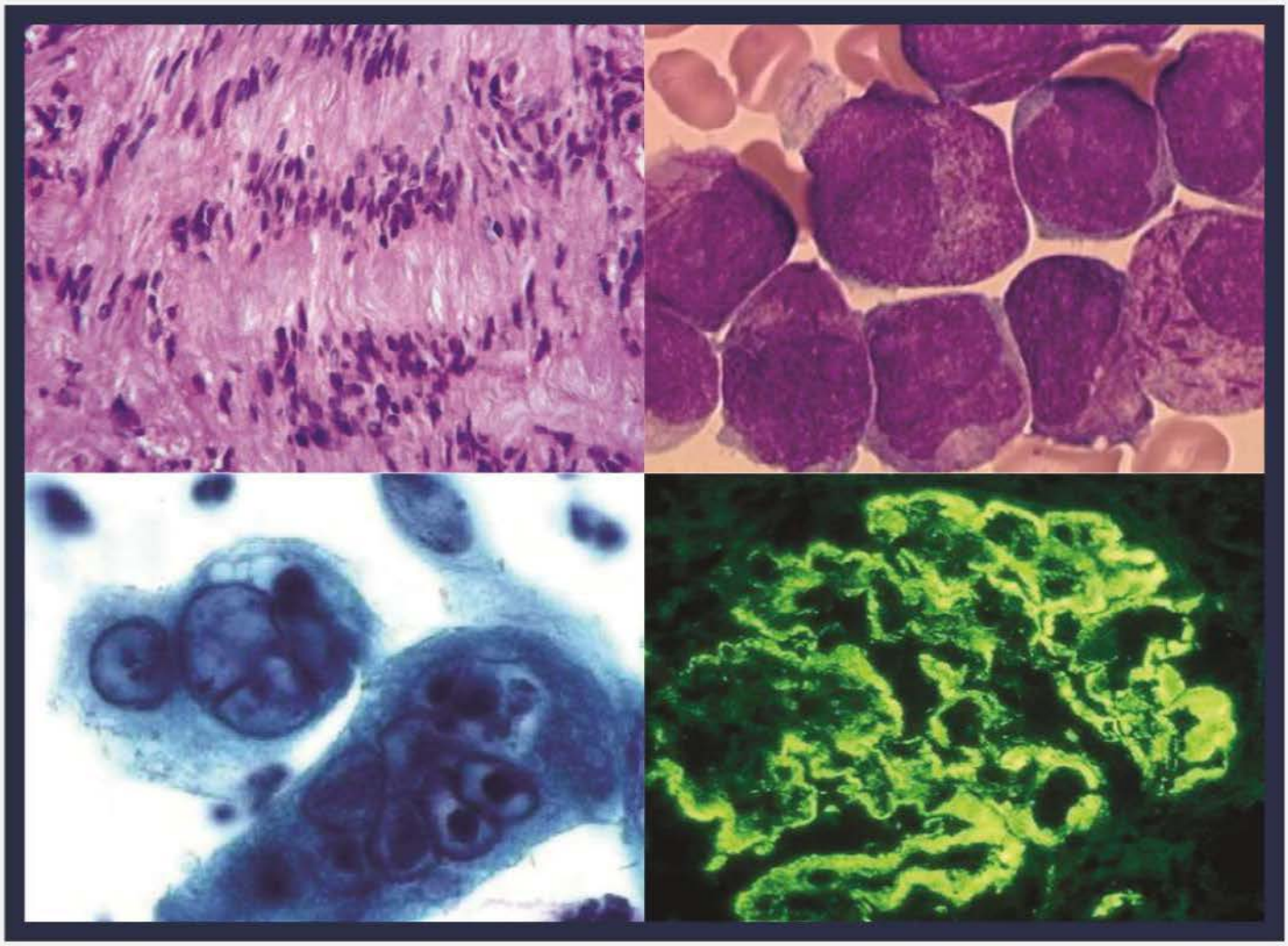
CRS

Comprehensive Review Series

PARACLINICALS

PATHOLOGY



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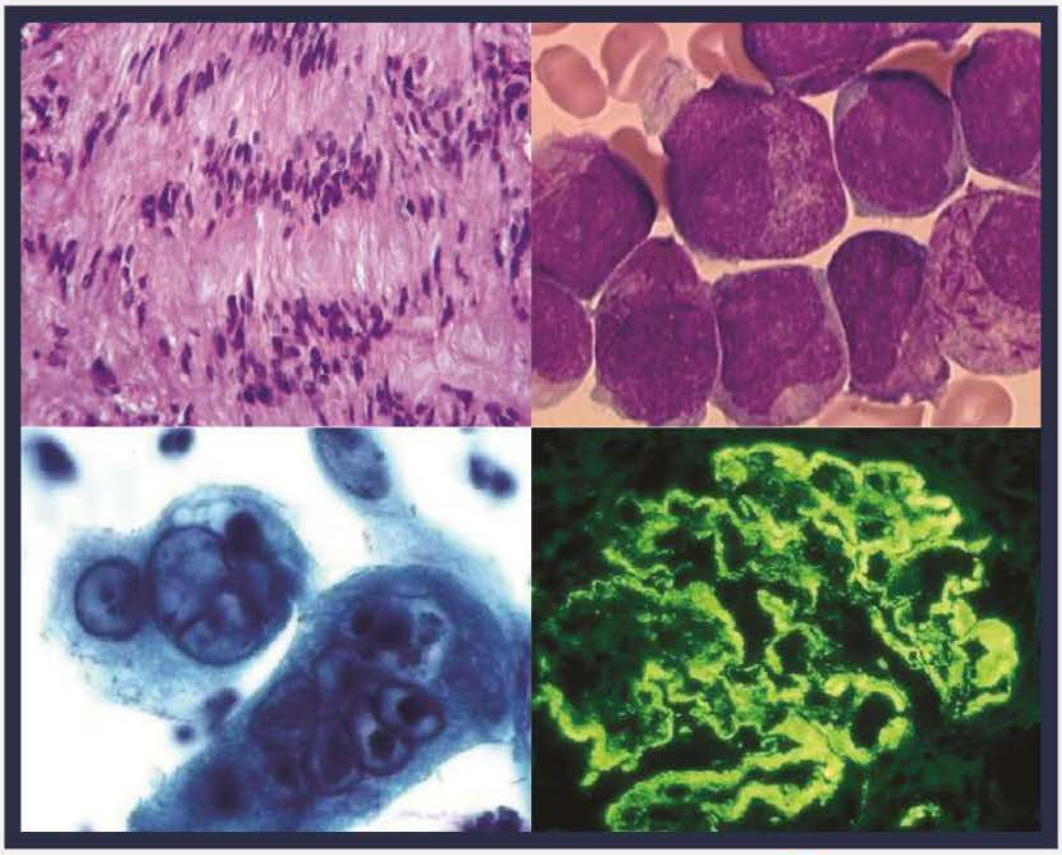

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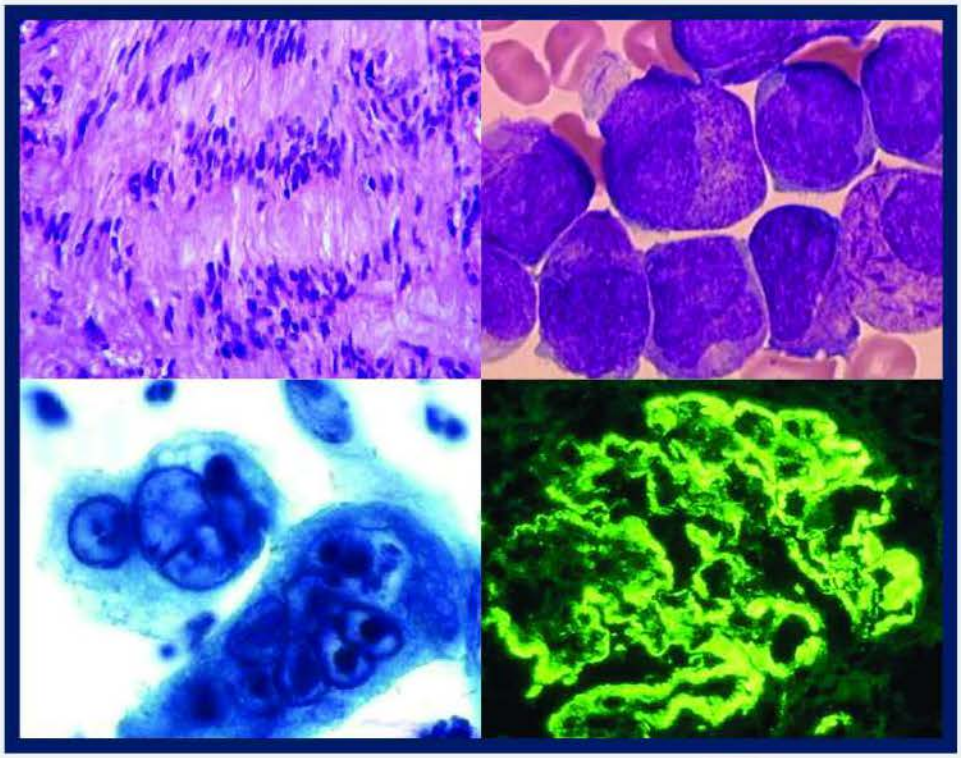

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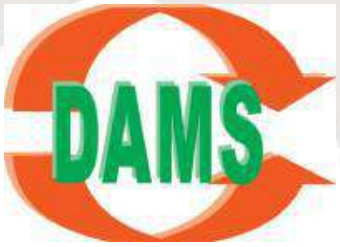


COMPREHENSIVE REVIEW SERIES

Paraclinicals

PATHOLOGY

For PG Medical Entrance


DAMS
Publications

Typeset by Delhi Academy of Medical Sciences Pvt. Ltd., New Delhi (India).

Contents

GENERAL PATHOLOGY

1 Cell as a Unit of Health and Disease

Human Genome:

- Published in draft form in 2001 and more completely detailed in 2003.
- Contains roughly 3.2 billion DNA base pairs.
- Within the genome there are about 20,000 protein-encoding genes, comprising only about 1.5% of the genome.
- 80% of the human genome either binds proteins, implying it is involved in regulating gene expression, or can be assigned some functional activity, mostly related to the regulation of gene expression, often in a cell-type specific fashion.

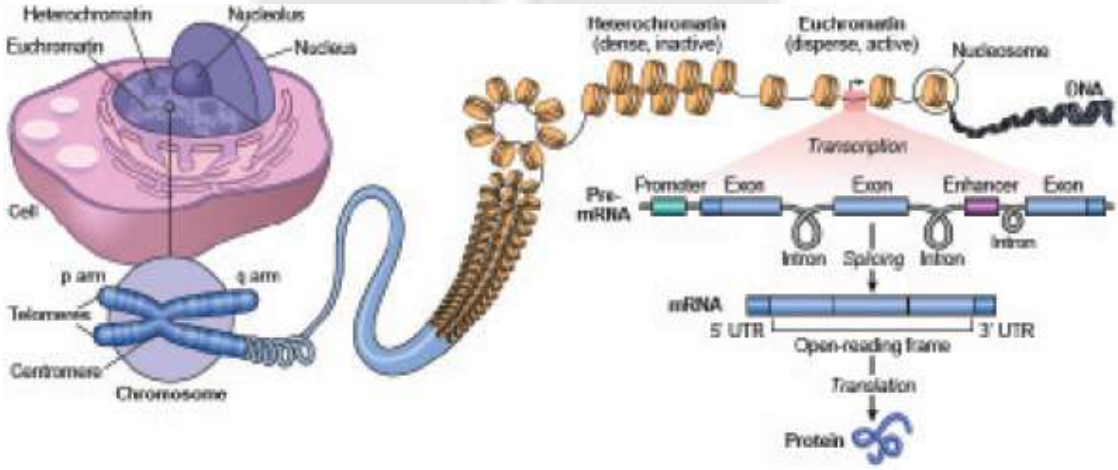


Fig. 1.1

The major classes of functional non–protein-coding sequences found in the human genome are the following.

1. **Promoter** and **enhancer** regions that provide binding sites for transcription factors.

2. Binding sites for factors that organize and maintain higher order **chromatin structures.**
3. **Noncoding regulatory RNAs.** More than 60% of the genome is transcribed into RNAs that are never translated into protein, but which nevertheless can regulate gene expression through a variety of mechanisms. The two best-studied varieties are micro-RNAs and long noncoding RNAs.
4. **Mobile genetic elements** (e.g., **transposons**). Remarkably, more than one third of the human genome is composed of these elements, popularly denoted as "jumping genes." These segments can move around the genome, exhibiting wide variation in number and positioning even amongst closely related species (i.e., humans and other primates). They are implicated in gene regulation and chromatin organization, but their function is still not well established.
5. Special structural regions of DNA, in particular **telomeres** (chromosome ends) and **centromeres** (chromosome "tethers").

The person-to-person variation, including differential susceptibility to diseases and in response to environmental agents and drugs, is encoded in less than 0.5% of our DNA. Though small when compared to the total nucleotide sequences, this 0.5% represents about 15 million base pairs.

The two most common forms of DNA variation in the human genome are single-nucleotide polymorphisms (SNPs) and copy number variations (CNVs).

Single Nucleotide Polymorphisms (Snps):

- Variants at single nucleotide positions and are almost always biallelic (i.e., only two choices exist at a given site within the population, such as A or T).
- Over 6 million human SNPs have been identified, many of which show wide variation in frequency in different populations.
- SNPs occur across the genome – within exons, introns, intergenic regions, and coding regions

- the SNP and the causative genetic factor are in **linkage disequilibrium**
- There is hope that groups of SNPs may serve as markers of risk for multigenic complex diseases such as type II diabetes and hypertension

Copy Number Variations (Cnvs):

- recently identified form of genetic variation consisting of different numbers of large contiguous stretches of DNA from 1000 base pairs to millions of base pairs.
- CNVs are responsible for between 5 and 24 million base pairs of sequence difference between any two individuals.
- Approximately 50% of CNVs involve gene-coding sequences; thus, CNVs may underlie a large portion of human phenotypic diversity.

EPIGENETICSs: is defined as heritable changes in gene expression that are not caused by alterations in DNA sequence

Different cell types are distinguished by lineage-specific programs of gene expression. Such cell type-specific differences in DNA transcription and translation depend on epigenetic factors (literally, factors that are “above genetics”) that can be conceptualized as follows.

1. **Histones and histone modifying factors.**

- **Nucleosomes** consist of DNA segments 147 base pairs long that are wrapped around a central core structure of highly conserved low molecular weight proteins called **histones.**
- The resulting DNA-histone complex resembles a series of beads joined by short DNA linkers and is generically called **chromatin.**
- At the light microscopic level, nuclear chromatin exists in two basic forms: (1) cytochemically dense and transcriptionally inactive **heterochromatin** and (2) cytochemically dispersed and transcriptionally active **euchromatin.**

- **Chromatin remodeling complexes** can reposition nucleosomes on DNA, exposing (or obscuring) gene regulatory elements such as promoters.
- "**Chromatin writer" complexes** , on the other hand, carryout more than 70 different histone modifications generically denoted as **marks** . Such covalent alterations include methylation, acetylation, or phosphorylation of specific amino acid residues on the histones.
- Histone marks are reversible through the activity of "chromatin erasers." Still other proteins function as "chromatin readers," binding histones that bear particular marks and thereby regulating gene expression.
- **Histone methylation** . Both lysines and arginines can b methylated by specific writer enzymes.
- **Histone acetylation.** Lysine residues are acetylated by histone acetyl transferases (HAT), whose modification tend to open up the chromatin and increase transcription. In turn, these changes can be reversed by histone deacetylases (HDAC), leading to chromatin condensation.
- **Histone phosphorylation** . Serine residues can be modified by phosphorylation.
- **DNA methylation.** High levels of DNA methylation in gene regulatory elements typically result in transcriptional silencing.
- **Chromatin organizing factors.** Much less is known about these proteins, which are believed to bind to noncoding regions and control long-range looping of DNA.

Micro Rna and Long Coding Rna:

Gene regulation also depends on the functions of noncoding RNAs.

As the name implies, these are encoded by genes that are transcribed but not translated.

Two important examples are discussed here: small RN molecules called **microRNAs,** and **long noncoding RNA** >200 nucleotides in length.

Micro RNA (miRNA):

The miRNAs do not encode proteins; instead, they function primarily to modulate the translation of target mRNA into their corresponding proteins.

Posttranscriptional silencing of gene expression by miRNA is a fundamental and well-conserved mechanism of gene regulation present in all eukaryotes (plants and animals).

Generation of microRNAs (miRNA) and their mode of action in regulating gene function. miRNA genes are transcribed to produce a primary miRNA (**pri-miRNA**), which is processed within the nucleus to form **pre miRNA** composed of a single RNA strand with secondary hairpin loop structures that form stretches of double-stranded RNA. After this pre-miRNA is exported out of the nucleus via specific transporter proteins, the cytoplasmic **Dicer enzyme** trims the pre-miRNA to generate mature double-stranded miRNAs of 21 to 30 nucleotides. The miRNA subsequently unwinds, and the resulting single strands are incorporated into the multiprotein **RNA-induced silencing complex (RISC)** . Base pairing between the single-stranded miRNA and its target mRNA directs RISC to either cleave the mRNA target or repress its translation. In either case, the target mRNA gene is silenced post transcriptionally.

Small interfering RNAs (siRNAs) are short RNA sequences that can be introduced experimentally into cells. These serves as substrates for Dicer and interact with the RISC complex in a manner analogous to endogenous miRNAs.

Synthetic siRNAs targeted against specific mRNA species have become useful laboratory tools to study gene function (so-called knockdown technology); they are also being developed as possible therapeutic agents to silence pathogenic genes, such as oncogenes involved in neoplastic transformation.

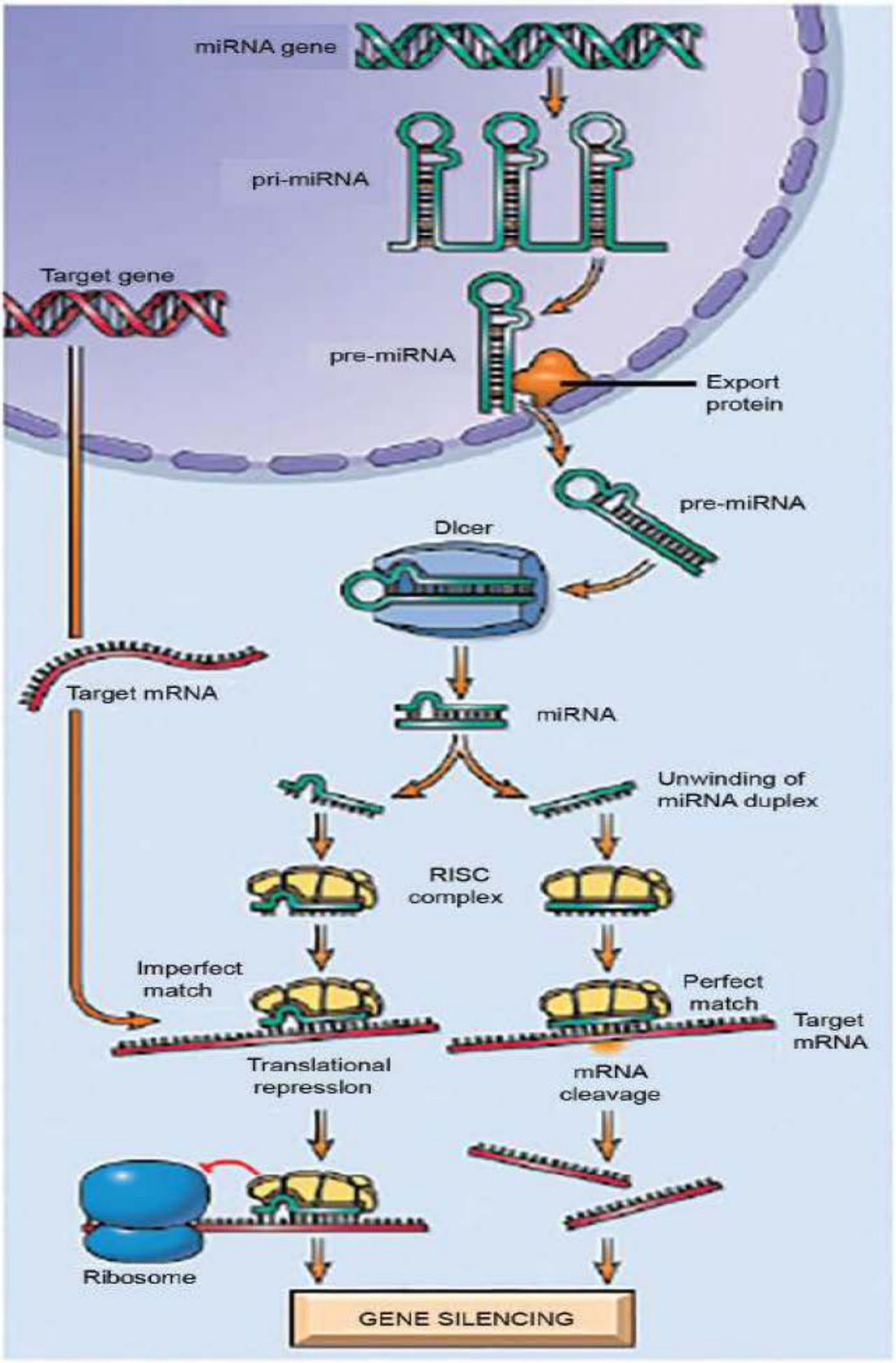

miRNA gene
pri-miRNA
Target gene
pre-miRNA
Export protein
pre-miRNA
Dlcer
Target mRNA
miRNA
Unwinding of miRNA duplex
RISC complex
Imperfect match
Perfect match
Target mRNA
Translational repression
mRNA cleavage
Ribosome
GENE SILENCING

Fig. 1.2

Long Noncoding RNA (lncRNA):

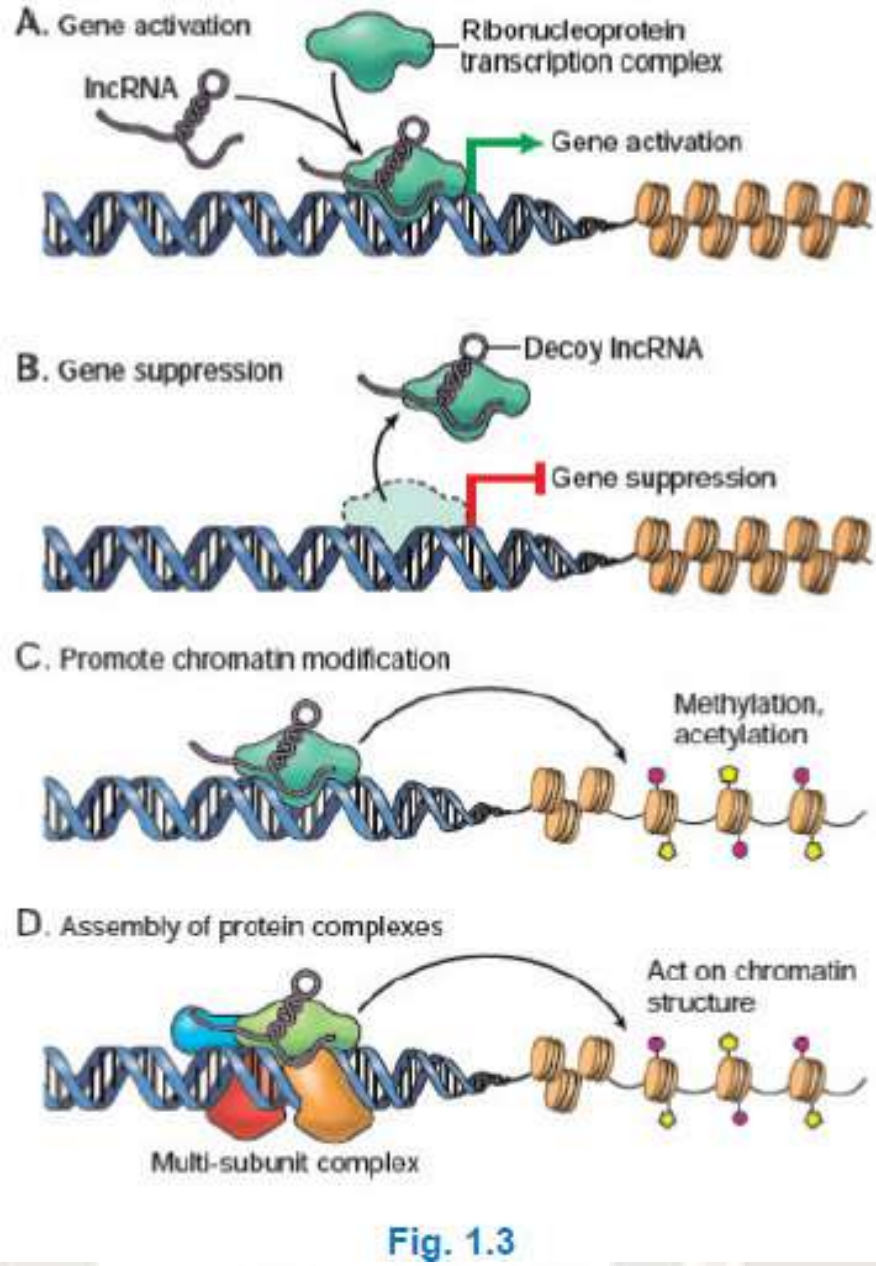


Fig. 1.3

Roles of long noncoding RNAs.

A. Long non-coding RNAs (lncRNAs) can facilitate transcription factor binding and thus promote gene activation.

B. conversely, lncRNAs can preemptively bind transcription factors and thus prevent gene transcription.

C. Histone and DNA modification by acetylases or methylases (or deacetylases and demethylases) may be directed by the binding of lncRNAs.

D. In other instances, lncRNAs may act as scaffolding to stabilize secondary or tertiary structures and/or multi subunit complexes that influence general chromatin architecture or gene activity.

The best known example of a repressive function involves XIST, which is transcribed from the X-chromosome and plays an essential role in physiologic X-chromosome inactivation. XIST itself escapes X inactivation, but forms a repressive "cloak" on the X chromosome from which it is transcribed, resulting in gene silencing. Conversely, it has recently been appreciated that many enhancers are sites of lncRNA synthesis, and these lncRNAs appear to often increase transcription from gene promoters through a variety of mechanisms.

Extra Cellular Matrix & Cell-Matrix Interactions:

Functions:

Sequesters H20 ; turgor

Sequesters minerals; rigidity

Reservoir for GF

Substrate for cells to adhere, migrate, proliferate.

Composition – Three groups:

a. Fibrous structural proteins – collagen & Elastin.
b. Adhesive glycoprotein- fibronectin lamina.
c. Gel of proteoglycans & hyaluronans.

Arrangement- Interstitial matrix / Basal membrane.

B/M: Amorphous; N.fibrillar collagen (Type IV).

Laminin, heparan sulfate proteoglycans

Interstitial matrix:

- Present in space between epithelial, endothelial & smooth muscle cells & in connective tissue.

- Fibrillar & N.F collagen, elastin, fibronectin, proteoglycans, hyaluronate

Collagen; tensile strength.

- Most common protein in animals.
- 3 alpha chains; form14 distinct collagen types.
- Type IV –non-fibrillar, BM.
- Pro collagens secreted by fibroblasts is subjected to peptidases → collagen (Deficiency of Amino peptidase in Ehlers Danlos Type III) → 'Tropocollagen.
- Enzyme Modification; Oxidation of lysine & hydroxylysine (vitamin C required) → Cross Linking – important for tensile strength.

Type 1: Skin (80%); Bones (90%); Tendons.

Type 2: Cartilage (90%), Vitreous Humor.

Type 3: Blood Vessels, Uterus Skin.

Type 4: Basement membrane.

Elastin, Fibrillin & Elastic Fibers

Elastic Fibers : Ability to recoil

Central core with peripheral microfibrillar network.

Central core Elastin (in large Blood Vessels; Aorta, Uterus, Skin Ligaments).

- Glycine, Alanine, proline amino acid rich
- Little hydroxyproline. No hydroxylysine Peripheral microfibrillar Fibrillin (glycoprotein).
- Associated with itself or ECM.
- Scaffolding for deposition of elastin & assembly of elastic fibers (Defect → Marfan’s syndrome).

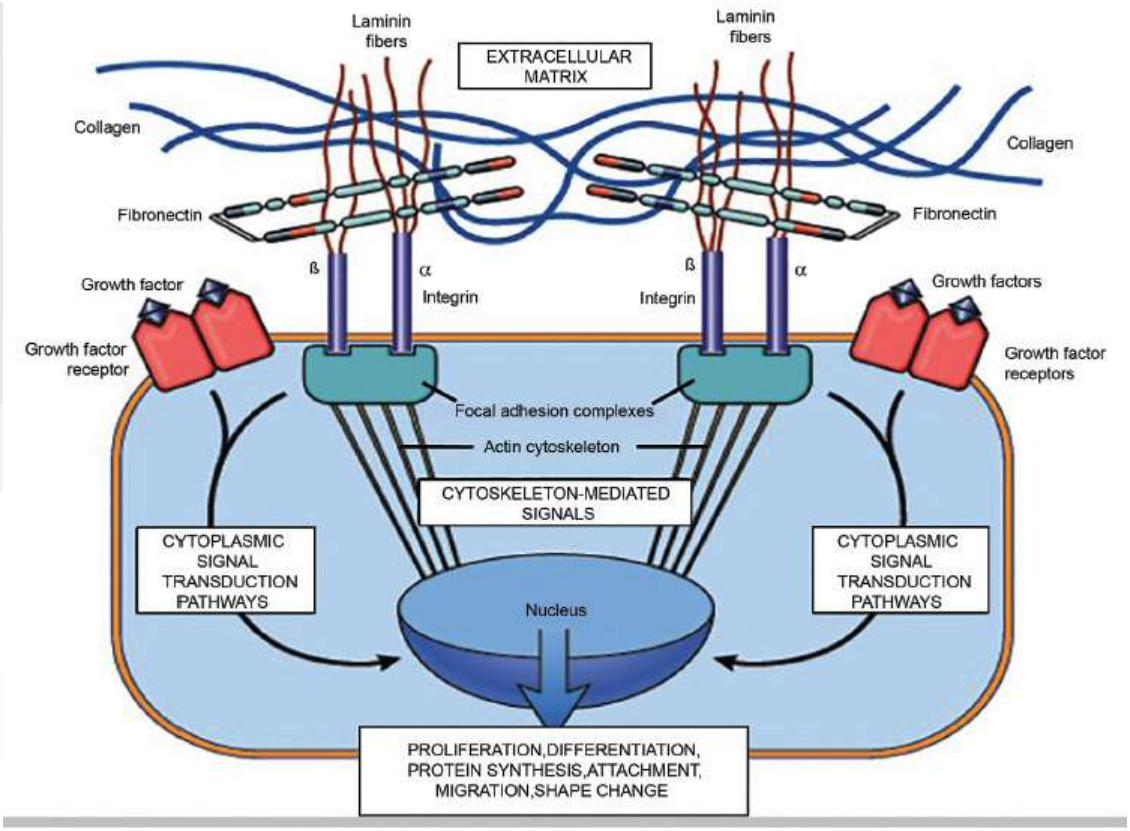


Fig. 1.4

Adhesive Glycoproteins & Integrins:

Property to bind ECM component with cell membrane proteins; link ECM components to one another and to cell surface.

Fibronectin; Multifunctional adhesive protein:

- Attaches cells to matrices.
- Produced by – fibroblasts monocytes endothelial cells other cells.
- Binds to ECM & cells via receptors which recognize RGD sequence (Arginine-glycine-Aspartic acid).
- Involved in attachment, spreading, migration of cells.
- Increased sensitivity of endothelial cell to proliferative effect of growth factor.
- Laminin.
- Most abundant Glycoprotein of basement membrane.
- Heterotrimeric, cross shaped structure.

- Span basal lamina; binds to cell surface & matrix comp (like Collagen Type IV , heparin sulfate).

Cell-cell adhesion molecules – Immunoglobulins, integrins cadherins selectins.

Integrins:

- Transmembrane glycoproteins, a (14) & β(8)Chains-20 type of integrins.
- Mediate cell attachment to ECM.
- E/C domain of integrins bind to many comp. in ECM (by recognizing RGD sequence).
- Important in organizing actin cytoskeleton of cell and in transduction of signals from.

ECM to cell interior:

Bind to ECM → clustering → focal adhesion (where integrins link to intra cellular cytoskeleton components).

Proteins in focal adhesions:-

- Talin, vinculin, a-actinin, tensin, paxillin.
- 'TENSEGRITY HYPOTHESIS'-Mechanical force → Biochemical signals.

Matricellular Proteins (other adhesion molecules):

- Non structural proteins.
- Interact with matrix protein cell surface receptor & other molecules.
- Can disrupt cell- matrix interactions.

1. SPARC (secreted protein acidic and rich in cysteine) (Osteonectin).

► Tissue remodeling.

► Angiogenesis inhibitor.

2. Thrombospondin: Angiogensesis inhibitor.
3. Osteopontin : regulates calcification.

- Mediator of leukocyte migration.

4. Tenacin: involved in morphogenesis & cell adhesion.

Proteoglycans & Hylauronans:

Protocoglycans: Core protein linked to polysaccharides called glycosaminoglycan

- Named according to principal repeating disacch e.g. Chondroitin sulfate, heparin sulfate. Dermatan sulfate.
- Can also be integral membrane? Proteins-Medicate cell growth & differentiation e.g. Syndecan family: spans plasma membranes, binds collagen, fibronectin, thrombospondin, FGF to heparin sulfate, and modulates GF activity.
- Associated with actin cyto skeleton which maintain morphology of epithelial sheets

Hyalunorans: in ECM.

Repeats of disaccharides stretched end to end.

Serve as ligand for core proteins. E.g. Cartilage link protein.

Associated with cell surface receptors.

Binds H2O: viscous gel.

Facilitates cell migration.

Cell Interactions:

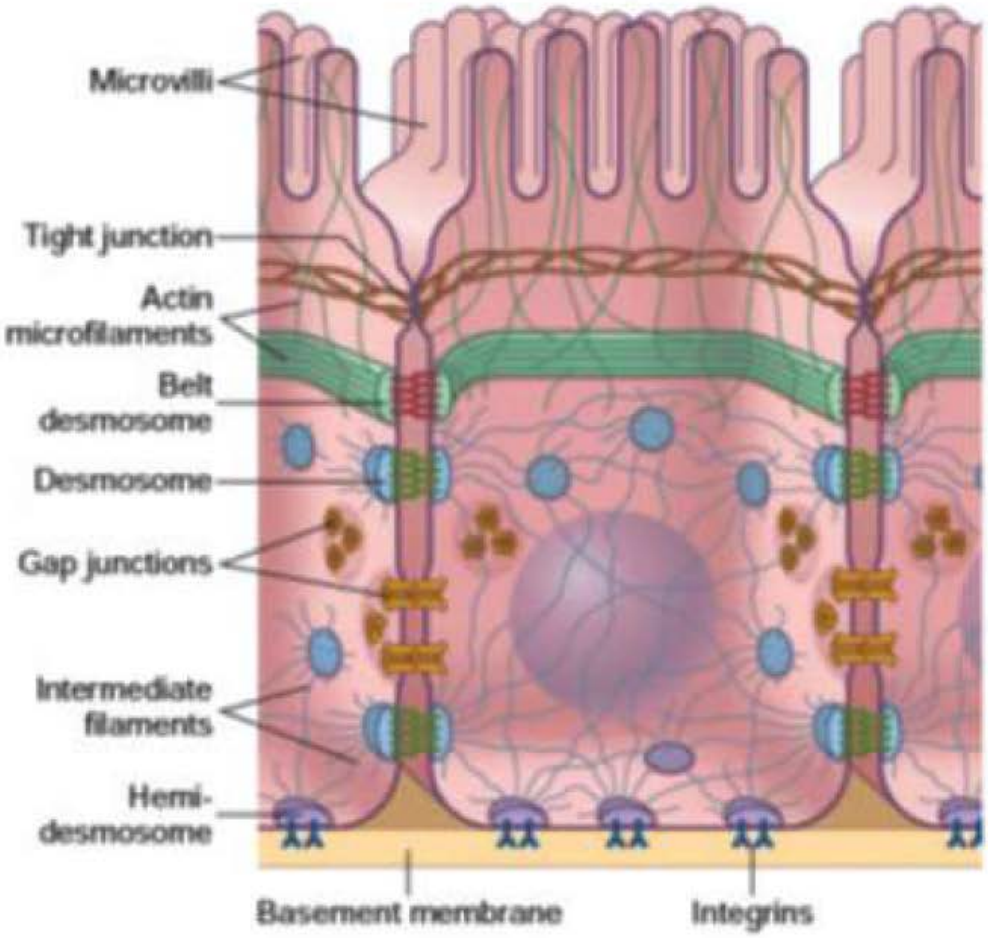


Fig. 1.5

Occluding junctions (tight junctions)	Seal adjacent cells together to create a continuous barrier that restricts the paracellular (between cells) movement of ions and other molecules. Viewed en face, occluding junction's forma tight mesh like network of macromolecular contacts between neighboring cells. The complexes that mediate the cell-cell interactions are composed of multiple trans membrane proteins, including occludin, claudin,zonulin, and catenin.
Anchoring junctions (desmosomes)	Mechanically attach cells—and their intracellular cytoskeletons—to other cells or to the extracellular matrix (ECM). When the adhesion focus is between cells, and is small and rivet-like, it is designated a spot desmosome or macula adherens. When such a focus attaches the cell to the ECM, it is called a hemi desmosome. Similar adhesion domains can also occur as broad bands between cells, where they are denoted as belt desmosomes.
Communicating junctions (gap junctions)	Mediate the passage of chemical or electrical signals from one cell to another. The junction consists of a dense planar array of1.5- to 2-nm pores (called connexons) formed by hexamersof trans membrane proteins called connexins. These pores permit the passage of ions, nucleotides, sugars, amino acids, vitamins, and other small molecules; the permeability of the junction is rapidly reduced by lowered intracellular pH or increased intracellular calcium.

Cell-cell desmosomal junctions are formed by homotypic association of Trans membrane glycoproteins called **cadherins.** In spot desmosomes, the cadherins are called **desmogleins** and **desmocollins** ; they are linked to intracellular intermediate filaments and allow extracellular forces to be mechanically communicated (and dissipated) over multiple cells. In belt desmosomes, the Trans membrane adhesion molecules are called **E-cadherins** and are associated with intracellular actin microfilaments, by which they can influence cell shape and/or motility. In hemi desmosomes, the Trans membrane connector proteins are called **integrins** ; like cadherins, these attach to intracellular intermediate filaments, and thus functionally link the cytoskeleton to the extracellular matrix. **Focal adhesion complexes** are large (>100 proteins) macromolecular complexes that can be localized at hemi desmosomes, and include proteins that can generate intracellular signals when cells are subjected to increased shear stress, such as endothelium in the bloodstream, or cardiac myocytes in failing heart.

Waste Disposal – Lysosomes and Proteosomes:

Extracellular Matrix:

ECM occurs in two basic forms: interstitial matrix and basement membrane.

- **Interstitial matrix** is present in the spaces between cells in connective tissue, and between parenchymal epithelium and the underlying supportive vascular and smooth muscle structures. Interstitial matrix is synthesized by mesenchyme cells (e.g., fibroblasts), forming a three-dimensional, relatively amorphous gel. Its major constituents are fibrillar and nonfibrillar collagens, as well as fibronectin, elastin, proteoglycans, hyaluronate, and other constituents (see later).
- **Basement membrane** . The seemingly random array of interstitial matrix in connective tissues becomes highly organized around epithelial cells, endothelial cells, and smooth muscle cells, forming the specialized basement membrane. The basement membrane is synthesized by contributions from the

overlying epithelium and underlyingmesenchymal cells, forming a flat lamellar “chicken wire” mesh (although labeled as a **membrane** , it is quite porous). The major constituents are amorphous non fibrillar type IV collagen and lamina.

Components of the Extracellular Matrix: The components of ECM fall into three groups of proteins.

- **Fibrous structural proteins** such as collagens and elastin that confer tensile strength and recoil.
- **Water-hydrated gels** such as proteoglycans and hyaluronan that permit compressive resistance and lubrication.
- **Adhesive glycoproteins** that connect ECM elements to one another and to cells.

Laminin is the most abundant glycoprotein in the basement membrane

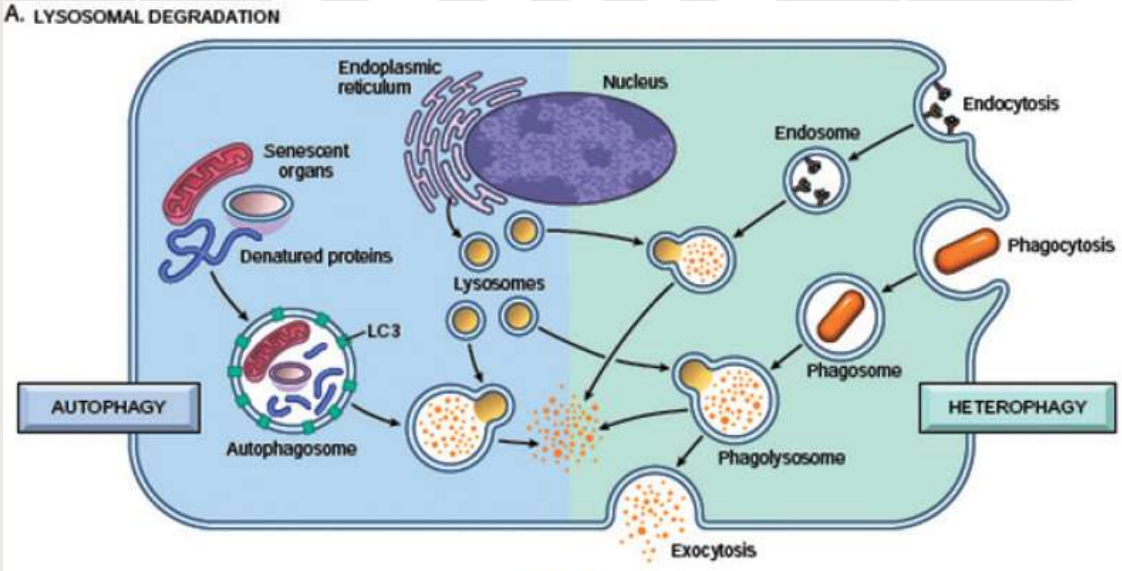


Fig. 1.6

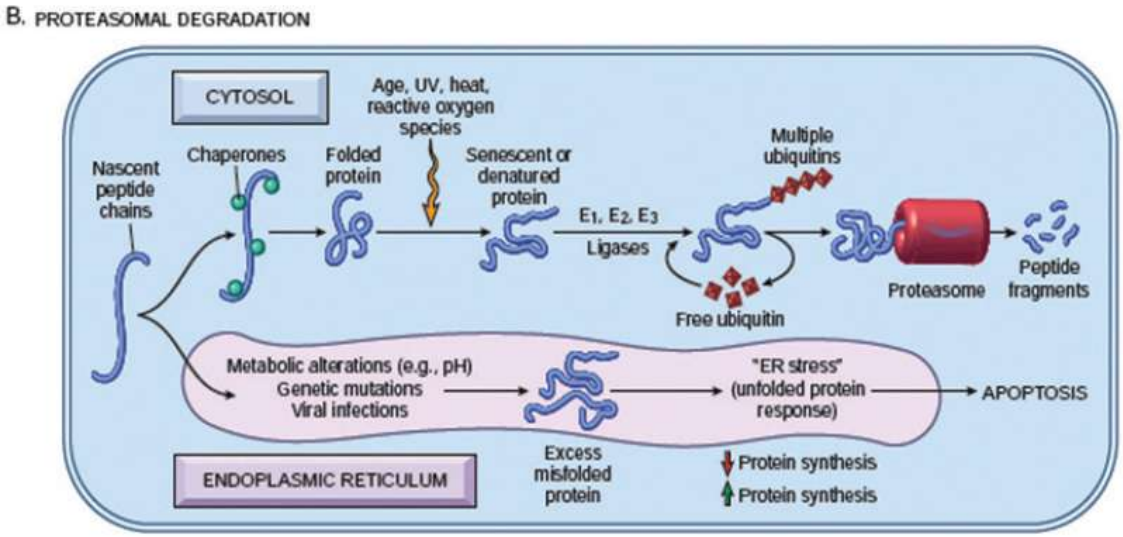


Fig. 1.7

Cell Cycle:

The sequence of events that results in cell division is called the **cell cycle;** it consists of G1 (presynthetic **growth**), S (DNA **synthesis**), G2 (premitotic **growth**), and M (**mitotic**)Phases.

Enforcing the cell cycle checkpoints is the job of **CDK inhibitors (CDKIs);** they accomplish this by modulating CDK-cyclin complex activity. There are several different CDKIs:

- One family—composed of three proteins called **p21** (CDKN1A), **p27** (CDKN1B), and **p57** (CDKN1C)—broadly inhibits multiple CDKs.
- The other family of CDKI proteins has selective effects on cyclin CDK4 and cyclin CDK6; these proteins are called **p15** (CDKN2B), **p16** (CDKN2A), **p18** (CDKN2C), and **p19** (CDKN2D).

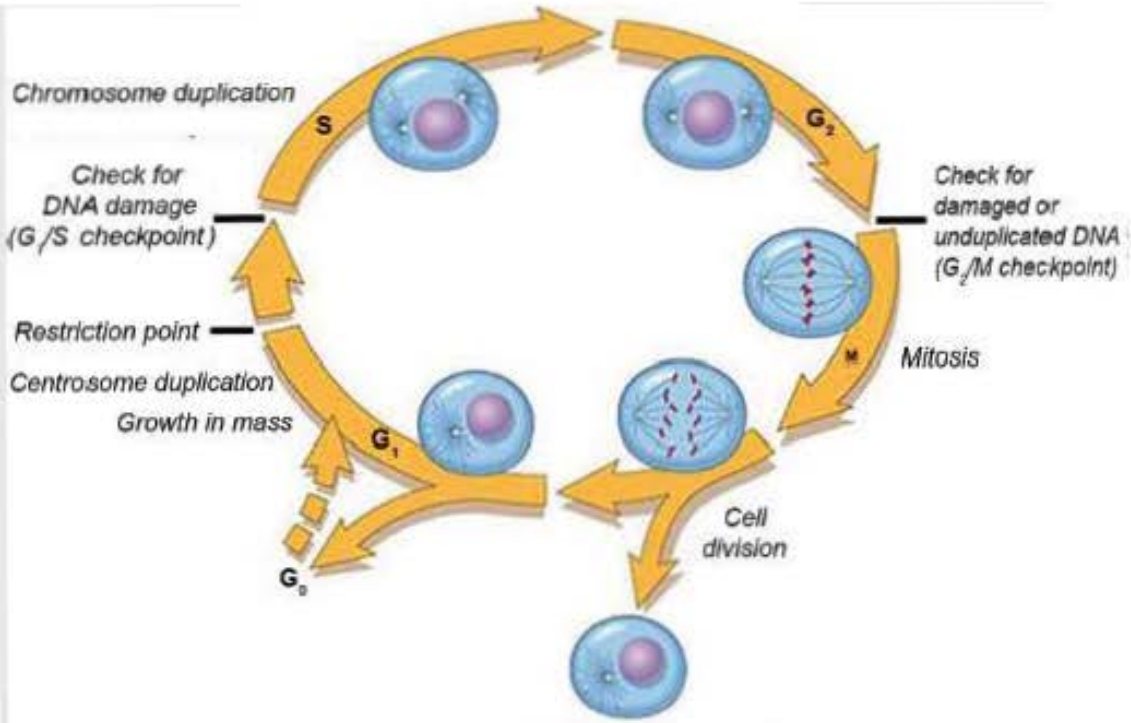

Chromosome duplication
S
G2
Check for
DNA damage
(G1/S checkpoint)
Check for
damaged or
unduplicated DNA
(G2/M checkpoint)
Restriction point
M
Mitosis
Centrosome duplication
Growth in mass
G1
Cell
division
G0

Fig. 1.8

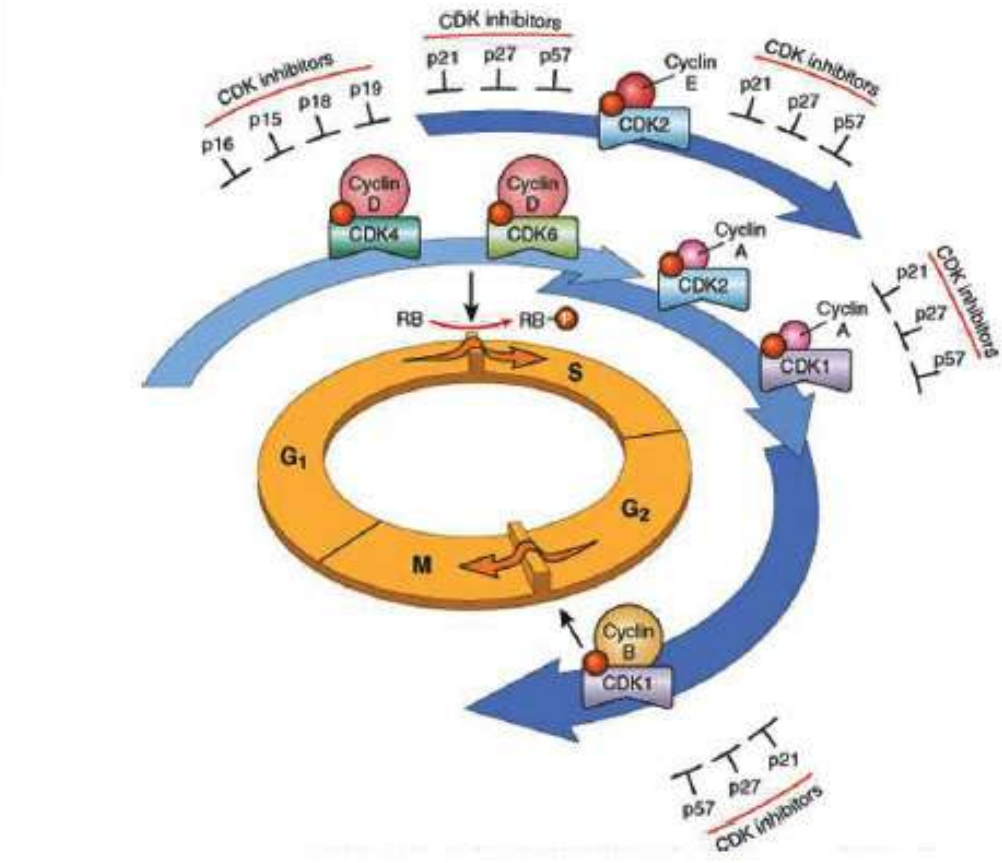


Fig. 1.9

Growth Factors Involved in Inflammation and Repair:

Growth Factor	Sources	Functions
Epidermal. growth factor (EGF)	Activated macrophages, salivary glands, keratinocytes, and many other cells.	Mitogenic for keratinocytes and fibroblasts; stimulates keratinocyte migration; stimulates formation of granulation tissue.
Transforming growth factor-α (FGF-α)	Activated macrophages, keratinocytes, many other cell types.	Stimulates proliferation of hepatocytes and many other epithelial cells.
Hepatocyte growth factor (HGF) (scatter factor)	Fibroblasts, stromal cells In the liver, endothelial cells.	Enhances proliferation of hepatocytes and other epithelial cells; Increases cell motility.
Vascular endothelial growth factor (VEGF}	Mesenchyme cells.	Stimulates proliferation of endothelial cells; Increases vascular permeability.
Platelet-derived growth factor IPOGF)	Platelets, macrophages, endothelial cells, smooth muscle cells, keratinocytes.	Chemotactic for neutrophils, macrophages, fibroblasts, and smooth muscle cells; activates and stimulates proliferation of fibroblasts, endothelial, and other cells; stimulates ECM protein synthesis.
Fibroblast growth factors (FGFs), Including acidic (FGF-1) and basic (FGF-2)	Macrophages, mast cells, endothelial cells, many other cell types.	Chemotactic and mitogenic for fibroblasts; stimulates angiogenesis and ECM protein synthesis.
Transforming growth factor-β (TGF-β)	Platelets, T lymphocytes, macrophages, endothelial cells, keratinocytes, smooth muscle cells, fibroblasts.	Chemotactic for leukocytes and fibroblasts; stimulates ECM protein synthesis; suppresses acute Inflammation.
Keratinocyte growth factor (KGF) (i.e., FGF-7)	fibroblasts.	Stimulates keratinocyte migration, proliferation, and differentiation.
ECM Extracellular membrane.		

Latest Update From Basics of Pathology, 10th Edition:

Gene Editing:

- Exciting new developments that permit exquisitely specific genome editing stand to usher in an era of molecular revolution. These advances come from a wholly unexpected source: the discovery of clustered regularly interspaced short palindromic repeats (CRISPRs) and Cas (or CRISPR-associated genes).
- These are linked genetic elements that endow prokaryotes with a form of acquired immunity to phages and plasmids.
- Bacteria use this system to sample the DNA of infecting agents, incorporating it into the host genome as CRISPRs.
- CRISPRs are transcribed and processed into an RNA sequence that binds and directs the nuclease Cas9 to a sequences (e.g., a

phage), leading to its cleavage and the destruction of the phage.

- Gene editing repurposes this process by using artificial guide RNAs (gRNAs) that bind Cas9 and are complementary to a DNA sequence of interest.
- Once directed to the target sequence by the gRNA, Cas9 induces double-strand DNA breaks.
- Repair of the resulting highly specific cleavage sites can lead to somewhat random disruptive mutations in the targeted sequences (through nonhomologous end joining [NHEJ]), or the precise introduction of new sequences of interest (by homologous recombination).
- Both the gRNAs and the Case enzyme can be delivered to cells with a single easy-to-build plasmid. Which is substantially better than other previous editing systems.Applications include inserting specific mutations into the genomes of cells to model cancers and other diseases, and rapidly generating transgenic animals from edited embryonic stem cells.
- On the flip side, it now is feasible to selectively “correct” mutations that cause hereditable disease, or – perhaps more worrisome – to just eliminate less “desirable” traits.

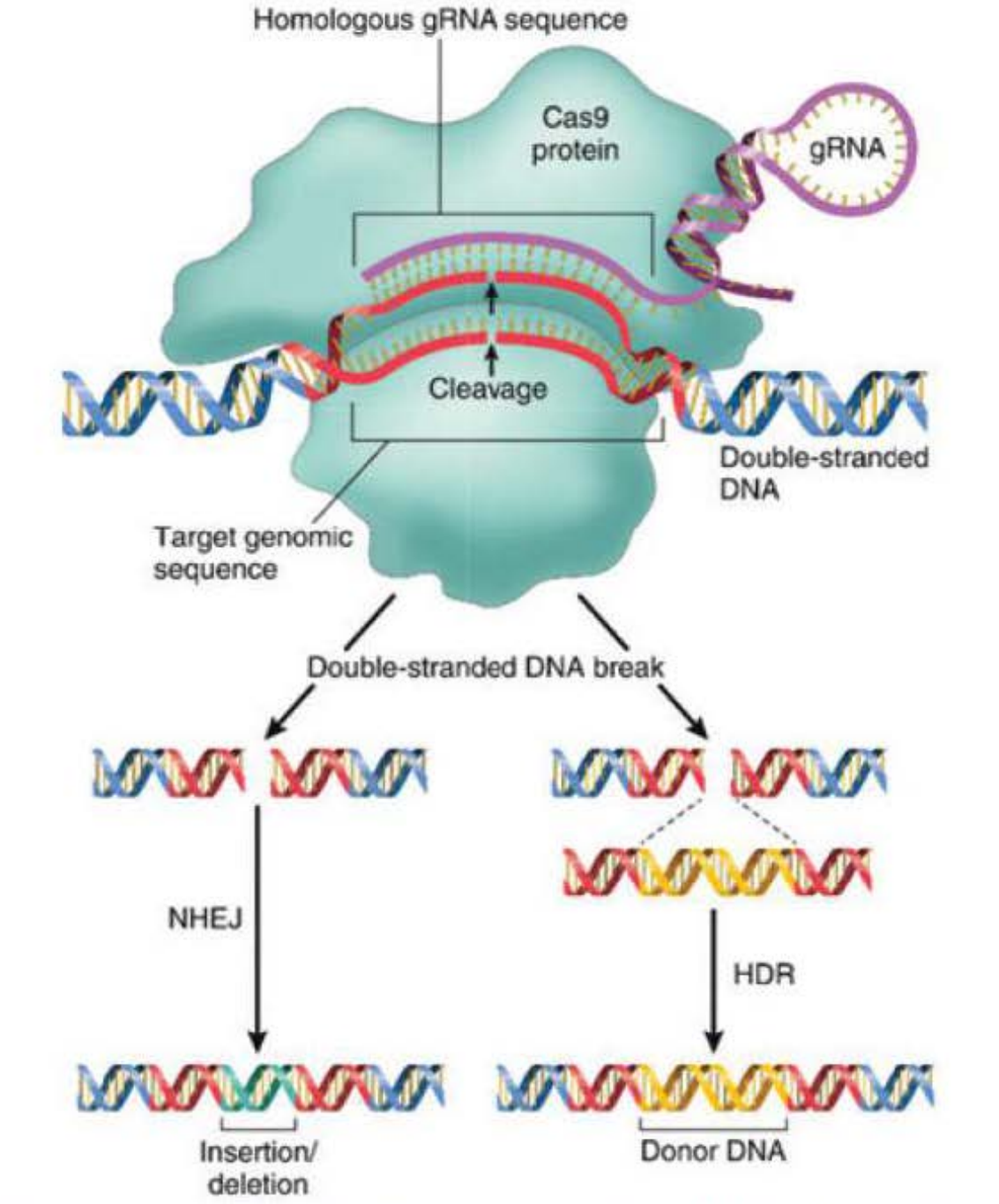
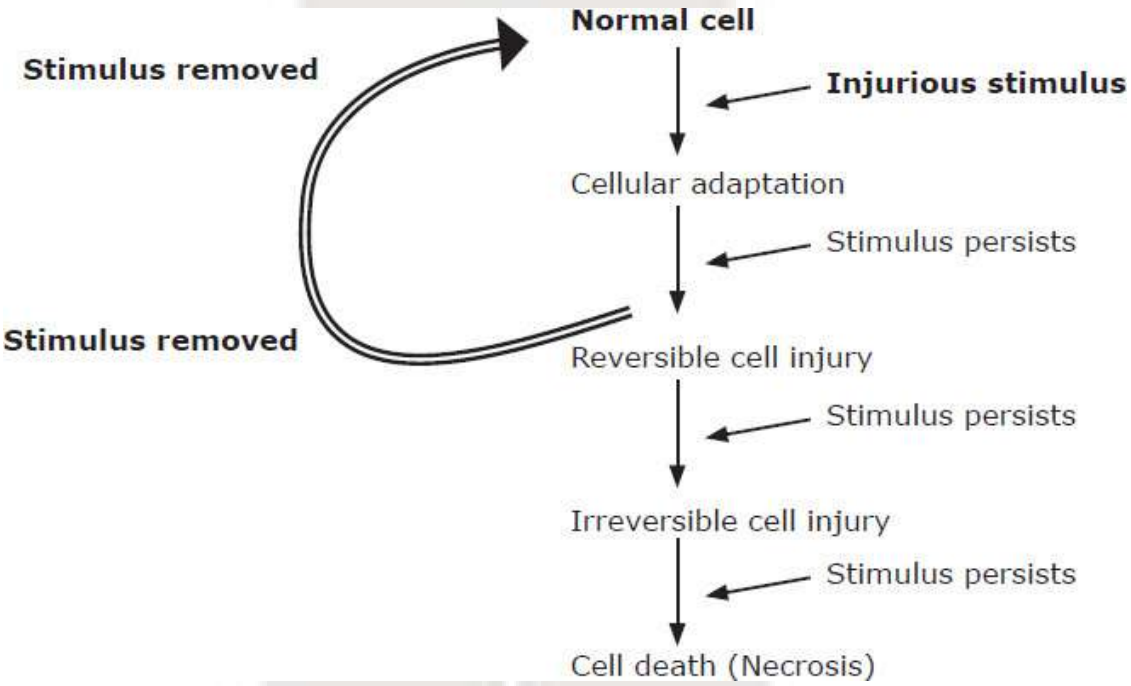

Homologous gRNA sequence
Cas9 protein
gRNA
Cleavage
Double-stranded DNA
Target genomic sequence
Double-stranded DNA break
NHEJ
HDR
Insertion/ deletion
Donor DNA
DNA with random mutation
DNA with specific mutation

Fig. 1.10

2 Cellular Adaptations, Injury and Death

Cellular Changes During Injury:

Cellular response to injury:



Adaptation	Process	Pathogenesis	Extra
Hypertrophy	Increase in size of cells due increase in cellular contents (organelles).	Induced by mechanical sensation, growth factors and vasoactive agents.	Phosphoinositide 3 kinase/ Akt pathway (physiological) and Signaling downstream of G protein coupled receptors (pathological).
Hyperplasia	Increase in number of cells.	Physiological- compensatory/ hormonal, Pathological- excess hormones or growth factors.	No genetic mutations like in Neoplasia; characteristic response to some viral infections like HPV.

Atrophy	Decrease in size	Increased protein degradation (Ubiquitin Proteasome Pathway) and decreased protein synthesis.	Causes DIPMED (mnemonic)*
Metaplasia	Reversible change in which one adult cell type (epithelial or mesenchymal) is replaced by another adult cell type.	Due to reprogramming of stem cells that exist in normal tissue or of undifferentiated mesenchymal cells in connective tissue.	Columnar to Squamous- MOST COMMON- occurs in respiratory epithelium in response to smoking and Vitamin A deficiency. Squamous to Columnar- in Barrett's esophagus in response to acid reflux.

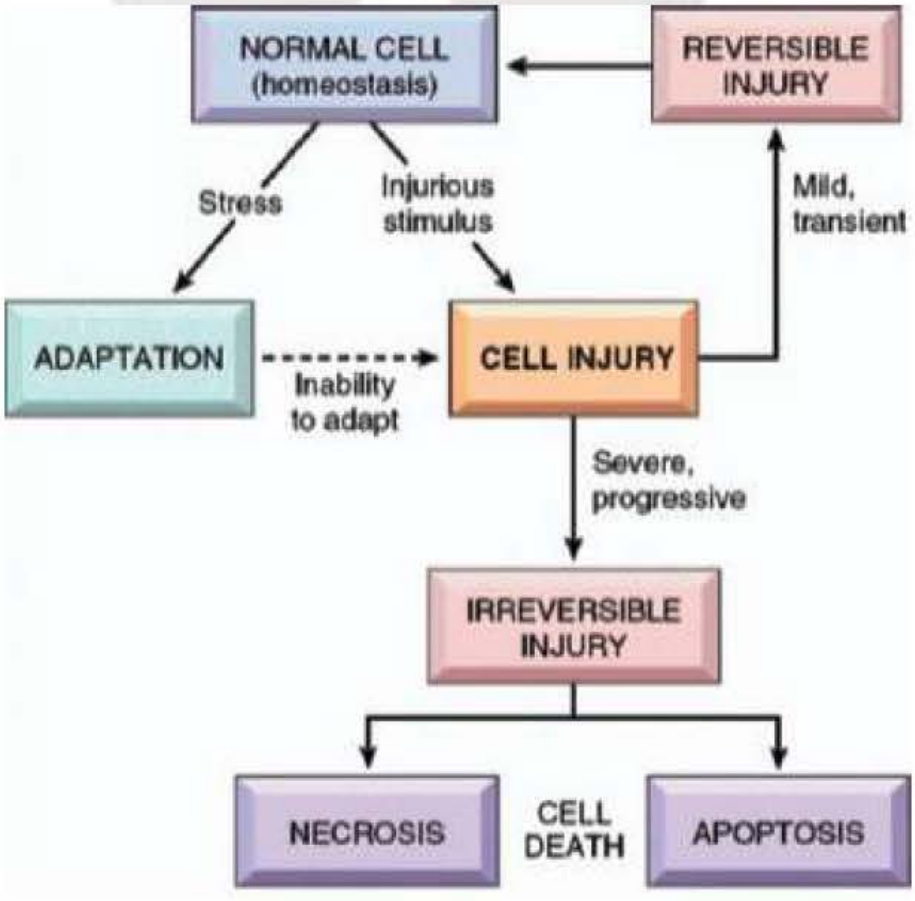


Fig. 2.1

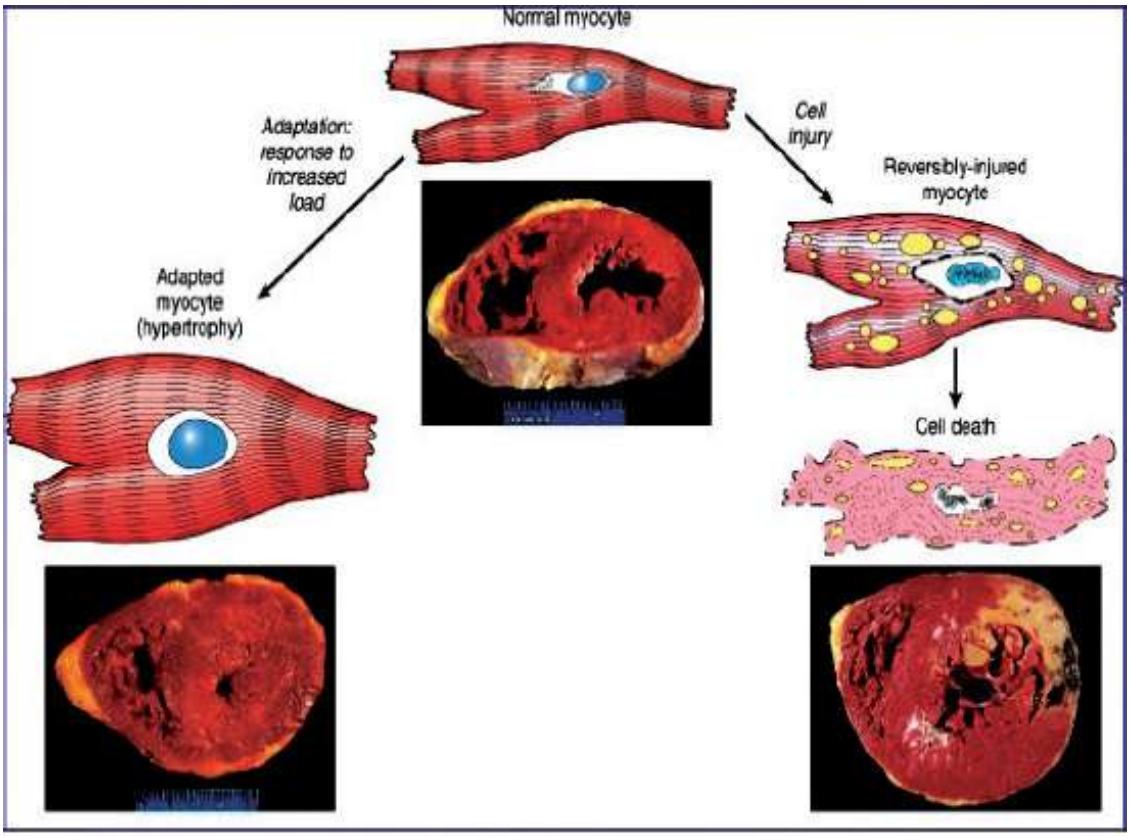


Fig. 2.2

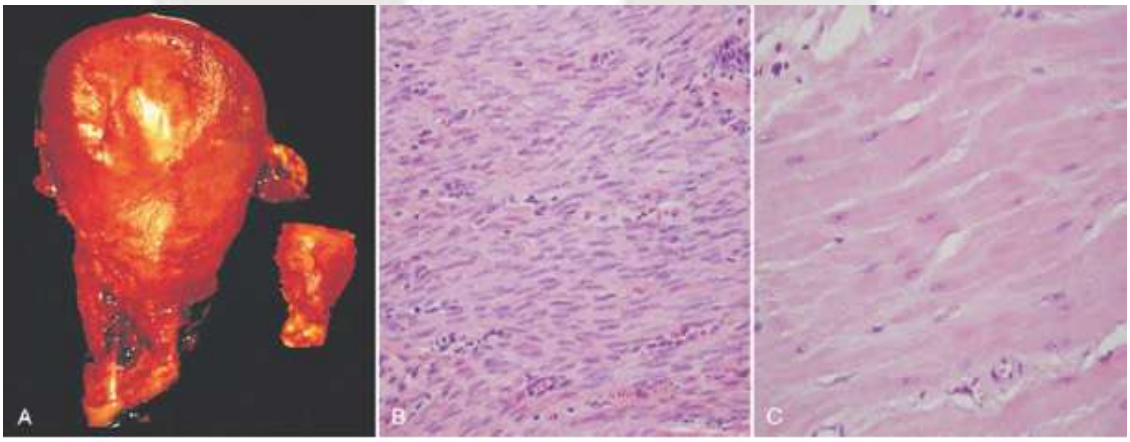


Fig. 2.3

Physiological hypertrophy of uterus during pregnancy:

A. Gross appearance of a normal uterus and a gravid uterus.

B. Small spindle shaped uterine smooth muscle from a normal uterus.

C. Large plump cells from a gravid uterus.

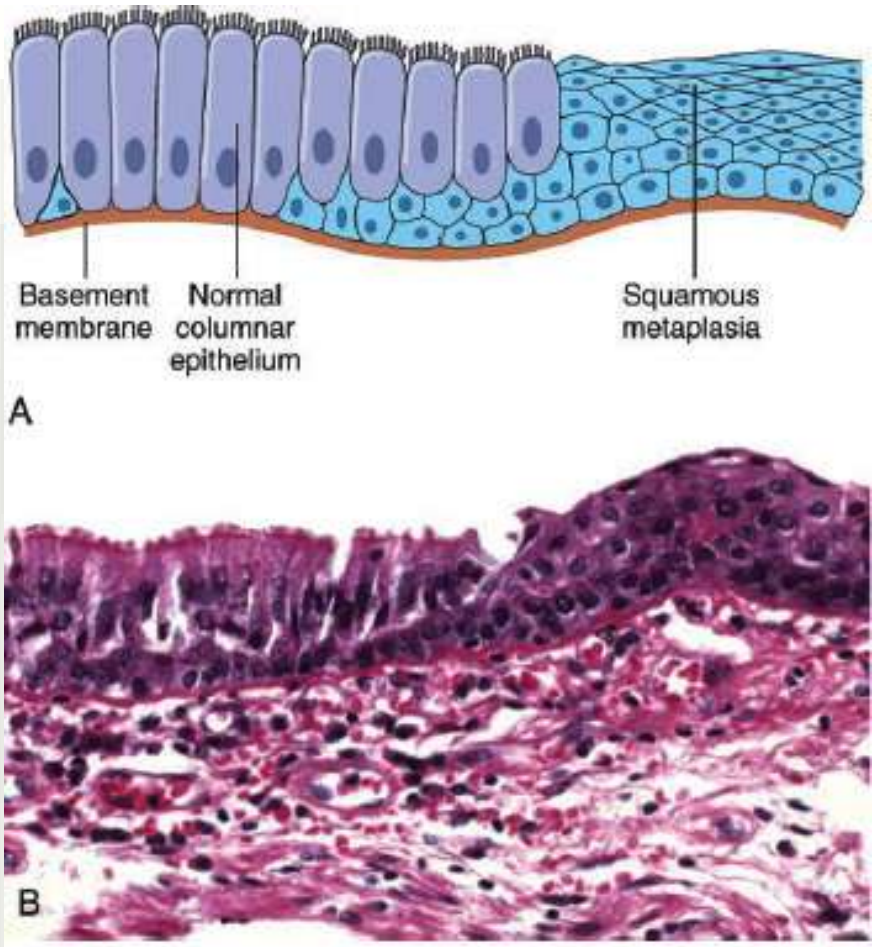


Fig. 2.4

Metaplasia of columnar to squamous epithelium:

A. Schematic diagram.

B. Metaplasia of columnar epithelium (left) to squamous epithelium (right) in a bronchus.

	Uterine Smooth Muscles	Breast
Puberty	hyperplasia	Hyperplasia (dominant)+ hypertrophy
Pregnancy	Hypertrophy (dominant) + hyperplasia	Hyperplasia (dominant)+ hypertrophy
Lactation	Involution	Hypertrophy
Menopause	Atrophy	Atrophy

Cellular Response to Injury Depends on:

a. Type of injury.

b. Duration of injury.
c. Type of cell injury.
d. Cell's metabolic state.
e. Cell's ability to adapt.

Intracellular systems vulnerable to injury:

a. Cell membrane.
b. Production of ATP via Aerobic respiration in mitochondria.
c. Protein synthesis by Endoplasmic reticulum & Ribosomes.
d. DNA in nucleus.

Timescale of Reversible and Irreversible Injury:

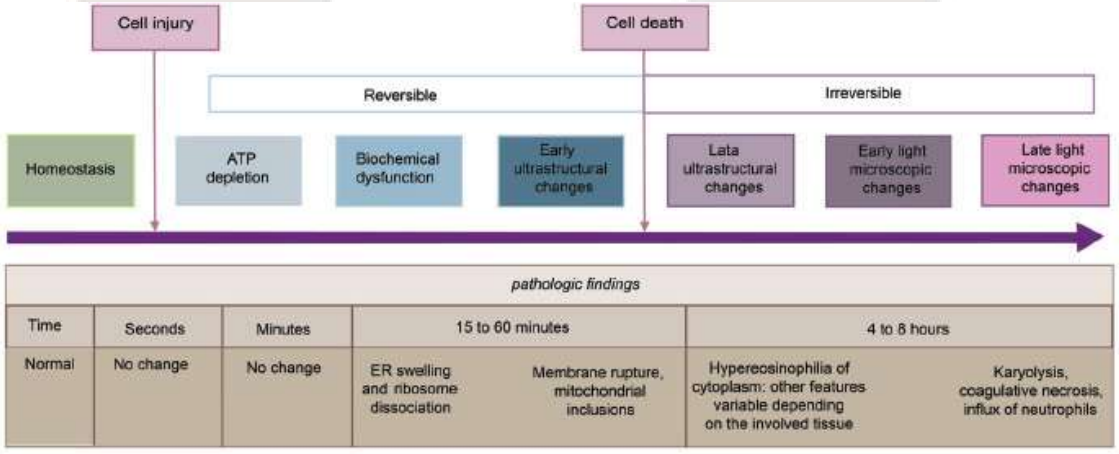


Fig. 2.5

Important mechanisms of cell injury

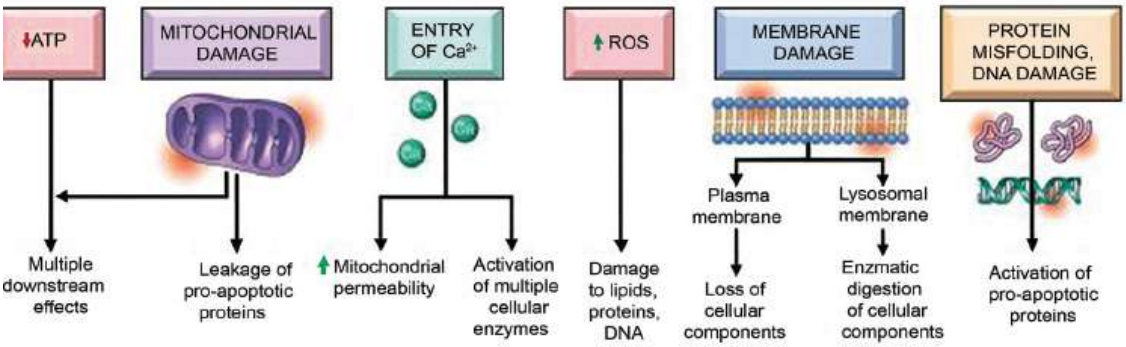


Fig. 2.6

i. Free radical injury.

ii. ATP depletion- seen in hypoxic cell injury.

iii. Influx of calcium: Important mediator of cell injury especially in ischemic and toxic.

- Second messenger.
- Normal free cytosolic Ca- low conc. Intracellular calcium is sequestered in mitochondria and ER.
- Ischemic and toxins - increases cytosolic Ca due to influx of extra cellular Ca and release of Ca from mitochondria and ER.
- Increase cytosolic Ca activates wide spectrum of enzymes.

Proteases – protein break down.

ATP ases – ATP depletion.

Phospholipids – cell membrane injury.

Endonucleases – DNA damage.

iv. Increased cell membrane permeability.

- Seen in most forms of cell injury.
- Biochemical mechanisms that contribute to membrane damage.
- Mitochondrial dysfunction → ↓ phospholipids synthesis.
- ↑cytosolic Ca associated with ATP depletion → activation of phospholipases → depletion of phospholipids from all membranes.
- Reactive oxygen species → lipid per oxidation of membranes.
- Lipid breakdown products that accumulate in injured cell like unesterified FFA, acyl Carnitine and lyso phospholipids have detergent effect on membranes and cause changes in membrane permeability.

v. Mitochondrial dysfunction – Targets for virtually all types of injurious stimuli

- Decrease oxidative phosphorylation
- Formation of mitochondrial permeability transition (MPT) channels.
- Release of cyto chrome c, a trigger for apoptosis.

Mechanism of cell injury: Reversible cell injury.

a. Decreased synthesis of ATP by oxidative phosphorylation.

b. Decreased function of Na+K+ATPase membrane pumps.

I. Influx of Na+ and water

II. Efflux of K+

III. Cellular swelling – earliest morphological feature of cell injury.

iv. Swelling of the endoplasmic reticulum- hydropic change (degeneration) - vacuoles in the cytoplasm.

c. Switch to glycolysis.

I. Depletion of cytoplasmic glycogen.

II. Increased lactic acid production.

III. Decreased intra cellular pH.

d. Decreased protein synthesis.

I. Detachment of Ribosomes form the rough endoplasmic reticulum.

e. Plasma – membrane blebs and myelin figures may be seen.

Mechanism of cell injury; Irreversible cell injury:

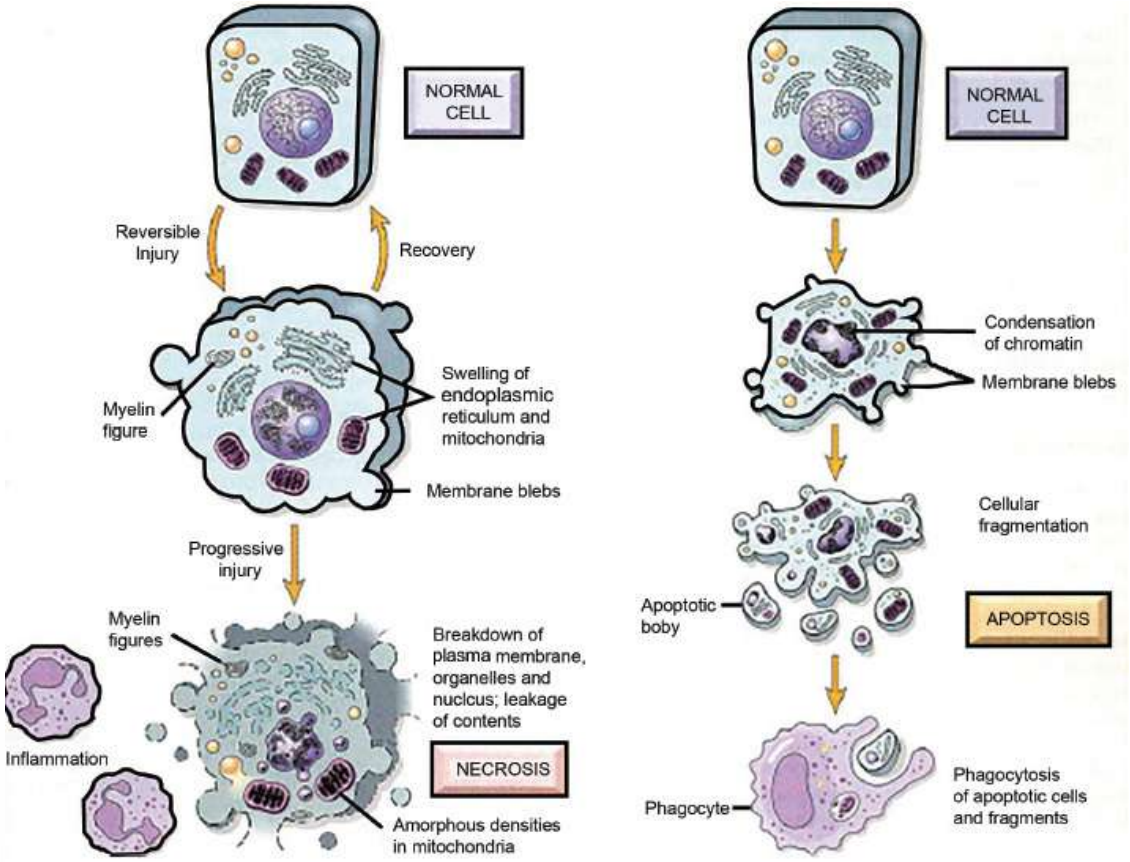


Fig. 2.7

a. Severe membrane damage.

I. Membrane damage plays a critical role in irreversible injury.

II. Massive influx of calcium.

III. Efflux of intracellular enzymes and proteins into the circulation.

b. Marked mitochondrial dysfunction

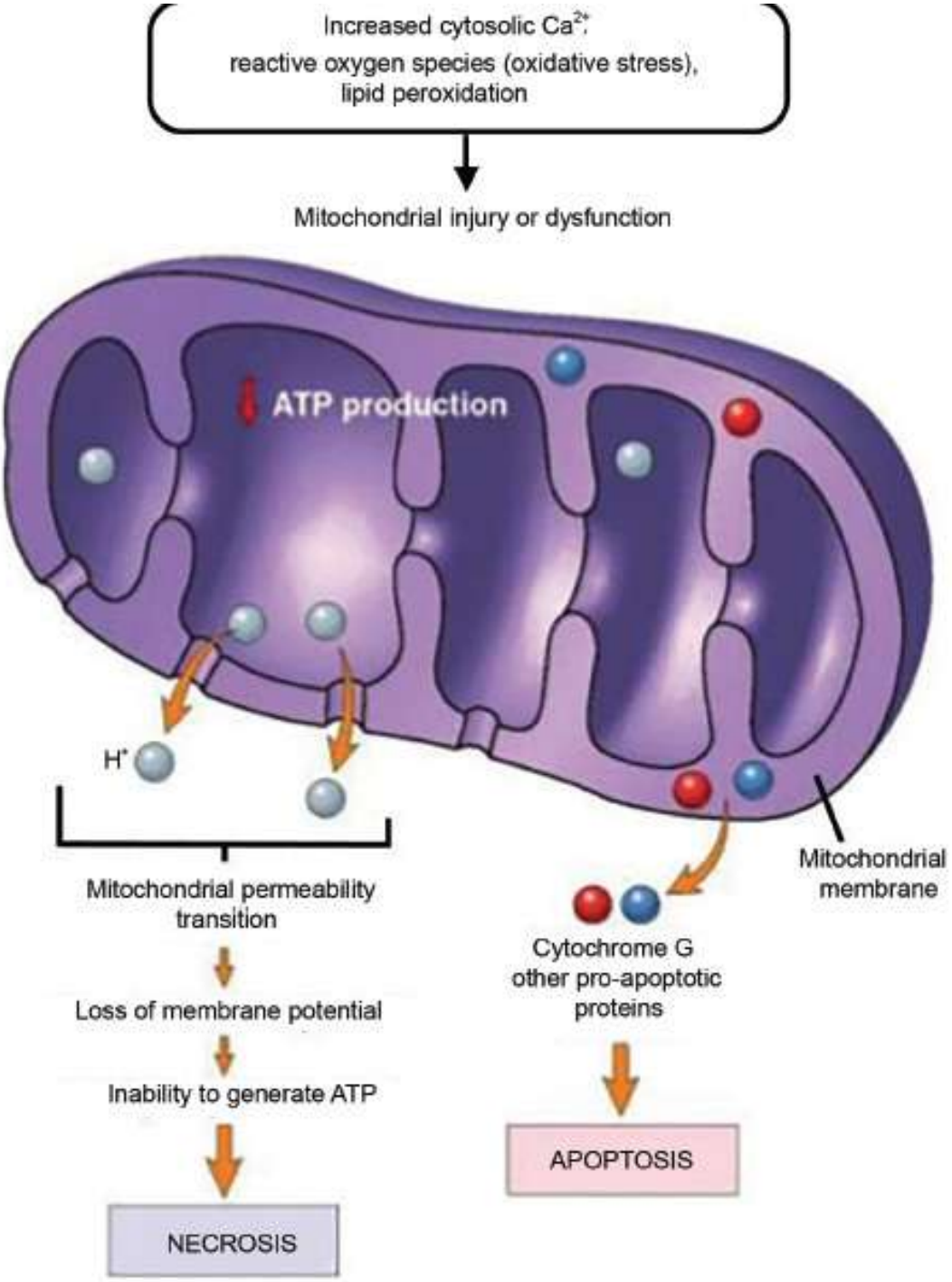


Fig. 2.8

I. Mitochondrial swelling.

II. Large flocculent densities are seen within the mitochondrial matrix (small densities can be seen in reversible injury also).

III. Irreparable damage of the oxidative phosphorylation pathway.

IV. Inability to produce ATP.

c. Rupture of the lysosomes.

I. Release of lysosomal digestive enzymes into the cytosol.

II. Activation of acid hydrolases followed by autolysis.

d. Nuclear changes.

I. Pyknosis: degeneration and condensation of nuclear chromatin.

II. Karyorrhexis: nuclear fragmentation.

III. Karyolysis: dissolution of the nucleus.

Free Radical Injury:

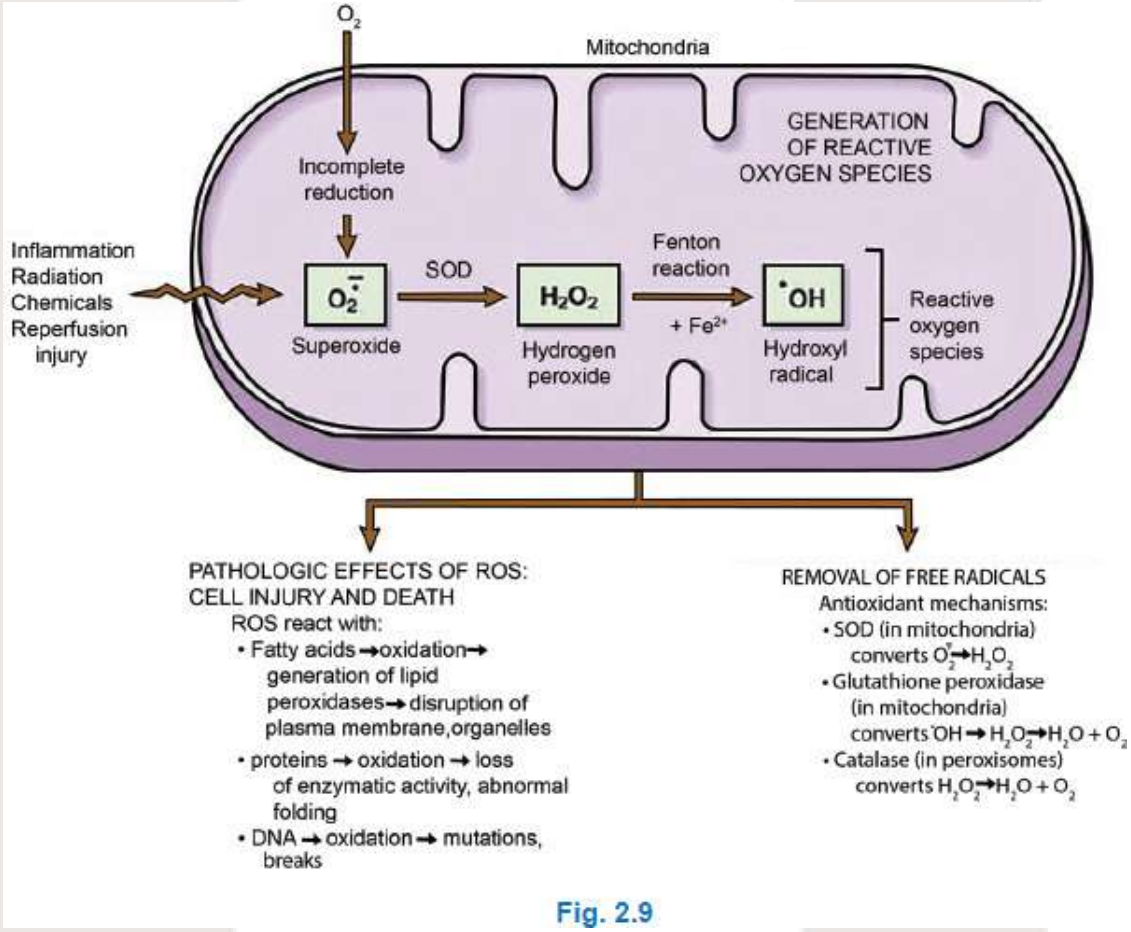


Fig. 2.9

a. Definition: Molecules with unpaired electrons in the outer orbit.

I. Extremely reactive, enter into reactions with lipids, proteins, carbohydrates.

II. Associated with oxygen, carbon, nitrogen.

III. Oxygen associated are: Super oxide, hydroxyl. Hydrogen peroxide

IV. Carbon associated are: CCL_3 .

V. Nitrogen associated are: NO_2 .

Reactive Oxygen Species (ROS)	
Molecule	**Attributes**
Hydrogen peroxide (H_2O_2)	Forms free radicals via Fe^{2+}– catalyzed Fenton reaction Diffuses widely within the cell.
Superoxide anion (O_2–)	Generated by leaks in the electron transport chain and some cytosolic reactions Produces other ROS Does not readily diffuse far from its origin.
Hydroxyl radical (OH•)	Generated from H_2O_2 by Fe^{2+}–catalyzed Fenton reaction The intracellular radical most responsible for attack on macromolecules (Most potent radical (*))
Peroxynitrite (ONOO•)	Formed from the reaction of nitric oxide (NO) with O_2 – Damages macromolecules.
Lipid peroxide radicals (RCOO•)	Organic radicals produced during lipid peroxidation.
Hypochlorous acid (HOCI)	Produced by macrophages and neutrophils during respiratory burst that accompanies phagocytosis Dissociates to yield hypochlorite radical (OCI–).

b. Mechanism of free radical damage:

I. Lipid peroxidation of membranes.

II. Oxidative damage to proteins.

III. DNA breaks.

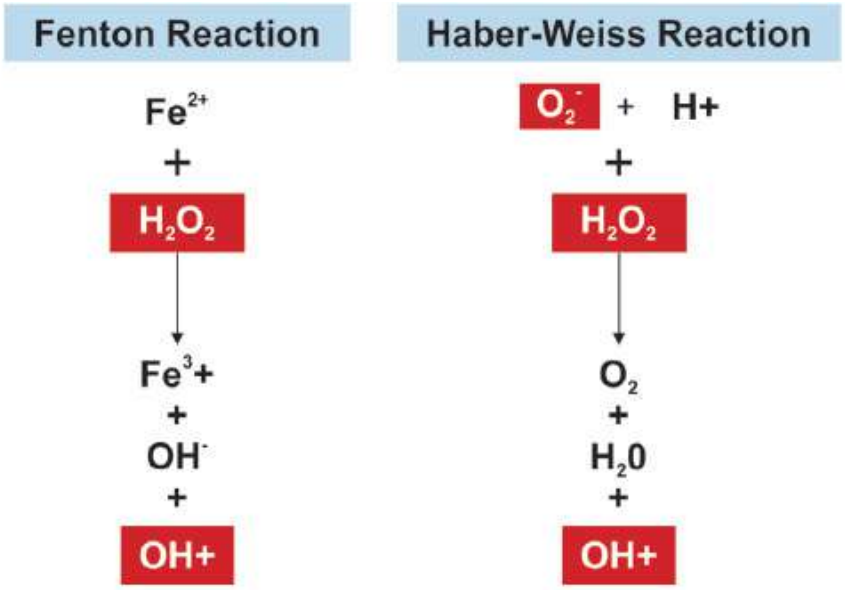


Fig. 2.10: Fenton and Haber-Weiss reactions to generate the highly reactive hydroxyl radical. Reactive species are shown in red. Fe^{2+} = ferrous iron; Fe^{3+} = Ferric iron; H+ = hydrogen ion; H2O2 = hydrogen peroxide; OH- = hydroxide; OH. = hydroxyl radical.

c. Inactivation of free radicals in the body:

I. Antioxidants: Vit A&C. Sulpha containing compounds like cysteine & Glutathione.

II. Serum proteins: Albumin, Ceruloplasmin, Transferrin.

III. Enzymes: superoxide Dismutase, Catalase, Glutathione Peroxidase.

Free radicals can cause cell injury and death by necrosis as well as apoptosis.

Cell Death:

Necrosis:

Morphological types of necrosis:

a. Coagulative Necrosis:

I. Most common form of necrosis.

II. Due to denaturation and coagulation of proteins in the cytoplasm.

III. Micro: Loss of nucleus but cellular outline is preserved.

IV. Common in infarct of solid organs like Hear, Liver, Kidney, limb (dry gangrene).

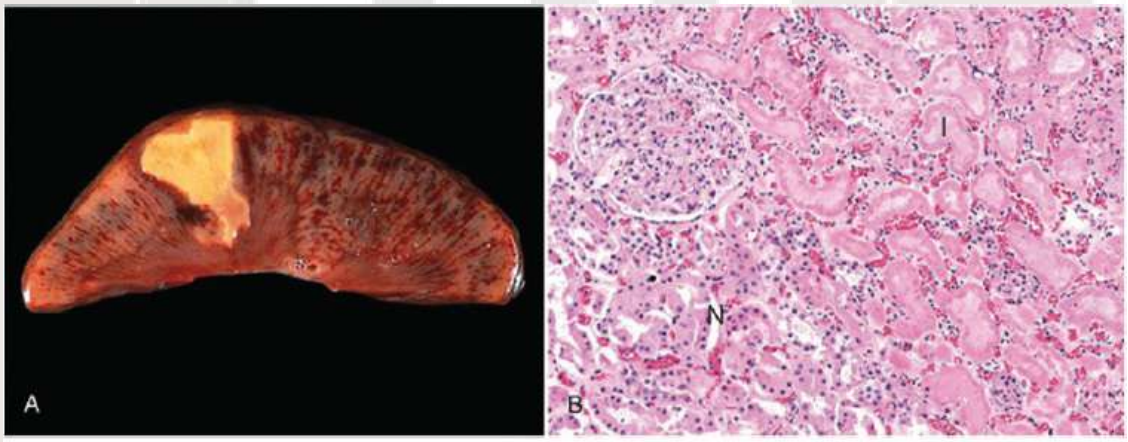


Fig. 2.11

Coagulative necrosis. A, A wedge-shaped kidney infarct (yellow). B, Microscopic view of the edge of the infarct, with normal kidney (N) and necrotic cells in the infarct (I) showing preserved cellular outlines with loss of nuclei and an inflammatory infiltrate (which is difficult to discern at this magnification).

b. Liquefactive necrosis:

I. Cellular destruction by hydrolytic enzymes.

II. Due to autolysis and heterolysis

III. Occurs in abscesses, brain infarct, pancreatic necrosis and wet gangrene.

c. Caseous necrosis:

I. Combination of liquefactive and coagulative necrosis.

II. Gross-friable, soft, and cottage – cheese like appearance.

III. Characteristic of tuberculosis.

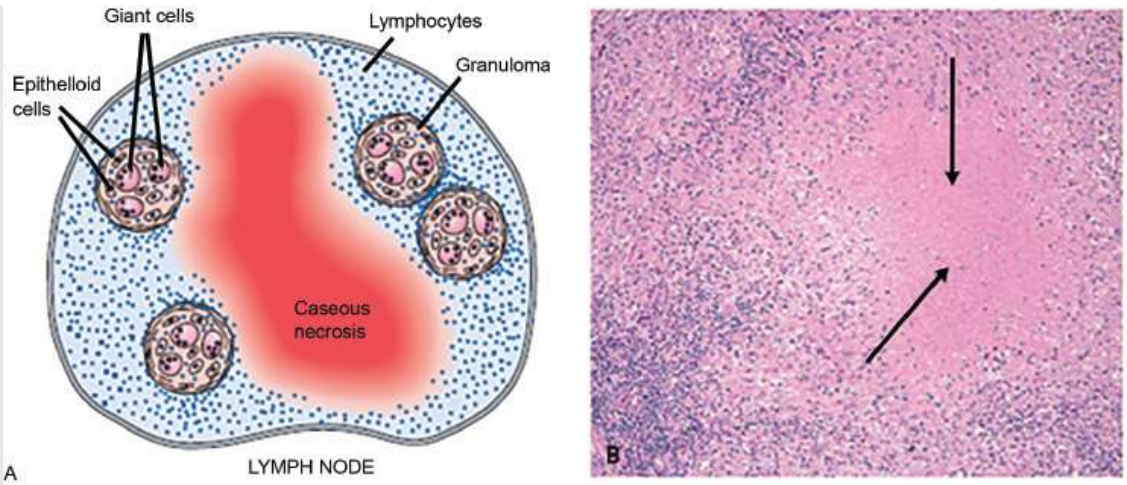


Fig. 2.12: Caseous necrosis in a tuberculous lymph node (A). the typical amorphous, granular, eosinophilic, necrotic center is surrounded by granulomatous inflammation. (B). Photomicrograph showing a tuberculosis granuloma with central caseous necrosis (arrows).

d. Fat necrosis:

I. Caused by action of lipases on fatty tissue.

II. Grossly: Chalky white in appearance.

III. Seen in breast and pancreatitis.

e. Fibrinoid Necrosis

I. Necrotic tissue that histologically resembles fibrin.

II. Micro: Has an eosinophilic (pink) homogenous appearance.

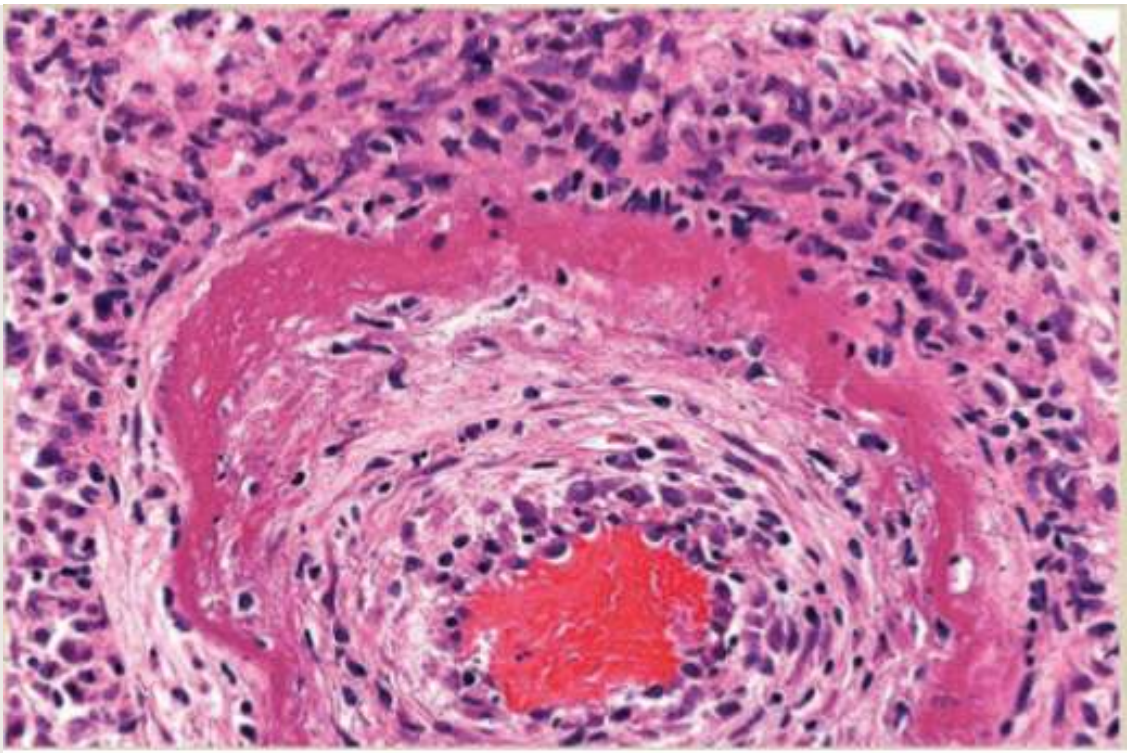


Fig. 2.13

III. Seen in malignant hypertension, type II and III hypersensitivity reactions, vasculitis, hyper acute and acute humoral graft rejections.

Fibrinoid necrosis in an artery. The wall of the artery shows a circumferential bright pink area of necrosis with inflammation (neutrophils with dark nuclei).

f. Gangrenous necrosis:

I. Gross term used to describe dead tissue.

II. Common sites: Lower limbs, Gl tract, testes.

III. Dry gangrene: microscopic pattern is coagulative necrosis.

IV. Wet gangrene: microscopic pattern is liquefactive necrosis.

Necrosis morphology:

1. **Increased eosinophilia**
2. **Myelin figures**
3. **Nuclear changes (karyolysis, pyknosis, karyorrhexis)**

Apoptosis:

a. Specialized form of programmed cell death.

b. Apoptosis is and active process regulated by genes and involves RNA and Protein synthesis.

c. Often affects only single cells or small groups of cells.

Physiological Apoptosis:

- During Embryogenesis- Implantation, Organogenesis, Developmental Involution, and Metamorphosis (*)
- Involution of hormone dependent tissues upon hormone withdrawal (endometrium after menstrual cycle, ovarian follicles after menopause, breast after weaning and prostrate after castration).
- Cell loss in proliferating cell populations like immature lymphocytes in bone marrow and thymus which fail to express useful antigen receptors.
- Elimination of potentially harmful self-reactive lymphocytes.
- Death of host cells which have completed their function (such as neutrophils in acute inflammatory response).

Pathological Apoptosis:

- DNA damage.
- Accumulation of misfolded proteins in ER leads to a condition called ER stress.
- Cell death in certain infections (viral mostly) and transplant rejection and tumours.
- Pathological atrophy.

Morphological Appearance:

- Cell shrinks in size with tight packing of organelles.
- Nuclear chromatin condensation.
- Formation of cytoplasmic membrane blebs.
- Break down of the cell into fragments or apoptotic bodies.

- Phagocytosis of apoptotic bodies by macrophages.
- A lack of inflammatory response.

Stimulus for Apoptosis:

- DNA damage by radiation toxins and free radicals stimulates p53.
- Lack of hormones, cytokines, or growth factors start the intrinsic pathway (mitochondrial pathway) of apoptosis.
- Receptor ligand signals start the extrinsic (death receptor initiated pathway).
- Fas binding to the Fas ligand.
- Tumor necrosis factor binding to TNF Receptor1.

