Immunology 2017: Lecture 12 handout

Secondary lymphoid organs

Dr H Awad

INTRODUCTION

So far we discussed the cells of the immune system and how they recognize their antigens and get stimulated. The number of these cells is small compared to the body surface. So how can this small number guard the whole body? How can a B cell find the Th needed for its activation? Where can the dendritic cell find the correct T cell that can recognize the antigen it is presenting? This is what we will discuss in this handout.

The presence of secondary lymphoid organs gives these cells a chance to meet. **Secondary lymphoid organs are the lymph nodes, the spleen and mucosa associated lymphoid tissue (MALT).**

Note that primary lymphoid organs are the bone marrow and thymus.

Before describing the secondary lymphoid organs and their functions, we will start by talking about two histological features common to all the secondary lymphoid organs : lymphoid follicles (found in all these organs) and high endothelial venules (in all organs except spleen)

LYMPHOID FOLLICLES

All secondary lymphoid organs share one histological feature: They all contain lymphoid follicles, which are critical for the functioning of the adaptive immune system

Lymphoid follicles start as "**primary" lymphoid follicles**: loose networks of follicular dendritic cells (FDCs) embedded in regions of the secondary lymphoid organs that are rich in B cells.
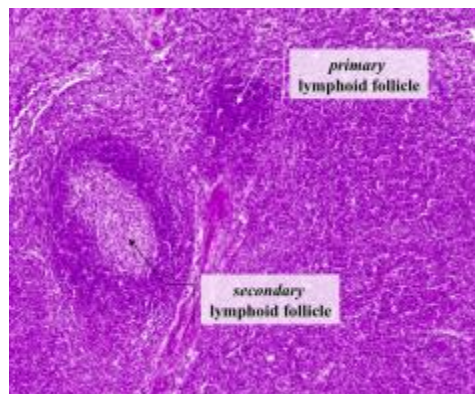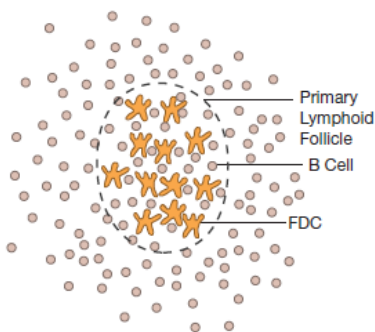
PLEASE NOTE: Follicular dendritic cells (FDC) are not the same as antigen presenting dendritic cells!

FDCs are white blood cells produced in the bone marrow. They then migrate to their positions in tissues and take up their final positions in the secondary lymphoid organs as the embryo develops (already are in place during the second trimester of gestation).

The function of follicular dendritic cells is to display antigen to B cells. (note: they display the antigen which means they make it easier to be seen by the B cells, they don't present the antigen because B cells do not need Ag presentation. This means B cells can directly recognize the antigen but FDC put the antigen in an accessible location)

Early in an infection, complement proteins opsonize the pathogen, and some of this complement-opsonized antigen will be delivered by the lymph or blood to the secondary lymphoid organs. Follicular dendritic cells that reside in these organs have receptors on their surface which bind complement fragments, and as a result, **follicular dendritic cells pick up and retain complement-opsonized antigen**. In this way, follicular dendritic cells become coated with antigens that are derived from the battle being waged out in the tissues. Moreover, **by capturing large numbers of antigens and by holding them close together, FDCs display antigens in a way that can crosslink B cell receptors**.

Later during the battle, when antibodies have been produced, **invaders opsonized by antibodies also can be retained on the surface of follicular dendritic cells – because FDCs have receptors that can bind to the constant region of antibody molecules**. OK! Do you remember that IgG can opsonize antigens??
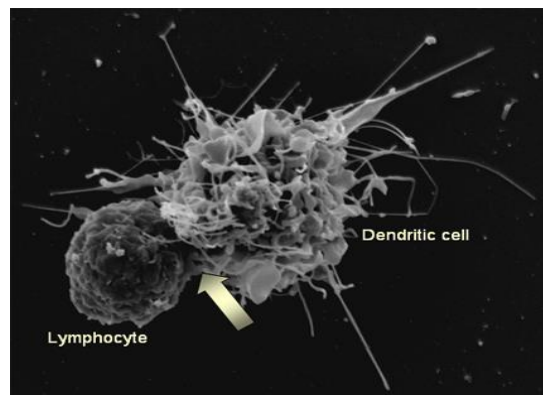

