B cell Immunophenotyping for PID diagnosis and Classification

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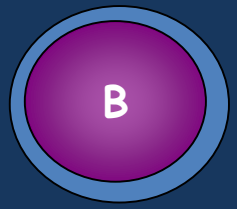
Basic B cell development pathway

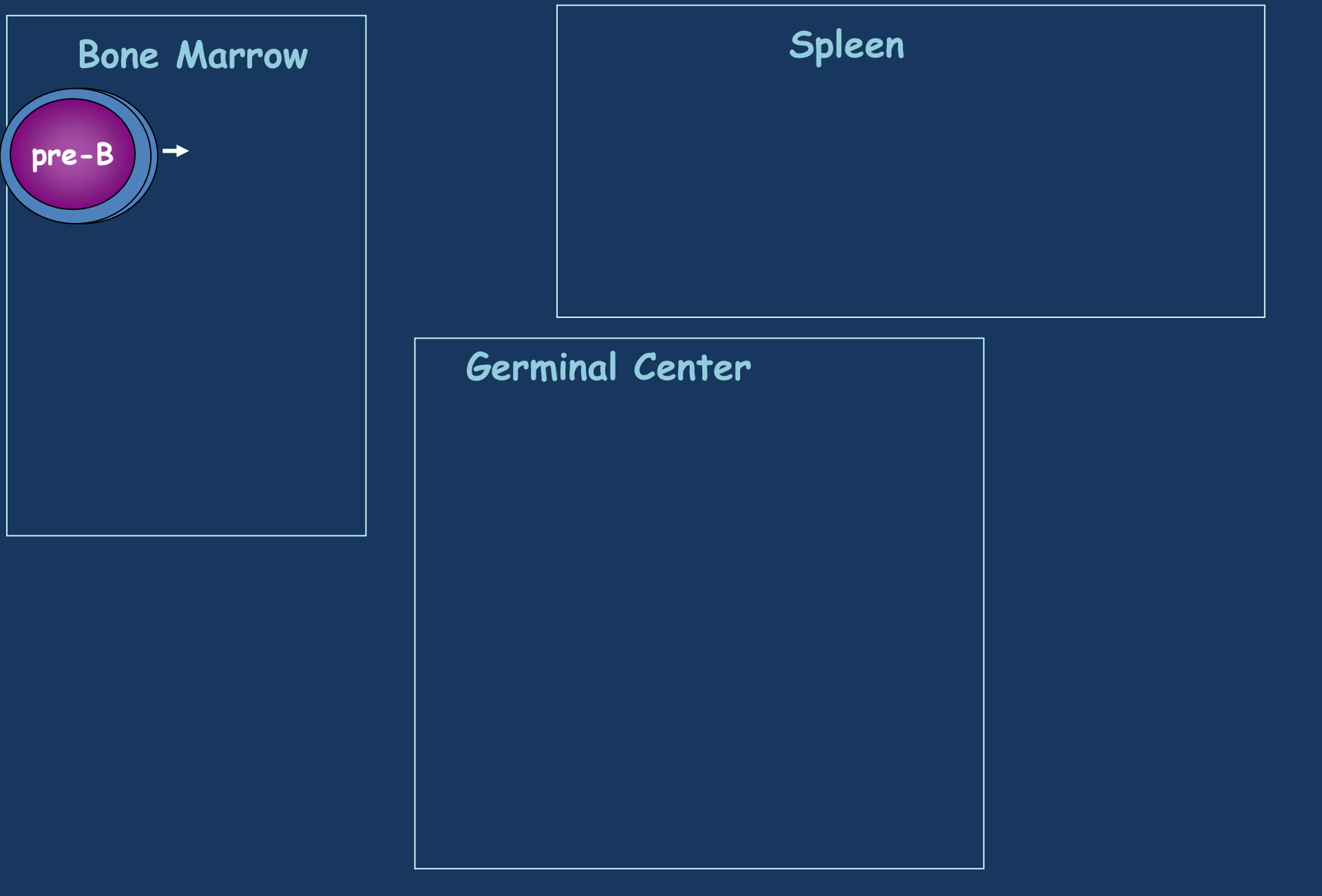
Bone Marrow



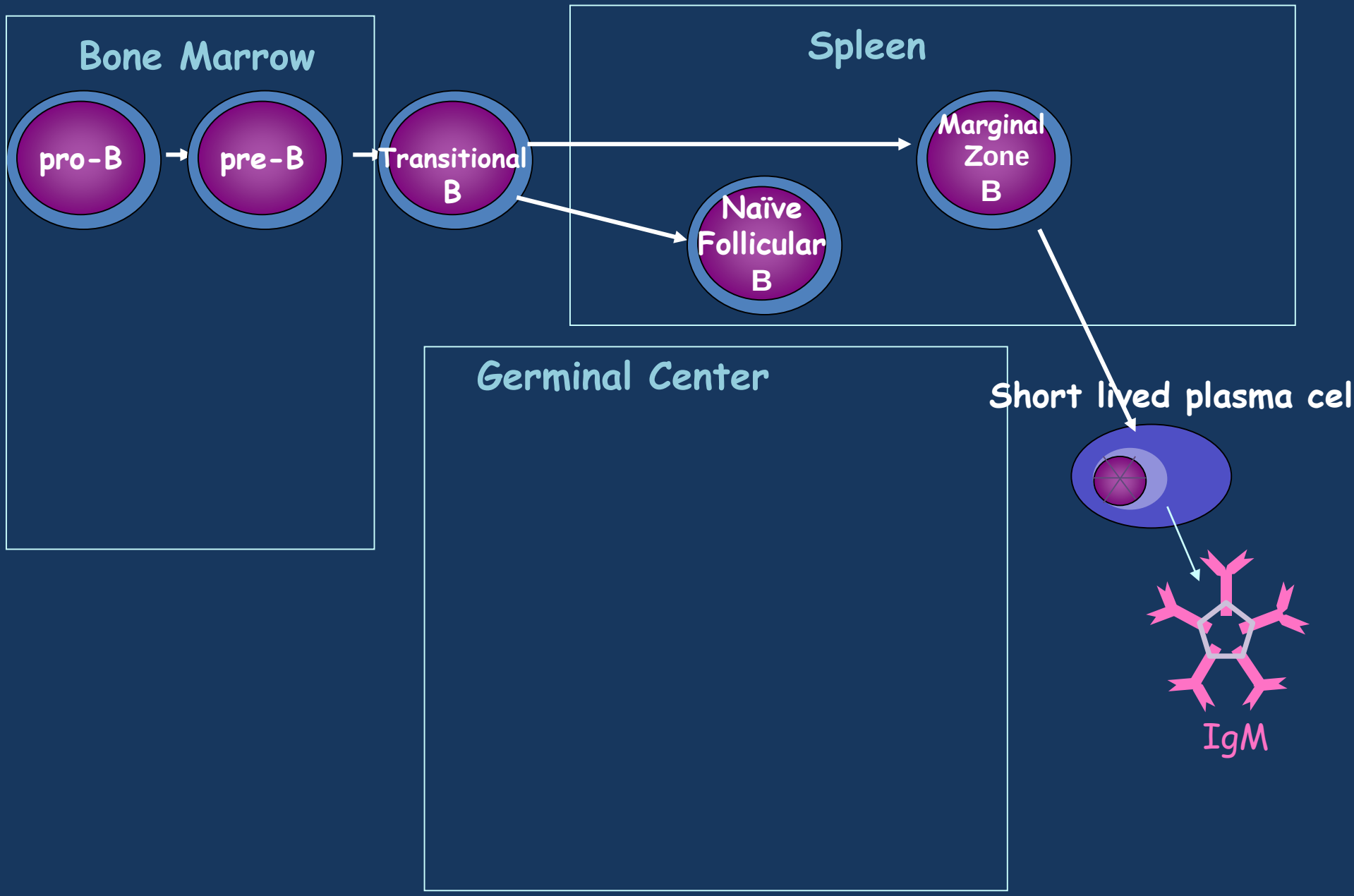
Spleen

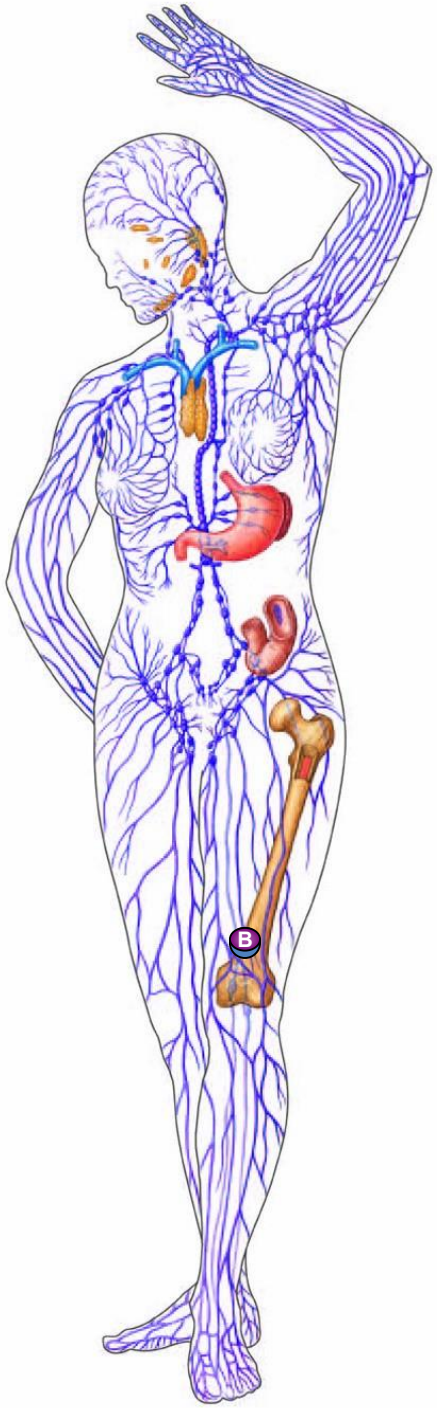
Germinal Center

Basic B cell development pathway

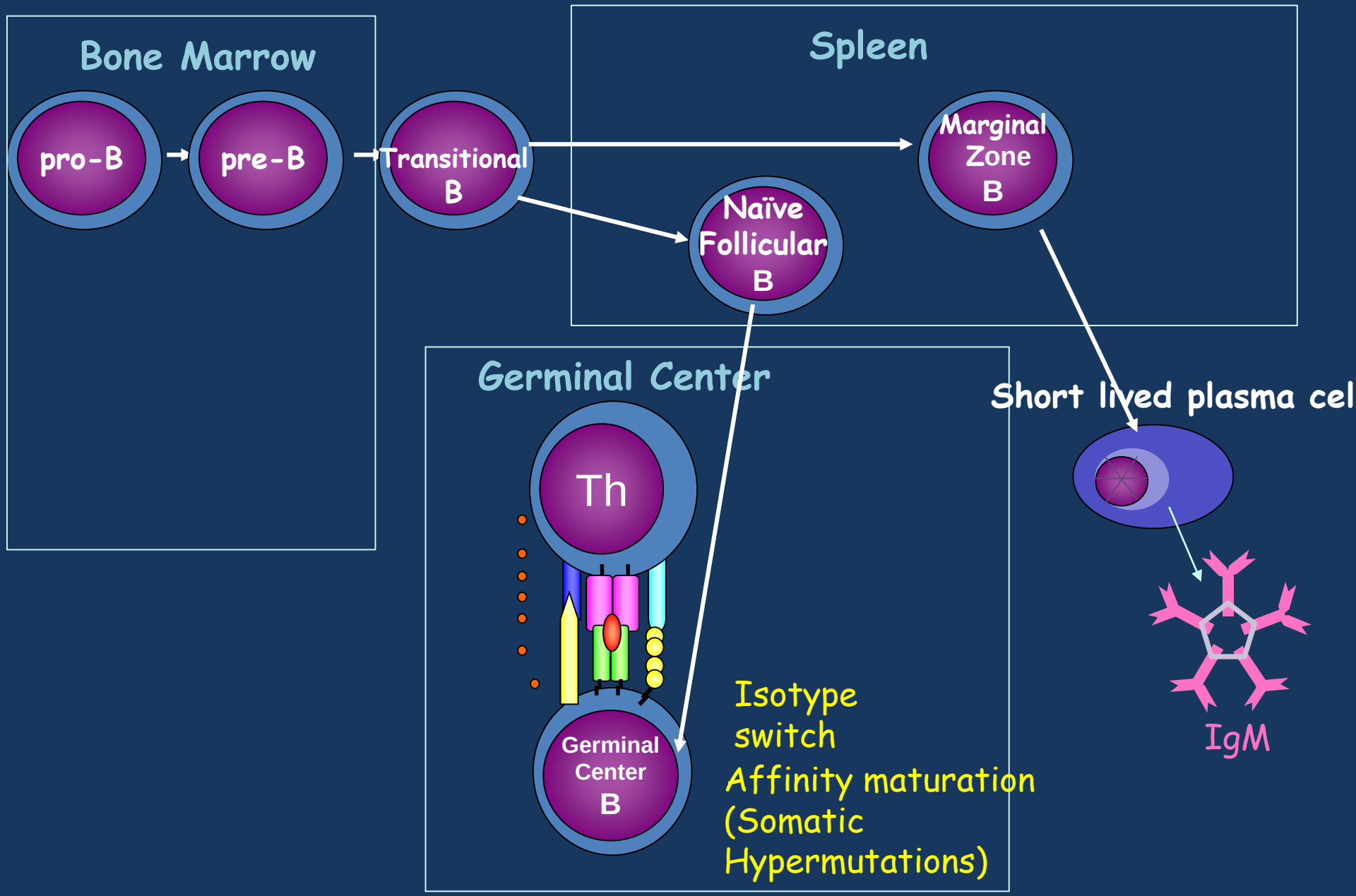


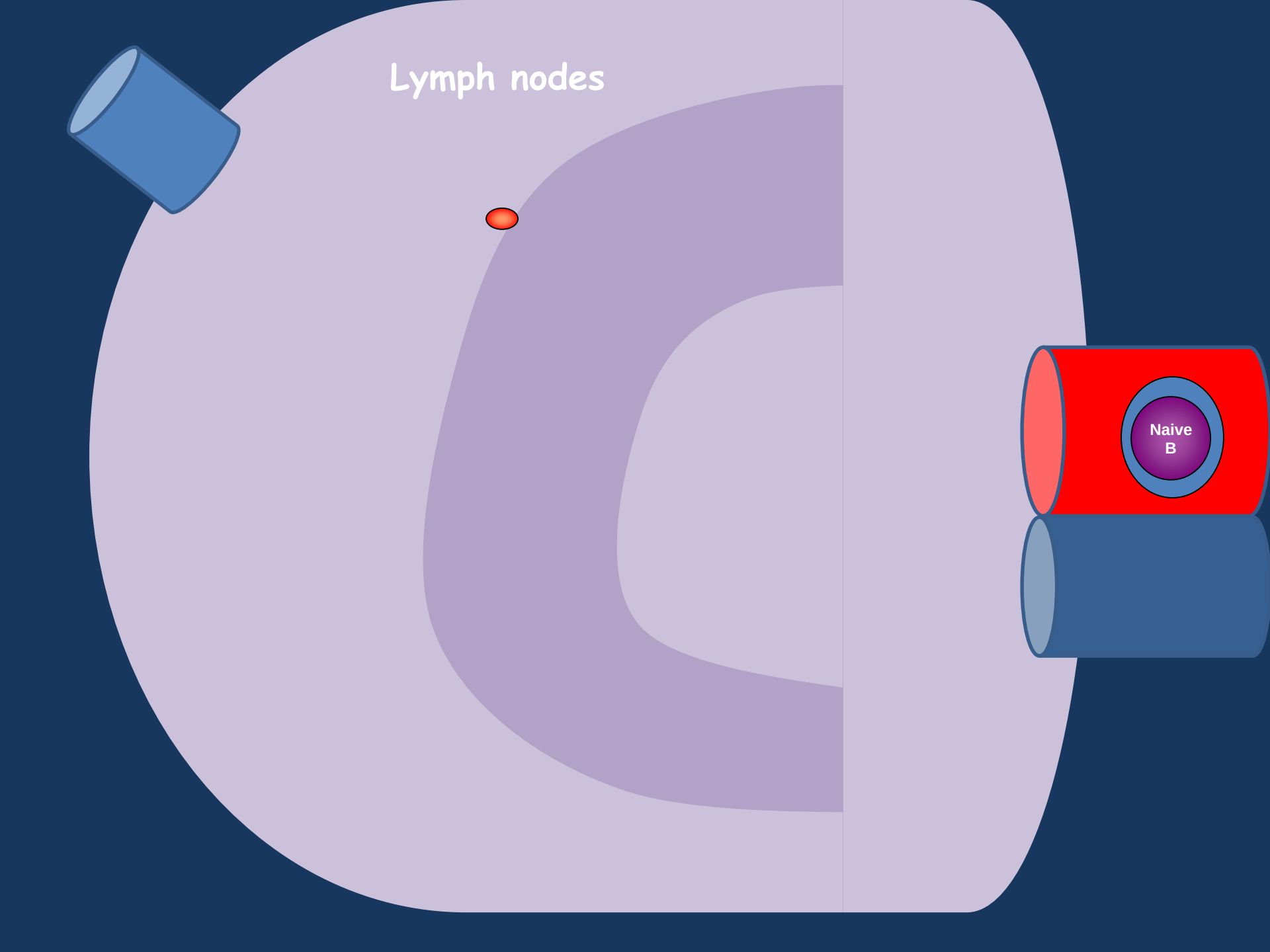
Basic B cell development pathway

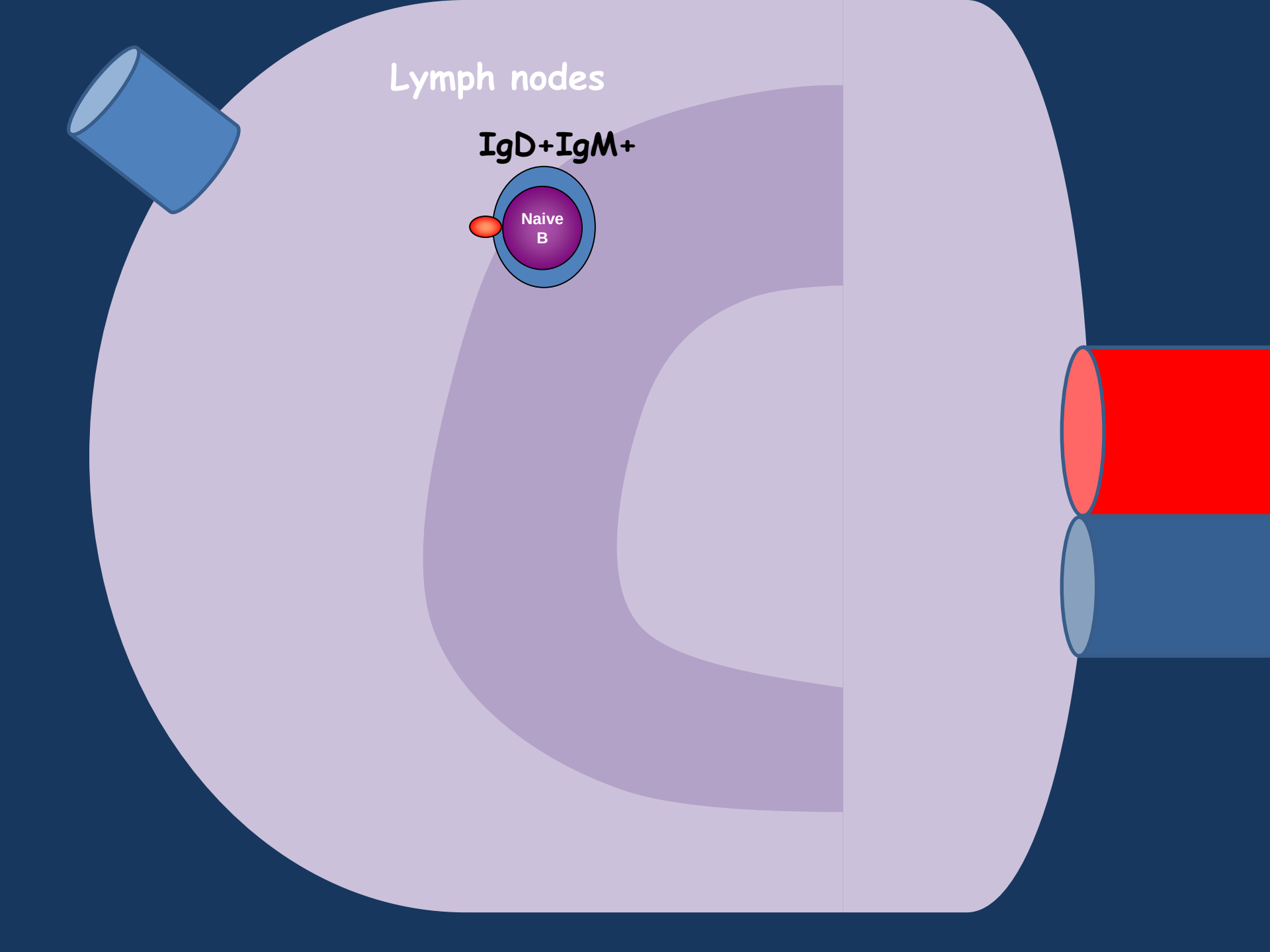


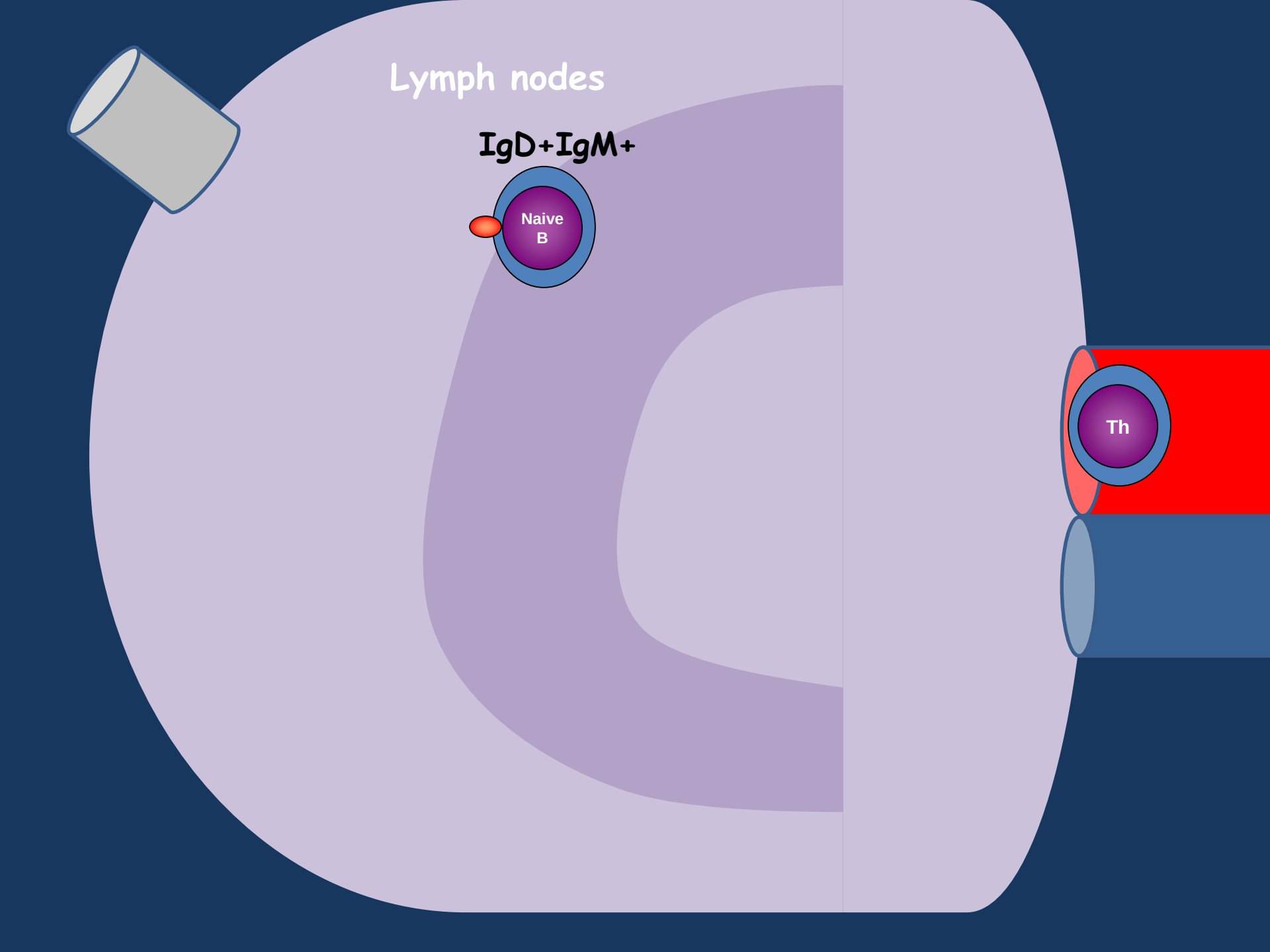

B

Basic B cell development pathway



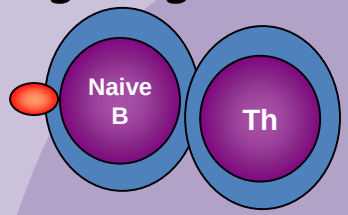

Lymph nodes
