B cells activation & antibody production,

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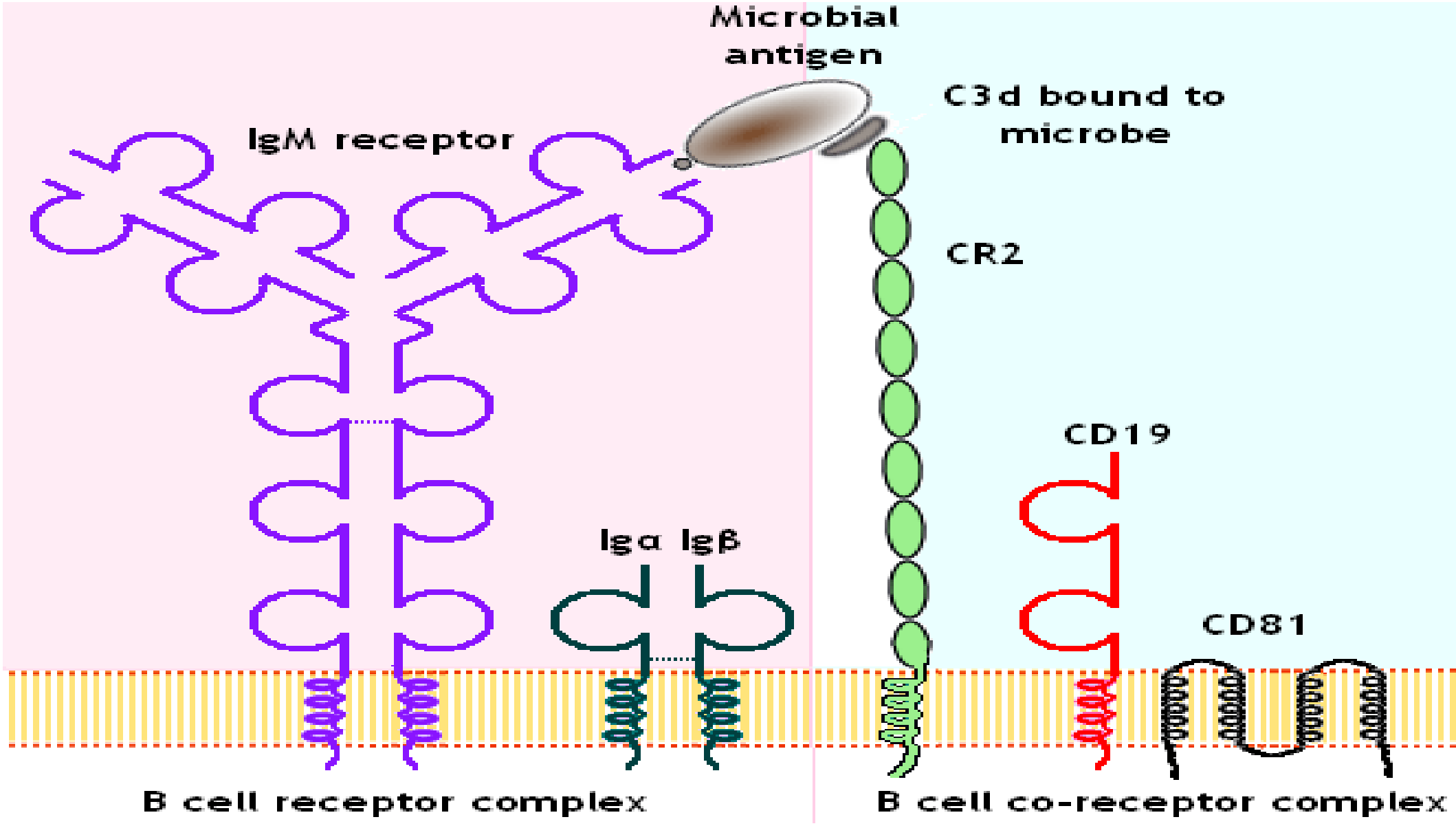
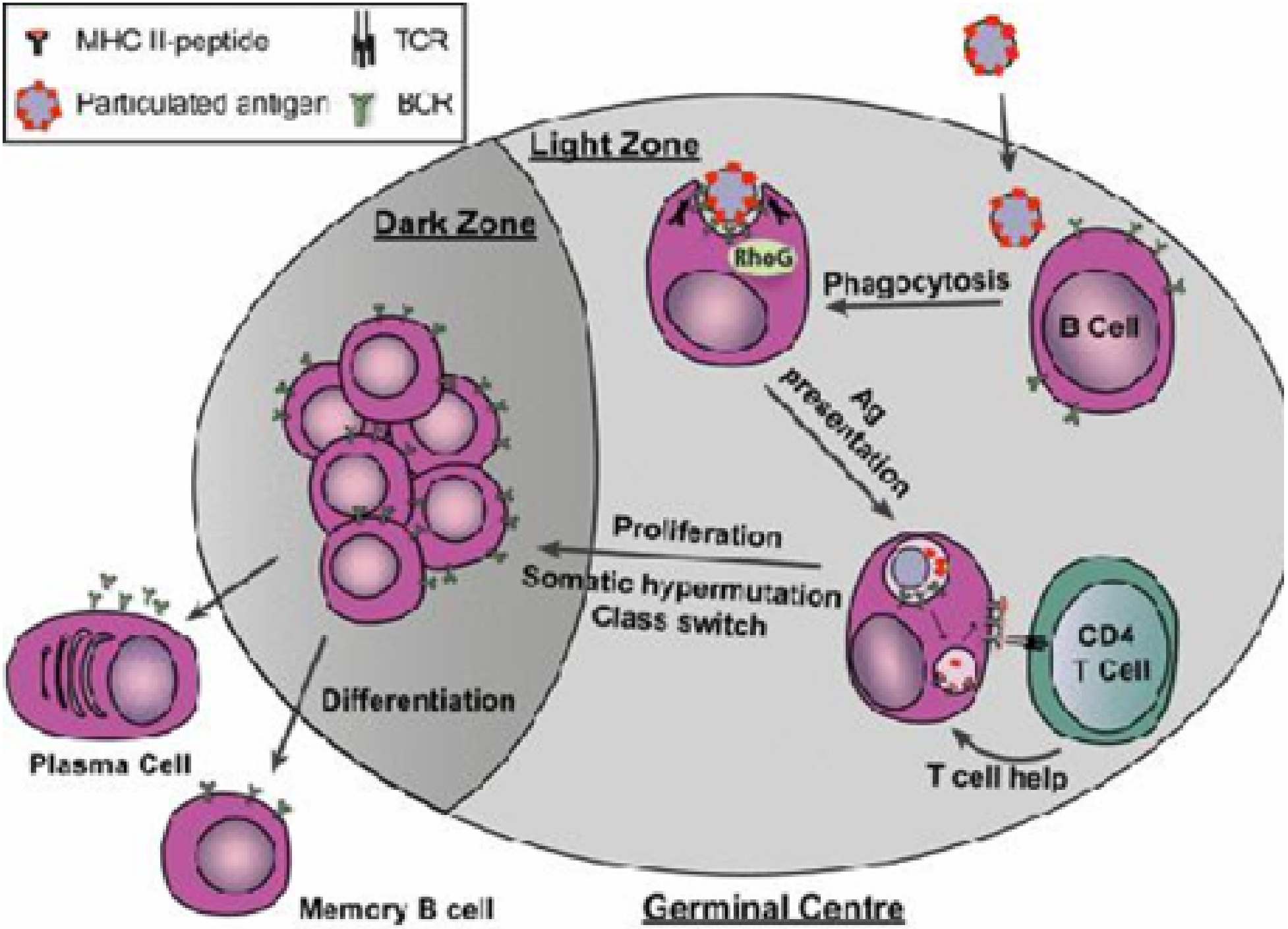
Antigen binding in B cells

