Activation of B lymphocytes and Antibody Production (II)

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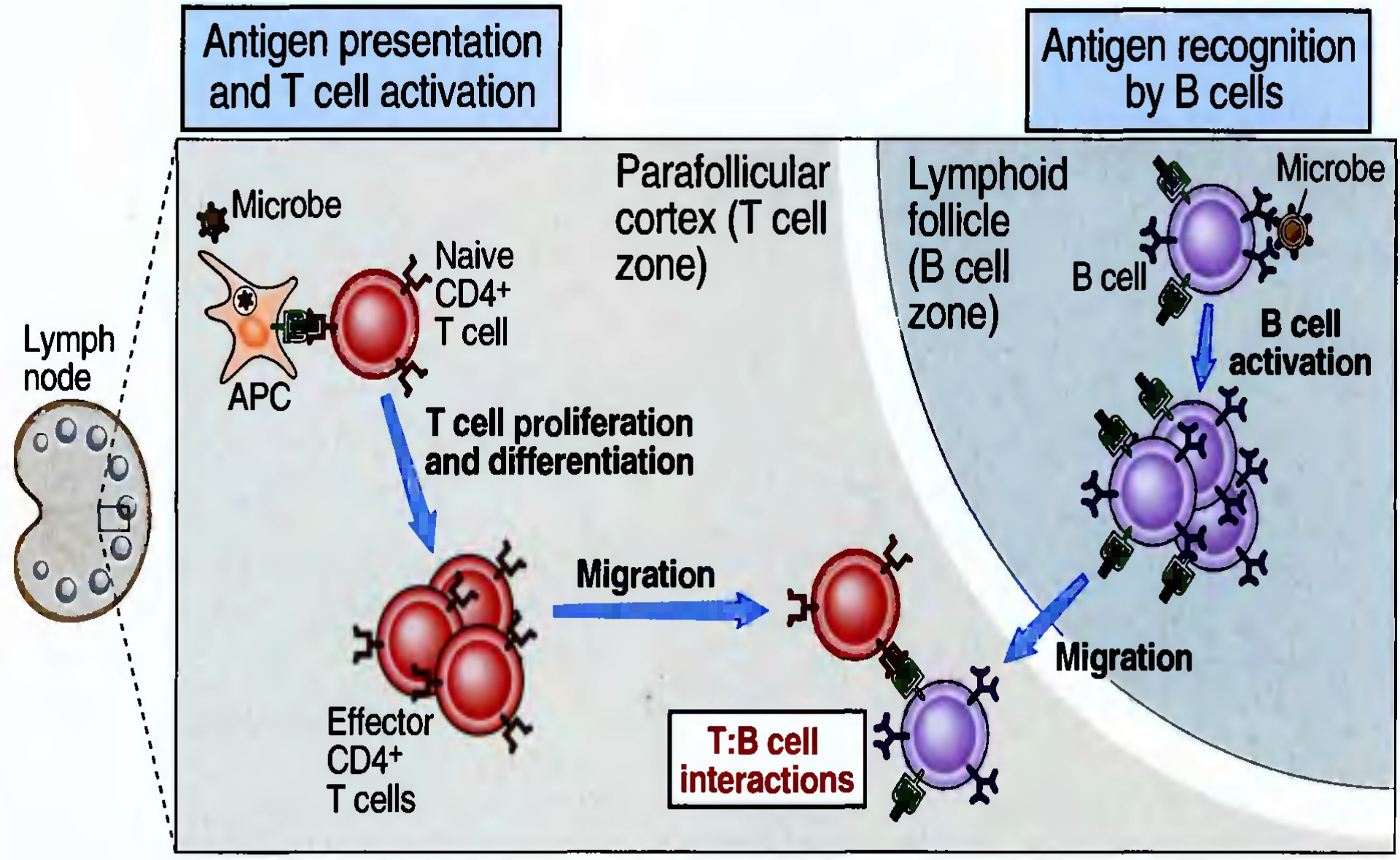
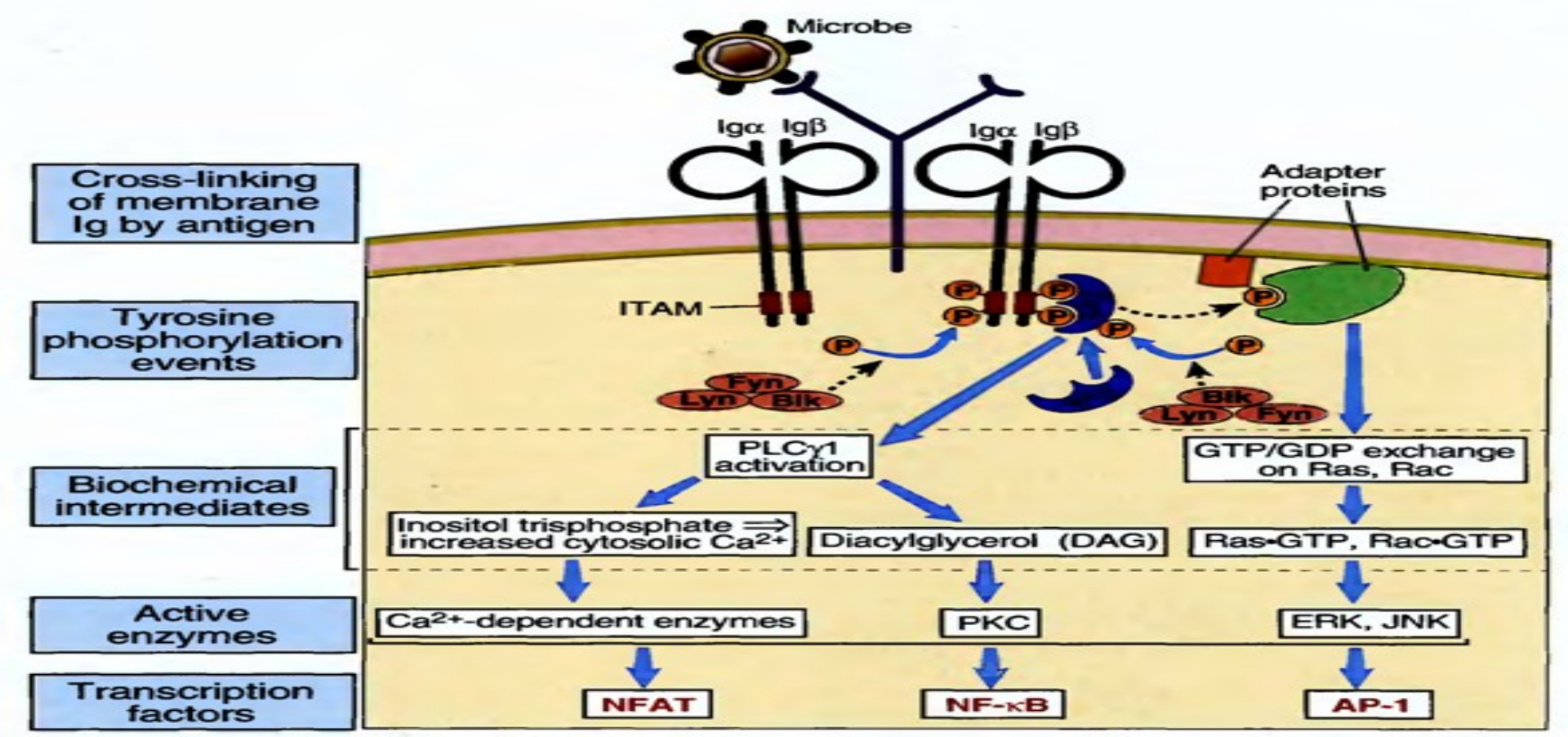
Stimulation of B lymphocytes by Antigens

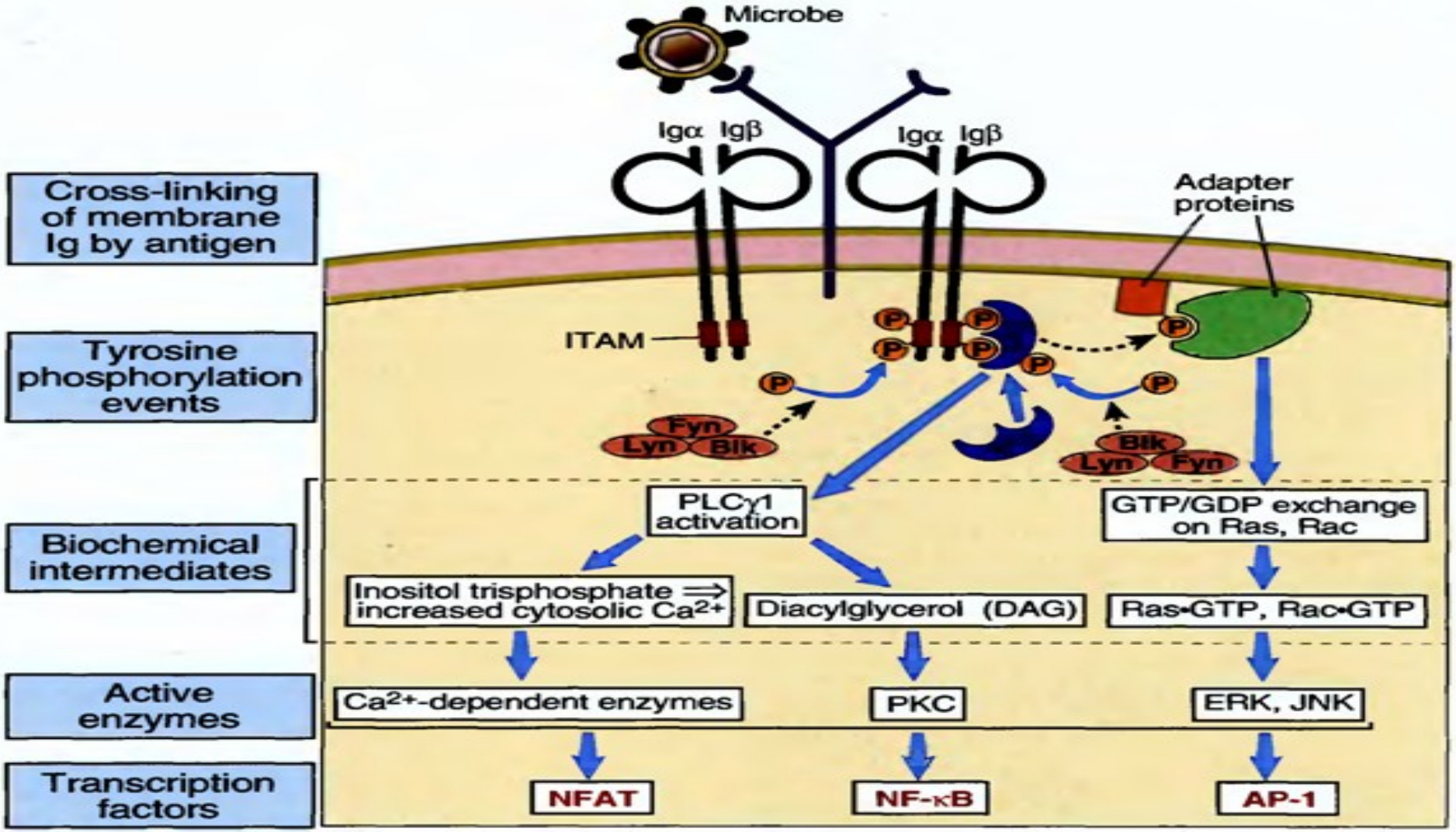
- **Humoral immune responses begin with the recognition of the antigen by antigen-specific B lymphocytes in the spleen, lymphoid follicles, lymph nodes and mucosal lymphoid tissues.**

- Some of the microorganism antigens that reach the tissues or are found in the blood are carried to the follicles of the peripheral lymphoid organs rich in B cells and accumulate in this region.

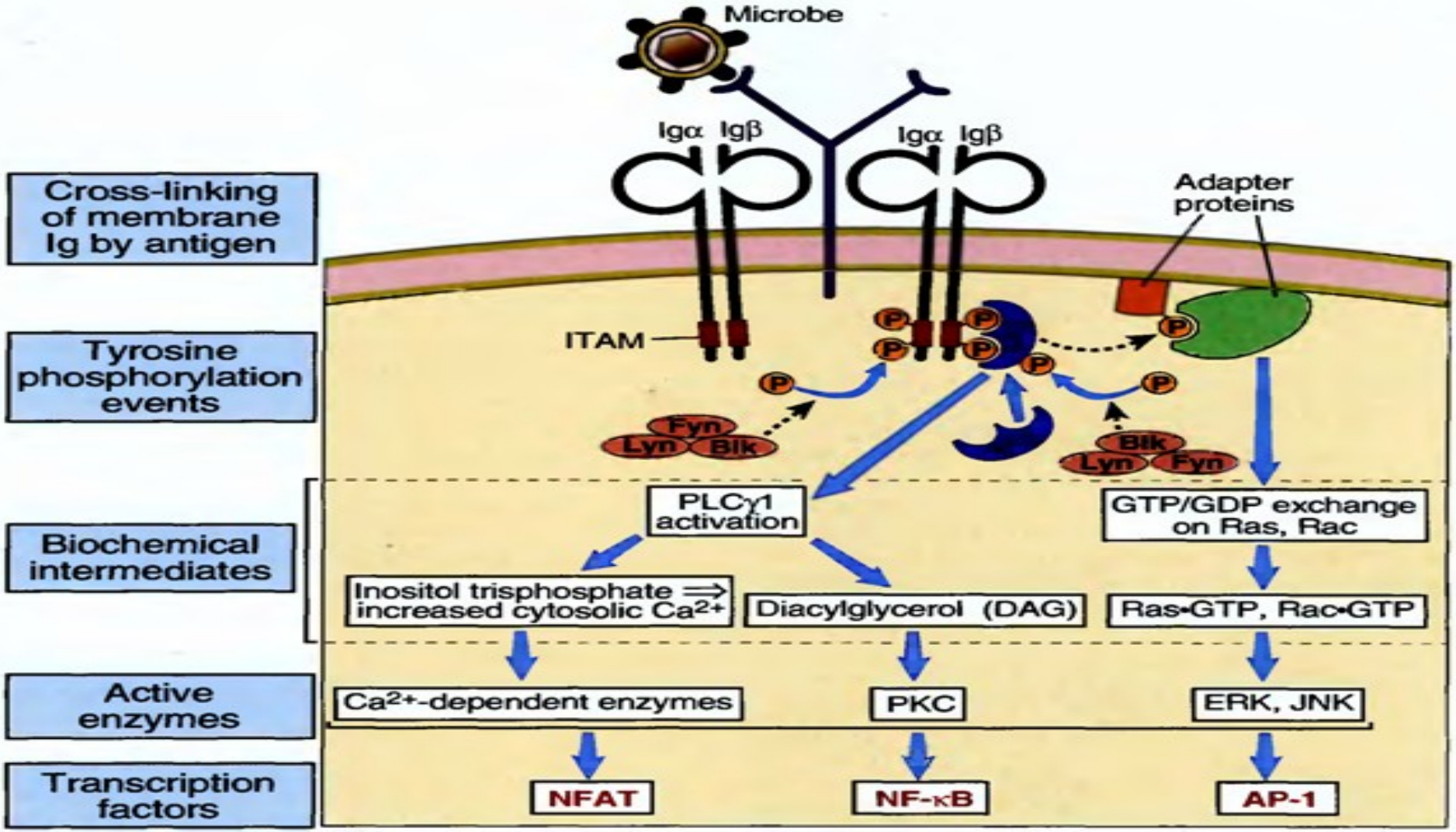

Antigen presentation and T cell activation
Antigen recognition by B cells
Lymph node
Parafollicular cortex (T cell zone)
Lymphoid follicle (B cell zone)
Microbe
Naive CD4+ T cell
APC
T cell proliferation and differentiation
Effector CD4+ T cells
Migration
T:B cell interactions
Microbe
B cell
B cell activation
Migration

B lymphocytes specific to an antigen, thanks to the membrane-bound immunoglobulin (Ig) receptors take the antigen in its natural state; that is, they recognize it without the need for processing.

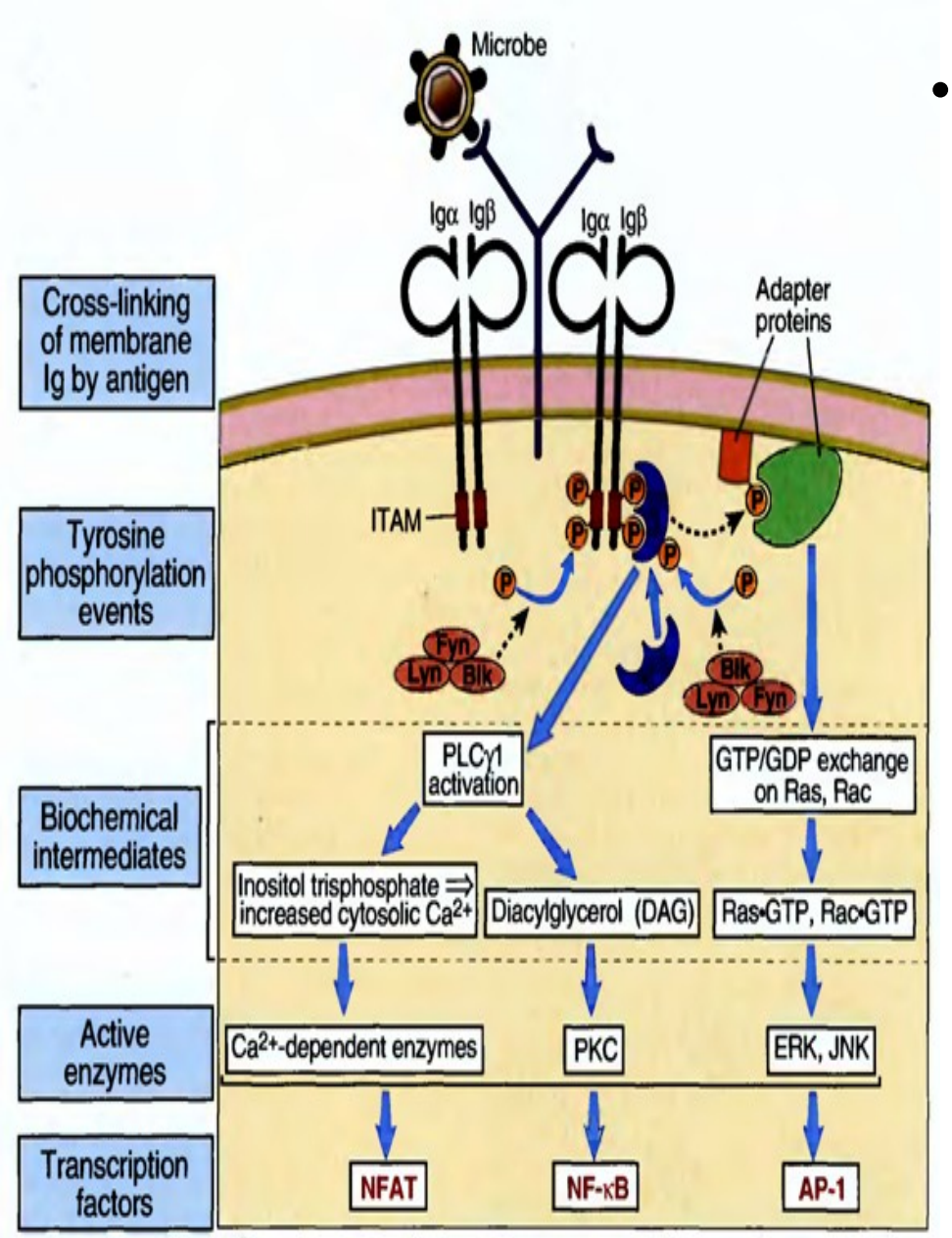




- Recognition of the antigen triggers signal transduction that initiates the activation of the B cell. Like T lymphocytes, B cell activation often requires **secondary signals** generated during innate immune responses to microorganisms.

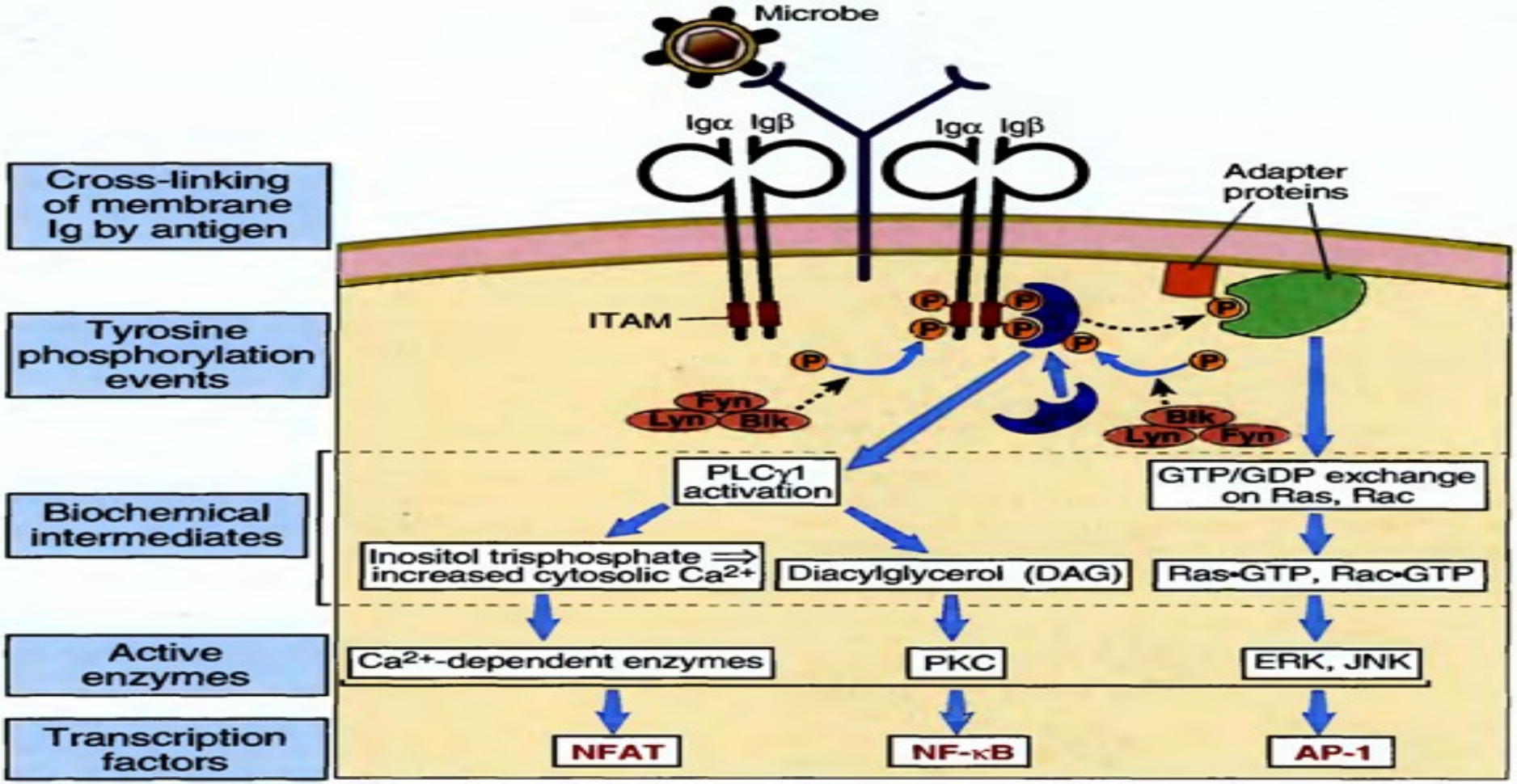


- B cell activation is basically similar to T cell activation. Ig receptor mediated signal transduction in B cells requires the assembly of two or more receptor molecules **(cross linking).**

