GENERAL PATHOLOGY

Rudolf Virchow is known as the Father of Modern Pathology

CELL INJURY

Homeostasis:

- Normally, cell function requires a balance between physiological demands and the constraints of cell structure and metabolic capacity; the result is a steady state or homeostasis.

Causes of cell injury

1.O_2 deprivation :Most important **Hypoxia**. Hypoxia can be due to ischemia, inadequate oxygenation (e.g., cardiorespiratory failure) or loss of oxygen carrying capacity of blood.

2. Physical agent – mechanical trauma, extremes of temperature (burns and deep cold),radiation, and electric shock
3. Chemical agents: arsenic, cyanide, or mercuric salts,
4. Infectious agents: viruses, rickettsiae, bacteria, fungi, and higher forms of parasites
5. Genetic derangements: cell injury because of deficiency of functional proteins, such as enzyme defects in inborn errors of metabolism, or accumulation of damaged DNA or misfolded proteins, both of which trigger cell death when they are beyond repair
6. Nutritional imbalance: Protein-calorie deficiencies

- In case of alteration in demands / foreign stimulus, cell elicits a CELLULAR RESPONSE

Cell Response to injury:

Nature of Injurious Stimulus	Cellular Response
ALTERED PHYSIOLOGICAL STIMULI;	**CELLULAR ADAPTATIONS:**

SOME NONLETHAL INJURIOUS STIMULI: • Increased demand, increased stimulation (e.g., by growth factors, hormones) • Decreased nutrients, decreased stimulation • Chronic irritation (physical or chemical)	• Hyperplasia, hypertrophy • Atrophy • Metaplasia
REDUCED OXYGEN SUPPLY; CHEMICAL INJURY; MICROBIAL INFECTION: • Acute and transient • Progressive and severe (including DNA damage)	**CELL INJURY:** • Acute reversible injury Cellular swelling fatty change • Irreversible injury → cell death -Necrosis -Apoptosis
METABOLIC ALTERATIONS, GENETIC OR ACQUIRED; CHRONIC INJURY	**INTRACELLULAR ACCUMULATIONS; CALCIFICATION**
CUMULATIVE SUBLETHAL INJURY OVER LONG LIFE SPAN	**CELLULAR AGING**

Determinants of Cell Injury:

- Type of injury
- Duration of injury
- Severity of injury
- Type of cell injured
- State and adaptability of cell

Intracellular mechanisms of cell Injury:

1. ATP depletion
2. Irreversible mitochondrial damage
3. ↑ intracellular Ca++
4. Free radial induced damage
5. Membrane permeability errors

Reversible Cell Injury	Irreversible Cell Injury
Morphological changes associated with reversible injury are • Cell swelling/hydropic change/cloudy swelling/vacuolar degeneration • Fatty change **<u>Mechanism of Reversible Cell Injury</u>** 1) Decreased synthesis of ATP by oxidative phosphorylation leads to decreased function of Na^+K^+-ATPase membrane pumps leading to influx of Na^+ and water into the cell and efflux of K^+ from the cell. The result is cellular swelling (hydropic swelling or cloudy change). 2) Switching to anaerobic glycolysis occurs due to lack of ATP synthesis by oxidative phosphorylation which results in depletion of cytoplasmic glycogen and increased lactic acid production. 3) Increased lactic acid lowers intracellular pH and leads to clumping of nuclear chromatin. 4) Lack of ATP results in detachment of ribosomes from the rough endoplasmic reticulum. This results in decreased protein synthesis and lipid deposition. 5) Plasma membrane blebs and myelin figures may be seen.	**Morphological changes** associated with irreversible injury is ***necrosis or apoptosis***. **<u>Mechanism of Irreversible Cell Injury</u>** Two important phenomena that consistently characterize irreversibility are severe mitochondrial dysfunction and increased cell membrane permeability. a) Severe membrane damage leads to ***massive influx of calcium and efflux of intracellular enzymes a***nd proteins into the circulation. b) Marked mitochondrial dysfunction is associated with mitochondrial swelling and formation of ***large flocculent densities*** within the mitochondrial matrix. There is irreparable damage of the oxidative phosphorylation pathway and inability to produce ATP. c) Rupture of the lysosomes occurs resulting in release *of lysosomal acid hydrolases* followed by autolysis. d) Nuclear changes seen are I. **Pyknosis:** degeneration and condensation of nuclear chromatin H. I. **Karyorrhexis:** nuclear fragmentation I. **Karyolysis:** dissolution of the nucleus

Profound membrane damage is due to

1) Mitochondrial dysfunction that results in decreased phospholipids synthesis, which affects all cellular membranes including the mitochondria.
2) Loss of membrane phospholipids due to activation of phospholipases.
3) Cytoskeletal abnormalities due to elaboration of proteases.
4) Reactive oxygen species that lead to lipid peroxidation of membranes.
5) Lipid breakdown products which have a detergent action on membranes.

Free radical damage:

- Definition – Molecules with single unpaired electron in outer obit
- Oxidative stress – imbalance between free radical generating and scavenging system

- Contributes to chemical & radiation injury, ischemia – reperfusion injury, cell ageing
- Reactive Oxygen Species (ROS) – O_2 H_2O_2, OH
- Free radical formation occurs within cells by
    1. Reduction / Oxidation reaction in body (physiological)
    2. Absorption of radiant energy → H_2O →H + OH
    3. Transition metals – Fe, Cu Fenton reaction : $H_2O_2 + Fe_2 + Fe_3^+ + OH^- + OH^-]$
    4. Metabolism of exogenous drugs → eg. $CC1_4 \rightarrow CC1_3$
    5. Nitric oxide (NO) – ONOO, NO_2, NO_3

Effect on cell by

a) Lipid Peroxidation of membranes → auto catalytic reaction
b) Oxidative change in protein with protein fragmentation
c) DNA breaks
d) Promote mitochondrial membrane permeability transition

Free radical removal mechanisms :

Antioxidants either block the initiation of free radical formation or inactivate (e.g., scavenge) free radicals

Enzymatic	Non enzymatic
• ***Glutathione peroxidase***^{Q}catalyzing free radical breakdown (H_2O_2 + 2GSH ➞GSSG [glutathione homodimer] + $2H_2O$, or 2OH + 2GSH ➞GSSG + $2H_2O$). • ***Catalase***^{Q}**p**resent in peroxisomes, decomposes H_2O_2 ($2H_2O_2$➞O_2 + $2H_2O$ • ***Superoxide dismutases*** **(SODs)**^{Q}in mitochondria converts superoxide ion to H_2O_2	• vitamin E, &A^Q • vitamins C ^{Q} • carotene, • ubiquinone, • uric acid • dietary polyphenols • selenium • glutathione^Q • proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin),
Except in people who were initially deficient, intervention trials *of vitamin E and beta-carotene have generally shown increased mortality among those taking the supplements*. beta-Carotene is only an antioxidant at low concentrations of oxygen; at higher concentrations of oxygen it *is an autocatalytic pro-oxidant*. Vitamin E forms a stable radical that is capable of either undergoing reaction with water-soluble antioxidants or penetrating further into lipoproteins and tissues, so increasing radical damage.	

Ischemic Injury / Hypoxic Injury

- Ischemia tends to injure tissue faster than hypoxia as glycolysis is also impaired unlike in hypoxia
- Most susceptible cell to ischemic injury →Neurons
- If cells can recover after recover after removal of ischemia → reversible injury

Reperfusion / Free radical Injury

Reperfusion causes death of cells which might have recovered otherwise by

a) Generating oxygen free radical
b) Promote mitochondrial permeability transition by reactive oxygen species
c) Recruitment of polymorphonuclear leucocytes in injured tissue →inflammation
d) Complement mediated damage via IgM
e) Myocardial / cerebral infarct – Ischemic reperfusion injury

Chemical Injury

a) Direct Injury →eg.
   1) mercury →affect GIT / Kidney
   2) cyanide →inhibits mitochondrial cytochrome oxidase

b) Indirect : Due to modification by P450 oxidase

- Covalent binding to membrane protein – acetaminophen
- Free radical → Lipid Peroxidation of membrane phospholipids
  Eg CC14 results in fatty change (damage to RER membrane - ↓ apoprotein synthesis
  → lipid accumulation)
  Hepatocyte death (damage to cytoplasmic membrane → irreversible injury

Irreversible Cell Injury:

A. Necrosis	**B. Apoptosis**

Necrosis:

- Spectrum of morphologic changes that follows cell death in living tissue
- Involves denaturation of intra – cellular proteins and enzymatic digestion of cells
- Morphology - Increased eosinophilia of cytoplasm
-EM – discontinuous membranes , large densities in mitochondria
- Nuclear changes – Karyolysis / Karyorrhexis / Pyknosis

Patterns of Tissue Necrosis:

Coagulative necrosis:

- Characterized by protein denaturation and preservation of cellular and tissue framework.
  - Preservation of outline of cells – TOMB STONE appearance
- It is seen in solid organs like heart, kidney and spleen and is characteristic of hypoxic death in all tissues **except brain.**

Liquefactive necrosis:

- Occurs when enzyme digestion predominates.
- It can be due to autolysis or heterolysis.
- Total destruction of cells, No outline of cells
- Seen in brain, abscess and wet gangrene.

Caseous necrosis:

- White cheesy area of necrosis
- On microscopy – amorphous granular debris of fragmented cells with in a granuloma
- Seen in tubercular infections.

Fat Necrosis:

- Lipases → breakdown of triglyceride / esters to fatty acids → combine with calcium (saponification)
- Necrotic fat cells with basophilic calcium deposits
- E.g. acute pancreatitis, breast

Fibrinoid necrosis:

Necrotic tissue that histologically resembles fibrin and microscopically has an eosinophilic (pink) homogenous appearance. Seen in blood vessels in malignant hypertension and vasculitis. It is also seen in Aschoff nodules in Rheumatic heart diseases, rheumatoid nodules and in Peptic ulcer.

Gangrenous necrosis-gross term used to describe dead tissues

Common sites: lower limbs, GI tract, testes.

Types

1. Dry gangrene: microscopic pattern is coagulative necrosis
2. Wet gangrene: microscopic pattern is liquefactive necrosis

APOPTOSIS

- Apoptosis is ***programmed***[Q]cell death
- Word **"apoptosis" is** named after the Greek designation for : "**Falling off**"[Q]

Physiological	Pathological
• Embryogenesis • Hormone dependent involution – menopause, lactating breast • Death of Immune cells (T/B cells) , Neutrophils • Self reacting lymphocytes • Cell deletion in rapidly dividing cells with DNA damage (e.g. intestinal epithelia)	• Cell injury- viral diseases eg. Councilman bodies in viral hepatitis • Heat, hypoxia, radiation etc. • Cell death in tumor

Morphology :

- Cell shrinkage
- **Chromatin condensation and DNA fragmentation**[Q]**(**The *chief morphologic* features of apoptosis)
- Cellular blebbing and fragmentation into apoptotic bodies
- Phagocytosis of apoptotic bodies by macrophages

On H/E

a) Round oval mass of intensely eosinophilic cytoplasim with dense nuclear chromatin
b) Absence of *inflammatory response*[Q]

Biochemical Alteration

1. Protein cleavage (hydrolysis) in apoptosis involves activation of : **caspases (cysteine proteases)**[Q][cleave nuclear & Cytoskeletal proteins]
2. Protein cross linking – transglutaminase
3. DNA cleavage at inter – nucleosomal position by endonuclease into 180-200 bp long fragments → seen **as DNA step Ladder pattern on agarose gel electrophoresis (cf-necrosis – diffuse smear pattern**). (Biochemical hallmark of apoptosis : Fragmentation **of DNA into 180-200 bp fragments**[Q])

4. Phagocytic recognition of apoptosis cells occurs because of surface expression of : **phosphatidylserine and thrombospondin**[Q]

- Gene playing a regulatory role in induction of apoptosis is : **P53**[Q]
- Major breakthrough in knowledge of apoptosis came from observations made in : **caenorrhabditiselegans (nematode)**
- Fraction of cells in apoptotic pathways can be assessed by : **In situ DNA nick end labeling**[Q]

- The extrinsic (Death-receptor-initiated) pathway of apoptosis is initiated by 2 important receptors – **Type-1 TNF receptor (TNFR-1) and FAS (CD 95)**[Q]
- Extrinsic pathway of apoptosis is inhibited by a protein called: **FLIP**[Q]

Anti-Apoptic(Prevent cell death)	Pro-Apoptic(Promote cell death)
• Bcl-2 [Q] • Bcl-x,[Q] • Mcl-1	• Bim, • Bid, • Bad[Q], • Puma, • Noxa

	• p53,[Q]

- Caspase – 8 and caspase – 9 are : **initiator caspases[Q]**
- Caspase -3 and caspase – 6 are **: executioner caspases[Q]**
- **Cytosolic cytochrome – C** is used in elution chain in : Apoptosis
- In apoptosis, **Apaf – 1 is activated by release of : Cytochrome – C[Q]**
- Gene which serves as critical "Life or death" switch in the case of genotoxic stress
- DNA agarose – gel electrophoresis – identify apoptotic cells by '**ladder pattern"[Q](but in necrosis,smearaed pattern is seen)**
- ***Annexin V[Q]***staining is commonly used to identify apoptotic cells

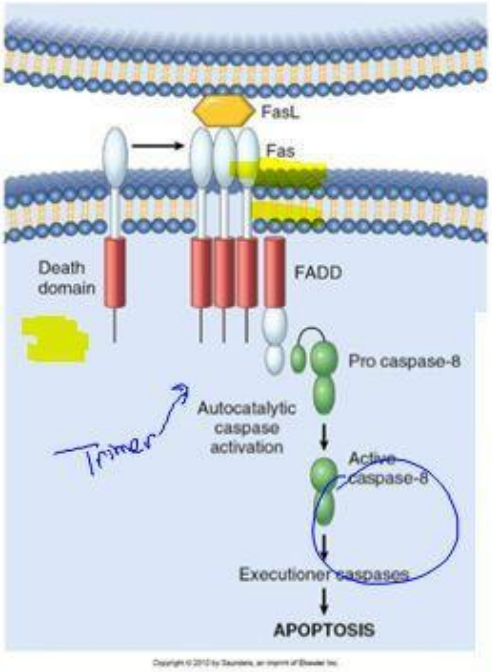
Mechanism of apoptosis - 3 stages

- **Initiation phase** – initiator / upstream caspases
  - extrinsic (death receptor associated) pathway
  - Intrinsic (mitochondrial) pathway
- **Execution phase –** executioner / downstream caspases
- **Removal of dead cells**

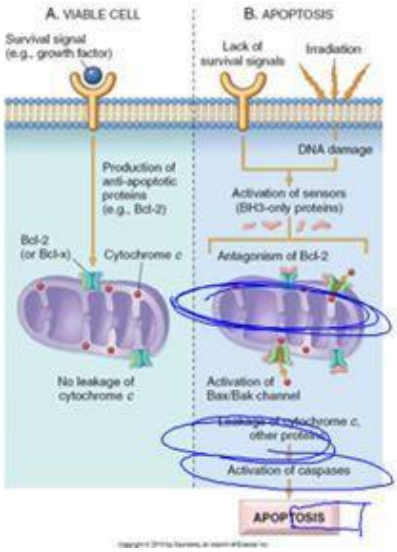
Extrinsic pathway:

- Death receptors contain death domains on cytoplasmic side
- Ligand binding activates Caspases 8 and 10
- E.g. Fas (CD95) via FADD, TNF-R1 via TRADD and then by FADD
- FLIP is a protein which inhibits apoptosis as it fails to activate pro-Caspase 8

Intrinsic pathway



Apoptosis:
Extrinsic pathway



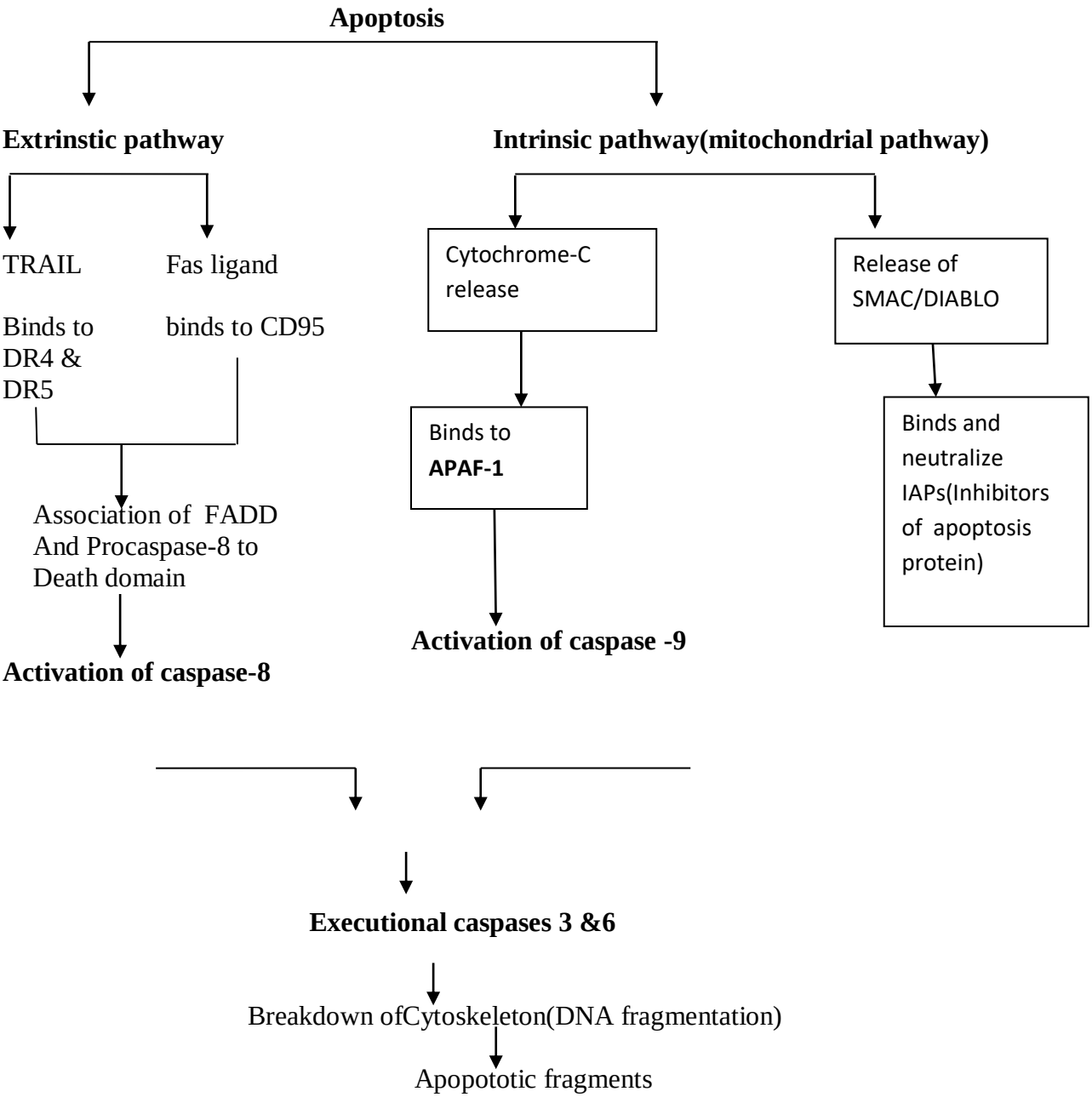
Apoptosis:
Intrinsic (mitochondrial) pathway

Robbins, fig. 1-25, p. 29

- Normally, growth factors stimulate production of anti apoptotic proteins e.g. bcl-2, bcl-x
- Lack of growth factors replaces them in mitochondrial membrane by pro-apoptotic factors e.g bak, bax, bid, AIF
- Increased mitochondrial permeability releases cytochrome c into cytoplasm
- Involves caspase 9

Execution phase.

- Execution Caspases – 2,3,6
- Cleave Cytoskeletal proteins
- Activates endonucleases



Examples :

1) Apoptosis after growth factor deprivation – mitochondrial pathway
2) Radiation → DNA damage →p53 activation of execution caspases by ↑ bax, bad etc.
3) FAS – FAS ligand →removal of activated lymphocytes from immune system.

4) TNFR1 – proapoptotic → by binding TRADD & FADD (adapter proteins)
   - Anti apoptotic (usually) – binding TRAFF (Adapter protein) + NFkB.
5) Cytotoxic T cell → perforin granzyme mediated or FasL expression

Dysregulated Apoptosi

Inhibited apoptosis - a) Cancer – P53 mutation, bc12 over – expression

b) Autoimmune disease

1) Increased apoptosis a) Neurodegenerative disease e.g. spinal muscular atrophy
b) Ischemic injury
c) Virus induced lymphocyte depletion eg. AIDS

Differences between apoptosis and necrosis

Necrosis	Apoptosis
• Adjacent inflammation is frequent • Plasma membrane disruption present • Cell size is enlarged • Nuclear changes are Pyknosis→ karyorrhexis→ karyolysis • Has a pathological role	• Adjacent inflammation is not seen • Plasma membrane intact • Cell size is shrunken • Fragmentation into nucleosome size fragments • Often physiologic, may be pathologic after some forms of cell injury

Cellular Adaptive Response to Injury:

Cellular adaptation is the result of a persistent stress or injury

Adaptive responses are

A. **Hyperplasia**:

An increase in the number of cells in a tissue or organ
Cause of hyperplasia
a. Physiologic causes of hyperplasia
- Compensatory (e.g., after partial hepatectomy)
- Hormonal stimulation (e.g., breast development at puberty)
- Antigenic stimulation (e.g., lymphoid hyperplasia)

b. Pathologic causes of hyperplasia
- Endometrial hyperplasia

Some cell type unable to exhibit hyperplasia (e.g., nerve, cardiac, skeletal muscle cells)

B. **Hypertrophy**

An increase in size and functional ability due to increased synthesis of intracellular components.

Cause of hypertrophy:

Increased mechanical demand

- Striated muscle of weight lifters
- Puberty (growth hormones, androgens/estrogens, etc.)
- Lactating breast (Prolactin and estrogen)
- Gravid uterus, (estrogen) hypertrophy and hyperplasia can occur together in gravid uterus

C. **Atrophy**-

Decrease in cell size and functional ability

Causes of atrophy:

- Decreased workload/disuse e.g. limb in a plaster cast
- Decreased blood supply e.g. ischemia (atherosclerosis).
- Lack of hormonal/neural stimulation e.g. muscle paralysis is polio
- Malnutrition

Aging

Microscopy: small shrunken cell with Lipofuscin granules-Brown atrophy

EM: Decreased intracellular components and increased autophagosomes.

D. **Metaplasia**:

A reversible change of one cell type to another, usually in response to irritation. It has been suggested the replacement cell is better able to tolerate the environment stresses

Example: Bronchial epithelium undergoes squamous metaplasia in response to the chronic irritation of tobacco smoke. Proposed mechanisms: the reserve cells (stem cells) of the irritated tissue differentiate into a more protective cell type due to the influence of growth factors, cytokines and matrix components.

INTRACELLULAR ACCUMULATIONS

Types:

a. Normal—water, fat, carbohydrates, proteins

b. Abnormal—mineral or infectious agents, pigments

- **Fatty change**—Deposition of triglycerides seen in liver, heart, kidney and muscle. Caused by toxins (alcohol, CC14), PEM, obesity, diabetes, anoxia. Earliest sign is formation of perinuclear vacuoles. Special stains for fat are oil red 0, Sudan Black, Nile blue sulphate. Fat can be demonstrated only on frozen section.
- **Cholesterol and esters**—seen in atherosclerotic plaques, xanthomas, cholesterolosis of gall

bladder, etc.

- **Protein**—Hyaline droplet change is seen due to excess protein reabsorption in proximal tubular cells of kidney during proteinuria. Russell bodies are homogenous inclusions seen in plasma cells in multiple myeloma.
  - Chaperones are involved in protein folding and transport.
  - Ubiquitin is a heat shock protein, which helps to degrade abnormal proteins
  - Proteinopathies are protein aggregation diseases which include neurodegenerative diseases like Alzheimer, Parkinson's and Huntington disease. Ubiquitin is a component of neurofibrillary tangles and Levy bodies.
- **Glycogen**—Seen as clear vacuoles. Demonstrated by Best Caramine or PAS with diastase sensitivity. Best fixative to demonstrate glycogen is absolute alcohol. Seen in diabetes mellitus and glycogen storage disorders.
- **Pigments**

I. Lipofuscin, which is an aging pigment, is seen peri-nuclearly as fine yellow brown granules. Pigment of Brown atrophy of heart.
II. Melanin which is only endogenous dark brown pigment
III. Hemosiderin—which is seen as coarse golden brown granules mainly in macrophages. Demonstrated by Perls or Prussian blue reaction.
IV. Bilirubin—Greenish yellow. Seen in liver in cholestatic states. Demonstrated by Fouchet's reaction.

PATHOLOGICAL CALCIFICATION

- Abnormal calcification
- Gross – white, chalky
- HE –basophilic, granular
- Spl Stains – von Kossa, Alizarin Red S
- 2 types

Dystrophic calcification	Metastatic calcification
a) Dead, necrotic tissue without hypercalcemia • Dystrophic calcification occurring in dead and dying tissues in presence of normal serum calcium levels, e.g., • -atheromas, • TB LN , • damaged heart valves, • tumors, • necrotic foci. • Michealix Guttmann bodies – Malakoplakia	Metastatic calcification may occur in normal tissues whenever there is hypercalcemia. There are four principal causes of hypercalcemia: (1) Increased secretion of parathyroid hormone (PTH) with subsequent bone resorption, as in ***hyperparathyroidism*** due to parathyroid tumors, and ectopic secretion of PTH-related protein by malignant tumors (2) ***Destruction of bone tissue,*** secondary to primary tumors of bone marrow (e.g., multiple myeloma, leukemia) or diffuse skeletal metastasis (e.g., breast cancer), accelerated bone turnover (e.g., Paget disease), or immobilization; (3**)** ***Vitamin D–related disorders***, including vitamin D intoxication**, sarcoidosis (in which macrophages activate a vitamin D**

	precursor), and **idiopathic hypercalcemia of infancy (Williams syndrome**), characterized by abnormal sensitivity to vitamin D; and (4) ***Renal failure,*** which causes retention of phosphate, leading to secondary hyperparathyroidism. Less common causes include aluminum intoxication, which occurs in patients on chronic renal dialysis, and milk-alkali syndrome, which is due to excessive ingestion of calcium and absorbable antacids such as milk or calcium carbonate.

HYALINE CHANGE

Alteration in cell / extracellular pace giving it a homogenous glassy pink appearance
- intra -cellular → Russel body, Mallory hyaline
- extra-cellular →benign hypertension, amyloidosis

Sub-cellular Responses to Injury

- Lipofuscin granules are residual bodies.
- Defective organization of microtubules leads to immotile cilia syndrome.
- Megamitochondria are seen in hepatocytes in alcoholic liver diseases.
- Parking lot mitochondria are seen in mitochondrial myopathies.
- Oncocytes are tumors that contain abundant enlarged mitochondria.
- Mallory bodies or alcoholic hyaline is an eosinophilic intracytoplasmic inclusion composed of intermediate filaments, seen in alcoholic liver diseases, Indian childhood cirrhosis, Hepatitis C, Primary biliary cirrhosis and Wilson's disease.

CLASSIFICATION OF INTERMEDIATE FILAMENTS

1) Keratin (epithelial cells) – Mallory hyaline
2) Neuro filament (neurons) – Neurofibrillary tangles
3) Desmin (muscle)
4) Vimentin (connective tissue)
5) Glial filament / GFA[(glial cells)

CELLULAR AGING

Aging

- Genetic factors and environmental insults combine to produce the cellular abnormalities that characterize aging.

Biochemical changes	**Morphological alterations**

• Decreased oxidative phosphorylation • Decreased uptake of nutrients • Decreased repair of chromosomal damage • Decreased synthesis of structural, enzymatic and regulatory proteins • Lipofuscin deposits implicating the role of free radical mediated cell damage • Accumulation of advanced glycosylation end products e.g. in lens protein leading to senile cataract	• Irregular and abnormally lobed nuclei of cells • Pleomorphic and vacuolated mitochondria • Decreased endoplasmic reticulum • Distorted Golgi apparatus

Time of aging:

- **Telomere shortening (**incomplete replication of chromosome ends) occurs with age due to decreased telomerase activity.
- Telomeres are short repeated sequences of DNA that compose the linear ends of chromosomes.
- They are important to ensure complete replication of chromosome ends.
- When cells replicate a small section of telomere is not replicated.
- As cells repeatedly divide, telomeres become progressively shortened ultimately signaling a growth checkpoint and cells become senescent.
- Telomerase is an enzyme that can maintain the length of telomeres by nucleotide addition.
- Telomerase activity is high in ***actively dividing cells, germ cells, stem cells and cancer cells but is usually absent in most somatic tissues.***

Werner syndrome is characterized by premature aging due to defect in the enzyme DNA helicase.

Wiskott Aldrich Syndrome

- WAS Protein is defective
- Function – link lymphocyte antigen receptor to cytoskeleton
- X recessive, eczema, thrombocytopenia, micro platelets
- Repeated infections - ↓ IgM, ↑ IgE, IgG & IgA WNL

INFLAMMATION AND REPAIR

- Inflammation is the reaction of vascularized connective tissue to injury .

- Four cardinal signs of inflammation given by CELSUS are: **Rubor, Calor, Dolor Tumor**.
- Fifth sign was added by VIRCHOW, that is **Functio Laesa.**
- ELIE METCHNIKOFF discovered phagocytosis.
- SIR THOMAS LEWIS discovered Histamine.

Acute: immediate onset and short duration. Microscopically characterized by edema and neutrophilic infiltration.	**Chronic**:late onset and long duration. Microscopically characterized by mononuclear cells and features of healing.

ACUTE INFLAMMATION

Events are:

1. **Vascular changes**:	2. **Cellular changes**:
• Transient vasoconstriction followed by massive dilation of arterioles and opening of new capillary beds. • Increased vascular permeability and edema formation. • Increased viscosity of blood leading of stasis. • Peripheral orientation of leukocytes (margination).	• Adhesion and Transmigration. • Chemotaxis. • Phagocytosis.

Mechanisms of Increased Vascular Permeability:

Endothelium becomes leaky by the following mechanisms

- **Immediate Transient Response:**
1. Most common mechanism of vascular leakage.
2. Occurs rapidly, is reversible and short lived.
3. Mediated by histamine, bradykinin, leukotrienes and substance P.
4. Affects venules only.
5. Gaps are intercellular and due to endothelial cell contraction.

- **Immediate Sustained Response:**
1. Seen after burns or severe bacterial infections.
2. Mediated by direct injury to endothelium leading to necrosis and endothelial cell detachment.
3. Affects arterioles, capillaries and venules.

- **Delayed Prolonged leakage:**
1. Seen in delayed sunburn, thermal injury, X-rays or UV rays.
2. Affects venules as well as capillaries.
3. Begins after 2-12 hours and lasts for days.
4. Due to cell damage by apoptosis.

- **Endothelial Retraction:**
1. Endothelial retraction is due to cytoskeletal reorganization.
2. Response is delayed (4-6 hours) and lasts for more >24 hours.
3. Mediated by cytokines like IL-1 and TNF-alpha.

- **Increased Transcytosis:**
1. VEGF induced. Occurs through Vesiculovacuolar organelle.

- **Leukocyte Mediated Endothelial Injury:**
1. By release of enzymes and toxic oxygen species.
2. Mostly seen in venules, pulmonary and glomerular capillaries.

- **Leakage from New Vessels:**
  1) New vessels are leaky initially till intercellular junctions are formed.

