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Unit-II

Hematopoiesis

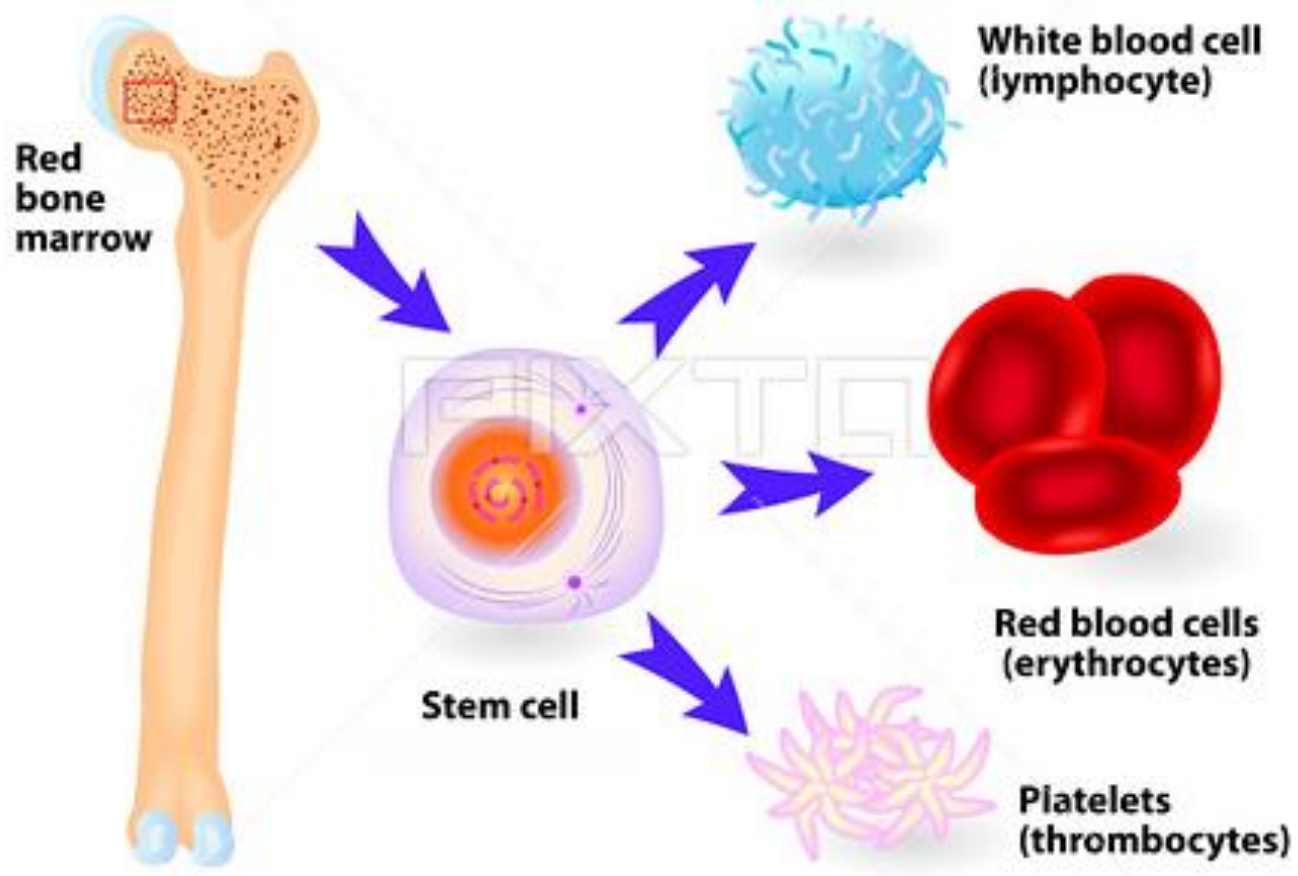
Dr. R. POORNIMA
Guest Faculty
Department of Biomedical Science

Unit II:

Hematopoiesis– T and B lymphocytes ontogeny and development, Activation and Effector mechanism of innate immune system – Phagocytosis - Respiratory burst - inflammation, T lymphocyte activation and downstream signaling following activation. Granzyme and perforin pathways, Fas-FasL pathway, T cell and B cell interaction - B cell activation and downstream signaling following activation and Immunoglobulin class switching. Complement mediated cytotoxicity and Antibody dependent cytotoxicity (ADCC).