- B cell (B2) bind Antigens (always proteins) then B cell activation is T cell dependent
- First, the antigen that is presented to B cells in follicle by follicular DC and bind BCR, it is generally in its intact, native conformation and is not processed by antigen-presenting cells,
- Second, the antigen carry C3d that bind another receptor on B cell (CR2).
- third, the receptor internalizes the bound antigen into endosomal vesicles, and if the antigen is a protein, it is processed into peptides that is presented on the B cell surface for recognition by helper T cells (B cell is antigen presenting cell to Th).


Microbial antigen
C3d bound to microbe
IgM receptor
CR2
CD19
Igα Igβ
CD81
B cell receptor complex
B cell co-receptor complex

T- dependent (TD) B cell activation Humoral immune response

- Activation of B cells by antigen results in increased expression of
  - class II major histocompatibility complex (MHC) molecules and B7 costimulators. To bind their ligands on Th
  - express the receptor CD40 which engage CD40 ligand (CD40L), on T cells (needed for isotype switch).
  - Increase in cytokine receptors on activated B cells
- Helper T cell–dependent B cell activation require initial activation of naive T cells by same antigen as B cell in the T cell zones.
- The activated lymphocytes migrate toward one another and interact at the edges of follicles, where the B cells present the antigen to helper T cells..
- Bidirectional activation (Th activation result in Th2 cells and B cell activation result in plasma cells)


MHC II-peptide
TCR
Particulated antigen
BCR
Light Zone
Dark Zone
RheG
Phagocytosis
B Cell
Ag presentation
Proliferation
Somatic hypermutation
Class switch
CD4 T Cell
T cell help
Differentiation
Plasma Cell
Memory B cell
Germinal Centre

In germinal center

--activated B cells by T cells migrate to germinal centers,

- The proliferation of each B cell in response to one antigen result in one clone of cells with receptors of identical specificities.

-Then B cells differentiate into antibody secreting plasma cells by switching membrane form IgM to secreting IgM,

- -B cell performs immunoglobulin isotype switching secreting different types of antibodies

-Differentiation to memory B cells

-Then increase affinity of produced antibody by Somatic hyper mutation ;

