CELL INJURY

Rudolf Virchow – Father of Pathology- gave concept that disease begins at Cellular Level. As cell is the basic functional unit of life, any insult or injury to the cell through any of the internal or external causes could lead to Cell Injury.

Any normal cell lives in a relatively hostile environment. For example - the concentration of calcium ions outside the cell is 10,000 times higher than that inside. If all this calcium were to enter the cell, it will prove toxic, and kill the cell.

QUESTION: Define and Discuss Cell Injury and its Types

The normal cell has to live within a fairly narrow range of function and structure, so that it is able to handle its normal physiological demands, so-called normal homeostasis.. Somewhat more excessive physiological stresses, or some pathological stimuli or injury/insult, bring about **ADAPTATION** in cell. Cells constantly adapt to physiological demands to maintain a homeostatic steady state, by way of performing excess work, replicating, decreasing functions, changing its differentiated properties etc. That is, the cell modifies its structure and functions in response to changing demands and stresses, with objective to maintain homeostasis.

If the adaptive capability is exceeded, or in certain cases when adaptation is not possible, a sequence of regressive changes occurs,collectively known as cell injury. The fundamental pathogenesis of cell Inury is disturbance of homeostasis. Cell Injury is initiated at the molecular level, and basic mechanism is categorized as follows:

1. Hypoxia (including Ischemia) and Depletion of ATP
2. Permeabilization of cell membranes
3. Disruption of Biochemical Pathways, mainly of Protein Synthesis
4. DNA Damage

The term **CELL INJURY** is used to indicate a state in which the capacity for physiological adaptation is exceeded. This may occur when the stimulus is excessive or when the cell is no longer capable to adapt without suffering some form of damage. The capacity for adaptation and the sensitivity to different types of injury varies according to cell type (i.e. myocardial cells and neurons are highly sensitive to ischemic injury; hepatocytes are more sensitive to chemical than ischemic injury). Cell injury may be Reversible (non-lethal damage) or Irreversible (lethal damage). The Adaptation, Reversible injury, Irreversible injury, and Cell Death are states of progressive encroachment on the cell's normal function and structure.

On functional basis, the Cell Injury is classified into 2 types:

REVERSIBLE CELL INJURY: is a non-lethal injury or damage, where the injured cell can regain homeostasis and return to morphologically and functionally NORMAL State. It was previously referred as Degeneration.

Hypoxia, ATP Depletion, Damage to ion channels, Acute cell Swelling, Free radical led injury and cell membrane damage play important part in cell injury, and (if damage to plasma membranes/mitochondria) within certain limits, the injury is reversible and cells could return to a normal state.

If the injury is severe or sustained, with persistent stress, the cell reaches a 'point of no return', known as **IRREVERSIBLE CELL INJURY** and it most often leads to Cell Death. The transition between reversible and irreversible damage, commonly referred to as the "point of no return" is important to be understood in cell injury. Irreversible cell injury is associated with severe swelling of mitochondria, extensive damage to plasma membranes and swelling of Lysosomes. Due to high calcium levels, activated Phospholipases and accumulated free fatty acids, together cause changes in the permeability of the inner mitochondrial membrane, often called as **mitochondrial permeability transition.** ***Large 'Amorphous Densities' (Calcium rich)*** accumulate in the mitochondrial matrix. The falling pH (due to accumulation of lactic acid and inorganic phosphates) causes injury to the lysosomal membranes. This is followed by leakage of their lysosomal enzymes into the cytoplasm and activation of acid hydrolases leading to enzymatic digestion of cytoplasmic and nuclear components.

QUESTION: DISCUSS IN DETAIL THE MECHANISMS INVOLVVED IN CELL INJURY

MECHANISM of CELL INJURY

BIOCHEMICAL Mechanisms Involved in CELL INJURY

A. HYPOXIA and DEPLETION of ATP:

The major cause of ATP depletion is reduced supply of Oxygen and nutrients, Mitochondrial Damage and action of some Toxins. Depletion of ATP to less than 5% - 10% of normal levels has widespread effects on many critical cellular systems.

Hypoxia (loss of oxygen supply) must be differentiated from ischaemia which is a loss of blood supply. In contrast to hypoxia, during which glycolytic energy production can continue (although less efficiently than by oxidative pathways),

iscahemia affects the delivery of substrates for glycolysis (e.g., glucose) supplied by the flowing blood.