Naive
B


Lymph nodes
IgD+IgM+
Naive
B


Lymph nodes
IgD+IgM+
Naive B
Th

Lymph nodes

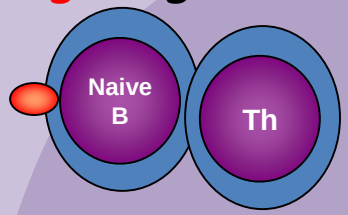
IgD+IgM+



T cell help (CD40-CD40L)
Class switch

Fate depends on BCR affinity!!!!

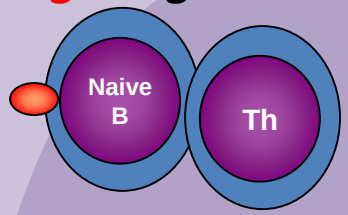
IgD-IgM+



T cell help (CD40-CD40L)
Class switch

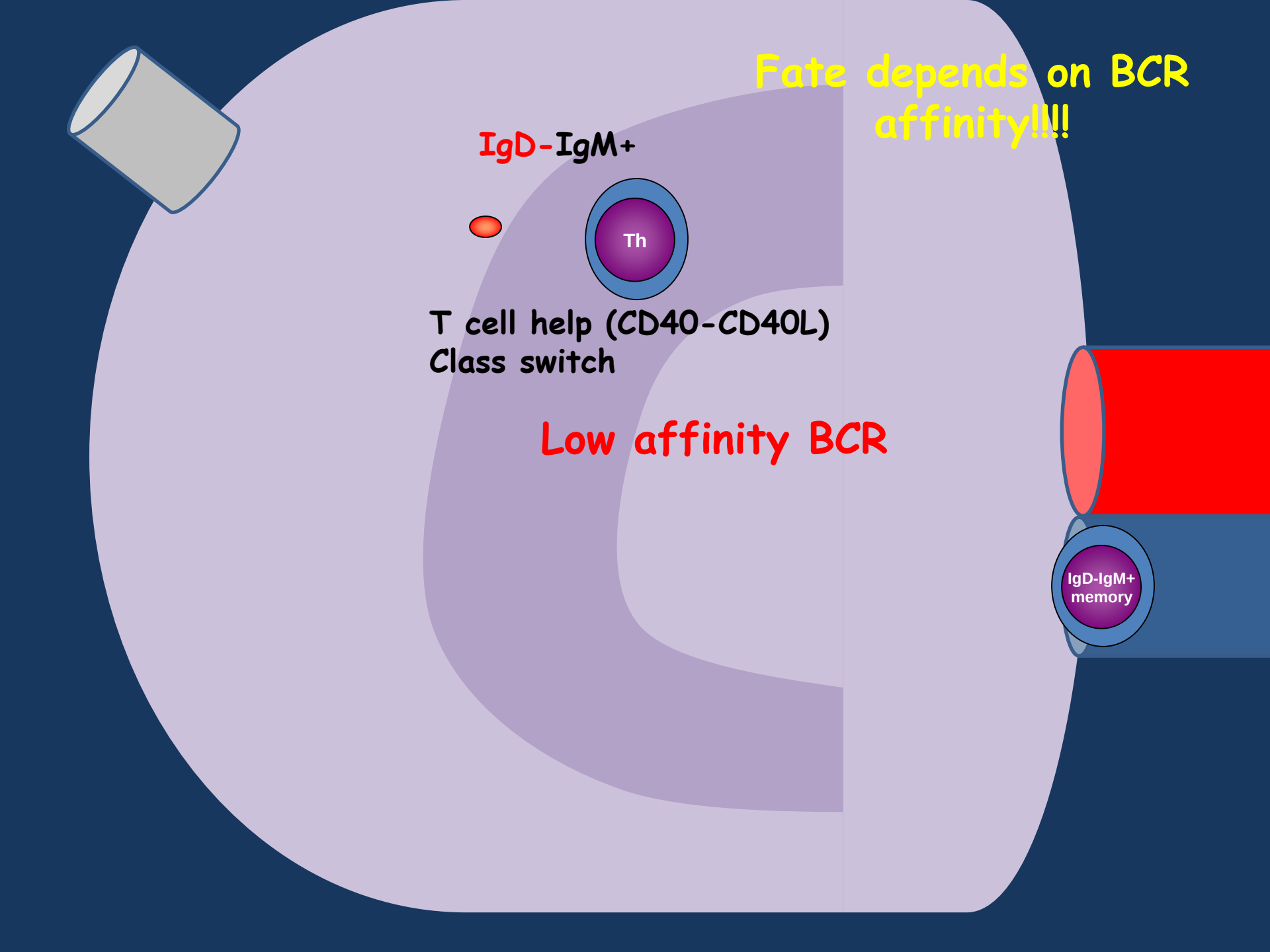
Fate depends on BCR affinity!!!!

IgD-IgM+



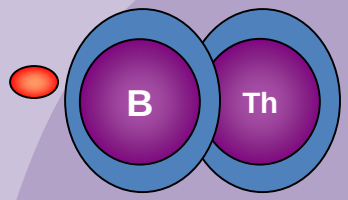
T cell help (CD40-CD40L)
Class switch

Low affinity BCR


Fate depends on BCR affinity!!!!
IgD-IgM+
Th
T cell help (CD40-CD40L)
Class switch
Low affinity BCR
IgD-IgM+ memory

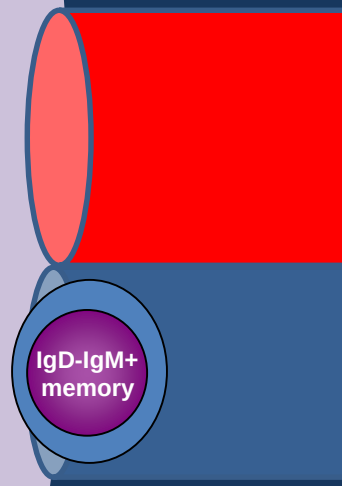
Fate depends on BCR affinity!!!!