PRESENTATION: 1

HAEMATOPOIESIS



- Hematopoiesis is the formation of blood cellular components.
- All cellular blood components are derived from hematopoietic stem cells.
- In a healthy adult human, roughly ten billion to a hundred billion new blood cells are produced per day, in order to maintain steady-state levels in the peripheral circulation.

T Cell and B Cell Ontogeny and Development

It is a complex processes involving the maturation and differentiation of these immune cells from their precursors to fully functional entities.

T Cell Ontogeny and Development

1. Origin

- **Bone Marrow**: T cells originate from hematopoietic stem cells in the bone marrow.

2. Development Stages

1.Migration to Thymus:

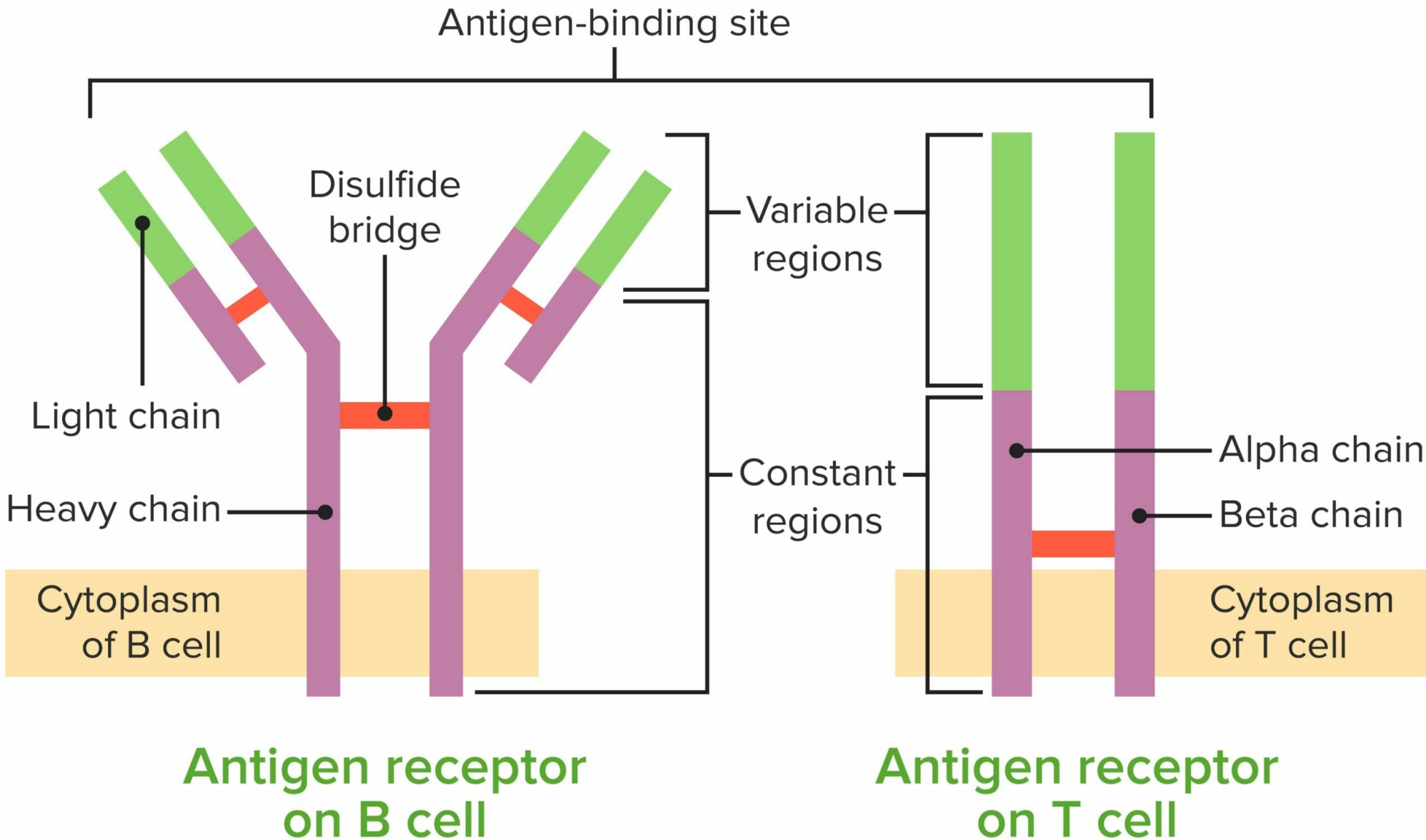
1. **Progenitor Cells**: Immature T cell precursors (thymocytes) migrate from the bone marrow to the thymus, a specialized lymphoid organ.

