Antigen Recognition and Humoral Immunity

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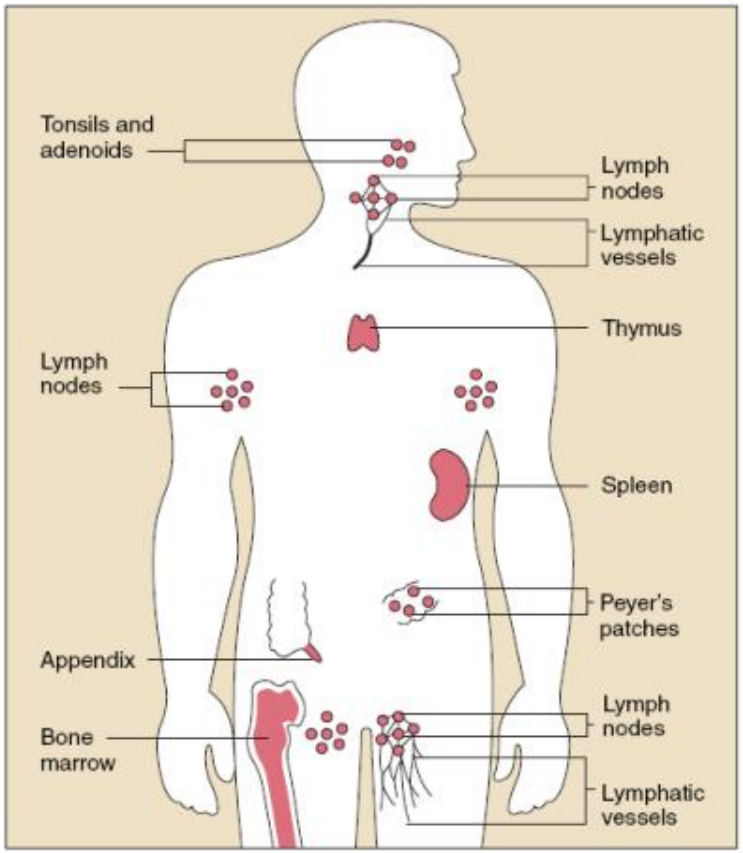
!!

LYMPHOID ORGANS

Primary lymphoid organs:

- Bone marrow
- Thymus

the cells of the immune system originate in and mature here

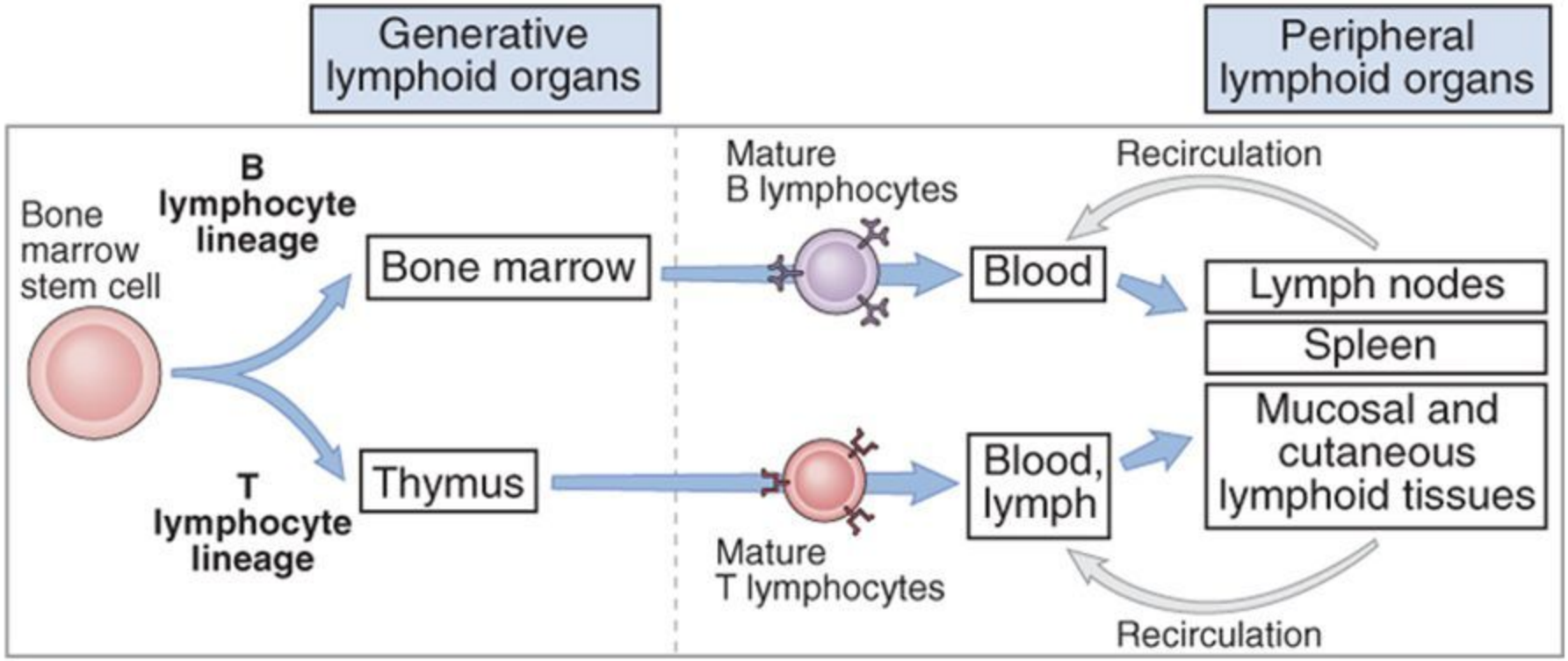


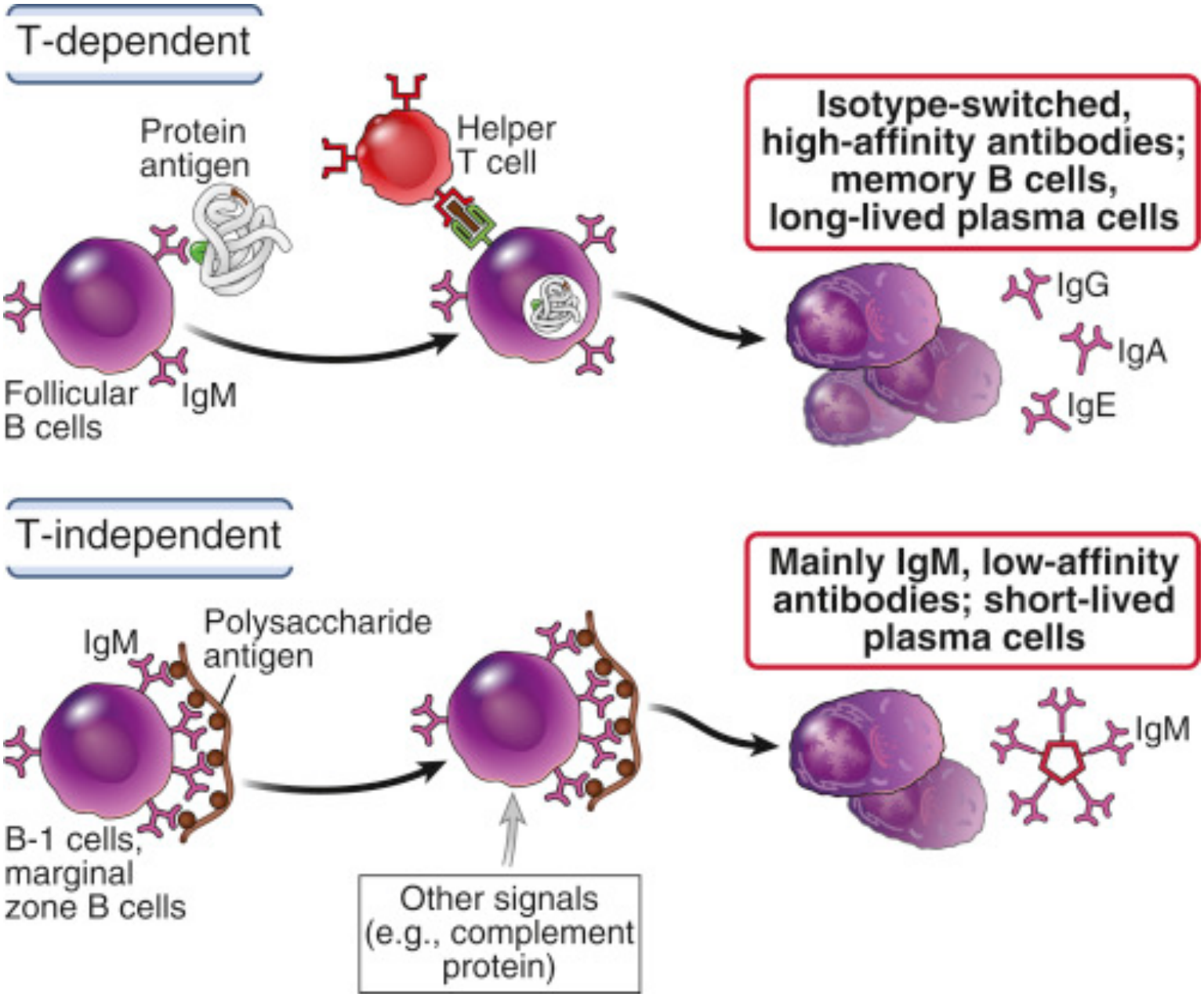
Secondary lymphoid organs:

- Spleen
- Lymphatic vessels
- Lymph nodes
  - Adenoids and tonsils
  - MALT (Mucosal Associated Lymphoid Tissue)
    - GALT (Gut Associated Lymphoid Tissue)
    - BALT (Bronchus Associated Lymphoid Tissue)
    - SALT (Skin Associated Lymphoid Tissue)
    - NALT (Nasal Associated Lymphoid Tissue)

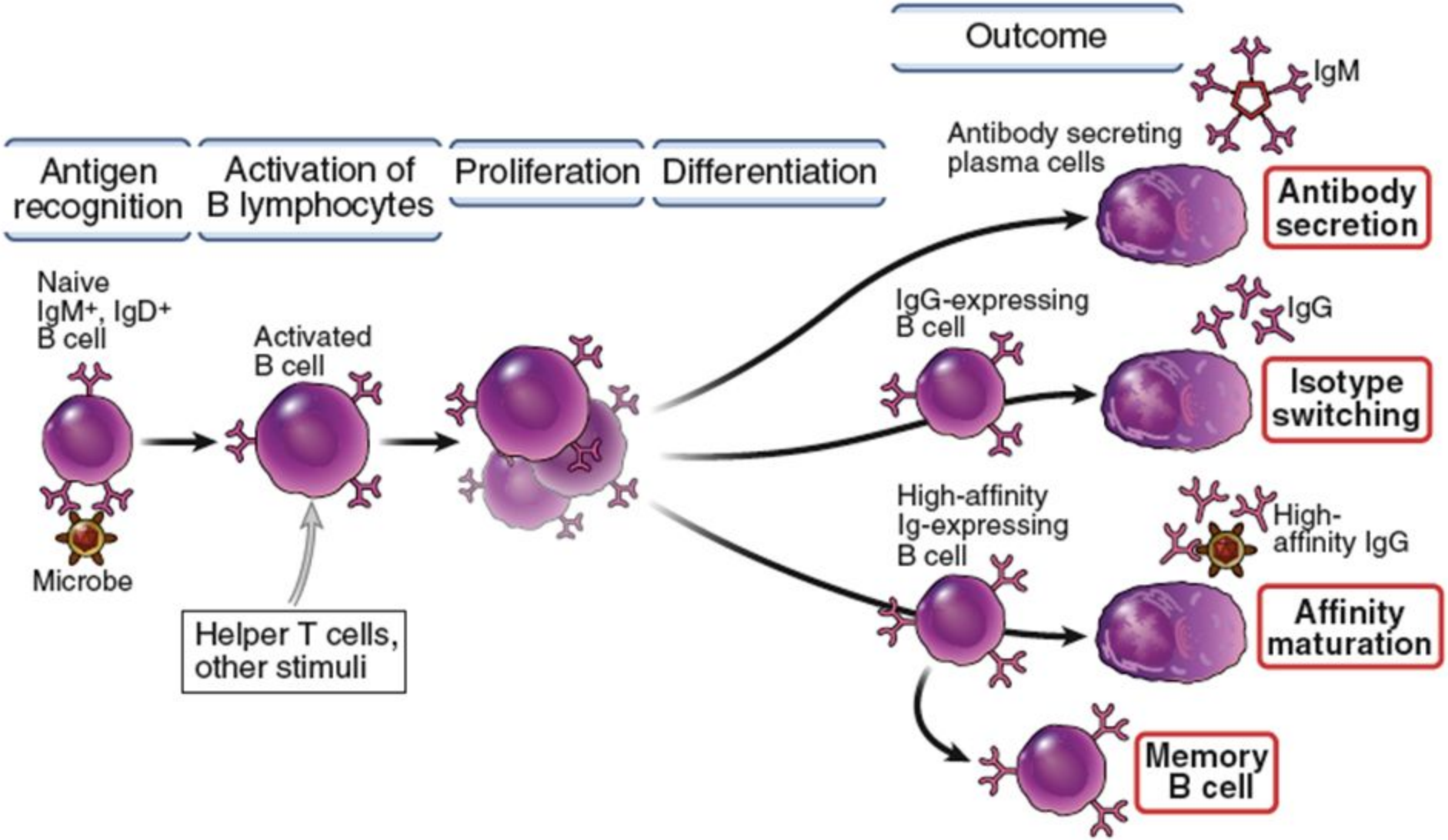
not for cell development. (final differentiation, activation may be performed) The cells of the adaptive immune system recognize here the pathogens

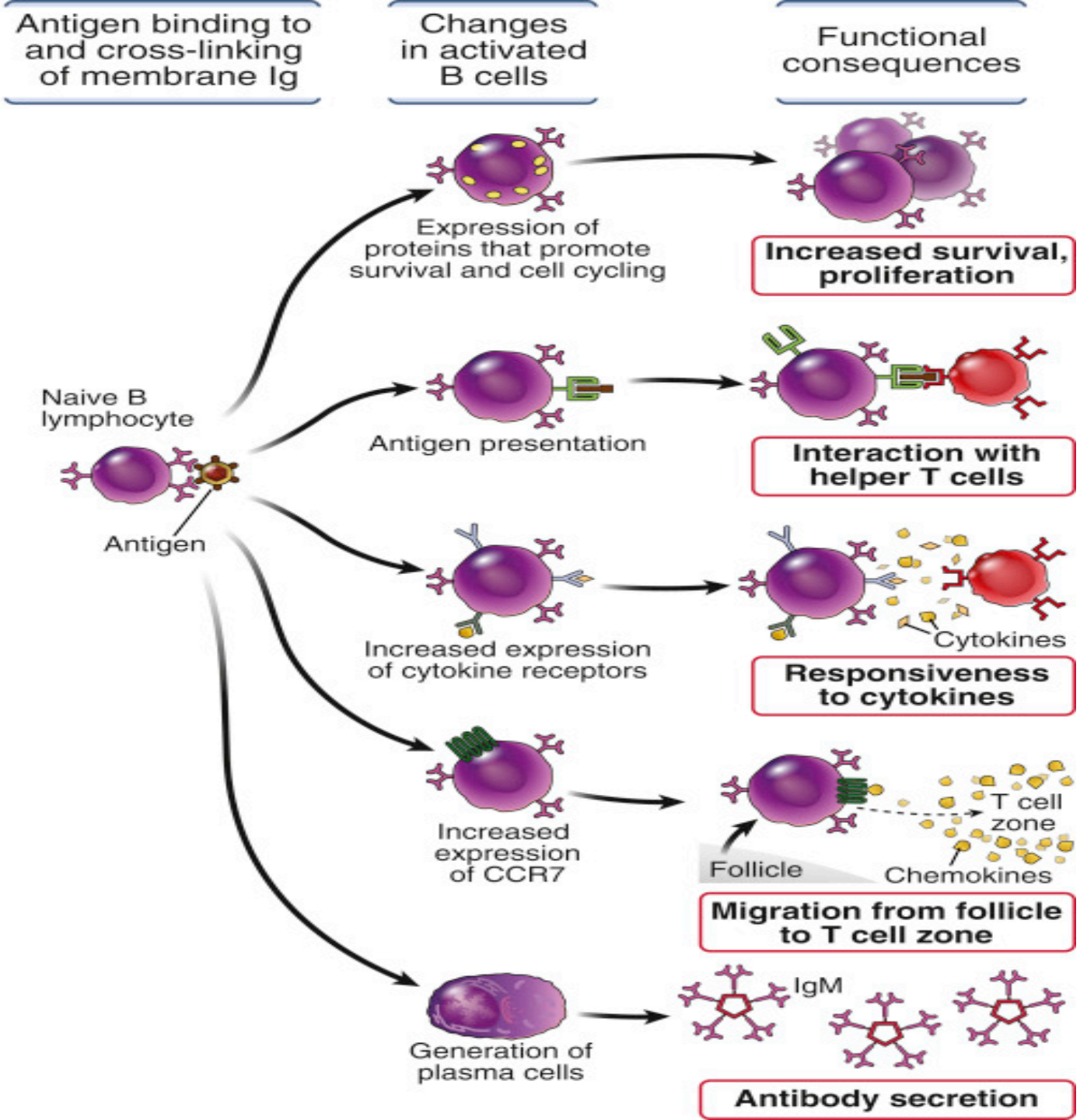
Lymphocyte maturation




T-dependent
Protein antigen
Helper T cell
Isotype-switched, high-affinity antibodies; memory B cells, long-lived plasma cells
Follicular B cells
IgM
IgG
IgA
IgE
T-independent
IgM
Polysaccharide antigen
Mainly IgM, low-affinity antibodies; short-lived plasma cells
B-1 cells, marginal zone B cells
Other signals (e.g., complement protein)
IgM

B cell activation and antibody production




Antigen binding to and cross-linking of membrane Ig
Changes in activated B cells
Functional consequences
Naive B lymphocyte
Antigen
Expression of proteins that promote survival and cell cycling
Increased survival, proliferation
Antigen presentation
Interaction with helper T cells
Increased expression of cytokine receptors
Cytokines
Responsiveness to cytokines
Increased expression of CCR7
T cell zone
Follicle
Chemokines
Migration from follicle to T cell zone
Generation of plasma cells
IgM
Antibody secretion

B Cell Receptors

B lymphocytes can recognize

- Proteins
- Polysaccharides
- Lipids
- Nucleic acids
- Toxins

B Cell Receptors

- B cells **differentiate into antibody producing cells in response to antigen and other signals.**
- Secreted antibodies can pass into the **circulation** and mucosal fluids and bind antigens, leading to their neutralization and elimination.

B Cell Receptors

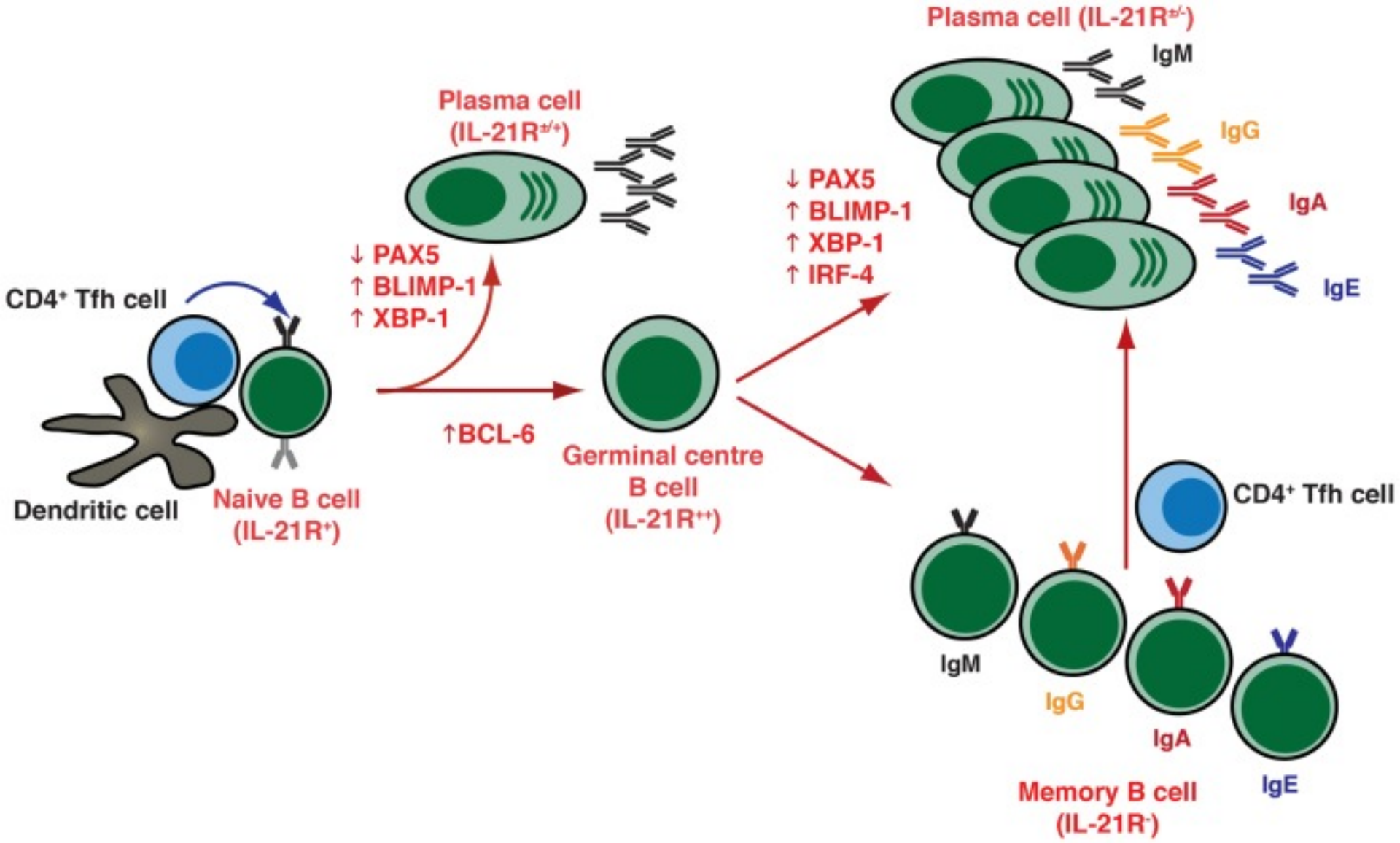
- B cell receptors and secreted antibodies **recognize antigens in their natural form.**
- **It is not necessary to have a specialized ASH group to present antigens to naive B cells.**

