Cours Immuunologie Fondamentale-2eme annee Medecine

LES LYMPHOCYTES B

Développement et activation

Dr. Naci

21-02-2024

PLAN

- ❑ **INTRODUCTION**
- ❑ **DÉVELOPPEMENT DES LYMPHOCYTES B (LB) :**

1) Phase médullaire de la lymphopoïèse

- ❖ Origine des LB (CSH): rappel sur l'hématopoïèse
- ❖ La niche hématopoïétique et le microenvironnement médullaire
- ❖ Phases de développement du lymphocytes B

2) Phase splénique de la lymphopoïèse

- ❑ **CIRCULATION DES LYMPHOCYTES B**
- ❑ **SOUS POPULATIONS DES LB**
- ❑ **ACTIVATION DES LB**

References:

- Textbooks d'immunologie Abul K. Abbas
- Textbook d'immunologie, Manuel Roitt's
- Pr. Ben Yahia, Cours de Résidanat en Immunologie, 2eme année Medecine, Univ Alger 1
- Dr. Ouikhlef N. Cours d'immunologie EHU Oran.
- Pr. Barker B. Basic Immunology lectures

INTRODUCTION

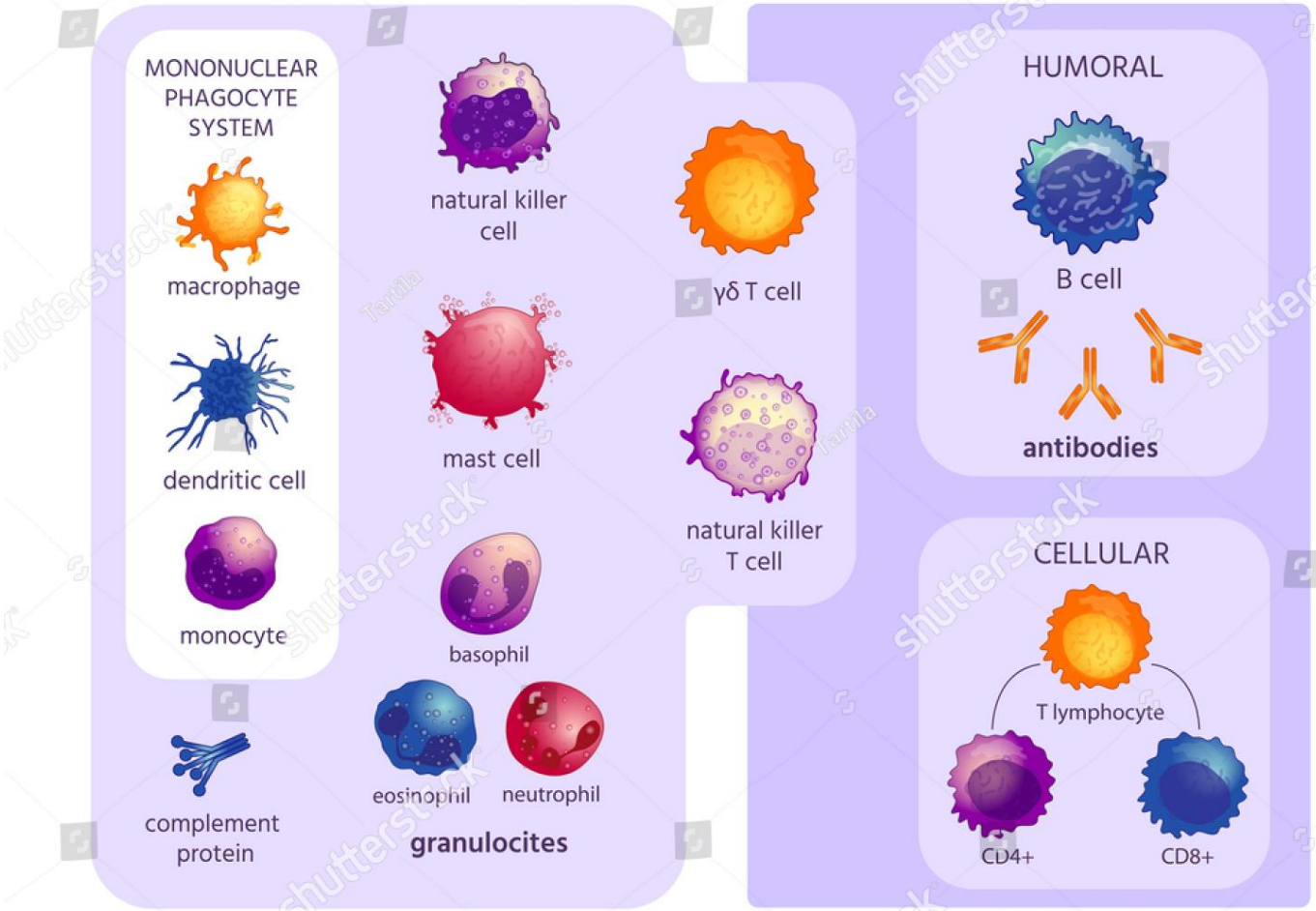
IMMUNITY

INNATE

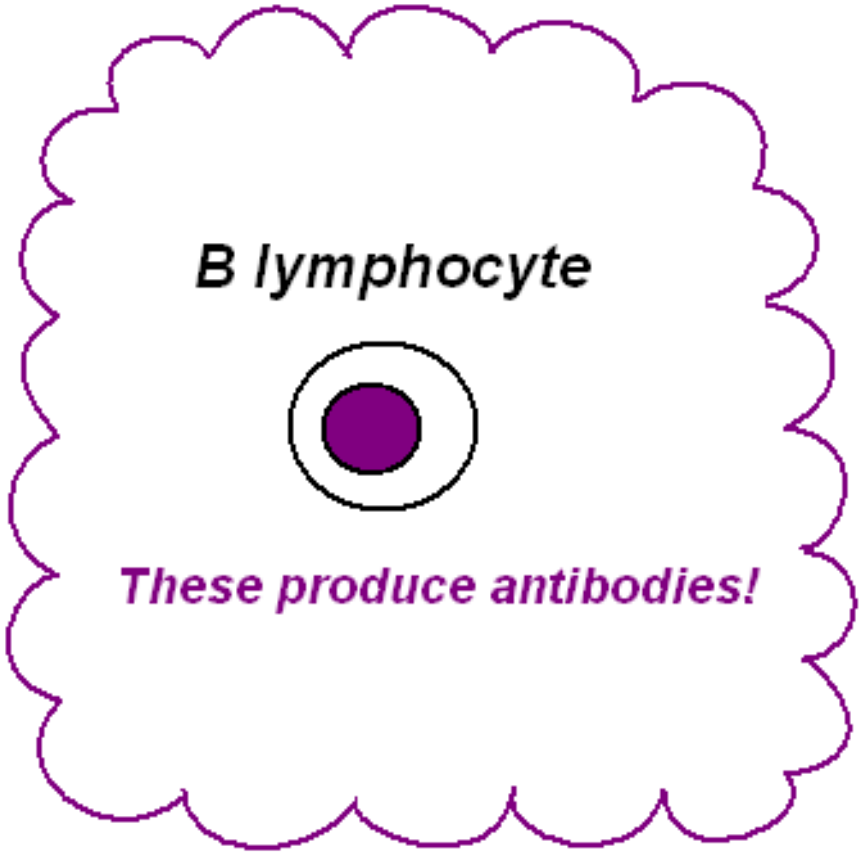
NONSPECIFIC
fast response (0-4 hours)

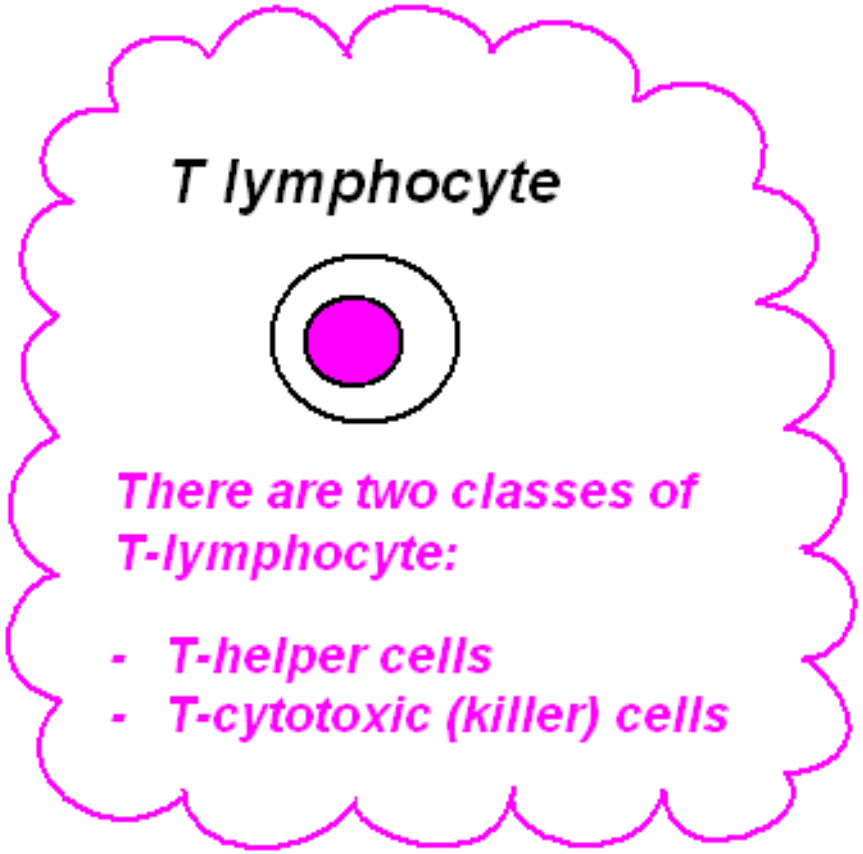
ADAPTIVE

SPECIFIC
slow response (4-14 days)



Cellules de l'immunité adaptative



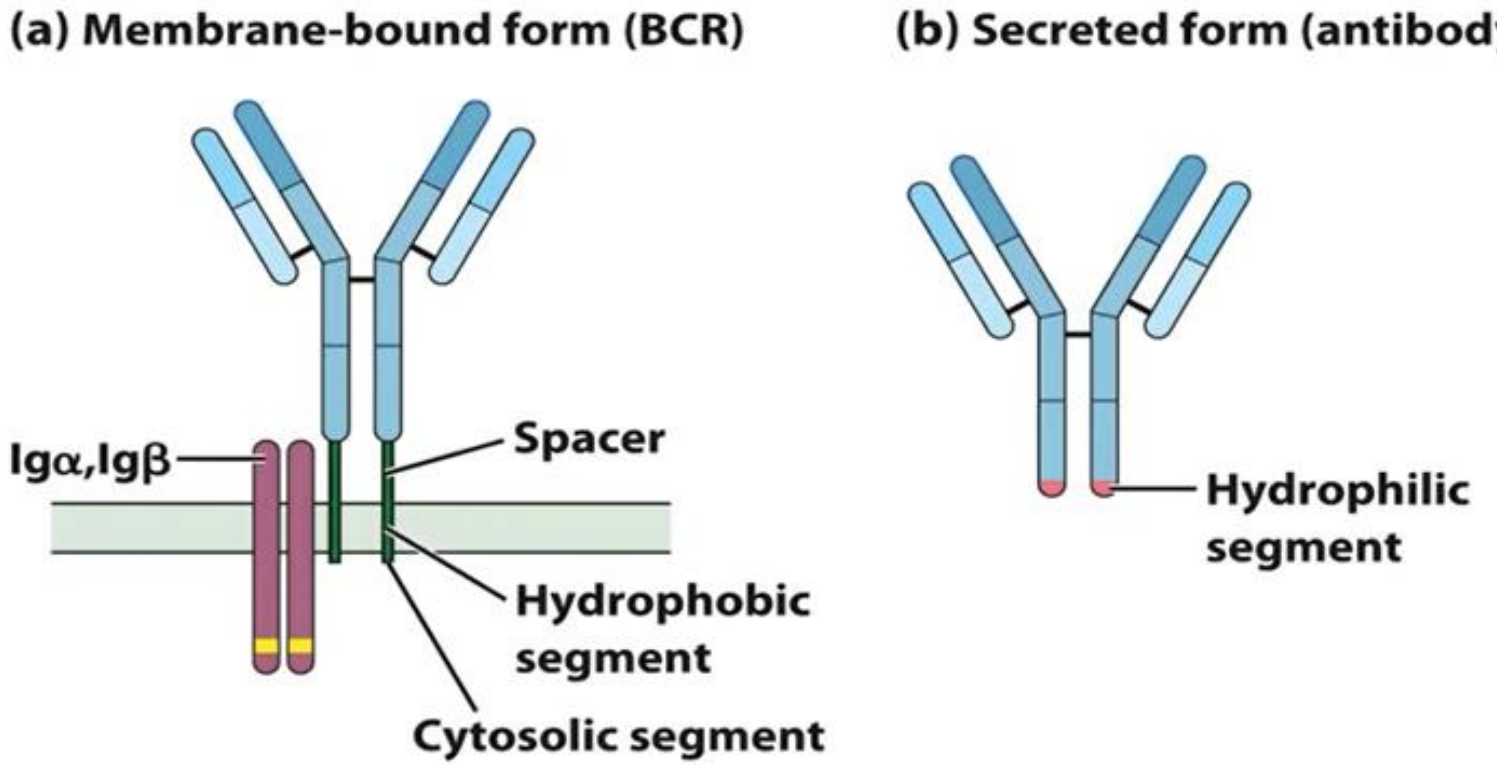


F Trimoreau, V Leymarie, CHU Limoges
Congrès des Hôpitaux Généraux, Angers, septembre 2011

Les Lymphocytes B (LB)

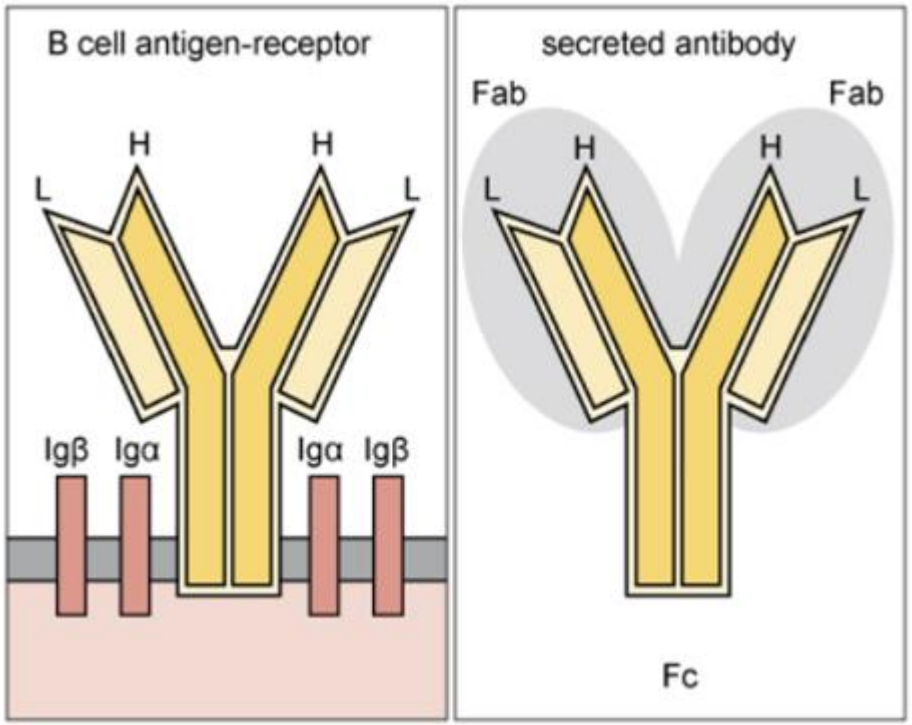
- Se développent dans : Bone Marrow chez l'homme
  Bourse de Fabricius de l'oiseau
- Représentent **5% – 15 %** lymphocytes circulants
- Effecteurs de l'immunité adaptative humorale
- Synthétisent des immunoglobulines
- Cellules Presentatrices d'Antigenes (CPA): peuvent présenter l'Ag aux lymphocytes T déjà actives
- Diversité clonale des LB: Expriment des immunoglobulines (ig) de surface = récepteur spécifique pour l'antigène (BCR)

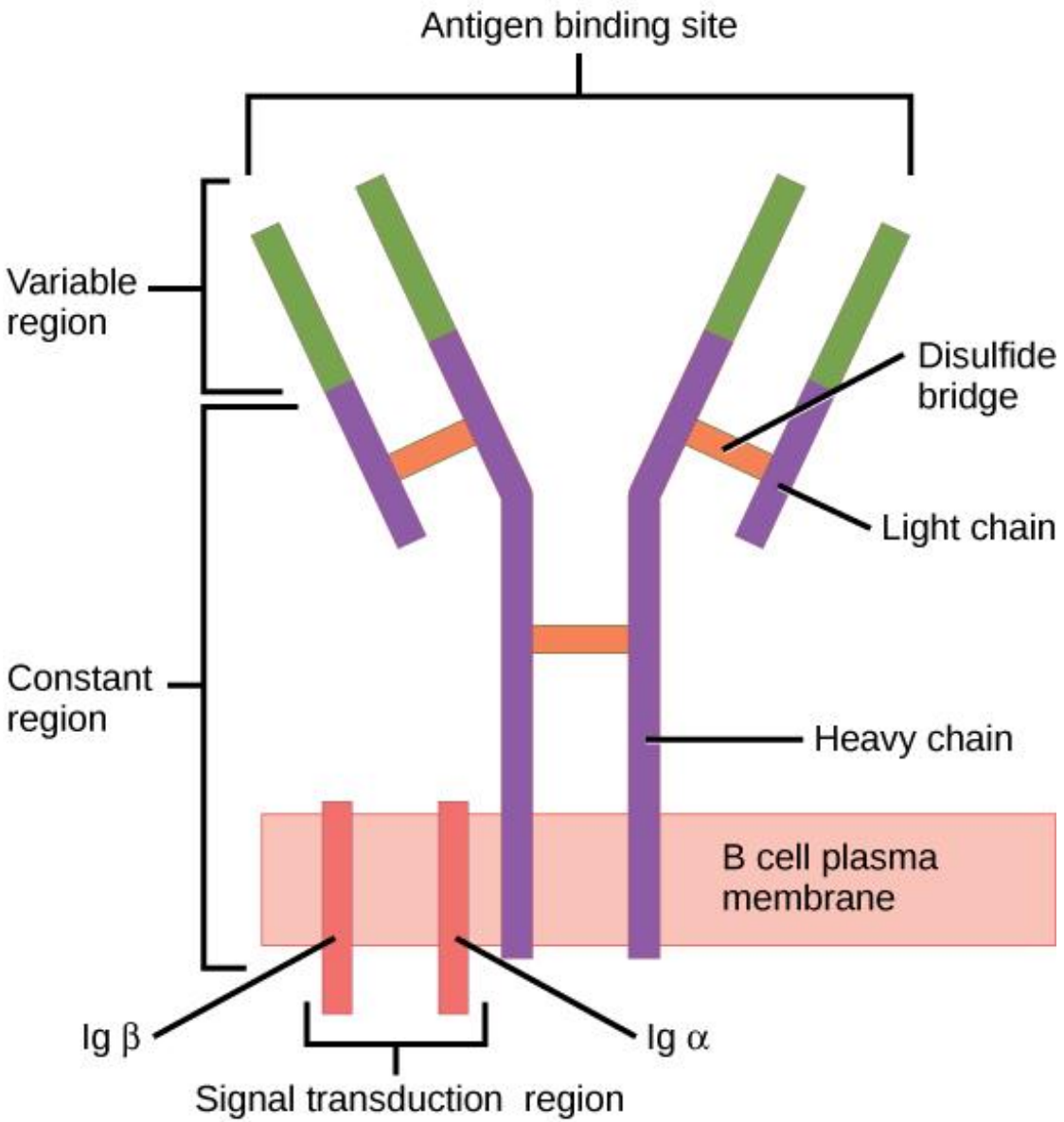
B cell receptor is an antibody with a transmembrane domain



