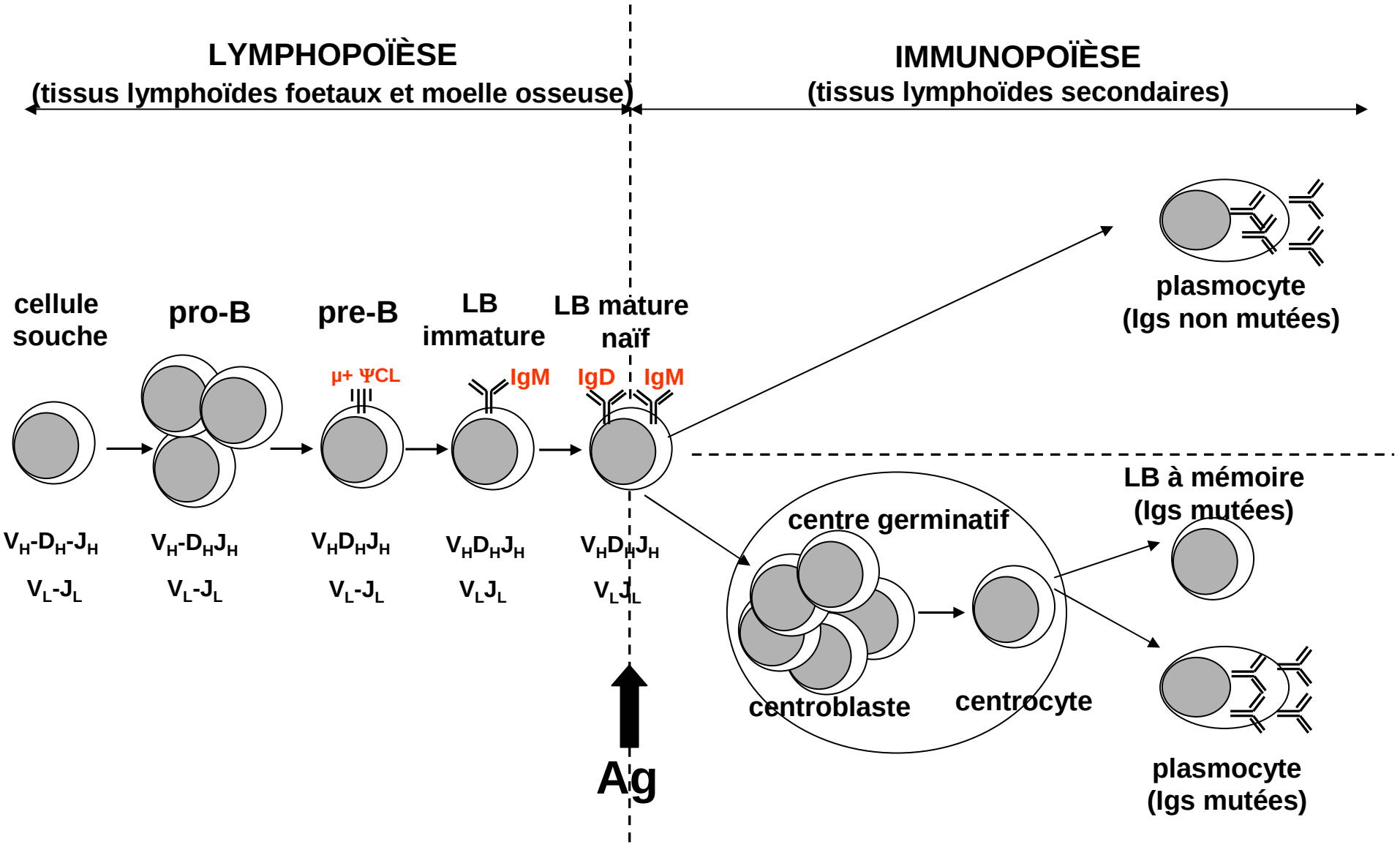
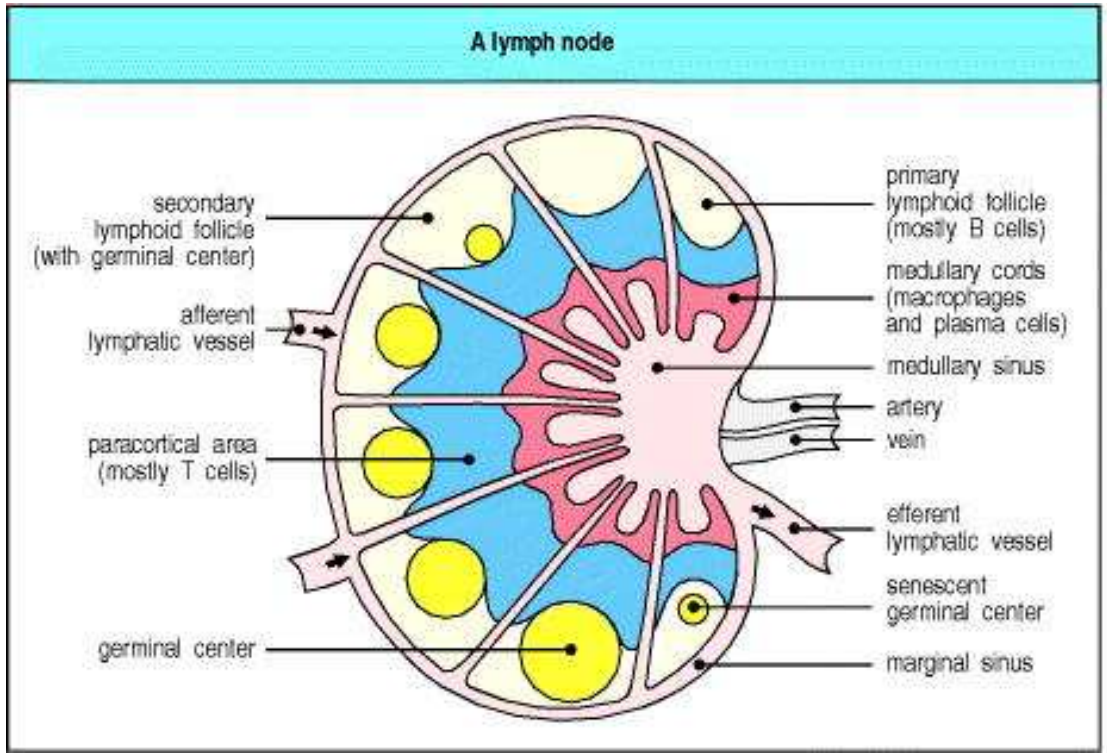
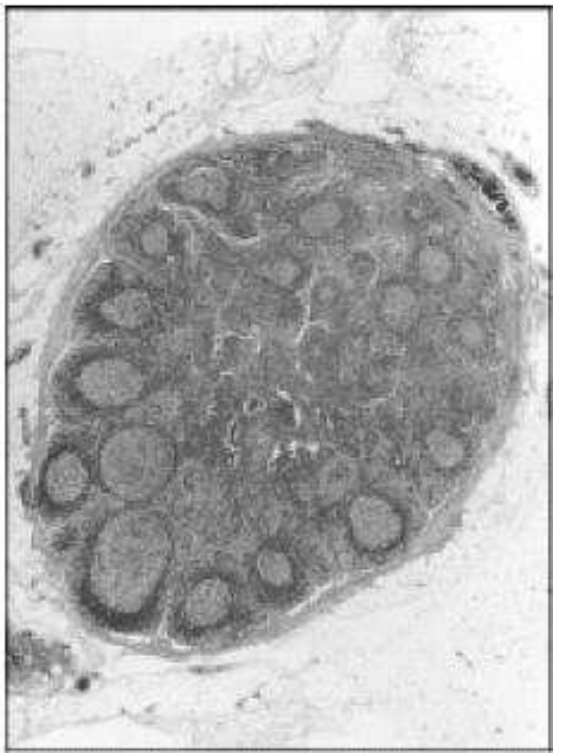
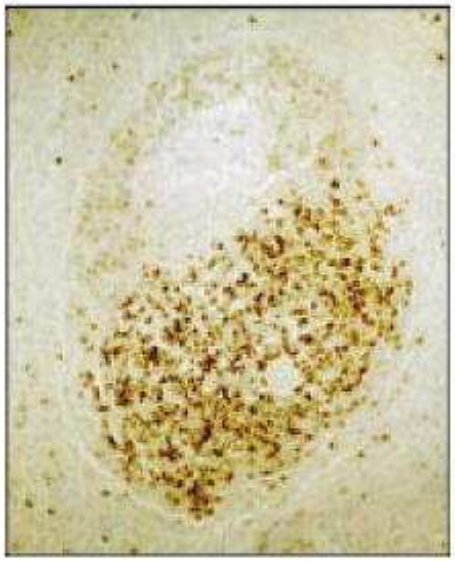
MICRO-ANATOMIE DES TISSUS LYMPHOÏDES SECONDAIRES

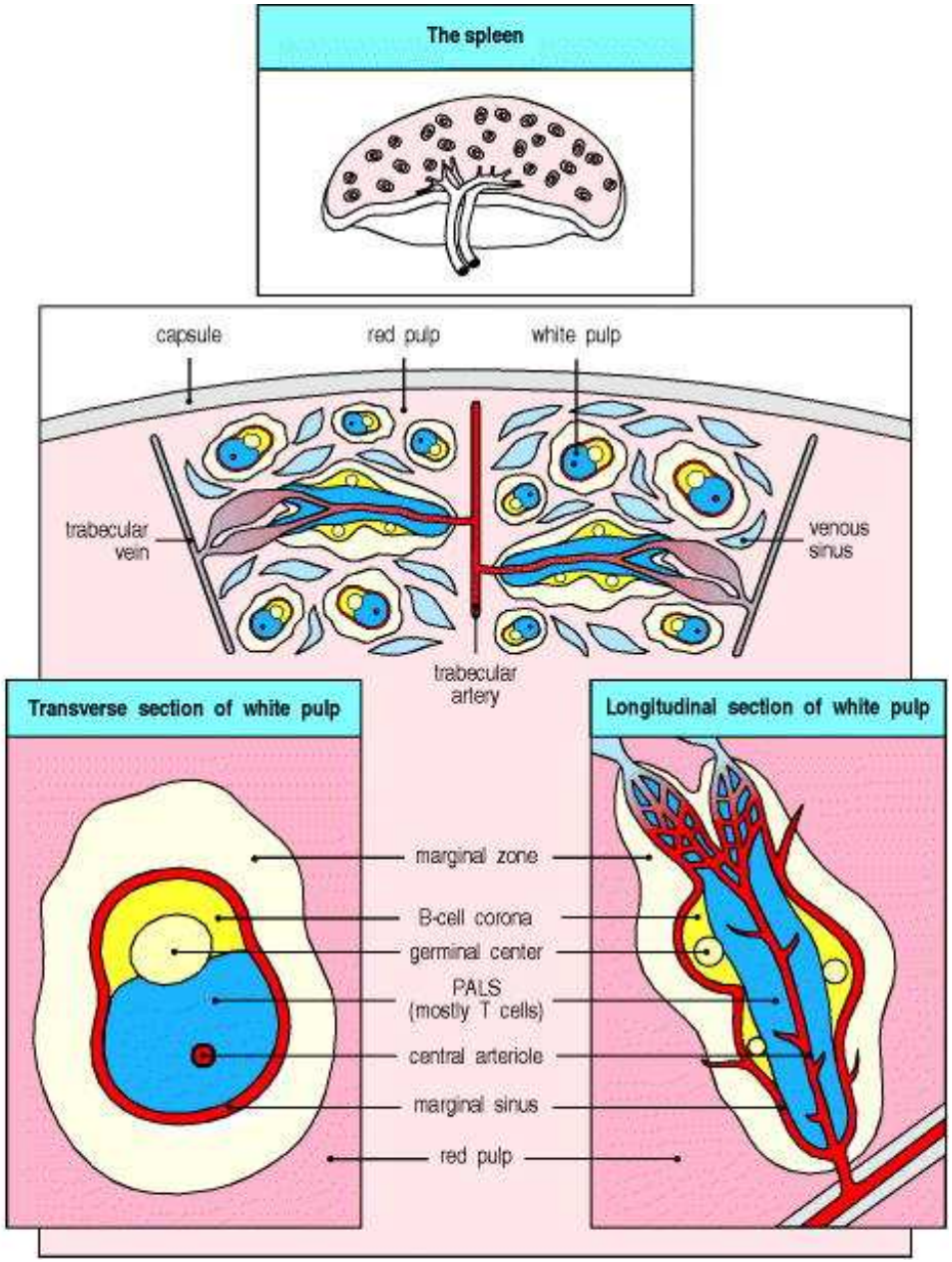


LES DEUX PHASES DU DÉVELOPPEMENT LYMPHOCYTAIRE B


A lymph node
secondary lymphoid follicle (with germinal center)
afferent lymphatic vessel
paracortical area (mostly T cells)
germinal center
primary lymphoid follicle (mostly B cells)
medullary cords (macrophages and plasma cells)
medullary sinus
artery
vein
efferent lymphatic vessel
senescent germinal center
marginal sinus

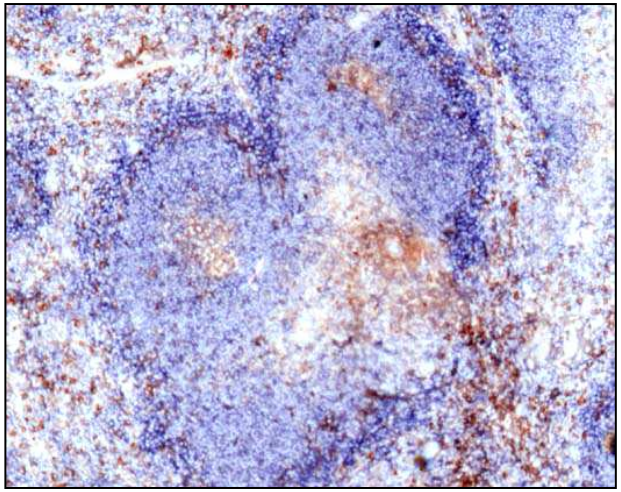


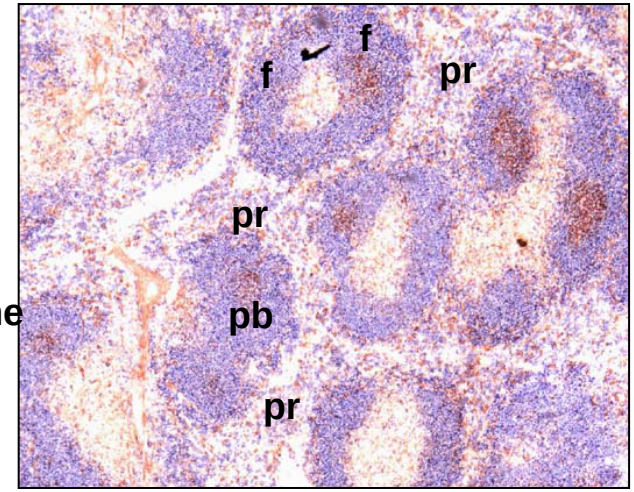



The spleen
capsule
red pulp
white pulp
trabecular vein
venous sinus
trabecular artery
Transverse section of white pulp
Longitudinal section of white pulp
marginal zone
B-cell corona
germinal center
PALS
(mostly T cells)
central arteriole
marginal sinus
red pulp

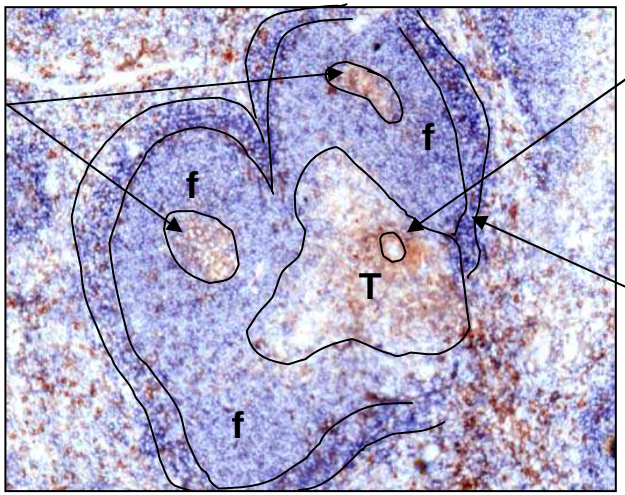
PNA/CD38

PNA/B220





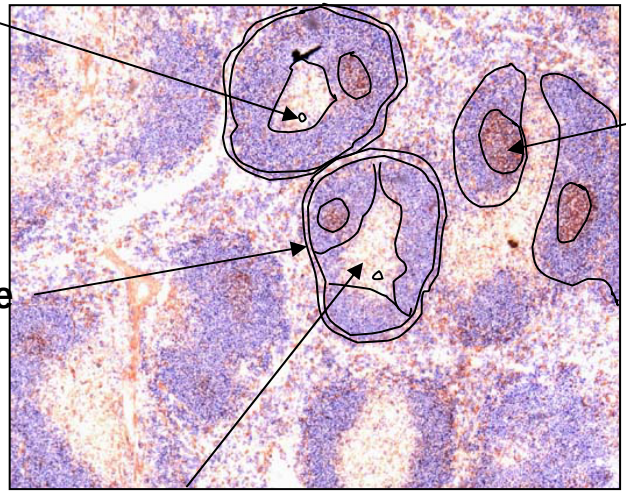
pr: pulpe rouge
pb: pulpe blanche
follicule: f



GC

artériole centrale

zone marginale

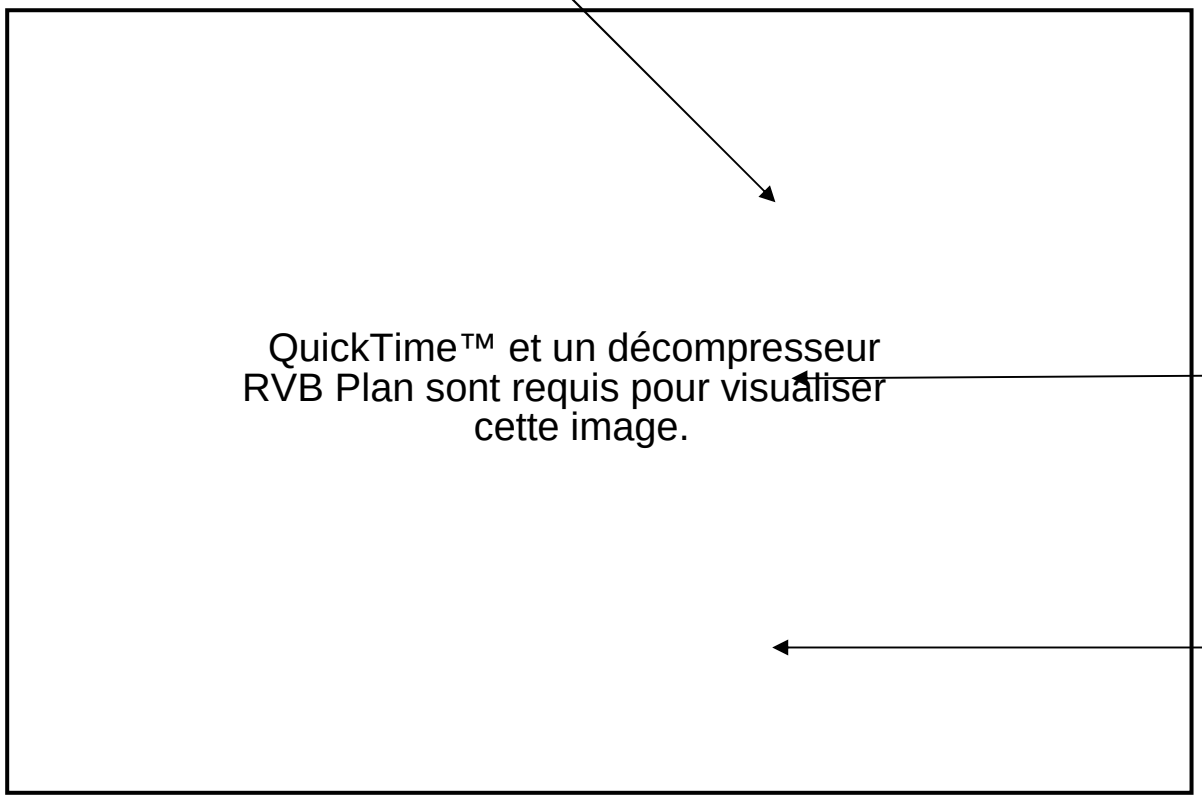


GC

zone T

MICROANATOMIE DU TISSU LYMPHOÏDE SPLÉNIQUE

manteau folliculaire
(LB naïfs IgM+/IgD+)



zone claire
- sélection
- différenciation

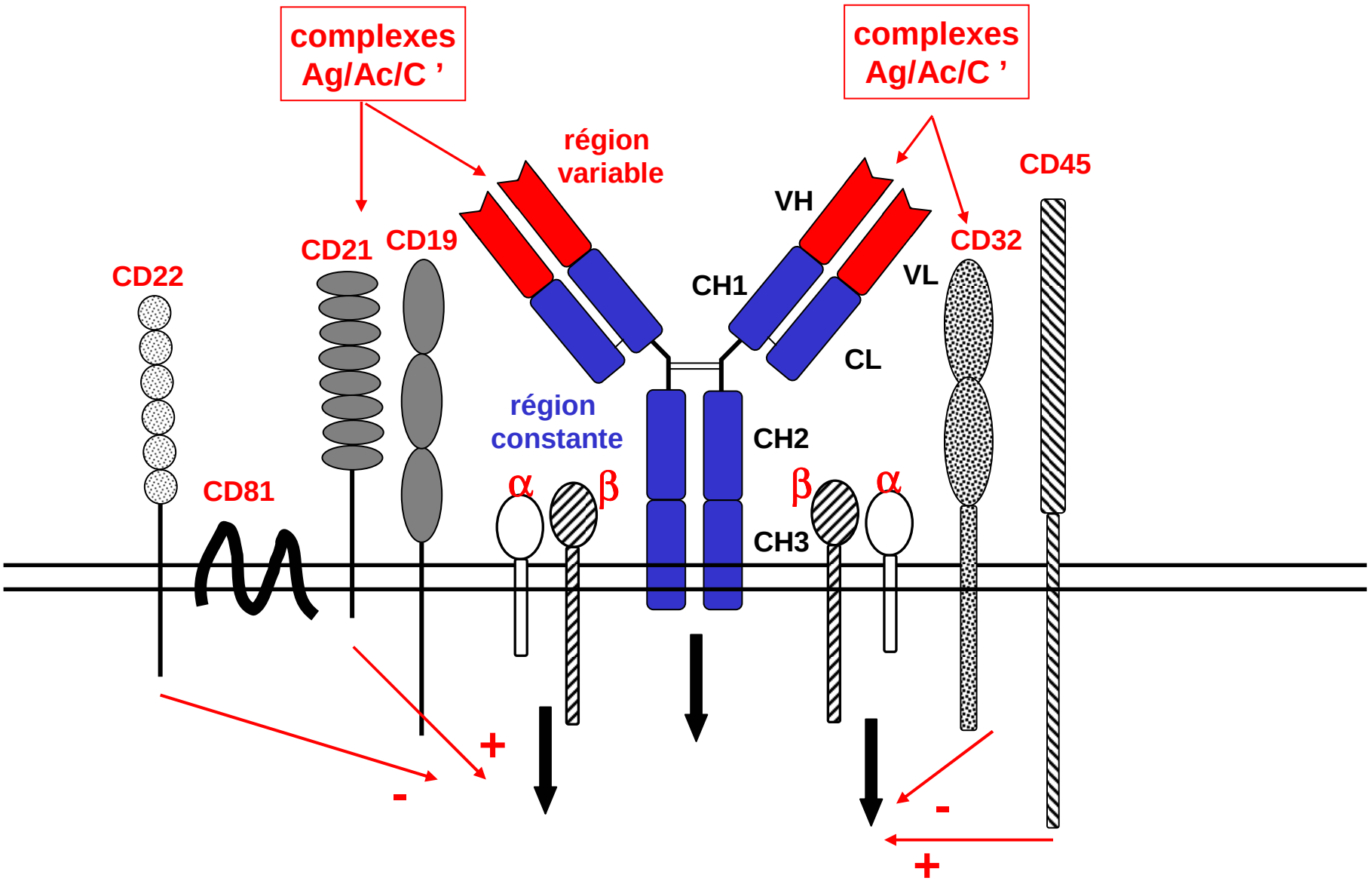
zone sombre
- expansion clonale
- diversification

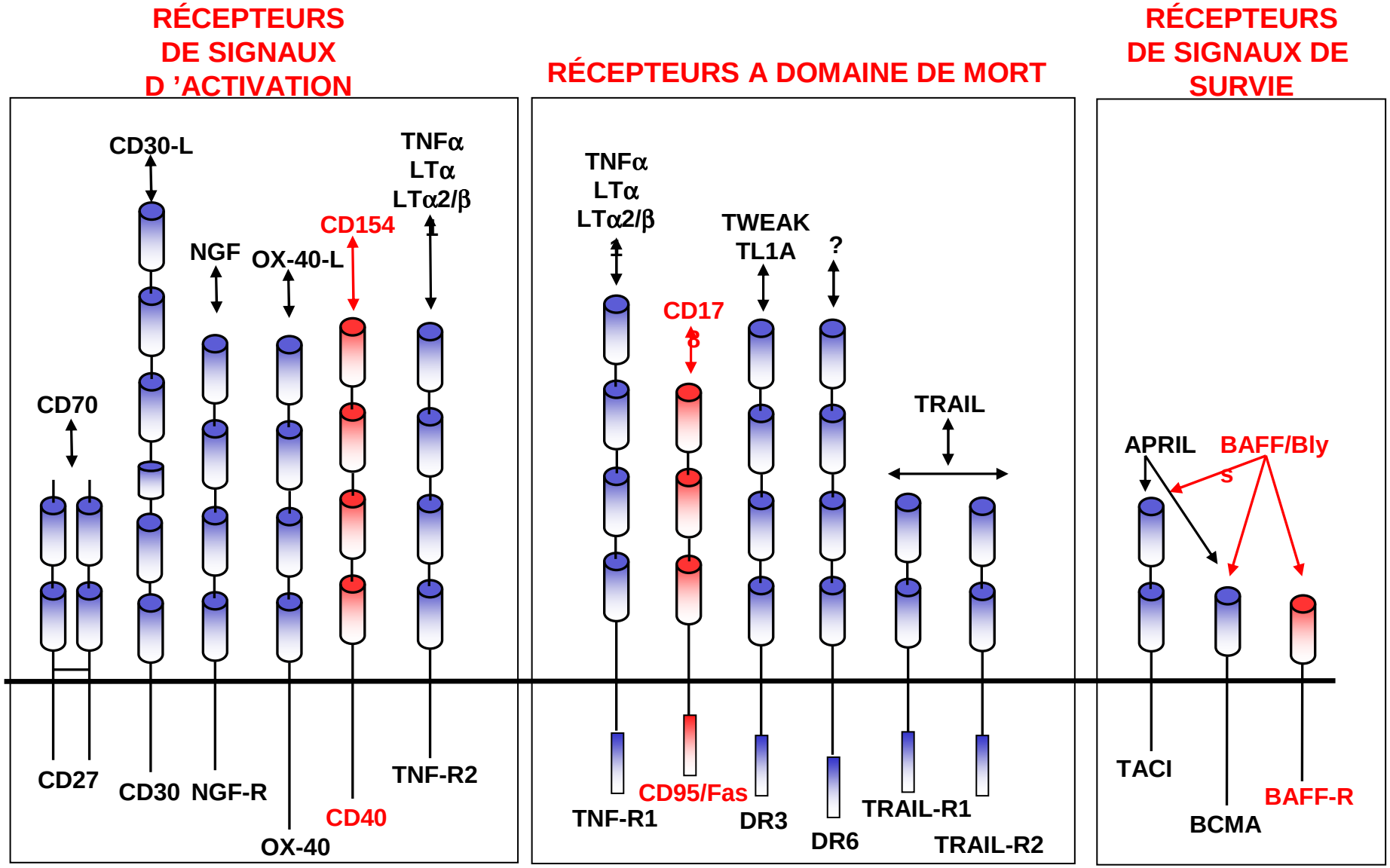
CG

bleu: anti-Ki67 (marqueur de prolifération)
rouge: anti-IgD (marqueur cellules naïves)

MICRO-ANATOMIE DES CENTRES GERMINATIFS (AMYGDALES)

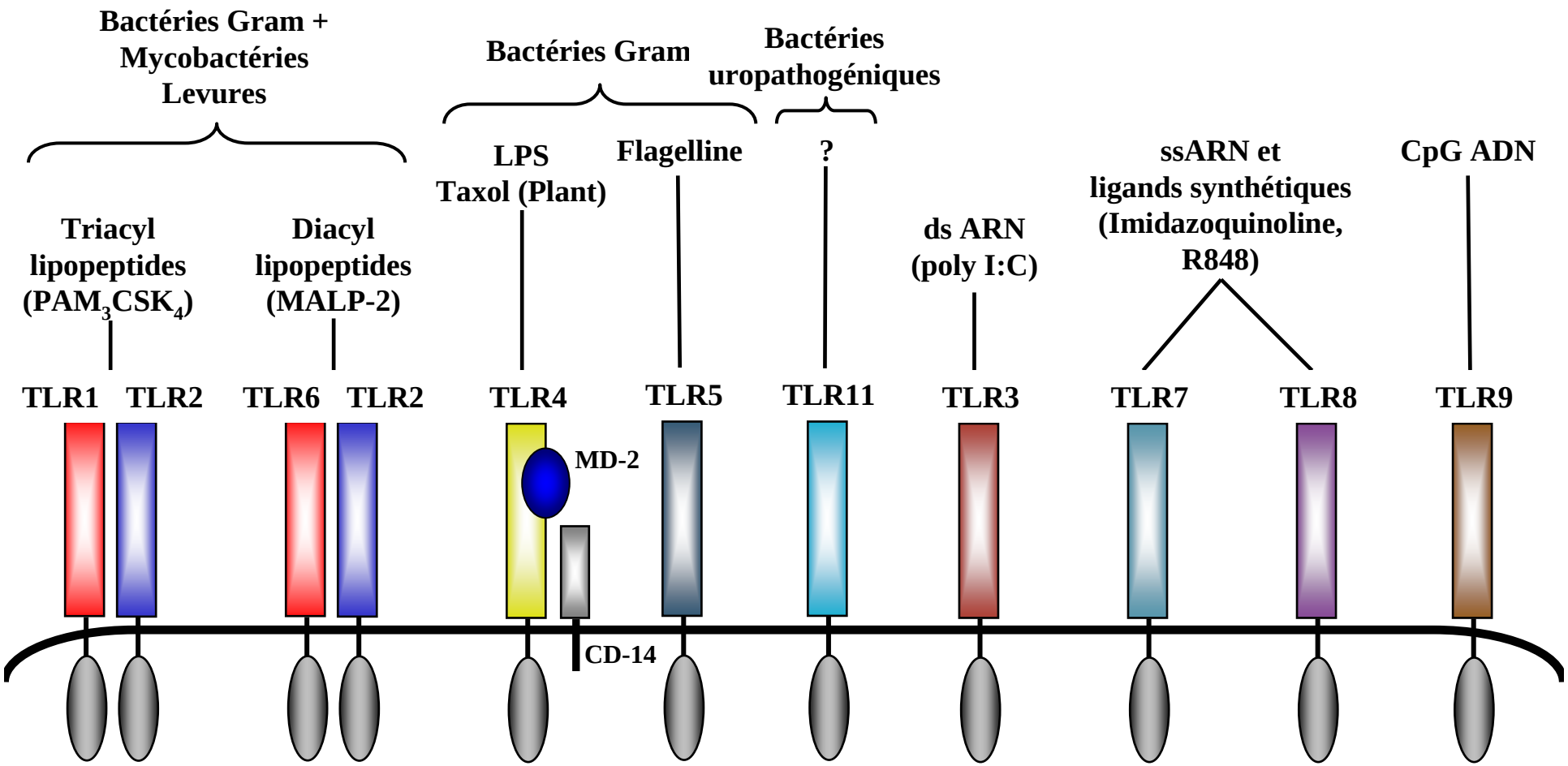
RÉCEPTEURS MEMBRANAIRES IMPLIQUÉS DANS L ’ACTIVATION DES LYMPHOCYTES B


complexes
Ag/Ac/C ’
complexes
Ag/Ac/C ’
région
variable
VH
CD45
CD32
CD21
CD19
CD22
CH1
VL
CL
région
constante
CH2
α
β
β
α
CD81
CH3
+
-
-
+

