MUNI
SCI

# C8116 Immunochemical techniques

## Immune system, part I

## Spring semester 2024

Hans Gorris
Department of Biochemistry
February 20th, 2024

# 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…

# Awarding reserach


**Novozymes:**
the world‘s market leader for the production of industrial enzymes:
*Enzyme Assay Scientist Award 2016*
https://www.novozymes.com/en/news/news-archive/2016/09/award

# Overview of the lecture

| | Date | Topic |
|---|---|---|
| 1 | Feb. 20th | Immune system I |
| 2 | Feb. 27th | Immune system II |
| no lecture | March 5th | - |
| 3 | March 12th | Antibodies as immunological tools |
| 4 | March 19th | Immunoassays I |
| 5 | March 26th | Immunoassays II |
| no lecture | April 2nd | - |
| no lecture | April 9th | - |
| 6 | April 16th | Immunoaffinity techniques |
| 7 | April 23rd | Guest lecture: Prof. Tero Soukka |
| 8 | April 30th | Advanced microscopy I |
| 9 | May 5th | Advanced microscopy II |
| 10 | May 14th | Advanced microscopy III |
| 11 | May 21st | Advanced microscopy IV |
| 12 | find 1 additional date | Electron microscopy |
| | During exam period | Individual oral exams (30 min) |

# Guest lecture on April 23rd

**Prof. Tero Soukka**
University of Turku, Finland
Department of Life Technologies/Biotechnology

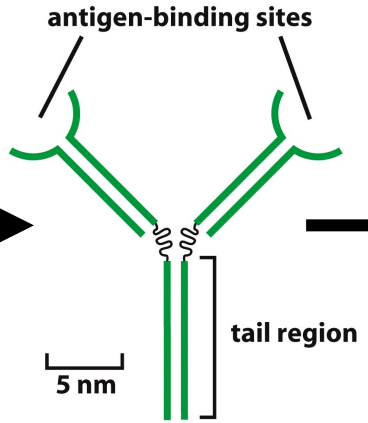
1 pm: Evolution of lanthanide-based labels for immunoassays

2 pm: Research talk open for all

# The idea behind the lecture

Immunology


The “tools“: antibodies

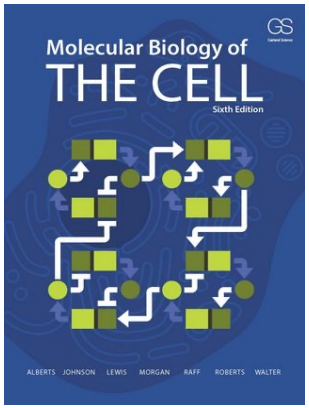



Immunoassay

# The immune system (2 days)

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

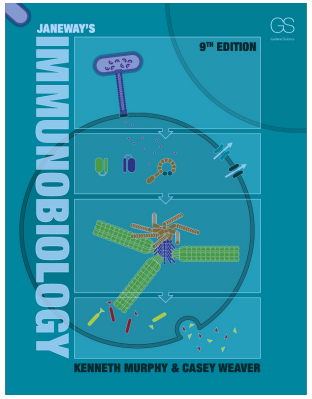
# Recommended reading




**Basic text book**

**Molecular Biology of the Cell** (6th edition)
Alberts, Johnson, Lewis, Morgan, Raff, Roberts & Walter
Garland Science, London 2014
Chapter on Immunity

New (7th) edition




**In depth reading**

**Immunobiology** (7th edition)
Murphy & Weaver
Garland Science, London 2017

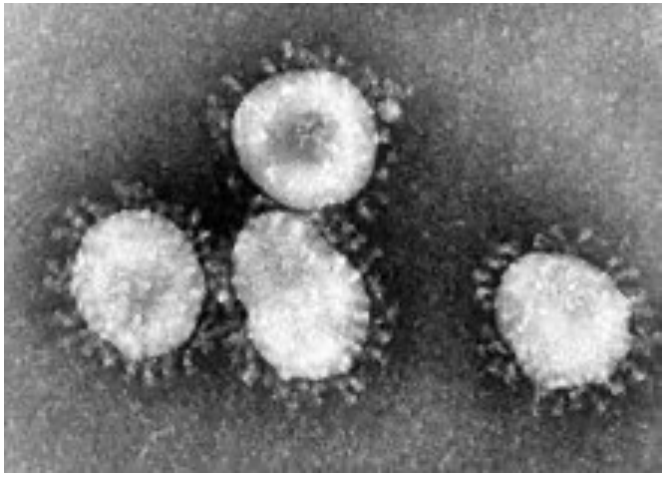
In online folder

Slides of the lecture are available online (Learning Materials)

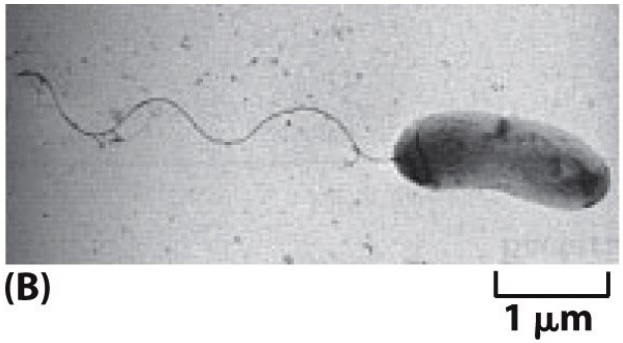
# Overview on our body‘s defenses against an infection

# Challenge: Great variability of infectious diseases

Coronavirus, intracellular



Diameter: ca. 100 nm



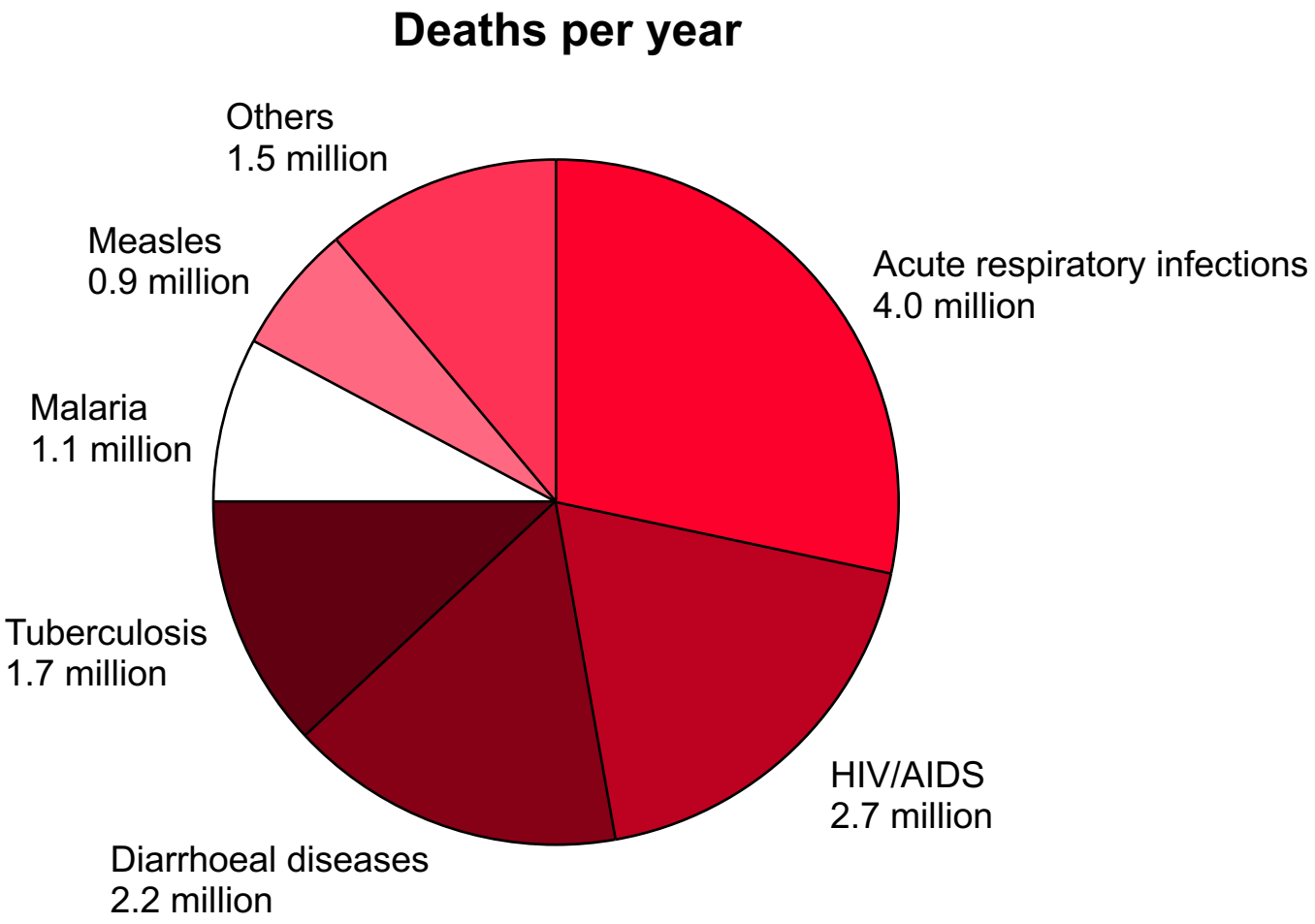

Cholera: bacterium, in the intestine

Toxoplasmosis:
single-celled, eukaryotic parasite, intracellular



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

# Infectious diseases

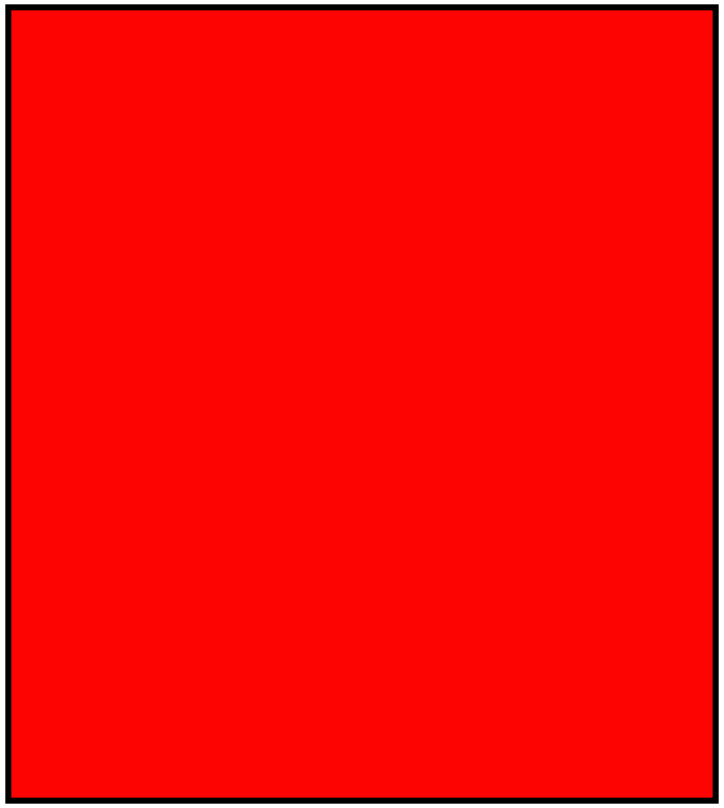



Source:
The World Health Report 2000, WHO

red colors:
Pathogens that enter our body
over mucosal surfaces

# Surface areas of human body

Mucosa

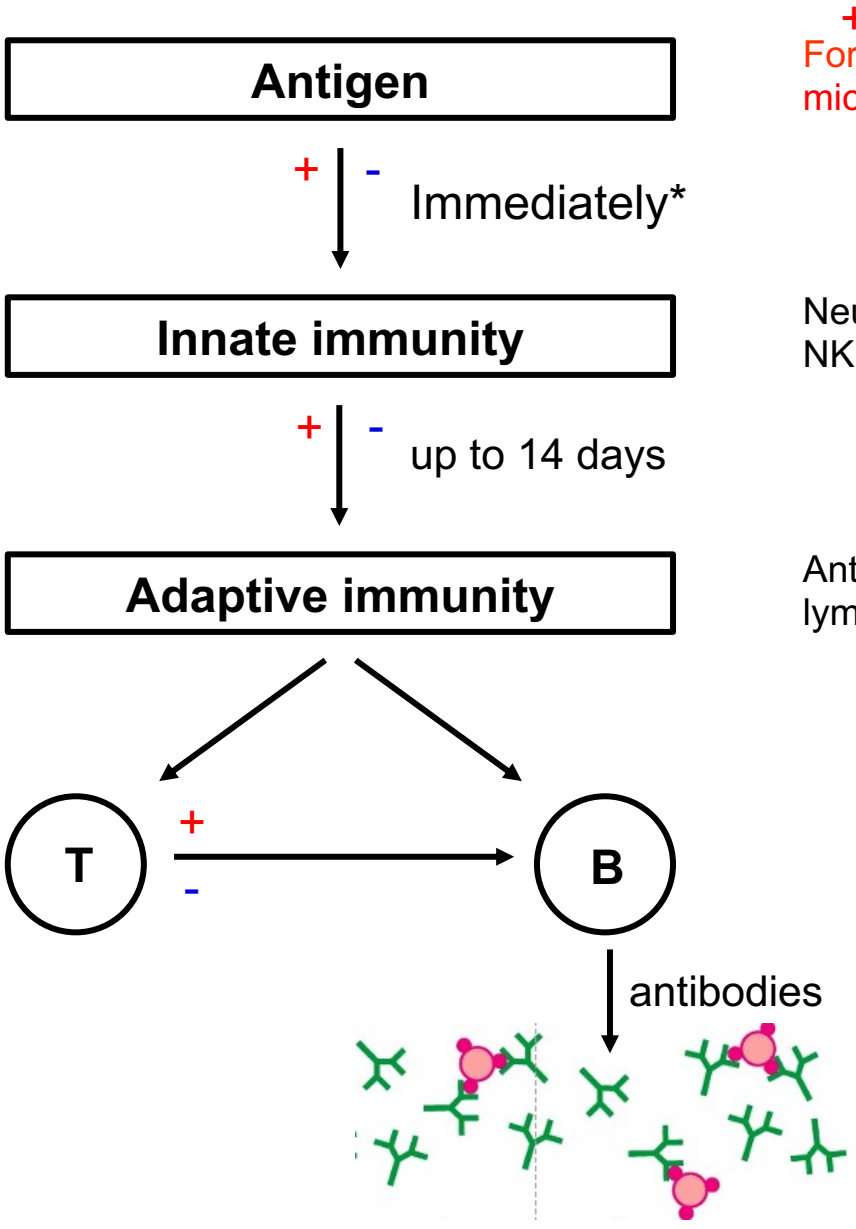


400 $m^2$

Skin

2 $m^2$

# Two lines of defence




+ Foreign and - own structures, microorganisms, viruses

Neutrophil granulocytes, macrophages, NK cells, diverse humoral factors

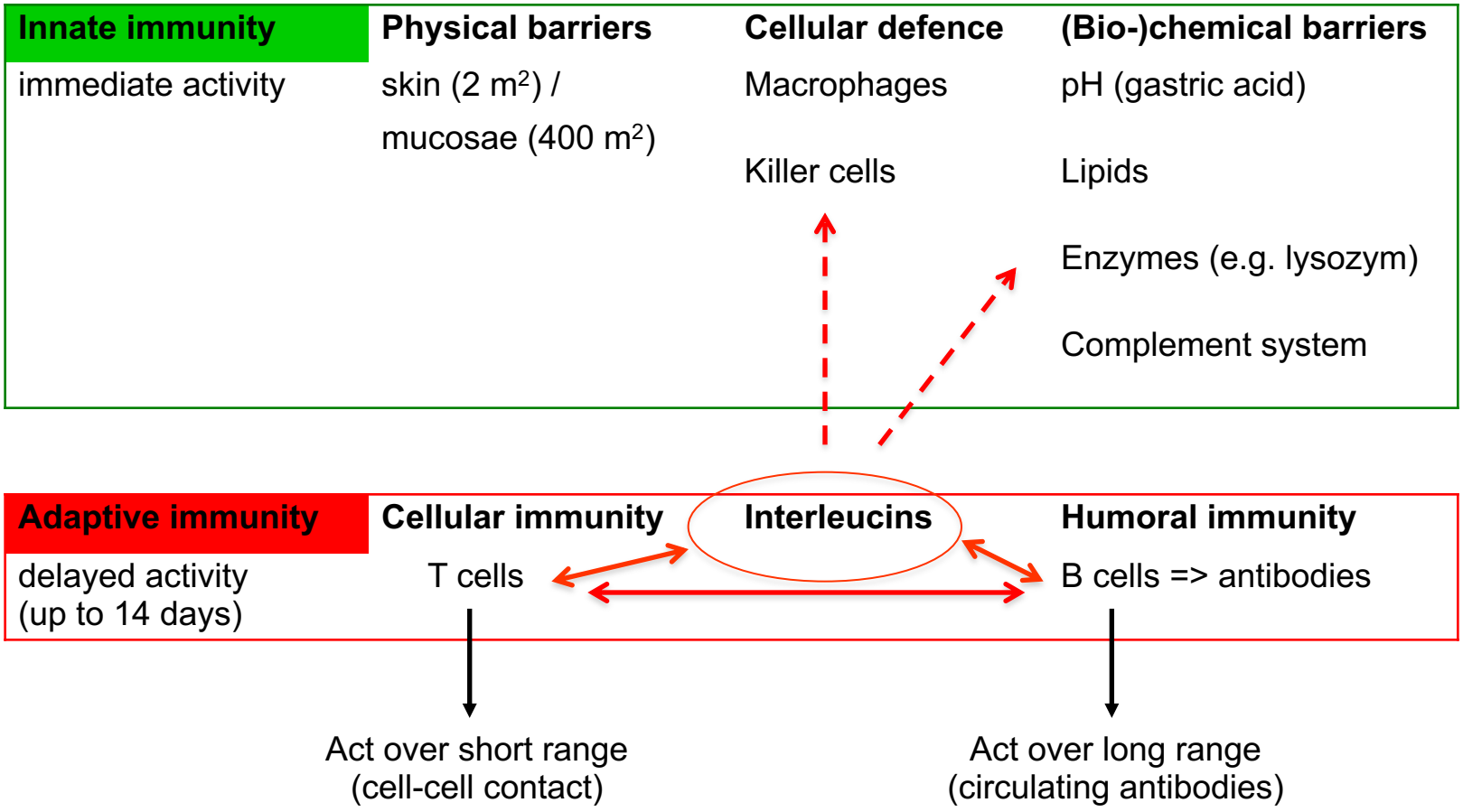
Antigen-presenting cells, T and B lymphocytes, plasma cells, cytokines

