Cells of Immunity – III

(Adaptive cells - Lymphocytes)

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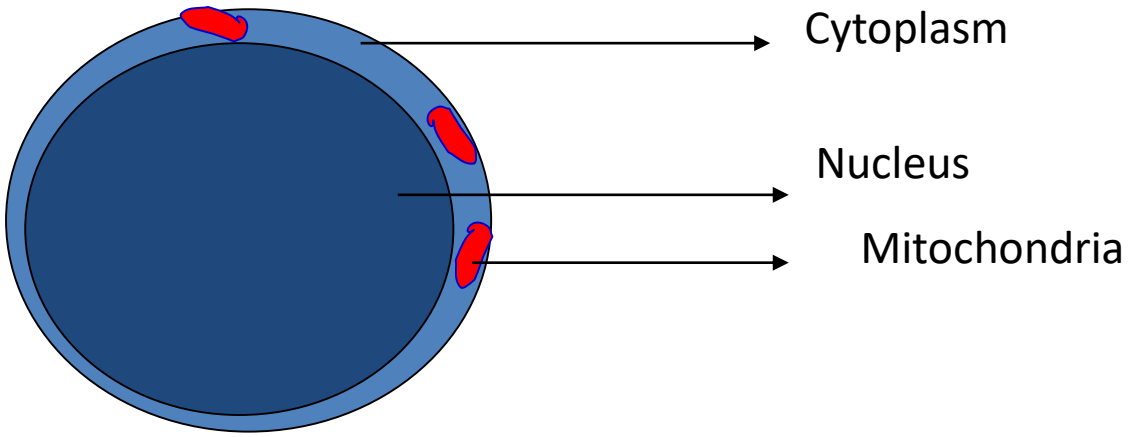
LYMPHOCYTES

Morphology of Lymphocytes

- Located in the blood, lymph and lymphoid tissues
- 5 to 15 µM in diameter
- Most of the cell contains nucleus (thin rim of cytoplasm containing free ribosomes, mitochondria)



Types of Lymphocytes

Most lymphocytes may LOOK ALIKE, but they are Diverse Subpopulations – T cells, B cells, and ILCs including NK cells

HOW DOES ONE IDENTIFY????

- Characteristic Surface Proteins (CD molecules)
- Functions

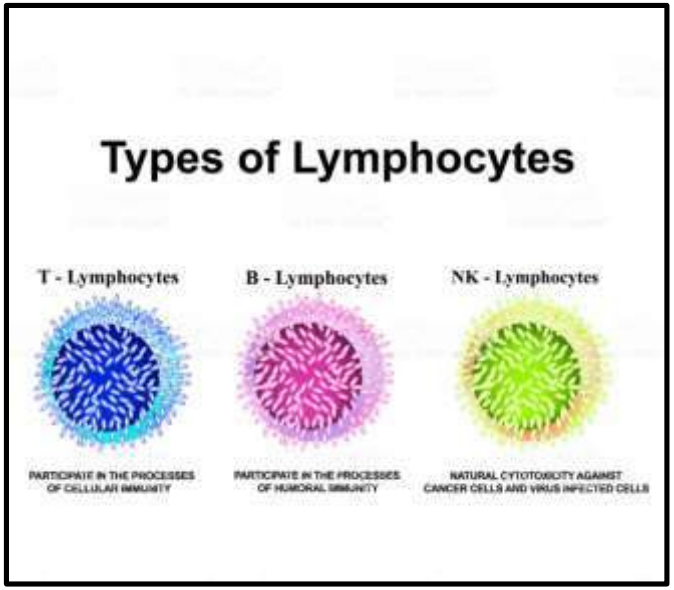
Antigens (CD molecules/surface markers)

Table 1. Main Distinguishing Markers of T and B cells			
Marker	B cells	Tc	Th
CD3	-	+	+
CD4	-	-	+
CD8	-	+	-
CD19 and/or CD20	+	-	-
CD40	+	-	-
Ag Receptor	BCR (surface Ig)	TCR	TCR

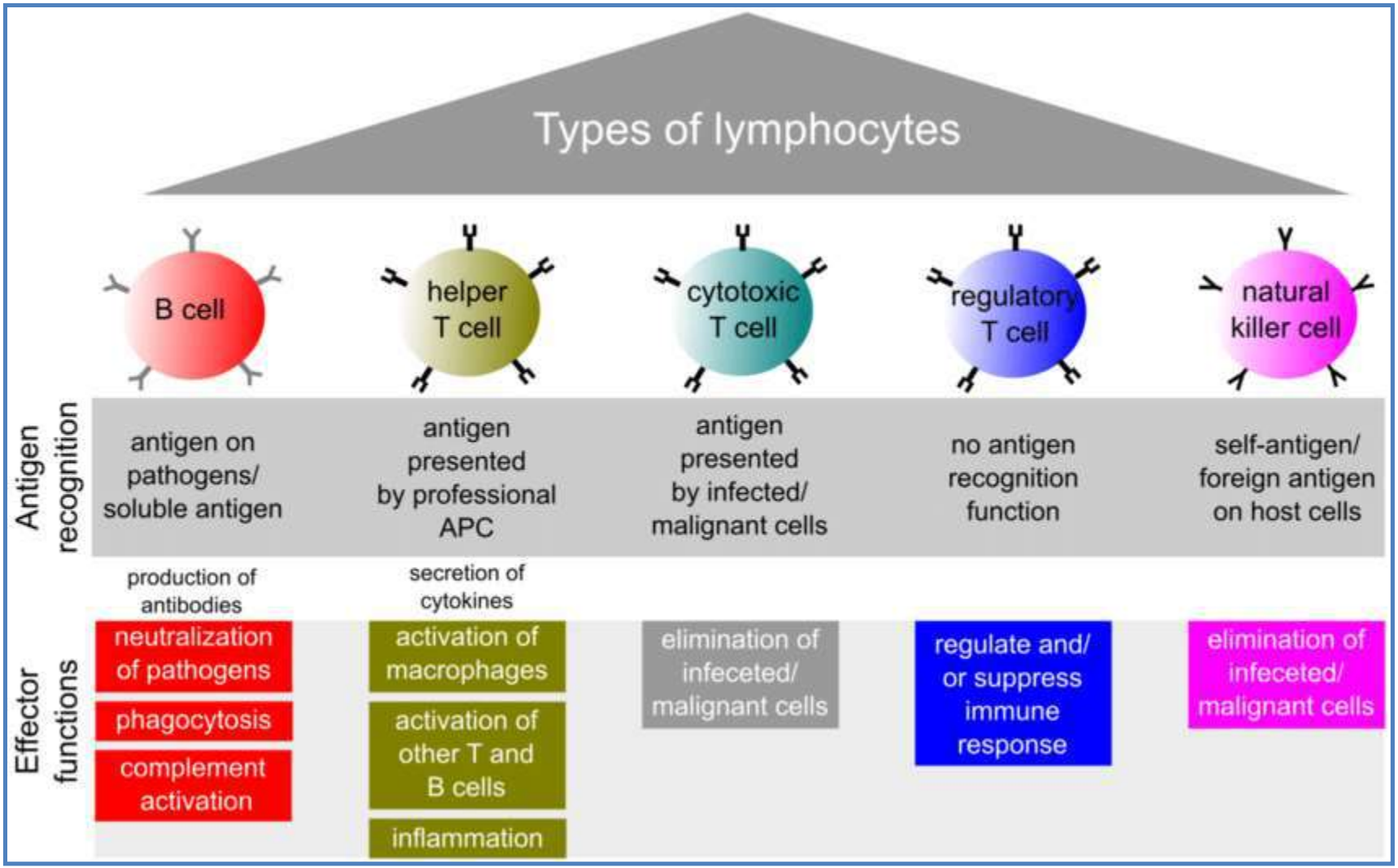
Functions of Different Types of Lymphocytes

Lymphoid Leukocytes and Their Properties

Total Lymphocytes	$1.3\text{-}3.5 \times 10^9/L$		Effector Function
B Cell	monocytic	Adaptive	Humoral immunity
Plasma Cell	monocytic	Adaptive	Terminally differentiated, antibody secreting B cell
T Cell	monocytic	Adaptive	Cell-mediated immunity
Natural Killer Cell	monocytic	Innate	Innate response to microbial or infection



Detailed Functions of Different Sub-types of Lymphocytes

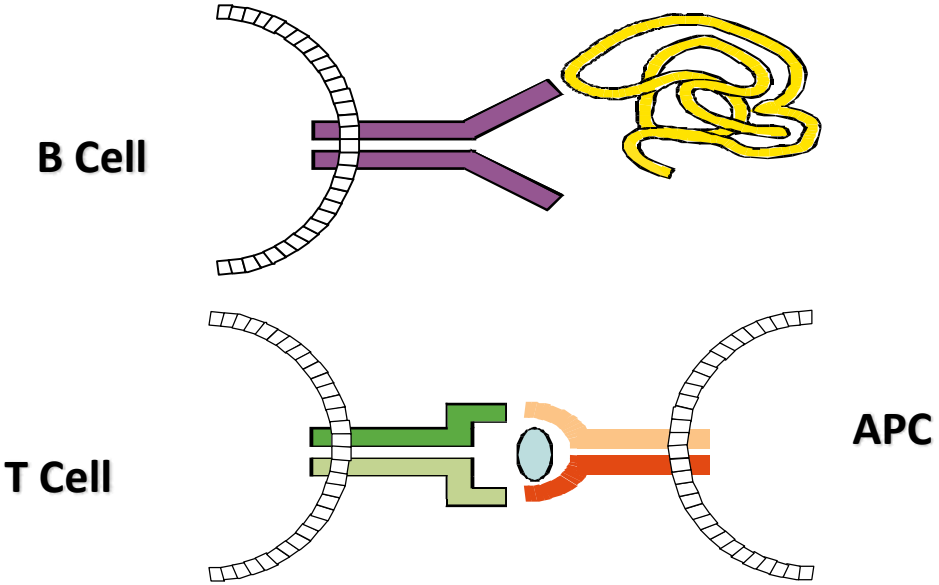


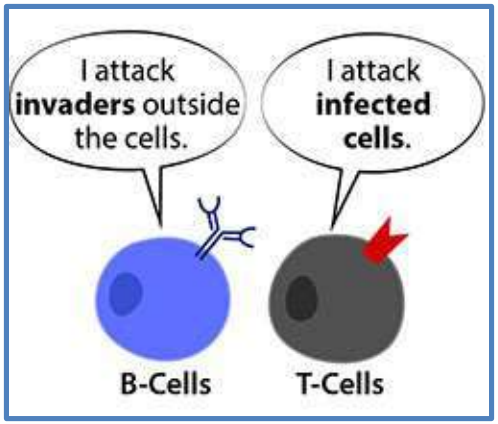
Types of Lymphocytes (general features)

- **B cells and T cells**
  - Adaptive immunity
  - Small lymphocytes
  - Those that have not interacted with antigen are called naïve
  - Possess BCR or TCR for interaction with antigen
  - Interaction with antigen – proliferation into effector cells (i.e. plasma cells) and memory cells

- **Natural Killer Cells**
  - Innate immune response
  - Large, granular
  - Activated by macrophages (IFN-γ)
  - Recognize tumor or virus-infected cells
  - CD16 – which can recognize a region of antibody that has attached to cell infected by virus (ADCC)
  - No BCR or TCR; instead possess KAR/KIR

T and B cells see different kinds of epitopes
and
are effective against antigens at different locations





Characteristic	T cell	Bcell
Origin	Bone marrow	Bone marrow
Maturation	Thymus	Bursa, bone marrow, Peyer's patches
Life span	Long lived	Short lived/long lived
Mobility	Highly mobile	Fairly mobile/Stationary
Surface Ig (sIg)	No	Yes
Complement receptors	No	Yes
Products	Cytokines, IFN-γ, cytolysins (perforins, granynzymes), growth factors	Imunoglobulins
Effective against	Intracellular antigens (viruses, tumor cells)	Extracellular antigens
Helper function	Yes (Th cells)	No
Cytotoxicity	Yes (Tc cells)	No
Antibody secretion	No	Yes
Antigen presentation	Yes	No
MHC restriction	Yes	No
Rosette formation with srbc	Yes (CD2)	No

T-LYMPHOCYTES

T Lymphocytes

- Site of maturation
  - Thymus
- Possess CD3 molecule as a surface marker
- T cell receptor
  - Only recognize antigen that is bound to cell membrane proteins called MHC
  - Once antigen is encountered with MHC:
    - Differentiation
      - Effector T cells
      - Memory T cells
- 3 major subpopulations
  - T helper (T_H)
  - T cytotoxic (T_C)
  - T regulatory (T_{reg})
- 4 major subpopulations of Th cells
  - Th1
  - Th2
  - Th9
  - Th17

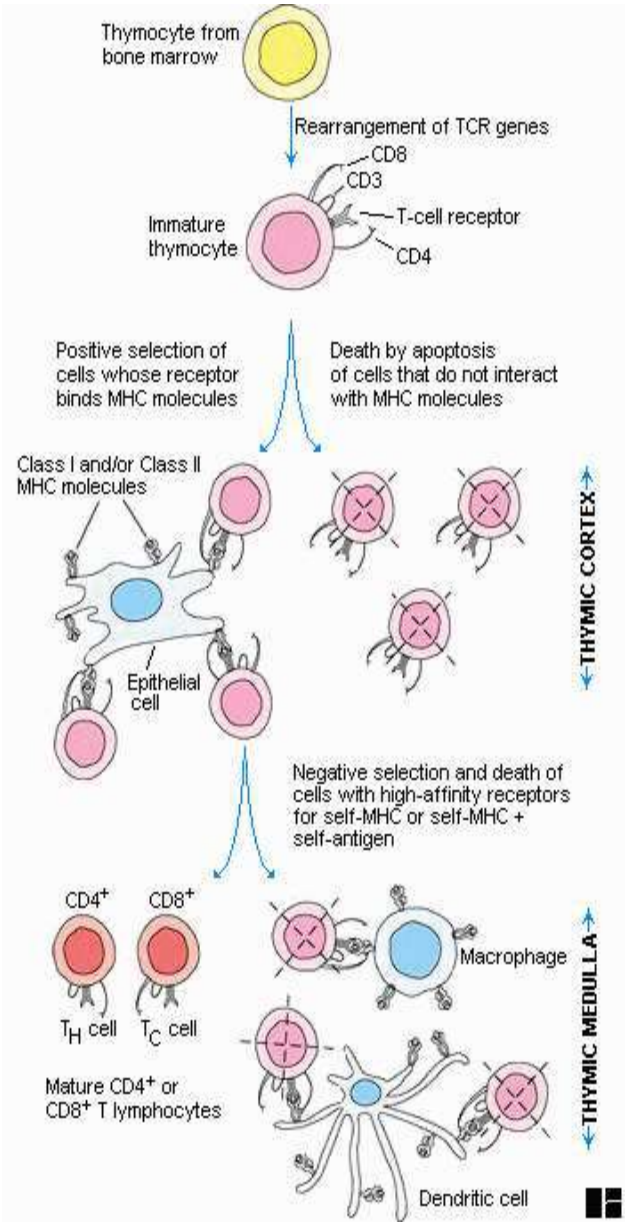
Functions of T Lymphocytes

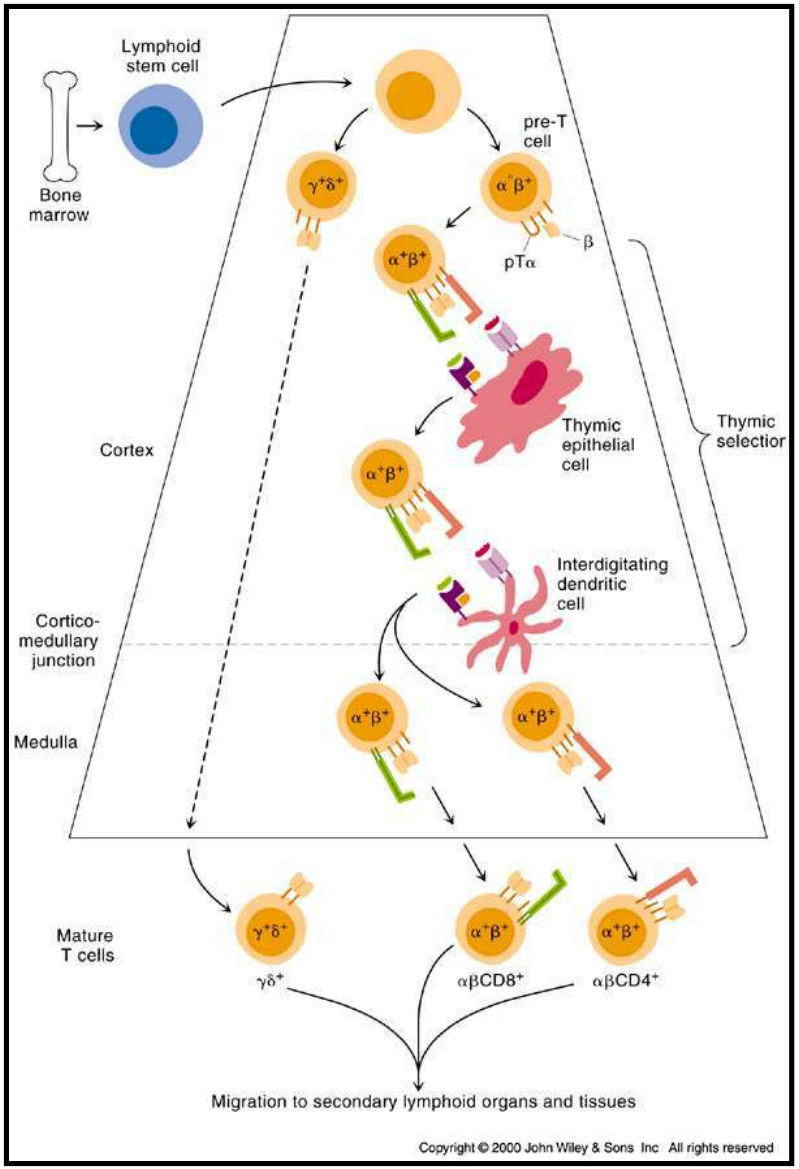
T lymphocytes play or facilitate a central role in the orchestration of all functions of the adaptive immune system and perform four important tasks:

- promotion of inflammation and CMI by cytokine production (Th1 and Th17 cells)
- helping B lymphocytes for AMI production (Th2 cells)
- killing of unwanted target cells (CTL)
- Regulating/suppressing immune responses (T-regulatory/suppressor cells)

Maturation (Ontogeny) of T-cells

- **Thymocytes mature by positive and negative selection**
  - **positive selection – cells whose receptor binds to MHC molecules (in cortex)**
  - **negative selection – (in medulla)**
    a) cells whose receptor does not bind to MHC molecules;
    b) cells whose receptors bind with high affinity to MHC and/or self antigens
- **Thymic hormones, such as thymosin and thymopoeitn, appear to play role in maturation**


Thymocyte from bone marrow
Rearrangement of TCR genes
CD8
CD3
T-cell receptor
CD4
Immature thymocyte
Positive selection of cells whose receptor binds MHC molecules
Death by apoptosis of cells that do not interact with MHC molecules
Class I and/or Class II MHC molecules
Epithelial cell
THYMIC CORTEX
Negative selection and death of cells with high-affinity receptors for self-MHC or self-MHC + self-antigen
CD4+
CD8+
Macrophage
TH cell
TC cell
Mature CD4+ or CD8+ T lymphocytes
Dendritic cell
THYMIC MEDULLA


Lymphoid stem cell
Bone marrow
pre-T cell
γ+δ+
α+β+
pTα
β
Thymic epithelial cell
Thymic selection
Cortex
Interdigitating dendritic cell
Cortico-medullary junction
Medulla
Mature T cells
γδ+
αβCD8+
αβCD4+
Migration to secondary lymphoid organs and tissues
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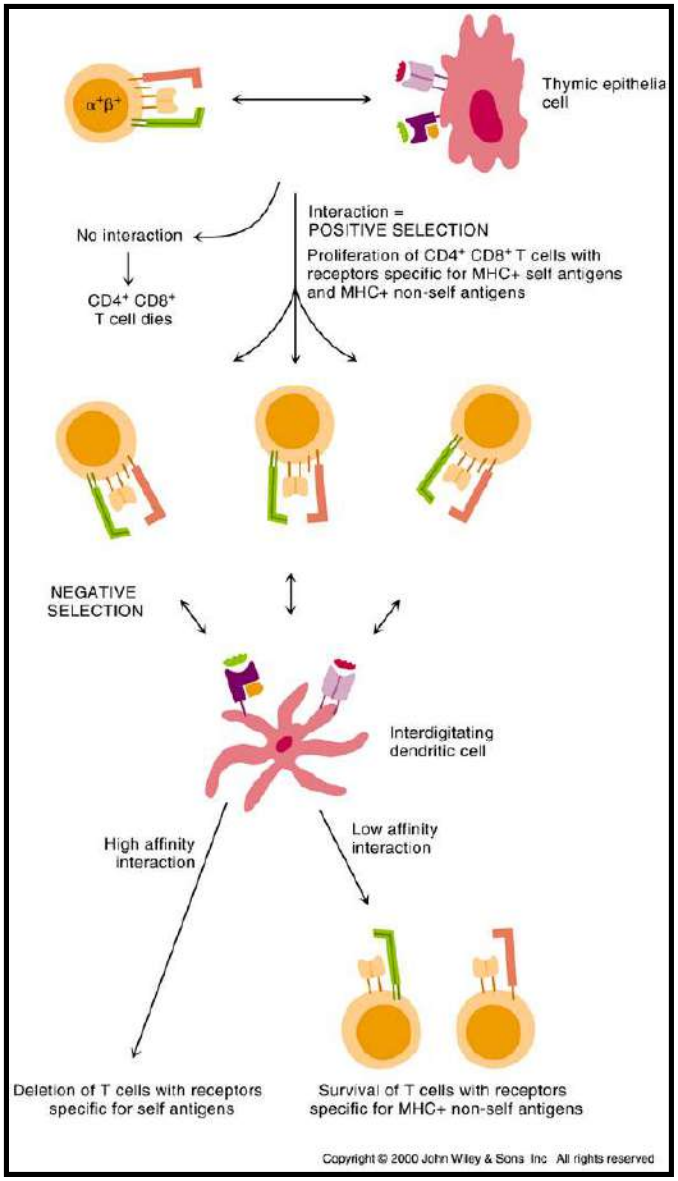
$\alpha^+\beta^+$
Thymic epithelial cell
No interaction
$CD4^+$ $CD8^+$ T cell dies
Interaction = POSITIVE SELECTION
Proliferation of $CD4^+$ $CD8^+$ T cells with receptors specific for MHC+ self antigens and MHC+ non-self antigens
NEGATIVE SELECTION
Interdigitating dendritic cell
High affinity interaction
Low affinity interaction
Deletion of T cells with receptors specific for self antigens
Survival of T cells with receptors specific for MHC+ non-self antigens
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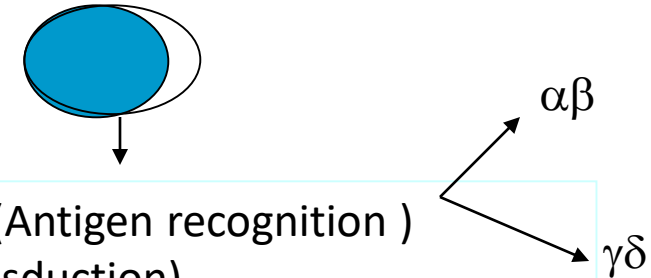
Key cell surface molecules on T Lymphocytes



T cell Receptor (Antigen recognition)
CD3 (Signal transduction)

CD4 (helper) or CD8 (cytotoxic)

