SHEET NO. 10

IMMUNOLOGY

DOCTOR 2019 | MEDICINE | JU

DONE BY : **Doctor 2018**

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

DOCTOR : **Dr.Anas**

In this lecture we will discuss:

1-B- Cell response

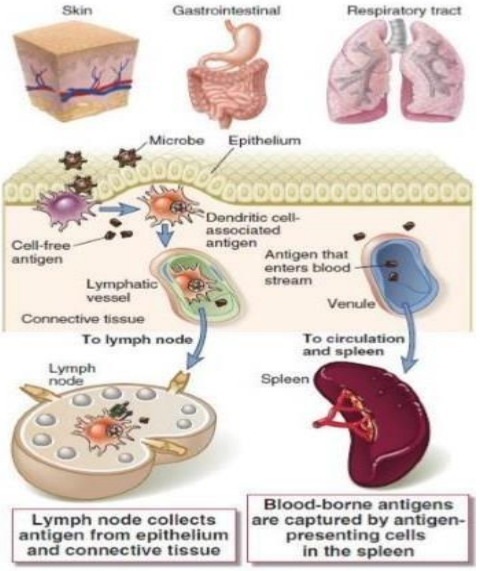
2-Active and passive immunity

B-cell Response to antigens

As we already know, we can acquire Antigens from the skin, GIT, Respiratory tract, and depending on where they enter, they take 2 paths:

1- If they enter the tissues, the Antigens will move through the lymphatic vessels towards the LNs either carried by APCs (like a Dendritic cell) or as free Antigens, it enters through the Afferent vessel of the LN.

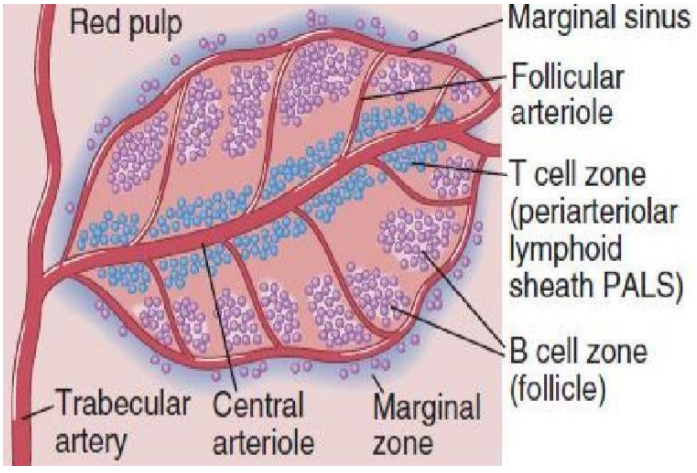
2-Antigens present in blood will go towards the spleen.

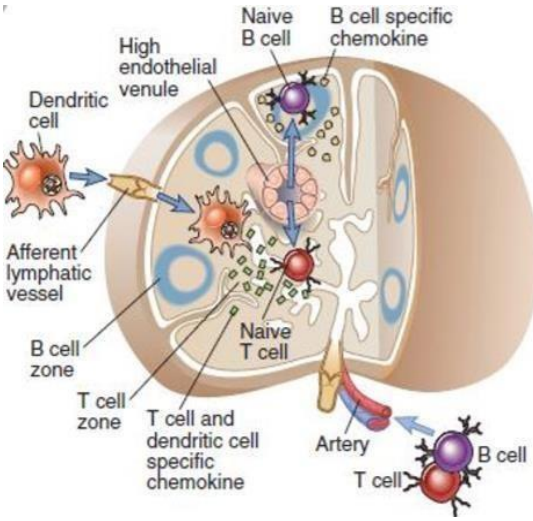


Regarding the LN, as we studied previously, it can be divided into separate compartments and in each compartment resides a specific type of cell, this segregation is due to the secretion of specific chemokines from the lymph node, so for example: Naïve B- cells follow Chemokine 13 (CCL-13), and move towards the follicles, T-cells reside in the paracortex by following other Chemokines.

Lymphocytes enter the LN through the "High endothelial venule" (in the Paracortex region) or Afferent lymph vessels.

And similar to what happens in the LN, in the spleen, Different Lymphocytes reside in different places, so for example the T-cells reside in the PALS, and B-Cells in the follicles and **also in the marginal zone of white pulp**.





