MVST BOD & NST Part 1B **Tues. 25th & Wed. 26th Oct. 2011**
Pathology Practical Class 5

LYMPHOID SYSTEM: CELLS OF THE ADAPTIVE IMMUNE SYSTEM

AIMS

1. To develop an understanding of the locations and arrangements of lymphoid tissue around the body and to relate this to the function of the individual cells within the adaptive immune system.
2. To recognise the individual cell types found within lymphoid tissue.
3. To explore the development and maturation of B and T lymphocytes.

ANATOMY OF THE LYMPHOID SYSTEM

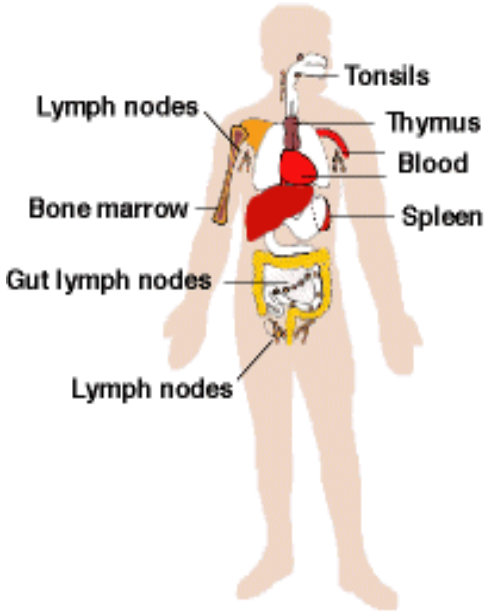
LYMPHOID TISSUE normally occurs as **lymph nodes,** as nodules in the **spleen** and as focal collections along **mucosal** surfaces, such as the upper respiratory and intestinal tracts, where they are called **mucosal associated lymphoid tissue (MALT)**. **ANTIGENS** enter **lymph nodes** via the afferent **lymph**. **ANTIGENS** reach the **SPLEEN** from the **blood** and they reach **MALT** from the **adjacent mucosal surface**. Most **LYMPHOCYTES** enter lymph nodes, MALT or spleen **from the circulating blood**.

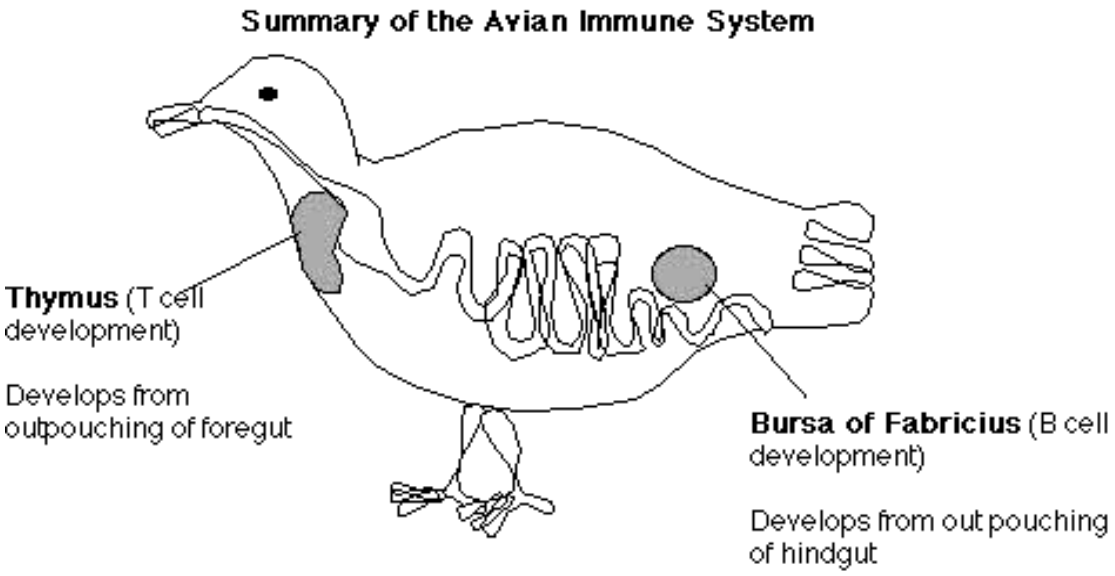
Lymphocytes originate from the bone-marrow but they can proliferate after leaving it. Different populations home to particular sites in the body.