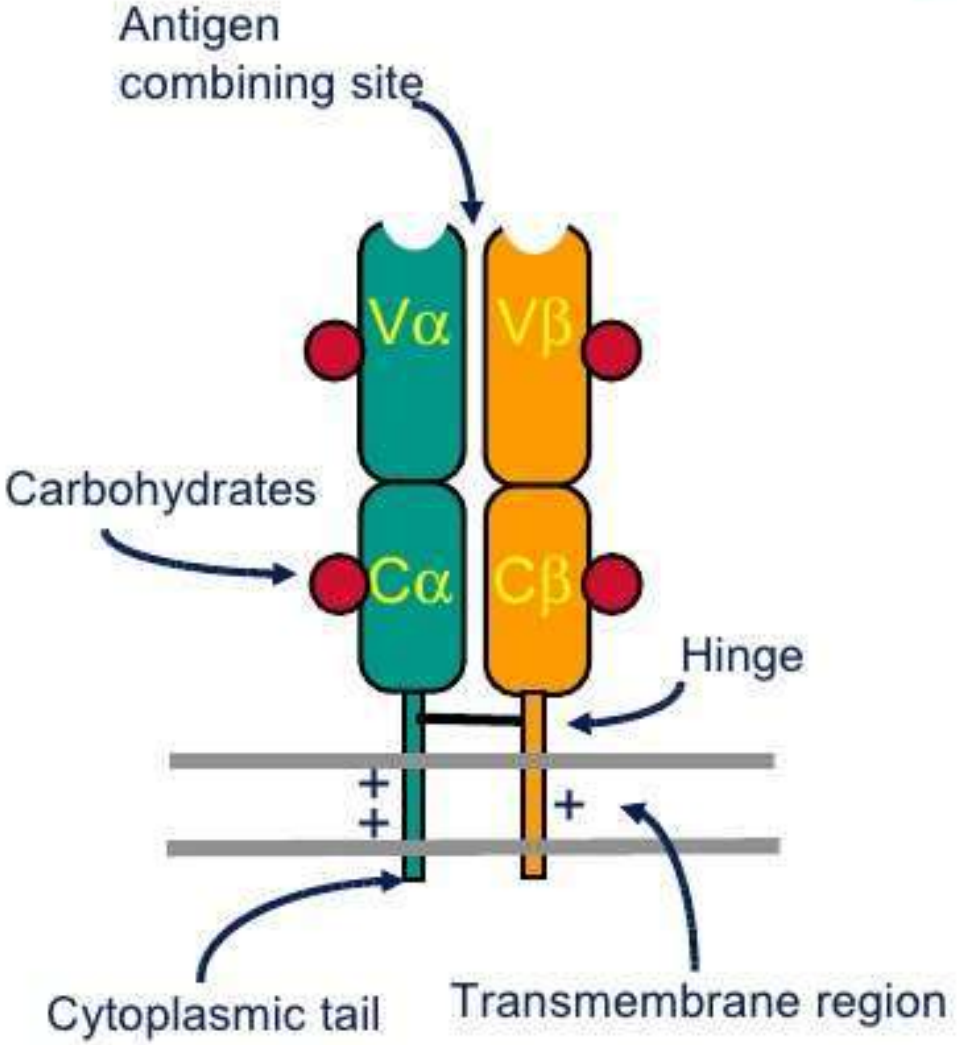
CD28 (co-stimulatory molecule)
CD40L (co-stimulatory molecule)
Cytokine receptors (IL-2, INF γ,etc)

Activation Markers (e.g. Transferrin receptor)

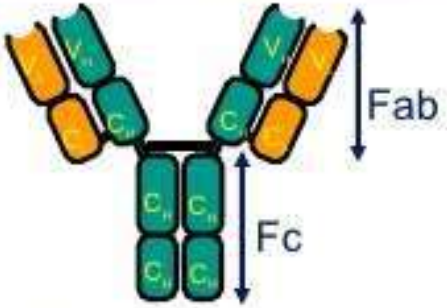
The T cell Receptor

- A heterodimer consisting of two polypeptide chains, α and β, of roughly equal length
- Both chains consist of a variable (V) and a constant (C) region
- Vα region has a joining (J) segment
- Vβ region has both a J and a diversity (D) segment
- Hypervariable sub-regions in V region contribute to diversity of TCR
- TCR recognizes MHC associated peptide bound in the agerotpe
- Approximately 30,000 TCR molecules of identical specificity are present per cell.
- TCR is cell associated, not secreted.
- **Small population of T cells has a TCR comprised of γ and δ chains – γδ TCR specificity differs from αβ TCR**

The T cell antigen receptor



Resembles an Ig Fab fragment



Domain structure: Ig gene superfamily

Monovalent

No alternative constant regions

Never secreted

Heterodimeric, chains are disuphide-bonded

Very short intracytoplasmic tail

Positively charged amino acids in the TM region

Antigen combining site made of juxtaposed Vα and Vβ regions

30,000 identical specificity TcR per cell

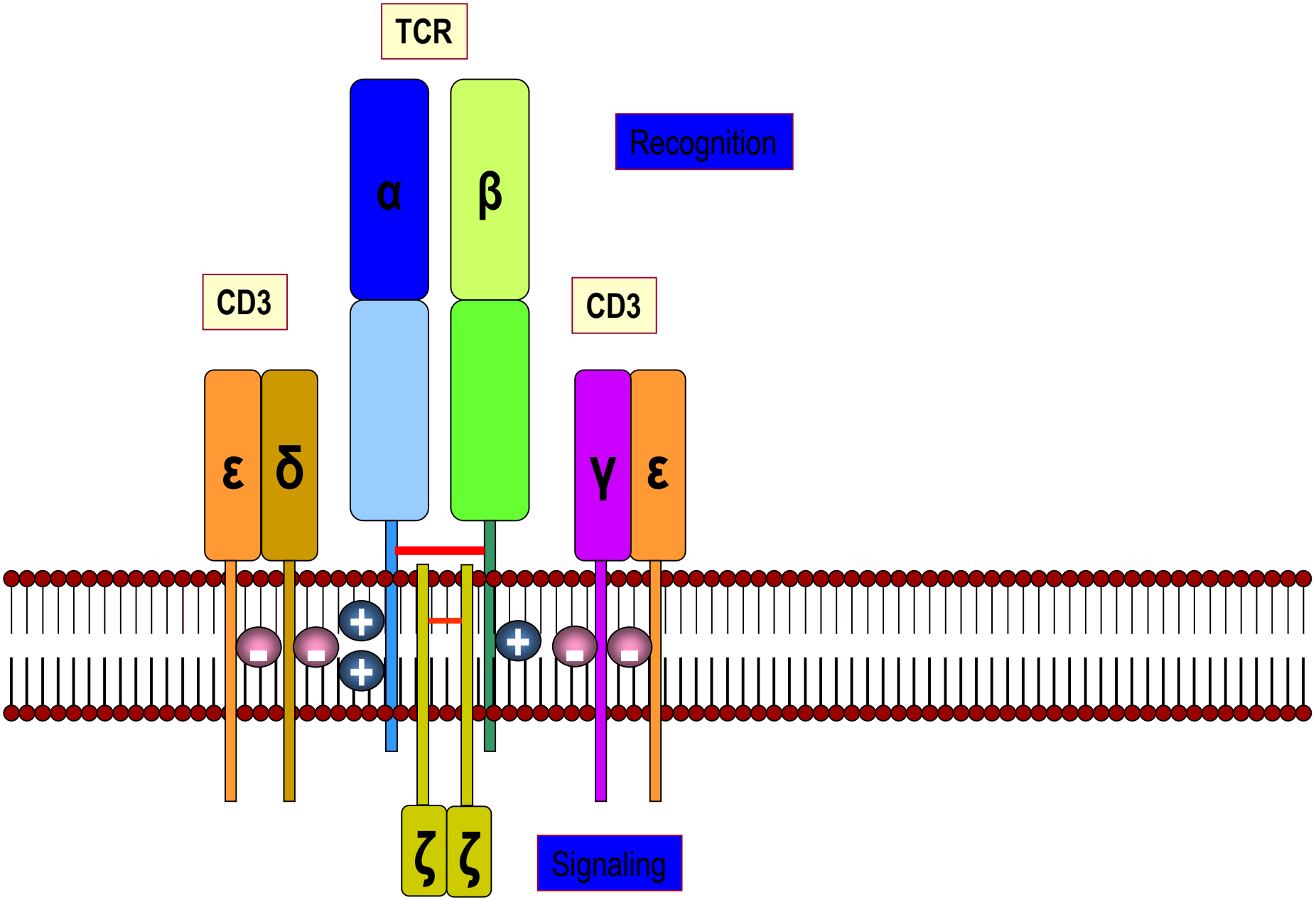
BCR vs TCR

BCR	TCR
• Four peptides	• Two peptides
• Has two antigen biding region	• Has one antigen binding region
• Has variable and constant regions	• Has variable and constant regions
• Has gene segments and gene rearrangement	• Has gene segments and gene rearrangement
• CD79 mol. As signal transducers	• CD3 mol. As signal transducers
• CD19, CR21, CD81 co-receptors	• CD4 or CD8 mol. As co-receptor
• Can recognize free unprocessed antigen without MHC	• Recognize processed antigen in association with MHC on Antigen presenting cells
• Somatic hypermutation of the variable region (affinity maturation)	• No somatic hypermutation
• **Recognize protein, carbohydrate, ipids and nucleic acid antigens**	**Recognize protein antigens, rather peptide**

CD3 Complex

- Group of four proteins associated with TCR
- Consists of one γ, one δ, two ε, and two ζ chains
- All four proteins are invariant
- Functions:
  - 1) synthesized co-ordinately with TCR, required to bring TCR to surface
  - 2) transduces activating signals to T cell when TCR TCR recognizes MHC-peptide

CD3 Complex With TCR



T cell subsets

αβ-TCR bearing T cells are heterogeneous and are of 4 main types:

1. CD4+ helper cells - help in the activation of B cells and Tc cells into effector and memory cells
2. CD8+ cytotoxic cells - cause lysis of virus-infected and tumour cells
3. Memory T cells.
4. Natural Killer T cells.

Few $CD4^-CD8^-$ (double negative) and $CD4^+CD8^+$ (double positive) cells also have been reported

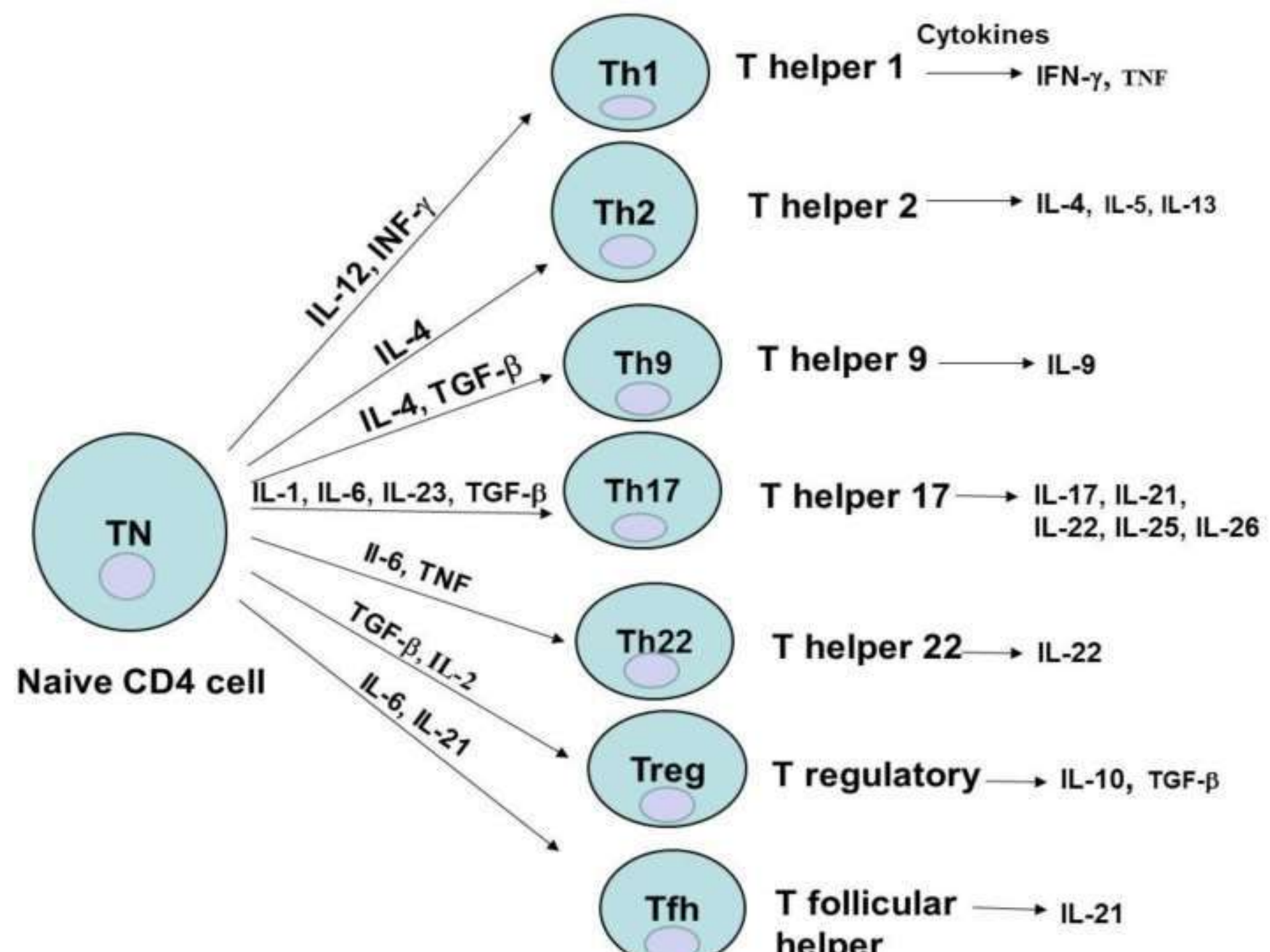
CD4+ Cell Subsets

- CD4+ T cell activation results in the secretion of cytokines that help and regulate other cells

- The pattern of cytokine expression defines the subsets of CD4+ T cells: Th1, Th2, Th9, Th17, Th22, Treg (regulatory T cells - Treg1 and Th3), and Tfh (follicular helper T cells).

- These different $CD4^+$ subsets play a critical role in the immune and effector response functions of T cells.

- All $CD4^+$ Th subsets are differentiated from naive $CD4^+$ Th0 cells by specific cytokines, and each Th subset releases specific cytokines that can have either pro- or anti-inflammatory functions, survival or protective functions.


Cytokines
Th1
T helper 1
IFN-γ, TNF
Th2
T helper 2
IL-4, IL-5, IL-13
Th9
T helper 9
IL-9
Th17
T helper 17
IL-17, IL-21,
IL-22, IL-25, IL-26
Th22
T helper 22
IL-22
Treg
T regulatory
IL-10, TGF-β
Tfh
T follicular
helper
IL-21
TN
Naive CD4 cell
IL-12, INF-γ
IL-4
IL-4, TGF-β
IL-1, IL-6, IL-23, TGF-β
Il-6, TNF
TGF-β, IL-2
IL-6, IL-21

CD4 Cell Subsets (contd.)

- **T helper type 1 (Th1) cells** are required for host defense against intracellular viral and bacterial pathogens and anti-tumor immunity as well as graft rejection **(CMI)**. These cells are characterised by their ablitiy to secrete cytokines **IFN-gamma and TNF-alpha**.
- **T helper type 2 (Th2) cells** are important for host defense against extracellular pathogens and are responsible for allergic responses **(AMI)**. Among the cytokines **(IL-4,5 and 13)** secreted by Th2 cells, **IL-4** is the most commonly used marker for Th2 cell identification.
- **T helper type 9 (Th9) cells** protect against **parasitic helminth infections**, but can also cause asthma symptoms and induce experimental autoimmune encephalomyelitis. **IL-9** production, together with a lack of IL-4, IL-5, and IL-13 production, is most commonly used as a marker for Th9 cells.
- **T helper type 17 (Th17) cells** recruits PMNs and are involved in **mucosal immunity** and autoimmune disorders. These cells are pro-inflammatory as they can inhibit the expansion of regulatory T (Treg) cells. Th17 cells are most commonly identified by **IL-17** production..

CD4 Cell Subsets (contd.)

- **T helper type 22 (Th22) cells** are recruited to **skin** where they defend against microbial pathogens, but are also associated with inflammatory skin disorders. High **IL-22** production, along with low IL-17 production, is utilized most commonly as a marker for Th22 cells.
- **Follicular helper T (Tfh) cells** are involved in the **regulation and development of antigen-specific B cell immunity**. Located in follicles of spleen and tonsils, stimulates B-cell to produce high-affinity antibodies. The most common surface markers for Tfh cell identification are **CXCR5** along with ICOS and/or PD-1.
- **Regulatory T (Treg) cells** comprise 5 - 10 % of total $CD4^+$ cells with the phenotype **CD4+CD25+** and are **anti-inflammatory** (regulation of immune response). These cells are responsible for maintaining **immune homeostasis** via inhibition of differentiation and activity of pro-inflammatory T helper cells. **FoxP3 expression** is the most commonly used marker for Treg cells. Treg cells usually secrete **IL-10** and transforming growth factor beta **(TGF-B).** **Include Treg1 and Th3 cells.**

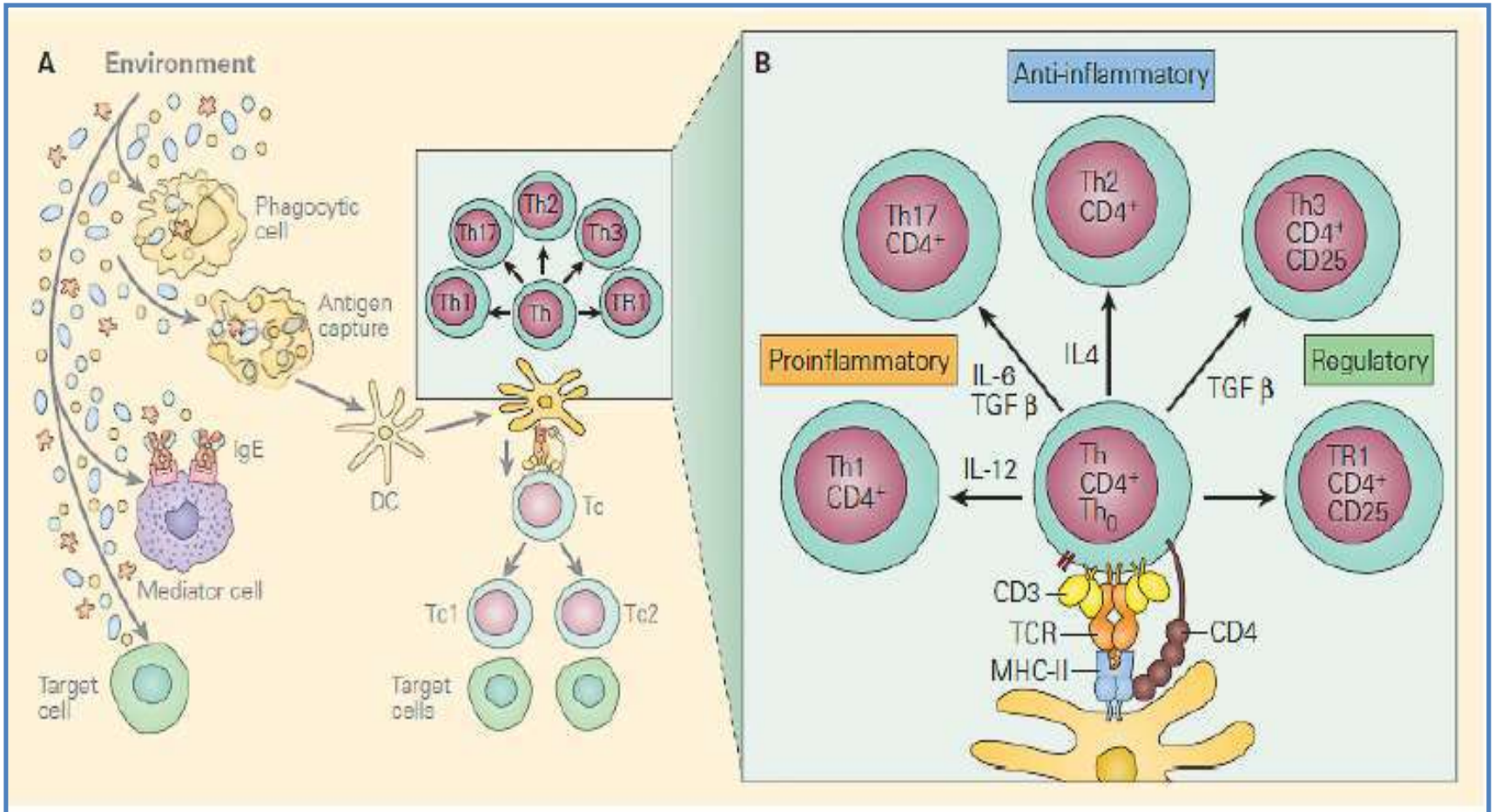
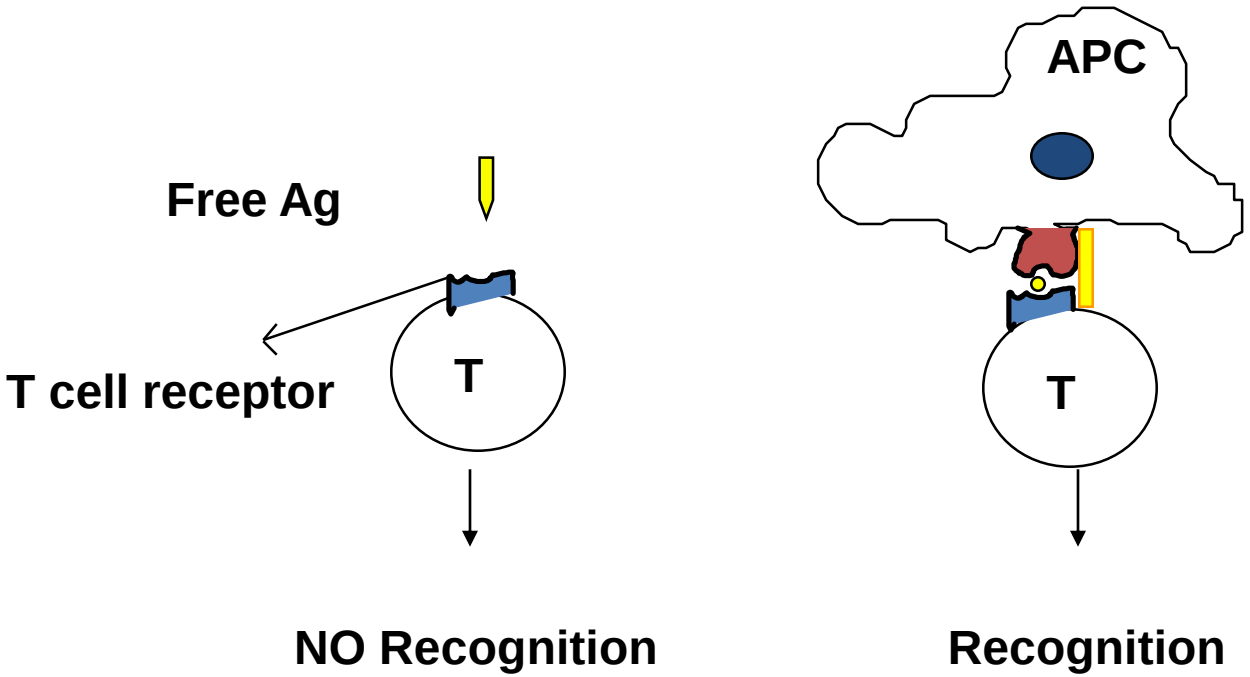


Figure 2. Panel A: The two main T cell populations are CD4+ and CD8+ cells. The CD4 are helper T cells and are shown highlighted with the CD4+ subsets Th1, Th17, Th2, Th3, and Tr1 and shown below are the CD8 cytotoxic T cells (faded). Panel B: Shows the molecular events in the immunologic synapse at the CD4+/dendritic cell interface together with the cytokines that induce the Th0 differentiation into each of the subsets. [Reproduced with permission from Bellanti, JA (Ed). Immunology IV: Clinical Applications in Health and Disease. I Care Press, Bethesda, MD, 2012].

