The lymphoid organs

- The immune system consists of immune cells that continuously circulate between the blood and lymphoid organs.

- Lymphoid organs are divided into:
  - **Primary lymphoid organs** are the sites where leukocytes are generated and include:
    - the bone marrow
    - the thymus
  - **Secondary lymphoid organs** are the sites where adaptive immune responses are initiated and include:
    - the spleen
    - the lymph nodes
    - the mucosa-associated lymphoid tissue
  - **Tertiary lymphoid organs** are the sites where adaptive immune responses are initiated and include:
    - the spleen
    - the lymph nodes
    - the mucosa-associated lymphoid tissue
Lymphoid organs are divided into:

- **Primary lymphoid organs** are the sites where leukocytes are generated and include:
  - the **bone marrow** in which hematopoiesis takes place and B lymphocytes are generated
  - the **thymus** in which T cells proliferate; differentiate and mature

- **The bone marrow**
  - Spongy (cancellous) bone consists of a lattice of bone trabeculae adjacent to small, irregular cavities containing bone marrow
  - The bone marrow is the main site of hematopoiesis.
  - The site of lymphopoiesis and generation of the B cell repertoire

- **The thymus**
  - serves as the primary lymphoid organ for the development of T cells.
  - The adult mammalian thymus is a pyramid-shaped organ formed of two structurally identical lobes
  - Each thymic lobe is surrounded by a capsule of connective tissue.
  - Lobules consist of a cortex and a medulla.
  - The thymic stroma consists of a network of epithelial cells that participate at the positive and negative selection.
Generation of the leukocyte lineages

- All leukocytes circulating in blood originate from stem cells in the bone marrow.
- The process that allows differentiation and maturation of leukocytes from stem cells is called hematopoiesis.
- Hematopoiesis is divided in two main arms:
  - Lymphopoiesis that generates lymphocytes
  - Myelopoiesis that generates granulocytes, monocytes, dendritic cells, platelets, erythrocytes.
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- Myelopoiesis that generates granulocytes, monocytes, dendritic cells, platelets, erythrocytes.

All components present in the peripheral blood and most of the cells that circulate in primary, secondary and tertiary lymphoid tissues are the progeny of a single cell which, by its functional capacity, has been designated the hematopoietic stem cell (HSC).
B Lymphopoiesis

- The development of B cells can be divided into several stages designated:
  - pro-B,
  - pre-B
  - immature B cells,

- This process is based on:
  - the *recombination status* of the heavy chain (H) and light chain (L) genes
  - the developmentally regulated expression of cell surface markers.
**B Lymphopoiesis**

**T cell development**

- CSH
- Stem cell
- pro-myelo-lymphoid
- pro-lymphoid
- TAL-1
- GATA-2
- PU-1
- Ikaros

**B cell development**

- EDF
- E2A
- IL-7R
- RAG-1
- RAG-2
- Pax 5, syk

**Pro B**

- B220+
- CD19+
- TdT+
- µ-

**Pre B I**

- Large
- heavy chain VDJH
- in frame
- µ+

**Pre B II**

- Small
- heavy chain VDJH
- out of frame
- µ-

**Immature B**

- Light chain
- in frame
- µ+

**Mature B**

- Light chain
- out of frame
- µ+

**NK cell**

**Monocyte/macrophage**

**TdT**: terminal deoxynucleotidyl transferase

**Negative selection**

**Positive selection**

**Apoptosis**
B Lymphopoiesis

- Reflects the expression of a gene from only one of the 2 parental chromosomes in each single cell.
  - Applies to both the heavy- and the light-chain genes.
  - Ensures that mature B cells express a single B-cell receptor.
  - Allelic exclusion acts by preventing the rearrangement of the second allele when the rearrangement of the first one (either maternal or paternal) has been successful.