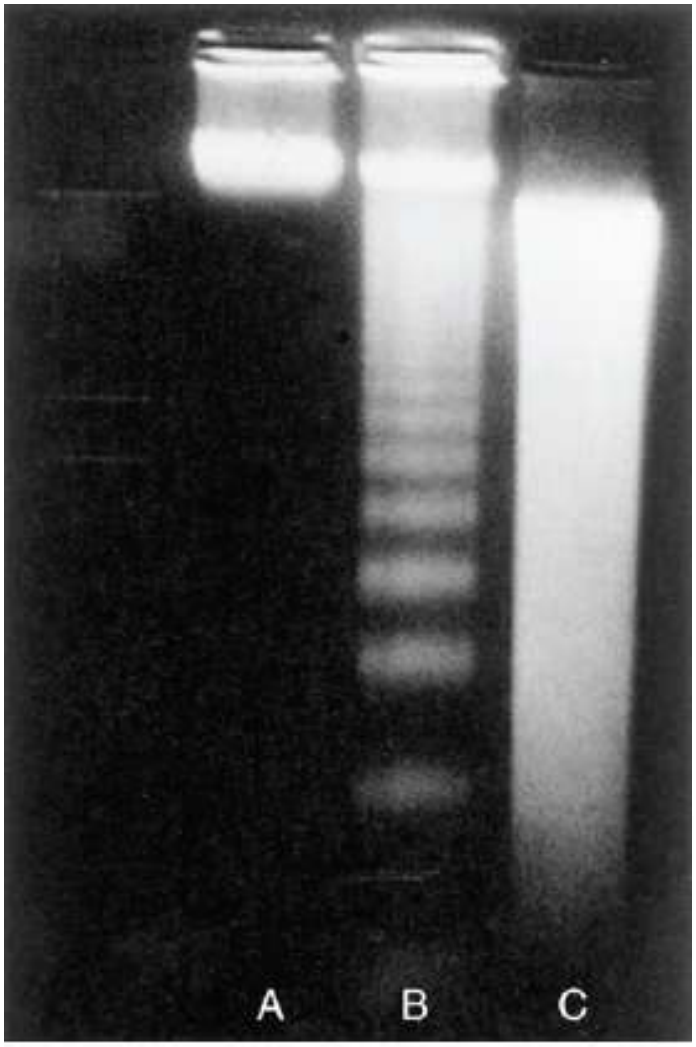


Fig. 2.14

Agarose gel electrophoresis of DNA extracted from culture cells. Ethidium bromide stain; photographed under ultraviolet illumination. **Lane A** , Viable cells in culture. **Lane B** , Culture of cells exposed to heat showing extensive apoptosis; note ladder pattern of DNA fragments, which represent multiples of oligonucleosomes. **Lane C** , Culture showing cell necrosis; note diffuse smearing of DNA.

Apoptosis is regulated by genes:

I. Bcl-2 family regulates apoptosis. Bcl-2 and bcl-X reside in the inner mitochondrial membrane and they inhibit apoptosis. Bak, Bax, and Bim stimulate apoptosis. When cell does not get hormones or growth factors, bcl-2 and bcl-X are replaced by Bax,

bak and Bim. They increase the permeability of mitochondrial membrane which results in leakage of cytochrome c of respiratory chain. This blinds Apaf-1 (Apoptosis activating factor -1). Which in turn activates caspases.

- **Anti-apoptotic proteins are Bcl-2, Bcl-x, and Mcl-1.**
- **Sensors are also members of the Bcl family, and they include proteins called Bim, Bid, and Bad and are also called as "BH3-only proteins." They activate two critical (pro-apoptotic) effectors, Bax and Bak, which form oligomers that insert into the mitochondrial membrane and create channels that allow proteins from the inner mitochondrial membrane to leak out into the cytoplasm.**
- **Extrinsic and intrinsic pathways for initiating apoptosis are distinct because they involve fundamentally different molecules for their initiation, but there may be interconnections between them. For instance, in hepatocytes and several other cell types, Fas signaling activates a BH3-only protein called Bid, which then activates the mitochondrial pathway.**

Representative Bcl- 2 Family Members and Their Roles		
Proapoptotic		**Antiapoptotic**
Bind Antiapoptotic Proteins	**Displace Bax and Bak**	
Bax	Bad	BcI-2
Bak	Bid	Bcl-X_L
	Bik	Bcl-X
	Nox	A1
	Puma	Ku70
	Noxa	Mcl-1

II. **P-53** (stimulates apoptosis): Elevated by DNA injury and arrests the cell in G_1 phase of cell cycle. If DNA repair is impossible. **p-53** stimulates apoptosis.

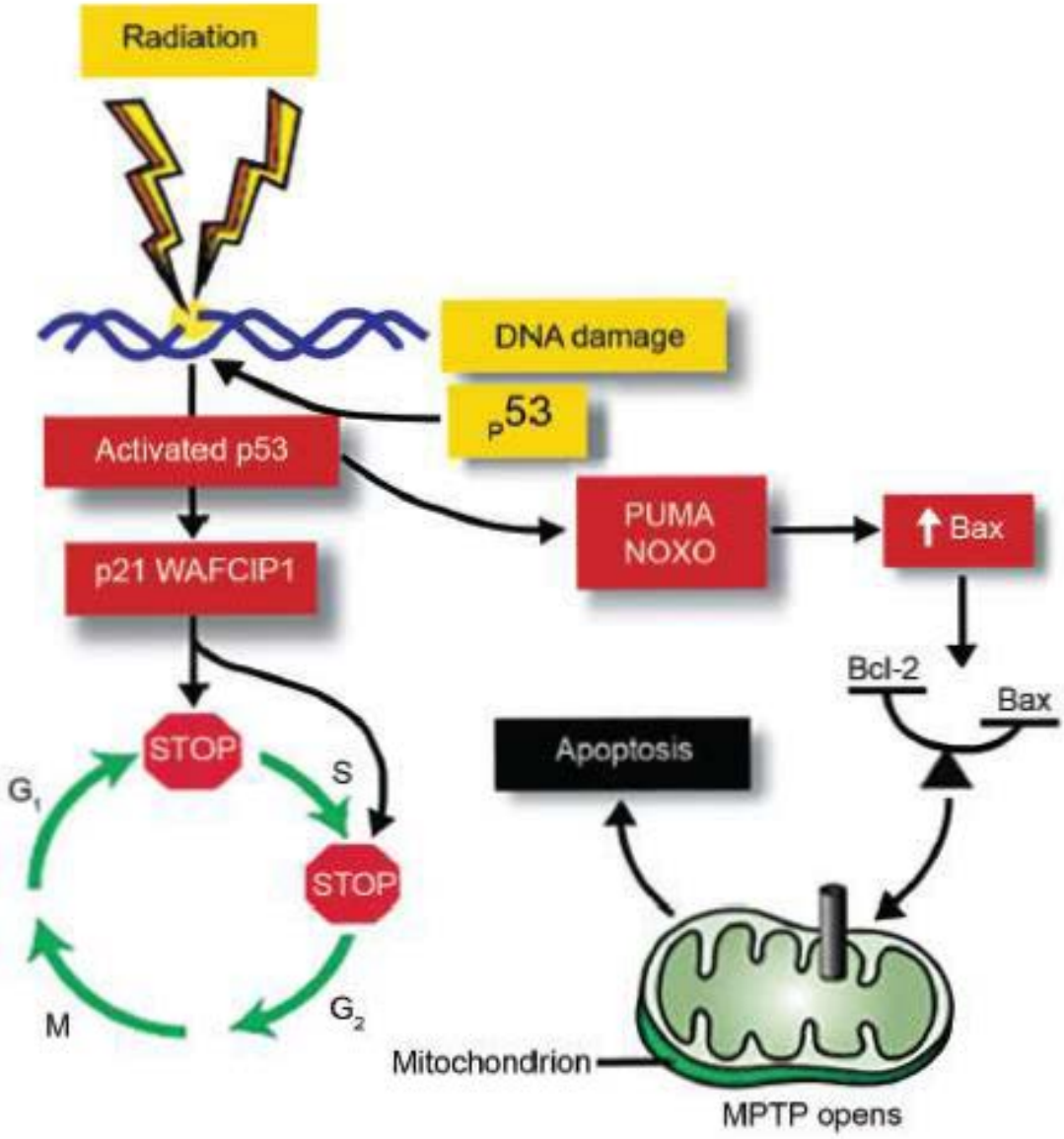

Radiation
DNA damage
pp53
Activated p53
p21 WAFCIP1
PUMA
NOXO
Bax
Bcl-2
Bax
STOP
STOP
G1
S
G2
M
Apoptosis
Mitochondrion
MPTP opens

Fig. 2.15

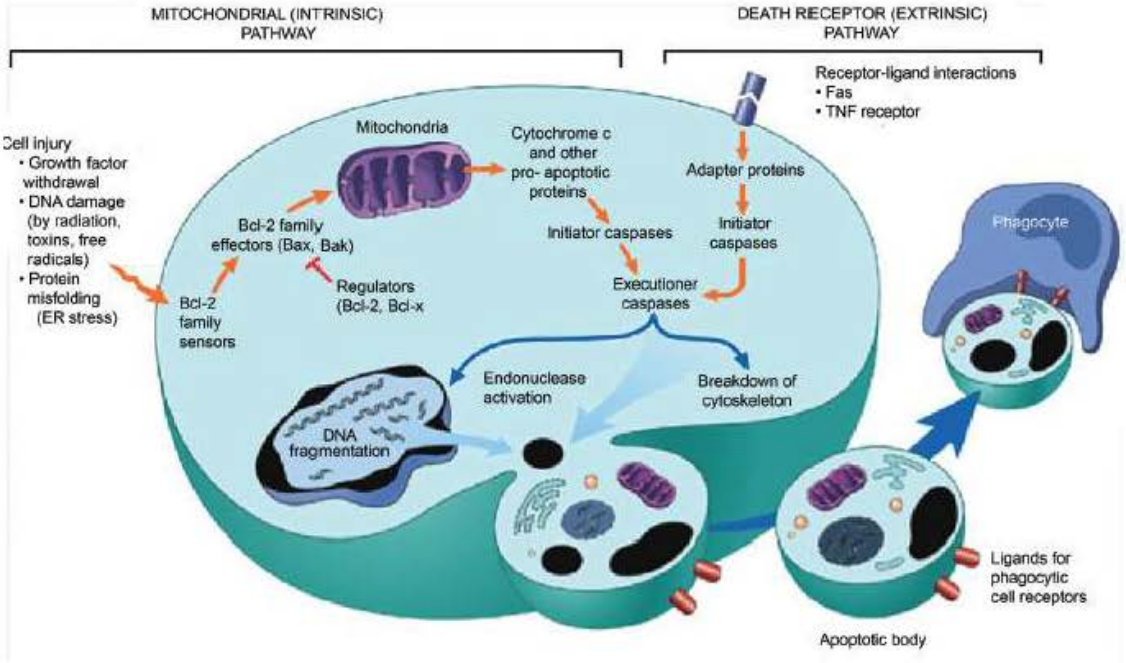

MITOCHONDRIAL (INTRINSIC) PATHWAY
DEATH RECEPTOR (EXTRINSIC) PATHWAY
Receptor-ligand interactions
• Fas
• TNF receptor
Cell injury
• Growth factor withdrawal
• DNA damage (by radiation, toxins, free radicals)
• Protein misfolding (ER stress)
Mitochondria
Cytochrome c and other pro- apoptotic proteins
Adapter proteins
Bcl-2 family effectors (Bax, Bak)
Initiator caspases
Initiator caspases
Phagocyte
Regulators (Bcl-2, Bcl-x
Bcl-2 family sensors
Executioner caspases
Endonuclease activation
Breakdown of cytoskeleton
DNA fragmentation
Ligands for phagocytic cell receptors
Apoptotic body

Fig. 2.16

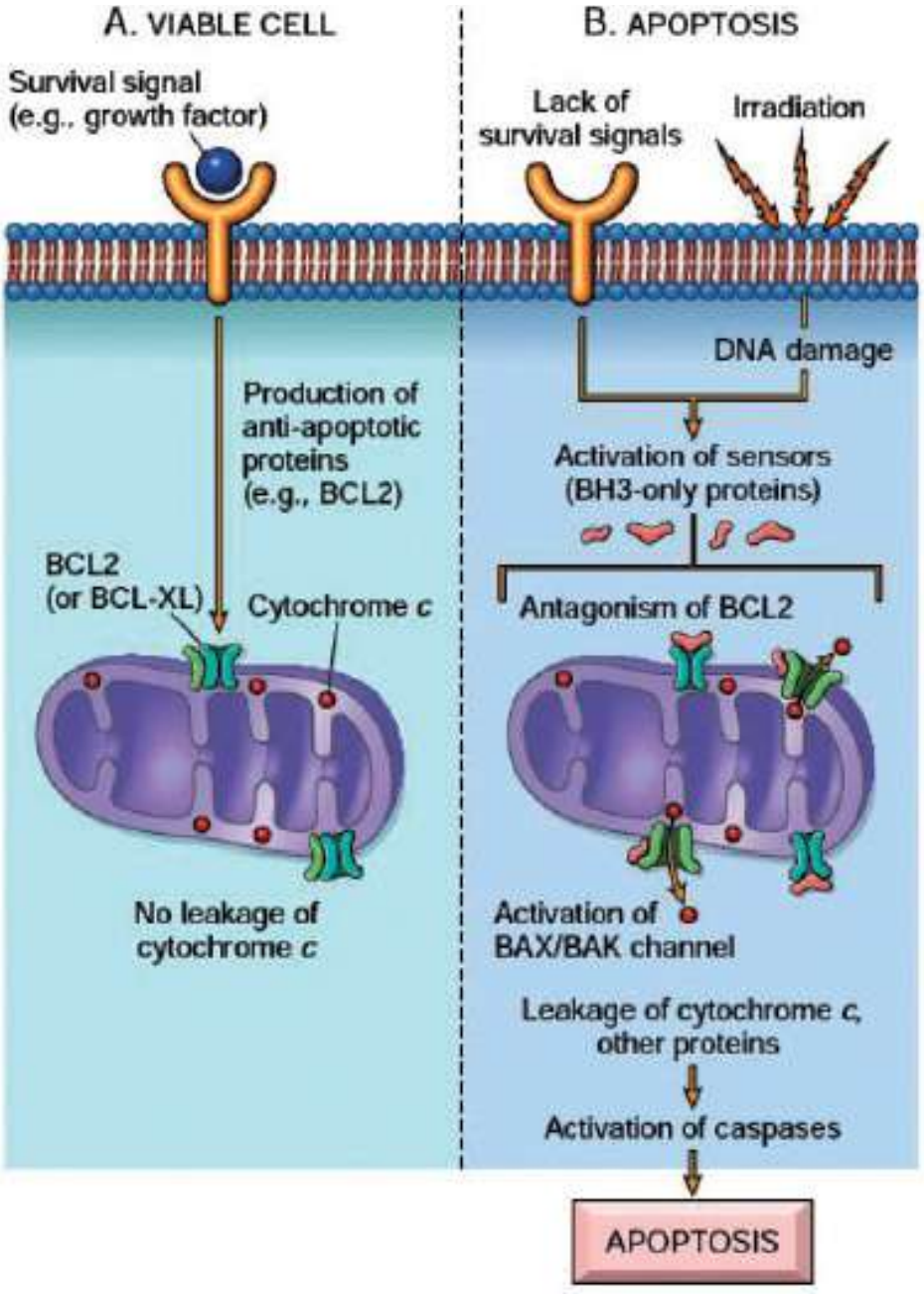


Fig. 2.17

The intrinsic (mitochondrial) pathway of apoptosis. A, Cell viability is maintained by the induction of anti-apoptotic proteins such as Bcl-2 by survival signals. These proteins maintain the integrity of mitochondrial membranes and prevent leakage of mitochondrial proteins. B, Loss of survival signals, DNA damage, and other insults activate sensors that antagonize the anti-

apoptotic proteins and activate the pro-apoptotic proteins Bax and Bak, which form channels in the mitochondrial membrane. The subsequent leakage of cytochrome c (and other proteins, not shown) leads to caspase activation and apoptosis.

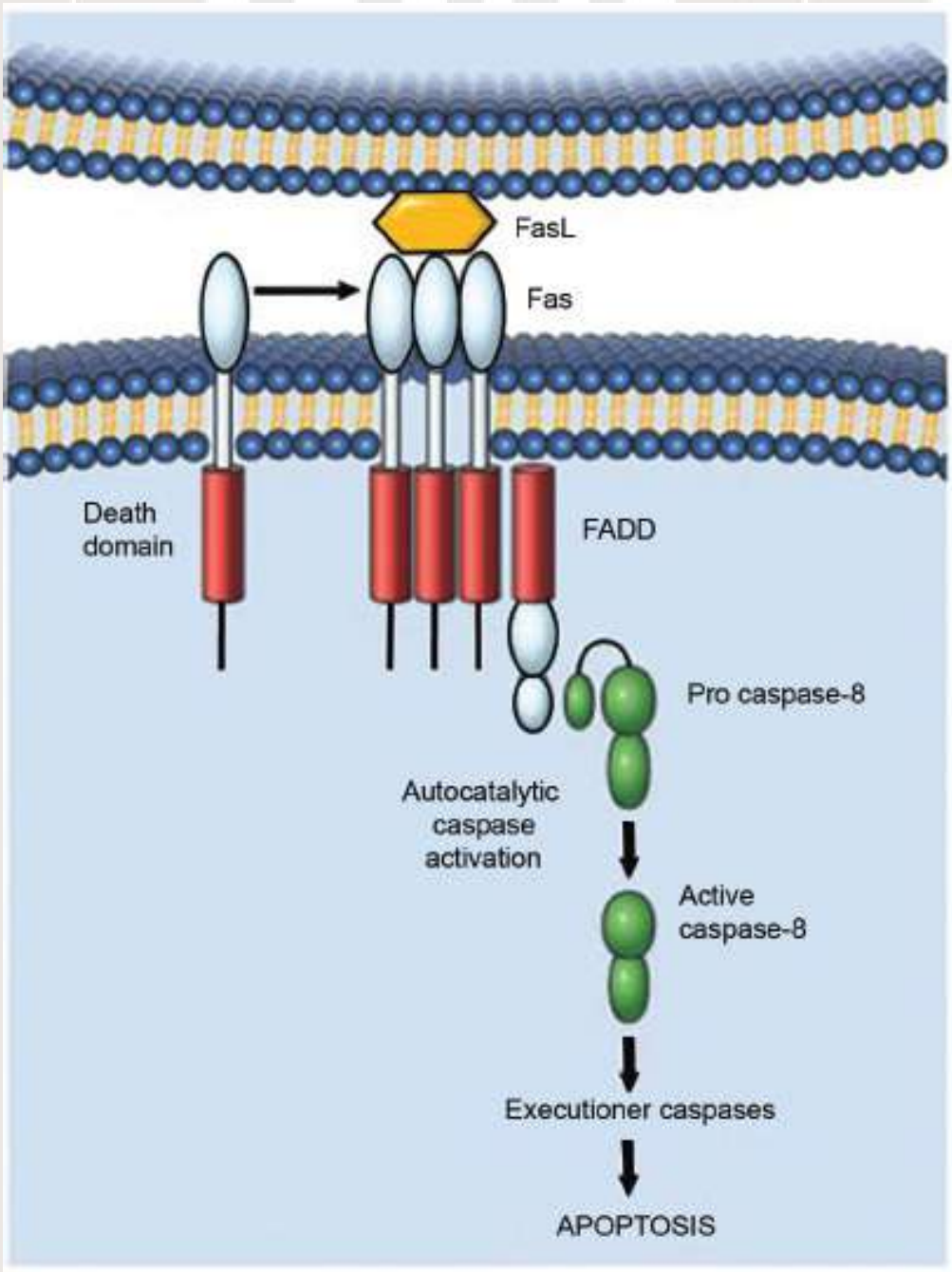


Fig. 2.18

The extrinsic (death receptor–initiated) pathway of apoptosis, illustrated by the events following Fas engagement. FAAD, Fas-associated death domain; FasL, Fas ligand.

Features of Necrosis and Apoptosis:

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

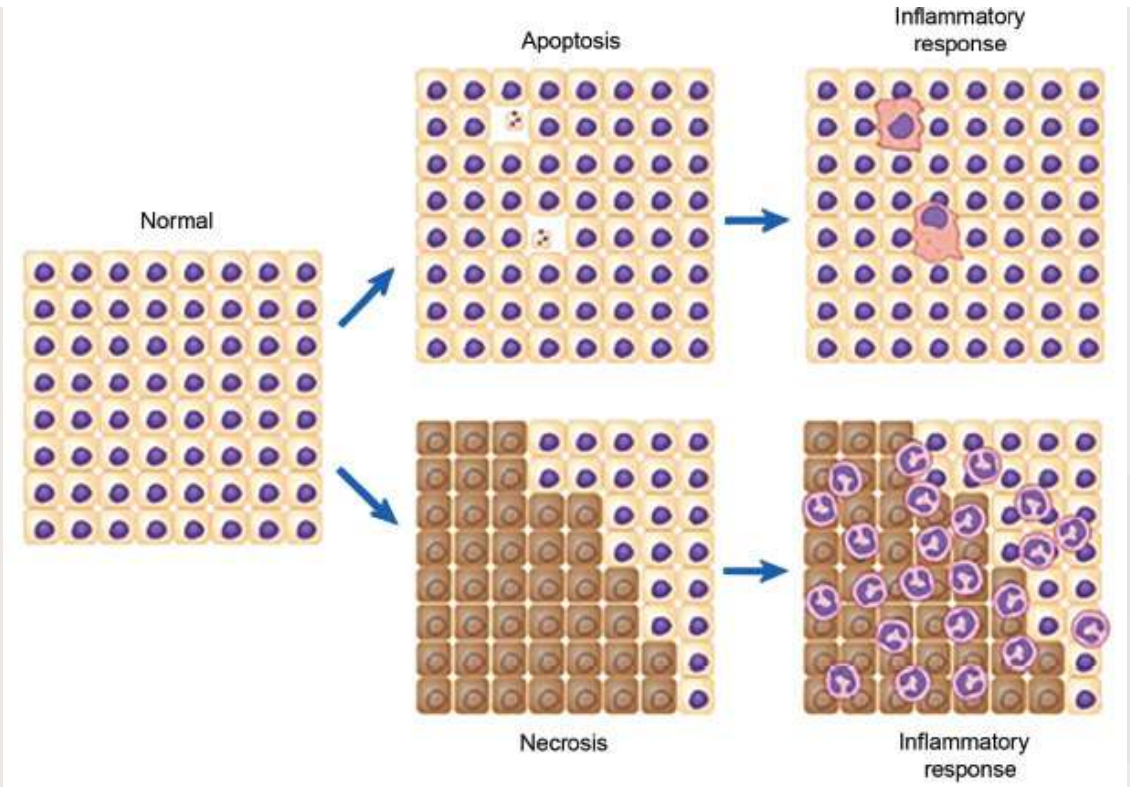


Fig. 2.19

f. Execution of apoptosis:

I. Mediated by Caspases.
II. Caspases digest nuclear and cytoplasmic proteins.
III. Caspases also activate endonucleases..

g. Examples of apoptosis:

I. Embryogenesis: organogenesis and development.
II. Hormone dependent Apoptosis e.g. menstrual cycle.
III. Thymus: selective death of lymphocytes.
IV. Viral diseases: Viral hepatitis (Councilman Bodies).
V. Cystic fibrosis: duct obstruction and pancreatic atrophy.

NECROPTOSIS (Robbins 9th edition- NEW TOPIC):

As the name indicates this form of cell death is a hybrid that shares aspects of both necrosis and apoptosis.

In sharp contrast to apoptosis, the genetic program that drives necroptosis does not result in caspase activation and hence it is also known as “caspase independent” programmed cell death.

Necroptosis and Pyroptosis:

- Necroptosis resembles necrosis morphologically and apoptosis mechanistically as a form of programmed cell death.
- Necroptosis is triggered by ligation of TNFR1, and viral proteins of RNA and DNA viruses.
- Necroptosis is caspase-independent but dependent on signaling by the RIP1 and RIP3 complex.
- RIP1-RIP3 signaling reduces mitochondrial ATP generation, causes production of ROS, and permeabilizes lysosomal membranes, thereby causing cellular swelling and membrane damage as occurs in necrosis.
- Release of cellular contents evokes an inflammatory reaction as in necrosis.
- Pyroptosis occurs in cells infected by microbes.lt involves activation of caspase-1 which cleaves the precursor form of IL-1 to generate biologically active IL-1. Caspase-1
  along with closely related caspase-11 also cause death of the infected cell.

Molecular mechanism of TNF mediated necroptosis. Cross linking TNFR1 by TNF causes recruitment of RIP 1 and RIP 3 along with caspase 8. Activation of caspase leads to apoptosis. Inhibition of caspase 8 as may occur in some viral infections, allows RIP 1 and RIP 3 to initiate signals that affect mitochondrial generation of ATP and ROS. This is followed by events typical of necrosis.

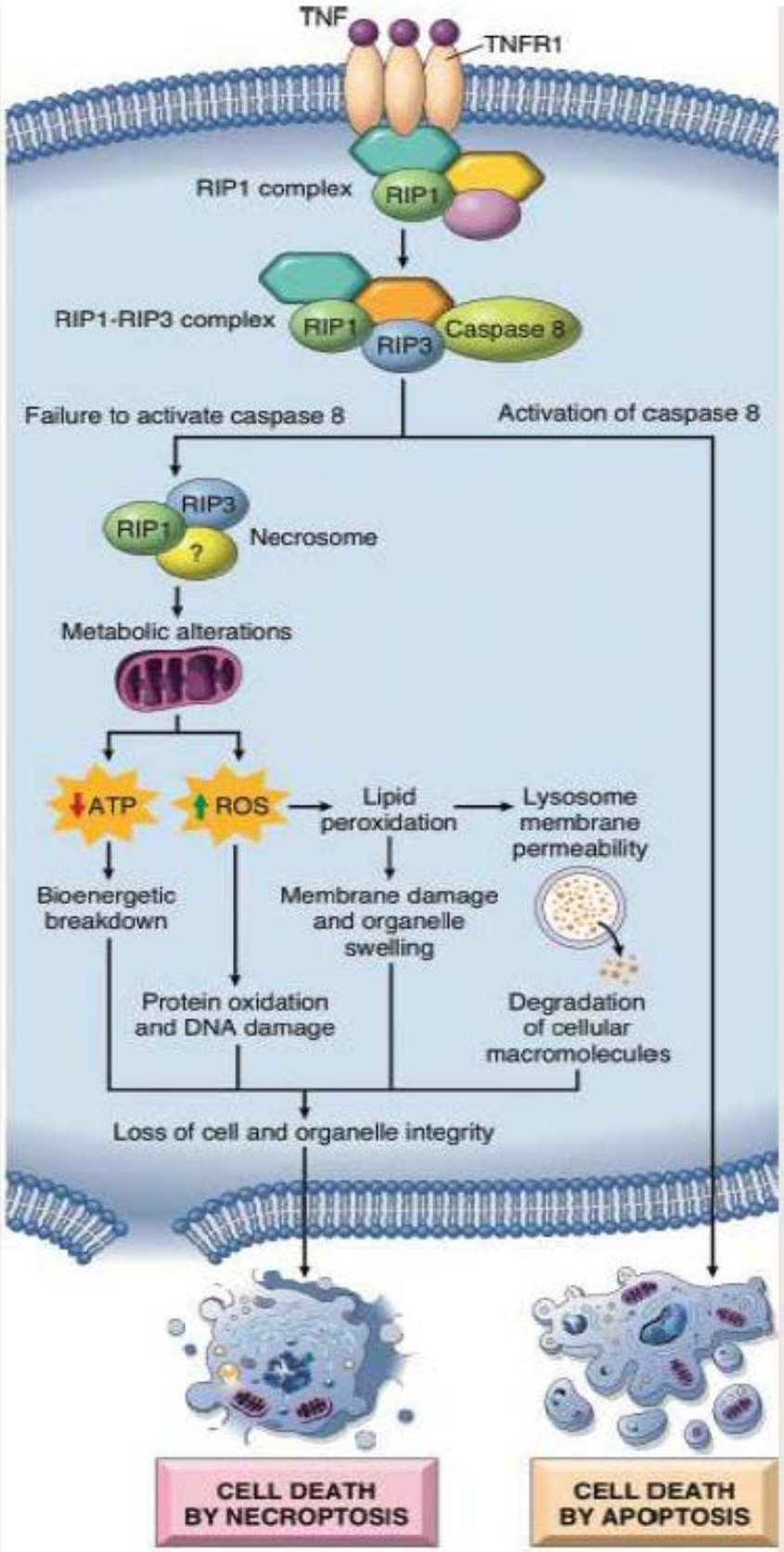

TNF
TNFR1
RIP1 complex
RIP1
RIP1-RIP3 complex
RIP1
RIP3
Caspase 8
Failure to activate caspase 8
Activation of caspase 8
RIP3
RIP1
?
Necrosome
Metabolic alterations
ATP
ROS
Lipid peroxidation
Lysosome membrane permeability
Bioenergetic breakdown
Membrane damage and organelle swelling
Protein oxidation and DNA damage
Degradation of cellular macromolecules
Loss of cell and organelle integrity
CELL DEATH BY NECROPTOSIS
CELL DEATH BY APOPTOSIS

Fig. 2.20

PYROPTOSIS (Robbins 9th edition- New Topic):

Another form of programmed cell death

Accompanied by release of fever inducing cytokine IL1 and because it bears some biochemical similarities with apoptosis

Microbial products enter cytoplasm à cytoplasmic immune receptors recognize them → activate the multiprotein complex called INFLAMMASOME à activates caspase 1 → cleaves a precursor form of IL 1

Unlike classical apoptosis, this pathway is characterized by swelling of cells, loss of plasma membrane integrity and release of inflammatory mediators.

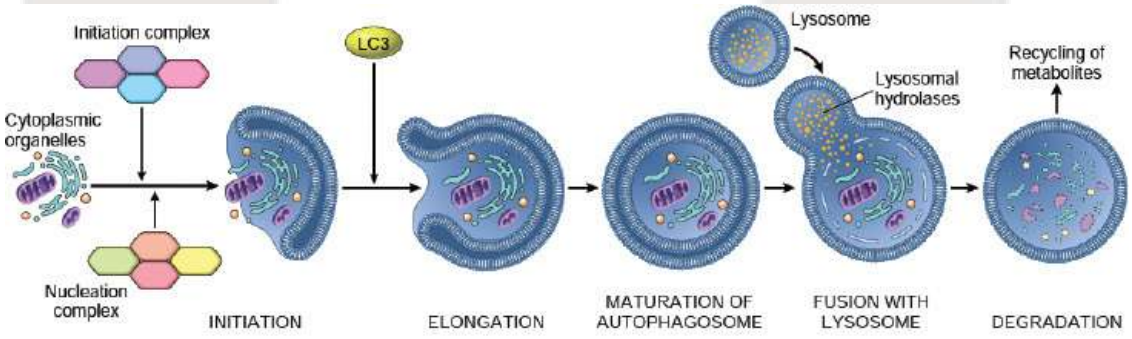


Fig. 2.21

Intracellular accumulation:

Lipids:

i. Triglycerides (e.g. Fatty change in liver cells).
ii. Cholesterol (e.g. Atherosclerosis, xanthomas).
iii. Complex lipids (e.g. Sphingolipids accumulation of immunoglobulins in carbon dust).

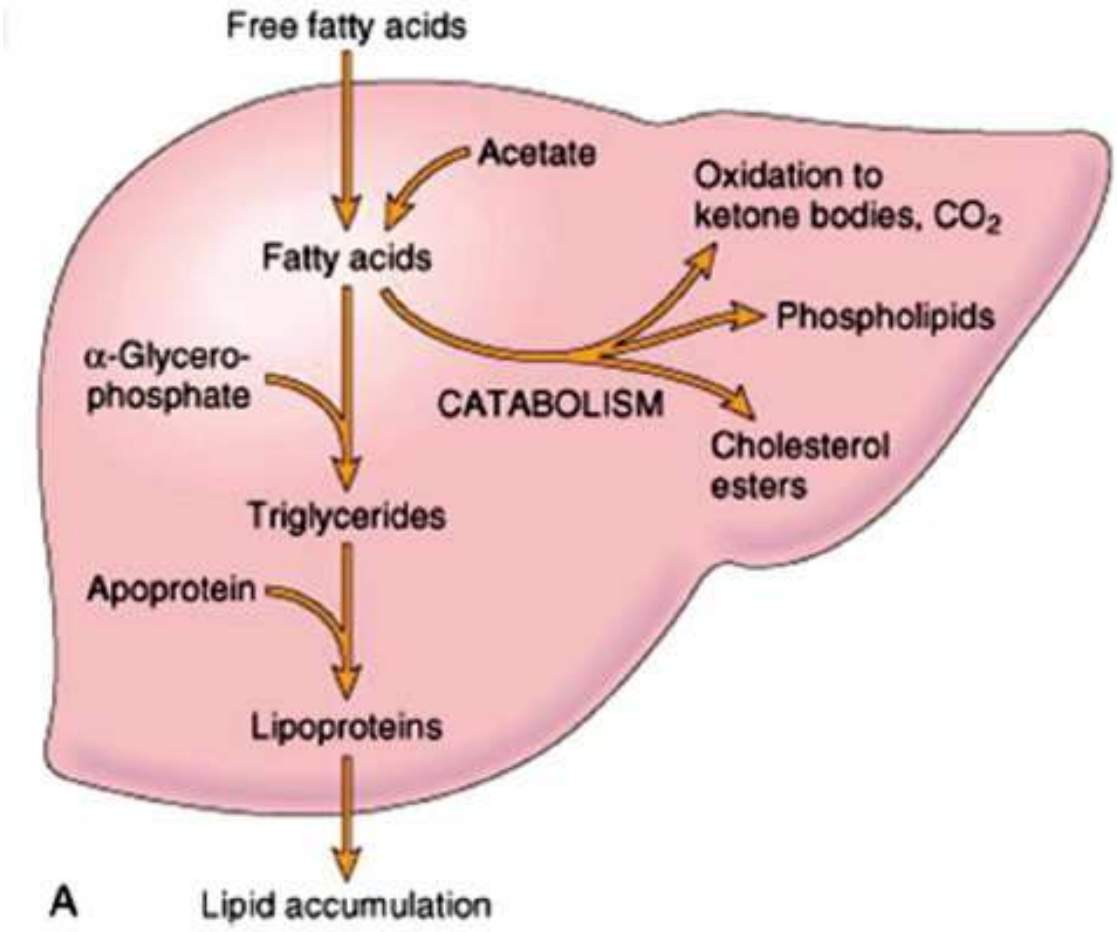


Fig. 2.22

Proteins:

i. Protein accumulates in proximal renal tubules in proteinuria.

ii. Russell bodies: intracytoplasmic accumulation of immunoglobulins in plasma cells.

iii. Defective protein folding can also result in protein accumulation.

Normal Protein Synthesis and folding:

- Proteins synthesized as polypeptide chains on ribosomes.
- Chains arranged into α helices / β sheets and folded properly.
- Protein folding and transportation across E.R., Golgi and beyond is aided by protein c. a chaperones.
- Chaperones:

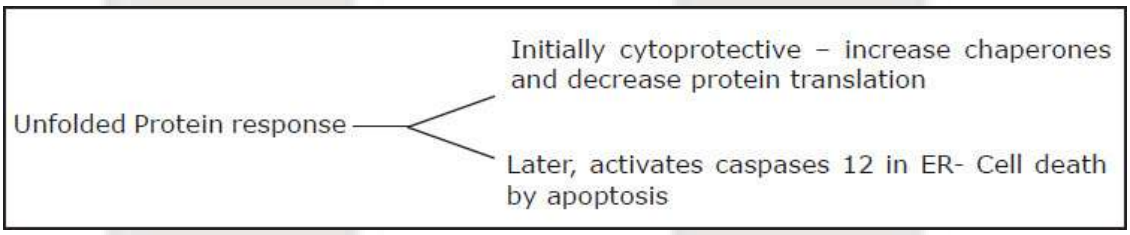
- Synthesized constitutively, affect normal intracellular protein trafficking
- Induced by stress and heat shock proteins e.g. hsp 70, hsp 90.
- 'Rescue' shock stressed proteins from misfolding.
- If folding is not successful, ubiquitin (a type of chaperones) facilitate degradation of damaged proteins.

Involvement of the Ubiquitin-Proteasome System in Disease		
Disease	**Ubiquitin-Proteasome System Activity**	**Anatomic Effect**
Neurologic Diseases (Diseases Associated With Neuron Loss)		
Parkinson disease	Decreased	Lewy bodies
Alzheimer disease	Decreased	Amyloid plaques, neurofibrillary tangles
Amyotrophic lateral sclerosis	Decreased	Superoxide dismutase aggregates in motor neurons
Huntington disease	Decreased	Polyglutamine inclusions
Autoimmune Diseases		
Sjogren syndrome	Decreased	Chronic inflammation
Metabolic Diseases		
Type II diabetes mellitus	Increased	Insulin insensitivity
Cataract formation	Decreased	Aggregated oxidized proteins
Muscle Wasting		
Aging	Increased	Atrophy
Cancer and other chronic disease	Increased	Atrophy
Cardiovascular		
Ischemia/reperfusion	Decreased	Myocyte apoptosis
Pressure overload	Decreased	Myocyte apoptosis

a. Defect in intracellular transport and secretion of critical proteins.

- α IAT deficiency – Mutations in protein slow the folding, partially folded protein accumulates in ER of Liver and is not secreted – Emphysema.
- Cystic fibrosis.
- Familial hyper cholesterolemia.

b. ER stress induced by misfolded / unfolded protein. Misfolded protein in ER → Unfolded protein response.



E.g. Neurodegenerative diseases- Alzheimer's disease, Huntington, Parkinson's disease? Type II Diabetes.

Exogenous pigments:

- Anthracotic pigment of lung is secondary to inhalation of carbon.
- Tattoos cinnabar, India ink dyes used.

Endogenous pigments:

- Lipofuscin:
- Wear and tear pigment tell tail sign of free radical injury; peri nuclear. Yellow brown pigment; indigestible material within lysosomes common in liver and heart Brown atrophy; atrophy of organ with lipofuscin pigment.
- Melanin: Brown black pigment found in melanocytes and substantia nigra
- Hemosiderin: Golden yellow brown pigment found in areas of hemorrhage / bruises systemic iron overload Prussian blue positive – Perl's reaction

Hyaline change:

Nonspecific term used to describe any intracellular or extra cellular alteration that has pink homogenous appearance on H and E.

- E.g. of intracellular hyaline Resorption droplets in proximal tubules of kidney Russel bodies Alcoholic hyaline.

- E.g. of extra cellular hyaline Hyaline arterioles clerosis Amyloid Hyaline membrane disease of new born.

Pathological calcification:

A. Dystrophic calcification:

- Precipitation of calcium phosphate in dying or necrotic tissue.
- S. calcium levels is normal with normal calcium metabolism.
- Examples: calcification in areas of fat necrosis, calcification in areas of coagulative and caseous necrosis; psammoma bodies-laminated concretions that occur in meningiomas, papillary carcinoma of thyroid and ovary; Monckebergs medial calcific stenosis and atherosclerotic plaques.

B. Metastatic calcification:

- Precipitation of calcium phosphate in normal tissues due to hypercalcemia.
- Causes: Hyper parathyroidism.
  Parathyroid adenomas.
  Renal failure.
  Paraneoplastic syndrome.
  - Vitamin D intoxication.
  Milk-alkali syndrome.
  Sarcoidosis.
  Paget's disease.
  Multiple myeloma.
  Meta static cancer to the bone.
- Location of calcification: Begins in mitochondria in all the organs except kidney where it begins in the basement membrane of the tubules.
- Stains used for demonstration of calcium: Von Kossa & Alizarin Red –S
- Metastatic calcification occurs widely throughout the body but principally affects the interstitial tissues of the gastric mucosa, kidneys, lungs, systemic arteries and pulmonary veins.

AGEING:

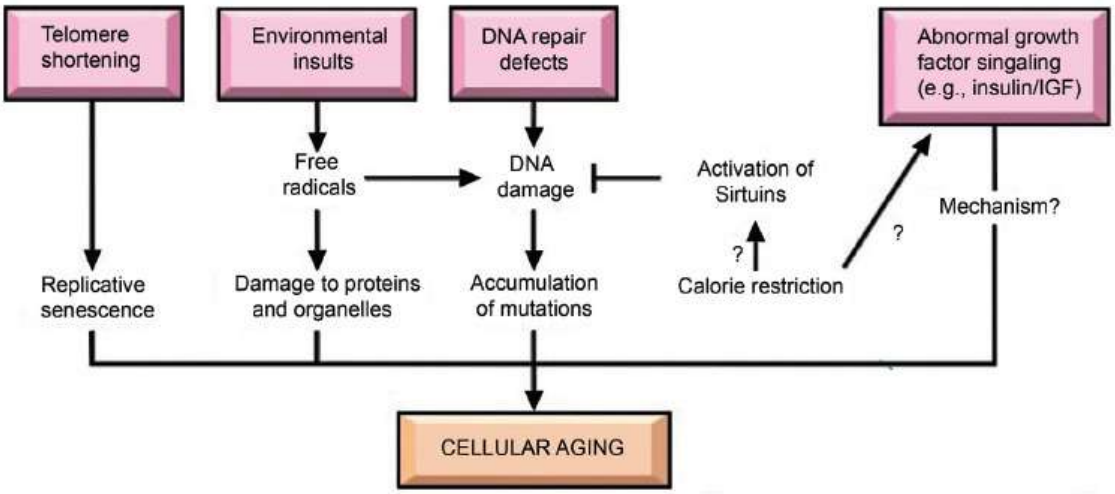


Fig. 2.23

Mechanisms of cellular aging. Genetic factors and environmental insults combine to produce the cellular abnormalities characteristic of aging. How calorie restrictions prolong life span is net established. IGF, insulin-like growth factor.

Experimental Factors That Influence Biological Aging (*)	
Factors That Increase Longevity	Factors That Decrease Longevity
Mutations in p53, $p14^{ARF}$, $p16^{INK4A}$, etc.	Increased p53 activation
Decreased metabolic rate	Increased metabolic rate
Caloric restriction	Increased oxidative stress
Increased Sirt 1	Increased mTOR activity
Age mutations	Increased cell cycle control proteins
Increased antioxidant defenses Episodic stress Genetic factors	Genetic factors

Cellular ageing results from a combination of accumulating cellular damage, reduced capacity to divide, reduced ability to repair damaged DNA and defective protein homeostasis.

1. Accumulation of DNA damage: defective DNA repair mechanisms; conversely, caloric restriction activates DNA repair and is known to prolong ageing in model organisms.

2. Replicative senescence: reduced capacity of cells to divide secondary to progressive shortening of chromosomal ends (telomeres).
3. Defective protein homeostasis: resulting from defective proteasome and chaperone functions.
4. Nutrient sensing system: caloric restriction inreases longevity. Mediators may be reduced IGF-1 signalling and increase in sirtuins.

3 Inflammation and Wound Healing

Inflammation:

Response of vascularized connective tissue to injury.

Fundamentally protective response, may be potentially harmful.

Acute:

Rapid onset (sec. – min) short duration: lasts for min →hrs. → day.

Features exudation of fluid & PP (oedema) extravasation of leukocytes (Neutrophils).

Chronic:

- Longer duration.
- associated with presence of lymphocytes & macrophages.
- Prolif. Of BVS, fibrosis, tissue necrosis.

Celsus:

(3000 B.C.)- Rubor, Tumor, Color, Dolor, Virchow- Functio laesa.

Elie Metchnikoff → Phagocytosis.

Sir Thomas Lewis → Histamine.

Acute Inflammation:

Events:

Vascular Events:

Cellular Events:

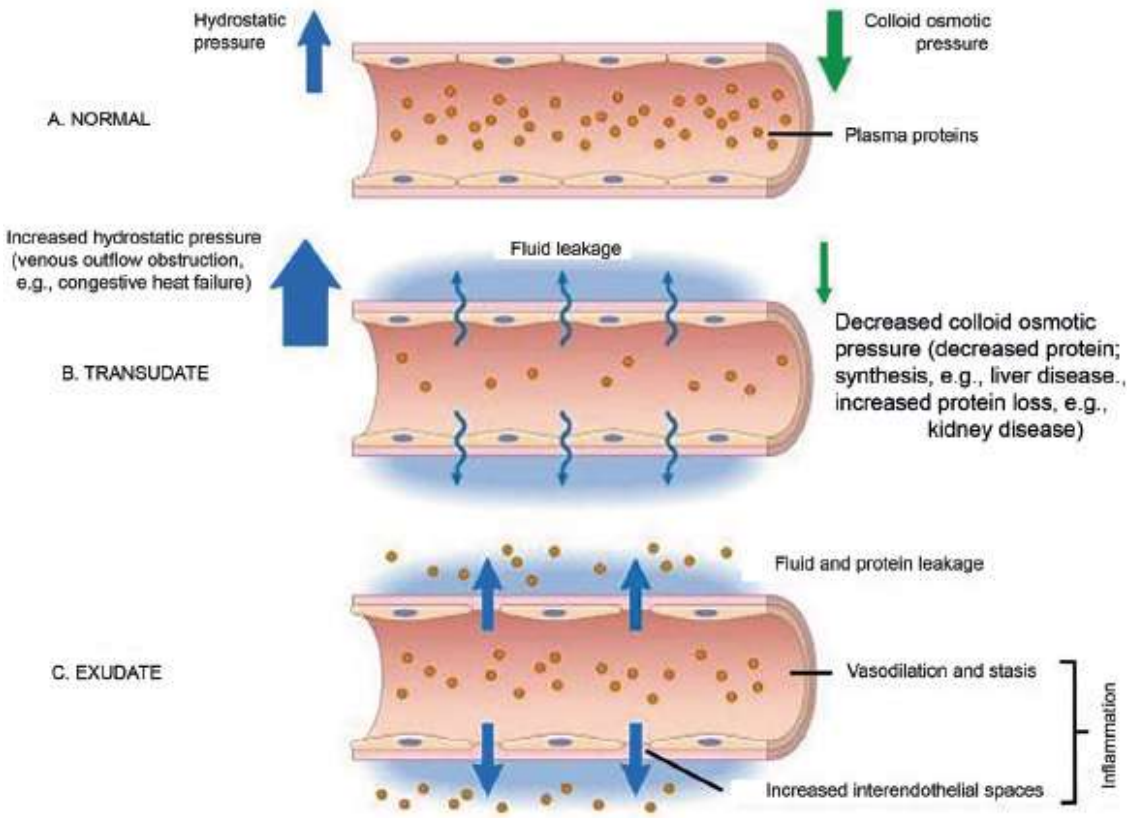


Fig. 3.1

Exudate	Transudate
Inflamm. Oedema	N.inflamm.
↑ Permeability	Hydrostatic. Imbalance
Escape of fluids	Ultra – filtrate of plasma
Proteins, cells	↓ / No proteins (albumin)
Sp. Gravity > 1.020	< 1.012

Formation of transudates and exudates. A, Normal hydrostatic pressure (blue arrows) is about 32 mm Hg at the arterial end of a capillary bed and 12 mm Hg at the venous end; the mean colloid osmotic pressure of tissues is approximately 25 mm Hg (green

arrows), which is equal to the mean capillary pressure. Therefore, the net flow of fluid across the vascular bed is almost nil. B, A transudate is formed when fluid leaks out because of increased hydrostatic pressure or decreased osmotic pressure. C, An exudate is formed in inflammation, because vascular permeability increases as a result of increased interendothelial spaces.

Pus:

Purulent Exudate.

Stimuli for acute inflammation:

- Infections (bacterial, viral , parasitic) and microbial toxins.
- Trauma (blunt, penetrating).
- Physical and chemical agents (thermal injury, radiation, chemicals).
- Tissue necrosis.
- Foreign bodies (splinters, dirt, sutures)
- Immune reactions.

The steps of inflammatory response can be remembered as the five Rs-

1. Recognition of the injurious agent.
2. Recruitment of leucocytes.
3. Removal of the agent.
4. Regulation (control) of the response.
5. Resolution (repair).

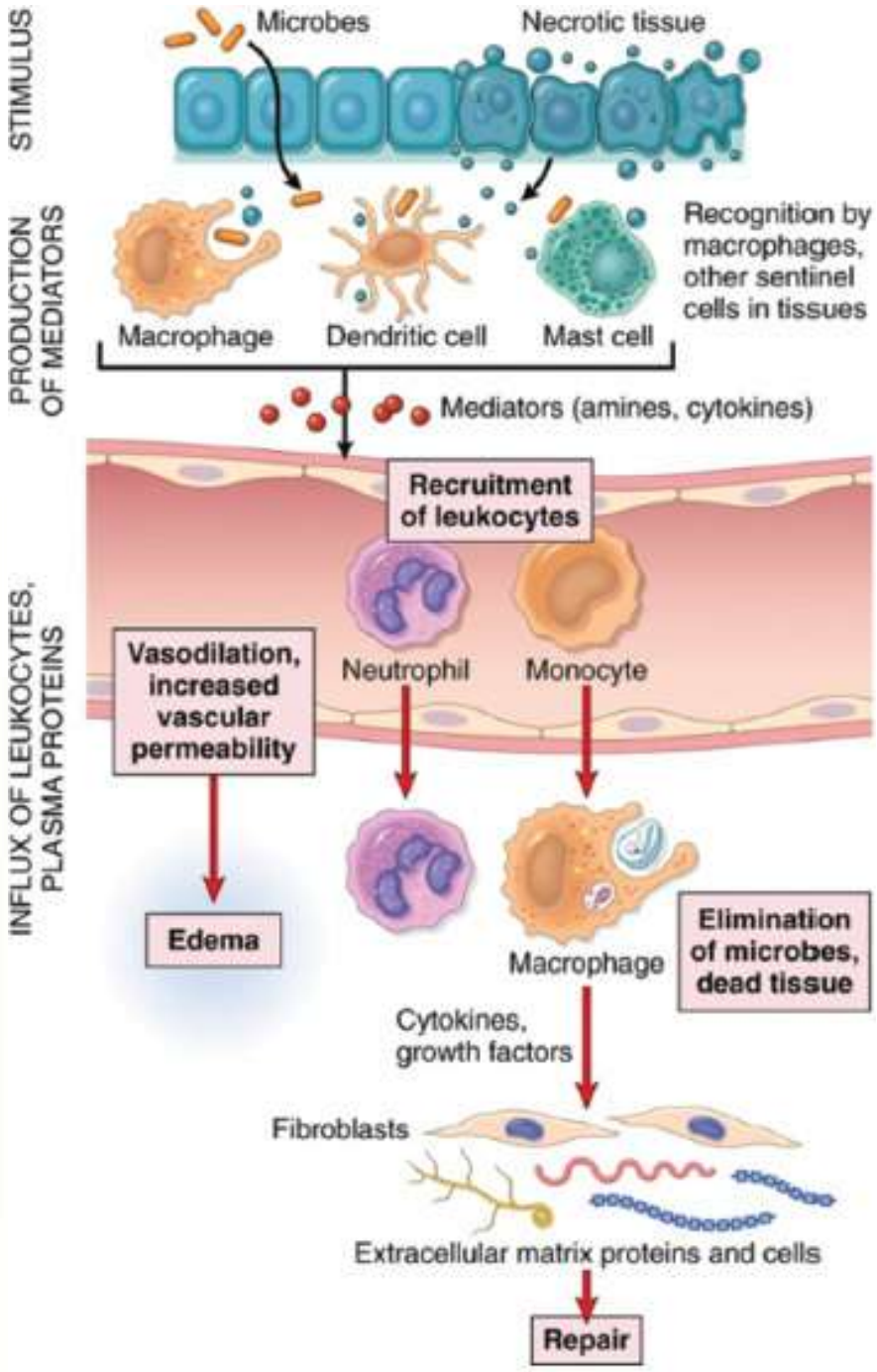
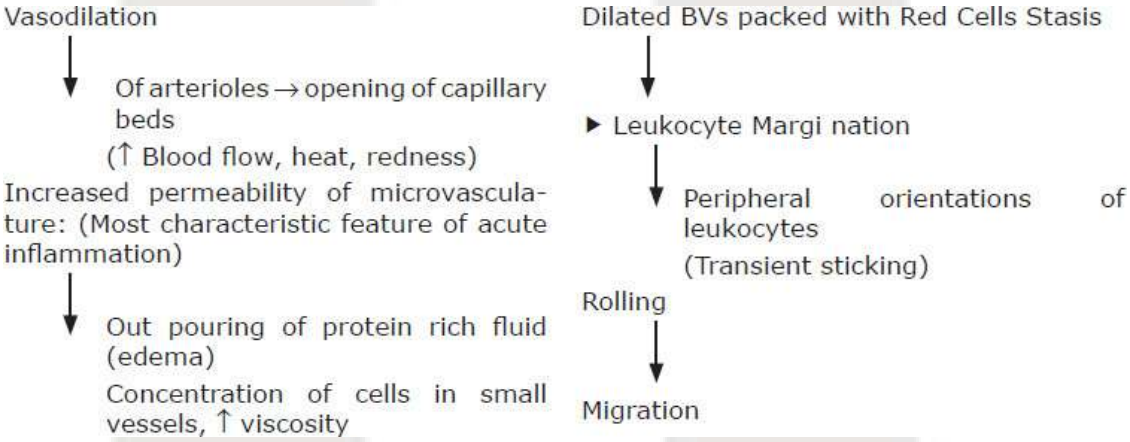


Fig. 3.2

Vascular changes:

Vasodilation is one of the earliest manifestations of acute inflammation; sometimes it follows a transient constriction of arterioles, lasting a few seconds.

Vasodilation first involves the arterioles and then leads to opening of new capillary beds in the area. The result is increased blood flow, which is the cause of heat and redness (erythema) at the site of inflammation.



Mechanism of increased Vascular Permeability:

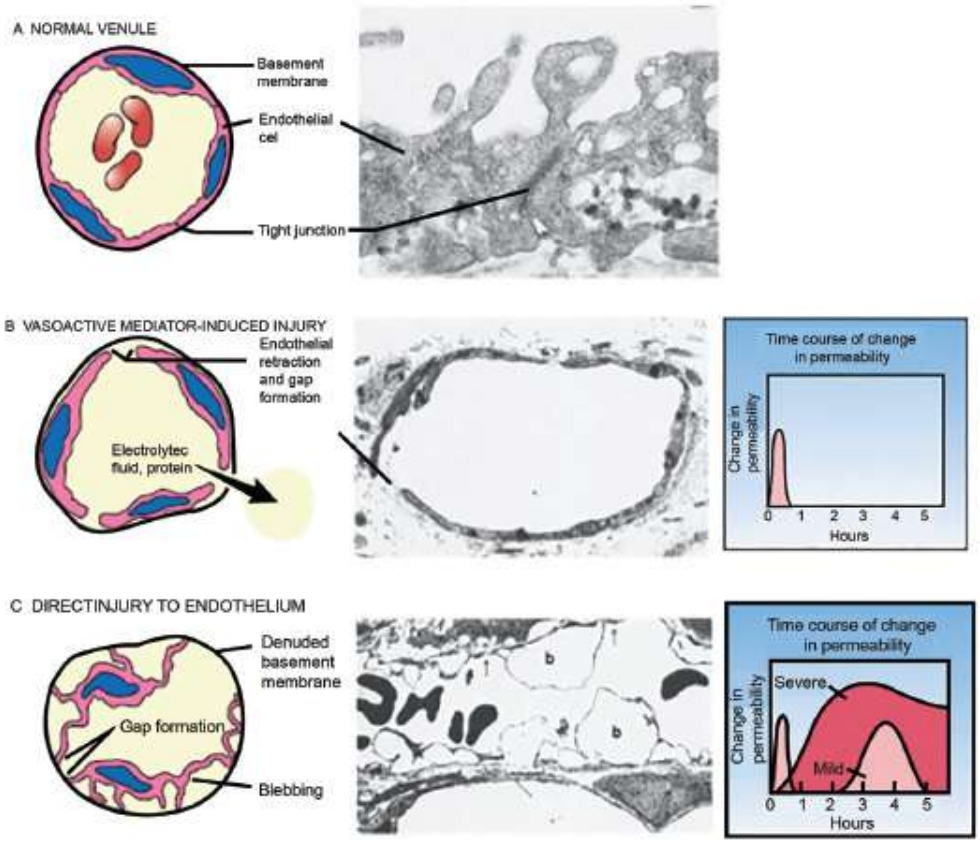


Fig. 3.3: Responses of the microvasculature to injury. A. The wall of the normal venule is sealed by tight junctions between adjacent endothelial cells. B. During mild vasoactive mediator induced injury, the endothelial cells separate and permit the passage of the fluid constituents of the blood. C. With severe direct injury, the endothelial cells form blebs (b) and separation the underlying basement membrane. Areas of denuded basement membrane (arrows) allow a prolonged escape of fluid elements from the microvasculature.

Cellular Events:

- Adhesion & Transmigration.
- Chemo taxis.
- Phagocytosis.

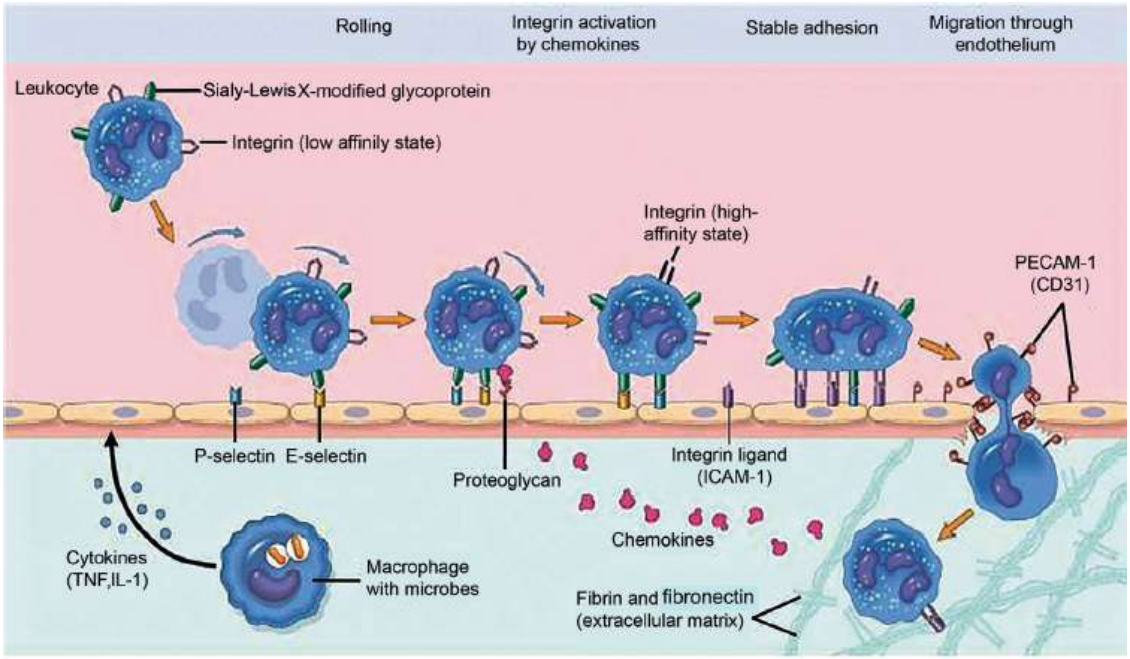


Fig. 3.4

Adhesion & Transmigration:

Steps:

- Lumen: Margination.
- Rolling – transient adhesion.
- Transmigration across endothelium (diapedesis).
- Migration in interstitial tissue (to chemo tactic stimulus).

Stasis → Margination → Rolling

↓

Firm adhesions (pebbles / marbles pavementing).

E/C Space ← Insert pseudopodia b/w endothelial cells.

Adhesion and transmigration require:

- Complementary adhesion molecules binding.

- Chemical mediators.

Adhesion Molecules:

- Selection – E.Selectin, P.Selectin, L.Selectin.
- Immunoglobulins – ICAM-1, VCAM-1.
- Integrins – Heterodimeric glycoproteins (α & β chain).
  - β 2 integrin LFA-1, MAC-1 (binds ICAM-1).
  - β 1 integrin VLA4 (binds VCAM-1).
- Mucin like Glycoproteins – gly CAM-1.
- CD 31.

Endothelial Molecule	Leukocyte Receptor	Major Role
• P selectin (on endothelium & platelets) (GMP 140/ PADGEM) CD 62P	Sialyl Lewis X PSGL-1 PSGL-1	• Rolling
E selectin (CD 62 E/ELAM-1) (on endothelium)	Sally Lewis X	• Rolling • Adhesion to activated endothelium
ICAM-1	β – integrins (CD 11/CD 18)	• Adhesion, arrest, transmigration
VCAM-1	(LFA-1, MAC-1) $\alpha_4\beta_1$ (VLA-4)	• Adhesion
Glycam-1	$\alpha_4\beta_7$ (LPAM -1) L selectin (LAM-1)	• Lymphocyte homing to high endoth. Venules.
CD 31	CD 31	• Leucocytes migration through endothelium.

Mechanism:

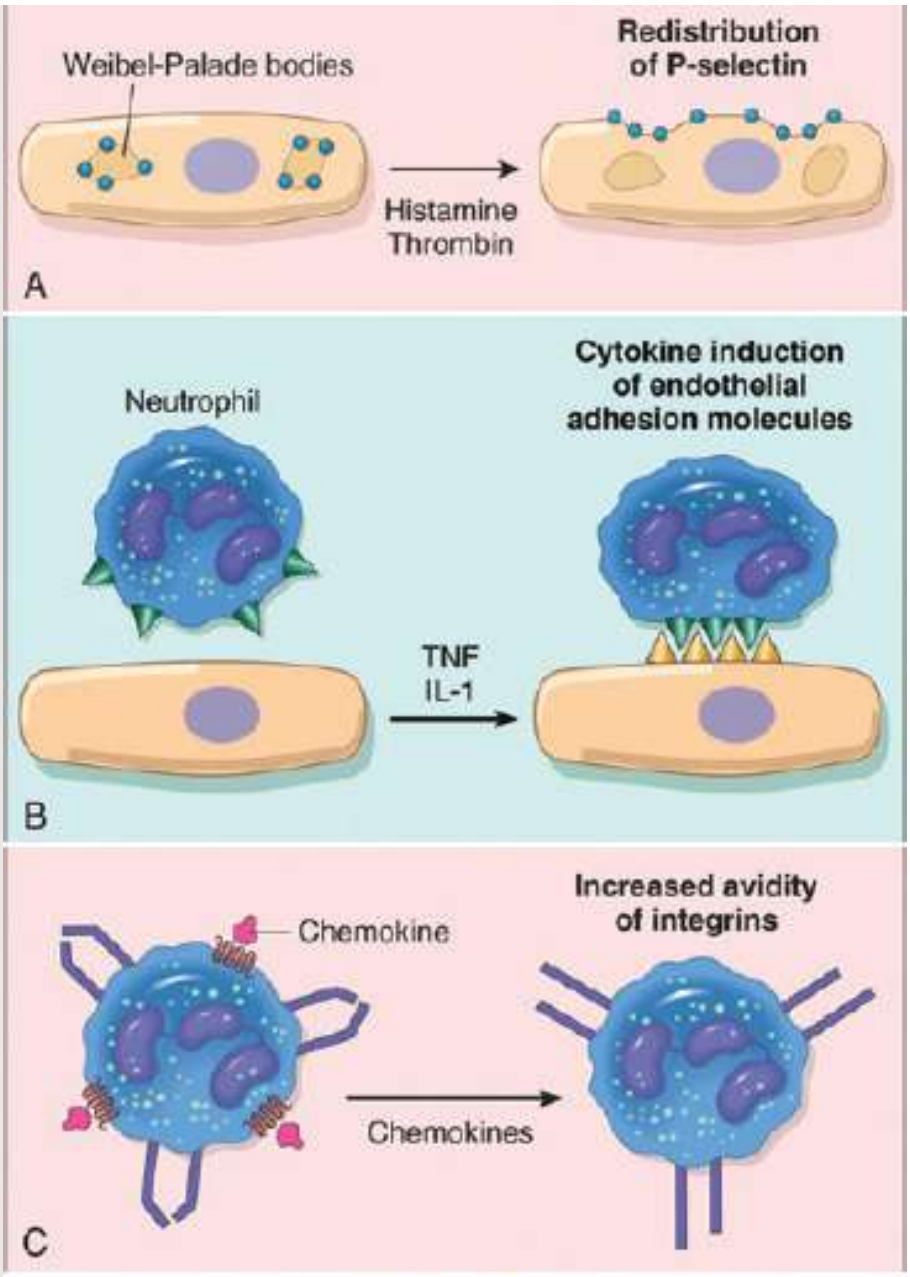


Fig. 3.5

Regulation of expression of endothelial and leukocyte adhesion molecules. A, Redistribution of P-selectin from intracellular stores to the cell surface. B, Increased surface expression of selectins and ligands for integrins upon cytokine activation of endothelium. C, Increased binding avidity of integrins induced by chemokines. Clustering of integrins contributes to their increased

binding avidity (not shown). IL-1, interleukin-1; TNF, tumor necrosis factor.

Impaired Leukocyte adhesion →

Recurrent bacterial infection

Defect.

LAD I: Absent β2 chain of LFA-1 & MAC-1 intergrins.

LAD II: Absent sialyl lewis X- defect in fucose metabolism.

Transmigration (Predominantly venules):

- Intercellular junctions – PECAM-1 / CD 31.
- Pierce the basement membrane by? Secreting collagenases.

1. 6-24 hrs- Neutrophils.

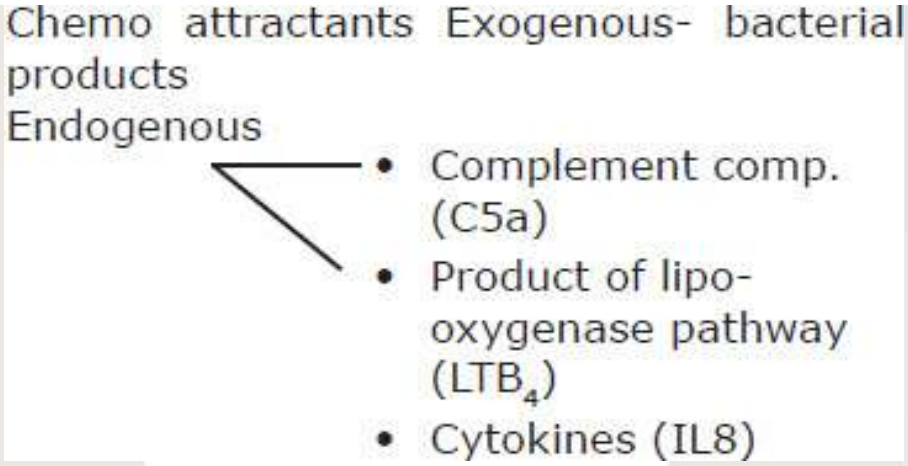
24-48 hrs- Monocytes Because of

- Induction / activation of different adhesion Molecules.
- Specific chemotactic factors in different Phase.
- Neutrophils- short life (apoptosis after 24-28 hrs).

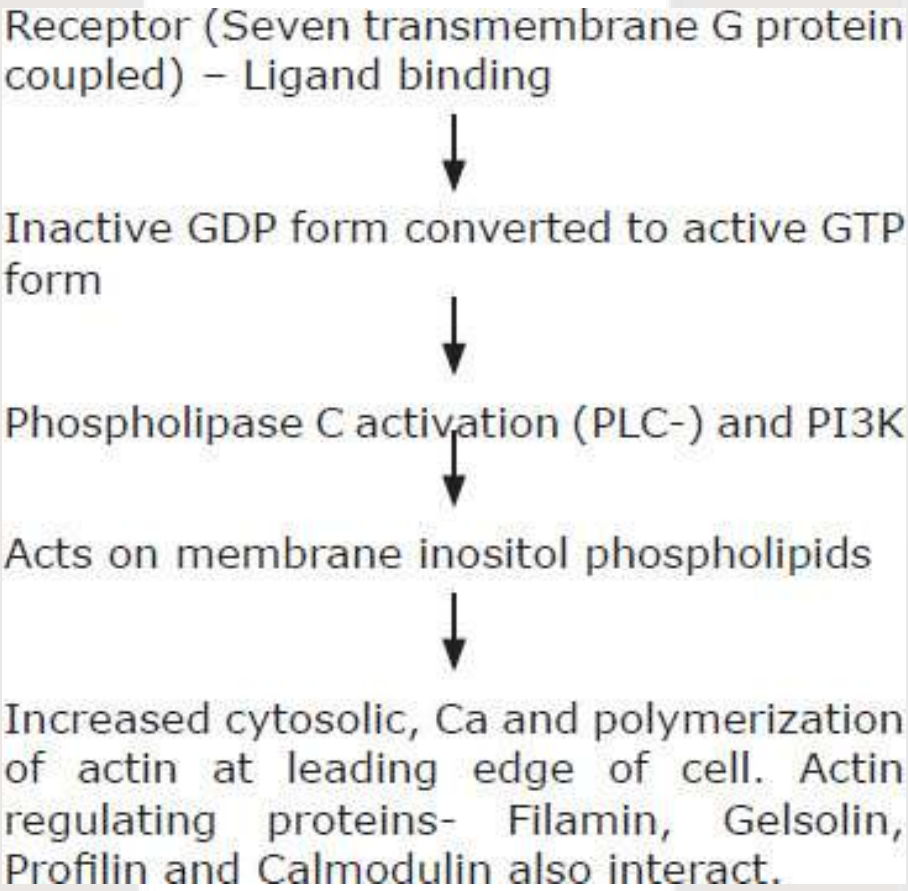
2. Pseudomonas: Neutrophils (2-4 days).
3. Viral infection Lymphocytes (First to arrive).
4. Hypersensitivity reactions: Eosinophils.

Chemotaxis:

locomotion oriented along a chemical gradient.

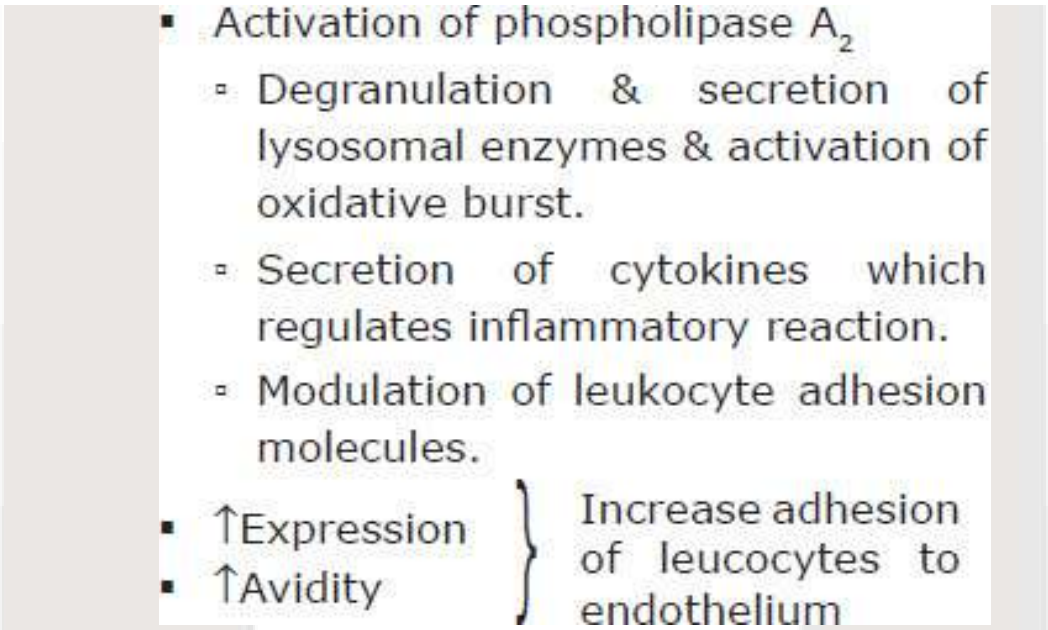


How Does A Leucocyte Move?



Leukocytle Activation:

- Production of arachidonic acid metabolites.



- Priming: ↑ rate & extent of leukocyte activation by mediator that itself causes little activation (e.g., TNF).
- Toll like receptors (TLRs) activate leucocytes in response to different types and components of microbes.
  - 10 TLRs identified till date
  - TLR- ligand binding → production of microbicidal substances and cytokines in leucocytes.
- Seven transmembrane 6 couples receptors.
  - Have 7 transmembrane α helical domain.
  - Ligands are-short acting peptides with N-Formyl methioyl residue, chemokines, and lipid mediators – PAF, PGE, and LTB4.
  - Result in chemotaxis.
- Receptors for cytokines like IFN-GAMMA
  - Major macrophage activating cytokine.
- Promote phagocytosis.

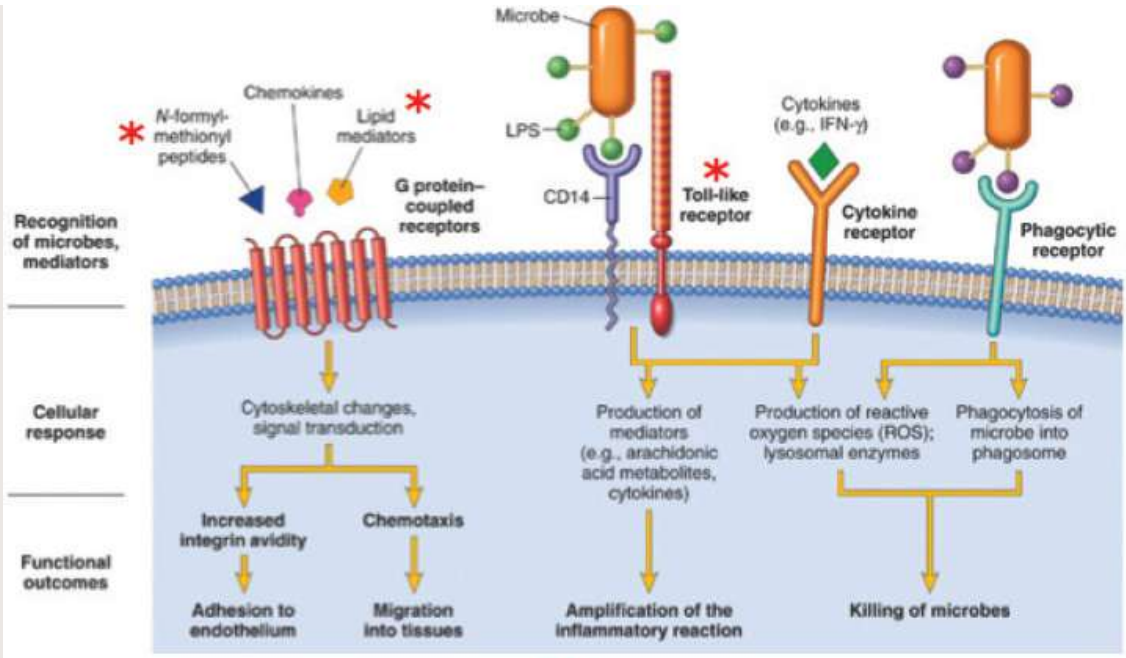


Fig. 3.6

Phagocytosis: 3 Steps:

1. Recognition & attachment of particle to be ingested.
2. Engulfment- formation of phagocytic vacuole.
3. Killing or degradation.

Recognition & attachments:

Leucocytes recognize microbes and dead cells by receptors.

- Mannose receptor- bind mannose and fucose residues of glycoprotein in microbial cell wall.
- Scavenger receptors- originally defined as molecules that bind modified LDL particles. Also bind microbes.
- Mac 1 integrins.

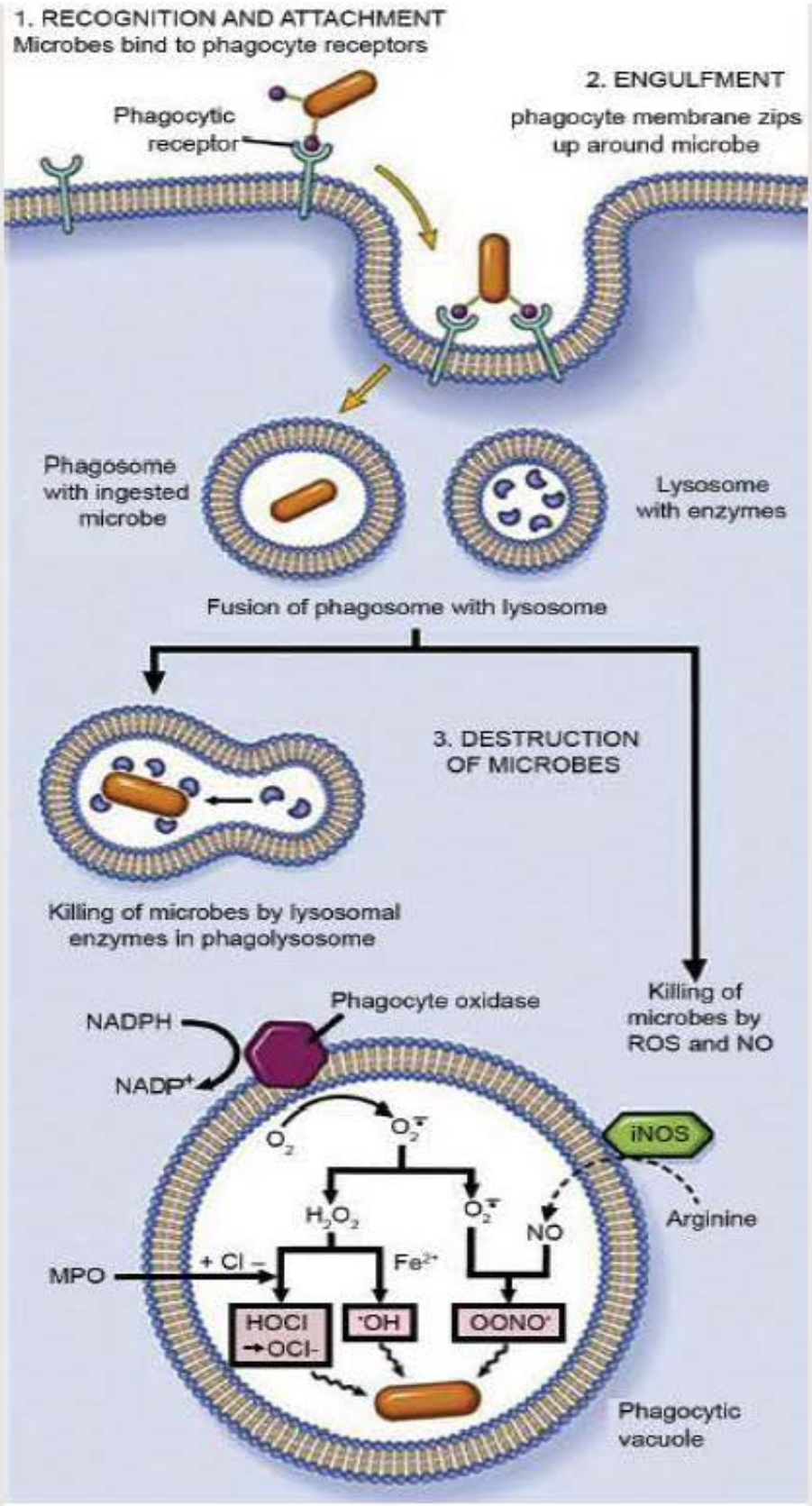

1. RECOGNITION AND ATTACHMENT
Microbes bind to phagocyte receptors
Phagocytic receptor
2. ENGULFMENT
phagocyte membrane zips up around microbe
Phagosome with ingested microbe
Lysosome with enzymes
Fusion of phagosome with lysosome
3. DESTRUCTION OF MICROBES
Killing of microbes by lysosomal enzymes in phagolysosome
Killing of microbes by ROS and NO
Phagocyte oxidase
NADPH
NADP+
O2
O2-
iNOS
Arginine
H2O2
O2-
NO
MPO
+ Cl –
Fe2+
HOCl →OCl-
·OH
OONO-
Phagocytic vacuole

Fig. 3.7

Efficiency of phagocytosis increased by opsonistion.

Opsonins:

bind to specific receptors on leukocytes.

- F_C fragment of IgG → F_C gamma R1
- C_{3b} & C_{3bi} → CR 1, 2,3

Plasma proteins:

Mannose binding lectins → C1q

Fibrinogen

Fibronectin → Integrins

C-Reactive proteins.

Neutrophils & macrophages: can recognize engulf bacteria & extraneous matter in absence of opsonins (Non- Opsonic phagocytosis).

Engulfment:

Steps:

- Pseudopods of phagocyte flow around the microbe to be engulfed.
- Microbe enclosed in a phagosome.
- Phagosome fuses with lysosome- phagolysosome.
- Degranulation into phagolysosome and bacterial killing.

Biochemical events of engulfment same as chemotaxis.

Killing / Degradation:

A. Mainly: O2 dependent mechanisms

- Activation of NADPH oxidase (found in neutrophil membrane).
- Requires MPO also.

HOCl: - Halogenation:

- Perioxidation.

1. H_2 O_2 - MPO-halide : most efficient bactericidal system.

- effective against fungi, viruses, protozoa, helminthes.

Dead organisms → lysosomal hydrolases.

2. MPO deficient leukocytes: superoxide, hydroxyl singlet oxygen.

B. O2–independent mechanisms: through action of substances in leukocyte granules.

- Bactericidal permeability increasing protein (BPI) – phospholipase activation, ↑ permeability of bacterial wall.
- Lysozyme –hydrolyses the glycopeptide coat of bacteria.
- Lactoferrin.
- Major basic protein: Eosinophils.
- Cytotoxic to many parasites.
- Defensins: pH of phagolysosome: 4-5.

Release of leukocyte products:

- Lysosomal enzymes: present in granules.
- Oxygen derived active metabolites.
- Products of arachidonic acid metabolism (PGs, LTs).

Cause:

- Endothelial injury.
- Tissue damage.
- Regurgitation during feeding: If Phagocytic vacuole remains transiently open to outside.
- Frustrated phagocytosis: on flat surface (e.g. GBM where immune complexes are deposited. The leukocyte is unable to phagocytosis the fixed immune complexes and lysosomal enzyme are released.

- Surface phagocytosis: Mech. by which phagocytes facilitate ingestion of bacteria and other foreign material by trapping it against resistant surface.
- Cytotoxic release: After phagocytosis of potentially membranolytic substances (e.g. crystals).

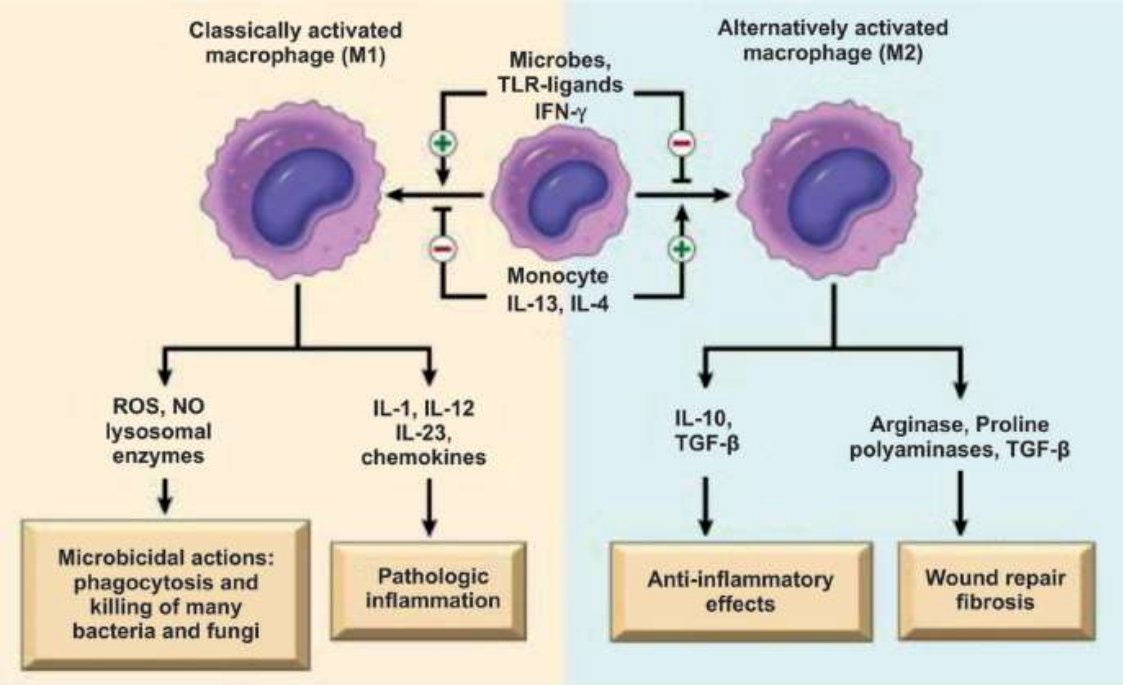


Fig. 3.8

Neutrophil Extracellular Traps (Robbins 9th edition- New Topic):

NETs are extracellular fibrillar networks that provide a high concentration of antimicrobial substances at sites of infection and prevent the spread of microbes by trapping them in the fibrils

They are produced by neutrophils in response to infectious pathogens and inflammatory mediators.

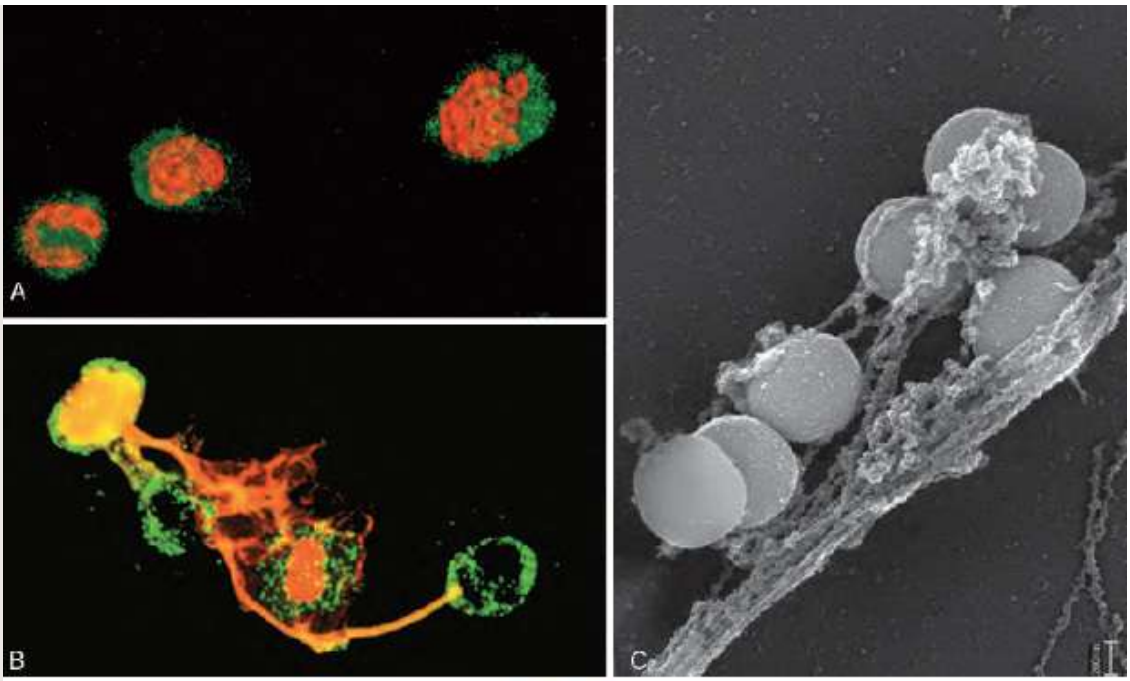


Fig. 3.9

A. Healthy neutrophils with nuclei stained red and cytoplasm stained green.

B. Release of nuclear material from neutrophils (note that two have lost their nuclei) forming extracellular traps.

C. An electron micrograph of bacteria (staphylococci) trapped in NETs.

Defects in Leukocyte Function:

Defects in leukocyte adhesion:

LAD 1: β chain of CD 11/ CD 18 integrins- repeated bacterial infection/ impaired wound healing.

LAD 2: Absent Sialyl Lewis X.

(Milder) (Defective fucosyl transferase).

Defects in Phagocytosis:

- Chediak Higashi Syndrome.
  - AR
- Defective degranulation & delayed microbial killing

- Neutrophils: Giant granules (in P/S) (aberrant organelle fusion), neutropenia.
- Disorder in membrane associated protein which is involved in organelle membrane docking & fusion.
- ↓ transfer of lysosomal enzymes.

1. To phagocytic vacuoles: - ↑ infections
2. Melanocytes - Albinism
3. Cells of CNS - Nerve defects
4. Platelets - Bleeding disorders

Defects of Microbicidal Activity:

Chronic granulomatous diseases: inherited defect in genes encoding several, components of NADPH oxidase (which generates super oxide).

- X linked: most common.
- AR

Others:

- Neutrophil specific granule deficiency.
- Myeloperoxidase deficiency.

Acquired: Thermal injury, malignancy, DM, sepsis, immunodef, etc.

Chemical Mediators:

Mediator	Principal Sources	Actions
Cell-Derived		
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation.
Serotonin	Platelets	Vasodilation, increased vascular permeability.
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever.
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion and activation.
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst.
Reactive oxygen species	Leukocytes	Killing of microbes, tissue damage.
Nitric oxide	Endothelium, macrophages	Vascular smooth muscle relaxation, killing of microbes.
Cytokines (TNF, IL-1)	Macrophages, endothelial cells, mast cells	Local endothelial activation (expression of adhesion molecules), fever/pain/anorexia/hypotension, decreased vascular resistance (shock).
Mediator	Principal Sources	Actions
Chemokines	Leukocytes, activated macrophages.	Chemotaxis, leukocyte activation.
Plasma Protein–Derived		
Complement products (C5a, C3a, C4a)	Plasma (produced in liver)	Leukocyte chemotaxis and activation, vasodilation (mast cell stimulation).
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain.
Proteases activated during coagulation	Plasma (produced in liver)	Endothelial activation, leukocyte recruitment.

IL-1, interleukin-1; MAC, membrane attack complex; TNF, tumor necrosis factor.

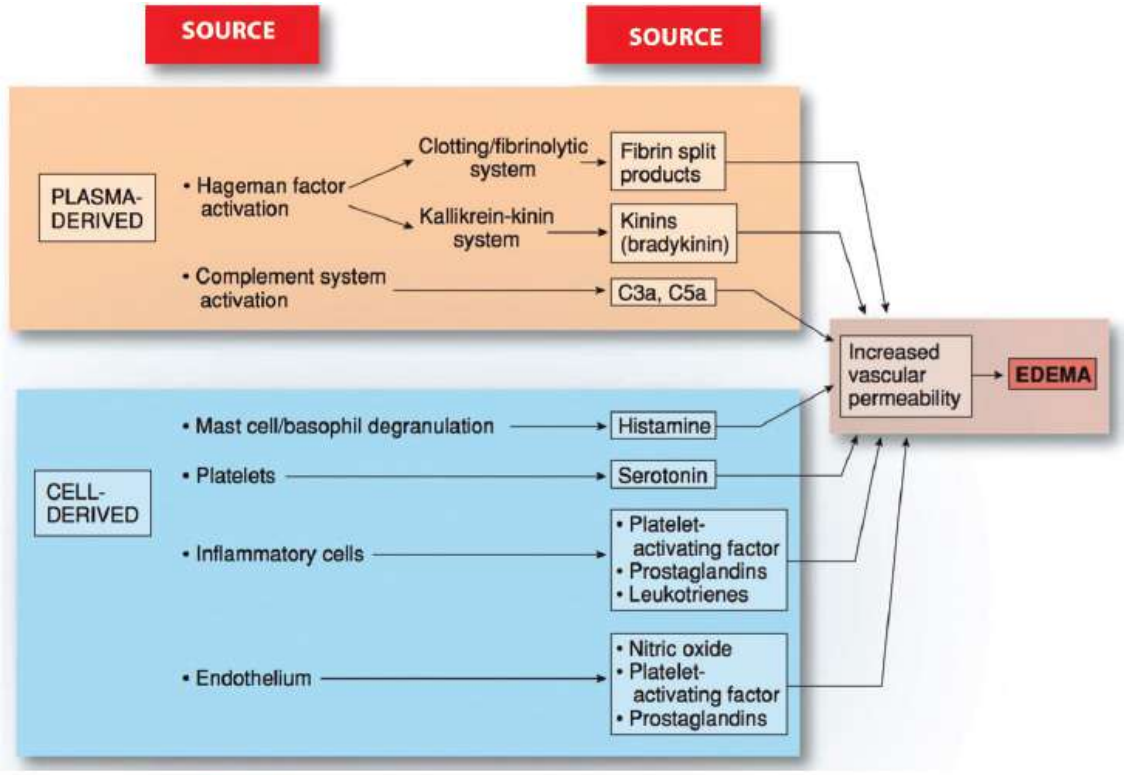


Fig. 3.10: Inflammatory mediators ofn increased vascular permrability.

Histamine:

- Performed stores.
- First to be released.
- Widely distributed.
- Richest source: mast cells (present in connective tissue adjacent to BVs).
- Basophils, Platelets.

Release:

- Physical injury (trauma, heat, cold).
- Immune reacts. (Ab. Binding to mast cells).
- C.comp. (C3α & 5α).
- Histamine releasing proteins.
- Neuropetides (substance P).
- Cytokines (IL-1, IL-8).

Function:

- Dilatation of arterioles (constricts large arteries).
- ↑ vascular permeability of venules.
- Immediate phase of ↑ vasc. Perm (via H_1 receptors).

Serotonin: (5HT):

Actions similar to histamine.

- present in platelets enterochromaffin cells.
- (Platelet aggregation and Release).

Plasma Proteases:

Complement System.

20 component proteins.

- Present in greatest concentration plasma.
- Present as inactive forms (C_1 to C_9).
- Most critical step:

Activation of C_3 by 3 pathways- classical, lectin and alternate

- C3 and C5 can also be activated by Plasmin and lysosomal enzymes also.
  - C_{3a} C_{5a} – Anaphylotoxins.
- Release histamine form mast cells.
  - C_{5a} – Chemotactic to Neutrophils, monocytes, eosinophils, basophils.
  - $C_{3b,}$ & C_{3bi} – Opsonins.

Deficiency of complement:

1. C_3 deficiency – susceptibility to infections which are fatal if not treated.

2. C_2 and C_4 Deficiency. – Association with Autoimmune Diseases e.g. SLE.
3. MAC Deficiency increase susp. To Neisseria organisms.

- C system: closely controlled by protein inhibitors.
- present in host cell membrane.
- Regulation of C_3 & C_5 convertase:
- DAF (Decay accelerating factor).
- Binding to active C comp. by specific proteins in plasma:
  - C1 INH (absent C1 binding to immune complex and also inhibits serine proteases like kallikrein and Hageman factor).
  - MIRL (Membrane. Inhibitor of reactive lysis) – inhibit Membrane attack complex (C_5 -9)

Hereditary Complement Deficiencies	
Complement Deficiency	Clinical Association
C3b, iC3b, C5, MBL	Pyogenic bacterial infections Membranoproliferative glomerulonephritis
C3, properdin, MAC proteins	Neisserial infection
C1 inhibitor	Hereditary angioedema
CD59	Hemolysis, thrombosis
CI q, C1r and C1s, C4, C2	Systemic lupus erythematosus
Factor H and factor I	Hemolytic-uremic syndrome Membranoproliferative glomerulonephritis

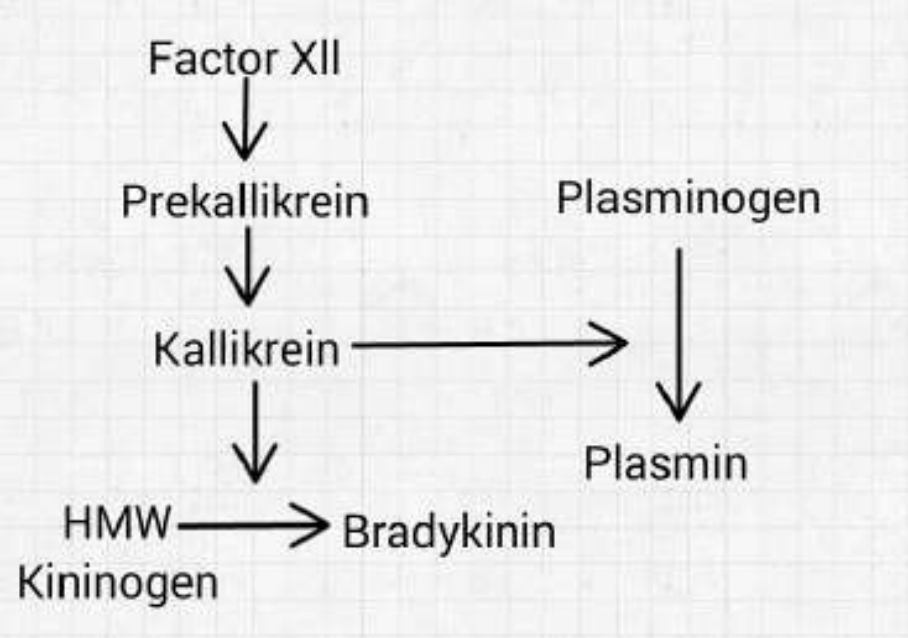
PNH: inability to express phosphatidyl-inositol-linked membrane proteins (DAF, MIRL).

Hereditary Angioneurotic edema : Deficiency of C1 INH (pptd. by emotional stress / trauma).

Episodic edema-skin, extremities, larynx and intestinal mucosa.

Mediator of edema – C2 kinin (proteolytic fragment of C_2) and bradykinin.

Kinin System:



Bradykinin:

- Contraction of smooth muscle.
- Dilation of blood vessels (venules).
- Pain.

Kallikrien:

- Activation of F XII → Autocataytic action.
- Chemotactic activity.
- Converts C_5 to C_{5a}

Clotting System:

- Thrombin: Main link between coagulation system and inflammation.

Thrombin binds to PARs – mobilization of P selectin, production of

Chemokines, expression of endothelial adhesion molecules for integrins,

Induction of COX2.

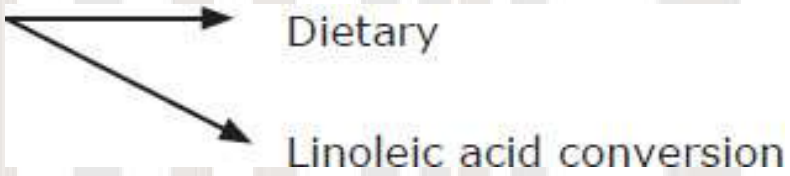
- Fibrinogen Fibrin.
  - vascular permeability.
  - Chemo taxis.

Hageman factor activates:

- Kinin cascade.
- Coagulation Cascade.
- Fibrinolytic cascade.
- Complement cascade.

Arachidonic Acid Metabolites:

AA: 20 C polyunsaturated FA (5, 8, 11, 14- eicostatetraenoic acid).



- Found normally esterified in membrane phospholipids.

AA metabolites → Eicosanoids- bind to G-protein coupled receptors on many cells, and mediate inflammation.

- Cyclo- oxygenase → PG_5 & TX – inhibited by NSAID.
- Lipoxygensae → Leukotriene & Lipoxins.

Cyclooxygenase pathway: - initiated by 2 enzymes COX 1 & COX 2 produce – prostaglandins.

COX1- constitutively expressed in most tissues, also produced in response to inflammatory stimuli. In addition to role in inflammation, also has homeostatic function (e.g. Fluid and electrolyte balance in kidneys, cytoprotection of G1 tract)

COX2 – produced only in response to inflammatory stimuli.

TXA 2: in Platelets (Thromboxane synthase).

- Causes Platelet aggregation and vasoconstriction.
- Unstable, converted to TXB2.

PGI2: in endothelium (Prostacyclin synthetase).

- Vasodilator, inhibits platelet aggregation: ↑ vascular permeability chemo tactic effects.

PGE2 – hyperalgesic.

Lipoxins: Most recent addition. Involve transcellular biosynthesis (involving 2 cell population).

Platelets alone can't form lipoxins (interact with leukocytes).

LXA_4 & LXB_4 → generated by action to platelet 12- Lipoxygenase on neutrophil LTA_4 .

LX: Proinflammatory & anti inflammatory actions.

- O inhibit neutrophil chemotaxis & adhesion.
- Stimulate monocyte adhesion.
- Vasodilatation.
- Endogenous negative regulators of leukotriene activity.

Resolvins: New class of AA mediators.

- inhibit leucocytes recruitment and activation by inhibiting cytokine production.
- Aspirin Also acts by stimulating resolvin production.

Anti-inflammatory therapy:

- Cyclooxygenase inhibitors Aspirin & NSAIDS – inhibit COX (not LO) COX2 inhibitors- newer class of drugs, produce less toxicity than COX1 inhibitors.
- Lipoxygenase inhibitors –newer drugs that inhibit leukotriene production / block leukotriene receptor (Cyst LT1 & cystLT2) used in treatment of asthma.
- Broad spectrum inhibitors – Glucocorticoids:

Down regulate expression of genes for

- COX2.
- Proinflammatory CKs (IL-1 & TNF α).
- Phospholipase A2.
- iNOS.
- Upregulate gene for anti- inflammatory proteins such as lipocortin I (inhibit release of AA from membrane phospholipids).

- Modify dietary lipids: fish oil.
  {(LTs from F.A. in fish oil (Linoleic acid) are less potent than those derived from AA found in most animal/vegetable fats)}

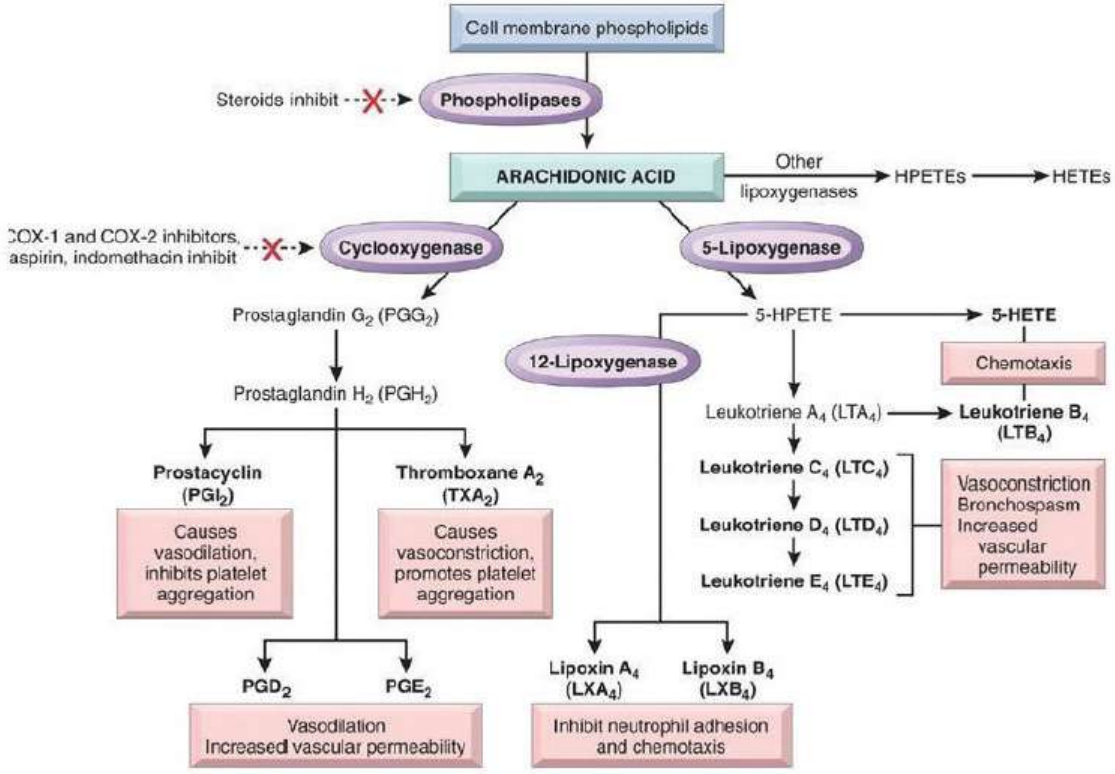


Fig. 3.11

Cytokines:

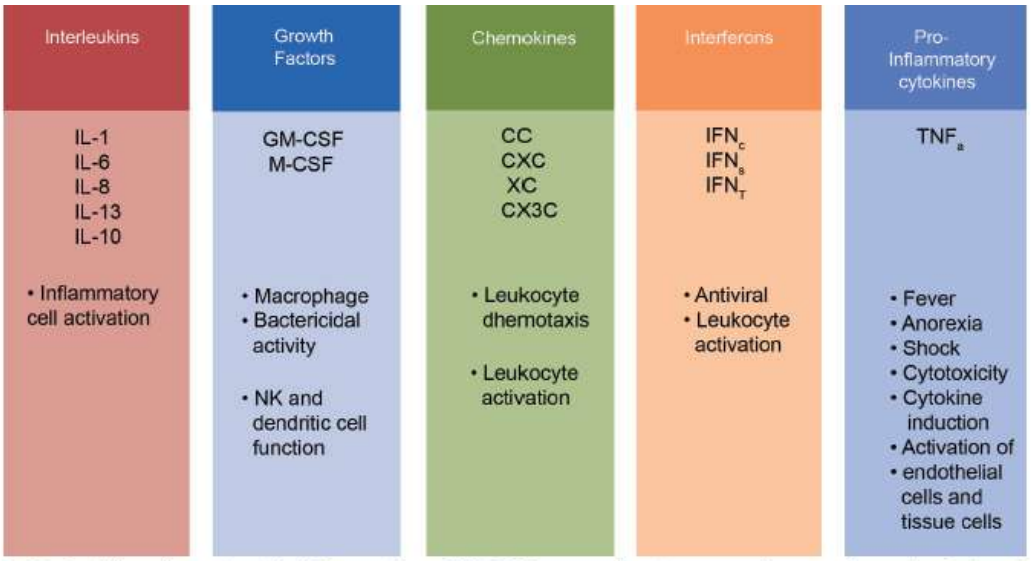


Fig. 3.12: Cytokines important in inflammation. GM-CSF = granulocyte-macrophage colony-stimulating factor, IL= interleukin; NK natural killer; IFN = interferon; TNF = tumor necrosis factor.

