B cells activation & antibody production,

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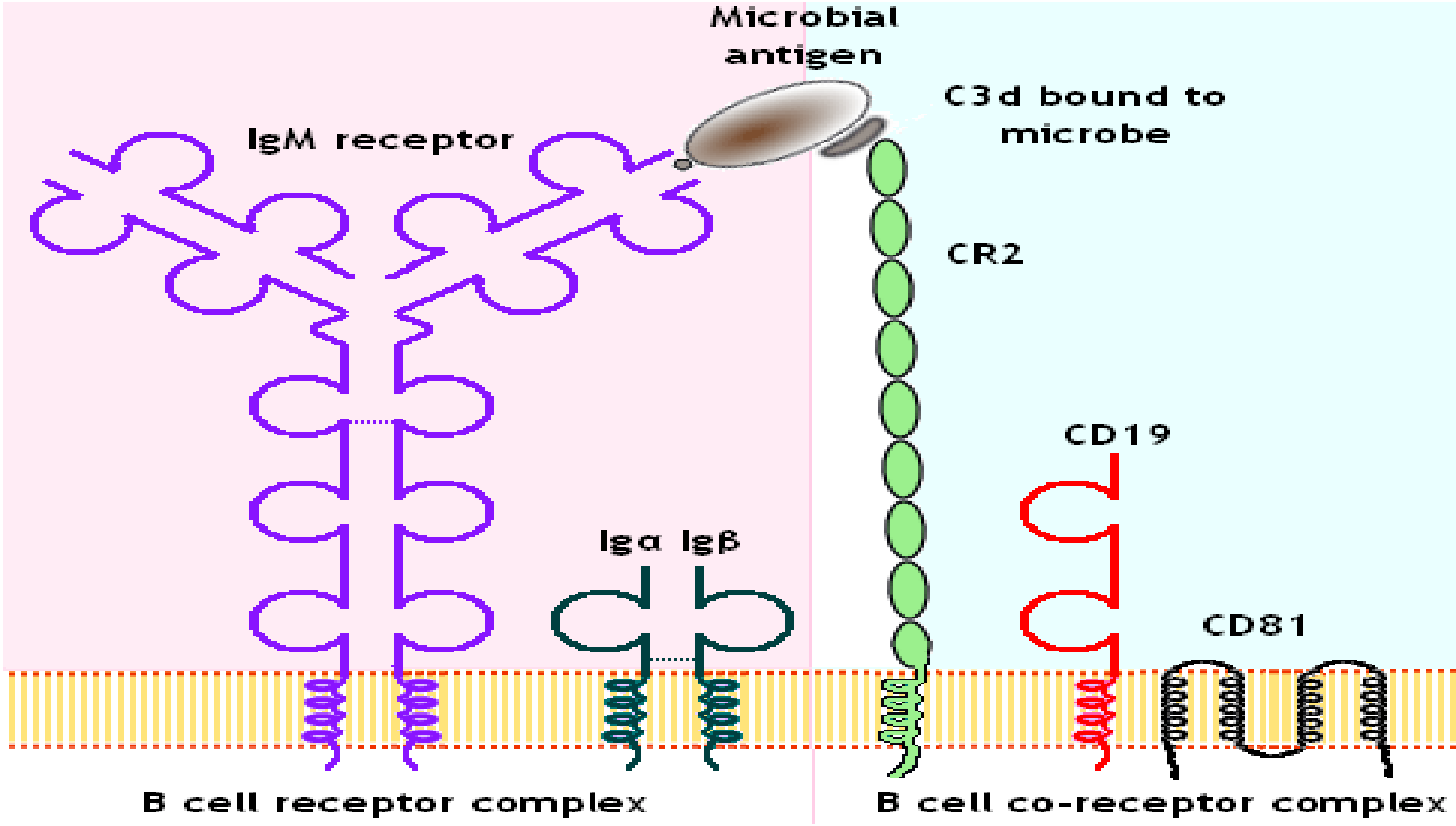
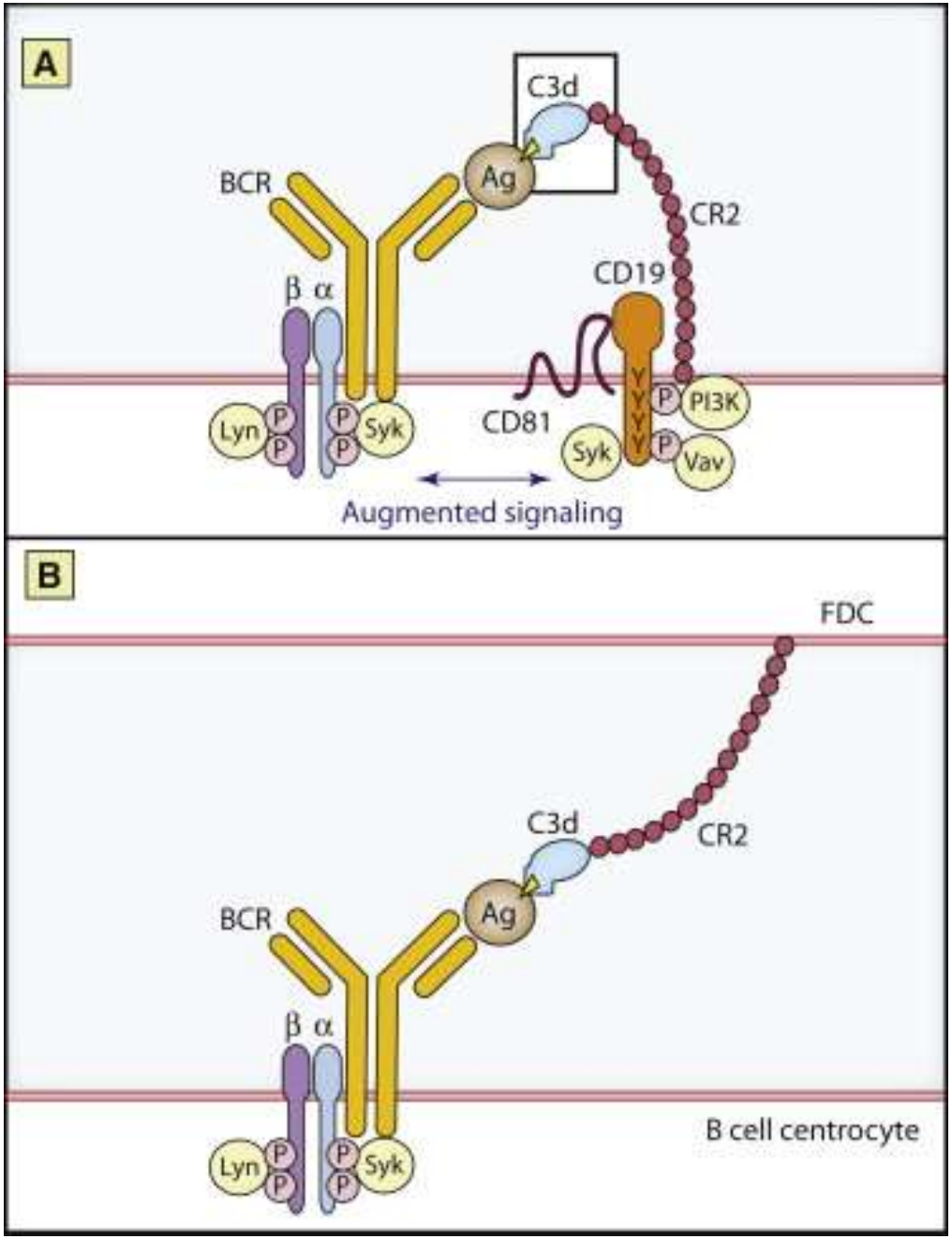
Antigen binding in B cells

