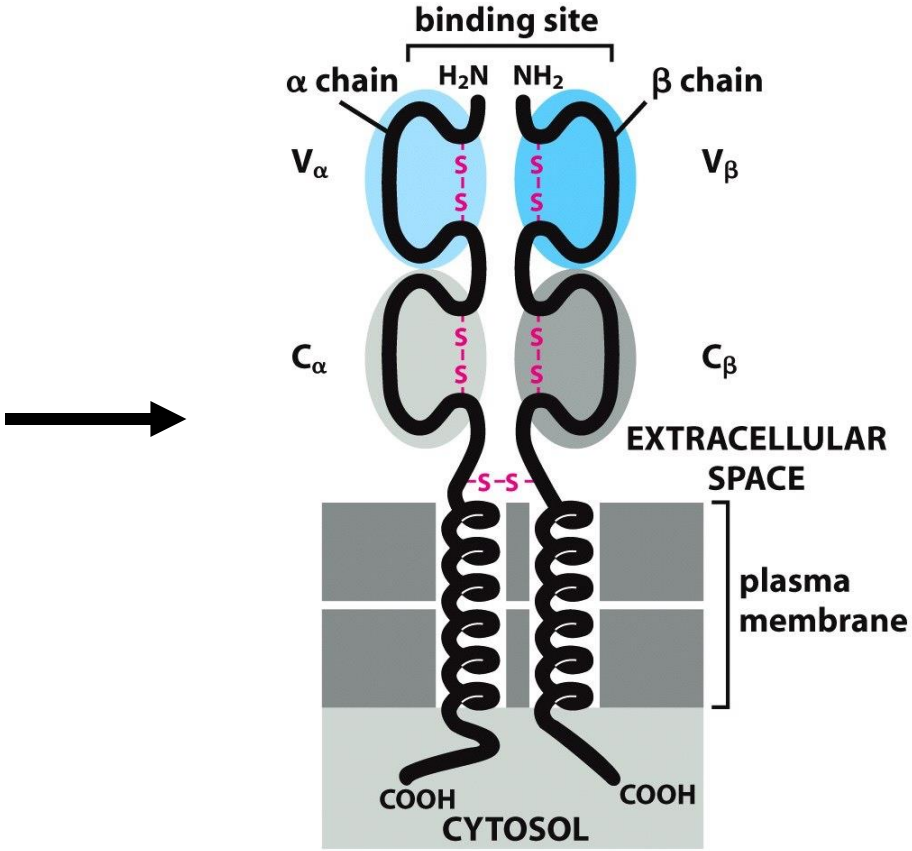
Complement system: pore formation/lysis



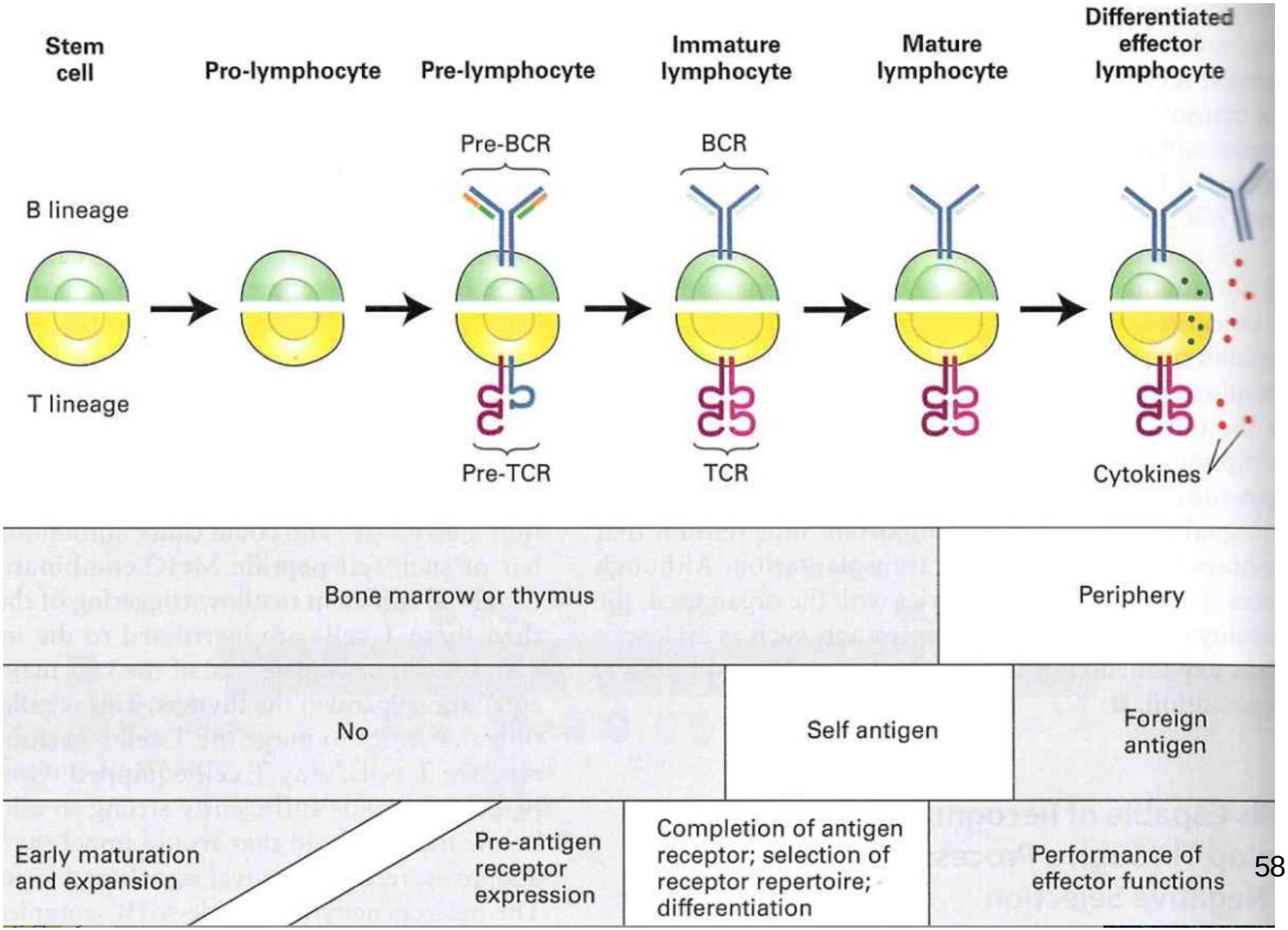


T cells and T cell receptor (TCR)

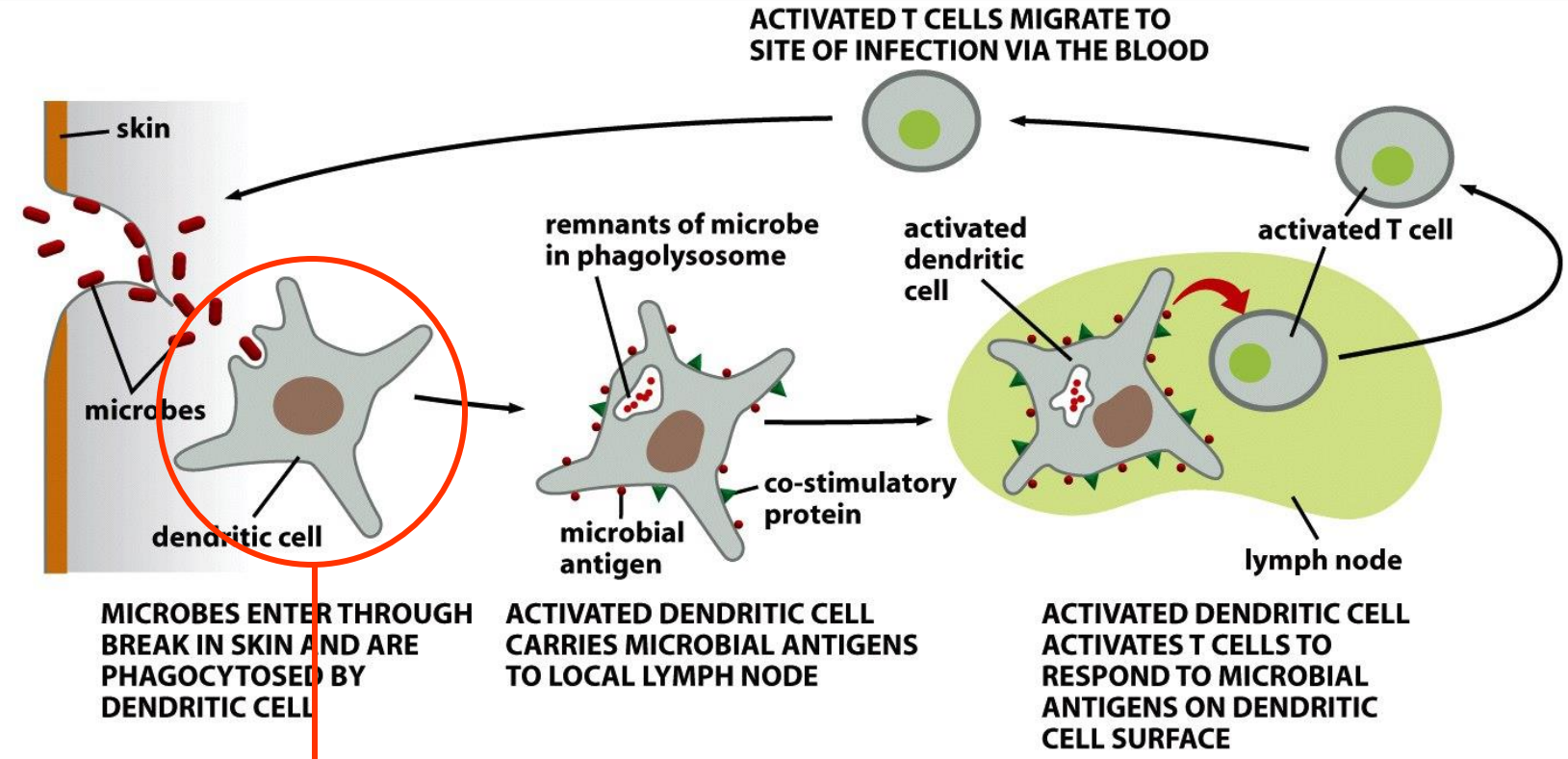


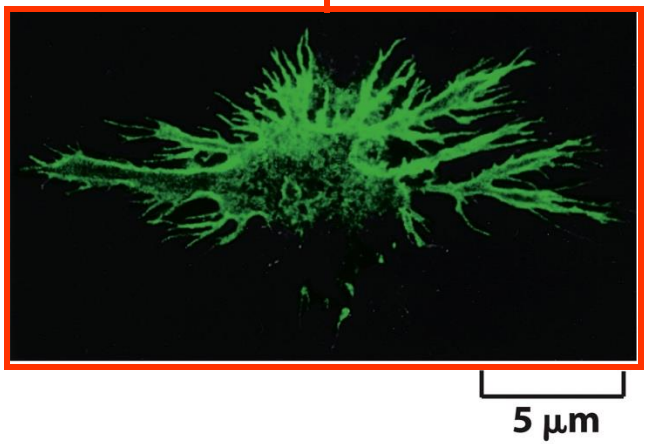


B and T cell maturation follow a similar course

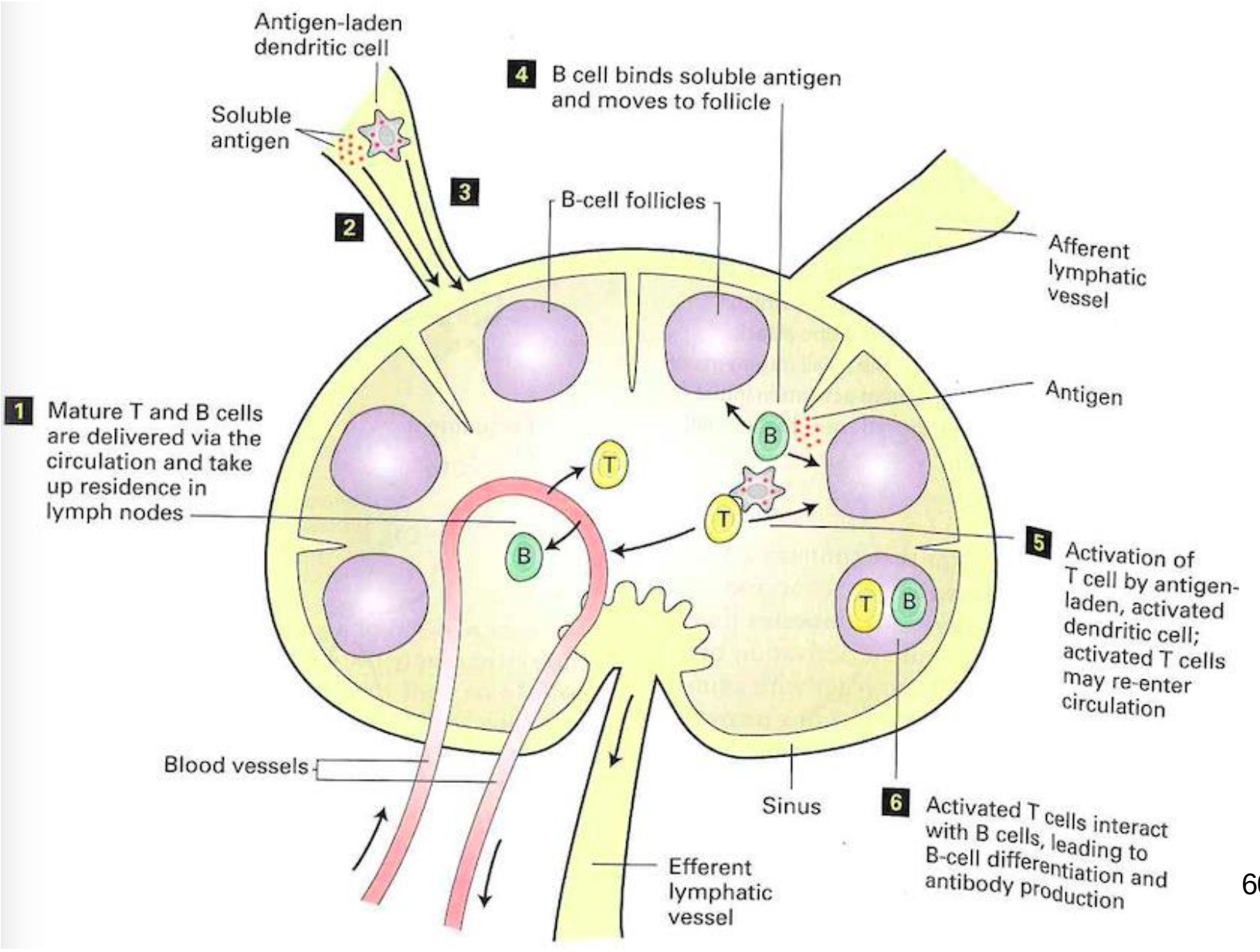


We take a larger picture: Antigen presentation



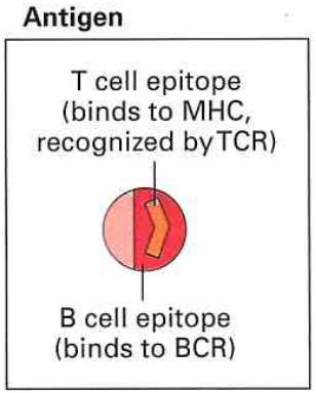


Larger picture: initiation of immune response



Better double check!