Chemokines: - Family of small proteins. Chemo attraction to leucocytes.

Four major classes.

CXC or α chemokine - act on neutrophils eg. $IL_{8.}$

- **C-C or β chemokines** – monocytes chemo attractant protein (MCP -1) macrophage.

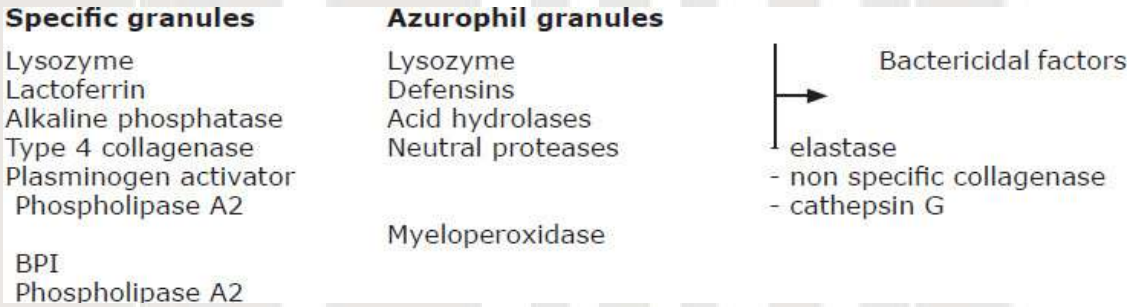
Inflammatory protein 1 α (MIP-1α) eotaxin, RANTES.

Acts on eosinophils, monocytes, basophils and lymphocytes.
Eotaxin selectively recruits eosinophilis

- C - or y chemokines- specific for lymphocyte.
- CX_3 C
- Fractalkine- both adhesion & chemotactic agent.
- Mediate their activities by binding to G protein linked receptor (CXCR/CCR).
- Serpentine receptors.
- Act as viral Co- receptor for HIV ($CXCR_4$, CCR-5).

Nitrous oxide: - 3 different types endothelial, neuronal, cytokine inducible causes. Vasodilator, ↓ platelet adhesion, regulates recruitment of.

- Lysosomal constituents: Specific granules & Azurophil granules.



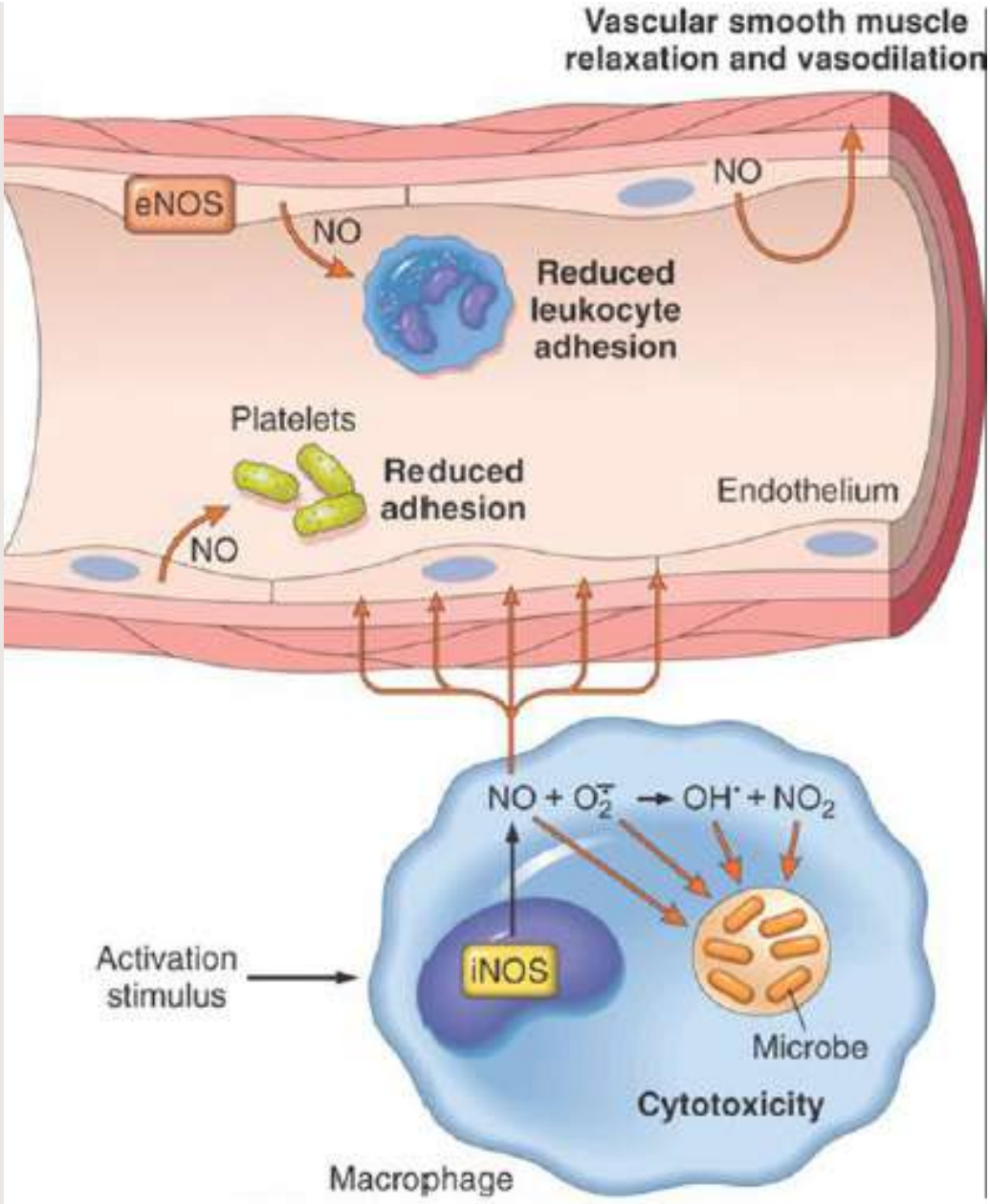


Fig. 3.13:

Harmful proteases kept in check by antiproteases- α1 antitrypsin & α2 macroglobulin.

Outcomes of acute inflammation:

- **Resolution** – clearance of injurious stimuli.
  - Clearance of mediators & acute inflammatory cells.

    - Replacement of injured cells.
    - Normal function.
- **Pus formation (Abscess).**
- **Healing with fibrosis.**
- **Chronic inflammation.**

Chronic Inflammation:

- Prolonged duration with inflammation, tissue repair and destruction.
- Arise during.
    - Persistent infection by micro organisms.
    - Prolonged exposure to potentially toxic agents .
    - Autoimmunity.

Histologic features:

- Infiltration by mononuclear cells- macrophage lymphocyte plasma cell.
- Tissue destruction.
- Healing by connective tissue replacement.

Macrophage- Dominant cell of chronic inflammation.

- Part of mononuclear phagocyte system.
    - Macrophages in different tissues – microglia (CNS), Kupffer cells (Liver), Alveolar macrophages (Lung), osteoclasts (Bone).

Bone marrow stem cell ——
Blood monocyte —— Tissue macrophage

Mechanism of macrophage accumulation in tissues:

- Recruitment of monocytes from circulation- chemotactic stimuli- chemokines (MCP-1), C5a, PDGF, TGF alpha etc.
- Local proliferation in tissue.
- Immobilization at the site of inflammation.

- After accumulation, macrophages get activated. Activated macrophages release a variety of substances, which:
- Kill the injurious agent.
- Cause tissue destruction- hall mark of chronic inflammation.
- Initiate repair.

Chronic Granulomatous Inflammation:

- Distinctive type of chronic inflammation.
- Granuloma is a microscopic aggregation of macrophages that are transformed into epithelium like cells (Epithelioid cells) surrounded by a collar of mononuclear leucocytes principally lymphocytes. Old granulomas may have an enclosing rim of fibrosis.
- Granuloma also has giant cells- Langhans type with horse shoe shaped nuclear arrangement or foreign body type with haphazard nuclear arrangement.
- 2 Types of granulomas.

Foreign body Granuloma:

- Inert foreign bodies – sutures, fibers, talc.
- Foreign material in centre of Granuloma.

Immune Granuloma:

- Insoluble microbes that can induce cell mediated immune response.
- INF gamma important in transformation of activated macrophages into epithelioid cell.

Common Causes:

- Tuberculosis.
- Leprosy.
- Syphilis.
- Cat Scratch disease.

- LGV.
- Sarcoidosis.
- Some fungal infection.
- Berylliosis.

Regeneration And Repair

Cell Surface Receptors and Associated Signal Transduction Systems:

Ceel Surface Receptors:

1. Receptors with intrinsic tyrosinase enzyme activity.
2. Receptors without intrinsic tyrosinase enzyme activity.
3. G protein coupled receptors (Seven spanning receptors).

Signal Transduction Systems:

1. MAP - Kinase pathway-Ras protein.
2. Phosphoinositide – 3 Kinase pathway – Akt protein.
3. Inositol Lipid pathway – Phospholipase C.
4. cAMP pathway → ↑ Protein kinase A.
5. JAK/STAT pathway – JAKS (Janus kinases) phosphorylate STAT (signal transducers activators of transcription).

Transcription Factors:

Signal transduction ⇒ Transfers information to nucleus ⇒ Controlled by transcription factors ⇒ DNA binding domain & Regulatory domain

Activation domain – e.g. cmyc

Repression Domain – e.g. p53 → ↑ CDKI (p21)

Cell Cycle Regulators:

Molecular controls:

- Cyclins (proteins) & Cyclin Dependent Kinases (CDKs).

After completion of function: Cyclins→ degraded by ubiquitin – proteasome pathway.

Restriction point: Surveillance mechanism – G1/S.

Cheek points; Sense problem in DNA replication repair, segregation – G1/S and G2/M.

Growth Inhibition:

- Contact inhibition.
- Growth suppression.

E.g. TGF-β ↑ CDK.

Repair By Fibrosis:

- Formation of new blood vessel.
- Migration and proliferation of fibroblasts.
- Deposition of ECM.
- Maturation and organization of fibrous tissue: Remodeling.
- Granulation tissue pink soft granular appearance on surface of wounds – fibroblastic & vascular endothelial cell proliferation.
- Wound contraction.

Angiogenesis:

- Vasculogenesis: Primitive vascular network during embryonic life; dev from angioblasts.
- Angiogenesis / Neovascularization: Pre – existing vessels send out capillary buds/ sprouts to produce new vessels.
    - Chr. Inflammation.
    - Tissue repair.
    - Malignancies.

Steps:

1. Proteolytic degradation of BM of vessel.
2. Migration of endothelial cells towards angiogenic stimulus.

3. Proliferation of endothelial cells behind leading front.
4. Maturation of endothelial cells: Capillary tube forming.
5. Recruitment of periendothelial cells to support endothelial tubes.

VEGF & Angioproteins (secreted by mesenchymal & stromal cells) – Receptors on endothelial cells.

VEGF + VEGF R2 – new capillary formation (endothelial proliferation).

VEGF + VEGF R1 – Mobilization of endo. Stem cells /? tube formation.

Angiopoietin 1 + tie 2 – recruits periendothelial cells.

Angiopoietin 2 + tie 2 stop signal in absence of VEGF.

PDGF + R – recruitment of smooth muscle cells.

FGF + R – angiogenic factor.

E/c Matrix proteins as regulators of angiogenesis.

1. Integrins – stabilize.
2. matricellular proteins – SPARC, tensacin. Thrombospondin → destabilise C – M interaction, promote angiogenesis.
3. Proteases.
4. Endostatin → (-) angiogenesis.

Fibrosis:

1. Emigration and proliferation of fibroblasts at site of injury - TGF, PDGF, EGF, FGF.
2. Deposition of ECM – Affected by collagen deposition / degradation.

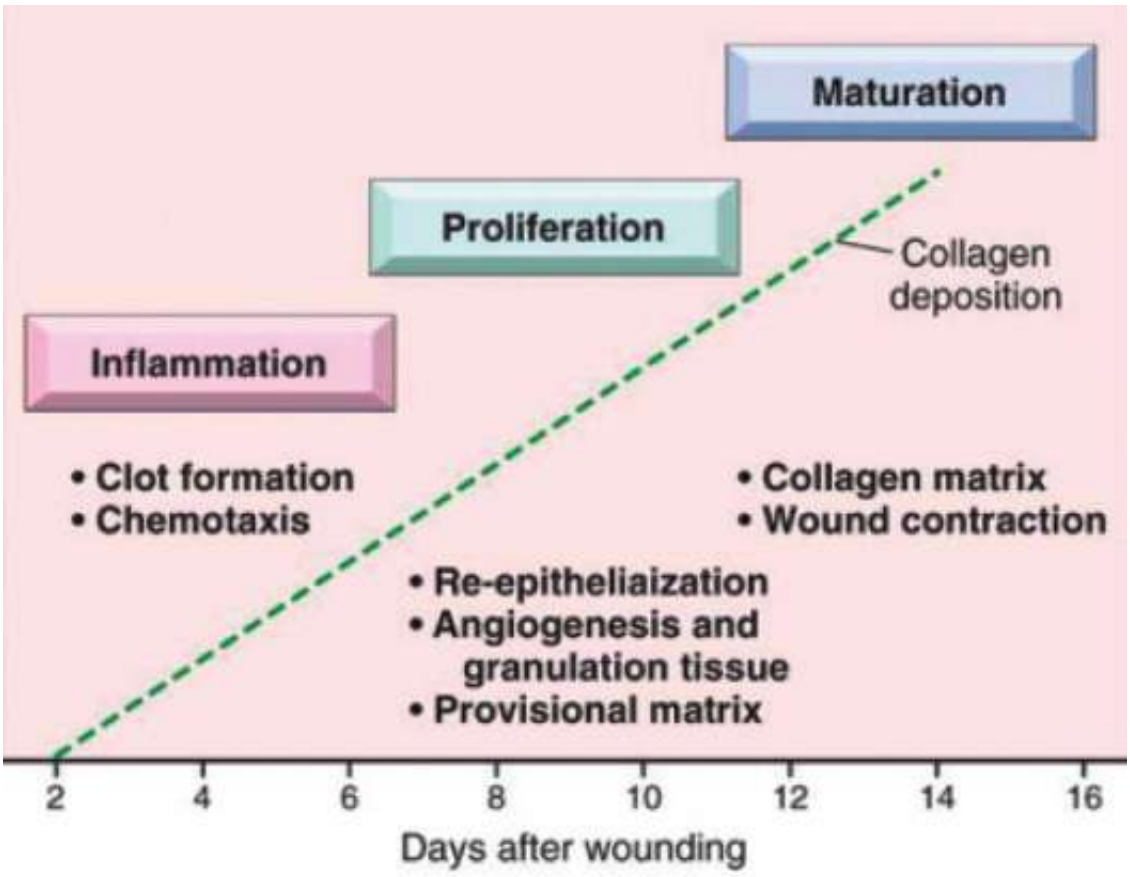


Fig. 3.14

Days after wounding:

1. Wound healing:

a. Regeneration and repair of damaged cells tissues starts as soon as the inflammatory process begins.

b. Wound healing involves two separate processes.

i. Regeneration of the damaged tissue by cells of the same type.

ii. Tissue repair with replacement by connective tissue.

2. Regeneration:

a. Different tissues have different regenerative caacities.

b. Labile cells.

i. Regenerate throughout life.

ii. Examples: surface epithelial cells (skin and mucosal lining cells), hematopoietic cells, stem cells etc.

c. Stable cells.

i. Replicate at a low level throughout life.

ii. Have the capacity to divide if stimulated by some initiating event. They are in Go phase and can be stimulated to enter G_1 phase.

iii. Examples: hepatocytes, proximal tubule cells, endothelium etc.

d. Permanent cells.

i. Cannot replicate.

ii. Example: neurons and cardiac muscle.

3. Tissue repair:

a. Replacement of a damaged area by a connective tissue scar.

b. Tissue repair is mediated by various growth factors and cytokines.

i. Transforming growth factor (TGF-β).

ii. Platelet derived growth factor (PDGF).

iii. Fibroblast growth factor (FGF).

iv. Vascular endothelial growth factor (VEGF).

v. Epidermal growth factor (EDF).

vi. Tumor necrosis factor (TNF-α) and IL-1.

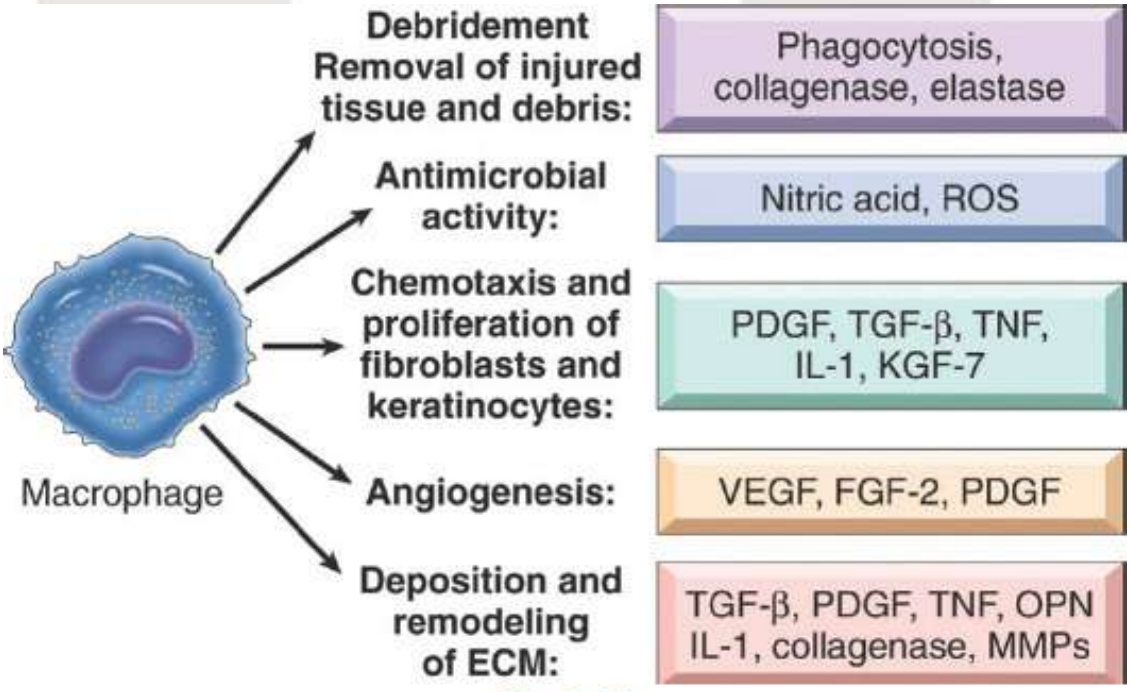


Fig. 3.15

c. Granulation tissue.

i. Synthetically active fibroblasts.

ii. Capillary proliferation.

d. Wound contraction is mediated by myofibroblasts.

e. Scar formation.

Primary union (healing by first intention):

a. Definition: 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.

Description of healing wounds:

A. Healing of a clean, uninfected surgical incision approximated by surgical sutures is k/a.

Primary Union- Healing by first intention.

Involves following changes:

i. O hours- incision filled with clot (fibrin + blood cells).

ii. Within 24 hrs-.

- Neutrophils from margins infiltrate the clot.
- Mitosis begins in epithelial basal cells.

iii. 24 to 48 hrs.

- Below scale a continuous, but thin epithelial layer is formed.

iv. Day 3.

- Neutrophils are replaced by macrophages (MCQ).
- Granulation tissue begins to appear (MCQ).
- Epithelial cell proliferation continues

v. Day 5.

- Incision space is filled with granulation tissue.

- Neovascularisation is maximum (MCQ).
- Collagen fibrils more abundant.
- Epidermis recovers normal thickness with surface keratinization MCQ.

vi. WK2.

- Accumulation of collagen and proliferation of fibroblasts.
- (↓ Leukocyte, ↓ edema, regression of vascular channels).

vii. End of 1 st month or 2 month-Scar comprises of cellular connective tissue.

Tensile Strength:

- 1st wk-Sutures removed – 10%. ↑s over next 4 wks.
- 3rd month – Plateau 70-80% of unwounded skin (through life).
- Collagen – adult skin – type 1; early Granulation tissue type III.

Healing by Secondary intention:

Occurs when there is more extensive loss of tissue as in infarction, inflammatory, ulceration, abscess and large wounds.

Common:

- Large tissue defect.
- Inflammatory reacton more intense.
- Large amount of granulation tissue formed.
- Large scar.

- Wound contraction – Most imp difference b/w 1° and 2° intention.

Remodeling: Balance between collagen deposition and collagenase secretion.

Degradation by Zinc metalloproteinases or collagenase- important for tissue. Remodeling, angiogenesis and cancer metastasis.

- Collagenase produced by – fibroblasts, macrophages, neutrophils, synovial cell and some epithelial cells.
- Activated collagenase inhibited by tissue inhibitor of metalloproteinases.

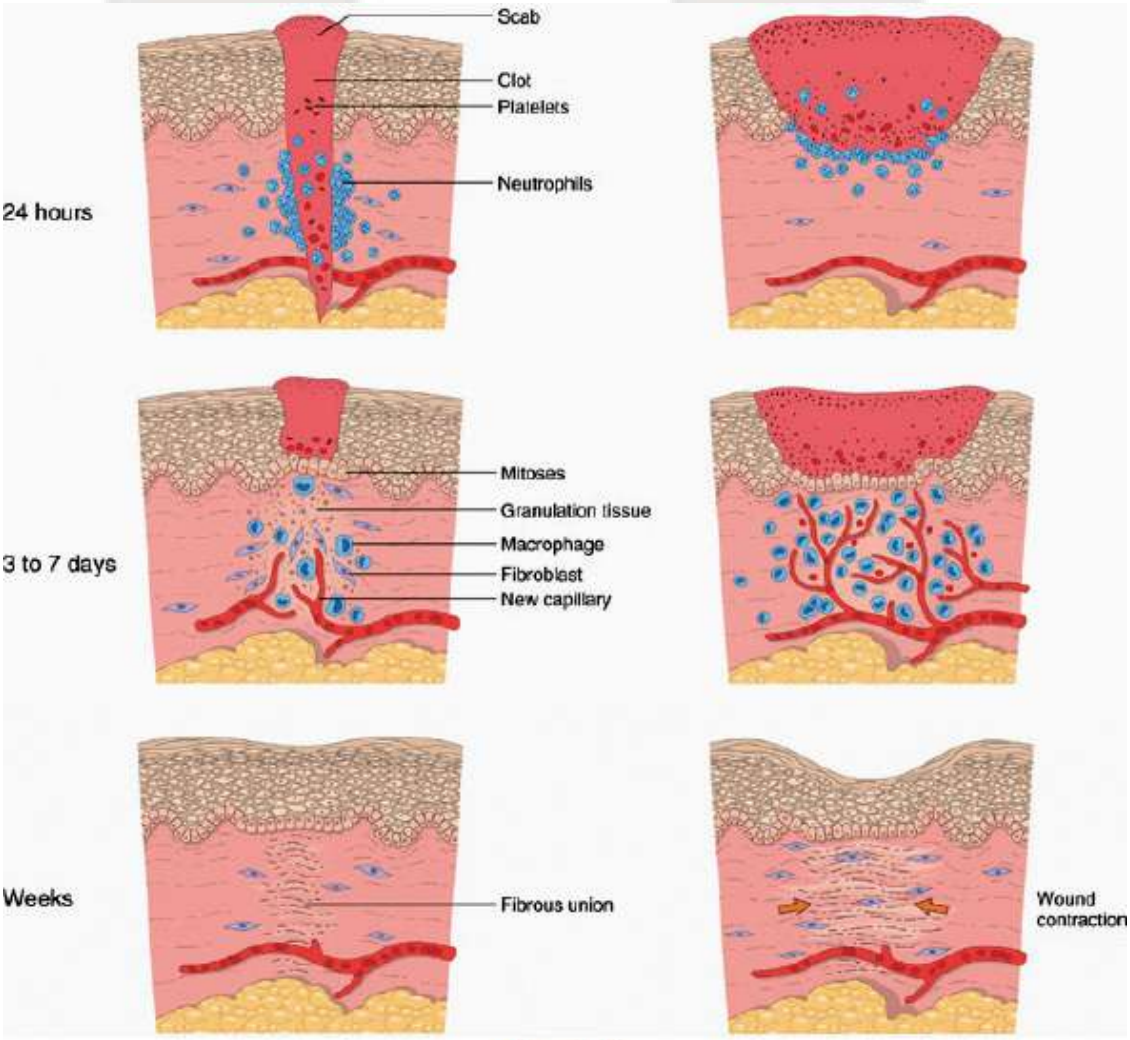


Fig. 3.16

Pathological Aspects of Repair:

Factors promoting Healing	Factors Delaying Wound Healing	
	Systemic	Local
• Vit C	• PEM	• Ischemia
• EGF	• ↓Vit C	• Infection
• PDGF	• Zn ↓	• Irradiation
• MDGF (mph)	• Severe anemia	• Irritant discharge
• Lymphokines	• Bleeding disorder	• Foreign body
• Fibrin	• ↑↑ corticosteroid	• Dead tissue
• Fibronectin	• DM	• Movement of edges
• GH	• Anticancer drugs	• Tension suture
• Estrogen	• Ehler Danlos Syndrome	

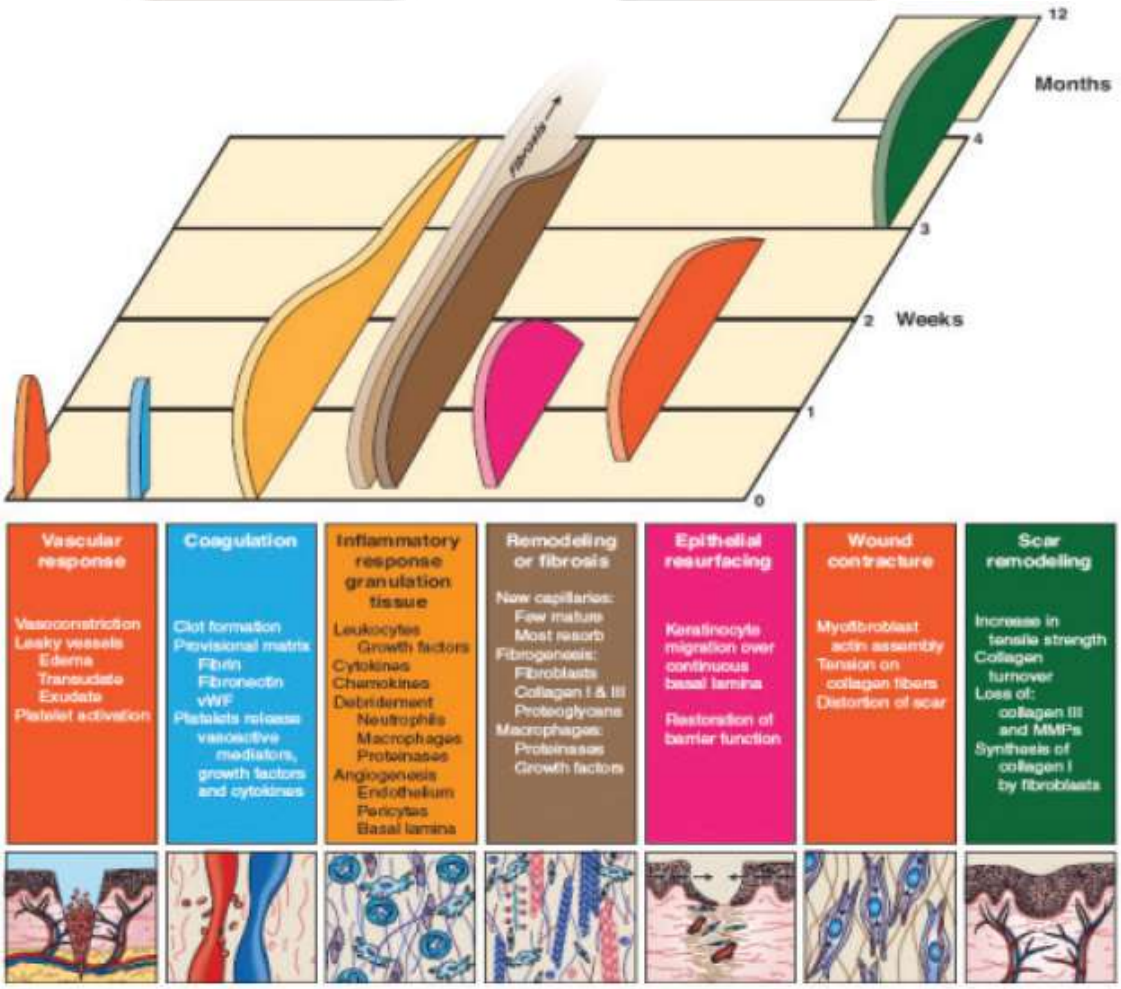


Fig. 3.17: The Sequential phases of the heaing process.

Aberrations in Wound Healing:

1. Delayed wound healing:

a. Wound healing may be prolonged by foreign bodies, infection, ischemia, diabetes, malnutrition, or scurvy.

2. Hypertrophic scar:

a. Results in a prominent scar that is localized to the wound.

b. Excess production of granulation tissue and collagen.

3. Keloid:

a. Genetic predisposition.

b. More common in African Americans.

c. Tends to affect the earlobes, face, neck, sternum, and forearms.

d. May produce large tumor like scars, which often extend beyond the injury site.

e. Excess production of collagen that is predominantly type III.

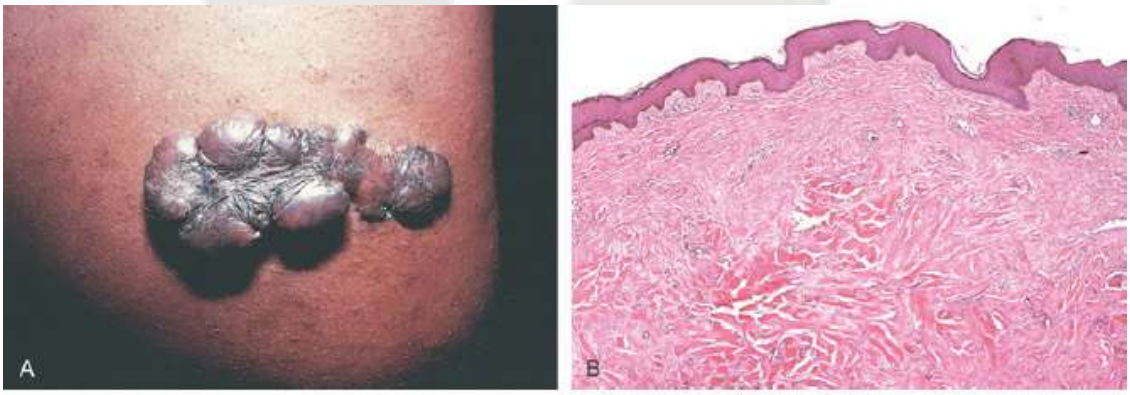


Fig. 3.18

Tissue Expression of Extracellular Matrix Molecules				
Tissue or Body Fluid	Primary Mesodermal Cell	Prominent Collagen Types	Noncollagenous Matrix Proteins	Glycosaminoglycans Proteoglycans (PGs)
Plasma			Fibronectin, fibrinogen, vitronectin	Hyaluronan
Dermis Reticular/ papillary Epidermal junction	Fibroblast	1, III, V, VI, XII VII, XVII (BP 180), anchoring fibrils, hemidesmosome	Fibronectin, elastin, fibrillin	Hyaluronan, decorin, biglycan,versican
Muscle Peri-, epimysium Aortic media/ adventitia	Muscle cell Fibroblast	1, III, V, VI, VIII, XIII	Fibronectin, elastin, fibrillin	Aggrecan, biglycan, decorin, fibromodulin
Tendon	Fibroblast	1, III, V, VI, XII	Fibronectin, tenascin (myotendon junction), elastin, fibrillin	Dacorin, biglycan, fibromodulin, lumican, varsican
Ligament	Fibroblast	I, III, V, VI	Fibronectin, elastin, fibrillin	Dacorin, biglycan, versican
Cornea	Fibroblast	1, III, V, VI, XII		Lumican, keratocan, mimecan, biglycan, decorin
Cartilage	Chondrocyte hypertrophic cartilage	III, IX, VI, VIII, X, XI	Anchorin Cll,fibronectin, tenscin	Hyaluronan, aggrecan, biglycan, decorin, fibromodulin, lu mican, perlecan (minor}
Bone	Osteocyte	l, V	Osteocalcin, ostaoporrtin, bone sialoprotain, SPARC {osteonectin}	Decorin, fibromodulin, biglycan
Basement membrane zones	Epithelial, endothelial adipocytes, Schwann cell, muscle cells (endomysium), pericytes	IV, XV, XVIII	Laminin, nidogen/ entactin	Heparan sulfate proteoglycans, perlecan Collagen XVIII (vascular), agrin

4 Neoplasia

In the premolecular era, the eminent British oncologist Willis came closest: “A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change.”

All tumors have two basic components:

1. Neoplastic cells that constitute the tumor parenchyma and
2. Reactive stoma made up of connective tissue, blood vessels, and variable numbers of cells of the adaptive and innate immune system.

Benign Tumors: A tumor is said to be **benign** when its gross and microscopic appearances are considered relatively innocent, implying that it will remain localized, will not spread to other sites, and is amenable to local surgical removal.

Malignant Tumors: Malignant tumors are collectively referred to as **cancers** , derived from the Latin word for crab, because they tend to adhere to any part that they seize on in an obstinate manner. Malignant tumors can invade and destroy adjacent structures and spread to distant sites (metastasize) to cause death.

Most important differentiating feature of benign vs. malignant (*).

Presence of metastasis > invasiveness.

Differentiation refers to the extent to which neoplastic parenchymal cells resemble the corresponding normal parenchymal cells, both morphologically and functionally; lack of differentiation is called **anaplasia.** Lack of differentiation, or anaplasia, is considered a hallmark of malignancy. The term anaplasia means “to form

backward," implying a reversal of differentiation to a more primitive level.

Lack of differentiation, or anaplasia, is often associated with many other morphologic changes:

1. Pleomorphism and tumor giant cells.
2. Abnormal nuclear morphology: a N:C ratio approaching 1:1 instead of usual 1:4 or 1:6; macro nucleoli and hyper chromatic nuclei.
3. Mitoses: atypical bizarre mitotic figures
4. Loss of polarity: orientation to the basement membrane is disturbed.
5. Ischemic necrosis, mostly in central areas.

The first step toward neoplasia is cellular transformation. The chronic irritation from cigarette smoke has led to an exchanging of one type of epithelium (the normal **respiratory epithelium** at the right) for another (the more resilient **squamous epithelium** at the left). Thus, there is metaplasia of normal respiratory laryngeal epithelium to squamous epithelium in response to chronic irritation of smoking.

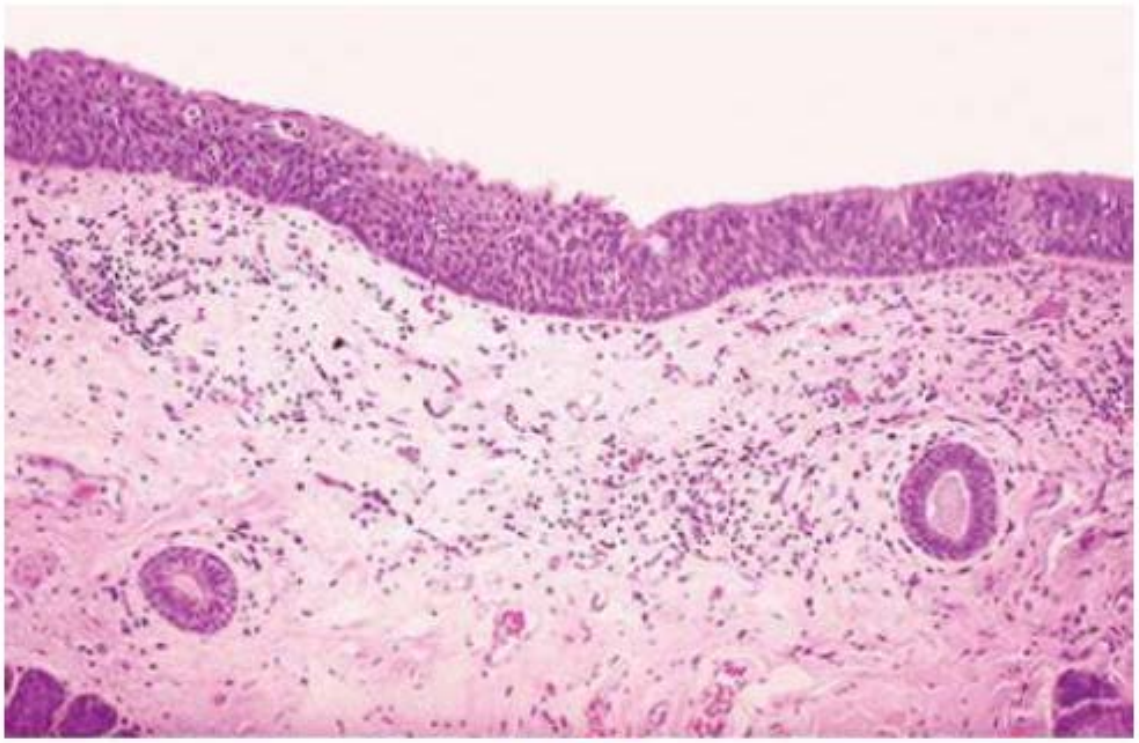


Fig. 4.1

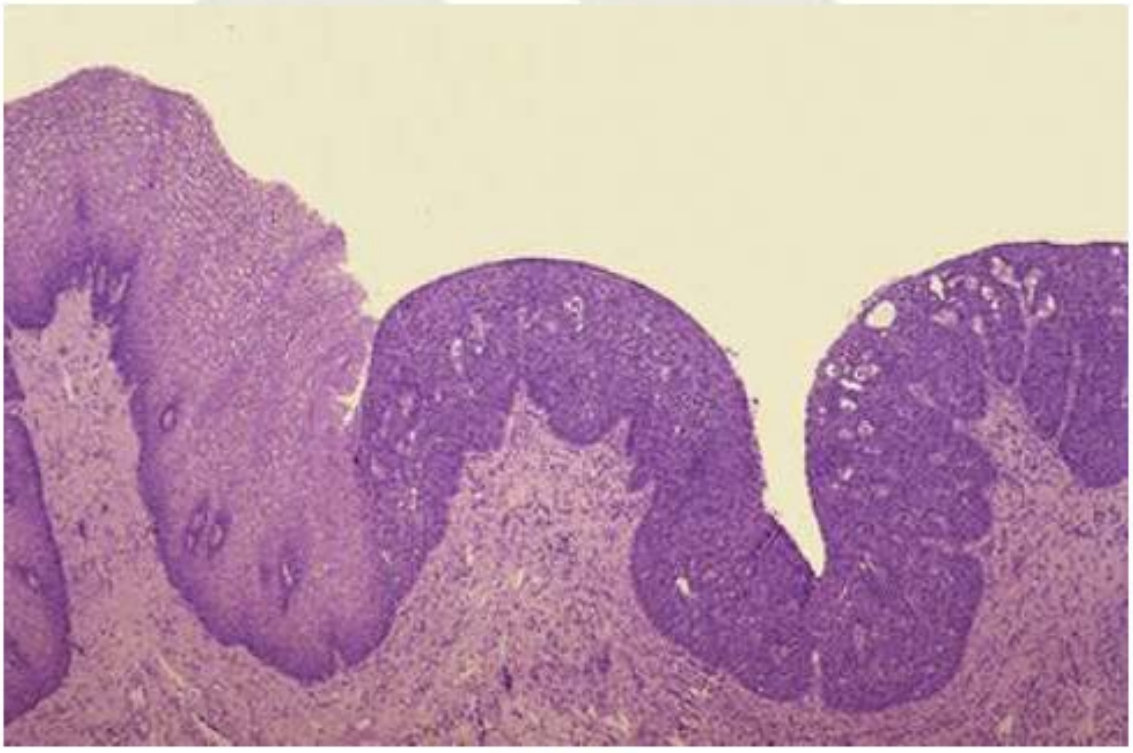


Fig. 4.2

This is the next step toward neoplasia. Here, there is **normal cervical squamous epithelium** at the left, but **dysplastic squamous epithelium** at the right. The dysplastic epithelial cells are darker, smaller, and more crowded, without an orderly process of maturation. Dysplasia is a disorderly growth of epithelium, but still confined to the epithelium. Dysplasia is still reversible.

Dysplasia: a term that literally means “disordered growth.”

It is encountered principally in epithelia and is characterized by a constellation of changes that include loss in the uniformity of the individual cells as well as loss in their architectural orientation**.**

Dysplastic cells may exhibit considerable pleomorphism and often contain large hyper chromatic nuclei with a high nuclear-to-cytoplasmic ratio.

The architecture of the tissue may be disorderly.

For example, in dysplastic squamous epithelium the normal progressive maturation of tall cells in the basal layer to flattened squares on the surface may fail in part or entirely, leading to replacement of the epithelium by basal-appearing cells with hyper chromatic nuclei. In addition, mitotic figures are more abundant than in the normal tissue and rather than being confined to the basal layer may instead be seen at all levels, including surface cells.

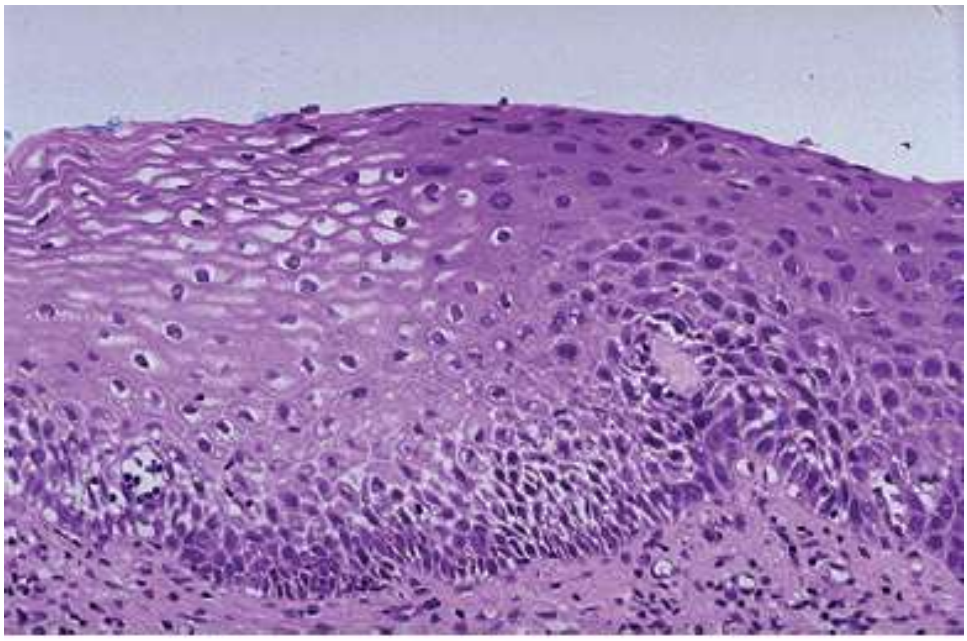


Fig. 4.3

At high magnification, the **normal cervical squamous epithelium** at the left merges into the **dysplastic squamous epithelium** at the right in which the cells are more disorderly and have darker nuclei with more irregular outlines.

Although dysplasia may be a precursor to malignant transformation, it does not always progress to cancer. With removal of the inciting causes, mild to moderate dysplasia that do not involve the entire thickness of epithelium may be completely reversible.

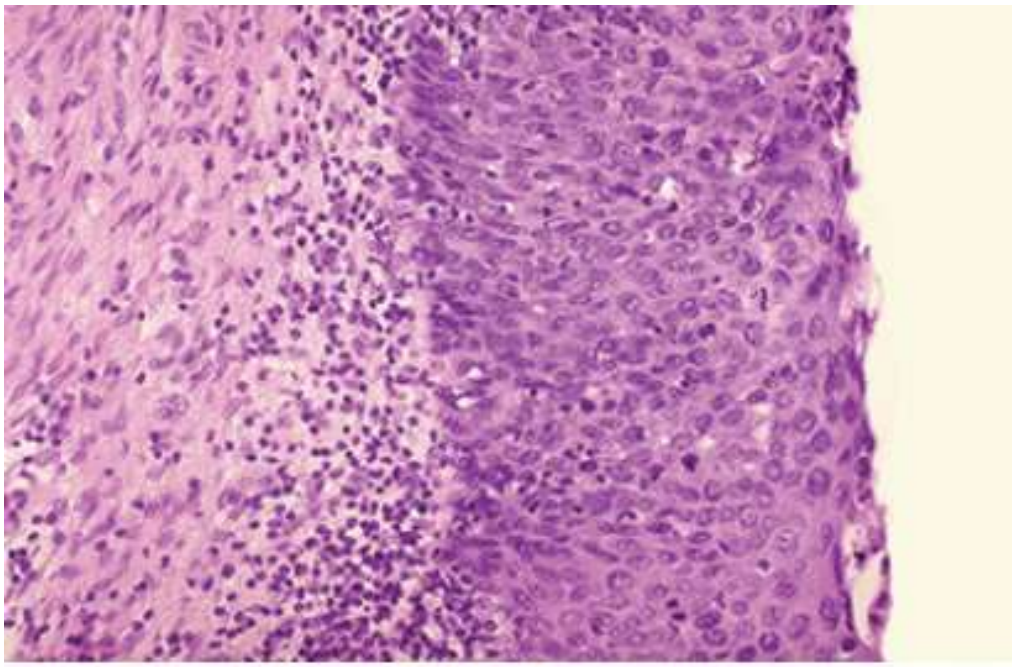


Fig. 4.4

When an entire portion of epithelium is composed of abnormal cells and no normal epithelial cells remain, and the process is not potentially reversible, then the process has gone beyond dysplasia and is now neoplasia, which is loss of control of the cellular proliferative process. If the **basement membrane** is still intact, as shown here, then the process is called «carcinoma in situ» because the carcinoma is still confined to the epithelium. A neoplasm arising in epithelium is termed as a carcinoma.

A benign neoplasm looks a lot like the tissue with normal cells from which it originated, and has a slow growth rate. Benign neoplasms do not invade surrounding tissues and they do not metastasize. Thus, characteristics include:

- Slow growth.
- Resemblance to tissue of origin (well differentiated).
- Circumscription.
- Lack of invasion.
- Absence of metastases.

A hamartoma is a peculiar benign neoplasm which is a localized but haphazard growth of tissues normally found at a given site (pulmonary hamartoma has jumbled cartilage, bronchial epithelium, and connective tissue).

A choristoma is a benign neoplasm consisting of tissue that is not normal to the site of origin (e.g., salivary gland choristoma of the middle ear).

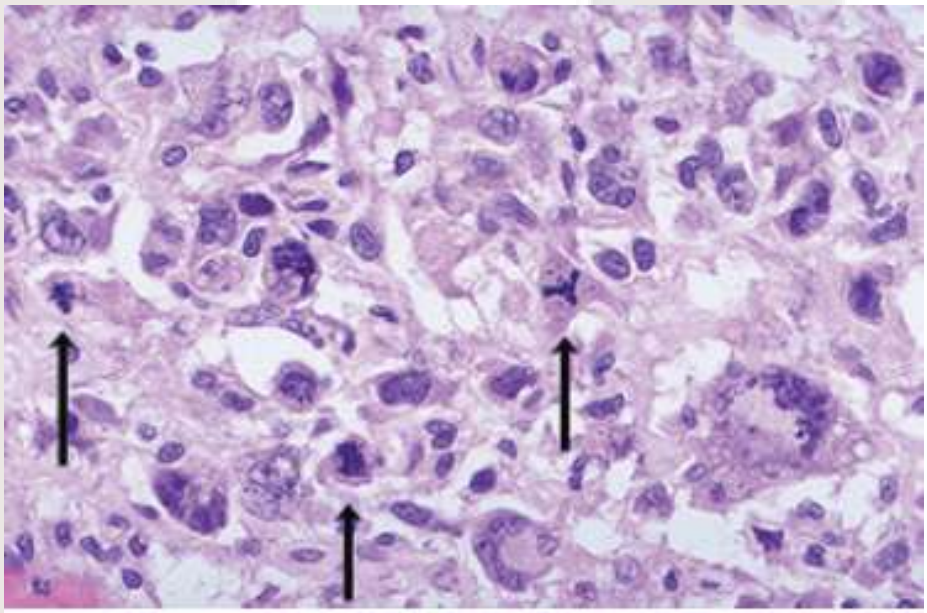


Fig. 4.5

Here are three abnormal mitoses. Mitoses by themselves are not indicators of malignancy. However, abnormal mitoses are highly indicative of malignancy. The marked pleomorphism and hyperchromatism of surrounding cells also favors malignancy.

Metastasis:

Metastasis is defined by the spread of a tumor to sites that are physically discontinuous with the primary tumor, and unequivocally marks a tumor as malignant, as by definition benign neoplasms do not metastasize**.**

All malignant tumors can metastasize, but some do so very infrequently.

Examples include malignant neoplasms of the glial cells in the central nervous system, called **gliomas** , and basal cell carcinomas of the skin.

Pathways of Spread:

Dissemination of cancers may occur through one of three pathways:

1. Direct seeding of body cavities or surfaces,
2. Lymphatic spread (**most common**), and
3. Hematogenous spread.

A sentinel lymph node is defined as “the first node in a regional lymphatic basin that receives lymph flow from the primary tumor.”

Sentinel node mapping can be done by injection of radiolabeled tracers or colored dyes, and examination of frozen sections of the sentinel lymph node performed during surgery can guide the surgeon to the appropriate therapy. Sentinel node examination has also been used for detecting the spread of melanomas, colon cancers, and other tumors.

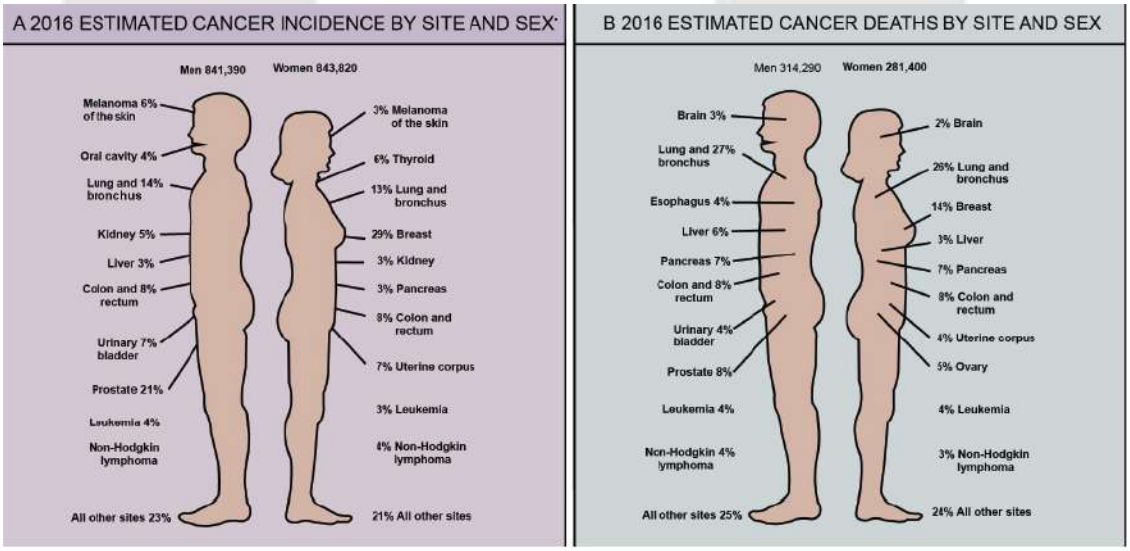


Fig. 4.6

Arteries, with their thicker walls, are less readily penetrated than are veins.

Understandably the liver and the lungs are most frequently involved in such hematogenous dissemination, because all portal area drainage flows to the liver and all caval blood flows to the lungs. Cancers arising in close proximity to the vertebral column often embolize through the paravertebral plexus, and this pathway is involved in the frequent vertebral metastases of carcinomas of the thyroid and prostate.

Occupational cancers:

Agents or Groups of Agents	Human Cancers for Which Reasonable Evidence is Available	Typical Use or Occurrence
Arsenic and arsenic compounds	Lung carcinoma skin carcinoma.	By-product of metal smelling; component of alloys. electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips.
Asbestos	Lung, esophageal, gastric, and colon carcinoma; mesothelioma.	Formerly used for many applications because of fire, heal and friction resistance: still found in easting construction as well as fire-resistant testes, friction materials (i.e., brake linings), underlayment and renting papers, and floor ties.
Benzene	Acute myeloid leukemia	Principal component of light oil; despite known risk many applications east in printing and lithography. paint rubber, dry clearing, adhesives and coatings, and detergents; formerly widely used as soften) and fumigant.
Beryllium and beryllium compounds	Lung carcinoma	Missile fuel and space vehicles. Hardener for lightweight metal alloys. particularly in aerospace applications and nuclear reactors.
Cadmium and cadmium compounds	Prostate carcinoma	Uses include yellow pigments and phosphors; found In solders; used in batteries and as alloy and in metal plating's and coatings.
Chromium compounds	Lung carcinoma	Component of metal alloys, paints, pigments, and preservatives.
Nickel compounds	Lung and oropharyngeal carcinoma.	Nickel plating: component of ferrous alloys. ceramics, and batteries; by-product of stainless-steel and welding.
Radon and its decay products	Lung carcinoma	From decay of minerals containing uranium; potentially serious hazard in quarries and underground mines.
Vinyl chloride	Hepatic angiosarcoma	Refrigerant; monomer for vinyl polymers: adhesive for pasties; formerly inert aerosol propellant in pressurized containers.
Modified from Stellman JM, Stelman SD: Cancer and workplace, CA Cancer J Cin 1996; 46:70.		

Chronic inflammatory states and cancer:

Pathologic Condition	Associated Neoplasm(s)	Etiologic Agent
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibers, silica particles
Inflammatory bowel disease	Colorectal carcinoma	
Lichen sclerosis	Vulvar squamous cell carcinoma	
Pancreatitis	Pancreatic carcinoma	Alcoholism, germ line mutations {e.g., in (ho trypsinogen gene}
Chronic cholecystitis	Gallbladder cancer	Bile acids, bacteria, gallbladder atones
Reflux esophagitis, Barrett esophagus	Esophageal carcinoma	Gastric acid
Sjögren syndrome, Hashimoto thyroiditis	MALT lymphoma	
Opistorchis, cholangitis	Cholangiocarcinoma, colon carcinoma	Liver flukes (Opisthorchis viverrini)
Gastritis/ulcers	Gastric adenocarcinoma, MALT lymphoma	Helicobacter pylori
Hepatitis Hepatocellular carcinoma		Hepatitis B and/or C virus
Osteomyelitis	Carcinoma in draining sinuses	Bacterial infection
Chronic cervicitis	Cervical carcinoma	Human papillomavirus
Chronic cystitis	Bladder carcinoma	Schistosomiasis
Adapted from Tlsty TD, Coussens LM: Timor stroma and regulation of cancer development. Ann Rev Pathol Mech Dis 2006; 1:119.		

Molecular Basis of Cancer:

Certain “genomic themes” haveemerged that are likely relevant to every cancer.

1. Nonlethal genetic damage lies at the heart of carcinogenesis.
2. Tumor is formed by the clonal expansion of a single precursor cell that has incurred genetic damage (i.e., tumors are clonal).

3. Four classes of normal regulatory genes—the growth promoting proto-oncogenes, the growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes involved in DNA repair—are the principal targets of cancer causing mutations.
4. Carcinogenesis results from the accumulation of complementary mutations in a stepwise fashion over time.

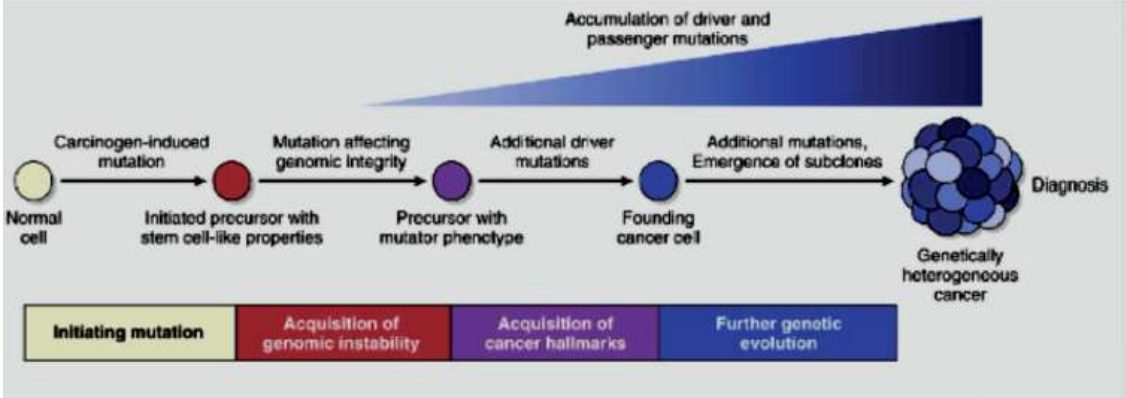


Fig. 4.7

Hallmarks of Cancer:

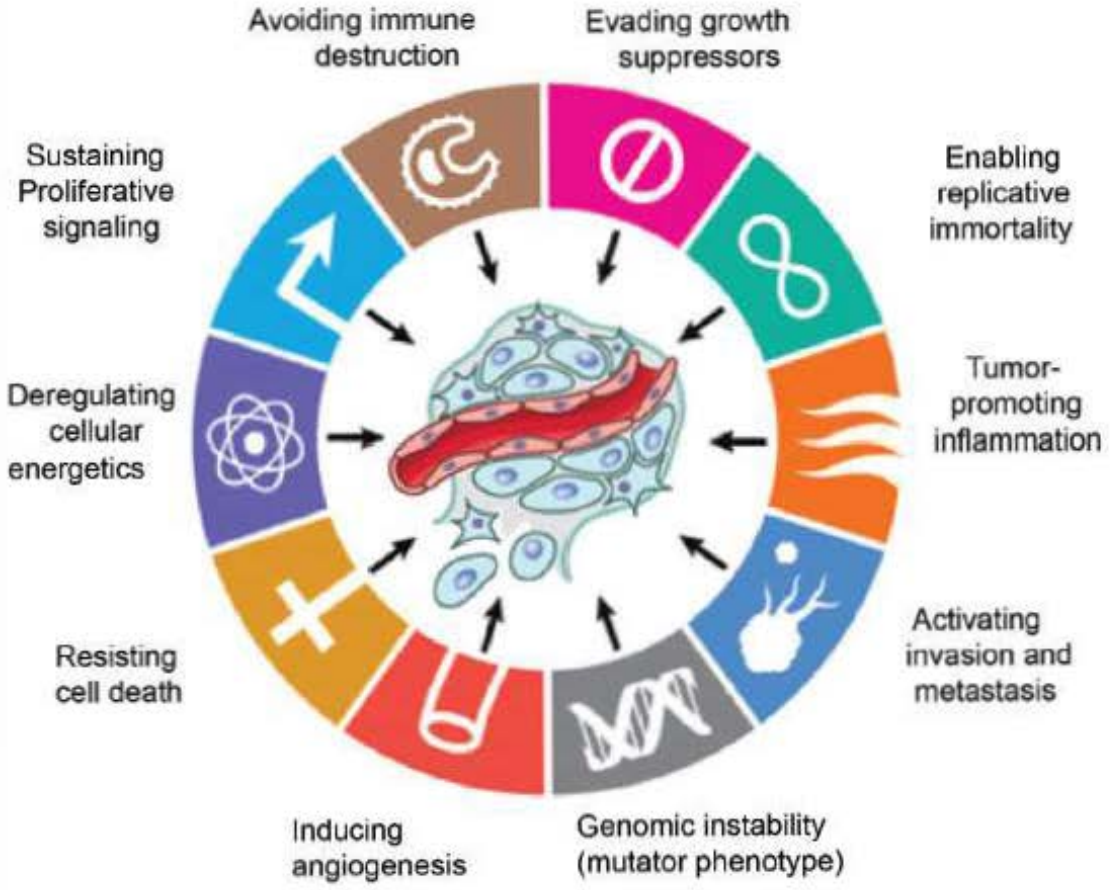

Avoiding immune destruction
Evading growth suppressors
Sustaining Proliferative signaling
Enabling replicative immortality
Deregulating cellular energetics
Tumor-promoting inflammation
Resisting cell death
Activating invasion and metastasis
Inducing angiogenesis
Genomic instability (mutator phenotype)

Fig. 4.8

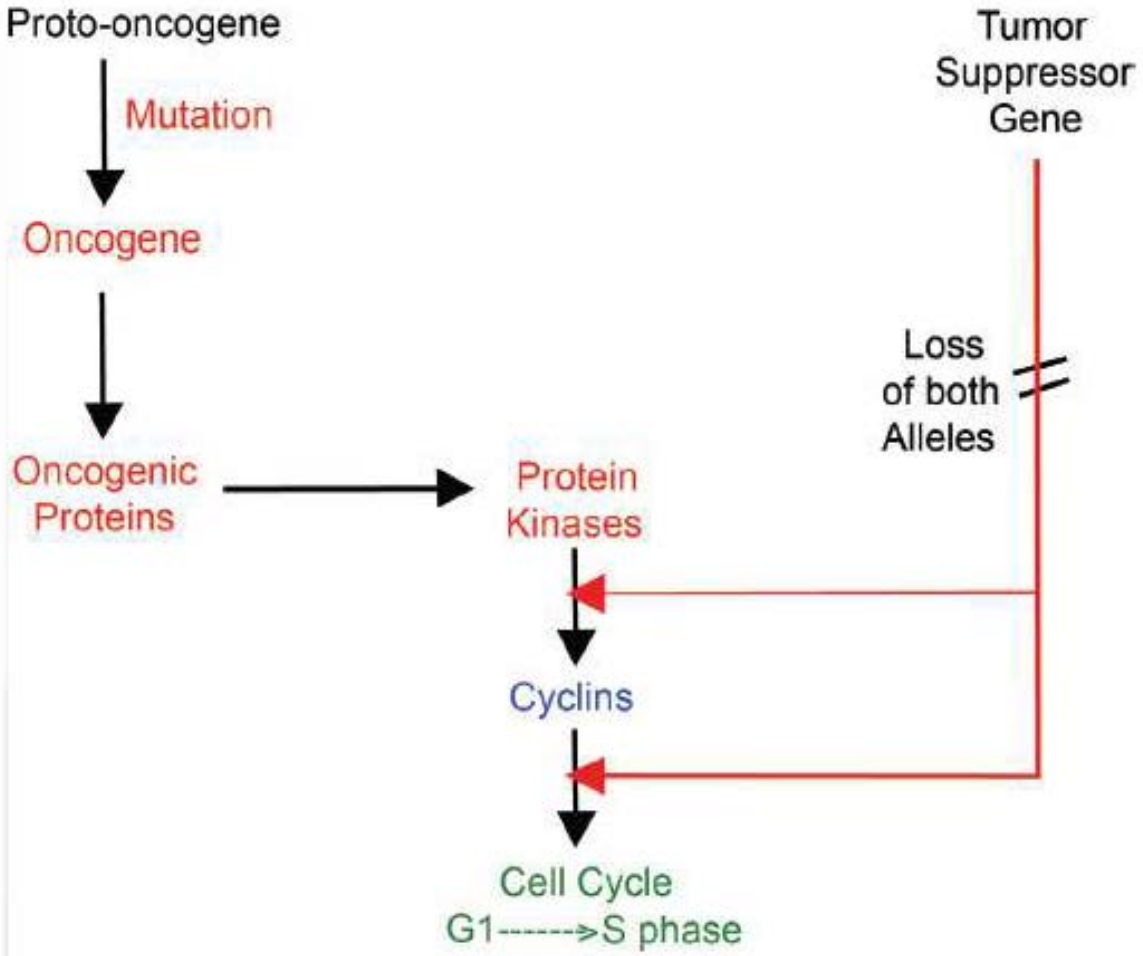

Proto-oncogene
Mutation
Oncogene
Oncogenic Proteins
Protein Kinases
Tumor Suppressor Gene
Loss of both Alleles
Cyclins
Cell Cycle
G1------>S phase

Fig. 4.9

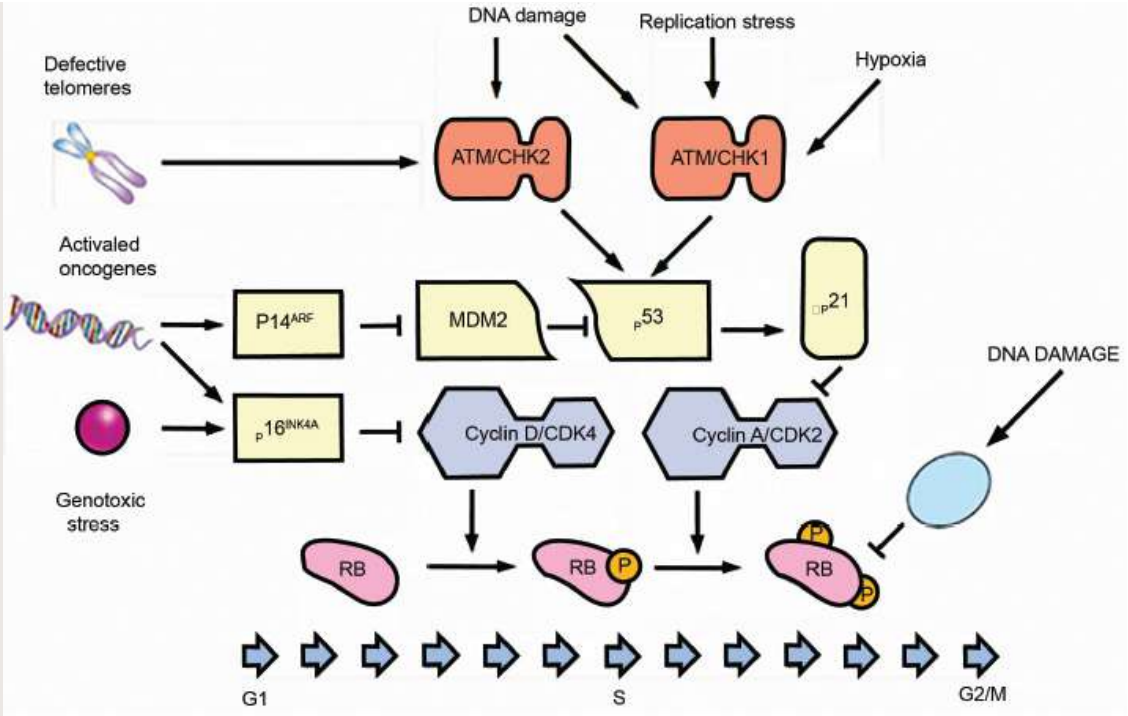


Fig. 4.10

Gl pathways that can trigger cell cycle arrest, senescence, or apoptosis. A variety of threats to genomic integrity lead to activation of pathways that result in cell cycle arrest. Signalling via ATM/CHK2 and ATR/CHKl leads to p53 activation, among other effects. One of the principle downstream effects of p53 is activation of p2 1 expression, with resultant cyclin E (A)/CDK2 inhibition and cell cycle arrest. Senescence, which results in a more sustained cell cycle exit, also involves the up-regulation of p 1 4ARF and p 1 61NK4A. Both proteins ultimately lead to retinoblastoma protein (RB) activation and Gl arrest via cyclin/CDK inhibition. In response to DNA damage during S phase, activation of PP2A can lead to dephosphorylation of RB and inhibition of DNA synthesis.

Inherited Genome Maintenance Defects with Cancer Predisposition:

Maintenance Mechanism	Syndrome	Gene Defect
Checkpoint response	Li Fraumeni Familial breast cancer	p53, CHK2
	Retinoblastoma	BRCA1, CHK2
	Familial melanoma	RB
		$p16^{INK4A}$
Mismatch repair	HNPCC/Lynch syndrome	MLH1, MSH2,
		PMS2, MSH6
Nucleotide excision repair	Xeroderma pigmentosa	XP genes
DSB response/repair	Ataxia telangiectasia	ATM
	AT-like disorder	MR Ell
	Nijmegen breakage	NBS1
	Fanconi anemia	Pane genes
	Familial breast cancer	BRCA1, BRCA2
		CHK2, PALB2
SCID, rare lymphoma Artemis		
	SCID, rare leukemia	LigaseIV
Helicase activity	Bloom	BLM
	Werner	WRN
	Rothmund Thomson	RECQ4
Mitotic checkpoint	Mosaic variegated aneuploidy	BUB1B
HNPCC, hereditary nonpolyposis colorectal cancer; DSB, double-strand break; AT, ataxia telangiectasia; SCID,severe combined immunodeficiency.		

Oncogenesis		
Mechanism	Action	Example
Growth Promotion	Overexpression of growth factor receptors (such as epidermal growth factor, or EGF) making cells more sensitive to growth stimuli	HER2 (c-erb-B2)
	Increased growth factor signal transduction by an oncogene that lacks the GTPase activity that limits GTP induction of cytoplasmic kinases that drive cell growth	RAS
	Overexpression of a gene product by stimulation from an oncogene (such as RAS)	C-SIS
	Lack of normal gene regulation through translocation of a gene where it is controlled by surrounding genes to a place where it is no longer inhibited	BCR-ABL
	Binding of oncogene product to the nucleus with DNA transcriptional activation to promote entry into the cell cycle	C-MYC
Loss of Tumor Suppressor Gene Function	Loss of normal growth inhibition	BRCA-1
	Lack of regulation of cell adhesion with loss of growth control through cell interaction	APC
	Loss of down-regulation of growth promoting signal transduction	NF-1
	Loss of regulation of cell cycle activation through sequestration of transcriptional factors	RB
	Loss of regulation of cell cycle activation through lack of inhibition of cell proliferation that allows DNA repair	p53
Limitation of Apoptosis	Overexpression of gene, activated by translocation, prevents apoptosis	BCL-2

Oncogenes:

Genes that promote autonomous cell growth in cancer cells are called oncogenes, and their unmutated cellular counter parts are called proto-oncogenes. Oncogenes are created by mutations in proto-oncogenes and encode proteins called oncoproteins that have the ability to promote cell growth in the absence of normal growth-promotingsignals.

Oncogenes most commonly and importantly implicated (**very important table for exams**).

Growth Factors			
PDGF-βchain	PDGFB	Overexpression	Astrocytoma
Fibroblast growth factors	HST1	Overexpression	Osteosarcoma
	FGF3	Amplification	Stomach cancer
			Bladder cancer
			Breast cancer
			Melanoma
TGF-α	TGFA	Overexpression	Astrocytomas

HGF Overexpression Hepatocellular carcinomas Thyroid cancer:

Growth Factor Receptors			
EGF-receptor family	ERBB1 (EGFR) ERBB2 (HER)	Mutation Amplification	Adenocarcinoma of lung Breast carcinoma.
FMS-Like tyrosine kinase 3	FLT3	Point mutation	Leukemia.
Receptor tor neurotrophic factors	RET	Point mutation	Multiple endocrine neoplasia 2A and B. familial medullary thyroid carcinomas.
PDGF receptor	PDGFRB	Overexpression, translocation	Gliomas, leukemias.
Receptor for KIT ligand	KIT	Point mutation	Gastrointestinal stromal tumors, seminomas, leukemias.
ALK receptor	ALK	Translocation, fusion gene formation Point mutation	Adenocarcinoma of lung, certain lymphomas Neuroblastoma.

Proteins Involved in Signal Transduction			
GTP-binding (G| proteins	KRAS	Point mutation	Colon, lung, and pancreatic tumors.
	HRAS	Point mutation	Bladder and kidney tumors.
	WHS	Point mutation	Melanomas, hematologic malignancies.
	GNAO	Point mutation	Uveal melanoma.
	GNAS	Point mutation	Pituitary adenoma, other endocrine tumors.
Nonreceptor tyrosine	ABL	Translocation	Chronic myelogenous leukemia.
kinase		Point mutation	Acute lymphoblastic leukemia.
RAS signal transduction	BRAF	Point mutation, Translocation	Melanomas, leukemias, colon carcinoma, others.
Notch signal transduction	N0TCH1	Point mutation, Translocation	Leukemias, lymphomas* breast carcinoma.
		Gene rearrangement	
JAK/STAT signal	JAK2	Translocation	Myeloproliferative disorders.
transduction			Acute lymphoblastic leukemia.

Growth factor signaling pathways in cancer. Growth factor receptors, RAS, PI3K, MYC, and D cyclins are oncoproteins that are activated by mutations in various cancers. GAPs apply brakes to RAS activation, and PTEN serves the same function for PI3K.

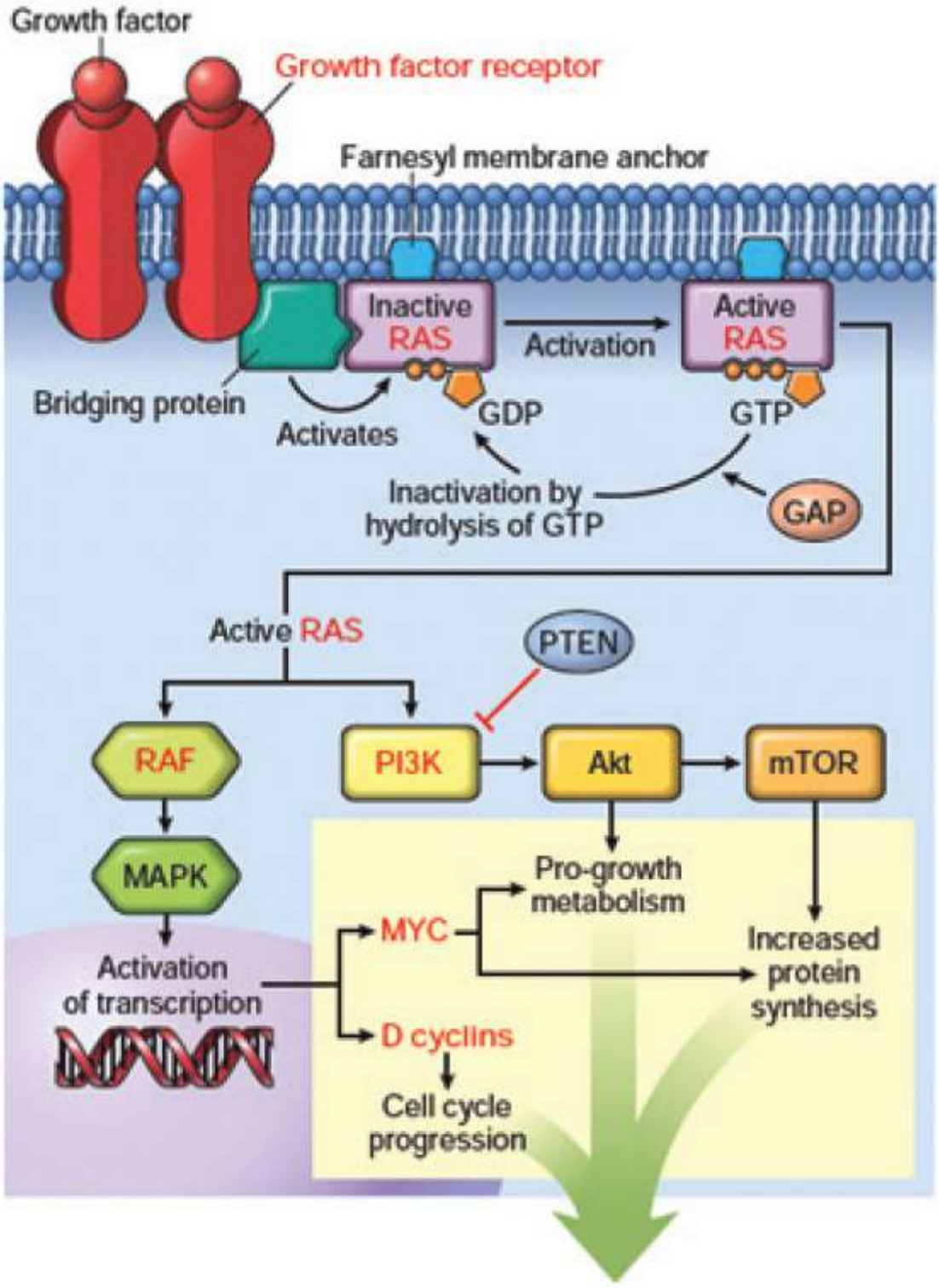


Fig. 4.11

Growth factor signaling pathways in cancer. Growth factor receptors, RAS, PI3K, MYC, and D cyclins are oncoproteins that are activated

by mutations in various cancers. GAPs apply brakes to RAS activation, and PTEN seres the same function for PI3K.

RAS Mutations:

- Point mutations of **RAS** family genes constitute the most common type of abnormality involving proto-oncogenes in human tumors.
- Approximately 15% to 20% of all human tumors express mutated RAS proteins.
- RAS proteins are members of a family of membrane-associated small G proteins that bind guanosine nucleotides (guanosine triphosphate [GTP] and guanosine triphosphate [GDP]), similar to the larger trimolecularG proteins. They normally flip back and forth between an excited signal-transmitting state in which they are bound to GTP and a quiescent state in which they are bound to GDP.
- Stimulation of receptor tyrosine kinases by growth factors leads to exchange of GDP for GTP and subsequent conformational changes that generate active RAS, which in turn stimulates both the MAPK and PI3K/AKT arms of the receptor tyrosine kinase signaling pathway.
- Activation of RAS is transient because RAS has an intrinsic GTPase activity that is accelerated by **GTPase-activating proteins (GAPs)** , which bind to the active RAS and augment its GTPase activity by more than 1000-fold, thereby terminating signal transduction. Thus, GAPs prevent uncontrolled RAS activity.
- The consequences of gain-of-function mutations in RAS proteins should be mimicked by loss-of-function mutations in GAPs that normally restrain RAS activity.
- Indeed, disabling mutations of neurofibromin 1, a GAP encoded by the **NF1** gene, are associated with the inherited cancer syndrome **familial neurofibromatsis type 1.**

Oncogenic BRAF and PI3K Mutations:

- **Mutations in BRAF,** a member of the **RAF** family, have been detected in close to 100% of hairy cell leukemias, more than 60% of melanomas, 80% of benign nevi, and a smaller percentage of a wide variety of other neoplasms, including colon carcinomas and dendritic cell tumors.
- BRAF is a serine/threonine protein kinase that sits at the top of a cascade of other serine/threonine kinases of the MAPK family.
- Like activating RAS mutations, activating mutations in BRAF stimulate each of these downstream kinases and ultimately activate transcription factors. Mutations in other MAPK family members downstream of BRAF are uncommon in cancer, suggesting only mutations affecting factors near the top of the RAS/MAPK cascade produce significant pro-growth signals in most cell types.
- PI3K is heterodimer comprised of a regulatory subunit and a catalytic subunit, of which several tissue-specific isoforms exist. Under normal circumstances, PI3K is recruited by receptor tyrosine kinase activation to plasma membrane associated signaling protein complexes.

Alterations in Nonreceptor Tyrosine Kinases:

Mutations that confer oncogenic activity occur in several non receptor tyrosine kinases that normally localize to the cytoplasm or the nucleus.

In many instances the mutations take the form of chromosomal translocations or rearrangements that create fusion genes encoding constitutively active tyrosine kinases.

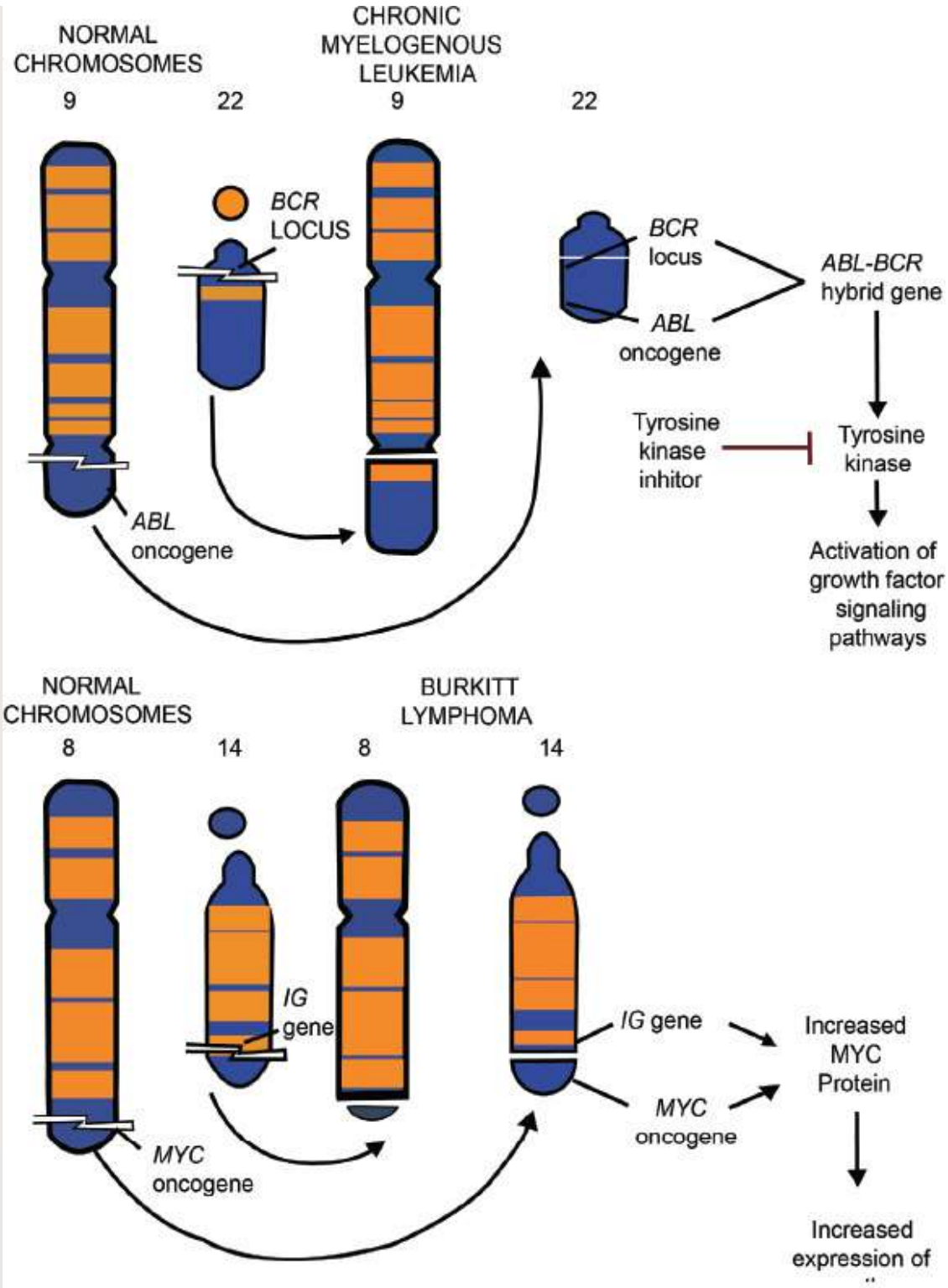


Fig. 4.12

Transcription Factors:

Transcription factors of this class include the products of the **MYC** , **MYB** ,**JUN** , **FOS** , and **REL** proto-oncogenes.

Of these, **MYC** is most commonly involved in human tumors.

Myc Oncogene:

- MYC activates the expression of many genes that are involved in cell growth
- In some contexts, MYC up regulates expression of telomerase
- MYC is one of a handful of transcription factors that can act together to reprogram somatic cells into pluripotent stem cells.

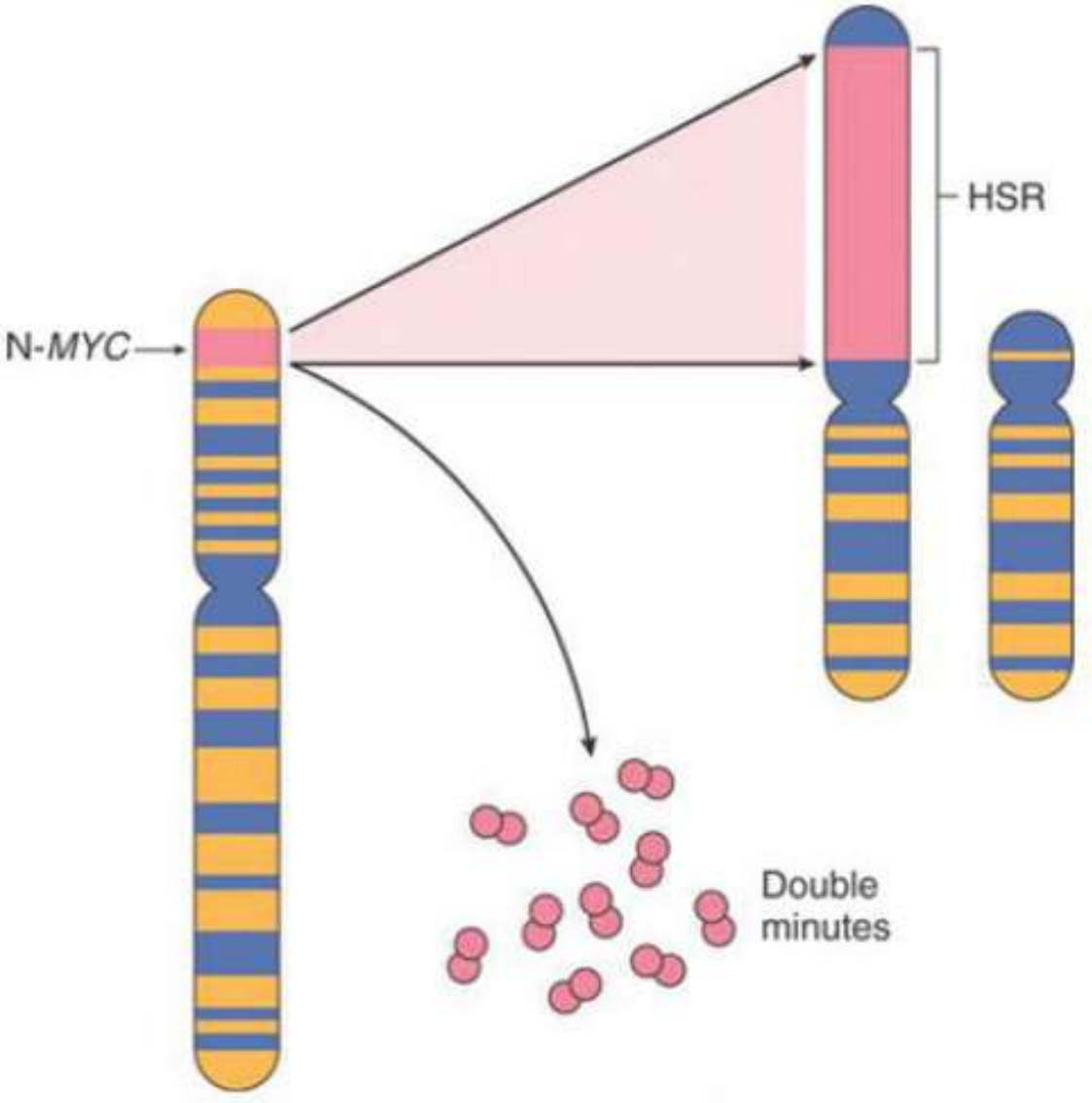


Fig. 4.13

Amplification of the **NMYC** gene in human neuroblastomas.

The **NMYC** gene, normally present on chromosome 2p, becomes amplified and is seen either as extra chromosomal double minutes or as a chromosomally integrated, homogeneous staining region (HSR). The integration involves other autosomes, such as 4, 9, or 13.

Cyclins and Cyclin-Dependent Kinases:

Cell Cycle Component	Main Function
Cyclins and Cyclin-Dependent Kinases	
CDK4; D cyclins	Form a complex that phosphorylates RB, allowing the cell to progress through the G1 restriction point.
Cell Cycle Inhibitors	
CIP/KIP family: p21, p27 {CDKN1A-D)	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-|J.
INK4/ARF family {CDKN2A-C}	p16/INK4a binds to cyclin D-CDK4 and promotes the inhibitory effects of RB p14/ARF increases p53 levels by Inhibiting MDM2 activity.
Cell Cycle Checkpoint Components.	
Rb	Tumor suppressive "pocket" protein that binds E2F transcription factors in Its hypophosphorylated state, preventing G,/S transition; also Interacts with several transcription factors that regulate differentiation.
p53	Tumor suppressor 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 tor the G1/S checkpoint and is a main component of the G2/M checkpoint.

Tumor suppressor genes:

Whereas oncogenes drive the proliferation of cells, the products of most tumor suppressor genes apply brakes to cell proliferation, and abnormalities in these genes lead to failure of growth inhibition, another fundamental hallmark of carcinogenesis.

Approximately 40%of retinoblastomas are familial, with the predisposition to develop the tumor being transmitted as an autosomal dominant trait. Carriers of the retinoblastoma trait have a10,000-fold increased risk of developing retinoblastoma (often in both eyes) as compared to the general population, and are also at greatly increased risk of developing osteosarcoma (*) and other soft-tissue sarcomas. About 60% of retinoblastomas occur sporadically (virtually always in only one eye), and such patients are not at increased risk for other forms of cancer. To explain these two patterns of occurrence of retinoblastoma, Knudson proposed his now canonic **"two-hit"**

hypothesis of oncogenesis. In molecular terms, Knudson's hypothesis can be stated as follows.

1. Two mutations (hits), involving both alleles of **RB** at chromosome locus 13q14, are required to produce retinoblastoma.
2. In familial cases, children inherit one defective copy of the **RB** gene in the germ line (the first hit), and the other copy is normal.
3. Retinoblastoma develops when the normal **RB** allele is mutated in retinoblasts as a result of a spontaneous somatic mutation (the second hit).
4. In sporadic cases both normal **RB** alleles must undergo somatic mutation in the same retinoblast (two hits). The probability of this event is low (explaining why retinoblastoma is an uncommon tumor in the general population), but the end result is the same: a retinal cell that has completely lost **RB** function and becomes cancerous.

Note that a child carrying an inherited mutant RB allele in all somatic cells is perfectly normal (except for the increased risk of developing cancer); it follows that one defective RB gene does not affect cell behavior. Thus, while the genetic trait (increased cancer risk) associated with germ line mutations in RB is inherited in an autosomal dominant fashion, at the level of the individual cell, loss of function mutations in the RB gene behave in a recessive fashion.

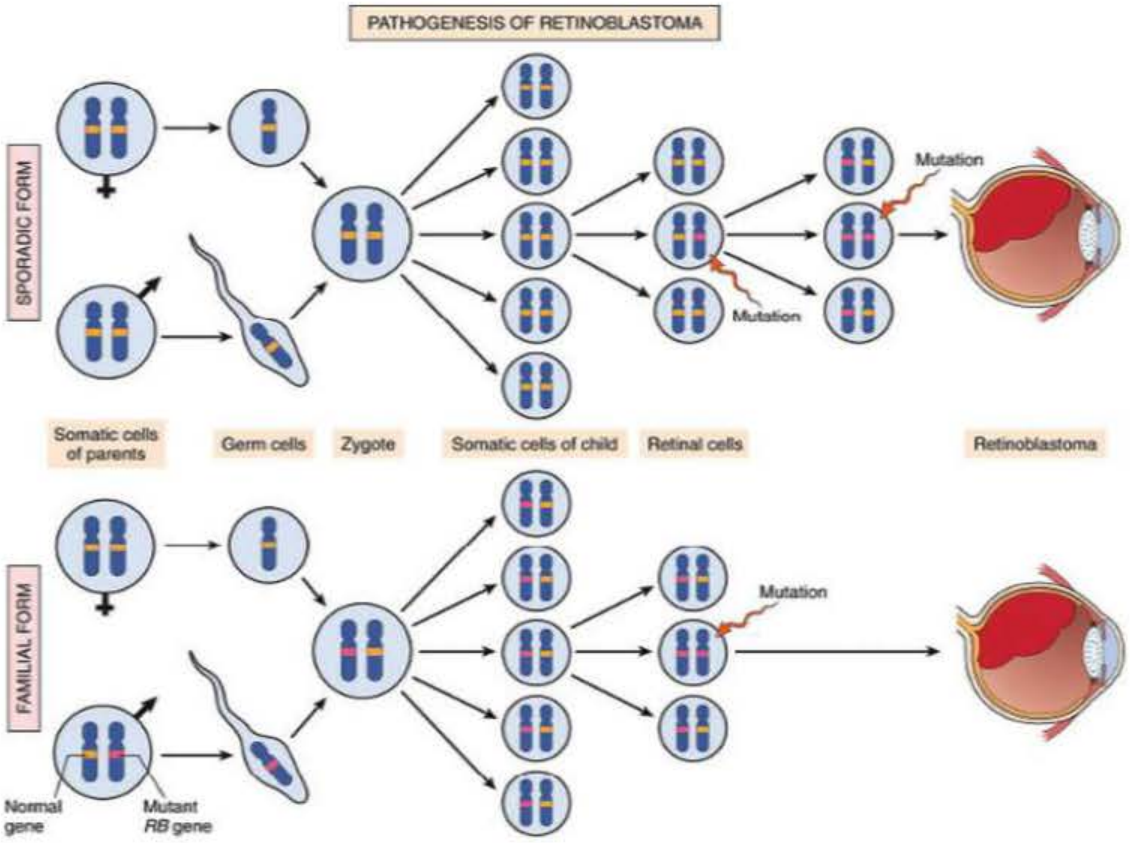


Fig. 4.14

Inhibitors of Mitogenic signaling pathways				
APC	Adenomatous polyposis coli protein.	Inhibitor of WNT signaling.	Familial colonic polyps and carcinomas.	Carcinomas of stomach, pancreas; melanoma.
NF-1	Neuro-fibromin-1.	Inhibitor of RAS/ MAPK Signaling.	Neurofibromatosis type 1 (neurofibromas and Malignant peripheral Nerve sheath tumors) .	Neuroblastoma, juvenile myeloid leukemia.
NF2	Merlin	Cytoskeletal stability, Hippo Pathway signaling.	Neurofibromatosis type 2 (acoustic schwannoma and meningioma).	Schwannoma, meningioma.
PTCH	Patched	Inhibitor of Hedgehog signaling.	Gorlin syndrome (basal cell carcinoma, medulloblastoma, several benign tumors).	Basal cell carcinoma, medulloblastoma.

PTEN	Phosphatase and tensin homologue.	Inhibitor of PI3K/ AKT signaling.	Cowden syndrome (variety of being skin, GI, and CNS growths; breast, endometrial, and thyroid carcinoma).	Diverse cancers, particularly carcinomas and lymphoid tumors.
SMAD2, SMAD4	SMAD2, SMAD4	Component of the TGFβ signaling pathway, repressors of MYC and CDK4 expression, inducers of CDK inhibitor expression.	Juvenile polyposis.	Frequently mutated (along with other components of the TGFβ signaling pathway) in colonic and pancreatic carcinoma.

Inhibitors of Cell Cycle Progression				
RB	Retinoblastoma (RBI protein.	Inhibitor of G,/S transition during cell cycle progression.	Familial retinoblastoma syndrome (retinoblastoma, osteosarcoma, other sarcomas).	Retinoblastoma; osteosarcoma carcinomas of breast, colon, lung.
CDKN2A	p16/INK4a and p14/ARF.	p16: Negative regulator of cyclln-dependent kinases; p14, Indirect activator of p53.	Familial melanoma.	Pancreatic, breast, and esophageal carcinoma, melanoma, certain leukemia.

Inhibitors of "Pro-growth" Programs of Metabolism and Angiogenesis				
VHL	Von Hippel Llndau (VHL) protein.	Inhibitor of hypoxia-lnduced transcription factors (e.g., HF1α.	Von Hippel Lindau syndrome (cerebellar hemangloblastoma, retinal angioma, renal cell carcinoma).	Renal cell carcinoma.
STK11	Liver kinase B1 (LKB1) or STK11.	Activator of AMPK family of kinases; suppresses cell growth when eel nutrient and energy levels are low.	Peutz-Jeghers syndrome (Gl polyps, Gl cancers, pancreatic carcinoma and other carcinomas).	Diverse carcinomas (5%-20% of cases, depending on type).
SDHB, SDHD	Succinate dehydrogenase complex submits B and D.	TCA cycle, oxidative phosphorylation.	Familial paraganglioma, familial pheochromocytoma.	Paragan glioma.

Inhibitors of invaslon and Metastasis				
CDH1	E-cadherin	Cell adhesion, inhibition of cell motility.	Familial gastric cancer.	Gastric carcinoma, lobular breast carcinoma.

Enablers of Genomlc Stability				
TP53	p53 protein	Cell cycle arrest and apoptosis in response to DNA damage.	Li-Fraumeni syndrome (Diverse cancers).	Most human cancers.

DNA Repair Factors				
BRCA1, BRCA2	Breast cancer-1 and breast cancer-2 (BRCA1 and BRCA2).	Repair of double-stranded breaks in DNA.	Familial breast and ovarian carcinoma; carcinomas of male breast; chronic lymphocytic leukemia (BRCA2).	Rare.
MSH2, MLH1, MSH6	MSH1, MLH1, MSH6.	DNA mismatch repair.	Hereditary nonpolyposis colon carcinoma.	Colonic and endometrial carcinoma.

Unknown Mechanisms				
WT1	Wilms tumor-1 (WT1).	Transcription factor.	Familial Wilms tumor.	Wilms tumor, certain leukemias.
MEN1	Menin	Transcription factor.	Multiple endocrine neoplasia-1 (MEN1; pituitary, parathyroid, and pancreatic endocrine tumors).	Pituitary, parathyroid, and pancreatic endocrine tumors.

RB: Governor of Proliferation:

RB, a key negative regulator of the G1 /S cell cycle transition, is directly or indirectly inactivated in most human cancers

RB function may be compromised in two different ways:

- Loss-of-function mutations involving both **RB** alleles.
- A shift from the active hypophosphorylated state to the inactive hyper phosphorylated state by gain-of-function mutations that up regulate CDK/cyclin D activity or by loss-of-function mutations that abrogate the activity ofCDK inhibitors.