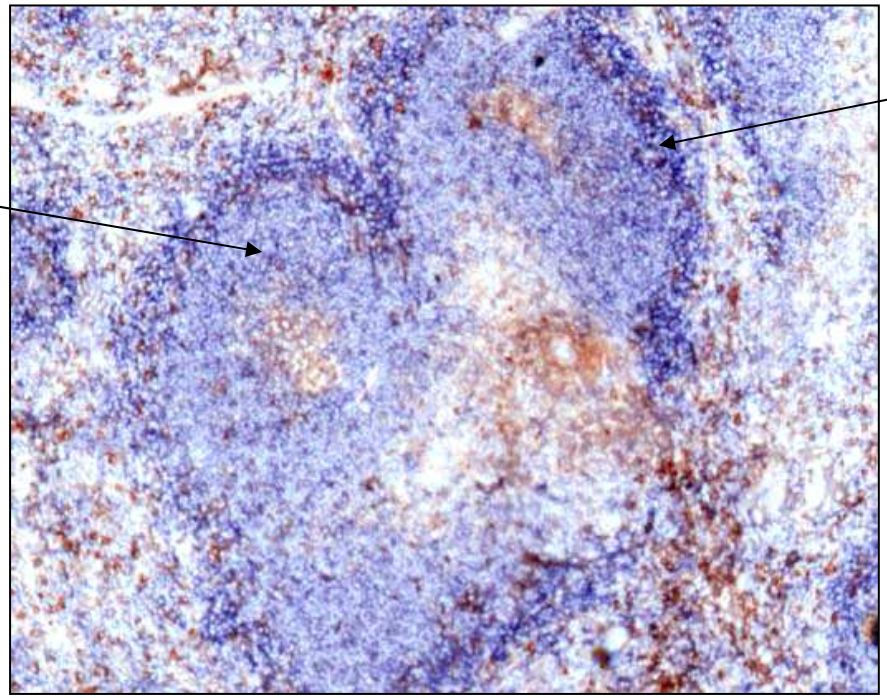


LA SUPERFAMILLE DES RÉCEPTEURS DU TNF

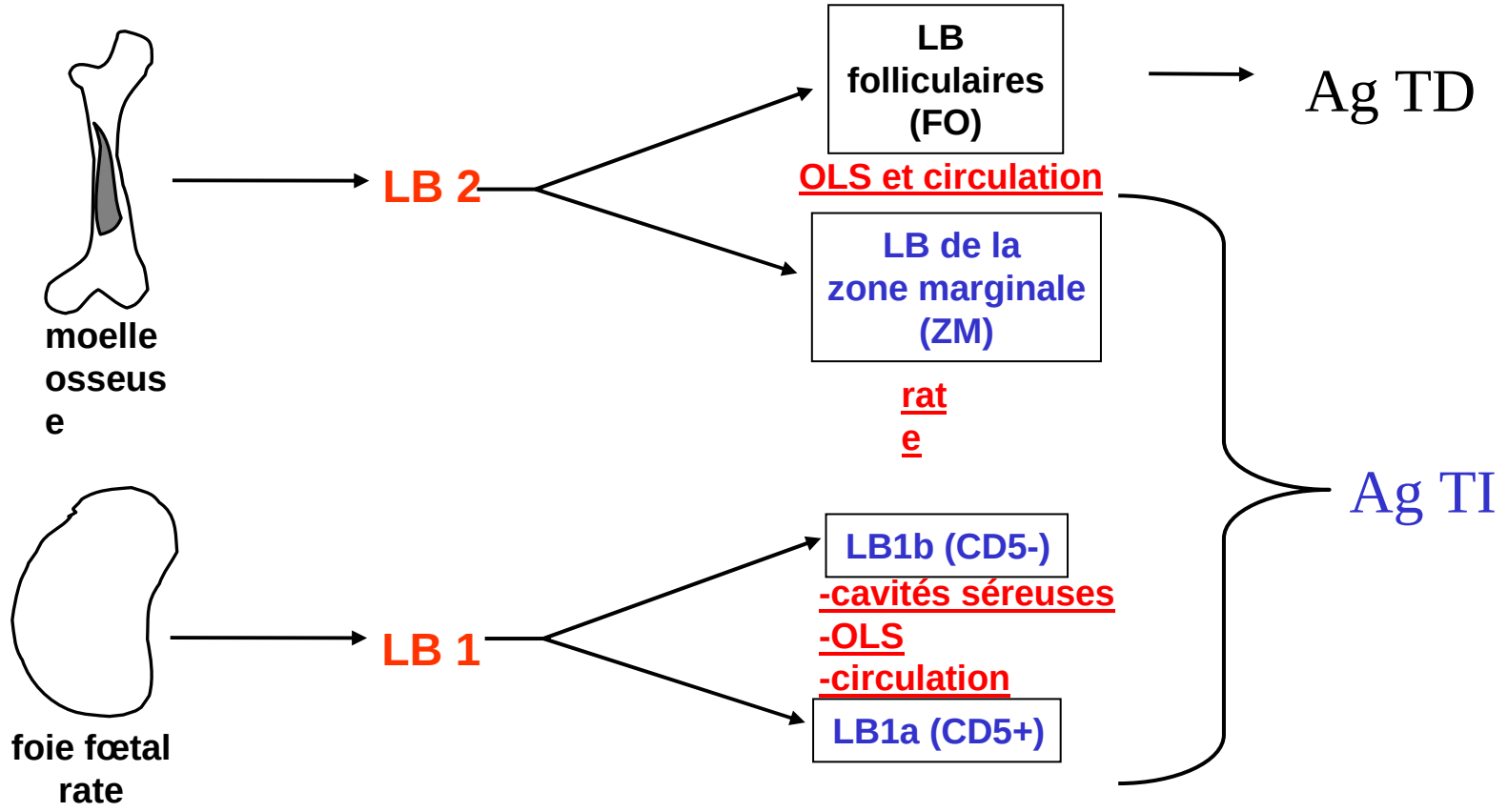
Les TLR et leurs ligands



LA RÉPONSE AUX Ag THYMO-DÉPENDANTS



	Ag TD	Ag TI
- Réponse Ac chez des souris athymiques	-	+
- Maturation d ’affinité de la réponse Ac	+	-
- Formation de centres germinatifs	+	+/-
- Production de LB à mémoire (rate)	+	-
- Production de plasmocytes à longue durée de vie (moelle)	+	-



2 lignages B (LB1 et LB2) forment le compartiment des LB naïfs. Ce compartiment se subdivise en 4 sous-populations:

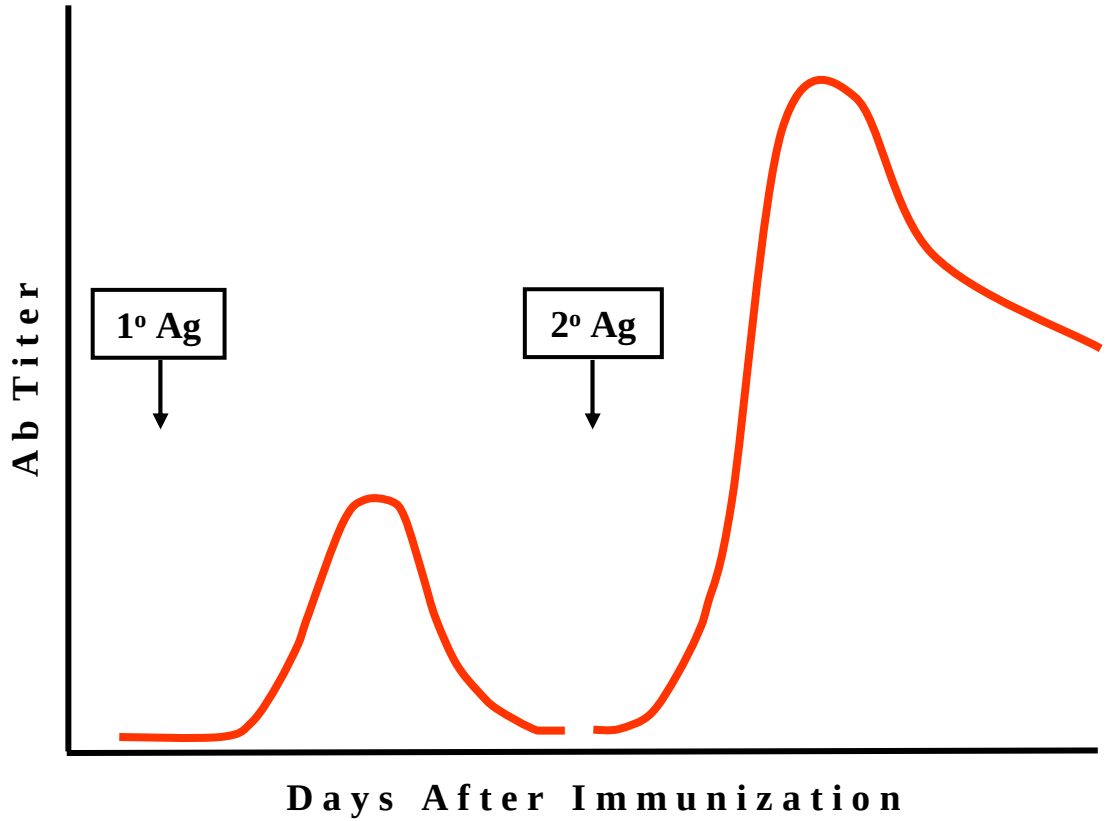
1. **les LB folliculaires (FO)**
2. **les LB de la zone marginale (ZM)**
3. **les LB1a (CD5+)**
4. **les LB1b (CD5-)**

Les LB1 et les LB ZM sont « spécialisés » dans la réponse aux Ag TI

LES LIGNAGES LYMPHOCYTAIRES B

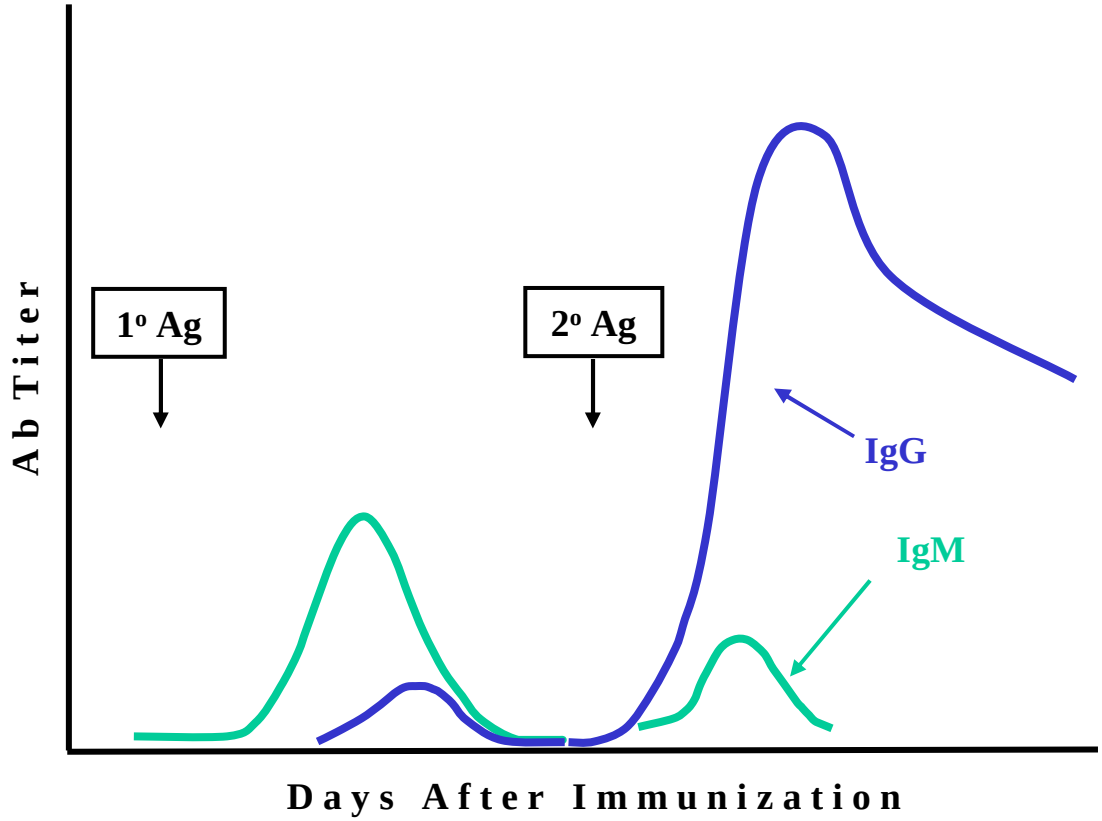
REPONSES B PRIMAIRES ET SECONDAIRES AUX Ag TD: ASPECTS QUANTITATIFS

- **amplitude de la réponse**
  - **– 1º - faible**
  - **– 2º - forte**



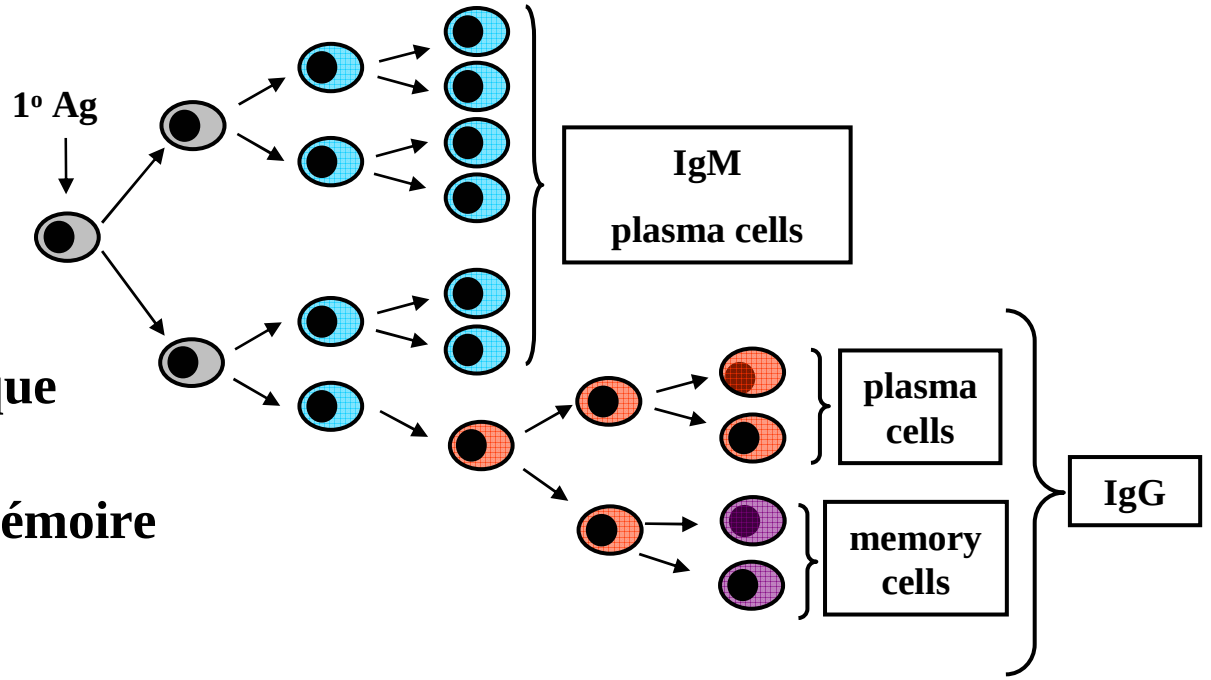
REPONSES B PRIMAIRES ET SECONDAIRES AUX Ag TD: ASPECTS QUALITATIFS

- **commutation isotypique**
  - **– 1º - IgM**
  - **– 2º - IgG, IgA or IgE**
- **affinité**
  - **– 1º - faible**
  - **– 2º - forte**



REPONSE PRIMAIRE AUX Ag TD

- **expansion clonale et différenciation (IgM)**



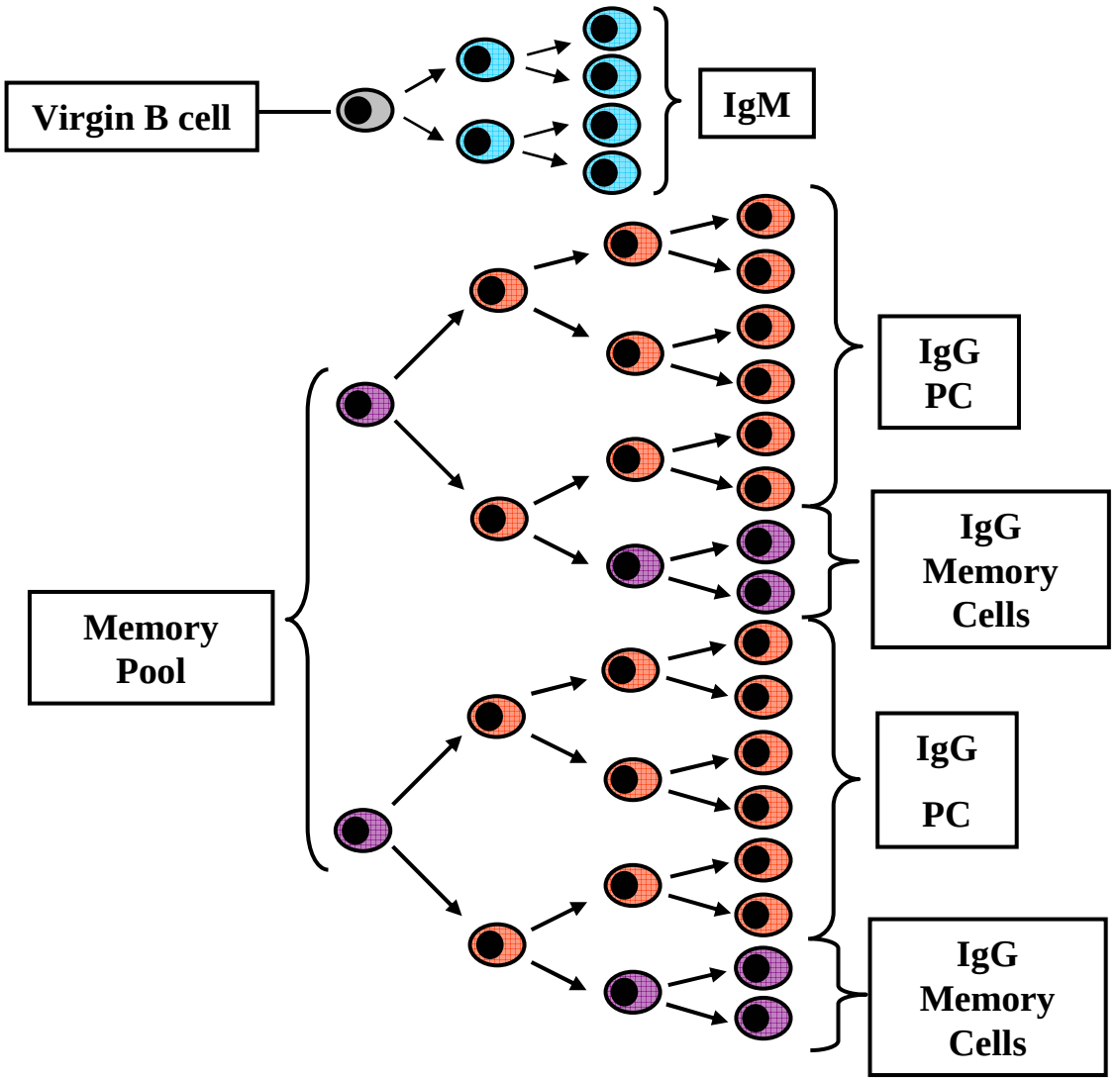
- **commutation isotypique**

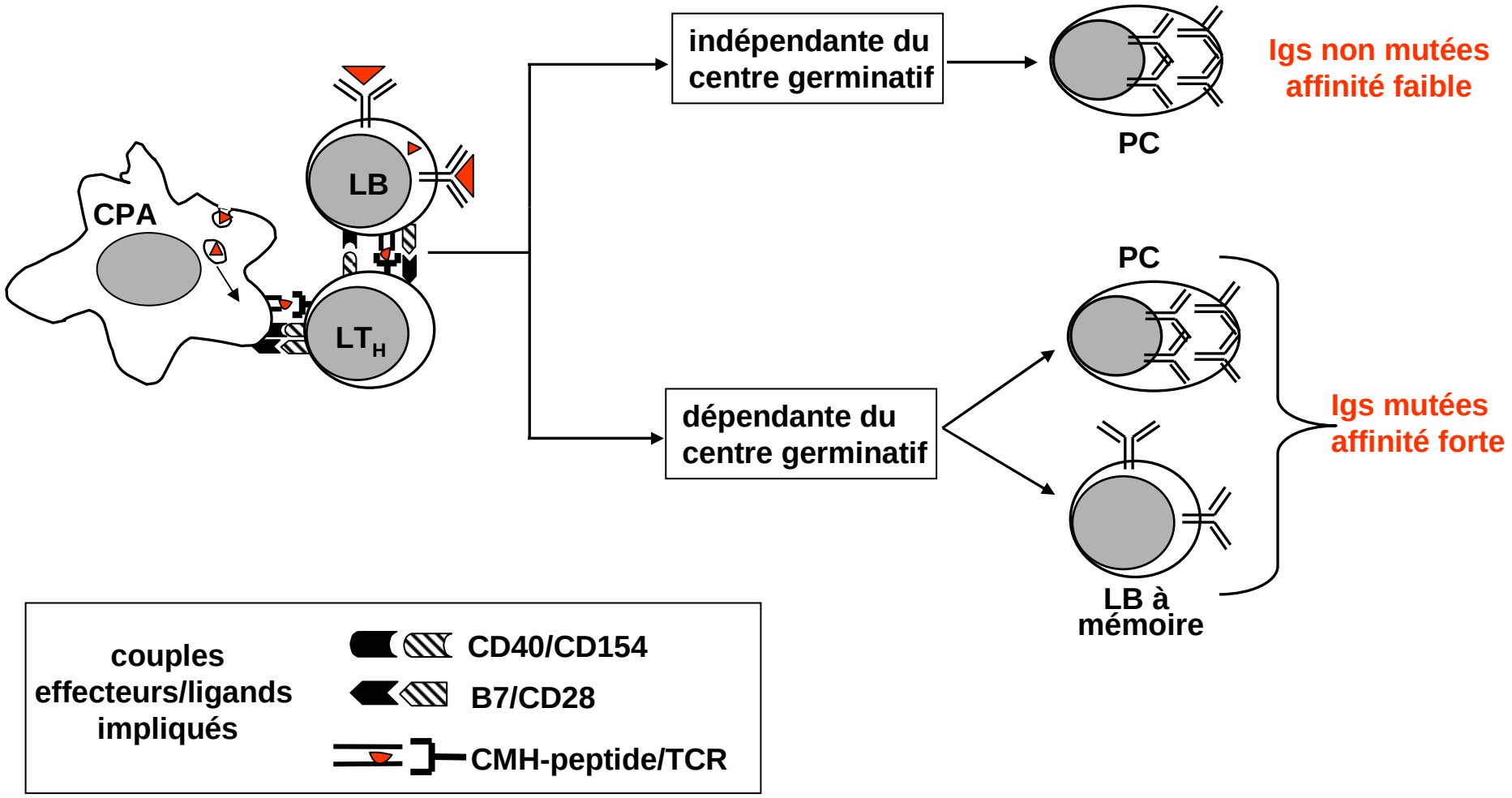
- **production de LB à mémoire**

REPONSE SECONDAIRE AUX Ag TD

-expansion et différenciation modeste des LB naïfs

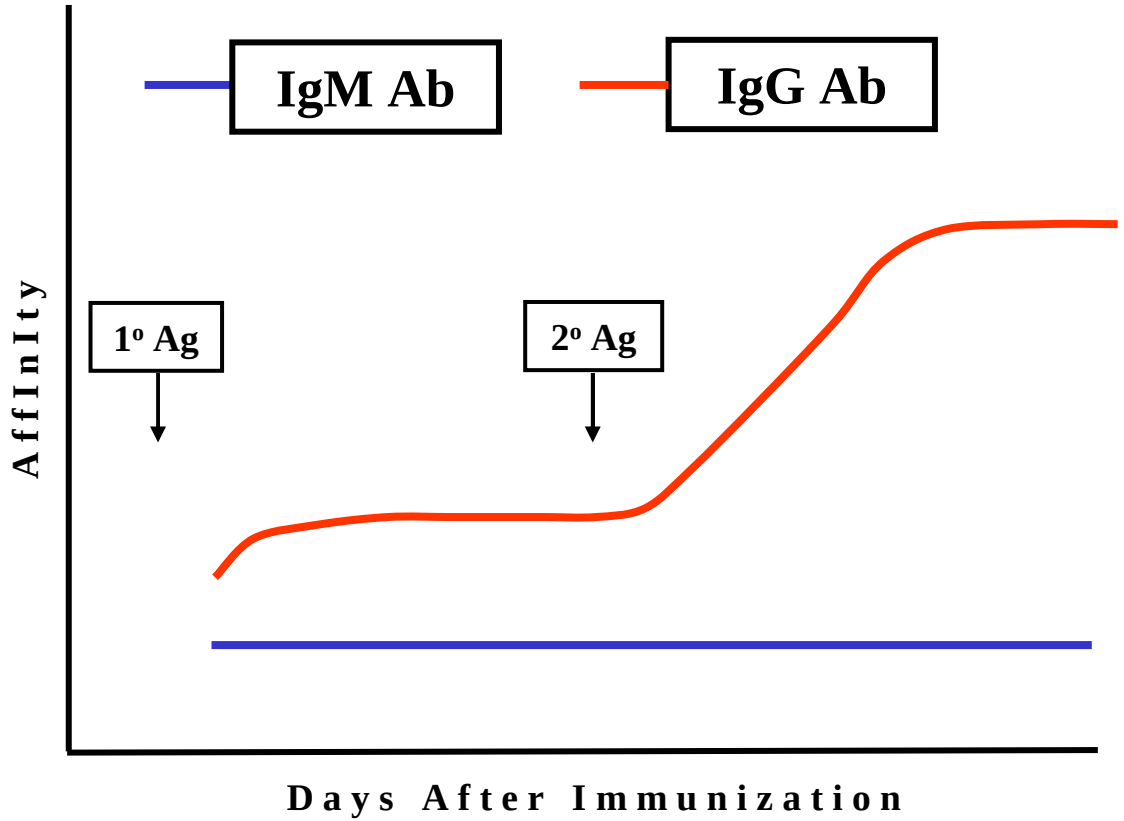
-expansion et différenciation massive des LB à mémoire