Now let's dig deeply into our topic of B- Cell response;

Mature B lymphocytes migrate from one secondary lymphoid organ to the next in search of antigen, while naïve B- Cell sits in the follicle waiting for its Antigen otherwise they die.

Most B cells enter **follicles** guided by the chemokine CXCL13 secreted **by follicular dendritic cells*** and are called follicular B cells or recirculating B cells.

*Follicular dendritic cells are not related and have nothing to do with the dendritic cell, it is a completely different type of cell and its named FDC only because it has similar morphology to dendritic cells.

CXCL13 binds to the CXCR5 chemokine receptor on recirculating naive B cells and attracts these cells into the follicles.

Naive follicular B cells have a specific lifespan and survive for limited periods until they encounter antigen, if they don't meet their Antigen, they die.

Survival of the B- Cells depends on signals from the BCR (B- Cell receptor), so if an Ig on the surface of the B-Cell recognizes its cognate Antigen, it will signal inside the cell prolonging its survival.

Survival of B- Cells also depends on inputs received from a **cytokine** called BAFF (B cell–activating factor of the TNF family, also known as BLyS, for B lymphocyte stimulator), which provides maturation and survival signals through the BAFF receptor.

Before going on with the lecture, there is a key point that must be understood:

Immature B- Cells have Ig-M on their surface, while mature B-Cells have both Ig-M & Ig-D on their surface.

Mature naïve B-Cells are cells that have not yet encountered their cognate Antigen.

Immature B-cells leave the Bone marrow and complete maturation in secondary lymphoid organs, while fully mature T-cells leave the thymus and are naïve until activated by a dendritic cell.

Naïve means that this cell has not yet met its cognate Ag.

Antigen Delivery to B-Cells

(please look at the below picture before& while reading for better understanding)

We have three mechanisms in the LNs:

1- Soluble antigens, generally smaller than 70 kD, may reach the B cell zone through conduits (passage) that extends between the subcapsular sinus and the follicle and interact directly with specific B cells. (B- Cells, unlike T-cells, DON'T NEED MHC and can bind soluble (free) Antigens)

What if the Antigen Is larger than 70kD or is opsonized?

2- Subcapsular sinus macrophages capture large microbes, antigen-antibody complexes and also opsonized antigens (for example by CD3 which binds to the CR2 (complement receptor)) and deliver these to follicles, which lie under the sinus.

3- Medium sized antigens may be captured in the medullary region by **resident dendritic cells** and transported into follicles, where they can activate B cells.

In the spleen, antigens in immune complexes may bind to complement receptors (in particular the complement receptor type 2 or CR2) on marginal zone B cells (spleen), and these cells can transfer the immune complex–containing antigens to follicular B cells.

In all these cases, the Antigen that is presented to B cells is generally in its intact, native conformation **and is not processed by antigen-presenting cells** (this is different than what happens in Antigen presentation to T-cells which requires processing the Antigen into a linear peptide, while here, the Antigen is not altered.)