CONDITIONS FOR ACTIVATION OF T CELLS

CONDITION # 1: T cells DO NOT recognize FREE ANTIGEN (Ag)

An antigen MUST be MEMBRANE BOUND (eg. on APC)



B cells can recognize free antigens

Condition #2:

T Cells recognize only processed antigens.
Processing takes place in antigen presenting cells.

T cells recognize peptide sequence
B cells recognize the shape of an epitope

Condition #3:

The processed antigen must associate with MHC molecules (MHC restriction) present on the membrane of cells (eg. APC)

Th cells requires help of MHC-II and Tc cells of MHC-I antigens
B cells are not MHC-restricted

No Response

Response

Condition # 4:

Antigen presenting cells should secrete cytokines, such as Interleukin-1 (IL-1) or other factors such as interleukin 12 (IL-12) which are necessary for activation of T cells.

Condition # 5:

Activation of T cells require at least 2 signals:

- **First, is the primary signal that is antigen-specific (delivered through TCR/CD complex), and**
- **a second, co-stimulatory signal through molecular contacts between T cells and APC and/or cytokines**

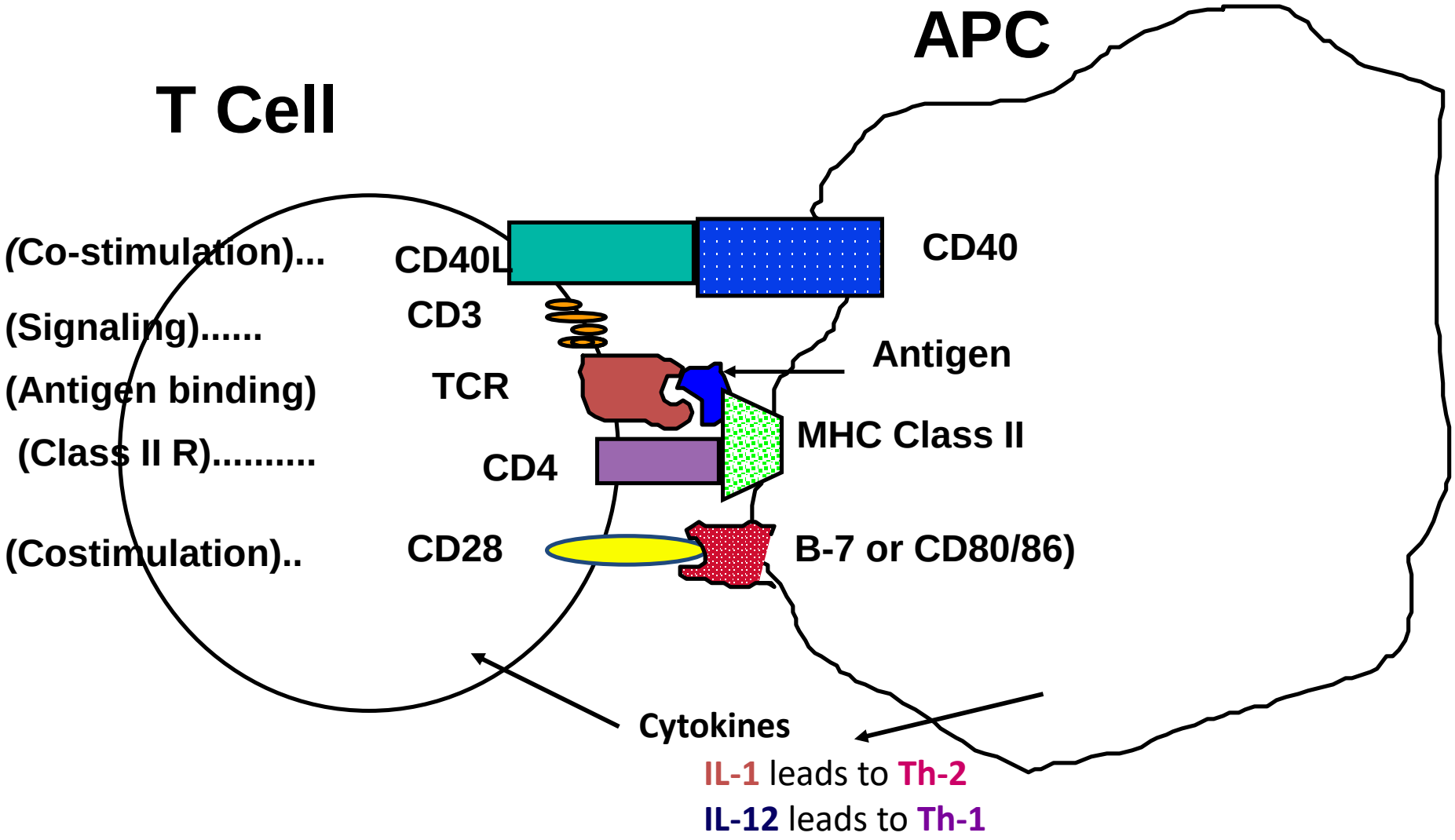
•Condition # 5 (contd.):

Co-stimulation of Th cells

- **Co-stimulatory contacts -** interactions of
  - **CD28 on T cells** with **CD80/86 molecules on APC.**
  - above binding is promoted and sustained by **CD40L on T cells** and **CD40 on APC**

- **Co-stimulatory Cytokines** secreted by APC that act on T cells include:
  - **IL-1 :** It leads to a bias in clonal expansion of **Th-2-type** of cells which are involved in helping antibody secretion, extracellular infections (AMI)
  - **IL-12:** It leads to a bias in clonal expansion of **Th-1**-type of cells which are important in immunity against intracellular infections and inducing inflammatory conditions (CMI)

SUMMARY: Interacting molecules of T cells and APC



$CD8^+$ Tc-Cells (CTLs)

- CTLs are cytotoxic cells responsible for inducing cell mediated immunity.
- These cells express the CD8 co-receptor and destroy infected cells in an antigen- speicific manner that depends on the expression of MHC-molecules on APCs and, like T_H cells, are fully activated by accessory co-stimulatory molecules.
- An activated Tc cell has no lytic powers at all; only its mature effector CTL progeny develop cytotoxicity
- Target cells of CTLs include cells infected with intracellularly replicating pathogens, tumor cells and foreign cells of a tissue transplant.
- CTLs induce apoptosis only in the target cell, not the neighboring normal cells
- The processes of antigen recognition, CTL activation, and delivery of apoptotic signals to the target cell can be accomplished within 10 minutes.
- The apoptotic process in the targeted cell may take 4 hours or more, and continue after the CTL has moved on to interact with other potential targets.
- The $CD8^+$ cells may be inactivated to an anergic state through IL-10, adenosine, and others molecules secreted by T_{reg} cells, to prevent autoimmune diseases.

CD8 Cell Subsets

Two subpopulations of CD8 cells can be differentiated by patterns of cytokine production:

- **CD8Tc1 cells**
  - secrete IL-2, IFN-γ, and TNF-β.
  - provide defence against tumors and viral infections,
- **CD8Tc2 cells**
  - secrete IL-4, IL-5, and IL-10
  - two sub-populations: *Tc2a* and *Tc2b*
  - biologic function of CD8Tc2a cells is ill defined: strongly cytotoxic and may play a role in neurologic and autoimmune diseases
  - CD8Tc2b cells are only weakly cytotoxic, but secrete proteins that prevent intracellular viral replication.
- **Tc17 cells –** The naïve Tc cells exposed to specific antigen in the presence of cytokines TGF-β, IL-6, and IL-21 can result in the development of Tc17 cells. Tc17 cells show greatly repressed cytotoxic functions and instead secrete excessive IL-17.

Activation of CD8 T cell

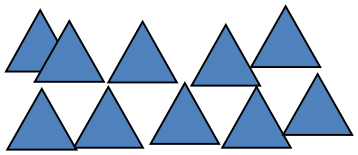
- **CONDITION # 1:** An antigen **MUST be MEMBRANE BOUND** (e.g. on APC)
- **CONDITION # 2:** An antigen **MUST be PROCESSED** (e.g. by APC)
- **CONDITION # 3:** The processed antigen **must associate with MHC-I** present on the membrane of cells (e.g. on APC)
- **CONDITION # 4:** Activation of Tc cells **require at least 2 signals**:

  First, is the **primary signal** that is antigen-specific (delivered through TCR/CD complex), and

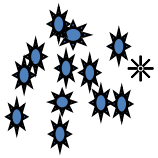
  Second, **co-stimulatory signal** (through molecular contacts between Tc cells and APC and/or cytokines)

What after activation? – T-Effector and Memory Cells

- After the naive T cell encounters an antigen (peptide in association with MHC by APC or any cell) through TCR and signal is transduced by CD3 molecule, it becomes activated and begins to proliferate, undergoes clonal expansion, and differentiate into the following **(the "On-Off-On" model)**:
  - **effector cells**, and
  - **memory cells**
- The effector cells are short-lived cells; include:
  - **Effector-inducer cells (Th/Tc)**, which will induce an active adaptive immunity
  - **Effector-regulatory cells (Tr)**, which will regulate the adaptive immunity
- The **effector Th** cells migrate to the site of infection and in turn activate B or Tc cells to eliminate the pathogen by AMI or CMI, respectively
- The **effector Tc** cells will induce apoptosis in the target cell carrying non/altered self antigen on its surface
- The **memory T cells** (Tcm) will survive in an inactive state in the host for a long period of time until they re-encounter the same antigen and gets reactivated to induce an anamnestic immune response.
- IL-7 induces survival and thus directs the memory T-cell pool; naïve and memory T-cells has high expression of IL-7R as compared to effector T-cells

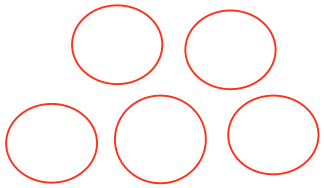


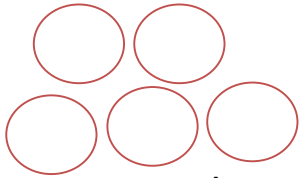
Immune response

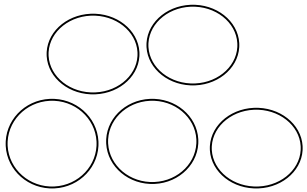


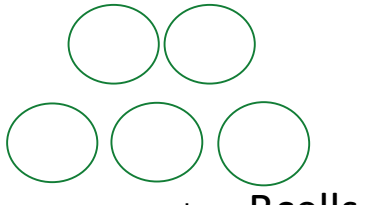
Antigen elimination

Clonal expansion of immune cells

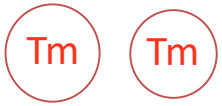


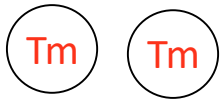






Majority of cells die and a few become memory cells



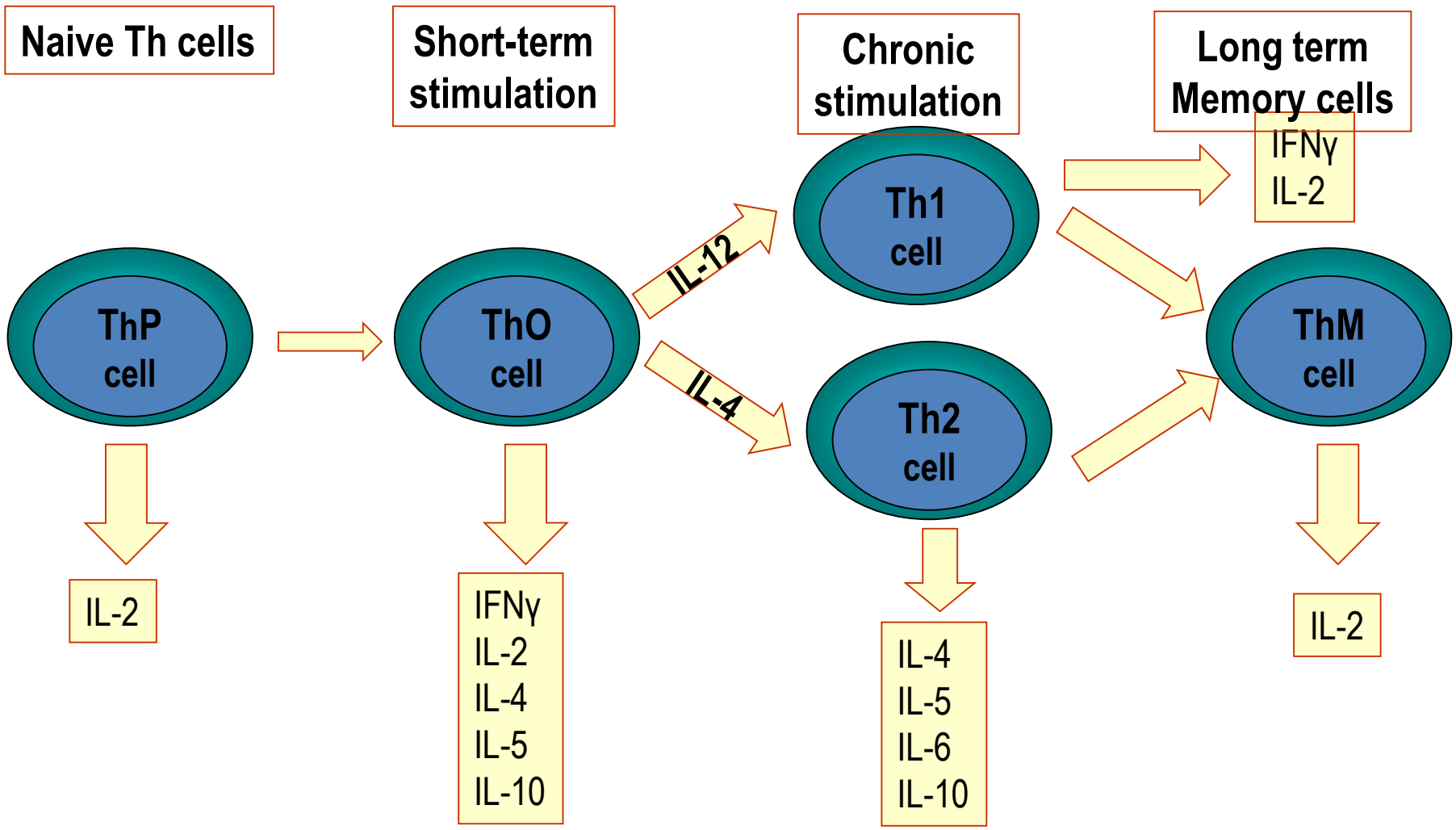


Functions of Effector Tc cells

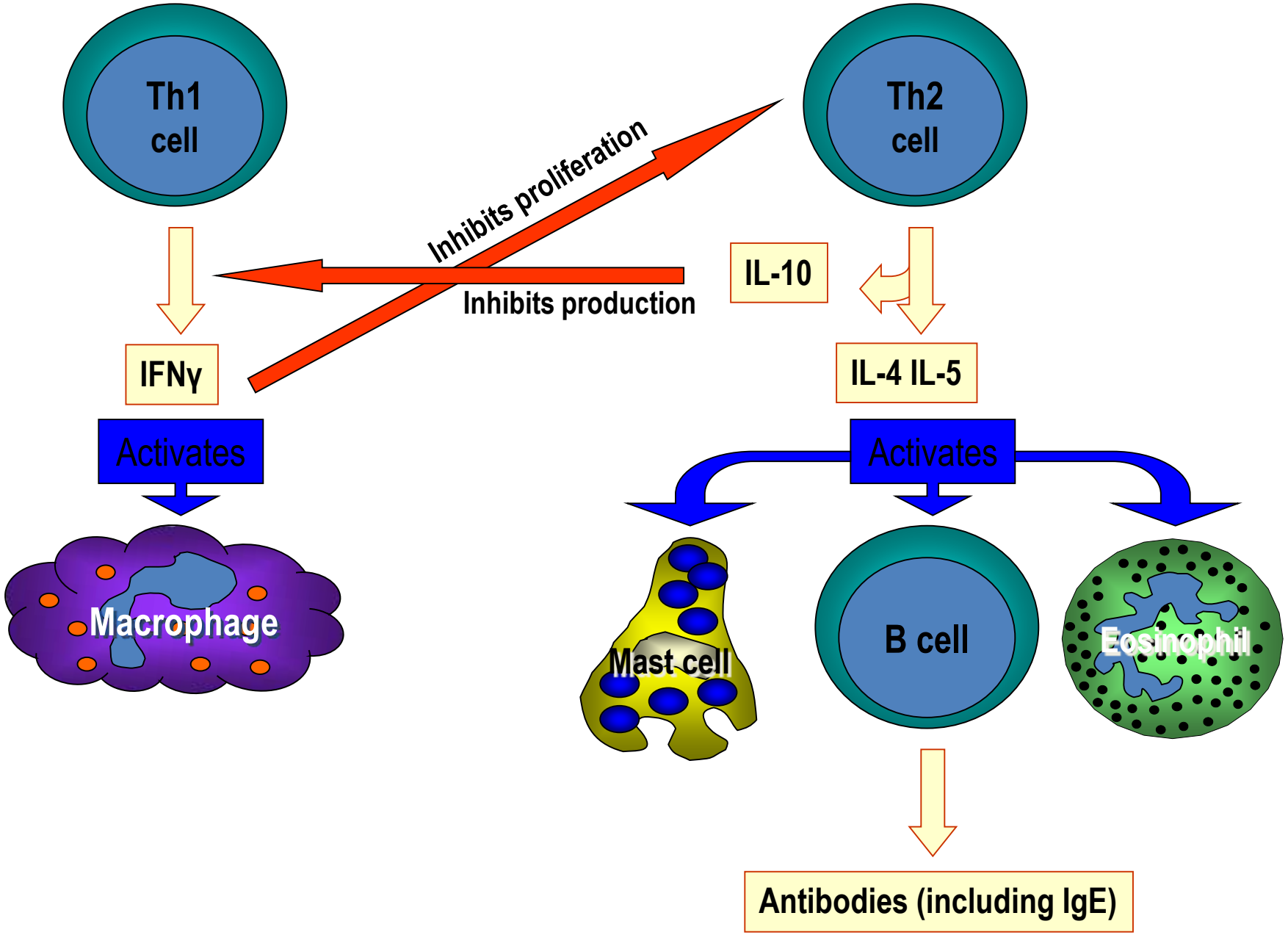
- Killing of target cells showing presence of non-self (e.g. viral) or altered self antigens (e.g. tumor) by inducing apoptosis through Perforin-granyzyme and/or Fas-FasL pathways
- Prevention of excessive tissue injury by secretion of the immunosuppressive cytokine IL-10

T_H 1 versus T_H2 cells

Naïve Th Cells Can Differentiate Into Th1 or Th2 Cells

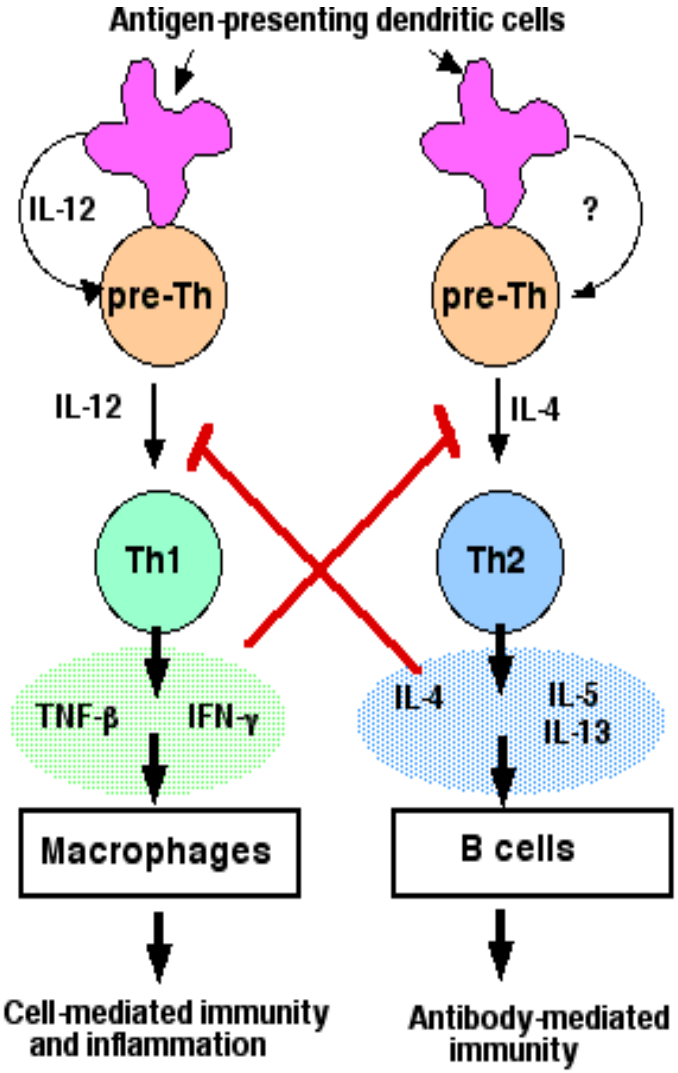


Functions of Th1 and Th2 Cells



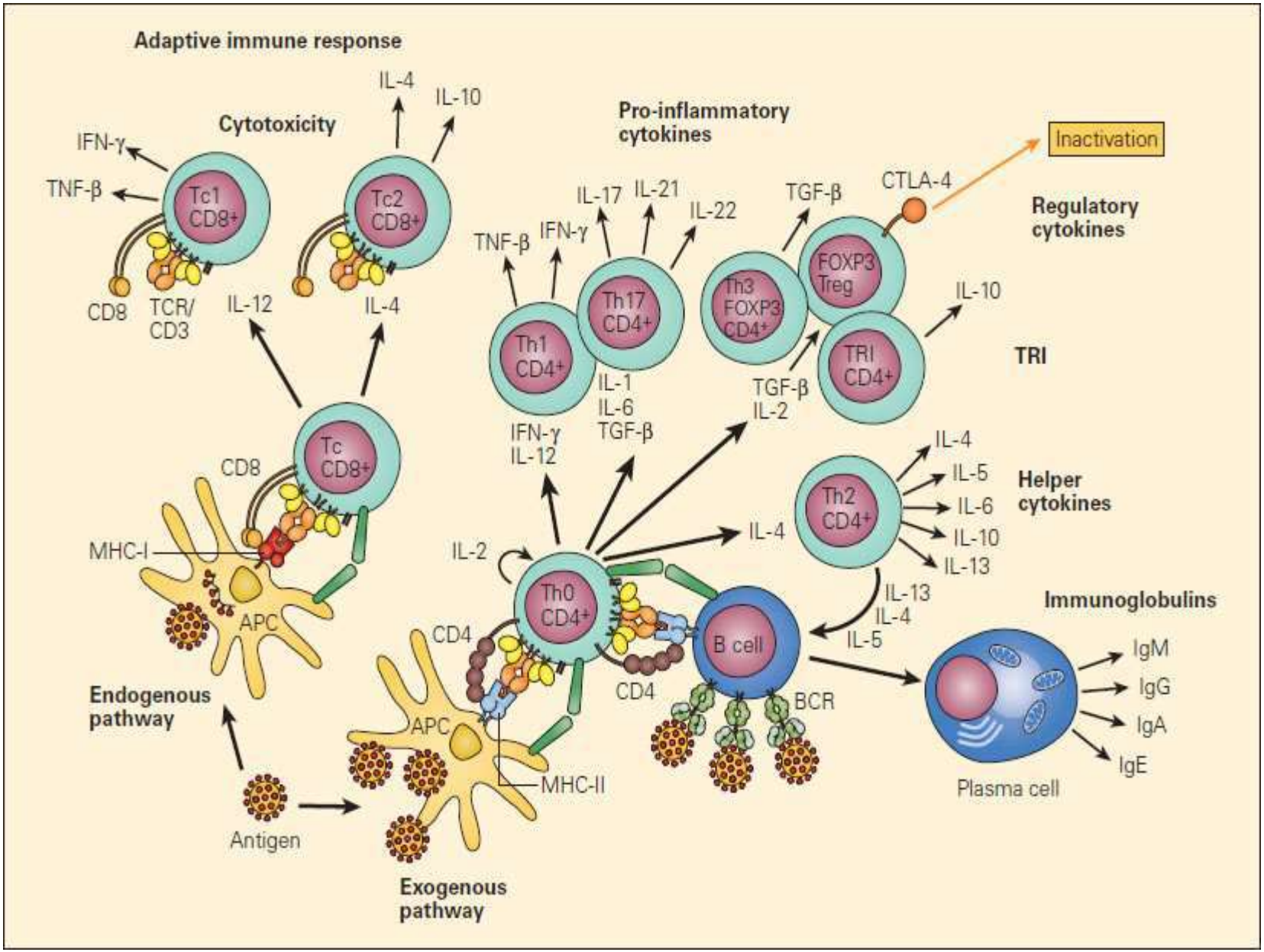
Mutual regulation of activities: T_H1 versus T_H2