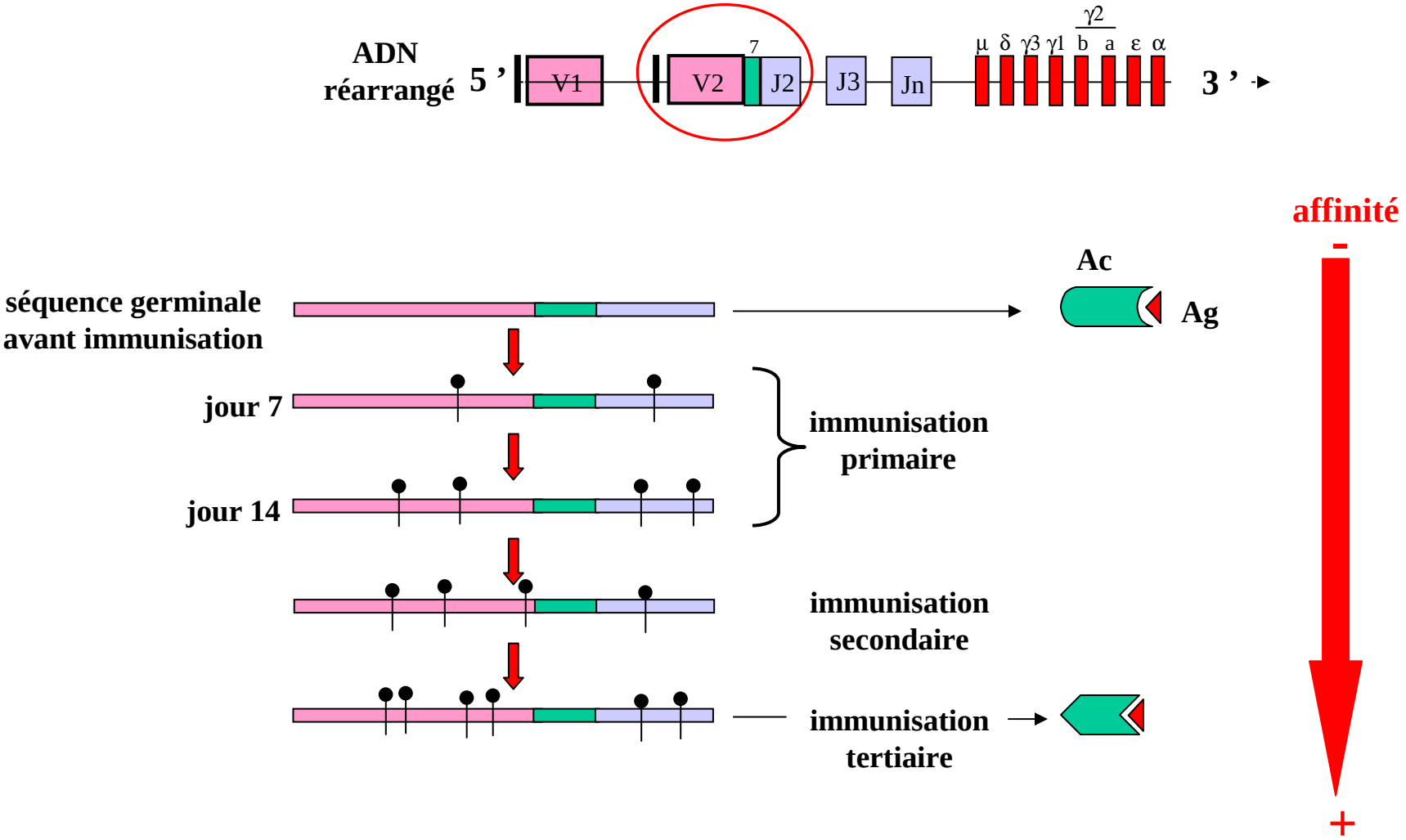


LES DEUX VOIES DE DIFFÉRENCIATION B EN RÉPONSE A UN Ag TD

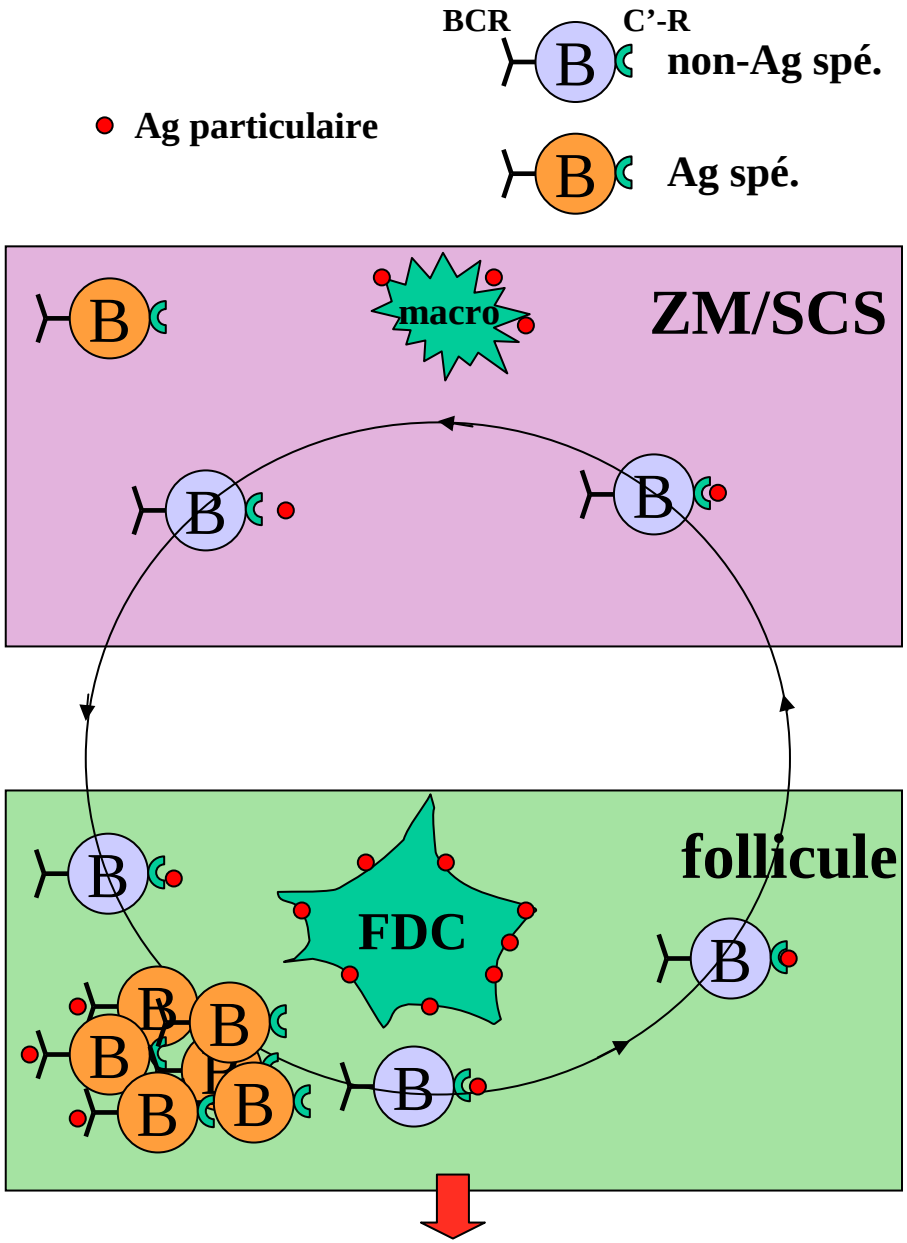
REPONSES B PRIMAIRES ET SECONDAIRES AUX Ag TD: LA MATURATION D'AFFINITE

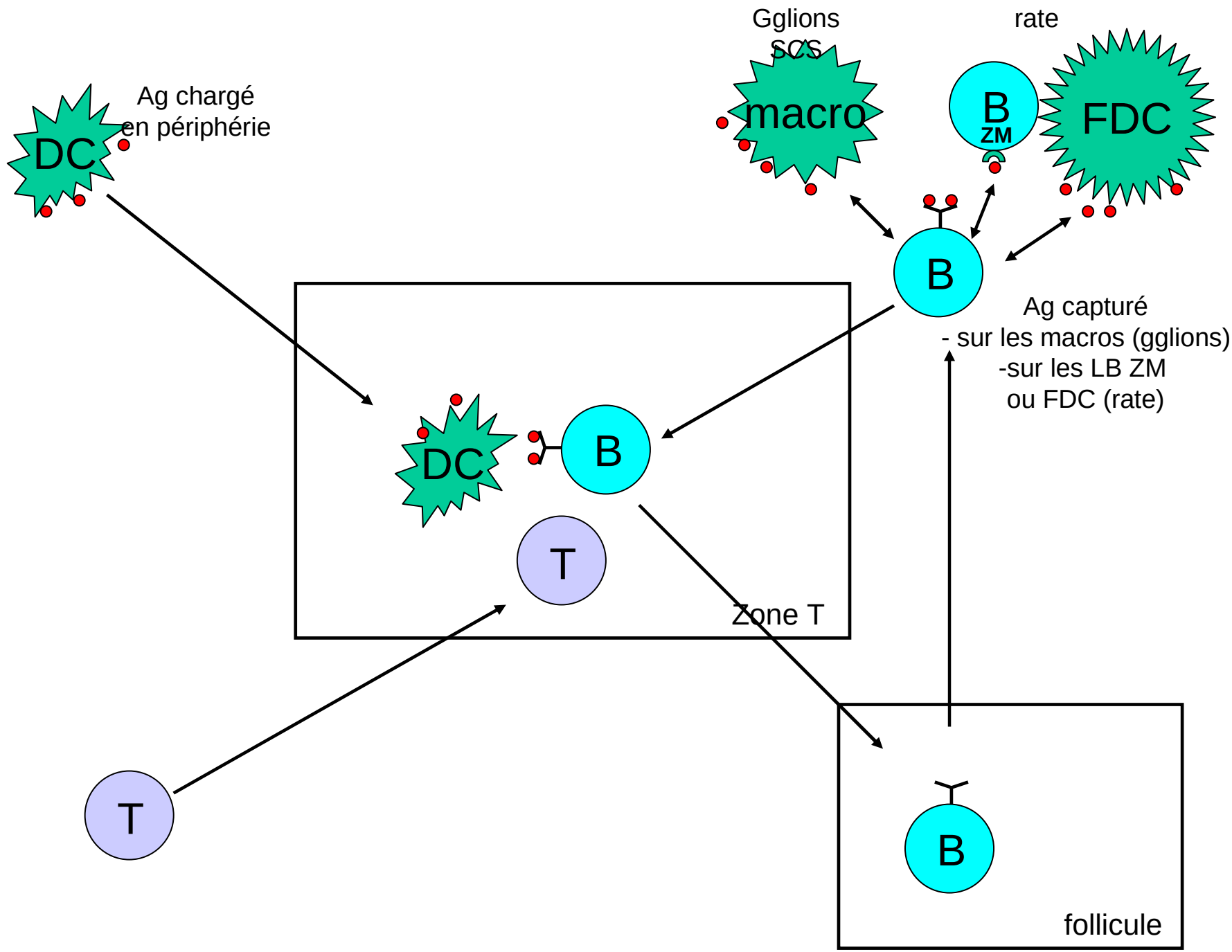


LA MATURATION D ’AFFINITÉ DES AcS PAR INTRODUCTION DE MUTATIONS PONCTUELLES (HYPERMUTATIONS SOMATIQUES)



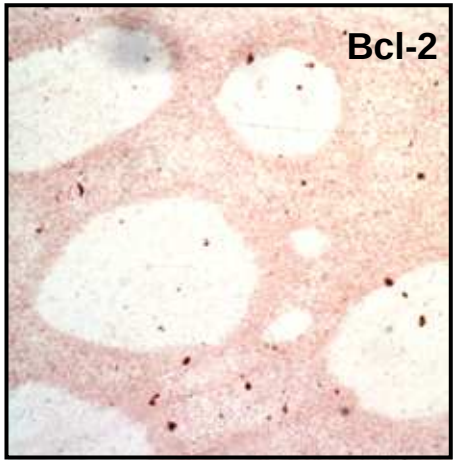
CAPTURE DE L'ANTIGENE PAR LES LYMPHOCYTES B


BCR
C'-R
non-Ag spé.
Ag particulaire
Ag spé.
ZM/SCS
macro
follicule
FDC
réponse TD

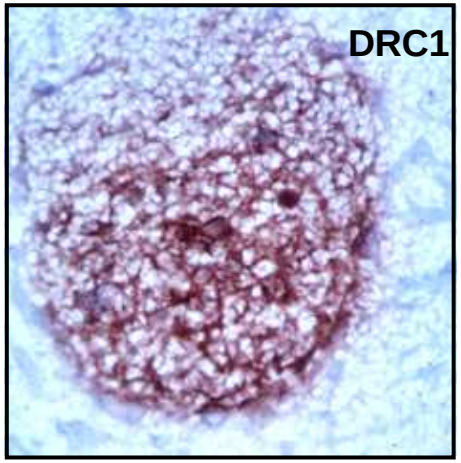

Gglions
SCS
rate
Ag chargé
en périphérie
DC
macro
B
ZM
FDC
B
Ag capturé
- sur les macros (gglions)
-sur les LB ZM
ou FDC (rate)
DC
B
T
Zone T
T
B
follicule

LA MATURATION D ’AFFINITÉ DE LA RÉPONSE ANTICORPS DANS LES CENTRES GERMINATIFS

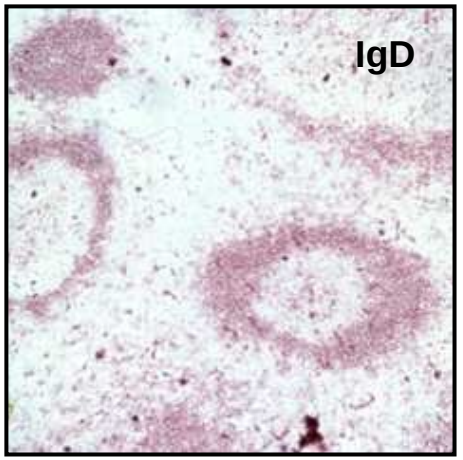
Apoptose



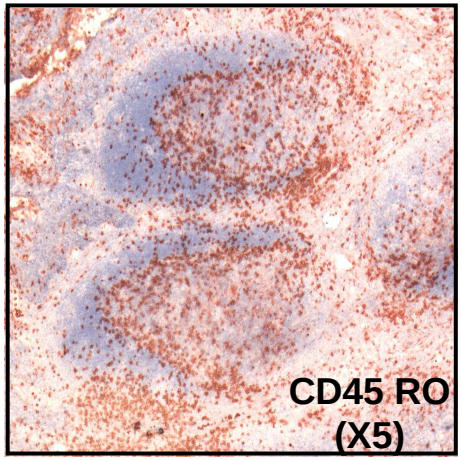
FDC

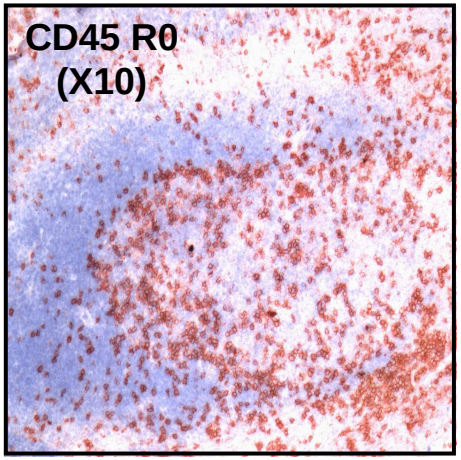


lymphocytes B naïfs



lymphocytes T





prolifération

QuickTime™ et un décompresseu
RVB Plan sont requis pour visualise
cette image.

IgD/Ki67

MARQUAGES IMMUNOHISTOLOGIQUES SUR COUPES D ’AMYGDALES

I. Le CG et le siège de deux processus cruciaux pour la réponse immune humoral 1. la maturation d ’affinité des Acs, 2. la génèse des LB à mémoire.

La plupart des mutations aboutissant à la disparition des CG s ’accompagnent d ’un déficit de différenciation des LB à mémoire et de production d ’Acs mutés de forte affinité.

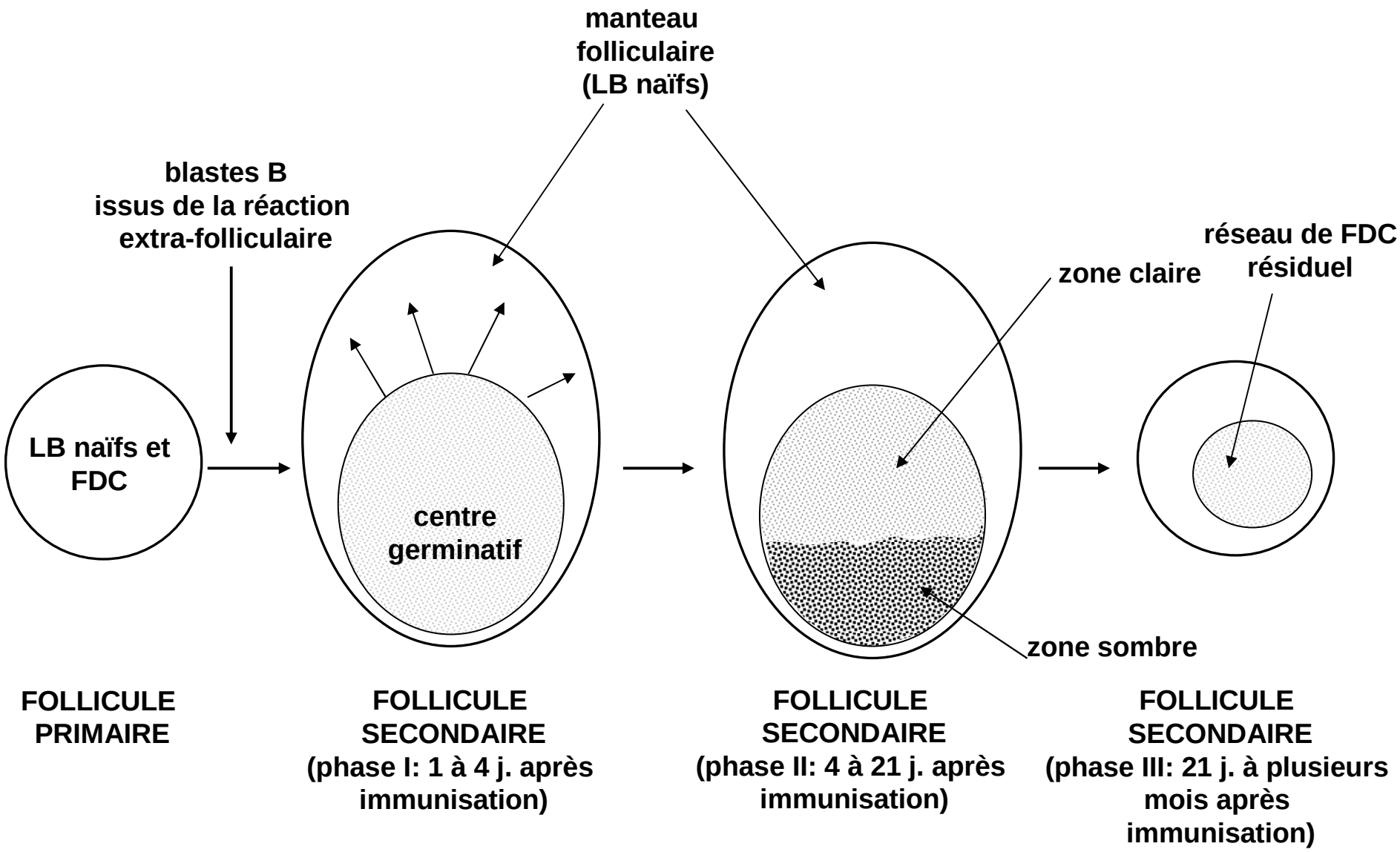
→ **Mais:**

1. il existe au moins 2 situations où les gènes V_H et V_L des LB sont hypermutés en l ’absence de formation des CG: a) les patients hyper IgM déficients pour l ’expression du CD40L, b)les souris KO pour Ltα.

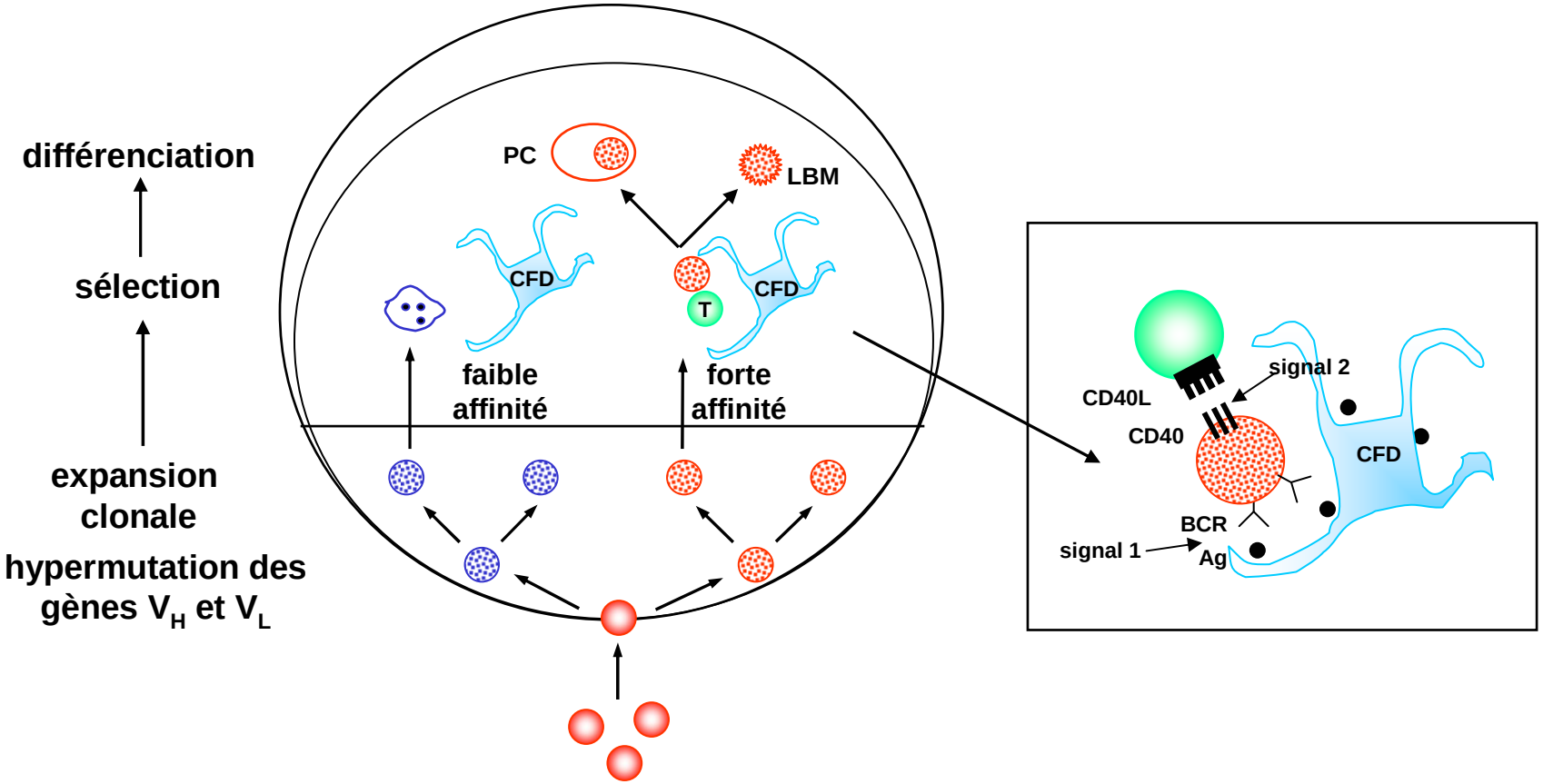
2. il existe des LB présentant les caractéristiques fonctionnelles des LB à mémoire chez les souris KO pour le répresseur de transcription bcl-6 ne formant pas de CG

II. Le CG abrite plusieurs lignages cellulaires:

1. des cellules hématopoïétiques: LB, LT CD4+, DC et macrophages
2. des cellules non-hématopoïétiques: les cellules dendritiques folliculaires (FDC

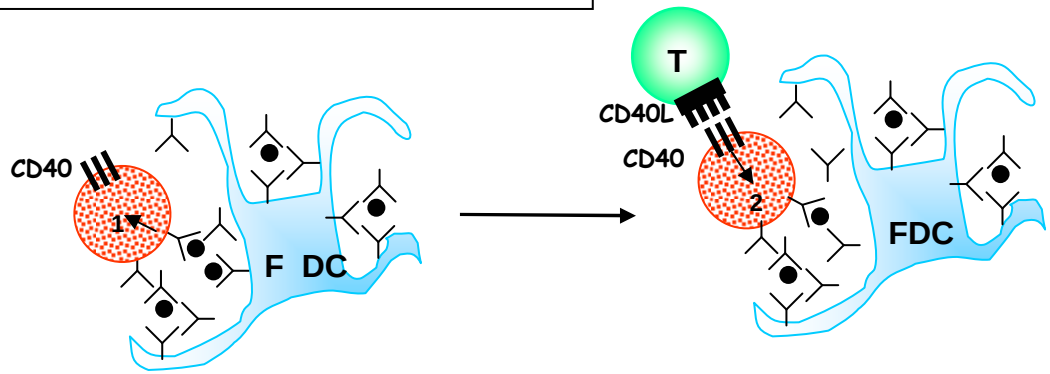


PHASES DE LA RÉACTION FOLLICULAIRE



DIVERSIFICATION ET SÉLECTION DU REPERTOIRE B DANS LES CENTRES GERMINATIFS

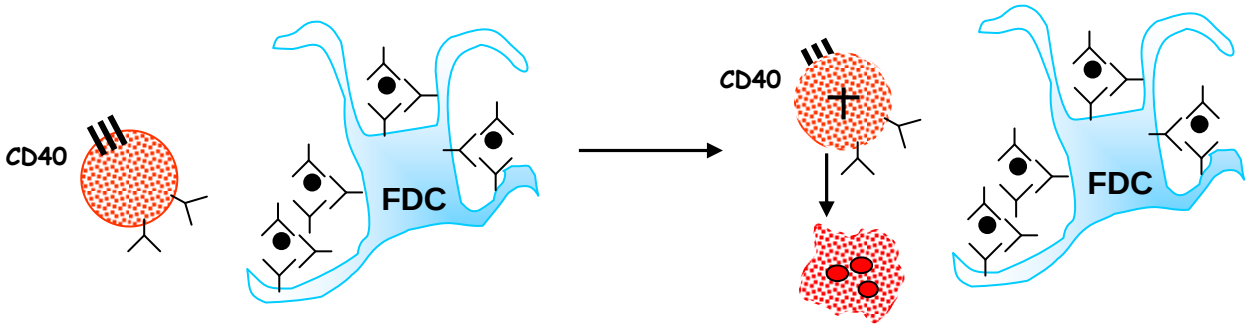
1. Mutant de forte affinité: affinité du BCR> celle des Acs des CI



- déplacement des Acs du complexe
- pontage du BCR par l 'Ag
- réception d 'un 1er signal de survie via le BCR
- capture et apprêtement de l'Ag

- présentation de l 'Ag aux LT dans un contexte MHC-restreint
- réception d 'un 2nd signal de survi via CD40

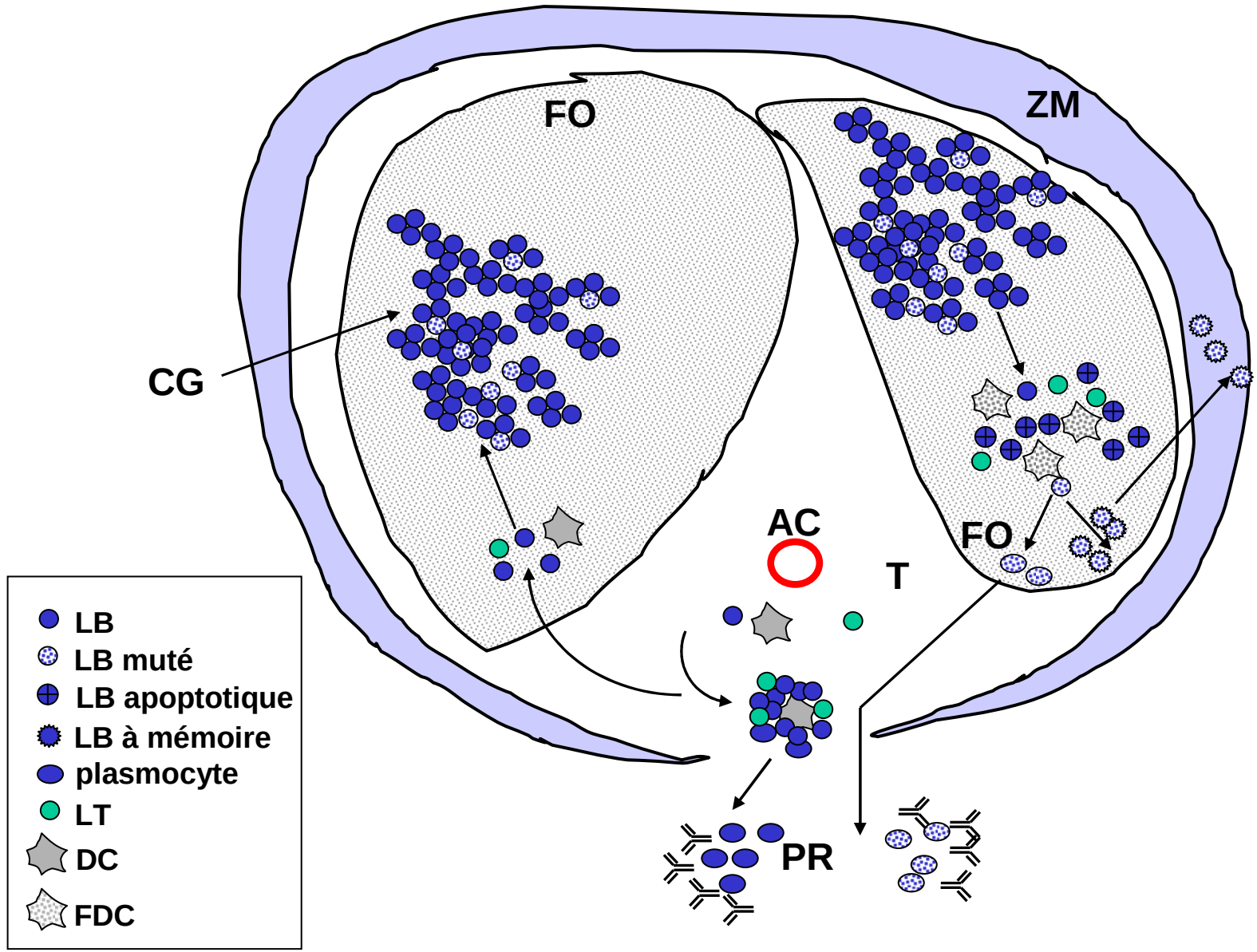
2. Mutant de faible affinité: affinité du BCR< ou = celle des Acs des CI



- signalisation faible ou absente via le BCR
- pas de signal de survie

exécution du programme apoptotique

MECANISME MOLÉCULAIRE DE LA SÉLECTION DES LB DANS LES CG (I)


FO
ZM
CG
AC
T
FO
PR
LB
LB muté
LB apoptotique
LB à mémoire
plasmocyte
LT
DC
FDC

LES CELLULES B À MÉMOIRE

CRITÉRES DE DÉFINITION DES LB A MÉMOIRE

1. PHÉNOTYPIQUES: **perte d ’expression des IgD**

2. FONCTIONNELS:

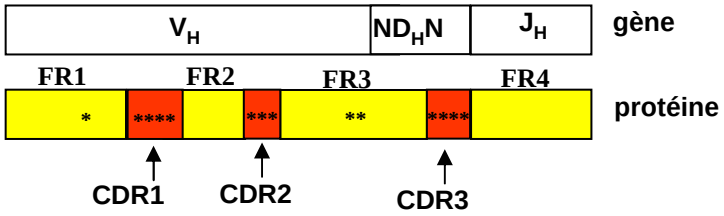
- capacité à générer une réponse Ac accélérée et de forte amplitude (susceptible de conférer une protection) après une injection de rappel

-cellule ayant proliféré à l’Ag et persistant à l’état quiescent dans les tissus lymphoïdes

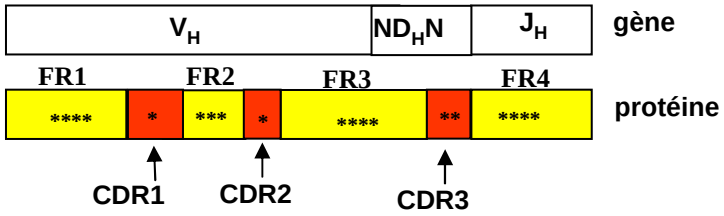
3. MOLÉCULAIRES: **gènes V_H et V_L mutés**

1. amplification de la région gènique VDJ
2. clonage des produits de PCR
3. séquençage
4. confrontation avec les banques de données contenant des séquences germinales

1. Fréquence et distribution des mutations somatiques



fréquence de mutations dans CDR>FR:
indice d 'une sélection par l 'antigène
ex: LB à mémoire



fréquence de mutations dans CDR<FR:
pas de sélection par l 'antigène
ex: LB du CG

2. Rapport mutations remplaçantes/mutations silencieuses

R: codon modifié avec changement d 'AA
S: codon modifié sans changement d 'AA
R/S >1= empreinte d 'une sélection antigènique

LB à mémoire:
1. gènes V_H/V_L mutés
2. mutations préférentiellement localisées dans les CDR
3. R/S >1

CRITÉRES MOLÉCULAIRES DE DÉFINITION DES LB A MÉMOIRE

Human Immunoglobulin (Ig)M$^+$IgD$^+$ Peripheral Blood B Cells Expressing the CD27 Cell Surface Antigen Carry Somatically Mutated Variable Region Genes: CD27 as a General Marker for Somatically Mutated (Memory) B Cells

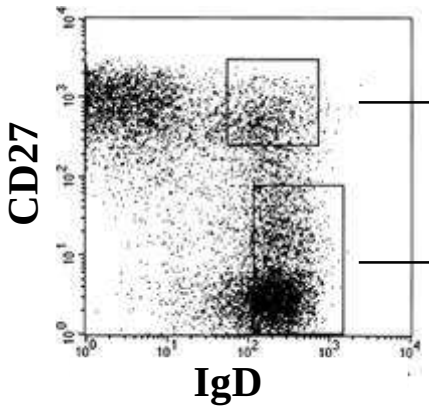
By Ulf Klein, Klaus Rajewsky, and Ralf Küppers

From the Institute for Genetics, University of Cologne, 50931 Cologne, Germany

Summary

Immunoglobulin (Ig)M$^+$IgD$^+$ B cells are generally assumed to represent antigen-inexperienced, naive B cells expressing variable (V) region genes without somatic mutations. We report here that human IgM$^+$IgD$^+$ peripheral blood (PB) B cells expressing the CD27 cell surface antigen carry mutated V genes, in contrast to CD27-negative IgM$^+$IgD$^+$ B cells. IgM$^+$IgD$^+$CD27$^+$ B cells resemble class-switched and IgM-only memory cells in terms of cell phenotype, and comprise ~15% of PB B lymphocytes in healthy adults. Moreover, a very small population (<1% of PB B cells) of highly mutated IgD-only B cells was detected, which likely represent the PB counterpart of IgD-only tonsillar germinal center and plasma cells. Overall, the B cell pool in the PB of adults consists of ~40% mutated memory B cells and 60% unmutated, naive IgD$^+$CD27$^-$ B cells (including CD5$^+$ B cells). In the somatically mutated B cells, V_H region genes carry a two- to threefold higher load of somatic mutation than rearranged V_κ genes. This might be due to an intrinsically lower mutation rate in κ light chain genes compared with heavy chain genes and/or result from κ light chain gene rearrangements in GC B cells. A common feature of the somatically mutated B cell subsets is the expression of the CD27 cell surface antigen which therefore may represent a general marker for memory B cells in humans.