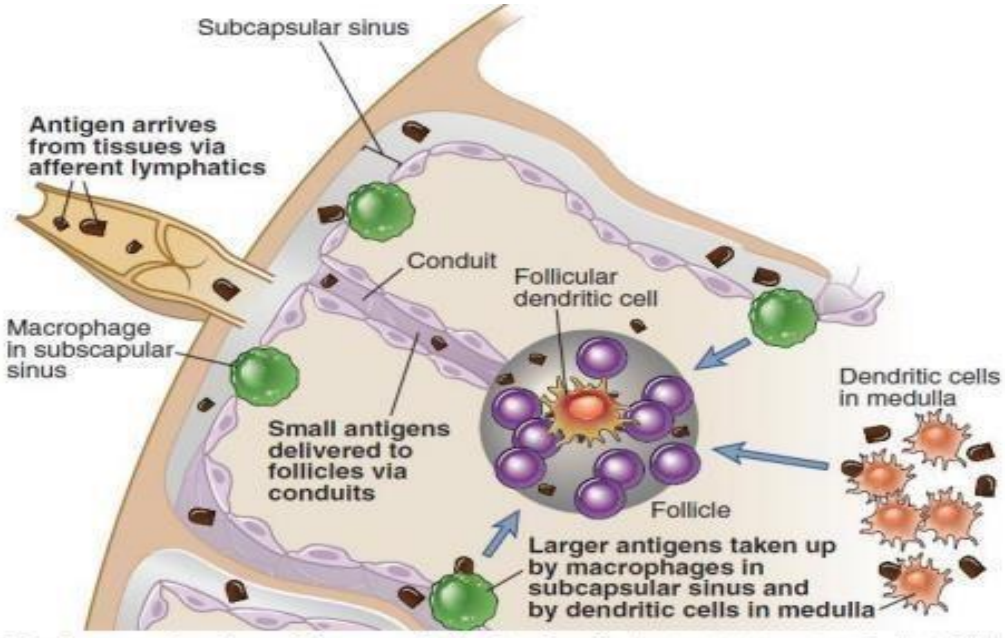


FIGURE 11–4 Pathways of antigen delivery to follicular B cells. Antigen is delivered to B cells in follicles largely through afferent lymphatics that drain into the subcapsular sinus of the lymph node. Small antigens may reach the follicle through conduits. Larger antigens may be captured by subcapsular sinus macrophages and delivered to the follicle, or they may directly access dendritic cells in the medulla that may be involved in delivering antigen not only to the T cell zone but also to B cell–containing follicles.

Activation of B-Cells

Membrane IgM (present as a MONOMER) and IgD, the antigen receptors of naïve B cells, have **short** cytoplasmic tails consisting of only three amino acids (lysine, valine, and lysine), thus, it can't relay the signal of Ag binding to the receptor to the inside of the cell, so it needs help by other molecules, which are Igα and Igβ proteins*- this additional receptor relays the signal through the "Immunoreceptor tyrosine-based activation motif (ITAM)"-.

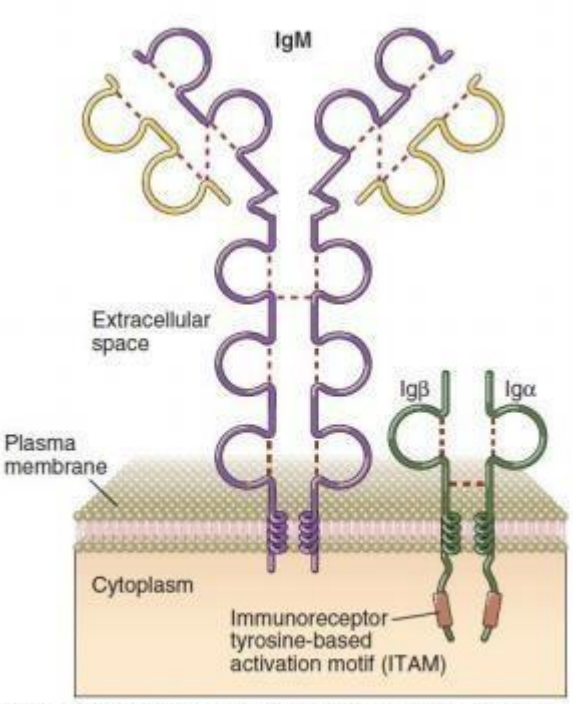


FIGURE 7–18 B cell antigen receptor complex. Membrane IgM (and IgD) on the surface of mature B cells is associated with the invariant Igβ and Igα molecules, which contain ITAMs in their cytoplasmic tails that mediate signaling functions. Note the similarity to the TCR complex.

So basically, the activation of antigen-specific B lymphocytes is initiated by the binding of antigen to membrane Ig molecules, which, in conjunction with the associated Igα and Igβ proteins, make up the antigen receptor complex of mature B cells.

So, when we say BCR complex we mean the Igα and Igβ proteins (which relays the signal) & the Immunoglobulin (which binds the Ag).

*Ig-mediated signals are transduced by two other molecules, called Igα and Igβ, that are disulfide linked to one another and are expressed in B cells noncovalently associated with membrane Ig.

B cell receptor complexes in class-switched B cells, including memory B cells, contain membrane immunoglobulins that may be of the IgG, IgA, or IgE classes.

Binding of antigen to the receptor **delivers biochemical signals to the B cells** that initiate the process of activation. And if the Antigen is a protein, **It also internalizes the bound antigen into endosomal vesicles**, it is processed into peptides that may be presented on the B cell surface for recognition by helper T cells, and if the Antigen is not a protein (Carbs, lipids…) binding to the receptor is enough to send signals to the inside of the cell.