Kuby Figure 3-9

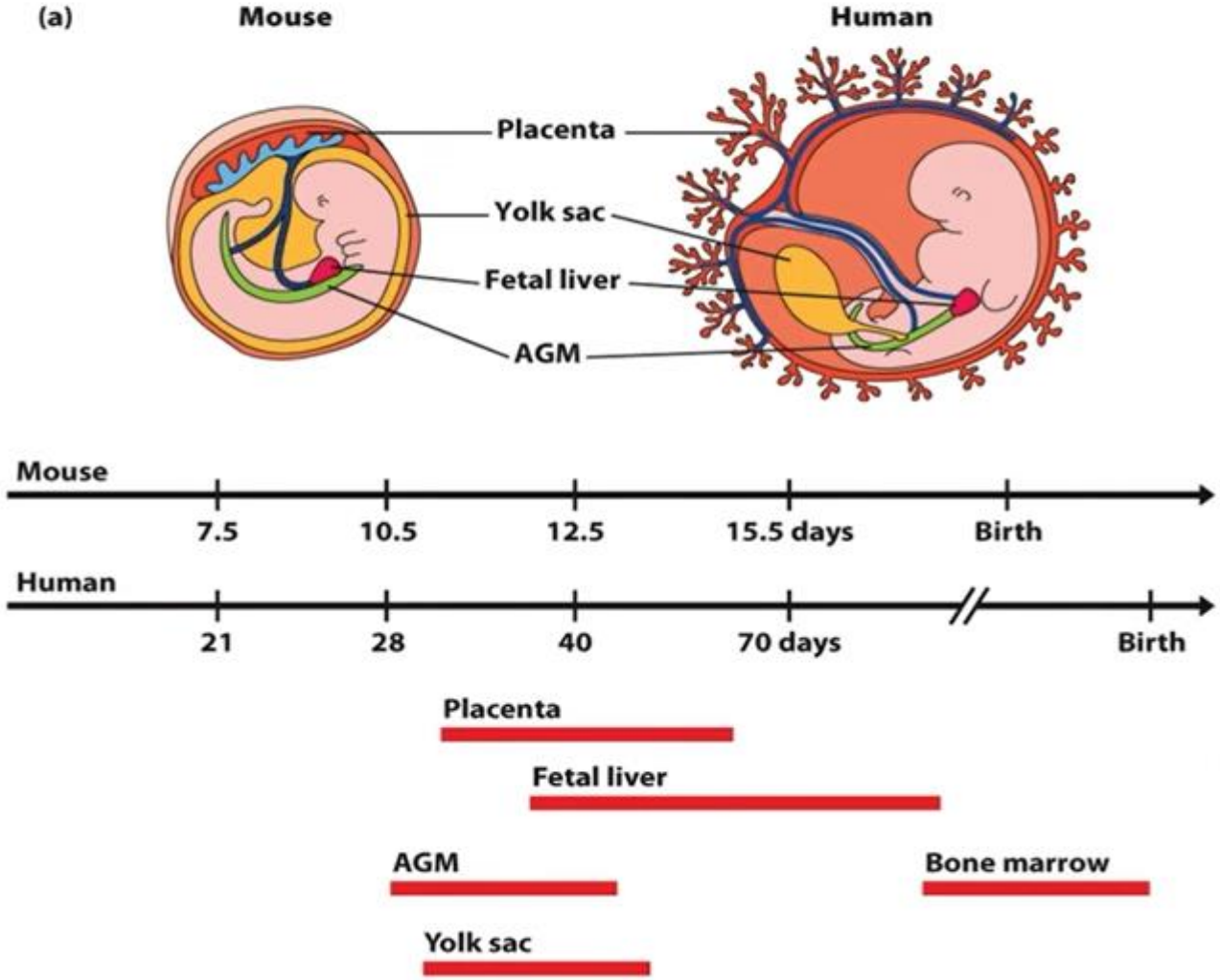
Structure du BCR

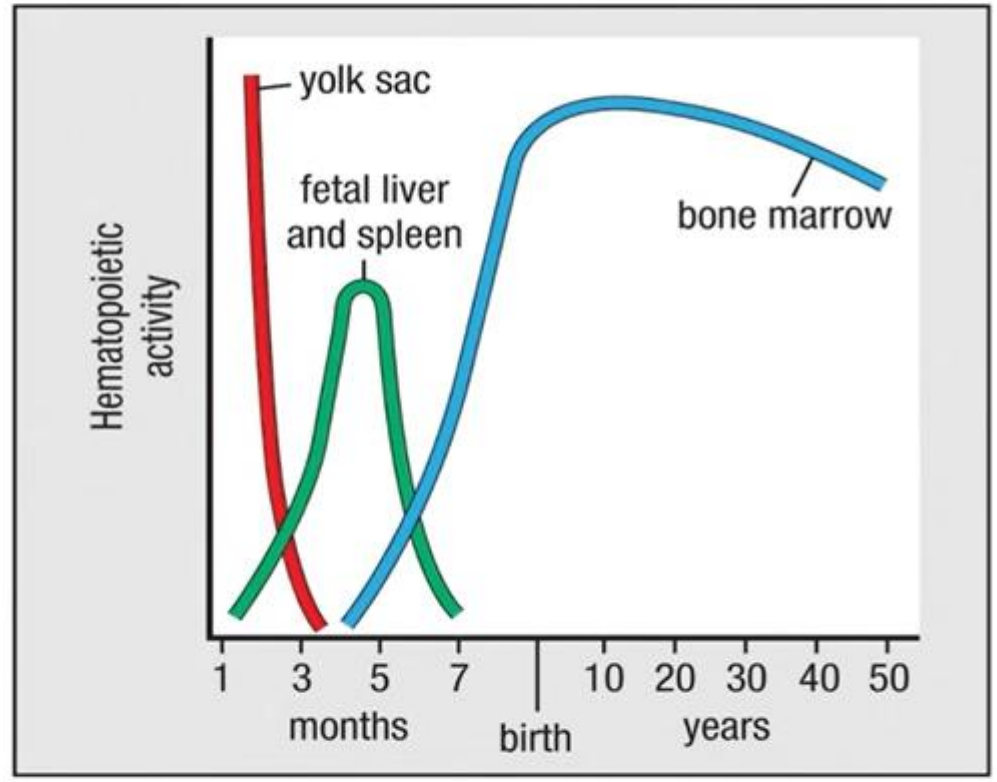




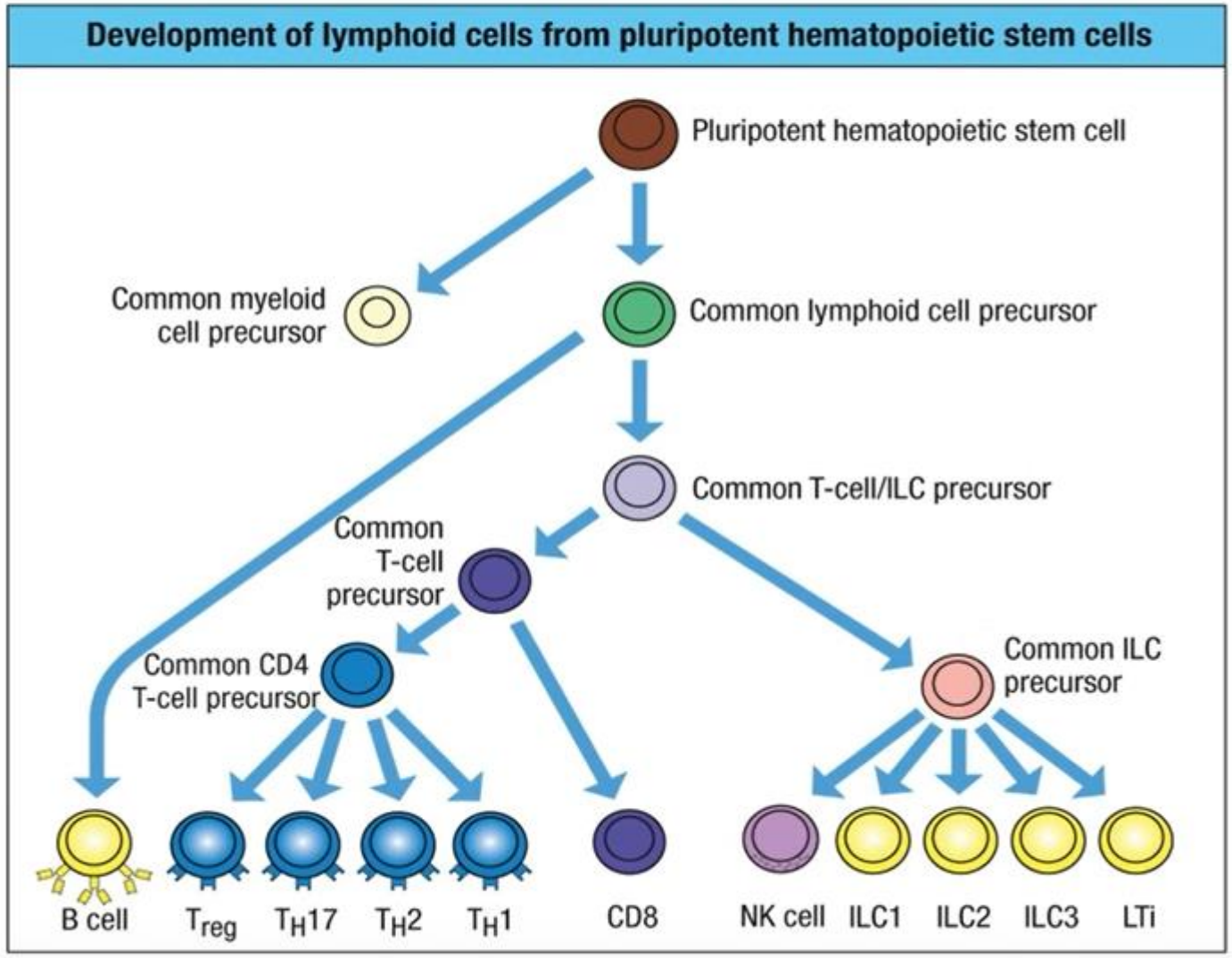
Different stades de la lymphopoiese B

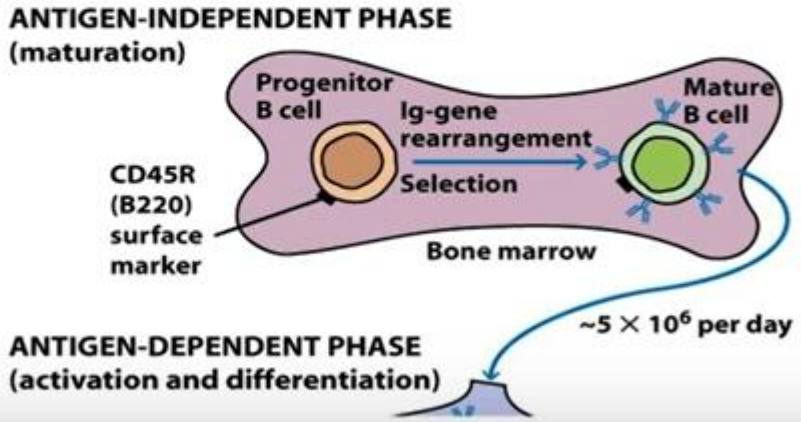
Locations of Hematopoiesis

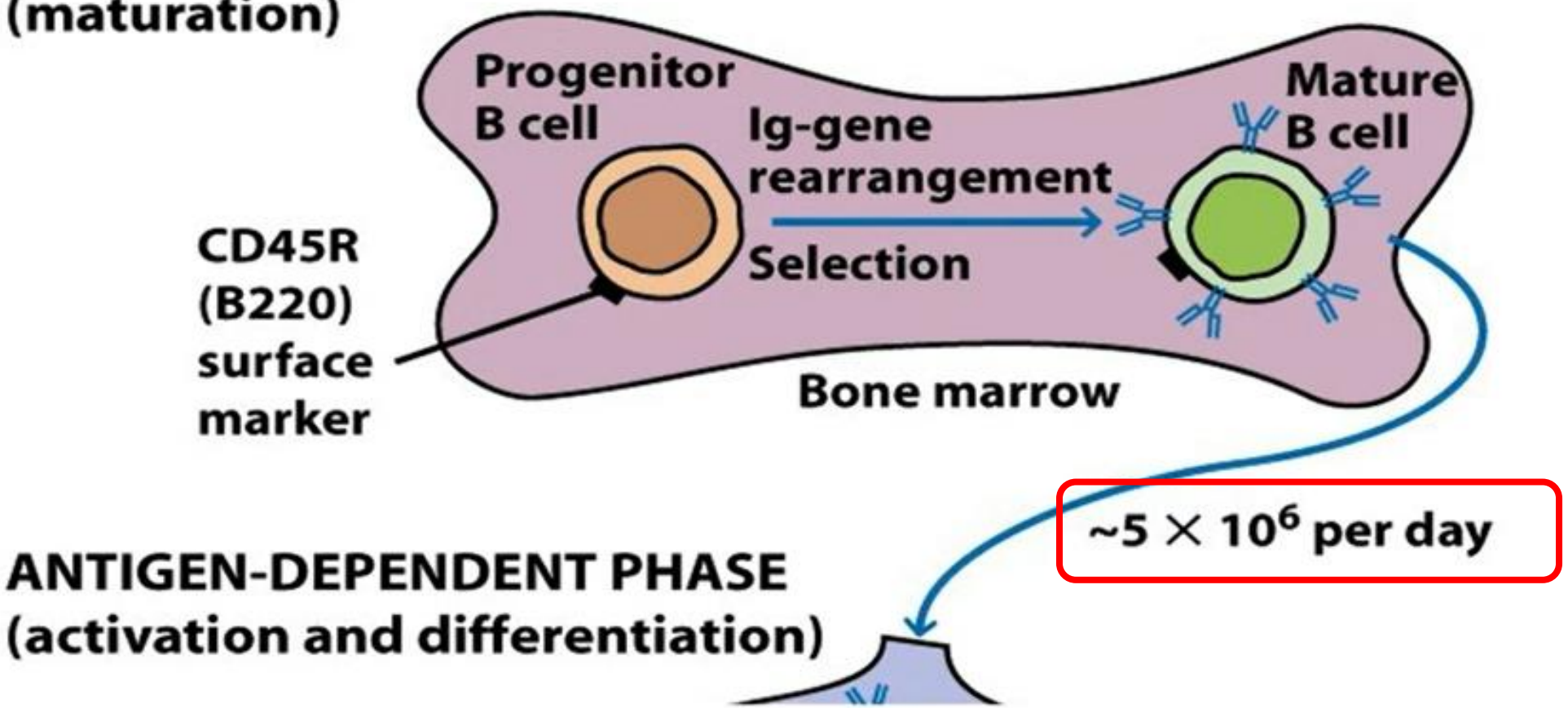




B Cell Development in the Bone Marrow

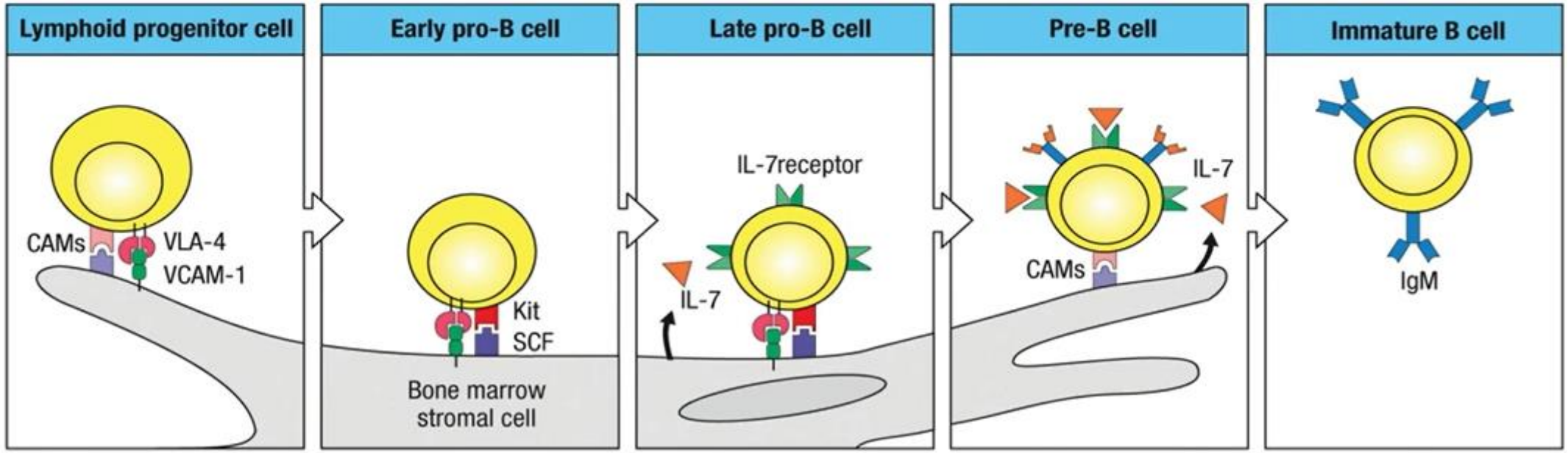




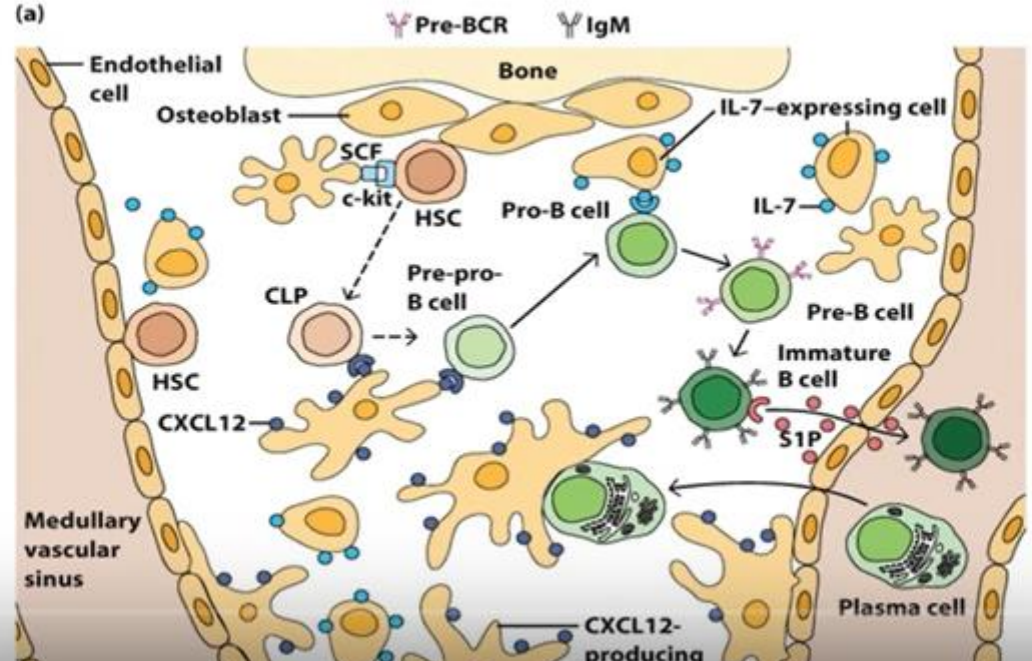


Kuby 7th Edition Figure 11-1

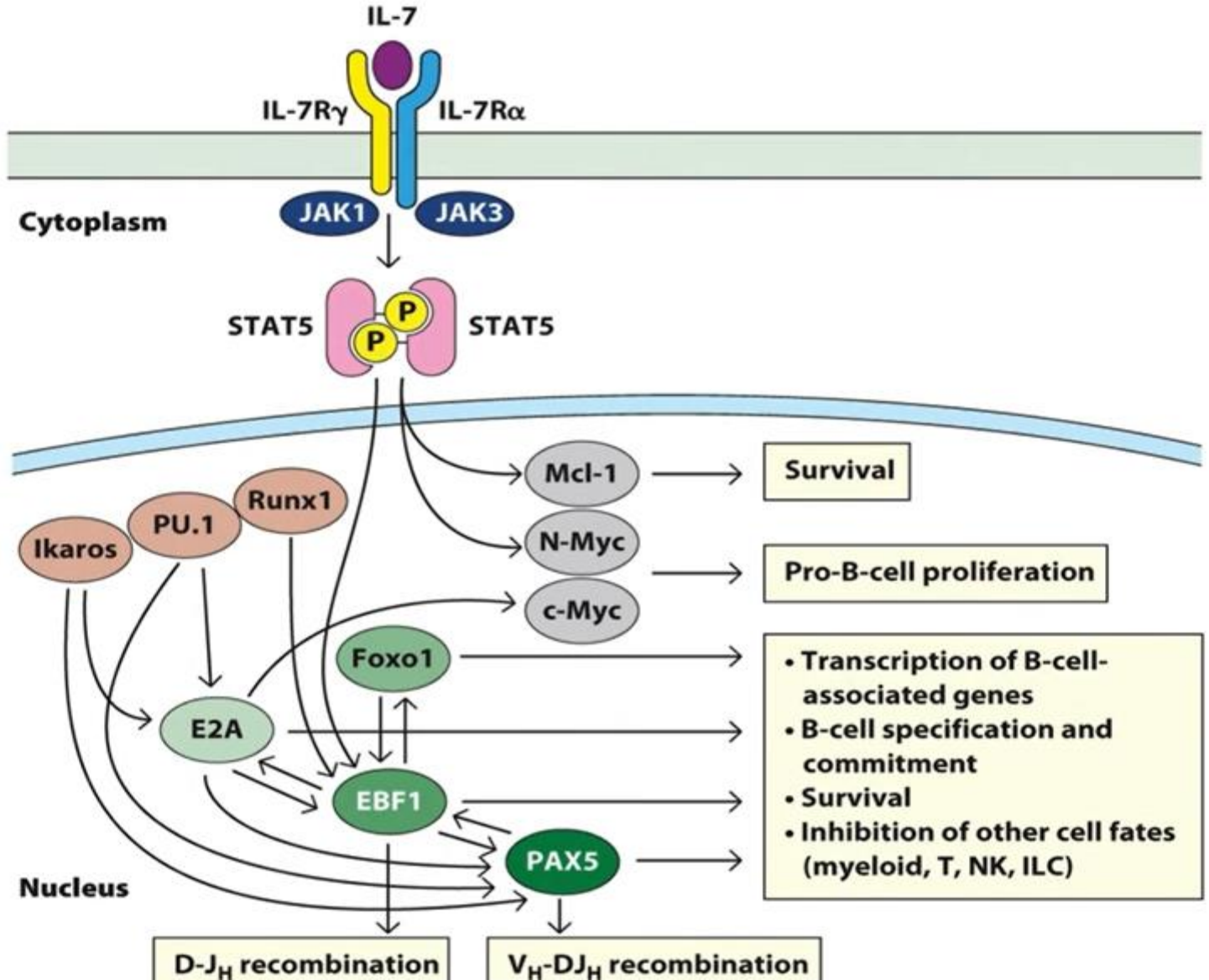
Why Bone Marrow?



Parham Figure 6.5



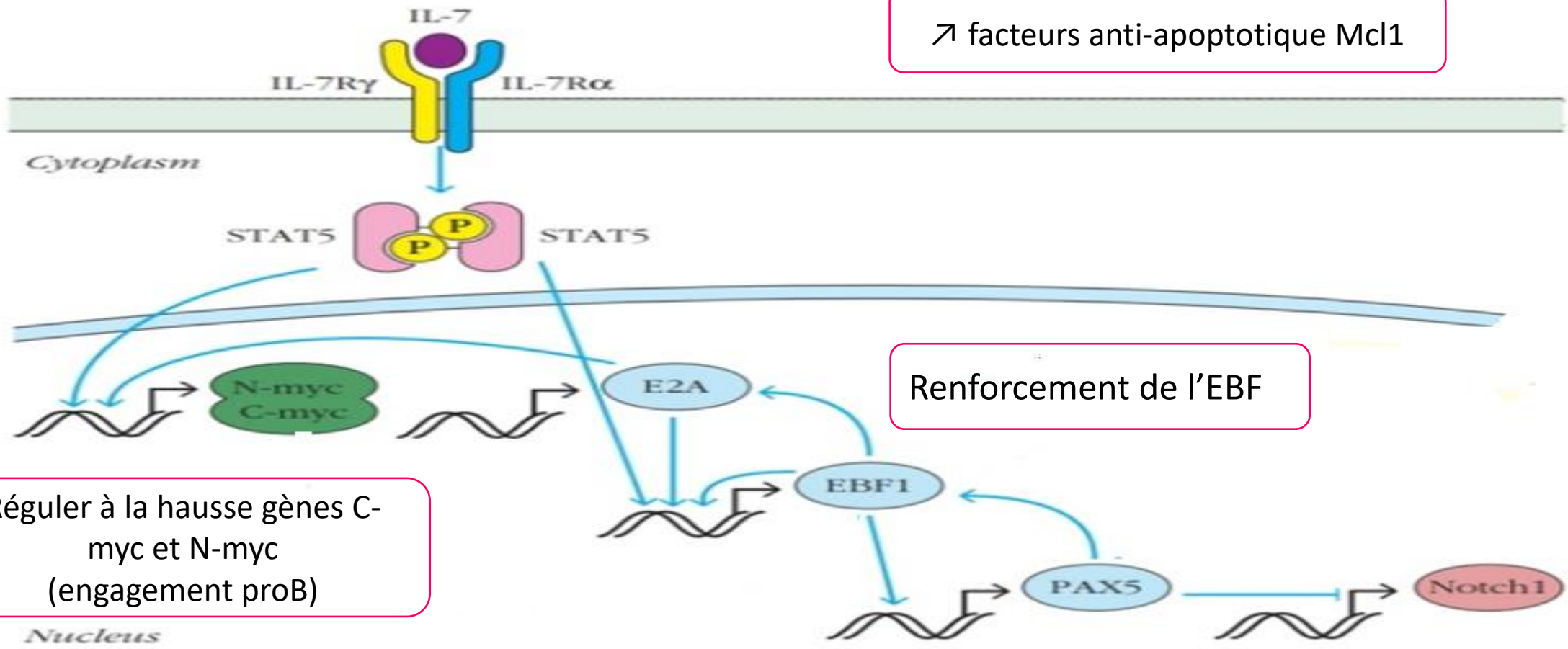
Why Bone Marrow? IL-7



Kuby Figure 9-3

Phase de développement du CLP

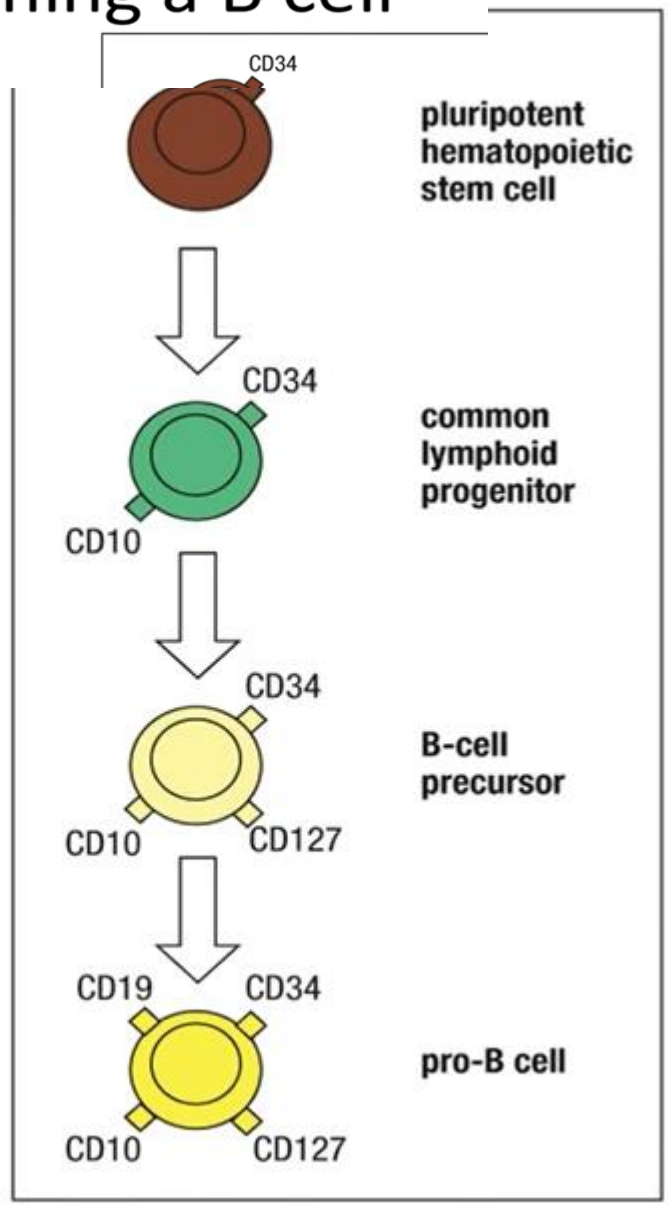
↗ facteurs anti-apoptotique Mcl1



Renforcement de l'EBF

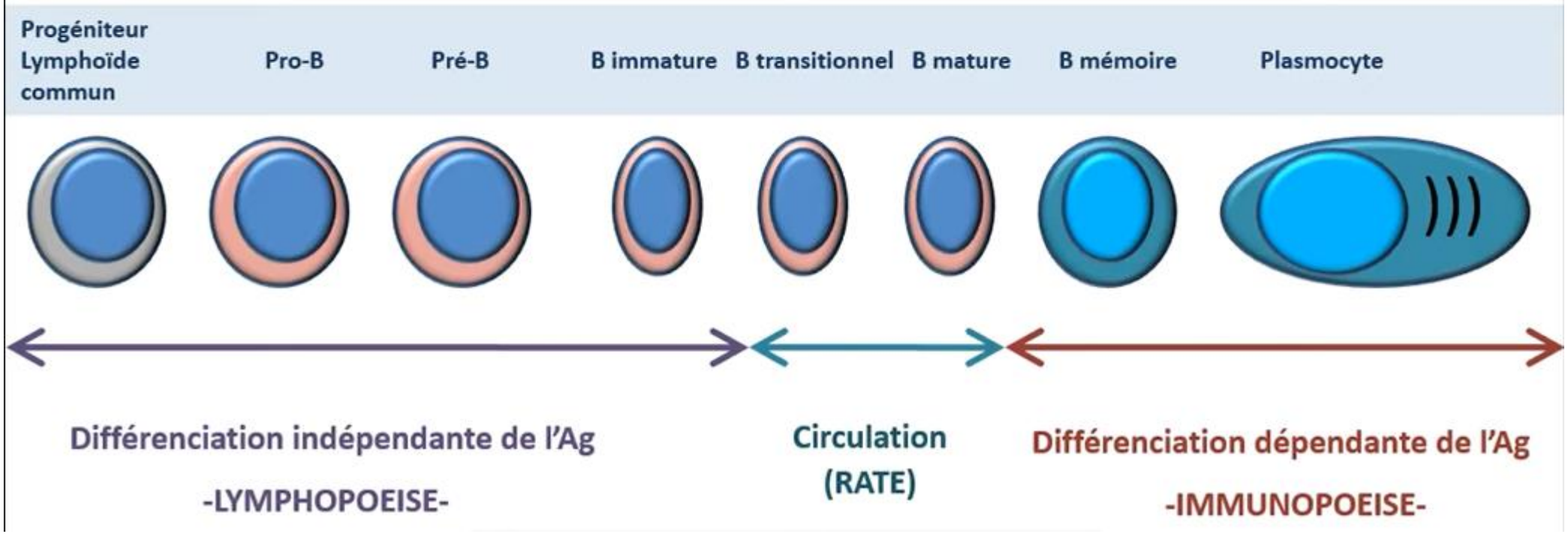
Réguler à la hausse gènes C-myc et N-myc (engagement proB)

Hematopoietic stem cell (HSC) commits to becoming a B cell



ONTOGENIE

- **Différenciation et maturation des LB :**



Immunophenotype des LB en cours de development

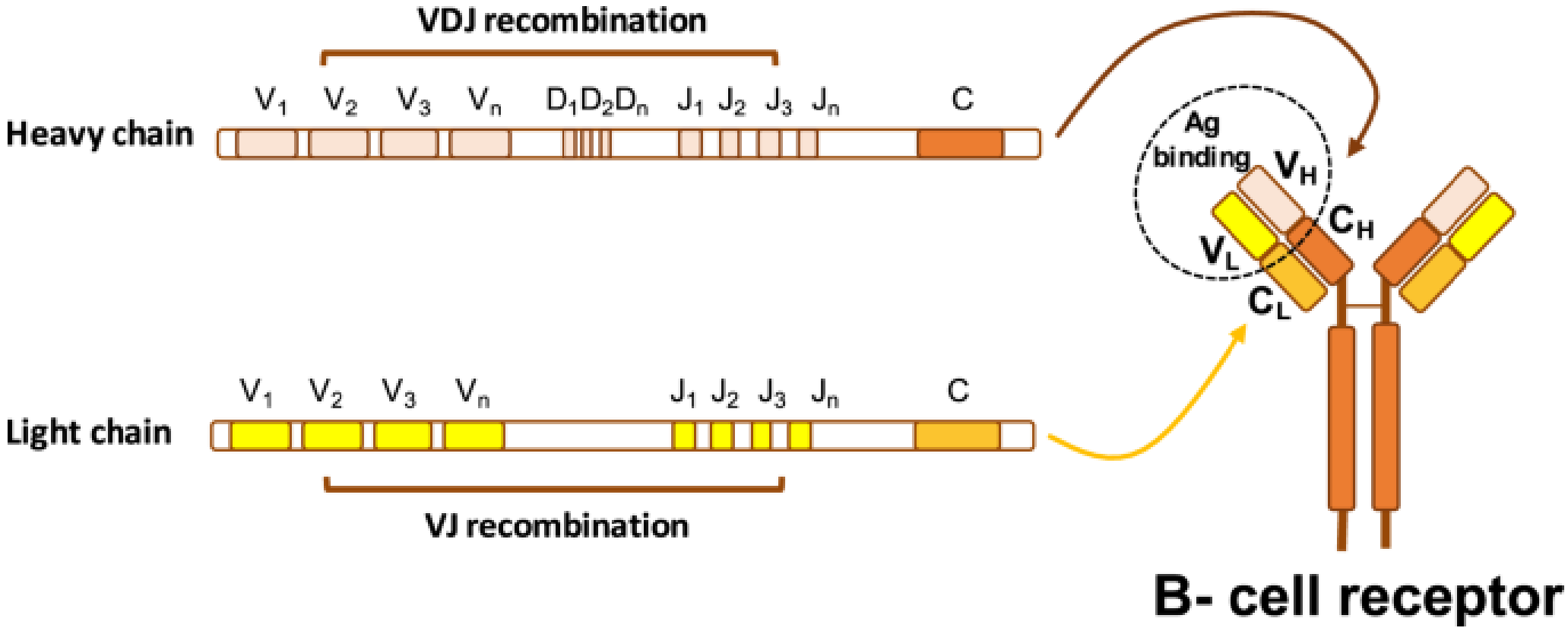
Table 14-2 Human B Cell Maturation Markers during B Cell Development

Marker	HSC	Pro-B	Pre-B	Immature	Transitional 1	Transitional 2	Plasma
CD34	+	+	–	–	–	–	–
CD19	–	+	+	+	+	+	+
CD10	–	+	+	+	+	+	–
CD20	–	+	+	+	+	+	–
CD21	–	–	–	–	–	+	–
CD22	–	–	+	+	+	+	–
CD23	–	–	–	–	–	+	–
CD38	–	+	+	+	+	+	+
CD40	–	+	+	+	+	+	–
CD45	–	+	+	+	+	+	+
CD138	–	–	–	–	–	–	+
RAG-1	–	+	+	+/–	+/–	+/–	–
RAG-2	–	+	+	+/–	+/–	+/–	–
Tdt	–	+	+	–	–	–	–
Igα	–	+	+	+	+	+	+
Igβ	–	+	+	+	+	+	+
Heavy chain	–	$-(D_H-J_H)$	$+ (V_H-D_H-J_H)$	+	+	+	+
Pre-BCR	–	–	+	–	–	–	–
Surface IgM	–	–	–	+	+	+	–
Surface IgD	–	–	–	–	–	+	–
Light chain	–	–	$+ (V_\kappa-J_\kappa\ V_\lambda-J_\lambda)$	+	+	+	+

When a CLP begins to transcribe RNA coding for proteins required for B cell maturation, E2A and EBF, the cell becomes an early B cell progenitor. Once these two transcription factors are expressed, they enable transcription of the proteins involved in the recombination machinery (RAG1/2). The beginning of D to J recombination marks the progress to a pro-B stage.

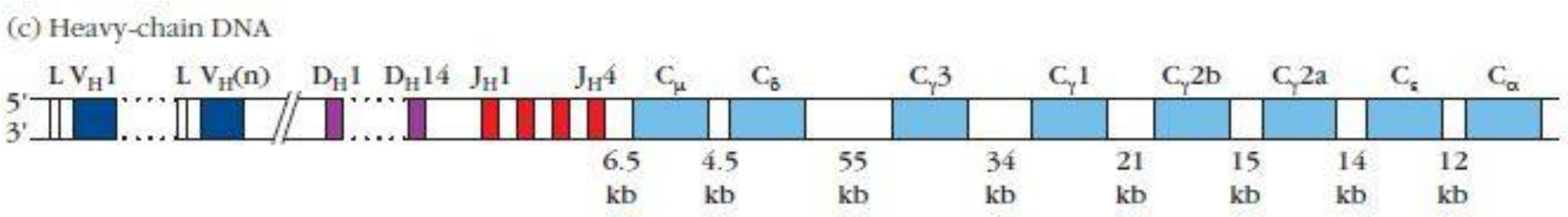
https://musculoskeletalkey.com/b-cells/

Diversite du BCR: Rearangement genique



Organisation des gènes de la chaine lourde

Cluster des gènes codant pour la région constante C des Ig



Ch 14

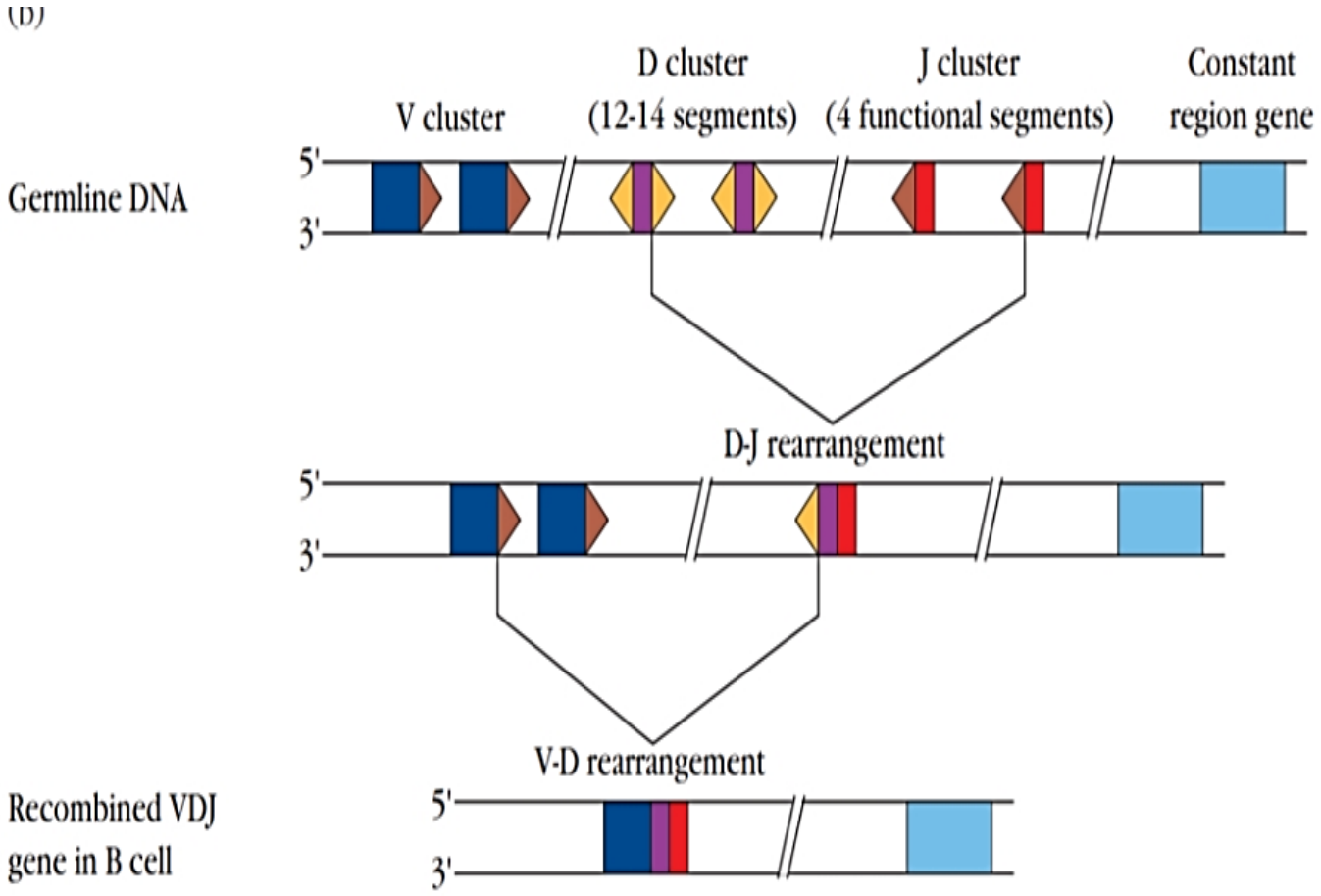
Kuby 7 Eme Edition

le cluster de la région variable est fait de 03 segments:

- ✓ V (variable) 38 segments fonctionnels
- ✓ D (diversité) 23 segments fonctionnels
- ✓ J (jonction) 6 segments fonctionnels

Réarrangement du cluster de la région variable

a) Chaine Lourde



Commence par la combinaison du segment D avec J au stade pre-proB suivit par la recombinaison du segment V avec D-J,

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Rearangement genique via les enzymes RAG, Tdt, complexe Artemis et autres enzymes

- **RAG1/2:** (gène activant la recombinaison) complexe de deux protéines spécifiques des lymphocytes catalysent le clivage de l'ADN et formation de jonction codante,

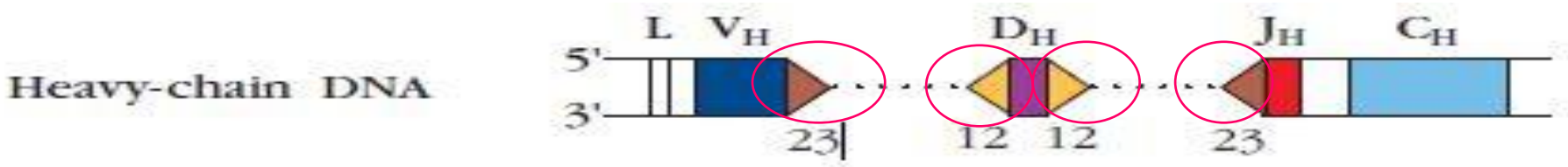
- **TDT :** (terminal deoxynucleotydil transférase) spécifique des lymphocytes accroit la diversité de la région CDR3 en ajoutant aléatoirement des nucléotides N (no templated ou hors matrice) aux extrémités 3' libres de la jonction de la chaine lourde,

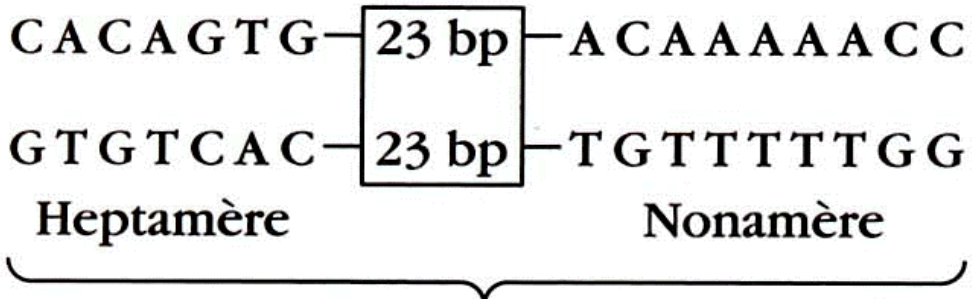
- **ARTEMIS :** complexe qui intervient dans la réparation des cassures de l'ADN clivé,

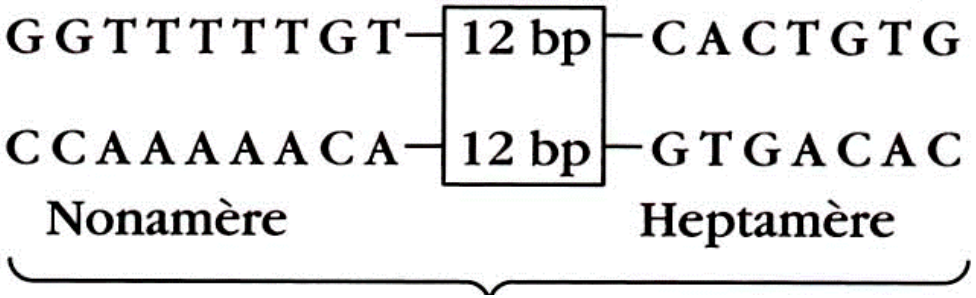
- **HMG1/2 :** stabilisent la liaison RAG1/2 aux segments signals SSR

- Autres proteines DNA ligase, XRCC4 .. Non specifiques aux lymphocytes

Mécanisme de réarrangements des gènes de la chaine lourde







RSS

RSS

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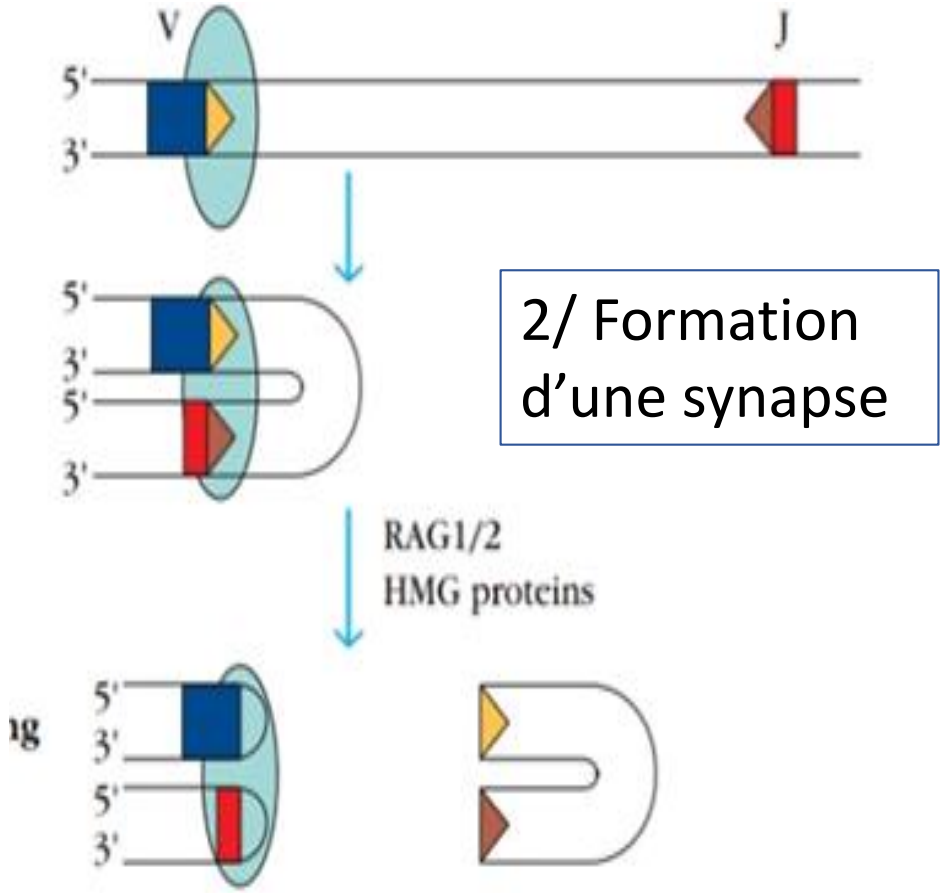
2-Mécanisme de réarrangement des gènes de la chaine lourde

1/ Liaison de RAG1/2 au niveau des RSS



2/ Formation d'une synapse

3/ Clivage simple brin et formation d'extremité en épingle à cheveux

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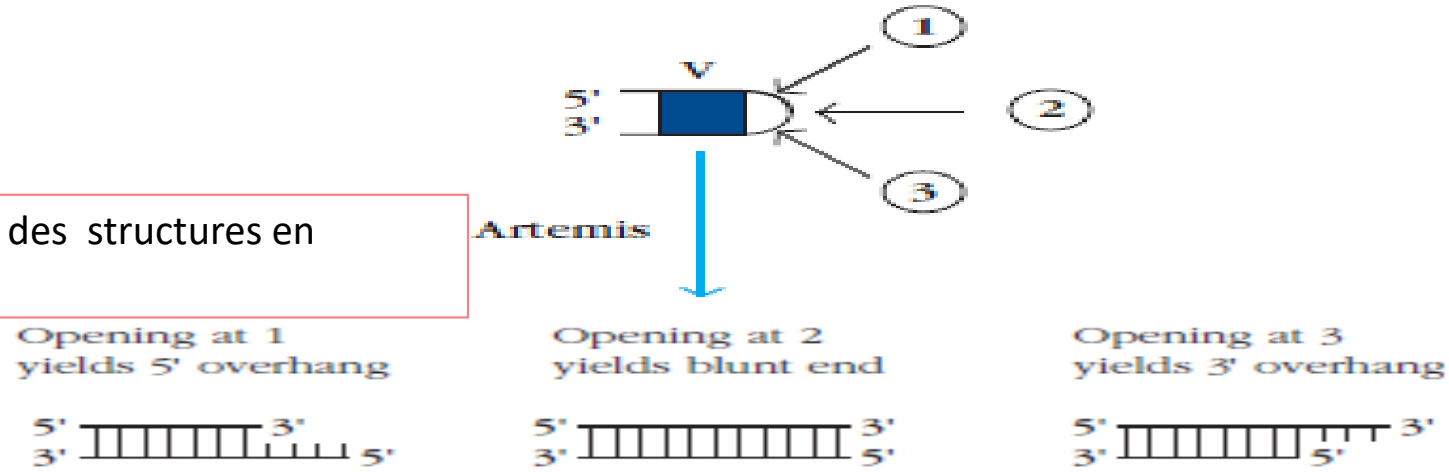
4/ Clivage asymétrique des structures en épingle à cheveux.



