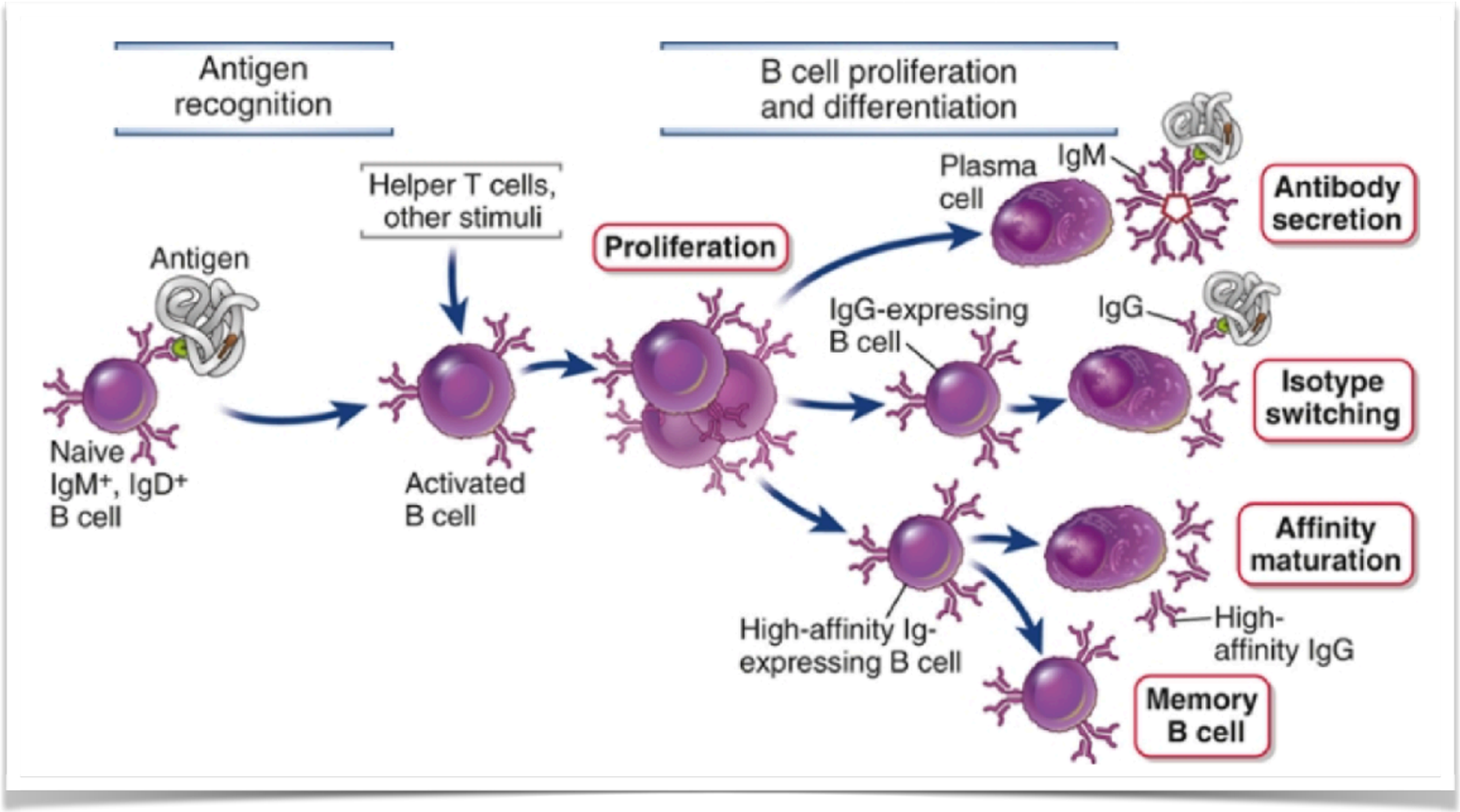
B cell Activation and Antibody Production

Overview of humoral immune responses

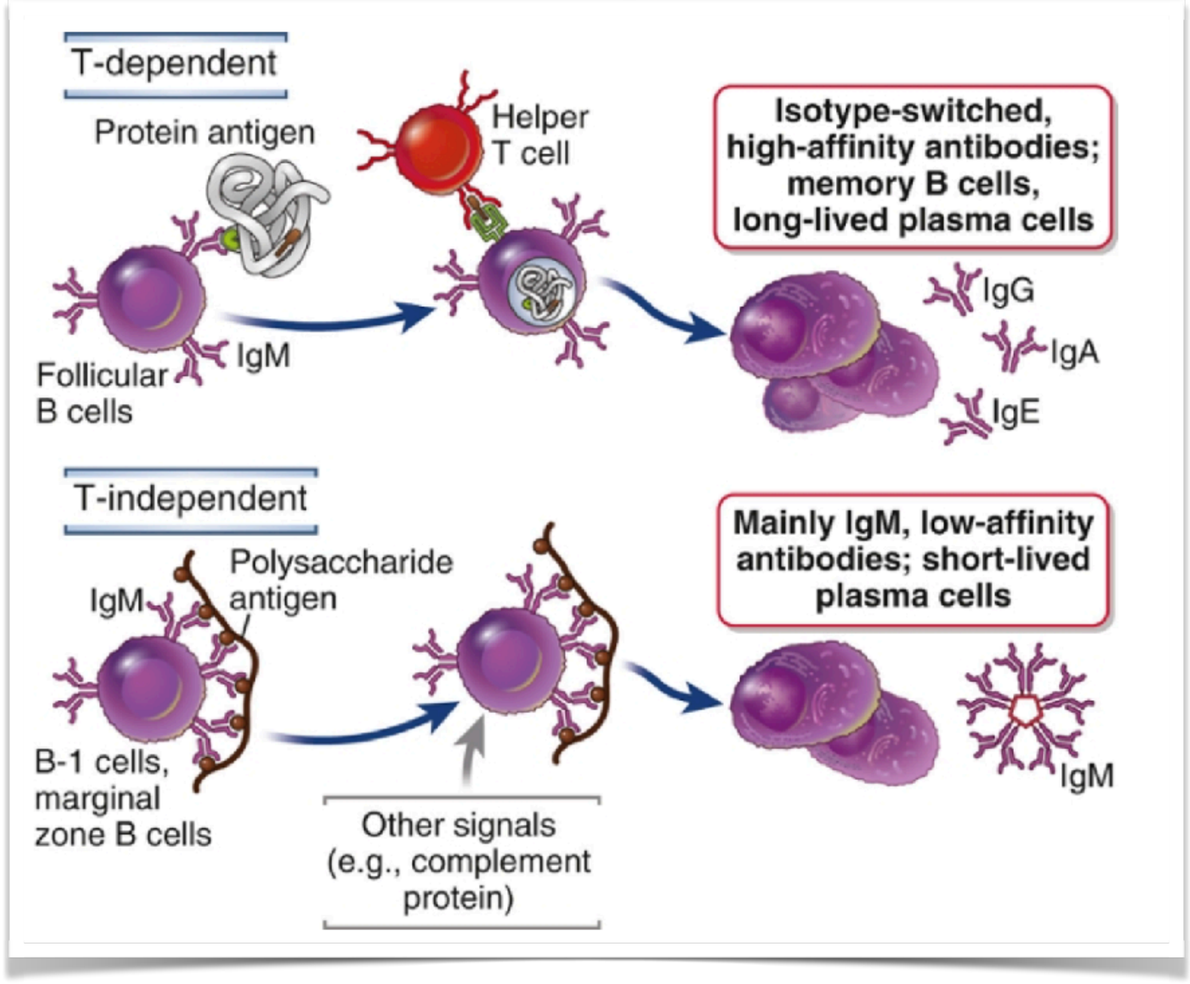
- Initiated by specific B cell recognition of antigen in secondary lymphoid organs
- Antibodies secreted by *plasma cells* have the *same specificity* as the original antibody that served as the antigen receptor on naive B cell surface
- A single B cell can give rise to 5000 secreting cells within a week which can produce 10^{12} antibodies per day, needed to *keep pace with rapidly dividing microbes*



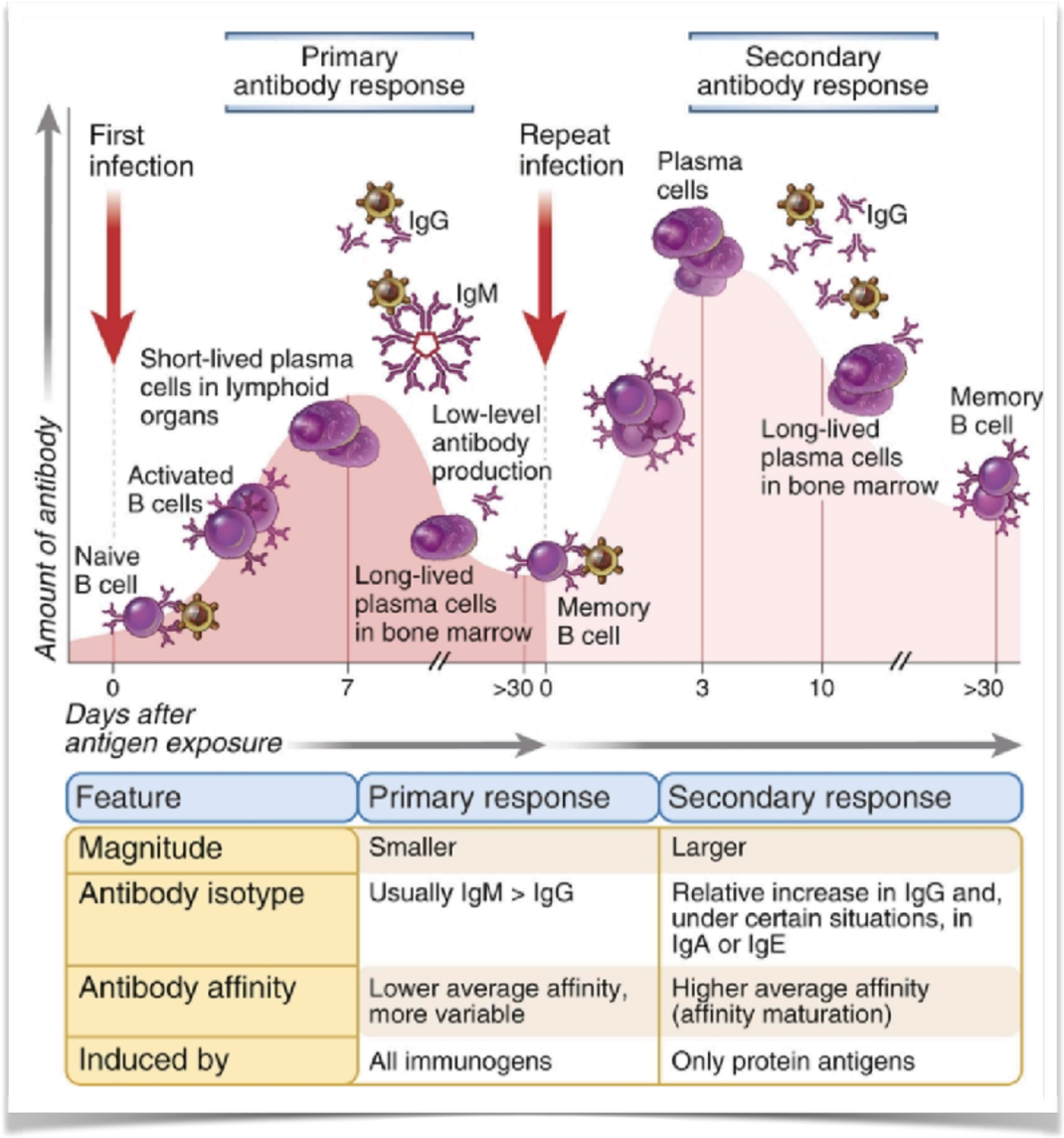
T-dependent and T-independent antibody responses