- For full responses to be induced, other stimuli cooperate with BCR engagement, including **complement proteins**, **pattern recognition receptors**, and, in the case of **protein antigens, helper T cells. (study the adjacent sketch**

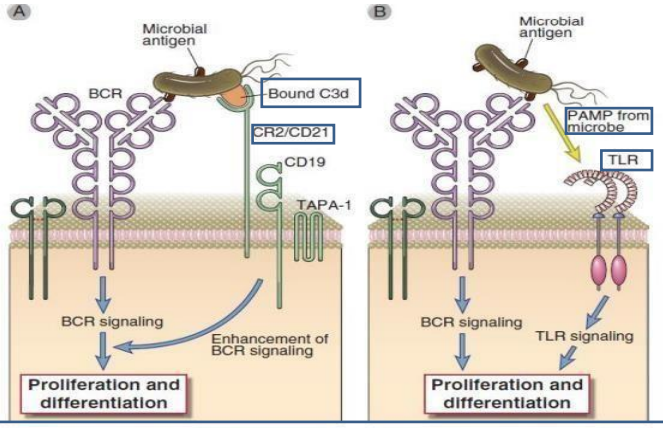


FIGURE 11–5 Role of CR2 and TLRs in B cell activation. In immune responses to microbes, activation of B cells through the BCR may be enhanced by complement-coated antigen that can simultaneously ligate the BCR and complement receptor 2 (CR2) **(A)**, and may also involve the contemporaneous activation of Toll-like receptors (TLRs) on B cells by molecules (so-called pathogen-associated molecular patterns [PAMPs]) derived from the microbe **(B)**.

An illustration of the previous figure (A): "Any antigen whether is a protein or carbohydrate or lipid will bind to the BCR complex and give a signal that usually isn't enough for the activation of the B-cell, so to enhance the BCR signaling we have another system consists of complement receptors CR2/CD21 that recognize another product of C3 fragmentation called C3D that binds to the antigen and this system relays another signal to enhance the BCR-signaling.

(B): Similarly, TLRs can recognize the PAMPs and send more signals to enhance the BCR signaling.

Binding of the Ag to BCR is not enough to activate the B-Cell to the maximum level, so, to enhance the binding and signal relay, other molecules are involved by the above-mentioned method.

The take-home message: signals activating the B-Cell can have different degrees/levels, so a signal can partially activate the B-Cell, meanwhile another stronger signal can fully activate this B-Cell and achieve maximum signaling, for this to happen, you need the help of other molecules mentioned before.

Functional Responses of B Cells to Antigens

Response of B-Cells varies with the nature of the antigen, Most T-independent antigens, such as **polysaccharides**, display **multiple identical epitopes** on each molecule or on a cell surface. Therefore, such multivalent antigens effectively **crosslink many B cell antigen receptors** and initiate responses even though they are not recognized by helper T lymphocytes, so because of cross-linking many BCRs, they produce enough signaling to activate the B-Cell, Unlike the Protein Ags which need T-Cells. بعزي الكثر من اشارة زي بعض