So follicular dendritic cells capture opsonized antigens and "advertise" these antigens to B cells in a configuration that can help activate them. Those B cells whose receptors are crosslinked by their cognate antigens coating the follicular dendritic cells proliferate to increase their numbers. And once this happens, the **follicle begins to grow and become a center of B cell development. Such an active lymphoid follicle is called a "secondary lymphoid follicle" or a germinal center.**

From the above description you can appreciate the importance of complement in triggering the development of a germinal center. **Lymphoid follicles in humans who have a defective complement system never progress past the primary stage**. This is an example of how adaptive immune system relies on the innate system to be able to function.

As B cells proliferate in germinal centers, they receive the activation signals from helper T cells and continues to proliferate. The rate at which B cells multiply in a germinal center is truly amazing: The number of B cells can double every 6 hours! These proliferating B cells push aside other B cells that have not been activated, and establish a region of the germinal center called the "dark zone" – because it contains so many proliferating B cells that it looks dark under a microscope. If B cells do not receive this costimulation, they will die by apoptosis (remember we said in the last lecture that B cells cannot function without Th and that's why people with T cell defect will actually have "combined" B and T cell defects… refer to handout 11 if you have already forgotten!)

After this period of proliferation, some of the B cells become plasma B cells and leave the germinal center. Others undergo somatic hypermutation to increase their affinity to the cognate antigen. Sometime during all this action, probably in the dark zone, B cells can switch the class of antibody they produce.
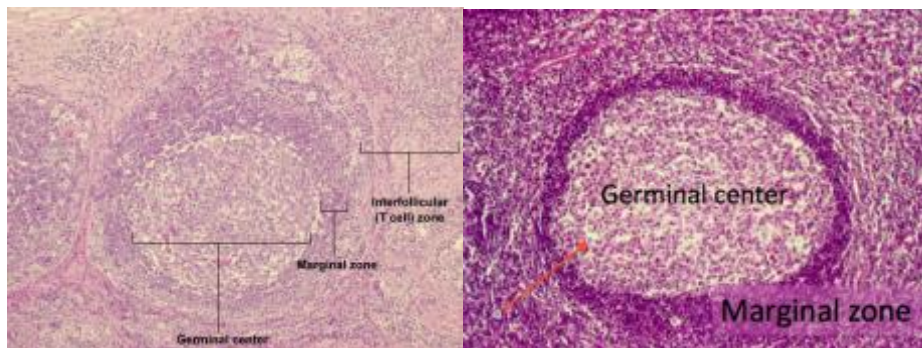

HIGH ENDOTHELIAL VENULES

A second feature common to all secondary lymphoid organs except the spleen is the high endothelial venule (HEV). Most endothelial cells flat cells that are tightly "glued" to the cells adjacent to them to prevent the loss of blood cells into the tissues. In contrast, within most secondary lymphoid organs, the small blood vessels that collect blood from the postcapillary venules **are lined with special endothelial cells that are columnar. These tall columnar cells are the high endothelial cells.**

So, high endothelial venule is **a special region in a small blood vessel (venule) where there are high endothelial cells**. Instead of being glued together, high endothelial cells have enough space between them for lymphocytes to enter. About 10 000 lymphocytes exit the blood and enter an average lymph node each second by passing between high endothelial cells.
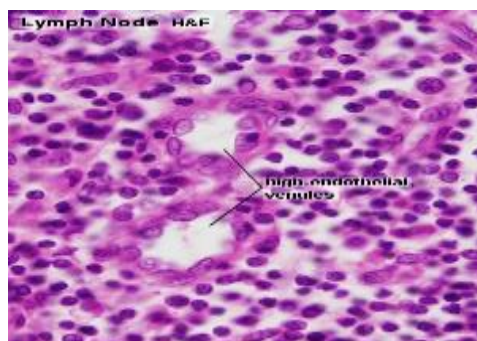
LYMPH NODES

