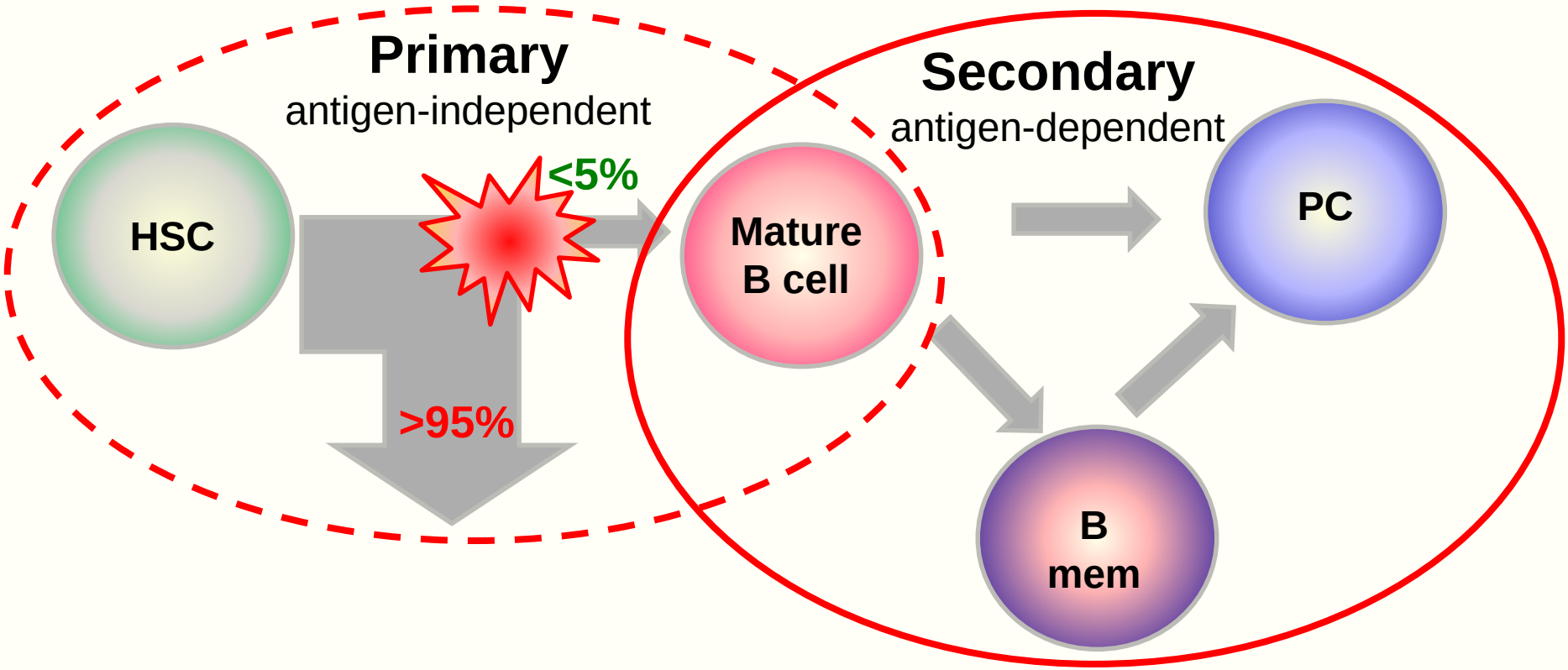
Basic Immunology

Lectures 13-14.

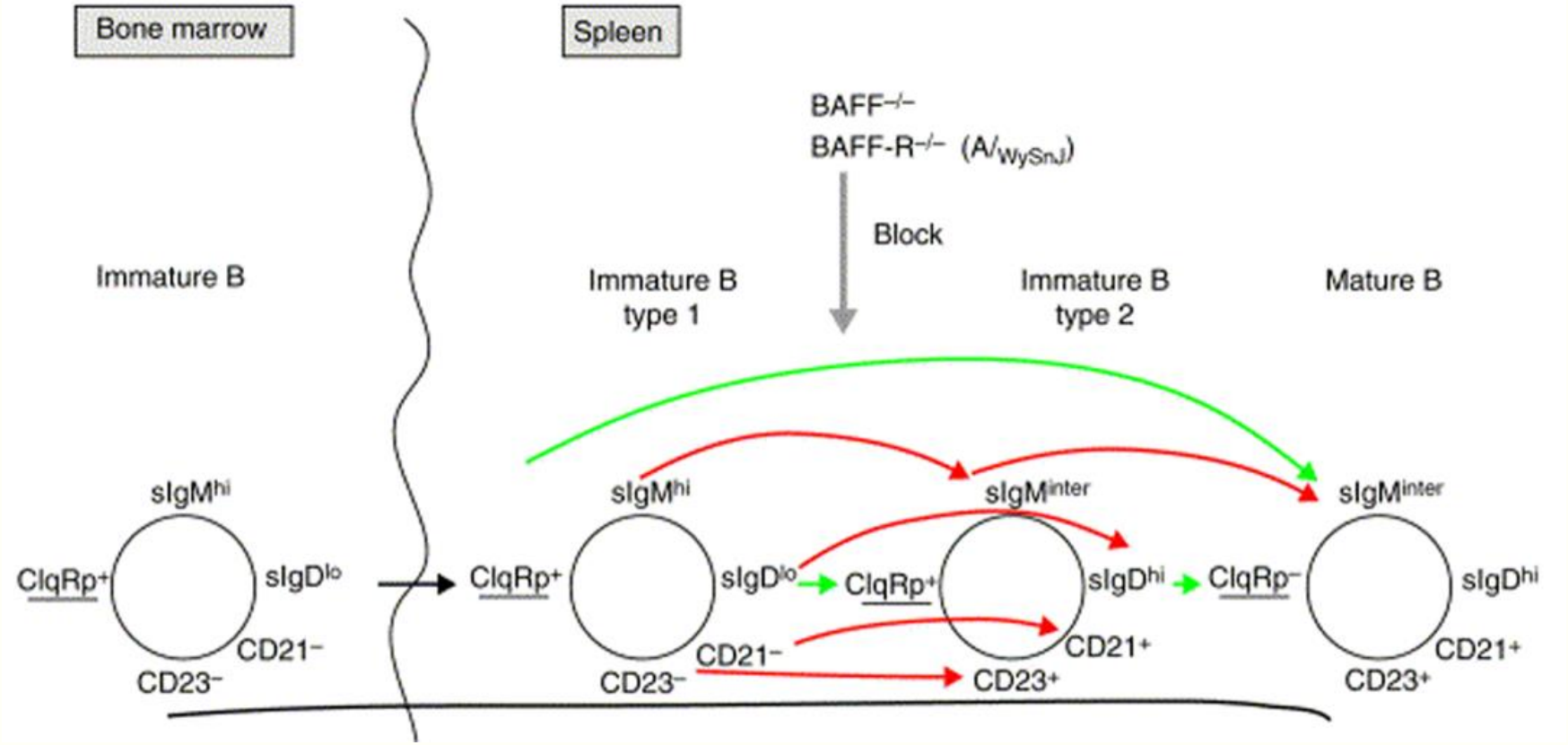
Humoral immune responses:

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).

