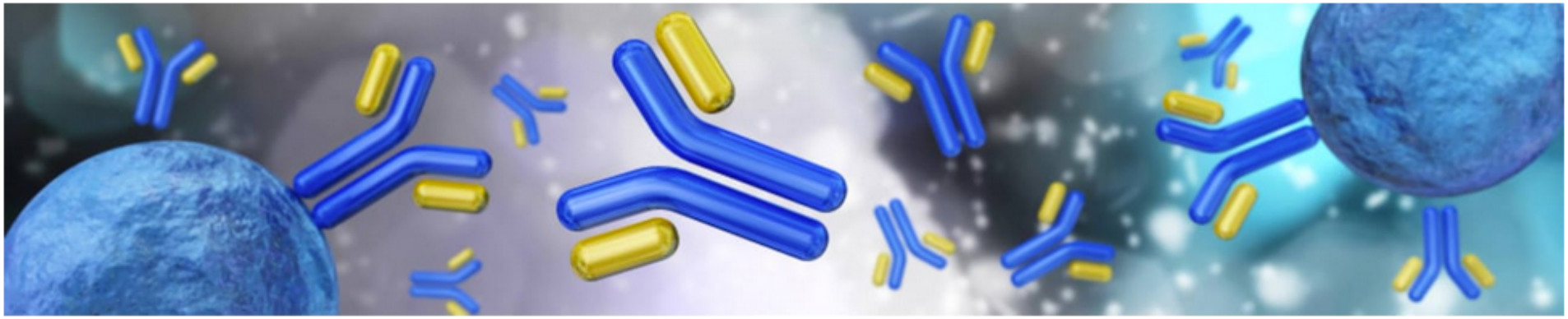
B Cell Activation and the Humoral Immune Response

Micro204 Molecular and Cellular Immunology

Lecturer: Jason Cyster



Vaccine Challenge

- Vaccines are needed that induce broadly neutralizing antibodies against flu and HIV
- How are we going to make them?
- Need to know how B cells see antigen, how they become activated to make antibody, what affects the range of antibodies they make and what ensures a long-lasting response

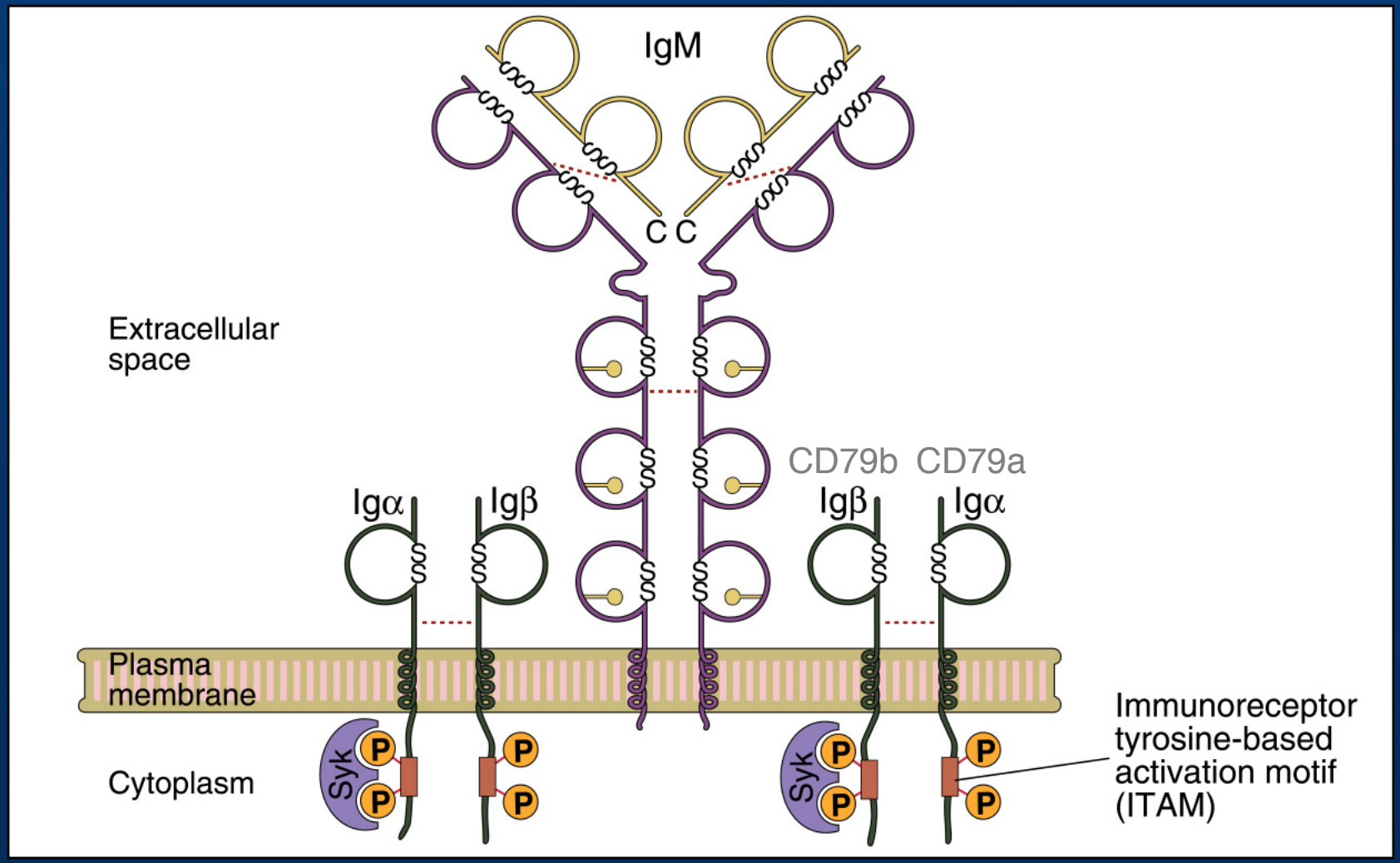
Questions

- What is the frequency of B cells specific for a typical viral antigen?

- What sets the number of B cells?

BCR

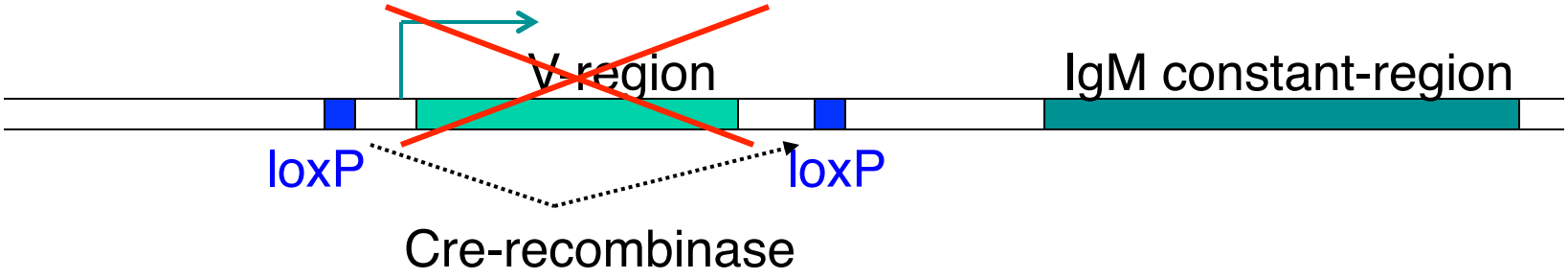
B cell antigen receptor complex

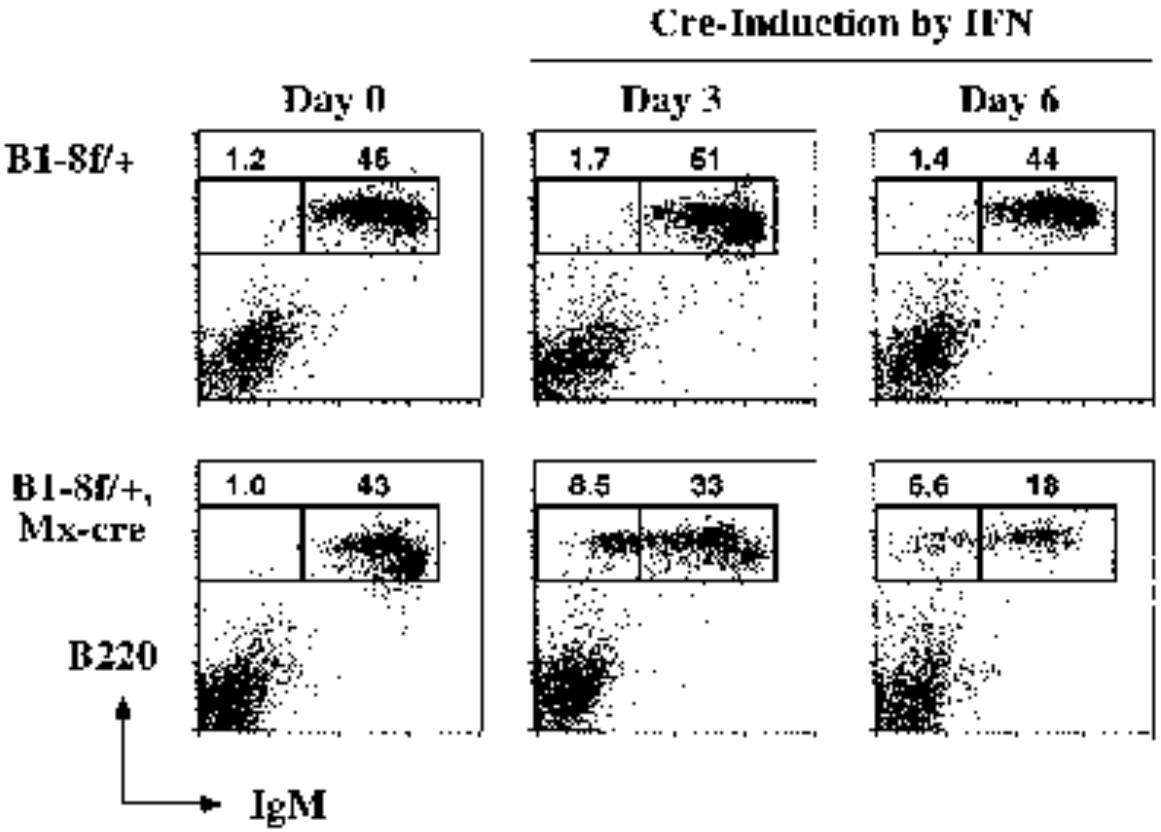


From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-3

Syk kinase is closely related to Zap70 in T cells (has 2 SH2 domains)

Constitutive BCR signaling is necessary for B cell survival

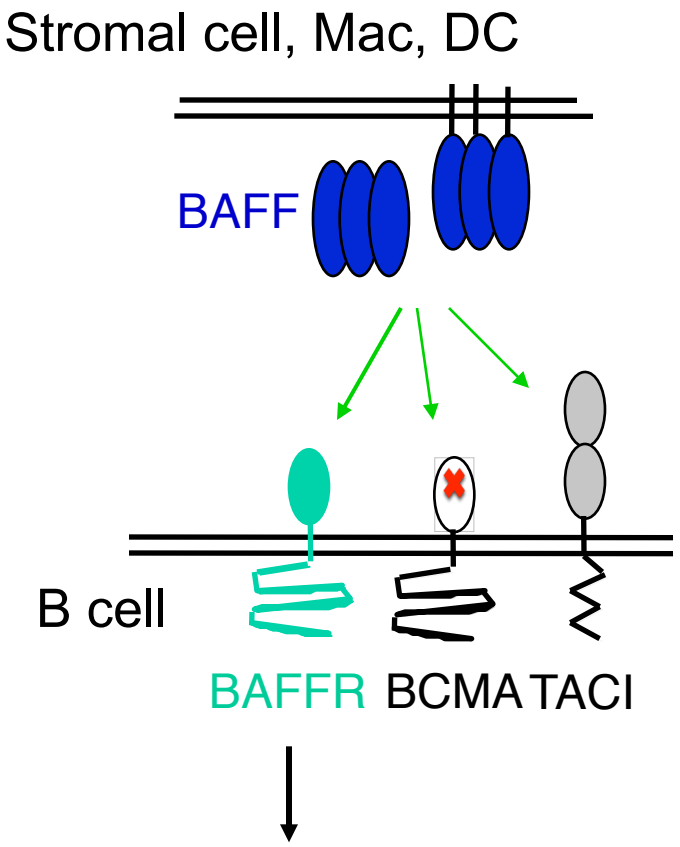




Lam / Rajewsky 1997 Cell

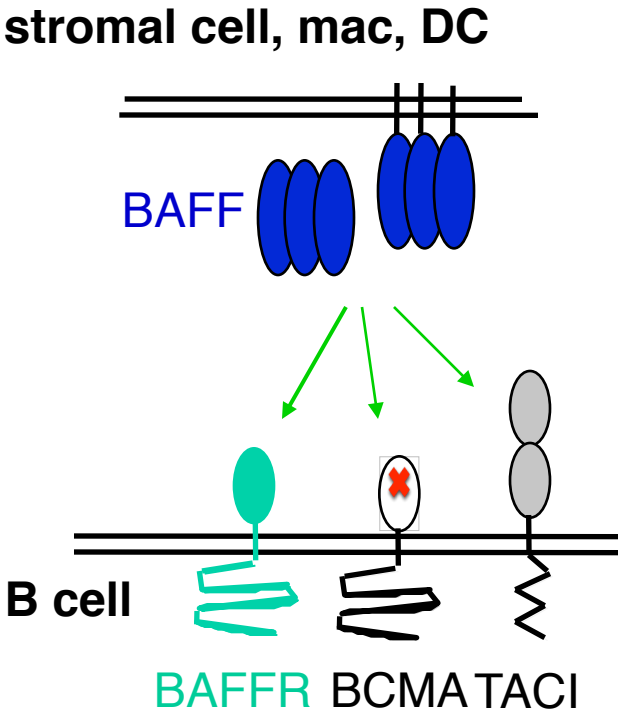
Srinivasan / Rajewsky 2009 Cell
PI3K signals BCR-dependent mature B cell survival

