Cell Injury Mediated By Free Radicals

• Lipid peroxidation of membranes. Double bonds in membrane polyunsaturated lipids are vulnerable to attack by oxygen-derived free radicals. The lipid radical interactions yield peroxides, which are themselves unstable and reactive, and an autocatalytic chain reaction ensues.
• Cross-linking of proteins. Free radicals promote sulfhydryl-mediated protein cross-linking, resulting in enhanced degradation or loss of enzymatic activity. Free-radical reactions may also directly cause polypeptide fragmentation.
• DNA fragmentation. Free-radical reactions with thymine in nuclear and mitochondrial DNA produce single-strand breaks. Such DNA damage has been implicated in cell death, aging, and malignant transformation of cells.

Remove Free Radicals

- Cells have developed many *mechanisms to* ***remove free radicals and thereby minimize injury***.
- *Free radicals are* inherently unstable and decay spontaneously.
- There are also several **nonenzymatic and enzymatic systems that contribute to inactivation of free-radical reactions**

The rate of spontaneous decay of superoxide is significantly increased by the action of superoxide dismutases (SODs) found in many cell types (catalyzing the reaction
$2O2^{\bullet} - 2H \rightarrow H2O2 + O2$.

Glutathione (GSH) peroxidase also protects against injury by catalyzing free-radical breakdown:
$2OH^{\bullet} - + 2GSH \rightarrow 2H2O + GSSG$ (glutathione homodimer)

The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of the oxidative state of the cell and an important aspect of the cell's

ability to catabolize free radicals.

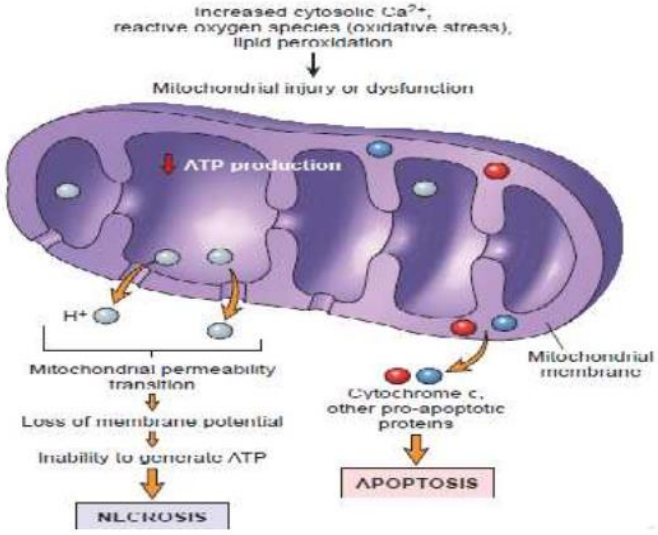
Catalase, present in peroxisomes, directs the degradation of **hydrogen peroxide (2H2O2 → O2 + 2H2O)**

Endogenous or exogenous antioxidants (e.g., vitamins E, A, and C, and β-carotene) may either **block the formation of free radicals or scavenge them once they have formed.**

***Iron and copper can catalyze* the formation of ROS.** The levels of these reactive metals are **reduced by binding of the ions to storage and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin), thereby decreasing the formation of ROS.**

There are two major consequences of mitochondrial damage:

1. Mitochondrial damage often results in the formation of a high-conductance channel in the mitochondrial membrane, called the **mitochondrial permeability transition pore**.
    - The opening of this channel leads to the **loss of mitochondrial membrane potential** and **pH changes, resulting in *failure of oxidative phosphorylation and progressive depletion of ATP, culminating in* necrosis of the cell.**

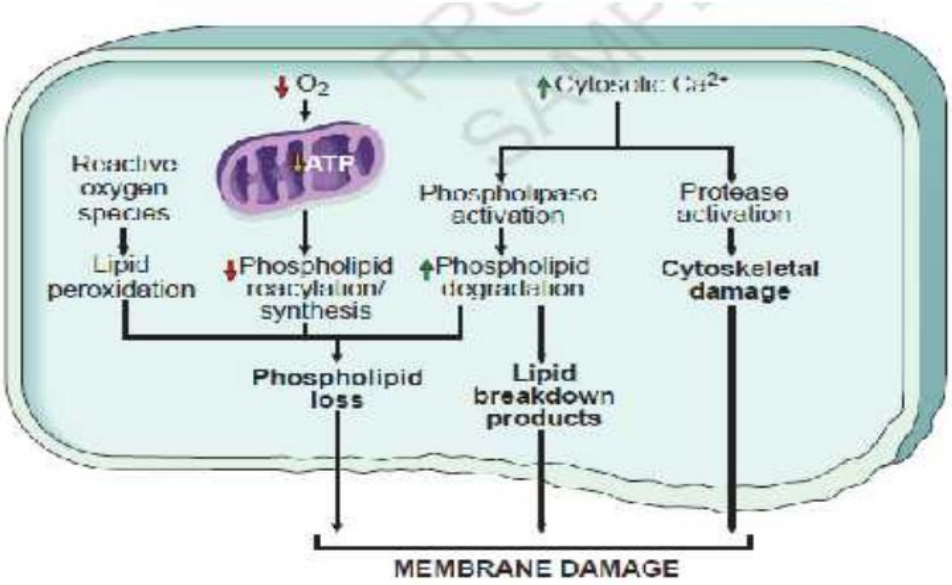


2.The mitochondria also **contain several proteins that are capable of activating apoptotic pathways,including cytochrome *c*** *(the major protein involved* in electron transport).

