

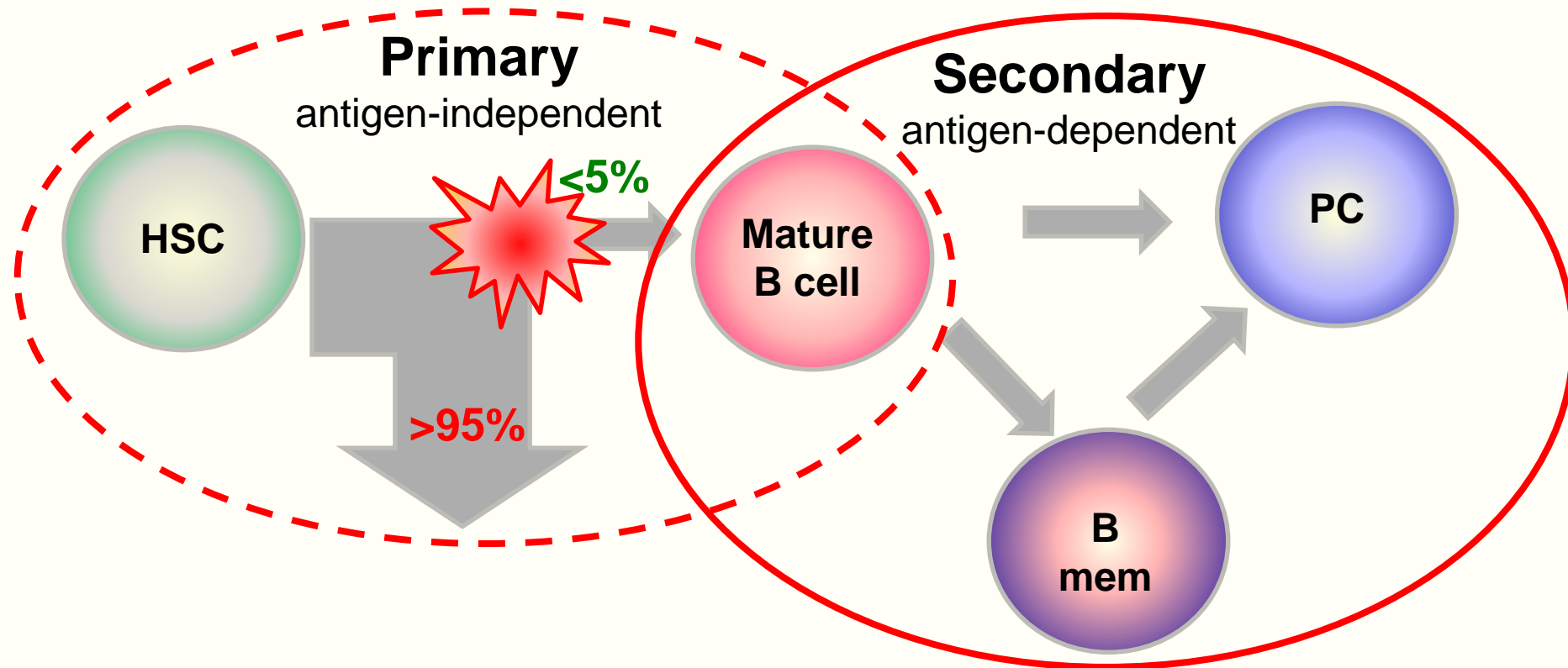
# Basic Immunology

*Lectures 13-14.*

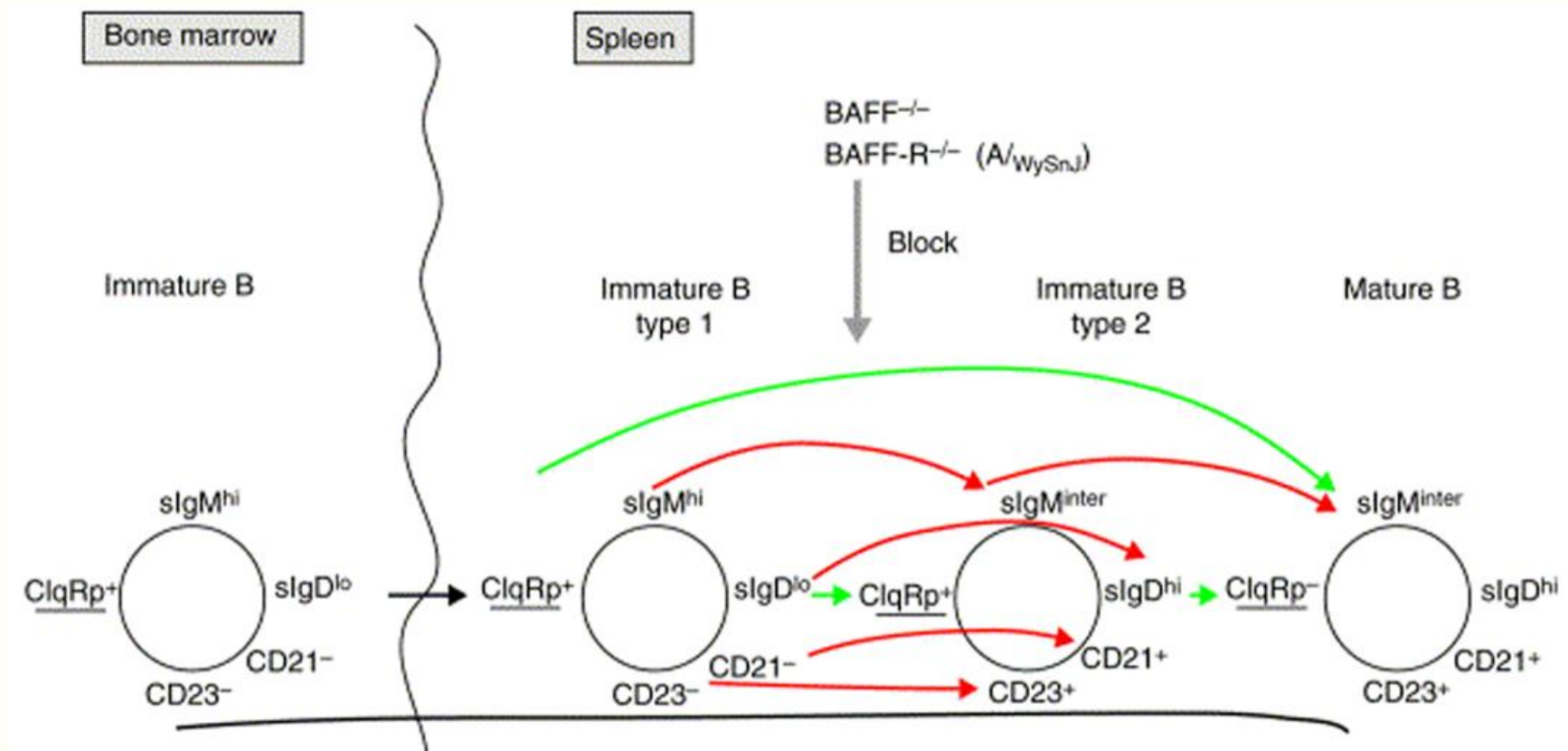
## **Humoral immune responses:**

Extrafollicular reaction and germinal center reactions - affinity maturation and isotype switch.