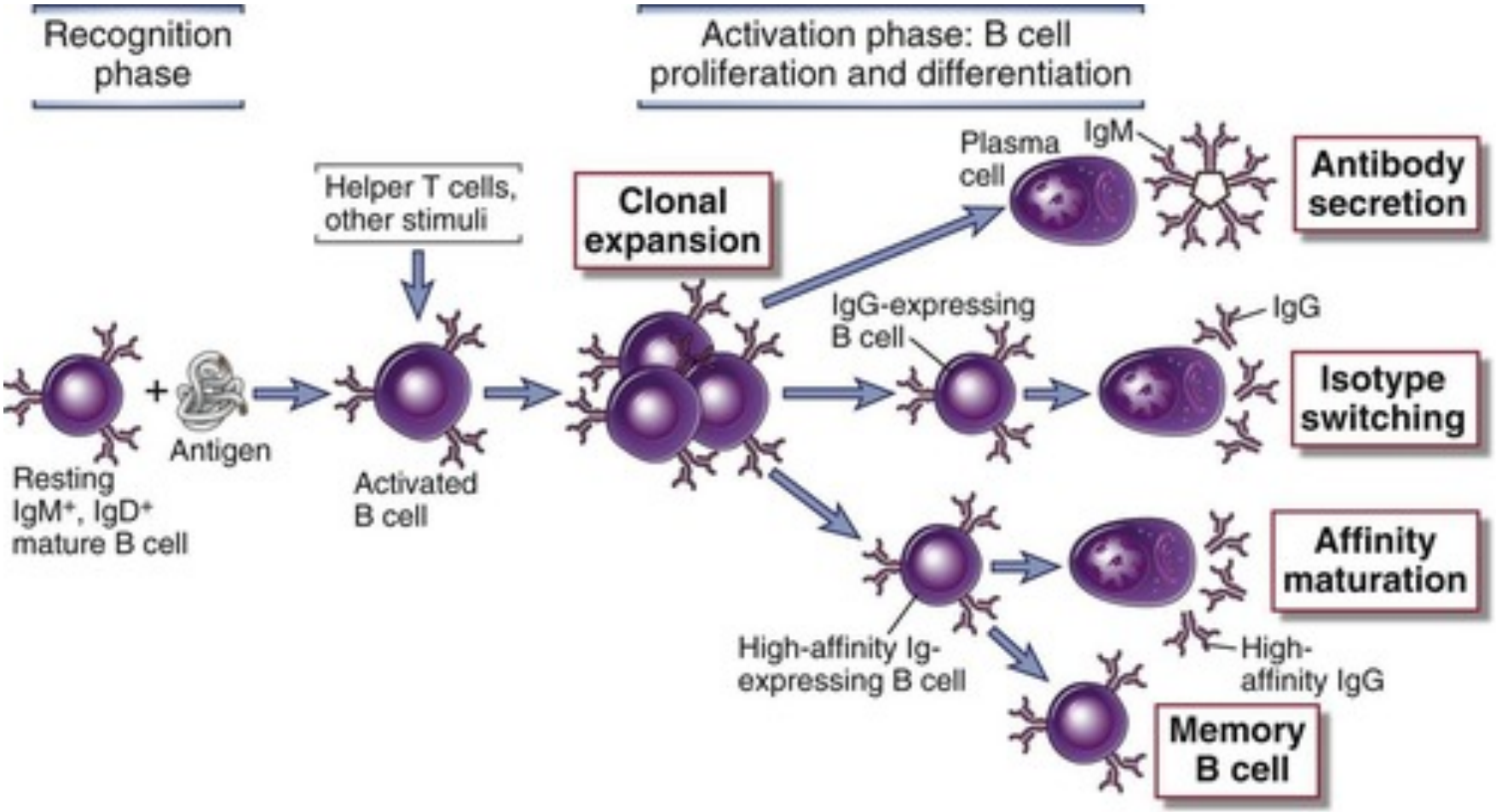
B Cell Receptors

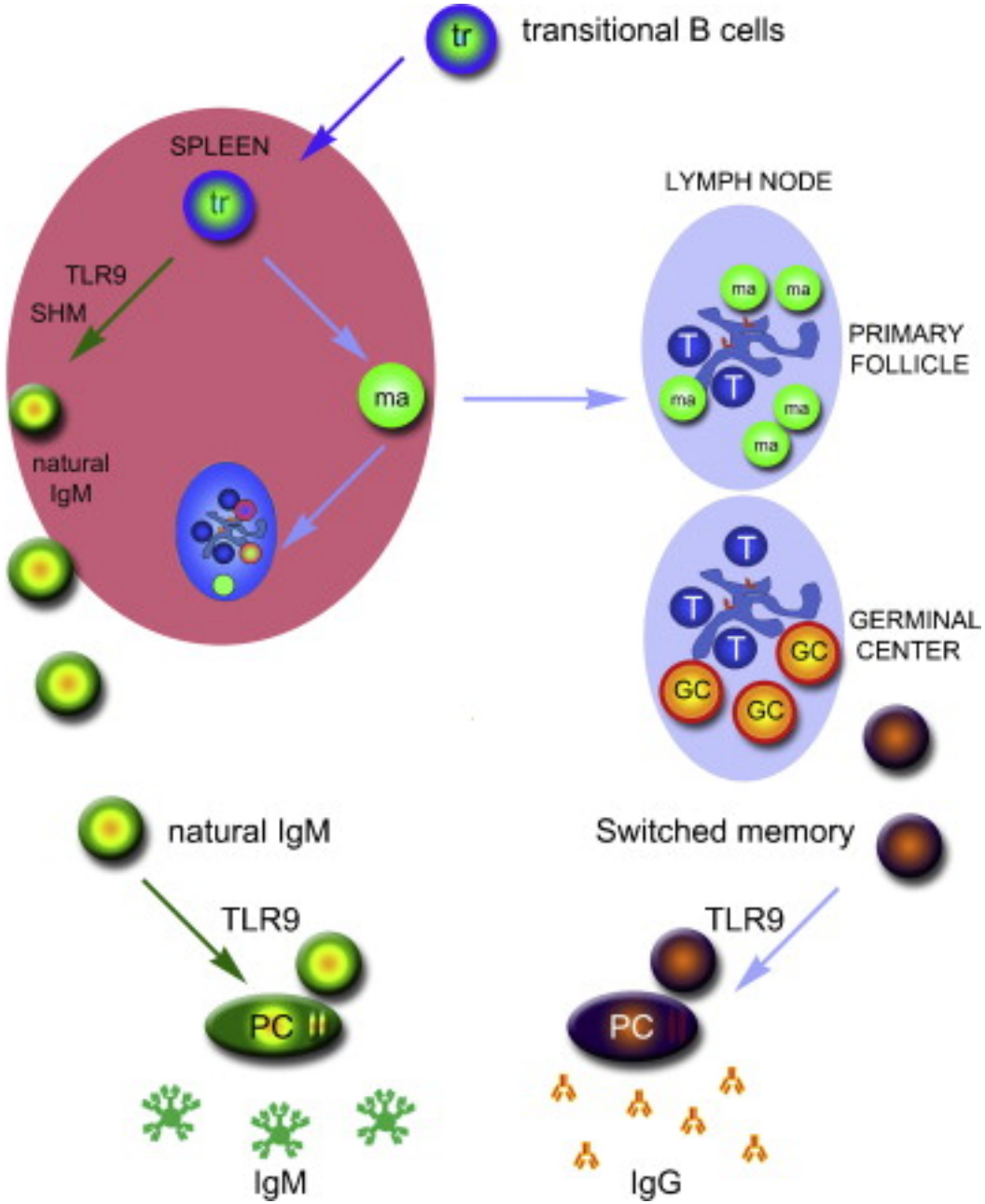
- A population of cells called **follicular dendritic cells (FDC)** is found in the lymphoid follicles rich in B cells of the lymph nodes and spleen.
- The function of these cells **is to show antigens to the activated B lymphocytes.**

B Cell Receptors

- FDCs take antibody-coated antigens using Fc receptors and it binds antigens covered by complement using C3d complement receptors.


Plasma cell (IL-21R+/-)
IgM
IgG
IgA
IgE
Plasma cell
(IL-21R+/+)
↓ PAX5
↑ BLIMP-1
↑ XBP-1
↓ PAX5
↑ BLIMP-1
↑ XBP-1
↑ IRF-4
CD4+ Tfh cell
↑BCL-6
Dendritic cell
Naive B cell
(IL-21R+)
Germinal centre
B cell
(IL-21R++)
CD4+ Tfh cell
IgM
IgG
IgA
IgE
Memory B cell
(IL-21R-)


Recognition phase
Activation phase: B cell proliferation and differentiation
Helper T cells, other stimuli
Clonal expansion
Plasma cell
IgM
Antibody secretion
IgG-expressing B cell
IgG
Isotype switching
Antigen
Resting IgM+, IgD+ mature B cell
Activated B cell
Affinity maturation
High-affinity Ig-expressing B cell
High-affinity IgG
Memory B cell

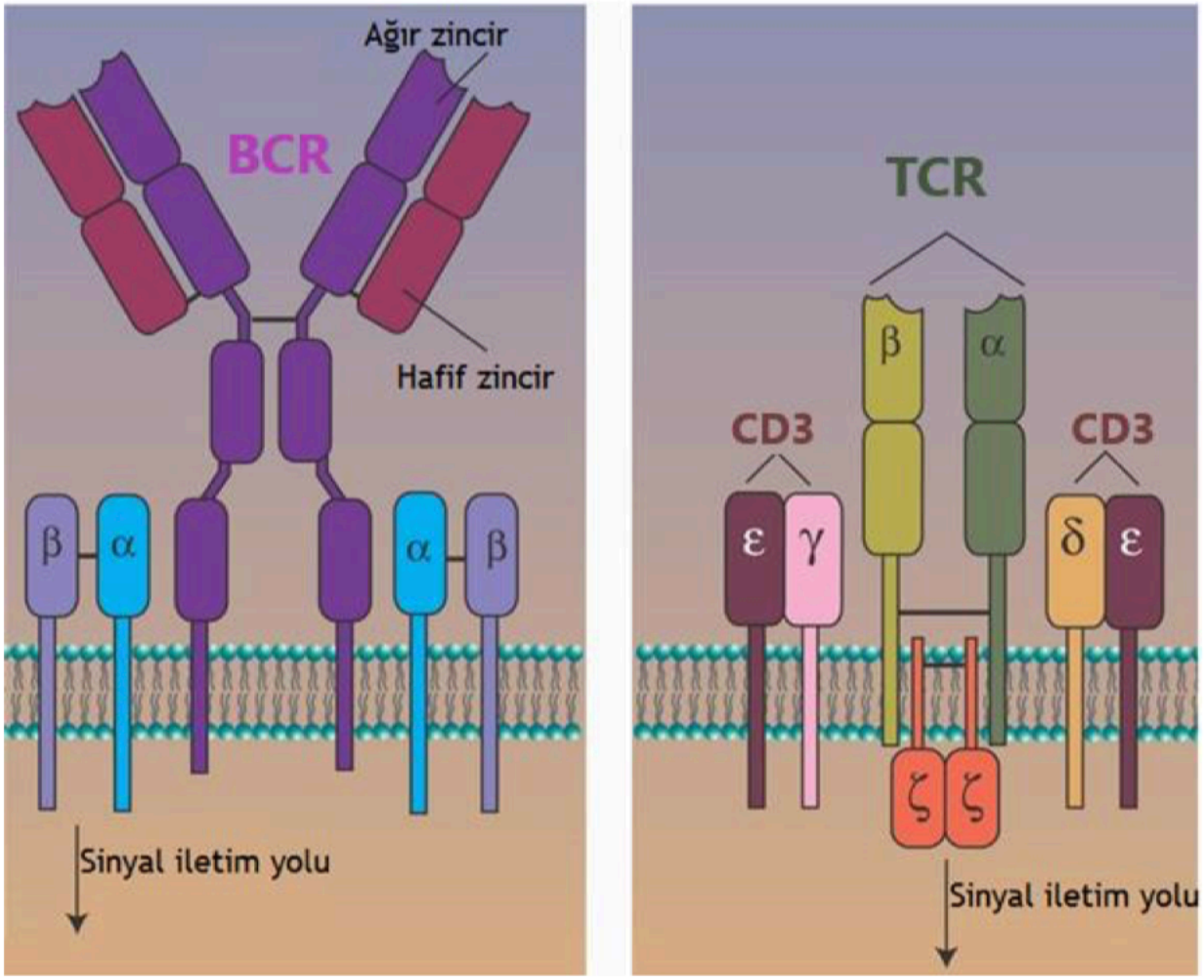

tr
transitional B cells
SPLEEN
tr
TLR9
SHM
ma
natural
IgM
LYMPH NODE
ma
ma
T
T
ma
ma
ma
PRIMARY
FOLLICLE
T
T
T
GC
GC
GC
GERMINAL
CENTER
natural IgM
TLR9
PC
IgM
Switched memory
TLR9
PC
IgG

Antigen Receptors of Lymphocytes

- Specific antigen recognition is the task of two structurally similar surface proteins of lymphocytes:

1. Membrane-bound antibodies of B cells

2. T cell receptors (TCR) on T lymphocytes


Ağır zincir
BCR
Hafif zincir
β
α
α
β
Sinyal iletim yolu
TCR
β
α
CD3
CD3
ε
γ
δ
ε
ζ
ζ
Sinyal iletim yolu

Antigen Receptors of Lymphocytes

- Cell receptors in the immune system have two functions:

1. detecting external stimuli
2. Triggering the immune response

Antigen Receptors of Lymphocytes

- Antigen receptors of lymphocytes can simultaneously recognize large amounts of different antigens.
- For this, **these receptors must have the ability to bind and distinguish very similar chemical structures.**

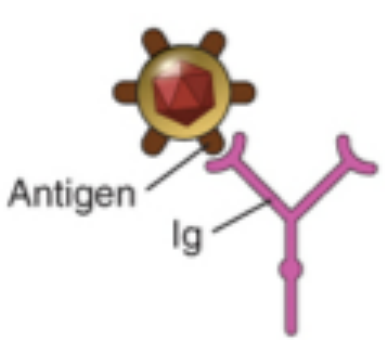
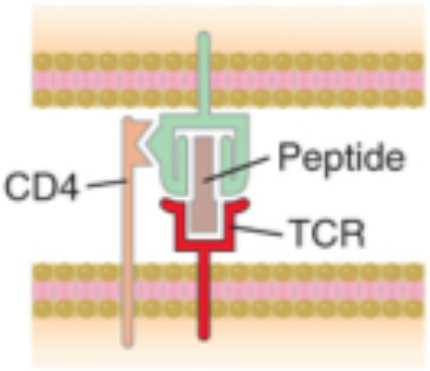
Antigen Receptors of Lymphocytes

- The antigen recognition regions of the receptors consist of 2 parts.;
- Variable (V) region
- Constant (C) region

T-cell Receptor (TCR) vs. Ab

	T cell receptor (TCR) 	Immunoglobulin (Ig) 
Components	a and b chains	Heavy and light chains
Number of Ig domains	One V domain and one C domain in each chain	Heavy chain; one V domain, three or four C domains Light chain; one V domain and one C domain
Number of CDRs	Three in each chain for antigen binding; fourth hypervariable region in b chain (of unknown function)	Three in each chain
Associated signaling molecules	CD3 and z	Iga and Igb
Affinity for antigen (K_d)	10^{-5}–10^{-7} M	10^{-7}–10^{-11} M (secreted Ig)
Changes after cellular activation		
Production of secreted form	No	Yes
Isotype switching	No	Yes
Somatic mutations	No	Yes

Abbreviations: C, constant; CDR, complementarity-determining region; K_d, dissociation constant; V, variable.

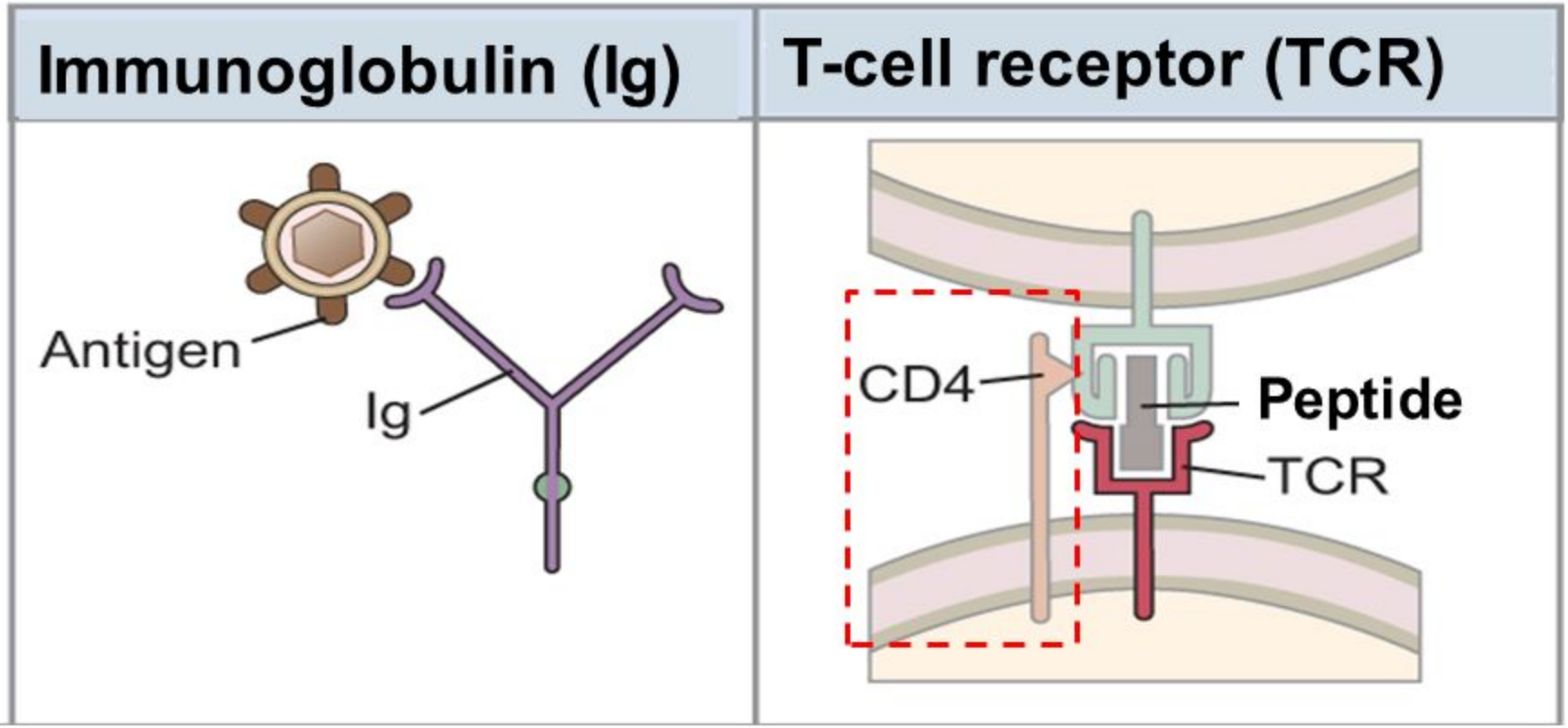
Feature	Antigen-binding molecule	
	Immunoglobulin (Ig)	T cell receptor (TCR)
		
Antigen binding	Made up of three CDRs in V_H and three CDRs in V_L	Made up of three CDRs in Vα and three CDRs in Vβ
Changes in constant regions	Heavy-chain class switching and change from membrane to secretory Ig	None
Affinity of antigen binding	K_d 10^{-7}-10^{-11} M; average affinity of Igs increases during immune responses to protein antigens	K_d 10^{-5}-10^{-7} M; No change during immune responses
On-rate and off-rate	Rapid on-rate, variable off-rate	Slow on-rate, slow off-rate

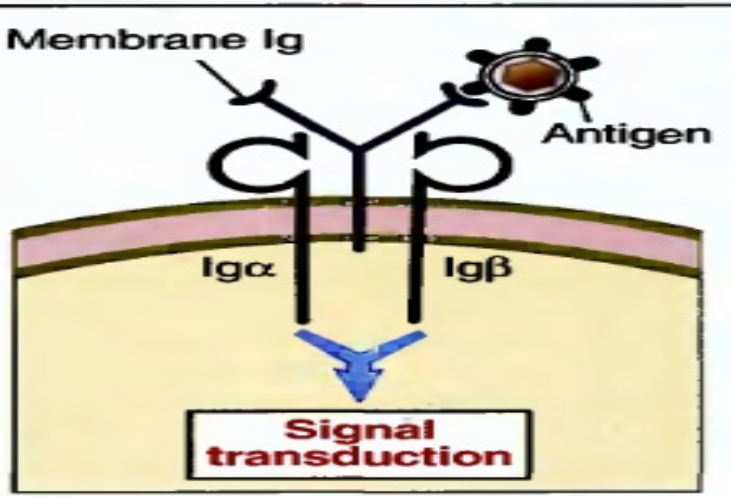
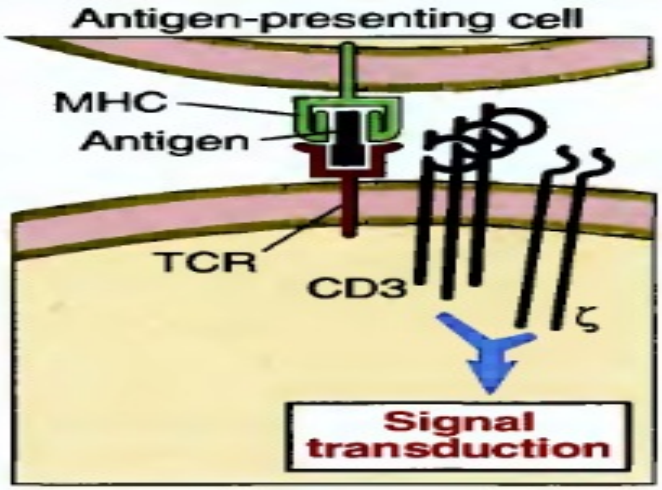
BCR