5/ Uniquement au jonctions VD et DJ de chaine lourde , la perte de nucléotides codants de par et d'autre de la jonction sous l'action d'une exonucléotidase

6/ Des nucléotides sont ajoutés aléatoirement au jonctions codantes par la Tdt

7/ Ligation de la chaine lourde par l'ADN ligase IV et les protéines NHEJ

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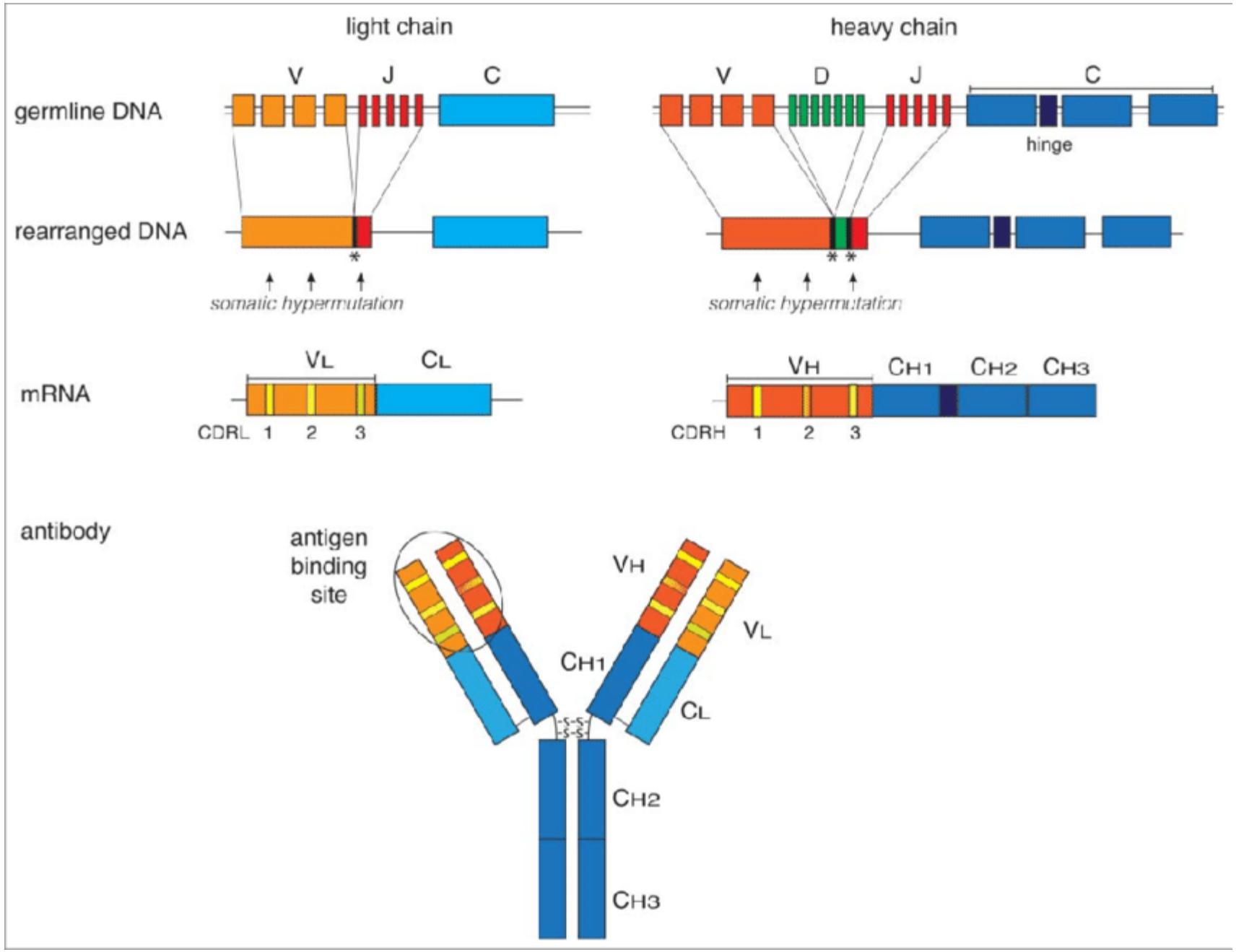


Molecular Biology of B Cells (Second Edition) 2015, Pages 13-34

Recombination-Activating Genes (RAGs)

encode parts of a protein complex that plays important roles in the rearrangement and recombination of the genes encoding immunoglobulin and T cell receptor molecules.

There are two recombination-activating genes RAG1 and RAG2, whose cellular expression is restricted to lymphocytes during their developmental stages.

RAG1 and RAG2 encoded on Chr. 11 *p13*

The enzymes encoded by these genes, RAG-1 and RAG-2, are essential to the generation of mature B cells and T cells

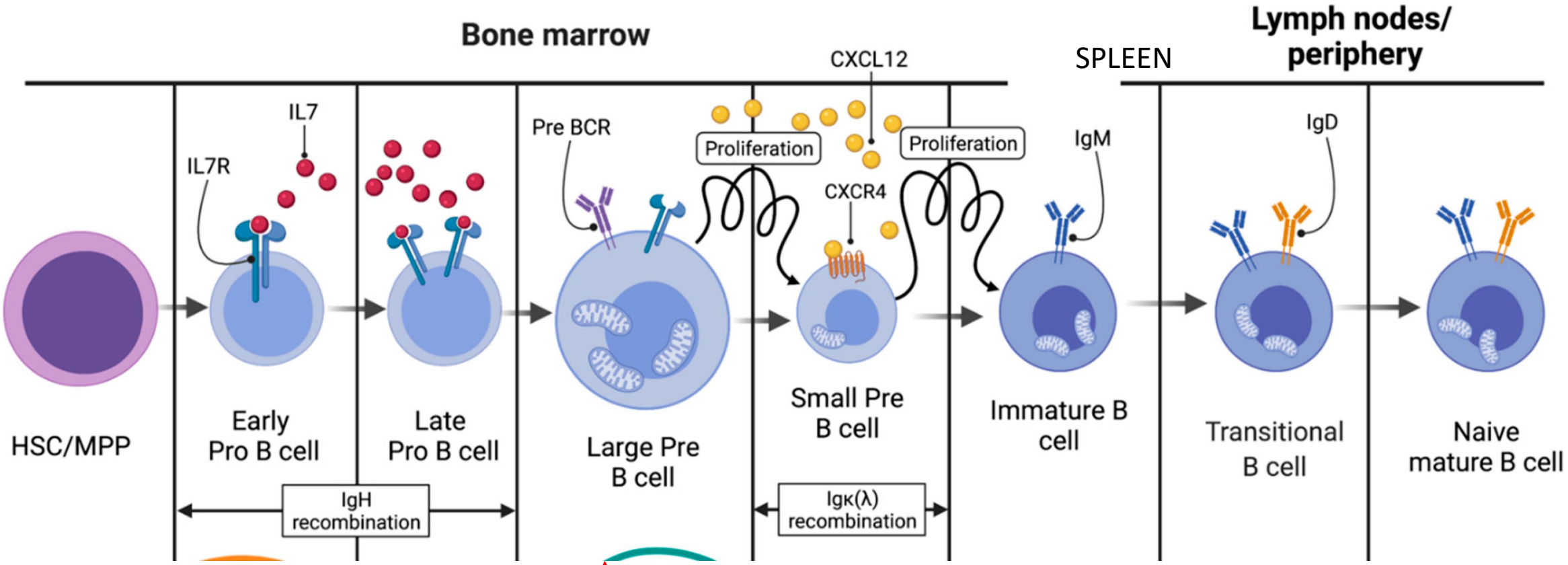
The immune system generates this diversity of antibodies by shuffling, cutting and recombining a few hundred genes (the VDJ genes) to create millions of permutations, in a process called V(D)J recombination.

RAG-1 and RAG-2 are proteins at the ends of VDJ genes that separate, shuffle, and rejoin the VDJ genes. This shuffling takes place inside B cells and T cells during their maturation.

20486_5-1.webp

T and B cell deficiency induced by RAG deficiency

SCID: SEVERE COMBINED IMMUNODEFICIENCY



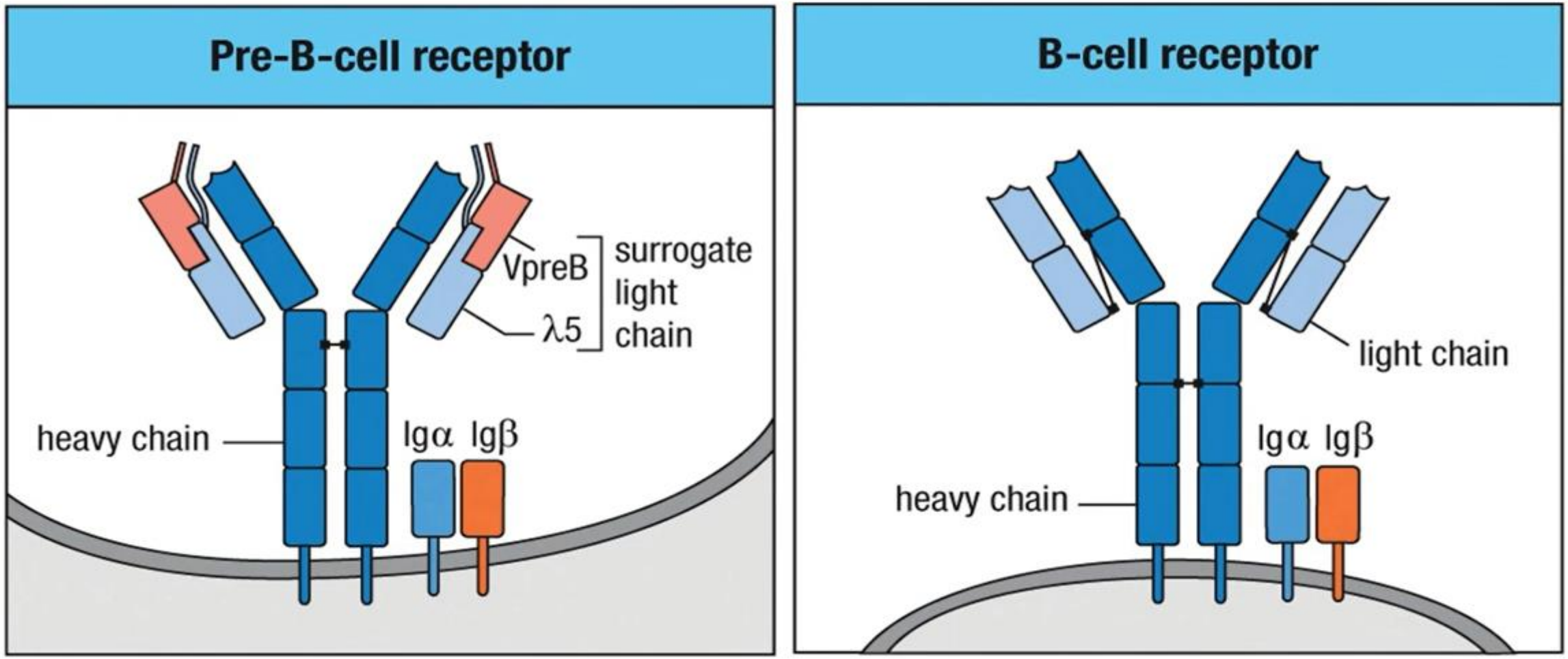
1st checkpoint

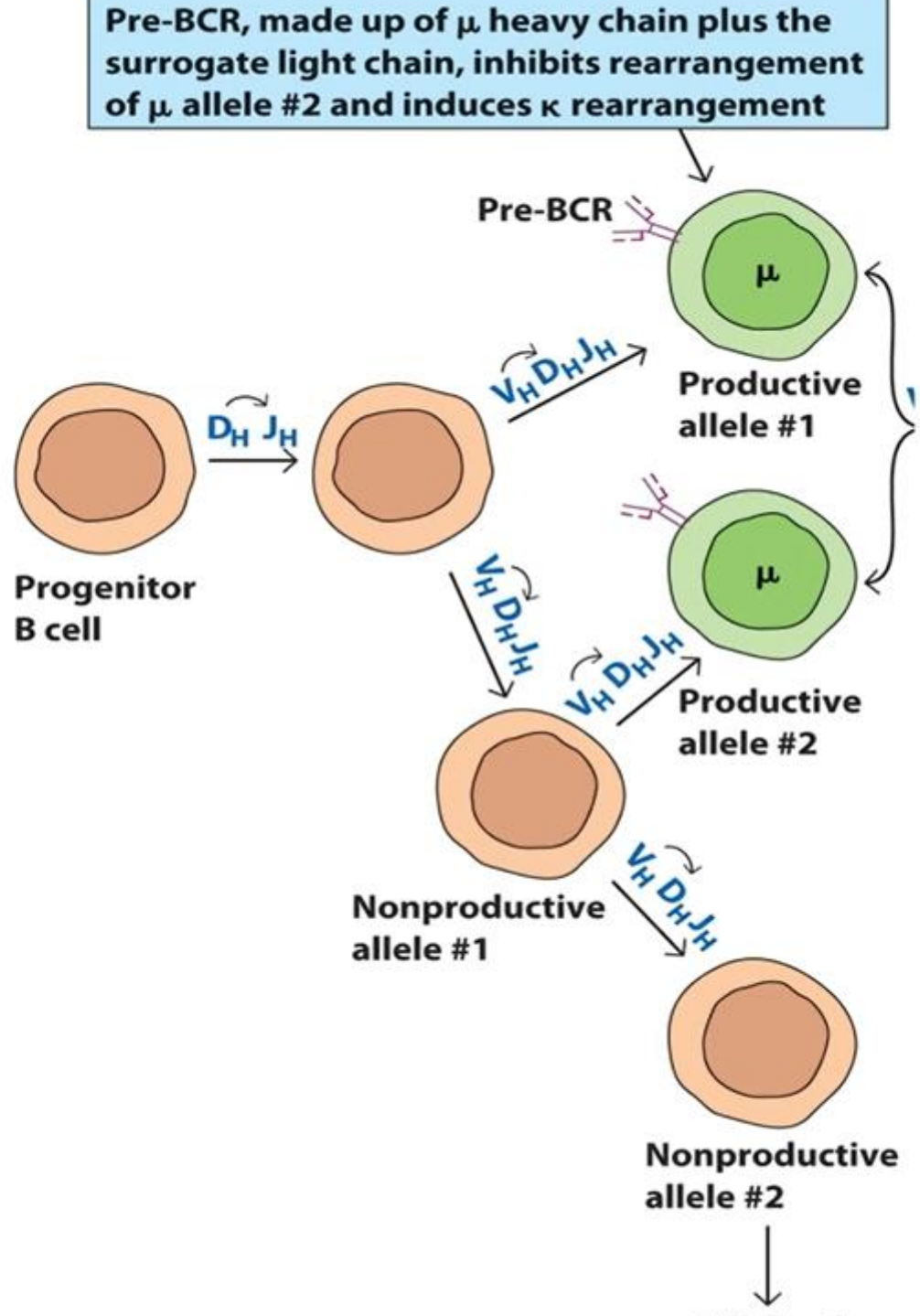
Fonctional Heavy chain

2st checkpoint

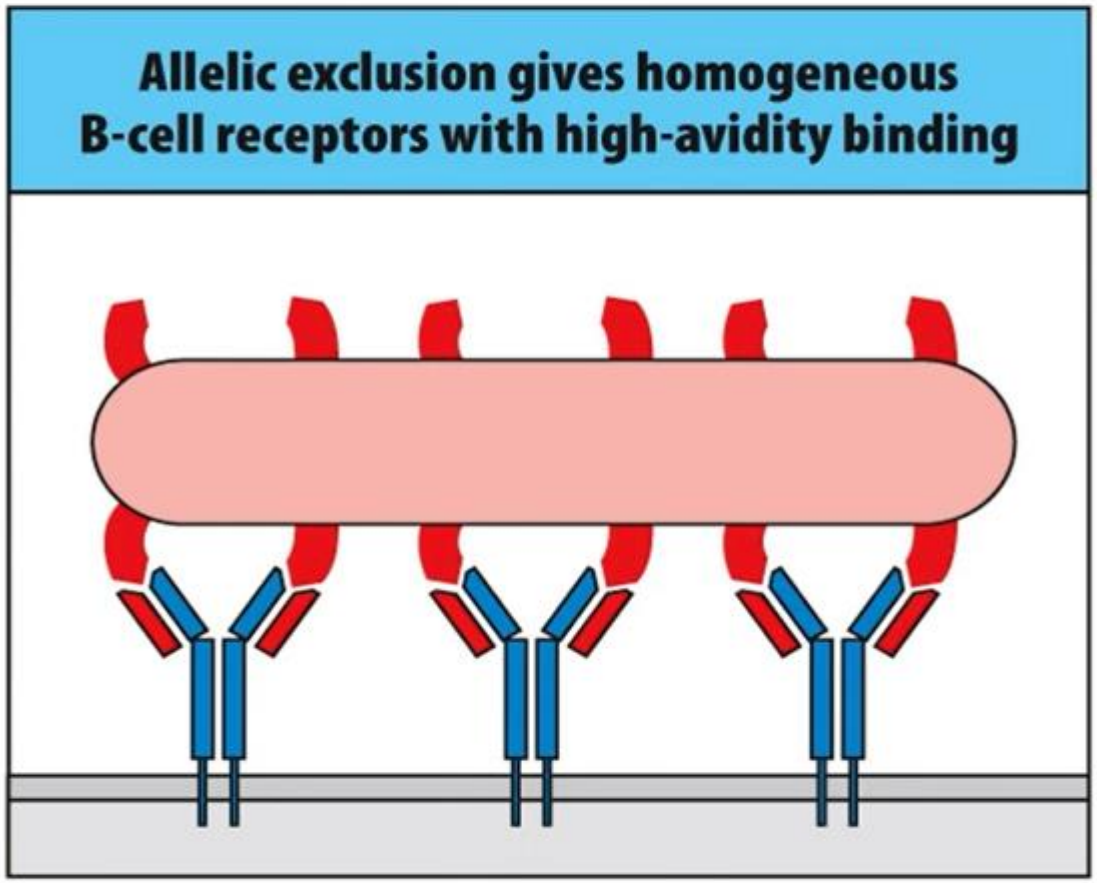
Deletion of autoreactive B-cells
1st Negative selection

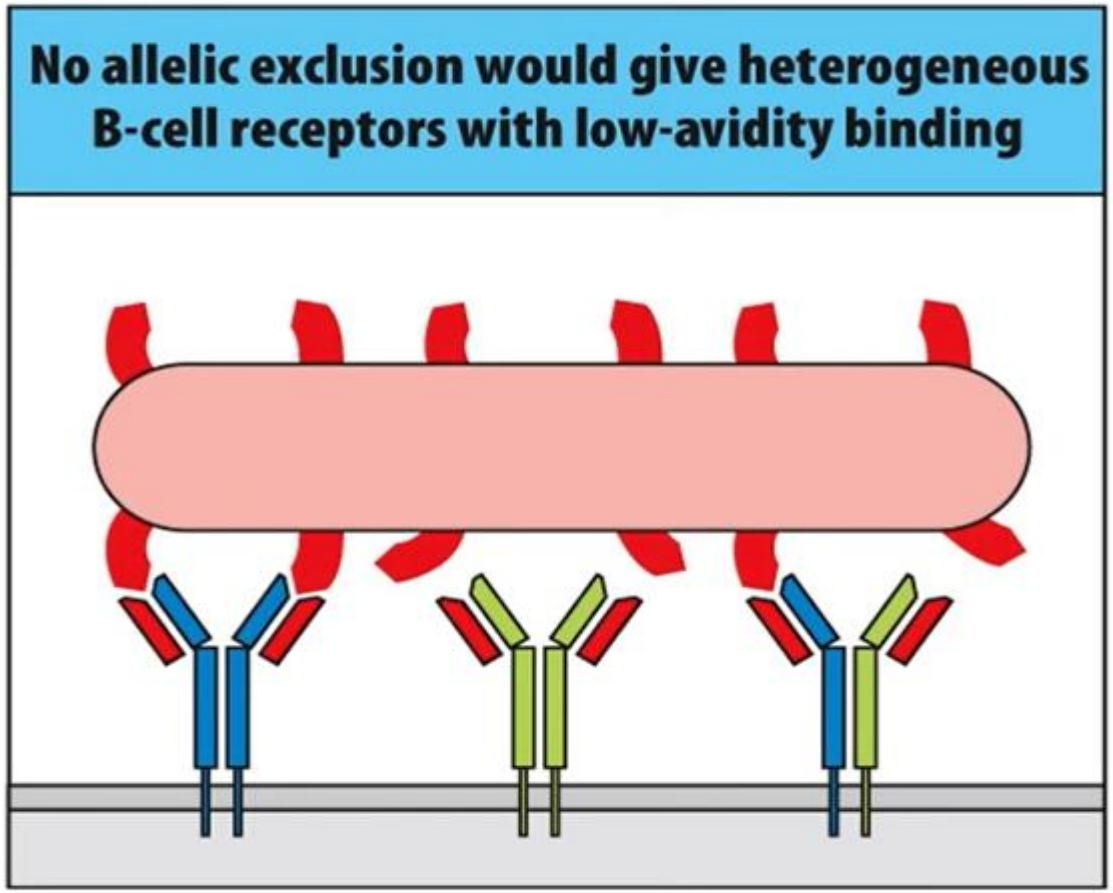
The Pre-B cell receptor and surrogate light chains




Pre-BCR, made up of μ heavy chain plus the surrogate light chain, inhibits rearrangement of μ allele #2 and induces κ rearrangement
Pre-BCR
μ
Productive allele #1
$V_H D_H J_H$
$D_H J_H$
Progenitor B cell
$V_H D_H J_H$
μ
$V_H D_H J_H$
Productive allele #2
Nonproductive allele #1
$V_H D_H J_H$
Nonproductive allele #2

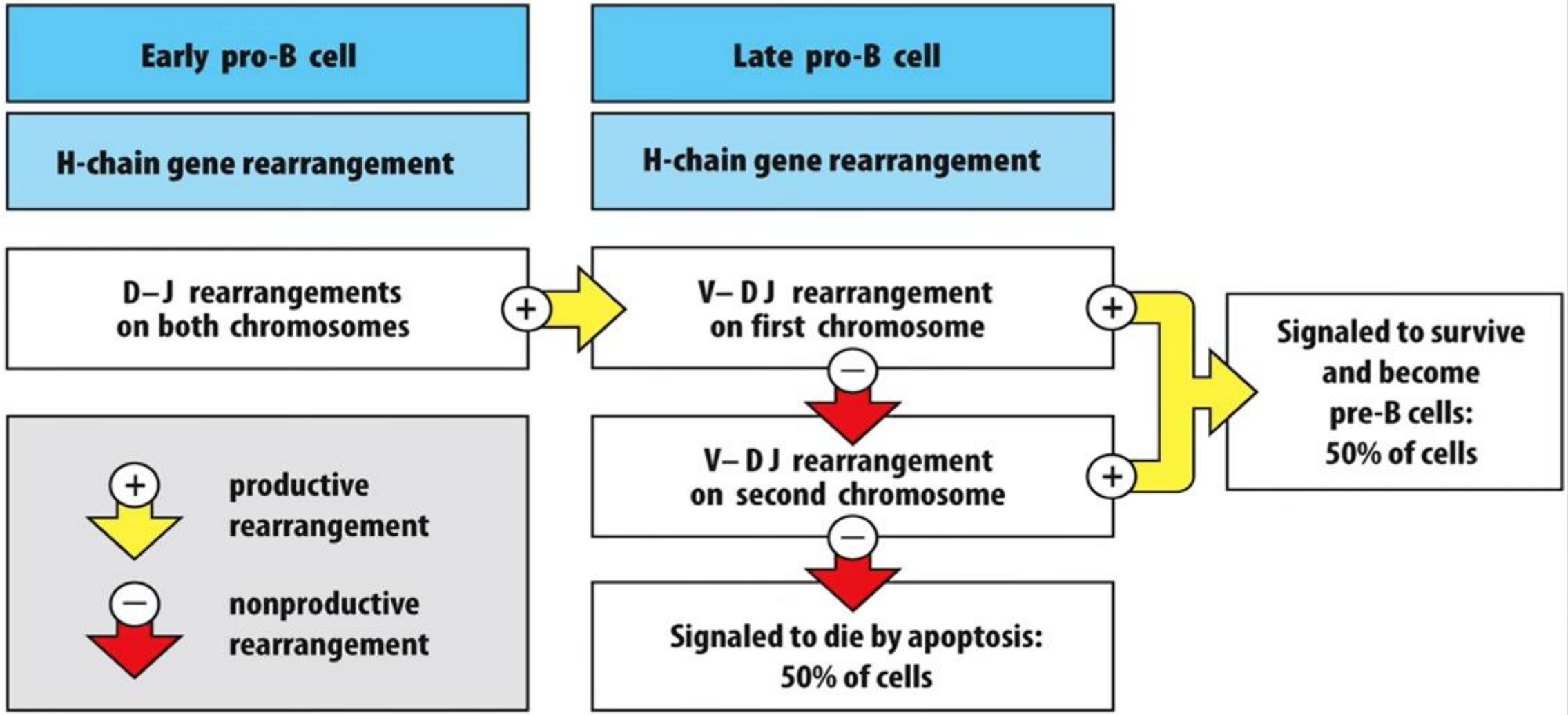
Allelic Exclusion





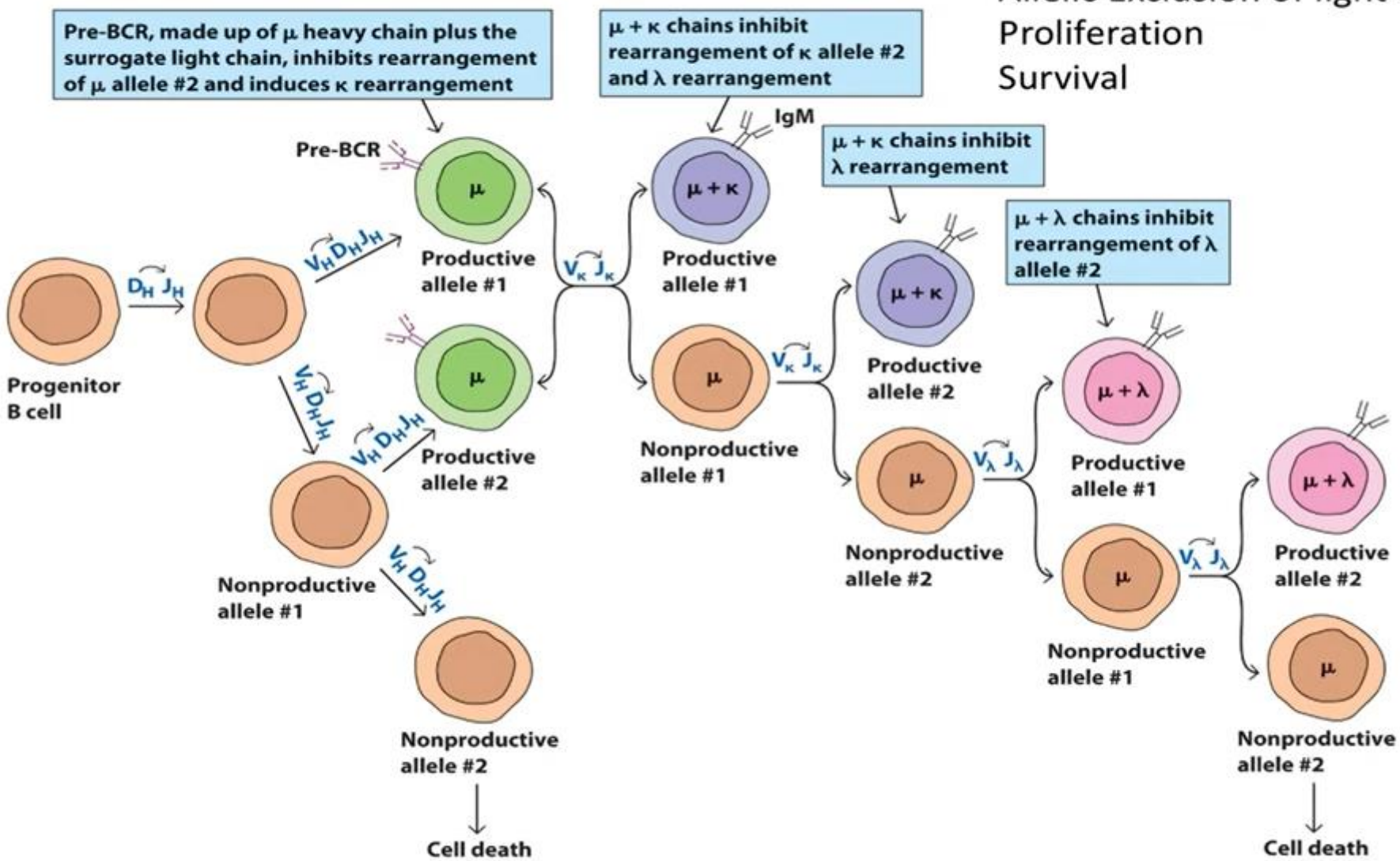
Parham 4th Edition Figure 6.8

Selecting for a Functional Heavy Chain



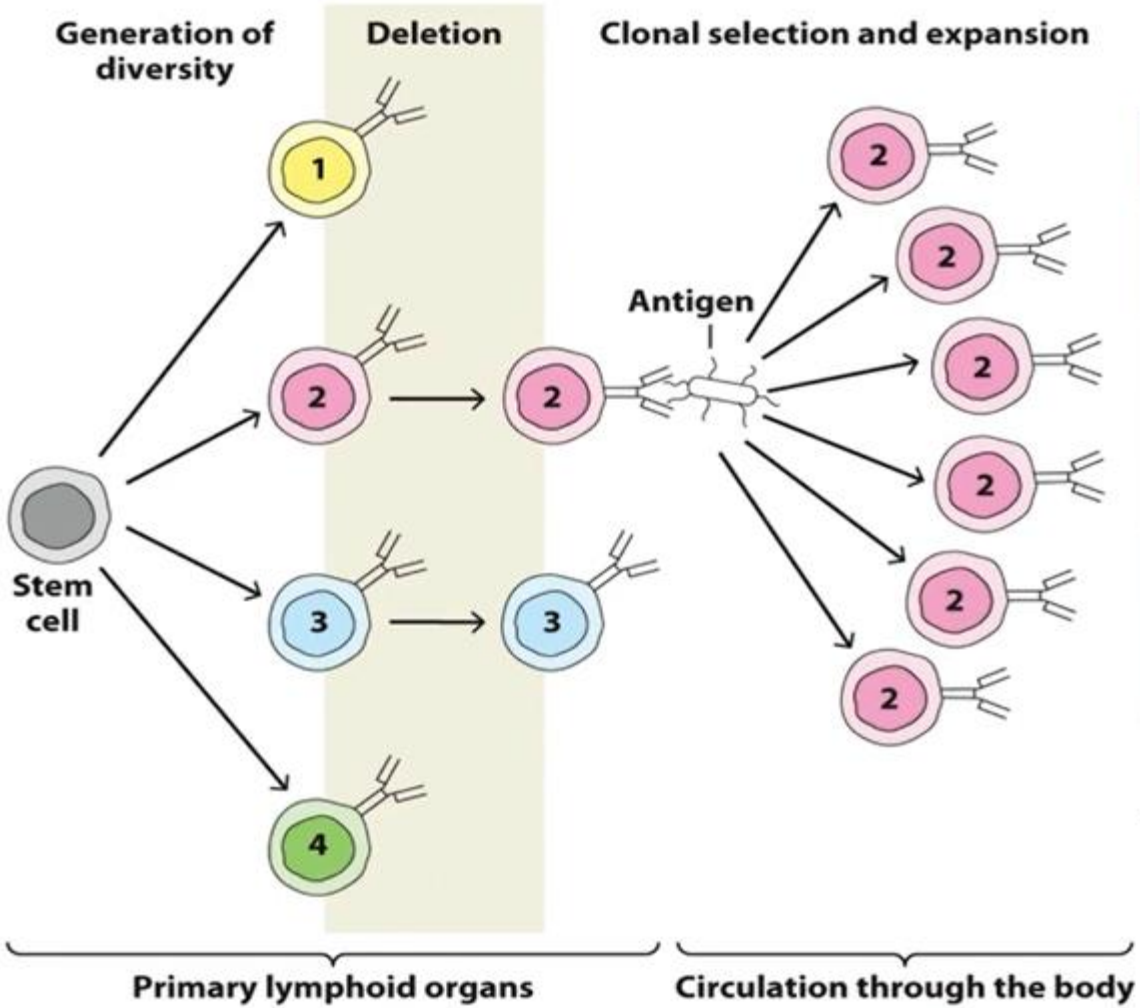
Testing the light chain

Allelic Exclusion of light chain
Proliferation
Survival



Kuby Figure 6-15

Central Tolerance



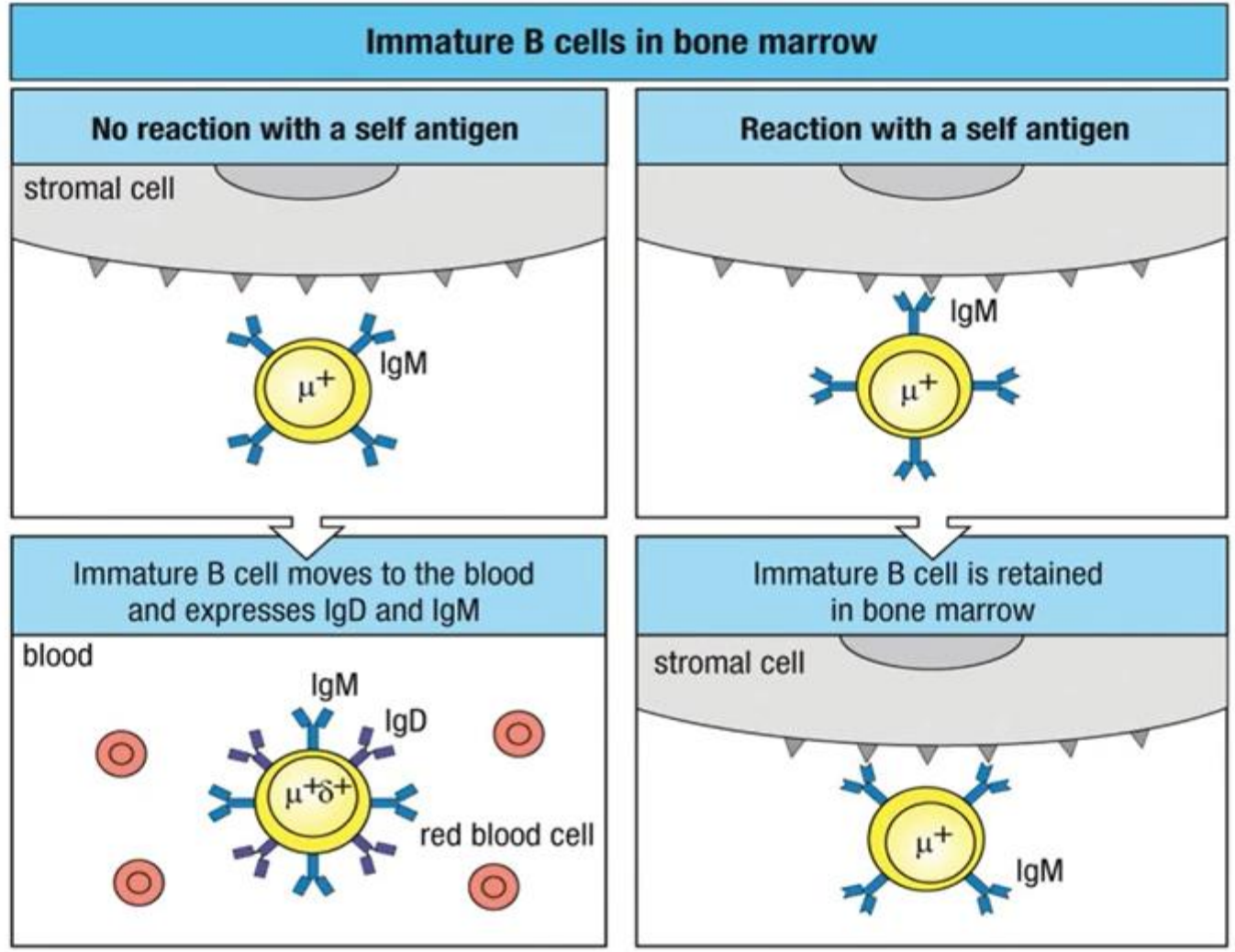
Kuby Figure 1-6

TABLE 11-1 Components of the clonal selection hypothesis

- Immature B lymphocytes bear immunoglobulin (Ig) receptors on their cell surfaces. All receptors on a single B cell have identical specificity for antigen.
- On antigen stimulation, the B cell will mature and migrate to the lymphoid organs, where it will replicate. Its clonal descendants will bear the same receptor as the parental B cell and secrete antibodies with an identical specificity for antigen.
- At the close of the immune response, more B cells bearing receptors for the stimulating antigen will remain in the host than were present before the antigenic challenge. These memory B cells will then be capable of mounting an enhanced secondary response.
- B cells with receptors for self antigens are deleted during embryonic development.