- **Increased permeability of the mitochondrial membrane may result in leakage of these proteins into the cytosol and *death by apoptosis*.**
- ***Thus,*cytochrome *c plays a key dual role in cell survival and* death; in its normal location inside mitochondria, it is essential for energy generation and the life of the cell, but when mitochondria are damaged so severely that cytochrome *c leaks out, it signals cells to die.***

Defects in Membrane Permeability

- Early loss of selective membrane permeability leading ultimately to overt membrane damage is a consistent feature of most forms of cell injury **(except apoptosis).**
- The plasma membrane can be damaged by **ischemia, various microbial toxins, lytic complement components, and a variety of physical and chemical agents**.
- Several biochemical mechanisms may contribute to membrane damage.



1. ***Decreased phospholipid synthesis.***

The ***production* of phospholipids** in cells may be **reduced** whenever there is a **fall in ATP levels**, leading to decreased energy dependent enzymatic activities. The reduced phospholipid synthesis may affect all cellular membranes including the mitochondria themselves, thus exacerbating the loss of ATP.

2. Increased phospholipid breakdown.

Severe cell injury is associated with increased degradation of membrane phospholipids, probably due to activation of **endogenous phospholipases by increased levels of cytosolic Ca2+.**

3. *ROS.*

Oxygen free radicals cause injury to cell ***membranes*** **by lipid peroxidation.**

4.Cytoskeletal abnormalities.

Cytoskeletal filaments serve as anchors connecting the plasma membrane to the cell interior. **Activation of proteases by increased cytosolic Ca2+ may cause damage to elements of the cytoskeleton**.

5.Lipid breakdown products

- *These include* ***unesterified*** **free fatty acids, acyl carnitine, and lysophospholipids, catabolic products** that are known **to accumulate in injured cells as a result of phospholipid degradation**.
- They **have a detergent effect on membranes**.
- They also either **insert into the lipid bilayer of the membrane or exchange with membrane phospholipids, potentially causing changes in permeability and electrophysiologic alterations.**

The most important sites of membrane damage during cell injury are the

1. ***Mitochondrial membrane damage.***

*D*amage to mitochondrial membranes results **in decreased production of ATP, culminating in necrosis, and release of proteins that trigger apoptotic death.**

2. *Plasma membrane damage.*

Plasma membrane **damage leads to loss of osmotic balance and influx of, fluids and ions as well as loss of cellular contents**. The cells may also leak metabolites that are vital for the reconstitution of ATP, thus further depleting energy stores.

3. *Injury to lysosomal membranes:*

Injury to lysosomal membranes results in leakage of their enzymes into the cytoplasm and activation of the acid hydrolases in the acidic intracellular pH of the injured (e.g., ischemic) cell.

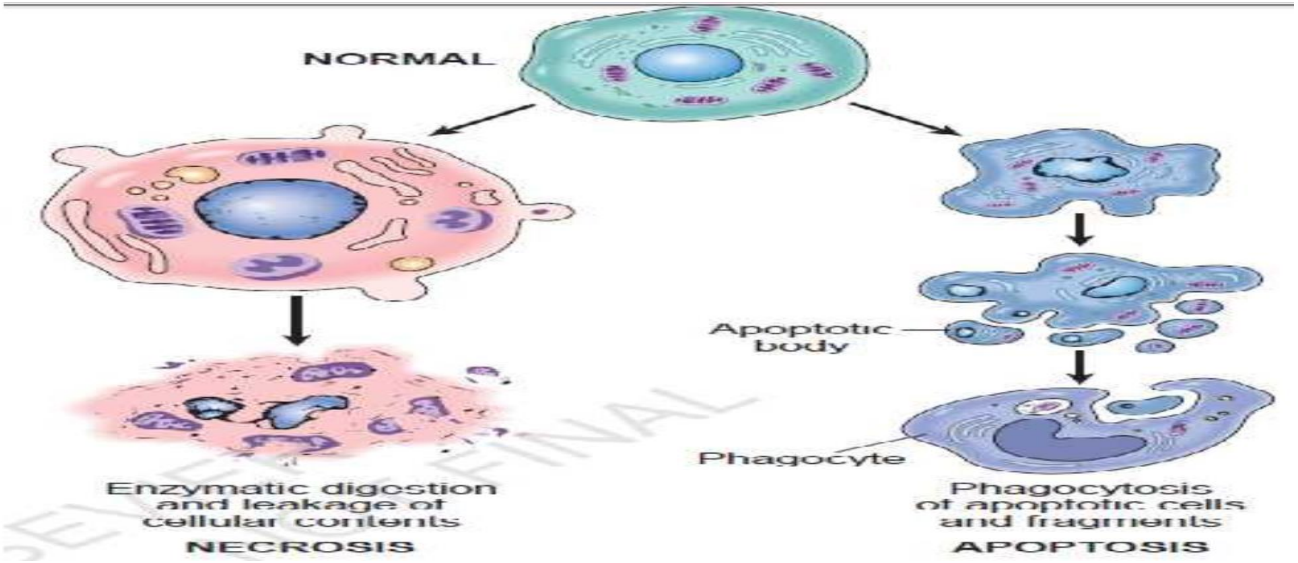
Lysosomes contain RNases, DNases, proteases, glucosidases, and other enzymes. Activation of these enzymes leads to enzymatic digestion of cell components, and the cells die by necrosis.