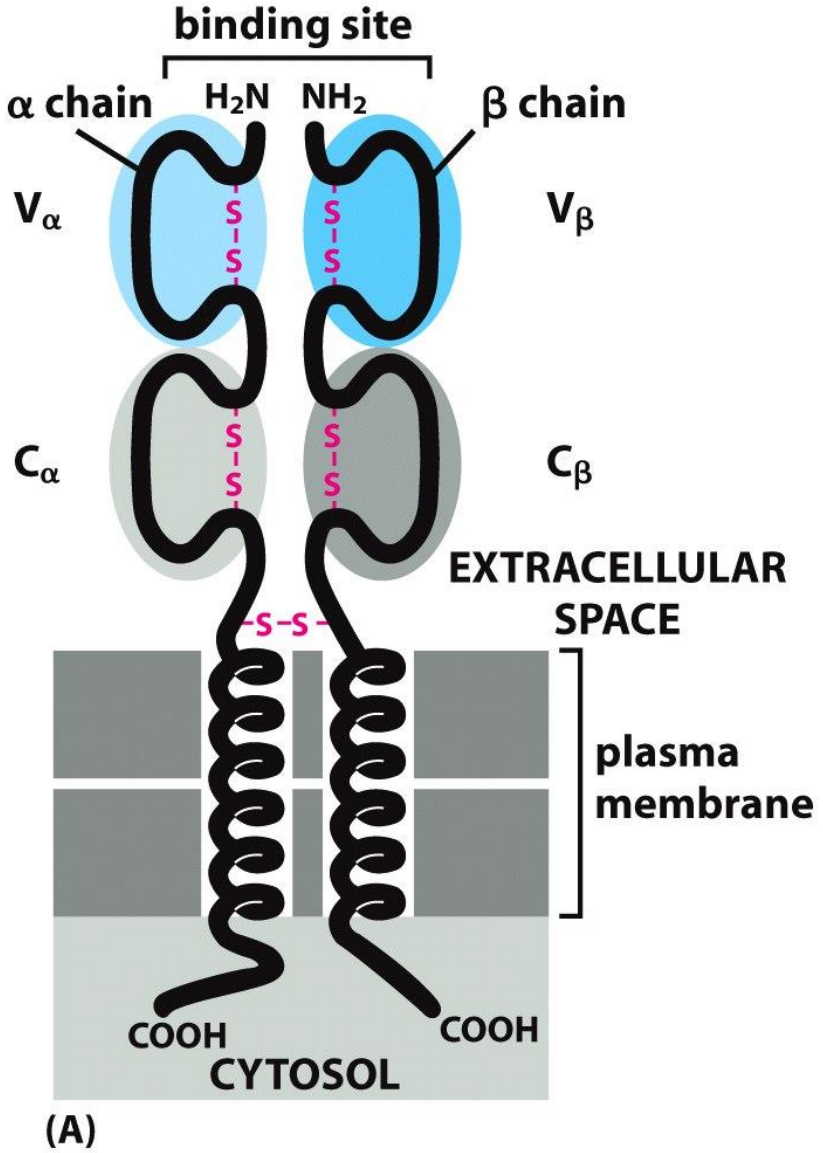
B cell epitope ⟷ BCR (recognition)

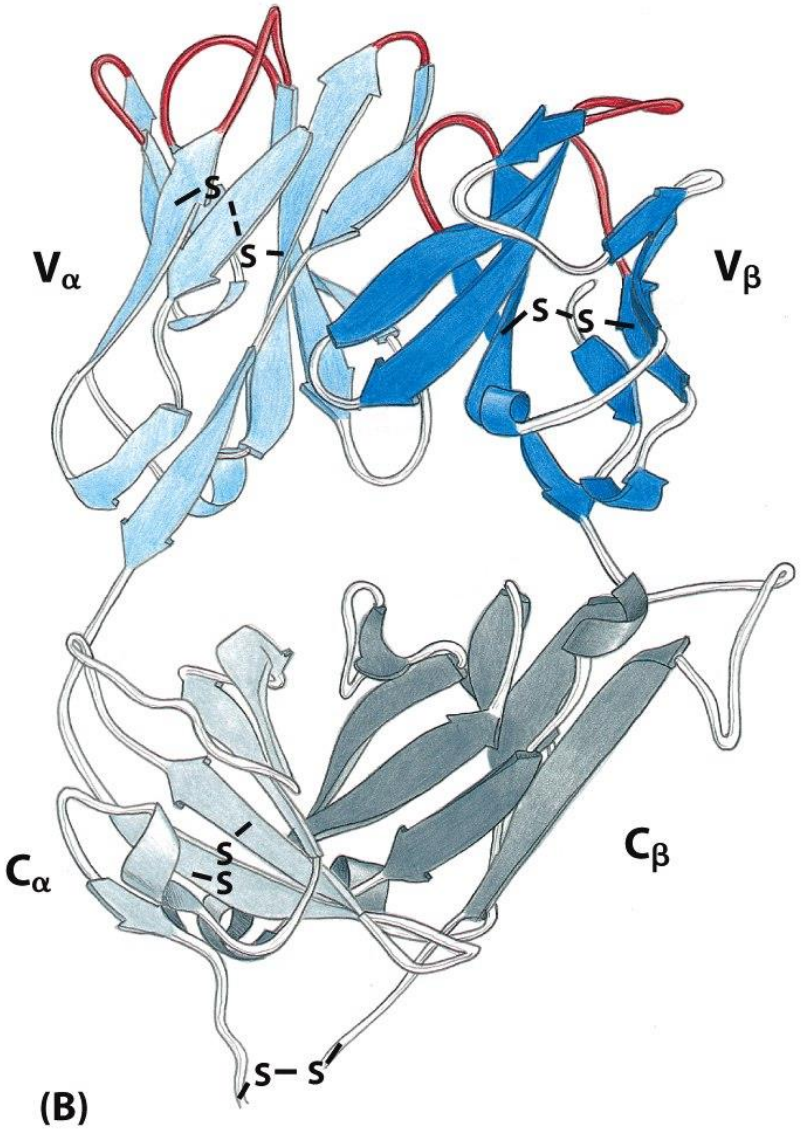


T cell eptiope ⟷ TCR (recognition)

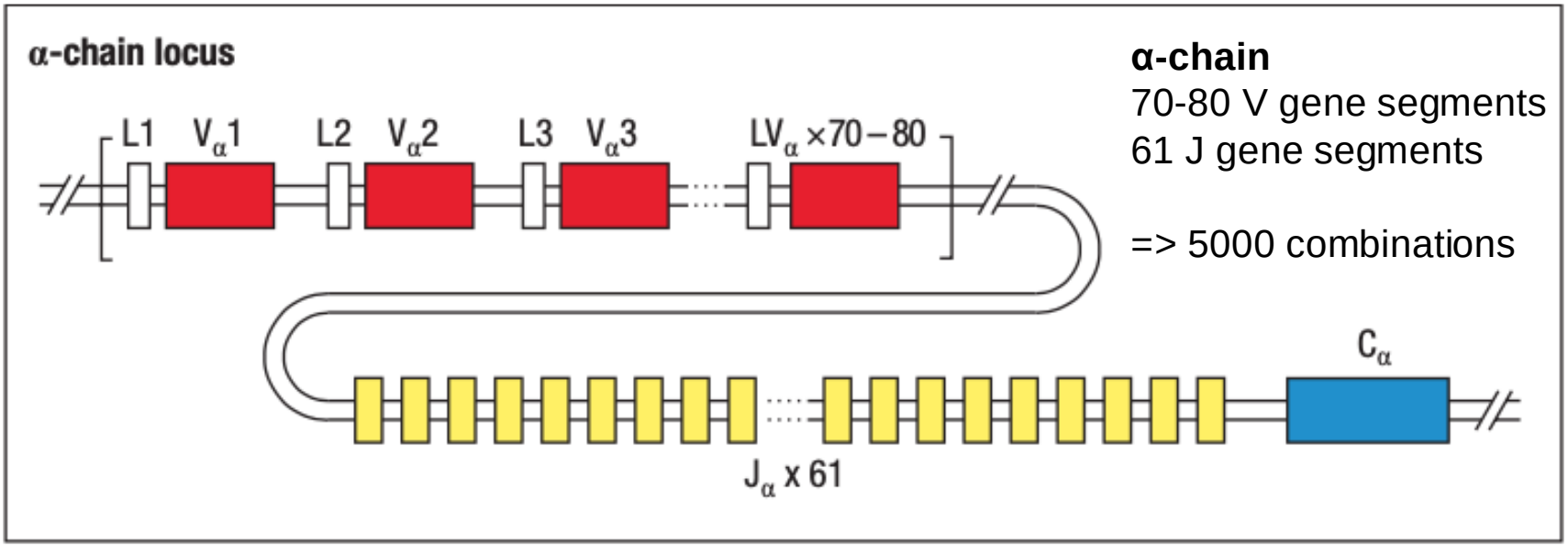
⇒ Minimizing the risk of wrong classification (friend/foe) to prevent e.g. autoimmune diseases, allergies

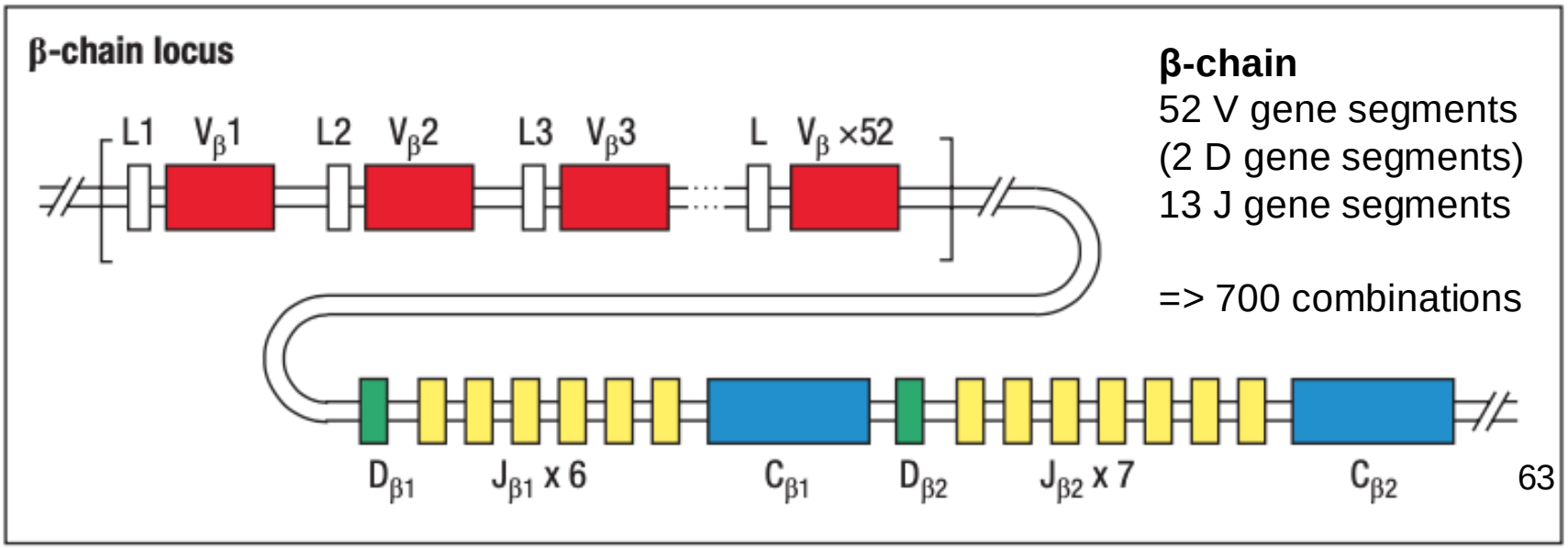
T cell receptor (TCR)