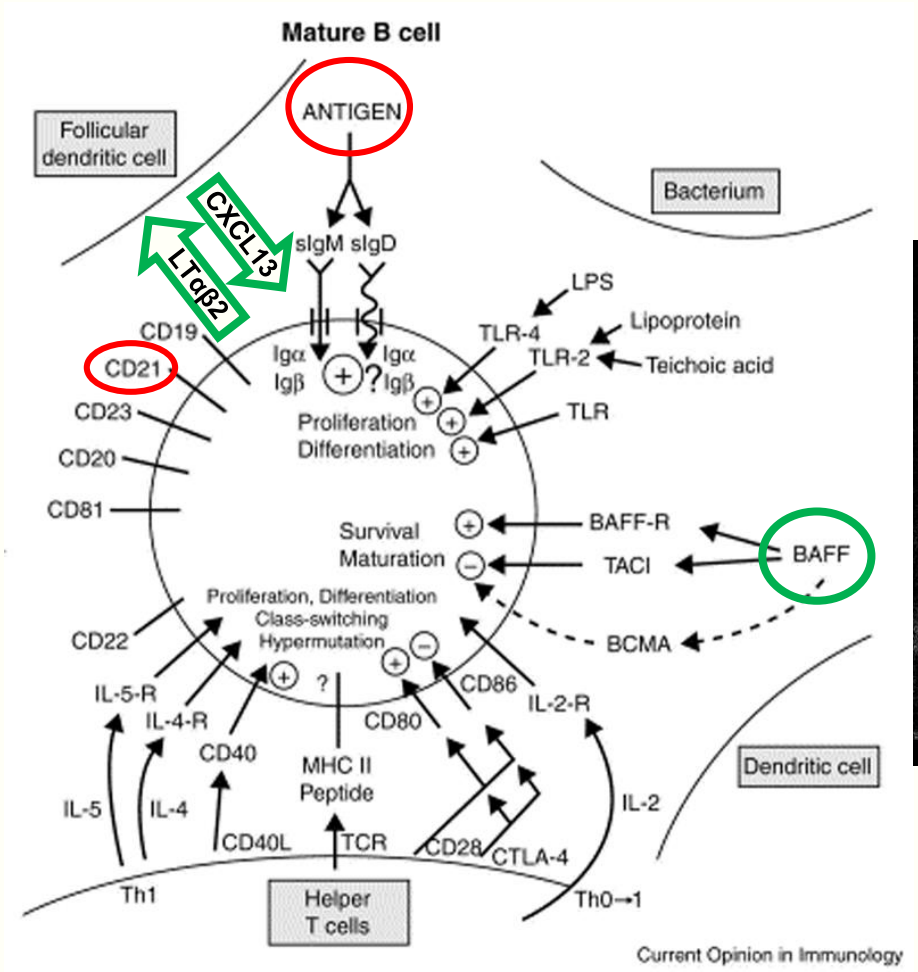
  (CD5+, CD43+, IgM++/IgD+)

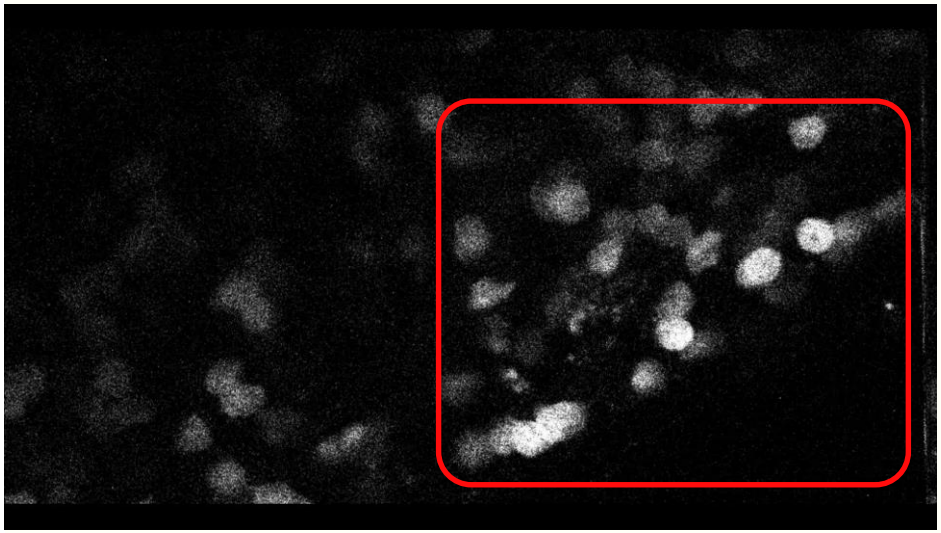
- **Marginal zone B cells**: Bone marrow origin, Ig isotype phenotype similar to B-1 B cells, different developmental requirements from follicular B cells, relatively sessile/non-migratory cells.

  (IgM++/IgD+, CD21++, CD23+/-)

- **Conventional follicular B cells.** Bone marrow origin, recirculate. (IgM+/IgD++, CD21+, CD23++).

B-cell sensors



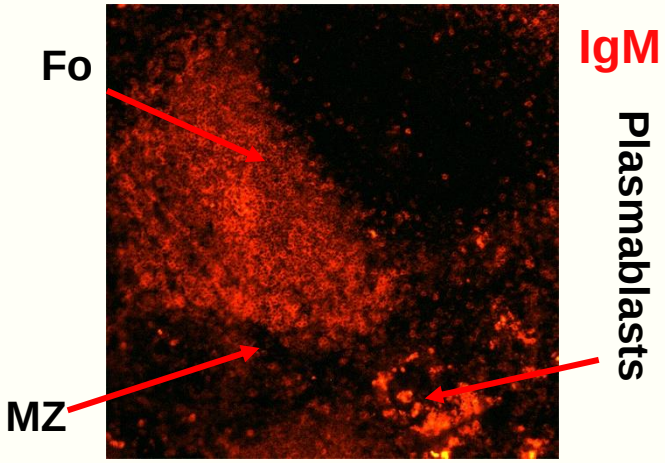


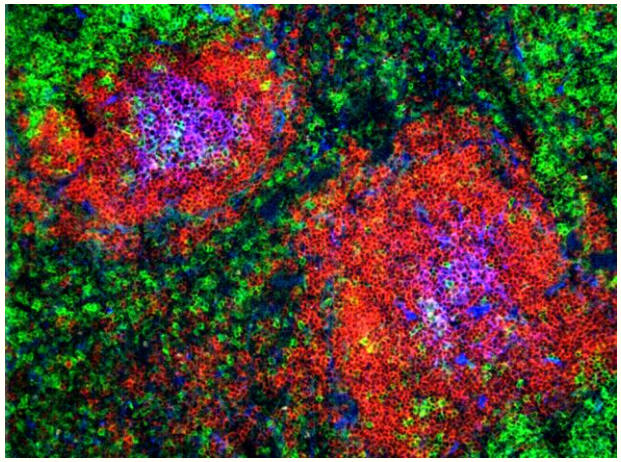
Forms of antibody production

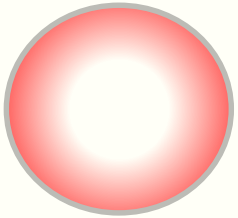
- **B-1 B cells:** IgM/IgA secretion (constitutive – gut/spleen axis: PEC B-1 cells ↔ spleen ↔ gut lamina propria PC?)
- **B-2 B cells:** differentiation following antigenic stimulation
  - *Extrafollicular (plasmacellular) reaction* (throughout T-independent & early stage of T-dependent humoral immune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