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

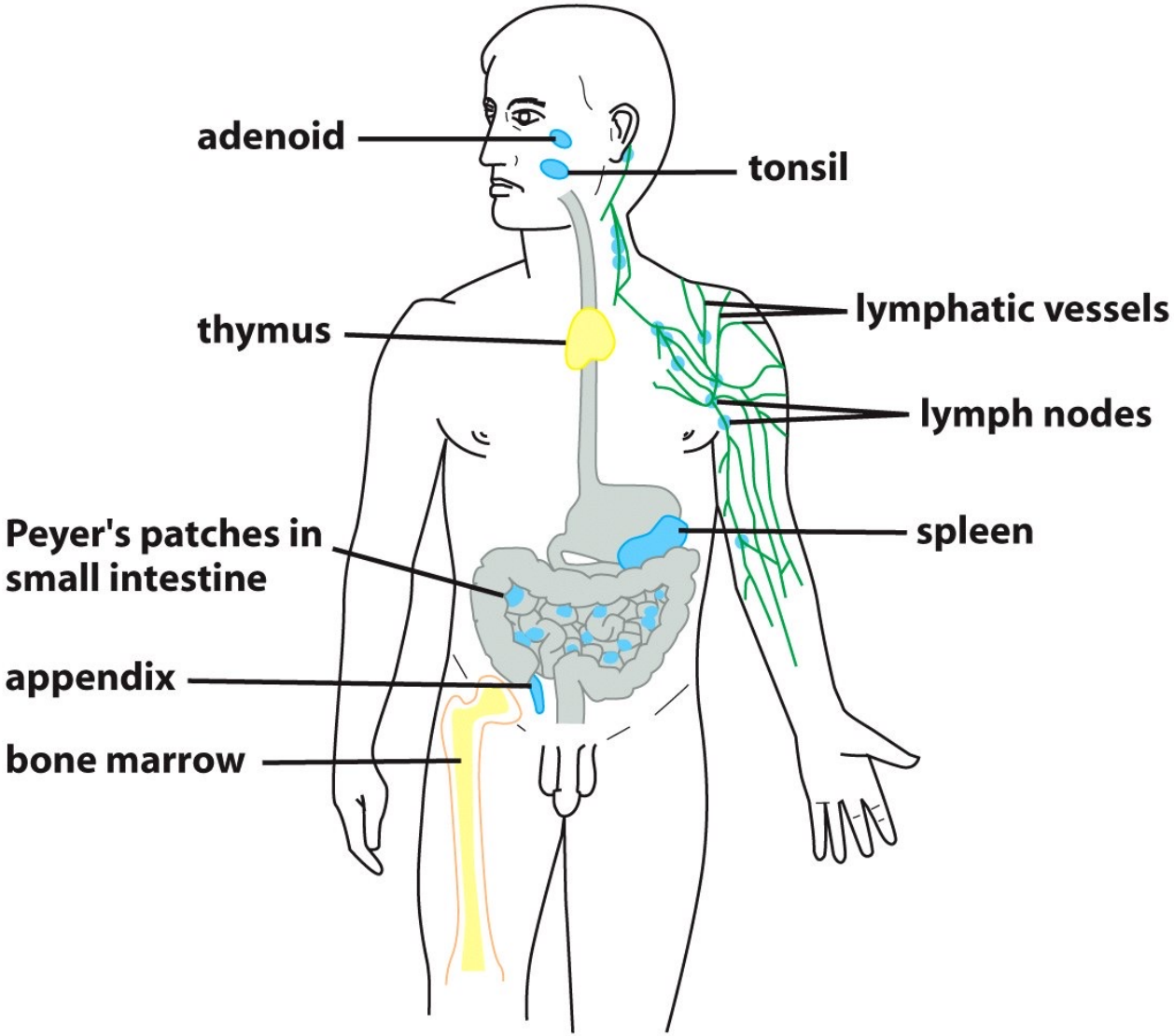
$x_t = x_0 * e^{(\mu * t)}$ ($\mu$=ln2/time for division (hrs))

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

# Innate / adaptive immunity



# Adaptive immunity: Human lymphoid organs

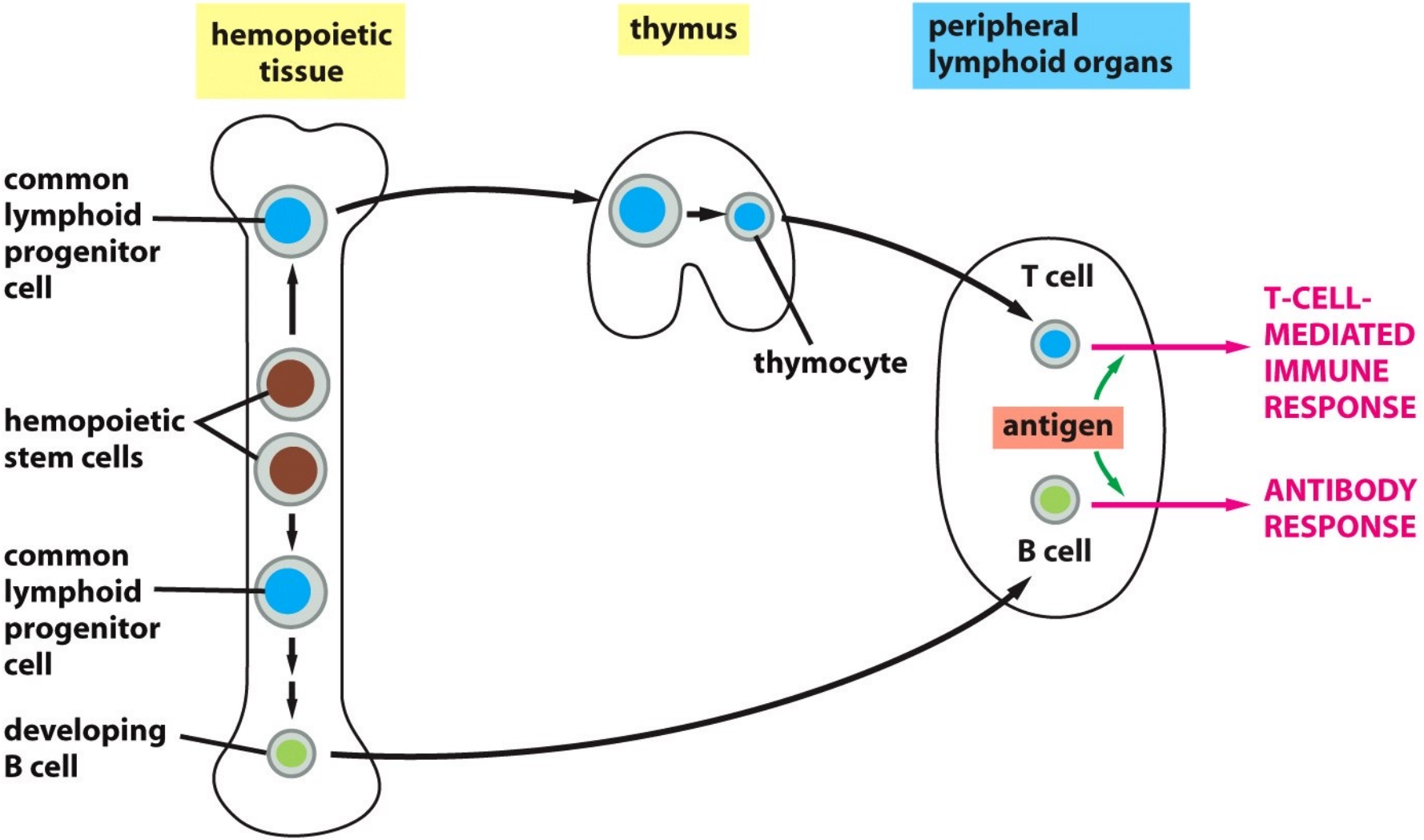



**Primary lymphatic organs (yellow):**
Bone marrow: B-cells
Thymus: T-cells

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

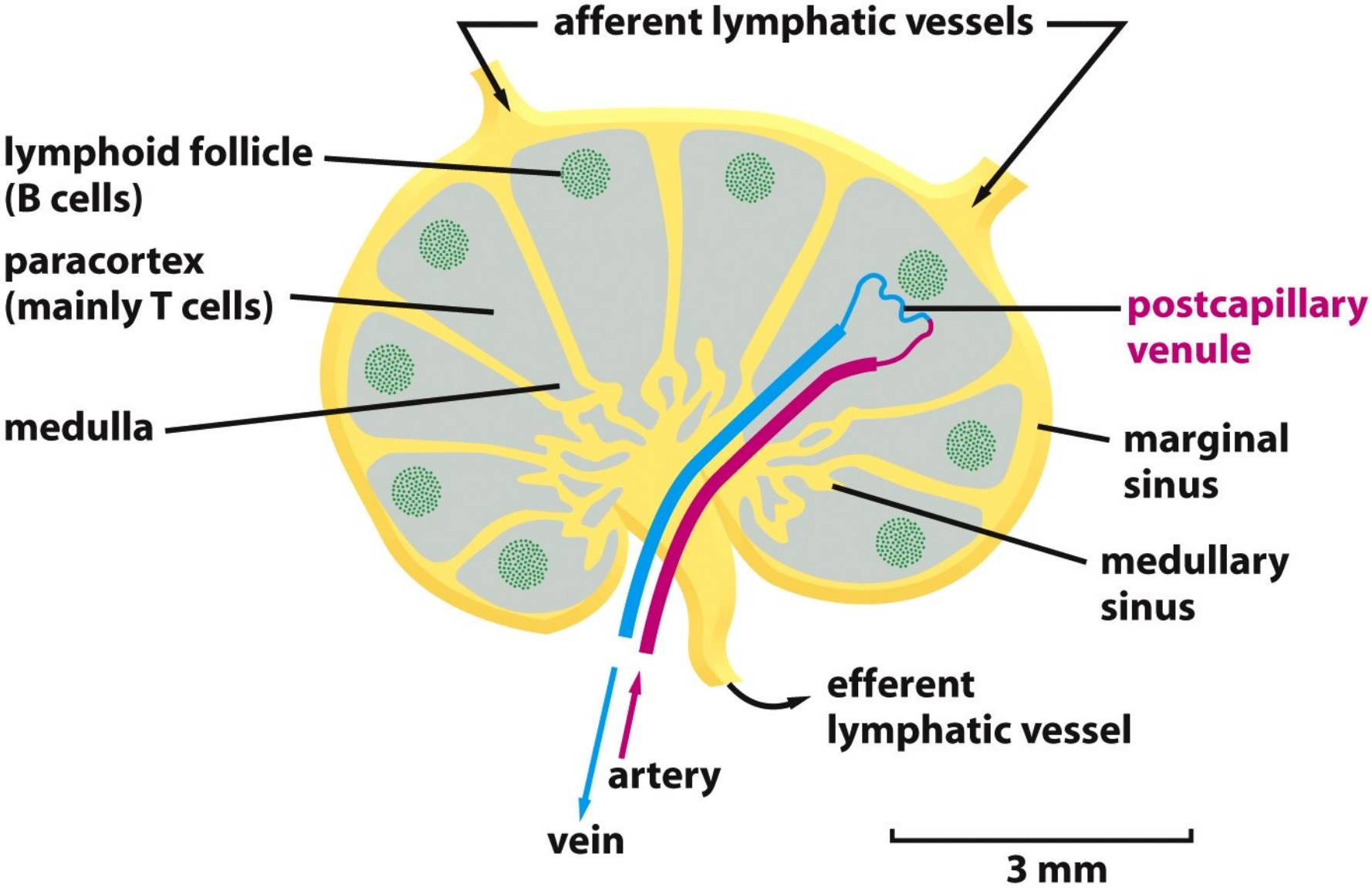
$10^{12}$ lymphocytes (ca. 1 kg)

# Development of B und T cells

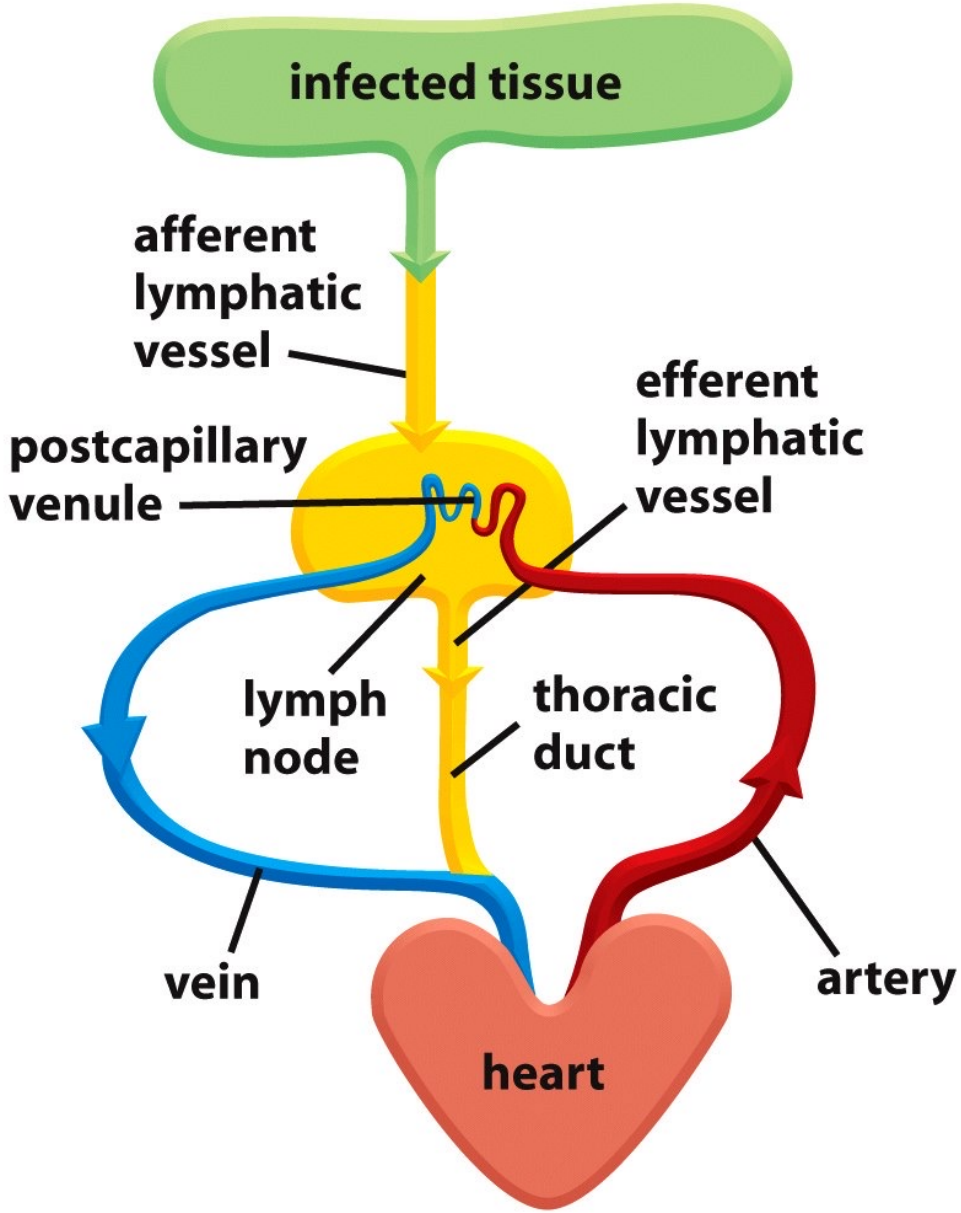



=> Bone marrow donation to reconstitute the immune system

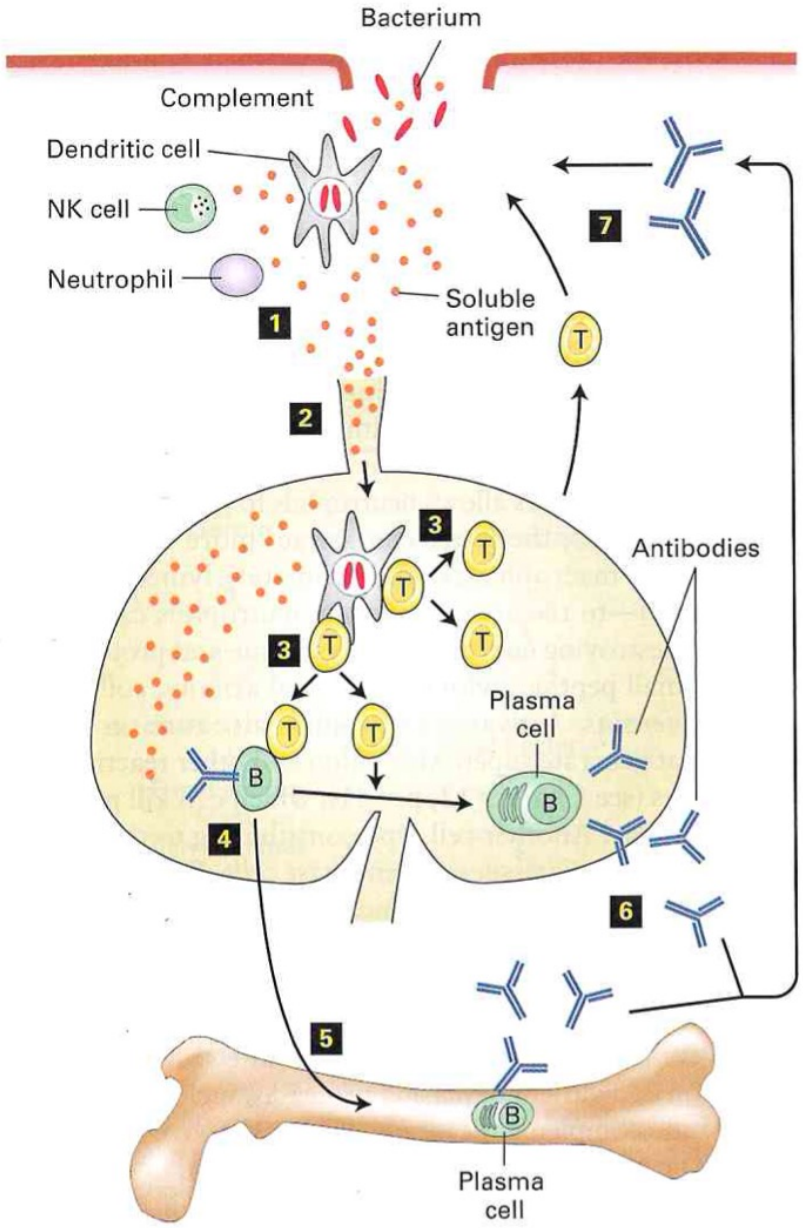
# Lymph node


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



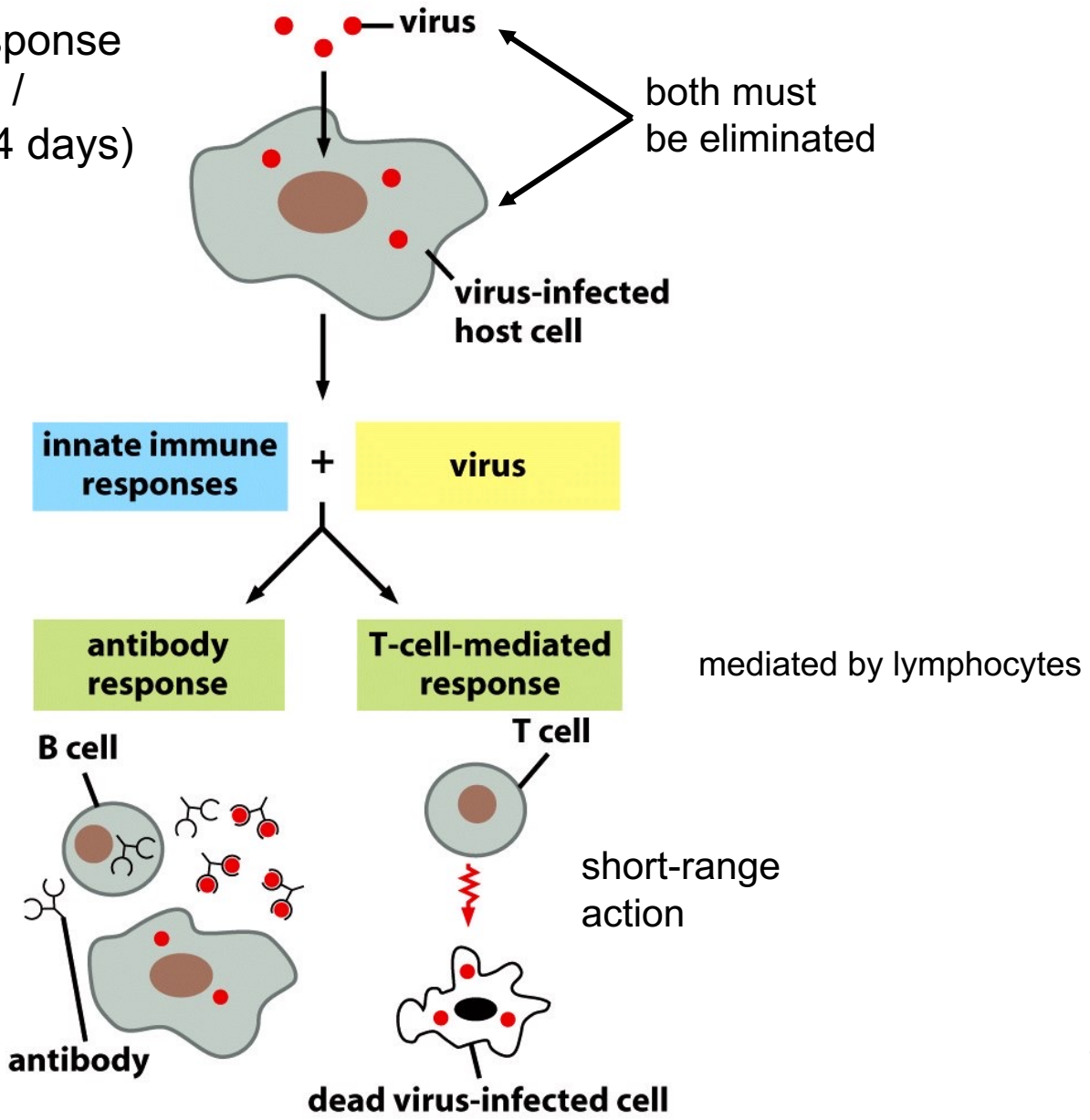
# Overview of an inflammatory response



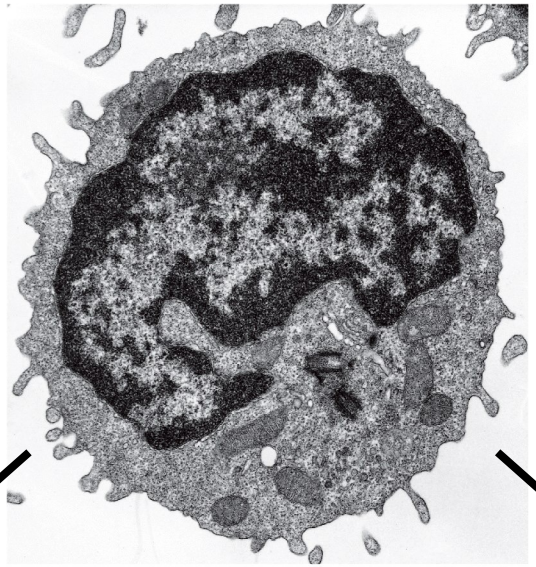

1) A bacterium encounters a first line of defense (innate immune response)
2) Breakdown of bacterium and release of antigens
3) Dendritic cells take up antigen and activate T cells
4) T cells proliferate and activate B cells
5) B cells differentiate into plasma cells
6) Plasma cells produce antibodies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

