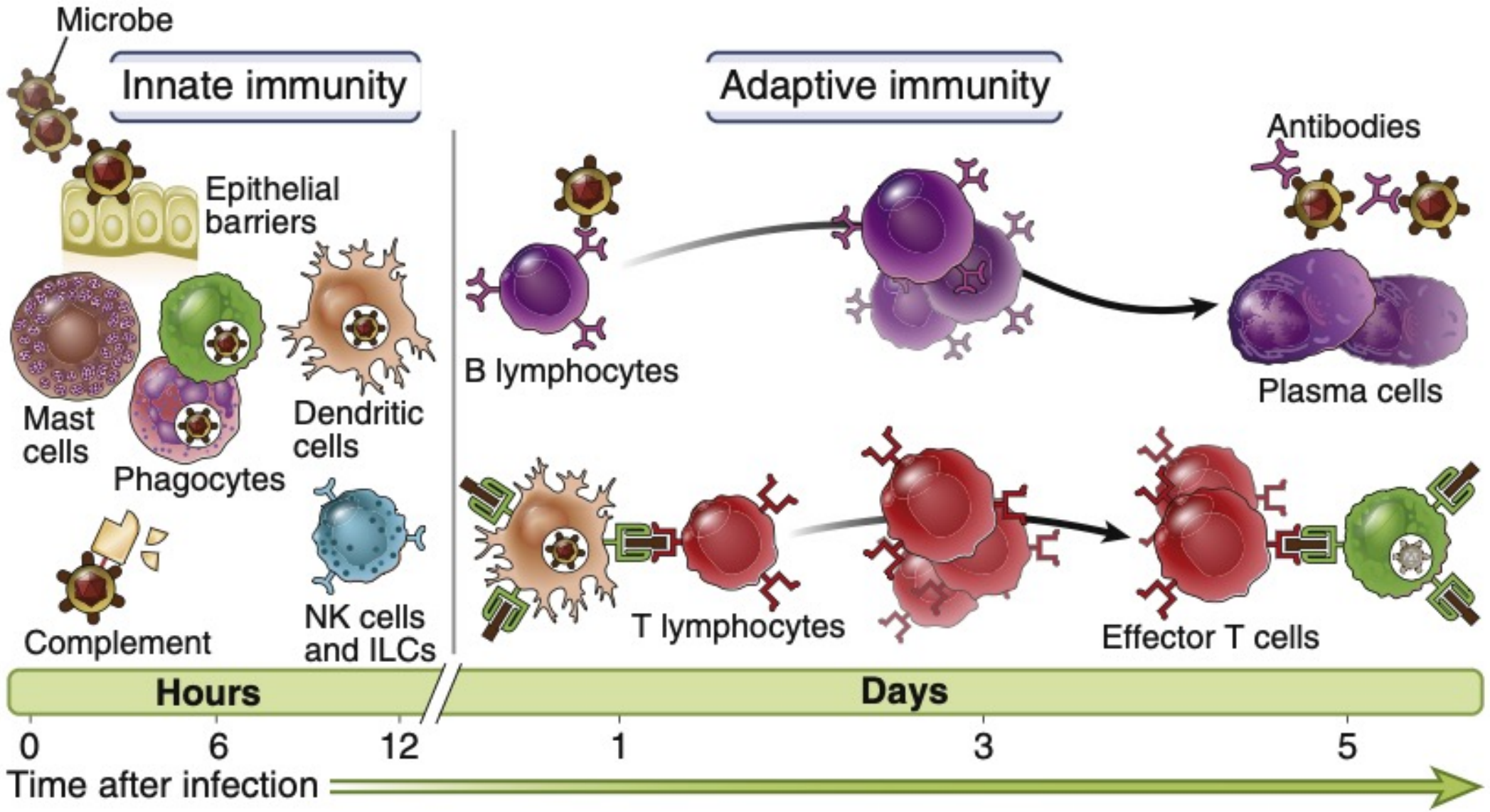
B-lymphocyte Development

R2 Yutthana/ Staff Yiwa
Allergy and Immunology PMK

Overview: Immune system

Protection		Surveillance	
Defect: immunodeficiency	Hyper-response: allergic disease	Abnormal cells: malignancy	Defect self detecting protein: autoimmune disease

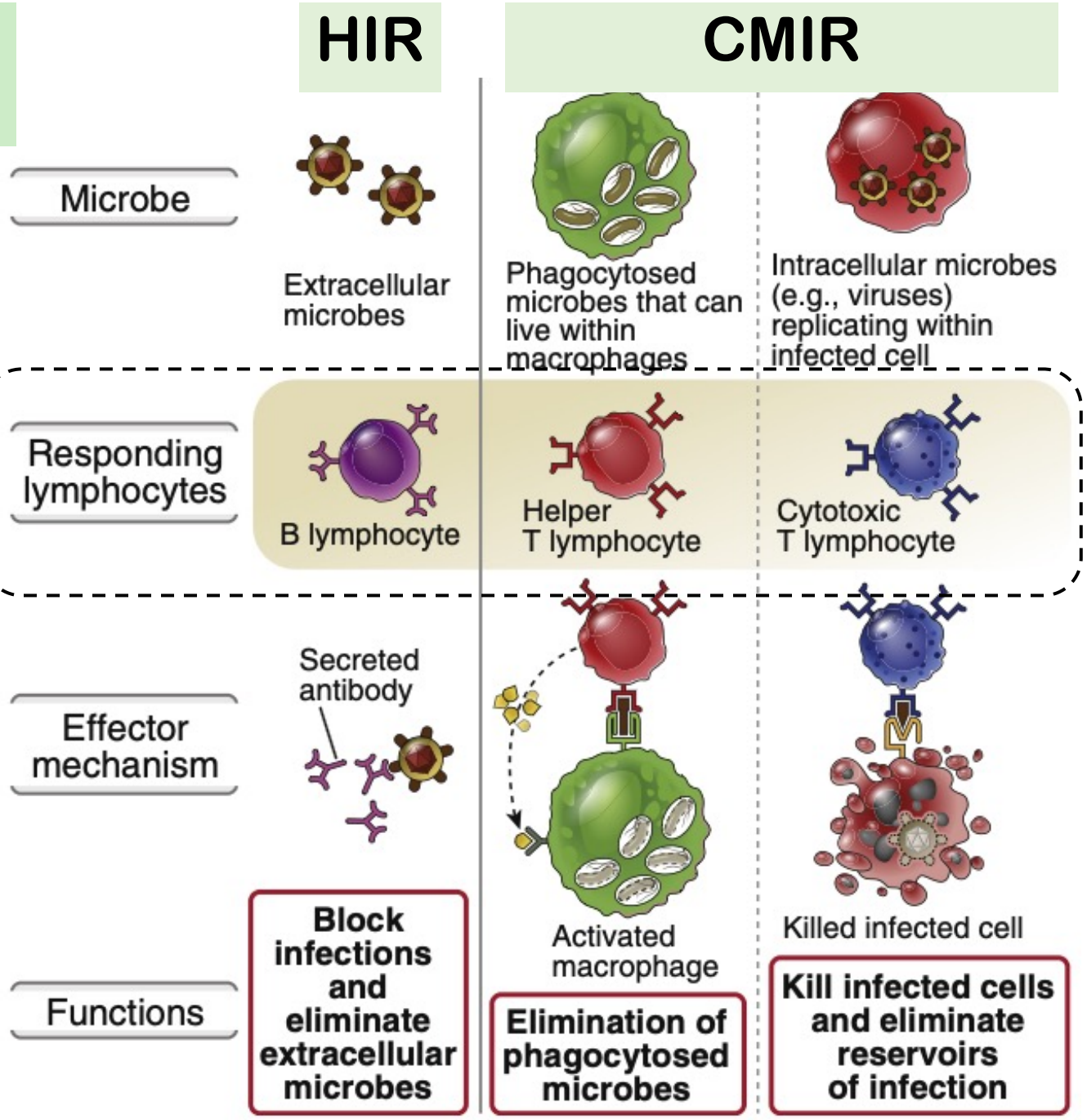
Principal mechanisms of innate and adaptive immunity

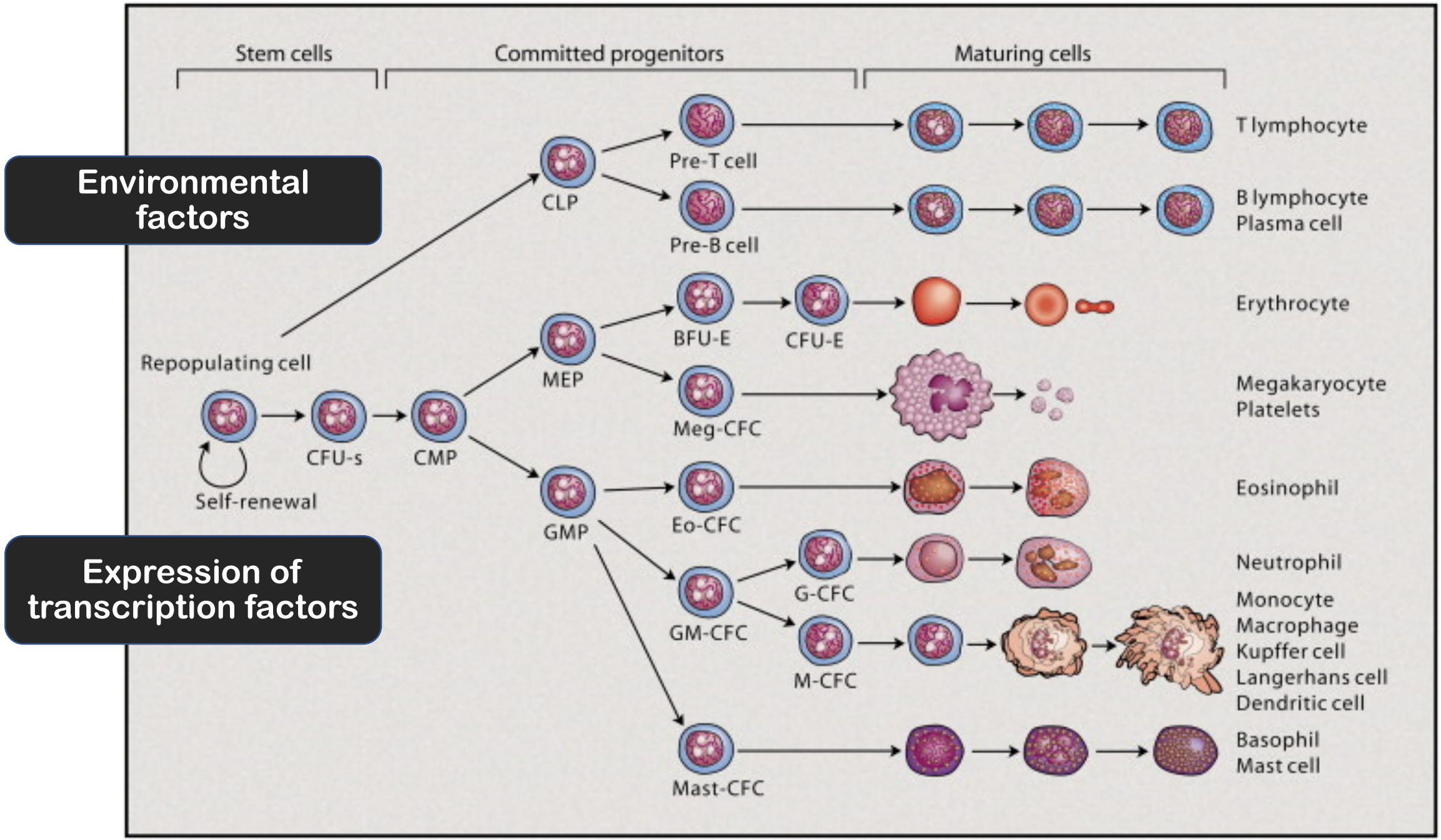


Types of adaptive immunity

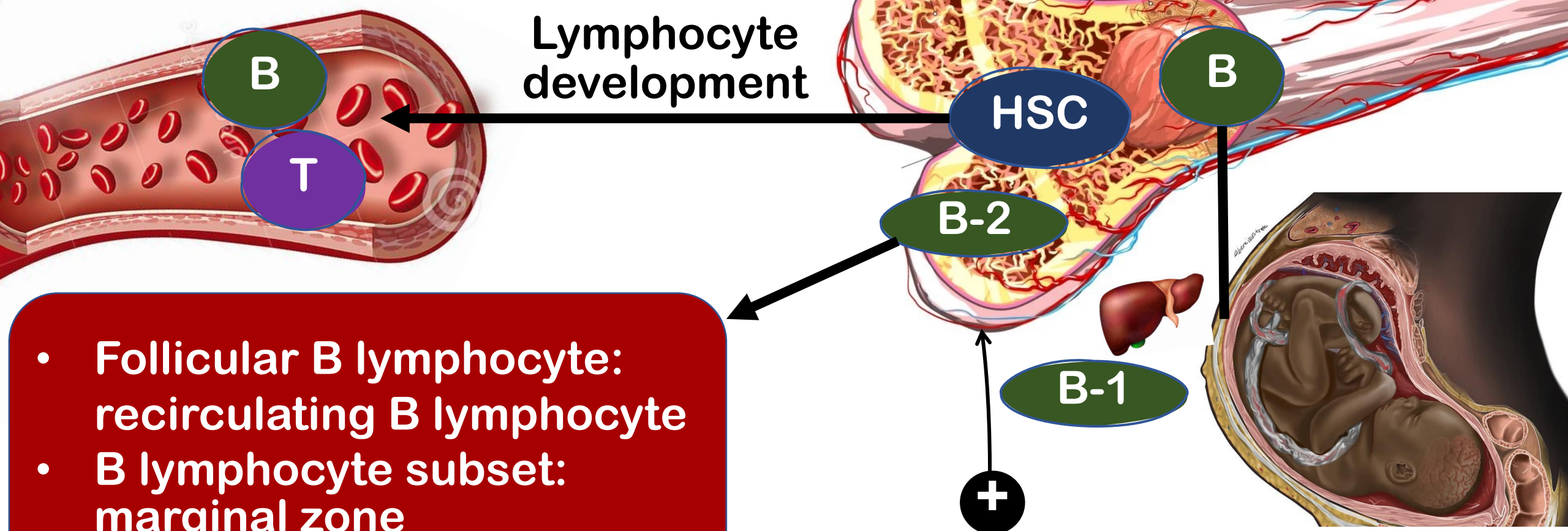
Lymphocytes play major role for adaptive immunity.