# Phases of B-cell development



# Peripheral primary B-cell differentiation



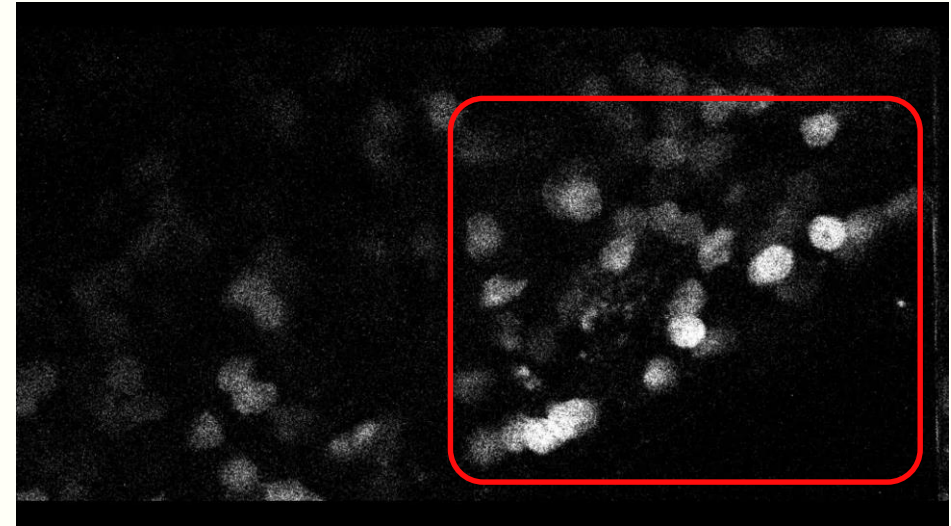
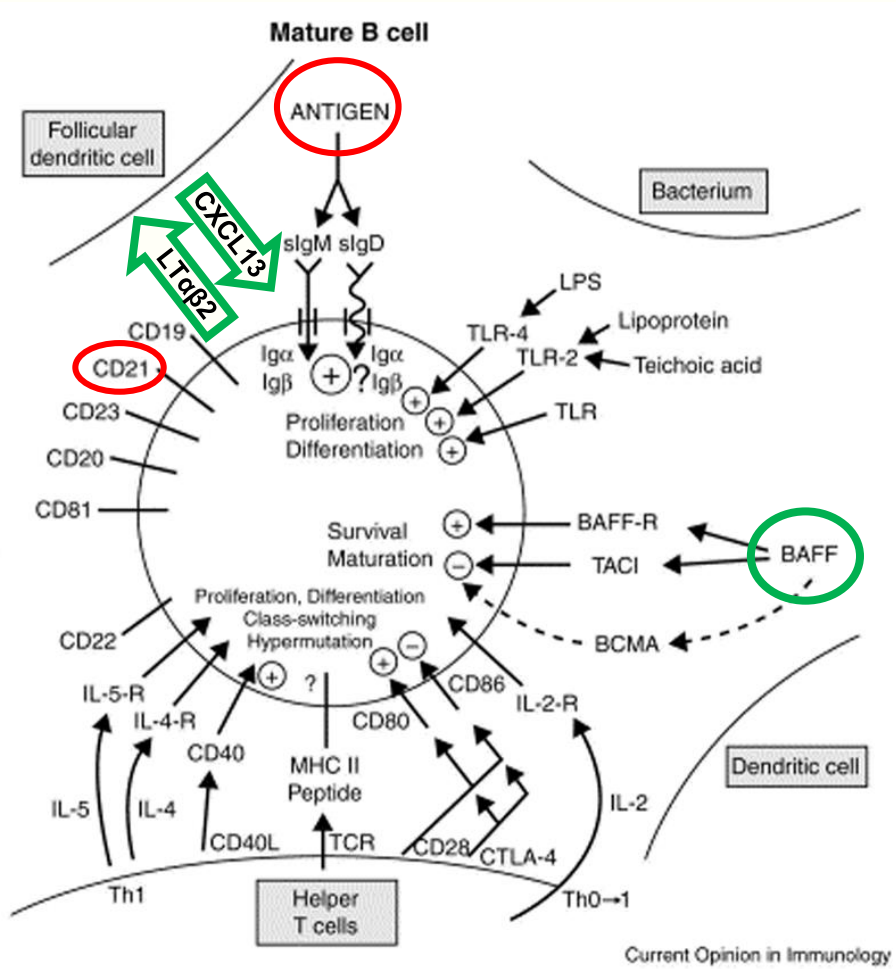
# Organization of the naive B-cell pool

Type of difference	Subset	Characteristics
Developmental origin	B-1	Fetal hematopoietic stem cell; self-renewal, low-affinity autoantibody production (TdT-independent BcR), dominance in neonates and CLL, located in body cavities. (CD5+, CD43+, IgM++/IgD+)
	B-2	Postnatal bone marrow-derived
Tissue compartmentalization (within the B-2 subset)	Follicular B cell (FoB)	Distributed in peripheral lymphoid tissues, recirculate (IgM+/IgD++, CD21+, CD23++).
	Marginal zone B cells (MZB)	Located in the splenic MZ (in humans also in MALT) with Ig phenotype similar to B-1 B cells, adult BM origin, distinct developmental regulation to Fo B cells, relatively sessile. (IgM++/IgD+, CD21++, CD23+/-)
Functional specialization	Regulatory and other B cells (Bregs)	Production of IL-10 and GM-CSF
Age-related appearance	Aging-associated B cells (ABC)	Increased presence in elderly and with autoimmune diseases (T-bet/CD11c)

# B cell subsets and their characteristics

- **B-1 B cells:** embryonic development, self-renewal, production of low-affinity autoantibodies, frequent occurrence in neonates and B-CLL patients, body cavity residence (in mice).  
(CD5+, CD43+, IgM++/IgD+)
- **Marginal zone B cells:** Bone marrow origin, Ig isotype phenotype similar to B-1 B cells, different developmental requirements from follicular B cells, relatively sessile/non-migratory cells.  
(IgM++/IgD+, CD21++, CD23+/-)
- **Conventional follicular B cells.** Bone marrow origin, recirculate. (IgM+/IgD++, CD21+, CD23++).

# B-cell sensors

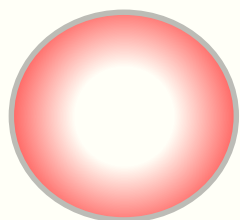
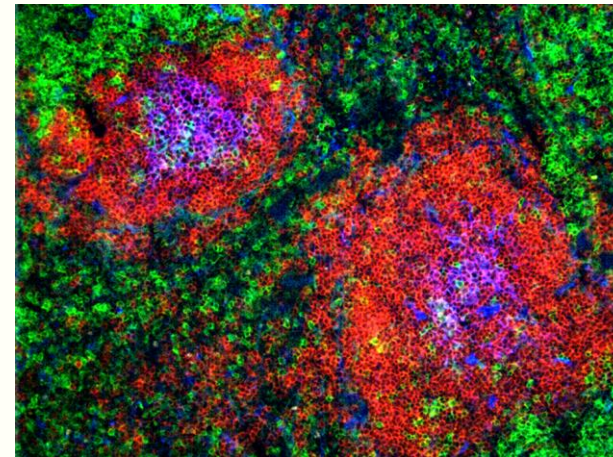
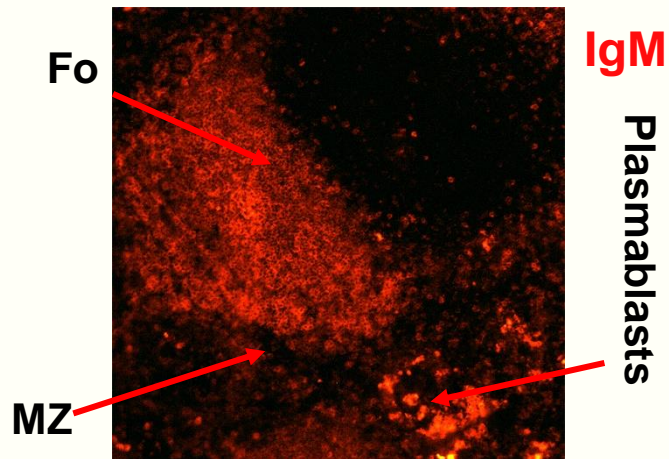


# Forms of antibody production

- **B-1 B cells:** IgM/IgA secretion (constitutive – gut/spleen axis: PEC B-1 cells ↔ spleen ↔ gut lamina propria PC?)
- **B-2 B cells:** differentiation following antigenic stimulation
  - *Extrafollicular (plasmacellular) reaction* (throughout T-independent & early stage of T-dependent humoral immune responses)
  - *Germinal center reaction* (advanced stage of T-dependent humoral immune responses)