# Two classes of adaptive immune responses

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

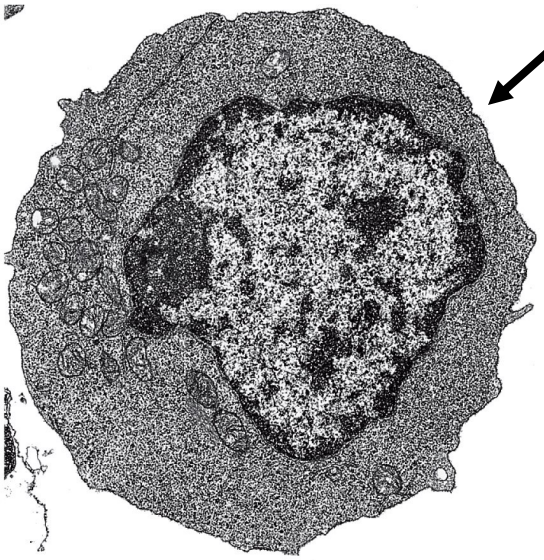


# Activation of lymphocytes



resting T or B cell

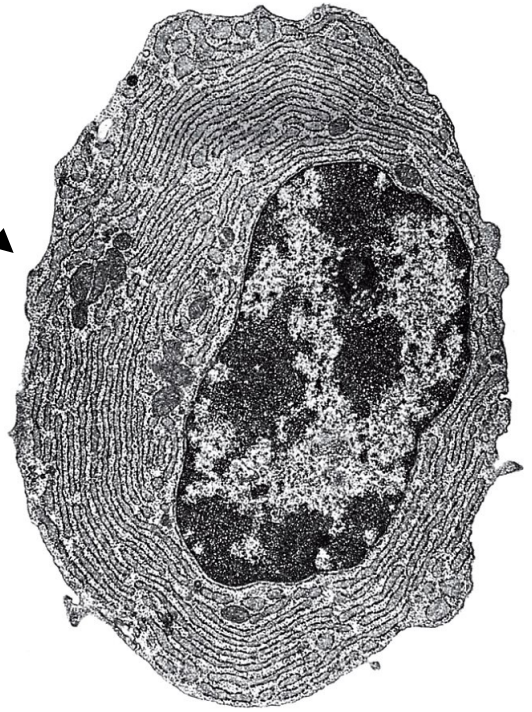
1 μm



effector T cell

1 μm

cytotoxic T cells
helper T cells
regulatory T cells

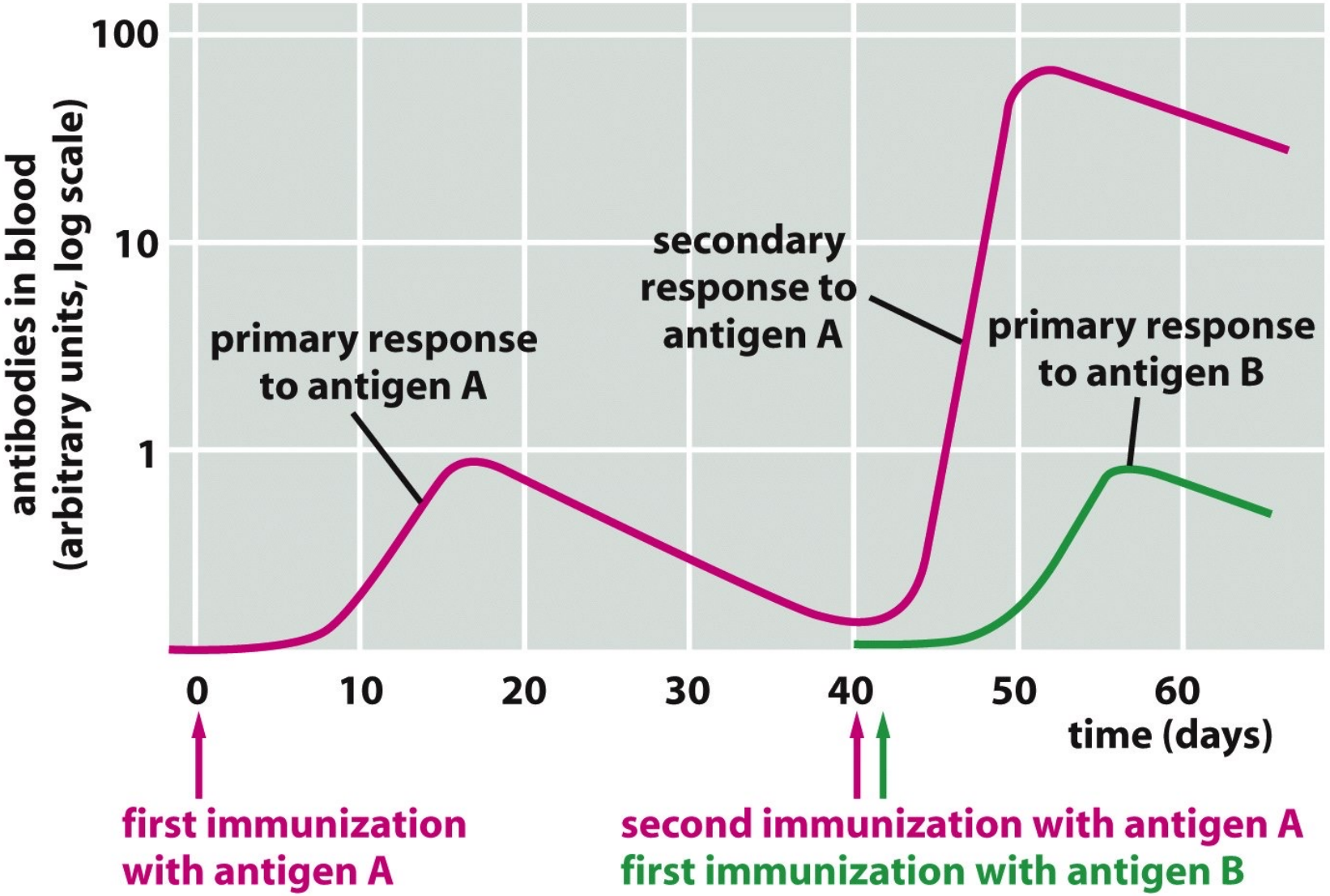


effector B cell (plasma cell)

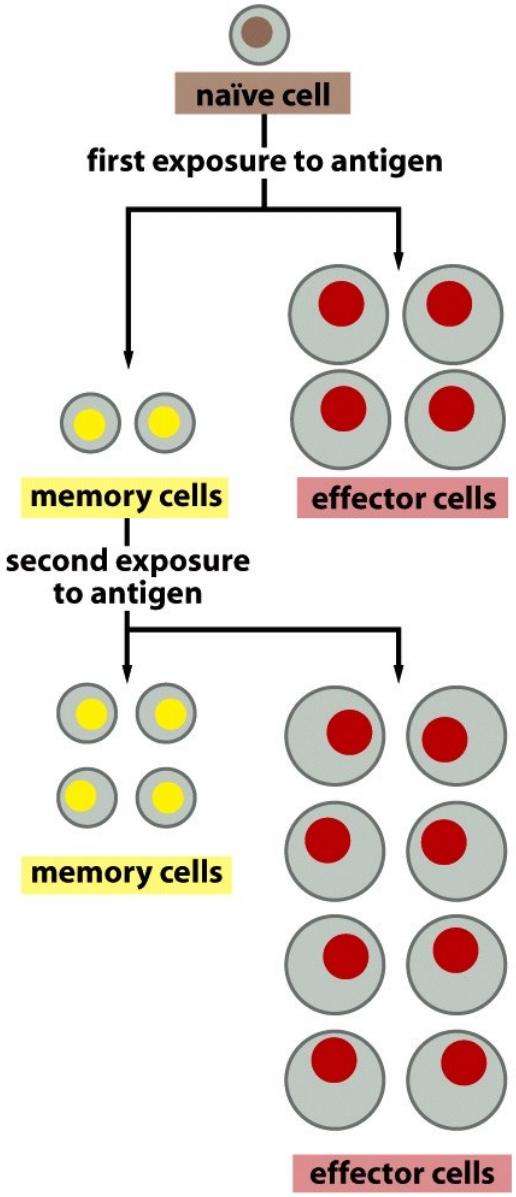
1 μm

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

# Progress of immune response

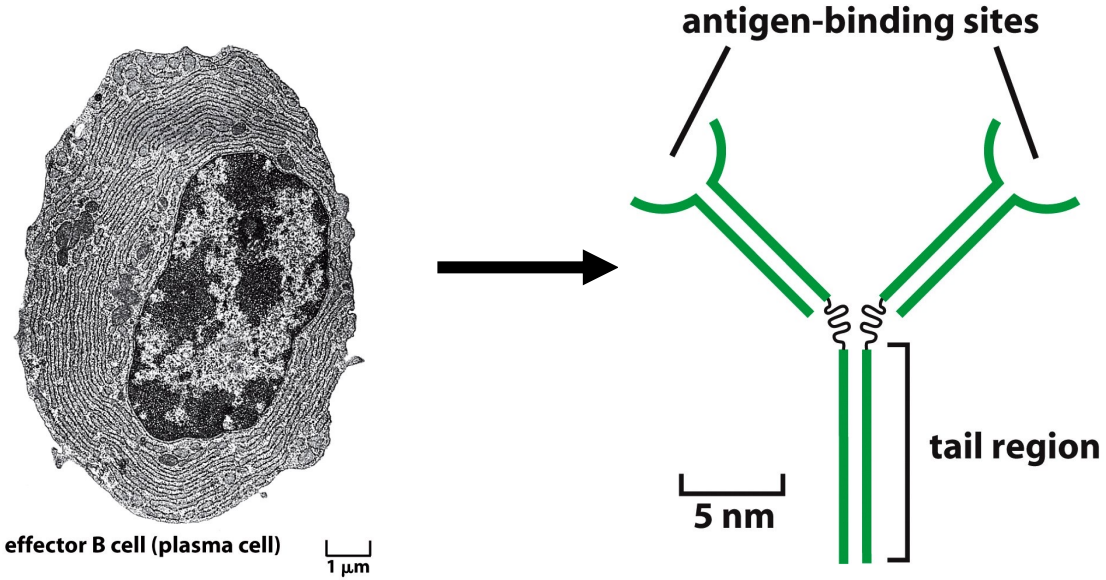


# Immunological memory

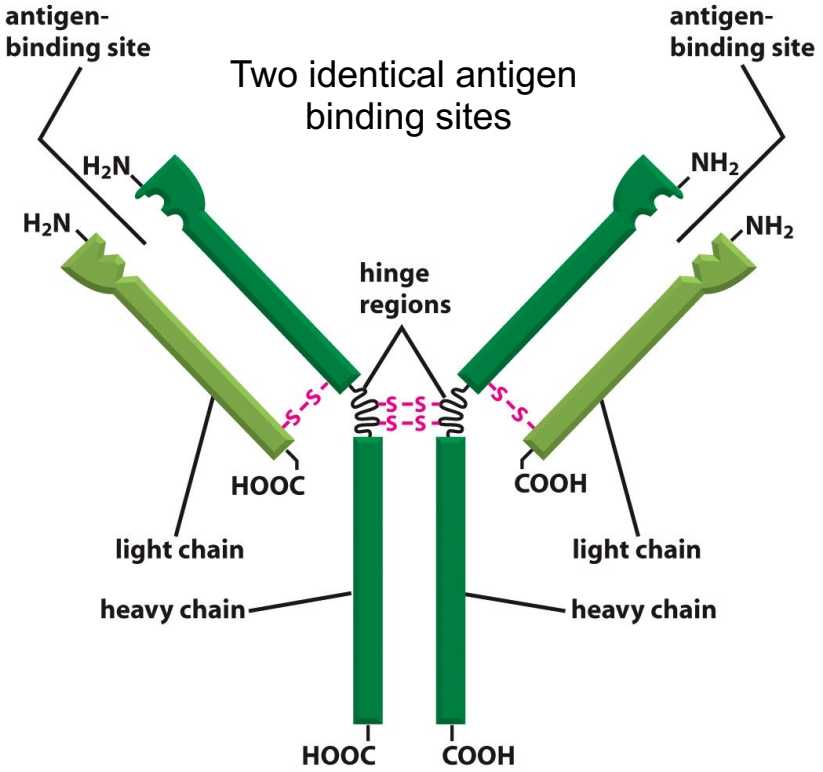


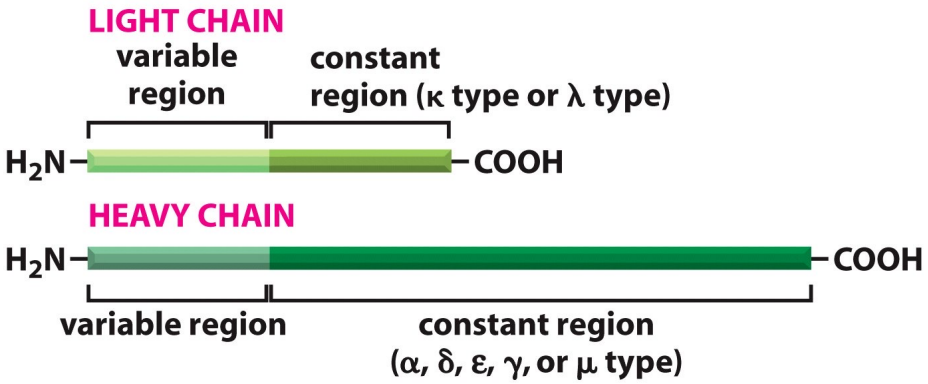

Vaccination!

# B cells and antibodies

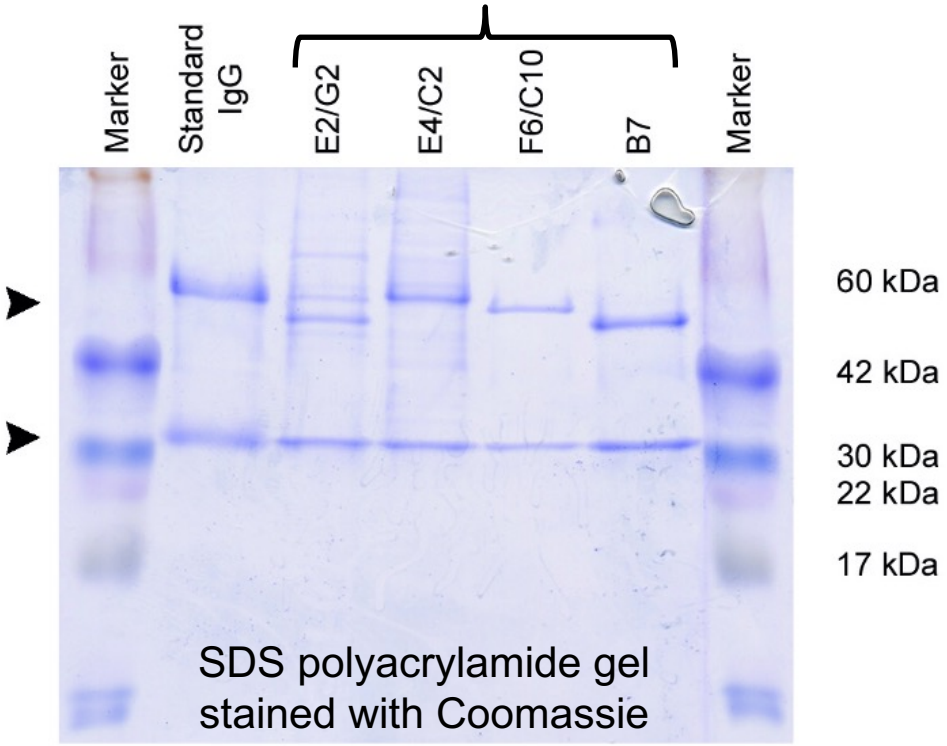


# Structure of IgG




4 different IgG antibody clones against the same antigen



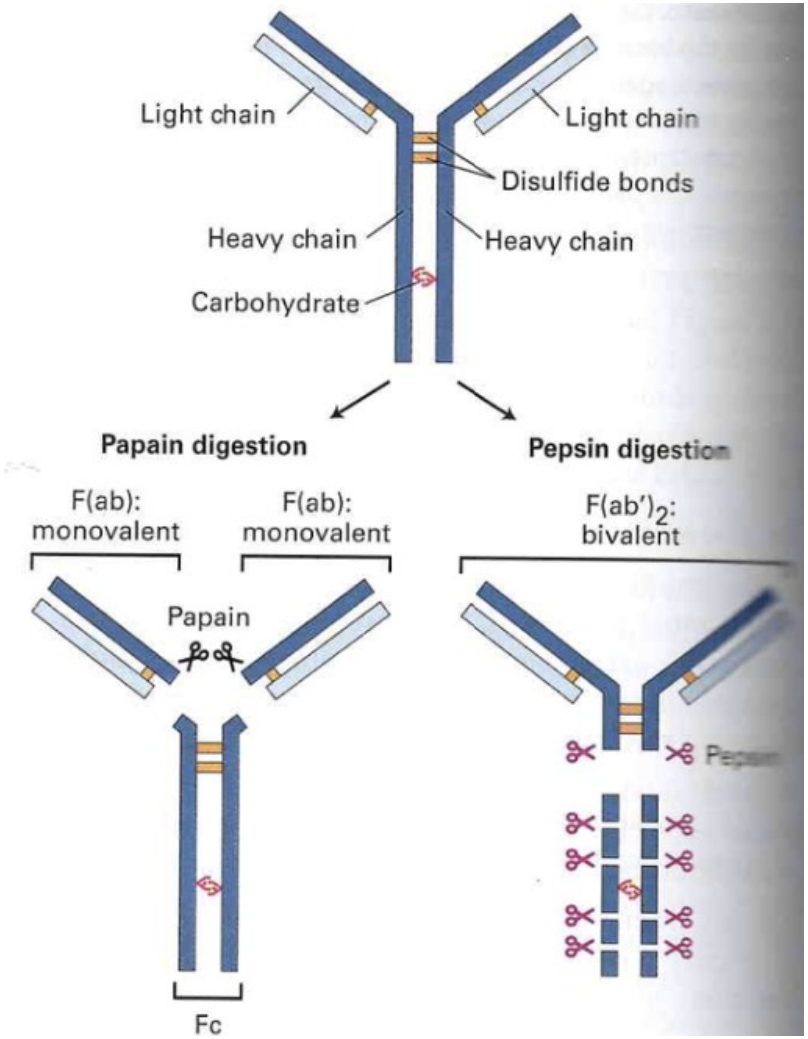

⇒ 2 light chains (25 kDa, light green)

⇒ 2 heavy chains (50 kDa, dark green)