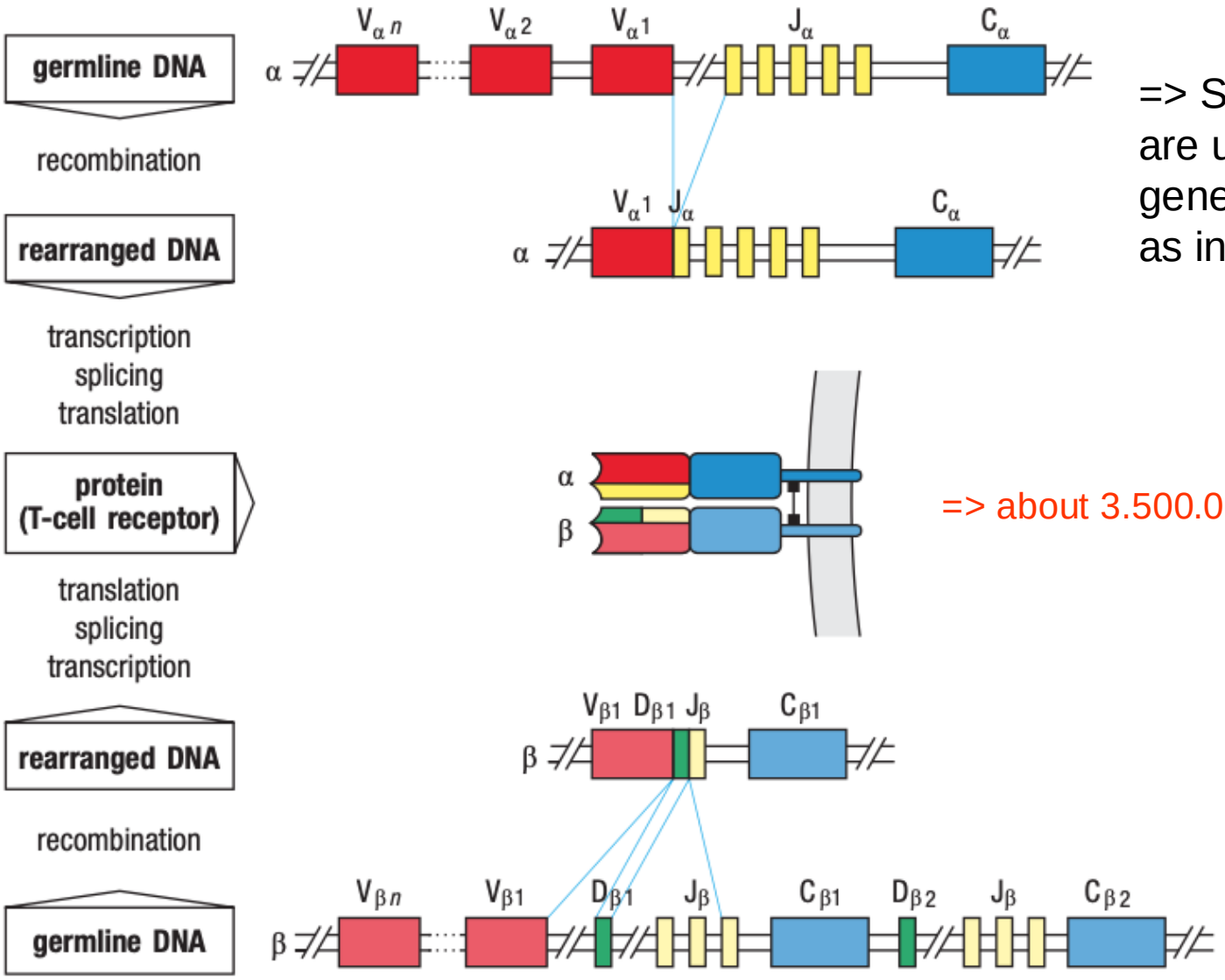


Generation of TCR diversity





Generation of TCR diversity

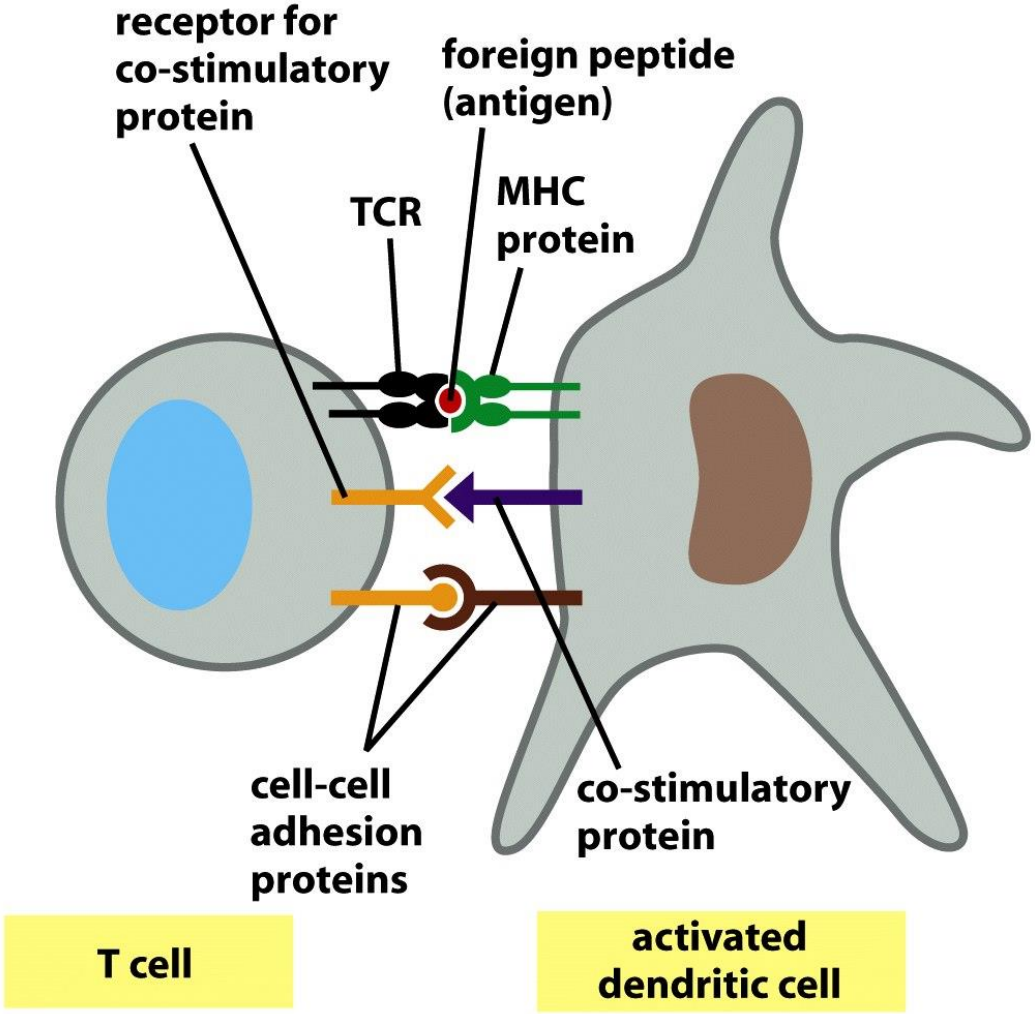


=> Same enzymes are used for the gene rearrangement as in B cells

=> about 3.500.000 combinations

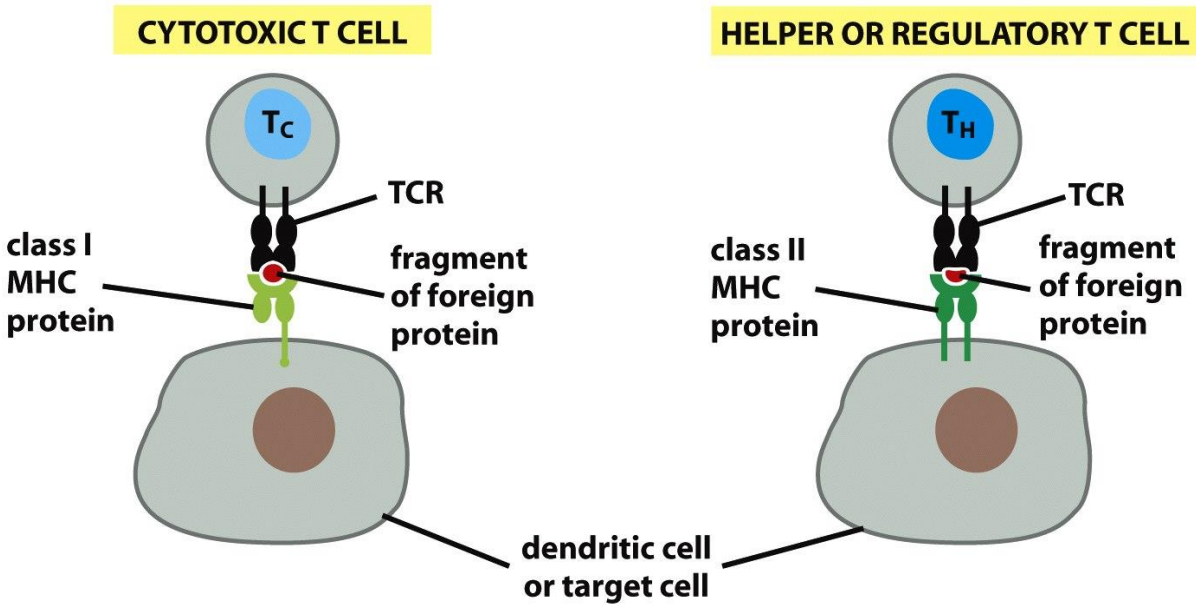
Unlike BCR no somatic hypermutation => only lower affinity (K_a = 10^5-10^7 M^{-1})

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

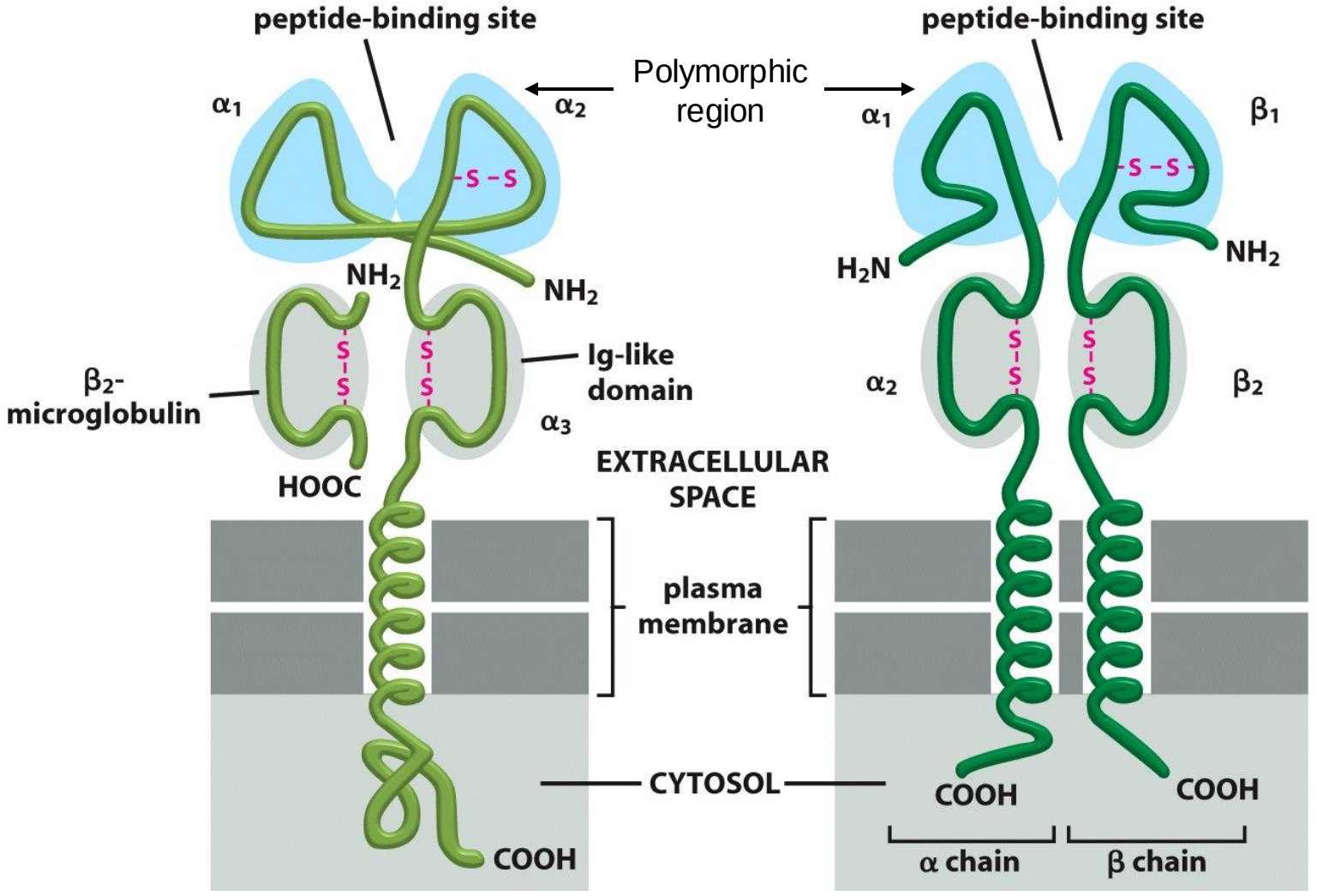
	CLASS I	CLASS II
Genetic loci	*HLA-A, HLA-B, HLA-C*	*DP, DQ, DR*
Chain structure	α chain + β_2-microglobulin	α chain + β chain
Cell distribution	most nucleated cells	dendritic cells, B cells, macrophages, thymus epithelial cells, some others
Presents antigen to	cytotoxic T cells	helper T cells, regulatory 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