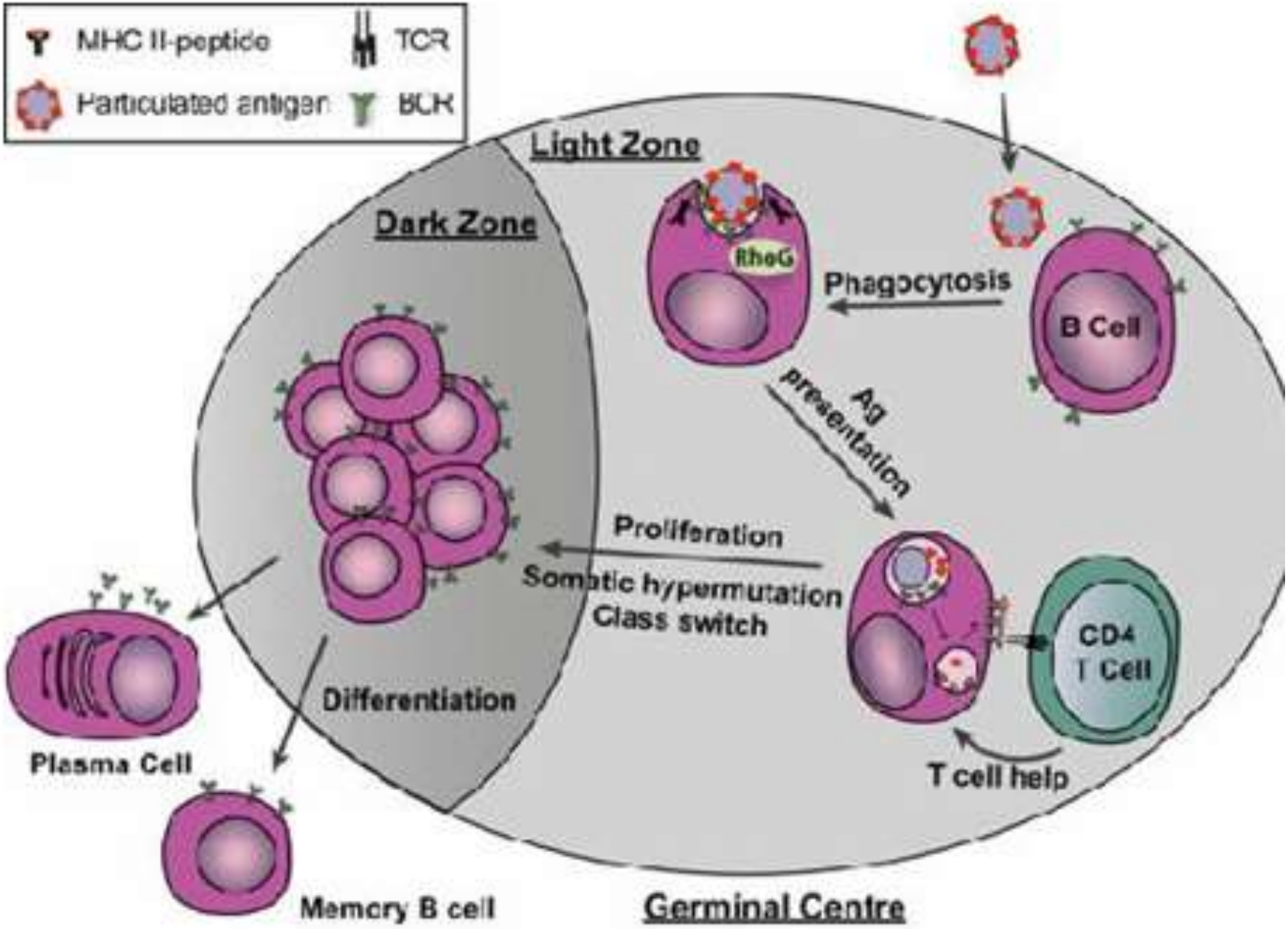
- B cell bind Antigens (always proteins) then B cell activation is T cell dependent
- First, the antigen that is presented to B cells in follicle by follicular DC and bind BCR, it is generally in its intact, native conformation and is not processed by antigen-presenting cells,
- Second, the antigen carry 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.