- **Whether the T_H precursor cell will develop into T_H1 or T_H2 is decided by cytokine ratio of IL-12 and IL-4**
- IL-12 is produced by macrophages and dendritic cells stimulated by certain microorganisms and it stimulates Th1 cells.
- IL-4 is produced by activated basophils and mast cells and it stimulates Th2 cells
- **T_H1 cytokines, mainly IFNγ, inhibit the development of T_H2 and stimulate the development of T_H1 cells.**
- **Cytokines produced by T_H2 (IL-4, IL-10) inhibit the development of T_H1 and stimulate the development of T_H2 cells**
- T_H3 development is stimulated by a specific cytokine environment (IL-4, IL-10, TGFβ); T_H3 produce TGFβ and cooperate with B cells in MALT


Antigen-presenting dendritic cells
IL-12
pre-Th
?
pre-Th
IL-12
IL-4
Th1
Th2
TNF-β
IFN-γ
IL-4
IL-5
IL-13
Macrophages
B cells
Cell-mediated immunity and inflammation
Antibody-mediated immunity

Differences between T_H1 and T_H2 cells

Character	Th1 cell	Th2cell
Transcription factors	STAT-4 and T-bet	STAT-6 and GATA
Type of Immunity	Mainly CMI	Mainly AMI
Induced by	IL-12	IL-4
Triggering cells	Macrophages, Dendritic cells	Basophils and Mast cells
Major cytokines secreted	IFN-γ, TNF-α, IL-12	IL-4, IL-5, IL-6, IL-10, and IL-13
Activates (Effector cells)	Tc cells, Macrophages, B cells	B cells


Adaptive immune response
Cytotoxicity
IL-4
IL-10
IFN-γ
TNF-β
Tc1 CD8+
Tc2 CD8+
CD8
TCR/ CD3
IL-12
IL-4
Tc CD8+
CD8
MHC-I
APC
Endogenous pathway
Antigen
Exogenous pathway
Pro-inflammatory cytokines
TNF-β
IFN-γ
IL-17
IL-21
IL-22
Th1 CD4+
Th17 CD4+
IL-1
IL-6
TGF-β
IFN-γ
IL-12
TGF-β
CTLA-4
Inactivation
Regulatory cytokines
Th3 FOXP3 CD4+
FOXP3 Treg
TRI CD4+
IL-10
TRI
TGF-β
IL-2
IL-2
Th0 CD4+
CD4
CD4
IL-4
Th2 CD4+
IL-4
IL-5
IL-6
IL-10
IL-13
Helper cytokines
IL-13
IL-4
IL-5
B cell
BCR
Immunoglobulins
IgM
IgG
IgA
IgE
Plasma cell
APC
MHC-II

Treg Cells

- Treg cells are immunoregulatory cells, which control induced immune response (AMI and CMI) by regulating the immunocompetent B and T cells.

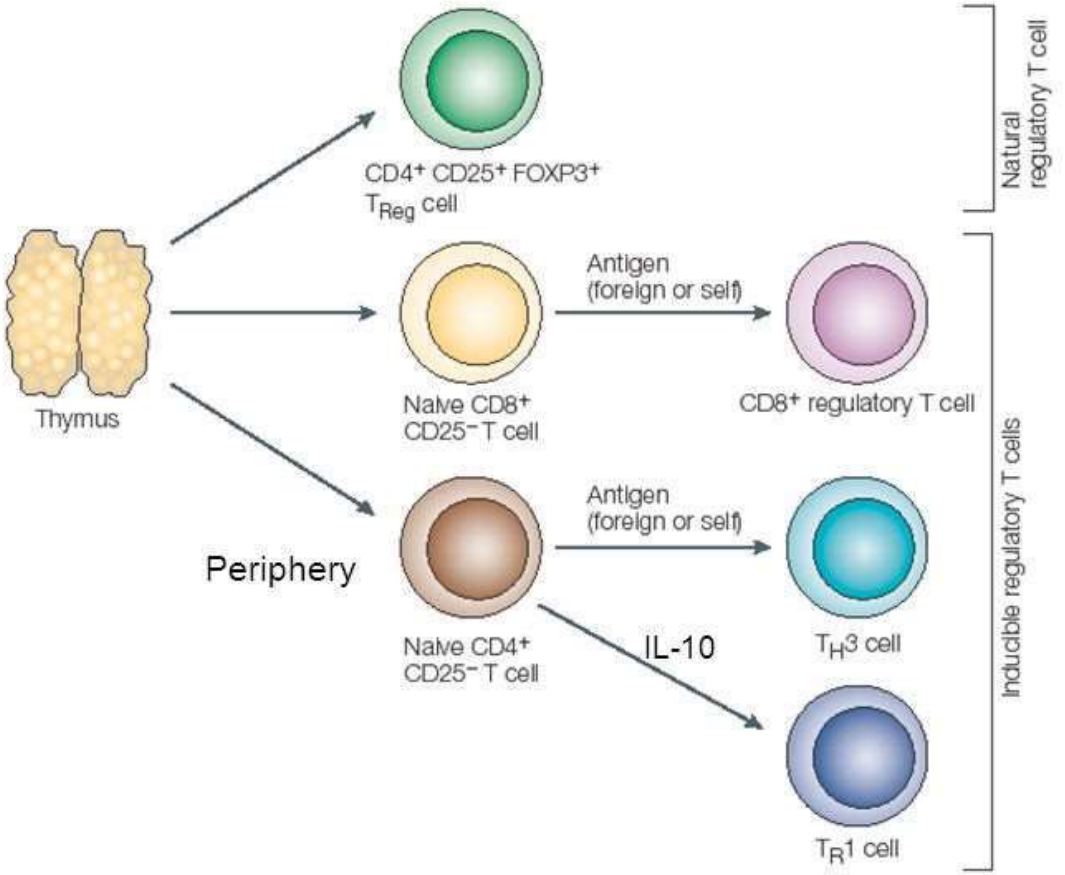
- Regulatory T (Treg) cells are responsible for maintaining immune homeostasis by mediating peripheral tolerance.

- There are two types of Tregs:
  - CD4+ Treg cells, and
  - CD8+ Treg cells

CD4+ Treg Cells

- **$CD4^+$ regulatory T cells** are a subset of $CD4^+$ cells, comprising of 5 - 10 % of total $CD4^+$ cells and are characterized with the phenotype CD4+CD25+.
- These cells are responsible for maintaining immune homeostasis via inhibition of differentiation and activity of pro-inflammatory T helper cells.
- **FoxP3 expression** is the most common marker for Treg cells in mice and humans.
- $CD4^+$ Treg cells usually secrete IL-10 and transforming growth factor beta (TGF- β).
- There are two types of $CD4^+$ Tregs primarily defined by where they develop:
  - natural Tregs (nTregs), and
  - induced Tregs (iTregs)

Types of regulatory T-lymphocytes



From: Nature Immunology

CD4+ Treg Cells Subsets

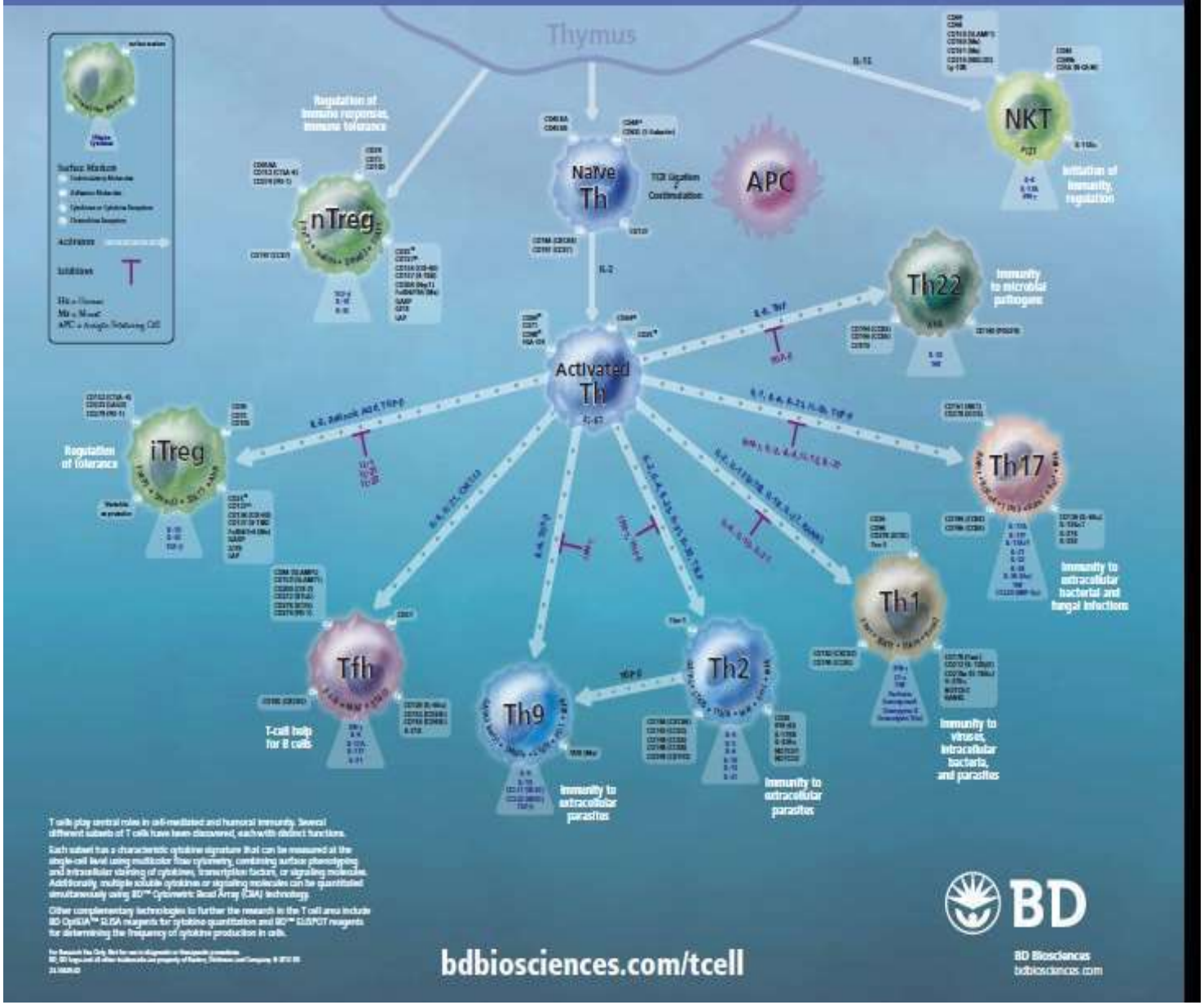
Natural Tregs (nTregs)

- develop in the thymus
- utilize the cytokines IL-10, IL-35 and TGF-β to exert their suppressive effects upon conventional Th cells

Induced Tregs (iTregs)

- derived from naïve $CD4^+$ T cells in the periphery.
- iTregs can be induced to become **$Foxp3^-$ Tr1** cells via IL-10 or **$Foxp3^+$ Th3** cells via TGF-β secreted by APCs, such as dendritic cells and macrophages.

CD4+ T Cell Differentiation



T cells play central roles in cell-mediated and humoral immunity. Several different subsets of T cells have been discovered, each with distinct functions.

Each subset has a characteristic cytokine signature that can be measured at the single-cell level using multicolor flow cytometry, combining surface phenotyping and intracellular staining of cytokines, transcription factors, or signaling molecules. Additionally, multiple soluble cytokines or signaling molecules can be quantitated simultaneously using BD™ Cytometric Bead Array (CBA) technology.

Other complementary technologies to further the research in the T cell area include BD OptEIA™ ELISA reagents for cytokine quantitation and BD™ ELISPOT reagents for determining the frequency of cytokine production in cells.

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CD8+ Treg Cells

- $CD8^+$Tregs block the overreacting cellular immune response and maintain the body's immune homeostasis.

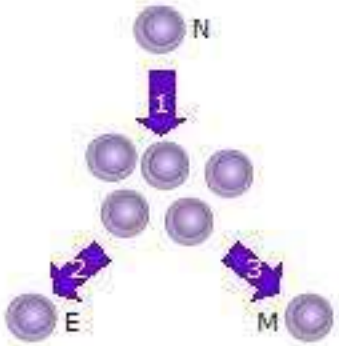
- $CD8^+$ Tregs in humans are predominantly $CD8^+CD28^-$

- Three categories of these cells have been identified :
  - Type-I cells act by influencing the expression (downregulation) of the co-stimulatory molecules CD80 and CD86 on DCs
  - Type-II cells exert inhibitory effect through cytokines, such as IFN-γ and IL-6, without direct contact with antigen-presenting cells (APCs)
  - Type-III cells function by secreting IL-10

T-memory cells

- are long-lived
- can be located in the secondary lymphoid organs (central memory cells, T_{CM}) or in the recently infected tissues (effecter memory cells as T_{EM} or T_{REM} cells).
- during re-exposure to antigen in the secondary immune response, memory T cells undergo fast expansion and cause more effective and faster immune response
- memory cells of each of the subset of Th, Tc, and Treg cells are produced
- The features of memory cells are:
  - 1. the presence of previous expansion and activation;
  - 2. persistence in the absence of antigen; and
  - 3. increased activity upon re-exposure to antigen.

T Cell Differentiation - Memory and Effector Cells



CD25- TN

APC + Pathogen Antigen

T EFF

T EM

Naive T cells
CD25-, CD45RA+
CD45RO-, CD127+

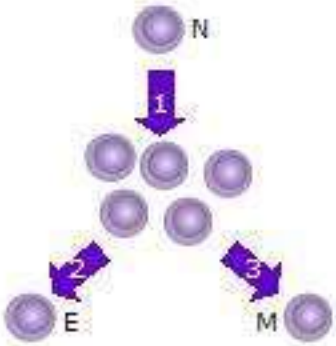
Effector T cells
CD25+, CD45RA+/-
CD45RO+/-, CD127-

Effector Memory T cells
CD25-, CD45RA-
CD45RO+, CD127+

T CM

Central Memory T cells
CD25+, CD45RA-, CD45RO+, CD127+

Central Memory T reg cells
CD25+, CD45 RA-, CD45RO+, CD127-



CD25+ Resting Treg

APC + Pathogen Antigen

T reg EFF

T reg EM

Resting T reg cells
CD25+, CD45RA+
CD45RO-, CTLA4-, CD127-

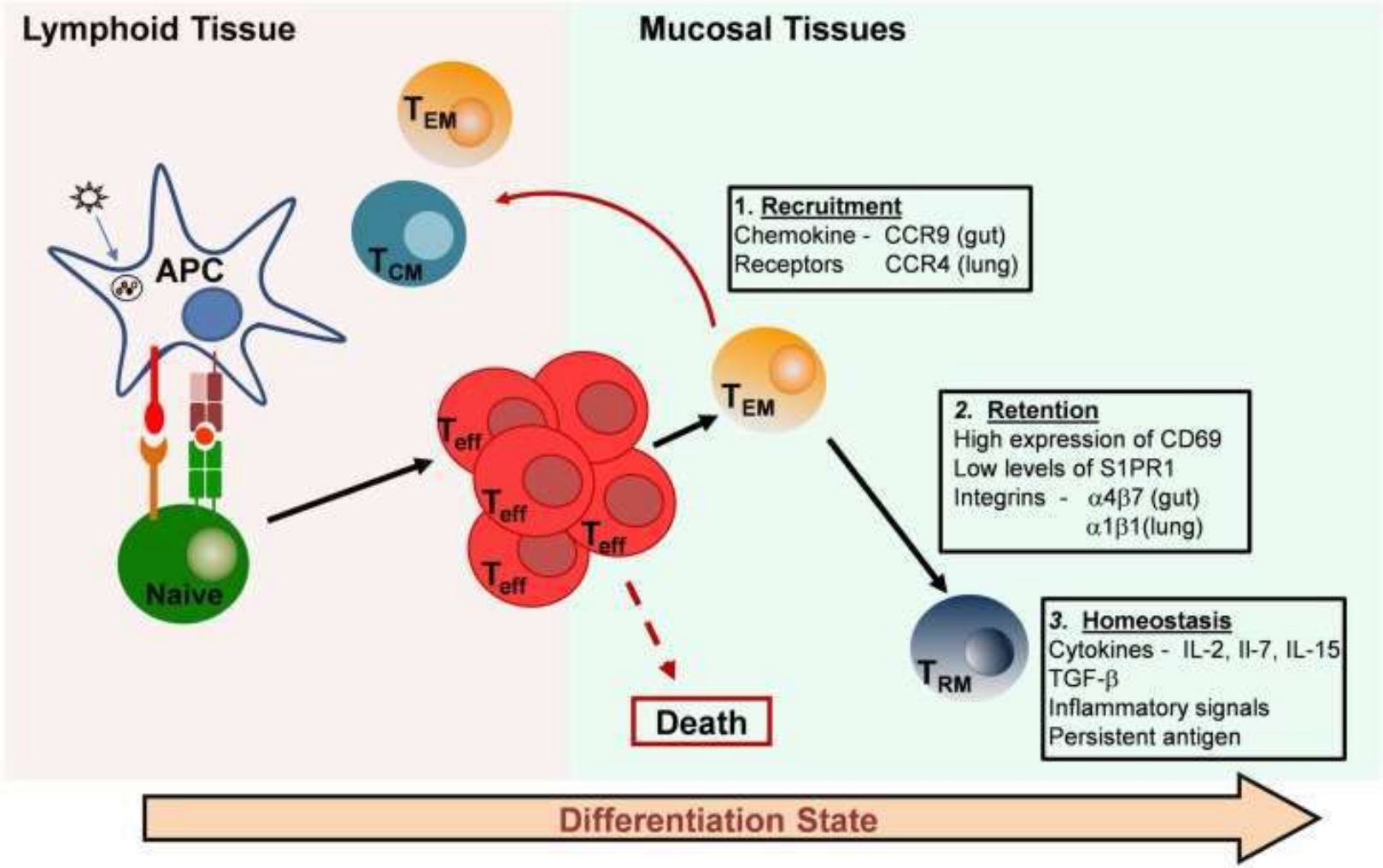
Effector T reg cell
CD25+/-, CD45RA-
CD45RO+, CTLA-4+, CD127-

Effector Memory T reg cells
CD25+, CD45RA-
CD45RO+, CTLA-4+,CD127+

T-memory cells: types

- The effecter T-cells carry specific chemokine receptors that direct their migration to individual tissues
- Most of the effector cells die, but a proportion of the effector or primed T cells differentiates into long-lived resting memory T cells.
- There are three major types:
- central-memory T cells (TCM), which migrate back to lymphoid tissue,
- effector-memory T cells (TEM), which circulate through peripheral tissues, and
- tissue-resident memory T cells (TRM), which are retained in mucosal tissue sites and take up long-term residence there without recirculating.

Types of T-memory cells



Central memory T cells (T_{CM})
($CD25^{+}CD45RO^{+}$ $CD45RA^{-}CCR7^{+}$)

- home to lymph nodes and in the peripheral circulation
- produce only low levels of effector cytokines, except IL-2 and IL-21
- expand rapidly to generate secondary waves of effector cells during subsequent exposure to homologous antigen
- have several attributes in common with stem cells, the most important being the ability of self-renewal.
- express CD45RO, C-C chemokine receptor type 7 (CCR7), and L-selectin (CD62L).

Effector memory T cells (T_{EM})

($CD25^{-}CD45RO^{+}$ $CD45RA^{-}$ $CCR7^{-}$)

- are more differentiated cells
- provides immediate immune response by converting into effecter cells
- produce high levels of effecter cytokines
- lack lymph node-homing receptors and are thus found in the peripheral circulation and home preferentially to inflamed non-lymphoid tissues
- express CD45RO but lack expression of CCR7 and L-selectin.
- A sub class of these cells are T_{EMRA} (terminally differentiated effecter memory) re-expressing CD45RA

Tissue-resident memory T cells (T_{RM})

- a population of non-circulating memory T cells in mucosal tissues likely derived from recruited effecter T cells that originated in lymphoid organs.
- pivotal in the protection of mucosal surfaces and epithelia (skin, lung, gastrointestinal tract, etc.) from invading pathogens, thus maintaining immuno-homeostasis at these strategically important barriers
- Maintenance of T_{RM} cells in mucosal tissues may depend on pro-survival cytokines, constitutive low-level inflammation, and the persistence of antigen at the site
- T_{RM} cells are identified in mice and in humans by the expression of CD69
- are crucial for quick response to barrier breach and response to any relevant pathogen present.

