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Programme: M.Sc., Biomedical Science

Course Code: BM35C6
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Unit-II

Hematopoiesis

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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: 2

THE GRANZYME AND PERFORIN PATHWAYS

The granzyme and perforin pathways are part of the immune system's response to infected or cancerous cells. They are mainly utilized by cytotoxic T cells and natural killer (NK) cells to induce apoptosis (programmed cell death) in target cells.

Perforin Pathway

1. **Recognition**: Cytotoxic T cells or NK cells recognize and bind to a target cell that is infected or cancerous. This recognition typically occurs through the interaction of specific receptors on the immune cell with antigens presented on the target cell.

2. **Release of Granules**: Upon recognition, the cytotoxic cells release granules containing perforin and granzymes from their cytoplasmic granules.

3. Perforin Action: Perforin is a protein that forms pores in the membrane of the target cell. It essentially punches holes in the cell membrane.

4. Entry of Granzymes: Through these pores, granzymes (serine proteases) enter the target cell. Granzymes are enzymes that trigger apoptosis by cleaving specific substrates inside the cell.

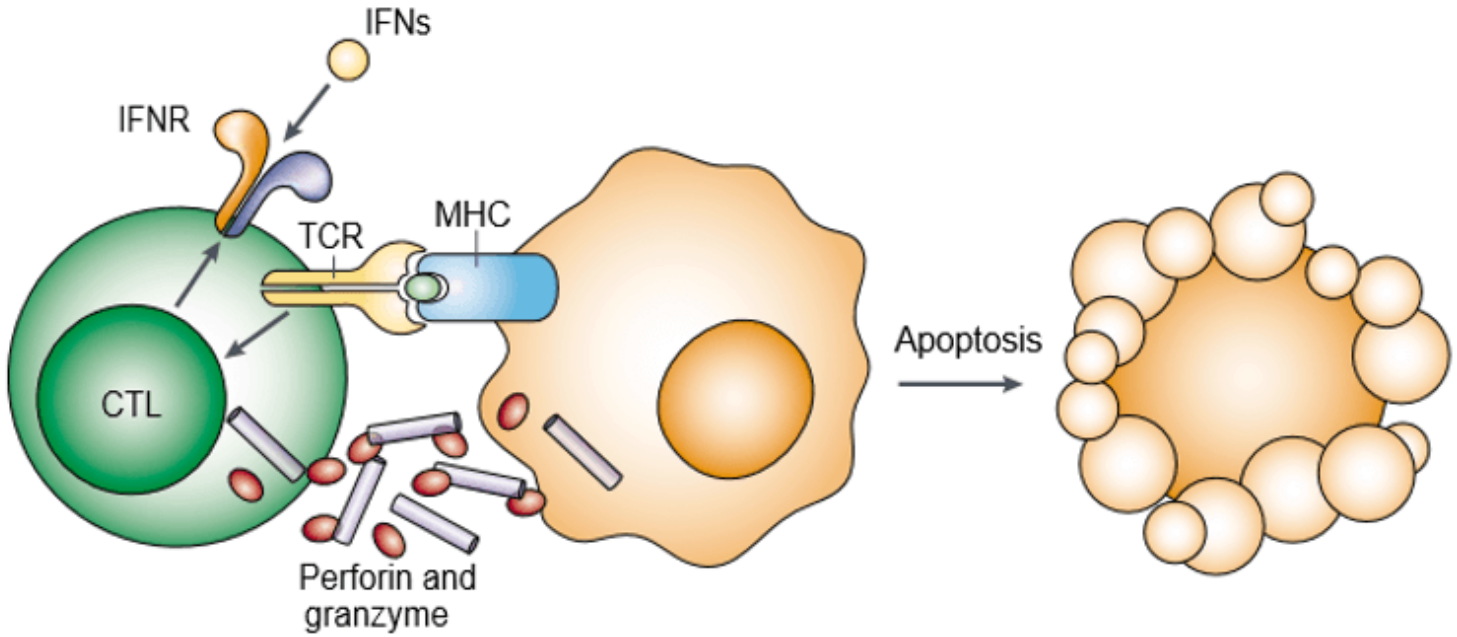
5. Induction of Apoptosis: Once inside the target cell, granzymes activate the apoptotic machinery, leading to cell death. This process involves the activation of caspases (a family of protease enzymes) and other proteins that orchestrate the cell death program.