2. Thymic Development:

- **Positive Selection**: Thymocytes with TCRs that recognize self-MHC molecules with moderate affinity receive survival signals and proceed to the next stage.
- **Negative Selection**: Thymocytes with TCRs that bind too strongly to self-MHC molecules presenting self-antigens undergo apoptosis to avoid autoimmunity.

3. Mature T Cells:

1. **Single-Positive (SP) Stage**: Thymocytes that successfully undergo positive and negative selection mature into CD4+ helper T cells or CD8+ cytotoxic T cells, depending on the specificity of their TCRs.
2. **Exit Thymus**: Mature T cells exit the thymus and enter the peripheral blood and lymphoid tissues, ready to encounter antigens.


Antigen-binding site
Disulfide bridge
Variable regions
Light chain
Heavy chain
Constant regions
Cytoplasm of B cell
Alpha chain
Beta chain
Cytoplasm of T cell
Antigen receptor on B cell
Antigen receptor on T cell

B Cell Ontogeny and Development

1. Origin

- **Bone Marrow**: B cells originate from hematopoietic stem cells in the bone marrow.

2. Development Stages

1. Bone Marrow Development:

1. **Pro-B Cell Stage**: Early B cell precursors undergo somatic recombination of immunoglobulin heavy chain genes. Successful rearrangement leads to the expression of the pre-B cell receptor (pre-BCR).
2. **Pre-B Cell Stage**: Cells express a functional heavy chain and undergo light chain rearrangement to form a complete BCR. The pre-BCR signals for further maturation.
3. **Immature B Cell Stage**: Cells express a complete BCR on their surface and undergo selection for self-tolerance. Immature B cells that strongly react with self-antigens undergo apoptosis or receptor editing to avoid autoimmunity.

2. Migration to Peripheral Lymphoid Organs:

Mature Naive B Cells: Cells that pass the self-tolerance test leave the bone marrow and migrate to peripheral lymphoid organs (spleen, lymph nodes).

3. Activation and Differentiation:

Antigen Encounter: Mature B cells encounter specific antigens in lymphoid organs. Upon activation, B cells proliferate and differentiate into plasma cells (which produce antibodies) or memory B cells (which provide long-term immunity).

Activation of the Innate Immune System

1.Recognition of Pathogens

1. **Pattern Recognition Receptors (PRRs)**: PRRs on innate immune cells detect pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs).
    1. **Toll-like Receptors (TLRs)**: Recognize various microbial components like lipopolysaccharides (LPS), flagellin, and viral RNA.
    2. **NOD-like Receptors (NLRs)**: Detect intracellular bacterial products.
    3. **RIG-I-like Receptors (RLRs)**: Recognize viral RNA in the cytoplasm.
    4. **C-type Lectin Receptors (CLRs)**: Bind to carbohydrate structures on pathogens.
2. **Complement System**: A group of plasma proteins that recognize and bind to pathogens, leading to their elimination.

2. Cellular Activation

- **Macrophages**: Activated by PRR engagement, they increase phagocytosis, produce cytokines, and generate reactive oxygen species (ROS).
- **Dendritic Cells**: Process and present antigens to adaptive immune cells, and secrete cytokines that influence T cell responses.
- **Neutrophils**: Activated to perform phagocytosis, release granules, and generate NETs (neutrophil extracellular traps).

3. Inflammatory Response

- **Cytokine Release**: Cells release cytokines (e.g., TNF-α, IL-1, IL-6) that recruit additional immune cells to the site of infection and mediate inflammation.
- **Acute Phase Response**: Liver produces acute phase proteins (e.g., C-reactive protein) that aid in pathogen elimination and inflammation.

Effector Mechanisms of the Innate Immune System

Effector Mechanisms of the Innate Immune System

1.Phagocytosis

Macrophages and Neutrophils: Engulf and digest pathogens and debris.

Process:

1. **Recognition and Binding**: Pathogens are recognized via PRRs or opsonins (complement proteins or antibodies).
2. **Internalization**: Pathogens are engulfed into phagosomes.
3. **Digestion**: Phagosomes fuse with lysosomes to form phagolysosomes where pathogens are degraded.