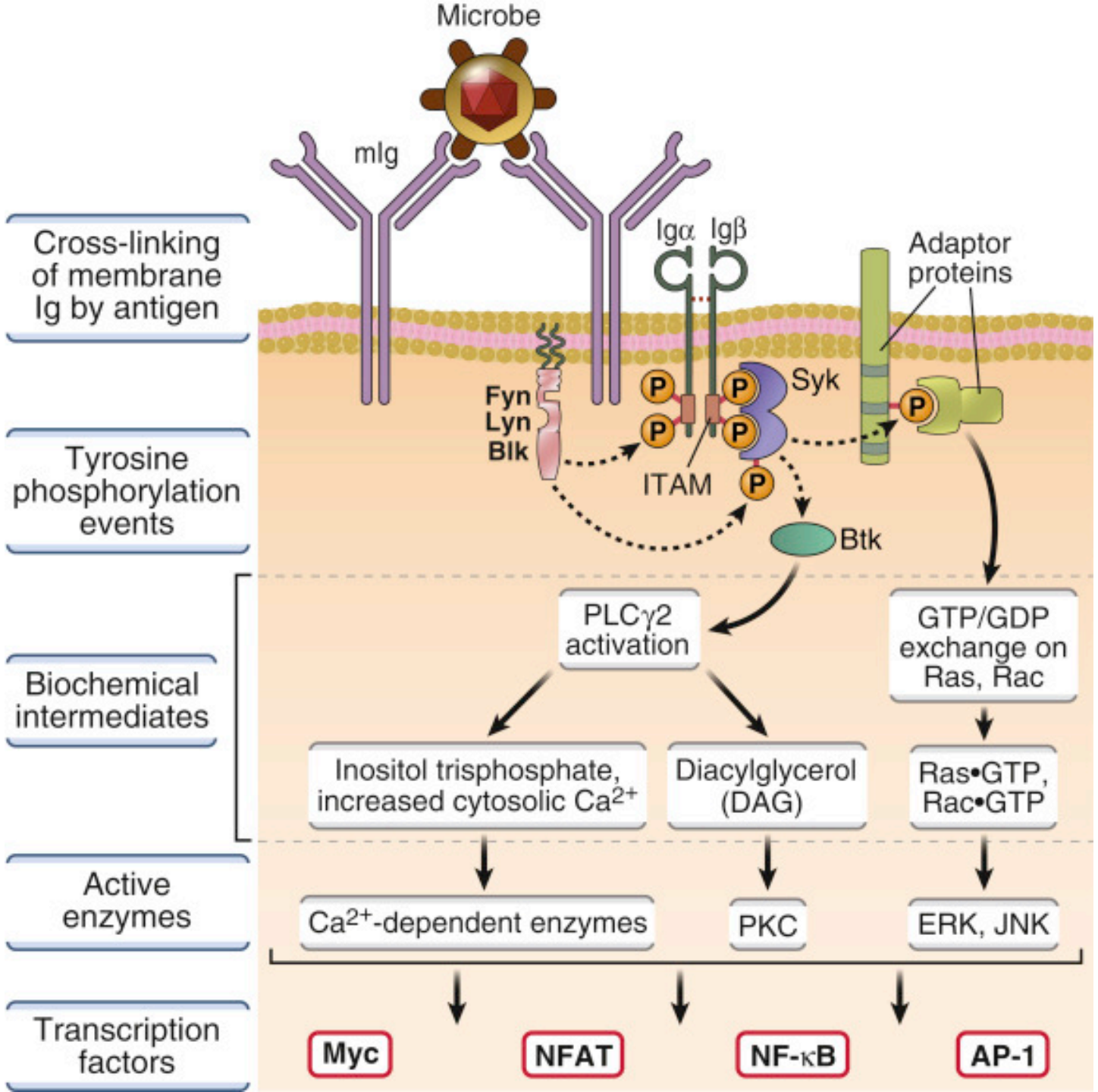
- Recognition without requirement for co-receptor molecules

TCR

- Co-receptor molecules (CD4/CD8) required

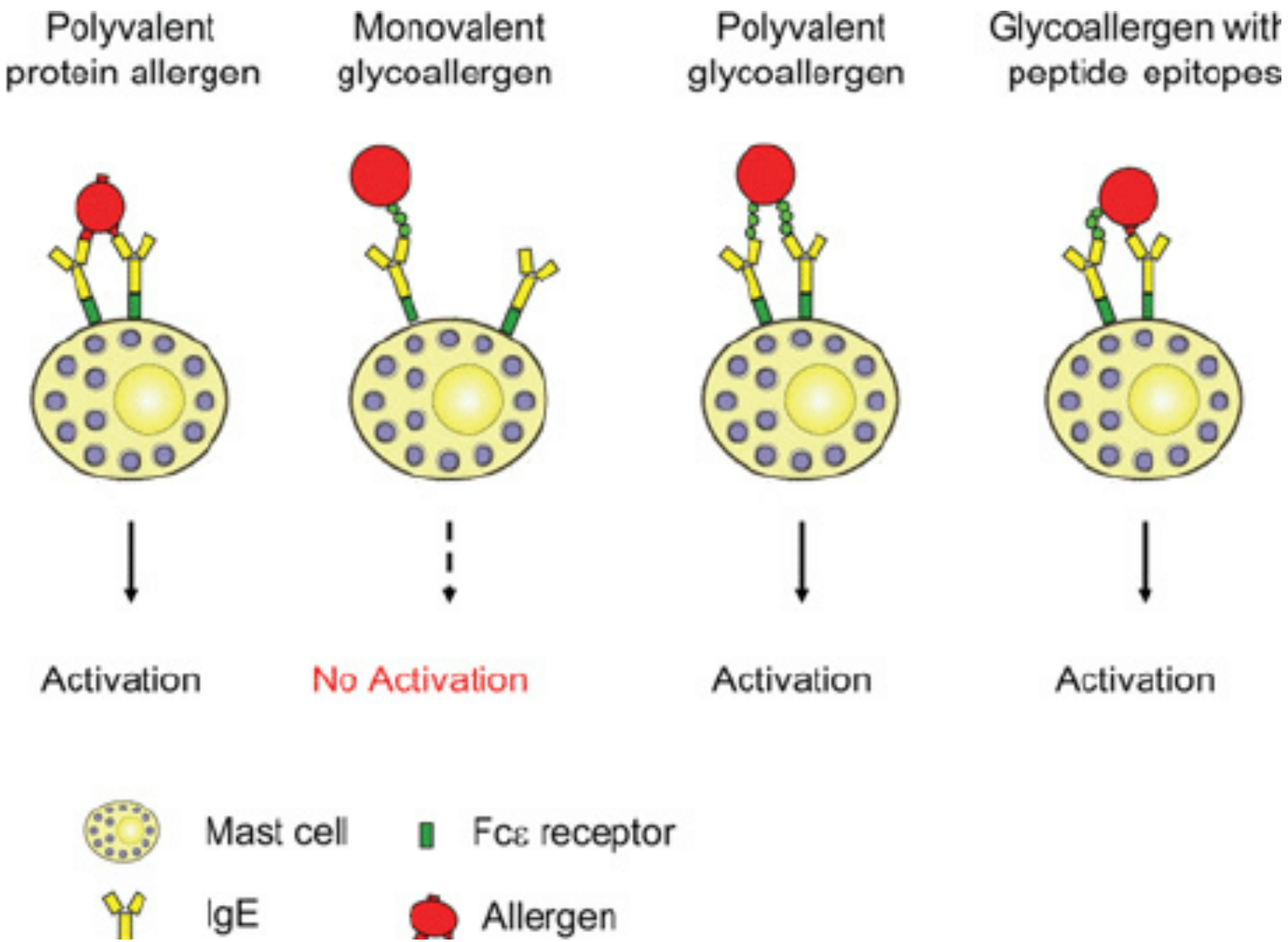


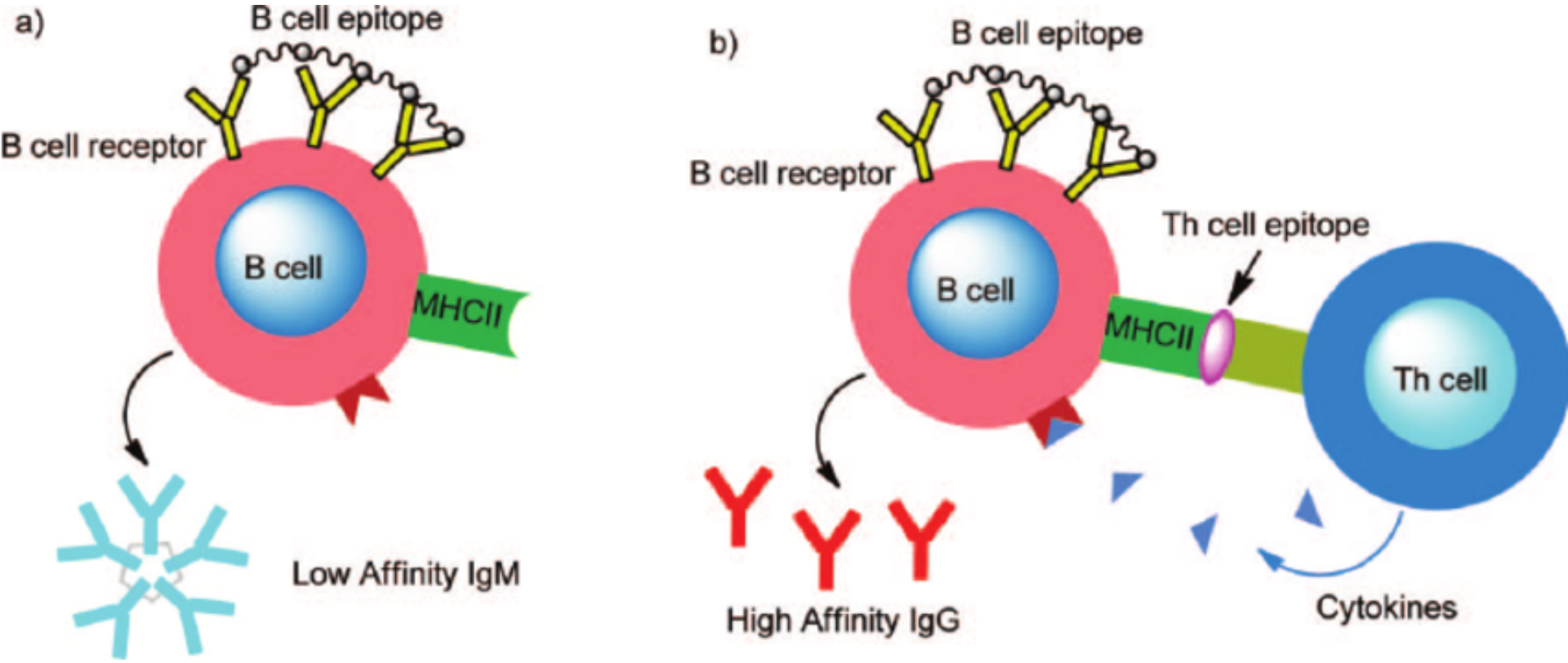
Feature or function	Antibody (Immunoglobulin)	T cell receptor (TCR)
		
		
Forms of antigens recognized	Macromolecules (proteins, polysaccharides, lipids, nucleic acids), small chemicals Conformational and linear epitopes	Peptides displayed by MHC molecules on APCs Linear epitopes
Diversity	Each clone has a unique specificity; potential for $>10^9$ distinct specificities	Each clone has a unique specificity; potential for $>10^{11}$ distinct specificities
Antigen recognition is mediated by:	Variable (V) regions of heavy and light chains of membrane Ig	Variable (V) regions of α and β chains
Signaling functions are mediated by:	Proteins (Igα and Igβ) associated with membrane Ig	Proteins (CD3 and ζ) associated with TCR
Effector functions are mediated by:	Constant (C) regions of secreted Ig	TCR does not perform effector functions


Microbe
mIg
Igα
Igβ
Adaptor proteins
Fyn
Lyn
Blk
Syk
ITAM
Btk
Cross-linking of membrane Ig by antigen
Tyrosine phosphorylation events
Biochemical intermediates
Active enzymes
Transcription factors
PLCγ2 activation
GTP/GDP exchange on Ras, Rac
Inositol trisphosphate, increased cytosolic Ca2+
Diacylglycerol (DAG)
Ras•GTP, Rac•GTP
Ca2+-dependent enzymes
PKC
ERK, JNK
Myc
NFAT
NF-κB
AP-1

Antigen Receptors of Lymphocytes

- When the antigen receptors of B lymphocytes bind to the antigen at the same time, the receptors cluster together.
- This process is called **cross-linking** and it keeps the respective signal proteins of the receptor complex in close link.


Polyvalent protein allergen
Monovalent glycoallergen
Polyvalent glycoallergen
Glycoallergen with peptide epitopes
Activation
No Activation
Activation
Activation
Mast cell
Fcε receptor
IgE
Allergen


a)
B cell epitope
B cell receptor
B cell
MHCII
Low Affinity IgM
b)
B cell epitope
B cell receptor
Th cell epitope
B cell
MHCII
Th cell
High Affinity IgG
Cytokines

Antigen Receptors of Lymphocytes

- Thus, enzymes attached to the cytoplasmic parts of the signal proteins provide **phosphorylation** of other proteins.
- Phosphorylation results in the production of many molecules that mediate lymphocyte responses by triggering complex signal cascades.

Antigen Receptors of Lymphocytes

- Antibodies are active molecules of humoral immunity.
- They are also called immunoglobins.
- Secreted antibodies recognize microbial antigens and toxins by their variable regions, just like membrane-bound B lymphocyte antigen receptors.

Antigen Receptors of Lymphocytes

- The constant regions of some secreted antibodies have the ability to bind to other molecules and thus serve to remove the antigen.
- **These molecules include receptors of phagocytes and proteins of the complement system.**

Antibodies

- An antibody molecule consists of two **heavy (H)** and two **light (L)** polypeptide chains
- Each chain contains a **variable (V)** and a **constant (C)** region
- These 4 chains combine to form a Y-shaped molecule

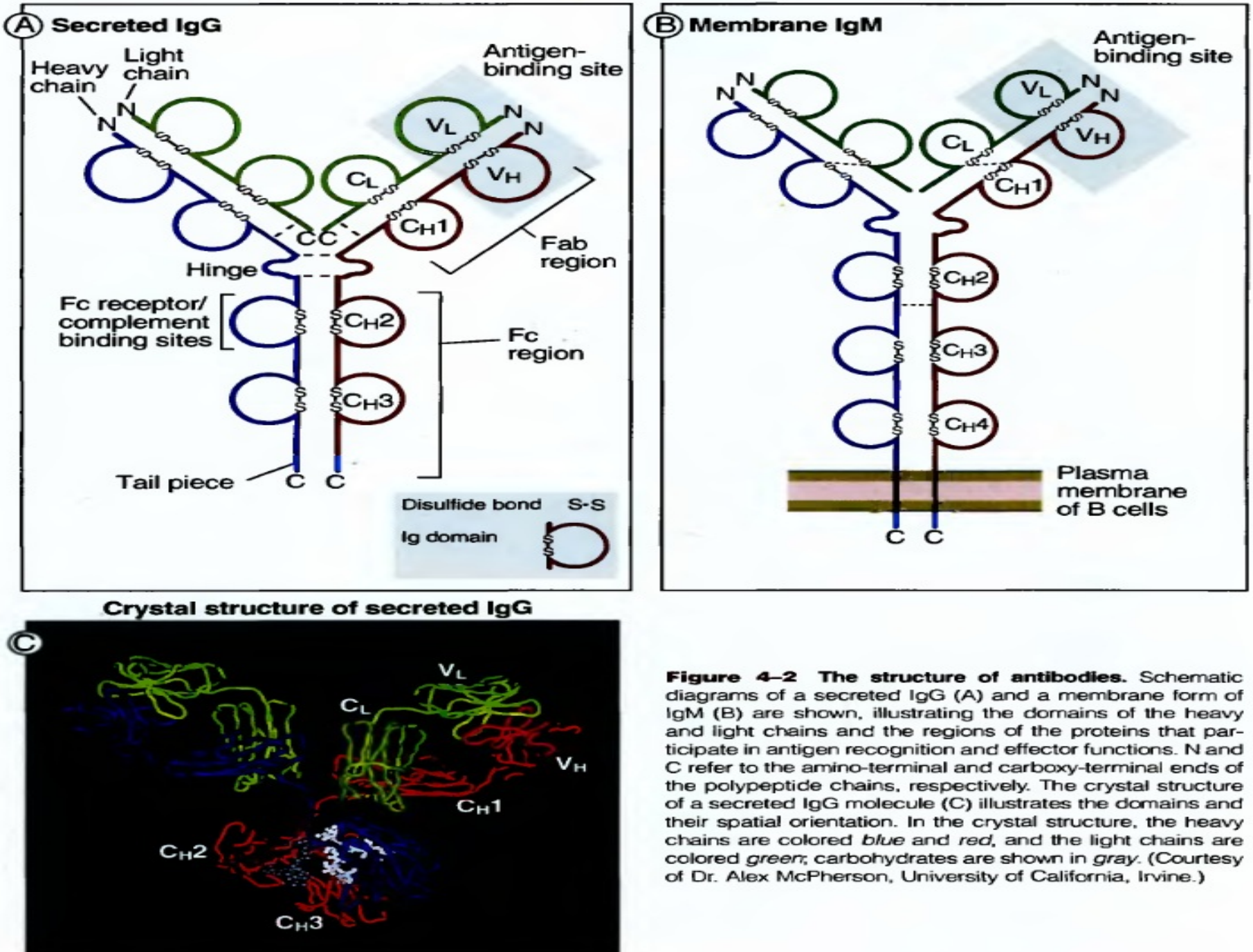
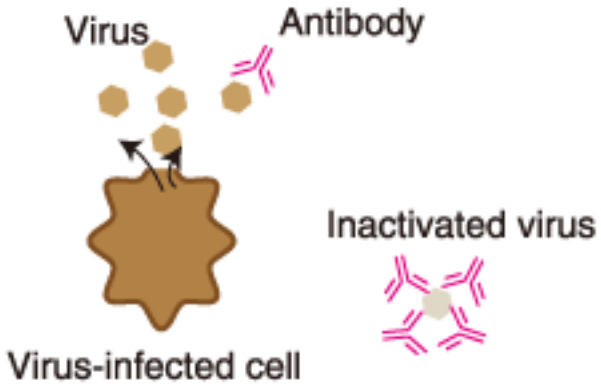


Figure 4–2 The structure of antibodies. Schematic diagrams of a secreted IgG (A) and a membrane form of IgM (B) are shown, illustrating the domains of the heavy and light chains and the regions of the proteins that participate in antigen recognition and effector functions. N and C refer to the amino-terminal and carboxy-terminal ends of the polypeptide chains, respectively. The crystal structure of a secreted IgG molecule (C) illustrates the domains and their spatial orientation. In the crystal structure, the heavy chains are colored *blue* and *red*, and the light chains are colored *green*; carbohydrates are shown in *gray*. (Courtesy of Dr. Alex McPherson, University of California, Irvine.)

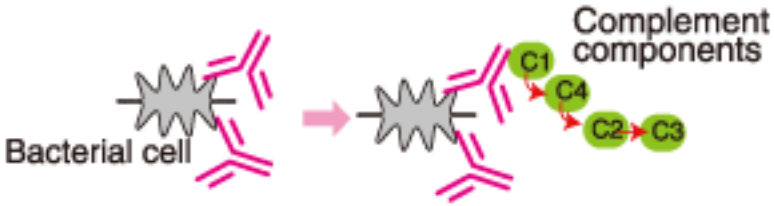
Functions of Antibodies

Neutralization



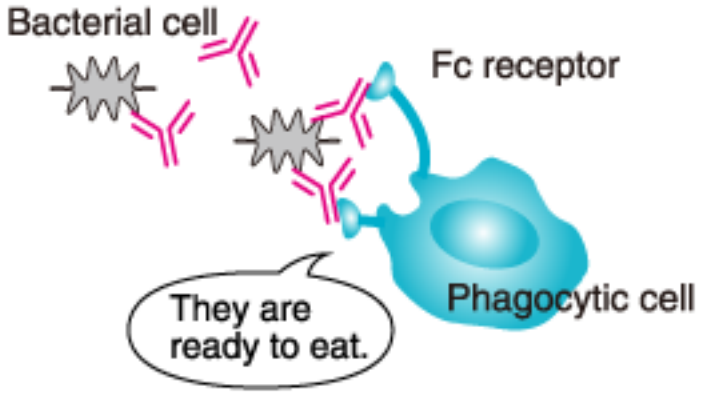
Antibodies bind to and inactivate viruses and toxins.
These antibodies are called "neutralizing antibodies."