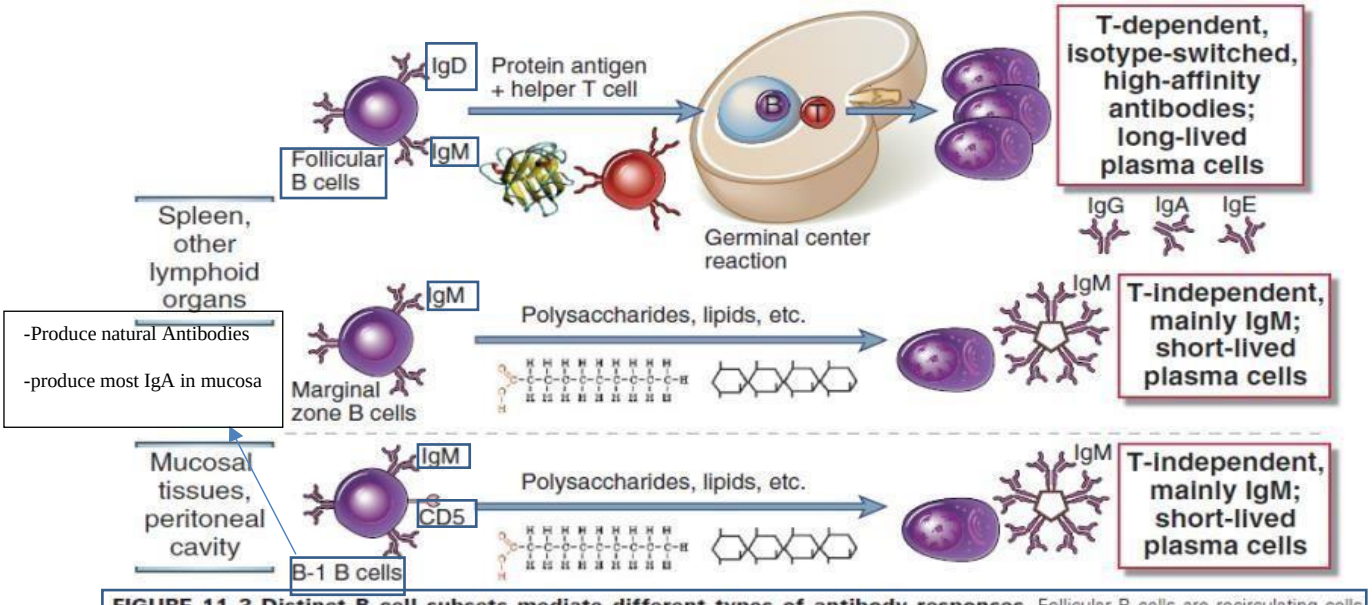


FIGURE 11–3 Distinct B cell subsets mediate different types of antibody responses. Follicular B cells are recirculating cells that receive T cell help when they respond to protein antigens and thus initiate T-dependent antibody responses. These responses can lead to the formation of germinal centers, where class switching and somatic mutation of antibody gene occur, resulting in specialized high-affinity antibody responses. T-independent responses to multivalent antigens such as lipids, polysaccharides, and nucleic acids are mediated mainly by marginal zone B cells in the spleen and B-1 cells in mucosal sites. These functional distinctions between subsets are not absolute.

The types of B-cells are discussed in the next page.

Please pay attention to every part of the above picture.

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B- Cell Subsets:

Follicular B Cells: Most mature B cells belong to the follicular B cell subset and produce IgD in addition to IgM. Follicular B cells are also often called recirculating B cells because they migrate from one lymphoid organ to the next, residing in specialized niches known as B cell follicles.

B-1 B cells, differs from the majority of B lymphocytes and develops in a unique manner. These cells develop from fetal liver–derived HSCs. B-1 cells as well as marginal zone B cells spontaneously secrete IgM antibodies that often react with microbial polysaccharides and lipids. B-1 cells contribute to rapid antibody production against microbes in particular tissues, such as the peritoneum. At mucosal sites, as many as half the IgA-secreting cells in the lamina propria may be derived from B-1 cells.

Marginal zone B cells are located primarily in the vicinity of the marginal sinus in the spleen and are similar to B-1 cells in terms of their limited diversity and their ability to respond to polysaccharide antigens and to generate natural antibodies.

-What happens after the binding of the Antigen to the receptor and the signal is relayed to the inside?

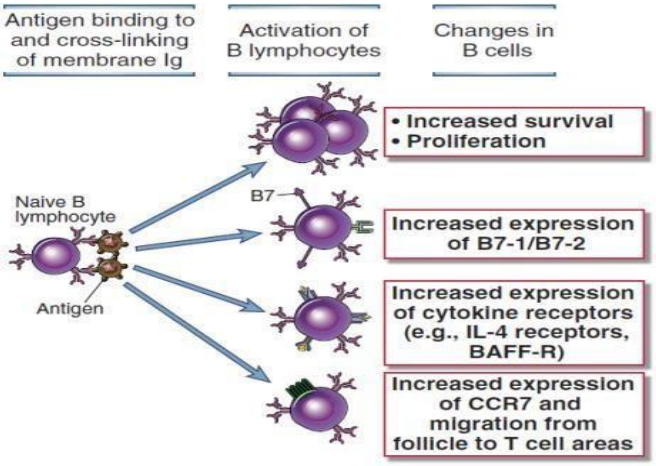
Antigen receptor cross-linking by some antigens can stimulate several important changes in B cells. The previously resting cells enter into the G1 stage of the cell cycle, and this is accompanied by **increases in cell size, cytoplasmic RNA, and biosynthetic organelles such as ribosomes (for the production of many Abs)** . The survival of the stimulated B cells is enhanced as a result of the production of **various antiapoptotic proteins, notably Bcl-2**, and the cells may proliferate and secrete some antibody.