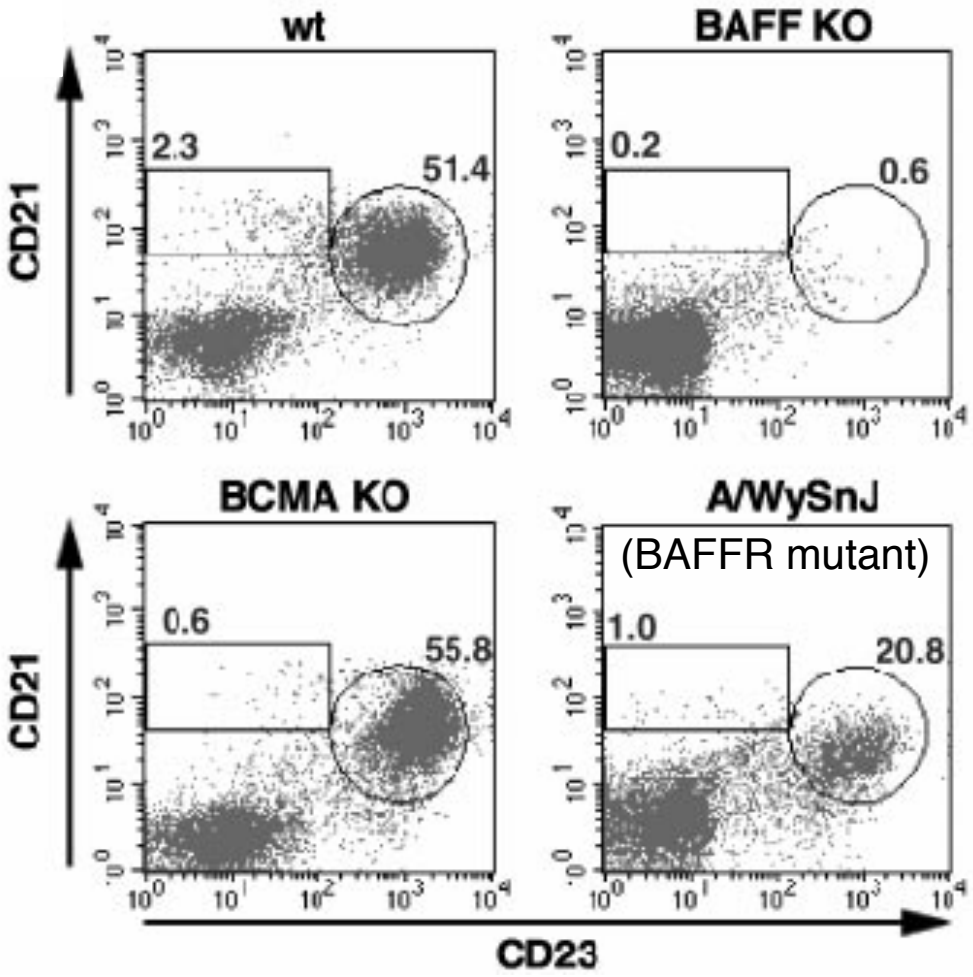
BAFF / Blys and B cell survival



- Member of TNF family
- Made by stromal cells, macrophages, DCs
- BAFF-receptor constitutively expressed on B cells
  - BAFF also binds TACI and BCMA
  - APRIL is a second TACI, BCMA ligand
- Critical role in B cell homeostasis
  - BAFF knockout has ~100x less B cells and is less able to mount antibody responses
  - BAFF over-expression increases total B cell numbers and promotes autoantibody production
  - BAFF serum levels elevated in lupus

BAFF/Blys is necessary for B cell survival





CD21 (Cr2) and CD23 are markers of mature (follicular) B cells
CD21^{hi}CD23^{lo} B cells in the spleen are marginal zone (MZ) B cells

from Schiemann et al., Science 293, 2111 (2001)

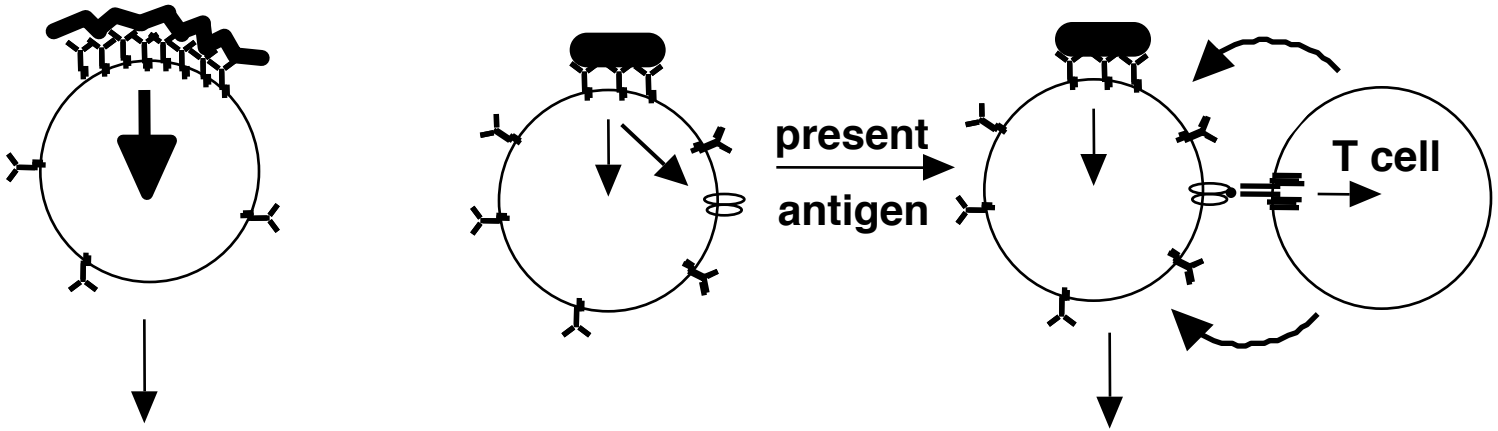
Questions

- Flu and HIV antigens are heavily glycosylated. Can we make antibodies against carbohydrate antigens? Can we make them against the ones on viruses?

Types of B cell Antigens

T-cell independent (TI)

T-cell dependent (TD)



mitogenic
BCR signal

'activation' signal
but not mitogenic

mitogenesis,
differentiation

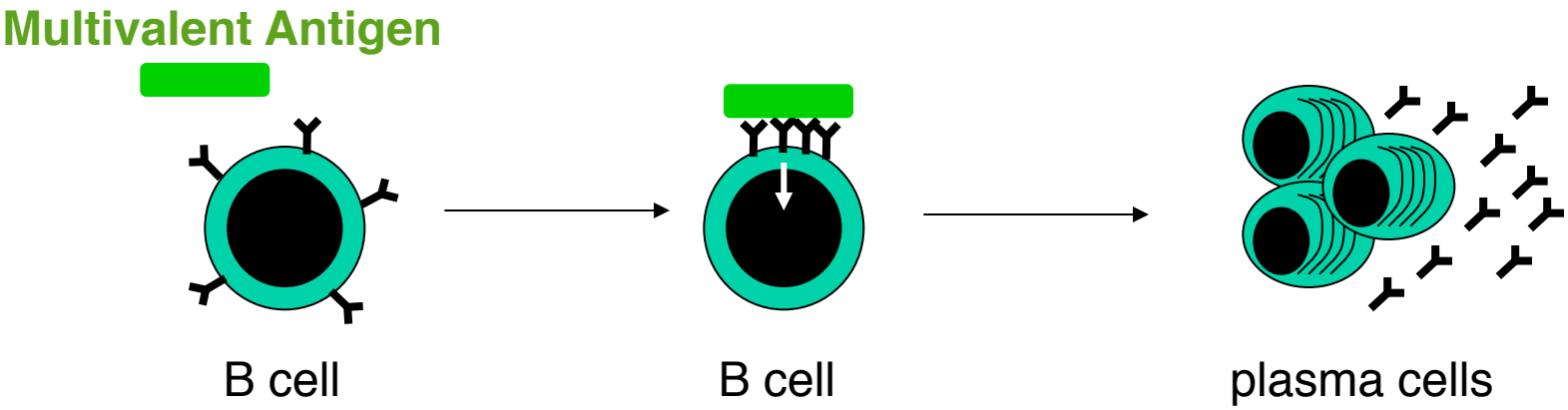
Mitogenic means promotes mitosis (proliferation) – clonal expansion

Types of antigen

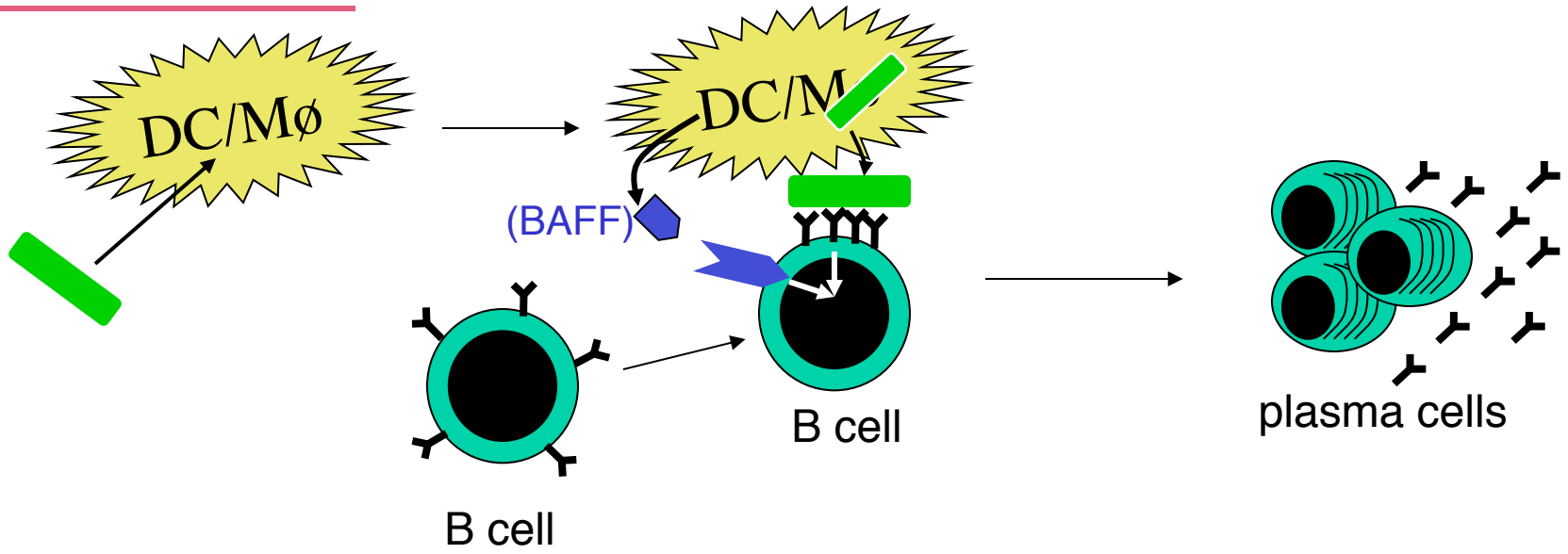
- T-independent (TI) antigens
  - induce division/differentiation by BCR signaling alone
    - bacterial polysaccharides, repeating surface molecules on viruses
- T-dependent (TD) antigens
  - activate via BCR but depend on additional signals from helper T cells to cause division/differentiation
    - any antigen containing protein
- Most pathogens contain both TI and TD antigens
- Only TD antigens can induce Germinal Center (GC) responses

T-Independent (type II) Responses

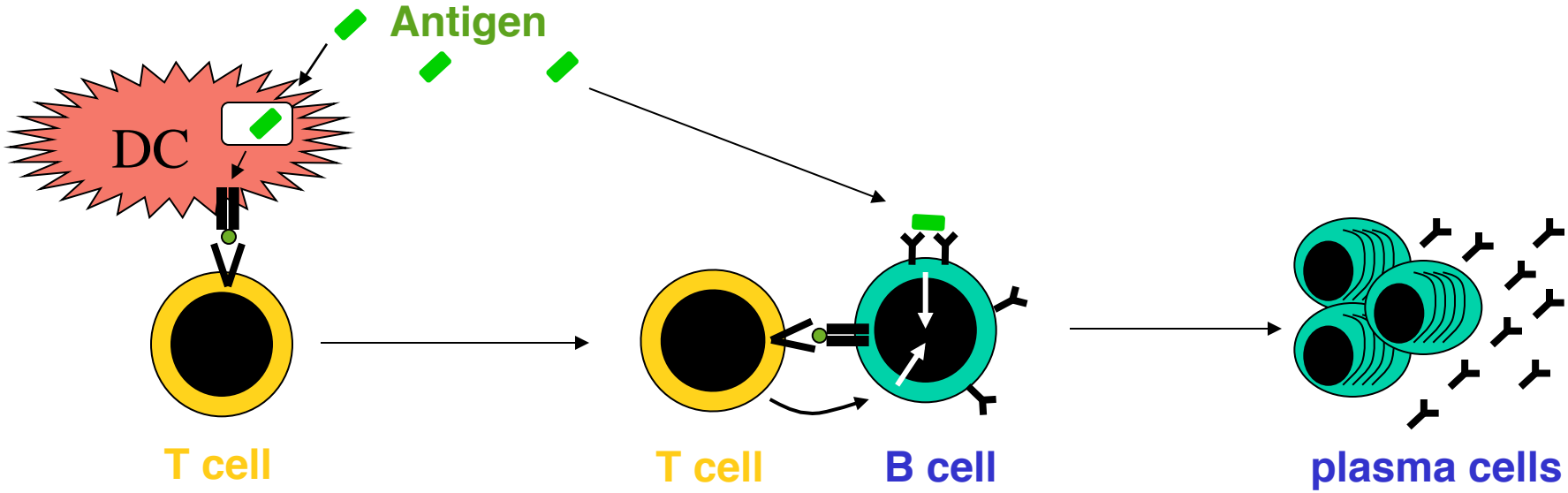
Old Paradigm:



Newer Model:



T-Dependent Responses



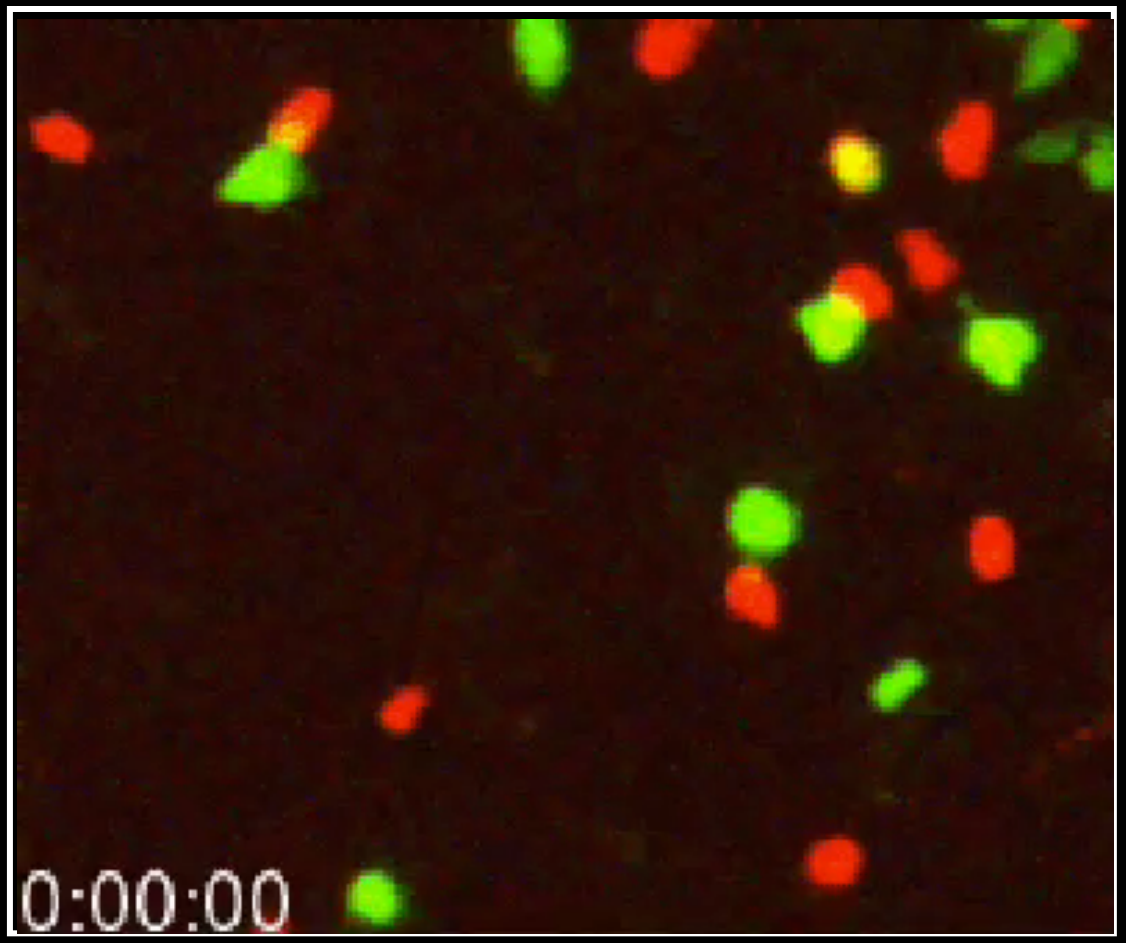
Dendritic Cell (DC) internalizes antigen (Ag), processes into peptides, presents peptides together with MHC molecules to T cells

B cell binds Ag via surface Ig, transmits BCR signals and presents peptides to T cells, receives T cell help (growth and differentiation factors)

Secretes Antibody (Ab)

Interactions between antigen-specific B and T cells

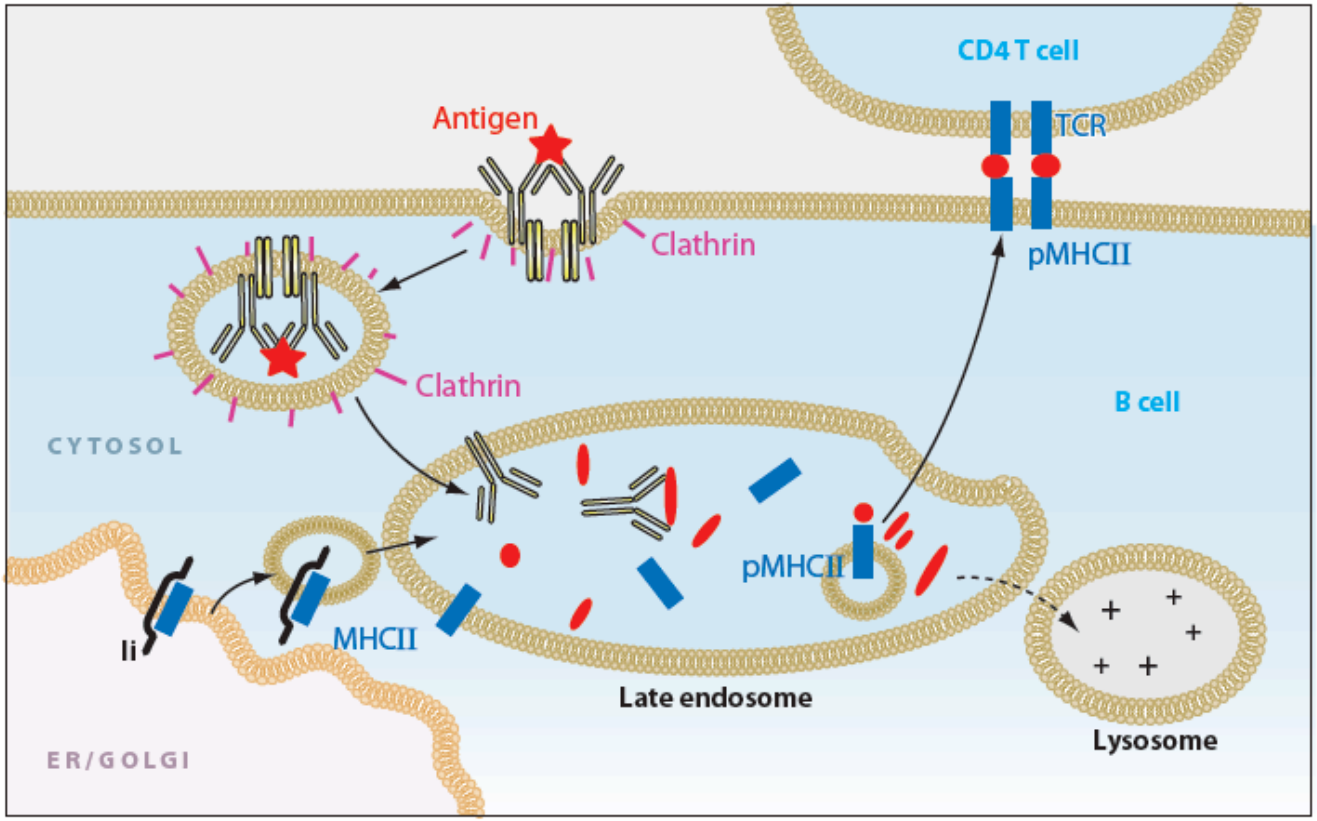
1 day after Hen Egg Lysozyme (HEL) antigen injection



HEL-specific (Ig-tg) B cells

HEL-specific (TCR7 tg) T cells

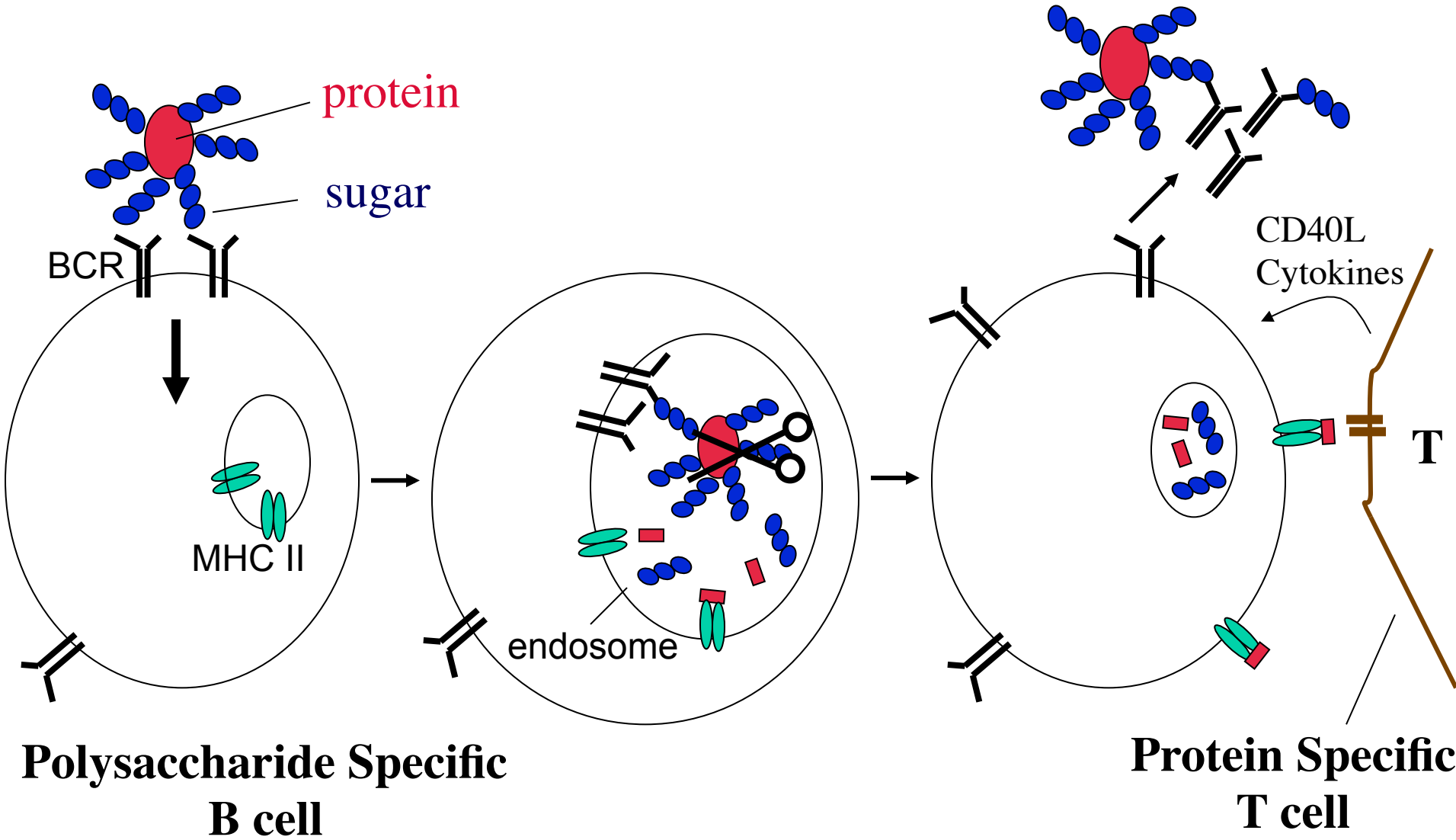
B cells are antigen-presenting cells



- BCR cross-linking induces antigen internalization to endosomes
- antigen is proteolysed to peptides
- peptides associate with MHC class II
- MHC class II-peptide complexes traffic to surface of B cell

➢ *B cells present antigen recognized by their BCR ~10^5 times more efficiently than other antigens*

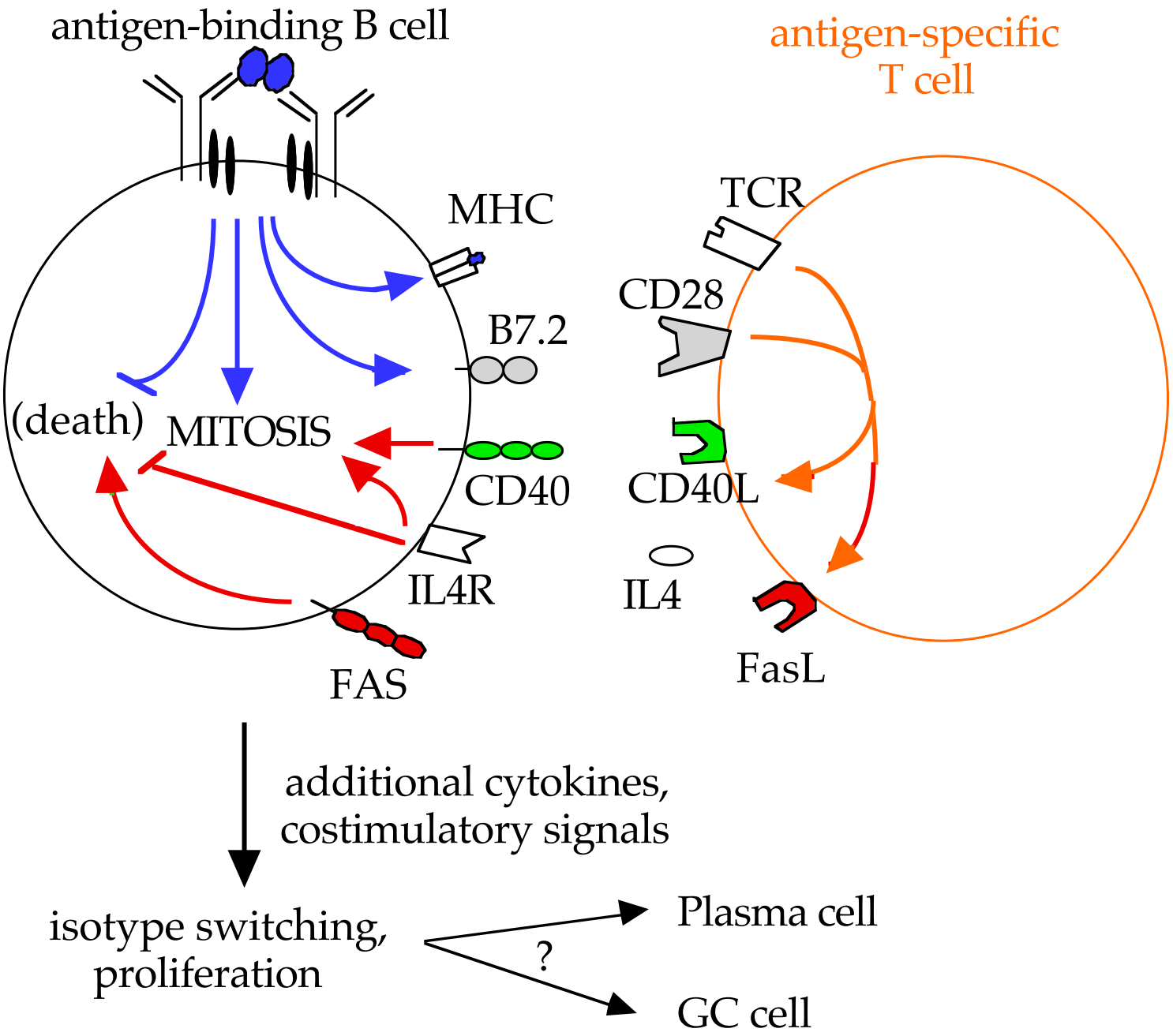
B cell antigen presentation and the concept of linked help



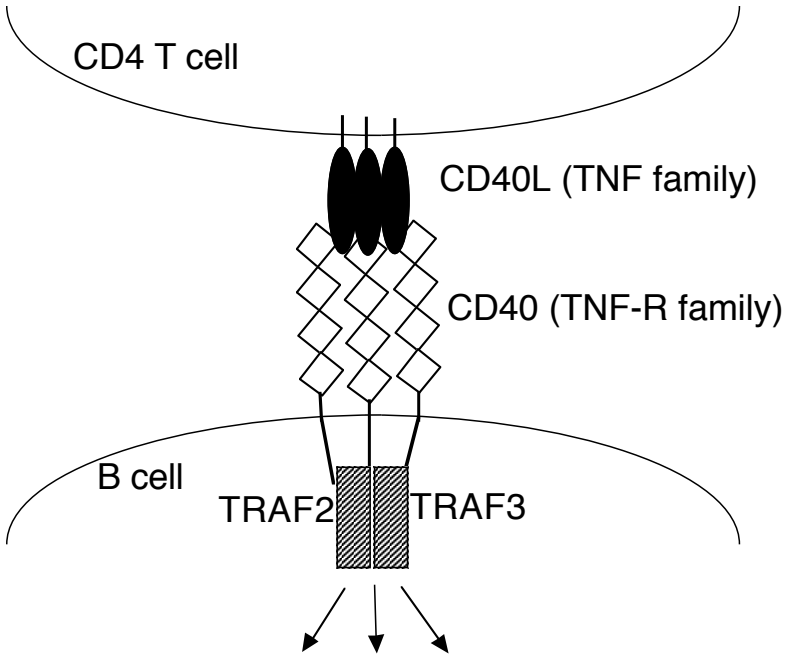
Questions

- Do we want the vaccine to drive a T-independent or T-dependent response?