In **mammals**, **B lymphocytes** undergo some maturation in the **bone marrow,** but in birds maturation occurs in a specialised diverticulum of the cloaca known as the **Bursa of Fabricius**.

The **THYMUS** is important for the development and maturation of **T lymphocytes.**

Please refer to the diagrams showing the anatomy of the human and avian immune system below. You are not required to memorise these. They are included for reference.





As in mammals, a **spleen**, **liver** and **lymph nodes** are present. Similarly, all blood cells develop initially in the **bone marrow**, which is the site of the stem cells which produce them.

ENTRY OF ANTIGEN TO THE LYMPHOID SYSTEM

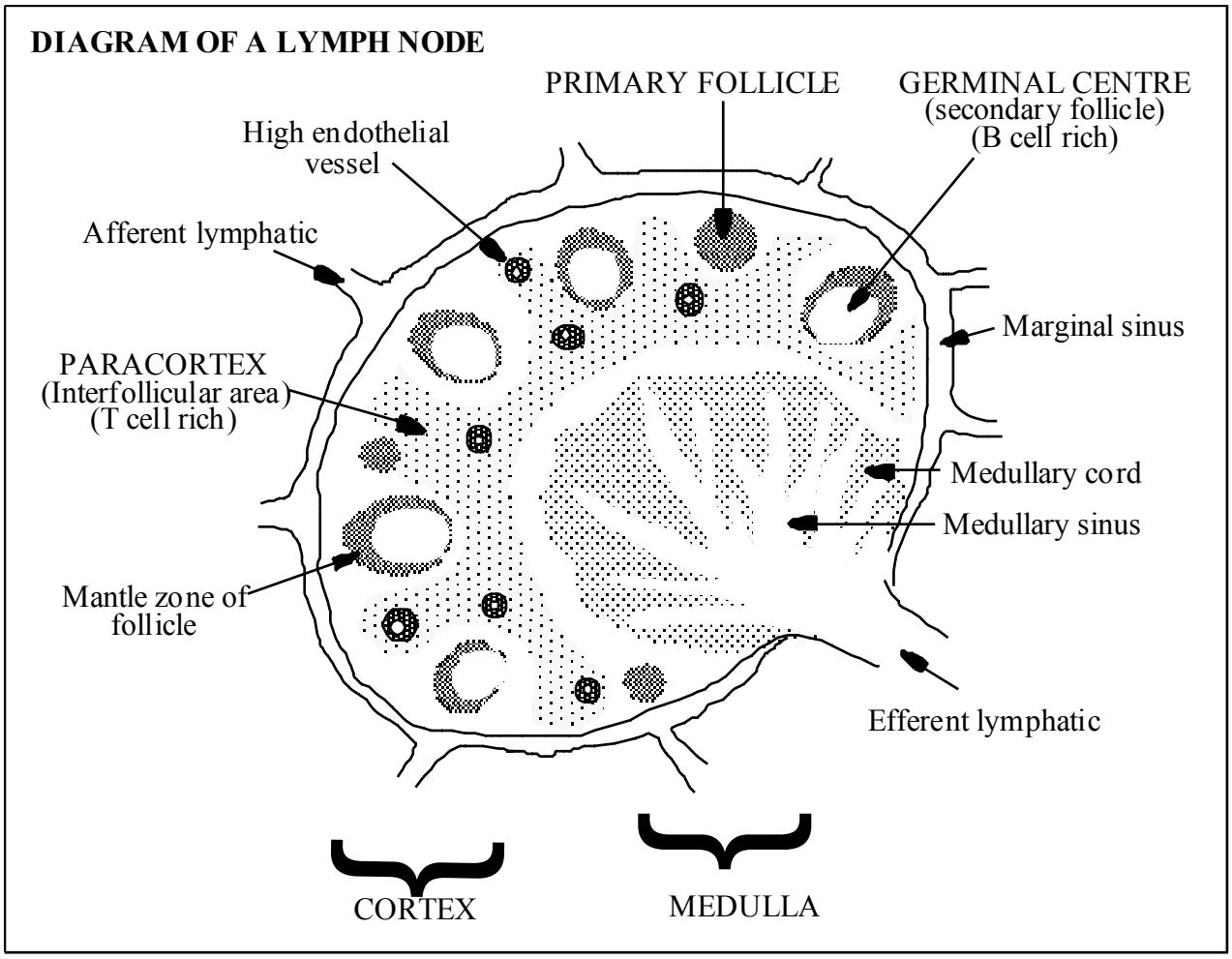
The distribution of lymphoid tissue described above ensures rapid contact between **lymphocytes** and their **accessory cells** with potentially harmful agents. Lymphoid tissue is not static; normally abundant in early life, it decreases with old age. In response to stimulation it can **re-form** and may develop at most sites.