Key words: B cell • CD27 • immunoglobulin D • memory B cell • somatic hypermutation



CD27+ → mutées

- M+D+ 15%
- M+D- 10%
- M-D+ < 1%
- M-D- (G+/A+) 15%

CD27- → non mutées

- M+D+ 60%

1. Tous les LB CD27+ sont mutés
2. Il existe des LB mutés non switchés M+D+
3. mutations somatiques et commutation isotypique dissociées

Le compartiment B à mémoire est hétérogène:

- fonctions biologiques différentes?
- induites en réponse à des Ag distincts?

Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire

Sandra Weller, Moritz C. Braun, Bruce K. Tan, Andreas Rosenwald, Corinne Cordier, Mary Ellen Conley, Alessandro Plebani, Dinakhanta S. Kumararatne, Damien Bonnet, Olivier Tournilhac, Gil Tchernia, Birte Steiniger, Louis M. Staudt, Jean-Laurent Casanova, Claude-Agnès Reynaud, and Jean-Claude Weill

The human peripheral B-cell compartment displays a large population of immunoglobulin M–positive, immunoglobulin D–positive CD27+ ($IgM^+IgD^+CD27^+$) "memory" B cells carrying a mutated immunoglobulin receptor. By means of phenotypic analysis, complementarity-determining region 3 (CDR3) spectratyping during a T-independent response, and gene-expression profiling of the different blood and splenic B-cell subsets, we show here that blood $IgM^+IgD^+CD27^+$ cells correspond to circulating splenic marginal zone B cells. Furthermore, analysis of this peripheral subset in healthy children younger than 2 years shows that these B cells develop and mutate their immunoglobulin receptor during ontogeny, prior to their differentiation into T-independent antigen-responsive cells. It is therefore proposed that these $IgM^+IgD^+CD27^+$ B cells provide the splenic marginal zone with a diversified and protective preimmune repertoire in charge of the responses against encapsulated bacteria. (Blood. 2004;104:3647-3654)

Introduction

The human peripheral B-cell compartment displays, in contrast to the mouse, a large population of CD27+ memory B cells that carry a mutated immunoglobulin receptor and represent up to 40% of circulating B cells. These memory B cells include the classical isotype-switched B cells and a population of immunoglobulin M–positive (IgM^+) B cells, which were originally divided into an IgM-only subset, an IgM^+IgD^+ subset, and a minor IgD-only subpopulation.[1-4]

The splenic marginal zone (SMZ) is a unique B-cell compartment that contains B cells with a high surface density of IgM and complement receptor 2 (Cr2 or CD21), and which exhibits a rapid activation and immunoglobulin secretion in response to blood-borne T-independent (TI) antigens.[5-7] Human SMZ B cells have been shown to carry somatic mutations, and mutated antibodies can be raised after immunization with T-independent polysaccharidic vaccines.[8-11]

We hypothesized previously that blood $IgM^+IgD^+CD27^+$ B cells might form a B-cell subset distinct from the classical germinal center–derived memory B cells. This proposition was based on the fact that hyper-IgM (HIGM) patients who carry an invalidating mutation of the *CD40L* gene and do not possess normally developed germinal centers and switched memory B cells[12] still presented a subpopulation of circulating $IgM^+IgD^+CD27^+$ B cells.[13,14] These B cells, moreover, carried a mutated immunoglobulin receptor, which led us to suggest that they could represent a different pathway of diversification that did not require a cognate T-B interaction and could thus be involved in T-independent immune responses.[14] By means of phenotypic analysis, complementarity-determining region 3 (CDR3) spectratyping during a T-independent vaccination, and gene-expression profiling of the different blood and splenic B-cell subsets, we show here that the blood $IgM^+IgD^+CD27^+$ B cells indeed correspond to circulating splenic marginal zone B cells in charge of T-independent responses, which is in accordance with a recent report[15] and our previous proposition.

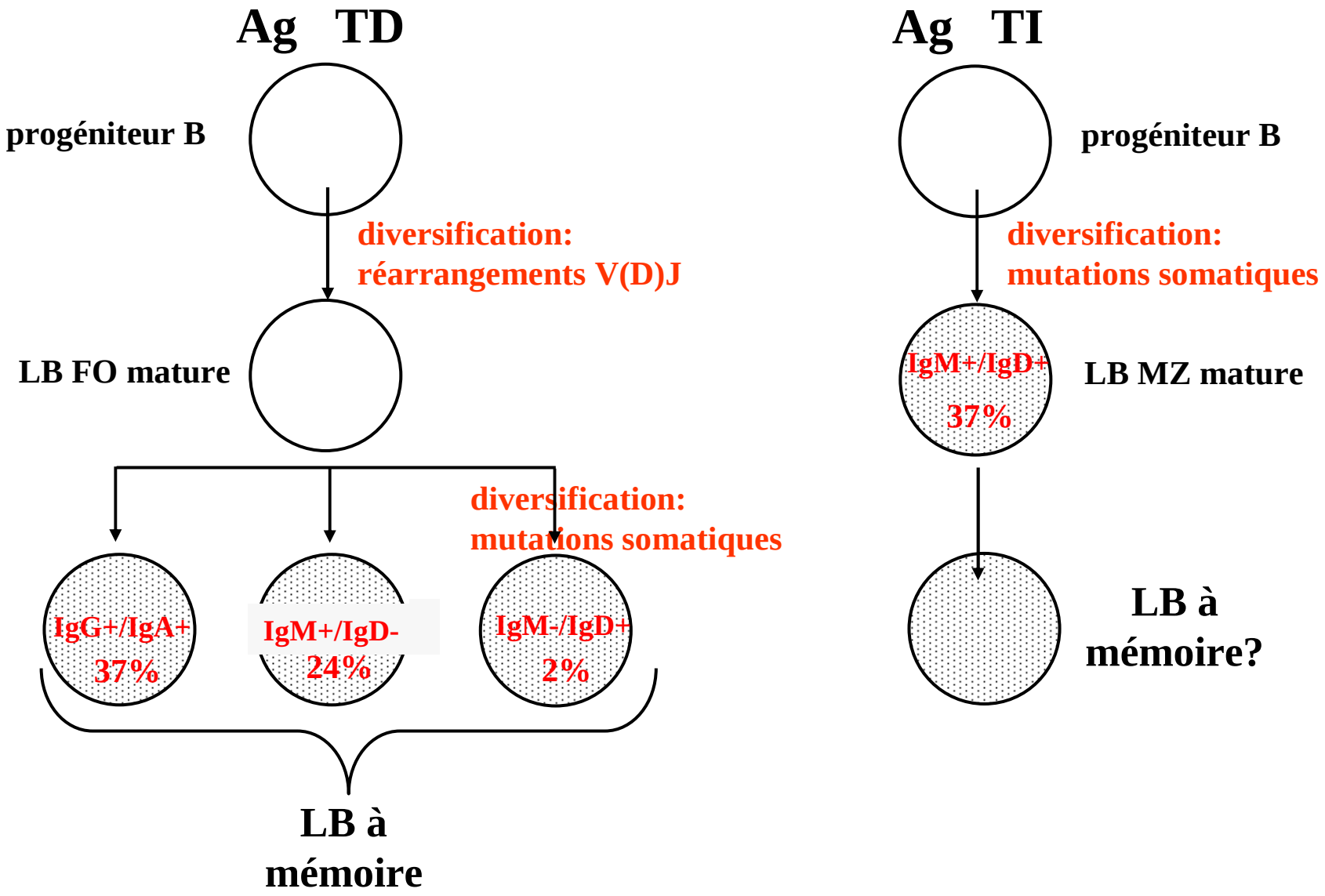
Materials and methods

Biologic samples

Fresh spleen samples were obtained from patients undergoing splenectomy owing to spherocytosis. Blood and spleen samples were obtained after

From the Institut National de la Santé et de la Recherche Médicale (INSERM) U373; Actions Concertées Incitatives (ACI) "Microbiologie, maladies infectieuses et

LES MUTATIONS SOMATIQUES COMME INSTRUMENT DE DIVERSIFICATION DU REPERTOIRE POST-IMMUN ET PRE-IMMUN



CRITÉRES DE DÉFINITION DES LB A MÉMOIRE

1. PHÉNOTYPIQUES:

a) perte d ’expression des IgD et expression du marqueur CD27
b) perte d’expression d’un transporteur ABCB1/efflux de drogues hors de la cellul

→ CD27 est aussi exprimé sur les LB naïfs de la ZM chez l’Homme

→ pas applicable chez la souris

2. FONCTIONNELS: capacité à générer une réponse Ac accélérée et de forte amplitude (susceptible de conférer une protection) après une injection de rappel

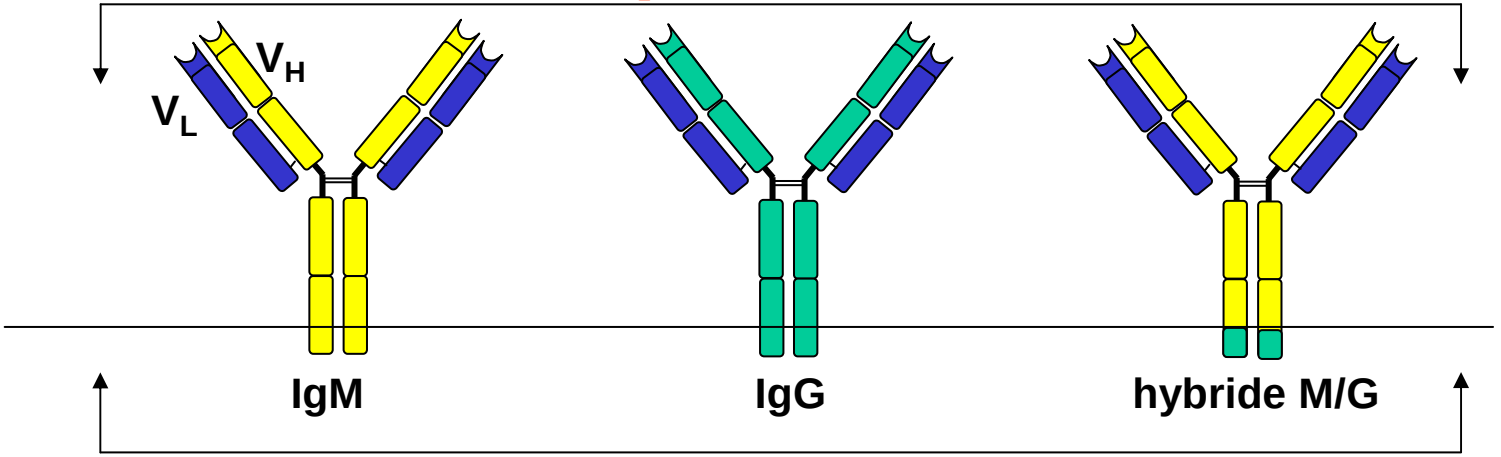
→ nécessite l ’isolement et le transfert adoptif de sous-populations B

3. MOLÉCULAIRES: gènes V_H et V_L mutés

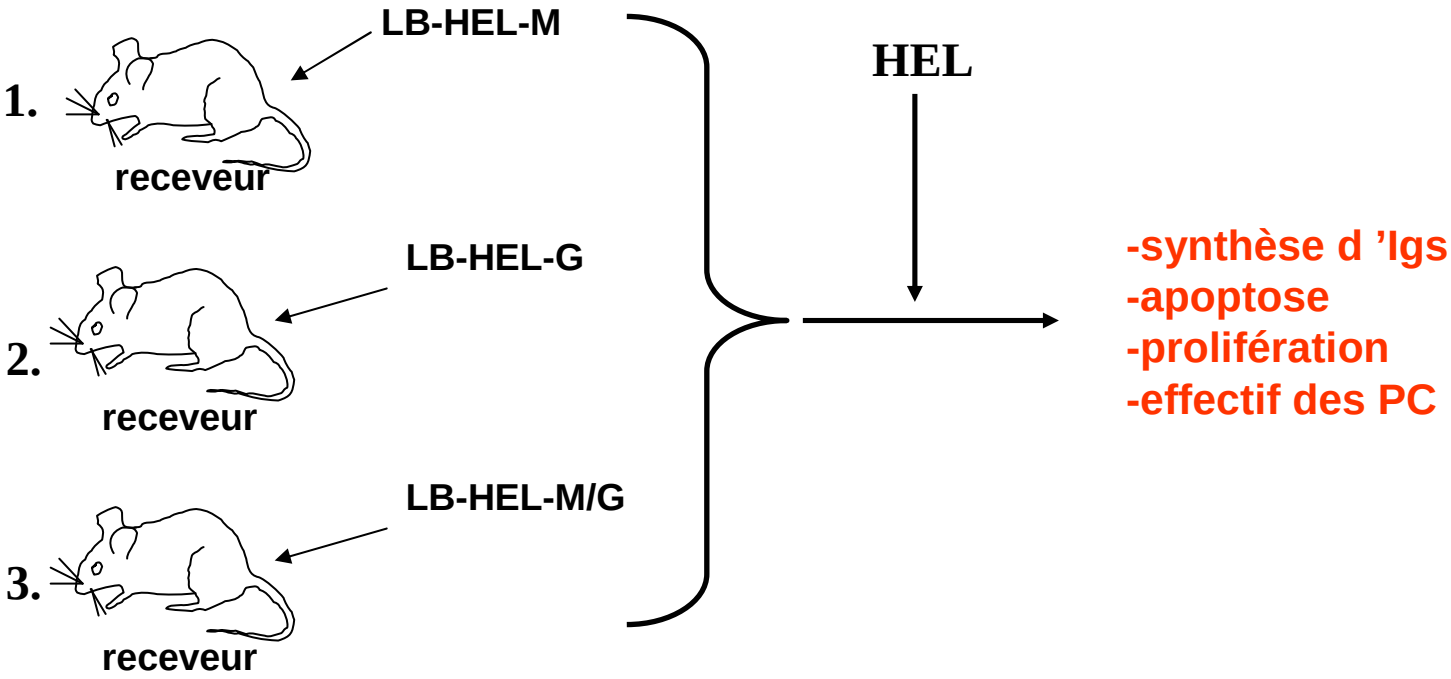
→ le processus de mutations somatiques peut être utilisé pour la diversification du répertoire B préimmun des LB de la ZM chez l’Homme

PROPRIETES FONCTIONNELLES DES LYMPHOCYTES B A MEMOIRE

mêmes VJ et VDJ codant pour un BCR anti-HEL de forte affinité



portions intracytoplasmiques différentes

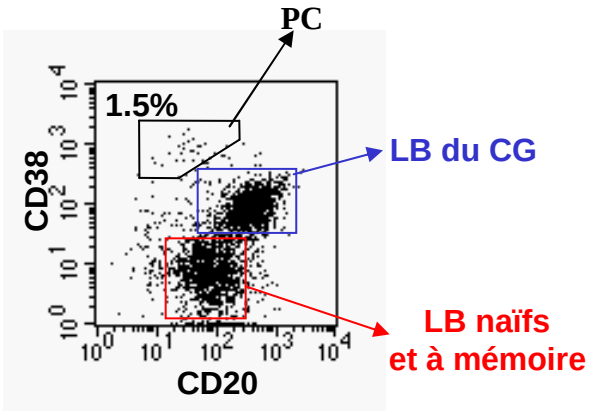


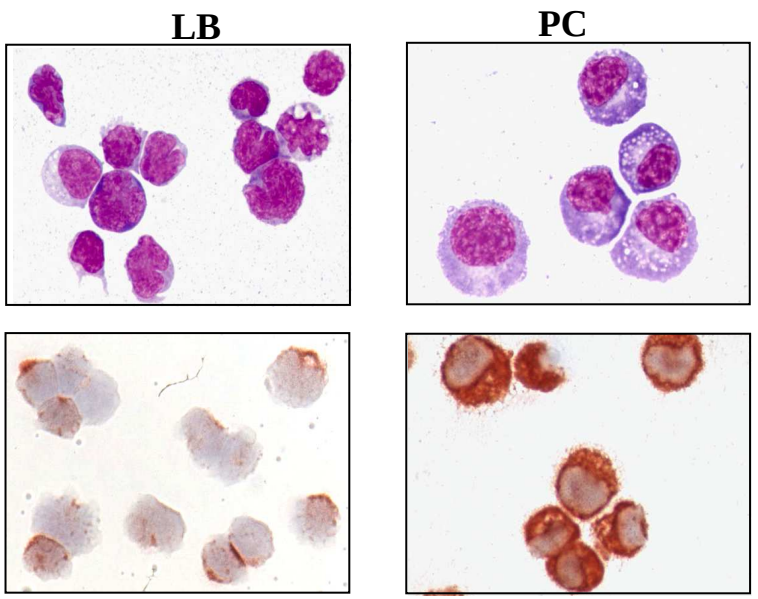
		Ac sériques anti-HEL	ASC anti-HEL	nombres de LB anti-HEL
1.	 LB-HEL-M receveur	+	+	+
2.	 LB-HEL-G receveur	++++	++++	++++
3.	 LB-HEL-M/G receveur	++++	++++	++++

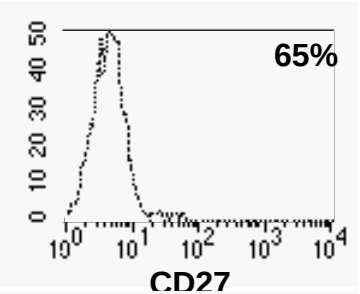
la portion cytoplasmique des IgG confère une plus grande capacité d 'expansion aux LB. Elle agirait en limitant l 'apoptose cellulaire au cours des divisions succcessives

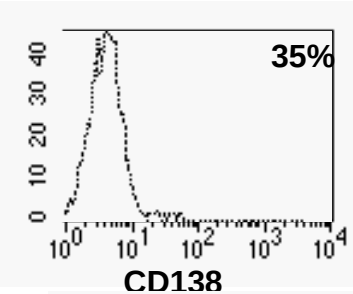
LA PORTION CYTOPLASMIQUE DES IgG EST RESPONSABLE D 'UNE PARTIE DES CARACTÉRISTIQUES FONCTIONNELLES DES LB A MÉMOIRE