Depend on the *nature of the antigen* and the involvement of other *T cells.*

➔ **Multivalent antigens** with repeating determinants such as polysaccharides can activate B cells without T cell help (*rapid but simple* response)



Primary and secondary humoral immune responses



Feature	Primary response	Secondary response
Magnitude	Smaller	Larger
Antibody isotype	Usually IgM > IgG	Relative increase in IgG and, under certain situations, in IgA or IgE
Antibody affinity	Lower average affinity, more variable	Higher average affinity (affinity maturation)
Induced by	All immunogens	Only protein antigens

During secondary responses, **memory cells** that have already undergone isotope switching and affinity maturation produce *more IgG with higher affinity*.

Distinct B cells subset and AG preferences

- Follicular B cells in peripheral lymphoid organs: responses to *protein* antigens and require *T cell help*

- Marginal zone B cells in the spleen and B-1 cells in mucosal tissues and the peritoneum: recognise *multivalent antigens* (blood-borne polysaccharides) and mount *T-independent* responses

Antigen capture and delivery to B cells

- **Small soluble antigens** can enter via afferent lymphatic vessels and reach the B cell zone in the lymph node though extended *conduits*

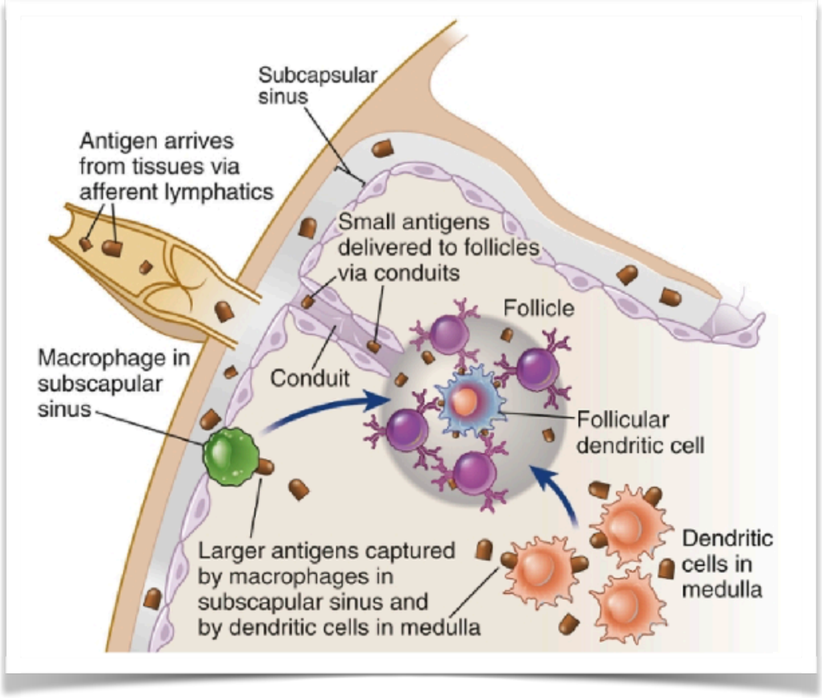
- **Large microbes and antigen-antibody complexes** may be captured by *macrophages* or *medulla resident DCs* and delivered to the follicles

- **Antigens in immune complexes** may bind to *complement receptor* on *marginal zone* B cells or follicular DCs and transferred to follicular B cells

- **Polysaccharide antigens** can be captured by macrophages in the *spleen* and displayed to B cells

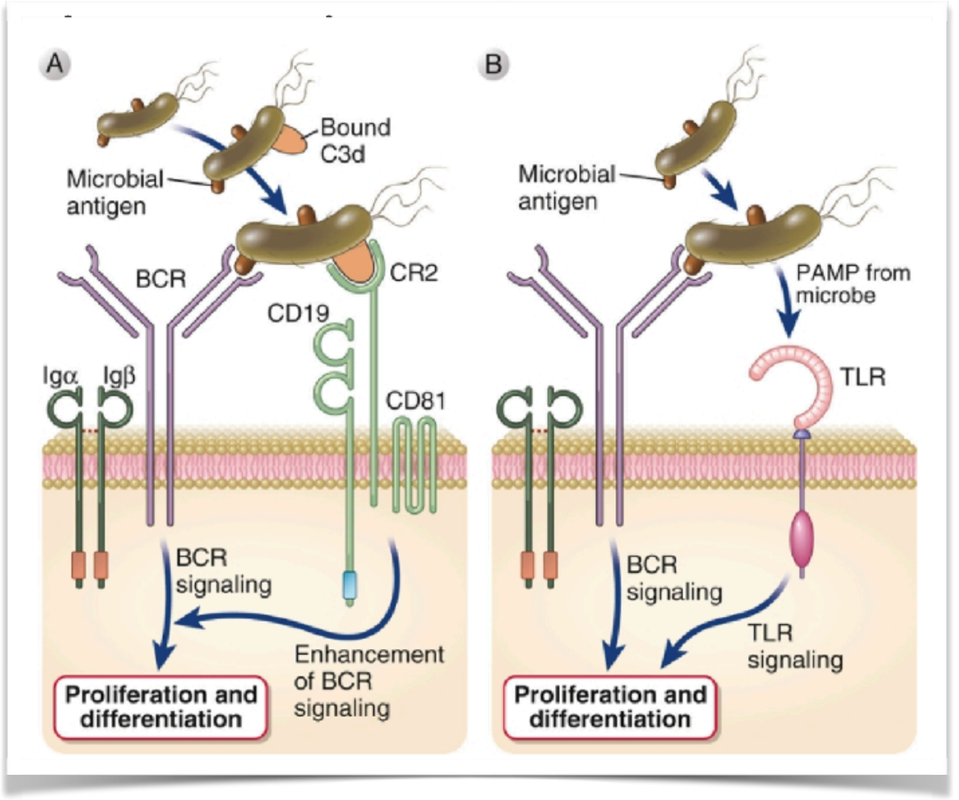
- Antigens vary in *size* and *composition* and may be *free* or *bound to antibodies*

- Antigens are presented to B cells in *intact native conformation* and not processed by APCs



Activation of B cells

- Signaling is more *robust with multivalent* T-independent antigens.
- The receptor internalises the bound antigen into *endosomal vesicles* and if it is a protein, it is processed into peptides that may be presented on the B cell surface for recognition by helper T cells
- Even for T-independent antigens, B cell response require the *cooperation of other stimuli* (complement proteins, PRR etc)



Activation of B cells

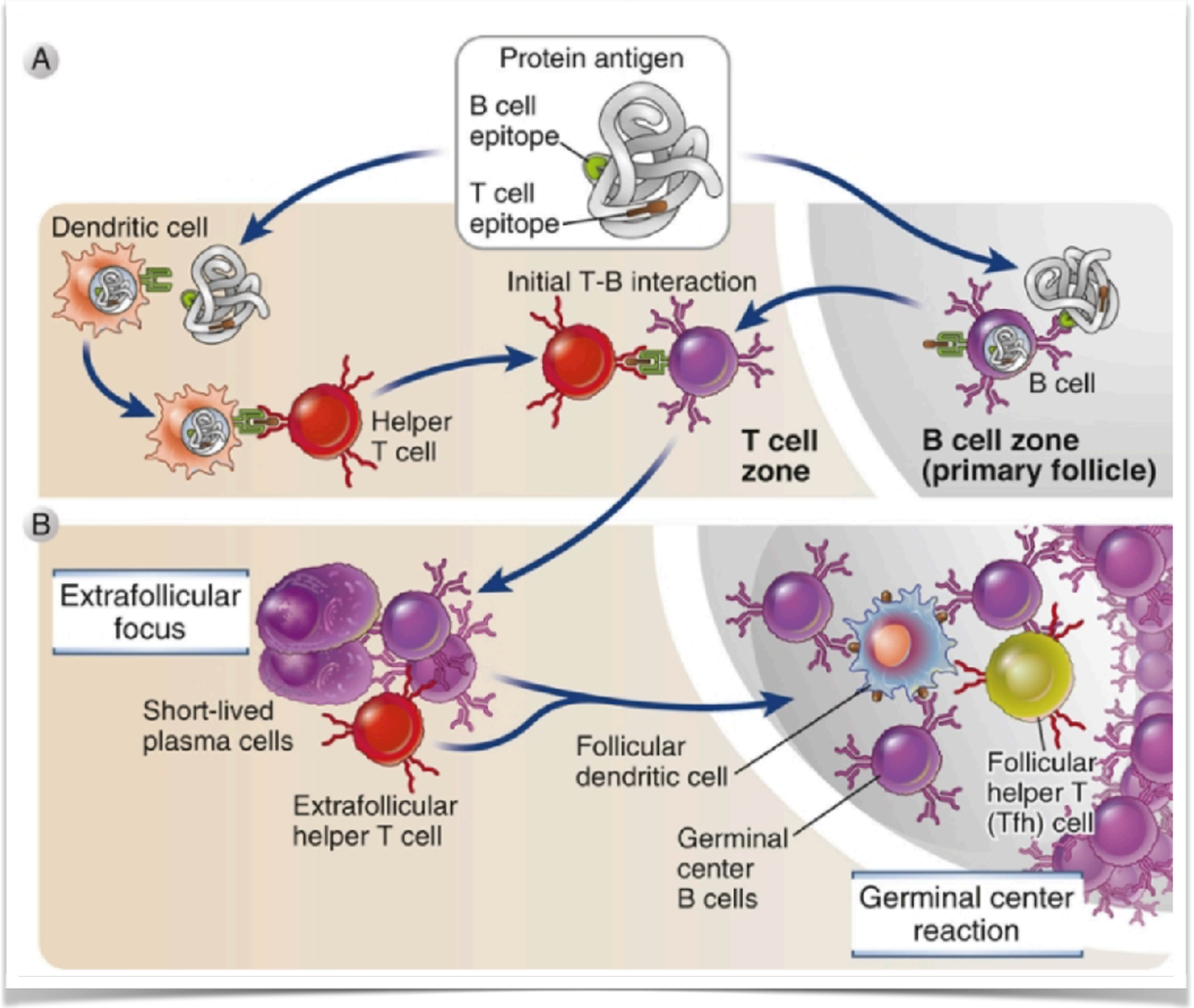
- **CR2/CD21 coreceptor:** recognises *complement fragments (C3d)* attached to antigens and enhances the strength of BCR signalling