Complement recruitment by antibodies

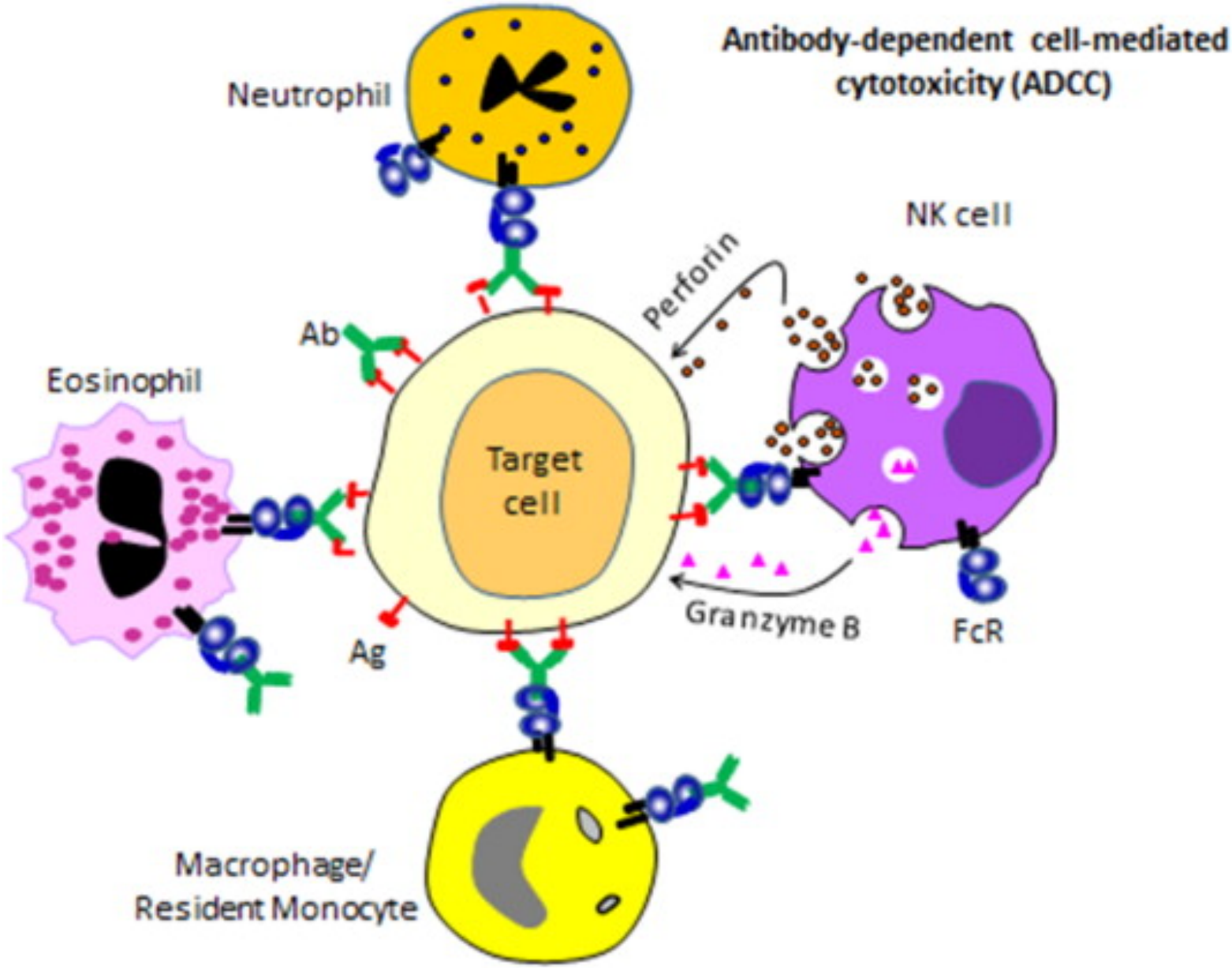


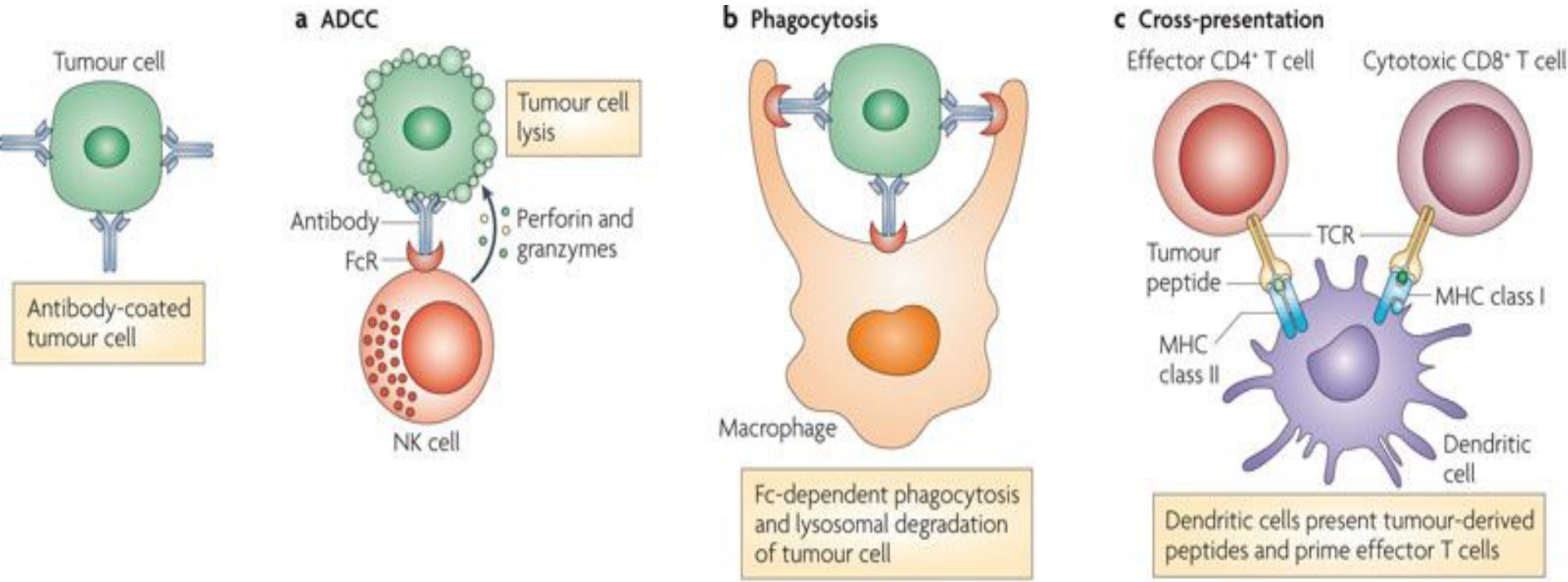
Antigen-antibody complexes activate the complement system (the classical pathway), triggering its antibacterial activity.

Opsonization



Phagocytic cells grab the antibodies bound to the surface of foreign substances, for efficient phagocytosis.


Antibody-dependent cell-mediated cytotoxicity (ADCC)
Neutrophil
NK cell
Perforin
Ab
Eosinophil
Target cell
Granzyme B
FcR
Ag
Macrophage/
Resident Monocyte


Tumour cell
Antibody-coated tumour cell
a ADCC
Tumour cell lysis
Antibody
FcR
Perforin and granzymes
NK cell
b Phagocytosis
Macrophage
Fc-dependent phagocytosis and lysosomal degradation of tumour cell
c Cross-presentation
Effector CD4+ T cell
Cytotoxic CD8+ T cell
TCR
Tumour peptide
MHC class I
MHC class II
Dendritic cell
Dendritic cells present tumour-derived peptides and prime effector T cells

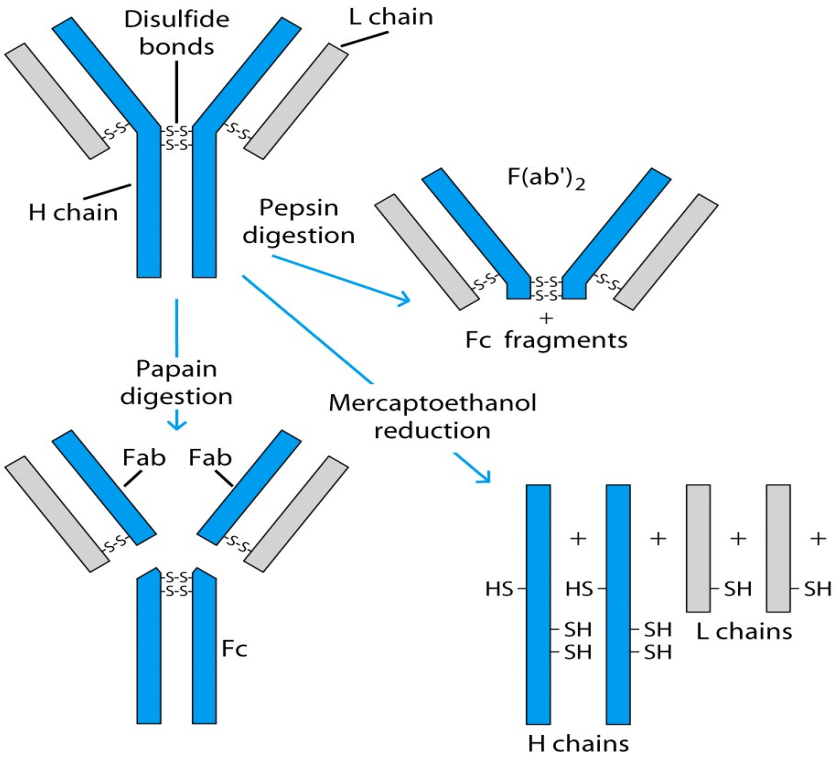
Functions of Antibodies

- Binding specific antigens
- Complement activation (Ig G and Ig M)
- Opsonization (Ig G)
- Toxin and virüs neutralization (Ig G)
- Antigen-dependent cellular toxicity (Ig G and Ig E)
- Histamin secretion from mast cells (Ig E)

Structures of Antibodies

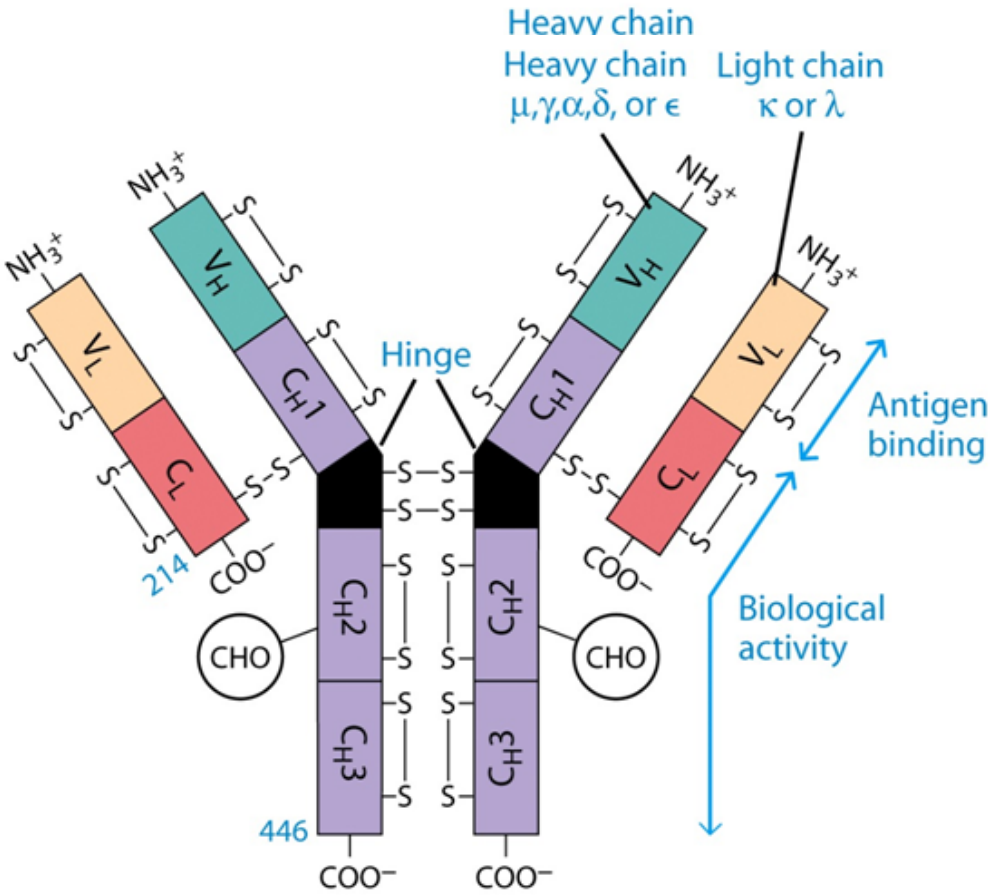
Two main parts:

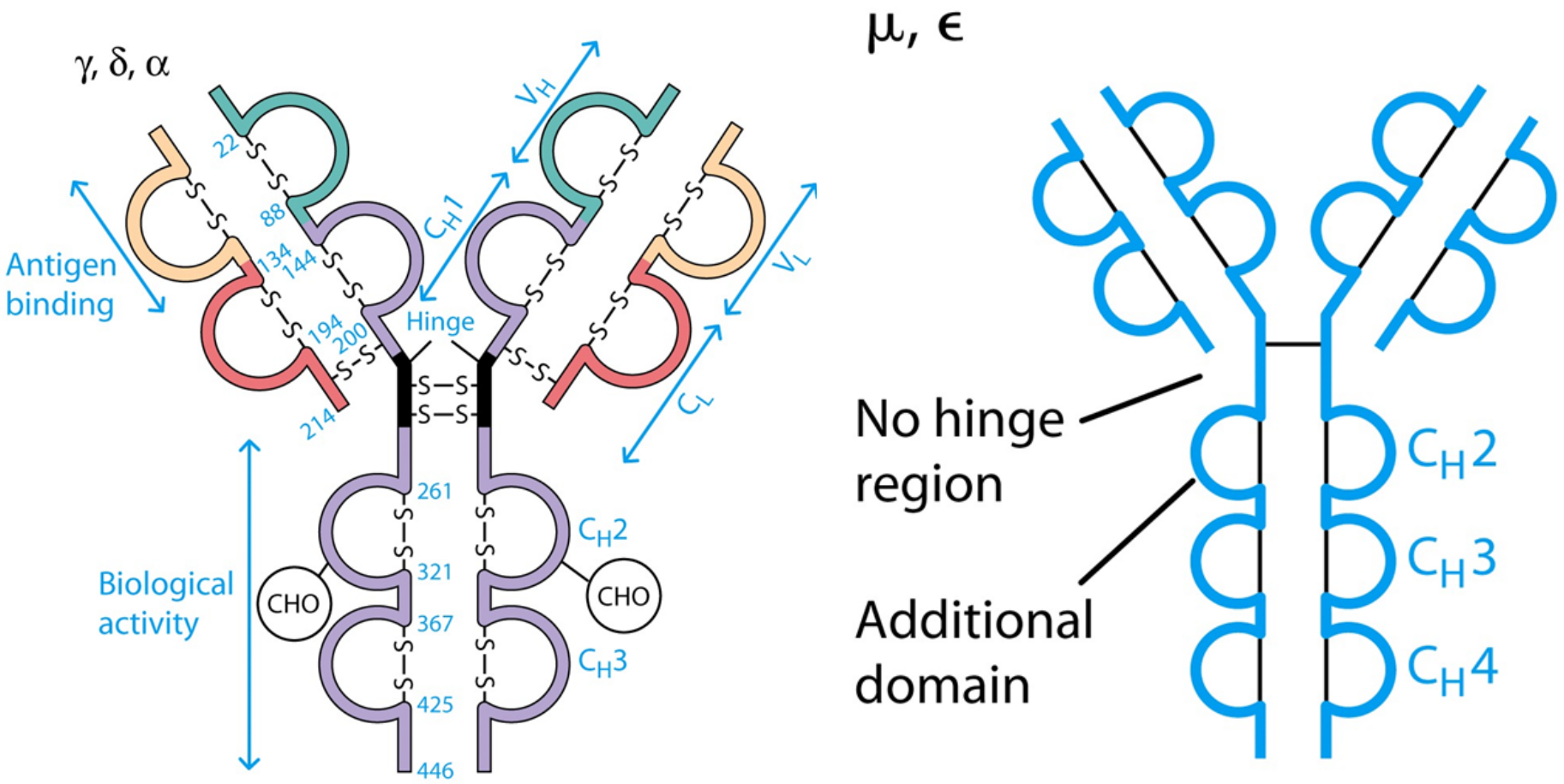
1. Fragment antigen binding (Fab)
2. Fragment crystaline



Antibodies are heterodimeric molecules. It consists of four peptide chains.

- **2 identical heavy chains (50kDa)**
- **2 similar light chains (25kDa)**




γ, δ, α
Antigen binding
Biological activity
V_H
C_H1
V_L
C_L
Hinge
22
88
134
144
194
200
214
261
321
367
425
446
CHO
CHO
C_H2
C_H3
μ, ϵ
No hinge region
Additional domain
C_H2
C_H3
C_H4

Antibodies

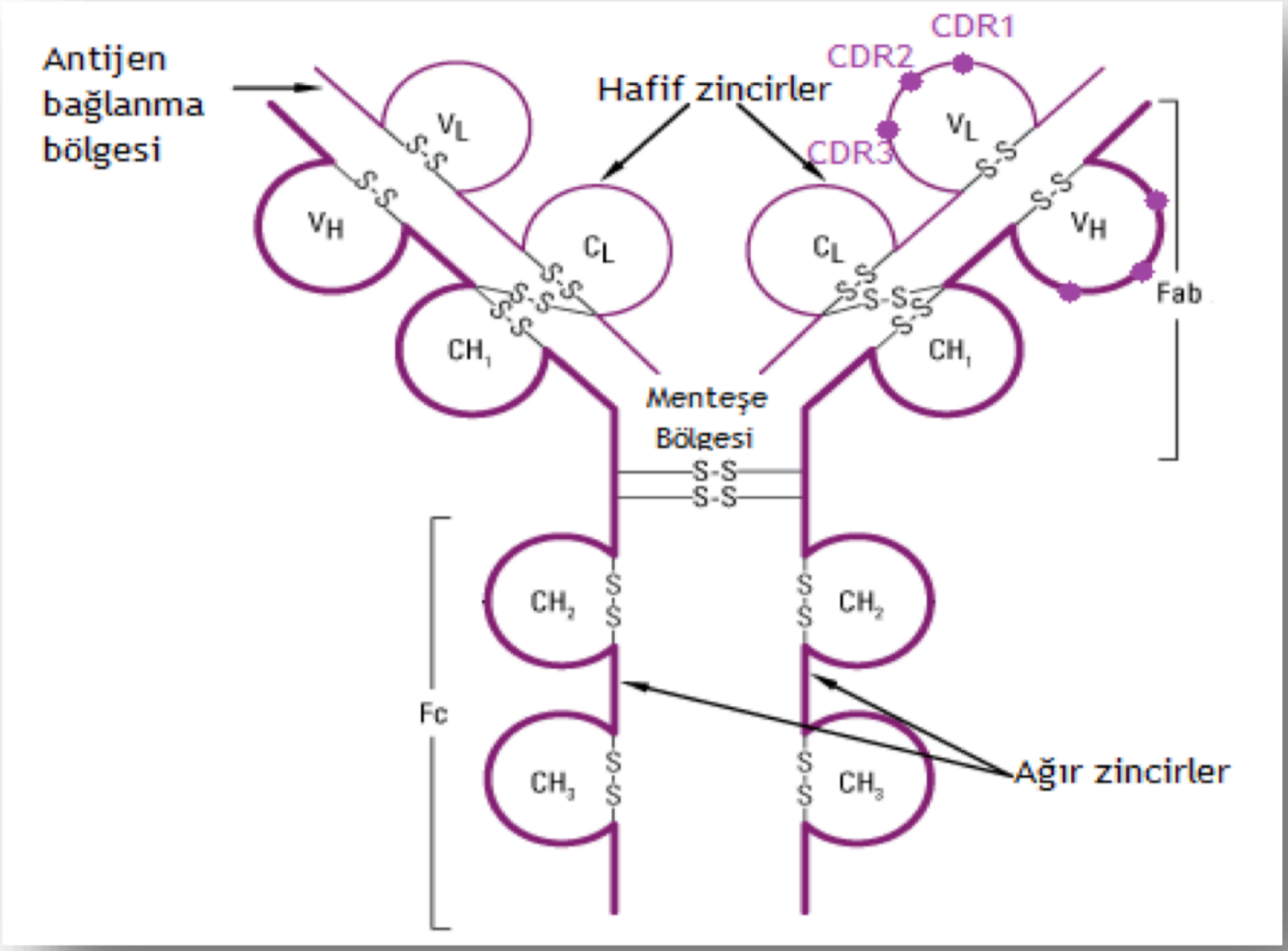
- **Each light chain is attached to a heavy chain by disulfide bonds**
- **Each heavy chain is linked to each other by disulfide bonds**
- One light chain: one V and one C regions,
- One heavy chain: one V and three or four C regions

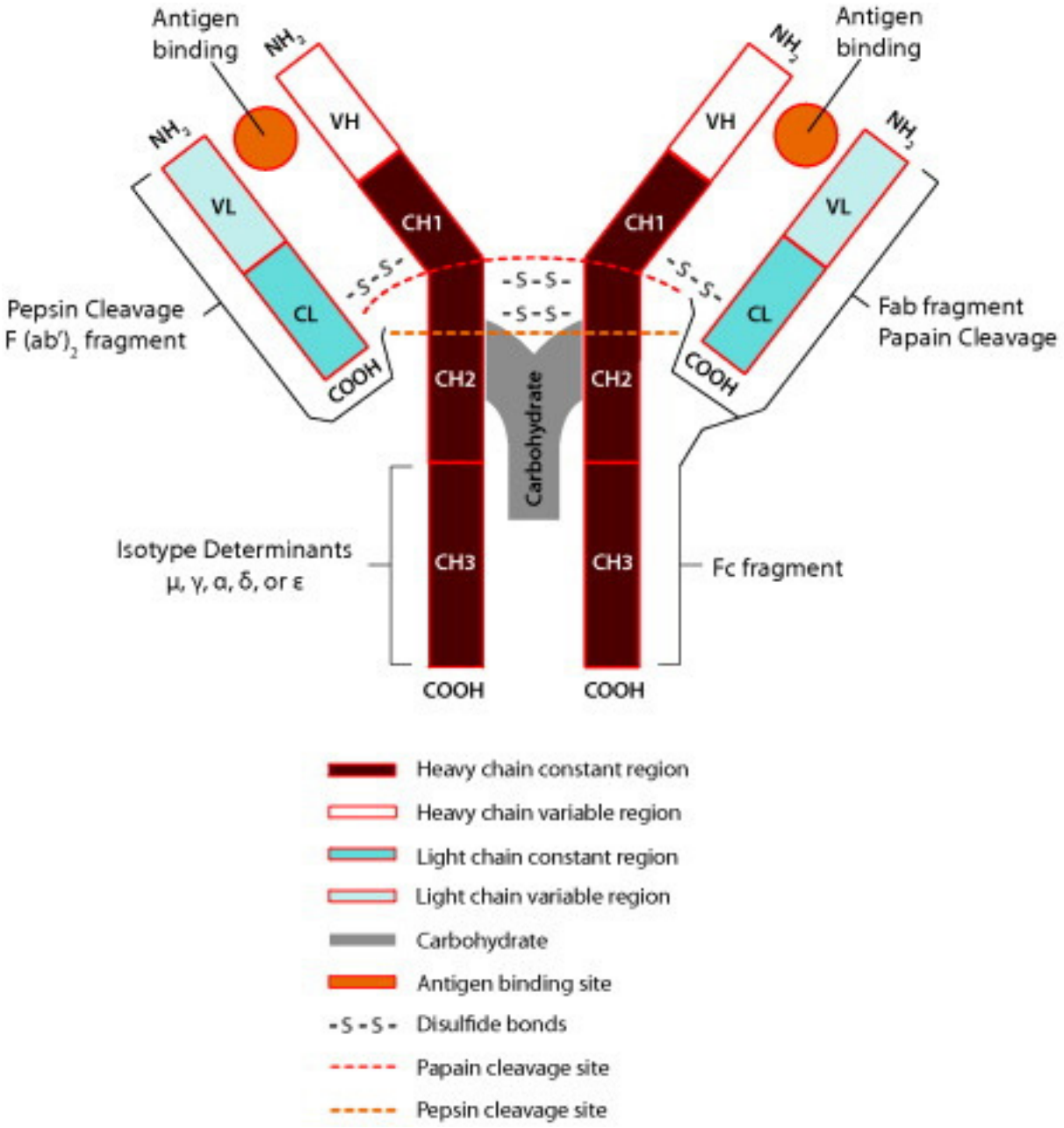
Antibodies

- Each variable region of heavy and light chains carries 3 hypervariable regions or CDRs.
- The most variable of these triads is the **CDR3** region located at the junction of the V and C regions.

