

# TEACHING UNIT 07

## Humoral Immune Response

# Humoral Immune Response



*Activation of B Lymphocytes and  
Antibody Production*

*... ...we know that humoral immunity...*

*...involves the action of B lymphocytes and antibodies  
in acquired immunity...*

*...is effective in protection against extracellular  
microorganisms and their toxins.*

*...is more important than cellular immunity in defense against  
microorganisms that possess polysaccharide and lipid-rich  
capsules.*

*... ...we also know that B lymphocytes...*

*...recognize macromolecules (proteins, lipids, polysaccharides, lipopolysaccharides, nucleic acids), as well as small molecules in solution or on the surface of particulate antigens.*

*...while T lymphocytes mainly respond to portions of protein antigens and do not participate in the response to other antigens.*

*Today, we need to answer two questions.*

- 1. How do naive B lymphocytes get activated and become antibody-secreting cells?*
- 2. How is it ensured that the response to different types of microorganisms results in the production of the most suitable classes of antibodies?*

*... We have learned...*

*...that receptors on naive B lymphocytes are IgM and IgD.*

*...that naive B lymphocytes get activated by an antigen (FIRST SIGNAL) and another signal.*

*...resulting in the proliferation and differentiation of B lymphocytes specific to the antigen (clonal expansion).*

*...this process gives rise to effector B lymphocytes – plasma cells (10,000 plasma cells, i.e.,  $10^{12}$  antibody molecules per day).*

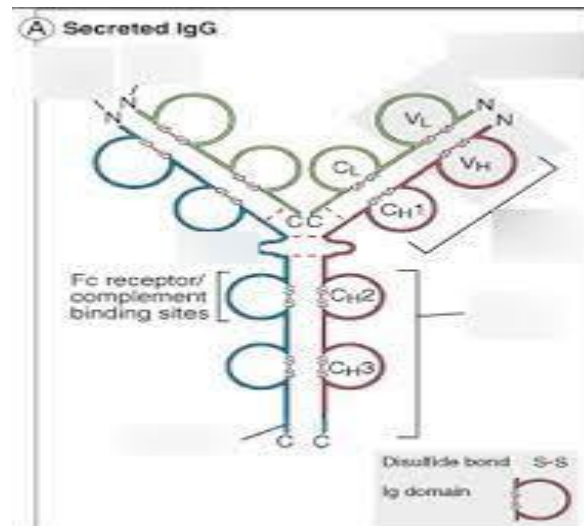
*...which is important due to the speed at which microorganisms replicate..*

*...we should know by now...*

*...if a B lymphocyte responds to protein antigens, during its differentiation, some effector cells begin to produce antibodies of different classes (class switching), while maintaining the same specificity.*

*...these antibodies have the same specificity but different effector functions because different Fc portions of Ig bind to different receptors on cells.*

*...which enables specialization for combating various microorganisms.*



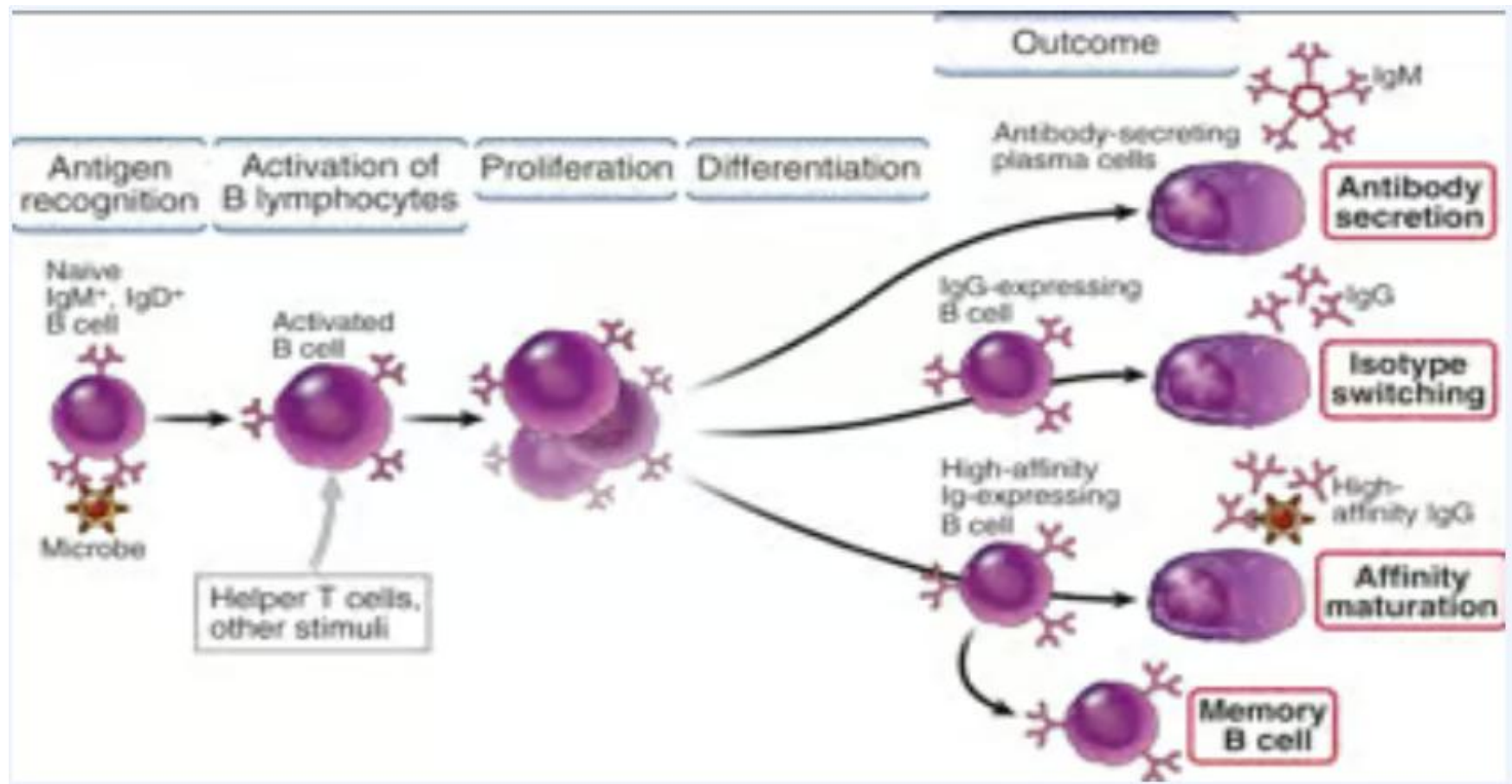
*...we should know by now...*

*repetitio mater studiorum est*

*...due to repeated exposures to the same protein antigen,  
antibodies with increasing affinity for that antigen are formed  
– AFFINITY MATURATION.*



# Phases and Types of Humoral Immune Response



**Humoral response to T-  
dependent antigens:**

**PROTEINS**

**Antibody class switching  
Affinity maturation of  
antibodies  
Formation of memory B  
cells**

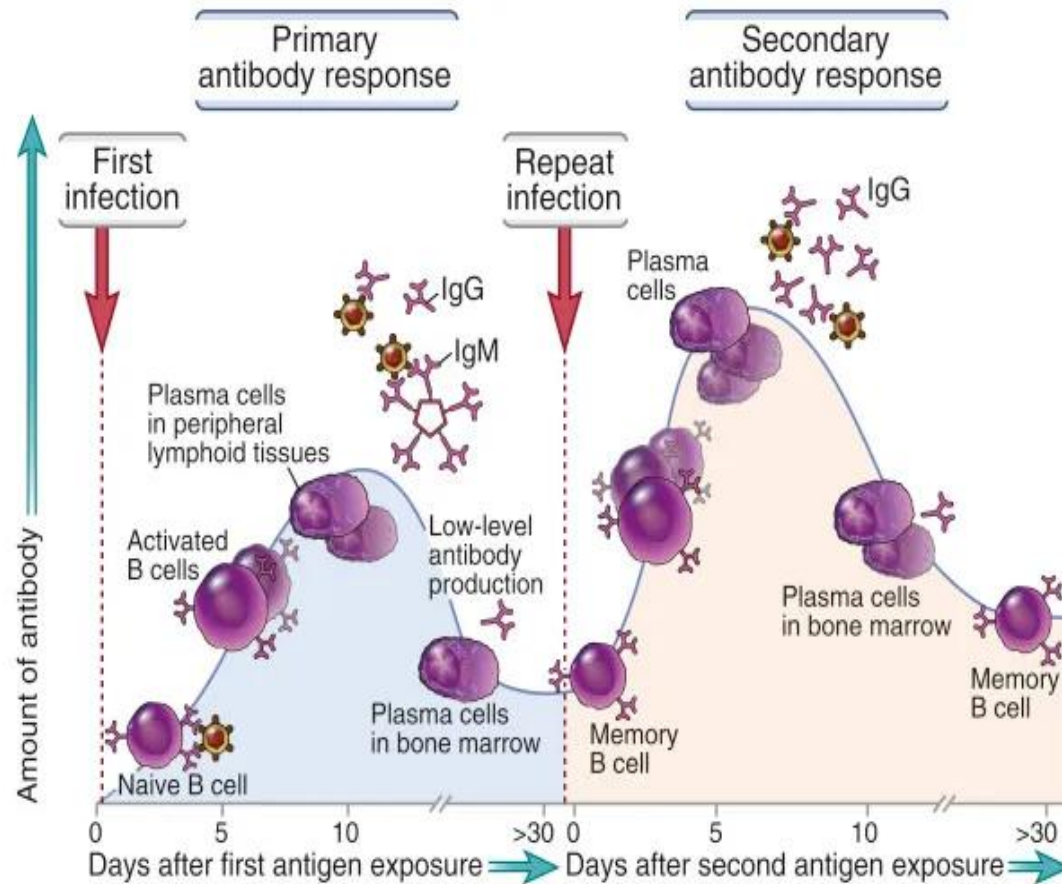
**Humoral response to T-  
independent antigens:**