Isotype switch

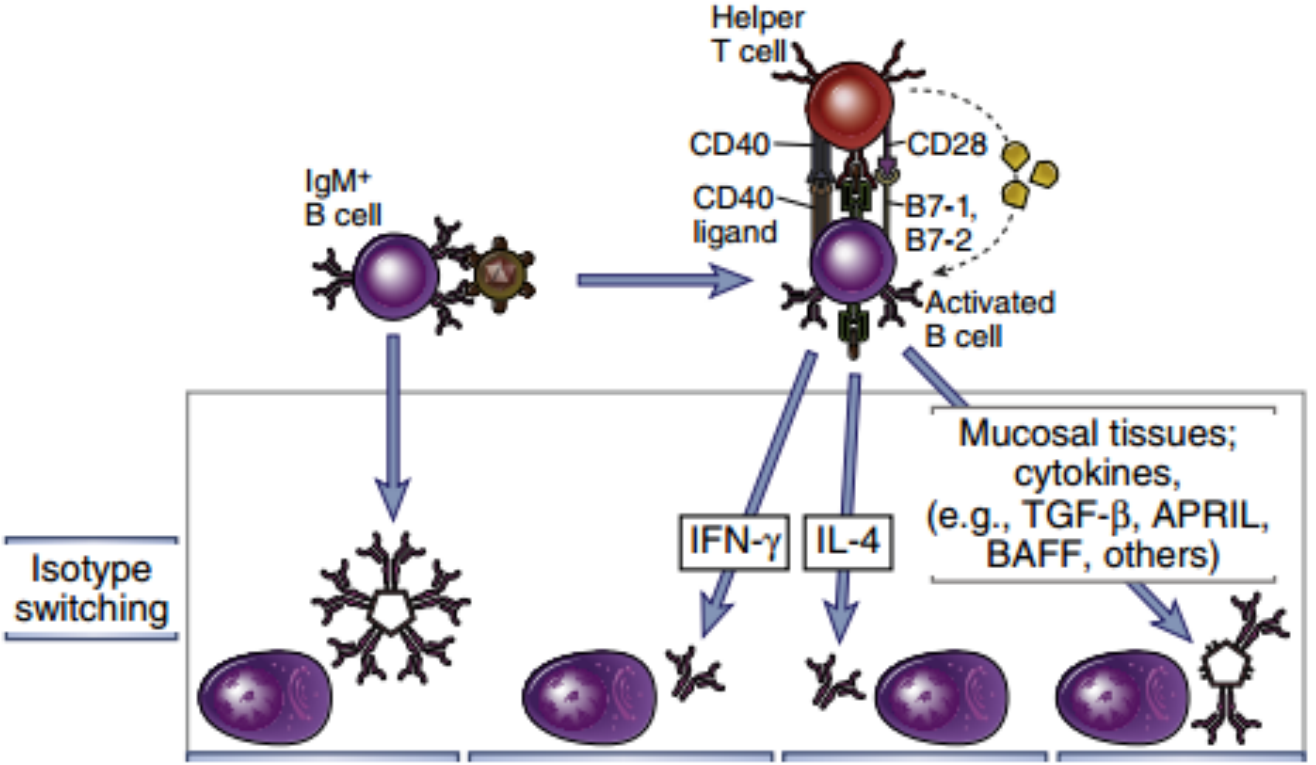
During B cell development in BM, naïve B cells receptors (IGM) formed by combining of Cμ to the V-D-J of the heavy chains;

where as in activated B cells on germinal center, isotype switch happen to other antibody isotypes, by combining other constant as Cγ for IGG, Cα for IGA and Cε for IGE to the variable part of heavy chain. but the specificity of the antibodies (which is determined by the variable regions) remains unaltered

- The molecular mechanism of isotype switching is a process called DNA recombination (just change constant and keep variable by allelic exclusion), in which B cells change the isotypes of the antibodies they produce
- The key enzyme required for isotype switching (and affinity maturation) is activation induced cytidine deaminase (AID)
- Deficiencies of AID underlie some forms of the hyper-IgM syndrome
- CD40 expression on B cells and its binding to CD40L on Th work to induce isotype switching.
- Mutations in the CD40L gene result in a disease called the X-linked hyper-IgM syndrome, which is characterized by defects in antibody production,

Isotype determinants

- Isotype switching in response to different types of microbes needs
  - protein antigens
  - T-dependent B cell activation.
- In addition, B cells in different anatomic sites switch to different isotypes. Specifically, B cells in mucosal tissues and secretory glands switch to IgA,
- a prior history of antigen exposure, first exposure more IGM, 2nd more IGG
- Microbe type
  - The response to most viruses and bacteria involves the production of IgG antibodies
  - The humoral response to many helminthic parasites and allergens is mainly driven by IgE antibodies,



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.

Somatic hyper mutation

- Or Affinity maturation is the process that leads to increased affinity of produced antibodies,
- In proliferating germinal center B cells, Ig V genes undergo point mutations at an extremely high rate (hypermutation) to produce high affinity Antibody (binding strength) (no change on specificity). For this reason, by increase duration of infection or repeated infections the produced antibody becomes more strong and specific
- cells exposed to antigens on follicular dendritic cells ,B cells producing high affinity Ab proliferate and become (antibody secretors) plasma cells and (non antibody secretors) memory B cells. While cells producing low affinity Ab die. This is called selection.

