DOCTOR 2020 | JU

IMMUNOLOGY

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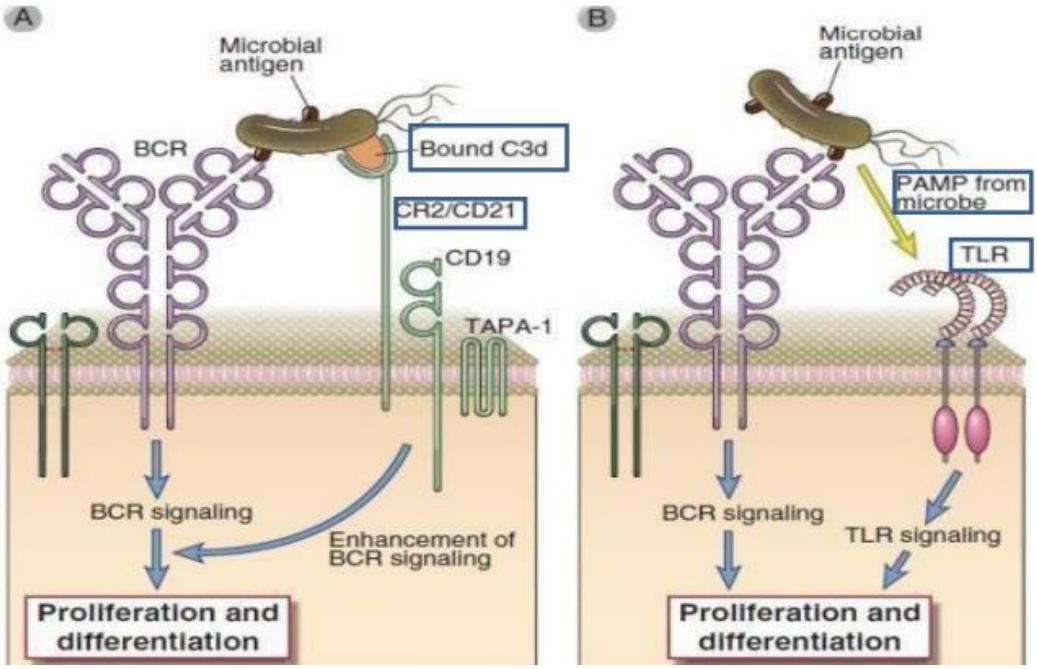
Random past information you should know :

-Naïve B cells after early maturation in the bone marrow are guided with chemokine signals towards the lymph node's follicles by expressing more CXCR5 and less CCR7.
-B cells can be activated through 2 ways, T cell **DEPENDENT** B cell activation and T cell **INDEPENDENT** B cell activation .
T cell INDEPENDENT B cell activation which was the main topic of our last lecture, here is a quick revision :

As the name implies, this mechanism doesn't involve T cells, B cells are activated through recognition of antigens but of certain types like carbohydrates and lipids, because they include repeated epitopes, thus cross linking B cell receptors, and as a result of more receptors activated → B cell activation
Also we mentioned that for full B cell activation complement receptors and proteins have to bind each other, like CR2/CD21 or TLR binding to PAMPS, all of these enhance the activation because BCR activation is not enough for full activation of B cells .

REMEMBER : As we said, this type of activation is mainly weak and will give rise to short lived plasma cells that produce lower affinity antibodies with no class switching (IgM) , the reasons will be obvious for you after we complete the T DEPENDENT B cell activation.



Now let's focus on T cell DEPENDENT B cell activation .
This type of activation is required to get LONG LIVED plasma cells that produce higher

affinity antibodies with different isotypes (with class switching), require recognition and processing of the antigen by B cells, followed by presentation of a linear peptide fragment of the antigen to helper T cells ,leading to cooperation between the antigen specific B and T lymphocytes, but the problem is that the frequency of naive B cells or T cells specific for a given epitope of an antigen is as low as 1 in 10^5 to 1 in 10^6 lymphocytes, and both populations have to be activated and the specific B and T cells have to find each other and physically interact to generate strong antibody responses.

First, an APC captures an antigen in a certain tissue and migrates with it towards the nearest lymph node, then presents it to a helper T cell as a linear peptide on MHC class 2 , **now after that helper T cell is activated**, it **increases** the expression of chemokine receptor CXCR5 and **decreases** CCR7 that will guide it towards the follicle where it meets with the B cell in the edge of the follicle , (as we mentioned in the first page these chemokine receptors are the same type found in naïve B cells guiding them towards the follicle!)
Also, the same antigen has to reach a B cell in the same lymph node, it reaches it in the native form either directly or 1) subcapsular macrophages,
2) resident dendritic cell ,3) follicular dendritic cell .

Now the B cell is activated, it will :

1) Engulf that antigen, processes it and presents it on MHC class 2
2) **Increase** expression of CCR7 and **decrease** CXCR5 thus guiding it outwards of the follicle.