Cellular Events:

1) **Adhesion and Transmigration:**
   Margination→ Rolling and transient adhesions →Firm adhesion→Transmigration.
   It brought about by complementary adhesion molecule binding.

Adhesion molecules:

Categories of adhesion molecules are:

1) Selectins–e.g., E. Selectin, P. Selectin and L-Selectin
2) Integrins–e.g., 12 integrins and f3i integrins
3) Immunoglobulin super family e.g., ICAM-1 and VCAM-1
4) Mucinlike glyco proteins e.g., gly CAM-1
5) CD31

Endothelial Molecule	Leukocyte Receptor
1) P. selectin (on endothelium and platelets) (GMP 140/PADGEM), CD 62P	Silayl Lewis X PSGL-1
2) E. selectin (CD 62 E/ELAM-1)	Sialyl Lewis X
3) ICAM-1	β_2 integrins (CD 11/CD 18) (LFA-1, MAC-1)
4) VCAM-1	$\alpha_4\beta_131$ (VLA-4) $\alpha_4\beta_7$ (LPAM -1)
5) Glycam-1	L selectin (LAM 1)
6) CD 31	CD 31

2. **Chemotaxis** is locomotion oriented along a chemical gradient.

Nentrophilic chemotactic factors are:

1. C5a
2. LTB4
3. IL8 and other chemokincs
4. Bacterial products
5. 5 HETE

Chemotactic agents can also cause leukocyte activation by acting through G protein coupled receptors and Toll Ii receptors etc.

How does a leukocyte move?

Receptor (Seven transmembrane G protein coupled) – Ligand binding

↓

Inactive GDP form converted to active GTP form

↓

Phospholipase C activation (PLC-) and PI3K

↓

Acts on membrane inositol phospholipids

↓

Increased cytosolic Ca and polymerization of actin at leading edge of cell.
Actin regulating proteins: Filamin, Gelsolin, Profilin and Calmodulin also interact.

3. **Phagocytosis** is the recognition, engulfment and killing of organisms.
   i) Recognition is brought about by Mannose receptors, scavenger receptor and Mac 1 integrins. Efficiency of phagocytosis is increased by opsonization.

   Opsonins are:

- Fc portion of IgG binds to Fc gamma R1.
- Complement component C3b and C3bi bind to CR 1, 2, 3.
- Plasma proteins like Mannose binding lectin, Fibrinogen, Fibronectin and C-Reactive protein

   ii) Engulfment leads to formation of phagolysosome.
   iii) Killing mechanisms are:

- 02 dependant: H202—MPO-Halide (HOCL) system. Enzymes important for this are NADPH oxidase and Myeloperoxidase (azurophilic granules of neutrophils).
- Oxygen independent: Bactericidal permeability increasing protein (BPI), Lysozyme, Lactoferrin, Major basic protein (eosinophil). Defensins.
  Killing is followed by lysis by hydrolases. Tissue damage is mediated by leakage of lysosomal enzymes, oxygen species and arachidonic acid metabolites.

DEFECTS IN LEUKOCYTE FUNCTION:

a) In **Defects in Leukocyte Functions**

Disease	**Defect**
GENETIC	
Leukocyte adhesion deficiency 1	AR disorder .Defective leukocyte **adhesion** because of mutations in β chain of CD11/CD18 integrins
Leukocyte adhesion deficiency 2	Defective leukocyte **adhesion** because of mutations in fucosyl transferase required for synthesis of sialylated oligosaccharide (ligand for selectins)
Chronic granulomatous disease **X-linked** Autosomal recessive	• Decreased oxidative burst Phagocyte oxidase (membrane component) Phagocyte oxidase (cytoplasmic components)
MPO (myeloperoxidase)deficiency	Decreased **microbial killing** because of defective MPO—H_2O_2 system
Chédiak-Higashi syndrome	• Defect In **Phagocytosis**: • Autosomal recessive disorder. • Decreased leukocyte functions because of mutations affecting protein involved in lysosomal

Disease	Defect
	membrane traffic • Fusion of phagosome with lysosome is impaired. • Neutropenia, defective degranulation and killing. • **Giant granules** are seen in neutrophils and neutropenia on peripheral smear Bleeding, silvery gray hair and nerve conduction defects are also seen.
ACQUIRED	
Bone marrow suppression: tumors, radiation, and chemotherapy	Production of leukocytes
Diabetes, malignancy, sepsis, chronic dialysis	Adhesion **and chemotaxis**
Leukemia, anemia, sepsis, diabetes, malnutrition	Phagocytosis and microbicidal activity

CHEMICAL MEDIATORS:

Preformed	Newly Synthesized
• Histamine-Mast cell, Basophil, Platelets • Serotonin-Platelets • Lysosomal-Neutrophils,macrophage enzyme	• PGs-All leukocytes • LTs-All leukocytes • PAF-All leukocytes • Activated O_2 species-All leukocytes • Nitric oxide-Macrophages • Cytokines-Lymphocytes, Macrophages,

1. **Vasoactive Amines**

Histamine and Serotonin (5 hydroxytryptamine)

- First mediators to be released.
- Cause dilatation of arterioles.
- Increase vascular permeability (immediate transient phase).
- Histamine is found in mast cells, basophils and platelets.
- Serotonin is found in platelets and enterochromaffin cells.

2. **Arachidonic Acid Metabolites (Eicosanoids)**

- Cyclooxygenases generate prostaglandins and thromboxanes.
- Lipooxygenases generate leukotrienes and lipoxins.
- Leukotriene C4, D4 and E4 - Increase vascular permeability, and causes vasodilation.
- Leukotriene B4 is a powerful chemotactic agent.
- PGI 2 (prostacyclin) and PGE 2 cause vasodilation and inhibits platelet aggregation.
- PGE2 causes pain, hyperalgesia, and fever.

- Lipoxins are formed by transcellular biosynthetic mechanisms.
  - They are endogenous negative regulators of leukotriene action.
  - LXA4 and LXB4 inhibit adhesion and chemotaxis of neutrophils.
- Thromboxane A2 causes vasoconstriction and promotes platelet aggregation.

3. **Cytokines**: Production mainly by lymphocytes and macrophages.
   Role in inflammation-IL 1, TNF alpha and beta, IFNy, IL5, IL10, IL12.
   Chemokines - cytokines that stimulate chemotaxis. Short chain polypeptides.
   Serpentine receptors.

   Four major classes :

Subtype	**Example**	**Target cell**
CXC or $^{\alpha}$	IL_8	neutrophils
C-C or β	Monocytes chemo attractant protein (MCP-1), macrophage inflammatory protein 1 α (MIP-lα), eotaxin, RANTES. Eotaxin selectively recruits eosinophils.	Eosinophils, monocytes/ macrophage
C or γ	Lymphotactin	lymphocyte
CX_3C	Fractalkine	Monocytes, Th cells

IMPORTANT - Fractalkine receptors act as viral Co- receptor for HIV (e.g. $CXCR_4$, CCR- 5)

4. Neuropeptides: e.g., Substance P causes vasodilation and increases vascular permeability.
5. **Nitric oxide**:

- Produced by endothelium, macrophages and neurons.
- Precursor is L arginine.
- Causes vasodilation.
- Cytotoxic, regulates leukocyte entry (inhibits adhesion).

6. **Lysosomal constituents**:
   Neutrophils

- Specific granules (lysozyme, lactoferrin).
- Azurophilic granules (MPO, elastase).

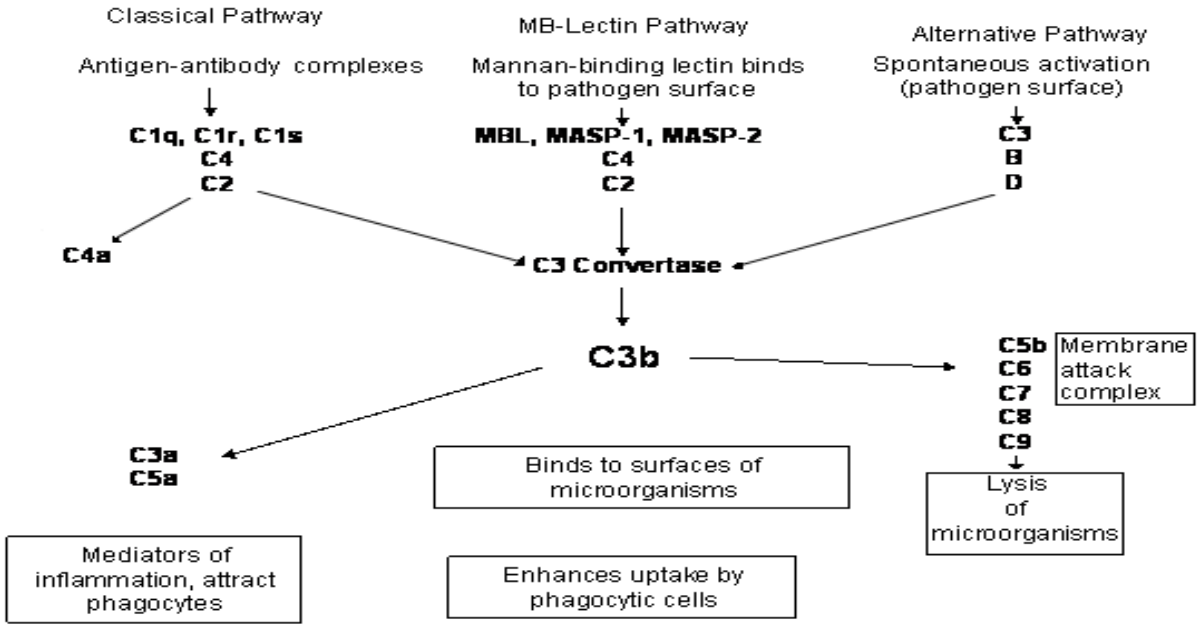
Plasma Proteases:

1. Kinins- Bradykinin

- Arteriolar dilation
- Increased venule permeability due to contraction of endothelial cells.
- Pain.

2. Complement system: consists of 20 proteins found in plasma in inactive form and numbered C1 to C9.

- Critical step is activation of third
- Activated through
  - Classical pathway- fixation of C1 to antigen antibody complex.
  - Alternate pathway- Endotoxins, aggregated Ig A, cobra venom activate C3
  - Lectin pathway- Mannose Binding Lectin binds microbe carbohydrate.



Functions of various complement protiens:

- C_{3a} and C_{5a} are also called anaphylotoxins which are chemicals causing vasodilatation and increased vascular permeability[Q]
- C_{3b} and inactive C_3 (C_i) used for opsonization[Q]
- C_{5a} also has important role in chemotaxis[Q]
- $C_{5b\text{-}9}$ (Membrane Attack Complex; MAC)[Q] attacks and kills the antigen

Regulation of complement system

- Decay accelarating factor (DAF) increases the dissociation of C_3 convertase[Q]
- Factor I proteolytically cleaves C_{3b}
- C_1 inhibitor (C_1 INH) blocks binding of C_1 to immune complexQ
- CD_{59} (Membrane inhibitor of reactive lysis) inhibits formation of MAC[Q]

Deficiency of complement component	Disease/ syndrome
1. C_1 Inhibitor	*Hereditary angioneurotic edema* [Q](subcutaneous edema because of extensive

	complement activation)
2. Early complement proteins C_1, C_2, C_4	SLE and collagen vascular disease
3. C_{3a} and C_{3b} inactivator	*Recurrent pyogenic infections*^{Q}
4. C_5 to C_8	Bacterial infections with Neisseria and Toxoplasmosis
5. C_9	No particular disease

Note: *Deficiency of C_2 is the most common.*

3. **Clotting System-** Activation by Hageman factor and plays role in inflammation.
   - **Fibrinopeptides** (Formed during cleavage of fibrinogen) – Induce vascular permeability and are chemotactic for leukocytes.
   - **Thrombin-** causes leukocyte adhesion to endothelium via binding to protease-activated receptors (PARS)

CHRONIC INFLAMMATION

- Chronic inflammation is characterized by
- Infiltration by mononuclear cells-macrophage, lymphocytes, plasma cells.
- Tissue destruction.
- Healing by connective tissue replacement

Macrophage is a dominant cell of chronic inflammation. Part of mononuclear phagocyte system.

- Macrophages in different tissues are — microglial cells (CNS), Kupffer cells (Liver), Alveolar macrophages (Lung) osteoclasts (Bone).
- Macrophages arise from bone marrow stem cell.

Chronic Granulomatous inflammation

- Distinctive type of chronic inflammation characterized by granuloma formation.
- Granuloma is a microscopic aggregation of macrophages that are transformed into epithelium like cells (Epithelioid, cells) surrounded by a collar of mononuclear leukocytes principally lymphocytes.
- Old granulomas may have an enclosing rim of fibrosis.
- Granuloma also has giant cells - Langhans type with horse shoe shaped nuclear arrangement and foreign body type with haphazard nuclear arrangement.
  Others giant cells found in various diseases

Touton giant cell	Xanthogranulomas
Warthin Finkeldey cells	Measles
Reed-Sternberg	Hodgkins
Aschoff body (Anitschkow cell)	Rheumatic fever
Schaumann	Sarcoidosis
Syncytiotrophoblastic	Chorionic tissue, Choriocarcinoma
Tumor giant cells	Malignancies, e.g. osteosarcoma
Osteoclast like	Osteoclastomas

Common Causes of granuloma formation

- Tuberculosis (Soft or caseating granuloma).
- Tuberculoid Leprosy (Caseation only in nerves).

- Syphilis (Gumma).
- Cat Scratch disease (Stellate granuloma).
- Schistosomiasis.
- Sarcoidosis (Noncaseating granulomas with asteroid and Schaumann bodies).
- Some fungal infection (Cryptococcus, Coccidioides immitis).
- Silicosis.
- Berylliosis.

Stellate abscesses are seen in LGV and Cat scratch disease.

A. REGENERATION AND REPAIR

1. **Wound healing involves two separate processes**: regeneration and repair.

I. Regeneration is replacement of the damaged tissue by cells of the same type.

II. Tissue repair involves replacement by connective tissue.

2. **Regeneration**: different tissues have different regenerative capacities.

a. Labile cells: regenerate throughout life.
Examples: surface epithelial cells (skin and mucosal lining cells), hematopoietic cells, stem cells etc.

b. Stable cells: replicate at a low level throughout life have the capacity to divide if stimulated by some initiating event. They are in G_o phase and can be stimulated to enter G_1 phase.
Examples: hepatocytes, proximal tubule cells, endothelium etc.

c. Permanent cells: cannot replicate.
Example: neurons and cardiac muscle cells.

3. **Tissue repair**

a. Repair by connective tissue occurs when there is extensive destruction of stromal framework and in tissues composed of permanent cells.

b. Tissue repair is mediated by various growth factors and cytokines like TGF, PDGF, FGF, VEGF, etc., and Tumor necrosis factor (TNF-a) and IL-1.

c. Four components of repair are

- Migration and proliferation of fibroblasts
- Deposition of collagen
- Formation of new blood vessels (angiogenesis)
- Maturation and organization of scar (remodeling).

d. Granulation tissue is formed, which is the hallmark of healing. It usually appears by 3rd day and derives its name from pink granular appearance due to buds of proliferating capillaries and synthetically active fibroblasts. It also contains macrophages and lymphocytes.

e. Wound contraction is mediated by myofibroblasts

B. PRIMARY UNION (HEALING BY FIRST INTENTION)

a. Occur with clean wounds when there has been little tissue damage and the wound edges are closely approximated.

b. The classic example is a surgical incision.

I. O hours - incision filled with blood clot.

II. Within 24 hrs - Neutrophils from margins infiltrate the clot Mitosis begins in epithelial basal cells.

III. 24-48 hrs - Below scale a continuous, but thin epithelial layer is formed.

IV. Day 3 Neutrophils are replaced by macrophages.
Granulation tissue begins to appear.
Collagen fibres present in the margins of the incision and are vertically oriented. Do not bridge the incision.

V. Day 5 –

- Incision space is filled with granulation tissue.
- Neovascularization is maximum
- Collagen fibrils more abundant and begin to bridge the incision.
- Epidermis recovers normal thickness with surface keratinization.

VI. WEEK 2 –

- Accumulation of collagen and proliferation of fibroblasts.
- Leukocytic infiltrate, edema and increased vascularity disappear.

VII. End of 1th month - Scar comprises of cellular connective tissue.
Tensile strength of the wound at the end of first week is 10% and increases over next 4 weeks.
At the end of 3rd month, the tensile strength reaches a plateau and is 70-80% of unwounded skin (remains so through life).

C. HEALING BY SECONDARY INTENTION

Occurs when there is more extensive loss of tissue as in infarction, inflammatory ulceration, abscess and in case of large wounds. Wound contraction occurs brought about by myofibroblasts.

Wound contraction- Most important difference between healing by primary and secondary intention.

- **Remodeling** is balance between collagen deposition and collagenase secretion.
- Degradation by zinc metalloproteinases of collagenase- important for tissue remodeling angiogenesis and cancer metastasis.
- Collagenase is produced by fibroblasts, macrophages, neutrophils, synovial cell and some epithelial cell.
- Activated collagenase is inhibited by tissue inhibitor of metalloproteinase.

D. CONNECTIVE TISSUE COMPONENTS

Extracellular Matrix:

Divided into basal membrane and interstitial matrix.

Composed of

- Fibrous proteins - Collagen, Elastin
- Adhesive glycoproteins - Laminin, Fibronectin, Integrins.
- Gel ofproteoglycans and hyaluronan.

Basal membrane is produced by epithelial and mesenchymal cells. Composed of Type IV collagen, Laminin, Heparan stulphate, Proteoglycans, Glycoproteins.

Interstitial matrix is composed of fibrillar collagen types I, III, V Elastin, fibronectin, Proteoglycan, **hyaluronan.**

Collagen:

Triple helix structure is composed of three polypeptide alpha chains with gly-x-y repeats.

14 types are known.

Contains high content of hydroxyproline and lysine. Vitamin C is required for hydroxylation.

Cross linkages due to oxidation of lysine and hydroxylysine residues lead to tensile strength.

a. Type I: most common, has high tensile strength and is found in skin, bone, tendons and most organs.
b. Type II: cartilage and vitreous humor.
c. Type III: granulation tissue, embryonic tissue, uterus, keloids.
d. Type IV: basement membranes

Elastin

Rich in glycine, proline, and alanine.

Seen in walls of great vessels, uterus, skin, ligaments.

Fibrillin forms scaffold of microfibrillar network which surrounds elastin core.

Fibronectin attaches cells to matrix via RGD recognition element, involved in attachment, spread, and migration of cells.

Laminin spans basal lamina and attaches and aligns cells to it.

Matricellular proteins e.g., SPARC, Thrombospondin, Osteopontin, tenascin.

Proteoglycans are heparin sulfate, dermatan sulfate, chondroitin sulfate and syndecan.

Disorders of healing

↑↑↑ Collagen- Keloid (Hypertrophied scar)

↑↑↑ Granulation tissue = exuberant granulation or proud flesh

↑↑↑ Fibroblasts + CT + recurs after excision = desmoid tumor or aggressive fibromatosis.

EDEMA

- Edema is increased fluid in the interstitial space or body cavities.

Pathophysiologic Categories of Edema:

INCREASED HYDROSTATIC PRESSURE

Impaired venous return

- Congestive heart failure
- Constrictive pericarditis
- Ascites (liver cirrhosis)

Venous obstruction or compression

- Thrombosis
- External pressure (e.g., mass)
- Lower extremity inactivity with prolonged dependency

Arteriolar dilation

- Heat
- Neurohumoral dysregulation

REDUCED PLASMA OSMOTIC PRESSURE (HYPOPROTEINEMIA)
• Protein-losing glomerulopathies (nephrotic syndrome) • Liver cirrhosis (ascites) • Malnutrition • Protein-losing gastroenteropathy
LYMPHATIC OBSTRUCTION
• Inflammatory • Neoplastic • Postsurgical • Postirradiation
SODIUM RETENTION

• Excessive salt intake with renal insufficiency
• Increased tubular reabsorption of sodium
  • Renal hypoperfusion
  • Increased renin-angiotensin-aldosterone secretion

INFLAMMATION
• Acute inflammation • Chronicinflammation • Angiogenesis

Transudate Versus Exudate :

• Transudates are protein-poor fluids due to hydrodynamic derangements and have specific gravity <1.012.	• Exudates are protein-rich and cellular fluids due to increased capillary permeability in inflammation and have gravity >1.020.

Sites for Edema

- Edema may be localized or systemic.
- Severe systemic edema is called Anasarca.
- Subcutaneous edema may be diffuse or occur where hydrostatic pressures are greatest (dependant edema).
- Dependent edema is typical of Congestive Cardiac Failure.
- Edema resulting from hypoproteinemia is generally more sever and diffuse: it is most evident in loose connective tissues (e.g. eyelids, causing periorbital edema).
- Pulmonary edema could be hemodynamic (Left Ventricular Failure, nephrotic syndrome, pulmonary vein obstruction) or due to microvascular injury (Adult Respiratory Distress

Syndrome).

- Brain edema may be localized to the site of injury (e.g. abscess or neoplasm) or may be generalized (encephalitis, hypertensive crisis or obstruction to venous outflow).

Mechanism of Edema:

- Sodium and water retention are important in edema due to hypertension and **poststreptococcal glomeruloneph**
- Inflammation resulting in increased vascular permeability is important in edema due to cellulitis.
- Lymphatic obstruction plays an important role in edema occurring post-MRM and irradiation for breast cancers
- Decreased plasma colloid osmotic pressure is the cause of edema in cases of tropical sprue, Kwashiorkor, Menetrier's disease and nephrotic syndrome.
- Increased hydrostatic pressure and sodium retention both play roles in edema due to congestive heart failure and cirrhosis of liver. Congestive Cardiac Failure is the most common cause of systemic edema.
- Increased hydrostatic pressure also results in edema in constrictive pericarditis and pregnancy.

HYPEREMIA AND CONGESTION

HYPEREMIA	CONGESTION
• Active process due to dilation of arterioles. It is seen at the sites of inflammation, blushing and exercise.	• Passive process due to impaired venous outflow and appears cyanotic. It occurs due to heart failure. • Left Ventricular Failure: Lung shows heart failure cells which are hemosiderin-laden macrophages (brown induration of lung). • Right Ventricular Failure: Nutmeg liver shows centrilobular necrosis and gradually progresses to cardiac cirrhosis.

HEMORRHAGE

- Rupture of blood vessels with extravasation of blood. In conditions with an increased tendency to bleeding, it may result from insignificant trauma.
- Rupture of large vessels is usually due to trauma, atherosclerosis, inflammatory or neoplastic erosion.
- Size wise: Petechia (1-2 mm) < purpura (>3 mm) < ecchymosis (1-2 cm) < hematoma.

Clinical effects — Shock with sudden and massive loss of blood. Anemia with slower and less loss of blood.

Hemostasis

Hemostasis is a normal physiologic process maintaining blood in a fluid, clot-free state in normal vessels, while inducing a rapid. localized hemostatic plug at sites of vascular injury. Thrombosis represents a pathological state.

Normal Hemostasis:

After Injury there is a characteristic hemostatic response:

- Reflex neurogenic arteriolar vasoconstriction mediated by endothelin (potent endothelium-derived vasoconstrictor).
- Platelet adhesion and activation (shape change and secretory granule release) leading to the formation of a temporary
- Activation of the coagulation cascade leads to the formation of a permanent plug.
- Activation of counter-regulatory mechanisms (e.g. tissue plasminogen activator {t-PA]) restricts the hemostatic plug to the site of injury.

THROMBOSIS:

Pathogenesis: **Virchow's triad**

1.Endothelial cell injury

- Atherosclerosis
- MI
- Vasculitis
- Hypercholesterolemia
- Homocysteinema
- Radiation
- Smoking

2.Stasis or turbulence of blood flow

- Brings platelets near the endothelium
- Prevents dilution of activated coagulation factors
- Retards inflow of coagulation inhibitors
- Promotes endothelial cell activation

Examples: Ulcerated atherstic plaques, aneurysm, mitral valve stenosis, hyperviscosity syndrome (Polycythemia, myeloma, heavy chain disease), sickle cell anemia

3.Hypercoagulable States

PRIMARY (GENETIC)

Common

- Factor V mutation (G1691A mutation; factor V Leiden)(Most common)
- Prothrombin mutation (G20210A variant)
- 5,10-Methylenetetrahydrofolate reductase (homozygous C677T mutation)
- Increased levels of factors VIII, IX, XI, or fibrinogen

Rare

- Antithrombin III deficiency
- Protein C deficiency
- Protein S deficiency

Very Rare

Fibrinolysis defects

Homozygous homocystinuria (deficiency of cystathione β-synthetase)

SECONDARY (ACQUIRED)

High Risk for Thrombosis

- Prolonged bedrest or immobilization
- Myocardial infarction
- Atrial fibrillation
- Tissue injury (surgery, fracture, burn)
- Cancer
- Prosthetic cardiac valves
- Disseminated intravascular coagulation
- Heparin-induced thrombocytopenia
- Antiphospholipid antibody syndrome

Lower Risk for Thrombosis

- Cardiomyopathy
- Nephrotic syndrome
- Hyperestrogenic states (pregnancy and postpartum)
- Oral contraceptive use
- Sickle cell anemia
- Smoking

Hypercoagulability:

1) **Factor V Leiden** is a mutated factor V (substitution of Glutamine for normal Arginine at position 506 that is more resistant to protein C cleavage and hence promotes unchecked coagulation). It is the most common cause of thrombophilia.
2) **Prothrombin gene mutation**- A single nucleotide change (G to A transition) in the 3' untranslated region of the prothrombin gene which is associated with elevated prothrombin levels and an almost three-fold increased risk of venous thrombosis.
3) **Elevated levels of homocysteine** contribute to arterial and venous thrombosis and the development of atherosclerosis. This effect is most likely due to inhibition of antithrombin III and endothelial thrombomodulin. Hyperhomocysteinemia may be inherited or acquired. Homozygosity for the C677T mutation in the methyltetrahydrofolate reductase gene causes mild homocysteinemia in 5-15% white and East Asian populations.

4) **Antiphospholipid antibody syndrome:**

Essentials of Diagnosis

- Hypercoagulability, with *recurrent thromboses*[Q] in either the venous or arterial circulation.
- T*hrombocytopen*ia is common. [Q]
- Pregnancy complications, specifically pregnancy losses after the first trimester.
- *Lifelong anticoagulation with warfarin is recommended*[Q] currently for patients with serious complications of this syndrome, as recurrent events are common.

Pathophysiology:

Many patients with lupus have circulating antibodies specifically directed either against phospholipids or against phospholipid-binding proteins such as ***β2-glycoprotein I (apolipoprotein H***). Anticardiolipin antibodies apparently bind directly to β_2-glycoprotein I, and this protein acts as a co-factor in this antigen-antibody reaction

Clinical Findings:

. Classification Criteria for the Antiphospholipid Antibody Syndrome	
Criteria	
Clinical	
Thrombosis	Unexplained venous, arterial, or small vessel thrombosis in any organ or tissue
Pregnancy	One or more unexplained fetal losses after 10 weeks; three or more consecutive miscarriages before 10 weeks; or preterm delivery for severe preeclampsia or placental insufficiency before 34 completed

	weeks
Laboratory	
Anticardiolipin antibodies	IgG or IgMisotypes in medium to high titers at least 6 weeks apart[Q]
Lupus anticoagulant	Identified twice, at least 6 weeks apart[Q]

One of two clinical criteria *must be present. In addition,* ***at least two*** *laboratory criteria that include LAC activity or medium- to high-positive specific IgG- or IgM-ACAs must be confirmed on two occasions 6 weeks apart.*

Thrombosis		**Pregnancy related**
Venous thrombosis (More common)[Q] • *DVT is the most common manifestation[Q]* • Pulmonary embolism • *Pulmonary hypertension*	Arterial thrombosis • CNS: - *Storke*[Q] • Lungs: Pulmonary Hypertension • Heart : infarction • Bones: Avascular necrosis[Q] • Kidney : Renal artery thrombosis	• *Recurrent miscarriages*[Q] • *Severe preeclampsia*[Q] • *Placental insufficiency*[Q] • *IUGR*[Q]

Laboratory features:

- Elevated anticardiolipinantibodies[Q] (IgG or IgM)[Q]
- Elevated anti β-2 glycoprotein 1 antibody[Q]
- Lupus anticoagulant: A finding more sensitive for a lupus anticoagulant is prolongation of a specialized coagulation assay known as the Russell viper venom time (RVVT).[Q]
- A clue to the presence of a lupus anticoagulant, which may occur in individuals who do not have SLE, may be detected by a prolongation of the ***partial thromboplastin time[Q]*** (which, paradoxically, is associated with a thrombotic tendency rather than a bleeding risk)
- antibody associated with a biologic false-positive test for syphilis .a positive rapid plasma reagin (RPR), but negative specific anti-treponemal assays
- Thrombocytopenia

Treatment:Guidelines indicate that patients with APS should be treated with anticoagulation for life to maintain an INR of 2.0–3.0

5) Heparin-Induced thrombocytopenia (HIT)

Thrombocytopenia occurs in 5% of patients receiving heparin.
Type I- Most common, occurs rapidly after starting therapy
Not very severe, may resolve despite continuation of heparin therapy.
Cause –Direct platelet aggregation effect of heparin
Type II- Severe, occurs 5-14 days after starting the therapy.
Praradoxically leads to arterial and venous thrombosis.
Cause- Antibodies produced against the complex of heparin and PF4 on the platelets. Binding of antibodies to the complex activates the platelets and promotes thrombosis even in the setting of thrombocytopenia.
Rx discontinues heparin therapy.

6) Disseminated intravascular coagulation (DIC)

a) **DIC** is an acute, subacute or chronic thrombohemorrhagic disorder occurring as a secondary complication in a number of diseases.
DIC is characterize by activation of the coagulation sequence resulting in the formation of widespread microthrombi throughout the microcirculation of the body.
As a result of thrombotic diathesis, there is consumption of platelets, fibrin, and clotting factors (causing hemorrhages) and activation of fibrinolytic mechanisms.

b) **Pathogenesis**
Two main mechanisms that trigger DIC are:

- Release of the tissue factor or thromboplastic substances into the circulation.
- Widespread injury to the endothelial cells.

c) **Causes**

I. **Obstetric complications** (placental tissue factor activated clotting)
- Abruption placentae
- Retained dead fetus
- Septic abortion
- Amniotic fluid embolism

II. **Neoplasms**
AML M3 (Cytoplasmic granules in neoplastic promyelocytes activate clotting)
Carcinomas of prostate, pancreas, pancreas, lung and stomach (mucus released may be thromboplastic).

III. **Infections**
Gram-negative sepsis (endotoxin-activated monocytes release of IL-1 and TNF-alpha both of which increase the tissue factor expression on endothelial cell membranes)
- Meningococcemia
- Rickettsia
- Aspergillosis
- Malaria

iv) **Massive tissue injury**

- Traumatic
- Bruns
- Extensive surgery

v) **Miscellaneous**

Acute intravascular hemolysis, snakebite, giant hemangioma, shock vasculitis, aortic aneurysm, liver disease.

d) **Morphology**

- Kidneys- Thrombi in renal glomeruli may be associated with microinfarcts or renal cortical necrosis.
- Brain- Microinfarcts and fresh hemorrhages may occur.
- Adrenals- Massive hemorrhages give rise to the Waterhouse-Friderichsen syndrome seen in meningococcemia.
- Placenta- Widespread thrombi occur associated with cytotrophoblast syncytitrophoblast atrophy.

Clinical Significance of thrombi:

Thrombi are significant because they: (1) cause obstruction of arteries and veins; and (2) are possible sources of emboli.