=> More information in online folder / doi: 10.1111/tan.14626

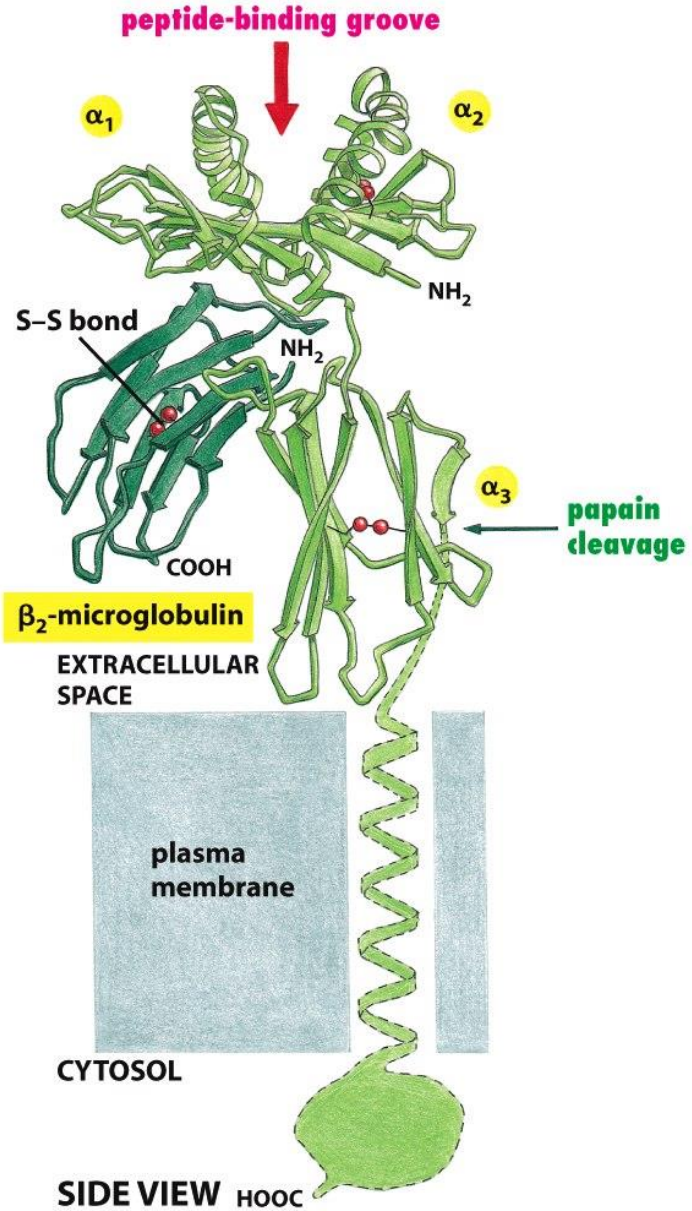
Major histocompatibility complex (MHC)

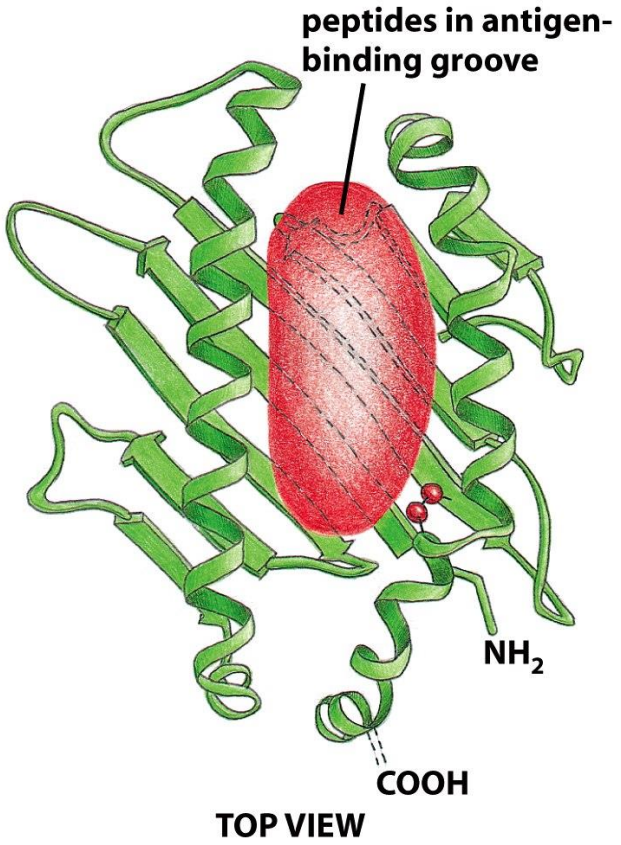
Class I MHC protein
=> on (almost) all cells

Class II MHC protein
=> Only on professional antigen-presenting cells (e.g. dendritic cells)

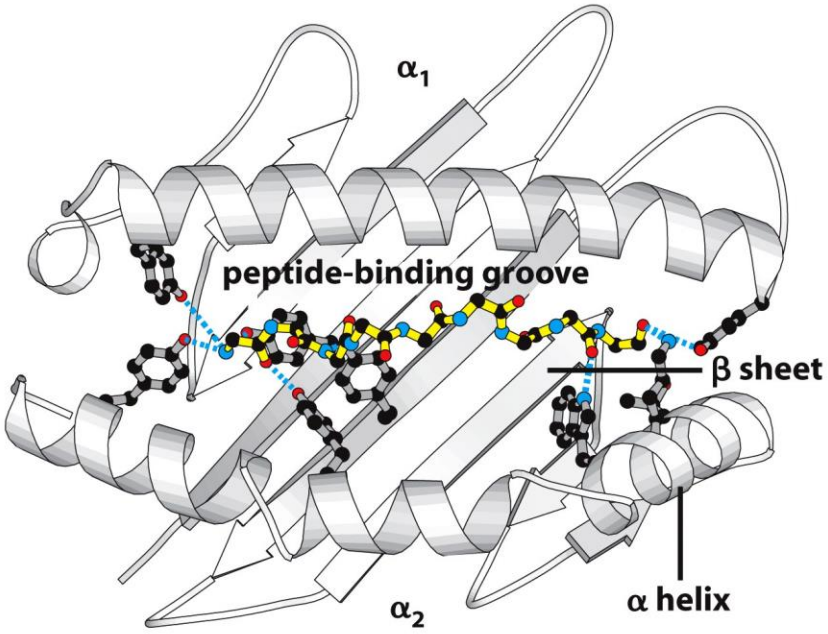


Peptide bound to MHC

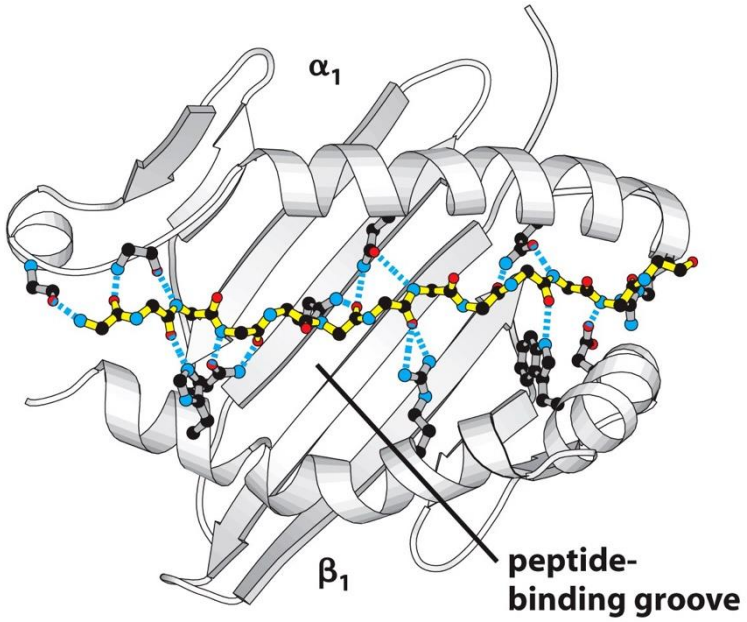




Peptide bound in the groove of MHC

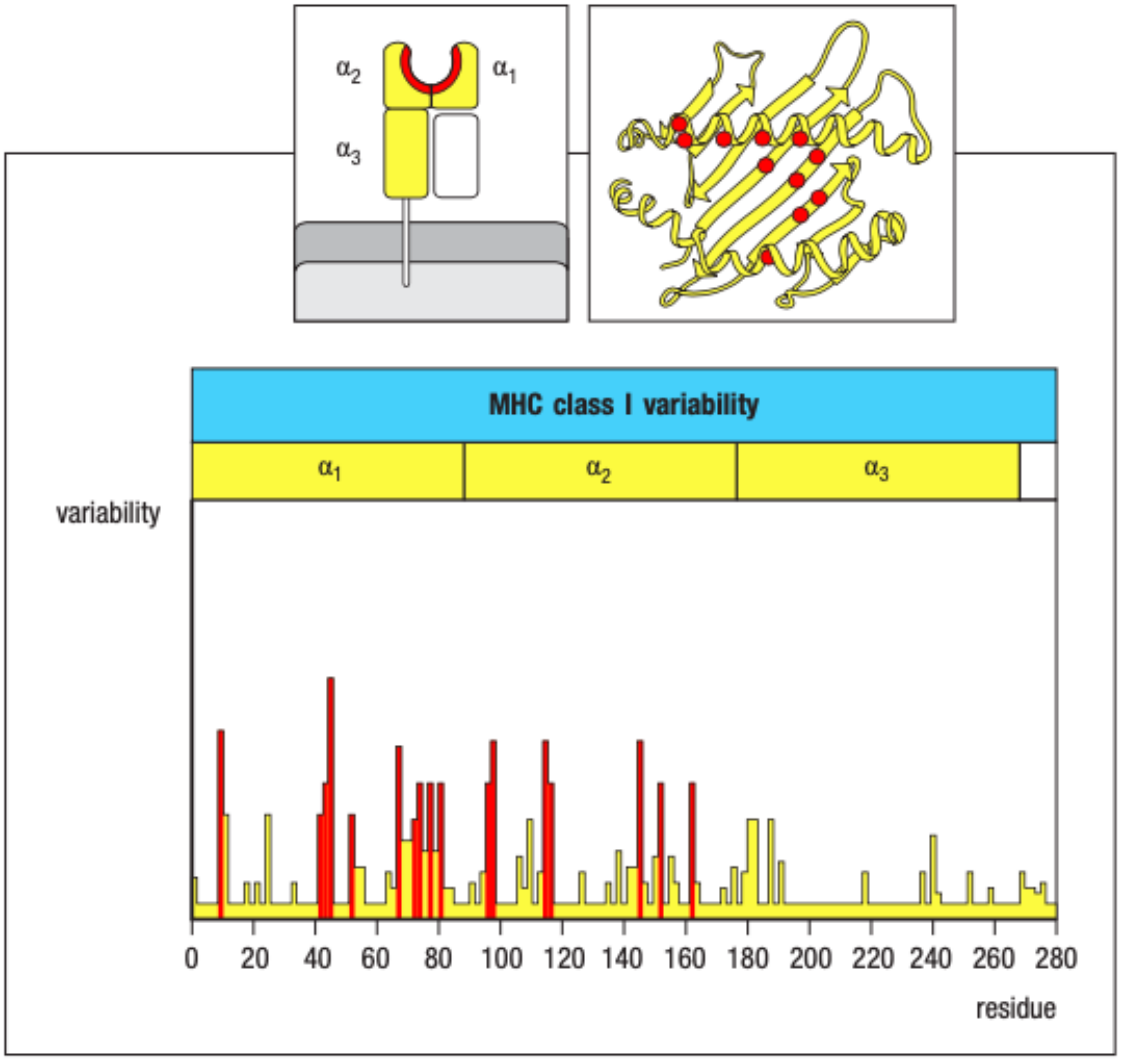


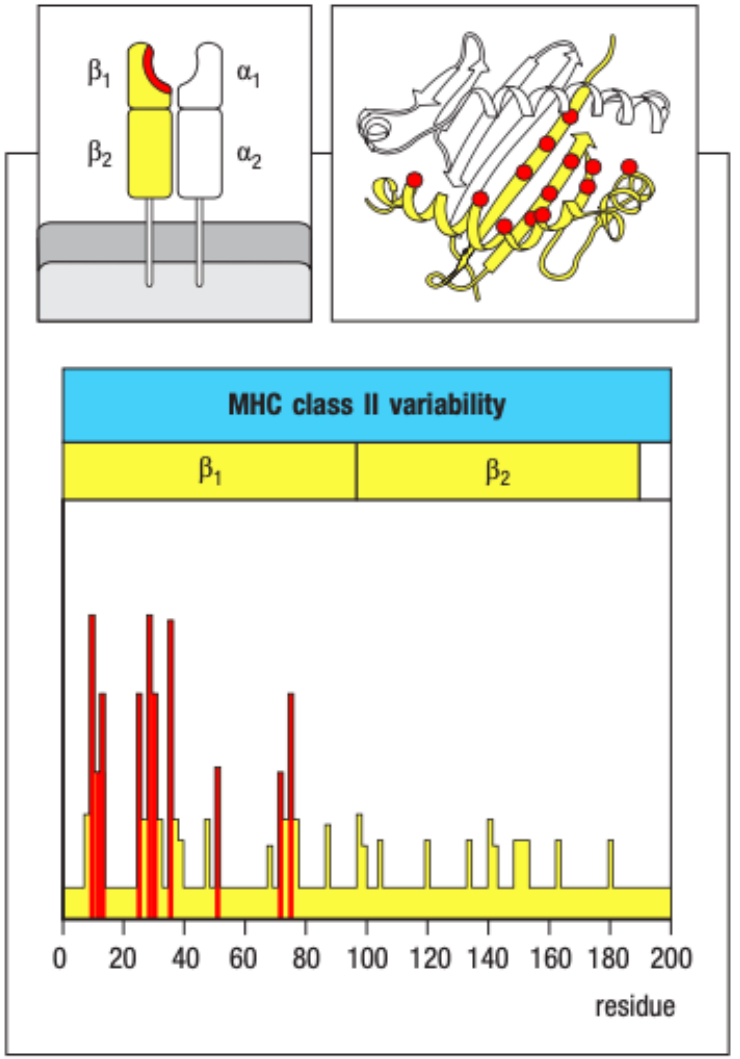
Peptides bound to **MHC-I**:
8-10 amino acids long