Where are lymphocyte come from ???



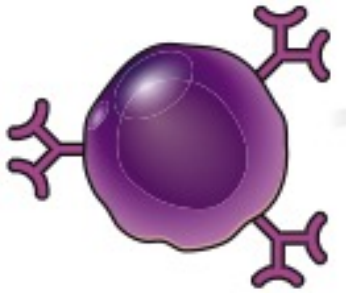


Metcalf, D. (2007, June 22). On hematopoietic stem cell fate. Immunity.



- Follicular B lymphocyte: recirculating B lymphocyte
- B lymphocyte subset: marginal zone

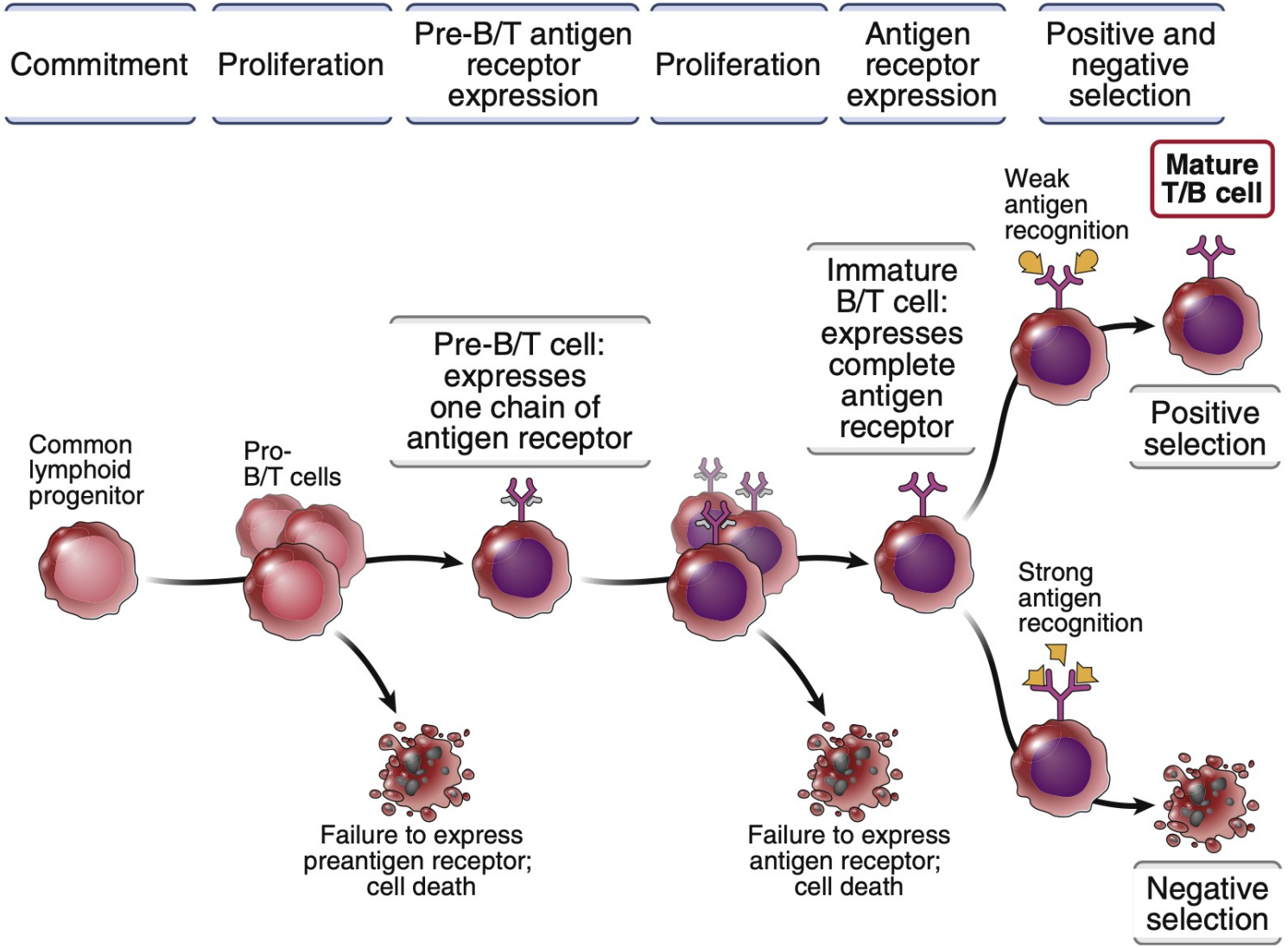
Surface receptor



Transcription factors

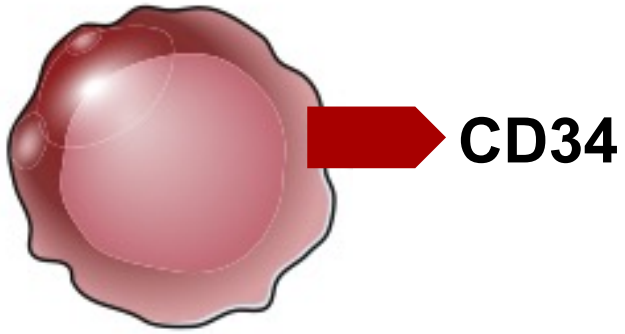
Lymphocyte development

Steps in maturation of lymphocytes

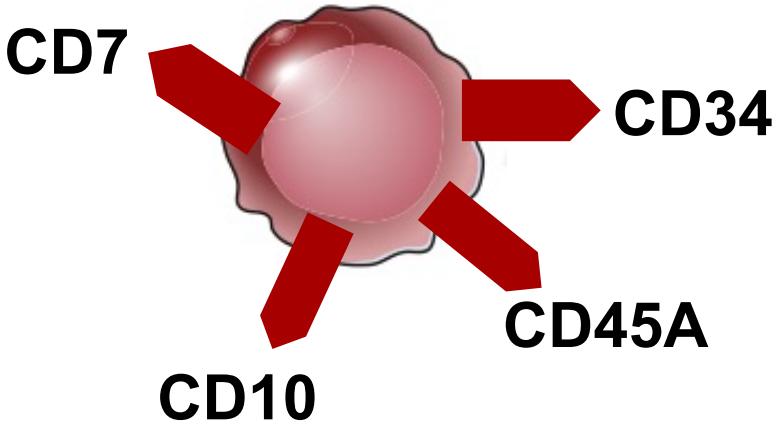


First Steps is commitment

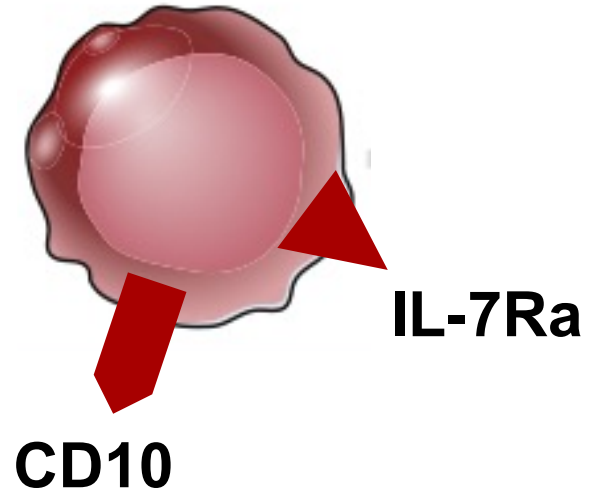
Hematopoietic stem cells



Common lymphoid progenitor

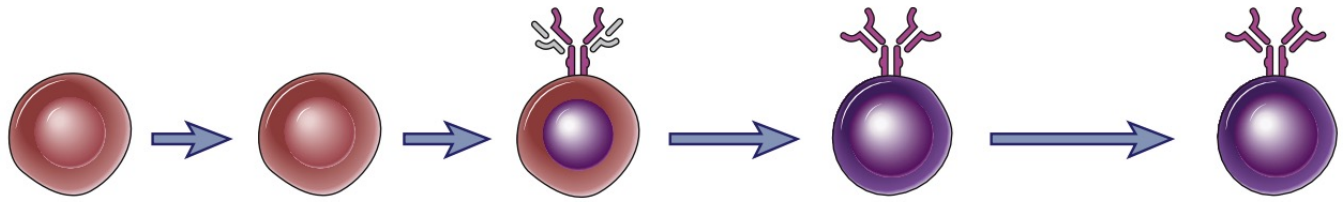


B-cell precursor



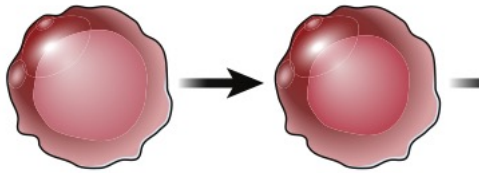
The cluster of differentiation (CD) is group of surface protein used for the identification and investigation of cell surface molecules present on leukocytes.

Steps in maturation of lymphocytes



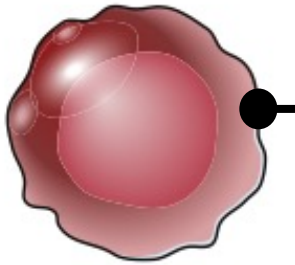
Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
RAG expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgM^{hi}
Anatomic site	Bone marrow				Periphery
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

Early B Lymphocytes Maturation



	Stem cell	Pro-B
Ig DNA, RNA	Germline DNA	Germline DNA
Ig expression	None	None

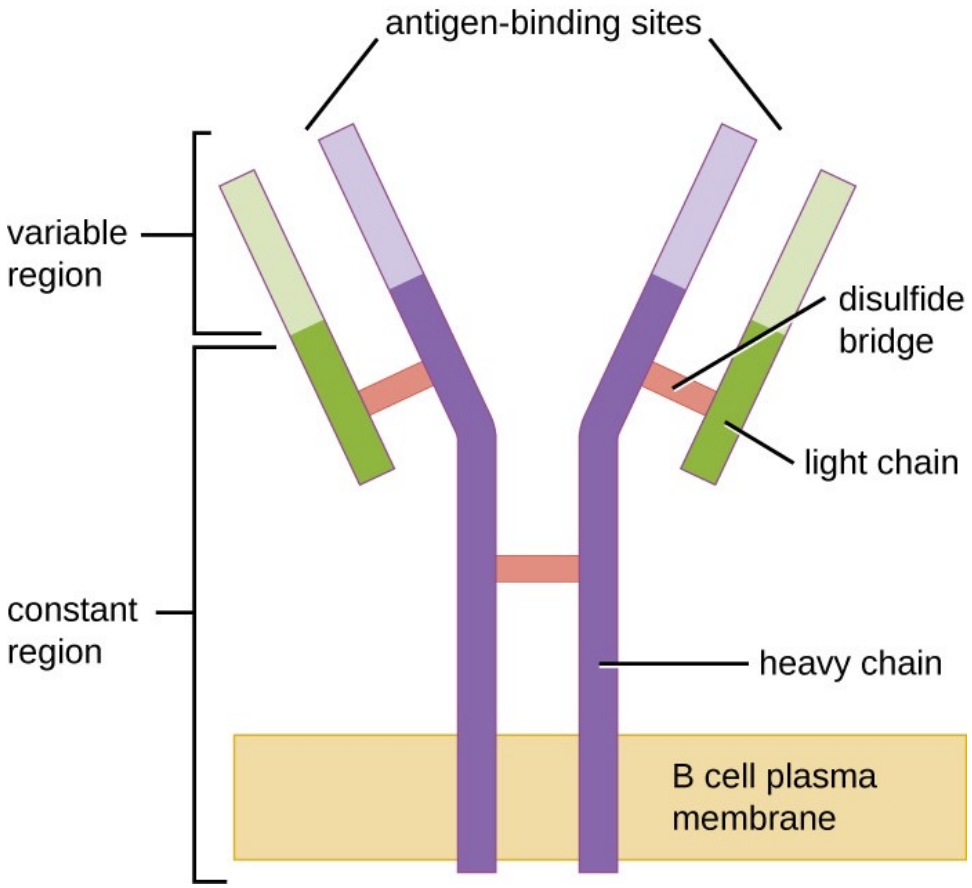
- The Ig **heavy-** chain locus rearranges **first**
- Only cells that are able to make an Ig μ heavy-chain protein are selected to survive and become pre-B cells.
- Require IL-3, IL-7, IGF-1