- **TLRs (TLR5, TLR7, TLR9)**: recognise *microbial products* and enhance B cell activation
  ➔ *also indirectly: myeloid cells PRR activation contributes to helper T cell (T-dependent B cell activation) and cytokine secretions (that can promote T-independent B cell responses)*

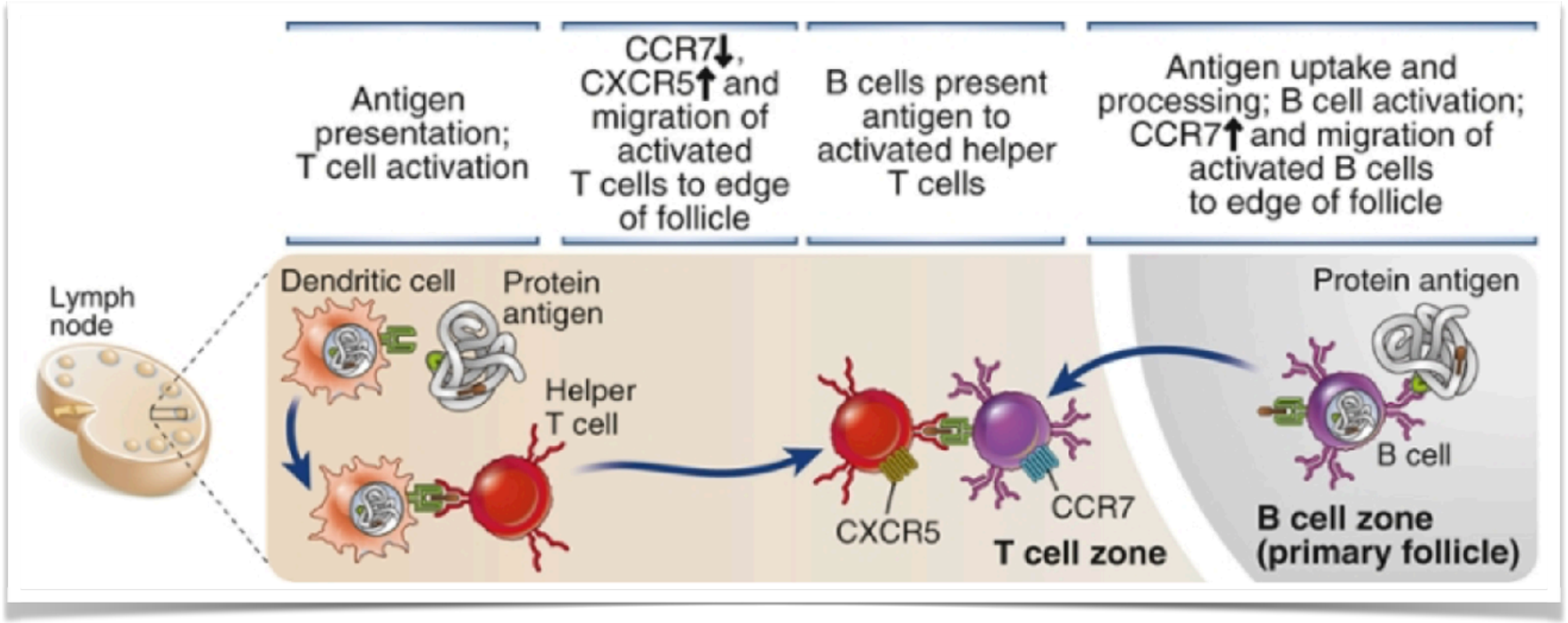
TABLE 12.1 Effects of B Cell Antigen Receptor Engagement on B Cells

Phenotypic Change	Functional Consequence
Increased expression of CCR7	Migration toward T cell zone
Increased expression of B7 costimulators	Enhanced ability to activate helper T cells
Increased expression of receptors for T-cell cytokines	Increased responsiveness to signals from helper T cells
Increased expression of anti-apoptotic proteins	Increased survival of B cells
These changes may be induced by binding of protein antigens to the B cell receptor (BCR) and prepare B cells to respond to T cell help. Protein antigens are also internalized, processed, and presented to helper T cells. With multivalent T-independent antigens, in addition to the changes listed above, the B cells proliferate and differentiate into IgM antibody-secreting plasma cells.	

Helper T cell-dependent antibody responses



Migration of B cells and helper T cells allowing T-B interaction



- Changes in *chemokine receptor expression* draw activated T and B cells towards each other.
- Protein antigens are *internalised and presented* by B cell in a form that can by recognised by helper T cells

Antigen presentation by B cells

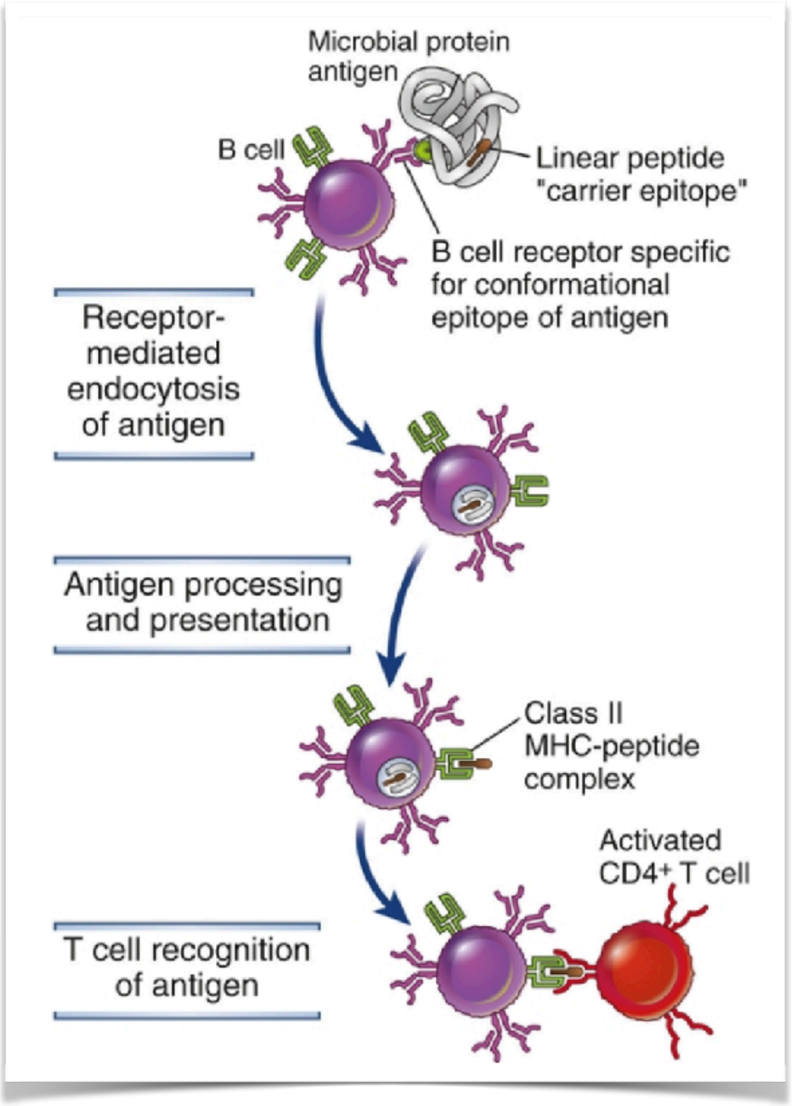
- **Presentation** follows the *class II MHC pathway*

➔ A B cell specific for a given antigen is much more efficient at presenting peptides derived from that antigen than other B cells because it *binds the antigen much more efficiently*

2 epitopes participate in the process:

- Surface epitope on native protein recognised by the B cell
- Linear peptide binds class II MHC and is recognised by helper T cell

- **Antibodies** produced are usually *specific for conformational determinant* (antibody response remains specific for the native protein)