A lymph node is bean-shaped organ which has incoming lymphatics that bring lymph into the node, and outgoing lymphatics through which lymph exits. In addition, there are arterioles that carry the blood that nourishes the cells of the lymph node, and veins through which this blood leaves the node.
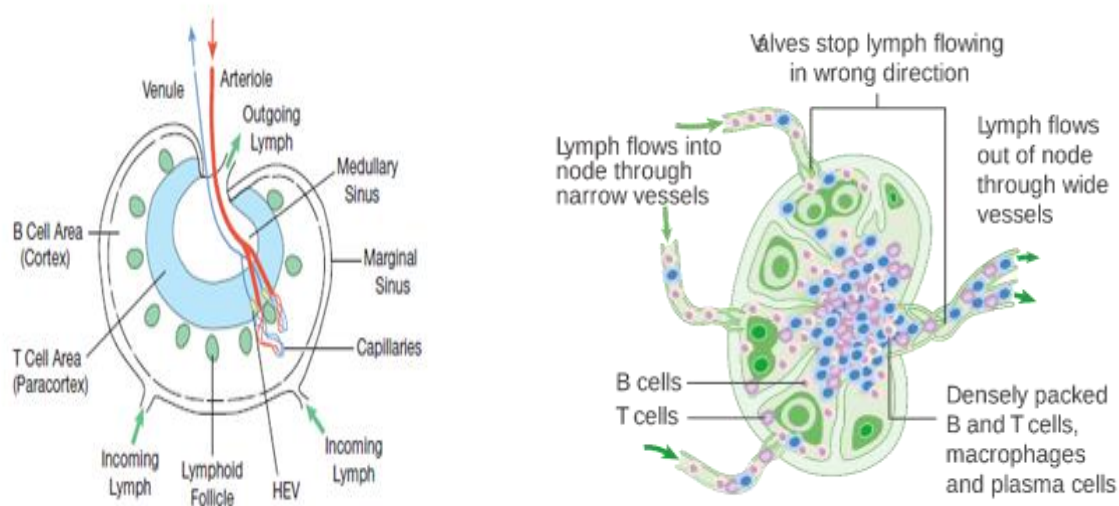
Lymphocytes can enter the lymph node via the lymph or the blood but they only exit via the lymph. Lymph nodes are places where lymphocytes find their cognate antigen, so how can antigens enter lymph nodes? When dendritic cells recognize antigens, they leave the tissues via the lymph, and carry the antigen into the secondary lymphoid organs. In addition, antigen which has been opsonized, either by complement or by antibodies, can be carried by the lymph into the node.

When lymph enters a node, it percolates through holes in the marginal sinus through the cortex and paracortex, and finally into the medullary sinus – from where it exits the node via the outgoing lymphatic vessels.

The walls of the marginal sinus are lined with macrophages which capture pathogens as they enter a lymph node. This substantially reduces the number of invaders that the adaptive immune system will need to deal with. So one of the functions of a lymph node is as a "**lymph filter.**"

The high endothelial venules are located in the paracortex, so B and T cells pass through this region of the node when they arrive from the blood. **T cells tend to accumulate in the paracortex**, being retained there by adhesion molecules. This accumulation of T cells makes sense, **because dendritic cells also are found in the paracortex** (so T cells can meet the APC they need!)

On the other hand, **B cells entering a lymph node accumulate in the cortex, the area where lymphoid follicles are located.** This localization of B cells works well, because the follicular dendritic cells that display opsonized antigen to B cells are located in this region of the lymph node.

So each cell has a specific place in the lymph node: T in paracortex, B in cortex, macrophages in sinuses. This localization is mediated by chemokines.
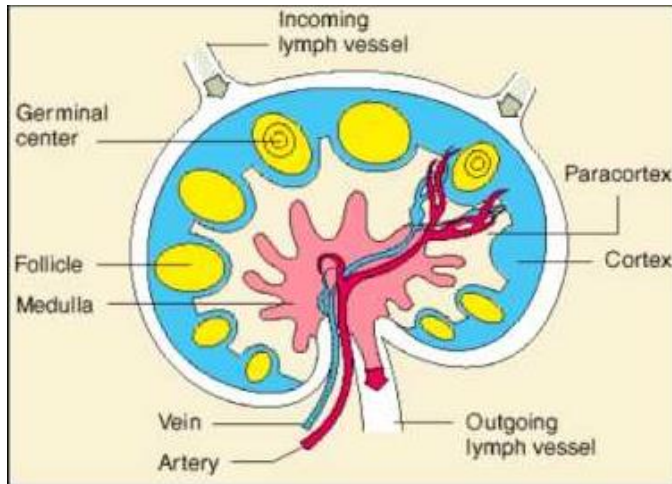
CHEMOKINES