Allelic exclusion reflects the expression of a gene from only one of the two parental chromosomes (allele #1 or #2) on each single cell. Allelic exclusion takes place for both the heavy- and the light-chain genes.
Stem cell  Pro B cell  Pre B-I cell  Large Pre B-II cell  Small Pre B-II cell  Immature B cell

**Transcription factors**
- Pax 5
- E2A 8(bHLH)
- EBF (early B cell factor)

**Markers**
- B220 (CD45R)
- CD19
- CD25 (IL2R α chain)

**Receptors**
- Igα, Igβ
- surrogate light chain
- surface IgM

**Rearrangement machinery**
- RAG-1, RAG-2
- TdT
Bone marrow: summary and objectives

- found in the bone cavities;
- made of a stroma consisting of a trimentional network of reticular fibers containing hematopoietic cell;
- in which hematopoiesis takes place in the adult;
- in which B lymphocytes differentiate and acquire antigen-specific receptors;
- in which autoreactive B cells are eliminated.

Your objectives

- describe the structure of the bone marrow
- describe the main steps involved in the generation of the B-cell repertoire and how autoreactive B cells are eliminated.
- The thymus serves as the primary lymphoid organ for the development of T cells.

- The adult mammalian thymus is a pyramid-shaped organ formed of two structurally identical lobes.

- Each thymic lobe is surrounded by a capsule of connective tissue.

- Lobules consist of a cortex and a medulla.

- The thymic stroma consists of a network of epithelial cells that participate at the positive and negative selection.
Thymic stroma

Epithelial cells specifically labeled with cytokeratin 14

Epithelial cells express MHC class I and MHC class II
Thymocyte compartmentalization

- Cortex
- Medulla

Double negative

Double positive

Single positives

CD4: yellow
CD8: blue
T lymphocyte progenitor produced in the bone marrow enter the thymus via the post capillary venules.

Progenitor migrate to the cortex where they proliferate and rearrange their TCR genes.

They become double positive and express CD4 and CD8 co-receptors.

Double positive thymocyte undergo positive selection.

They become single positive and express either CD4 or CD8 co-receptors.

Single positive thymocyte migrate to the medulla where they undergo negative selection.

Mature CD4 and CD8 T cells leave the thymus through the post capillary venules.
Thymocyte development

- Cortex
  - Double positive small resting thymocyte
  - CD4+8+
  - Double negative large and active thymocyte
  - CD4-8-

- Medulla
  - Single positive small, resting thymocyte
  - CD8+
  - CD4+
  - Export to the periphery
  - Mature naive T cells

- Selection
  - Negative selection
  - Positive selection 95%
  - Apoptosis 5%
The thymus is

- an epithelial stroma colonized by lymphocytes
- the organ in which T lymphocytes differentiate into helper (CD4) and cytotoxic T (CD8) cells
- the organ in which the generation of the T cell repertoire takes place
- the organ in which autoreactive T cells are eliminated

Objectives:

- Describe the structural features of the thymus and the compartmentalization of the lymphocytes
- Identify the cells that participate in the selection of the T lymphocytes
- Identify the sites where T cell progenitors enter the thymus and the site where mature T cells leave the thymus
The secondary lymphoid organs

- **Secondary lymphoid organs** are the sites where adaptive immune responses are initiated and include:
  - the lymph nodes
  - the spleen
  - the mucosa-associated lymphoid tissue

- secondary lymphoid organs in which naïve lymphocytes encounter antigens drained by afferent lymphatics.

- where immune responses are mounted against antigens derived from skin and internal organs
The lymph nodes

Structure of the lymph nodes

- The stroma
- The lymphatics and sinuses
- The blood vessels
- The parenchyma

Initiation of the immune response

- Antigen sampling & presentation
- T and B cell activation
- Differentiation into effector and memory cells
- Migration & homing to effector sites
Antigen sampling

- Antigens encountered in the skin are sampled by DC and transported through the afferent lymphatics to the draining LN for presentation to lymphocytes.

- Antigens encountered in MALT structures are sampled by M cells which pass on intact antigen to underlying DC. From there, DC move to the paracortical region of MALT for presentation to local lymphocytes.

- Antigens that enter the bloodstream are sampled by DCs in the spleen.

- Antigens that enter epithelial tissues (skin and mucosae) and escape local sampling by DC may be transported in soluble form through the afferent lymphatics to the draining LN. There antigens are sampled by local DC for presentation to recirculating naive T cells.
The trafficking of the immune cells within the lymph node
Adaptive immunity is mediated by two cell types: B and T cells.