# Role of antigen in defining the form of antibody productions

Type of antigen	Type of early reaction	Type of late reaction	Result
T-independent	Plasmacellular reaction (3-14 days)	-	Serum IgM + Memory -
T-dependent	Plasmacellular reaction (3-7 days)	Germinal center reaction (7-14 days)	Serum IgM/G + Memory +



*T-independent response*

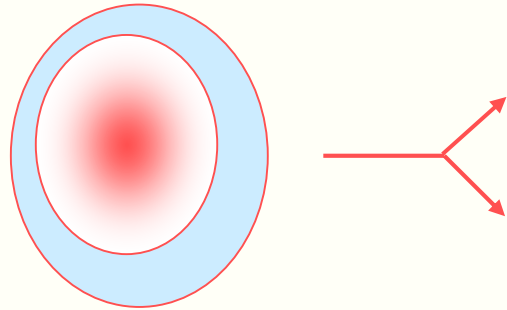
→ *Extrafollicular reaction - PC*

→ *Germinal center reaction – PC/Bmem*

*T-dependent response*

# Follicular or extrafollicular pathway: balance between Bcl-6 & Blimp-1

T/B boundary



Activated B cells

## Follicular pathway:

Bcl-6 ↑ : Blimp-1 inhibition

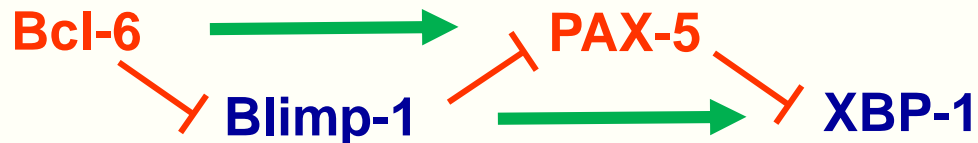
PAX-5: XBP-1 inhibition

*Result: Centroblast*

## Extrafollicular pathway:

Blimp-1 ↑: inhibition of PAX-5 target elements  
(BcR, CD19, etc)

*Result: Plasmablast*



# Main cellular components involved in germinal center reaction

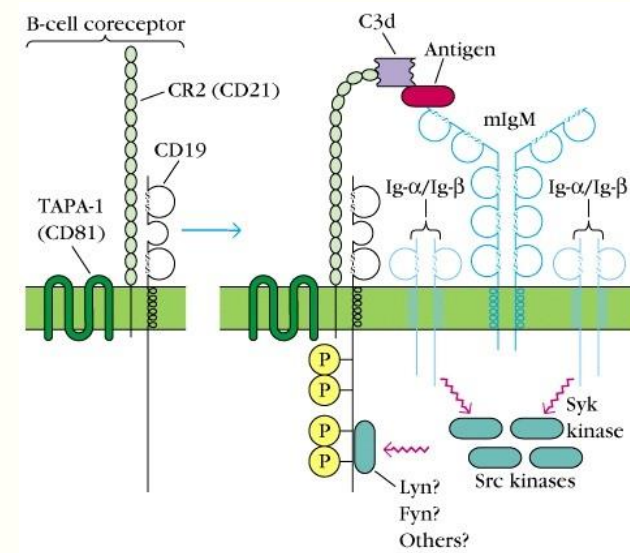
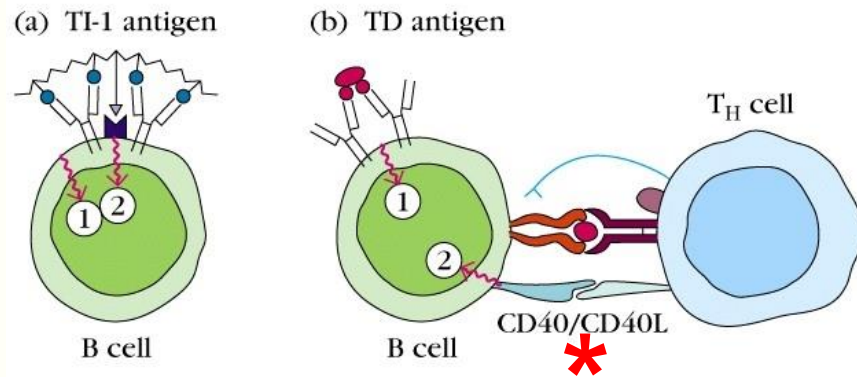
- **B-2 B cells**
  - **MZ B cells:** early response/IgM/Ag-transport
  - **Fo B cells:** quasi-clonal expansion
- **T cells:** T<sub>FH</sub> differentiation
- **„Tingible body” macrophages (TBM):** phagocytosis of dead cells
- **FDC:** Ag-retention
- **(LTi:** remodeling of lymphoid tissue following immune responses??)

# Main events during humoral immune responses I. B-cell activation and relocation

## 1. Activation:

„1. signal”: BcR antigen recognition

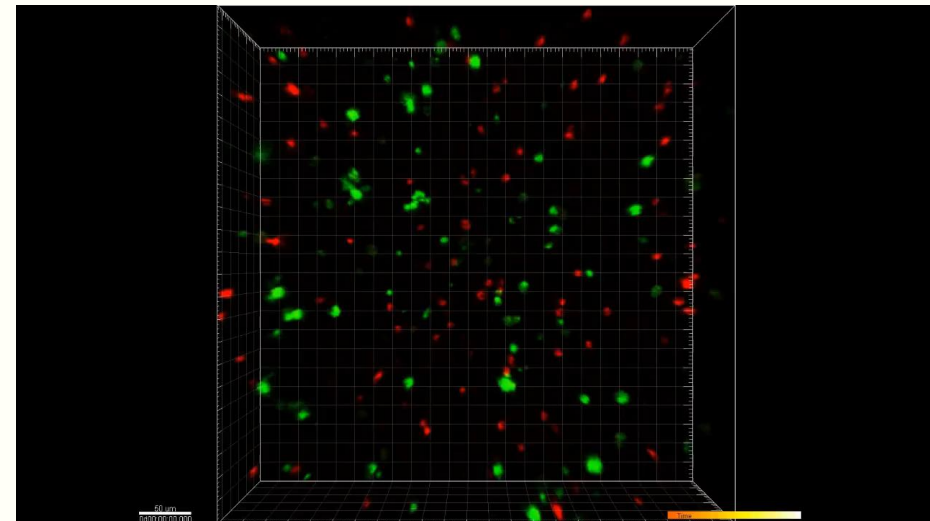
„2. signal”: Ligand binding of co-receptors