Antibodies

- CDR3 is the part of the immunoglobulin molecule most involved in antigen binding.
- The V regions of the heavy and light chain form the antibody region required to recognize the antigen and is called the **Fab (fragment antigen binding).**


Antijen bağlanma bölgesi
CDR1
CDR2
CDR3
Hafif zincirler
V_L
V_H
C_L
CH_1
Fab
Menteşe Bölgesi
S-S
CH_2
CH_3
Fc
Ağır zincirler


Antigen binding
NH_2
VH
NH_2
VL
CH1
-S-S-
CL
Pepsin Cleavage
F (ab')$_2$ fragment
COOH
-S-S-
-S-S-
CH2
Carbohydrate
CH3
Isotype Determinants
μ, γ, α, δ, or ε
COOH
Antigen binding
NH_2
VH
NH_2
VL
CH1
-S-S-
CL
Fab fragment
Papain Cleavage
COOH
CH2
CH3
Fc fragment
COOH
Heavy chain constant region
Heavy chain variable region
Light chain constant region
Light chain variable region
Carbohydrate
Antigen binding site
-S-S- Disulfide bonds
Papain cleavage site
Pepsin cleavage site

Antibodies

- The remaining C regions of the heavy chain are called crystalline fragment **Fc (fragment crystaline).**
- Because this region tends to form crystals in solution.

Antikorlar

- Most antibody molecules have an elastic region between the Fab and Fc regions.
- **This region is called the hinger region.**
- The hinge allows each antibody molecule to move the antigen-binding Fab region and **freely capture the antigen epitopes** that stand apart.

Antibodies

- Antibodies are named according to their heavy chain type (IgM, IgG, IgD,IgE, IgA)

Type of heavy chain in each Ig class	
Immunoglobulin class	Heavy chain type
IgG	γ (gamma)
IgA	α (alpha)
IgM	μ (mu)
IgD	δ (delta)
IgE	ε (epsilon)

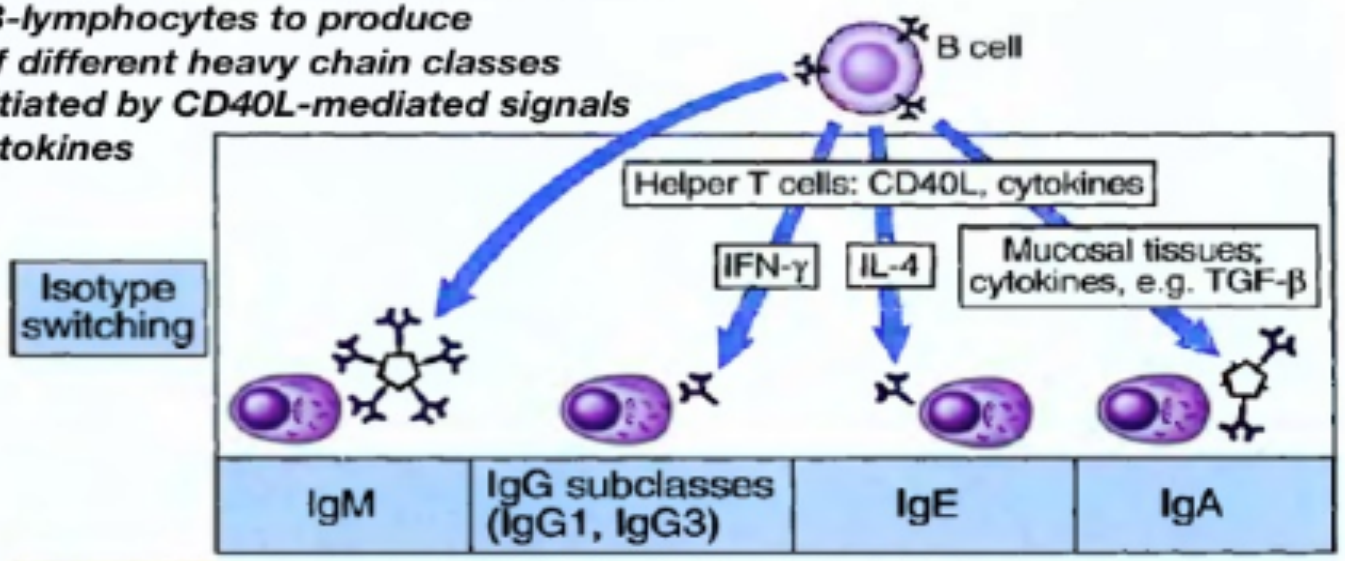
Antibodies

- The physical and biological properties of different isotypes are different from each other. **The antigen receptors of naive B lymphocytes are in the structure of IgM and IgD.**
- As a result of exposure to antigen or stimulation with T helper cells, the antigen-specific B-lymphocyte clone expands and differentiates into plasma cells that secrete antibodies.

Heavy chain isotype switching

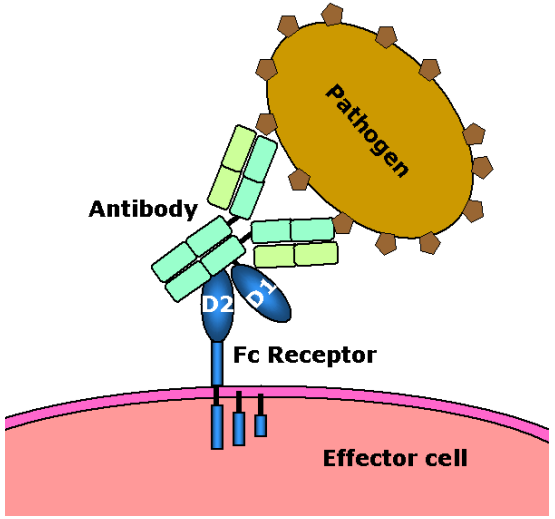
ISOTYPE SWITCHING

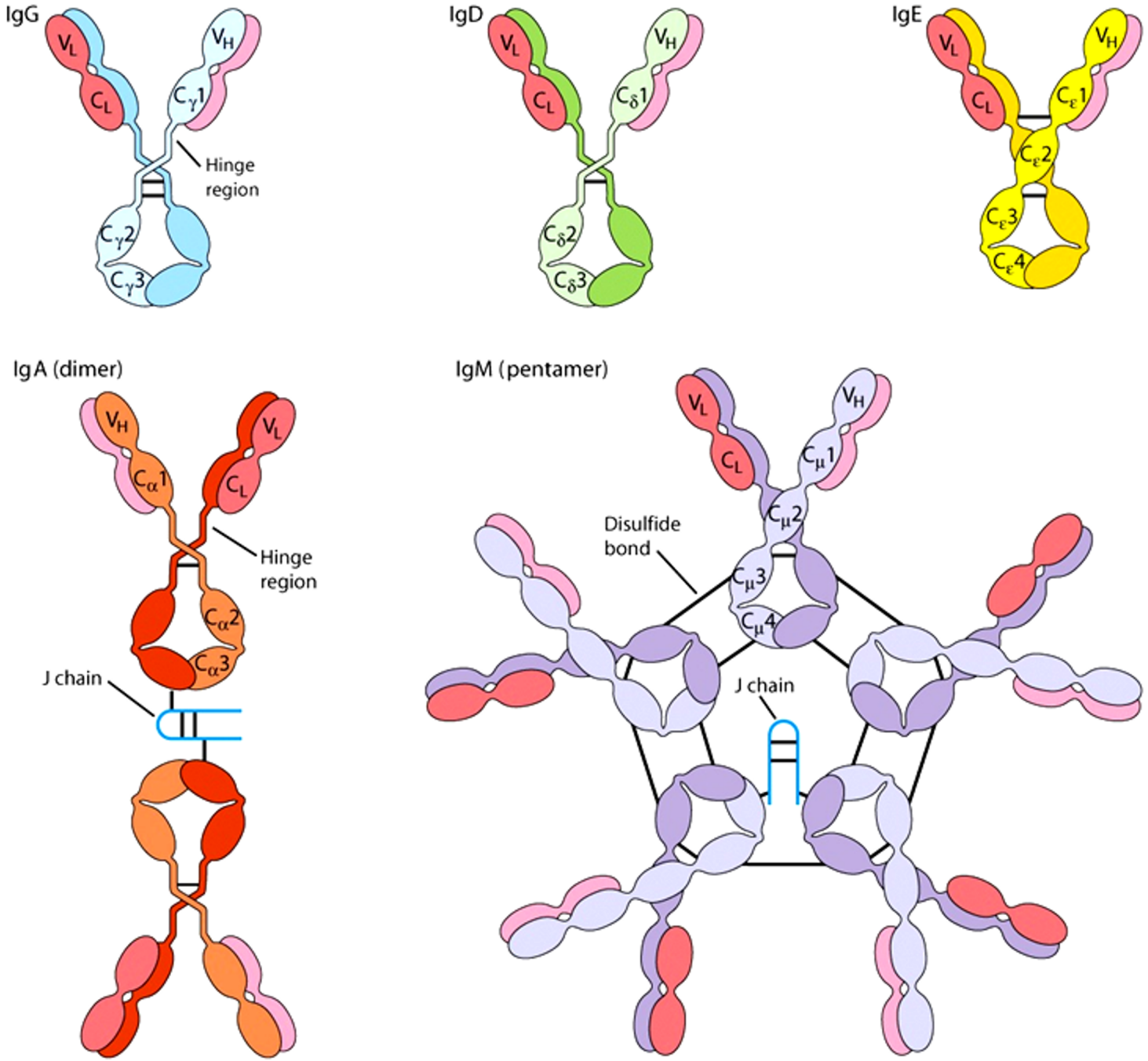
Helper T-cells stimulate the progeny of IgM+ IgD expressing B-lymphocytes to produce antibodies of different heavy chain classes (isotypes) initiated by CD40L-mediated signals and other cytokines



	IgM	IgG subclasses (IgG1, IgG3)	IgE	IgA
Principal effector functions	Complement activation	Fc receptor–dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)	Immunity against helminths Mast cell degranulation (immediate hypersensitivity)	Mucosal immunity (transport of IgA through epithelia)

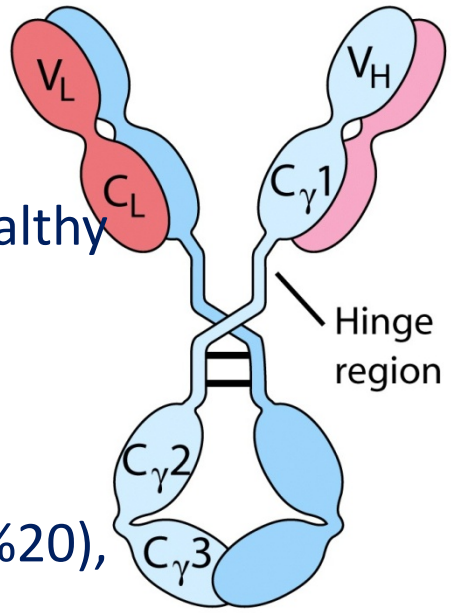
- **Activation of Complement proteins**
  - IgM, IgG3, IgG1, IgG2
- **Placenta transmission**
  - IgG1, IgG3, IgG4, Ig A
- **Fc Receptor binding**
  - IgG1, IgG3, IgG4, IgG2




IgG
VL
VH
CL
Cγ1
Hinge region
Cγ2
Cγ3
IgD
VL
VH
CL
Cδ1
Cδ2
Cδ3
IgE
VL
VH
CL
Cε1
Cε2
Cε3
Cε4
IgA (dimer)
VH
VL
Cα1
CL
Hinge region
Cα2
Cα3
J chain
IgM (pentamer)
VL
VH
CL
Cμ1
Cμ2
Disulfide bond
Cμ3
Cμ4
J chain

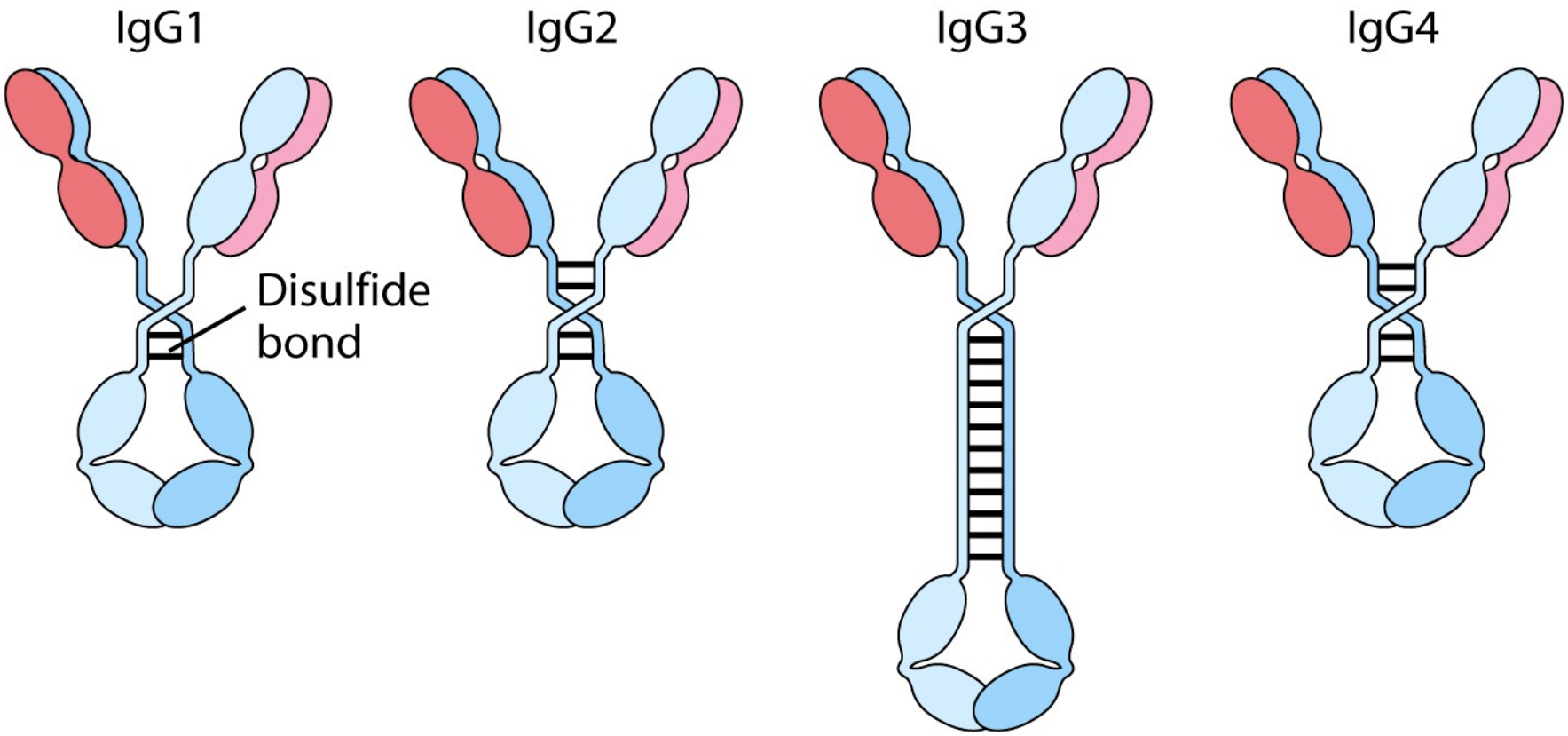
IgG

- Molecular weight nearly 150-170 kDA.



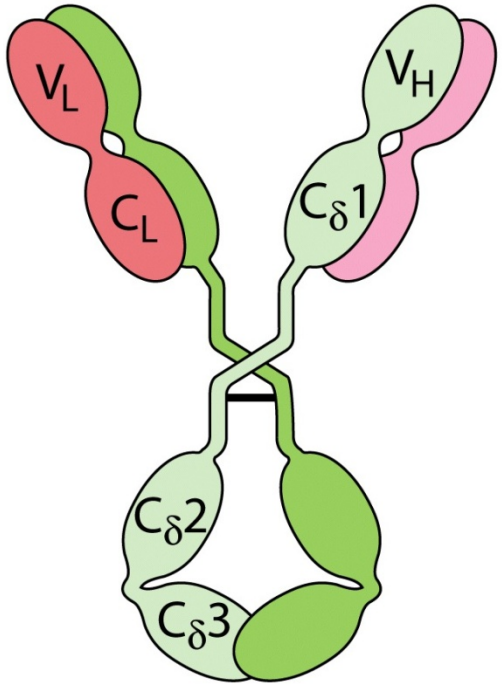
- It covers 80% of the antibodies in the serum of a healthy person.
- It is divided into four sub-classes: IgG1(%68), IgG2(%20), IgG3(%8),IgG4(%4).
- IgG3 is active in classical complementpathway activation.
- IgG1 and IgG3bind to Fc receptors with high affinity.

IgG



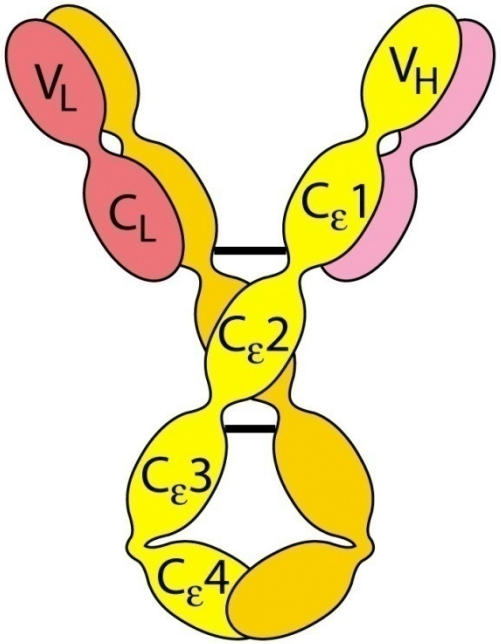
IgD

1. Molecular weight nearly 180kDa.
2. It covers 1 % of the antibodies in the serum of a healthy person.
3. Active in B cell differentiation



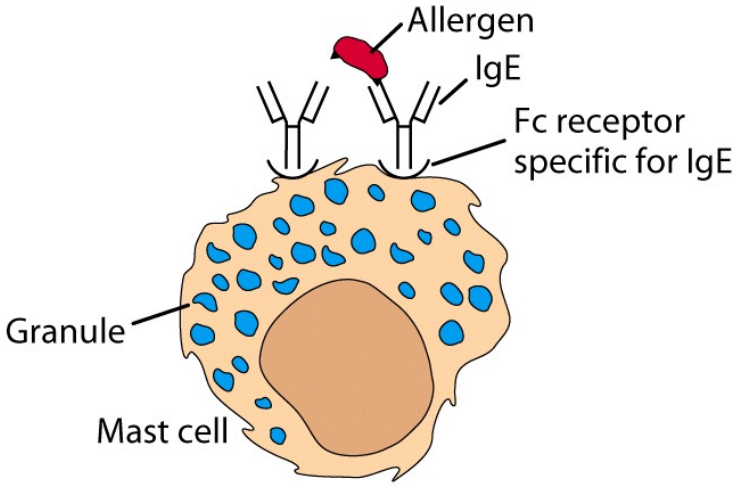
IgE

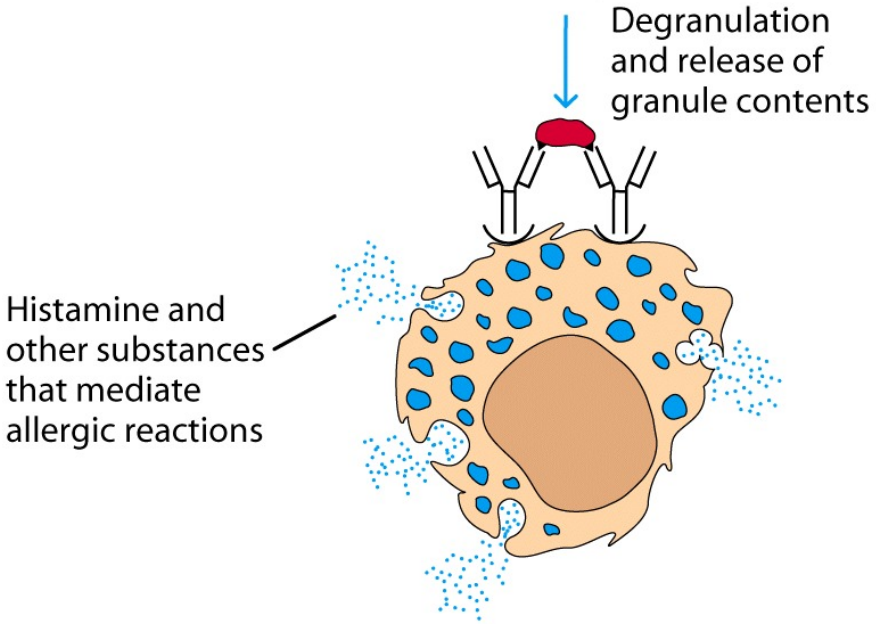
1. Molecular weight nearly 200 kDA.
2. It is found in extremely small amounts in the serum of a healthy person.
3. It is seen at a high rate in those with allergies and helminth infections.