Cardinal features of B - T interaction



Role of CD40 in B cell activation



increased expression of cell cycle molecules,
survival molecules, cytokines,
promotes isotype switching

- TCR triggering up-regulates CD40L on T cell
- CD40 signaling promotes B cell activation
- CD40L-deficiency = 'hyper-IgM syndrome' (no isotype switching, no Germinal Centers)
- CD40 signaling also important in DC, Mac function

B-T interaction summary

- BCR signals induce costimulatory molecules (CD86, ICAM1 etc), entry to G1, antigen presentation
- CD40L triggers CD40 -> synergizes with BCR signals to promote mitosis
- T cell derived cytokines promote proliferation, differentiation, isotype switching:
  - IL6 promotes differentiation
  - IL4 -> IgG1, IgE
  - IFNγ -> IgG2a, IgG3
  - TGFβ and IL5 -> IgA
- Many other molecules involved in T-B interactions, including:
  - Ly108 (SLAM family) homotypic adhesion molecules and SLAM-associated protein (SAP) promote prolonged B-T interaction essential for GC response
  - ICOSL on GC B -> ICOS on Tfh (essential for GC response)

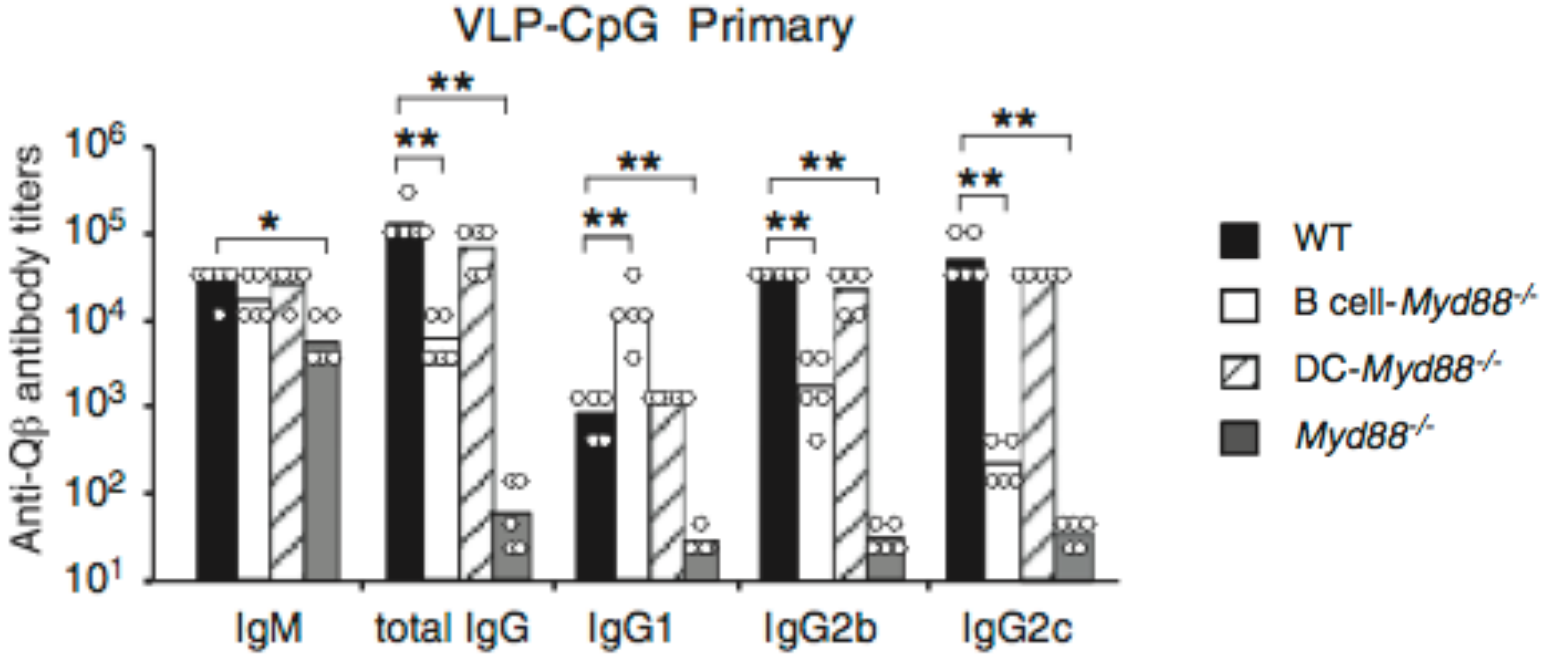
Questions

- Do viruses promote the B cell response solely be engaging the BCR or can other receptors on the B cell be involved? Which ones?

Innate features of pathogens act as B cell costimulators

- Pathogen multivalency
  - provides a level of BCR crosslinking optimal for activation
- Many pathogens activate TLRs
  - TLR signaling synergizes with BCR signal
- Some induce type I IFNs
  - IFNα/β receptor signaling synergizes with BCR
- Many activate the complement cascade and become C3d coated
  - complement receptor (CR) crosslinking synergizes with BCR signal

TLR signaling in B cells via adaptor Myd88 augments Ab response

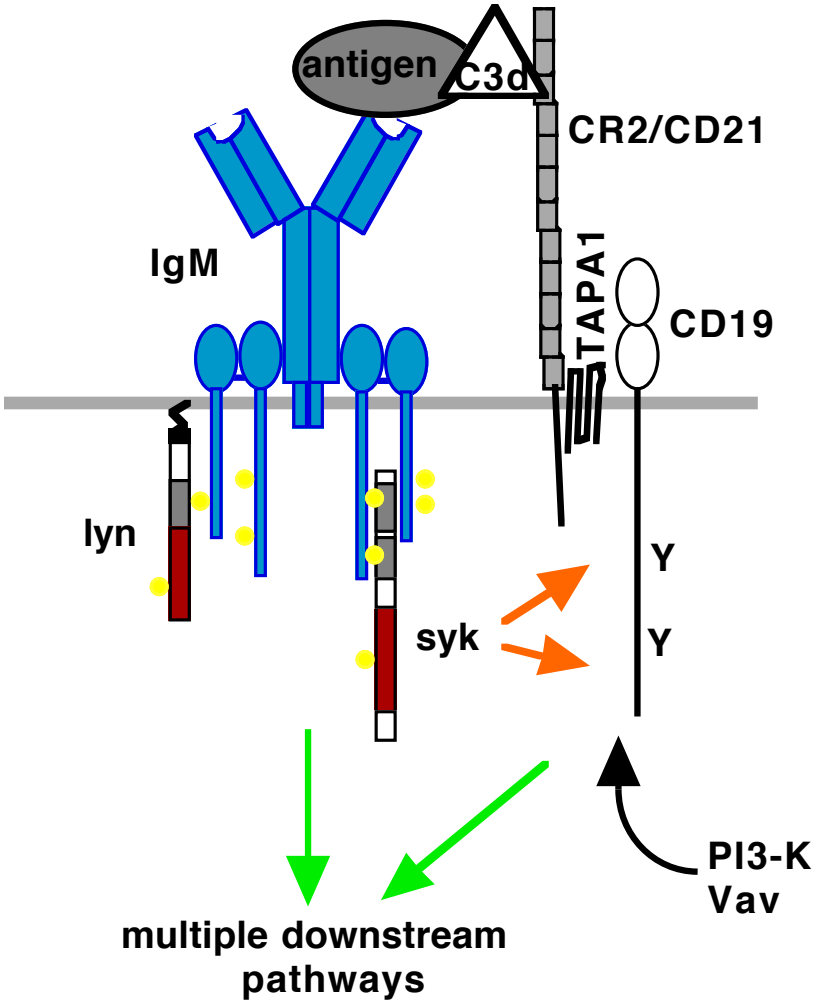


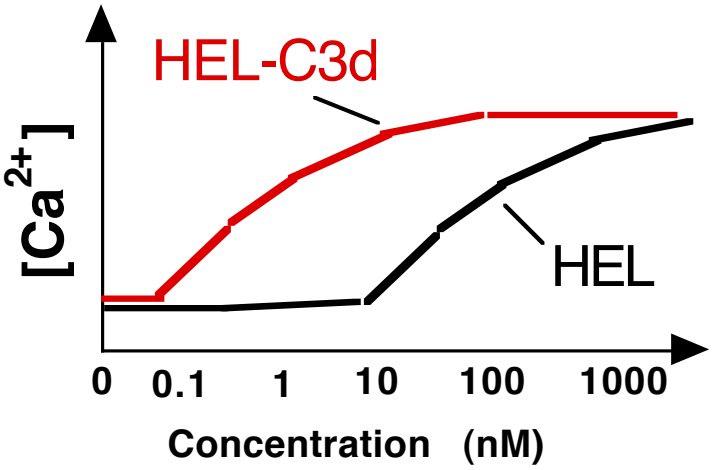
VLP – viral-like particle
Myd88 – signaling adaptor needed for TLR9 signaling

- Antigen internalized via BCR colocalizes TLR-ligand (CpG DNA) with endosomal TLR9 in B cell -> costimulatory signaling of antigen specific cell

Hou et al., *Immunity* 2011

Antigen-C3d complexes cross-link BCR and CR2-CD19 -> increase sensitivity to antigen





Dempsey et al. (1996) Science 271, 348

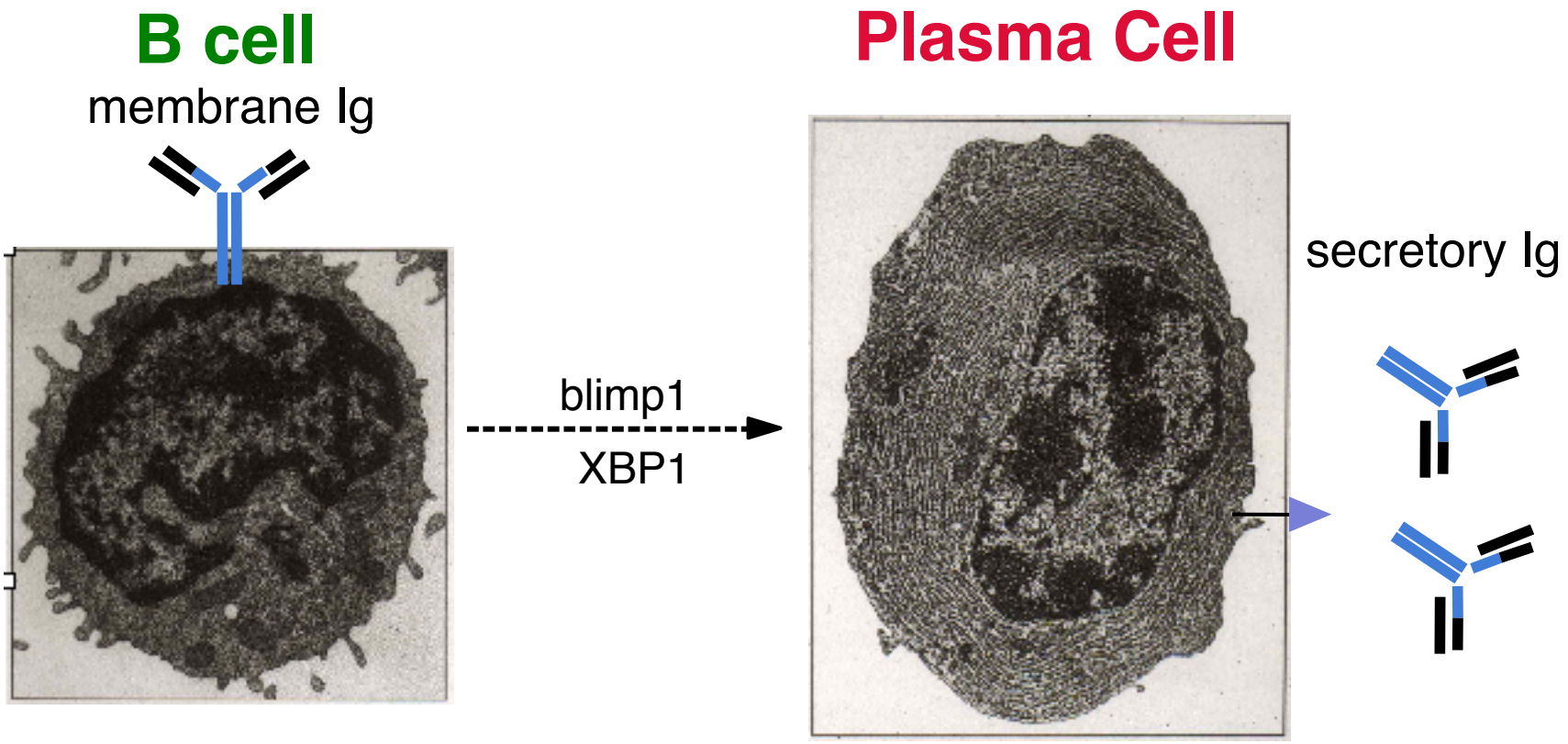
Sensitivity of anti-HEL Ig-transgenic B cells to HEL and HEL-C3d

HEL – hen egg lysozyme (model antigen)

Questions

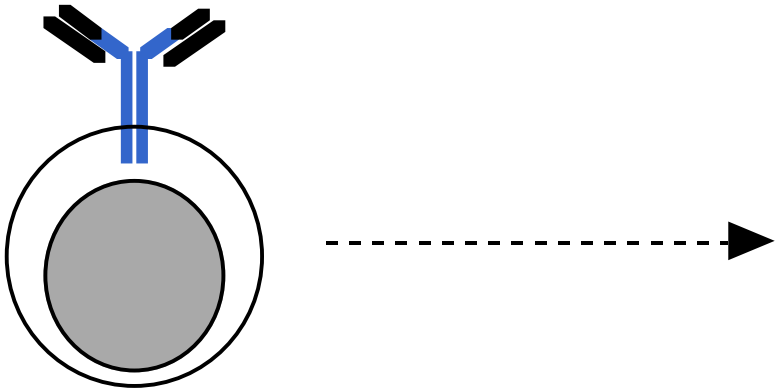
- Ultimately the vaccine needs to induce plasma cell differentiation. Does it matter what type of plasma cell is induced?