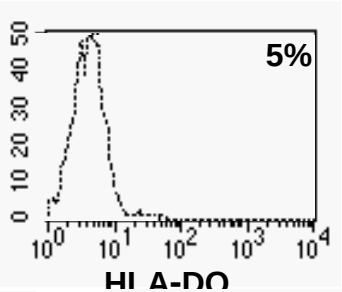
LES CELLULES B EFFECTRICES

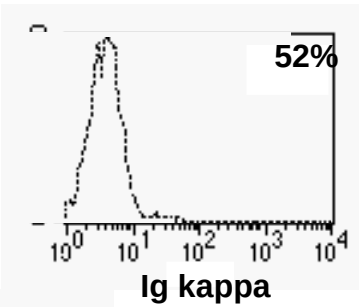


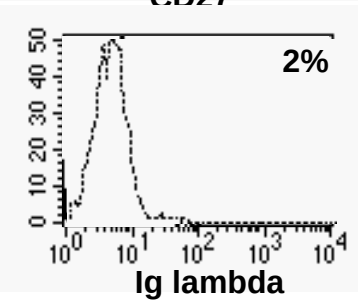


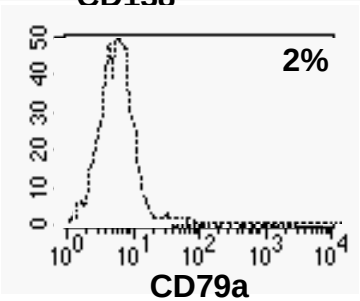


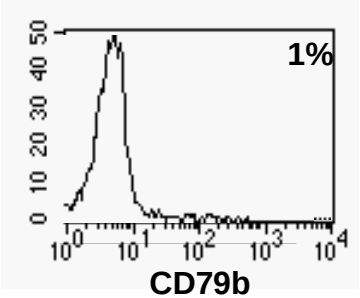








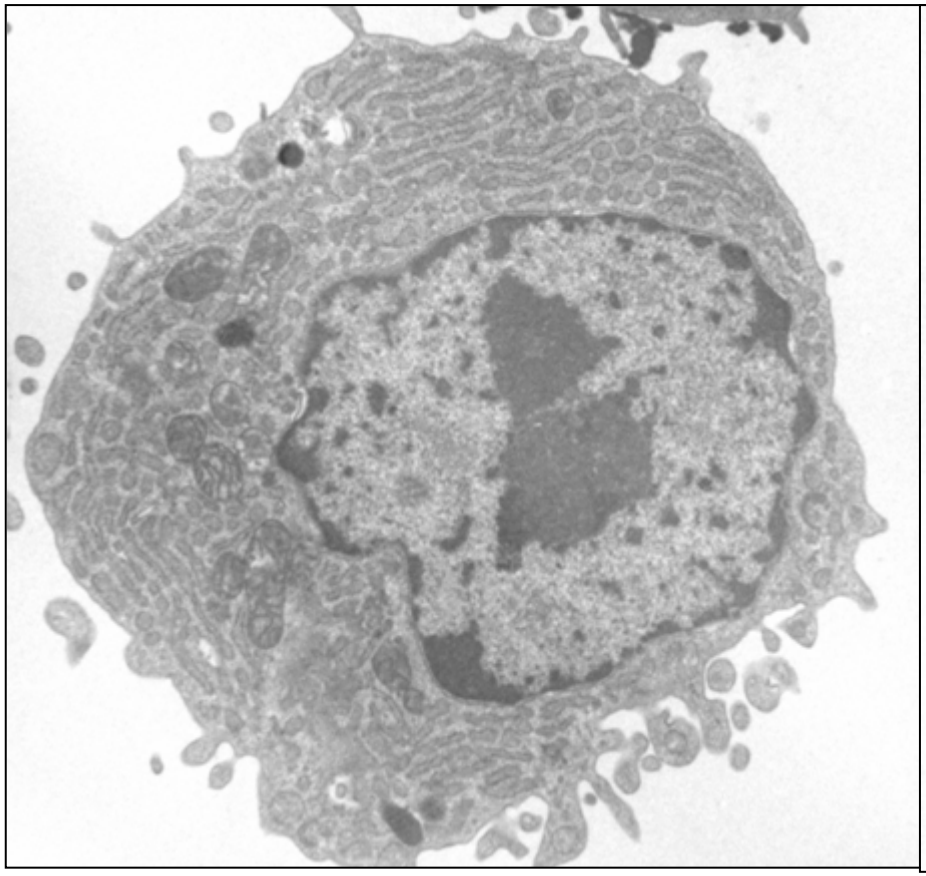


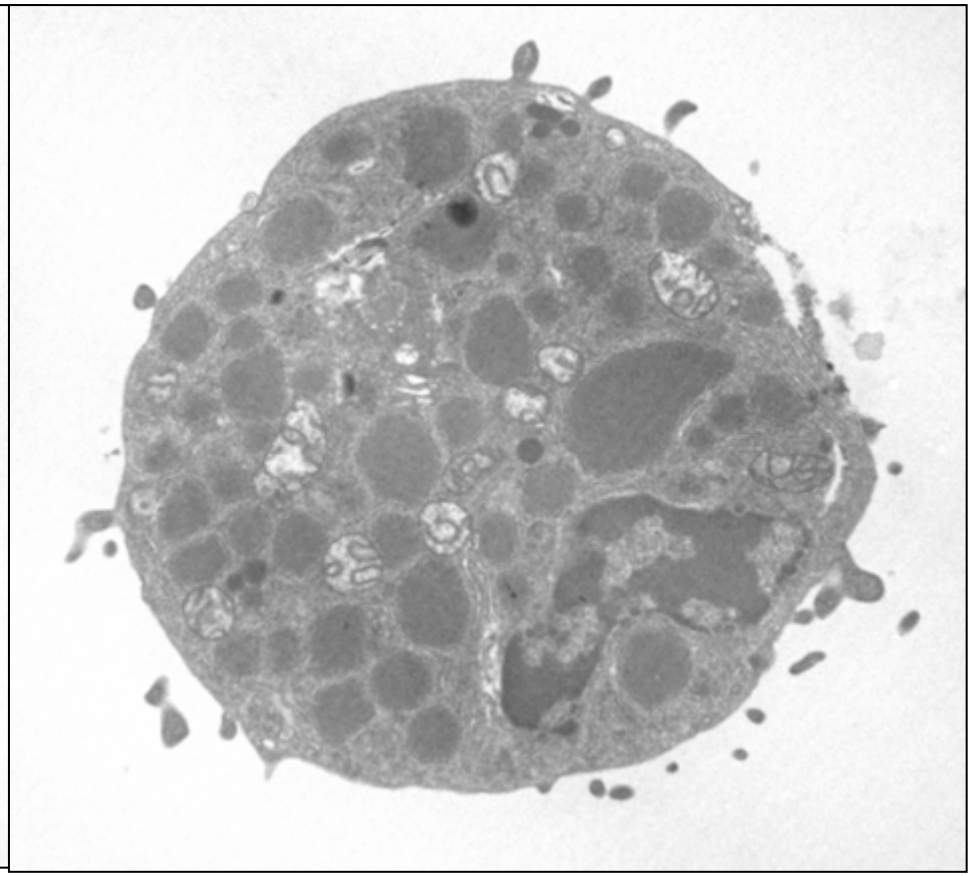


CARACTÉRISTIQUES PHÉNOTYPIQUES ET MORPHOLOGIQUES DES CELLULES PLASMOCYTAIRES

PC

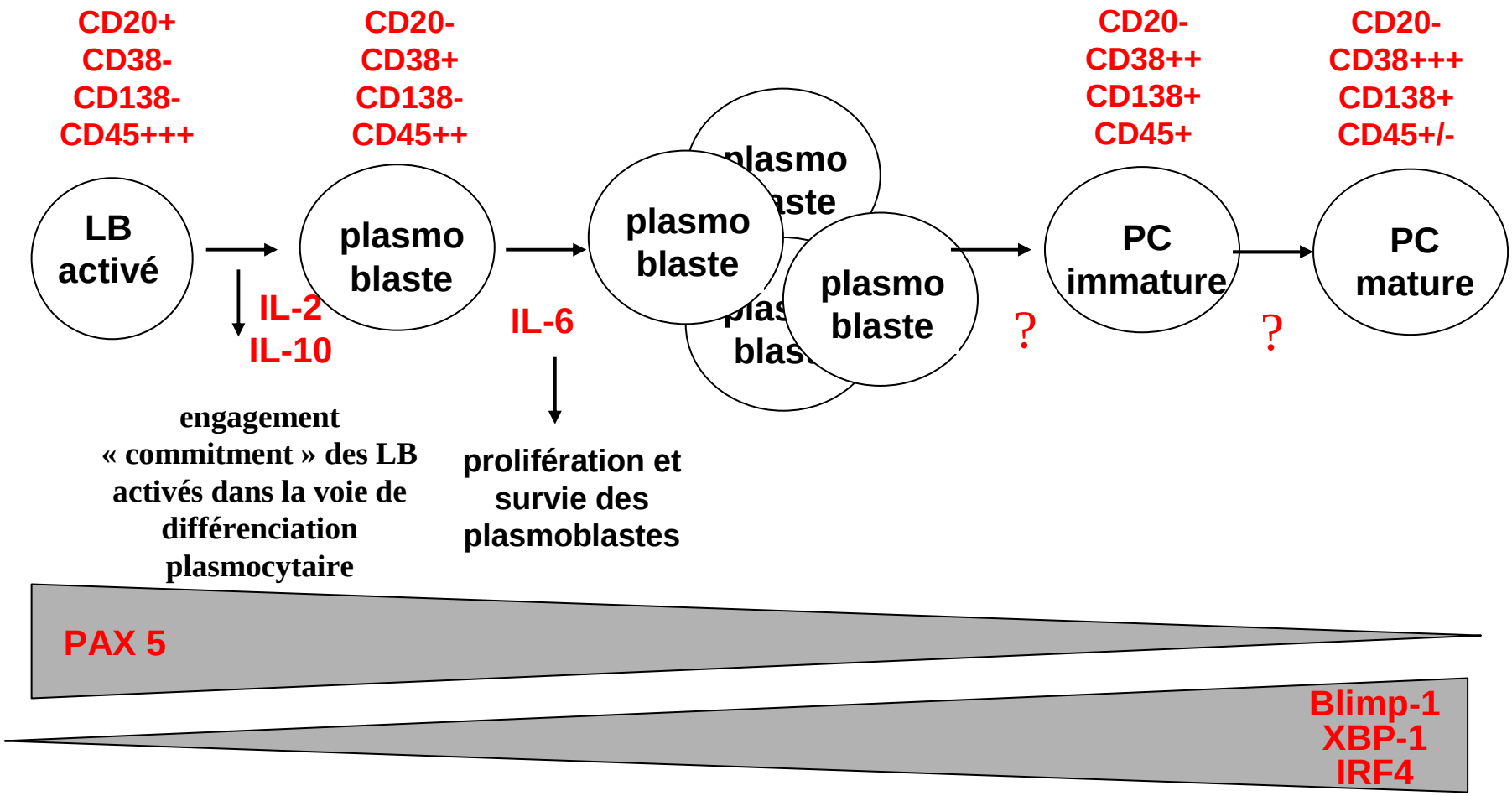
Mott Cell





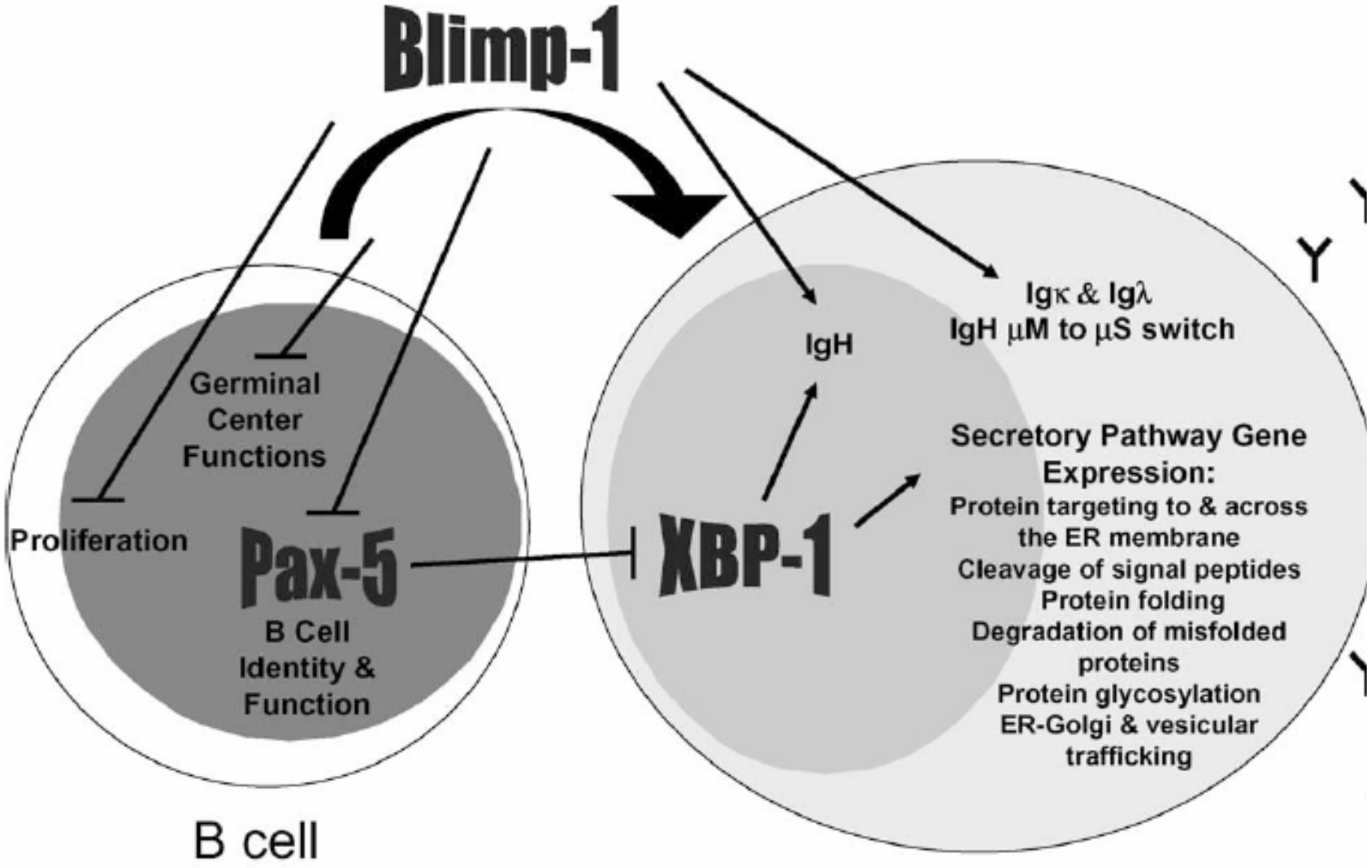
PC with Russel bodies

LA DIFFÉRENCIATION PLASMOCYTAIRE



Autres facteurs potentiellement impliqués:

1. Le couple OX40/OX40L : le blocage de cette interaction inhibe la diff. plasmo dans les foyers extra folliculaires
2. Le couple CD27/CD70: des Tf CD70 potentialisent la différenciation plasmocytaire in vitro
3. Les TLR-L


Blimp-1
Germinal
Center
Functions
Proliferation
Pax-5
B Cell
Identity &
Function
IgH
XBP-1
Igκ & Igλ
IgH μM to μS switch
Secretory Pathway Gene
Expression:
Protein targeting to & across
the ER membrane
Cleavage of signal peptides
Protein folding
Degradation of misfolded
proteins
Protein glycosylation
ER-Golgi & vesicular
trafficking
B cell

Immunity, Vol. 8, 363–372, March, 1998, Copyright ©1998 by Cell Press

Humoral Immunity Due to Long-Lived Plasma Cells

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Atlanta, Georgia 30322

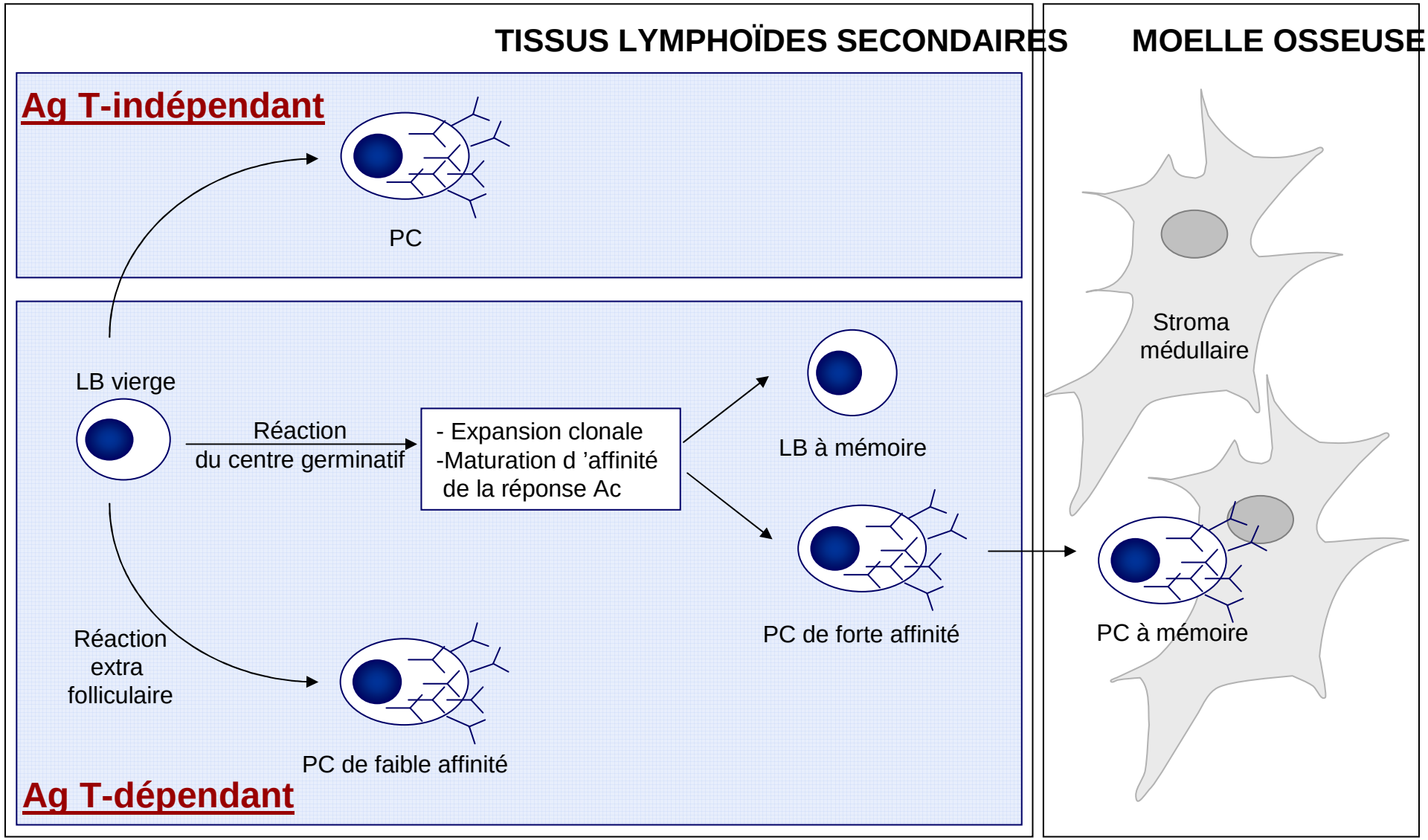
Summary

Conventional models suggest that long-term antibody responses are maintained by the continuous differentiation of memory B cells into antibody-secreting plasma cells. This is based on the notion that plasma cells are short-lived and need to be continually replenished by memory B cells. We examined the issue of plasma cell longevity by following the persistence of LCMV-specific antibody and plasma cell numbers after in vivo depletion of memory B cells and by adoptive transfer of virus-specific plasma cells into naive mice. The results show that a substantial fraction of plasma cells can survive and continue to secrete antibody for extended periods of time (>1 year) in the absence of any detectable memory B cells. This study documents the existence of long-lived plasma cells and demonstrates a

unlike memory B cells, mature plasma cells are unlikely to participate in antigen processing and presentation. Instead, the main function of a plasma cell is to continuously secrete large quantities of specific antibody. Plasma cell secretion rates have been estimated to be as high as 10,000 molecules/second (Helmreich et al., 1961; Hibi and Dosch, 1986). In contrast, memory B cells do not spontaneously secrete antibody; rather, following appropriate stimulation, these cells proliferate and differentiate into antibody-secreting cells (ASC). Because protection against microbial infection often relies on the level of preexisting antibody in the serum or mucosal surfaces, the number and specificity of preexisting plasma cells are critical components of protective immunity.

The mechanisms underlying long-term antibody production are not fully understood, but the conventional model postulates that the maintenance of serum antibody requires the continuous proliferation and differentiation of memory B cells into antibody-secreting plasma cells (Szakal et al., 1989; Tew et al., 1990; MacLennan et al., 1992; Stites et al., 1994; Gray et al., 1996; Zinkernagel et al., 1996). This model is based on the belief that plasma cells are short-lived. Although an early study (Miller, 1964) had suggested that some plasma cells may survive for several months, current immunological dogma holds that plasma cells have a half-life of only

Développement des LB lors de la réponse immunitaire

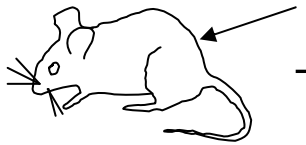


PC à courte durée de vie

PC à longue durée de vie

1. Le modèle expérimental

immunisation

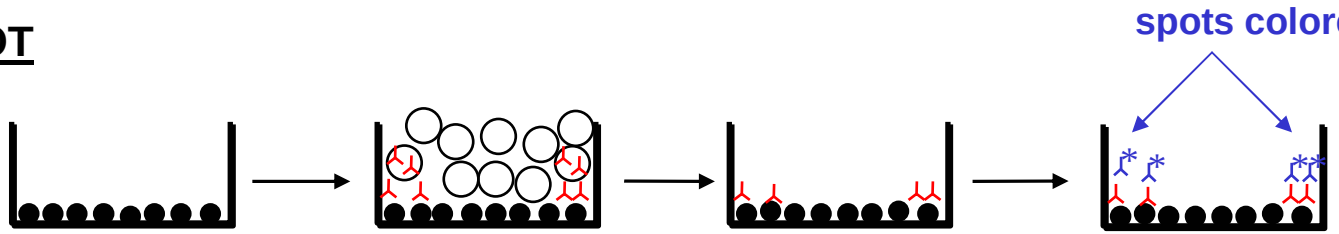


LCMV

LBM: sécrétion d ’Acs après réexposition à l ’Ag in vitro

PC: sécrétion spontanée d ’Acs sans réexposition à l ’Ag

ELISPOT



adsorption de l’Ag sur une membrane de nitrocellulose

addition des cellules

élimination des cellules

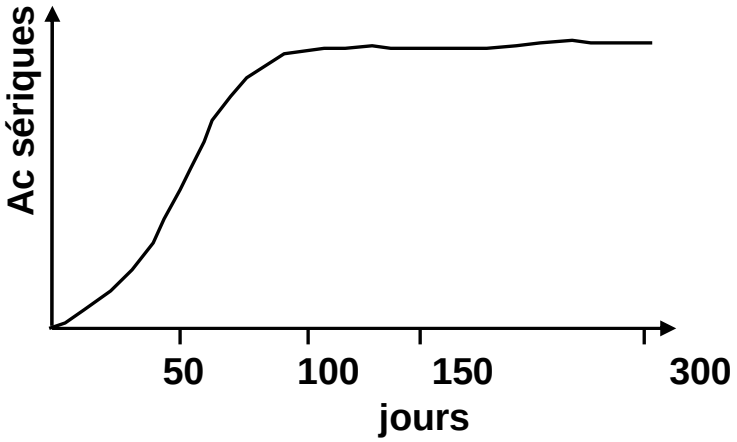
addition d ’un système de révélation

numération des « spots » colorés
estimation de la fréquence des cellules sécrétrices

LES PC À LONGUE DURÉE DE VIE (I)

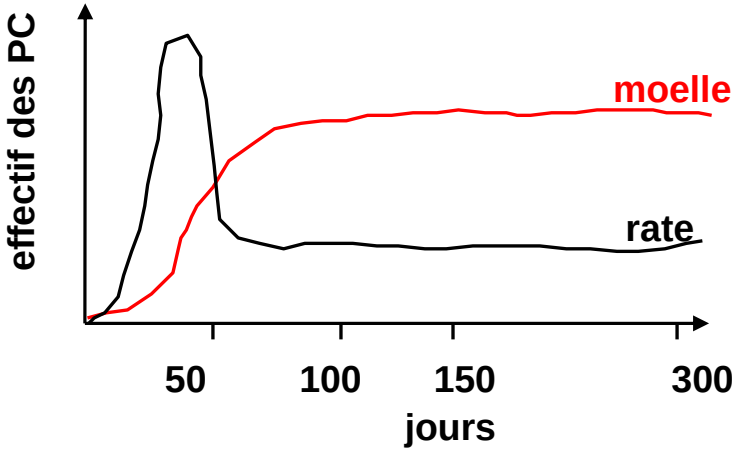
Slifka et Ahmed (Immunity, 8: 363, 1998)

2. Résultats expérimentaux (I)



le taux d 'Acs sériques après immunisation LCMV reste stable au-delà de 300 jours alors que la 1/2 vie des Acs sériques est de 20 jours

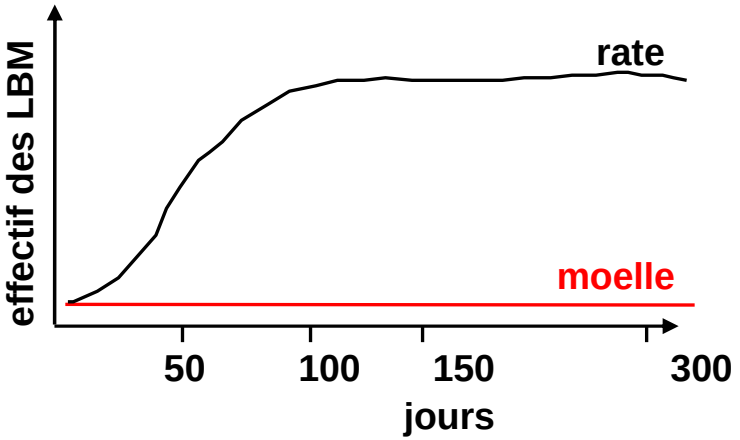
→ Il existe un mécanisme assurant le maintien à long terme du taux d 'Acs sériques



après immunisation, les PC apparaissent successivement dans la rate puis dans la moelle. Leur effectif se maintient à long terme dans la moelle

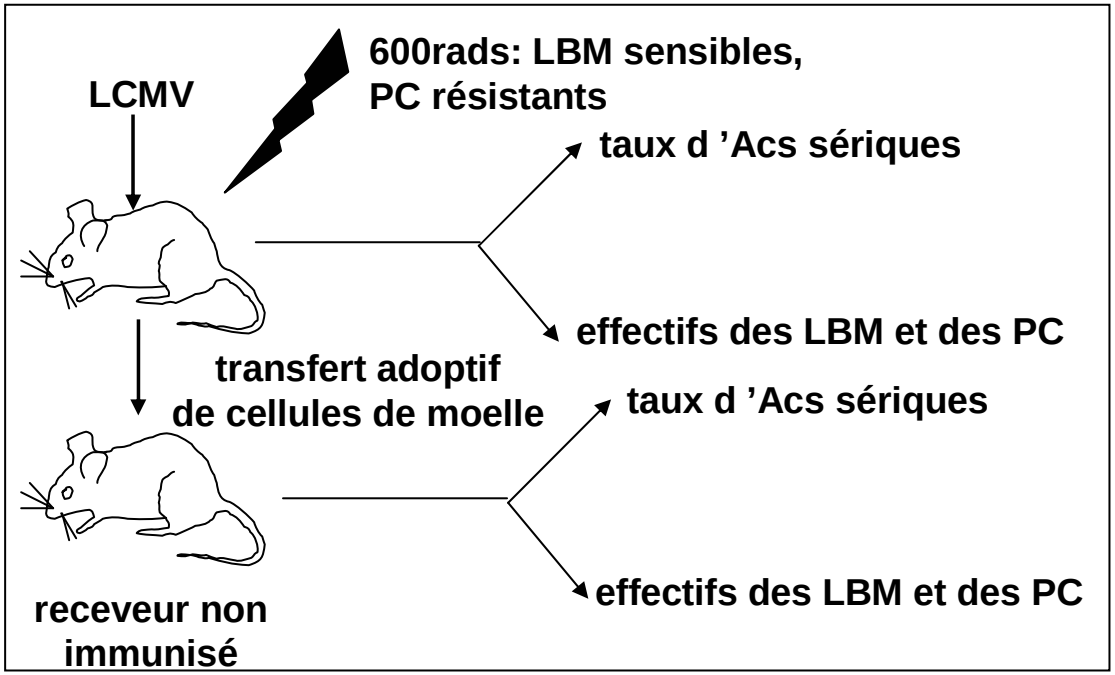
Comment se maintient l 'effectif des PC médullaires?

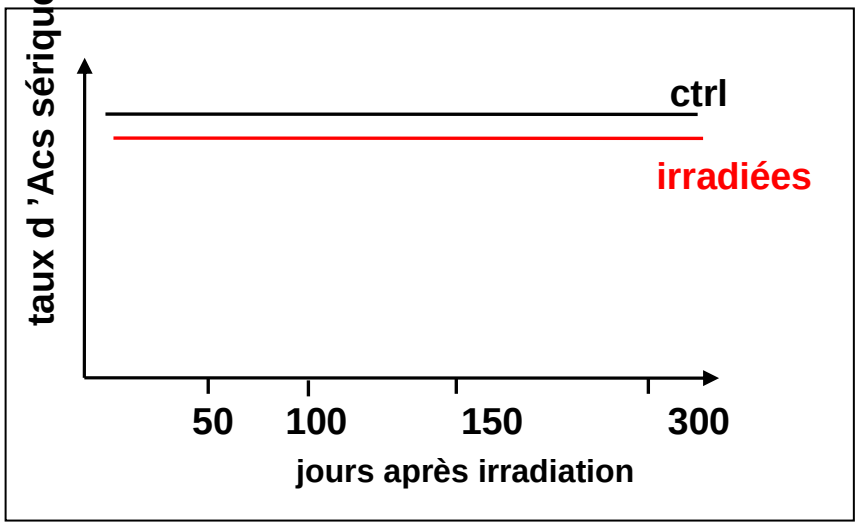
1. MBC ⇉ PC ?
2. PC ↔ PC ?

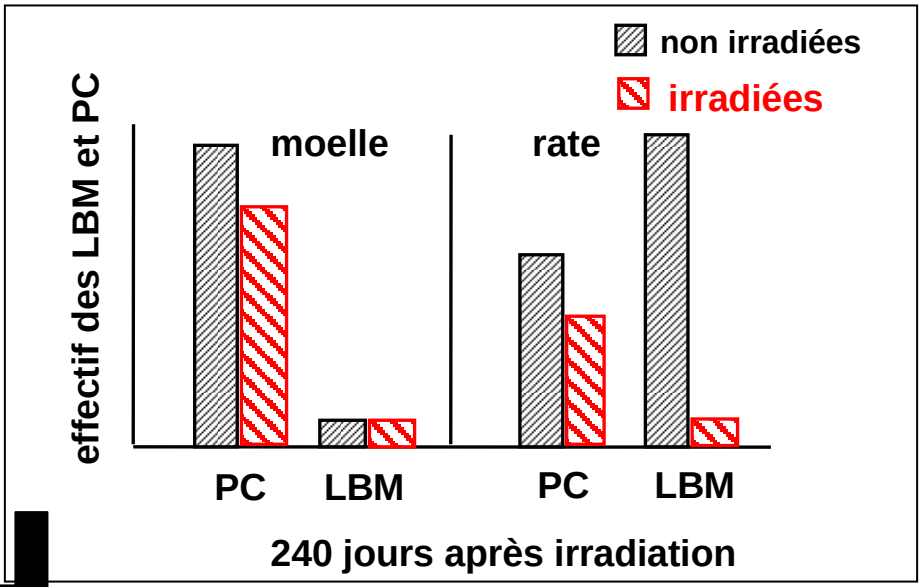


L 'immunisation génère des LBM. Au contraire des PC , les LBM persistent dans la rate et pas dans la moelle

2. Résultats expérimentaux (II)







L 'effectif des PC médullaires n 'est pas renouvellé par des apports du compartiment des LBM.

Les deux composantes cellulaires de la mémoire B aux Ag TD

	LB à mémoire conventionnels	PC à mémoire
Expression du BCR	**+**	**-**
Expression des molécules de classe II	**+**	**+/-** (DR^+ DQ^-)

Les capacités de présentation antigènique et de réactivation par l 'Ag sont réduites chez les PC à mémoire

Localisation	Tissus lymphoïdes secondaires	Moelle osseuse
Production d'Acs Protecteurs	Induite par l'antigène	Constitutive
Fonction	Protection après réinfection	Prévention de la réinfection

LA PERSISTANCE DE LA MÉMOIRE B

L'ÉVOLUTION DES CONCEPTS SUR LA PERSISTANCE DE LA MÉMOIRE B

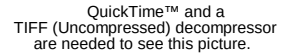
1. La longévité de la mémoire B est conditionnée par la persistance de l'Ag
Gray D. 1988.

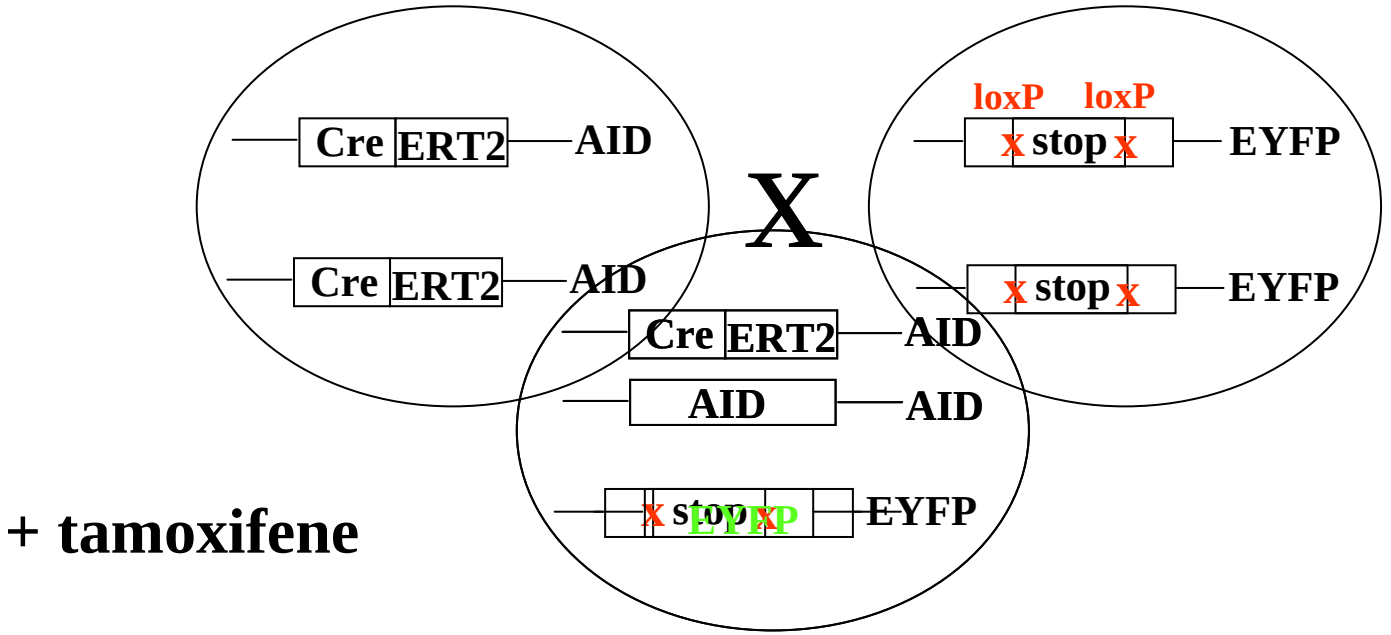
transfert de LB à mémoire chez des receveurs naïfs, en présence ou en l'absence de l'Ag:
pas de survie à long terme des LBM si l'Ag n'est pas co-transféré

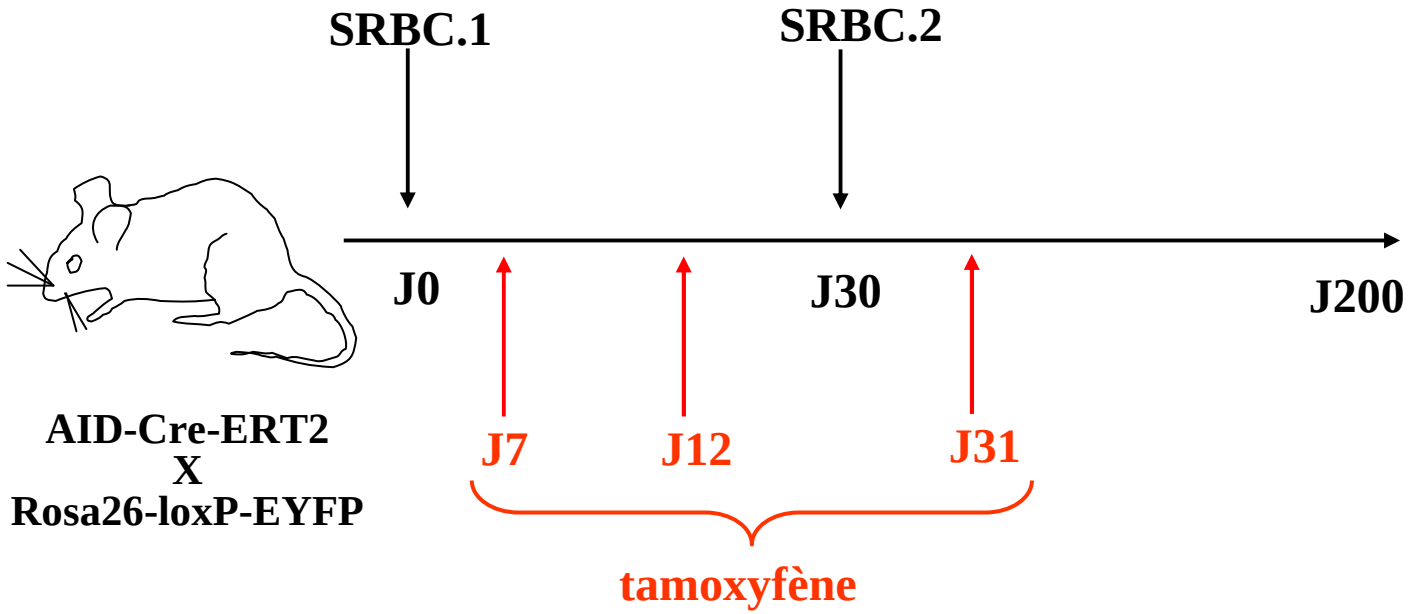
2. La longévité de la mémoire B n'est pas conditionnée par la persistance de l'Ag
Rajewsky K. 2000.