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

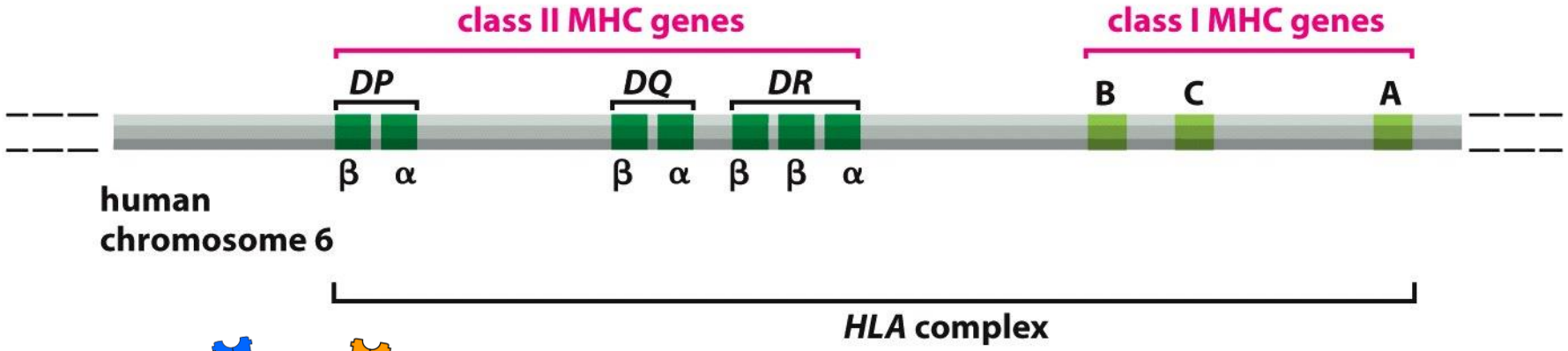
Allelic variation in MHC genes

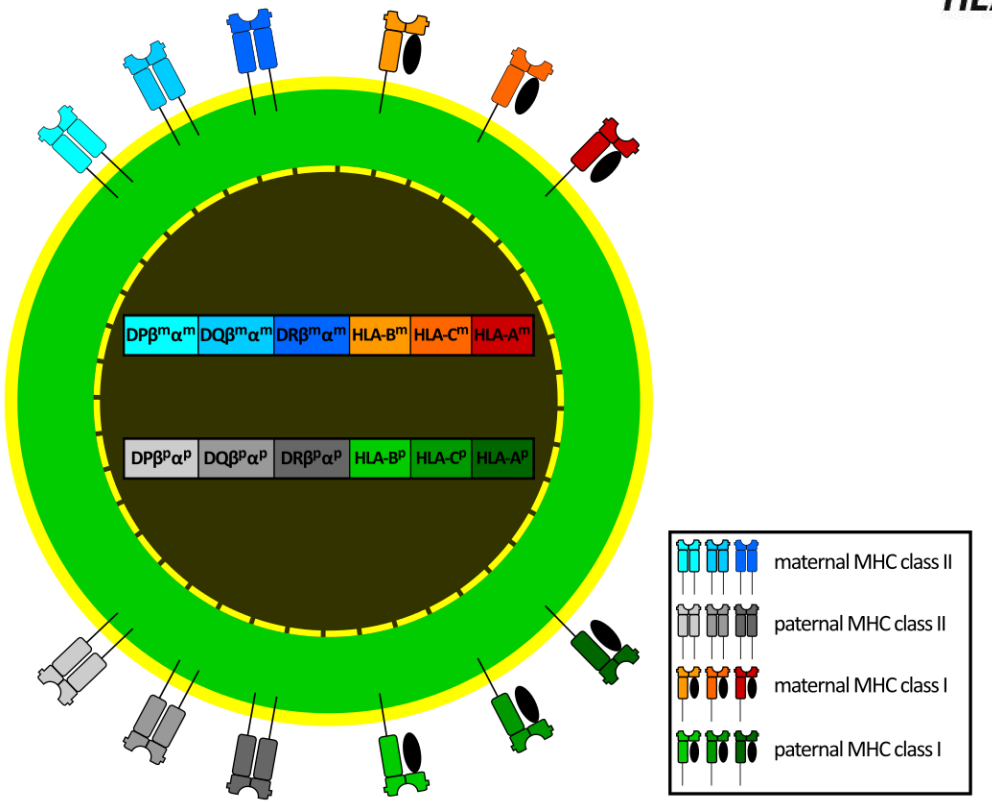




Red: peptide binding regions

Human MHC genes

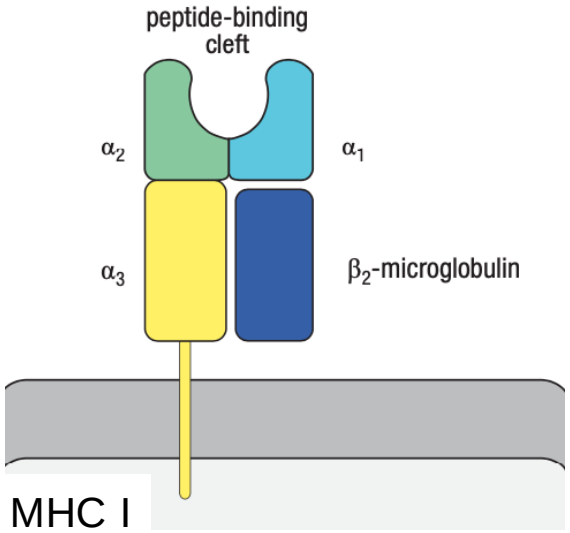




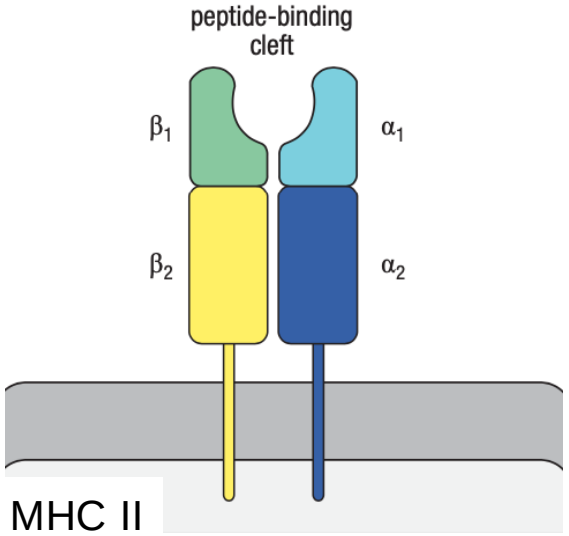
There is a large variability of MHC molecules in a population, but in each individual:

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

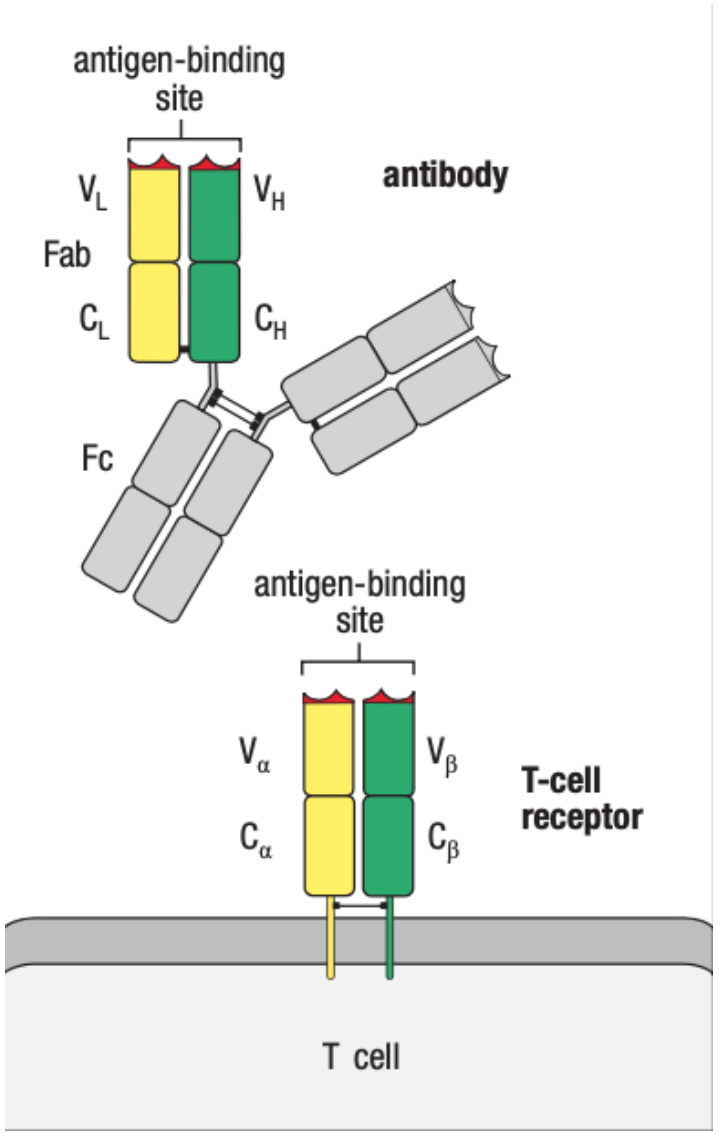
Structural comparison: antibody, MHC and TCR



MHC I



MHC II



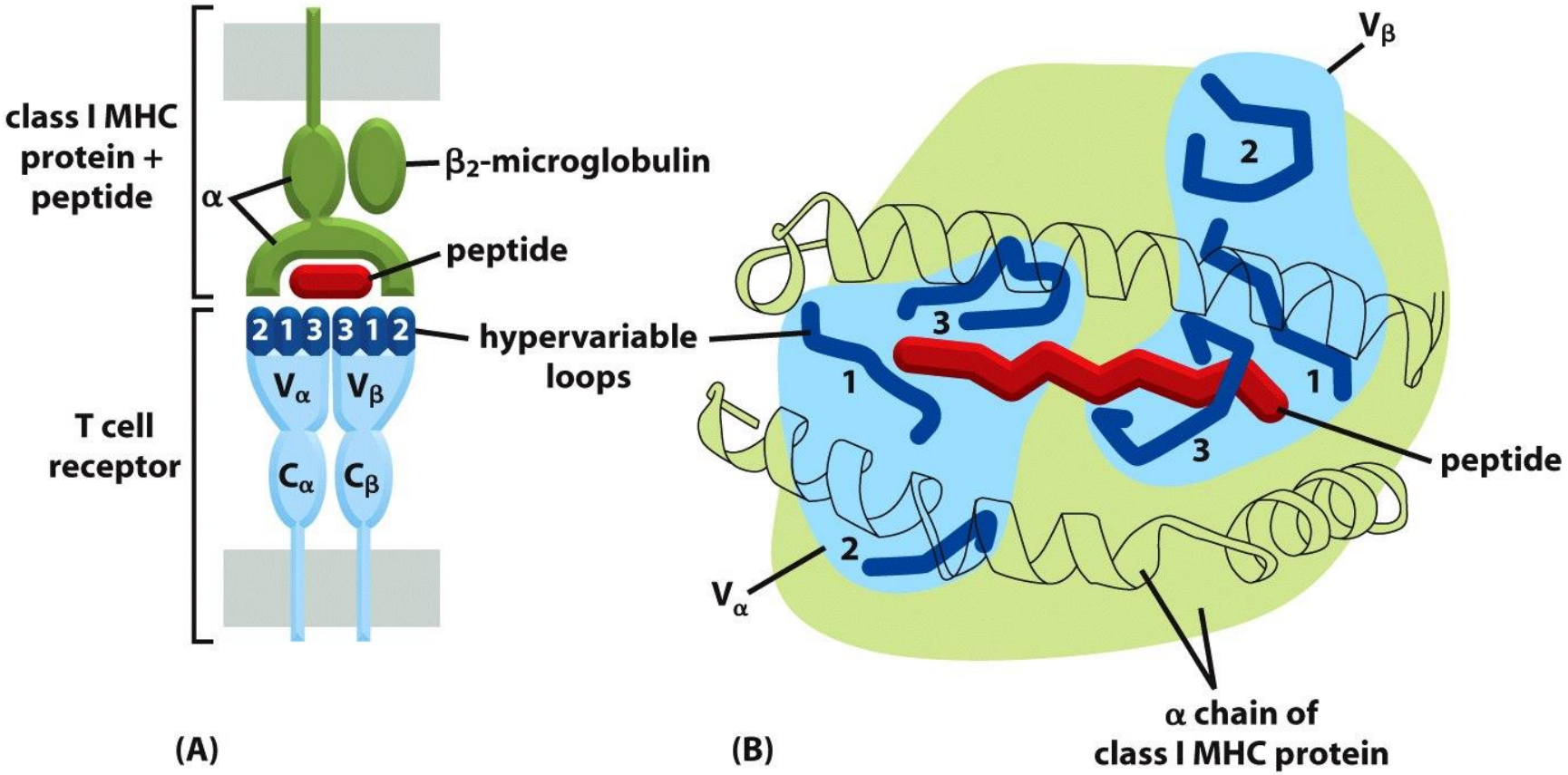
Large diversity in the recognition of antigens