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


A
C3d
Ag
BCR
CR2
CD19
β α
CD81
Lyn
P
Syk
PI3K
Vav
Augmented signaling
B
FDC
C3d
CR2
Ag
BCR
B cell centrocyte

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

- Helper T cell–dependent B cell responses to protein antigens require initial activation of naive T cells by same antigen 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..
- Activation of B cells by antigen and Th 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


MHC II-peptide
TCR
Particulated antigen
BCR
Light Zone
Dark Zone
RhoG
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 Ig to secreting Ig,

- Differentiation to memory cells

-and at the same time B cell performs immunoglobulin isotype switching

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

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 as in Bone marrow, 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)

- 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,

- Activation-induced cytidine deaminase (AID) plays a key role in both class switch and Somatic hyper mutation
- Deficiencies of AID underlie some forms of the hyper-IgM syndrome

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,

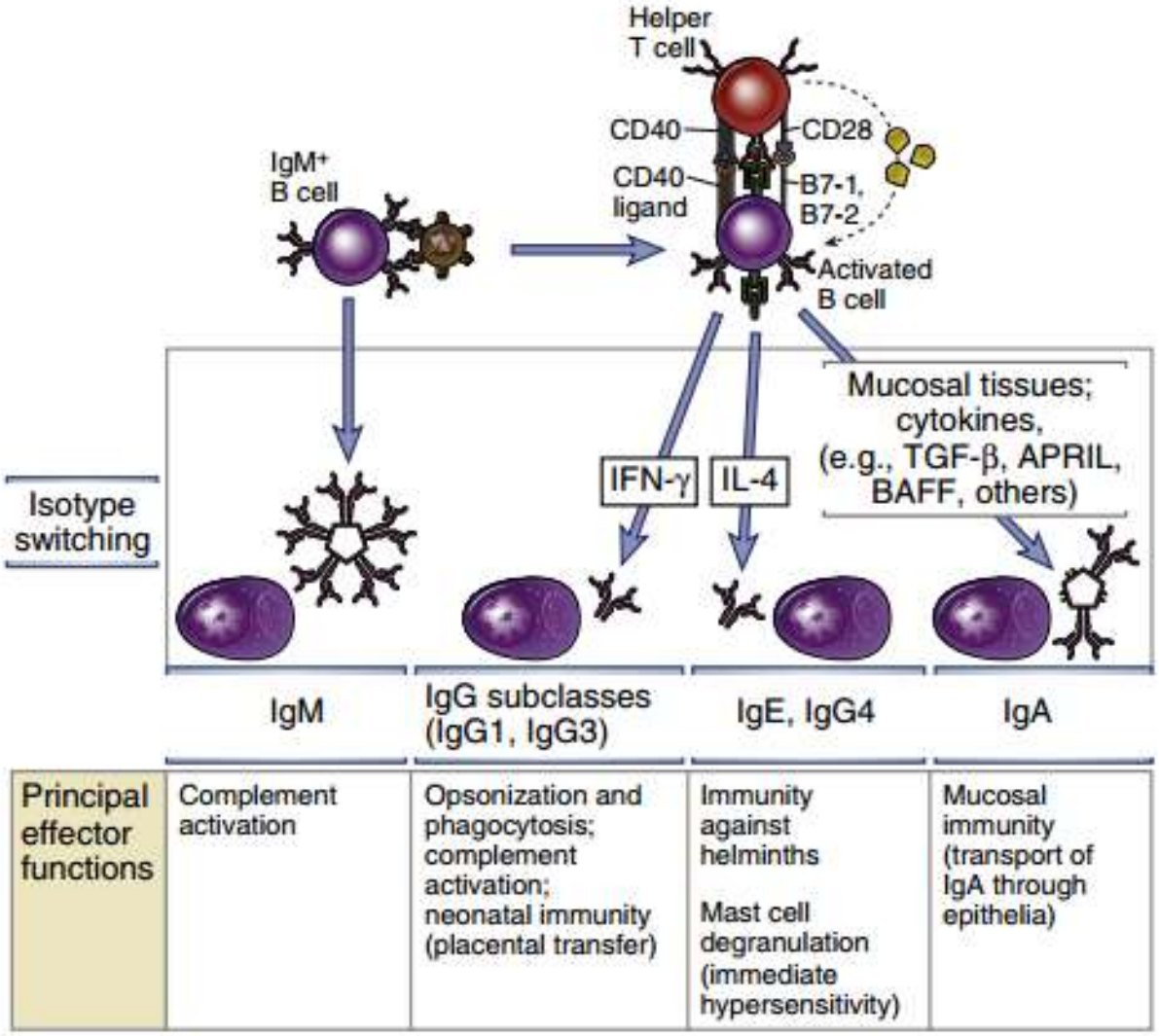


FIGURE 12-14 Ig heavy chain isotype switching. B cells activated by helper T cell signals (CD40L, cytokines) undergo switching to different Ig isotypes, which mediate distinct effector functions. Selected examples of switched isotypes are shown. The role of IFN-γ in directing specific isotype switching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

somatic hyper mutation

- Affinity maturation or somatic hyper mutation 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. For this reason, by increase duration of infection or repeated infections the produced antibody becomes more strong and specific

selection

- The antibodies produced have variable affinities (binding strength) to the antigen so that 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