IgE

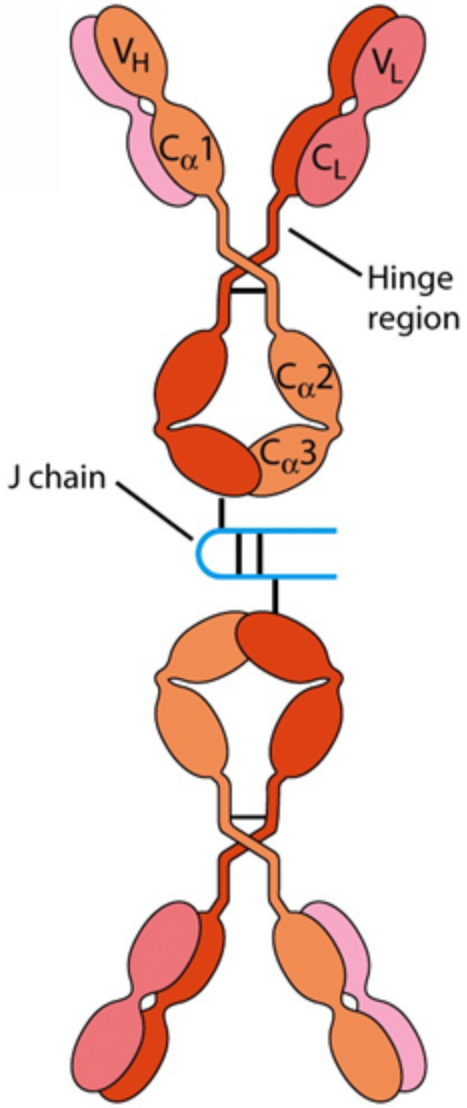
Degranulation





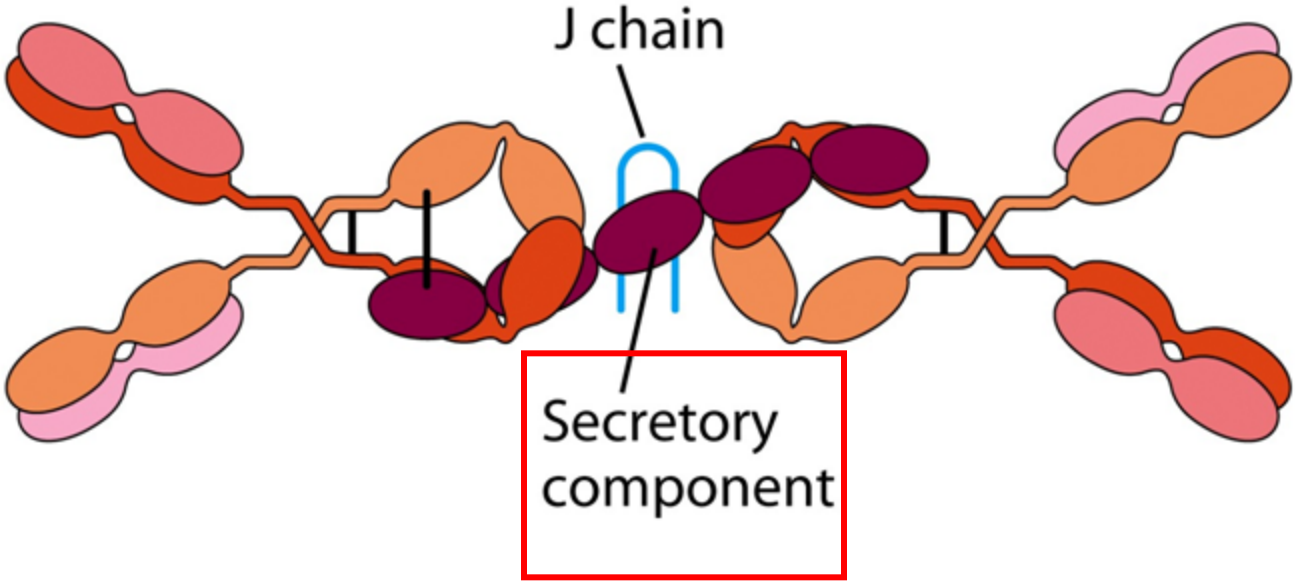
Ig A

- Molecular weight of monomer form: 160kDa.
- Monomer, dimer, trimer and tetramer
- It constitutes 10-15% of total antibodies in healthy human serum.
- It contains J chain that stabilizes the dimeric structure.

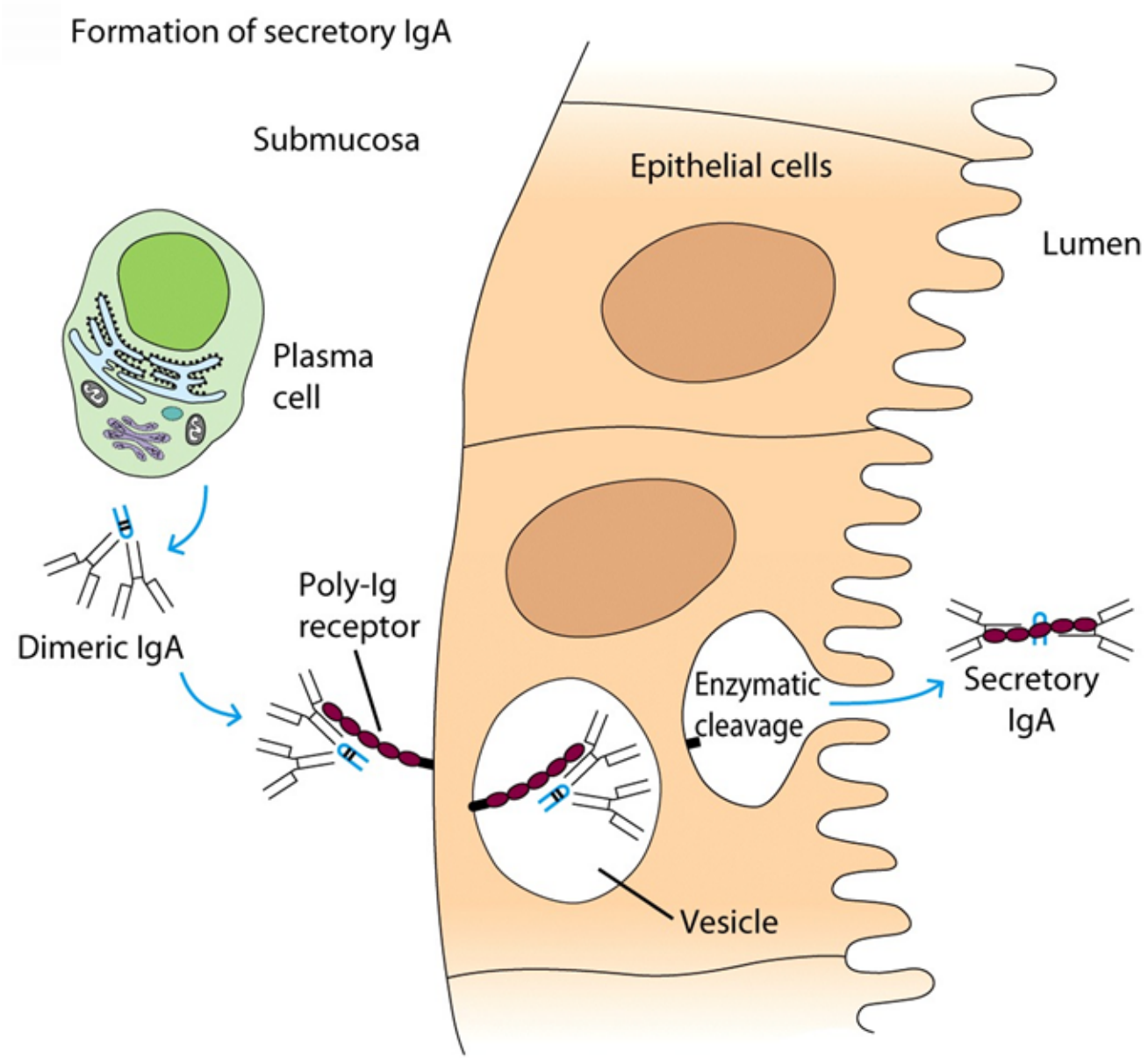


Ig A

In addition to being present in serum, it crosses the epithelial barrier with the secretory part and is found **in saliva, tears, urine, milk and intestinal secretions.**

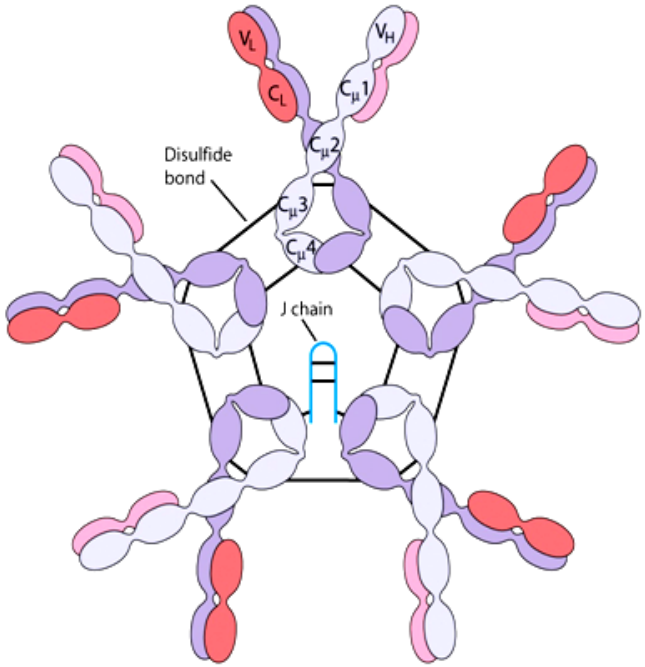


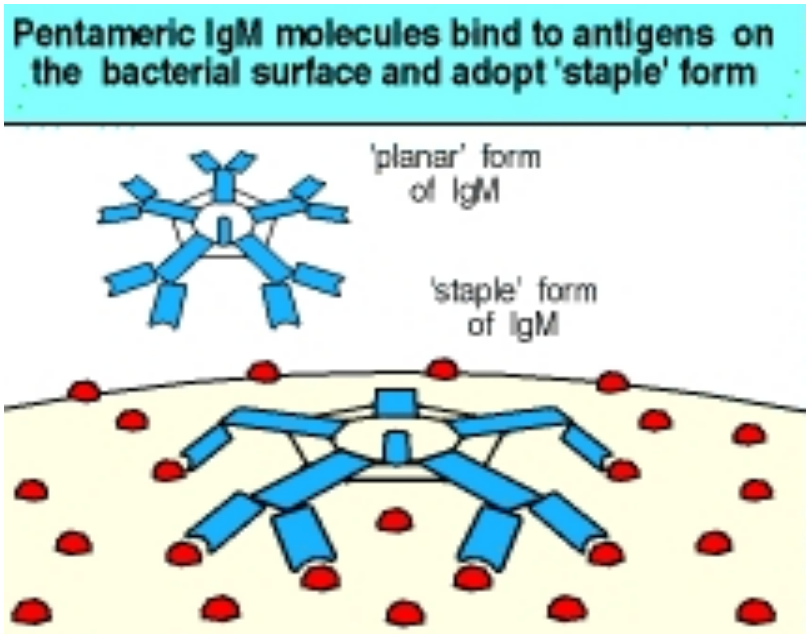
Ig A

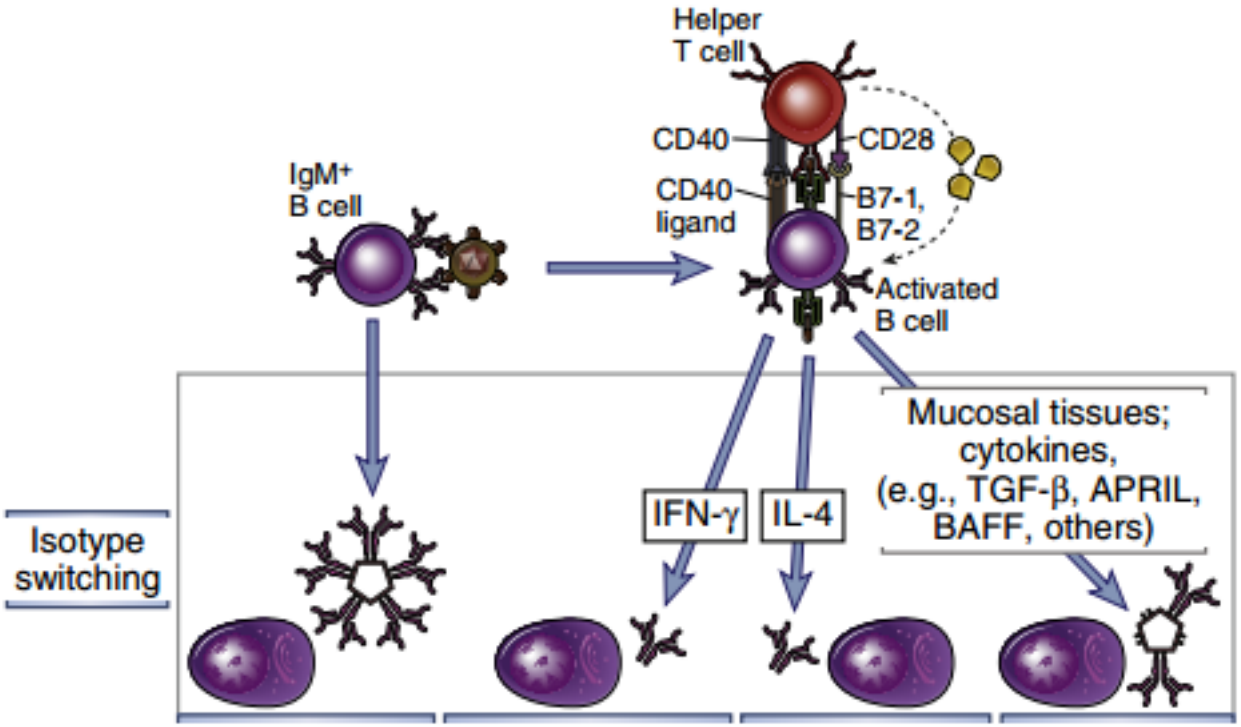

Formation of secretory IgA
Submucosa
Epithelial cells
Lumen
Plasma cell
Dimeric IgA
Poly-Ig receptor
Enzymatic cleavage
Secretory IgA
Vesicle

Ig M

1. Molecular weight 900kDa.
2. %6 of total Immunoglobulines
3. Effectively binding complement proteins
4. Pentameric structure
5. Monomeric IgMs are found on the surface of B-lymphocytes.



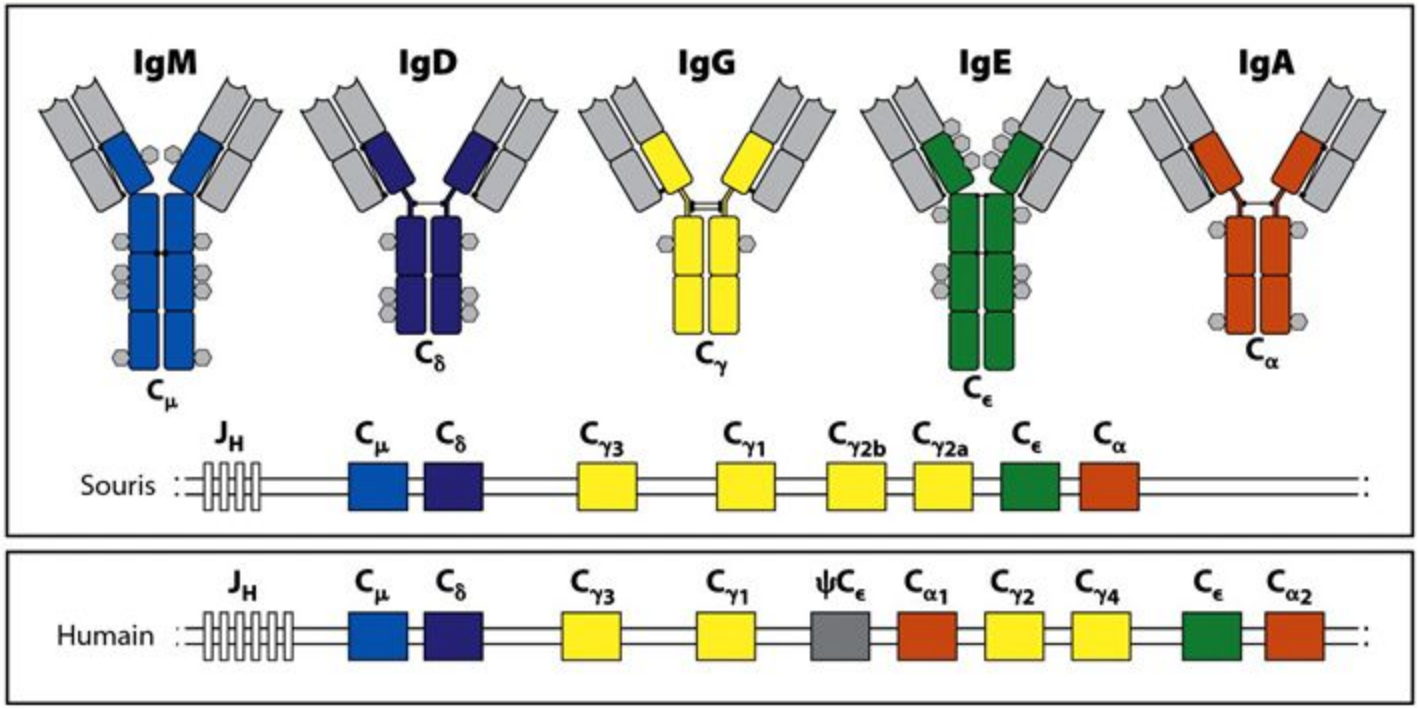




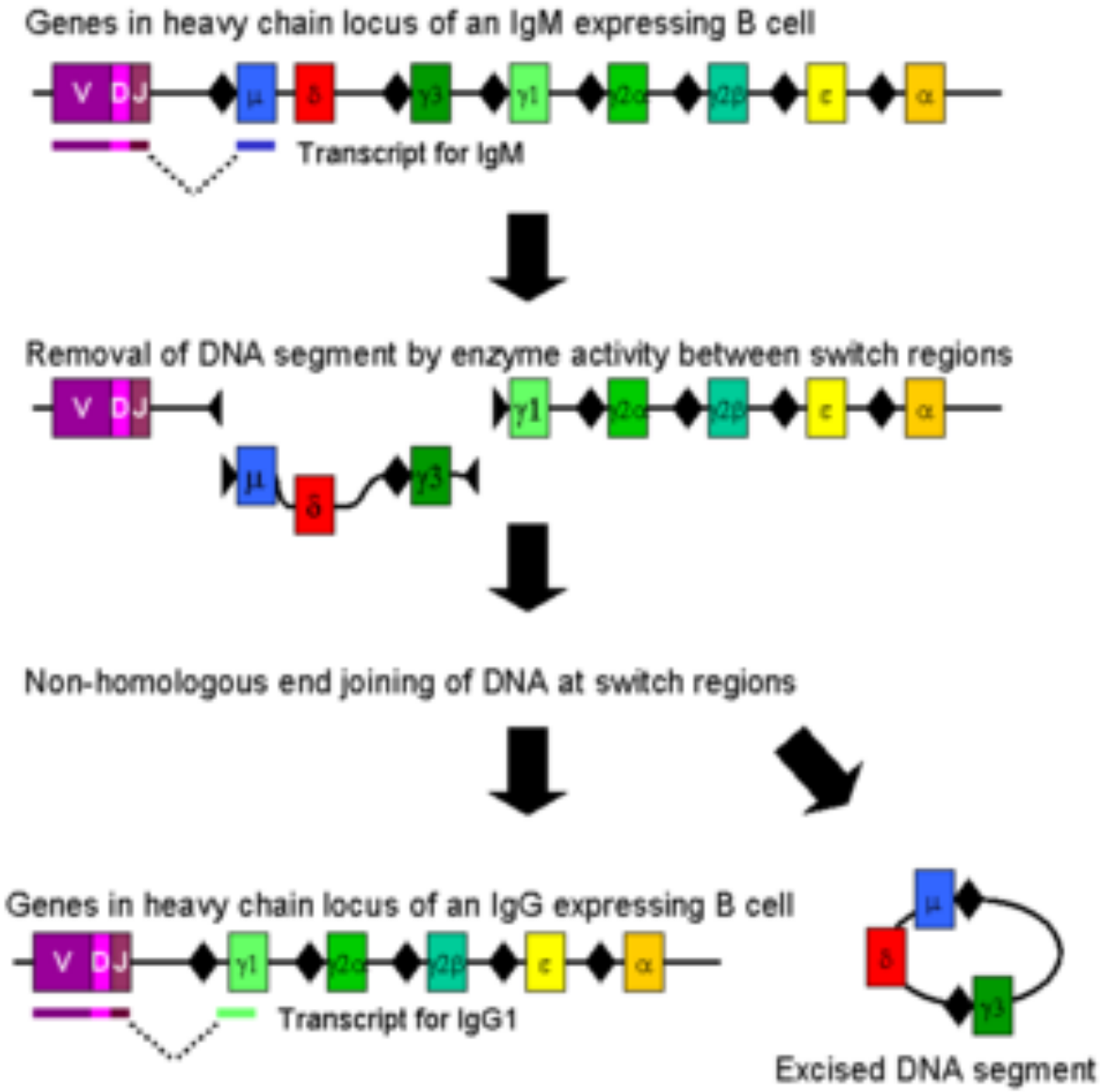
Principal effector functions	Complement activation	Opsonization and phagocytosis; complement activation; neonatal immunity (placental transfer)	Immunity against helminths Mast cell degranulation (immediate hypersensitivity)	Mucosal immunity (transport of IgA through epithelia)

FIGURE 12-14 Ig heavy chain isotype switching. B cells activated by helper T cell signals (CD40L, cytokines) undergo switching to different Ig isotypes, which mediate distinct effector functions. Selected examples of switched isotypes are shown. The role of IFN-γ in directing specific isotype switching events has been established only in rodents.