Damage to DNA and Proteins

- Cells have mechanisms that repair damage to DNA, but if this damage is too severe to be corrected (e.g., after radiation injury or oxidative stress), the cell initiates **its suicide program and dies by apoptosis**.
- A similar reaction is triggered by **improperly folded proteins, which may be the result of inherited mutations** or **external triggers such as free radicals**.

APOPTOSIS

- *Apoptosis is a **pathway of cell death that is induced by a** **tightly regulated suicide program in which cells destined to die activate enzymes capable of degrading the cells'**own nuclear DNA and nuclear and cytoplasmic proteins.
- Fragments of the apoptotic cells then break off, giving the appearance that is responsible for the name (*apoptosis,* "falling off").
- The **plasma membrane of the apoptotic cell remains intact**, but the **membrane is altered in such a way that the cell and its fragments become avid** targets for phagocytes.
- The **dead cell is rapidly cleared before its contents have leaked out,** and therefore cell death by this pathway **does not elicit an inflammatory reaction in the host.**
- Thus, apoptosis differs from necrosis, which is characterized by loss of membrane integrity, enzymatic digestion of cells, leakage of cellular contents, and frequently a host reaction.



Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome-size fragments
Plasma membrane	Disrupted	Intact; altered structure, especially orientation o lipids
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic, means of eliminating unwant cells; may be pathologic after some forms of cell injury, especially DNA damage

Causes of Apoptosis

1. Apoptosis in Physiologic Situations
2. Apoptosis in Pathologic Conditions

Apoptosis in Physiologic Situations: *Death by apoptosis is a* ***normal phenomenon that serves to eliminate cells that are no longer needed and to maintain a steady number of various cell populations*** *in tissues.*

1. ***The programmed destruction of cells during embryogenesis,*** including implantation, organogenesis, developmental involution, and metamorphosis.
2. ***Involution of hormone-dependent tissues upon hormone deprivation,*** *such as endometrial cell breakdown* during the menstrual cycle, and regression of the lactating breast after weaning.
3. ***Cell loss in proliferating cell populations,*** *such as* intestinal crypt epithelia, so as to maintain a constant number.

4. ***Death of cells that have served their useful purpose***, such as **neutrophils** in an acute inflammatory response,and **lymphocytes** at the end of an immune response. In these situations, cells undergo apoptosis because they are deprived of necessary survival signals, such as growth factors.

5.*Elimination of potentially harmful self-reactive lymphocytes,* either before or after they have completed their maturation, in order to prevent reactions against one's own tissues.

6.*Cell death induced by cytotoxic T lymphocytes*, *a* defense mechanism against viruses and tumors that serves to kill and eliminate virus-infected and neoplastic cells.

Apoptosis in Pathologic Conditions: *Apoptosis eliminates cells that are genetically altered or injured beyond repair without eliciting a severe host reaction, thus keeping the damage as contained as possible.*

1. ***DNA damage.***

- *Radiation, cytotoxic anticancer drugs,* extremes of temperature, and even hypoxia can damage DNA, either directly or via production of free radicals.
- If **repair mechanisms cannot cope with the injury, the cell triggers intrinsic mechanisms that induce apoptosis.**
- In these situations, elimination of the cell may be a better alternative than risking mutations in the damaged DNA, which may progress to malignant transformation.
- These injurious stimuli cause apoptosis if the insult is mild, but **larger doses of the same stimuli result in necrotic cell death.**

Inducing apoptosis of cancer cells is a desired effect of chemotherapeutic agents, many of which work by damaging DNA.

2. *Accumulation of misfolded proteins.*

- *Improperly* folded proteins **may arise because of mutations in the genes encoding these proteins or because of extrinsic factors, such as damage caused by free radicals.**
- Excessive accumulation of these proteins in the ER leads to a condition called ***ER stress****, which culminates in apoptotic* death of cells.

- *3.Cell injury in certain infections, particularly viral* infections, in which loss of infected cells is largely due to apoptotic death that may be induced by the virus (as in **adenovirus and human immunodeficiency virus infections**) or by the host immune response (as in **viral hepatitis**)
- 4.*Pathologic atrophy in parenchymal organs after duct obstruction, such as occurs in the pancreas, parotid* gland, and kidney.

Mechanisms of Apoptosis

- The fundamental event in apoptosis is the activation of enzymes called ***caspases*** *(so named because they are* ***cysteine proteases*** *that cleave proteins after aspartic* residues).
- Activated caspases cleave numerous targets, **culminating in activation of nucleases that degrade DNA and other enzymes that presumably destroy nucleoproteins and cytokeletal proteins**.

- The activation of caspases **depends on a finely tuned balance between pro- and anti apoptotic molecular pathways**.
- Two distinct pathways converge on caspase activation, called the ***mitochondrial pathway and the death receptor pathway***.

The Mitochondrial (Intrinsic) Pathway of Apoptosis

- Other **related sensors inhibit the anti-apoptotic molecules Bcl-2 and Bcl-xL (B-cell lymphoma-extra large)**, with the same end **result—the leakage of mitochondrial proteins**.
- **Cytochrome *c,*** *together with some* **cofactors, activates caspase-9,** while other proteins **block the activities of caspase antagonists** that function as physiologic inhibitors of apoptosis.
- The net result is the **activation of the caspase cascade, ultimately leading to nuclear fragmentation.**
- **If cells are exposed to growth factors** and other survival signals, **they synthesize antiapoptotic members of the Bcl-2 family, the two main ones of which are Bcl-2 itself and Bcl-xL.**
- These **proteins antagonize Bax and Bak,** and thus limit the escape of mitochondrial pro-apoptotic proteins.