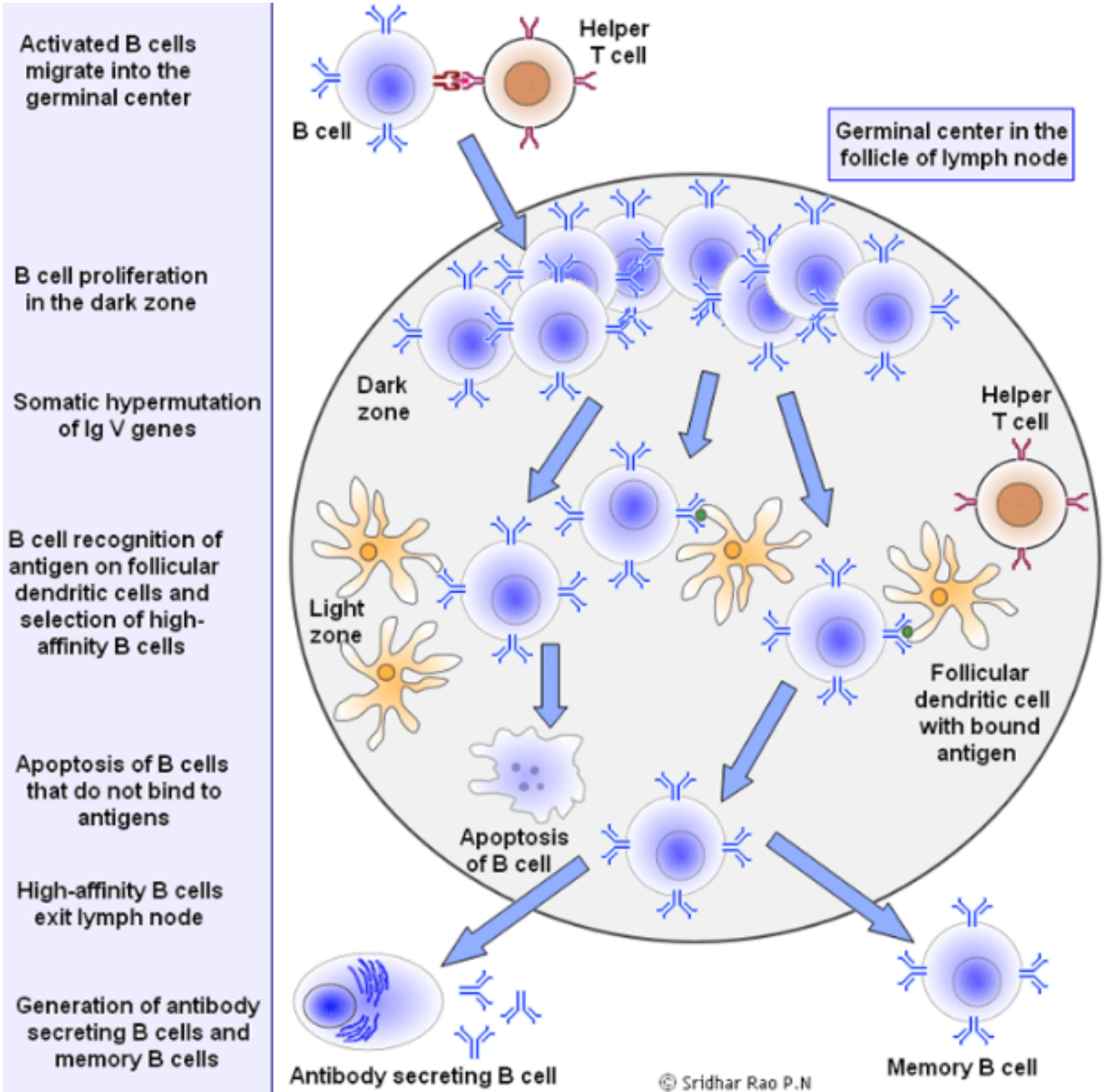

Activated B cells migrate into the germinal center
B cell proliferation in the dark zone
Somatic hypermutation of Ig V genes
B cell recognition of antigen on follicular dendritic cells and selection of high-affinity B cells
Apoptosis of B cells that do not bind to antigens
High-affinity B cells exit lymph node
Generation of antibody secreting B cells and memory B cells
B cell
Helper T cell
Germinal center in the follicle of lymph node
Dark zone
Light zone
Helper T cell
Follicular dendritic cell with bound antigen
Apoptosis of B cell
Antibody secreting B cell
Memory B cell
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TABLE 11-2 Properties of thymus-dependent and thymus-independent antigens

Property	TD antigens	TI antigens	
		Type 1	Type 2
Chemical nature	Soluble protein	Bacterial cell- wall components (e.g., LPS)	Polymeric protein antigens; capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No

Table 11-2
Kuby IMMUNOLOGY, Sixth Edition

Plasma cells

- Long-lived plasma cells are generated in T-dependent germinal center responses to protein antigens.
- they maintain antibody production for decades or even for the lifetime of an individual, and, unlike B cells, Long-lived plasma cells do not need antigen restimulation to generate antibodies
- Signals from the B cell antigen receptor and IL-21 cooperate in the generation of plasma cells; plasma cells are identified as antibody- secreting cells that do not express CD20, a marker of mature B cells.
- Some of (plasma cells) generated in germinal centers stay in medulla of secondary LN. some enter the circulation and home to the bone marrow
- short-lived plasma cells are rapidly formed in secondary lymphoid organs, where they undergo apoptosis after a few days

Memory B cells

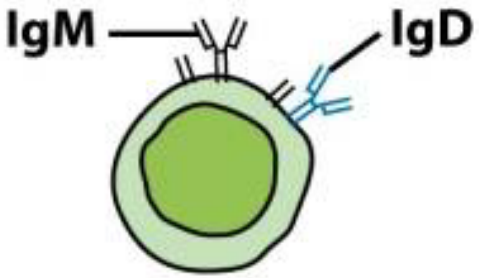
- B cells activated only in a T-dependent manner may differentiate into memory cells.
- These memory B cells survive in a resting state in peripheral lymph nodes or in bone marrow without secreting antibodies for many years, but they mount rapid responses on subsequent encounters with the same antigen (secondary immune response)
- high levels of the anti-apoptotic protein Bcl-2, which contributes to their long life span, high in CD27 protein

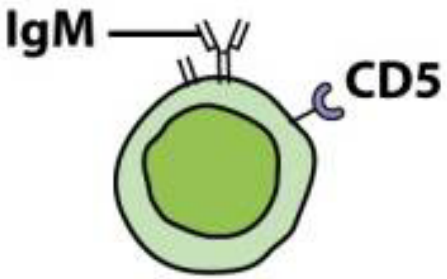
Memory B cells

- Infections or Effective vaccines against microbes and microbial toxins must induce long lived plasma cells and memory B cell formation, and these events will occur only if the helper T cells activated.
- This concept has been applied to the design of vaccines for some bacterial infections in which the target antigen is a capsular polysaccharide or hapten, which is incapable of stimulating T cells.
- In these cases, the polysaccharide is covalently linked to a foreign protein to form the equivalent of a conjugate, which does activate helper T cells. Such vaccines, which are called conjugate

B1 cells (CD5+ B cells)

- B-1 cells 5-10% of blood B cells naturally found from fetal life
- B1 cells response to non-protein antigens (T-independent antigens) with repeating determinants, such as polysaccharides, some lipids, and nucleic acids. These cells are self renewing, present in the peritoneum and in mucosal sites.
- do not require antigen-specific helper T lymphocytes.
- Their responses are elicited by engagement of the B cell receptor (BCR) with the antigen and by activation of Toll-like receptors (TLRs) on B cells by molecules [PAMPs]) derived from the microbe
- Some activated B cells differentiate into short-lived antibody-secreting plasma cells, do not do isotype switch or do affinity maturation
- some antibody switch to IGM, IGA and IGG2 occurs