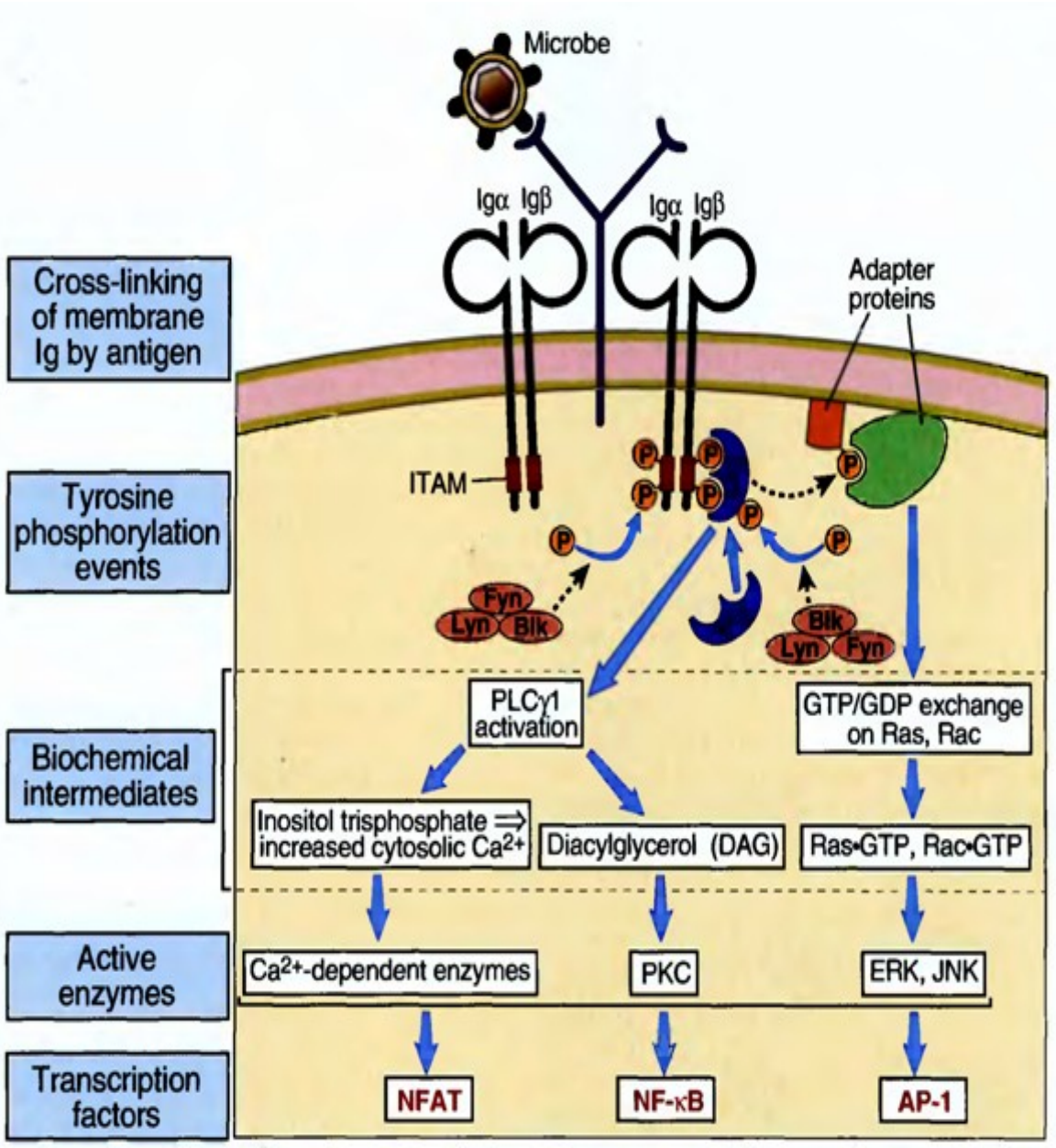


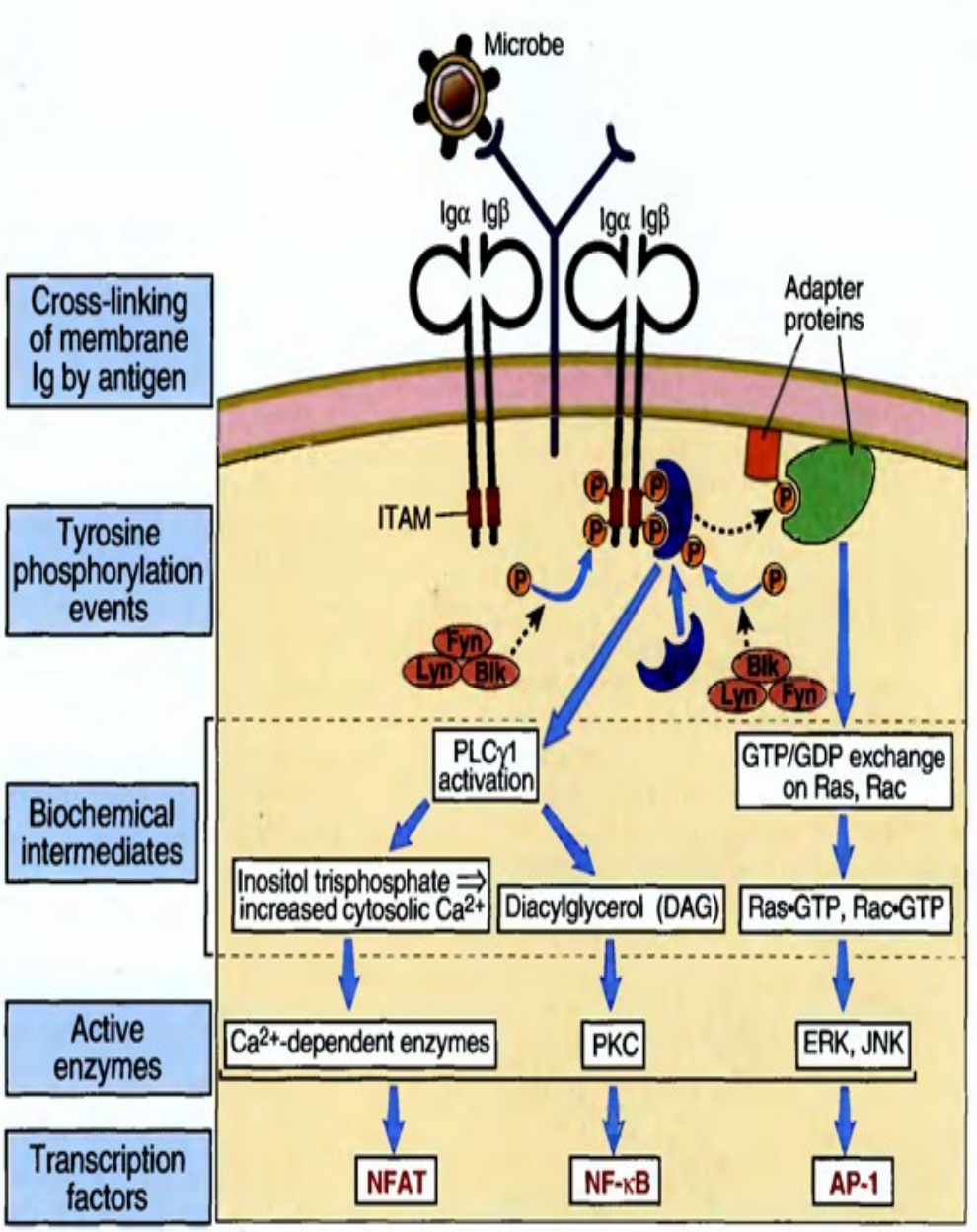
- Receptor cross-linking occurs when two or more antigen molecules or repetitive epitopes in an antigen molecule bind to Ig molecules located closely together on the membrane of a B cell.

- **Signals initiated by the antigen receptor cross-linking are transferred into the cell by receptor-related proteins.**



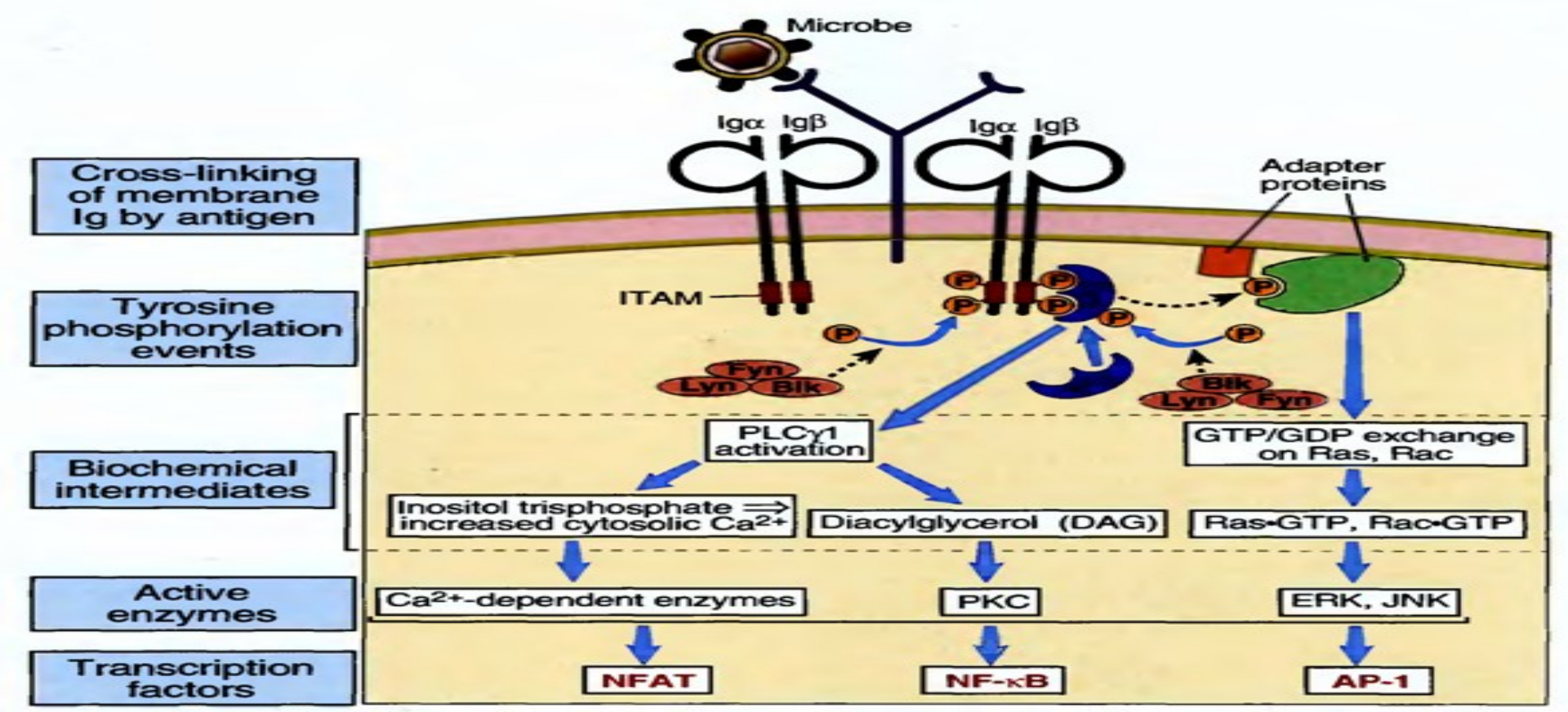
- **IgM and IgD**, antigen receptors of naive B cells, are highly diverse proteins with short cytoplasmic segments (domains).

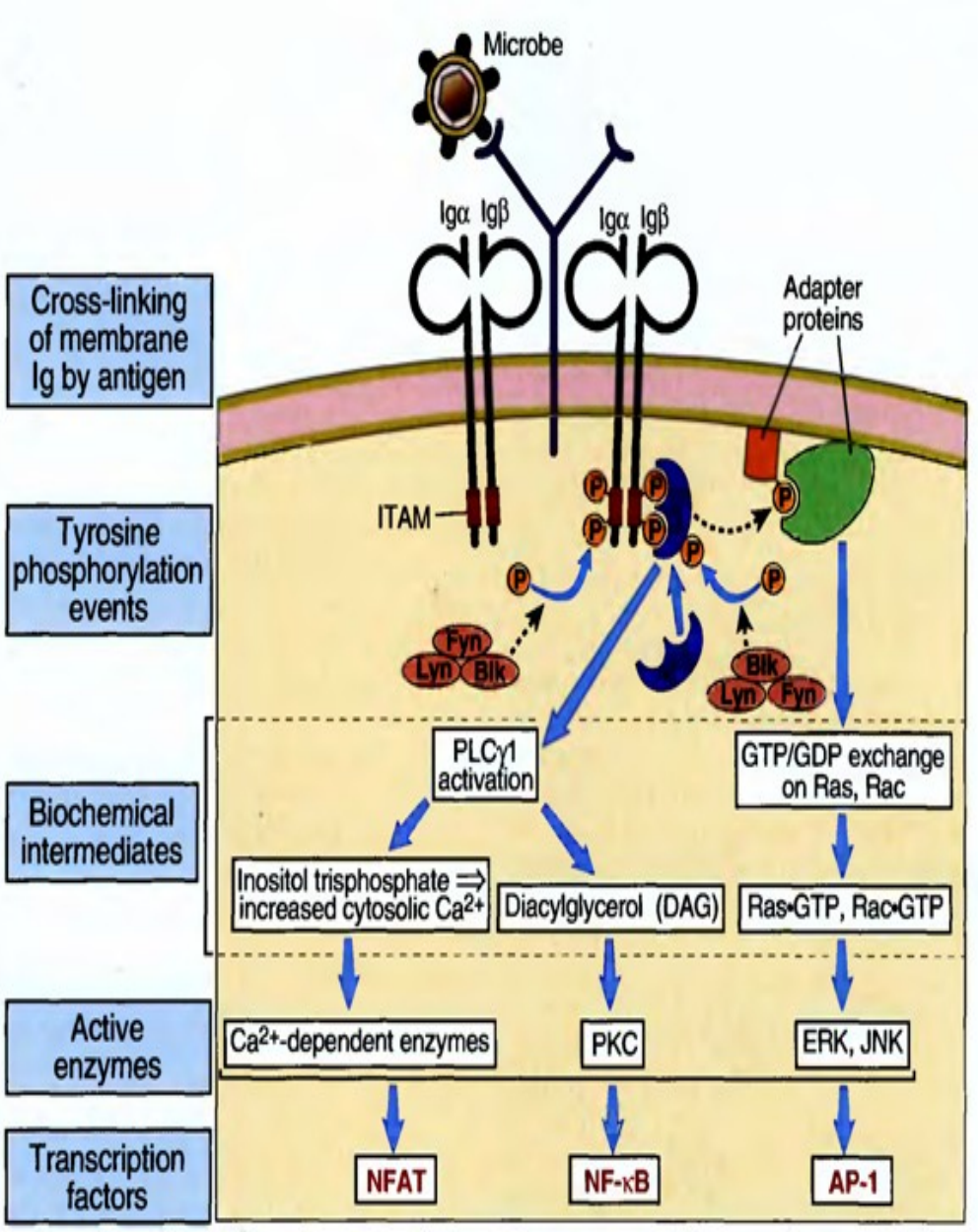




- These membrane receptors recognize antigens but do not transmit signals themselves. The receptors bind noncovalently (weak bond) to two proteins called Igα and Igβ, forming the **B cell receptor BCR complex.**

- Cytoplasmic loops of Igα and Igβ carry immunoreceptor tyrosine-based activation motifs **(ITAM, immunoreceptor tyrosine-based activation motifs),** which are also found in signal transmitting subunits of most activating receptors in the immune system.

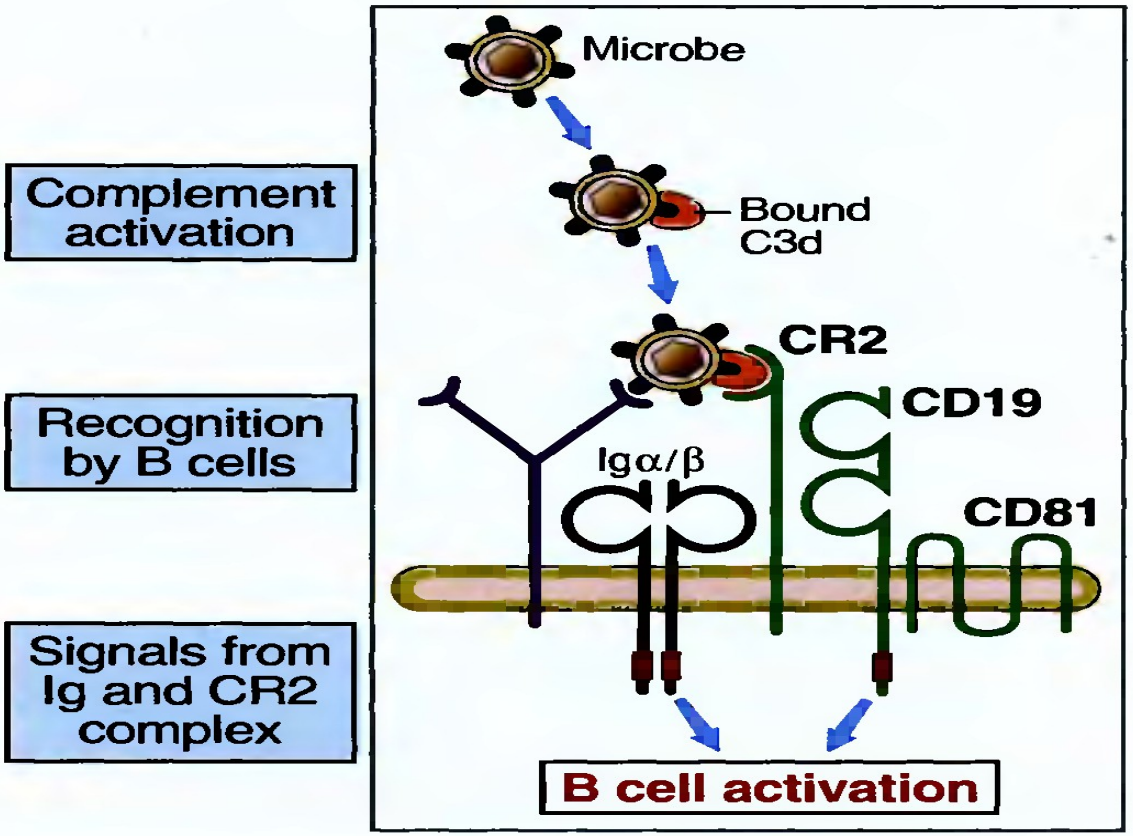




- When two or more antigen receptors come together in a B cell, the tyrosines in the ITAMs of Igα and Igβ are phosphorylated by kinases associated with the BCR complex.

Role of complement proteins in B cell activation

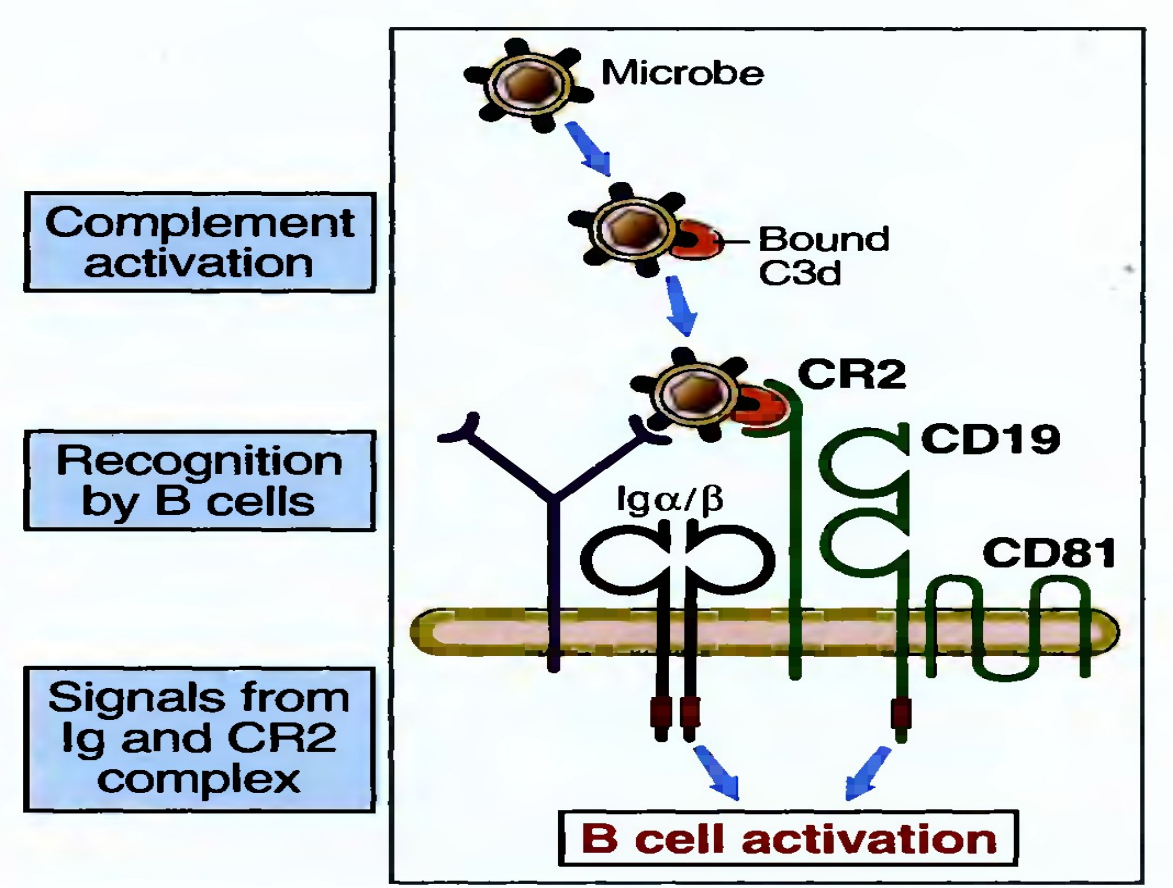
- B lymphocytes express a specific receptor for a protein of the complement system that signals for their activation.



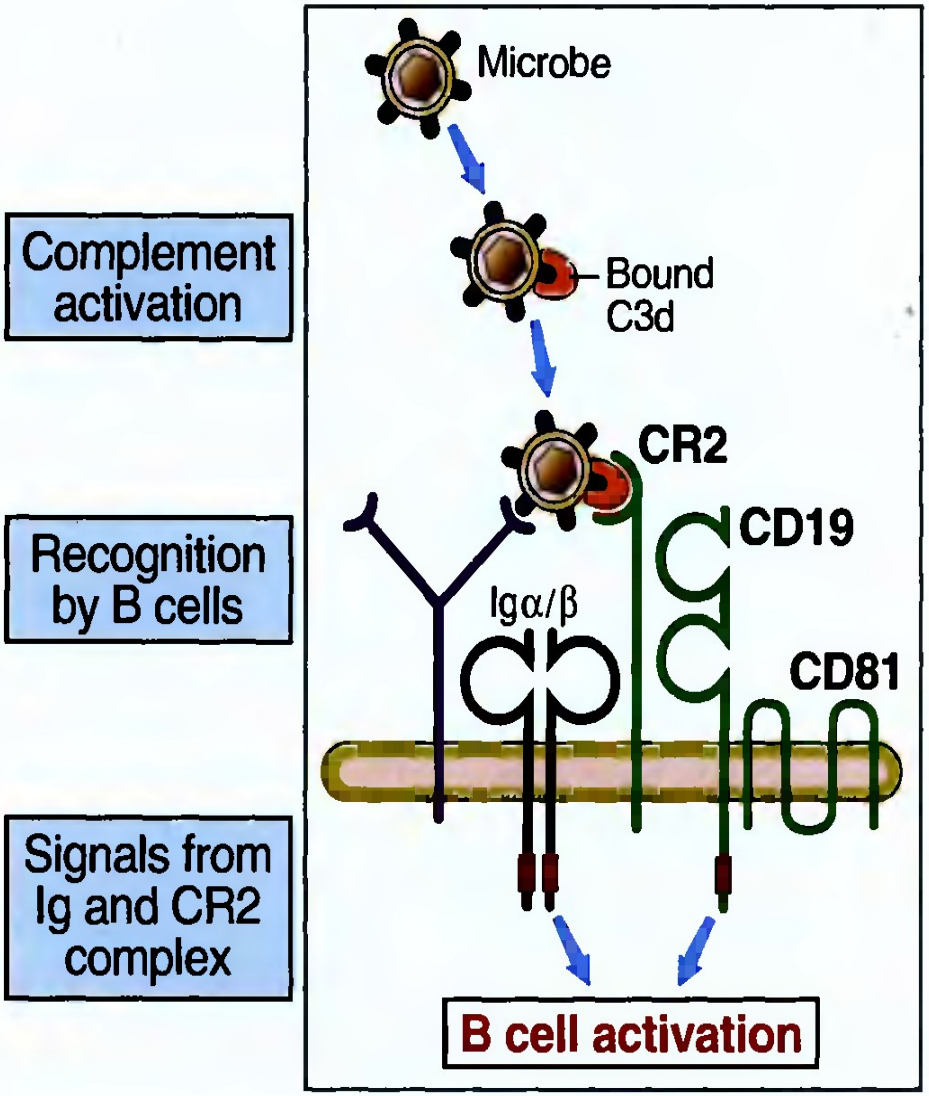
- The complement system is a plasma protein assembly activated by microorganisms and antibodies attached to microorganisms, acting as an effector mechanism in host defense.

Role of complement proteins in B cell activation

- When the complement system is activated by a microorganism, this microorganism is covered with the degradation products of **C3**, the most abundantly produced complement protein.