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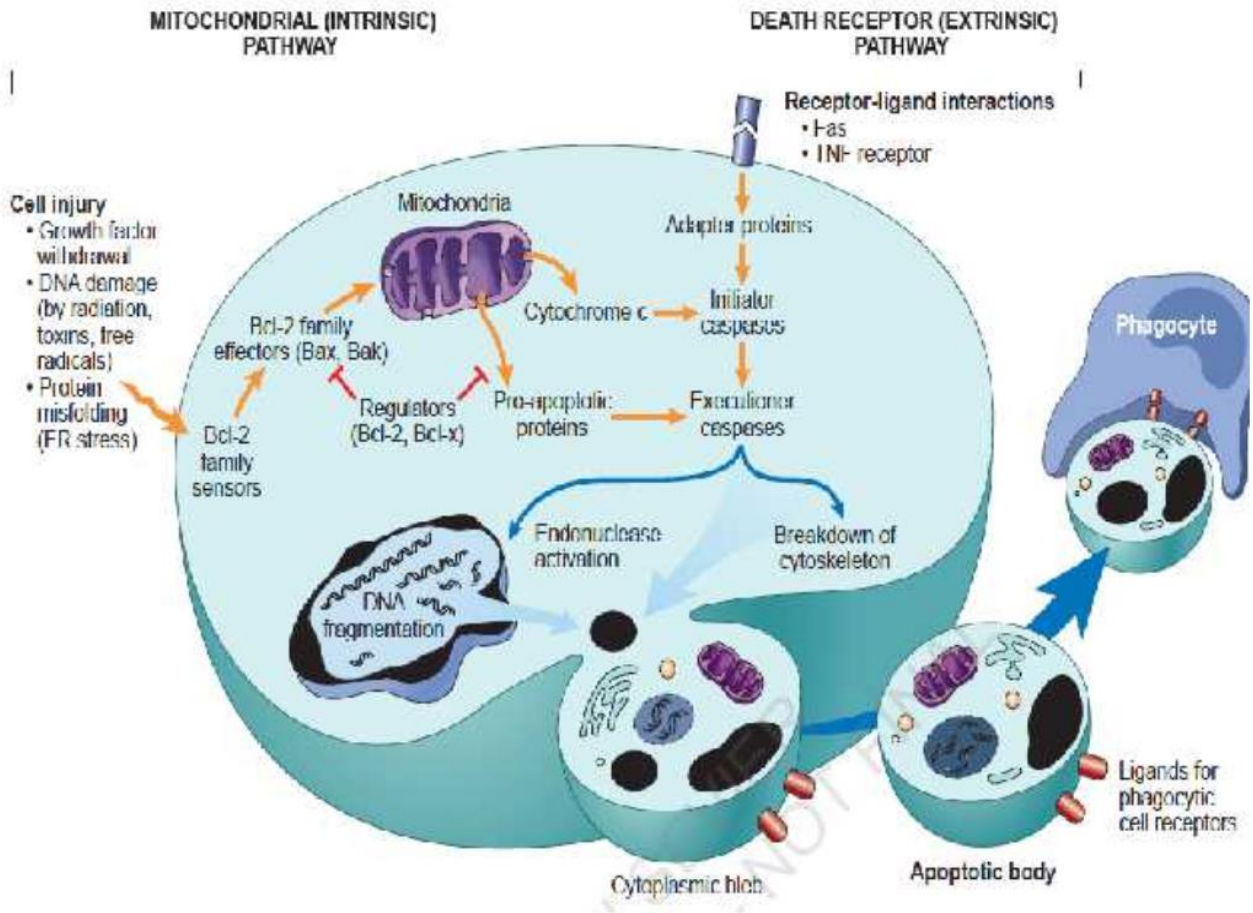


The Death Receptor (Extrinsic) Pathway of Apoptosis

- Many cells express surface molecules, called death receptors, that trigger apoptosis. Most of these are members of the tumor necrosis factor (TNF) receptor family that contain in their cytoplasmic regions a conserved "death domain," so named because it mediates interaction with other proteins.
- The prototypic death receptors are the type I TNF receptor and Fas receptor (CD95). Fas-ligand (FasL) is a membrane protein expressed mainly on activated T lymphocytes.
- When these T cells recognize Fas-expressing targets, Fas molecules are cross-linked by the FasL and they bind adapter proteins, which in turn bind caspase-8.
- Clustering of many caspase molecules leads to their activation, thus initiating the caspase cascade.

- ❑ In many cell types **caspase-8 may cleave and activate a pro-apoptotic member of the Bcl-2 family called Bid(BH3 interacting-domain)**, thus feeding into the mitochondrial pathway.
- ❑ The **combined activation of both pathways delivers** a lethal blow to the cell.
- ❑ The death receptor pathway is involved in elimination of **self-reactive lymphocytes** and in killing of target cells by some cytotoxic T lymphocytes.