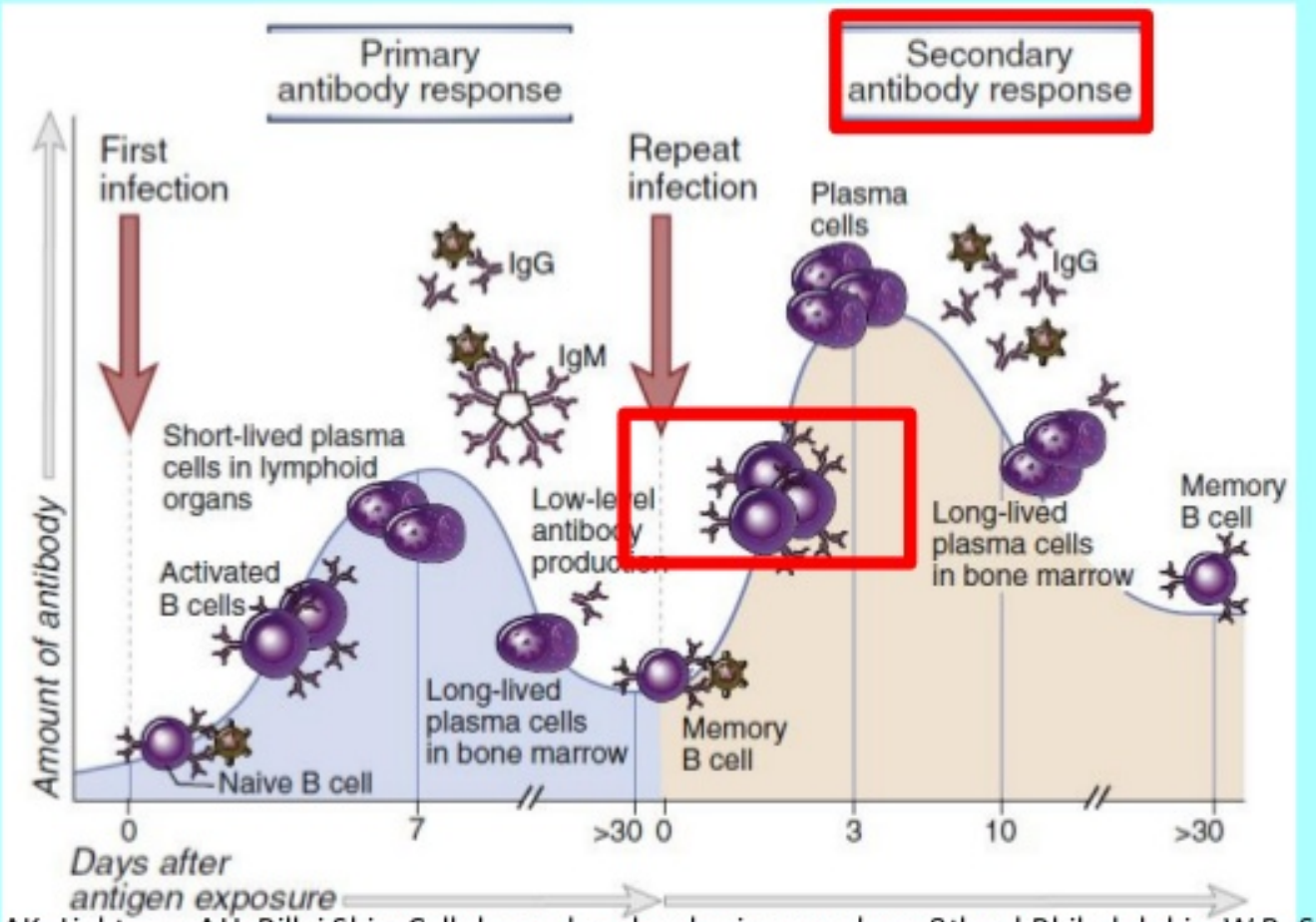
Attribute	Conventional B cells (B-2 B cells)	B-1 B cells
Major sites	Secondary lymphoid organs	Peritoneal and pleural cavities
Source of new B cells	From precursors in bone marrow	Self-renewing (division of existing B-1 cells)
V-region diversity	Highly diverse	Restricted diversity
Somatic hypermutation	Yes	No
Requirements for T-cell help	Yes	No
Isotypes produced	High levels of IgG	High levels of IgM
Response to carbohydrate antigens	Possibly	Definitely
Response to protein antigens	Definitely	Possibly
Memory	Yes	Very little or none
Surface IgD on mature B cells	Present on naive B cells	Little or none

Figure 11-5
Kuby IMMUNOLOGY, Sixth Edition

Primary and secondary immune response

- Primary and secondary antibody responses to protein antigens differ qualitatively and quantitatively.
- Primary responses result from the activation of previously unstimulated naive B (production of IGM more) and T cells, whereas secondary responses are due to the stimulation of memory B and T cells.
- Therefore, the secondary response develops more rapidly than does the primary response, and larger amounts of antibodies (more IGG) are produced in the secondary response. isotype switching and affinity maturation also increase with repeated exposure to protein antigens.
- (studies showed that IgG+ memory B cells preferentially generate plasma cells, whereas IgM+ cells re-initiate germinal center reactions).

FIGURE 12-2 .Primary and secondary humoral immune responses.