2. Inflammatory Response

- **Vasodilation**: Blood vessels expand to increase blood flow to the infected area, allowing more immune cells to reach the site.
- **Increased Permeability**: Blood vessel walls become more permeable, allowing immune cells and proteins to pass into tissues.
- **Recruitment of Immune Cells**: Chemokines and cytokines attract neutrophils, macrophages, and other immune cells to the infection site.

3. Complement Activation

- **Classical Pathway**: Activated by antibody-antigen complexes.
- **Alternative Pathway**: Activated directly by pathogen surfaces.
- **Lectin Pathway**: Activated by binding of lectins to pathogen carbohydrates.
- **Outcomes**:
  - **Opsonization**: Pathogens are marked for phagocytosis by complement proteins.
  - **Lysis**: Formation of the membrane attack complex (MAC) leads to pathogen lysis.
  - **Inflammation**: Complement fragments (e.g., C3a, C5a) induce inflammation.

4. Cell-Mediated Killing

- **Natural Killer (NK) Cells**: Recognize and kill infected or abnormal cells (e.g., cancer cells) that have reduced expression of MHC class I molecules.
- **Mechanisms**:
  - **Cytotoxic Activity**: NK cells release perforin and granzymes to induce apoptosis in target cells.
  - **Cytokine Production**: NK cells produce cytokines (e.g., IFN-γ) that enhance the overall immune response.

PHAGOCYTOSIS

- **Phagocytosis -**immune system will ingest and eliminate pathogens, dead cells, and other debris. It involves the engulfment of particles or microorganisms by specialized cells called phagocytes.

Steps:

Recognition and Binding

1. **Phagocyte Receptors**: Phagocytes, such as macrophages, neutrophils, and dendritic cells, have various receptors on their surface that recognize and bind to pathogens or particles. These receptors include:
    1. **Pattern Recognition Receptors (PRRs)**: Such as Toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs).
    2. **Opsonin Receptors**: Such as those for complement proteins (e.g., C3b) or antibodies (e.g., Fc receptors) that enhance recognition and binding of pathogens through opsonization.
2. **Opsonization**: The process by which pathogens are coated with opsonins (complement proteins or antibodies) to enhance their recognition and uptake by phagocytes.

2. Engulfment

- **Phagocyte Extension**: The phagocyte extends its membrane around the bound pathogen or particle, forming pseudopodia (protrusions of the cell membrane).
- **Phagosome Formation**: The pseudopodia fuse around the pathogen to enclose it in a membrane-bound vesicle called a phagosome.

3. Phagosome Maturation

- **Phagosome-Lysosome Fusion**: The phagosome fuses with lysosomes (organelles containing digestive enzymes and antimicrobial substances) to form a phagolysosome.
- **Phagolysosome Formation**: The phagolysosome is an acidic and enzymatically active environment where the pathogen or particle is subjected to degradation.

4. Pathogen Destruction

- **Enzymatic Digestion**: Inside the phagolysosome, enzymes such as proteases, lipases, and nucleases break down the pathogen’s components.
- **Reactive Oxygen Species (ROS)**: Phagocytes produce ROS (e.g., superoxide, hydrogen peroxide) and reactive nitrogen species (e.g., nitric oxide) to kill pathogens through oxidative stress.
- **Antimicrobial Peptides**: Phagocytes can also release antimicrobial peptides and proteins (e.g., defensins, lysozyme) that further aid in pathogen destruction.

5. Exocytosis and Resolution

- **Residual Body Formation**: After digestion, any indigestible material forms a residual body within the phagocyte.
- **Exocytosis**: The residual body, containing the remaining debris, is expelled from the phagocyte by fusion with the cell membrane, releasing the waste material into the extracellular space.

RESPIRATORY BURST

Respiratory Burst

It is a process in the immune response, primarily performed by phagocytes like neutrophils and macrophages. It involves a rapid increase in oxygen consumption and the production of reactive oxygen species (ROS) to help kill pathogens and facilitate inflammation.

Mechanism of Respiratory Burst

1.Activation

1. **Phagocyte Activation**: When phagocytes encounter pathogens, they are activated through various receptors, such as Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs), or by opsonins (e.g., complement proteins or antibodies).
2. **Signal Transduction**: Activation leads to intracellular signaling pathways that initiate the respiratory burst. This includes the activation of NADPH oxidase, a key enzyme complex involved in ROS production.