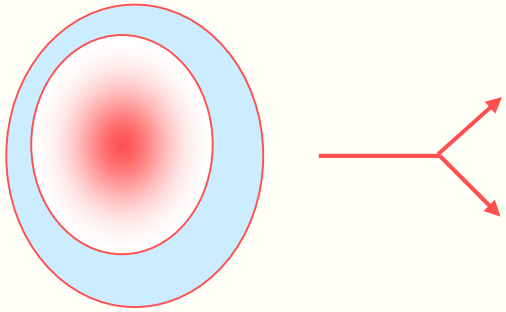
Extrafolliicular reaction - PC

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

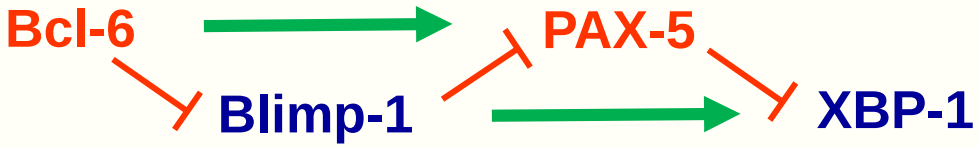
PAX-5: XBP-1 inhibition

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

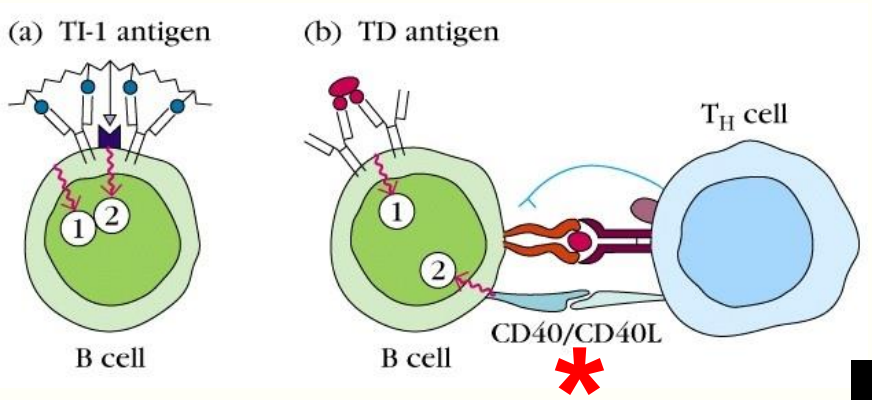
- **B-2 B cells**
  - **MZ B cells:** early response/IgM/Ag-transport
  - **Fo B cells:** quasi-clonal expansion
- **T cells:** T_{FH} differentiation
- **„Tingible body" macrophages (TBM):** phagocytosis of dead cells
- **FDC:** Ag-retention
- **(LTi:** remodeling of lymphoid tissue following immune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

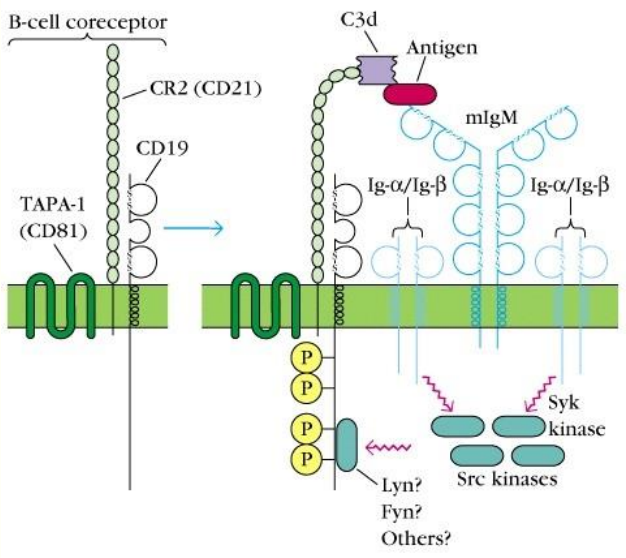
Main events during humoral immune responses I. B-cell activation and relocation