Clearance of Apoptotic Cells

- **Apoptotic cells undergo** several changes in their membranes that promote their **phagocytosis.**
- In normal cells **phosphatidylserine is present on the inner leaflet of the plasma membrane, but in apoptotic cells this phospholipid "flips" out** and is expressed on the outer layer of the membrane, **where it is recognized by macrophages**.
- Cells that are **dying by apoptosis also secrete soluble factors** that **recruit phagocytes.**This **facilitates prompt clearance of the dead cells before they undergo secondary membrane damage and release their cellular contents (which can result in inflammation).**
- Some **apoptotic bodies express adhesive glycoproteins** that are **recognized by phagocytes**, and **macrophages** themselves may **produce proteins that bind to apoptotic cells (but not to live cells)** and target the dead cells for engulfment.

Examples of Apoptosis

1.Growth Factor Deprivation.

1. **Hormone-sensitive cells** deprived of the relevant hormone,
2. Lymphocytes that are not stimulated by antigens and cytokines,
3. Neurons deprived of nerve growth factor.

These conditions initiate apoptosis in those cells.

In all these situations, **apoptosis is triggered by the mitochondrial pathway and is attributable to activation of proapoptotic members of the Bcl-2 family and decreased synthesis of Bcl-2 and Bcl-xL**.

2.DNA Damage.

- **Exposure of cells to radiation or** chemotherapeutic agents induces DNA damage, and if this is too severe to be repaired it triggers apoptotic death.
- When DNA is damaged, the **p53 protein accumulates in cells**. It first **arrests the cell cycle (at the G1 phase) to allow time for repair** .
- However, if the damage is too great to be repaired successfully, **p53 triggers apoptosis,** mainly by activating **sensors that ultimately activate Bax and Bak**, and by **stimulating synthesis of proapoptotic members of the Bcl-2 family.**
- When **p53 is mutated or absent** (as it is in certain cancers), it is incapable of inducing apoptosis, so that **cells with damaged DNA are allowed to survive.** In such cells, the DNA damage may result in mutations or translocations that lead to neoplastic transformation .

3.Accumulation of Misfolded Proteins.

During normal protein synthesis, chaperones in the ER control the proper folding of newly synthesized proteins, and **misfolded polypeptides are ubiquitinated and targeted for proteolysis**.

If, however, **unfolded or misfolded proteins accumulate in the ER because of inherited mutations or stresses, they induce "ER stress"** that triggers a number of cellular responses, collectively called the ***unfolded protein response***. *This response activates signaling pathways* that increase the **production of chaperones** and **retard protein translation,** thus reducing the levels of misfolded proteins in the cell.

However, if this response is unable to cope with the accumulation of misfolded proteins,the **result is the activation of caspases that lead to apoptosis.**

- **Intracellular accumulation of abnormally folded proteins, caused by mutations, aging, or unknown environmental factors, is now recognized as a feature of a number of neurodegenerative diseases, including Alzheimer, Huntington, and Parkinson diseases, and possibly type II diabetes.**
- **Deprivation of glucose and oxygen, and stress such as heat, also result in protein misfolding, culminating in cell injury and death.**

4.Apoptosis of Self-Reactive Lymphocytes.

- **Lymphocytes** capable of recognizing self antigens are normally produced in all individuals.
- If these **lymphocytes encounter self antigens, the cells die by apoptosis**. Both the **mitochondrial pathway and the Fas death receptor pathway** have been implicated in this process .
- Failure of apoptosis of self-reactive lymphocytes is one of the causes of autoimmune diseases.

5.Cytotoxic T Lymphocyte–Mediated Apoptosis

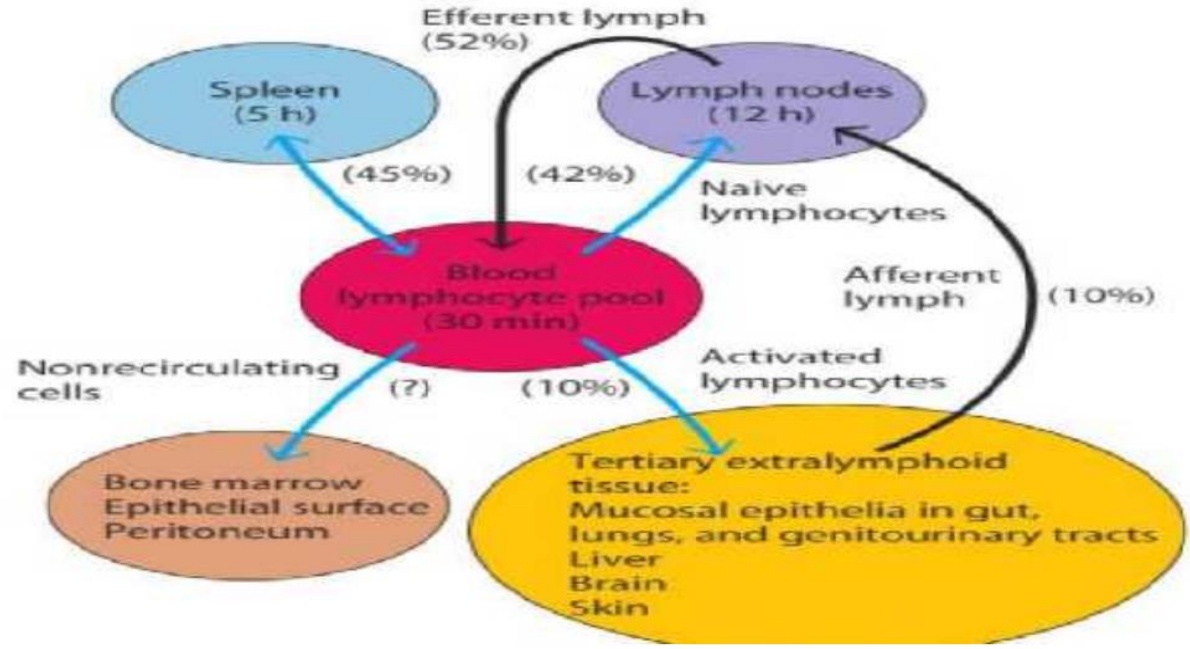
- **Cytotoxic** T lymphocytes (CTLs) recognize **foreign antigens presented on the surface of infected host cells and tumor Cells** .
- Upon activation, **CTL granule proteases called *granzymes*** *enter the target cells.* ***Granzymes*** *cleave* proteins at aspartate residues and are able to activate **cellular caspases**.
- In this way, the **CTL kills target cells by directly inducing the effector phase of apoptosis, without engaging mitochondria or death receptors**.
- CTLs also express FasL on their surface and **may kill target cells by ligation of Fas receptors**