Follicular dendritic cells in a lymph node produce a chemokine called CXCL13. Naive B cells which enter the node express receptors for this chemokine, and are attracted to the area of the node where FDCs are displaying opsonized antigen. If a B cell finds its cognate antigen advertised there, it downregulates expression of the receptors for CXCL13, and upregulates expression of another chemokine receptor, CCR7. This receptor detects a chemokine produced by cells in the region of the lymph node where activated Th cells and B cells meet – the border between the B and T cell areas. Consequently, once a B cell has found its antigen, it is attracted by this chemokine to the correct location to receive help from activated Th cells.

Meanwhile, activated Th cells downregulate expression of the chemokine receptors that have been retaining them in the T cell areas. At the same time, they upregulate expression of CXCR5 chemokine receptors, which cause them to be attracted to the border of the follicle – where antigen-activated B cells are waiting for their help.

So the movement of immune system cells through a lymph node is orchestrated by the up- and downregulation of chemokine receptors, and the localized production of chemokines that can be detected by these receptors

INTERACTION BETWEEN B AND T CELLS IN THE LYMPH NODE

Th and B cells need to interact and this happens within the lymph nodes. When B cells recognize Ag they dislocated it inside the cell and process it to present it to Th via MHCII (we already discussed this in the lecture of Ag presentation). This allows Th to recognize the invader which B cell is fighting. **Note that the Ag the B and T recognize are different, although they came from the same pathogen.**. B recognizes a certain epitope specific to BCR.. But T recognizes a processed Ag that fits MHC molecule.

During this interaction between B and T in the lymph node, Th stimulates B via CD40-CD40l interaction (which results in affinity maturation and class switching). Note that also B cells help Th to fully mature. This involves cell–cell contact during which B7 proteins and proteins called ICOSL on the B cell surface bind to CD28 and ICOS proteins, respectively, on the Th cell surface.

This means that at the border of the lymphoid follicle, an activated Th cell and an activated B cell perform a bidirectional activation important for their maturation. Th cells provide the CD40L that B cells need. And B cells provide the B7 and ICOSL that helper T cells require for their full maturation. Such fully mature Th cells are called follicular helper T cells (Tfh). These Tfh cells are now able to help these B cells switch classes or undergo somatic hypermutation (affinity maturation).
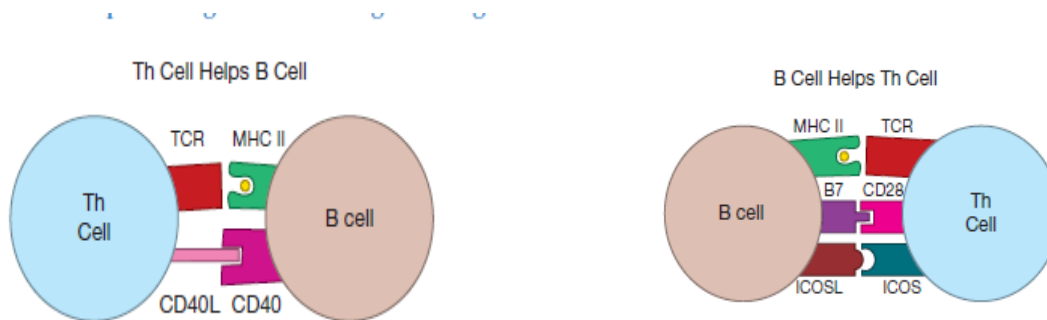