**NOT PROTEINS**

**No or weak antibody class  
switching  
No or weak affinity  
maturation of antibodies  
Mostly no memory B cells**

**NO SECONDARY  
RESPONSE**

(A)



## Characteristics of primary and secondary immune response

(B)

### Primary response

### Secondary response

Lag after immunization

Usually 5–10 days

Usually 1–3 days

Peak response

Smaller

Larger

Antibody isotype

Usually IgM>IgG

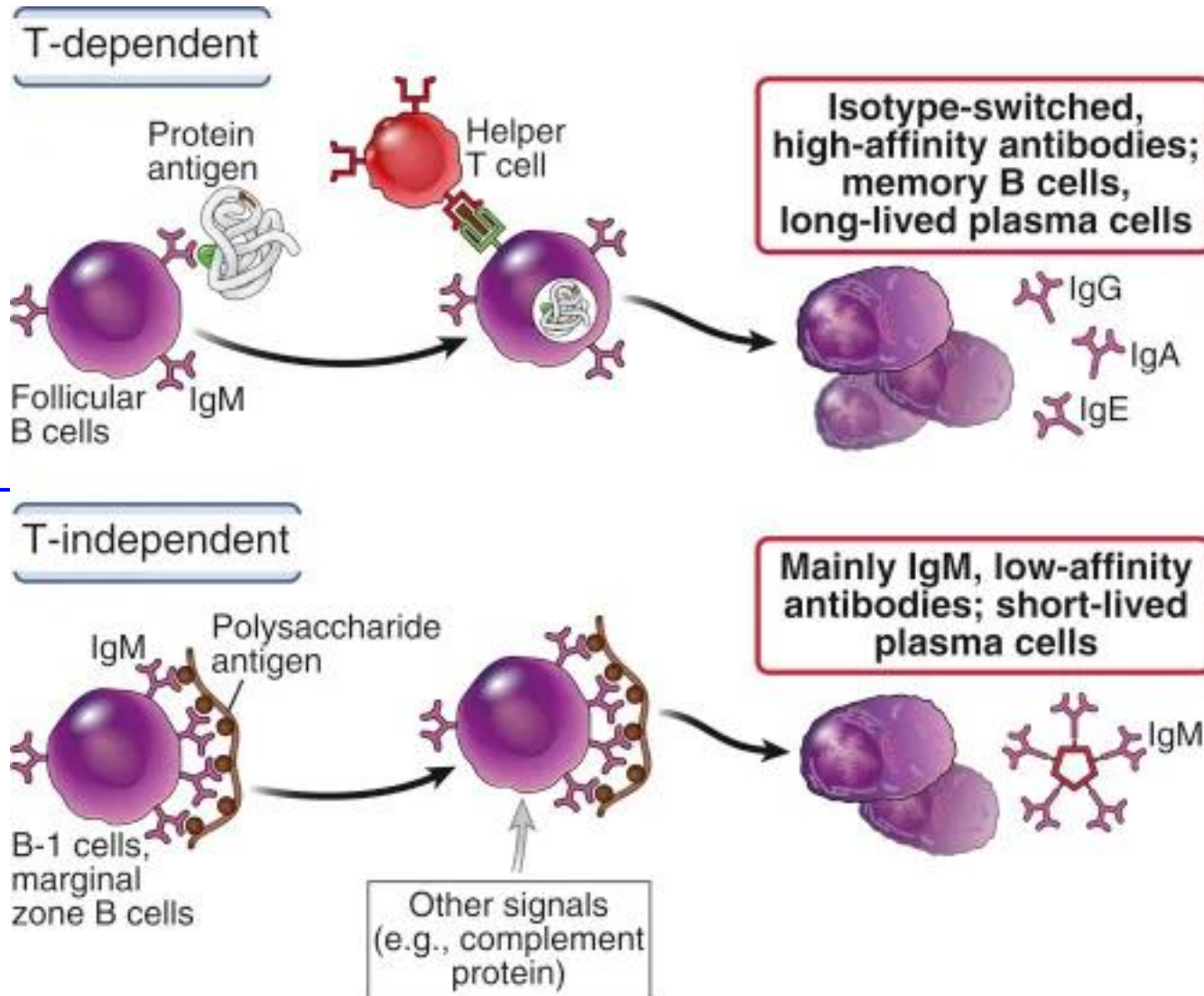
Relative increase in IgG and, under certain situations, in IgA or IgE (heavy-chain isotype switching)

Antibody affinity

Lower average affinity, more variable

Higher average affinity (affinity maturation)

# Subpopulations of B lymphocytes



# Humoral response to (T-dependent) antigens...

...begins in lymphoid tissue when a B lymphocyte recognizes the NATIVE antigen.

<http://www.youtube.com/watch?v=hQmaPwP0KRI>

# **Stimulation of B lymphocytes by antigen.**

**Antigens of microorganisms are transported from the lymphoid tissue or via the blood and concentrate in the follicles and marginal zones of peripheral lymph organs...**

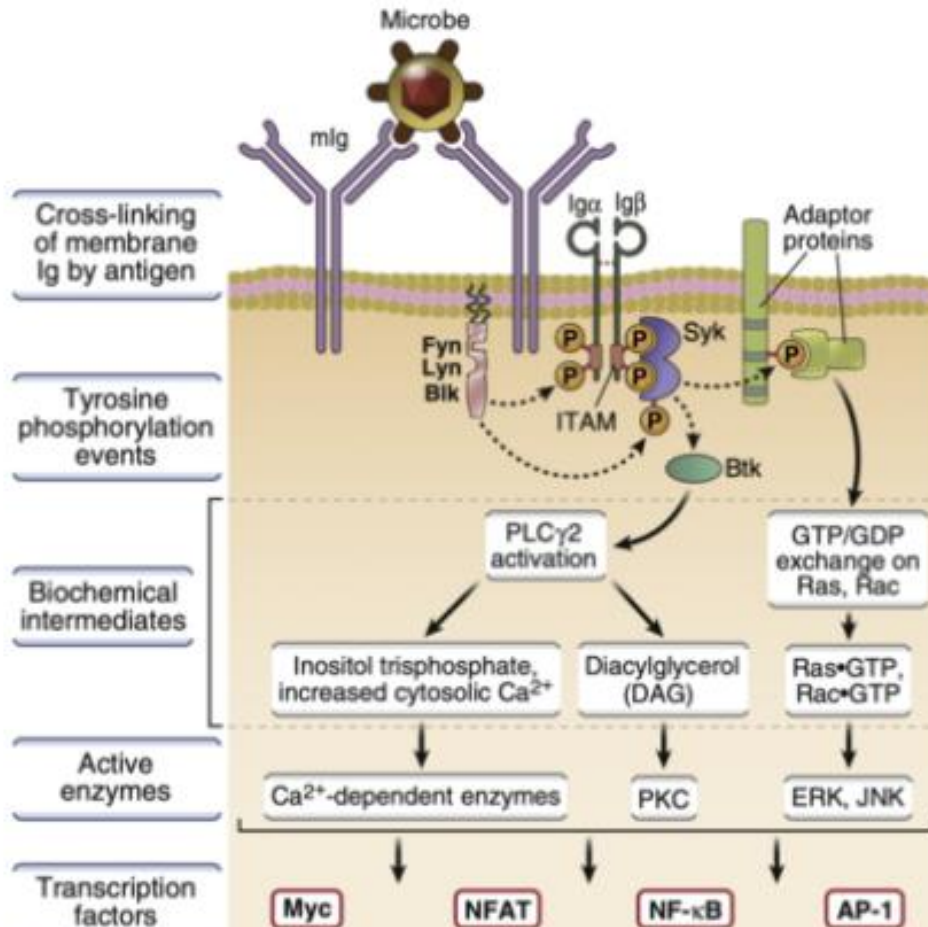
**...where they are recognized by antigen-specific B lymphocytes...**

**...initiating signal pathways or cell activation...**

**In response to both protein and non-protein antigens, lymphocyte activation requires additional (secondary) signals.**



The antigen induces the activation of B lymphocytes by clustering their receptors with its determinants, initiating signal transduction towards the nucleus.



The BCR complex consists of BCR (Ig), Igα, and Igβ chains.