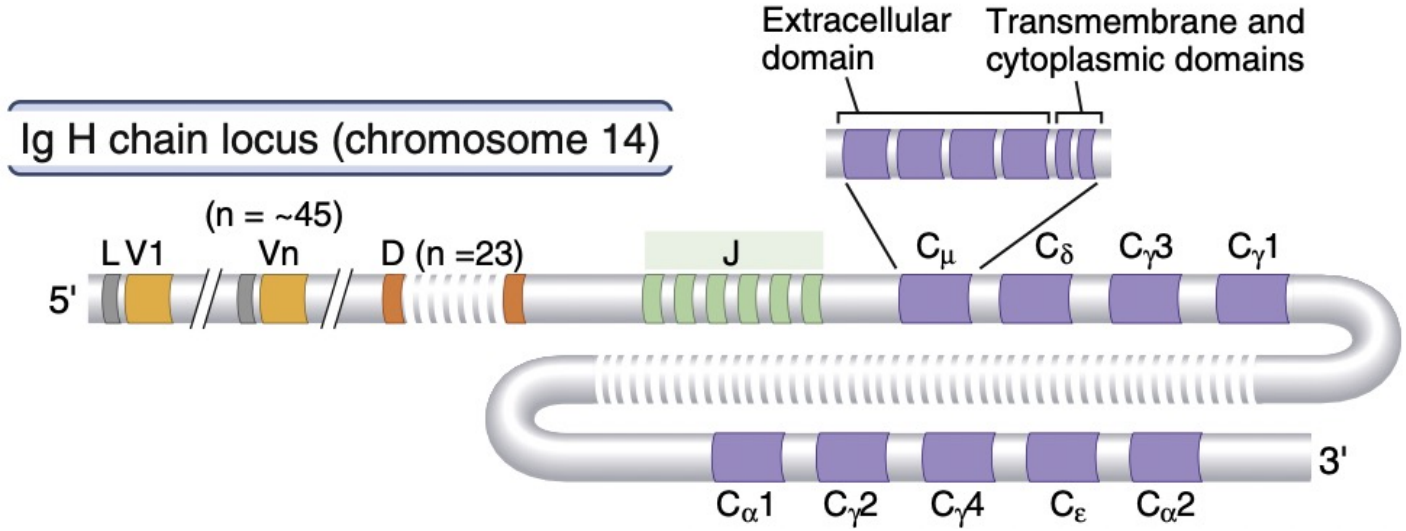


No surface Ig or BCR

Pro-B cells

B Cell Receptor or surface immunoglobulin structure

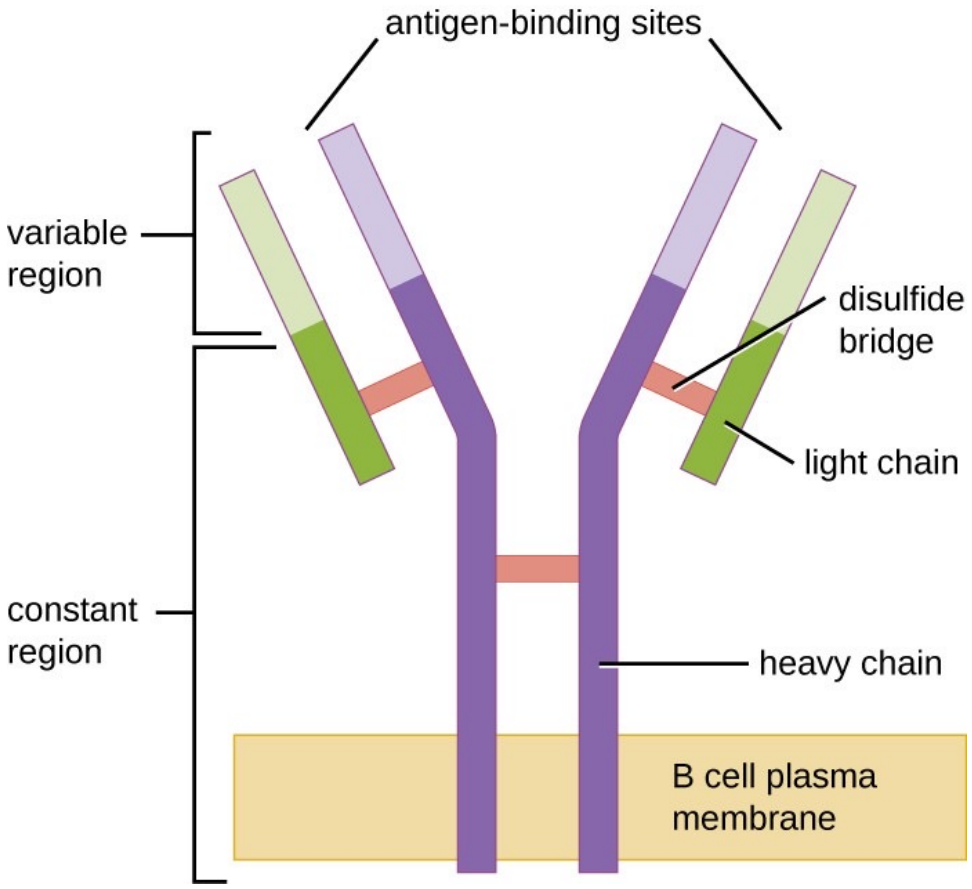


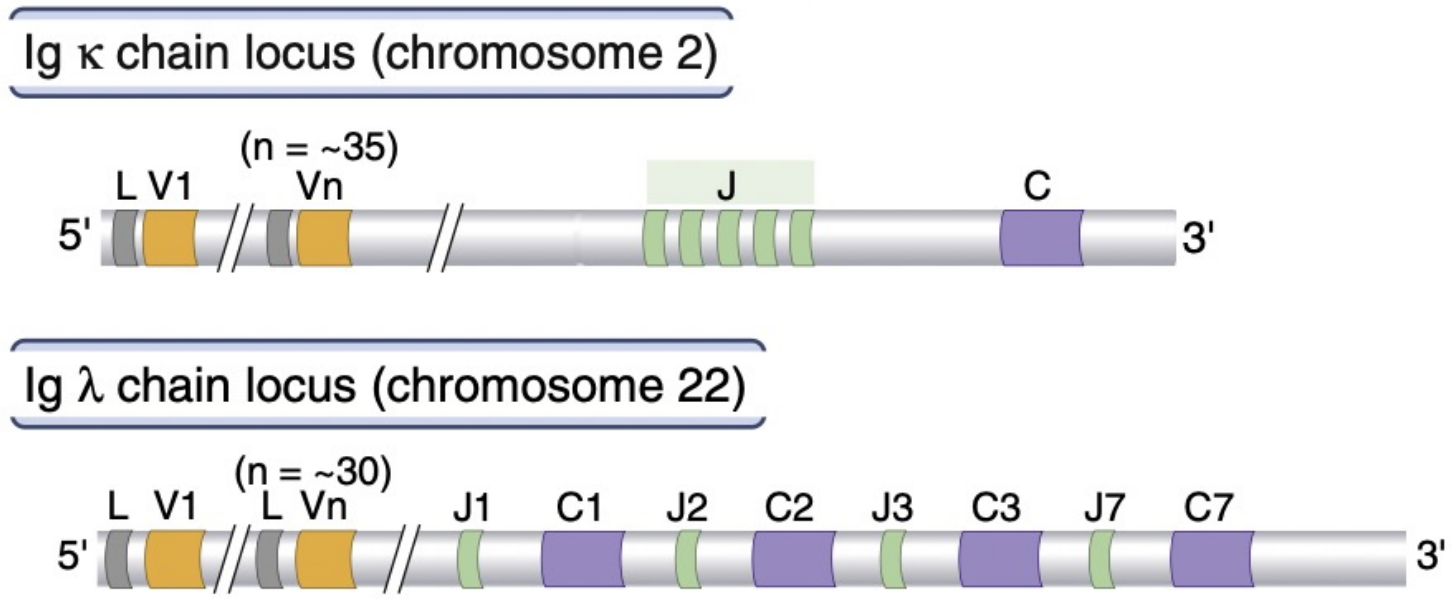


- **Heavy chain:** 300 V, 20 D, 5J segments
  - D-J recombination
  - V-DJ recombination

Two heavy chains and two light chains

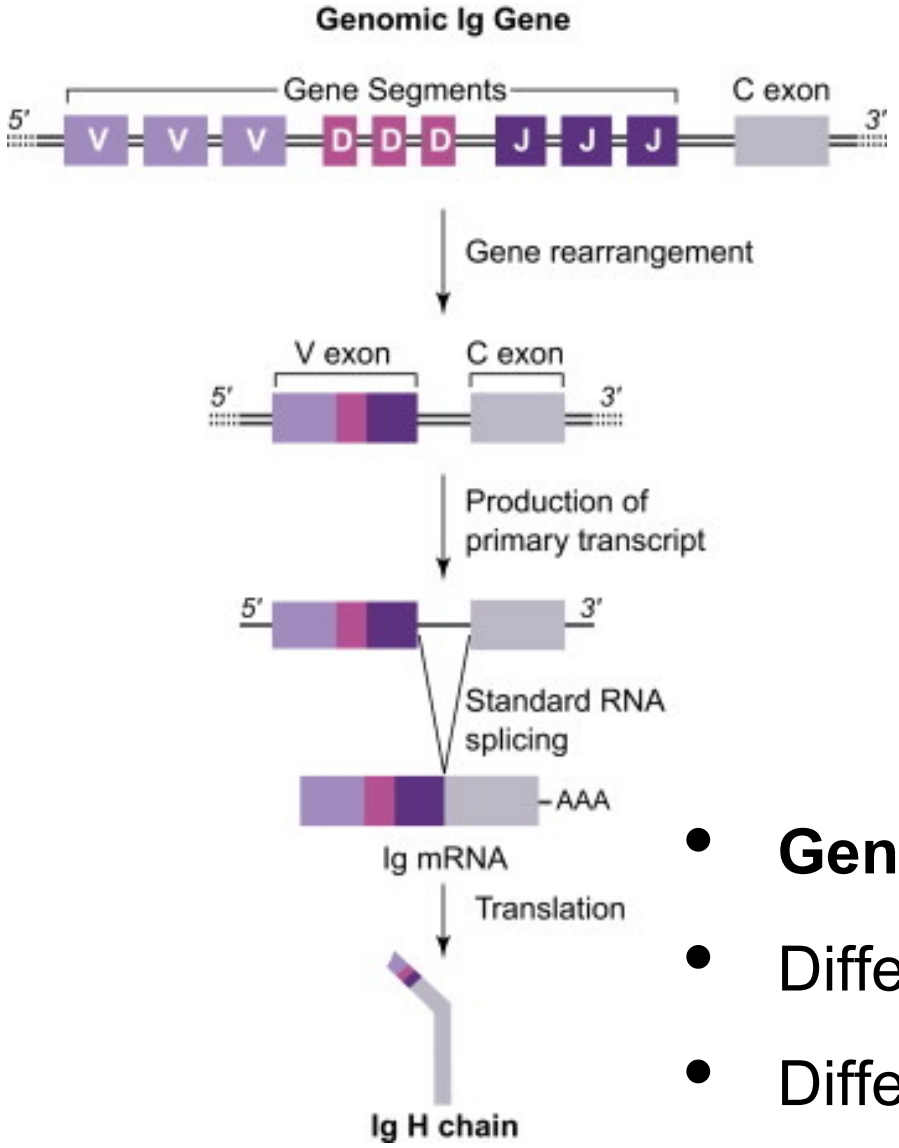
B Cell Receptor or surface immunoglobulin structure





- **Light chain:** V-J recombination
  - 100 V, 4J segments

Gene rearrangement



Removal unwanted D and J segment

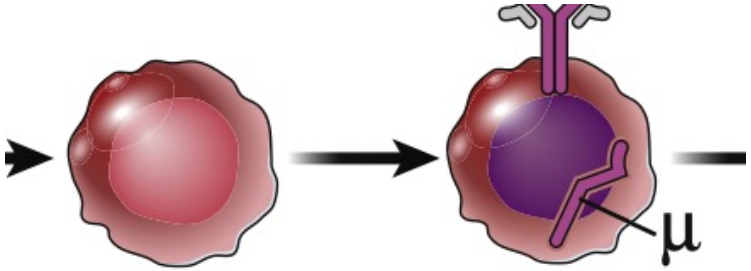
Recombination D and J exon

Removal unwanted V and D segment

Recombination V and DJ exon

- **Gene rearrangement** make different protein "**Diversity**"
- Different heavy chain 300x20x5= 30,000 heavy chains
- Different light chain 100x4= 400 light chains

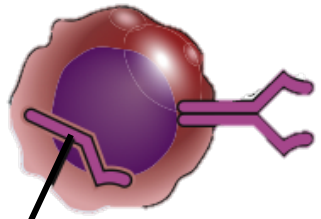
Pro-B lymphocyte to Pre-B lymphocyte



Pro-B	Pre-B
Germline DNA	Recombined H chain gene (VDJ); μ mRNA
None	Cytoplasmic μ and pre-B receptor-associated μ

- Expressed by μ, RAG1, RAG2, Igα, Igμ, λ5, *BLNK* gene
- Defective cell-surface expression of Igμ: arrest of B-cell differentiation at the CD19+,CD34+, TdT+ pro-B-cell stage.