Antigens can be transported free, or phagocytosed and carried by **macrophages,** or minute quantities can be endocytosed by specialized **antigen presenting cells** (APCs). These antigen-carrying cells use the lymph flow to enter nodes. Within lymph nodes they become closely associated with **T lymphocytes**, thus promoting antigen presentation and recognition.

Lymphocytes mainly enter lymph nodes from the blood by means of specialized venules known as **high endothelial vessels,** but they occasionally enter via the afferent lymph. High endothelial vessels are lined by enlarged endothelial cells with an increased level of activation. The cells express cell adhesion molecules.

Afferent lymph arrives from nodes upstream, as well as from the tissues. Lymph carries lymphocytes, albeit a smaller number than those that enter via high endothelial vessels, and also carries free antigens. Most importantly, however, it carries dendritic cells (a type of leucocyte specialized for antigen presentation). Cells destined to recirculate exit via the medulla and efferent lymphatic vessel and return to the bloodstream.

HISTOLOGY OF THE LYMPHOID SYSTEM



General structure of lymph nodes: Lymph nodes can be regarded as having a 'cortex' (outer part) and a 'medulla' (inner part). B lymphocytes **aggregate around** follicular dendritic cells to form distinct **FOLLICLES**, which are found in the **cortex**. Within the follicles, some T cells are also present.

Germinal Centre Formation: If any of the B cells within the follicles are activated by antigen in an appropriate environment which includes T cell help, they enlarge and proliferate. The antigen is recognized by their immunoglobulin receptors (i.e. cell surface antibody). Many of the B cells undergo **somatic hypermutation**. Eventually, plasma cells (and also some memory cells) are produced. When this B cell activation occurs, the centre of the follicle appears paler and is now called a **germinal centre**. Here, enlarged B lymphocytes show considerable mitotic activity. B lymphocytes which are *not* selected undergo cell death by apoptosis, and macrophages phagocytose their remnants.

Follicular dendritic cells: The follicular dendritic cells (FDCs) hold **antigen/antibody complexes** distributed along their dendritic (branching) processes. Antigen in this form can only be accessed by B cells with highly specific immunoglobulin receptors on their cell surfaces. Increased affinity for antigen is achieved by the process of hypermutation; appropriate contact with the FDCs' complementary receptors and stimulation by T cells all contribute to **selection** of B cells with increased affinity for antigen. (N.B. You are not able to see the follicular dendritic cells in H&E sections.)

B lymphocytes: Besides the B lymphocytes (described above) found in follicles, small B lymphocytes surround the germinal centres as a dark rim which is referred to as a '**mantle**'. These B-cells are described as "resting" and are not thought to be taking part in the current response. During a humoral (antibody) response, the B lymphocytes become **plasma cells**

(plasma cells may occasionally be seen in the medulla of the node, but the medullary cords and sinuses are very difficult to identify in the sections). Their antibodies pass into the efferent lymph and reach the bloodstream. Plasma cells themselves do not circulate in significant numbers and most die after a few days, but memory cells do circulate and a significant number migrate to the bone marrow, where longer lived plasma cells develop. B lymphocytes can also circulate in the blood to reach a site of infection and mature into plasma cells at that site.

T lymphocytes: A small but important number of T lymphocytes (T helpers) are found in the B cell follicles. On the whole, however, T cells predominate in the **interfollicular areas**, sometimes called the **paracortex**. This region is where the antigen presenting cells settle and become interdigitating cells.