Venous Thrombosis (Phlebothrombosis)

Occurs in most instances in the superficial or deep leg veins.

- Superficial thrombi usually occur in varicose saphenous veins, causing local congestion and pain but rarely embolizing.
- Deep thrombi in larger leg veins above the knee (e.g. Popliteal, femoral and iliac veins) embolize more readily. Deep vein thrombosis is entirely asymptomatic in approximately 50% of patients and are recognized only after embolization.

Arterial Thrombosis

- Cardiac and aortic mural thrombi can also embolize peripherally
- The brain, kidneys and spleen are prime targets.
- **Causes of Mural Thrombi**

Arterial thrombi can be seen in:

- Myocardial infarction with dyskinesis and endocardial damage.
- Rheumatic valvular heart disease like mitral stenosis with left atrial dilatation and thrombus formation.
- Atherosclerosis

Comparison of a thrombus with a post-mortem clot

	Thrombus	**Blood clot**
Location	Intravascular	Extravascular or intravascular
Composition	Platelets Fibrin RBCs and WBCs	Lacks platelets and fibrin
Lines of Zahin	Present	
Shape	Has Shape	Absent
Attachment to the vessel wall	Present	Lacks shape Absent

Common Locations of Arterial Thrombosis

- Coronary and cerebral arteries
- Heart chambers, atrial fibrillation or post-MI (mural thrombi)
- Aortic aneurysms
- Heart valves (vegetations)
- Deep leg veins (DVTs)

Outcomes of Thrombosis

- Vascular occlusion and infarction
- Embolism
- Thrombolysis
- Organization and recanalization

EMBOLISM

1) Definition — Any intravascular mass that has been carried down the bloodstream from its site of origin, resulting in the occlusion of a vessel.
2) Composition of emboli

- Thromboemboli — most common.
- Atheromatous emboli — severe arthrosclerosis.
- Fat emboli — bone fractures and soft tissue trauma.
- Bone marrow emboli — bone fractures and cardiopulmonary resuscitation (CPR)
- Gas emboli — decompression sickness ("the bends" and Caisson disease).
- Amniotic fluid emboli — complication of labor
- Tumor emboli — metastasis
- Talc emboli — intravenous drug abuse (IVDA)
- Bacterial/septic emboli — infectious endocarditis

3) Pulmonary emboli

- Often clinically silent
- Mostly arise in the deep veins of the legs
- Other sources include pelvic venous plexus of prostrate and uterus and right side of the heart.

- Outcomes — no sequela (75%), infarction (15%), sudden death (5%). chronic pulmonary hypertension (3%)

4) **Systemic arterial emboli**

- Mostly arise in the heart and causes infarction
- Common sites of infarction include lower extremities, brain, intestine, kidney and spleen

Paradoxical Emboli

Any venous embolus that gains access to the systemic circulation by crossing over from the right to the left side of the heart through a septal defect.

INFARCTION

- Infarction is a localized area of necrosis secondary to ischemia.
- Most infarcts (99%) result from thrombotic or embolic occlusion of an artery or vein. Other causes include vasopasm and torsion of arteries and veins (e.g. volvulus, ovarian torsion)
- Common sites of infarction are heart, brian, lungs and intestines.
- Infarcts often have a wedge shape and apex of the wedge tends to point to the occlusion.
- There are two type of infarcts:
  a) Anemic infarcts (pale or white color): Occur in solid organs with a single blood supply such as the spleen, kidney and heart.
  b) Hemorrhagic infarcts (red color): Occur in organs with a dual blood supply or collateral circulation, such as the lung and intestines. Also occur with venous occlusion (e.g. testicular torsion)

  Microscopic Pathology of Infarction

  - coagulative necrosis – most organs
  - liquefactive necrosis- brain
  - general sequence of tissue changes after infarction:

    Ischemia→Coagulative necrosis→inflammation→Granulation tissue→Fibrous Scar

SHOCK

Shock is systemic hypoperfusion resulting from reduction in either cardiac output or effective circulation blood volume: the result is hypotension, followed by impaired tissue perfusion and cellular hypoxia.

Cellular injury is initially reversible.

If the hypoxia persists, the cellular injury becomes irreversible, leading to death of cells and patients.

Three Major Types of Shock

Type of Shock	Clinical Example	Principal Mechanisms
CARDIOGENIC		
	Myocardial infarction Ventricular rupture Arrhythmia Cardiac tamponade Pulmonary embolism	Failure of myocardial pump resulting from intrinsic myocardial damage, extrinsic pressure, or obstruction to outflow
HYPOVOLEMIC		
	Fluid loss (e.g., hemorrhage, vomiting, diarrhea, burns, or trauma)	Inadequate blood or plasma volume
SEPTIC		
	Overwhelming microbial infections (bacterial and fungal) Superantigens (e.g., toxic shock syndrome)	Peripheral vasodilation and pooling of blood; endothelial activation/injury; leukocyte-induced damage, disseminated intravascular coagulation; activation of cytokine cascades

Neurogenic Shock (Generalize Vasodilatation)

- Anesthesia
- Brain or spinal cord injury

Anaphylactic Shock (Generalized Vasodilation)

Type I hypersensitivity reaction

Stages of Shock

a) Stage I: compensation, in which perfusion to vital organs is maintained by reflex mechanisms. Neurohormonal mechanisms maintain cardiac output.
    - Increased sympathetic tone.
    - Release of catecholamines.
    - Activation of the rennin-angiotensin system
    - ADH release
    - Baroreceptor reflexes
b) Stage II: Decompensation
    - Progressive decrease in tissue perfusion
    - Potentially reversible tissue injury occurs
    - Development of a metabolic acidosis, electrolyte imbalances, and renal insufficiency
c) Stage III: Irreversible
    - Irreversible tissue and organ failure, ultimately resulting in death

Pathology

a. Kidneys
    - Acute tubular necrosis
    - Oliguria and electrolyte imbalances occur

b. Lungs undergo diffuse alveolar damage ("shock lung")

c. Intestines
    - Superficial mucosal ischemic necrosis and hemorrhagesj
    - Prolonged injury may lead to sepsis with bowel flora

d. Liver undergoes centrilobular necrosis ("shock liver")

e. Adrenals undergo the Waterhouse-Friderichsen syndrome
    - Commonly associated with meningococcal septic shock
    - Bilateral hemorrhagic infarction
    - Acute adrenal insufficiency
    - Brain shows hypoxic encephalopathy

f. Heart shows coagulation necrosis and contraction band necrosis.

IMMUNITY

IMMUNE DYSFUNCTION

Types of immunity

Innate – Present before birth	Adaptive- Stimulated by microbes
- Broad, non antigen dependent, common to all microbes - Cells – neutrophils, macrophages, NK cell - Biological molecules - Complement, CRP , lung surfactant	- Cells – lymphocytes - T lymphocytes - CMI – cell mediated immunity - B lymphocytes – HI – humoral immunity - Proteins – Complement (Classical pathway)

CELLS OF THE IMMUNE SYSTEM

A. Lymphocytes

T – lymphocytes (T – cells)

- 60-70% of circulating lymphocytes are T – cells
- Are found predominantly in the paracortex of lymph nodes
- They also make up most of the white pulp of the spleen (peri – arteriolar sheath). And occupy the thymus gland.
- Recognize antigens by TCR (Tcell Receptor) – MHC restricted mostly
- 95% of TCR are α/β combinations, only 5% are γ/δ type (do not need MHC)
- TCR rearrangement distinguishes polyclonal T cell proliferation from monoclonal T cell proliferation
- Super antigens – can stimulate large no of T lymphocytes by binding to TCR at sites different from the usual antigen binding groove
- Nalve T cell marker – CD 45 RA

- **Memory T cell marker – CD 45 RO**

Activation of T cells : Two signals

1. TCR binding to MHC bound Ag, activation of CD 3 – Signal 1 CD4/CD8 – co-receptors
2. Interaction of CD – 28 with co-stimulatory molecules B7-1 (CD80) & B7-2 (CD86) – Signal 2

In absence of signal 2; T cells → Apoptosis)

T-cell markers :

CD3 – Pan T marker, signal transduction function

CD4 – Helper cells / master regulator cells of immunity, Recognize class II MHC antigens

- T_H1's produce IL- 2 and IFN – gamma and act on macrophages; associated with type IV HSR
- T_H2's produce IL-4, 5, 13; stimulate IgE synthesis, activate eosinophils; associated with type I HSR

CD8- marker for killer cells, recognize class 1 MHC antigens

B-lymphocytes (B-cells)

- Around 15% of circulating lymphocytes in the peripheral blood are B –cells.
- B-cells predominate in the follicles (germinal centers) of the lymph nodes, white pulp of spleen and are more common than T-cells in the red pulp of the spleen and in the bone marrow.
- B cells → (Ag stimulus) →Plasma cells →Igs.
- Recognize Ag via B-cell antigen receptor complex (BCR)
- IgM (present on surface of all B cells) and less often IgD constitutes Ag binding comp. of BCR
- BCR has unique antigen specificity, THEREFORE Rearranged Ig gene – used as molecular Marker of B lineage cells

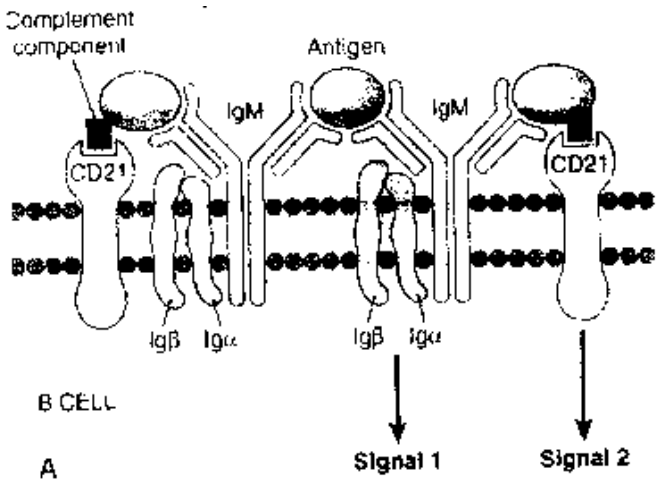
B cell associated markers : CD10(CALLA), CD19,CD20, CD21 (EBV receptor), CD22, CD23, CD24

Activation of B cells : Two signals

- Ig α & Ig β on B cell membrane → required for signal

transduction 1 (~to CD3)

- CD 21 – complement binding – signal 2



A

Natural Killer Lymphocytes :

- ✓ NK cells are bigger than other lymphocytes, and have cytoplasmic granules.
- ✓ Also called "Large Granular Lymphocytes", Null cell, Non T Non B cell
- ✓ Class I MHC molecules give inhibitory signals to NK cells
- ✓ NK cells attack and cause apoptosis in cells with altered or devreased MHC I expression, without being " previously sensitized".
- ✓ Component of innate immunity
- ✓ CD 16 (receptor for IgG Fc), CD56+ve, CD –ve
- ✓ Share some T cell markers (e.g.CD2)
- ✓ Kill tumor cells, fungi, and cells altered by viral infection.
- ✓ Involved in "antibody dependent cell – mediated cytotoxicity"

B. **Mononuclear phagocytes**

-Members of the "reticuloendothelial system" found throughout the body.

- Process & present Ag to T cells
- Effector cells in CMI
- Effector cells in humoral immunity (Phagocytose opsonized microbes)

C.**Dendritic cells : antigen presenting cells**

- Interdigitating dendritic cells (IDC) – T cells
  Eg.Langerhans cells of skin
    EM – Birbeck granules (tennis racket shaped cytoplasmic structures)
    Markers – S-100, HLA- DR, CD 1a, CCR6
  Neoplasia – Langerhan cell Histiocytosis (previously – Histiocytosis X)
    - Letterer – Siwe syndrome, Hand-Schuller – Christian disaease, Eosinophilic granuloma
- Follicular dendritic cells – B cells, role in AIDS

CYTOKINES

- Soluble proteins secreted by lymphocytes (lymphokines), Monocytes (monokines) and NK cells as well as other cell types

-Act as effector molecules influencing the behavior of B cells, T cells, NK cells, hemopoietic cells etc.

Classification of cytokines

A.Interleukins (IL) 1-26

B.Interferons (IFN)- β, and Ɣ

C.Colony stimulating factors (CSF)

1. GM-CSF (granulocyte – macrophage- csf)
2. M-CSF (macrophage – CSF)
3. G-CSF (granulocyte – CSF)

D.Tumor necrosis factor – alpha (TNF – α)

E.Lymphotoxin (TNF – β)

F.Transforming growth factor-beta (TGF – β)

G.Platelet – derived growth factor (PDGF)

Role of cytokines in pathophysiologic processes

IL- 1 stimulates T cell proliferation and IL-2 production

IL-2 stimulates proliferation of T cells, B cells and NK cells; activates monocytes.

IL-3 acts as growth factor for tissue mast cells and hemopietic stem cells
IL-4 promotes growth of B and T cells; enhances expression of HLA class II antigen
IL-5 promotes end stage maturation of B-cells in to plasma cells
IL-6 promotes maturation of B and T cells; inhibits growth of fibroblasts
IFN-α, TNF-β has antiviral activity
IFN-Ϋ has antiviral activity; activates macrophages; enhances expression of HLA class II antigens
TNF-α, TNF-β stimulates T cell proliferation and IL – 2 production; cytotoxic to some tumor cells.

HLA/MHC SYSTEM

- consists of a group of related proteins called HLA antigens
- Physiologic function – antigen presentation to specific T cells, also – Role in transplant rejection
-The genes for these antigens (histocompatibility genes)are localized on chromosome 6

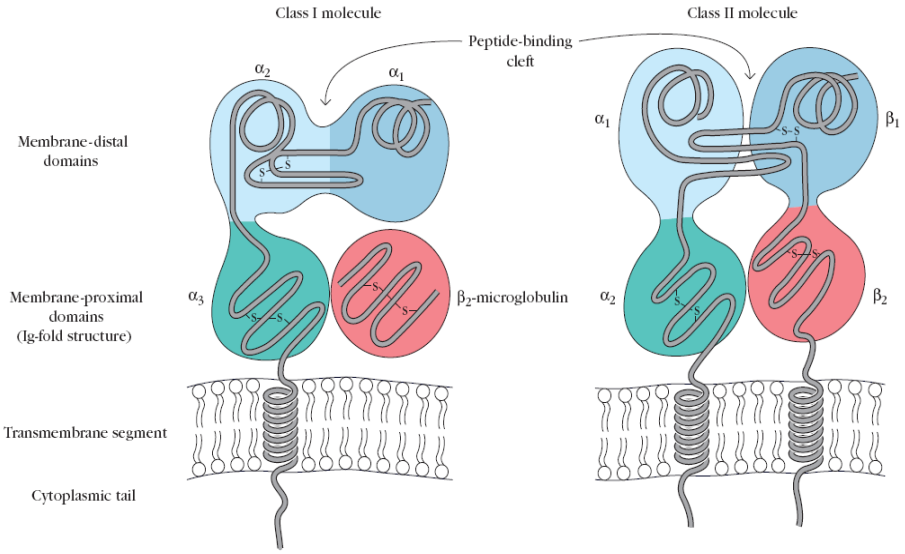


FIGURE 7-5 Schematic diagrams of a class I and a class II MHC molecule showing the external domains, transmembrane segment, and cytoplasmic tail. The peptide-binding cleft is formed by the membrane-distal domains in both class I and class II molecules. The membrane-proximal domains possess the basic immunoglobulin-fold structure; thus, class I and class II MHC molecules are classified as members of the immunoglobulin superfamily.

HLA antigens

- There are two major classes

Class I antigens

- Encoded by HLA-A, HLA-B, HLA-C genes
- Composed of α component and β2 macroglobulin (non-polymorphic)
- Found on almost all nucleated human cells and platelets
- Present the processed antigens to CD8+ cytotoxic T cells (CD8+T cells show MHC restriction to class I antigens)
- Present exogenous antigens and present them to CD4+T cells (CD4+T cells show MHC restriction to class II antigens)

Class – II antigens

- Encoded by HLA-DP, HLA-DQ,HLA-DR
- Composed of α and β components (both polymorphic)
- Found on immunocompetent cells such as macrophages, dendritic cells,Langerhans cells, B-cells and some T-cells

- Present exogenous antigens and present them to CD4+ T cells (CD4 + T cells show MHC restriction to class II antigens)

Class III proteins

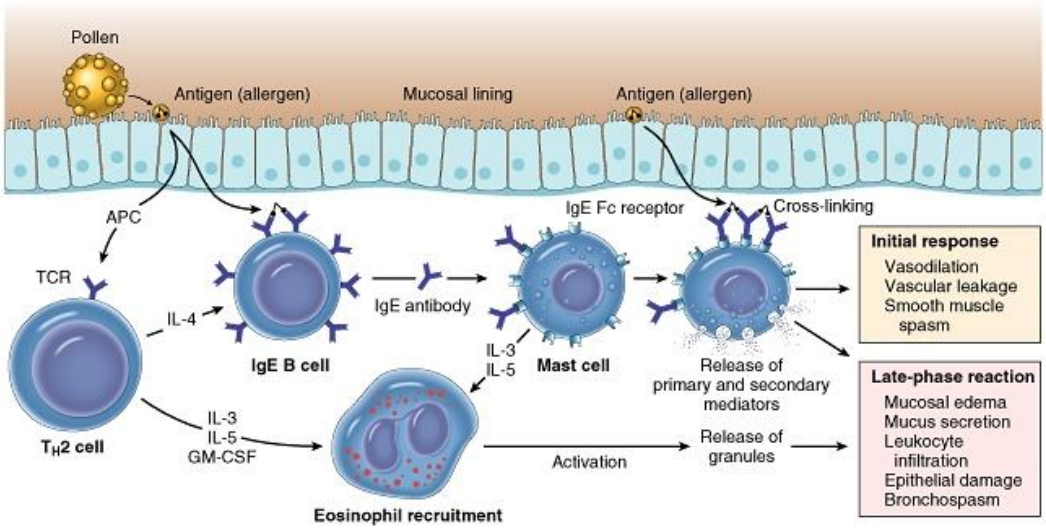
- **E**ncoded by non MHC genes in MHC region
- Not histo-compatibility antigens
- Some components of complement system (C2, C4, Bf), TNFα, TNFβ, Hsp 70, 21 hydroxylase
- No role in transplant rejection

Diseases having association with HLA:

Disease	HLA Allele	Relative Risk
Ankylosing spondylitis	B27	90
Postgonococcal arthritis	B27	14
Acute anterior uveitis	B27	14
Rheumatoid arthritis	DR4	4
Chronic active hepatitis	DR3	13
Primary Sjogren syndrome	DR3	9
Type 1 diabetes	DR3	5
	DR4	6
	DR3/DR4	20

HYPERSENSITIVITY REACTIONS

1. Type I HS (Anaphylactic type)
   Rapidly developing immunologic reaction occurring within minutes after the combination of an antigen with antibody bound to mast cell or basophils in individual previously sensitized to antigen
   Systemic (anaphylaxis) e.g. hormones, anti sera , drugs,
   Local – skin allergy, hives Allergic Rhinitis, Allergic conjunctivitis, Hay fever, Bronchial Asthma
   Allergic Gastroenteritis



<u>Two phses:</u>

- **Initial Response :**
  - Vasodilation & Vascular leakage, smooth muscle spasm, Glandular secretions

- Evident within – 5-30 min, subside in 60 min

- **<u>2nd late phase Reaction</u>**
  - More intense infiltration of tissues with neutrophils, eosinophils, eosinophils, basophils, monocytes, CD4 + T cells – Tissue destruction
  - Sets in 2-8 hour, Lasts for several days

Mast cell & Basophils:

- Central to dev. Of Type I HS
- Activated by cross linking of IgE Fc receptors
- Other activators (anaphylactoid reaction)

C3a & C5a (Anaphylotoxins) Macrophages derived CKs (IL-8)
Codeine, Morphine

Mellitin (Bee venom) Physical Stimuli (heat, cold, sunlight)

Primary & secondary mediators

Primary Mediators : smooth muscle contraction

- Biogenic Amines – Histamine ↑vascular permeability
  ↑secretions
  - Adenosine – enhances Mast cell mediator release
  - causes bronchoconstriction
  - platelet aggregation
- Chemotactic mediators – eosinophil chemotactic factor
  - neutrophils chemotactic factor
- Enzymes – (in granule matrix)
  - Proteases (chymase, tryptase), Acid hydrolases
- Proteoglycans – heparin
  - chondroitin sulfate

Secondary Mediators :

- Lipid Mediators → Arachidonic acid pathway
  LTB_4 : chemotacic for neutrophils, eosinophils, monocytes
  LTC_4 &LTD4 : Vasoactive, Spasmogenic
  PGD2 – most abundant mediator derived by COX pathway in mast cells
  - Bronchospasm, ↑edema

- Platelet activating factor (PAF)

- Causes platelet aggregation, histamine release, bronchospasm, ↑ Vascular permeability, vasodilation

- Chemo tactic for neutrophils & eosinophils

Cytokines

- TNF α, IL- 1, IL-3, IL-4, IL-5, IL-6, GM-CSF, MIP-1α, MIP-1β

In late phase : Eosinophils are particularly important

- Survival favored by IL-3, IL-5, GM-CSF
- Eotaxin & RANTES – Chemotaxis

ATOPY:

- Genetically determined predisposition to develp localized anaphylactic reactions to inhaled or ingested allergens.
- 10% of population, Positive family h/o of allergy in 50%
- ↑ Th2 cell responsiveness

2. Type II HS – mediated by antibodies directed towards antigens present on surface of cells or other tissue **components.**

Following mechanisms

1. **Complement dependent reactions :**
   2 Pathways a) Direct Lysis → by MAC (membrane attack complex) → drills holes in cell membrane
   b) Opsonization with Ab or **C_3b**
   Examples :
   i. Transfusion reactions
   ii. Erythroblastosis fetalis
   iii. Autoimmune hemolytic Anemia agranulocytosis or thrombocytopenia
   iv. Pemphigus Vulgaris (Ab against desmosomes)
   v. Certain drug reactions

2. Antibody dependent cell mediated cytotoxicity : (ADCC)
   - No complement required

- Target cells, coated with low concentration of IgG Ab → killed by Non sensitized cells that have Fc receptors
- Cells lysis without phagocytosis
- Mediated by Monocyted, neutrophils, eosinophils, Nk cells
- (Eosinophil mediated ADCC→ IgE Ab used
  Examples :
  - Destruction of parasites
  - Destruction of tumor cells
  - Role in graft rejection

3. Antibody mediated cellular dysfunction :

- Ab against cell surface receptor impair or dysregulate function without causing cell injury or inflammation
- Examples :
- Myasthenia gravis : Ab reactive with acetyl choline receptors in motor end plate of skeletal muscle
  impair neuromuscular transmission →weakness
- Graves diseases : Ab against TSH receptor on thyroid epithelial cells stimulate the cells→
  Hyperthyroidism (?type V HS reaction)

4. <u>Complement & Fc receptor mediated inflammation</u>
   - Antibodies deposit in ECM/BM
   - C5a, C4b, C3a recruit neutron & mono which are activated via Fc receptors
   - E.g.glomerulonephritis (Goodpasture's syndrome) , vascular rejection

Other type II HS disorders :

- Bullous pemphigoid
- Pernicious anemia
- Vasculitides
- Thrombotic phenomena
- Acute rheumatic fever

3. Type III HS (Immune Complex mediated)

- induced by Ag-Ab complexes that produce tissue damage as a result of their capacity to activate the complement systems

Exogenous Ag – Bacteria /virus/ fungi/ parasite/ drugs

Endogenous Ag →e.g.

Nuclear Ag : SLE
Immunoglobulins : Rheumatoid arthritis
Tumor antigens : Glomerulonephritis

Generalized e.g Acute Serum Sickness (After administration of large amounts of horse anti- tetanus serum)

Localized (e.g. local Arthus reactions

Phase I – Immune complex formation Ab. Produced approx. 5 days after introduction of Ag
Phase II – Immune complex deposition I/C bind inflammatory cells (thro' Fc or C3b receptors) →Release of vasoactive mediators →↑ vascular permeability
Deposits in Glomeruli, joints, skin, heart, serosal surfaces, BV'S
→**Factors :**

- Size : Larger I/C (Ab excess) →rapidly removed , relatively harmless
- Functional status of MPS (mononuclear phagocyte system)
- Charge of I/C
- Hemodynamic factors
- Valency of Ag
- Avidity of Ag
- Affinity of Ag to various tissue comp.
- 3-D, structure of I/C

Phase III – Immune Complex mediated inflammation

1. Acitvation of C' CASCADE (complement cascade)
2. Activation of neutrophils, macrophages through their Fc receptors
   → Release of C' components & release of proinflammatory substances : PGs, Vasodilator peptides, lysosomal enzymes, chemotactic substances
   - Also platelet aggregation
   - Hageman factor activation
   →Vasculitis / Glomerulonephritis / Arthritis
   Morphology : Acute necrotizing vasculitis with Fibrinoid necrosis, intense neutrophilic exudate

→Single, large exposure to Ag →Lesion resolves (e.g. Acute serum sickness, Ac, post streptococcal Glomerulonephritis)

→ Chronic Antigenemia → e.g SLE, Rheumatoid arthritis, PAN, Membranous Glomerulonephritis

Local Immune complex Disease (Arthus Reaction) :

→Localized area of tissue necrosis resulting from acute immune complex vasculitis
→ Experimentally – In animals with circulating Antibody

↓

Large I/C (Ab excess)

Precipitate locally & trigger the inflammatory reaction
Morphology : Fibrinoid necrosis
Hemorrhage
Thrombosis

4. Type IV Hypersensitivity (Cell mediated)

- Initiated by specifically sensitized T lymphocytes

2 types

Classic delayed type HS- by CD4 T Cells

Direct cell cyto toxicity – by CD8 T cells

Delayed type HS e.g. tuberculin reaction

exposure to tubercle bacillus

↓ (with MHC Class II molecules on Ag pres. Cell)

Naïve CD4 → T_H1 cell

T cell

- Some T_H1 cells enter the circulation → Memory pool

- **Intracut. Inj. Of tuberculin to prev. exposed individual**

  ↓

  TH1 cell interaction with Ag on surface of Ag presenting cell

  Cytokines involved – IL2, IL12, TNFα

  TNF-α & Lymphotoxins:

  - ↑ secretion of PGI_2 →local vasodilation
  - ↑ expression of E-selectin
  - Induction & secretion of chemotactic factors IL-8

<u>Tuberculin Reactions</u> : ↑ microvascular permeability, edema, deposition of fibrin

Macrophages →Epithelioid cells

With GC_s lymphocytes → (GRANULOMA)

- TB, fungi, certain parasites, transplant rejection, tumor immunity
- Contact Dermatitis : Contact with Urshiol (Ag component of

poison ivy or poison oak) – Delayed
Type HS reaction

T cell mediated cytotoxicity :

(CTL_s) CD8+ T cells kill antigen bearing target cells

- **Role in viral inf., tumor immunity**

Mechanisms :

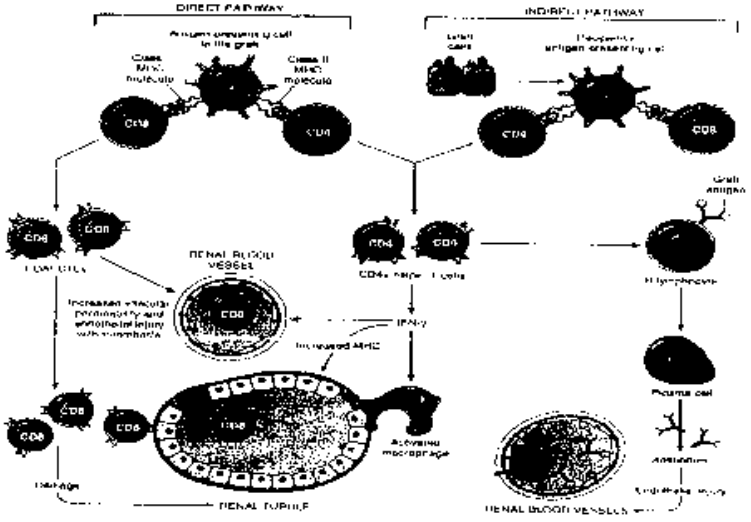
a) Perforin granzyme dependent killing:
- contained inlysosome like granules of CTL_5
- Perforin → drilling holes → pore formation
- Granzymes → Proteases

b) Fas- Fas ligand dependent killing – apoptosis
Activated CTLs express fas ligand ; which bind to Fas expessing target cells

TRANSPLANTATION IMMUNOLOGY

Mechanisms – Cellular (T cell mediated)

- **Humoral (B cell mediated)**



T CELL MEDIATED REACTIONS

- Direct – Ag presenting cells of donor present Ag to CD4 & CD8 Cells, role in acute rejection
- Indirect – Ag presenting cells of recipient present Ag to CD4 cells, role in chronic rejection

ANTIBODY MEDIATED REACTIONS

- Acute rejection
  - complement dependent cytotoxicity, inflammation, ADCC
  - initial target is graft vasculature (rejection vasculitis)
- Hyperacute rejection
  - Preformed anti donor antibodies present
  - e.g. already rejected transplant, multiparous lady , prior BT
  - vessel thrombosis & ischemia

MORPHOLOGY OF TRANSPLANT REJECTION

There are three basic types of graft rejection:

1. Hyperacute rejection
   - The recipient is previously sensitized to antigens in the graft
   - Occurs within minutes of transplantation due to preexisiting antibody to donor antigens
   - Is a localized Arthus reaction marked by acute inflammation, Fibrinoid necrosis of small vessels, and extensive thrombosis
2. Acute rejection
   - Occurs within months of rejection; may occur after 1-2 years after cessation of immunosuppressive therapy.
   - Two types
     a) Acute cellular rejection

- T cell mediated, responds to immunosuppressive therapy
- There is infiltration by lymphocytes and macrophages

b) Acute vascular rejection
- antibody mediated
- marked by arteritis and thrombosis

3. Chronic rejection

- Primary caused by antibody mediated vascular damage and subsequent ischemia
- May occur months to years after transplantation
- Histologiclly marked by vascular fibrointimal proliferation (graft arteriolosclerosis,) tubulr atrophy, duplication of glamerular BM, interstitial fibrosis and inflammation.

GRAFT – VERSUS HOST DISEASE

- Major complication of bone marrow transplantation
- Immunocompetent cells in the graft attack the foreign host cells

Acute GVHD

- Occurs 20-100 days after transplant
- Primarily involves:
- a)Skin (dermatitis)
- b)Intestine (diarrhea, malabsorption)
- c)Liver (jaundice, raised, serum alkaline phosphatase
- Reactivation of CMV infection, especially in the lung

Chronic GVHD

- Occurs after 100 days of transplantation
- Produces skin changes akin to scleroderma
- Git – Strictures
- Liver – cholestatic jaundice

GRAFT – VERSUS LEUKEMIA EFFECT

- GVL is effected by donor T cells & NK cells
- Immune destruction of leukemia cells
- Depletion of donor T cells eliminates GVH and there is
- Increased incidence of graft failure & recurrence of disease in leukemic patients

AUTOIMMUNE DISEASES

- Autoimmune disease may be defined as an immune reaction against self – antigens
- Secondary to a loss of self - tolerance.