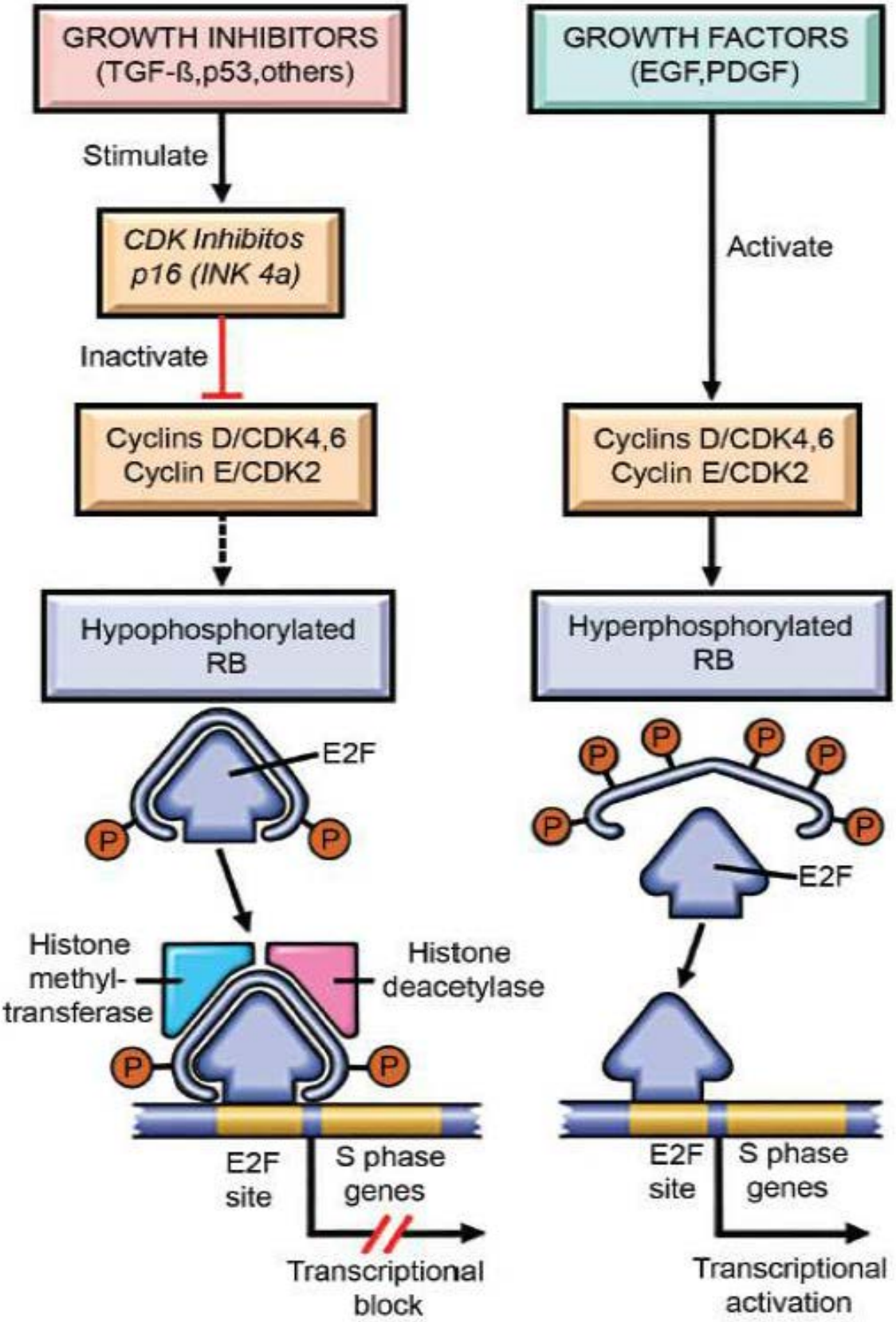

GROWTH INHIBITORS
(TGF-ß,p53,others)
Stimulate
CDK Inhibitos
p16 (INK 4a)
Inactivate
Cyclins D/CDK4,6
Cyclin E/CDK2
Hypophosphorylated
RB
E2F
P
Histone
methyl-
transferase
Histone
deacetylase
E2F
site
S phase
genes
Transcriptional
block
GROWTH FACTORS
(EGF,PDGF)
Activate
Cyclins D/CDK4,6
Cyclin E/CDK2
Hyperphosphorylated
RB
E2F
E2F
site
S phase
genes
Transcriptional
activation

Fig. 4.15

The Role of RB in Regulating the G1-S Checkpoint of the Cell Cycle:

Hypophosphorylated RB in complex with the E2F transcription factors binds to DNA, recruits chromatin-remodeling factors (histone deacetylases and histone methyl-transferases), and inhibits transcription of genes whose products are required for the
S phase of the cell cycle. When RB is phosphorylated by the cyclin D-CDK4, cyclin D-CDK6, and cyclin E-CDK2 complexes, it releases E2F. The latter then activates transcription of S-phase genes. The phosphorylation of RB is inhibited by cyclin-dependent kinase inhibitors, because they inactivate cyclin-CDK complexes. Virtually all cancer cells show dysregulation of the G1-S checkpoint as a result of mutation in one of four genes that regulate the phosphorylation of RB; these genes are **RB, CDK4,** the genes encoding cyclin D proteins, and **CDKN2A.**

TP53: Guardian of the Genome:

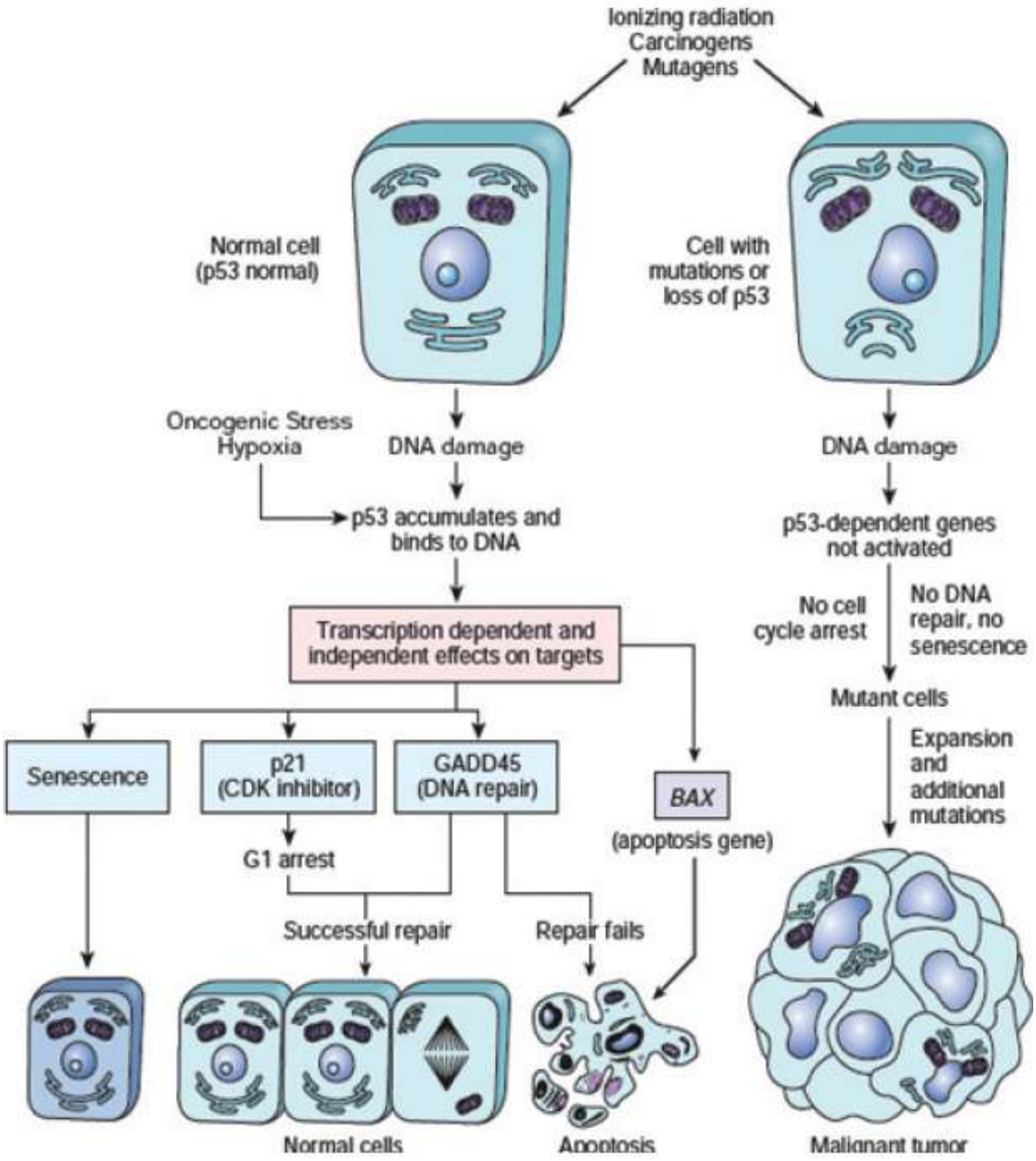


Fig. 4.16

TP53 , a tumor suppressor gene that regulates cell cycle progression, DNA repair, cellular senescence, and apoptosis, is the most frequently mutated gene in human cancers

Loss-of-function mutations in **TP53** , located on chromosome 17p13.1, are found in more than 50% of cancers.

Inheritance of a mutated copy of **TP53** predisposes individuals to malignant tumors because only one additional “hit” in the lone

normal allele is needed to abrogate**TP53** function. 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.

APC: Gatekeeper of Colonic Neoplasia:

Germ line loss-of-function mutations involving the **APC** (5q21) locus are associated with familial adenomatous polyposis, an autosomal dominant disorder in which individuals born with one mutant allele develop thousands of adenomatous polyps in the colon during their teens or 20s.

APC is a component of the WNT signaling pathway, which has a major role in controlling cell fate, adhesion, and cell polarity during embryonic development

WNT signals through a family of cell surface receptors called frizzled (FRZ), and stimulates several pathways, the central one involving β-catenin and APC.

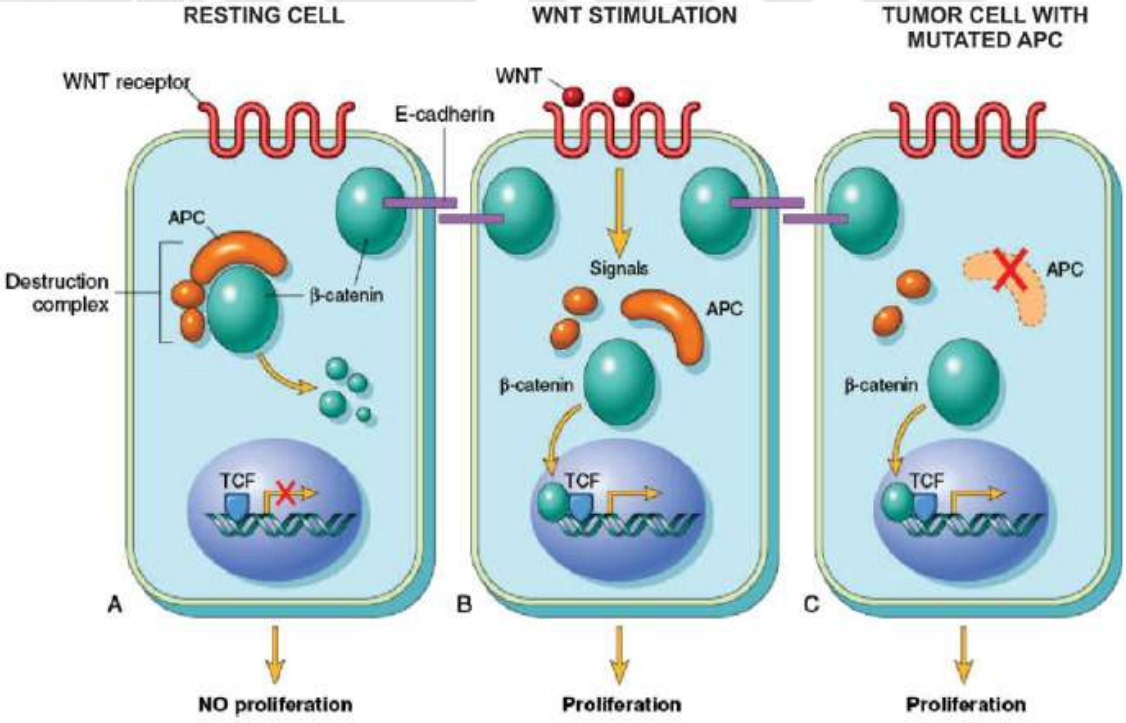


Fig. 4.17

The role of APC in regulating the stability and function of β-catenin.

APC and β-catenin are components of the WNT signaling pathway.

A, in resting colonic epithelial cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low.

B, when normal colonic epithelial cells are stimulated by WNT molecules, the **destruction complex** is deactivated, β-catenin degradation does not occur, and cytoplasmic levels increase. β-catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates genes involved in cell cycle progression.

C, When **APC** is mutated or absent, as frequently occurs in colonic polyps and cancers, the destruction of β-catenin cannot occur. β-catenin translocates to the nucleus and coactivates genes that promote entry into the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

E-Cadherin:

β-catenin binds to the cytoplasmic tail of E-cadherin, a cell surface protein that maintains inter cellular adhesiveness.

Loss of cell-cell contact, such as in awound or injury to the epithelium, disrupts the interaction between E-cadherin and β-catenin, and also promotes increased translocation of β-catenin to the nucleus, where it stimulates genes that promote proliferation; this is an appropriate response to injury that can help repair the wound.

Loss-of contact inhibition, by mutation of the E-cadherin/β-catenin axis, or by other changes, is a key characteristic of carcinomas.

Germ line loss of-function mutations of the E-cadherin gene, known as **CDH1** , cause familial gastric carcinoma, and a variable proportion of sporadic gastric carcinomas are also associated with loss of E-cadherin expression.

CDKN2A:

The **CDKN2A** gene locus encodes two protein products:

1. p16/INK4a cyclin-dependent kinase inhibitor, which blocks CDK4/cyclin D-mediated phosphorylation of RB, thereby reinforcing the RB checkpoint; and
2. p14/ARF, which activates the p53 pathway by inhibiting MDM2and preventing destruction of p53.

Thus, mutation or silencing of **CDKN2A** impacts both the RB and p53 tumor suppressor pathways.

TGF-β Pathway:

In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of proliferation.

It regulates cellular processes by binding to TGF-β receptors I and II.

Dimerization of the receptor upon ligand binding initiates intracellular signals that involve proteins of the SMAD family

Under normal circumstances, these signals turn on antiproliferative genes (e.g., genes for cyclin-dependent kinase inhibitors) and turn off genes that drive cell growth

Mutations affecting the type IITGF-β receptor are common in cancers of the colon, stomach, and endometrium, while mutational inactivation of SMAD4 is common in pancreatic cancers.

PTEN:

PTEN (**p** hosphatase and **ten** sin homologue) is a membrane-associated phosphatase encoded by a gene on chromosome 10q23 that is mutated in Cowden syndrome, an autosomal dominant disorder marked by frequent benign growths.

PTEN acts as a tumor suppressor by serving as a brake on the PI3K/AKT arm of the receptor tyrosine kinase pathway.

NF1:

Individuals who inherit one mutant allele of the **NF1** gene develop numerous benign neurofibromas and optic nerve gliomas as a result of inactivation of the second copy of the gene. This condition is called **neurofibromatosis type 1.**

NF2:

Germ line mutations in the **NF2** gene predispose to the development of **neurofibromatosis type 2.**

The product of the **NF2** gene, called **neurofibromin2** or **merlin** , is structurally similar to the red cell membrane cytoskeletal protein 4.1.

WT1:

Loss-of-function mutations in the **WT1** gene, located on chromosome 11p13, is associated with the development of Wilms tumor.

The WT1 protein is a transcriptional activator of genes involved in renal and gonadal differentiation. It regulates the mesenchymal-to-epithelial transition that occurs in kidney development.

Interestingly, although WT1 is a tumor suppressor in Wilms' tumor, a variety of adult cancers, including leukemias and breast carcinomas, overexpress WT1.

PATCHED (PTCH):

PTCH1 is a tumor suppressor gene that encodes a cell membrane protein called PATCHED1.

PATCHED proteins are negative regulators of the Hedgehog signaling pathway.

Germ line loss-of-function mutations in **PTCH1** cause Gorlin syndrome, an inherited condition also known as nevoid basal cell carcinoma syndrome.

VHL:

Encodes a component of a ubiquitin ligase that is responsible for degradation of hypoxia-induced factors (HIFs), transcription factors that alter gene expression in response to hypoxia.

- Germ line loss-of-function mutations cause von Hippel-Lindau syndrome, autosomal dominant disorder associated with a high risk of renal cell carcinoma and pheochromocytoma.

- Acquired biallelic loss-of mutations are common in sporadic renal cell carcinoma.

The Warberg Effect:

Even in the presence of ample oxygen, cancer cells demonstrate distinctive form of cellular metabolism characterized by high levels of glucose uptake and increased conversion of glucose to lactose (fermentation) via the glycolytic pathway.

This phenomenon, called the **Warburg effect** and also known as **aerobic glycolysis** , has been recognized for many years (Otto Warburg received the Nobel Prize in 1931 for discovery of the effect that bears his name).

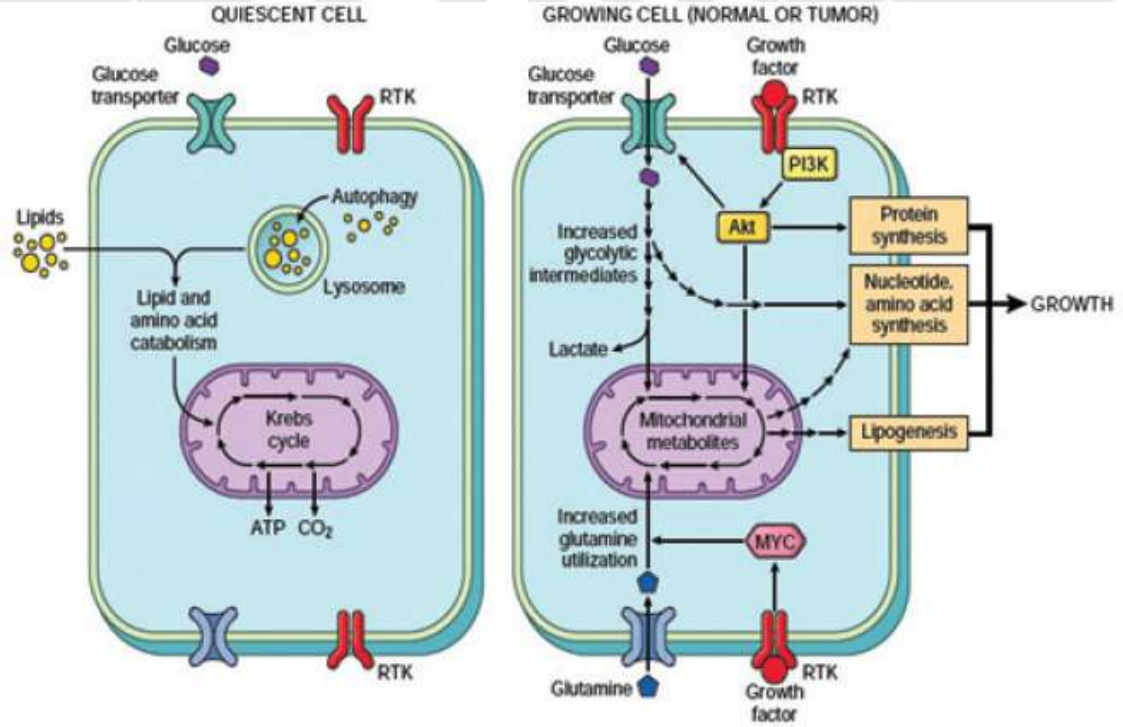


Fig. 4.18

Quiescent cells rely mainly on the Krebs cycle for ATP production; if starved, autophagy (self-eating) is induced to provide a source of fuel. When stimulated by growth factors, normal cells markedly up regulate glucose and glutamine uptake, which provide carbon sources for synthesis of nucleotides, proteins, and lipids. In cancers,

oncogenic mutations involving growth factor signaling pathways and other key factors such as MYC deregulate these metabolic pathways, an alteration known as the **Warburg effect.**

Evasion of Apoptosis:

- Apoptosis can be initiated through intrinsic or extrinsic pathways, both of which result in the activation of a proteolytic cascade of caspases that destroys the cell.
- Abnormalities of both pathways are found in cancer cells, but lesions that incapacitate the intrinsic (mitochondrial) pathway appear to be most common.
- in greater than 85% of follicular B-cell lymphomas, the anti-apoptotic gene **BCL2** is overexpressed due to a (14; 18) translocation.
    - Overexpression of other BCL2 family members such asMCL-1 is also linked to cancer cell survival and drug resistance.

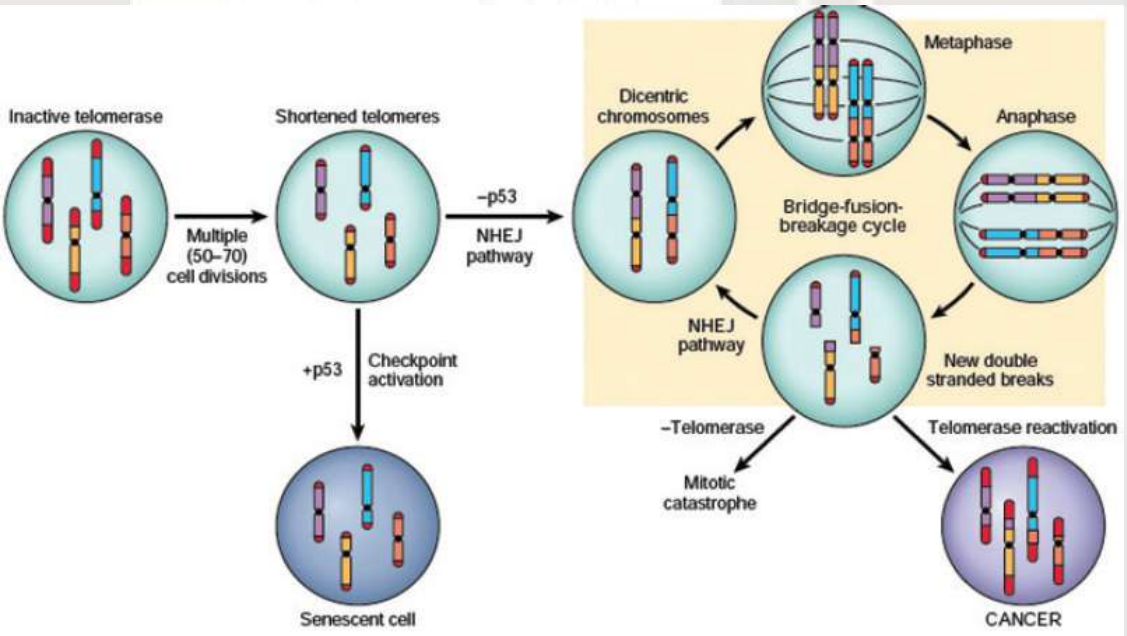


Fig. 4.19

Escape of cells from senescence and mitotic catastrophe caused by telomere shortening. Replication of somatic cells, which do not express telomerase, leads to shortened telomeres. In the presence of

competent checkpoints, cells undergo arrest and enter non replicative senescence. In the absence of checkpoints, DNA repair pathways, such as the non homologous end-joining (NHEJ) pathway are inappropriately activated, leading to the formation of dicentric chromosomes. At mitosis the dicentric chromosomes are pulled apart, generating random double-stranded breaks, which then activate DNA-repair pathways, leading to the random association of double-stranded ends and the formation, again, of dicentric chromosomes. Cells undergo numerous rounds of this bridge-fusion-breakage cycle, which generates massive chromosomal instability and numerous mutations. If cells fail to reexpress telomerase, they eventually undergo mitotic catastrophe and death. Reexpression of telomerase allows the cells to escape the bridge-fusion-breakage cycle, thus promoting their survival and tumorigenesis.

Angiogenesis:

Even if a solid tumor possesses all of the genetic aberrations that are required for malignant transformation, it cannot enlarge beyond 1 to 2 mm in diameter unless it has the capacity to induce angiogenesis.

The current paradigm is that angiogenesis is controlled by a balance between angiogenesis promoters and inhibitors; in angiogenic tumors this balance is skewed in favor of promoters.

Hypoxia triggers angiogenesis through the actions of HIF-1α on the transcription of the proangiogenic factor VEGF.

Many other factors regulate angiogenesis; for example, p53 induces synthesis of the angiogenesis inhibitorthombospondin-1, while RAS, MYC, and MAPK signaling all up regulate VEGF expression and stimulate angiogenesis.

VEGF inhibitors are used to treat a number of advanced cancers and prolong the clinical course, but are not curative.

Invasion and Metastasis:

Invasion of the ECM initiates the metastatic cascade and is an active process that can be resolved into several steps:

- “Loosening up” of tumor cell–tumor cell interactions.
- Degradation of ECM.
- Attachment to novel ECM components.
- Migration and invasion of tumor cells.

A. Loosing of Intercellular Junction.

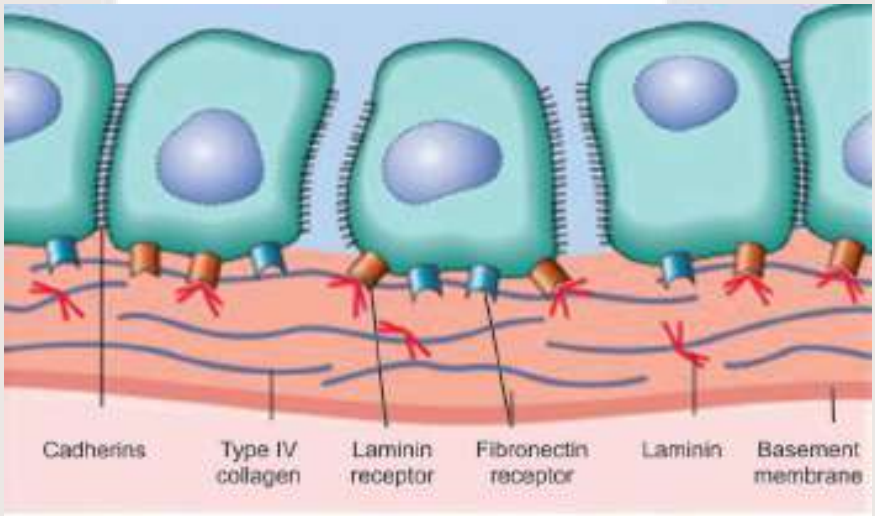


Fig. 4.20

B. Degradation of ECM

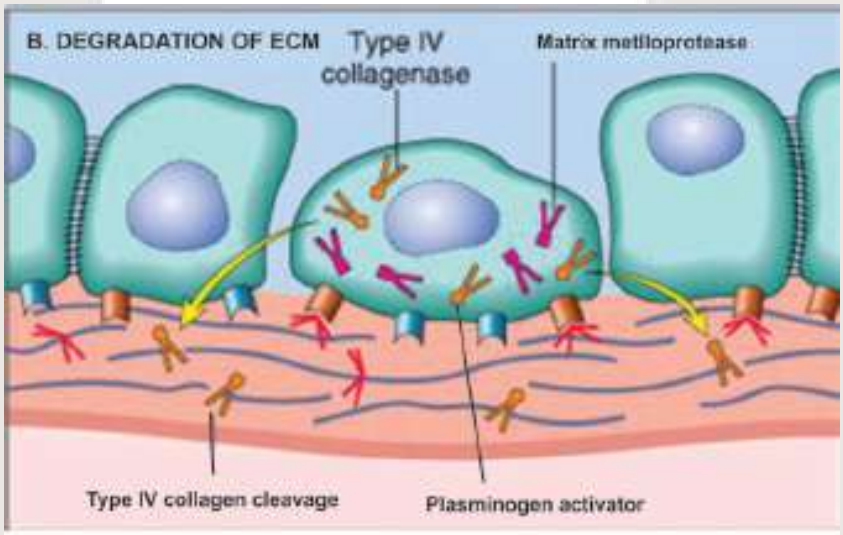


Fig. 4.21

C. Migration and Invasion

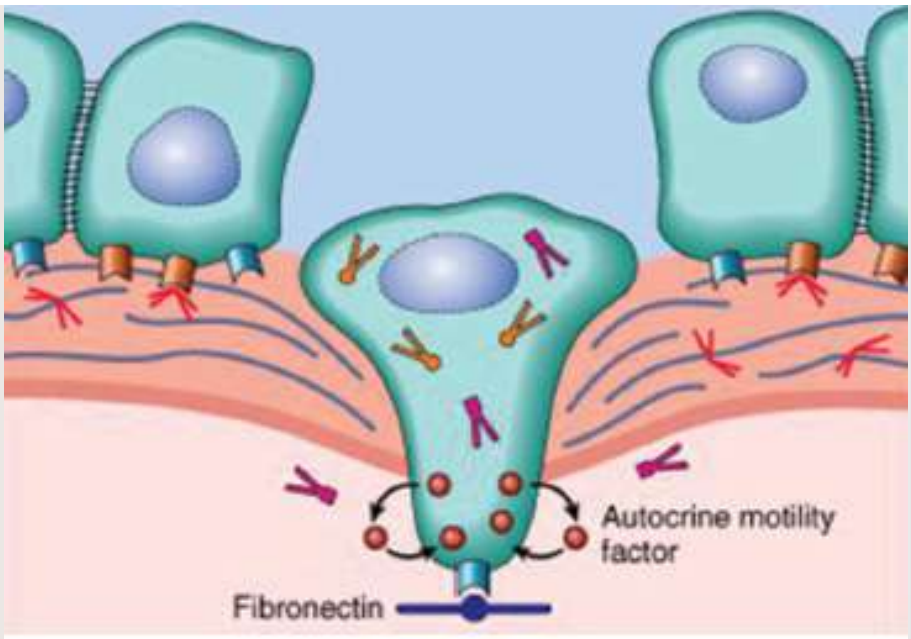
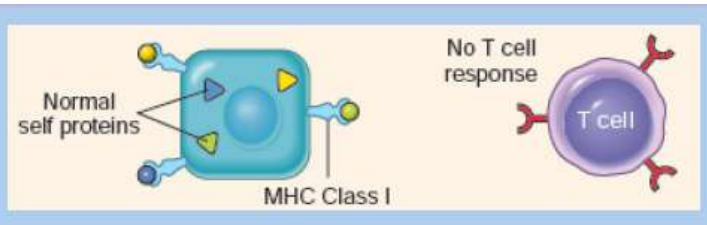
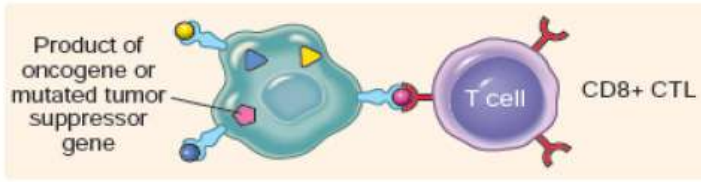
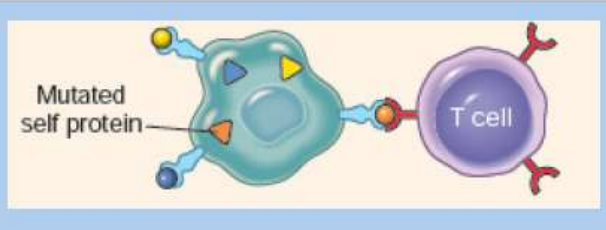
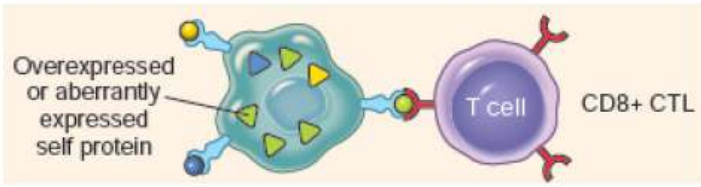
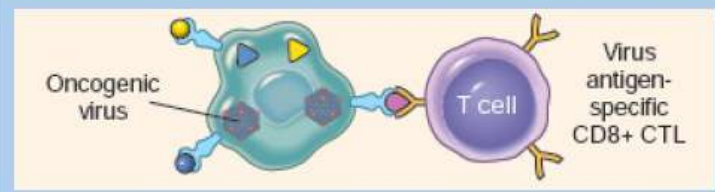


Fig. 4.22

Dissociation of cancer cells from one another is often the result of alterations in intercellular adhesion molecules and is the first step in the process of invasion.

- The metastatic site of many tumors can be predicted by the location of the primary tumor. Many tumors arrest in the first capillary bed they encounter (lung and liver, most commonly).
- Some tumors show organ tropism, probably due to expression of adhesion or chemokine receptors whose ligands are expressed by endothelial cells the metastatic site.
- Genes that promote epithelial-mesenchymal transitions, like **TWIST** and **SNAIL** , may be important metastasis genes in epithelial tumors.

Normal host displaying multiple MHC-associated self antigens		Examples
Tumor cells expressing different types of tumor antigens		Oncogene products: mutated RAS, BCR/ABL fusion proteins Tumor suppressor gene products: mutated p53 protein
		Various mutant proteins in carcinogen, or radition, induced animal tumors; various mutated proteins in melanomas
		Overexpressed: tyrosinase, gp 100, MART in melanomas Aberrantly expressed: cancer-testis antigens (MAGE, BAGE)
		Human papilloma virus E6, E7 proteins in cervical carcinoma; EBNA proteins in EBV-induced lymphoma

Evading Host Immunity:

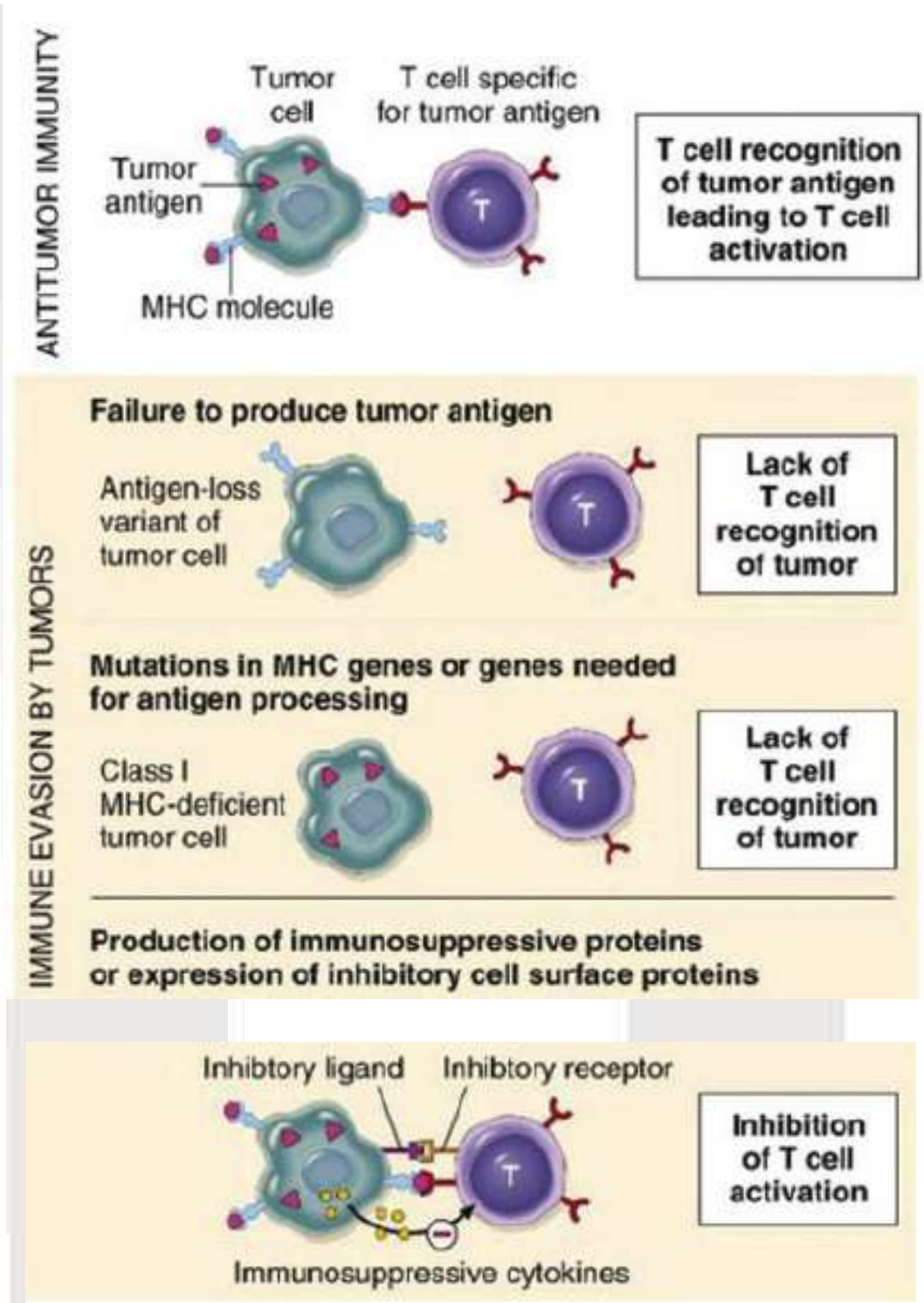


Fig. 4.23

Mechanisms by Which Tumors Evade The Immune System:

Tumors may evade immune responses by losing expression of antigens or major histocompatibility complex (MHC) molecules or by producing immune-suppressive-cytokines or ligands such as PD-L1 for inhibitory receptors on T cells.

Genomic Instability as Enabler of Malignancy:

- Persons with inherited mutations of genes involved in DNA repair systems are at greatly increased risk for the development of cancer.
- Patients with HNPCC syndrome have defects in the mismatch repair system, leading to development of carcinomas of the colon. These patients' genomes show microsatellite instability, characterized by changes in length of short repeats throughout the genome.
- Patients with xeroderma pigmentosum have a defect in the nucleotide excision repair pathway and are at increased risk for the development of cancers of the skin exposed to UV light, because of an inability to repair pyrimidine dimers.
- Syndromes involving defects in the homologous recombination DNA repair system constitute a group of disorders—Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia—that are characterized by hypersensitivity to DNA-damaging agents, such as ionizing radiation. **BRCA1** and **BRCA2,** which are mutated in familial breast cancers, are involved in DNA repair.
- Mutations incurred in lymphoid cells due to expression of gene products that induce genomic instability (RAG1, RAG2, AID) are important causes of lymphoid neoplasms.

Genetic Lesions in Cancer:

- Tumor cells may acquire several types of oncogenic mutations, including point mutations and other nonrandom chromosomal abnormalities, such as translocations, deletions, and gene amplifications.
- Balanced translocations contribute to carcinogenesis by overexpression of oncogenes or generation of novel fusion

proteins with altered signaling capacity. Deletions frequently cause loss of tumor suppressor gene function, and occasionally activate proto-oncogenes. Gene amplification.

- Generally increases the expression and function of oncogenes.
- Genomic sequencing has revealed numerous “cryptic” (sub cytogenetic) rearrangements, mainly small deletions and insertions (“indels”), as well as chromothrypsis, in which a chromosome is “shattered” and then reassembled in a haphazard way.

Malignancy	Translocation	Affected Genes*
Chronic myelogenous leukemia (CML)	(9;22) (q34; q11)	ABL 9q34 BCR 22q11
Acute myeloid leukemia (AML)	(8;21) (q22; q22) (15;17)(q22;q21)	AML 8q22 ETO 21q22 PML 15q22 RARA 17q21
Burkitt lymphoma	(8;14)(q24;q32)	MYC 8q24 IGH 14q32
Mantle cell lymphoma	(11;14)(q13;q32)	CCND1 11q13 IGH 14q32
Follicular lymphoma	(14;18)(q32;q21)	IGH 14q32 BCL2 18q21
Ewing sarcoma	(11;22)(q24;q12)	FLI1 11q24 EWSR1 22q12
Prostatic adenocarcinoma	(7:21) (p22; q22) (17:21) (p21; q22)	TMPRSS2 (21q22.3) ETV1 (7p21.2) ETV4 (17q21)

Gene(s)	Function	Tumor (Approximate Frequency of Mutation)
DNMT3A	DNA methylation	Acute myeloid leukemia (20%)
MLL1	HI stone methylation	Acute leukemia in Infants (90%)
MLL2	HI stone methylation	Follicular lymphoma (90%)
CREBBP/ EP300	HI stone acetylation	Diffuse large B cell lymphoma (40%)
ARID 1A	Nucleosome positioning/chromatin remodeling	Ovarian clear cell carcinoma (60%), endometrial carcinoma (30%-40%}
SNF5	Nucleosome positioning/chromatin remodeling	Malignant rhabdoid tumor (100%)
PBRM1	Nucleosome Positioning/chromatin remodeling	Renal carcinoma (30%)

Chemical Carcinogenesis:

- Chemical carcinogens have highly reactive electrophile groups that directly damage DNA, leading to mutations and eventually cancer.
- Direct-acting agents do not require metabolic conversion to become carcinogenic, while indirect-acting agents are not active until converted to an ultimate carcinogen by endogenous metabolic pathways. Hence, polymorphisms of endogenous enzymes such as cytochrome P-450 may influence carcinogenesis.
- After exposure of a cell to a mutagen or an initiator, tumorigenesis can be enhanced by exposure to promoters, which stimulate proliferation of the mutated cells.
- Examples of human carcinogens are direct-acting agents (e.g., alkylating agents used for chemotherapy), indirect acting agents (e.g., benzo[a]pyrene, azo dyes, aflatoxin), and promoters or agents that cause pathologic hyperplasias of the endometrium or regenerative activity in the liver.

The UV portion of the solar spectrum can be divided into three wavelength ranges: UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm). Of these, UVB is believed to be responsible

for the induction of cutaneous cancers. UVC, although a potent mutagen, is not considered significant because it is filtered out by the ozone layer surrounding the earth (hence the concern about ozone depletion).

Viral and Bacterial Oncogenesis:

HTLV-1: a retrovirus that is endemic in Japan, the Caribbean, and parts of South America and Africa that causes adult T-cell leukemia/lymphoma

- HTLV-1 encodes the viral protein Tax, which turns on pro-growth and pro-survival signaling pathways (PI3K/AKT, NF-κB), leading to a polyclonal expansion of T cells.
- After a long latent period (decades), a small fraction ofHTLV-1–infected individuals develop adult T-cell leukemia/lymphoma, a CD4+ tumor that arises from an HTLV-1infected cell, presumably due to acquisition of additional mutations in the host cell genome.

HPV: an important cause of benign warts, cervical cancer, and oropharyngeal cancer

- Oncogenic types of HPV encode two viral oncoproteins, E6 and E7 that bind to Rb and p53, respectively, with high affinity and neutralize their function.
- Development of cancer is associated with integration of HPV into the host genome and additional mutations needed for acquisition of cancer hallmarks.
- HPV cancers can be prevented by vaccination against high-risk HPV types.

EBV: ubiquitous herpes virus implicated in the pathogenesis of Burkitt lymphomas, B-cell lymphomas in patients with T-cell immunosuppression (HIV infection, transplant recipients), and several other cancers.

- The EBV genome harbors several genes encoding proteins that trigger B cell signaling pathways; in concert, these signals are potent inducers of B cell growth and transformation.
- In the absence of T-cell immunity, EBV-infected B cells can rapidly “grow out” as aggressive B-cell tumors.
- In the presence of normal T-cell immunity, a small fraction of infected patients develop EBV-positive B-cell tumors (Burkitt lymphoma, Hodgkin lymphoma) or carcinomas (nasopharyngeal, gastric carcinoma).

Hepatitis B virus and hepatitis C virus: cause of between70% and 85% of hepatocellular carcinomas worldwide

- Oncogenic effects are multifactorial; dominant effect seems to be immunologically mediated chronic inflammation, hepatocellular injury, and reparative hepatocyte proliferation.
- HBx protein of HBV and the HCV core protein can activate signal transduction pathways that also may contribute to carcinogenesis.

H. pylori: implicated in gastric adenocarcinoma and MALT lymphoma

- Pathogenesis of **H. pylori** -induced gastric cancers is multifactorial, including chronic inflammation and reparative gastric cell proliferation.
- **H. pylori** pathogenicity genes, such as **CagA,** also may contribute by stimulating growth factor pathways.
- Chronic **H. pylori** infection leads to polyclonal B-cell proliferations that may give rise to a monoclonal B-cell tumor (MALT lymphoma) of the stomach as a result of accumulation of mutations.

Paraneoplastic Syndromes:

Hypercalcemia is probably the most common paraneoplastic syndrome; in fact, symptomatic hypercalcemia is more often related to some form of cancer than to hyperparathyroidism.

Two general processes are involved in cancer-associated hypercalcemia:

1. **Osteolysis** induced by cancer, whether primary in bone, such as multiple myeloma, or metastatic to bone from any primary lesion, and
2. The production of **calcemic humoral substances** by extra osseous neoplasms.

Only the second mechanism is considered to be paraneoplastic; hypercalcemia due to primary or secondary involvement of the skeleton by tumor is not a paraneoplastic syndrome.

Endocrinopathies		
Cushing syndrome	Small-cell carcinoma of lung pancreatic carcinoma Neural tumors	ACTH or ACTH-like substance
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 Breast carcinoma Renal carcinoma Adult T-cell leukemia/lymphoma	Parathyroid hormone-related protein (PTHRP), TGF-α, TNF, IL-1
Hypoglycemia	Ovarian carcinoma Fibrosarcoma Other mesenchymal sarcomas	Insulin or insulin-like substance
Polycythemia	Renal carcinoma Cerebellar hemangioma Hepatocellular carcinoma	Erythropoietin

Nerve and Muscle syndromes		
Myasthenia	Bronchogenic carcinoma Thymic neoplasms	Immunologic
Disorder of the central and peripheral nervous system	Breast carcinoma	
Dermatologic Disorders		

Acanthosis nigricans	Gastric carcinoma Lung carcinoma Uterine carcinoma	Immunologic; secretion of epidermal growth factor
Dermatomyositis	Bronchogenic carcinoma Breast carcinoma	Immunologic

Osseous, Articular, and Soft Tissue Changes		
Hypertrophic osteoarthropathy and clubbing of the fingers	Bronchogenic carcinoma Thymic neoplasms	Unknown
Vascular and Hematologic Changes		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma Bronchogenic carcinoma Other cancers	Tumor products (mucins that activate clotting)
Disseminated intravascular coagulation	Acute promyelocytic leukemia Prostatic carcinoma	Tumor products that activate clotting
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Red cell aplasia	Thymic neoplasms	Unknown
Others		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

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	
Oncofetal Antigens	
α-Fetoprotein	Liver cell cancer, non seminomatous germ cell tumors of testis

Carcinembryonic 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
Cell-Free DNA Markers	
TP53, APC, RAS mutants in stool and serum	Colon cancer
TP53, RAS mutants in stool and serum	Pancreatic cancer
TP53, RAS mutants in sputum and serum	Lung cancer
TP53 mutants in urine	Bladder cancer

5 Disorders of Immune System

Introduction to Immunity:

- Immunity is a systemic response to stimulus (usually antigenic).
- Two types of immunity are there Innate and acquired.
- Innate immunity can occur without antigenic sensitization, usually is from birth and lacks antigenic memory.
- While acquired immunity occurs after antigenic sensitization only. This will have an antigenic memory and usually is acquired later in life.

Hypersensitivity Reactions:

Type of Hypersensitivity	Pathologic immune Mechanisms	Mechanism of Tissue Injury and Disease
Immediate: type 1	IgE antibody, TH2 cells.	Mast cells, eosinophils, and their mediators (vasoactive amines, lipid mediators, cytokines).
Antibody-mediated: type 2	IgM, IgG antibodies against cell surface or extracellular matrix antigens.	Opsonization and phagocytosis of cells Complement- and Fc receptor-mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular function, e.g., hormone receptor signaling, neurotransmitter receptor blockage.
Immune complex-mediated: Type 3	Immune complexes of circulating antigens and IgM or IgG\antibodies.	Complement-and Fc receptor—mediated recruitment and activation of leukocytes.
T cell-mediated: type 4	CD4+T cells (TH 1 and TH17 cells) CD8+ CTLs.	Cytokine-medicated inflammation Direct target cell killing, cytokine-medicated inflation .

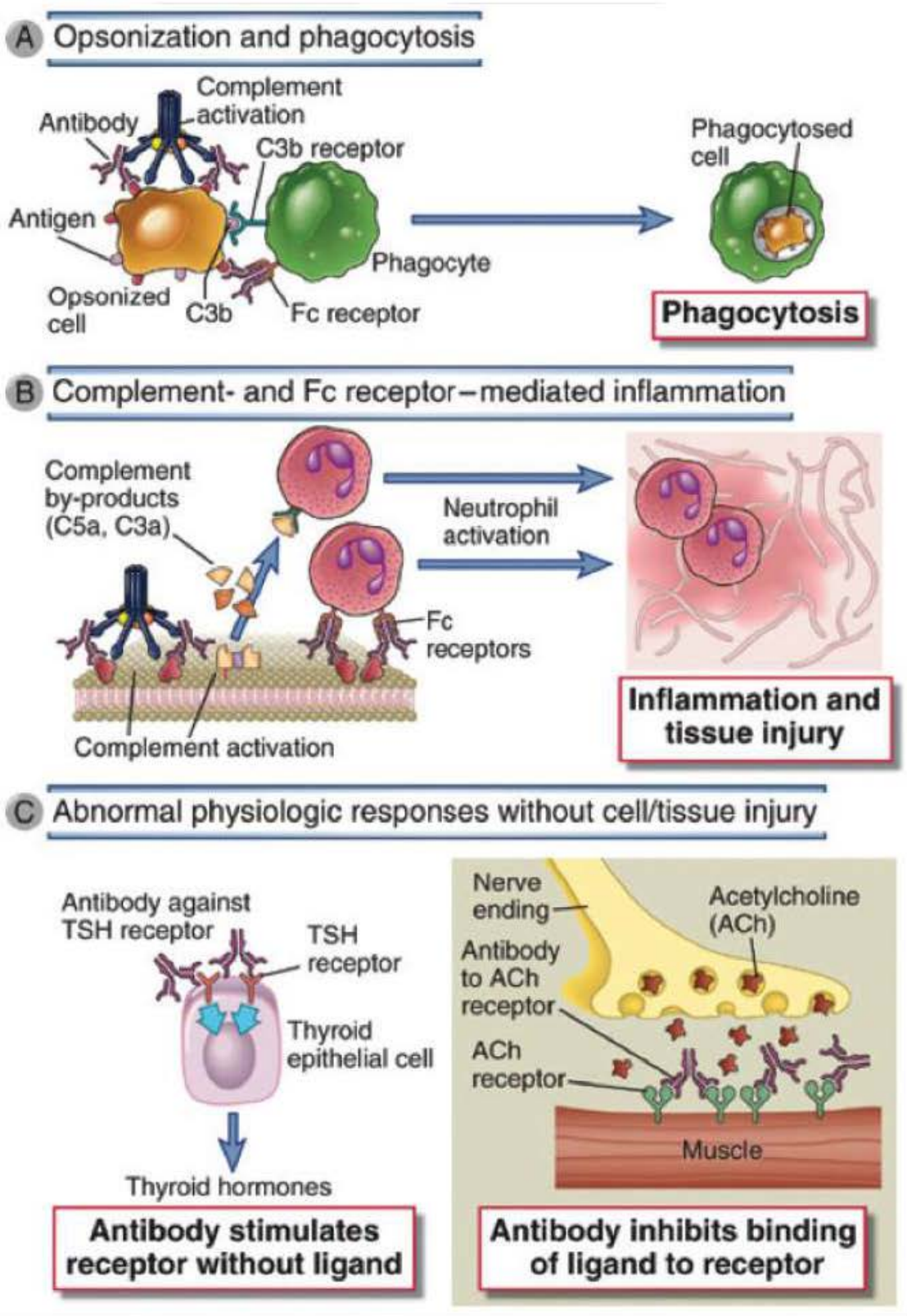

A Opsonization and phagocytosis
Antibody
Complement activation
C3b receptor
Antigen
Phagocytosed cell
Phagocyte
Opsonized cell
C3b
Fc receptor
Phagocytosis
B Complement- and Fc receptor–mediated inflammation
Complement by-products (C5a, C3a)
Neutrophil activation
Fc receptors
Complement activation
Inflammation and tissue injury
C Abnormal physiologic responses without cell/tissue injury
Antibody against TSH receptor
TSH receptor
Thyroid epithelial cell
Thyroid hormones
Antibody stimulates receptor without ligand
Nerve ending
Acetylcholine (ACh)
Antibody to ACh receptor
ACh receptor
Muscle
Antibody inhibits binding of ligand to receptor

Fig. 5.1

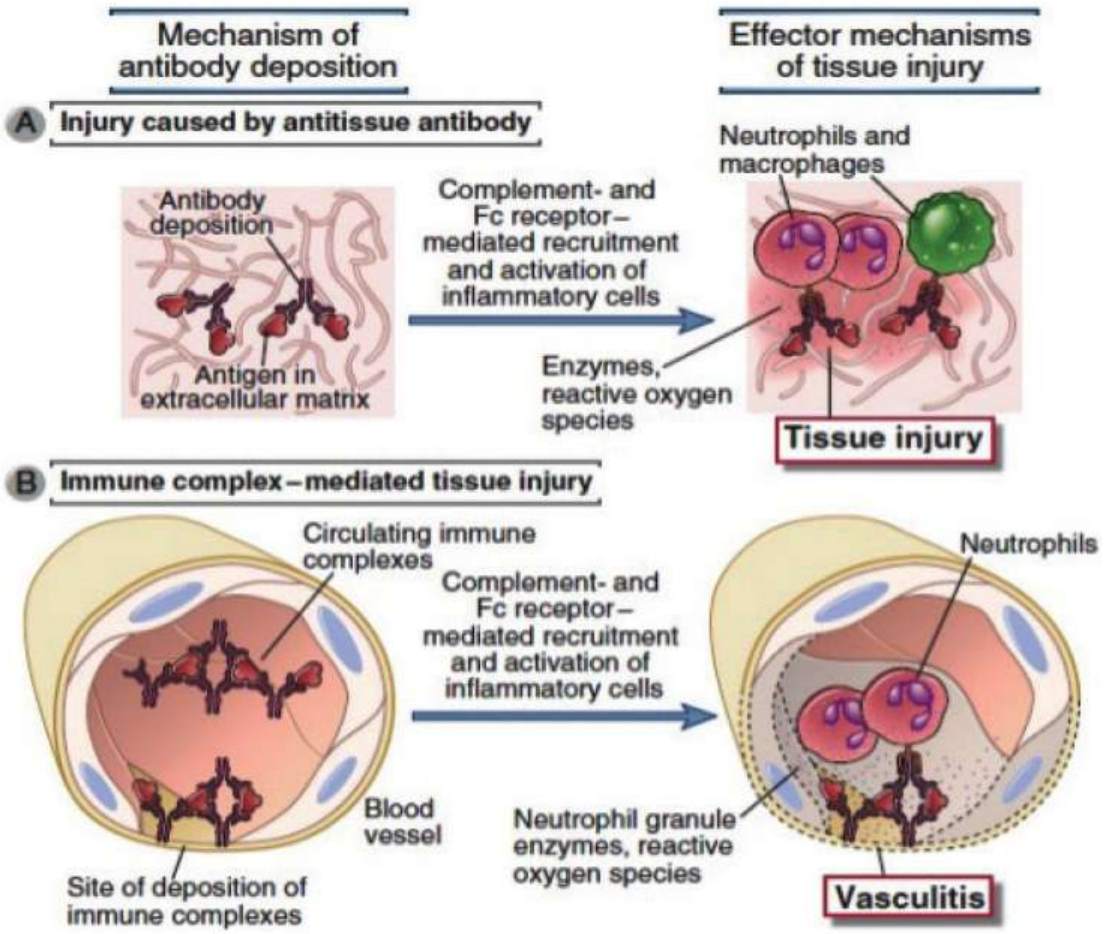


Fig. 5.2

Type I Hypersensitivity Reaction:

Anaphylaxis: Prior sensitization has resulted in an immune response initially mediated by CD4 lymphocytes (of the Th2 variety) that promote mast cell proliferation and plasma cell production of IgE. The IgE becomes bound to mast cells in places such as respiratory tract mucosa. Encountering the allergen again leads to mast cell degranulation with release of primary mediators (such as histamine, serotonin) which cause vasodilation, bronchoconstriction, etc. and release of secondary mediators (such as leukotrienes, prostaglandin) which lead to inflammatory cell infiltrates.

Laboratory Findings:

- Type 1 hypersensitivity reactions may be accompanied by an increase in eosinophils, as noted with differential count of peripheral white blood cells.
- The serum tryptase may be increased in the hour following mast cell activation.
- Measurement of serum total IgE and levels of specific IgE for certain antigens may be undertaken when allergy therapies are planned. Testing for total or specific IgE should be done only when the history is consistent with allergy and specific allergens are suspected as the cause.

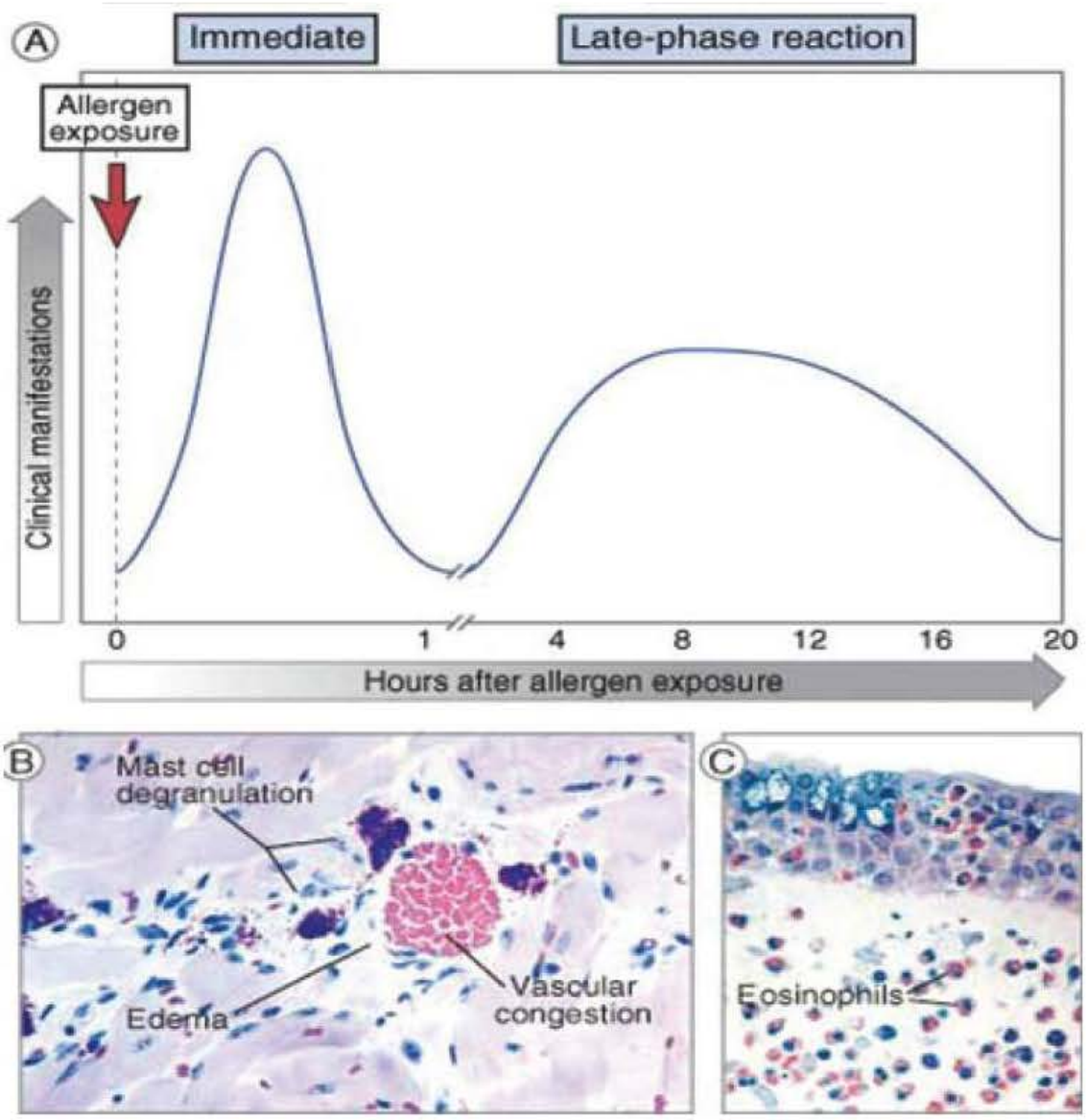


Fig. 5.3: Phases of immediate hypersensitivity reactions. A, Kinetics of the immediate and late-phase reactions. The immediate vascular and smooth muscle reaction to allergen develops within minutes after challenge (Allergen exposure in a previously sensitized individual), and the late-phase reaction develops 2 to 24 hours later. The immediate reaction (B) is characterized by vasodilation, congestion, and edema, and the late-phase reaction (C) is characterized by an inflammatory infiltrate rich in eosinophils, neutrophils, and T cells. (Courtesy Dr. Daniel Friend, Department of Pathology, Brigham and Women's Hospital, Boston, Mass.)

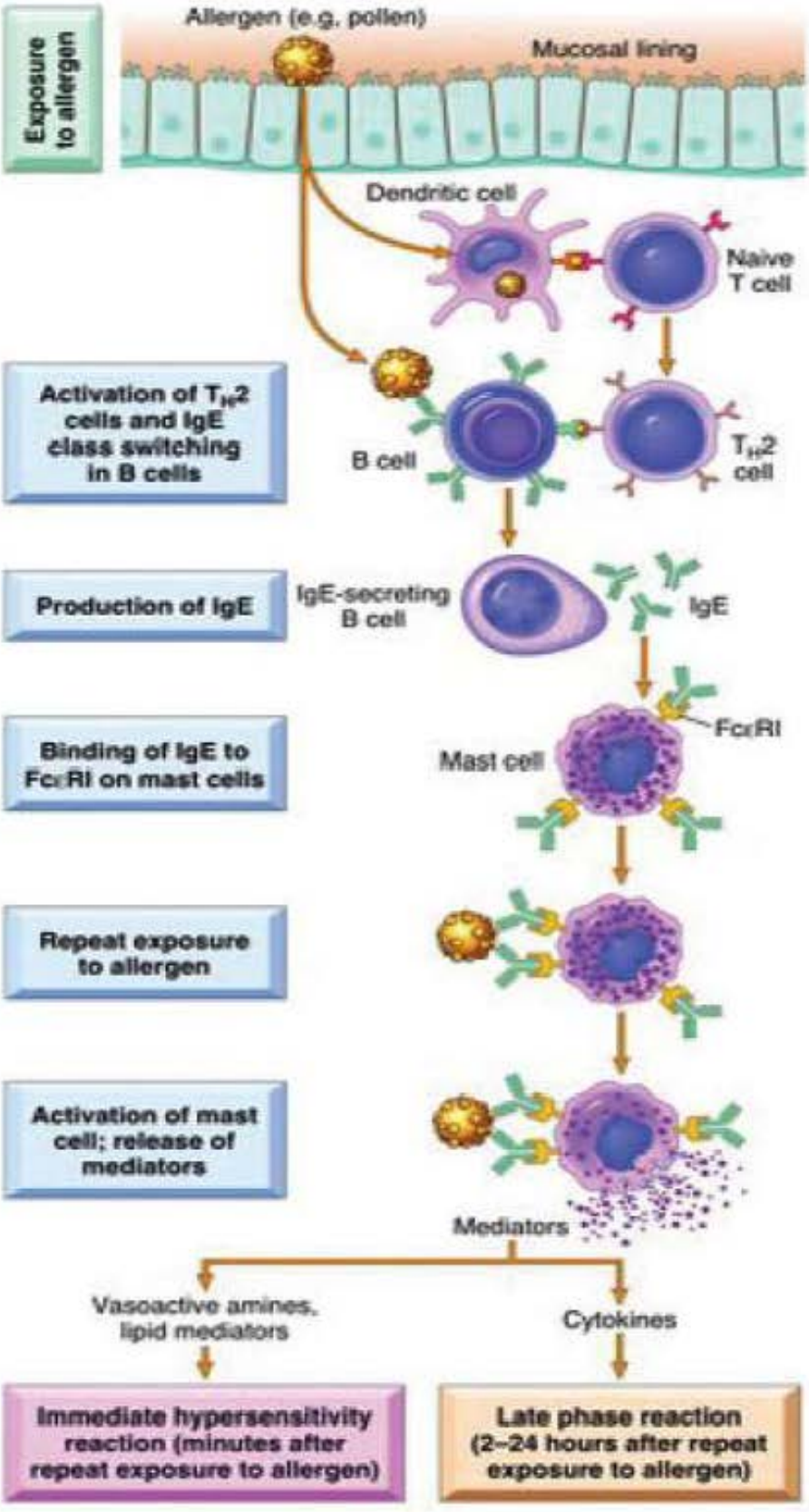

Exposure to allergen
Allergen (e.g. pollen)
Mucosal lining
Dendritic cell
Naive T cell
Activation of T_H2 cells and IgE class switching in B cells
B cell
T_H2 cell
Production of IgE
IgE-secreting B cell
IgE
Binding of IgE to FcεRI on mast cells
FcεRI
Mast cell
Repeat exposure to allergen
Activation of mast cell; release of mediators
Mediators
Vasoactive amines, lipid mediators
Cytokines
Immediate hypersensitivity reaction (minutes after repeat exposure to allergen)
Late phase reaction (2–24 hours after repeat exposure to allergen)

Fig. 5.4

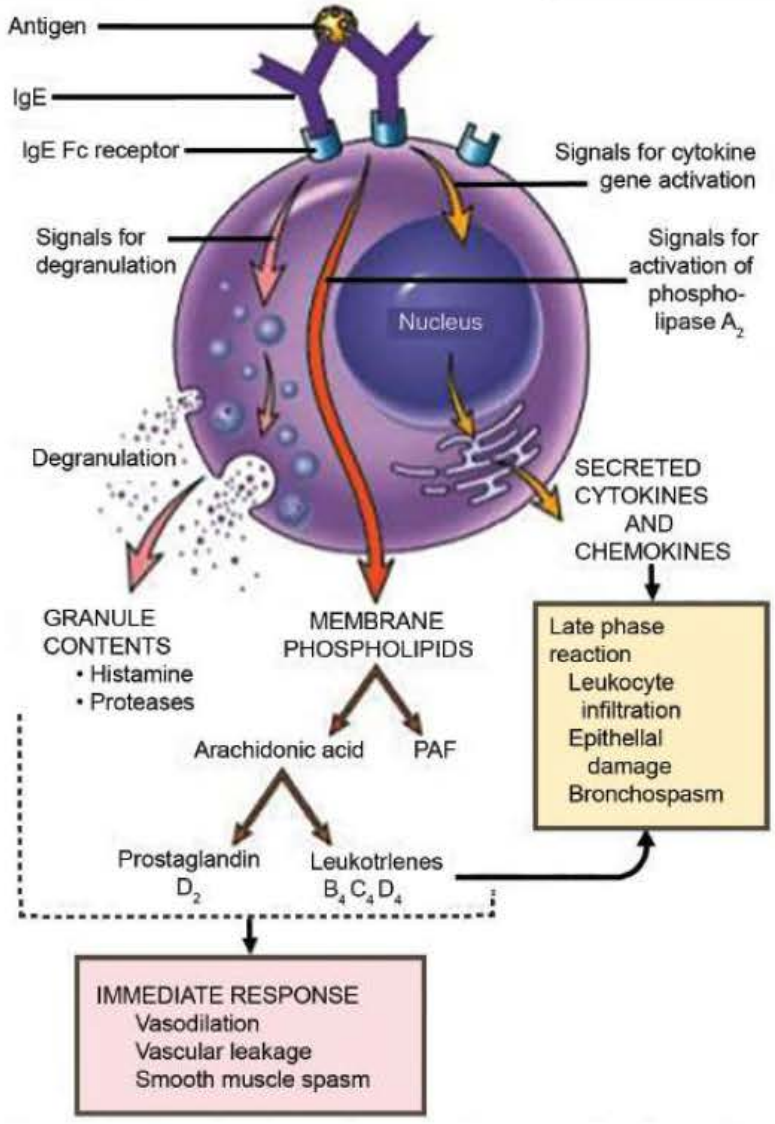


Fig. 5.5: Mast cell mediators. Upon activation, mast cells release various classes of mediators that are responsible for the immediate and late-phase reactions. PAF, Platelet-activating factor.

Clinical Syndrome	Clinical and pathologic Manifestations
Anaphylaxis (may be caused by drugs, bee sting, food)	Fall in blood pressure (shock) cause by vascular dilation; airway obstruction due to laryngeal edema
Bronchial asthma	Airway obstruction caused by bronchial smooth muscle hyperactivity; inflammation and tissue injury caused by late-phase reaction
Allergic rhinitis, sinusitis (hay fever)	Increased mucus secretion; Inflammation of upper airways, sinuses
Food allergies	Increased peristalsis due to contraction of intestinal muscles

Type II Hypersensitivity Reaction:

Complement dependent reactions: Antibody is directed against antigen on cells (such as circulating red blood cells) or extracellular materials (basement membrane). The resulting Ag-Ab complexes activate complement (via the classic pathway), leading to cell lysis or extracellular tissue damage.

Antibody-dependent cell-mediated cytotoxicity (ADCC): Low concentrations of IgG or IgE (in the case of parasites) coat target cells. Inflammatory cells such as NK (natural killer) cells, monocytes, and granulocytes then bind to the immunoglobulin Fc receptors and lyse, but do not phagocytize, the target cells.

Antireceptor antibodies: IgG antibody is directed against receptors in target cells, resulting in complement-mediated destruction of the receptors.

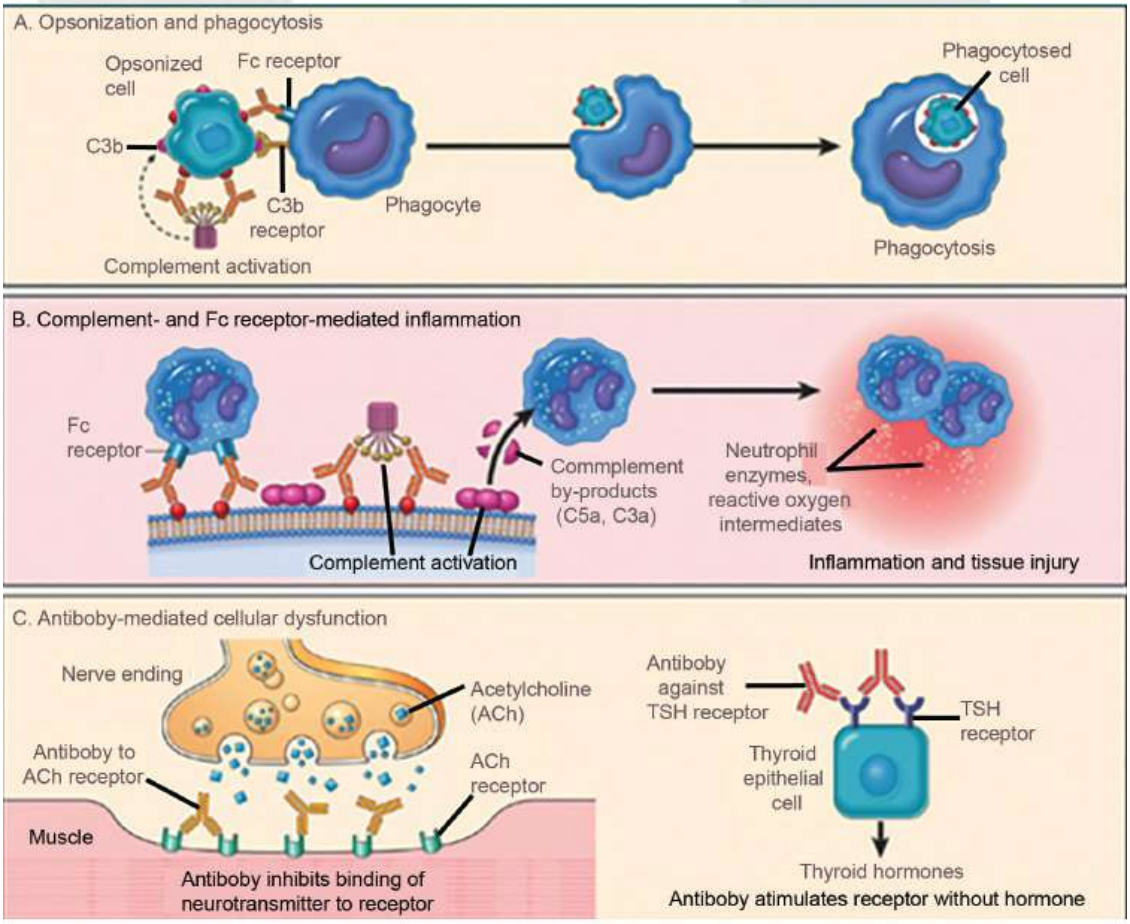


Fig. 5.6

Disease	Target Antigen	Mechanisms of Disease	Clinic pathologic Manifestations
Autoimmune hemolytic anemia.	Red cell membrane proteins (Mi blood group antigens. 1 antigen}.	Opsonization and phagocytosis of red cells.	Hemolysis, anemia.
Autoimmune thrombocytopenic purpura.	Platelet membrane proteins (Gpllb illa integrin).	Opsonization and phagocytosis of platelets.	Bleeding.
Pemphigus vulgaris	Proteins In Intercellular Junctions of epidermal cells .	Antibody-mediated activation of proteases, disruption of Intercellular adhesions.	Skin vesicles (bullae).
Vasculitis caused by ANCA.	Neutrophil granule contents, presumably released from activated neutrophils.	Neutrophil degeneration and Inflammation.	Vasculitis.
Goodpasture syndrome.	Noncollagenous protein In basement membranes of Kidney glomeruli and lung alveoli.	Complement- and Fc receptor-mediated Inflammation.	Nephritis, lung hemorrhage
Acute rheumatic fever.	Streptococcal cell wall antigen; antibody cross-reacts with myocardial antigen.	Inflammation, macrophage activation.	Myocarditis, arthritis.
Myasthenia gravis.	Acetylcholine receptor.	Antibody Inhibits acetylcholine binding, down-modulates receptors.	Muscle weakness, paralysis.
Graves' disease (hyperthyroidism!.	TSH receptor.	Antibody-mediated stimulation of TSH receptors.	Hyperthyroidism.
Insult-resistant diabetes.	Insulin receptor.	Antibody inhibits binding of insulin.	Hyperglycemia, ketoacidosis.
Pernicious anemia.	Intrinsic factor of gastric parietal cells.	Neutralization of Intrinsic factor, decreased absorption of vitamin B1z.	Abnormal erythropoiesis, anemia

Type III Hypersensitivity Reaction:

This reaction is mediated by immune (Ag-Ab) complexes which promote tissue damage primarily through complement activation (alternate pathway). C3b as an opsonin attracts neutrophils, which

then release lysosomal enzymes. C5a as a chemoattractant brings in neutrophils. Serum complement is reduced as it is used up in this process.

Immune complexes can be deposited systemically or locally:

Systemic immune complex disease: Ag-Ab complexes form in the circulatory system and are deposited in tissues, typically near basement membranes in places such as blood vessels, glomeruli, skin, joints, pleura, and pericardium. Larger immune complexes are quickly phagocytized by macrophages and removed, but small to intermediate complexes formed with antigen excess may escape removal leading to:

- Glomerulonephritis.
- Serum sickness.
- Vasculitis.

Local immune complex disease: Also called an “Arthus” reaction, it occurs with local injection of the antigen and leads to focal vasculitis. This kind of immune reaction also plays a role in the development of hypersensitivity pneumonitis (so-called “farmer’s lung”).

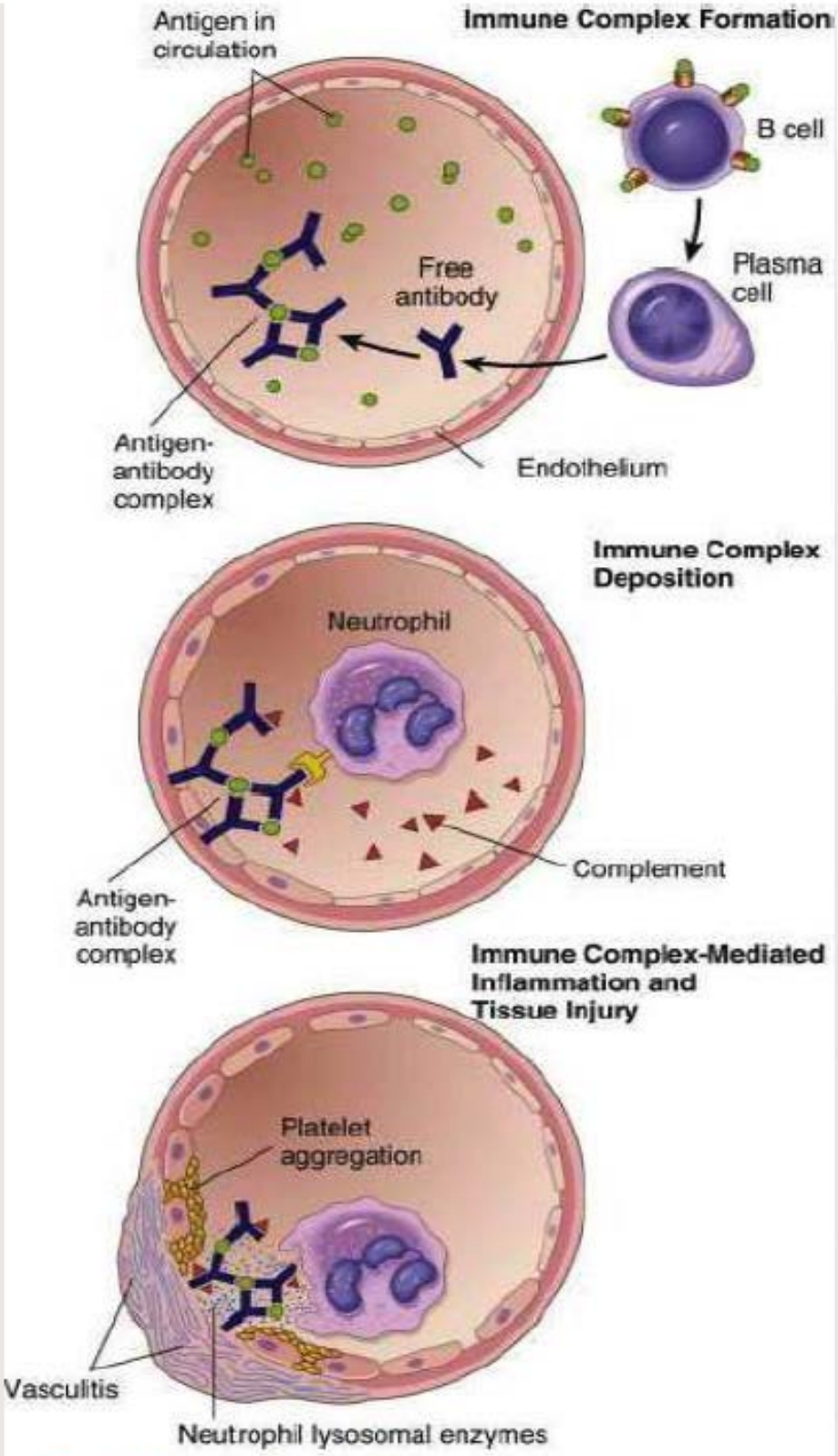


Fig. 5.7: Immune complex disease. The sequential phases in the induction of systemic immune complex-mediated diseases (type III hypersensitivity).

Disease	Antigen Involved	Clinic pathologic Manifestations
Systemic lupus erythematosus	Nuclear antigens (circulating or "planted" In kidney)	Nephritis, skin lesions, arthritis, others
Post streptococcal glomerulonephritis	Streptococcal cell wall antigen(s); maybe "planted" In glomerular basement membrane	Nephritis
Polyarteritis nodosa	Hepatitis B virus antigens In some cases	Systemic vasculitis
Reactive arthritis	Bacterial antigens (e.g., Yersinia)	Acute arthritis
Serum sickness	Various proteins, e.g., foreign serum protein (horse antithymocyte globulin!	Arthritis, vasculitis, nephritis
Arthus reaction (experimental!	Various foreign proteins	Cutaneous vasculitis

Type IV Hypersensitivity Reaction:

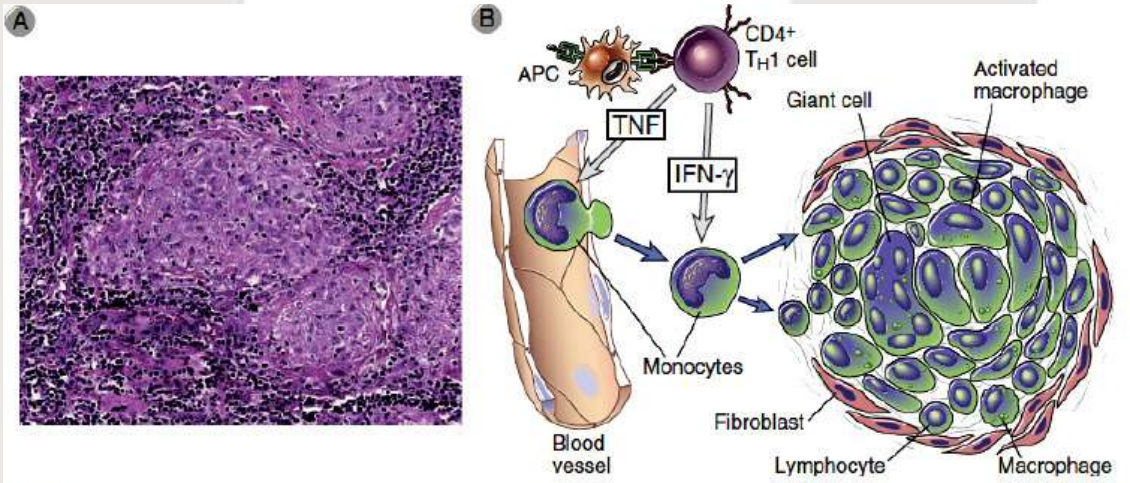


Fig. 5.8: Granulomatous inflammation. A, Lymph node from a patient with tuberculosis containing granulomas with activated macrophages, multinucleate giant cells, and lymphocytes. In some granulomas, there may be a central area of necrosis. Immunohistochemmical studies would identify the Lymphocytes as T cells. B, Mechanisms of granuloma formation. Cytokines are involved in the generation of T_H1 cells, activation of macrophages and recruitment of leukocytes. Prolonged reactions of the type lead to the formation of granulomas.

This reaction is called "delayed hypersensitivity" because it is mediated by sensitized CD4+ T lymphocytes which process antigens in association with class II HLA molecules and release lymphokines. The lymphokines promote a reaction (especially mediated through macrophages) beginning in hours but reaching a peak in 2 to 3 days.

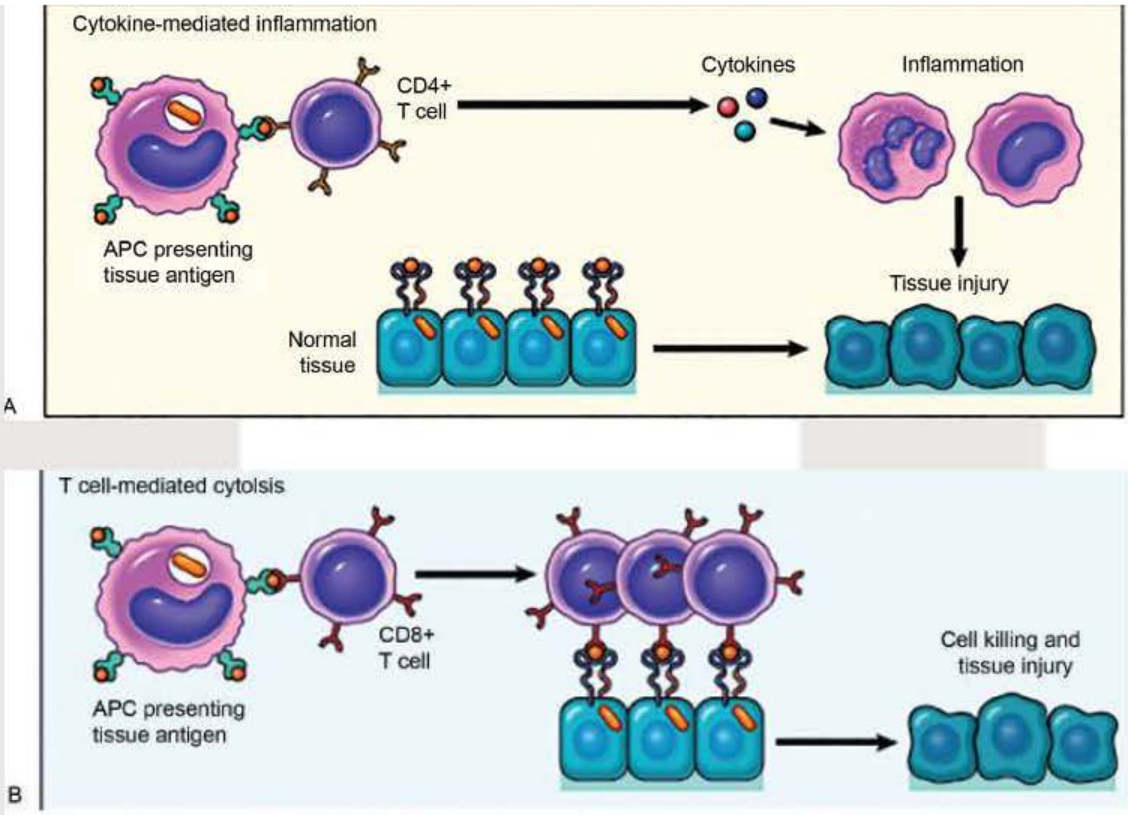

Cytokine-mediated inflammation
CD4+
T cell
Cytokines
Inflammation
APC presenting
tissue antigen
Tissue injury
Normal
tissue
A
T cell-mediated cytolsis
CD8+
T cell
Cell killing and
tissue injury
APC presenting
tissue antigen
B

Fig. 5.9

Disease	Specificity of Pathogenic T cells	Principal Mechanisms of tissue Injury	Cllnicopathologic Manifestations
Rheumatoid arthritis	Collagen? Cirtullinated self-proteins?	Inflammation mediated by TH 17 (and T H1?) cytokines; role of antibiotics and immune complexes?	Chronic arthritis with inflammation, destruction of articular cartilage.
Multiple Sclerosis	Protein antigens in myelin (e.g., myelin basic protein).	Inflammation medicated by TH1 and TH17 cytokines, myelin destruction by activated macrophages.	Demyelination in CNS with perivascular Inflammation; paralysis,
Type 1 diabetes Mellitus	Antigens of pancreatic islet ß cells (Insulin, glutamic acid decarboxylase, Other).	T cell-medicated inflammation, destruction of islet cells by CTLs.	Insulitis (chormic inflammation, in islet) destruction of ß cells; diabetes.
Inflammatory bowel diseases	Enteric bacteria: self-antigens?	Inflammation mediated by TH1 and TH17 cytokines.	Chronic intestinal inflammation, obstruction.
Psoriasis	Unknown	Inflammation mediated mainly by TH17 cytokines.	Destructive plaques in the skin.
Contact sensitivity	Various environmental chemicals (e.g., urushiol from poison ivy or poison oak).	Inflammation mediated by TH1(and TH17?) cytokines.	Epidermal necrosis, dermal inflammation, causing skin rash and blisters.

Examples of human T cell-mediated diseases are listed. In many cases, the specificity of the T cells and the mechanisms of tissue Injury.

Amyloidosis:

- Pathologic proteinaceous substance, deposited between cells in various tissues and organs Amorphous, Eosinophilic, Hyaline, extracellular substance.

Progressive accumulation → pressure atrophy of adjacent cells.

Physical nature of Amyloid:

- Non branching fibrils of indefinite length
- Diameter: 7.5 -10 nm.

X-ray crystallography and Infra red spectroscopy:

- Characteristic cross β – pleated sheet confirmation. (responsible for birefringence).

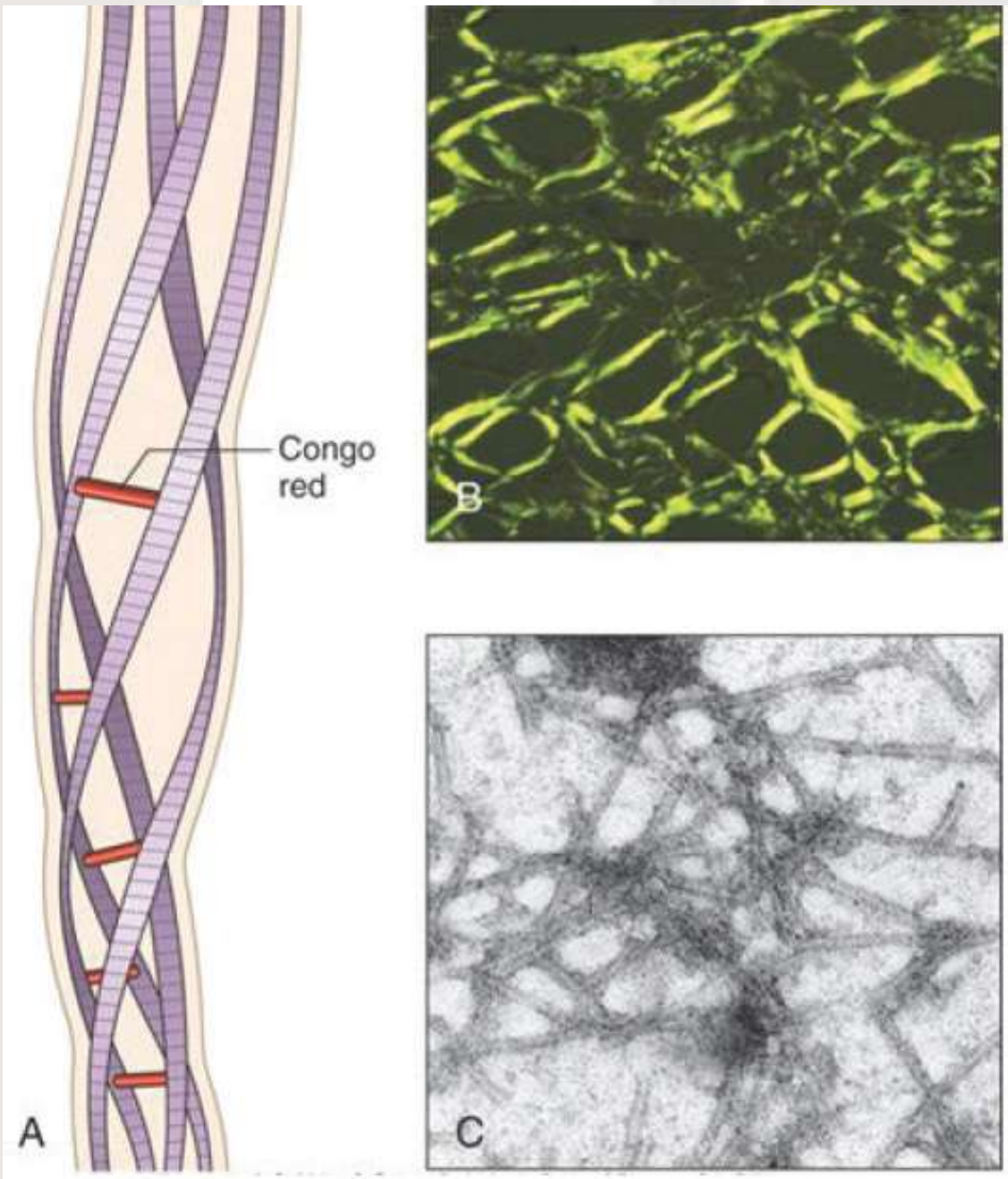


Fig. 5.10

Structure of amyloid. A**, an amyloid fiber schematically showing four fibrils (there can be as many as six in each fiber) wound around one another with regularly spaced binding of the Congo**

red dye. B, Congo red staining shows apple-green birefringence under polarized light, a diagnostic feature of amyloid. C, Electron micrograph of 7.5- to 10-nm amyloid fibrils.

15 biochemically distinct forms.

3 more common forms:

a. AL (amyloid light chain).

- derived from plasma cells (most AL-LAMBDA VI).
- Contains Ig light chains.

b. AA (Amyloid associated).

Non – immunoglobulin protein synthesized by liver.

c. Aβ amyloid: In cerebral lesion of Alzheimer disease.

Classification of Amyloidosis:

Clinicopathologic Category	Associated Diseases	Major Fibril Protein	Chemically Related Precursor Protein
Systemic (Generalized) Amyloidosis			
Immunocyte dyscrasias with amyloidosis (primary amyloidosis).	Multiple myeloma and other monoclonal plasma cell proliferations.	AL	Immunoglobulin light chains, chiefly λ type.
Reactive systemic amyloidosis (secondary amyloidosis).	Chronic inflammatory conditions.	AA	SAA
Hemodialysis-associated amyloidosis.	Chronic renal failure.	Aβ2m	β2-microglobulin.
Hereditary Amyloidosis			
Familial Mediterranean fever.		AA	SAA
Familial amyloidotic neuropathies (several types).		ATTR	Transthyretin
Systemic Senile Amyloidosis.		ATTR	Transthyretin
Localized Amyloidosis.			
Senile cerebral	Alzheimer disease	Ab	APP
Endocrine		A Cal	Calcitonin
Medullary carcinoma of thyroid.	Type 2 diabetes	AIAPP	Islet amyloid peptide.
Islets of Langerhans.		AANF	Atrial natriuretic factor.
Isolated atrial amyloidosis.			

Types:

A. SYSTEMIC

B. LOCALIZED

A. SYSTEMIC:

1. PRIMARY AMYLOIDOSIS / Immunocyte dyscrasias: systemic amyloidosis.

AL: Complete Ig light chain - NH_2 terminal fragment both.

Most common – Lambda or kappa.

- Associated with plasma cell dyscrasia.
- Systemic in nature.

5-15% of pts, With Multiple Myeloma develop AL Amyloidosis.

2. Reactive Systemic Amyloidosis / Secondary Amyloidosis.

- AA protein deposited.
- Secondary to associated inflammations.
- Systemic disorder.

Association:

Prev: TB, bronchiectasis, chronic osteomyelitis.

Now, most common: Rheumatoid Arthritis (13% of pts. Dev. AA).

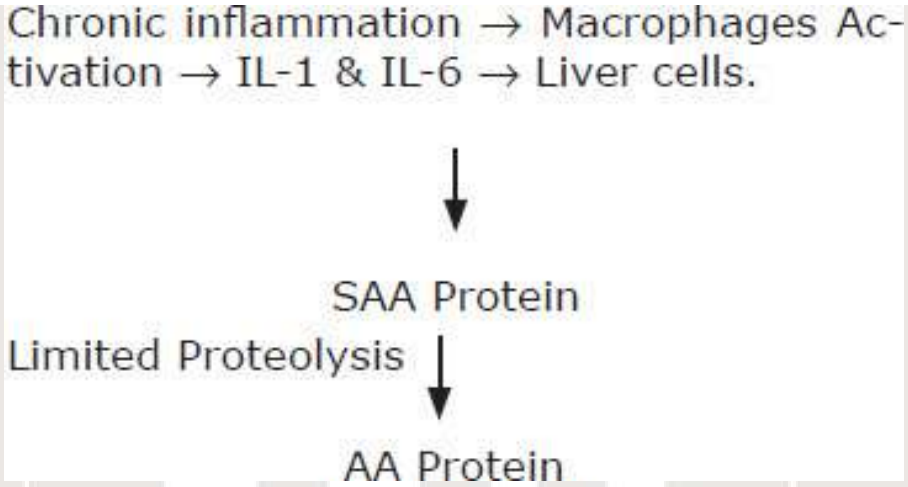
Ankylosing spondylitis.

Inflammatory bowel disease.

Others: Heroine abuses.

RCC.

Hodgkin’s disease.



3. **Hemodialysis associated amyloidosis:**

- Deposition of β_2 Microglobulin (component of MHC class I molecule) (Can't be filtered through cuprophane dialysis membranes)
- Deposits is synovium, joints & tendon sheaths.

4. **Heredofamilial Amyloidosis:**

a. **Familial Mediterranean fever** : Fever with inflammation of serosal surface (Pleura, peritoneum & 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 & autonomic nerves involved

►AD

►Deposits of ATTR
(Trans thyretin)
(Mutant form)

c. **Systemic senile Amyloidosis:**

►Deposits of ATTR (structurally normal).

►Deposits in heart of aged individuals (70-80 years).

d. **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.

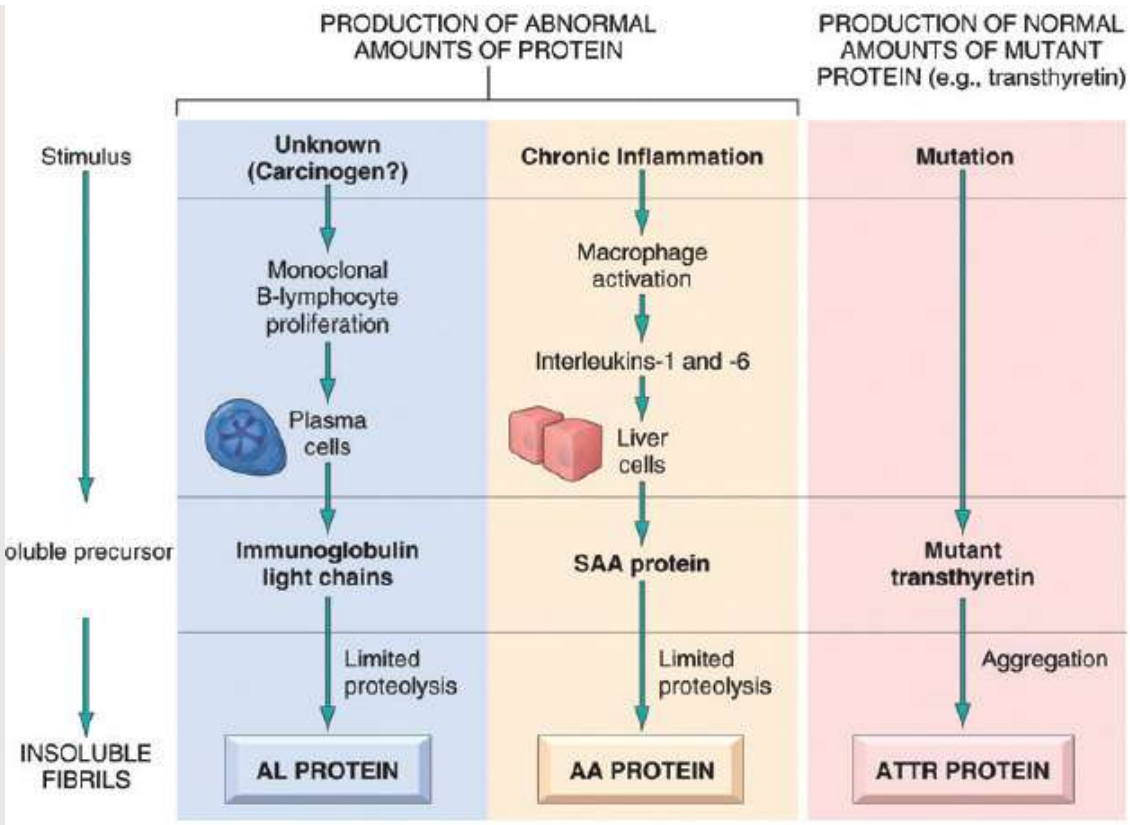


Fig. 5.11

Morphology:

AA: M. severe systemic involvement

- Kidneys, Liver, Spleen, Lymph nodes, Adrenal, Thyroid

AL: Heart, kidney, GIT, Peripheral nerves, Skin, Tongue

Gross: Organ → enlarged, firm, waxy

Cut surface paint with H_2 SO_4 yellow color → → Iodine ----- Blue violet Colour

Staining:

1. PAS+ (: of P component → glycoproteins)

2. Congo red — on light Microscopy it appears Light pink

Polarized Light ----- Green Birefringence

After t/t with KMnO4 AA protein losses its affinity:

3. Crystal violet/ methyl violet
4. Thioflavin ‘T & S’
5. Immunohistochemical staining
6. Electron Microscopy

Kidney	Most common and most serious form of organ involvement. • In interstitial peritubular tissue, arteries & arterioles.
Spleen	Sago spleen: In splenic follicles On gross: tapioca like granules Lardaceous spleen: In walls of splenic sinuses & in red pulp . • Large, map like areas.
Liver	First in space of Disse = pressure atrophy • Also vascular involvement & Kupffer cell deposition.

Heart	Focal subendocardial accumulation and between the muscle fibres (May damage conduction = ECG abnormalities) • May present as CHF, arrhythmias
Adrenals	Initially in Zona glomerulosa
GIT	Any level: Gingiva to anus Tongue = macroglossia • Tumor forming amyloid of the tongue.

Most common organ involved in amyloidosis- Kidney.
Most common cause of mortality in primary amyloidosis- cardiac.
Most common cause of mortality in secondary amyloidosis- renal.
Most common cause of mortality overall- cardiac.

Prognosis: Poor for generalized amyloidosis.

AL (not including MM) → median 2 years survival.

In MM → Worse Prognosis.

AA → Control the cause → Better Prognosis.

Transplant Rejection:

- Graft rejection depends on recognition of grafted tissue as foreign by host.
- Both cellular and humoral immunity play a role.

- Hypersensitivity type II, III, IV involved.

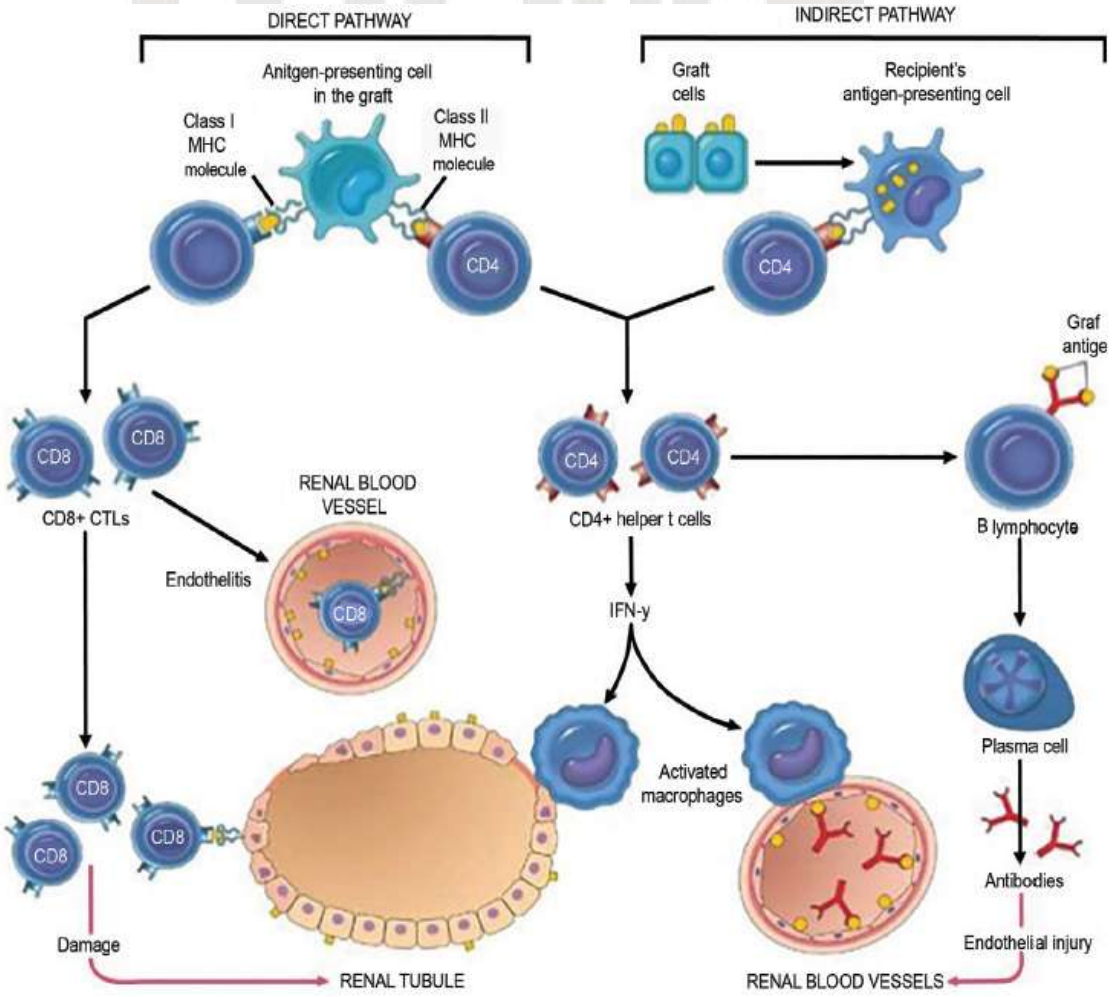


Fig. 5.12

1. Hyperacute - Mediated by preformed antidonor antibodies.

- Within minutes or hours after transplantation.
- Initial target- Graft vasculature.
- Type II HR at the level of vascular endothelium.
- Commonly seen in patients who have received multiple blood transfusion / transplant before.

Morphology:

Hyperacute: Gross- cyanotic mottled, flaccid organ within minutes of joining vasculature – identified by surgeon himself- earliest feature of

rejection.

M/E – Neutrophilic vasculitis with thrombosis.

2. **A. Acute Cellular rejection** – Most common type of graft rejection seen within few days to months or after removal of immunosuppressive drag.

- Interstitial inflammatory infiltrate (CD4, CD8).
- Glomerular and peritubular capillaries, show large number of mononuclear cells.
- Tubulitis is most characteristic feature of this.
- After removal of immunosuppressive drugs.
- Most frequent form of rejection.
- Responds to immuno suppressive therapy.
- Type IV HR.

2. b. Acute humoral rejection:

- Necrotizing vasculitis, intimal thickening of arteries.
- Endothelial necrosis
- Deposition of immunoglobulin, fibrin and thrombosis.
- Responds poorly to increased doses of immuno suppression.

3. **Chronic Rejection** : – Occurs when acute rejection is revolved by parenchymal fibrosis.

- * Vascular changes – Obliterative intimal fibrosis especially in cortical arteries.
- Interstitial mononuclear infiltrate – lymphocytes, plasma cells and eosinophils.
- Tubular atrophy and global Glomerulosclerosis (post trans plant Glomerulo pathy).

To summarize:

Hyperacute- type II.

Acute humoral- type II.

Acute cellular – type IV.

Chronic- fibrotic reaction > type IV.

Transplantation of Hematopoetic organs: Three major problems in allogenic BM transplant.