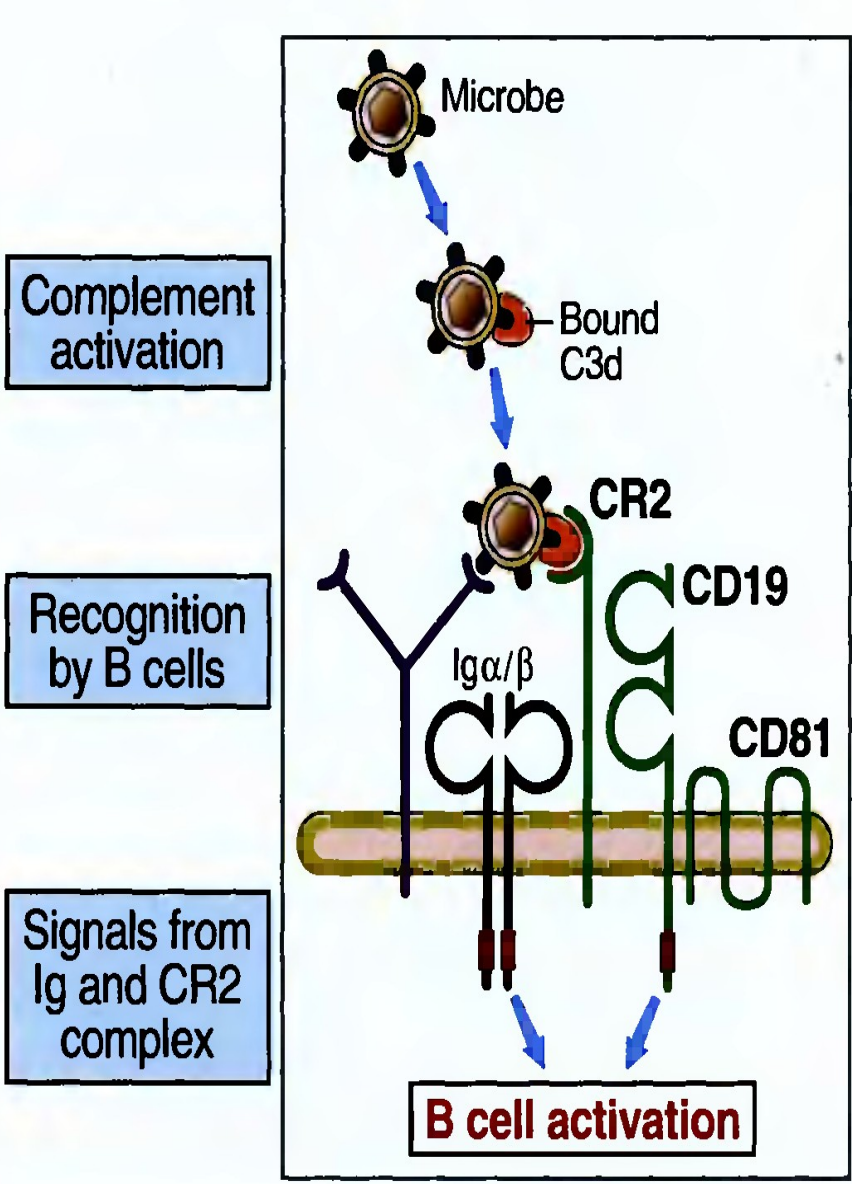
Role of complement proteins in B cell activation



- One of these destruction products is C3d.
- B lymphocytes express a receptor called type 2 complement receptor (CR2 or CD21) that binds C3d.
- **Thus, B cells specific to the antigens of a microorganism recognize the antigen with the Ig receptor and C3d with the CR2 receptor together.**

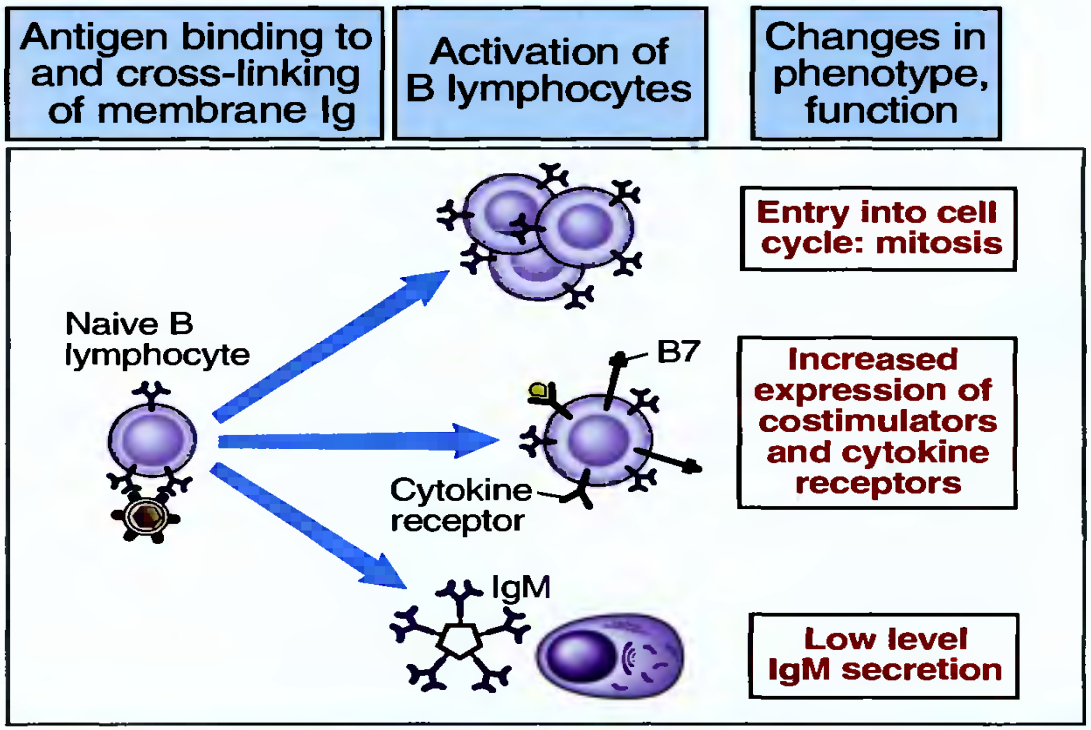
Role of complement proteins in B cell activation

- The binding of CR2 with C3d also triggers signals that activate B cells.
- Second signal !!!



Functions of antigen-mediated B cell activation

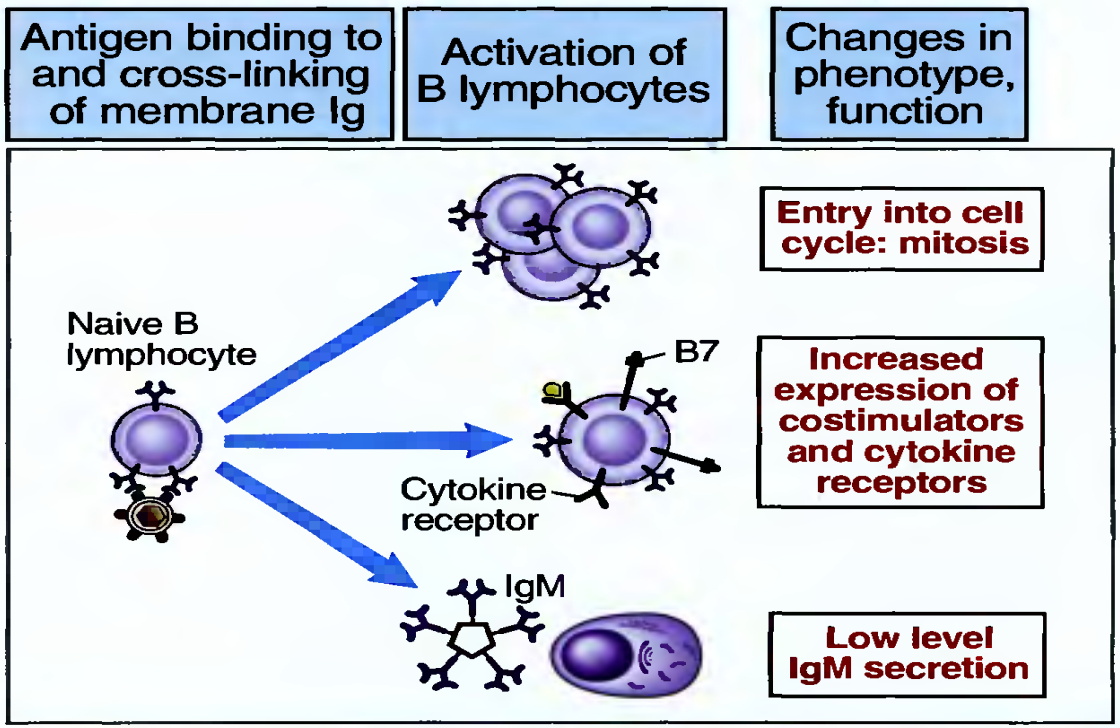
- As a result of the activation of the B lymphocytes through the antigen and the second signal, their interaction with the helper T lymphocytes takes place, and the proliferation and differentiation phases of these cells begin.



B cell response to antigen	Significance
Entry into cell cycle, mitosis	Clonal expansion
Increased expression of B7 costimulators	Ability to activate helper T cells
Increased expression of cytokine receptors	Ability to respond to cytokines produced by helper T cells
Migration out of lymphoid follicles	Interaction with helper T cells
Secretion of low levels of IgM	Early phase of humoral immune response

Functions of antigen-mediated B cell activation

- Activated B lymphocytes enter the cell cycle and B lymphocyte proliferation occurs, resulting in the increase of antigen-specific clones.



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Antigen stimulation causes at least three changes in B lymphocytes, thus increasing the ability of B cells to interact with helper T cells.

- B cell activation;
- B7 co-stimulus expression that generates the second signal for T cell activation
- Receptor expression for cytokines, mediators involved in the maintenance of helper T cell functions.

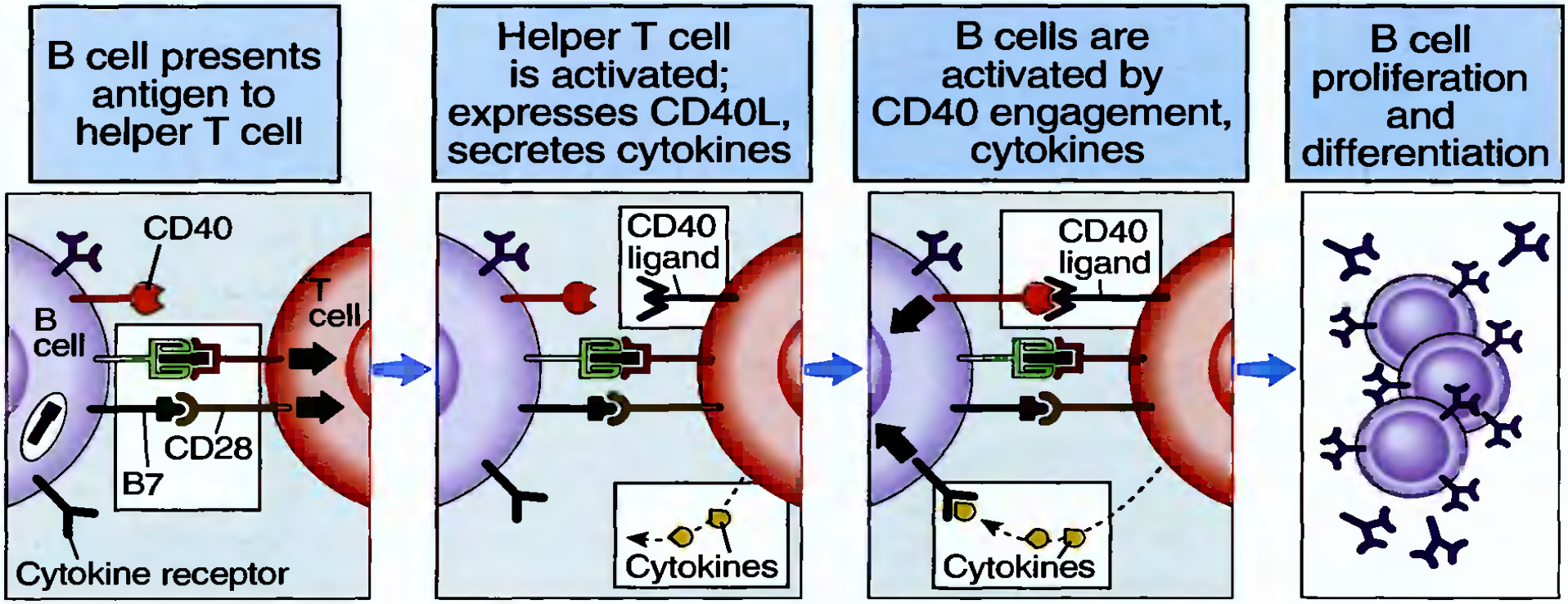
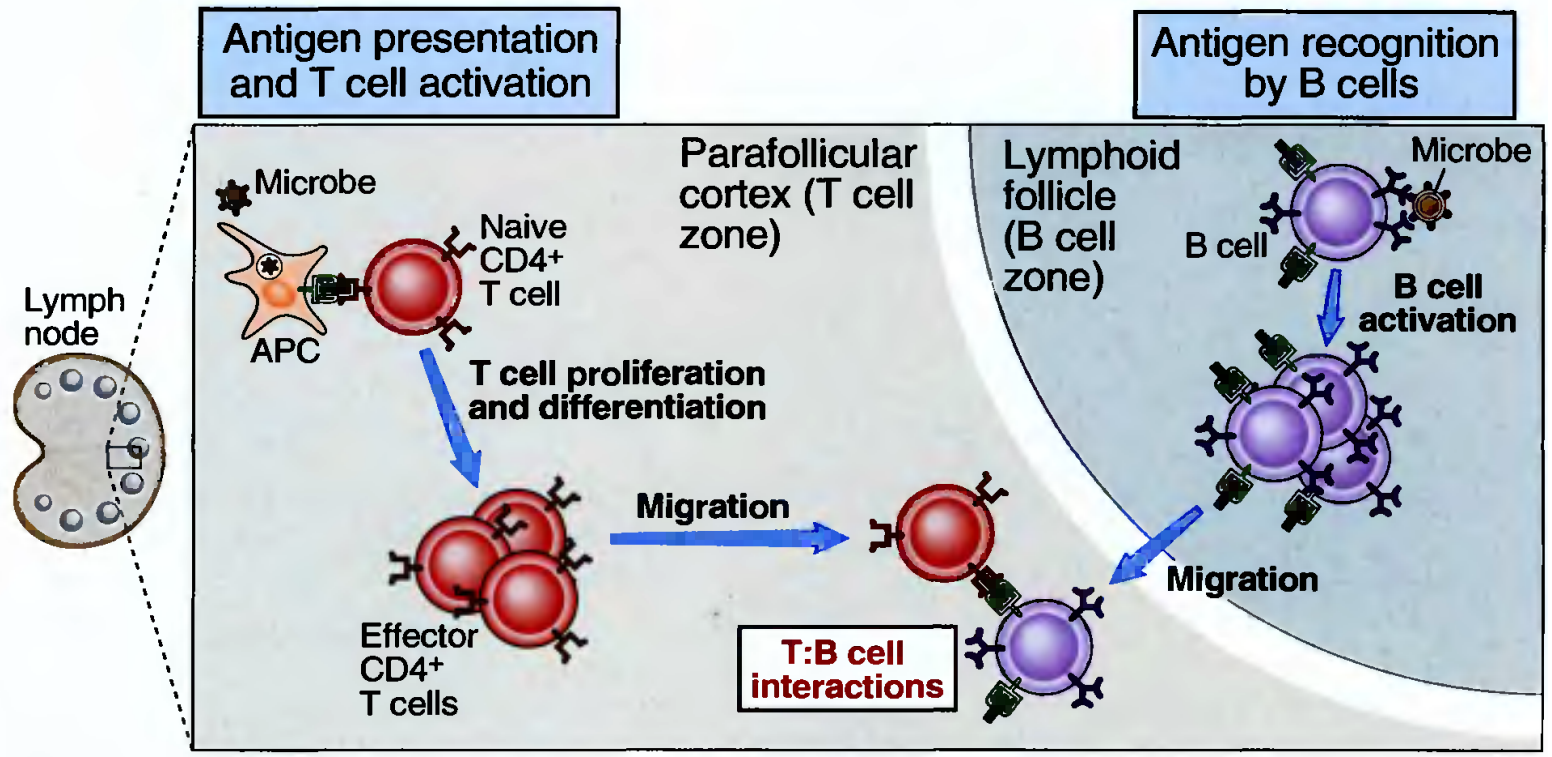


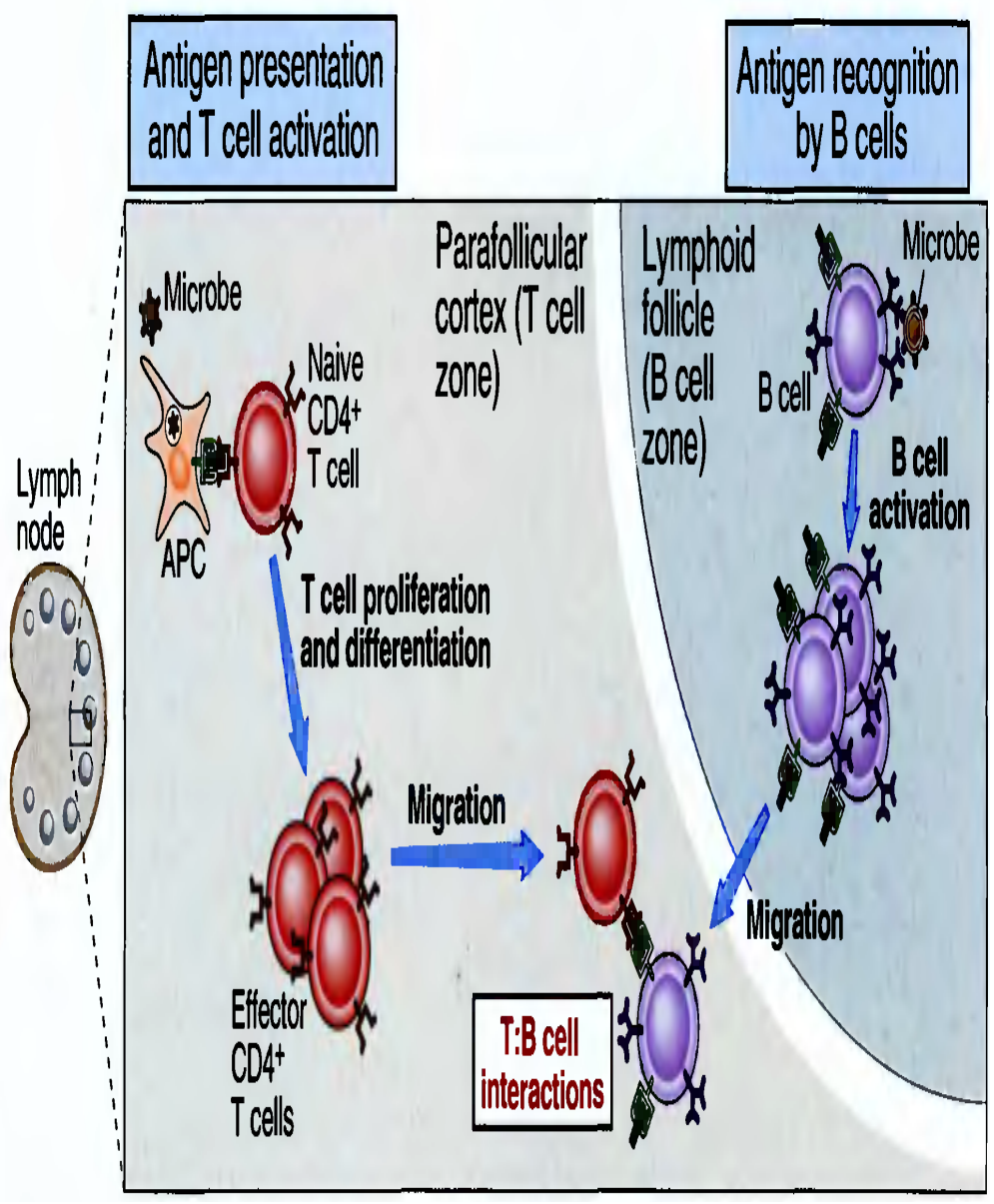
Figure 7–8 Mechanisms of helper T cell–mediated activation of B lymphocytes. Helper T cells recognize peptide antigens presented by B cells and costimulators (e.g., B7 molecules) on the B cells. The helper T cells are activated to express CD40L and secrete cytokines, both of which bind to their receptors on the same B cells and activate the B cells.

The role of helper T lymphocytes in the humoral immune response to protein antigens

- In order for a protein-structured antigen to stimulate the antibody response, the antigen-specific B lymphocytes and helper T lymphocytes must come together in lymphoid organs and interact to stimulate B cell proliferation and differentiation.

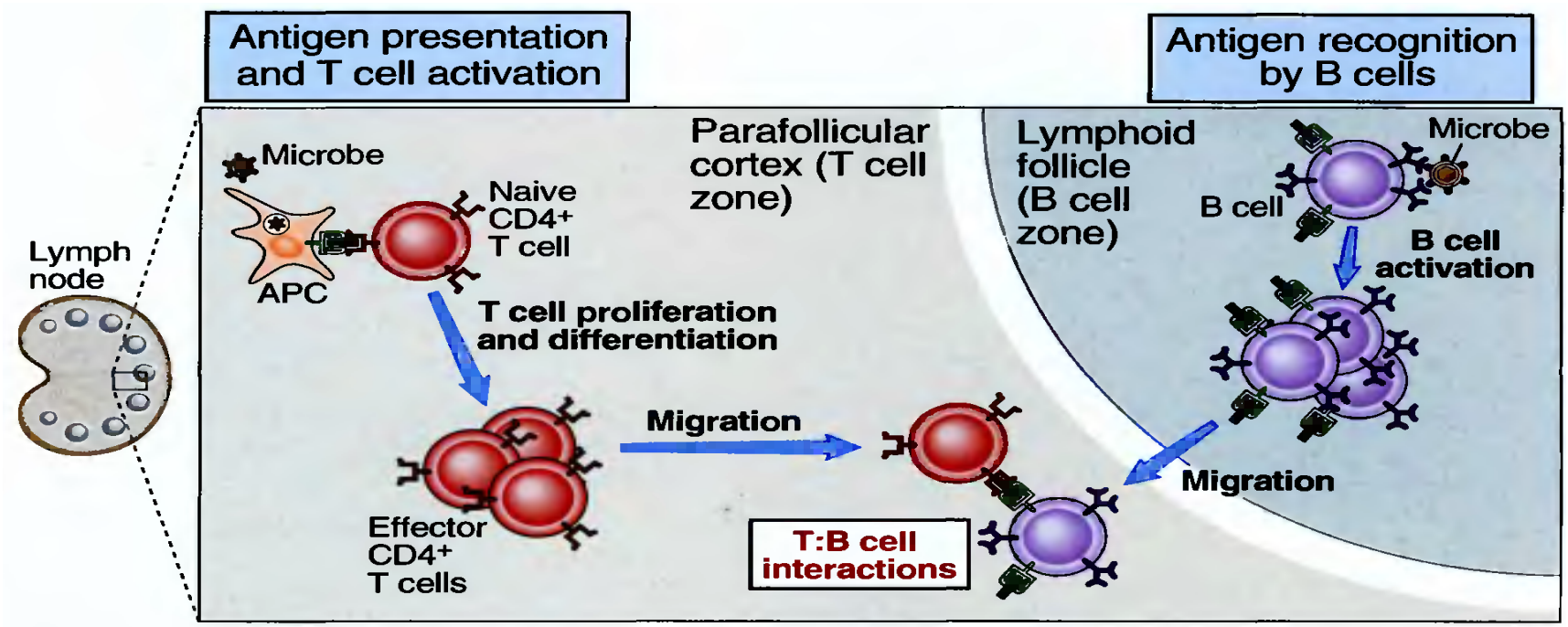


- Activated B cells reduce the receptor expression of chemokines produced in lymphoid follicles and whose task is to retain B cells in these follicles.
- As a result, activated B cells migrate out of the follicle and collect in the anatomical section where helper T cells are densely located.



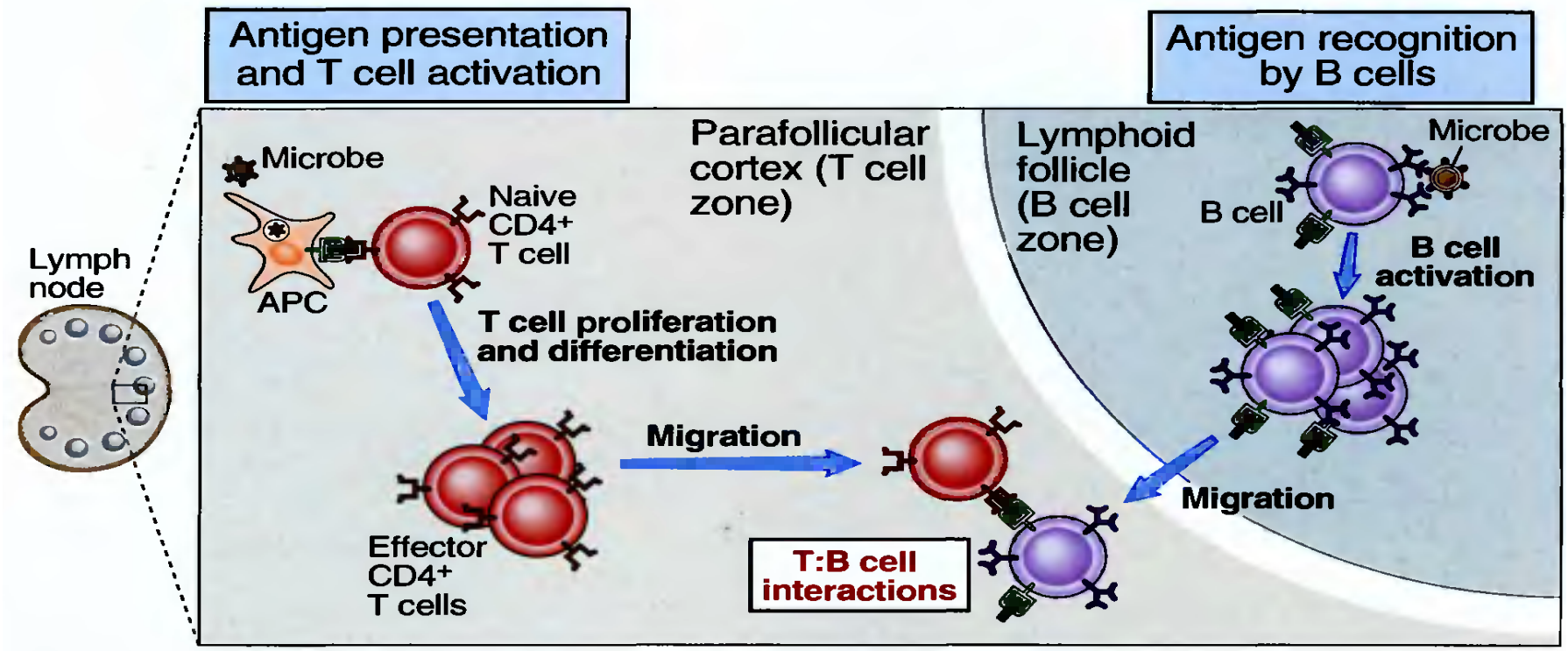
Activation and migration of helper T cells

- Helper T cells that are activated to differentiate into effector cells interact with antigen-stimulated B lymphocytes at the margins of lymphoid follicles in peripheral lymphoid organs.