Mechanisms of tissue injury in autoimmunity

Autoimmune diseases result from *breakdown* of 'self tolerance' which may occur by any of the following methods:

- Central – Deletion of self reactive T & B cell clones during maturation (negative selection)
- Peripheral –
    - Breakdown of T – cell anergy
    - Failure of activation – induced cell death
    - Failure of T cell mediated suppression
    - Molecular mimicry
    - Polyclonal lymphocyte activation
    - Release of sequestered antigens (thyroglobulin, lens, protein , spermatozoa)

Determining factors in the onset of autoimmune disease

A. Genetic factors
    1. Association with HLA phenotypes

2. Clustering in families

B. Microbiologic agents – role in autoimmunity is suspected, expecially in regards to viruses, but no clear demonstration established

C. Drugs – certain drugs are known to induce autoimmune disease in susceptible hosts (e.g. drug - induced SLE)
e.g. systemic lupus erythematosus (sle), drug induced lupus, systemic sclerosis [scleroderma] & crest syndrome, sjogren's syndrome, inflammatory muscle disease (polymyositis, dermatomyositis, and inclusion body myositis)

HEREDITARY (PRIMARY) IMMUNODEFICIENCIES

- **T cell defect**
  - **Viral & fungal infections**
  - **Bacterial sepsis**
  - **Opportunistic infections**
- **B cell defect**
  - **Bacterial infections**
  - **Intestinal giardiasis**

B CELL DEFECTS

1. X linked Agammaglobulinemia of Bruton
   - Failure of pro B & pre B to differentiate into B cells
   - Deficient Btk gene (X chromosome)
   - Presents as recurrent bacterial infections (staph, H. influenza, Strep. Pneumoniae), Giardia lamblia infection, viral infections – beginning after 6-months of age
   - All immunoglobulin levels are reduced
   - B cell development improper – UNDER DEVELOPED germinal centeres, absent plasma cells

- Normal no of B cells in BM
- CMI is intact- Normal T cell number **and function**

2. **Hyper IgM syndrome**
    - Deficient synthesis of IgG, IaG, IgA, IgE
    - Abnormal isotype switching
    - CD40 signals needed for IgM → IgG / A / E – absent
    - 70% - X linked (T cell defect – CD 40 L / CD 154 defect)
    - 30% - A Recessive (B cell defect – CD 40 defect)
    - Recurrent pyogenic infections (IgG)
    - P.carinii pneumonia (defective CMI)
    - Autoimmune cytopenias
    - Normal / ↑IgM, ↓IgA, IgE, IgG
    - T & B cells – normal numbers
3. **Common variable Immunodeficiency (CVID)**
    - Hypogammaglobulinemia (IgG – always)
    - B cells cannot differentiate to plasma cells
    - Molecular defect not known
    - Sporadic / Familial – F/H/O – IgA deficiency
    - Both sexes, onset – adolescence
    - Germinal centers HYPERPLASTIC
4. **Isolated IgA deficiency**
    - Most common
    - Low levels of both secretory and plasma IgA
    - Familial - related to CVID /Acquired
    - Antobodies to IgA in some cases

T CELL DEFECTS

Di George syndrome

a) Thymic hypoplasia

- The third and fourth branchial pouches fail to form properly.
- Deletion of 22q 11 is seen in 90% cases
- T cell deficiency with lack of CMI (to fungi and viruses)
- B cells and Ig are normal

b) Parathyroid hypoplasia – hypocalcemic tetany
c) Congenital defects of heart and great vessels
d) Dysmorphic facies

- 22q 11 deletion is also ass with velofaciocardial syndrome i.e. CATCH 22 * **Nezelof's syndrome'** is a possibly related syndrome; these patients have very little thymic tissue but normal parathyroid hormone levels; some of these patients lack purine nucleoside phosphorylase

SEVERE COMBINED IMMUNODEFICIENCY (SCID)

1. **AR**

   Adenosine deaminase deficiency
   ADA deficiency was he first disease to be cured by introduction of the normal gene into cells. (Gene therapy)

2. **<u>X linked</u>**

   In 50-60 % cases
   Defective interleukin 2 (IL2) receptor (*d/t defective common gamma chain (IL- 2RG), which it shares with the interleukin 4 and 7 receptors)

Others

Wisckott- Aldrich syndrome

- X linked recessive

- Immunodeficiency variable losses of cellular immunity (especially against viruses)
- Thrombocytopenia
- IgM ↓(↑catabolism of immunoglobulin),
- There is a moderate increase in lymphomas

Complement defects

- C2 deficiency – commonest
- Neisserial infections common
- C1 inhibitor deficiency → hereditary. Angioneurotic edema

AMYLOIDOSIS

Pathologic proteinaceous substance, deposited between cells in various tissues and organs which on Heinatoxylin and Eosin in is amorphous, eosinophilic, hyaline, extracellular substance

progressive accumulation →pressure atrophy of adjacent cells

Physical nature of Amyloid

Electron microscopy

- Non-branching fibrils of indefinite length and diameter of 7.5-10 nm

X-ray crystallography and Infrared spectroscopy C

Chracteristic cross β-pleated sheet confirmation (responsible for birefringence)

Chemical Nature: → 95% - Fibril protein

→ 5% - P-component and other glycoproteins

15 biochemical distinct forms:

3 more common: 1) AL (amyloid light chain)

- derived from plasma cells (most AL-Lambda chains)
- Contains Ig light chains

b) AA (Amyloid associated)
Non-immunoglobulin protein synthesized by liver

c) Aβ amyloid: In cerebral lesion of Alzheimer disease

Types:

A) SYSTEMIC :ALIZED
B) LOCALIZED

a) Systemic

1) **Primary Amyloidosis/Immunocyte dyscrasias associated**: systemic amyloidosis characterized by deposition of light chains
   (AL). Complete Ig light chain or NH, terminal fragment or both)
   Most common — Lambda or kappa
   Associated with plasma cell dyscrasia
   5-15% of patients, with multiple myeloma develop AL Amyloidosis
2) **Reactive Systemic Amyloidosis/Secondary Amyloidosis**
   - AA protein deposited

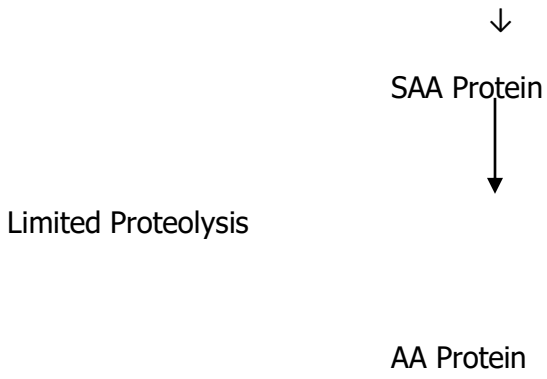
- Secondary to associated inflammations
- Systemic disorder

Association:

Previously. TB, bronchiectasis, chronic osteomyelitis Now, most common:

- Rheumatoid arthritis (13% of patients develop AA)
- Ankylosing spondylitis
- Inflammatory bowel disease
- Others: Heroine abuses
- RCC
- Hodgkin's disease

Chronic inflammation →Macrophages Activation →IL-1 and IL-6 →Liver cells



3) **Hemodialysis associated amyloidosis**:
   - Deposition of P2 Microglobulin (component of MHC class I molecule)
     (Can't be filtered through cuprophane dialysis membranes)
   - Deposits is synovium, joints and tendon sheaths
4) **Heredofamilial Amyloidosis**
   a) **Familial Mediterranean fever**: Fever with inflammation of serosal surface (Pleura, peritoneum and synovial membrane)
      - Deposits of AA proteins
      - AR Gene product →'Pyrin': Exact function not known? Regulates acute inflammations
   b) **Familial amyloidotic neuropathies (several types)**:
      - Both peripheral and autonomic nerves involved
      - AD
      - Deposits of ATTR (Transthyretin), mutant form
   c) **Systemic senile Amyloidosis**
      - Deposits of ATTR (structurally normal)
      - Deposits in heart of aged individuals (70-80 years)

B) **LOCALIZED AMYLOIDOSIS:** Nodular deposits most often in lung, larynx, skin, urinary bladder, tongue around etc.
   1) **Senile cerebral amyloidosis**
      - Found in Alzheimer's disease
        Deposits: - β— amyloid protein (Aβ)
        Precursor: Amyloid precursor protein

2) **Endocrine**

a) Medullary carcinoma of thyroid Deposits of A cal (Precursor: calcitonin)

b) Islet of Langerhans (in Type II DMA)
Deposits: AIAPP
(Precursor: Islet Amyloid Peptide)

c) Isolated Atrial Amyloidosis: Deposits: AANF
(Precursor- Atrial Natriuretic factor)

d) Prion Disease — Mis folded Prion protein

Morphology: .

AA: More severe systemic involvement

- Kidneys, Liver, Spleen, Lymph nodes, Adrenal, Thyroid
  AL: Heart, kidney, GIT, Peripheral nerves, Skin, Tongue
  Grossly organs are enlarged, waxy and firm. Iodine gives mahogany brown color which turns blue violet after sulfuric acid application.

Stains

1. Congo red: Ordinary light - pink or red color
   Polarized light - apple green birefringence
   This is due to beta pleated configuration of amyloid fibrils
   AA protein loses its affinity for Congo red after treatment with potassium permanganate
2. Metachromatic stains like methyl violet and crystal violet (violet to pink)
3. Secondary fluorescence in UV light with dyes like thioflavin S and T.
4. PAS positive due to P component, Diastase Resistant
   Kidney- most common and more serious form of organ involvement.
   Grossly enlarged pale kidney. Later on may become contracted.
   Microscopy-Deposits in glomeruli, interstitium, blood vessels. In glomeruli the deposits are mesangial and suben-dithelial.

Spleen- Deposits in white pulp (follicles) - Sago Spleen

- Involvement of splenic sinuses and red pulp - Lardaceous spleen.

Liver -Amyloid appears first in space of Disse.

Heart: Commonest site of involvement in primary amyloidosis.

Localized pink gray subendocardial droplets especially in atrial chambers

Adrenals: Initially in Zona glomerulosa

GIT: Any level: Gingiva to anus

Tongue = macroglossia (tumor forming amyloid of the tongue)

Patients on long-term hemodialysis patients develop deposits in carpal ligament producing carpal tunnel syndrome

Clinical

Renal damage is dominating and most life threatening.

Cause of death - renal failure or cardiac failure, conduction disturbances and restrictive cardiomyopathy.

Prognosis of reactive systemic amyloidosis is better than primary.

Diagnosis: Kidney, rectal or gingival biopsies - 75% cases +ve in generalized amyloidosis. Abdominal fat aspiration can also be stained with Congo red

Amyloid Fibril Proteins and Their Clinical Syndromes			
Term	**Precursor**	**Clinical Syndrome**	**Clinical Involvement**
	Systemic Amyloidoses		
AL	Immunoglobulin light chain	Primary or myeloma associated[a]	Any
AH	Immunoglobulin heavy chain	Primary or myeloma associated (rare)	Any
AA	Serum amyloid A protein	Secondary; reactive[b]	Renal, any
Abeta $_2$M	beta$_2$-Microglobulin	Hemodialysis-associated	Synovial membrane, bone
ATTR	Transthyretin	Familial (mutant) Senile systemic (wild type)	Cardiac, peripheral and autonomic nerves
AApoAI	Apolipoprotein AI	Familial	Hepatic, renal
AApoAII	Apolipoprotein AII	Familial	Renal
AGel	**Gelsolin**	Familial	Corneas, cranial nerves, renal
AFib	Fibrinogen Alpha	Familial	Renal
ALys	Lysozyme	Familial	Renal
ALECT2	Leukocyte chemotactic factor 2	?	Renal

	Localized Amyloidoses		
A beta	Amyloid beta protein	Alzheimer's disease;	Down syndrome
ACys	Cystatin C	Cerebral amyloid angiopathy	CNS, vascular
APrP	Prion protein	Spongiform encephalopathies	CNS
AIAPP	Islet amyloid polypeptide (amylin)	Diabetes-associated	Pancreas
ACal	Calcitonin	Medullary carcinoma of the thyroid	Thyroid
AANF	Atrial natriuretic factor	Age-related	Cardiac atria
APro	Prolactin	Endocrinopathy	Pituitary

[a]Localized deposits can occur in skin, conjunctiva, urinary bladder, and tracheobronchial tree.

[b]Secondary to chronic inflammation or infection, or to a hereditary periodic fever syndrome, e.g., familial Mediterranean fever

NEOPLASIA

Neoplasia is an abnormal growth which is uncoordinated and persists after the stimulus that caused id.

NOMENCLATURE

a. **Benign:** 'oma' e.g. fibroma, adenoma, papilloma, polyp (projects above the mucosal surface), cystadenoma (adenomas producting large cystic masses).

b. **Malignant (cancers)**
- Malignant neoplasm of mesenchymal tissues- sarcomas
- Malignant neoplasm of epithelial origin – carcinomas

c. Can be derived from any of the germ layers.
Ectodermal-Squamous cell carcinoma
Mesodermal-Renal cell carcinoma
Endodermal- Adeno carcinoma

d. Some tumors have more than one parenchymal cell type:
Mixed tumor of parotid- Derived from one germ layer that differentiates into more than one parenchymal cell type.
Teratoma- Made up of variety of parenchymal cell types representative of more than one germ layer.

e. Two non-neoplastic lesions that resemble tumors:
- **Hamartoma –** Excess of normal tissues native to the site (malformation).
- **Choristoma –** Heterotopic rest of cells. Excess of normal tissues at abnormal sites.

f. Others:
- **Chordoma-** Malignant tumor notochord
- **Chloroma-** Tumor of hemopoietic cells (CML)
- **Chondroma-** Benign tumor of cartilage

	BENIGN	**MALIGNANT**
Differentiation	Well differentiated, may be typical of the tissue of origin	some lack of differentiation with anaplasia; structure is often atypical
Rate of growth	Usually progressive and slow. May stop/regress. Mitotic figures- rare and normal	erratic growth-slow/rapid mitotic figures- numerous and abnormal Spontaneous regression-rentinoblastoma., neuroblastoma choriocarcinoma, melanoma
Local invasion	Usually cohesive and expansile. Well demarcated and do not invade or infiltrate surrounding tissues, often encapsulated.	locally invasive; infiltrating the surrounding normal tissue; may seem cohesive and expansile with pseudocaps-capsule gliomas and Basal cell carcinoma-locally malignant locally invasive; infiltrating the surrounding normal may seen cohesive and expansile

Metastasis	Unencapsulated benign tumors are hemangioma and neurofibroma Not seen, rarely lelomyonas meaasize.	withpseudocaps- capsule. Gliomas and basal cell carcinoma-locally malignant

NOTES

Presence of metastasis is the most important differentiating feature between benign and malignant.

Anaplasia is a hallmark of malignant. Anaplastic cells are undifferentiated

Pleomorphism implies variation in size and shape of cells, more often seen in malignant tumors**.**

Features of malignant cell are:-

- **Nuclei** are hyperchromatic
- **High N.**C. ratio.
- Nuclei are variable and bizarre.
- Coarse andclumped chromattinmitosis numors sow-
- Prominent nucleoli.
- Mitosis numerous atypical (multiple spindles, etc).

Dysplasia may show similar features but mitosis is usually typical and it is limited to the basement membrane. It does not necessarily progress to carcinoma

METASTASIS

Pathways

1) Seeding within body cavities- carcinoma colon, lung, ovary, medulloblastoma.
   Ependymoma (penetrates cerebral ventricles and carried by CSF to the meningeal surface)
2) **Lymphatic spread** more typical of carcinomas. Enlargement of lymphnodes in proximity of primary neoplasm may not imply cancerous involvement. Necrotic products of neoplasms and possibly tumor antigens often evoke reactive changes in nodes such hyperplasia of follicles.
3) **Hematogenous:** Favored by sarcomas, but cancers often use it. Venous invasion frequently involves portal areas-liver, caval flow- lungs. Carcinomas close to vertebral column- paravertebral plexus e.g. carcinoma thyroid and porstate.

Note: Renal cell carcinoma-Renal Veins- IVC-Rt. Side of heart

Hepatocellular carcinoma involves portal vein radicals.

Note (Frequently asked)

1. Bronchogenic carcinomas is the commonest cancer metastasizing to brain and heart
2. Carcinomas metastasizing to bone-
   PUBLIK T- Prostate, Lung, Breast, Liver, Intestines, Kidney and Thyroid
3. Carcinomas producing osteoblastic secondaries: Carcinoma prostate, breast, thyroid, carcinoid
4. Blood-borne metastasis usually occurs in osteo sarcoma, choriocarcinoma and renal cell carcinoma

Epidemiology of Cancer

- Most (90%) neoplasms arise from epithelium.
- The remainder arises from mesenchymal cells.
- Cancer is more common in those older than 55 years of age and is the main cause of death in women aged 40-79 and in men aged 60-79.
- Certain cancers are particularly common in children younger than 15 years of age:
- Tumors of the hemoatopoietic system (leukemias and lymphomas)
- Neuroblastomas

- Wilms tumors
- Retinoblastomas
- Sarcomas of bone and skeletal muscle

Heredity predisposition

Heredity plays a role in the development of cancer even in the presence of clearly defined environmental factors. Nevertheless, less than 10% of the cancer patients have inherited mutations the predispose to malignancy; the frequency is even lower (0.1%) for certain cancers.

Inherited Cancer Syndrome (Autosomal dominant)

RB	Retinoblastoma
P53	**Li-Fraumeni** syndrome (various tumors)
P16INK4A	Melanoma
APC	Familial adenomatous polyposis/colon cancer
NF1, NF2	Neurofibromatosis 1 and 2
BRCA-1, BRCA-2	Breast and ovarian tumors
MEN1, RET	Multiple endocrine neoplasia 1 and 2
MSH2, MLH1, MSH6	Hereditary non-polyposis colon cancer
PATCH	Nevoid basal cell carcinoma syndrome
PTEN	Cowden syndrome (epithelial cancers)
LKBI	Peutz-Jegher syndrome (epithelial cancers)
VHL	Renal cell carcinoma

Familial Cancers

Familial clustering but the role of inherited predisposition is not clear:

- Breast cancer
- Ovarian cancer
- Colon cancer other than FAP
- Pancreatic carcinoma

AR Syndrome of Defective DNA Repair

- Xeroderma pigmentation
- Ataxia telangiectasia
- Bloom syndrome
- Fanconi anemia

Prenoplastic Conditions:

- Endometrial hyperplasia
- Cirrhosis in Liver
- Chronic gastritis
- Ulcerative colitis
- Solar keratosis
- Leukoplakia

MOLECULLAR BASIS OF CANCER

1) Non-lethal genetic damage lies at the heart of carcinogenesis. The genetic damage may be acquired in the somatic cells by environmental agents or inherited in the germline.
2) Tumors develop as a clonal progeny of single genetically damaged progenitor cell.
3) Four classes of normal regulatory genes are the targets of genetic damage:
    - Growth-promoting proto-oncogenes
    - Growth-inhibiting tumor suppressor genes

- Genes that regulate apoptosis.
- Genes that regulate DNA repair

4) Carcinogenesis multistep process: The attributes of malignancy (e.g. invasiveness, excessive growth, and escape from the immune system) are acquired in a stepwise fashion called tumor progression. At the genetic level, progression results from accumulation of successive mutations.

Essential alterations for malignant transformation :

- Self-sufficiency in growth signals (proliferation without external stimuli)
- Insensitivity to growth inhibitory signals.
- Evasion of apoptosis.
- Defects in DNA repair
- Limitless replicative potential (related to telomere maintenance).
- Sustained angiogenesis.
- Ability to invade and metastasize.
- Ability to escape immune recognition and regulation

Normal cell cycle

Cyclins and cyclin-dependent kinases

- The orderly progression of cells through the cell cycle is orchestrated by cyclins and cyclin-dependent kinases (CDK) and their inhibitors.
- **Cyclins:** Synthesized during specific phases of cell cycle, activate CDKs
- Cyclin D, E, A, B appear sequentially during cell cycle.
- **CDK:** Drive cell cycle by phosphorylating critical target proteins. Expressed constitutively during cell cycle, but in an inactive form, activated by phosphorylation (after binding to cyclins)

Cyclin D and Rb protein

a) Cyclin D- First cyclin to appear (mid G1)
   - Disappears in S phase
   - Three forms: D1, D2, D3
   - Degraded by ubiquitin- proteasome pathway
   - Cyclin D activates CDK4
   - Cyclin D-CDK4 complex promotes cell replication by phosphorylating Rb protein

b) **Rb protein-** Encoded by Rb gene (tumor suppressor gene on 13q14).
   Main barrier to cell cycle progression from the G-S phase. Molecular on-off switch applies breaks to the cell cycle.
   - Active form of Rb-Hypophosphorylated Rb. Found in quiescent cells.
   - Binds E2F/DP1 factors and inhibits DNA transcription
   - E2F/DP1/Rb complex recruits histone deacetylase which causes compaction of nuclear chromation and inhibition of DNA transcription.

- When quiescent cells are stimulated by growth factors, cyclin D/CDK4 complex phosphorylates Rb protein.
  Hyperphosphorylated Rb dissociates from E2F factors→Activation of transcription of E2F target genes→ progression through S phase

Cyclin E/CDK2

- G1/S transition and progression through S phase

Cyclin A/CDK2

- Initiates G2 M transition
- Regulation events at mitotic prophase

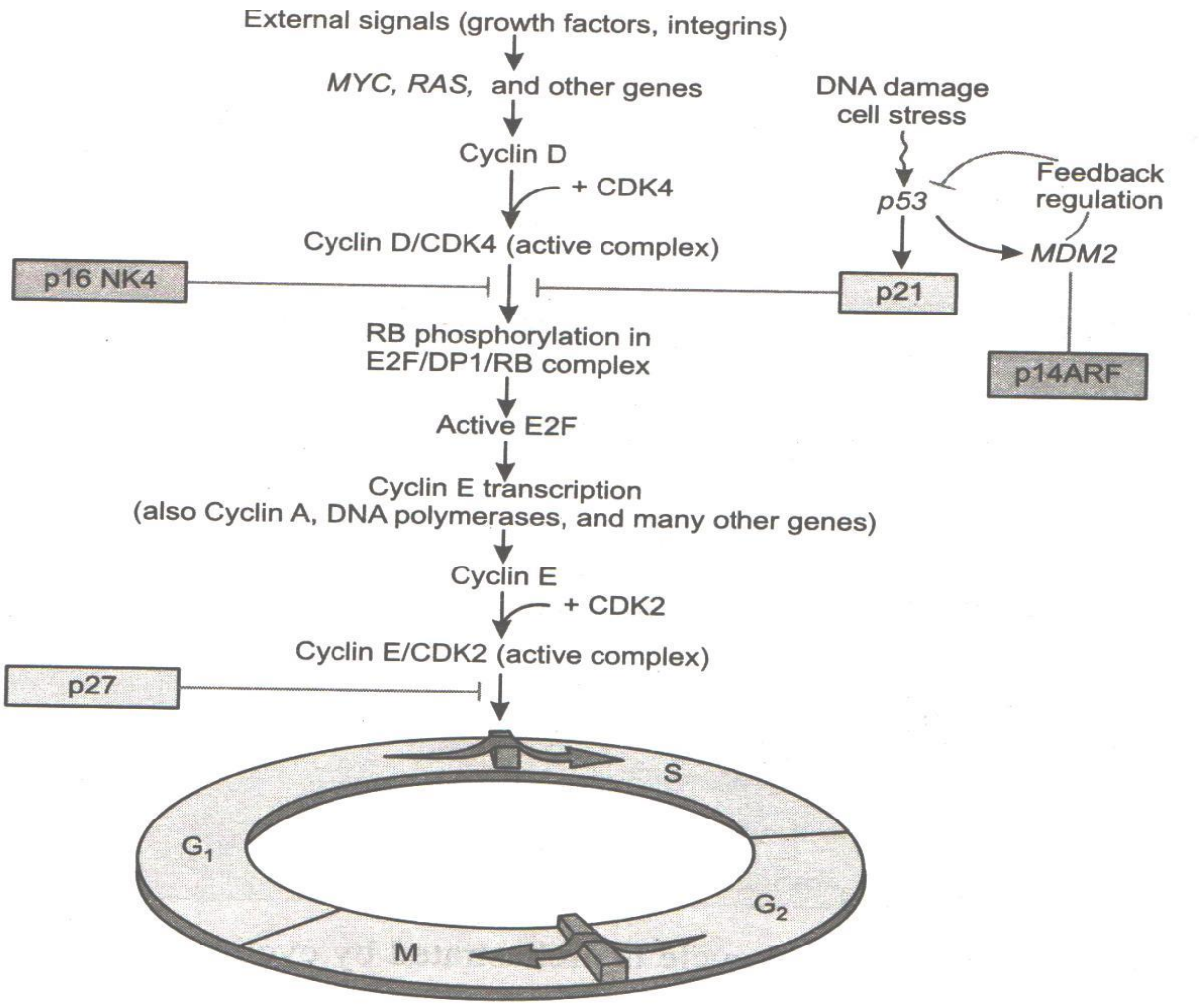
Cyclin B/CDK1-Activated by protein phosphatase (cdc25)

- Responsible for G2M transition and progression beyond prophase
- Regulates critical events at G2M transition

Main Cell Cycle Components and Their Inhibitors

Cell Cycle Component	Main Function
CYCLIN-DEPENDENT KINASES	
CDK4	Forms a complex with cyclin D that phosphorylates RB, allowing the cell to progress through the G_1 restriction point.
CDK2	Forms a complex with cyclin E in late G_1, which is involved in G_1/S transition. Forms a complex with cyclin A at the S phase that facilitates G_2/M transition.
CDK1	Forms a complex with cyclin B that facilitates G_2/M transition.
INHIBITORS	
CIP/KIP family: p21, p27 (CDKN2A-C)	Block the cell cycle by binding to cyclin-CDK complexes; p21 is induced by the tumor suppressor p53; p27 responds to growth suppressors such as TGF-β.
INK4/ARF family (CDKN1A-D)	p16/INK4a binds to cyclin D–CDK4 and promotes the inhibitory effects of RB; p14/ARF increases p53 levels by inhibiting MDM2 activity.
CHECKPOINT COMPONENTS	
p53	Tumor suppressor gene altered in the majority of cancers; causes cell cycle arrest and apoptosis. Acts mainly through p21 to cause cell cycle arrest. Causes apoptosis by inducing the transcription of pro-apoptotic genes such as *BAX*. Levels of p53 are negatively regulated by MDM2 through a feedback loop. p53 is required for the G_1/S checkpoint and is a main component of the G_2/M checkpoint.
Ataxia-	Activated by mechanisms that sense double-stranded DNA breaks.

Cell Cycle Component	Main Function
telangiectasia mutated	Transmits signals to arrest the cell cycle after DNA damage. Acts through p53 in the G_1/S checkpoint. At the G_2/M checkpoint, it acts both through p53-dependent mechanisms and through the inactivation of CDC25 phosphatase, which disrupts the cyclin B–CDK1 complex. Component of a network of genes that include *BRCA1* and *BRCA2*, which link DNA damage with cell cycle arrest and apoptosis



SELF-SUFFICIENCY IN GROWTH SIGNALS (PROLIFERATION WITHOUT EXTERNAL STIMULI)

- Tumor growth autonomy occurs when the normal steps of cell proliferation occur in the absence of growth-promoting signals.
- Normal cell proliferation involves the following steps that can be potentially subverted by oncogenes:
- Binding of GF to receptor
- Transient and limited activation of the growth factor receptor→activation of signal transducing proteins
- Transmission of signal to nucleus
- Activation of nuclear regulatory factors
- Entry and progression of the cell through the cell cycle
- Proto-oncogenes are normal cellular genes that affect growth and differentiation.
- Oncogenes: Derived from proto-oncogenes which promote autonomous cell growth in cancer cells. Discovered in acute-transforming retrovirus by Varmus and Bishop.
  Many p oncs named after viral homologues.
  Encode proteins-Oncoproteins

Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

Category	Proto-oncogene	Mode of Activation	Associated Human Tumor
GROWTH FACTORS			
PDGF-β chain	*SIS* (official name *PBGFB*)	Overexpression	Astrocytoma
			Osteosarcoma
Fibroblast growth factors	*HST1*	Overexpression	Stomach cancer
	INT2 (official name *FGF3*)	Amplification	Bladder cancer
			Breast cancer
			Melanoma
TGF-α	*TGFA*	Overexpression	Astrocytomas
			Hepatocellular carcinomas
HGF	*HGF*	Overexpression	Thyroid cancer
GROWTH FACTOR RECEPTORS			
EGF-receptor family	*ERBB1* (*EGFR*), *ERRB2*	Overexpression	Squamous cell carcinoma of lung, gliomas
FMS-like tyrosine kinase 3	*FLT3*	Amplification	Breast and ovarian cancers
Receptor for neurotrophic factors	*RET*	Point mutation	Leukemia
		Point mutation	Multiple endocrine neoplasia 2A and B, familial medullary thyroid carcinomas
PDGF receptor	*PDGFRB*	Overexpression, translocation	Gliomas, leukemias

Category	Proto-oncogene	Mode of Activation	Associated Human Tumor
Receptor for stem cell (steel) factor	*KIT*	Point mutation	Gastrointestinal stromal tumors, seminomas, leukemias
PROTEINS INVOLVED IN SIGNAL TRANSDUCTION			
GTP-binding	*KRAS*	Point mutation	Colon, lung, and pancreatic tumors
	HRAS	Point mutation	Bladder and kidney tumors
	NRAS	Point mutation	Melanomas, hematologic malignancies
Nonreceptor tyrosine kinase	*ABL*	Translocation	Chronic myeloid leukemia
			Acute lymphoblastic leukemia
RAS signal transduction	*BRAF*	Point mutation	Melanomas
WNT signal transduction	β-catenin	Point mutation	Hepatoblastomas, hepatocellular carcinoma
		Overexpression	
NUCLEAR-REGULATORY PROTEINS			
Transcriptional activators	C-*MYC*	Translocation	Burkitt lymphoma
	N-*MYC*	Amplification	Neuroblastoma, small-cell carcinoma of lung
	L-*MYC*	Amplification	Small-cell carcinoma of lung
CELL CYCLE REGULATORS			
Cyclins	Cyclin D	Translocation	Mantle cell lymphoma
		Amplification	Breast and esophageal cancers
	Cyclin E	Overexpression	Breast cancer
Cyclin-dependent kinase	*CDK4*	Amplification or point mutation	Glioblastoma, melanoma,

Singal-transducing proteins

Ras- 15-20% of human tumors contain mutated ras proteins.
Ras is attached to cell membrane by farnesyl anchor
Inactive ras – GDP-bound, active ras- GTP bound
Normal ras protein flips back and forth between an activated (GTP-bound) signal transmitting form and an inactive (GDP-bound) quiescent form.

- The conversion of active ras to inactive ras is mediated by its intrinsic GTPase activity and is augmented by

a famil of GTPase activating proteins (GAPs) encoded by NF-1 gene.

- Mutant ras proteins bind GAPs, but still lack GTPase activity, and hence remain trapped in the signal-transmitting GTP-bound form.
- In this state, the activate ras turns on the MAP kinase pathway and promotes mitogenesis.

Nuclear transcription proteins

- All signal transduction pathways enter nucleus.
- Regulated by family of genes products which control transcription of growth-related e.g. myc. Myb. Jun.fos

C-myc

- Expressed in virtually all eukaryotic cells
- Belongs to immediate early growth response genes.
  Signal
  ↓ translation
  ↓
  c-myc protein enters the nucleus
  ↓
  myc-max heterodimer
  Transient in c-myc m RNA
  ↓
  E-boxes → Transcription activation
  (DNA Sequences) (Genes for Ornithine decarboxylase, CDKs)
- Myc activation in absence of survival signals →Apoptosis (Conflict Model)-observed in vitro.
  Burkitt's lymphoma: t(8:14), chromosome 8 has c-myc

Activation of oncogenes

I. **Point mutation:** e.g. Ras
II. **Chromosomal rearrangement**

- Translocation
- Inversion

Translocation:

a) Overexpression of p-onc by placing them under regulatory element of immunoglobulin or T cell receptor loci e.g.