The first point of attack of hypoxia is the cell's aerobic respiration, *i.e.*, oxidative phosphorylation by mitochondria. As the oxygen tension within the cell decreases, there is loss of oxidative phosphorylation and decreased generation of adenosine triphosphate (ATP).

Due to depletion of ATP, the activity of Plasma membrane's "**Energy-Dependent Sodium Pump**" is reduced, resulting in intra-cellular accumulation of Sodium and efflux of Potassium. The net gain of Solute is accompanied by iso-osmotic gain in water, causing CELL SWELLING and Dilation of ER.

Decreased pH and ATP levels cause detachment of ribosomes from the granular endoplasmic reticulum and dissociation of polysomes into monosomes, causing consequent 'reduction in Protein Synthesis'. Ultimately, there is irreversible damage to mitochondria & lysosomal membranes and cell undergo NECROSIS.

Compensatory increase in **ANAEROBIC GLYCOLYSIS** in an attempt to maintain the cell's energy sources. As consequence, intracellular Glycogen Stores are rapidly depleted AND **LACTIC ACID Accumulates**, leading to the decreased **Intra-cellular pH** and Decreased activity of many Cellular Enzymes.

Failure of Ca+ Pump leads to the Influx of Ca+ with damaging effects on numerous cellular components, mainly plasma membranes and mitochondria, as described later.

B. INFLUX OF CALCIUM

Cytosolic Free Ca+ is maintained by ATP-dependent Ca-transporters, at concentration that are 10,000 times lower than the extra-cellular Ca+ Conc. By any damage. The INCREASED CYTOSOLIC Ca+ activates a Number of Enxymes with potentially deleterious effects . These Enzymes include (i) PhosphoLipase (cause membrane damage), (ii) Proteases (breakdown membrane & cytoskeleton proteins), (iii) Endonucleases (responsible for DNA & Chromatin Fragmentation), and (iv) Adenosine Phophatases (ATPases, depleting ATPS).

Increased Cytosolic Ca+ levels also result in Induction of release of "Mitochondria outer membrane Pores (MOMPs)", release of mitochondria Cytochrome-C and thereby APOPTOSIS by direct activation of the Caspase Enzymes due to increased mitochondrial permeability/damage.

C. DAMAGE to MITOCHONDRIA

These can be damaged by Increase in Intra-Cellular CYTOSOLIC Ca+, reactive Oxygen species, Oxygen deprivation, and virtually all types of injurious stimuli, including Hypoxia and Toxins. There are 2 major consequences of Mitochondrial Damage:

i. Mitochondrial damage results in formation of high-conductance Channels in Mitochondria membrane, called the MITOCHONDRIAL PERMEABILITY TRANSITION PORE. Opening of this channel, leads to Loss of Mitochondrial membrane Potential AND pH changes resulting in the failure of OXIDATIVE PHOPHORYLATION and Probgresisve depletion of ATP, culminating into Necrosis of cell.

ii. Mitochondria also contain several proteins, that are capable of activating Apoptotic Pathways, including Cytochrome-C. Increased Permeability of mitochondrial membrane may result in Leakage of these Proteins into the Cytosol and Death by Apoptosis. Thus, Cytochrome-C plays a key dula role in Cell Survival & Death. When Mitochondria are damaged so severely, that Cytochrome-C leaks out, it signals the cell to die.

D. ACCUMULATION OF OXYGEN DERIVED FREE-RADICALS (Oxidative Stress)

Free radical Chemical states are extremely unstable and readily react with inorganic / organic chemicals due to free electron in their outer orbit. When generated in cells, they avidly attack nucleic acids as well as variety of Cellular proteins & Lipids. In addition, molecules that react with these Free Radicals, further themselves in turn are converted to Free-radicals thus propagating a chain of damage.

Reactive Oxygen Species (ROS) are free-radicals that are produced normally in cells, during Mitochondrial Respiration and Energy generation, BUT are also removed routinely. When ROS production increases to high levels, its scavenging systems become saturated & ineffective, resulting in excess accumulation of Free Radicals, leading to a condition called OXIDATIVE STRESS. Main Free Radicals includes (i) Superoxides (O_2^-), (ii) Hydrogen peroxide (H_2O_2), (iii) Hydroxyl Radicals (OH^-), (iv) Singlet Oxygen (O), and (v) Nitric Oxide (NO).