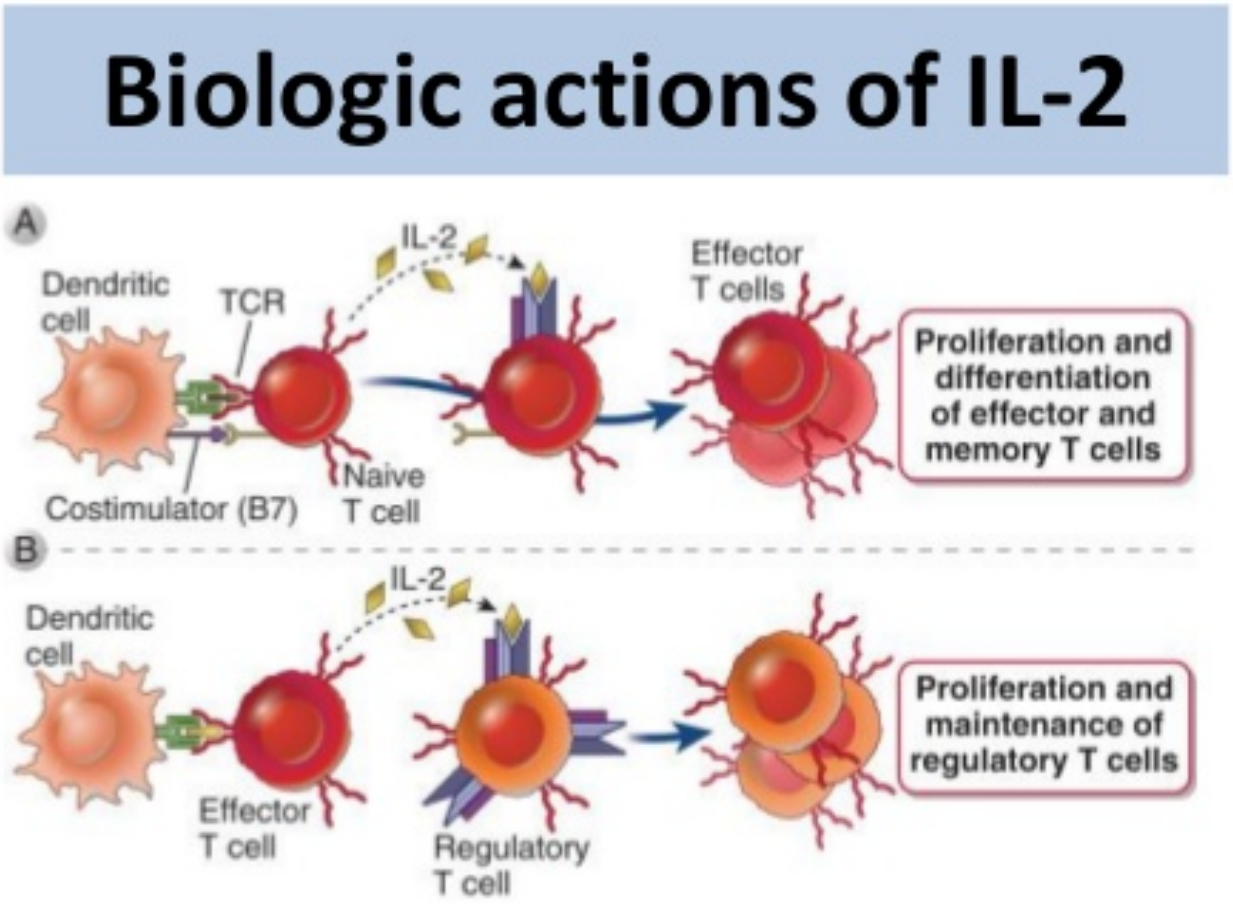


Activation and migration of helper T cells

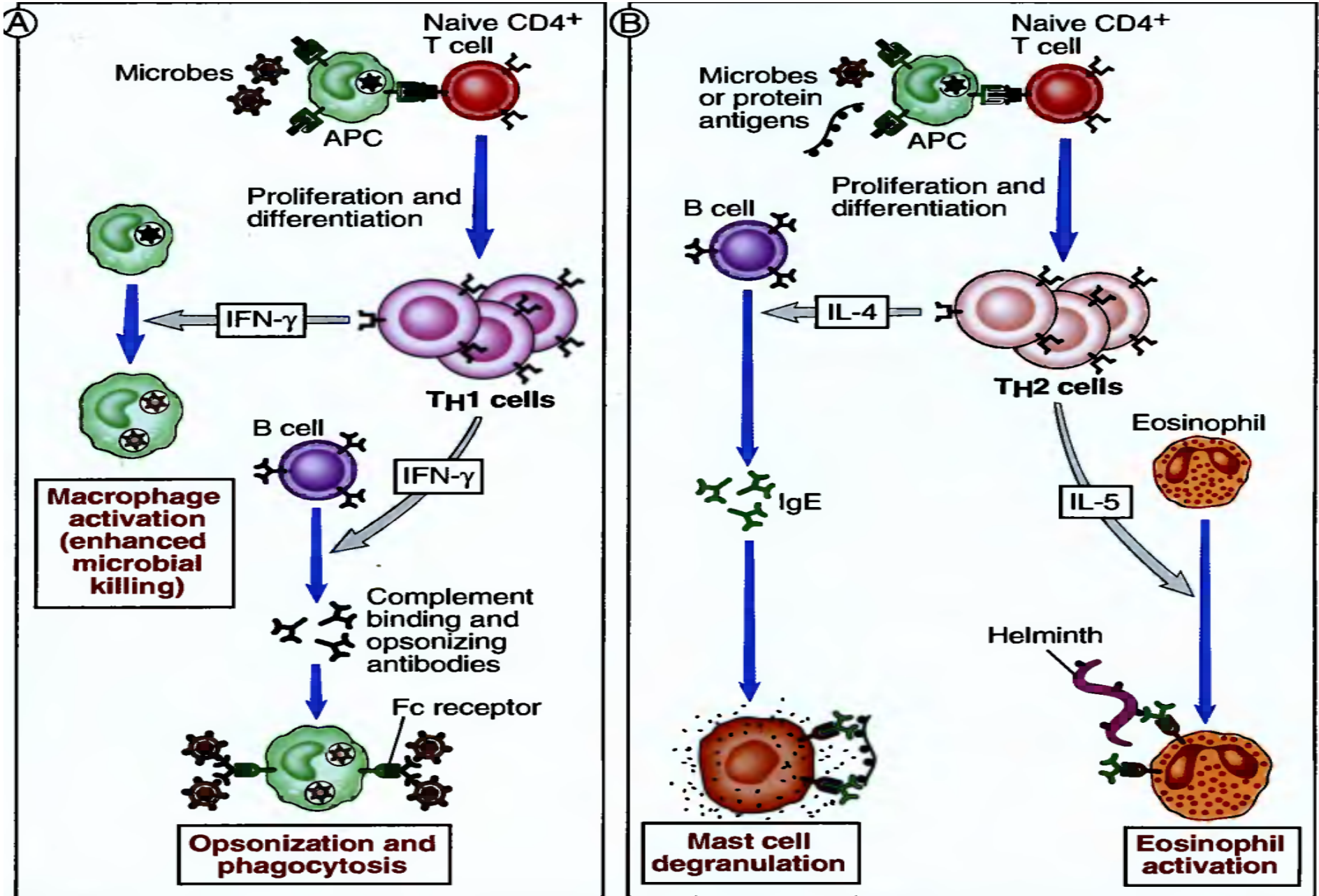
- **Naive CD4 + helper T cells are stimulated to differentiate and proliferate into cytokine-producing effector cells as a result of professional antigen presenting cells (APCs) in lymphoid organs to recognize and present the antigen.**

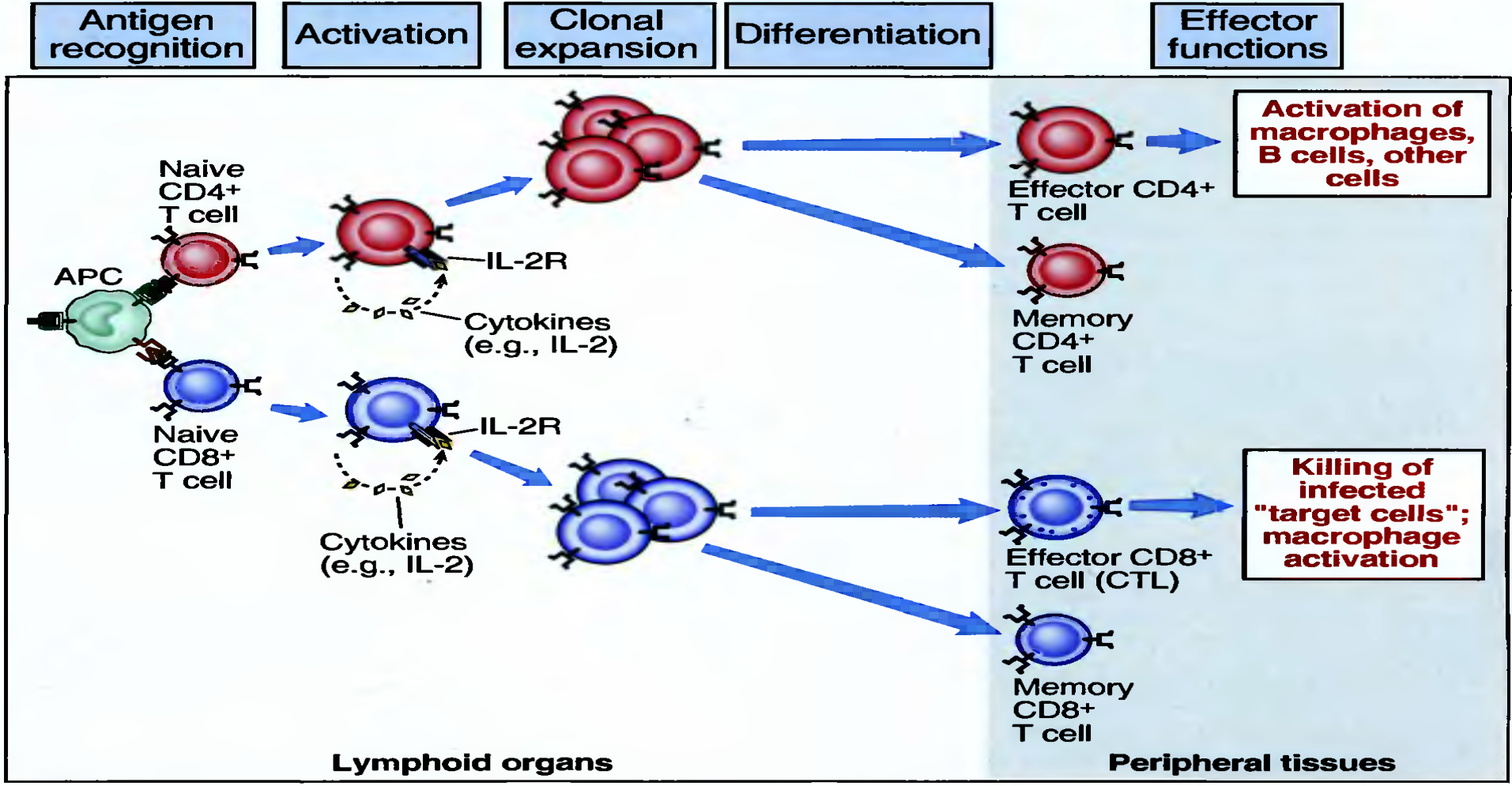


Activation and migration of helper T cells



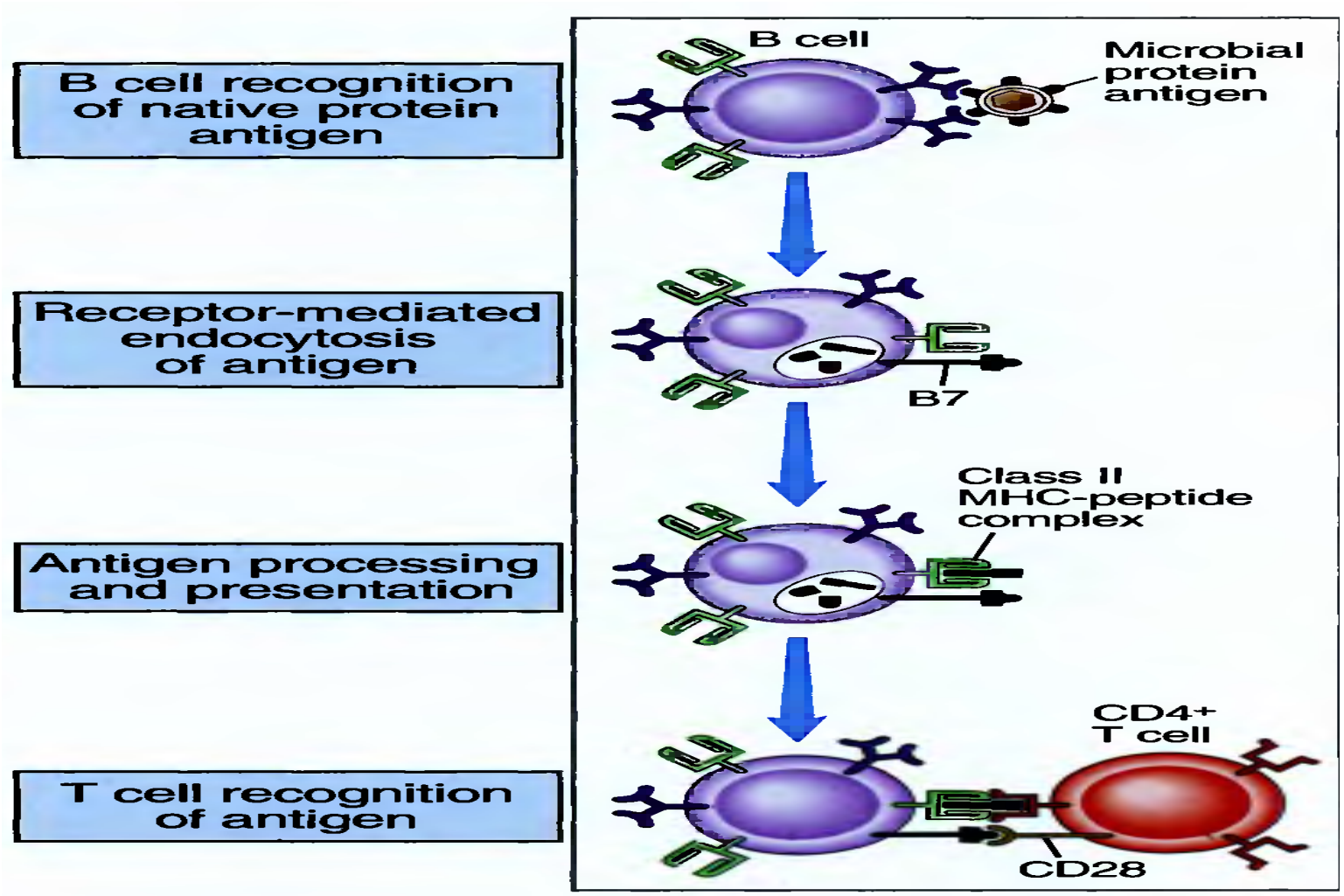
Activation and migration of helper T cells





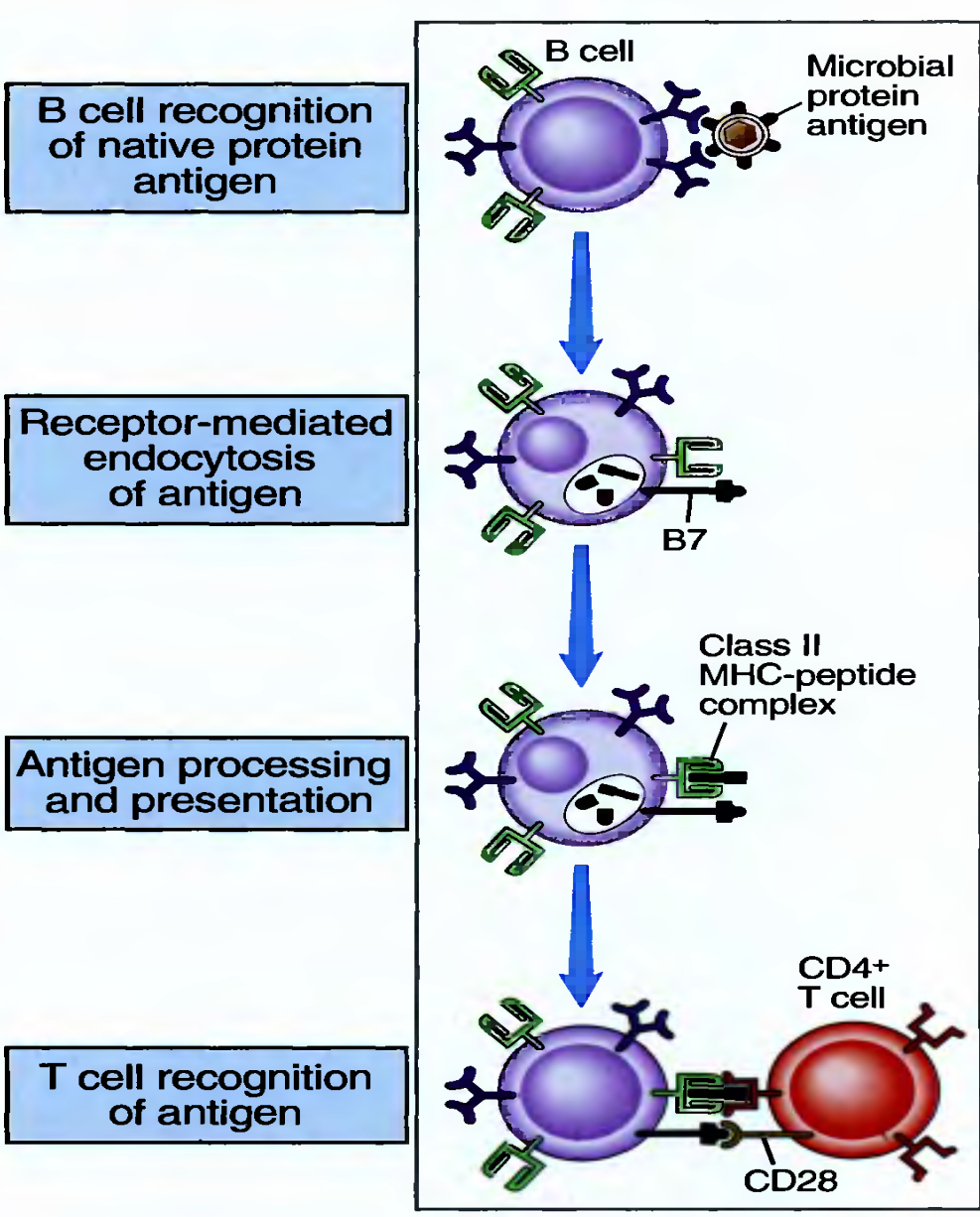
- Differentiated effector T cells migrate out of their normal accommodation. Some of these T lymphocytes circulate, find microbial antigens in distant regions and eliminate these microorganisms through cell-mediated immunity.

Presentation of antigens by B lymphocytes to helper T cells



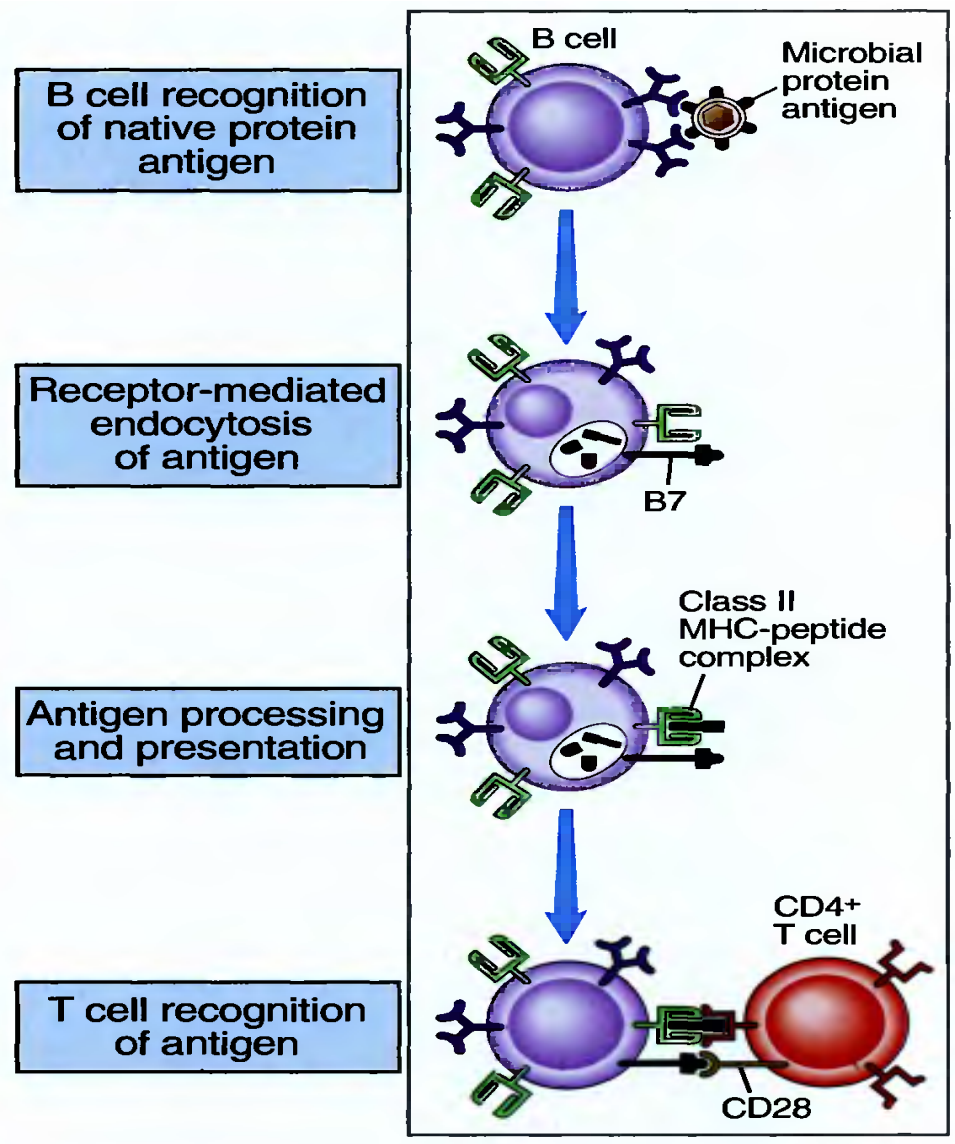
Presentation of antigens by B lymphocytes to helper T cells

- **B cells are very effective APCs for antigens they specifically recognize.**
- Any B cell binds to, incorporates and processes an epitope of a protein-structured antigen, and expresses various peptides of this protein on its surface for T cell recognition.



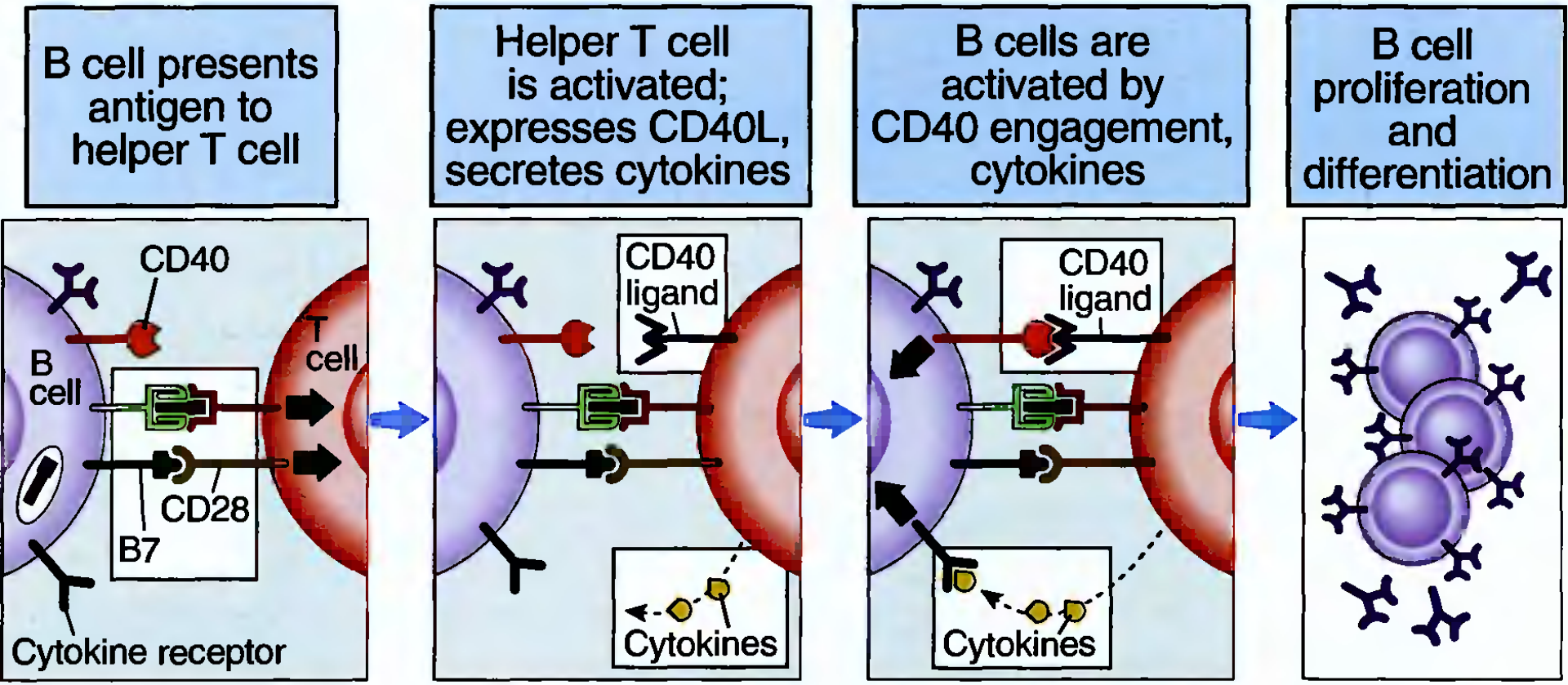
Presentation of antigens by B lymphocytes to helper T cells

- Antigen-activated B lymphocytes express co-stimuli such as **B7 molecules** and stimulate helper T cells that recognize the antigen expressed by B cells.