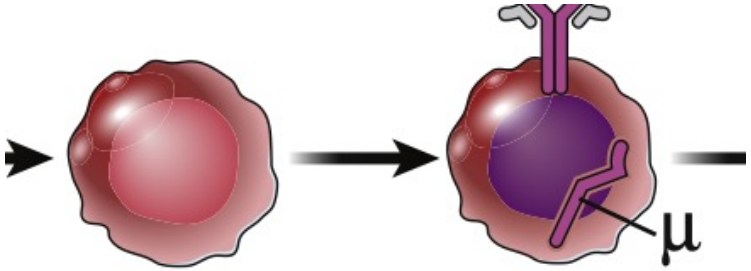
Agammaglobulinemia



Pre-B Cell Receptor

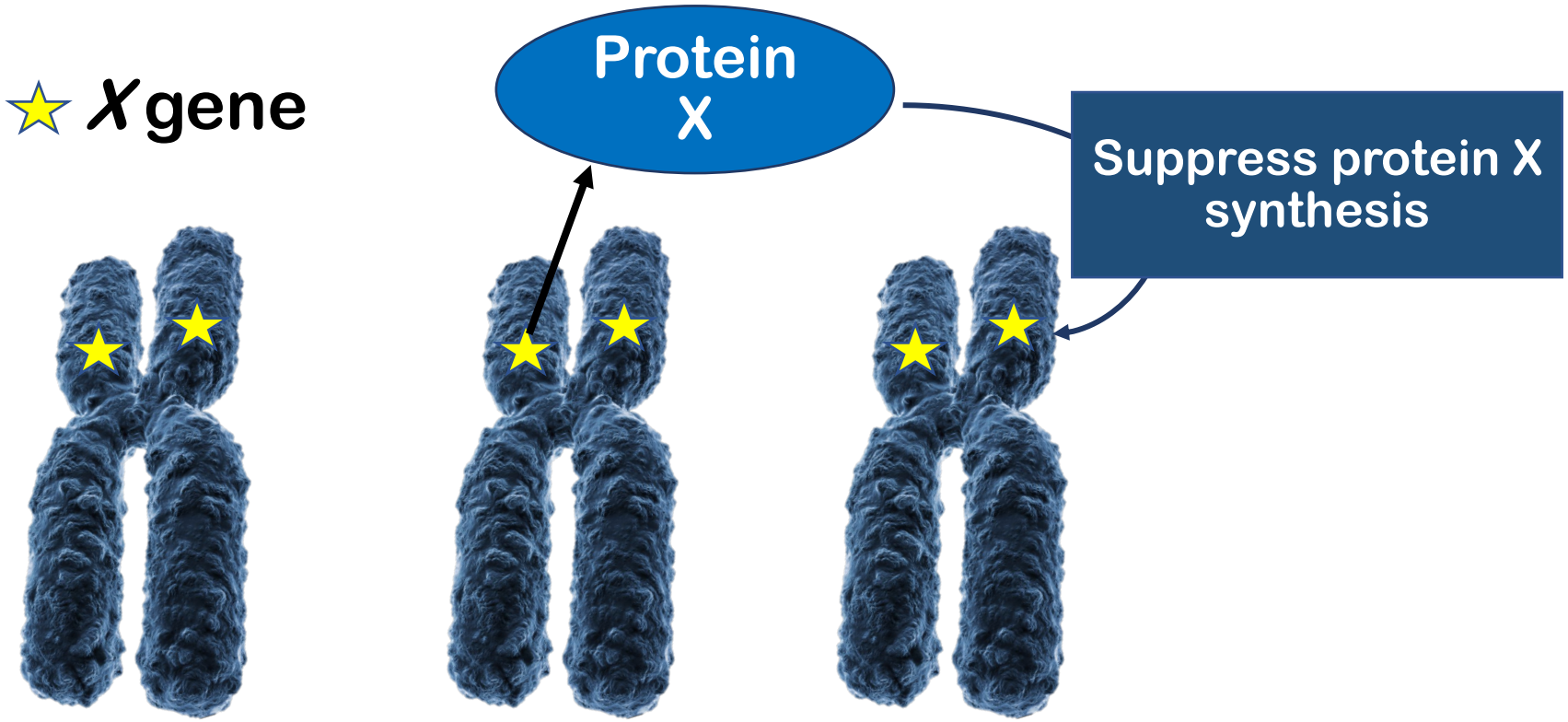
Pre-B cells

Pro-B lymphocyte to Pre-B lymphocyte



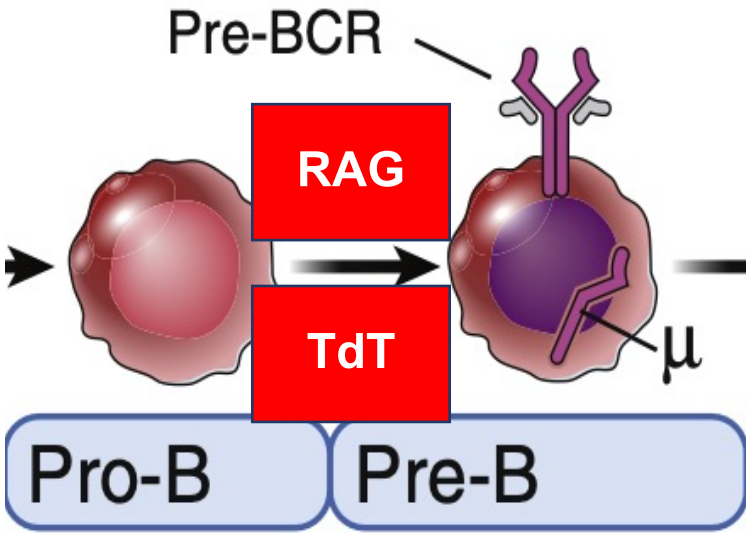
Pro-B	Pre-B
Germline DNA	Recombined H chain gene (VDJ); μ mRNA
None	Cytoplasmic μ and pre-B receptor-associated μ

- μ mRNA to μ chain
- **Allelic exclusion** plays major role.
- No light chain



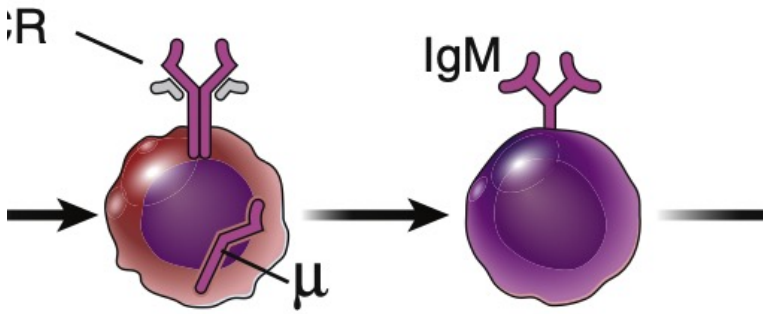
Gene rearrangement

- **“Diversity of B/T lymphocytes”**
- **RAG: Recombinant-activating gene**
  - RAG1/ RAG2
  - Gene rearrangement at antigen-specific B lymphocyte receptor
- **TdT: Terminal deoxynucleotidyl Transferase**
  - Add nucleotide at terminal DNA



μ chain and surrogate light chain **= Pre-B cell receptor**

Pre-B lymphocyte to Immature B lymphocyte

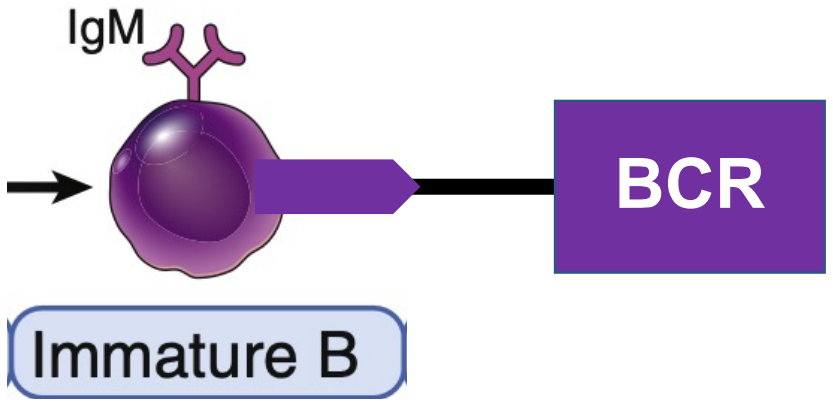


Pre-B	Immature B
Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene, κ or λ genes; μ and κ or λ mRNA
Cytoplasmic μ and pre-B receptor-associated μ	Membrane IgM (μ+κ or λ light chain)

- Gene rearrangement of **light chain**
- **Membrane IgM and BTK gene**
- Allelic exclusion
- **Decrease** surrogate light chain
- **Increase Pre-B** lymphocyte

Light chain

μ gene



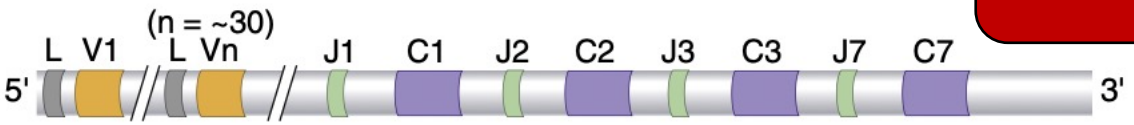
Pre-B lymphocyte to Immature B lymphocyte

Ig κ chain locus (chromosome 2)

κ chain

Suppress λ chain

Ig λ chain locus (chromosome 22)



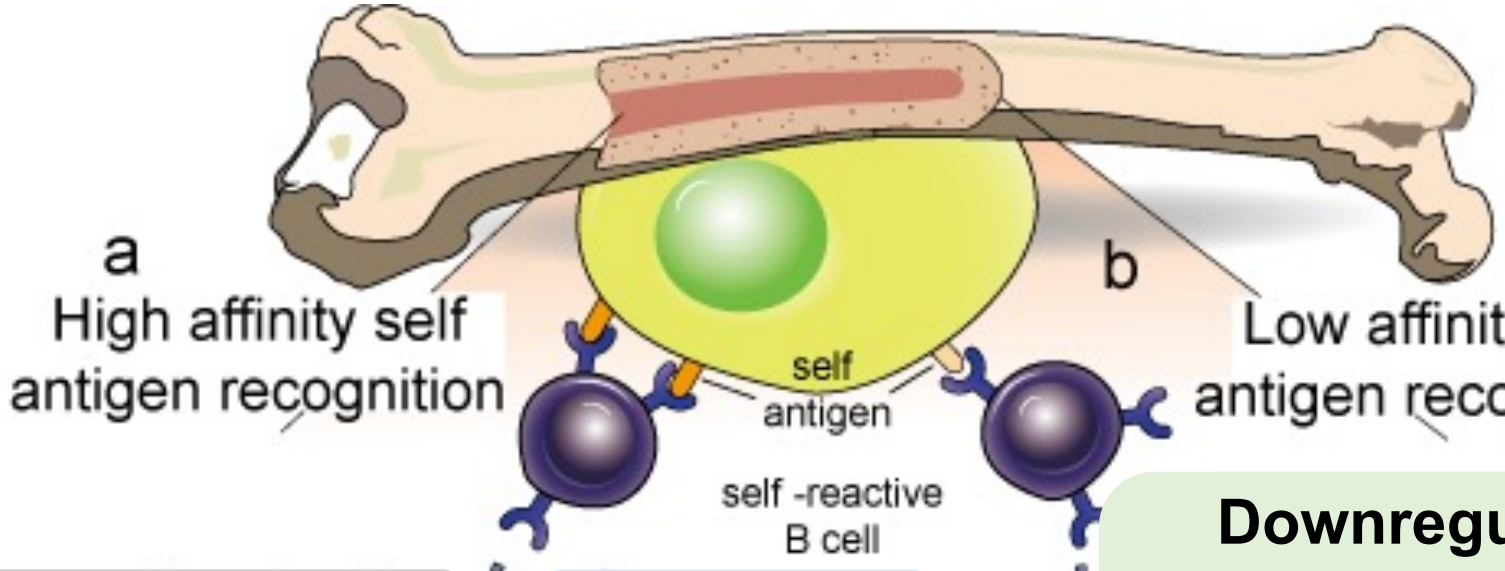
"Light chain isotype exclusion"

Impaired κ Chain

"Receptor editing"

λ Chain rearrangement

Self recognized antigen B lymphocyte



Receptor editing

Apoptosis

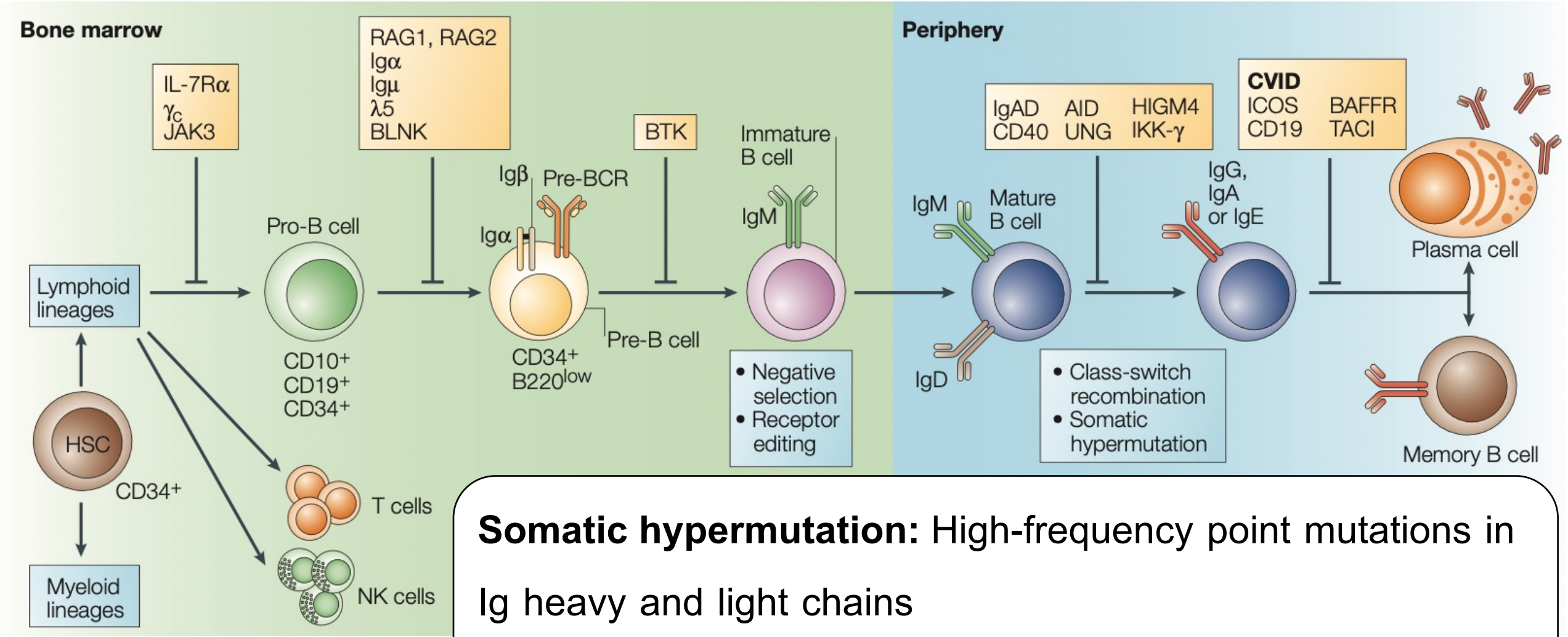
Downregulate IgM expression and upregulate IgD

Anergy

Non-self reactive B cells

Negative selection

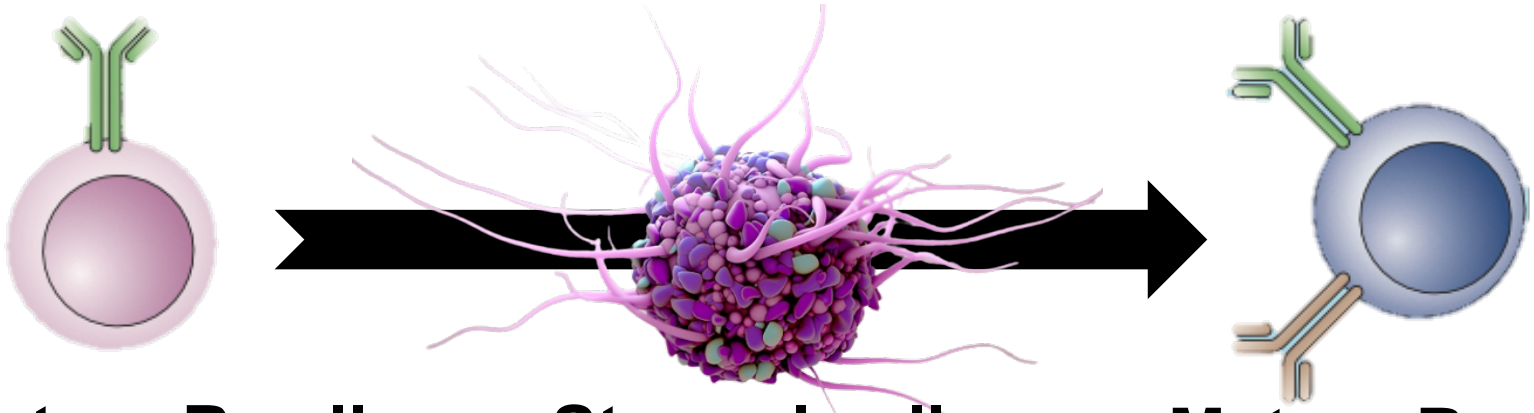
Steps in maturation of lymphocytes



Somatic hypermutation: High-frequency point mutations in Ig heavy and light chains