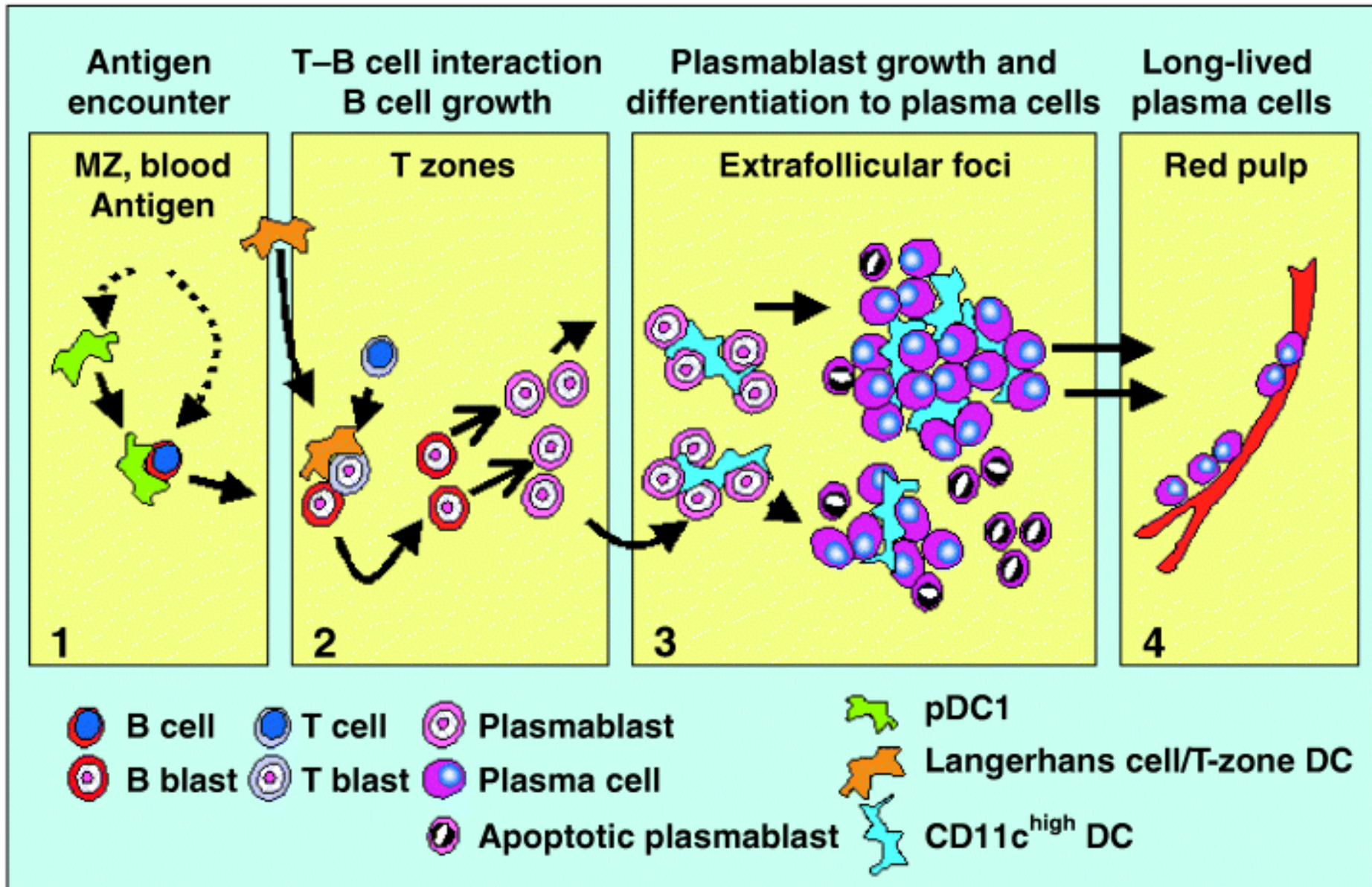
## 2. Relocation: Follicle → T/B boundary