LYMPH NODES

(Remember to look first with the naked eye)

You are provided with some sections of normal lymph node stained with H&E, and also immunostained with several different antibodies. In order to immunostain a tissue section, the section is first incubated with an antibody (usually from a mouse) against the protein of interest. Then, this antibody is detected by a second antibody (e.g., a goat antibody raised against mouse antibodies). Thirdly, an enzyme can be coupled to this second antibody. This enzyme, in turn, converts a substrate into visible brown insoluble crystals. These brown crystals are deposited specifically in the areas where the (initial) antibody is bound on the tissue section.

H5 Lymph nodes (H&E) 76.661

5.1 Lymph nodes (H&E) 01.229 & 02.153

Catalogue Number	Small Image	Image Map	Large Image
N_HL_LN2_36.jpg	Lymph node (H&E)		Lymph node (H&E)
N_HL_LN2_26.jpg	Lymph node (H&E)	Image map	Lymph node (H&E)
N_HL_LN2_27.jpg	Lymph node (H&E)		Lymph node (H&E)
N_HL_LN2_02.jpg	Lymph node (H&E)	Image map	Lymph node (H&E)

Look carefully at this section with the naked eye and then under the microscope, moving from low power (x4 objective) up to the x10 and then the x40 objective. Using the diagram on page 3 and the annotated photosheet available (P1) on your bench to identify the different areas in the lymph node. Draw a labeled sketch of the slide in your notes, ensuring that you include the **cortex**, the **medulla**, the **sinuses**, the **paracortex** and several **lymphoid follicles**/ **germinal centres**. (N.B. The immunostained sections may not be from the same lymph node, so your sketch is to remind you of the principles rather than the exact features of your specific lymph node.)

5.2 Lymph nodes: CD3 Immunostaining (T lymphocyte marker) 01.230 & 02.154

Catalogue Number	Small Image	Image Map	Large Image
N_HL_LN2_08.jpg	Lymph node (CD3)	Image map	Lymph node (CD3)
N_HL_LN2_31.jpg	Lymph node (CD3)		Lymph node (CD3)

Look carefully at this slide (naked eye and gradually increasing power objectives). Annotate the main areas that are CD3+ (brown) on your labeled sketch.

Q.1. Which areas of the lymph node have most CD3 staining?
Q.2. Are there any CD3+ cells in the germinal centres? If so, why are these CD3+ cells present here?
Q.3. At high power, which parts of the cells seem to express CD3 (nucleus or cytoplasm/ membrane)?

N.B. CD3 is a protein complex expressed on T cells, which binds to the T cell receptor at the cell surface and plays an essential role in T cell receptor signal transduction.

5.3 Lymph nodes: CD20 immunostaining (B lymphocyte marker) 01.231 & 02.155

Catalogue Number	Small Image	Image Map	Large Image
N_HL_LN2_13.jpg	Lymph node (CD20)	Image map	Lymph node (CD20)
N_HL_LN2_33.jpg	Lymph node (CD20)		Lymph node (CD20)

Look carefully at this slide (naked eye and gradually increasing power objectives). Annotate the main areas that are CD20+ (brown) on your labeled sketch.

Q.4. Which areas of the lymph node have most CD20 immunostaining?

N.B. CD20 is a protein expressed on the surface of B cells. Its function is unknown.

5.4 Lymph nodes: CD68 Immunostaining (Macrophage/dendritic cell marker) 01.232 & 02.156

Catalogue Number	Small Image	Image Map	Large Image
N_HL_LN2_22.jpg	Lymph node (CD68)	Image map	Lymph node (CD68)

Look carefully at this slide (naked eye and gradually increasing power objectives). Annotate the main areas that are CD68+ (brown) on your labeled sketch.

Q.5. Where do you see CD68 positive cells in the lymph node?
Q.6. Associated with B cell areas, specialised macrophages are present. Where can you see these?
Q.7. What is the main function of these specialised macrophages?
Q.8. In T cell areas, CD68+ cells are present. What are these likely to be? What is their main function?
Q.9. At high power, can you see any differences in morphology (shape) between the CD68+ cells in T cell areas and those elsewhere in the lymph node? Explain any differences you see.
Q10. Some CD68+ cells are present in the sinuses of the lymph node. Where have these come from and what is their function?