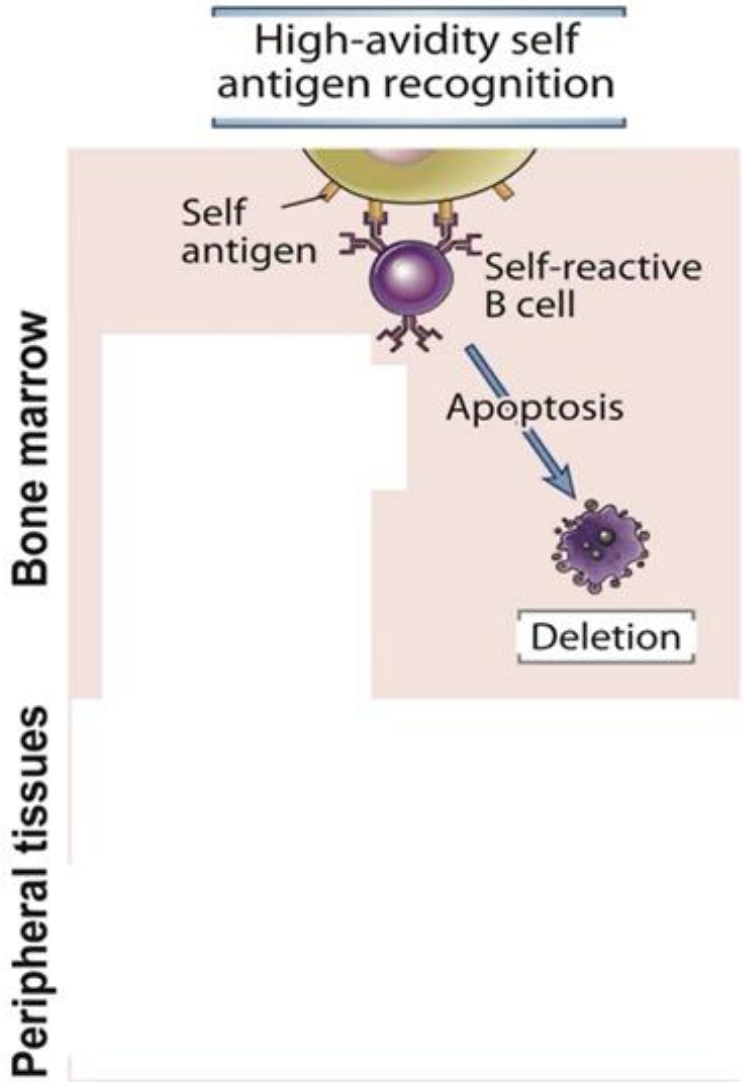
Kuby Table 11-1

B Cell Central Tolerance



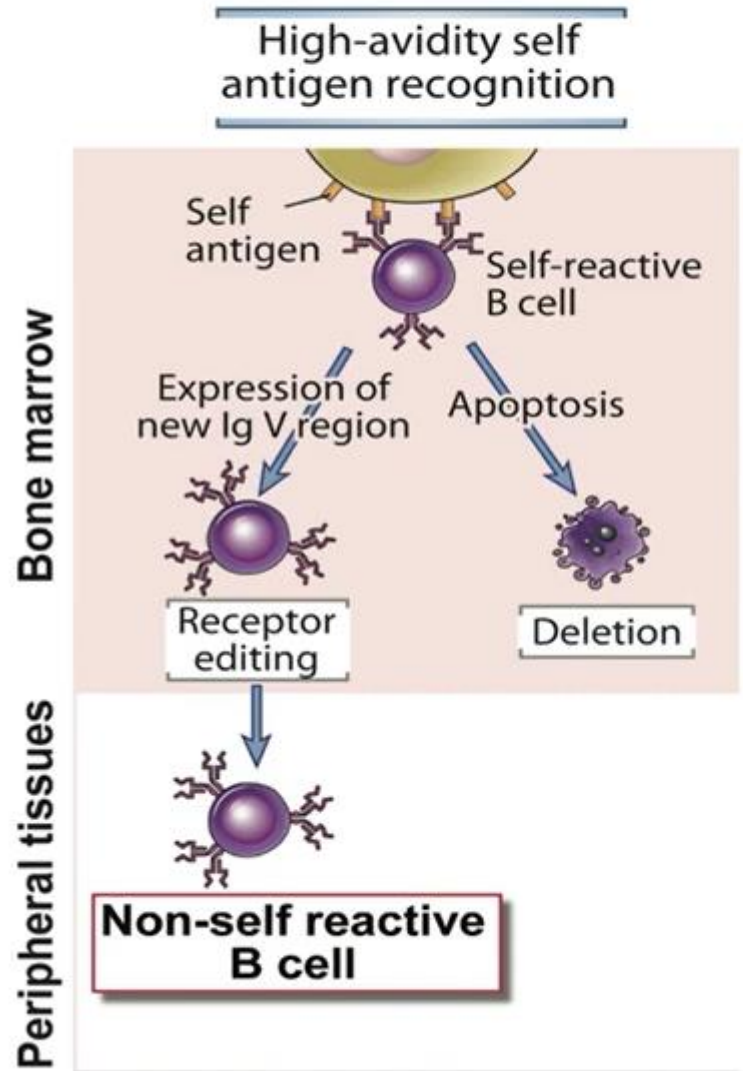
Parham Figure 6.16

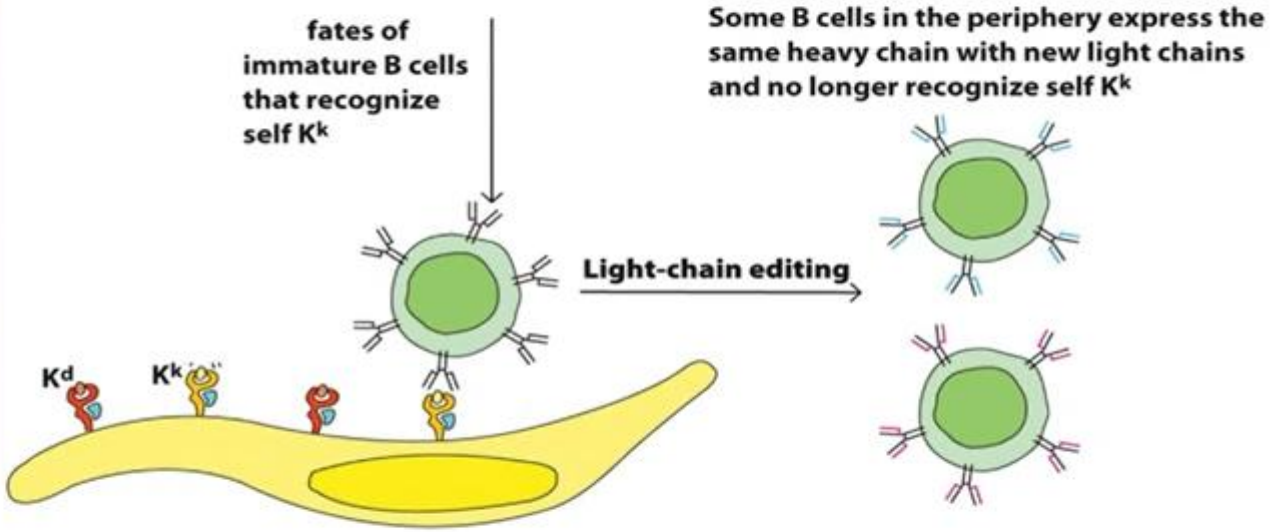
Option 1: Deletion



Abbas Fig. 14-8
See Kuby Figure 9-6

Option 2: Receptor editing

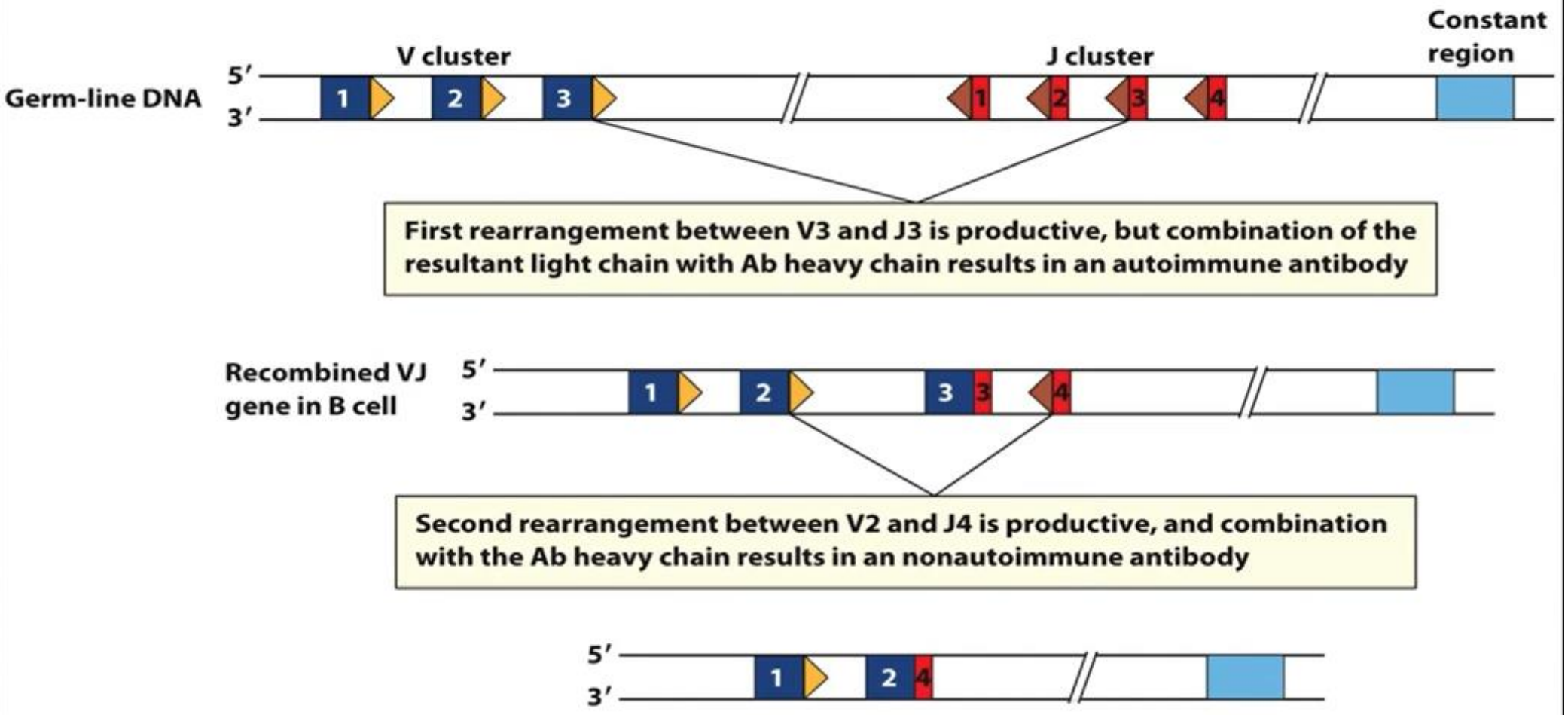




Abbas Fig. 14-8

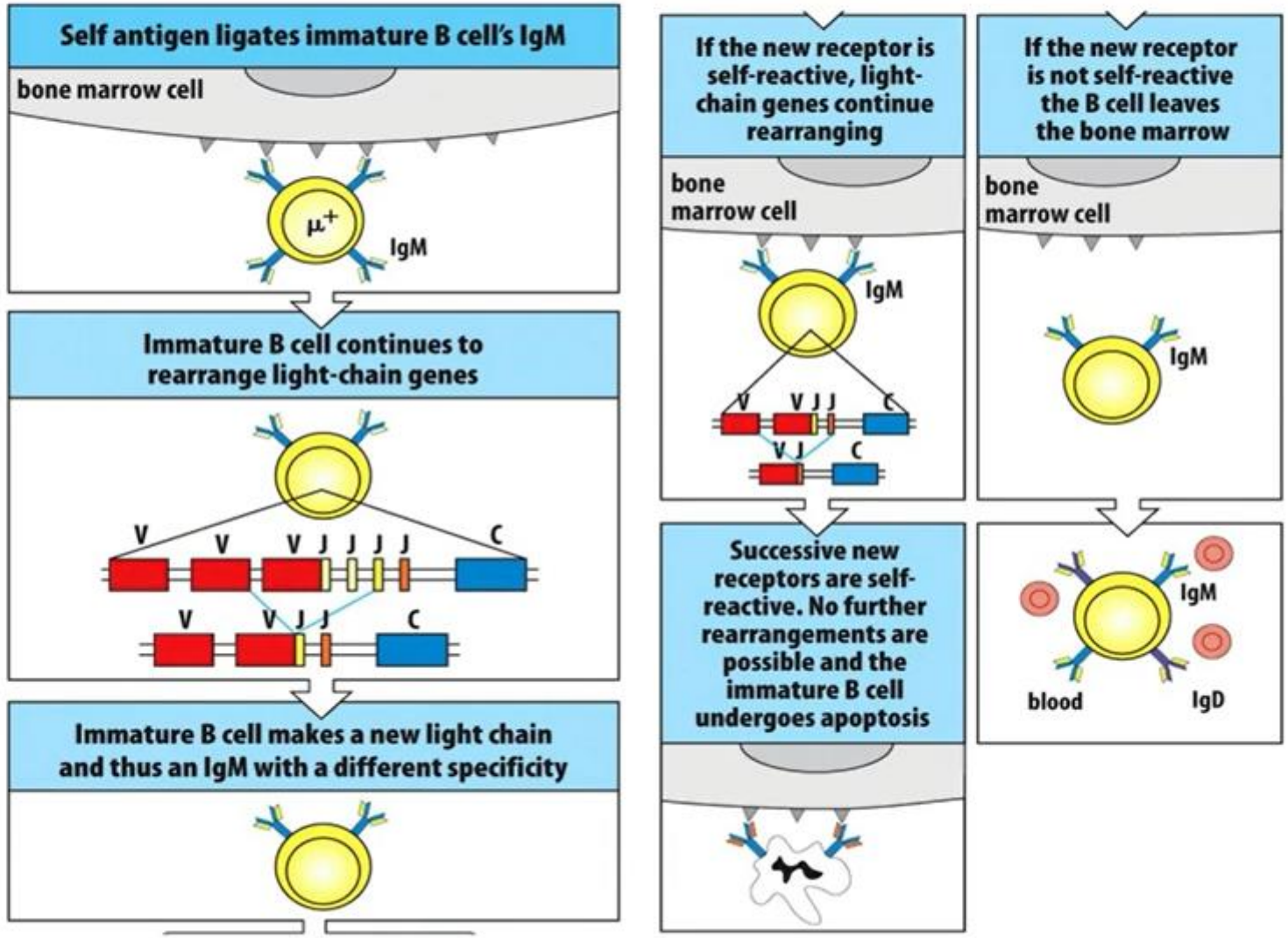
Kuby Figure 9-6

Receptor editing



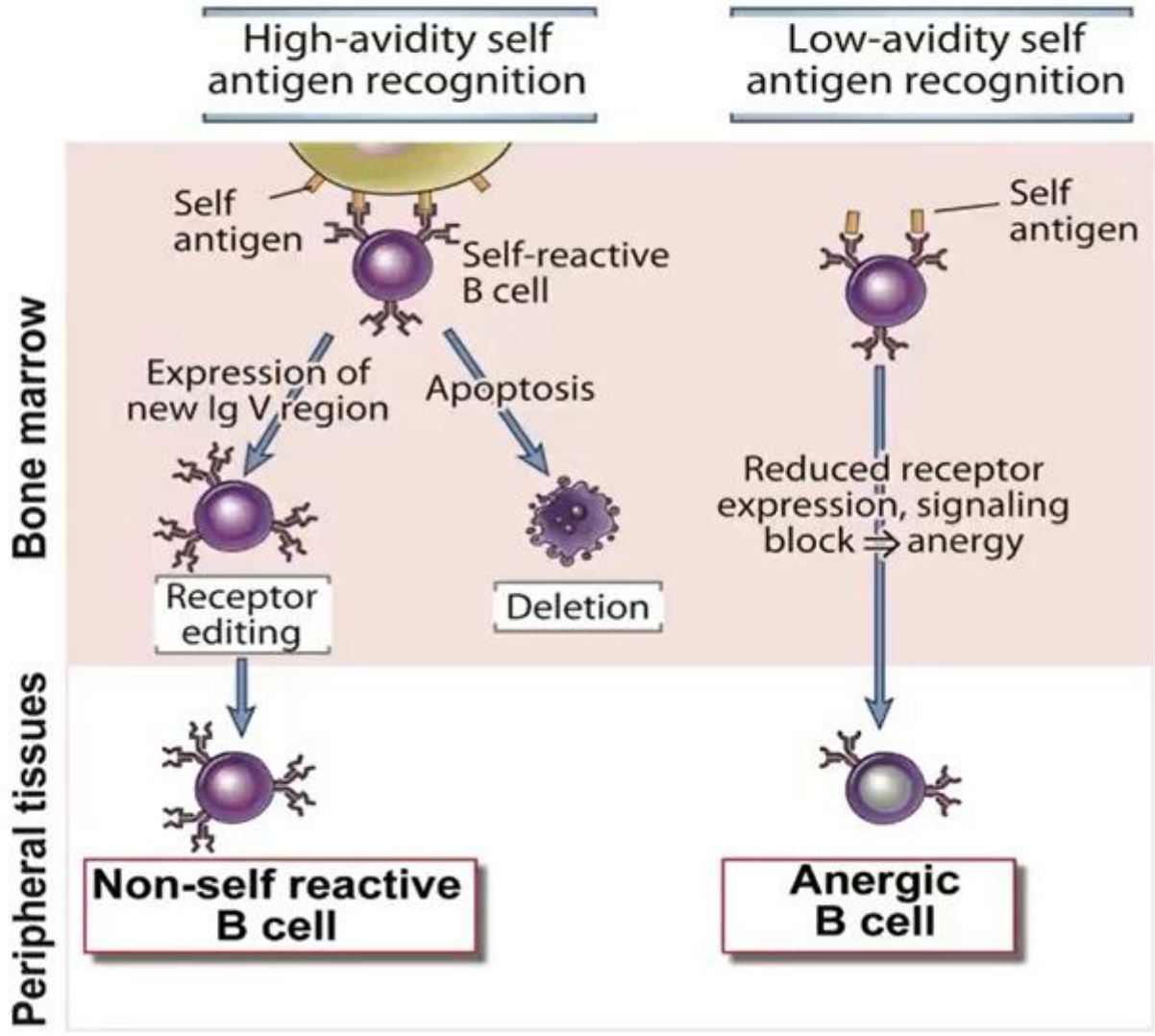
Kuby Figure 6-16

Auto-reactive B cells receptor edit



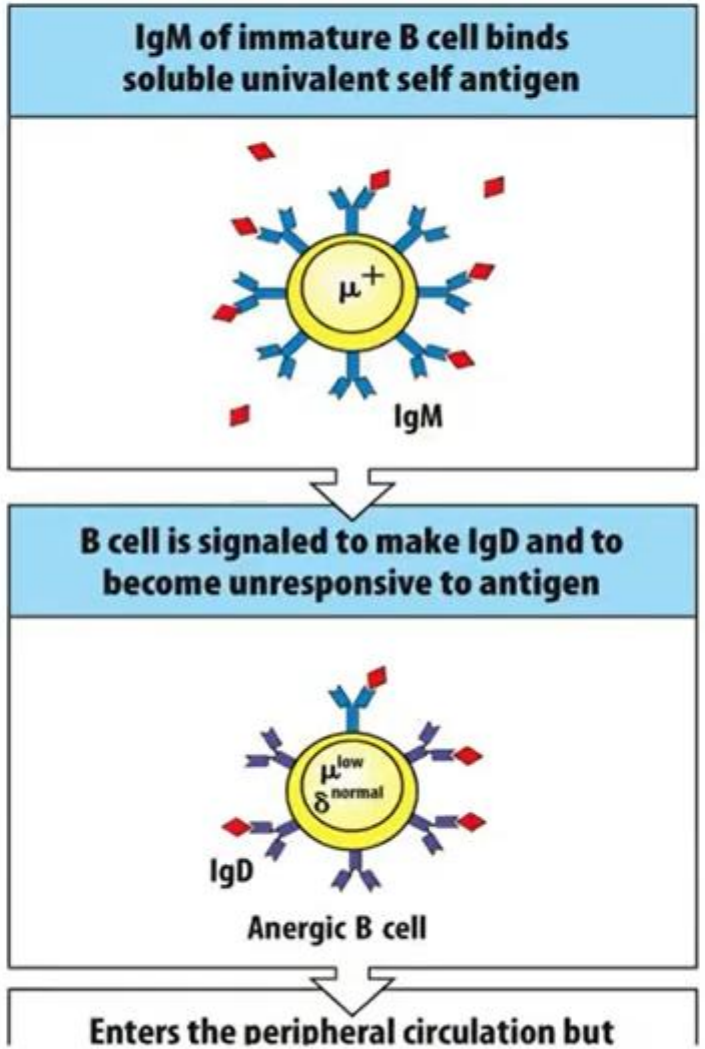
Parham Figure 6.17

Option 3: Anergy



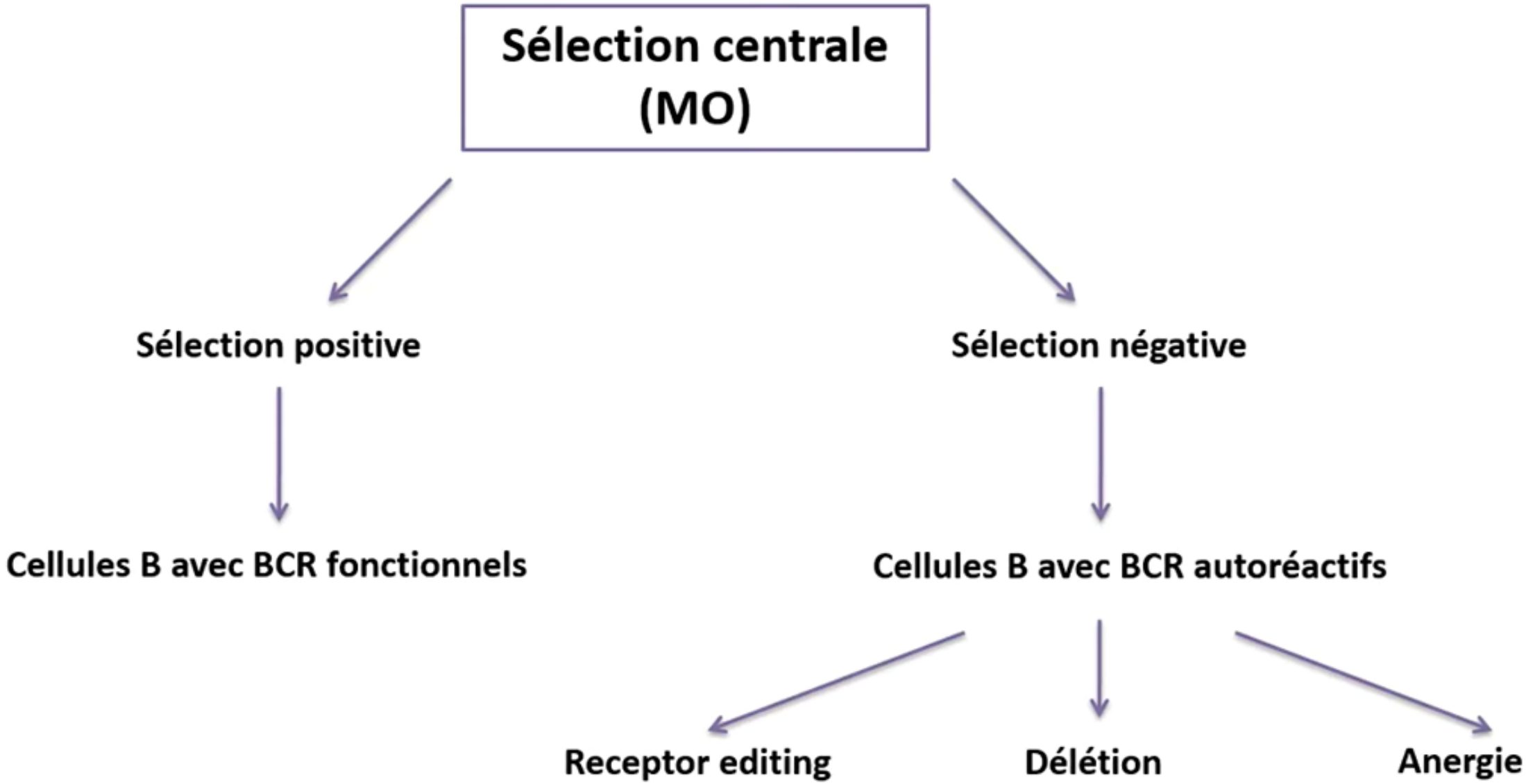
Abbas Fig. 14-8

Auto-reactive B cells can be anergized



Parham Figure 6.18

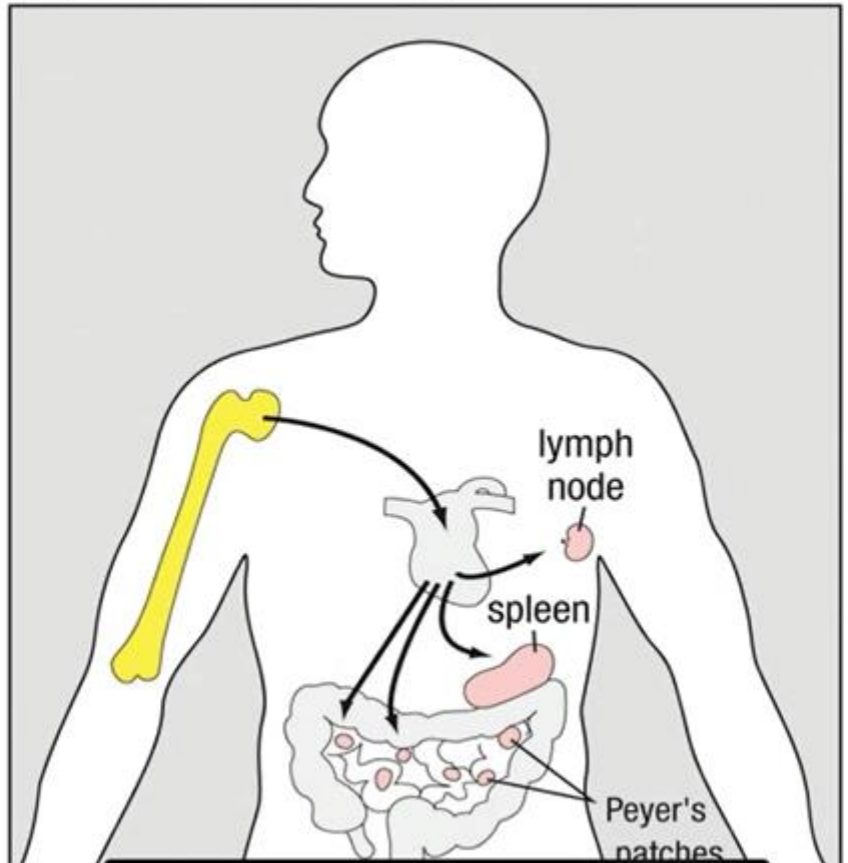
Is unresponsive


Sélection centrale (MO)
Sélection positive
Sélection négative
Cellules B avec BCR fonctionnels
Cellules B avec BCR autoréactifs
Receptor editing
Délétion
Anergie

General phases in the life of a B cell

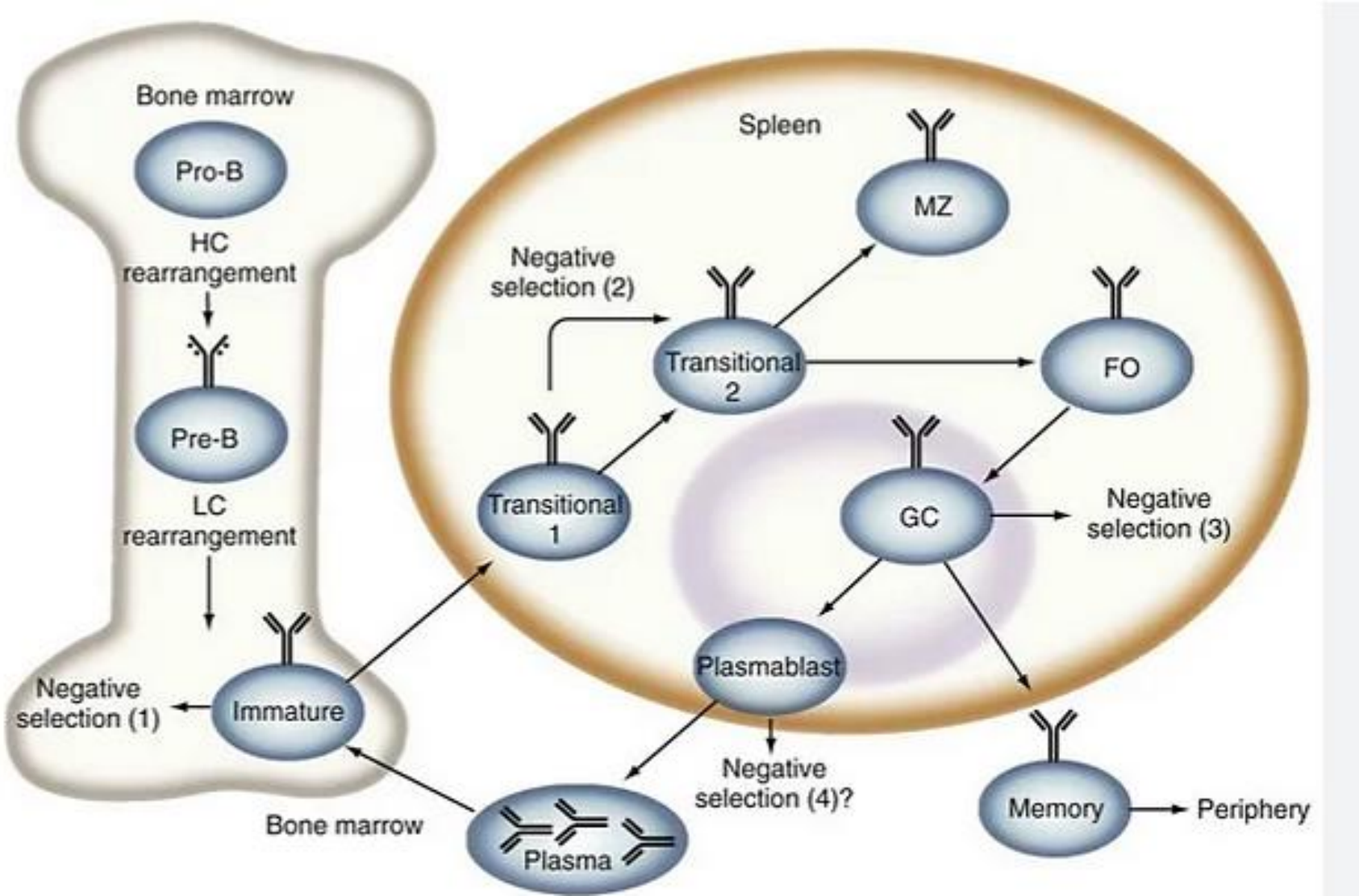
Phase 1	Phase 2	Phase 3	Phase 4	Phase 5	Phase 6
Generation of diverse and clonally expressed B-cell receptors in the bone marrow	Alteration, elimination, or inactivation of B-cell receptors that bind to components of the human body	Promotion of a fraction of immature B cells to become mature B cells in the secondary lymphoid tissues	Recirculation of mature B cells between lymph, blood, and secondary lymphoid tissues	Activation and clonal expansion of B cells by pathogen-derived antigens in secondary lymphoid tissues	Differentiation to antibody-secreting plasma cells and memory B cells in secondary lymphoid tissue
Repertoire assembly	Negative selection	Positive selection	Searching for infection	Finding infection	Attacking infection