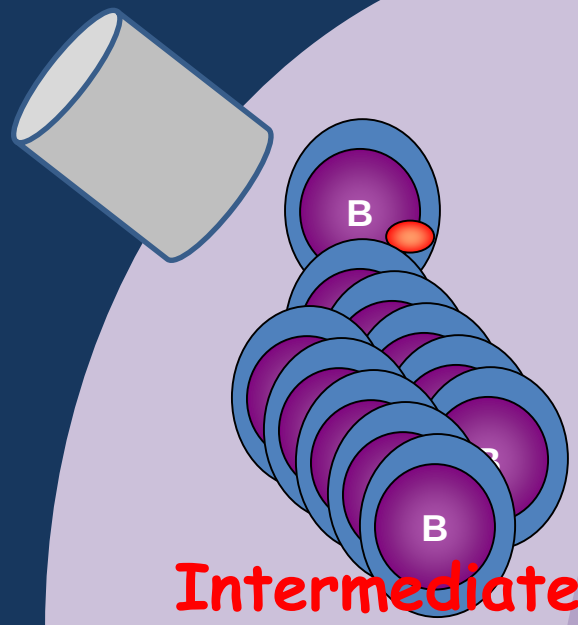
IgD-IgM+



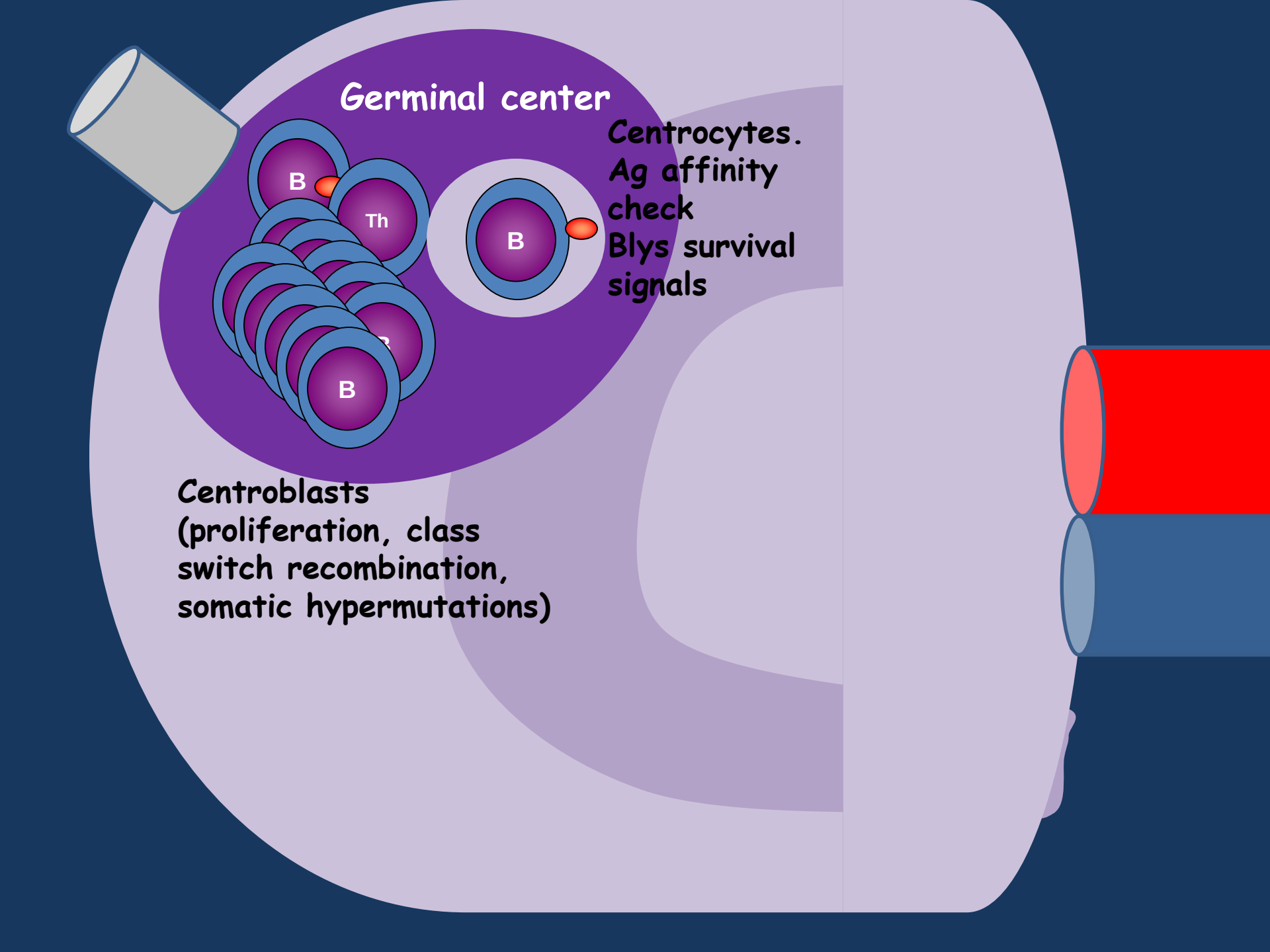
T cell help (CD40-CD40L)
Class switch

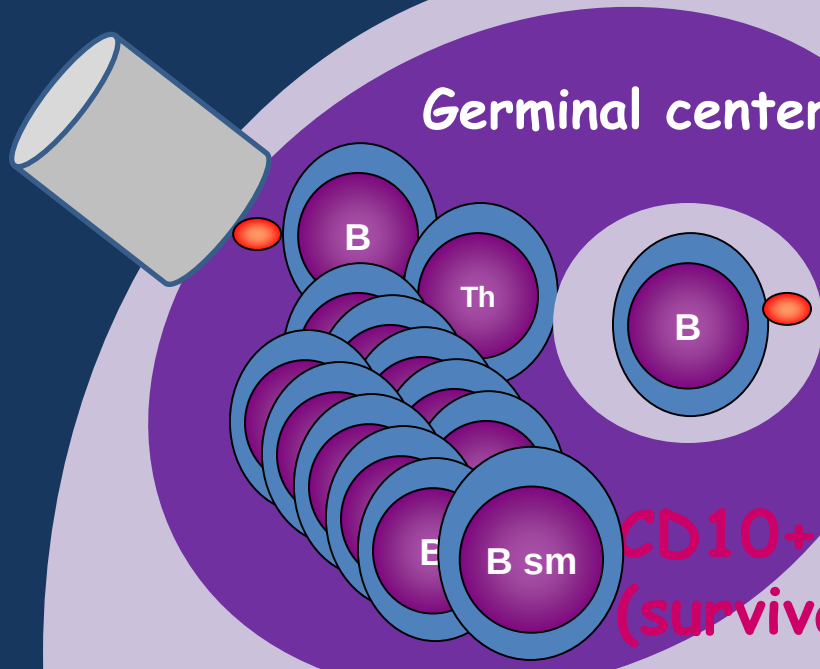
Intermediate affinity
+ IL21 signals





Intermediate affinity
+ IL21 signals


Germinal center
B
Th
B
B
Centrocytes.
Ag affinity
check
Blys survival
signals
Centroblasts
(proliferation, class
switch recombination,
somatic hypermutations)

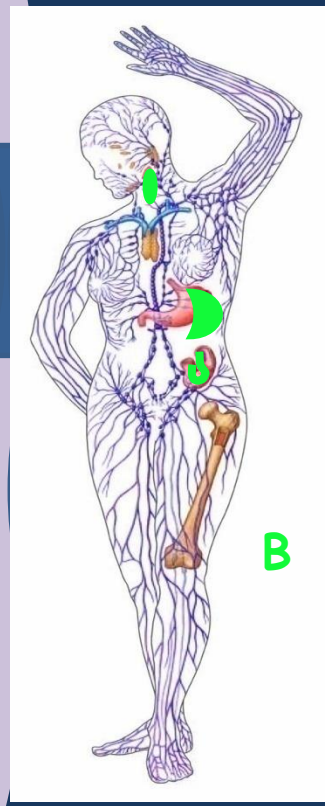


Centrocytes.
Ag affinity
check
Blys s
signal

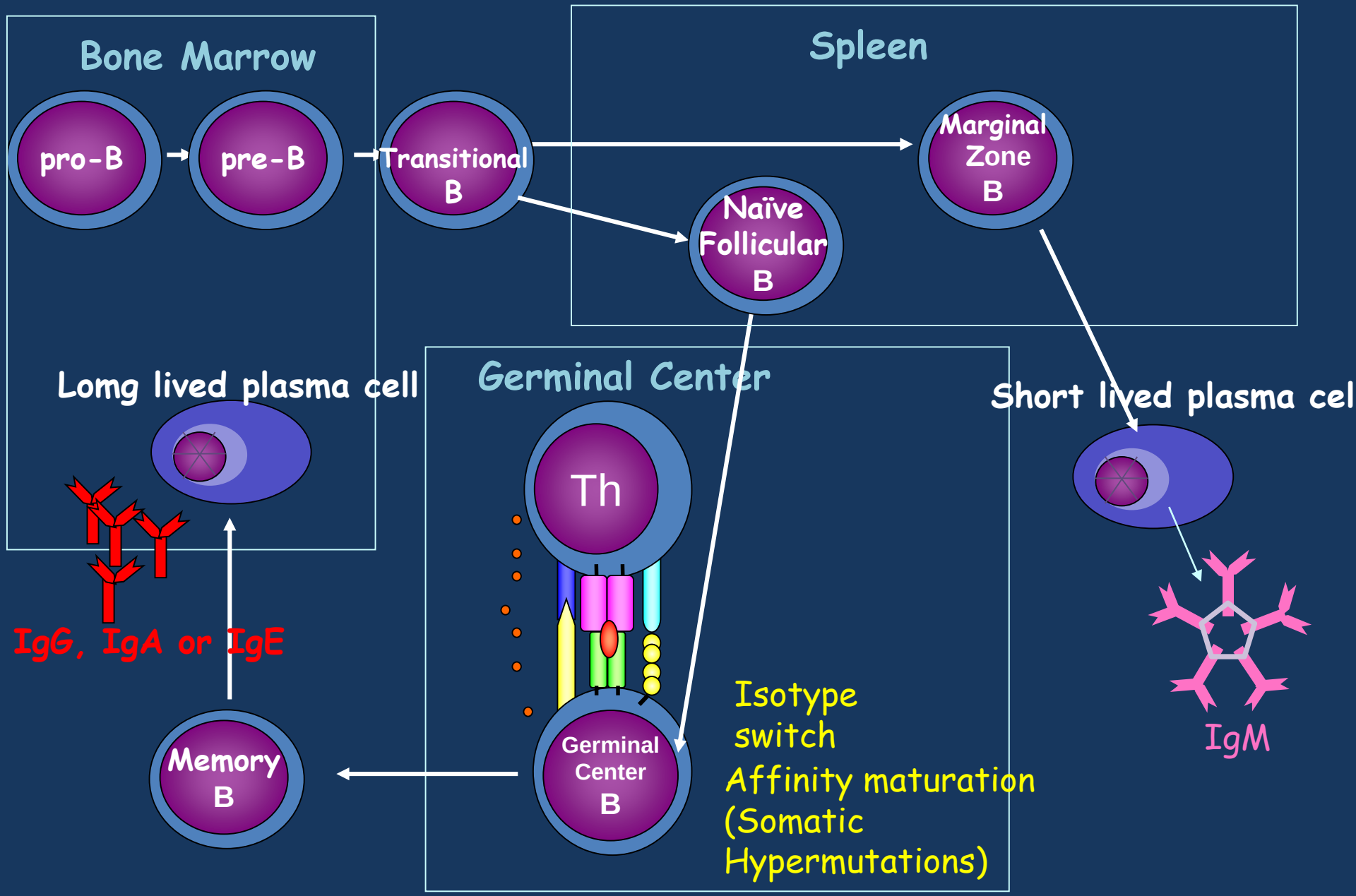
CD10+, CD
(survival)

Centroblasts
(proliferation,
class switch
recombination,
somatic hypermutations)