The mechanism of cell injury *mediated by Free-radicals* includes:

i. **Lipid Peroxidation of Membranes**: Free radicals in the presence of oxygen may cause ***peroxidation of lipids*** in plasma and organellar membranes. Oxidative

damage begins when double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen derived free radicals, particularly by hydroxyl radicals (OH'). Double bonds are vulnerable to attack by free radicals. The lipid-radical interactions yield peroxides, which are themselves unstable and reactive. Therefore, an autocatalytic chain reaction follows (called propagation), which can result in extensive membrane, organellar and cellular damage.

ii. **Cross-Linking of Proteins**: Free radicals act on the sulphydryl bonds (-SH-HS-) of proteins and promotes Sulfhydryl-mediated Protein Cross-Linking, resulting in enhanced degradation of Stuctural Proteins or Loss of Enzymatic Activity. Cross-linking of proteins by the formation of disulphide bonds (-S-S-) in labile amino acids such as methionine, histidine, cystine, and lysine causes extensive damage throughout the cell.

iii. **DNA Fragmentation**: Free radical reactions with THYMINE in Nuclear and Mitochondrial DNA, produces single-strand breaks.This induces mutations in the genetic code. Such DNA damage has been implicated in both cell killing and malignant transformation of cells.

E. DEFECTS IN MEMBRANE PEMEABILITY:

It may be due to:

i. Decreased Phopholipid Synthesis: with fall in ATP, reduced phospholipid synthesis is there, affecting all Cellular Membranes.

ii. Increased Phospholipid Breakdown: Increased Degradation of Membrane Phospholipids by activation of ENDOGENOUS PHOSPHOLIPASES, by increased Cytosoli Ca+ levels.

iii. ROS / Free Radical Injury: causes injury to Plasma Mmbranes by Lipid Peroxidation

iv. Cytoskeletal Abnormalities: Cytoskeletal filaments serve as Anchors, connecting the Plasma Membrane to the Cell Interior. Activation of Proteases by increased Cytosolic Ca+ may cause damage to the elements of Cytoskeleton.

v. LIPID Breakdown Products including Unesterified Free fatty acids, Acyl Carnitine and Lysophospho-lipids, catabolicproducts etc that are known to accumulate in injured cells-as a result of Phospholipid degradation. They have a Detergent Effect on the Plasma membrane.

The most important site of MEMBRANE DAMAGE during Cell Injury is the Mitochondrial Membrane, Plasma Membranes and Membrane of Lysosomes.

MITOCHONDRIAL MEMBRANE DAMAGE: results in decreased ATP production, culminating in Necrosis; and release of Proteins and enzyme Cytochrome-C that triggers Apoptotic cell death.

PLASMA MEMBRANE DAMAGE: leads to the losss of osmotic Balance & influx of Fluid & ions, as well as loss of Cellular Contents.

LYSOSOMAL MEMBRANE DAMAGE: leads to leakage of Enzymes into Cytosol & activation of Acid Hydrolases in acidic intracellular pH conditions of injured cell. Lysosomes contain RNAases, DNAases, Proteases, Glucosidases that may lead to enzymatic digestion of cell components & Cell die by NECROSIS.

F. DAMAGE TO DNA & PROTEINS: If DNA / Protein damages are too severe to be corrected, the Cell Initiates its suicide program and dies by Apoptosis. Similar events takes place by Improperly folded proteins, that could result of either mutations or free-radical injury.

QUESTION: Write in Brief about **Cytoskeletal and Biochemical** Changes in Cell Injury

CYTOSKELETAL AND BIOCHEMICAL CHANGES IN CELL INJURY

The Cytoskeleton is a structural network that regulates SHAPE & MOVEMENT of the Cells and its Organelles, Cell Division and also Biochemical Pathways.

The Biomolecules including proteins and Enzymes mainly are involved in the Cell Signalling Pathways, that directly govern the Cell Hoemostasis and Controls Cell Growth, Division, and/or Cell Death.

The CYTOSKELETON consists of 3 integral components: (i) The ACTIN Filaments (6-7nm diameter), (ii) INTERMEDIATE Filaments (10nm), and , (iii) MICROTUBULES (upto 25 nm diameter).

Functions of most cell organelles require their interaction with cytoskeleton. The general concept is that:

i. **The Microfilaments** facilitate Cell Motility (eg: Amoebic movements, Chemotaxis, Cilia, Pseudopodia etc).
ii. **The Intermediate Filaments** facilitate the Physical Strength and Shape of Cell & Tissues, and often via Junction Complexes, and
iii. **The Microtubules**, move the Organelles and Vesicles within the Cytosol and Chromosomes too – via Mitotic Spindles during Cell Division.