N.B. CD68 is a protein expressed on the surface of macrophages. It is not, however, entirely specific for this cell type. It is also present on monocytes and dendritic cells. The function of CD68 is unknown.

P5.1 Lymph node Photosheet

Catalogue Number	Small Image	Image Map	Large Image
N_HL_LN_92.jpg	Photo sheet		Photo sheet

THE SPLEEN

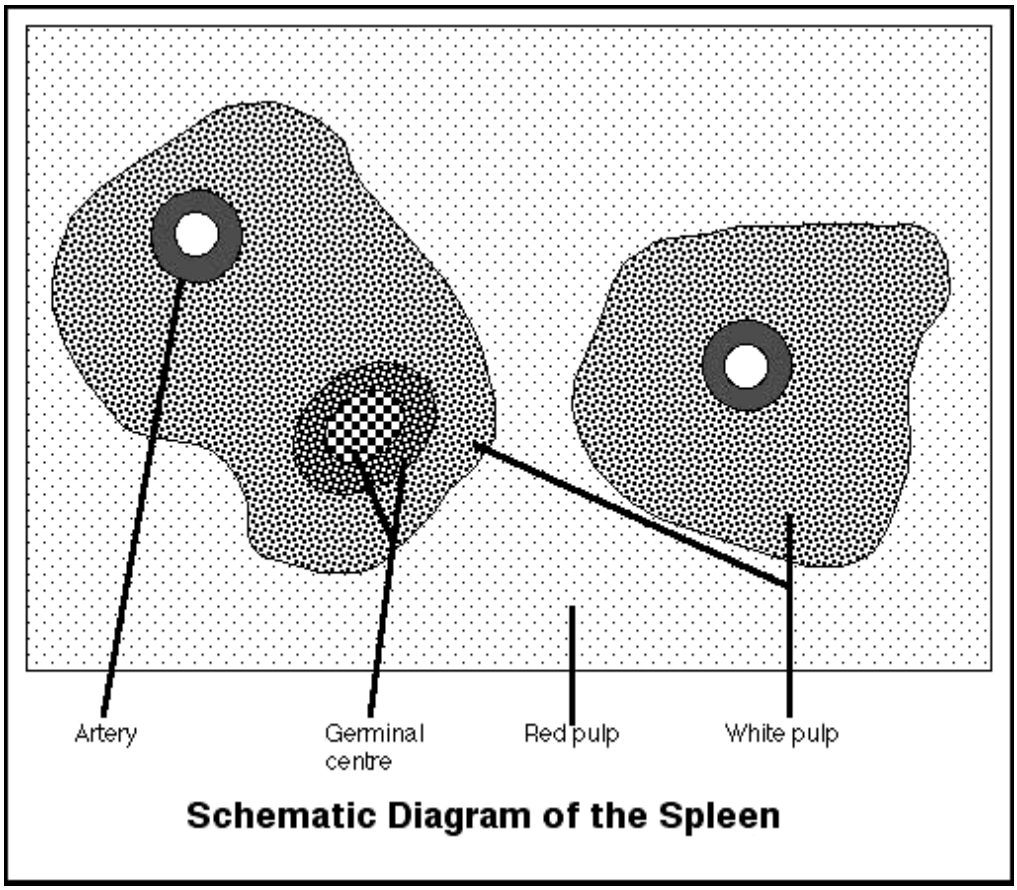
Functions: The **spleen filters the blood and can mount an immune response to blood-borne antigens**. It removes particulate debris (such as bacteria) from the blood and effete ("worn out") red blood cells are phagocytosed by its **reticulo-endothelial system** (see below).

Structure: The spleen has a rich blood supply. Two major areas are recognized within it. These are known as **red pulp** and **white pulp** (appears as small white spots to the naked eye).

Red Pulp: This is composed of sinusoids (passageways) through which the blood flows. The sinusoids are lined by a type of macrophage and they are said to be part of the "Reticulo-Endothelial System".