T-cell type	T-cell receptor type	Glycoprotein coreceptor	Antigen-presenting molecule	Foreign stimulus	Function or defining characteristics
Naive	Any	Any	Any	Any	Has not encountered an antigen
Th1	αβ	CD4	MHC II	Virus/intracellular bacteria	Activates macrophages; causes other cells to go on guard against a virus, quarantining it
Th2	αβ	CD4	MHC II	Parasites	Stimulates eosinophils, basophils, and mast cells to eliminate parasite; stimulates B cells to produce IgE and IgA antibodies
Th9	αβ	CD4	MHC II	Parasites	Supports CD4 + T-cell expansion and survival; recruits mast cells
Th17	αβ	CD4	MHC II	Extracellular bacteria/fungi	Recruits neutrophils, which kill many bacteria and fungi
T-follicular helper (Tfh)	αβ	CD4	MHC II	Any	In follicles of spleen and tonsils, stimulates B-cell production of high-affinity antibodies
Regulatory T cell (Treg)	αβ	CD4	MHC II	NA	Regulates T-cell activation and proliferation
Cytotoxic T lymphocyte (CTL)	αβ	CD8	MHC I	Any	Releases vesicles containing perforin, which punctures the target cell, and granzyme, which induces apoptosis, into the vicinity of infected cells, destroying them

T-cell type	T-cell receptor type	Glycoprotein coreceptor	Antigen-presenting molecule	Foreign stimulus	Function or defining characteristics
Central memory (Tcm)	αβ	CD4 or CD8	MHC II or MHC I, respectively	Any	Responds to secondary infections by proliferating; also circulates in blood, peripheral organs, and lymphoid organs, fighting secondary infections, but less so than Tem; that is, focuses on proliferating
Effector memory (Tem)	αβ	CD4 or CD8	MHC II or MHC I, respectively	Any	Travels around in tissues fighting secondary infections; also circulates through blood supply but avoids lymphoid organs (spleen, lymph nodes, lymphatic vessels); less proliferative than Tcm
Tissue-resident memory (Trm)	αβ	CD4 or CD8	MHC II or MHC I, respectively	Any	Stays in the tissue where it previously fought an infection and fights secondary infections there; does not recirculate in blood or revisit lymphoid organs
Virtual memory	αβ	CD8	Responds to cytokines, not antigens	Any	Antigen-inexperienced cell that leaves the thymus and becomes an activated memory cell without first encountering an antigen; cytokines can activate this cell type; particularly important early in life, when immune system has not seen many antigens, and late in life, when it is weakened

T cell subset	Phenotype	Characteristic cytokines	Characteristic transcription factors	Function
Naïve	$CD45RA^+CCR7^+$	IL-2		Precursor cells, protection against new pathogens
T_{CM} (central memory)	$CD45RA^-CCR7^+$	IL-2, IL-21		Secondary expansions, help
T_{EM} (effector memory)	$CCR7^-$	IFN-γ, IL-4, IL-5, IL-17		Protection in tissues, help
T_{RM} (tissue-resident memory)	$CD103^+CD69^+$	IFN-γ		Immediate protection in tissues
T_{FH} (follicular helper)	$CXCR5^+ICOS^+$	IL-21	BCL6	B cell help
Th1	$CXCR3^+$	IFN-γ	T-bet	Protection against intracellular pathogens
Th2	$CRTH2^+$	IL-4, IL-5, IL-13	GATA-3	Protection against extracellular parasites
Th9	?	IL-9	PU.1	Protection against extracellular parasites
Th17	$CCR6^+CD161^+$	IL-17, IL-22, IL-26	RORC2	Protection against extracellular bacteria and fungi
Treg	$CD25^+CD127^-$	TGF-β	FOXP3	Maintenance of self-tolerance
Tr1 (type 1 regulatory)	$CD25^-CD127^-$ or $CD49b^+LAG3^+$	IL-10	?	Inhibition of immunopathology

Natural killer T (NKT) cells

- Natural killer T (NKT) cells are a specialized population of T cells that express a semi-invariant TCR-αβ, besides surface molecules typically associated with NK cells.
- The TCR on NKT cells recognizes glycolipid antigens presented by the MHC-I like molecule CD1d.
- NKT cells contribute to anti-bacterial and anti-viral immune responses, promote tumor-related immunosurveillance or immunosuppression, and inhibit or promote the development of autoimmune diseases.
- Like NK cells, NKT cells can also induce perforin-, Fas-, and TNF-related cytotoxicity

Gamma delta T cells

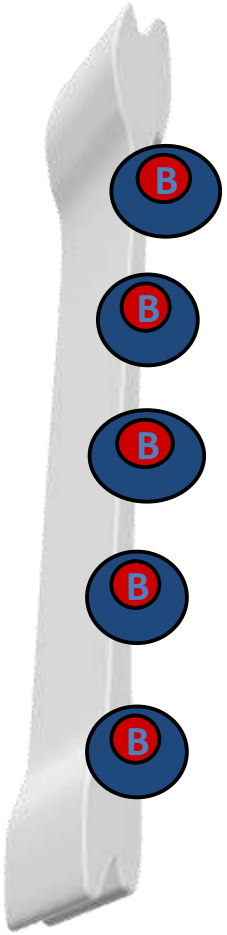
- Gamma delta T cells are a subset of T cells that express TCR chains encoded by the gamma and delta gene loci.
- Usually double-negative (display neither CD8 nor CD4).
- Gamma delta T cells represent a small fraction (1 - 5 %) of the overall T cell population in peripheral blood.
- Gamma delta T cells constitutes more than 50 % of the T cell population in epithelial cell-rich compartments like skin, the digestive tract, and reproductive organ mucosa (IELs).
- These cells have roles in both innate and adaptive immune responses
- Recognizes pyrophosphate intermediates of bacterial lipid synthesis

B-LYMPHOCYTES

B-lymphocytes (B-cells)

- **B-lymphocytes** refer to lymphocytes that are produced in the bone marrow and mature either in bone marrow (mammals) or Bursa of Fabricus (avians).
- During its maturation, each B-lymphocyte acquires **an antibody molecule on its surface (sIg/mIg) as an antigen binding receptor (BCR) with a unique specificity** through a series of gene splicing and rearrangement reactions.
- sIg belongs to IgD and/or monomeric IgM (mIgM) isotype
- The BCR has a specific 3-dimensional shape capable of binding a specific epitope of an antigen.
- The body produces 10^7 or more B-lymphocytes, each with a unique BCR.
- Antibodies, secreted by plasma cells derived from antigen-stimulated terminal B-lymphocytes, are of identical specificity as that of BCR present on surface of parent B-cell.
- **B cells also express MHC-II molecules on their surface and can thus act as an APC** (MHC-I is also there)

Role of bone marrow in the development of B cells



Regulates construction of an antigen receptor (diversity)

Ensures each cell has only one specific BCR (specificity)

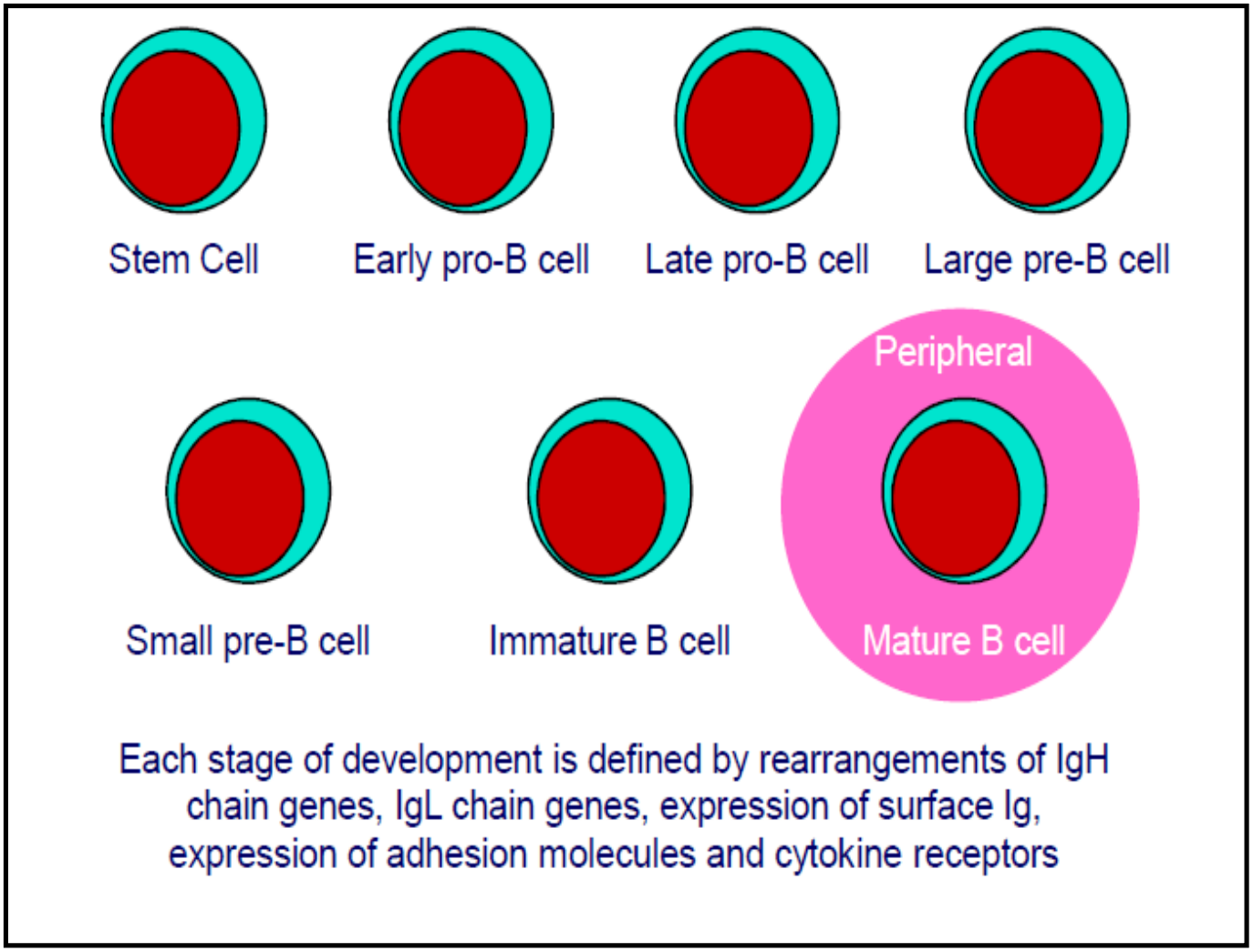
Checks and disposes of self-reactive B cells (tolerance) (negative selection)

Exports useful cells to the periphery

Provides a site for antibody production (induction of immunity)

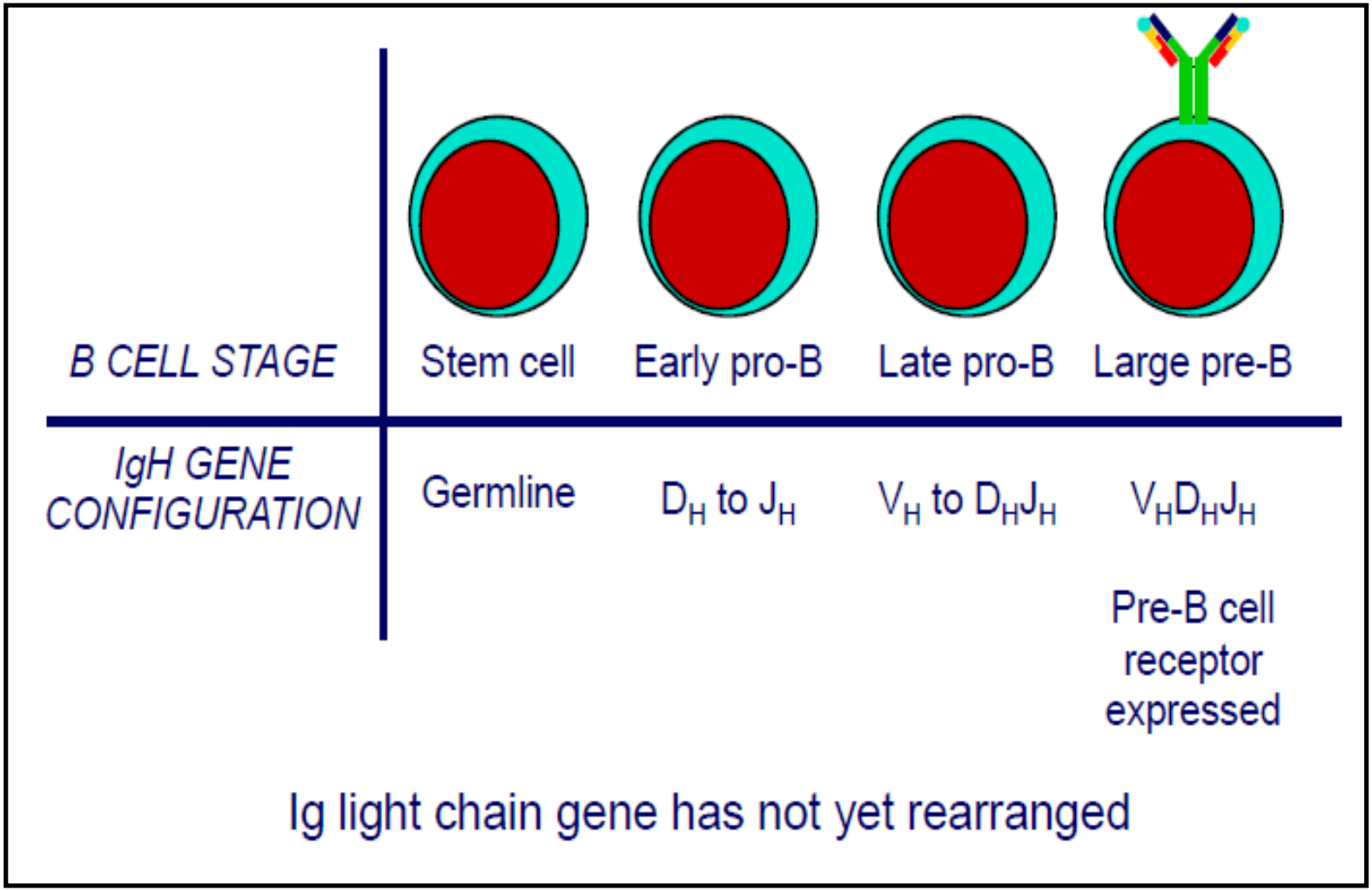
Bone Marrow provides a
MATURATION & DIFFERENTIATION MICROENVIRONMENT
for B cell development

Stages of B cell development (Ontogeny of B cells)

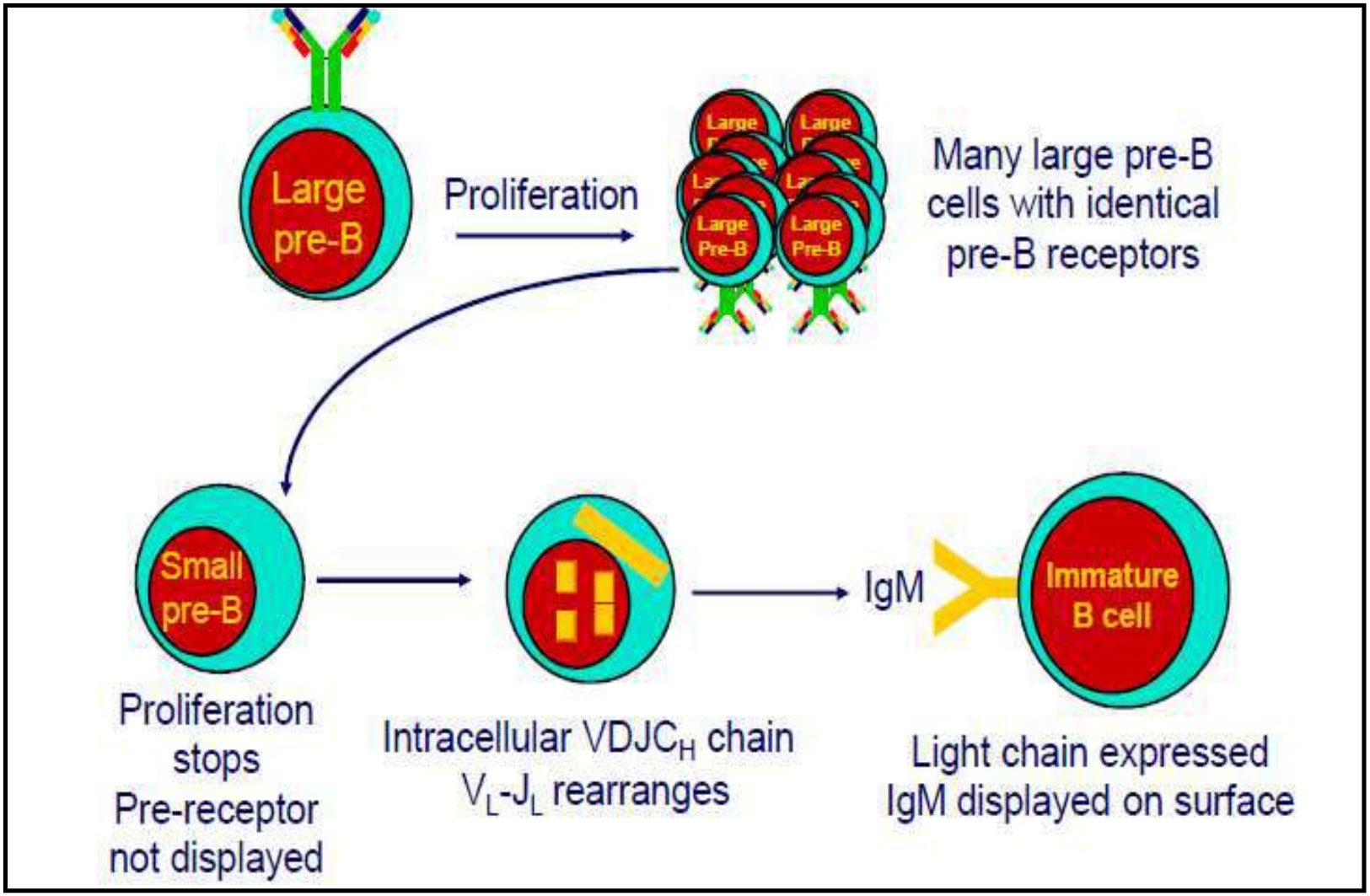


Each stage of development is defined by rearrangements of IgH chain genes, IgL chain genes, expression of surface Ig, expression of adhesion molecules and cytokine receptors

Stages of differentiation of B cells in the bone marrow



Formation of Immature B cell



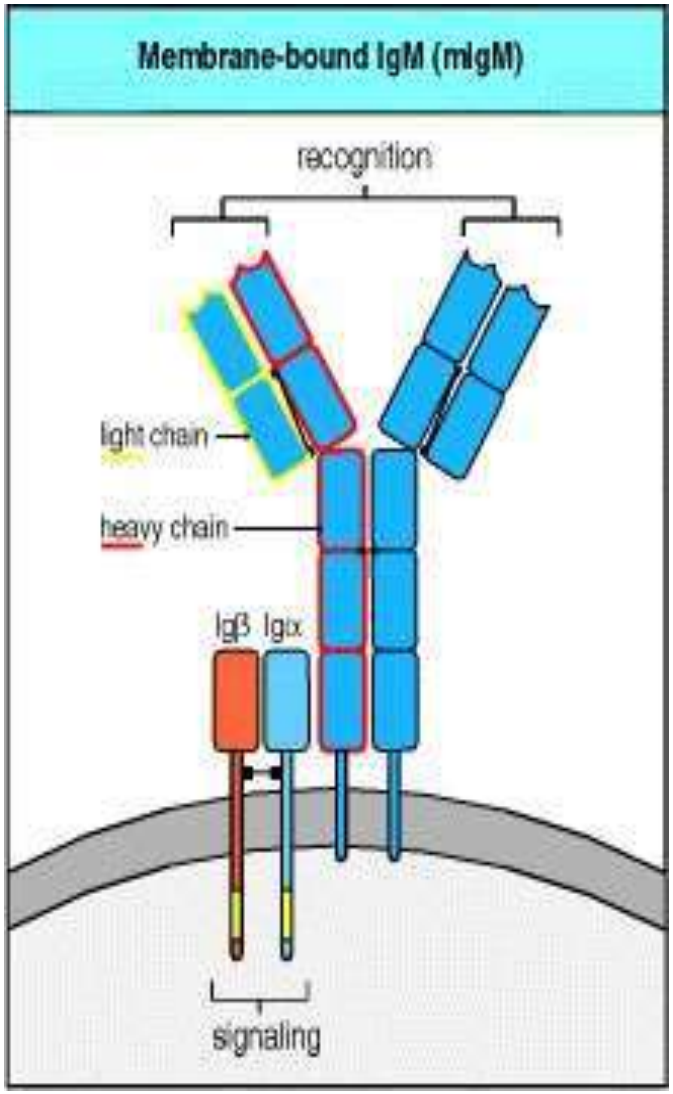
Stages of differentiation of B cells in the bone marrow

- Large pre-B cells that do not express pre-BCR die by apoptosis
- Cells expressing pre-BCR undergo "positive selection" to form small B cells → immature B cells
- Immature B Cells
  - Light chains pair with heavy chains of membrane-bound Ig molecule
  - Immature B cells can recognize and respond to foreign Ag, but this interaction results in long-lasting inactivation **(Anergy)**
  - those cells with potential reactivity to self-antignes are prevented from responding → **"negative selection" (Deletion by apoptosis)**
  - those cells which are neither inactivated nor deleted undergo **"positive selection" to form mature B cells**
- Mature B Cells
  - Development of IgM^+ /IgD^+ mature B cells
  - Predominantly in bone marrow
  - Can also occur in secondary lymphoid organs

The B cell Receptor (BCR) complex

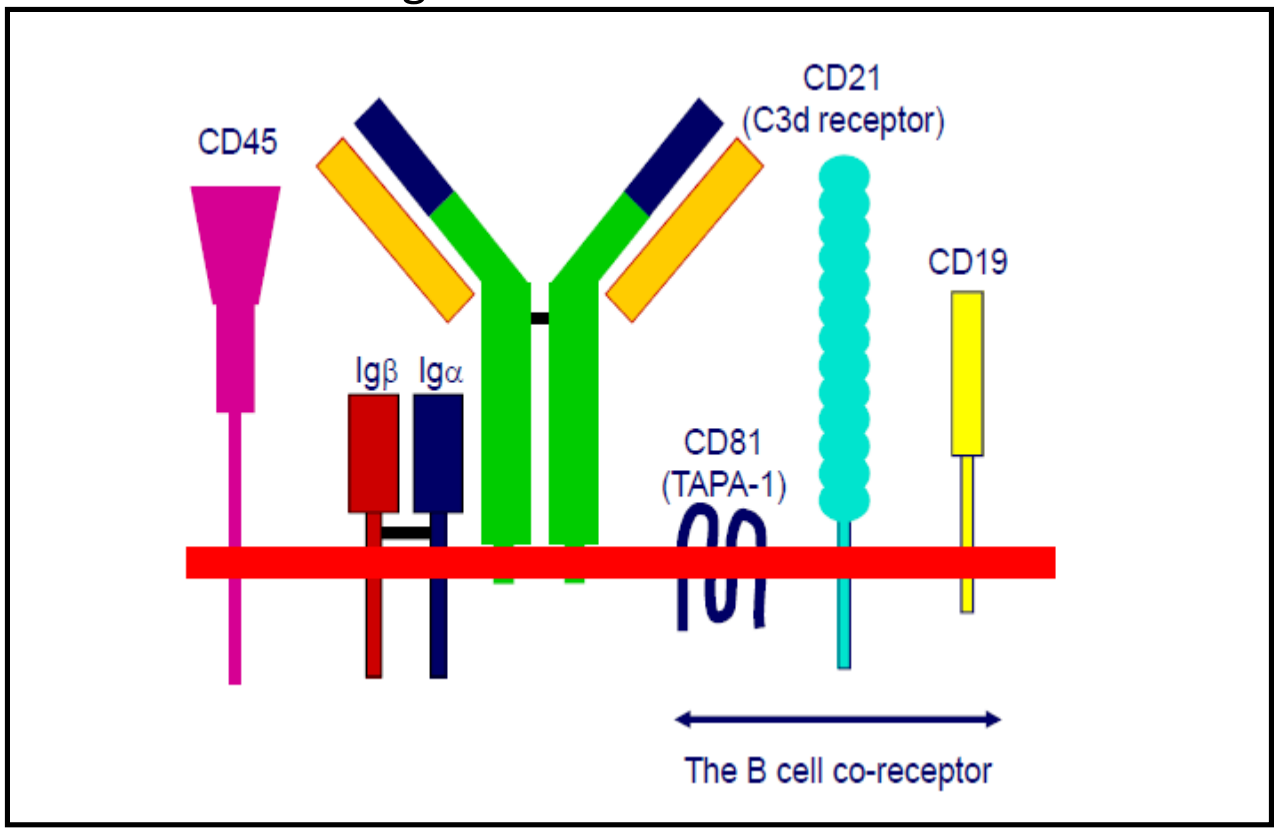
- **The BCR complex is made up of cell-surface immunoglobulin associated with two invariant proteins.**
- The cell-surface immunoglobulin is a monomeric immunoglobulin (mIgM or IgD), attached to the membrane through the carboxyl termini of the paired heavy chains.
- The carboxyl terminus of the protein also has a transmembrane domain and a very short cytoplasmic tail.
- The antigen-binding portion of the cell-surface immunoglobulin (Fab) has the same antigen specificity as the secreted antibodies that the B cell will eventually produce and is of unique specificity
- Two proteins associated with heavy chains of sIg on the B-cell surface are **Igα** and **Igβ**.
- The invariant proteins have a single immunoreceptor tyrosine-based activation motif (ITAM) in their cytosolic tails that enables them to transduce signal when the B-cell receptor interact with corresponding antigen.