And after that, antigen-activated helper T cells and B cells will meet and make physical contact in (The edge of primary follicle) which is the meeting point for both activated lymphocyte.
Once they meet, we have what we call an activation loop, where the B cell captured antigen presented on MHC class 2 activates T cell further, and that activated helper T cell in response will produce different activating signals for the B cell.
B cell constitutively express CD40
Activated T cell upregulates the ligand for this receptor CD40L
These 2 will bind each other beside the binding of TCR with its antigen on MHC class 2
Immediately, the B cell will start to proliferate just outside the follicle, we call this “extrafollicular focus”
NOTE: This results in short-lived plasma cells generation with early isotype switching only, but why do we need this extrafollicular focus?
-To produce short lived plasma cells → producing antibodies QUICKLY*

so we handle the situation of the current infection as fast as possible .
Of course this is not the final result we are trying to achieve .

This extrafollicular foci of T-dependent B cell activation are generated relatively early. (remember, short lived plasma cells , low affinity AB's, **IgM** and low IgG)

B cell response/ HELPER T CELL–DEPENDENT ANTIBODY RESPONSES TO PROTEIN ANTIGENS

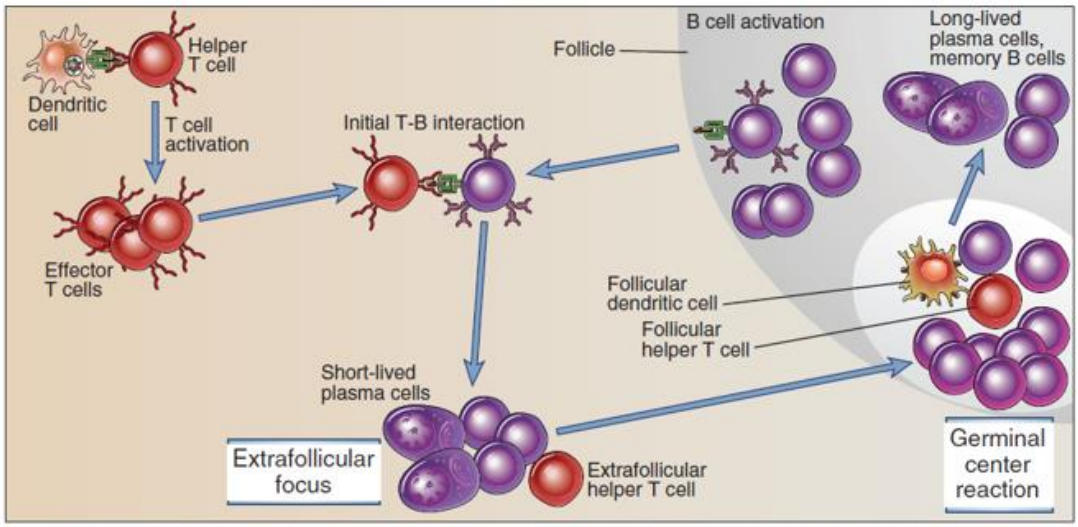


FIGURE 11–7 Sequence of events in humoral immune responses to T cell–dependent protein antigens. Immune responses are initiated by the recognition of antigens by B cells and helper T cells. The activated lymphocytes migrate toward one another and interact, resulting in B cell proliferation and differentiation. Restimulation of B cells by helper T cells in extrafollicular sites leads to early isotype switching and short-lived plasma cell generation. The late events occur in germinal centers and include somatic mutation and the selection of high-affinity cells (affinity maturation), additional isotype switching, memory B cell generation, and the generation of long-lived plasma cells.

Our final result that we want, requires the formation of GERMINAL CENTER reaction, which appears after a few days.
The characteristic events of helper T cell–dependent antibody responses, including **affinity maturation, isotype switching, generation of memory B cells, and long-lived plasma cell differentiation**, occur primarily in the germinal centers of lymphoid follicles, And Each fully formed germinal center contains cells derived from only one or a few antigen-specific B cell clones.

B and T cells will go back to the follicle and form a germinal center there, this germinal center has two regions, Dark zone with proliferating B cells and the other is the Light zone, and its also called the test region where we have follicular dendritic cells and follicular helper T cells (This cell is the same helper T cell but after entering the follicle it's given this name .