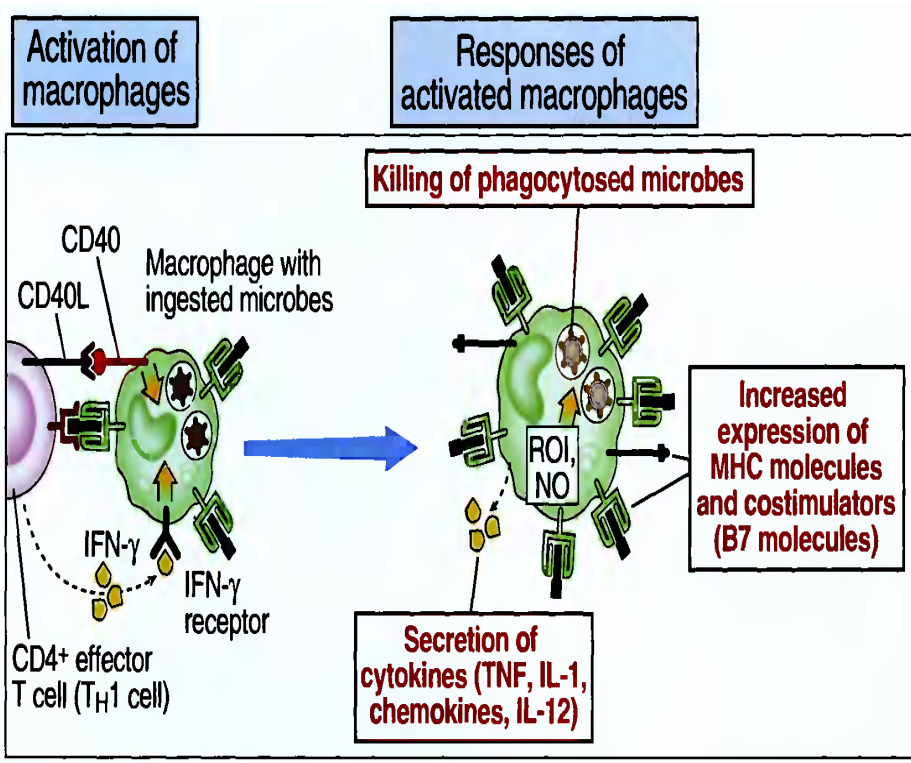
B cell activation mechanisms by T helper cells

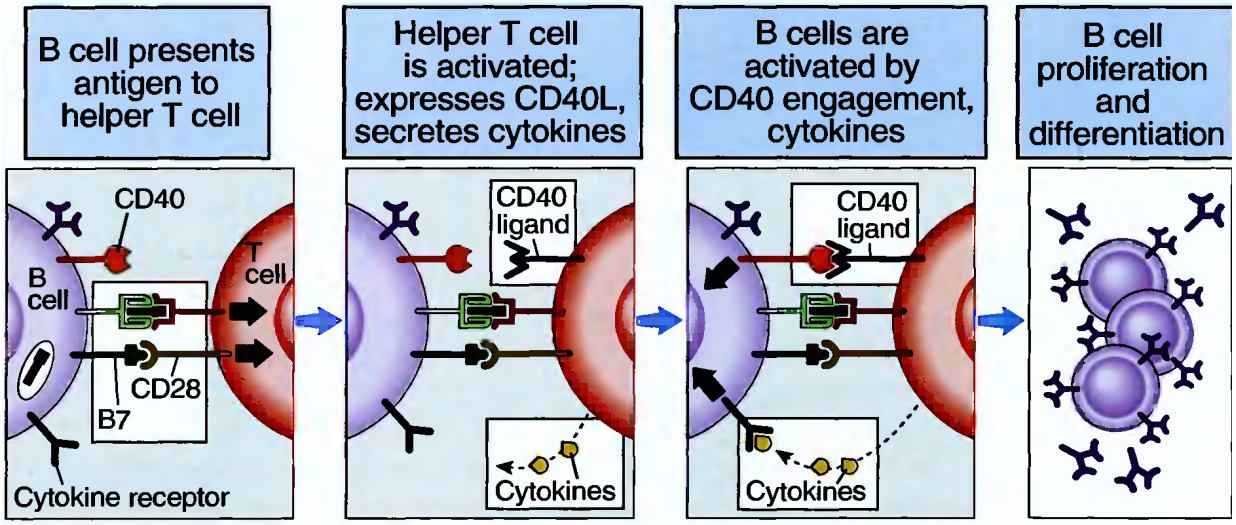
- Helper T cells that recognize the antigen presented by B cells activate B cells by expressing **CD40 ligand (CD40L)** on their surface and secreting cytokines.



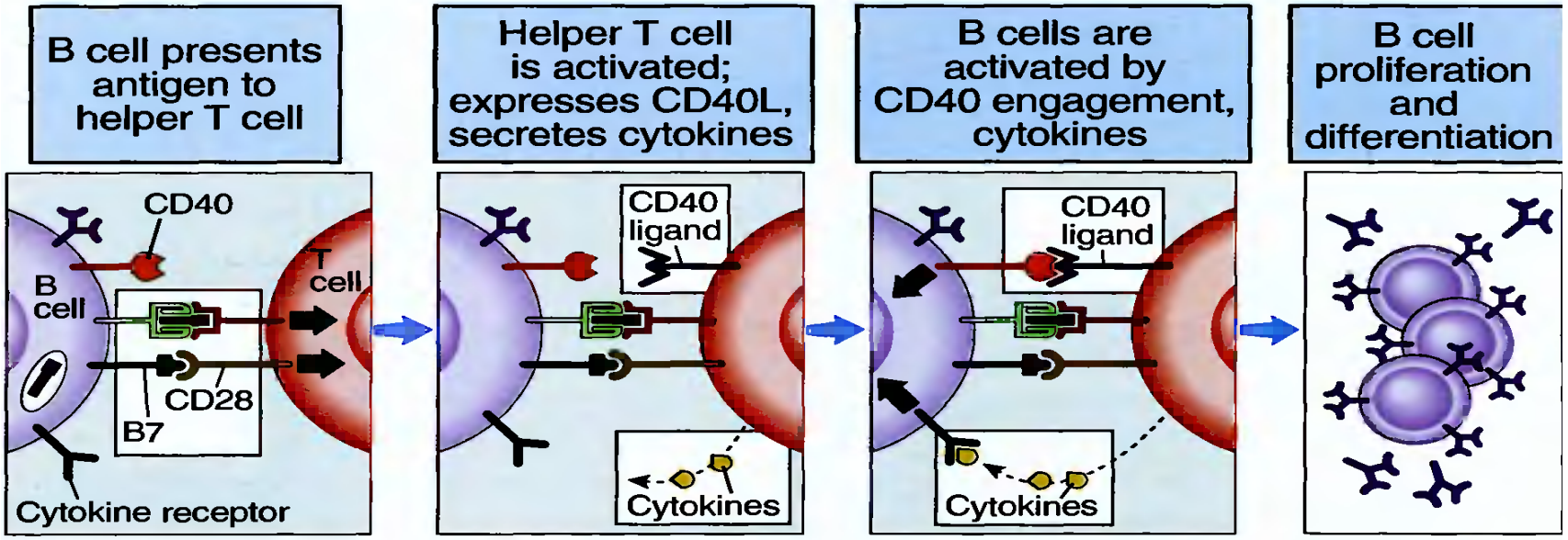
B cell activation mechanisms by T helper cells

- **Helper T cell mediated B lymphocyte activation It is similar to T cell mediated macrophage activation.**





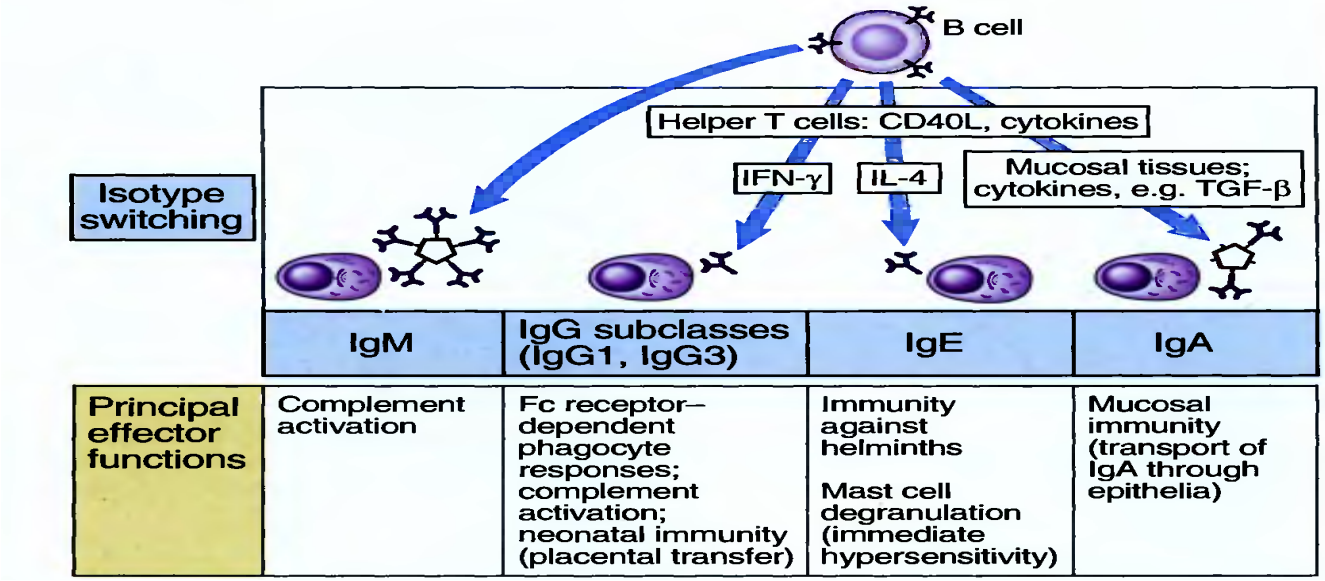
B cell activation mechanisms by T helper cells



- **At the same time, cytokines produced by helper T cells bind to cytokine receptors on B lymphocytes, stimulating further B cell proliferation and antibody production. The CD40L-CD40 interaction indicates that T and B lymphocytes can reach the stage where they can produce antibodies only by physical contact.**

Heavy chain isotype switching

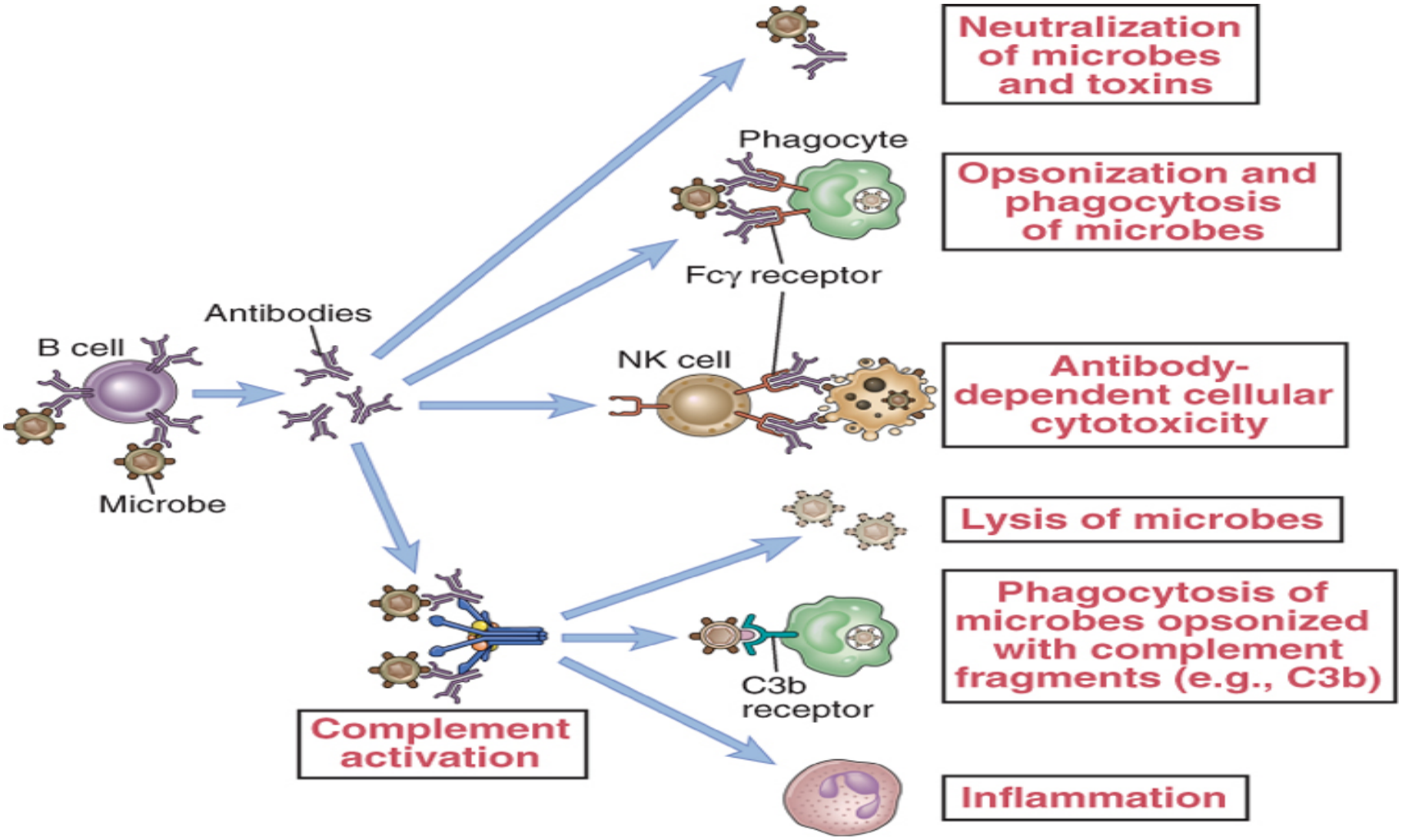
- **Helper T cells stimulate B lymphocytes expressing IgM + IgD that will produce antibodies among different heavy chain classes (isotypes).**



	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)

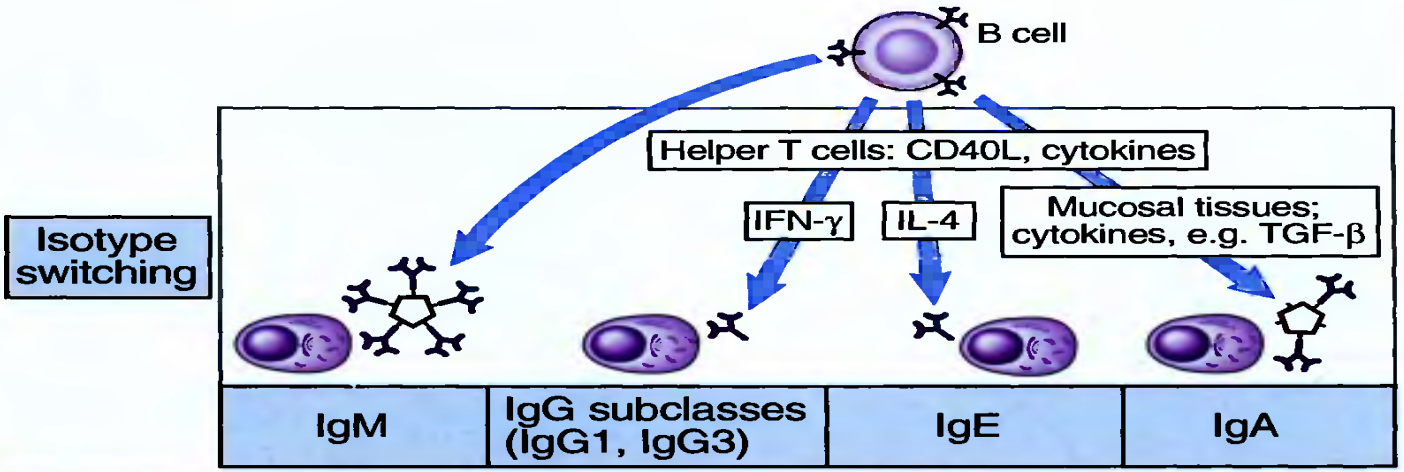
- The importance of isotype conversion is that it makes humoral immune responses suitable for combating various microorganisms in the most convenient way.

Heavy chain isotype switching



Heavy chain isotype switching

- **Heavy chain isotype conversion is initiated by CD40-mediated signals, and conversion to different Ig isotypes is induced by different cytokines. The signals mediated by CD40L and cytokines affect activated B cells and trigger isotype transformation.**



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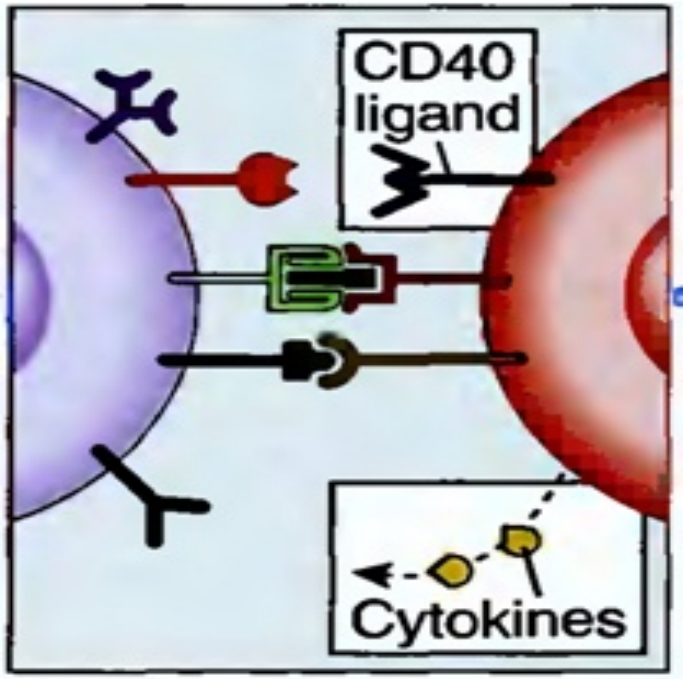
Principal effector functions	Complement activation	Fc receptor–dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)	Immunity against helminths Mast cell degranulation (immediate hypersensitivity)	Mucosal immunity (transport of IgA through epithelia)

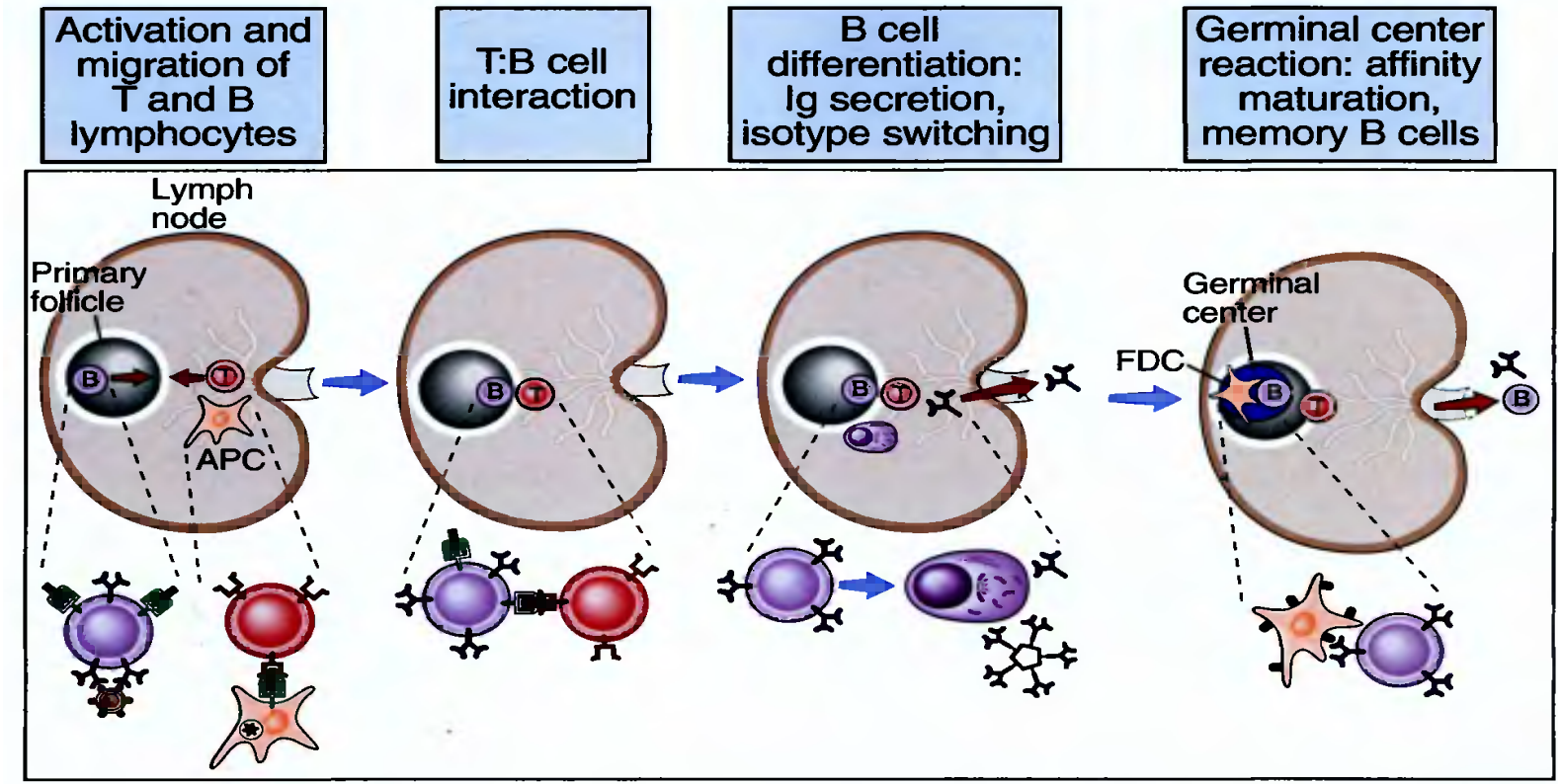
- **In the absence of CD40 or CD40L**, B cells secrete only IgM and are unable to convert to other isotypes, indicating how necessary the receptor-ligand pair is in isotype conversion.