3. La longévité de la mémoire B est conditionnée par des signaux non-spécifiques d'Ag (cytokines, agents microbiens)
Lanzavecchia A. 2003.

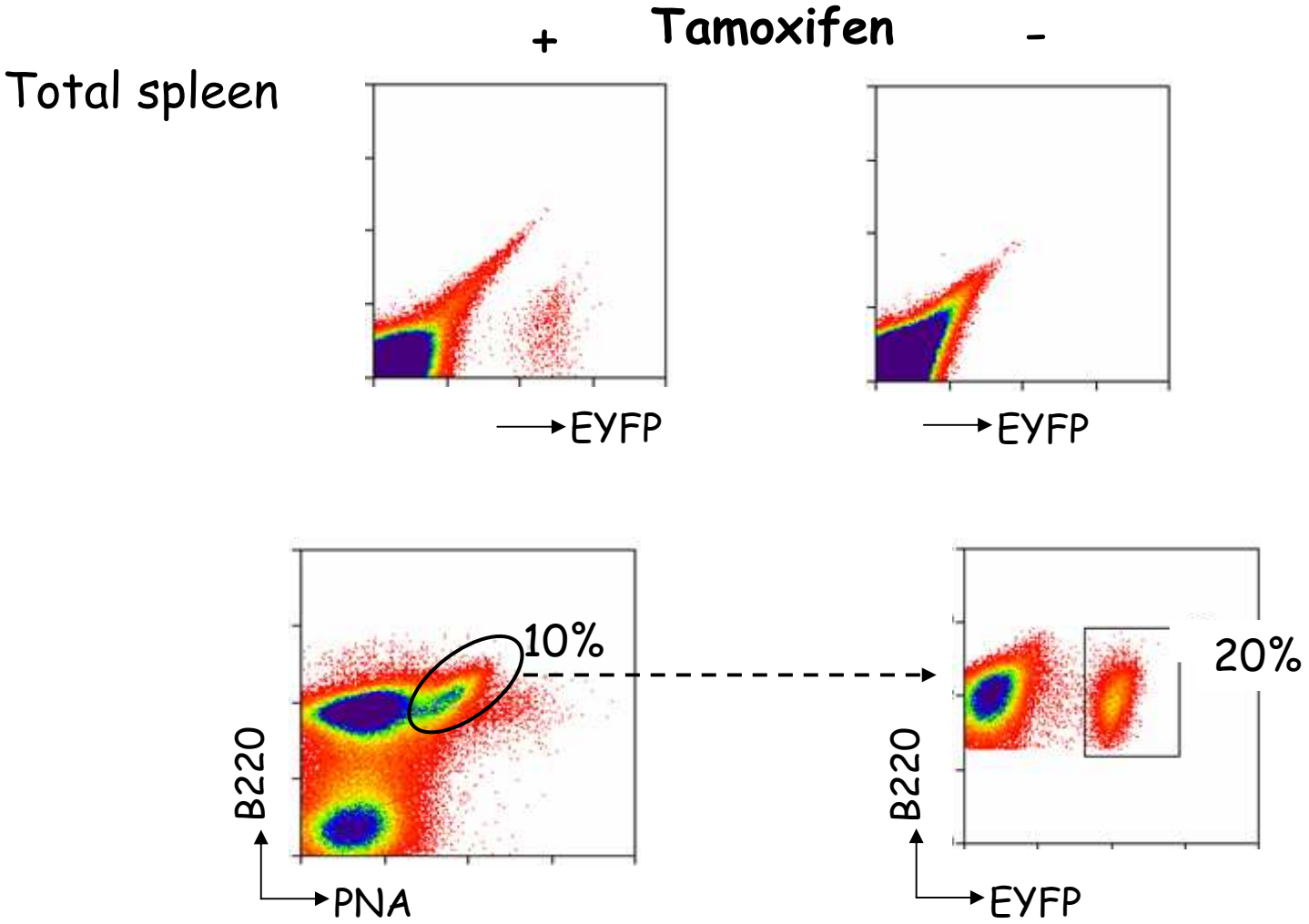
4. La longévité de la mémoire B est en partie conditionnée par la persistance de l'Ag,
Weill JC & Reynaud CA. 2009.


QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

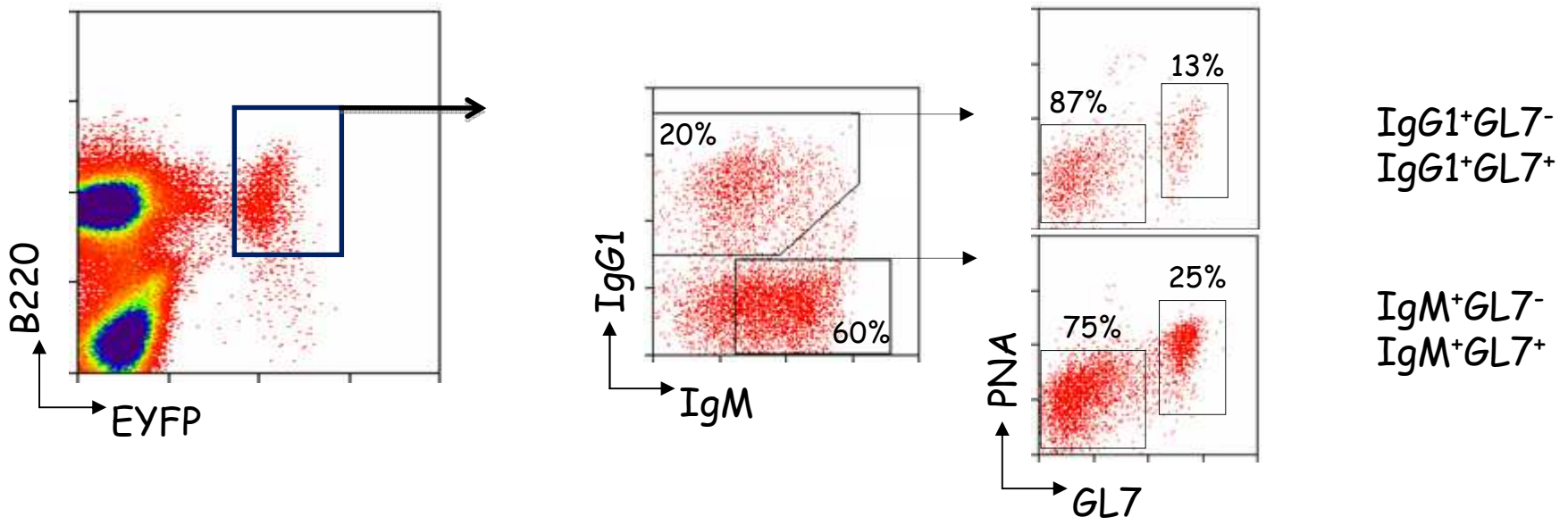

Cre ERT2
AID
Cre ERT2
AID
X
loxP loxP
x stop x
EYFP
x stop x
EYFP
Cre ERT2
AID
AID
AID
x stop x
EYFP
+ tamoxifene


SRBC.1
SRBC.2
J0
J30
J200
J7
J12
J31
tamoxyfène
AID-Cre-ERT2
X
Rosa26-loxP-EYFP

Tamoxifen-dependent induction of EYFP expression 4 days after a secondary immunization with SRBC

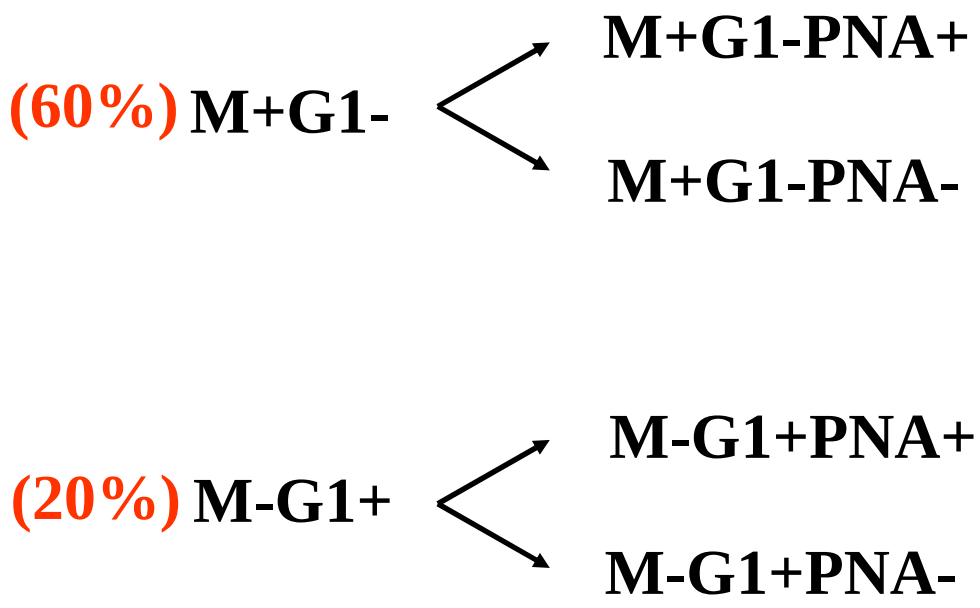


Four EYFP+ « memory » B cell subpopulations

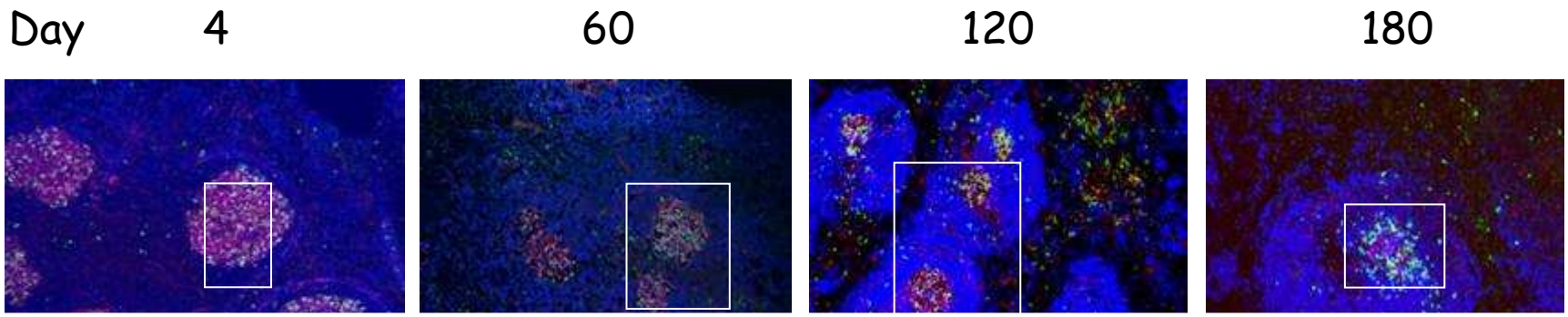


Analysis 2 months after secondary SRBC challenge

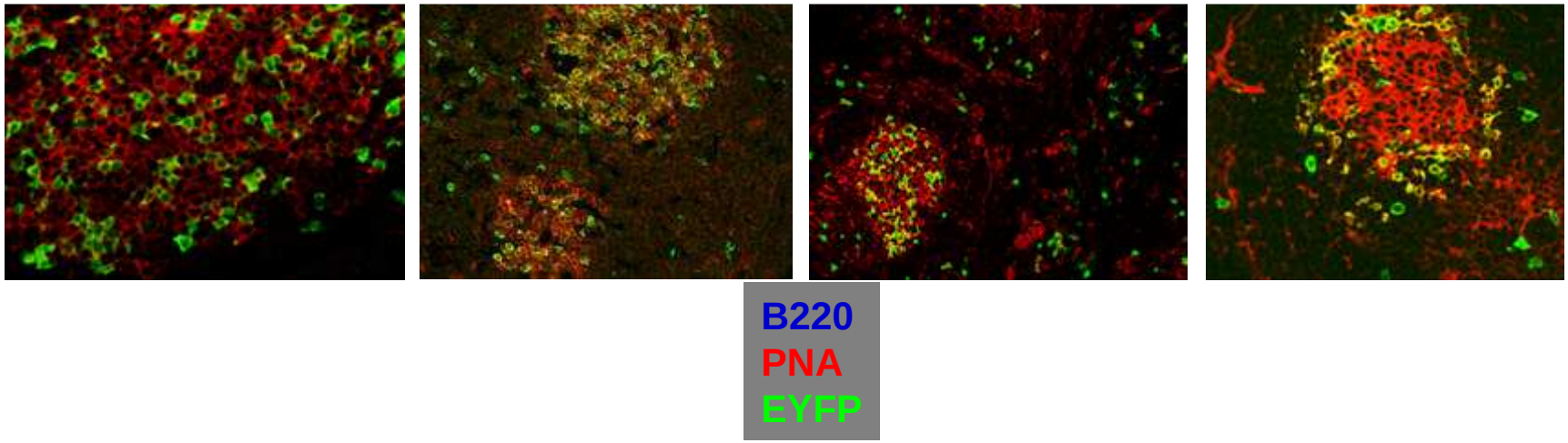
4 sous-populations LB mémoire (EYFP+)



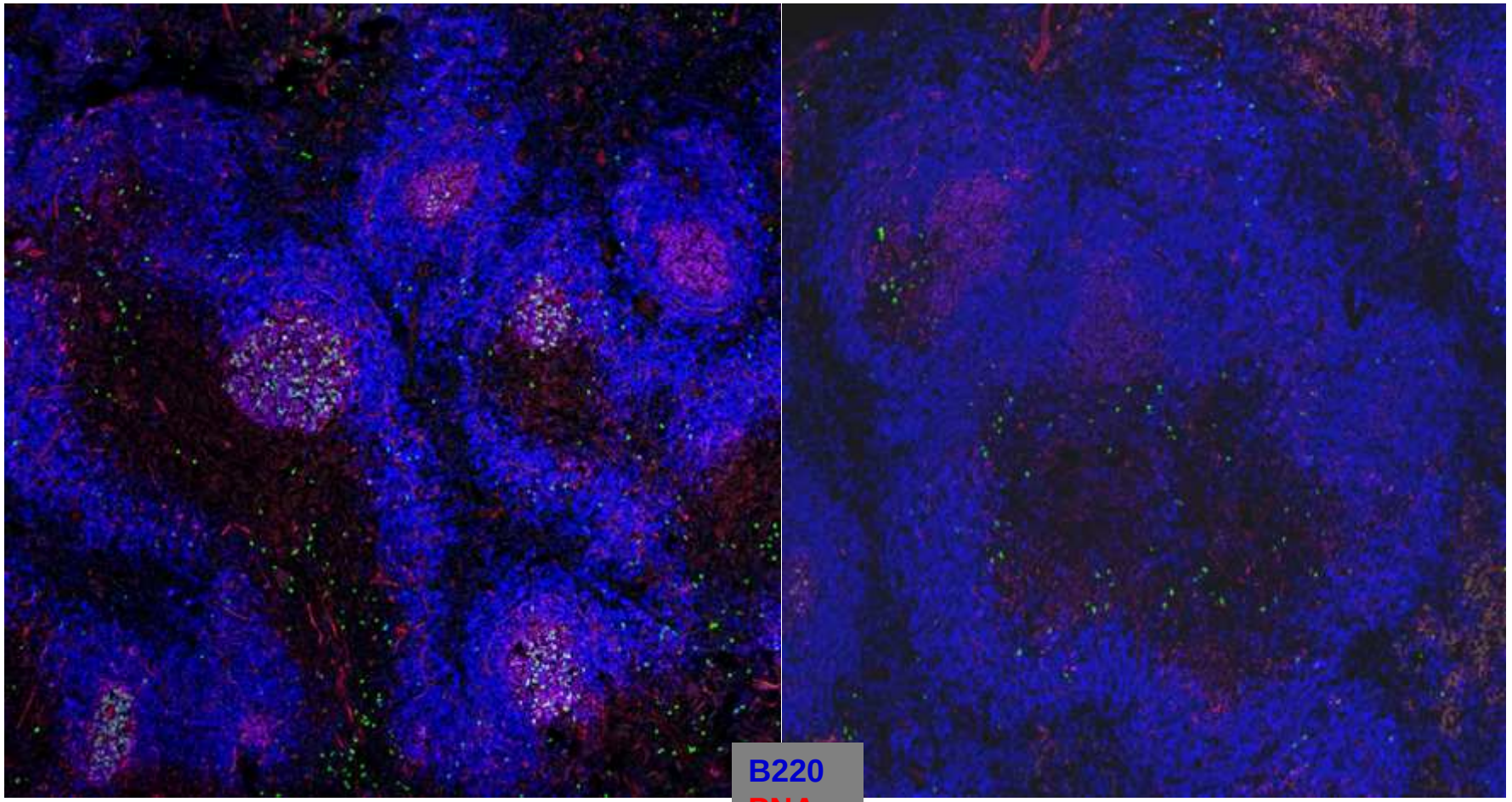
EYFP^{+}PNA^{+} B cells are associated
with persistent germinal center-like structures (up to 8 months)



Il persiste après immunisation des structures de type CG contenant des LT et des FDCs dans lesquelles sont localisés une partie des LB à mémoire

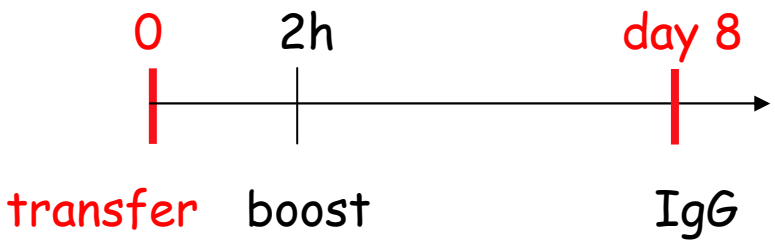


Mice immunized with NP-*CGG* display EYFP+ memory B cells
in the red pulp at 4 months
but not PNA^{high} B cells whithin germinal center structures



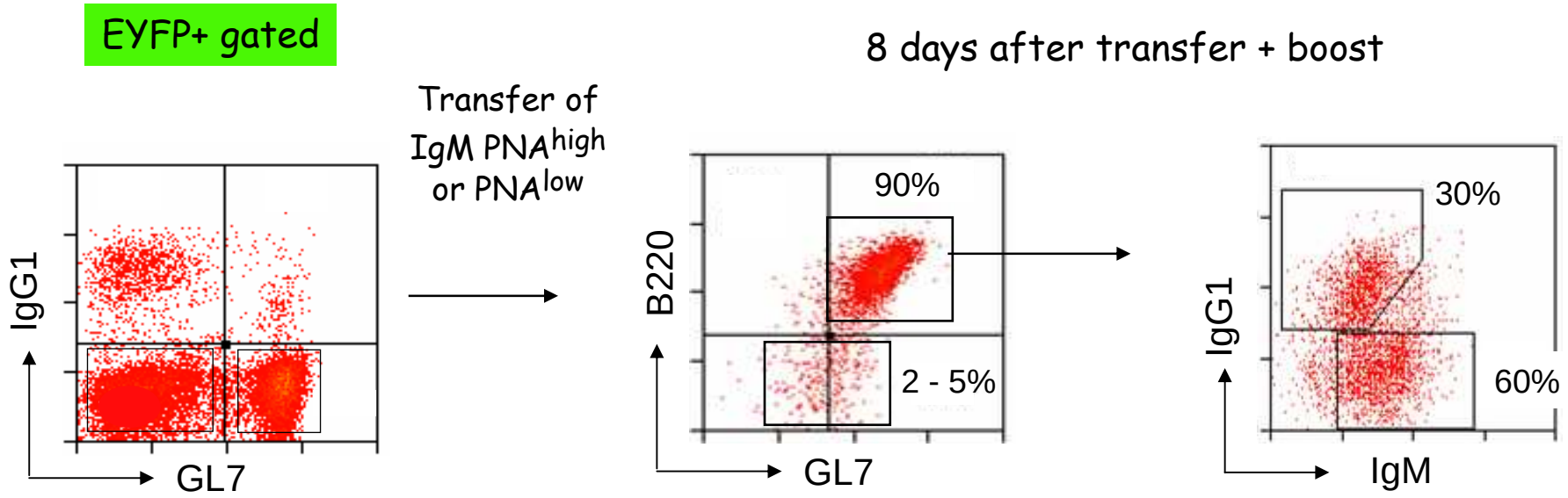
Recall response after cell transfer in SRBC-preimmunized mice:

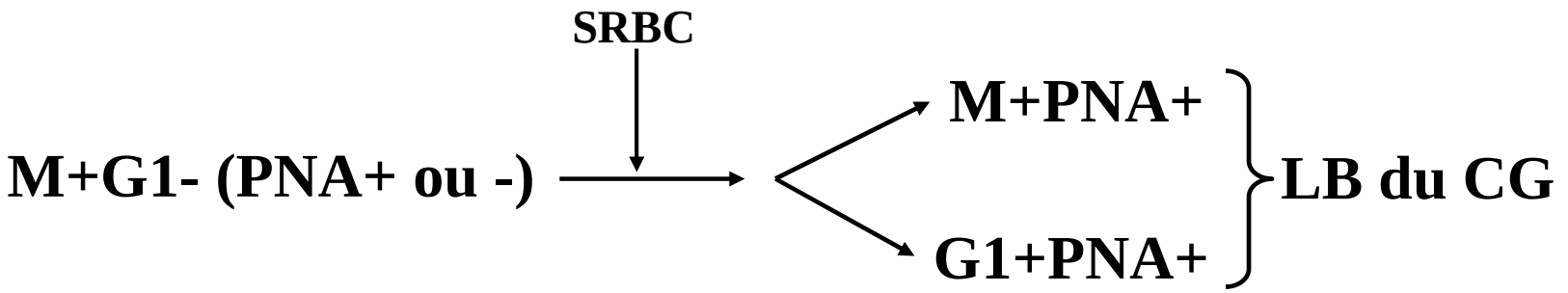
10,000 EYFP+ memory B cells taken from mice
6 months after SRBC immunization



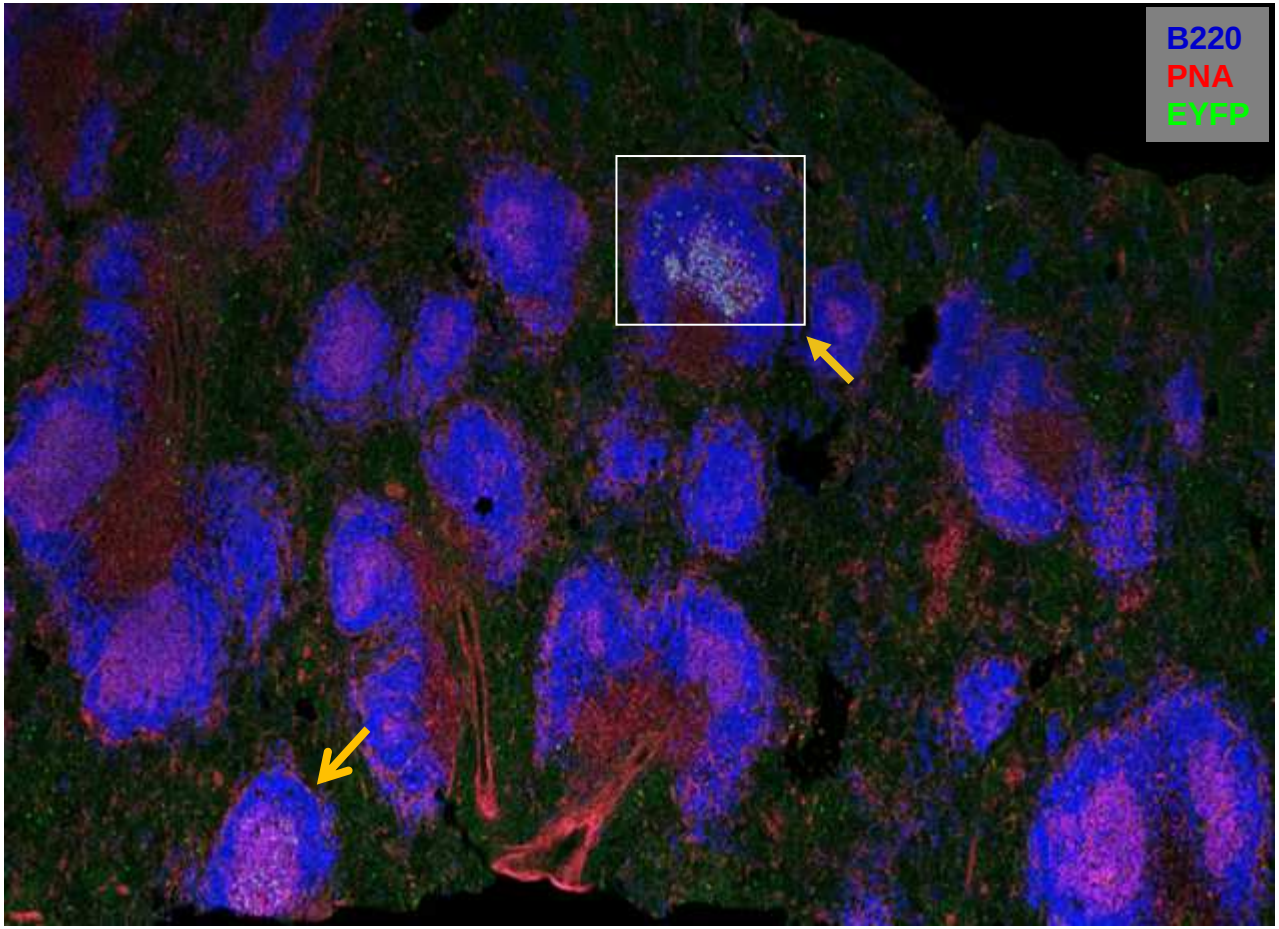
Analysis by immunohistochemistry, flow cytometry,
anti-SRBC elispot assay

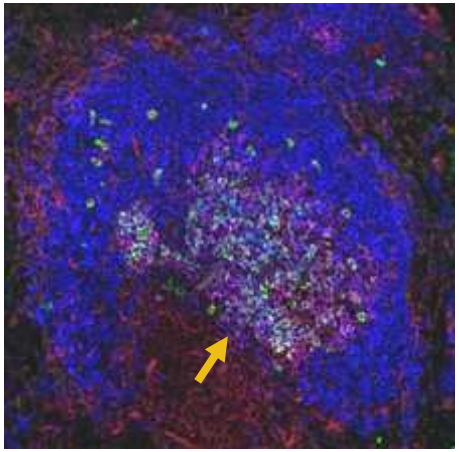
Transfer of IgM^{+}PNA^{high} or PNA^{low} EYFP^{+} B cells at 6 months gives rise after a boost to IgM and IgG1 PNA^{high} B cells





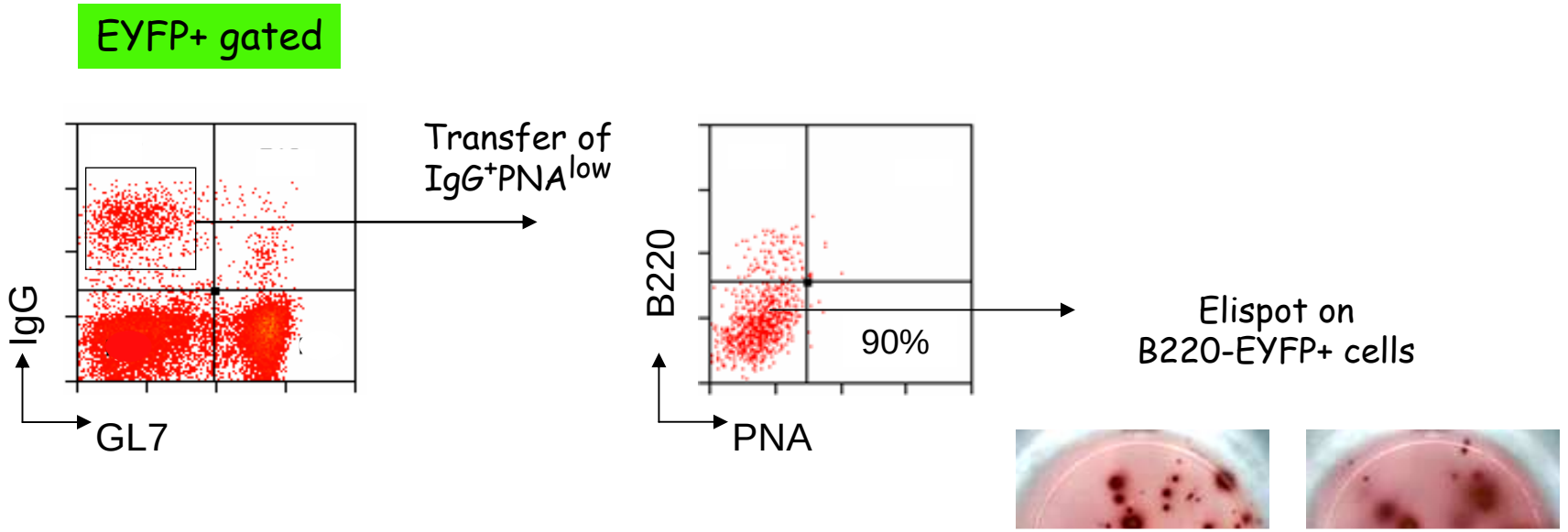
Transfer of IgM$^+$ PNA^{high} or PNA^{low} EYFP$^+$ B cells at 6 months gives rise after a boost to germinal center B cells





germinal center
EYFP+ cells

Transfer of IgG$^+$ PNA^{low} memory B cells at 6 months gives rise after a boost to plasma cells (anti-SRBC) and not to germinal center B cells