Resting B cell: CXCR5 > CCR7

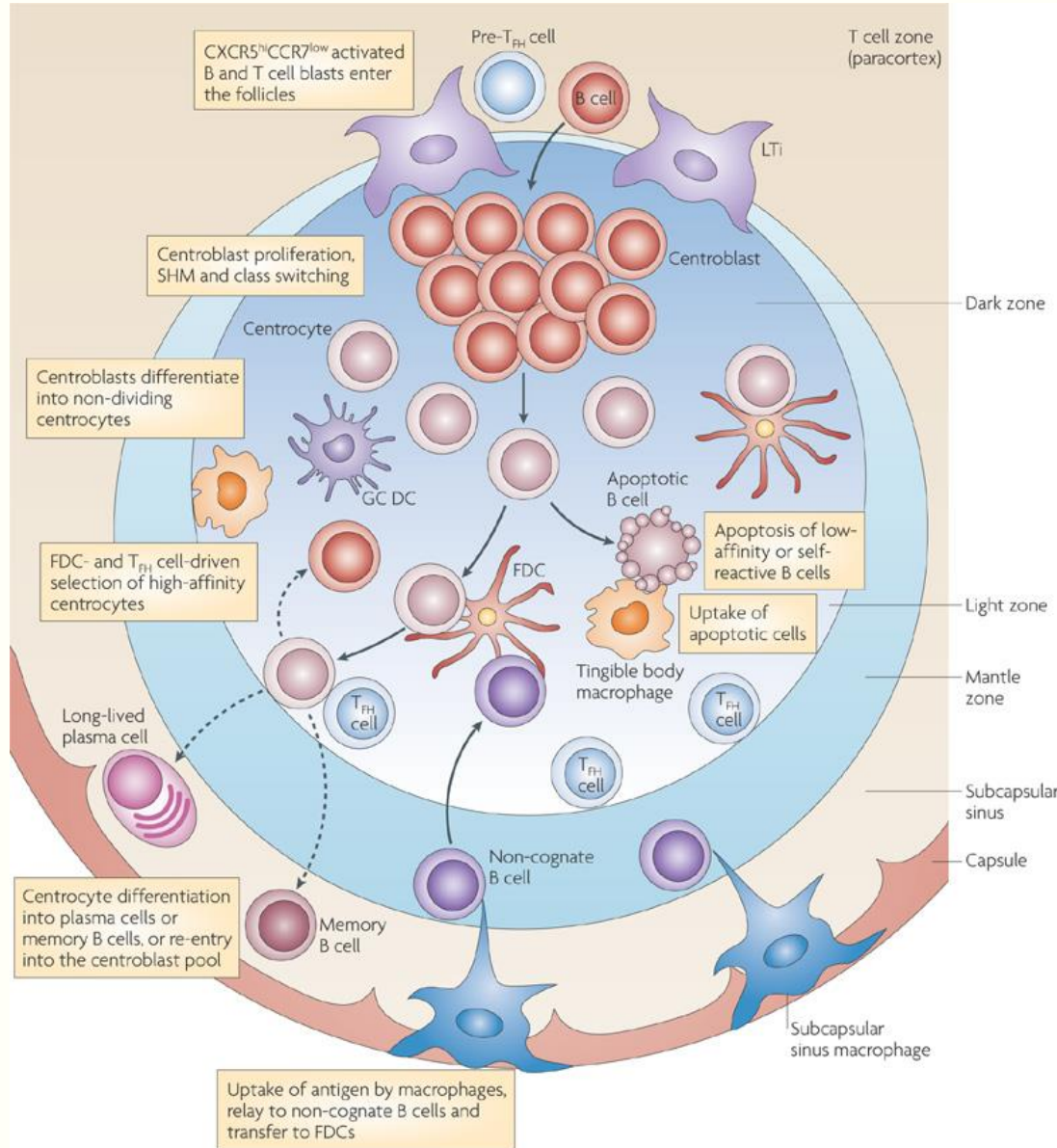
Activated B cell: CXCR5 < CCR7



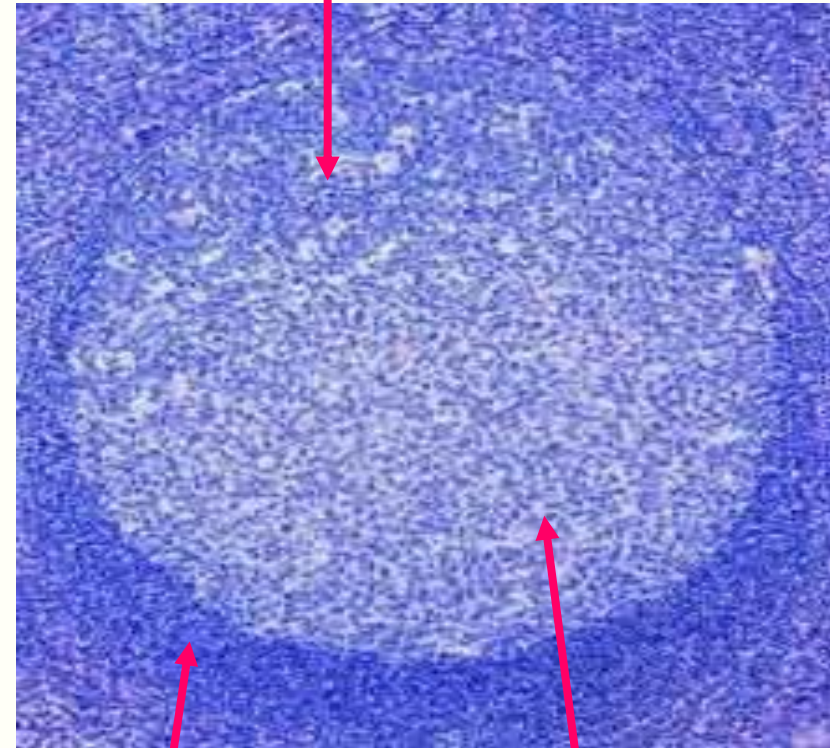
# I. phase: Extrafollicular reaction



# II. phase: Formation of germinal center



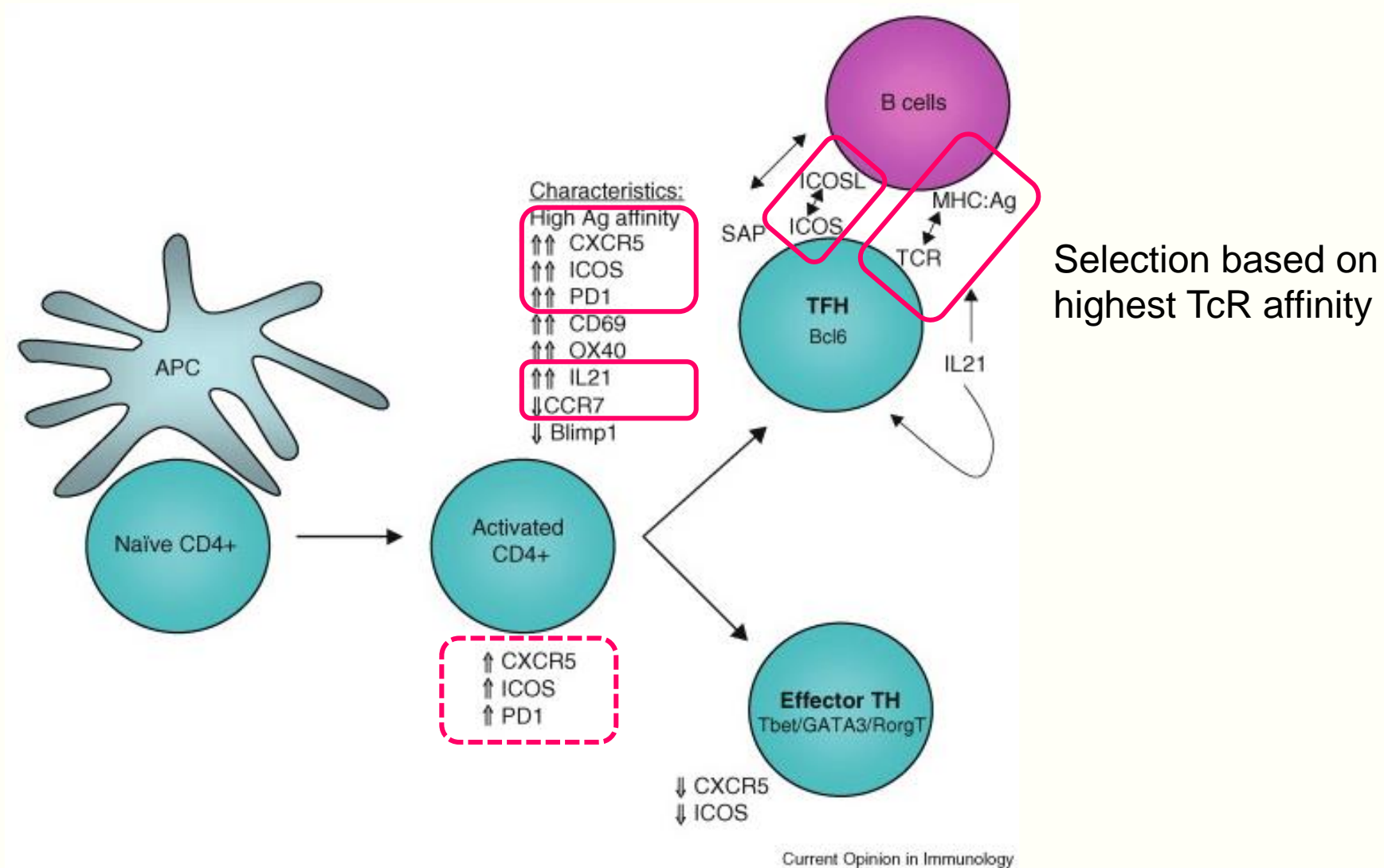
**Dark zone: centroblasts + TBMs**



**Light zone: centrocytes + FDCs**

**Mantle zone: residual resting B cells**

# T<sub>FH</sub> differentiation: T-B communication in activation



# GC structure: dynamic DZ/LZ polarization, centroblast/centrocyte segregation

Paradox: motile cells  $\leftrightarrow$  fixed GC/DZ

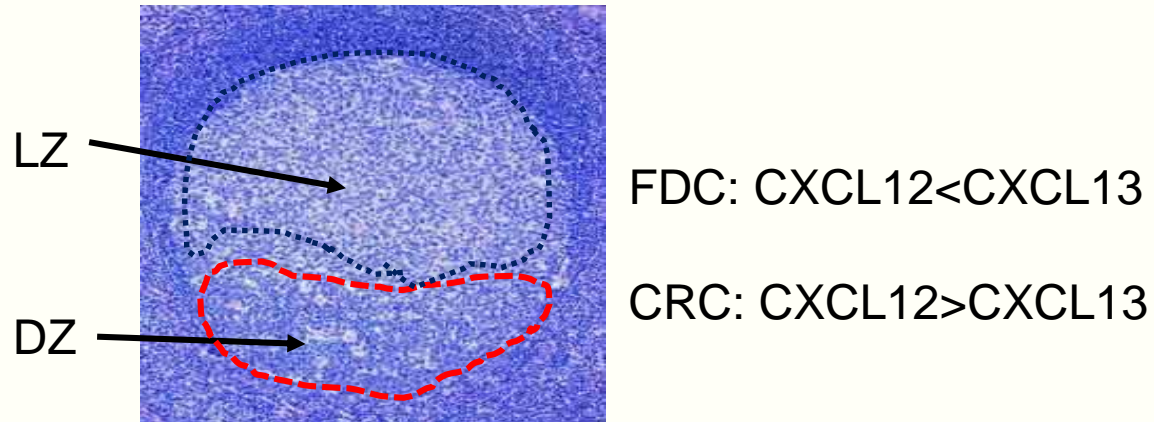
(1) T-zone/Follicle: CCR7/CXCR5 - CCL21/CXCL13

(2) LZ:DZ: CXCR4<sup>-</sup>/CXCR5<sup>+</sup> : CXCR4<sup>+</sup>/CXCR5<sup>+</sup> - CXCL12/CXCL13

Partner cells: GC LZ: FDC (CXCL13), GC DZ: CXCL12-producing stromal cell (CRC)

Others: S1P (sphingosine-1-phosphate) - S1PR3, GGG (geranylgeranyl glutathione) - P2RY8

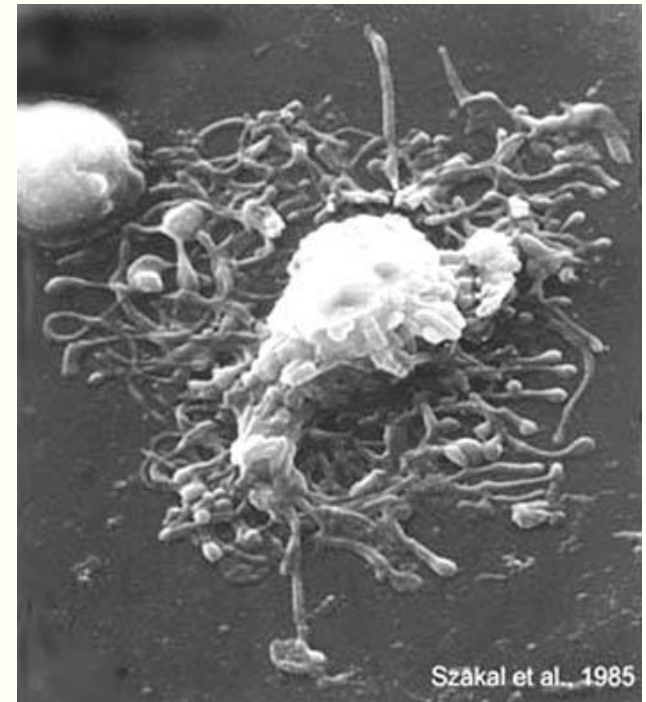
*(Significance: mutations in aggressive B-cell lymphomas  $\rightarrow$  dissemination)*