- Burkitt's lymphoma t(8,14): chromosome 8-c myc gene. Chromosome 14→IgH locus
- Mantle cell lymphoma t (11:14): chromosome 11→cyclin D; chromosome 14-IgH locus)
- Follicular lymphoma t (14:18): chromosome 18→bcl 2 locus, chromosome 14-IgH locus)

b) Recombination of two loci→ formation of hybrid gene→chimeric gene product e.g. ph chromosome (9:22)
Ph chromosome:

- In CML: P 210
- In ALL: P 180

In Ewing's sarcoma:

- T (11:22): EWS gene at chromosome 22 and FL-1 gene at chromosome 11
- Cheimeric EWS –FL-1→Transactivator of c-myc promoter

iii) **Gene amplification**

- Detected by molecular hybridization with appropriate DNA probe
- Two patterns
  Dms-double minutes-multiple, small, chromosome like structures
  HSRs-homogenous staining regions

e.g. N-myc	Neuroblastoma	Poor
	Breast Cancers	Prognosis

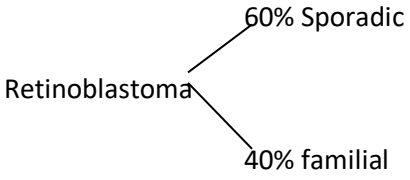
INSENSITIVITY TO GROWTH INHIBITORY SIGNALS

Cancer-suppressor genes

- Cancers can arise not only by activation of growth promoting oncogenes, but also by inactivation of genes that normally suppress cell proliferation (tumor-suppressor genes).
- Tumor-Suppressor genes apply brakes to cell proliferation and their loss leads to neoplastic growth.

Rb Gene

Most commonly studied tumor-suppressor gene is Rb gen (chromosome 13q 14).
First tumor-suppressor gene to be discovered.
Expressed in every cell type examined
Underphosphorylated (active) Rb protein serves as brake on advancement of cell from G1 to S phase
Phosphorylation Rb protein releases the brake
Mutations of Rb gene occur in 'Rb pocket'
Loss of Rb gene is associated with Retinoblastoma and osteogenic sarcoma



To account for familial and sporadic occurrence of retinoblastomas, a two hit hypothesis has been proposed called **Knudson's "two-hit hypothesis"**
Both normal alleles of the Rb locus must be inactivated (two hits) for the development of a retinoblastoma.
In hereditary cases - I hit is inherited (present in all somatic cells).
- II hit occurs in one of many retinal cells
In sporadic cases - Both hits occur somatically in a single retinal cell.

Pathways coverging on pRb

- Cyclin D/CDK4, p16
- TGF β which is a growth-inhibiting cytokine that upregulates CDK inhibitors (e.g.. p27) thus preventing Rb hyperphosphorylation.
- Various viruses SV 40, polyoma virus, adenovirus, HPV→bind in Rb pocket.
- P53 GENE

Selected Tumor Suppressor Genes Involved in Human Neoplasms

Subcellular Locations	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Assocated with Inherited Mutations
Cell surface	TGF-β receptor	Growth inhibition	Carcinomas of colon	Unknown
	E-cadherin	Cell adhesion	Carcinoma of stomach	Familial gastric cancer
Inner aspect of plasma membrane	*NF1*	Inhibition of RAS signal transduction and of p21 cell cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	*NF2*	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromastosis type 2, acoustic schwannomas, and meningiomas
Cytosol	*APC*/β-catenin	Inhibition of signal transduction	Carcinomas of stomach, colon, pancreas; melanoma	Familial adenomatous polyposis coli/colon cancer
	PTEN	PI3 kinase signal transduction	Endometrial and prostate cancers	Cowden syndrome
	SMAD2 and *SMAD4*	TGF-β signal transduction	Colon, pancreas tumors	Unknown
Nucleus	*RB1*	Regulation of cell cycle	Retinoblastoma; osteosarcoma carcinomas of breast, colon, lung	Retinoblastomas, osteosarcoma
	p53	Cell cycle arrest and apoptosis in response to DNA damage	Most human cancers	Li-Fraumeni syndrome; multiple carcinomas and sarcomas
	WT1	Nuclear transcription	Wilms' tumor	Wilms' tumor
	P16/INK4a	Regulation of cell cycle by inhibition of cyclindependent kinases	Pancreatic, breast, and esophageal cancers	Malignant melanoma
	BRCA1 and *BRCA2*	DNA repair	Unknown	Carcinomas of female breast and ovary; carcinomas of male

Subcellular Locations	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Assocated with Inherited Mutations
				breast

PI3 kinase, phosphatidylinositol 3-kinase.

p53: Guardian of the Genome.

- The *p53* gene is located on chromosome 17p13.1, and it is the most common target for genetic alteration in human tumors.
- As with the *RB* gene, inheritance of one mutant allele predisposes individuals to develop malignant tumors because only one additional "hit" is needed to inactivate the second, normal allele. Such individuals, said to have the *Li-Fraumeni syndrome*, have a 25-fold greater chance of developing a malignant tumor by age 50 than the general population.
- it is evident that p53 acts as a "*molecular policeman*" that prevents the propagation of genetically damaged cells
- short half-life (20 minutes) because of its association with MDM2, a protein that targets it for destruction
- *To summarize, p53 links cell damage with DNA repair, cell cycle arrest, and apoptosis.*
- The discovery of p53 family members p63 and p73 has revealed that p53 has collaborators. Indeed, p53, p63, and p73 are players in a complex network with significant cross-talk that is only beginning to be unraveled

DNA REPAIR AND INSTABILITY IN CANCER CELLS

Genes for DNA Repair

DNA damage → Environmental agents
DNA damage → During replication

Repair → Mismatch repair
Repair → Nucleotide excision repair
Repair → Recombination repair

1) Mismatch repair genes
   - Act as spell characters when a strand of DNA is replicating.
   - Without these, errors slowly accumulate in several genes (protoncogenes/tumor suppressor genes)→

Replication error phenotype.

- Replication error phenotype is documented by microsatellite instability.
- Microsatellites are tandem repeats of one-two nucleotides scattered through out genome, mixed for an individual, fixed for life.
- HNPCC or Lynch syndrome-Patients develop predominantly caecum and proximal colonic carcinoma without an adenomatous polyp pre-existing stage.
- Defect in 'mismatch repair genes' MSH2 (2p 16) and MLH (3p21) is implicated

2) Nucleotide excision Repair (NER)

U.V. Light →cross=linking of pyrimidine residues→Repaired by NER pathway.

- Mutations in NER genes-xeroderma pigmentosa.

3) **Repair by homologous recombination**

- Repaired double-stranded DNA breaks by homologous recombination
- Genes involved are:
    - ATMegne-senses double stranded DNA breaks. ATM mutations-Ataxia Telangiectasia
    - Mutation in BLM helicase → Blooms syndrome
    - **BRCA-1 and BRCA-2**
- Participate in repair of double stranded DNA breaks by homologous recombination
- Genes involved are:
    - ATMgene – senses double stranded DNA breaks. ATM mutations-Ataxia telangiectasia
    - Mutation in BLM helicaseα→Blooms syndrome
    - **BRCA-1 and BRCA-2**
- Participate in repair of double stranded DNA breaks by homologous recombination.
- ATM and CHEK2 phosphorylate BRCA-1and RAD51 which colocalize to nucleus
- Associated with hereditary breast and ovarian cancers, not associated with sporadic carcinomas
- **BRCA-1** — 17q21, high risk of breast and epithelial ovarian carcinomas. Slightly ↑risk of prostate and colonic carcinoma.

BRCA-2— 13 q12 Risk of carcinoma ovary, carcinoma male breast

DEVELOPMENT OF SUSTAINED ANGIOGENES1S

Angiogenesis: in absence of angiogenes, tumor cells can grow upto 1-2 mm.

Angiogenesis helps in supply of nutrients and O_2 and in metastasis (related to Microvessel Density)

Promoters of Angiogenesis	**Inhibitors of Angiogenesis**
VEFG	- Thrombospondin 1
	- Angiostatin
Bfgf	- Vasculostatin
	- Endostatin

Invasion and metastasis

Tumor progression

- Subclone with metastatic potential appears.
- Shows decreased expression of E-cadherins and abnormal catcnin
- Attachment to matrix components by laminin and f-ibronectin receptors (all around cell membrane),↑ expression of integrins.
- Degradation of extracellular matrix by scrine proteases, eysteine proteases (cathepsin D), MMP (Type IV collagenase)
- Locomotion aided by factors derived from tumor cells: β thytnosin (+ve in prostate cancer, -ve in BHP), insulin-like growth factors I and II, autocrine motility factors, etc.
- Vascular dissemination and homing (tumor emboli formed with platelets and CD44 + T cells).
- Arrest and egress.

Genes for metastasis

- E. cadherin
- TIMP
- Nm 23
- KAI-1 in prostate Ca
- KISS 1 human malignant melanoma

CARCINOGENIC AGENTS

1) **Chemical**
   John Hill: Snuff →polyps
   Sir Percival Pott; Soot → scrotal skin carcinoma
   Neoplastic transformation brought about by cancer cells is a multistep process. It can be broadly divided into two
   Stages: initiation and promotion
   Initiation — induction of irreversible change (mutations) in the genome of cells.
   Initiated cells are not transformed cells but they can give rise to tumors when appropriately stimulated by promoting agents.
   Promotion — Process of tumor induction in previously initiated cells by chemicals referred to as promotion.

Effect of promoters is short-lived and reversible.

Complete carcinogens are capable of both initiation and propagation.

Promoters: Phorbol esters, phenols, hormones. drugs, okadaic acid. saccharin.

Initiators

Direct acting e.g. Alkylating agents.

Indirect — Cyto p450 dependent mono-oxygenases convert them into active form e.g. Benzopyrene and aromatic amines.

Ames test: Because vast majority of chemical carcinogens arc mutagenic. this test is a simple in vitro test for carcinogenicity using the ability of potential carcinogens to induce mutations in selected strains of bacterium S. typhimurium.

Major Chemical Carcinogens

DIRECT-ACTING CARCINOGENS

Alkylating Agents
β-Propiolactone Dimethyl sulfate Diepoxybutane Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others)
Acylating Agents
1-Acetyl-imidazole Dimethylcarbamyl chloride
PROCARCINOGENS THAT REQUIRE METABOLIC ACTIVATION
Polycyclic and Heterocyclic Aromatic Hydrocarbons
Benz[*a*]anthracene Benzo[*a*]pyrene Dibenz[*a*,*h*]anthracene 3-Methylcholanthrene 7,12-Dimethylbenz[*a*]anthracene
Aromatic Amines, Amides, Azo Dyes
2-Naphthylamine (β-naphthylamine) Benzidine 2-Acetylaminofluorene Dimethylaminoazobenzene (butter yellow)
Natural Plant and Microbial Products
Aflatoxin B_1
Griseofulvin Cycasin Safrole Betel nuts
Others
Nitrosamine and amides Vinyl chloride, nickel, chromium

Insecticides, fungicides Polychlorinated biphenyls

2.Microbes:Viruses

DNA
HPV
EBV
HBV and KSHV

HPV:~ 70 types

Causes warts, squamous cell carcinoma of cervix, and anogenital, oral and larynx carcinoma.

- Low risk-6, 11
- Intermediate risk— 31, 33
- High risk — 16, 18

Benign warts and preneoplastic lesions — viral DNA is not integrated, remains in episom.al form.

In Carcinoma→ integration of viral DNA occurs in host genome. Site of integration is random but clonal.

HPV virus has E_1/E_2 ORF which is interrupted by integration into host genomc.

E_7-- binds to under phowhorvlated pR1) and inactivates it

E_6-binds to and degrades p53.

Ebstein Barr virus: Associated with

- Burkitt's lymphoma,
- B-cell lymphoma in immunosuppressed. (In HIV infection)
- Nasopharyngeal carcinoma
- Hodgkin's disease
- Oral Harry Leukoplakia
- Infections mononucleosis (benign condition)
- CD21 is the Kati receptor
- Virus remains in the host cells in episotnal form and the cells become immortalized.

HBV: Viral DNA is integrated in the host genome and the integration is clonal.

- No consistent pattern of integration
- No oncoproteins encoded by HBV genome

- Injury and regenerative hyperplasia causes the pool of mitotically active cells to be subjected to mutational damage by environmental agents.
- HBV encodes HBx protein which causes transcriptional activation of several proto-oncogenes.
- HBx binds to and inactivates p53

RNA virus

HTLV-I virus: Causes T cell leukemiallymphoma, tropical spastic paraparesis, uveitis; arthritis.

Bacteria

H. pylori: Gastric carcinoma, lymphomas (B cells)

3.Radiation:

Biological effects

1. Dose rate: single dose> divided doses
2. Target: DNA of rapidly dividing cells — Most sensitive (Hematopoietic. germ cells, GI epithelium; squamous epithelium; endothelial cells, lymphocytes)
3. G2 and M: Most sensitive to ionizing radiation

a. UV rays UVA (320-400 mm)

- UVB (280-320 mm) →= Induction of cutaneous carcinoma (SCC. MM, BCC)
- UVC (200-280mm) →= Filtered by ozone
- UV light leads to formation of pyrimidine dimmers in DNA which are normally removed by NER pathway.
- Xeroderma pigmentosum: defective NER genes characterized by:
  - ↑Photosensitivity
  - 200 times increased risk of skin cancers
  - Neurologic abnormalities

Ionizing radiation

Electro magnetic (X-ray, y-rays) etc.)

Participate (α, β etc.)

Lonising radiations cause cancer by their ability to induce mutations.

Mutations result from direct of the radiant energy or an indirect effect mediated by generation of free radicals from water or oxygen.

Radiation-induced neoplasms:

- Most frequent: Leukemias (except CLL); papillary carcinoma thyroid
- Intermediate: Breas, lung, salivary gland
- Least: Skin, bone, GIT

Paraneoplastic Syndromes

- That cannot be explained by local or distant spread of tumor or by elaboration of hormones indigenous to tissue form which tumor arose.
- Are seen in 10% of malignant disease
- May be the earliest manifestation
- Can cause significant clinical problem
- May mimic metastatic disease

Paraneoplastic Syndromes

Clinical Syndromes	Major Forms of Underlying Cancer	Causal Mechanism
ENDOCRINOPATHIES		
Cushing syndrome	Small-cell carcinoma of lung	ACTH or ACTH-like substance
	Pancreatic carcinoma	
	Neural tumors	
Syndrome of inappropriate antidiuretic hormone secretion	Small-cell carcinoma of lung; intracranial neoplasms	Antidiuretic hormone or atrial natriuretic hormones
Hypercalcemia	Squamous cell carcinoma of lung	Parathyroid hormone–related protein (PTHRP), TGF-α, TNF, IL-1
	Breast carcinoma	
	Renal carcinoma	
	Adult T-cell leukemia/lymphoma	
Hypoglycemia	Ovarian carcinoma	
	Fibrosarcoma	Insulin or insulin-like substance
	Other mesenchymal sarcomas	
Carcinoid syndrome	Hepatocellular carcinoma	
	Bronchial adenoma (carcinoid)	Serotonin, bradykinin
	Pancreatic carcinoma	
Polycythemia	Gastric carcinoma	
	Renal carcinoma	Erythropoietin
	Cerebellar hemangioma	
	Hepatocellular carcinoma	
NERVE AND MUSCLE SYNDROMES		
Myasthenia	Bronchogenic carcinoma	Immunological

Clinical Syndromes	Major Forms of Underlying Cancer	Causal Mechanism
Disorders of the central and peripheral nervous system	Breast carcinoma	
DERMATOLOGIC DISORDERS		
Acanthosis nigricans	Gastric carcinoma	Immunological; secretion of epidermal growth factor
	Lung carcinoma	
	Uterine carcinoma	
Dermatomyositis	Bronchogenic, breast carcinoma	Immunological
OSSEOUS, ARTICULAR, AND SOFT-TISSUE CHANGES		
Hypertrophic osteoarthropathy and clubbing of the fingers	Bronchogenic carcinoma	Unknown
VASCULAR AND HEMATOLOGIC CHANGES		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma	Tumor products (mucins that activate clotting)
	Bronchogenic carcinoma	
	Other cancers	
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Red cell aplasia	Thymic neoplasms	Unknown
OTHERS		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

ACTH, adrenocorticotropic hormone; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

Selected Tumor Markers

HORMONES	
Human chorionic gonadotropin	Trophoblastic tumors, nonseminomatous testicular tumors
Calcitonin	Medullary carcinoma of thyroid
Catecholamine and metabolites	Pheochromocytoma and related tumors

Ectopic hormones	See “Paraneoplastic Syndromes” (Table 7-11)
ONCOFETAL ANTIGENS	
α-Fetoprotein	Liver cell cancer, nonseminomatous germ cell tumors of testis
Carcinoembryonic antigen	Carcinomas of the colon, pancreas, lung, stomach, and heart
ISOENZYMES	
Prostatic acid phosphatase	Prostate cancer
Neuron-specific enolase	Small-cell cancer of lung, neuroblastoma
SPECIFIC PROTEINS	
Immunoglobulins	Multiple myeloma and other gammopathies
Prostate-specific antigen and prostate-specific membrane antigen	Prostate cancer
MUCINS AND OTHER GLYCOPROTEINS	
CA-125	Ovarian cancer
CA-19-9	Colon cancer, pancreatic cancer
CA-15-3	Breast cancer
NEW MOLECULAR MARKERS	
p53, APC, RAS mutants in stool and serum	Colon cancer
p53 and RAS mutants in stool and serum	Pancreatic cancer
p53 and RAS mutants in sputum and serum	Lung cancer
p53 mutants in urine	Bladder cancer

HEMATOLOGY

Red Blood Cell Pathology

Abnormal size: Anisocytosis (aniso means unequal).
Abnormal shape: Poikilocytosis (poikilo means various)

Red Cell Shapes

1. Elliptocytes may be seen in hereditary elliptocytosis
2. Spherocytes result from decreased RBC membrane.

I. May be seen in hereditary spherocytosis
II. Autoimmune hemolytic anemia
III. G6PD deficiency

3. **Target cell** result from increased RBC membrane. May be seen in hemoglobinopathies like HbC, thalassemia, and liver disease
4. Acanthocytes **have irregular** spicules on their surfaces. Numerous acanthocytes can be seen in abetalipoproteinemia.
5. **Echinocytes** (or burr cells) have smooth undulations on their surface. They may be seen in uremia or more commonly as an artifact.
6. **Schistocytes** are RBC fragments (helmet cell is a type of schistocyte). Can be seen in microangiopathic hemolytic anemias or traumatic hemoloysis.
7. **Bite cells** are RBC's with "bites" of cytoplasm being removed by splenic macrophages. Bite cells may be seen in
8. **Teardrop cells (dacrocytes)** may be seen in thalassemia and myelofibrosis.
9. **Sickle cells (**drepanocytes) are seen in sickle cell anemia.
10. **Rouleaux** ("stack of coins") refer o RBCs lining up in a row. Rouleaux are characteristic of multiple myeloma. Red cell inclusions

- **Basophilic** stippling results from cytoplasmic remnants of RNA. May indicated reticulocytosis or lead poisoning.
- **Howell-joly bodies are remnants of nuclear chromatin. May occur in severe anemias or patient without spleens.**
- Ring sideroblasts have iron trapped abnormally in mitochondria, forming a ring around nucleus. Can be seen in sideroblastic anemia.
- Heinz bodies result from denatured hemoglobin. Can be seen with G6PD (glucose-6-phosphate dehydrogenase) deficiency.

All tissues of the body that have normal cycles of cell replacement and repair are dependent upon resident cells for ongoing cell production, and these "special" cells are termed ***stem cells*.**

The stem cells generally reside in specific locations **(niches),** are not fully differentiated (may not display the appearance or all of the functions of the mature cells of the tissue), possess controlled but robust proliferative potential (for the lifetime of the host tissue), and have the capacity to divide into daughter cells in which one of the daughter cells retains all the properties of the parental cell (self-renewal) whereas the other daughter cell adopts a differentiated fate specific for the needs of that tissue

Thus, most **tissue stem cells** residing in vivo are defined as clonal, self-renewing, multipotent cells sustaining the homeostatic cellular requirements of a tissue or organ for the lifetime of the host.

Vertebrate stem cells can be classified as **embryonic stem (ES) cells** or **adult stem cells (also called somatic or postnatal stem cells).**

The fertilized oocyte (zygote) is the "mother" of all stem cells

This cell has the potential for forming all the cells and tissues of the body plan, including the placenta and extraembryonic membranes. Thus, the zygote is a **totipotent cell**.

<u>Hematopoiesis:</u>

Origin of definitive hemopoietic stem cells- not settled (bust most – AGM – mesoderm of intraembryonic aorta / gonad / mesonephros)

Haematopoiesis

3rd week – yolk sac (New data ⟶ intrembryonic gonad / aorta / mesonephros and germ cells)

8 week – FETAL LIVER – chief site of blood cell formation till birth

At birth – BM – sole source of blood cells

At 18 – year – old only vertebrae, rib, sternum, skull, pelvis & proximal epiphyseal region of humans & femur retain red marrow

From about gestational week 30 onward, β-globin synthesis steadily increases so that, by term, 50% to 55% of hemoglobin synthesized is HbA. HbF level gradually declines so that normal levels (<1%) are achieved by 200 days after birth.

Extramedullary hematopoiesis – First site ⟶ liver

Second site ⟶ spleen

⟶ Lymph node or thymus

The **earliest** recognizable erythroid cell is the *proerythroblast*, which after four to five mitotic divisions and serial morphologic changes gives rise to mature erythroid cells. Its progeny include *basophilic erythroblasts*, which are the earliest daughter cells, followed by *polychromatophilic* and *orthochromatic erythroblasts*. Their morphologic characteristics reflect the accumulation of erythroid-specific proteins (i.e., hemoglobin) and the decline in nuclear activity (Fig. 25-2). After the last mitotic division, the inactive dense nucleus of the orthochromatic erythroblast moves to one side of the cell and is extruded, encased by a thin cytoplasmic layer. Expelled nuclei are ingested by marrow macrophages, and the resulting enucleated cell is a *reticulocyte*.

The bone marrow microenvironment consists of **three broad components**: stromal cells (e.g., fibroblasts, endothelial cells, osteogenic cells), accessory cells (monocytes, macrophages, T cells), and extracellular matrix (a protein–carbohydrate scaffold). **Accessory cells are progeny of hematopoietic stem cells**; hence, after marrow transplantation, these cells are of donor origin, whereas stromal cells are host derived.[1] Extracellular matrix molecules are synthesized and secreted by microenvironmental cells and include collagens (types I, III, IV, and V), glycoproteins (fibronectin, laminins, thrombospondins, hemonectin, and tenascin), and glycosaminoglycans (hyaluronic acid, chondroitin, dermatan, and heparan sulfate). Besides providing structure to the marrow space and a surface for cell adhesion, the microenvironment is important for hematopoietic cell homing, engraftment, migration, and the response to physiologic stress and homeostasis.

Bone marrow: Ratio = fat cells / hemopoietic element = 1:1 (nl adult) $\rightarrow \therefore$ adult only half of marrow space is active in hematopoiesis.

Origin of hemepoeitic elements – PLURIPOTENT STEM Cells – property of self renewal & differentiation

Granulocytes – 60%
Erythropoid precursors – 10%

Lymphocyte & monocyte precursors – 10%

} (in adult)

Unidentified / disintegrating cells – 10%

Factors acting on early stem cell s are – stem cell factor (c-kit ligand) & FLT 2 ligand
Recombinant factors being used to stimulate hematopiesis – (i) E.P.O. (ii) GM-CS F
(ii) G-CSF 9 (iii) Thrombopoietin
Stem cells can differentiate from – Endothelial cells, hepatocytes, bile duct, myocardium, dia, neuron skeletal muscle

Certain genes, such as **SCL**, are absolutely required for hematopoietic development, whereas other genes, such as **GATA2, c-myb, CBF, TEL**, and some downstream signal transducing molecules, such as gp30 and Shp-2, are responsible for expansion and maintenance of a normal pool of fetal liver and adult hematopoietic progenitors.

Maturation of multipotent progenitor stem cells into specialized blood cells (lymphocytes, erythrocytes, neutrophils, monocytes, and eosinophils, among others) is regulated by a well-orchestrated interplay of transcription factors that are capable of instructing the expression of a specific set of genes within a specified lineage

The **first category** includes factors such as stem cell leukemia transcription factor (SCL), GATA2, and acute myeloid leukemia transcription factor-1 (AML-1) that influence differentiation to all of the hematopoietic lineages; the **second category** comprises the master regulators of lineage development, including GATA1, PU.1, and CCAAT enhancer-binding protein-α (C/EBPα). These factors not only promote lineage-specific gene expression but also suppress alternative lineage pathways.

Abnormalities in molecular pathway:
The transcription factor found most frequently altered in inherited and acquired human diseases of the erythroid and megakaryocytic lineage is **GATA1**

Diamond-Blackfan anemia, a rare red cell aplasia characterized by anemia, bone marrow erythroblastopenia (lack of late erythroid forms), and congenital anomalies. The disease is associated with heterozygous mutations in the ribosomal protein S19 gene (RPS19) in approximately 25% of probands

Stages of Neutrophil Differentiation :

Granulocytes differentiate from early progenitors in the bone marrow in a process that takes 7 to 10 days. The cells pass through several identifiable maturational stages, during which they acquire the morphologic appearance and granule contents that characterize the mature granulocyte.

Neutrophil Granules: Major Classes and Contents

Primary (Azurophilic)	**Secondary (Specific)**	**Tertiary**
Microbial Agents		

Lysozyme Myeloperoxidase Defensins Cationic proteins Bactericidal permeability–increasing agent (BPI)	Lysozyme	
Proteases		
Elastase Cathepsin G Other proteases	Collagenase	Gelatinase
Acid Hydrolases		
N-acetylglucuronidase Cathepsins B and D β-Glucuronidase β-Glycerophosphatase α-Mannosidase		
Other		
Kinin-generating enzyme C5a-inactivating factor	Lactoferrin Vitamin B_{12}–binding protein Plasminogen activator Cytochrome *b*[*]	

	CD11/1B complex[*] Formyl peptide receptor[*] Histaminase[*] NGAL

LEUCOPENIA

Neutropenla – Reduction in granulocyte number in peripheral blood
Agranulocytosis – marked reduction in neutrophil counts – predisposition to infection

ANC <1000/NL ⟶ ↑ Susceptibility to infection
<500/NL ⟶ difficult to control endogenous microbial flora – gut, mouth. Serious infection
< 200/NL s ⟶ inflammatory process absent

Clinical features - agranulocytosis ⟶ characteristic feature ⟶ infections ⟶ Agranulocytic angina

a. Ulcerating lesion of gingival floor of mouth, buccal mucosa floor of mouth/anywhere
   i. Deep. Undermined with grey green black membrane
   ii. Numerous bacterial / fungi

Therapy – at first sign of infection – broad spectrum antibiotic later – G-CSF

Neutrophila

1. ↑↑ Production
   - Idiopathic
   - Drugs – glucocortiocoids
   - Infections -> II & TND (Ac. Infection) (D/d chr. Infection – CSF)
   - Inflammations – thermal injury, MI, CVD, hypersensitivity
   - Myeloproliferative disorders
2. ↑ Marrow mobilization – steroids, endotoxins, thermal injury
3. Defective margination
   - Drugs – epinephrine, steroids, NSAIDS
   - Stress, excitement, virgorous exercise
   - LAD TYPE 1
   - LAD TYPE 2
4. Miscellaneous – metabolic
   - Ketoacidosis, ARF, eclamplsia
   - Li
   - Metastatic Ca, acute hemorrhage or hemolysis

Toxic granules – persistent primary granules – severe infection & inflammation (Kawasaki disease)
Dohle bodies – infection – dialted E.R. (ribosome rich)

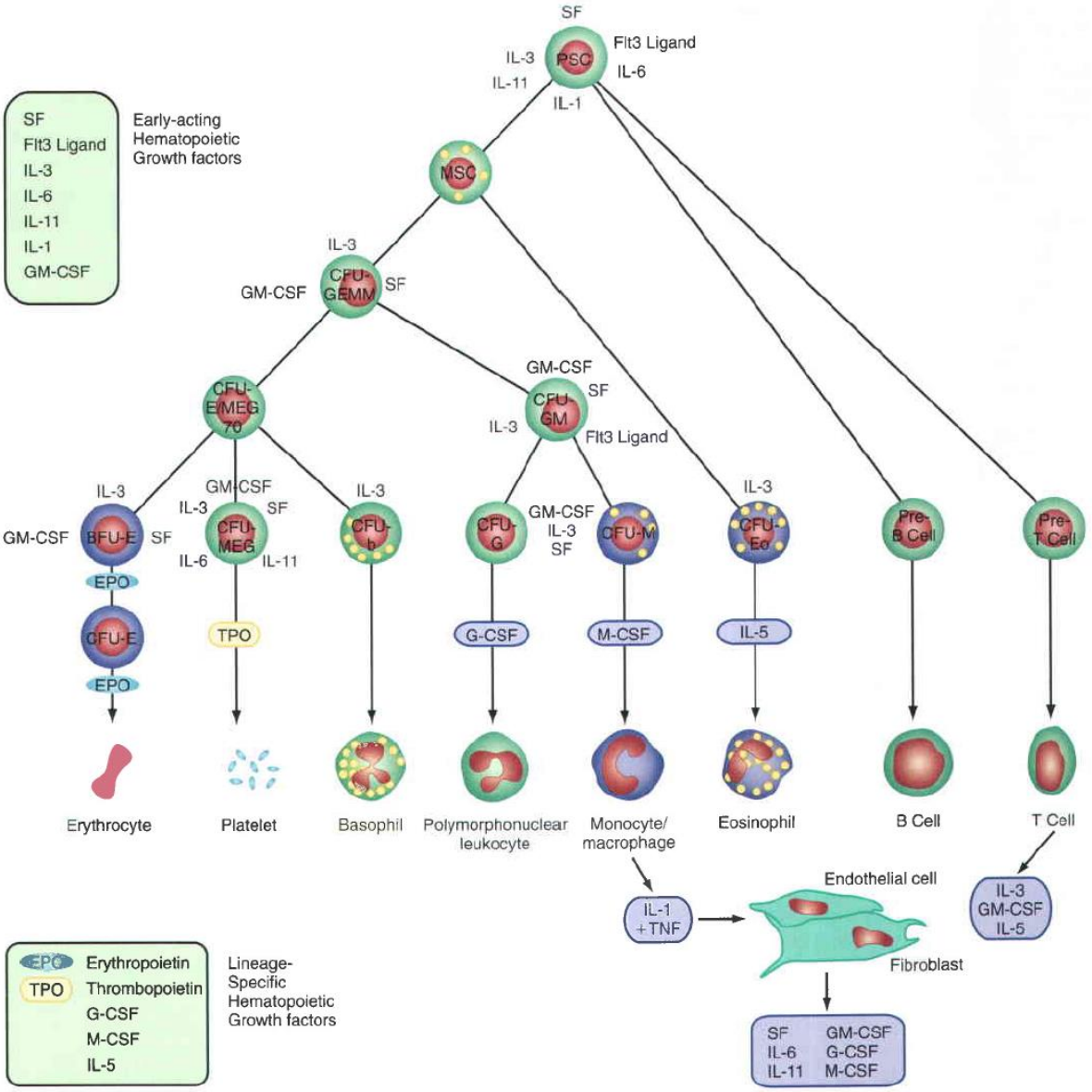


Figure 446-1. Major cytokine sources and actions to promote hematopoiesis. Cells of the bone marrow microenvironment, such as macrophages, endothelial cells, and reticular fibroblasts, produce macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and granulocyte colony-stimulating factor (G-CSF) after stimulation. These cytokines and others listed in the text have overlapping interactions during hematopoietic differentiation, as indicated; for all lineages, optimal development requires a combination of early- and late-acting factors. BFU, burst forming unit; CFU, colony forming unit; EPO, erythropoietin; MSC, myeloid stem cells; PSC, pluripotent stem cell. (From Sieff CA, Nathan DG, Clark SC: The anatomy and physiology of hematopoiesis. In Orkin SH, Nathan DG [editors]: *Hematology of Infancy and Childhood,* 5th ed. Philadelphia, WB Saunders, 1998, p 168.)