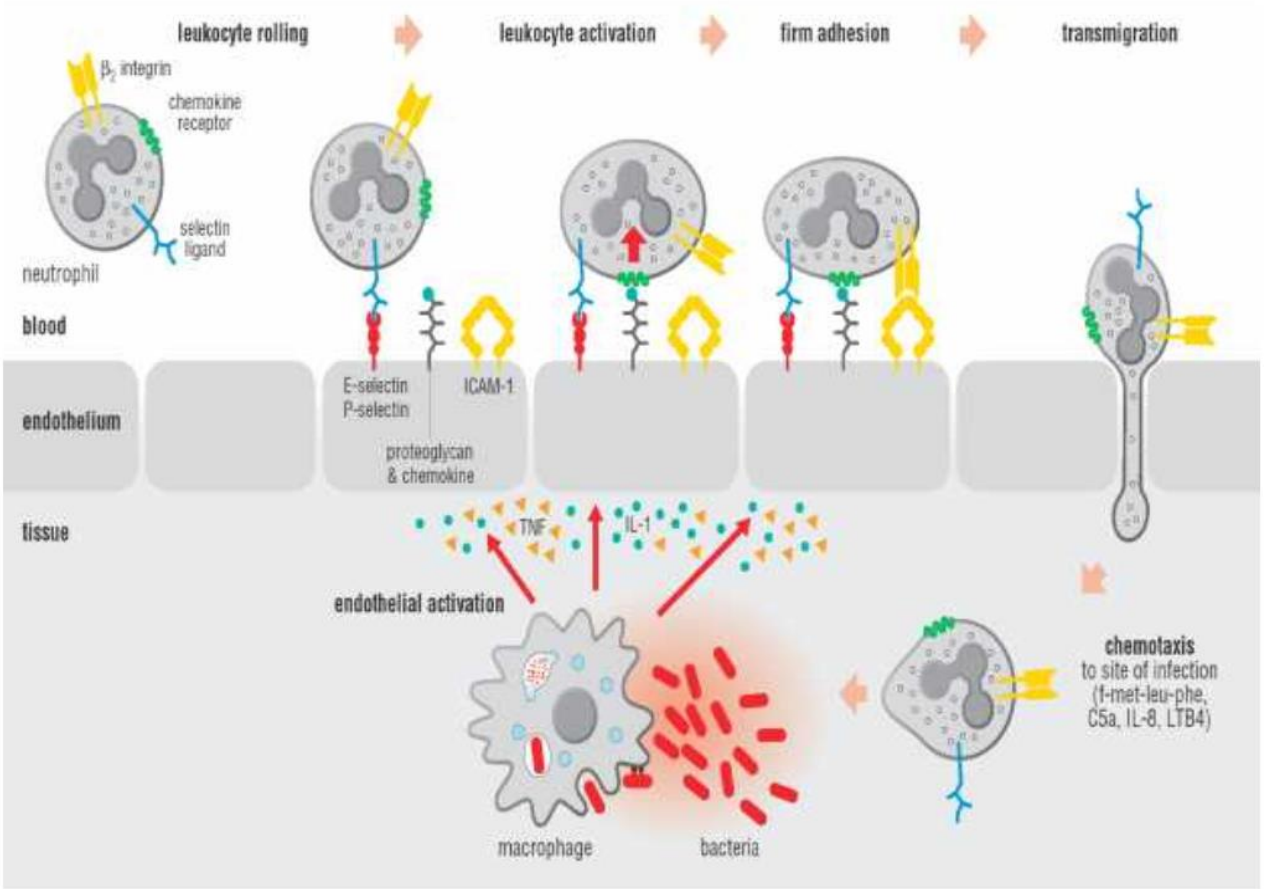
Inflammation-Innate Immunity

- Invaders infect our bodies
  - Cells of innate immune system arrive first
  - Adaptive immune response provides longer protection
  - Leukocytes are constantly monitoring for infection
    - When detected, cells cross the blood barrier and travel to site of infection

Lymphocyte recirculation

Lymphocytes continuously moving through the blood and lymph to various to various lymphoid organs.