SRBC

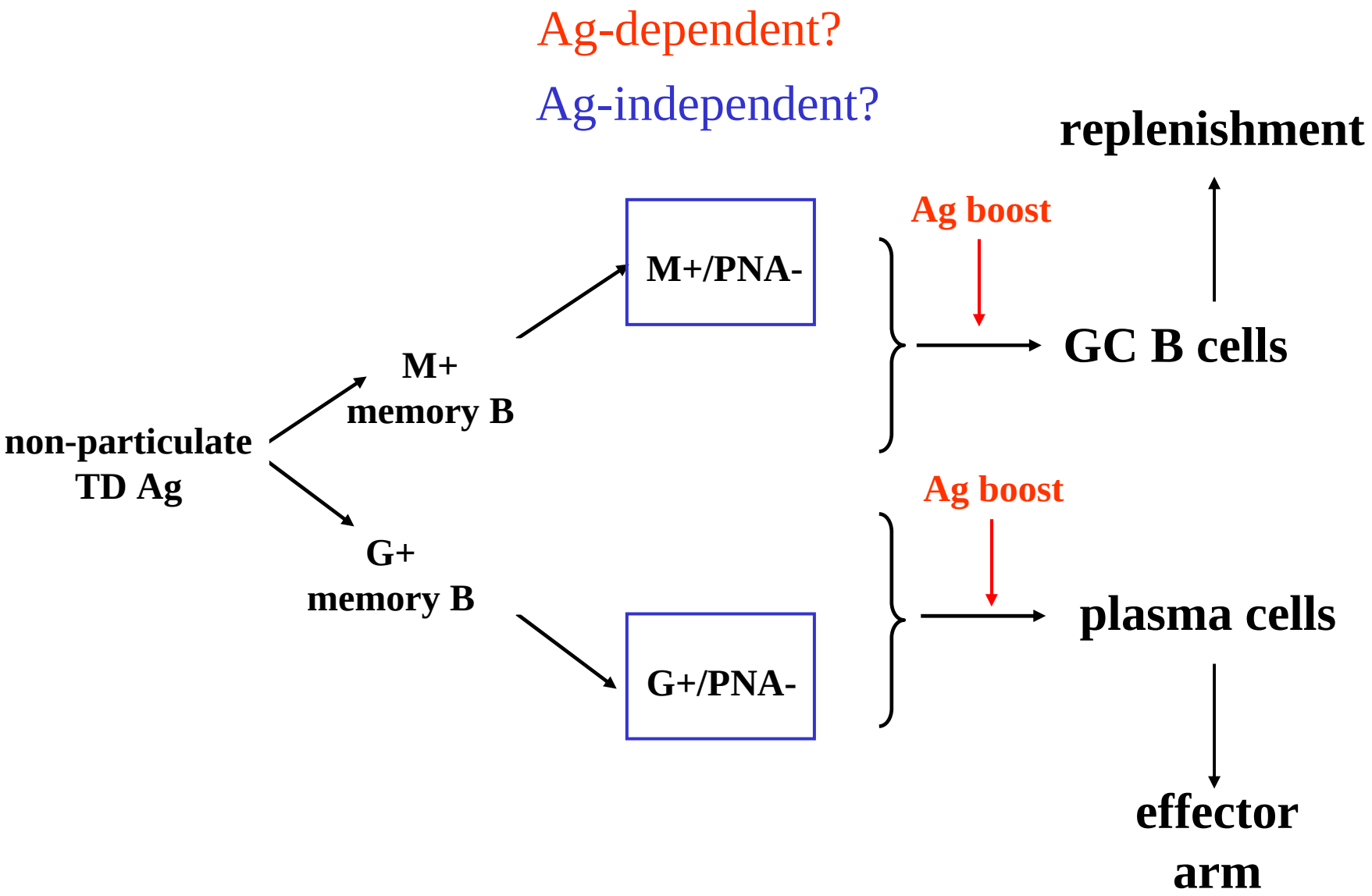
M-G1+ (PNA+ ou -) → LB B220-/PNA- = plasma cells

CONCLUSIONS

1) GC like structures containing centroblasts and FDCs can persist
for a long time after immunization with a particulate antigen(SRBC)
but not with a soluble protein + adjuvant (NP-CGG)

2) B cell memory against SRBC is composed of two long-lived populations:
an « antigen-dependent subset » of centroblasts
and an « antigen-independent » subset of memory B cells
Both subsets are composed of an IgM and an IgG component

3) Upon a boost:
the IgG subset has immediate effector functions and does not self maintain :
« effector function »
the IgM subset resplenishes the memory pool and produces effector B cells in
prevision of a next encounter with the antigen :
« central function »


Ag-dependent?
Ag-independent?
replenishment
Ag boost
M+/PNA-
M+
memory B
non-particulate
TD Ag
GC B cells
Ag boost
G+
memory B
G+/PNA-
plasma cells
effector
arm

LA RÉPONSE AUX Ag THYMO-INDÉPENDANTS

Immunité adaptative

↓

Reconnaissance par le récepteur d'antigène (BCR)

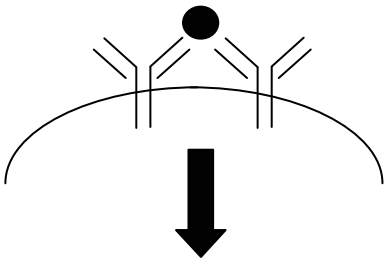
Immunité innée

↓

Reconnaissance par un récepteur distinct du BCR

Ag TD (divalent)

Protéines



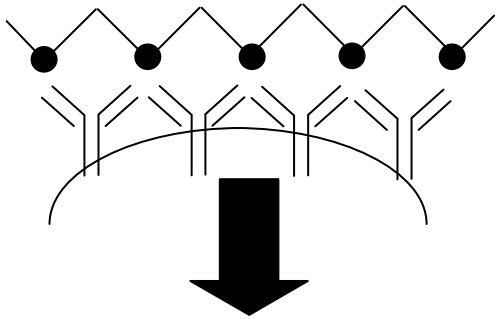
LB folliculaires

↓

Follicule

Ag TI-2 (multivalent)

Polysaccharides bactériens



LB1 (CD5+)

↓

Péritoine et rate

LB ZM

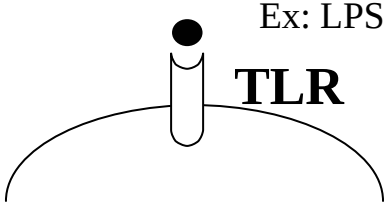
↓

Zone marginale

Ag TI-1

Pathogen Associated Molecular Patterns

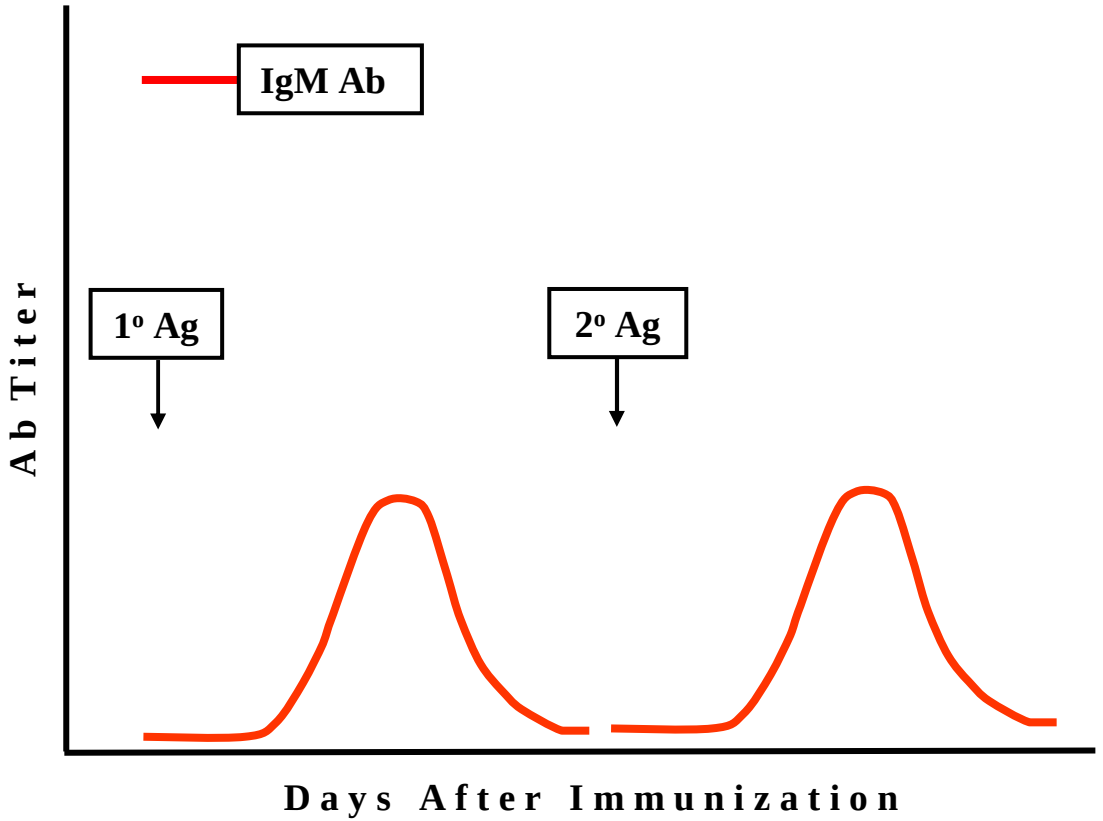
Ex: LPS



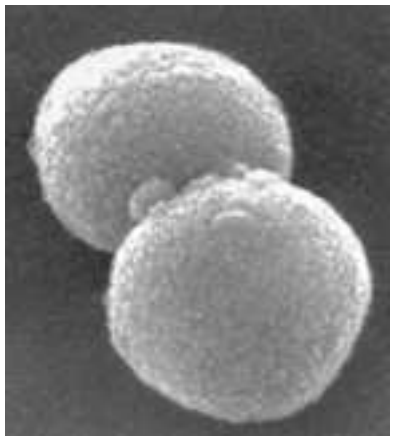
?

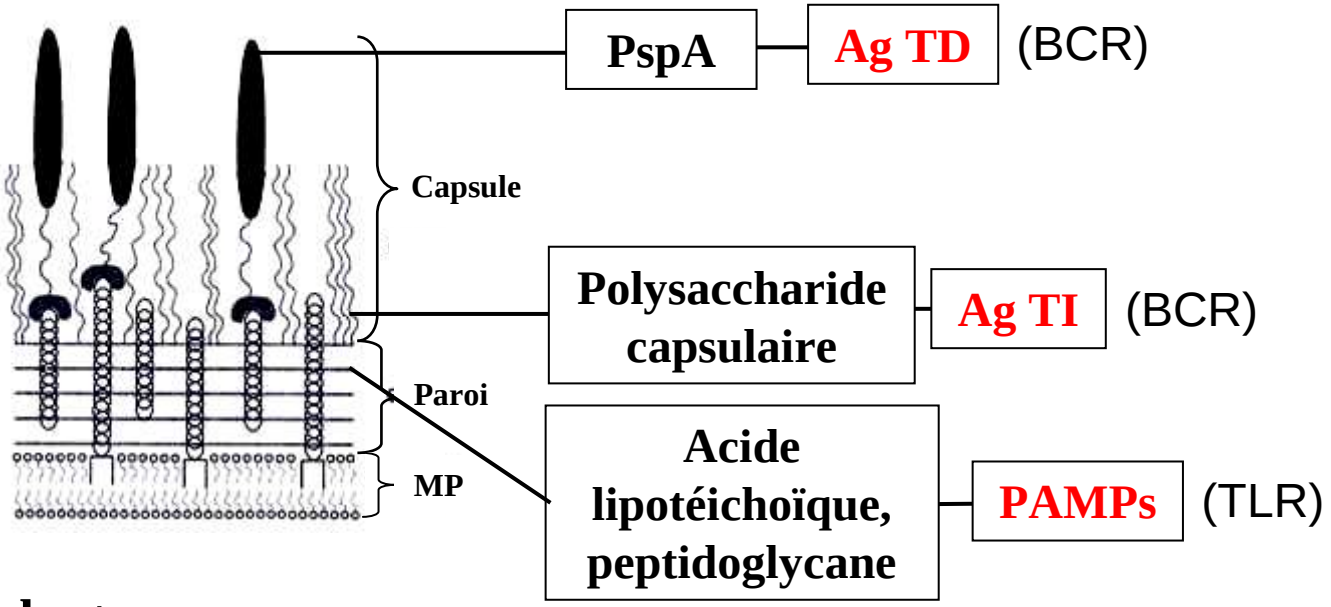
Kinetics of Ab Response to T-independent Ags

- IgM antibody
- No secondary response



Généralités sur les PS bactériens

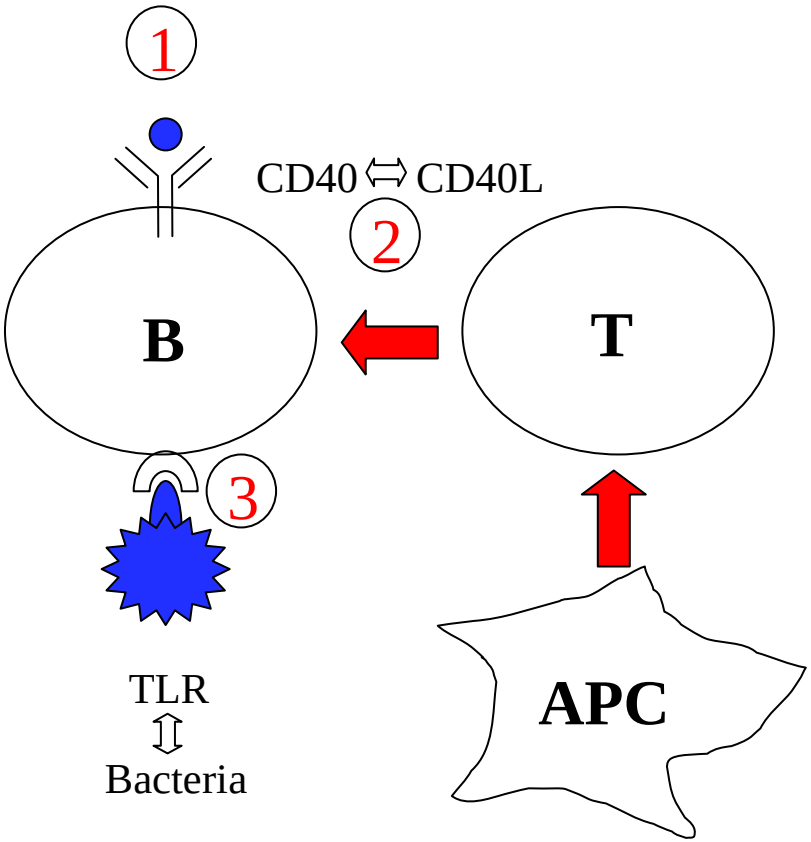




- ✓ **Ag thymo-indépendants**
- ✓ **pas de présentation via molécules du CMH**
- ✓ **Induisent une réponse Ac protectrice**
- ✓ **N'induisent pas de réponse Ac «secondaire» accélérée et amplifiée**
- ✓ **N'induisent pas de «mémoire immunologique B»**

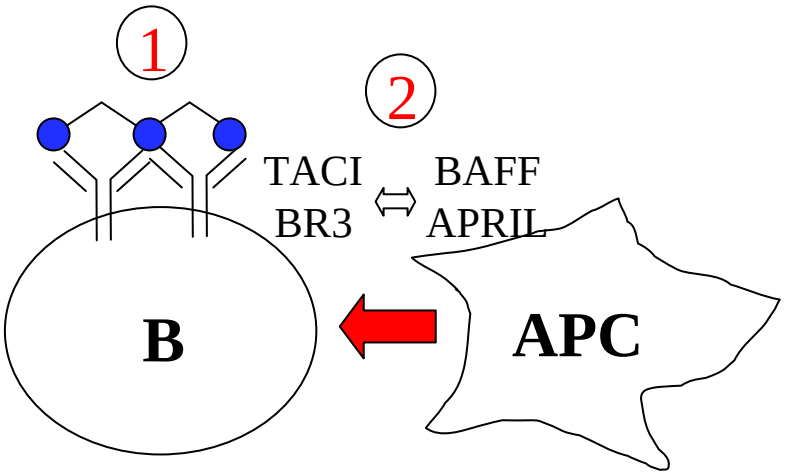
TD Ag

Signal 1: BCR
Signal 2: T cells
Signal 3: TLR

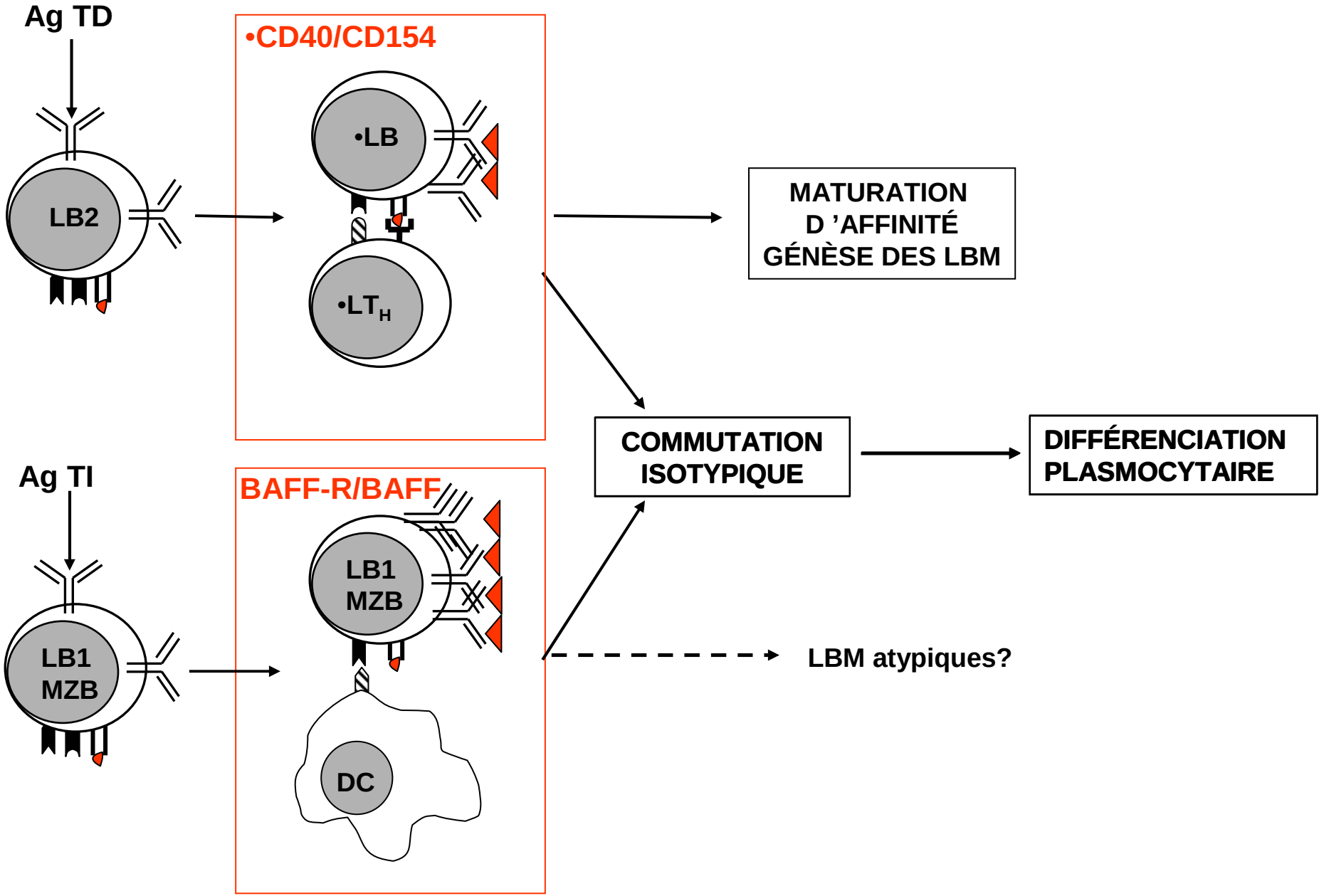


TI Ag

Signal 1: BCR
Signal 2: APC



LES PARTENAIRES CELLULAIRES ET MOLÉCULAIRES DES LB LORS DE LA RÉPONSE A UN Ag TI



LE PARADOXE IMMUNOLOGIQUE DE LA REPONSE B AUX PS BACTERIENS

les PS bactériens n'induisent ni réponse Ac « secondaire » accélérée et amplifiée ni « mémoire immunologique B »

les vaccins polysaccharidiques purs sont protecteurs

mémoire B non conventionnelle en réponse aux Ag TI?

Les cellules B-1b sont le support de la mémoire B aux Ag TI

Immunity, Vol. 21, 379–390, September, 2004, Copyright ©2004 by Cell Press

B1b Lymphocytes Confer T Cell-Independent Long-Lasting Immunity

Kishore R. Alugupalli,[1,*] John M. Leong,[1]
Robert T. Woodland,[1] Masamichi Muramatsu,[2]
Tasuku Honjo,[2] and Rachel M. Gerstein[1]
[1]Department of Molecular Genetics and Microbiology
University of Massachusetts Medical School
Worcester, Massachusetts 01655
[2]Department of Medical Chemistry
Graduate School of Medicine
Kyoto University
Kyoto 606-8501
Japan

les LB-1b confèrent la protection après immunisation par la bactérie *B. hermsii*

Immunity, Vol. 23, 7–18, July, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.04.011

B-1a and B-1b Cells Exhibit Distinct Developmental Requirements and Have Unique Functional Roles in Innate and Adaptive Immunity to *S. pneumoniae*

Karen M. Haas, Jonathan C. Poe,
Douglas A. Steeber, and Thomas F. Tedder*
Department of Immunology
Box 3010
Duke University Medical Center
Durham, North Carolina 27710

les LB-1b confèrent la protection après immunisation par le PS3 de *S. pneumoniae*

LES COMPOSANTES CELLULAIRES DE LA MÉMOIRE B

- lymphocytes B à mémoire « conventionnels »
- plasmocytes à mémoire

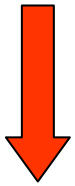
→ **Ag TD**
LB-2

- lymphocytes B à mémoire « atypiques » → **Ag TI**
LB-1b

LE SECOND PARADOXE DE LA REPONSE B AUX Ag TI

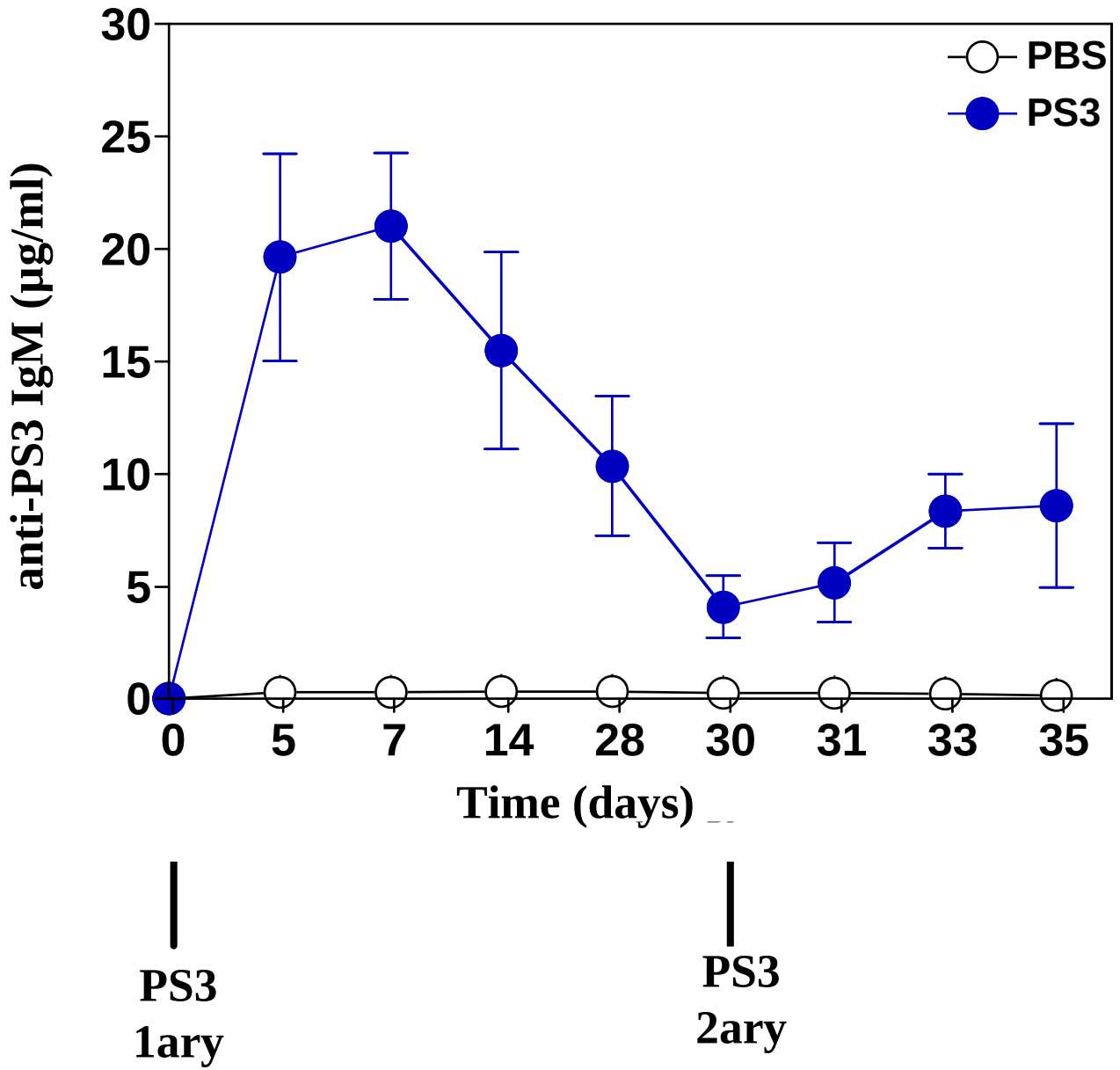
les Ag TI (PS bactériens) induisent la production de LB-1b « à mémoire »

une immunisation secondaire avec un Ag TI n'induit pas de réponse Ac amplifiée et/ou accélérée



par quel mécanisme les Ag TI peuvent-ils conférer une protection?

PS3 DOES NOT GENERATE AN AMPLIFIED SECONDARY RESPONSE IN IMMUNOCOMPETENT MICE



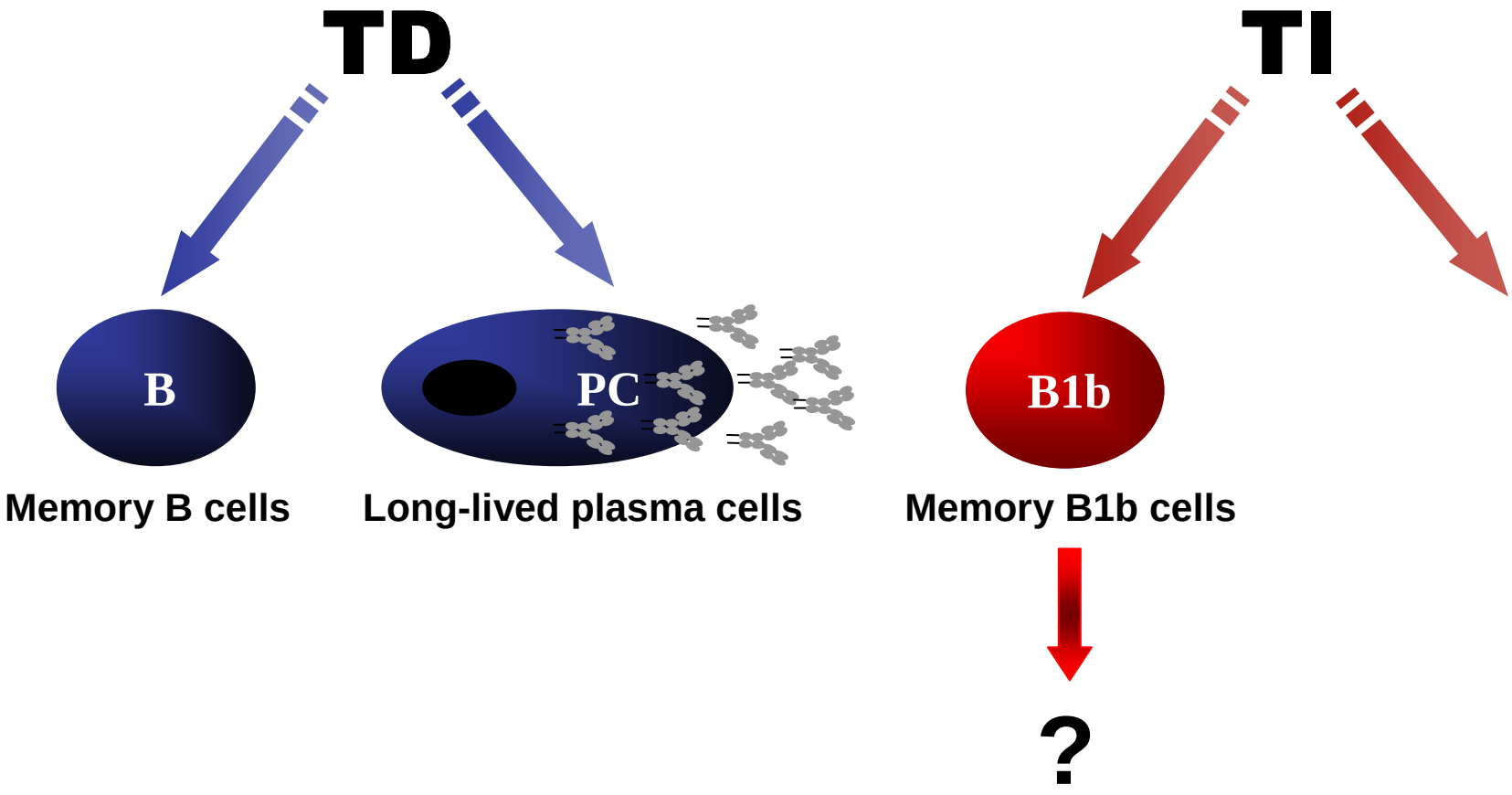
BRIEF DEFINITIVE REPORT

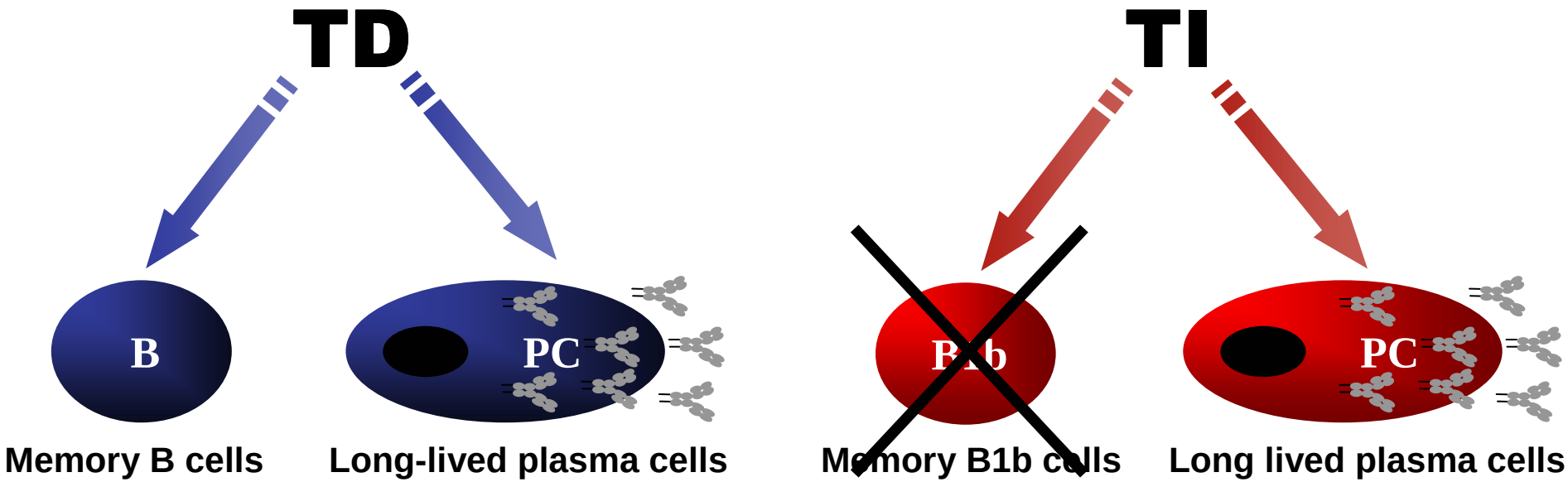
T-independent type II immune responses generate memory B cells

Tetyana V. Obukhanych[1] and Michel C. Nussenzweig[1,2]

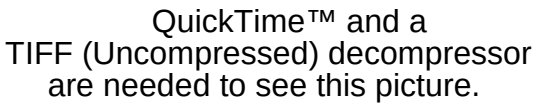
[1]Laboratory of Molecular Immunology and [2]Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021

Unlike T-dependent immune responses against protein antigens, T-independent responses against polysaccharides confer long-lasting humoral immunity in the absence of recall responses and are not known to generate memory B cells. Here we report that polysaccharide antigens elicit memory B cells that are phenotypically distinct from those elicited by protein antigens. Furthermore, memory B cell responses against polysaccharides are regulated by antigen-specific immunoglobulin G antibodies. As the generation and regulation of immunologic memory is central to vaccination, our findings help explain the mode of action of the few existing polysaccharide vaccines and provide a rationale for a wider application of polysaccharide-based strategies in vaccination.