Also, the expression of receptors for several T-cell derived Cytokines is also increased.

• Damage to the Bcl-2 gene has been identified as a cause of a number of cancers, including **chronic lymphocytic leukemia**.

The activation of B cells results in their proliferation, leading to clonal expansion, followed by differentiation, culminating in the generation of memory B cells and antibody-secreting plasma cells.

A single B cell may, within a week, give rise to as many as 5000 antibody secreting cells, which produce more than 10^12 antibody molecules per day!!!!!



*B7-1/B7-2 are ligands for T-Cell receptors.

*CCR7(Chemokine receptor) is usually present on T-Cells to lead them to the follicle of the LNs, but B-Cells upregulate these receptors so that they can follow the T-Cells towards the Follicle.

HELPER T CELL–DEPENDENT ANTIBODY RESPONSES TO PROTEIN ANTIGENS

Antibody responses to protein antigens 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. (Please refer to the video for the following part: (timestamp 30:15)

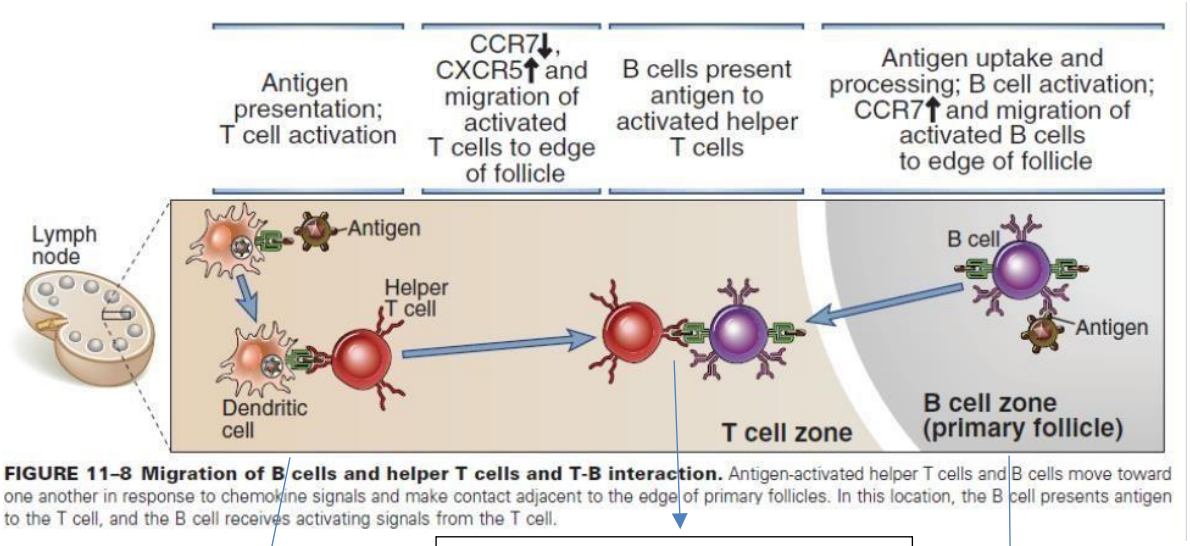


FIGURE 11–8 Migration of B cells and helper T cells and T-B interaction. Antigen-activated helper T cells and B cells move toward one another in response to chemokine signals and make contact adjacent to the edge of primary follicles. In this location, the B cell presents antigen to the T cell, and the B cell receives activating signals from the T cell.

Meet in between the T and B cell zones

APCs take the antigen present it to T-helper cells and co-stimulation is induced to proliferate, express CD40L (it's a ligand, it binds to CD40 on B-Cells), and secrete cytokines. They also **downregulate** the chemokine receptor CCR7 and **increase** the expression of CXCR5 and as a result leave the T cell zone and migrate toward the follicle. CXCL13, the ligand for CXCR5, is secreted by **follicular dendritic cells** and other follicular stromal cells, and it contributes to the migration of activated CD4+ T cells toward the follicle.