Increased affinity of antibodies and maturation of HIR

Trafficking to lymphoid follicles

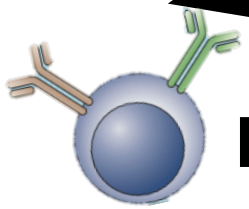


Immature B cell **Stromal cells** **Mature B cell**

Express IgD and IgM

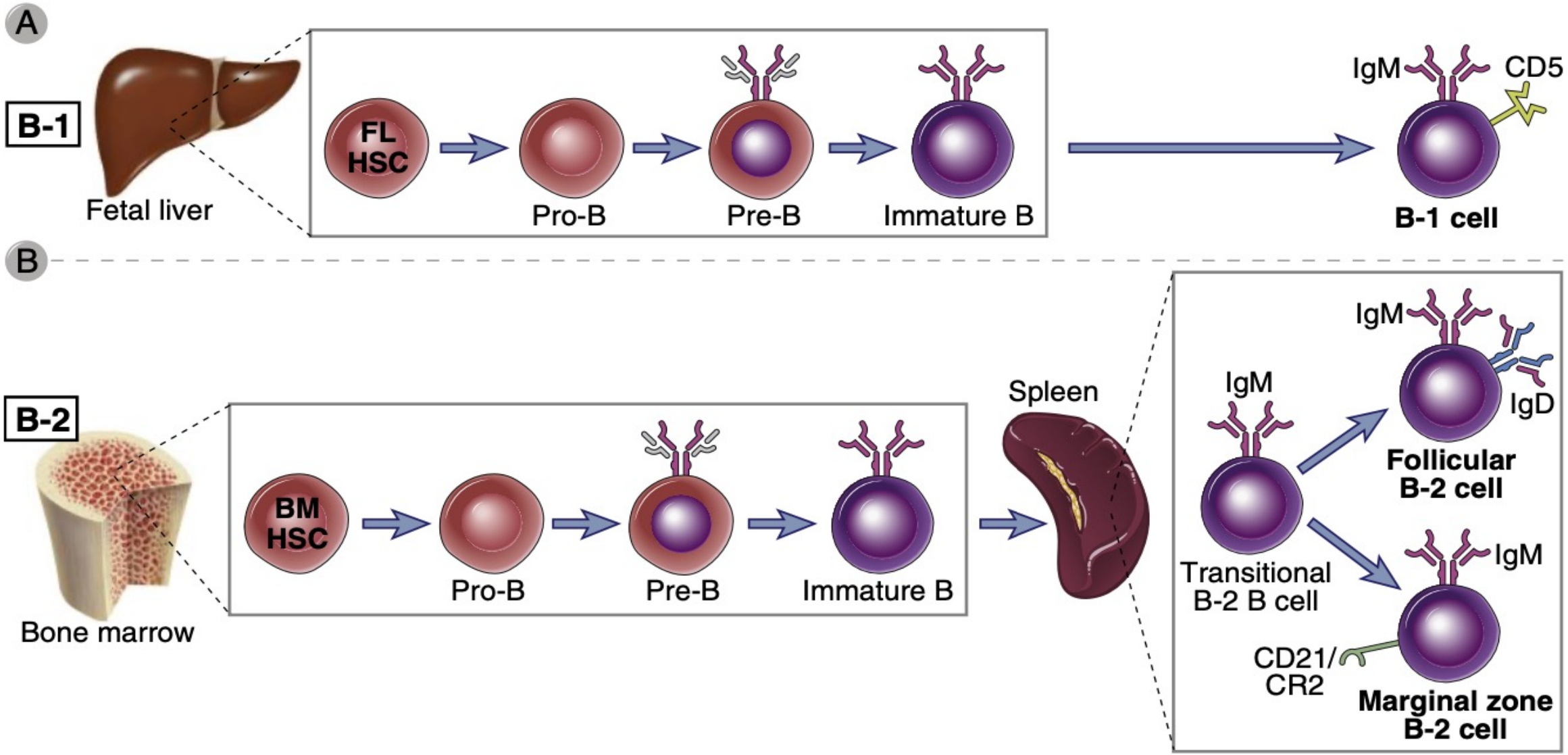
- CCL21: high endothelial venules
- CCL19: lymph nodes
- CXCL13: primary follicles (B-Cell area)