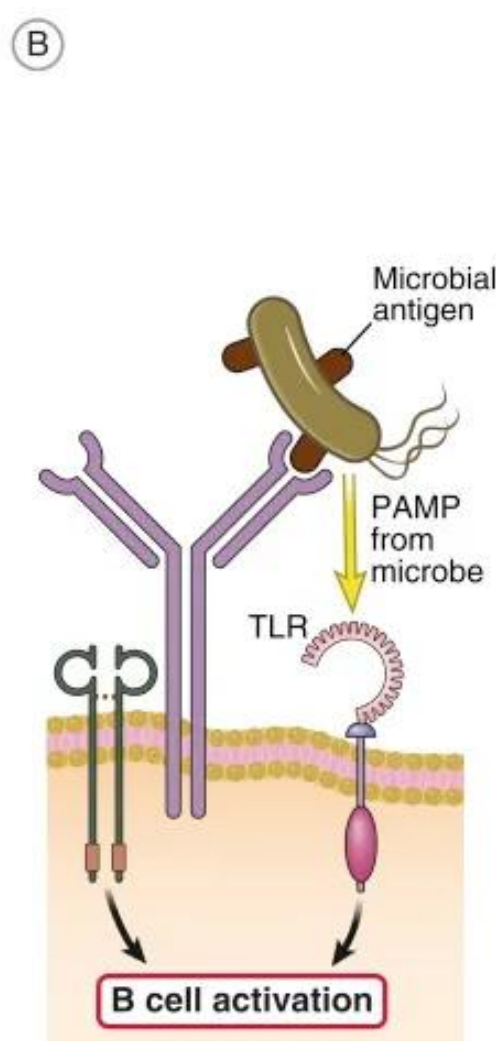
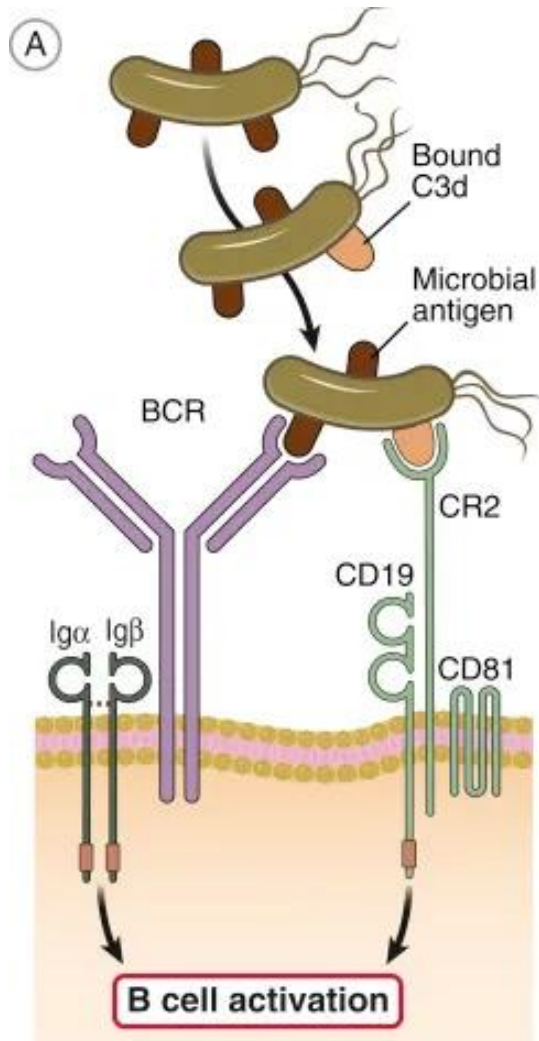
Signal transduction requires the cross-linking of two or more BCRs.

Igα and Igβ chains in B lymphocytes are analogous to CD3 and ζ chains in T lymphocytes.

Igα and Igβ chains contain ITAM sequences.

The ultimate result is the activation of transcription factors.

# The role of C3d in the activation of B lymphocytes.



**SECOND SIGNAL**

**TLR**

**CD40**

**PART OF THE FIRST SIGNAL (similar to co-receptors)**

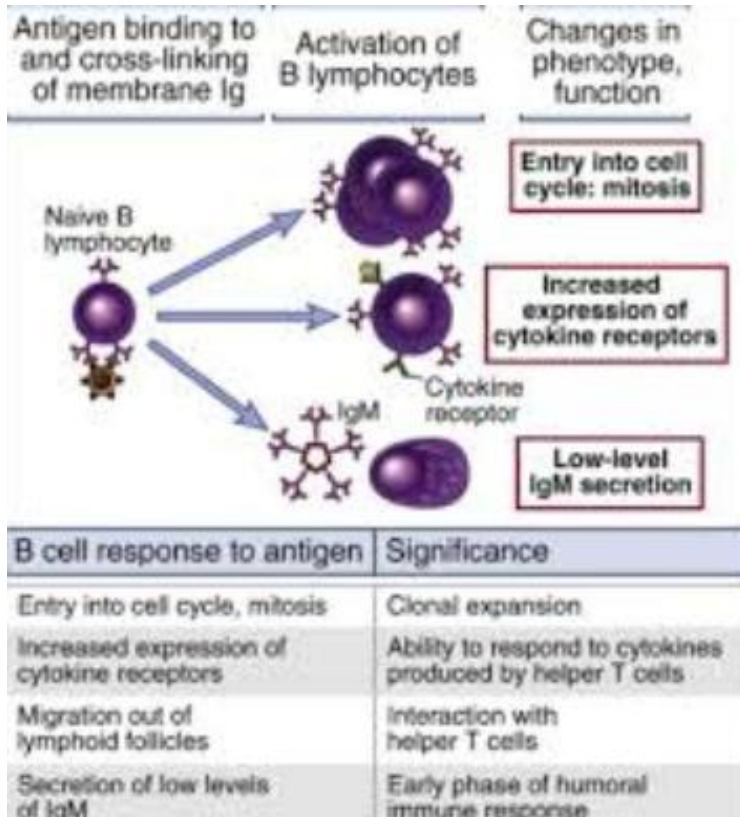
**CR2 = CD21**

**EB virus**

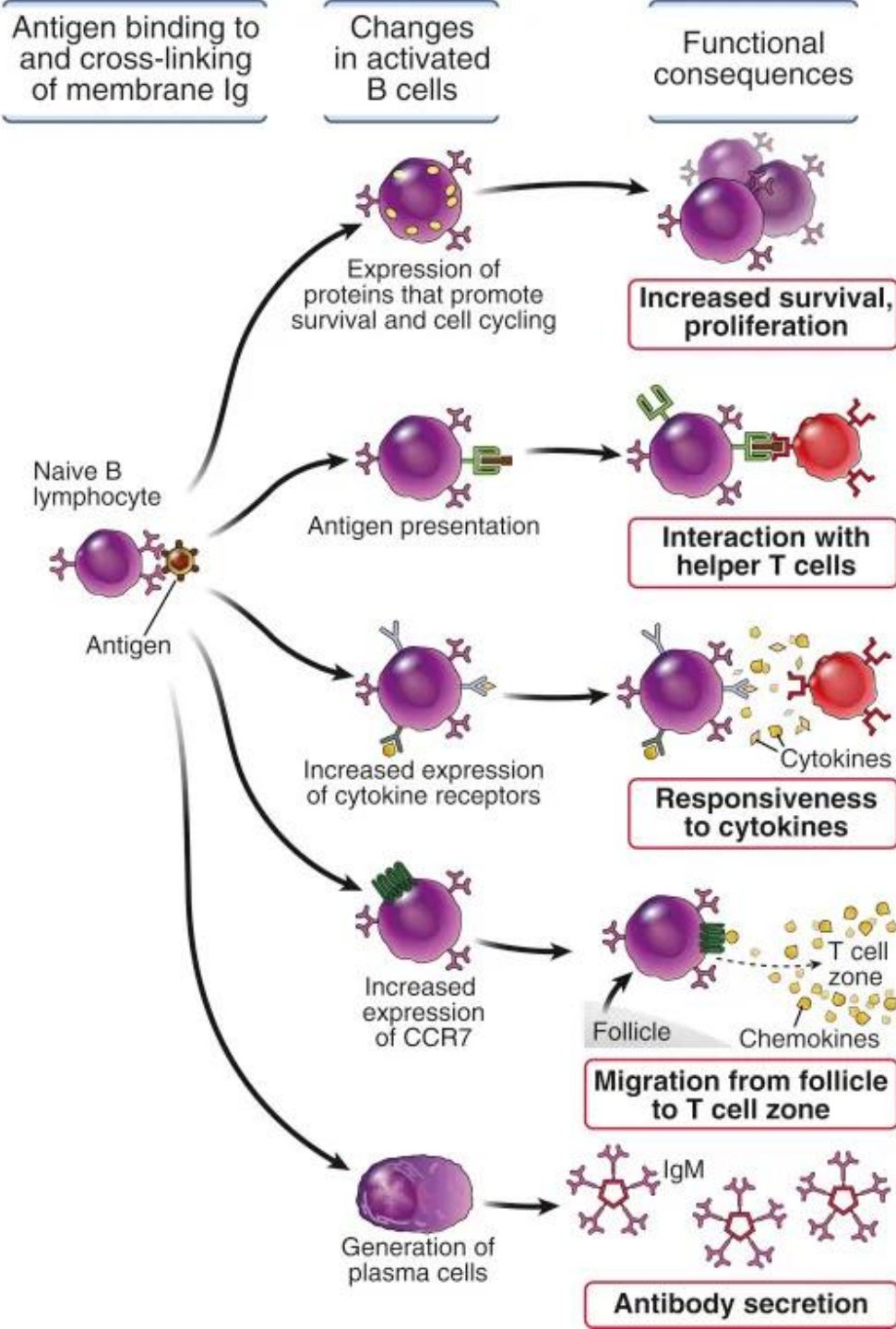


# PROLIFERATION and DIFFERENTIATION of B lymphocytes

**B lymphocytes in response to T-independent antigens synthesize more IgM and begin to secrete them, initiating the early humoral immune response.**