The B cell Receptor



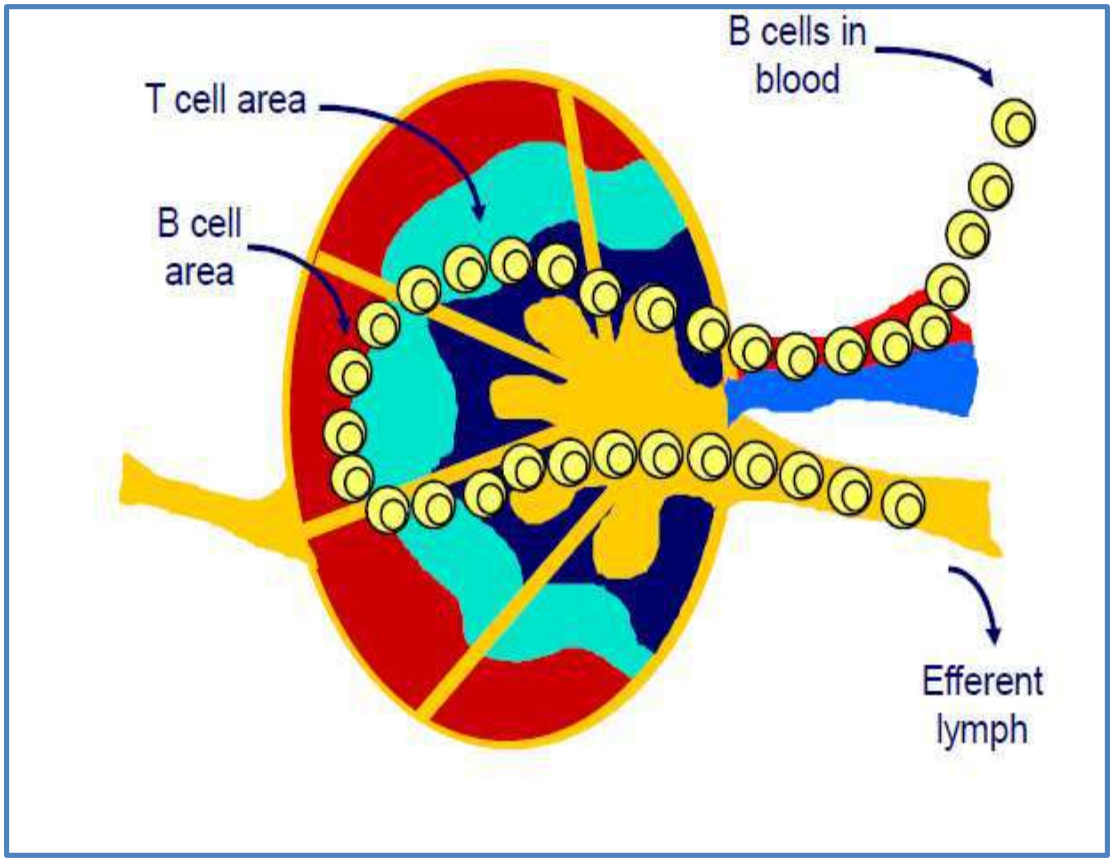
The B-cell co-receptor

- The B-cell co-receptor is expressed on mature B cells as a complex of the cell-surface molecules CD19, CD21, and CD81.
- Co-ligation of the B-cell receptor with its co-receptor amplifies signal 1000- to 10,000-fold.
- CD21 molecule is a complement (C3d) receptor – CR2.
- CD19 is also a B cell lineage marker



Activation of B cells

Recirculating B cells normally pass through lymphoid organs



Steps in activation of B cells

- Activation of naïve virgin mature B cell
  - Response to foreign Ag (interaction)
  - Occurs primarily in secondary lymphoid organs (lymph node and spleen) in the germinal centers
  - Enlarge to become B-cell "*blasts*" (activation)
  - Proliferate (divide) and differentiate (clonal expansion and differentiation)
    - Plasma cells → class switching
    - Memory B cells → class switch and affinity maturation, but non-proliferating, long-lived

Activation of a Naive B-lymphocyte (contd…)

- Initial B cell encounter with antigen may rely on several methods:
  - First, **small soluble antigens** may gain access to the follicles **directly** from afferent lymph vessels and be accessible to B cells as free diffusing antigen.
  - Second, **particulate opsonized antigen** (via ICs or C3d) can be presented to B cells as they migrate through the follicles, either **on the surface of specialized macrophages** located in the subcapsular sinus of lymph nodes or on the **surfaces of FDCs.**
  - Third, **dendritic cells may migrate into lymph nodes carrying unprocessed intact antigen** that antigen-specific B cells are able to remove from their cell surface

Activation of a Naive B-lymphocyte

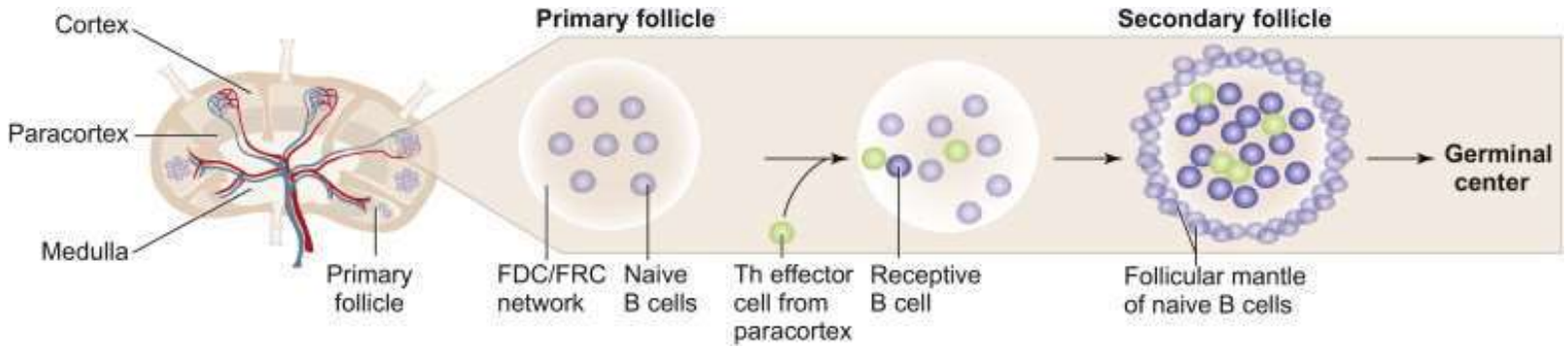
- Naïve B cells in primary follicles bind either free antigen or antigen trapped in the FDC/FRC network (ICs).

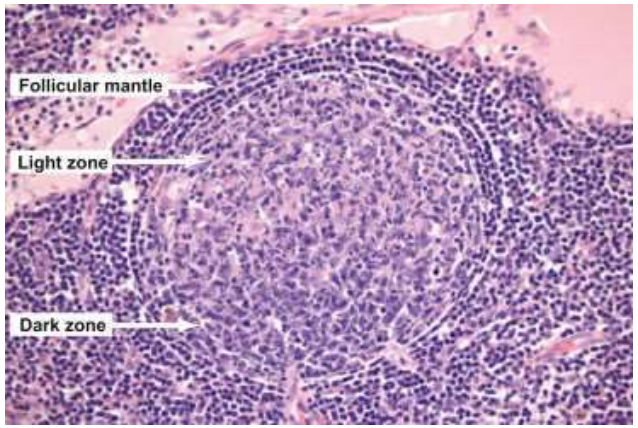
- Simultaneously, the same antigen is processed and presented as pMHCs by local APCs on their surface to appropriate Th cells in the paracortex, which in turn gets activated following interaction of its TCR with specific pMHCs

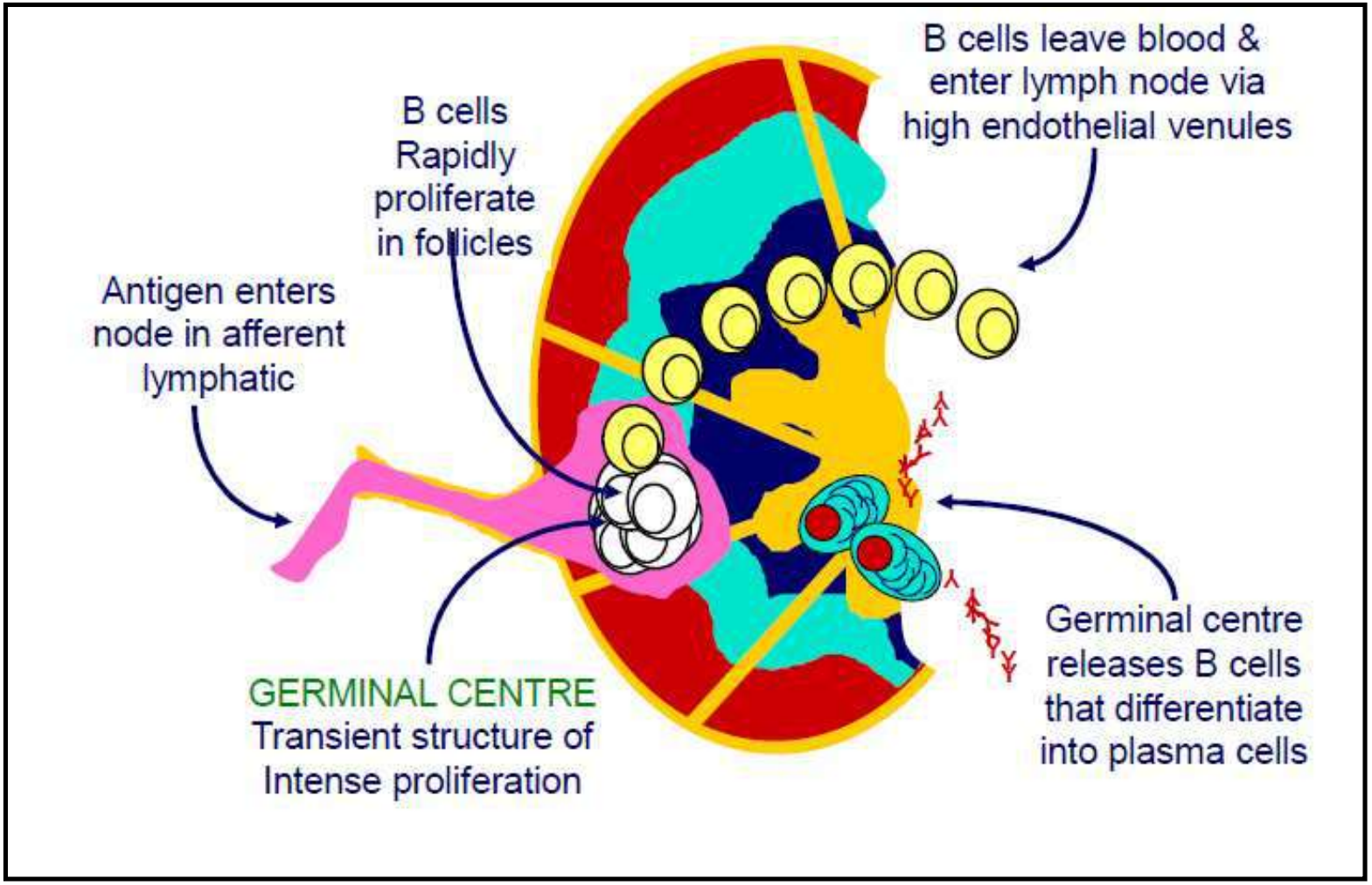
- The Th effector cell and receptive B cell meet at the outer edge of the primary follicle, where the B cell receives Th cell help through co-stimulatory molecules and cytokines.

- The B cell thus becomes fully activated, moves into the center of the follicle and proliferates generating **secondary follicles with germinal center**.

- The proliferating B-cells push naïve B cells to the edge of the follicle to form the **follicular mantle zone** of secondary follicles


Cortex
Paracortex
Medulla
Primary follicle
Primary follicle
Secondary follicle
FDC/FRC network
Naive B cells
Th effector cell from paracortex
Receptive B cell
Follicular mantle of naive B cells
Germinal center


Follicular mantle
Light zone
Dark zone


B cells leave blood & enter lymph node via high endothelial venules
B cells Rapidly proliferate in follicles
Antigen enters node in afferent lymphatic
GERMINAL CENTRE
Transient structure of Intense proliferation
Germinal centre releases B cells that differentiate into plasma cells

Signals for activation of a Naive B-lymphocyte

- The **first signal** in the activation of a naive B-lymphocyte by a T-dependent antigen is the **binding of an epitope of an antigen to a corresponding BCR** (sIg) on the surface of a B cell; naïve B cells in primary follicles bind free antigen or antigen trapped in the FDC/FRC network as IC

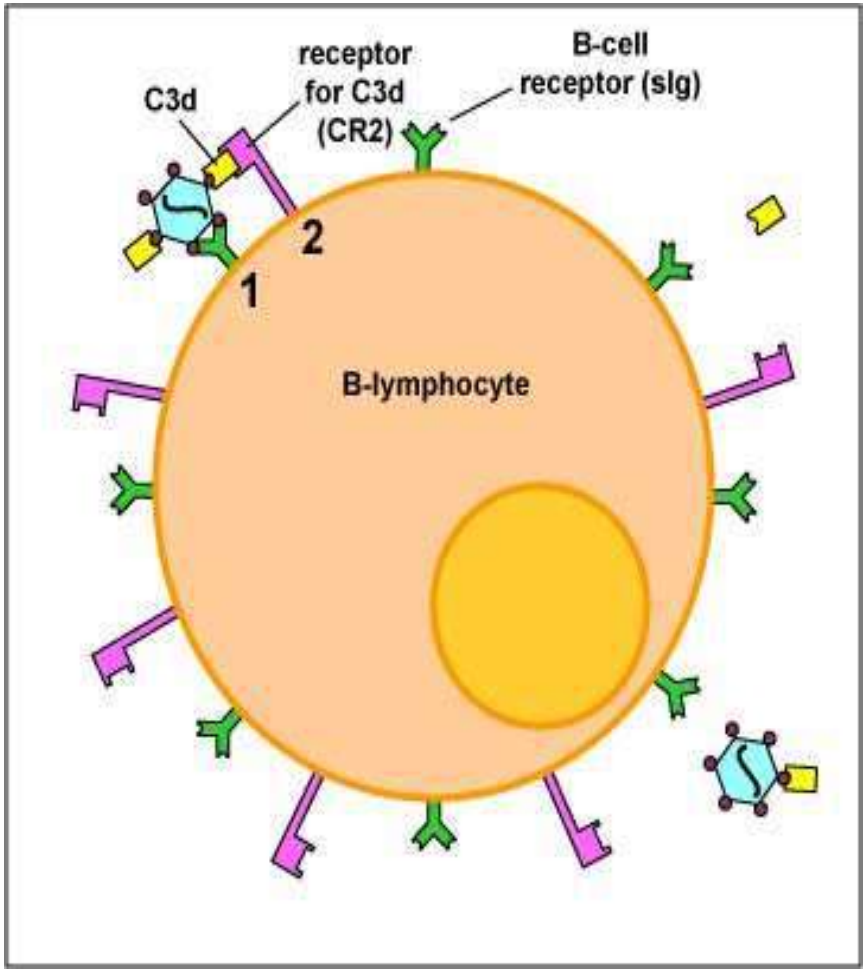
- The **second signal** is provided when a component of the complement system **C3b binds to the microbial surface, subsequently degraded to C3d** which, in turn, **binds to a complement receptor called CR2 on the surface of the B-cell**.

- **These events activate the naive B-lymphocyte** and enable it to produce increased amounts of MHC-II, co-stimulatory molecules such as B7/CD80 and CD40, and receptors for T-lymphocyte derived cytokines (e.g. IL-4R).

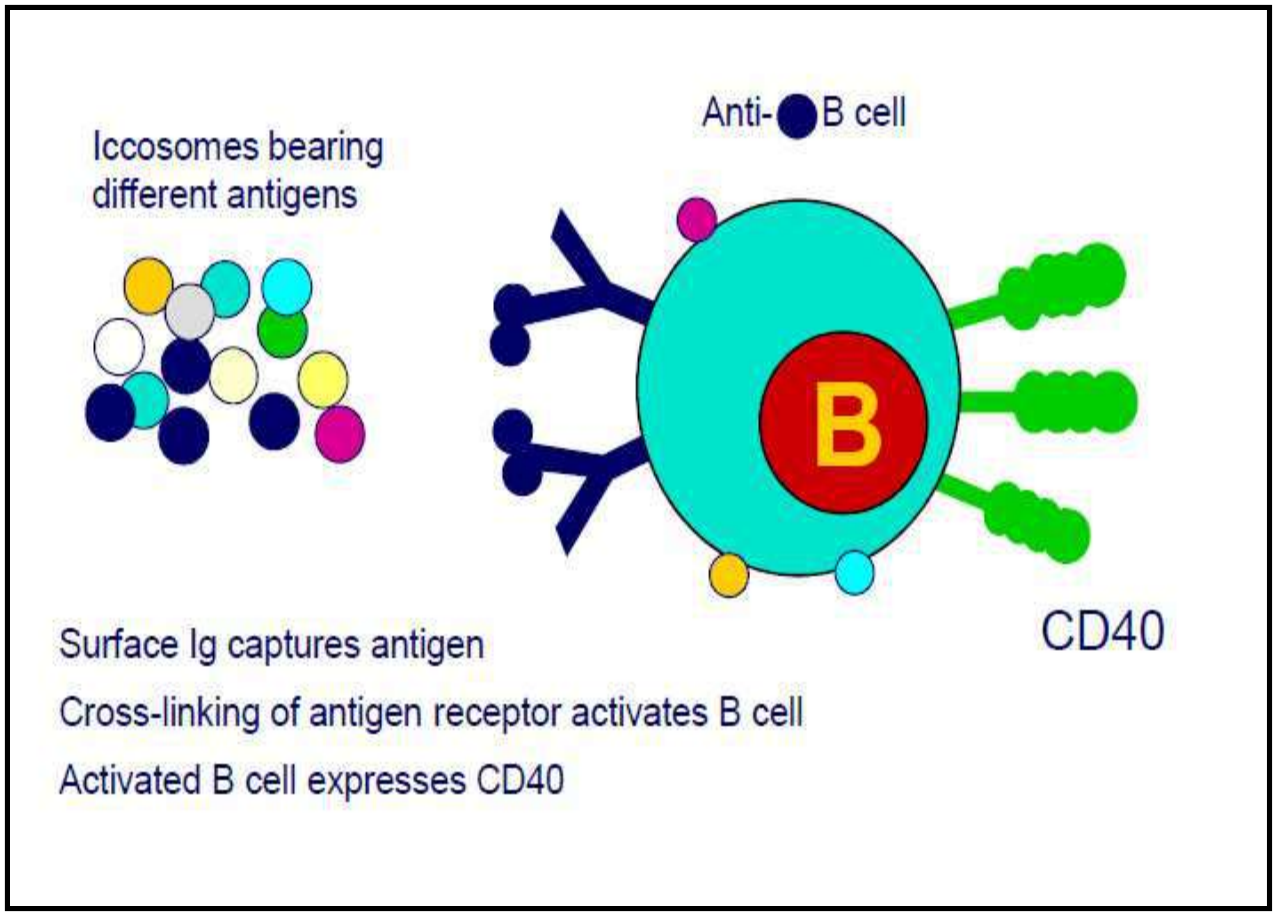
- Simultaneously **Th cells are in turn activated following interaction of its TCR with specific pMHCs on the surface of APCs** (e.g., DCs, B cells) and co-stimulation by latter.