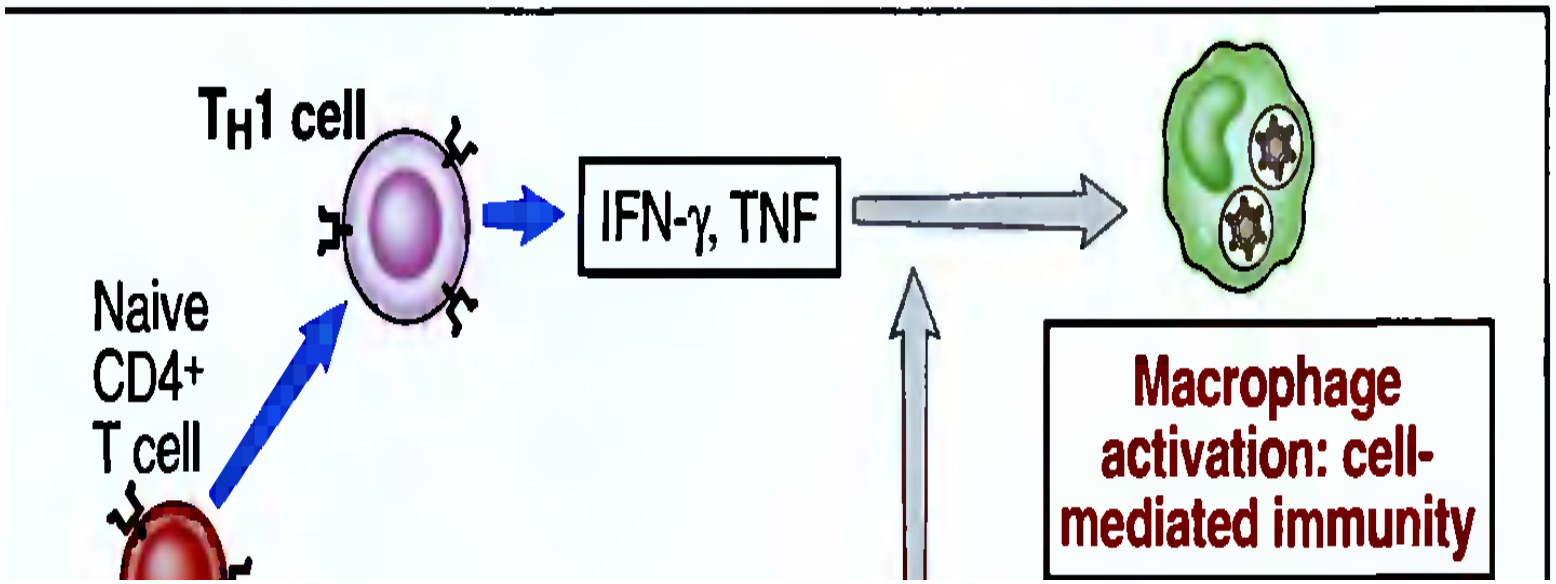


X-linked hyper IgM syndrome

- The disease caused by the syndrome that causes inactivation in the CD40L gene located on the X chromosome is called **X-linked hyper IgM syndrome**.
- Since heavy chain transformation cannot be performed in this disease, **most of the serum antibodies are in the IgM structure.**

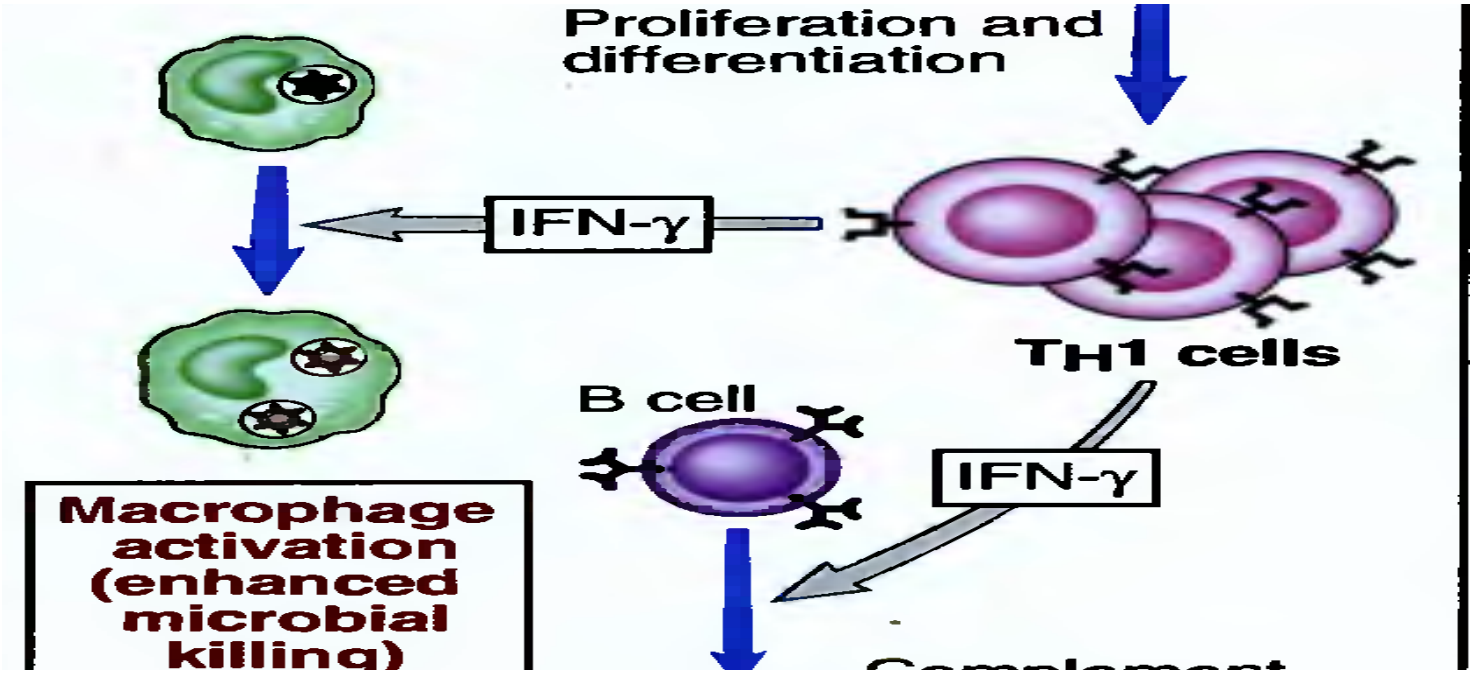
- Since CD40L plays an important role in T cell mediated immunity, the cellular immune response given by patients to intracellular microorganisms is also impaired.
- Cytokines determine which heavy chain isotype to convert from a B cell.



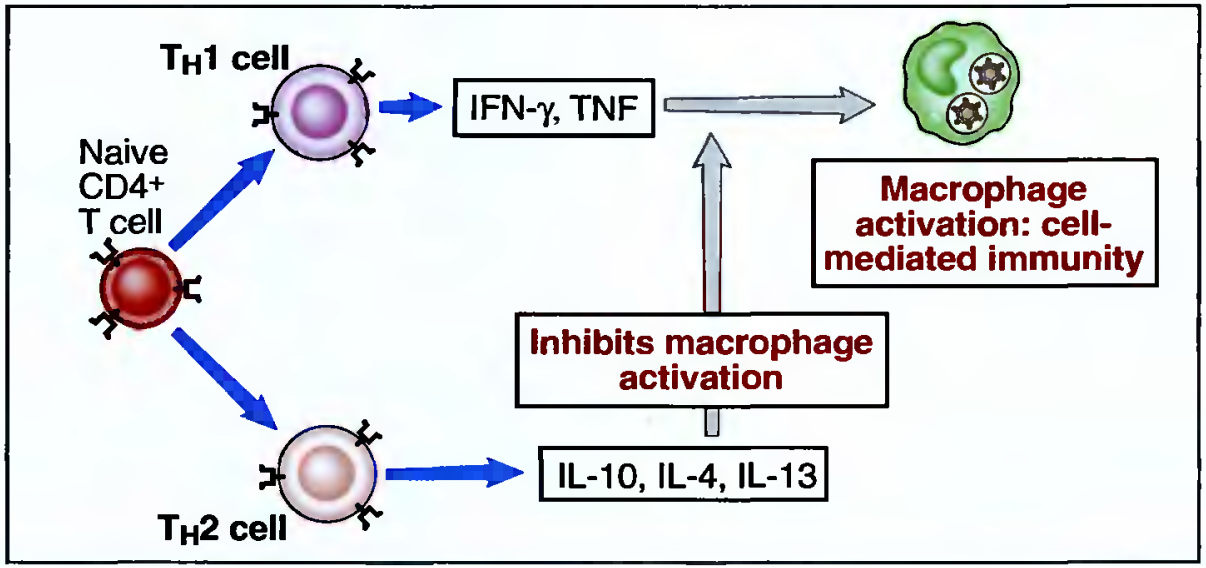


- For example, the production of opsonin antibodies that bind to the phagocyte FC receptor is stimulated by interferon- γ (IFN-γ), the key cytokine of Th1 cells.

- Opsonin-bearing antibodies support phagocytosis. IFN-γ is also the cytokine that activates phagocyte cells and stimulates the microbicidal functions of phagocytes. IFN-γ is a cytokine with activity on both B cells and phagocyte cells.

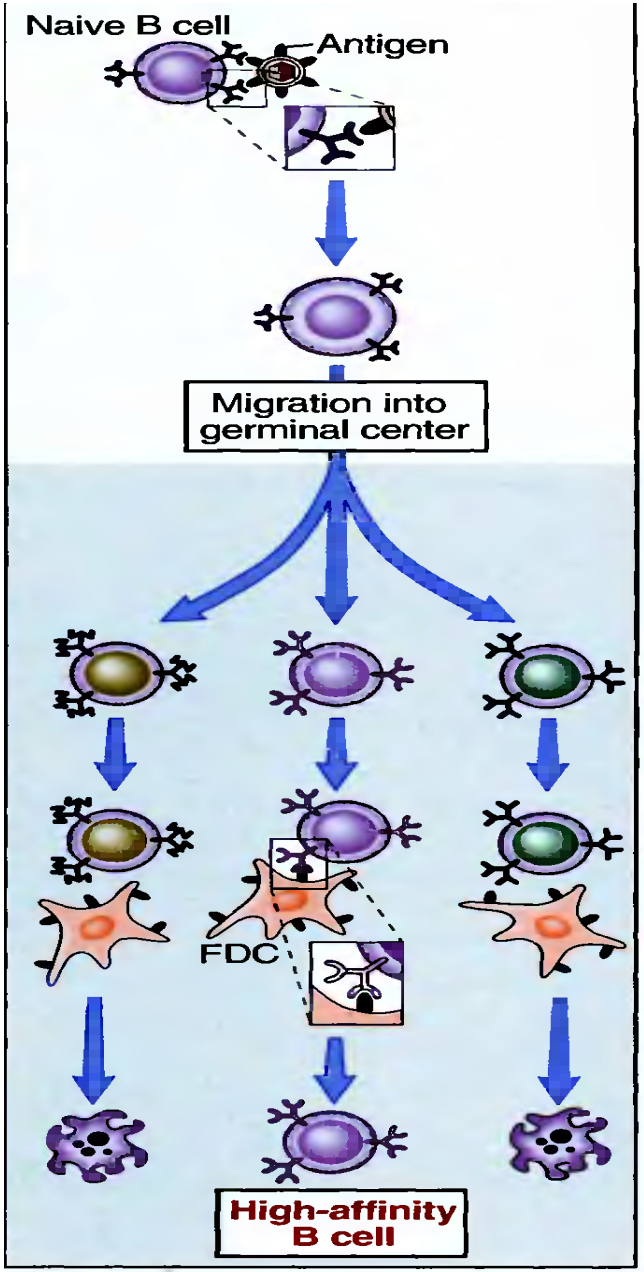


- Most of the microorganisms in the form of **bacteria and viruses** activate **Th1 responses**, which is the effector mechanism that best eliminates them.
- Conversion to IgE isotype is stimulated by the **interleukin IL-4**, which is the key cytokine of **Th2 cells**.



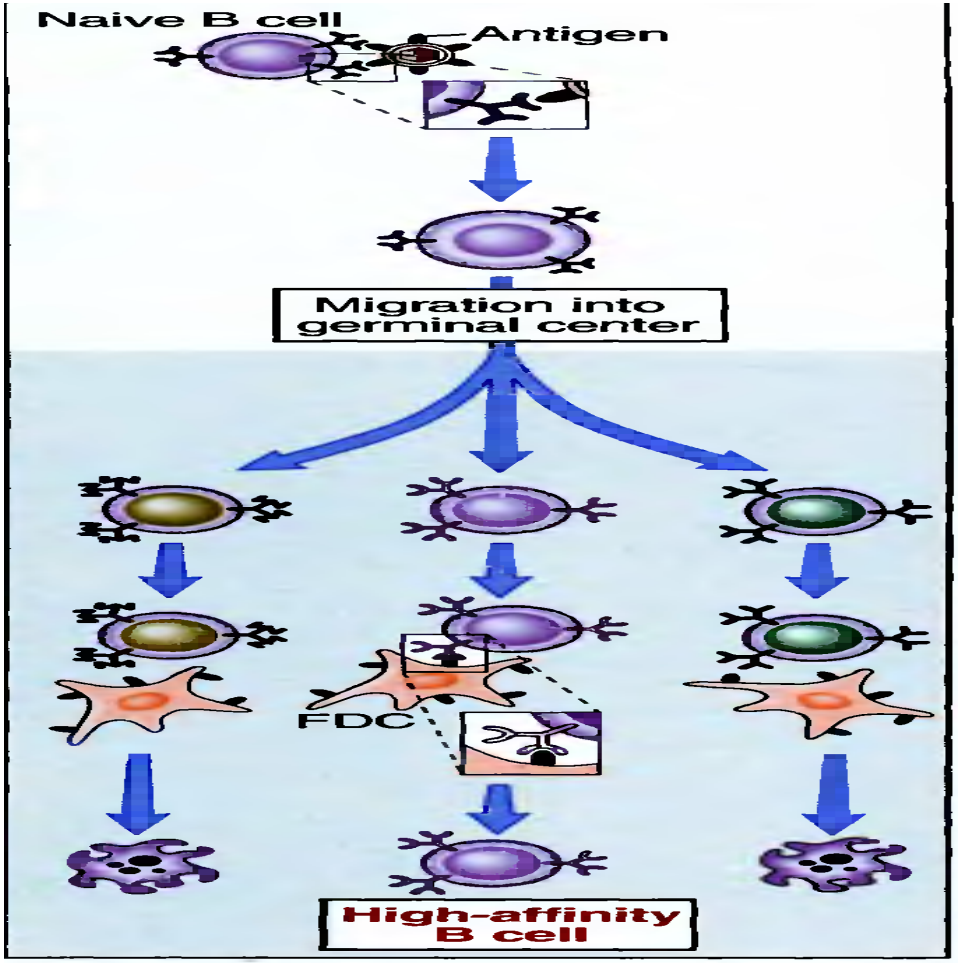
Affinity Maturation

- Affinity maturation is the process by which the affinity of antibodies produced against an antigen in protein structure increases with prolonged or repeated encounters with that antigen.
- Due to affinity maturation, the ability of antibodies to bind to a microorganism or microbial antigen increases if the infection is persistent or recurrent.

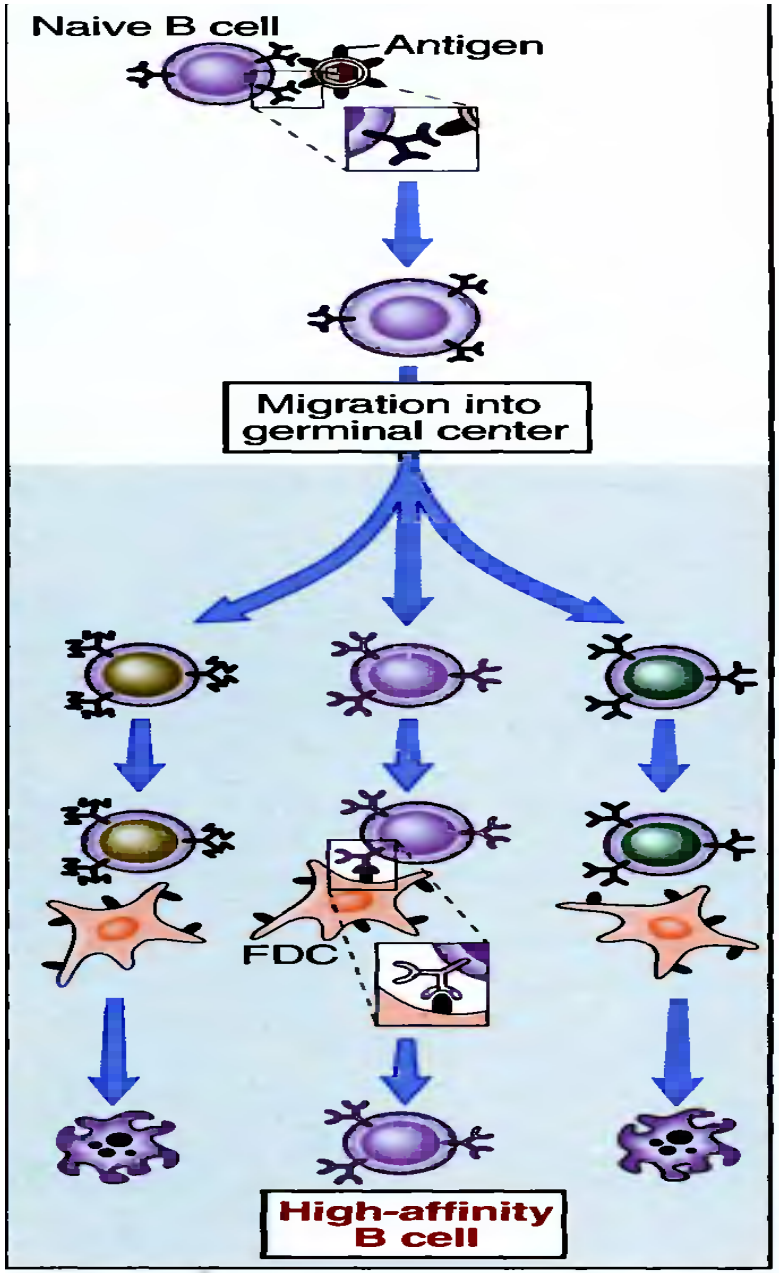


Affinity Maturation

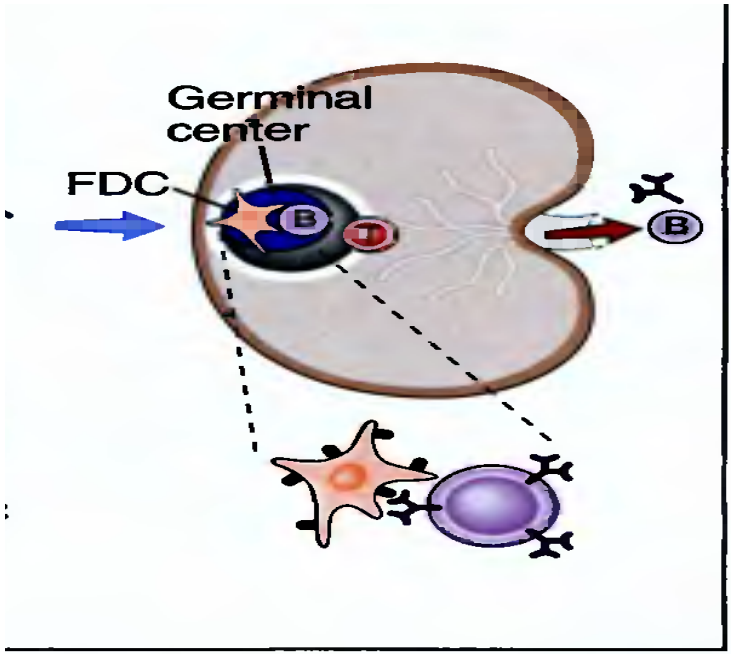
- Affinity maturation is seen only in the immune response that develops through **protein-like antigens** and as helper **T-cell dependent.**



- Affinity maturation occurs **in the germinal centers of lymphoid follicles.**
- It occurs as a result of **somatic hypermutation of Ig genes** in B cells with high affinity selected by the antigen presented by **follicular dendritic cells.**

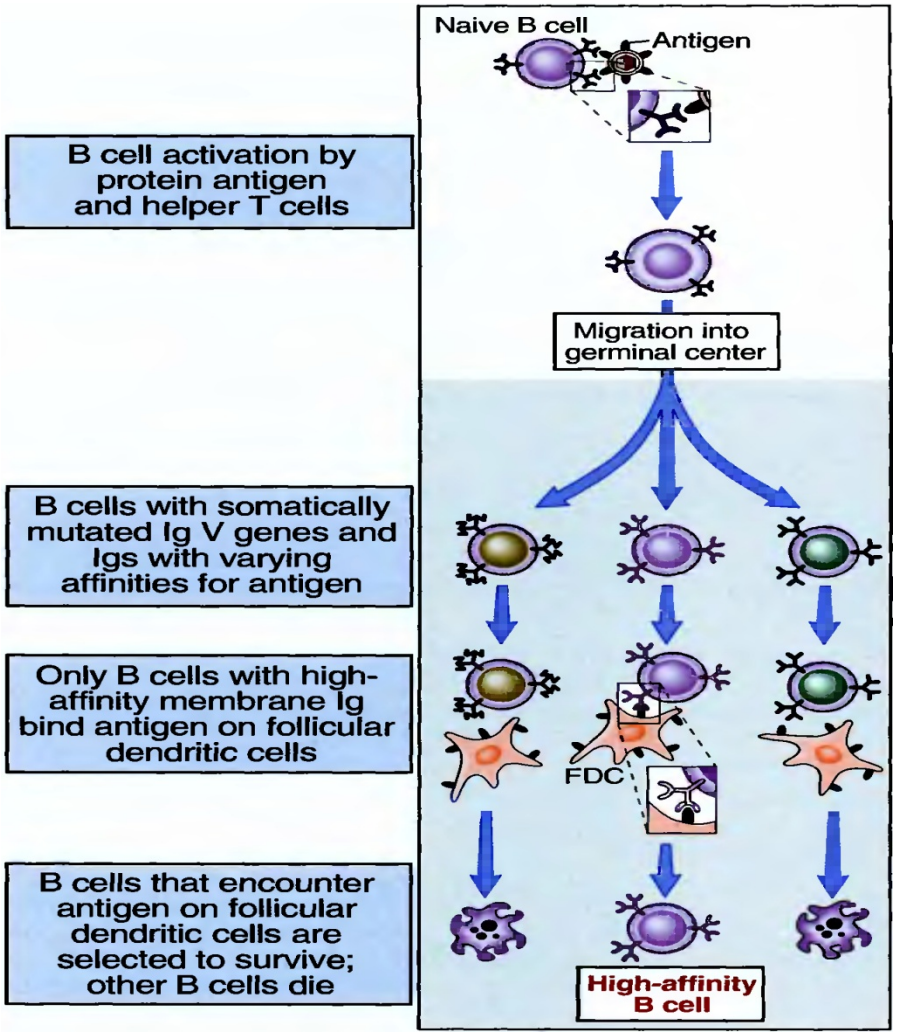


- Thus, B cells tending to somatic hypermutation find the opportunity to bind to the antigen on follicular dendritic cells and avoid death.



- As the immune response develops or as a result of repeated encounters with the same antigen (repeated immunization), the amount of antibody produced increases.

- Antibodies produced by B cells pass into the peripheral blood. Some of the plasma cells that secrete antibodies migrate to the bone marrow, **where they can live for months for years and continue to produce antibodies even after the antigen is removed.**

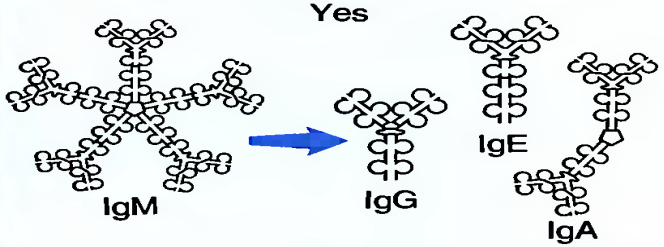
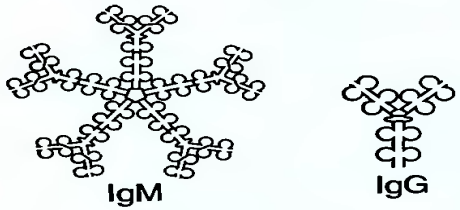


Memory B cells

- Memory B cells do not secrete antibodies, remain in circulation and live for months and years unless additional contact with the antigen occurs. They are ready to respond quickly when the antigen is encountered again.

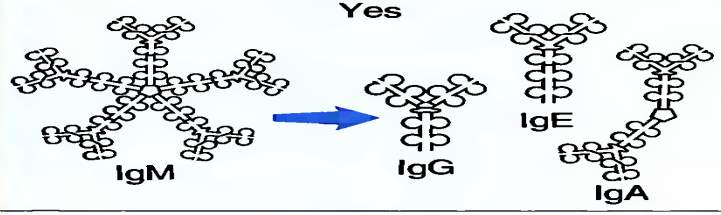
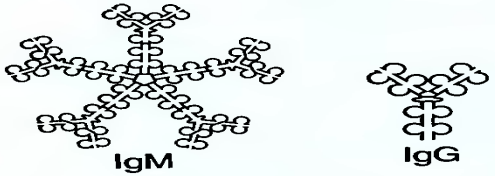
Antibody responses to T-independent antigens

- Polysaccharide, lipid, and other non-protein antigens generate antibody responses without the participation of helper T cells.

	Thymus-dependent antigen	Thymus-independent antigen
Chemical nature	Proteins 	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids 
Features of antibody response		
Isotype switching	Yes 	Little or no: may be some IgG 
Affinity maturation	Yes	Little or no
Secondary response (memory B cells)	Yes	Only seen with some antigens

Antibody responses to T-independent antigens

- Crosslinking can activate B cells without the need for T cell assistance and enough to induce differentiation-proliferation.

	Thymus-dependent antigen	Thymus-independent antigen
Chemical nature	Proteins 	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids 
Features of antibody response		
Isotype switching	Yes 	Little or no: may be some IgG 
Affinity maturation	Yes	Little or no
Secondary response (memory B cells)	Yes	Only seen with some antigens

Control of humoral immune responses: antibody feedback

- Some B lymphocytes live for a long time after differentiating into antibody secreting cells and memory cells.
- Most of the activated B cells, on the other hand, are destroyed by a process of programmed cell death.
- This progressive loss of activated B cells causes the humoral immune response to decrease physiologically.

