INTRODUCTION-IMMUNE SYSTEM

- When an animal is born, it emerges from mother's womb to a new external environment where microorganisms are present.
- To combat the micro- organisms, Immune system must be developed.
- In some species like mice, gestation period is short and immune system is not fully developed at the time of birth but in major domestic animals, gestation period is long and immune system is fully developed at birth although not fully functional.
- Development of immune capability depends on antigenic stimulation but in very early days they are protected by passive transfer of antibody from mother.
- The development of immune system in mammalian fetus follows a consistent pattern.
- Thymus is the first lymphoid organ *(primary lymphoid organ)* to be developed followed by *secondary lymphoid organs.*
- Primary lymphoid organs are the organs where differentiation and maturation of lymphocytes takes place without antigenic stimulation. In secondary lymphoid organs, antigenic stimulation of B and T lymphocytes results in specific immune reaction.
- Immunoglobulin containing cells develop soon after the appearance of spleen and lymph nodes but antibodies are found in late stage of fetal life. Capability to respond to antigen develops after the lymphoid organs appear.

ORGANS OF THE IMMUNE SYSTEM

- Lymphoid organs are the important organs of the immune system.
- Lymphocytes are the highly dynamic cells and large number of them recirculating as individual cells in blood, lymph and tissue fluids.
- Lymphocytes are the predominant cells in lymphoid organs. They can recognize, respond and eliminate antigens.
- Lymphocytes either produce humoral or cell mediated immune (CMI) response or both. Lymphoid organs can be classified into two groups
    - Primary lymphoid organs
    - Secondary lymphoid organs

Primary lymphoid organs

- The organs that regulate the production and differentiation of lymphocytes *into immunocompetent lymphocytes* without antigenic stimulation are called primary lymphoid organs.
- Primary lymphoid organs are also called *generative organs or central lymphoid organs.*
- In primary lymphoid organ, lymphocytes first express antigenic receptors and mature both phenotypically and functionally without any antigenic stimulation.
- Mature lymphocytes fall into two major populations, T cells and B cells depending on at which primary organ they were matured.
- T cells mature in thymus where as B cells in Bursa of Fabricius (in birds) or bone marrow (primates and rodents) or Peyer's patches (in ruminants and pigs).
- *Examples of primary lymphoid organs:* bone marrow, thymus, Bursa of Fabricius, some Peyer's patches and fetal liver.

Secondary lymphoid organs

- They are also called *peripheral lymphoid organs.*
- In these organs antigens are trapped and immune response is generated based on foreign antigenic stimulation.
- These organs are rich in macrophages and dendritic cells (which trap and process antigen) and also T and B cells (which mediate immune response).
- *Examples of secondary lymphoid organs:* Tonsil, spleen, lymph nodes, mucosal associated lymphoid tissues (MALT), gut associated lymphoid tissues (GALT).

COMPARISON OF PRIMARY AND SECONDARY LYMPHOID ORGANS

Characteristics	Primary Lymphoid organs	Secondary Lymphoid organs
Origin	Ecto-endodermal junction or endoderm	Mesoderm
Time of development	Early in embryonic life	Late in foetal life
Persistence	Involutes after puberty	Persists through adult life
Effects of removal	Loss of lymphocytes and immune response	Minimal or no effects
Response to antigen	Not dependent	Fully reactive
Examples	Bone marrow, thymus, bursa, Some peyer's patches	Spleen, lymph nodes, MALT etc.

PRIMARY LYMPHOID ORGANS

- Thymus
- Bursa of Fabricius
- Peyer's patches
- Bone marrow
- Lymphoepithelial glands
- Fetal liver

THYMUS

- Thymus is the first lymphoid organ to develop in mammals.
- The thymus is located in the anterior mediastinum and develop from invaginations of the ectoderm.
- In horse, cattle, sheep, pigs, and chickens, it extends up to the neck as far as to the thyroid gland.
- In human it develops from 3rd bronchial clefts (paired structure).
- The size of thymus varies considerably. It increases progressively during fetal and neonatal life and attains maximum size during puberty. After puberty it atrophies but remnants do persists in old age.