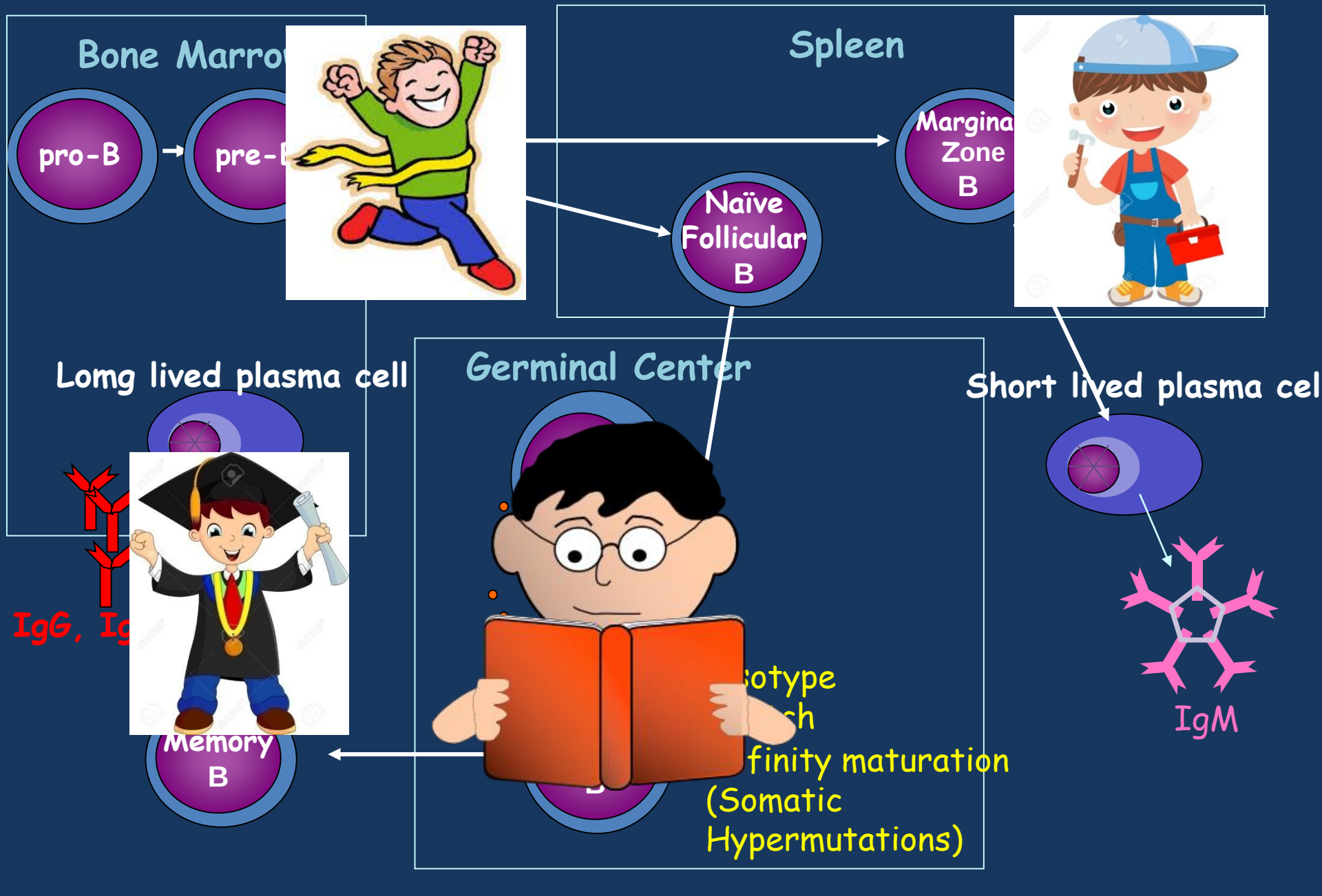
CD27+ IgD-IgM-



Basic B cell development pathway



Basic B cell development pathway

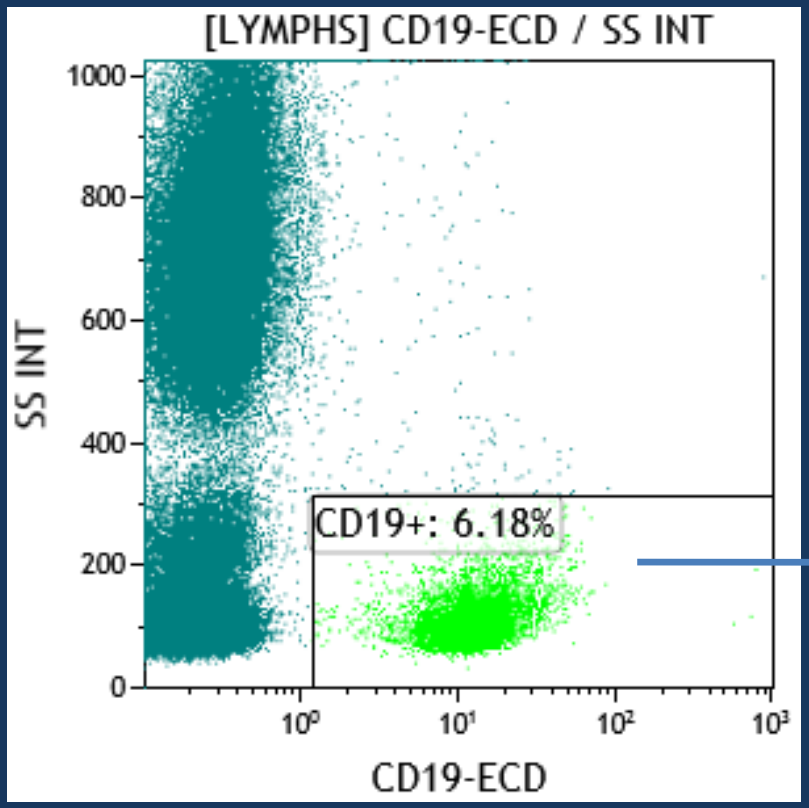


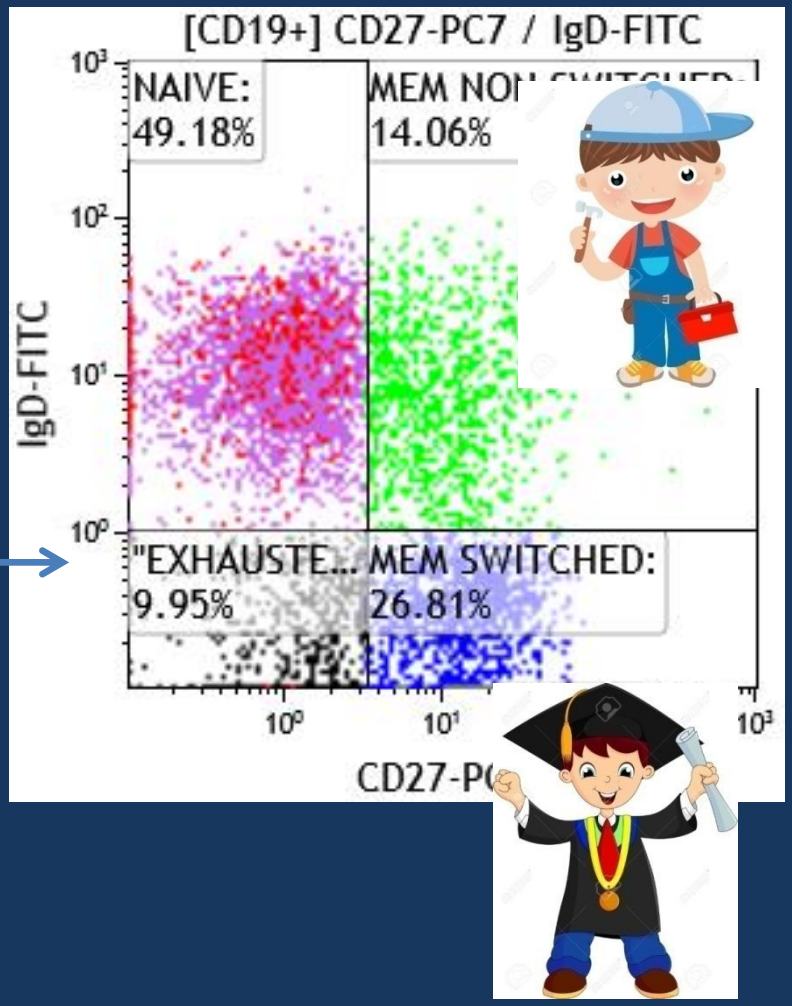
Which markers are useful to identify these subsets??

Basic immunophenotypic markers of Peripheral Blood B cell characterization

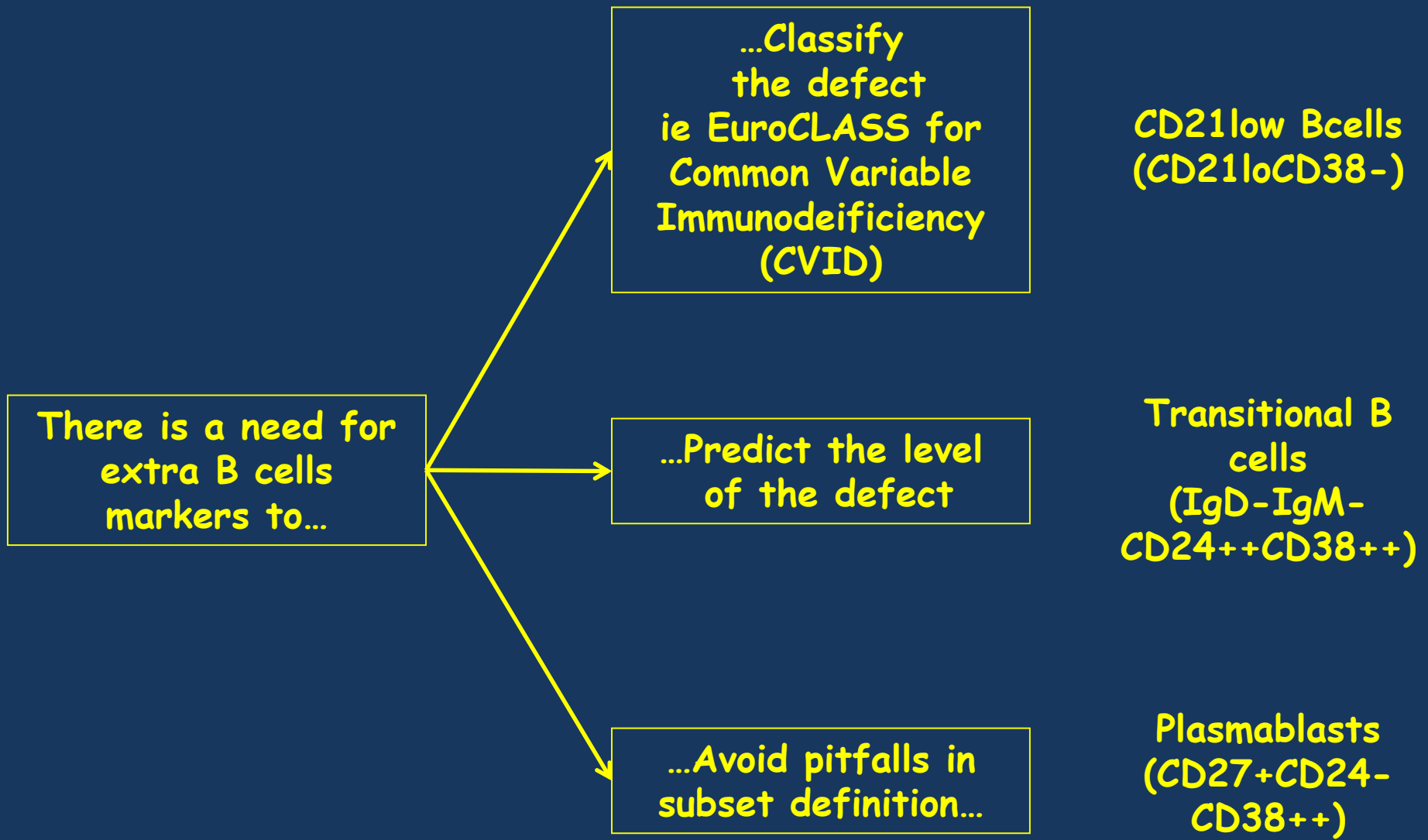
Marker	Role	B cell subset					
		...ve ...tional	...aïve Follicular	...emory ...tched	Ex...d	Plasmablasts	...arginal Zone like
CD19	forms complex with CD21 and CD81- co stimulation	+	+	+	+	+low	+
CD21	Complement Receptor- Co receptor	med	+	+	.ow	med	++
CD27	TNF-R family- co stimulation	-	-	+	-	++	+
CD23	FcεR- IgE coated Ag capture in GC	-	+	-	-	-	-
CD24	GPI anchored- Cell adhesion +...	++	+	++		-	++
IgM		++	low/+	-	-	-	++
IgD		low	++	-	-	-	low
CD38	cyclic ADP ribose hydrolase-222	++	+	+		+++	-

Basic gating strategies on CD19+ cells (I)



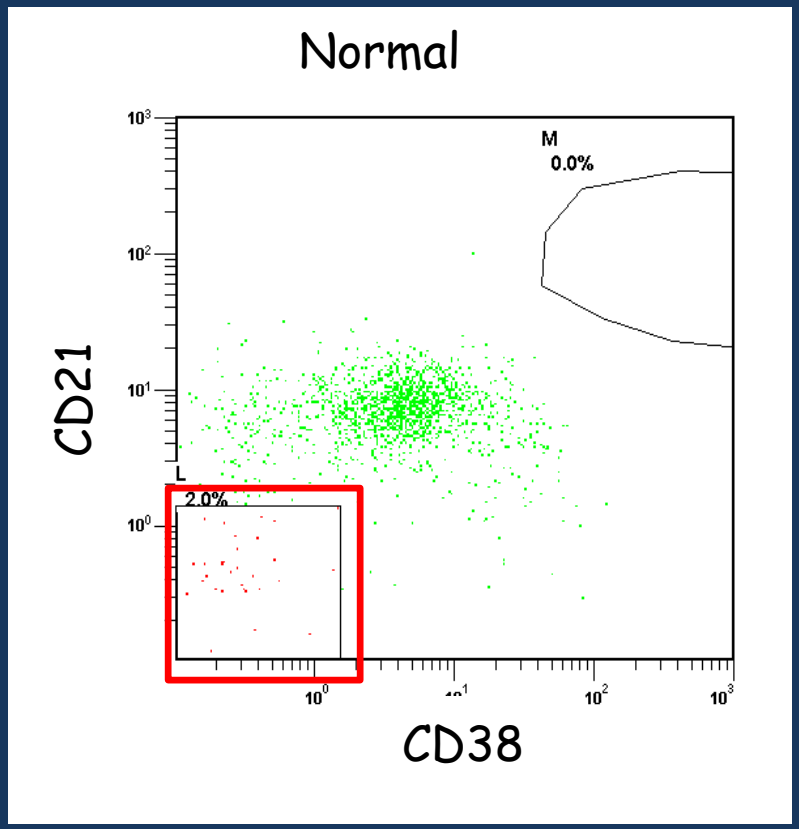


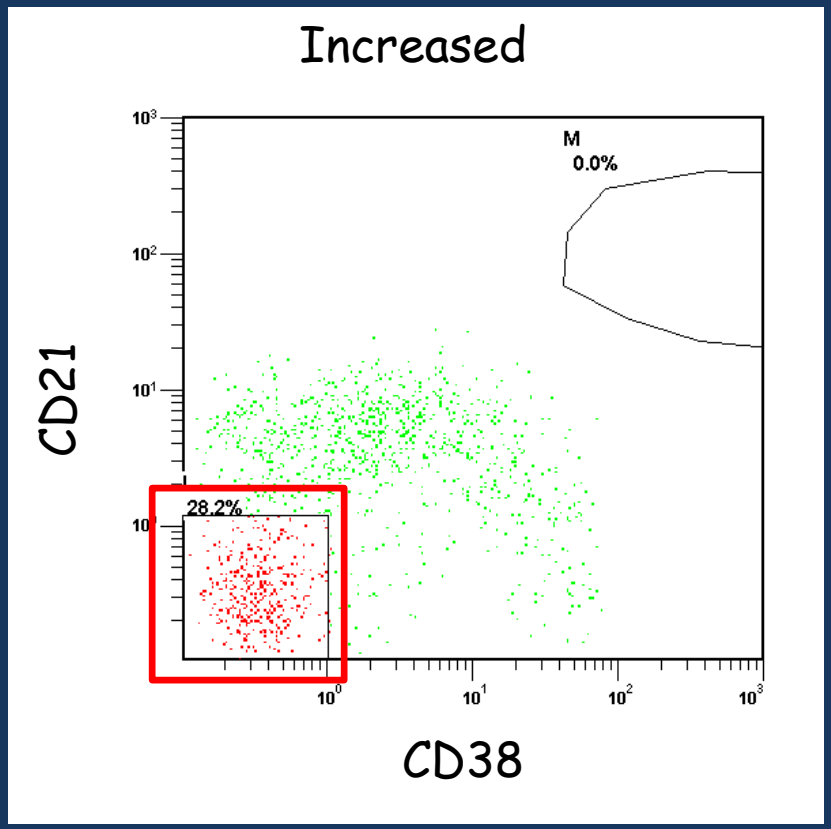
Is this enough??



Basic gating strategies on CD19+ cells (II)

CD21low

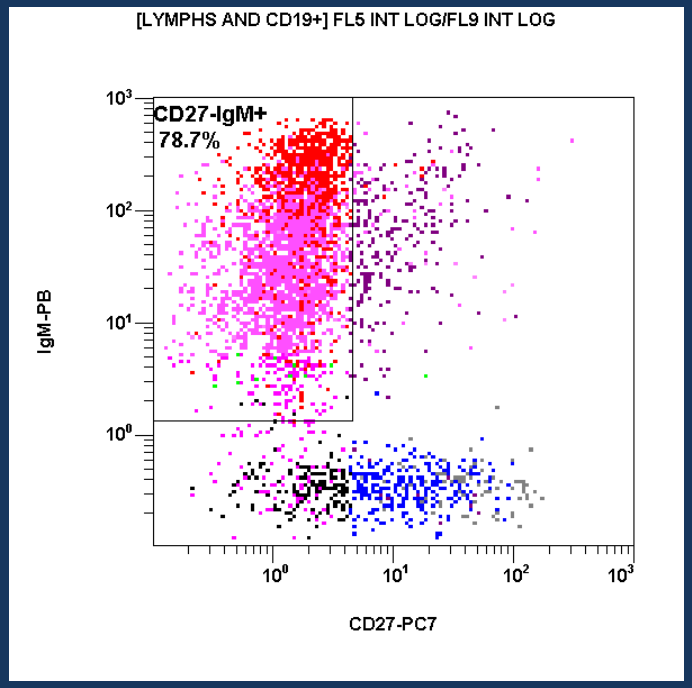


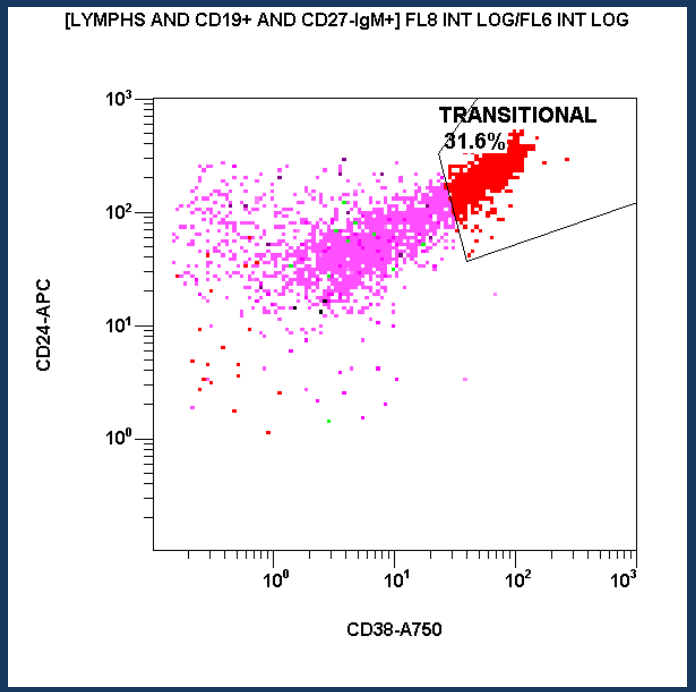


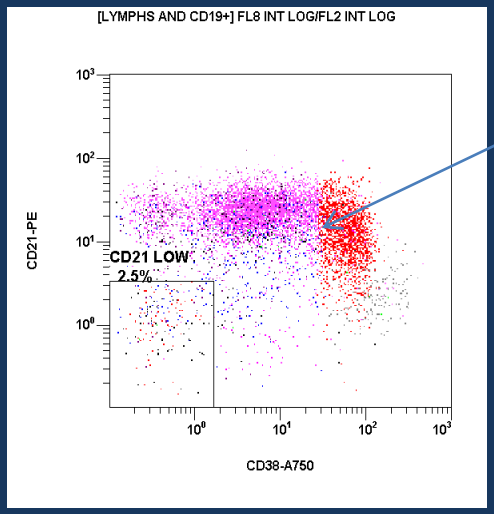
CD19+

Basic gating strategies on CD19+ cells (III)