The hapten-carrier effect

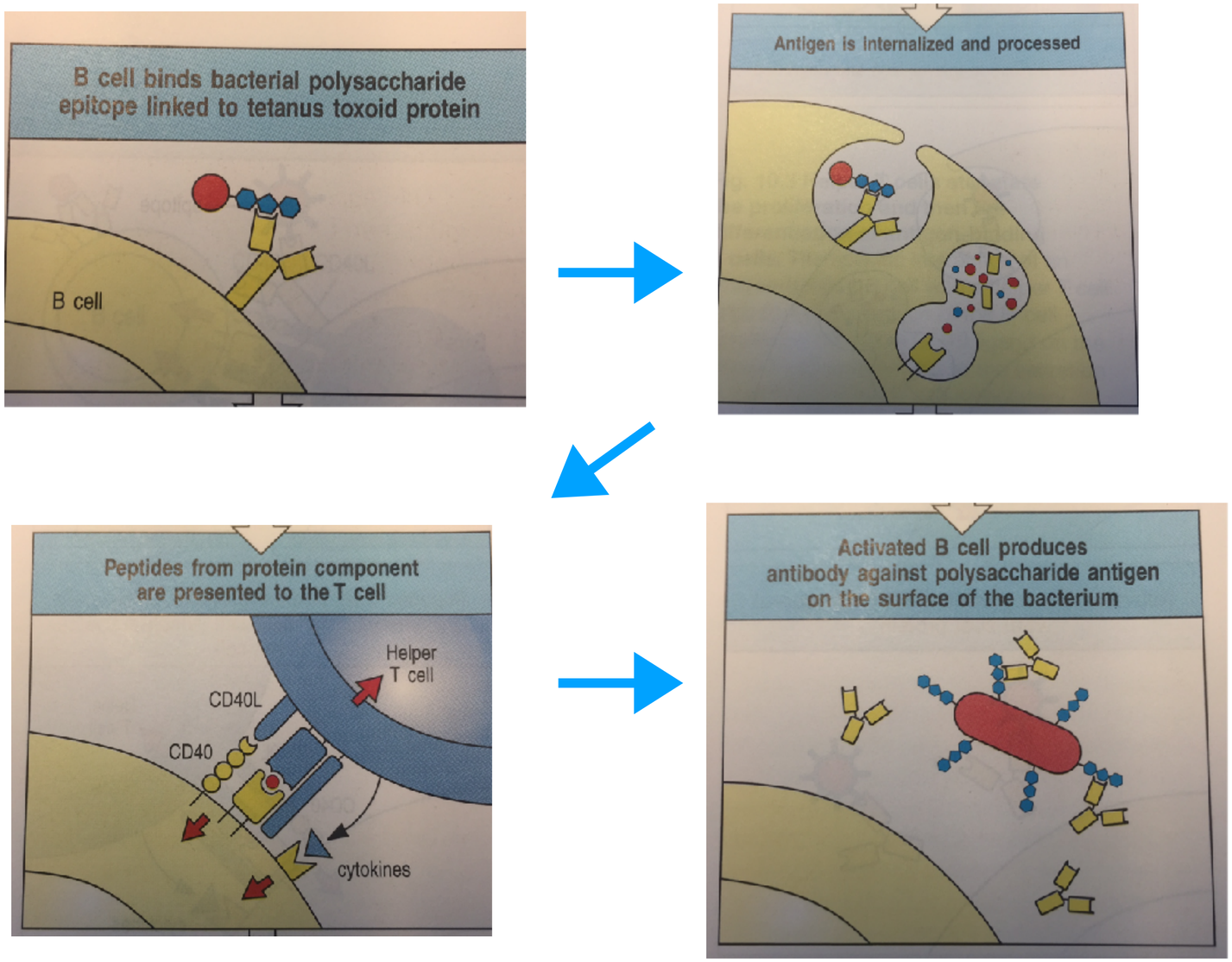
Small chemicals can be *recognised by antibodies without being immunogenic*.
➔ If coupled to protein *carriers*: conjugates can induce a response against the *hapten*

- Requires hapten specific **B cell** and carrier specific helper **T cell**
- Hapten and carrier portions need to be *physically linked*
- Interaction is *class II MHC* restricted

NB: these characteristics apply to all protein antigens in which one intrinsic determinant is recognised by B cells and another determinant (in the form of a class II MHC associated linear peptide) is recognised by helper T cells.

➔ Basis for the development of *conjugate vaccines* against encapsulated bacteria = carbohydrate recognised by B cells attached to proteins recognized by T cells

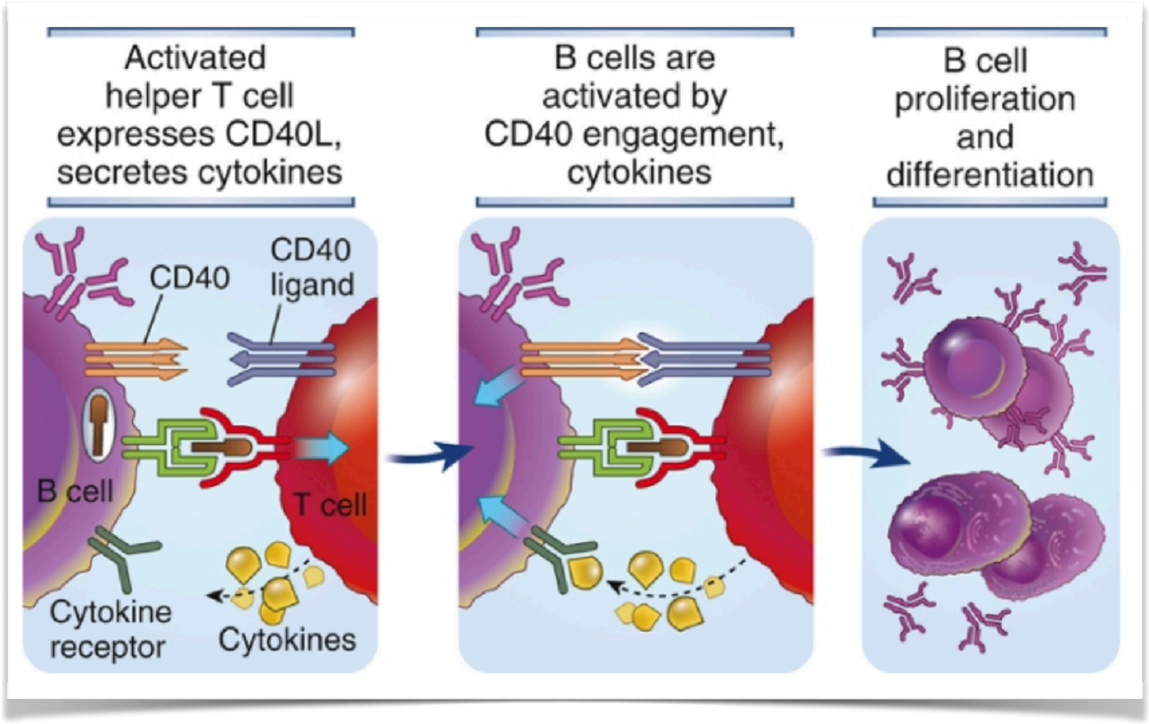
Linked recognition/Hapten-carrier effect is important for vaccine design



CD40L:CD40 interaction in T-dependent B cell activation

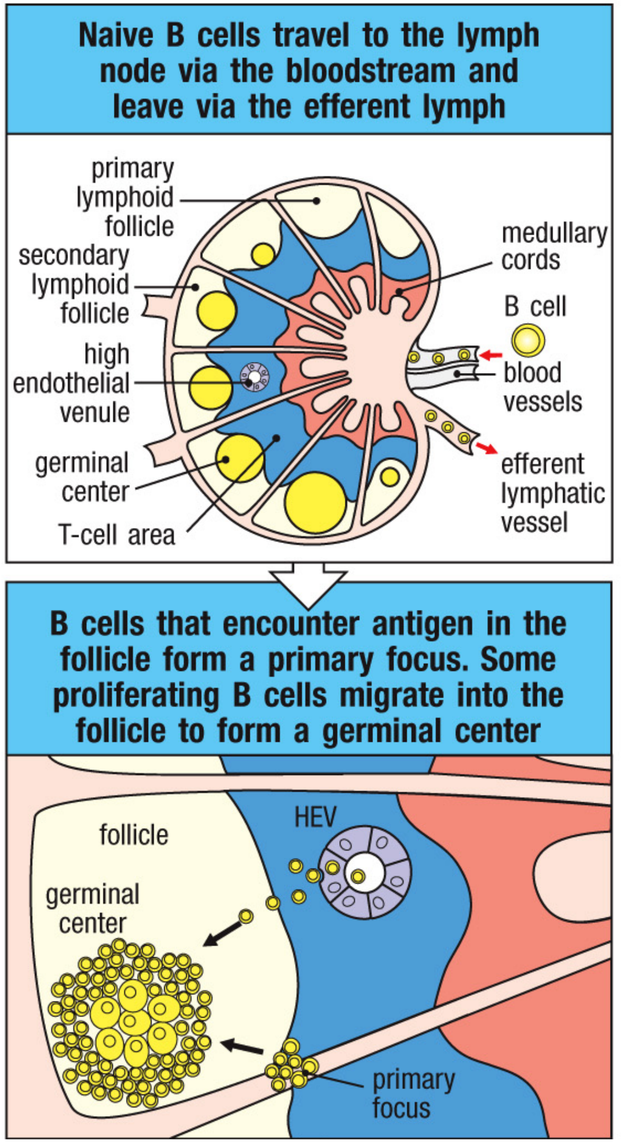
- CD40: *constitutively expressed on B cells*
- CD40L: expressed on *recently activated helper T cells* by antigen and costimulation

- Interaction induces signaling pathways in B cell that stimulates *proliferation* and *synthesis/secretion of Ig.*
- Several other cytokines from helper T cells contribute to B cell response



Extrafollicular B cell activation

- *Early* antibody response to *protein* antigens
- Generates *low-affinity antibodies* that circulate and *limit the spread* of infection
- B cells that are activated by helper T cells through CD40L can undergo some isotype switching
- The generated antibody-secreting cells are mostly *short-lived* and do not migrate to distant sites
- Helps to *generate follicular helper T* cells that migrate into the follicle and are reared for germinal center formation



The germinal center reaction

- **Organised structure** within lymphoid follicles during *T-dependent* responses
- Site of complex processes for *genetic diversification* and *survival of the fittest B cells*
- Develop within *4-7 days* after initiation of T-dependent B cell responses
- Rapid proliferation of few B cells migrating back from extrafollicular foci into the follicle
- Distinct region containing cells derived from one/few antigen-specific B cell clones
  - Dark zone: densely packed with *proliferating B cells* undergoing mutational process (doubling time 6-12h)
  - Light zone: *differentiation and selection* process of proliferating B cells

Follicular dendritic cells (FDC): express complement receptors and Fc receptors, *display antigens* for selection, no MHCII expression (not classical DCs)

Sequential process:

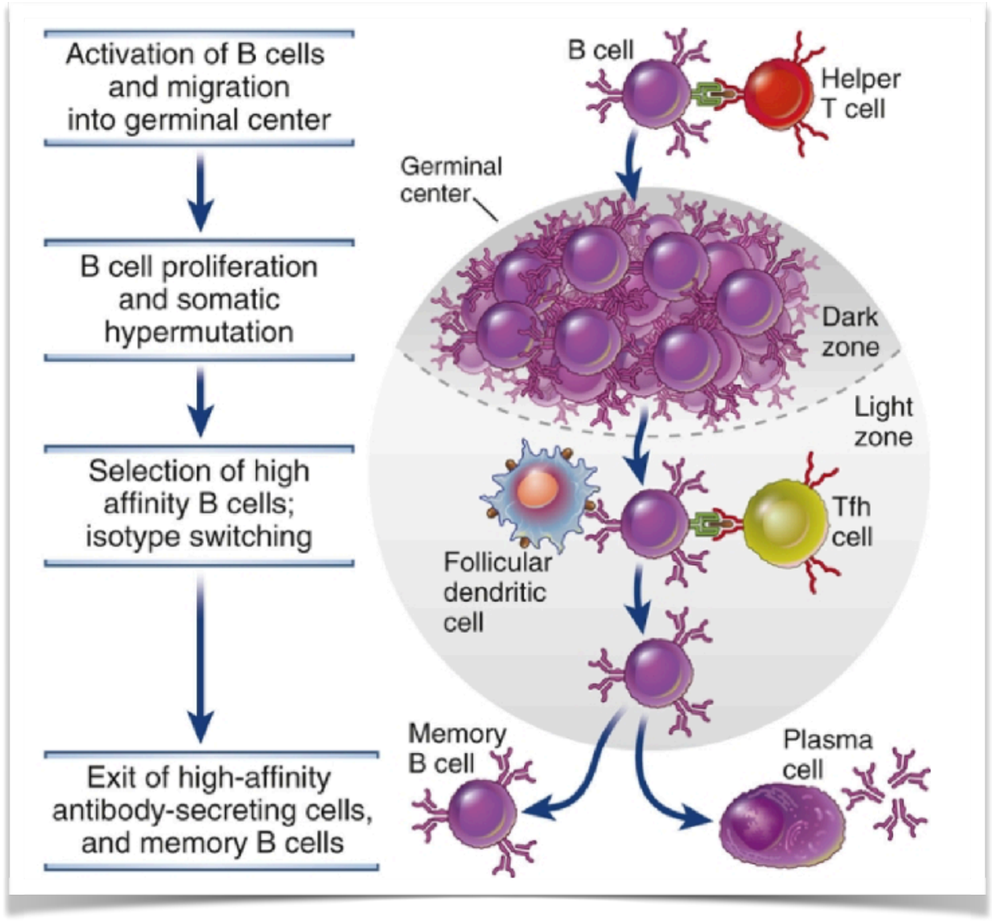
- **Hypermutation** in the dark zone
- Nonproliferating progeny enters the light zone for **selection** events
- Selected cells *return to the dark zone* for **repeated rounds of mutation** and selection
- Selected **high affinity B cells** differentiate into *plasma cells and memory* B cells and exit the germinal center

The germinal centre reaction

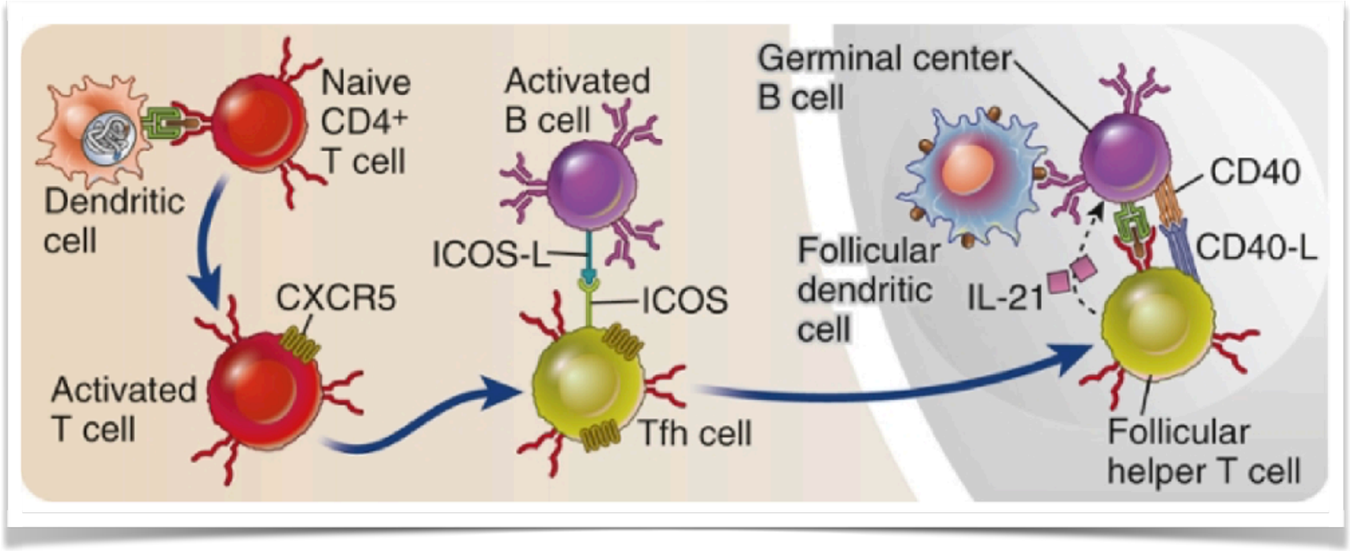
Formation is dependent on *Tfh CD40L interaction with CD40 on B cells*

➔ critical for B cell proliferation and expansion

➔ induces the expression of enzyme activation-induced deaminase =*AID*, required for *isotope switching and affinity maturation*



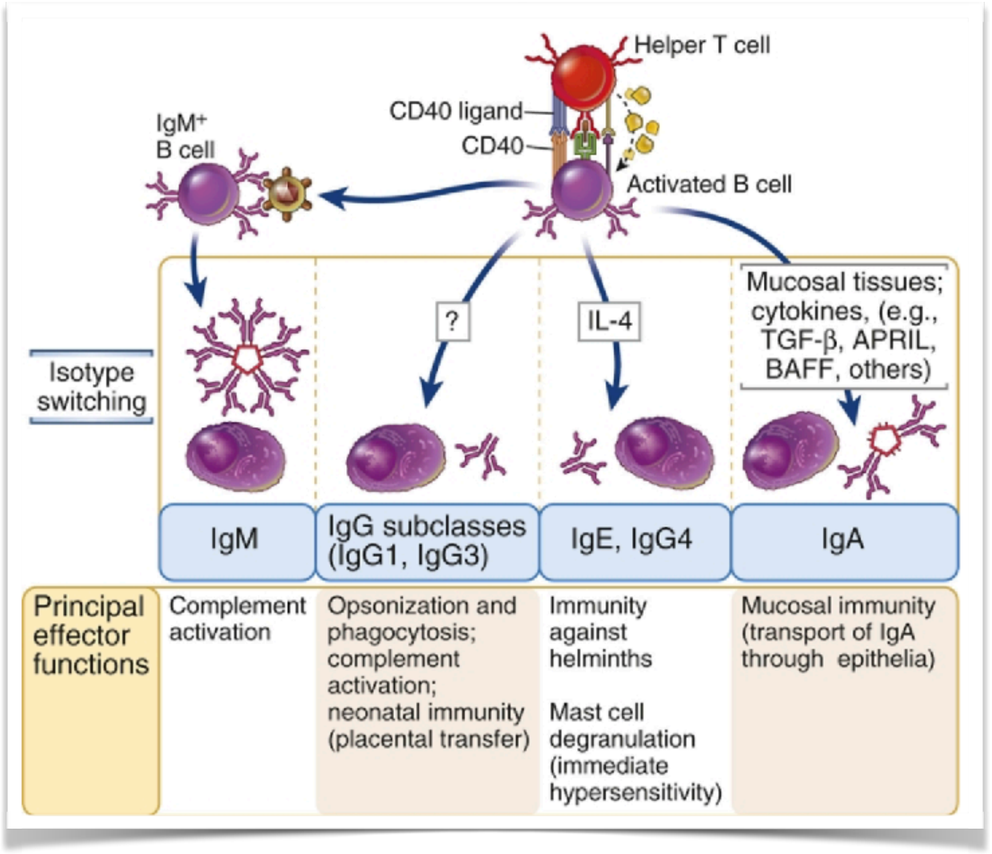
The induction of follicular helper T cells (Tfh)



- Induced *4-7 days* after antigen exposure
- Differentiate from naive $CD4^+$ T cell
- *Strong interaction* between DC peptide-class II MHC complex and TCR induces *Bcl-6* expression which *represses IL-2R* transcription (and thereby Th1, Th2 and Th17 differentiation)
- Express CXCR5 and ICOS: important costimulator in *interaction with activated B cell*
- Play a critical role in *germinal center formation and function*
- Effector cytokine: mainly *IL-21*, contributes to generation of plasma cells in germinal centres and facilities B cell selection and differentiation into plasmablasts

Heavy chain isotype (class) switching

- Can occur in B cells in extrafollicular foci by extrafollicular helper T cells
- In **germinal centers,** the process is driven by Tfh cells *in the light zone*
- Contributes to the *plasticity* in humoral immune responses by generating AB with distinct effector functions to defend against different types of infectious agents
- Obtained via changes in *constant regions* of the heavy chains without altering the specificity = determined by variables regions



Heavy chain isotype switching regulation

Different cytokines produced by helper T cells activated by <u>different microbes:</u>

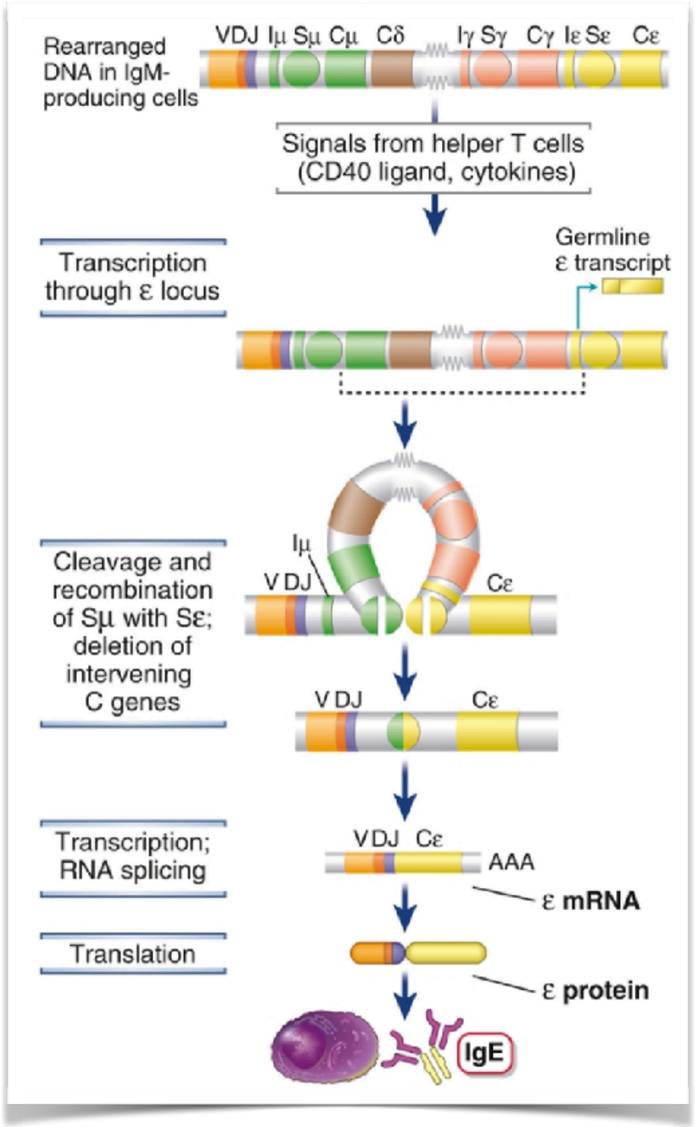
- **Bacteria and virus :** IgG is predominant
  ➔ long half-live in the blood, contributes to the protective capacity of the *humoral immunity*