It is widely known that it is the BIOCHEMICAL CHANGES that precede the Appearance of Lesions due to cell INJURY/Death. They lead to Functional disturbances – damaging the cell functionally (biochemically)- AND yet may have No Morphologic Alterations. Cell are injured by variety of Intrinsic / Extrinsic causes and these are likely to activate One or More of FOUR Final common BIOCHEMICAL MECHANISMS leading to the Cell Injury. These Fundamental Biochemical changes are:

1. ATP Depletion
2. Defects / Permeabilization of Cell Membrane
3. Influx of Calxium
4. Damage to Mitchondria
5. Oxidative Stress (Free-Radical accumulation)/ Disruption of Biochemical Pathways, and
6. Damage to DNA / Proteins.

The cellular derangement in 'reversible Injury' can be repaired & if such Injurious stimuli abates, the Cell will return to Normalcy. Persistent or Excessive injury, however causes cell to pass the nebulous "point of No return" into Irreversible Injury and/or Cell Death. Although there is non-definitive morphologic or biochemical correlates of IRREVERSIBILITY, There are TWO Phenomenon consistently characterize irreversibility i.e.

(i) The Inability to Reverse Mitochondrial Dysfunction (lack of Oxid. Phosphorylation & ATP generation), and

(ii) Profound disturbance in Membrane Functions.

QUESTION: Write in Brief about **major Sub-Cellular Responses** to Cell Injury

The Major Sub-cellular Response to cell Injury are:

1. **AUTOPHAGY**: it refers to Lysosomal digestion of cell's own components and is contrasted with Heterophagy in which cell ingests substances from outside. Autophagy is a survival mechanism in times of Nutrient Deprivation. In this process, Intracellular Organelles and portions of Cytosol are first sequestrated from Cytoplasm in an **Autophagic-vacoule**, formed from Ribosome-free regions of the rough-ER. The vacuole fuses with Lysosomes to form an **Autophagolysosomes**, and and the Cellular Components are digested by Lysosomal Enzymes. Autophagy is initiated by several proteins that sense the Nutrient deprivation.

The enzymes in Lysosomes, can breakdown most proteins and CHO, although some Lipids remain undigested. Lysosomes with undigested debris may persist within Cells, **as RESIDUAL BODIES** or may be extruded. **LIPOFUCHSIN Pigment Granules** represent undigestible material resulting from Free-radical mediated Lipid Peroxidation.

Hereditary Lysosomal Storage Disorders caused by Deficiency of Enzymes that degrade various macromolecules, result in abnormal collection of intermediate metabolites in Lysosomes of cells, all over the Body.

2. **INDUCTION OF Smooth Endoplasmic Reticulum**: The sER is involved in metabolism of various chemicals and cells exposed to these chemicals show hypertrophy of ER as an Adaptive Response.

Eg.: Barbiturates are metabolized in Liver by – Cytochrome P 450 MFO System – found in the sER. Protracted use of barbiturates, leads to state of tolerance, with decrease in Drug effect AND need to use Increased dosage. This adaptation is due to increased volume / Hypertrophy of sER of hepatocytes and increased P-450 activity.

3. **MITOCHONDRIAL ALTERATIONS**: these play important role in Acute Cell Injury and death.
   - In the Cellular Hypertrophy - there is Increase in No. of Mitochondria in Cells;
   - In Cellular Atrophy – Mitochondria Nos. decrease in cells.

   Mitochondria may assume extremely large and Abnormal Shapes (Megamitochondria) as seen in Hepatocytes in various Nutritional Deficiency and Alcoholic Liver Diseases.

4. **CYTOSKELETAL ABNORMALITIES**: The cytoskeleton is important for various functions.
   - ✓ Cell Mobiltiy & Intracellular transport of Organelles;
   - ✓ Maintenance of Cell Architechture & Maintenance of Mechanical Strength for tissue integrity;
   - ✓ Transmission of Cell- Cell and Cell-Extracellular matrix signals to the Nucleus;;
   - ✓ Phagocytosis;

Abnormality of Cytoskeleton occurs in various Pathologic states. These may be manifested as Abnormal Appearance & Function of cells, Aberrant movement of intracellular organelles, Defective Cell Locomotion or Intracellular Accumulation of Fibrillar material.

Disturbed organization of Microtubules can lead to Sterility by inhibiting Sperm Motility. Defective mobility of Respiratory Cilia leads to Chronic Respiratory Infections.

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The cellular response to injury depends on:

(1) the type of cell injured and its susceptibility and/or resistance to hypoxia and direct membrane injury and

(2) the nature, severity, and duration of the injury.