Transitional B cells





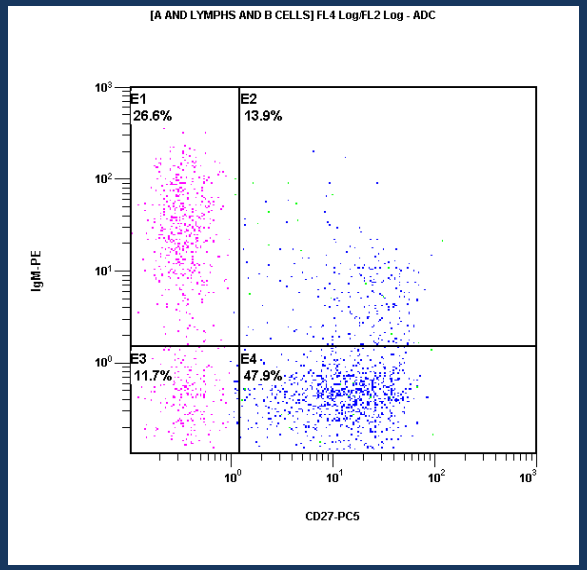


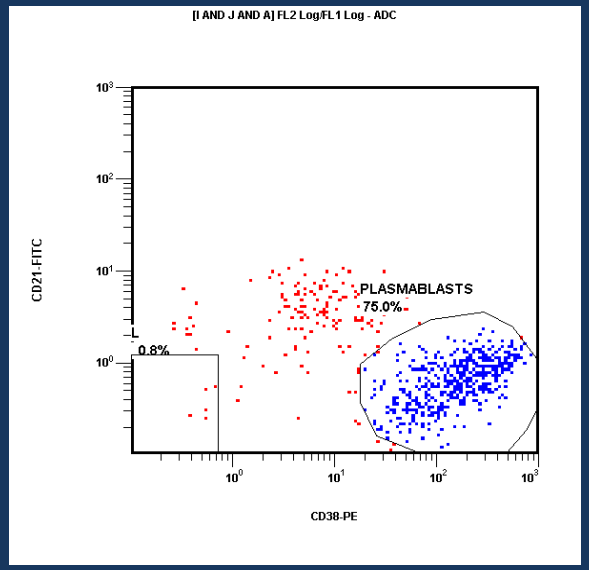
HELPS DEFINE TRANSITIONAL REGION

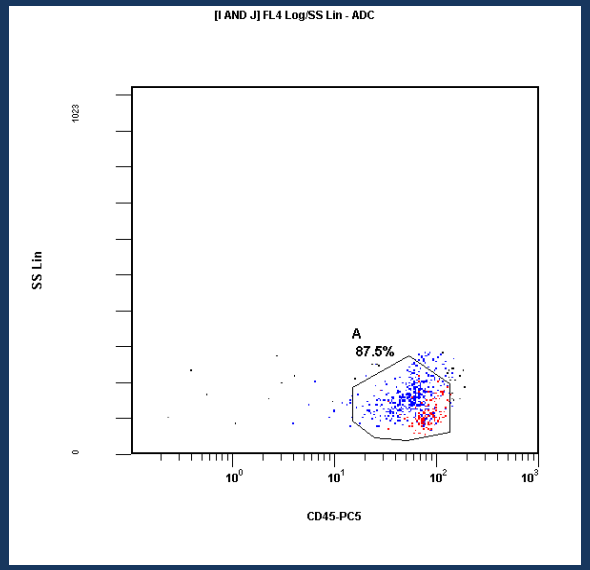
Gate	Number	Logic
All	399,696	Ungated
LYMPHS	66,565	LYMPHS
CD19+	7,915	CD19+ AND LYMPHS
CD27-IgM+	6,492	CD27-IgM+ AND CD19+ AND LYMPHS
TRANSITIONAL	1,788	TRANSITIONAL AND CD27-IgM+ AND CD19+ AND LYMPHS

Basic gating strategies on CD19+ cells (IV)

a quite common pitfall





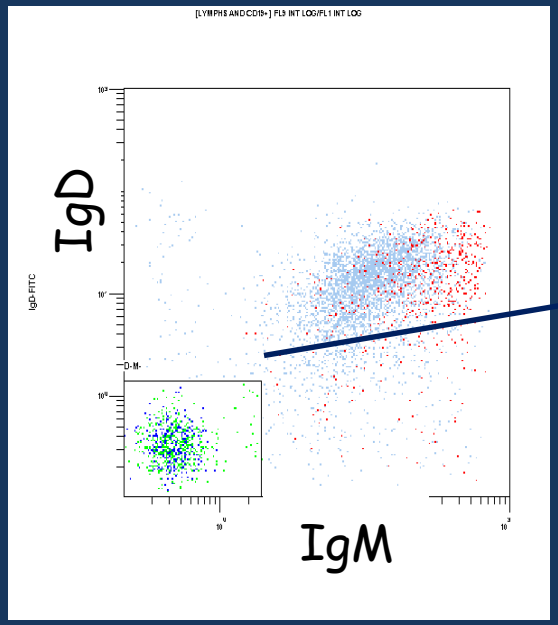


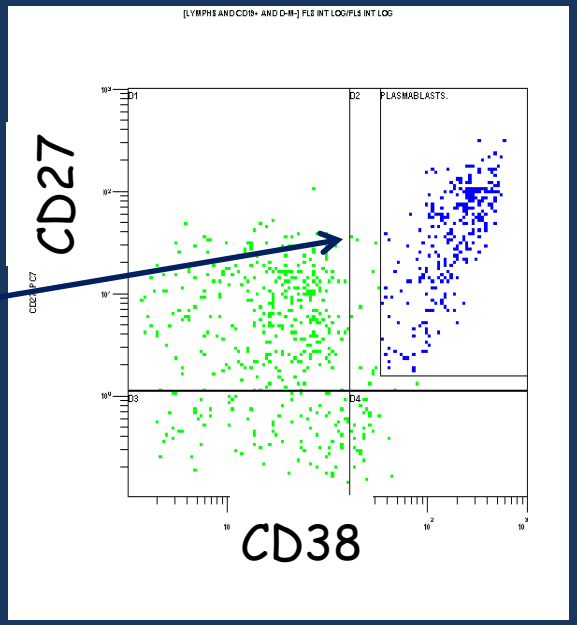
Does this patient have so many memory switched B cells?

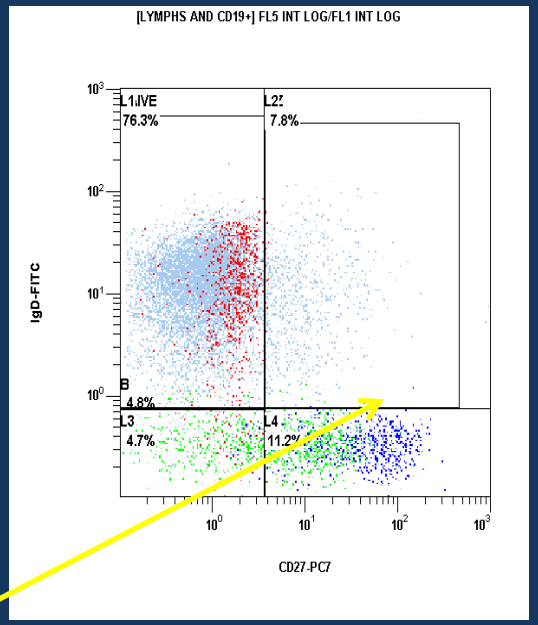
Most of them are plasmablasts!

Basic gating strategies on CD19+ cells (IV)

Plasmablasts



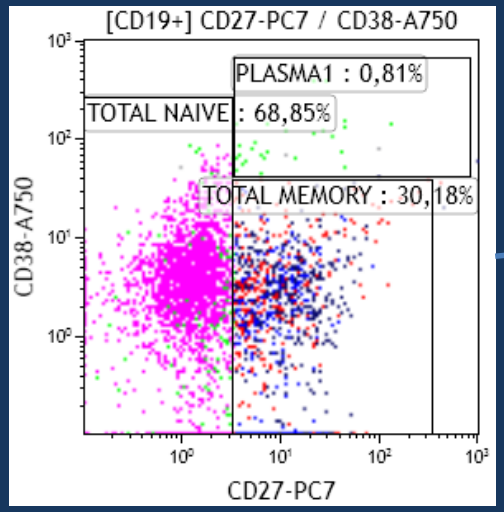


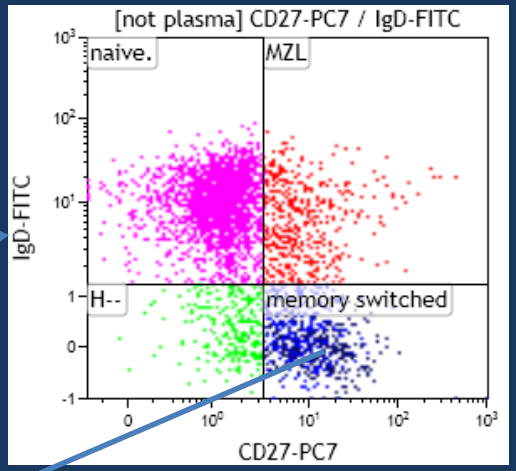


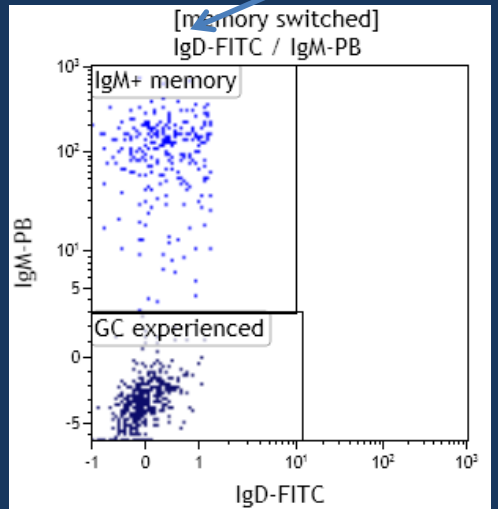
They increase the % of the Memory switched region

Basic gating strategies on CD19+ cells (IV)

Exclude Plasmablasts when estimating memory B cells







Be careful
with normal values!

B cell subset values for adults

Cytometry PART A
Journal of the International Society for Advancement of Cytometry

MIFlowCyt

Age and Gender Leucocytes Variances and References Values Generated Using the Standardized ONE-Study Protocol

Anders H. Kverneland,[1,2] Mathias Streitz,[1] Edward Geissler,[3] James Hutchinson,[3] Katrin Vogt,[1] David Boës,[1] Nadja Niemann,[1] Anders Elm Pedersen,[2] Stephan Schlickeiser,[1†] Birgit Sawitzki[1†*]

ALL CELL SUBSETS FIRST COHORT

DESCRIPTION	PHENOTYPE	DENOMINATOR	MEDIAN (RANGE) PROPORTIONAL (%)	MEDIAN (RANGE) ABSOLUTE (CELLS/μL)
B cells	$CD19^+$ $CD3^-$	Lymphocytes	11.3 (3.8–21.5)	205 (51–728)
Naïve B cells	$CD19^+$ $CD27^-$ IgD^+	B cells	53.6 (17.9–75.1)	43 (5–401)
Transitional B cells	$CD19^+$ $CD27^-$ $CD38^{high}$ IgM^+ $CD24^+$	B cells	2.1 (0.1–6.3)	2 (0–20)
MZ B cells	$CD19^+$ $CD27^+$ IgD^+	B cells	12.5 (3.8–35.6)	8 (1–84)
Memory B cells	$CD19^+$ $CD27^+$ $CD38^{dim}$	B cells	21 (11–46.6)	18 (3–80)
Class-switched B cells	$CD19^+$ $CD27^+$ $CD38^{dim}$ IgD^- IgM^-	B cells	15.9 (4.6–35.5)	14 (1–53)
Non-switched B cells	$CD19^+$ $CD27^+$ $CD38^{dim}$ IgM^+	B cells	6 (1.9–23.7)	5 (1–28)
Plasmablasts	$CD19^+$ $CD27^{high}$ $CD38^{high}$ IgD^- IgM^-	B cells	1.2 (0.3–7.8)	1 (0–6)
CD21low B cells	$CD19^+$ $CD38^{low}$ $CD21^{low}$	B cells	6.2 (1.9–19.3)	5 (1–37)

Cytometry PART A
ISAC
Journal of Quantitative Cell Science

MIFlowCyt

OMIP-047: High-Dimensional Phenotypic Characterization of B Cells

Thomas Liechti,[1*] Huldrych F. Günthard,[1,2] Alexandra Trkola[1*]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland

[2]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland

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Grant sponsor: Clinical Priority Research Program of the University of Zurich (Viral infectious diseases: Zurich Primary HIV

Purpose and Appropriate Sample Type

This 16-color, 18-parameter panel was designed to allow a detailed dissection of human B cell subsets and their phenotype in peripheral blood mononuclear cells (PBMC) in healthy donors and in the context of chronic viral diseases such as Human Immunodeficiency Virus 1 (HIV-1) infection. The panel encompasses a range of backbone markers for the accurate definition of common B cell subsets with a focus on memory B cells and a unique collection of phenotypic markers (chemokine receptors, cytokine receptor, B cell receptor isotypes, and proliferation marker) not combined in multicolor flow cytometry B cell phenotyping thus far. This new panel allows highly detailed phenotypic and functional investigations of B cell subsets. The panel was validated using cryopreserved PBMC from healthy and HIV-1 infected donors allowing the retrospective analysis of clinical samples (Table 1).

Cytometry Part B (Clinical Cytometry) 74B:261–271 (2008)

Review Article

Flowcytometric Phenotyping of Common Variable Immunodeficiency

Klaus Warnatz* and Michael Schlesier
Division of Rheumatology and Clinical Immunology, University Medical Center Freiburg, Freiburg, Germany

Table 3
Reference Values of B-Cell Subpopulations in Peripheral Blood

B-cell population		Reference range[a] (%)
CD19+ in CD45+ lymphocytes	B cells	4.9–8.4
IgM− IgD− in CD19+ B cells	Class switched B cells	7.6–31.4
IgD+ CD27− in CD19+ B cells	Naive B cells	42.6–82.3
IgD+ CD27+ in CD19+ B cells	Marginal zone-like B cells	7.4–32.5
IgM−IgD− CD27+ in CD19+ B cells	Class switched memory B cells (and Plasmablasts)	6.5–29.1
$CD21^{low}$ CD38− in CD19+ B cells	$CD21^{low}$ B cells	0.9–7.6
M++ D++ CD24++ CD38++ in CD19+ B cells	Transitional B cells	0.6–3.4
IgM-(+) CD24-CD38+++CD27++ in CD19+ B cells	Plasmablasts	0.4–3.6

[a]5. to 95. percentile, based on 54 healthy donors (age range 19–61 years).

B cell subset values for children
(Beware of subset definitions!!)