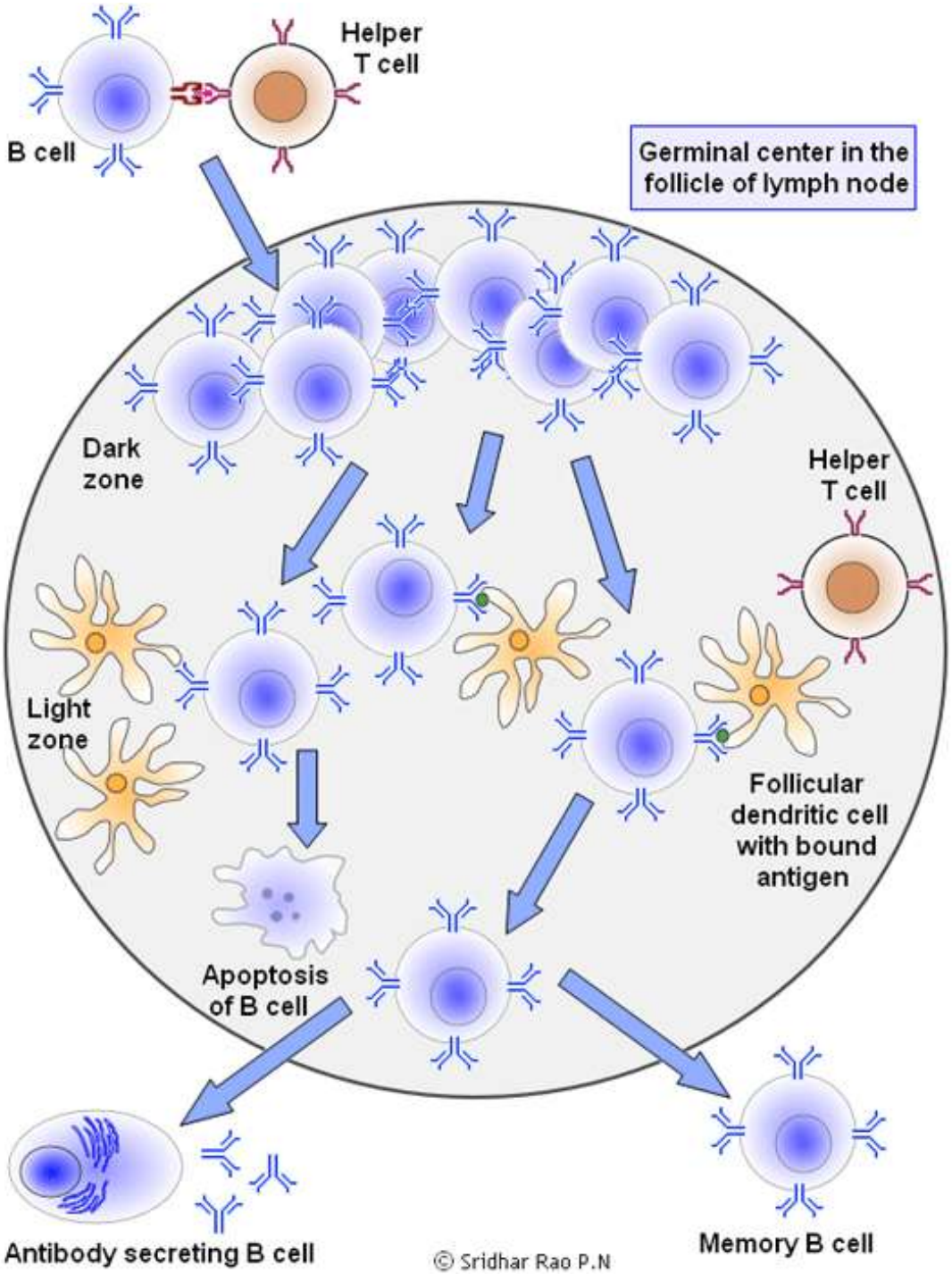


TABLE 11-2 Properties of thymus-dependent and thymus-independent antigens

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

- Short-lived plasma cells are generated during T-independent responses and early during T cell–dependent responses. These cells are generally found in secondary lymphoid organs and in peripheral tissues.
- Long-lived plasma cells are generated in T-dependent germinal center responses to protein antigens. 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 enter the circulation and home to the bone marrow where they differentiate into long-lived plasma cells. Some stay in medulla of secondary LN.

Memory 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 antigen.
- high levels of the anti-apoptotic protein Bcl-2, which contributes to their long life span