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

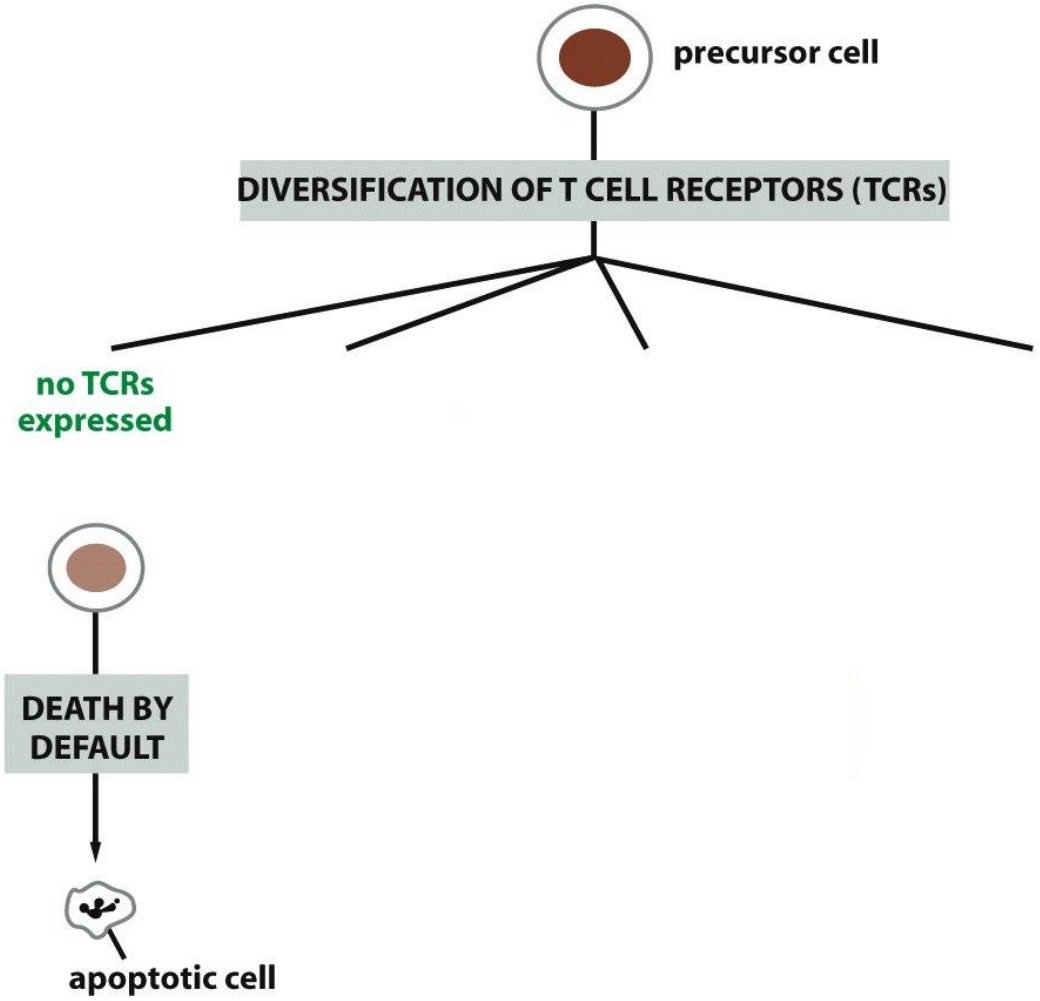
MHC: no gene rearrangement but 3 genes and several thousand alleles in a population
=> Can bind a large variety of peptides (but not all)
=> a whole population is well protected but there is an individual risk of missing some pathogenic peptides
=> populations with a large gene pool are more resistant to an epidemic

Interaction of TCR with a peptide on MHC class I

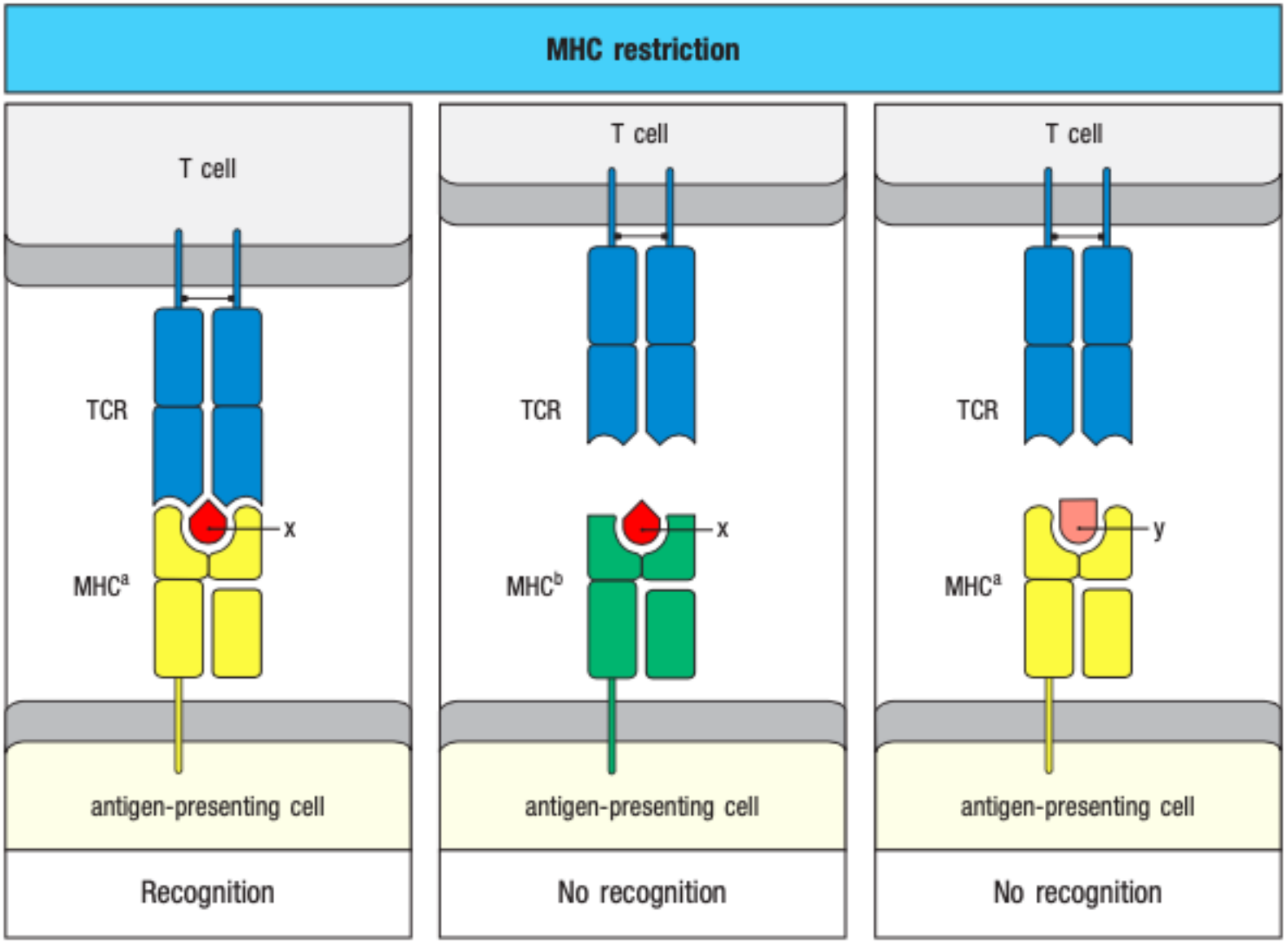


=> Only linear peptide epitopes

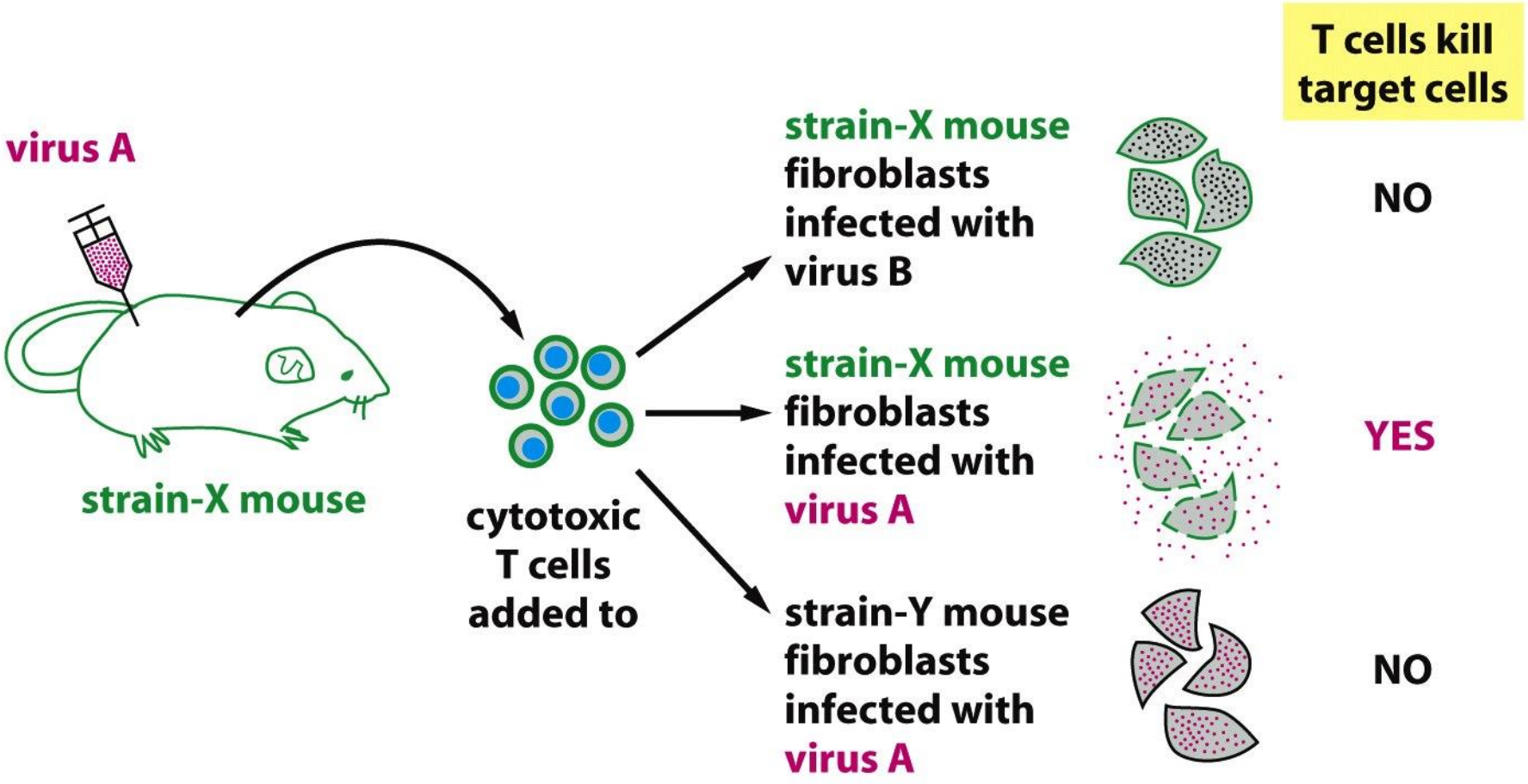
Friend / foe recognition by T cells



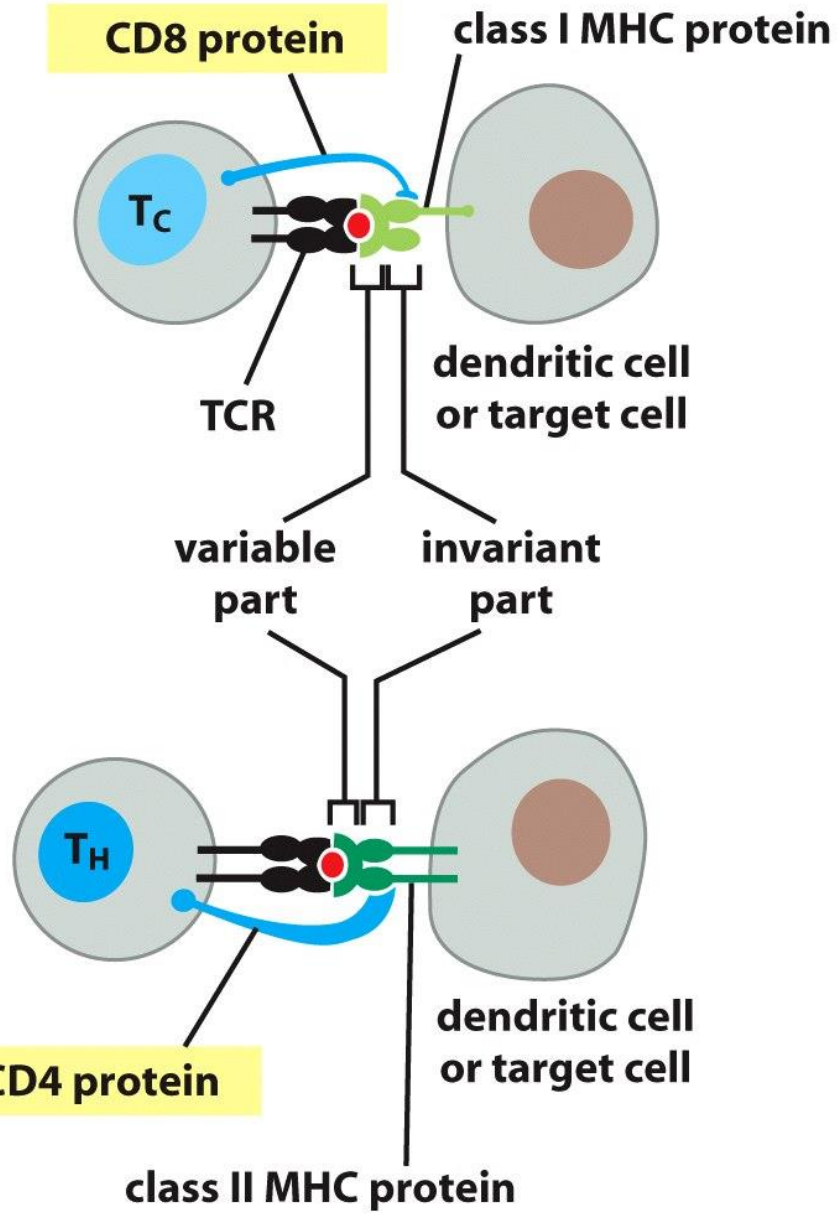
T cell recognition of antigens is MHC restricted