Clinical Immunology (2009) 131, 50–59

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yclim

Memory B-cells in healthy and antibody-deficient children

Kirsten Huck [a], Oliver Feyen [a], Sujal Ghosh [a], Kathrin Beltz [b], Sven Bellert [a], Tim Niehues [b,*]

[a] Department of Pediatric Oncology, Hematology and Clinical Immunology, Centre for Child and Adolescent Health, Heinrich Heine University of Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany
[b] Centre for Child and Adolescent Health, HELIOS Klinikum Krefeld, Academic Hospital, Heinrich Heine University of Düsseldorf, Lutherplatz 40, 47805 Krefeld, Germany

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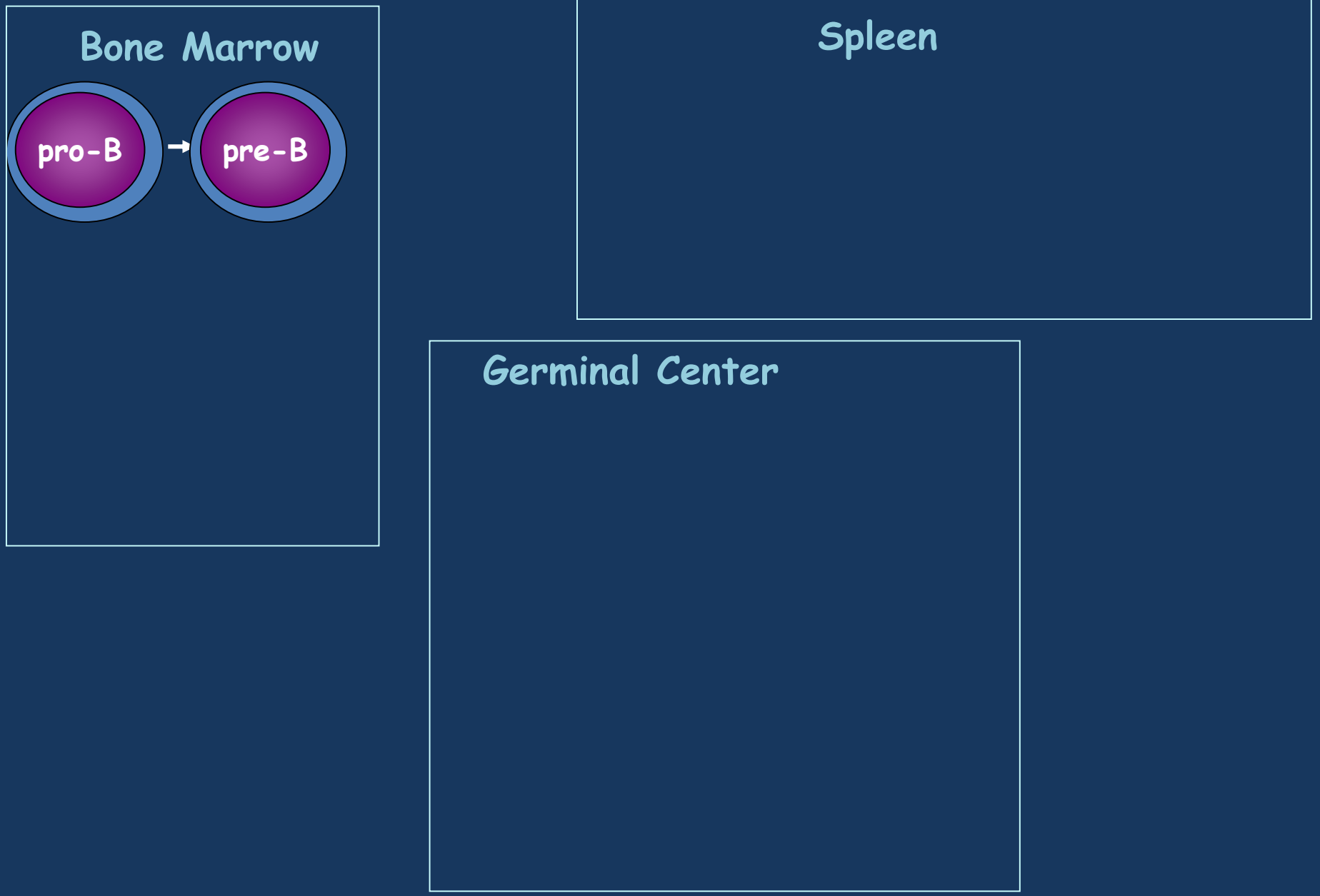
Available online 21 January 2009

CD27-IgD+ in %CD20	88	85	78.5	78	74,5
naive	88,4	84.2	77.8	77.03	75.27
	(84.5–93)	(77.4–90)	(70.7–85)	(66.1–87.6)	(63.3–87.9)
	(83.25–93.75)	(74.7–90.5)	(89.9–85.6)	(63.1–89.15)	(60.15–88.95)
CD27+IgD+ in %CD20	7	8	9.5	10.75	11
non-switched memory	6.81	8.7	10.1	10.76	11
	(4–9.5)	(5–14.3)	(7–14)	(4.7–17)	(6.1–16.9)
	(3.25–10.75)	(4.9–14.2)	(7–15.2)	(2.93–19)	(5.05–17.95)
CD27+IgD- in %CD20	3	5	8	8	9
switched memory	3.03	5.2	8.6	8.72	10.32
	(1.35–5)	(3–8)	(5–12.3)	(4–14)	(4.1–18.7)
	(1–5)	(2.9–9.2)	(3.9–16.2)	(3.85–16.5)	(4–22.8)

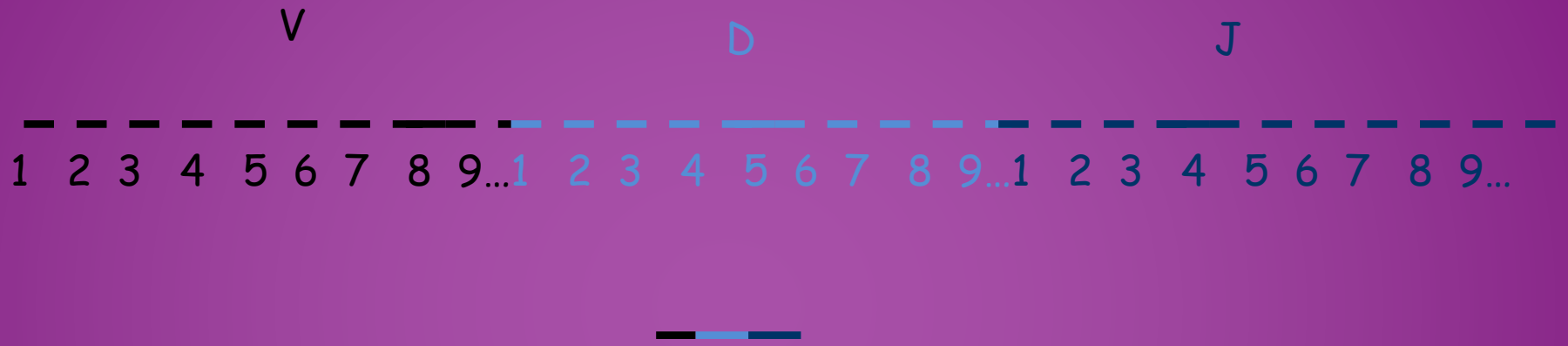
Which molecules are essential for the process??

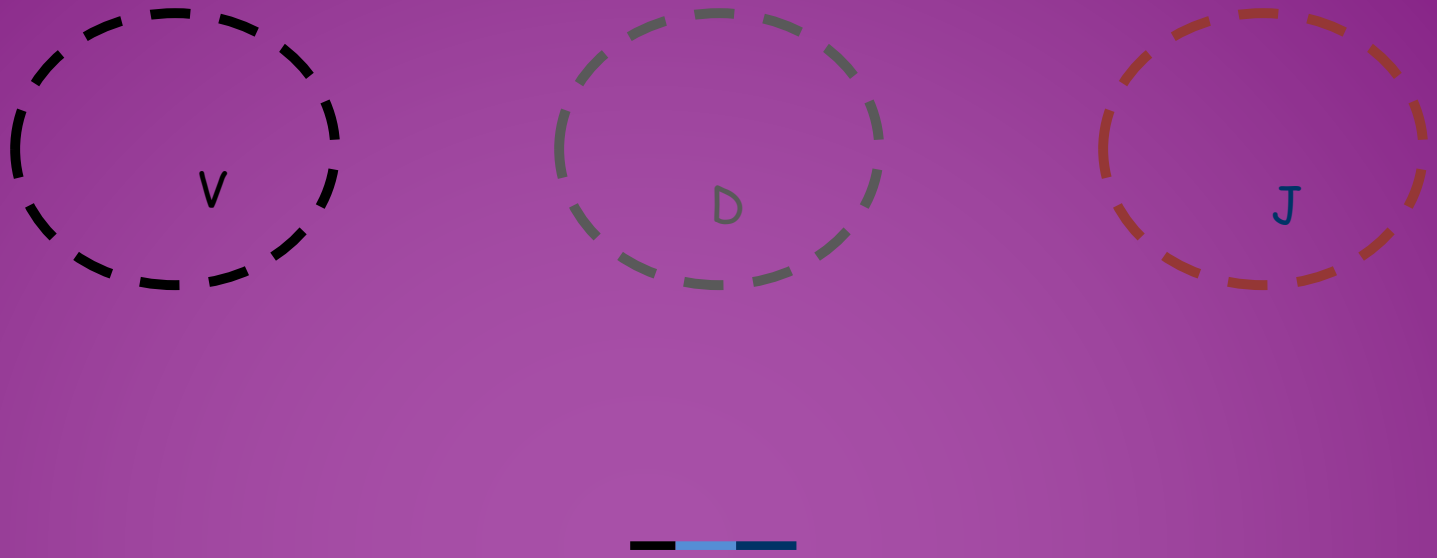
Basic B cell development pathway




V
D
J
1 2 3 4 5 6 7 8 9…1 2 3 4 5 6 7 8 9…1 2 3 4 5 6 7 8 9…


V
D
J
1 2 3 4 5 6 7 8 9…1 2 3 4 5 6 7 8 9…1 2 3 4 5 6 7 8 9…

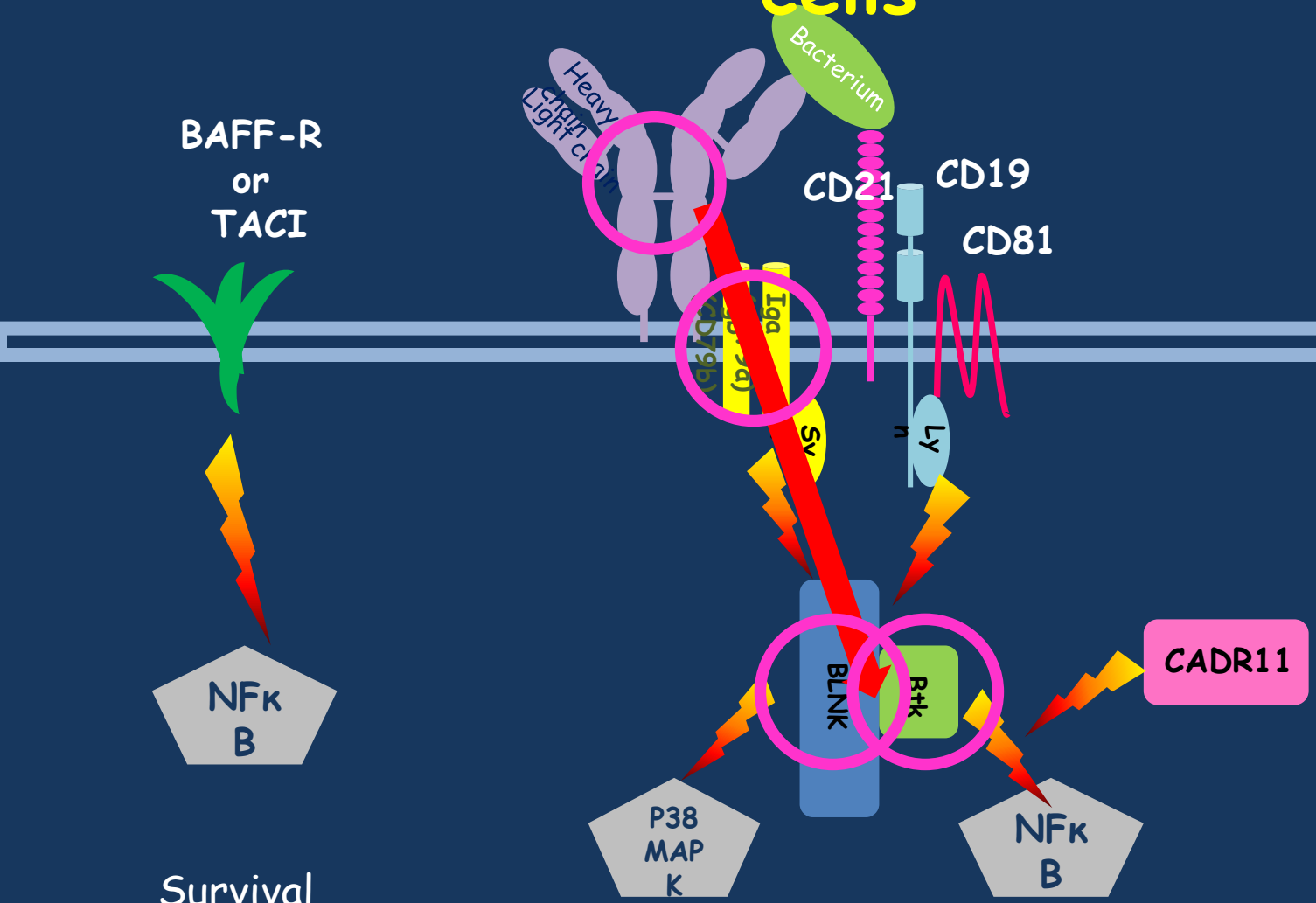

V
D
J
1 2 3 4 5 6 7 8 9…1 2 3 4 5 6 7 8 9…1 2 3 4 5 6 7 8 9…



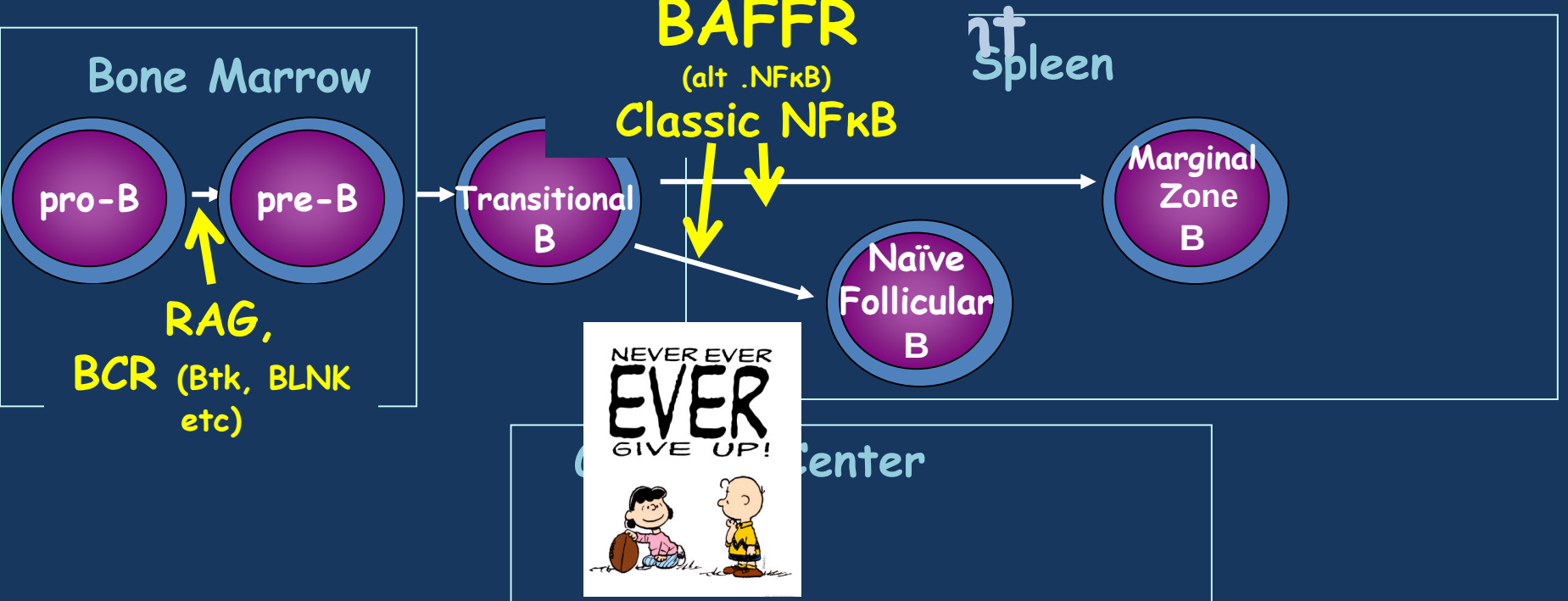
VDJ Recombination

RAG!!!
Artemis, DNAligase IV, DNA PKcs…

Basic receptors and signal transduction in B cells



Key molecules and signals in B cell



Basic receptors and signal transduction in B cells

BAFF-R
or
TACI

Heavy chain
Light chain

Bacterium

CD21

CD19

CD81

Iga (CD79a)