- Contribute to secondary immune response

Memory cells

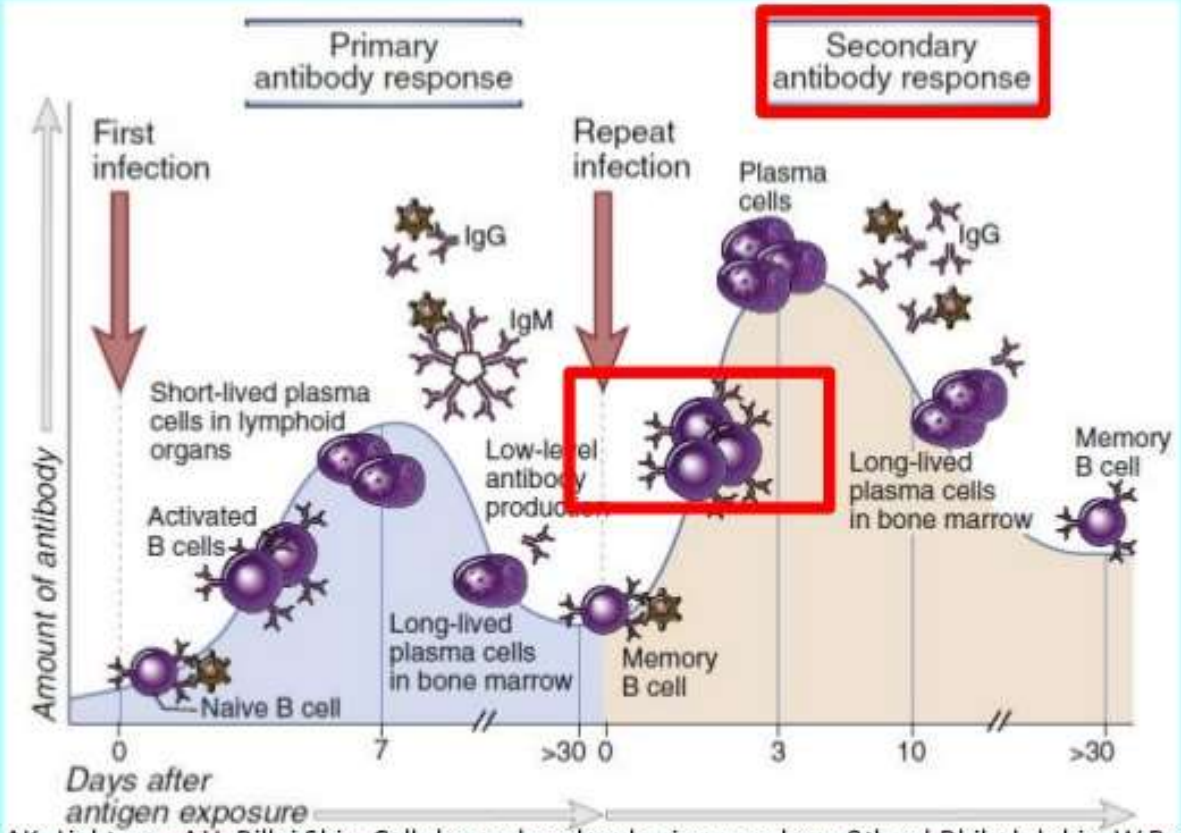
- Infections or Effective vaccines against microbes and microbial toxins must induce memory B cell formation, and these events will occur only if the helper T cells activated. This concept has been applied to the design of vaccines for some bacterial infections in which the target antigen is a capsular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

- B1 cells response to non-protein antigens with repeating determinants, such as polysaccharides, some lipids, and nucleic acids, do not require antigen-specific helper T lymphocytes. These antigens are therefore called T-independent antigens. These 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, some antibody switch to IGA and IGG2 occurs
- T cell independent Ags (LPS, lipids, nucleic acid and protein)