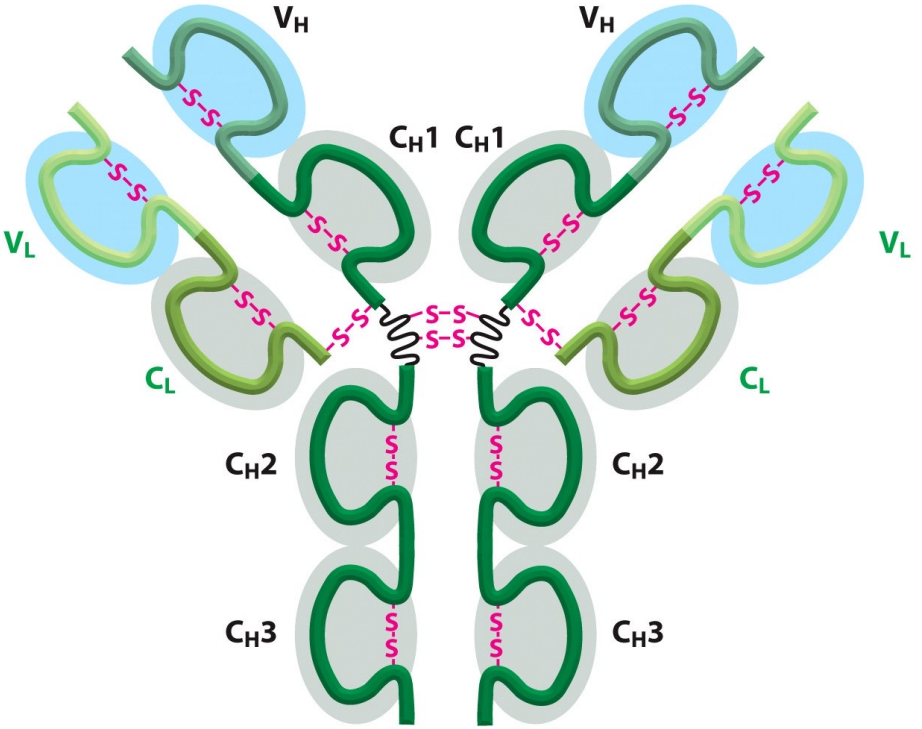
total mass: 150 kDa

# Structure of IgG

Fragmentation of antibody

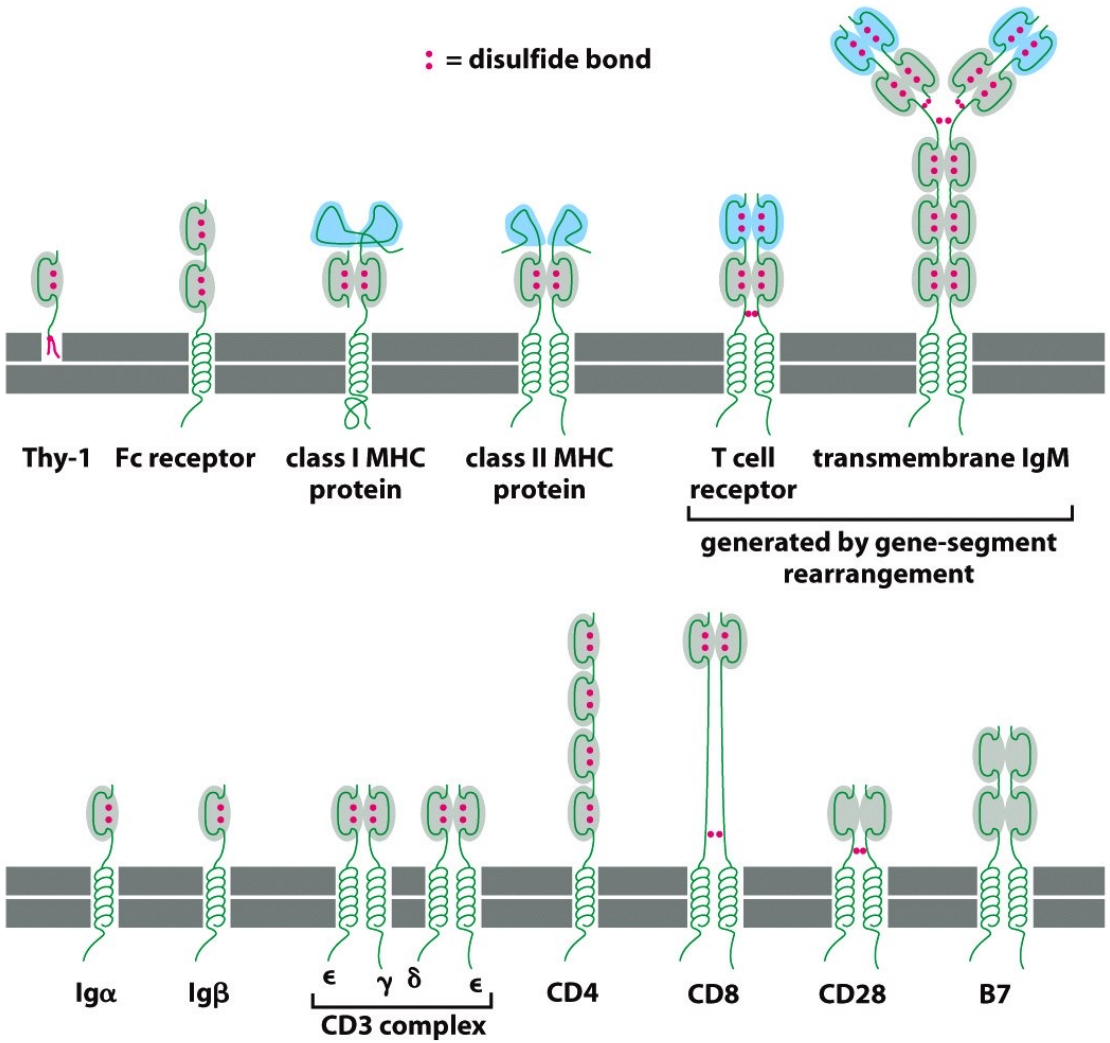



Immunoglobulin domains



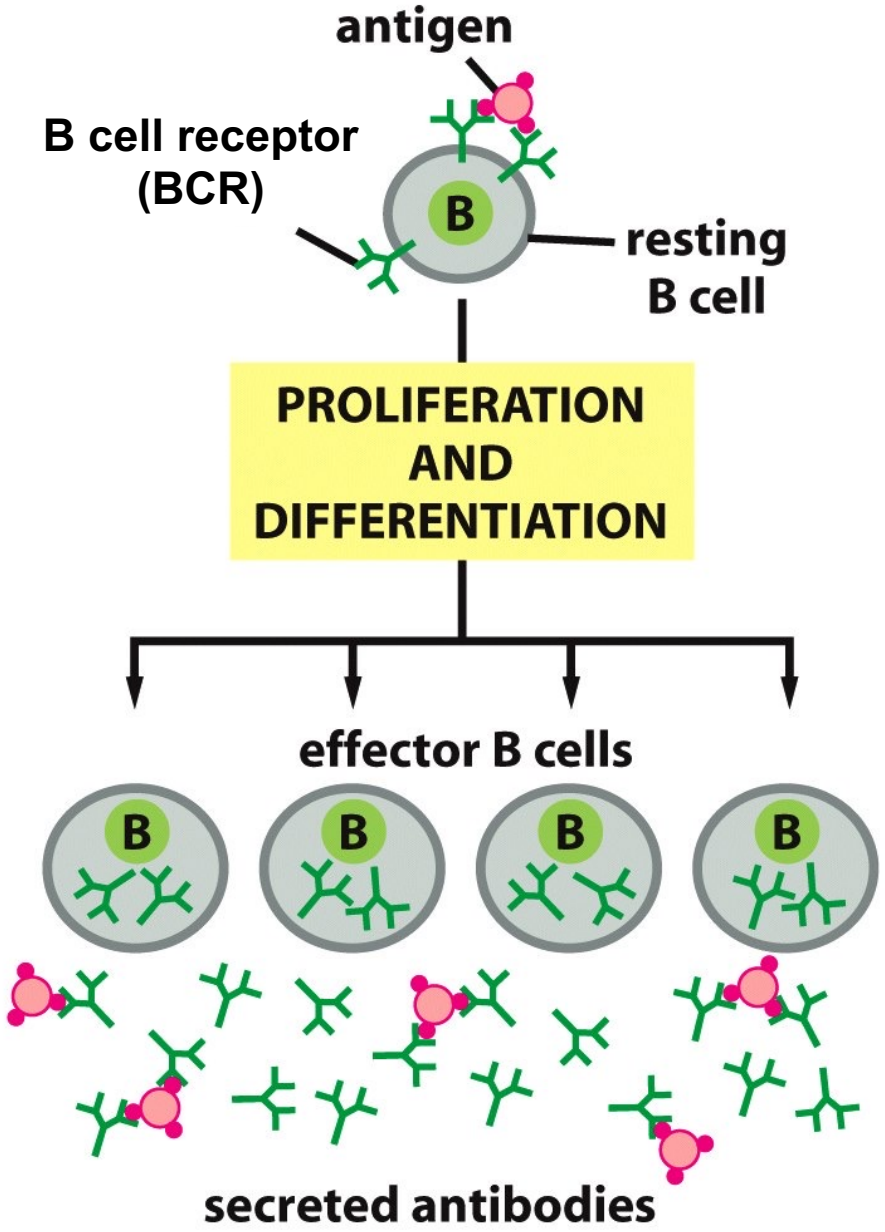

F: fragment
ab: antigen binding
c: crystallizable (constant)

# 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

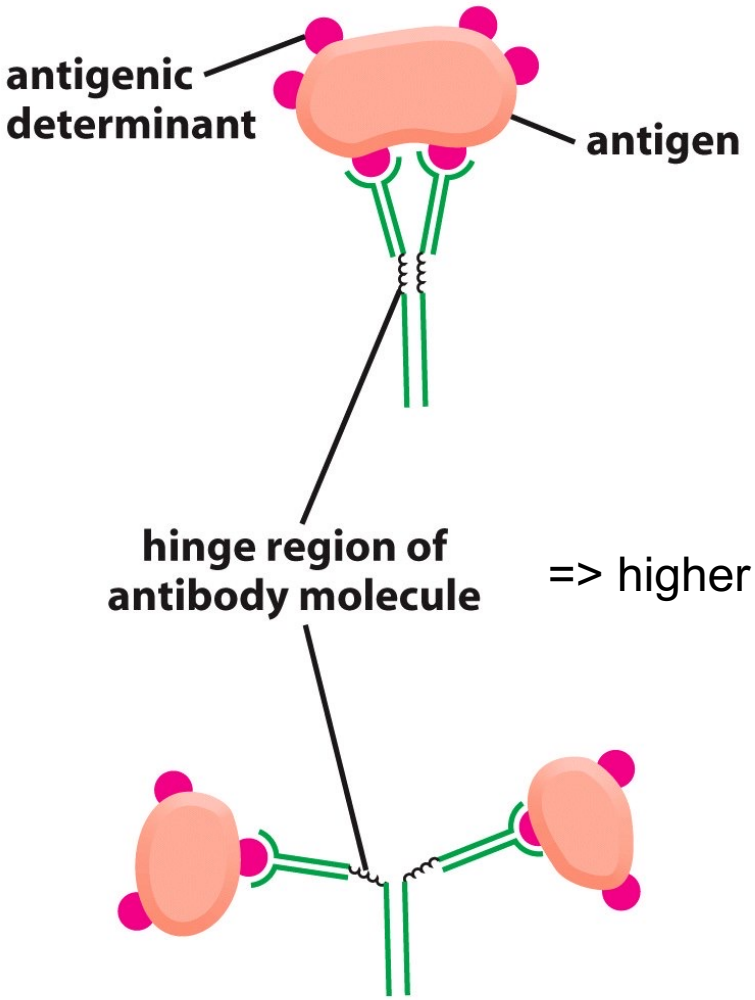



a single clone
of B cells

⬇

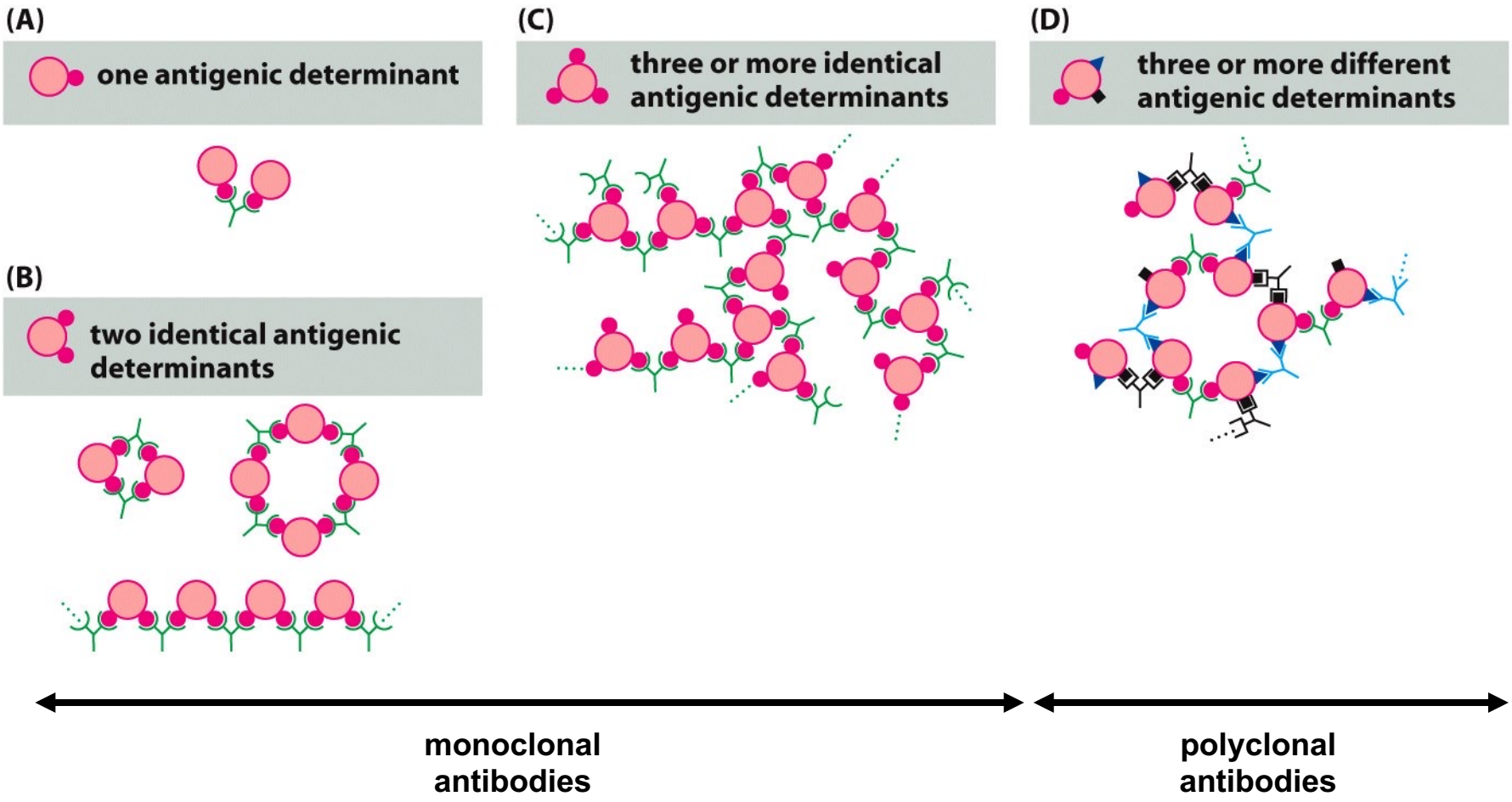
“monoclonal
antibodies“

# The hinge region



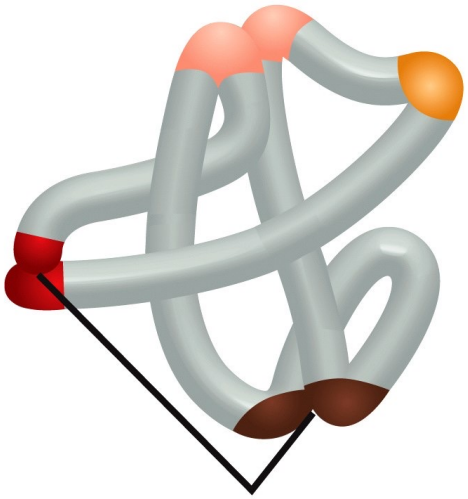

=> higher flexibility

# Interactions of antibody and antigen



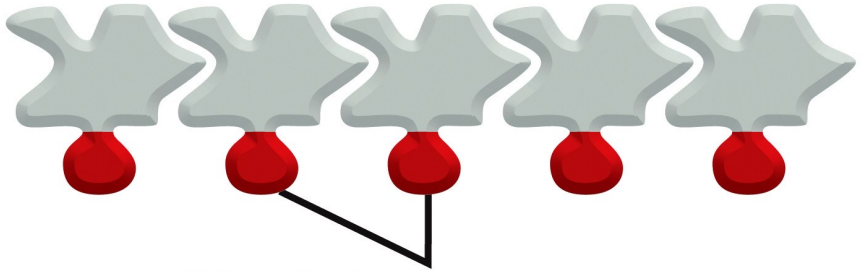
# Multiple antigenic determinants: epitope

**MULTIVALENT ANTIGEN**



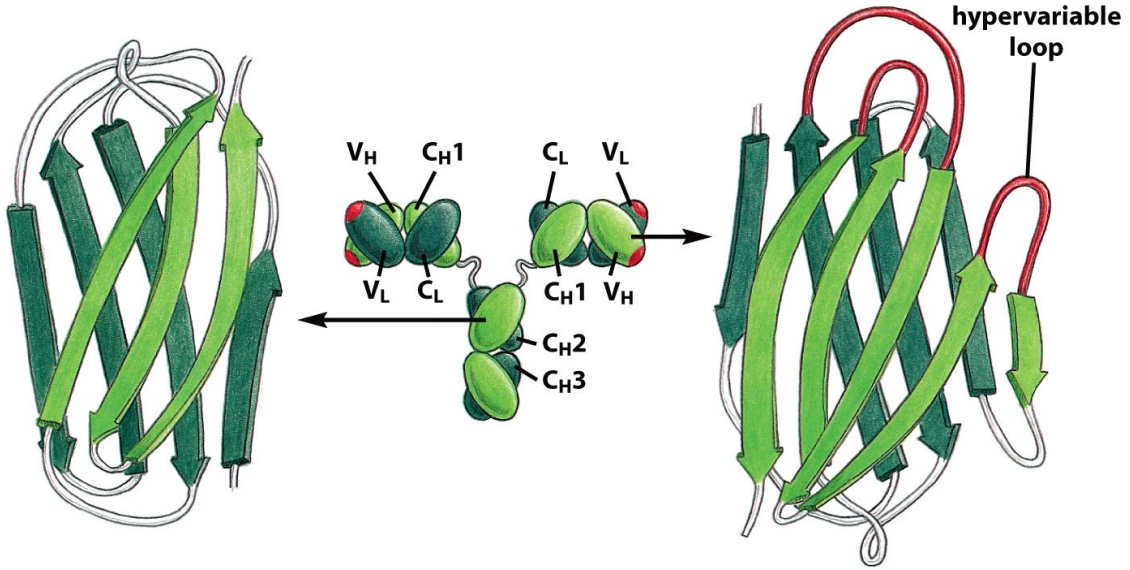
multiple **different** antigenic determinants

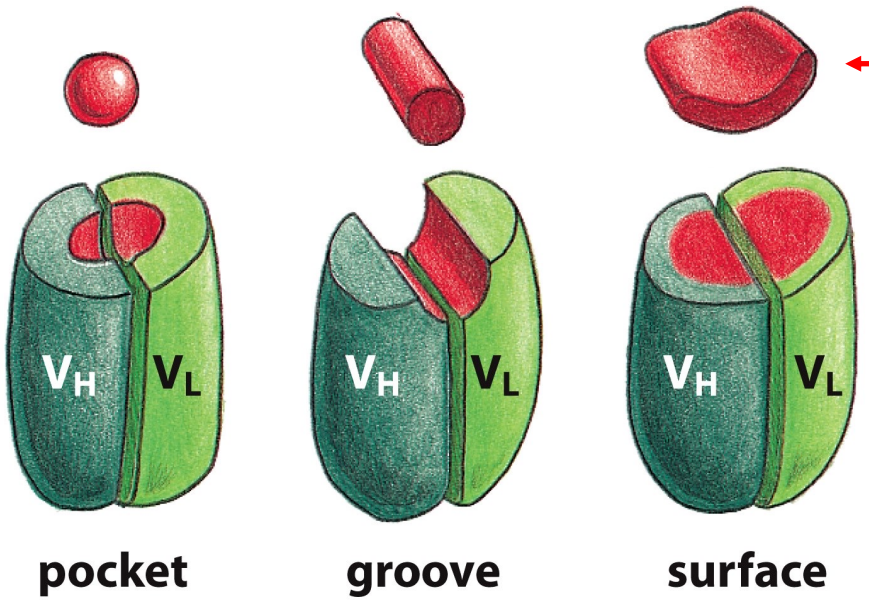
**POLYVALENT ANTIGEN**



multiple **identical** antigenic determinants

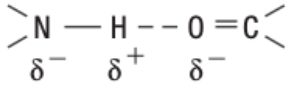
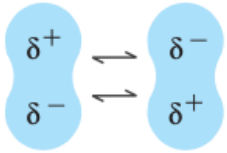
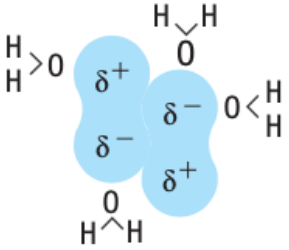
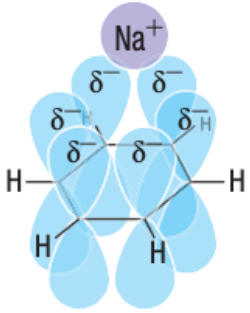
# Antigen-binding sites of antibodies