1. **Graft versus host disease: -** immunologically competent cells or precursors are transplanted into immunosuppressed host. Recipients of bone marrow transplant are immuno deficient because of primary disease or treatment. When such recipients receive normal bone marrow cells from allogenic donors, the immuno competent T cells (CD_4 , CD_8 T cells) recognize recipients HLA antigens as foreign and attack the host tissue. Present as:
   Acute GVH – Involvement of immune system & epithelia of skin (rashes), liver (jaundice) & GIT (diarrhea) by lymphocytic infiltration.
   Chronic GVH: May follow acute GVH or occurs insidiously. Severe fibrotic damage of skin, immune system GIT (oesophageal strictures), lungs, xerophthalmia and musculoskeletal system. There is involution of thymus and depletion of lymphocytes from lymph nodes. Allogenic T cells in the graft lead to GVHD but there are required for engraftment of graft and control of leukemia. This is known as graft versus leukemia
2. **Transplant rejection:** Mediated by NK cells & T cells of host.
3. **Immunosuppressive:** CMV infection is most common (lung, GIT), others- HSV, parasite and fungal infections can also occur.

Autoimmune Disease:

Immune reaction against self antigens.

Implies loss of immunologic tolerance (clonal deletion, clonal anergy and peripheral suppression)

Mechanism of auto immunity:

1. Bypass of T helper cell tolerance

Modification of molecule (drug induced hemolytic anemia, RA).

Molecular mimicry (Rheumatic carditis)

2. Polyclonal lymphocyte activation- By endotoxin or EBV. Anergic clones get stimulated.
3. Imbalance of suppressor- helper function- Decreased supp and increased helper activity.
4. Emergence of sequestered antigen (spermatozoa, Lens crystalline, Myelin basic protein).
5. Genetic factors – Linkage with HLA.
6. Microbial agents- Mechanism 1, 2, and 3.

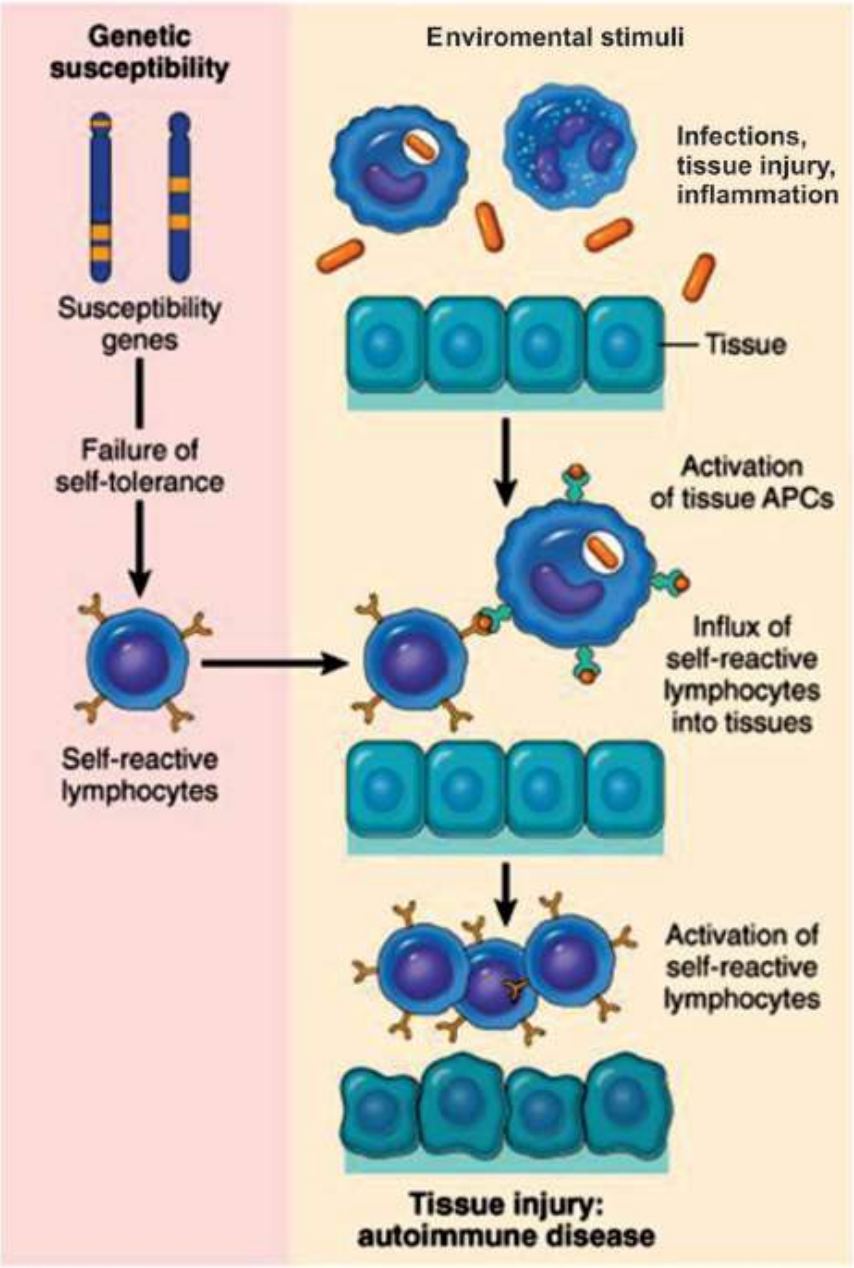


Fig. 5.13

Pathogenesis of autoimmunity. Autoimmunity results from multiple factors, including susceptibility genes that may interfere with self-tolerance and environmental triggers (such as infections, tissue injury, and inflammation) that promote lymphocyte entry into tissues, activation of self-reactive lymphocytes, and tissue damage

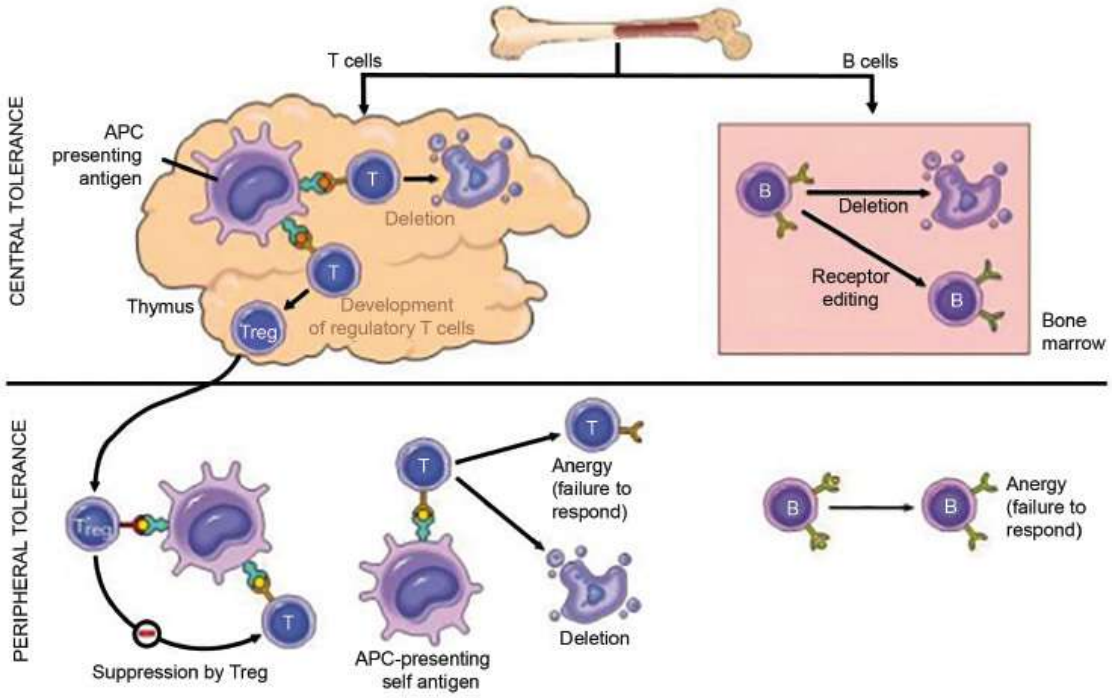


Fig. 5.14

Mechanisms of immunologic tolerance to self antigens. The principal mechanisms of central and peripheral self-tolerance in T and B cells are illustrated.

Antinuclear Antibodies in Various Autoimmune Diseases:

Nature of Antigen	Antibody System	Disease, % Positive					
		SLE	Drug-Induced LE	Systemic Sclerosis –Diffuse	Limited Scleroderma –CREST	Sjögren Syndrome	Inflammatory Myopathies
Many nuclear antigens (DNA, RNA, proteins)	Generic ANA (indirect IF)	>95	>95	70–90	70–90	50–80	40–60
Native DNA	Anti–double-stranded DNA	40–60	<5	<5	<5	<5	<5
Histones	Ant histone	50–70	>95	<5	<5	<5	<5

Core proteins of small nuclear RNP particles (Smith antigen)	Anti-Sm	20–30	<5	<5	<5	<5	<5
RNP (U1RNP)	Nuclear RNP	30–40	<5	15	10	<5	<5
RNP	SS-A(Ro)	30–50	<5	<5	<5	70–95	10
RNP	SS-B(La)	10–15	<5	<5	<5	60–90	<5
DNA topoisom-erase I	Scl-70	<5	<5	28–70	10–18	<5	<5
Centromer-ic proteins	Anti-centromere	<5	<5	22–36	90	<5	<5
Histidyl-tRNA synthetase	Jo-1	<5	<5	<5	<5	<5	25

ANA, antinuclear antibodies; IF, immunofluorescence; LE, lupus erythematosus; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus.

Disease	HLA allele
Rheumatoid arthritis (anti-CCP fit positive)	DRB1,1 SE allele DRB1, 2 SE alleles
Type 1 diabetes	DRB1*0301-DQA1*0501 - DQB1*0201 haplotype DRB1*0401 -DQA1*0301 - DQB1*0302 haplotype DRB1*0301/0401 haplotype heterozygotes
Multiple sclerosis	DRB1 1501
Systemic lupus erythematosus	DRB1*0301 DRB1*1501
Ankylosing spondylitis	B*27 (mainly B*2705 and B*2702)
Celiac disease	DQA1 *0501 -DQB1 *0201 haplotype

Association of Non-MHC Genes with Autoimmune Diseases:

1. Polymorphisms in a gene called PTPN22, which encodes a protein tyrosine phosphatase, are associated with rheumatoid arthritis, type 1 diabetes, and several other autoimmune diseases. Because these disorders have a fairly high prevalence (especially rheumatoid arthritis), **PTPN22 is said to be the gene that is most frequently implicated in autoimmunity** . It is postulated that the disease-associated variants encode a phosphatase that is functionally defective and is thus unable to fully control the activity of tyrosine kinases, which are involved in many responses of lymphocytes and other cells. The net result is excessive lymphocyte activation.
2. Polymorphisms in the gene for **NOD2** are associated with Crohn disease, a form of inflammatory bowel disease, especially in certain ethnic populations. NOD2, a member of the NOD-like receptor (NLR) family, is a cytoplasmic sensor of microbes that is expressed in intestinal epithelial and other cells. According to one hypothesis, the disease-associated variant is ineffective at sensing gut microbes, including commensal bacteria, resulting in entry of and chronic inflammatory responses against these normally well tolerated organisms.
3. Polymorphisms in the genes encoding the **IL-2 receptor (CD25)** and **IL-7 receptor** α chains are associated with multiple sclerosis and other autoimmune diseases. These cytokines may control the maintenance of regulatory T cells.

Organ specific:

	Disease	Autoantibodies
Thyroid	Hashimoto's thyroiditis	• Thyroglobulin • Thyroid microsomes • TSI, LATS • TSH receptor Abs. Therefore, • Action like TSH
	Grave's Disease	
	1 myxedema	• TSH receptor Abs which Block TSH action
Stomach	Chr atrophic gastritis	• Gastric parietal cells
	Leads to PA	• Microsomes
		• Surface
		• IF
		• IF / B12 complex
		[Blocks interaction with B12 prevents absorption]
Adrenal cortex	Addison's disease	• Adrenal cell microsomes
		• leads to atrophy
		ACTH receptor
		-inhibits binding of
		• ACTH to receptor

Pancreatic islet cell	Diabetic mellitus	• Antibodies to Beta cells
	Type I	↓ Insulin
Skeletal Muscle	Myasthenia gravis	• Acetyl choline receptor
		↓ Acetylcholine binding
		• Cross reactive abs to thymic
		Epithelium + Skeletal muscle

Non organ specific:

Systemic Lupus Erythematoses:

Male: Female = 9:1; Age peak – third decade.

Classic prototype of multisystem autoimmune disease.

1997 Revised Criteria for Classification of Systemic Lupus Erythematosus:

Criterion	Definition
1. Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds.
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions.
3. Photosensitivity	Rash as a result of unusual reaction to sunlight, by patient history or physician observation.
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician.
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion.
6. Serositis	Pleuritis—convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion, or
	Pericarditis—documented by electrocardiogram or rub or evidence of pericardial effusion.
7. Renal disorder	Persistent proteinuria >0.5 gm/dL or >3 if quantitation not performed or
	Cellular casts—may be red blood cell, hemoglobin, granular, tubular, or mixed.
8. Neurologic disorder	Seizures—in the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance), or
	Psychosis—in the absence of offending drugs or known metabolic derangements (e.g., uremia, ketoacidosis, or electrolyte imbalance).
9. Hematologic disorder	Hemolytic anemia—with reticulocytosis, or
	Leukopenia—<4.0 × 109 cells/L (4000 cells/mm3) total on two or more occasions, or
	Lymphopenia—<1.5 × 109 cells/L (1500 cells/mm3) on two or more occasions, or
	Thrombocytopenia—<100 × 109 cells/L (100 × 103 cells/mm3) in the absence of offending drugs
10. Immunological disorder	Anti-DNA antibody to native DNA in abnormal titer, or
	Anti-Sm—presence of antibody to Sm nuclear antigen, or
	Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test for lupus anticoagulant using a standard test, or (3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by negative Treponema pallidum immobilization or fluorescent treponemal antibody absorption test.
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with drug-induced lupus syndrome.
	This classification, based on 11 criteria, was proposed for the purpose of identifying patients in clinical studies. A person is said to have systemic lupus erythematosus if any 4 or more of the 11 criteria are present, serially or simultaneously, during any period of observation.

Antinuclear antibodies (ANAs):

These are directed against nuclear antigens and can be grouped into four categories:

1. Antibodies to DNA,
2. Antibodies to histones,
3. Antibodies to nonhistone proteins bound to RNA, and
4. Antibodies to nucleolar antigens.

The most widely used method for detecting ANAs is indirect immunofluorescence, which can identify antibodies that bind to a variety of nuclear antigens, including DNA, RNA, and proteins (collectively called generic ANAs). The pattern of nuclear fluorescence suggests the type of antibody present in the patient's serum (potential exam question).

Homogeneous or diffuse nuclear staining	antibodies to chromatin, histones, and, occasionally, double-stranded DNA.
Rim or peripheral staining	antibodies to double-stranded DNA and sometimes to nuclear envelope proteins.
Speckled pattern	presence of uniform or variable-sized speckles. This is the most commonly observed patterns of fluorescence and therefore the least specific. It reflects the presence of antibodies to non-DNA nuclear constituents such as Sm antigen, ribonucleoprotein, and SS-A and SS-B reactive antigens.
Nucleolar pattern	presence of a few discrete spots of fluorescence within the nucleus and represents antibodies to RNA. This pattern is reported most often in patients with systemic sclerosis.
Centromeric pattern	Patients with systemic sclerosis often contain antibodies specific for centromeres, which give rise to this pattern.

Mechanism of Injury:

Most visceral lesions are mediated by **Type III HS.** Low level of serum complements and granular deposits of lg and complement support this.

Hematologic manifestations are mediated via **Type II HS.**

Clinical and Pathologic: Manifestations of Systemic Lupus Erythematosus:

Clinical Manifestation	Prevalence in Patients (%) [*]
Hematologic	100
Arthritis	80–90
Skin	85
Fever	55–85
Fatigue	80–100
Weight loss	60
Renal	50–70
Neuropsychiatric	25–35
Pleuritis	45
Myalgia	35
Pericarditis	25
Gastrointestinal	20
Raynaud phenomenon	15–40
Ocular	15
Peripheral neuropathy	15

Morphology:

Characteristic lesion is an acute necrotizing arteritis involving small arteries and arterioles in skin and muscle.

Spleen- perivascular fibrosis- **Onion Skin Lesion**

Kidney - Diffuse proliferative glomerulonephritis - most common form of renal lesion.

Heart – Libman Sacks endocarditis - non bacterial verrucous endocarditis.

- 1 to 3 mm warty deposits on either side of leaflets of valve Bread and butter pericarditis.
- Increased predisposition to coronary atherosclerosis esp. in cases treated with corticosteroids.

Chronic DLE- Manifestations limited to skin.

Drug induced lupus – Hydralazine, isoniazid, procainamide and D-penicillamine.

High frequency of anti-histone antibodies.

Sjogren's Syndrome:

- Females, in age grp of 40-60 years present with dry mouth and dry eye due to destruction of lacrimal and salivary glands.
- Anti SS A and SSB are seen in 90% pts with SjS. 75% are positive for RF, 50 -80% pts have ANA and 25% have a positive LE cell test.
- EBV have role in pathogenesis.
- Morphologically show periductal and perivascular lymphocytic infiltration in involved glands. Lip biopsy used to diagnose Sjogrens. Glomerular l; lesions and extremely rare.
- Miculicz syndrome refers to enlargement of lacrimal, other tumorous.
- Lymph nodes show picture of pseudo lymphoma. Also increases risk of development of lymphomas.

Systemic Sclerosis:

- Scleroderma because skin is most commonly affected.
- Diffuse scleroderma is characterized by widespread fibrosis throughout the body, esp. skin, GIT, kidneys, heart, muscles and heart.
- Localized – CREST syndrome. Calcinosis, Raynaud's phenomenon, Oesophageal dysmotility, Sclerodactyly, Telangiectasia
- Antinuclear antibodies seen in virtually all patients.

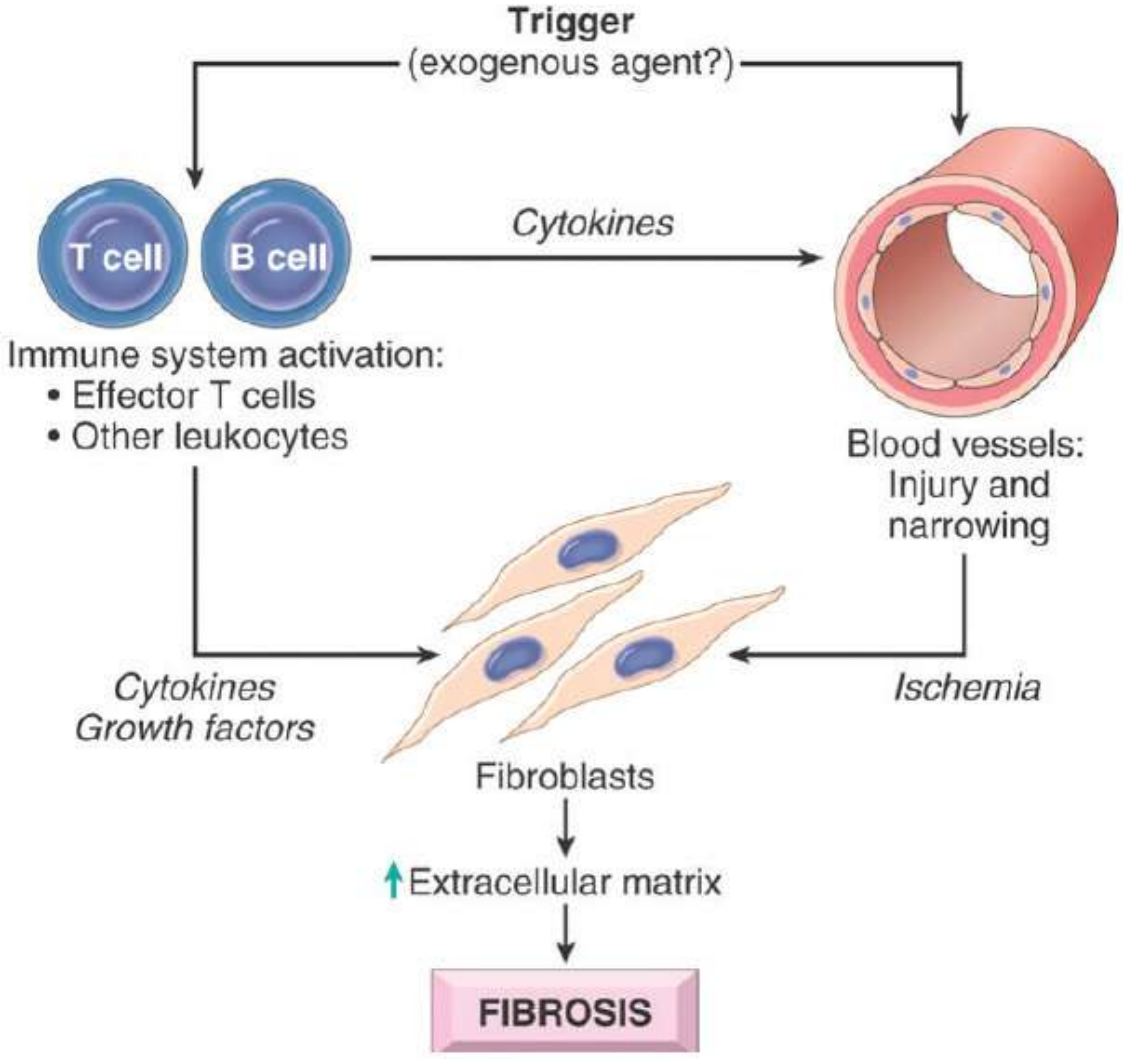


Fig. 5.15

- **Anti DNA topoisomerase I** (ScI 70) unique to Diffuse systemic sclerosis.
- **Anticentromere Ab seen** in CREST syndrome. Both Abs do not coexist.
- Morphologically, **skin** shows diffuse sclerotic atrophy, **Rubber hose esophagus,** malabsorpion syndrome, pulmonary fibrosis. **Renal** involvement involves interlobular vessels which show changes similar to malignant HT.

Rheumatoid Arthritis:

Non suppurative, proliferative synovitis- leads to destruction of articular cartilage+ progressive disabling arthritis (symmetrical) [Prox IP + MP joints affected – DIP spared]

- 25 -55 yrs
- Females > Males
- HLA DR 4

Serum of most patient's **rheumatoid factor** i.e. autoantibodies against Fc piece of lg, autoantibodies are usually lgM type

Morphologically, synovial proliferation with pericascular lymphoid follicles, rice bodies in synovial fluid, pannus formation over articular surface which cause erosion of underlying cartilage. Rheumatoid nodules seen in subcutaneous tissue.

Mechanism - type III + Type IV

Variants:

Juvenile Rheumatoid arthritis- larger joints. Rh factor negative + subcutaneous nodules not frequent.

Stills disease- Acute febrile onset with Leukocytosis (15,000- 28,000/ cynn),

Hepatosplenomegaly, lymphadenopathy + skin rash. 1/3rd monoarticular.

Felty's syndrome Triad of Polyarthritis, Splenomegaly and Leukopenia.

Spondyloarthropathles:

Sero – ve for Rheumatoid factor

Ankylosing spondylosis also called Marie – Stumpell disease.

- Affects ligamentous attachment to bones
- Sacroiliac joint most commonly involved (MCQ)
- Ass HLA B27 especially ankylosing spondylitis – 95%.
- Also uveitis, conjunctivitis, urethritis, skin + mucosal involved.

Reiter's syndrome – triad of arthritis, nongonococcal urethritis / cervicitis and conjunctivitis.

Inflammatory Myopathies:

Dermatomyositis, Polymyositis and inclusion – body myositis.

DM – Skin and muscle involvement. Lilac or heliotrope discoloration of upper eyelid with periorbital oedema. Grotton's lesions over the knuckles, elbows, knees.

Typically affect proximal muscles. Higher risk of developing visceral malignancies:

Microvasculature is attacked by Abs and complement.

PM- No cutaneous involvement. Cell mediated injury by cytotoxic T cells.

Features Associated with Inflammatory Myopathies			
Characteristic	Polymyositis	Dermatomyositis	Inclusion Body Myositis
Age at onset	>18 years	Adulthood and childhood	>50 years
Familial association	No	No	Yes, in some cases
Extramuscular manifestations	Yes	Yes	Yes
Associated conditions			
Connective tissue diseases	Yesa	Scleroderma and mixed connective tissue disease (overlap syndromes)	Yes, in up to 20% of casesa
Systemic autoimmune diseasesb	Frequent	Infrequent	Infrequent
Malignancy	No	Yes, in up to 15% of cases	No
Viruses	Yesc	Unproven	Yesc
Drugsd	Yes	Yes, rarely	No
Parasites and bacteriae	Yes	No	No

Immunodeficiency Diseases:

Features of Immunodeficiencies Affecting I or B Lymphocytes		
Feature	B Cell Deficiency	T Cell Deficiency
Susceptibility to infection.	Pyogenic bacteria (otitis, pneumonia, meningitis, osteomyelitis), enteric bacteria and viruses, some parasites.	Pneumocystis proved, many viruses, atypical mycobacteria, fungi.
Diagnosis		
Serum Ig levels DTH reactions to common antigens.	Reduced Normal.	Normal or reduced.
Morphology of lymphoid tissues.	Absent or reduced follicles and germinal centers (B cell zones).	Usually normal follicles, may be reduced parafollicular cortical regions (T cell zones).
DTHr delayed-type hypersensitivity.		

Examples of Infections In Immunodeficiencies:

Pathogen Type	T-Cell Defect	B-Cell Defect	Granulocyte Defect	Complement Defect
Bacteria	Bacterial sepsis	Streptococci, staphylococci, Haemophilus.	Staphylococci, Pseudomonas.	Neisserial infections, other pyogenic infections.
Viruses	Cytomegalovirus, Epstein-Barr virus, severe varicella, chronic infections with respiratory and intestinal viruses.	Enteroviral encephalitis.		
Fungi and parasites	Candida, Pneumocystis jiroveci.	Severe intestinal giardiasis.	Candida, Nocardia, Aspergillus.	
Special features	Aggressive disease with opportunistic pathogens, failure to clear infections.	Recurrent sinopulmonary infections, sepsis, chronic meningitis.		

Congenital Disorders of Innate Immunity		
Disease	Functional Deficiencies.	Mechanism of Defect.
Chronic granulomatous disease.	Defective production of reactive oxygen species by phagocytes; recurrent intracellular bacterial and fungal infections.	Mutation in genes of phagocyte oxidase complex; phox-91 (cytochrome subunit) is mutated in X-linked form.
Leukocyte adhesion deficiency type 1.	Defective leukocyte adhesion to endothelial cells and migration into tissues linked to decreased or absent expression of p21; recurrent bacterial and fungal infections.	Mutations in gene encoding the p chain (CD18) of p2 integrins.

Leukocyte adhesion deficiency type 2.	Defective leukocyte rolling and migration into tissues linked to decreased or absent expression of leukocyte ligands for endothelial E- and P-selectins, causing failure of leukocyte migration into tissues; recurrent bacterial and fungal infections.	Mutations in gene encoding GDP-fucose transporter-1, required for transport of fucose into the Golgi and its incorporation into sialyl Lewis X.
Leukocyte adhesion deficiency type 3.	Defective leukocyte adhesion and migration into tissues linked to defective chemokine-stimulated inside-out signaling and therefore defective integrin activation.	Mutations in gene encoding KINDLIN-3, a cyto- skeletal protein linked to inside-out signaling.
Chediak-Higashi syndrome.	Defective vesicle fusion and lysosomal function in neutrophils, macrophages, dendritic cells, NK cells, cytotoxic T cells, and many other cell types; recurrent infections by pyogenic bacteria.	Mutation in LYST leading to defect in secretory granule exocytosis and lysosomal function.
NK cell deficiencies.	Reduced or absent NK cells.	Mutations in the gene encoding the GATA-2 transcription factor and in the gene encoding the MCM-4 DNA helicase.
Toll-like receptor signaling defects.	Recurrent infections caused by defects in TLR and CD40 signaling and defective type 1 interferon production.	Mutations in TLR3, TRIE, TBK1, NEMO, UNC93B, MyD88, kBa, and IRAK-4 compromise NF-kB activation downstream of Toll-like receptors.
Mendelian Susceptibility to Mycobacterial Diseases.	Severe disease caused by non-tuberculous environmental mycobacteria and BCG.	Mutations in IL-J2piO, IL-12RB, IENGR1, IFNGR2, STAT1, NEMO, and ISG15.
BCG. bacillus Calmette-Guerin; IRAK-4. IL-1 receptor-associated kinase 4; LYST. lysosomal trafficking protein; NEMO. NF-ktB essential modulator..		

Severe Combined Immunodeficiencies		
Disease	Functional Deficiencies.	Mechanism of Defect.
Defects in Cytokine Signaling.		
X-linked SCID.	Marked decrease in T cells; normal or increased B cells; reduced serum Ig.	Cytokine receptor common y chain mutations; defective T cell development in the absence of IL-7-derived signals.
Autosomal recessive forms.	Marked decrease in T cells; normal or increased B cells; reduced serum Ig.	Mutations in IL2RA, IL7RA, JAK3.
Defects in Nucleotide Salvage Pathways.		

ADA deficiency	Progressive decrease in T cells, B cells, arid NK cells; reduced serum Ig.	Mutations in the ADA gene, leading to accumulation of toxic metabolites in lymphocytes.
PNP deficiency.	Progressive decrease in T cells, B cells, and NK cells; reduced serum Ig.	Mutations in the PAfPgene, leading to accumulation of toxic metabolites in lymphocytes.
Defects in V(D)J Recombination.		
RAGl or RAG2 deficiency recombination*.	Decreased T cells and B cells; reduced serum Ig; absence or deficiency of T and B cells.	Cleavage defect during V(0)J recombination; mutations in RAG J or RAG2.
Double-stranded break repair and checkpoint.	Decreased T and B cells; reduced serum Ig; absence or deficiency of T cells and B cells.	Failure to resolve hairpins during V(D)J recombination; mutations in ARTEMIS, DNA-PKcs, CERNUNNQS, LIG4, NBS1, MRE11,ATM.
Defective Thymic Development.		
Defective pre-TCR checkpoint.	Decreased T cells; normal or reduced B cells; reduced serum Ig.	Mutations in CD45, CD3D, CD3E, ORAIJ (CRAC channel component), STIM1.
DiGeorge syndrome.	Decreased T cells; normal B cells; normal or reduced serum Ig.	22q11 deletion;T-box 1 (TBX1) transcription factor mutations.
FoxNI deficiency.	Thymic aplasia with defective T cell development.	Recessive mutation in FOXN1.
TCFt a chair deficiency.	No op T cells; y6 T cells normal; recurrent infections and autoimmunity.	Autosomal recessive deletion in C region of TCR a chain.
Defective T cell thymic egress and defective T cell signaling.	Marked reduction in all peripheral T cells.	Mutations in RHOHand MSTJ.
Selective loss of CD4+ T cells and defective T cell signaling.	Decreased CD4+T cells.	Mutations in LCKand UNCI 19.
Other Defects.		
Reticular dysgenesis .	Decreased T cells, B cells, and myeloid cells.	Mutation in AK2.
ADA, adenosine deaminase; AK2, adenylate kinase 2; ATM, ataxia-telangiectasia mutated; CRAC. calcium release activated channel; DNA-PKcs, DN A-dependent protein kinase catalytic subunit; UG4, DNA ligase 4; MRE11, meiotic recombination homologue 11; NBS1, Nijmegert breakpoint syndrome 1; PNP, purine nucleoside phosphory- "Hypomorphic mutations in /MGgenes and in ARTEMIS can contribute to Omenn's syndrome..		

Defects in T Cell Activation		
Disease	Functional Deficiencies.	Mechanism of Defect.
Defects in MHC Expression.		
Bare lymphocyte syndrome.	Defective MHC class II expression and deficiency in CD4+ T cells; defective cell-mediated immunity and T-dependent humoral immune responses.	Defects in transcription factors regulating MHC class II gene expression, including CliTA, RFXANK, RFX5, and RFXAP.
MHC class I deficiency.	Decreased MHC class I levels; reduced CDS+T cells.	Mutations in TAP1, TAP2, and TAPASIN.
Defective T Cell Signaling.		
Proximal TCR signaling defects.	Defects in cell-mediated immunity and T-cell— dependent humoral immunity.	Mutations in CD3 genes, CD45, ST1M1,0RAI1.
Wiskott-Aldrich syndrome Autosomal recessive WAS-1 ike disease.	Defective T cell activation and leukocyte mobility Defective T cell activation and leukocyte mobility.	TCR-dependent actin-cytoskeletal rearrangements are defective because of mutations in WAS, an- Xlinked gene mutation in WIP.
Familial Hemophagocytic Lymphohistiocytoses.		
X-linked lymphoproliferative syndrome.	Uncontrolled EBV-induced B cell proliferation, uncontrolled macrophage and CTL activation, defective NK cell and CTL function.	Mutations in SAP Mutations in X-IAP.
Perforin deficiencies.	Uncontrolled macrophage and CTL activation, defective NK cell and CTL function.	Mutations in PERFORIN.
Granule fusion.	Uncontrolled macrophage and CTL activation, defective NK cell and CTL function.	Defective cytotoxic granule exocytosis; mutations in RAB27A, MUNC13-4, SYNTAX- IN, >5A?jand in iTSfin Chediak-Higashi syndrome–see Table 21-2).
AP3, adaptor-related protein complex 3; LYST, lysosomal trafficking regulator protein; SAP, SLAM-assoc iated protein; TAP, transporter associated with antigen processing; WASP, Wiskott-Aldrich syndrome protein.		

Immune-Mediated Inflammatory Diseases:

Diseases Mediated By Antibodies And Immune Complexes

Organ-specific autoimmune diseases.

Autoimmune hemolytic anemia

Autoimmune thrombocytopenia

Myasthenia gravis

Graves disease

Goodpasture syndrome.

Systemic autoimmune diseases.

Systemic lupus erythematosus (SLE).

Diseases caused by autoimmunity or by reactions to microbial antigens

Polyarteritis nodosa.

Diseases Mediated By T Cells.

Organ-specific autoimmune diseases.

Type 1 diabetes mellitus

Multiple sclerosis.

Systemic autoimmune diseases.

Rheumatoid arthritis[*]

Systemic sclerosis[*]

Sjogren syndrome[*]

Diseases caused by autoimmunity or by reactions to microbial antigens .

Inflammatory bowel disease (Crohn disease, ulcerative colitis)

Inflammatory myopathies.

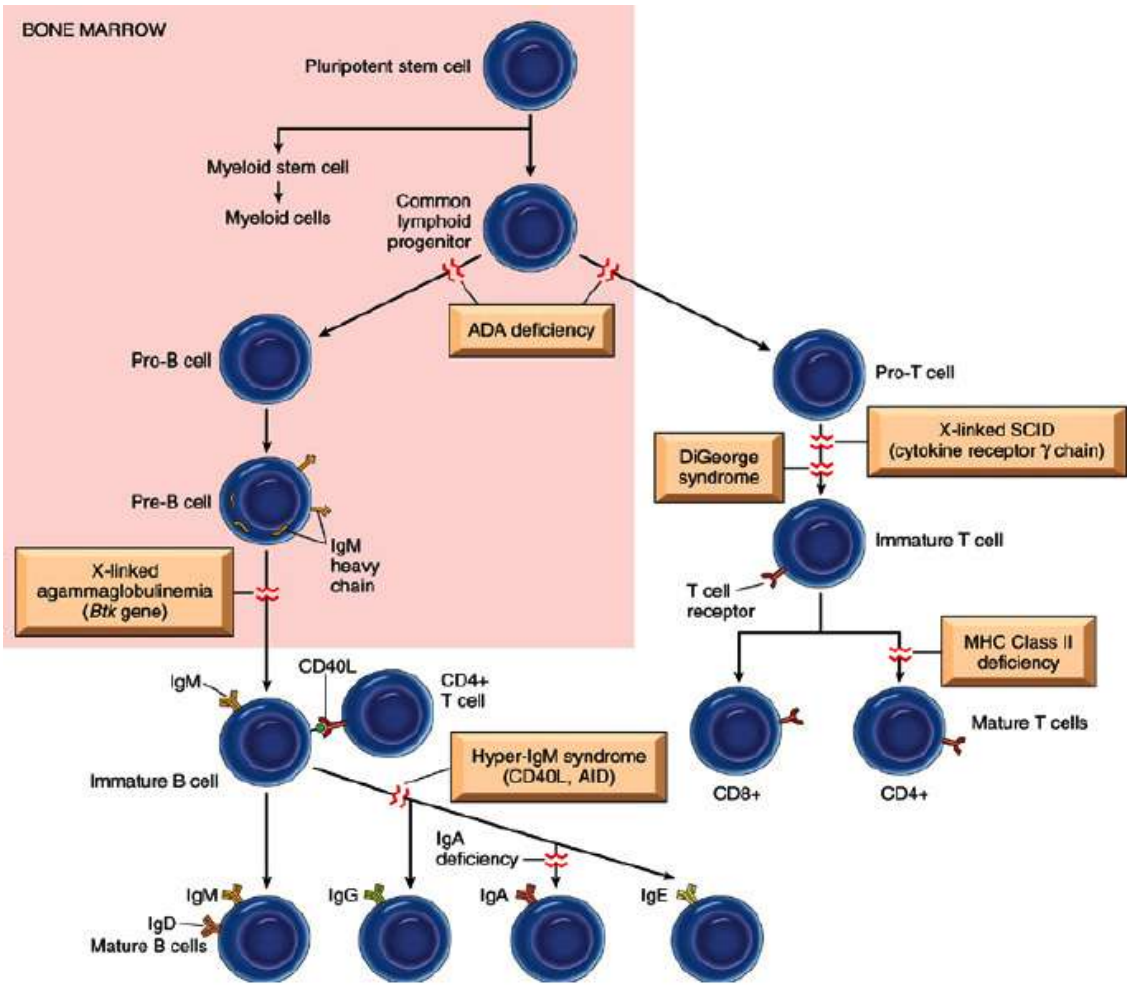


Fig. 5.16

1. Deficiencies of B cell functions:

a. Infantile X linked agammaglobulinemia ~ Burton type agammglobulinemia

Lack of mature B cells due to mutation in B cell tyrosine Kinase gene (B TK gene).

- Absence of lgs, plasma cells & B cells.
- Presents after 8- 9 months of birth (after maternal lgG has been catabolized).
- Septicemia + recurrent pyogenic infection (Staph, H. influenza).
- Diarrhea – Giardia.
- Fungal + Viral infection handled normally.

b. Transient Hypogammaglobulinemia –

- Familial.
- Males + Females.
- Affects lgG alone.
- More severe in premature infants (as lgG crosses late in pregnancy) disappears in first 3 yrs.

c. Common variable immunodeficiency:

- All have hypogammaglobulinema.
- Affecting all classes sometimes lgG alone.
- Recurrent sinopulmonary pyogenic infections.
- Recurrent herpes virus infection.
- Recurrent bacterial infections + giardiasis.
- ↓ Autoimmunity – RA, P.A, Hemolytic Anemia, lymph reticular malignancies (esp. in females) and gastric carcinoma.
- Normal or near normal number of B cells in blood and lymphoid tissue but no plasma cells and antibodies.

d. Complement deficiency: C2 deficiency is the commonest.

- Recurrent bacterial infections are common complications of most complement deficiency with C3 deficiency– opsonisation – severe pyogenic infection (Pneumonia, meningitis, septicemia).
- Deficiency of C5, 6, 7, 8, 9, - recurrent Neisseria bacteremia.
- Properdin deficiency – meningococcal septicemia.

2. Combined T cell + B cell: Severe combined immunodeficiency Disease (SCID) – develops within just 2 yrs – infants show delayed growth with recurrent bacterial, viral and fungal infections.

Types:

a. Most common type is X-linked characterized by mutation in gamma chain subunit of several cytokine receptors (I12, I14, IL7, IL9, IL11, IL15) resulting in failure of lymphoid progenitors to be stimulated by many Cytokines especially IL-7.

b. Swiss type SCID – Failure of lymphoid stem cell development, no lymphocytes, and AR disorder.

c. Adenosine deaminase deficiency (ADA). AR disorder, characterized by deficiency of enzyme adenosine deaminase Accumulation of dioxyadenosine and deoxyadenosine tri PO4 – both are toxic to lymphocytes.

d. The Bare lymphocyte syndrome – HLA ags – Class I or II not expressed.

e. Ataxia Teleangiectasia, Defective T and B cell function ↓ cell mediated immunity.

+ ↓ lgA + lgE, **Lymphoma**

3. Deficiencies of T cell function:

A. Di George's Syndrome – due to failure of development of 3 rd + 4 th branchial arches (thyroid, parathyroid, parafollicullar cells of thyroid + ultimobranchial body).

- Infants – total absence of CMI, hypocalcemic telangiectasia + congenital defects of heart + great vessels.
- Circulating – B cells can deal with pyogenic infection but suffer from opportunistic infection (pneumocystis carinii) fungal + viral infection.

b. Nezelof 's Syndrome – X linked disorder.

- thymic hypoplasia.
- B cells + lg levels are normal.

c. PNP deficiency: Purine nucleoside phosphorylase deficiency – DNA synthesis in T cells is diminished. Severe T cell deficiency – especially susceptible to CMV + Varicella.

Autoimmune dyscrasias + lymphomas occur.

d. Wiskott – Aldrich Syndrome – X linked.

- Thrombocytopenia, eczema and recurrent infection.

↓ cell mediated immunity, lgA + lgE ↑ , lgM ↓

- Development of lymphoreticular neoplasms.

6 Genetics

Genetic Disorders:

Genetics – Study of single / few genes & their phenotypic effects.

Genomics – Study of all genes in the genome and their interactions.

Proteomics – Measurement of all proteins expressed in a cell / tissue.

Bioinformatics – Biologists, Computer scientists, Mathematicians.

Pharmacogenomics – Individualized drug therapy.

Two strategies of characterize involved genes:

1. Functional cloning / Classics approach:

Done in inborn errors of metabolism e.g. phenylketonuria, disorders of Hb synthesis.

Clinical phenotypes → Biochemical abnormality → abnormal protein → abnormal gene identified & studied.

2. Positional cloning / Candidate gene approach:

Initially ignores biochemical clues relies on mapping disease phenotypes to particular chromosomal location by cytogenetic studies or Linkage analysis e.g. Cystic fibrosis, Neurofibromatosis, Duchenne muscle dystrophy, PCKD, Huntington disease.

Mutant mice: Transgenic – molecularly cloned DNA introduced into mice →manifest human disease e.g. c-myc →↑tumors.

Gene knock out: replace (n) genes by inactive genes. E.g. Loss of both LDL receptor gene → hypercholesterolemia Benefits of genetic engineering.

1. Production of biologically active agents e.g. TPA, GH, Insulin, CSF.

2. Gene therapy.

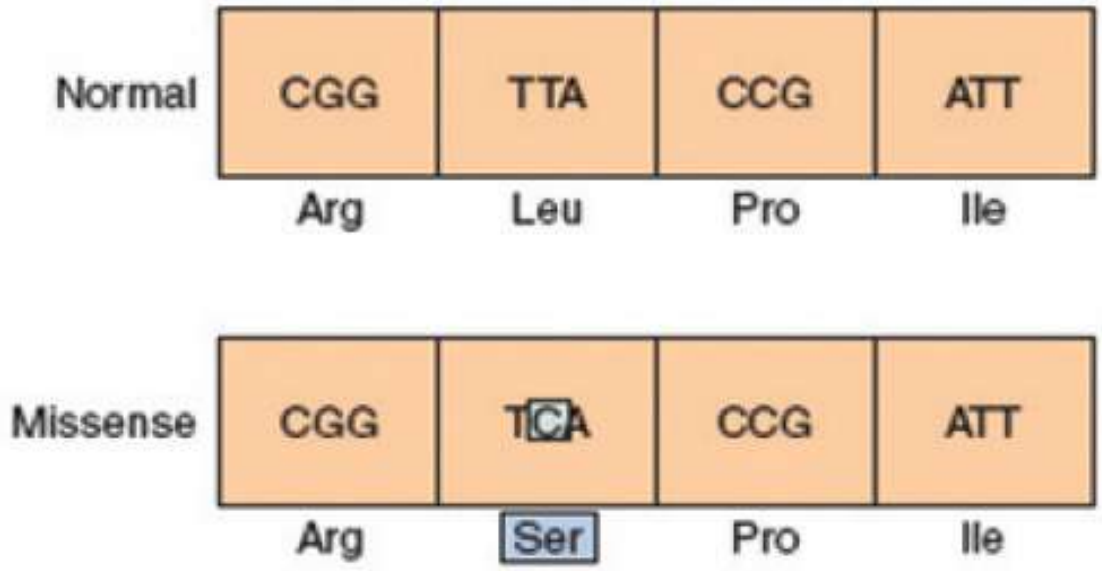
3. Disease diagnoses: by molecular probes

Mutation: Permanent change in DNA.

Genome mutation: loss or gain of whole chromosome.

Chromosome mutation: rearrangement of genetic material, give rise to visible structural and changes in chromosome.

Gene mutations: Affecting gene as single base pair e.g. point mutation. Insertion/ deletion, microdeletions, trinucleotide repeat mutation.



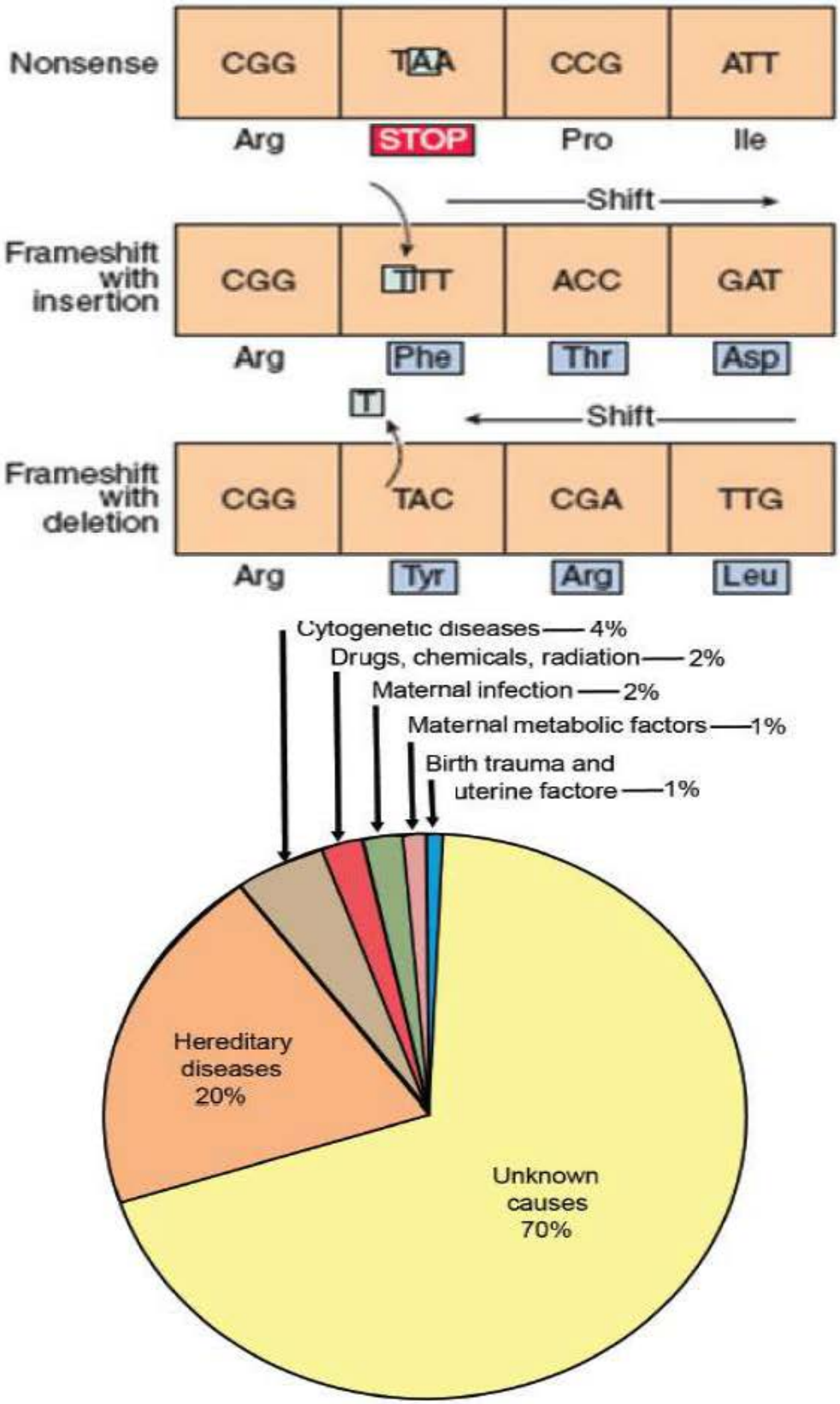


Fig. 6.1: Causes of birth defects in humans. Most birth defect have unknown causes

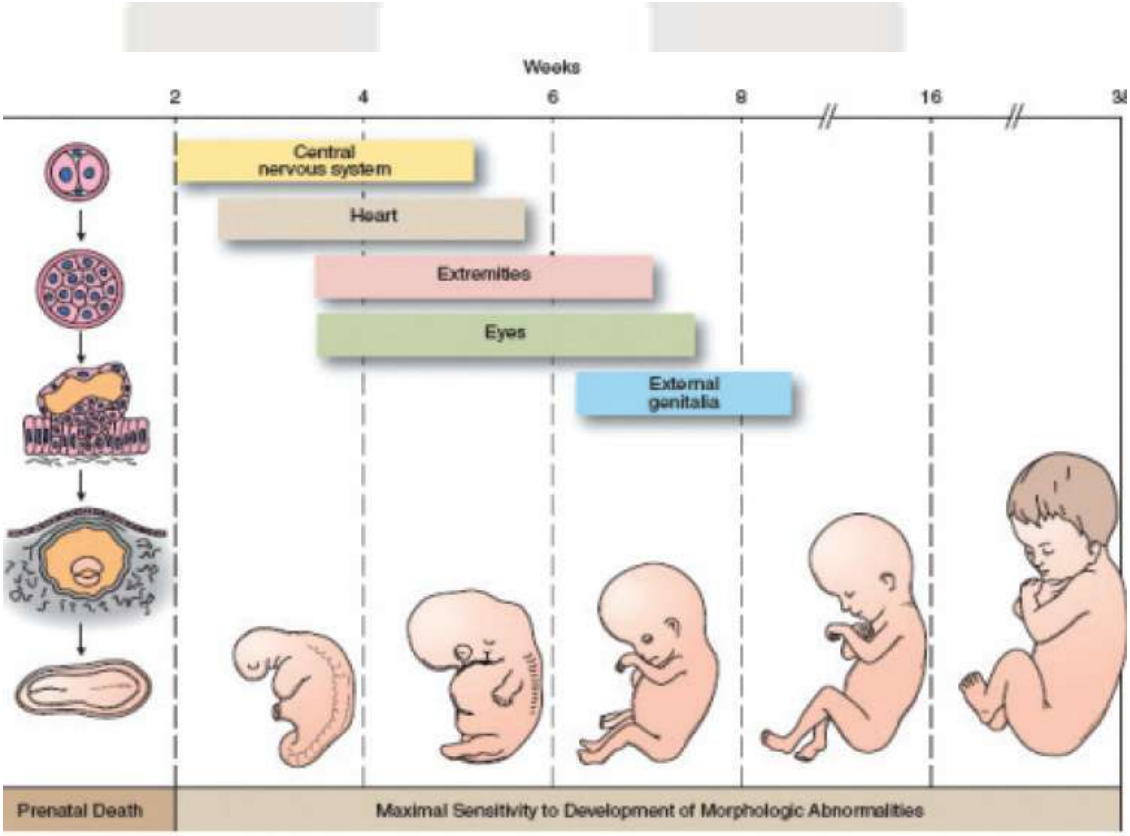


Fig. 6.2: Sensitivity of specific organs to teratogenic agents at critical stages of human embryogenesis. Exposure to adverse influences in preimplantation and early post implantation stages of development (far left) leads to prenatal death. Periods of maximum sensitivity to teratogens (horizontal bars) vary for different organ systems but overall are limited to the first weeks of pregnancy.

Genetic Disorders:

1. Disorders related to mutant genes of large effect (Mendelian disorder).
2. Disorders with multi-factorial inheritance.
3. Chromosome disorders.
4. Single gene disorders with non classic pattern of inheritance.

Mendelian Disorders:

Dominant.

Recessive.

Co- dominance e.g. histocompatibility and BG Ag.

Single genes: 3 patterns of transmission: AD, AR, X Linked.

Reduced penetrance Individuals inherit the mutant gene but are phenotypically normal.

Variable expressivity: Trait seen in all individuals but expressed differently.

Pleiotropic Single mutant gene → many end effects.

Genetic heterogeneity: Mutations at several loci producing the same trait.

Autosomal Dominant Inheritance:

Representative Autosomal Dominant Disorders		
Disease	**Frequency**	**Chromosome**
Familial hypercholesterolemia	1/500	19p
von Willebrand disease	1/8000	12p
Hereditary spherocytosis (major forms)	1/5000	14, 8
Hereditary elliptocytosis (all forms)	1/2500	1, 1p, 2q, 14
Osteogenesis imperfecta (types l-IV)	1/10,000	17q, 7q
Ehlers-Danlos syndrome type III	1/5000	2q
Marfan syndrome	1/10,000	15q
Neurofibromatosis type 1	1/3500	17q
Huntington chorea	1/15,000	4p
Retinoblastoma	1/14,000	13q
Wilms tumor	1/10,000	11p
Familial adenomatous polyposis	1/ 10,000	5q
Acute intermittent porphyria	1/15,000	11q
Hereditary amyloidosis	1/100,000	I8q
Adult polycystic kidney disease	1/1000	16p

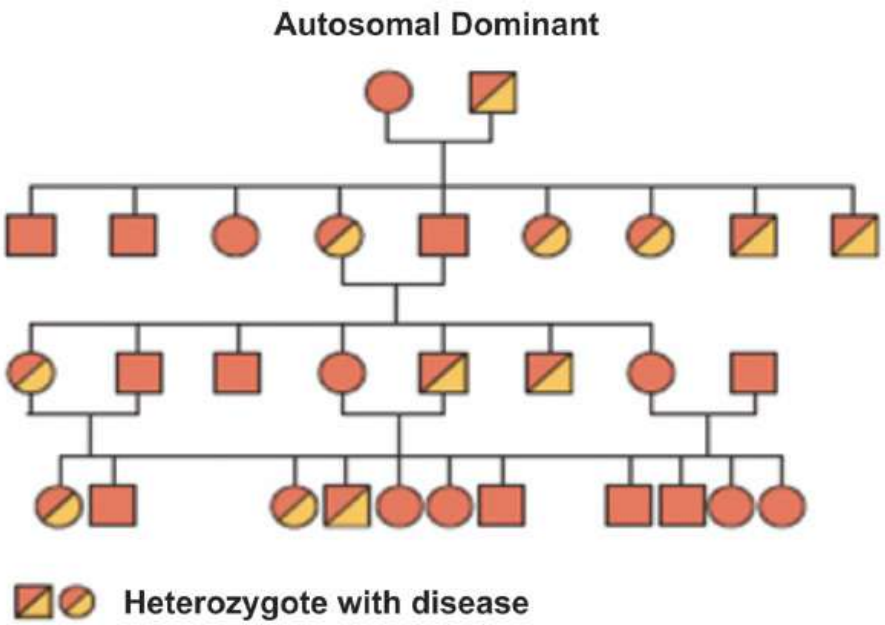


Fig. 6.3: Autosomal dominant inheritance. Only symptomatic persons transmit the trait to the next generation, and half the children have a chance of being symptomatic. Bath males and females are affected.

1. An affected person usually has at least one affected parent
2. Affects either sex;
3. Transmitted by either sex;
4. A child of an affected x unaffected mating has a 50% chance of being affected (assuming the affected person is heterozygous)

Autosomal Recessive:

Representative Autosomal Recessive Disorders		
Disease	Frequency	Chrouosome
Cystic fibrosis	1/2500	7q
α-Thalassemia	High	16p
β-Thalassemia	High	11p
Sickle cell anemia	High	lip
Myeloperoxidase deficiency	1/2000	17q
Phenylketonuria	1/10,000	12q
Gaucher disease	1/1000	1q
Tay-Sachs disease	1/4000	15q
Hurler syndrome	1/100,000	22p
Glycogen storage disease la (von Gierke disease)	1/100,000	17
Wilson disease	1/50,000	13q
Hereditary hemochromatosis	1/1000	6p
a1-Antitrypsin deficiency	1/7000	14q
Oculocutaneous albinism	1/20,000	11q
Alkaptonuria	<1/100,000	3q
Metachromatic leukodystrophy	1/100,000	22q

Phenylketonuria, Galactosemia, Homocystinuria,

Lysosomal storage disease, a1 – AT deficiency, Wilson disease, Hemochromatosis. Glycogen storage disease

Cystic fibrosis, Sickle cell anemia. Thalassemias,

Congenital adrenal hyperplasia, E – D syndrome (some variety), Alkaptonuria Neurogenic muscular atrophies

1. Affected people are usually born to unaffected parents;
2. Parents of affected people are usually asymptomatic carriers;
3. There is an increased incidence of parental consanguinity;
4. Affects either sex;
5. After the birth of an affected child, each subsequent child has a 25% chance of being affected (assuming both parents are phenotypically normal carriers).

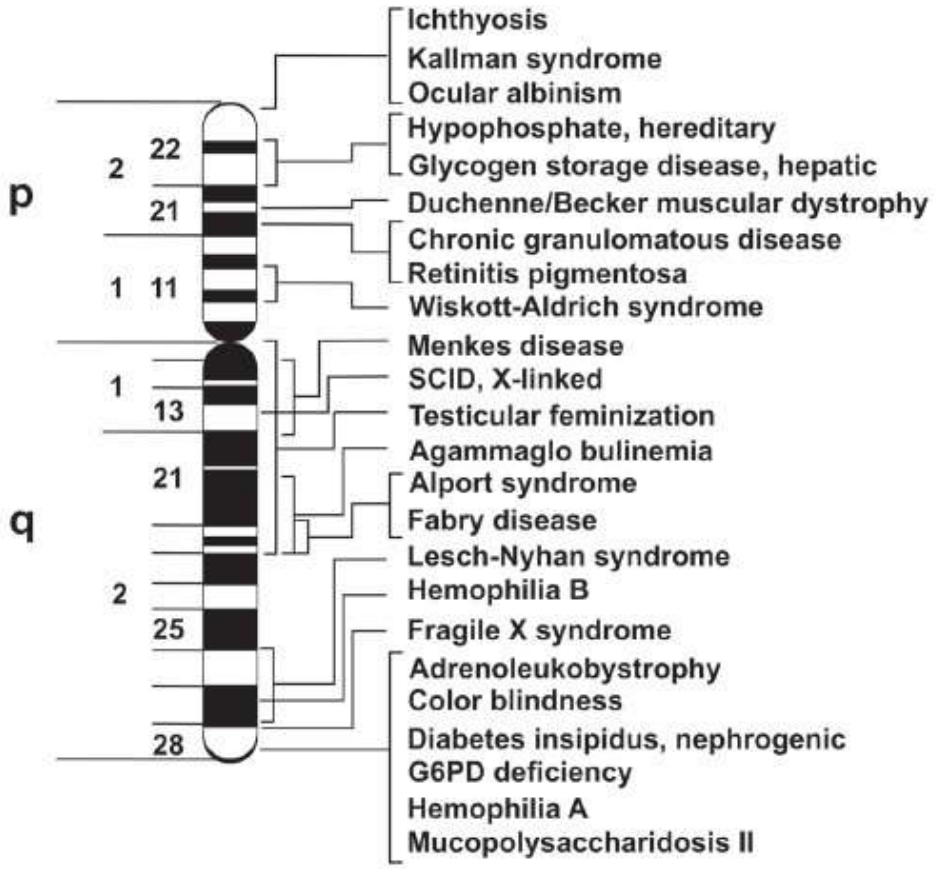


Fig. 6.4: Localization of representative inherited diseases on the X chromosome GGPD.

X Linked Recessive:

Duchenne muscular dystrophy, Hemophilia A&B Chronic granulomatous disease, G6PD deficiency Agammaglobulinmia, Wiskott Aldrich syndrome Diabetes insipid us, Leach- Nyhan syndrome,

1. Affects mainly males;
2. Affected males are usually born to unaffected parents: the mother is normally an asymptomatic carrier and may have affected male relatives;
3. Female may be affected if the father is affected and the mother is a carrier, or occasionally as a result of nonrandom X- inactivation.
4. There is no male –to –male transmission in the pedigree.

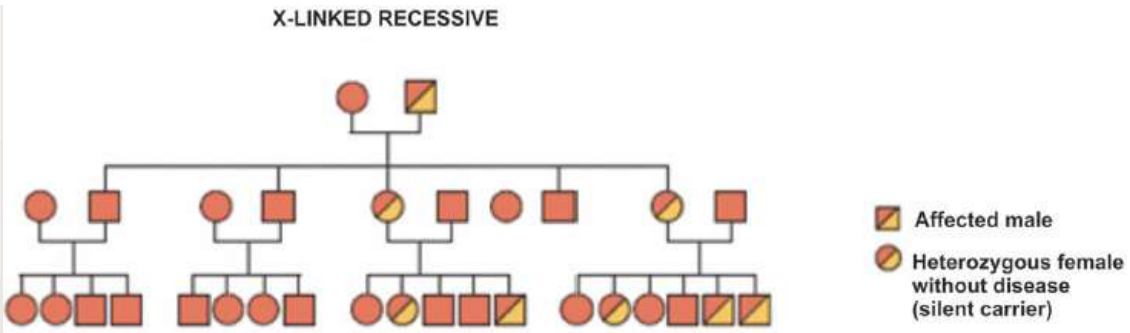


Fig. 6.5

X – Linked Dominant:

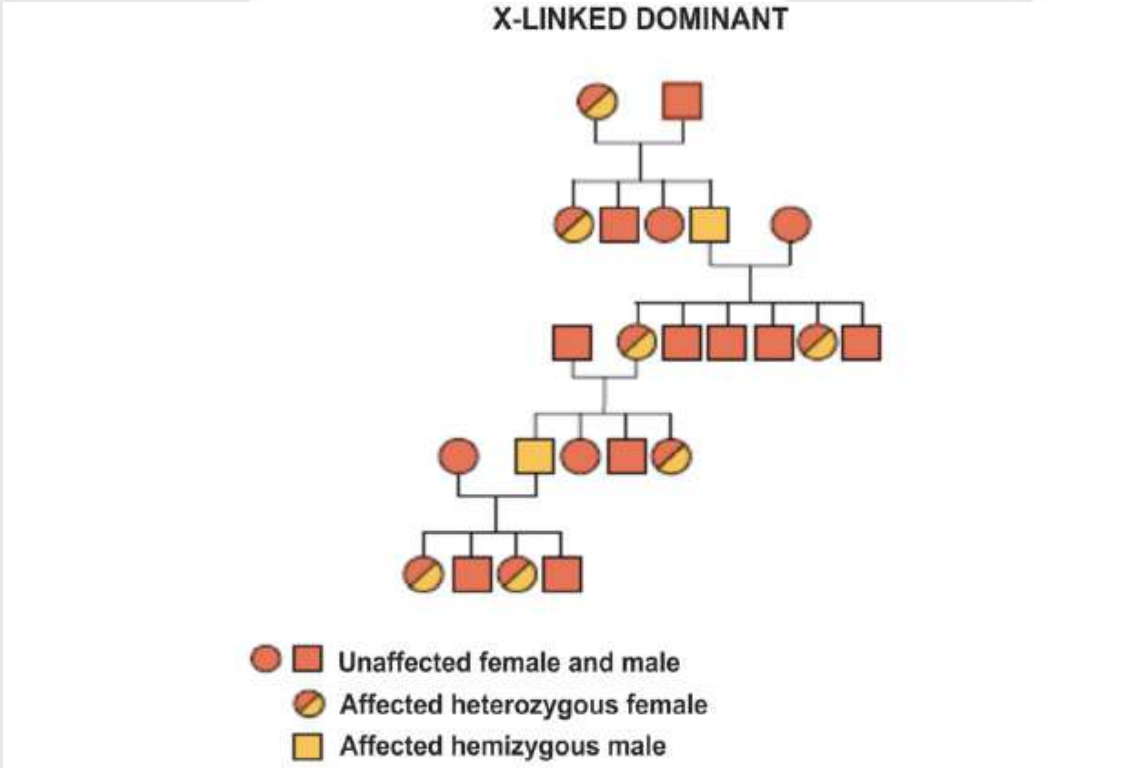


Fig. 6.6: X-linked dominant inheritance-.

1. Affects either sex, but more females than males;
2. Females are often more mildly and more variably affected than males;
3. The child of an affected female, regardless of its sex, has a 50% chance of being affected
4. For an affected male, all his daughters but none of his sons are affected

Y Linked Inheritance:

- **No Y-linked diseases are known as yet:**

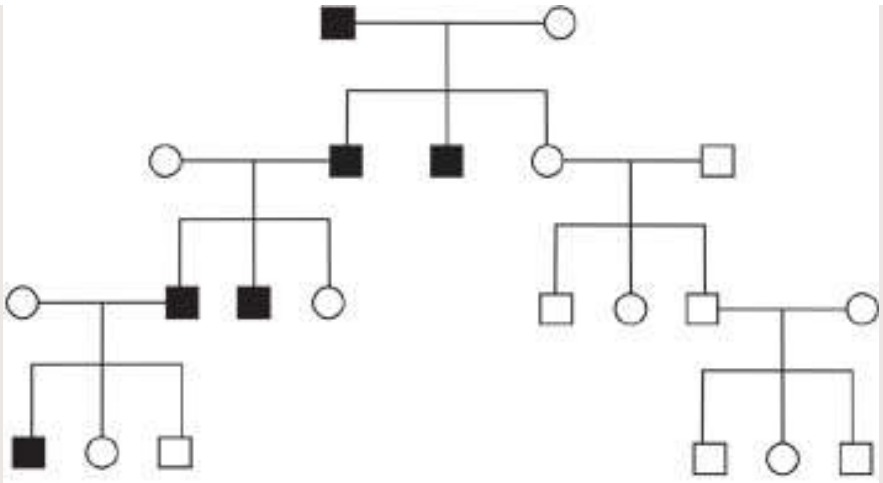


Fig. 6.7

Save for determinants that dictate male differentiation, the only characteristic that may be located on the Y chromosome is the attribute of hairy ears, which is not altogether devastating.

1. Affects only males.
2. Affected males always have an affected father (unless there is a new mutation).
3. All sons of an affected man are affected.

Mitochondrial Inheritance:

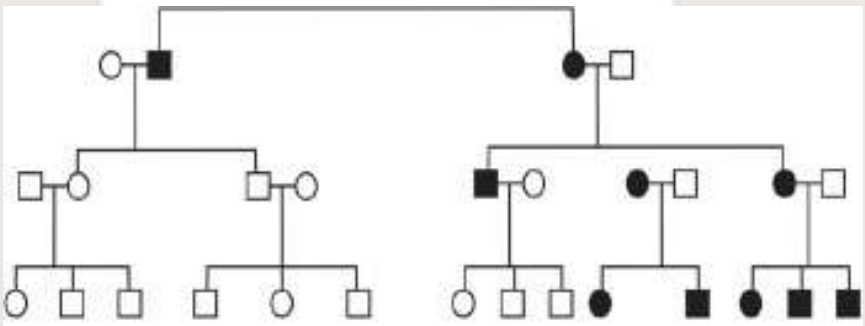


Fig. 6.8

Leber Hereditary optic neuropathy, MERRF (Myoclonic epilepsy with ragged red fibers) Leigh disease, mitochondrial

encephalomyopathy, lactic acidosis and stroke llike episodes (MELAS).

1. Both males and females affected.
2. Affected females transmits to all – males and females.
3. Affected male do not transmit the disease.

Disorders associated with defects in structural proteins:

Marfan syndrome:

- Connective tissue disorders, affecting skeleton, eyes &CVS, AD.
- Mutation of FBN 1 (chromosome ,5q21) → Marfan syndrome.
- Mutation of FBN 2 (chromosome 5q3) → congenital contractual arachnodactyly (AD).
- Mutation fibrillin – disrupts assembly of (n) alleles also →Dominant negative.
- Morphology:
- Skeletal abnormality → most striking features: Tall, long extremities, long tapering fingers and toes, US/LS ratio ↓, (LS-tall).
- Lax 'joint ligaments, double jointed, long headed (dolichocephalic), prominent supraorbital ridges, bossing of frontal eminences deformed chest (as seen in Abraham Lincoln).
- Ocular: B/L outward and upward subluxation and dislocation of lens (Ectopia lentis).
- CVS : Most life threatening features: Mitral valve prolapse , Cystic medial necrosis ⇒ dilatation of aortic valve ring and root →Aortic incompetence.
- MV lesions are more frequent but clinically less important than aortic lesion, Death from Rupture of aortic dissection / Cardiac failure.

Ehlers – Danlos syndrome:

- Result from defect in collagen synthesis or structure.
- 14 collagen types; 9 EDS variants, all 3 patterns of inheritance known.

- Gen – skin – hyper extensible fragile hypermobile joints, joint dislocation.
- Various c/o Rupture of colon and large arteries: EDS type IV.
  - Ocular fragility: EDS type VI.
  - Diaphragmatic hernia: EDS type I.
- Type VI: most common. AR from EDS.
  - Mutation in enzyme encoding lysyl hydroxylase (cross linking).
- Type IV: AD. mutation affects gene for collagen Type III (abundant in blood vessels & intestine).
- Type VII: (a, b, c): abnormality in conversion of type I procollagen to collagen.
- Type IX: Defect of Cu metabolism (mutation in copper binding protein ↓ action of Copper lysyl oxidase.

Disorders Associated with Defects in Receptor Proteins: Familial Hypercholesterolemia:

Most frequent Mendelian disorder.

Abnormal LDL receptor →↑LDL in plasma → alternate scavenger pathway → atheroscelerosis, xanthomas.

LDL receptor gene: Chromosome 19.

Class I – rare, absent synthesis.

Class II – defect in transport of R.

Class III - ↓ Binding of LDL with R.

Class IV – Failure to form coated Pits (clustering).

Class V - Recycling.

Disorders Associated with Defects in Enzyme:

A. Lysosomal storage Disease.

Lysosomal enzyme.

Synthesized in ER, transported to Golgi apparatus and undergo translation post translational modifications, Attachment of mannose – 6 phosphate groupings (address label), which is recognized by specific receptor on inner surface of Golgi membrane. Helps in.

Segregation from other secretory proteins. Abnormal address label → LSD.

Mucopolysaccharidoses			
Type	Eponym	Location of Gene	Clinical Features
1 H	Hurler	4p16.3	Organomegaly, cardiac lesions, dysostosis multiplex, corneal clouding, death in childhood
I S	Scheie	4p16.3	Stiff joints, corneal clouding, normal intelligence, longevity
II	Hunter	X	Organomegaly, dysostosis multiplex, mental retardation, death earlier than 15 years of age
III	Sanfilippo	12q 14	Mental retardation
IV	Morquio	16q24	Skeletal deformities, corneal clouding
V	Obsolete	–	–
VI	Maroteaux Lamy	5q13–14	Dysostosis multiplex, corneal clouding, death in second decade/
VII	Sly	7q21.t-22	Hepatosplenomegaly, dysostosis multiplex

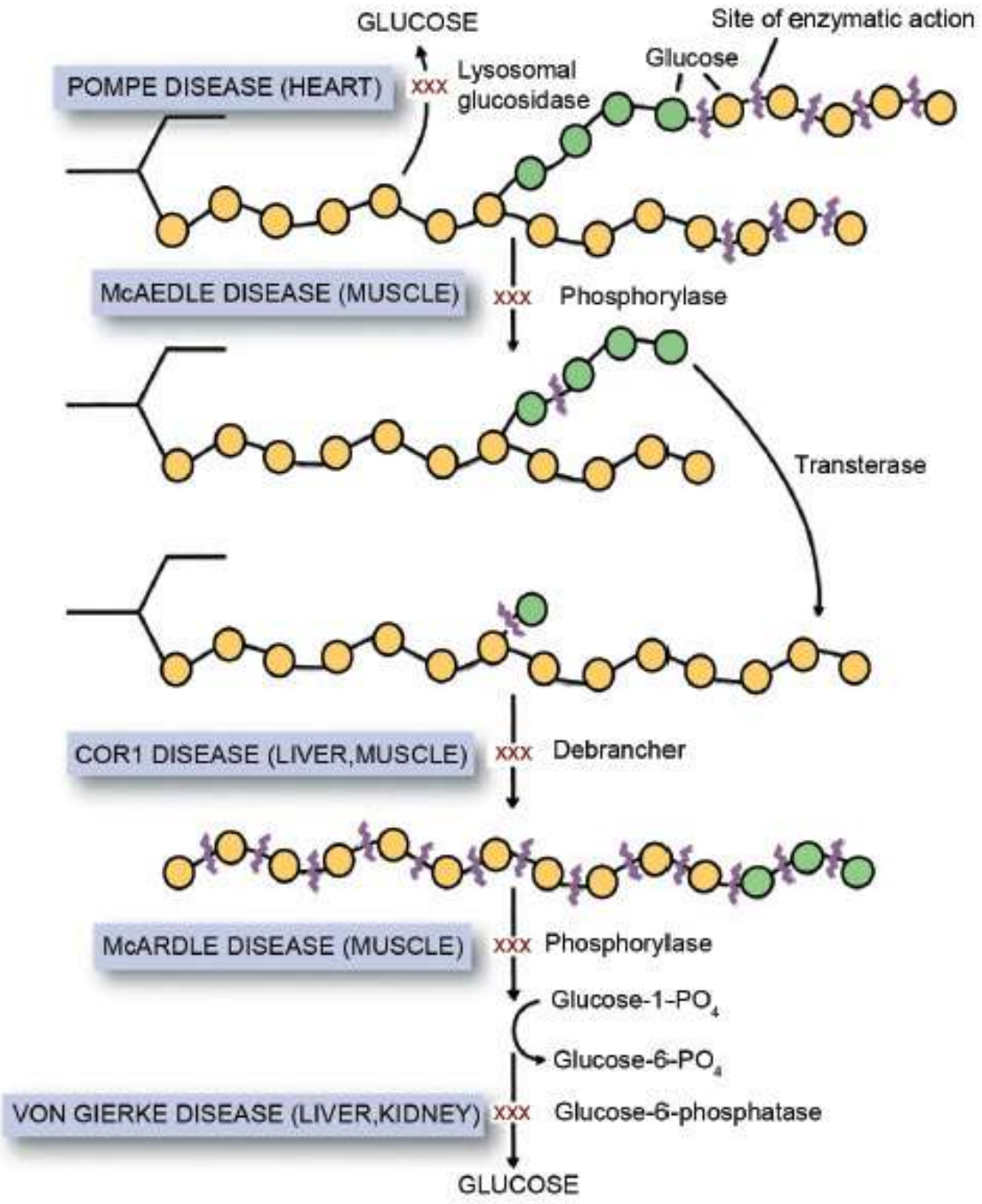


Fig. 6.9: Sequential catabolism of glycogen

TAY – Sachs Disease:

- Most common form of GM_2 gangliosidosis.
- Hexosaminidase α- unit deficiency.

- High prevalence in Jews: carrier rate is 1 in 30.
- GM_2 ganglioside accumulates in heart, liver spleen etc.
- Main clinical presentation: CNS & ANS & Retina involved.
- Ganglion cells in retina : swollen particularly in margins of Macula ⇒Cherry Red spot.
- L/M: Neurons – ballooned with cytoplasmic vacuoles followed by destruction (Oil Red O, Sudan Black B positive).
- E/M: Cytoplasmic inclusions: whorled configuration within lysosomes composed of onion skin layers of membrane.
- C/F: Infants – (n) at birth.
- S/S - at 6 months of age (depend on severity of deficiency).
- Motor incoordination. Mental obtundation, muscle flaccidity, Blindness, dementia. Death 2-3 years of age.

Niemann Pick Disease:

- Type A&B: (N/P Disease)
- Deficiency of sphingomyelinase ⇒accumulation of sphingomyelin
- (Previous grouped with N/P disease Type C – primary defect in intracellular cholesterol esterification and transport).
- Common in Jews:
- Type A: severe, infantile from with extensive neurologic involvement.
- Progressive wasting and early death within first 3 years of life.
- Type B: No CNS involvement, survives into adulthood.
- L/M: affected cells are enlarged (upto 90 um in diameter) with innumerable small vacuoles foamy appearance of cytoplasm, SBB/ Oil Red O positive.
- E/M: Vacuoles are enlarged secondary lysosomes.
- Contain membranous cytoplasmic bodies resembling lamellated myelin figures parallel palisades – Zebra bodies.
- Gross: Splenomegaly, hepatomegaly, lymphadenopathy.
- Brain: Gyri are shrunken, sulci – widened.
- Retinal cherry spot – positive in 1/3 – ½ pts.

Gaucher Disease:

Lysosomal storage disorder.

Deficiency of glucocerebrosidase, accumulation of glucocerebrosied in phagocytic cells.

3 Clinical subtypes:

Type 1	Type II	Type III
• Chronic non – neuropathic • Limited to phagocytic cells • Splenic & skeletal inv. Dominate • Jews + • Longevity – slightly ↓ • ↓ (not absent) enzyme	Acute neuronopathic (infantile acute cerebral pattern) CNS inv + HSM CNS manifestation dominant No predilection for Jews Death at early age No detectable enzyme in tissue	Intermediate pattern Usually juveniles CNS disease begins At teens to twenties

L/M: Gaucher cells seen in Virchow – Robin spaces.

Diagnosis:

- For homozygote: measurement of glucocerebrosidase activity in peripheral blood leukocytes of cultured skin fibroblasts.
- Heterogotes: Detection of specific mutation.
- To differentiate from Pseudo Gaucher cells- iron (AIIMS question).

Alkaptonuria (Ochronosis):

- First inborn error of metabolism to be discovered, AR.
- Lack of Homogentisic oxidase, block metabolism of phenylalanine tyrosine → HA accumulation.
- Gene for enzyme: chromosome 3q 21.
- Homogentisic acid binds to collagen in connective tissue, tendons and cartilage → Blue black pigment → ochronosis.
- Cartilage become brittle and fibrillated.
- Prime site of attack: vertebral column (intervertebral disc) later knees, shoulder hips.
- Small joints of hands and feet →spared.

Disorders associated with defects in proteins that regulate cell growth:

1. Neurofibromatosis type I (Von Recklinghausen disease):

- 1 in 3000.
- AD, chromosome 17q 11.2 (nerofibromin).
- Expressivity – variable , pentrance : 100%.
- 3 major features.
- Multiple neurofibromas dispersed anywhere on the body.
- Pigmented skin lésions – café lait spots.
- Pigmented iris hamatomas – Lisch nodules.
- Neurofibromas 3 types.
- Cutaneous.
- Subcutaneous.
- Plexiform → diffusely involves subcutaneous tissues contain numerous torturous thickened nerves (become malignant in 5 % of pts with NF -1.
- Cutaneous pigmentation: seen in 90% patients.
- Café au lait spots → round to ovoid, smooth border, over trunk parallel to underlying nerve (>6 in number, > 1.5 cm diameter).
- Pigmented hamartomas / Lisch nodules: seen in 94% patients older than 6 years.
- Other lesion:
- Skeletal lesion erosive defect, scoliosis intraosseous cystic lesion sub periosteal bone cyst, pseudoarthrosis of tibia.
- ↑ risk of developing Wilm's tumor Rhabdomyosarcoma: Meningioma: Optic glioma; Pheochromocytoma
- ↑ risk of developing CML.
- ↓IQ.

2. Neurofibromatosis – Type: 2

- AD 1 in 40,000 to 50,000.
- B/L acoustic Schwannomas and multiple meningiomas
  - Gliomas (esp. ependymoma) of spinal cord Schwannosis, Meningio angiomatosis glial harmartomas
  - Lisch nodules are not found

- Chromosome 22q 12 (product –merlin)

Disorders with multifactorial inheritance:

Combined action of environmental influences and two or more mutant genes having additive effects e.g. of normal / phenotypic characters: hair color, eye color, height, intelligence.

Cleft lip or cleft palate - Congenital heart disease

Coronary heart disease - Hypertension

Gout - Diabetes mellitus

Pyloric stenosis - Club foot

Normal karyotype:

Karyotype is standard arrangement of photographed or imaged stained metaphase spread in which chromosome pairs are arranged in order of decreasing length.

Staining G banding (400 – 800 bands per haploid set).

Autosomes: grouping → (according to length, decreasing order & shape).

Group A: Chromosome 1to 3

Group B: Chromosome 4, 5

Group C: Chromosome 6 to 12

Group D: Chromosome 13-15

Group E: Chromosome 16-18

Group F: Chromosome 19-20

Group G: Chromosome 21, 22

Metacentric chromosomes: Centromere in middle: GP – A&F.

Sub metacentric centromere toward one end: GP - B, C E.

Acrocentric chromosomes: Centromere near tip: GP -D, G.

Sex chromosomes: X with C group

Y with G group (or placed in Right hand corner).

FISH – study chromosomes in inter –phase nuclei also.

Subtle microdeletions and complex translocations detected with DNA probes, localize gene to a specific site.

Chromosome painting: Type of FISH: Whole chromosome labeled with fluorescent DNA probes at multiple sites. Spectral Karyotyping (SKY): ALL 46 chromosomes visualized simultaneously using 5 fluorochromes & computer aided signals.

Cytogenetic disorders:

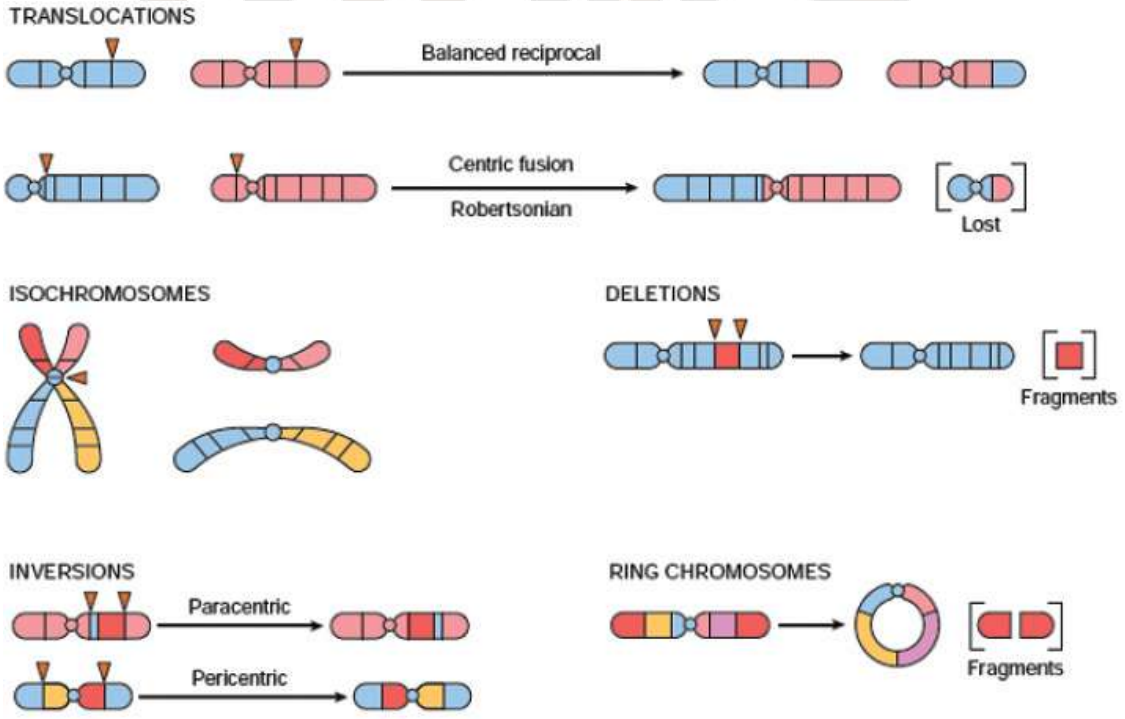


Fig. 6.10

Any exact multiple of haploid is called ‘Euploid’.

Not an exact multiple of haploid:’ Aneuploid’ (Causes: Non disjunction & Anaphase lag)

Monosomy or Trisomy of sex chromosome – Compatible with life – associated with variable degrees of phenotypic abnormality

Monosomy of Autosome - gen. do not permit survival.

Trisomy of Autosome → (with exception or Down syndrome) severally handicapped, die at early age.

Mosaicism → 2 or more cell populations in same individual. d/t mitotic errors in early development.

Autosomal mosaicism is much less commo

Changes / Abnormality in structure.

Studied using FISH / SKY:

1. Deletion: loss of a portion of chromosome
   a. Terminal / Interstitial e.g. .46X4, Del (16) P (14).
   b. Ring chromosome: special form of deletion occurring at both ends with fusion of damaged ends.
2. Inversion: Two breaks within a single chromosome with inverted reincorporation of segment.
   Paracentric – inv only one arm.
   Pericentric – breaks on opposite sides of centromere.
3. Isochoromosome: One arm is lost, remaining arm is duplicated.
4. Translocation: segment from one chromosome is translocated to other balanced reciprocal translocation.
5. Robertsonian translocation / or centric fusion: B/W two acrocentric chromosome

Forming abnormally long chromosome and abnormally short chromosome (lost)
↑ risk of producing abnormal gametes

- 7.5 % of all conceptions have a chromosomal abnormality, most of which are not compatible with survival or live birth.
- Chromosomal abnormalities are identified in 50 % of early spontaneous abortuses and in 5% of still births and infants who die in immediate postnatal period.
- In live born infant, free is 0.5 -1%.

Trisomy 21 (Down syndrome):

Most common chromosomal disorder

Major cause of mental retardation

Incidence: 1 in 700

Maternal age: Incidence of 1 in 25 live births for mother > 45 years

Karyotypes:

→ 95% – Trisomy 21 (Meiotic non - disjunction in ovum).

4% - Translocation and 1% - Mosaic non disjunction).

Morphology :

- Epicanthic fold flat facial profile.
- Mental retardation: 80% IQ = 25-50.
- Abundant neck skin.
- Simian crease.
- Congenital heart defect (in 40% of patients):

Most common are defects of endocardial cushion. Including ostium primum, ASD, A / V valve malformation VSD.

- intestinal stenosis.
- umbilical hernia.
- predisposition to leukemia (All and AML –M7) (10-20 times ↑) Most common leukemia is ALL and most specific leukemia is AML M7.
- Hypotonia.
- Gap between 1st and 2nd toe.
- After 40 years → Alzheimer's disease.
- 80% survival till 30 years.

Trisomy 18 (Edward syndrome): 1 in 8000:

90% – trisomy 18 – Maternal age ↑

10% mosaic – Maternal age (n).

Morphology:

- Prominent occiput.
- Mental retardation.
- Micrognathia.
- Low set ears, overlapping fingers.
- Short neck.
- Cardiac, renal, intestinal defects.
- Rocker bottom feet.
- Limited hip abduction.
- Rarely survive after 1 years of age.

Patau syndrome (Trisomy 13)1 in 15.000:

Trisomy 13

Translocation type (maternal age not implicated)

Mosaic type.

- Morphology.
- Microcephaly, micropthalmia. mental retardation, Holoprosencephaly.
- Polydactyly.
- Cleft lip and palate.
- Cardiac and Renal defect.
- Umbilical hernia.
- Rocker bottom feet.
- Rarely survival after 1 year of life.

Cri du chat (cat cry) syndrome : 1 in 50,000:

46XX, 5P – Maternal age in normal.

46XY, 5P –

- mental retardation.
- mewing cry.
- microcephaly and round facies.
- Epicanthic folds.

Chromosome 22q 11 deletion syndrome:

Clinical feature: Cong. HD, Abnormality of palate, facial dysmorphism, development delay, variable degree of T cell immunodef. & hypocalcaemia.

Previously thought to be 2 disorder – Di George syndrome.

Velocardiofacial syndrome

Di George syndrome: Thymic hypoplasia → T cell immunodef.

Parathyroid hypoplasia → hypocalcemia

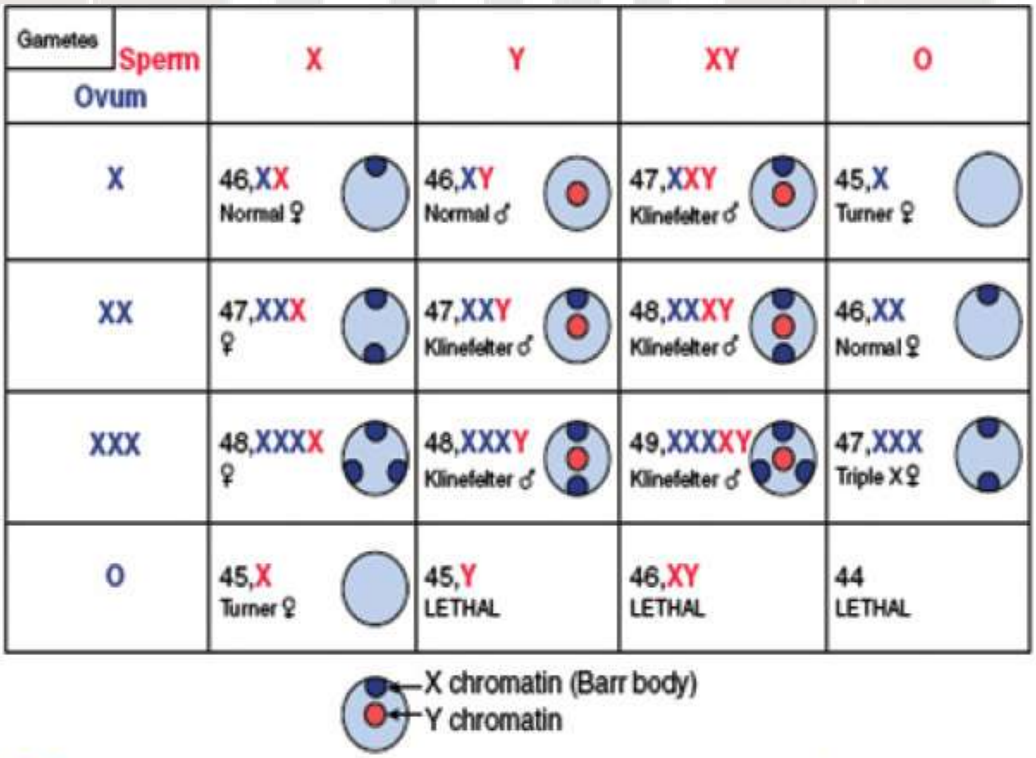


Fig. 6.11: Numerical aberrations of sex chromosomes Nondisjunction in either the male or female gametes is the principal cause of these abnormalities.

Velocardiofacial syndrome: Facial dysmorphism cleft palate, CVS abnormality, learning disability acronym ‘catch 22

C – Cardiac abnormality

A – Abnormal defect

T – T Cell defect

C - Cleft plate

H – Hypocalcemia

Diagnosis – FISH, Molecular basis –NK

Cytogenetic disorders inv. sex chromosomes:

More common than autosomal.

Better tolerated.

Lyon hypothesis:

1. only one X chromosome is genetically active.
2. inactivation of other X (maternal / paternal) occurs at random in all cells of blastocysts on day 16 of embryonic life.
3. Inactive X chr seen as Barr body or X chromatin.
4. Many genes may escape X inactivation (i.e. why 45X →Turner syndrome).
5. Also inactive X selectivity reactivity before first meiotic division.

Sex chromosomal abnormality:

Subtle chronic problems related to fertility and sex development.

Difficult to diagnose at birth, recognized at time of puberty.

Higher the number of X chromosome, the greater the likehood of mental retardation.

Klinefelter syndrome: Male hypogonadism:

≥ 2X ≥ 1Y chromosome (47 XXY, 46 XY/ 47 XXY).

1 in 850 live births.

Morphology: testicular atrophy & hyalinization, Eunuchoid body habitus: ↑ sole to os publis length gynecomastria

Mean IQ: Lower than (n) (but not MR).

Plasma FSH ↑ Testosterone ↓ Estradiol ↑

Cause: maternal non dysjunction / paternal non disjunction (ass with maternal age ↑)

Variant: 48, xxxy ,49, xxxxy, 48 xxyy

Turner syndrome:

1 in 3000 females birth, 99% of 45 X conceptuses – Non viable.

Complete or partial monosomy of x chromosome Hypogonadism in phenotypic females.

57% - 45 XO

14 % - structural abnormality

46, X, I (X) (q 10)

46, X, r (X)

46, X, del (Xq)

46, X del (XP)

29% - Mosaics

45x/ 46XX

45X/46 XY

45X/ 47XXX

45X/ 46 X, 1 (X) (q 10).

Morphology – short stature, commonest cause of primary amenorrhea, infertility, webbing of neck, cubitis valgus, peripheral lymphoedema at birth, board chest and wide spaced nipples, low posterior hairline pigmented nevi, coarctation of aorta preductal) and bicuspid aortic valves, streak ovaries.

CVS anomalies → single most important cause of mortality.

Single gene disorders with non – classic inheritance:

Disease caused by triple repeat mutations

Disease caused by mutations in mitochondrial genes.

Disease associated with genomic imprinting.

Disease associated with gonadal mosaicism.

Triple repeat mutation:

Disease	Gene	Locus	Protein	Repeat	Normal	No. of Repeats Disease
Expansions Affecting Noncoding Regions						
fragile X syndroms	FMRI (FRAXA)	Xq27.3	FMR-1 Protein (FMRP)	CGG	6–55	55–200 (pis); >230 (full)
Friedreich ataxia	FXN	9q21.1	Frataxin	GAA	7–34	34–80 (pre); >100 (full)
Myotonic dystrophy	DMPX	19q13.3	Myotonic dystrophy protein kinase (DMPK)	CTG	5–37	34–80 (pre); >100 (full)
Expansions Affecting Coding Regions						
Spinobulbar muscular atrophy (Kennedy disease)	AR	Xq12	Androgen receptor (AR)	CAG	9–36	38–62
Huntington disease	HTT	4p16.3	Huntingtin	CAG	6–35	36–121
Dentatorubral-pallidoluysian atrophy (Haw River syndroms)	ATNL	12p13.31	Atrophin–1	CAG	6–35	49–33
Spinocerebellar ataxia type 1	ATXN1	6p23	Ataxin–1	CAG	6–44	39–32
Spinocerebellar ataxia type 2	ATXN2	12q24.1	Ataxin–2	CAG	15–31	36–63
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	ATXN3	14q21	Ataxin–3	CAG	12–40	55–84
Spinocerebellar ataxia type 6	CACNA2A	19p13.3	α_{1A}–Voltage–dependent calcium channelsubunit	CAG	4–18	21–33
Spinocerebellar ataxia type 7	ATXN7	3p14.1	Ataxin–7	CAG	4–35	37–306

Mutation is characterized by long repeating sequence of 3 nucleotides.

Specific nucleotide sequence undergoing amplification differs in various disorders, in most cases, share nucleotides G and C.

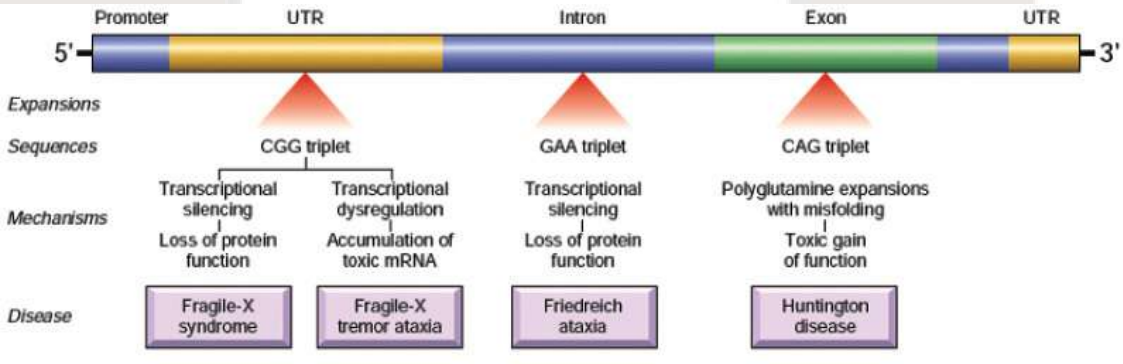


Fig. 6.12

Fragile X syndrome:

2nd most common cause of MR after Down syndrome.

X linked characterized by unusual mutation within FMR – 1 gene (Familial mental retardation-1).

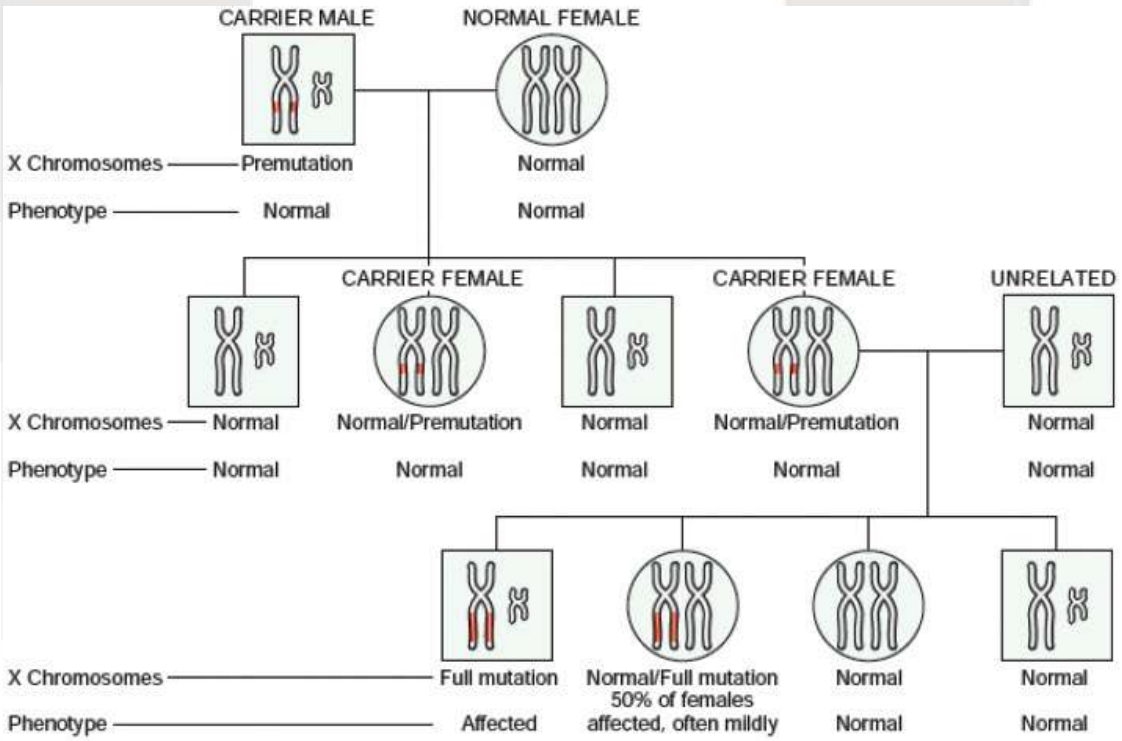


Fig. 6.13

Note that in the first generation all sons are normal and all females are carriers. During oogenesis in the carrier female, premutation expands to full mutation; hence, in the next generation all males who inherit the X with full mutation are affected. However, only 50% of females who inherit the full mutation are affected, and only mildly.

Alteration seen as discontinuity of staining or constrictions in long arm of X chromosome → appears broken → fragile site.

Males, IQ:20 -60; long faces, large mandible, large ears hyper extensibility

Macro – orchidism (→distinctive feature)

- X linked disorder.
- Presence of carrier males (transmitting male).
- Affected females :50% of carrier females are affected.

- Risk of phenotype effects - Risk depends of position of individual in pedigree “Sherman’s paradox.
- Anticipation: clinical feat worsens with successive generation.
- Mutation: Xq 27.3, FMR -1 gene – contains CGG multiple tandem repeats

(N) pop; CGG repeats;6-46.

Permutation: 50- 230 CGG repeats (N) transmitting males & carrier females.

Full mutation; 230-4000 CGG repeats.

During oogenesis (but not in spermatogenesis) permutation→ mutations by triplet amplification.

Other diseases associated with nucleotide repeats:

Not always triplets:

Expansion:

Oogenesis→ Fragile X syndrome.

Spermatogenesis→ Huntington’s disease.

Affecting non – coding regions	affecting coding regions
Fragile X syndrome	Spinobulbar muscular
Myotonic dystrophy	atrophy (Kennedy disease)
Freidrichs ataxia	Huntington’s disease
Progressive myoclonus epilepsy	Dentorubropallidolu-sian atrophy

Mutations in mitochondrial genes:

- Maternal inheritance (Ova contain MT within their abundant cytoplasm).
- (enzymes inv.in oxidative phosphorylation).

E.g. Leber Hereditary optic neuropathy-progressive B/L loss of central vision. Neurodegenerative disease other mitochondrial encephelomyopathies:

Leigh disease.

Myoclonic epilepsy and ragged red fibres-mit DNA.

Mitochondrial encephalopathy, lactic acidosis and stroke like episodes.

Genomic imprinting:

Imprinting; Selective inactivation of either maternal or paternal allele e. g

Maternal imprinting – Inactivation /Silencing of maternal allele.

1. Prader willi syndrome: MR obesity, short stature, hypotonia, hypogonadism

-Deletion on chromosome 15 band q 12 (affects paternally derived chromosomes).

2. Angelman syndrome; deletion of same region on maternally derived chromosomes.

MR, ataxia of inappropriate laughter- ‘happy’ puppets.

Also because of uniparental disomy.

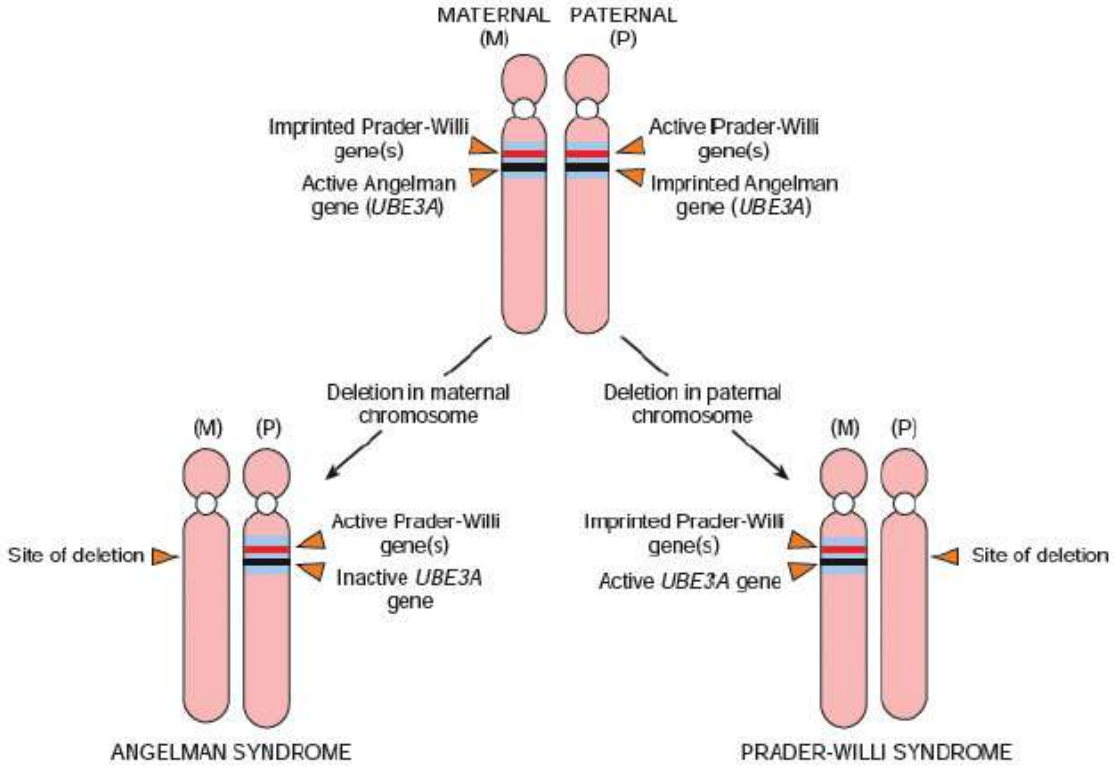


Fig. 6.14

The molecular basis of these two syndromes lies in the genomic imprinting. Three mechanisms are involved.