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 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 are produced in the secondary response. isotype switching and affinity maturation also increase with repeated exposure to protein antigens.
- Secondary immune response is mediated by memory cells

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



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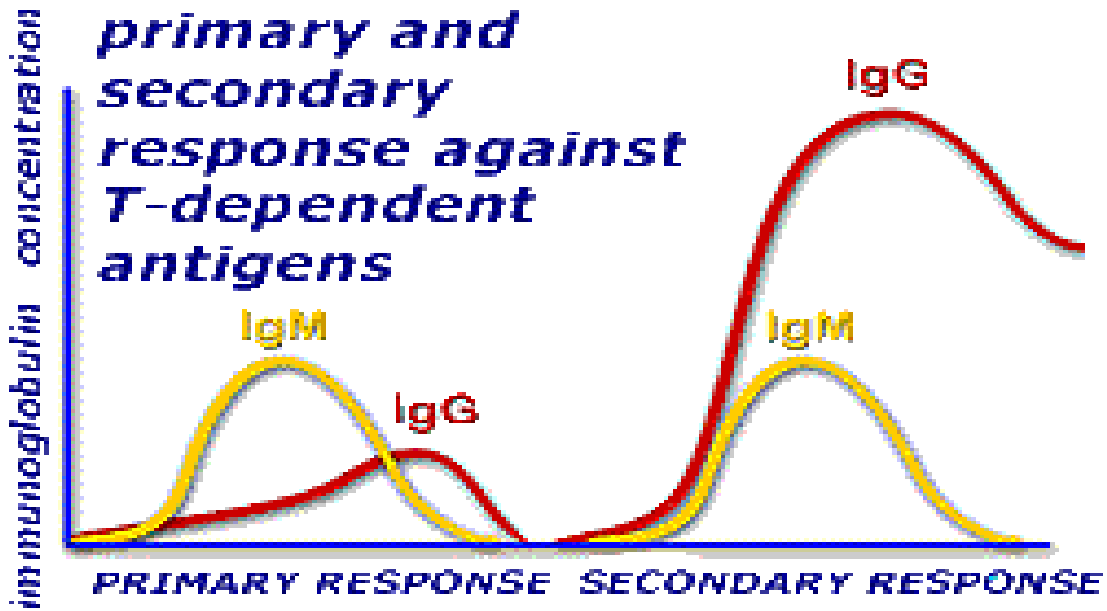

primary and secondary response against T-dependent antigens
immunoglobulin concentration
IgM
IgG
IgG
IgM
PRIMARY RESPONSE
SECONDARY RESPONSE

Difference Between Primary Response and Secondary Response.

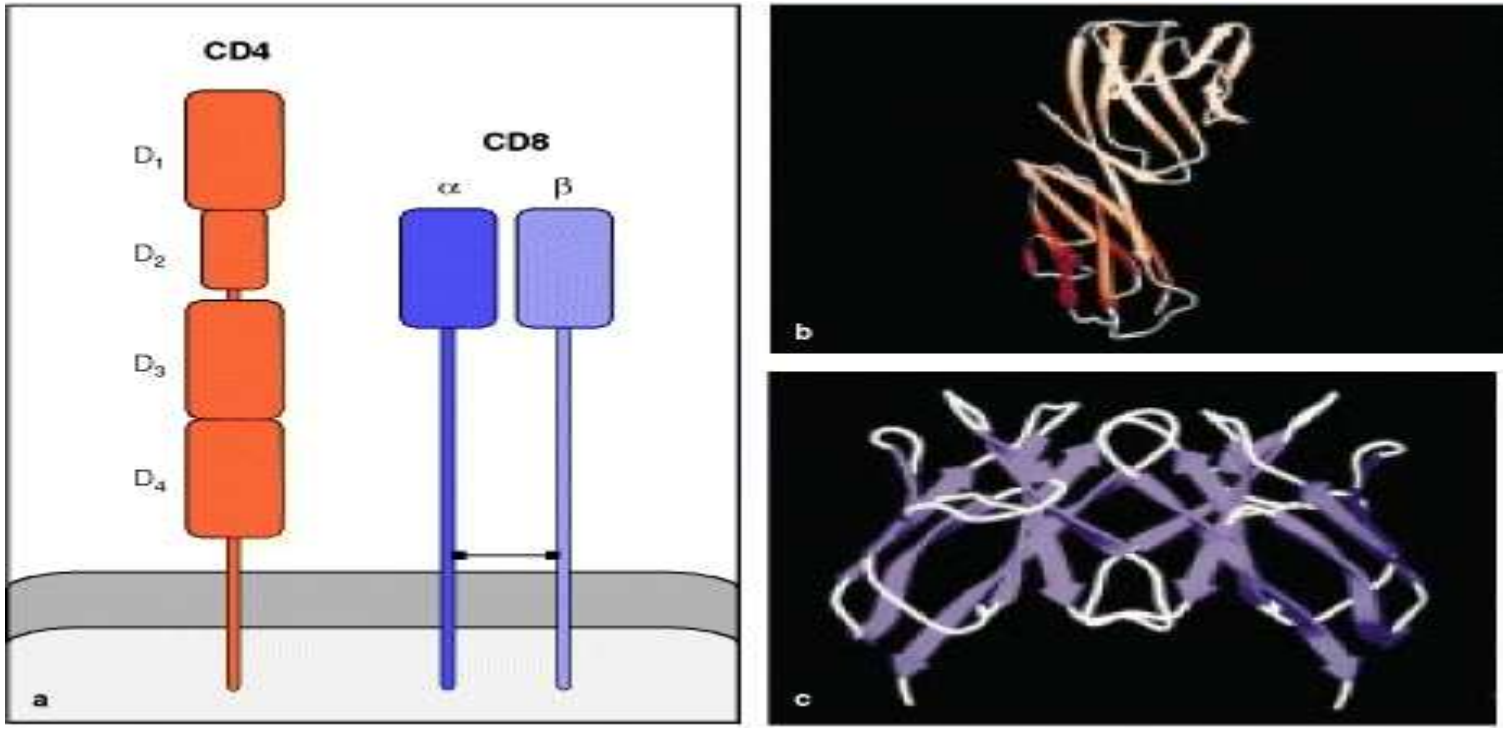
	Primary Response	Secondary Response
Exposure to antigen	first exposure to a specific antigen	*after second exposure to the same a*ntigen
Time of onset	1-week delay	Within hours
Strength	weak potency	more potent
Duration	Short life , for only a few weeks	forms antibodies for many months
Type of antibody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