Granzyme Pathway

- The granzyme pathway is actually part of the perforin pathway but focuses on the role of granzymes:

1. **Entry into Target Cell**: As described above, granzymes enter the target cell through perforin-induced pores.
2. **Activation of Apoptosis**: Granzymes activate various apoptotic pathways within the target cell. They can cleave and activate procaspases into active caspases, which then initiate a cascade of events leading to apoptosis. Granzymes can also cleave other proteins that are crucial for cell survival.
3. **Execution of Cell Death**: The activation of caspases and other apoptotic factors leads to the characteristic features of apoptosis, including DNA fragmentation, membrane blebbing, and ultimately the death of the target cell.

Perforin and granzyme induce target-cell apoptosis cooperatively.



FAS-FASL pathway

The FAS-FASL pathway, also known as the extrinsic apoptosis pathway, is another important mechanism for inducing programmed cell death (apoptosis). It is particularly significant in regulating immune responses and maintaining cellular homeostasis.

FAS-FASL Pathway

1.FAS Ligand (FASL) Binding:

- **FAS Ligand (FASL)** is a protein expressed on the surface of various cells, including cytotoxic T lymphocytes and other immune cells.
- **FAS** (also known as CD95 or APO-1) is a death receptor present on the surface of the target cells that will undergo apoptosis.

2. Receptor-Ligand Interaction:

- When a cell expressing FASL comes into contact with a cell expressing FAS, FASL binds to FAS.
- This binding induces receptor aggregation and triggers the formation of a death-inducing signaling complex (DISC).

3. Formation of the Death-Inducing Signaling Complex (DISC):

- Upon binding, the FAS receptor undergoes a conformational change that recruits adapter proteins such as FADD (Fas-Associated protein with Death Domain) to the receptor.
- FADD, in turn, recruits and activates initiator caspases, particularly **caspase-8** and **caspase-10**.

4. Caspase Activation:

- The activated initiator caspases (caspase-8 and caspase-10) cleave and activate downstream effector caspases, such as **caspase-3** and **caspase-7**.