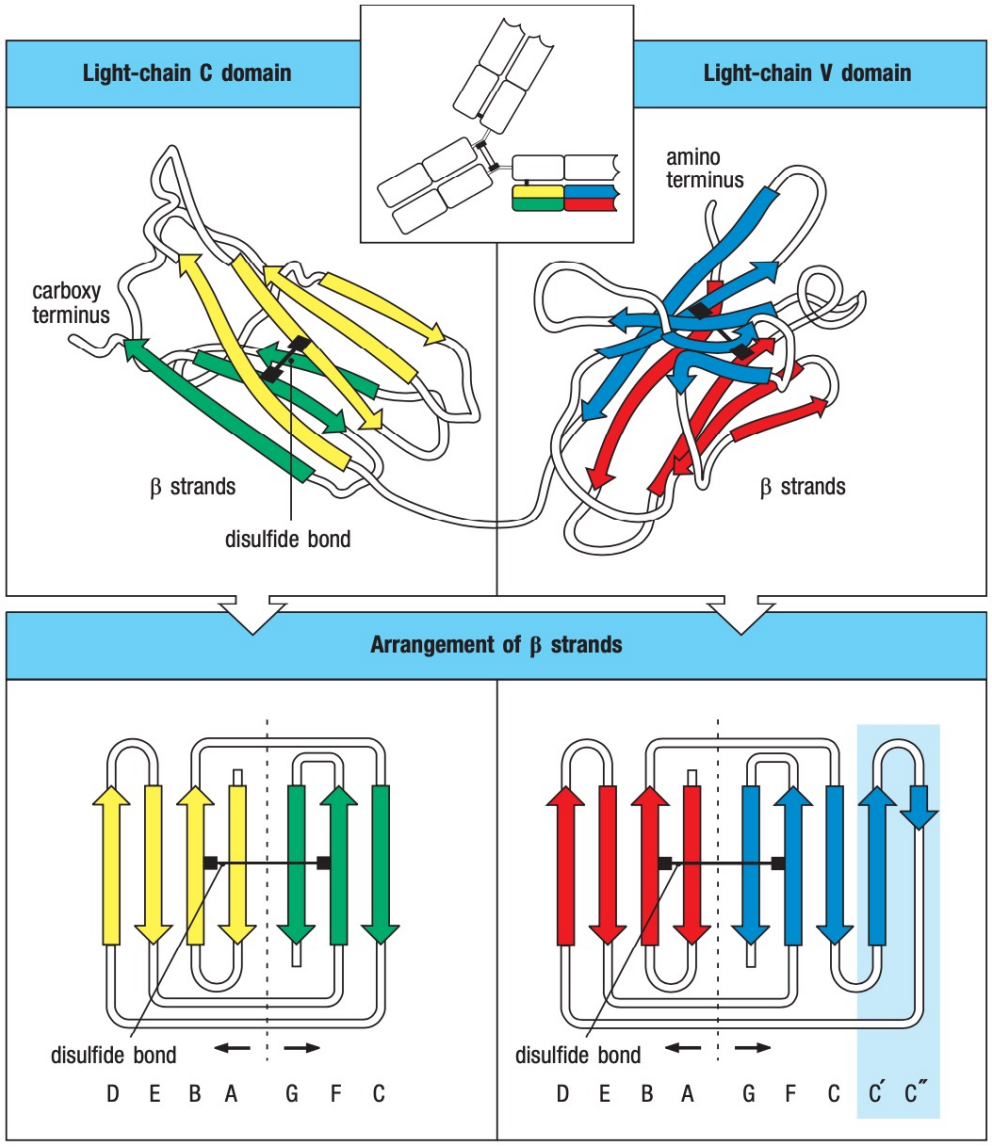




Different antigenic structures

# 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



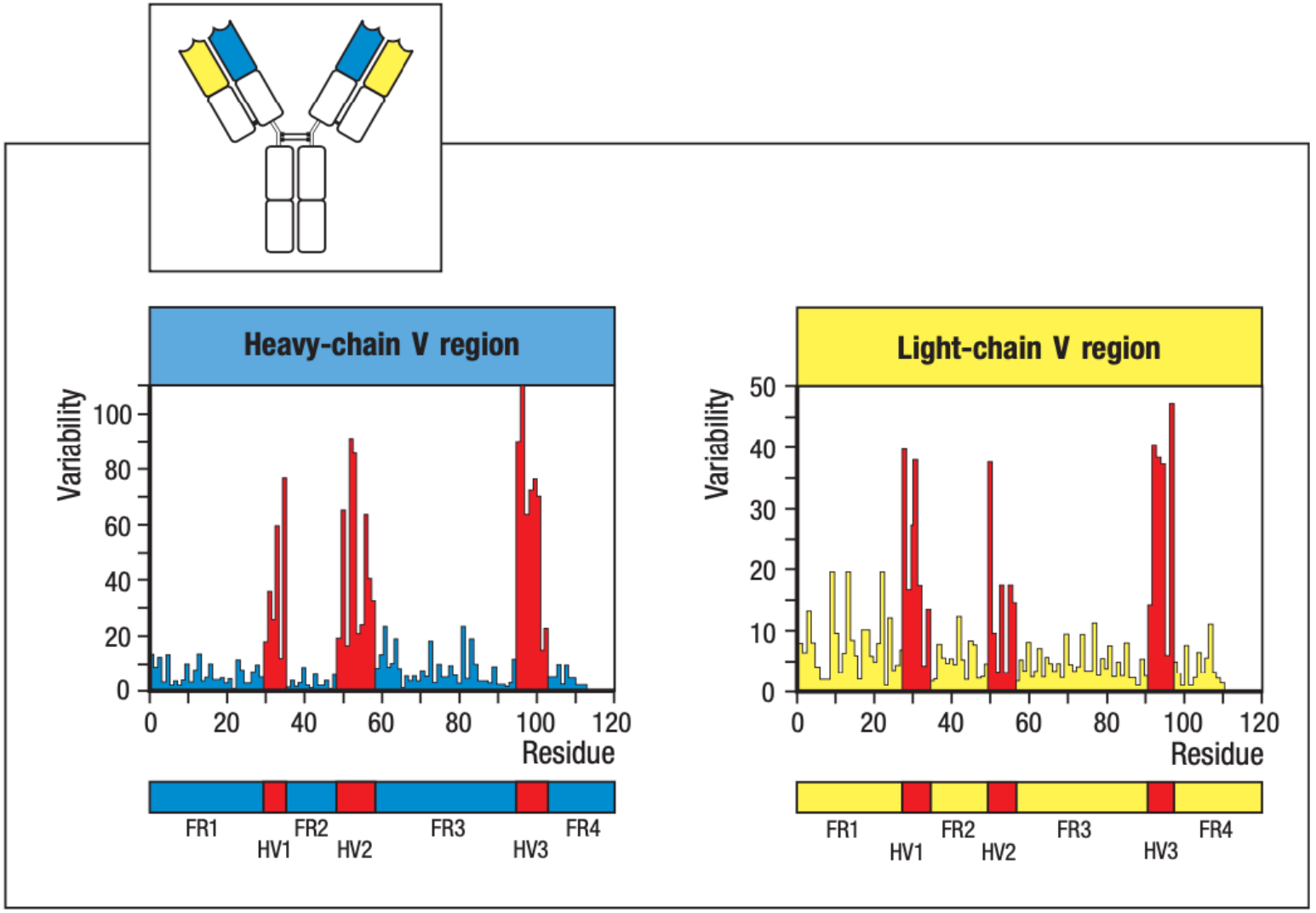

4 strand + 3 strand

4 strand + 5 strand

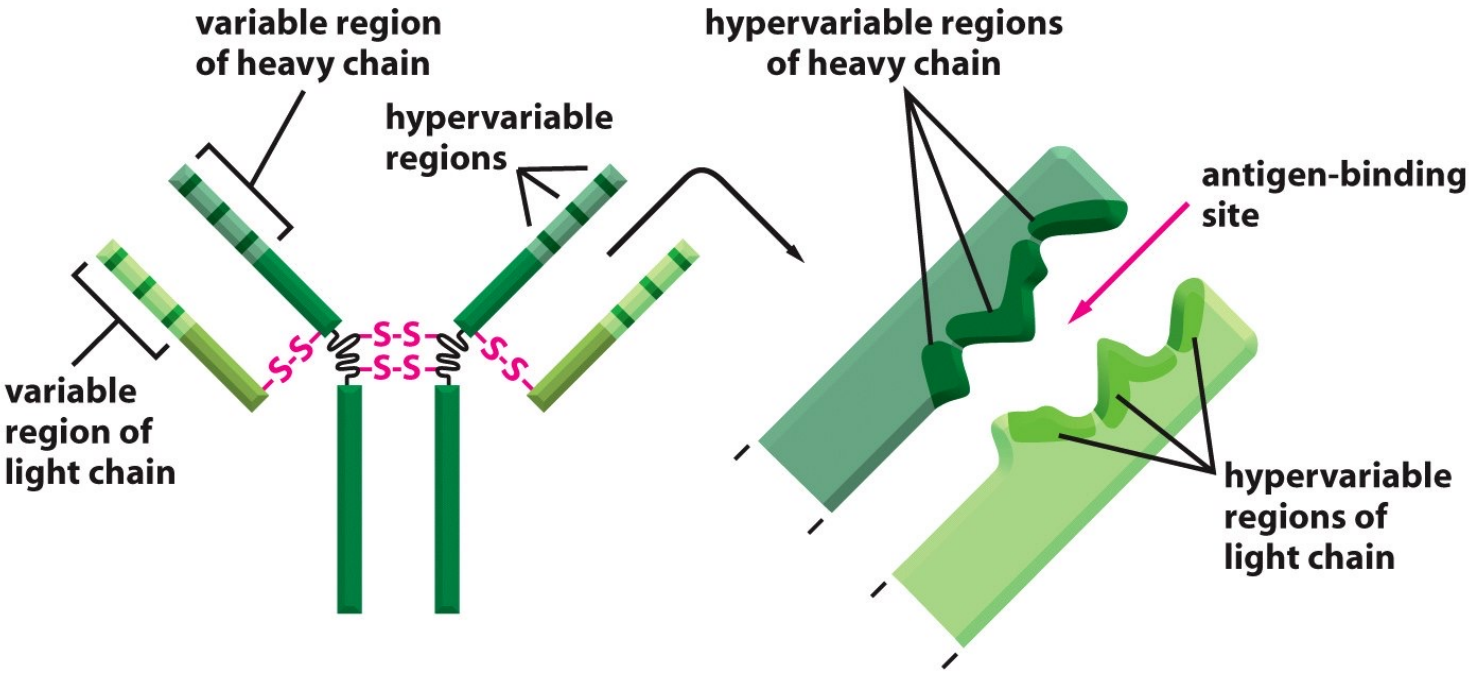
anti-parallel β sheets form a β barrel

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

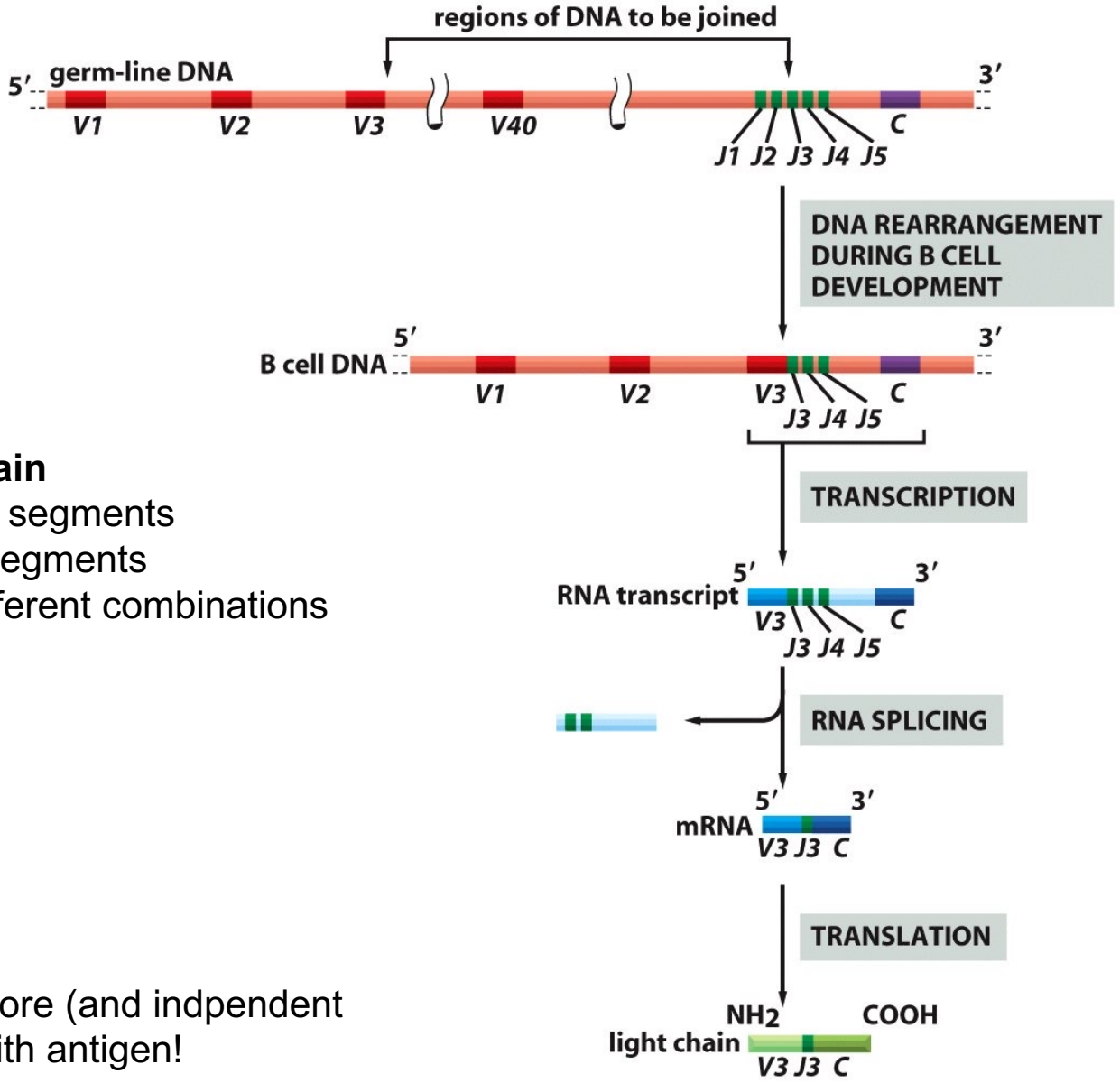
# Hypervariable regions of binding sites



# Hypervariable regions of binding sites



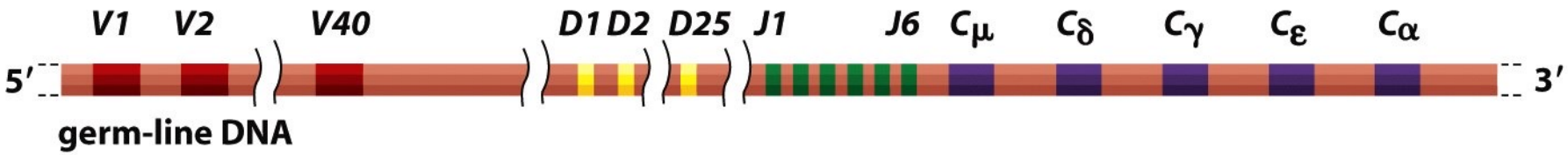
# Generation of antibody diversity: light chain




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

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

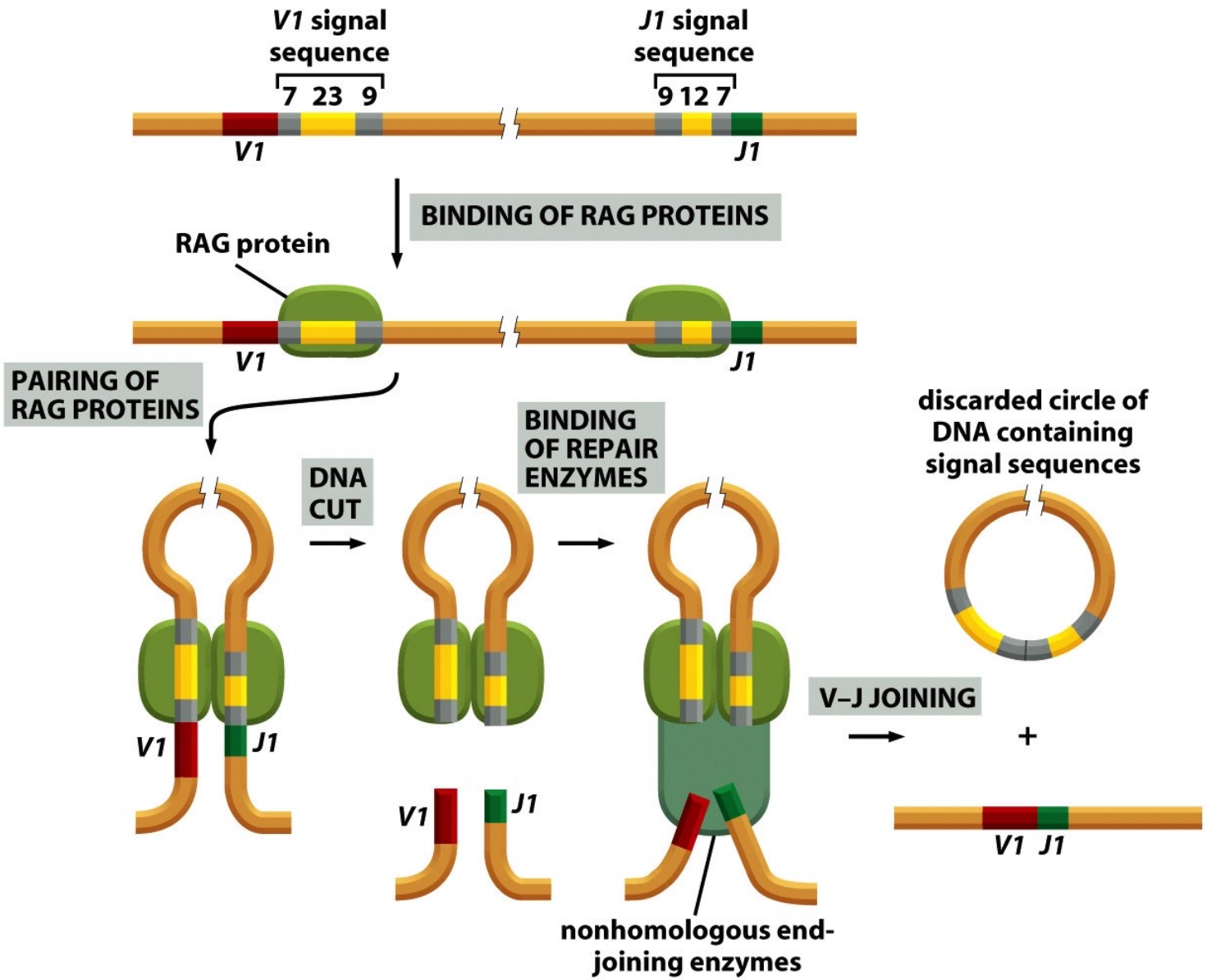
# Generation of antibody diversity: heavy chain