1. **Deletions.** It is known that a gene or set of genes on maternal chromosome 15q12 is imprinted (and hence silenced), and thus the only functional allele(s) are provided by the paternal chromosome. When these are lost as a result of a deletion, the person develops Prader-Willi syndrome. Conversely, a distinct gene that also maps to the same region of chromosome 15 is imprinted on the paternal chromosome. Only the maternally derived allele of this gene is normally active. Deletion of this maternal gene on chromosome 15 gives rise to the Angelman syndrome. Deletions account for about 70% cases.
2. **Uniparental disomy.** Molecular studies of cytogenetically normal patients with the Prader-Willi syndrome (i.e., those without the deletion) have revealed that they have two maternal copies of chromosome 15. Inheritance of both chromosomes of a pair from one parent is called uniparental disomy. The net effect is the same

(i.e., the person does not have a functional set of genes from the [nonimprinted] paternal chromosomes 15). Angelman syndrome, as might be expected, can also result from uniparental disomy of paternal chromosome 15. This is the second most common mechanism responsible for 20% to 25% cases.

3. **Defective imprinting** . In a small minority of patients (1% to 4%), there is an imprinting defect. In some patients with Prader-Willi syndrome, the paternal chromosome carries the maternal imprint and conversely in Angelman syndrome the maternal chromosome carries the paternal imprint (hence there are no functional alleles).

Gonadal Mosaicism:

- Results from mutation that occurs post zygotically during early (embryonic) development.
- If mutation affects only cells destined to form gonads, gametes carry mutation but the somatic cells are completely normal.

Molecular Diagnostics:

Genomic probes – derived from a region of DNA.

CDNA probe-derived from RNA by reverse transcriptase.

Oligonucleotide probe – synthetic probe (Genomic & cDNA probes from cellular material).

Rib probe – prepared by in vitro transcription system.

1. In situ Hybridization (ISH).
   Localizes DNA or RNA directly in an intact cell by hybridization and radiolabelled probing FISH.
2. Filter hybridization.
   Target DNA/RNA is extracted immobilized on nitrocellulose filter or nylon & hybridized with labeled probe
   a. Slot and dot blots: DNA is not fractionated before immobilizing.
   b. Southern blot – DNA fractionation followed by gel electrophoresis.
   c. Northern blot – Similar to southern blot but involves RNA.

d. Western blot – Protein fractionation and antibodies are used as probes.

Polymerase chain reaction (PCR):

Several millions of copies are formed from a single DNA fragment using a primer, heat stable DNA polymerase (Taq polymerase) d-NTP (decoy nucleotide. d ATP, d CTP, d TTP) MgC12 and buffer in a thermo cycler.

Each cycle consists of 3 steps.

Heat denaturation of DNA (at 94˚C for 60-90 sec).

Annealing of primers (at 55ºC for 30-120 sec).

Extension using DNA polymerase (at 72°C for 60-180 sec).

Repeated cycles done in automated thermal cycler.

Advantages:

Can be done on living or dead tissue.

Small amounts of initial DNA template.

↑sensitivity.

Rapidity.

Amenability of automation.

No need for radionucliotide probes.

Indirect DNA diagnosis linkage analysis:

A. Site Polymorphisms (RELP) – Natural DNA variations like single base pair changes may abolish or create recognition sites for restriction enzymes, thereby altering the length of DNA fragments product after digestion with certain restriction enzymes. Using appropriate DNA probes that hybridize with sequences in the vicinity of the polymorphic sites, DNA fragments.

B. Length polymorphisms – satellites are short repetitive sequences of noncoding DNA.

Microsatellites – 2to6 pairs.

Minisatellites – larger, usually 15-70 base pair.

Antenatal Diagnosis:

Chorionic villus sampling (CVS).

Amniocentesis.

Cordocentesis (PUBS).

Maternal serum AFP.

Fetal cell in maternal blood.

Gene therapy:

Disease	Target cell	Gene product
ADA deficiency	Lymphocytes ,stem cell	ADA
X-SCID	Stem cell	Gamma – ILR
Hemophilia B	Hepatocyte , fibroblasts	FIX
Familial Hypercholesterolemia	Hepatocytes	LDL rec
Cystic fibrosis	Airway epithelial cells	CFTR
Duchenne muscular	Myoblasts	Dystrophin
Gaucher disease	Macrophages	Glucocerebrosidase
Ischemic heart disease	Cardiomyocytes	VEGF, FGF

Methods of Gene therapy:

Cell fusion.

Co precipitation / Transfection.

Electroporation.

Liposome fusion.

Direct introduction of naked DNA.

Viral vectors – Adenovirus, retrovirus.

SYSTEMIC PATHOLOGY

7 Blood Vessels

Blood Vessels:

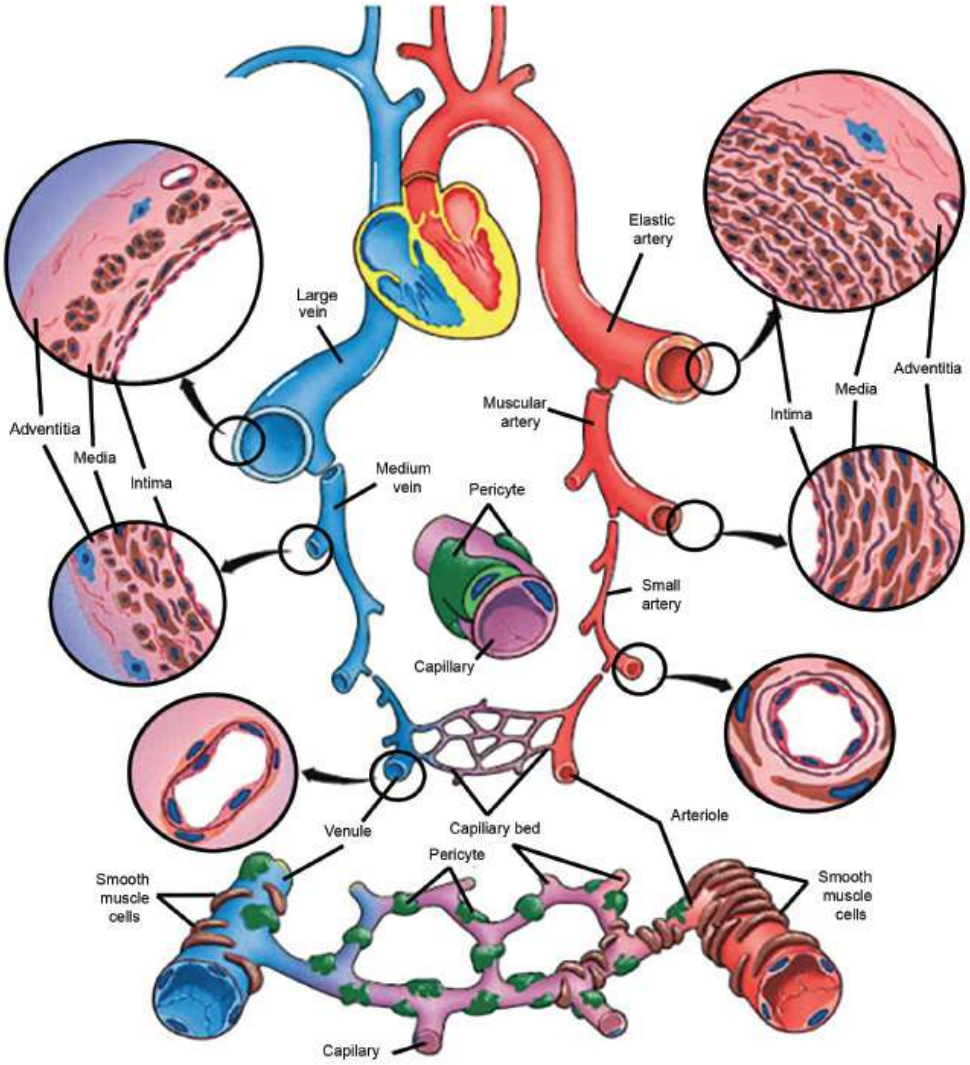


Fig. 7.1: Subdivisions and histologic structure of the vascular system. Each subdivision is subject to a set of pathologic changes conditioned by the structure-function relationship of that part of the system. For example, the aorta, an elastic artery subject to great pressure, frequently shown a pathologic dilation (aneurysm) if the supporting elastic media is damaged. Muscular arteries are the most significant site of atherosclerosis. Small arteries, particularly arterioles are sites hypertensive changes. Capillary beds, venules and veins each display their own types of pathologic changes.

Diseases of Blood Vessels:

Endothelial cells contain **Weibel Palade bodies** that are the storage organelle for VWF. Markers for endothelial cells are Factor 8 related antigen (Ab to VWF) and CD 31.

Arteriosclerosis means hardenings of arteries.

Most prevalent and clinically significant arterial disease is **Atherosclerosis** , which is characterized by formation of intimal

fibrous plaques with lipid core.

Arteriolosclerosis effects small arterioles and arterioles. Whole vessel wall is affected. May be hyaline or hyperplastic.

- Hyaline – Benign hypertension, DM, benign nephrosclerosis.
- Hyperplastic – Onion skin, concentric, laminated thickening microscopically seen in malignant hypertension, malignant nephrosclerosis.

Monckeberg's medial sclerosis seen in patients above 50 years. Ring like dystrophic calcification within the media of medium to small sized muscular arteries. May undergo ossification. Intima + adventitia normal. No narrowing or inflammation seen. Femoral, tibial, radial + ulnar arteries are affected.

Atherosclerosis:

Atheroma (fibro fatty plaque) is fundamental lesion. Affects elastic arteries and large and medium muscular arteries. Major consequences are ME, Stroke, aortic aneurysm and gangrene of extremities.

Sites in descending order of involvement are abdominal aorta, coronary artery, popliteal a, descending thoracic aorta, internal carotid artery, circle of Willis.

Vessels spared are upper extremity, mesenteric A, renal A.

Complications of plaque are thrombosis, rupture, hemorrhage, weakening of wall and development of aneurysm, calcification and atheroemboli.

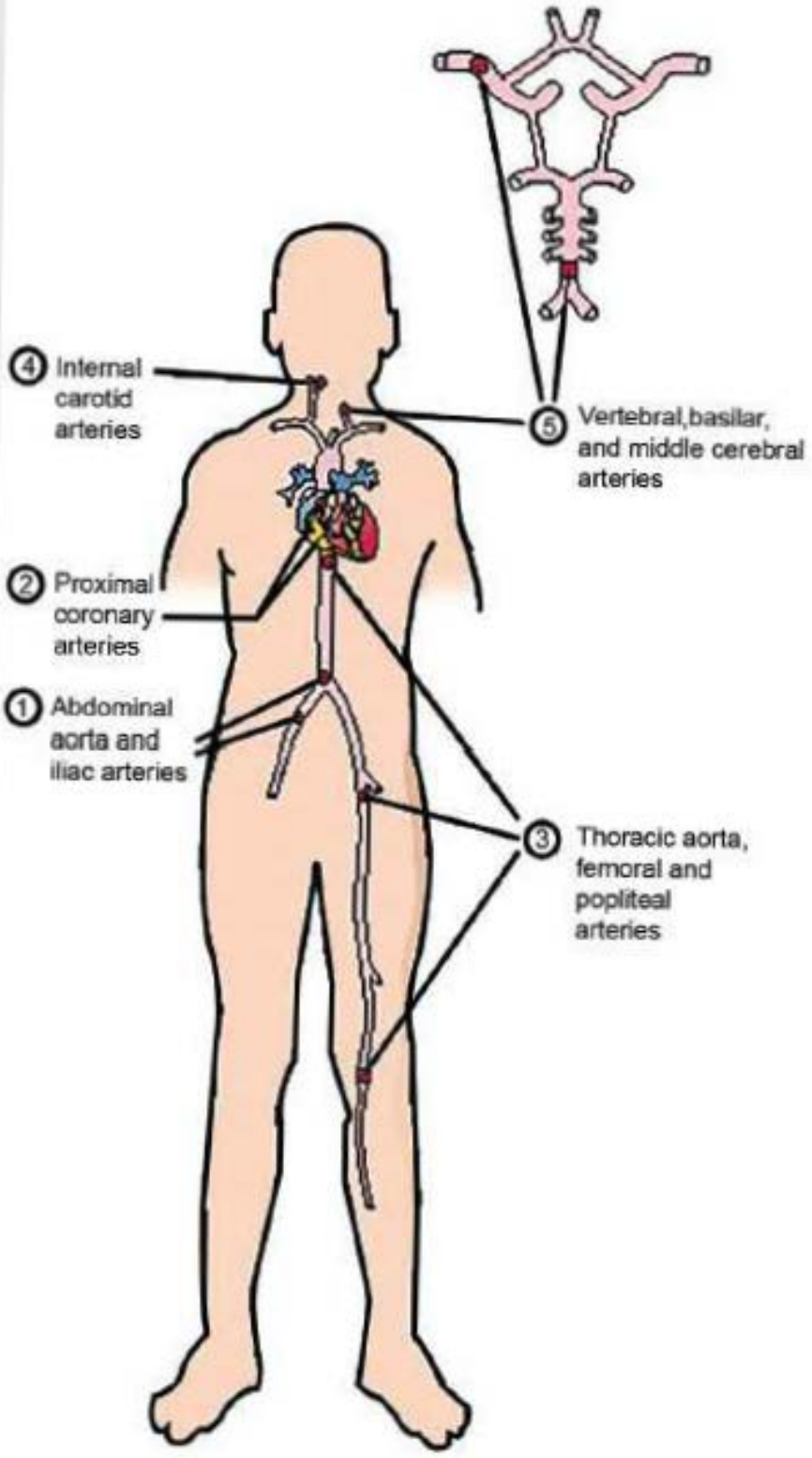


Fig. 7.2: Sites of servere atherosclerosis in order of frequency.

Fatty streaks may be precursors of AS plaques. Fatty dots are less than 1 mm in size. Both are sub- intimal foam cell collections. Not all fatty streaks become plaques.

American Heart association defines six types of lesions.

Type 1- Fatty dot	Clinically silent
Type 2- Fatty streak	Clinically silent
Type 3-2 + Small extra cellular lipid pools	Clinically silent
Type 4-2 + Core of extra cellular lipid pools	Increased collagen and smooth muscle. Complication present
Type 5- Fibroa atheroma	Increased collagen and smooth muscle. Complication present
Type 6- Complicated lesions (ulceration / haemorrhage / thrombosis	Increased collagen and smooth muscle. Complication present

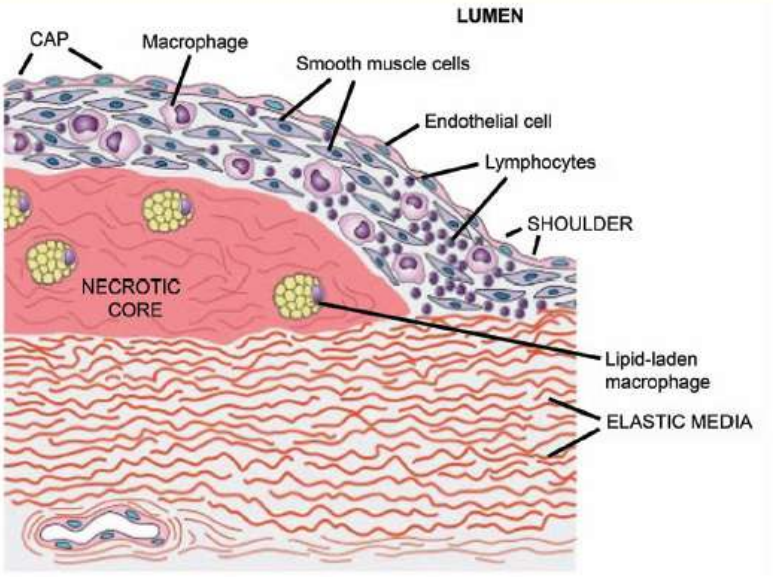


Fig. 7.3: Fibrofatty plaque of atherosclerosis. A in this fully developed fibrous plaque, the core contains lipid-filled macrophages and necrotic smooth muscle cell debris. The "fibrous" cap is composed largely of smooth muscle cells, which produce collagen, small amounts of elastin, and glycosaminoglycans. Also shown are infiltrating macrophages and lymphocytes. Note that the endothelium over the surface of (the fibrous cap frequently appears intact. B. Adaptive stage with atherosclerotic plaque and vessel wall dilatation to maintain the normal size of the lumen. Normal artery wall is at the top.

Risk factors:

Disease of middle age. Men> women. Familial disposition.

Major risk factor are- * Smoking * Hypertension * Hyperlipidemia * Diabetes.

Lipid – LDL and TG are bad.

HDL is good. Involved in reverse transport of cholesterol.

HDL increases with exercise and moderate consumption of ethanol.

HDL decreases with obesity and smoking.

Dyslipoproteinemia- Acquired – nephrotic syndrome, alcoholism, DM, hypothyroidism Genetic.

Commonest is Type 2(and b included), or Type 4.

Type 1 has no risk of atherosclerosis.

Cut off points for Cholesterol are < 200 mg%.

Triglycerides < 100 mg%.

LDL < 150 mg%.

High intake of fish oils rich in omega 3 fatty acids leads to decreased LDL, and Platelet aggregability.

Type	Increased lipoprotein class	Increased lipid class	Incidence	Genetic defect
Type 1	Chylomicrons	TG	Less than 1%	Abn lipoprotein lipase, non atherogenic.
Type 2a	LDL	Cholesterol	10%	Mutation in the LDL receptor gene and Apo lipoprotein B gene.
Type 2b	LDL and VLDL	Cholesterol and triglycerides	40%	Mutation in the LDL receptor gene and Apo lipoprotein B gene.
Type 3	Remnant Chylomicrons and IDL	Triglycerides and Cholesterol	<1	Mutations in Apo lipoprotein E genes.
Type 4	VLDL	Triglycerides	45%	Mutation of Lipoprotein lipase.
Type 5	VLDL and Chylomicrons	Triglycerides and Cholesterol	5%	Apo lipoprotein C2 abnormalities.

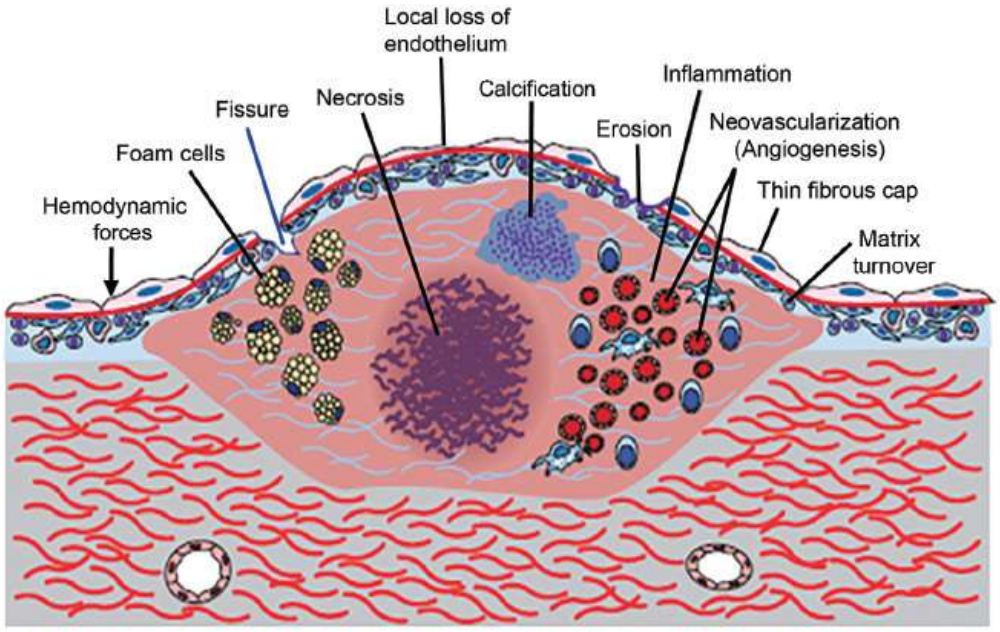


Fig. 7.4: Complicated lesions of atherosclerosis. The luminal surface of the abdominal aorta and the common iliac arteries shows numerous fibrous plaques and raised, ulcerated lesions containing friable, atheromatous debris. The distal portion of the aorta displays a small aneurysmal dilation.

HYPERTENSION:

Stronger risk above 45 years of age.

Minor-

- Obesity.
- OCPs.
- Sedentary habits.
- Stress.
- Family history.
- Age.
- Male.
- High carbohydrate diet.
- Hyperhomocysteinaemia (causes endothelia dysfunction).
- Type A personality.

Markers of atherosclerosis are increased plasminogen activator, CRP, LP (a) which is an abnormal altered LDL

2 risk factors increase risk 4 fold

3 risk factors increase risk 7 fold.

Types and Causes of Hypertension (Systolic and Diastolic):

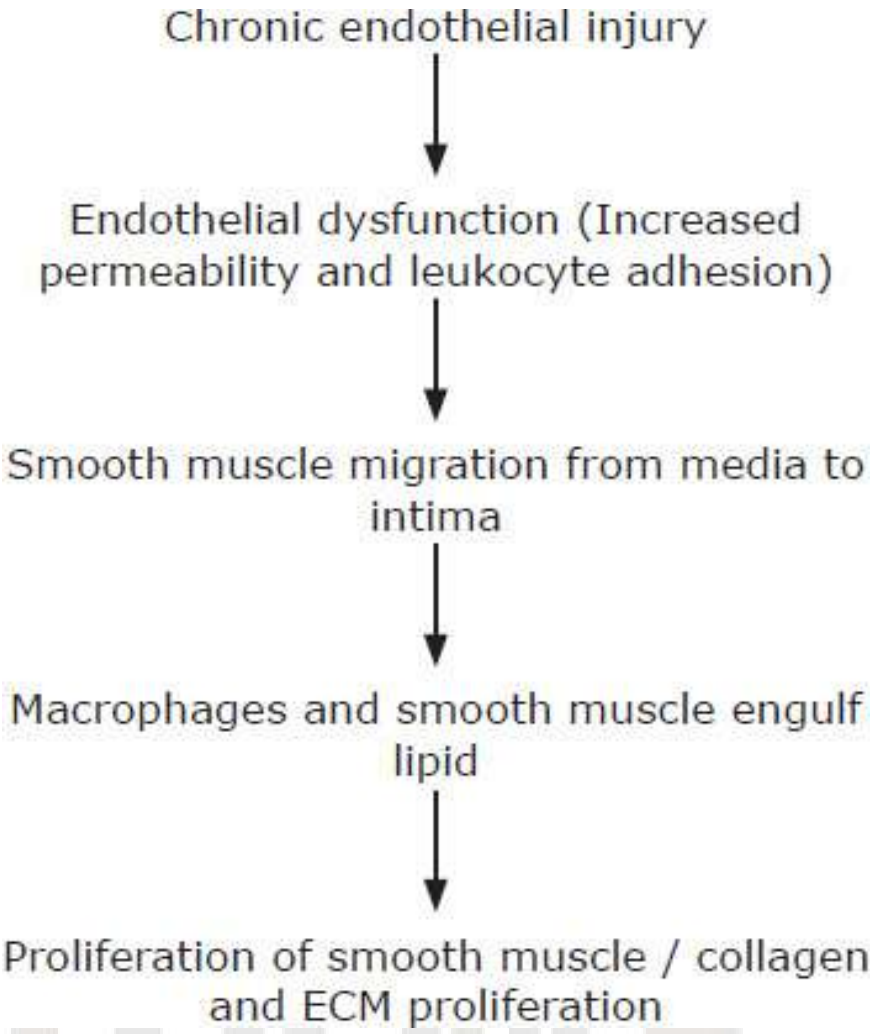
Essential Hypertension (90% To 95% of Cases)
Secondary Hypertension.
Renal
Acute glomerulonephritis. Chronic renal disease. Polycystic disease. Renal artery stenosis. Renal vasculitis. Renin-producing tumors.
Endocrine
Adrenocortical hyperfunction (Cushing syndrome, primary aldosteronism, congenital adrenal hyperplasia, licorice ingestion). Exogenous hormones (glucocorticoids, estrogen [including pregnancy-induced and oral contraceptives], sympathomimetics and tyramine-containing foods, monoamine oxidase inhibitors) Pheochromocytoma.

Acromegaly.
Hypothyroidism (myxedema).
Hyperthyroidism (thyrotoxicosis).
Pregnancy-induced.
Cardiovascular
Coarctation of aorta.
Polyarteritis nodosa.
Increased intravascular volume.
Increased cardiac output.
Rigidity of the aorta.
Neurologic
Psychogenic.
Increased intracranial pressure.
Sleep apnea.
Acute stress, including surgery.

Pathogenesis:

1. Reaction to injury hypothesis. As is a chronic inflammatory response of arterial wall to endothelial injury.

Endothelial injury may be due to hyperlipidemia, HT, Smoking, Homocysteine, hemodynamic stresses, toxins, virus, immune.



2. Monoclonal hypothesis – Smooth muscle proliferation is the primary event.
3. Infective – Chlamydia pneumonia, HSV, CMV are implicated.

Hypertensive vascular disease:

90-95% are idiopathic, 5 – 10% are renal

Renal dysfunction is essential for development and maintenance of hypertension.

Essential HT- Polygenic inheritance / environmental.

Single gene disorders associated with HT are:

1. Abnormal aldosterone synthase.
2. 11 β hydroxylase deficiency.

3. 17 α hydroxylase deficiency.
4. Liddle syndrome (β or γ subunit of epi Na channel).
5. Gillman syndrome (Na Cl contransporter def).
6. Pseudohypoaldosteronism (α or β subunit of Epi Na channel).

Environmental factors include stress, obesity, smoking, physical inactivity, increased salt intake.

Mechanisms are renal retention of sodium and vasoconstriction and vascular hypertrophy.

Vessel changes:

Large – Accelerated atherogenesis, weakens the wall and predisposes to aneurysm and dissection.

Small – Hyaline and hyperplastic arteriolosclerosis.

Hyaline – Homogeneous pink thickening of arteriolar wall with loss of structural detail and narrowing of lumen. Due to leakage of plasma proteins and ECM production by smooth muscles.

Hyperplastic – Seen in malignant hypertension (Diastolic BP> 100). Onion skin, concentric laminated thickening, reduplicated basement membrane, fibrinoid necrosis (Necrotising arteriloitis). Changes esp seen in kidney.

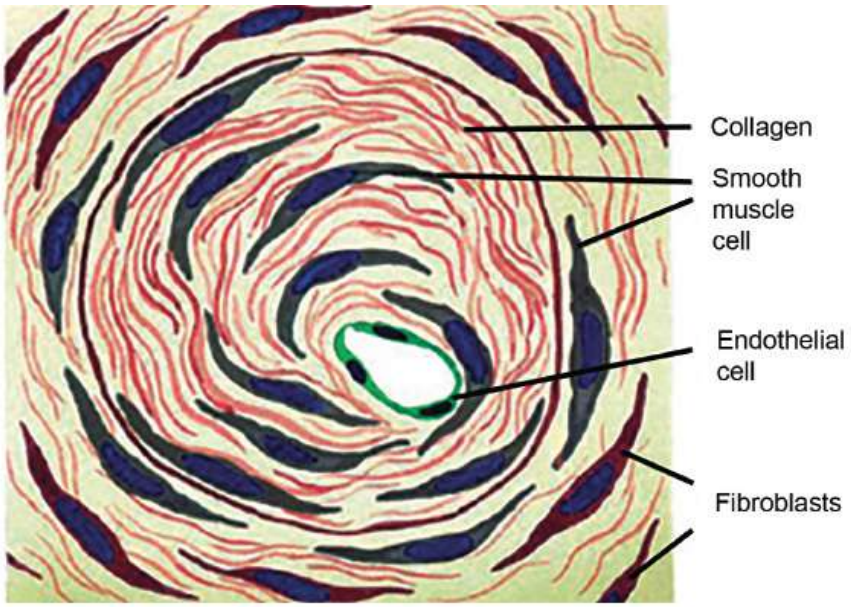


Fig. 7.5: Arteriolosclerosis. In **cams** of hypertension, the arterioles exhibit smooth muscle cell proliferation and increased amounts of intercellular collagen and glycosaminoglycans, resulting in an 'onionskin' appearance. The mass of smooth muscle and associated elements tends to fix the size of the lumen and restrict the arteriole's capacity to dilate.

Vasculitis:

Denotes inflammation of vessel wall.

Two mechanisms. Direct (Toxins, microbes, irradiation, mechanical trauma) a and immunologic.

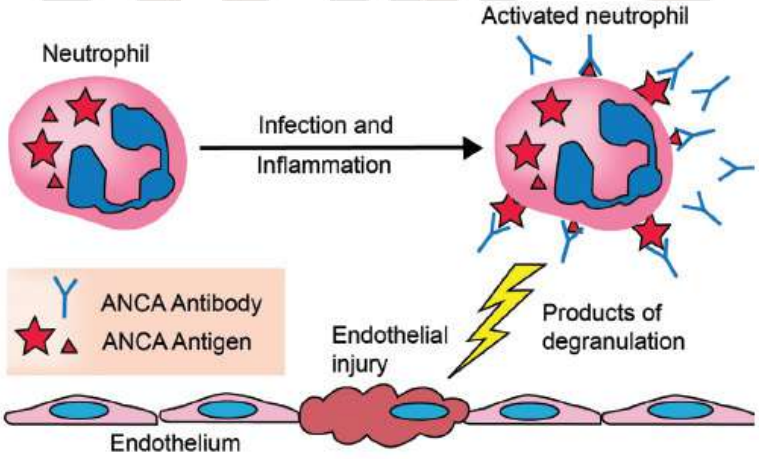


Fig. 7.6: Model of the pathogenesis of antineutrophil cytoplasmic antibodies (ANCA) vasculitis. ANCA antigens are normally found in the neutrophil cytoplasm with very little surface expression. In inflammation and infection, increased cell surface expression of ANCA antigens is induced in the neutrophils. ANCA present in the circulation due to previous formation through unknown mechanisms binds to these ANCA antigens on the surface, leading to neutrophil activation and interaction with including reactive oxygen species, PR3 and MPO, and other granule enzymes cause endothelial cell apoptosis and necrosis, leading to endothelial injury.

Infections associated with vasculitis are:

- Bacterial – Neisseria.
- Rickettsial – RMSF.
- Spirochetes – Syphilis.
- Fungal – Aspergillus / Mucor.
- Viral – Varicella.

Non infectious vasculitis.

Immune complex mediated – SLE, Drug induced, Viral (HBSAg, HCV, RNA).

ANCA associated – cANCA seen with Wagener's granulomatosis.

PANCA seen with microscopic polyangiitis and Churg Strauss syndrome.

- Anti endothelial antibodies – Kawasaki's.

Classification:

Large vessel	Medium sized vessels	Small vessel
Giant cell (Temporal arteritis) Takayasu's disease.	Polyarteritis nodosa Kawasaki's disease.	Wegener's granulomatosis Churg Strauss. Microscopic polyarteritis. Henoch Schonlein purpura. Essential cryoglobulinemia.

Vasculitis Type[*]	Examples	Description
Large-Vessel Vasculitis	Giant-Cell (Temporal) Arteritis.	Granulomatous Inflammation; Frequently Involves The Temporal Artery. Usually Occurs In Patients Older Than Age 50 And Is Associated With Polymyalgia Rheumatica..
Aorta And Large Branches To Extremities, Head, And Neck.	Takayasu Arteritis	Granulomatous Inflammation Usually Occurring In Patients Younger Than Age 50.
Medium-Vessel Vasculitis	Polyarteritis Nodosa	Necrotizing Inflammation Typically Involving Renal Arteries But Sparing Pulmonary Vessels.
Main Visceral Arteries And Their Branches	Kawasaki Disease	Arteritis With Mucocutaneous Lymph Node Syndrome; Usually Occurs In Children. Coronary Arteries Can Be Involved With Aneurysm Formation And/Or Thrombosis.
Small-Vessel Vasculitis	Wegener Granulomatosis.	Granulomatous Inflammation Involving The Respiratory Tract And Necrotizing Vasculitis Affecting Small Vessels, Including Glomerular Vessels. Associated With Pr3-Ancas.
Arterioles, Venules, Capillaries, And Occasionally Small Arteries	Churg-Strauss Syndrome	Eosinophil-Rich Granulomatous Inflammation Involving The Respiratory Tract And Necrotizing Vasculitis Affecting Small Vessels. Associated With Asthma And Blood Eosinophilia. Associated With Mpo-Ancas.
	Microscopic Polyangiitis	Necrotizing Small-Vessel Vasculitis With Few Or No Immune Deposits; Necrotizing Arteritis Of Small And Medium-Sized Arteries Can Occur. Necrotizing Glomerulonephritis And Pulmonary Capillaritis Are Common. Associated With Mpo-Ancas.

Giant Cell arteritis:

Most Common:

- Granulomatous inflammation of aorta and major branches with a predilection for the extra cranial branches of carotid artery. Often involves the temporal artery.
- Seen in patients older than 50 years.
- Associated with polymyalgia rheumatica. Hematological malignancies appear in 2 to 4% of those with concurrent polymyalgia rheumatica.
- Unknown etiology.
- Giant cells seen in 2/3 of cases.
- Clinically – Female > 50 years with history of fever and weight loss, c/o severe unilateral head ache with transient to permanent vision loss. ESR is markedly elevated.

- Diagnosis is by biopsy.

Takayasu's arteritis:

- Granulomatous vasculitis of aorta and its major branches.
- Occurs below 40 years.
- Classically involves aortic arch and pulmonary vessels.
- Also called PULSELESS disease or REVERSE COARCTATION.

Classical PAN:

- Necrotising arteritis affecting small and medium sized muscular arteries, esp renal arteries. Arterioles, capillaries and venules are spared. No Glomerulonephritis seen. Commonest sites are kidney, heart, liver, GIT, pancreas. **Pulmonary vessels are spared.** Vessels show irregular aneurismal dilatation, nodularity and obstruction.
- Transmural inflammation of arterial wall is seen with fibrinoid necrosis. Fibrosis may occur later. No **granulomas seen.** Lesions are in different staged, acute, healing and healed.
- PANCA associated

Kawasaki's disease:

- Arteritis involving large, medium and small sized arteries.
- Coronary arteries are often involved
- Occurs in **children.** 80% cases occur below 4 years.
- Cause of acquired heart disease in children.
- **Associated with mucocutaneous lymph node syndrome.**
- Due to anti-endothelial and anti-smooth muscle antibodies. May be virally triggered. Vessels show Tran mural inflammation with less prominent necrosis and no granulomas.

Wegener's granulomatosis:

- Triad of

- Acute necrotizing granulomas in respiratory tract.
- Focal necrotizing or granulomatous vasculitis.
- Focal or diffuse necrotizing Crescentric Glomerulonephritis.
- Vasculitis affects capillaries, venules and arterioles and also small and medium sized arteries most prominent in the upper airways and lungs.
- Peak incidence in fifth decade.
- CANCA associated in up to 90% patients and is a good marker of activity.

Churg Strauss syndrome:

- Also called allergic granulomatosis and angiitis.
- Lesions are identical to PAN. Seen to involve small to medium sized vessels.
- Strong association with bronchial asthma, allergic rhinitis and eosinophilia.
- Pulmonary and splenic veins and peripheral nerves are frequently involved with intra and extravascular granulomas and eosinophilic infiltration.
- Renal disease is infrequent.
- PANCA is seen in 70% cases.

Microscopic polyangiitis:

- Also called Leukocytoclastic / hypersensitivity vascultits or microscopic polyarteritis.
- Necrotizing vasculitis affecting small vessels esp of skin and mucous membranes.
- Isolated cutaneous leukocytoclastic vasculitis presents with palpable purpura.
- Necrotising arteritis of small and medium arteries, necrotizing Glomerulonephritis and pulmonary capillaritis is common.
- Sites involved are skin, mucous membranes, lung, brain, heart, GIT, Kidney, muscles.
- Associated with p ANCA in 80% cases.

- Pauci immune lesions with no immune deposits. Leukocytoclasia is seen.
- Disseminated vascular lesions of hypersensitivity angiitis may also appear in a number oil syndromes including Henoch Schonlein purpura, Essential cryoglobulinemia, vasculitis with connective tissue disorders and malignancies.

Henoch Schonlein purpura:

Vasculitis with IgA dominant immune deposits affecting small vessels typically of skin, gut and glomeruli. Associated with arthritis and arthralgias.

Essential cryoglobulinemia:

Cryoglobulin deposits in vessels along with cryoglobulins in serum. Skin and glomeruli often involved.

Buerger's disease:

- Also called thromboangiitis obliterans.
- Segmental thrombosing acute and chronic inflammation of medium and small arteries. Associated with smoking
- Affects tibial and radial arteries most often.
- Histologically shows thrombus with abscesses and granulomatous reaction with acute and chronic inflammation of wall.

Aneurysm:

Localized abnormal dilatation of blood vessels.

True- bounded by complete arterial wall components.

False – or pseudoaneurysm is haematoma communicating with the vessel lumen. Dissecting aneurysms are false aneurysms.

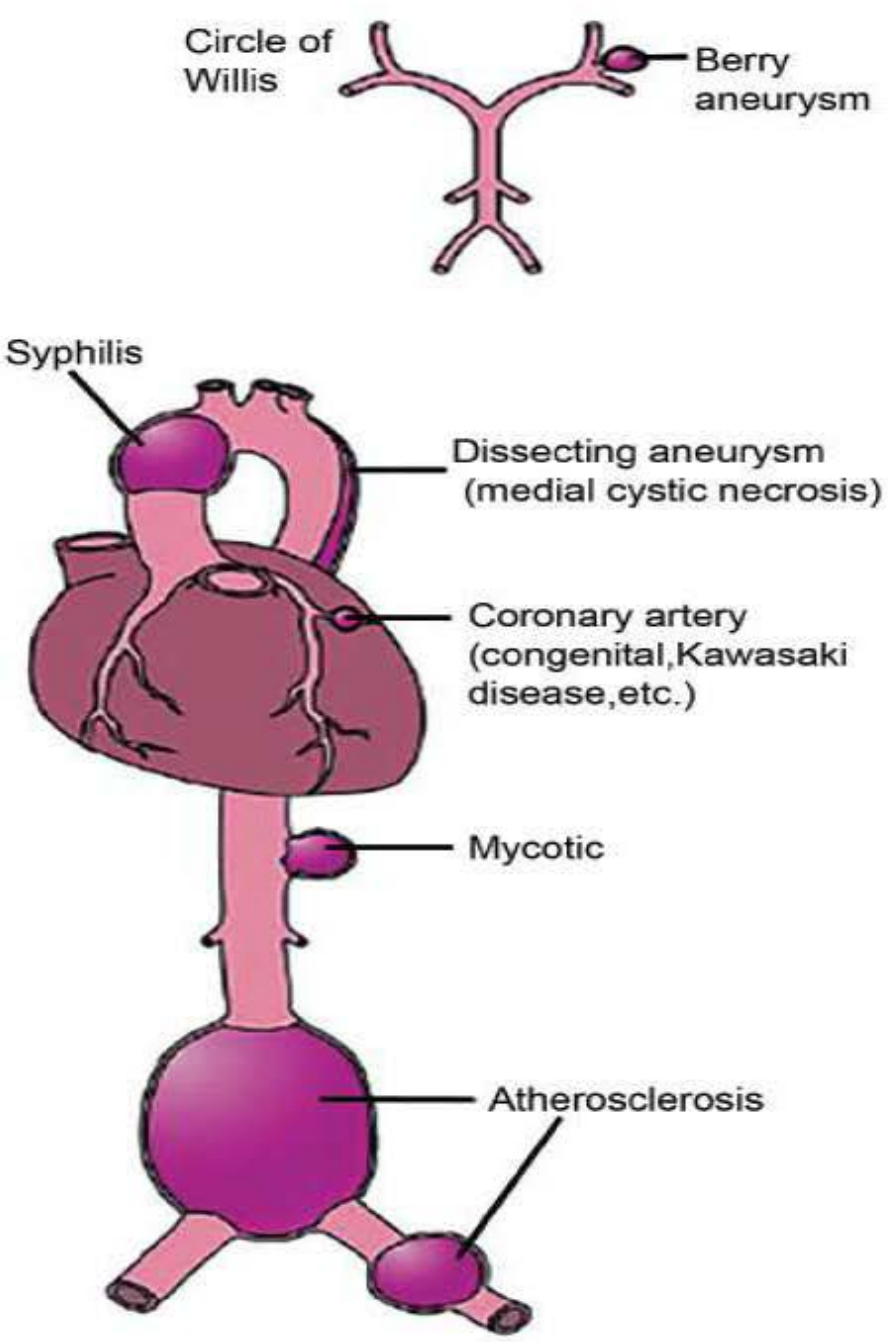


Fig. 7.7: The locations of aneurysms. Syphilitic aneurysms are the common variety in the ascending aorta, which is usually speared by the atherosclerotic process. Atherosclerotic aneurysms can occur in the abdominal aorta or muscular arteries, including the coronary and popliteal arteries and other vessels. Berry aneurysms are seen in the circle of Willis, mainly at branch points; their rupture leads to subarachnoid hemorrhage. Mycotic aneurysms occur almost anywhere that bacteria can deposit on vessel walls.

Aortic aneurysm:

Most frequently due to atherosclerosis. Other causes are cystic medial degeneration, Syphilis, trauma.

Arterial aneurysms are due to vasculitides, trauma or congenital (Berry).

Aneurysms are fusiform, cylindroid or saccular.

Commonest site **atherosclerotic aneurysm** is abdominal aorta below renal arteries and above iliac bifurcation. Other sites are common iliac, descending thoracic aorta and arch of aorta. Until proved otherwise an abdominal aneurysm is assumed to be atherosclerotic in origin.

Syphilitic aneurysms:

- (Luetic aneurysm) are seen in tertiary syphilis. Involve ascending aorta.
- Due to obliterative endarteritis of vasa vasora.
- Tree barking appearance is linear wrinkled appearance of intimal surface.
- Lead to aortic regurgitation and dilated heart called cor bovinum.

Dissecting aneurysms:

- Are due to dissection of blood between and along laminar planes of media (Intra mural haematoma). Dissections almost always originate with intimal tears. 90% located within 10cms of aortic valve. Blood propagates between outer + middle third of media.
- 40-60 years Associated with hypertension.
- Younger patients Associated with Marfan’s syndrome.
- Type I + II – Ascending aorta (Type A) Type III- Descending aorta (Type B).
- Histologically detectable lesion is cystic medial degeneration, characterized by elastic tissue fragmentation with separation by

small cleft like spaces filled with amorphous EMC. This change is seen in Marfan's.

- Annulo aortic ectasia is also seen in Marfans.

Veins:

Commonest site of varicose veins – Superficial veins of legs.

Thrombosis – Deep Veins of lower extremities.

S.V.C. Syndrome - Bronchogenic C_A Mediastinal lymphoma

IVC Syndrome - Hepatocellular C_A Renal cell C_A

Milroy's Disease = Heredofamilial congenital lymphodema.

Tumors:

Classification:

Benign	Borderline	Malignant
Haemangioma Lymphangioma Glomus Ectasias Bacillary angiomatosis	Haemangioendothelioma Kaposi's sarcoma.	Angiosarcoma Haemangiopericytoma.

Cystic hygroma – Cavemous lymmphangioma.

Glomus – Commonest site is distal digits, esp sub ungula location. Tumor of neuromyoarterial receptor cells.

Pyogenic Granuloma – Lobular capillary haemangioma. Granuloma gravid arum of pregnancy commonly located in the gums is this type of tumor.

Nevus flammeus – Salmon patch and port wine stain.

Bacillary angiomatosis:

Associated with AIDS.

Opportunistic infection due to Bartonella henslae, a gram negative bacillus causing cat scratch disease.

Shows reactive vascular proliferation with epithelioid endothelial cells, nuclear dust, neutrophils and granular material which is the causative organism.

Cat is reservoir and flea is vector.

Erythromycin and macrolide antibiotics are effective.

Kaposi's sarcoma – Commonest AIDS associated neoplasm.

Fibromuscular Dysplasia

- Focal irregular thickening of the walls of medium and large muscular arteries, including renal, carotid, splanchnic, and vertebral vessels.
- The cause is unknown but is probably developmental; first-degree relatives of affected individuals have an increased incidence.
- Segments of the vessel wall are focally thickened by a combination of irregular medial and intimal hyperplasia and fibrosis; this results in luminal stenosis, and in the renal arteries may be a cause of renovascular hypertension.
- Vascular outpouchings **(aneurysms)** may develop in the vessel segments with attenuated media and in some cases can rupture.
- Fibromuscular dysplasia can manifest at any age, although it is seen most frequently in young women; there is no association with use of oral contraceptives or abnormalities of sex hormone expression.

8 Heart

Heart:

Cardiac hypertrophy:

Hypertrophy of heart is due to volume overload or pressure overload. Pressure overload leads to concentric hypertrophy. Volume overload causes eccentric hypertrophy.

Hypertrophy involved re- induction of immediate early genes and fetal gene programming.

Mild - pulmonary hypertension, IHD
2 times.

Moderate - hypertension, Aortic stenosis, MR, DCM 2-3 times.

Severe - AR, HCM Greater than 3 times.

Causes of Left sided Heart Failure:

1. IHD.
2. Hypertension.
3. Aortic valvular disease + MR (RHD +Mitral prolapse).
4. 1° myocardial disease.

LUNGS- Pulmonary edema, CVC lung – Hear failure cells + **brown induration of lungs** dyspnea, orthopnea, paroxysmal nocturnal dyspnea, and cough – frothy + blood tinged sputum.

KIDNEYS – Pre renal azotemia.

CEREBRAL – Hypoxia – irritability + restlessness.

Causes of Right sided Heart Failure:

1. MS.
2. Left to right shunts.
3. Intrinsic disease of lung or pulm. Vasculations -↑ resistance in pulmonary circulation + Cor Pulmonale.
4. Less commonly cardiomyopathy + myocarditis.

Diseases of Lung – COPD, Diffuse pulmonary interstitial fibrosis, extensive persistent atelectasis.

Cystic fibrosis.

Diseases of Pulmonary vessels – Pulmonary embolism, 1^0 pulmonary vascular sclerosis. Extensive pulmonary arteritis (WG), Drugs + toxin induced vasc sclerosis.

Disorders affecting chest movement – Kyphoscoliosis marked obesity = Pick Wickian Syndrome neuromuscular disorders.

Disorder inducing pulmonary arteriolar constriction- chronic altitude sickness, obstruction to major airways, idiopathic alveolar hypoventilation.

LIVER - Nutmeg Liver

SPLEEN - Congestive splenomegaly

KIDNE - More pronounced pre- renal azotemia

Peripheral edema and effusions.

Ischaemic Heart Disease:

Also called **Coronary artery disease** . Critical factor is reduction in coronary blood flow resulting in insufficiency of oxygen and nutrients and accumulation of metabolites. Until proven otherwise IHD is due to advanced stenosing coronary atherosclerosis.

Four ischemic syndromes are:

1. Angina pectoris.
2. Myocardial infarction.
3. Chronic ischemic heart disease.
4. Sudden cardiac death – Death within 1 hour from cardiac causes.

Pathogenesis:

A. Role of fixed coronary obstruction – 90% cases of IHD have fixed critical (> 75% of cross section) obstruction of at least one major epicardial artery.

B. Role of acute plaque change - Hemorrhage/ rupture of fissuring / erosion or ulceration of plaque triggers thrombosis.

Disrupted plaque is markedly eccentric, with large soft core of necrotic debris and lipid high density of macrophages and a **thin fibrous cap.**

C. Role of coronary thrombosis – Critical role in acute coronary syndromes. In MI- complete stenosis due to thrombosis. In Unstable angina, there is transient mural thrombosis.

D. Role of vasoconstriction – Transient vasospasm is induced at the site of plaque disruption and thrombosis.

Syndrome	Pathology
A. Stable angina	Fixed critical stenosis.
B. Unstable angina	Plaque rupture with mural thrombus, often with thromboemboli, with vasoconstriction leading to decreased coronary flow.
C. MI	Plaque rupture with complete thrombosis.
D. Sudden death	Severe multivessel disease, often plaque rupture, often thrombus or thromboemboli, triggering a fatal arrhythmia.

Sudden cardiac death – Is most commonly due to **IHD.** Ultimate mechanism is development of fatal arrhythmias. Others causes are aortic valve stenosis, abnormalities of conduction system, mitral valve prolapse, myocarditis and dilated or Hypertrophic cardiomyopathies.

Chronic ischemic heart disease – Is used for patients who develop insidious **CHF** as a consequence of IHD. Also called ischemic cardiomyopathy OR atherosclerotic coronary artery and ischemic heart disease with heart failure.

Angina pectoris – is paroxysmal attacks of chest discomfort caused by transient ischemia that falls short of inducing infarction.

Types:

- Stable angina – ischemia induced by increased demand. Pain is relieved by rest ECG shows ST depression. Due to Fixed coronary obstruction
- Variant or Prinzmetal angina – Pain is present at rest. Due to coronary artery spasm Relieved by vasodilators. ECG shows ST elevation.
- Unstable / Crescendo /Preinfarction angina – pain occurs with progressively increasing frequency and less effort. Associated with acute plaque change in a preexisting coronary thrombus. Platelet activation and aggregation are important in its pathogenesis.

Mycoardial Infarction:

Infarcts can be transmural or Subendocardial.

Transmural infarcts involve full thickness of myocardium and are associated with coronary atherosclerosis, plaque rupture/ fissure/ sudden change in morphology with activation of coagulation leading to occlusive thrombosis.

Subendocardial infarcts involve inner 1/3 to ½ of wall. Are associated with diffuse stenosing coronary atherosclerosis with reduction in blood flow. No plaque rupture or thrombosis is seen. **10% Infarcts seen without coronary atherosclerosis** - Due to Vasospasm and platelet aggregation, Emboli from mural thrombus or vegetative endocarditis / Paradoxical emboli, No abnormality seen an angiography in one third of these cases.

Myocardial response to ischemia:

Ischemia is most pronounced in the sub endocardium.

Approximate Time of Onset of Key Events in Ischemic Cardiac Myocytes

Feature	Time
Onset of ATP depletion	Seconds
Loss of contractility	<2 min
ATP reduced	
to 50% of normal	10 min
to 10% of normal	40 min
Irreversible cell injury	20–40 min
Microvascular injury	>1 hr

Evolution of Morphologic Changes in Myocardial Infarction

Time	Gross Features	Light Microscope	Electron Microscope
Reversible Injury			
0–½ hr	None	None	Relaxation of myofibrils; glycogen loss; mitochondrial swelling.
Irreversible Injury.			
½–4 hr	None	Usually none; variable waviness of fibers at border.	Sarcolemmal disruption; mitochondrial amorphous densities.
4–12 hr	Dark mottling (occasional).	Early coagulation necrosis; edema; hemorrhage.	
12–24 hr	Dark mottling.	Ongoing coagulation necrosis; pyknosis of nuclei; myocyte hypereosinophilia; marginal contraction band necrosis; early neutrophilic infiltrate.	
1–3 days	Mottling with yellow-tan infarct center.	Coagulation necrosis, with loss of nuclei and striations; brisk interstitial infiltrate of neutrophils.	
3–7 days	Hyperemic border; central yellow-tan softening.	Beginning disintegration of dead myofibers, with dying neutrophils; early phagocytosis of dead cells by macrophages at infarct border.	
7–10 days	Maximally yellow-tan and soft, with depressed red-tan margins.	Well-developed phagocytosis of dead cells; early formation of fibrovascular granulation tissue at margins.	
10–14	Red-gray depressed	Well-established granulation tissue with new	
2–8 wk	Gray-white scar, progressive from border toward core of infarct.	Increased collagen deposition, with decreased cellularity.	
>2 mo	Scarring complete.	Dense collagenous scar.	

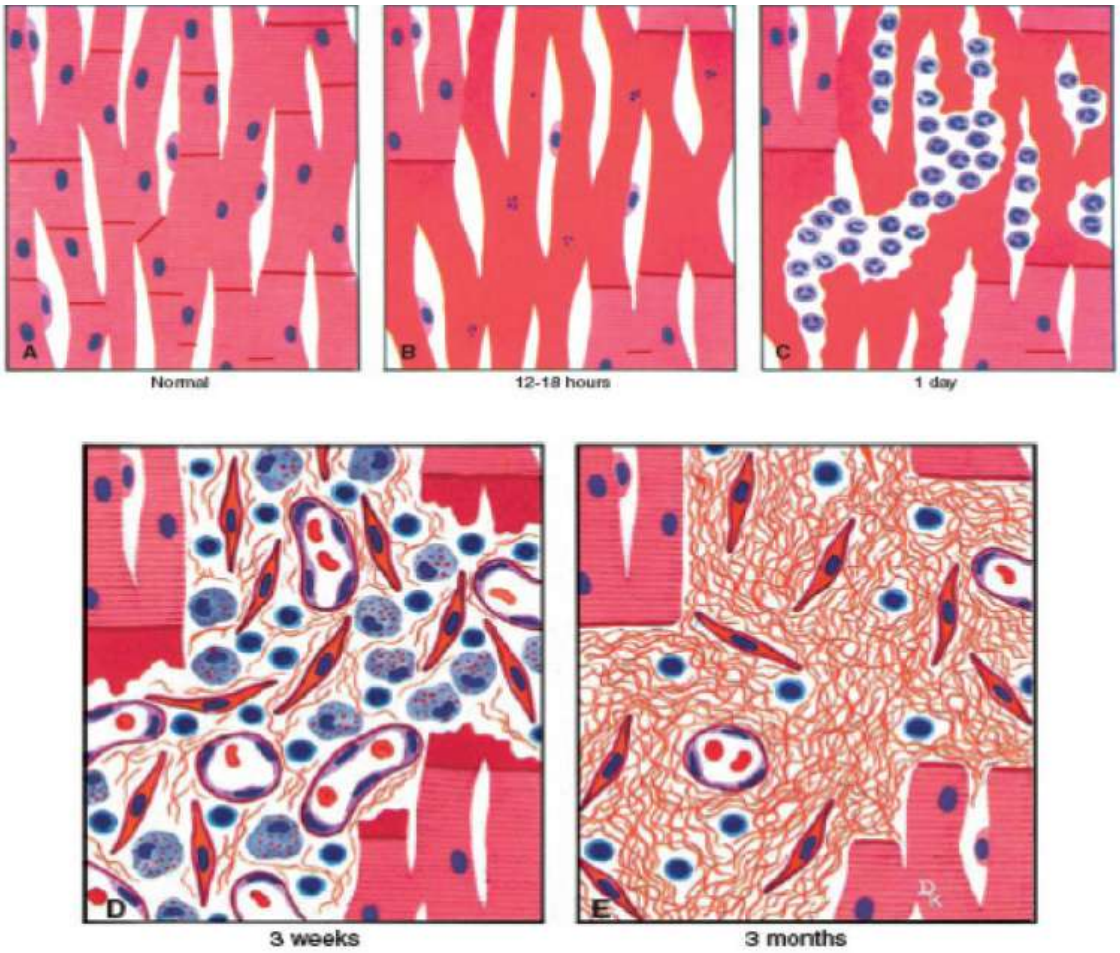


Fig. 8.1: Development of a myocardial infarct. A. Normal myocardium. B. After about 12 to 18 hours, the infarcted myocardium shown eosinophilia (red staining) in section of the heart stained with hematoxylin and eosin. C. About 24 hours after the onset of infarction, polymorphonuclear neutrophils infiltrate necrotic myocytes at the periphery of the infarct. D. After about 3 weeks, peripheral portions of the infarct are composed of granulation tissue with prominent capillaries, fibroblasts, lymphoid cells and macrophages. The necrotic debris has been largely removed from this area, and a small amount of collagen has been laid down. E. after 3 months or more, the infarcted region has been replaced by scar tissue.

Contraction band necrosis represents reperfusion injury. They are intensely eosinophilic transverse bands that traverse the involved myocyte. Due to hyper contraction of myofibrils in the dying cell.

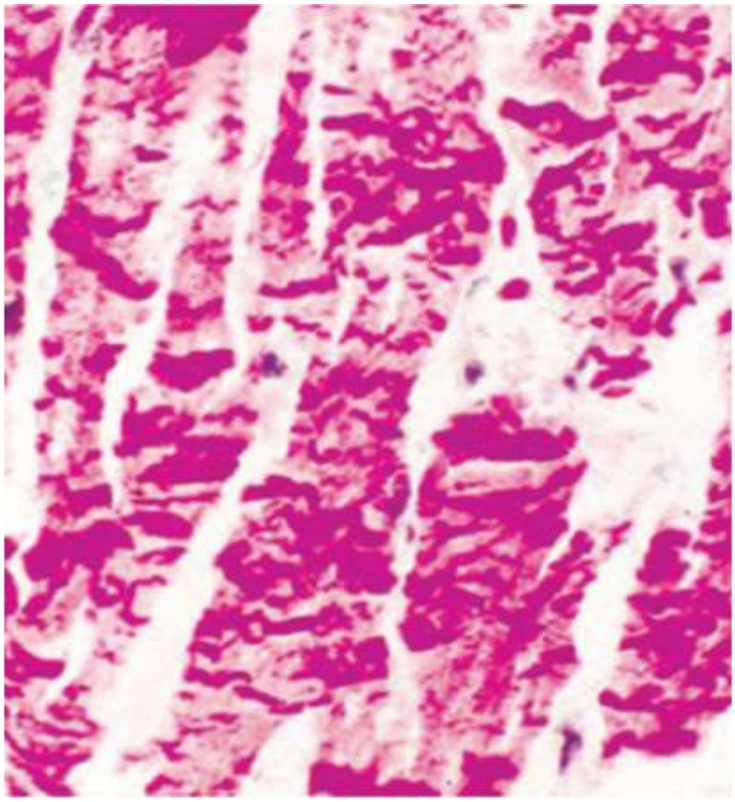


Fig. 8.2: Contraction band necrosis. A section of infarcted myocardium shows prominent thick, wavy, transverse bands in myofibers.

Stunned myocardium is prolonged post ischemic ventricular dysfunction in the reperfused myocardium.

Complications of MI are:

- Sudden cardiac death in 20% patients.
- No complication in 10-20% patients.
- Complications in 80-90% cases which include.
  - Cardiac arrhythmias (Commonest).
  - LVF with pulmonary edema.
  - Cardiogenic shock.
  - Thromboembolism.
  - Cardia rupture syndromes (due to weakening of the necrotic myocardium) which include.
- Rupture of the ventricular free wall with heamopericardium and cardiac tamponade (commonest).
- Rupture of interventricular septum leading to Left to right shunt.

- Papillary muscle rupture leading to severe MR.
  - Fibrinous or fibrinohaemorrhagic pericarditis.
  - Ventricular aneurysm is a late complication mostly following a large anteroseptal transmural infarct.

Pericarditis, cardiac rupture and ventricular aneurysms rarely develop after sub endocardial infarcts.

Hypertensive Heart Disease:

Left sided concentric hypertrophy seen in pressure overload. Wall thickness more than 2 cm and weight more than 500 grams.

Acute cor pulmonale is seen after massive pulmonary embolism.

Chronic cor pulmonale is seen due to prolonged pressure over load in lung / vessel / chest wall diseases.

Rheumatic Heart Disease:

Jones Criteria

2 major or 1 major + 2 minor

Major	Minor
1. Polyarthritis-75%	Arthralgia – Fever
2. Carditis – 35%	Previous history of RF
3. Subcutaneous nodules – 10%	Raised ESR or + CRP
4. Erythema marginatum – 10%	Prolonged PR interval in ECG
5. Chorea -10%	

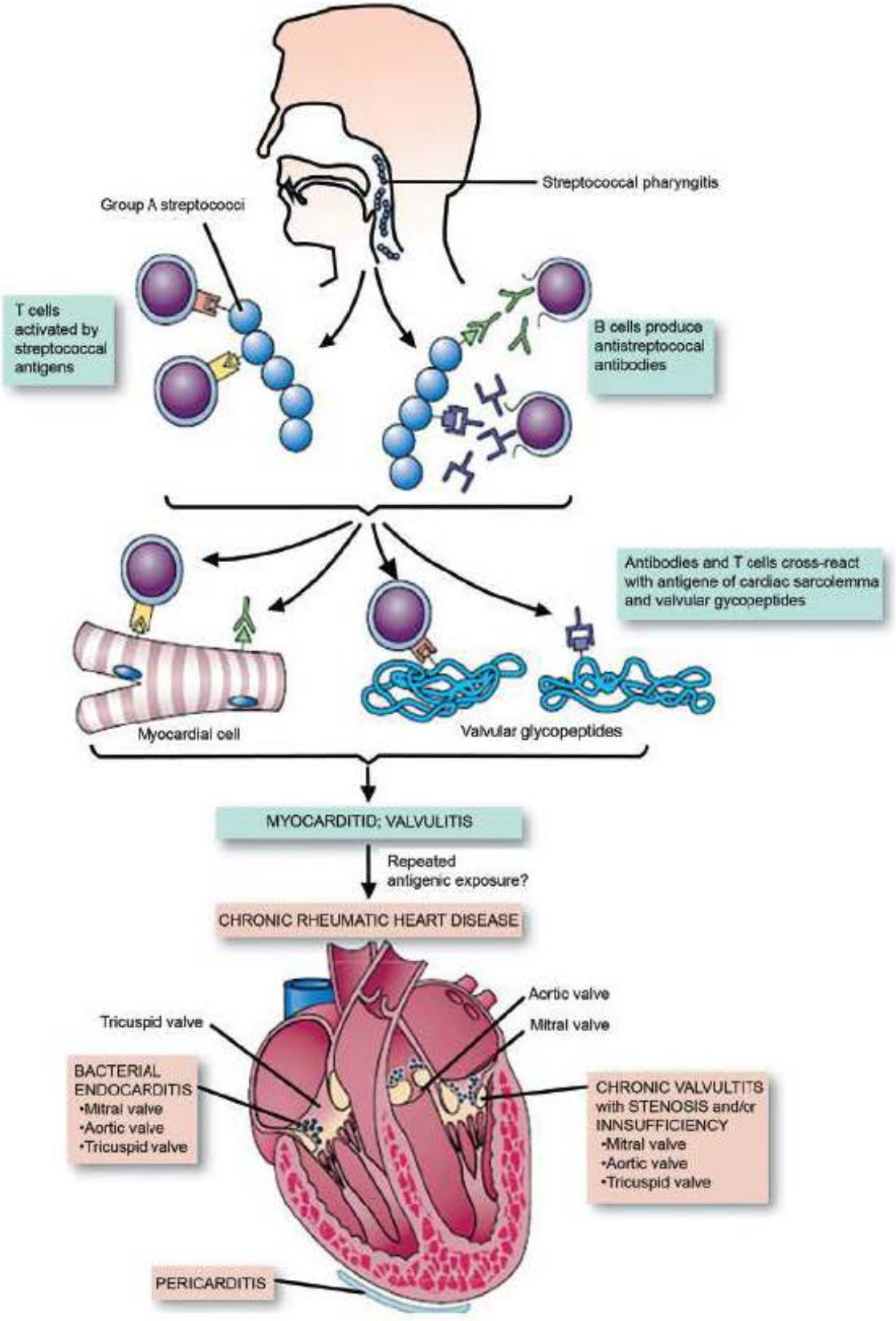

Streptococcal pharyngitis
Group A streptococci
T cells activated by streptococcal antigens
B cells produce antistreptococcal antibodies
Antibodies and T cells cross-react with antigene of cardiac sarcolemma and valvular gycopeptides
Myocardial cell
Valvular glycopeptides
MYOCARDITID; VALVULITIS
Repeated antigenic exposure?
CHRONIC RHEUMATIC HEART DISEASE
Aortic valve
Mitral valve
Tricuspid valve
BACTERIAL ENDOCARDITIS
•Mitral valve
•Aortic valve
•Tricuspid valve
CHRONIC VALVULTITS with STENOSIS and/or INNSUFFICIENCY
•Mitral valve
•Aortic valve
•Tricuspid valve
PERICARDITIS

Fig. 8.3

Rheumatic carditis – Pancarditis.

- **Endocarditis** – Mitral valve above 75%; Mitral + Aortic – 25%.
- Vegetations along line of closure, which are verrucous, sterile and small.
  - **Mc callum's patch - commonest in post wall of left atria.**
  - MR, Chronic – fish mouth / button hole stenosis – MS.
  - **Myocarditis** – Aschoff bodies are diagnostic – non caseating fibrinoid necrosis surrounded by Anitschkow myoocytes (caterpillar cells), Aschoff's giant cells, mononuclear cells + fibroblasts.
  - **Pericarditis** – Fibrinous; Bread + Butter appearance.

In Acute Rheumatic fever, Myocarditis is most dangerous.

In chronic RHD, Endocardial and valvular involvement dominate.

Infective Endocarditis:

May be Bacterial, Fungal, Rickettsia or Chlamydial.

Predisposing factors are preexisting cardiac abnormalities like congenital HD< Damaged valves, neutropenia, immunodeficiency, indwelling vascular catheters, diabetes mellitus, alcohol, intravenous drug abuse.

Organisms - Strept viridans, Staph aureus (commonest cause of acute endocarditis and endocarditis in drug abusers), Strept pneumoniae, gram negative rods, fungi.

Acute Bacterial endocarditis occurs in normal valves due to high virulence organisms and has a high mortality.

Sub – acute Bacterial endocarditis is due to organisms of low virulence affecting previously damaged valves and is a more treatable condition.

Vegetations are bulky, friable, and destructive and composed of fibrin, inflammatory cells and microorganisms.

Complications of IE include.

1. Cardiac.

2. Embolic.

3. Renal.

Cardiac include.

- Valvular insufficiency / stenosis and failure.
- Myocardial ring abscess, perforation of aorta / heart.
- Suppurative pericarditis.
- Partial dehiscence of artificial walls.

Embolic phenomena result in abscess / infarcts.

- To brain, kidney and spleen in left sided lesions.
- To lungs in right sided lesions.

Renal complications include.

- Focal Glomerulonephritis.
- Diffuse glomerulonephritis.
- Multiple abscesses.
- Embolic infarction.

Non bacterial thrombotic endocarditis

Marantic endocarditis, occurs in debilitated patients.

Vegetations are small sterile masses of fibrin and blood elements on normal valves. No organisms seen, lesions are non destructive.

Associated with hypercoagulable states, mucinous adenocarcinomas of pancreas. GIT and ovary, Indwelling Catheters.

Libman Sach's endocarditis

Associated with SLE.

Vegetations are small sterile 1-4 mm on either side of valve leaflets, most frequent in the undersurface of AV valve.

Associated with vasculitis. Haematoxylin bodies may be seen.

Myocardial Disease:

Divided into Cardiomyopathies and Specific heart muscle diseases.

Cardiomyopathies:

Cardiomyopathy and Indirect Myocardial Dysfunction: Functional Patterns and Causes:

Functional Pattern	Left Ventricular Ejection Fraction[*]	Mechanisms of Heart Failure.	Causes of Phenotype.	Indirect Myocardial Dysfunction (Mimicking Cardiomyopathy).
Dilated	<40%	Impairment of contractility (systolic dysfunction).	Genetic; alcohol; peripartum; myocarditis; hemochromatosis; chronic anemia; doxorubicin (Adriamycin); sarcoidosis; idiopathic.	Ischemic heart disease; valvular heart disease; hypertensive heart disease; congenital heart disease.
Hypertrophic	50% to 80%	Impairment of compliance (diastolic dysfunction).	Genetic; Friedreich ataxia; storage diseases; infants of diabetic mother.	Hypertensive heart disease; aortic stenosis.
Restrictive	45% to 90%	Impairment of compliance (diastolic dysfunction).	Amyloidosis; radiation-induced fibrosis; idiopathic.	Pericardial constriction.

DCM:

- Seen with infective myocarditis, Haemochromatosis, Chronic anemia, Alcoholism, Adriamycin toxicity, Sarcoidosis.
- Systolic failure.
- Hear is enlarged 2-3 times and is flabby. Chambers are dilated. Thickness of left ventricle is <, = or > normal.

HCM:

- Associated with Friedrich’s Ataxia. Glycogen storage disease, Infants of diabetic mothers, Hypertensive heart disease.
- Diastolic failure.

- Heavy, muscular hyper contracting heart. Commonest site is sub aortic followed by mid ventricular. Histology shows myofiber disarray.

RCM:

- Seen in amyloidosis, radiation associated fibrosis.
- Diastolic failure due to impaired diastolic relaxation.
- Normal sized ventricles. Patchy or diffuse interstitial fibrosis

Specific Heart Muscle Disease:

Myocarditis:

Inflammation of the heart muscle with leukocyte infiltration and non ischemic necrosis of myositis. Leads to heart failure with fever and sudden appearance of ECG changes.

Commonest cause is viral (Coxsackie B virus):

Causes of Myocarditis
Idiopathic
Infectious
• Viral: Coxsackievirus,, adenovirus, echovirus, influenza virus, human immunodeficiency virus and many others.
Rickettsia: Typhus, Rocky Mountain spotted fever.
Bacterial: Diphtheria, staphylococcal, streptococcal, meningococcal, Borrelia (Lyme disease) and leptospiral infection.
Fungi and protozoan parasites: Chagas disease, toxoplasmosis, aspergillosis, cryptococcal and candidal infection.
Metazoan parasites: Echinococcus, Trichina.
Noninfectious.
• Hypersensitivity and immunologically related diseases: Rheumatic fever, systemic lupus erythematosus, scleroderma, drug reaction (e.g.to penicillin or sulfonamide) and rheumatoid arthritis.
• Radiation.
• Miscellaneous: Sarcoidosis, uremia.

Pericardial Disease:

Pericarditis is inflammation of the pericardium. Causes are

- Infections – Virus, Pyogenic bacteria, TB, Fungi, Bacteria.
- Immunologic – Rheumatic fever, SLE, Scleroderma, Post cardiotomy, Dressler's syndrome, Drug hypersensitivity.
- Miscellaneous – Post MI, Uraemia, Post surgical, Neoplastic, Traumatic, Post irradiation.
- Types are
- Acute.
    - Serous – Non infectious inflammation, RF, SLE, Scleroderma, Tumours, / Uraemia, Viral.
    - Serofibrinous / Fibrinous – MOST FREQUENT FORM. Acute MI, Post MI, Post Surgical and later stage of Serous.
    - Purulent – Infective organisms.
    - Haemorrhagic – MOST COMMONLY Tubercular. Other causes are Neoplasia or Post-surgical.
    - Caseous – Tubercular or mycobacterial infections.
    - Healed – Follows suppurative, caseous, surgery and irradiation.
    - Adhesive pericarditis and mediastinopericarditis. Thin strands of fibrosis obliterating pericardial sac and adhering it to the mediastinum. DCM like heart.
    - Constrictive pericarditis – Encased in a dense fibrous calcific scar which limits diastolic expansion. Quiet heart with reduced output.
    - Most common heart finding in Rheumatoid arthritis is fibrinous pericarditis.

Tumours:

- Myxoma- Commonest, In Left atrium (Fossa ovalis), Obstructs out flow in a ball valve fashion
- Lipoma – Most often in left ventricle
- Papillary fibroelastoma- Incidental finding on Valves and Atrial surfaces of AV valves.
- Rhabdomyoma – Most frequent in infants. Contain Spider cells. Increased frequency in Tuberous sclerosis.

- Secondary – Most common from Lung, Breast, melanoma, Leukemias, Lymphomas.

Most Common Tumor of Heart: Secondaries.

Most Common Heart Tumor of Infants: Rhabdomyoma.

Most Common Heart Tumor of Adults: Myxoma.

Selected Examples of Gene Defects Associated With Congenital Heart Disease*:

Disorder	Gene(s)	Gene Product Function
Nonsyndromlc		
ASD or conduction defects	NKX2.5	Transcription factor
ASD or VSD	GATA4	Transcript on factor
Tetralogy of Fallot	ZFPM2 or N/012.5	Transcription factors
Syndromic[1]		
Alagille syndrome – pulmonary artery stenosis or tetralogy of Fallot	JAG1 or N0TCH2	Signaling proteins or receptors
Char syndrome – PDA	TFAP2B	Transcription factor
CHARGE syndrome – ASD, VSD, PDA, or hypoplastic right side of the heart	GHD7	Helicase-bindlng protein
DIGeorge syndrorne ASD, VSD, or outflow tract obstruction	TBX1	Transcription factor
Holt-Oram syndrome – ASD, VSD, or conduction defect	TBX5	Transcript on factor
Noonan syndrome pulmonary, valve stenosis vsd, or hypertrophic cardiomyopathy	PTPN11 KRAS, S0S1	Signaling proteins

9 Hepatobiliary System

Liver & Gall Bladder:

Weight: 1400-1600gm 2.5% of Body weights.

Incoming blood.

- Portal vein (60-70%).
- Hepatic artery (30%-40%).

Outgoing- Hepatic vein → IVC.

Microanatomy:

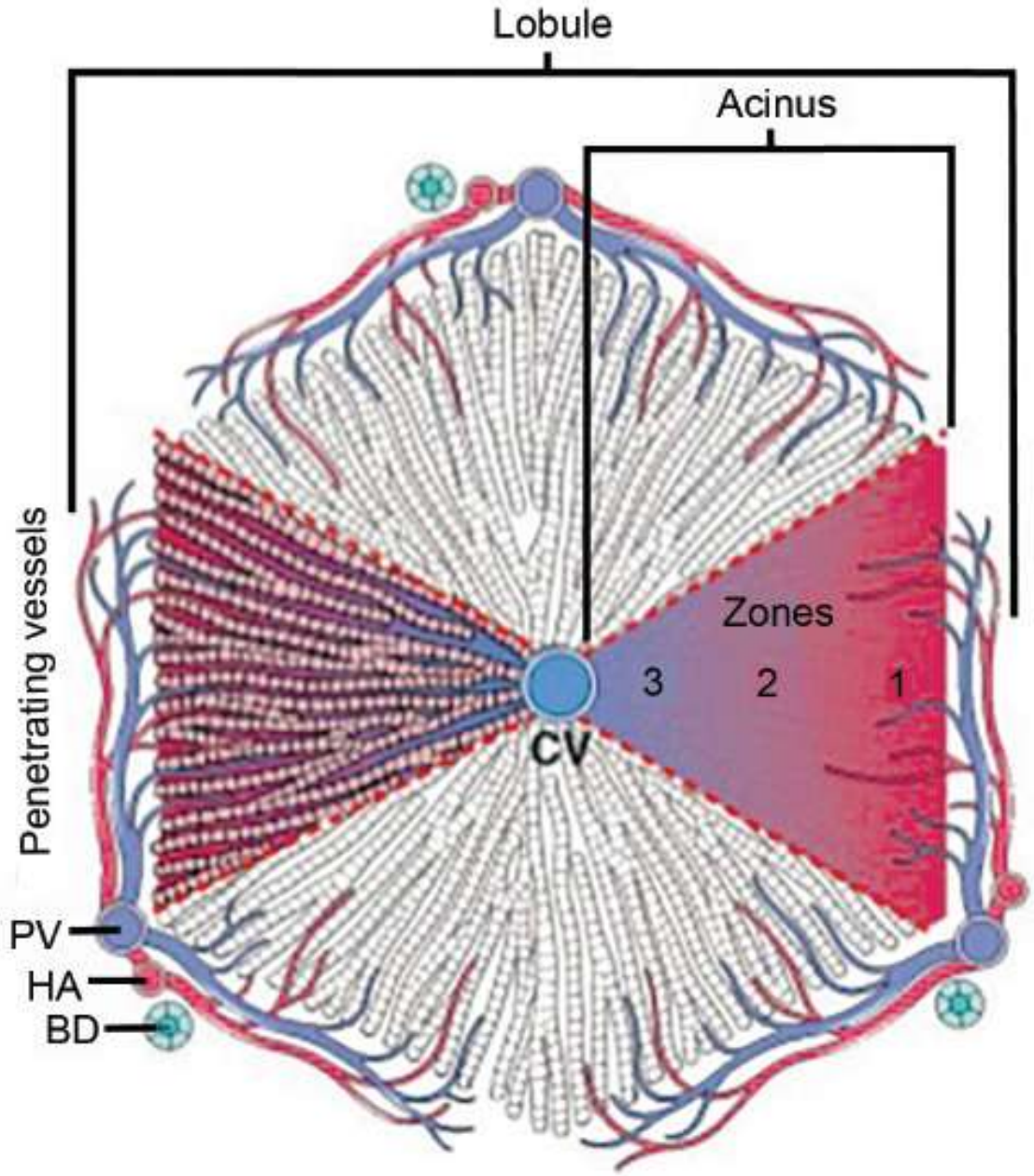


Fig. 9.1

Microscopic anatomy of the liver; the two models, hepatic lobular model and acinar model, are illustrated. In the lobular model the terminal hepatic vein (CV) is at the center of a "lobule," while the portal tracts (PV) are at the periphery. Pathologists refer to the regions of the parenchyma as "periportal and centrilobular." In the acinar model, on the basis of blood flow, three zones can be

defined, zone 1 being the closest to the blood supply and zone 3 being the farthest. BD, bile duct; HA, hepatic artery.

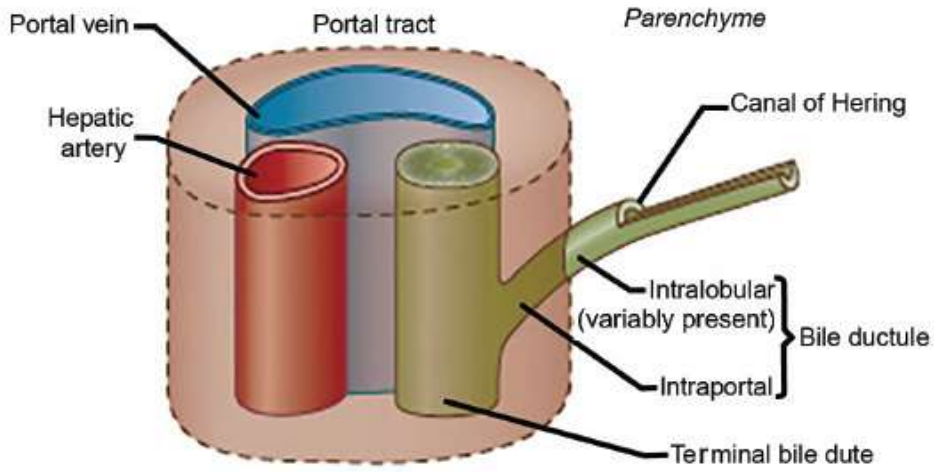


Fig. 9.2: Schematic diagram of relationship between bile ducts, ductules and canals of Hering.

Fatty Liver:

Causes of fatly changes in liver.

- DM.
- Alcoholism (Most Common).
- Anoxia.
- Toxins- CCI_4

Drugs: Corticosteroids, salicylates tetracyclines, Na Valproate.

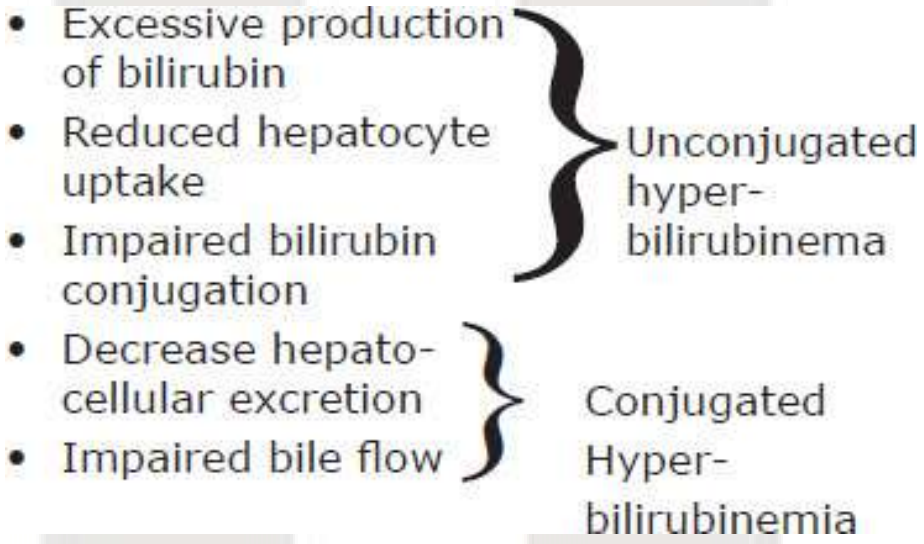
- Starvation.
- Obesity.
- Chronic illness.
- Acute fatly liver of pregnancy.
- Reyes syndrome.
- chronic HCV infection.

$CC1_4$ & Protein malnutrition: ↓ synthesis of Apo protein, drugs: Corticosteroids salicylates tetracyclines Na Valproate ↓ FA oxidation.

Alcohol- shunting of (n) substances away from catabolism & towards lipid biosynthesis (because of ↑ NADH).

- Impaired assembly & secretion of Lipoproteins due to Apo protein deficiency.
- Increased peripheral catabolism of fat.

Jaundice: Elevated serum bilirubin (>2mg/dl)



Causes of Jaundice:

1. Predominantly Unconjugated Hyperbilirubinemia.

Excess production of bilirubin.

- Hemolytic anemias.
- Resorption of blood from internal hemorrhage (e.g. alimentary tract bleeding, hematomas).
- Ineffective erythropoiesis syndromes (e.g. pernicious anemia, thalassemia).

Reduced hepatic uptake:

- Drug interference with membrane carrier systems eg. rifampicin.
- Some cases of Gilbert syndrome.

Impaired bilirubin conjugation:

- Physiologic jaundice of the newborn (decreased UGT1A1 activity, decreased excretion.
- Breast milk jaundice (β- glucouronidases in milk).
- Genetic deficiency of UGT1A1 activity (Criggler Najar syndrome types I and II).
- Gilbert syndrome (mixed etiologies).
- Diffuse hepatocellular disease (e.g. viral or drug- induced hepatitis, cirrhosis).

2. Predominantly conjugated Hyperbilirubinemia:

- Deficiency of canalicular membrane transporters (Dubin-Johnson syndrome, Rotor syndrome).
- Impaired bile flow – stone, stricture, carcinoma (surgical jaundice).

3. Neonatal Jaundice (physiological jaundice of newborn).

- Transient.
- Conjugating /excretory mechanism matures at 2 wks. of age.
- Breast milk contains β glucuronidase, which deconjugates bilirubin in intestine.

4. Hereditary hyperbilirubinaemias

Unconjugated Hyperbilirubinemia:

a. Criggler Najar syndrome Type I.

- AR.
- Absent UGT1A1 activity.
- Fatal (within 19 months of birth).
- (N) Liver morphologically.

b. Criggler Najjar syndrome Type II.

- AD.
- ↓ UGIA1 – less severe, non-fatal, activity (↓).

- Liver (N).

c. Gilbert's syndrome? AD.

- Benign; mild fluctuating hyper bilirubinemia.
- ↓ UGTA1 activity (to 30% of (n).
- Liver – (n) - typically detected in adolescent / adult life in association with stress, strenuous exercise.
- 6% of population.

Conjugated hyper bilirubinemia:

a. Dubin Johnson syndrome:

Impaired excretion of bilirubin glucoronides

(canalicular membrane carrier protein MRP2 absent).

- Liver: Darkly pigmented (coarse pigmented granules within cytoplasm of hepatocytes).
- Electron microscopy? Epinephrine metabolite in lysosomes.
- Asymptomatic / chronic & recurrent jaundice.
- AR.

b. Rotor syndrome:

- AR.
- Multiple defects in hepatic uptake and excretion of bilirubin.
- Liver (N).
- Jaundice, Asymptomatic.

Cholestasis: Causes are:

- Hepatocellular dysfunction.
- Intrahepatic biliary obstruction.
- Extrahepatic biliary obstruction.

C/F:

1. (Pruritus, skin xanthomas.

2. ↑ S alkaline phosphates ↑ Gamma- glutamyltranspeptidase, ↑ 5' nucleotidase).

3. ↓ bile flow ⇒ malabsorption.

Familial intrahepatic cholestasis

2 groups of disorders:

Group I- Disorders with ↓ GGT.

- ↓ Bile salts and bile acids, ↓ cholesterol and ↓ phosphatidylcholine secretion in bile.
- ↑ S. bile acids pruritus. ↑ S. Cholesterol. ↓ Gamma- glutomyl transpeptidase (GGT).

a. Benign recurrent intrahepatic cholestasis

- Intermittent attack of cholestasis over life.
- No progression to chronic liver disease.

b. Progressive familial intra hepatic cholestasis 1 (PFIC-1).

- Cholestasis in infancy.
- Liver failure by adult hood.
- Also c.a byler syndrome (family members affected) or byler disease (unrelated individuals).
- Mutation in ATP8 B1 gene on chromosome 18q21 encodes canalicular P-type ATPase.

c. Progressive familial intra hepatic cholestasis 2 (PFIC-2)0

- Cirrhosis by 1st decade of life.
- Mutation in canalicular bile salt export pump (BSEP) encoded by ABCB11 gene on chromosome 2q24.

Group II - ↑ SGGT levels.

- Progressive familial intrahepatic cholestasis 3 (PIFC-3).
- Mutation in ABC B4 gene on chromosome 7q21.

- Encodes MDR3- canalicular transport protein responsible for flipping phosphatidyl choline from internal to external leaflet of canalicular membrane.
- No phosphatidylcholine in bile.

H/P: Bile in hepatocytes, canaliculi, kupffer cells.

- Feathery/ foamy degeneration (wispy appearance).
- Parenchymal destruction ⇒Bile lakes obstruction ⇒ Bile stasis ↑ back pressure.
- Proliferation of duct epithelial cell ⇒ looping and reduplication ⇒ bile lakes.
- PT fibrosis ⇒ Biliary Cirrhosis. (Bile stained cirrhotic liver).

Extrahepatic cholestasis curable by surgery, intrahepatic cholestasis requires liver transplant.

Hepatic Failure:

- 80% -90% of hepatic functional capacity loss.

Causes:

a. Massive hepatic necrosis: Fulminant viral hepatitis, drugs and chemicals (acetaminophen, halothane, ATT (Rifampicin, INH) MAO inhibitors, CCI_4 , Amantia phalloides).

b. Chronic Liver disease – chronic hepatitis, cirrhosis.

c. Hepatic dysfunction without overt necrosis: Reye syndrome, tetracylcline toxicity, acute fatty liver of pregnancy.

Clinical features:

- Jaundice.
- Hypoalbuminemia leads to peripheral edema.
- Fetor hepaticus- musty body odour due to mercaptans.
- Palmar Erythema.
- Hypogonadism - impaired metabolism of estrogen.
- Gynaecomastia.

- Portal H.T.

Life threatening complications.

- Multiple organ failure esp. lung and kidney.
- Coagulopathy due to impaired hepatic synthesis of factors II, VII, IX & X resulting in bleeding tendency.
- Hepatic encephalopathy: EEG- n, specific, Asterixis- characteristic.
- Cause- disorder of neurotransmission in the CNS and neuromuscular system due to ↑ levels of ammonia that impairs neuronal function and promotes brain edema.
- Hepatorenal Syndrome.
  Renal failure in severe liver disease with no cause for renal failure.
- Hepatocellular carcinoma:

Cirrhosis:

Three characteristics-

- Bridging fibrous septae.
- Parenchymal Nodules.
  < 3mm- Micronodular.
  > 3 mm- Macronodular.
- **Disruption of architecture ⇒** Abnormal vascular interconnection.
- Diffuse.
- Irreversible.

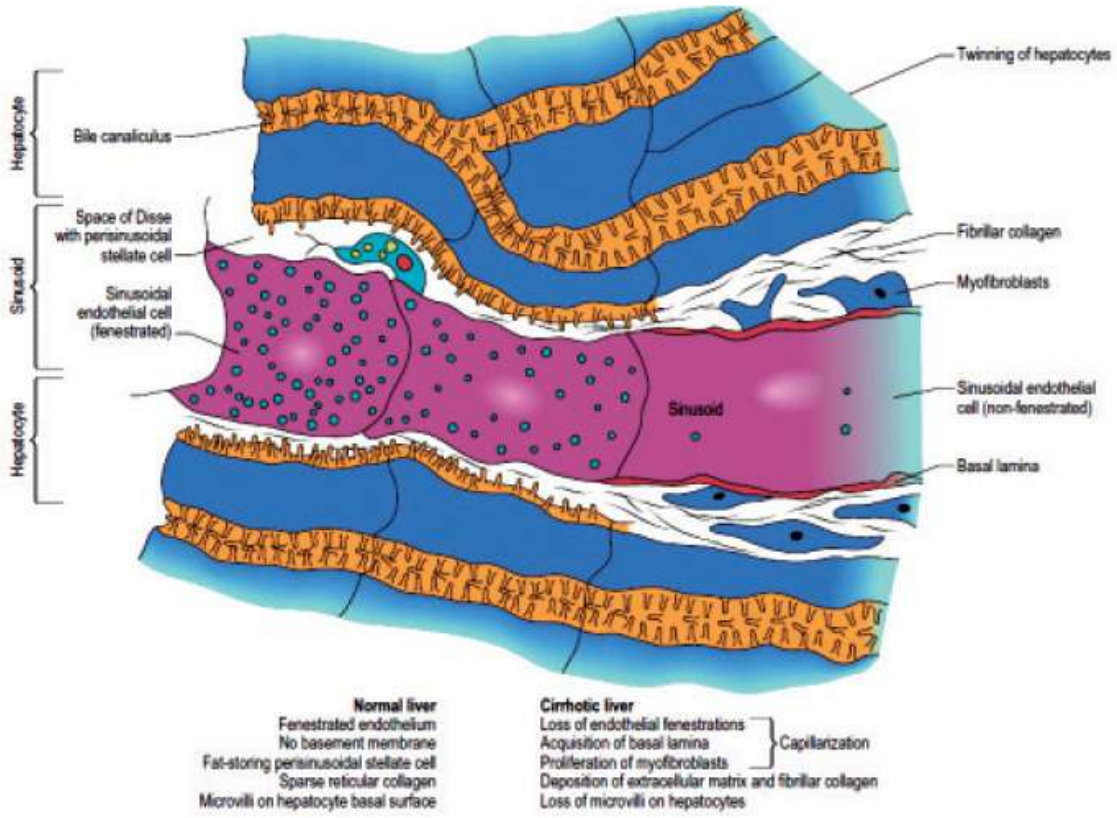


Fig. 9.3

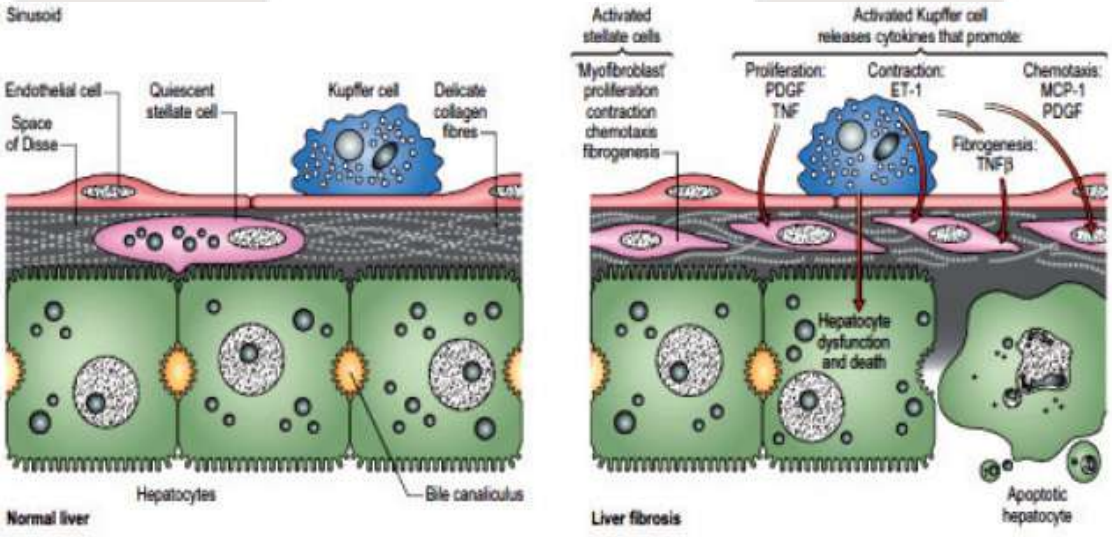


Fig. 9.4

Regression in fibrosis is seen in:

• Schistosomiasis • Hemochromatosis	rare case

Causes:

1. Alcoholic liver disease- 60-70%.
2. Viral Hepatitis 10%.
3. Biliary Cirrhosis 5-10%.
4. Primary Hemochromatosis rare.
5. Wilson Diseased Rare.
6. α-1 AT def Rare.
7. Drug induced α methyl dopa.
8. Cryptogenic 10-15%.

Normally:

Type I & III (interstitial collagen – PT and around CVs.

Type IV – in space of Disse (alongside hepatocytes).

Cirrhosis – Type I and III collagen in lobules.

- Loss of (n) fenestrations in sinusoidal endothelial cells due to deposition of collagen in the space of Disse.
  Source of collagen: Perisinusoidal hepatic stellate cells.
- Transformed into ‘myofibroblast like cells.

Cause of death:

- Progressive Liver failure.
- Portal hypertension.
- Hepato cellular carcinoma.

Infectious Disorders of Liver:

VIRAL HEPATITIS – caused by viruses having special affinity for liver; other viruses (hepatinophic) like Epstein Barr virus.

Cytomegalovirus (newborn/ immuno suppressed) and yellow fever virus can also infect the liver.

Cell type	Comment
Antigen-presenting cells Kupffer cells (KG) Dendritic cells (DC)	When activated, secrete TNF-n, IL-2: IL-12 and leukotriene B3 Express toll-like receptors (TLR); secrete IL-12, TIMF-m, IFN-m, and IL-10.
Innate immune system Natural killer cells (MK) Natural killer T cells (NKT)	'Pit cells'; can be Th-1* or Th-2**
Adaptive immune system B lymphocytes T lymphocytes CD4+ T cells CD4+ T helper cells CD4+ CD25+ T cells (T-regs) CD8+ T cells Cytotoxic T Cells (CTL)	Secrete immunoglobulin, generate plasma cells Multiple subsets Can be Th-1* or Th-2** Regulate activation of CD4+ and CD8+ T cells Activity is enhanced by Th-1 cytokines; car be cytolytic or non-cytolytic.
*Th-1: proinflammatory, IFN-y and IL-2 secreting **Th-2: anti-inflammatory, IL-4 and IL-10 secreting	

Viral agents	Characteristics
Hepatitis A fHAV)	RIMA picornavirus Sporadic or epidemic occurrence with faecal-oral transmission, resulting in acute disease only.
Hepatitis B fl-EV)	DNA hepadnavirus Sporadic or endemic occurrence through sexual, perinatal and parenteral transmission Chronic disease persists in 5% of adults and in up to 90% of infants Chronic infection is associated with hepatocellular carcinoma.
Hepatitis C (HCV)	RIMA flavi-like virus Sporadic occurrence with parenteral transmission Perinatal and sexual spread is less common Chronic disease develops in 60-30% of persons infected and cirrhosis is associated with hepatocellular carcinoma.
Hepatitis D (HDV)	RIMA defective virus Sporadic or endemic disease occurs as coinfection with HBV Transmission is parenteral and sexual Chronic disease is seen in patients with chronic HBV HDV worsens the clinical severity of HBV infection.
Hepatitis E (HEV)	RIMA virus Sporadic or epidemic occurrence Transmission is faecal-oral, resulting in acute disease Mortality rate is 25% in pregnant women.

1. Hepatitis A Virus:

- Infections hepatitis, benign, self- limited disease.
  - IP: 2-6 weeks.
  - Chronic hepatitis – None.
  - Carrier state – None.
  - Fulminant hepatitis – Rarely.
  - Fatality: 0.1%.
- Worldwide, bad hygiene.
- Accounts for 25% of clinically evident cases of acute hepatitis world wide.
  - Unenveloped, SSRNA virus (Picorna virus) → Hepatovirus.
- Icosachedral capsid: 27mm in diameter
  Spread: Feco-oral Route. In developed countries raw or steamed shell fish may spread the infection
  Present in stools: 2-3 weeks before and 1 week after onset of

jaundice
Transient Viremia.

- Serodiagnosis- IgM antibody appears in blood with onset of symptoms – marker of acute infection.
  IgG antibody – lifelong immunity.

2. Hepatitis B Virus:

Serum Hepatitis.

- Acute hepatitis.
- Non –progressive chronic hepatitis → cirrhosis.
- Fulminant hepatitis.
- Carrier state.
- Back drop for HDV infection.
- HCC.

IP 30-180 days (1-6 months).

- HBV remains in blood: last stage of IP and Active disease (Acute and chronic).
- Present in all physiologic and pathologic body fluids, except in stools (unlike HAV).

MOI: Blood products, needle sticks etc. (30%).

- Sexual transmission.
- Vertical → Carrier state.
- Belongs to Hepadnaviridae family.
- Spherical, double layered ‘Dane particle’ 42 mm in size.
- Core of virus contains double stranded DNA and enzyme DNA polymerase.

Genome:

HBc Ag: Nucleo capsid core protein.

HBe Ag: Both precore and core region.

HBs Ag: Envelope glycoproteins Synthesized and secreted by infected hepatocytes.

DNA polymerase that exhibits reverse transcriptase activity.

HBX: Protein form X region:

- Transcriptional transactivation of viral genes and host gene promoter.
- Role in HCC.

→ **Phase of Infections:**

Proliferative Phase: HBV DNA in episomal form.

Formation of complete virion with associated antigens.

MHC class I → CD8+ T cells activation → Infected hepatocyte destruction.

Integrative phase:

- Viral DNA incorporated into host DNA. Occurs in hepatocytes not destroyed by immune response.
- With cessation of viral replication within hepatocytes and appearance of antibodies, infectivity ends and liver damage subsides.

Serological Diagnosis:

HBs Ag:

- Appears before onset of symptoms.
- Peaks during overt disease.
- ↓ To undetected levels in 3-6 months.

HBe Ag, HBV DNA, DNA polymerase appear:

- After HBs Ag.
- Signify active viral replication.

IgM Anti HBC:

- Appear shortly before onset of symptoms
- Concurrent with onset of ↑ S. aminotransferases.
- Marker of window period.
- IgM → IgG.

Anti HBe:

- Shortly after disappearance of HBe Ag (i.e. acute infection has peaked and disease is on its wane).

Anti Hbs:

- Doesn't rise till acute disease is over.
- Not detectable for few weeks to several months after disappearance of HBs Ag (window period).
- Persists for life, conferring protection.

CARRIER STATE: HBs Ag>6 months (doesn't necessarily indicate replication)

Chronic replication.

- Persistence of circulating HBs Ag, HBV DNA usually with ant HBc and occasionally with anti Hbs.
- Progressive liver damage can occur.

3. Hepatitis C Virus:

- MOI: Inoculation and Blood transfusion
  - Sexual and vertical transmission → Infrequent causes: (risk of perinatal transmission is much lower with hepatitis C-6% births to infected mother than with hepatitis B-20-60% of births to infected mothers)
- Most important Cause of transfusion associated hepatitis.
- Acute HCV infection is generally undetected clinically.

- In contrast HBV, chronic disease occurs in majority of infected individuals and cirrhosis develops in 20% patients.
- Leading infectious cause of chronic Liver disease world wide.

Virus: Flaviviridae:

- SSRNA.
- Unstable ⇒ Types and sub types.

Difficulty in vaccine development.

- ↑ IgG. Anti HCV → No effective immunity.
- Cirrhosis – in 5-10 years.

Serology:

IP: 2-26 weeks.

HCV RNA: present in blood for 1-3 weeks (with ↑ S. transaminase).

IgM Anti HCV → IgG Anti HCV.

Chronic infection: Episodic elevations in S. transminase with intervening (n) period.

HCV RNA persists in blood.

4. Hepatitis D Viurs:

- Delta agent 35 nm SSRNA virus.
- Replication defective.
- Infection when encapsulated by HBs Ag
  1. Co-infection.
- Acute C- infection:
- Simultaneous exposure to HBV and HOV.
- HBV- must establish first.
  - Recovery – 90%.
  - Fulminant Hepatitis:3-4%.
  - Chronic Hepatitis: Rare.

1. Super infection: In chronic carriers of HBV followed by, HDV infection.

Disease after 3-50d.

Acute disease → Recovery: 10%-15%

Fulminant hepatitis → 7% - 10%

Chronic HBV/HDV hepatitis → 80%

Serology:

HDVRNA+ in blood and liver just before and in early days of acute symptomatic disease IgM Anti HDV- most reliable marker (late and short lived)

5. Hepatitis E Virus:

- Enterically transmitted.
- Sporadic infection.
  Young – middle age adults.
  (Rare in children).
- Accounts for over 50% of cases of acute hepatitis in India.
- Pregnancy →high mortality (20%).
- Self-limited (not associated with chronic disease).

IP: 2-8 weeks: not associated with persistent viremia & chronic liver disease

Virus: SSRNA, Calciviridase unenveloped

Serology: HEV RNA & Virions + in stool & liver- before onset of clinical illness.

- ↑ S. transaminases.
- IgM anti HEV → IgG (in 2-4 wks).

6. Hepatitis G Virus:

- SSRNA.
- Flaviviridae.

MOI: Parenteral- Contaminated blood/ blood products.

Possibly sexual.

- Prevalence of HGV RNA in blood donors 1-4%.
- In up to 75% of infections, HGV cleared from plasma, in remainder, infection becomes chronic.
- Site of HBV replication is mononuclear cells.
- No rise in S. amino transferases. Non- pathogenic.
- Co- infects patients with HIV, dual infection protective against HIV disease

Clinico Pathologic Syndromes:

A. Asymptomatic infections with recovery:

- ↑ S. transminase/presence of antiviral antibodies.

B Acute viral Hepatitis: 4 phases.

Phase:

I. IP (Incubation period).

II. Symptomatic pre icteric phase. Non- specific, constitutional symptoms 10% have serum sickness like picture.

III. Symptomatic icteric phase- Usual in adults with acute HAV (not children). Absent in about half cases of HBV and in majority of cases of HCV. Jaundice is predominantly conjugated hyperbilirubinemia.

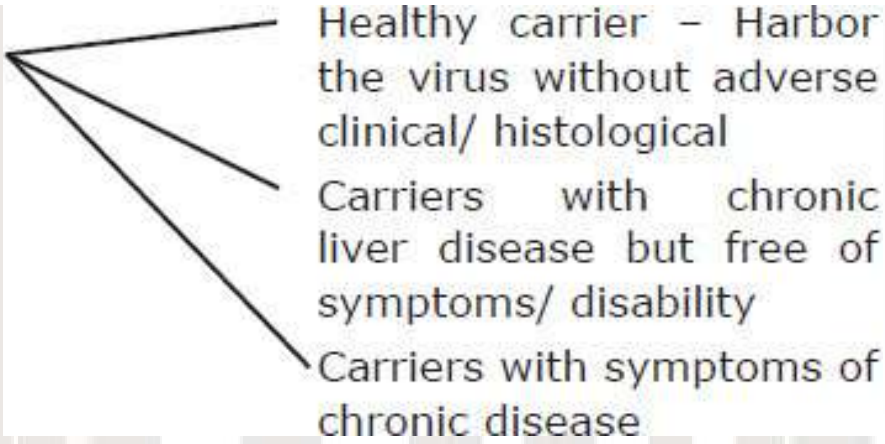
IV. Convalescence.