1. Activation:

„1. signal”: BcR antigen recognition

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

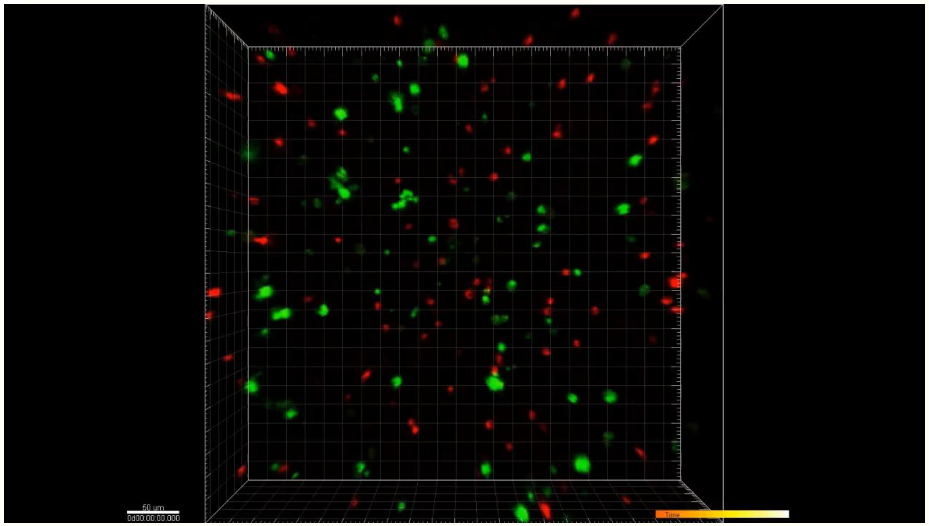




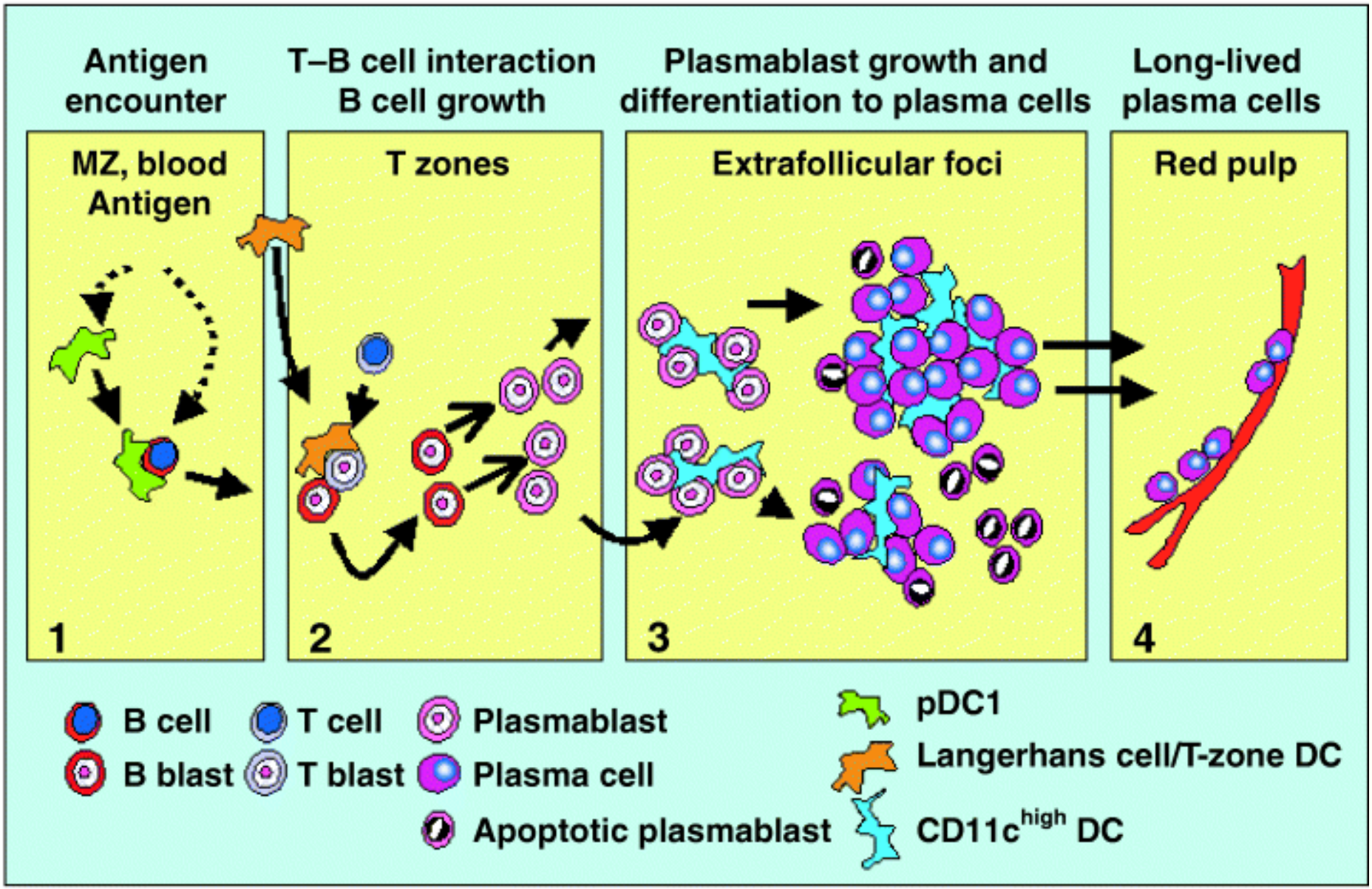
2. Relocation: Follicle → T/B boundary

Resting B cell: CXCR5 > CCR7

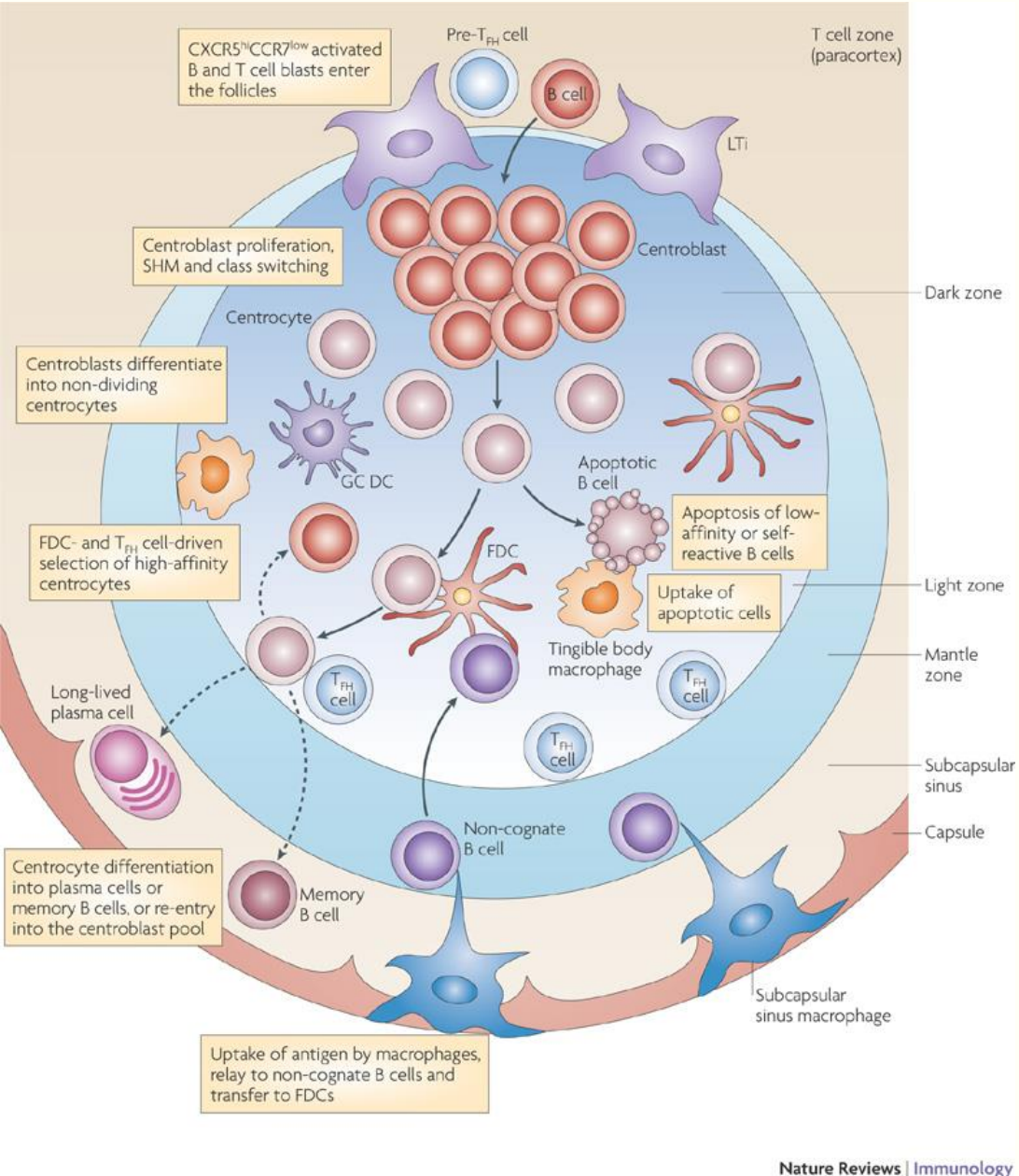
Activated B cell: CXCR5 < CCR7



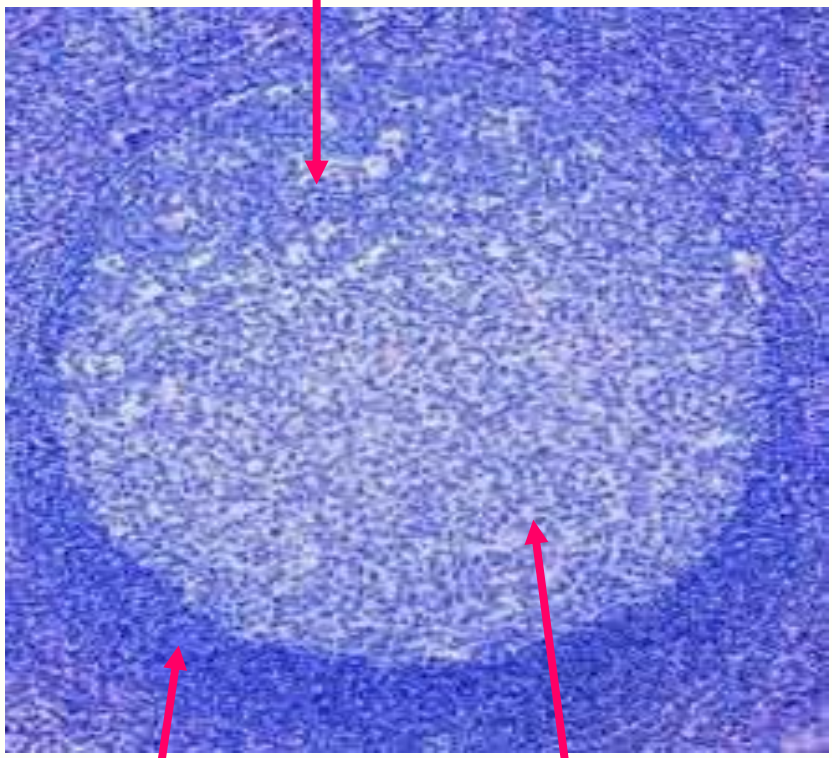
I. phase: Extrafollicular reaction



II. phase: Formation of germinal center



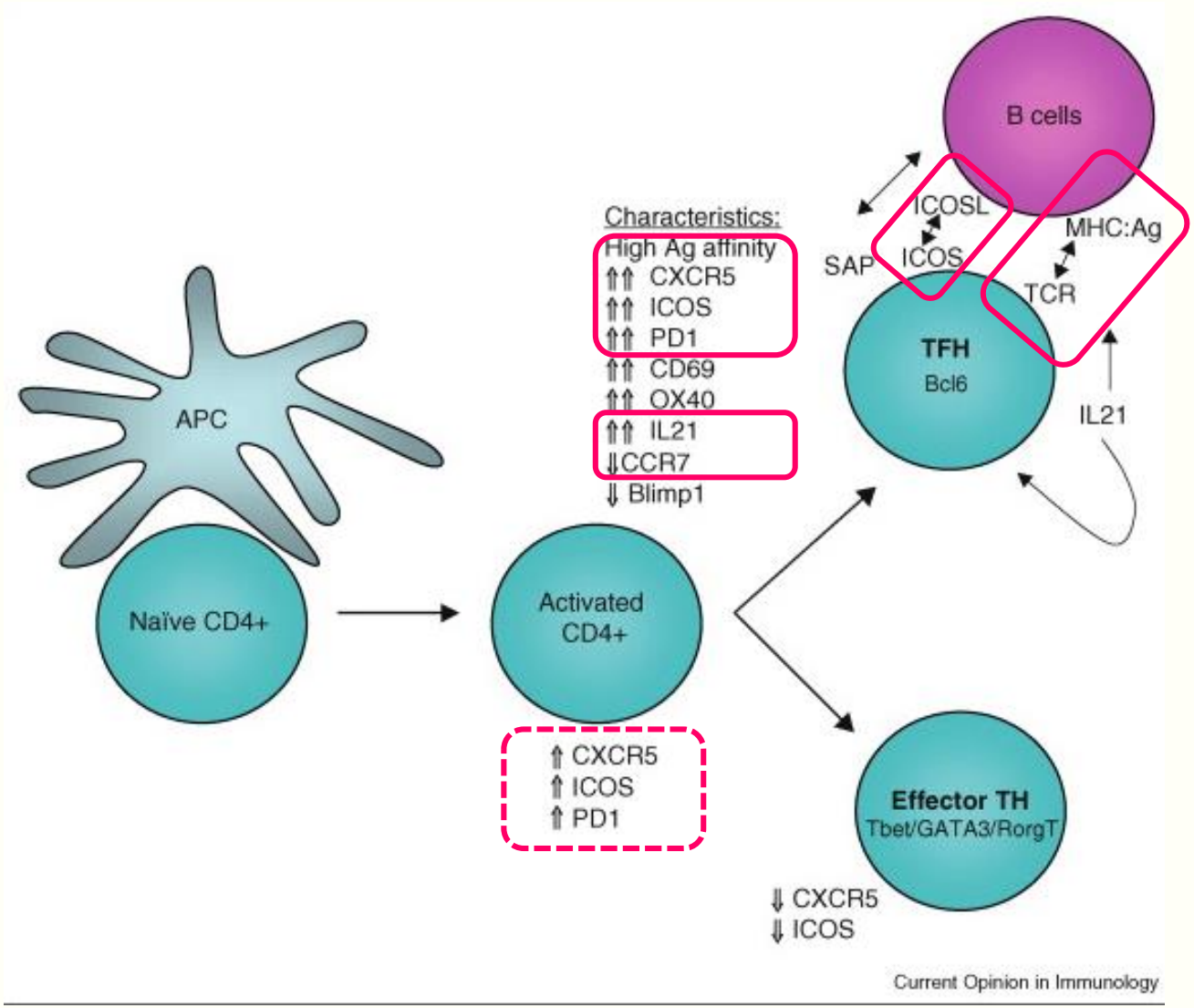