Reticulo-Endothelial System: When **monocytes** from the blood enter the tissues, they are called **macrophages**. Macrophages have a wide range of important roles in acute and chronic inflammation, the process of healing and the immune system. Some monocyte/ macrophage populations move about freely, while others (**sessile macrophages**) are attached to the lining of wide blood vessels (**sinuses or sinusoids)** within organs and in lymph vessels in which the **flow is slow**. Such sinuses or sinusoids are found in the **liver** and **spleen** and are known as the **reticulo-endothelial system**. Since the cells of the monocyte-macrophage system capture antigen, it is unsurprising that areas of **lymphoid tissue** exist adjacent to them. Phagocytosis of particulate debris and effete red blood cells by macrophages occurs in the sinusoids (sometimes called the cords of Billroth) in the red pulp of the spleen**.** Macrophages are also resident in many other tissues, for instance the alveolar macrophages in lung and the microglia in the brain.

White Pulp: The white pulp consists of cells of the immune system: lymphocytes and macrophages. Both T and B lymphocyte areas are present within the white pulp. Immune responses can be generated here to antigens taken up from the blood.



Schematic Diagram of the Spleen

H7 Spleen: normal (Refer to diagrams above & below and to the annotated image of spleen on P5.2) 76.659

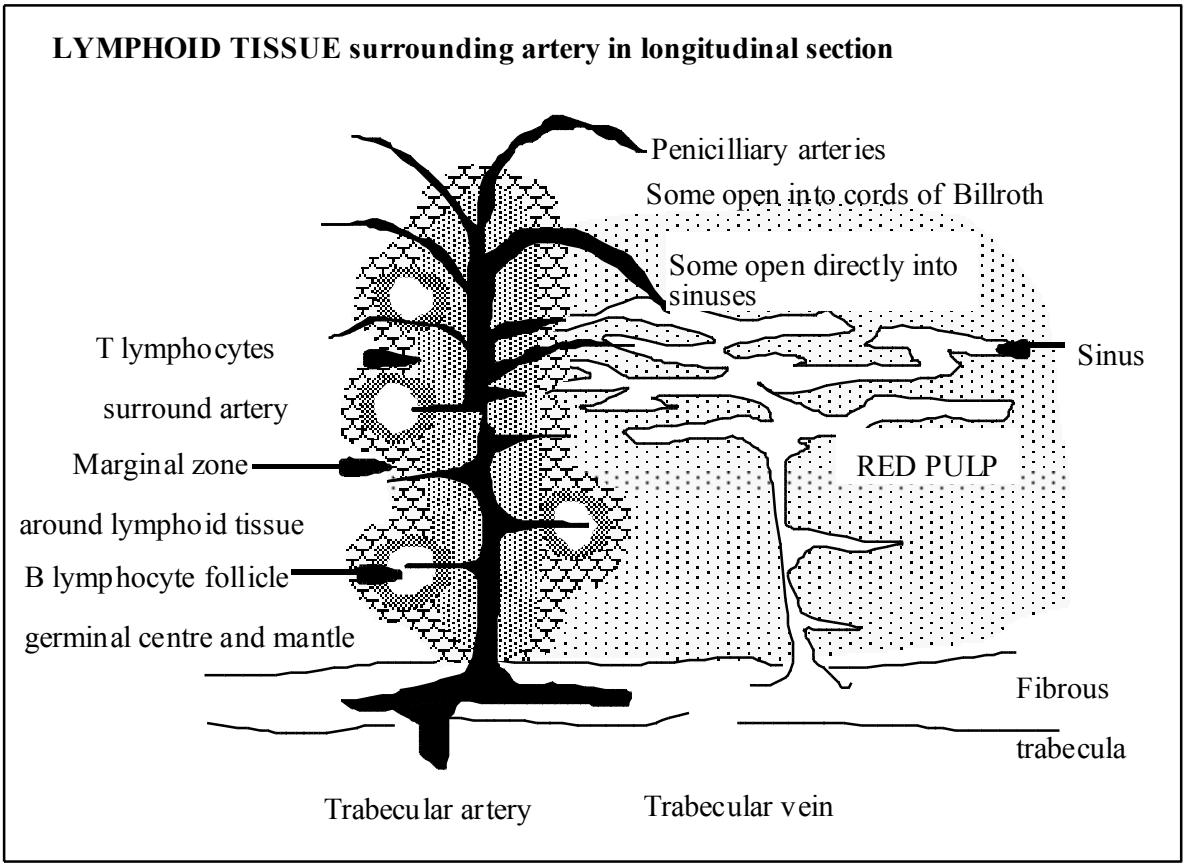
Scanned images on computer can also be viewed: Spleen 76.659 or 72.1199