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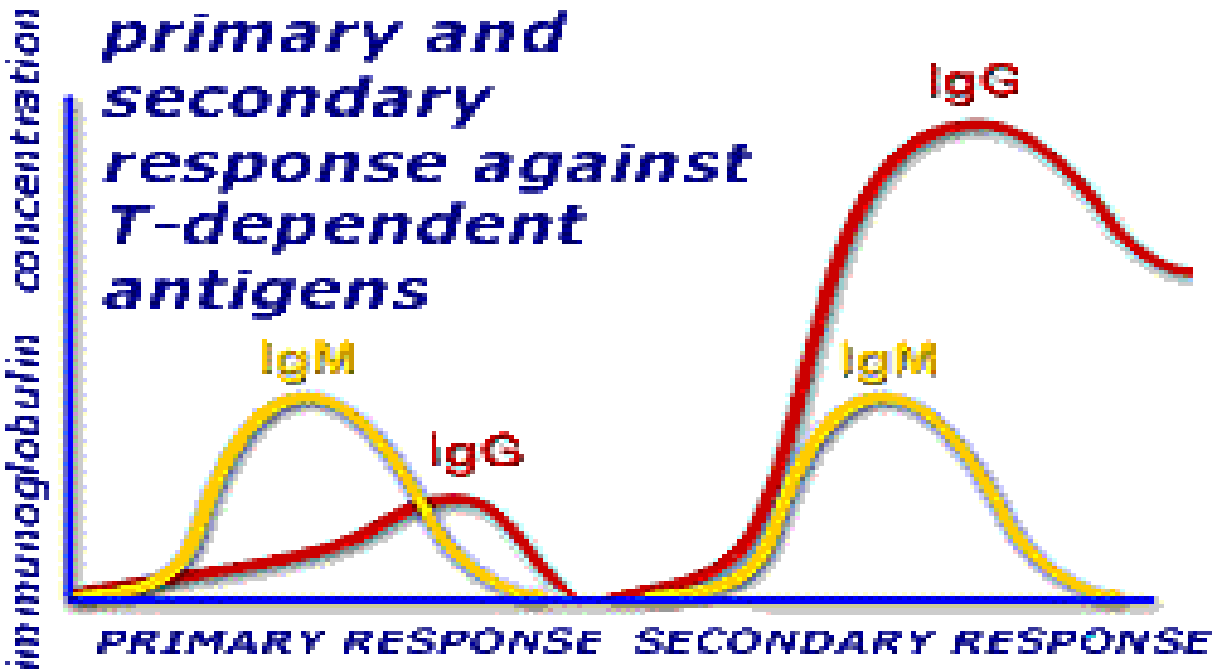

primary and secondary response against T-dependent antigens
immunoglobulin concentration
IgM
IgG
IgG
IgM
PRIMARY RESPONSE
SECONDARY RESPONSE

Difference Between Primary Response and Secondary Response.

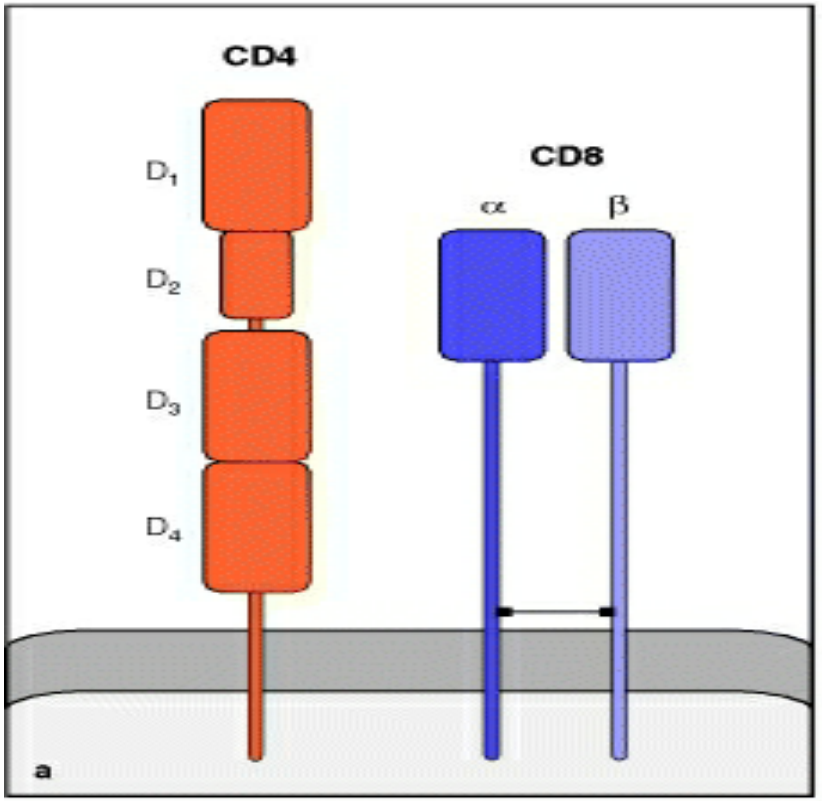
	Primary Response	Secondary Response
Exposure to antigen	first exposure to a specific antigen	*after second exposure to the same a*ntigen
Time of onset	1-week delay	Within hours
Strength	weak potency	more potent
Duration	Short life , for only a few weeks	forms antibodies for many months
Type of antibody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

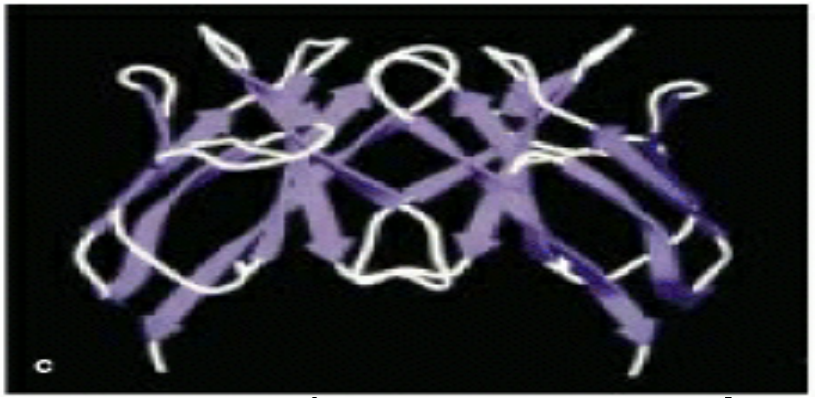
Factors influencing the Strength binding of the T Cell and B Cell receptor