- The T lymphocyte response is characterized by:
  - an induction phase
  - an effector phase
  - a contraction phase

- The B lymphocyte response is characterized by:
  - an induction phase
  - somatic hypermutation
  - affinity maturation
  - isotypic switch
  - differentiation into effector cells
  - production of antibodies
The T cell response: the induction phase

T lymphocytes that recognize antigen are activate to proliferate extensively, provided they receive also a co-stimulatory signal.

After recognition of a new antigen, about a week is required for the clonal expansion of the antigen-specific lymphocytes.

Once clonally expanded the T cells differentiate into functional effector T cells.
The spleen

- The red pulp that participates in the clearance of cell debris and aged erythrocytes and leukocytes
- The white pulp that acts as a secondary lymphoid organ able to trigger immune responses against blood borne antigens.

- Structure of the spleen
- Cell trafficking in the spleen
Mature naïve T and B lymphocytes are produced in primary lymphoid organs. Once distributed through the blood stream, naïve T and B lymphocytes continuously recirculate between the secondary lymphoid organs where they encounter antigens.

The first interaction of a naïve T or B cell with its specific antigen is called **priming** (primary immune response induction). Primed T and B lymphocytes differentiate into an **effector** T or B cell, capable of:
- cytotoxic activity (CD8+ T cells)
- cytokine secretion (CD4+ T helper cells)
- antibody secretion (B cells).

Upon first antigen recognition, a few T and B cells also give rise to **memory** T or B cells, capable of responding with greater intensity and faster kinetics upon reencounter of the same antigen (secondary or memory immune responses).
T cell responses serve three purposes:

- Eliminate intracellular microbes, such as viruses, that infect and replicate inside various cell types, including non-phagocytic cells;
- Defend against intracellular microbes that evolved to survive within phagocytes;
- Assist the development of B cell responses, activate macrophages, mast cells, and eosinophils.

T cell immune responses can be thought of occurring as three phases:
T cell activation

- T-cell activation allows antigen-specific T cells to proliferate and differentiate into:
  - CD8+ cells: CTL effector cells capable of cytotoxic activity.
  - CD4+ cells: helper T cells capable of secreting cytokines.

- T-cell activation typically requires two events:
  - antigen recognition by T cell, a specific event that involves the ligation of the TCR/CD3 complex with antigen fragments bound to MHC molecules on APCs
  - antigen-nonspecific interactions between costimulatory molecules and their ligands on the APC and the T cell.
T cell activation: Role of antigen

- Antigen can regulate the induction of T cell activation dependent upon
  - **Antigen localization**
    Antigen has to be transported by APCs from the periphery to the organized lymphoid tissue.
  - **Antigen dose**: an optimal antigen dose is required.
    If the amount of antigen is too low, no immune response can be induced. On the other hand, if antigen is overwhelming, all specific T cells will be induced within a few days and will die off because of their limited life-span.
  - **Antigen kinetics (availability)**:
    The duration of antigen presentation plays a critical role in T cell activation.

1. **IGNORANCE**: If antigens remain in peripheral solid organs and never reach lymphoid tissues or only for a too short period of time or at too low quantities, they are usually ignored by the immune system. This is the case of peripheral self antigens.

2. **INDUCTION OF AN IMMUNE RESPONSE**: If antigens reach secondary lymphoid organs in high enough quantities and for sufficient long time periods, naive T cells will be activated.

3. **EXHAUSTION**: If antigens spread within a few days throughout the entire organism the T cell response becomes exhausted due to deletion or death of all specific T cells.

4. **PROTECTIVE IMMUNITY**: if antigens are not completely eliminated, they can persist in the periphery and may spread periodically to lymphoid organs and restimulate the immune response.
Initial contacts between APCs and T cells are antigen-independent and mediated by cell-adhesion molecules.
- LFA1 and CD2 on T cells
- ICAM and LFA-3 (CD58) on APCs

Upon antigen recognition
- Interactions occur between the TCR and MHC
- Specialized junctions at the cell surface, the immunological synapses (IS) are formed
  - IS contains
    - The TCR/MHC-peptide complex,
    - The co-receptors
    - The costimulatory molecules
    - Peripheral cell adhesion molecules.
- The adhesion ring is itself surrounded by a ruffling membrane.
- An IS is maintained for up to 48 h enabling the prolonged signal transduction required for T cell activation

Signaling pathways involved in T cell activation
- The TCR with its coreceptors.
- The costimulatory receptors.
- The adhesion molecules.
The primary activation signal is induced when specific TCRs on naïve T cells recognize antigenic peptides bound to the MHC/peptide complex on APCs.