B cell response/ HELPER T CELL–DEPENDENT ANTIBODY RESPONSES TO PROTEIN ANTIGENS

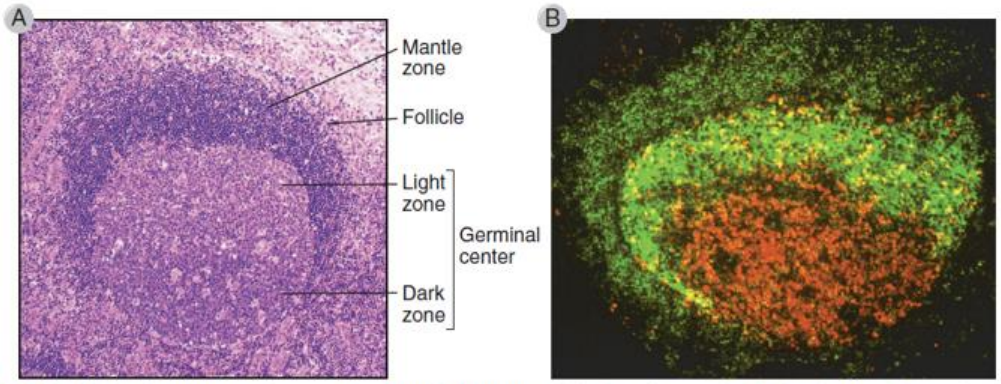
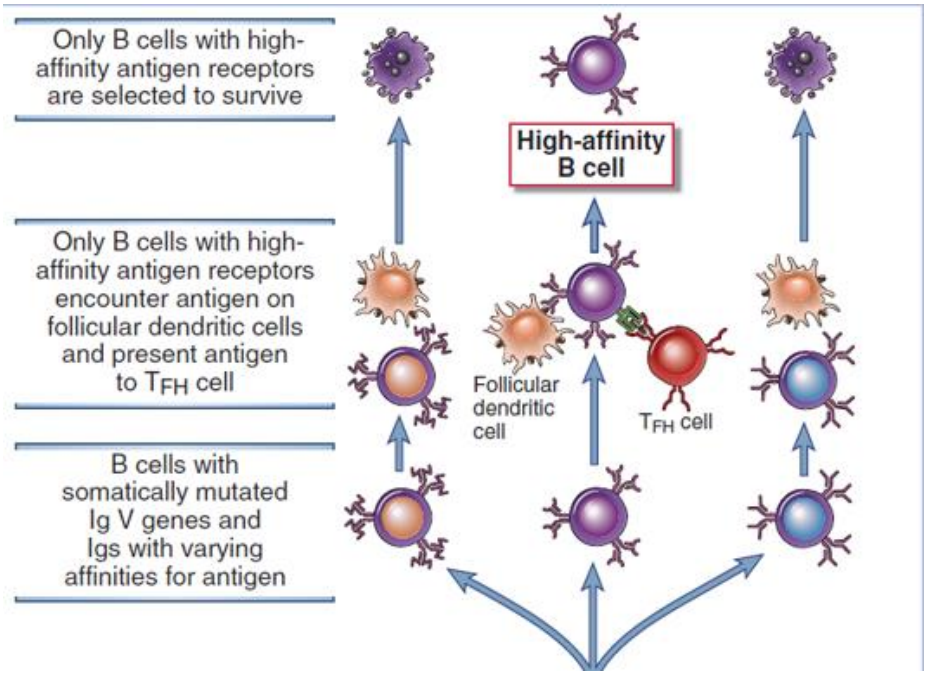


FIGURE 11–11 Germinal centers in secondary lymphoid organs. A, Histology of a secondary follicle with a germinal center in a lymph node. The germinal center is contained within the follicle and includes a basal dark zone and an adjacent light zone. The mantle zone is the parent follicle within which the germinal center has formed. *(Courtesy of Dr. James Gulizia, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.)* **B,** Cellular components of the germinal center. A secondary follicle has been stained with an anti-CD23 antibody (green), which brightly stains follicular dendritic cells in the light zone and dimly stains naive B cells in the mantle zone. Anti-Ki67 (red), which detects cycling cells, stains mitotically active B cell blasts in the dark zone. *(Modified from Liu YJ, GD Johnson, J Gordon, and IC MacLennan. Germinal centres in T-cell–dependent antibody responses. Immunology Today 13:17-21, Copyright 1992, with permission from Elsevier.)*

On the left, we have a histological section of a follicle in a lymph node, the bigger circle is the Follicle which the germinal center was formed inside, the dots inside are the cells, B cells, Follicular helper T cells and follicular dendritic cells. The smaller circle is the germinal center, 2 regions, darker one is the proliferating B cells region, (stains darker because of more cells), the light zone is the other region (test zone) we will talk about it in a second.

On the right, we have an immunofluorescence section for the same region.
RED is the result of using a TAG for an antibody directed against a protein that is responsible for cell cycle progression (proliferating cells have faster cell cycle thus more of this protein = more red appearance .
while the GREEN zone is a marker for follicular dendritic cells, the YELLOW is the overlap between the two, a follicular dendritic cell and next to it a proliferating B cell.
During the proliferation of B cells, they undergo something called “Somatic Hyper Mutation” in which fine-tuning occurs, which is similar to the recombination that B cells underwent to get their unique B cell receptor, but here we are just “fine tuning” the hyper variable regions (CDR’s = complementarity determining regions) which assign the affinity to the epitope.
After a B cell is formed from the proliferating region (Dark region) , it goes to the test region(Light region) .
The Light region has some characteristics that you have to know :
1) Its an environment which has plenty of Proapoptotic signals that should induce cell death unless enough antiapoptotic signals are shown.
2) Its rich in dendritic cells that should have that antigen which the germinal center was formed to attack.
3) IL-21 secreted by TFH cells induces the expression of proteins that induce apoptosis and reduces the expression of proteins that prevent apoptosis
Here, we are testing our fine tuning, to check if it’s successful (produced higher affinity