Not bind non-self Ag

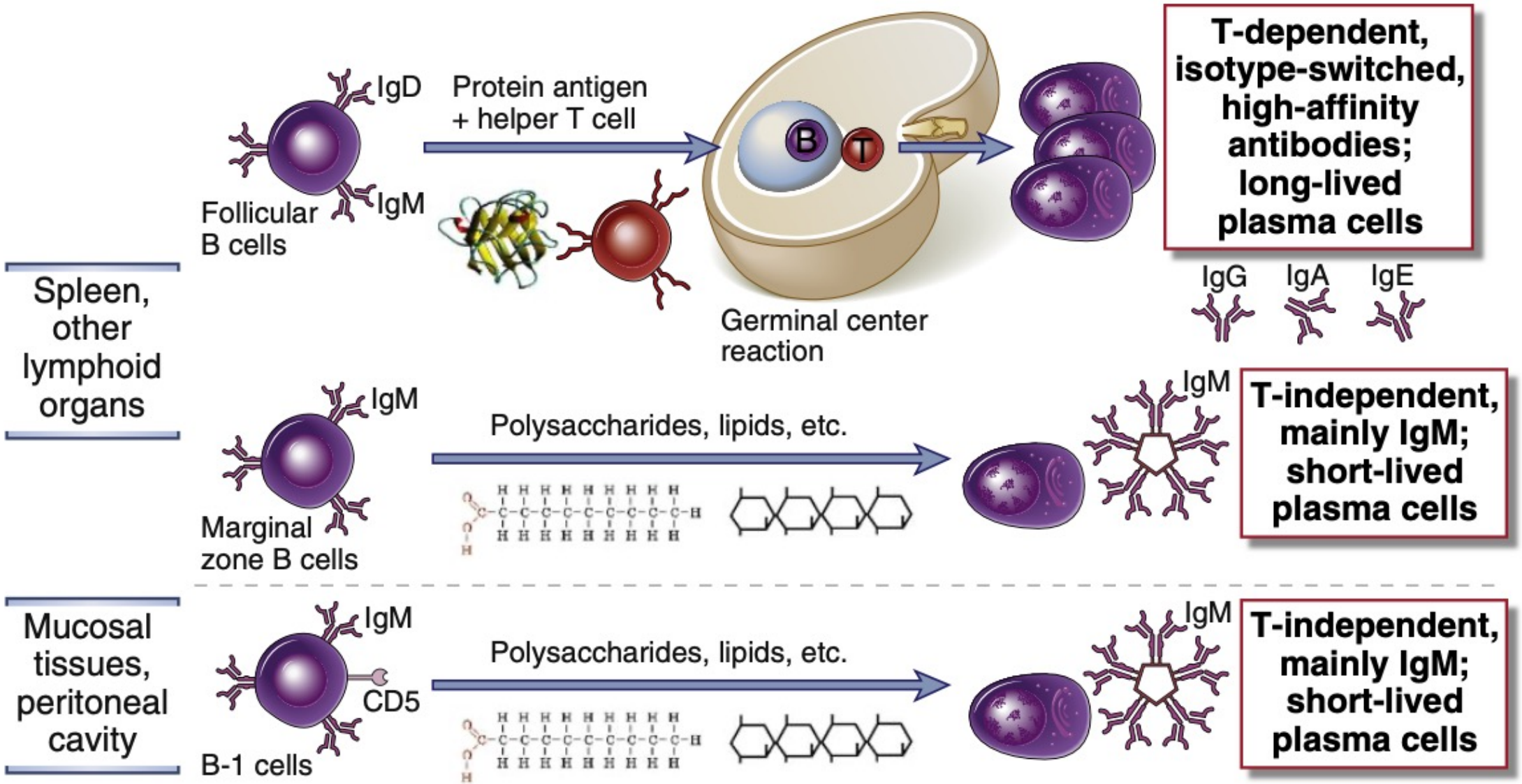


Naive B cell

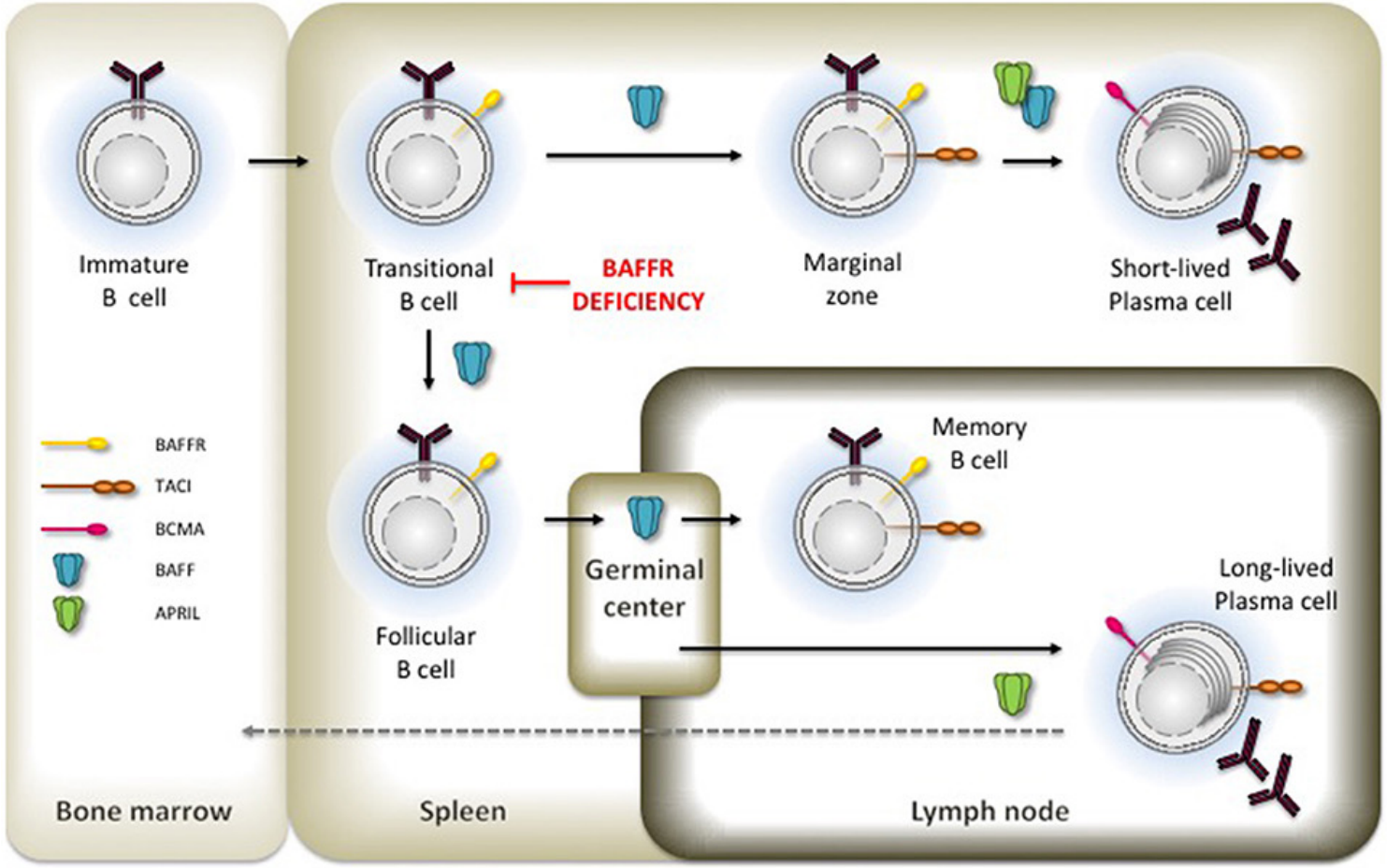
Mature B lymphocyte subsets



T-dependent and T-independent antibody responses



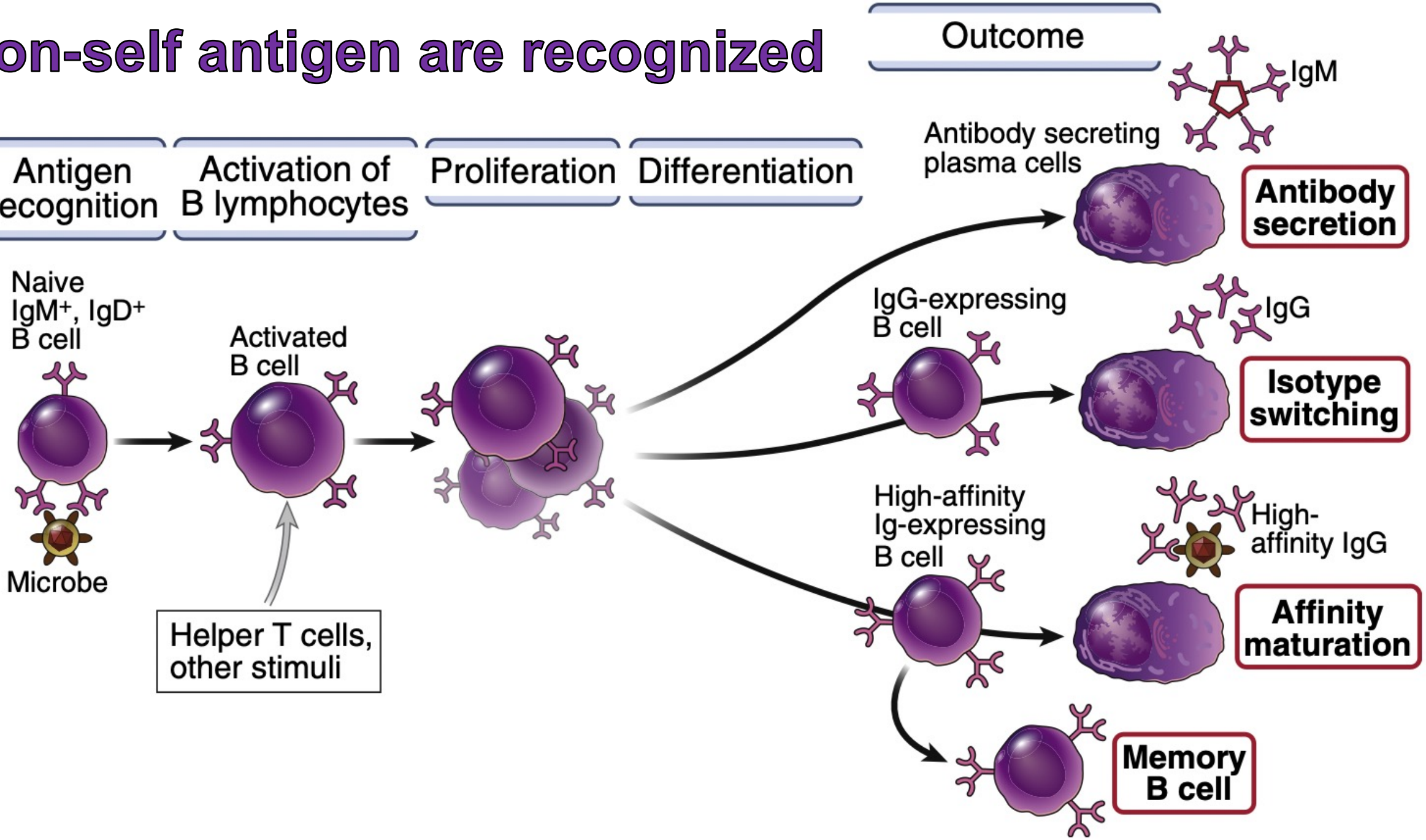
Follicular B cell survival



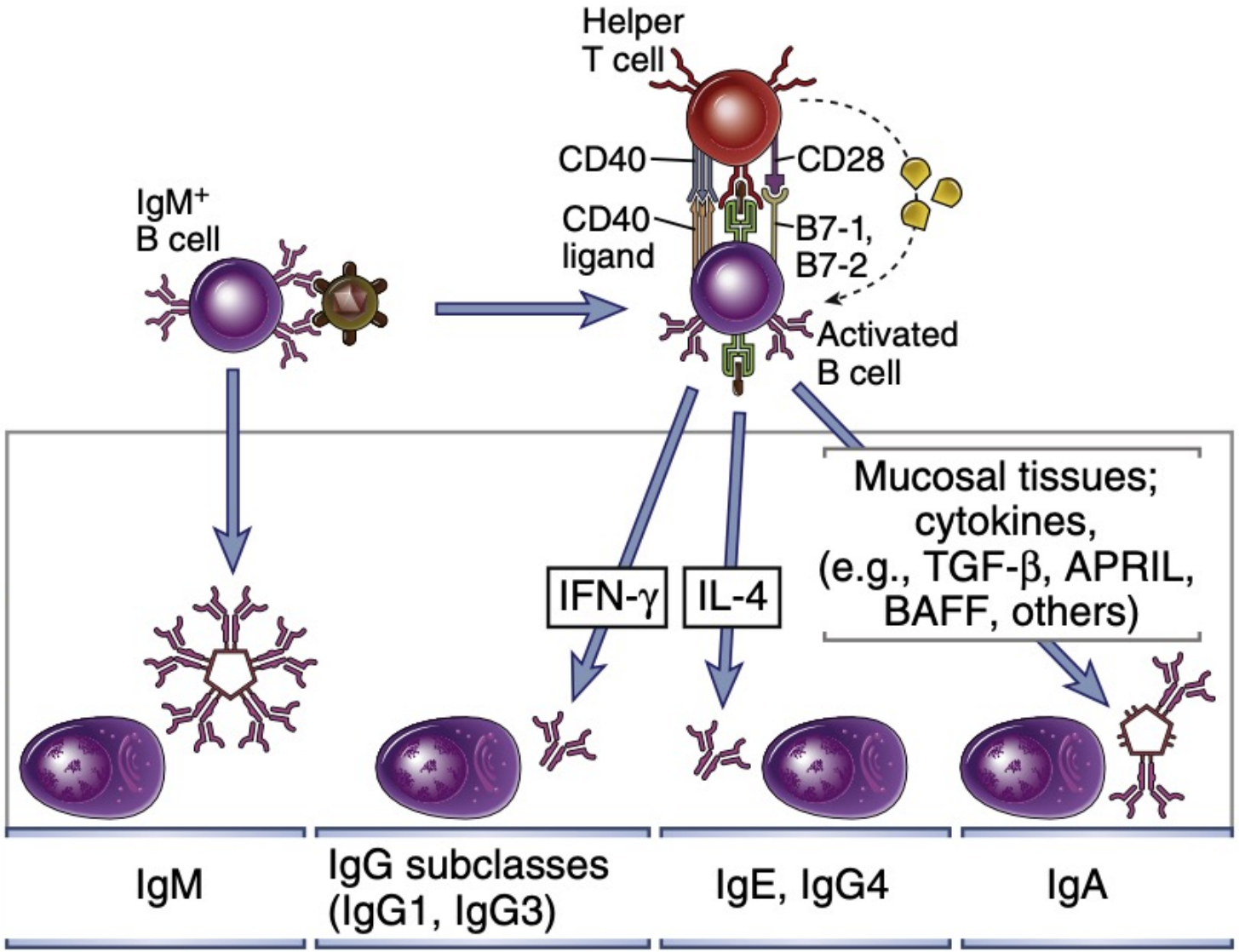
- **BAFF and APRIL** produced by myeloid cells in lymphoid follicles and bone marrow
- **Activated BAFF**: B-cell activation and differentiation

Smulski, C. R., & Eibel, H. (2018). BAFF and BAFF-Receptor in B Cell Selection and Survival. *Frontiers in Immunology*, *9*.

If non-self antigen are recognized

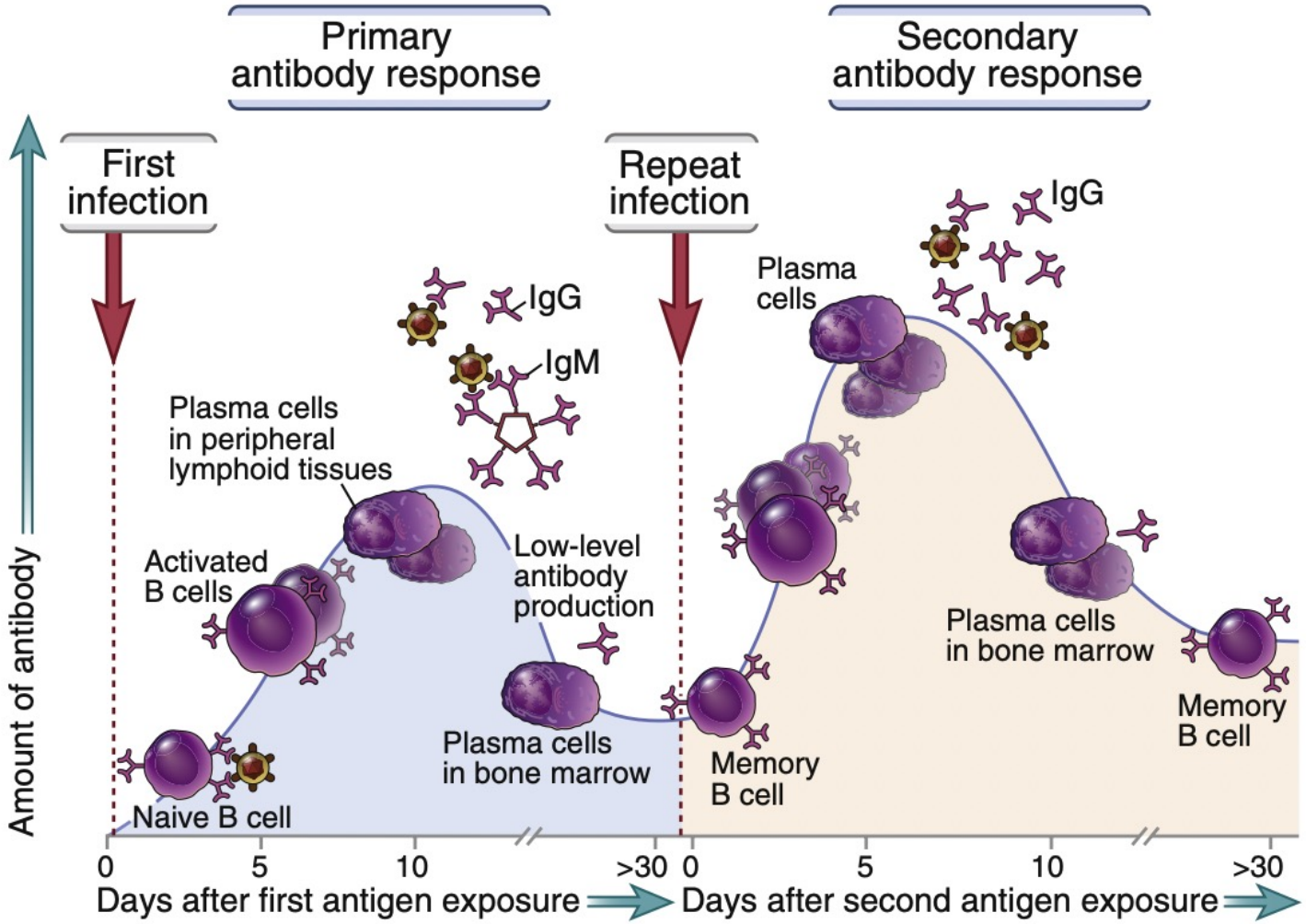


Immunoglobulin (Ig) heavy-chain isotype (class) switching

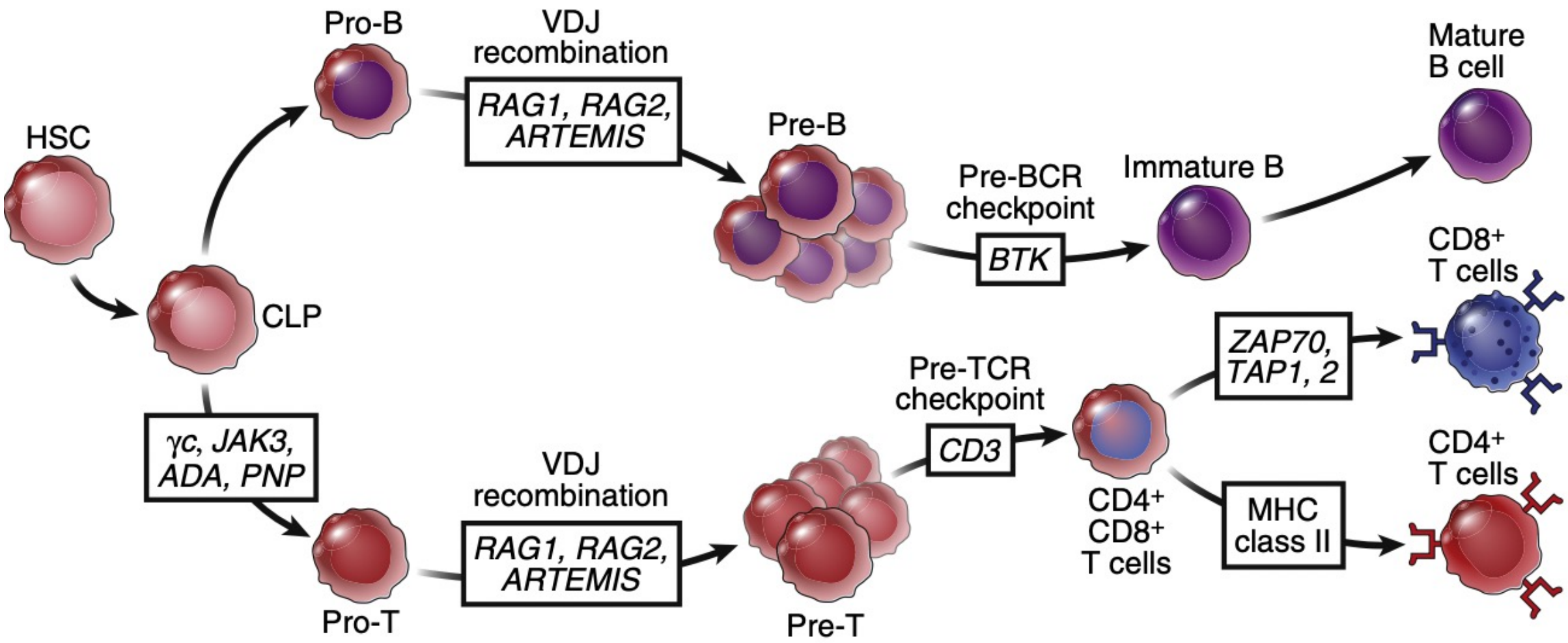


Type	Principal effector functions
IgM	Complement activation
IgG subclasses	Opsonization and phagocytosis, neonatal immunity
IgE, IgG4	Immunity against helminths, Mast cell degranulation
IgA	Mucosal immunity