- The activated Th cells provides the **third signal** to the B cells through the co-stimulatory molecules (e.g., CD40:40L) and cytokines (e.g., IL-4,5,13) and result in formation of B-Th cell conjugate (immune synapse); **without this signal activated B cell becomes anergic**

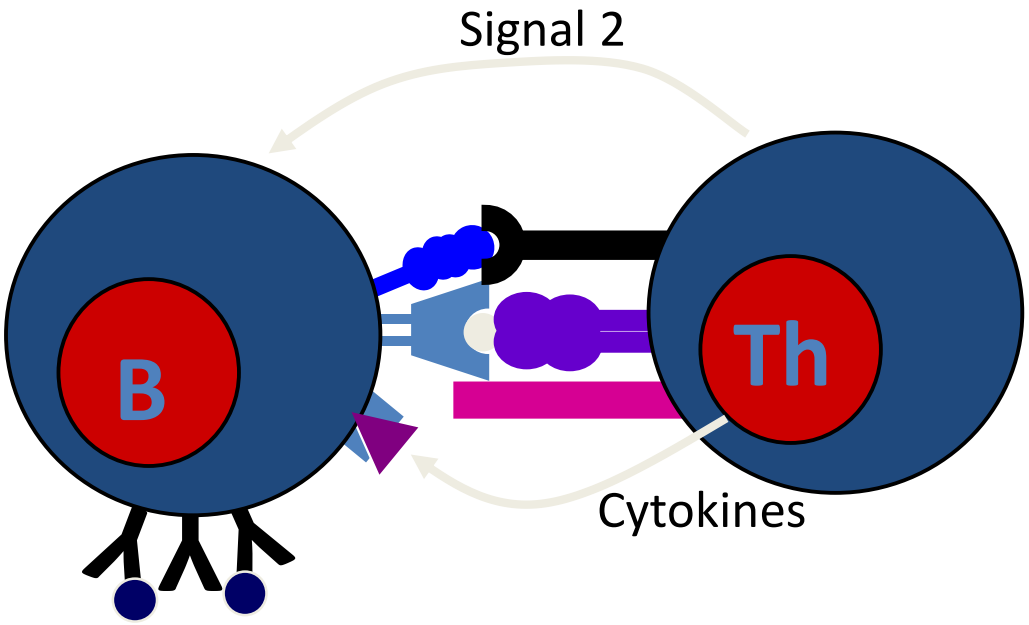
Activation of a Naive B-lymphocyte



Signal 1 and 2 - Uptake of Iccosomes/native Antigen by B cells via sIg (BCR) and CD21 (C3dR)



Signal 3 - Th cell help



Cytokines	Costimulation
IL-4	CD40:CD40L
IL-5	B7:CD28
IL-6	
IFN-γ	
TGF-β	

B cells are inherently prone to die by apoptosis

Signal 1 & 2 upregulate Bcl-X_L in the B cell and Bcl-X_L prevents apoptosis

Th cells regulate the survival of B cells and control the clonal selection of B cells

Th cells activates hypermutation in B cell clones

Only B cells with high affinity for antigen can express CD40 and thus can receive signal 2 from Th cell **(affinity maturation)**

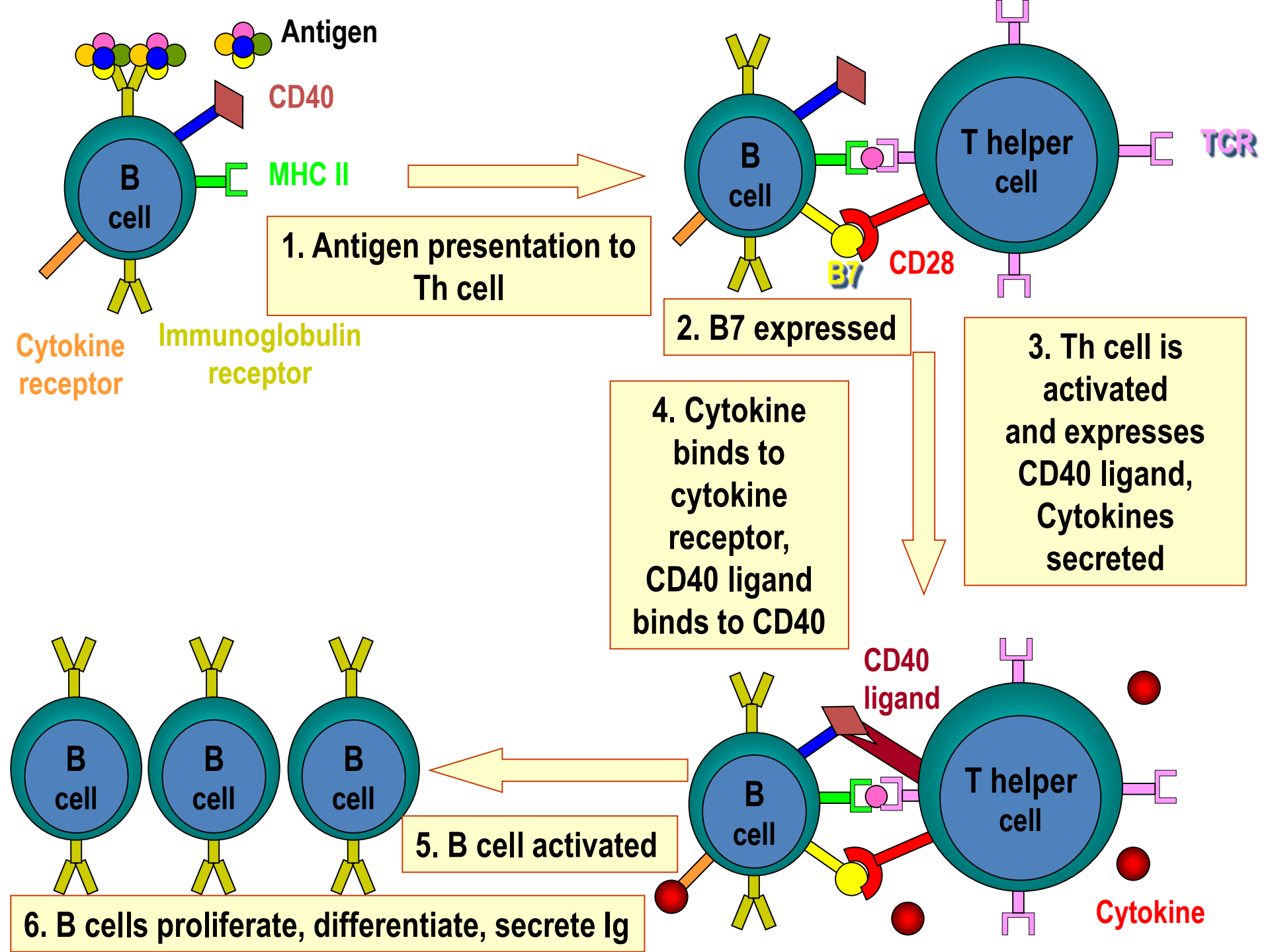
Only these B cells are rescued from apoptosis i.e. clonally selected

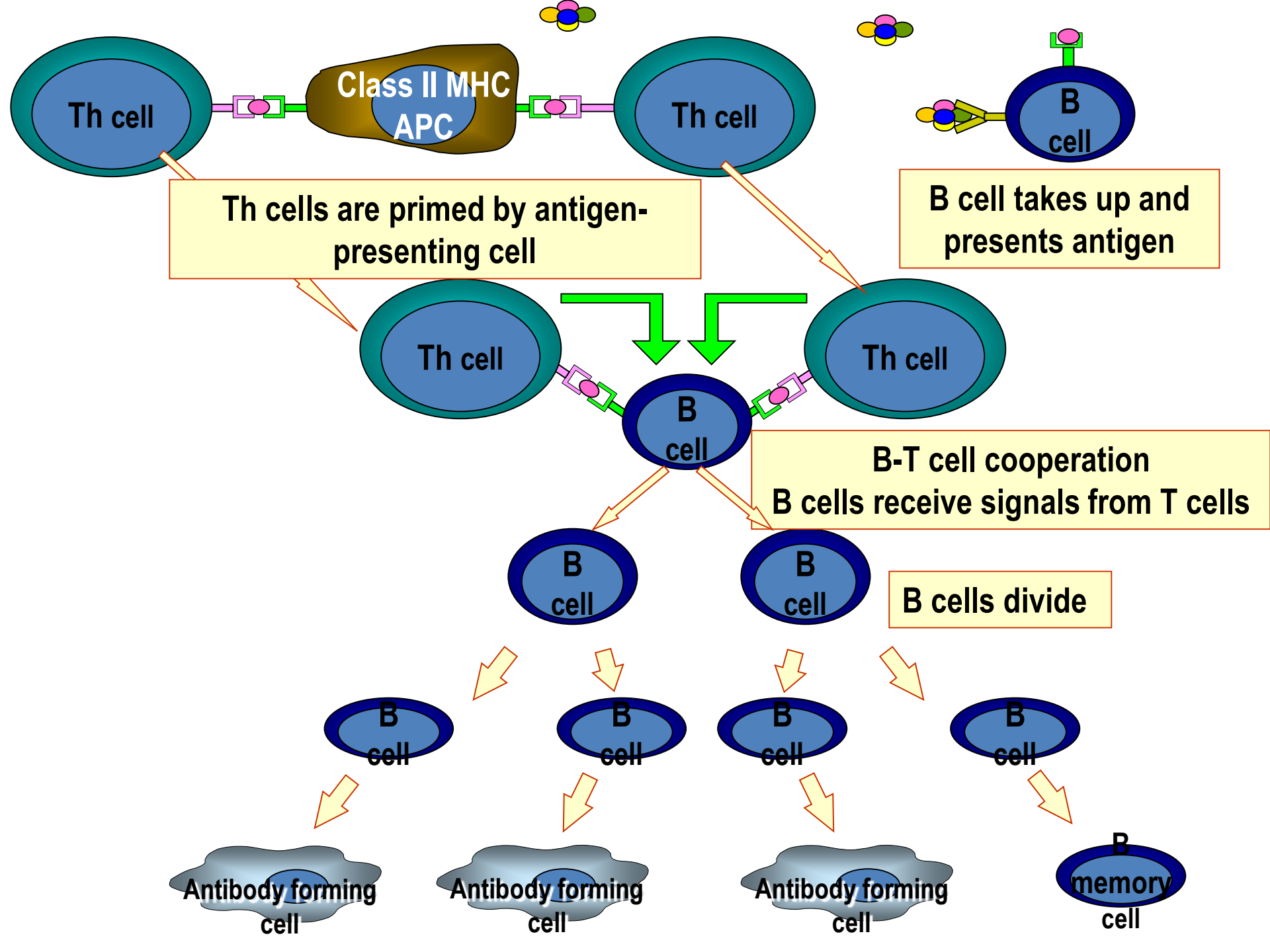
Activation of a Naive B-lymphocyte (contd…)

- About 4–6 days after antigen contact, the activated B cell undergoes one of two fates.
  - In some cases, the B cell on the edge of the follicle immediately proliferates and terminally differentiates into a population of **short-lived plasma cells** without undergoing isotype switching or somatic hypermutation.
  - In other cases, the B cell drags back into the center of the follicle where they proliferate, undergoes clonal expansion and few become **long-lived plasma cells** while the remainder will become memory B cells.
- T cells regulate the survival of B cells and control the **clonal selection of B cells**
- T cells activates **Somatic Hypermutation** (SMH) in B cell clones
- Only B cells with high affinity for antigen can express CD40 and can receive signal 2 from Th cell **(affinity maturation)**

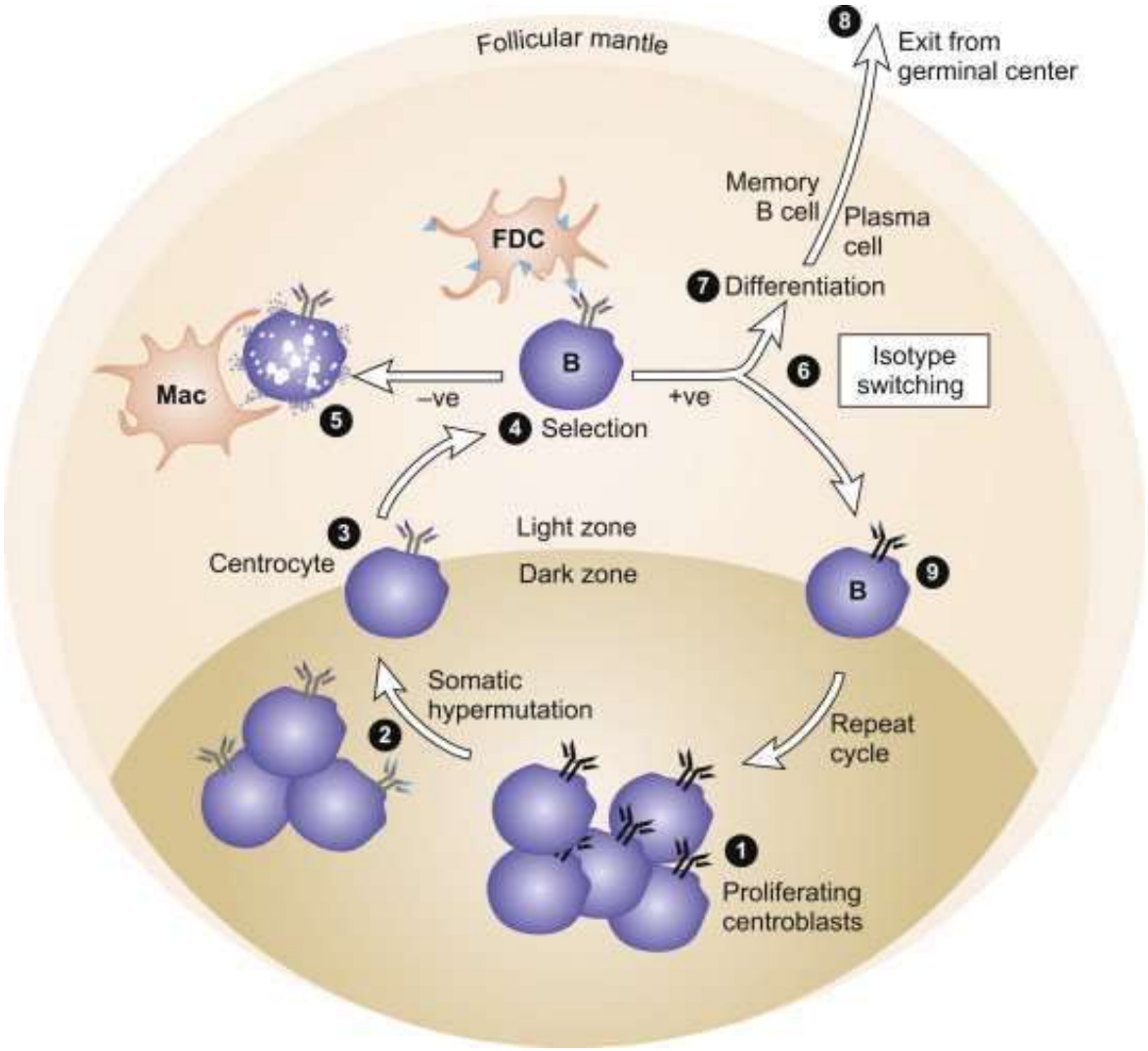
Activation of a Naive B-lymphocyte (contd…)

- A subset of these activated B cells from the germinal center will become **memory cells**, ready to respond faster and better than a naïve B cell the next time an individual encounters the same or a similar pathogen.
- Other activated B cells will terminally differentiate into **plasma cells**, which are unable to respond to T-cell help or antigen but possess specific characteristics:
  - (1) reduced surface immunoglobulin expression;
  - (2) lack of MHC class II expression;
  - (3)the inability to undergo class switching or somatic hypermutation;
  - (4) expanded endoplasmic reticulum reflecting;
  - (5) secretion of vast amounts of immunoglobulin.

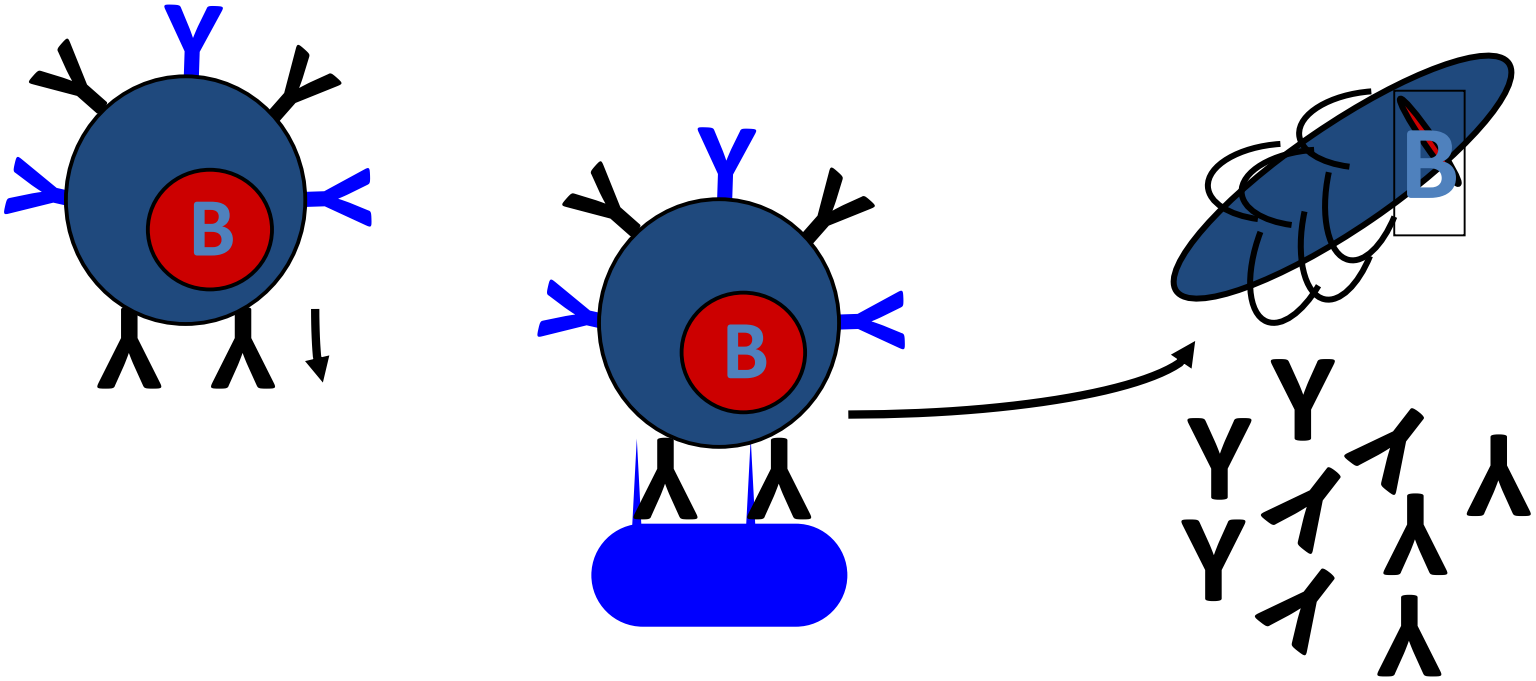

Antigen
CD40
MHC II
B cell
Cytokine receptor
Immunoglobulin receptor
1. Antigen presentation to Th cell
B cell
T helper cell
TCR
B7
CD28
2. B7 expressed
3. Th cell is activated and expresses CD40 ligand, Cytokines secreted
4. Cytokine binds to cytokine receptor, CD40 ligand binds to CD40
CD40 ligand
B cell
T helper cell
Cytokine
5. B cell activated
B cell
B cell
B cell
6. B cells proliferate, differentiate, secrete Ig


Th cell
Class II MHC
APC
Th cell
B
cell
Th cells are primed by antigen-presenting cell
B cell takes up and presents antigen
Th cell
Th cell
B
cell
B-T cell cooperation
B cells receive signals from T cells
B
cell
B
cell
B cells divide
B
cell
B
cell
B
cell
B
cell
Antibody forming cell
Antibody forming cell
Antibody forming cell
B
memory
cell

Activation and maturation of B cells



Differentiation in the periphery

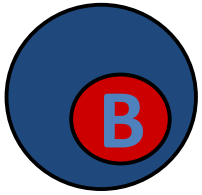
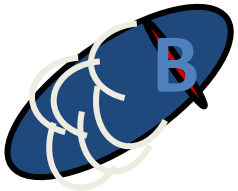


Mature peripheral B cell

B cell recognises non-self antigen in periphery

Ig-secreting plasma cell

Mature B cell versus Plasma cells

	Surface Ig	Surface MHC II	High rate Ig secretion	Growth	Somatic hypermution	Isotype switch
 Mature B cell	High	Yes	No	Yes	Yes	Yes
 Plasma cell	Low	No	Yes	No	No	No

Memory B-Cells

- Memory B-cells are created during primary immune response.
- Have high-affinity Ig receptors following affinity maturation during late phase of primary immune response.
- Therefore take up antigens at much lower concentrations than other antigen presenting cells that lack Ig antigen receptors.
- Memory B cells respond promptly and much more efficiently upon subsequent exposure to minute amounts of homologous antigens and rapidly differentiate into ASCs that produce substantially higher titres of protective antibodies (secondary immune response) than naïve B cells.

Memory B-Cells (contd…)

- After challenge, memory B cells enter into pre-existing GCs that were formed in the primary response, where they can expand and accumulate somatic hyper mutations.
- $CD4^+$ Th cells (effector and memory cells) play a pivotal role in humoral immunity by controlling the terminal differentiation of memory B cells.
- Class-switched (IgM→IgG/IgA) memory B cells express high levels of the co-receptors required for interaction with Th cell compared with naïve and GC B cells, improving memory cell response potency.
- Memory B cells also have a potent APC activity as compared with naïve B cells, which provides an effective activation of cognate helper T cells, resulting in increased efficacy of memory B cell activation.

SOMATIC HYPERMUTATION (SHM)

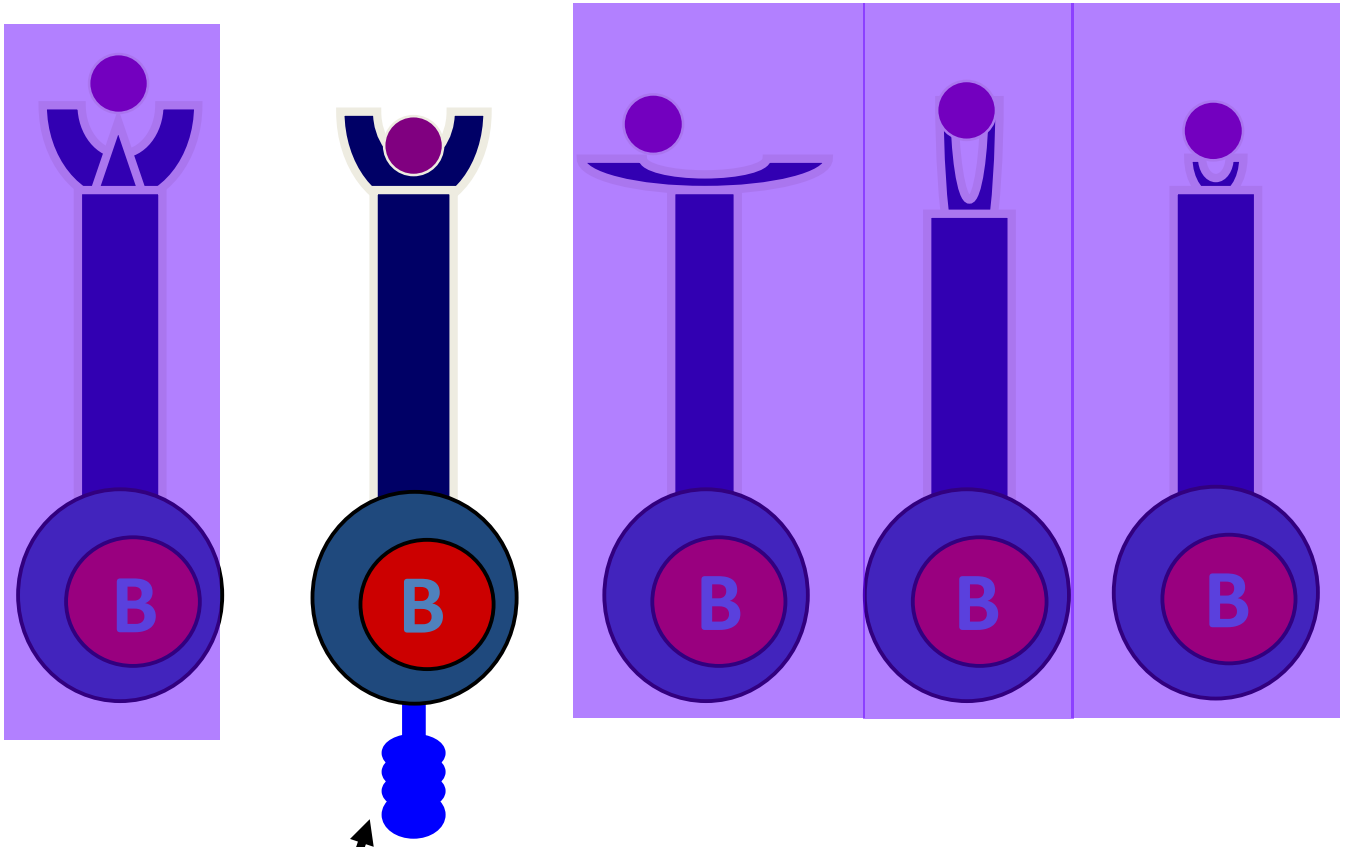
- **Somatic hypermutation** is a process in which **point mutations accumulate in the variable (V) regions** of both the heavy and light chains of an antibody molecule
- Somatic hypermutation occurs at the centroblast stage of B-cell differentiation in the germinal centers of secondary lymphoid organs.
- The overall goal of this process is **to produce high-affinity antibodies.**
- Somatic hypermutation is a key mechanism in generating antibody diversity

AFFINITY MATURATION

- Affinity maturation is the process by which by which Tfh cell-activated **B cells produce antibodies with increased affinity** for antigen during the course of an immune response.
- Affinity maturation is the result of somatic hypermutation coupled to clonal selection.
- Affinity maturation occurs within the GC, where somatically mutated BCRs undergo clonal selection on antigen retained on FDCs (antigen is retained in the form of ICs and involves the interaction of both complement receptors - CD21 and FcγRIIB – CD19 on the surface of B cells with these ICs on FDCs).
- With repeated exposures to the same antigen, a host will produce antibodies of successively greater affinities.