The TCR complex consists of

- The TCR
  - α (or δ) chain
  - β (or γ) chain
- CD3: the signal transduction complex
  - γ chain
  - δ chain heterodimers
  - ε chain
  - ζ chain homodimer
There are two major classes of co-stimulators:

- The CD28/B7 family members (Ig superfamily):
  - CD 80 or B7-1
  - CD 86 or B7-2
  - The family members (Ig superfamily) interacts with CD28 & CTL4 on T cells
  - provides the major co-stimulus to resting naïve T cells by reducing the threshold for TCR signaling

- The tumor necrosis factor receptor (TNF-R) family members
  - control the absolute number of effector T cells that are generated late in the primary immune response
  - dictate the frequency of memory T cells that subsequently develop.
The TCR interacts with the peptide present in the groove of MHC class II.

- If the TCR recognizes the peptide, it activates with the help of the CD4 molecule the tyrosine protein kinase of the Src family Lck or fyn.

- Src-kinases are kept inactive by Csk (C-terminal Src-kinase) which phosphorylates the C-terminal inhibitory tyrosine residue of Lck. Csk is regulated by the phosphoprotein PAG/CBP which is dephosphorylated upon TCR stimulation.

- Src-kinases can then be activated by removal of the inhibitory phosphate ($P_i$) by CD45 and addition of an activating phosphate ($P_a$) by autophosphorylation.

- Src-kinases phosphorylates the $\zeta$ chain which becomes a docking site for ZAP70, a kinase.

- ZAP70 in turn phosphorylates an adaptor protein LAT which now recruits effector molecules that activates signal transduction pathways.
The TCR is part of a complex signaling machinery which includes:

- TCR αβ dimer
- the accessory molecules CD4 or CD8
- a signal transduction module made up of various chains (CD3),
  - γ, δ, ε,
  - ζ chains that contain ITAM motifs

(immunoreceptor tyrosine-based activation motifs).
T cell activation: Signal transduction

- Engagement of the TCR triggers 4 distinct transduction pathways leading to:
  - T cell proliferation
    - MAP kinase
  - T cell differentiation
    - NFAT pathway
    - NFκB pathways
  - T cell motility
    - Rho/CDC42 pathway

As a T cell encounters cognate antigen several signaling pathways become activated in the immunological synapse. These include the NF-AT, NFκB and MAP kinase pathways. As a result transcription factors are activated that regulate the
Proliferation of clones of antigen-specific T cells is one of the most important events during a primary immune response.

The key molecule that regulates T cell proliferation is the soluble cytokine IL-2.

- IL-2 produced by activated T cells induces expansion through interaction with its receptor (IL-2R)
  - autocrine pathway (promoting its own clonal expansion)
  - paracrine pathway (inducing proliferation of other IL-2R^+ cells).

- TCR signaling induces naïve T cells to progress from the G0 to the G1 cell cycle stage
- IL-2 induces cell cycle progression, from G1 through S, G2, and M.
The different types of differentiated effector T cells

- Cytotoxic T lymphocytes (CTL), for CD8+ T cells
- T helper (Th) cells, for CD4+ T cells.
  - Th1 cells
  - Th2 cells.
- The polarization into Th1 or Th2 phenotypes, which relies on different gene signaling pathways, is dependent on
  - the cytokine microenvironment
  - interactions with DCs via co-receptors
  - the nature and concentration of antigen presented.
- DCs are responsible for the initial production of cytokines involved in CD4+ T cell differentiation
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DCs are responsible for the initial production of cytokines involved in CD4+ T cell differentiation.
Effector T cells can be activated by signals generated through the TCR alone, in the absence of co-stimulation.