- **Helminths**: dominated by IgE
  ➔ *eosinophil* and mast-cell activation

B cells are influenced by various cytokines present in <u>different anatomic sites:</u>

e.g. in **mucosal tissues** : TGF-b/BAFF mediated induction of IgA
➔ most efficiently *transported though epithelia* to mucosal secretions, not necessarily T-cell dependent

Molecular mechanism of isotype switching



- *Recombination* of Ig heavy chain (cut and recombined, with loss of intervening DNA)
- Involves sequences called *switch regions* present in introns of J and C segments
- Previously formed VDJ exon is placed adjacent to a *downstream C region*
- *Cytokines* determine which constant regions will undergo germline transcription
- AID is a key enzyme required to generate double-stranded breaks allowing switch regions to be joined together ➔ its expression is induced in B cells via CD40 signal from Tfh cells

Role of cytokines in regulating expression of antibody classes

Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces
IL-21		Induces	Induces				Induces

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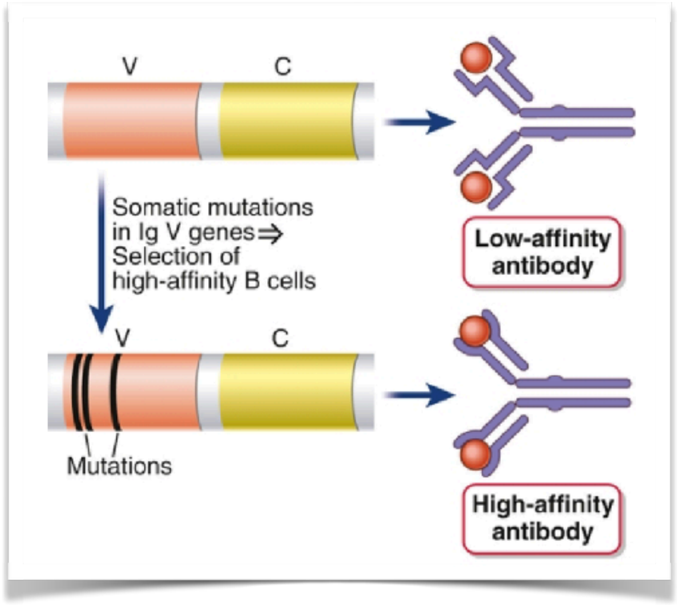
Affinity maturation selects for high-affinity B cells

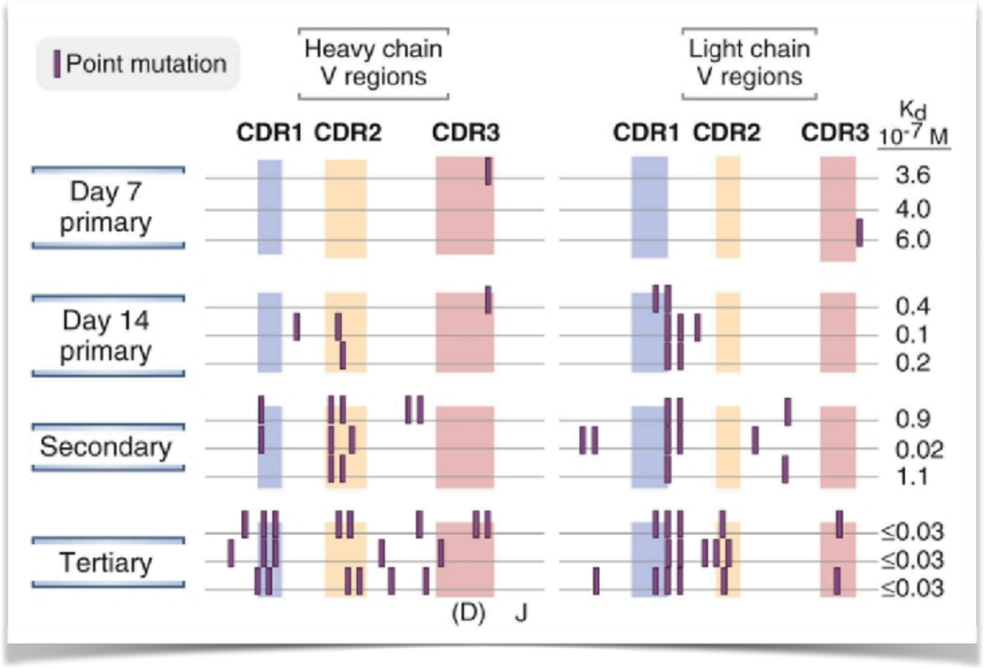
- Increased ability to *bind* antigens
- More *efficiently* neutralise and eliminate microbes and their toxins
- *Helper T cell* (T-dependent) and *CD40:CD40L* interactions required for initiation



Affinity maturation selects for high-affinity B cells

- **Somatic hypermutation**: Ig V genes rearrangements in germinal center dark zone undergo *point mutations* at extremely *high rate*

- **Mutations**: clustered in *V regions*, mostly in antigen-binding to complementary-determining regions *CDRs* ➔ the presence of mutations correlated with *increased antibody affinity*



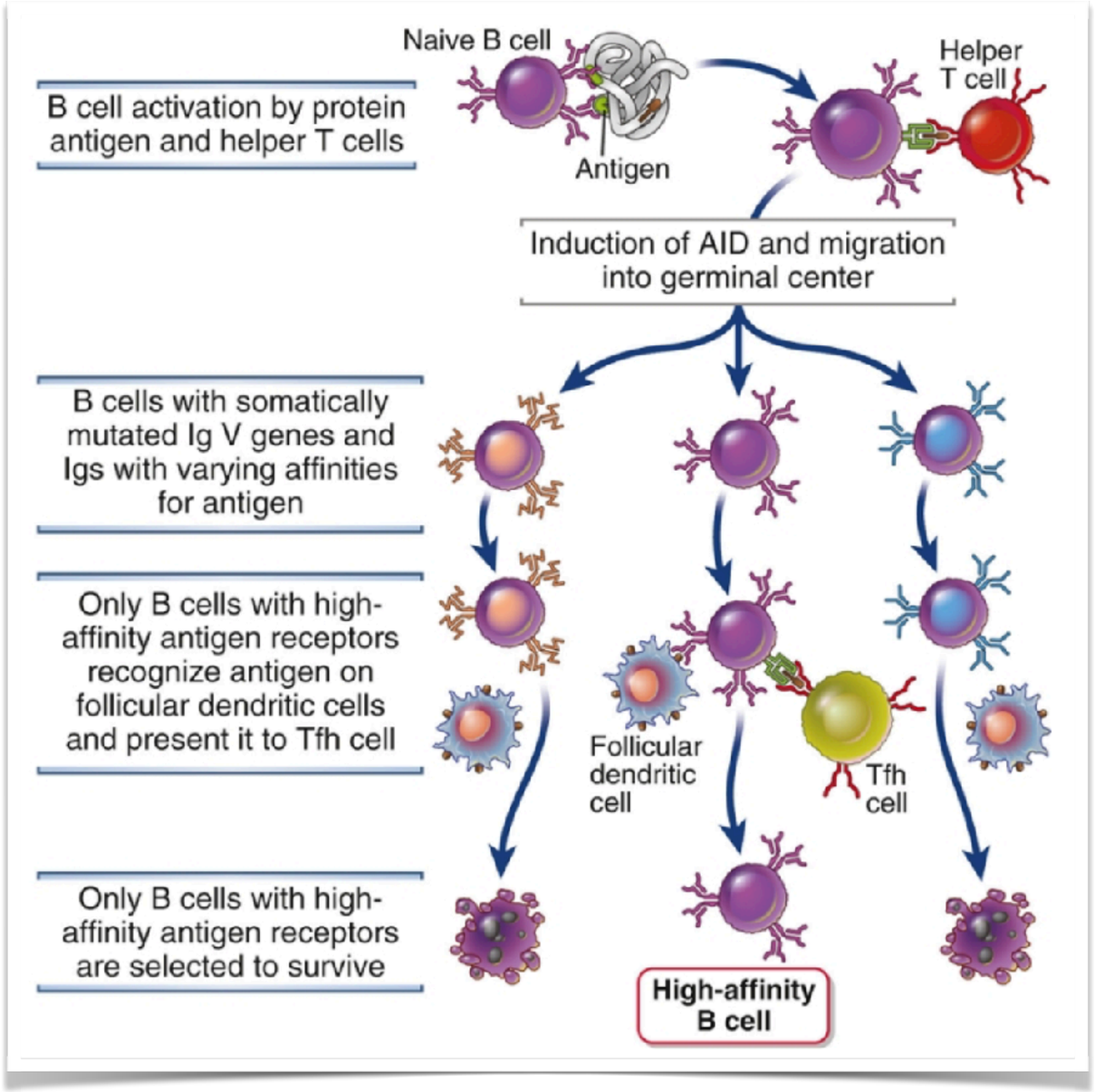
- AID deaminase activity targets primarily rearranged V regions and contributes to the *generation of mutations*
- MSH2, MSH6: DNA mismatch repair enzymes that recruit error prone DNA polymerase in germinal peter B cells for somatic hypermutation

Affinity maturation selects for high-affinity B cells

Many mutations are *not useful* ➔ darwinian natural selection ensures *survival of the best B cells* = fittest in terms of antigen binding