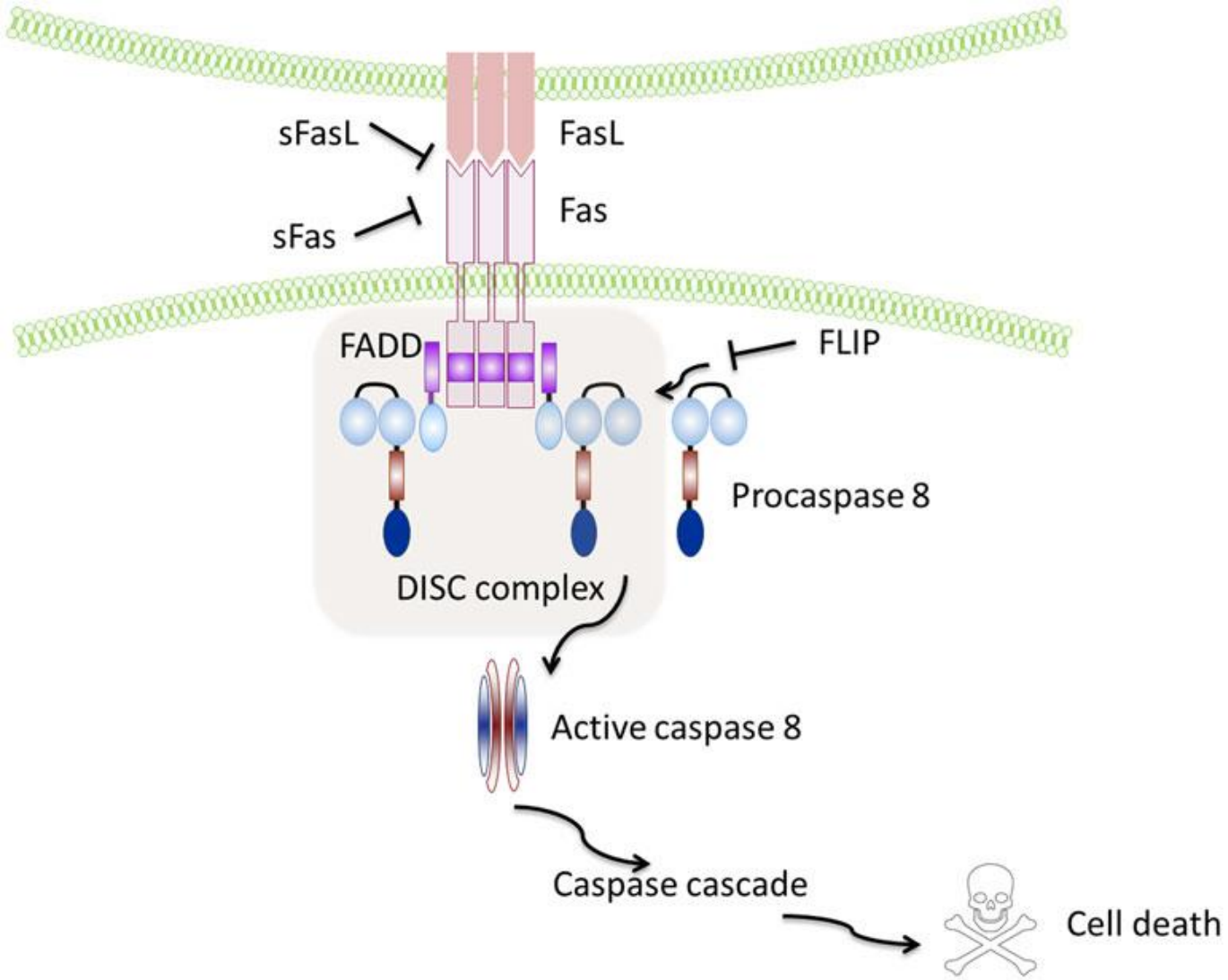
5. **Execution of Apoptosis**:

- The effector caspases execute the apoptotic program by cleaving various cellular substrates, leading to the characteristic features of apoptosis: DNA fragmentation, membrane blebbing, and cell death.

6. **Cell Death**:

- The cell undergoes controlled death, and the remains are typically phagocytosed by neighboring cells or macrophages, preventing inflammation.

FAS-FASL pathway



T CELL AND B CELL INTERACTIONS

- T cell and B cell interactions are central to the adaptive immune response, helping to coordinate the body's defense against pathogens. These interactions involve several key steps and mechanisms:
- **1. Antigen Presentation**
- **Antigen-Presenting Cells (APCs)**: Dendritic cells, macrophages, and B cells can act as APCs. They process and present antigens on their surface using Major Histocompatibility Complex (MHC) molecules.
- **MHC Class II**: This is specifically used by APCs to present processed antigen fragments to CD4+ T helper cells. B cells can also act as APCs, but they primarily present antigens they have internalized and processed.

2. T Cell Activation

- **Recognition**: CD4+ T helper cells recognize the antigen-MHC Class II complex through their T cell receptor (TCR).
- **Co-stimulation**: For full activation, T helper cells also require additional signals provided by co-stimulatory molecules on the APC, such as CD80/86 binding to CD28 on the T cell.

3. B Cell Activation

- **Antigen Binding**: B cells can directly recognize and bind to free antigens via their B cell receptors (BCRs), which are membrane-bound immunoglobulins.
- **Helper T Cell Assistance**: For most antigens, especially protein antigens, B cells require help from T helper cells. This interaction is crucial for the activation and differentiation of B cells.