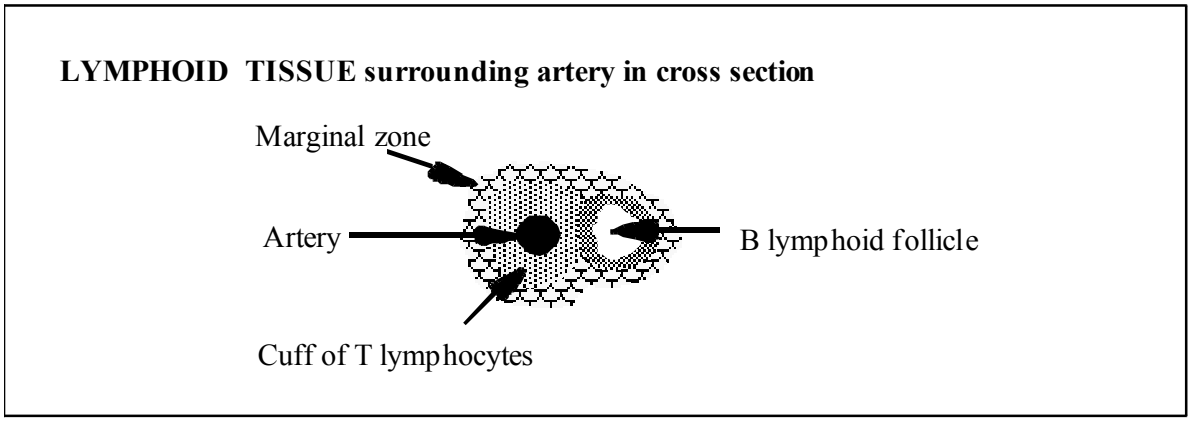
Catalogue Number	Small Image	Image Map	Large Image
N_HL_SP_23.jpg	Normal Spleen	Image map	Normal spleen

In this section of spleen, try to identify the separate areas of red pulp, in which large numbers of red blood cells are visible within the sinusoids, and white pulp in which numerous small deeply haematoxyphilic (blue staining) lymphocyte nuclei are seen.

THE SPLEEN

Diagrams to indicate the pattern of lymphoid tissue and red pulp





CONCEPTUAL DIAGRAM OF BLOOD FLOW THROUGH THE SPLEEN

THYMUS

Function of the thymus: T lymphocytes become activated following the recognition of an antigenic peptide, which is presented to the T lymphocyte on a major histocompatability complex (MHC) molecule by an antigen presenting cell (APC). The antigen presenting cell is usually a dendritic cell, although it is sometimes a B lymphocyte or macrophage. The antigenic peptide is derived from a protein, which has been endocytosed and broken down by the antigen presenting cell into small fragments (peptides). If this protein was a host protein, it is important that T lymphocytes do not exist which could recognize such a self peptide.

The thymus is responsible for the selection of T lymphocytes that have the ability to recognize host MHC (antigen presenting molecules) with adequate affinity to be useful, but which do not have a *very high* affinity for host MHC and/or self peptides from host proteins.

Structure of the thymus: A cortex and medulla can be distinguished in the thymus. Like all lymphoid organs, there is a rich vascular supply to allow movement of lymphocytes to and from the thymus. Besides precursors of lymphocytes, specialized thymic epithelium, dendritic cells and macrophages are also present. The thymus is large in the foetus and child, but involutes during adolescence, becoming difficult to identify in the majority of adults.

Processes in the thymus: Precursors of T lymphocytes develop in the bone marrow and make their way to the thymus via the blood. Once in the thymus, they are known as **thymocytes**. These cells are not yet committed to being CD4+ or CD8+ T lymphocytes, but start to express both CD4 and CD8 in the thymic cortex.

Positive selection occurs mainly in the thymic cortex on **thymic epithelial cells** and some thymic dendritic cells. T lymphocyte precursors (thymocytes) are "tested" for their ability to bind to host MHC (regardless of the peptide) to some extent. Those that bind class I MHC retain only CD8 expression, while those that bind class II MHC retain only CD4 expression. Thymic epithelium may be seen in both the cortex and medulla. However, it is believed mainly to play a role in the cortex.