Structure

- It is a bilobed lympho- epithelial organ.
- Each lobe consists of lobules of loosely packed epithelial cells and covered by a connective tissue capsule.
- The outer part of each lobule is cortex and inner part is called medulla.
- Cortex
    - It is composed of numerous lymphocytes (called thymocytes) of various stages of development , many epithelial cells (called reticular cells, which are stellate shaped with abundant cytoplasm and contact with other cells by desmoses) and a few macrophages.
    - Yolk sac, Bone marrow or foetal liver derived immature T (thymus dependent) cell lineage enter the thymic cortex through the blood vessels.
    - The capillaries that supply thymic cortex are surrounded by an abnormally thick basement membrane and a continuous layer of epithelial cells, which prevent antigen from entering thymic cortex.
    - Enroute the thymocytes mature and express receptor for antigens and surface markers.
    - Maturation begins in the cortex and as the immature thymocytes migrate towards medulla they come in contact with epithelial cells, macrophages and dendritic cells where they undergo positive selection (imposes self MHC-restriction on T cells) and negative selection (results into central tolerance).
    - Because of the positive and negative selection of thymocytes, 90-95% thymocytes die in the thymus by process of apoptosis and only5-10% of thymocytes mature as mainly either $CD4^+$ or $CD8^+$ T-cells.

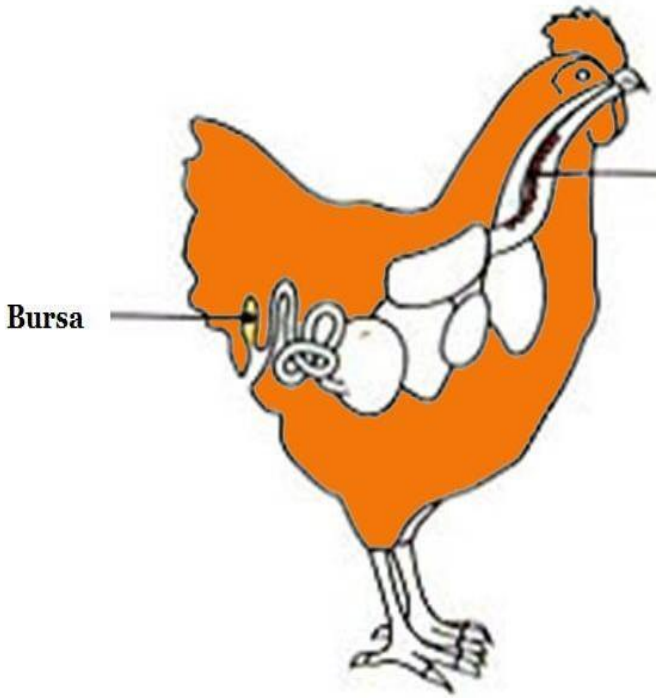
    - Thymic epithelial cells secrete hormones like thymopoietins, thymosins, thymulin, and thymostimulins.
    - Under the influence of these hormones the cells mature. Thus the medulla contains mostly mature T cells.
- Medulla
    - It contains few epithelial cells (reticular cells) and at places Hassall's corpuscle (which are composed of tightly packed whorls of epithelial cells that may be remnants of degenerating cells).
    - Medulla also contain small thymocytes but majority are matured with surface markers ($CD4^+$, $CD8^+$) and receptors expressed over it.
    - Only mature T cells from medulla exit the thymus through efferent vessels into blood circulation and peripheral lymphoid tissues.
- Function
    - Differentiation of immature thymocytes to immunologically competent T lymphocytes
    - Development of secondary lymphoid organs like spleen, lymph nodes and other lymphoid tissues. As thymic dependent lymphocytes colonize in these organs

BURSA OF FABRICIUS

- Bursa of Fabricius is found only in birds and equivalent organs in mammals are gut associated lymphoid tissues (GALT) in Peyer's patches of small intestine, lymphoid tissue of colon and appendix or tonsil.
- Bursa is a round sac like structure (elongated in case of ducks) originates from the dorsal epithelium diverticulum of the cloaca (located just above the cloaca).
- The bursa reached its maximum size by 3 weeks after hatch and then undergoes gradual involution.