4. T-B Cell Interaction

- **Formation of the Immunological Synapse**: When a B cell presents an antigen to a T helper cell, the interaction between the TCR and the antigen-MHC Class II complex is stabilized by additional molecules (e.g., CD40L on T cells binds to CD40 on B cells).
- **Cytokine Signaling**: Activated T helper cells secrete cytokines that provide signals to B cells, promoting their proliferation, differentiation, and class switching. For instance, cytokines like IL-4, IL-5, and IL-21 are involved in B cell activation and antibody production.

5. B Cell Differentiation

- **Clonal Expansion**: Activated B cells proliferate and form a clone of cells that are specific for the antigen.
- **Antibody Production**: These B cells differentiate into plasma cells, which secrete large amounts of antibodies (immunoglobulins) specific to the antigen.
- **Memory B Cells**: Some B cells differentiate into memory B cells, which persist long-term and can respond more rapidly upon subsequent exposure to the same antigen.

B cell activation and Downstream Signaling Following Activation

B Cell Activation

1.Antigen Binding:

1. **Recognition**: B cells have membrane-bound immunoglobulins (B cell receptors, or BCRs) that recognize and bind to specific antigens. This can be a free-floating antigen or an antigen that has been processed and presented by another cell.
2. **Internalization**: Upon binding to the antigen, the BCR-antigen complex is internalized by the B cell, processed, and presented on Major Histocompatibility Complex (MHC) Class II molecules.

2. T Cell Help (for Protein Antigens):

1. **Antigen Presentation**: B cells present processed antigen-MHC Class II complexes to CD4+ T helper cells.
2. **T Cell Recognition**: T helper cells recognize the antigen-MHC Class II complex via their T cell receptors (TCRs) and provide additional signals.
3. **Co-stimulation**: Interaction between CD40L (on T helper cells) and CD40 (on B cells) is important for B cell activation. Cytokines secreted by T helper cells, such as IL-4, IL-5, and IL-21, further support B cell activation.

Downstream Signaling Following Activation

1.BCR Signaling:

1. **Signal Transduction**: Antigen binding to BCRs initiates a signaling cascade inside the B cell. This involves the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic tails of the BCR.
2. **Syk Kinase Activation**: The phosphorylation of ITAMs recruits and activates spleen tyrosine kinase (Syk), which further propagates the signal inside the cell.

2. Activation of Signaling Pathways:

1. **Phosphatidylinositol 3-Kinase (PI3K) Pathway**: Activated Syk leads to the activation of PI3K, which produces inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 increases intracellular calcium levels, while DAG activates protein kinase C (PKC).
2. **MAPK Pathway**: The mitogen-activated protein kinase (MAPK) pathway, including ERK (extracellular signal-regulated kinase), is also activated. This pathway regulates gene expression and cell proliferation.
3. **NF-κB Pathway**: NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is activated by signaling cascades, including those involving IκB proteins. NF-κB translocates to the nucleus and activates genes involved in cell survival and proliferation.

3. B Cell Proliferation and Differentiation:

1. **Clonal Expansion**: Activated B cells undergo clonal expansion, producing numerous cells that are specific for the same antigen.
2. **Differentiation**: B cells differentiate into plasma cells, which are specialized for producing large quantities of antibodies (immunoglobulins) against the antigen. They can also form memory B cells, which persist long-term and provide faster responses upon re-exposure to the same antigen.

4. Class Switching and Affinity Maturation:

1. **Class Switching**: Activated B cells can undergo class switching, changing the type of antibody they produce (e.g., from IgM to IgG, IgA, or IgE) while maintaining specificity for the same antigen. This is guided by cytokines and interactions with T helper cells.
2. **Affinity Maturation**: In germinal centers of lymph nodes, B cells undergo affinity maturation, a process that improves the binding affinity of antibodies for their antigen through somatic hypermutation.

ACKNOWLEDGEMENT

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