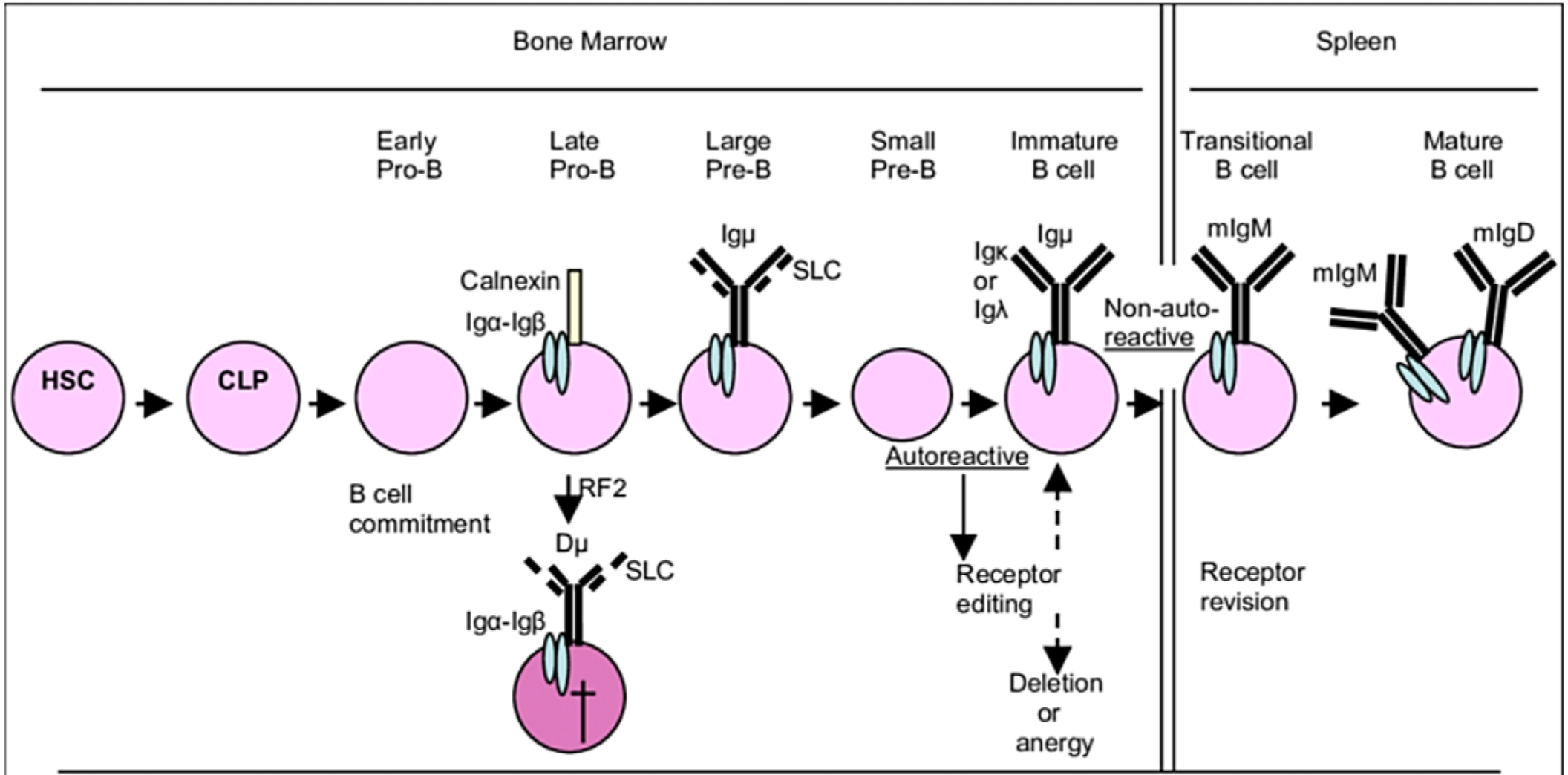
Parham Figure 6.1



Parham Figure 6.2

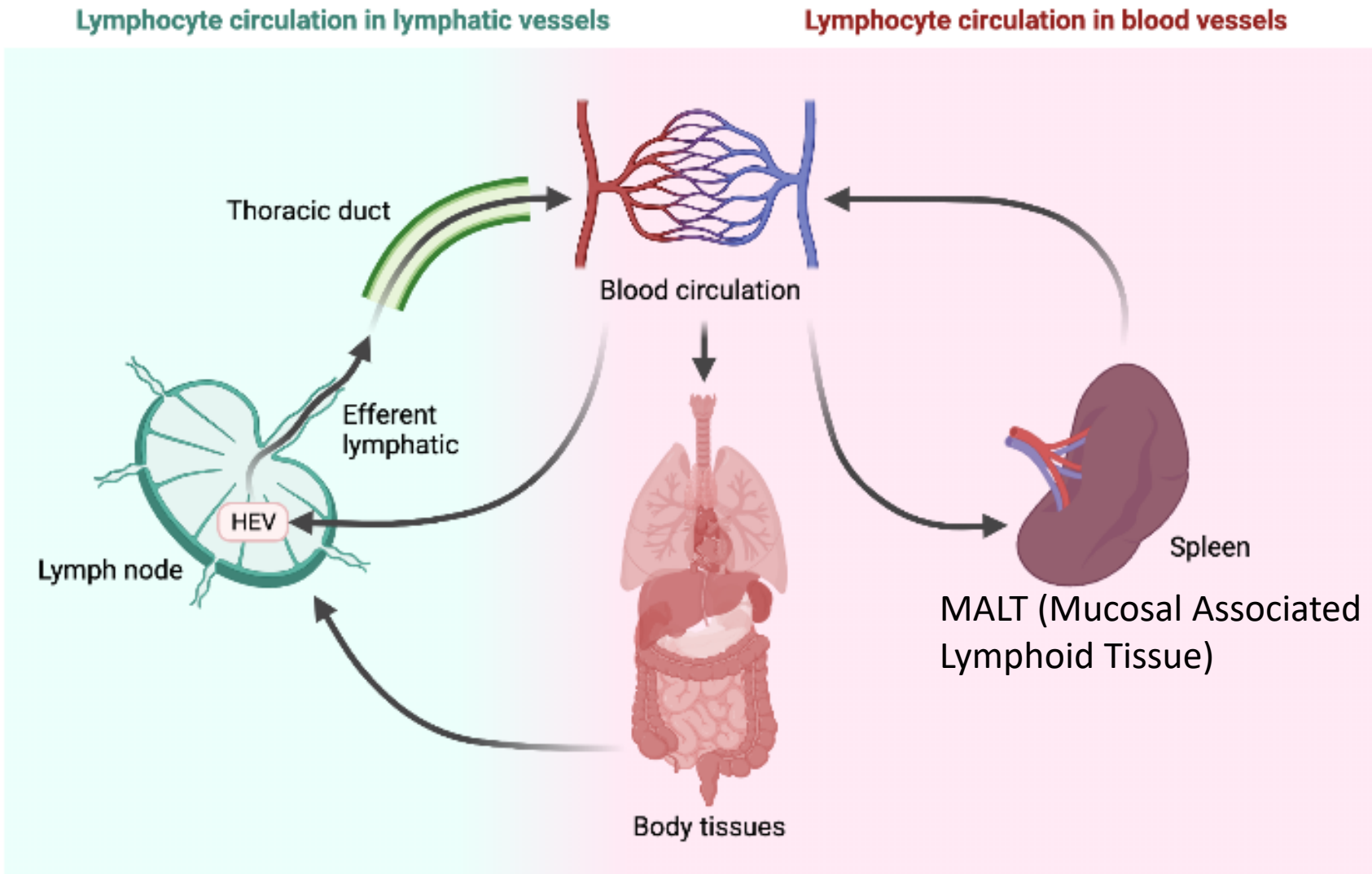
Phase splenique du development des lymphocytes B





RAG-1/2	-	Low	+	-	+	Low	Low	-
TdT	-	Low	+	+	+	-	-	-
IgH	GL	DJ	VDJ	-	-	-	-	-
IgL	GL	GL	GL	GL	VJ	VJ?	VJ?	-
Mb-1 (Igα)	-	Low	+	+	+	+	+	+
B29 (Igβ)	-	Low	+	+	+	+	+	+
V-pre-B	-	Low	+	+	-	-	-	-

Lymphocyte Circulation Pattern



MALT (Mucosal Associated Lymphoid Tissue)

MARQUEURS DE SURFACE DU LB

Le complexe BCR est formé par:

- **Un module de reconnaissance :**

Ig de membrane.

- **Un module de transduction des signaux :**

Hétérodimère Igα (CD79a) et Igβ (CD79b).

Codées respectivement par b29 et mb1.

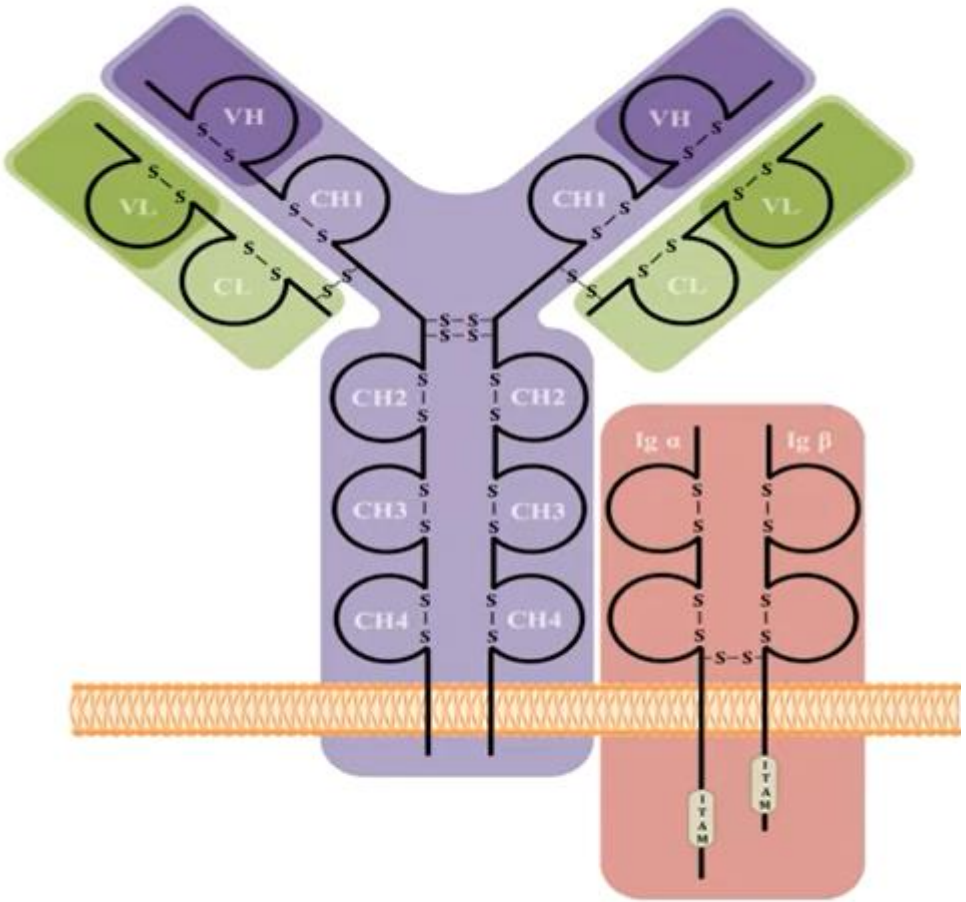
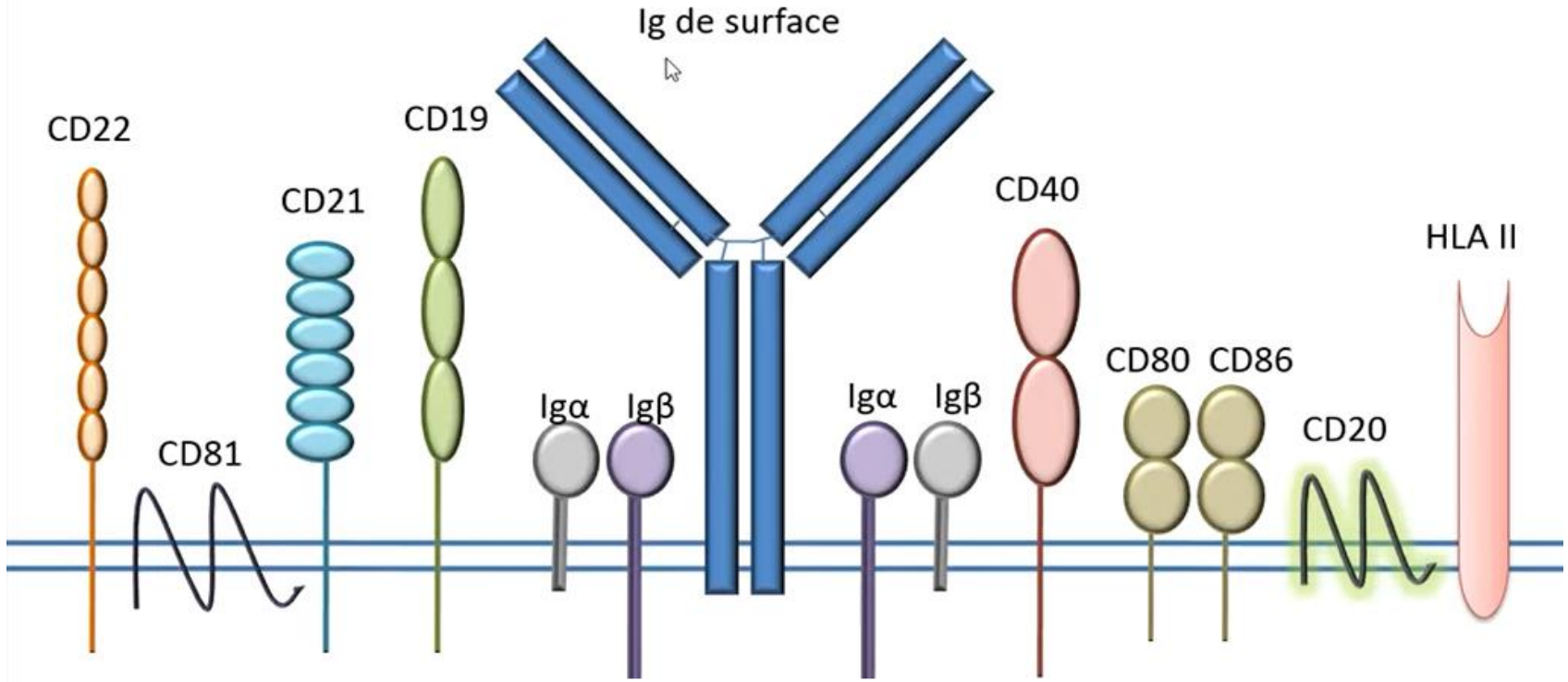


Fig. : Complexe BCR

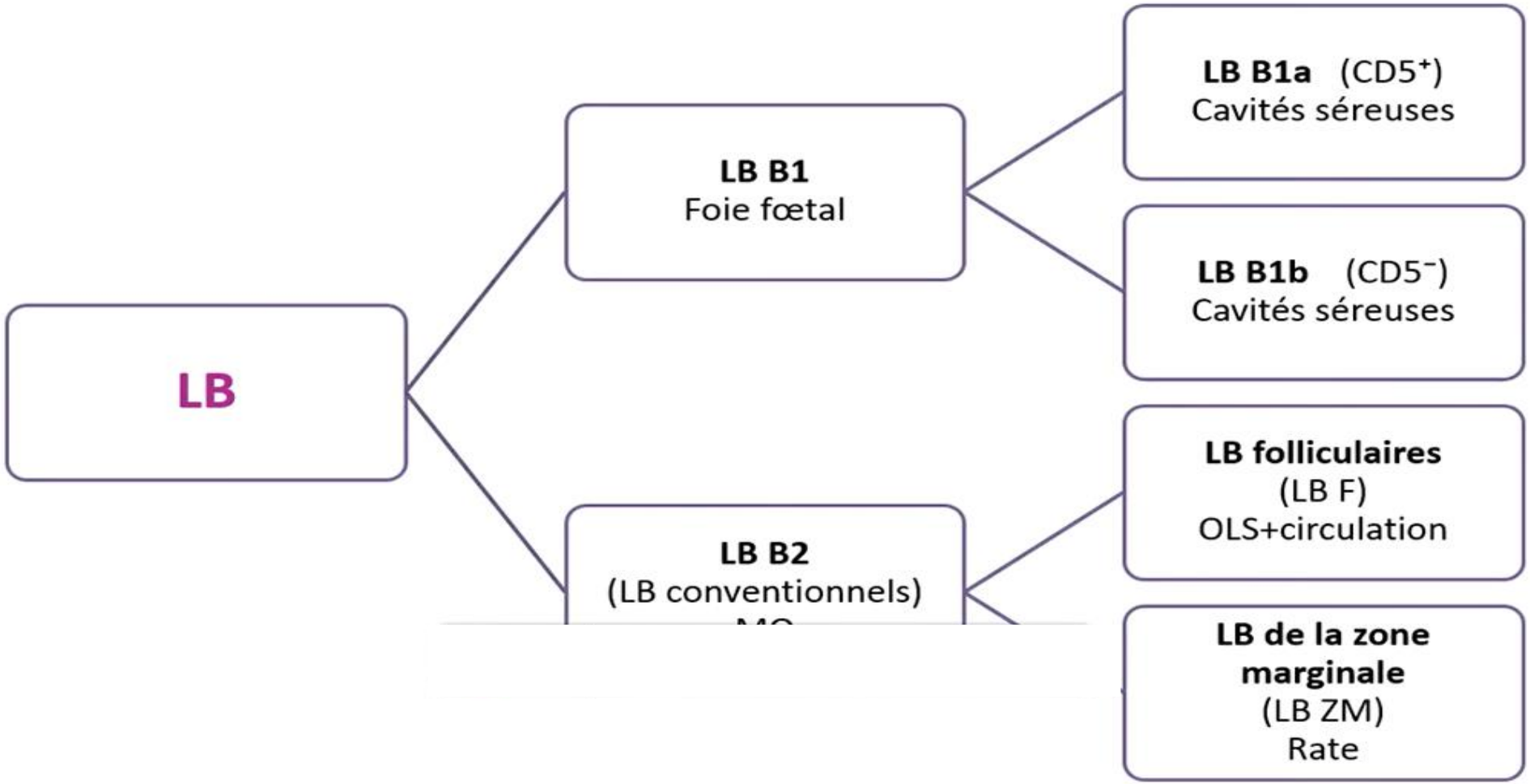
MARQUEURS DE SURFACE DU LB



Le LB exprime différents TLR :
TLR2, TLR-4 et TLR-6.

SOUS POPULATIONS DES LB

- **Classification 2:**



Fonction:

- B1a ⟶ Ac naturels
- B1b ⟶ Ac contre les Ag thymo-indépendants.

5% de la population totale des LB

Cellules innate-like.

Les LB B1

Localisation:

Cavités séreuses (pleurales et péritonéales).

Phenotype: **IgM high, IgD low**
Origine: **Foetale**
Autorenouvellement

SOUS POPULATIONS DES LB

LB de la zone marginale

- Fonction : réponse aux polysaccharides bactériens.

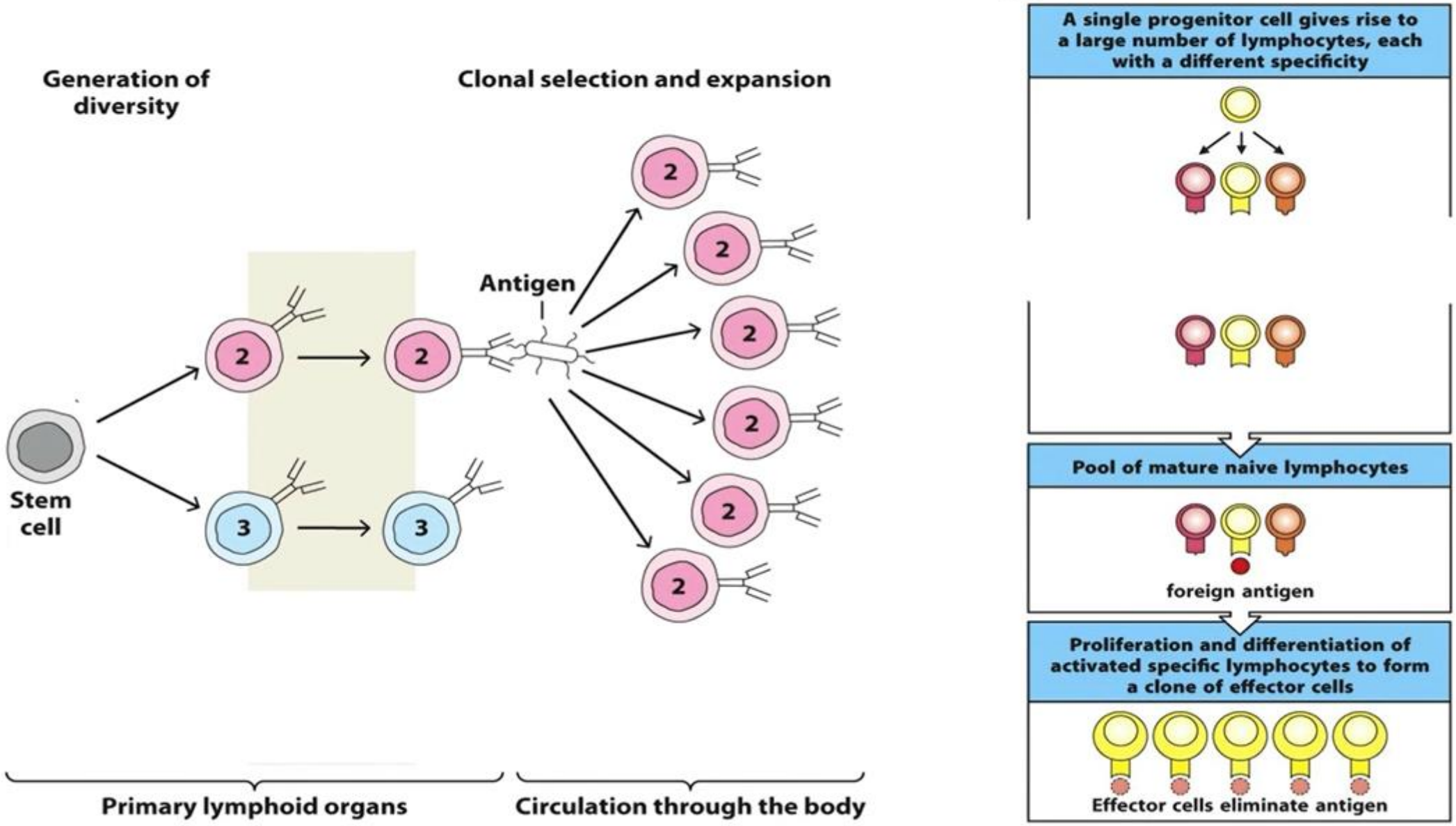
LB Folliculaires

- Fonctions:
  - Réponse aux antigènes thymo-dépendants.
  - Formation des centres germinatifs.
    - Plasmocytes
    - Lymphocytes B memoires

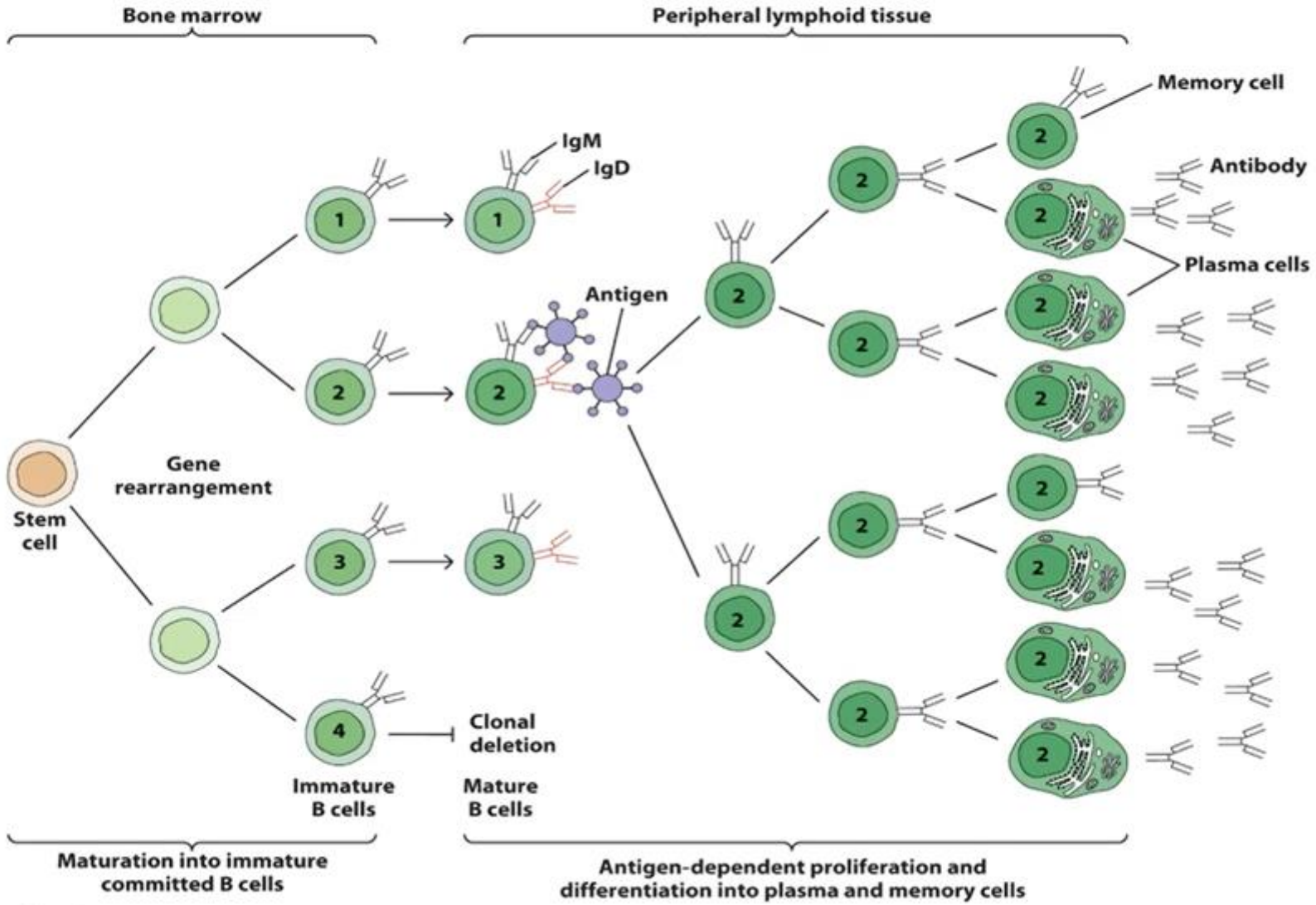
Réponse aux antigènes thymo-indépendants (Ag TI)	Réponse aux antigènes thymo-dépendants (Ag TD)
Pas de mémoire	Mémoire immunologique
Pas de centre germinatif	Formation decentre germinatif
IgM+++	IgM, puis IgG ou IgA ou IgE ++
Pas de switch	Switch vers les autres classes d'Ig
Pas d'hypermutation somatique	Hypermutation somatique >> forte affinité

B-cell activation

VDJ Recombination happens in the absence of antigen



What happens in the periphery? (2^0 LO)

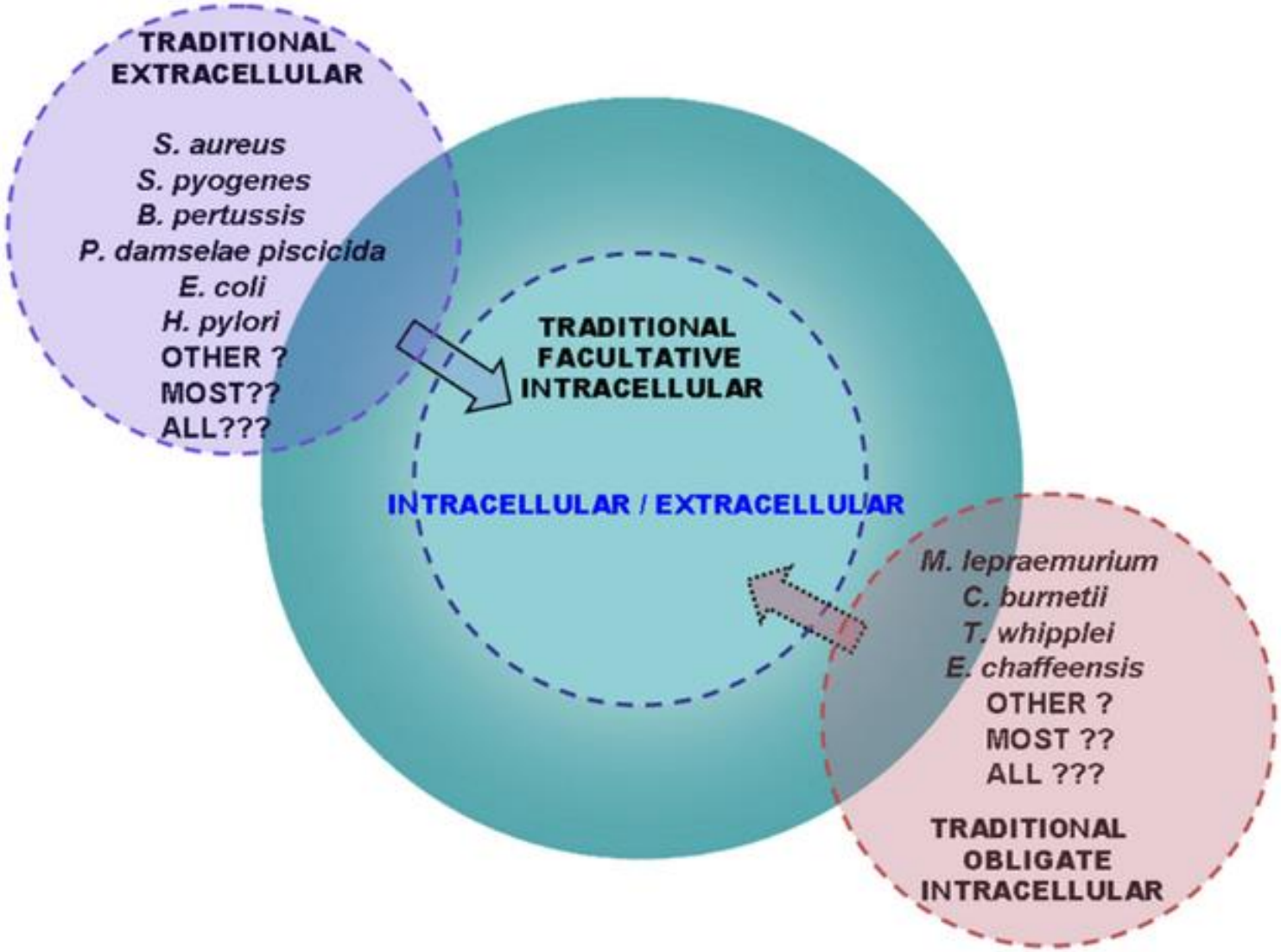


Kuby Figure 11-2

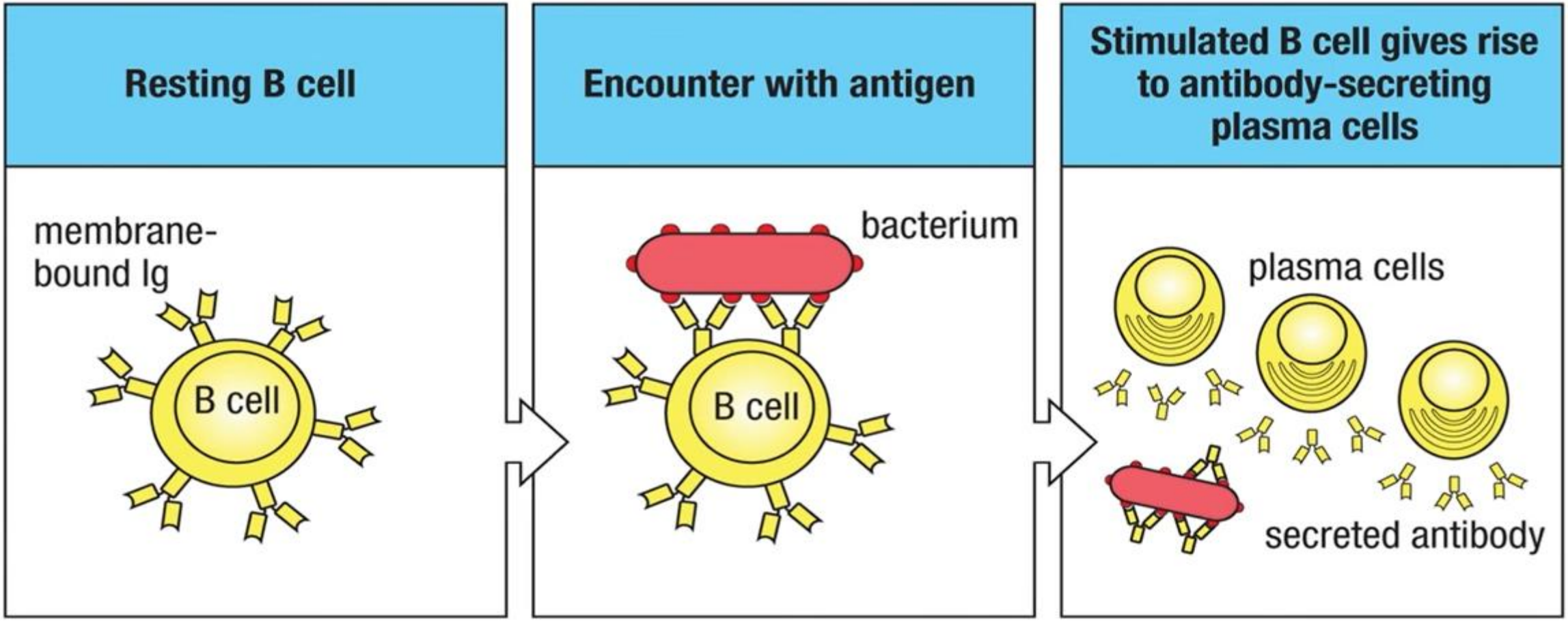
Les lymphocytes B (LB) sont le support de l'immunité humorale adaptative.