ANTIBODY FEEDBACK: REGULATION OF HUMORAL IMMUNE RESPONSES BY Fc RECEPTORS

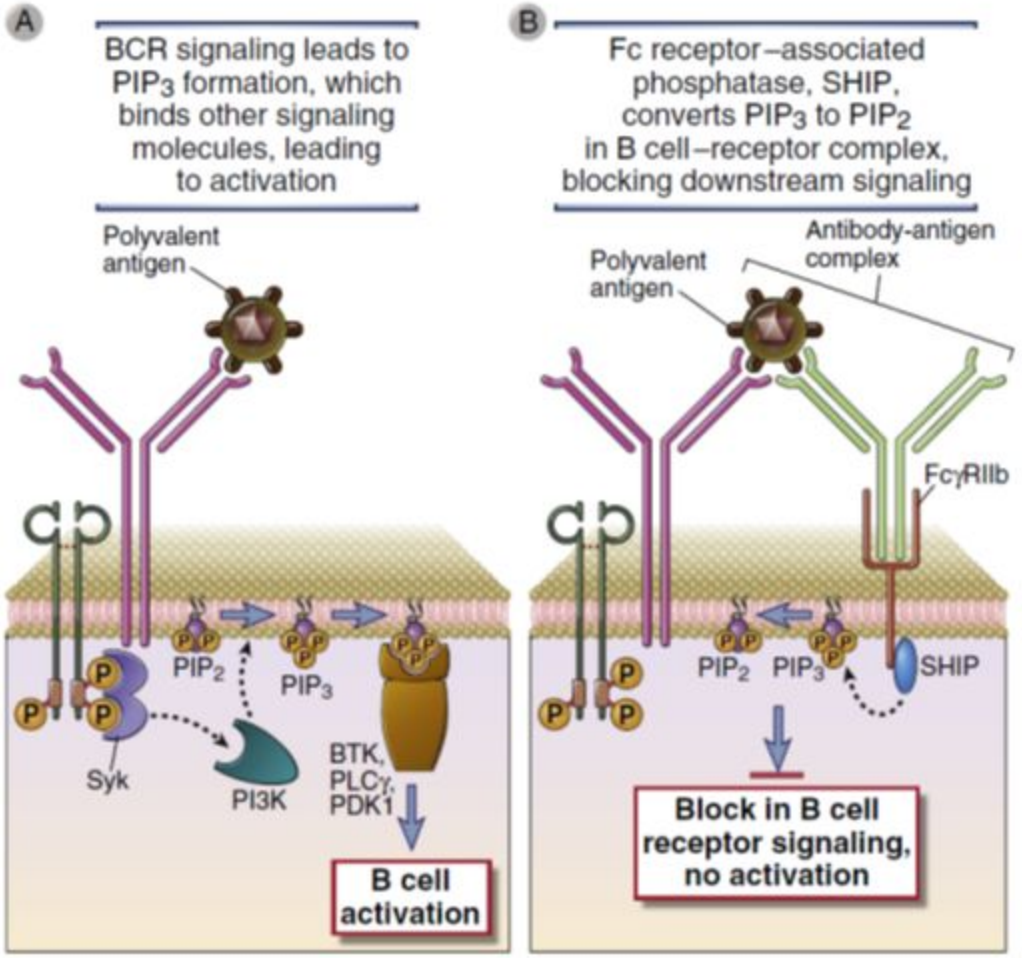
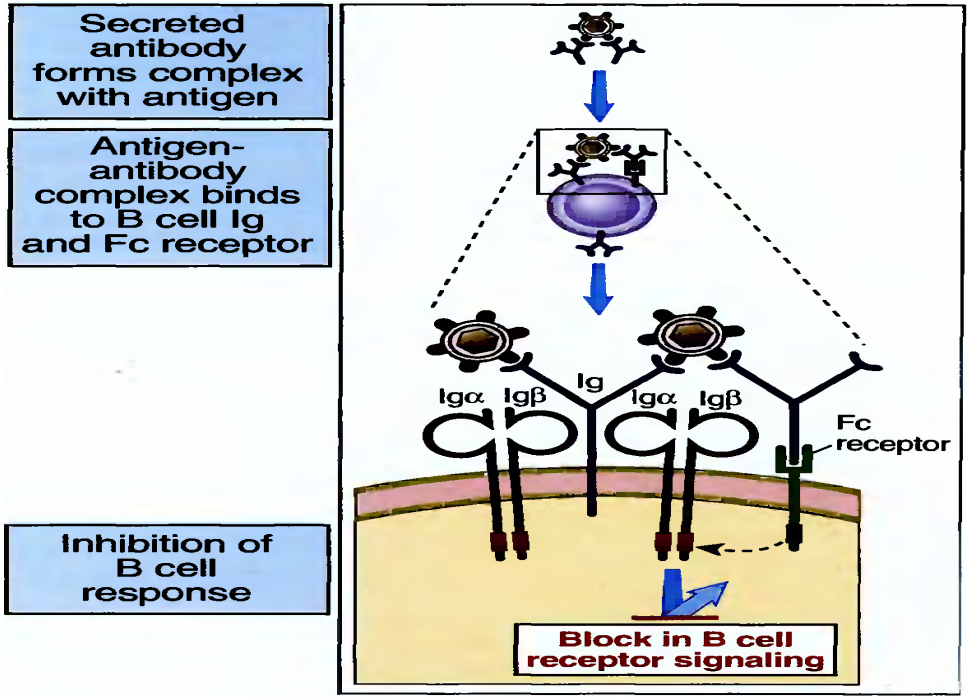
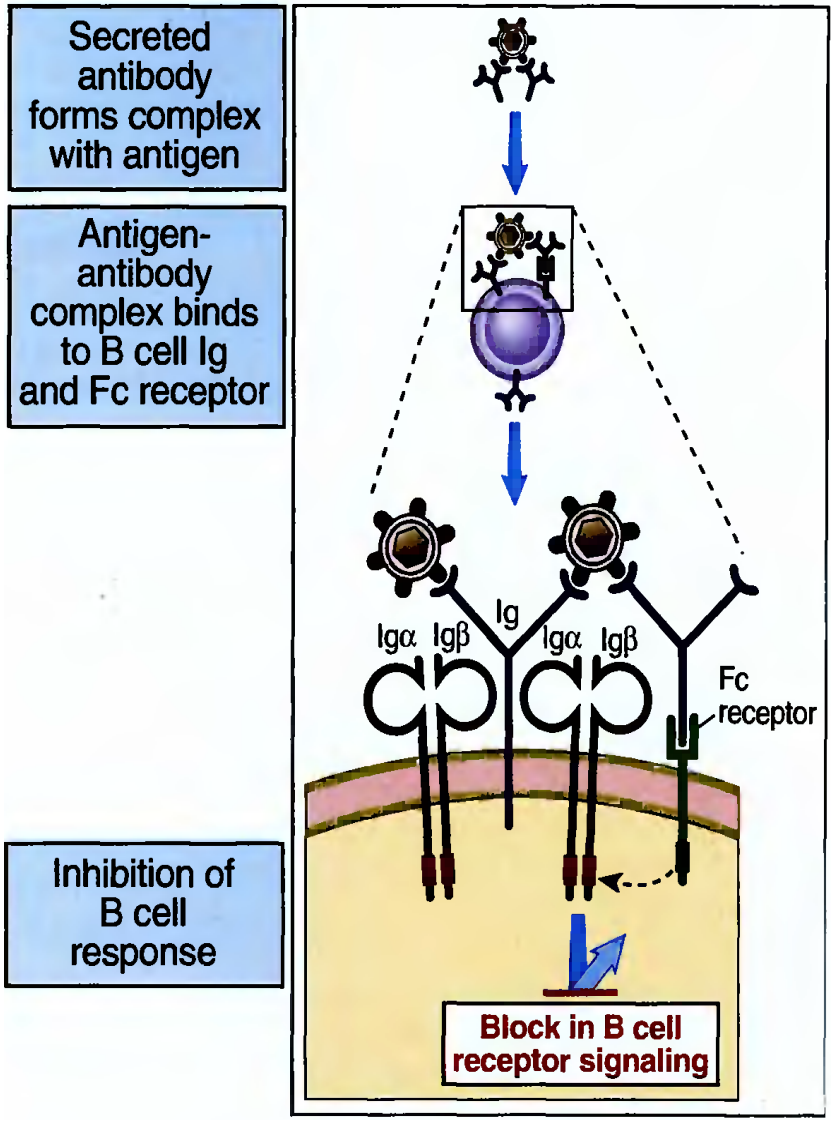


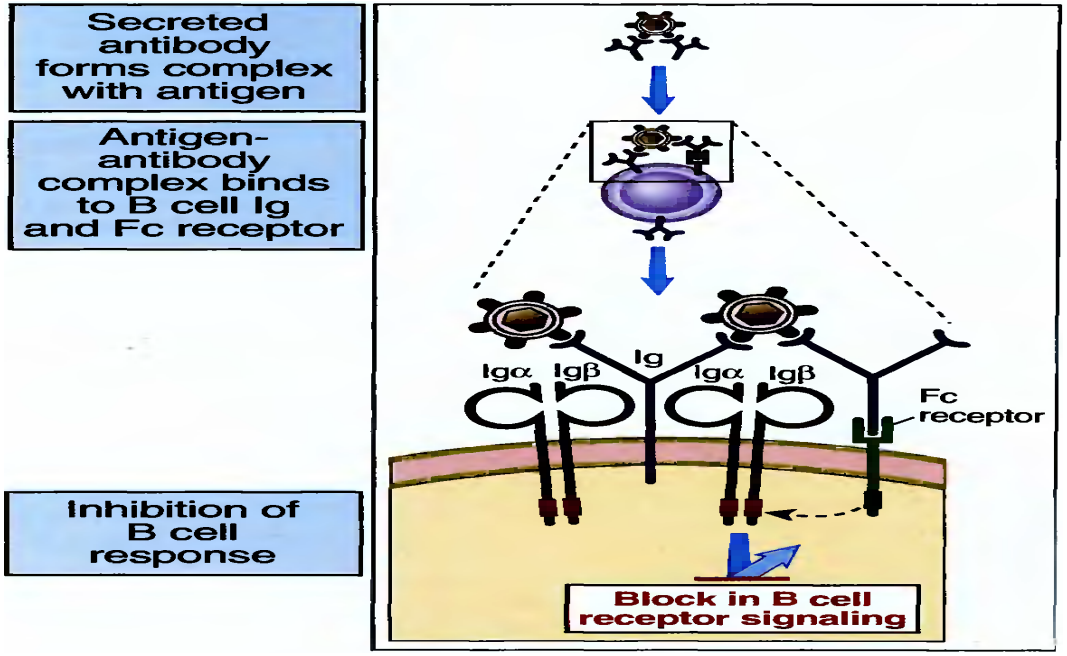
FIGURE 12-21 Regulation of B cell activation by FcγRIIB. A, Antigen-antibody complexes can simultaneously bind to membrane Ig (through antigen) and the FcγRIIB receptor through the Fc portion of the antibody. **B,** As a consequence of this simultaneous ligation of receptors, phosphatases associated with the cytoplasmic tail of the FcγRIIB inhibit signaling by the BCR complex and block B cell activation.

- B cells use another mechanism to stop antibody production. While the IgG antibody produced circulates throughout the body, it binds to the antigen still present in blood and tissues and forms immune complexes. Antigen-specific B cells can bind to the antigen part of the immune complex with Ig receptors.



- Also, the Fc "tail" of the antigen-binding IgG antibody is recognized by the Fc receptor expressed on B cells.
- **This Fc receptor terminates the B cell response by shutting off the signals stimulated by the antigen receptor.**





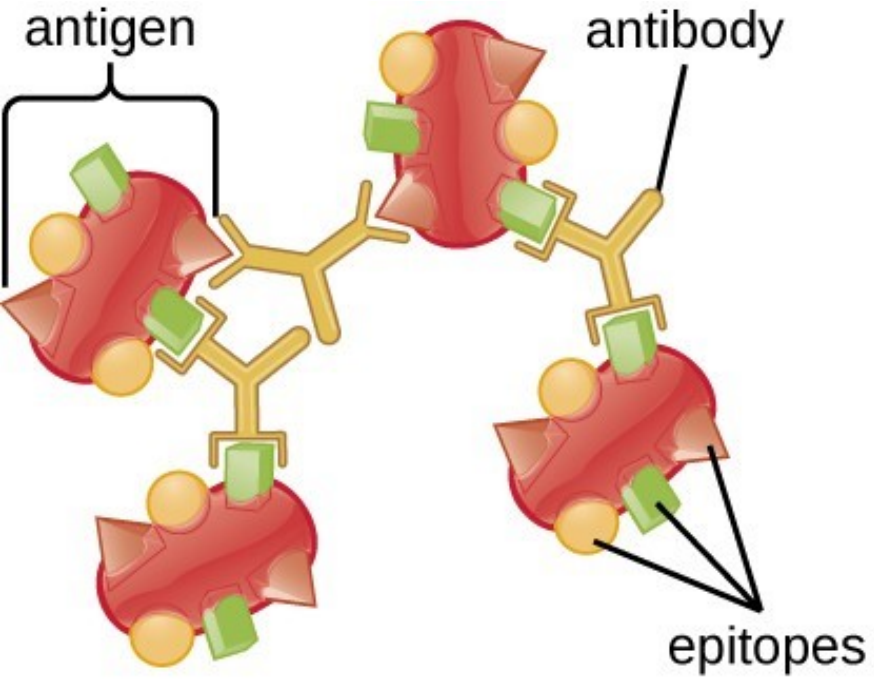
- This process in which the antibody that binds to the antigen inhibits the production of new antibodies is called **antibody feedback**. Thus, the humoral immune responses that enable sufficient IgG antibodies to be produced are terminated.

Monoclonal antibodies

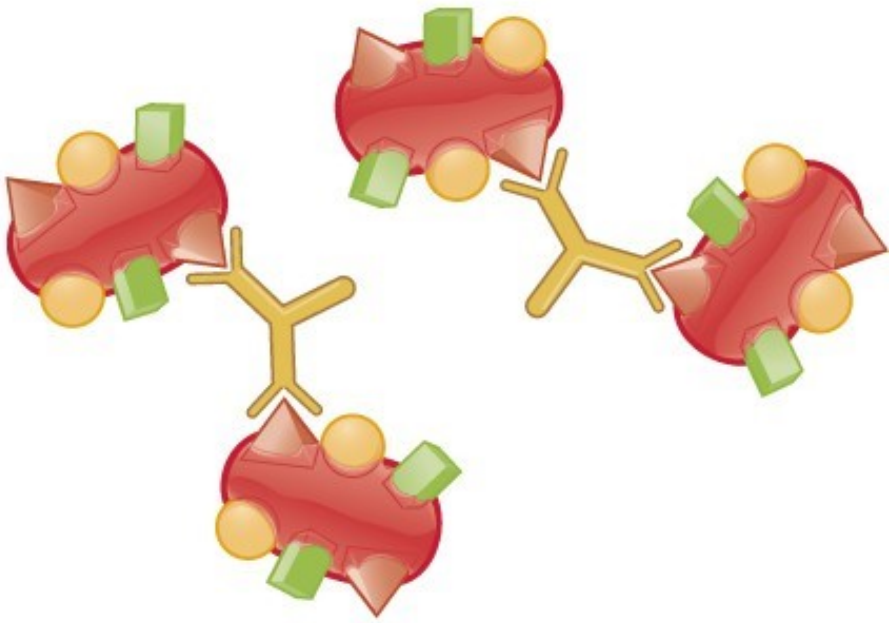
- products of a single B-lymphocyte clone
- homogeneous (antigene-specificity, affinity, isotype)

- in human body: only under pathological circumstances
  e.g. in gammopathy
  (malign growth of a certain plasma-cell clone)

-their advantage versus polyclonal antibodies:
antibodies of the given specificity and isotype can be produced in **large amount** and of the **same quality**



polyclonal antiserum

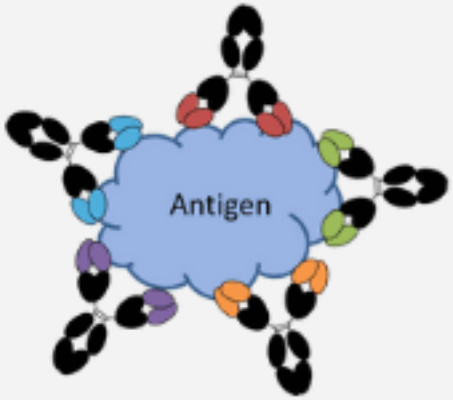


monoclonal antibodies

Polyclonal Antibody

- Cheap to produce
- Mixed population of antibodies
- May bind to different areas of the target molecule
- Tolerant of small changes in protein structure

Polyclonal antibody

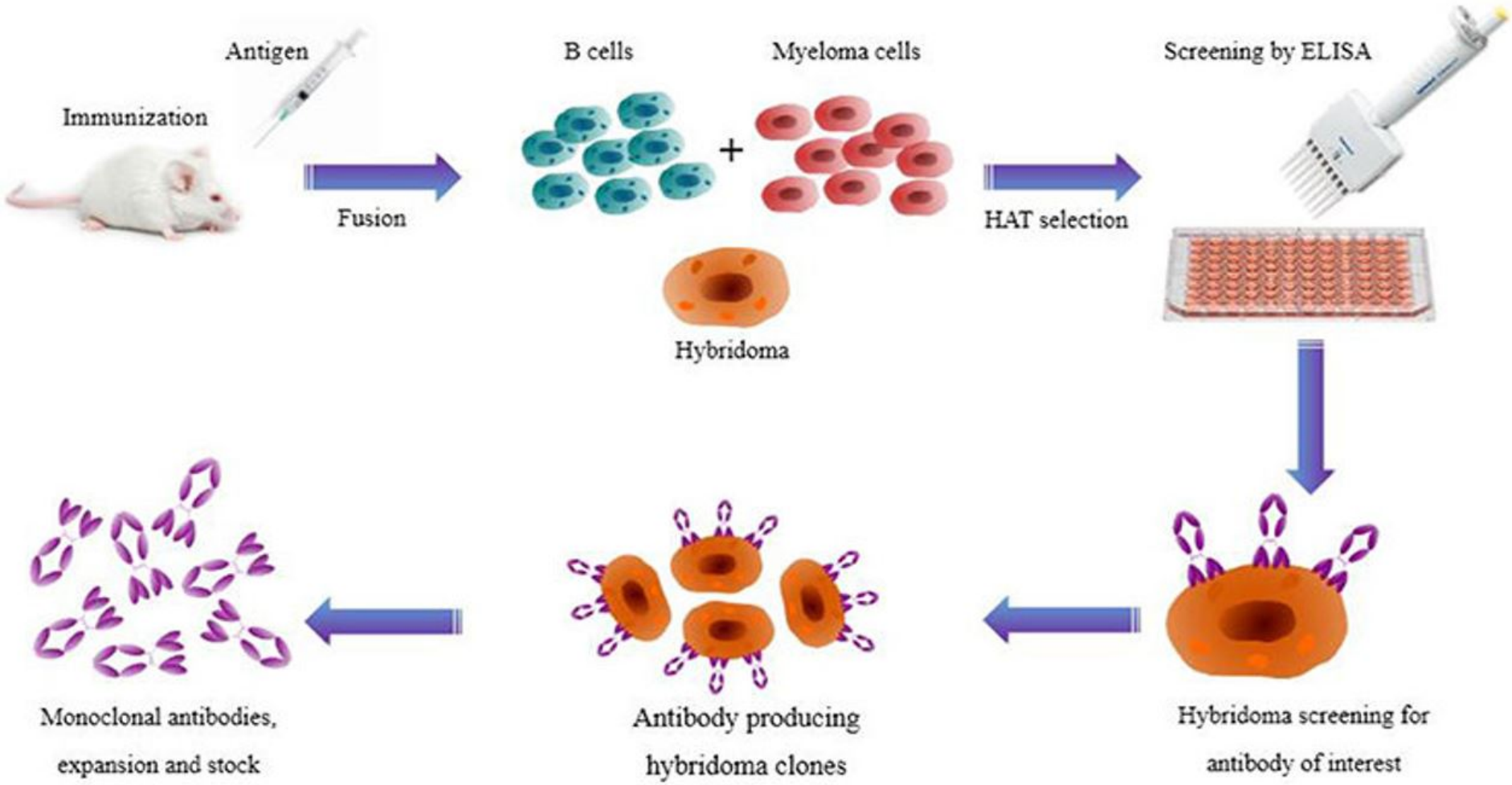


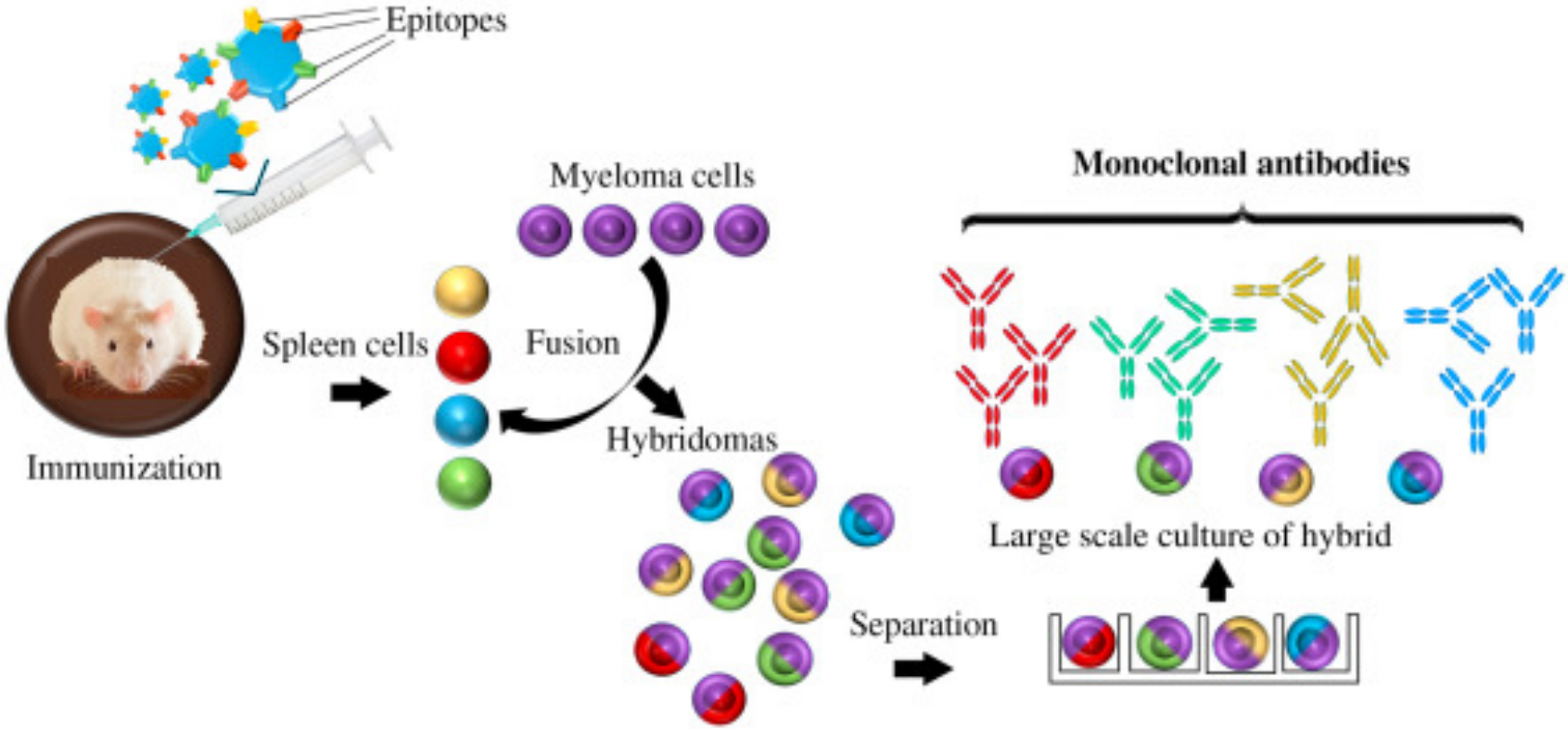
Monoclonal Antibody

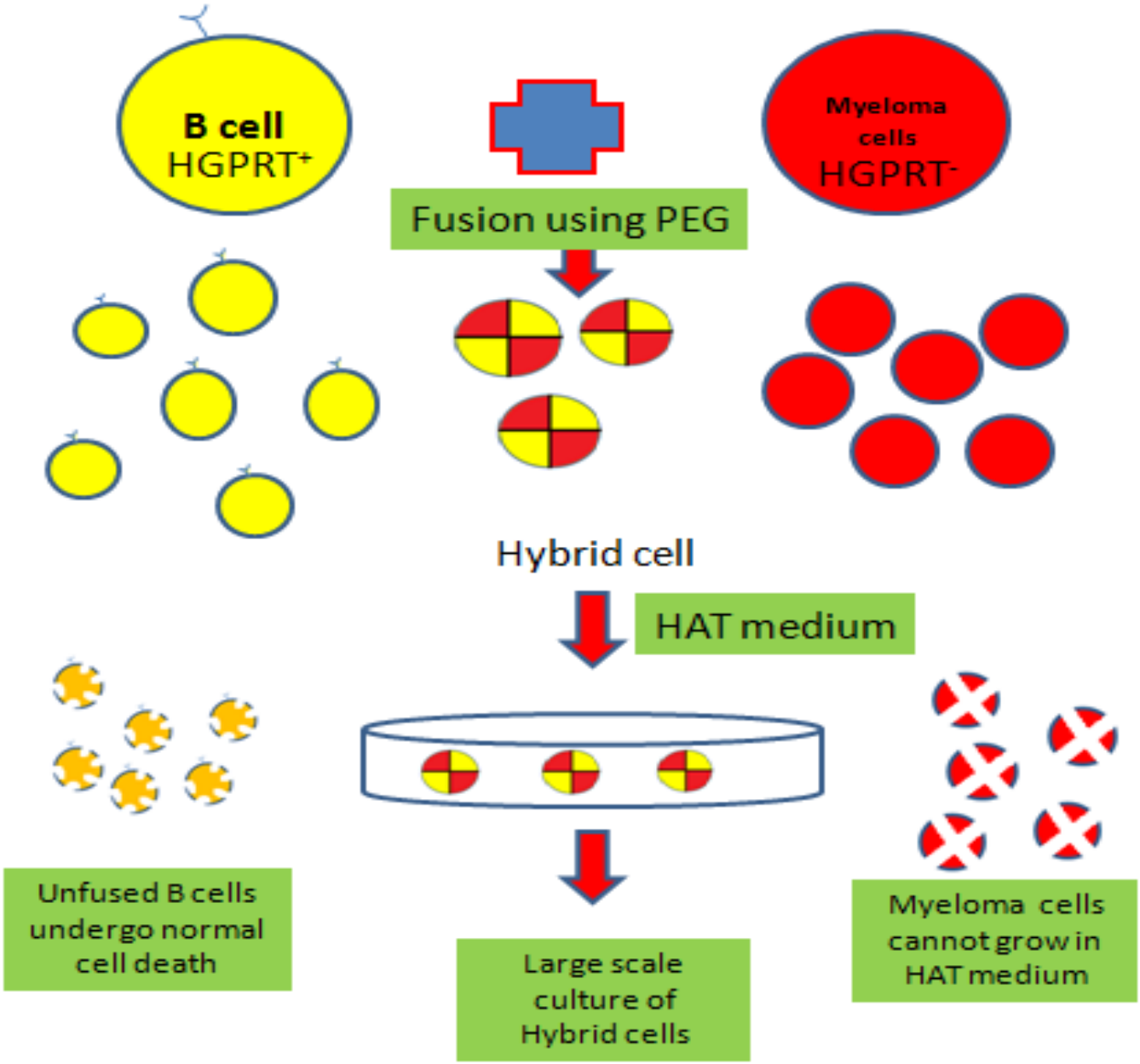
- Expensive to produce
- Single antibody species
- Will only bind single specific site
- May recognise a particular protein form