binding region) or not (produced low affinity binding regions or a region that can't bind the antigen). But how do we test them ?
1) As we said the test zone is rich in pro-apoptotic signals, so the formed B cell would have to bind strongly to its antigen from the plenty of follicular dendritic cells, because this binding ,if you remember from the last sheet, gives anti apoptotic signals thus that B cell with high affinity survives the test zone ☑
2) BUT if the formed B cell binds weakly or doesn't bind it's antigen → no anti-apoptotic signals → cell death by apoptosis in the test zone.



So only high affinity to antigen B cells will give enough anti-apoptotic signals to survive and that whole stage is called "AFFINITY MATURATION".
NOTES on affinity maturation : Only hyper variable regions are mutated, out of every 1000 nucleotide we change only 1 so it's very fine tuning.
Other than this somatic hyper mutations, we have isotype switching, in which we change the heavy region of the antibody to be produced.

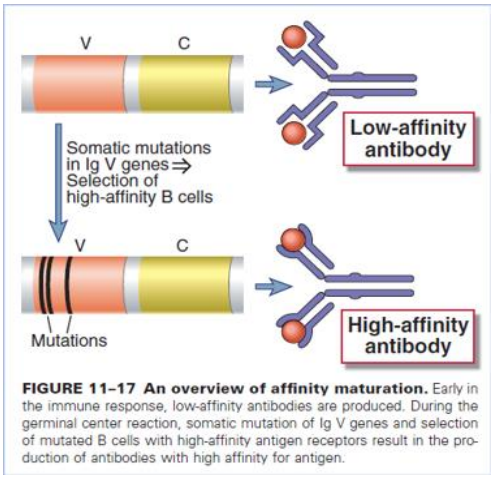


FIGURE 11–17 An overview of affinity maturation. Early in the immune response, low-affinity antibodies are produced. During the germinal center reaction, somatic mutation of Ig V genes and selection of mutated B cells with high-affinity antigen receptors result in the production of antibodies with high affinity for antigen.

Isotype switching is not random and determined by the cytokines secreted by follicular helper T cells and as each class serves a special function, then Each cytokine these follicular helper T cells produce is for a different purpose, so if we have a germinal centered formed near a mucosal tissue then certain cytokines by FTHC will be produced to switch IgM to IgA (dimer ~ aids in mucosal humoral immunity).
If the antigen is from a parasite, by certain mechanisms, Presentation of the antigen by dendritic cells or B cells will lead FTHC to produce IL4 that switch the immunoglobulin to IgE (the isotype against helminths and parasites), and if the antigen is from a virus or a bacteria, then that FTHC will produce Interferon-gamma, that will switch IgM to IgG and IgG3 which are best against viral and bacterial infections.

IgA = forming dimers and mucosal immunity
IgM = pentamers they're good at agglutination
IgG = opsonization
IgE = immunity to helminths +parasites +allergic reactions