Peak infectivity: Last days of IP & early days of acute infection.

C. Chronic Viral Hepatitis: Symptomatic, Biochemical or serologic evidence of continuing or relapsing disease for > 6 months with histological documentation of inflammation/necrosis.

Etiology most important indicator of likelihood at progress to cirrhosis.

CARRIER STATE- Chronic hepatitis constitutes a “Carrier State”.



Early infection (Particularly vertical) → 90-95%

Adult infection → 1-10%

Old classification:

- Chronic Persistent Hepatitis (CPH).
- Chronic Active Hepatitis (CAH).
- Chronic Lobular Hepatitis (CLH).

CPH: In. in PT, no piecemeal necrosis.

CLH: Within Lobules.

Newer classification:

- Etiology.
- Grading.
- Staging.

Morphology:

HBV infection.

Ground glass hepatocytes → HbsAG in the form of spheres & tubules in cytoplasm.

Sanded nuclei → HBc Ag in nucleus.

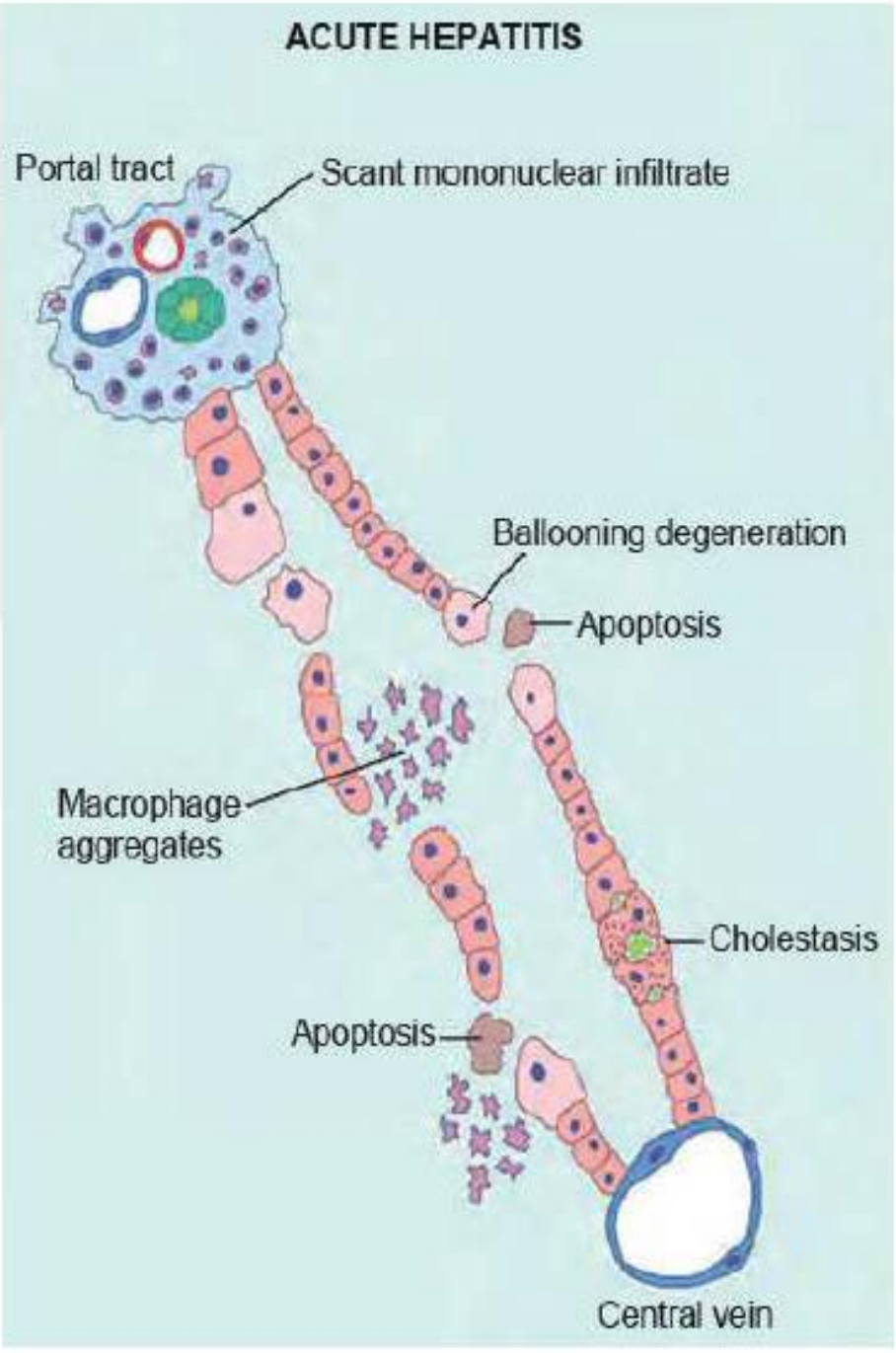

ACUTE HEPATITIS
Portal tract
Scant mononuclear infiltrate
Ballooning degeneration
Apoptosis
Macrophage aggregates
Cholestasis
Apoptosis
Central vein

Fig. 9.5

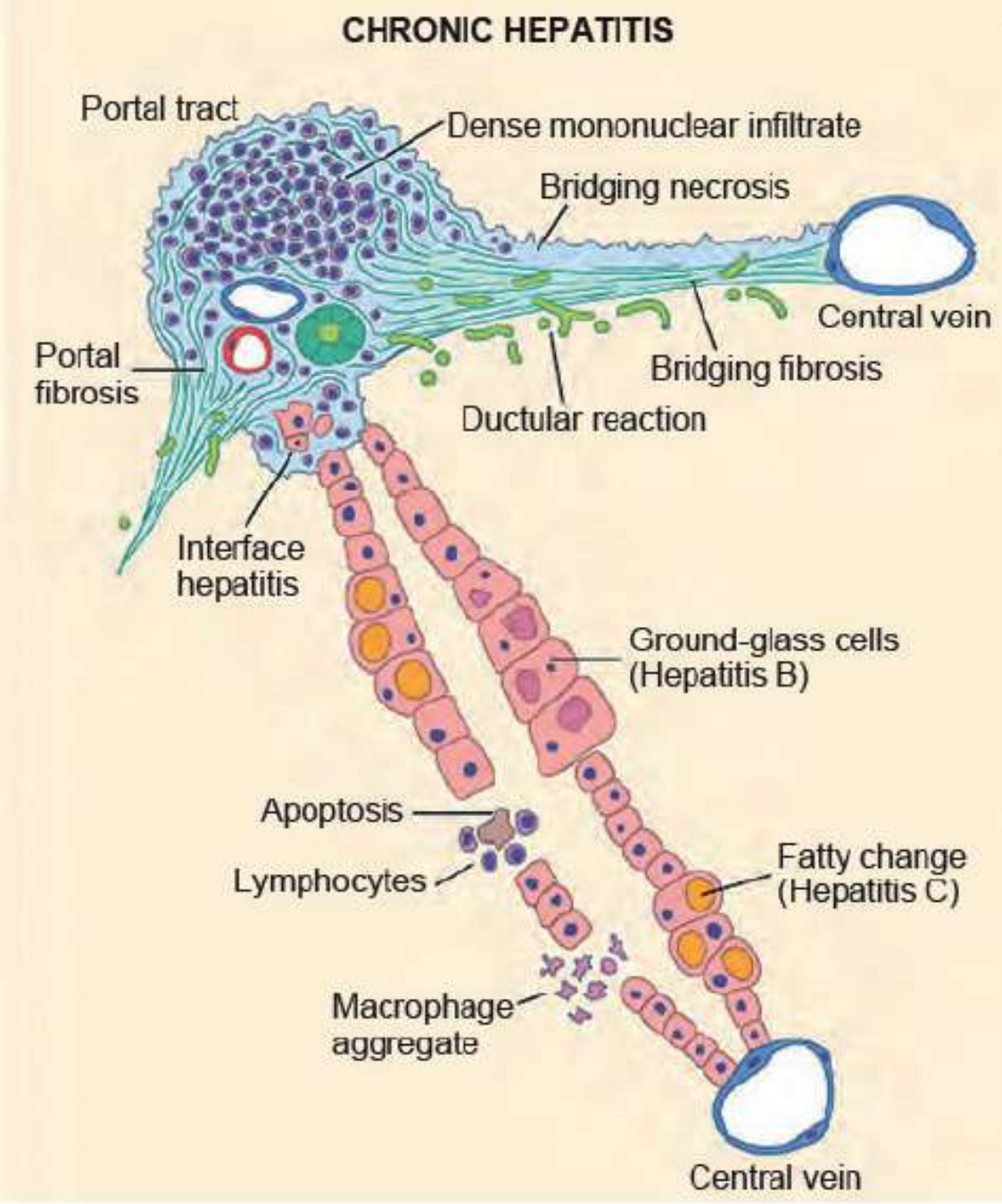


Fig. 9.6

Acute hepatitis:

- Ballooning degeneration (Swelling).
- Cholestasis.
- Cell death- Cytolysis apoptosis- councilman bodies.

Bridging necrosis:

- Hepatocyte swelling and regeneration, loss of radial array.
- Kupffer cell hyperplasia and hypertrophy.
- Portal tracts inflammatory infiltrate.
- Interface hepatitis, spillage of inflammatory cells from portal tracts into adjacent parenchyma with necrosis

Chronic hepatitis: Mild to severe.

- Continued interface hepatitis and bridging necrosis.
- Bridging fibrosis.
- Periportal fibrosis, Portal fibrosis

Eventually cirrhosis (post necrotic cirrhosis).

HCV infection: Special features:

- Bile duct damage.
- Steatosis- Macro vesicular type.
- Portal lymphoid aggregates.

Quick Summary:

- The vowels (hepatitis A and E) never cause chronic hepatitis, only **acute** hepatitis, except HEV in immunocompromised hosts and pregnant females.
- Only the consonants (hepatitis B, C, D) have the potential to cause chronic disease (C for consonant and for chronic).
- Hepatitis B can be transmitted by blood, birthing, and “bonking” (as they say in the United Kingdom).
- Hepatitis C is the single virus that is more often chronic than not (almost never detected acutely; 80% or more of patients develop chronic hepatitis, 20% of whom will develop cirrhosis).
- Hepatitis D, the delta agent, is a defective virus, requiring hepatitis B co-infection for its own capacity to infect and replicate.

- Hepatitis E is endemic in equatorial regions and frequently epidemic.
- The inflammatory cells in both acute and chronic viral hepatitis are mainly T cells; it is the pattern of injury that is different between the two time courses, not the nature of the infiltrate.
- Biopsy assessment in chronic viral hepatitis is most important for grading and staging of disease, which are used to decide whether a patient undergoes often arduous antiviral treatments.
- Patients with long-standing HBV or HCV related cirrhosis are at increased risk for the development of hepatocellular carcinoma.

Fulminant Hepatitis:

- Progression from onset to hepatic encephalopathy/death.

  Within 2-3 weeks – fulminant hepatitis

  Within 3 months – sub fulminant failure

  Cause of 50-65% cases of hepatic failure.

Causes:

- Viral infections: HAV, HBV, HCV, HDV, HEV.
- Drugs and chemicals: Acetaminophens, INH, α- methyl DOPA, Amantia phalloides.
- Ischemic hepatitis necrosis.
- Obstruction of hepatic vein.
- Massive malignant infiltration of liver.
- Wilson' disease, stroke, fatty liver pregnancy.

Morphology: Liver Shrunk – 500-700 gm's red, limp organ covered by wrinkled

Capsule Necrotic areas: Muddy red, mushy appearance.

Collapsed Reticulum.

Prognosis: 25-90% mortality in absence of liver transplant.

Most common causes in Hepatitis:

- Acute viral hepatitis in children: Hep A.
- Acute viral hepatitis in adults: Hep E.
- Acute viral hepatitis (prevalence): Hep B.
- Acute viral hepatitis leading to fulminant hepatitis: Hep D.
- Fulminant hepatitis: Hep E.
- Epidemic: Hep C.
- Viral cause of cirrhosis: Hep C.
- Chronic hepatitis: Hep B.
- Acute viral hepatitis leading to chronic hepatitis: Hep C.
- Carrier state: Hep B.
- Acute viral hepatitis leading to carrier state: Hep B.
- Viral cause of HCC: Hep B.

Autoimmune Hepatitis:

Histologically similar to chronic hepatitis.

		Points
Autoantibodies	ANA or ASMA or LKM > 1:80 ANA or ASMA or LKM > 1:40 SLA/LP Positive (>20 units)	2 1 0
IgG (or gamma-globulins)	>1.10 times normal limit Upper normal limit	2 1
Liver histology1	Typical tor autoimmune hepatitis Compatible with autoimmune hepatitis Atypical for autoimmune	2 1 0
Absence of viral hepatitis	Yes No	2 0

Features:

- Female predominance (78%).
- Absence of viral serologic markers.
- Elevated serum IgG and gamma globulin levels (>1.5 times).
- High serum titer of auto antibodies (in 80%of cases) antinuclear, anti-smooth muscle, and /or antiliver/ kidney microsomes antibodies (anti LKMI).
- Negative antimitochondrial antibodies.
- Untreated severe disease leads to death in 40% patients and cirrhosis develops in at least 40% of survivors.
- Rx- Immuno suppressive therapy, liver transplantation.
- Frequency of HLAB of HLA DRW3.
- Other Auto immune disease present 60% patients like R.A, thyroiditis, sjogrens, UC.
- Two subgroups.
- Type I- Most common has ANA and / or SMA serum marker.
- Type II- Younger patients, anti-liver/ kidney micro some antibodies.

Quick Revision:

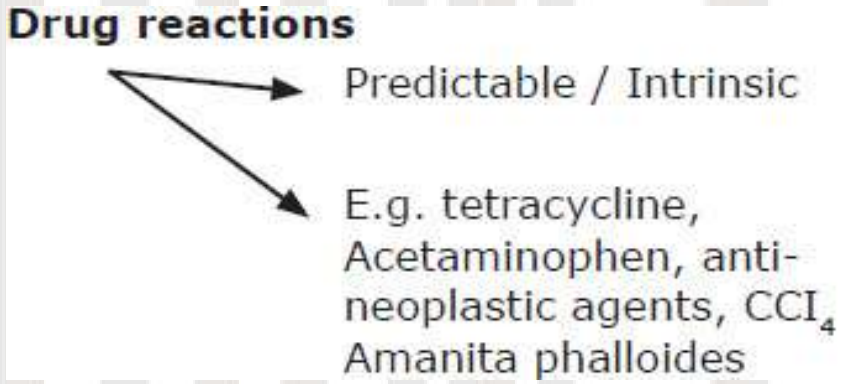
- there are two primary types of autoimmune hepatitis:
- Type 1 autoimmune hepatitis is most often seen in middle-aged women and is most characteristically associated with antinuclear and anti–smooth muscle antibodies (ANA and ASMA).
- Type 2 autoimmune hepatitis is most often seen in children or teenagers and is associated with anti-liver kidney microsomal autoantibodies (anti-LKM1).
- autoimmune hepatitis may either develop with a rapidly progressive acute disease or follow a more indolent path; if untreated, both are likely to lead to liver failure.
- Plasma cells are a prominent and characteristic component of the inflammatory infiltrate in biopsy specimens showing autoimmune hepatitis.

Drug Injury:

- Direct injury.
- Conversion of xenobiotic to an active toxin.
- Through immune mechanism.

Drug- or Toxin-Induced Liver Injury:

- most drugs or toxins affecting the liver may be classified as:
- Predictable hepatotoxins, acting in a dose-dependent manner and occurring in most individuals.
- Unpredictable or idiosyncratic hepatotoxins, which happen in rare individuals and which are often independent of dose.
- Hepatotoxins may cause harm from direct cell toxicity, through hepatic conversion of a xenobiotic to an active toxin, or by immune mechanisms, such as by the drug or a metabolite acting as a hapten to convert a cellular protein into an immunogen.
- the most common hepatotoxin causing acute liver failure is acetaminophen.
- the most common hepatotoxin causing chronic liver disease is alcohol.



Unpredictable / Idiosyncratic e.g. Chlorpromazine, sulfonamides, methyldopa, allopurinol.

Pattern of Injury	Morphologic Findings	Examples of Associated Agents
Cholestasis	Bland hepatocellular cholestasis, without inflammation	Contraceptive and anabolic steroids, antibiotics, HAAFT
Cholestatic hepatitis	Cholestasis with lobular necro inflammatory activity; may show bile duct destruction	Antibiotics, phenothiazine, statins
Hepatocellular necrosis	Spotty hepatocyte necrosis Massive necrosis Chronic hepatitis	Methyldopa, phenytoin Acetaminophen, halothane Isoniazid
Fatty liver disease	Large and small droplet fat "Micro vesicular steatosis" (diffuse small droplet fat) Steatohepatitis with Mallory-Denk bodies	Ethanol, corticosteroids, methotrexate, total parenteral nutrition Valproate, tetracycline, aspirin (Reye syndrome), HAART Ethanol, amiodarone
Fibrosis and cirrhosis	Periportal and pericellular fibrosis	Alcohol, methotrexate, enalapril, vitamin A and other retinoids
Granulomas	Noncaseating epithelioid granulomas Fibrin ring granulomas	Sulfonamides, amiodarone, isoniazid Allopurinol
Vascular lesions	Sinusoidal obstruction syndrome (veno-occlusive disease): obliteration of central veins Budd-Chiari syndrome Peliosis hepatis: blood-filled cavities, not lined by endothelial cells	High-dose chemotherapy, bush teas Oral contraceptives Anabolic steroids, tamoxifen
Neoplasms	Hepatocellular adenoma Hepatocellular carcinoma Cholangiocarcinoma Angiosarcoma	Oral contraceptives, anabolic steroids Alcohol, thorotrast Thorotrast, vinyl chloride

Alcoholic Liver Disease:

- Hepatic Steatosis.
- Alcoholic hepatitis.
- Cirrhosis.

Steatosis Micro vesicular → Macro vesicular

- Initially Centrilobular.

Liver: Enlarged, yellow, greasy. Easily fractured.

- Reversible- till fibrosis appears.

Hepatitis:

- Hepatocyte swelling and necrosis.
- Mallory bodies: Tangled skeins of cytokeratin intermediate filaments and other protein Also seen in-

a. Alcoholic Liver disease.

b. Primary Biliary cirrhosis.

c. Wilson's disease.

d. Chronic cholestasis syndrome.

- Neutrophilic reaction.
- Centrilobular fibrosis and perivenular fibrosis.

Cirrhosis:

Final and irreversible form

Liver: Initially: Yellow, fatty, enlarged.

Later: Brown, Shrunken non – fatty uniform with micro nodules → broad expanses of tough, pale, scar tissue.

(Laennec cirrhosis):

Pathogenesis: Short term, <80gm: → Mild, reversible fatty change.

>80gm, daily → ↑ risk of severe hepato injury.

>160 gm, 10-20 years → severe injury

10-20% Alcoholics have cirrhosis.

Women More susceptible.

Cause of alcohol induced liver damage:

1. **Hepatocellular steatosis** - Cause

- Shunting of normal substrates from catabolism to lipid synthesis due to increase NADH by alcohol dehydrogenase and acetaldehyde dehdrogenase.
- Increased peripheral catabolism of lipid, impaired assembly and secretion of lipoprotein.

2. Induction of cytochrome P 450.
3. Free radical generation.
4. Direct effect on micro tubular and mitochondrial function and membrane fluidity.
5. Immunologic attack of hepatic neoantigens.
6. ↓ Intrahepatic glutathione levels (GSH) sensitizing the liver to oxidative injury.

Cause of Death:

- Hepatic coma.
- Massive GIT bleeding.
- Intercurrent infection.
- Hepatorenal infection.
- HCC: 3%-6%.

Non Alcoholic steato hepatitis.

- Obesity – most important Risk factor.
- Type II DM.
- Hypertriglyceridemias.

Metabolic Disease of Liver:

1. Hemochromatosis.

- Excessive body Fe.
- Genetic / hereditary – AR condition.
- Chromosome 6 (HFE gene- regulates intestinal absorption of dietary iron).
- Linked to HLA- A3 halotype.

Cysteine → Tyrosine at AA282 of HFE gene in H/C.

- Secondary.
  - Parenteral Fe overload.
  - Ineffective erythropoiesis.
  - Increased oral Fe (Bantu disease).

- Congenital A tranferinemia.
- Chronic liver disease.

Total body Fe pool: 2-6 gm.

Liver: 0.5gm (98% in hepatocytes).

In genetic H/C: Fe > 50 gm (>1/3 in liver)

Male…>Female….5^{th} – 6^{th} decade.

Triad:

- Micro nodular cirrhosis (all patients).
- Skin pigmentation.
- DM (75%-80%).

→ Defect in intestinal Fe absorption … Fe accumulation of 0.5-1gm / years.

Disease manifestation > 20gm.

Iron Causes:

- Lipid peroxidation (by Fe catalyzed free radical reactions).
- Stimulation of collagen formation.
- Direct interaction of iron with DNA.

Morphology:

- Deposition of hemosiderin in Liver, pancreas, myocardium, pituitary, adrenal, thyroid, parathyroid, joints and skin.
- Cirrhosis (Micro nodular).
- Pancreatic fibrosis.

  Liver: Periportal hepatocytes: golden yellow hemosiderin granules Pearls+ ve reaction.
- No inflammation.

Hepatitis Fe content:

- (N) :< 1000 µg/gm dry weight of liver
  Genetic hemochromatosis :> 10,000 µg/gm weight of liver.
  Cirrhosis > 22,000 µg/gm weight of liver.

Pancreas: Pigmented.

Fibrosis in interstitial tissue.

Parenchymal atrophy.

H ‘siderin’ in acinar and islet cells.

Heart: H ‘siderin’ in myocardial fibres Heart is enlarged.

Skin: H ‘siderin’ in dermal macrophages and fibroblasts.

↑ epidermal melanin production (results n pigmentation) state gray color Bronze diabetes.

Testes – Small and atrophic.

- Cirrhosis.
- Cardiac disease.
- HCC risk increase 200 times.

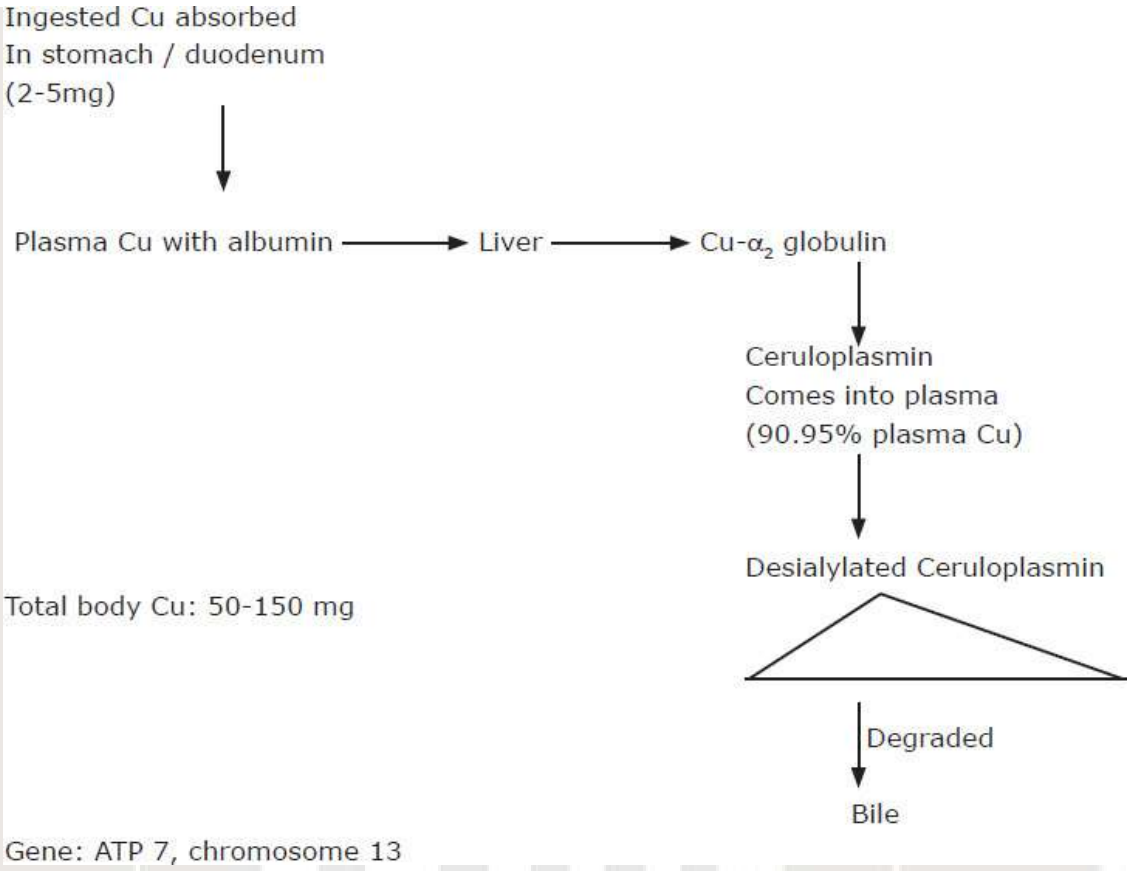
Screening technique for family members of probands.

- S ferritin & iron.
- HLA gene molecular analysis.
- Liver biopsy.

WILSON’S Disease:

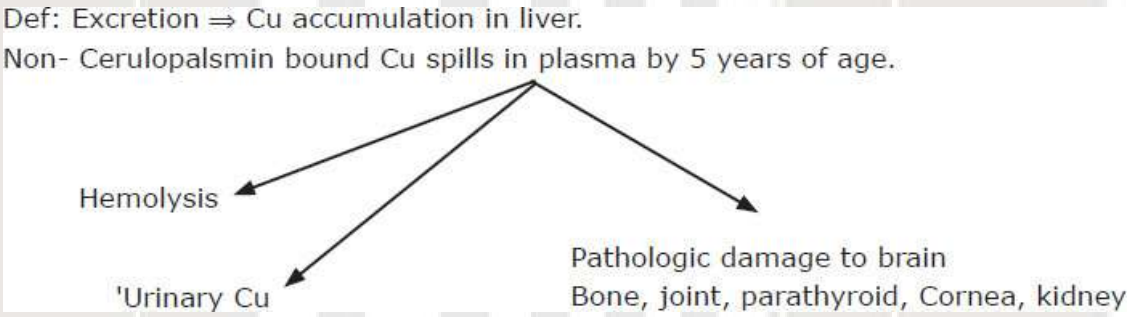
Accumulation of toxic level of Cu in liver, brain, eye (Hepatolenticular degeneration).

AR



Gene: ATP 7, chromosome 13

- Encodes transmembrane Cu transporting ATPase.
- Located at hepatocyte canalicular membrane.



Morphology: Fatty change.

- Acute and chronic hepatitis .
- Mallory bodies – Acute stage.
- Cirrhosis.

- Massive liver necrosis – Rare.

Excess Cu: Rhodanine / Orcein Stain.

Hepatic CU> 250µg/gm dry weight of Liver

S. Cerulopasmin< 20 mg/dl.

U. Cu>30-50 µg/24 hours

Brain: Toxic injury to basal ganglia: Putamen.

Eye: Kayser Fleischer Rings (deposit in Descemet membrane).

Usually manifest after 6 years of age.

- Neuropsychiatric / hepatic manifestations.

Lab diagnosis:

- "Decrease serum ceruloplasmin.
- 'Hepatic copper.
- 'Urinary copper.

3. α_1 **– Antitrypsin Deficiency.**

(Protease inhibitor).

AR

- α_1 AT: synthesized by hepatocytes
- Gene on chromosome 14
- Most common genotype Pi MM (90%)

Deficiency Var: Pi S variant - ↓ S-α1 AT.

PiZZ: 10% of (n)

PiMZ→ intermediate

Pi null → no α_1 AT

Def. Variants have defect in movement of secretory proteins from ER to Golgi apparatus.

Mutant Polypeptide: abnormally folded → hindered movement of α_1 ATZ peptide 1 retention in E.R.

Morphology:

Round to oval cytoplasmic inclusion.

- Eosinophilic, PAS+ve diastase resistant

C/F → Neonatal hepatitis with cholestatic jaundice in 10-20% newborns with this deficiency. Cirrhosis by middle to late life.

HCC: 2-3%

Neonatal Hepatitis / Cholestasis: 1 in 2500 births:

Clinical data and Liver biopsy are crucial in D/D of neonatal hepatitis and biliary atresia.

Neonatal hepatitis – Prolonged, conjugated hyper bilirubinemia.

Histopathology:

- Lobular disarray with focal liver necrosis.
- Panlobular giant cell formation of hepatocytes.
- Hepatocellular & canalicular cholestasis.
- Mononuclear infiltration (periportal) with kupffer cell hyperplasia).
- Extramedullary hematopoesis.

Neonatal cholestasis – Causes:

1. Extra hepatic biliary Atresia (20%).
2. Idiopathic Neonatal hepatitis (50%).
3. Neonatal infectious CMV, Bacterial Sepsis, UTI etc.
4. Toxic – Drugs.
5. Metabolic diseases-

- α IAT Deficiency (15%).
- Niemann – Pick disease.
- Galactosemia.

- Cysticfibrosis.

6. Miscellaneous shock, ICC.

Quick Revision:

- the most common metabolic disorder is nonalcoholic fatty liver disease, which is associated with the metabolic syndrome, obesity, type 2 diabetes mellitus or other impairments of insulin responsiveness, dyslipidemia, and hypertension.
- Nonalcoholic fatty liver disease may show all the changes associated with alcoholic liver disease: steatosis, steatohepatitis, and steatofibrosis, even though the features of steatohepatitis (e.g., hepatocyte ballooning, Mallory-Denk bodies, and neutrophilic infiltration) are often less prominent than they are in alcohol-related injury.

Reye's syndrome:

Encephalopathy following viral infection. H/O Aspirin intake (no role in pathogenesis) Micro vesicular steatosis- characteristic feature (AIIMS question).

Quick Revision:

Inherited Metabolic Liver Disease.

- The inherited metabolic diseases include hemochromatosis, Wilson disease, and α1-antitrypsin deficiency.
- hereditary hemochromatosis is caused by a mutation in the **HFE** gene, whose product is involved in intestinal iron uptake by its effect on hepcidin levels. It is characterized by accumulation of iron in liver and pancreas.
- Wilson disease is caused by a mutation in the metal ion transporter **ATP7B,** which results in accumulation of copper in the liver, brain (particularly basal ganglia), and eyes ("Kayser-Fleisher rings").
- Wilson disease effects on the liver are protean, presenting as acute massive hepatic necrosis, fatty liver disease, or chronic

hepatitis and cirrhosis.

- α1-Antitrypsin deficiency is a disease of protein misfolding that results in impaired secretion of α1 Antitrypsin into the serum.
- The Z variant of α1-Antitrypsin is the most likely to impair secretion by hepatocytes and cause disease, particularly when homozygous, that is, the **PiZZ** genotype; the main consequences are pulmonary emphysema caused by increased elastase activity and liver injury caused by the accumulation of abnormal α1-Antitrypsin.

Intra Hepatic Biliary Tr. Disease:

1. SEC- BILIARY CIRHOSIS:

Etiology: Extra hepatic bile duct obstruction: stones, atresia, structure, carcinoma.

Conjugated bil. ',' S. Alkaline P0 $_4$ ase ' Bile acids 'cholesterol.

C/F Pruritis, jaundice, dark urine, light stools.

M/C bile stasis with bile duct proliferation

Jigsaw pattern due to coarse fibrous septae Small and Large bile ducts in septae.

2. Primary Biliary CIRRHOSIS:

Female: Male = 6:1 middle age (40-50 yrs).

- Chronic, progressive, often fatal cholestatic liver disease.
- Destruction of intra hepatic bile dusts- Medium sized ducts.
- Serum Auto antibodies: Anti mitochondrial Ab (in 90%) against E2 subunit of pyruvate.
  Dehydrogenase complex of (IgM type).
- Extra hepatic manifestation – sjogrens syndrome, scleroderma, Thyroiditis, Membranous Glomerulonephritis, celiac disease Reynauds phenomenon.

Morphology: Focal and variable disease

Diff. Degrees of severity in different portion of liver).

Stages:

- Florid duct lesion (by granulomatous inflammation) - Destruction of terminal and conducting bile ducts.
- Ductular proliferation.
- Scarring.
- Cirrhosis.

Bile stained green liver.

Treatment: Liver transplantation.

3. **Primary Sclerosing Cholangitis:** Inflammation and obliterative fibrosis of intra and extrahepatic bile ducts, with dilatation of preserved segments.

Male: Female ….3-5 th decade.

- Obliterative, fibrosing cholangitis (onion skin).
- Segmental obliteration of intra and extra hepatic bile duct.
- Beading of Barium column.
- Associated with IBD (ulcerative colitis in 70%).
- 'risk of cholangiocarcinoma.
- Treatment: Liver transplantation.

Anomalies of Biliary Tree:

1. Von Meyenberg complex/ Bile duct microhamartomas:

Incomplete involution of embryonic bile duct remnants.

- Within PT: small clusters of dilated bile ducts embedded in fibrous, stroma.
- May communicate with biliary tree.

2. Polycystic Liver Disease:

- Multiple diffuse cystic lesions.
- No pigmented material. Detached from biliary tree.

3. Cong. Hepatic Disease:

- Pt enlarged by irregular Broad bands of collagen.

- Divide liver into irregular Islands.
- Variable no. of abnormal, shaped bile ducts embedded in fibrous tissue.
  - In continuity with biliary tree.
  - May dev. PHT, 'risk of cholangio Carcinoma.

4. Caroli's Disease:

Larger ducts of intra hepatic biliary tree are segmentally dilated and contain inspissated bile.

- C/O: Intra hepatic cholelithiasis, Cholangitis.
- 'Risk of cholangiocarcinoma, Portal H.T

5. Alagille syndrome:

- Paucity of bile ducts.
- Un common AD condition.
- Liver (n) but PT bile ducts absent.

Peculiar facies, vertebral abnorm, CVS defects.

Risk of hepatic failure & HCC.

Mutation in gene Jagged 1 on chromosome 20p: encoded ligands for notch 1 (Role in development of organ systems).

Circulatory Disorders:

1. Portal vein obstruction and thrombosis- Extrahepatic / Intrahepatic:

a. Banti Syndrome: Extrahepatic PB thrombosis.

Subclinical occlusion (neonatal umbilical sepsis/ umbilical vein.

Catheterization) presents as ascites and v aricealbleeding years later.

b. Intraabdominal sepsis:

c. Thrombogenic disorders (post- surgical):

d. Trauma:

- Acute intra hepatic PV thromboses produced sharply demarcated area of red- blue discoloration – infarct of zahn.

Budd- Chiari Syndrome:

- Hepatic vein thrombosis Hepatomegaly, weight gain, ascites, abdominal pain.
- Associated with polycythemia vera, pregnancy, post partum state, OCs, PNH intra abd. Ca Idiopathic (30%).

Treatment: Portosystemic shunt.

Veno-Occlusive Disease:

- Jamaican drinkers of pyrrolizidine alkaloid containing bush tea.
- Now. After BM transplant 25% of patients.

→ Obliteration of hepatic vein radicals by endothelial swelling and fine collagen.

Diagnosis by H/O tender hepatomegaly, jaundice ascites; Do not to biopsy as. It can be Catastrophic Pathogenesis? Toxic damage to endothelium.

Peliosis Hepatitis:

- Sinusoidal dilatation.
- Hepatic efflux impeded associated with Anabolic steroids, rarely OCs and danazol.
- HIV infection → Bartonella hensealse infection → Peliosis.

Heart Failure:

- (R) Heart failure- Passive congestion of liver.
- (L) Heart failure / shock – centrilobular necrosis.
- Combination of hypo perfusion and retrograde congestion → centrilobular hemorrhagic necrosis (Nut Meg liver).
- Complication – Cardiac sclerosis /cardiac cirrhosis.

Liver Disease with Pregnancy:

1. Pre eclampsia & Eclampsia:

HELLP syndrome.

On liver: Small, red, hemorrhagic patches.

- Periportal necrosis; periportal fibrinoid deposits.
- Hematoma.
- Hepatic Rupture (blood under glissons capsule).
- T/T- Termination of pregnancy.

2. Acute fatty liver of pregnancy:

- Latter half of pregnancy (3^{rd} trimester).
- Defect in intra mitochondrial FA oxidation.
- Micro vesicular Steatosis with lobular disarray.
- Clinical features of Hepatic failure.

3. Intra hepatic cholestasis of pregnancy:

- Mild cholestasis without necrosis.
- 3^{rd} trimester.

TUMORS:

1. Most common benign lesions: Cavernous hemangioma – 2cm under capsule.

2. Nodular hyperplasia:

Focal nodular hyperplasia.

Nodular regenerative hyperplasia.

- Affects the whole liver spherical nodules without fibrosis.
- Plump hepatocytes are surrounded by rims of atrophic hepatocytes.
- Associated with portal hypertension.

→ Focal nodular Hyperplasia:

- Young to middle age adults female preponderance.
- Asymptomatic / abd. Mass / Discomfort
- No malignant potential.

Path: Well demarcated non- encapsulated, Nodule Lighter in color than liver.

- Central scar with large vessels (arteries).
- Septae show bile duct proliferation.

Types of hepatocellular adenomas.

1. HNF1 α inactivated type:

- 90% of these tumors have inactivating mutations which are somatic; 10% are germline.
- Heterozygous germline mutations are responsible for Autosomal dominant MODY 3.
- Most commonly found in women; OCPs are implicated in some.
- These tumors are devoid of cellular and architectural atypia and are fatty
- Almost no risk of malignant transformation.
- Liver fatty acid binding protein (LFABP) [downstream regulated protein of HNF1 α is constitutively expressed in all normal hepatocytes but is absent in these tumors.
- IHC for LFABP showing absence is diagnostic.

2. β catenin activated type:

- associated with neoplasia and malignancy in many organs
- Very high risk for malignant transformation in liver
- should be resected even when asymptomatic.
- associated with oral contraceptive and anabolic steroid use.
- found in both men and women.
- High degree of cytological and architectural dysplasia.
- IHC for β catenin shows nuclear translocation indicative of its activated state.
- Glutamine synthetase (target of beta catenin) is also diffusely positive in these tumors.

3. Inflammatory type:

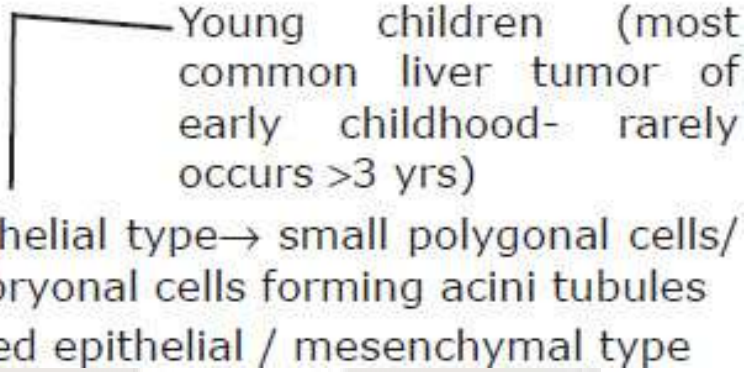
- Both men and women.

- associated with NAFLD.
- Small but definite risk of malignant transformation.
- characterized by activating mutations in gp130, a coreceptor for IL-6, that leads to constitutive JAK-STAT signaling and overexpression of acute phase reactants.
- have areas of fibrotic stroma, mononuclear inflammation, ductular reactions, dilated sinusoids, telangiectatic vessels
- Most of these tumors overexpress acute phase reactants such as CRP, serum amyloid A.
- Some have β catenin activating mutations too.

Neoplastic condition	Risk factors
Adenoma	Oral contraceptive exposure Glycogen storage disease type la [Von Gierke disease).
Hepatocellular carcinoma	Viral hepatitis. Hepatitis B infection. Hepatitis C infection. Cirrhosis from other causes. Alcoholic liver disease. Hereditary haemochromatosis. Hereditary tyrosinaemia. al -antitrypsin storage disorder. Wilson disease (rare). Primary biliary cirrhosis (rare). Inherited disorders without obligate cirrhosis. Glycogen storage disease type 1a (Von Gierke disease).
Hepatoblastoma	Familial adenomatous polyposis (FAP)
Cholangiocarcinoma	Primary sclerosing cholangitis. Fluke infection of the biliary tract.
Angiosarcoma	Toxin expo5LreJ Vinyl chloride Arsenic
'Historicity' Thorotrast exposure was a risk Factor for hepatocellular carcinoma, cholangiocarcinoma and angiosarcoma.	

Malignant Tumors:

1. Hepatoblastoma



- Contain primitive mesenchyme

2. Angiosarcoma:

- Vinyl chloride, Arsenic, thorotrast

3. HCC: 90% of primary carcinoma of Liver:

- HBB and HCV infection.
- Aflatoxins.
- Cirrhosis (alcoholic cirrhosis)/ hemo chromatosis.
- Hereditary tyrosinemias → 40% dev. HCC.
- Universal HBV vaccination may decrease incidence of HCC.

Unifocal – single large mass.

- Multi focal multiple nodules.
- Diffuse Involves entire liver.
- Cells are bile stained.
- Tendency of invade vascular channels.
- Well → poorly differentiated.
- Fibrolamellar Ca: 20-40 years.
- No associated with cirrhosis / HBV.
- Better prognosis.
- H/P – Schirrous tumor- fibrous bands and well differentiated polygonal cell in nests.
- ↑ α FP levels.

4. Cholangio Carcinoma – Ca of extra / intra hepatic biliary tree.

- Thorotrast.
- Opisthorchis sinensis.
- Caroli's disease.
- Prim. Cholangitis.
- Cong. Hepatic fibrosis.
- Lymphatic and vascular invasion prominent.
- Extensive intra hepatic metastases.
- LN mets → Perihilar, Peripancreatic, para- aortic LNs.
  Hematog. Mets → lung, bones, adrenals, brain.

(Less with HCC than cholangio Ca).

Most common – Adenocarcinomas with marked desmoplasia.

- Not bile stained.

Death: Cachexia.

GIT bleeds/ variceal bleed.

Liver failure.

Rupture of tumor.

Premalignant lesions for cholangiocarcinoma are also known, the most important of which are biliary intraepithelial neoplasias (low to high grade, BilIN-1, -2 or -3).

Metastatic tumors: More common in liver than primary tumors (most common neoplasm of the liver).

MC primary: colon > Breast.

- Multiple nodular implants with umblilcation.

Quick Revision:

- **Hepatocellular adenomas** are benign tumors of neoplastic hepatocytes. Most can be subclassified on the basis of molecular changes:

- **HNF1-** α **inactivated adenomas,** with virtually no risk of malignant transformation, often associated with oral contraceptive pill use or in individuals with MODY-3.
- β-**Catenin activated adenoma,** with mutations in the β-catenin gene leading to marked atypia and associated with a very high risk for malignant transformation.
- **Inflammatory adenomas,** the hallmark of which is up-regulation of C-reactive protein and serum amyloid A (often derived from gp130 mutations); 10% of these have concomitant β-catenin activating mutations. Risk for malignant transformation is intermediate.
- The main **primary malignancies are HCCs and cholangiocarcinomas;** HCCs are by far the most common.
- HCC is a common tumor in regions of Asia and Africa, and its incidence is increasing in the United States.
- The main etiologic agents for HCC are chronic hepatitis B and C, alcoholic cirrhosis, non-alcoholic fatty liver disease, and hemochromatosis. In the Western population, about 90% of HCCs develop in cirrhotic livers; in Asia, almost 50% of cases develop in noncirrhotic livers.
- The chronic inflammation and cellular regeneration associated with viral hepatitis or the activation of IL-6/ JAK STAT pathway may be predisposing factors for the development of carcinomas.
- HCCs may be unifocal or multifocal, tend to invade blood vessels, and recapitulate normal liver architecture to varying degrees.
- **Cholangiocarcinoma** is endemic in areas where liver flukes such as **Opisthorchis** and **Clonorchis** species are endemic. Chronic inflammatory diseases of bile ducts are also risk factors. The tumors may arise from extra hepatic or intrahepatic bile ducts. They have uniformly poor prognosis.

10 Gastrointestinal Tract

Hirschsprung Disease:

- Results when the normal migration of neural crest cells from cecum to rectum is arrested prematurely or when the ganglion cells undergo premature death.
- This produces a distal intestinal segment that lacks both the Meissner submucosal and the Auerbach myenteric plexus (“aganglionosis”). Coordinated peristaltic contractions are absent and functional obstruction occurs, resulting in dilation proximal to the affected segment.

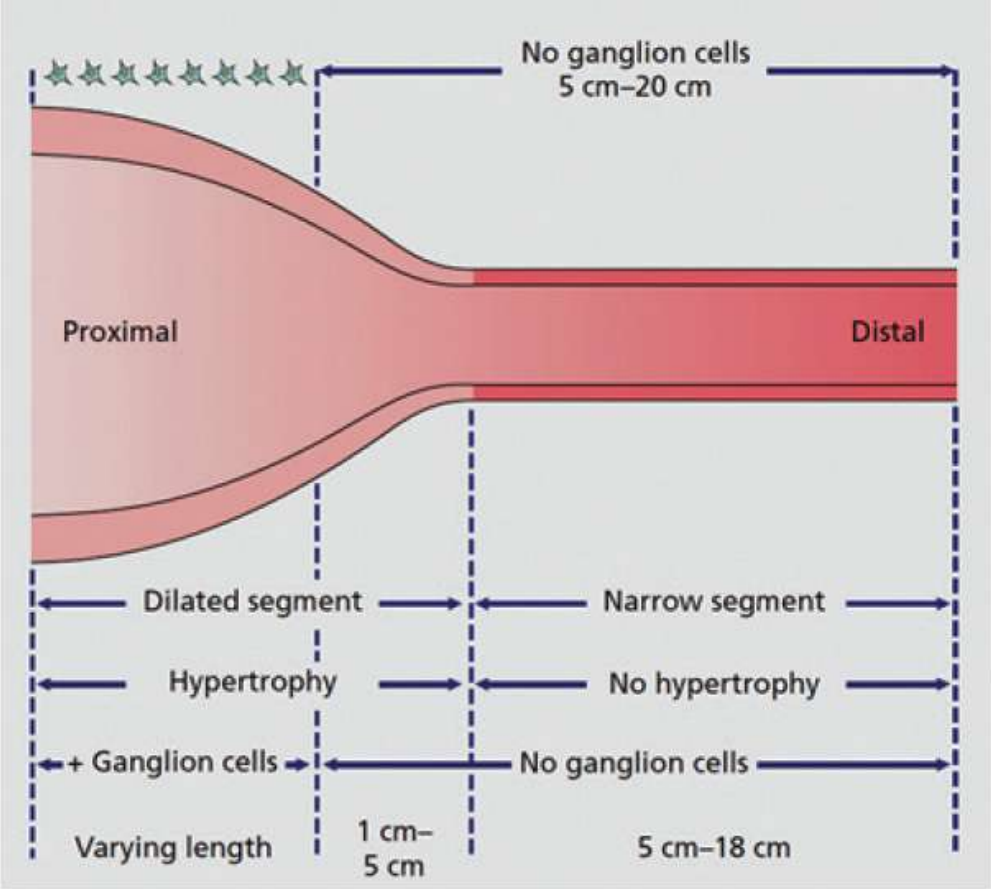


Fig. 10.1: Schematic diagram of gross and microscopic changes in 15 cases of Hirschsprung disease.

- Heterozygous loss of- function mutations in the receptor tyrosine kinase **RET** account for the majority of familial cases.
- **Diagnosis of Hirschsprung disease requires documenting the absence of ganglion cells within the affected segment.**
- In addition to their characteristic morphology in hematoxylin and eosin–stained sections, ganglion cells can be identified using immunohistochemical stains for acetylcholinesterase.
- The rectum is always affected.
- Intraoperative frozen-section analysis is commonly used to confirm the presence of ganglion cells at the anastomotic margin.

Barret's Esophagus:

- **Barrett esophagus is a complication of chronic GERD that is characterized by intestinal metaplasia within the esophageal**

squamous mucosa.

- **The greatest concern in Barrett esophagus is that it confers an increased risk of esophageal adenocarcinoma.**
- Barrett esophagus is most common in white males and typically presents between 40 and 60 years of age.
- The presence of dysplasia, a preinvasive change, is associated with prolonged symptoms, longer segment length, increased patient age, and Caucasian race.
- recognized as one or several tongues or patches of red, velvety mucosa extending upward from the gastroesophageal junction. This metaplastic mucosa alternates with residual smooth, pale squamous (esophageal) mucosa and interfaces with light-brown columnar (gastric) mucosa distally.
- Available data suggest that the risk of dysplasia correlates with length of esophagus affected.
- Diagnosis of Barrett esophagus requires endoscopic evidence of metaplastic columnar mucosa above the gastroesophageal junction.
- Microscopically, intestinal-type metaplasia is seen as replacement of the squamous esophageal epithelium with goblet cells.
- These are diagnostic of Barrett esophagus, and have distinct mucous vacuoles that stain pale blue by hematoxylin and eosin and impart the shape of a wine goblet to the remaining cytoplasm.
- Special stains like PAS and alcian blue at low pH can be used to demonstrate mucin in goblet cells characteristically.

Mechanisms of Gastric Injury:

H. pylori and Autoimmune Gastritis:

	H. pylori-Associated	Autoimmune
Location	Antrum	Body
Inflammatory infiltrate	Neutrophils, subepithelial plasma cells	Lymphocytes, macrophages
Acid production	Increased to slightly decreased	Decreased
Gastrin	Normal to decreased	Increased
Other lesions	Hyperplastic/inflammatory polyps	Neuroendocrine hyperplasia
Serology	Antibodies to H. pylori	Antibodies to parietal cells (H+,K+-ATPase, intrinsic factor)
Sequelae	Peptic ulcer, adenocarcinoma, MALToma	Atrophy, pernicious anemia, adenocarcinoma, carcinoid tumor
Associations	Low socioeconomic status, poverty, residence in rural areas	Autoimmune disease; thyroiditis, diabetes mellitus, Graves disease

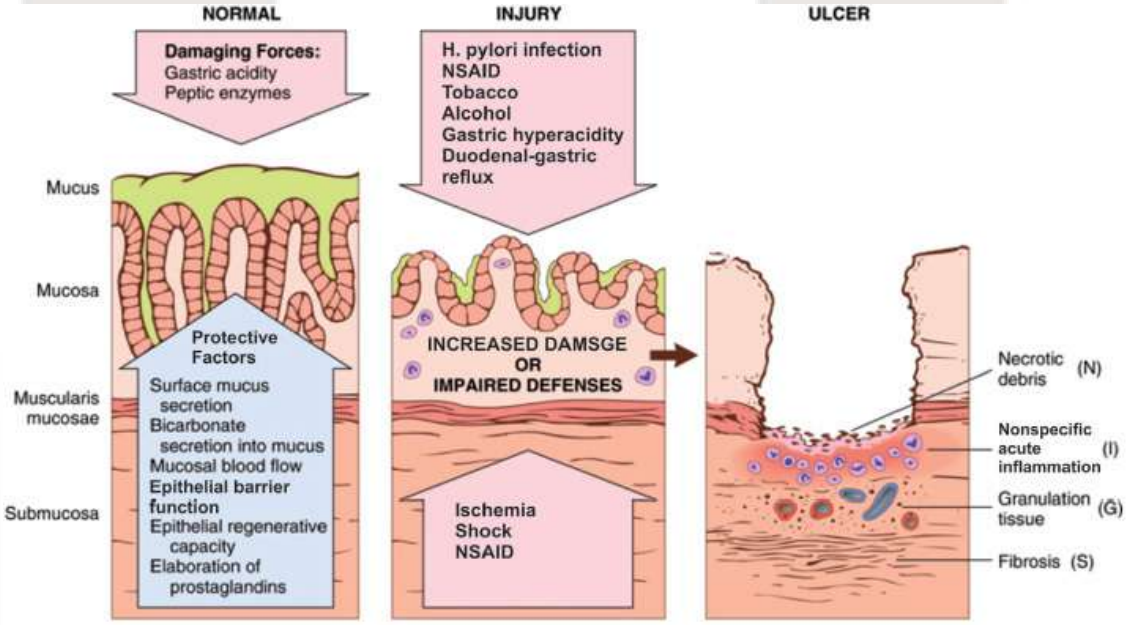


Fig. 10.2

Complications of Peptic Ulcer Disease:

Bleeding
Occurs in 15% to 20% of patients Most frequent complication May be life-threatening Accounts for 25% of ulcer deaths May be the first indication of an ulcer
Perforation
Occurs in up to 5% of patients Accounts for two thirds of ulcer deaths Is rarely first indication of an ulcer

Obstruction
Mostly in chronic ulcers Secondary to edema or scarring Occurs in about 2% of patients Most often associated with pyloric channel ulcers May occur with duodenal ulcers Causes incapacitating, crampy abdominal pain Can rarely cause total obstruction and intractable vomitin

Hypertrophic Gastropathies and Gastric Polyps:

Parameter	Menetrier Disease (adult)	Zollinger Ellison Syndrome	Inflamma-tory and Hyper-plastic Polyps	Gastritis Cystica	Fundic Gland Polyps	Gastric Adenomas
Mean patient age (yr)	30-60	50	50-60	Variable	50	50-60
Location	Body and fundus	Fundus	Antrum > body	Body	Body and fundus	Antrum > body
Predominant cell type	Mucous	Parietal > mucous, endocrine	Mucous	Mucous, cyst-lining	Parietal and chief	Dysplastic, intestinal
Inflamma-tory infiltrate	Limited, lymphocytes	Neutrophils	Neutrophils and lymphocytes	Neutrophils and lymphocytes	None	Variable
Symptoms	Hypo-proteinemia weight loss, diarrhea	Peptic ulcers	Similar 1o chronic gastritis	Similar to chronic gastritis	None, nausea	Similar to chronic gastritis
Risk factors	None	Multiple endocrine neoplasia	Chronic gastritis, H. pylori	Trauma, prior surgery	PPIs, FAP	Chronic gastritis, atrophy, intestinal metaplasia
Association with adeno-carcinoma	Yes	No	Occasional	No	Syndromic (FAP} only	Frequent

Gastrointestinal Stromal Tumor:

- Most common mesenchymal tumor of the abdomen arise from the interstitial cells of Cajal, or pacemaker cells, of the gastrointestinal muscularis propria of the uncommon GISTs in children, some are related to the **Carney triad** , a nonhereditary syndrome of unknown etiology seen primarily in young females that includes gastric GIST, paraganglioma, and pulmonary chondroma. There is also an increased incidence of GIST in individuals with neurofibromatosis type 1.
- Approximately 75% to 80% of all GISTs have oncogenic, gain-of-function mutations in the receptor tyrosine kinase KIT.
- Approximately 8% of GISTs have mutations that activate a closely related receptor tyrosine kinase, platelet-derived growth factor receptor α (PDGFRA).

- For unknown reasons, GISTs bearing PDGFRA mutations are overrepresented in the stomach.
- **KIT** and **PDGFRA** gene mutations are mutually exclusive.
- These mutations, which cause loss of SDH function, are often inherited in the germline and confer an increased risk for GIST and paraganglioma (**Carney- Stratakis syndrome** , not to be confused with Carney triad); with the second copy of the affected gene being either mutated or lost in the tumor.
- The mechanisms by which SDH mutations lead to GIST are unclear; one hypothesis is that the accumulation of succinate leads to dysregulation of hypoxia inducible factor-1α (HIF-1α), which results in increased transcription of the vascular endothelial growth factor (**VEGF**) and insulin-like growth factor-1 (**IGF1R**) genes.
- **Mutation of KIT or PDGFRA is an early event in sporadic.**
- **GISTs and is detectable in lesions as small as 3 mm.** Therefore, **KIT** or **PDGFRA** mutations alone are insufficient for tumorigenesis.
- Changes associated with progression to overt GIST are not well-defined, but loss or partial deletion of chromosomes 14 and 22 is common and losses and gains at other chromosomes also occur.
- In particular, deletion of 9p results in loss of the cell cycle regulator **CDKN2A** , a tumor suppressor that is involved in many cancers. In addition to potentially being related to progression, increased numbers of chromosomal alterations correlate with poor prognosis.
- GISTs composed of thin elongated cells are classified as spindle cell type, whereas tumors dominated by epithelial appearing cells are termed epithelioid type; mixtures of the two patterns also occur.
- The most useful diagnostic marker is KIT, which is detectable in Cajal cells and 95% of gastric GISTs by immunohistochemical stains.
- Most sensitive marker- CD 117.
- Most specific marker- DOG1.

Neoplastic and Non neoplastic proliferations of the stomach:

- **Ménétrier disease** is a rare disorder caused by excessive secretion of transforming growth factor α (TGF-α) and characterized by diffuse foveolar hyperplasia and protein losing enteropathy.
- **Zollinger-Ellison syndrome** is caused by gastrin-secreting tumors that cause parietal cell hyperplasia and acid hyper secretion; 60% to 90% of gastrinomas are malignant.
- The majority of gastric polyps are **inflammatory or hyperplastic polyps,** reactive lesions that are associated with chronic gastritis.
- **Fundic gland polyps** occur sporadically, most often as a consequence of proton pump inhibitor therapy, and in familial adenomatous polyposis (FAP) patients.
- **Gastric adenomas** develop in a background of chronic gastritis and are particularly associated with intestinal metaplasia and mucosal (glandular) atrophy. Adenocarcinoma is frequent in gastric adenomas, which therefore require more aggressive therapy than adenomas of the colon.
- **Gastric adenocarcinoma** incidence varies markedly with geography. Individual tumors are classified according to location, gross, and histologic morphology. Gastric tumors with an **intestinal histology tend to form bulky tumors** and may be ulcerated, while those composed of **signetring cells typically display a diffuse infiltrative growth pattern** that may thicken the gastric wall without forming a discrete mass. Gastric adenocarcinomas are linked to **H. pylori** induced chronic gastritis.
- **Primary gastric lymphomas** are most often derived from mucosa-associated lymphoid tissue (MALT), whose development is induced by chronic gastritis that is most often induced by **H. pylori** .
- **Carcinoid tumors** (well-differentiated neuroendocrine tumors) arise from diffuse components of the endocrine system and are most common in the GI tract, particularly the small intestine.

Prognosis is based on location; tumors of the small intestine tend to be most aggressive, while those of the appendix are typically benign.

- **Gastrointestinal stromal tumor (GIST)** is the most common mesenchymal tumor of the abdomen, occurs most often in the stomach, and is related to benign pacemaker cells, or interstitial cells of Cajal. Tumors generally have activating mutations in either KIT or PDGFRA tyrosine kinases and respond to specific kinase inhibitors.

Defects in Malabsorptive and Diarrheal Diseases:

	Intraluminal	Terminal	Transepithelial	Lymphatic
Disease	Digestion	Digestion	Transport	Transport
Celiac disease		+	+	
Environmental enteropathy		+	+	
Chronic pancreatitis	+			
Cystic fibrosis	+			
Primary bile acid malabsorption	+		+	
Carcinoid syndrome			+	
Autoimmune enteropathy		+	+	
Disaccharidase deficiency		+		
Whipple disease				+
Abetailpoproteinemia			+	
Viral gastroenteritis		+	+	
Bacterial gastroenteritis		+	+	
Parasitic gastroenteritis		+	+	
Inflammatory bowel disease	+	+	+	
+ indicates that the process is abnormal in the disease indicated. Other processes are not affected.				

Celiac Disease:

- Celiac sprue or glutensensitive enteropathy.
- It is an immune-mediated enteropathy triggered by the ingestion of gluten-containing foods, such as wheat, rye, or barley, in genetically predisposed individuals.
- Celiac disease is triggered by ingestion of gluten, which is the major storage protein of wheat and similar grains. The alcohol-soluble fraction of gluten, **gliadin** , contains most of the disease-producing components. Gluten is digested by luminal and brush-border enzymes into amino acids and peptides, including a 33-amino acid α-gliadin peptide that is resistant to degradation by gastric, pancreatic, and small intestinal protease.
- Some gliadin peptides may induce epithelial cells to express IL-15, which in turn triggers activation and proliferation of CD8+ intraepithelial lymphocytes.
- These gliadin peptides interact with HLA-DQ2 or HLA-DQ8 on antigen-presenting cells and, in turn, can stimulate CD4+ T cells to produce cytokines that contribute to tissue damage.
- Associations between celiac disease and other immune diseases, including type 1 diabetes, thyroiditis, and Sjögren syndrome, IgA nephropathy, as well as neurologic disorders, such as ataxia, autism, depression, epilepsy, Down syndrome, and Turner syndrome.
- Biopsy specimens from the second portion of the duodenum or proximal jejunum, which are exposed to the highest concentrations of dietary gluten, are generally diagnostic in celiac disease.
- The histopathology is characterized by increased numbers of intraepithelial CD8+ T lymphocytes (intraepithelial lymphocytosis), crypt hyperplasia, and villous atrophy.
- This loss of mucosal and brush-border surface area probably accounts for the malabsorption.
- In addition, increased rates of epithelial turnover, reflected in increased crypt mitotic activity, may limit the ability of absorptive enterocytes to fully differentiate and express proteins necessary for terminal digestion and transepithelial transport.
- Other features of fully developed celiac disease include increased numbers of plasma cells, mast cells, and eosinophils,

especially within the upper part of the lamina propria.

- The most sensitive tests are the measurement of IgA antibodies against tissue transglutaminase. IgA anti-endomysial antibodies can also be present. IgG anti-tissue transglutaminase antibodies may be detected in patients with IgA deficiency.
- The most common celiac disease-associated cancer is enteropathy-associated T-cell lymphoma, an aggressive lymphoma of intraepithelial T lymphocytes.
- Small intestinal adenocarcinoma is also more frequent in individuals with celiac disease.

Inflammatory Bowel Disease:

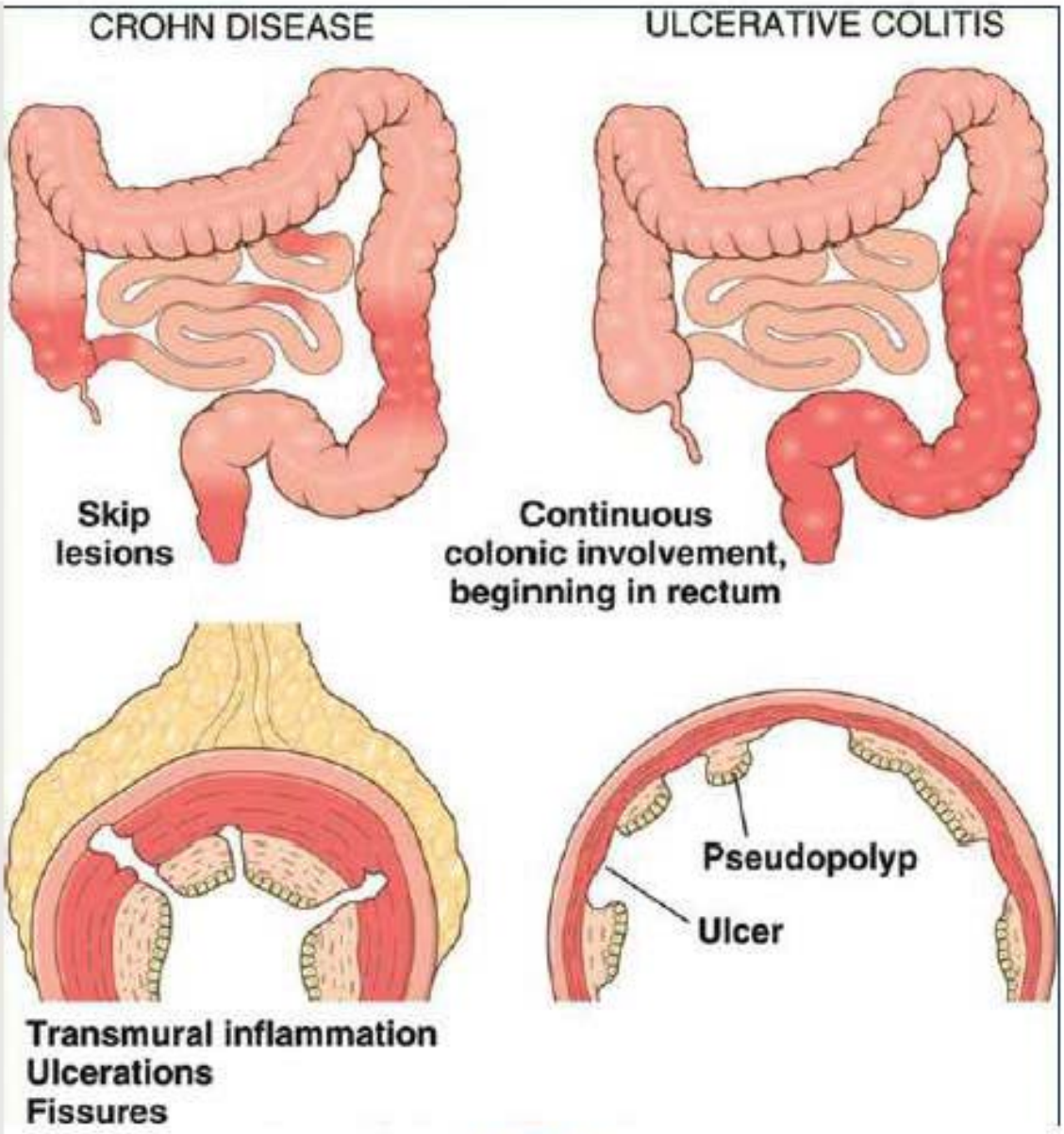

CROHN DISEASE
ULCERATIVE COLITIS
Skip lesions
Continuous colonic involvement, beginning in rectum
Pseudopolyp
Ulcer
Transmural inflammation
Ulcerations
Fissures

Fig. 10.3

Features	Ulcerative Colitis	Crohn Disease
Clinical		
Rectal bleeding	Common	Inconspicuous
Abdominal mass	Practically never	10-15%
Abdominal pain	Usually left-sided	Usually right-sided
Sigmoidoscopy	Abnormal in 95%	Abnormal in less than 50%
Free perforation	12%	4%
Colon carcinoma	5-10%	Very rare
Anal complications	Rare; minor	75%; fissures, fistulas, ulceration
Response to steroid therapy	75%	25%
Results of surgery	Very good	Fair
Ileostomy dysfunction	Rare	Common
Radiographic		
Sparing of rectum	Exceptional	90%
Involvement of ileum	Rare; dilated ('backwash ileitis')	Common; constricted
Strictures	Absent	Often present
Skip areas	Absent	Common
Internal fistulas	Absent	May be present
Longitudinal and transverse ulcers	Exceptional	Common
Fissuring	Absent	Common
Thumb printing	Absent	Common
Morphologic		
Distribution of involvement	Diffuse; predominantly left-sided; mucosal and submucosal	Focal; predominantly right-sided transmural
Mucosal atrophy and regeneration	Marked	Minimal
Cytoplasmic mucin	Diminished	Preserved
Lymphoid aggregates	Rare	Common
Edema	Minimal	Marked
Hyperemia	May be extreme	Minimal
Granulomas	Absent	Present in 60%
Fissuring	Absent	Present
Crypt abscesses	Common	Rare
Rectal involvement	Practically always	50%
Ileal involvement	Minimal; dilated not more than 10 cm	50%; constricted; transmural inflammation
Lymph nodes	Reactive hyperplasia	May contain granulomas

Intestinal Polyps:

- Polyps are most common in the colo-rectal region but may occur in the esophagus, stomach, or small intestine.
- Most, if not all, polyps begin as small elevations of the mucosa.
- As sessile polyps enlarge, proliferation of cells adjacent to the mass and the effects of traction on the luminal protrusion, may combine to create a stalk.
- Polyps with stalks are termed pedunculated.
- In general, intestinal polyps can be classified as nonneoplastic or neoplastic in nature.
- The most common neoplastic polyp is the adenoma, which has the potential to progress to cancer.
- The non-neoplastic polyps can be further classified as inflammatory, hamartomatous, or hyperplastic.

Gastrointestinal Polyposis Syndromes:

Syndrome	Mean Age at Presentation (yr)	Mutated Gene(s); Pathway	Gastrointestinal Lesions	Selected Extra-Gastrointestinal Manifestations
Juvenile polyposis	<5	SMAD4.BMPR1A; TGF-|S signaling pathway.	Juvenile polyps; risk of gastric, small Intestinal, colonic, and pancreatic adenocarcinoma.	Congenital malformations, digital clubbing.
Peutz-Jeghers syndrome	10-15	STK11: AMP kinase-related pathways.	Arborizing polyps; Small intestine > colon > stomach; colonic adenocarcinoma.	Pigmented macules; risk of colon, breast, lung, pancreatic, and thyroid cancer.
Cowden syndrome, Bannayan-Ruvalcaba-Riley syndrome*	<15	PTEN. PI3K/AKT pathway	Hamartomatous/ inflammatory intestinal polyps, lipomas, ganglioneuromas.	Benign skin tumors, benign and malignant thyroid and breast lesions; no Increase In Gl cancers.
Cronkhite-Canada syndrome	>50	Nonheredltary, unkn own cause.	Hamartomatous polyps of stomach, small intestine colon; abnormalities in nonpolypoid mucosa.	Nail atrophy, hair loss, abnormal skin pigmentation, cachexia, and anemia. Fatal In up to 50%.
Tuberous sclerosis		TSC1 (hamartin), TSC2 (tuberin); mTOR pathway.	Hamartomatous polyps.	Mental retardation, epilepsy, facial angiofibroma, cortical (CNS) tubers, renal angiomyolipoma
Familial adenomatous polyposis |FAP| Classic FAP Attenuated FAP Gardner syndrome	10-15 40-50 10-15	APC APC APC	Multiple adenomas Multiple adenomas Multiple adenomas.	Congenital RPE hypertrophy Osteomas, thyroid and desmoid tumors, skin cysts .
Turcot syndrome MW-associated polyposis	10-15 30-50	APC APC MYH	Multiple adenomas Multiple adenomas.	Medulloblastoma, glioblastoma.

Sporadic and Familial Colonic Neoplasias:

Etiology	Molecular Defect	Target Gens (s)	Transmission	Predominant Site(s)	Histology
Familial adenomatous polyposis (70% of FAP)	APC/WNT pathway	APC	Autosomal dominant	None	Tubular, villous; typical adenocarcinoma
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	MSH2, MLH1	Autosomal dominant	Right side	Sessile serrated adenoma; mucinous adenocarcinoma
Sporadic colon cancer (80%)	APC/WNT pathway	APC	None	Left side	Tubular, villous; typical adenocarcinoma
Sporadic colon cancer {10%-15%)	DNA mismatch repair	MSH2, MLH1	None	Right side	Sessile serrated adenoma; mucinous adenocarcinoma
FAP, Familial adenomatous polyposis.					

Molecular Carcinogenesis Of Colorectal Cancer:

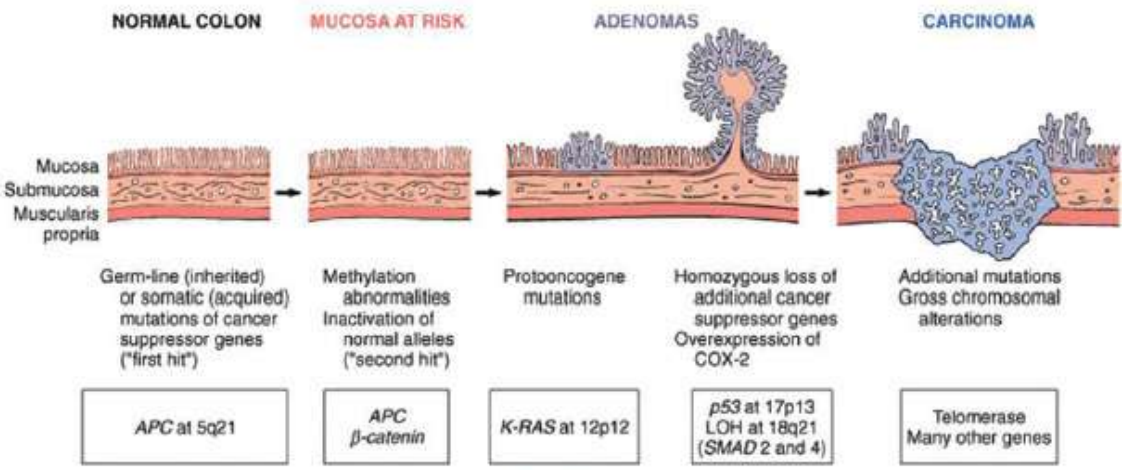


Fig. 10.4

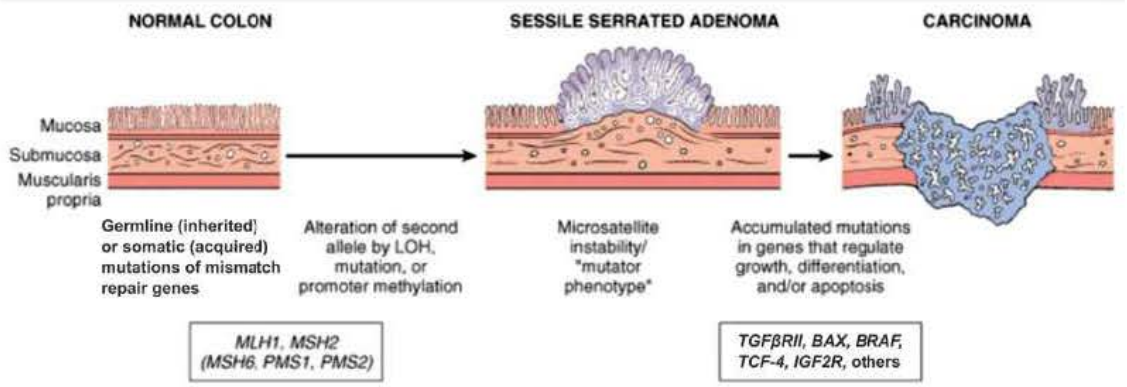

NORMAL COLON
SESSILE SERRATED ADENOMA
CARCINOMA
Mucosa
Submucosa
Muscularis propria
Germline (inherited) or somatic (acquired) mutations of mismatch repair genes
Alteration of second allele by LOH, mutation, or promoter methylation
Microsatellite instability/ "mutator phenotype"
Accumulated mutations in genes that regulate growth, differentiation, and/or apoptosis
MLH1, MSH2 (MSH6, PMS1, PMS2)
TGFβRII, BAX, BRAF, TCF-4, IGF2R, others

Fig. 10.5

11 Kidney and Urinary Bladder

Kidney:

Weight – 150 gm (0.5% of TBW)-25%
Size =11 x 6 x 3cm

Major Calyx (2-3) /Kidney.
Minor calyx-3-4/major calyx, total max-12

C.O. — 90% cortex
C.O. — 10% Medulla

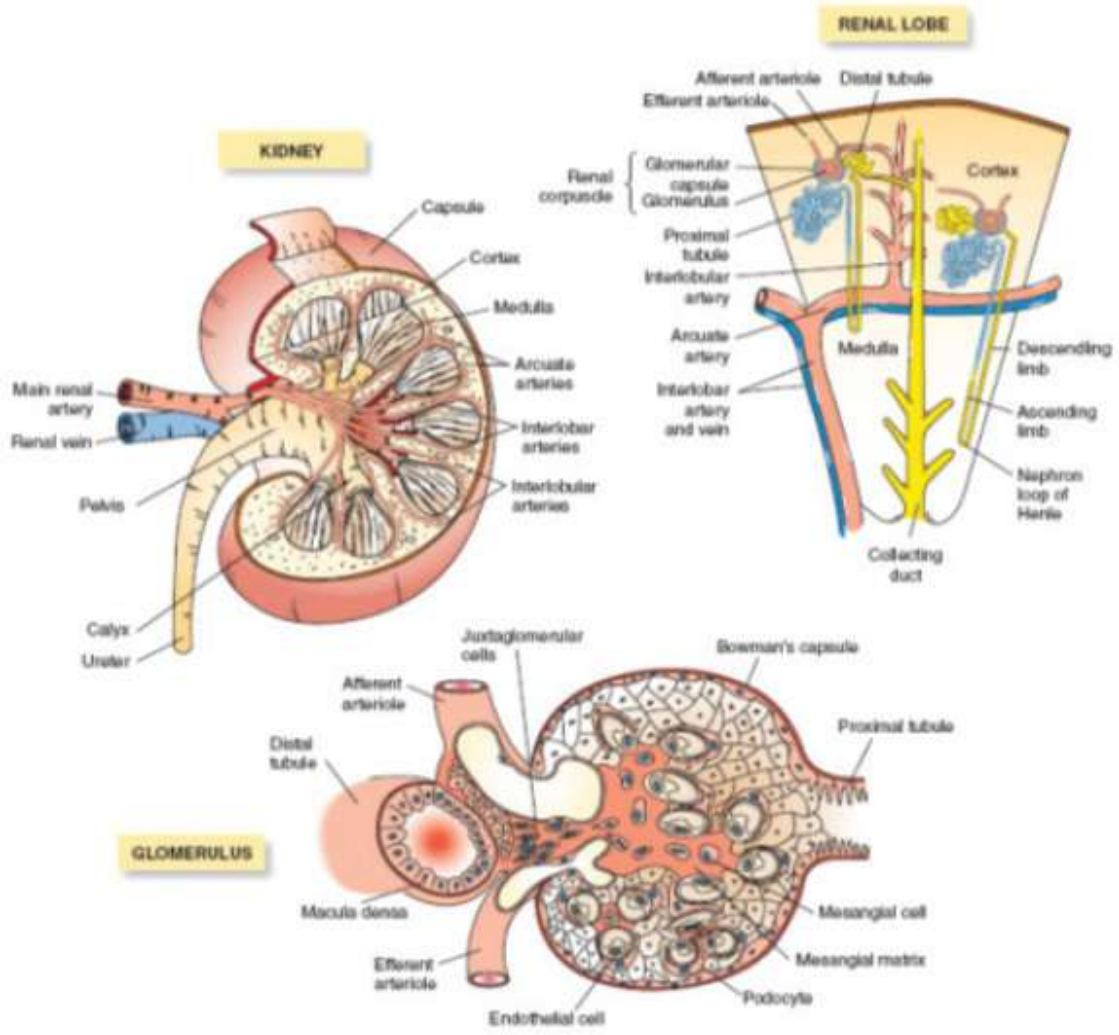


Fig. 11.1

Congenital Anomalies:

1. Agenesis: Total B/L.
Incompatible with life.

- Ass. With limb defects, hypoplastic lungs.

U/L

- Uncommon Compensatory hypertrophy (opposite kidney).

2. Hypoplasia:

- D/D: Acquired Atrophic kidney.
- No Scars.

≤ 6 Renal lobes/ Pyramid
Oligomeganepheronia.

- Kidney is small but nephrons are hypertrophied.

3. Ectopic kidney"

- C/o kinking Obstruction. Most common abnormal location- pelvic brim.

4. Horse shoe shaped:

- 1 in 500-1000 pelvic brim.
- Midline anterior to great vessels.
  - 90%: Lower pole.
- 10%: Upper pole.

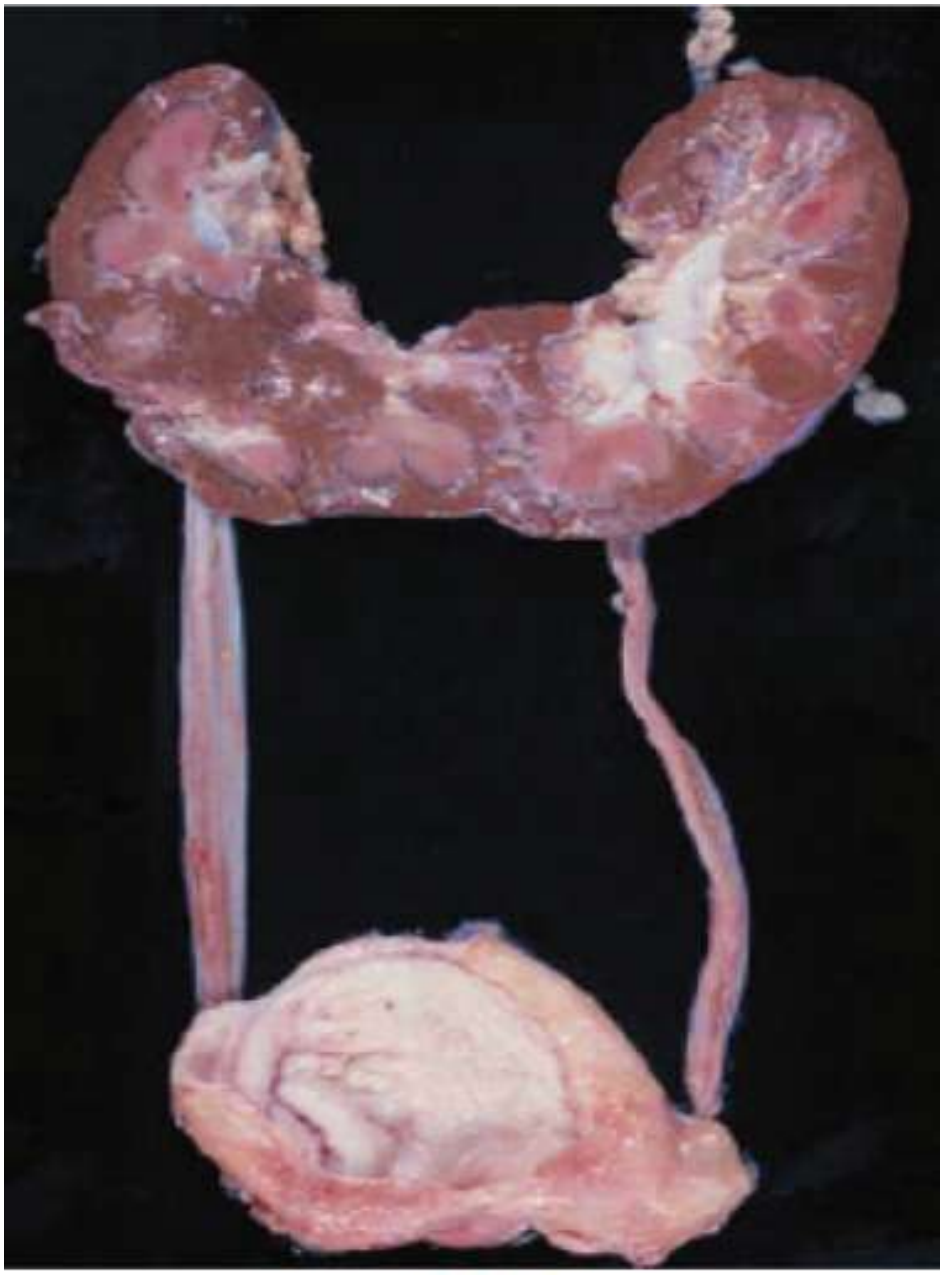


Fig. 11.2

Cystic Diseases:

Disease	Inheritance	Pathologic Features	Clinical Features of Complications	Typical Outcome	Diagrammatic Representation
Adult polycystic	Autosomal dominate	Large multicystic kidneys, liver cysts, berry aneurysms.	Hematuria, flank pain, urinary tract infection, renal stones, hypertension.	Chronic renal failure beginning at age 40-60 years.	
Childhood polycystic kidney disease	Autosomal recessive	Enlarged, cystic kidneys at birth.	Hepatic fibrosis	Variable, death in infancy of childhood.	
Medullary sponge kidney	None	Medullary cysts on excretory urography.	Hematuria, urinary tract infection, recurrent renal stones.	Bening	
Familial juvenile nephronophthisis	Autosomal recessive	Corticomedullary cysts, shrunken kidneys.	Salt wasting, polyuria, growth retardation, anemia.	Progressive renal failure beginning in childhood.	
Adult-onset medullary cystic disease	Autosomal dominate	Corticomedullary cysts, shrunken kidneys.	Salt wasting, polyuria.	Chronic renal failure beginning in adulthood.	
Multicystic renal dysplasia	None	Irregular kidneys with cysts to variable size.	Association with order renal anomalies.	Renal failure if bilateral, surgically curable if unilateral.	
Acquired renal cystic disease	None	Cystic degeneration in end-stage kidney disease.	Hemorrhage, erythrocytosis Neoplasis.	Dependence on dialysis.	
Simple cysts	None	Single or multiple cysts in normal-sized kidneys.	Microscopic hematuria.	Bening	

Classification:

1. CRD.
2. PCKD.
3. Medullary CD.

4. Dialysis associated.
5. Simple cysts.
6. Hereditary malformation syndromes (e.g. tuberous sclerosis).
7. Glomerulocystic disease.
8. Extraparenchymal renal cysts (pyelo calyceal cysts etc).

Cystic Renal Dysplasia:

Sporadic with out familial clustering.

- Abnormal Metanephric differentiation.
- Persistence of abnormal structures (undifferentiated mesenchyme, immature CD, cartilage) & abnormal, **lobar organization.**
- U/P Obstructions, ureteral agenesis, or atresia.
- Kidney cystic, irregular, enlarged.

PCKD:

- Adult (AD) PCKD- potters II.
- Childhood (AR) PCKD- potters I.

Child hood PCKD (AR):

i. Perinatal / Neonatal — common, serious manifestations at birth

ii. Infantile / Juvenile — Assoc. with congenital hepatic fibrosis

iii Gross: B.L. enlarged kidney. External surface smooth.

- Cysts oriented in a radial fashion with their long axis at right angles to the capsule.
- Cysts arise from collecting ducts

iv. Associations – Hepatic cysts, Congenital hepatic fibrosis.

v. Genes – PKHD1 gene 6 p21-23- encodes fibrocystin Role in collecting duct and biliary differentiation.

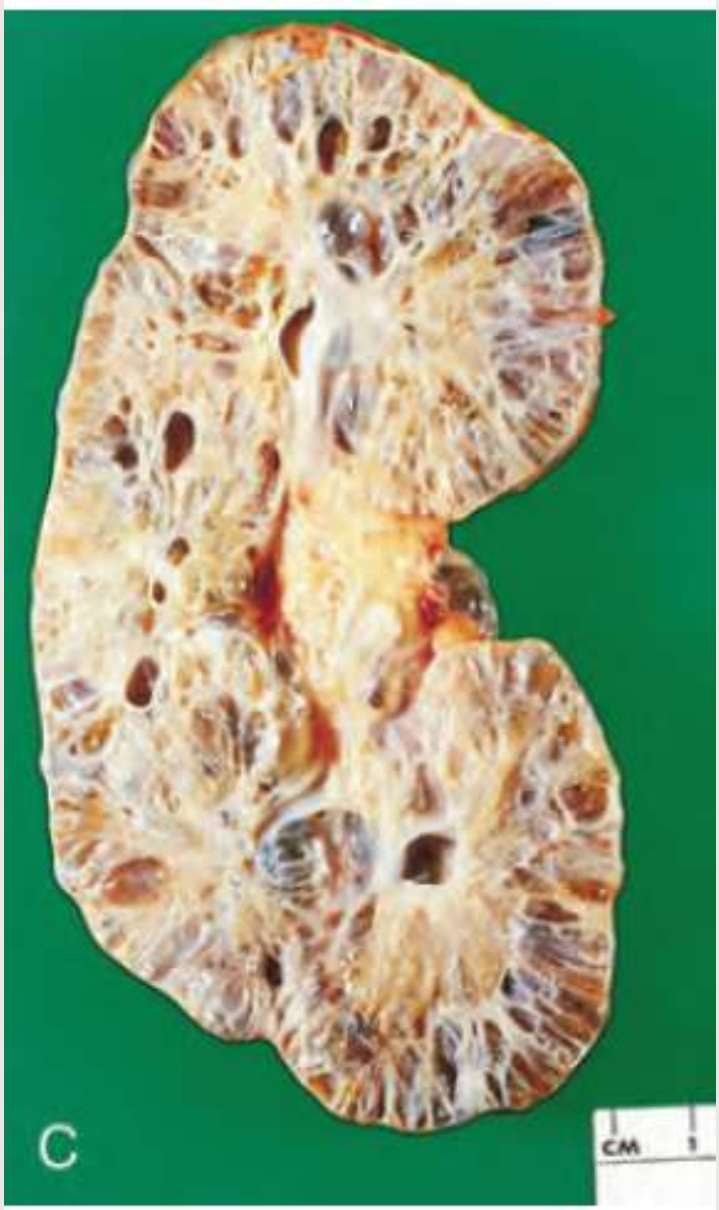


Fig. 11.3

Adult PCKD (AD):

1. Genes-

PKD1- 16p 13.3- Poly cystin I-85%

Found on tubular epithelia cells especially DCT.

Involved in cell – cell and cell – matrix interactions.

PDK2- 4Q 21- Polycystin II -10%.

Found in all tubular epithelia cells.

Involved in intracellular Ca2+regulation.

2. Chances of developing renal failure-

	PKD1 Mutations	PKD 2 Mutations
By 40 yrs	<5%	-
By 50 yrs	>35%	< 5%
By 60 yrs	>70%	15%
By 70 yrs	>95%	45%

3. C/F:

- Asymptomatic with normal renal function until middle age.
- Presents with renal insufficiency, haematuria and HT.
- Abdominal mass with flank pain.
- Most patients develop end- stage renal failure by their seventh decade.

4. Gross:

- Massive bilateral kidney enlargement, bulging cysts.
- Cysts filled with serous, turbid or haemorrhagic fluid.

5. Microscopy: Functioning nephrons are present between the cysts:

6. Extra renal manifestations:

- Liver cysts (40%) (most common extrarenal manifestation) (*). Cysts in spleen, pancreas, lung.
- I/C berry anenrysm.
- Mitral valve prolapse (20-25%).
- Colonic diverticulae.

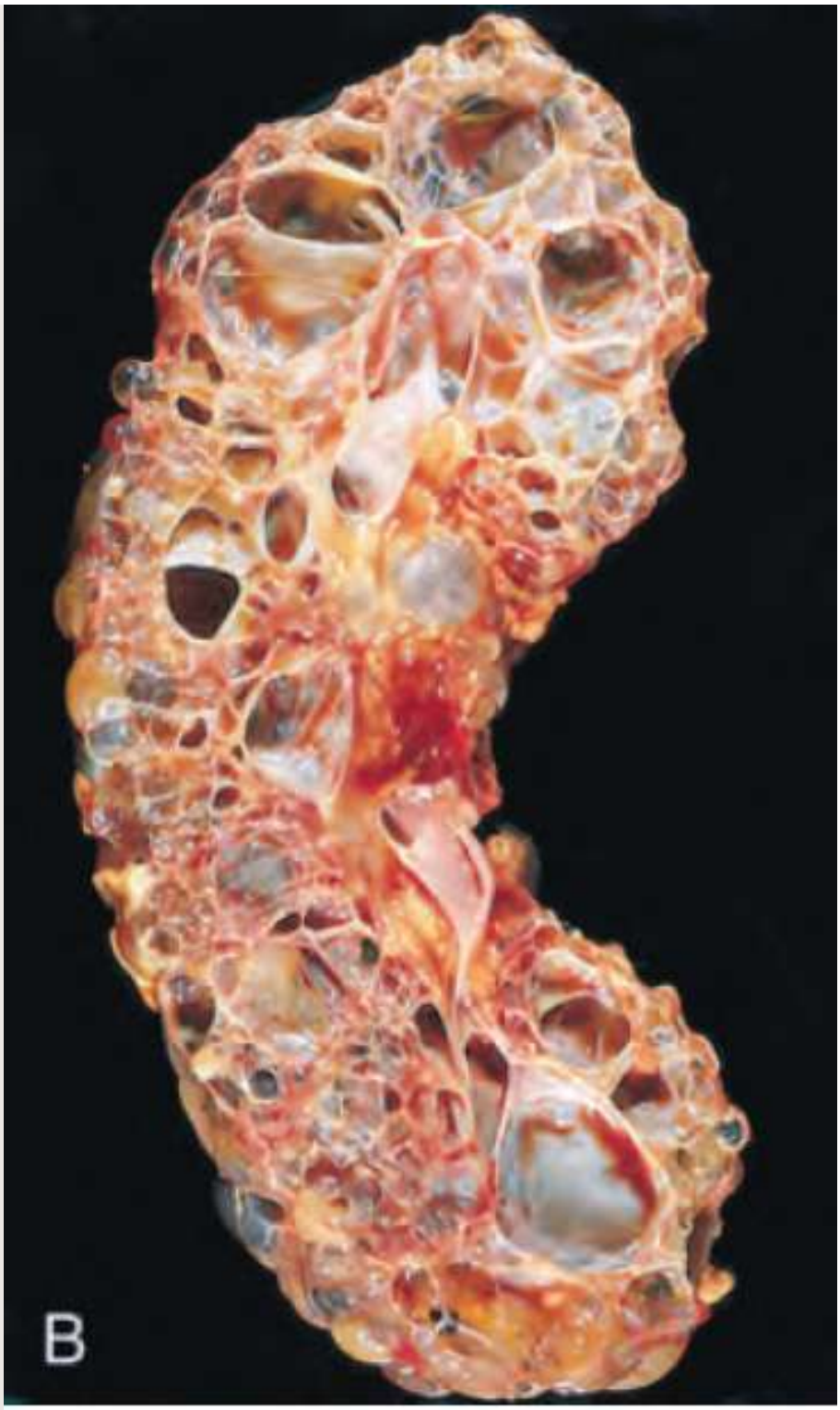


Fig. 11.4

Medullary Cystic Disease:

1. Medullary sponge kidney.

- Multiple cysts in medulla.
- Arise from collecting ducts Pathogenesis – NK.

C/O: Hematuria, UTI, Calcification stones.

2. Nephonophthisis – Uremic Medullary cystic disease complex-

4. Variants:

a. Sporadic Non familial (20%).

b. Familial Juvenile Nephronothisis (40%-50%) AR.

c. Renal – Retinal dysplasia (AR with Retinitis pigmentosa) (15%).

d. Adult onset Medullary Cystic disease (AD) (15%).

Clinical Features:

Polyuria, defect in concentrating Ability

Tubular Acidosis.

Salt wasting.

RF in 5-10 years.

Cysts in medulla with cortical tubular atrophy & interstitial fibrosis.

Gross: Small, Contracted with cysts in Medulla, Corticomedullary junction; some in cortex.

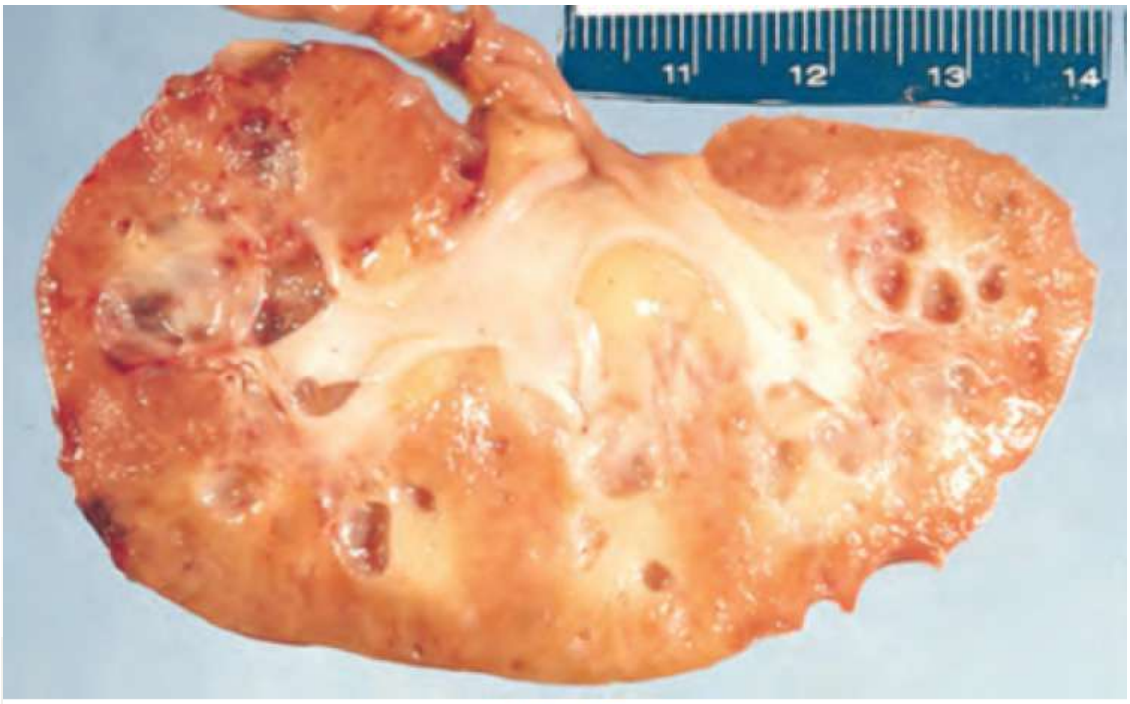


Fig. 11.5

3. Dialysis Associated Cysts:

- Prolonged Dialysis.
- Numerous cortical & Medullary cysts
- 0.5 – 2cm, clear fluid.
- Obstruction by oxalate crystal and interstitial fibrosis.
- Clinically: Asymptomatic, haematuria in some cases.
- **RCC**- 7% of patients

4. Simple Cyst:

Single or X: in normal sized kidney – cortical cyst.

- 1.5 cm, translucent, lined by gray, glistening smooth membrane, Clear fluid Clinically: Asymptomatic; hemorrhage, pain, calcification D/D – Renal tumors.

Glomerular Diseases:

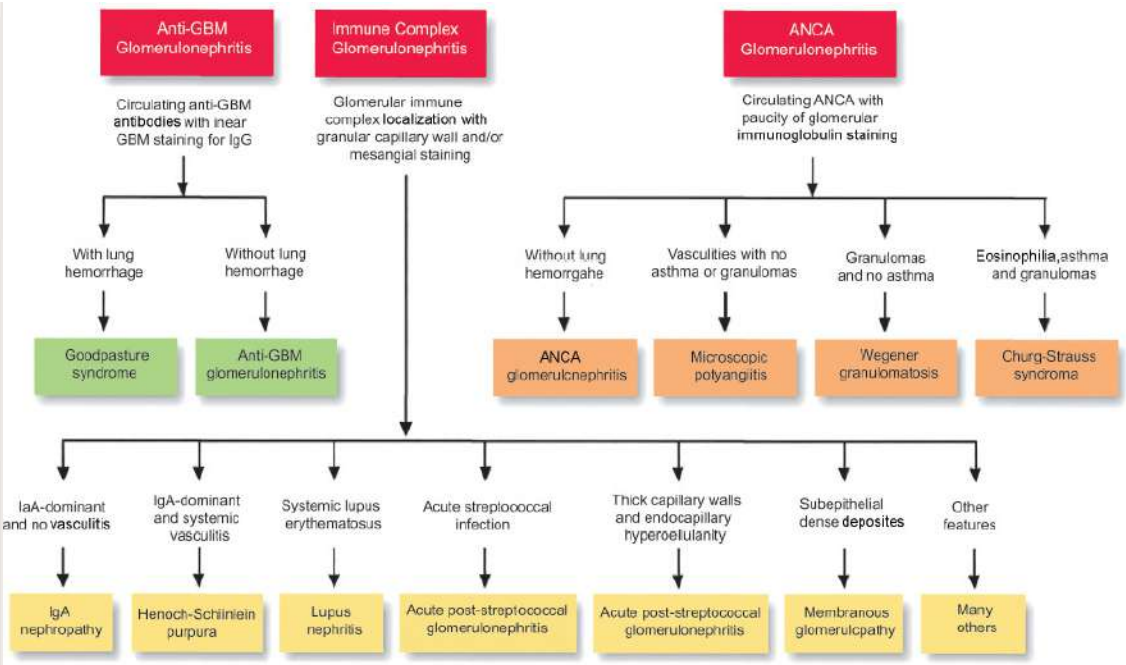


Fig. 11.6

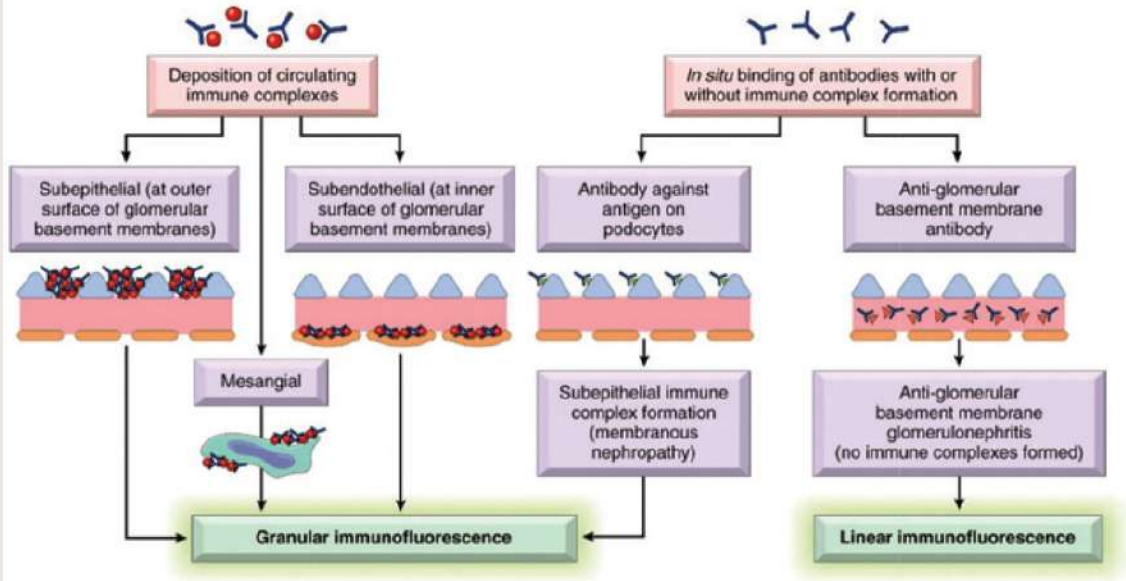


Fig. 11.7

Primary glomerulopathies:

- Acute diffuse proliferative Glomerulonephritis.
- Poststreptococcal.
- Non- poststreptococcal.

- Rapidly progressive (crescentic) glomerulonephrtitis.
- Membranous glomerulopathy.
- Minimal change disease.
- Focal segmental glomerulosclerosis.
- Membranoproliferative Glomerulonephritis.
- Iga nephropathy.
- Chronic Glomerulonephritis.

Systemic Diseases with Glomerular Involvement:

- Systemic lupus erythematsus.
- Diabetes mellitus.
- Amyloidosis.
- Goodpasture syndrome.
- Microscopic polyarteritis / polyangiitis.
- Wegener granulomatosis.
- Hench- schonlein purpura.
- Bacterial endocarditis.

Hereditary Disorders:

- Alport syndrome.
- Thin basement membrane disease.
- Fabry disease.