- Efficace contre les germes à multiplication extracellulaire
- Certaines phases des infection par des germes à multiplication intracellulaire

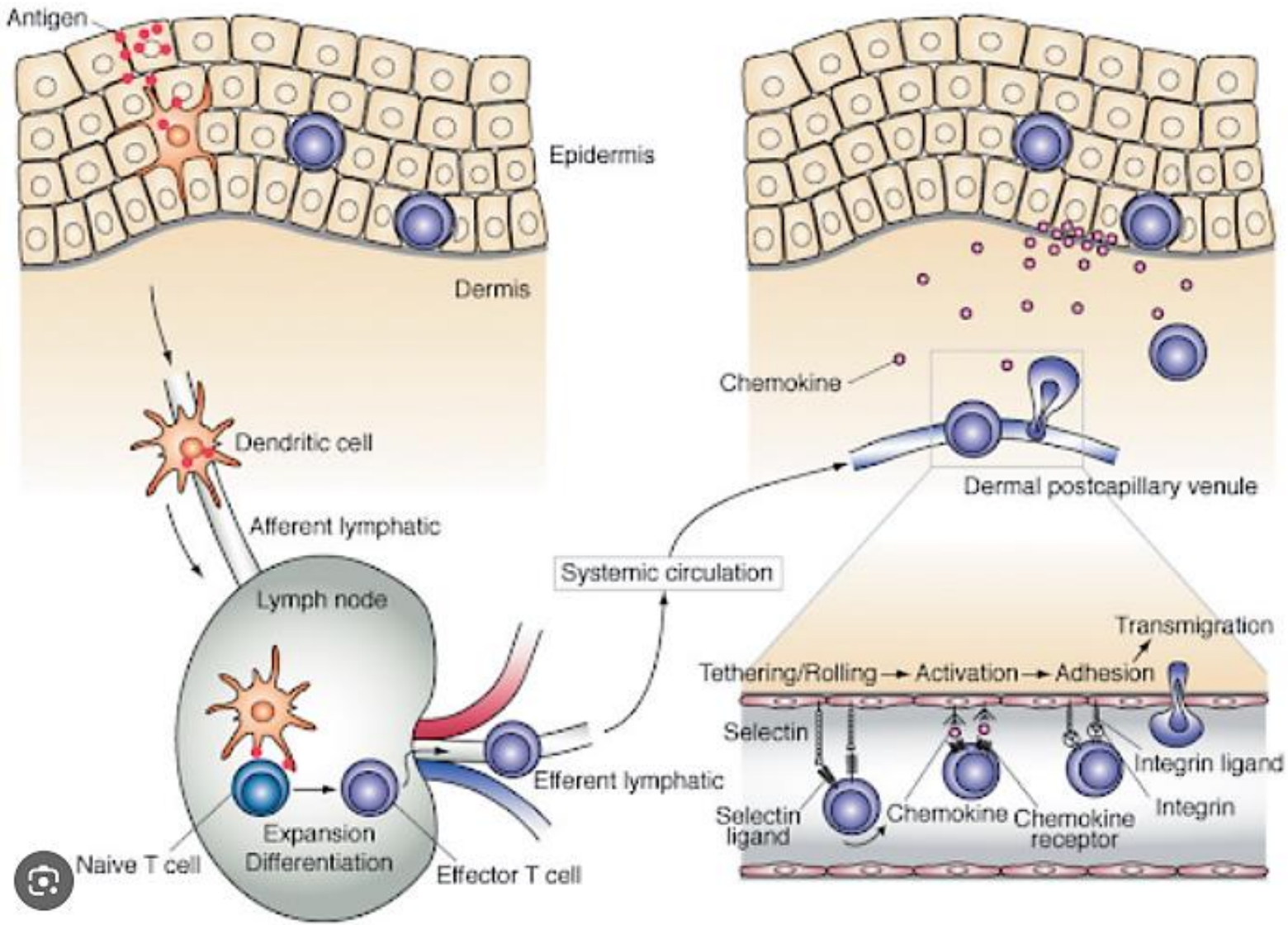
Example: infection par le virus du COVID19



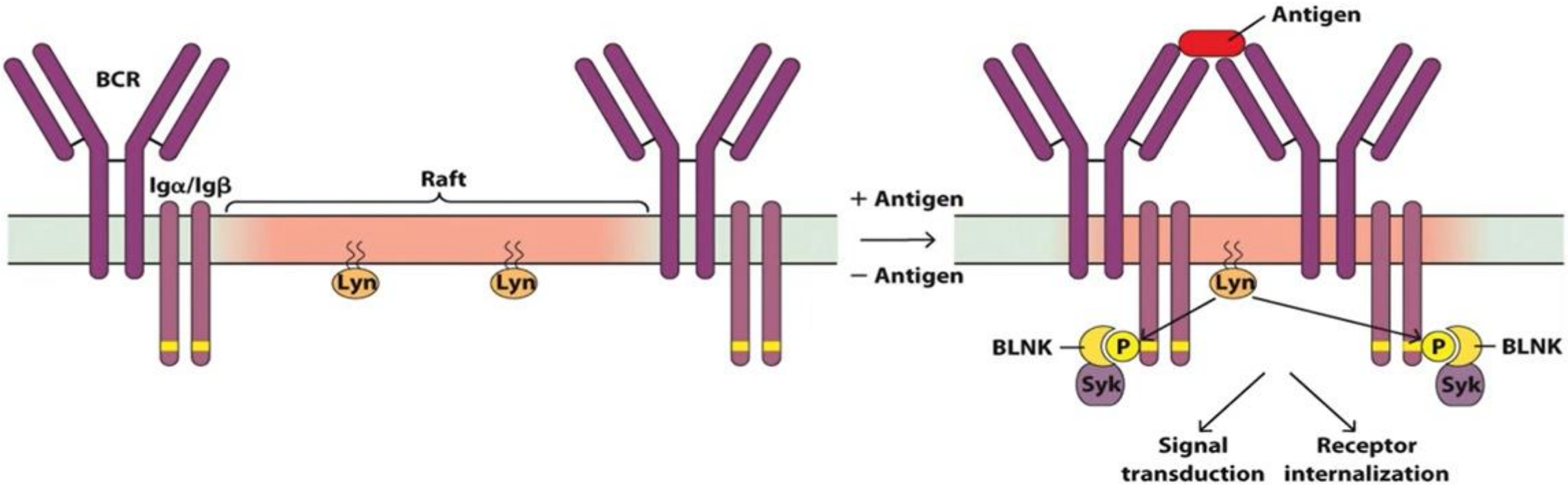
How do you activate a B cell?



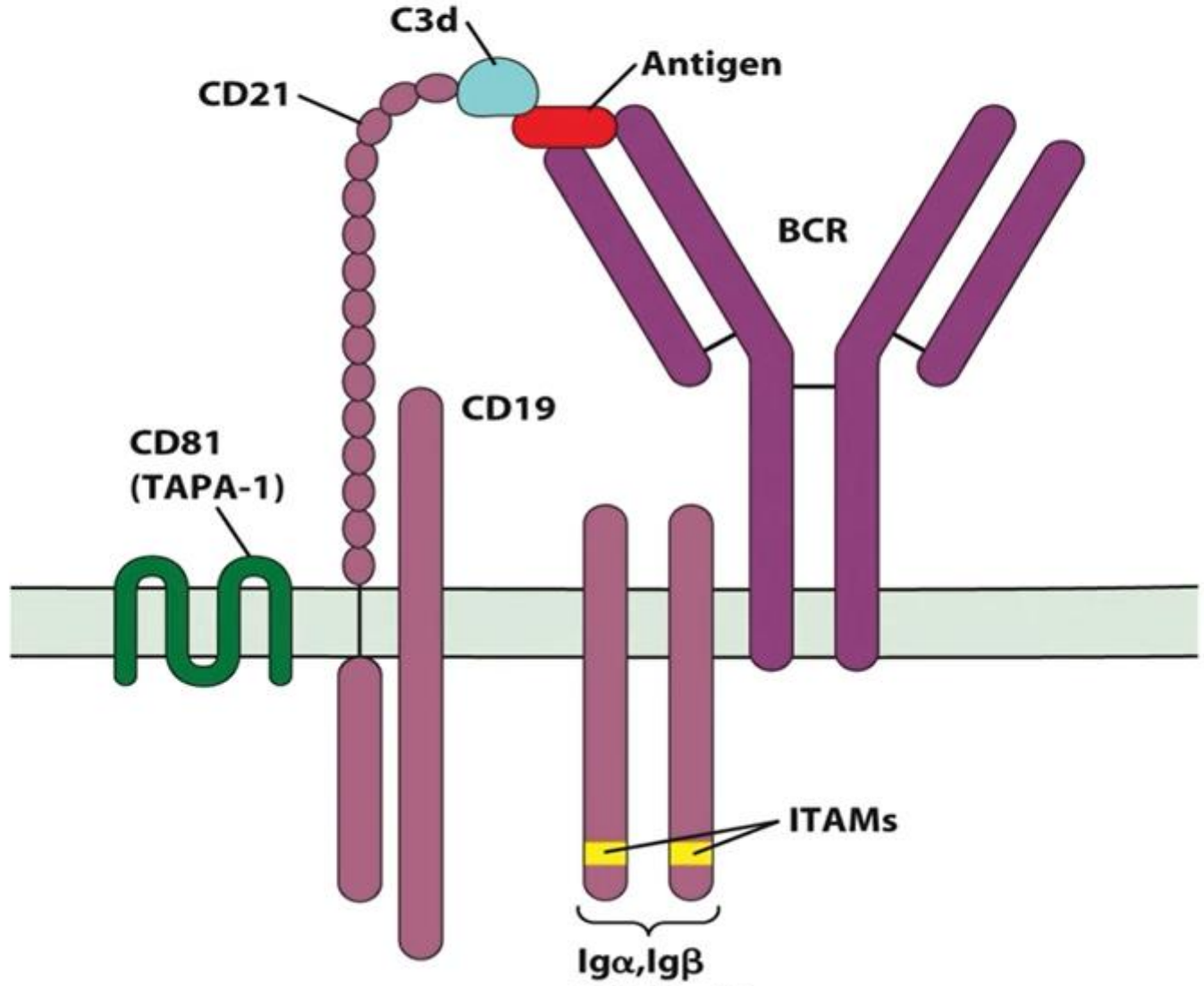
Kesri clinic



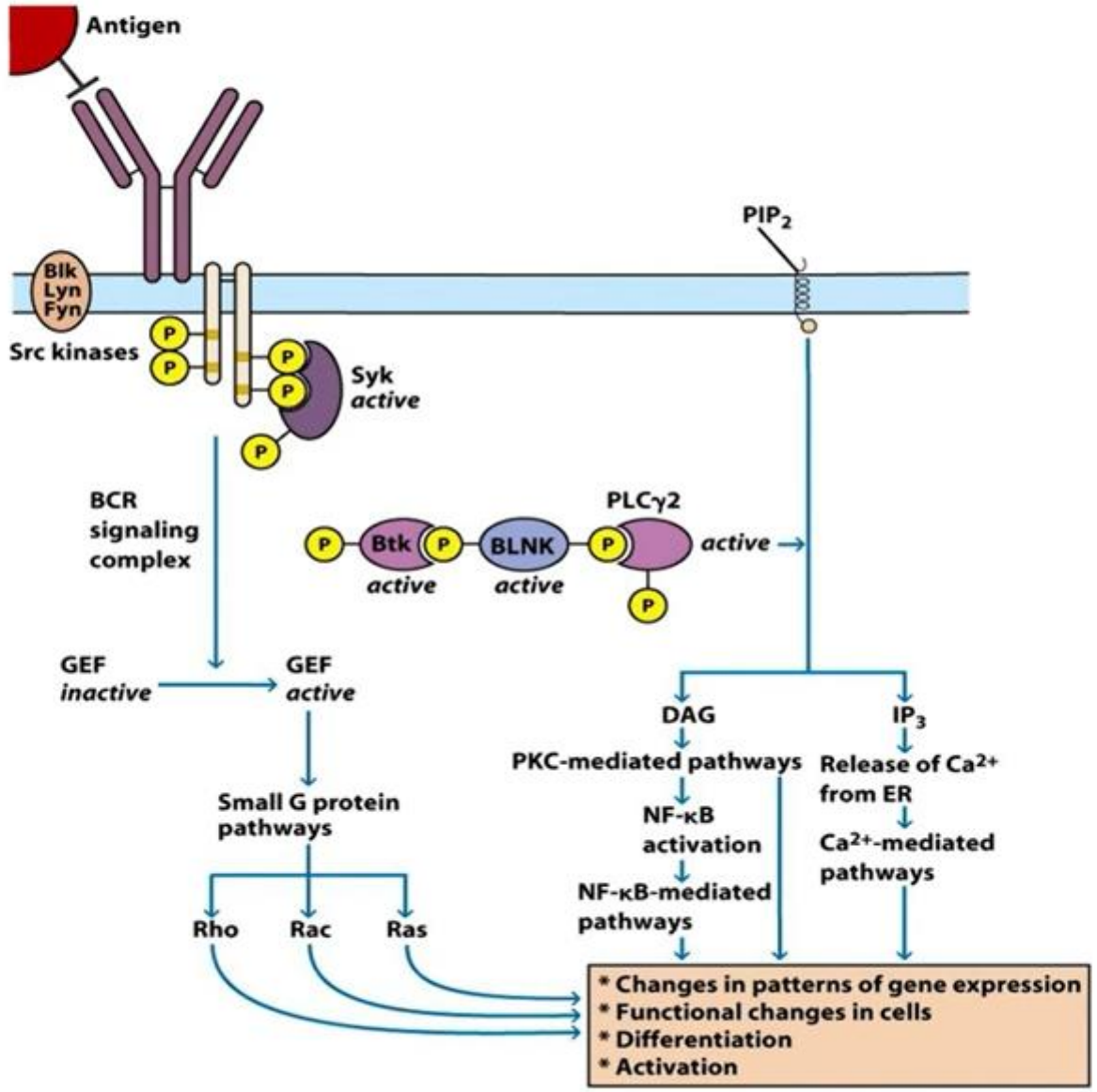
BCRs signal when they are cross-linked (Induced proximity)

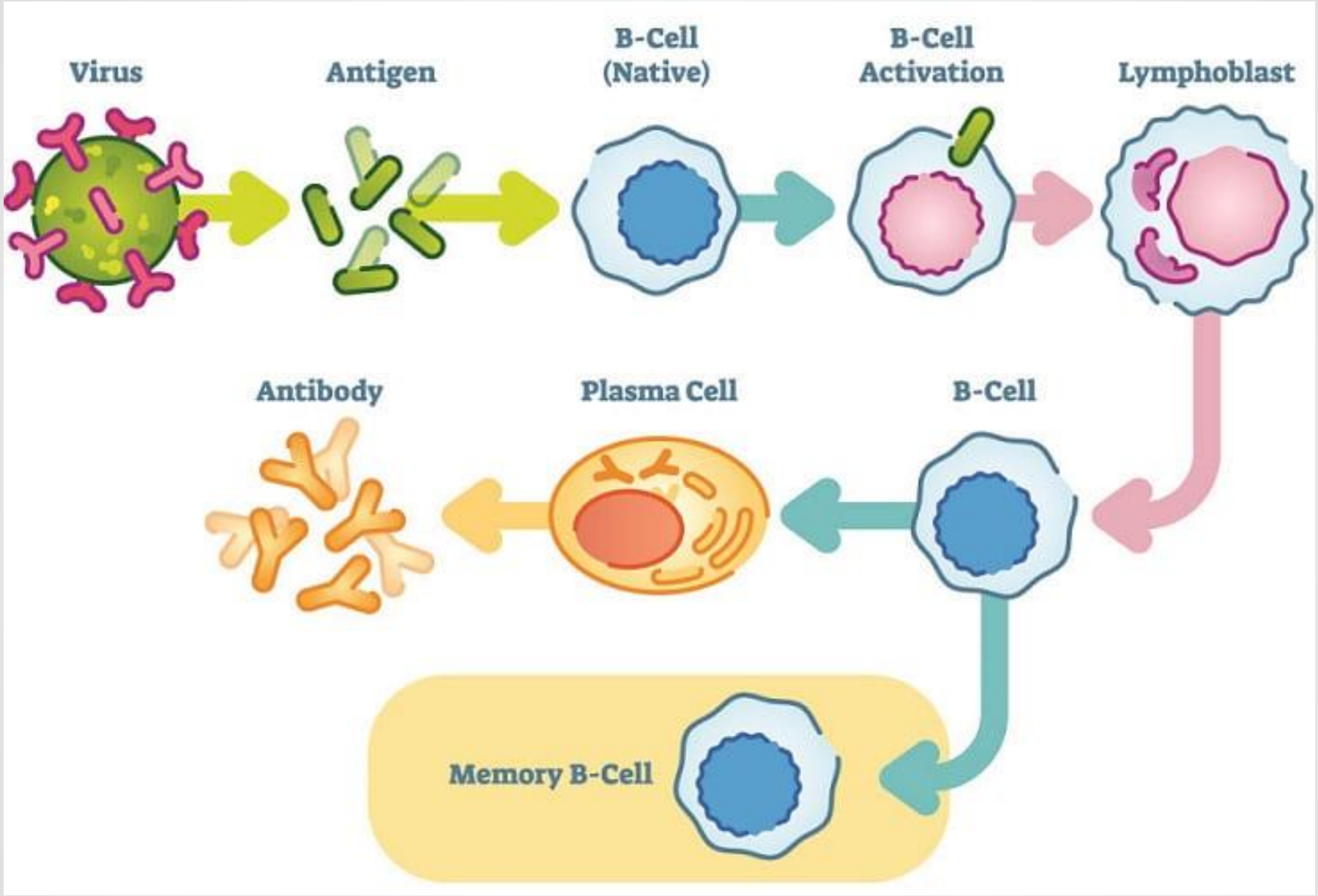


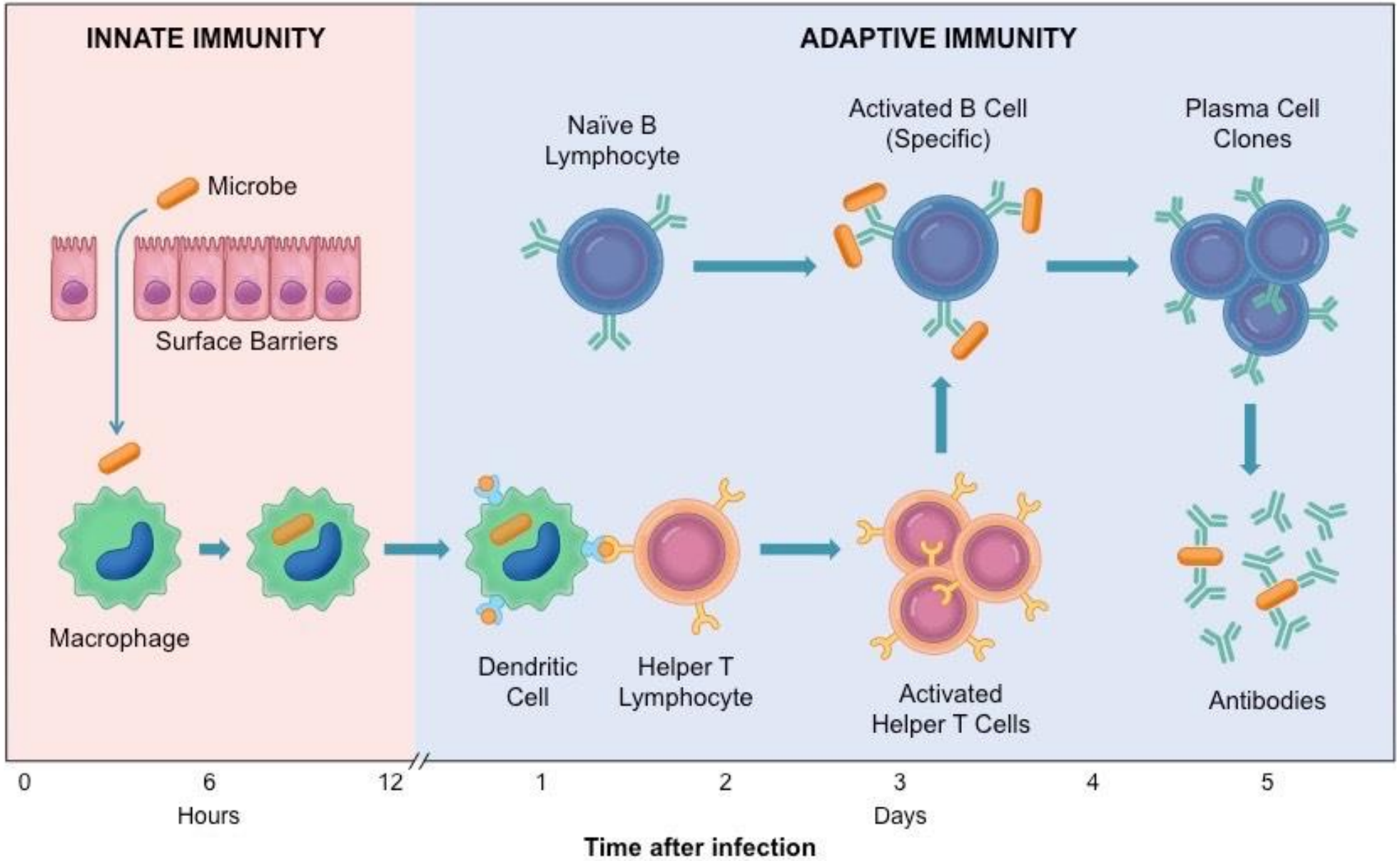
Co-receptors on B cells cooperate in B cell activation



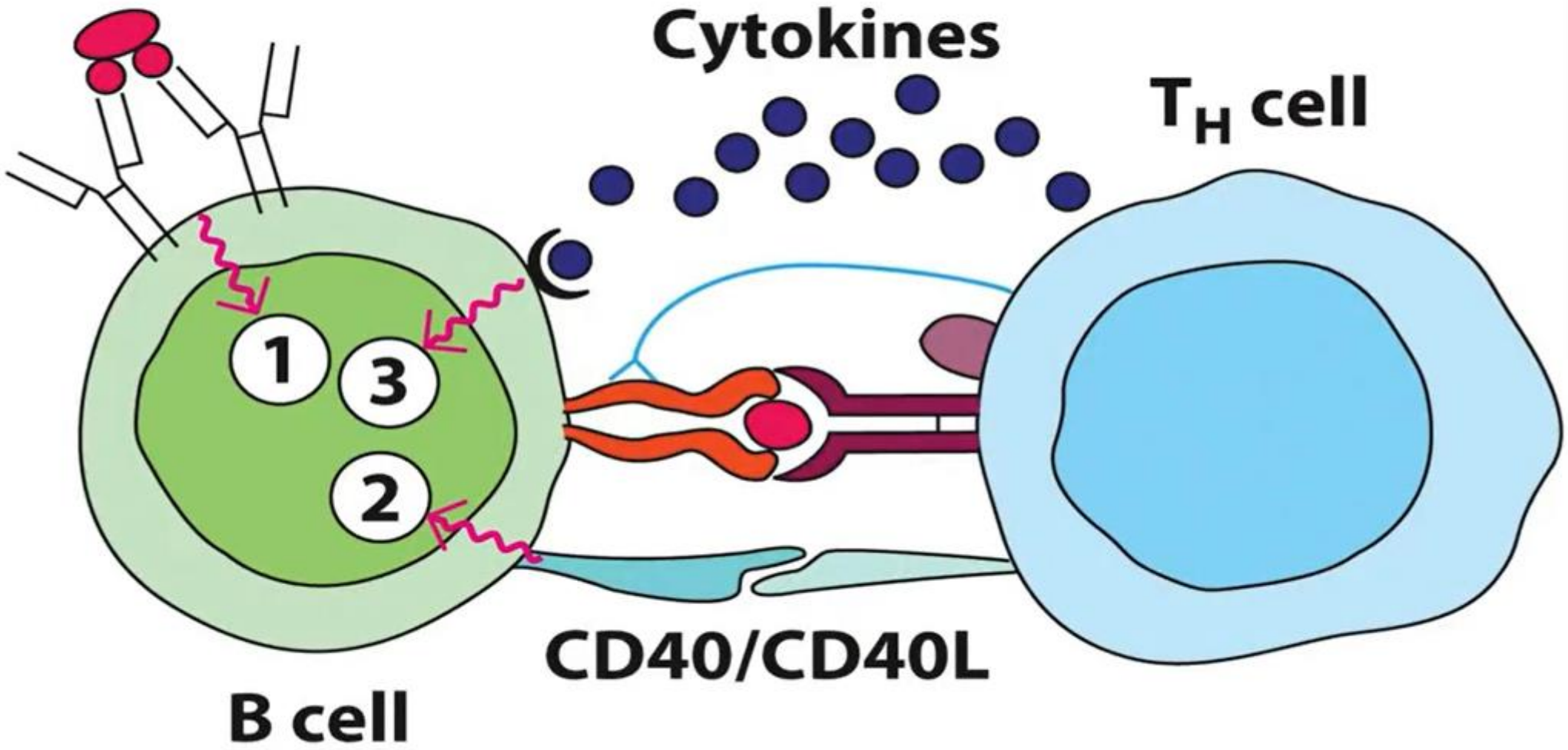
Signaling downstream of the BCR




Virus
Antigen
B-Cell (Native)
B-Cell Activation
Lymphoblast
Antibody
Plasma Cell
B-Cell
Memory B-Cell

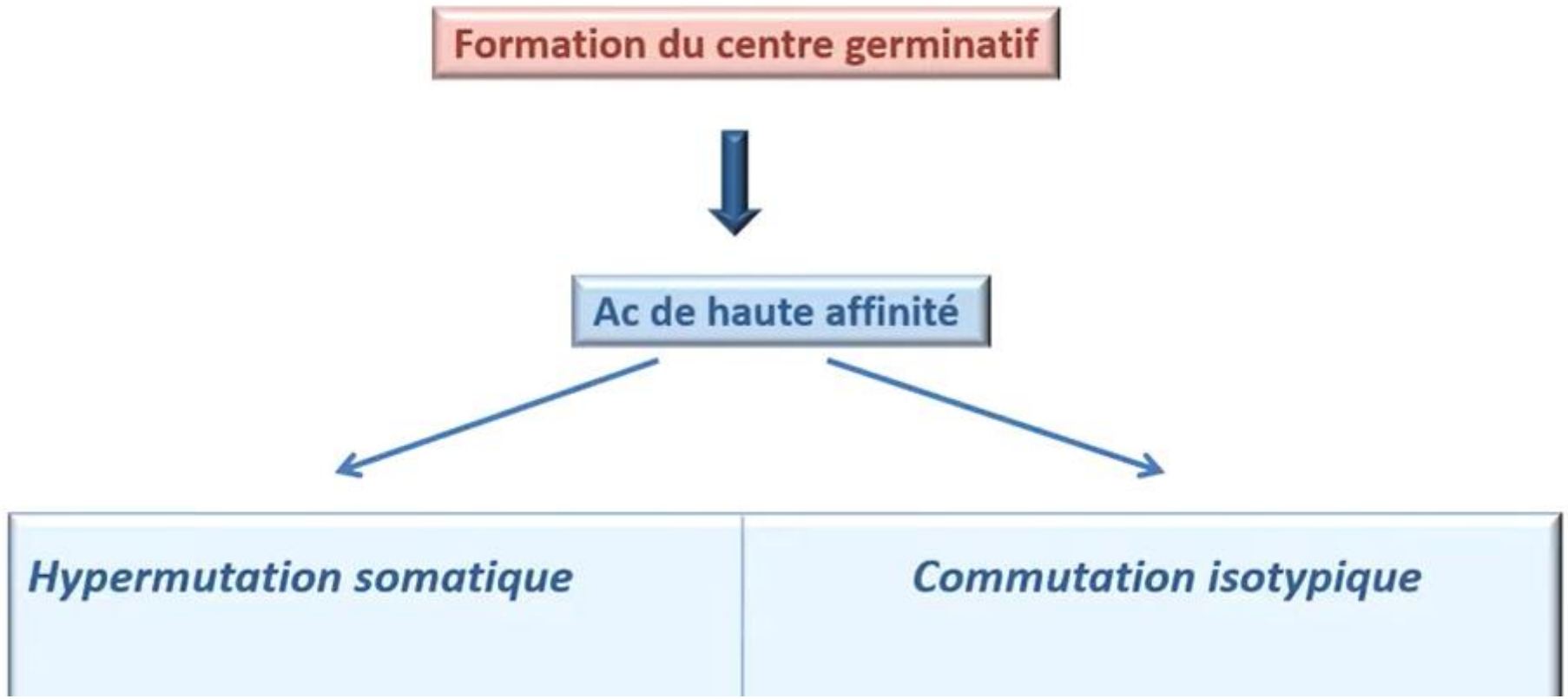

INNATE IMMUNITY
ADAPTIVE IMMUNITY
Naïve B Lymphocyte
Activated B Cell (Specific)
Plasma Cell Clones
Microbe
Surface Barriers
Macrophage
Dendritic Cell
Helper T Lymphocyte
Activated Helper T Cells
Antibodies
0
6
12
1
2
3
4
5
Hours
Days
Time after infection