Structure

- Bursa consists of lymphocytes embedded in epithelial tissue. This epithelial tissue lines a hollow sac connected to the cloaca by a duct.
- Inside the sac, folds of epithelium (plicae) extend into the lumen. Each plica contains many lymphoid follicles (bursal follicle).
- Bursal follicles contain follicle associated epithelial cells, lymphocytes, macrophages and plasma cells
- Each follicle is divided into a cortex and medulla. The cortex is the outer part packed with lymphocytes, plasma cells and macrophages.
- The inner part is medulla. These stem cells under the influence of bursal micro environment (hormonal influence of bursepoietin/ bursin) mature and differentiate into bursal lymphocytes or B cells.
- B cells appear in the bursa between12-15 days of embryonic development.
- Bursa is not a ***pure primary lymphoid organ*** because it can trap antigen and some antibody synthesis takes place. It also contains a small focus of T cells just above the bursal duct opening.



Bursal follicles

Function

- Differentiation of immature B- cells into immunocompetent B- cells (antibody producing cells)
- It can also trap antigen and produce antibody.

PEYER'S PATCHES

- Some of the Peyer's patches (PP) present in ruminants intestine function as primary lymphoid organs.
- There are two types of PP in sheep; one in jejunum and the other one is in the ileum and cecum.
- The PP in jejunum persists through out the animal's life.
- They are pear shaped follicles, separated by extensive inter follicular tissue and contain up to 30% T cells. But ileo-cecal PP reach maximum size before birth, disappears by 15 months of age and cannot be detected in adult sheep.
- The ileo-cecal PPs resembles bursa in birds. B cell differentiation and maturation take place in ileo-cecal PPs but many cells self reactive B-cells die of apoptosis (negative selection) and only very few cells are released into the circulation.

BONE MARROW

- The specialized ileo-cecal Peyer's patches (PP) have been described in ruminants and pigs. In other species, bone marrow serves as the primary lymphoid organ for B cell development.
- The bone marrow is the site of generation of all circulating blood cells in the adults including immature lymphocytes and also the site of B cell maturation.
- In the embryonic life, hematopoiesis (generation of blood cells) starts in blood island of yolk sac and the para-aortic mesenchyme and latter in the liver and spleen. Gradually this is taken over by the bone marrow after birth.
- At puberty, hematopoiesis mostly occurs in the flat bones (sternum, vertebrae, iliac bone, ribs etc.).
- The red marrow consists of sponge like reticular framework lined in the endothelium and found between long tuberculae.

- The spaces in this framework are filled with fat cells, stem cells and hematopoietic cells.
- These precursors mature and exit through the network of vascular sinuses to the vascular circulation.
- All blood cells except T lymphocytes are produced in the bone marrow.

LYMPHOEPITHELIAL GLANDS

- Structures located throughout the large intestine and caecum of horses, dogs, pigs and several other mammals.
- These consists of lymphoid aggregates that surround elongated intestinal or caecal glands to form submucosal diverticula.
- The arrangement resembles avian bursa and it contains many plasma cells suggesting that they are major sites of antibody production.

SECONDARY LYMPHOID ORGANS

Learning objectives

This module deals with

- Role of secondary lymphoid organs in immunity,
- Antigen presenting cells and their role in immunity,
- Lymphocyte circulation and their role in immunity.