- Antigen binding increase by (First signal for cells activation)
  - Receptor binding to antigen
  - Coreceptors binding and transmembrane signaling protein
  - Coreceptor is protein on surface of cell bind with the antigen at the same time as receptor
    - T cells coreceptors are the CD4 and CD8 proteins that demarcate two functionally distinct subsets (Th or Tc respectively). CD8 and CD4 interact with class I and class II MHC molecules, respectively.

- B cell co-receptor, CR2 (CD21) is expressed on mature B cells as a complex (TAPA-1) with two other membrane proteins, CD19 and CD81. The CR2-CD19-CD81 complex is often called the B cell coreceptor complex. CD21 bind complement proteins C3d on the microbe, C19 transduces the signal and CD81 stabilizes both molecules
- CD3 and zeta chain that do signal transduction to inside T cells
- immunoglobulin alpha and beta beside

CD4 and CD8





- Cluster of differentiation (CD) are proteins expressed on T cells (CD4 or CD8) have a role in binding the MHC and used to differentiate the cells by binding to monoclonal antibodies. CD8 T cells are Tc, CD4 T cell is Th1 or Th2

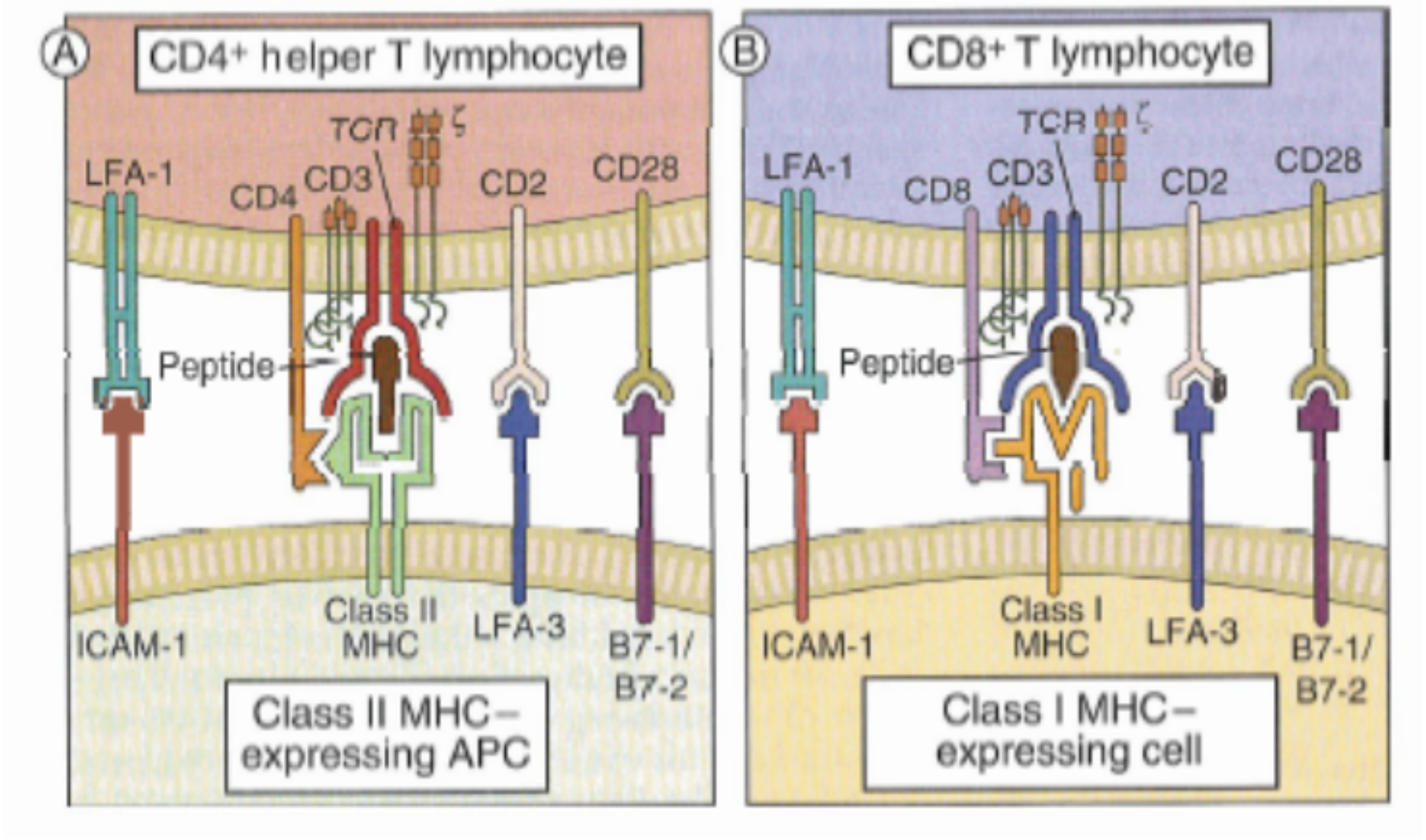
Costimulatory receptors on T cells

- Provide so-called second signals for lymphocytes and ensure that immune responses are optimally triggered by infectious pathogens.
  - CD28, the earliest accessory molecules induce signaling after TCR CD4/8 binding to MHC and antigen. when it bind B7 on APC, it initiate T cell proliferation by expression of IL-2 cytokine and its receptor.
  - CD28 is replaced by CTLA-4 on T cell when the antigen is cleared So that T cell is regulated, lead to T cell death.
  - CD2 is a glycoprotein present on more than 90% of mature T cells, and on NK cells. The principal ligand for CD2 in humans is a molecule called leukocyte function associated antigen 3 (LFA-3, or CD58), CD2 functions as a signal transducer

The immunologic synapse.