**In addition to proliferation and differentiation, B lymphocytes prepare for cooperation with T lymphocytes (protein antigens)...**

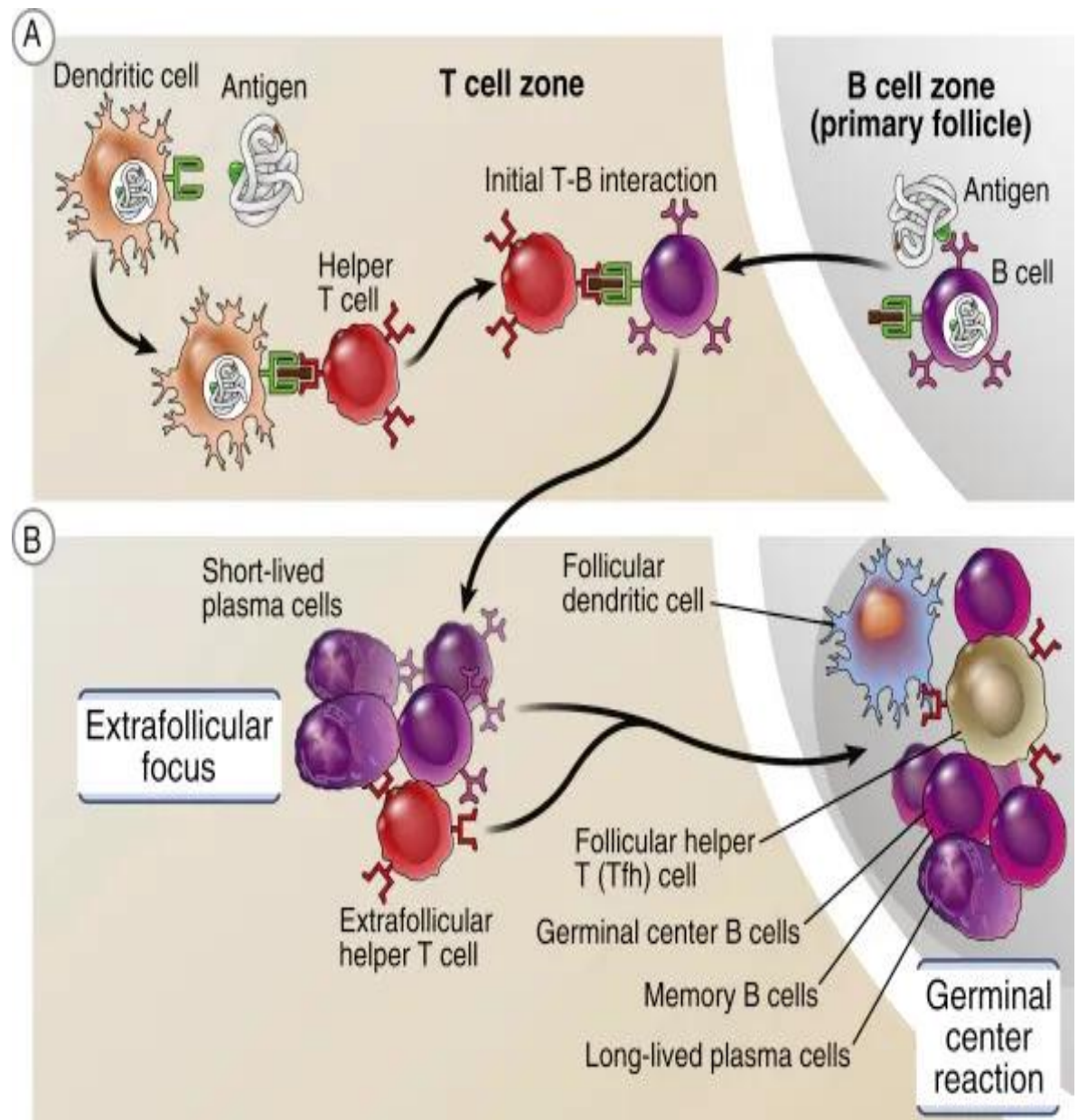


...

Most soluble protein antigens are not capable of simultaneously engaging a larger number of receptors – this is not sufficient for proliferation and differentiation. However, they induce higher expression of B7, providing a second signal to T lymphocytes, and also...

...to enhance their own expression of cytokine receptors and reduce the expression of chemokine receptors present in the follicles, allowing them to migrate towards the edges of the follicles, i.e., towards the T cell zones of lymphoid organs..

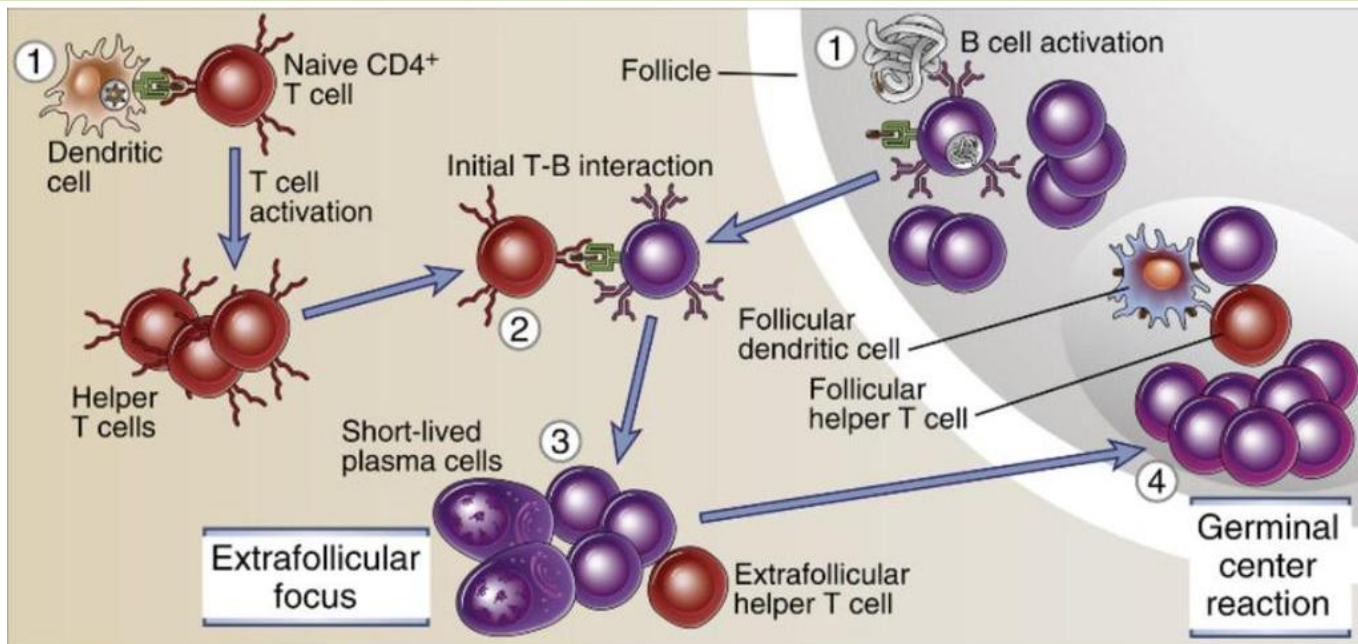
## Order of events in T-dependent humoral immune response





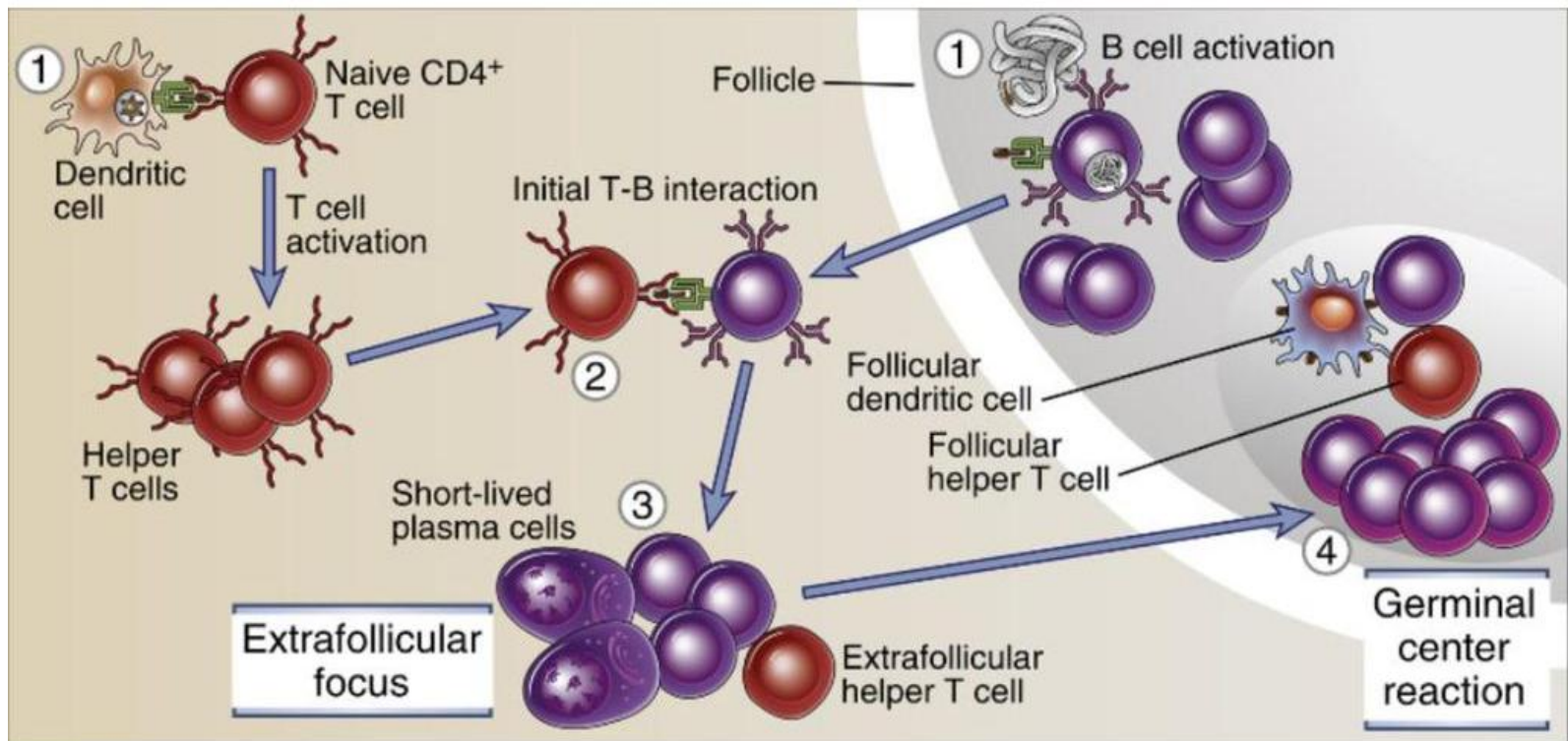
# Interaction of CD4<sup>+</sup> Th and B lymphocytes in response to protein (T-dependent) antigens