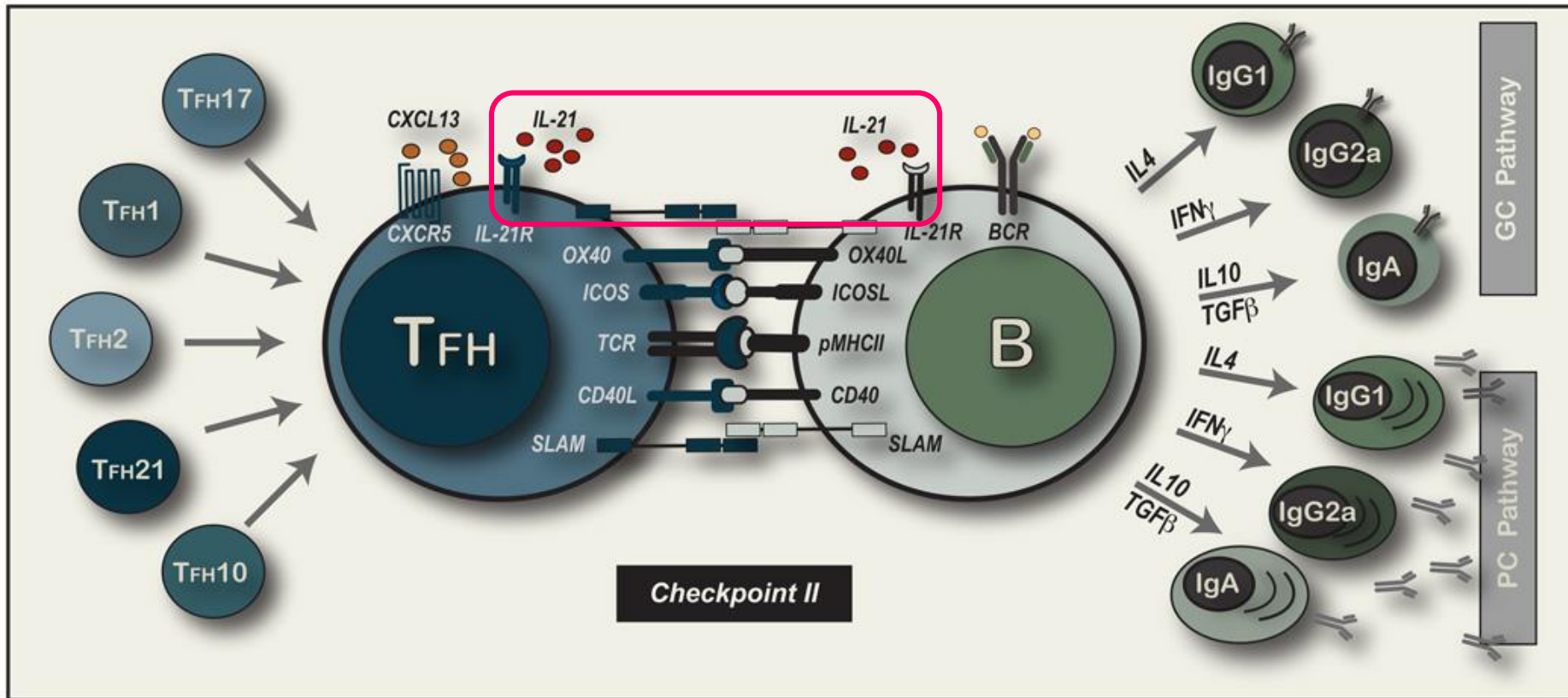
# Follicular dendritic cells: GC organizers and cellular mediators of B-cell selection

## FDC:

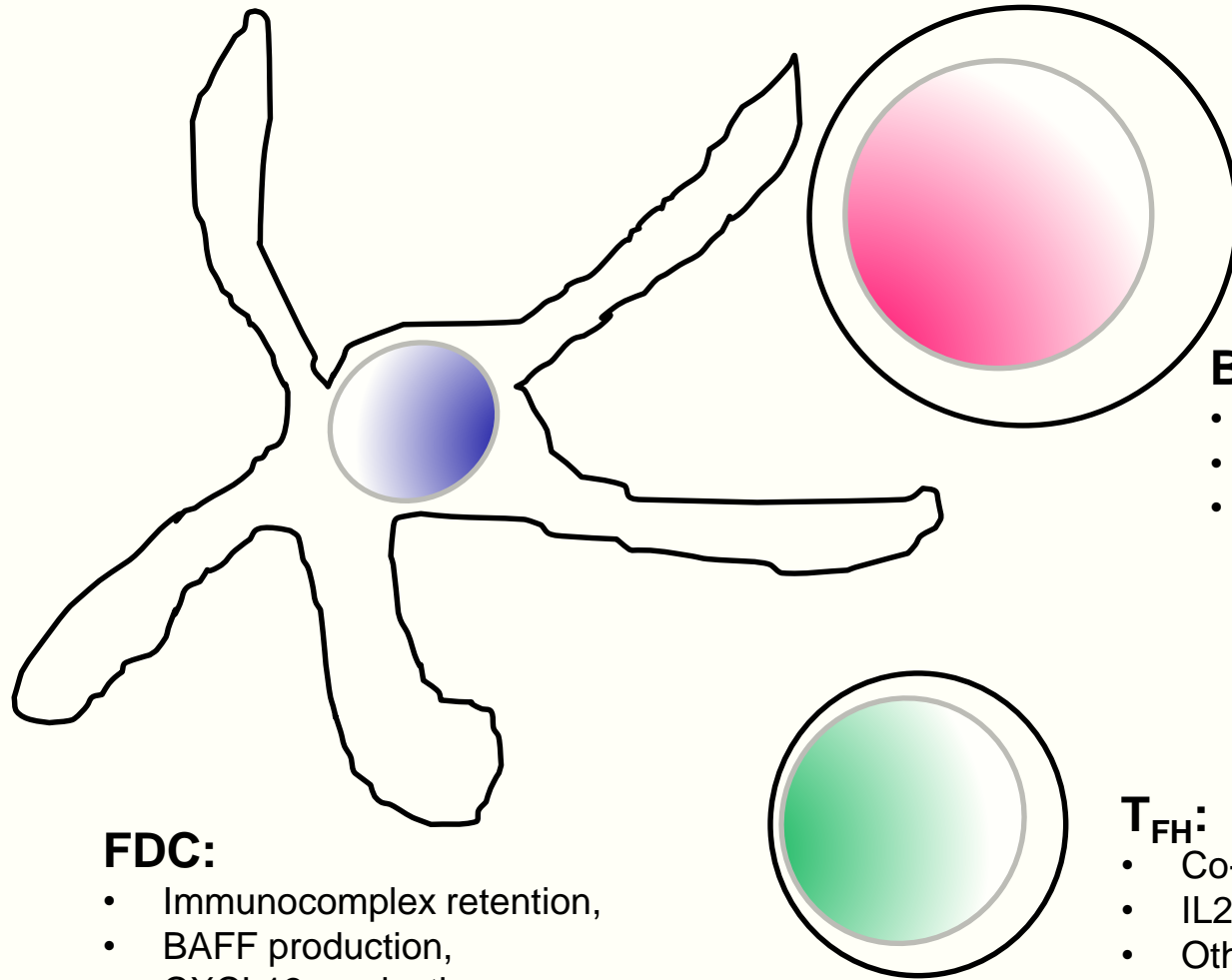
- Local mesenchymal origin – organ-specific differences of precursors
- Non-phagocytic, non-adherent
- Surface markers: CD21/35, FcγR, inducible VCAM-1 – *Centroblast binding*
- LTβR-dependent differentiation, TNF-dependent tissue location
- Long-term retention of antigens as immune complexes (*iccosome*)