2. NADPH Oxidase Activation

- **Enzyme Complex**: NADPH oxidase is composed of several subunits, including membrane-bound and cytosolic components . Upon activation, these subunits assemble to form a functional enzyme complex.
- **ROS Production**: NADPH oxidase catalyzes the reduction of oxygen to produce superoxide anion (O_2^-). This superoxide is subsequently converted into other ROS, including hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and hypochlorous acid (HOCl) through various enzymatic reactions.

3. Generation of Reactive Oxygen Species (ROS)

- **Superoxide Anion (O_2^-)**: The primary ROS produced by NADPH oxidase. It is rapidly converted to hydrogen peroxide.
- **Hydrogen Peroxide (H_2O_2)**: Can diffuse through membranes and is further converted to more reactive forms of ROS, such as hydroxyl radicals.
- **Hydroxyl Radicals (•OH)**: Highly reactive and capable of damaging cellular components, including proteins, lipids, and DNA.
- **Hypochlorous Acid (HOCl)**: Produced by the enzyme myeloperoxidase (MPO) in neutrophils, it has potent antimicrobial properties and contributes to pathogen killing.

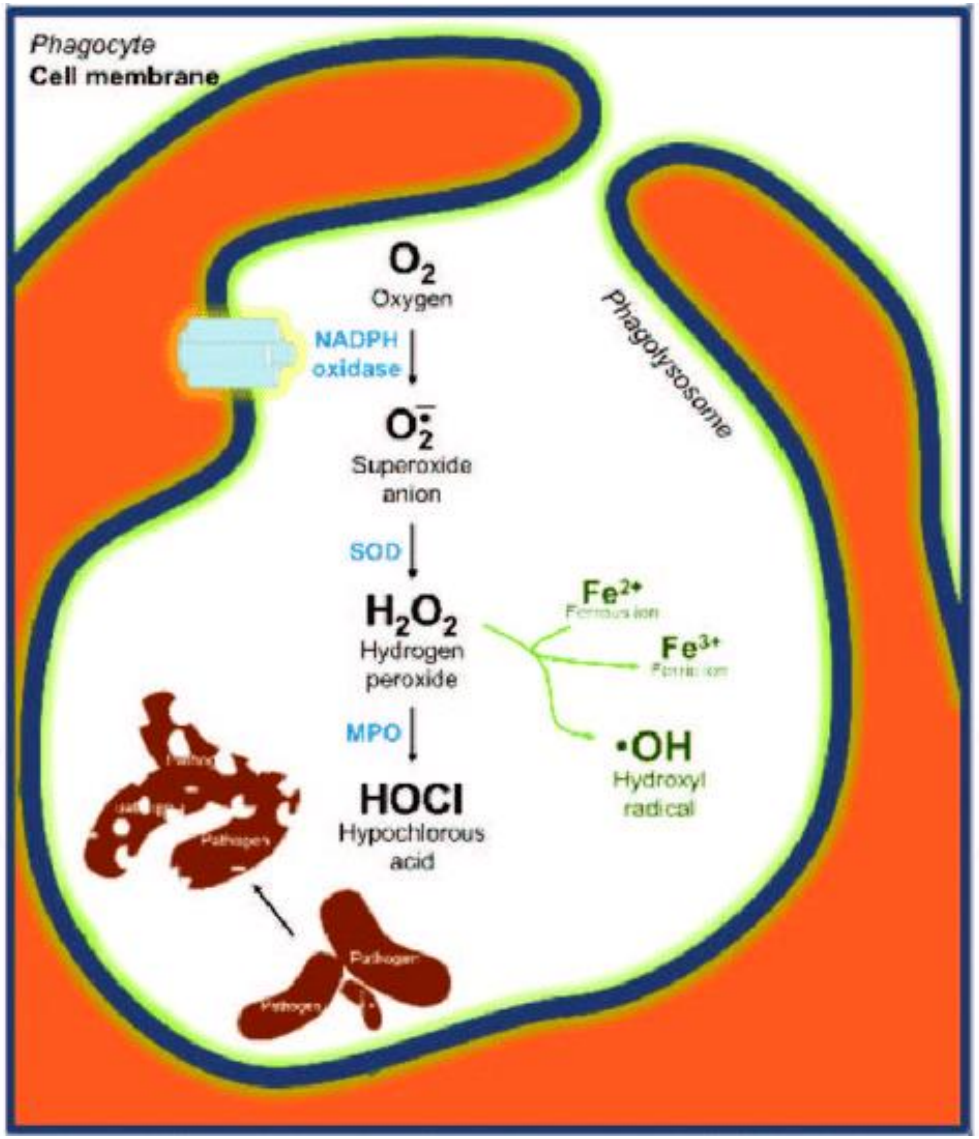
4. Pathogen Killing and Degradation

- **Microbial Killing**: ROS produced during the respiratory burst damage and kill pathogens by oxidizing their cellular components.
- **Degradation**: Phagocytes also use ROS to enhance the breakdown of engulfed pathogens within phagosomes, contributing to efficient digestion and clearance.

5. Resolution of Inflammation

- **Anti-inflammatory Effects**: Once the respiratory burst subsides, mechanisms are in place to control and resolve inflammation. Excessive ROS can lead to tissue damage, so regulatory processes ensure a balance between pathogen killing and tissue protection.

Respiratory Burst



INFLAMMATION

Inflammation is a fundamental biological response to injury or infection, characterized by a complex interplay of cells, proteins, and signaling molecules. It is designed to protect the body by removing harmful stimuli, initiating repair processes, and restoring homeostasis.

Types of Inflammation

1.Acute Inflammation

1. **Duration**: Short-term, usually lasting from minutes to a few days.
2. **Characteristics**: Rapid onset, characterized by redness, heat, swelling, pain, and loss of function.
3. **Examples**: Response to acute injuries, infections (e.g., bacterial infections), and tissue damage.

2. Chronic Inflammation

- **Duration**: Long-term, lasting weeks to years.
- **Characteristics**: Persistent inflammation, often with less obvious signs than acute inflammation. It involves ongoing tissue damage and repair.
- **Examples**: Chronic diseases like rheumatoid arthritis, inflammatory bowel disease, and chronic infections.