RECIRCULATION THROUGH LYMPH NODES

When a T cell enters a lymph node, it checks the dendritic cells, trying to find one which is presenting its cognate antigen. If a T cell is not successful in this search, it leaves the node and continues to circulate through the lymph and blood. If a helper T cell does encounter a dendritic cell presenting its cognate antigen in the paracortex, the Th cell will be activated and will begin to proliferate. This proliferation phase lasts a few days while the T cell is retained in the lymph node by adhesion molecules. Expanded population of T cells then leaves theT cell zone. Most newly activated Th cells exit the node via the lymph, recirculate through the blood, **and re-enter** lymph nodes via high endothelial venules. This process of *recirculation* is fast – it generally takes about a day – and it is extremely important. We need four major ingredients which must be "mixed" before the adaptive immune system can produce antibodies: **APCs to present antigen to Th cells, Th cells with receptors that recognize the presented antigen, opsonized antigen displayed by follicular dendritic cells, and B cells with receptors that recognize the antigen.** Early in an infection, there are very few of these ingredients around, and naive B and T cells just circulate through the secondary lymphoid organs at random, checking for a match to their receptors. So the probability is small that the rare Th cell which recognizes a particular antigen will arrive at the very same lymph node that is being visited by the rare B cell with specificity for that same antigen. However, **when activated Th cells first proliferate to build up their numbers, and then recirculate to lots of lymph nodes and other secondary lymphoid organs, the Th cells have a much better chance of encountering those rare B cells which require their help.**

B cells and CTL have similar circulation within the lymph node.

MALT

Mucosa associated lymphoid tissue is composed of lympoid aggregates (lymph nodules) under mucosal surfaces.Peyer's patches are examples of MALT which function as secondary lymphoid organs. Peyer's patches begin to develop before birth, and an adult human has about 200 of them.
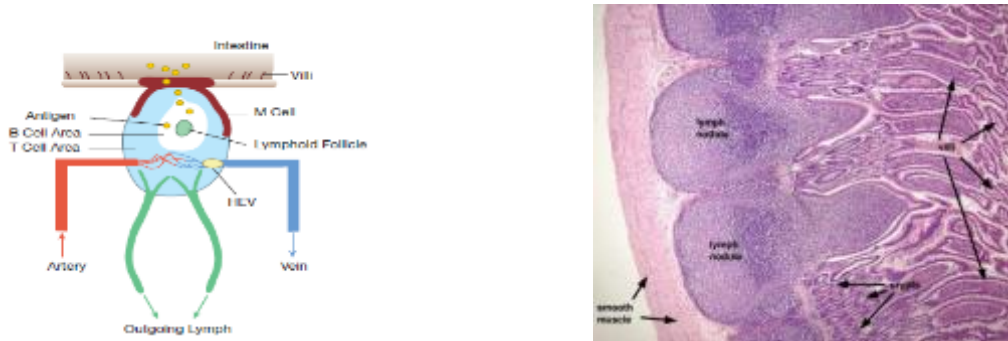
Peyer's patches have high endothelial venules through which lymphocytes can enter from the blood, and outgoing lymphatics that drain lymph away from these tissues. However, there are **no incoming lymphatics** . So the Ag can not enter throhg lymphatics because they are not available. Antigens enter throgh cells within the epithelium called **M cells**.. see figures below .

These M cells are not coated with mucus, so they are easily accessible to microorganisms that inhabit the intestine. They are "sampling" cells which specialize in **transporting antigen from the interior (lumen) of the small intestine into the tissues beneath the M cell**. To accomplish this, M cells enclose intestinal antigens in vesicles (endosomes). These endosomes are then transported through the M cell, and their contents into the tissues that surround the small intestine. Except for its unusual method of acquiring antigen, a Peyer's patch is quite similar to a lymph node.

Recently it was discovered that M cells are **selective** about the antigens they transport, they transport antigens that can bind to molecules on the surface of the M cell.
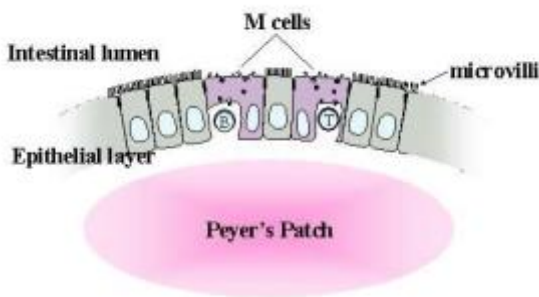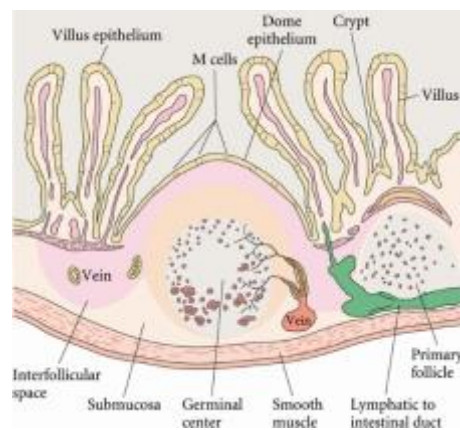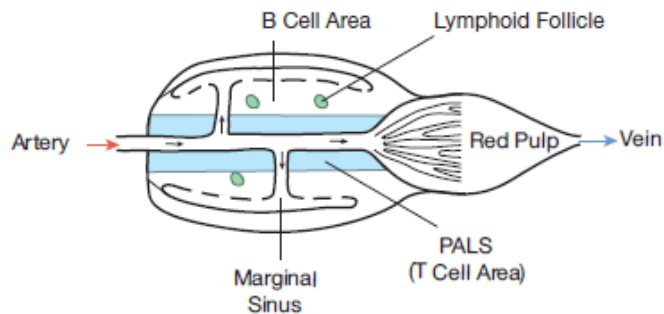