Dark zone: centroblasts + TBMs



Light zone:
centrocytes + FDCs

Mantle zone:
residual resting B cells

T_{FH} differentiation: T-B communication in activation



Selection based on highest TcR affinity

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

Paradox: motile cells ↔ fixed GC/DZ

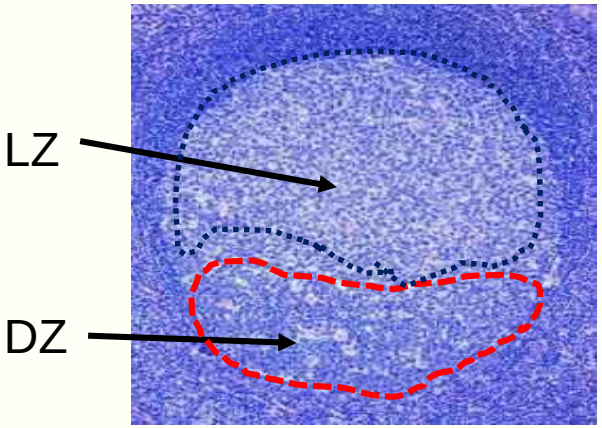
(1) T-zone/Follicle: CCR7/CXCR5 - *CCL21/CXCL13*

(2) LZ:DZ: $CXCR4^-/CXCR5^+$: $CXCR4^+/CXCR5^+$ - *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 → dissemination)