Phases of Acute Inflammation

1.Initiation

1. **Injury or Infection**: Inflammation is triggered by damage to tissues, infection by pathogens, or exposure to harmful agents (e.g., chemicals, toxins).
2. **Recognition**: The immune system recognizes damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) on immune cells.

2.Vascular Response

1. **Vasodilation**: Blood vessels in the affected area expand to increase blood flow. This causes redness and heat.
2. **Increased Permeability**: Blood vessel walls become more permeable, allowing immune cells, proteins, and fluids to enter the tissue. This leads to swelling (edema).

3.Cellular Response

1. **Leukocyte Recruitment**: White blood cells (leukocytes) like neutrophils and macrophages are recruited to the site of inflammation. They migrate from the bloodstream into the tissue in a process called chemotaxis.
2. **Phagocytosis**: Phagocytes engulf and digest pathogens, dead cells, and debris.

4. Resolution

1. **Cessation of Inflammation**: Once the harmful stimuli are removed, anti-inflammatory signals and mediators are produced to downregulate the inflammatory response.
2. **Tissue Repair**: Damaged tissue is repaired and normal function is restored. This includes the removal of apoptotic cells and tissue regeneration.

Key Mediators of Inflammation

1.Cytokines

1. **Pro-inflammatory Cytokines**: Such as TNF-α, IL-1, IL-6, which promote inflammation and recruit immune cells.
2. **Anti-inflammatory Cytokines**: Such as IL-10 and TGF-β, which help resolve inflammation and promote healing.

2.Chemokines

1. **Function**: Small proteins that attract immune cells to the site of infection or injury.

3. Prostaglandins and Leukotrienes

1. **Origin**: Produced from arachidonic acid by the action of cyclooxygenase (COX) and lipoxygenase enzymes.
2. **Function**: Involved in vasodilation, increasing vascular permeability, and attracting immune cells.

4. Histamine

1. **Source**: Released by mast cells and basophils.
2. **Function**: Causes vasodilation and increased vascular permeability.

5. Complement System

1. **Function**: Enhances the ability of antibodies and phagocytic cells to clear pathogens, and promotes inflammation.

T Lymphocyte Activation

T Lymphocyte Activation

1.Antigen Presentation

1. **Antigen-Presenting Cells (APCs)**: Dendritic cells, macrophages, and B cells present antigens to T cells.
2. **Major Histocompatibility Complex (MHC) Molecules**:
    1. **MHC Class I**: Presents endogenous antigens to CD8+ cytotoxic T cells.
    2. **MHC Class II**: Presents exogenous antigens to CD4+ helper T cells.
3. **T Cell Receptor (TCR)**: On T cells, the TCR binds to the peptide-MHC complex on the APC.