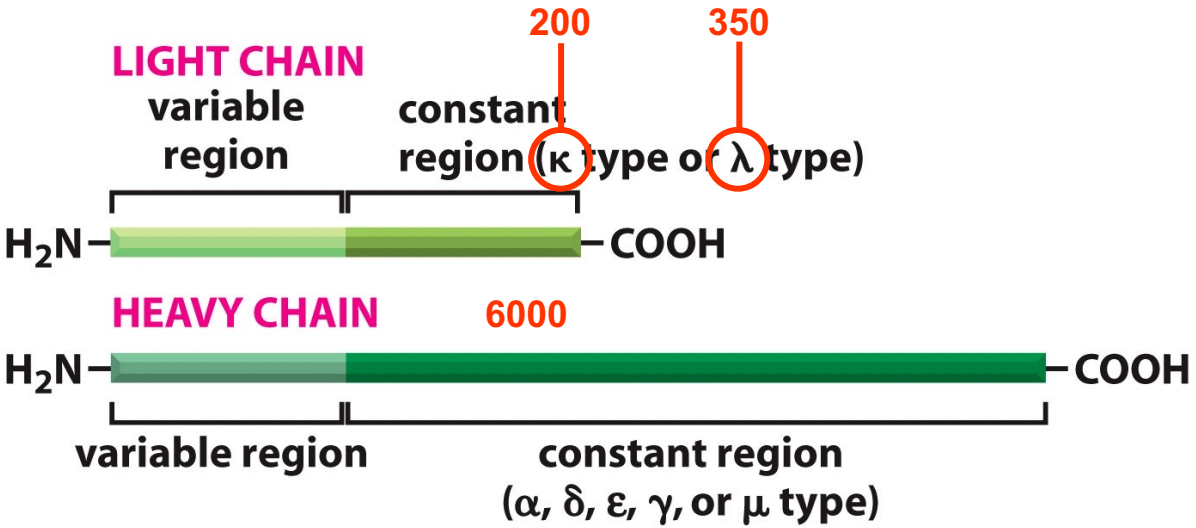

**heavy chain**
40 V gene segments
25 D gene segments
6 J gene segments

=> 6000 combinations

# Gene segment joining



# Generation of antibody diversity

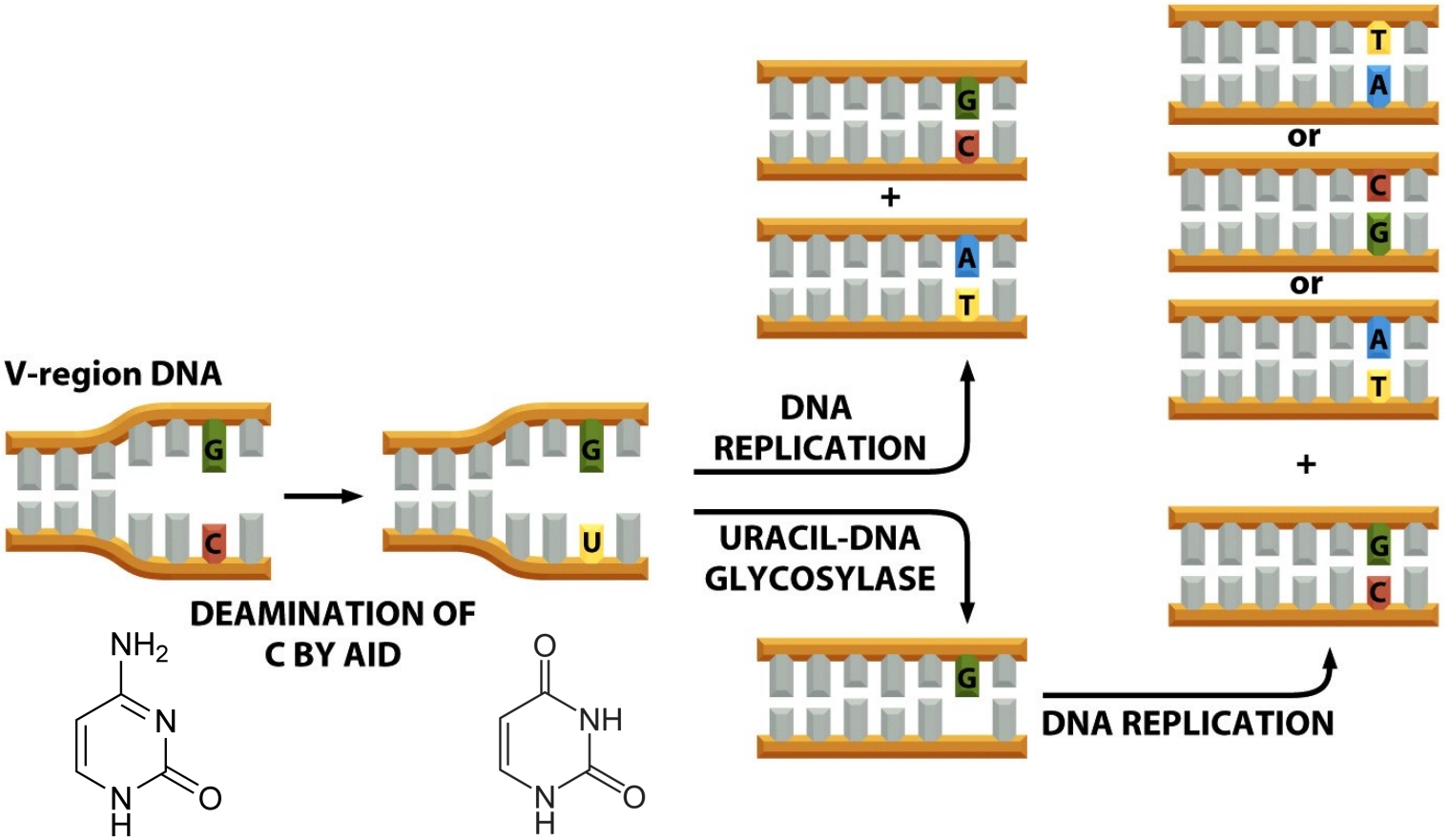



=> about 2.000.000 combinations

# Affinity maturation of antibodies

Somatic hypermutation by activity-induced deaminase (AID)

=> 1 mutation per V region per cell cycle





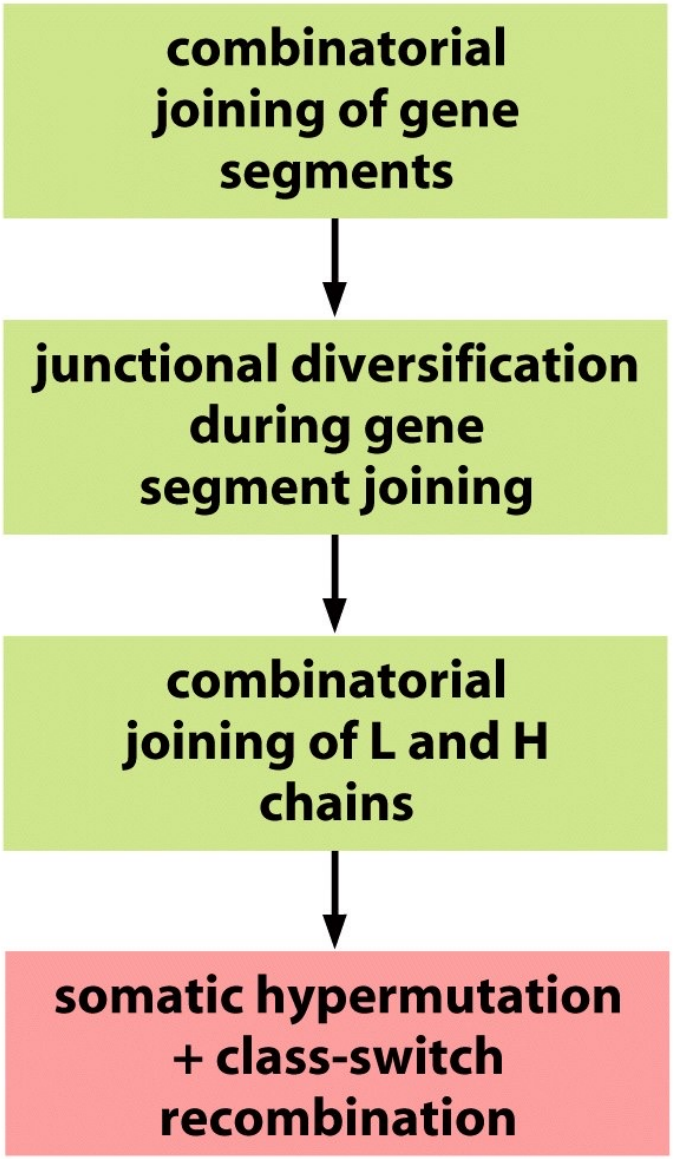


Cytosin:
forms 3 hydrogen bonds




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

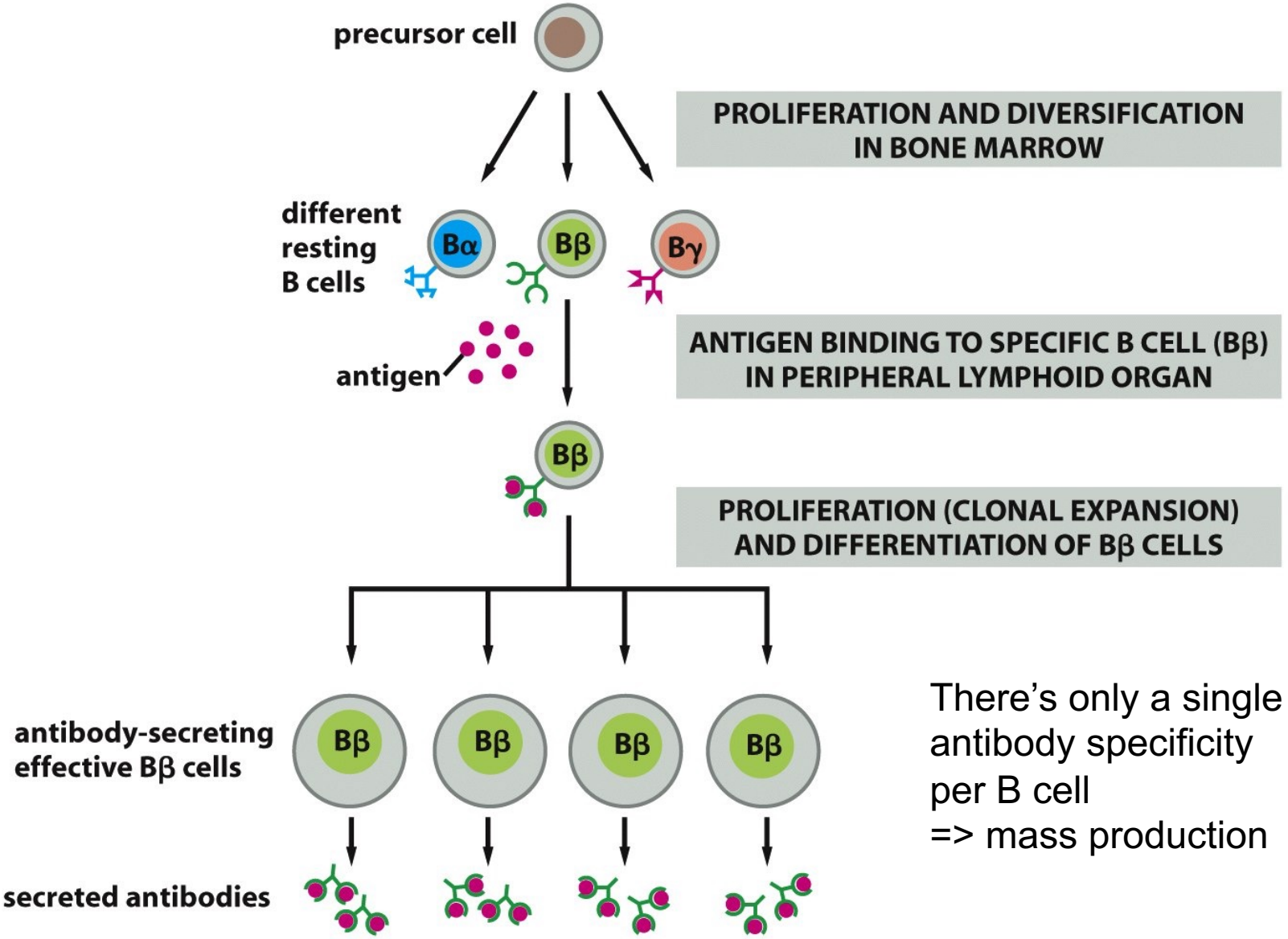
Release rate $k_{off}$: $10^{-3}$-$10^{-4}$ $s^{-1}$
=> controlled by time for signal transduction/endocytosis
after antigen binding to cell surface receptors

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)

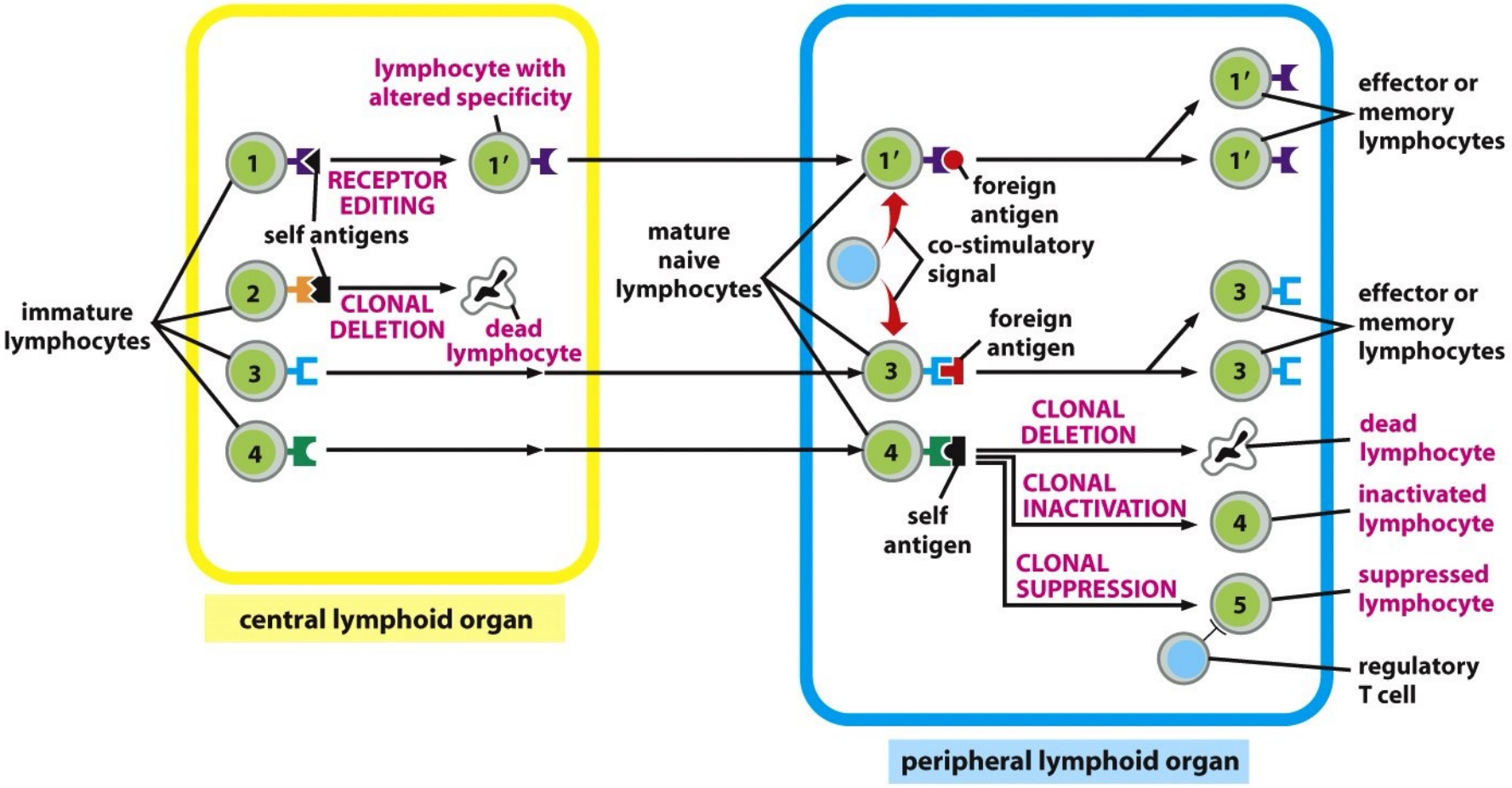
*for comparison: biotin-strepatividin: $K_a = 10^{14}$ $M^{-1}$

# 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

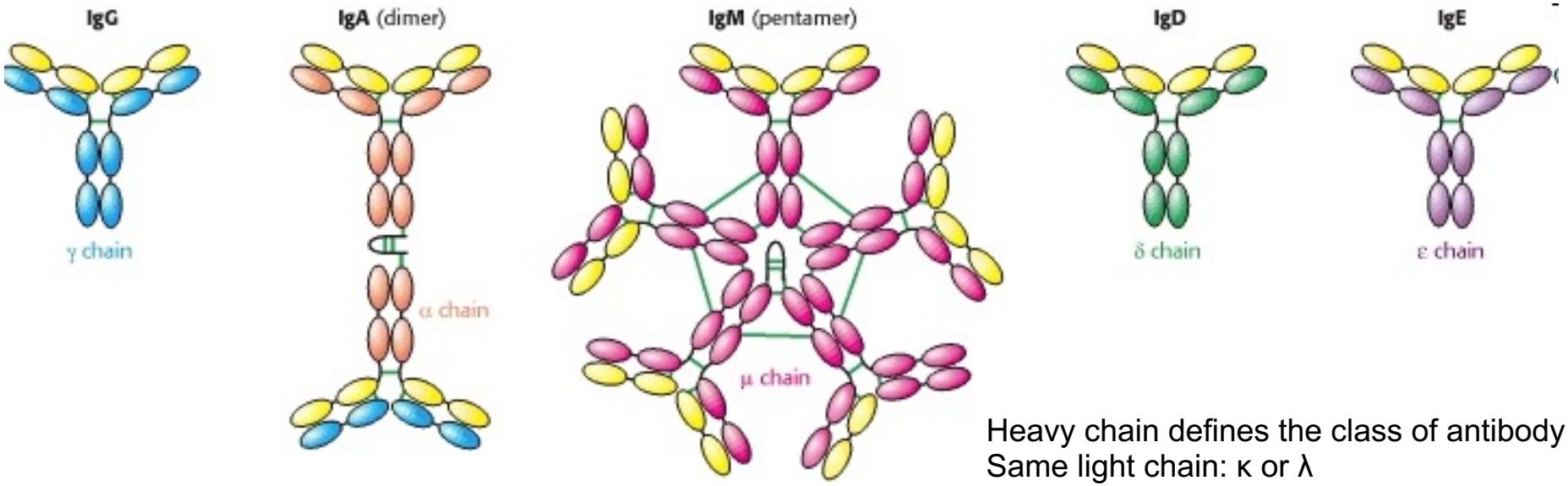
Problem: through natural evolution,
a pathogen can adapt to hide
or change its distinct antigenic
signatures
(pathogens have a big evolutionary
advantage because they have a much
shorter lifecycle (bacteria > 20 min) and
larger populations than animals (> 1 year)
in principle they can adapt 30,000x faster!)