As examples, neurons, cardiac myocytes, endothelium, and epithelium of the proximal tubule of the kidney are cells that are extremely susceptible to hypoxia, whereas fibroblasts, adipocytes, and other mesenchymal structural cells are less susceptible.

The response to injury can be degenerative, adaptive, or completely reversible with restoration of normal structure and function for the affected cell; **however, with more severe or persistent injury**, acute cell swelling can progress to irreversible cell injury and cell death.

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QUESTION: Write Short Notes on **(i)** Heat Shock Proteins / Chaperones, **(ii)** Acute Phase Proteins.

ACUTE PHASE PROTEINS

Under the influence of IL-1, IL-6, and TNF-alpha, **Liver Cells** synthesize and secrete acute-phase proteins. Because their synthesis accompanies acute infections and inflammation and also because their concentrations rise very rapidly, they are known as **'acute-phase proteins'**.

Acute phase proteins are plasma proteins synthesized in the liver whose concentrations increase (or decrease) by 25% or more during inflammation. These proteins serve as inhibitors or mediators of the inflammatory processes and include **C-reactive protein (CRP), serum amyloid-A (SAA), Serum Amyloid P (SAP), α1-acid glycoprotein, haptoglobin, mannose-binding protein, Fibrinogen, α1-antitrypsin, and complement components C3 and C4**. The concentration of these acute phase proteins usually increases during inflammation, whereas the concentration of prealbumin, albumin (also acute phase proteins) and trasferrins decreases in inflammation and these are therefore also known as Negative acute-phase proteins..

CRP is the major acute-phase protein in humans, monkeys, pigs, rabbits and dogs, whereas SAP is the major protein in mice. CRP is not an acute-phase protein in cattle or horses, even though it is found in normal serum. **Fibrinogen** concentration in the blood of cattle is used clinically as an indicator of systemic inflammation.

Once concentrations of acute phase proteins and systemic concentrations of inflammatory cytokines are elevated, they affect the heart rate, blood pressure, and the hypothalamic regulation of temperature by directly or indirectly stimulating neurons within specific hypothalamic nuclei.

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Physiological adaptations (adjustments) usually represent responses of cells to normal stimulation by hormones or endogenous chemical substances, for example, enlargement of the mammary gland (breast in humans) and induction of lactation by pregnancy. **Pathological adaptations** share the same underlying mechanisms but they allow the cells to modulate (change) their internal environment, and thus escape injury. Cellular adaptation, then, is a state that lies between the normal (unstressed) cell and the injured (over-stressed) cell.

HEAT SHOCK PROTEINS (HSPs) / Chaperones:

One of the most important adaptive responses within the cell is **formation of stress proteins** after injurious stimuli. These were originally called 'heat shock proteins (HSPs)' as these are observed after slight rises (4° to S°C) in temperature associated with process of acute inflammation, but now this term is misnomer..

In any normal cell, newly synthesized polypeptide chains of proteins made on ribosomes are arranged, either into alpha helices or beta sheets. Their proper arrangement (protein folding) is extremely important for the function of individual protein and its transport across the cell organelles. It is here that heat shock proteins play important roles in protein folding and in the transport of proteins into various intracellular organelles (protein kinesis). Thus, HSPs are also called **CHAPERONES** (*French word; chaperones (HSPs) look after proteins within the cell*).

In the normal process of protein folding, partially-folded intermediates are formed and these are very prone to form intracellular aggregates among themselves, or by involving with other proteins. These intermediates are stabilized by a number of molecular chaperones that interact with proteins directly. Some HSPs are synthesised physiologically in cell, eg **Hsp60** and **Hsp90.** However others are formed after injurious stimuli, e.g. **Hsp70** - protect (rescue) shock-stressed proteins from misfolding.

Thus, chaperones (HSPs) help in (i) proper folding of proteins and (ii) in their transport across the endoplasmic reticulum (ER), Golgi complex, and (iii) handling the denatured/damaged proteins. The Acute-Phase Proteins (Chaperones) handle the damaged (denatured) protein in two ways:

i. By re-folding the damaged proteins to restore their function before they can cause serious cell dysfunction or death, and

ii. When re-folding is not successful, permanently damaged proteins are bound to the **ubiquitin HSP molecule**. Ubiquitin binding makes these proteins as targets for intracellular degradation (destruction) by **proteasomes**, (a particulate cluster of non-lysosomal proteinases).