2. Co-Stimulation

1. **Second Signal**: In addition to antigen recognition, T cells require a second signal for full activation, provided by co-stimulatory molecules.
    1. **CD28**: On T cells, interacts with B7 molecules (CD80/CD86) on APCs.
    2. **Additional Co-Stimulatory Signals**: Various other molecules, such as ICOS and 4-1BB, also contribute to T cell activation.

3. Activation

1. **Signal 1**: The binding of the TCR to the peptide-MHC complex initiates the activation process.
2. **Signal 2**: The interaction between CD28 and B7 molecules provides the necessary co-stimulatory signal.

Downstream Signaling Pathways Following Activation

(Upstream signaling refers to the initial events in a signaling pathway that occur when a signaling molecule, such as a ligand (e.g., a hormone or growth factor), binds to its receptor on the surface of a cell.

Downstream signaling refers to the subsequent events that occur after the initial activation of a signaling pathway. These events often involve a series of protein interactions, modifications (such as phosphorylation), and the activation of transcription factors that lead to changes in gene expression or other cellular responses.**)**

1.TCR Signaling Pathway

1. **TCR Complex**: The TCR is associated with CD3 and ζ-chain (Zeta) proteins, which are involved in signaling.
2. **Lck**: A protein tyrosine kinase (PTK) associated with the TCR complex, phosphorylates ITAMs (Immunoreceptor Tyrosine-based Activation Motifs) on the CD3 and ζ-chains.
3. **ZAP-70**: Another PTK that binds to phosphorylated ITAMs and becomes activated.

2. Activation of Key Signaling Pathways

- **PLC-γ1 Pathway**:
  - **Phospholipase C-γ1 (PLC-γ1)**: Activated by ZAP-70, PLC-γ1 hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to produce inositol trisphosphate (IP3) and diacylglycerol (DAG).
  - **IP3**: Leads to the release of calcium ions (Ca^{2+}) from the endoplasmic reticulum.
  - **DAG**: Activates protein kinase C (PKC), which further activates NF-κB (Nuclear Factor kappa B) and AP-1 (Activator Protein 1) transcription factors.

- **MAPK Pathway**:
  - **Mitogen-Activated Protein Kinases (MAPKs)**: Include ERK (Extracellular signal-Regulated Kinase), JNK (c-Jun N-terminal Kinase), and p38 MAPK.
  - **Activation**: MAPKs are activated through a cascade involving Ras and Raf kinases.
  - **Transcription Factors**: Activated MAPKs lead to the activation of transcription factors like AP-1, which regulate gene expression.
- **PI3K-Akt Pathway**:
  - **Phosphoinositide 3-Kinase (PI3K)**: Activated by the TCR signaling complex, leading to the production of phosphatidylinositol-3,4,5-trisphosphate (PIP3).
  - **Akt (Protein Kinase B)**: Activated by PIP3, Akt promotes cell survival, growth, and metabolism.
  - **mTOR**: A downstream target of Akt, mTOR regulates cell growth and proliferation.

3. Transcriptional Changes

- **NF-κB**: Translocates to the nucleus and drives the expression of genes involved in inflammation, survival, and proliferation.
- **AP-1**: Regulates genes related to cell proliferation and differentiation.
- **NFAT (Nuclear Factor of Activated T-cells)**: Activated by increased intracellular Ca^{2+} levels, NFAT translocates to the nucleus and promotes the expression of cytokines like IL-2.

4. T Cell Proliferation and Differentiation

- **IL-2 Production**: Activated T cells produce IL-2, a growth factor that drives their proliferation.
- **Differentiation**: T cells differentiate into specific subsets based on cytokine signals:
  - **CD4+ T Helper Cells**: Differentiates into Th1, Th2, Th17, or Treg cells, depending on the cytokine environment.
  - **CD8+ Cytotoxic T Cells**: Develop into cytotoxic T lymphocytes (CTLs) capable of killing infected or cancerous cells.

5. Effector Functions

- **CD4+ T Cells**: Assist other immune cells, including B cells and macrophages, through cytokine production and co-stimulation.
- **CD8+ T Cells**: Directly kill infected or cancerous cells through the release of perforin and granzymes.

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