T cell recognizes viral antigen *and* host target cell

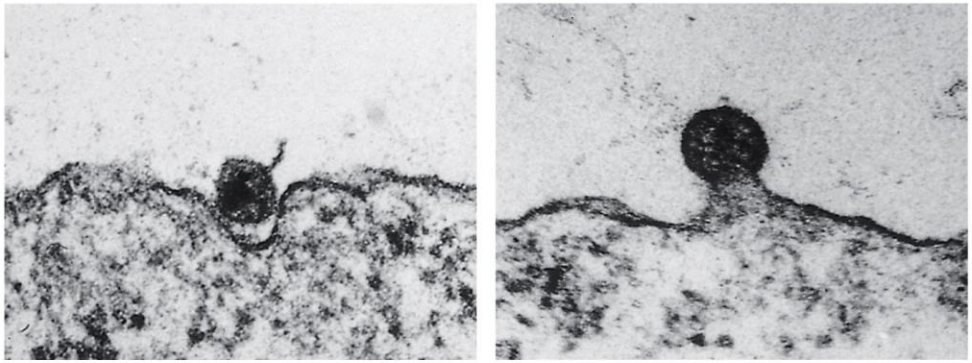


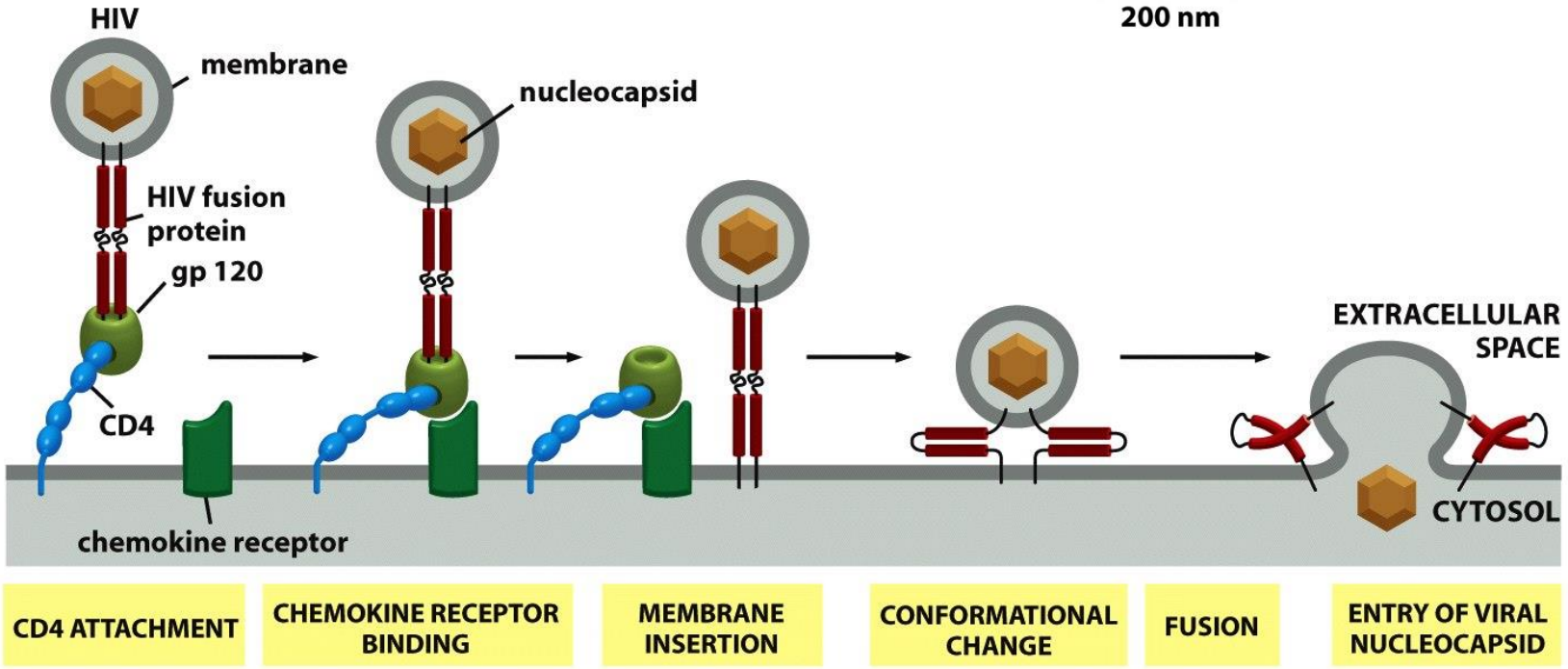
Co-receptors on T cells



Serves as a receptor
for HI virus binding

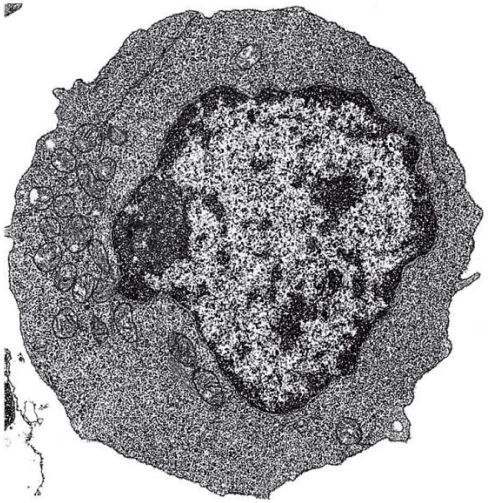
Excursion: HI virus infecting T cell





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

Classification of T cells



Cytotoxic T cells (T_C)
($CD8^+$ cells)
=> recognize peptides on MHC I

T helper cells (T_H)
($CD4^+$ cells)
=> recgonize peptides on MHC II

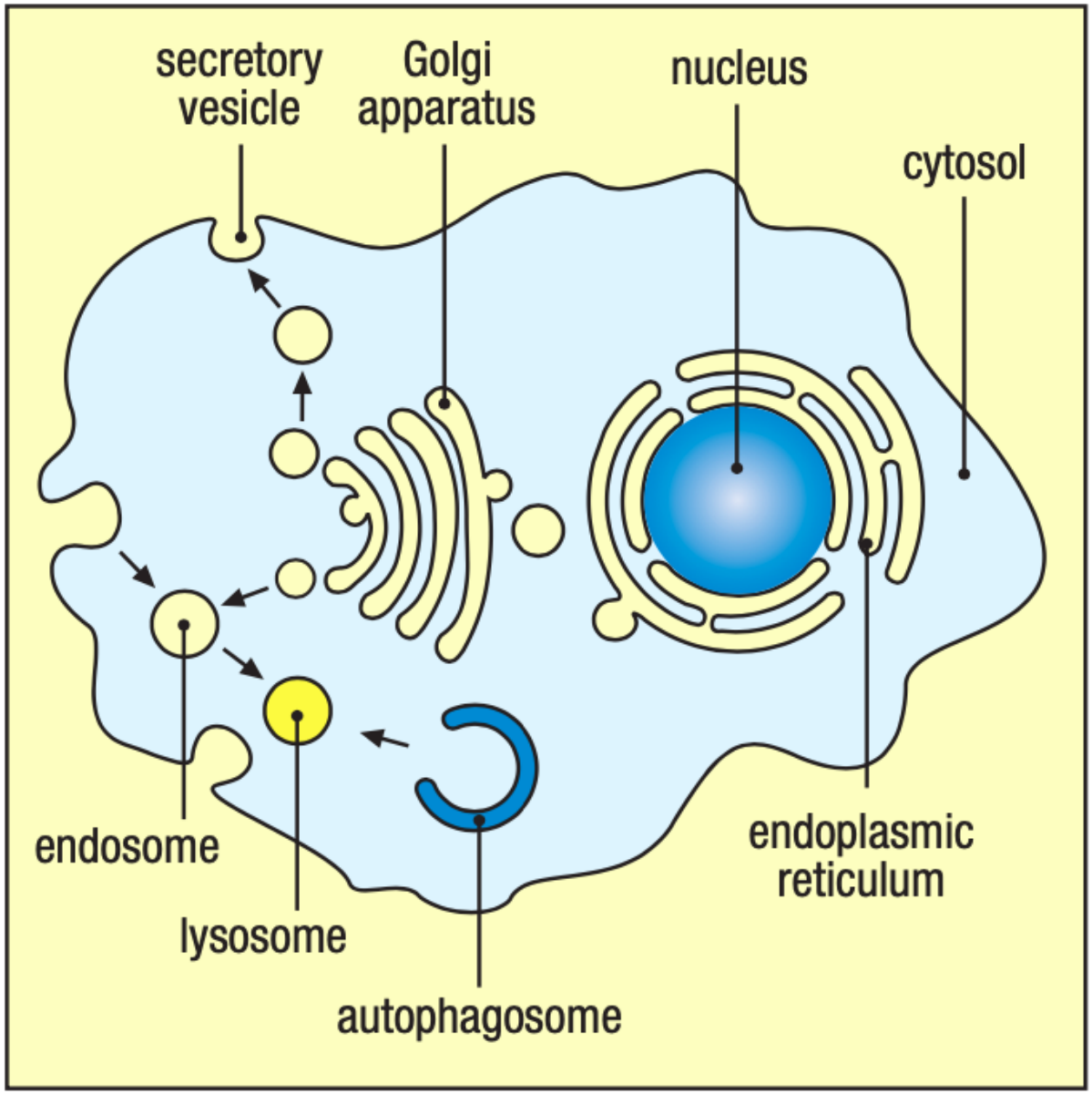
T_H0

T_H1
infections
by microbes

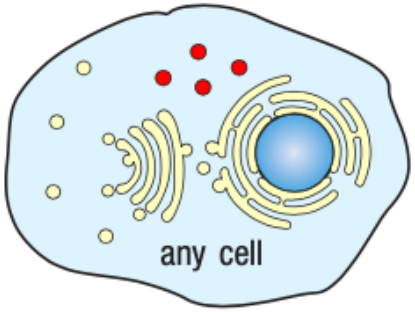
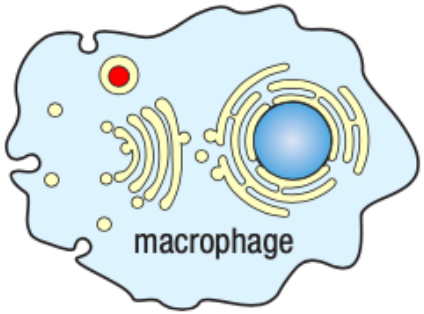
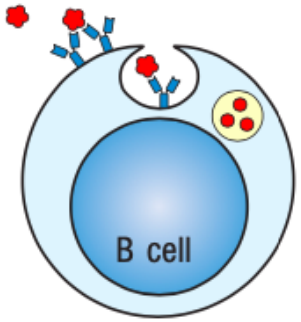
T_H2
infections
by parasites

Suppressor T cells
regulatory function

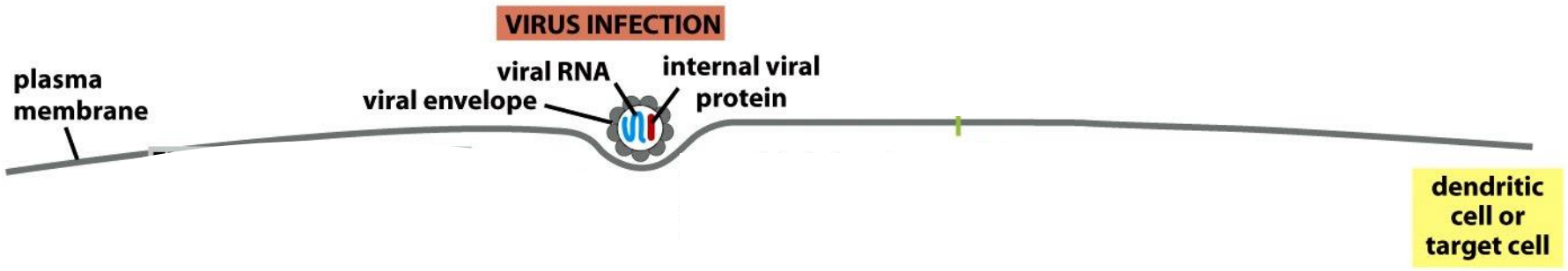
Topologically equivalent compartments



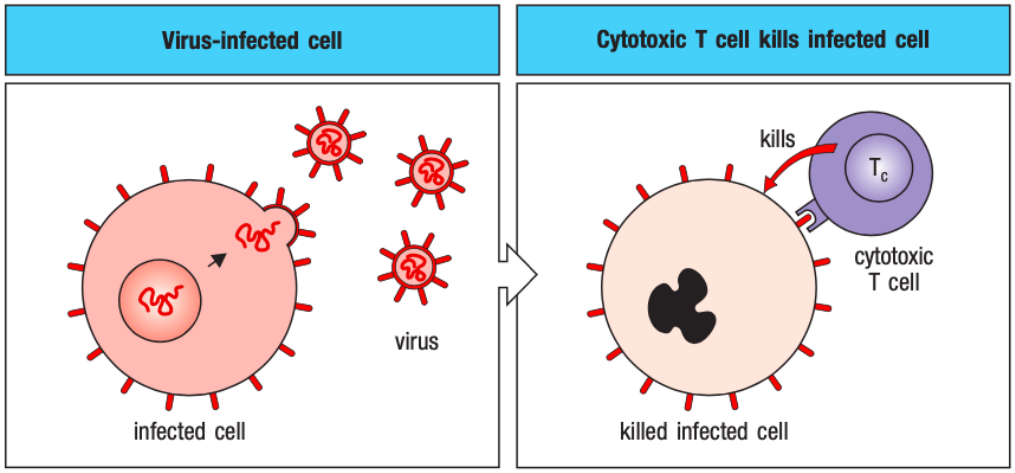
Antigen acquisition sites

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
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

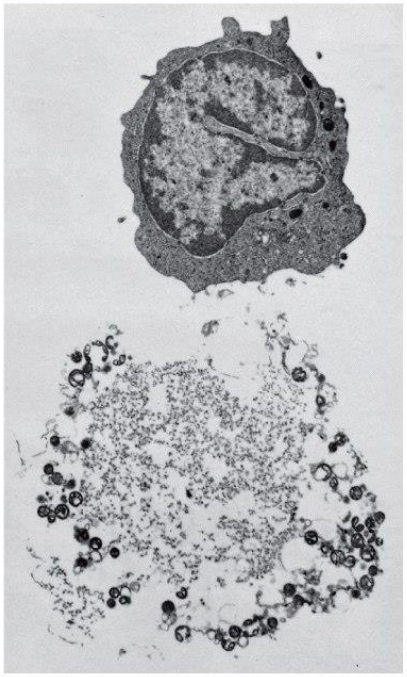
Antigen presentation by MHC-I



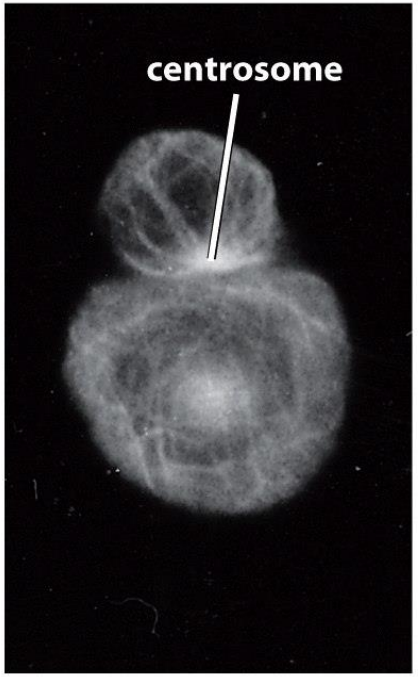
Activation of cytotoxic T cells




(A)