1. CD4 T lymphocytes are activated in the paracortex in contact with dendritic cells; B lymphocytes are activated in the follicles.
2. T and B lymphocytes interact outside the follicles (CD40:CD40L interaction).
3. Extrafollicular foci; B lymphocytes differentiate into plasma cells and produce a smaller quantity of antibodies (limited isotype switching may occur), and some T lymphocytes differentiate into follicular helper T cells (T<sub>fh</sub>).



...

4. Activated B lymphocytes and Tfh migrate to the follicle, where after their interaction, germinal centers form (sites of intense B lymphocyte proliferation, class switching, somatic mutations, and affinity maturation, as well as the generation of memory B lymphocytes and the development of long-lived plasma cells).



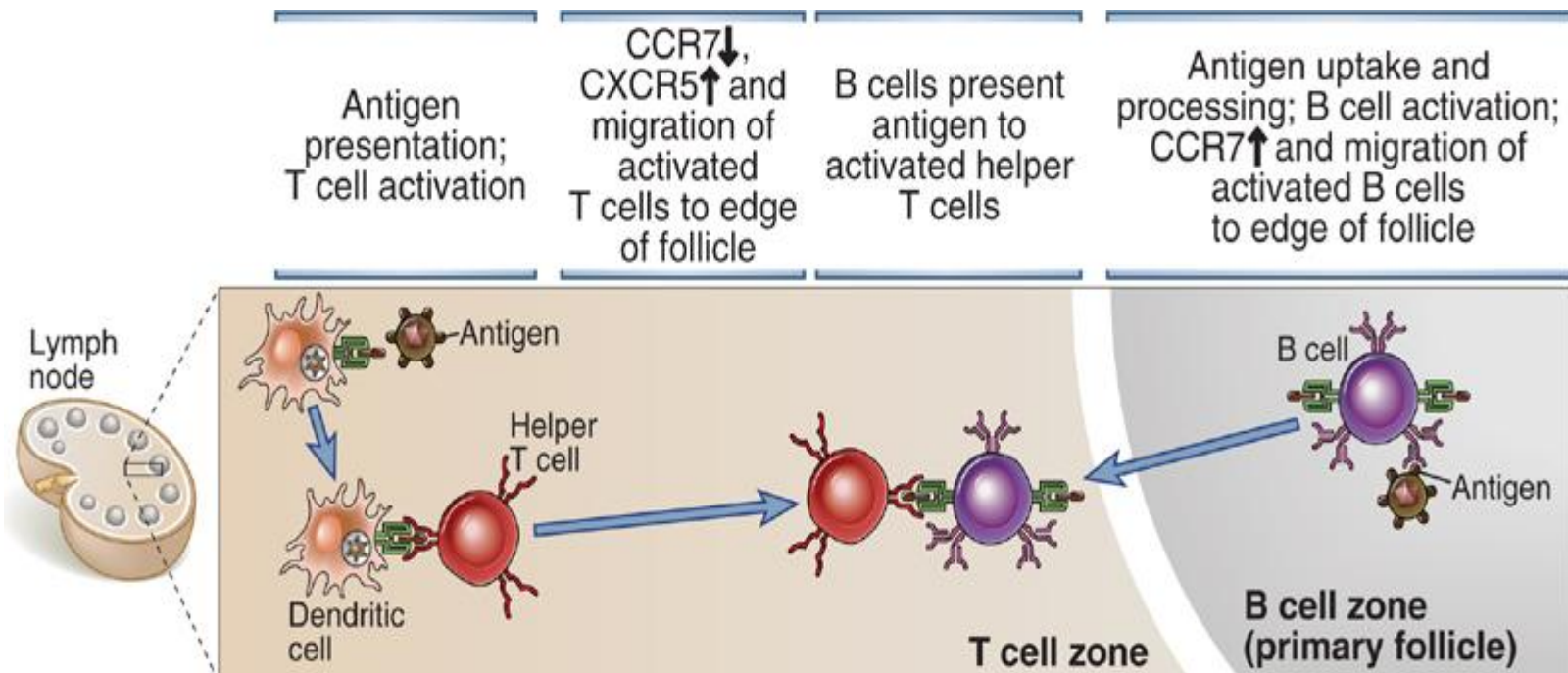
Effector CD4<sup>+</sup> T lymphocytes lose the expression of CCR7...

...most of them migrate to the site of antigen entry...

...while a smaller number migrate towards B lymphocytes, near the edges of lymph follicles.

# Initial Activation of T and B Lymphocytes

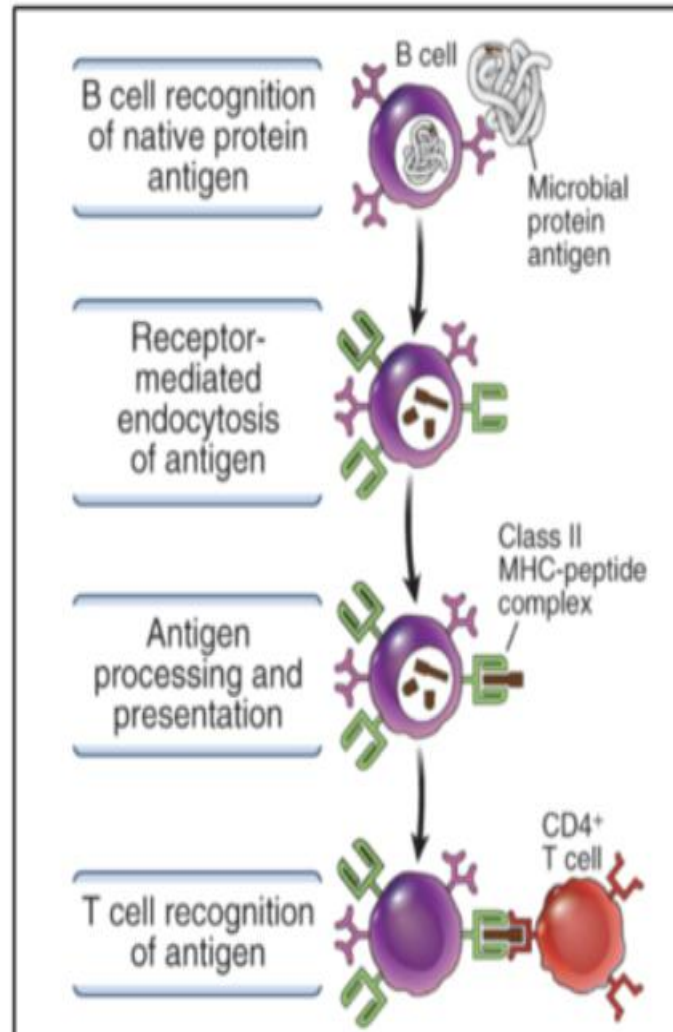
Directed migration of activated B and T lymphocytes towards each other depends on changes in the expression of specific chemokine receptors.



**\*\*T lymphocytes: expression of CD40L, CXCR5, reduced expression of CCR7.\*\***

**\*\*B lymphocytes: increased expression of CCR7, decreased CXCR5; increased expression of CD69 (preventing exit from the lymph node); promoting all B lymphocyte functions crucial for the antigen presentation process.\*\***

# B lymphocyte presents peptides to CD4+Th lymphocytes



B lymphocytes are highly efficient APCs, but only for antigens they specifically recognize and are capable of activating (expressing B7) effector but not naive Th lymphocytes.