Features of primary and secondary antibody responses

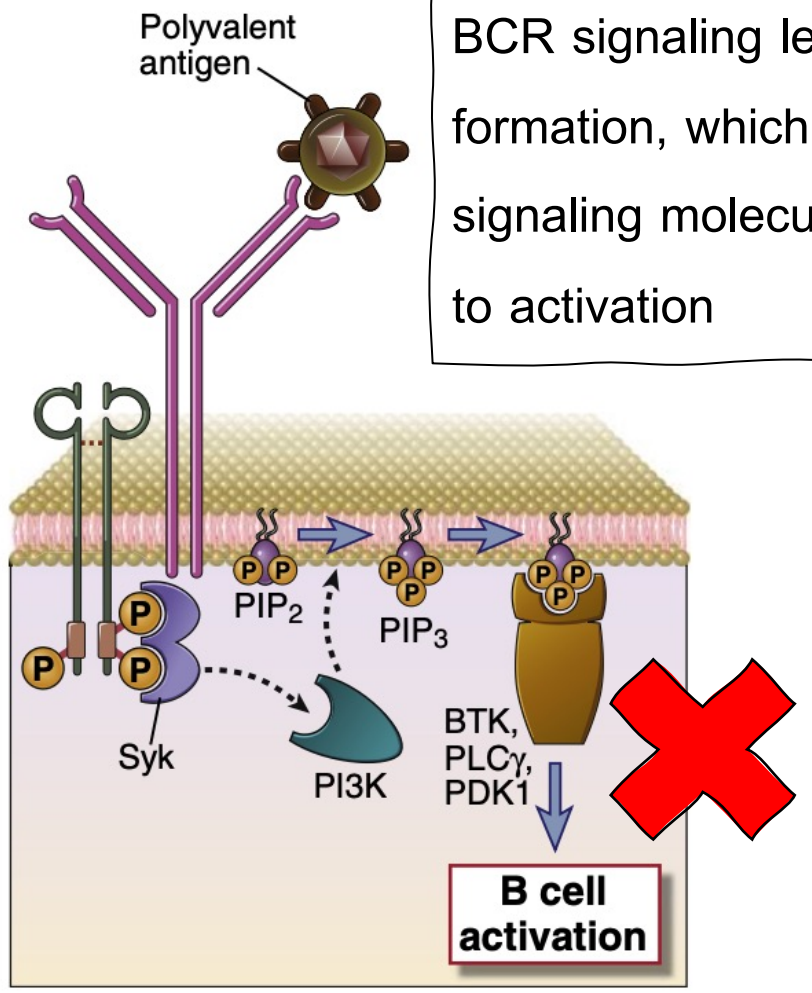


Clinical relevant: Congenital immunodeficiencies



X-Linked Agammaglobulinemia

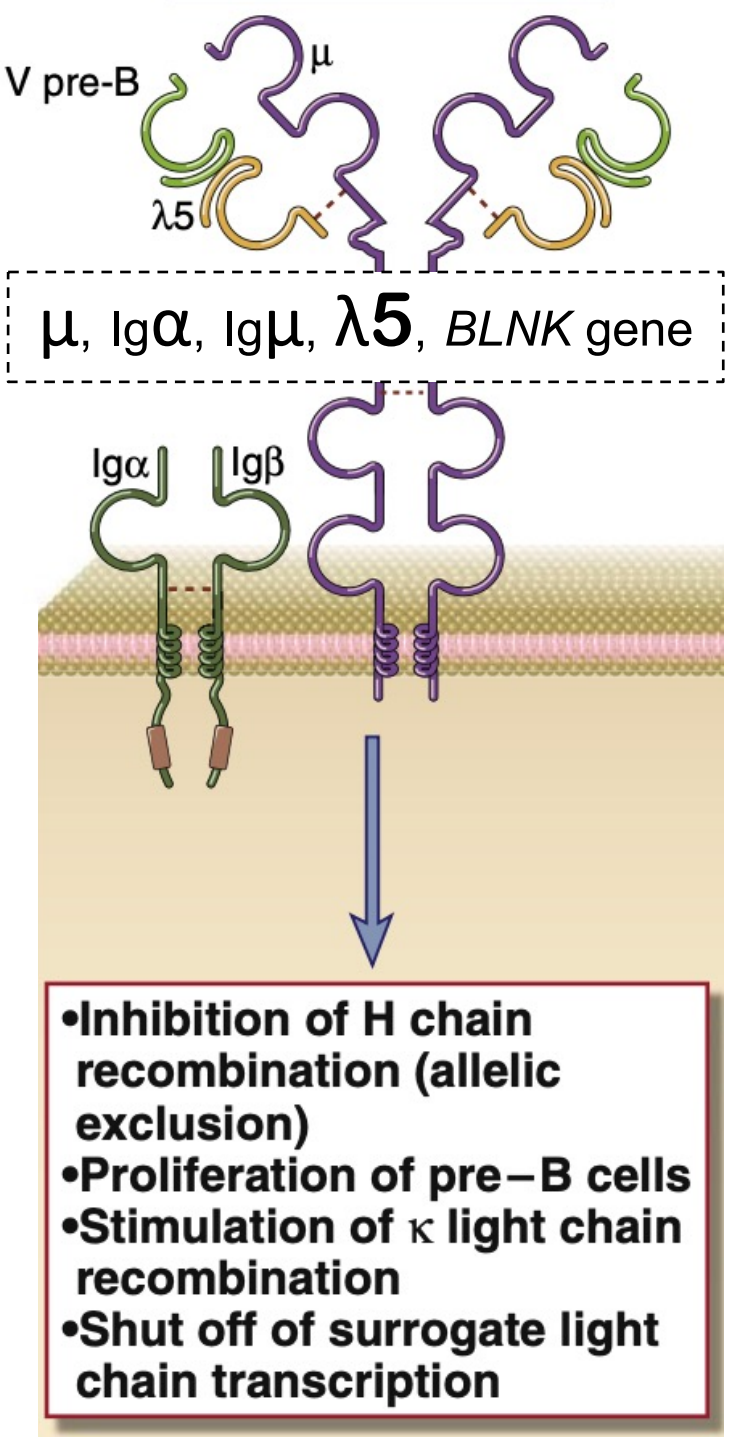
- Block in maturation beyond pre–B cells, because of mutation in Bruton tyrosine kinase (BTK)
- Chromosome X: Xq21.3–Xq22 **"X-linked Recessive"**
- Decrease in all serum Ig isotype and reduced B cell numbers
- Investigation: absence CD19, CD20 (circulating B cells) and decrease Ig levels



BCR signaling leads to PIP_3 formation, which binds other signaling molecules, leading to activation

Vetrie, D. *et al*. The gene involved in X-linked agammaglobulinaemia is a member of the *src* family of protein-tyrosine kinases. *Nature* **361**, 226–233 (1993).

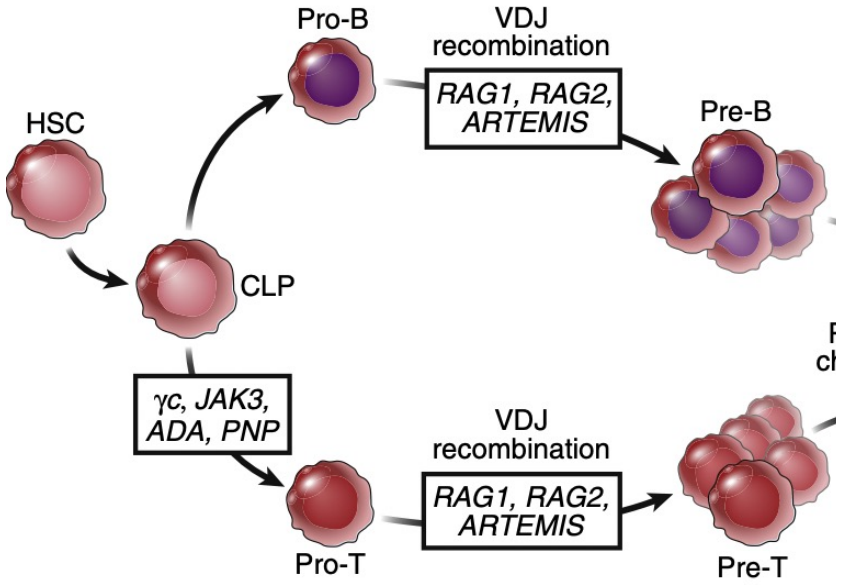
Autosomal Recessive Agammaglobulinemia



- Females with XLA-like phenotype
- Males with presumed XLA but not have a pathogenic variant in BTK.
- History of consanguinity
- **Rare** AR forms of agammaglobulinemia

Schwarz, K. *et al*. RAG mutations in human B cell-negative SCID. *Science* **274**, 97–99 (1996).

Autosomal Recessive Severe Combine Immunodeficiency



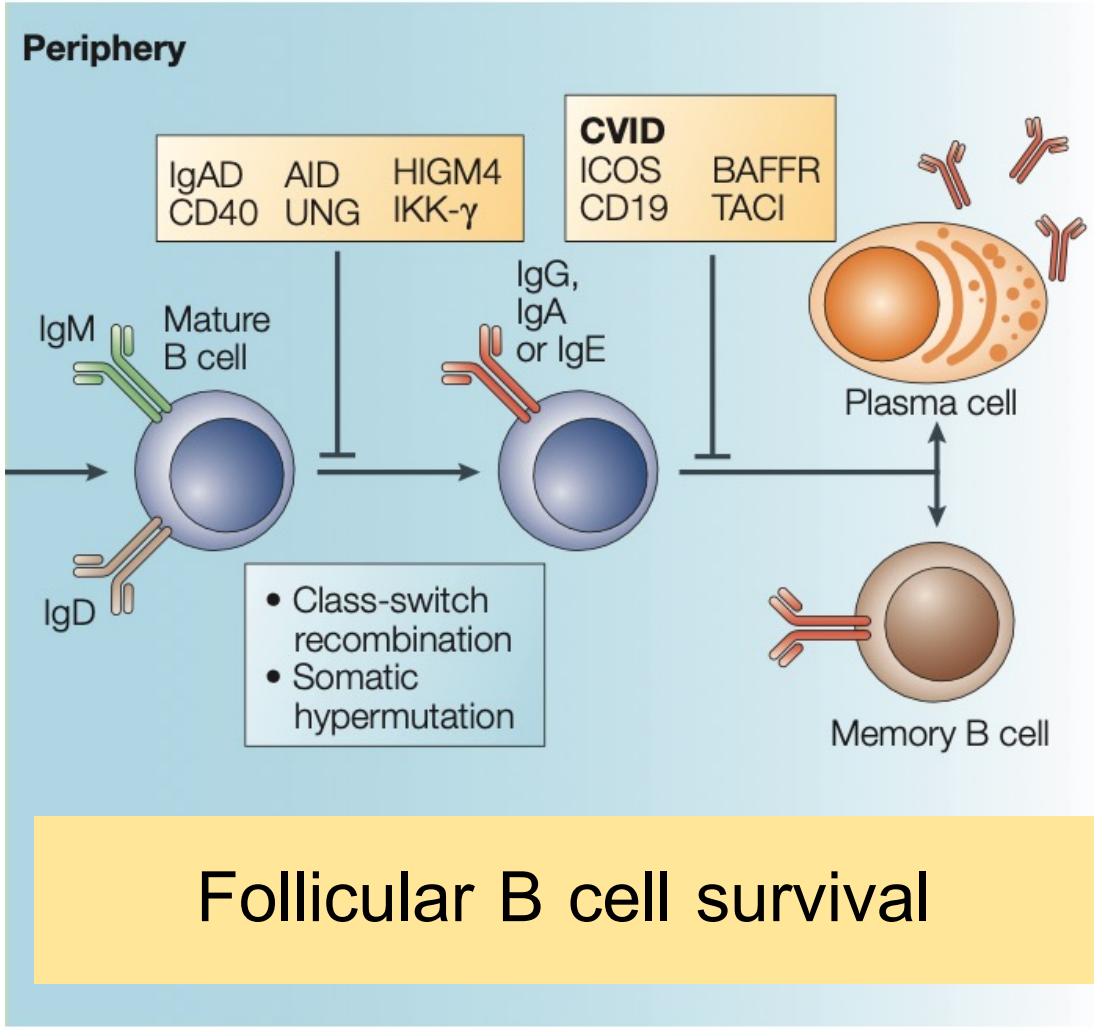
- **SCID** is impairment of humoral and cellular functions.
- Infants with opportunistic infection (CMV, PCP), fatal infections after live-attenuated vaccine

- ***RAG1, RAG2*** have major role for gene rearrangement.
- Mutations cause **abnormal VDJ recombination**.
- Impaired B cell receptors (BCR)

Yel, L. *et al*. Mutations in the μ heavy-chain gene in patients with agammaglobulinemia. *N. Engl. J. Med.* **335**, 1486–1493 (1996).

Common variable immunodeficiency

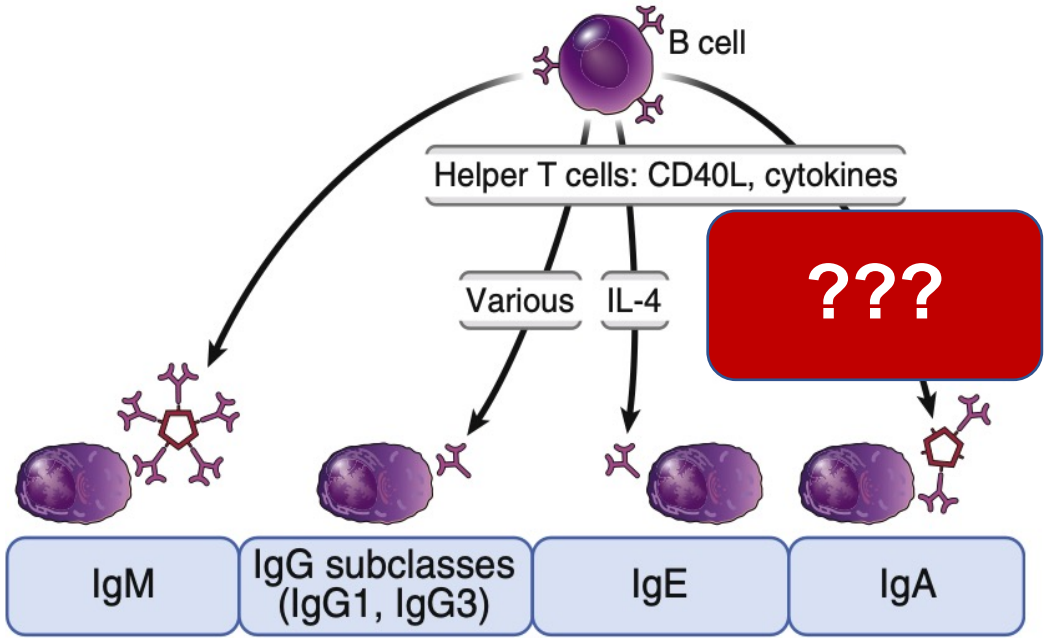
- **Defect antibody production**
- Recurrent pyogenic sinopulmonary and gastrointestinal infections
- Low IgG and IgA levels
- Normal B-cell numbers
- *CD19, **BAFFR, TACI**, ICOS* mutation
- Bimodal age distribution (first and third decade)



Salzer, U. *et al*. ICOS deficiency in patients with common variable immunodeficiency. *Clin. Immunol.* **113**, 234–240 (2004).