**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)

Time to acquire 2% difference in genome sequences
Humans: 8 million years
Poliovirus: 5 days

# Antibody classes




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

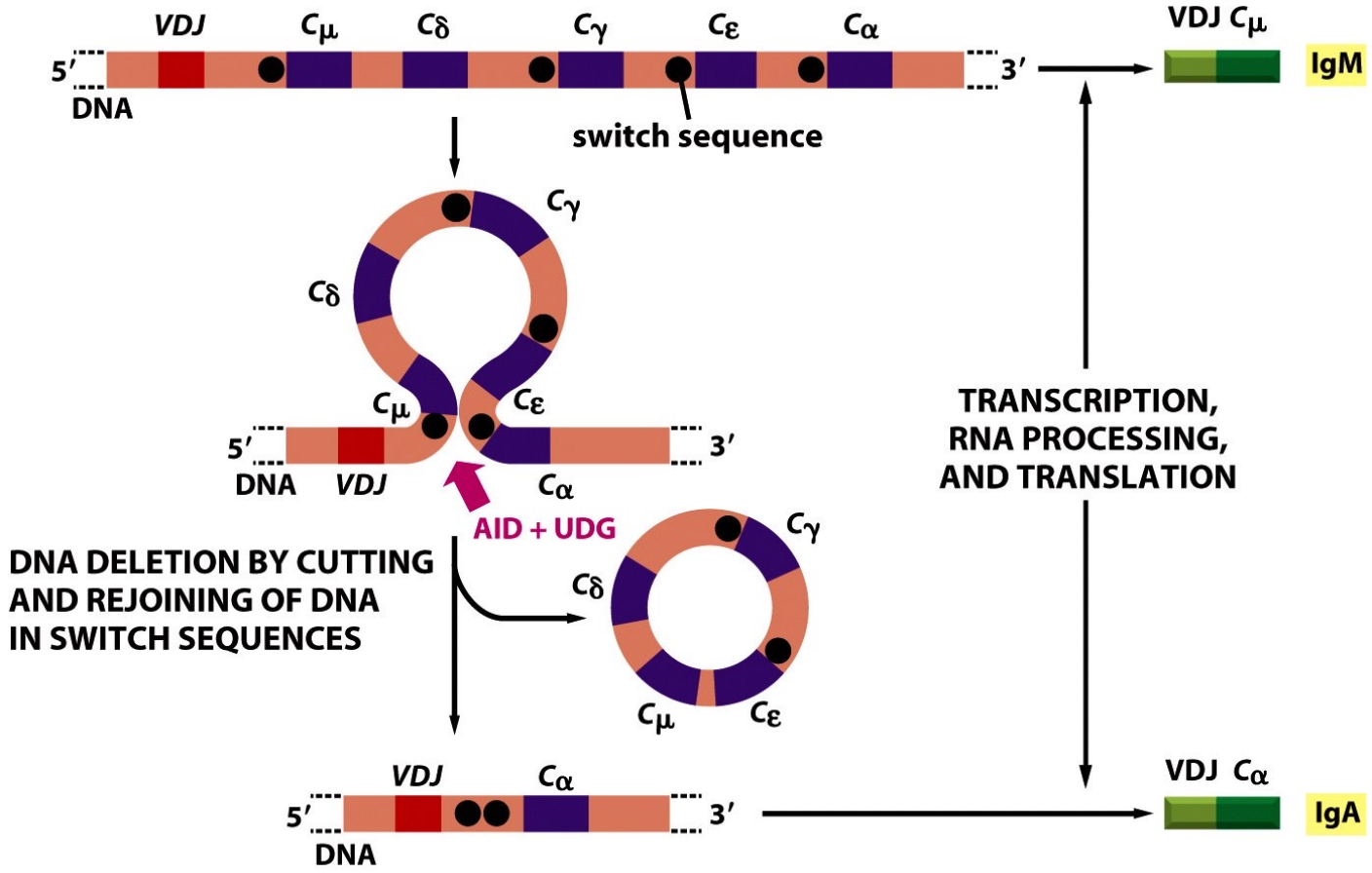
| PROPERTIES | CLASS OF ANTIBODY | | | | |
|---|---|---|---|---|---|
| | IgM | IgD | IgG | IgA | IgE |
| Heavy chains | μ | δ | γ | α | ε |
| Light chains | κ or λ | κ or λ | κ or λ | κ or λ | κ or λ |
| Number of four-chain units | 5 | 1 | 1 | 1 or 2 | 1 |
| Percentage of total Ig in blood | 10 | <1 | 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

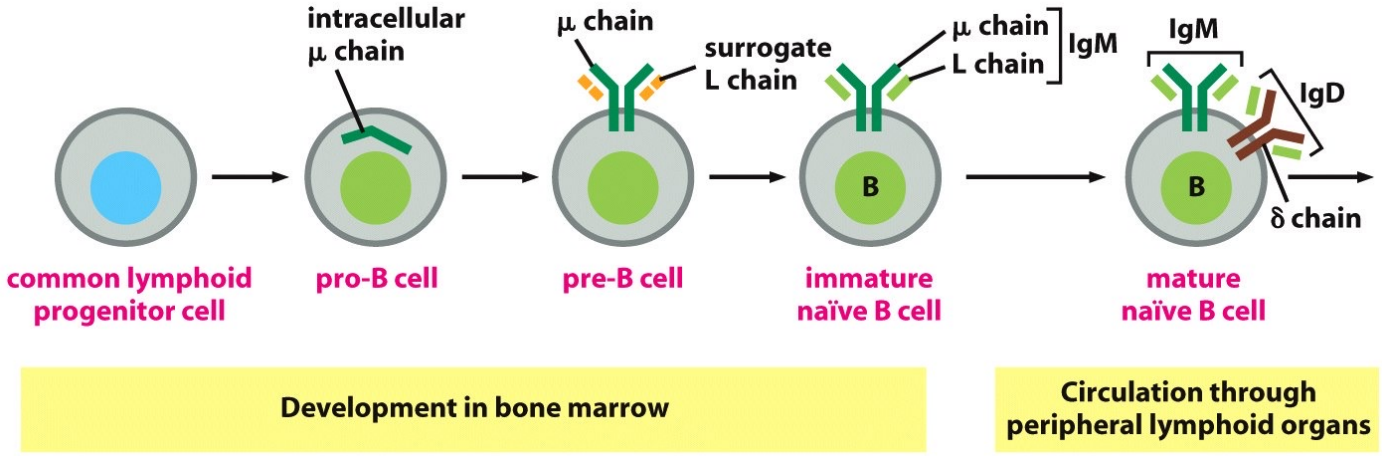
# Class switch mediated by DNA rearrangement



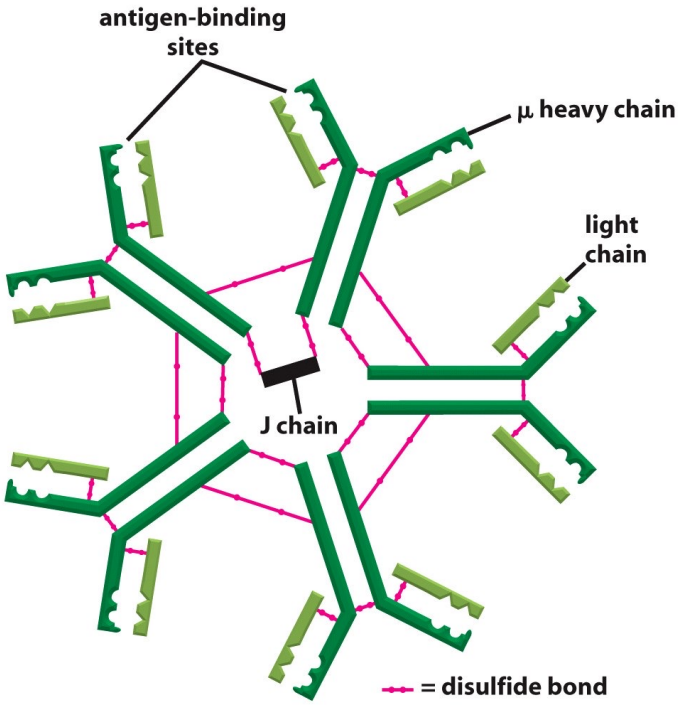

Class switch DNA recombination (not splicing!) => irreversible
depends on switch sequences (consisting of tandem repeats) and
the enyzmes activiation indcued deaminase (AID) + uracil-DNA glycosylase (UDG)