(B)

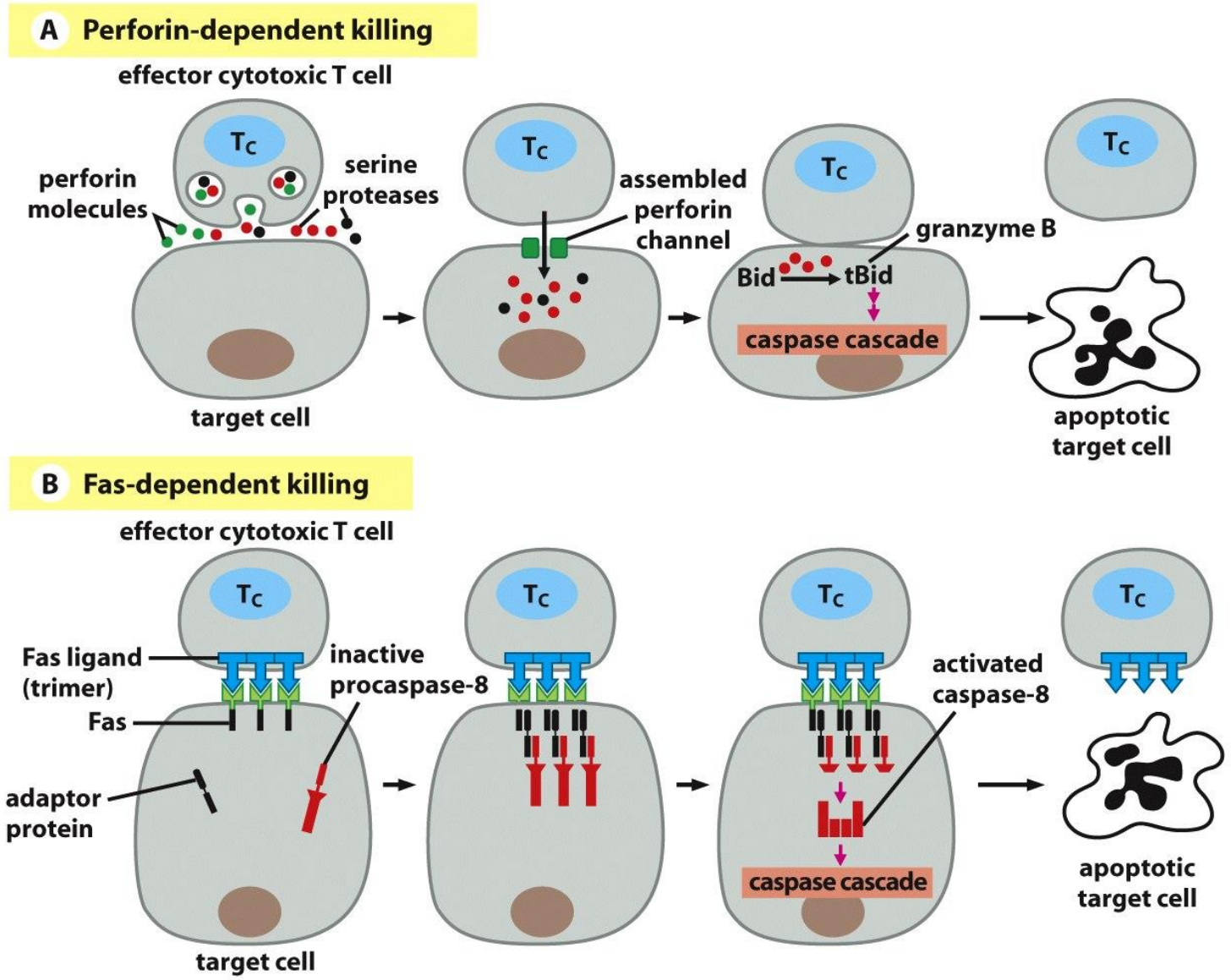
5 μm



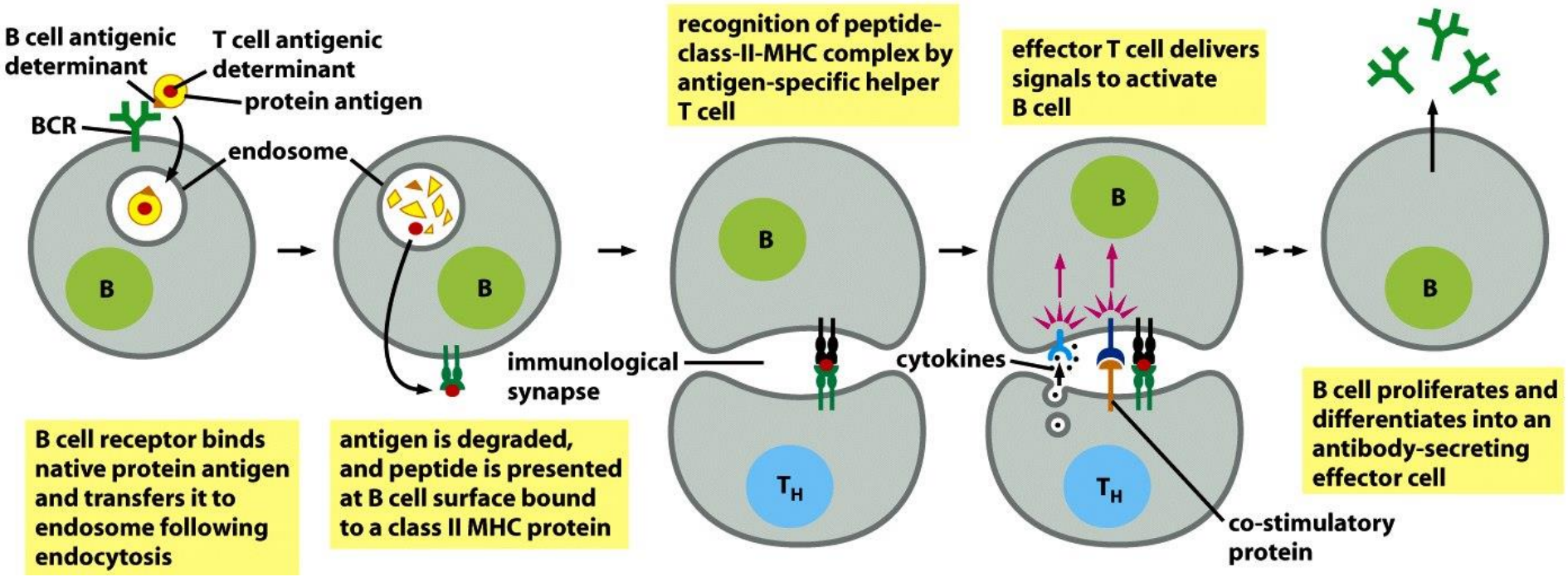
(C)

10 μm

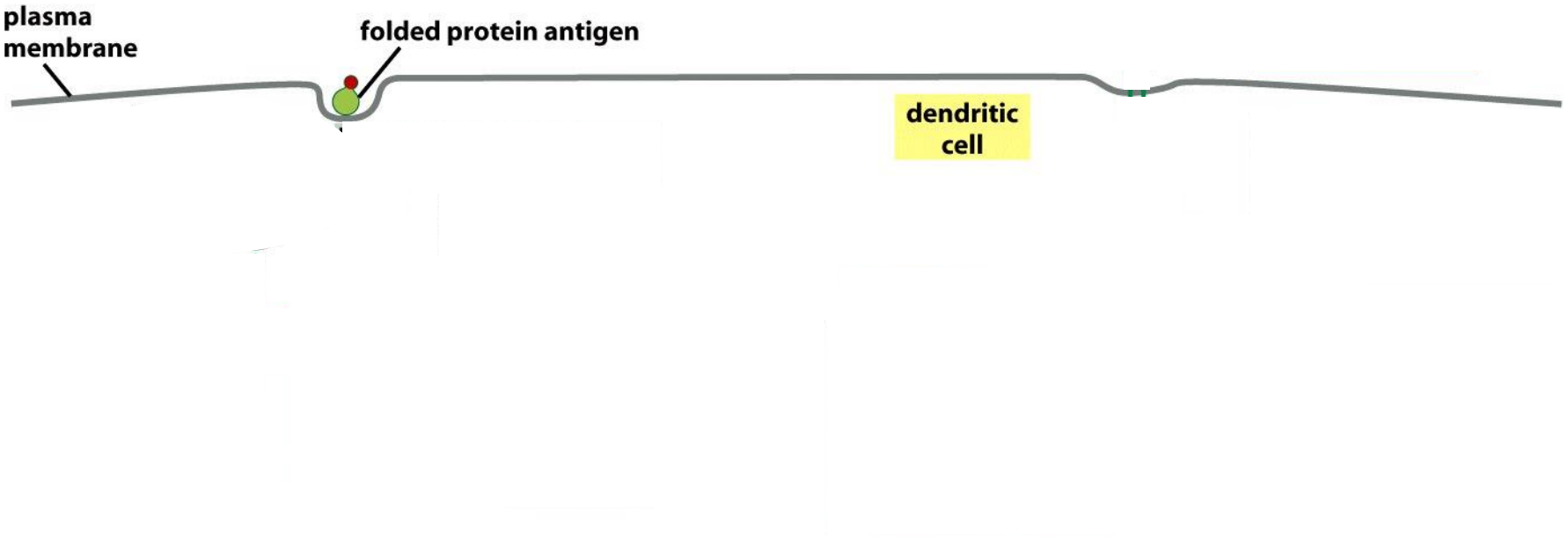
Cytotoxic T cells induce apoptosis



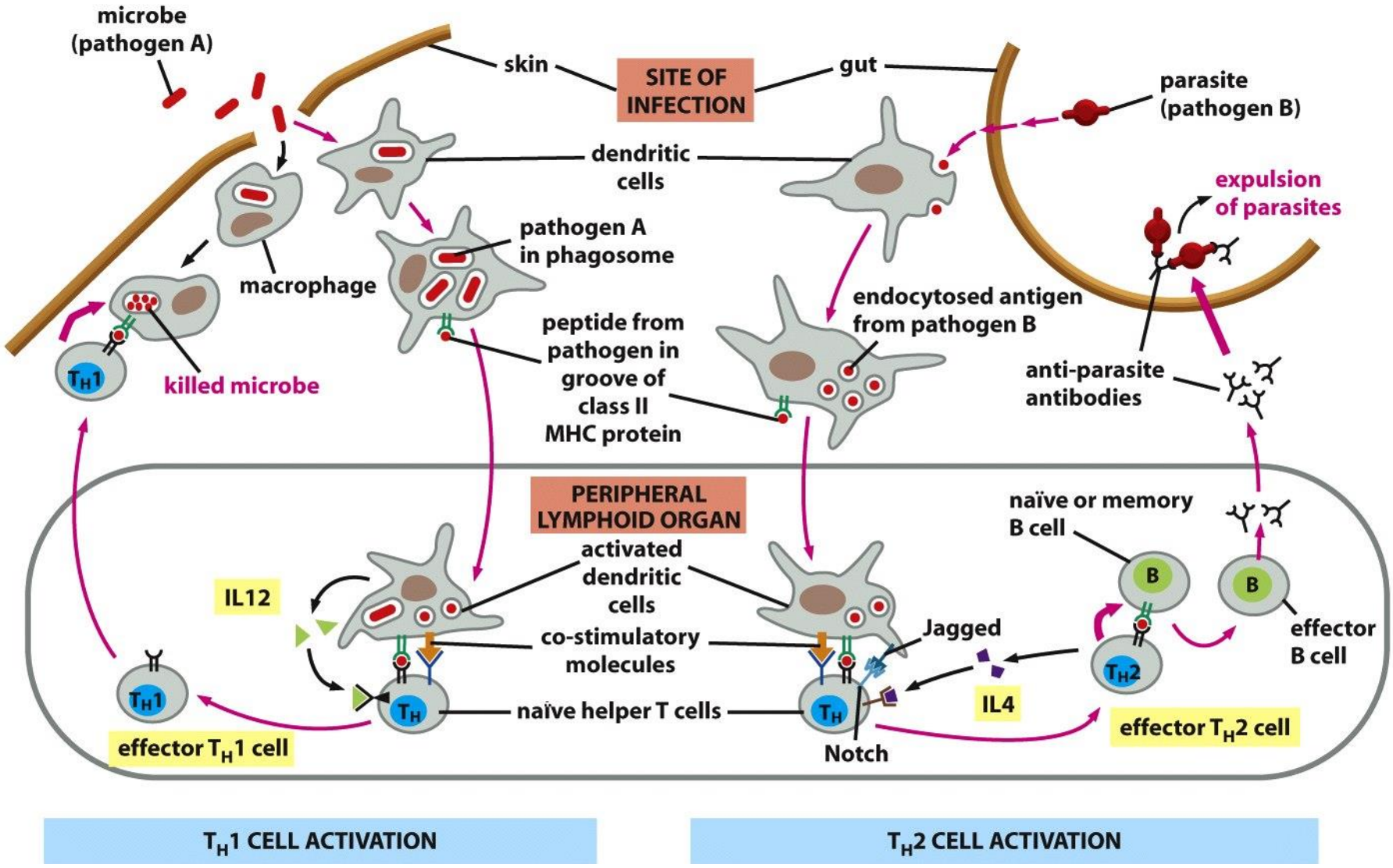
Activation of a B cell by an antigen *and* T_H cell



Antigen presentation by MHC-II



Activation of T_H1 and T_H2 cells



Summary of interplay between T_H and B cells