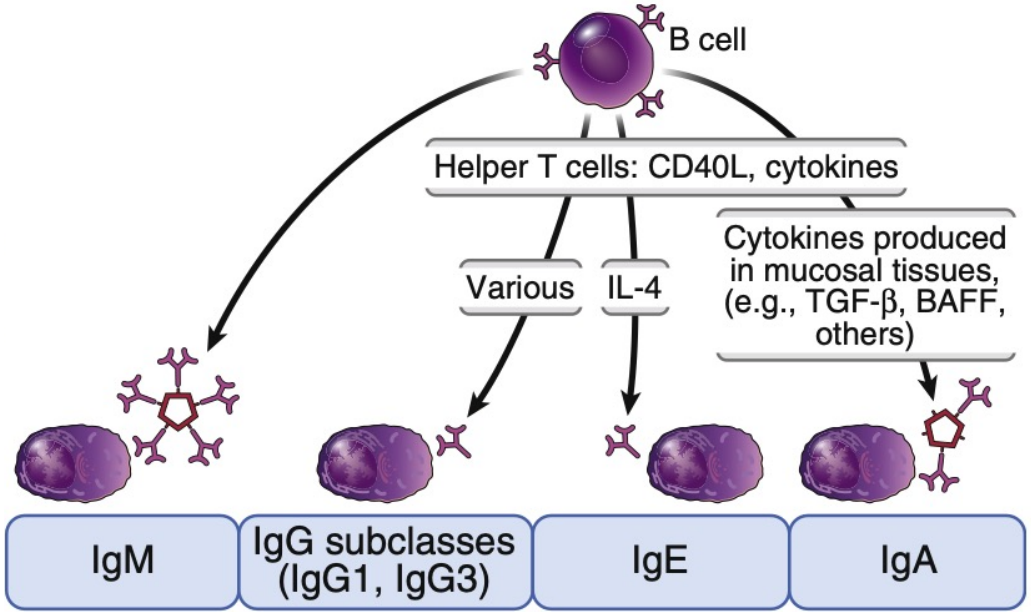
Selective IgA deficiency

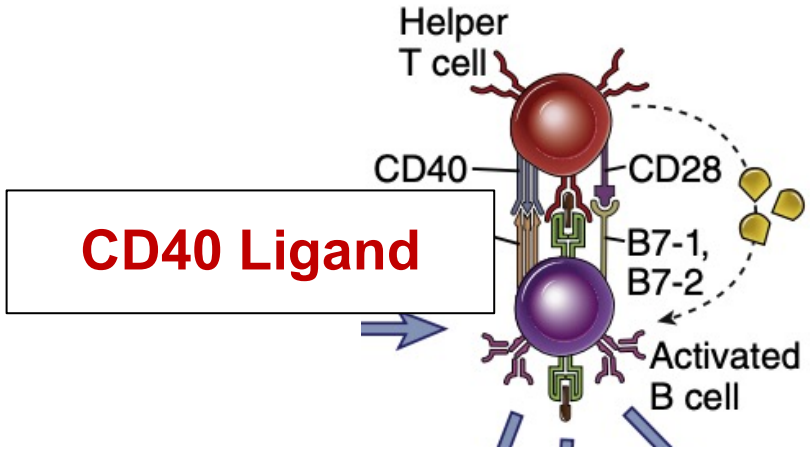
- **The most common primary immunodeficiency**
- Recurrent pyogenic sinopulmonary and gastrointestinal infections
- **Absence of class switching to IgA**
- The molecular defect is unknown in most cases.
- *TACI*, *IGAD1* (HLA-DQ and HLA-DR) mutation



Am. J. Hum. Genet. **64**, 1096–1109 (1999).

X-linked Hyper-IgM syndrome

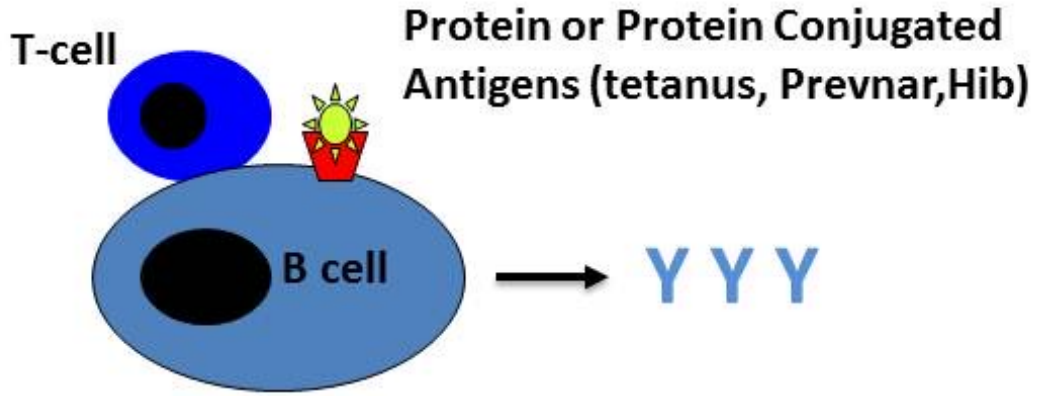




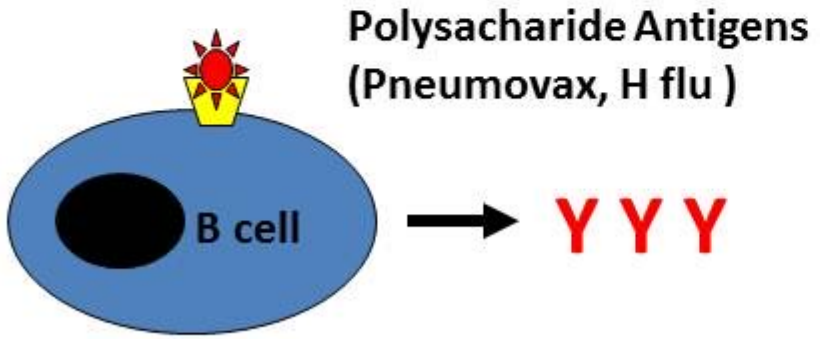
- Immunoglobulin **class switch** recombination defect
- *CD40L* mutation on Xq26–Xq27
- Susceptible to *Pneumocystis jiroveci, Cryptosporidium spp.*, CMV infection
- Defect both humoral and cell-mediated immunities

Specific Antibody Deficiency

- T-cell independent antibodies response defect
- Normal immunoglobulins levels and recurrent infection
- Diminished antibody responses to polysaccharide antigens following vaccination



T-cell dependent antibody proactive

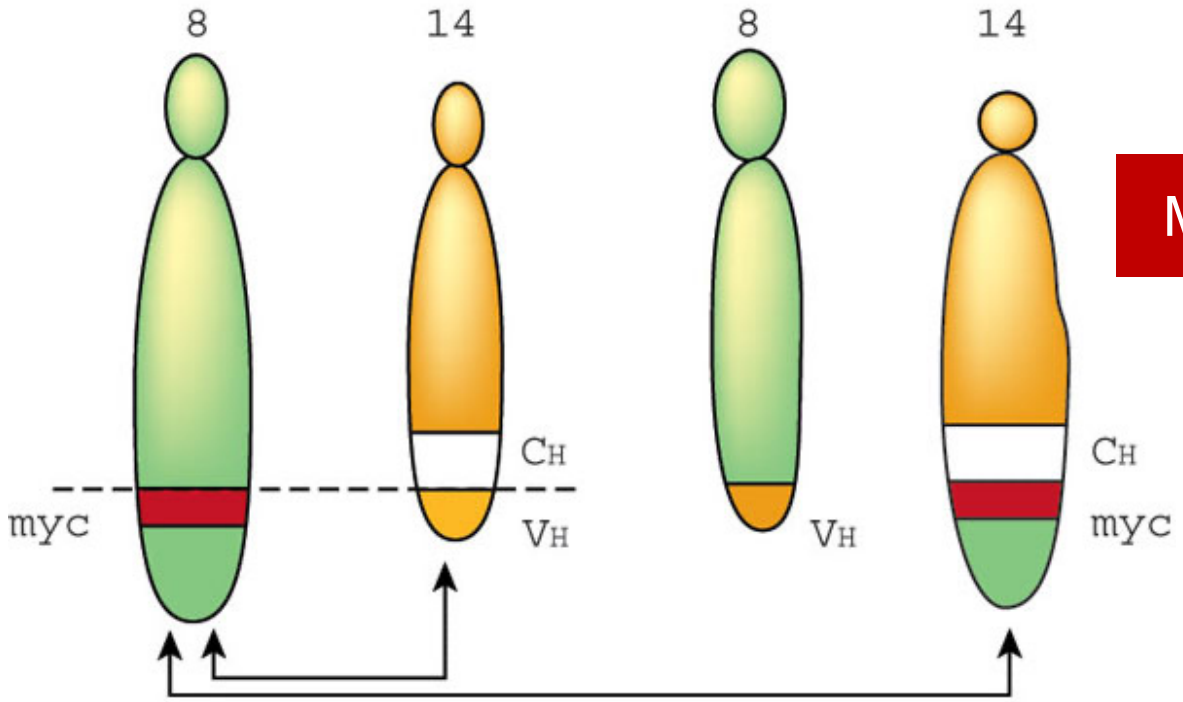


T-cell independent antibody proactive

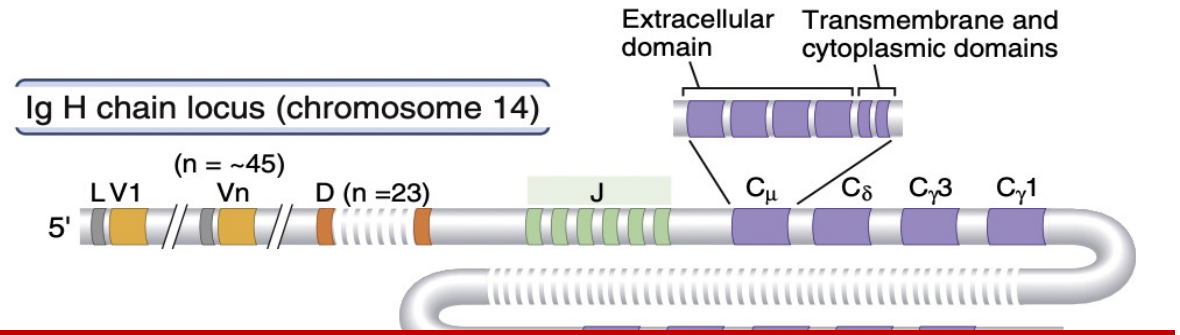
Perez, E., Bonilla, F. A., Orange, J. S., & Ballow, M. (2017). Specific Antibody Deficiency: Controversies in Diagnosis and Management. *Frontiers in immunology*, *8*, 586.

Burkitt Lymphoma

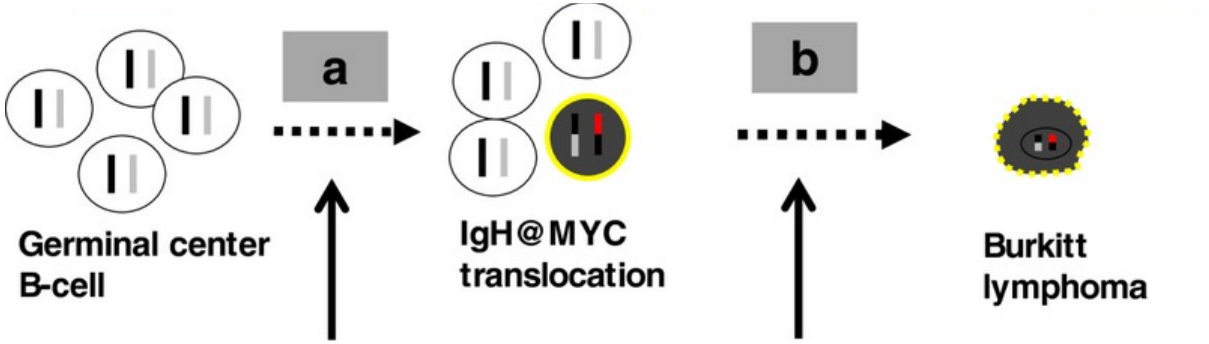
Normal chromosome **Burkitt Lymphoma**

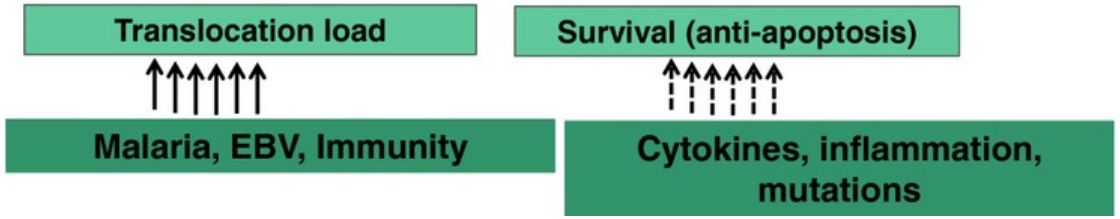


Translocation of Myc gene



Mutation of *Myc* cause strong heavy chain promoter





Take home message

- B lymphocyte is the part of adaptive immunity.
- Main function is antibody secretion for against pathogen.
- In fetus B lymphocyte occur in liver but afterbirth occur in bone marrow and trafficked to lymphoid tissue.
- All steps require genes that regulate immunoglobulin expression, differentiation, diversity of antibodies
  - *BTK* mutation cause blocking in maturation beyond pre–B cells.
  - *RAG1*, *RAG2* mutation cause abnormal VDJ recombination.