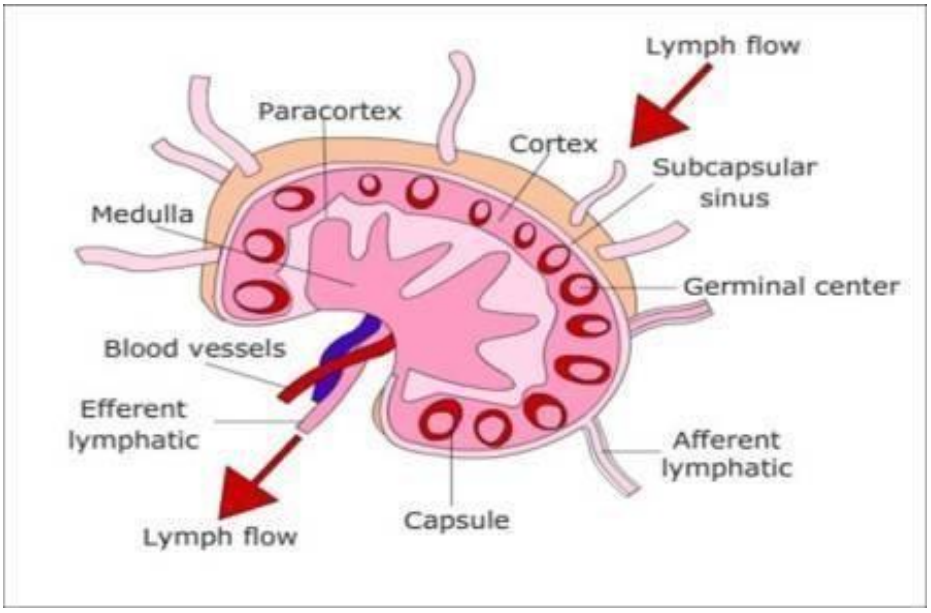
SECONDARY LYMPHOID ORGANS

- The secondary lymphoid organs arise late in fetal life and persist through adult life. These organs develop well depending on antigenic stimulation to facilitate antigen trapping and development of immune response.
- The secondary lymphoid organs are
    - Lymph nodes
    - Spleen
    - Cutaneous immune system
    - Mucosal immune system
    - Bone marrow

LYMPH NODES

- Lymph nodes are bean shaped structure found in large number along the course of lymphatic vessels and connected to each other by lymphatic to trap antigen carried by lymph.
- Lymph nodes are covered with fibrous capsule and each one has an outer cortex, inner medulla and para cortical zone in between them.
- Lymph node consists of a reticular network filled with lymphocytes, macrophages, dendritic cells through which lymphatic sinuses penetrate.
- Cortex
    - Beneath the sub capsular sinus, the outer cortex is area of B. lymphocytes which are present as aggregate in the form of primary folicles (containing mainly naive mature B-cells) and secondary folicles (germinal centre).
    - Naive B cells are attracted to the follicle in response to chemokines produced in follicles and expressed chemokine receptor.
    - Some follicles contain central *germinal centre,* which develop on antigenic stimulation, and they are called secondary follicles.

- *Germinal centres* are sites of B cell maturation (somatic maturation), immunoglobulin class switching and memory cell formation. Primary follicle mostly contains mature, naïve B-lymphocytes.
- The *follicular dendritic cells (FDCs)* present in the germinal center, trap antigen and present to B cells.
- A few T cells are found in the cortex, mainly between follicles. About 70% of these T cells are CD4+ (helper T cells) and relatively less CD8+cells.



- Para cortex
  - The paracortical area of the lymph node is the site for homing of T-cells and interdigitating dendritic cells, where DC capture antigen, process and present them to the T-cells.
- Medulla
  - Predominant cells are B cells, macrophages, reticular cells and plasma cells.
  - They are arranged in cellular cords between the lymphatic sinuses.
  - Activated T and B cells migrate towards one another.
- Activated T cells ultimately exit lymph node and enter the circulation where as activated B cells migrate into germinal centre or medulla and secrete antibodies.
- *Other animals:* In pigs, elephants, hippopotamuses, rhinoceroses and dolphins, lymph nodes consist of several aggregated nodules. Cortex is located toward the centre and medulla is at the periphery.