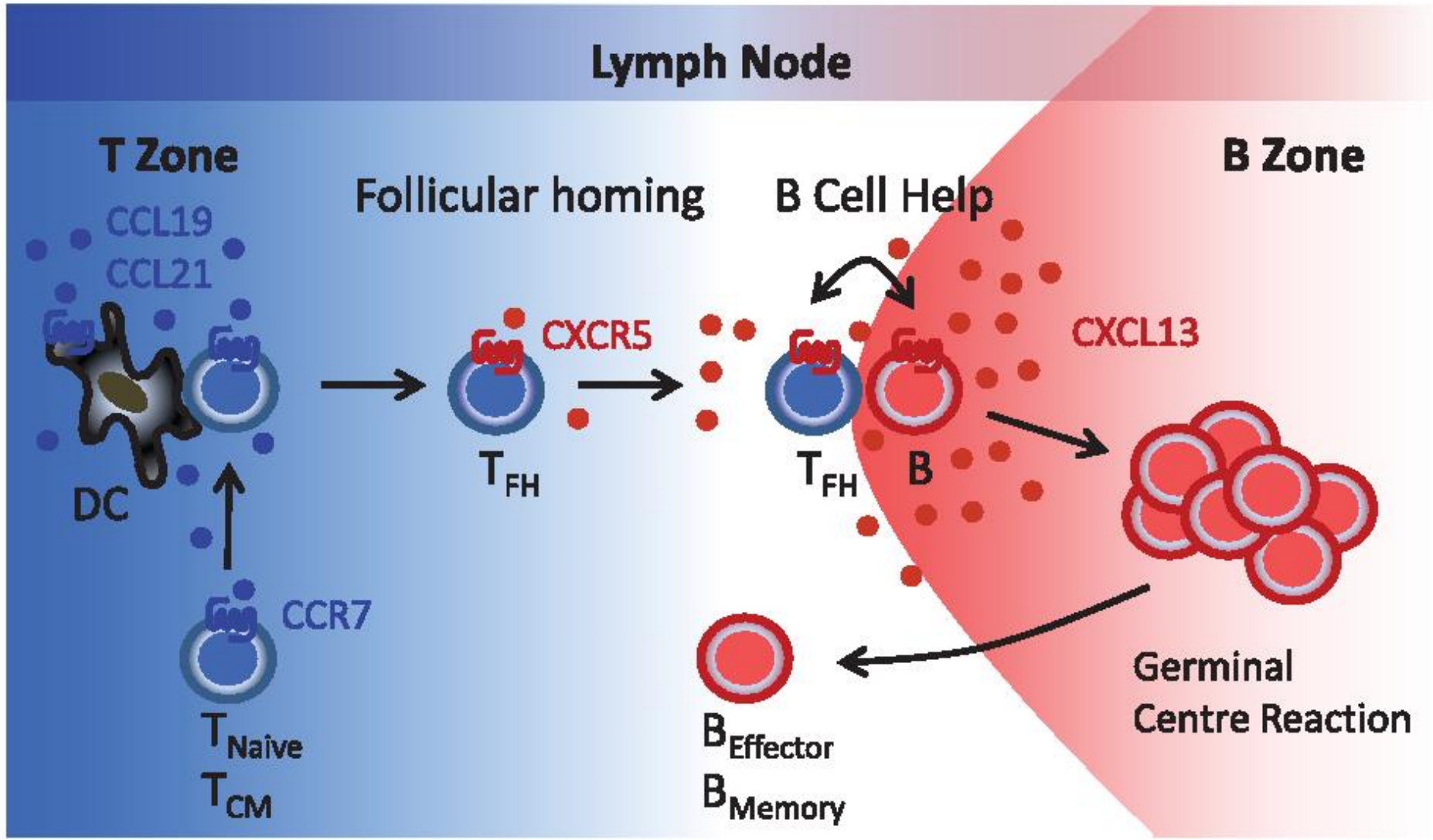
Activating B Cells in the Periphery



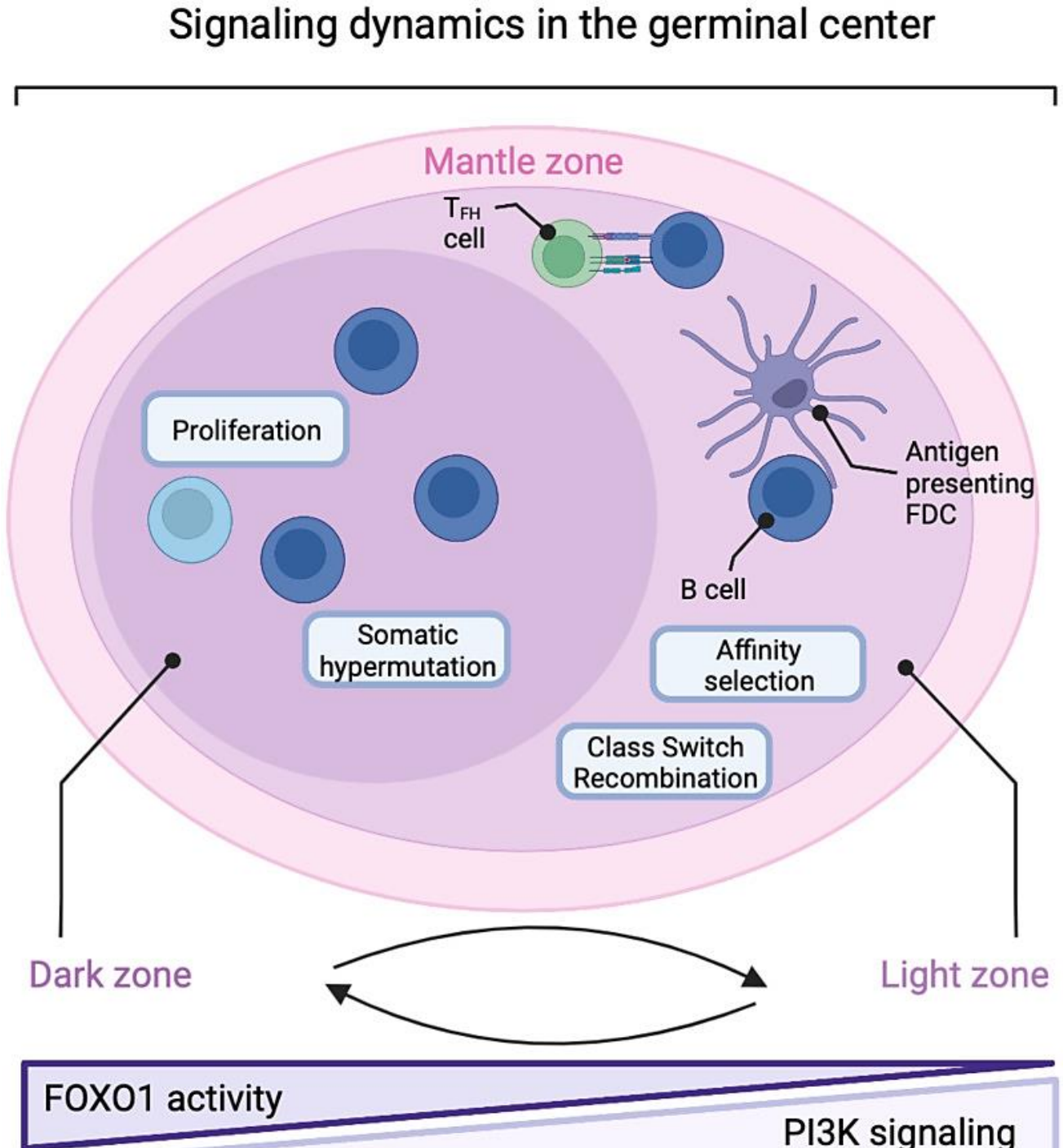
Kuby Figure 11-3

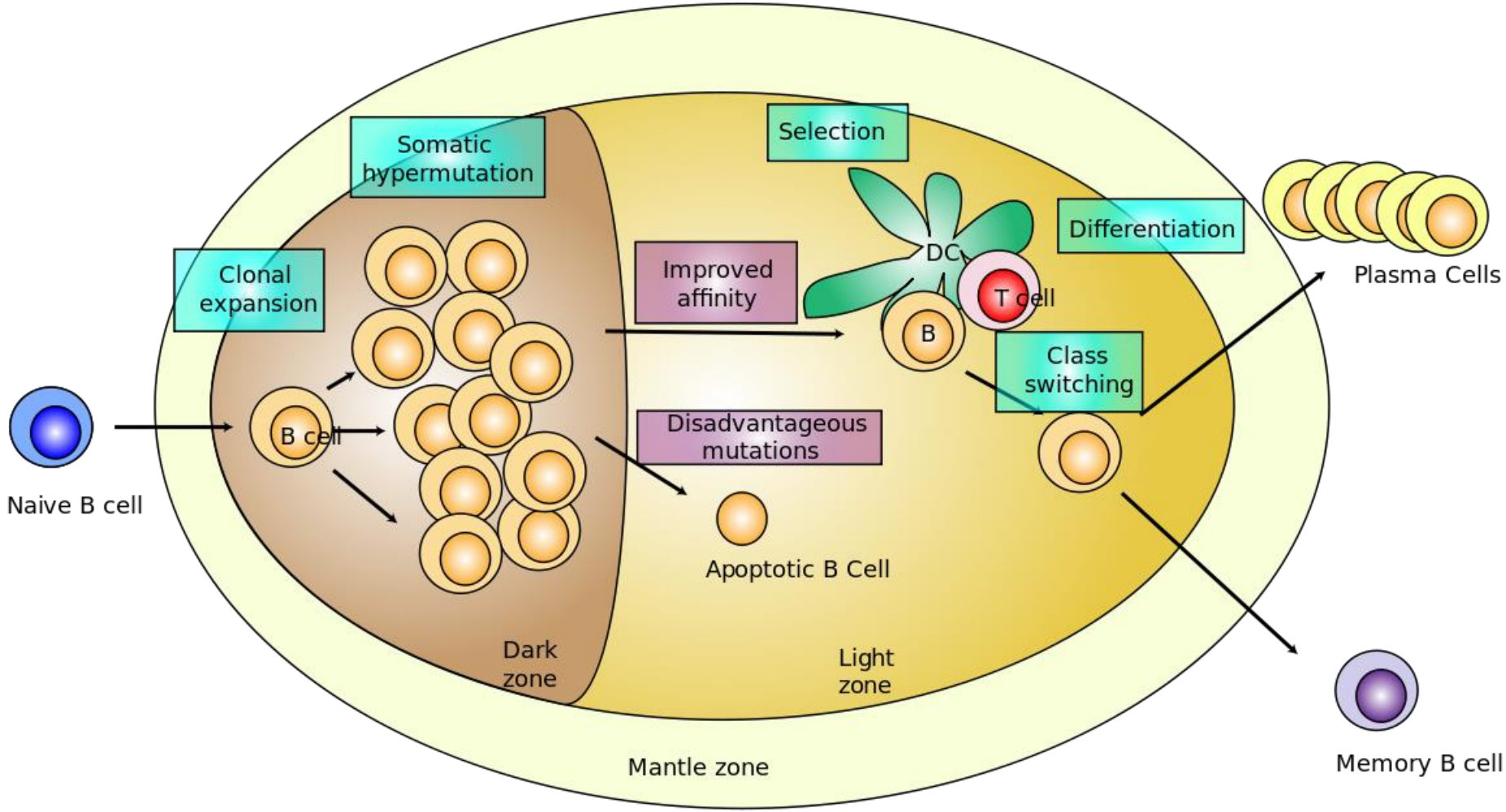
- **Réponse aux Ag TD « *Formation du centre germinatif* »**




Lymph Node
T Zone
B Zone
Follicular homing
B Cell Help
CCL19
CCL21
CXCR5
CXCL13
DC
T_{FH}
T_{FH}
B
CCR7
T_{Naive}
T_{CM}
$B_{Effector}$
B_{Memory}
Germinal
Centre Reaction

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fbooks%2FNBK585273%2Ffigure%2Fp13k.fig5%2F&psig=AOvVaw1_sIltofy13uc_bAdAgQif&ust=1708519801455000&source=images&cd=vfe&opi=89978449&ved=0CBMQjRxqFwoTCIDEju3MuoQDFQAAAAAdAAAAABAE




Somatic hypermutation
Selection
Clonal expansion
Improved affinity
DC
T cell
B
Differentiation
Plasma Cells
Class switching
B cell
Disadvantageous mutations
Naive B cell
Apoptotic B Cell
Dark zone
Light zone
Mantle zone
Memory B cell

La réponse adaptative

Mécanismes moléculaires et acteurs cellulaires

La vaccination

Commutation isotypique

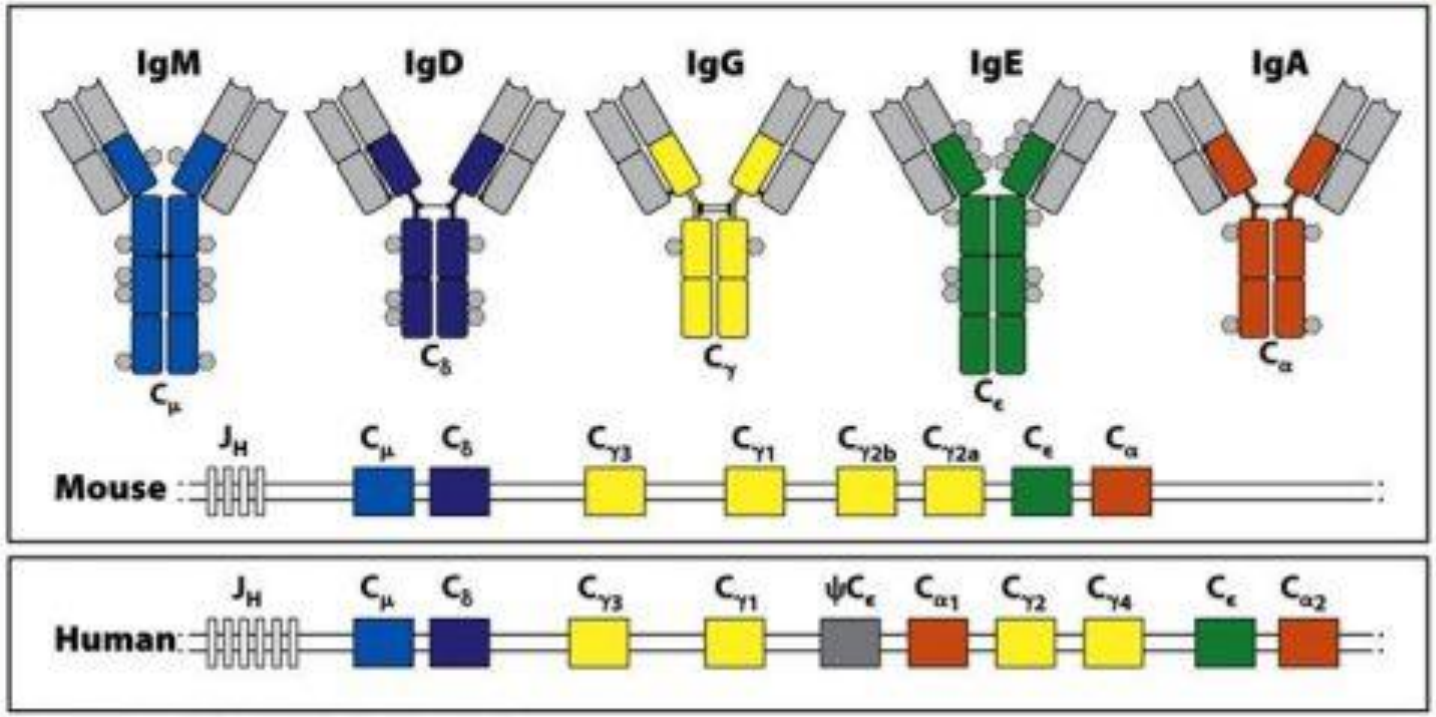


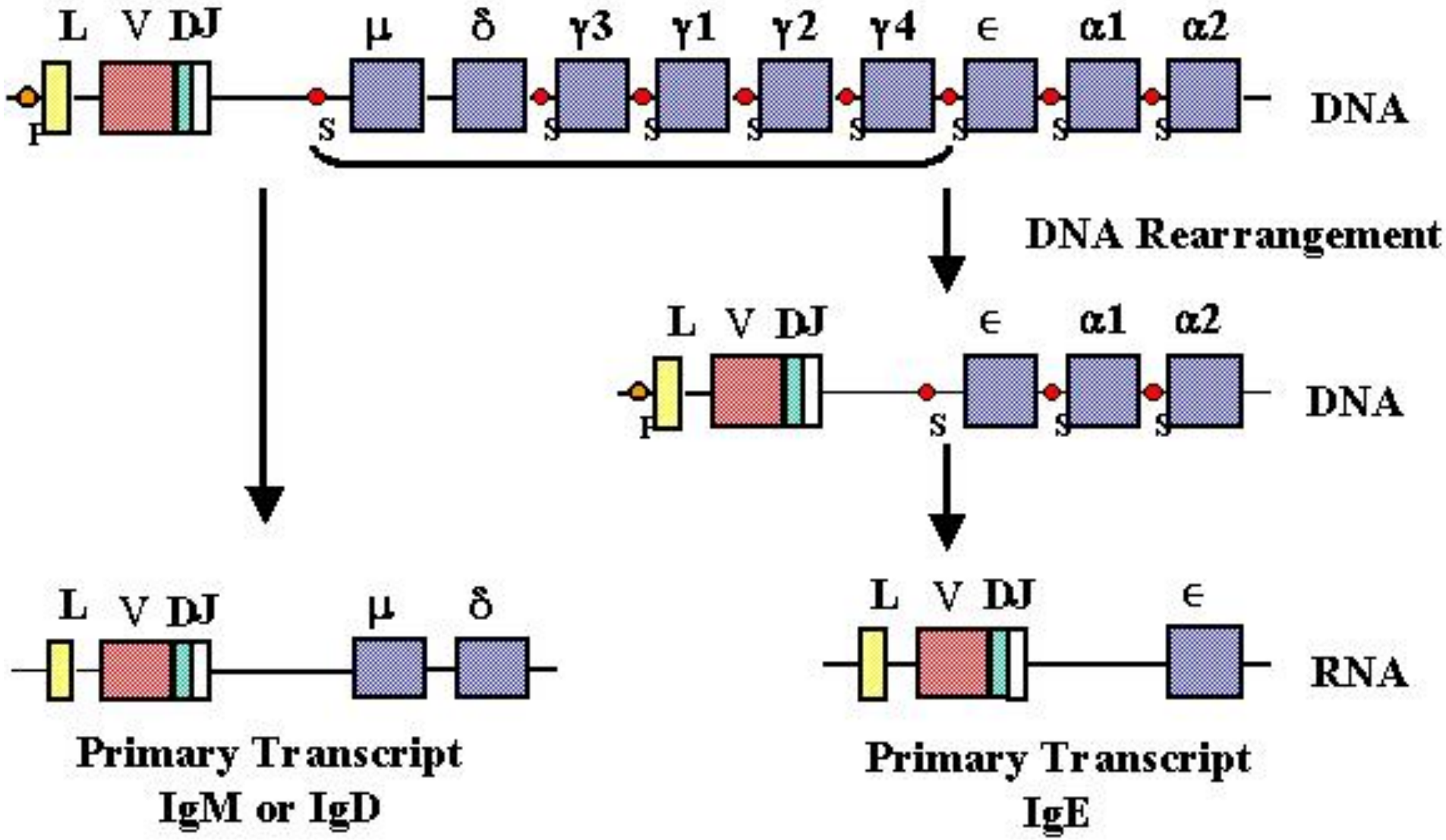
Figure 4-17 Immunobiology, 7ed. (© Garland Science 2008)

Activation du complément

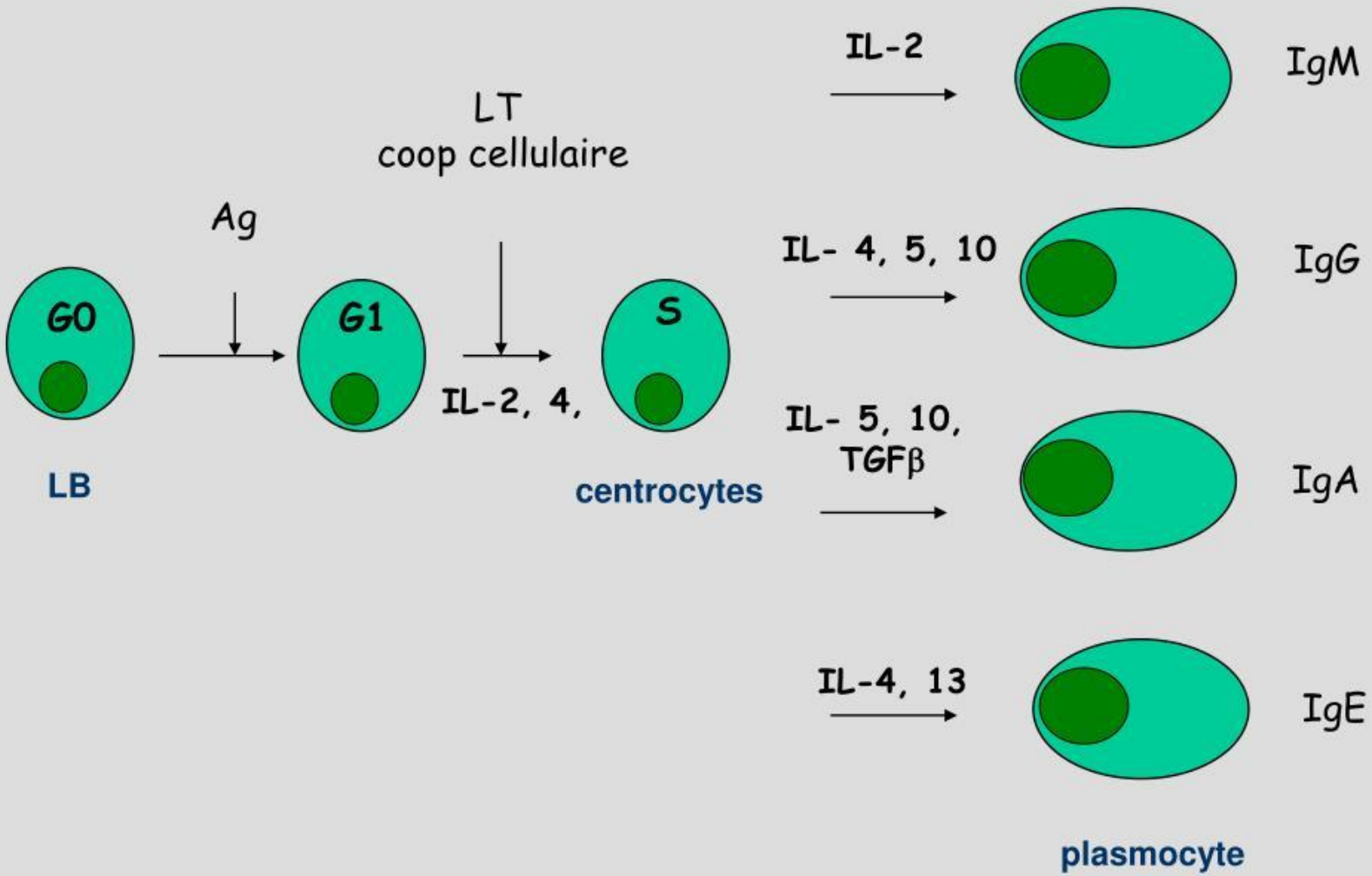
Réponse du phagocyte dépendante du récepteur de Fc
Activation du complément

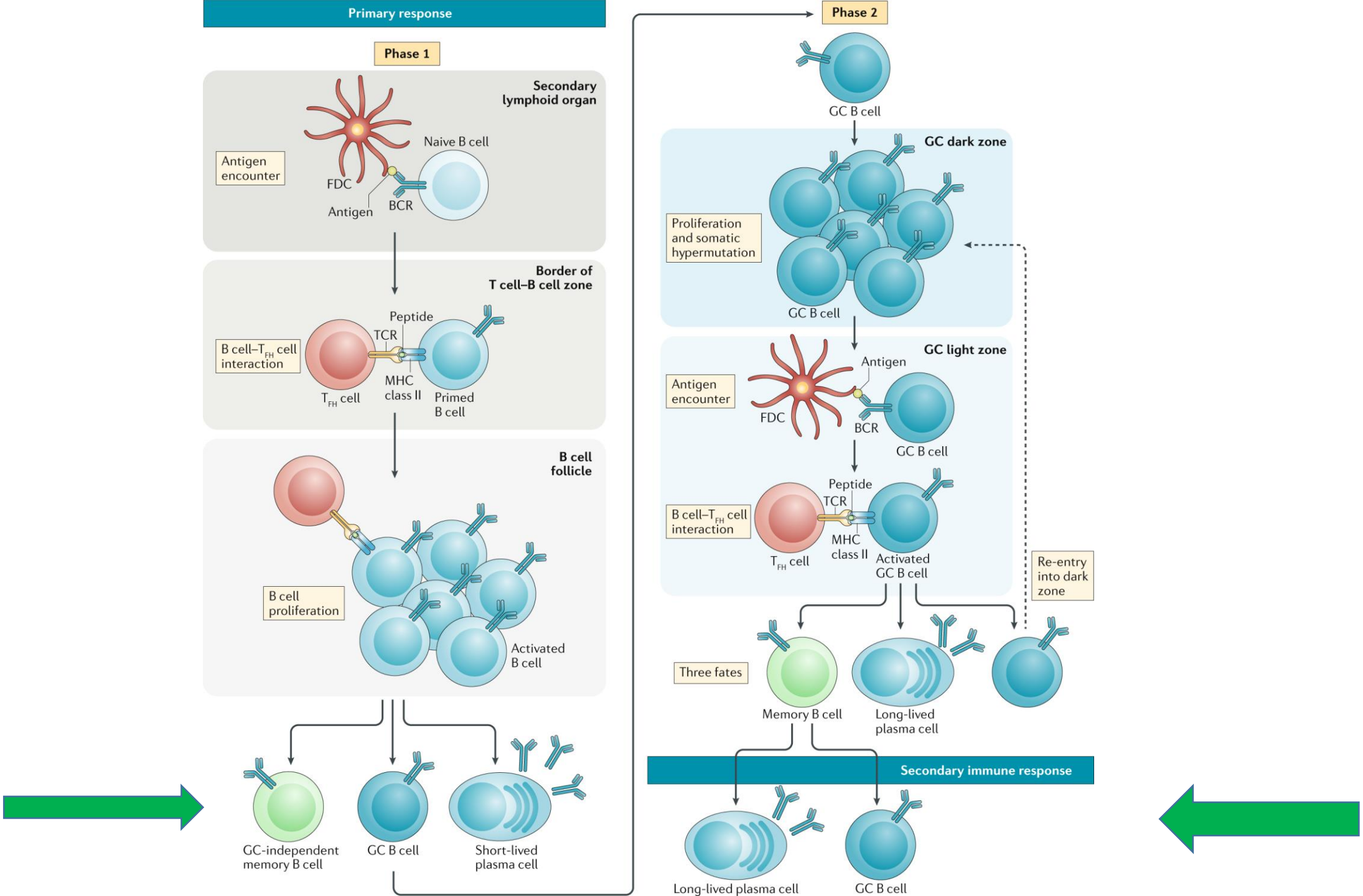
Immunité contre les helminthes
Dégranulation des mastocytes

Immunité des muqueuses


L V DJ
μ δ γ3 γ1 γ2 γ4 ∈ α1 α2
P
S
DNA
DNA Rearrangement
L V DJ ∈ α1 α2
DNA
L V DJ μ δ
Primary Transcript
IgM or IgD
L V DJ ∈
RNA
Primary Transcript
IgE

La commutation isotypique dépend des Interleukines




Primary response
Phase 1
Secondary lymphoid organ
Antigen encounter
Naive B cell
FDC
Antigen
BCR
Border of T cell–B cell zone
B cell–T_{FH} cell interaction
Peptide
TCR
MHC class II
T_{FH} cell
Primed B cell
B cell follicle
B cell proliferation
Activated B cell
GC-independent memory B cell
GC B cell
Short-lived plasma cell
Phase 2
GC B cell
GC dark zone
Proliferation and somatic hypermutation
GC B cell
GC light zone
Antigen encounter
Antigen
FDC
BCR
GC B cell
B cell–T_{FH} cell interaction
Peptide
TCR
MHC class II
T_{FH} cell
Activated GC B cell
Re-entry into dark zone
Three fates
Memory B cell
Long-lived plasma cell
Secondary immune response
Long-lived plasma cell
GC B cell

Notes conclusives

- Les LB sont les cellules pivot de la réponse immune adaptative humorale.

- Leurs ontogenèse commence dans la moelle osseuse et leurs maturation
- s'achève dans la rate.

- La compréhension de la physiologie et L'ontogenèse des LB à permis
- de comprendre certaines pathologies qui son liées à leurs développement.

- La cellule LB fait toujours l'objet des travaux de recherche.

DÉFICIT IMMUNITAIRE PRIMITIF À PRÉDOMINANCE HUMORALE

Bilan de 1ère intention	FNS		N						
	Sérologie post vaccinale		/	/	Altéré	Faible/N		N	
	G A M	IgG	0 ↓↓	0 ↓↓	↓ > 2g/l	↓	N	N	
		IgA	0 ↓↓	0 ↓↓	↓	N	N	↓	
		IgM	0 ↓↓	N ou ↑	N ou ↑ ou ↓	N	N	N	
Bilan de 2ème intention	LB (TBNK par CMF)		<2%	N	N ou ↓	N	N	N	
	Sous classes IgG	IgG1	/	/	/	↓	N	N	N
		IgG2	/	/	/	N	N ou ↓	N	↓
		IgG3	/	/	/	N ou ↓	N	N	N
		IgG4	/	/	/	N	N ou ↓	N	↓
IEI			Agamma globulinémie	Hyper IgM	CVID phénotype	D en IgG1/3	D en IgG 2/4	Isolé	associé
						Déficit en S/C d'IgG		Déficit en IgA	

ETUDE DE CERTAINS ASPECTS PATHOLOGIQUES

Affectant les lymphocytes B

1) **Lymphocytoses B**
2) **Deficits congenitales en lymphocytes B**

SIEMENS

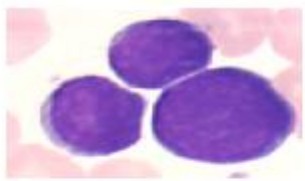
Réseau Cytolim CHU Limoges

MORPHOLOGIE DU LYMPHOCYTE B (I)

MOELLE OSSEUSE | SANG | RATE | GANGLION/TISSU LYMPHOÏDE SECONDAIRE | MOELLE OSSEUSE

C E L L U L E S B N O R M A L E S

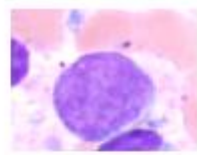
Lymphopoïèse → Immunopoïèse



Hématogones

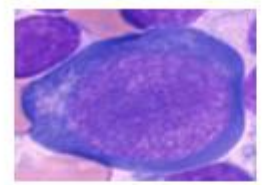
Lymphocyte trans[el] puis naïf

« Atypies » chez enfant

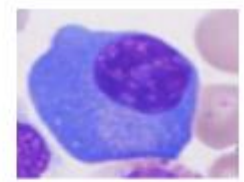


Centrocyte

Centroblaste



Immunoblaste



Plasmocyte

Lymphocytoses B

- Lymphocytoses B réactionnelles
- Lymphocytoses B malignes:

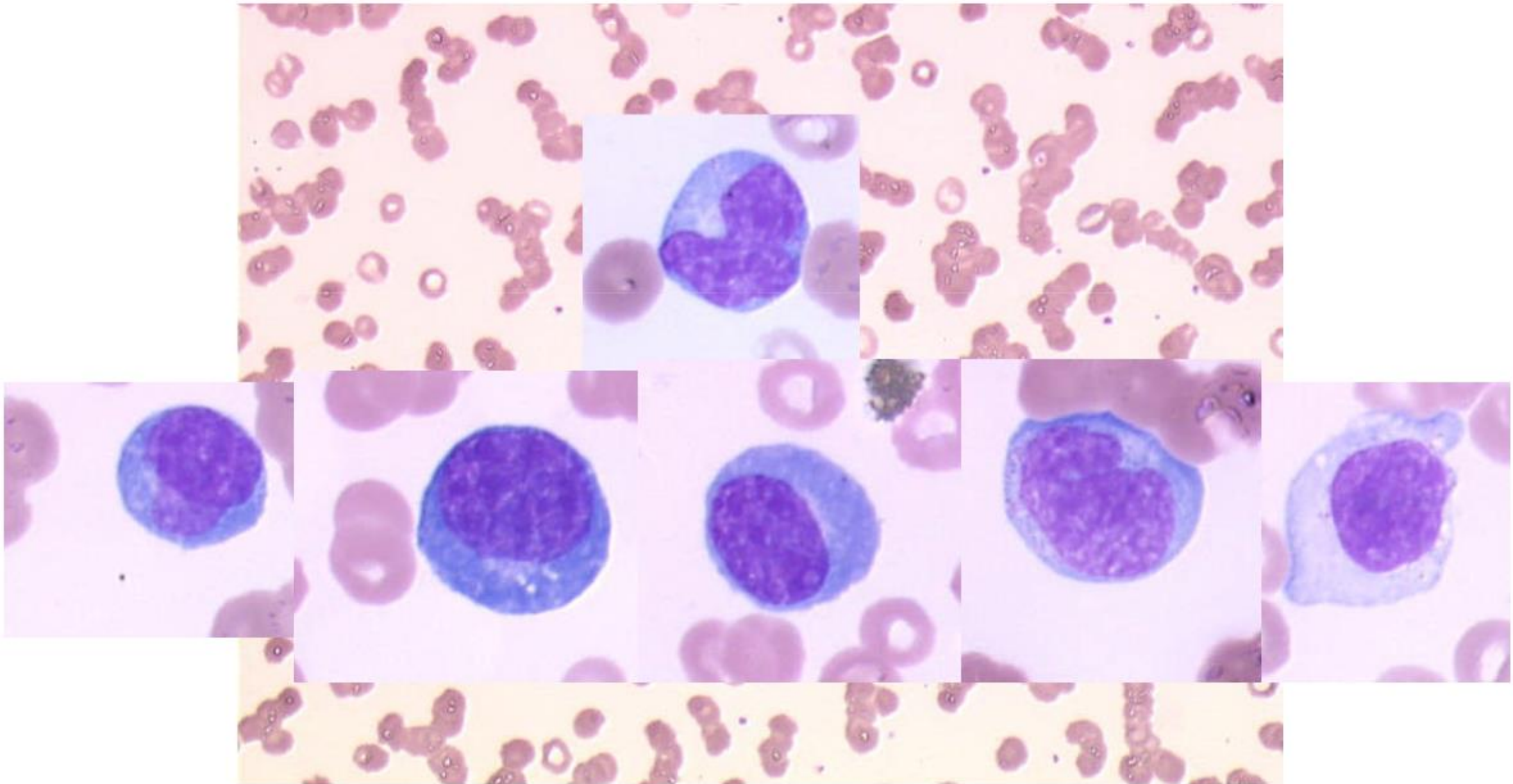
- Néoplasies B à « petites cellules », points difficiles

– Néoplasies B à « grandes cellules »

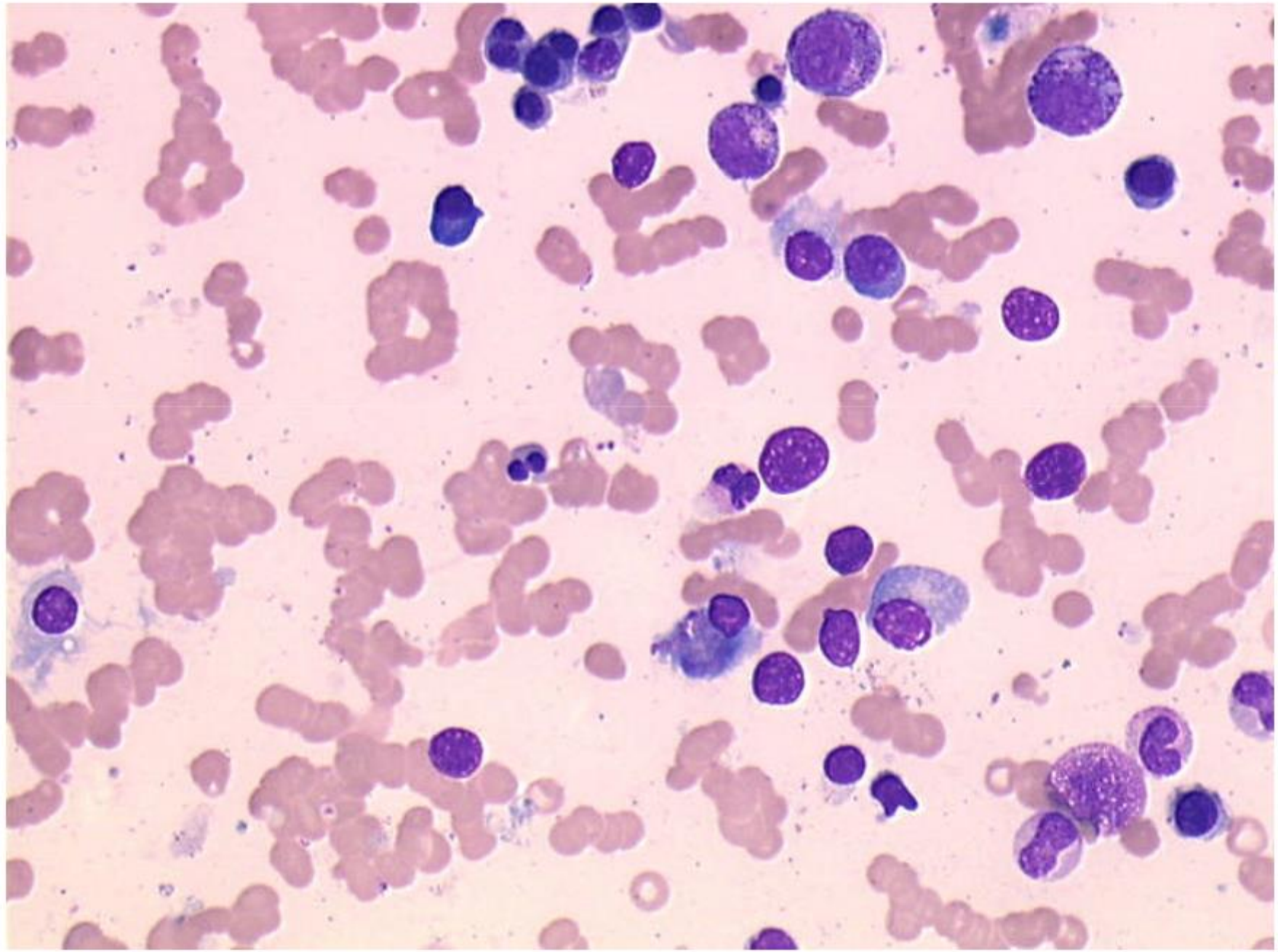
Cas 1 : Khe Lak

- Homme de 68 ans , AEG fièvre 40° hypergamma globulinémie 40 g/L, annoncée comme monoclonale, image hépatique

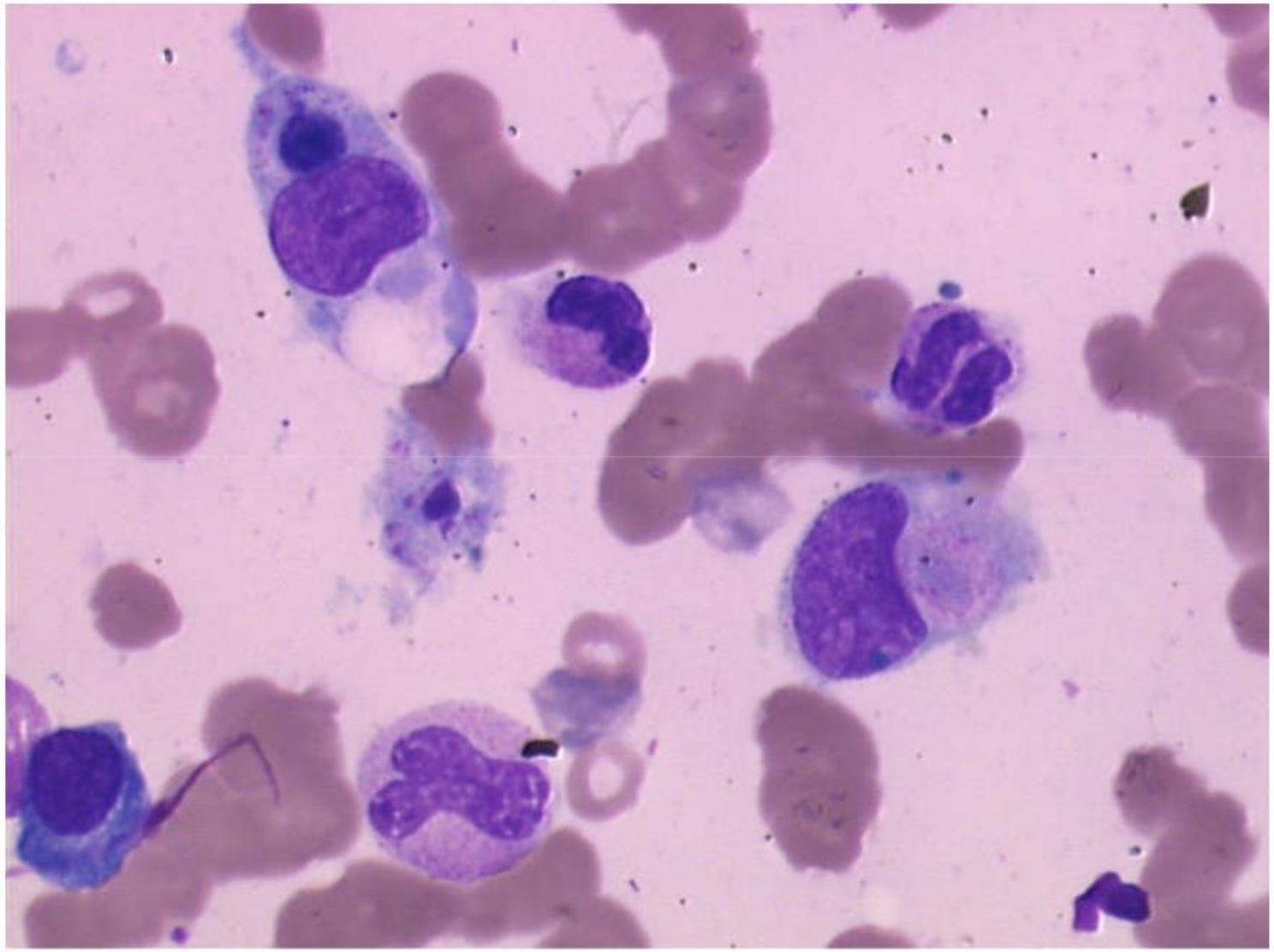
- Sang : rouleaux +++ Cellules activées ? Plasmocytes ? Cellules type LAI ?