Activation of a specific B lymphocyte clone:

Specificity of BCR for the antigen\*\*

Phagocytosis mediated by BCR

Cytokines and CD40L

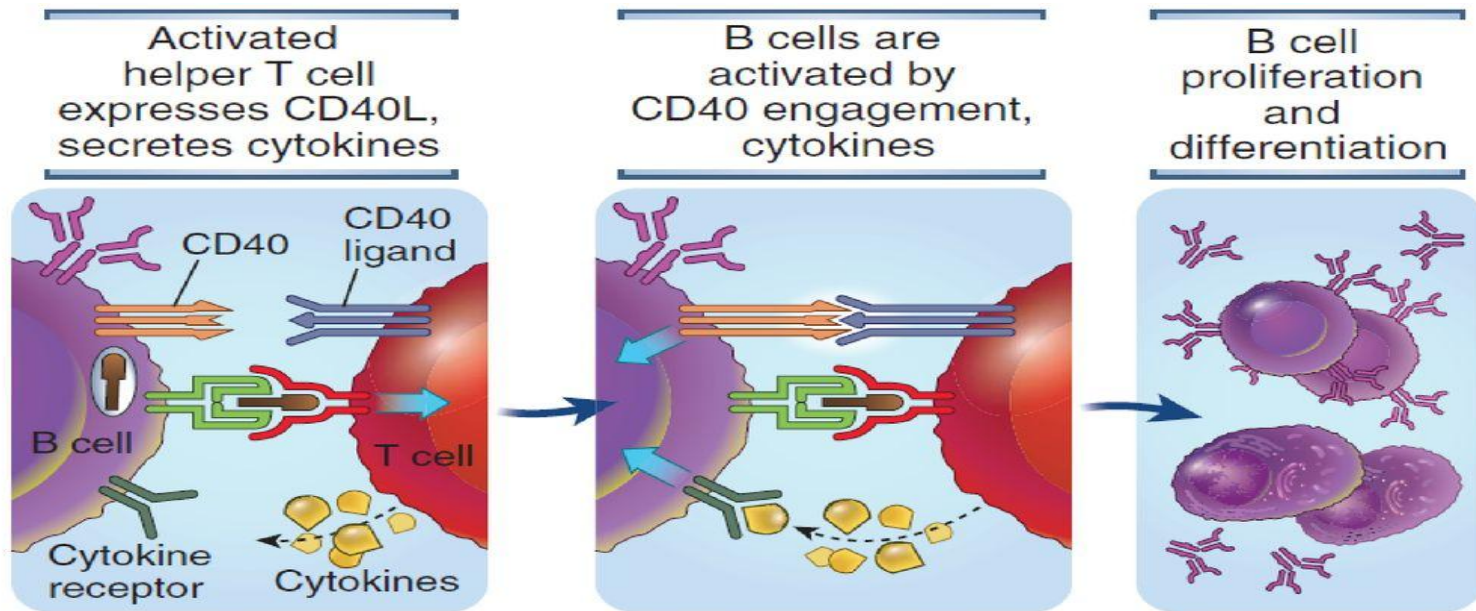


**CD4+Th lymphocytes, through costimulators and cytokines,  
ADDITIONALLY ACTIVATE B lymphocytes...**

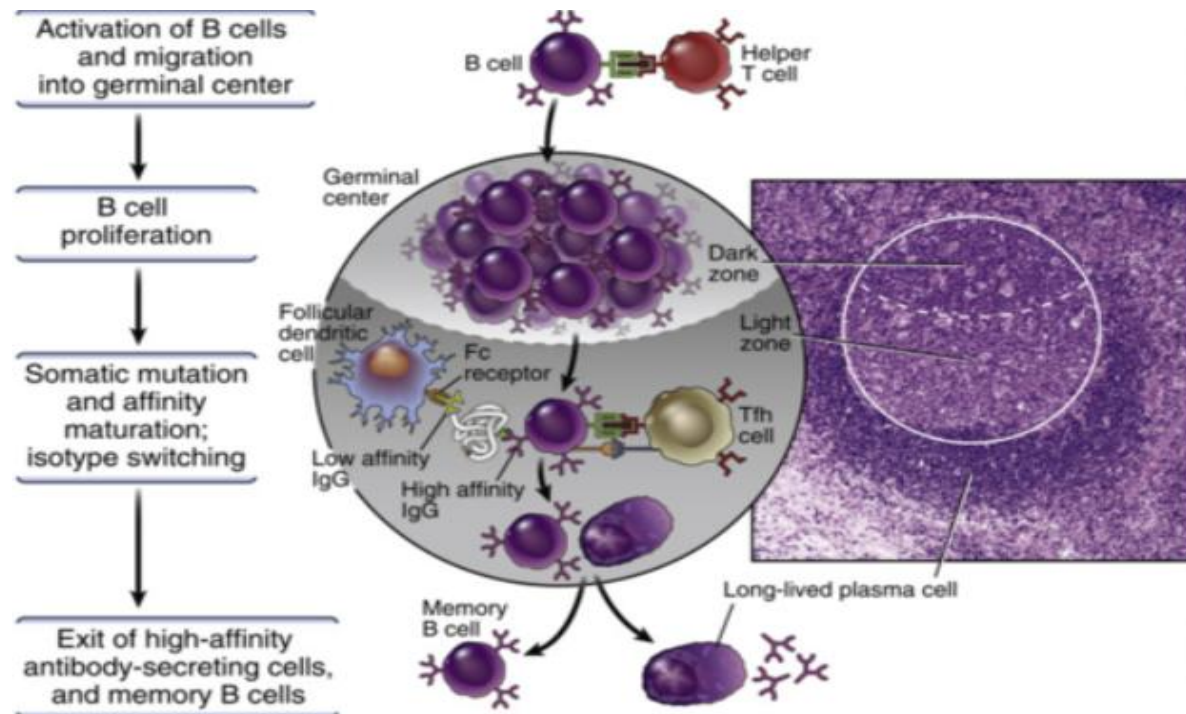
...by expressing CD40L  
...and by secreting cytokines

**Engagement with CD40 delivers to B lymphocytes a proliferative signal as well  
as a signal for antibody synthesis, class switching, and affinity maturation.**

**Cytokines enhance this signal but also modulate it by influencing antibody  
class and affinity maturation.**



For many events that occur in a fully developed humoral immune response, the participation of specialized helper T lymphocytes is necessary.



# Follicular helper T lymphocytes

Some of the activated helper T lymphocytes, after interacting with B lymphocytes, differentiate into follicular helper T lymphocytes (Tfh), which express a high level of the chemokine receptor CXCR5 guiding them to lymph follicles.

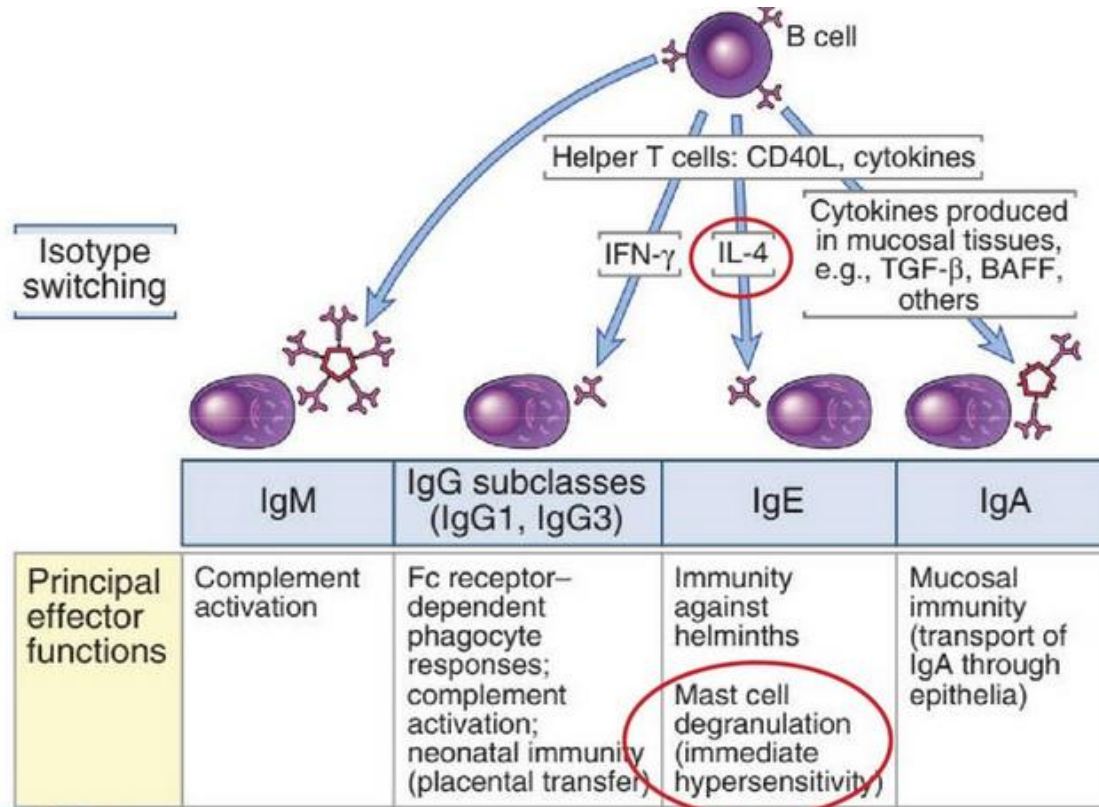
Tfh can develop from undifferentiated cells, as well as from Th1, Th2, or Th17 lymphocytes.