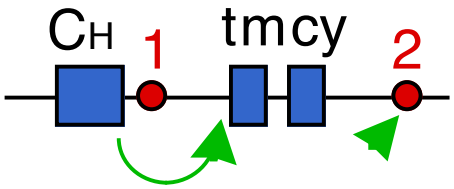
After appropriate activation the B cell differentiates into an antibody secreting cell, also known as a Plasma Cell



Production of membrane vs secreted Ig

membrane Ig





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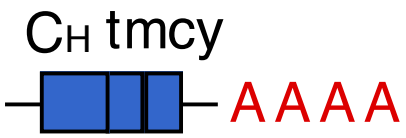
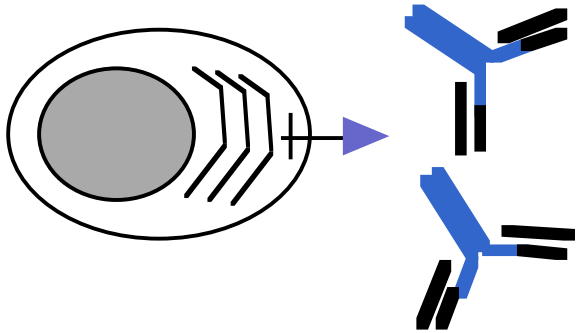
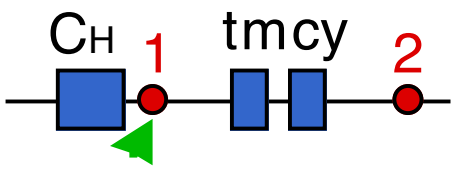


secretory Ig





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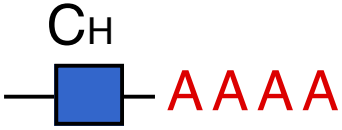
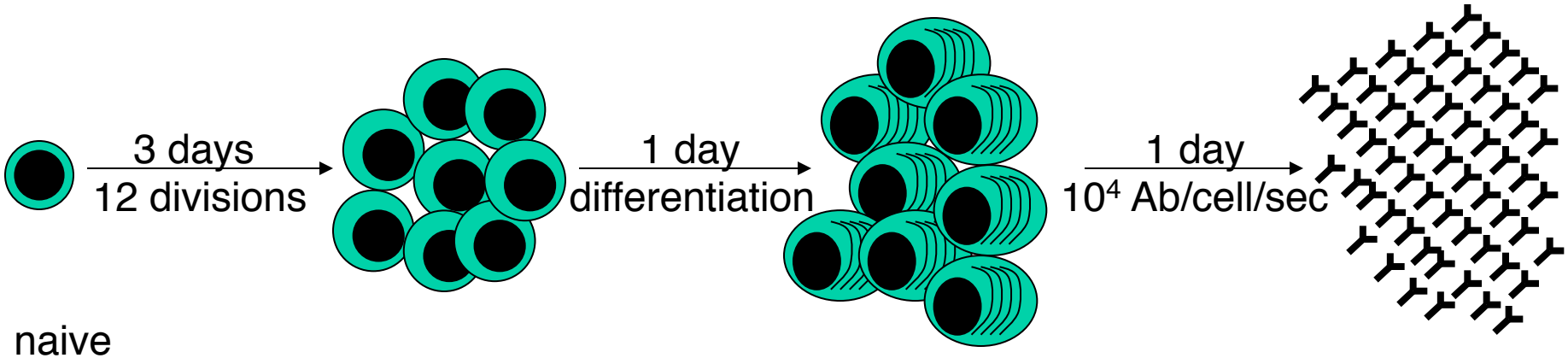


B cell antibody response - clonal replication enters into a higher order upon plasma cell differentation



(these are just rough numbers)

Plasma Cells are antibody secreting cells

Two types:

1. Plasma cells generated early in the primary response
   - short-lived (~ few days)
   - typically low affinity
   - form in T-independent and T-dependent responses
   - home to red pulp of spleen, medullary cords of lymph nodes
   - IgM but also IgG and other isotypes

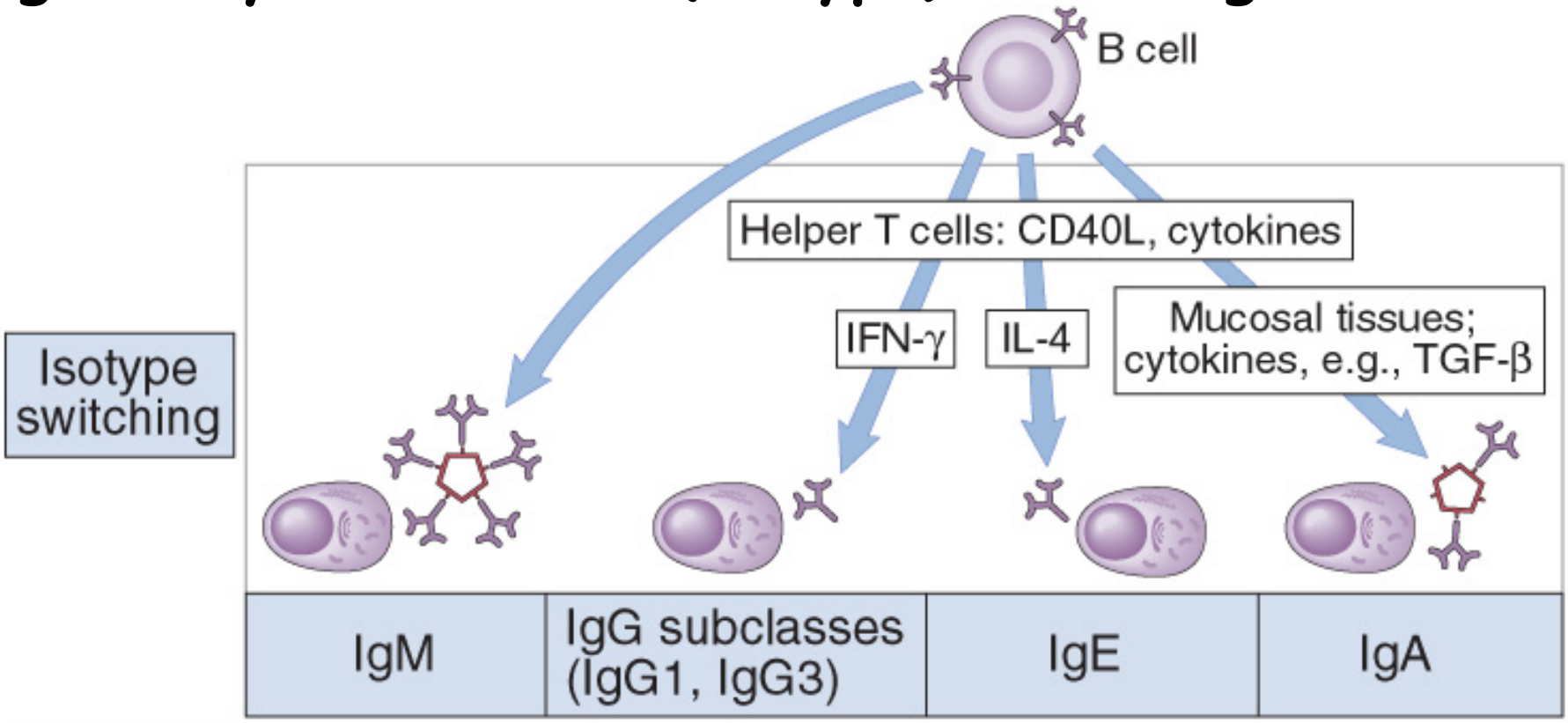
2. Plasma cells generated later in the primary response and that predominate in secondary responses
   - arise predominantly from germinal centers (in primary) or from memory B cells (in secondary)
   - long-lived (possibly several months)
   - often home to bone marrow, gut, lactating mammary gland
   - predominantly isotype switched

Questions

- Which Ig isotype would be best for an anti-Flu response? Or anti-HIV?

- What is meant by antibody-mediated opsonization? How does this differ from complement-mediated opsonization?

Ig Heavy chain class (isotype) switching



	IgM	IgG subclasses (IgG1, IgG3)	IgE	IgA
Principal effector functions	Complement activation neutralization	Fc receptor—dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)	Immunity against helminths and basophil Mast cell ^ degranulation (immediate hypersensitivity)	Mucosal immunity (transport of IgA through epithelia) neutralization

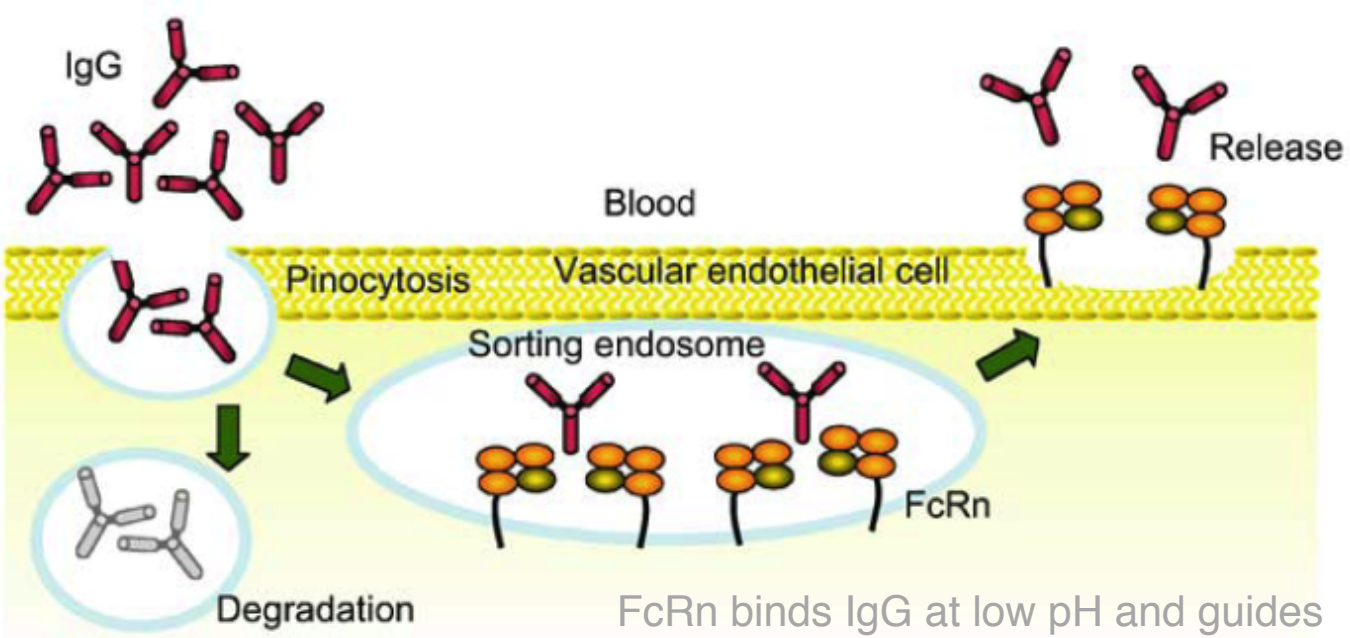
Neutralization
3wk t1/2

Neonatal Immunity

- Maternal IgG is transported by the neonatal Fc receptor (FcRn)
  - across the placenta to the fetus
  - from colostrum (first milk) across the gut epithelium
- Confers passive immunity in the newborn
- The duration of protection is 3-4 months (or ~5 IgG half-lives)
  - explains the high incidence of disease after this period by bacteria such as *Haemophilus influenzae*
- Human milk contains IgA
  - provides some protection against gut pathogens
- Neonatal protection is only as good as the titer of IgG (and IgA) in the mother against the specific organism

IgG half-life

- FcRn is present in endothelium in the adult where it protects IgG from degradation
- Accounts for the long (3 week) half-life of IgG compared to other Ig isotypes
- Therapeutic agents that are fused to IgG Fc regions take advantage of this property e.g. Enbrel (TNFR-Fc)



FcRn binds IgG at low pH and guides away from lysosomes and back to cell surface for release at neutral pH