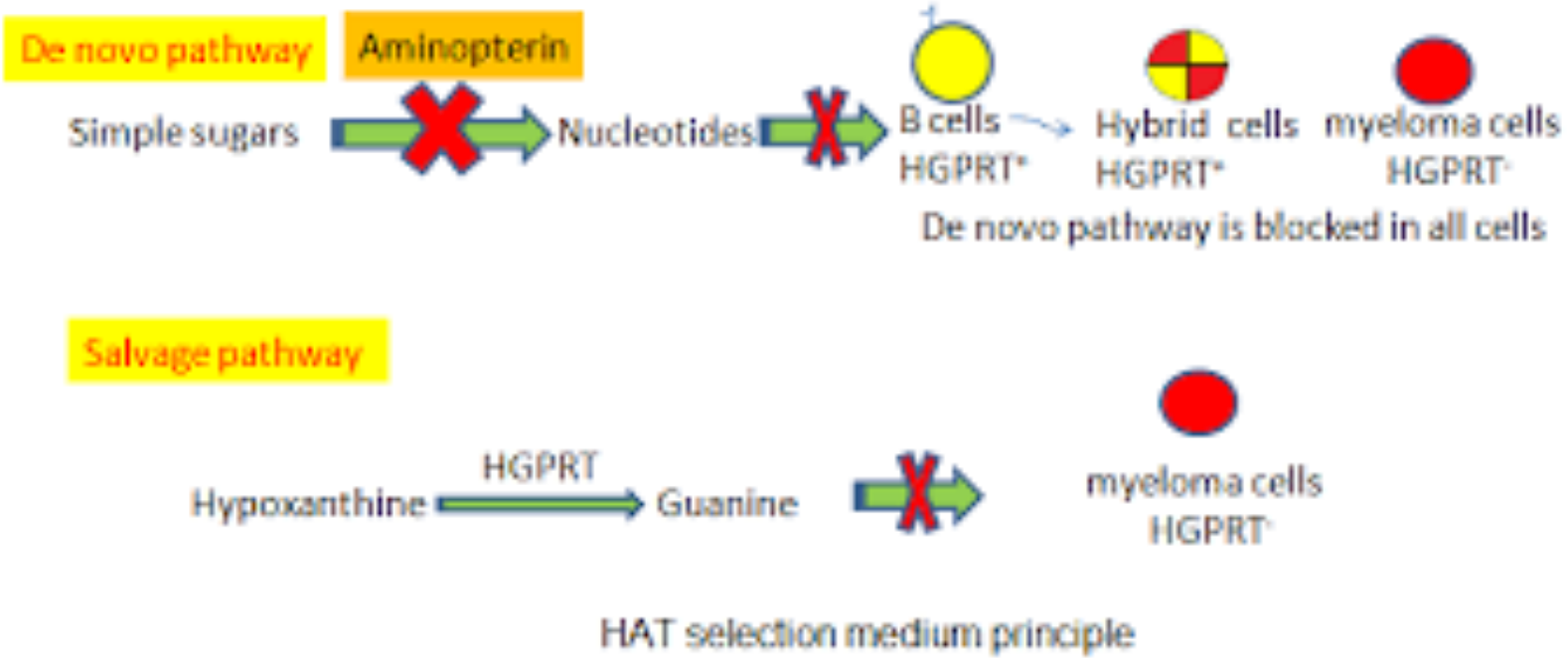
Monoclonal antibody

labclinics


Antigen
Immunization
Fusion
B cells
Myeloma cells
Hybridoma
HAT selection
Screening by ELISA
Hybridoma screening for antibody of interest
Antibody producing hybridoma clones
Monoclonal antibodies, expansion and stock


Epitopes
Immunization
Spleen cells
Myeloma cells
Fusion
Hybridomas
Separation
Large scale culture of hybrid
Monoclonal antibodies





HAT selection medium principle

Hybridomas

- myeloma cells are selected as HGPRT-
- HGPRT- myeloma cells are fused with B-lymphocytes taken from spleen of antigen-injected mouse

myeloma cells HGPRT- + B-lymphocytes HGPRT+

↓

Plate on HAT medium: hypoxanthine, thymidine (salvage pathway) and aminopterin (blocks de novo pathway)

↓

Unfused myeloma cells **die** (HGPRT-): can't use salvage pathway
Unfused B lymphocytes **die** (primary cells with short life span)
Fused hybridomas grow- each cell producing a colony secreting a unique monoclonal antibody

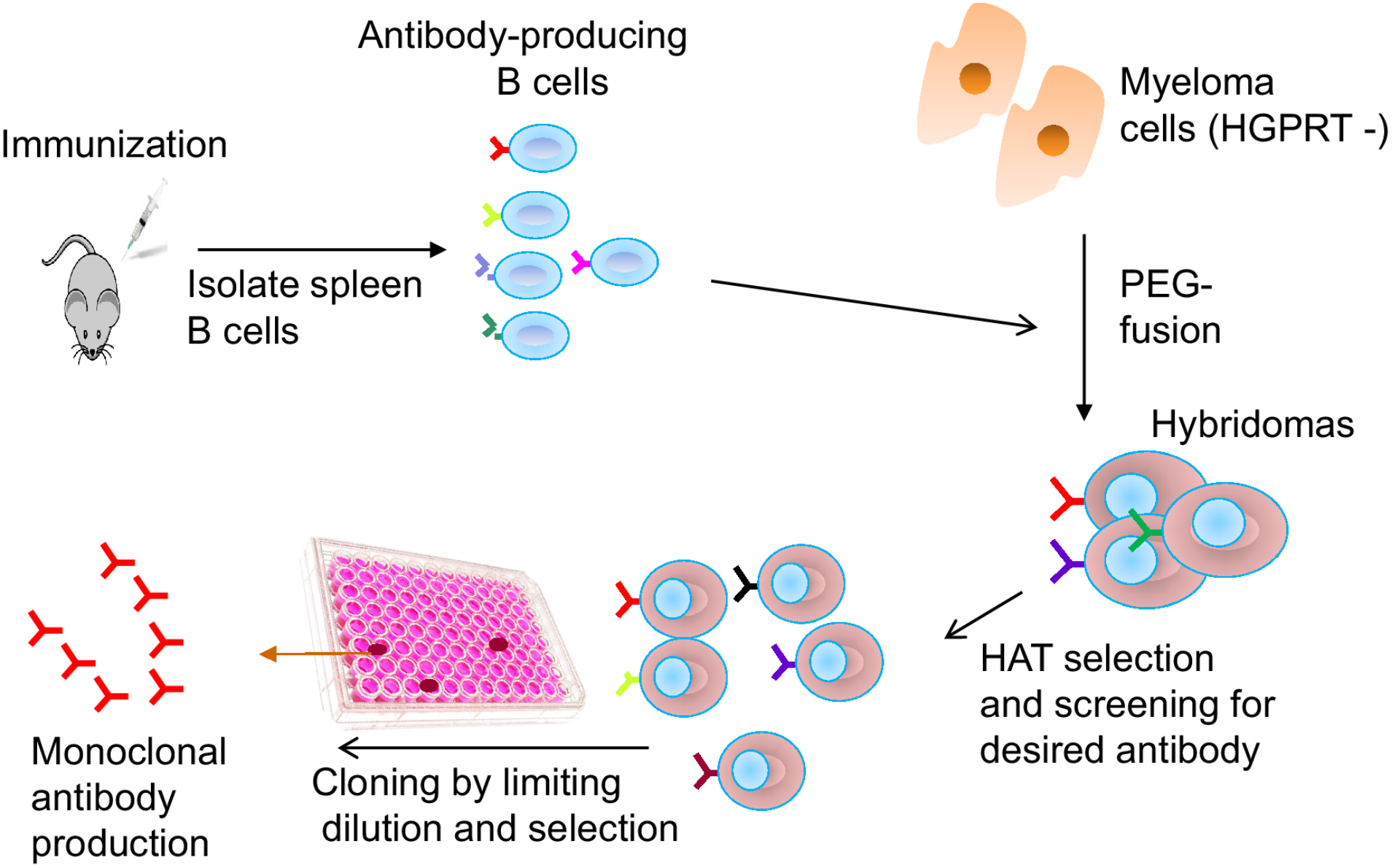
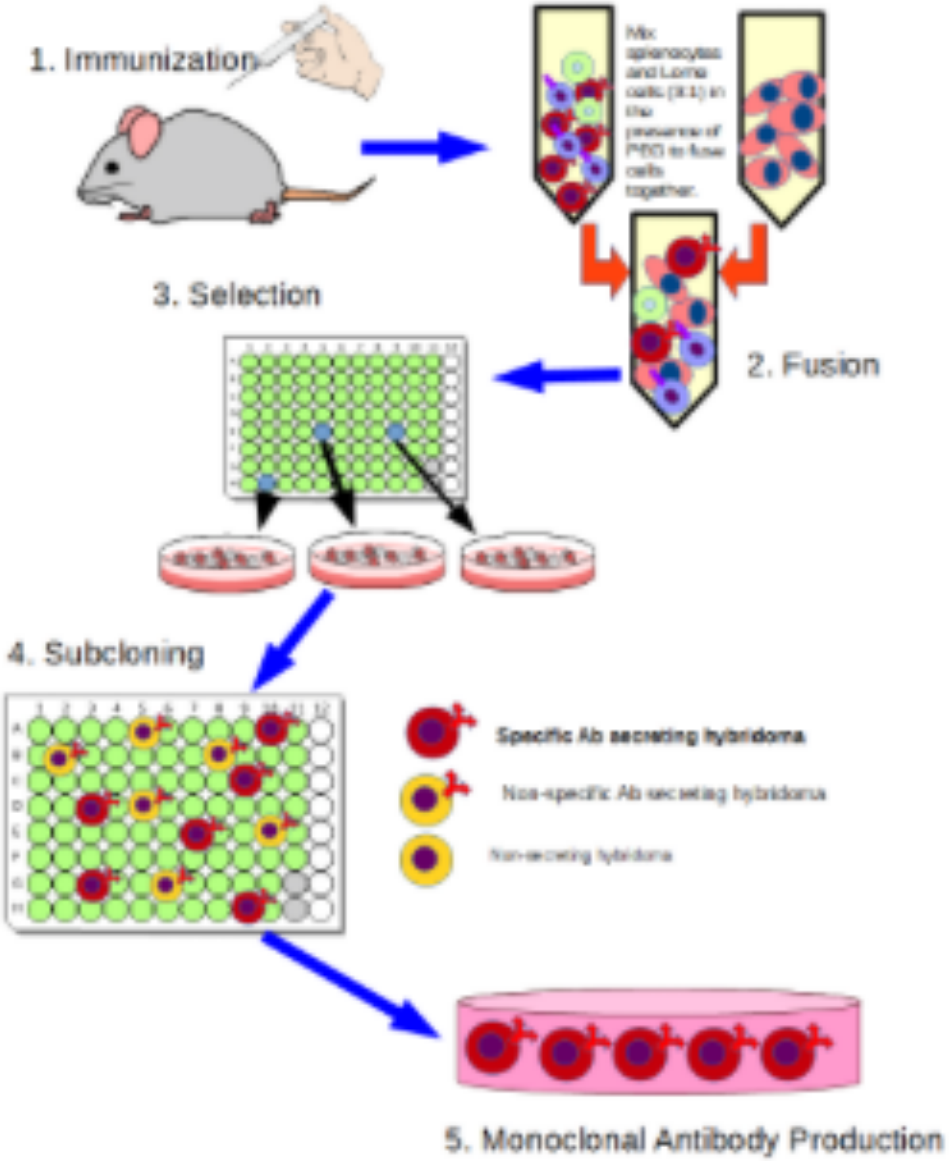
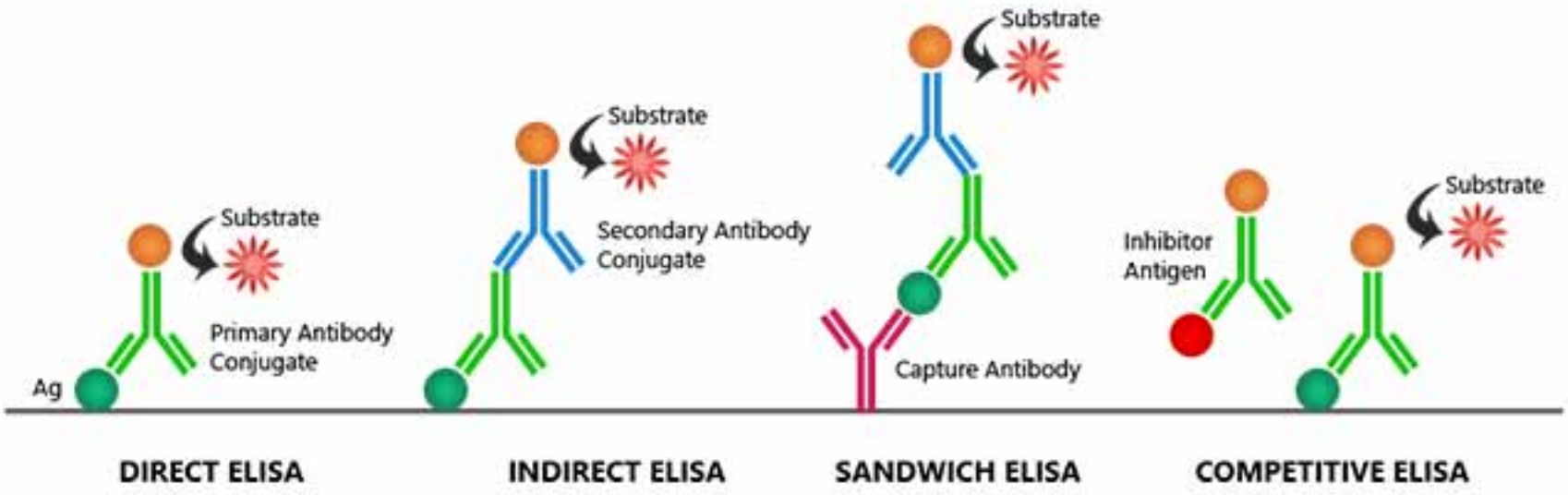

Antibody-producing
B cells
Myeloma
cells (HGPRT -)
Immunization
Isolate spleen
B cells
PEG-
fusion
Hybridomas
HAT selection
and screening for
desired antibody
Monoclonal
antibody
production
Cloning by limiting
dilution and selection

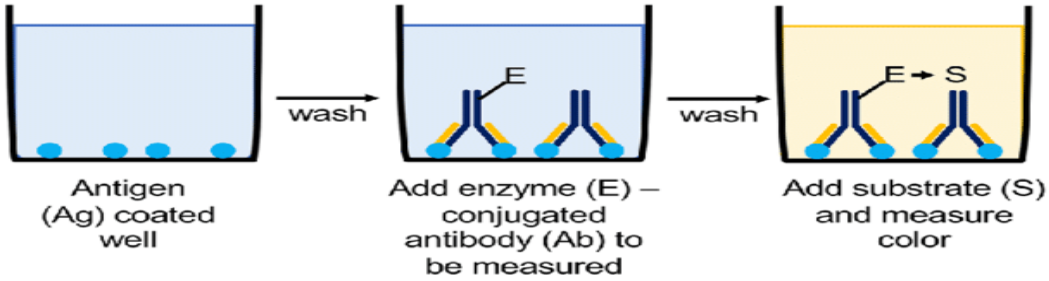
Figure 1: Hybridoma Development



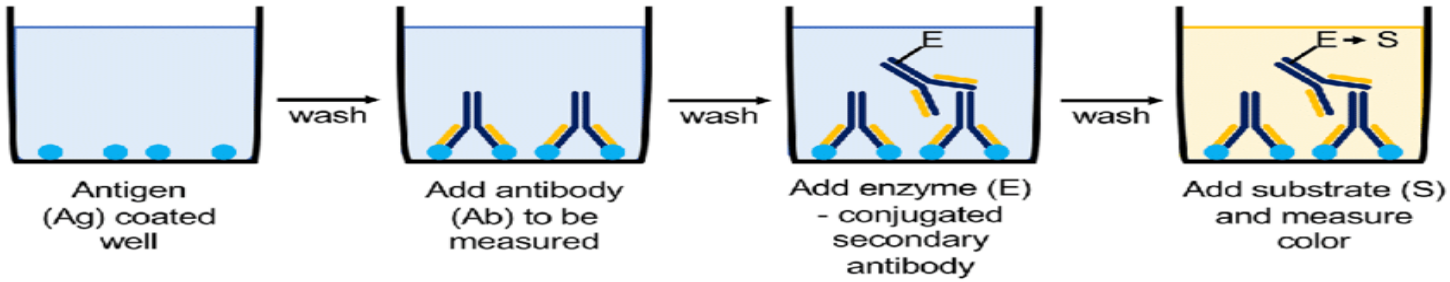
Types of ELISA



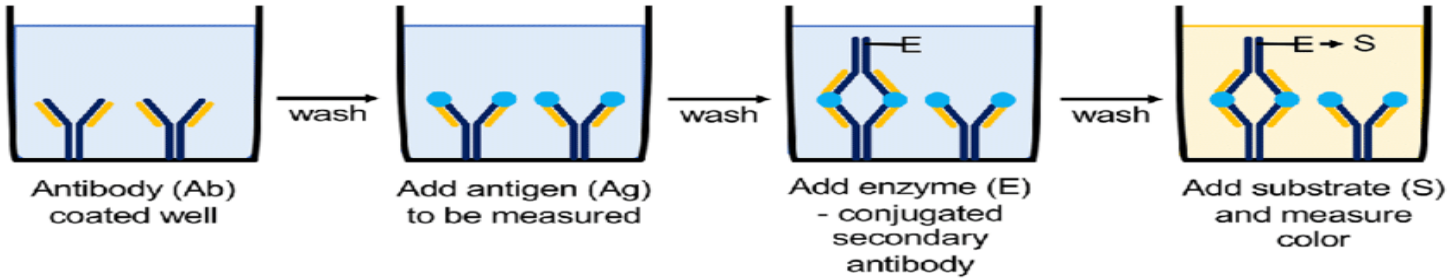
(a) Direct ELISA



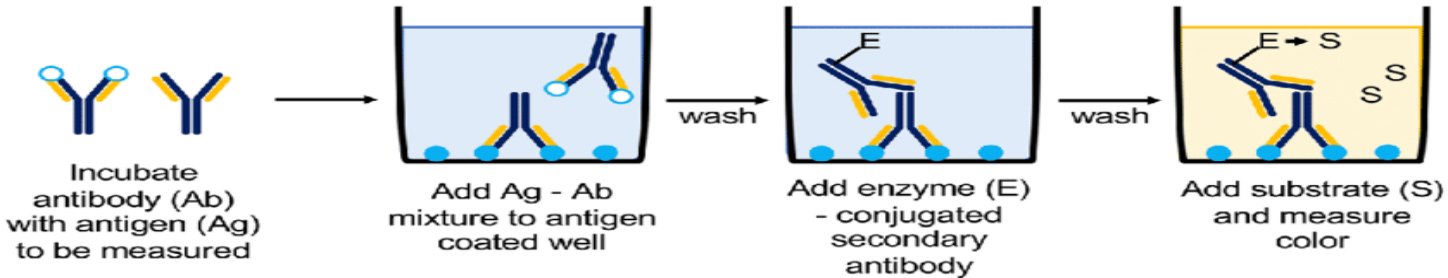
(b) Indirect ELISA



(c) Sandwich ELISA

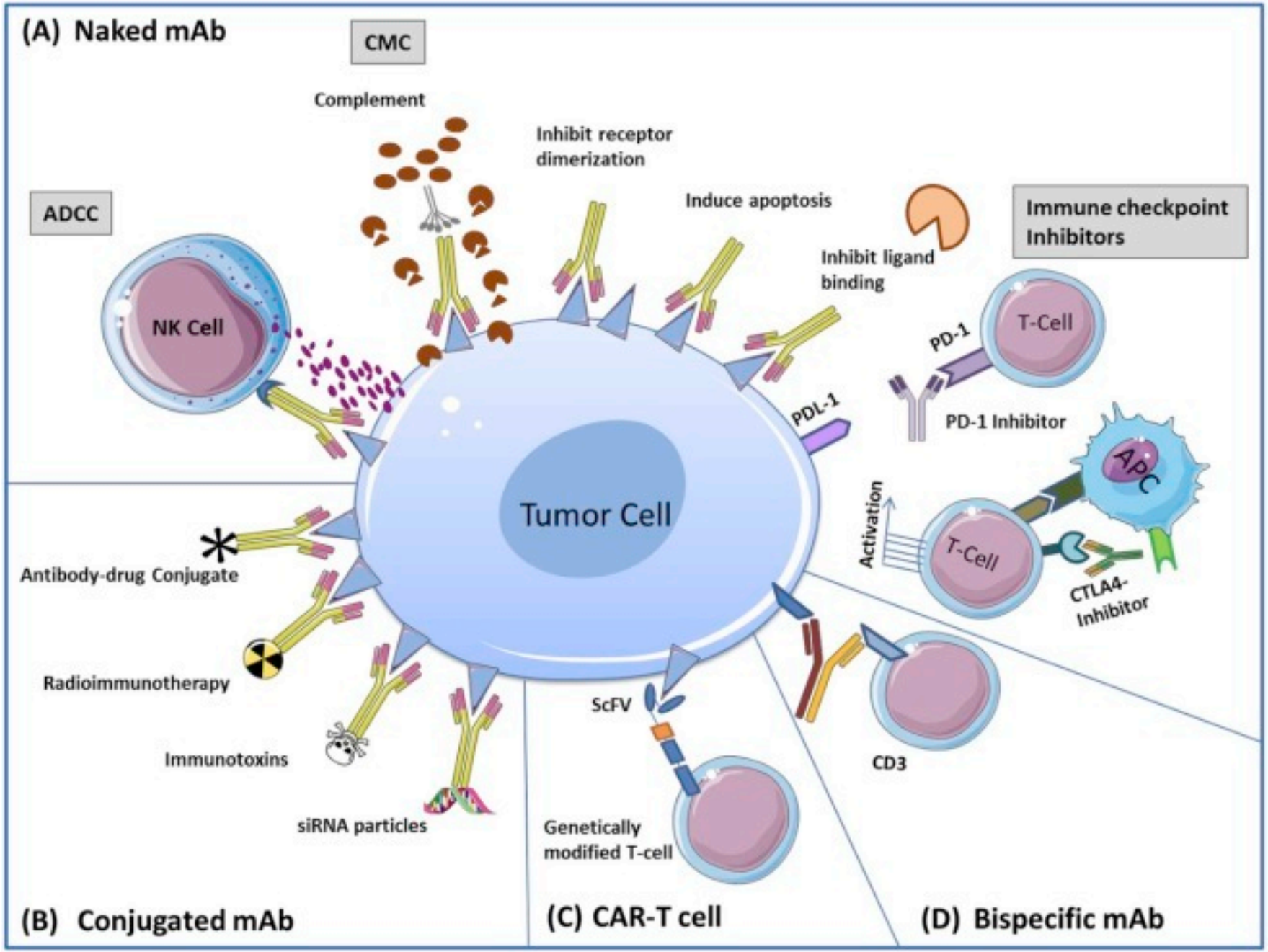


(d) Competitive ELISA



Clinical Uses for Monoclonal Antibodies

- *In vitro* diagnostic agents
- *In vivo* imaging agents
- Therapeutic agents (MAb works like a drug)
- Targeting agents (MAb linked to toxin)


(A) Naked mAb
CMC
Complement
Inhibit receptor dimerization
Induce apoptosis
ADCC
NK Cell
Inhibit ligand binding
Immune checkpoint Inhibitors
PD-1
T-Cell
PDL-1
PD-1 Inhibitor
Tumor Cell
APC
Activation
T-Cell
CTLA4-Inhibitor
Antibody-drug Conjugate
Radioimmunotherapy
Immunotoxins
siRNA particles
ScFV
CD3
Genetically modified T-cell
(B) Conjugated mAb
(C) CAR-T cell
(D) Bispecific mAb