Figure. Follicle-associated epithelia (FAE) and M cells

SPLEEN

spleen is located between an artery and a vein, and it functions as a blood filter. Each time the heartpumps, about 5% of its output goes through the spleen. As with Peyer's patches, there are no lymphatics that bring lymph into the spleen. There are no high endothelial vesels in the spleen.

When blood enters from the splenic artery, it is diverted out to the marginal sinuses from which it percolates through the spleen before it is collected into the splenic vein. The marginal sinuses are lined with macrophages that clean up the blood by phagocytosing cell debris and foreign invaders. As they ride along with the blood, naive B cells and T cells are temporarily retained in different areas – T cells in a region called the periarteriolar lymphocyte sheath (PALS) that surrounds the central arteriole, and B cells in the region between the PALS and the marginal sinuses.

Of course, since the spleen has no lymphatics to transport dendritic cells from the tissues, you might ask, "Where do the antigen presenting cells in the spleen come from?" The answer is that the marginal sinuses, where the blood first enters the spleen, is home to "resident" dendritic cells. These cells take up antigens from invaders in the blood and use them to prepare a class II MHC display. Resident dendritic cells also can be infected by pathogens in the blood, and can use their class I MHC molecules to display these antigens.

LYMPHOCYTE TRAFFICKING

about 500 billion lymphocytes circulate each day through the various secondary lymphoid organs. However, these cells don't just wander around. They follow a well-defined traffic pattern which maximizes their chances of encountering an invader.

Importantly, the traffic patterns of virgin and experienced lymphocytes are different. virgin T cells express **a mixture of cellular adhesion molecules on** their surface. These adhesion molecules direct T cells to the secondary lymphoid organs.

For example, virgin T cells have a molecule **called L-selectin on their surface that can bind to its adhesion partner, GlyCAM-1, which is found on the high endothelial venules of lymph nodes.** Virgin T cells also express **an integrin molecule, α4β7,** whose **adhesion partner, MadCAM-1, is found on the high endothelial venules of Peyer's patches and the lymph nodes that drain the tissues around the intestines (the mesenteric lymph nodes).**

Equipped with these adhesion molecules, inexperienced T cells circulate through all of the secondary lymphoid organs. There these T cells check the several hundred dendritic cells. If they do not see their

cognate antigens, they re-enter the blood either via the lymph or directly (in the case of the spleen), and continue to recirculate. Naive T cells make this loop about once a day, spending only about 30 minutes in the blood on each circuit. A naive T cell can continue doing this circulation thing for quite some time, but after about six weeks, if the T cell has not encountered its cognate antigen presented by an MHC molecule, it will die by apoptosis. In contrast, those T cells that do find their antigen are activated in the secondary lymphoid organs.

Experienced T cells also have adhesion molecules, but they are limited because, during activation, expression of certain adhesion molecules on the T cell surface is increased, whereas expression of others is decreased. This modulation of cellular adhesion molecule expression is not random, the cellular adhesion molecules that activated T cells express depend on where these T cells were activated. In this way, T cells are imprinted with a memory of where they came from.Thus, when activated T cells recirculate, they usually exit the blood and re-enter the same type of secondary lymphoid organ in which they originally encountered antigen.

T cells also have adhesion molecules to help them exit the blood at sites of infection.

B cell trafficking is roughly similar to T cell trafficking.

شُكراً جَزيلاً