HEMOLYMPH NODES AND DENTRITIC CELLS

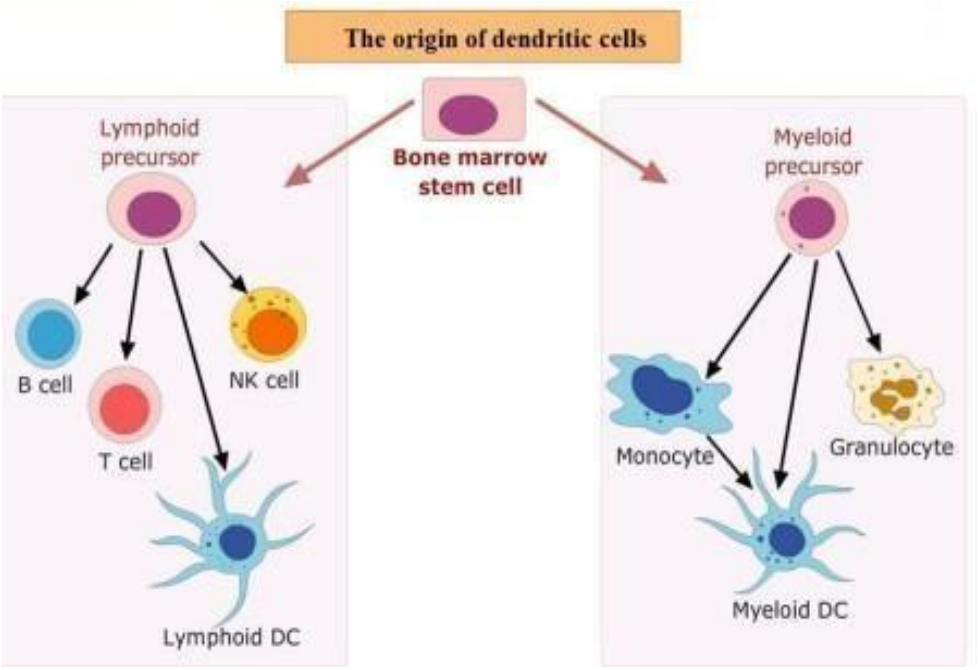
Hemolymph Nodes

- Similar to lymph nodes found in ruminants and other mammals.
- Function is not clear.

Dendritic cells

- These are specialized cells found in the epithelia of the skin, respiratory tract, gastro intestinal tract and in most parenchymal organs.
- Morphologically they have membranous or spine likes projections.
- They have lobulated nuclei and clear cytoplasm containing characteristic granules called Birbeck granules.

- They capture antigens and transport these antigens to peripheral lymphoid organs.
- All dendritic calls are thought to have originated from bone marrow and related to mononuclear phagocytes lineage.

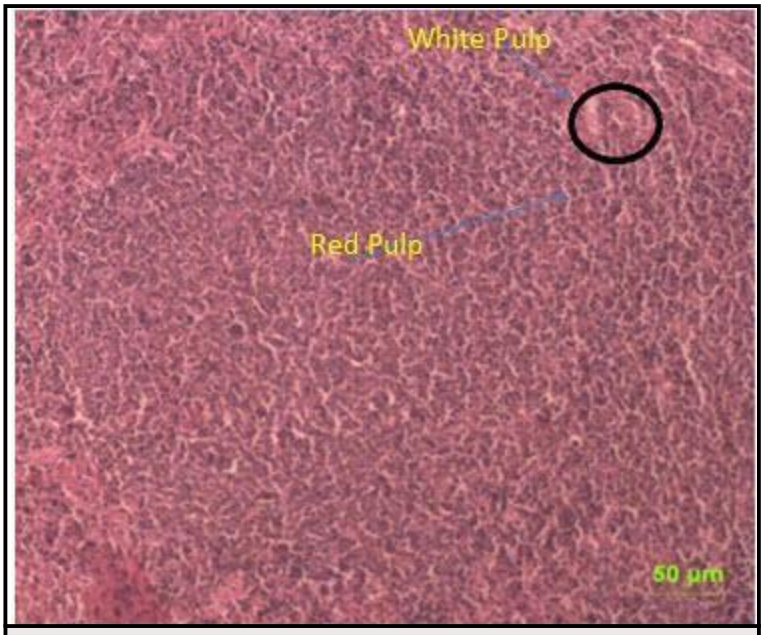


LYMPHOCYTE CIRCULATION

- The predominant lymphocytes in blood are T cells.
- They leave the blood by two routes.
- Those which have not encountered antigen previously bind to venules in the paracortex of lymph nodes.
- These are called high endothelial venules (HEV) because they possess tall rounded endothelial cells unlike the flattened ones found in other blood vessels.
- They are joined by discontinuous junction which facilitate passage of lymphocytes between them.
- HEVs are not normally found in sheep lymph nodes.
- In contrast to naïve T cells, memory T cells leave the blood via conventional blood vessels in tissues and are them carried to lymph nodes by afferent lymph.
- Ninety per cent of the lymphocytes leaving a node are derived form cells entering through HEVs, whereas 10% enter by way of afferent lymph.
- The lymph leaves lymphnode by way of efferent lymphatics which join together into large lymph vessels.
- The largest lymph vessel is thoracic duct. It collects the lymph from lower body and intestine and empties in to the anterior vena cava.
- If the thoracic duct is cannulated and the lymph removed, blood lymphocytes (mainly T cells) drop significantly.
- T cells also disappear from the paracortex of lymph nodes. This implies that thoracic duct lymphocytes normally circulate back to lymph node through the blood**.**
- In pigs, the circulating lymphocytes enter the lymph node through HEVs. But they leave the lymph node not through the lymphatics but migrate back in to the blood stream through the HEVs of the paracortex. Hence, very few lymphocytes are found in pig lymph**.**