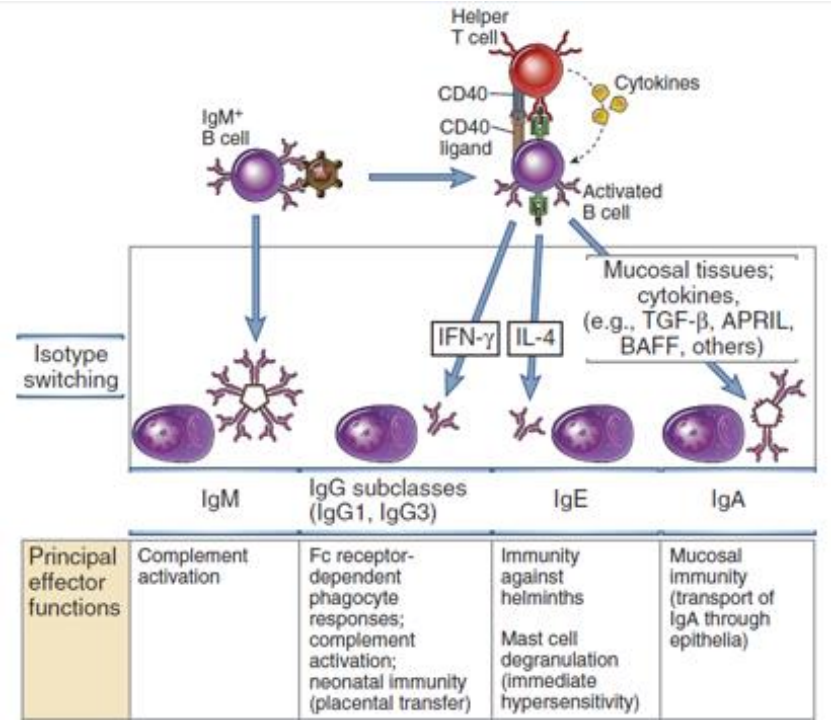
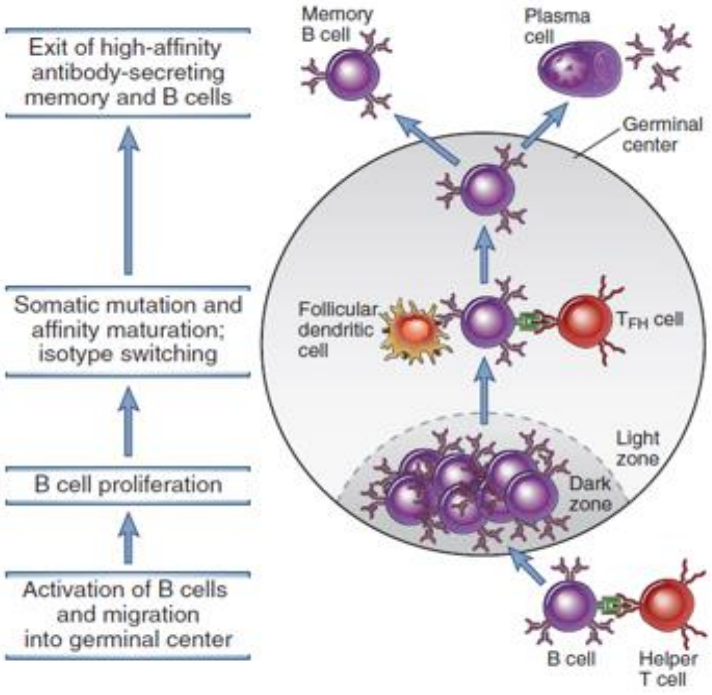


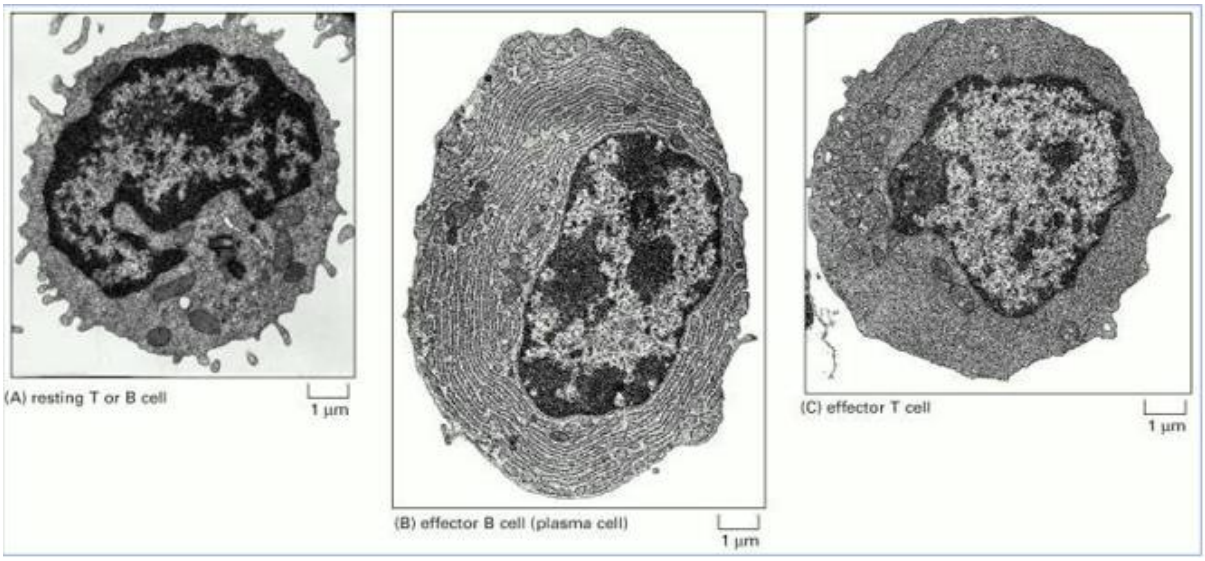
TABLE 12–1 Functions of Antibody Isotypes

Antibody Isotype	Isotype-Specific Effector Functions
IgG	Opsonization of antigens for phagocytosis by macrophages and neutrophils Activation of the classical pathway of complement Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells Neonatal immunity: transfer of maternal antibody across the placenta and gut Feedback inhibition of B cell activation
IgM	Activation of the classical pathway of complement Antigen receptor of naive B lymphocytes*
IgA	Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts Activation of complement by the lectin pathway or by the alternative pathway
IgE	Mast cell degranulation (immediate hypersensitivity reactions)
IgD	Antigen receptor of naive B lymphocytes*

*These functions are mediated by membrane-bound and not secreted antibodies.