The interaction of ICOS on CD4<sup>+</sup> T lymphocytes with the ICOS ligand on B lymphocytes is crucial for the formation of Tfh.

Tfh lymphocytes produce IL-21, which is important for antibody production.

Tfh can secrete IFN $\gamma$ , IL-4, and IL-17, directing the isotype switch.

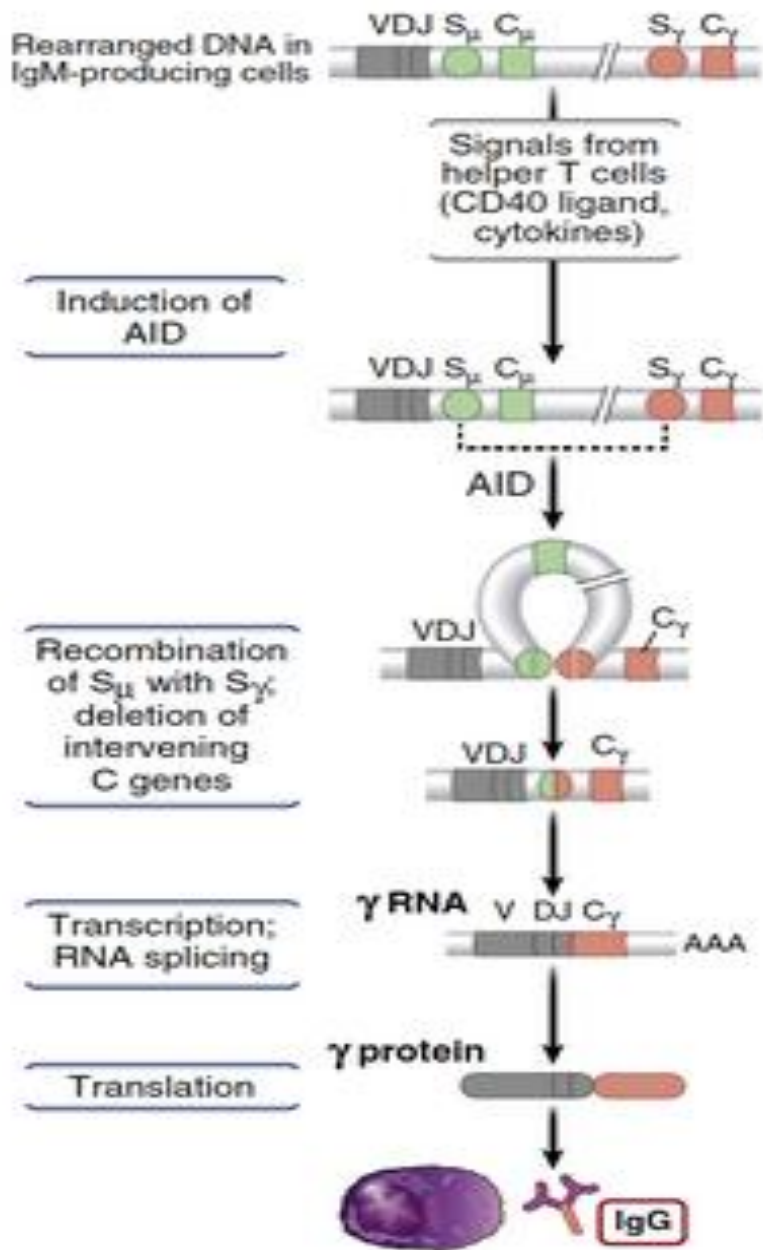
# CD4+Th induces CLASS SWITCHING of antibodies synthesized by B lymphocytes.



**Class switching enables adaptation of the humoral response to different types of microorganisms.**

**Class switching is initiated by the interaction of CD40 (on B cells) with CD40 ligand (on T cells) and is guided by various cytokines. If there is no contact, only IgM is synthesized (hyper-IgM syndrome, which is associated with the X chromosome – an inactive gene for CD40 ligand located on that chromosome).**

## The molecular basis of antibody class switching.



Recombination that triggers antibody class switching (switch recombination) is DNA chain recombination.

CD40-CD40L contact induces the expression of AID (activation-induced deaminase).

S (μγ $\epsilon$ α) (switch) regions - absent only in front of δ.



Naive B cell



5' region contains switch region that drives recombination

Rearranged DNA in IgM-producing cells

VDJ S<sub>μ</sub> C<sub>μ</sub> C<sub>δ</sub> S<sub>γ</sub> C<sub>γ</sub> S<sub>ε</sub> C<sub>ε</sub>

No signals from helper T cells

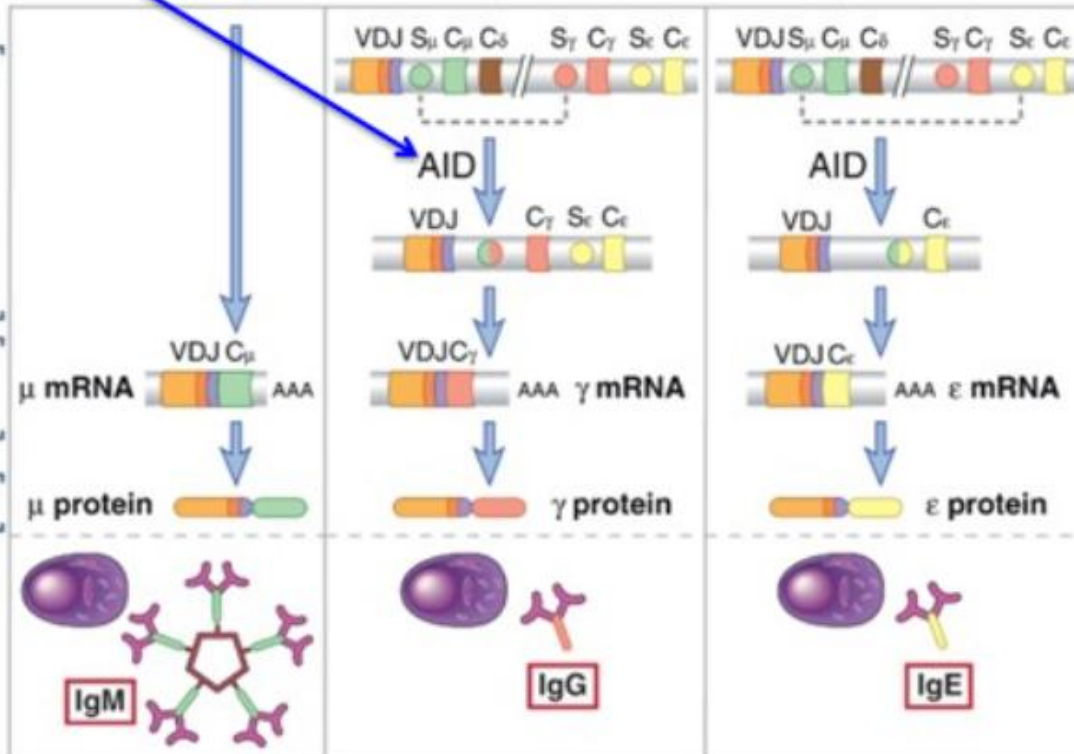
Signals from helper T cells (CD40 ligand, cytokines)

activation-induced cytidine deaminase (AID) induces DNA double-stranded breaks which drive switch recombination

In response to T cell signals, recombination of S<sub>μ</sub> with S<sub>γ</sub> or S<sub>ε</sub>; deletion of intervening C genes

Transcription; RNA splicing

Translation



# **AFFINITY MATURATION of antibodies...**

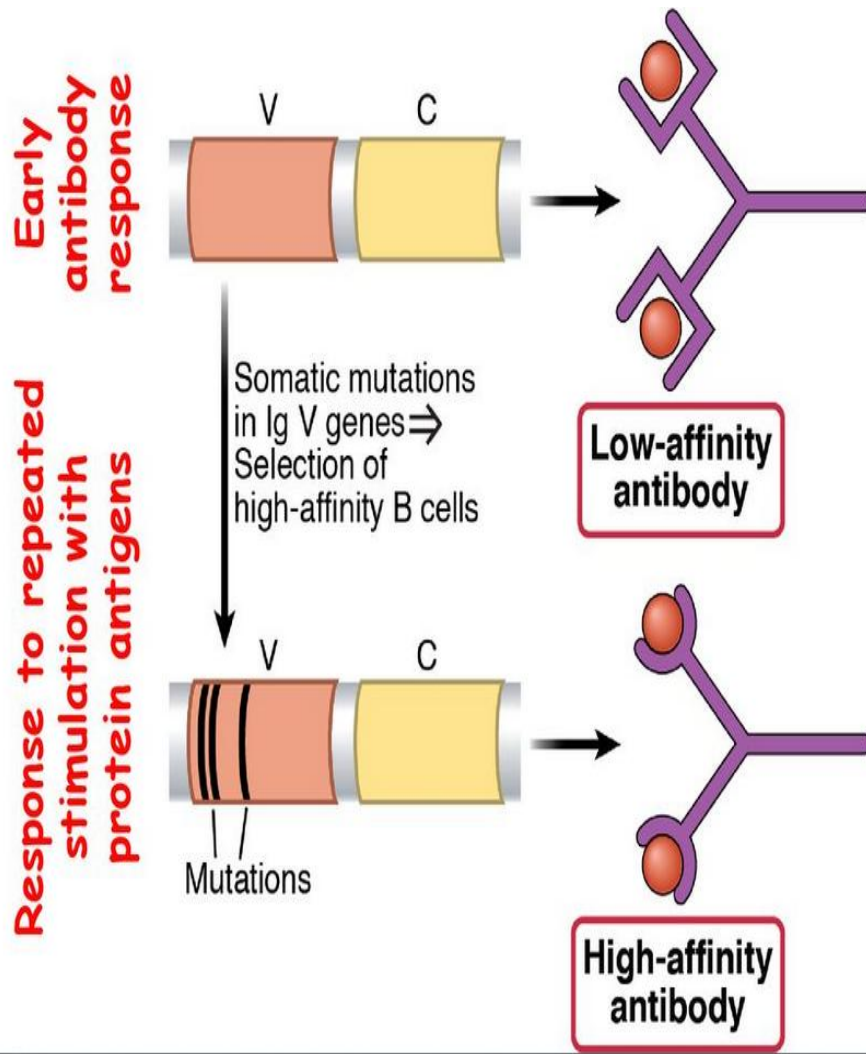
**...is a process by which the affinity of antibodies produced in response to protein antigens increases upon repeated or prolonged exposure to the same antigen.**