Factors influencing the Strength binding of the TC and BC receptor

- Antigen binding increase by
  - Coreceptors binding; coreceptors are transmembrane signaling protein
    - 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.
    - These besides co-receptors CD3 and zeta chain beside TCR do signal transduction to inside T cells

- B cell co-receptor, CR2 (CD21) is expressed on mature B cells as a complex with two other membrane proteins, CD19 and CD81 (also called TAPA-1). The CR2-CD19-CD81 complex is often called the B cell coreceptor complex. CD21bind complement proteins C3d on the microbe, C19 transduces the signal and CD81 stabilizes both molecules
- immunoglobulin alpha and beta beside BCR are also co-receptor do signal transduction to inside B cells

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

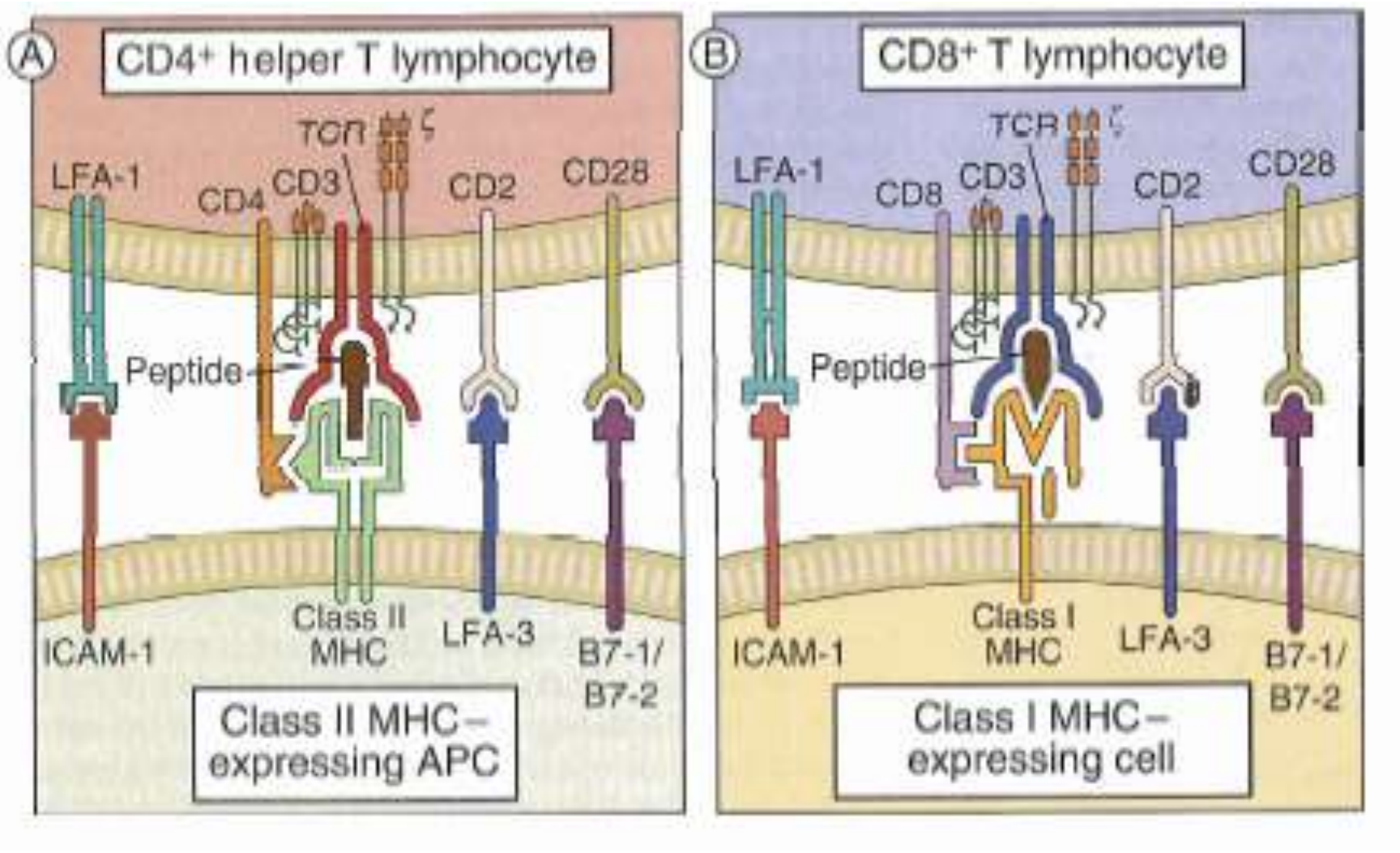
Costimulatory receptors on T cells

- provide so-called second signals for lymphocytes (antigen recognition provides the first signal) 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. it binds 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 that associate with the cytoplasmic tails of the transmembrane receptors.

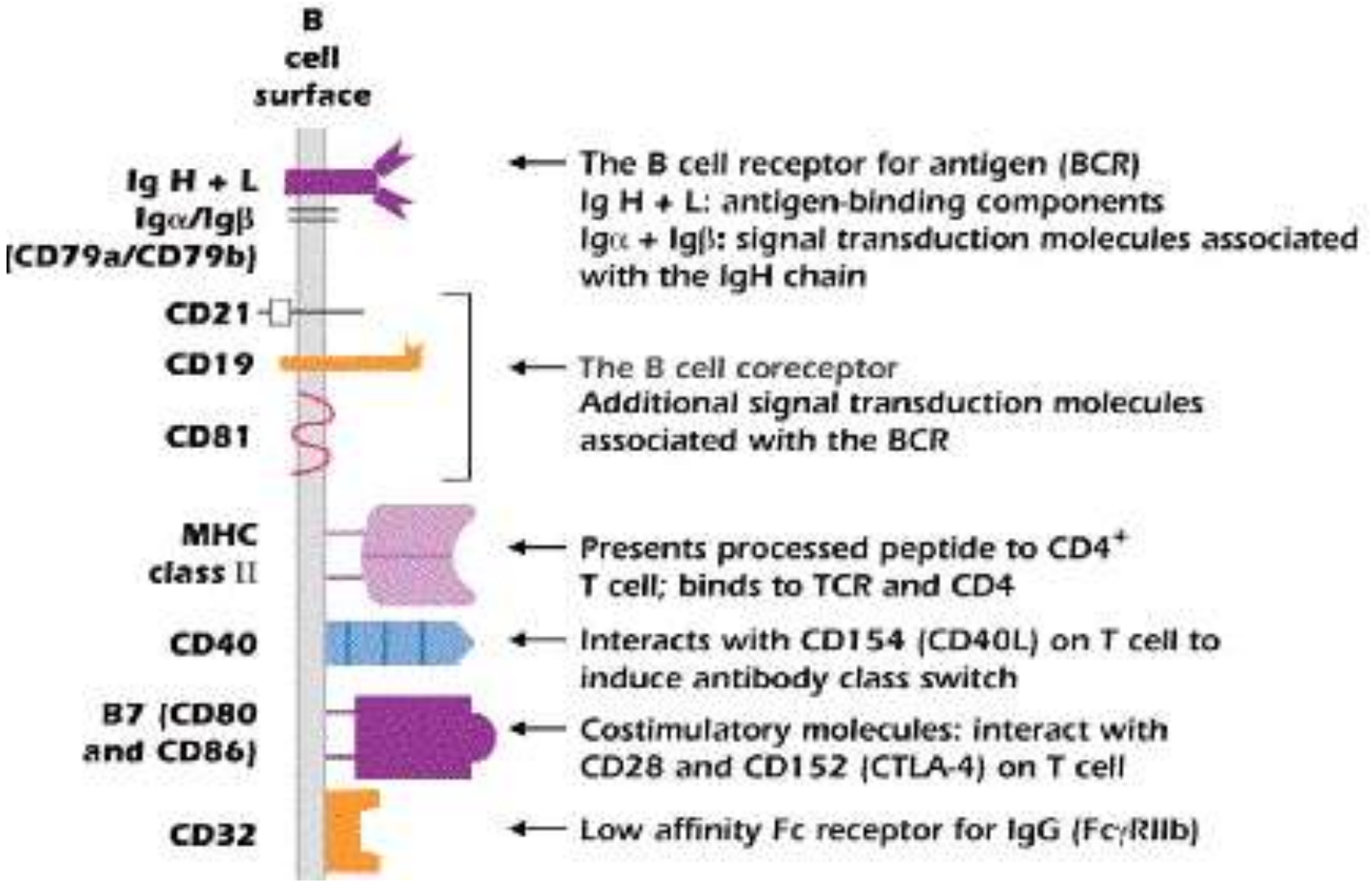
The immunologic synapse.



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)
- IgM antibodies (which activate complement) are involved in amplification

- 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,