# Role of polarized T<sub>FH</sub> cells in Ig isotype regulation



# Connection of T<sub>FH</sub> - FDC - B cell interactions during GC reactions



## FDC:

- Immunocomplex retention,
- BAFF production,
- CXCL13 production

## B cells:

- MHC/Ag presentation
- Co-stimulatory ligands (CD40, ICOSL)
- TNF/LT ligands – FDC maintenance

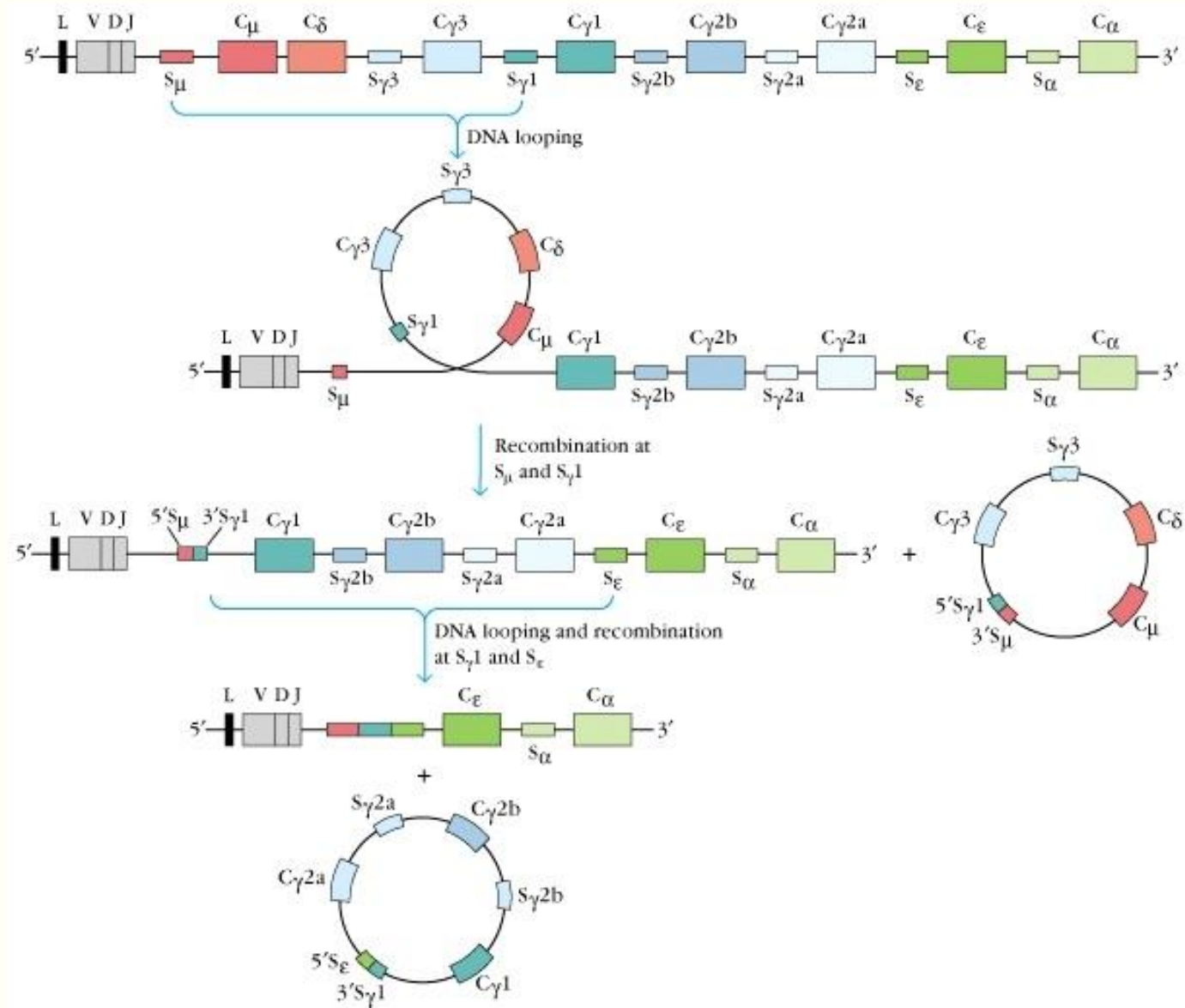
## T<sub>FH</sub>:

- Co-stimulatory molecules (CD40L, ICOS)
- IL21 production
- Other cytokines – Ig isotype switch

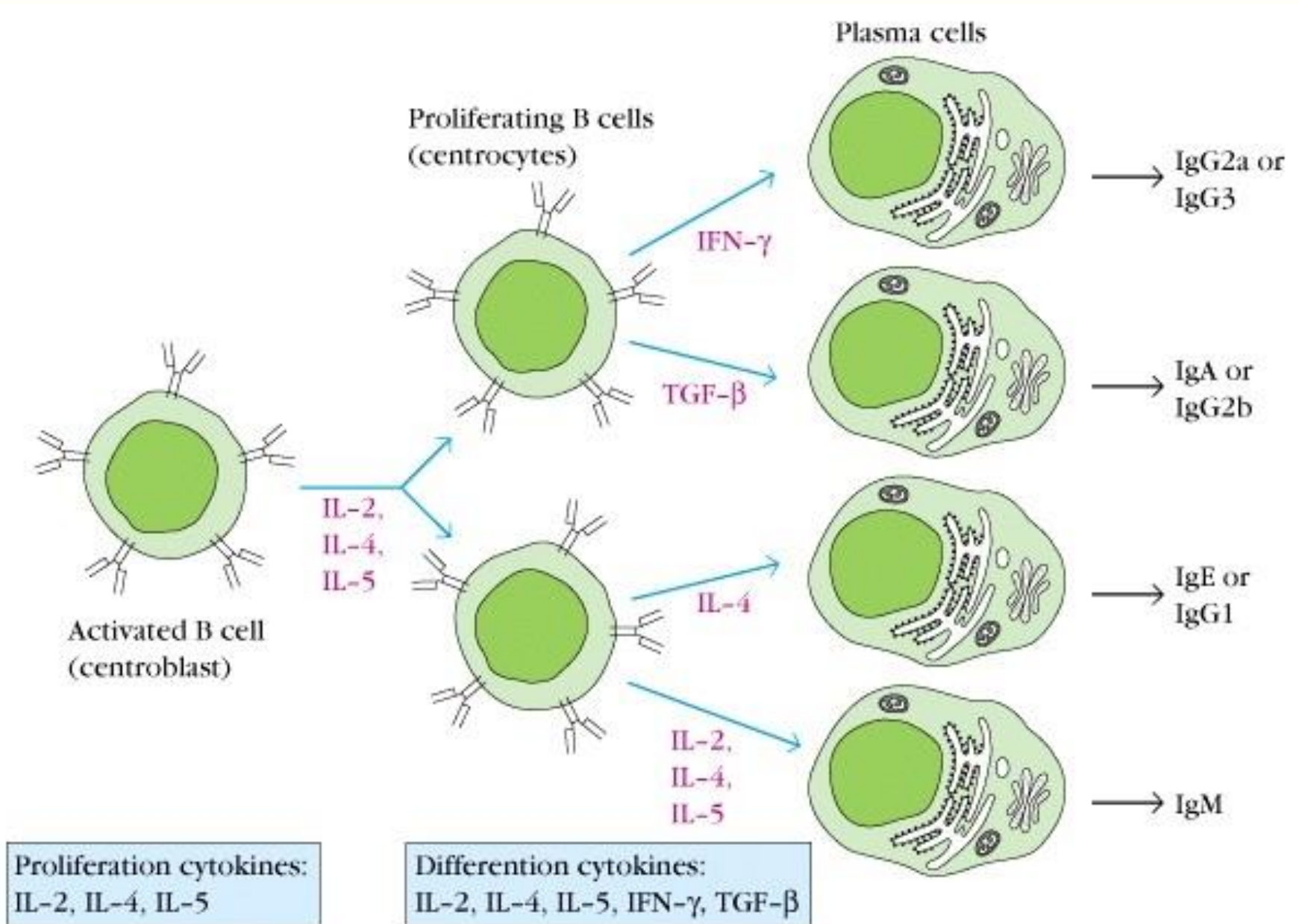
# Mechanism of affinity maturation

1. **BcR antigen-binding** – T-cell contacts are established.
2. **Double-strand DNA breakage** (blunt ends) at CDRs (and also within Bcl-6 & c-myc).
3. **AID (activation-induced cytidine deaminase)**, free 3' & 5' ends are formed
4. **Single (repeatable) nucleotide-exchange**: error-prone DNA-polymerase.