Anemias

Anemia is reduction below normal lits of the total circulating red cell mass.

Lab terms

I. MCV (mean cell volume) is the average volume of a red blood cell expressed in femotoliters.
II. MCH (mean cell hemoglobin) is the average content (mass) of hemoglobin per RBC expressed in pictogram.
III. MCHC (mean cell hemoglobin concentration) is the average concentration of hemoglobin in a given volume of packed RBCs Expressed in gm/dl.
IV. RDW (red cell distribution width) is the coefficient of variation of red blood cell volume (RDW is a measure of anisocytosis).

Adult Reference Ranges for Red Blood Cells

Measurements (Units)	Men		Women
Hemoglobin (gm/dl)	13.6-17.2		12.0-15.0
Hematocrit (1%)	39-49		33-43
Red cell count (10%	4.3-5.9		3.5-5.0
Reticulocyte count (%)		0.5-1.5	
Mean corpuscular hemoglobin (pg)		82-96	
Mean corpuscular hemoglobin Concentration (gm/dl)		27-33	
RBC distribution with		11.5-14.5	

CLINICAL MANIFESTATION (ANEMIA)

- If there is a blood loss < 20% - no clinical signs at rest
- If the blood Loss >30% - patient supine, postural hypotension and tachycardia at rest
- 40% (>2000 ml) – Hypovolemic shock.

- Earliest change in peripheral blood immediately after acute blood loss – leucocytosis
- Reticulocyte count may increase up to 10-15% after 7 days

- Early recovery from blood loss accompanied by thrombocytosis

- Anemia insidious in development and cardiopulmonary s/s occurs when Hb% reaches 8gm/dl
- Fe defiicney anemia – S/s – Hb < 6 gm / dl
- Common symptoms → weakness, malaise, easy fatigubility
- Cardio respiratory systems – Hyper dynamic cardiac failure, Na and H_2O retention, edema LVF.
- On correction of anemia – cardiac index falls fastest (6 months)

- LVH last to be corrected

ECG changes – MC – change – depression of ST J^n with u shape $deform^n$ of ST seg
Flat or inverted T waves
- Change in duration of electrical systole
- Abnormalities of AV conduction

SKIN → most evident sign of anemia – pallor
Pallor without Anemia – Myxedema
In hand skin of palm first become pale but crease pink Hb <7 gm/dl
Distinct sallow skin colour – chronic anemia
Waxy dead whiteness - Acute blood loss
Permicious anemia – Thinning, loss of lustre and early graying of hair

NEUROMUSCULAR

Anoxia can cause fatty change in liver, myocardium & kidney
Retinopathy
GI- System – Glossitis and atrophy of papillae (tongue) – occur in Pernicious anemia, Rarely due to iron deficiency anemia
Painful ulcerative and necrotic lesion mouth → Aplastic anemia
→ Acute leukemia

Dysphagia – iron deficiency anemia

Genito urinary complains

In female – mentstural flow excessive if-
- > 12 pad used / period
- Or clots passed after 1st day
- Total bleeding duration > 7 days

Reticulocytes

I. Reticulocytes are large cells (macrocytic cells) that are spherical and have a bluish colon (polychromasia) due to free ribosomal RNA.
II. Reticulocytes do not have nucleus.
III. Maturation to a mature RBC takes about 1 day.
IV. Reticulocyte count-Percent of reticulocytes present of reticulocytes present in peripheral blood. Normal - 0.5-2%

Classification of anemia based on color

I. Normochromic – Normal color (Central pallor of about a third the diameter of the RBC).
II. Hypochromic- decreased color (seen as increased central pallor of RBC).
III. Hyperchromic- increased color (loss of central pallor of RBC) e.g. macrocyes, spherocytes.

Classification of Anemia According to Underlying Mechanism

Blood Loss

- Acute –Trauma
- Chronic – lesions of gastrointestinal tract, gyneocologic disturbances

Increased Rate of Destruction (Hemolytic Anemias)

- Intrinsic (intracorpuscular) abnormalities of red cells
- Hereditary
- Red cell membrane disorders
- Disorders of membrane cytoskeleton-sphercytosis, elliptocytosis
- Red cell enzyme deficiencies.
- Glycolytic enzymes- pyruvate kinase deficiency, hexokinase deficiency
- Disorders of hemoglobin synthesis
- Deficient globin synthesis (hemoglobinopathies)- sickle cell anemia, unstable hemoglobins
- Acquired
- Membrane defect – paroxysmal nocturnal hemoglobinuria
- Extrinsic (extracorpuscular) abnormalities
- Antibody Mediated
- Isohemagglutinins- Transfusion reactions, erythroblastosis fetalis
- Autoantibodies- Idiopathic (primary), drug-associated, systemic lupus erythematosus, malignant neoplasms, mycoplasmal infection
- Mechanical trauma to red cells
- Microangiopathic hemolytic anemias- thrombotic thrombocytopenic purpura, disseminated intravascular coagulation
- Cardiac raumatic hemolytic anemia
- Infections- Malaria, hookworm
- Chemical injury- lead poisoning
- Sequestration in mononuclear phagocyte system-hypersplenism
- Impaired Red Cell Production
- Disturbance of proliferation and maturation of erythroblasts
- Defective DNA synthesis-deficiency or impaired use of vitamin B_{12} and folic acid (megaloblastic anemias)
- Defective hemoglobin synthesis
- Deficient heme synthesis-iron deficiency
- Deficient globin synthesis-thalassemias
- Unknown or multiple mechanisms-sideroblastic anemia, anemia of chronic infections, myelophthisic anemias due to marrow infiltrations

Classification of anemia based on RPI (Reticulocyte production index) i.e., kinetic classification

Normal reticulocyte count – 0.5-2%
Reticulocyte production index-two corrections required

a. Correction for lowering hematocrit
b. Correction for shift reticulocytosis (early release of reticulocyte from marrow)

RPI = Reticulocyte % X (Hematocrit/45) x (1/reticulocyte maturation time)
Maturation time of reticulocytes in peripheral blood

Hct%	45	-	1 day
	35	-	1.5 days
	25	-	2 days
	15	-	2.5 days

Normal RPI = 1

Hypoproliferative anemia (RPI <2.5)
Aplastic anemia
Anemia of chronic

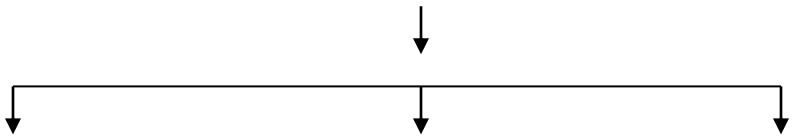
Maturation disorder (RPI <2.5)
Cytoplasmic defects – Iron deficiency anemia, thalassemia, sideroblastic anemia
Nuclear defects-Vitamin B_{12} deficiency, folate deficiency

Hemolysis/Hemorrhage (RPI >2.5)

- Blood loss – acute, chronic
- Inherited RBC membrane defects – Hereditary Spherocytosis, elliptocytosis
- Acquired membrane defects – PNH
- Enzyme deficiencies- pyruvate kinase hexokinase, G6PD, gluthathione synthetase
- Hemoglobinopathy – Hb S, unstable Hb
- AIHA
- MAHA
- Treated nutritional anemia

MICROCYTIC ANEMIA

Microcytic anemia



Disorders of iron metabolism 1. Iron deficiency anemia 2. Anemia of chr-disorders (Generally NCNC) 3. Atransferrinemia 4. Shahidi – Nathan diamond syndrome (Cong. Hypochromic microcytic anemia with iron overload) 5. Familial microcytic anemia with impaired absorption and metabolism of iron 6. Antibodies to transferring receptor 7. Aluminum intoxication	Disorder of Globin synthesis. Thalassesmias (Alpha and Beta) Hb E syndromes (AE, EE, Eβ thal) Hb C syndromes (AC, CC) Unstable hemoglobin disease	**<u>Disorders of porphyrin and heme synthesis sideroblastic anemia</u>** Hereditary SA X linked (XLSA) X linked with ataxia (XLSA / A) Presumed autosomal sporadic congenital associated with thiamine responsive megaloblastic anemia (TRMA) Associated with mitochondrial cytopathy (Pearson syndrome) 2. Acquired idopathtic SA (AISA) pure sideroblastic anemia (PSA) refractory amenia with ring siderolblast (RARS) associated with hematological malignancies myeloproliferative neoplasm 3. reversible- associated with alcoholism certain durgs (isonizid, chloramphenicol) copper deficiency, hypothermia

Iron deficiency anemia, most common anemia in the world.

a. Normal forms of iron (Fe) and metabolism – total body iron- 2 gm is females, up to 6 gm in male.
    I. Functional iron is found in hemoglobin, myoglobin, and enzymes (catalase and cytochromes).
    II. Ferritin is the physiological storage form found in liver, spleen, bone marrow, and skeletal muscle. In liver, ferritin is found in hepatocyte iron is derived from plasma transferring whereas phagocyic iron comes from break down of RBC.
    III. Iron is transported by transferring.
        - Transferring levels- total iron-binding capacity (TIBC) (normal = 300 μg/dl)
        - Normal % saturation = one-third saturation (as normal serum iron is 100μg/dl)

.

b. Daily requirement – 7-10 mg for males and 7-20 mg for females.
c. Causes of iron deficiency
    I. Dietary deficiency is seen in elderly, children, and poor.
    II. Increased demand is seen in children and pregnant wormen.
    III. Decreased absorption.
        - Generalized malabsorption
        - After gasrectomy. Due to decreased acid, which is needed to ferrous absorption; decreased small intestinal transit time (dumping syndrome)
    IV. Chronic blood loss due to gynecological (menstrual bleeding) or GI causes (carcinoma, hookworm)
d. Sequence of events during iron deficiency
    I. First is decreased storage irons, which produce
        - Decreased serum ferritin
        - Decreased bone marrow iron on Prussian blue stains (hemosiderin)
    II. Next is decreased circulating iron which causes
        - Decreased serum iron
        - Increased TIBC
        - Decreased % saturation
    III. Last is formation of microcytic hypochromic anemia with pencil shaped RBC.
        - Decreased MCV
        - Decreased MCH
        - High RDW
e. Other Symptoms of iron deficiency
    I. Decreased serum hepcidin levels
    II. Increased free erythrocyte protoprphyrin (FEP).
    III. Epithelial atrophy is seen in Plummer-Vinson syndrome.
    IV. Koilonychina – concave nails (spoon nails) with abnormal ridging and splitting
    V. Pica- eating unusual thing (e.g. dirt).
f. Response to iron therapy first indicated by subjective improvement

RESPONSES TO IRON THERAPY IN IRON-DEFICIENCY ANEMIA

TIME AFTER IRON ADMINISTRATION	RESPONSE
12-24 hr	Replacement of intracellular iron enzymes; subjective improvement; decreased irritability; increased appetite
36-48 hr	Initial bone marrow response; erythroid hyperplasia
48-72 hr	Reticulocytosis, peaking at 5-7 days
4-30 days	Increase in hemoglobin level
1-3 mo	Repletion of stores

Iron Panel for microcytic Anemias

	Iron deficiency	AOCD	Thalassemia	Sideroblastic anemia
Serum iron	↓	↓	Normal	↑
TIBC	↑	↓	Normal	↓
% saturation	↓	↓	Normal	↑
Serum ferritin	↓	↑	Normal	↑

2. **Anemia of chronic disease (AOCD)**
    a. Causes- Chronic microbial infections, osteomyelitis, SABE, lung abscess.
    Chronic immune disorders – rheumatoid arthirits, regional enteritis.
    Neoplasms-Hodgkin's lymphoma, carcinoma lung and breast.
    b. Characterized by iron being traped in bone marrow macrophages leading to impaired iron utilization.
    c. Lab-low S. iron, reduced TIBC, and increased S. ferritin (abundant iron stores)
    d. Pathogenesis-Impediment in ransfer of iron from storage pool to erythroid precursors.
    Low EPO levels leading to inadequate proliferation of erythroid progenitors. ↓EPO levels are due to IL-1, TNF-α, and IFN which are secreted in chronic inflammatory and neoplastic disorders.
3. **Thalassemia syndromes**
    a. General
    I. Thalassemias are quantitative, not qualitative, abnormalities of hemoglobin
        - Alpha –thalassemia has decreased alpha-globin chains with relative excess beta chains.
        - Beta-thalassemia has deceased beta-globin chains with relative excess alpha chains
    II. Thalassemia provides a protective advantage to carriers, such as against malaria.
    III. Types of Hb
        - Erythropoiesis – up to 8th week – yolk sac
        Embryonic Hb- Gower-I (ζ2Є2)
        Gower-I (ζ2Є2)
        Portland (ζ2γ2)
        - Fetal liver (after 8 weeks) – Hb F (α2γ2)
        - Bone marrow- at 20 weeks, the site erythropoiesis begins to switch from the liver and spleen to the bone marrow

Fetal (Hb F) to adult erythropoiesis **(**HbA)- witch over begins at 30th week.
Alpha-thalassemia- deficient alpha chain synthesis, non-alpha chains form aggregates.

a. **Genetics**
   I. There are a total of four alpha-globin chains genes, two pairs on chromosome 16
   II. Alpha chains are normally expressed prenatally and postantally; therefore, there is prentatal and postanatal disease
   III. Alpha-thalassemia is due to gene deletion
b. **Clinical disease sates**
   I. Normal: four alpha genes (alpha alpha/alpha alpha) and 100% alpha chains
   II. Silent carrier. One deletion
      - Total number of alpha genes: two, which produces 50% alpha chains.
      - Individuals are completely asymptomatic and all lab tests normal.
   III. Alpha- That trait: Two deletions
      - Total number of alpha genes: two, which produces 50% alpha chains
      - Genotype: cis(--alpha, alpha)types is seen in Asians.
      - Genotype: Trans(-alpha/-alpha) type is seen in African- American (offspring do not develop HbH disease or Hydrops).
   IV. HbH disease: three deletions
      Number of alpha genes: (alpha), which produces 25% alpha chains.
      Increased HbH (Beta4) forms Heinz bodies, which can be seen with crystal blue stains.
   V. Hydrops fetalis: Four deletions and is lethal in utero
      - Number of alpha genes:0 (--/--), and 0% alpha chains
      - Increased Barts hemoglobin (γ4)
      - Hydrops fetalis with severe pallor, edema, and hepatosplenomegaly.

Beta-Thalassemia

a. **Genetics**
   I. There are a total of two beta-globin chains genes on chromosome 11
   II. There are a total of two beta-globin chains.
   III. They are expressed postnatally only therefore only postnatal diseases is seen, no prentatla disease)
   IV. Mechanism; Mainly due to point tmutations that form either some beta chains (beta_) or none (betaO).
      Theses mutations are seen in splicing regions commonly or in promter regions
      RNA polymerase binding decrease-transcription
      Chain terminator mutations produce B0
      Splicing mutations produce β+ β0. Most common cause, mostly introns
      At splice junction-β0
      Ectopic splice site- β+
      Common Indian mutations of β thalassemia:
      - IVS 1-5 (most common)
      - Base pair deletion (619)
      - IVS 1-1
      - Frameshift mutation 8-9
      - Frameshift mutation 41-42
b. Beta-thal minor
   I. Asymptomatic
   II. Increased hemoglobin A_2(8%) and increased hemoglobin F (5%)
c. Beta-thal intermedia has asevere anemia, but no transfusion needed.
d. Beta-thal major (Cooley's anemia)
   I. Patients are normal at birth

II. Symptoms develop at about 6 months as hemoglobin F levels decline.

III. Severe hemolytic anemia results from decreased RBC life span.

- Intramedullary destruction results in "infective erythropoiesis."
- Hemolysis causes jaundice and an increased risk of pigment (bilirubin) gallstones.
- Lifelong transfusion is required, which result in secondary hemochromatosis.
- Congestive heart failure (CHF) is the most common cause of death.

IV. Erythroid Hyperplasia in the bone marrow causes "crewcut" skull X-ray and increased size of maxilla 'chipmunk face'.

V. Peripheral blood

- Microcytic hypochromic anemia with marked anisopoikilocytosis
- Numerous target cells, increased reticulocytes, and Nrbc IN PERIPHERAL SMEAR.

VI. Screening test-NESTROFT (naked eye single tube red cell osmotic fragility test)-Based on reduced fragility of RBCs in thalassemia.

VII. Confirmatory test: quantization of Hb A_2 increase (3.5-8% n2.5% + 0.3%) and Hb F (increased/normal) and family studies.

VIII. Antenatal diagnosis- PCR of fetal DNA (Aminocentesis/chorionic villus sampling) followed by hybridization to ASO probes.

4.

Sideroblastic anemia

Sideroblastic anemias are a heterogeneous group of disorders characterized by anemia of varying severity and diagnosed by finding ring sideroblasts in the bone marrow aspirateCharacteristic - Total body iron ↑

- Ringed siderobiasts in BM

- Hypochromic anemia

1. Hereditary – Sex linked

AR

2. Acquired →

i. Idiopathic refractory sideroblastic anemia or Refractory anemia with ringed sideroblast (RARS)

ii. Secondary to drugs toxin – lead, Alcohol, INH, Chloramphenicol

Secondary associated with thalassemia

Treatment

1. Vitamin B6 (particularly in reversible SA)
2. Recombinant erythropoietin
3. Phlebotomy
4. Deferoxamine

Pathophysiology

Hereditary – sex lined –↓ ALA synthase

Mutation in gene ALA synthase

↓ ALA synthase / ALA synthse with ↓ affinity for pyridoxine

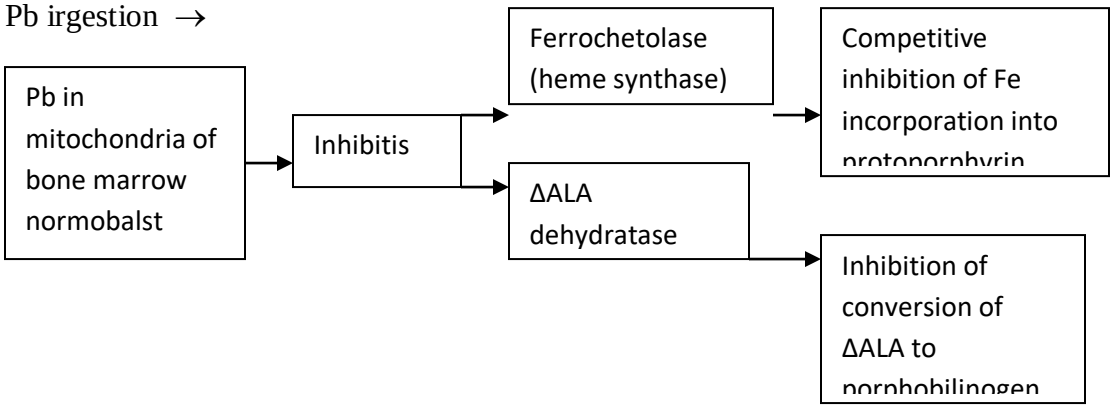
Rate limiting step glycine + succinyl Co A → ΔALA (Pyridoxine)

Acquired

1. Idiopathic Form (Stem cell disorder)- Most important abnormal mitochondrial iron metabolism + ↓ ALA synthase activity. Risk of development into AML.
2. Secondary to drug / toxin – interfere with acitivyt of heme enzymes (ATT, Alcohol, Pb, chemotherapy, chloramphenicol)

Lead poisoning (Plumbism)

Pb irgestion →



1. ↑ Urinary ΔALA
2. Erythrocyte protoporphyrin ↑→ RBC fluorescence
3. Urine coproporphyrin ↑
4. Fe accumulates in cells

Screening Test → Detects blood lead level measurement > 10 ng/dl

Clin. Features – Low IQ, concentration disorder, Hearing loss, impaired growth and development

Causes basophilic stippling – (∴ Aggregating ribosomes and mitochondria)

- EPP ↑

- Hyperplastic BM. But RPI < 2 (ineffective erythropores)

Normocytic Anemia

a. **Aplastic anemia**
   - Syndrome of marrow failure associated with pancytopenia due to suppression/disappearance of multi-potent myeloid stem cell.

b. **Etiology**

Most cases are idiopathic (65%)

Classification:

Acquired Aplastic Anemia
• Secondary aplastic anemia • Irradiation • Drugs and chemicals • Regular effects

- Cytotoxic agents
- Benzene
- Idiosyncratic reactions
- Chloramphenicol
- Nonsteroidal antiinflammatory drugs
- Antiepileptics
- Gold
- Other drugs and chemicals
- Viruses
- Epstein-Barr virus (infectious mononucleosis)
- Hepatitis virus (non-A, non-B, non-C, non-G hepatitis)
- Parvovirus (transient aplastic crisis, some pure red cell aplasia)
- Human immunodeficiency virus (acquired immunodeficiency syndrome)
- Immune diseases
- Eosinophilic fasciitis
- Hyperimmunoglobulinemia
- Thymoma and thymic carcinoma
- Graft-versus-host disease in immunodeficiency
- Paroxysmal nocturnal hemoglobinuria
- Pregnancy
- Idiopathic aplastic anemia

Inherited Aplastic Anemia

- Fanconi anemia
- Dyskeratosis congenita
- Shwachman-Diamond syndrome
- Reticular dysgenesis

- Amegakaryocytic thrombocytopenia
- Familial aplastic anemias
- Preleukemia (eg, monosomy 7)
- Nonhematologic syndromes (eg, Down, Dubowitz, Seckel)

- Chemical agents – whole body irradiation
- Viral infections- Hepatitis, CMV, EBV, Parvo B19
- Inherited-Fanconi's anemia

c. **Pathogenesis**
   - Suppression of stem cell function by activated T cells results in aplastic anemia
   - Stem cells antigenically altered by exposure to various, drugs, etc.→ T-cell produce TNFα - →↓EPO by kidney→marrow aplasia
   - Fundamental stem cell abnormality with Karyotypic aberration

d. **P/S-** normocytic normochromic blood picture with reticulocytopenia, pancytopenia

e. **BMA-** Dry tap

f. **BMB-** Hypocellular marrow, devoid of hematopoietic cells, increased fat and clusters of lymphocytes and plasma cells.

g. **Clinical features:** Anemia-Easy fati gability, weakness
   - Thrombocytopenia-Petechiae and ecchymoses
   - Granulocytopenia-Repeated infections.
   - Splenomegal is characteristically absent.

Fanconi (Aplastic) Anemia

FA is a chromosomal fragility disorder characterized by cytopenias, progressive bone marrow underproduction, variable developmental anomalies and a strong propensity for cancer.

Anomaly	**Frequency (%)**
Skin pigment changes and/or café au lait spots	55
Short stature	51
Upper limb abnormalities (thumbs, hands, radii, ulnae)	43

Anomaly	Frequency (%)
Hypogonadal and genitalia changes (mostly male)	35
Other skeletal findings (head/face, neck, spine)	30
Eyes/lids/epicanthal fold anomalies	23
Renal malformations	21
Gastrointestinal/cardiopulmonary malformations	11
Hips, legs, feet, toes abnormalities	10
Ear anomalies (external and internal), deafness	9

A major finding is abnormal chromosome fragility seen readily in metaphase preparations of peripheral blood lymphocytes cultured with phytohemagglutinin. The karyotype shows "spontaneously" occurring chromatid breaks, rearrangements, gaps, endoreduplications, and chromatid exchanges in cells from homozygote FA patients

A major feature of the FA phenotype is the propensity to develop cancer.

Anemia blood of loss

- Acute blood loss may cause shock or death.
- If the patient survives, the resulting hemodilution caused by shift of water form the interstitium will lower the hematocrit.
- Earliest change in P/S after acute blood loss is leukocytosis
- There will be a marked reticulocytosis in 5-7 days. Early recovery from bloos loss is accompanied by thrombocytosis
- Chronic blood loss, such as from the GI tract or from the gynecological problems, may result in iron deficiency anemia.

Hemolytic Anemias

Have following features:

- Shortened RBC life span due to premature RBC destruction
  I. ↑EPO levels and ↑ erythropoiesis in bone marrow (marrow erythroid hyperplasia) to compensate for loss of red cells.
  II. ↑RBC destruction leads to accumulation of products of hemoglobin catabolism
- Intravascular (IV) hemolysis

I. Release of hemoglobin into the blood cause hemogloinemia, hemoglobinuria, and hemosiderinuria.
II. Increased bilirubin from RCBs causes jaundice
III. Hemoglobin may be oxidized to methemoglobin, which causes methemoglobinemia and methemoglobinuria.
IV. Markedly decreased hemoglobin-binding proteins in the blood, such as haptoglobin and hemopexin are characteristic.
V. No splenomegaly.

- **Extravascular (EV) Hemolysis**

I. Splenomegaly results if the extravascular hemolysis occurs in spleen.
II. Hepatomegaly results if the extravscular hemolysis occurs in liver.
III. Increased bilirubin and decreased heptoglobin occur, but not as much as with IV hemolysis. Absence of hemoglobinemia, hemoglobinuria, and methemoglobin formation.

Classification of Hemolytic Anemias*		
	Intracorpuscular Defects	**Extracorpuscular Factors**
Hereditary	**Hemoglobinopathies** **Enzymopathies** **Membrane-cytoskeletal defects**	**Familial (atypical) hemolytic uremic syndrome**
Acquired	**Paroxysmal nocturnal hemoglobinuria (PNH)**	**Mechanical destruction (microangiopathic)** **Toxic agents** **Drugs** **Infectious** **Autoimmune**

Sickle Cell Disease

Genetics

I. Abnormality :Single nucleotide change in codon causes valine (neutral) to replace normal glutamic acid at the sixth position of the beta-globin chain.
II. Heteroxygous (AS): trait- have 40% Hb S and 60% normal Hb.
  - About 8% of African-Americans are heterozygous fro hemoglobin S.
  - Patients with sickle trait have fewer symptoms than those with sickle disease.
  - Have resistance of Plasmodium falciparum infection (malaria.

III. Homozygous (SS) disease (sickle cell anemia)- almost all their Hb is sickle Hb.

- Pathogenesis

I. When deoxygenated, Hb S molecules undergo aggregation and polymerization. Initially the RBC cytosol converts from free flowing liquid to viscous gel. With continued deoxygenation, Hb S molecules assemble into long needle like fibers within RBCs, membrane gets damaged and irreversibly sickle cells are formed. These cells are rigid, nondeformable and sticky and lead to microvascular occlusion.

II. Membrane damage results in increased entry of calcium into the cell which in turn activates potassium channel leading to efflux of K^+ and H_2O from the cell. This results in intracellular dehydration and ↑MCHC.

III. Average life span of sickle cells is only 20 days.

- Factor affecting formation of irreversibly sickle red blood cells.

I. ↑MCHC due to intracellular dehydration makes symptoms worse

II. Decreased pH decrease oxygen affinity and makes symptoms worse.

III. Increased hemoglobin F makes symptoms better (rationale for therapy with Hydroxyurea, which increases blood hemoglobin F levels).

IV. Presence of hemoglobin C (SC:double-heterozygote individual) makes symptoms better.

- Increased RBC destruction causes a severe hemolytic anemia.

I. Erythroid hyperplasia in the bone marrow causes:
    - Expansion of marrow→crew haircut appearance on Xray
    - Extra medullary hematopoiesis in liver, spleen

II. Increased bilirubin leads to jaundice and gallstone (pigment) formation.

- Lab tests for hemoglobins

I. Sickling test (metabisulfite test, which can't tell sickle cell disease from sickle cell trait)

II. Hemoglobin electrophoresis confirmatory

III. Prenatal diagnosis: genetic testing (Mst II endonuclease)

- Therapy includes ***Hydroxyurea (increases hemoglobin F)***

Hemoglobin C Disease

- Abnormality: single nucleotide change in a codon causes lysine (basic) to replace normal glutamic acid (acidic) at the beta 6 position.
- Signs: Mild normochromic — normocytic anemia, splenomegaly, target cell, and rod- shaped crystals in RBCs (the latter being characteristic).

Gucose-6-phosphate Dehydrogenase Deficiency

Glucose-6-phosphate

GRAPH

NADP

GSH H20,

Glutathione reductase

Glutathione peroxidase

6-Phosphogluconate

NADPH GSSG 1120

1g. 11.3: Role of glucose-6-phosphate dehydrogenase (G6PD) in defense against oxidant injury. The disposal of H202, a potential xidant, is dependent on the adequacy of reduced glutathione (GSH), which is generated by the action of NADPH. The synthesis of IADPH is dependent on the activity of G6PD. GSSG, oxidized glutathione.

- **Pathogenesis**

I. Deficiency of glucose-6-phosphate dehydrogenase (G6PD) results in decreased levels of the antioxidant glutathione (GSH).
II. RBCs are sensitive to injury by oxidant stresses leading to hemolysis
III. Deficiency of G6PD is not due to decreased synthesis but rather to defective protein folding, resulting in a protein having a decreased half-life

- X-linked inheritance, patient population include

a. African—Americans (A-type)
- Hemolysis is secondary to acute oxidative stress, such as oxidative drugs primaquine, sulfonamides, anti-TB drugs), and more typically by viral or bacterial infections.
- Hemolysis is intermittent (even if drugs to continued) because only older RBCs have decreased levels of G6PD. Reticulocytes have normal enzyme activity.

b. **Mediterranean type**
- Associated with favism due to ingestion of fava beans
- Has more severe hemolysis because all RBCs have decreased G6PD activity in that there is both decreased synthesis and decreased stability.

- Oxidation of hemoglobin forms Heinz bodies,

I. Heinz bodies cannot be seen with normal peripheral blood stains (Wright—Giemsa)
II. Need supravital stains (methylene blue and crystal violet) to see Heinz bodies.
III. Heinz bodies are 'eaten' by splenic macrophages (extravascular hemolysis), which may form bite cells and spherocytes.
IV. Diagnosis: Methylene blue reduction test

Hereditary Spherocytosis (HS)

a. Definition: Autosomal dominant disorder that is due to a defect involving ankyrin (most commonly), spectrin rarely protein 3 and protein 4.1 in RBC membrane, which causes a decrease in the RBC surface membrane (spherocytosis)
b. Spherocytes are not flexible and are removed in spleen by macrophages (i.e., extravascular hemolysis), which causes
    I. Splenomegaly with a mild to moderate hemolytic anemia.
    II. Chronic hemolysis produces increased bilirubin and an increased risk for jaundice and pigment gallstones.
    III. Increased risk for acute red cell aplasia due to parvovirus B_{19} infection
    IV. Hemolytic crisis due to recurrent infection. Massive wave of hemolysis may occur.
c. Lab tests
    I. Spherocytes and reticulocytes on P/S
    II. Increased osmotic fragility
    III. Normal MCH with increased MCHC
d. Treatment is splenectomy

Immune Hemolytic Anemia

Classification of Immunohemolytic Anemias

WARM ANTIBODY TYPE (IgG ANTIBODIES ACTIVE AT 37°C)
Primary (idiopathic) Secondary Autoimmune disorders (particularly systemic lupus erythematosus) Drugs Lymphoid neoplasms
COLD AGGLUTININ TYPE (IgM ANTIBODIES ACTIVE BELOW 37°C)
Acute (mycoplasmal infection, infectious mononucleosis) Chronic Idiopathic Lymphoid neoplasms
COLD HEMOLYSIN TYPE (IgG ANTIBODIES ACTIVE BELOW 37°C)
Rare; occurs mainly in children following viral infections

I. Diagnosed by direct Coombs, antiglobulin test

II. Classification

A. **Warm Antibody type**

a. Most common type of immune hemolytic anemia

b. Primary (idiopathic)
Secondary to Lymphomas (CLL) and leukemias, other neoplastic disease
Auto immune disorders — SLE, drugs -- penicillin, cephalosporins, α-methyldopa

c. Antigens against which antibodies are directed in most cases are Rh blood group antigens. The antibody is of IgG type, does not fix the complement and is active at 37°C.

d. Hemolysis produced is extravascular in the spleen. IgG coated red cells bind to Fc receptors on splenic macrophages, which results in loss of RBC membrane producing spherocytes.