- When the TCR complex recognizes MHC-associated peptides on an APC, several T cell surface proteins and intracellular signaling molecules are rapidly mobilized to the site of T cell–APC contact
- This region of physical contact between the T cell and the APC is called an immunologic synapse or a supramolecular activation cluster (SMAC).
- The T cell molecules that are rapidly mobilized to the center of the synapse include the TCR complex (the TCR, CD3, and ζ chains), CD4 or CD8 coreceptors, receptors for costimulators (such as CD28), enzymes, and adaptor proteins

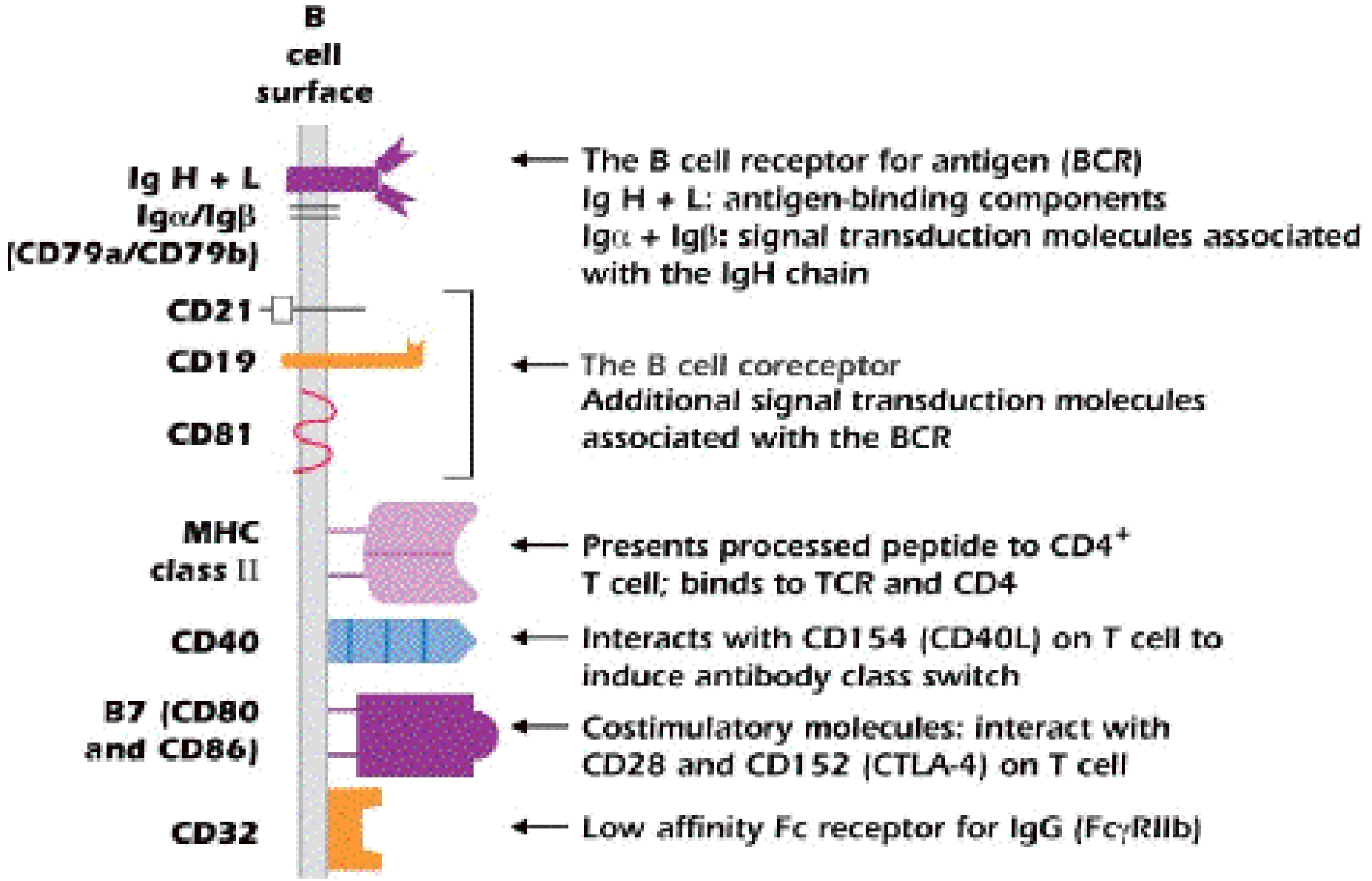
The immunologic synapse.



Costimulatory receptors on B cells

- B7-1 (CD80) and B7-2 (CD86), ligands on B cells and antigen presenting cells (APCs), they bind CD28 on Th cells (signal 2) in T-B cell binding lead to B cell activation
- CD40 is a glycoprotein present on B cells and bind CD40L on T cells. Lead to B cell activation and isotype switch,

BCR and co-stimulatory molecules



Inhibition of B cells

- Secreted antibodies (IGG) inhibit continuing B cell activation by binding to inhibitory CD32 (FcγRIIB) inhibitory receptor on B cells (negative feed back)
- A polymorphism in the FcγRIIB gene has been linked to susceptibility to the autoimmune disease systemic lupus erythematosus (SLE)
- B cells express another inhibitory receptor called CD22,