Questions

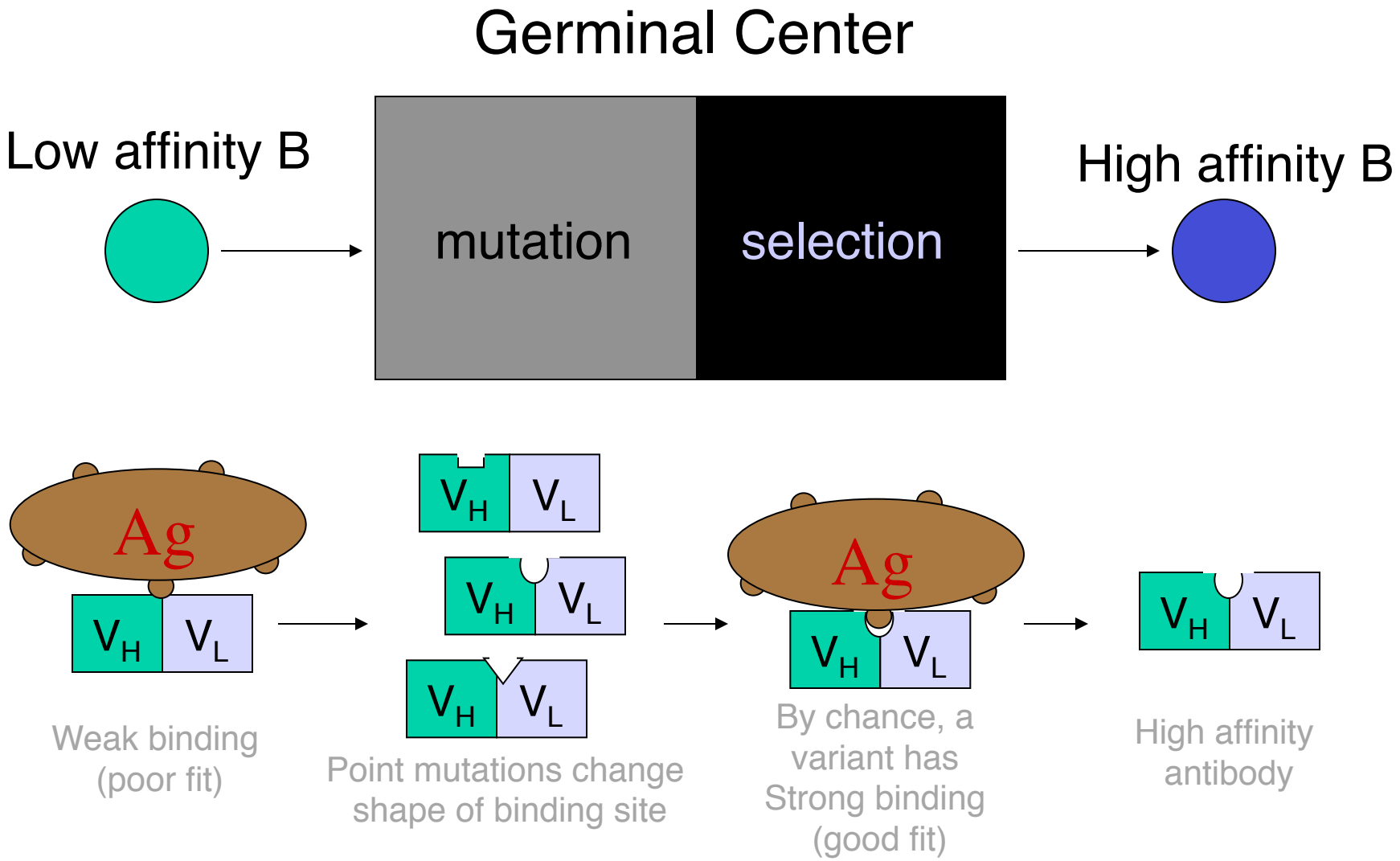
- Why do infants get repeated rounds of a vaccine within months of each other?

Affinity Maturation

- Affinity maturation occurs in germinal centers and is the result of somatic hypermutation of Ig-genes in dividing B cells followed by selection of high affinity B cells by antigen displayed by FDCs

- The high affinity B cells emerging in germinal centers give rise to long-lived plasma cells and memory B cells

Antibody Affinity Maturation



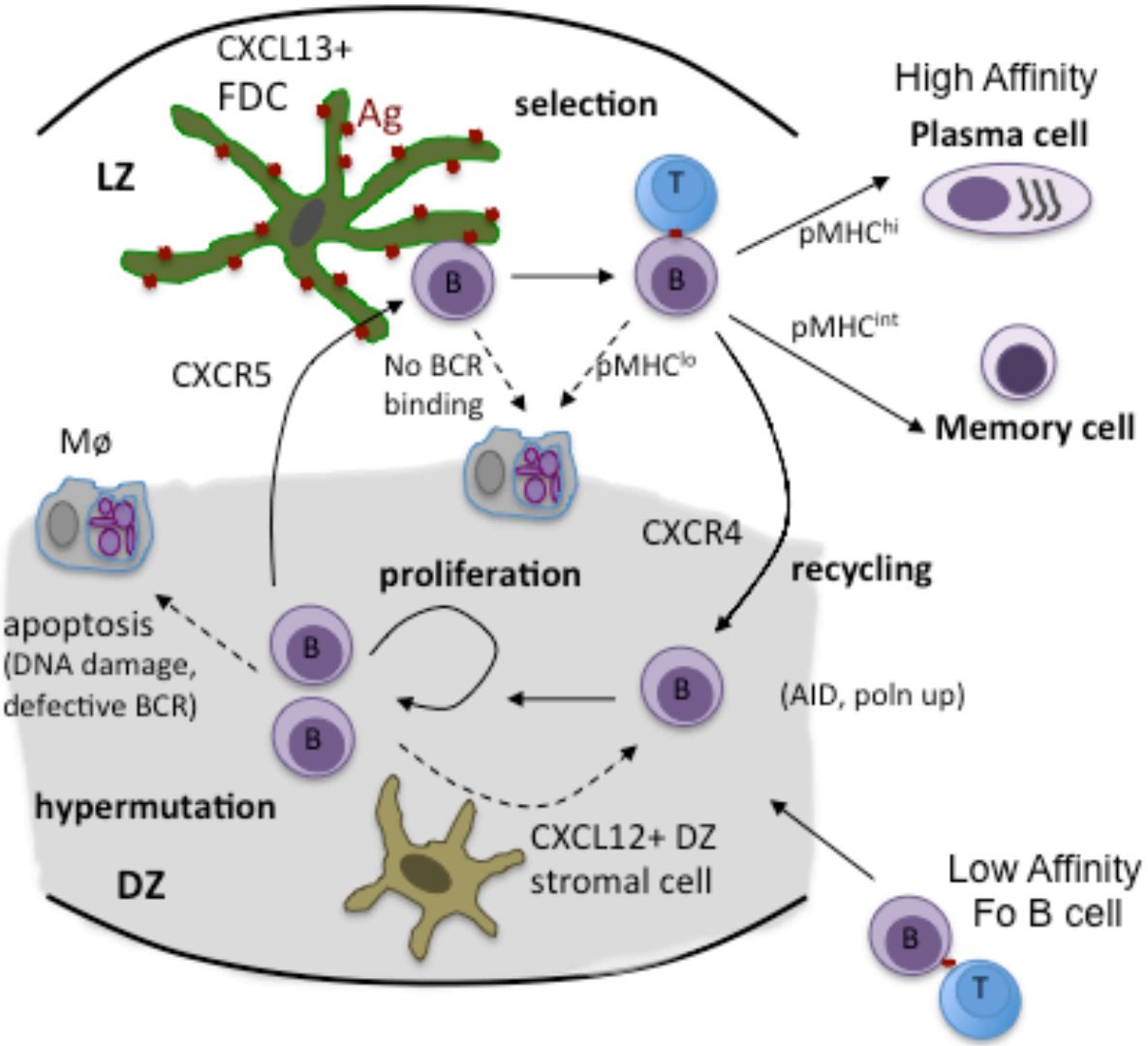
Germinal Center Organization

Light Zone

selection, differentiation

Dark Zone

proliferation, somatic hypermutation



Germinal Centers

Function: **to generate B cells that produce antibodies with increased affinity for the inducing antigen**

=> affinity maturation

Germinal Center Reaction:

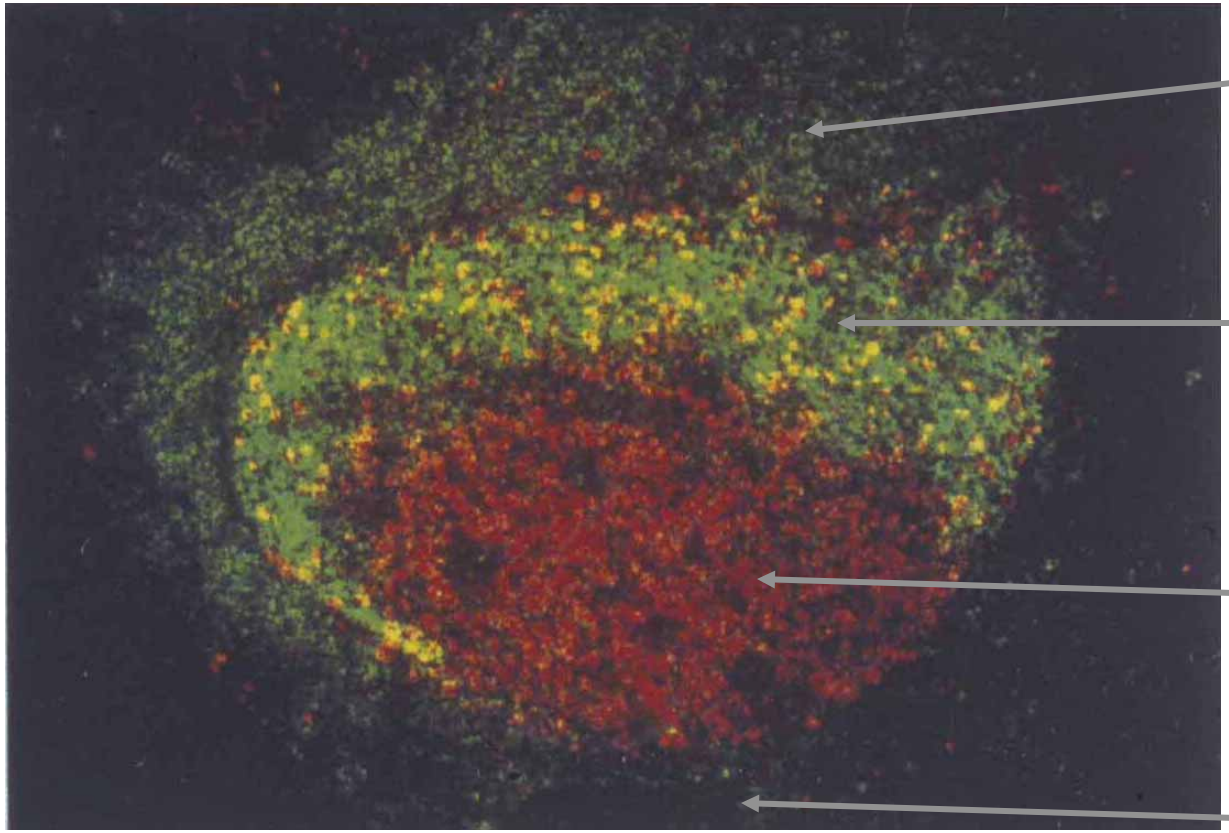
Activated B cells give rise to Dark zone cells (centroblasts)

- localize in follicle, undergo rapid cell division and turn on machinery that causes somatic mutation in V-regions

Centroblasts give rise to Light zone cells (centrocytes)

- migrate to the FDC-rich region of the Germinal Center
- survival is dependent on interaction with FDC-bound Ag and presentation of Ag to T cells
- centrocytes that successfully compete to bind antigen (e.g. by having higher affinity BCR) and to receive T cell help are positively selected and may differentiate into long-lived plasma cells or memory B cells

Germinal Center in Human Tonsil



Folliuclar mantle zone
(CD23^{+} naive B cells)

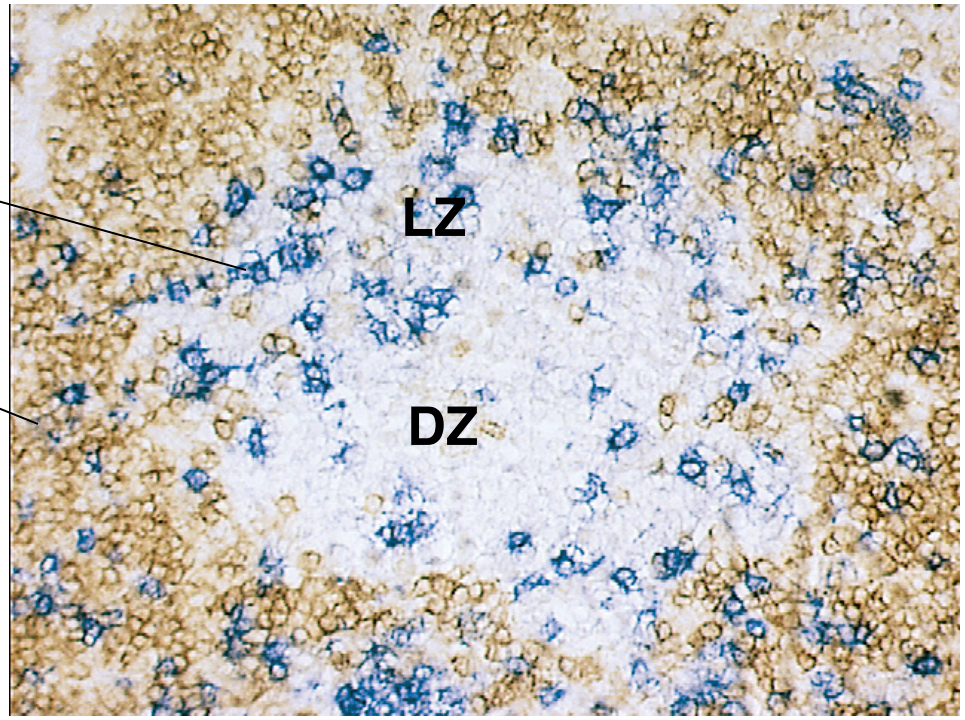
GC light zone
(CD23^{++} FDC
and 'centrocytes')

GC dark zone
(Ki67 cell cycle antigen^{+}
'centroblasts')