Efferent lymph
(52%)
Spleen
(5 h)
Lymph nodes
(12 h)
(45%)
(42%)
Naive
lymphocytes
Blood
lymphocyte pool
(30 min)
Afferent
lymph
(10%)
Nonrecirculating
cells
(?)
(10%)
Activated
lymphocytes
Bone marrow
Epithelial surface
Peritoneum
Tertiary extralymphoid
tissue:
Mucosal epithelia in gut,
lungs, and genitourinary tracts
Liver
Brain
Skin


leukocyte rolling
leukocyte activation
firm adhesion
transmigration
β2 integrin
chemokine
receptor
selectin
ligand
neutrophil
blood
endothelium
E-selectin
P-selectin
ICAM-1
proteoglycan
& chemokine
tissue
TNF
IL-1
endothelial activation
chemotaxis
to site of infection
(f-met-leu-phe,
C5a, IL-8, LTB4)
macrophage
bacteria

- Tissue damage caused by a wound or by an invading pathogenic micro organism induces a complex sequence of events collectively known as the Inflammatory response.
- **Eg:LPS of G-ve Bacteria** trigger an inflammatory response via interaction with immune cell surface receptors.

Inflammation is a physiological process

1. Acute
2. **Chronic-disease persistent immune activation-has pathological consequences**

- In first century Roman Physician Celsus described the four cardial sign of inflammation:
- Rubor(redness)
- Tumor(swelling)
- Calor(heat)
- Dolor(pain)
- In second century another physician added a fifth sign
- Functio laesa(loss of function)

Inflammation

Tissue damage

- 1) **Release of Vasoactive and chemotactic Mediators** → histamine, serotonin, etc
- 2) **Vasodilation**: ↑diameter of capillaries, ↑blood flow
- 3) **Increased Vascular Permeability**: ↑ Leakiness from blood vessels → ↑ recruitment of cells and fluid → edema
- 4) **Extravasation of Phagocytes** – recruitment of leukocytes → Chemotaxis (chemokines; C3a/C5a, N-formyl peptides)

Mediators of Inflammatory response

1. Chemokines-Key mediators of inflammation.
2. Kinin system
3. Fibrin
4. Plasmin
5. Complement
6. Lipids
7. Cytokines
8. Acute phase proteins-c reactive protein

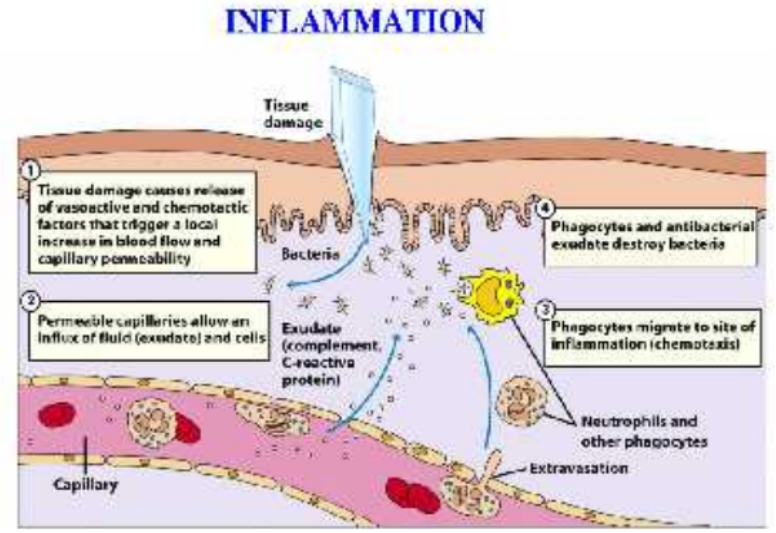
- Acute phase proteins-eg **Creactive proteins-If tissue damage is there means Produced by liver .**
- **Name coz of its recognition pattern-recognize C –polysaccharide cell wall component found on a variety of fungi and bacteria.This binding activates complement system-thus clears pathogen.**
- **Histamine-chemical released by cells(macrophages) in response to tissue damage,Histamine binds to receptors on nearby capillary and venules-cause vasodilation-increase permeability.**
- **Kinin-eg-Bradykinin-small peptides-(proform in blood plasma)-if tissue damage-vasodilation.**
- **Fibrin-component for blood clot-strands wall off the injured area from the rest of the body and serve to prevent the spead of infection.**
- Vasodilation
- An increase in the diameter of blood vessels of nearby capillaries occurs results in engorged capillaries are responsible for tissue redness and an increase in tissue temperature.

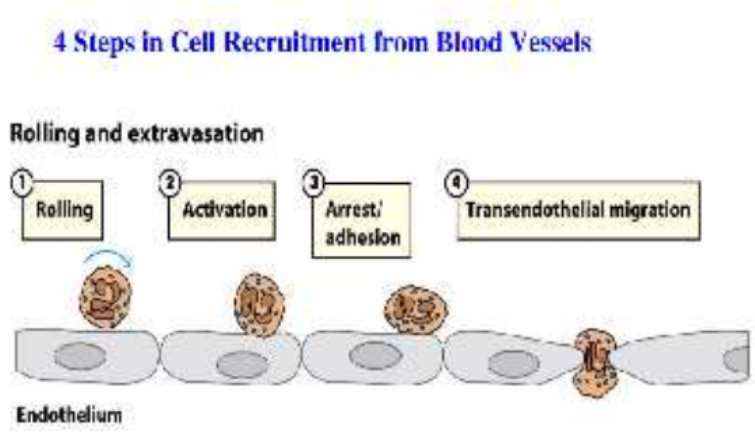
Increased vascular permeability

- Vascular permeability facilitated an influx of fluid and cells from the engorged capillaries into the tissue.
- The fluid accumulatess (Exudates) has much protein content (complement,c-reactive protein,antibody)than fluid of normal vascualture.
- Accumulation of exudates contribute to tissue swelling.(Edema)