- B cells die by *apoptosis* unless they recognise antigens which induces the anti-apoptotic proteins *Bcl-2*
- High-affinity B cell will preferentially endocytose and present the antigen and *interact with a limited number of Tfh* cells in the germinal center and these helper T cells may signal via CD40L to promote B cell survival
- As more antibodies are produced, the *antigen availability decreases*, thus only B cells with *higher-affinity receptor* capable of binding antigens in increasingly *lower concentrations will be rescued from death*
- Germinal centres are sites of tremendous apoptosis
- Selected cells *differentiate* either into memory or antibody-secreting plasma cell precursors and exit the germinal centre

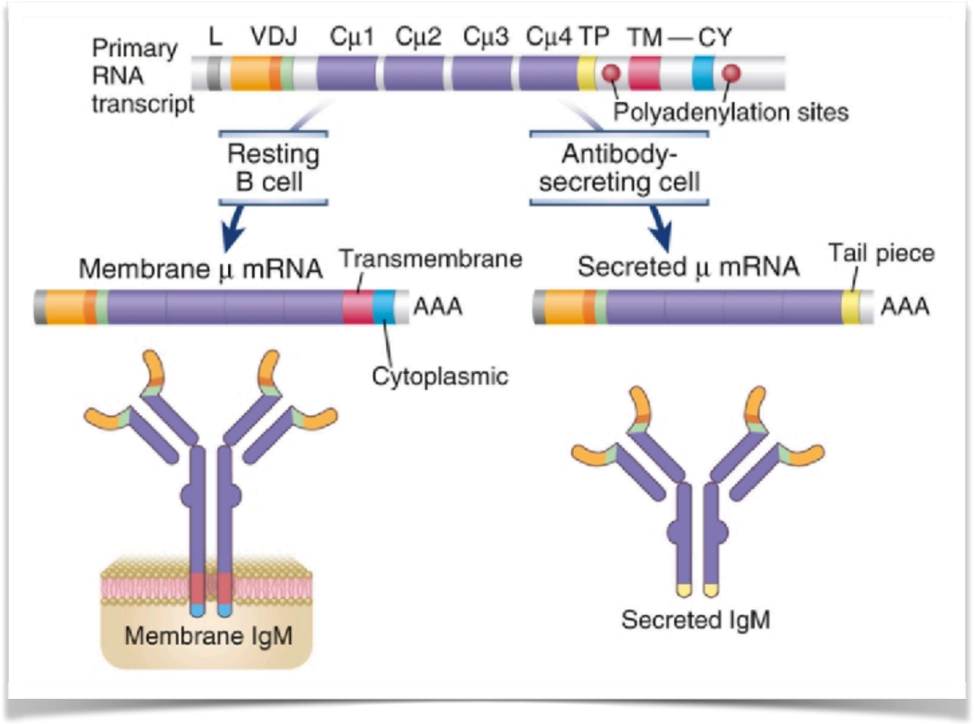
Affinity maturation selects for high-affinity B cells



B cell differentiation into antibody-secreting plasma cells

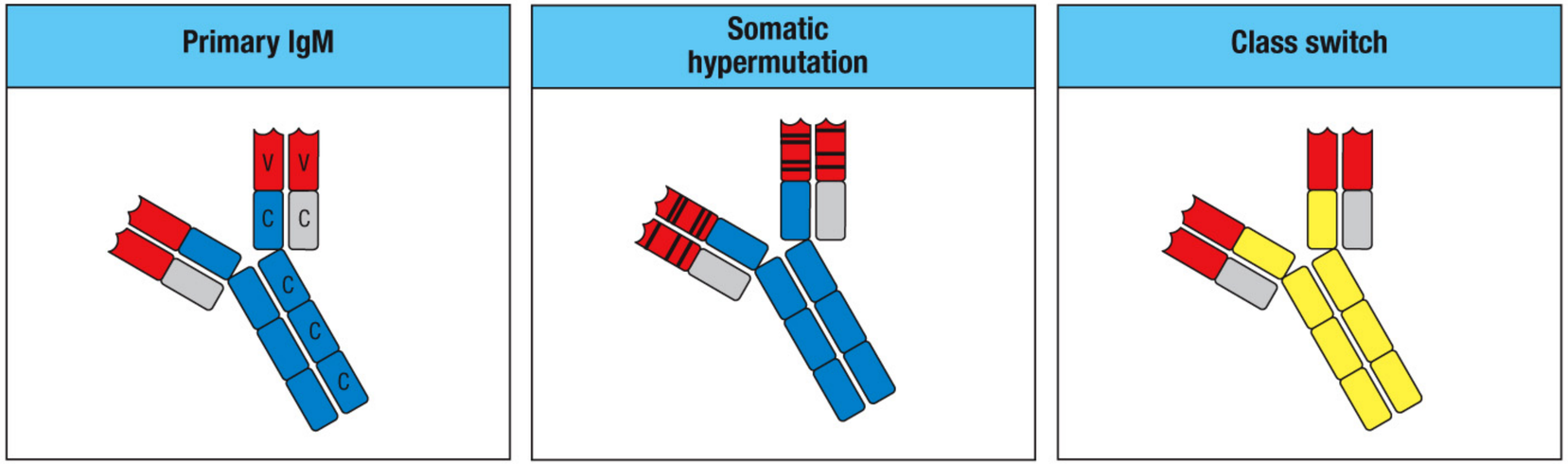
2 types of plasma cells:

- **Short-lived** : generated during *T-independent* responses and early in T-dependent response in extrafollicular B cell foci
- **Long-lived:** generated in *T-dependent* geminal center in response to *protein* antigens, 2-3 weeks after immunisation the BM becomes a major site of AB production, where plasma cells may continue to secrete antibodies for *decades*



<u>Morphological changes:</u>
cell enlargement, *ER becomes prominent* (AB production)

<u>Membrane versus secreted AB:</u>
regulated by *alternative RNA processing* of Ig mRNA and each B cell *can synthesise both* forms



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Generation of memory B cells

- **Location**
  - Generated mainly in *germinal centres*
  - Remain in *lymphoid organ* or *recirculate* between blood and lymphoid organs

- **Characteristics**
  - Acquire the ability to *survive for long periods* without continuous antigenic stimulation
  - Express high levels of anti-apoptotic protein *Bcl-2*
  - Express *high affinity antigen receptors* and *switched isotypes*

- **Main Function: c**ontribute to *accelerated production of antibodies* after secondary exposure

  NB: Effective vaccines must induce both affinity maturation and memory B cell formation, thus they need to activate *helper T cells* ➔ e.g. polysaccharides need to be linked to a protein to form a hapten-carrier conjugate, especially because young children are less able to make strong T-independent responses

Transcriptional regulators determine the fate of activated B cells

- Bcl-6: repressor that *maintains germinal center reaction*, supporting the massive proliferation of B cells, helps B cell to *tolerate DNA damage* during isotype switching and somatic hypermutation by preventing apoptosis and inhibits differentiation into plasma cells in germinal centres

- Blimp-1 and IRF4: commit B cells to a *plasma cell fate*, suppress Bcl-6, contribute to maturation and *enhanced Ig synthesis* in these cells

- TF that delineate memory B cell development remain poorly characterised

Antibody response to T-independent antigens

- Antigens
  - *Nonprotein* (polysaccharides, lipids, nucleic acids) that *cannot be processed* and presented in associated with MHC molecules
  - Often *multivalent* and may induce maximal *cross-linking of the BCR* complex, thus activating B cells without cognate T cell help
  - Polysaccharides can also *activate the complement* system by the alternative or lectin pathway, augmenting B cell activation
  - Many *bacterial cell wall* polysaccharides mount this type of response (major mechanism of defence against infections by encapsulated bacteria)

- Marginal zone and B-1 subsets: important for these responses, upon activation differentiate into *short-lived* plasma cells and produce mainly *IgM* with *low affinities* and limited *isotype switching*. Responses are mainly initiated in the *spleen*, peritoneal cavity and *mucosal* sites.
  ➔ Macrophages in the spleen marginal zone efficiently trap polysaccharides.

- Cytokines produced by non-T cells may stimulate some isotype switching (e.g. TGF-b mediated IgA switch at mucosal sites)

AGs that do not require T cell help to induce B cell responses

Thymus-independent (TI) AG induces AB w/o T cell help

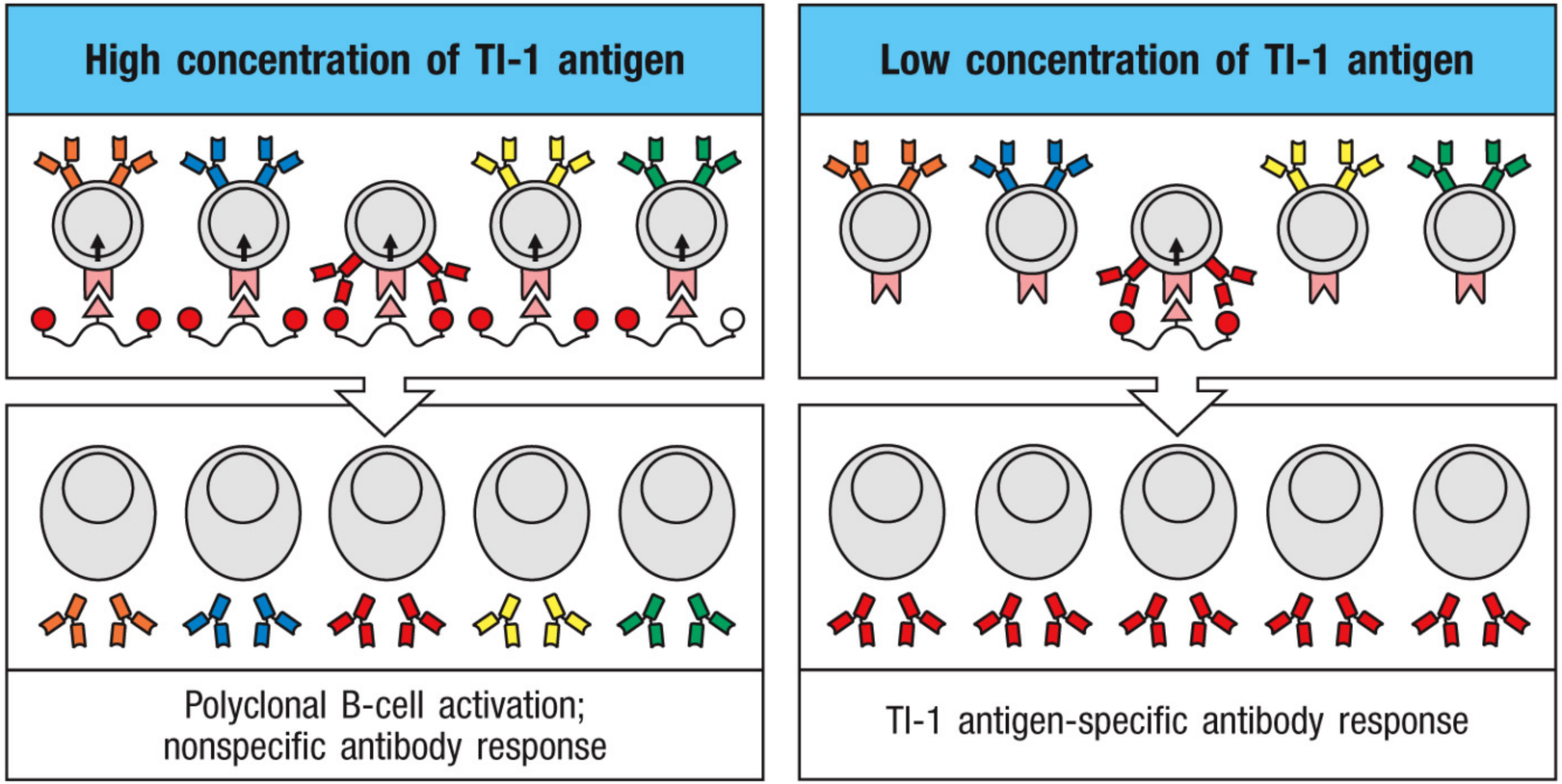
→ Polysaccharides
Polymeric proteins
Lipopolysaccharides