Isotype switch

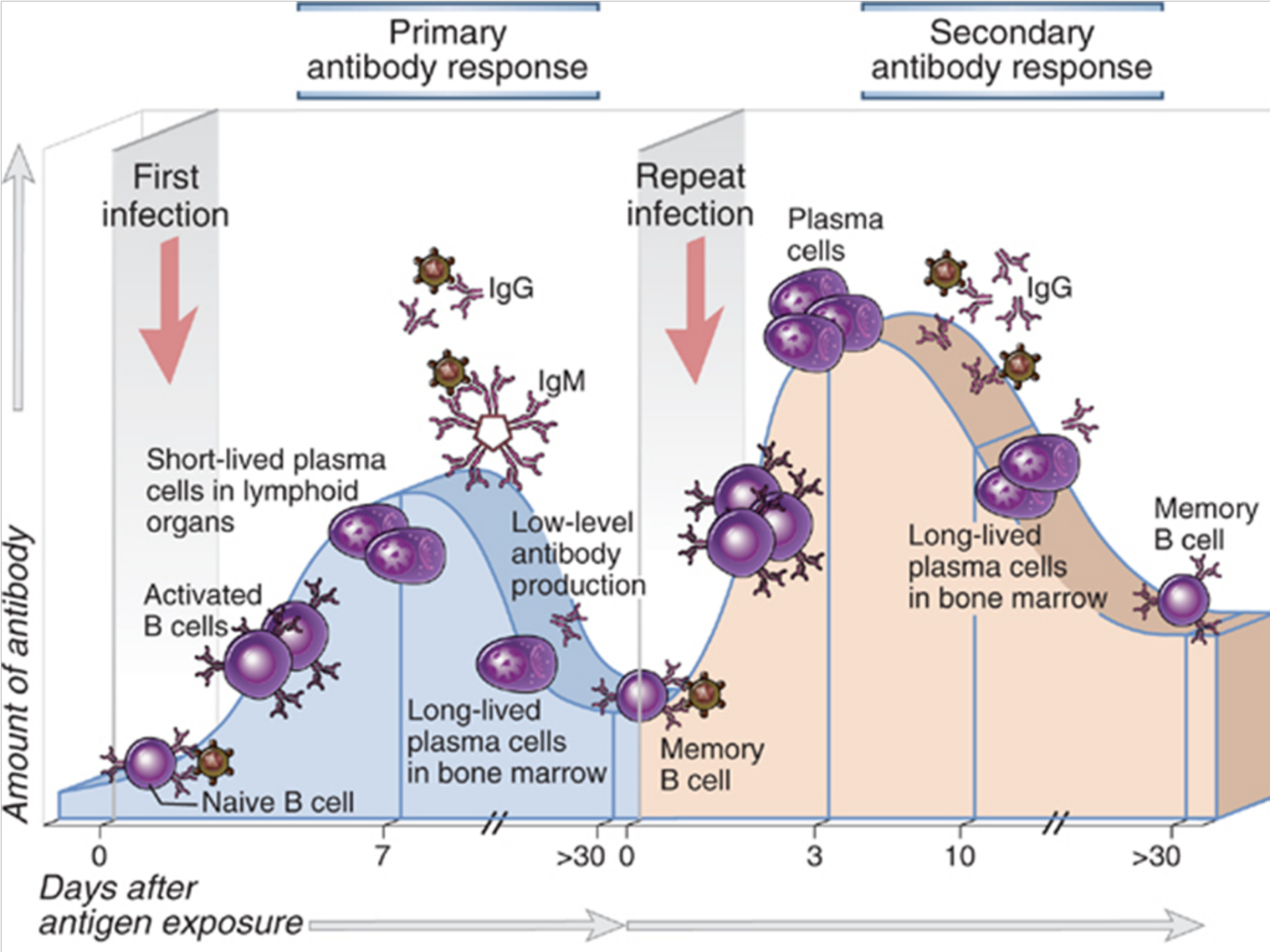


- Ig isotype doesn't change the specificity of antibody binding site (i.e. variable region)
- Switch only happens once B cell meets antigen.
- Process is regulated by a switch region in the DNA located upstream of the constant region of the heavy chain
- Switch happens thanks to many enzymes also involved in DNA repair


Genes in heavy chain locus of an IgM expressing B cell
V D J
μ
δ
γ3
γ1
γ2α
γ2β
ε
α
Transcript for IgM
Removal of DNA segment by enzyme activity between switch regions
V D J
γ1
γ2α
γ2β
ε
α
μ
δ
γ3
Non-homologous end joining of DNA at switch regions
Genes in heavy chain locus of an IgG expressing B cell
V D J
γ1
γ2α
γ2β
ε
α
Transcript for IgG1
μ
δ
γ3
Excised DNA segment

Difference Between Primary Response and Secondary Response.

	Primary Response	Secondary Response
Exposure to antigen	first exposure to a specific antigen	*after second exposure to the same a*ntigen
Time of onset	1-week delay	Within hours
Strength	weak potency	more potent
Duration	Short life , for only a few weeks	forms antibodies for many months
Type of antibody	IgM	IgG


Primary antibody response
Secondary antibody response
First infection
Repeat infection
IgG
IgM
Plasma cells
IgG
Short-lived plasma cells in lymphoid organs
Low-level antibody production
Activated B cells
Long-lived plasma cells in bone marrow
Memory B cell
Long-lived plasma cells in bone marrow
Memory B cell
Naive B cell
Amount of antibody
0
7
>30
0
3
10
>30
Days after antigen exposure

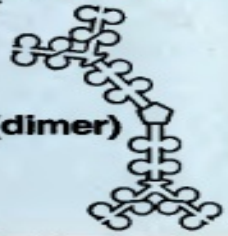
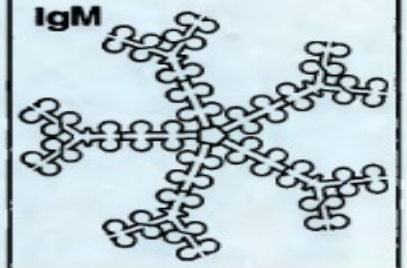
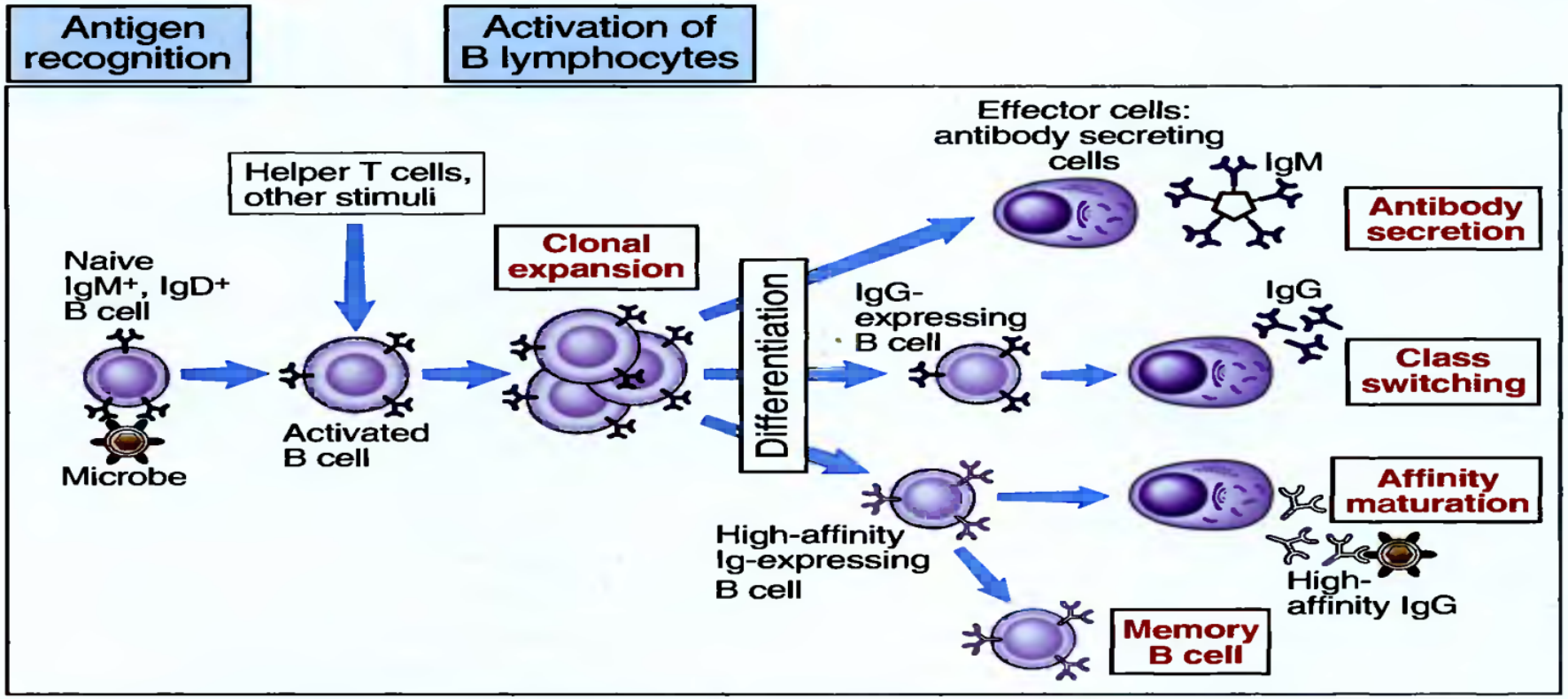
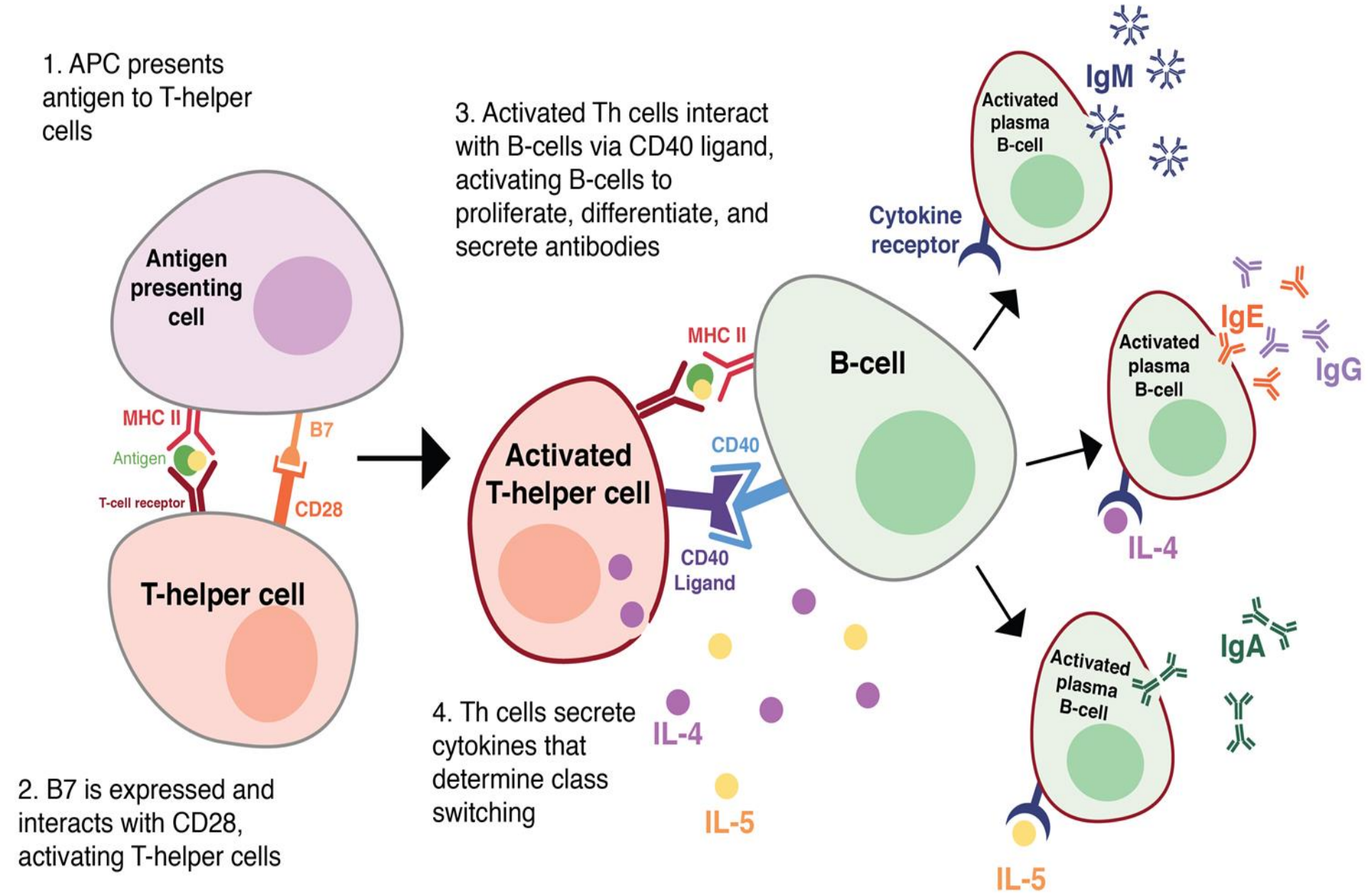
Isotype of antibody	Subtypes	H chain	Serum concentr. (mg/mL)	Serum half-life (days)	Secreted form	Functions
IgA	IgA1,2	α(1 or 2)	3.5	6	Monomer, dimer, trimer 	Mucosal immunity, neonatal passive immunity
IgD	None	δ	Trace	3	None	Naive B cell antigen receptor
IgE	None	ε	0.05	2	Monomer 	Mast cell activation (immediate hypersensitivity)
IgG	IgG1-4	γ (1,2,3 or 4)	13.5	23	Monomer 	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None	μ	1.5	5	Pentamer 	Naive B cell antigen receptor, complement activation

Figure 4–3 Features of the major isotypes (classes) of antibodies. The table summarizes some important features of the major antibody isotypes of humans. Isotypes are classified on the basis of their heavy chains; each isotype may contain either κ or λ light chain. The schematic diagrams illustrate the distinct shapes of the secreted forms of these antibodies. Note that IgA consists of two subclasses, called IgA1 and IgA2, and IgG consists of four subclasses, called IgG1, IgG2, IgG3, and IgG4. (IgG subclasses are given different names in other species, for historical reasons; in mice, they are called IgG1, IgG2a, IgG2b, and IgG3.) The serum concentrations are average values in normal individuals.