B. Cold Antibody type

a. Causes – Acute mycoplasmal infection, infectious mononucleosis, HIV, CMV infections, chronic idiopathic associated with lymphomas.

b. The antibodies produced are of IgM class and are most active at 0-4°C.

c. Binding of IgM antibodies to RBC antigen and complement fixation occur only in peripheral cool parts of the body e.g. fingers, ears, toes. As the blood recirculates, IgM antibody dissociates from red cells but C3b (opsonin) gets deposited on RBC resulting in removal of this RBC by macrophages in spleen.

C. **Cold Hemolysin Hemolytic Anemia**

- Cause paroxysmal cold hemoglobinuria – acute intermittent intravascular hemolysis after exposure to cold.
- Cause – Syphilis.
  Following infections like mycoplasma pneumonia, measles, mumps and ill-defined viral "flu" syndromes.
- Auto antibodies are of IgG class that bind to P blood groups antigens at low temperatures and fix the complement

- Complement mediated intravascular lysis does not occur until cells re-circulate to warm central regions as complement function occurs best at 37°C.
- IgG antibody of PCH is also called Donath—Landsteiner antibody or biphasic antibody.

Paroxysmal nocturnal hemoglobinuria (PNH)

a. Acquired intrinsic defect in red cell membrane.
b. Abnormality:- Acquired mutations in phosphatidyl inositol glycan A (PIGA) which is essential for synthesis of GPI anchors.
c. GPI anchors are responsible for anchoring certain important proteins to RBC, WBC and platelet membrane which protect the membrane from oxidative stress.
d. GPI linked protein: Decay accelerating factor (CD55),Membrane inhibitor of reactive lysis (CD59) and C8 binding protein which regulate complement activity and are deficient in PNH. Thus RBC, WBC and platelets are sensitive to complement mediated lysis.
e. Symptoms. Episodes of hemolysis at night.
f. Acidosis in vivo, which occurs during sleep (breathing slowly retains CO_2) and exercise (lactic acidosis) causes activation of complement.
g. PNH a clonal stem cell disorder that therefore affects all cell lines.
h. Pancytopenia in peripheral blood: anemia, leucopenia, thrombocytopenia.
i. Complications: increased risk for aplastic anemia, leukemia, and venous thrombosis (hepatic, portal, cerebral)
j. Decreased LAP scores.
k. Lab tests for PNH
   Sucrose lysis test
   Ham's test
   Flow cytometry—gold standard.

MACROCYTIC ANEMIAS

Causes of Macrocytic anemia
1. Vit. B12 deficiepcy
2. Folic acid deficiency
3. Orotic aciduria
4. Nitrous oxide inhalation
5. Liver disease
6. Hypothyroidism
7. Thiamine deficiency

Megaloblastic anemias

Vitamin B_{12} and Folic acid are essential for DNA synthesis.

a. IN megaloblastic anemia, basic cause is impaired DNA synthesis (delayed mitosis'?) while RNA synthesis is not impaired. This produces a nuclear- cytoplasmic asynchrony that affects all rapidly proliferating cell lines, including of bone marrow, GI tract, and female genital tract.
b. Bone marrow aspiration: Bone marrow is hypercellular

- Megaloblasts are seen in the bone marrow. Megaloblastic change is best seen in orthochromatic stage where the cells have pink well hemoglobinized cytoplasm but are large with immature nucleus.
- Myeloid series also shows nuclear cytoplasmic asynchrony with giant metamyelocytes and giant stab forms.
- Megakaryocytes are abnormally large with bizarre multilobated nuclei.
- Ineffective erythropoiesis in bone marrow causes increased S. bilirubin, LDH and pancytopenia. .

c. P.S Pancytopenia

- RBC are macrocytic and oval (macro-ovalocytes) with MCV > 100 fl. They also lack a central pallor, ↓Retic count
- Neutrophils larger than normal with hyper segmented nucleus (>5 lobes).
- Thrombocytopenia.

1. Megaloblastic anemia due to vitamin B12 deficiency

a. Causes of B_{12} deficiency

I. Dietary deficiency – Daily requirement 2-3 mg

- Rare because B_{12} is stored in liver and it takes years to develop dietary deficiency.
- Seen only in strict vegetarians (diet with no animal proteins, mild, or eggs)

II. Decreased absorption, which may be cause by any of the following:

- Decreased IF associated with gastrectomy or pernicious anemia
- Pancreatic insufficiency (pancreatic proteases normally break down B_{12}-R complexes in duodenum)
- Intestinal malabsorption due to parasites (fish tapeworm, a.k.a. Diphyllobothrium lattun), bacteria Blindloop syndrome), or Crohn's disease ileum.

b. Biochemical functions of vitamin B_{12}

II. Methylmalonyl CoA $\xrightarrow[\text{Methyl COA Mutase}]{\text{Adenosylcobalamin}}$ Succinyl co-enzyme A ↓ (Forms a part of neural lipids)

c. Signs and symptoms of B_{12} deficiency

I. Weakness due to anemia (megaloblasitc anemia)

II. Sore ‘beefy’ tongue due to generalized epithelia atrophy

III. Subacute combined degeneration of the spina cord (SCDSD): demyelination of the posterior column and lateral spinothalamic tract of the spinal cord
Posterior (sensory) tracts cause loss of vibration and position
Lateral involves dorsal spinocerebellar tracts (arm and leg dystaxia) and corticospinal tracts (spastic paralysis)

d. Lab test

I. Low serum B_{12} level and increased serum homocysteine levels.

II. Increased methylmalonic acid in urine.

III. Schilling test

- Inability to absorb an oral dose of cobalamin (assessed by urinary excretion of radio labeled cyanocobalamin given orally)

2. Megaloblastit anemia due to folate deficiency

a. Cause include

I. Decreased intake

- Dietary deficiency takes only months to develop.
- Seen n chronic alcoholics and elderly (“tea and toast” diet)

II. Decreased absorption: Intestinal malabsorption (folate is absorbed in the upper small intestine)

III. Increased requirement for folate
- Pregnancy (folate deficiency during pregnancy is an important cause of neural tube defects)
- Infancy

IV. Decreased utilization: Folate antagonists used in chemotherapy such as methotrexate

b. Signs and symptoms of folate deficiency

I. Megaloblastic anemia

II. But no neurological symptoms (i.e., No SCD of spinal cord)

c. Lab tests

I. Low serum folate level and increased serum homocysteine with IFIGLU excretion

d. Treatment: Folate

LEUKEMIAS AND LYMPHOMAS

Tumor of White Blood Cell

1. Lymphoid Neoplasms- Lympho cytic leukemia, lymphoma
2. Myeloid Neoplasms- Arise form hematopoietic stem cells that give rise to myeloid (erythroid, granulocytic, and/or thrombocytic) lineage Three categories
   a. Acute myelogenous leukemias — Immature blast cells accumulate in bone marrow; the diagnostic criteria is ≥20% blasts in bone marrow and/or peripheral smear.
   b. Myelodysplastic syndromes — Associated with dysplastic features and ineffective hematopoiesis and resultant peripheral blood cytopenias.
   c. Chronic myeloproliferative disorders — Associated with increased production of one or more terminally differentiated myeloid elements which lead to increased peripheral blood counts.
3. **Histiocytosis** – Tumors of macrophages and dendritic cells e.g. Langerhans cells histiocytoses.

Important Points:

I. Lymphocytic leukemia – Lymphoid neoplasms presenting primarily with bone marrow involvement (≥20% lymphoblasts) with or without presence of tumor cells in peripheral smear.

II. 'Lymphoma' – Lymphoid neoplasm presenting primarily as discrete tissue masses. Leukemias may also show soft tissue involvement and lymphomas may have a leukemic phase. The two terms merely describe the tissue distribution of disease at the time of clinical presentation.

III. Lymphomas have been divided into Hodgkins and non-Hodgkins lymphomas. 2/3rd NHL arise from extranodal sites (e.g. skin, stomach, brain)

IV. 80-85% lymphoid neoplasms are of B-cell origin. Most of the remaining are of T-cell origin. NK cell turn are very.

WHO Classification of Lymphoid Malignancies		
B Cell	**T Cell**	**Hodgkin's Disease**
Precursor B cell neoplasm	Precursor T cell neoplasm	Nodular lymphocyte-predominant Hodgkin's disease
Precursor B lymphoblastic leukemia/lymphoma (precursor B cell acute lymphoblastic leukemia)	**Precursor T lymphoblastic lymphoma/leukemia (precursor T cell acute lymphoblastic leukemia)**	

Mature (peripheral) B cell neoplasms	Mature (peripheral) T cell neoplasms	Classical Hodgkin's disease
B cell chronic lymphocytic leukemia/small lymphocytic lymphoma	T cell prolymphocytic leukemia	Nodular sclerosis Hodgkin's disease
B cell prolymphocytic leukemia	T cell granular lymphocytic leukemia	Lymphocyte-rich classic Hodgkin's disease
Lymphoplasmacytic lymphoma	Aggressive NK cell leukemia	Mixed-cellularity Hodgkin's disease
Splenic marginal zone B cell lymphoma (± villous lymphocytes)	Adult T cell lymphoma/leukemia (HTLV-I+)	Lymphocyte-depletion Hodgkin's disease
Hairy cell leukemia	Extranodal NK/T cell lymphoma, nasal type	
Plasma cell myeloma/plasmacytoma	Enteropathy-type T cell lymphoma	
Extranodal marginal zone B cell lymphoma of MALT type	Hepatosplenic T cell lymphoma	
Mantle cell lymphoma	Subcutaneous panniculitis-like T cell lymphoma	
Follicular lymphoma	**Mycosis fungoides/Sézary's syndrome**	
Nodal marginal zone B cell lymphoma (± monocytoid B cells)	Anaplastic large cell lymphoma, primary cutaneous type	
Diffuse large B cell lymphoma	**Peripheral T cell lymphoma, not otherwise specified (NOS)**	
Burkitt's lymphoma/Burkitt's cell leukemia	**Angioimmunoblastic T cell lymphoma**	
	Anaplastic large cell lymphoma, primary systemic type	

Note: Malignancies in bold occur in at least 1% of patients.

Abbreviations: HTLV, human T cell lymphotropic virus; MALT, mucosa-associated lymphoid tissue; NK, natural killer; WHO, World Health Organization

A. PRECURSOR B AND T-CELL NEOPLASMS

ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

a. Composed of immature precursor B or T cells called as lymphoblasts. Positive for terminal deoxytransferase (TDT) PAS and acid phosphatase.
b. 85% of all are precursor B cell tumor. Present typically in child hood as acute 'Leukemias'.
c. Precursor T-cell tumor present as 'Lymphomas 'in adolescent males with thymic involvement (mediastinal mass)
d. Immunologic markers

I. **Precursor B ALL**-Three types
Early pre B ALL - CD19, CD10 (CALLA). Most common ALL
Late Pre B - Cytoplasmic mu chains.
Mature B- CD 20, CD21
*CD19 is the first marker to appear and CD23, the last to appear.

II. **Precursor T ALL**
Early pre-T - CD1, CD2, CD5, CD7
Late Pre T - CD3, CD4, CD8

e. Cytogenetics - >90% ALL have numerical and structural changes in chromosomes:

- Hyperploidy (>50 chromosomes) - most common trisomy 4 and 10
- t(12:21)
- t(9:22) - Philadelphia chromosome
- t(4:11)

f. C/F OF ALL:

- Abrupt stormy onset
- Symptoms of bone marrow depression
  Anemia—fatigue
  Leukopenia - Fever, infections
  Thrombocytopenia - Petechial, ecchymosis, epistaxis, gum bleeding
- Bone pains and tenderness - Marrow expansions and subperiosteal infiltration
- Generalized lymphadenopathy, hepatosplenomegaly
- Testicular involvement, thymic involvement (T-ALL)
- CNS manifestations - headache, vomiting nerve palsies

g. Prognosis –
>90% children with ALL achieve complete remission with aggressive chemotherapy prognostic factors:

I. Age <2 years or presentation in adolescent/adulthood
II. >100,000 blasts/ul in P/S
III. T(9:22)

Good prognostic factors

I. Age between 2-10 years
II. Early pre-B phenotype
III. Hyperploidy, t(12:21)
IV. Low white cell count

B. PERIPHERAL B-CELL NEOPLASMS

1. Chrononic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). Most common leukemia of adults in

a. CLL is very similar to SLL, which is also called well differentiated lymphocytic lymphoma (WDLL)

I. If patients present with blood findings (absolute lymphocyte count >4000/mm3)
= CLL, If patients present with lymph node findings = SLL

II. Lymph node involvement is also common (50%) with CLL

b. Immunophenotype of CLIALL--B-cell markers such as CD 19 and CD20 One T-cell marker is also present CD5. Also important is that the cells are CD23 positive and CD10 negative.

c. Histology of affected lymph nodes reveals diffuse pattern of proliferation of small lymphocytes, with proliferation centers (with loose aggregates of prolymphocytes in the center).

d. Peripheral blood findings

I. Increased numbers of normal-appearing lymphocytes

II. Numerous smudge cells (parachute cells) are present, smudge cells result from the fact that the neoplastic lymphocytes are unusually fragile.

e. Bone marrow numerous normal-appearing neoplastic lymphocytes forming non-paratrabecular aggregates.

f. Clinical characteristics of CLL

I. CLL is the most indolent of all of the leukemias

II. Mean age at time of diagnosis is 60. M > F

III. Most patients with CLL/SLL are asymptomatic. When symptoms appear they are nonspecific.

IV. The malignant cells are nonfunctional →hypo gamma globulinemia increased risk of infections

V. CLL is associated with warm autoinununne hemolytic anemia (AIHA) (10% of cases), which spherocytes to be observed in peripheral blood.

VI. CLL rarely transforms into a worse disease, such as prolymphocytic leukemia or Large cell lymphoma (Richer's syndrome).

g. Chromosomal abnormalities - Deletions of 13q, 11q, 17p, and trisomy 12q.
Trisomy 12, 11q, and 17p deletions-poor prognosis

2. **Hairy cell leukemia**

a. Indolent disease of older males. Rare B-cell tumor.

b. Lymphocytes have 'hair-like' cytoplasmic projections ('dry tap" with bone marrow aspiration).

c. Diagnostic stain: positive tartrate resistant acid phosphatase (TRAP).

d. Physical exam: a markedly enlarged spleen (splenomegaly) due to infiltrate of red pulp by malignant cells Rarely hepatomegaly.

e. Treatment with 2-chlorodeoxyadenosine (2CDA), which inhibits adenosine deaminase (ADA) and increase level of toxic deoxyadenosine.

f. Immunopheno typing pan B marker CD19, CD20, Surface IgH and monocytic markers CD11C, CD25, CD103 and FMC7

g. C/F — Due to pancytopenia. Increased incidence of atypical mycobacterial infections.

3. **Follicular lymphomas**

a. All follicular lymphomas are derived from B-lymphocytes

b. Characteristic translocation is t(14:18)

I. Chromosome 14 has immunoglobin heavy-chain genes.

II. Chromosome 18 has bc1-2 (activation of bc1-2 inhibits apoptosis by blocking the bax channel).

c. Clinical features

I. Commonly present with painless generalized lymphadenopathy, extranodal involvement rare.

II. Although incurable, they usually follow an indolent course with overall median survival of 7-9 year

III. Does not respond to therapy (unlike the more aggressive diffuse lymphomas)

IV. Up to half of cases will progress to a diffuse large-cells NHL

d. Morphology: Nodular pattern of growth in lymph nodes. Two types of cells seen:
Small cleaved cells (centrocytes) and larger cells (centroblasts). Small cleaved cells make up majority of cell

e. Immunophenotype
CD 19, CD20, CD 10 and surface immunoglobin positive.

Bel 2 and Bel 6 are also positive in majority of the tumors.

4. **Diffuse large B-cell lymphoma**
   a. Constitute 20% NHLS. Median age of presentation is 60 years with male predominance
   b. Common features:
   I. Composed of large cells with diffuse growth pattern
   II. May present at extranodal sites e.g. stomach, CNS, etc.
   III. Aggressive rapidly proliferating tumor
   IV. Fatal if untreated with intensive combination chemotherapy.
   c. Immunophenotype
   CD 19, CD20 and Bel 6 positive
   d. Cytogenetics:
   - Various translocations that have in common a break point at chromosome 3q 27 (Bcl 6 dysregulation common.
   - Few tumors have t(14:18) like follicular lymphomas
   e. Special subtypes
   I. Immunodeficiency associated B-cell lymphoma (associated with EBV) e.g. in HIV patients
   II. Body cavity large B-cell Lymphoma (HHV-8 associated)-Also called primary effusion lymphomas.

5. **Small Noncleaved Lymphoma (Burkitt's Lymphoma)**
   a. Morphology
   I. Diffuse infiltrate of medium size lymphocytes with a high mitotic rate
   II. "Starry-sky" appearance is due to numerous reactive tangible body macrophages (phagocytosis of apoptotic tumor cells)
   b. Characteristic t (8:14)
   Chromosome 14 has immunoglobulin heavy-chain genes.
   Chromosome 8 has oncogene c-myc
   c. Three categories
   - African type (Endemic)-All EBV associated
   I. Involvement of mandible or maxilla is characteristic. Unusual predilection for abdominal viscera
   II. Found in children and young adults
   - Sporadic (non-endemic) - 15-20% show EBV association
   -Aggressive subtypes occurring in HIV positive individuals - 25% show EBV association
   * Endemic and sporadic forms account for 30% child hood NHLS.
   d. Immunophenotyping
   -Tumors of mature B cells
   Express surface Ig M, Kappa or Lambda light chain CD 19, CD20, CD 10, and BCL6

6. **Mantle zone lymphoma**
   a. Rare NHL
   b. Tumor cells resemble normal mantle zone B cells that surround germinal centers
   c. Morphology - Nodular/diffuse effacement of lymph node by small cleaved cells.
   d. The tumor cells arise from mantle zone B. lymphocytes (positive of CD19,CD20, CD5, negative for CD23)
   e. Characteristic translocation t(11;14)
   Chromosome 11 has bc1-1(cyclinD)
   Chromosome 14 has immunoglobulin heavy-chain genes.'
   f. C/F-Generalized lymphadenopathy
   Extranodal involvement- bone marrow, spleen, liver and GUT
   Multifocal small bowel and colonic involvement produces "Iymphomatoid Polyposis"

7. **Marginal zone lymphoma (MALTOMA)**
   a. May arise inside or outside lymph nodes (extranodal)

b. Associated with mucosa-associated lymphoid tissue: (MALTomas)
c. Begins as reactive polyclonal reaction and may be associated with previous autoimmune disorders, chronic inflammatory disorders or infections etiology
d. Remains localized for long periods of time.
e. May regress after the inciting agent is removed e.g. H. pylori
f. Extranodal lymphomas may occur in stomach (H pylori), orbit (Chlamydia Psittaci), intestine, lung, thyroid, skin (Borrelia sp.) salivary gland, etc.
g. First line of treatment is antibiotics. Tumors that don't respond to antibiotics often have cytogenetic abnormalities
Rx. Combination chemotherapy

8. **MULTIPLE MYELOMA**

a. Tumor of plasma cells arising in the bone marrow.
b. Characteristic involvement of axial skeleton at multiple sites. Can spread to lymph nodes and extranodal sites spleen, liver, skin, kidneys, etc.
c. Blood
I. Normocytic normochromic anemia
II. Rouleaux formation
III. Increased ESR
IV. Hypercalcemia
V. More than 20% plasma cells in peripheral blood is called as plasma cell leukemia
d. Bone marrow
i. More than 10% plasma cells in bone marrow
ii. Flame cells (fiery red cytoplasm), Mott cells (multiple blue grapes like cytoplasmic droplets) and Dutcher bodies (nuclear globules) may be seen.
e. Immunoglobulins
I. Increased serum proteins with normal albumin
II. M. Spike — Monoclonal immunoglobulin spike most commonly IgG (60%), next IgA (20%) rarely IgD. Or IgF. 1% myelomas are non-secretory
III. Light chains in serum and urine called as Bence Jones proteins.
IV. Normal polyclonal immunoglobulin is greatly reduced leading to repeated infections.
f. Clinical features:
I. Peak age – 50-60 years.
II. Common presentation- Bone pains and pathological fractures
III. Bones of axial skeleton commonly involved-vertebral column (most common), ribs, skull, pelvis FEMURS, clavicle, scapula etc.
IV. Excessive bone resorption leads to hypercalcemia — lethargy, confusion, weakness and metastatic calcification
V. Recurrent infections due to Streptococcus pneumoniae, Staph. aureus and E. coli (main cause of death
VI. Renal insufficiency due to Bence Jones proteins (toxic to renal tubular epithelial cells), nephrocalcim amyloidosis and amyloidosis.
VII. X-ray — Multiple punched out osteoporotic lesions of diffuse osteoporosis.
g. Multiple lytic bone lesions are due to osteoclast activating factors (OAF)
I. OAF is IL-6. Increased amounts of IL-6 are associated with poor prognosis because survival of myeloma cells ins dependent on IL-6 h.
h. Complications
I. Infections- Most common cause of death.
II. Renal disease (myeloma nephrosis)
III. Amyloidosis (10% patients) due to AL chains

Prognosis

(i) Total myeloma cell mass and clinico pathological features (Dune–Salmon Staging)

(ii) Serum 132 microglobulin levels correlate with stage of disease and survival. Also important in evaluation response to treatment.

Plasmacytoma: solitary aggregates of plasma cells, which may be located

1) Within bone : precursor lesion to later develop into myeloma
2) Outside bone (extramedullary): usually found within the upper respiratory tract and are not precursor lesion for myeloma

Monoclonal gammopathy of undetermined significance (MGUS) — Characterized by M spike in absence of associated disease of B cells.

I. Old name was benign monoclonal gammopathy.
II. M proteins is found in 1% of asymptomatic individuals over the age of 50 (the incidence increasesing age) and 10% over the age 75.
III. Multiple myeloma develops in 1% patients/year
IV. Serial serum and urine electrophoresis should be done every 6-12 months.

Poems Syndrome

Polyneuropathy, organomegaly, endocrinopathy, Multiple myeloma, skin changes
1/3-Diabetes (Type 2), Hypothyroidism, Adrenal insufficiency
Skin changes- Hyperpigmentation, Hypertrichosis, skin thickening, digital clubbing

9. **Lysmhoplasmacytic lymphoma (waldenstrom's macroglobulinemia)**
   a. Synonym: small lymphocytic lymphoma with plasmacytic differentiation
   b. Waldenstrom's macroglobulinemia (WM) is a cross between multiple myeloma and small lymphocytic lymphoma (SLL).
   I. Like myeloma, WM has an M spike (IgM).
   II. Like SLL (Unlike myeloma), the neoplastic cell infiltrate many organs, such as lymph nodes, spleen, and bone marrow
   III. Unlike multiple myeloma (MM), there are no lytic bone lesions, and serum calcium levels do not increase
   c. Russel bodies (cytoplasmic immunoglobulin) and Dutcher bodies (intranuclear immunoglobulin) may be present.

   B/M shows lymphocytes, plasma cells and plasmacytoid lymphocytes

   d. P/S - rouleaux formations and spherocytes (extravascular hemolysis)
   e. May have hyperviscosity syndrome (because IgM is large pentamer)
   I. Visual abnormalities due to vascular dilations and hemorrhages in the retina.
   II. Neurologic symptoms include headaches and confusion.
   III. Bleeding and cryoglobulinemia due to abnormal globulins, which precipitate at low temperature and may cause Raynaud's phenomenon. Positive Direct Coombs Test.

C. PERIPHERAL T-CELL AND NATURAL KILLER CELL NEOPLASMS

1. **Peripheral T-cell lymphoma, unspecified**
   a. This is a "wastebasket" diagnostic category.
2. Adult T- cell leukemia/lymphoma (ATLL)
   a. ATLL is a malignant T-cell disorder (CD4-T cells) due to HTLV-1 infection that is found in Japan and the Caribbean.

b. Clinical symptoms: skin lesions, hypercalcemia, enlarged lymph nodes, liver and spleen
c. Micro: hyperlobated "4 clover leaf' or flower cells in the peripheral blood

3. Mycosis fungoides (MF) and sezary syndrome (SS)
   a. MF is malignant T-cell disorder (postthymic CD4 cells) but has a better prognosis than ATLL.
   b. Clinical: Generalized pruritic erythematous rash (no hypercalcemia)
   c. Sequence of skin changes (stages): inflammatory eczematous stage →plague stage →tumor (nodule) stage.
   d. M/C reveals atypical PAS- positive lymphocytes in epidermis (epidermotropism), aggregates of these cells are called Pautrier microabscesses.
   e. Sezary syndrome - cerebri form sezary cells in peripheral blood with generalized exfoliative erythroderma.
4. Anaplastic Large Cell Lymphoma
   I. Tumor of cytotoxic - T cells.
   II. Large anaplastic cells with horse shoe shaped nuclei Hallmark cells)
   III. Tumor cells show Alk gene rearrangements on chromosome 2p23 in children or young adults. ALK gene rearrangement confers a good prognosis.

5. **Large Granular Cell Lymphoma**

I. Also called as CD 8 Lymphocytosis, CD8+T-CLL,TY lymphoproliferative disease.
II. Tumor of CD8+T-cells/NK cells Tumor cells
III. Tumor cells have abundant blue cytoplasm and coarse azurophilic granules.
IV. C/F are due to neutropenia and anemia.
V. Many patients have rheumatological disorder like Felty syndrome (RA, splenomegaly, neutropenia).

6. **Extranodal N.K/T Cell Lymphoma**

I. Also called as lethal midline granuloma/angiocentric lymphoma.
II. Presents commonly as destructive midline mass of nasopharynx, rarely skin, testis etc
III. Tumor of NK cell/cytotoxic T cells.
IV. Aggressive tumors

Hodgkin's lymphomas

1. Hodgkin's versus non-Hodgkin's lymphomas
   a. Characteristics of HD that are different form NHL
      I. Clinically, HD may present similar to infection (with fever)
      II. Most often localized to single axial group of nodes (cervical, mediastinal, para-aortic.)
      III. Spread is contiguous to adjacent node groups
      IV. No leukemic state
      V. Extranodal spread uncommon. Mesenteric LN and Waldeyer's ring rarely involved
2. **Hodgkin's disease**
   a. The malignant cells are the Reid—Sternberg (RS) cell.
      I. ‘Owl-eye" appearance. symmetric (mirror image) bibbed nucleus with prominent central nucleoli surrounded by clear space
      II. RS cells are positive for CD15 and CD30 in most subtypes.
      III. Except for lymphocyte predominate HD in which the malignant cells stain for B-cell markers (CD20)and have negative C15 and CD 30.
   b. Classification

Subtypes of Hodgkin Lymphoma

Subtype	Morphology and Immunophenotype	Typical Clinical Features

Subtype	Morphology and Immunophenotype	Typical Clinical Features
Nodular sclerosis	Frequent lacunar cells and occasional diagnostic RS cells; background infiltrate composed of T lymphocytes, eosinophils, macrophages, and plasma cells; fibrous bands dividing cellular areas into nodules. RS cells CD15+, CD30+; usually EBV-	**Most common subtype**; usually stage I or II disease; frequent mediastinal involvement; equal occurrence in males and females (F = M), most patients young adults
Mixed cellularity	Frequent mononuclear and diagnostic RS cells; background infiltrate rich in T lymphocytes, eosinophils, macrophages, plasma cells; RS cells CD15+, CD30+; 70% EBV+	More than 50% present as stage III or IV disease; M greater than F; biphasic incidence, peaking in young adults and again in adults older than 55
Lymphocyte rich	Frequent mononuclear and diagnostic RS cells; background infiltrate rich in T lymphocytes; RS cells CD15+, CD30+; 40% EBV+	Uncommon; M greater than F; tends to be seen in older adults
Lymphocyte depletion	Reticular variant: Frequent diagnostic RS cells and variants and a paucity of background reactive cells; RS cells CD15+, CD30+; most EBV+	Uncommon; more common in older males, HIV-infected individuals, and in developing countries; often presents with advanced disease
Lymphocyte predominance	Frequent L&H (popcorn cell) variants in a background of follicular dendritic cells and reactive B cells; RS cells CD20+, CD15-, C30-; EBV-	Uncommon; young males with cervical or axillary lymphadenopathy; mediastinal

L&H, lymphohistiocytic; RS cell, Reed-Sternberg cell.

MYELOBLASTS NEOPLASM

Major Subtypes of AML in the WHO Classification

Class	Prognosis	FAB Subtype	Morphology/Comments
I. AML WITH GENETIC ABERRATIONS			
AML with t(8;21)(q22;q22); *CBF*α/*ETO* fusion gene	Favorable	M2	Full range of myelocytic maturation; Auer rods easily found; abnormal cytoplasmic granules
AML with inv(16)(p13;q22); *CBF*β/*MYH*11 fusion	Favorable	M4eo	Myelocytic and monocytic differentiation; abnormal eosinophilic precursors with abnormal basophilic granules

Class	Prognosis	FAB Subtype	Morphology/Comments
gene			
AML with t(15;17)(q22;11-12); *RARα*/*PML* fusion gene	Intermediate	M3, M3v	Numerous Auer rods, often in bundles within individual progranulocytes; primary granules usually very prominent (M3 subtype), but inconspicuous in microgranular variant (M3v); high incidence of DIC
AML with t(11q23;v); diverse *MLL* fusion genes	Poor	M4, M5	Usually some degree of monocytic differentiation
AML with normal cytogenetics and mutated *NPM*	Favorable	Variable	Detected by immunohistochemical staining for NPM
II. AML WITH MDS-LIKE FEATURES			
With prior MDS	Poor	Variable	Diagnosis based on clinical history
AML with multilineage dysplasia	Poor	Variable	Maturing cells with dysplastic features typical of MDS
AML with MDS-like cytogenetic aberrations	Poor	Variable	Associated with 5q-, 7q-, 20q-aberrations
III. AML, THERAPY-RELATED	Very poor	Variable	If following alkylator therapy or radiation therapy, 2- to 8-year latency period, MDS-like cytogenetic aberrations (e.g., 5q-, 7q-); if following topoisomerase II inhibitor (e.g., etoposide) therapy, 1- to 3-year latency, translocations involving *MLL* (11q23)
IV. AML, NOT OTHERWISE SPECIFIED			
AML, minimally differentiated	Intermediate	M0	Negative for myeloperoxidase; myeloid antigens detected on blasts by flow cytometry
AML without maturation	Intermediate	M1	>3% of blasts positive for myeloperoxidase
AML with myelocytic maturation	Intermediate	M2	Full range of myelocytic maturation
AML with myelomonocytic maturation	Intermediate	M4	Myelocytic and monocytic differentiation

Class	Prognosis	FAB Subtype	Morphology/Comments
AML with monocytic maturation	Intermediate	M5a, M5b	In M5a subtype, nonspecific esterase-positive monoblasts and pro-monocytes predominate in marrow and blood; in M5b subtype, mature monocytes predominate in the blood
AML with erythroid maturation	Intermediate	M6a, M6b	Erythroid/myeloid subtype (M6a) defined by >50% dysplastic maturing erythroid precursors and >20% myeloblasts; pure erythroid subtype (M6b) defined by >80% erythroid precursors without myeloblasts
AML with megakaryocytic maturation	Intermediate	M7	Blasts of megakaryocytic lineage predominate; detected with antibodies against megakaryocyte-specific markers (GPIIb/IIIa or vWF); often associated with marrow fibrosis; most common AML in Down syndrome

AML, acute myeloid leukemia; DIC, disseminated intravascular coagulation; MDS, myelodysplasia; NPM, nucleophosmin; vWF, von Willebrand factor.