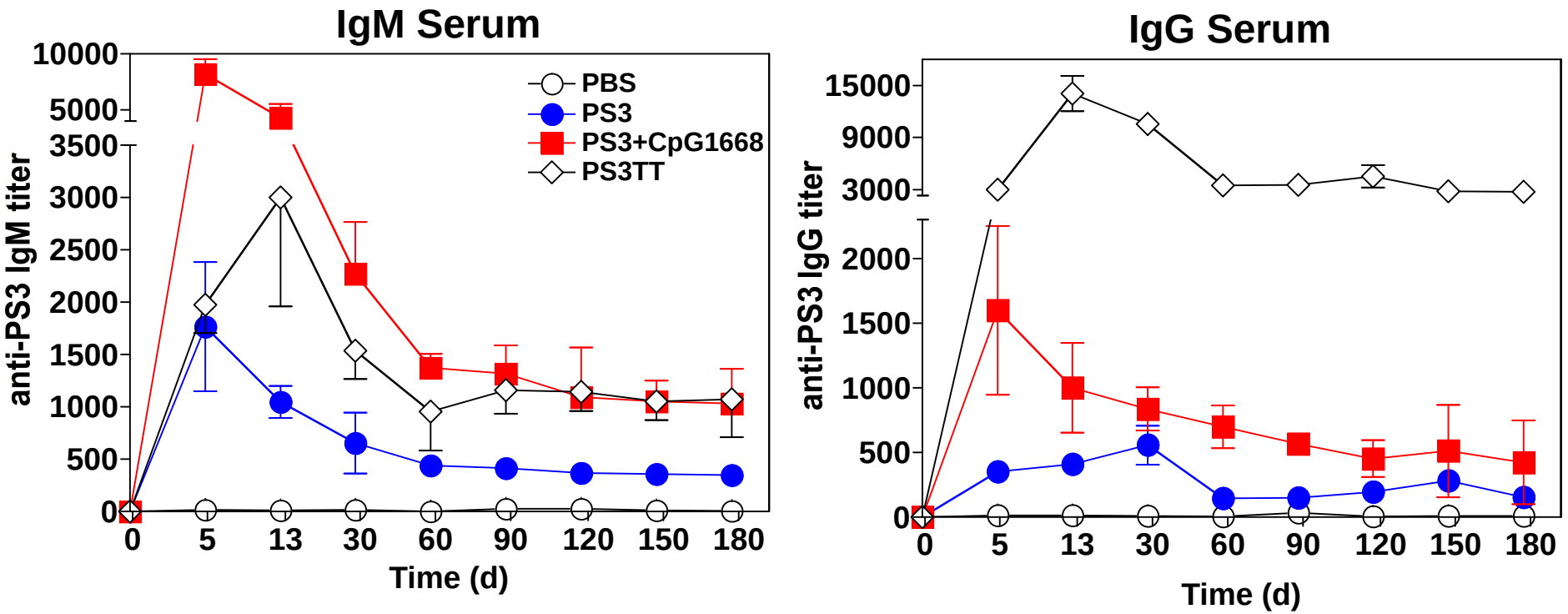

TD
TI
B
PC
B1b
Memory B cells
Long-lived plasma cells
Memory B1b cells
?



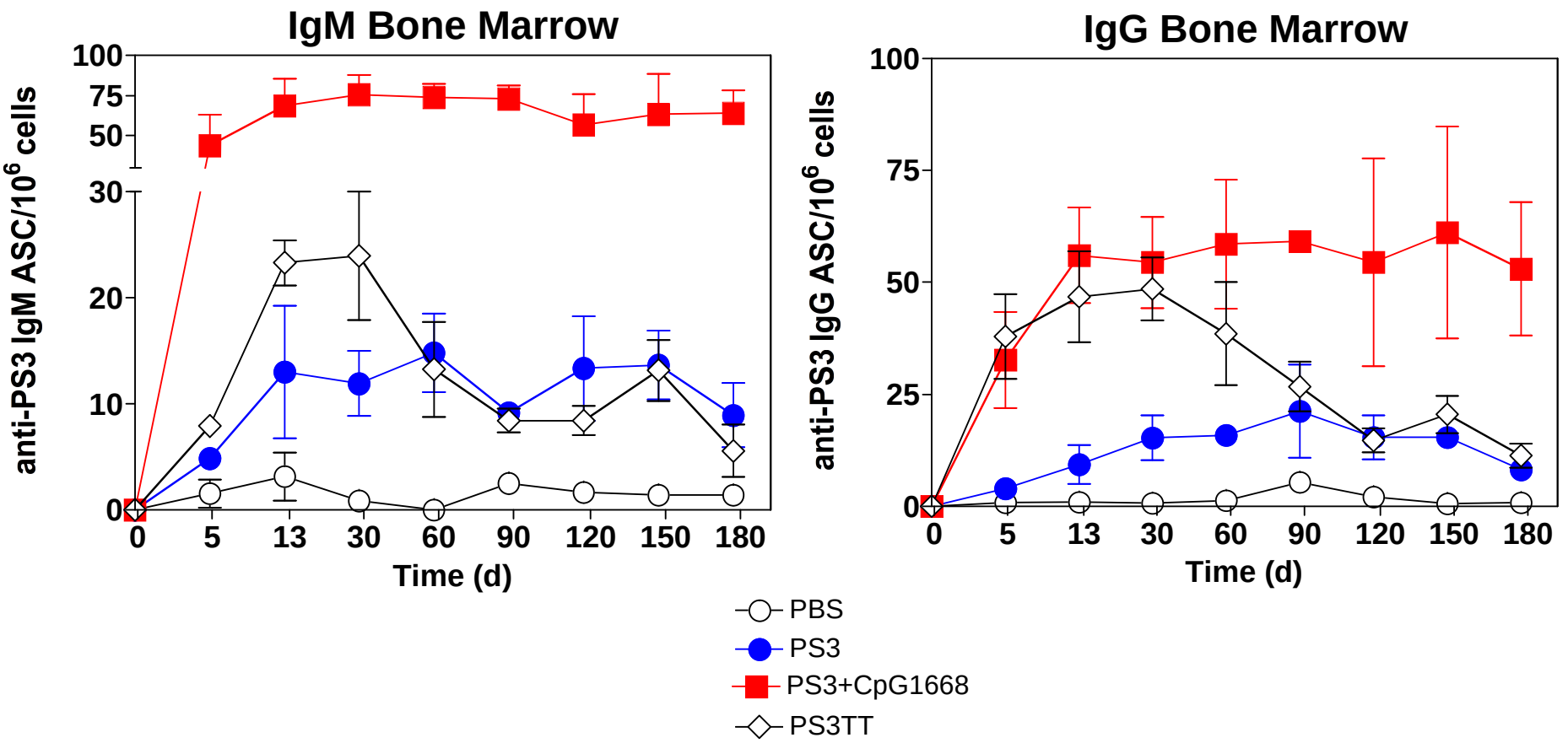
What are the mechanisms accounting for protection?

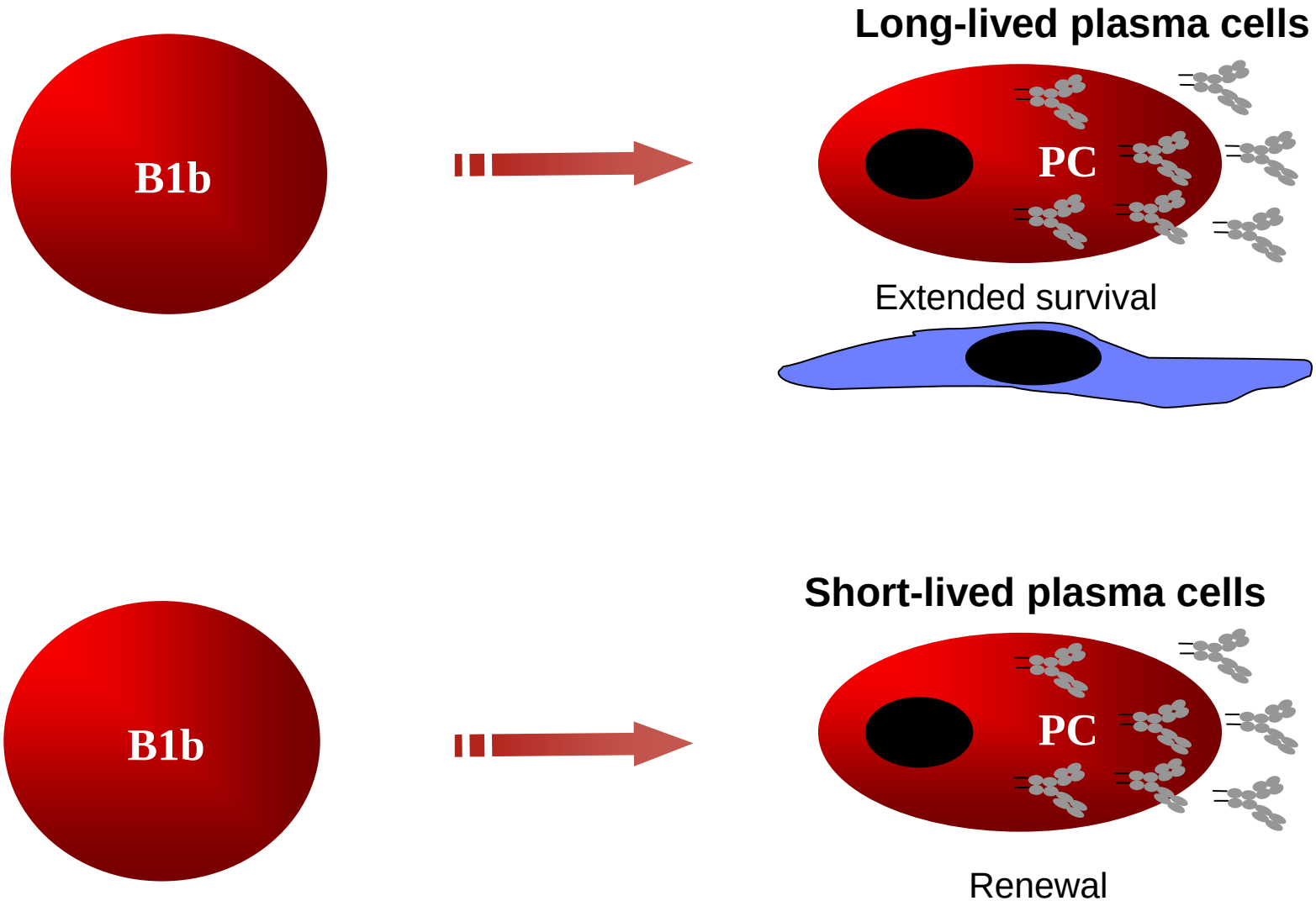

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

IMMUNIZATION WITH A PLAIN OR ADJUVANTED PS3 VACCINE GENERATES SERIC PS3 Ab THAT PERSIST 180 DAYS AFTER IMMUNIZATION.

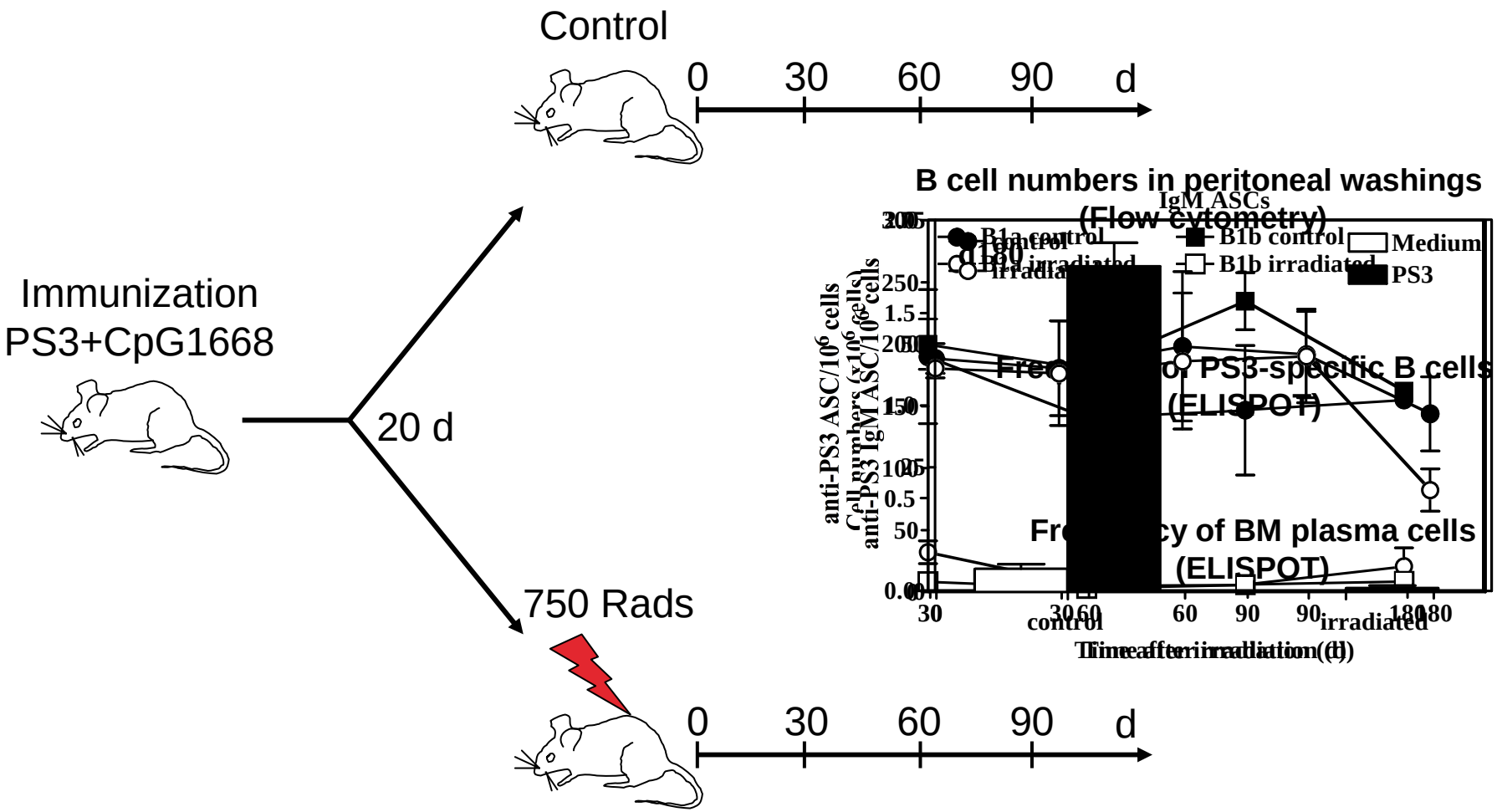


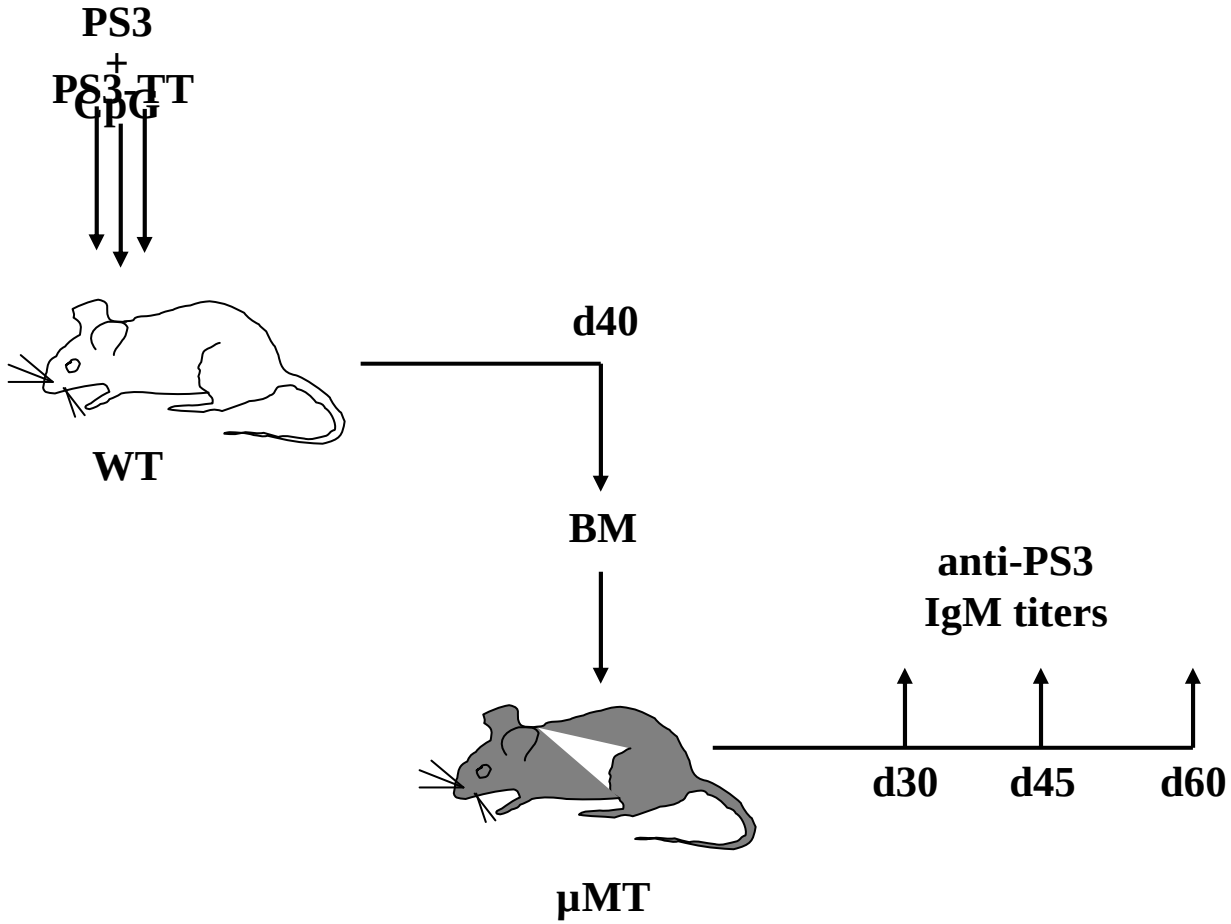
IMMUNIZATION WITH A PLAIN OR ADJUVANTED PS3 VACCINE INDUCES A PERSISTENT POOL OF PLASMA CELLS IN THE BM



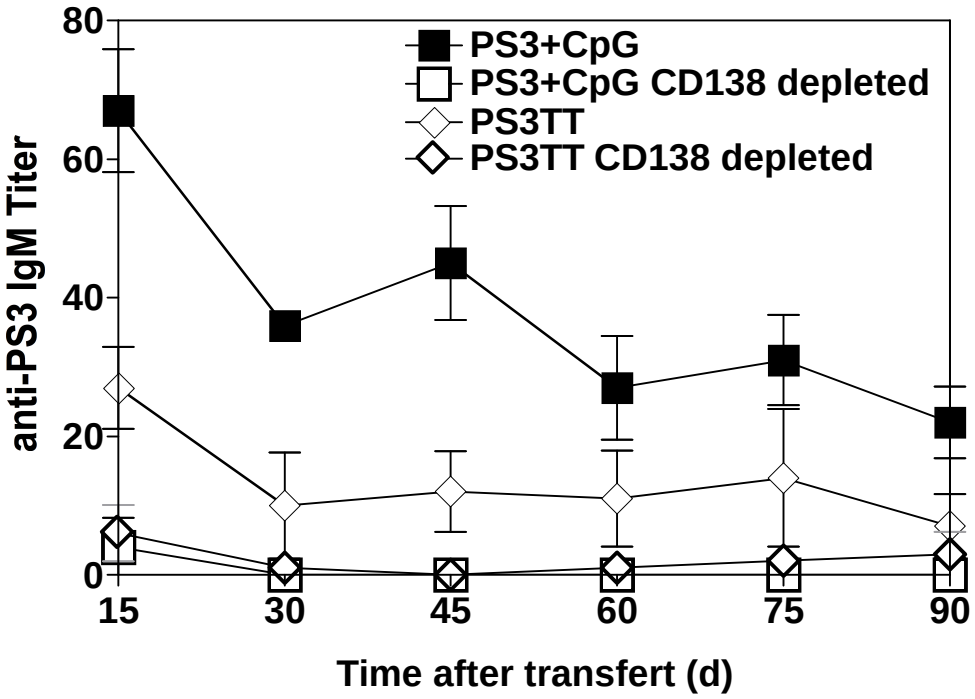

B1b
Long-lived plasma cells
PC
Extended survival
B1b
Short-lived plasma cells
PC
Renewal

IMMUNIZATION WITH AN ADJUVANTED PS3 VACCINE INDUCES LONG-LIVED BM PLASMA CELLS (1)

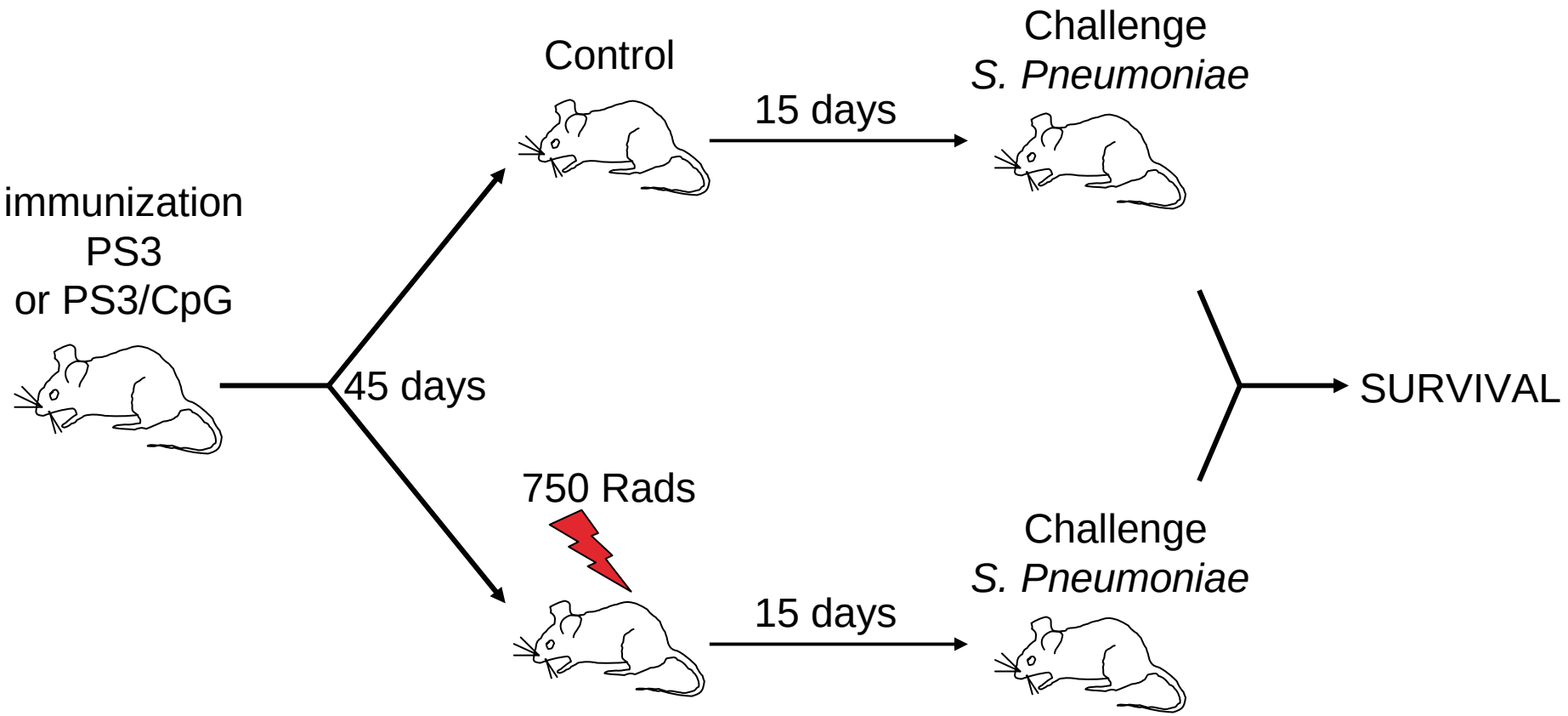



PS3
+
PS3-TT
CpG
WT
d40
BM
µMT
anti-PS3
IgM titers
d30
d45
d60

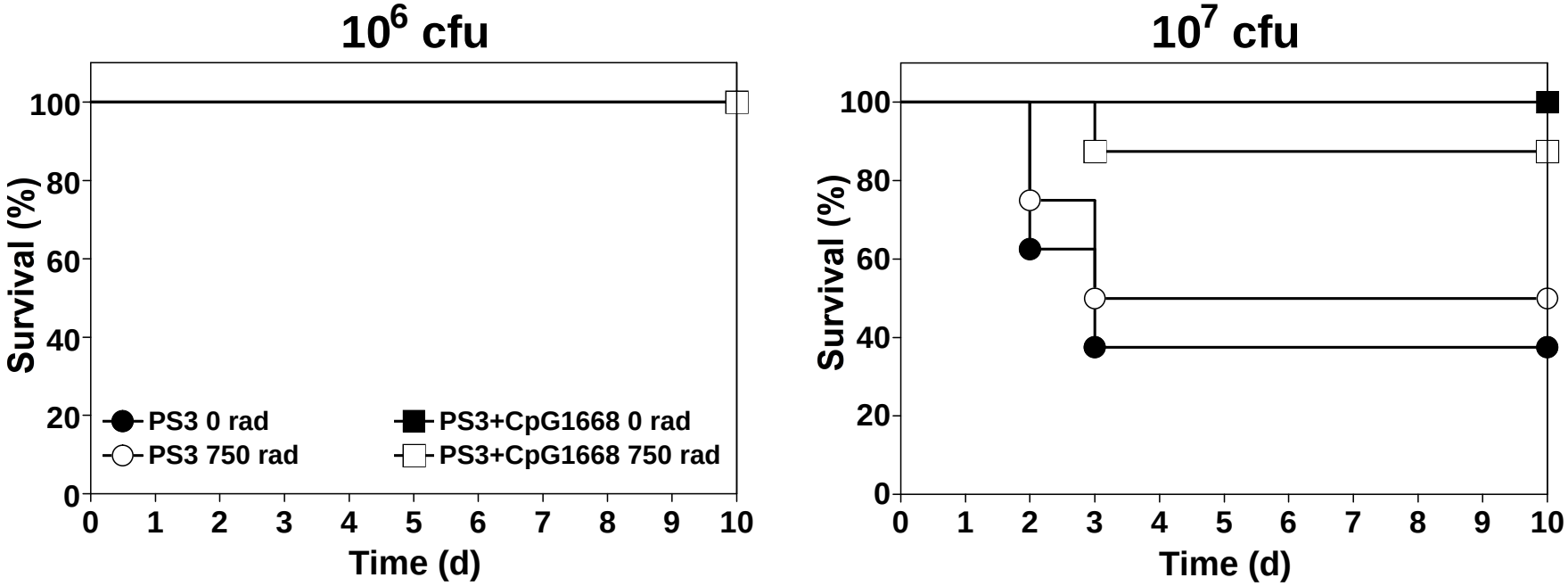
IMMUNIZATION WITH AN ADJUVANTED PS3 VACCINE INDUCES LONG-LIVED BM PLASMA CELLS (2)



ROLE OF PS3-SPECIFIC LONG-LIVED BM PLASMA CELLS IN PROTECTION AGAINST *S. PNEUMONIAE* INFECTION



LONG-LIVED BM PLASMA CELLS ARE RESPONSIBLE FOR THE IMMUNE PROTECTION CONFERRED BY A PS3 VACCINE



Paradigms on the B cell response to TI Ag have to be reconsidered

✓ **TI Ag do not generate B cell memory**

➡ **Adoptive transfer of B-1b cells from PS-immune mice protects naive mice**

✓ **TI Ag do not promote bona fide secondary responses**

➡ **Primed B-1 cells generate an amplified and accelerated Ab response after transfer in Rag2 recipients. TI Ag can induce a secondary Ab response in AID KO mice.**

✓ **TI Ag do not generate long-lived plasma cells**

➡ **High numbers of PS3-specific PC can be detected in the bone marrow of mice immunized with PS3 and TLR-L**

✓ **Young mice are unresponsive to TI Ag**

➡ **TLR-L can restore responsiveness of young mice to bacterial PS**