Humoral Immunity Phases

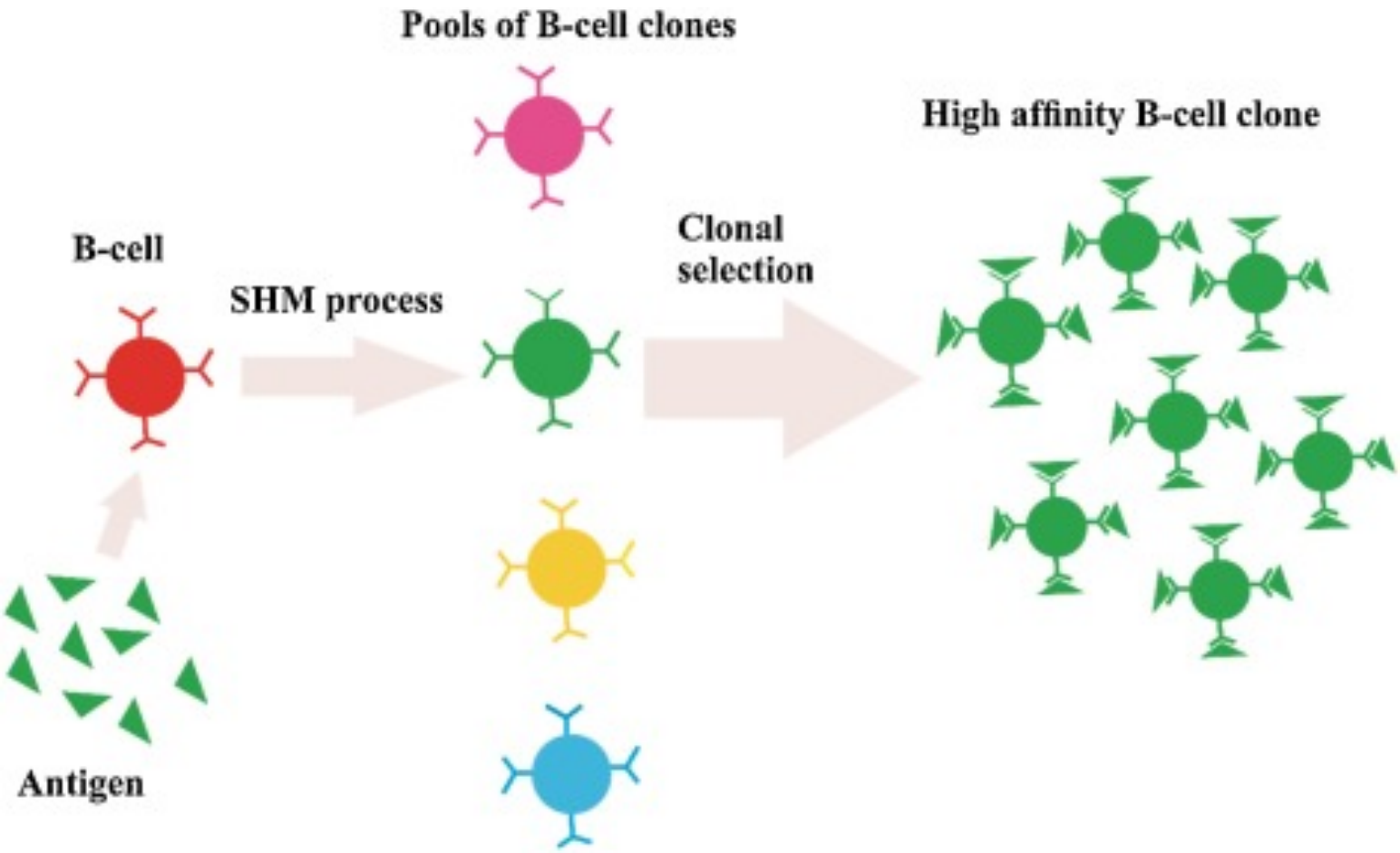


Activation and Class-switching of B-cells

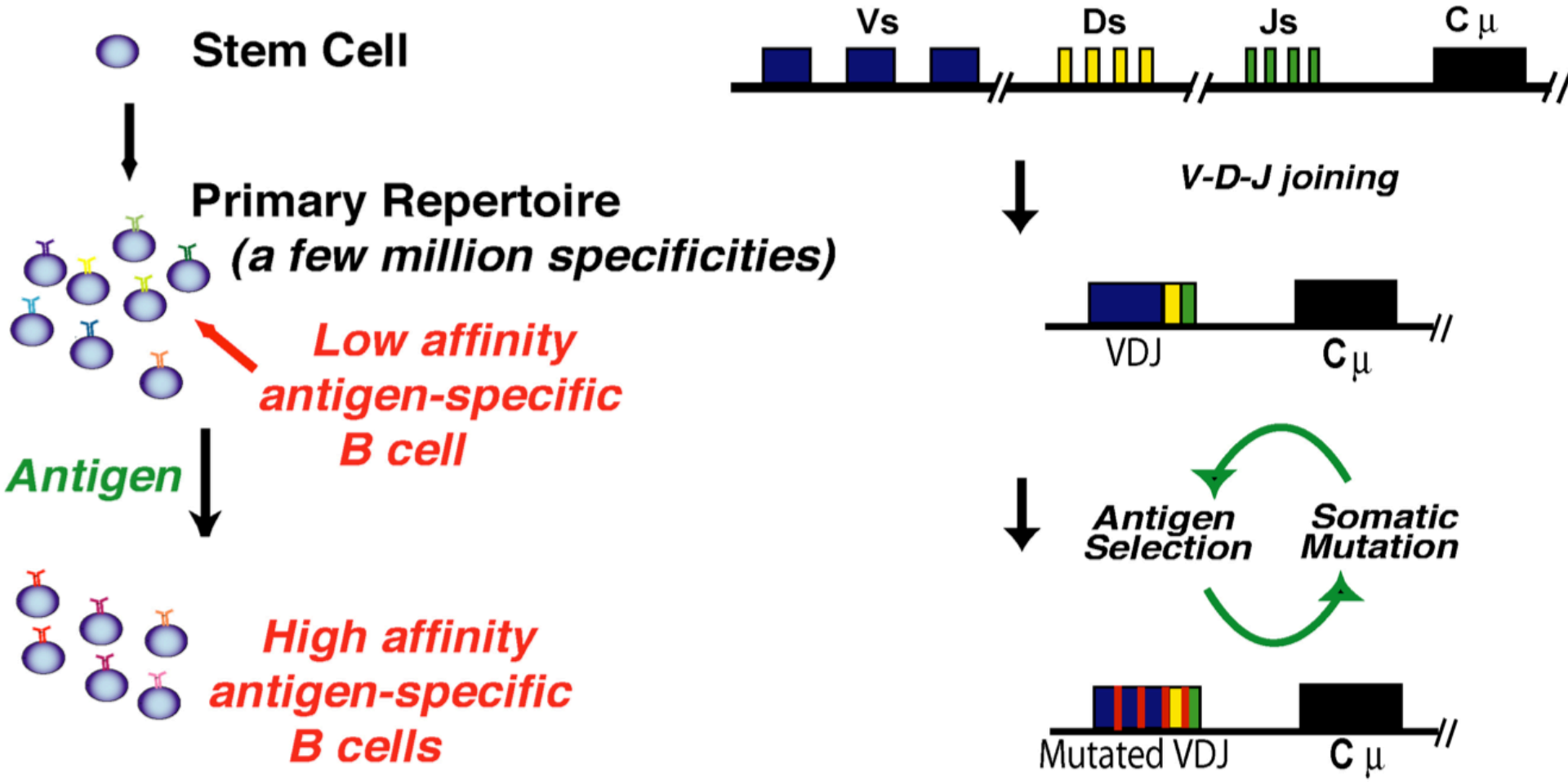


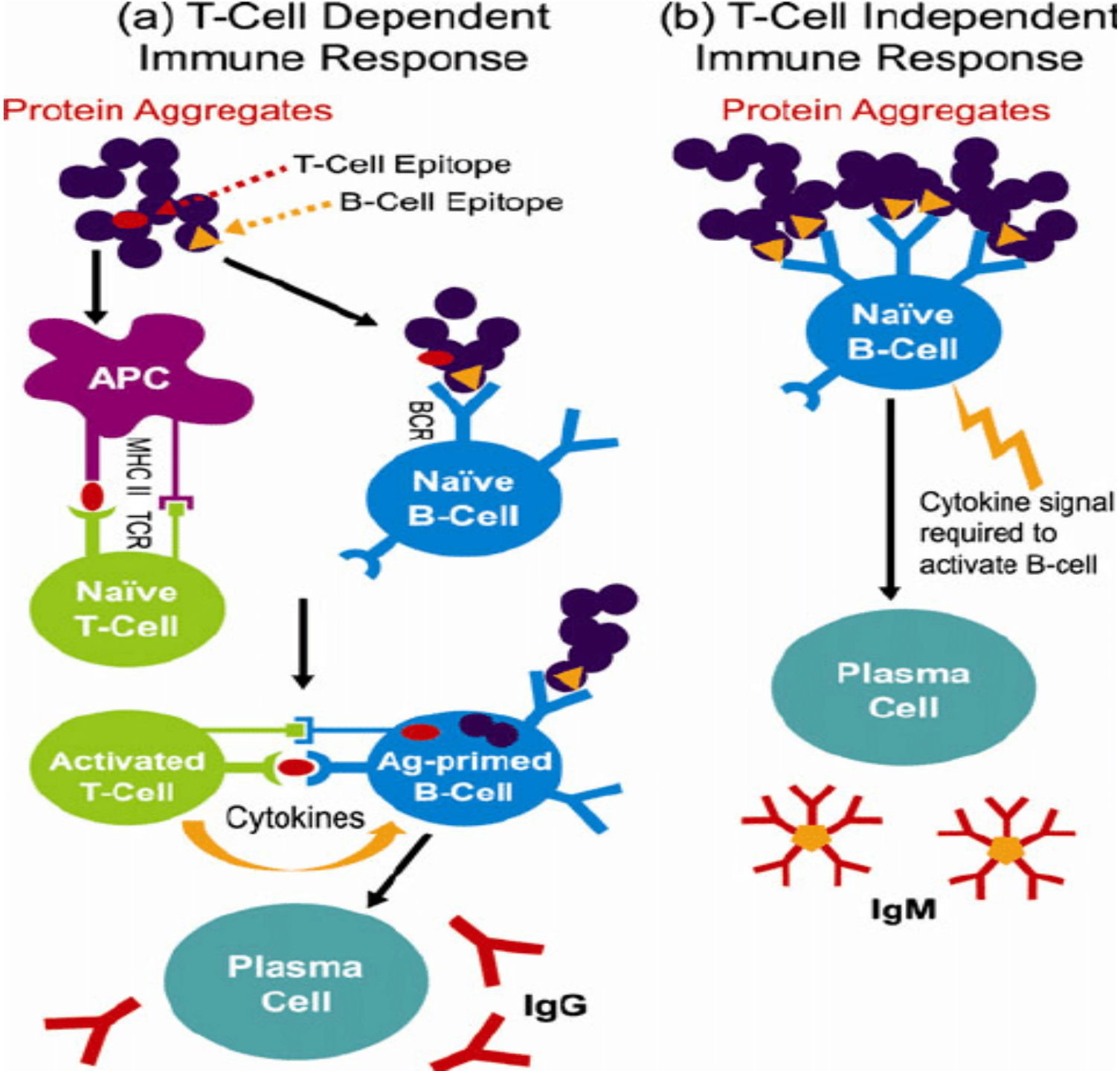
Lucy Liu

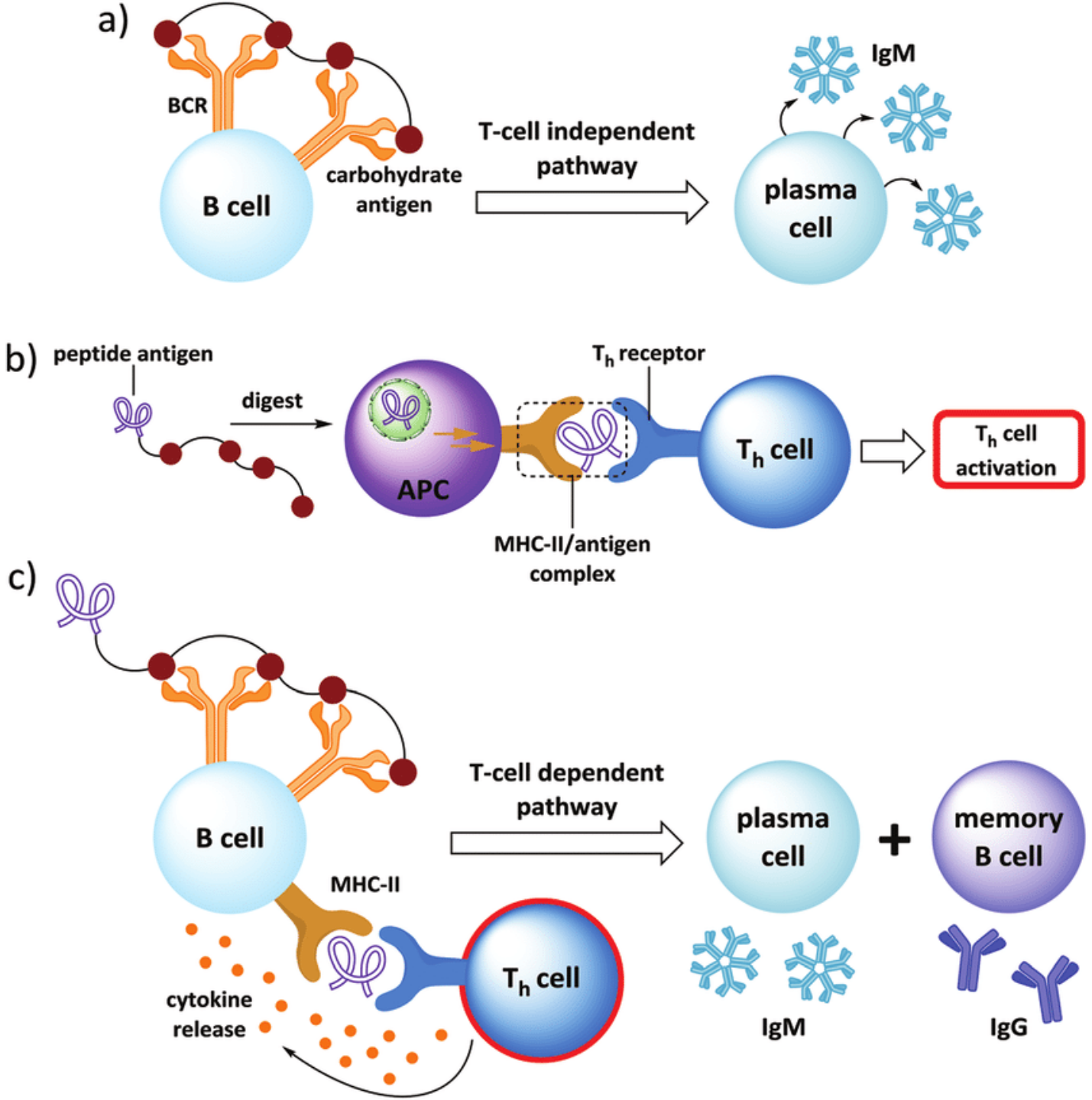
Affinity Maturation



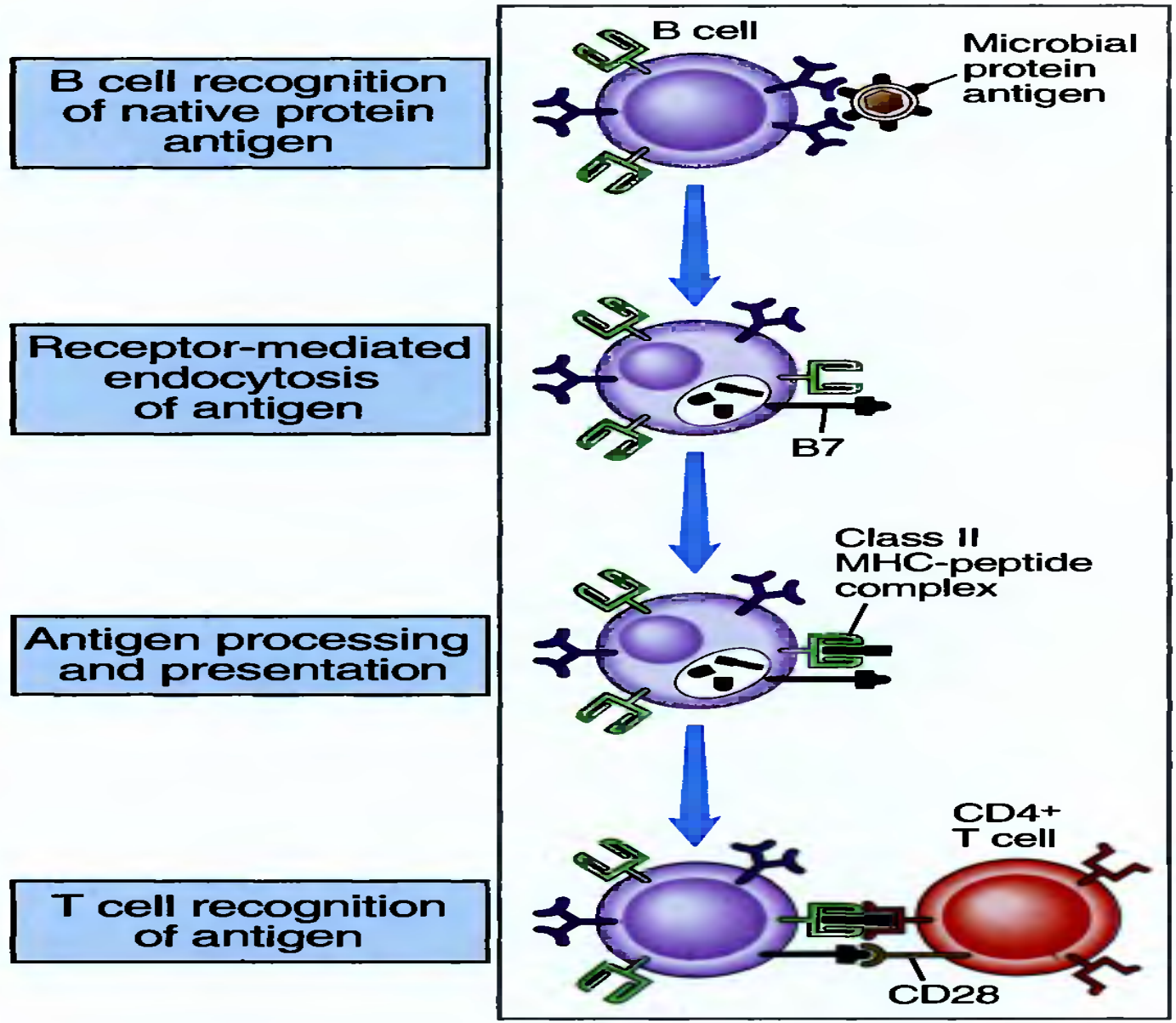
Affinity Maturation



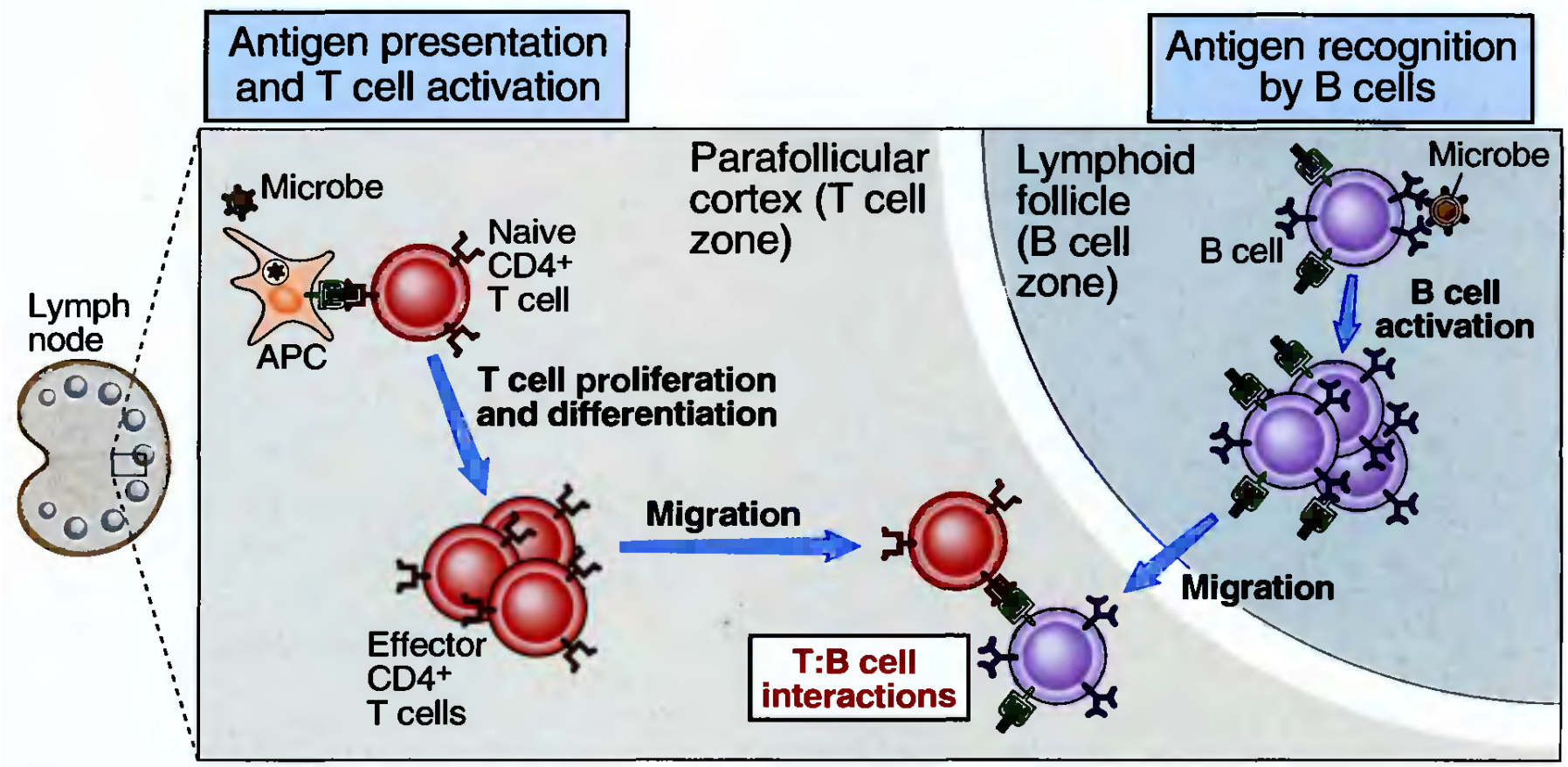

(a) T-Cell Dependent Immune Response
Protein Aggregates
T-Cell Epitope
B-Cell Epitope
APC
MHC II
TCR
Naïve T-Cell
BCR
Naïve B-Cell
Activated T-Cell
Ag-primed B-Cell
Cytokines
Plasma Cell
IgG
(b) T-Cell Independent Immune Response
Protein Aggregates
Naïve B-Cell
Cytokine signal required to activate B-cell
Plasma Cell
IgM

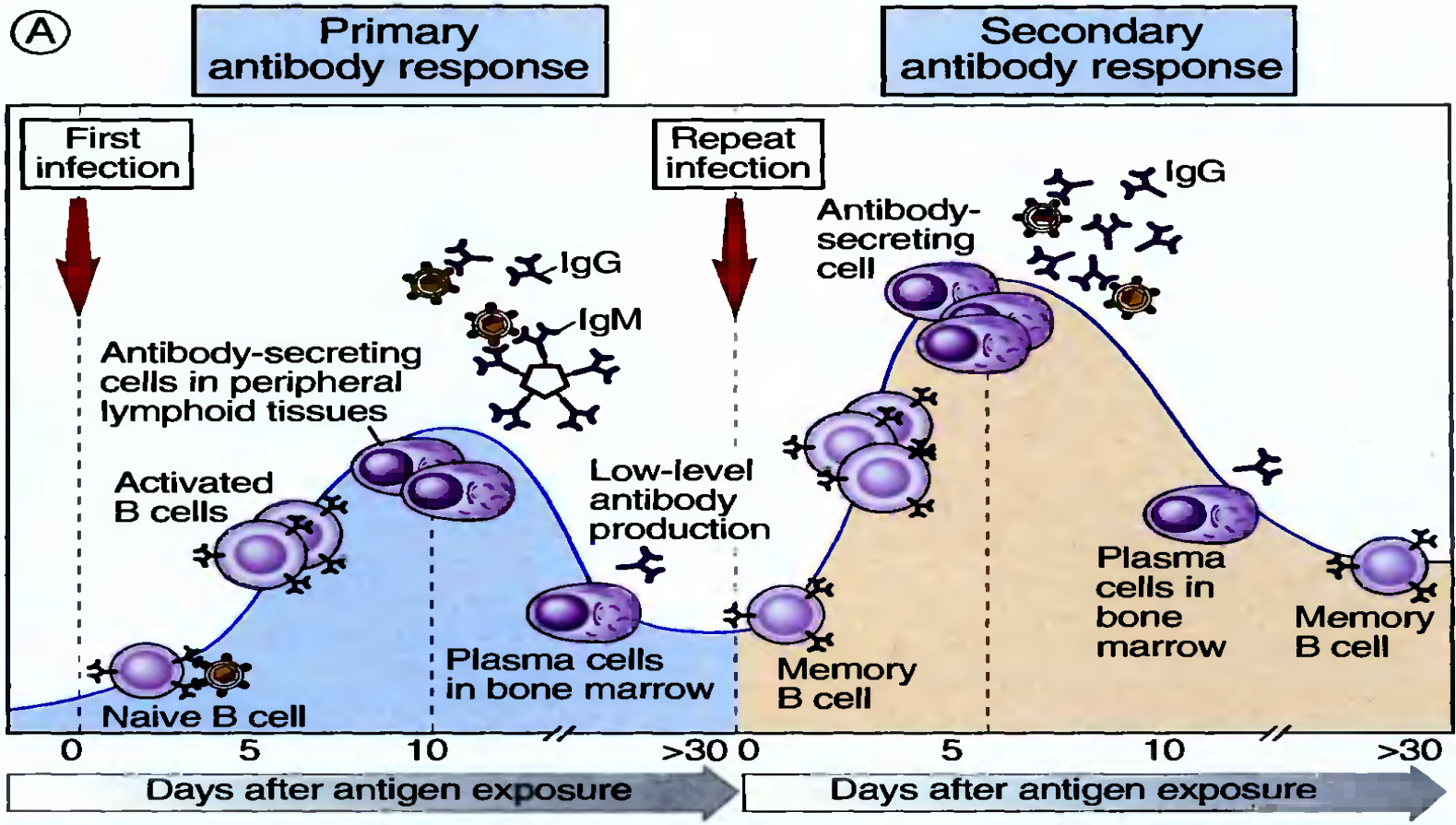

a)
BCR
B cell
carbohydrate antigen
T-cell independent pathway
IgM
plasma cell
b)
peptide antigen
digest
APC
Th receptor
Th cell
Th cell activation
MHC-II/antigen complex
c)
B cell
MHC-II
Th cell
cytokine release
T-cell dependent pathway
plasma cell
+
memory B cell
IgM
IgG

T-dependent B Cell Response



T-dependent B Cell Response





B

	Primary response	Secondary response
Lag after immunization	Usually 5-10 days	Usually 1-3 days
Peak response	Smaller	Larger
Antibody isotype	Usually IgM>IgG	Relative increase in IgG and, under certain situations, in IgA or IgE (heavy chain class switching)
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)

Secondary Immune Response

- Repeated stimulation with an antigen leads to an increase in the number of helper T lymphocytes, resulting in an increase in secondary response to **protein-structured antigens, heavy chain isotype switching and affinity maturation**