- Moelle : plasmocytose 13% non dystrophique



Rares atypies « dans les limites de la tolérance »

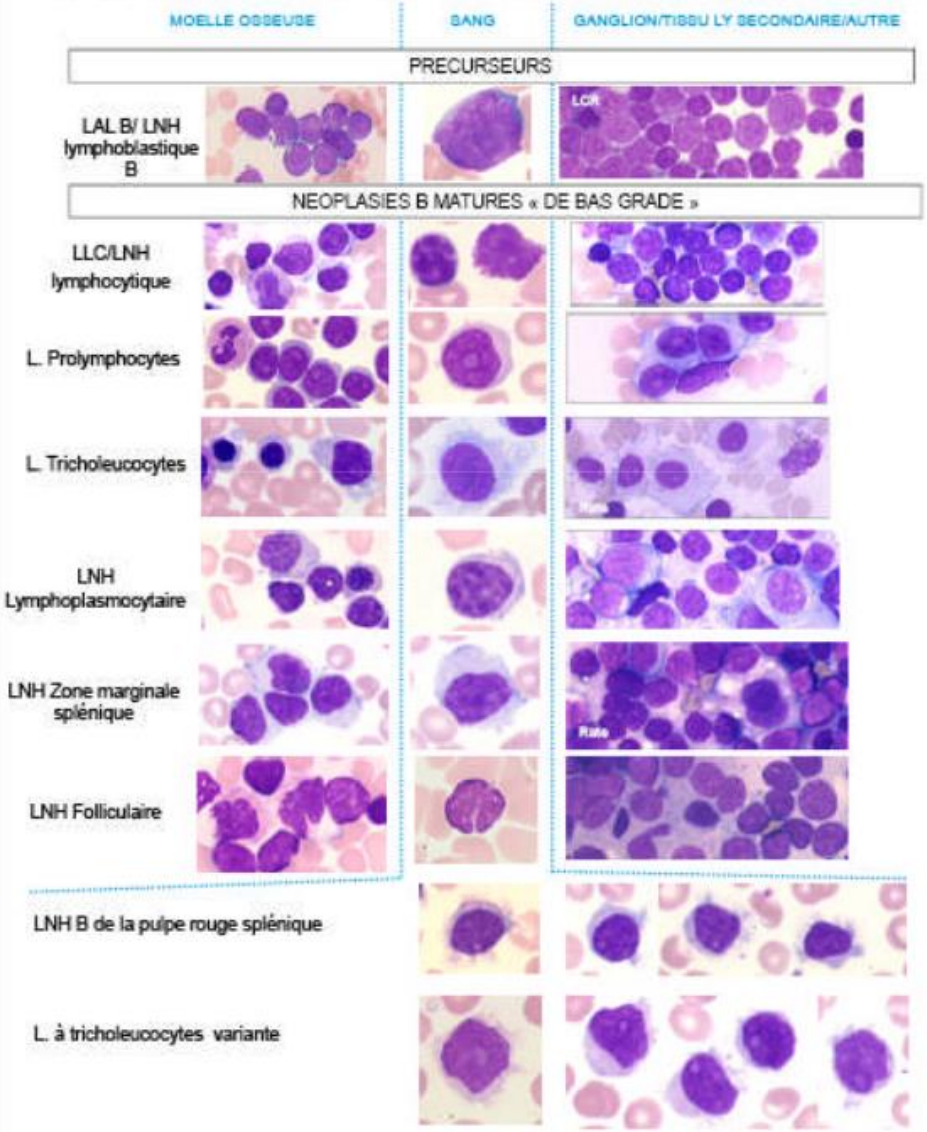


SIEMENS

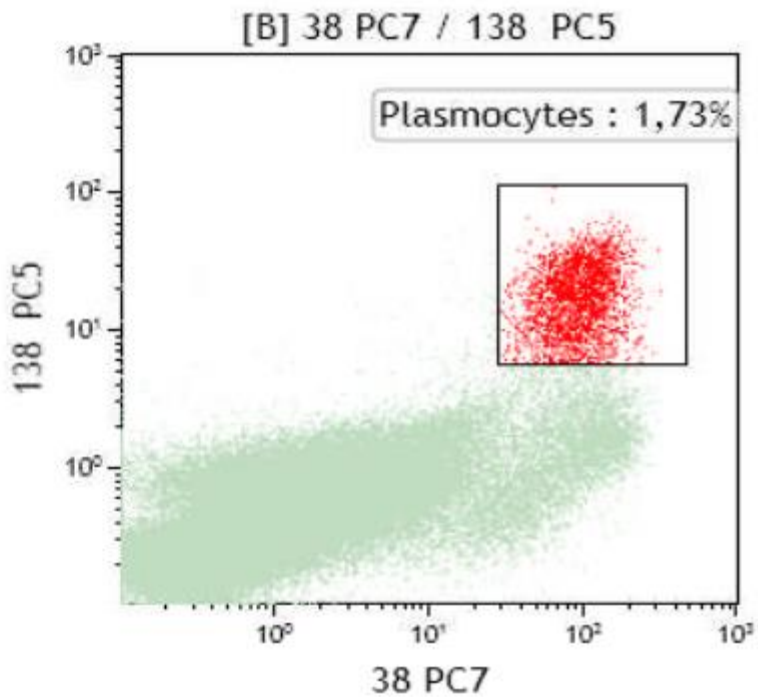
Réseau Cytolim CHU Limoges

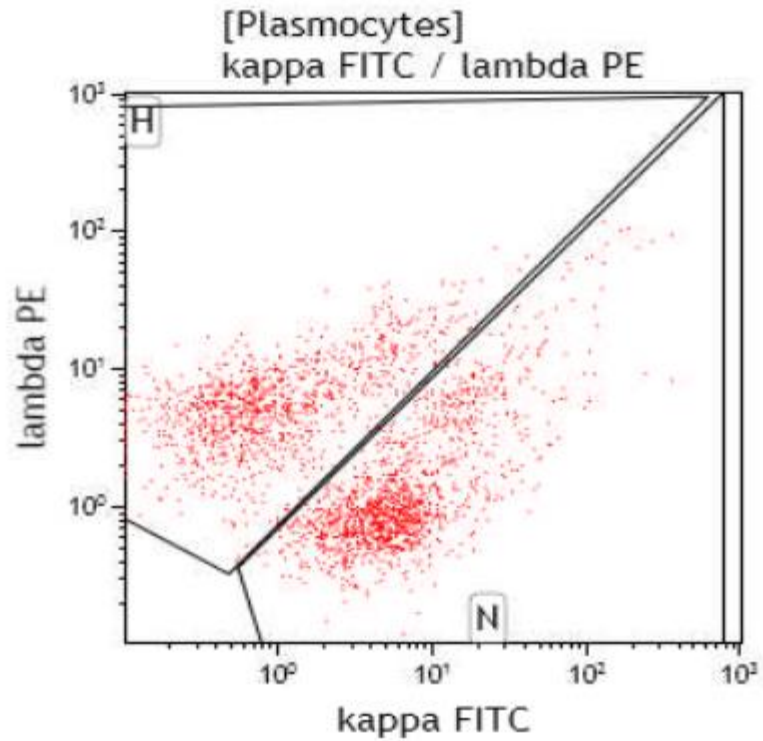
MORPHOLOGIE DU LYMPHOCYTE B (II)

C E L L U L E S B T U M O R A L E S

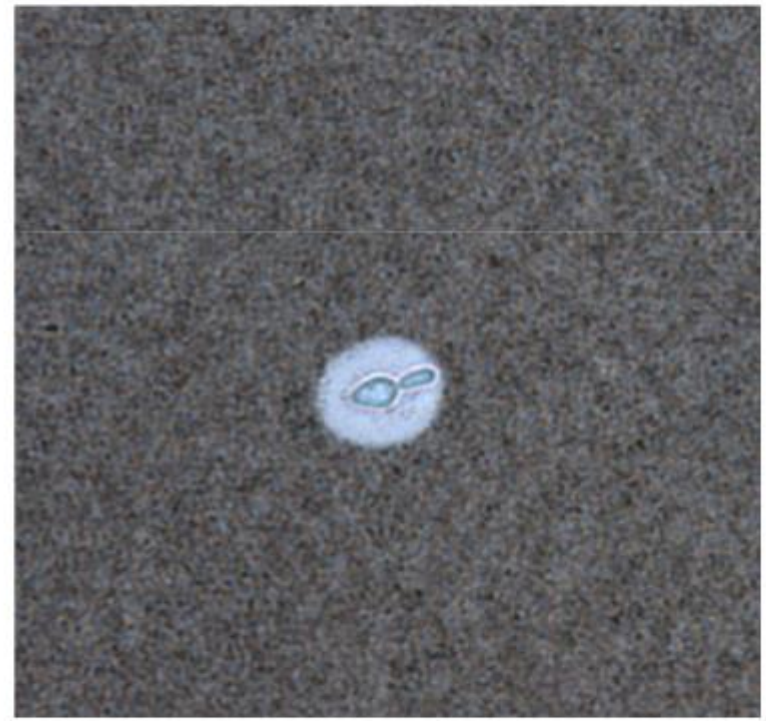


MOELLE


[B] 38 PC7 / 138 PC5
Plasmocytes : 1,73%
138 PC5
38 PC7


[Plasmocytes]
kappa FITC / lambda PE
H
N
lambda PE
kappa FITC

- CFM :
  - Moelle : plasmocytose polytypique
  - Sang : CD4=0



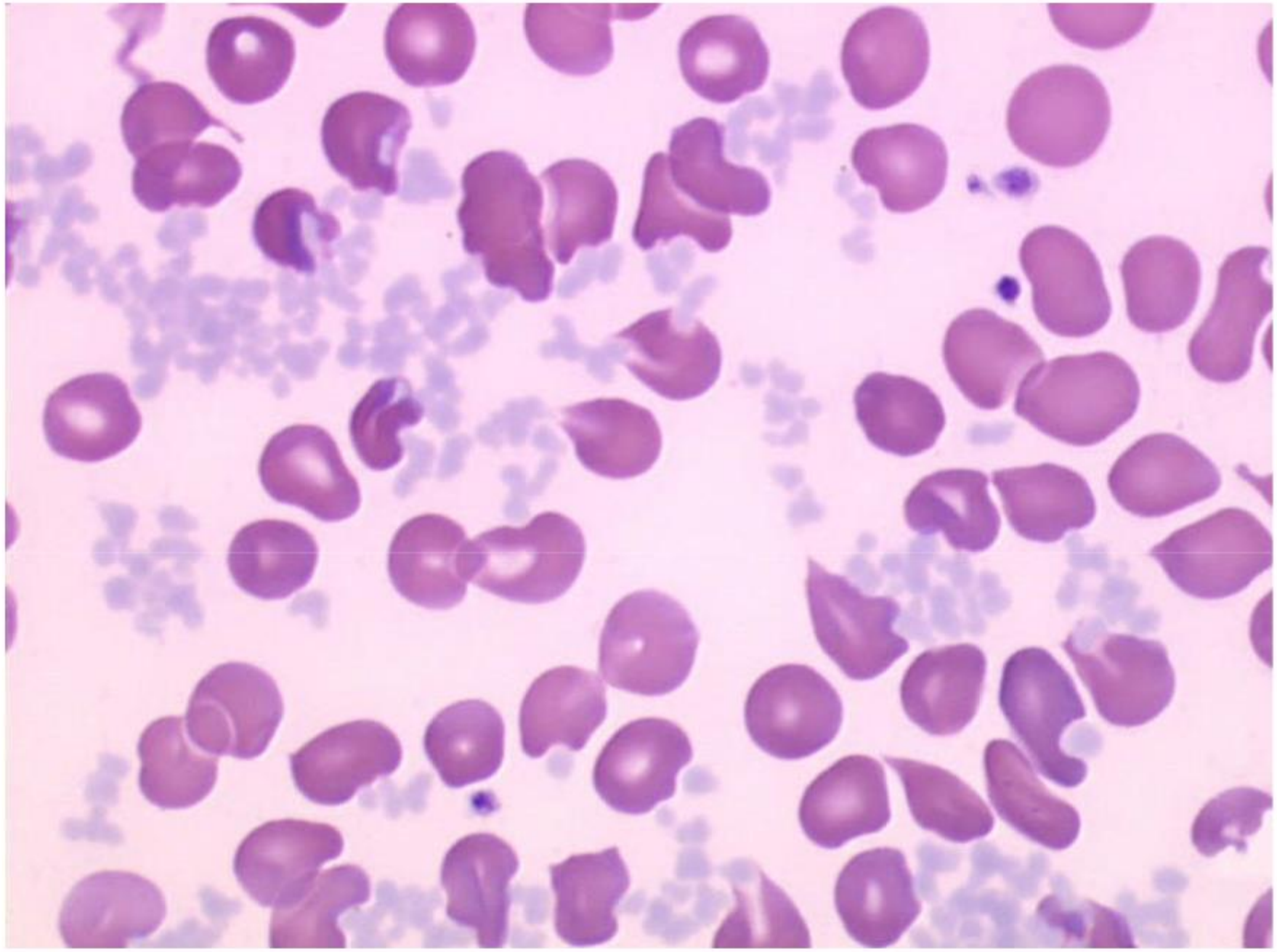
méningite à cryptocoque, SIDA

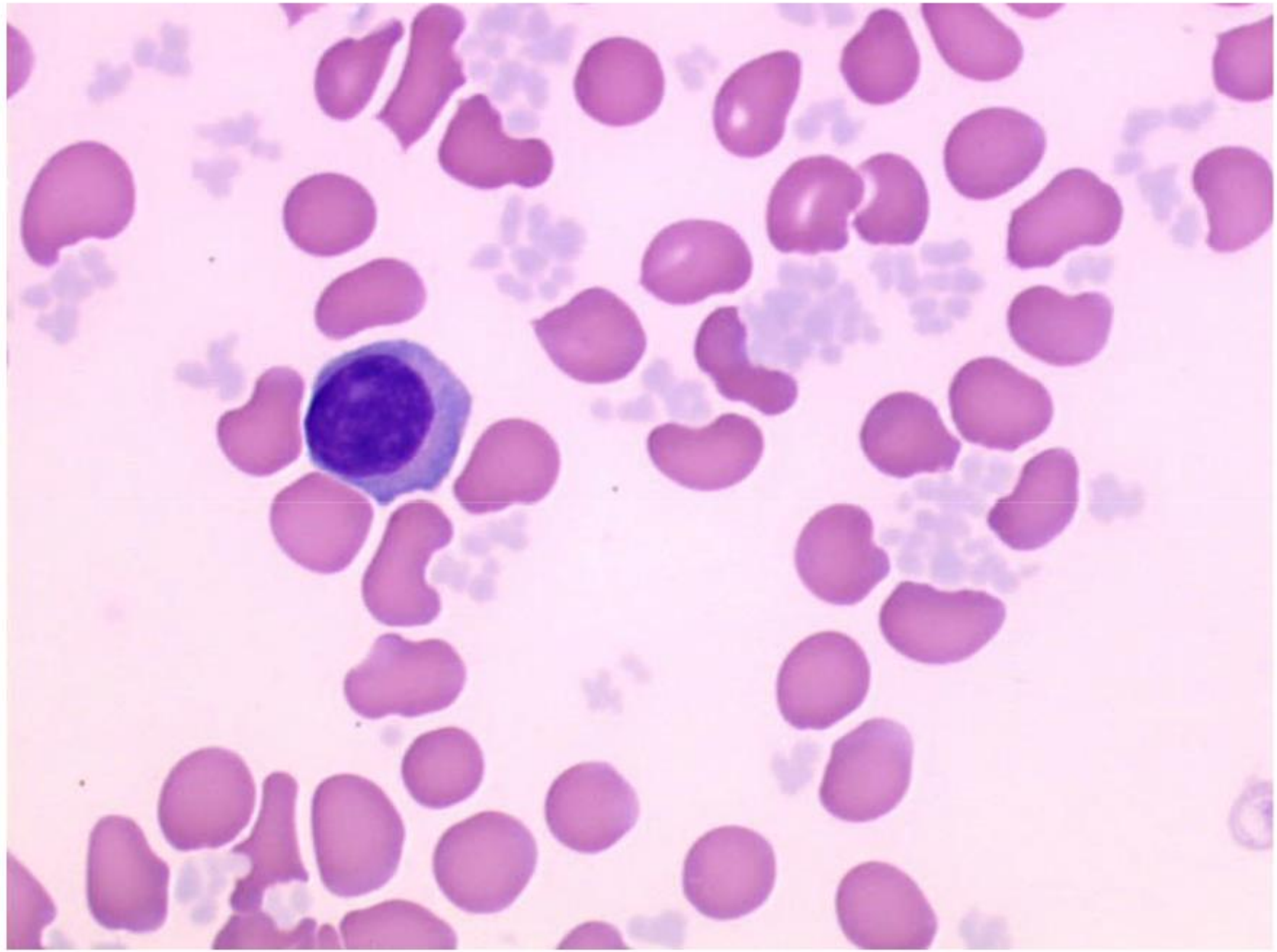
(NB : anomalies lymphoïdes liées au HIV)

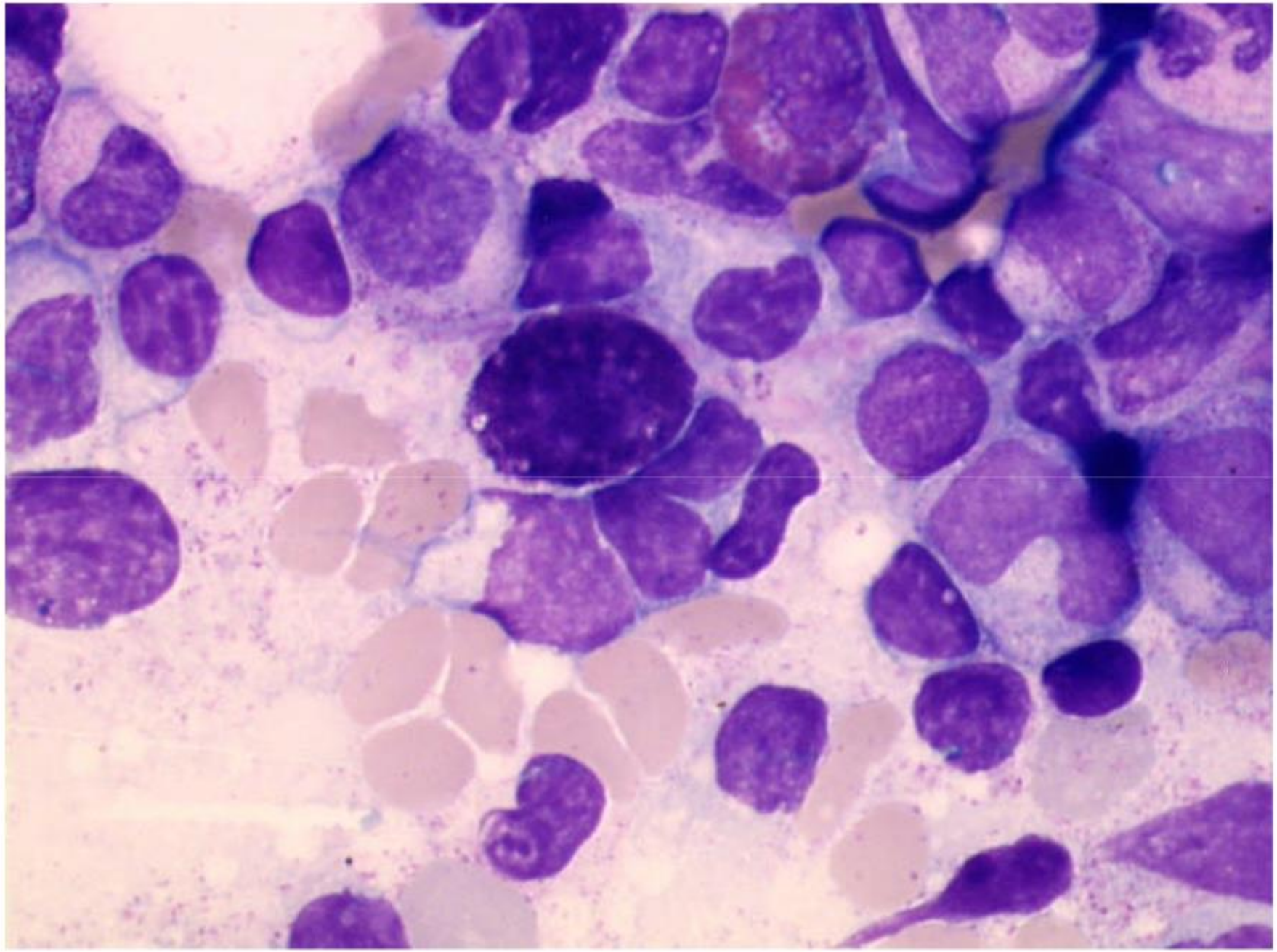
- Expansion d'hématogones
- Primo infection : syndromes mononucléosiques
- SIDA : lymphopénie (CD4)
- Parfois hyperlymphocytose CD8+, à LGL, persistantes
- Moelle : augmentation plasmocytes (corps de Russel) macrophages +/- activés
- Lymphomes : Burkitt, DLBCL (Lymphome B diffus à grandes cellules :immunoblastique, plasmablastique), primitif séreuses, primitifs du SNC, Hodgkin

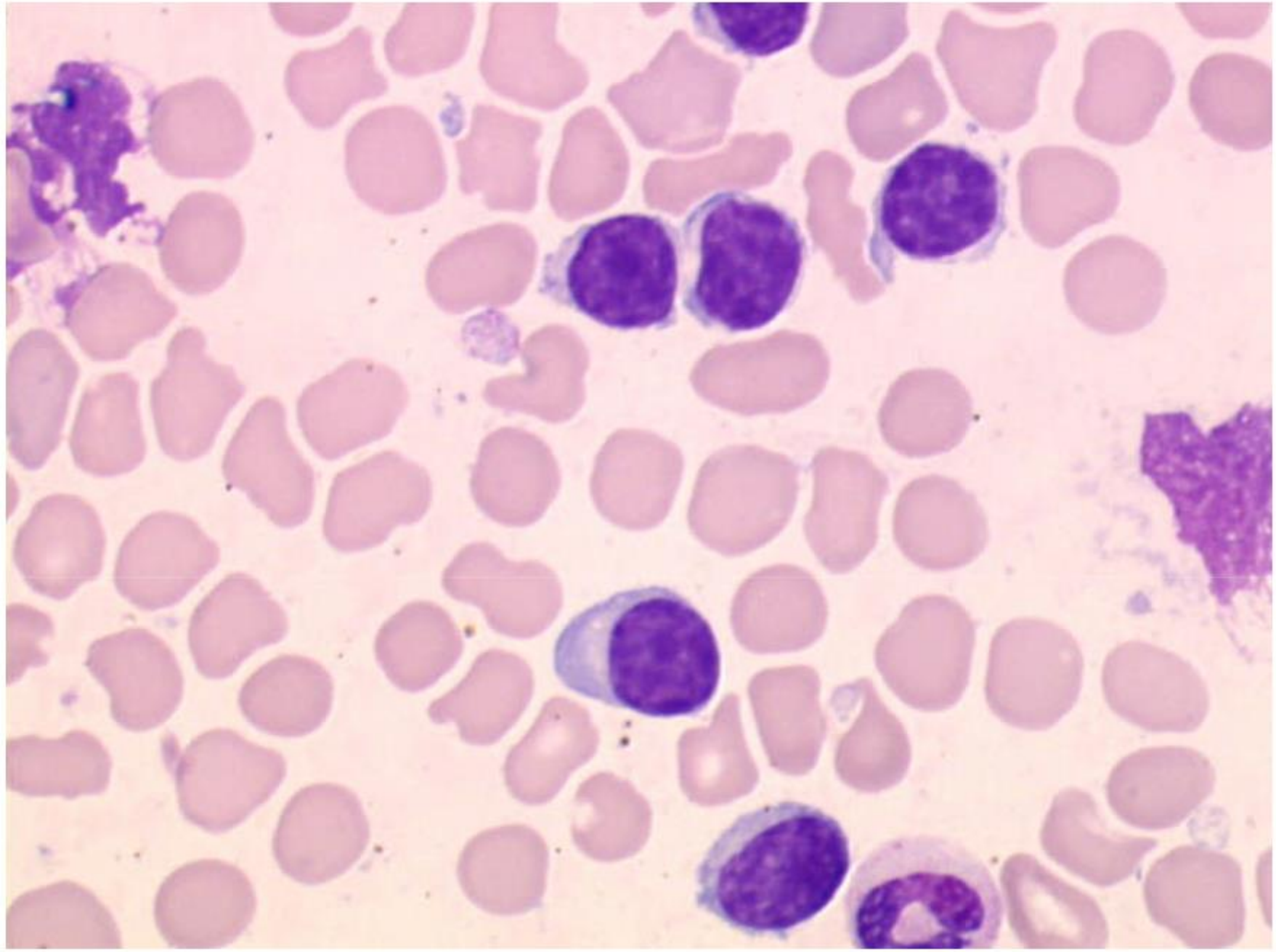
Cas 5 Dou je

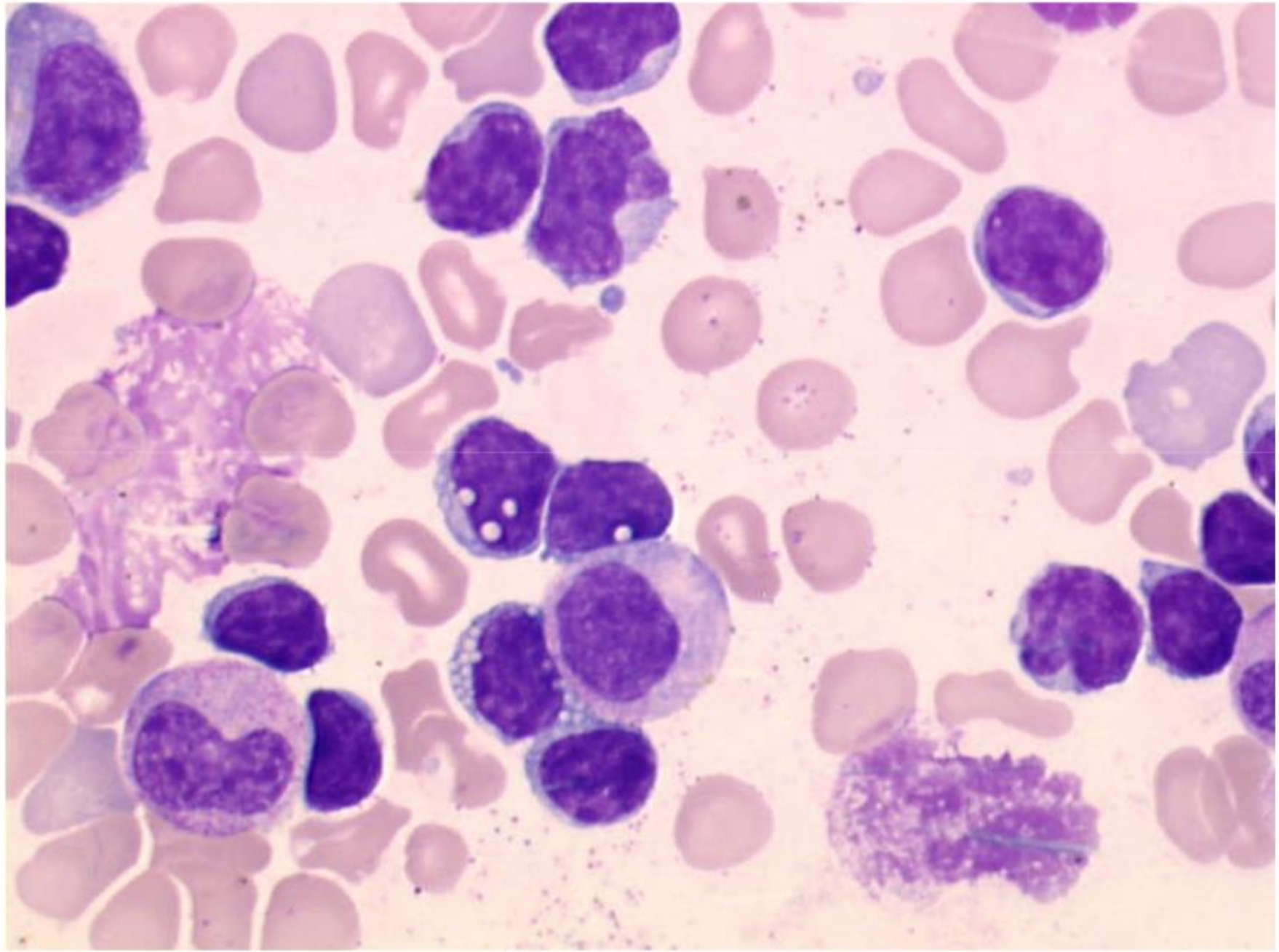
- Homme de 78 ans
- Clinique : amaigrissement
- NFS : plaq : 1000 avec le graphe suivant sur Siemens Advia 2120. Plaquettes : 81 sur le sang passé à 37°C. GB 7 PNN 2.5 Ly 4











Biochimie : IgM 5.3g/l Cryoglobuline de très forte activité
Frottis : cryoglobuline faux décompte de plaq sur Advia . MO
MO : infiltrat type Waldenström 86% CFM CD5- CD23- FMC7+ CD20+
DIAGNOSTIC : MALADIE DE WALDENSTROM AVEC CRYOGLOBULINEMIE