T zone

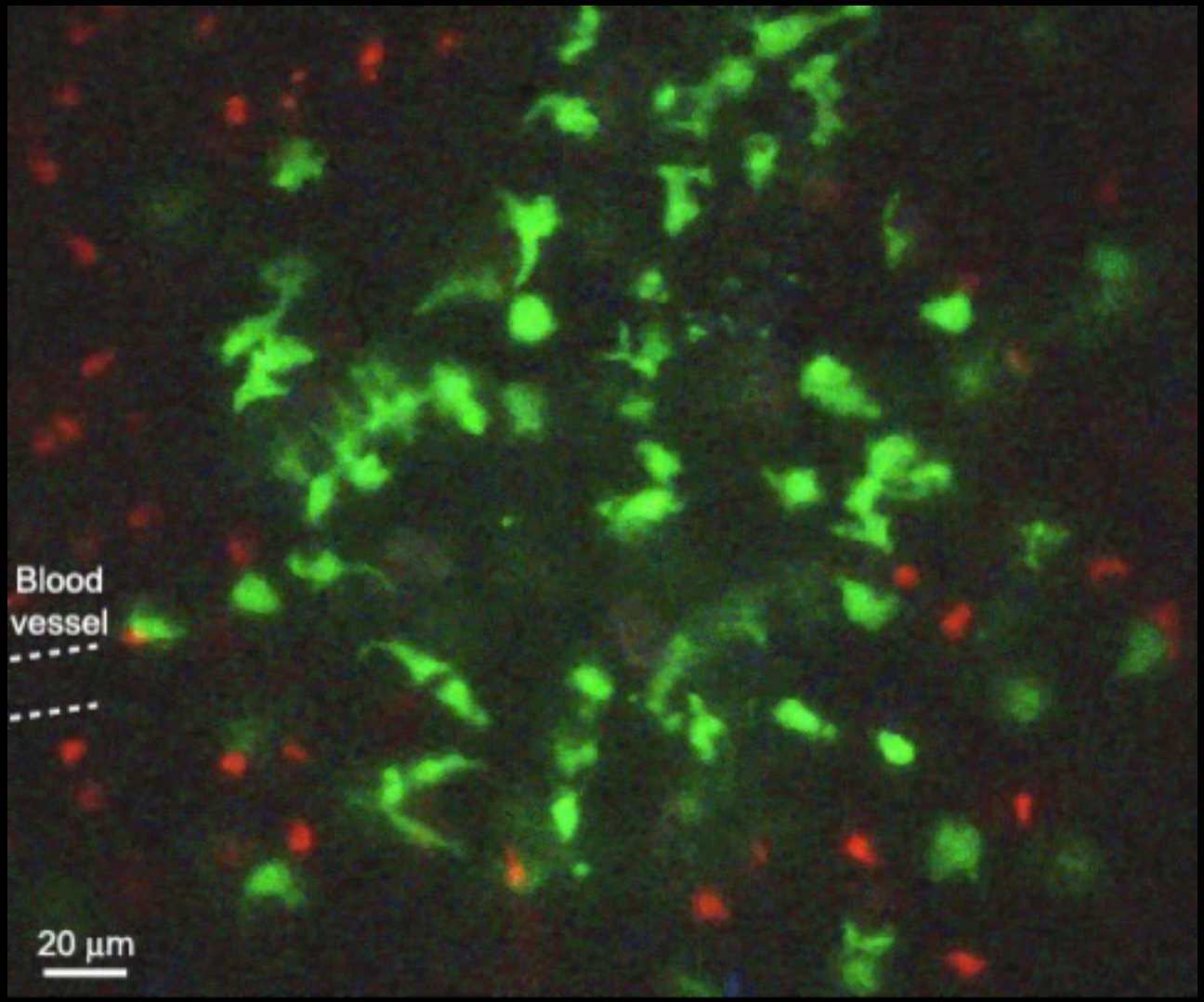
from Liu et al., Immunology Today 13, 17-21

Affinity maturation is T-dependent



- T follicular helper (Tfh) cells
  - Enriched in GC light zone
  - Make CD40L, IL21, IL4 and/or IFNγ
- Represent ~10% of GC cells

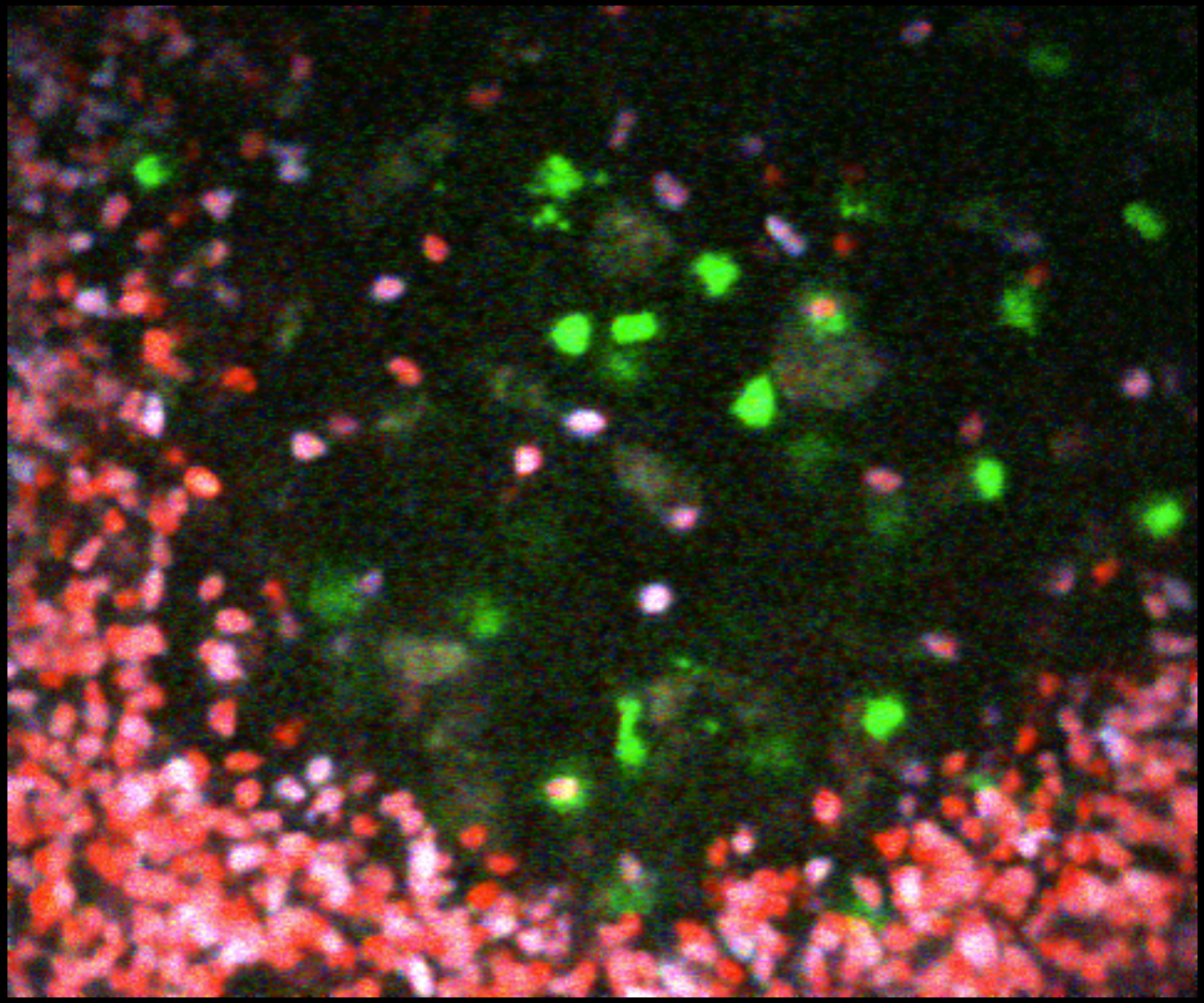
GC B cell migration dynamics (d7)



GC B naive B and T 00:00:00

21μm z-projection

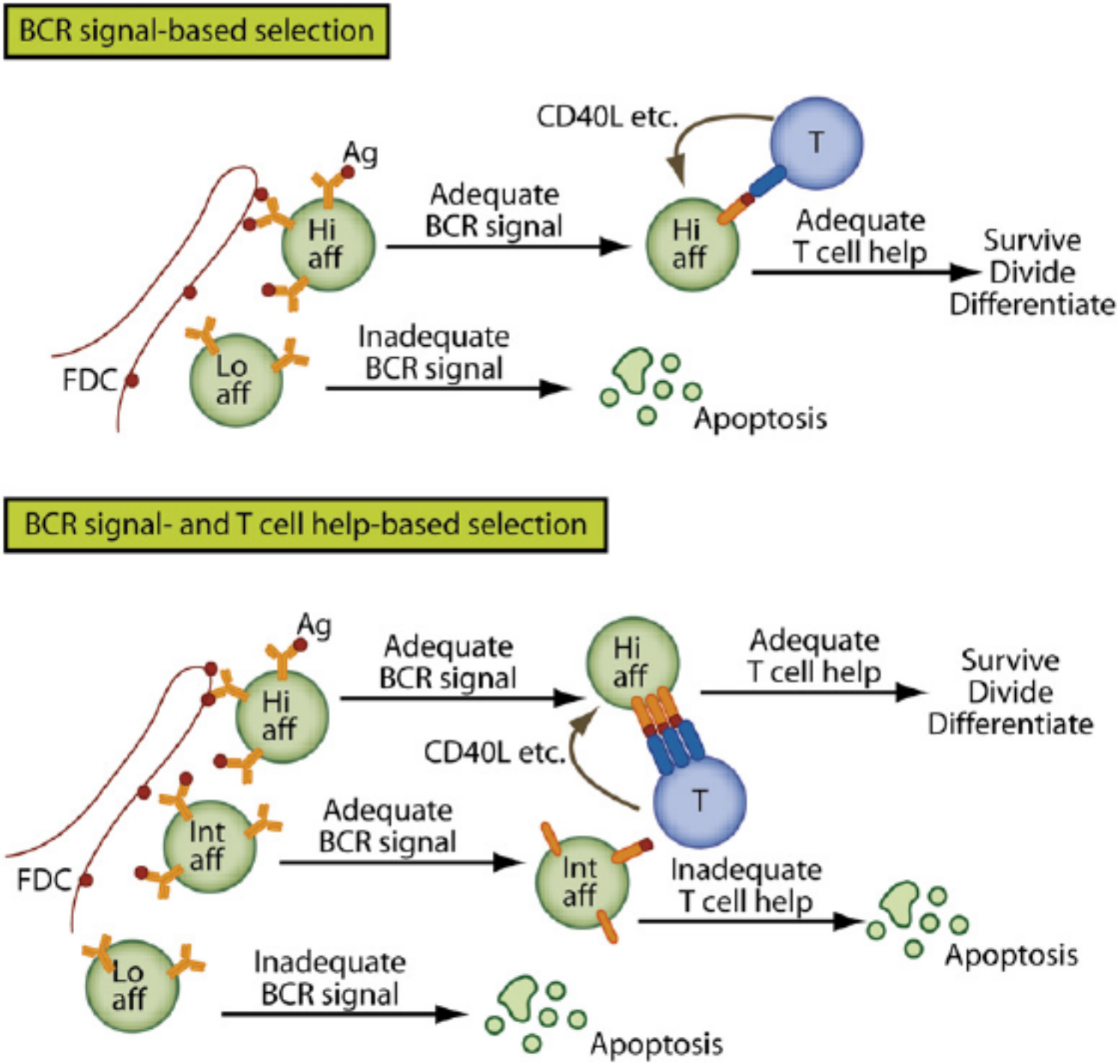
Germinal Center and Follicular Mantle B cell migration



Germinal center B cells Naïve B cells

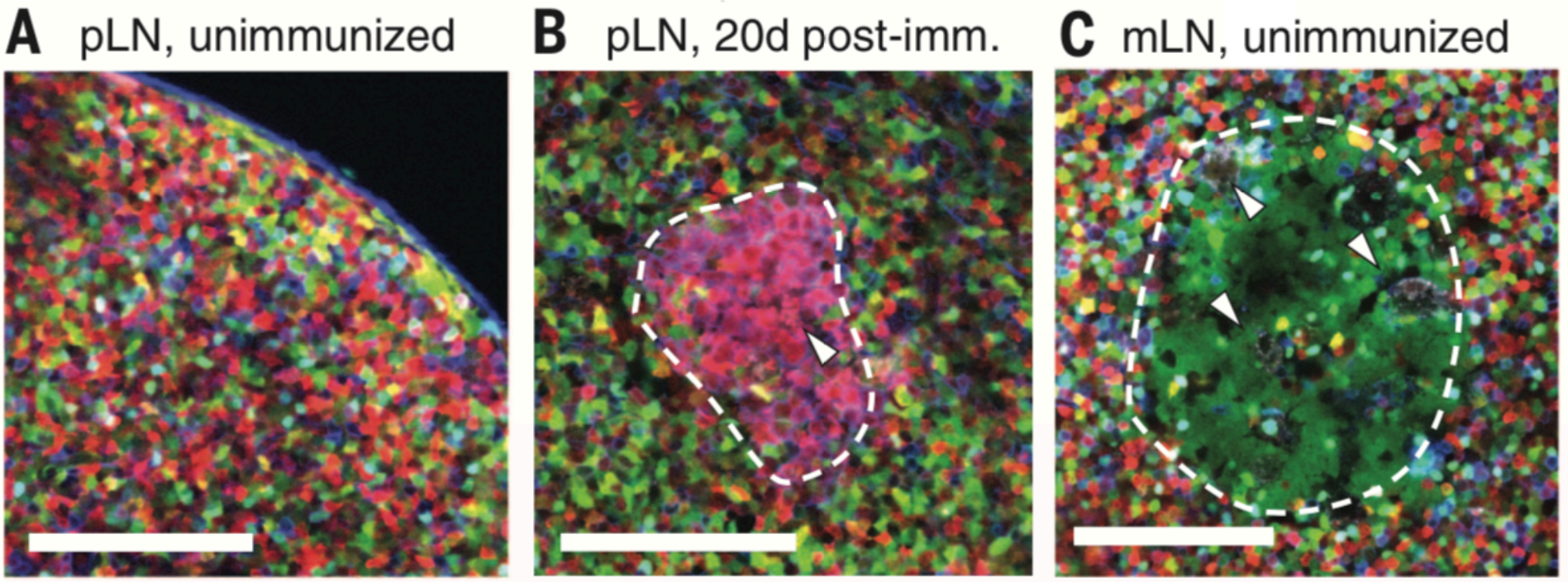
Selection Model

- GC B cells that have improved affinity capture more antigen in a given amount of time, receiving a stronger BCR signal and presenting more MHC-peptide complexes to GC T cells, outcompeting surrounding B cells for T cell help
- BCR critical for antigen uptake and presentation
- Importance of BCR signal for selection still being determined