# Ig-isotype expression : Mechanism of recombination



# Ig-isotype expression: Cytokine regulation



# Mechanism of cell death

## BcR & CD40 stimulation: Ag & CD40L

### Extrinsic path:

Fas expression enhances: Ligand binding

Caspase 8 induction

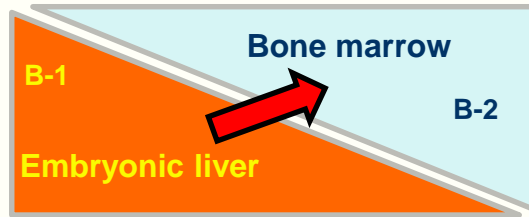


### Intrinsic path:

Repeated stimulation of BcR and CD21 leads to activation of anti-apoptotic Bcl-2 activation; stabilization of mitochondrion membrane



# Decision points in B-cell differentiation



## Primary B-cell differentiation:

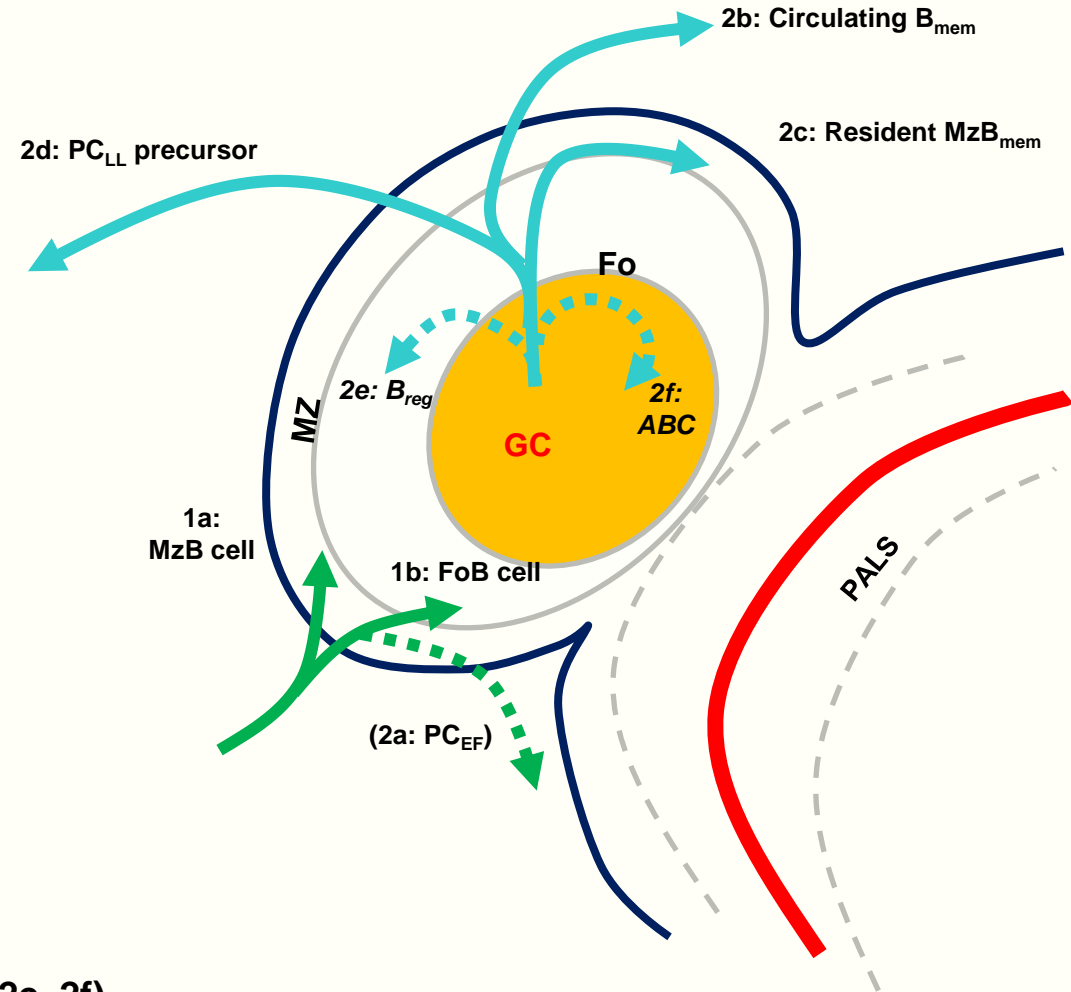
- B-1/B-2

## 1. Pre-immune B-cell differentiation:

- FoB/MzB (1a/1b)

## 2. Ag-induced secondary B-cell differentiation:

- *Extrafollicular plasma cell* (2a)
- *GC reaction*
  - Circulating  $B_{\text{mem}}$  (2b), MzB-resident  $B_{\text{mem}}$  (2c)
  - Long-lived plasma cell/precursor(2d)
  - Regulatory  $B_{\text{reg}}$  autoimmune/aging B cells (ABC) (2e, 2f)



# Alterations of Ig gene and their characteristics

<i>Ig gene alteration</i>	<b>Ag/AID</b>	<b>RAG-I/II</b>	<b>Promotes survival</b>	<b>Cytokine regulation</b>	<b>Effector mechanism</b>
<i>VDJ (H/L)</i>	-	+	+	-	-
<i>Affinity maturation</i>	+	-	+	-	-
<i>Isotype switch</i>	+	-	-	+	+
<i>Protein alteration</i>					mIg → sol. Ig

# Groups of B cells with regulatory functions

## **Breg: regulatory B cells (mediated by IL-10 and other factors)**

- *Identification:* Removal of B cells led to deterioration of autoimmune diseases and course of chronic inflammation
- *Origin:* Possibly a distinct differentiation group induced upon (a) BcR stimulation and TLR2/4/9 activation, (b) IFN $\alpha$ /CD40 engagement (c) upon IL-35/IL-1 $\beta$ /IL-6 effects.
- *Regulatory functions:* Mediated by (a) IL-10 (b) TGF $\beta$ , (c) FasL, PD-L1 in the early phase of immune responses
- *Therapeutic significance:* B-cell depletion in the early phase of rejection enhances, while in the later phase suppresses rejection.

## **ABC: Age-associated B cells/T-bet<sup>+</sup>/CD11c<sup>+</sup> (Th1 TF/DC-marker)**

- *Identification:* after immunization with intracellular pathogens, amongst splenic B cells based on cell surface features (CD11c expression)
- *Origin:* uncertain, after BcR/TLR7 stimulation of B cells
- *Regulatory functions:* Promotion of IL-10/TNF/Th17 commitment
- *Therapeutic significance:* Due to BAFFR<sup>+</sup> competition with Fo and MZ B cells and bone marrow primary B-cell formation → B-cell lymphopenia in the elderly with the relative dominance of ABC cells (CD21<sup>-</sup>/CD23<sup>-</sup>).