Effector T cells increase cell surface expression of cell adhesion molecules (CD2 and LFA-1, which bind to LFA-3 and ICAMs) to allow greater and prolonged interaction with
- APCs for CD4⁺ Th cells
- Target cells for CD8⁺ CTLs.

Effector T cells express many membrane-bound (FasL, CD40, and LT-β) and soluble effector molecules that are absent in naïve T cells

T cells alter their expression of lymphocyte homing receptors, allowing them to leave the lymphoid organ where they were activated, enter peripheral tissues, and migrate to the site of pathogen entry or inflammation.
CD4 T cell effector functions

- The effector functions of these two types of CD4+ T cells are primarily due to the biological activity of the cytokines that they produce.

- Differentiation of naïve CD4+ T cells into either the Th1 or Th2 phenotype is directed by the cytokines present at the time of antigen-specific activation.
  - Th1 cell development requires IL-12
  - Th2 cell development requires IL-4.
The main Th1 effector cytokines produced are IFN-γ, IL-2, and LT-α.

- IFN-γ plays a major role in activating macrophages, increasing their phagocytosis, and enhancing their ability to kill ingested microbes by inducing increased nitric oxide and superoxide production, increasing expression of MHC Class I and MHC Class II molecules, and production of pro-inflammatory cytokines, (TNF-α and IL-12).
Th1 effector functions

- Th1 cells also produce IL-2, the T cell growth factor and induces proliferation of T cells.
LTα activates macrophages, neutrophils, and inhibits B cells.
Th2 effector functions

- Th2 cells are primarily mediated through the cytokines that they secrete.
- Th2 cells produce cytokines that:
  - regulate B cell proliferation
  - isotype switching
  - haematopoiesis
  - recruitment of eosinophils & mast cells
  - stimulate mucus production
- Defense against extracellular parasites
  - gastrointestinal worms.
  - amoebia
- Th2 cells also play a role in suppressing the inflammation induced by Th1 cells.
Th2 effector functions

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CD8 T cell effector functions

- Naïve CD8 T cells express receptors required for migration through HEV, (CCR7, L-selectin (CD62L) & LFA-1.

- Acutely activated CD8 T cells can produce IL-2 and express CD25 (the α chain of IL-2 receptor).

- After several rounds of proliferation the CD8 T cells
  - loss of IL-2 production,
  - IFN-γ production
  - Lytic granules perforin & granzymes.
  - Homing CCR5 and β1 and β7 integrins or migration to non lymphoid tissues.
CD8 T cell effector functions

- Effector CTL induce apoptosis of infected cells through:
  - granule exocytosis pathway: a calcium-dependent release of specialized lytic granules.
  - Fas-FasL pathway

- CD8 T cells granules contain:
  - perforin, a pore-forming protein
  - granzymes: enzymes that catalyze caspases and induce apoptosis
The T cell response includes distinct steps:
- T cell activation
- T cell differentiation
- T cell migration
- T cell effector functions.

T cell activation requires:
- The engagement of the TCR
- The action of the co-receptors CD4 and CD8 that direct the activation of CD4 or CD8 T cells
- The engagement of the co-stimulatory receptors: CD80, CD86, and CD40 (2nd signal)
- The action of cytokines (3rd signal)

T cell differentiation
- CD4 T cells differentiate into helper cells
  - TH1 cells
  - TH2 cells
  - Treg cells
- CD8 T cells differentiate into CD8 cytotoxic or killer cells
Summary

- **CD4 T cell effector function**
  - CD4 Th1 T cells produce IFN-γ, IL-2, and LT-α that
    - activate macrophages
    - induce an inflammatory response
    - provide help to B cells to produce Th1 inflammatory antibodies
  - CD4 Th2 T cells produce IL-4, IL-13, IL-25 that
    - provide protection against extracellular parasites
    - provide help to B cells to produce Th2 non inflammatory antibodies
  - CT4 Treg cells induce tolerance preventing the activation of CD4 T cells

- **CD8 T cell effector function**
  - CD8 cytotoxic (or killer cells) induce apoptosis in target cells via
    - the grauly exocytotic pathway
    - the Fas-FasL pathway.
    - provide help to B cells to produce Th1 inflammatory antibodies