Igβ (CD79b)

Syk

Lyn

BLNK

Btk

CADR11

NFκB

P38 MAPK

NFκB

Survival

Proliferation
Transcription regulation

Regulation of
apoptosis vs survival
Inflammation

Basic B cell development pathway

Bone Marrow

pro-B → pre-B → Transitional B

Spleen

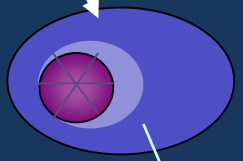
Marginal Zone B

Naïve Follicular B

TACI
TLRs

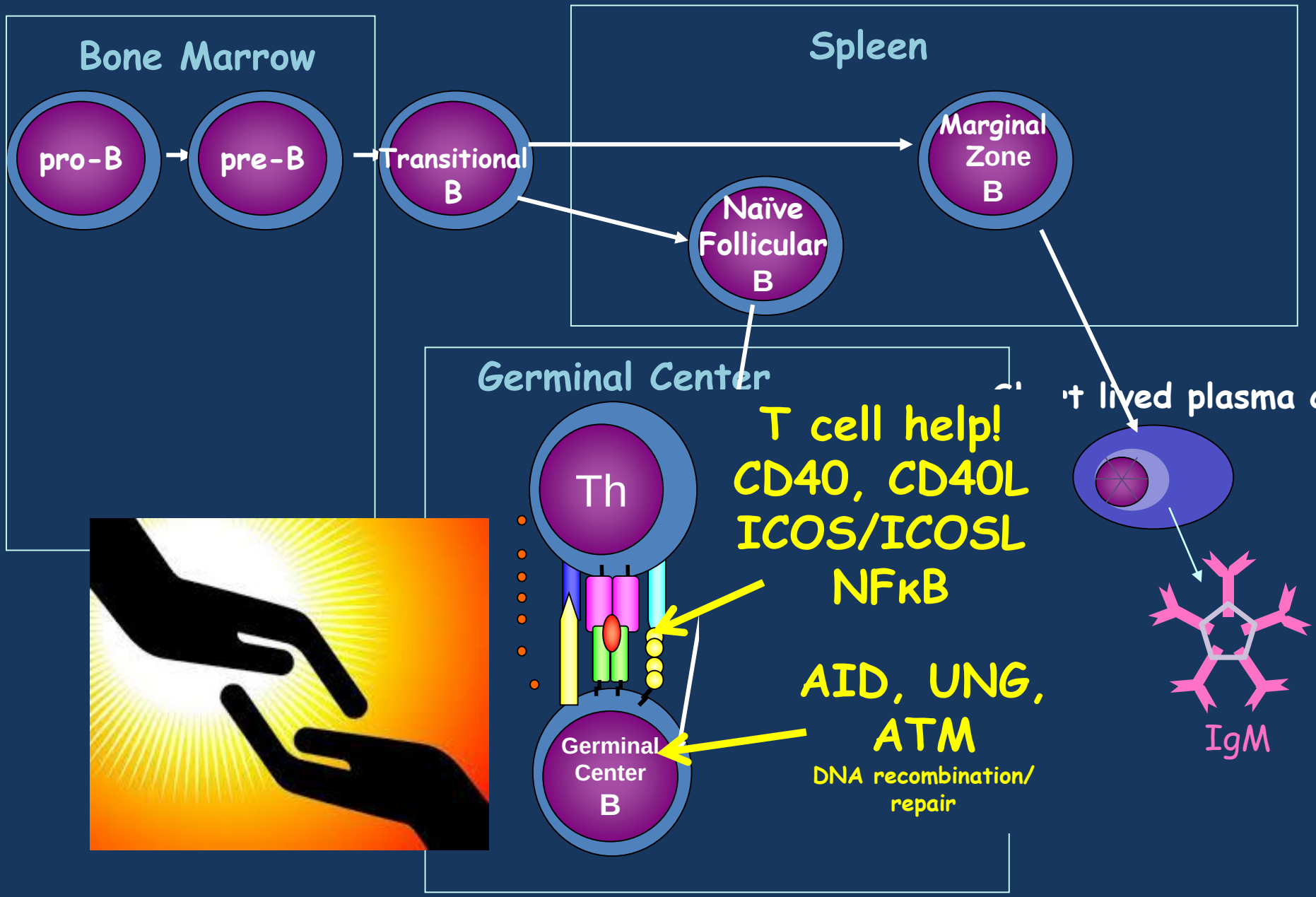
Germinal Center

Short lived plasma cel



IgM

Basic B cell development pathway




VDJ
Cμ
Cδ
Cγ3
Cγ1
Cγ2b
Cγ2a
Cε
Cα

IgE

CS Recombination

AID!!!

UNG, ATM,

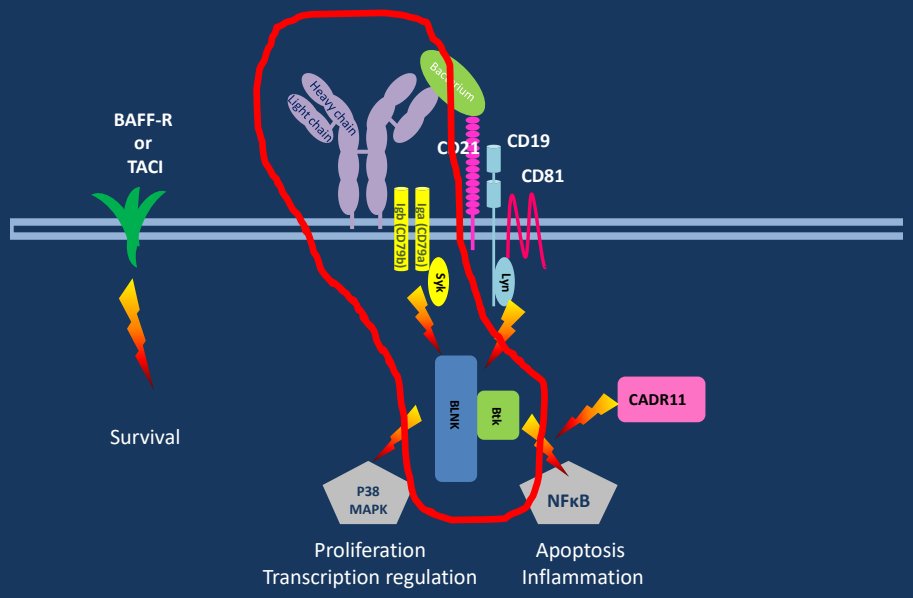
How can we spot the defect ?

No B cells in the Peripheral Blood

Block in

VDJ recombination (RAG1/RAG2) NHEJ (Artemis Cernunnos, LIG4, DNA-PKcs)

BCR signals



SCID phenotype

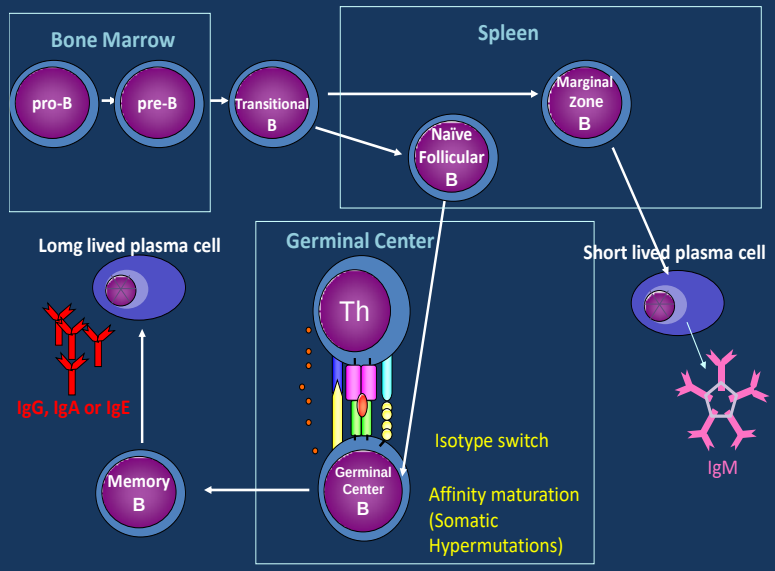
Agammaglobulinaemia

Detectable B cells in the Peripheral Blood with antibody deficiency

B cell subsets can give clues on underlying mechanisms

5 Patterns have been proposed

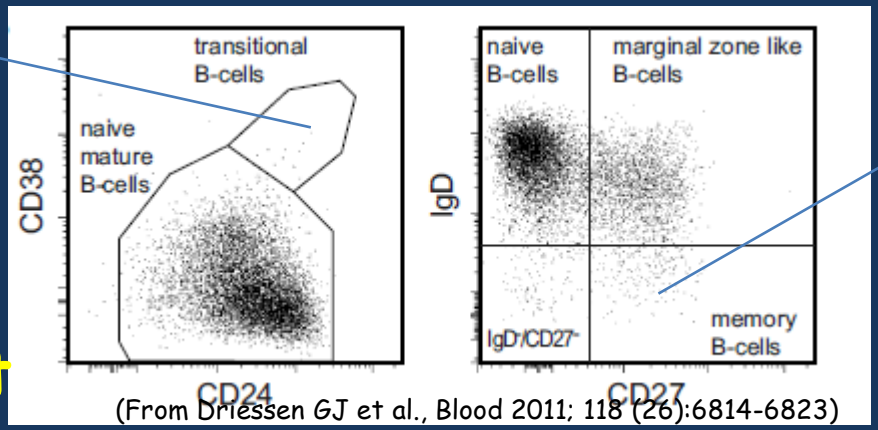
(Driessen GJ et al., Blood 2011; 118 (26):6814-6823)



Pattern 1

Low transitional B

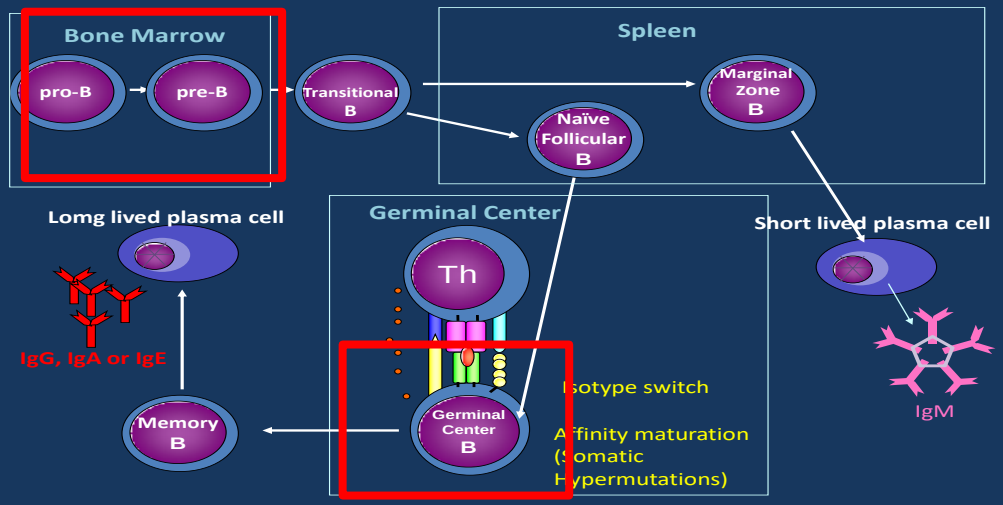
Low BM output



(From Driessen GJ et al., Blood 2011; 118 (26):6814-6823)

Low switched memory B

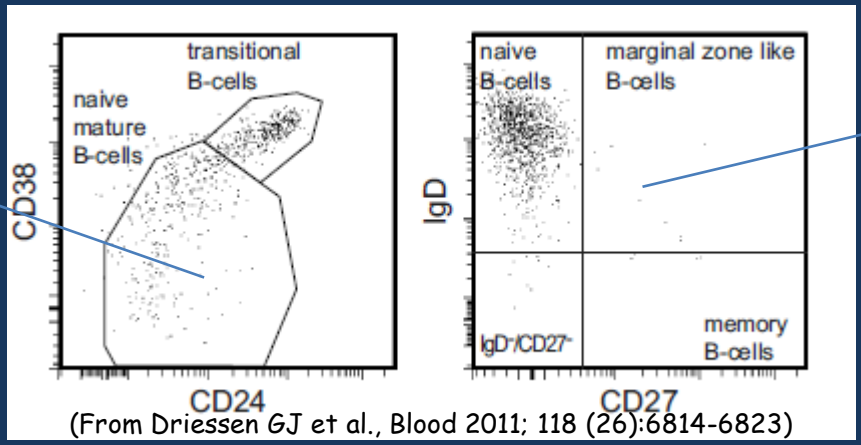
Germinal center deffect



Common mechanism: DNA repair (eg. Niimengen sy)

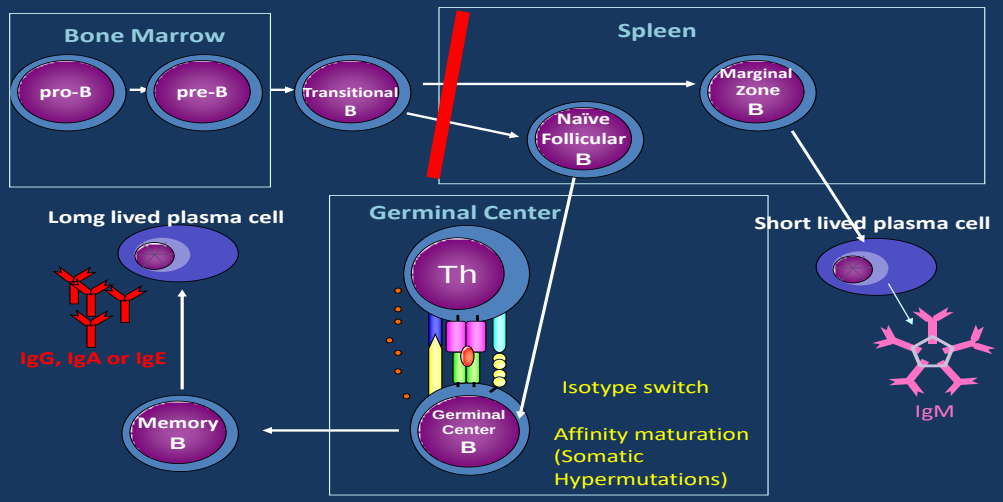
Pattern 2

Low % of cells beyond transitional B (incl, naïve)



(From Driessen GJ et al., Blood 2011; 118 (26):6814-6823)

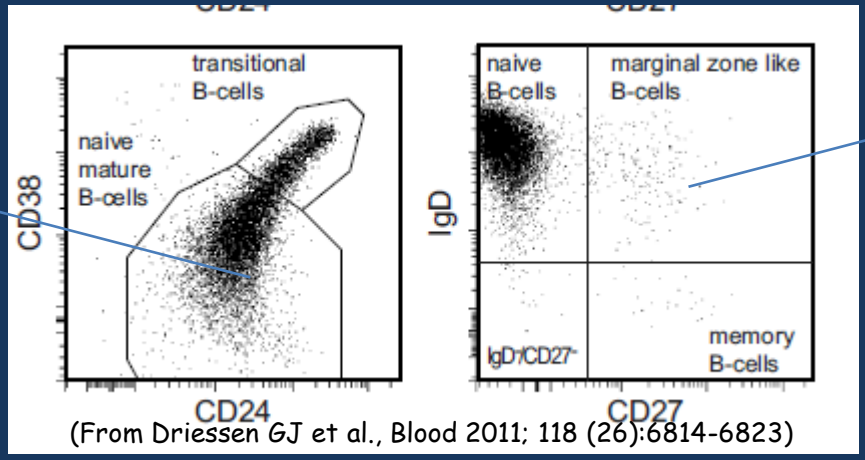
i.e. Low total memory B



Possible mechanism: lack of survival signals (e.g. BAFFR def. CARD11 def.)