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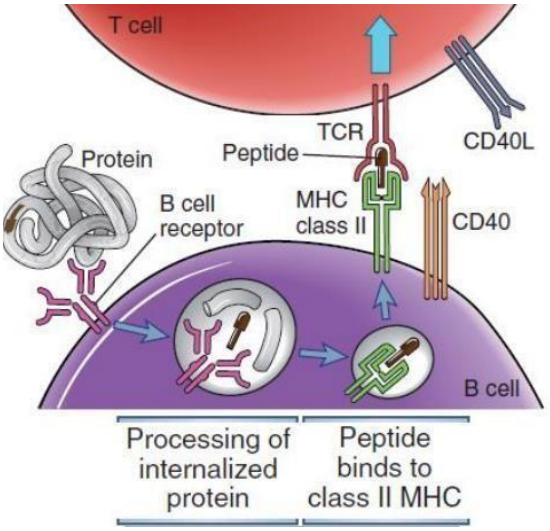
In the follicles, the B- cells bind to the antigen in its native form and then the cells get activated and the BCR engagement by these antigens results in **reduced** cell surface expression of the chemokine receptor CXCR5 and **increased** expression of CCR7 -back to the previous page-, which is normally expressed on T cells. As a result, activated B cells migrate toward the T cell zone drawn by a gradient of **CCL19** and **CCL21**, the ligands for CCR7.

Finally, they will meet in between the B and T cell zones.

Remember that when the B-Cell binds the Ag, it starts expressing Cytokine receptors to prepare itself for the cytokines that will come from T-helper, so, when the B-Cell meets the T-cell, it will ready for the Cytokines secreted by that T-Cell.

- Antigen is taken up by dendritic cells that have also been activated by microbial products and presented to naive helper T cells in the T cell zones of lymphoid organs.
- Helper T cells are initially activated by the dendritic cells presenting antigenic peptides on class II MHC molecules and also expressing costimulatory ligands such as the B7 molecules (see Chapters 6 and 9).
- Activated helper T cells express CD40L and also chemokine receptors that promote their migration toward the follicle following a chemokine gradient.
- B cells in the lymphoid follicles are activated by antigen, which may be in soluble form or displayed by other cells.
- B cells process and present the antigen, alter their cell surface chemokine receptor profile, and migrate toward the T cell zone.
- Activated helper T cells and B cells interact at the boundary of the T cell zone and follicle, where the B cells are activated by CD40L on the helper T cells and by cytokines that the T cells secrete.
- Small extrafollicular B cell foci form in the medulla of the lymph node or between the periarteriolar lymphoid sheath and the red pulp of the spleen. B cells in these foci undergo low levels of isotype switching and somatic mutation and generate short-lived plasma cells that secrete antibodies.
- Some activated helper T cells are induced during B:T interactions to differentiate into T follicular helper cells (T_{FH} cells).
- Activated B cells and T_{FH} cells migrate into the follicle, where the B cells are activated by T_{FH} cells. Germinal centers form within the follicles and are the sites of extensive B cell proliferation, isotype switching, somatic mutation, selection events that lead to affinity maturation, memory B cell generation, and induction of long-lived plasma cells that migrate to the bone marrow.

This was taken from the slides; it summarizes what was previously mentioned.



First, the protein in its native form will activate the B-cell, and it will be internalized and processed, then presented to T-Helper cell by MHC II, this T-Helper cell has already been activated by the same Ag through a dendritic cell that is why it has migrated towards the follicle.

• A protein antigen that elicits a T-dependent B cell response therefore makes use of at least two epitopes when activating specific B cells. A **surface epitope on the native protein is recognized with high specificity by a B cell**, and an **internal linear peptide epitope** is subsequently released from the protein, binds class II MHC molecules, and is **recognized by helper T cells**.

Do you think that the antibodies produced by the B-Cells are specific to Native form Ag, or the internal linear peptide epitope?

The antibodies that are subsequently secreted are usually **specific for conformational determinants of the native antigen** because that is the protein that was recognized by its original BCR .

The doctor said that more regarding this subject will be explained in the next lecture.

This brings us to the **Hapten-carrier effect**.

Haptens are small chemicals that can be bound by specific antibodies but are not very immunogenic by themselves, haptens are usually sugars or lipids (lack proteins), that is probably why they are not very immunogenic; as proteins are recognized, internalized, processed and presented to T-helper cells mounting a strong immune response (Check P.5).

Keep in mind that T-independent immune response is weak, produces short lived plasma cells and lacks isotype switching (only produces IgM), while T-dependent immune response produces the opposite.

If, however, haptens are coupled to proteins, which serve as carriers, the conjugates are able to induce antibody responses against the hapten-carrier molecule.