Now, survived B cells would either leave the germinal center in the form of :
1) Plasma cells
2) Memory B cells
we don't know what determines which type the B cell decides to go, it might just be random or 50-50 between either type .
First, if we get a plasma cell. They're morphologically distinct, terminally differentiated B cells committed to abundant antibody production.

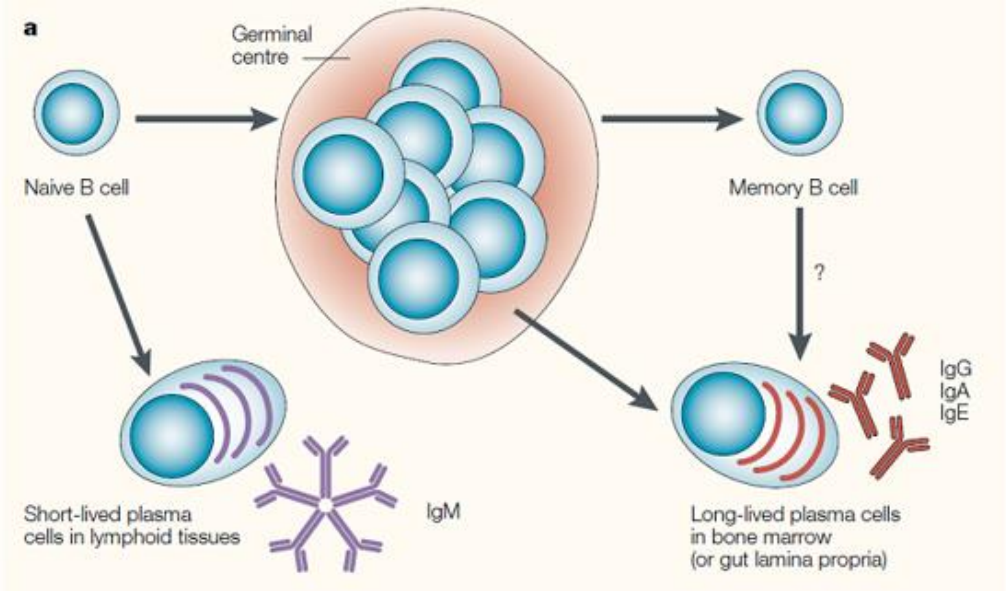




They’re much larger than a resting B cell, they look different compared to activated T cells and resting B cells, they have larger cytoplasm so smaller nucleus/cytoplasm ratio , also, they have larger Endoplasmic Reticulum due to the increasing demand of protein production.
We have 2 types of them , short-lived and long-lived plasma cells, short comes from : 1) multivalent antigens (carbs + lipids (T cell-independent B cell activation)
2) T cell-dependent B cell activation (extrafollicular focus)
The long lived comes from the Germinal centers (T-dependent)
they usually set in the bone marrow and don’t recirculate, and that’s okay because their function is limited to antibody production while being a safe environment from pathogens, also this environment (in bone marrow) helps them be long lived because its

rich in ANTI-apoptotic factors and that helps them survive for longer, they actually survive for years! They might even survive as long as the host does!

So the major site of antibody production is the bone marrow and that is 2-3 weeks after immunization with T cells, and even after not having the antigen the antibody production continues for months or years.
In our serum HALF of the antibodies come from these long lived plasma cells, the other half is either the natural antibodies (B1 B cells / marginal zone B cells) without any exposure to antigens, or antibodies from T cell independent activation / or dependent with extrafollicular foci .
Technically, every antigen should produce long-lived plasma cells, but some antigens just don't and we don't know why until now.

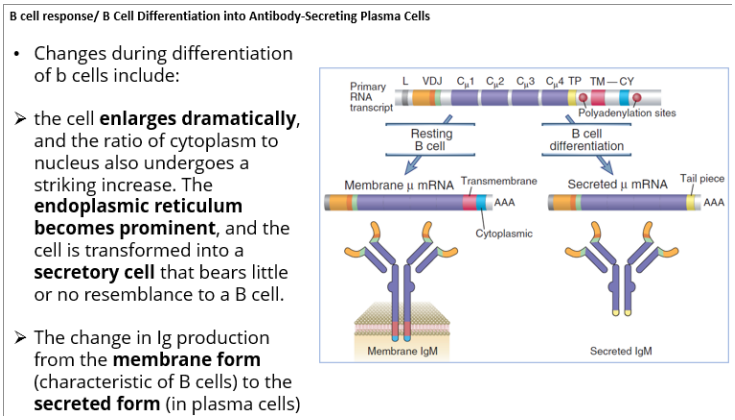


What determines whether the antibody is going to be a receptor or a secreted antibody?!