AML, acute myeloid leukemia;

I. Myeloblasts- delicate nuclear chromatin, 2--4 nucleoli and more cytoplasm than a lymphoblast
- Fine azurophilic granules in cytoplasm.
- Auer rods in cytoplasm. (Pathognomic of myeloblasts).
- Auer rods are dysplastic lysosomes and are most commonly found in large number in AML M3-
- Myeloblasts stain positive for MPO and sudan black
- Blasts are positive for CD13, CD15, CD33, CD117, HLADR

II. Monoblasts:
- Folder or lobulated nuclei, lack Auer rods and granules.
- MPO and Sudan black negative
- Non specific esterase positive
- CD14 and CD11b positive

III. Megakaryoblasts – CD41, CD42b and CD61 positive

IV. Erythroblasts — PAS positive, glycophorin A positive

d. **Classification of A.M.L**

FAB classification

i. MO — Minimally differentiated AML

ii. MI-AML without differentiation, little maturation beyond myeloblast stage

iii. M2-AML with maturation — Most common AML (30-40%). Full range of myeloid maturation seen. M2 often associated with t (8:21)

iv. M3 AML-- Acute hypergranular promyelocytic leukemia
- Most cells are hyper granular promyelocytes
- Numerous Auer rods per cell (faggots)
- May develop DIC due to release of thrombo plastic substances from granules (especially when therapy kills the leukemic cells)
- Characteristic t(15:17)
- 15 has PML gene and 17 has retinoic acid receptor a gene (RAR gene)
- Translocation forms an abnormal retinoic acid receptor; therefore therapy is all transretinoic acid

v. M4 AML—Acute myelomonocytic leukemia
- 15-20% AML cases
- Myelocytic and monocytic differentiation
- Inv (16) associated

vi. M5 Acute Monocytic leukemia
- Monoblasts and monocytes
- Older patients
- Tissue infiltration (gum bleeding), organomegaly, and lymphadenopathy.

vii. M6 Acute Erythroleukemia (Di Guglielmo's disease)
- Abnormal erythroid precursor with megalo blastoid feature present along with myeloblasts
- Old age, poor prognosis
- 20% therapy related and 1% denovo, AML's

viii. M7 Acute mega karyocytic leukemia
- Associated with acute myelofibrosis due to release of platelet derived growth factor (PDGF)
- FAB classification has now been replaced by the WHO classification described already.

B. MYELODYSPLASTIC SYNDROMES (MDS)- Clonal disorder with maturation defects associate with ineffective hematopoieses and pancytopenia and 20% blasts on BM.

World Health Organization (WHO) Classification of Myelodysplastic Syndromes/Neoplasms			
Name	**WHO Estimated Proportion of Patients with MDS**	**Peripheral Blood: Key Features**	**Bone Marrow: Key Features**
Refractory cytopenias with unilineage dysplasia (RCUD):			
Refractory anemia (RA)	10-20%	Anemia <1% of blasts	Unilineage erythroid dysplasia (in 10% of cells) <5% blasts
Refractory neutropenia (RN)	<1%	Neutropenia <1% blasts	Unilineage granulocytic dysplasia <5% blasts
Refractory thrombocytopenia (RT)	<1%	Thrombocytopenia <1% blasts	Unilineage megakaryocytic dysplasia <5% blasts
Refractory anemia with ring sideroblasts (RARS)	3-11%	Anemia No blasts	Unilineage erythroid dysplasia 15% of erythroid precursors are ring sideroblasts <5% blasts
Refractory cytopenias with multilineage dysplasia (RCMD)	30%	Cytopenia(s) <1% blasts No Auer rods	Multilineage dysplasia ± ring sideroblasts <5% blasts No Auer rods
Refractory anemia with excess blasts, Type 1 (RAEB-1)	40%	Cytopenia(s) <5% blasts No Auer rods	Unilineage or multilineage dysplasia
Refractory anemia with excess blasts, type 2 (RAEB-2)		Cytopenia(s) 5-19% blasts ± Auer rods	Unilineage or multilineage dysplasia 10-19% blasts ± Auer rods
MDS associated with isolated Del(5q) (Del(5q)	Uncommon	Anemia Normal or high platelet count <1% blasts	Isolated 5q31 chromosome deletion Anemia; hypolobated megakaryocytes <5% blasts
Childhood MDS, including refractory cytopenia of childhood (*provisional?*) (RCC)	<1 %	Pancytopenia	<5% marrow blasts for RCC Marrow usually hypocellular
MDS, unclassifiable (MDS-U)	?	Cytopenia	Does not fit other categories

		1% blasts	Dysplasia <5% blasts If no dysplasia, MDS-associated karyotype

Note: If peripheral blood blasts are 2–4%, the diagnosis is RAEB-1 even if marrow blasts are less than 5%. If Auer rods are present, the WHO considers the diagnosis RAEB-2 if the blast proportion is less than 20% (even if less than 10%), AML if at least 20% blasts. For all subtypes, peripheral blood monocytes are less than 1×10^9/L. Bicytopenia may be observed in RCUD subtypes, but pancytopenia with unilineage marrow dysplasia should be classified as MDS-U. Therapy-related MDS (t-MDS), whether due to alkylating agents, topoisomerase II (t-MDS/t-AML) in the WHO classification of AML and precursor lesions. The listing in this table excludes MDS/myeloproliferative neoplasm overlap categories, such as chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, and the provisional entity RARS with thrombocytosis.

a. The classification of myelodysplastic syndromes is based on the number of blasts in the marrowDysplastic changes include Pelger—Huet cells ("aviator glasses" nuclei), ring sideroblasts, nuclear building, and "pawn ball" megakafryocytes.
b. MDS patients have an increased risk of developing acute leukemia (preleukemias). 5q deletion is seen in post therapy MDS. 5q deletions associated with thrombocytosis.

C. MYELOPROLIFERATIVE SYNDROMES (MPS)

General

a. MPSs are clonal neoplastic proliferation of multipotent myeloid stem cells.

b. Bone marrow is usually markedly hypercellular (hence the name myeloproliferative)

i. All cell lines are increased in number (erythroid, myeloid, and megakaryocytes)
ii. Cannot tell the MPSs apart by the histologic appearance of the bone marrow.
iii. Homing of the neoplastic stem cells to secondary hematopoietic organs produces extramedullary hematopoiesis
iv. Variable transformation to spent phase characterized by marrow fibrosis and peripheral blood cytopenias.
v. Variable transformation to acute leukemias
vi. Presence of mutated, constitutively activated tyrosine kinase.

Tyrosine Kinase Mutations in Myeloproliferative Disorders

Disorder	Mutation	Frequency[¶]	Consequences[*]

Disorder	Mutation	Frequency[¶]	Consequences[*]
Chronic myeloid leukemia	*BCR-ABL* fusion gene	100%	Constitutive ABL kinase activation[†]
Polycythemia vera	*JAK2* point mutations	>95%	Constitutive JAK2 kinase activation
Essential thrombocythemia	*JAK2* point muations	50% to 60%	Constitutive JAK2 kinase activation
	MPL point mutations	5% to 10%	Constitutive MPL kinase activation
Primary myelofibrosis	*JAK2* point mutations	50% to 60%	Constitutive JAK2 kinase activation
	MPL point mutations	5% to 10%	Constitutive MPL kinase activation
Systemic mastocytosis	*c-KIT* point mutations	>90%	Constitutive c-KIT kinase activation
Chronic eosinophilic leukemia[‖]	*FIP1L1-PDGFR*α fusion gene	Common	Constitutive PDGFRα kinase activation
	*PDE4DIP-PDGFR*β fusion gene	Rare	Constitutive PDGFRβ kinase activation[†]
Stem cell leukemia[‡]	Various *FGFR1* fusion genes	100%	Constitutive FGFR1 kinase activation[§]

* All stimulate ligand-independent pro-growth and survival signals.

† Responds to imatinib therapy.

‡ Rare disorder originating in pluripotent hematopoietic stem cells that presents with concomitant myeloproliferative disorder and lymphoblastic leukemia/lymphoma.

§ Responds to PKC412 therapy.

¶ Refers to frequency within a diagnostic category.

‖ Associated with Loefflers endocarditis (Chapter 12).

1. **Chronic Myelogenous Leukemia (CML)**
   a. Clonal proliferation of pluripotent stem cells.
   b. A unique characteristic is the chromosomal translocation.
      I. Philadelphia (Ph), chromosome, which has t (9:22).
      II. 9 has c-abl (an oncogene), while 22 has bcr (breakpoint cluster region).
      III. This translocation forms a new protein (P210) that has increased tyrosine kinase activity.

CML

c. **Bone marrow**

- Hypercellular bone marrow with all cell lines increased in number especially granulocytic

- Sea blue histiocytes (storage histiocytes with wrinkled green blue cytoplasm)

d. **Peripheral blood** –
   - Marked leukocytosis often with >1 lakh cells/mm3
   - Predominant cells are granulocytes and myelocytes. Myeloblasts are <10%
   - Eosinophilia and Basophilia seen
   - Thrombocytosis present

e. Decreased leukocyte alkaline phosphatase (LAP) activity is diagnostic: (compared with leukemoid reaction, which has increased LAP).

f. C/F-Insidious onset
   - Mild to moderate anemia and hyper metabolism lead to easy fatigability, weakness, weigh loss and anorexia.
   - Dragging sensation in abdomen due to massive splenomegaly.
   - Left upper quadrant pain due to splenic infarction.
   - Natural History of CML
     Slowly progressive disease even with out treatment, median survival is 3 years.
     Accelerated phase — Seen in 50% patients after a variable period averaging 3 years.

Criteria for accelerated phase of CML
- Increasing spleen size unresponsive to therapy
- Increasing anemia
- Increasing thrombocytopenia (<1 lakh /u1)
- Blood or bone marrow blasts between 10-20%
- Blood bone marrow basophils — ≥20%
- Cytogenetic clonal evolution
  Blast crisis: Blood or bone marrow blasts ≥20% About 1/2 blasts are myeloid, one third lymphoid, few erthroid and rest undifferentiated.

h. Treatment
   Control with hydroxyurea
   Bone marrow transplant

2. **Polycythemia vera (P. vera)** clonal disorder of multipotent stem cell with increased proliferation granulocytes and thrornbocytes.
   a. Characteristic findings
   I. Increased erythroid precursors with increased red cells mass (primary)
   II. Increased hematocrit
   III. Increased blood viscosity

b. Decreased erythropoietin (EPO), but RBCs have increased sensitivity to EPO and over proliferate

c. Increased basophils and increased eosinophils (like all of the MPSs)

d. Histamine released from basophils causes intense pruritus and gastric ulcers (bleeding may deficiency)

e. Increased LAP score.

f. Clinical characteristics: plethora (redness) and cyanosis (blue)

g. Complications
   - Increased blood viscosity can cause deep vein thromboses and infarcts
   - High cell turnover can cause hyperuricemia
   - P. vera may develop into a "spent phase" with myelofibrosis

h. Increased risk of acute leukemia- seen in 2% patients treated with chemotherapy.
   - Criteria for diagnosis
   - Elevated red cell
   - Normal arterial oxygen saturation

- Splenomegaly

In absence of splenomegaly

- Leulcocytosis and thrombocytosis

I. Rx: Phlebotomy-mainstay of treatment
Chemotherapy with hydroxy urea used only in cases of symptomatic splenomegaly

3. **Essential thrombocythemia (ET)**
   a. Increased megakaryocytes (and other cell lines) in bone marrow
   b. Peripheral blood smear

I. Increased number of platelets more than 6 lalchs/rnm3 some with abnormal shapes

II. Also increased number of leukocytes

   c. Clinical signs include excessive bleeding and occlusion of small vessels.

4. **Myelofibrosis (MF) with myeloid metaplasia**
   a. Etiology is unknown (agnogenic)
   b. Bone marrow aspiration may be a 'dry tap'.
   c. Biopsy specimen shows hypocellular marrow with fibrosis (increased reticulin) in later stages of dim stages, marrow is hypercellular with increased granulocytic, erythroid and platelet precursors. Fibrosis is secondary to factors released from megakaryocytes, such as platelet derived growth factor (PDGF)
   d. Enlarged spleen due to extramedullary hematopoiesis (myeloid metaplasia)
      I. Note that the spleen is the most common site for extramedullary hematopoiesis.
   e. Peripheral smear
      I. Leukoerythroblastosis (immature white cell and nucleated red cells)
      II. Teardrop RBCs (dacryocytes)
   f. High cell turnover causes hyperuricemia and gout.

BLEEDING DISORDERS

Causes of abnormal bleeding

1. Vascular disorders
2. Platelet disorders – Deficiency (Thrombocytopenia) Dysfunction
3. Coagulation Disorders

VASCULAR DISORDERS

a. Vesse1 wall abnormality leads to bleeding
b. Also called as non-thrombocytopenic purpura
c. Relatively common, does not cause serious bleeding problems

I. Infections – Meningococcemia, septicemia, infective endocarditis and rickettsioses. Cause of bleeding is microbial damage to vasculature (vasculitis) or DIC ,

II. Drug reactions- vascular injury due to drug induced antibodies or deposition of immune complexes in vessel . wall (leukocytoclastic vasculitis)

III. Scurvy and Ehlers-Danlos syndrome — Microvascular bleeding due to impaired production of collagen needed for support of vessel walls.

IV. Henoch-Schonlein purpura — systemic disease of unknown cause, clinically characterized by pruritic rash, colicky abdominal pain, polyarthralgia and acute glomerulonephritis. Circulating immune complexes deposited in vessels and within glomerular capillaries. ,

V. Hereditary hemorrhagic telangiectasia – AD disorder, dilated tortuous thin walled vessels that bleed easily.

VI. Amyloid infiltration of blood vessels – seen in plasma cell dyscrasias.

PLATELET DISORDERS

Platelets

a. Derived from magakaryocytes in the bone marrow. They are disc shaped.
b. Formation of a hemostatic plug

Step 1: Platelet adhesion

I. First vWf adheres to sub endothelial collagen
II. Platelets then adheres to vWF by glycoprotein lb
III. Thus a monolayer of platelets is formed on injured vessel.

Step 2: Platelet activation

I. Platelets undergo a shape change from discs to spheres with pseudo pods and degranulation occurs Contents of Platelet Alpha Granules and Dense Bodies

Alpha Granules	**Dense Bodies**
Fibrinogen	ADP (Potent platelet aggregator)
Fibronectin	Calcium
Factor V and vwf	Histamine and serotonin
Platelet factor 4	Epinephrine
Platelet derived growth factors (PDGF)	

II. Platelet synthesis of thromboxane A2.
III. Membrane expression of the phospholipid complex glycoprotein IIb/IIIa which is an important plati coagulation cascade. Platelets can now bind fibrinogen.

Step 3: Platelet aggregation

I. Additional platelets are recruited from the blood stream.
II. ADP and thromboxane A2 are potent mediators of aggregation.
III. Platelets bind to each other by binding to fibrinogen using Gp IIb/IIIa.
IV. Platelets aggregation occurs and a primary hemostatic plug is formed.
V. Primary hemostatic plug is highly unstable and can be dislodged easily. So coagulation cascade is ac fibrin is deposited around the aggregated platelets resulting in secondary hemostatic plug formation.

c. Laboratory tests for platelets
   i) Platelets count (Norma 1.50 to 4.0 lacs)
   ii) Bleeding time test (normal 2 to 7 minutes)
   iii) Platelet aggregometry

Causes of Thrombocytopenia

1. Decreased production of platelets

I. Generalized diseases of bone narrow Aplastic anemia Marrow infiltration- leukemias
II. Selective impairment of platelet production
Drug – induced: alcohol, thiazides
Infections — measles, HIV
III. Ineffective megakaryopoiesis
Megaloblastic anemia
Myelodysplastic syndromes
TARR syndrome (Conga megakaryocytic hypoplasia + thrombocytopenia + absent radii)

2. **Decreased platelet survival**

I. Immunologic destruction
Autoimmune: idiopathic thrombocytopenic purpura, systemic lupus erythematosus
Isoimmune: Post-transfusion and neonatal
Drug – associated: quinidine, heparin.
Infections : Infectious mononucleosis, HIV infection, cytomegalovirus

II. Nonimmunologic destruction
Disseminated intravascular coagulation
Thrombotic thrombocytopenic purpura

HUS

Microangiopathic hemolytic anemias

3. **Sequestration: Hypersplenism**

Immune thrombocytopenic purpura (ITP)

a. Etiology

I. Antiplatelet antibodies against platelet antigens such as Gp 111a and Gp Ib/IX.

II. Antibodies are made in the spleen.

III. Platelets are destroyed peripherally in the spleen by macrophages which have Fc receptors that coated platelets.

b. Forms of ITP-Primary (Idiopathic)/secondary. Primary can be acute or chronic

I. Acute ITP

- Seen in children following a viral infection
- Self– limited disorder with abrupt onset
- 20% may develop chronic ITP

II. Chronic ITP

- Usually seen in women in their childbearing years.
- May be the first manifestation of systemic lupus erythematous (SLE).
- Petechiae, ecchymoses, menorrhagia, and nosebleeds.

c. Lab

I. Decreased platelet count and prolonged bleeding time.

II. Normal prothrombin time (PT) and partial thromboplastin time (PTTK).

III. Peripheral blood smear shows thrombocytopenia with enlarged immature platelets (megathrombocytes).

IV. Bone marrow biopsy shows increased number of megakaryocytes with immature forms.

d. Treatment

I. Corticosteroids which decrease antibody production

II. Immunoglobulin therapy, which floods Fc receptors on splenic macrophages

III. Splenectomy, which removes the site of platelet destruction and antibody production

IV. Splenomegaly and lymphadenopathy are not seen in primary ITP

Thrombotic thrombocytopenic purpura (TTP)

a. Pathology

I. Widespread formation of platelet thrombi with fibrin (hyaline thromb) in microvasculature

II. No activation of the coagulation system

b. Clinical findings

I. Most often affects adult females

II. Pentad of characteristic signs

- Fever
- Thrombocytopenia
- Microangiopathic hemolytic anemia
- Neurologic symptoms
- Renal failure

III. Cause — Deficiency of ADAMTS 13 (vWF metalloprotease) an enzyme that degrades high molecular weight multimers of vWF. In absence of the enzyme, the multimers accumulate in the plasma leading to platelet micro aggregate formations.

c. Lab

I. Decrease platelet count and prolonged bleeding time

II. Normal PT and PTTK

III. Peripheral smear shows thrombocytopenia schistocytes, and reticulocytosis

d. Plasma exchange is life saving, can provide the missing enzyme.

Hemolytic uremic syndrome (HUS)

I. Occurs most commonly in children, rarely adults
II. Follows a gastroenteritis with bloody diarrhea
III. Organism. Verocyto-toxin – producing E. coli 0157:h7
IV. Similar clinical pentad as TTP

Disorders of platelet function

a. Defect in adhesion

I. Bernard – Soulier syndrome AR disorder, deficiency of Gp Ib-IX, >60% platelet are large (giant platelet syndrome).
II. vW disease

b. Defect in Platelet aggregation

Glanzmann's thrombasthenia—AR disorder. platelets fail to aggregate in response to ADP, collagen, epinephrine etc due to deficiency of gp IIb-IIIa.

c. Defect in platelet secretion

I. Storage pool disease — Deficiency of Dense granules
II. Gray platelet syndrome-defect in alpha granules. Platelets appear agranular on P/S because alpha granules are most numerous of all platelet granules.

COAGULATION DISORDER

a. Coagulation factors
   I. The majority of the clotting factors are produced by the liver
   II. They are proenzymes that must be converted to the active form
   III. Some conversions occurs on a phospholipids surface
   IV. Some conversions require calcium
b. Intrinsic coagulation pathway is activated by the contact factors

I. Contact with sub-endothelial collagen
II. High molecular weight lcininogen (HMWK)
III. Kallikrein

c. Extrinsic coagulation pathway is activated by the release of tissue factor :
d. Laboratory tests for coagulation

I. Prothrombin time (PT)

- Test the extrinsic and common coagulation pathways
- VII, X, V prothrombin, fibrinogen

II. Partial thromboplastin time kaolin (PTTKJAPTT)

- Test the intrinsic and common coagulation pathways
- XII, XI, IX, VIII, X, V, prothrombin, fibrinogen

III. Thrombin time (TT) test for adequate fibrinogen levels
IV. Fibrin degradation products (FDP) test fibrinolytic system (increased in DIC)

Role of Vitamin K

- Essential role in hemostasis
- Absorbed from small intestine and stored in liver
- Serves as cofactor for enzymatic carboxylation (of glutamic acid residues on prothrombin complex). Factor VI and Protein C have the shortest half life, and therefore the deficiency of vitamin K manifests with prolongation of PT first. With severe deficiency, APTT is also prolonged

Three main causes of vitamin K deficiency:

- Poor dietary intake
- Intestinal malabsorption
- Liver Disease

C/F — bleeding from umbilicus/circumcision. Generalized ecchymosis, intracranial and intramuscular bleeding.

Hemophilia A (Classic Hemophilia)

a. Deficiency of factor VIII
b. X-linked recessive
c. Clinical features

I. Predominately affects males
II. Symptoms are variable dependent on the degree of deficiency
III. Spontaneous hemorrhages into joints (hemarthrosis)
IV. Easy bruising and hematoma formation after minor trauma
V. Severe prolonged bleeding after surgery
VI. No petechiae or ecchymoses

d. Lab

I. Normal platelet count and bleeding time
II. Normal PT and prolonged PTTK

e. Treatment Factor VIII concentrate

Hemophilia B (Christmas disease)

a. Deficiency of factor IX
b. X-linked recessive
c. Clinically identical to hemophilia A

Von Willebrand's disease

a. Definition: inherited bleeding disorder characterized by either a deficiency or qualitative defect in von Willebrand's, factor
b. vWF is normally produced by endothelial cells (Weibel Palade bodies) and megakaryocytes
c. Function of vWF-platelet adhesion to sub endothelial collagen and Carrier for factor VIII ((N) half life of factor VIII (N) half life of factor VIII with vWF is 12 h and without it is 2-4 hr only).
d. Clinical features

I. Spontaneous bleeding from mucosal membranes
II. Normal PT with often a prolonged PTTK
III. Abnormal platelet response to ristocetin (adhesion defect) is an important diagnostic test.
IV. Treatment: Treat mild cases (Type I) with desmopressin (ADH analog). Release vWF from Weibel Palade Bodies of endothelial cells

Disseminated intravascular coagulation (DIC)

a. DIC is always secondary to another disorder
b. Causes

I. Obstetric complications (placental tissue factor activated clotting)
II. AML M3 (cytoplasmic granules in neoplastic promyelocytes activate clotting)
III. Adenocarcinomas
IV. Gram-negative sepsis (tumor necrosis factor (TNF) activates clotting)
V. Microorganisms (especially meningococcus and rickettsia)

c. Pathology

I. Results in widespread microthrombi
II. Consumption of platelets and clotting factors causes hemorrhages

d. Lab

I. Platelet count is decreased
II. Prolonged PT/APTT
III. Decreased fibrinogen .
IV. Elevated fibrin split products (D-dimers)

e. Treatment: Treat the underlying disorder

Fibrinogen Deficiencies

I. A fibrinogenemia - AR disorder, homozygous condition Complete deficiency of fibrinogen

II. Hypofibrinogenemia - AR, heterozygous disorder plasma fibrinogen level between 20-100 mg/dl

III. Dysfibrinogenemia - AD condition Quantity of fibrinogen is normal but the molecule is qualitatively abnormal. C/F--Mild post traumatic bleeding.

Lab Tests — PT
APTT (PTTK)] Prolonged/Abnormal
TT

BT-Abnormal (fibrinogen is required for primary aggregation also)

Diagnostic Test — Functional assay for fibrinogen

Natural Anticoagulants

a. Once coagulation cascade is activated, it is restricted to local site of injury to prevent clotting in the entire vascular by:
   - Restricting factor activation to site of exposed phospholipids on platelets and injured endothelium.
   - Natural anticoagulants

I. Antithrombin III (AT III)
   - Activated by heparin like molecules on the endothelium
   - Combines with thrombin and inactivates it
   - Also inhibits IX a, Xa, XIa, Xlla

II. Thrombomodulin
   - Binds to thrombin and converts it from procoagulant to an anticoagulant which then activates Protein C

Thrombin + Thrombomodulin → Activation of Protein C (protein S) → Inactivates V a and VIII a

Protein C and Protein S are vit K dependent proteins.

III. Tissue Factor Pathway Inhibition (TFPI)]
   - Produced by endothelium
   - Complexes with T.F-VII a, and factor Xa and inactivates them.

Fibrinolytic Pathway — Responsible for break down of clot.

Plasminogen
↓
uPA — Plasma, tissues
tPA — Endothelial cells. Physiologically most active when attached to fibrin
Streptokinase (produced by bacteria)

Plasmin
↓
Inhibitors of Plasmin
α2 antiplasmin
α2 macroglobulin

Fibrinclot

Fibrin Split Products (FDP, D-Dimer

THROMBOSIS

Causes

Endothelial injury
Alteration in laminar blood flow
Hypercoagulable state

I. Hyper coagulable state

Primary (genetic)-common
   - Mutation factor V gene (factor V leiden)
   - Mutation in Prothrombin gene

- Mutation in methyltetrahydrofolate gene
- Antithrombin III deficiency (rare)
- Protein C deficiency (rare)
- Protein S deficiency (rare)

Secondary (Acquired)

→ High risk for thrombosis

- Prolonged bed rest/immobilization
- Myocardial infarction
- Atrial fibrillation
- Tissue damage (surgery, fracture, burns)
- Cancer
- DIC
- Heparin induced thrombocytopenia
- Prosthetic cardiac valves
- Antiphospholipids syndromes

→+ Low risk for thrombosis

- Nephrotic syndrome
- Hyper estrogenic states (pregnancy)
- Oral contraceptive usage idle cell anemia
- Smoking
- Primary causes must be considered in patients under the age of 50 years who present with thrombosis in the absence of any acquired predisposition.

PRIMARY

a. Factor V mutation (Leiden Mutation) —Most common among primary causes

- Gilutamine substituted for arginine at position 506 → mutated protein resistant to cleavage by protein C → unchecked coagulation.
- Accounts for 25% inherited prothrombotic states
  Heterozygotes-seven times increased risk of venous thrombosis
  Homozygotes--20 times increased risk of venous thrombosis

b. Prothrombus gene mutation - seen in 1-2% population.

- Single nucleotide change (G to A) in the 3 untranslated region of prothrombin gene
- Elevated prothrombin levels and 3-fold increased risk of venous thrombosis.

c. Hyper homocysteinemia

- Can be inherited or acquired.
- Leads to arterial and venous thrombosis. Also a risk factor for atherosclerosis.
- Increased levels of homocysteine inhibit AT III and thrombomodulin.
- Mutation in methyltetrahydrofolate gene also causes mild hyper homocysteinemia.

d. AT III deficiency Protein C deficiency
Protein S deficiency

- Affected individuals typically present with venous thrombosis and recurrent thromboembolism in adolescent and lirly adult life.
- AT III deficiency patients presenting with thrombosis cannot be treated with heparin Rx- Prophylactic Warfarin

ACQUIRED DISORDERS

- Drug induced- quinine, quinidine, sulfonamides, and heparin
  Heparin induced thrombocytopenia (HIT)
- Thrombocytopenia occurs in 5% patients receiving heparin

Type I - Most common, occurs rapidly after starting of therapy

- Not very severe, may resolve despite continuation of heparin therapy
- Cause- Direct platelet aggregation effect of heparin

Type II -Severe, occurs 5-14 days after starting the therapy.

- Paradoxically leads to arterial and venous thrombosis.
- Cause-Antibodies produced against complex of heparin and PF4 on the platelets. Binding of antibodies to the activates the platelets and promotes thrombosis even in the setting of thrombocytopenia.
- Rx discontinue heparin therapy

ANTI COAGULANT THERAPY

1. HEPARIN

a. Action

- Interacts with AT III →1000 times faster inactivation of serine proteases (XIIa, XIa, IXa,) Thrombin and Plasmin)
- Also inhibits interaction between factor X and prothrombin

b. Lab test to monitor heparin therapy: APTT

2. ORAL ANTICOAGULANTS: Dicoumarols and Coumarins (widely used- Warfarin and Indanediones)

a. Action — Prevents thrombin formation by inhibiting Vit. K. In presence of coumarins, liver cells are unable to utilize vit K to carboxy late the glutamic acid residues of factor II,VII, IX, X, Protein C and S.

Lab test the monitor Warfarin therapy — P.T

Some Immune Cell Antigens Detected by Monoclonal Antibodies

Antigen Designation	Normal Cellular Distribution
PRIMARILY T-CELL ASSOCIATED	
CD1	Thymocytes and Langerhans cells
CD3	Thymocytes, mature T cells
CD4	Helper T cells, subset of thymocytes
CD5	T cells and a small subset of B cells
CD8	Cytotoxic T cells, subset of thymocytes, and some NK cells
PRIMARILY B-CELL ASSOCIATED	
CD10	Pre-B cells and germinal-center B cells; also called CALLA
CD19	Pre-B cells and mature B cells but not plasma cells
CD20	Pre-B cells after CD19 and mature B cells but not plasma cells
CD21	EBV receptor; mature B cells and follicular dendritic cells
CD23	Activated mature B cells
CD79a	Marrow pre-B cells and mature B cells

Antigen Designation	Normal Cellular Distribution
PRIMARILY MONOCYTE- OR MACROPHAGE-ASSOCIATED	
CD11c	Granulocytes, monocytes, and macrophages; also expressed by hairy cell leukemias
CD13	Immature and mature monocytes and granulocytes
CD14	Monocytes
CD15	Granulocytes; Reed-Sternberg cells and variants
CD33	Myeloid progenitors and monocytes
CD64	Mature myeloid cells
PRIMARILY NK-CELL ASSOCIATED	
CD16	NK cells and granulocytes
CD56	NK cells and a subset of T cells
PRIMARILY STEM CELL–AND PROGENITOR CELL–ASSOCIATED	
CD34	Pluripotent hematopoietic stem cells and progenitor cells of many lineages
ACTIVATION MARKERS	
CD30	Activated B cells, T cells, and monocytes; Reed-Sternberg cells and variants
PRESENT ON ALL LEUKOCYTES	
CD45	All leukocytes; also known as leukocyte common antigen (LCA)

CALLA, common acute lymphoblastic leukemia antigen; CD, cluster designation; EBV, Epstein-Barr virus; NK, natural killer.