1) TI-1 = « B cell mitogens »

Can directly induce B cell proliferation *regardless of AG-specificity*
= **polyclonal activation** (e.g. TLR ligands)

Context: low concentration of TI-1 AGs, only the B cells expressing an *activated BCR* can be stimulated

Important: early stages of infection with *extracellular bacteria*



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10.14 - AGs that do not require T cell help to induce B cell responses

2) TI-2

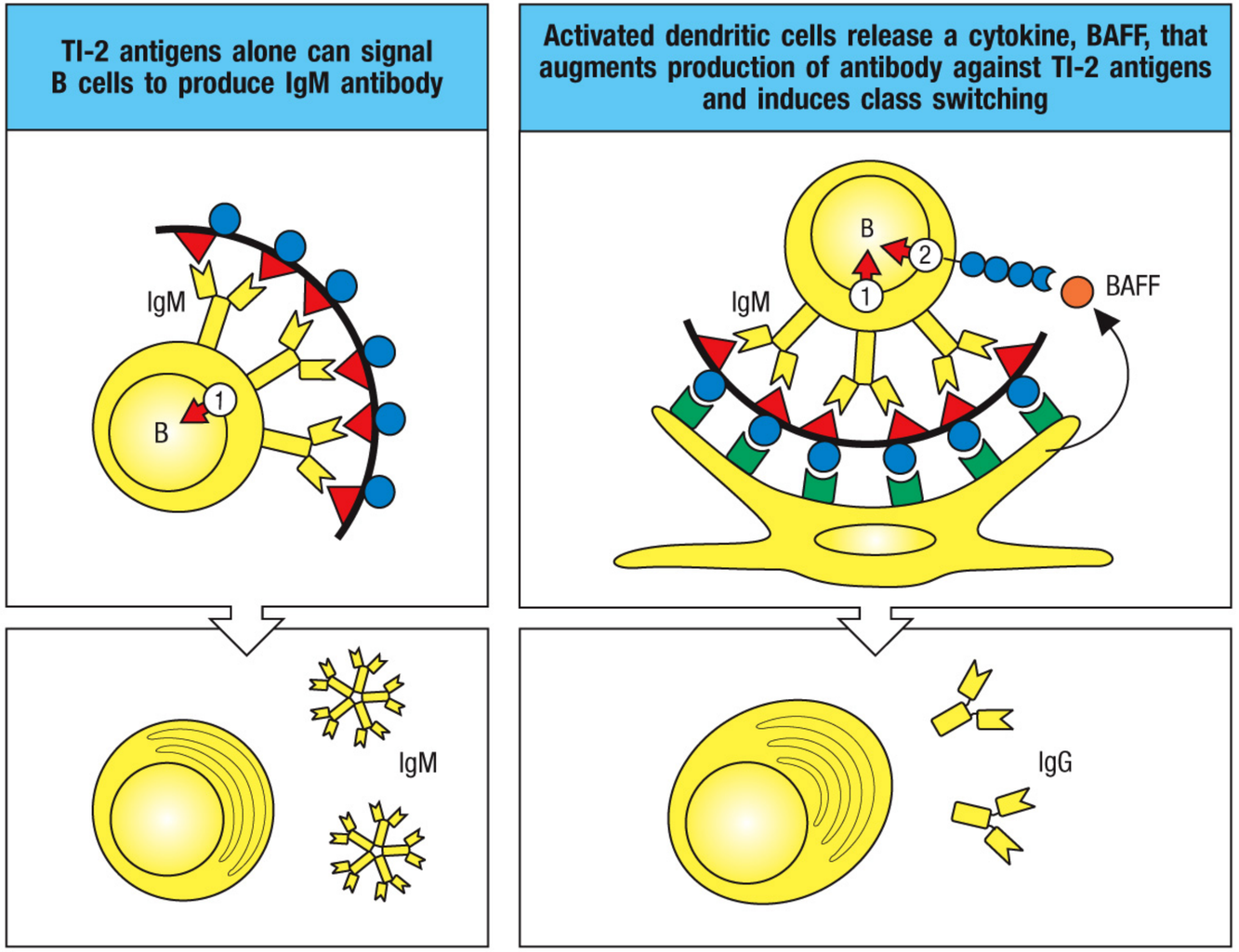
Molecules that have highly repetitive structure!

- Only mature B cells activated by TI-2 AGs (Polysaccharides ineffective in children, maybe because most B cells are immature)
- TI-2 AGs recognized by marginal zone B cells

Mechanism: cross-linking o BCRs on the surface of B cells

DCs and MΦ can provide co-stimulatory signals (BAFF) ➔ class-switching

Important: during infection with capsulated bacteria



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Properties of different classes of antigens

	TD antigen	TI-1 antigen	TI-2 antigen
Antibody response in infants	Yes	Yes	No
Antibody production in congenitally athymic individual	No	Yes	Yes
Antibody response in absence of all T cells	No	Yes	Yes
Primes T cells	Yes	No	No
Polyclonal B-cell activation	No	Yes	No
Requires repeating epitopes	No	No	Yes
Examples of antigen	Diphtheria toxin Viral hemagglutinin Purified protein derivative (PPD) of *Mycobacterium tuberculosis*	Bacterial lipopoly-saccharide *Brucella abortus*	Pneumococcal polysaccharide *Salmonella* polymerized flagellin Dextran Hapten-conjugated Ficoll (polysucrose)

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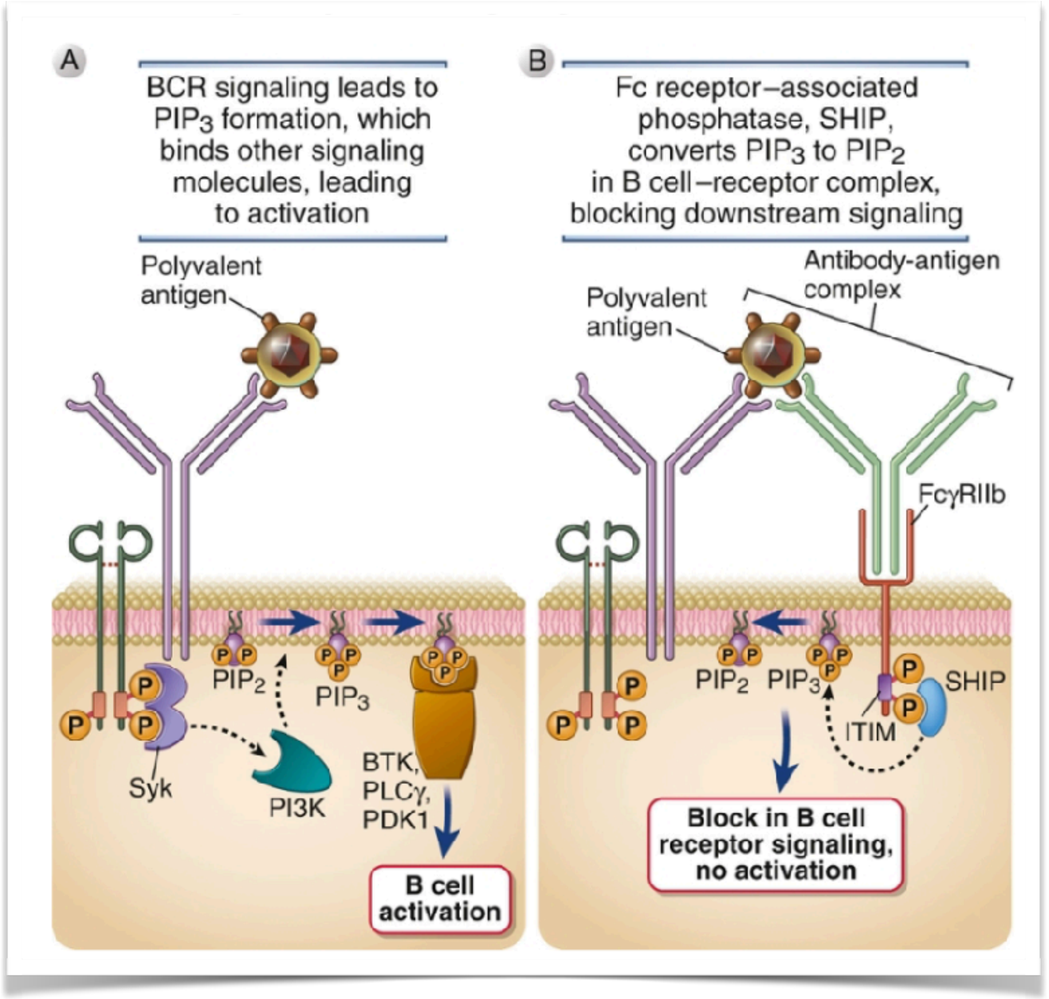
Regulation of humoral immune responses by Fc receptors

Secreted AB inhibit continuing B cell activation by forming *AB-AG complexes* that bind to antigen receptor and inhibitory Fs receptor on antigen-specific B cells.



Antibody feedback: Physiological control mechanism in humoral responses *preventing uncontrolled AB* production