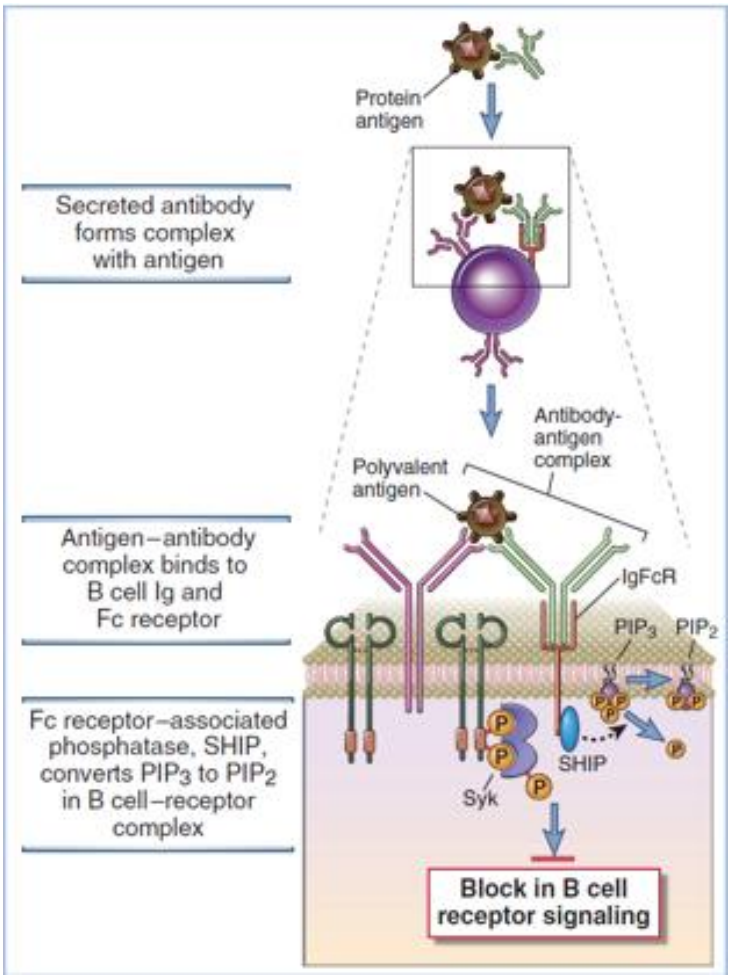
The mRNA sequence of that antibody, in secretory form, we splice a region that helps it to bind membrane (as a receptor) so now it doesn't bind the membrane → secreted Ig . And the resulting antibody will have certain amino acid sequence added that acts as a tag for it .

What if we get a Memory B cell ?!
These cells are special, because we need them for long time, and they actually survive for longer periods due to expression of anti-apoptotic Bcl-2, but they're different than plasma cells in terms of location, whereas plasma cells DON'T need to meet their antigen, memory cells DO need to! So they're located mainly in lymph nodes, and to a

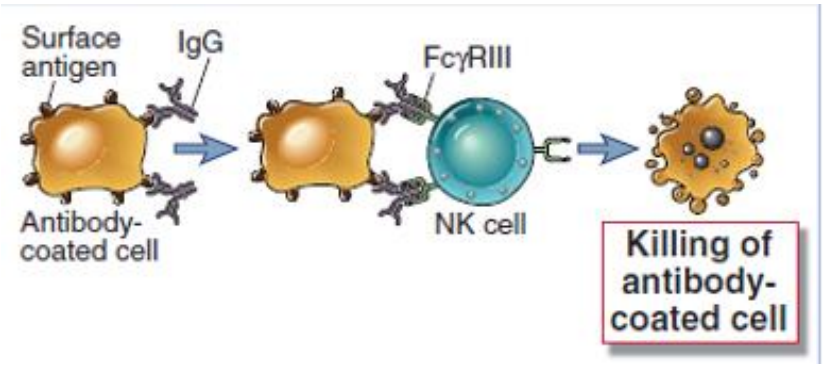
lesser extent circulation, and they keep cycling between the two until they meet their antigen.
The production of large quantities of isotype-switched, high-affinity antibodies is greatly accelerated after secondary exposure to antigens. Because :
After re-encountering the specific antigen they are able to reactivate very quickly, propagate themselves, create plasma cells and re-enter germinal centers to improve affinity of their antibodies.



-Let's talk quickly about antibody receptors (Fc receptors):
Fc-gamma-R2B – feedback inhibition, this type inhibits the antibody production, If we produce too much antibody, we have to inhibit this production and this inhibition occurs when a B cell gets its antigen while its already bound to an antibody (we have enough antibodies for this antigen) so that antibody's Fc portion will bind to this Fc gamma R2B receptor on the B cell and inhibits antibody production from this B cell by inhibition the activation of the B cell.