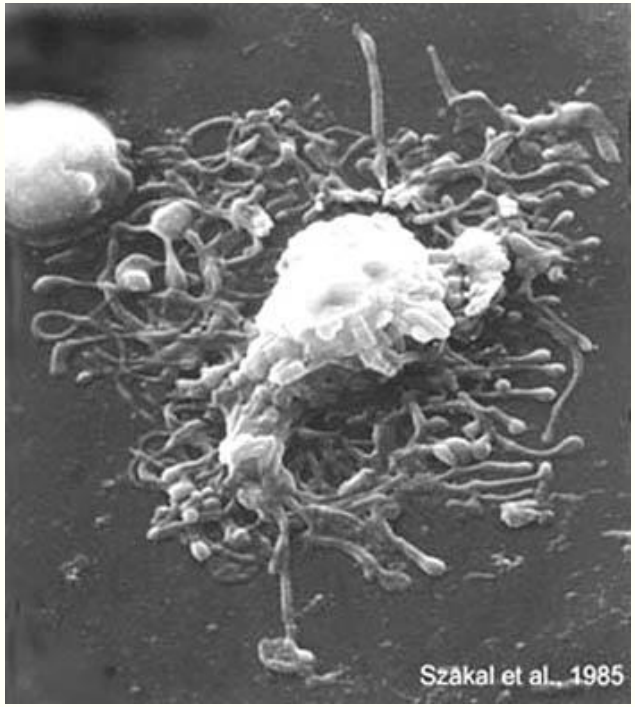
FDC: CXCL12<CXCL13

CRC: CXCL12>CXCL13

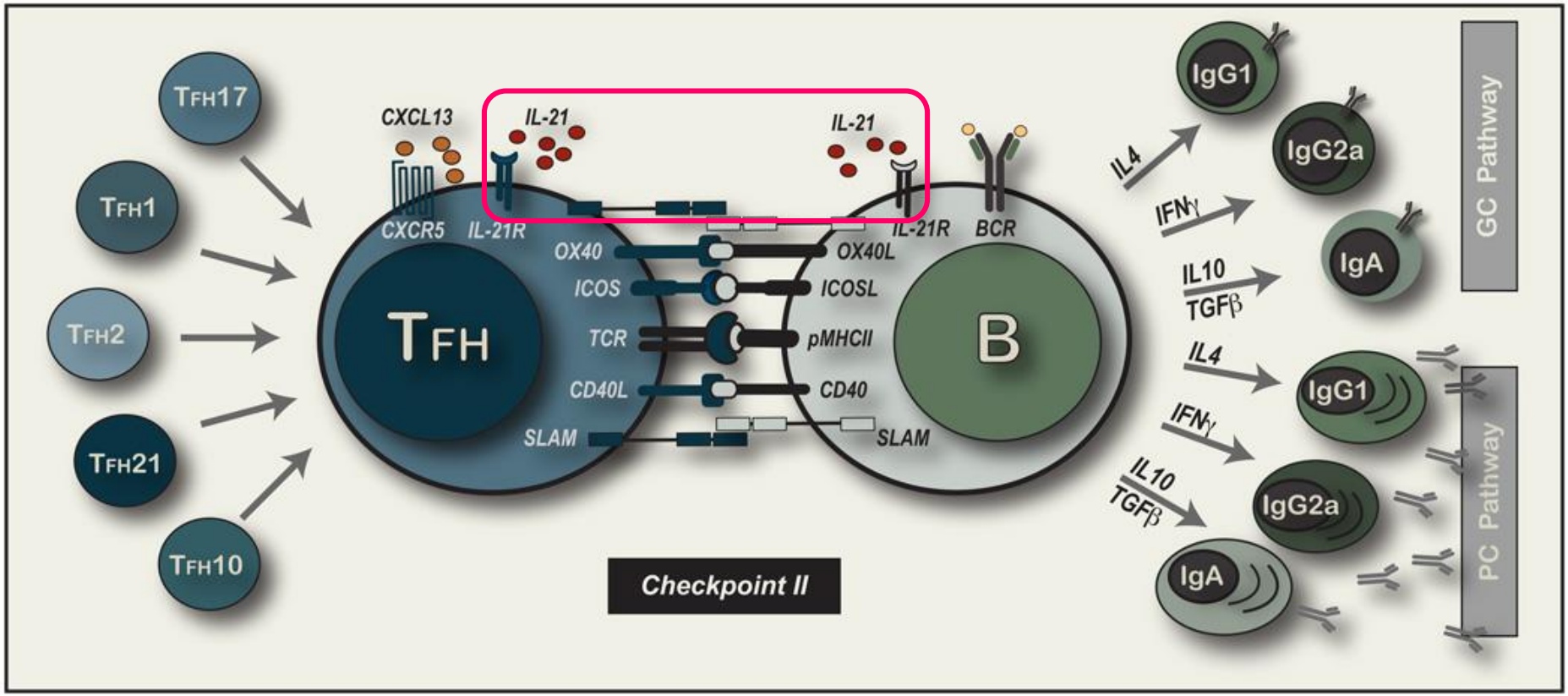
Follicular dendritic cells: GC organizers and cellular mediators of B-cell selection

FDC:

- Probably local mesenchymal origin
- 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)*