Control of Affinity & Affinity Maturation

Five B cell antigen receptors - all specific for ●, but with different affinities due to somatic hypermutation of Ig genes in the germinal centre



Only this cell, that has a high affinity for antigen can express CD40.
Only this cell can receive signal 2 from Th cell
Only this cell is rescued from apoptosis i.e. clonally selected

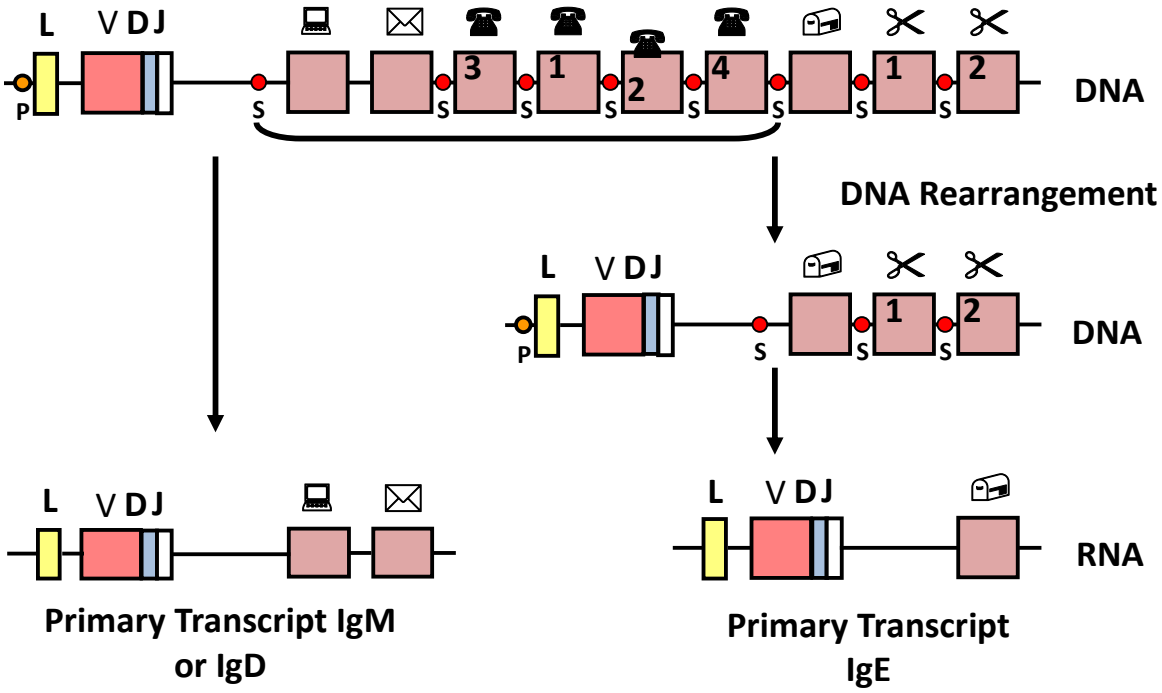
The cells with lower affinity receptors die of apoptosis by neglect

ISOTYPE SWITCHING

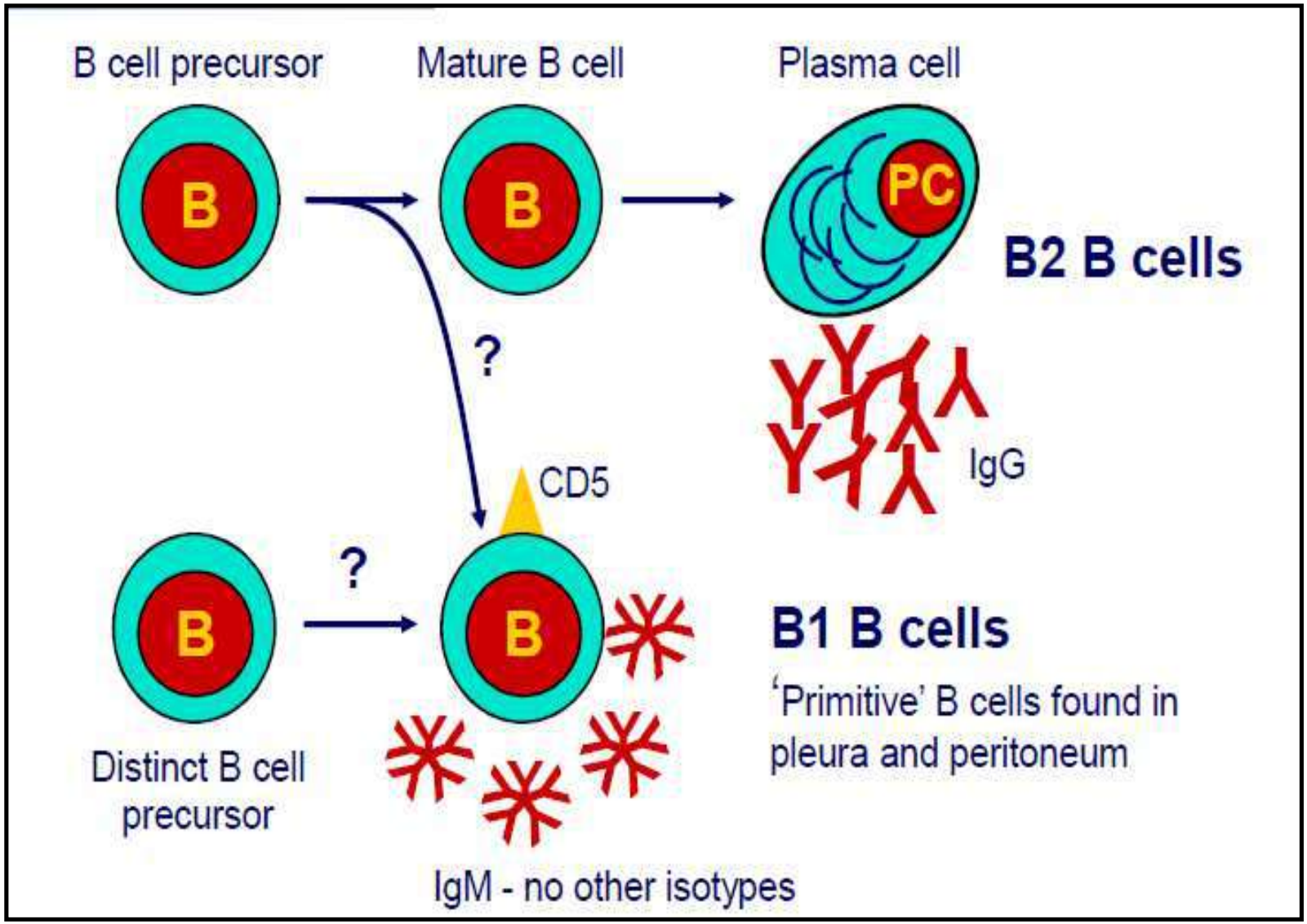
- **Isotype switching**, also known as **Immunoglobulin class switching,** isotypic commutation or **class-switch recombination** (CSR), is a biological mechanism that changes the production of immunoglobulin from one isotype to another, such as from the isotype IgM to the isotype IgG, by a B cell
- Antibody class switching occurs **in mature B cells** in response to antigen stimulation and co-stimulatory signals.
- **Isotype switching does not have any effect on specificity of antibody molecule**.
- It usually occurs either in **later stages of primary immune response or in secondary immune response**.
- It occurs by a unique type of **intrachromosomal deletional recombination** within special G-rich DNA sequences, called **switch, or S-regions**, located upstream of each of the heavy chain constant (C_H) region genes**, except Cδ (IgD)**

ISOTYPE SWITCHING

- DNA rearrangement
  - Antigen dependent
  - Switch site
  - Same VDJ
  - T_H cytokines



TYPES OF B-CELLS



B-1 (CD5) BCells

- First B cells appearing in development
- Express surface IgM, little or no IgD
- CD5 surface marker
- Antibodies are of IgM isotype only with low avidity and can bind multiple different antigens (polyreactive)
- Recognize repeating epitope antigens such as phospholipids and polysaccahrides
- Contribute most of the IgM in adult serum (natural antibodies)
- Do not develop into memory cells
- Are self-renewing
- Reside in peripheral tissues
- Predominant lymphocyte in peritoneal cavity
- Response mainly against TI antigens, i.e. do not require T cell help

CD5 B-1Cells and Conventional B-2 Cells

Properties	CD5 B-1 Cells	B-2 Cells
Development	Early	Later
Renewal	Self Renewal	Replaced from bone marrow
Production of Ig	High	Low
Isotype secreted	IgM**>>**IgG	IgG>IgM
Bind multiple different ligands	Yes	No

Comparison of B-1 and B-2 B cell properties

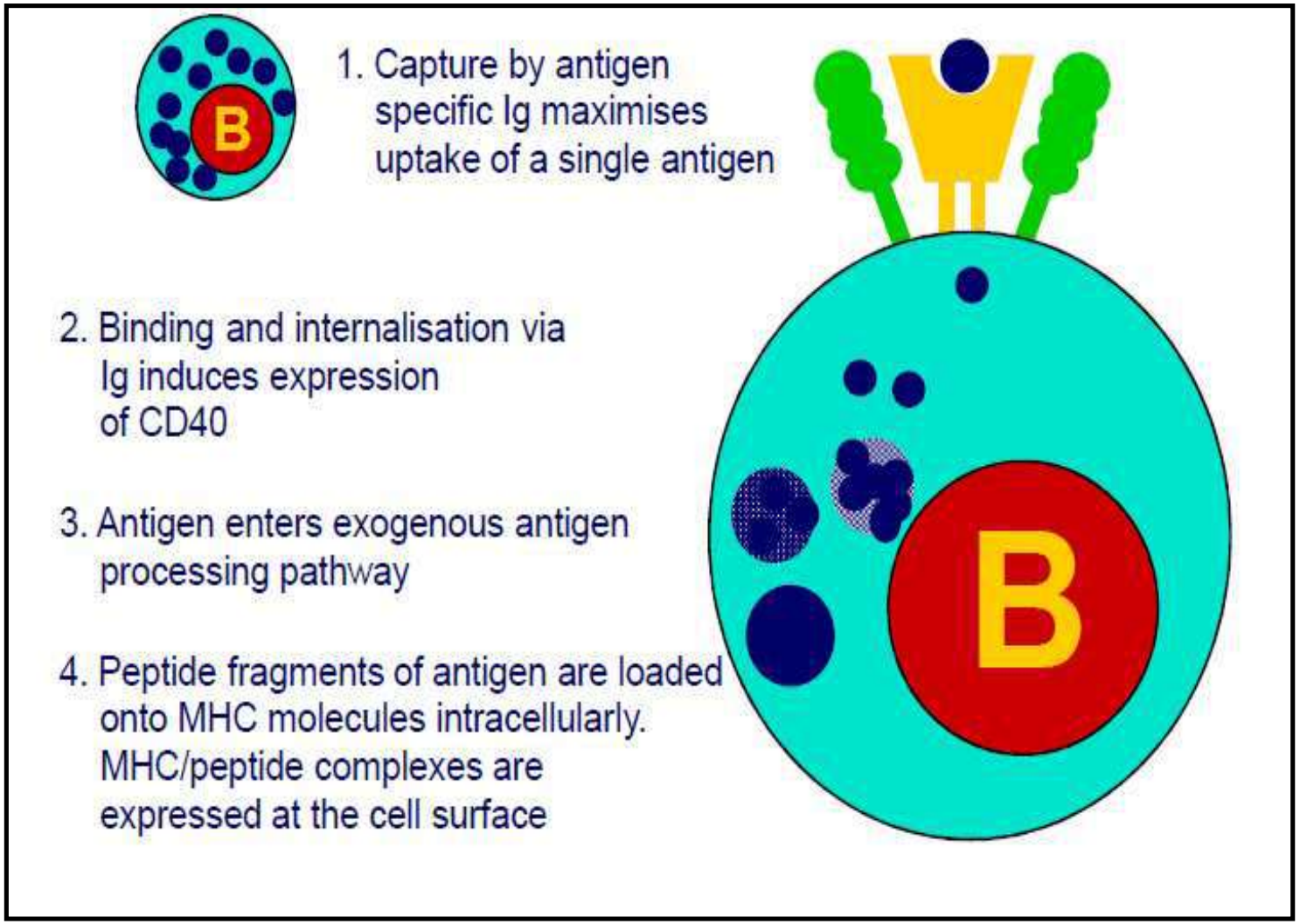
Property	B-1 cells	B-2 cells
N regions	Few	Extensive
V region repertoire	Restricted	Diverse
Location	Peritoneum/pleura	Everywhere
Renewal	Self renewal in situ	Bone marrow
Spontaneous Ig production	High	Low
Isotypes	IgM	IgM/G/A/D/E
Carbohydrate specificity	Yes	Rarely
Protein specificity	Rarely	Yes
Need T cell help	No	Yes
Somatic hypermutation of Ig	No	High
Memory development	No	Yes

Specificity & requirement for T cell help suggests strikingly different types of antigens are seen by B-1 and B-2 B cells

Marginal zone B cells

- MZ B cells are innate-like cells found in spleen and can be induced to differentiate into short-lived plasma cells in the absence of BCR ligation.
- MZ B cells can also mediate the transport of antigen as immune complexes into splenic follicles.
- In rodents, MZ B cells primarily appear to mediate T-independent responses to antigens in blood-born pathogens.
- MZ B cells may also participate in T-dependent immune responses to protein antigens, as well as in responses to lipid antigens.
- In human lymph nodes B cells in an outer extra-follicular rim have also long been called as MZ B cells. These cells could potentially also play a role in antigen capture and transport into lymph node B cell follicles
- MZ B cells also express high levels of CD1d, which could potentially be involved in the presentation of lipid antigens to NKT cells.

Processing and Presentation of internalised antigen (B-cells as APC)

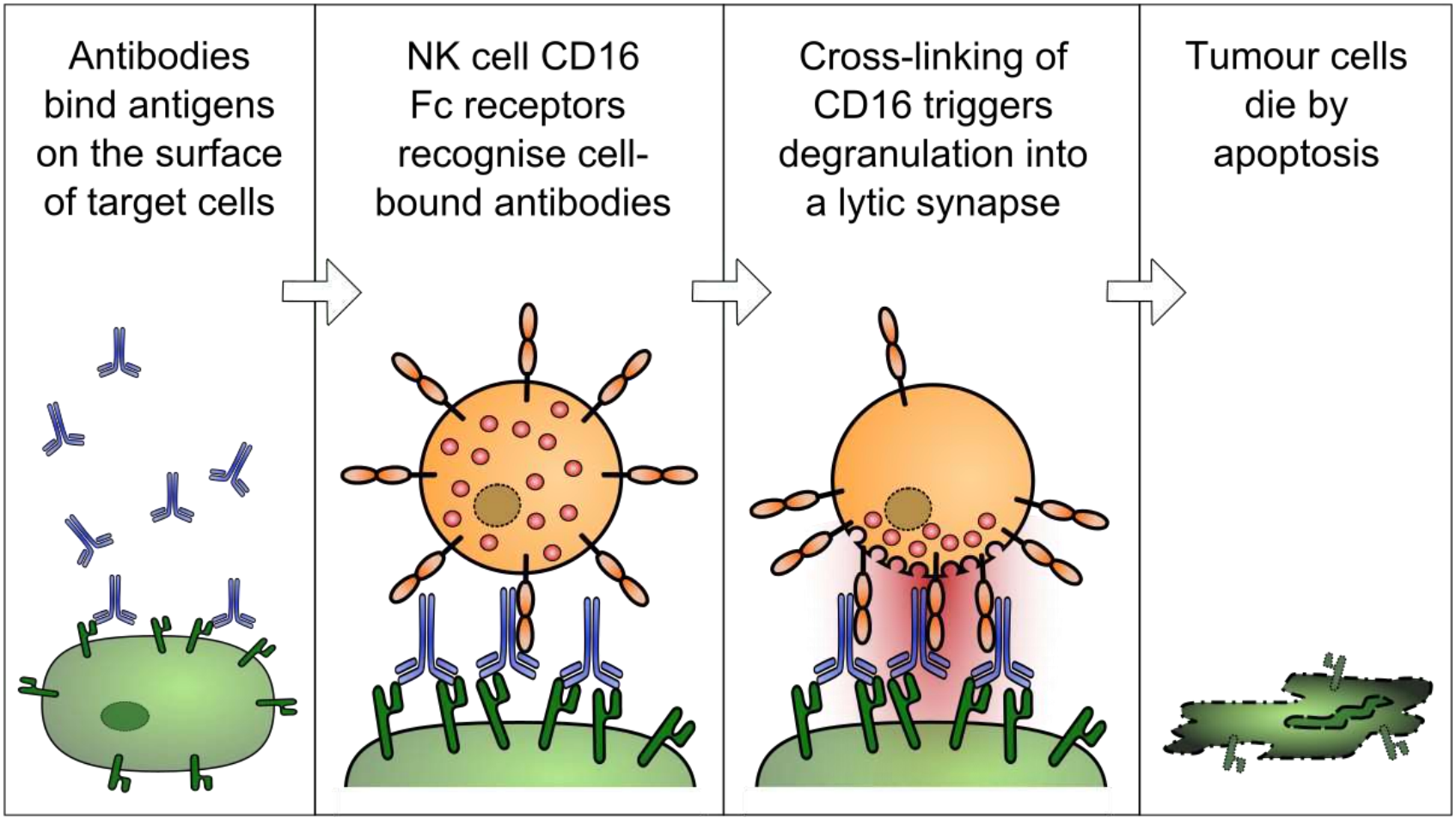


NATURAL KILLER CELLS
(ADCC)

Antibody dependent cell cytotoxicity (ADCC)

- **ADCC is a mechanism where effector cells (NK or NKT cells) secrete cytotoxic molecules and lyse antibody-coated target cells.**
- ADCC depends on the bifunctional structure of IgG molecules.
- The fragment antigen-binding (Fab) of the Ab molecule bind to a specific viral or TAA associated on the surface tumor or the target cell.
- The fragment Fc of Ab bind with FcγRIII (CD16) present on surface of NK cell.
- On engagement of both ADCC is initiated since this creates a bridge from the tumor/target cell to the effector NK cell.
- The recognition of target cells is then combined to a lytic attack on the target cell mounted by effector cells.
- ADCC does not depend on the immune complement system in which targets are also lysed but no other cell is required.

ADCC


Antibodies bind antigens on the surface of target cells
NK cell CD16 Fc receptors recognise cell-bound antibodies
Cross-linking of CD16 triggers degranulation into a lytic synapse
Tumour cells die by apoptosis

IMMUNE SYNAPSE

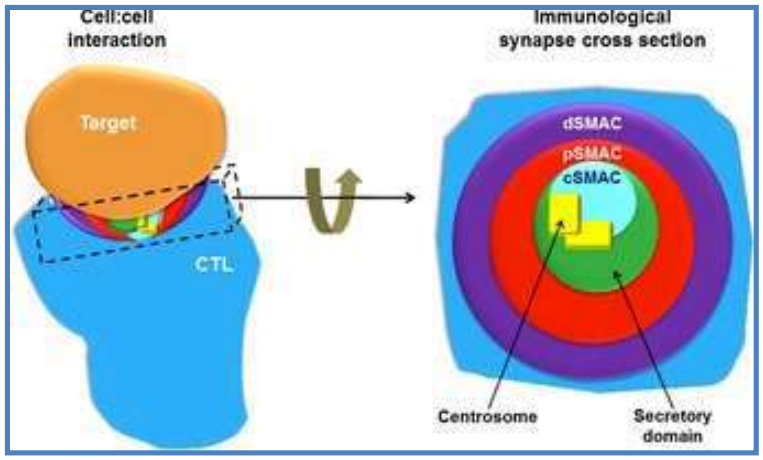
Definition and Introduction

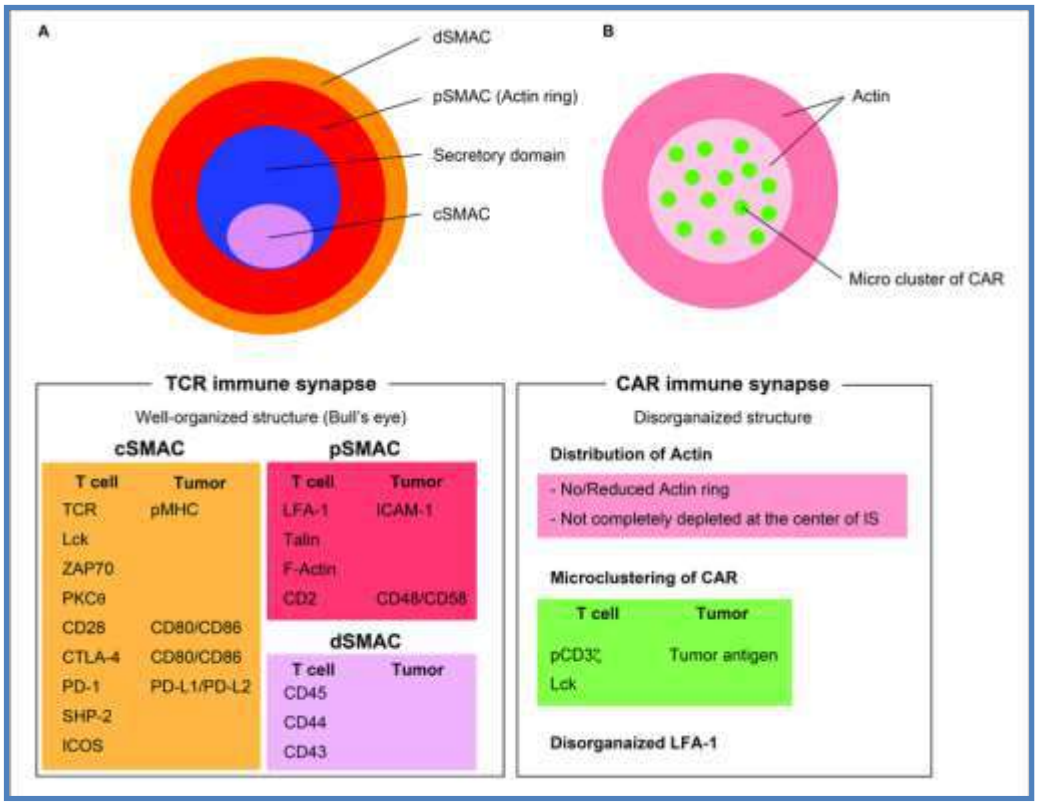
- Immune cells can communicate directly with each other by forming close cell–cell contacts that have become known as immune synapses.
- The immune synapse provides an important structure for communication with immune cells.
- In addition, the immune system makes use of the synapse during direct attack on infected and cancerous cells.
- Thus, it is only after the establishment of the synapse that cytotoxic T lymphocytes (CTLs) and natural killer cells deliver cytotoxic substances from cytolytic granules to destroy the target.
- Thus the immune synapse can be formed between an APC or target cell and a lymphocyte such as a T/B cell or NK cell
- This combination of a specialized junction, cell polarization, and positional stability bears a striking similarity to the classical synapse of the nervous system .

Structural features of the synapse

- The formation of immune synapses involves the reorganisation of receptors that are involved in recognition and adhesion to form specialised functional domains at the interface between two cells.
- The immune synapse is also known as the **supramolecular activation cluster** or **SMAC**.
- This structure is composed of concentric rings each containing segregated clusters of proteins—often referred to as the **bull's-eye model** of the immunological synapse:
  - **c-SMAC** (central-SMAC) composed of the TCR/BCR along with protein kinase C (PKC)-θ, CD2, CD4, CD8, CD28, and Fyn.
  - **p-SMAC** (peripheral-SMAC) - c-SMAC is surrounded by a ring of adhesion molecules – lymphocyte function-associated antigen 1 (LFA-1) and its adaptor cytoskeletal protein talin. This is called as the peripheral SMAC (pSMAC).
  - **d-SMAC** (distal-SMAC) - an accumulation of actin surrounding the pSMAC, enriched in CD43 and CD45 molecules

Structure of SMAC





Functions of the synapse

- Regulation of lymphocyte activation
- Transfer of peptide-MHC complexes from APCs to lymphocytes
- Enhancing signalling
- Directing secretion of cytokines or lytic granules
- Terminating signalling
- Balancing signalling