-Fc gamma receptors, they're found on all phagocytes such as : Macrophages, dendritic cells, B cells, neutrophils , all of these immune cells have mainly Fc gamma. Because gamma antibodies aid in OPSONIZING antigens, all phagocytic immune cells have these receptors to bind the antibody on the antigen thus easier phagocytosis .
-Natural killer cells use Fc gamma R3A , the fc portion of this antibody when its bound to its antigen is an activating signal for them (by increasing cytotoxicity of these NK cells)



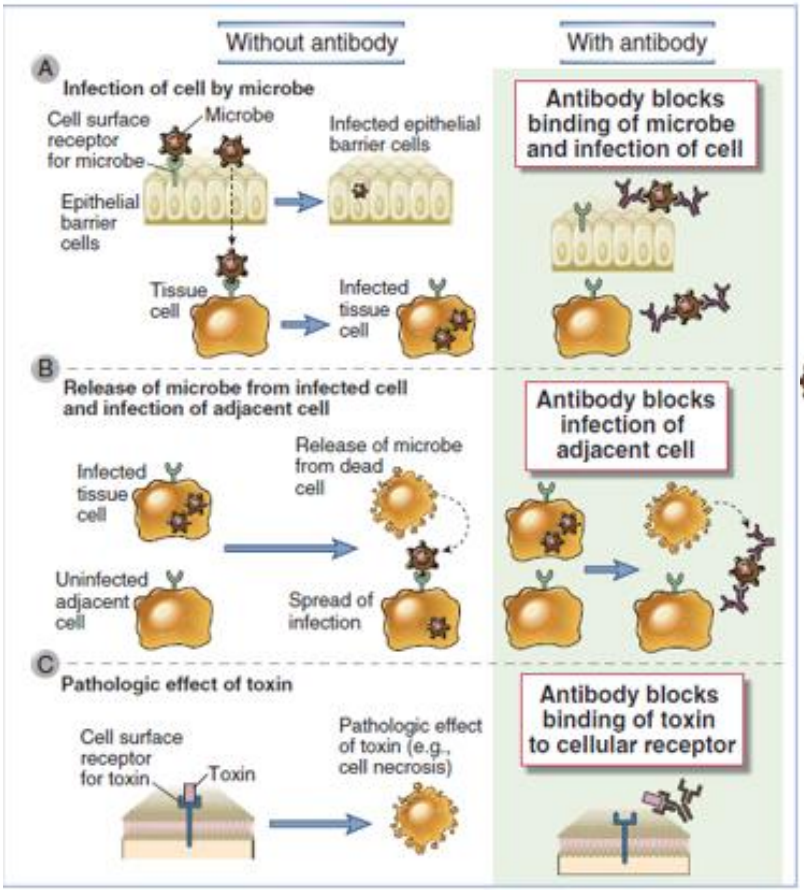
-Mast cells , eosinophils, basophils, have mostly IgE receptors (Fc epsilon receptors)

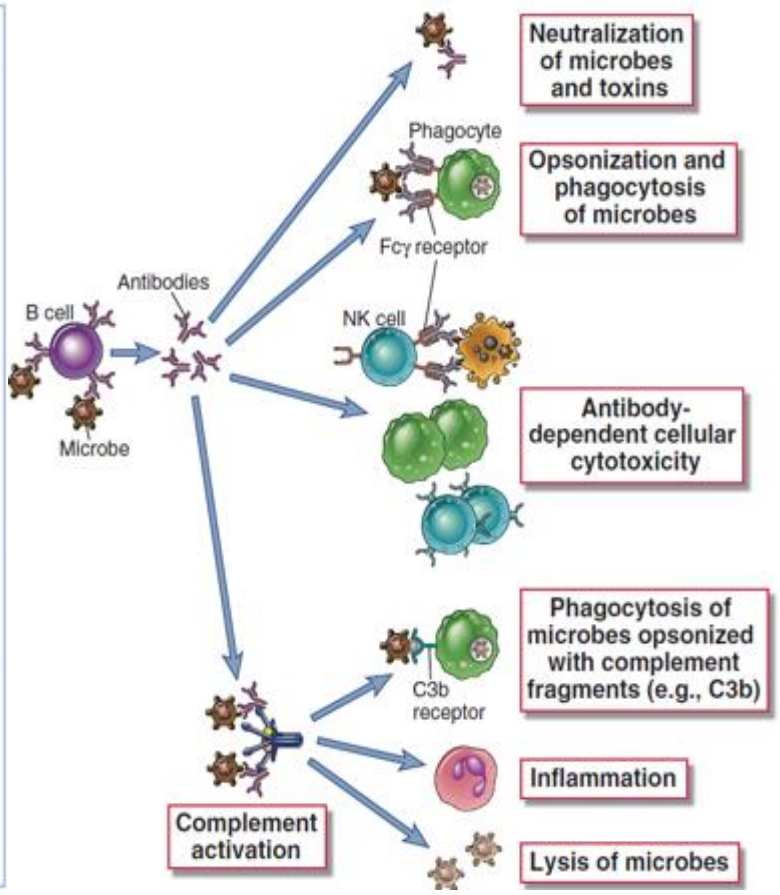
TABLE 12–3 Fc Receptors

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation (inefficient)
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes	Feedback inhibition of B cells
FcγRIIC (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-6}$ M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, glycophosphatidylinositol; NK, natural killer.

XX : not important





Having all of these Fc receptors on immune cells tells you how important is the role of antibodies in immune responses in our bodies .