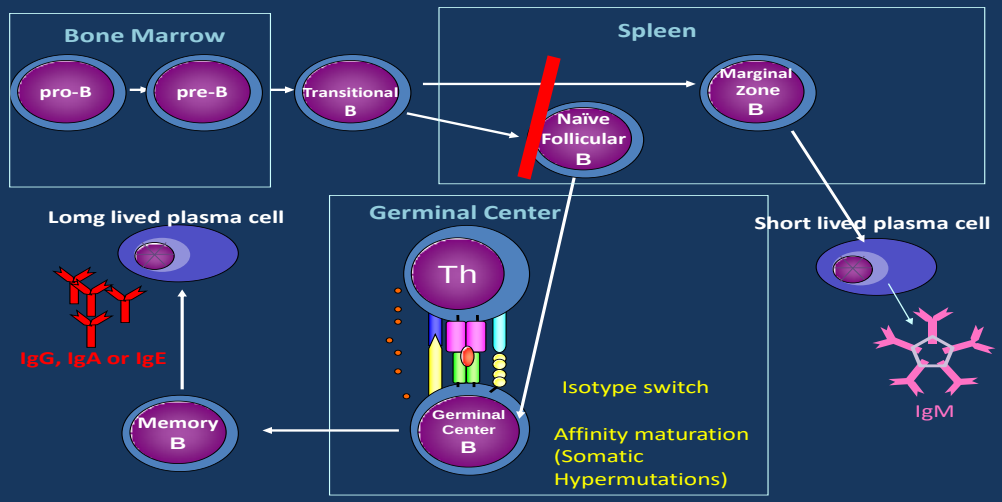
Pattern 3

Normal maturation from transitional B



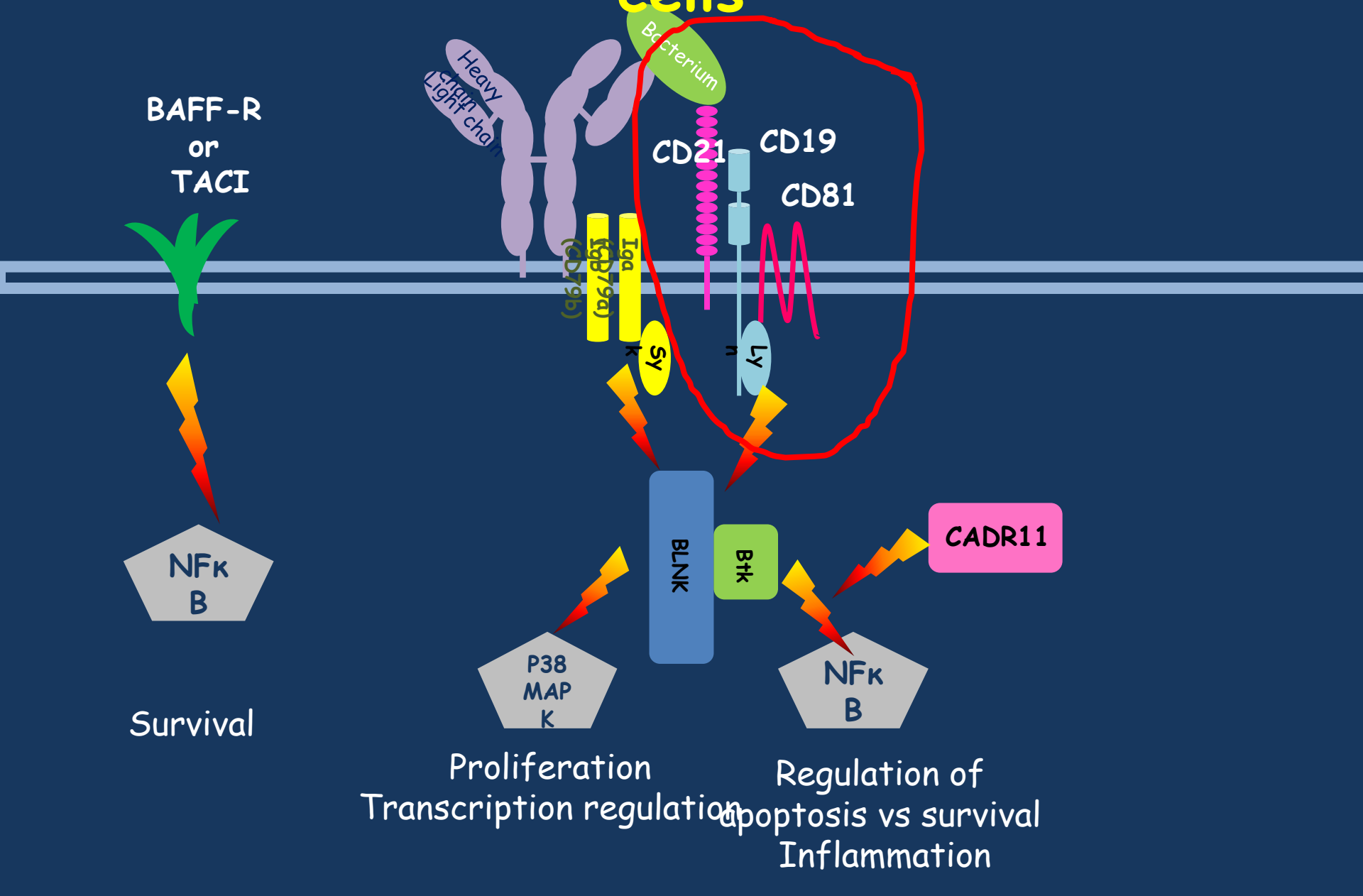
(From Driessen GJ et al., Blood 2011; 118 (26):6814-6823)

Low total memory B (MZ and switched)



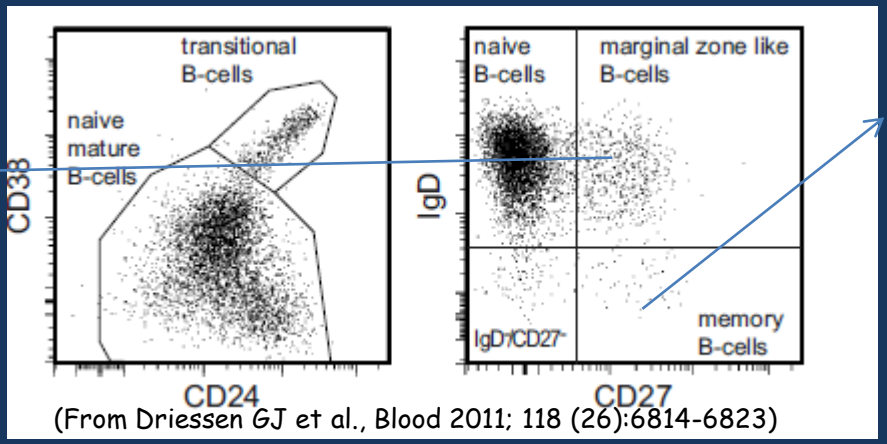
Possible mechanism: Impaired response to Ag: lack of costimulatory signals (e.g. CD19, CD81, TLR signal def.)

Basic receptors and signal transduction in B cells



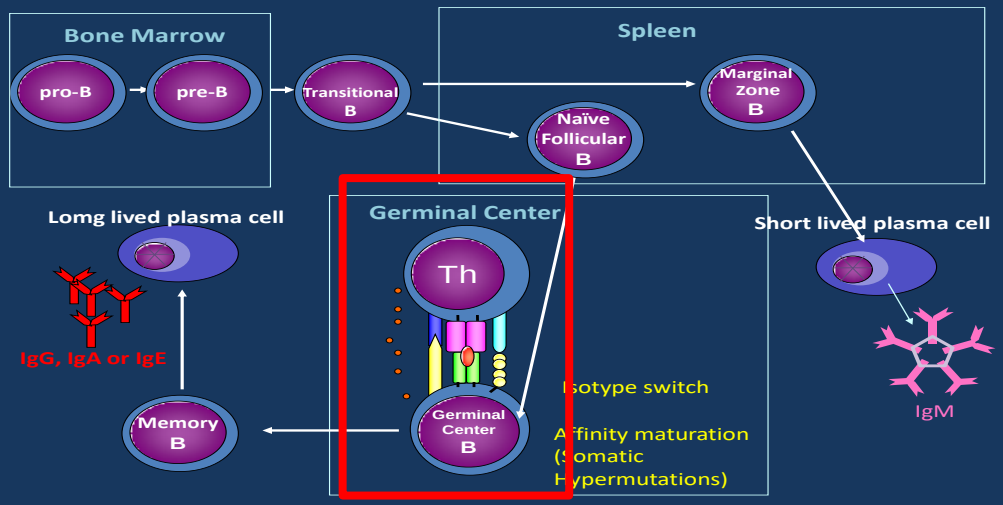
Pattern 4

Normal MZ B



(From Driessen GJ et al., Blood 2011; 118 (26):6814-6823)

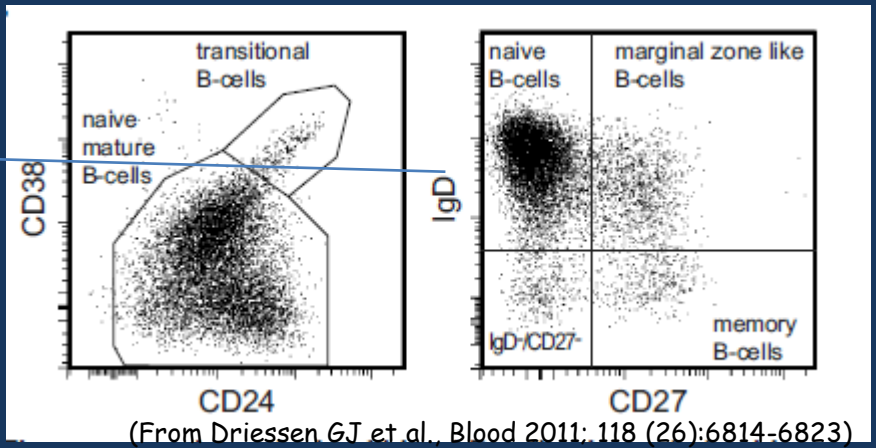
Low memory switched B



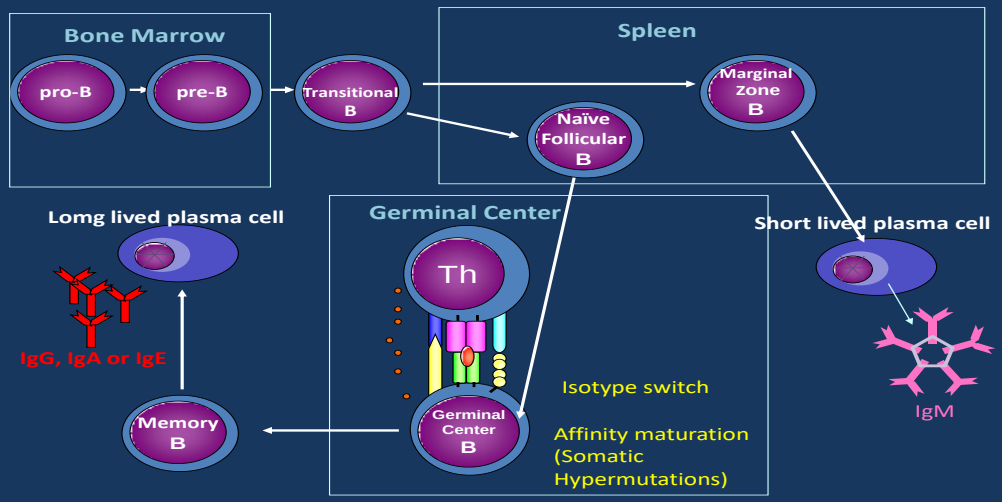
Possible mechanism: Germinal center dysfunction - NOT necessarily CVID -
(T cell deficiency, CD40def., DNA recombination and repair def. (AID, UNG,NEMO), PIK3δ GOF)

Pattern 5

Normal B cell subsets



(From Driessen GJ et al., Blood 2011; 118 (26):6814-6823)



Possible mechanism: ?
Post GC cell survival?

Generation-survival of memory B cells
is correlated to metabolism regulation

BCR, CD19,
TLRs

PI3K/mTOR/AKT/S6
pathway

anabolic state

PROLIFERATION

≠

NO survival
signals

AMPK

catabolic state
autophagy

SURVIVAL!

Generation-survival of memory B cells
is correlated to metabolism regulation

BCR, CD19, TLRs

PI3K/mTOR/AKT/S6 pathway

anabolic state

PROLIFE

≠

NO survival signals

AMPK

catabolic state
autophagy

SURVIVAL!

frontiers in Immunology

REVIEW
published: 09 May 2018
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Check for updates

"Immune TOR-opathies," a Novel Disease Entity in Clinical Immunology

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TOR-opathies

BCR, CD19, TLRs

PI3K/mTOR/AKT/S6 pathway

anabolic state

PROLIFE

NO survival signals

AMPK

catabolic state
autophagy

SURVIVAL!

TOROpathies

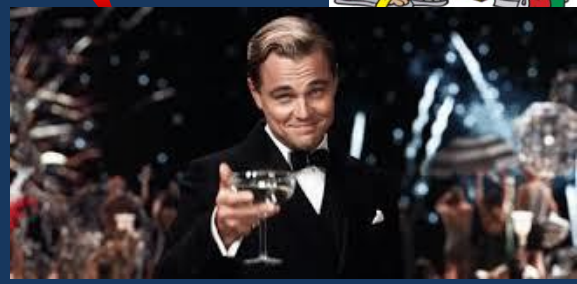
BCR, CD19, TLRs

PI3K/mTOR/AKT/S6 pathway

anabolic state

PROLIFE

NO survival signals



anabolic state

NO memory B cells

European Concencus Classification for CVID (EUROclass) 2008

>1% B cells = group B+

>2% switched memory B cells = group smB+

≤2% switched memory B cells = group smB-

≥10% CD21lo B cells = group smB+21 lo

<10% CD21lo B cells = group smB+21norm

≥10% CD21lo B cells = group smB-21 lo

<10% CD21lo B cells = group smB-21norm

≥9 Transitional B cells = group smB-Trhi

<9% Transitional B cells = group smB-Trnorm

Some, non CVID, PIDs correlated with abnormal B cell subsets

Wiskott Aldrich sy. → Low B

DOCK 8 def. → Low memory B

Defective actin polymerization/ reorganization. Poor B cell shuttling and interaction with T cells

Hyper IgE sy. → Low memory B

Useful for diff. diagnosis from allergy

XLP → Low switched memory B

Poor GC formation

Comel-Netherton sy. → Low MZ B


TEAM


Eureka!

Спасибо !!!