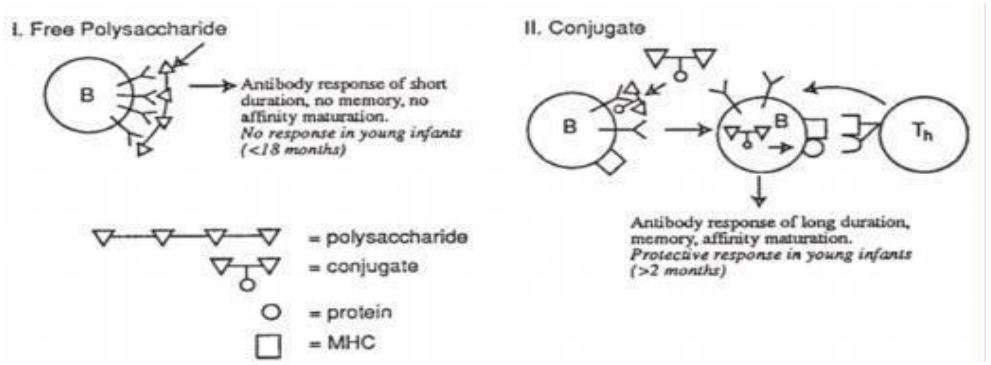
What is the significance of this topic?

-This can be used in the production of conjugate vaccines. A conjugate vaccine consists of a polysaccharide antigen (Hapten) that is conjugated to a carrier molecule.

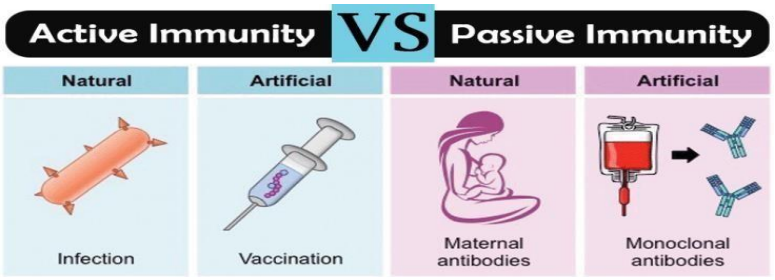
Clinical correlation: Streptococcus pneumoniae causes severe disease especially in children, one of the most important Virulence factors of this Bactria is its capsule, so unencapsulated strains are sometimes avirulent, so scientist tried making a vaccine by isolating sugars from the capsule of this bacteria, and after injecting it in the body, it

didn't elicit an immune response because it didn't not involve T-helper cells, so they then took a sugar molecule and conjugated it to a carrier (a protein from the bacteria), why is that?

So, when the B-cell recognizes the Hapten-carrier molecule, it will induce an antibody response against the hapten-carrier molecule and that will almost resemble what will happen during infection, so if infection occurs, antibodies will inhibit the pathogenies of the bacteria not only bind to its capsule.



Active vs Passive Immunity



Acquired immunity is attained through either passive or active immunization

Active immunity refers to the process of exposing the body to an antigen to generate an adaptive immune response: the response takes days/weeks to develop but may be long lasting—even lifelong (unlike passive immunity).

It can occur:

1) **Naturally** through infection with a certain pathogen.

2) **Artificially** through administration of vaccines containing weekend or inactive pathogen.

Examples: **Natural**: Wild infection with hepatitis A virus (HAV) and subsequent recovery gives rise to an active immune response usually leading to lifelong protection.

Artificial: In a similar manner, administration of two doses of hepatitis A vaccine generates an acquired active immune response leading to long-lasting (possibly lifelong) protection.

Passive immunization refers to the transfer of "ready- made" antibodies, from one individual to another.

It can occur:

1) **naturally** by transplacental transfer of maternal antibodies to the developing fetus (**IgG**), or through colostrum and breast milk rich in **IgA**.

2) it can be induced **artificially** by injecting a recipient with exogenous antibodies targeted to a specific pathogen or toxin. • Examples:

Natural: Maternal antibodies protect against some diseases such as measles, rubella, and tetanus for the first few months of life.

Artificial: Pooled human immunoglobulins used intravenously (IVIG) can be used prophylactically in the case of immunodeficiency diseases, or specific antibodies used in the treatment of several types of acute infections such as rabie

Please watch last 2 minutes of the lecture, I didn't feel it was important so I didn't include it here…