**...is therefore crucial for persistent and recurrent infections.**

**...at the core of this process is the selection of somatic point mutations in the genes for CDR regions.**

**Mutations in these regions depend on Th lymphocytes, and the selection of desirable mutations is performed by FDC.**

...



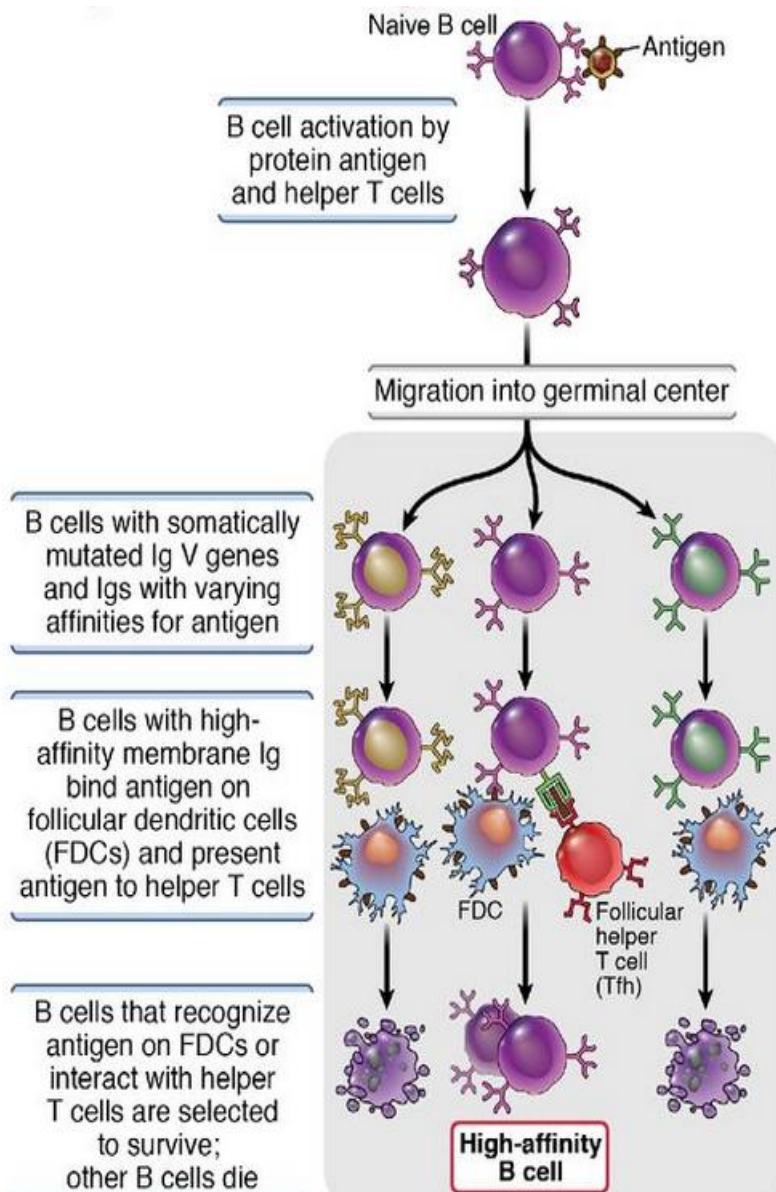
The mentioned enzyme AID, in addition to enabling class switching, also alters nucleotides at the site of the new junction.

The frequency of these mutations is 1000 times higher for these genes than for any other (somatic hypermutations).

This is how B cell clones with receptors of different affinities arise.

B lymphocytes in the germinal center are highly prone to apoptosis. Recognition of the antigen and/or signals from Th cells can protect them from apoptosis.





**Antibodies generated earlier during the response bind to the antigen, activating the complement.**

**Such complexes bind to the Fc receptors and complement receptors on FDC.**

**Only those B lymphocytes whose receptors bind to antigens presented on FDC with high affinity survive.**

**B lymphocytes can also present peptides of protein antigens to Th lymphocytes, which can also enable their survival.**


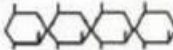
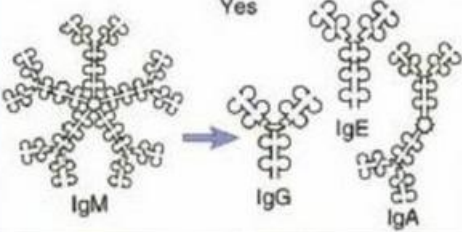
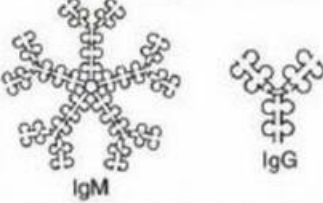
# Humoral response to T-independent antigens:

*We know that...*

*... T lymphocytes do not participate in the response to these antigens because they only recognize peptides.*

*... T-independent antigens include polysaccharides, lipids, lipopolysaccharides, nucleic acids...*

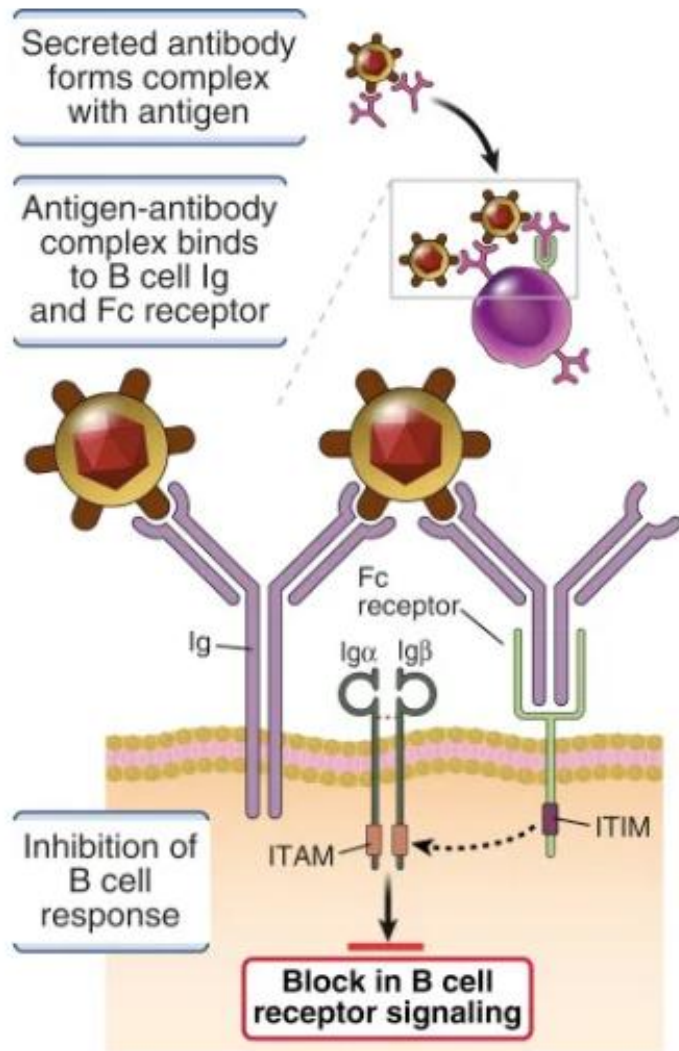
*... Many bacteria possess these antigens in their cell walls, and antibodies against them play a crucial role in defense by facilitating phagocytosis and activating the complement.*

	Thymus-dependent antigen	Thymus-independent antigen
Chemical nature	Proteins 	Polymeric antigens, especially polysaccharides; also glycolipids, nucleic acids 
Features of antibody response		
Isotype switching	Yes 	Little or no: may be some IgG 
Affinity maturation	Yes	No
Secondary response (memory B cells)	Yes	Only seen with some antigens (e.g., polysaccharides)

B lymphocytes in the marginal zone of the spleen are responsible for the humoral immune response to T-independent antigens originating from the blood.

B-1 lymphocytes respond to T-independent antigens in mucous membranes and the peritoneum.

# Regulation of the humoral immune response by antibodies (feedback regulation by antibodies)



**Fc $\gamma$ RIIb (CD32)**

This mechanism is used in the therapy of certain inflammatory diseases by administering intravenous immunoglobulins (IVIG).