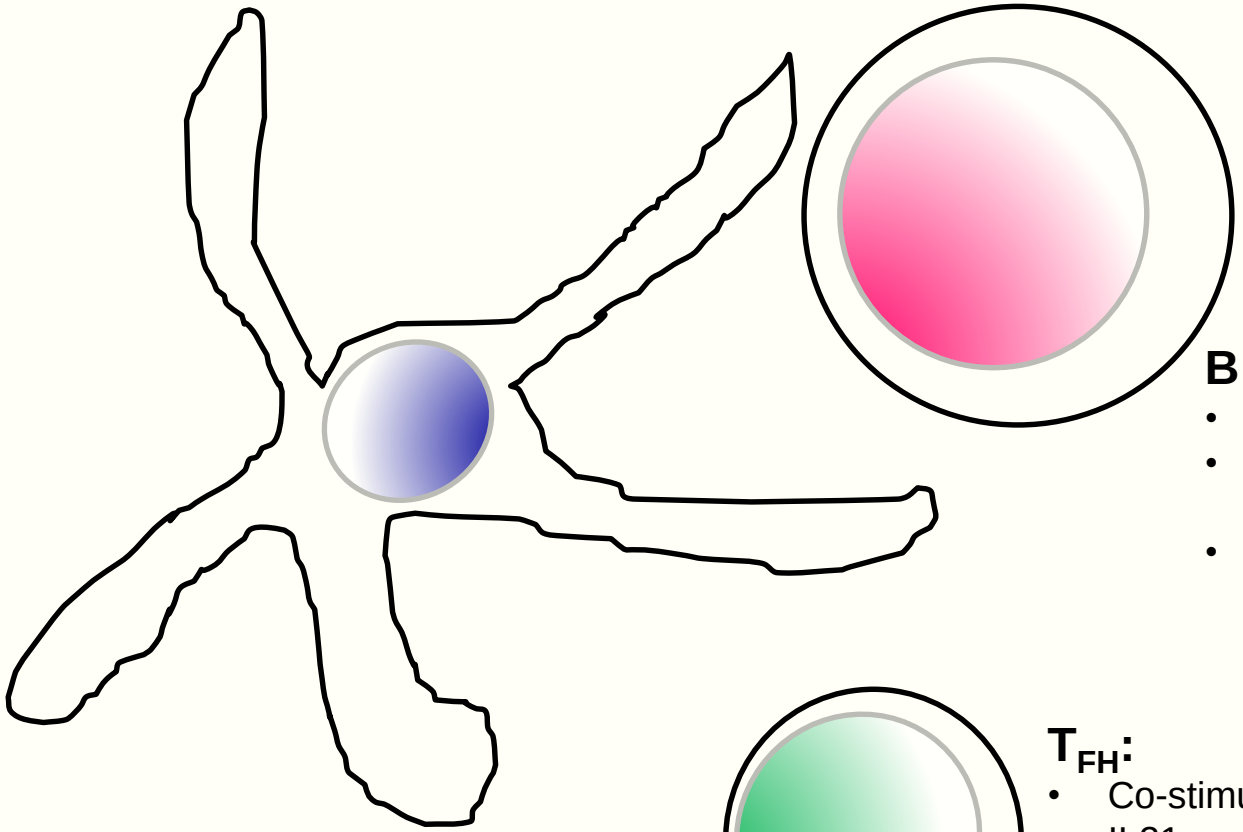


Role of polarized T_{FH} cells in Ig isotype regulation



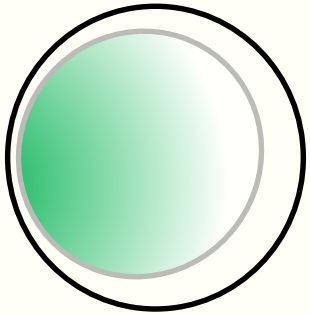
Fazilleau et al., 2009

Connection of T_{FH} - FDC - B cell interactions during GC reactions



B cells:

- MHC/Ag presentation
- Co-stimulatory ligands (CD40L, ICOSL)
- TNF/LT ligands – FDC maintenance



T_{FH}:

- Co-stimulatory molecules (CD40, ICOS)
- IL21 production
- Other cytokines – Ig isotype switch

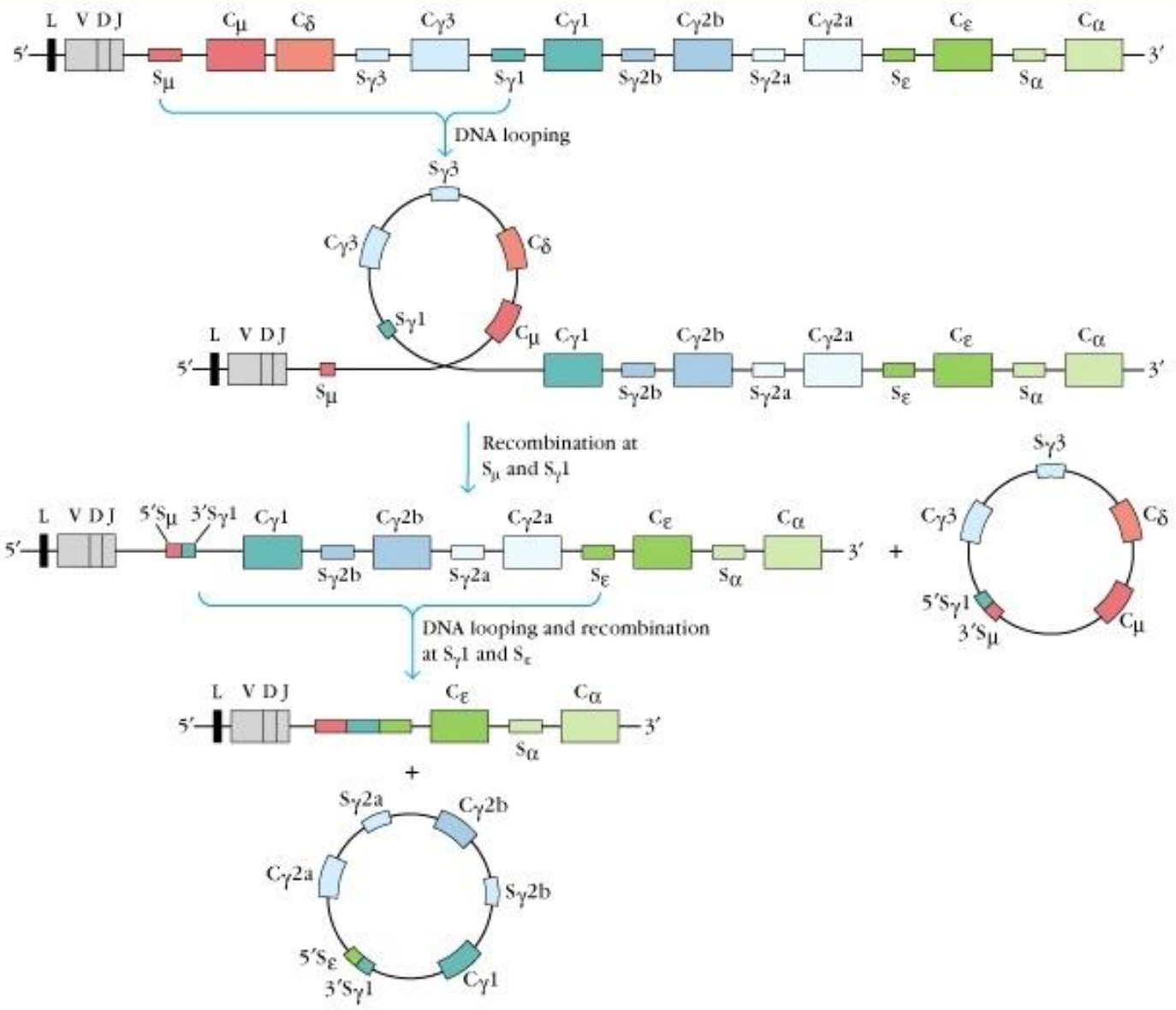
FDC:

- Immunocomplex retention,
- BAFF production,
- CXCL13 production

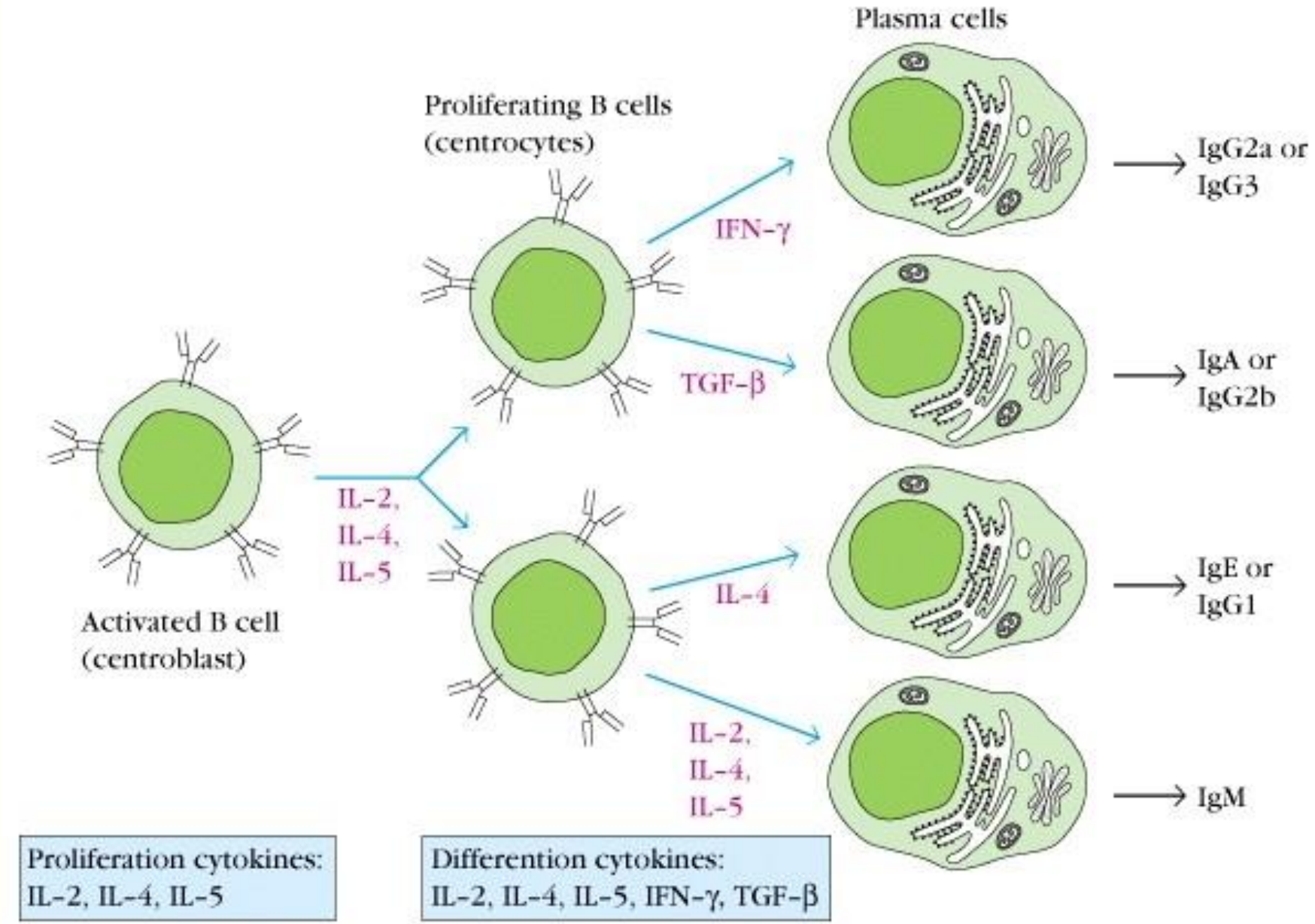
Mechanism of affinity maturation

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



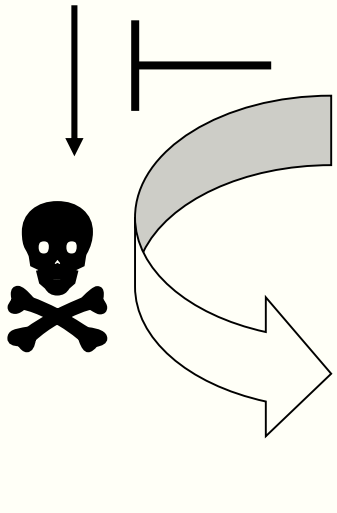
Mechanism of cell death

BcR & CD40 stimulation: Ag & CD40L

Extrinsic path:

Fas expression enhances: Ligand binding

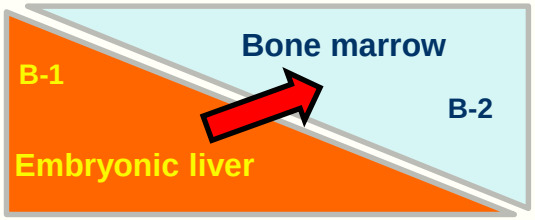
Caspase 8 induction

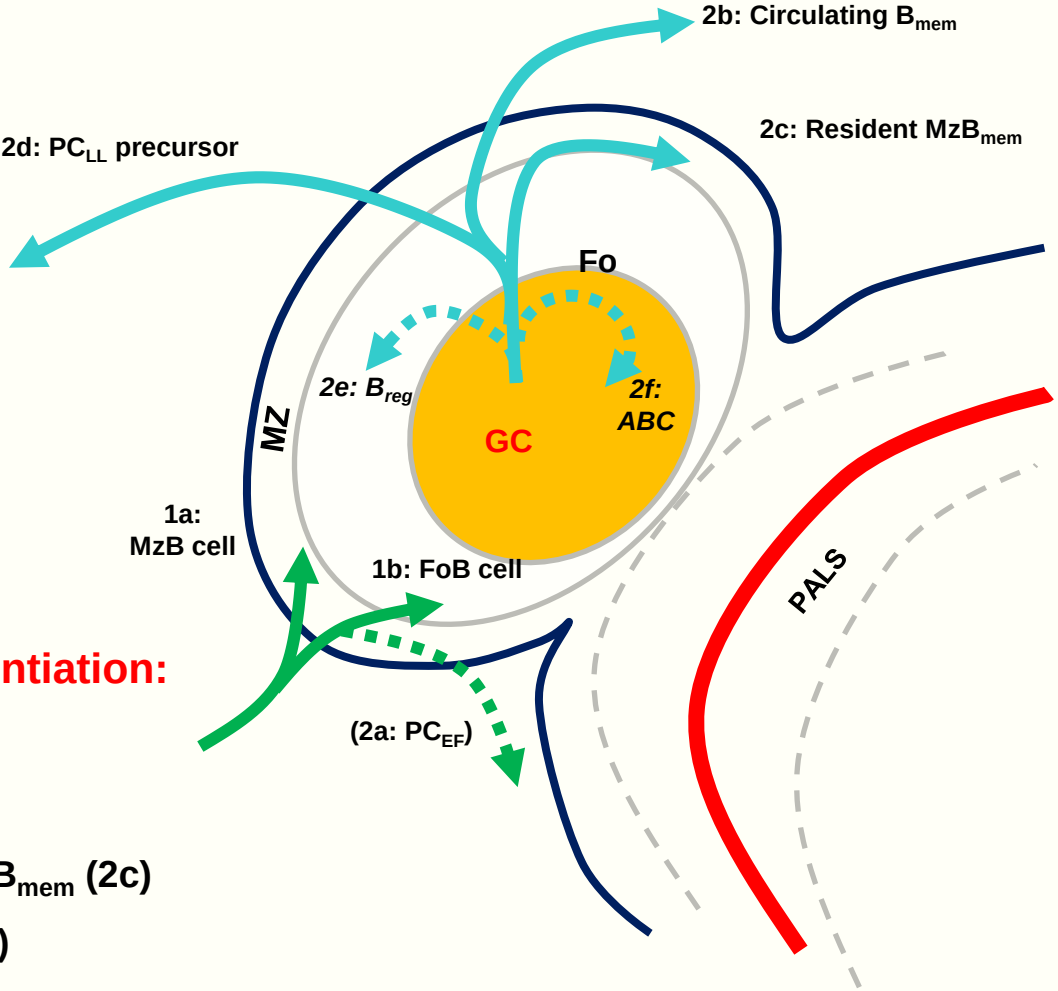


Intrinsic path:

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:

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

Alterations of Ig gene and their characteristics

Ig gene alteration	Ag/AID	RAG-I/II	Promotes survival	Cytokine regulation	Effector mechanism
VDJ (H/L)	-	+	+	-	-
Affinity maturation	+	-	+	-	-
Isotype switch	+	-	-	+	+
Protein alteration				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) IFNα/CD40 engagement (c) upon IL-35/IL-1β/IL-6 effects.
- *Regulatory functions*: Mediated by (a) IL-10 (b) TGFβ, (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$^+$/CD11c$^+$ (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$^+$ 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$^-$/CD23$^-$).