# IgM: First antibody class

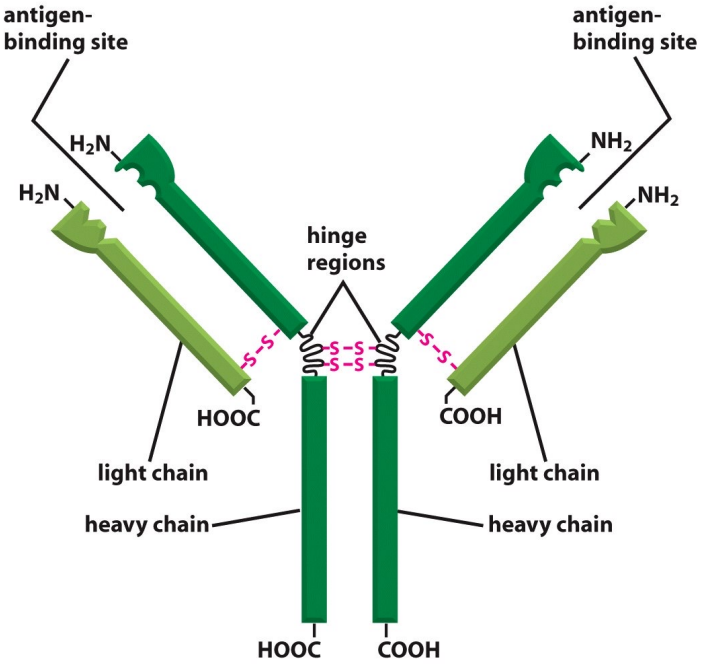
**on cell surface**

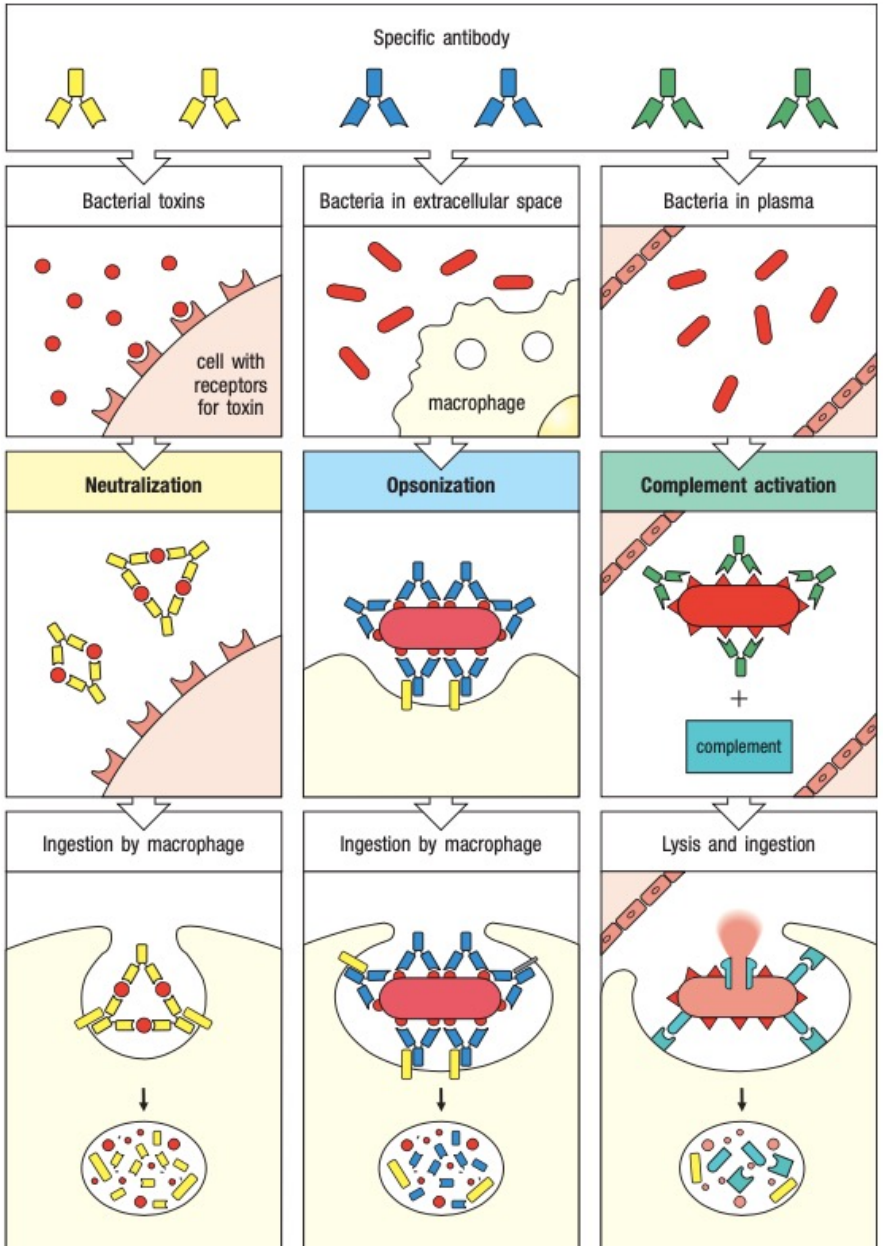



**circulating in blood**

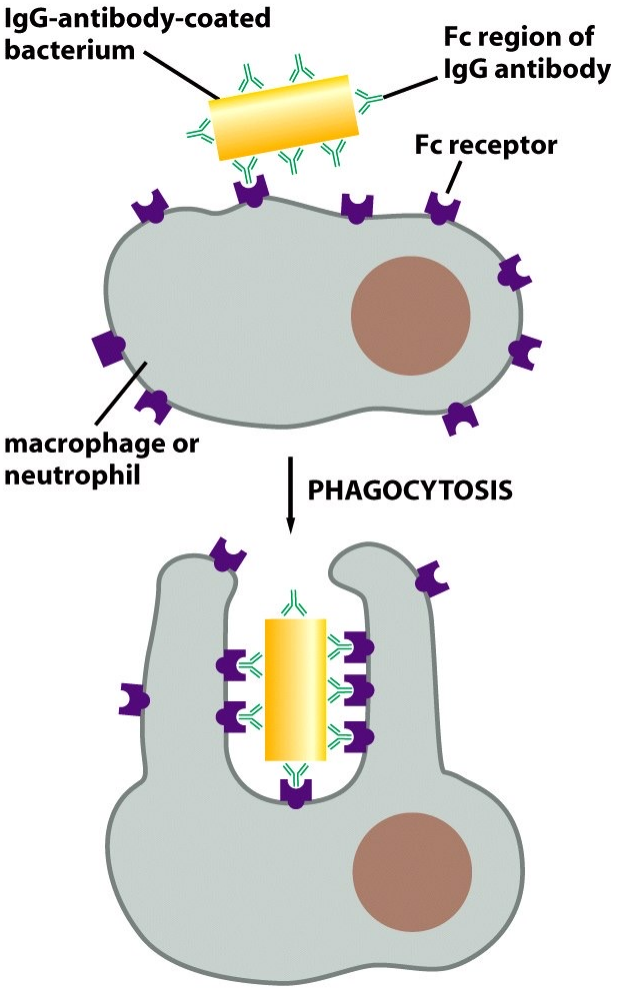


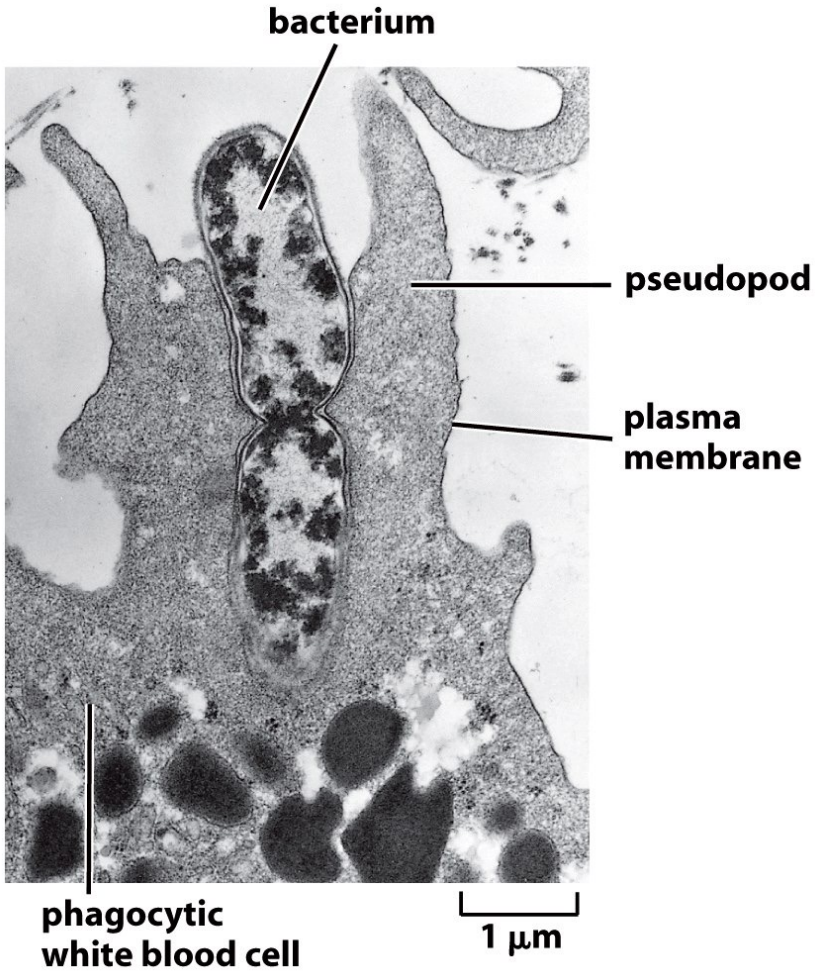
# IgG: Main class in blood



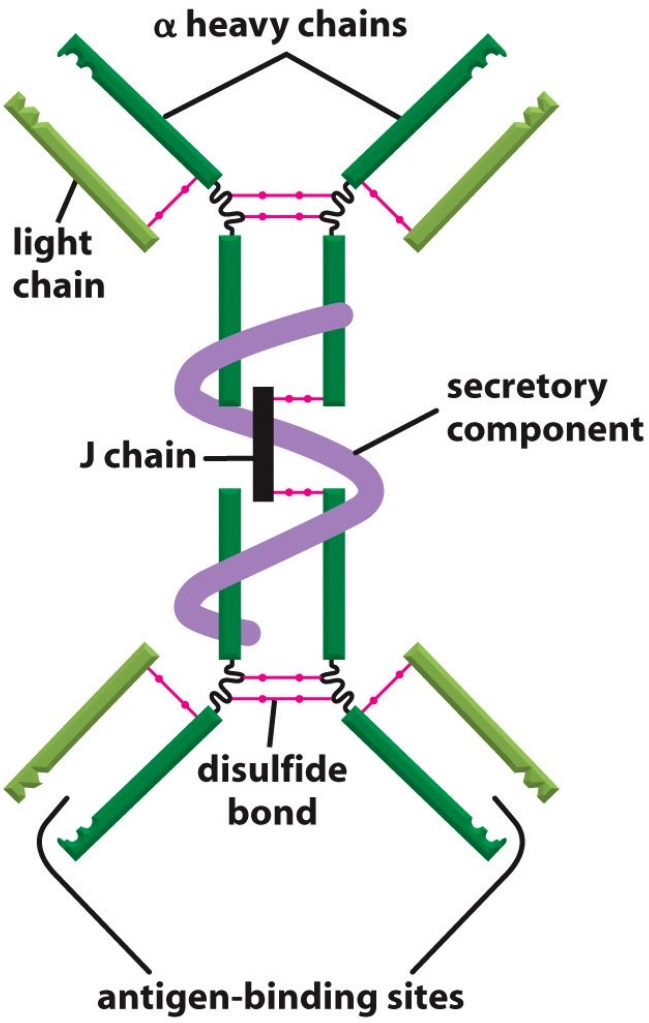
# Opsonization



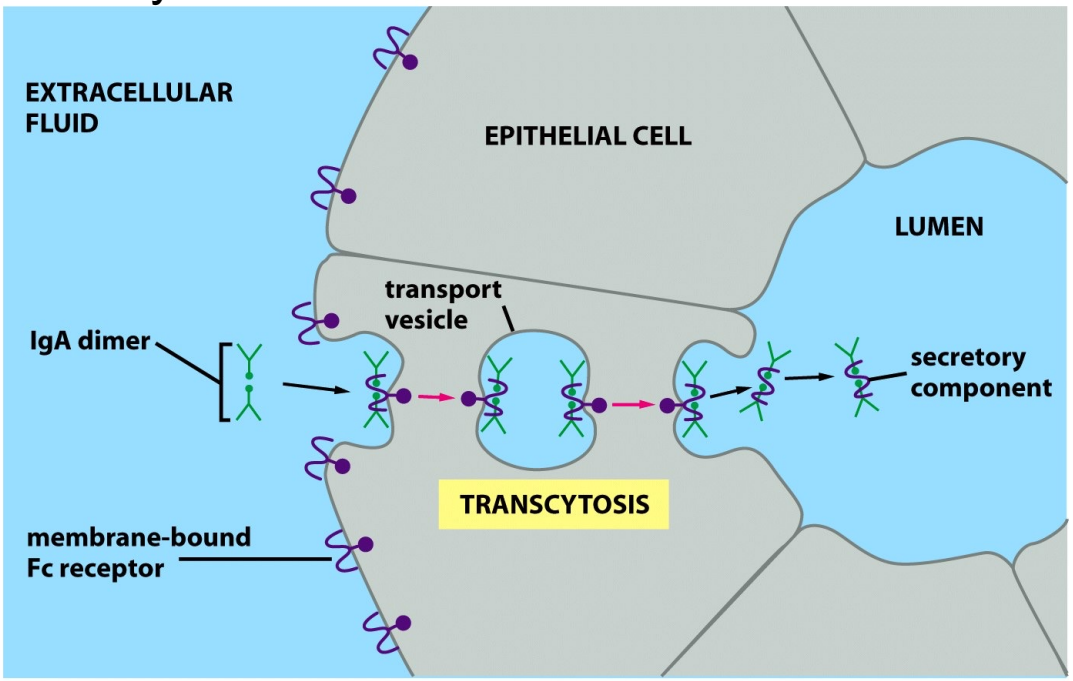




TEM image

# IgA: Defence of mucosal surfaces



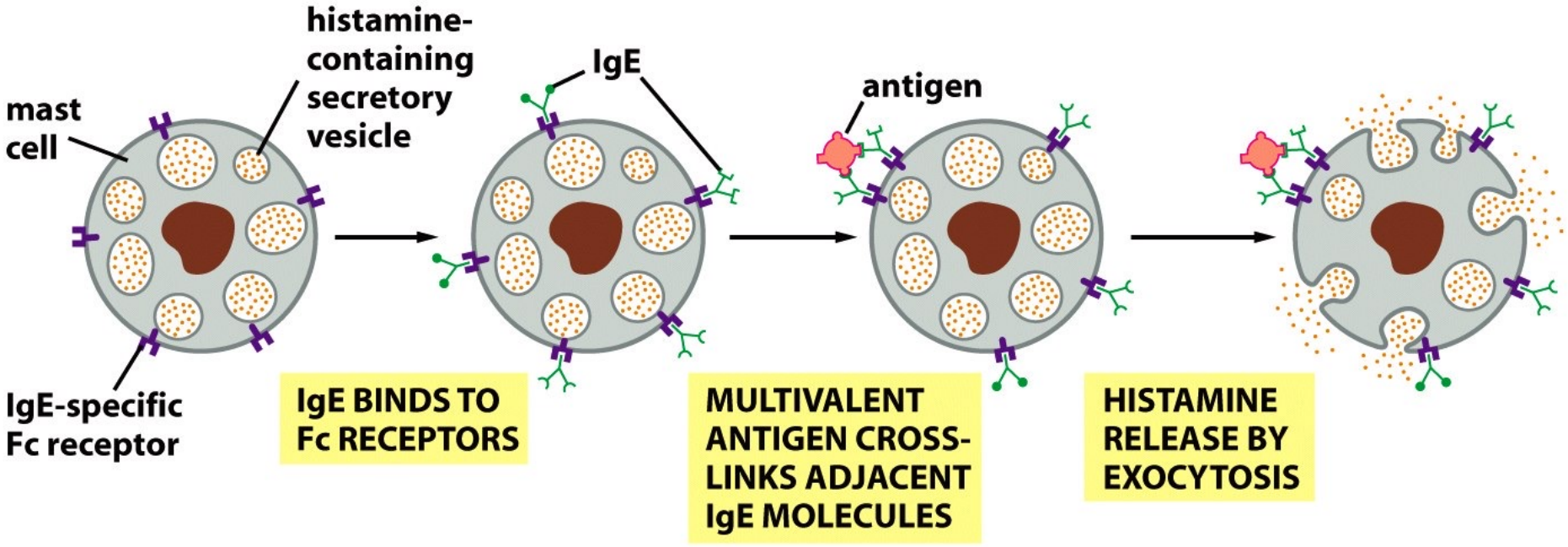

Transcytosis



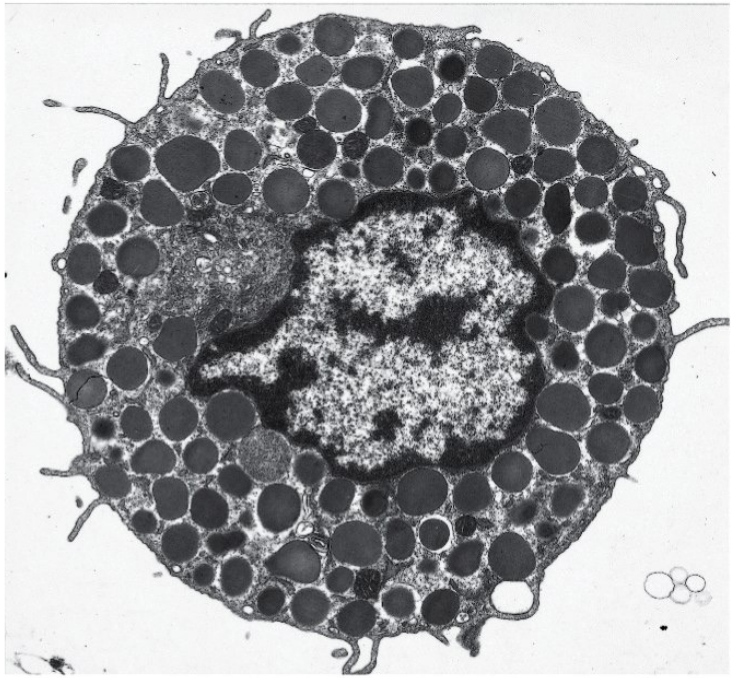


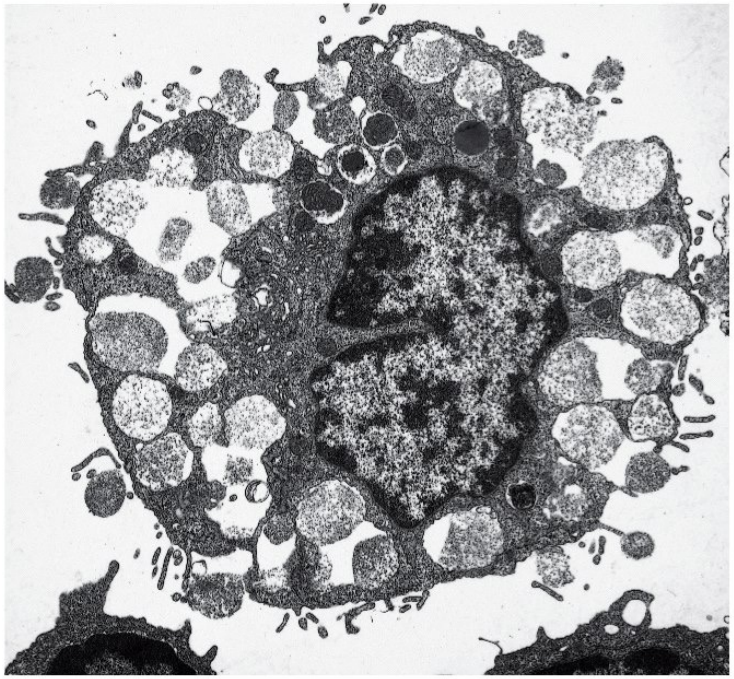

⇒ Similar mechanism of *IgG* transcytosis across the placenta to protect the fetus

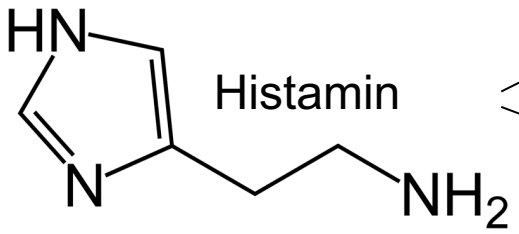
# IgE: Protection against large parasites



# Release of histamin by mast cells



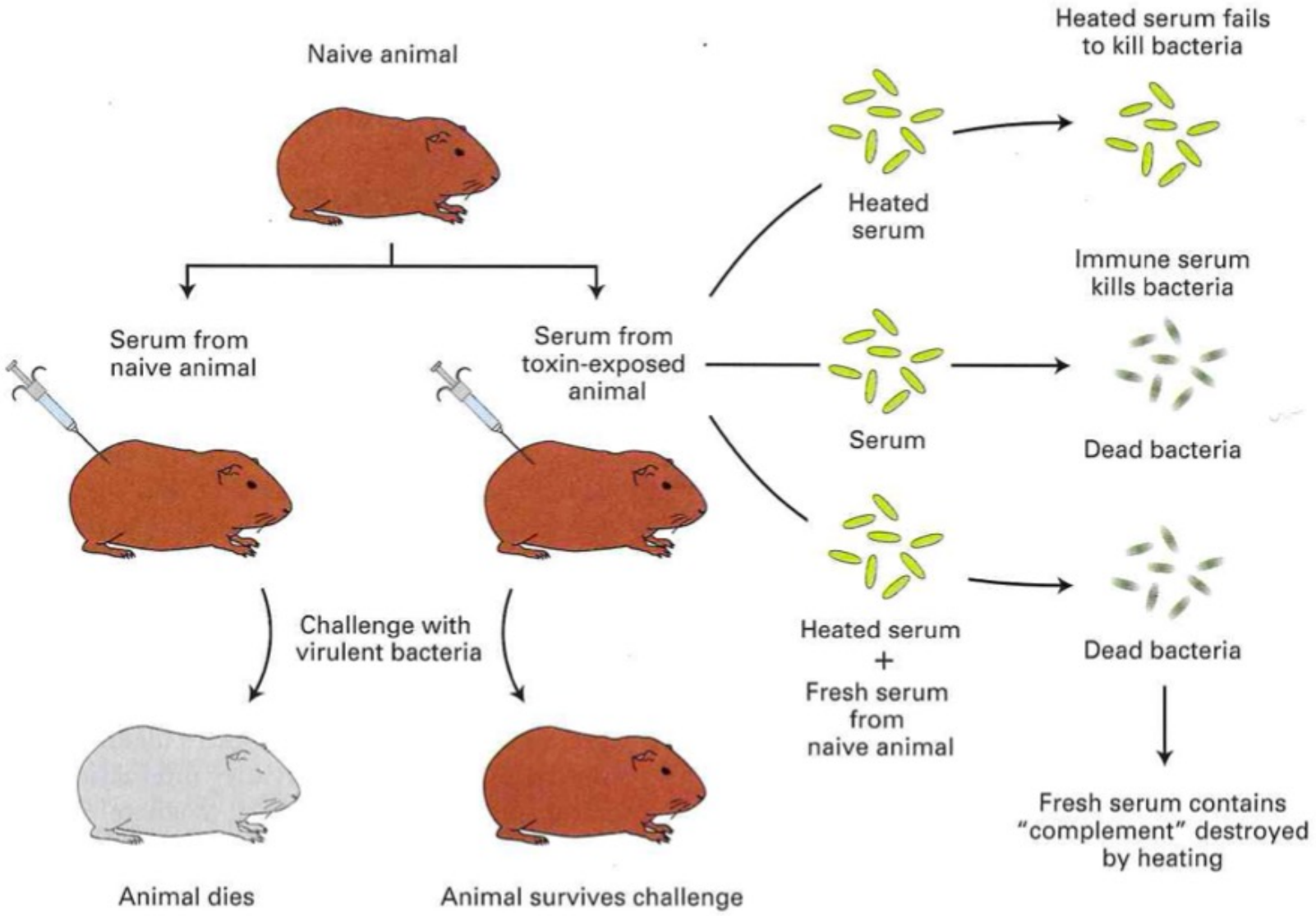




Histamin

→ Protection against parasites

→ Allergies

# Classic experiment

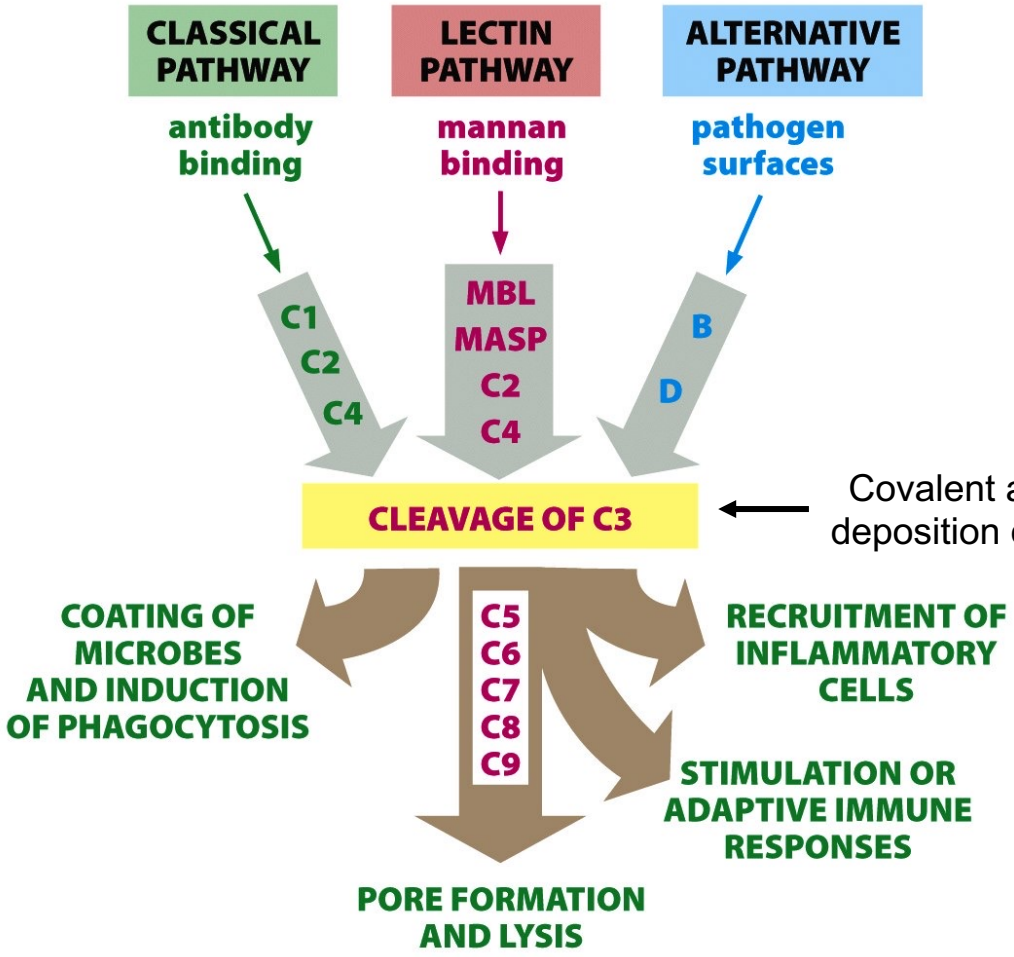




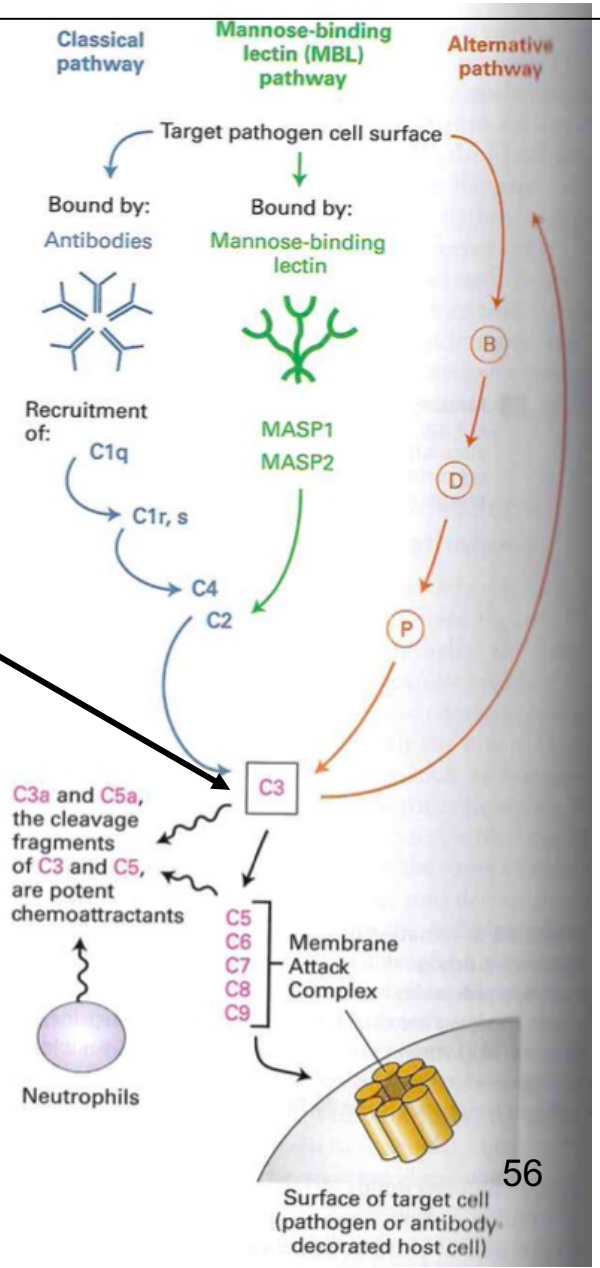

=> Behring/Kitasato (ca. 1890)

# Complement system

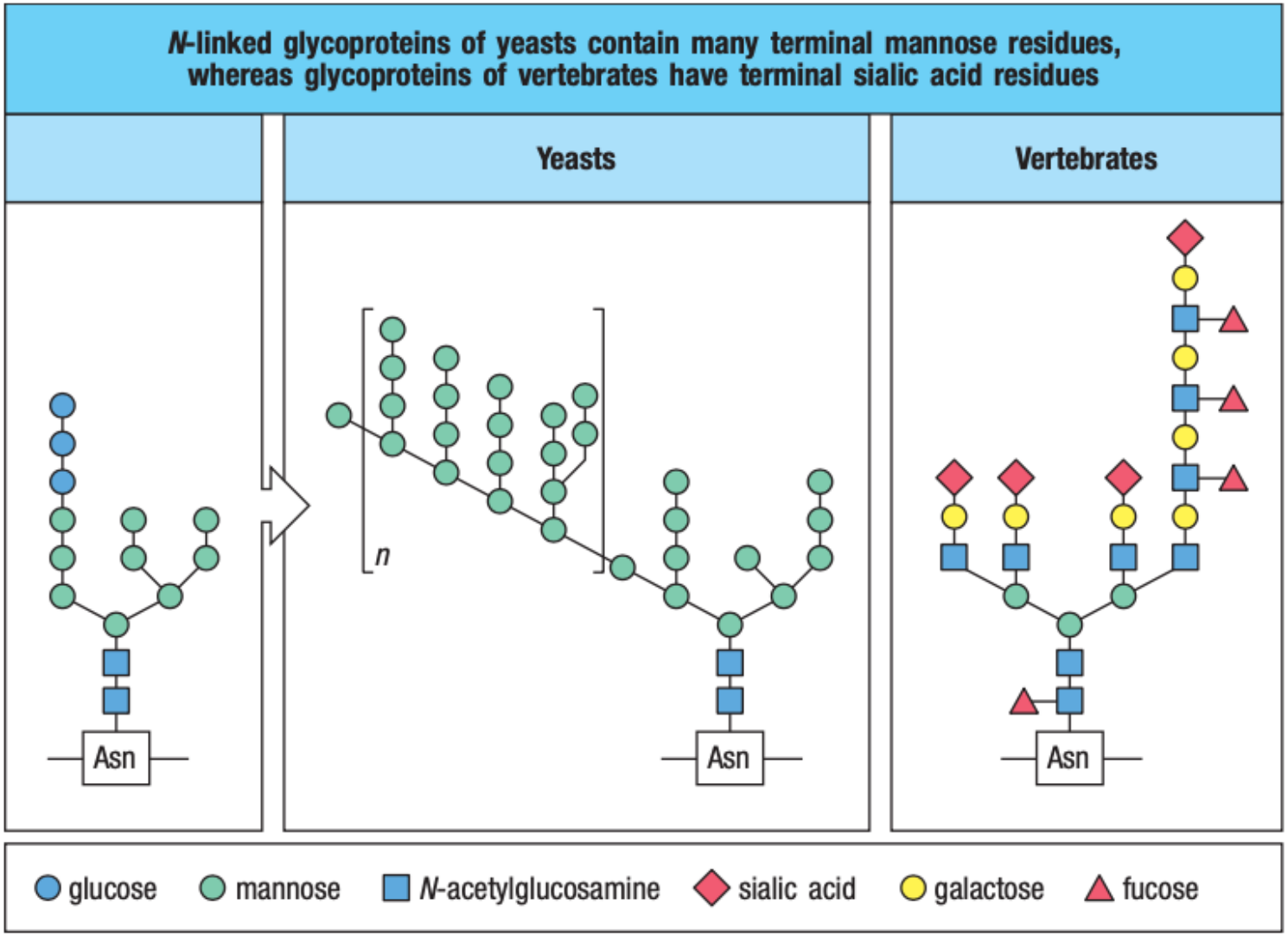
=> A protease cascade: amplification steps




Covalent attachment / deposition on target cell



# Lectin pathway



# Complement system: pore formation/lysis