Extravasation of phagocytes/diapedesis

- Influx of phagocytes facilitated by the increased permeability of the capillaries.
- The emigration of phagocytes is a multistep process :
- **Margination**:adherence of the cells to the endothelial wall of the blood vessels.
- **Extravasation**:emigration between capillary endothelial cells into the tissue.
- **Chemotaxis:**migration through the tissue to the site of the invasion.
- As the phagocytic cells accumulate at the site and begin to **phagocytose bacteria**,and release lytic enzymes which would damage nearby healthy cells.The accumulation of dead cells and digested material and fluids forms a substance called **pus.**





Role of CAM(cell adhesion molecules) in immune cell recruitment from blood vessels

1. The vascular endothelium serves as an important "**gate keepers"** regulating movement of blood borne immune molecules and cells into the tissues.
2. So in order to facilitate the leukocyte entry to injured tissue region the immune cells must adhere to and to pass between the endothelial cell linings of blood vessels .
3. Endothelial cell express leukocyte specific **cell adhesion molecules**

Types of CAM

1.Selectins(L(leukocyte),E,P(Endothelial cells)-membrane glycoprotein has a distal lectin like domain that enables the carbohydrate group(peptidoglycon) . Selectins primarily linked to **sialylated carbohydrate moieties, which are often linked to mucin** like molecules.
2.Mucins(by both endothelial and leckocytes)-group of serine and threonine rich proteins that are heavily glycosylated ,thus present sialylated carbohydrate ligands to **selectins.**
3.Integrins-Heterodinmeric proteins(alpha and beta) expressed by leckocytes and facilitate adherence to the vascular endothelium.
4.ICAM-Immunoglobulin CAM-1,2,3,VCAM-expressed on vascular endothelial cells bind integrin molecules.(MAdCAM-1 has both **Ig like domains** and **mucin like domains**-thus binds **integrins and selectins**)

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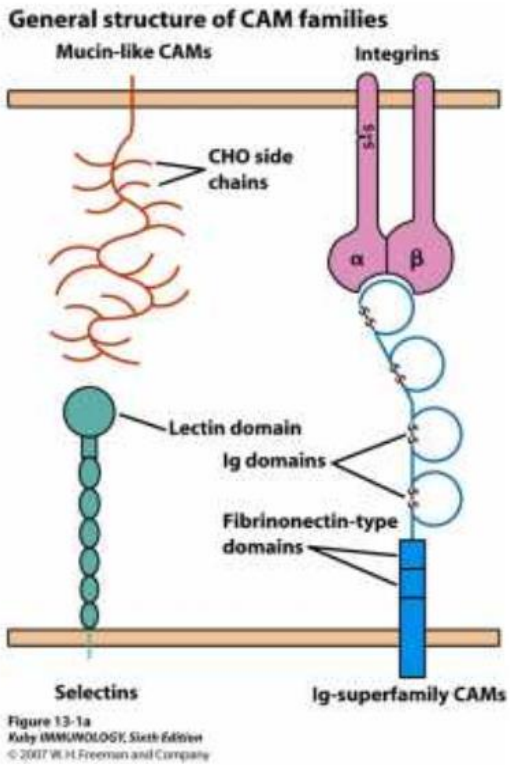

Selected CAMs belonging to each family

Mucin-like CAMs:	**Selectins:**
GlyCAM-1	L-selectin
CD34	P-selectin
PSGL-1	E-selectin
MAdCAM-1	
Ig-superfamily CAMs:	**Integrins:**
ICAM-1, -2, -3	α4β1 (VLA-4, LPAM-2)
VCAM-1	α4β7 (LPAM-1)
LFA-2 (CD2)	α6β1 (VLA-6)
LFA-3 (CD58)	αLβ2 (LFA-1)
MAdCAM-1	αMβ2 (Mac-1)
	αXβ2 (CR4, p150/95)

1. First step Neutrophils attach loosely to the endothelium by a low affinity selection carbohydrate interaction.During inflammatory response cytokines act upon endothelial cells and induce expression of CAM like selectins.Then selectins bind mucin molecules on the neurophil membrane.
2. Thus endothelial cells attach neutrophils(but soon get detached,eventhough another selectin attract neutrophil so neutrophil tumbles end over end along endothelium **(Rolling)**

2.As the neutrophil rolls it is activated by various chemo attractants (**any cytokines called chemokines- IL-8 and MIP-1beta** and **other chemoattractants- PAF-Platelet activatingfactor,complement split products-c5a,c3a,c5b67,N-formyl peptides of bacterial proteins**) and binds the leukocyte receptors and trigger activation of **G protein receptor** .

This signal enables the conformational changes in the **integrin** molecules in the neutrophil membrane and increasing the affinity for the **ICAM** on the endothelium.

3.Subsequent interaction between **integrins and ICAM** stabilizes the adhesion of neutrophils to the endothelial cells enables the neutrophils to adhere firmly to the endothelial cells.

4.Then neutrophils migrates through the vessels wall in to the tissues.Further **chemokines assist** to reach site of infection in tissue.

Chemokines

- Chemokine receptors on leukocytes mediate leukocyte activity