SPLEEN

- Lymph nodes filter antigen from lymph where as the spleen filters antigen from the blood.
- The spleen is the major site of immune responses to blood borne antigens.
- The filtering process removes both antigen particles and aged blood cells.
- Spleen stores red cells and platelets and produce blood cells in the fetus.
- Spleen is covered by a capsule and trabeculae pass into splenic pulp.
- The splenic pulp is divided into two compartments
    - Red pulp
    - white pulp



- The white pulp is circular in structure and is made up mainly of lymphocytes. It functions in a manner similar to the nodules of the lymph node.
- The red pulp surrounds the white pulp and contains mainly red blood cells and macrophages. The main function of the red pulp is to phagocytize old red blood cells

- The framework of the pulp is supported by the meshwork of reticular cells and fibres.
- Blood supply in spleen is by a single splenic artery that pierces the capsule at hilum and progressively divided into branches as trabecular artery.
- Small trabecular arterioles are surrounded by cuffs of lymphocytes (T cells and called T cell zone) in a cylindrical form and are called periarterial lymphoid sheath *(PALS)*.
- Within these PALS, lymphoid follicles are present and some of which contain germinal centre.
- The follicles are in B cell zone.
- Germinal centres develop on antigenic stimulation and they are also called *Malpighian corpuscles*.
- A layer of T cells forming a *mantle zone* surrounds each follicle.
- The white pulp consists of periarteriolar sheath, the B cell follicle and the mantle zone.
- Trabecular artery in PALS is known as central artery and branches into many.
- Some supply terminates in marginal zone as sheathed artery or penicilli.
- White pulp and red pulp are separated by *marginal zone*, which is formed by fine spongy work of reticular cells and their fibres.
- Both T cells and B cells are present in this zone.

- *The Red pulp* is formed by cords of a meshwork of reticular cells and sinuses. All arterial blood vesselsenter and terminate in cords. The cords contain RBCs, macrophages, platelets and plasma cells. The cords act as filter for RBCs, WBCs and sites for extra medulla hematopoiesis. Destruction of platelets and RBCs takes place in the red pulp and is referred to as *hemocatharesis*. The immune responses occur in the white pulp.
- Function - Site for the development of immune response. 2). Site for hematopoiesis (lymphopoiesisand erythropoiesis) . 3). Organ for filtration of RBCs and WBCs. 4). Trap antigen and develop immune response.

CUTANEOUS IMMUNE SYSTEM

- The skin contains a specialized cutaneous immune system consisting of lymphocytes and APCs.
- The epidermis contains keratinocytes, melanocytes, epidermal Langerhans cells and intraepithelial Tcells.
- Both keratinocytes and melanocytes are responsible for innate immune response.
- Langerhans cells capture antigen and because of antigenic stimulation move to dermis due to thestimulation by chemokines.
- They subsequently home to lymph nodes through lymphatic vessels.
- In epidermis, about 2% lymphocytes are present and majorities are CD8+ T cells.
- Dermis contains both CD8+ T cells and CD4+ T cells, predominantly in a pervascular location withscattered macrophages.

MUCOSAL IMMUNE SYSTEM

- The mucosal surfaces of the gastro intestinal tract, respiratory tract, genital tract and oral cavity represent a vast surface area which is vulnerable to invasion and colonization by many micro organisms. Defense in these membrane surfaces is provided by organized lymphoid tissue, collectively known as mucosa associated lymphoid tissue *(MALT)*.
    - In G-I tract, lymphocytes are found in large numbers in three main regions:
        - Within epithelial layer (intraepithelial lymphocytes),
        - Scattered throughout the lamina propria and
        - In payer's patches of lamina propria.
- Intra epithelial lymphocytes are mainly T cells ($CD8^+$).
- The follicles contain B cells, macrophages, dendrite cells. B cells express IgM in the periphery of the follicle and IgA in the germinal centre.
- T cells predominate in inter and para follicular regions.

BONE MARROW

- Bone marrow is probably the largest secondary lymphoid tissue in the body.
- If antigens are given intravenously, some antigen will be trapped in the bone marrow.
- During the primary immune response, antibodies are largely produced in the spleen and Lymphnode.
- Towards the end of that response, the memory cells leave the spleen and colonize in bone marrow.
- When a second dose of antigen is given, the bone marrow produces very large quantities of antibodiesand is the major sources of IgG in adult rodents.