Negative selection occurs mainly in the medulla on **dendritic cells**. Here thymocytes with dangerously high affinity for self peptides are deleted. They receive signals from the APC that cause them to undergo apoptosis.

P5.2 Thymus: Photosheet showing H&E, CD3, CD68 and cytokeratin immunostaining in the thymus.

Scanned images on computer can also be viewed: Thymus 56.0323 or 81.0858

Catalogue Number	Small Image	Image Map	Large Image
N_HL_TY_28.jpg	Photo sheet		Photo sheet

Look first at the H&E stained section on the photosheet. Make a sketch of this in your notes. Now look at the photographs of immunostained sections. Annotate on your sketch of the thymus the main areas/ features that are immunostained by each of the antibodies as you work through the next part.

Q.11. What process takes place in the cortex? Which immunostain supports this?
Q.12. What process takes place in the medulla? Which immunostain supports this?
Q.13. What are the different functions of mature CD4+ T cells and CD8+ T cells? How could you distinguish between them in tissues?

PROCESS IDENTIFICATION AND REPORT WRITING

A.13/15 Appendix: normal (for comparison) 84.50 or 76.554

Catalogue Number	Small Image	Image Map	Large Image
N_AR_AP_01.jpg	Appendix: normal		Appendix: normal
N_AR_AP_04.jpg	Appendix: normal		Appendix: normal

Note the plasma cells in the lamina propria, **normal lymphoid tissue** in the submucosa forming lymphoid follicles with germinal centres. The appendix may be regarded as a significant collection of lymphoid tissue for the intestinal tract.

5.5 Appendix: abnormal 79.558 & 55.229

Catalogue Number	Small Image	Image Map	Large Image
A_AI_AC_AP_30.jpg	Appendix: abnormal	Image map	Appendix: abnormal
A_AI_AC_AP_06.jpg	Appendix: abnormal		Appendix: abnormal
A_AI_AC_AP_07.jpg	Appendix: abnormal		Appendix: abnormal
A_AI_AC_AP_15.jpg	Appendix: abnormal		Appendix: abnormal
A_AI_AC_AP_17.jpg	Appendix: abnormal		Appendix: abnormal
A_AI_AC_AP_19.jpg	Appendix: abnormal		Appendix: abnormal

This is a transverse section of a vermiform appendix from a young person with abdominal pain.

Please draw a diagram, give a description and identify the pathological process.

ORIGINS OF WORDS USED TO DESCRIBE THE LYMPHOID SYSTEM

PREFIX / SUFFIX	ETYMOLOGY
lympho-	Latin: "*lympha*" – water
macro-	Greek: "*makro*" – large
-phage	Greek: "*phagein*" – eat
pino-	Greek: "*pinein*" – drink

CLASS 5 MUSEUM SPECIMENS

I SPLEEN : normal
P84.470

Catalogue Number	Small Image	Image Map	Large Image
N_HL_SP_25.jpg	Spleen		Spleen

From a boy of ten years who died following a brain haemorrhage.

P87.224

Catalogue Number	Small Image	Image Map	Large Image
N_HL_SP_24.jpg	Spleen		Spleen

II THYMUS : normal
P84.470

Catalogue Number	Small Image	Image Map	Large Image
N_HL_TY_29.jpg	Thymus		Thymus

From the same boy as above. The thymus atrophies in later life. (What is its role?)

III BURSA of FABRICIUS (from a hen)
R84.1003A & R84.1003B

Catalogue Number	Small Image	Image Map	Large Image
N_HL_BU_17.jpg	Bursa of Fabricius		Bursa of Fabricius

IV THYMUS, CERVICAL LYMPH NODES and TONSILS
P84.078

The pericardial sac has not been opened. From a nine year old boy who died of a cerebral haemorrhage and bronchopneumonia.

Catalogue Number	Small Image	Image Map	Large Image
N_HL_TY_30.jpg	Thymus, cervical, lymph nodes & Tonsils		Thymus, cervical, lymph nodes & Tonsils

V SPLEEN : normal
P84.078

Catalogue Number	Small Image	Image Map	Large Image
N_HL_SP_25.jpg	Spleen		Spleen

From a nine year old boy who died of a cerebral haemorrhage and bronchopneumonia.