Allen, Okada, Cyster *Immunity* 2007

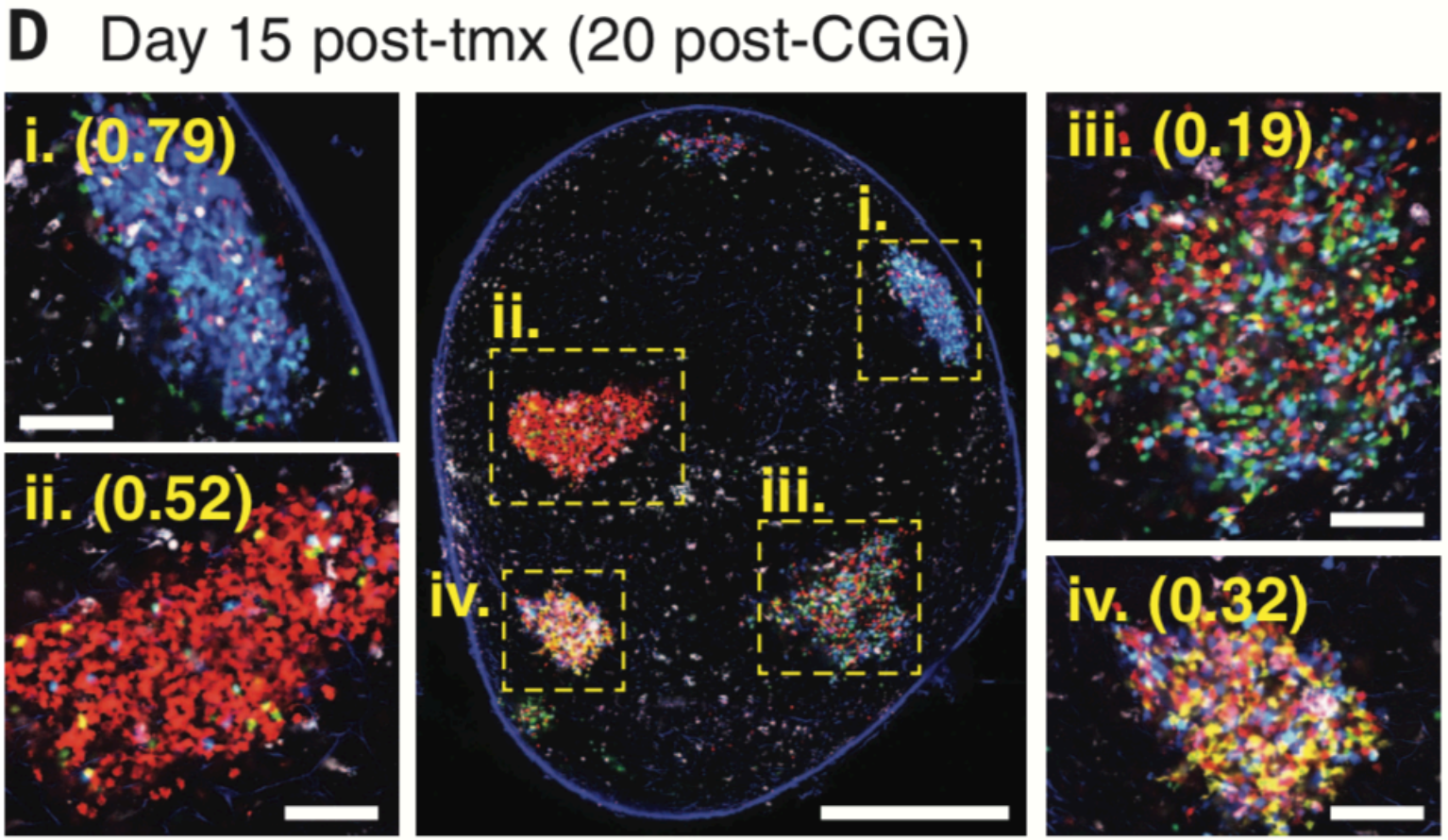
Visualizing GC clonal selection using ‘confetti’ reporter mice



Lymph nodes from Rosa26Confetti/Confetti Mx1-Cre mice (recombination triggered early in life, generating 10 different color combinations), imaged by multiphoton microscopy

Tas et al., Science 2016

Visualizing GC clonal selection using ‘confetti’ reporter mice



- Tens to hundreds of distinct B cell clones seed each GC
- GCs lose clonal diversity at widely disparate rates
- Efficient affinity maturation can occur in the absence of homogenizing selection

Numbers in parentheses are the normalized dominance score (clonality)

Tas et al., Science 2016

Questions

- Why do we get boosters of some vaccines and not others?

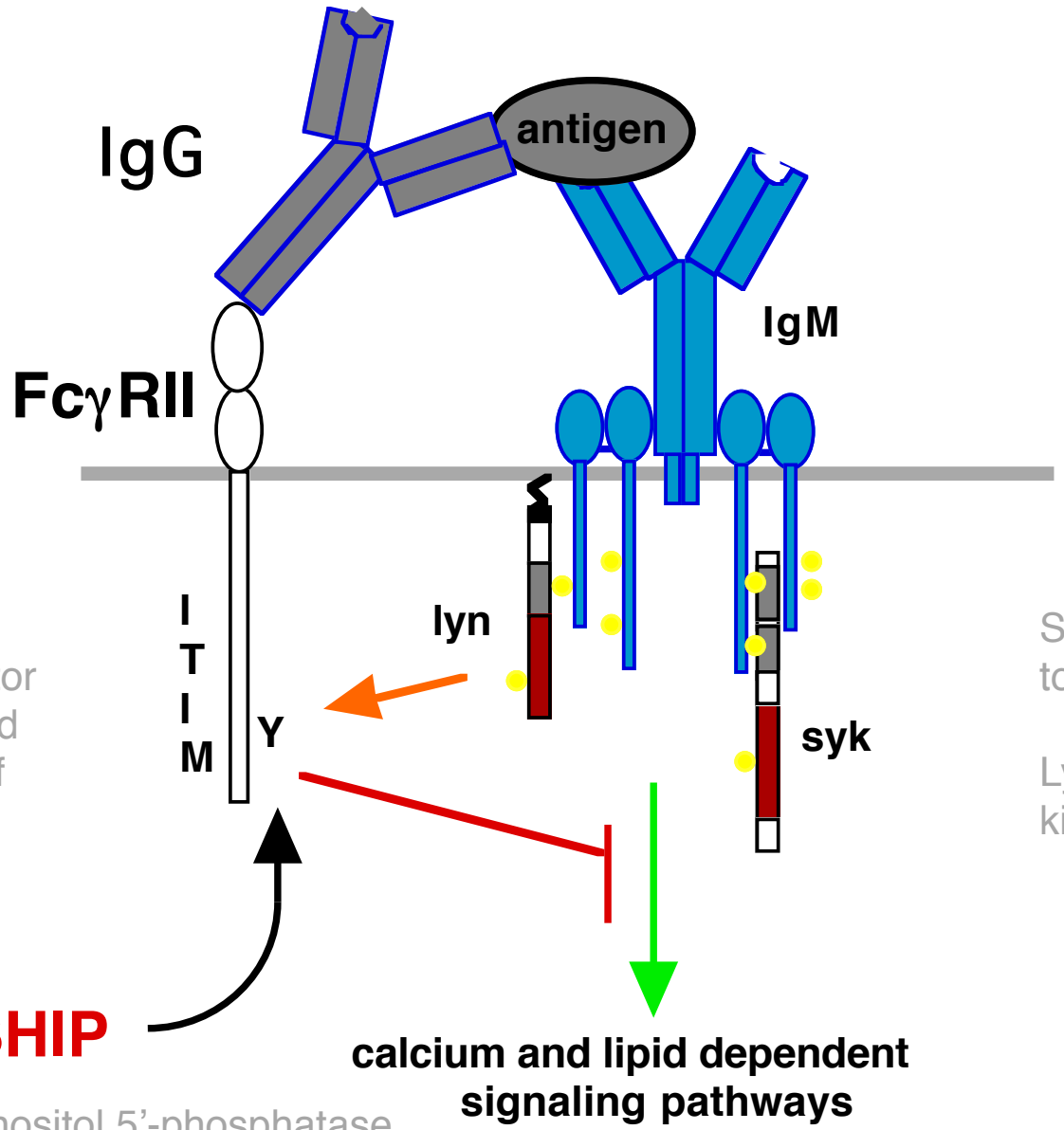
Memory B cells

- Generated during the primary response
  - best characterized for T-dependent responses involving GCs but some may arise independently of (and prior to) the GC response
- Small, recirculating cells
- Often isotype switched (e.g. IgG^+ or IgA^+) but some can be IgM+
- Typically have higher affinity for the inducing Ag (carry V-region mutations)
- Longer lived than naïve B cells
  - Persistence of memory B cells after an immune response ensures that we have increased numbers of B cells specific for the antigen and ready to respond on re-encounter
- May have intrinsic differences that promote greater clonal expansion and more rapid differentiation to plasma cells
  - differences in cytoplasmic domains of IgG vs IgM/D
  - upregulation of TLRs

Questions

- How does pre-existing antibody against one part of the virus affect the ability to make antibody against another?

Down-regulation of B cell response by pre-formed IgG



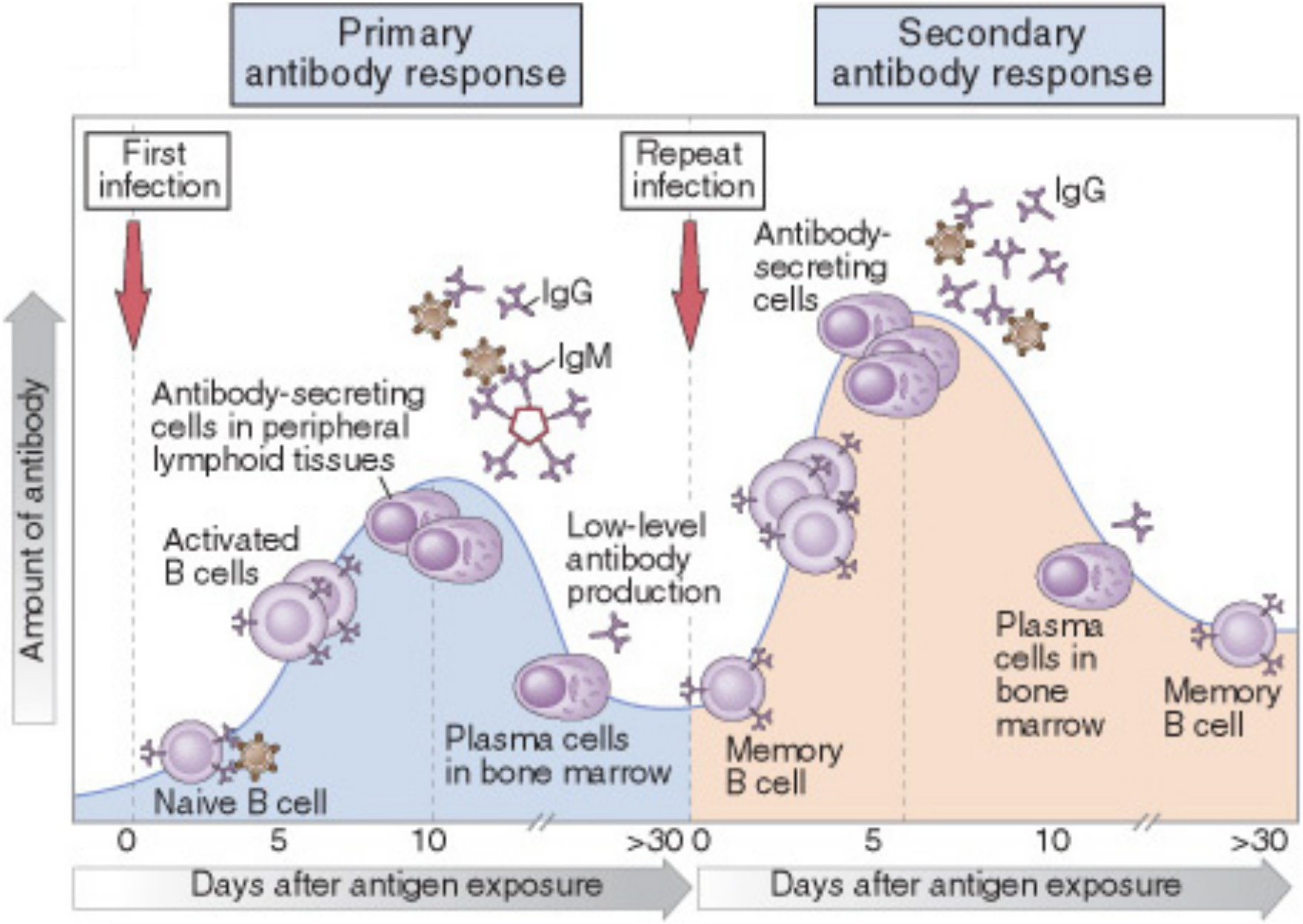
ITIM – Immunoreceptor Tyrosine-based Inhibitory Motif

Syk – kinase related to Zap70 in T cells

Lyn – Src family kinase

SH2-containing inositol 5'-phosphatase
- hydrolyzes PI3P preventing Ca^{2+} signaling etc

Features of primary and secondary antibody responses



Recommended Reading

Primary papers:

- Tas JM … Victora GD. (2016) Visualizing antibody affinity maturation in germinal centers. *Science* 351: 1048-54
- Hou B, Saudan P, Ott G, Wheeler ML, Ji M, Kuzmich L, Lee LM, Coffman RL, Bachmann MF, DeFranco AL. (2011) Selective utilization of Toll-like receptor and MyD88 signaling in B cells for enhancement of the antiviral germinal center response. *Immunity* 34:375-84
- Okada T, Miller MJ…Cahalan MD, Cyster JG (2005) Antigen-Engaged B Cells Undergo Chemotaxis toward the T Zone and Form Motile Conjugates with Helper T Cells. *Plos Biol.* 3, e150

Reviews:

- Weisel F, Shlomchik M (2017) Memory B cells of mice and humans. *Annu Rev Immunol*. 35: 255-284.
- Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM (2015) The generation of antibody-secreting plasma cells. *Nat Rev Immunol.* 15: 160-71.
- Victora GD, Nussenzweig MC (2012) Germinal Centers. *Annu Rev Immunol*.
- Kurosaki T, Shinohara H, Baba Y (2009) B Cell Signaling and Fate Decision. *Annu Rev Immunol*.

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