Disease	Clinically	Light Microscopy (H And E, Silver/ Pas Special Stain)	Immuno-fluorescence	Electron Microscopy
Minimal change disease	Nephrotic	No change	Negative	Effacement of foot processes.
Focal segmental glomerulosclerosis	Predominantly nephrotic	Focal and segmental hyalinosis and sclerosis.	Usually negative	Effacement of foot processes, epithelial denudation.
Membranous glomerulopathy.	Nephrotic	Capillary thickening, "spike and dome" appearance.	Granular IgG and C3	Subepithelial deposits.
Membranoprolifer-ative glomerulone-phritis type 1.	Nephrotic/ Nephritic	Mesangial and endocapillary proliferation, "tram track" appearance.	Granular IgG, C3, Clq, C4	Subendothelial deposits.
Acute infectious glomerulonephritis	Nephritic	Endocapillary proliferation, mesangial proliferation, leucocytic infiltration.	Granular IgG, C3	Primarily subepithelial (early-subendothelial).
IgA nephropathy	Recurrent hematuria	Mesangial proliferation	IgA +/- IgG, IgM, C3 in the mesangium.	Mesangial and paramesangial deposits.
Pauci immune glomerulonephritis (ANCA mediated).	Rapidly proliferative glomerulonephritis	Extra capillary proliferation, crescent formation.	Fibrin in crescents.	No deposits, fragmented GBM.

Nephritic Syndrome:

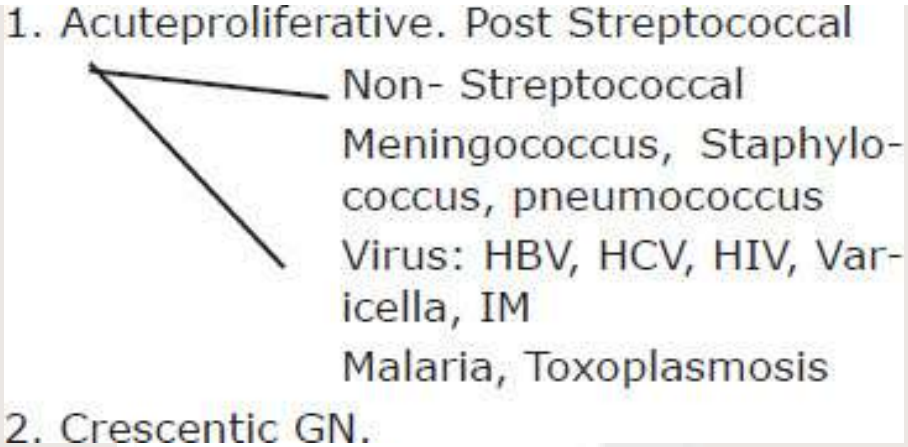
Acute Glomerulonephritis-

Inflammatory alterations in glomeruli with nephritic syndrome.

Clin: Acute nephritis.

Secondary – SLE, PAN.

Primary



1. Acute Proliferative (P. Streptococcal) GN:

Age: 6-10 Years

1-4 weeks after streptococcal infection.

Group A, β hemolytic streptococci.

(Type 12, 4, 1).

Antigen: Endostreptosin, Cationic antigens (proteinase), Nephritis strain associated protein (NSAP).

B/ Chem. ↑ ASO, ↓ serum complement esp C3.

Cryoglobulins (+)

H/P:

Glomeruli: Enlarged, hyprcellular (endothelial cells, mesangial cells, leucocytes)

E/M: Subepithelial, discrete electron dense humps (antigen – antibody complexes)

I/F: Granular deposits of IgG, IgM and C3 throughout the glomerulus.

Clin: Hematuria (Smoky urine/ Cocoa Coloured): Proteinuria ($<^{3}$ g/d).

HT, Qedema, Azotemia.

Spontaneou.

Recovery — Children: 95% (5% RPGN, CGN)

Adults: 60% (40% RPGN, CGN)

Complication- Chronic glomeruli nephritis.

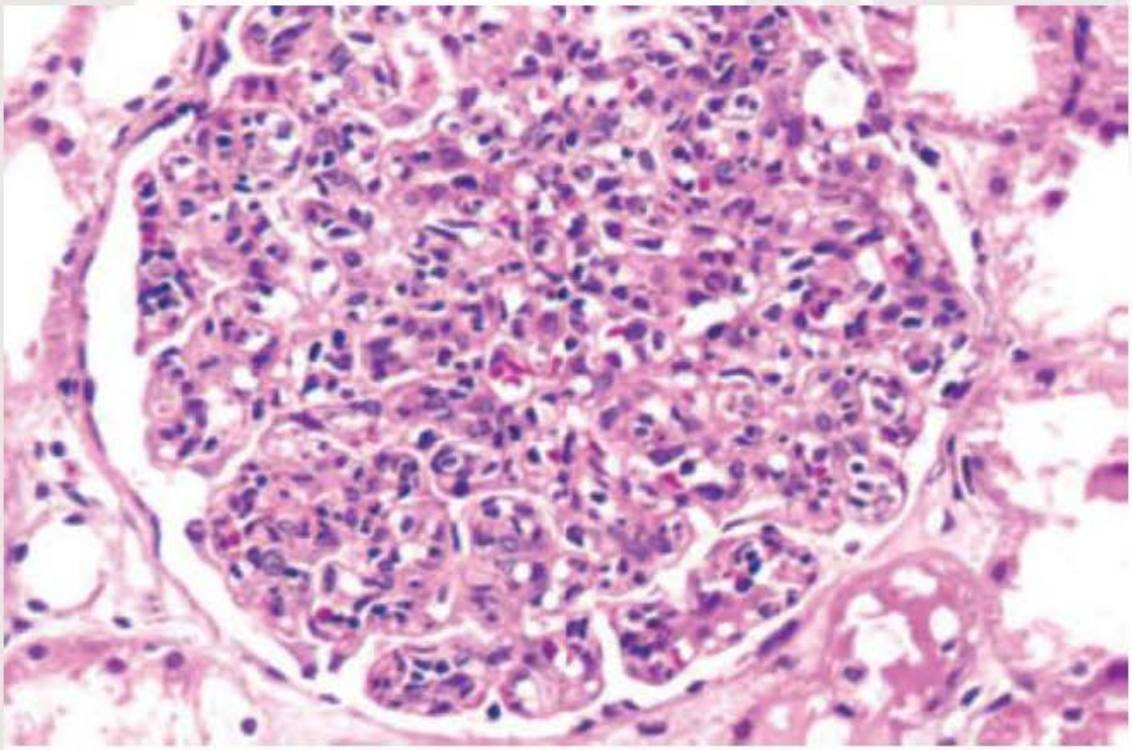


Fig. 11.8

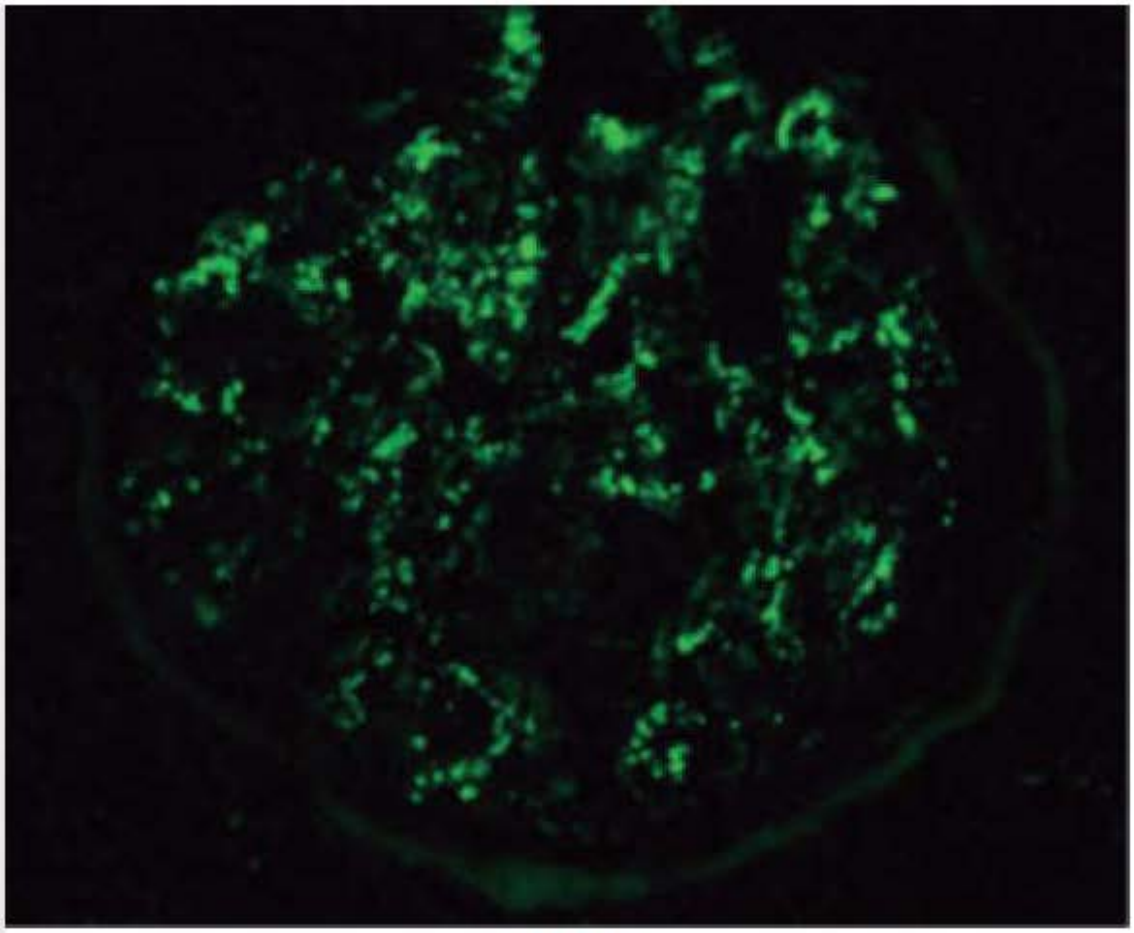


Fig. 11.9

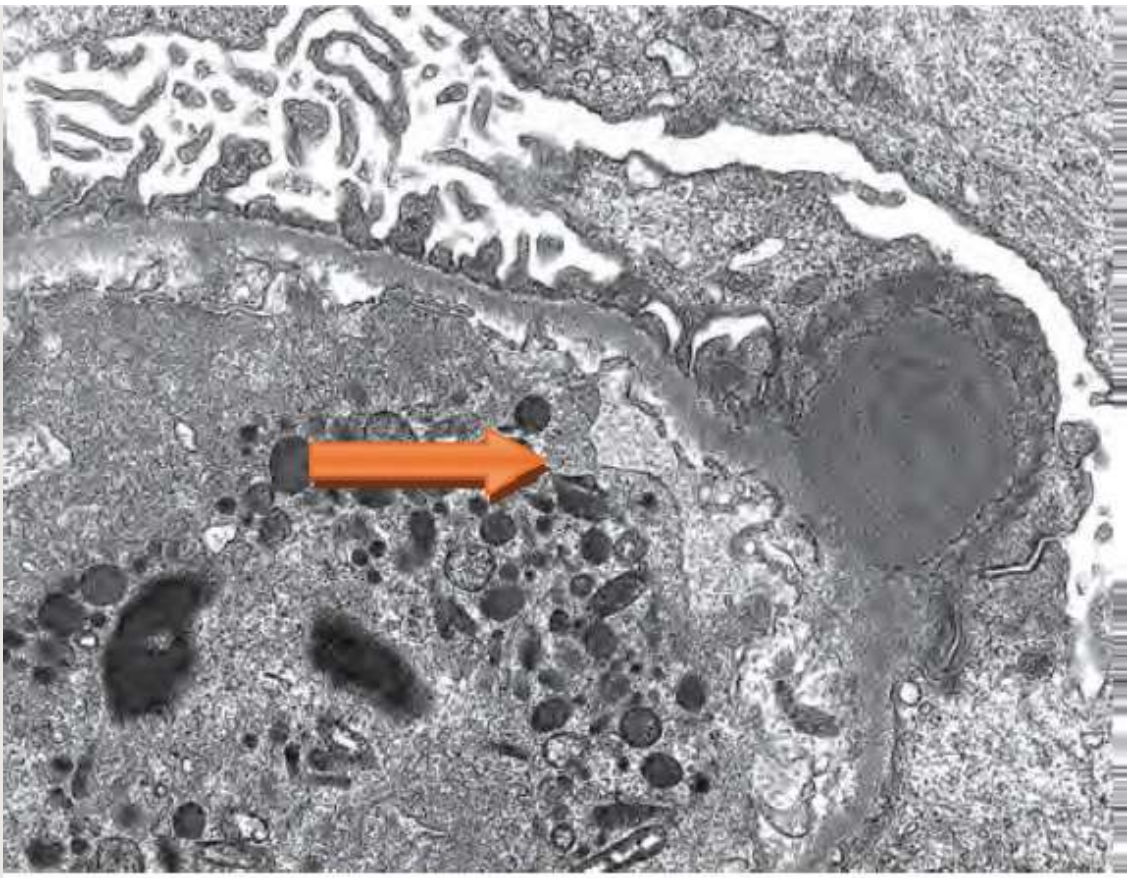


Fig. 11.10

2. RPGN (Crescentic):

Rapid loss of renal function and severe oliguria with renal failure within weeks to months if not treated.

Crescents formed in Bowman capsule.

Classification:

Type 1 RPGN / Anti GBM disease:

- Smooth and Linear deposits of IgG, C3 in GBM.
- Antigen resides in alpha 3 portion of Type IV collagen/diopathic.

Good Pasture Syndrome:

(Pulmonary hemorrhages with renal failure).

Anti GBM anti bodies cross react with pulmonary alveolar basement membrane. M>F, 20-40 yrs.

Pulmonary involvement preceded renal disease.

Type II RPGN: (Immune Complex Disease):

Idiopathic –granular pattern of deposits.

Post infectious.

SLE, H-S Purpura etc.

Type III RPGN:

Pauci immune / ANCA associated.

Idiopatic IF shows no deposis.

Wegner's granulomatosis (C-ANCA against proseinase 3 in azuroplilic granules).

Microscopic Polyarteritis nodosa (P-ANCA against MPO).

Gross: Kidney enlarged, pale with petechial hemorrhage (flea bitten kidney).

Histopathology:

- Crescents due to proliferation of epithelial cells.
- Infiltration by monocytes & macrophages
- Fibrin strands in between cellular layer in crescent.

Electron Microscopy:

- Variable, may or may not have electron dense deposits.
- Rupture in BM indicating severe glomerular injury.

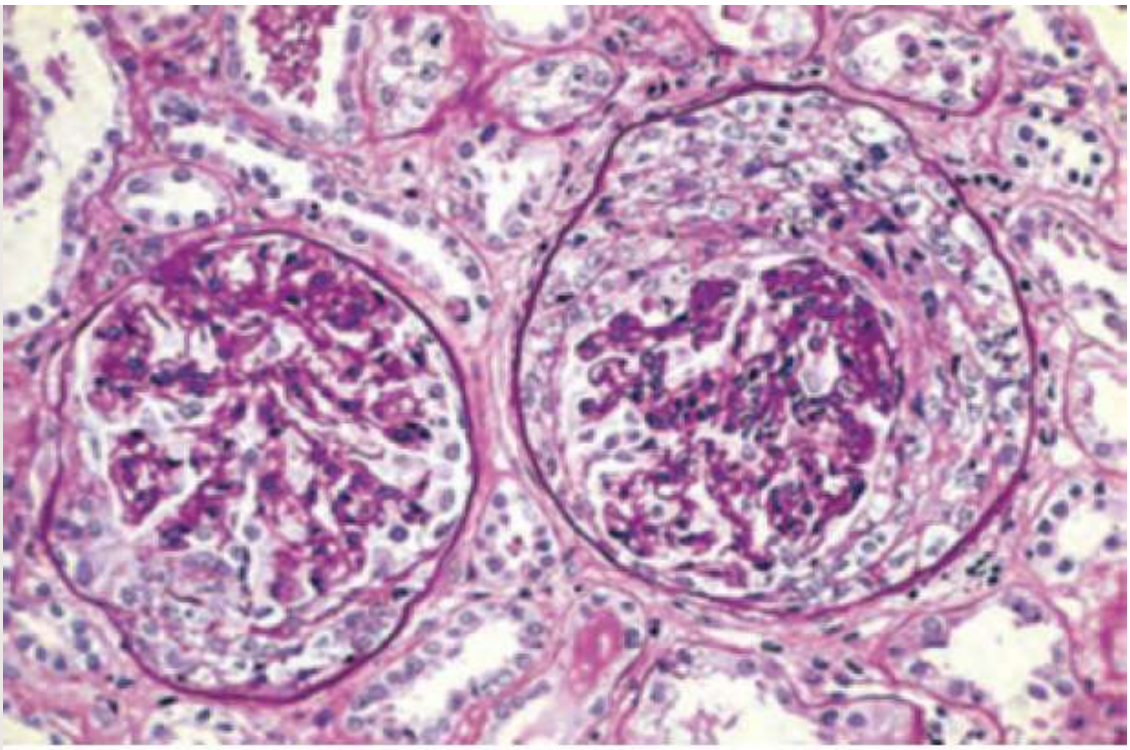


Fig. 11.11

Nephrotic Syndrome:

Most common cause:

Children - Lipoid nephrosis

Adults - Focal and segmental glomerulosclerosis.

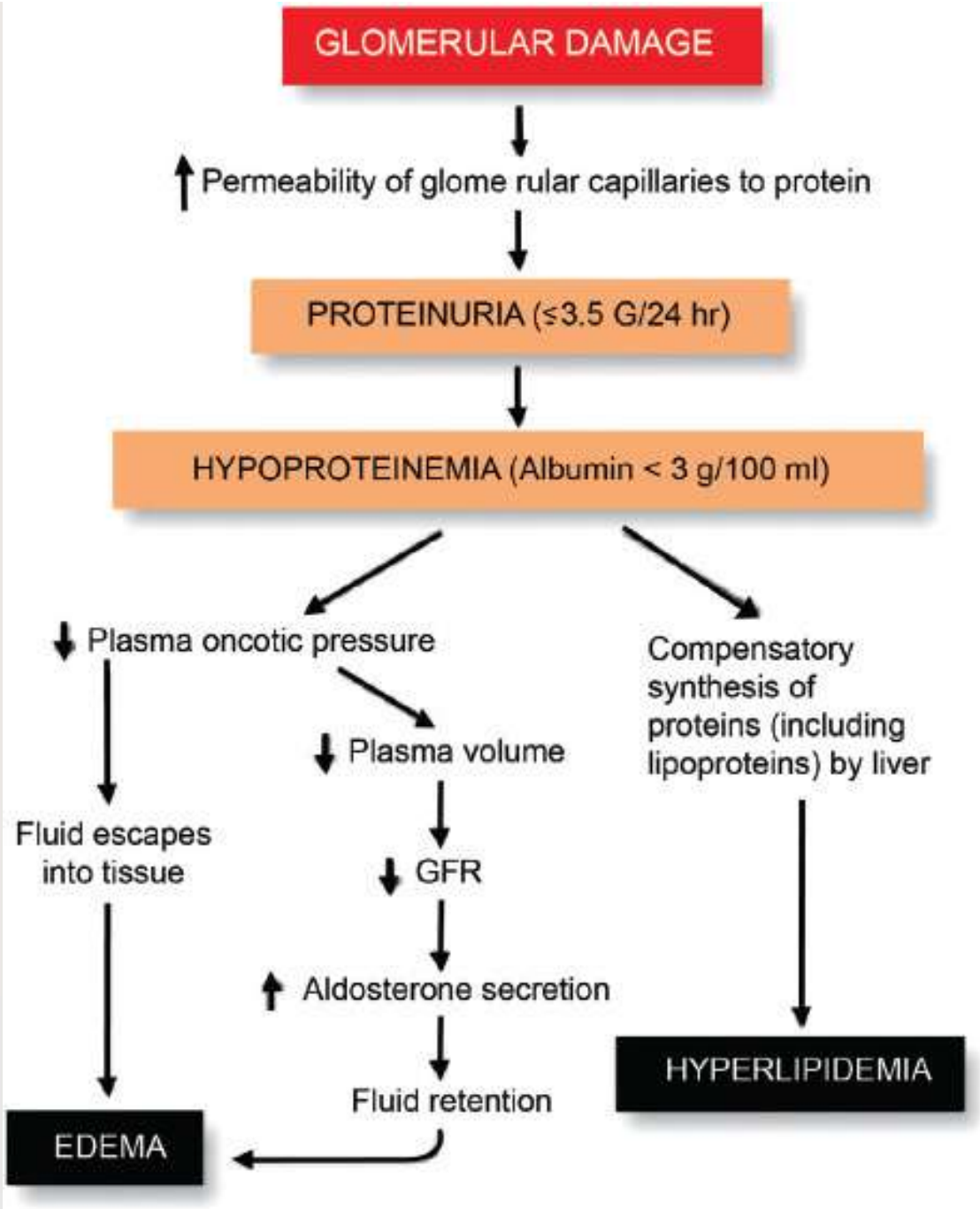


Fig. 11.12: Causes of Nephrotic Syndrome

Primary Glomerular Disease:

1. Membranous glomerulopathy.
2. Minimal change disease.
3. Focal segmental glomerulosclerosis.

4. Membranoproliferative GN.
5. Other prolifertive glomerulopathies.

Systemic Diseases:

Diabetes mellitus.

Amyloidosis.

Systemic lupus erythematosus.

Drugs (nonsteroidal anti-inflammatory, penicillamine, “street heroin”

Infections (malaria, syphilis, hepatitis B and C,

Acquired immunodeficiency syndrome)

Malignant disease (carcinoma, lymphoma)

Miscellaneous (bee-sting allergy, hereditary nephritis)

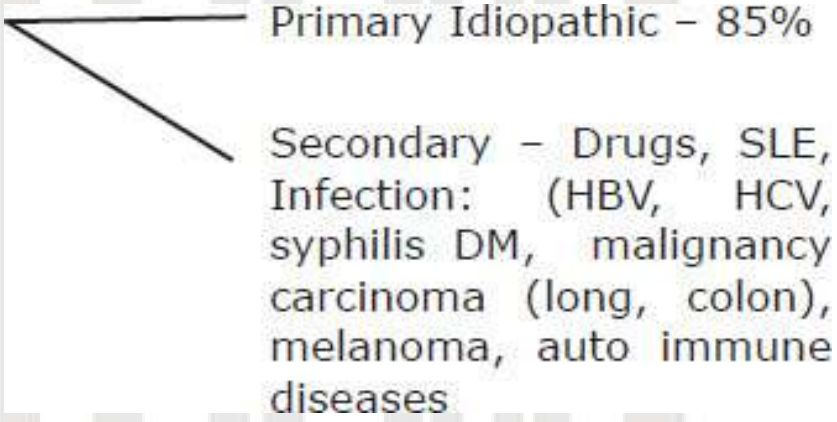
Children less than 15 years → Nephrotic syndrome due to primary kidney cause

In adults even systemic cause to be ruled out

Pathologic Features of Important Causes of the Nephrotic Syndrome				
	Minimal-Change Glomerulopathy	Focal Segmental Glome-rulosclerosis	Membranous Glome-rulopathy	Membrano-proliferative Glomerulone-phritis
Light microscopy	No lesion	Focal and segmental glomerular consolidation	Diffuse global capillary wall thickening	Capillary ml thickening and endocapillary hypercellularity
Immuno-fluorescence microscopy	No immune deposits	No immune deposits	Diffuse capillary wall immunoglobulin	Diffuse capillary wall complement
Electron microscopy	No immune deposits	No immune deposits	Diffuse subepithelial dense deposits	Subendothalial (type 1) dense deposits; intra membranous (type II) dense deposits

3. Membranous Glomerulonephritis:

- Characterized by diffuse thickening of glomerular capillary wall & electron dense subeptithelial deposit in BM
- MC cause of nephritic syndrome in elderly



Pathogenesis:

Heymann nephritis.

- Auto antibodies against renal glomerular protein megalin (gp 330)

Subepithelial deposits of Ig, Complement

↓

Basement membrane material laid down between deposits as spikes

↓

Spikes thicken to produce domes which bury the antibody deposits Membrane thickening encroaches on capillary lumen, sclerosis of mesangium.

Light microscopy:

- Diffuse membrane like thickening of capillary walls.
- Basement membrane projections (“spkes”) seen on silver strains.

Immunoflouroscence:

- Granular and linear pattern of IgG and C3.

Electron microscopy:

- Subepithelial deposits along the basement membranes.
- Effacement of foot processes of podocytes.

Prognosis:

- Variable course.
- 85% Nephrotic syndrome.
- 15% Non Nephritic proleinuria. Poor response to cortico steroids.
- Persistent Proteinuria in >60% patients 10% may progress to renal failure within
  10 years.

Minimal Change Disease:

(Lipoid Nephrosis, Nil Disease):

- Characterized by diffuse loss of foot processes of epithelial cells.
- 2-6 years.
- Selective proteniuria – good response to Csteroids.
- Most common cause of NS in children

  L/M: (Normal glomeruli, Lipid laden PCT cells).

  I/F – Negative; no immune complexes.

  E/M NO dense deposits.

Visceral epithelial cells have effacement of foot processes of podocytes.

- Retraction, Vasculization & microvillous transformation also seen.

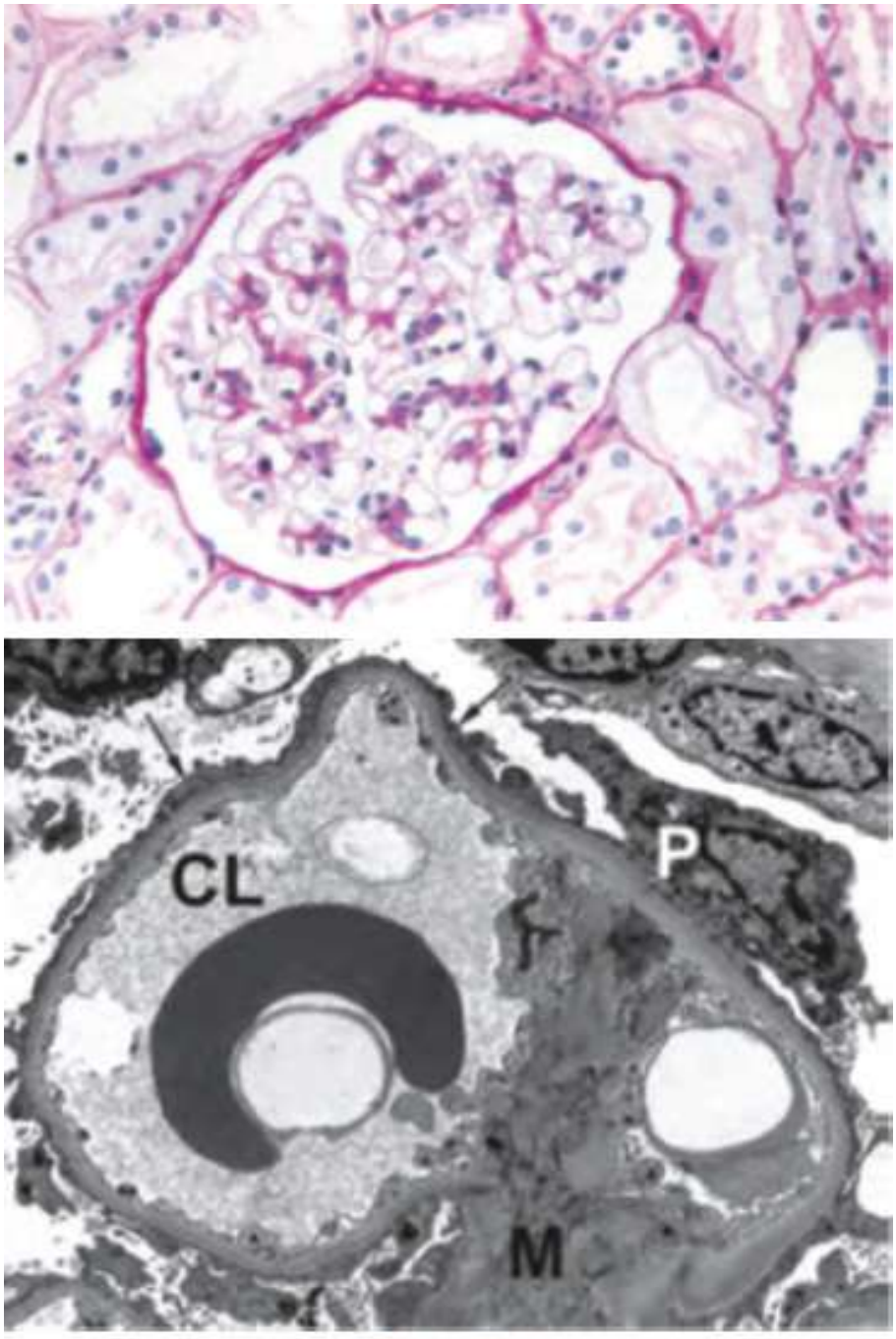


Fig. 11.13

Causes:

- Associated with respiratory tract infection; immunization; atopic diseases; Mutation in renal glomerular proteins ‘Nephrin’ (in hereditary: ’Finnish type’).

Sec:

- Hodgkin's disease.
- Leukemia / lymphoma.
- NSAID therapy.

Focal Segemental Golmerulosclerosis:

a. Clinical features:

i. African Americans> Caucasians.

ii. Occurs in all ages.

iii. Nephrotic syndrome / heavy proteinuria.

b. Etiology:

i. Idiopathic (primary) 10-15% cases.

ii. Associated with loss of renal tissue – U/L kidney agenesis.

iii. Superimposed on other gomerular diseases, such as IgA nephropathy.

iv. Sickle cell anemia.

v. Heroin abuse.

vi. AIDS.

vii. Morbid obesity.

viii.Inherited due to mutations ingenes encoding nephrin, podocin, α- actinin.

c. Light microscopy:

i. Focal segmental sclerosis and hyalinization of glomeruli.

ii. Initially affects the glomeruli along the medullary border.

Focal: only some of the glomeruli are affected.

Segmental: only a portion of the glomerular tuft shows sclerosis.

d. Immunoflurescence: IgM and C3 deposits in the sclerotic glomeruli are affected segments.

e. Electron microscopy:

i. Nonsclerotic regions exhibit effacement of foot processes.

ii. Sclerotic segments show increased mesangial matrix.

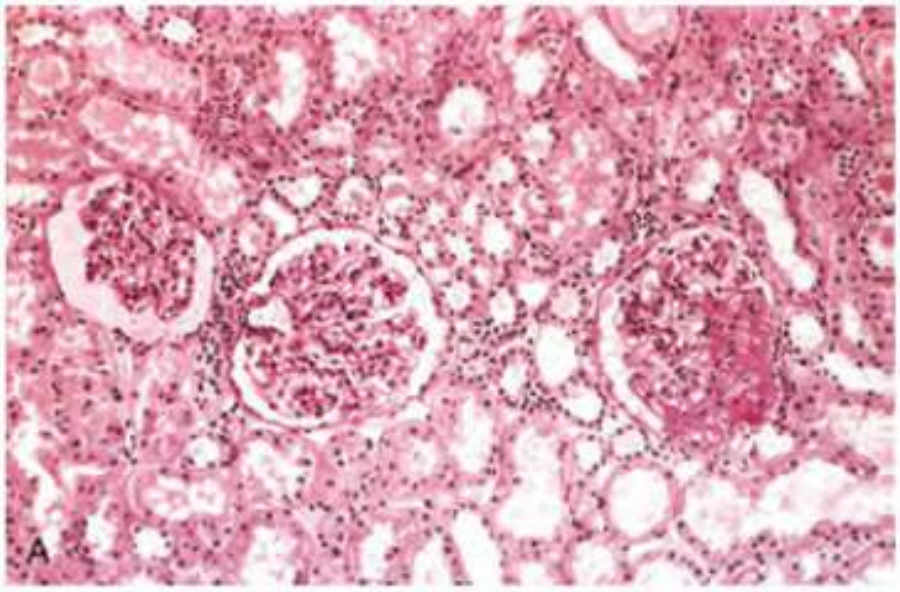


Fig. 11.14

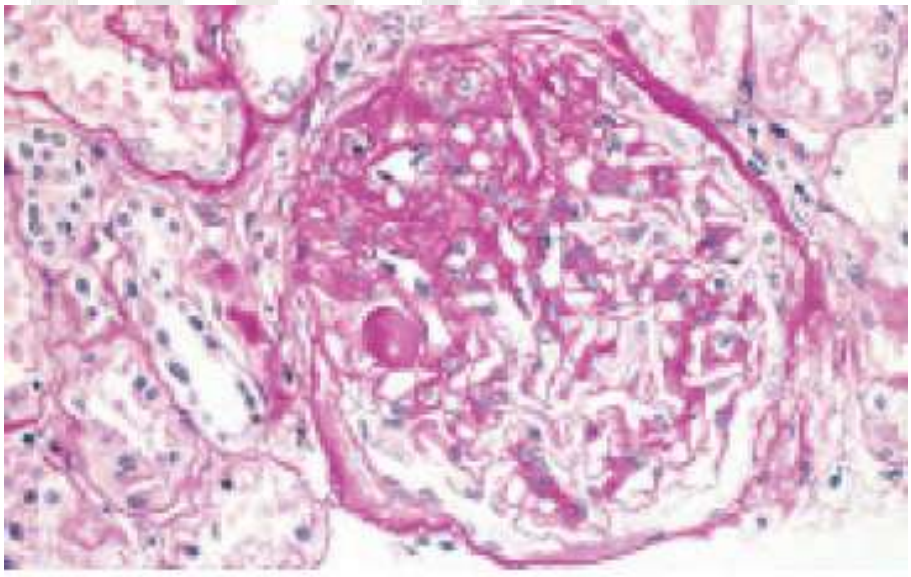


Fig. 11.15

f. Treatment:

i. Poor response to steroids, non – selective proteinuria

ii. High rate of recurrence in renal transplants

iii. 50% go to end stage renal disease

g. Prognosis

i. Poor; children do better than adults

ii. Most progress to chronic renal failure

h. Pathogeneses – Diffuse epithelial damage

Variants: collapsing variant, glomerular tip lesion, peri hilar variant, cellular variant fsgs.

Collapsing Variant:

Seen in HIV patients.

Rapid down hill course.

Collapse and sclerosis of entire tuft.

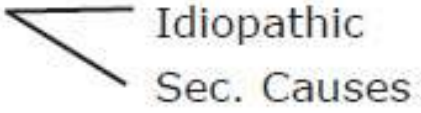
Cystic dilatation of tubules.

Tubuloreticular inclusions in endothelial cell.

Membrano Proliferative Gn/Mesangiocapiillary Gn:

10-20% of nephritic syndrome in children / adults.

- Double – contour, Tram track appearance.
- Characterized by:
  - alteration in BM.
  - proliferation of glomerular cells.
  - Leukocyte infiltration.



Causes:

1. Chronic immune complex disorder – SLE, HBV, HCV, Endocarditis, HIV, schis to somiasis.
2. α 1AT deficiency.
3. Malignant diseases (CLL, lymphoma).
4. Hereditary complement deficiency.

Primary:

Type I: Subendothelial: C3 & IgG.

Activation of classic & Alt. C pathway.

Type II: Lamina densa – irregular ribbon like.

- Dense deposit disease.
- IgG- Absent.

- Activation of alternate C pathway C_3 present but C_4 , C_{1Q} absent.
- IgG- Absent.
- ↓ Factor B & Properdins.
  - C3NeF → stabilizes C3 convertase: also seen in partial lipodystrophy.

L/M:

enlarged cellular glomeruli, (mesangial cell proliferation) thickened basement membrane, double contour or tram track due ot mesangial, endothelial or leukocyte interposition. PAS/ Silver stain- Double contour or Tram Track.

Clin:

Nephritic syndrome haematuria,

Proteinuria, RPGN.

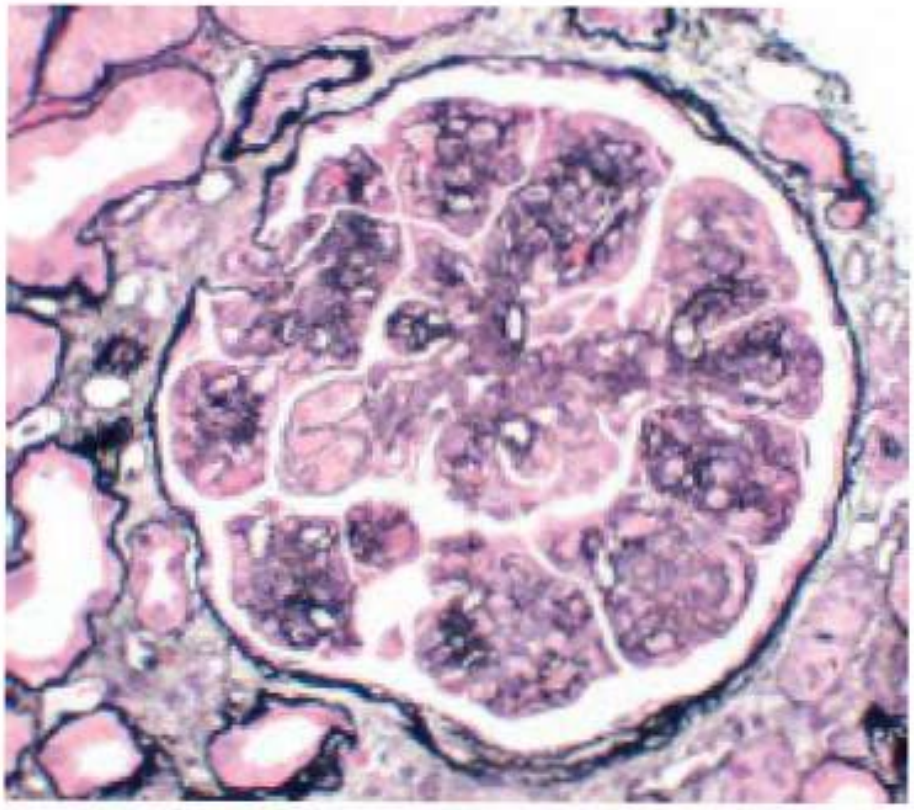


Fig. 11.16

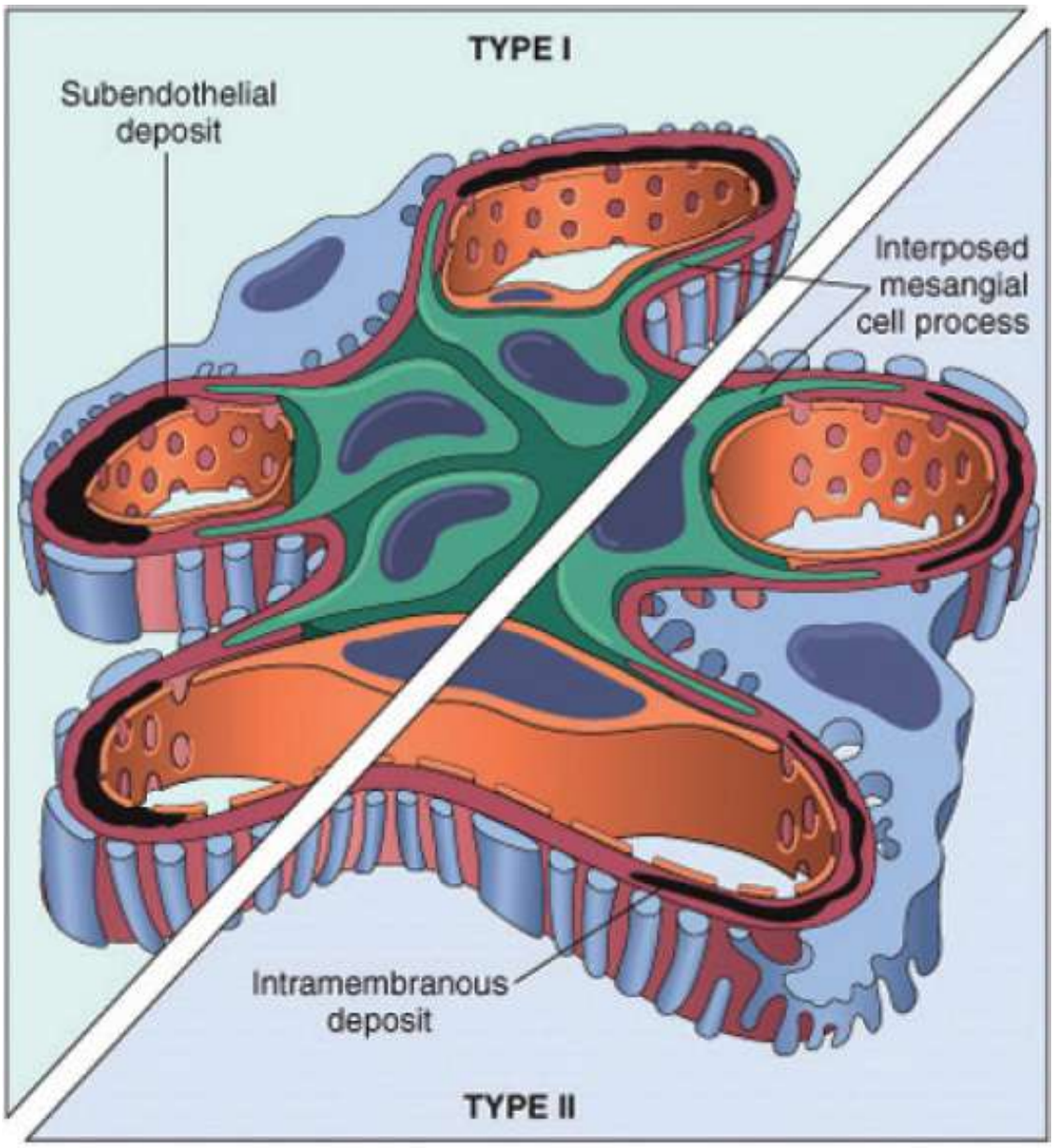


Fig. 11.17

IgA Nephropathy (Berger's Disease):

- Most common Glomerulonephritis world wide.
- IgA in mesangium, activation of alternative C pathway.
- Gross/Microscopic, haematuria, mild proteinuria.

IgA deposition seen in:

- Berger's (IgA nephropathy).
- H.S. Purpura.
- Secondary IgA nephropathy (Liver & Int. disease e.g. gluten enteropathy).

IgA Monomeric (n).

Polymeric: ↑ in Berger's disease- alternate complement pathway activation

Ag. Unknown

Qualitative alterations in IgA.

L/M: (N) / Mesangial widening & Proliferation or focal proliferation or crescentic. GN

I/F: Mesagial deposistion of IgA, C3.

25-50% progress of CRF.

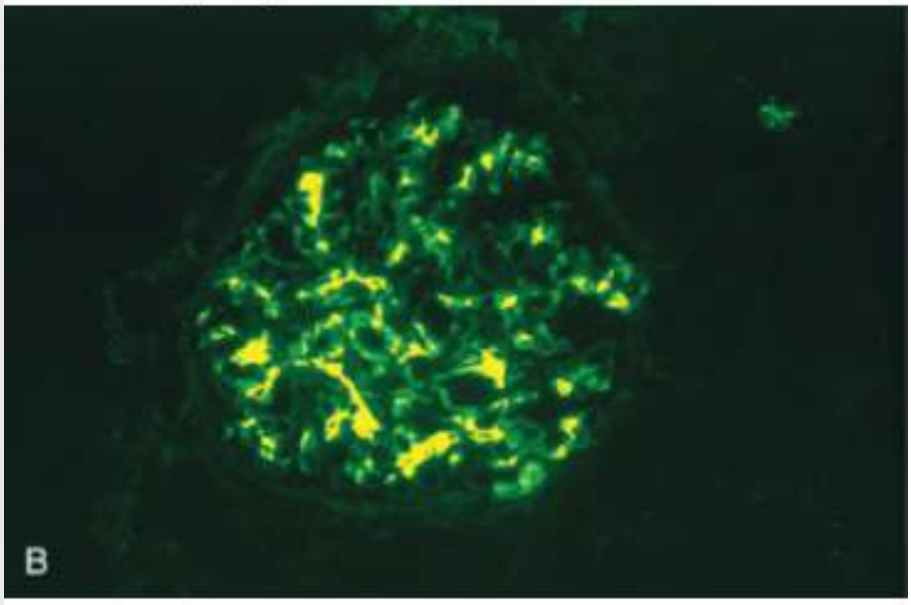


Fig. 11.18

Focal Proliferative & Necrotizing GN:

Associated with systemic disease: SLE, PAN, SABE, Wegner's

- IgA nephropathy.

Idiopathic.

Hereditary Nephritis:

Alport's Syndrome:

Nephritis and Nerve deafness.

X linked disease with females being carriers, there is increased incidence of alport in females explained by llyon hypothesis (inactivation of X chromosome). Eye Lens dislocation, post, cataract, corneal dystrophy.

M>F; 5-20 y;

E/M: GBM: Irregular foci of thickening & thinning with splitting & lamination of L densa.

IHC: Absent α3, α4, α5 collagen.

Defect in gene encoding α5 chain of collagen Type IV- defective GBM synthesis in X linked diseases.

Mutations of α3, α4 chains of type IV collagen in Autosomal recessive (15%)

Clinically: Gross microscopic haematuria

RBC casts, proteinuria.

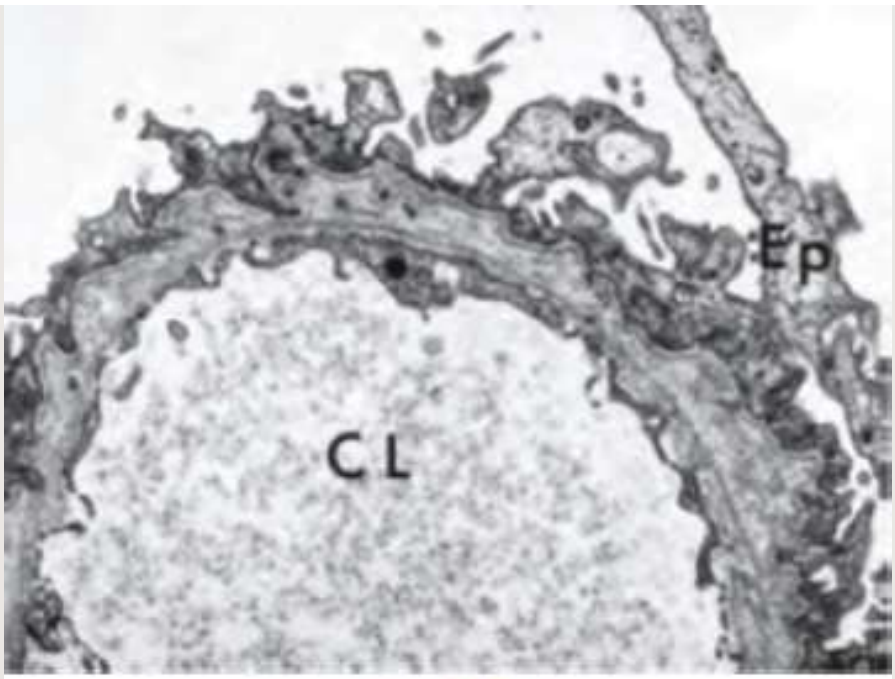


Fig. 11.19

Thin Membrane Disease (Benign Familial Hematuria):

GBM thinned to 150-225 nm (normal 300-400 nm).

- Hematuria /Proteinuria / Asymptomatic
- Abnormality in gene encoding α3 & α4 chains.

Chronic Glomerulonephrtitis:

Def: End stage disease characterized by progressive renal failure, uremia and ultimately death.

MGN (50%)
MPGN (50%)
IgA (30-50% } Slow progression

Focal sclerosis Fast progression

RPGN (90%).

Port streptococcal GN (1-2%)

Gross: - Symmetrically contracted Diffusely granular.

- Thin cortex: increase peripelvic fat.

M/S hyalinization of glomeruli, interstitial fibrosis, atrophy, of tubules and lymphocytic infiltrate.

Urine Analysis- broad waxy casts.

RX – Dialysis and renal transplantation.

Diabetes Mellitus:

End stage kidney disease – 30% of IDDM

Diabetes affects Glomeruli, arteries & interstitium.

Glomeruli:

- GBM thickening (2-5 years).
- Diffuse Glomerulosclerosis (10-12 years).
- **Kimmel Steil Wilson disease** Nodular Glomerulosclerosis/ Intercapillary / Glomerulosclerosis.

Ovoid, laminated hyaline mass-

Periphery of glomerulus.

Within Mesangium.

Surrounded by capillaries.

- Fibrin Cap: Eosinophilic crescents subendothelial (in peripheral capillaries).
- Arteriosclerosis.
- Athermoa of Renal Artery.
- Necrotizing papillitis.
- Pyelonephritis.

Hencoh – Schonlein Purpura:

Children 3-8 years following URI, atopy.

- Purpuric skin lesions (extensor- Arms, legs, buttocks).
- Abdominal pain, vomiting. Intestinal bleed.
- Non migatory Arthralgia.
- Renal.
- Gross / microscopic haematuria.
- Proteinuria, Nephrotic syndrome.
- RPGN with Crescents.

(C3+IgA+) in Mesangium.

M/S Focal/ Diffuse mesangial proliferation with crescents.

Amyloidosis:

First in mesangial matrix.

B/M thickening.

Tubules & Interstitium:

1. Acute tubular necrosis (ATN):

a. Definition: acute renal failure associated with reversible injury to the tubular epithelium.

b. Clinical features.

i. ATN is the most common cause of acute renal failure in the United States.

ii. Oliguria and elevation of blood urea nitrogen (BUN) and creatinine.

iii. Metabolic acidosis and Hyperkalemia.

iv. Urinalysis shows dirty brown granular casts and epithelial casts.

Causes of Acute Tubular Necrosis and Acute Tubular Injury
Ischemic Prerenal Acute Renal Failure or Ischemic Acute Tubular Injury
Massive hemorrhage
Septic shock
Severe burns
Dehydration
Prolonged diarrhea
Congestive heart failure
Volume redistribution (e.g. pancreatitis, peritonitis)
Nephrotoxin Acute Tubular Injury
Antibiotics (e.g., aminoglycosides, amphotericin B)
Radiographic contrast agents
Heavy metals (e.g., mercury, lead, cisplatin)
Organic solvents (e.g., ethylene glycol, carbon tetrachloride)
Poisons (e.g., paraquat)
Heme Protein Cast Nephropathies
Myoglobin from rhabdomyolysis, e. g., with crush injury)
Hemoglobin (from hemolysis, e.g., with transfusion reaction)

Types:

c. Ischemic ATN:

i. Is the most common cause of ATN.

ii. Is due to decreased blood flow caused by severe hemorrhage,

iii. Vasoconstriction, hypotension, dehydration, or shock.

d. Nephrotoxic ATN, Caused by:

i. Drugs (e.g. polymyxin, methicillin, gentamicin. Sulfonamides).

ii. Radiographic contrast agents.

iii. Heavy metals (e.g. mercury, Acidophilic inclusions), lead, gold).

iv. Organic solvents (e.g. carbon tetrachloride, fatty change, chloroform, methyl alcohol).

v. Ethylene glycol (antifreeze) – ballooning and vacuolar degeneration.

vi. Mushroom poisoning.

vii. Phenol.

viii. Pesticides.

ix. Myoglobin.

- Most vulnerable PCT:

Urinalysis in Acute Renal Failure	
Causes of Acute Renal Failure	Urinalysis Sediment Findings
Acute tubular injury	Dirty brown casts and epithelial cells
Acute glomerulonephritis	Red blood cell casts and proteinuria
Acute tubulointerstitial nephritis	White blood cell casts and pyuria

Pyelonephritis (PN):

Tubules, Intestitium & Renal Pelvis.

- Acute PN- associated with UTI.
- Chronic PN- infection associated with others (Vesicouretirc reflux, obstruction etc.) 85%: gram negative bacilli: E. coli, Proteus, Klebsiella- mostly from patients own faecal flora.
    - Small urethra.
    - Absence of antibacterial properties in vaginal fluid.
    - Hormonal changes.

Acute PN:

- Patchy interstitial neutrophilic infiltration.
- Tubular necrosis – Glomerular sparing.

Complications:

1. Papillary necrosis – bilateral, pyramids, distal 2/3 rd have grey white necrosis.

Other causes.

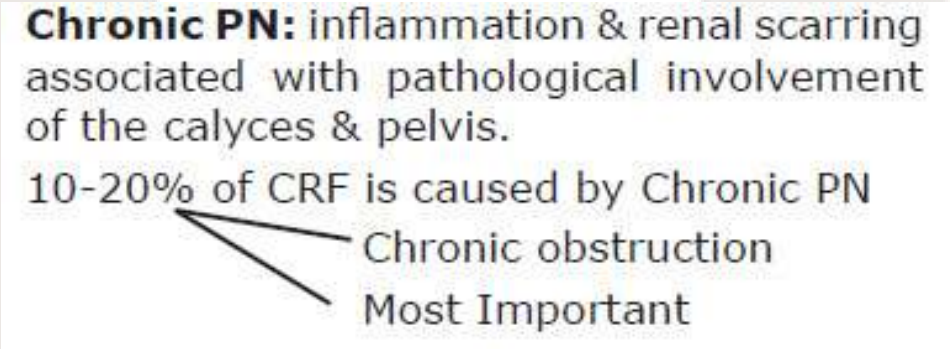
- DM.
- U tract obstruction.
- Analgesic Abuse Nephropathy.
- Sickle cell anemia Renal TB.

2. Pyonephrosis – complete obstruction.
3. Perinephric Abscess- pyelonephritic scar.

Associated with:

- U tract obstruction.
- Instrumentation.
- Vesicoureteric reflux.
- Pregnancy – 4-6% present as bacteruria – 20-40% have UTI.

Presence of Leucocyte casts indicate renal involvements.



10-20% of CRF is caused by Chronic PN

Drugs & Toxins:

1. Acute hypersensitivity nephritis eg. Sulfonamide penicillin, NSAID – fever, cosino plulia rash, renal abnormality.
2. ATH.
3. Chronic renal in sniff.

Gross – kidneys irregularly scarred:

Coarse, discrete, C/M scar, overlying blunted, dilated & deformed calyx

- More in upper & lower poles.

M/S – Thyroidization of tubules.

- Degeneration and regeneration of epithelial lining.
- Interstital fibrosis and chronic inflammation infiltrate.
- Periglomerular fibrosis.
- Xanthogranulomatous PN: Macrophages (foamy) with plasma cells & giant cells.
- Associated with Proteus infection & obstruction.

Analgesic Abuse Nephropathy:

All papillae are at same stage of necrosis important for D/D of diabetes.

- Papillary necrosis followed by tubulointerstitial nephritis.
- Phenacetin – Acetaminophen; covalent binding and oxidative damage.

C/O: Transitional Papillary Carcinoma of renal pelvis.

	DM	Analgesic	Sickle Cell Anaemia	Obstruction Uropathy
Male: Female	1:3	1:5	1:1	9:1
Time course	10Y	7Y	V	V
Infection	80%	25%	±	90%
Calcification	Rare	Freq.	Rare	Frequent
Number of Papilla	Several	Almost all,	Few different Stages of necrosis	V
Affected		All at same Stages		

NSAIDS :

- Ac Renal failure.
- Membranous GN.
- Lipoid Nephrosis.
- Acute HS interstitial nephritis.

Urate Nephropathy:

- Ac. Uric acid nephropathy-Precipitation of crystals in tubules, CD⇒obstruction & ARF

Precipitation favoured in acidic urine.

- Chronic Urate Nephropathy – Ppt. of crystals in DT. CD & interstitium (Birefringent, needle like crystals surrounded by, foreign body giant cell & fibrosis →Tophus) Cortical atrophy & scarring.
- Nephrolithiasis – 22% in gout.

Benign Nephroscleorsis:

Sclerosis of Renal arterioles & small blood vessels – focal ischemia of parenchyma.

- HT, DM, ↑ age.
  Grossly (N) to ↑ size.
- Grain leather appearance.

M/S:

- Hyaline arteriosclerosis & ↓ lumen
  Medial Hypertrophy.
- Fibro-elastic Hyperplasia (interloubular and arcuate arteries).
- Reduplication of elastic LaminaMyofbroblastic tissue in intima.

Malignant HT / Accelerated Nephrosclerosis:

Gross flea bitten kidney.

L/M: fibrinoid necrosis, Necrotizing arteriloitis intravascular thromobosis.

- Hyperplastic Arteriloitis / onion skinning (interlobular arteries) (Concentrically arranged smooth muscle cells & collagen).
- Necrotizing Glomerulitis Increase level of Renin.

Renal Artery Stenosis:

2-5% of HT, curable.

Most comm. Cause.

- www.Atheroma of Renal Artery
- Fibromuscular dysplasia -3 type intimal, medial, adventitial. (Medial most common).
  - Young females (30-40yrs).
  - Kidney small with atrophic tubule, crowded glomeruli, interstitial fibrosis.

Thrombotic Microangiopathies:

- Thrombosis in capillaries & arterioles.
- Microangiopathic hemolytic anemia.
- Thrombocytopenia.
- Renal failure.

Classification:

1. Classic childhood HUS verocytotoxin producing E. coli (0157: H7)].

   - sudden onset: GI influenza like, Prodromal episode hematemesis, malena, oliguria, MAHA neurologic changes.

2. Adult HUS, associated with.

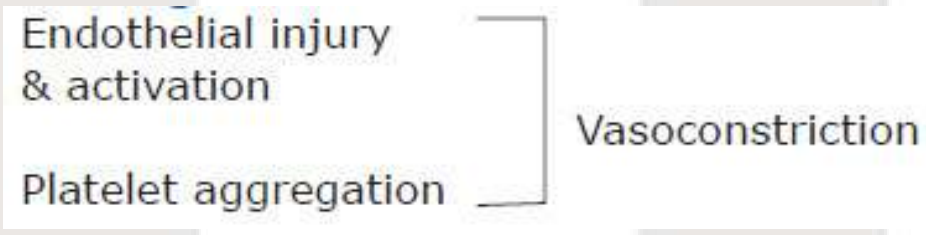
   - Infections- endotoxin – shigella, (shiga) E. coli septicemia, typhoid fever.
   - Antiphospholipid Ab syndrome.
   - C/O of pregnancy of Contraceptives.
   - Vascular renal disease – hypertension, scleroderma.
   - Chemotherapy.

3. Familial HUS- Inherited deficiency of complement regulatory protein factor H (breaks down C3 convertase).
4. Idiopathic TTP- cause – Acquired / genetic defect in ADAMTS -13 (protease that cleaves vwf). CNS involvement dominant renal involvement in 50% patients.

Gross – Kidney shows patchy / wide renal cortical necrosis.

Microscopy –glomruli with thickened capillary wall with fibrin deposits.

Pathogenesis:



Diffuse Cortical Necrosis:

- Following obstretical emergency; Abruptio placenta, Septic, Shock, Surgery.

D/D/ of Granular Kidney:

Arteriolar nephrosclerosis fine, regular >1 mm.

Chronic GN- coarse, 1-3 mm.

Diabetic GS.

Late stage of Amyloidosis.

Shrunken Kidney	Petechial Hemorrhage (9 causes)
CGN	Acute GN- Coagulation disorder
CPN	SABE- Leukemia
Diabetic	Malign. HT- Epidemic hemorrhagic fever
Late stages of Amyloid	Pyuria – Typhus
Cystic disease- Nephronophthisis	Recent infract

Renal Infarcts:

- Embolisation from LV/LA.
- Pale grey white infarct.
- Wedge shaped.

Tumors:

Benign:

1. Renal Papillary Adenoma < o.5 cms, cortical.

- Origin tubules.
- Gray yellow.

3. Angiomyolipoma associated with Tuberous Sclerosis.

TS: N/cut. Syndrome.

- Cortical tubers & Subependymal hamartomas.
- Renal Angiomyolipoma.
- Pulmonary & Cardiac Rhabdomyoma.
- Cysts in liver, kidney & pancreas.

Cutaneous: Angiofibromas.

- Shagreen patch (localized leathery Thickening).
- Ash Leaf patch (hypopigmented).
- Sub ungula fibrous.

Genes:

AD.

TSC 1- chromosome 9q34 – Hamartin.

TSC2- chromosome 16 p 13.3- Tuberin.

4. Oncocytoma:

Tan/ Mahogany brown colour and a central scar.

Origin: Intercalated cell of CD.

Cells: eosinophilic granular – (Because of Mitochondria).

Malignant Tumors:

RCC/ Hypernephroma:

Adeno carcinoma:

6-7th decade.

Risk factors: Tobacco, cigarette, obesity, HT, Estrogen therapy.

Asbestos, petroleum, Heavy metal.

↑ incid. In CRF, acq. Cystic disease.

ASSOCIATED Syndrome:

1. VHL Syndrome AD:

- Retinal angiomatosis.
- RCC.
- Adrenal pheochromocytoma.
- Cerebellar Hemangioblastoma (also in Retina, Brainstem spinal cord).

Gene: chromosome 3 p 25.3 → Elongin.

RCC: X, B/L.

1. Clear cell RCC:

Sporadic clear cell Ca.

- Deletion of chromosome 3.

Hered. Clear cell Ca.

Unbalanced Transl. of 3; 6, 3; 8, 3; 11

2. Papillary RCC:

Sporadic Papillary Ca: Not associated 3 p del.

Hered, Papillary Ca:

Trisomy 7 Activtn of MET.

(MET- p-oncogene; Tyrosine kinase receptor for HGF).

3. Chromophobe RCC: Excellent prognosis, Arises from I/C cells of collecting ducts.

Gross: Upper Pole.

Distort Renal Outline.

Bright Yellow – Variegated.

Invades Renal Vein.

Collecting duct Carcinoma: Hobnail pattern.

Clinically: Pain, lump, Hematuria.

Paraneoplastic syndrome: Polycythemia, Hypercalamia, Hepatic dysfunction, feminization, masculanization, Cushing, Leukemoid Reaction, Eosinophilia, Amyloidosis.

Wilm's Tumor:

- Most common primary renal tumor in children.

Age: 2-5 years.

Pathogenesis & Genetics:

1. WAGR syndrome: 33% chances of developing Wilm's.
(Wilm's Aniridia genital abnormal; MR)
WT-1 gene on chr. 11 p13 (Deletn. / FS/ Non. Sense Mutation).
2. Denys Drash Syndrome.

- Gonadal dysgensis (male pseudohermophrodite).
- Nephtopathy ⇒ Renal failure.
- WT-1: Missense Mutation.

3. Beckwith – Wiedemann syndrome.

- Enlargement of body organs.
- Hemihypertophy.
- Renal medullar cysts.
- Adrenal cytomegaly.
- WT-2⇒ chromosome 11p 15.5.
- ↑ risk of developing hepatoblastoma, Adrenocortical & Pancreatic tumors.

Rhabdomyosarcomas.

Premalignant: Nephroblastomatosis (X diffuse foci of immature nephritogenic elements).

Gross: Solitary, large, well circumscribed

Dwarf's the kidney fish flesh, homogenous

M/S: Blastema.

Epith- Abortive tubules & gomeruli.

Mesenchymal – Cartilage, muscle, fibrous myxoid.

Renal Pelvis, Ureters, Bladder, Urethra- Transitional Ephithelium (3-7 layers in bladder).

Brunn Nests:

- Cystic inclusion of transitional epithelium: Cystitis Cystica.
- Cystic inclusion of intestinal metaplastic epithelium: Cystitis Glandularis.

Cystitis:

Hemorrhagic cystitis: associated with chemotherapy, radiation, adeno virus.

- Supurative Cystitis.
- Chronic cystitis.
- Follicular cystitis.
- Eosinophilic cystitis.
- Interstitial Cystitis / Huner's Ulcer Persistent, painful form of chronic cystitis
- More frequent in women.
- Associated with inflammation and fibrosis of all layers.
- Ulceration and submucosal oedema.

Malacoplakia:

Grossly: Soft, yellow, slightly raised mucosal plaques; 3-4 cm in diameter.

M/S: Large, foamy macrophages, occasional multinucleate giant cells.

Michalis Gutman Bodies: Laminated mineralized concretions.

- *E. coli* / *Proteus* .

Tumors of Urinary Bladder:

95% → Epithelial.

1. Urothelial (transitional cell) tumors:

- Inverted papilloma.
- Papilloma (Exophytic).
- Urothelial tumour of low malignant potential.
- Urothelial Carcinoma.
- Carcinoma in situ.

2. Sq. cell Ca.
3. Mixed Ca.
4. Adeno Ca.
5. Small cell Ca.
6. Sarcoma.

WHO grading of urothelial tumors:

Papilloma – Rare, ≤ 1% bladder tumors, younger pts., stalked with finger like papillae. Normal looking transitional epithelium. Recurrence.

TCC Grade I

TCC Grade II

TCC Grade III

Grade I: Gross: similar to papilloma

- Cytologic and archet. Atypia (but well differentiated).
- ↑ Layers (only slight loss of polarity).
- Seldom invasive, 95% to 98% 10 yrs survival rate.

Grade II: Papillary with flat regions.

- ↑ Layers (>10).
- ↑ Mitosis.
- ↑ Loss of polarity.

(May be associated with invasion, low risk of progression).

Grade III: Papillary flat, or both.

- Larger extensive, most invasive.

- Disarray, Loosening and Fragmentation of superficial layers.
- Necrosis, hemorrhage.

Local spread: Bladder wall, prostate, vesicles, ureter, retroperitoneum.

LN: Regional LNS.

Hematogenous: Liver, lung, Bone Marrow.

Carcinoma in Situ:

High – grade flat abnormality confined to bladder mucosa.

- Appear as area of mucosal reddening/ granularity / thickening without intraluminal mass.
- Seen in surrounding areas of invasive carcinoma.

Staging:

Depth of Invasion	AJC / UICC
Noninvasive, papillary	Ta
Carcinoma in situ Noninvasive, flat	Tis
Lamina Propria invasion	T1
Muscularis propria invasion	T2
Microscopic extravesicle invasion	T3a
Grossly apparent extravesicle invasion	T3b
Invades adjacent Structures	T4

- LN: N 1-3.
- Distant metastasis → M1.

Epidemiology and Pathogenesis:

- M: F= 3:1, 50-80 yrs.
- Cigarette smoking - ↑ risk by 3-7 times.
- Arylamines: β – naphthyamine.
- Schistosomia haematobium.
- Long – term use of Analgesics.
- Heavy long- term exposure to cyclophosphamdies.
- Deletion of 9 p.
- Deletion of 17 p (Tumor supressor gene p16).
- Mutation of P53, Rb.
    - Two pathway mode: deletion of oncogene on
        - 9p → p53 mut
        - p 53 mutation
- 10 Years Survival Recurrence
- Papilloma & Grade I Ca 98% Low grade – 50%
- Grade 11 Ca-40% High grade – 80- 90%

Most common benign mesenchymal tumor – Leiomyoma:

12 Lung

Respiratory System:

Atelectasis –

Areas of airless lung parenchyma.

Reversible change.

- Obstructive- Bronchial asthma, COPD, Foreign bodies. Mediastinum moves towards the obstructed lung.
- Compressive – CHF, Air, fluid or blood into the pleural cavity. Mediastinum moves away from the affected lung.
- Patchy – In Hyaline membrane disease and ARDS.

Commonest source of **Pulmonary emboli** – Deep veins of leg.

Commonest cause of **Pulmonary hypertension** - COPD.

Other causes of pulmonary hypertension Recurrent thromboembolic, Endothelial dysfunction. Ingestion of Bush tea, Adulterated olive oil.

Pulmonary infarcts – $3/4^{th}$ of all infarcts affect the lower lobes. Extend to the periphery of the lung substance with the apex pointing towards the hilus of the lung. Are classically hemorrhagic. Diagnostic feature of pulmonary infarction is the ischemic necrosis of the lung substance within the area of hemorrhage.

Scimitar syndrome is a multifaceted malformation characterized by a large anomalous pulmonary vein that drains from one lung, usually the right, into the inferior vena cava.

Broad roentgeno- graphic shadow of this Vein forms the scimitar.

Bronchopulmonary dysplasia (BPD) found in infant treated for HMD with O_2 and artificial ventilation.

Wilson Mihity syndrome – neonatal hyperaeration pulm. Dysmaturity.

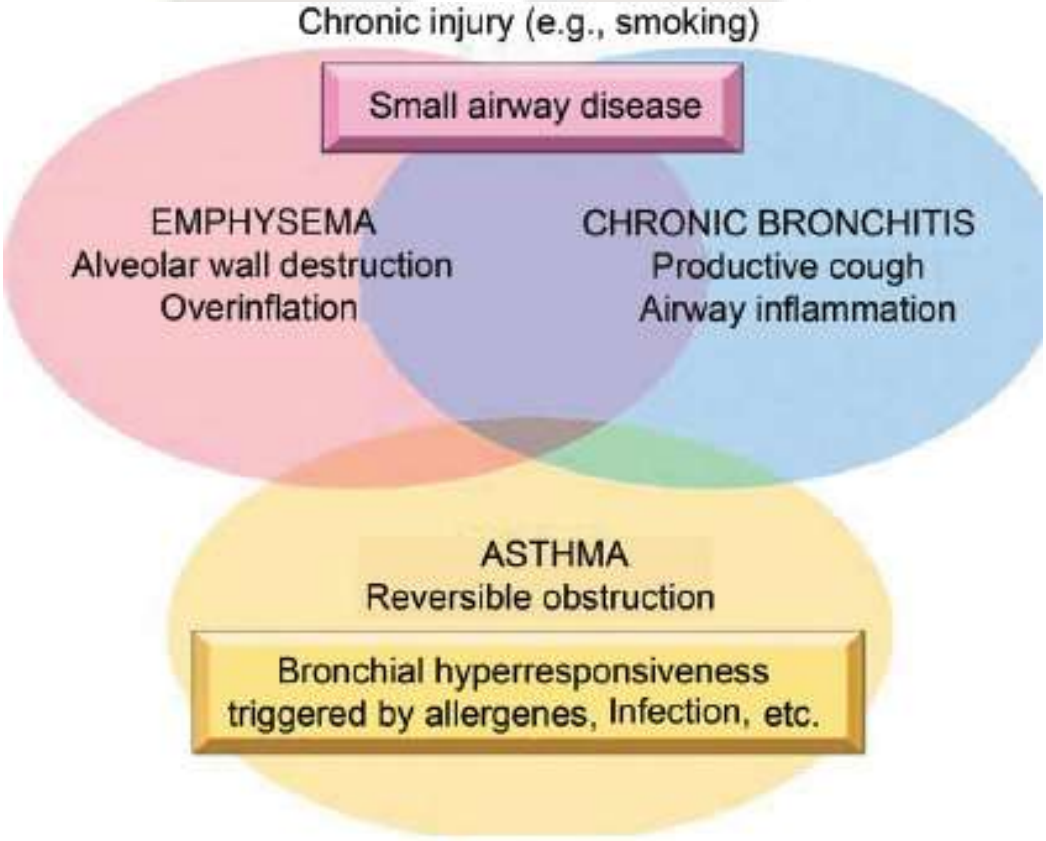


Fig. 12.1

Emphysema:

Abnormal permanent dilatation of acini with destruction of wall without fibrosis.

Pink puffers, 50-75 years of age, early dyspnea, Cough is non – productive and a late feature. Infections are rare. Rarely progress to Cor pulmonale.

Main pathology is loss of elastic recoil.

Types:

Centrilobar	Panacinar	Paraseptal	Irregular
• Resp bronchioles affected, distal alveoli spared.	• Acini enlarged from level of resp. bronchiole to terminal blind alveoli	• Strikingly adjacent to pleura along lobular C.T. septa and at margins of lobules.	• Invariably associated with scarring. Most common form.
• Upper lobes.	• Lower zones anterior margin of lungs.		
• Cigarette smoking, coal dust def. (functional).	• α – 1 antitrypsin.	• Adjacent to areas of fibrosis, scarring or atelectasis • Responsible for spontaneous pneumothorax.	

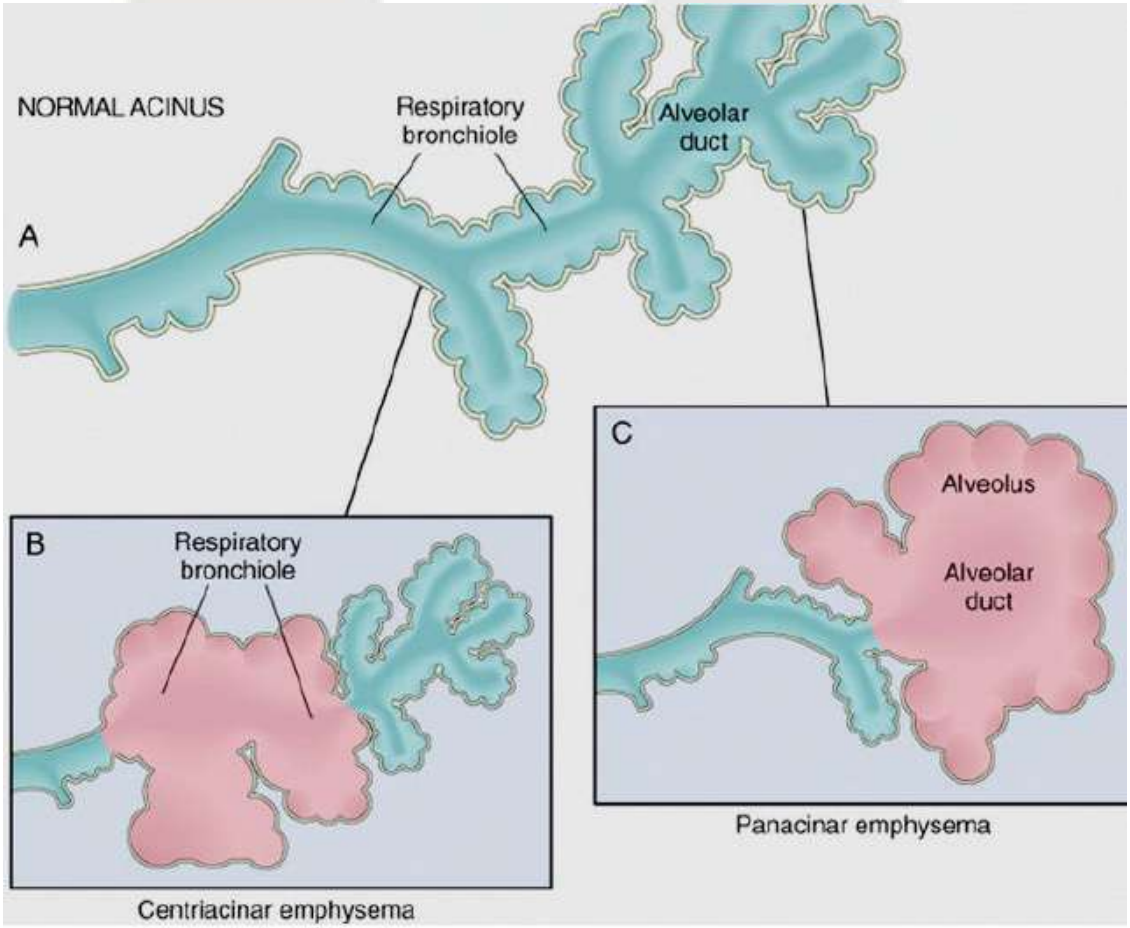


Fig. 12.2

Role of Smoking:

- Chemotactic for neutrophils + macrophages.

- Release of elastase and other proteases from neutrophils + macrophages.
- Oxidants and free radicals in smoke inhibit α – 1 antitrypsin.

Other types – Compensatory, Senile, Obstructive over inflation, Bullous emphysema.

Interstitial emphysema – Air in connective tissue of lung, mediastinum, subcutaneous tissues. If extensive it encroaches on blood supply. May be seen in:

- Rib fracture.
- Chest wound.
- Alveolar tears.
- Children with whooping cough.
- Patients with airway obstruction like blood clots, tissue, foreign body.
- Artificial ventilation.
- Inhalation of irritant gases.

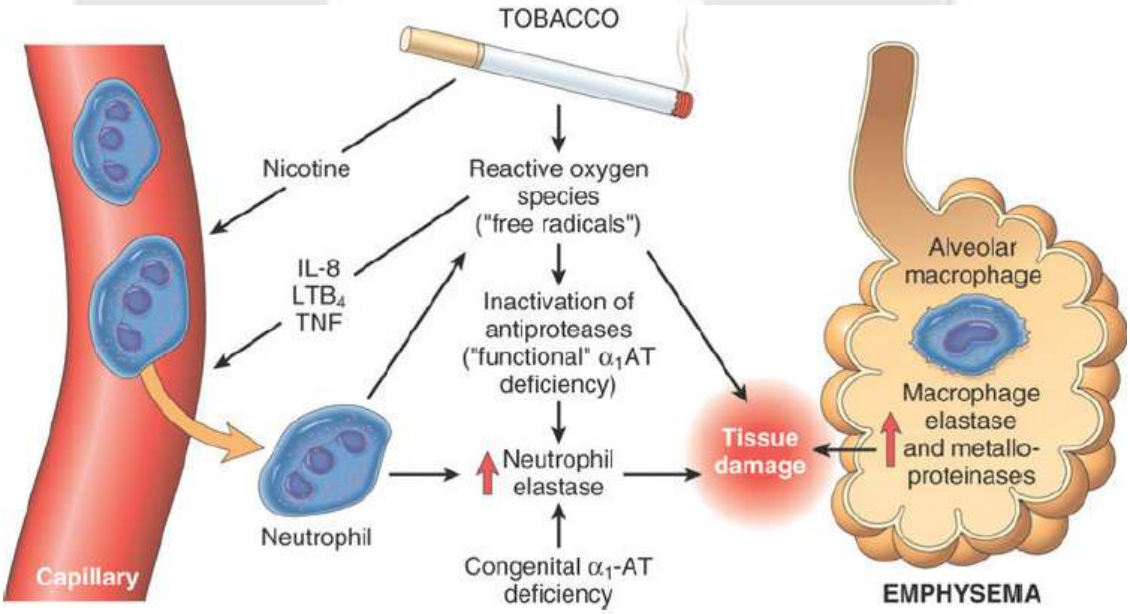


Fig. 12.3

Quick Revision:

- Emphysema is a chronic obstructive airway disease characterized by permanent enlargement of air spaces distal to terminal bronchioles. It is a component of COPD (chronic obstructive pulmonary disease) along with chronic bronchitis.
- Subtypes include centriacinar (most common, smoking related), panacinar (seen in α1-antitrypsin deficiency), distal acinar and irregular.
- Smoking and inhaled pollutants cause ongoing accumulations of inflammatory cells, releasing elastases and oxidants, which destroy the alveolar walls.
- Most patients with emphysema also have some degree of chronic bronchitis, which is to be expected since cigarette smoking is an underlying risk factor for both.

Chronic Bronchitis:

- Persistent cough with sputum for at least 3 months in al least two consecutive years.
- **Blue bloaters** , Productive cough is an early feature. Cor pulmonale is a more frequent complication.
- Associated with **heavy smoking.**
- Condition is caused by chronic irritation and maintained by recurrent infections.
- Bronchiolitis refers to inflammation of small airways.
- Hallmark feature- **hypersecretion of mucus associated with hypertrophy of submuscosal glands.**
- **Reid index normal** – o.44. In chronic bronchitis means is 0.52. Ratio of the thickness of the mucous gland layer to the thickness of the wall between epithelium and the cartilage links with severity and duration of the disease.
- Other changes include mucous plugs and goblet cell metaplasia in small airways, increased pigment laden macrophages and inflammatory cells, fibrosis, squamous metaplasia and dysplasia of lining of brochi.

Emphysema and Chronic Bronchitis:

	Predominant Bronchitis	Predominant Emphysema
Age (yr)	40–45	50–75
Dyspnea	Mild; late	Severe; early
Cough	Early; copious sputum	Late; scanty sputum
Infections	Common	Occasional
Respiratory insufficiency	Repeated	Terminal
Cor pulmonale	Common	Rare; terminal
Airway resistance	Increased	Normal or slightly increased
Elastic recoil	Normal	Low
Chest radiograph	Prominent vessels; large heart	Hyperinflation; small heart
Appearance	Blue bloater	Pink puffer

Bronchial Asthma:

Hyper responsiveness of tracheobronchial tree to various stimuli potentiating paroxysmal reversible constriction of the bronchial airaways. Usually mediated through type 1.

Hypersensitivity.

Types of Asthma.

- Atopic (allergic, Extrinsic) incited by allergen.
- Occupational Due to chemical dusts or lume.

Type 1 IgG mediated.

- Allergic bronchopulmonary aspergillosis (Type 1 and 3 reactions).
- Nonreaginic (Intrinsic).
  Viral respiratory infection (Hyper reactive airway).
- Pharmacologic Aspirin (Decreased PGs and Increased LTs)

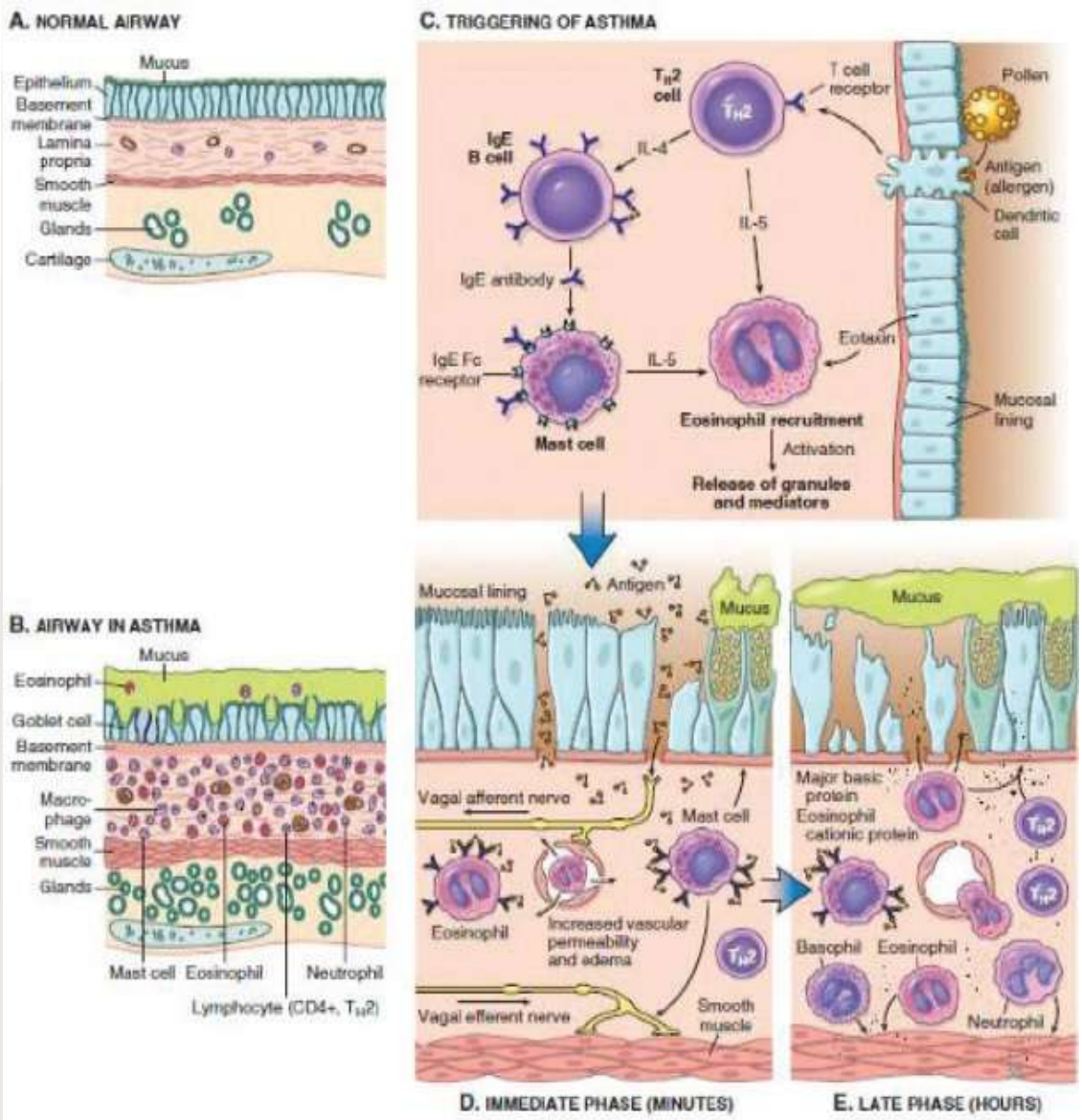


Fig. 12.4

Sputum findings- Increased eosinophils, Charcot Leyden crystals and Curschman's spirals.

Histologic features – Bronchial thickening and inflammation with eosinophlilic infiltration, mucous gland hypertrophy.

Quick Revision:

Asthma:

- Asthma is characterized by reversible bronchoconstriction caused by airway hyperresponsiveness to a variety of stimuli.
- Atopic asthma is caused by a TH2 and IgE-mediated immunologic reaction to environmental allergens and is characterized by acute-phase (immediate) and late-phasereactions. The TH2 cytokines IL-4, IL-5, and IL13 are important mediators. IL17 and IL9 are also being shown to be important in some asthmatics.
- Triggers for nonatopic asthma are less clear but include viral infections and inhaled air pollutants, which can also trigger atopic asthma.
- Eosinophils are key inflammatory cells found in almost all subtypes of asthma; other inflammatory cells include mast cells, neutrophils and T lymphocytes.
- Airway remodeling (sub-basement membrane fibrosis, hypertrophy of bronchial glands, and smooth muscle hyperplasia) adds an irreversible component to the obstructive disease.

Bronchiectasis:

Chronic necrotizing infection of the bronchi and bronchioles associated with abnormal dilatation (permanent) of these airways.

Causes:

a. Bronchial obstruction – Tumors, FB, mucous impaction, Atopic asthma, chronic bronchitis.

b. Hereditary – Cystic fibrossi, intralobar sequestration of the lungs, immune-deficiency. Kartagener's syndrome (lmmotile cilia, Autosomal recessive. Most common abnormality is defective or absent dyenin arms).

c. Necrotizing pneumonia – TB, Staphylococcal.

Pathology is vicious cycle of obstruction and infection.

Usually affect both lower lobes. Segmental involvement is seen in tumours or FB obstruction. Brochi are dilated upto 4 times normal and can be traced upto the pleural surface.

Types- Cylindroid, fusiform and saccular.

Complication- Dyspnoea, cyanosis, respiratory insufficiency, Cor pulmonale, Metastatic brain abscess, Amyloidosis.

Pneumonia:

Infection of lung parenchyma resulting in consolidation.

Predisposing factors are:

- Impaired host resistance due to chronic diseases, immunodeficiency, Leukopenia.
- Highly virulent organisms.
- Impaired cough reflex and mucociliary clearance.
- Pulmonary congestion and oedema.
- Accumulations of secretions.
- **The Pneumonia Syndromes.**

Community-Acquired Acute Pneumonia
Streptococcus pneumoniae. Haemophilus influenzae. Moraxella catarrhalis. Staphylococcus aureus. Legionella pneumophila. Enterobacteriaceae (Klebsiella pneumoniae) and Pseudomonas spp.
Community-Acquired Atypical Pneumonia
Mycoplasma pneumoniae. Chlamydia spp. (C. pneumoniae, C. psittaci, C. trachomatis). Coxiella burnetii (Q fever). Viruses: respiratory syncytial virus, parainfluenza virus (children); influenza A and B (adults); adenovirus (military recruits); SARS virus.

Hospital-Acquired Pneumonia
Gram-negative rods, Enterobacteriaceae (Klebsiella spp., Serratia marcescens, Escherichia coli) and Pseudomonas spp. Staphylococcus aureus (usually penicillin resistant).
Aspiration Pneumonia
Anaerobic oral flora (Bacteroides, Prevotella, Fusobacterium, Peptostreptococcus), admixed with aerobic bacteria (Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, and Pseudomonas aeruginosa).
Chronic Pneumonia
Nocardia Actinomyces Granulomatous: Mycobacterium tuberculosis and atypical mycobacteria, Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis
Necrotizing Pneumonia and Lung Abscess
Anaerobic bacteria (extremely common), with or without mixed aerobic infection Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes, and type 3 pneumococcus (uncommon).
Pneumonia in the Immunocompromised Host
Cytomegalovirus. Pneumocystis jiroveci. Mycobacterium avium-intracellulare. Invasive aspergillosis. Invasive candidiasis. "Usual" bacterial, viral, and fungal organisms (listed above).

Types are:

1. **Bronchopneumonia** – Patchy consolidation. Usually occurs in extremes of age. May be an extension of bronchitis or bronchitis. Causes are mostly Staphylococci, Streptococci.
2. **Lobar Pneumonia -** Consolidation of a lobe or part of a lobe. Common cause is Pneumococcus, klebsiella, Staphylococci, Streptococci, H influenzae.

Pseudomonas and Proteus.

Stages of lobar pneumonia are

Congestion, Red hepatization (neutrophils + fibrin + extravasation of RBCs); Grey hepatization (fibrin + disintegration of WBCs +RBCs) and Organization or Resolution Complications of Pneumonia are abscess formation, empyemia, Organization and dissemination to distant sites (endocarditis, arthritis, Meningitis).

3. **Interstitial pneumonia –** Patchy pneumonitis without consolidation. Pleura is smooth and effusions and pleuritis are uncommon. Causes are Viral, Chalmydial, Mycoplasma, Rickettsial. (InfluenzaA and B, RSV, Adenovirus, Rhinovirus, Rubeola and Varicells, Psittacosis and Q fever).

Histologically, alveolar septae are widened with mononuclear infilatration and hyaline membranes. Secondary bronchopneumonia due to secondary bacterial infection may be seen.

- Characteristic features in **Mycoplasma pneumonia** is the presence of **cold agglutinins** which are IgM antibodies that do not react at 37°C but cause agglutinations of patients RBCs at 4°C.Also seen in Measles and CMV infections.

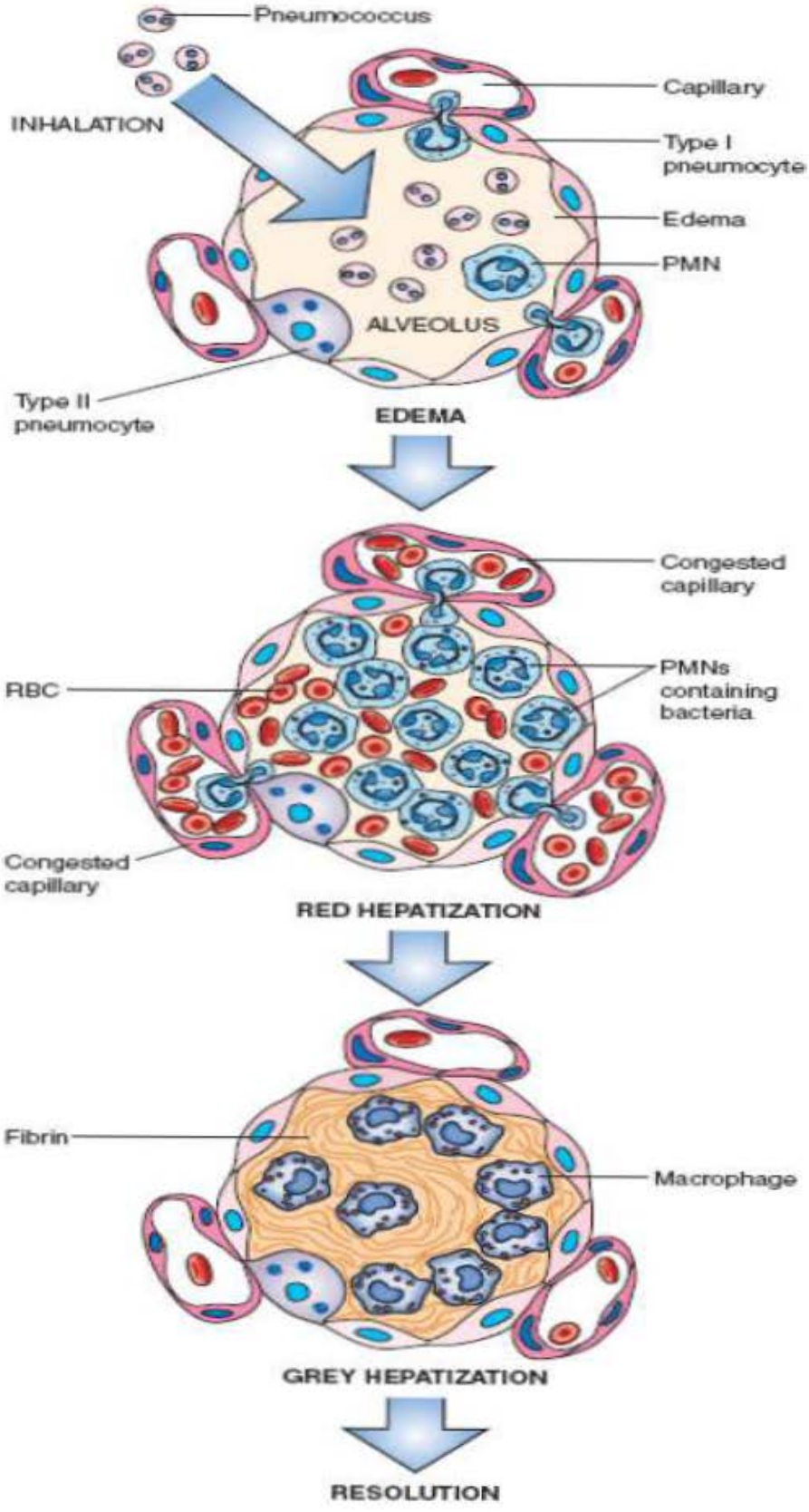

Pneumococcus
INHALATION
Capillary
Type I pneumocyte
Edema
PMN
ALVEOLUS
Type II pneumocyte
EDEMA
Congested capillary
PMNs containing bacteria
RBC
Congested capillary
RED HEPATIZATION
Fibrin
Macrophage
GREY HEPATIZATION
RESOLUTION

Fig. 12.5

Lung Abscess:

- Localised suppuration of lung. Associated with.
- Most frequent with **aspiration** of infective material (In comatose, Alcoholic, Anaesthetized and debilitated patients).
- Oropharyngeal surgery.
- Sino bronchial infections.
- Dental sepsis.
- Bronchiectasis.
- Around pneumonitic patches.
- Septic emboli.
- Neoplastioc obstruction.
- Direct spread or haematogenous spread.
- Primary cryptogenic abscess.

May be few mm to 5-6 cm. Aspiration associated abscess are seen in the right lung.

Chronic Interstitial Lung Diseases:

- Diffuse interstitial fibrosis of the lung gives rise to restrictive lung diseases characterized by reduced lung compliance and reduced forced vital capacity (FVC). The ratio of FEV1 to FVC is normal.
- Idiopathic pulmonary fibrosis is prototypic of restrictive lung diseases. It is characterized by patchy interstitial fibrosis fibroblastic foci and formation of cystic spaces (honeycomb lung). This histologic pattern is known as usual interstitial pneumonia.
- The cause of idiopathic pulmonary fibrosis is unknown, but genetic analyses point to roles for senescence of alveolar epithelium (due to telomere shortening), cell stress related to protein misfolding, abnormal signaling in alveolar fibroblasts, and altered mucin production. The resulting injury to alveolar epithelial cells set in motion event that lead to increase local production of fibrogenic cytokines such as TGF-β.

- The other diseases that cause diffuse interstitial fibrosis are heterogeneous poorly understood, but most have better prognoses that idiopathic pulmonary fibrosis.

Tuberculosis:

Primary TB- Ghons complex is a primary pulmonary parenchymal subpleural focus just above or below the interlobar fissure b/w the upper and lower lobes with enlarged caseous lyphnode draining it. Usually undergoes fibrosis and calcification. Exceptionally it may progress and cavilate, lead to TB pneumonia or military TB.

Secondary TB - Following primary TB bacilli establish themselves in lung apices (region of high Oxygen tension). Secondary TB may be either Reactivation or Reinfection type. Secondary lesions are common in the apices as small focus of consolidation. Histologic Hallmark- Caseating confluent granulomas with Langhan's giant cells.

Out come:

- Heal and form a fibrocalcific nodule.
- Progress to.
  - Cavitary Fibrocaseous TB.
  - Advanced Fibrocaseous TB.
  - Pleural effusion.
  - Tubercular empyema.
  - Endobronchial/ endotracheal/ intestinal/ Laryngeal TB.
  - Miliary TB due to lymphohaematogeneos seeding.
  - End organ TB.
  - TB bronchopneumonia.
  - Lobar exudative consolidation (Galloping consumption).

Restrictive Airway Diseases:

- Diffuse chronic infiltrative diseases.
- Clinically present with dyspnoea, tachypnoea and cyanosis.

- X ray shows ground glass shadows; diffuse infiltration by small nodules or irregular lines. Eventually progress to secondary pulmonary hypertension and cor pulmonale.
- Advanced forms of all entities show gross destruction and fibrosis and appear as Honey comb lung.

Known causes	Idiopathic
Environmental inhalants. • Inorganic- silicosis, Asbestosis, Berylliosis, Pneumoconiosis. • Organic- Hypersensitivity pneumonitis. • Gases – Oxygen toxicity, SO2, Toluene Drugs and toxins. • Busulphan, Bleomycin, Amiodarone, Gold, Penicillanmine, Paraquat infections. • Viral – influenza, CMV. • Widespread TB. • Fungal. • Pneumocustis pneumonia.	Sarcoidosis. Collagen vascular diseases. Good pasture's syndrome. Idiopathic pulmonary hemosiderosis. Eosinophilic pneumonia. Histiocytosis X. Alveolar proteinosis. Desquasmative interstitial pneumonia. Usual interstitial pneumonia (Idiopathic pulmonary fibrosis / Cryptogenic fibrosing alveolitis).

In the order of frequency Commonest is Pneumoconiosis followed by sarcoidosis IPF, Collagen vascular diseases.

Pneumoconiosis:

Non- neoplastic lung reaction to inhalation of mineral dusts. Terms broadened to include diseases caused by organic and inorganic particulates, chemical fumes and vapours.

Coal:

- Anthracosis is asymptomatic carbon pigmentation of lungs/ draining lymphonodes without any reaction. Seen in coat miners, smokers, urban dwellers. Coal pigment is seen inside alveolar macrophages.
- Simple coal worker's pneumoconiosis is characterized by COAL MACULES (1-2 mm) and larger coal nodules which are aggregates of carbon laden macrophages. Common in upper lobes. Can complicate into centrilobular emphysema.

- Complicated CWP is progressive massive fibrosis, which occurs after long periods of exposure. Lung show distinct nodular lesions, which develop rapidly.
- CWP does not predispose to lung cancer as an independent variable.

Silica:

- Most prevalent and takes years to develop.
- Crystaline forms are Quartz, Crystobalite, Tridymite. More fibrogenic
- Amorphous forms are Talc, vermiculte and mica.
- Silica reacts with membrane proteins and phospholipids and denatures and damages them. It also incites inflammation.
- Nodular fibrosing disease involving upper zone. More fibrosing and less cellular lesions.
- Grossly- Tiny discrete pale to blackened nodules in upper zone- Progress to collagenous scars. Polarizer reveals silica.
- Superimposed TB cause softening and cavitation.
- Egg shell calcification in lymphnode.
- No clear cut association with cancer.

Asbestos:

- Cyrstalline hydrated silicates. Associated with.
- Localized fibrous plaques in pleura (Most common manifestation).
- Pleural effusions.
- Diffuse interstitial fibrosis.
- Bronchogenic CA (5 fold risk).
- Mesothelioma (1000 fold risk).
- Laryngeal and colonic carcinoma.

2. Geometric forms.

- **Serpentines** - Curly and flexible, Eg crysotile. **Most common** type to exposure. More soluble and easily removed by

mucociliary clearance.

- **Amphiboles** – Straight, stiff and brittle. Eg Crocidolite, Amosite, Tremolite.
- Anthophyllite, Actionlyte. Are more pathogenic and lead to mesothelioms. Asbestos bodies or ferruginous bodies are golden brown, fusiform or beaded rods with translucent center of asbestos fibres coated with iron and calcium containing protein. Asbestosis begins with fibrosis around respiratory bronchioles and alveolar ducts. Begins in the **lower lobes** subpleurally.

Beryllium:

Heavy dose leads to Acute pneumonitis

Low dose for prolonged duration leads to a pulmonary and systemic granulomatous lesions mimicking sarcoid.

Non caseating graulomas in lung hilar lymphnodes which become progressively fibrotic. Chest X ray shows nodular irregular fine denisities.

Increased risk of Bronchogenic CA.

Others.

Iron – Siderosis.

Barium sulphate- Baritosis.

Tin oxide – Stannosis.

Quick Revision:

Pneumoconioses:

- Pneumoconioses encompass a group of chronic fibrosing diseases of the lung resulting from exposure to organic and inorganic particulates, most commonly mineral dust.
- Pulmonary alveolar macrophages play a central role in the pathogenesis of lung injury by promoting inflammation and producing reactive oxygen species and fibrogenic cytokines.

- Coal dust-induced disease varies from asymptomatic anthracosis to simple coal workers' pneumoconiosis (coalmacules or nodules, and centrilobular emphysema), to progressive massive fibrosis (PMF), manifested by increasing pulmonary dysfunction, pulmonary hypertension, and cor pulmonale.
- Silicosis is the most common pneumoconiosis in the world, and crystalline silica (e.g., quartz) is the usual culprit. The lung disease is progressive even after exposure stops.
- The manifestations of silicosis can range from asymptomatic silicotic nodules to large areas of dense fibrosis; persons with silicosis also have an increased susceptibility to tuberculosis. There is two-fold increased risk of lung cancer.
- Asbestos fibers come in two forms; the stiff amphiboles have a greater fibrogenic and carcinogenic potential than the serpentine chrysotiles.
- Asbestos exposure is linked with six disease processes: (1) parenchymal interstitial fibrosis (asbestosis); (2) localized pleural plaques (asymptomatic) or rarely diffuse pleural fibrosis; (3) recurrent pleural effusions; (4) lung cancer; (5) malignant pleural and peritoneal mesotheliomas; and (6) laryngeal cancer.
- Cigarette smoking increases the risk of lung cancer in the setting of asbestos exposure; even family members of workers exposed to asbestos are at increased risk for cancer and mesothelioma.

Sarcoidosis:

- Hard non- caseating granulomas in lung with bilateral hilar lymphadenopathy.
- Associated skin and eye lesions.
- Uveoparotid involvement (Miculicz syndrome).
- Female predominance.

Idiopathic Pulmonary Fibrosis:

- Also called Usual interstitial pneumonitis.
- Chronic interstitial fibrosis. Hamman Rich syndrome. Cryptogenic fibroising alveolitis.

- Characterized by thickened fibrosed inflamed septae with hyaline membranes. Honey comb lung ensues.

Note- Lung fibrosis is a feature of all endstage interstitial diseases like pneumoconiosis, Hypersensitivity pneumonitis, Scleroderma, Collagen vascular diseases, Radiation, Oxygen toxicity, Pulmonary hemosiderosis, Drugs like Bleomycin and busulfan. It is not a feature of Leffler's syndrome and Pulmonary alveolar proteinosis.

Desquamative Interstitial Pneumonitis:

Aggregates of macrophages in the alveoli. Ground glass infiltrates in lower lobes.

Considered an early stage of UIP. Good response to steroids.

Macrophages contain lipid and PASpositive granules.

Hypersensitivity Pneumonitis:

Also called extrinsic allergic alveolitis. Immunologically mediated interstitial lung disorder due to inhaled organic dusts.

- Farmer's Lung – Due to mouldy hay, Spores of thermophilic actinomycetes.
- Pigeon breeder's disease- Due to proteins from serum, droppings and feathers.
- Baggasosis- Sugar cane baggasse.
- Humidifier lung – Thermophilic bacteria.
- Duck fever – Feathers.
- Mushroom picker's Lung.
- Maple bark disease.

All are characterized by interstitial pneumonitis, fibrosis, obilterative bronchioliitis and granulomas in lung.

Mediated via type 3 and 4 reactions.

Pulmonary Eosinophilia:

Simple or Loeffler's syndrome – Benign

Tropical eosinophilia due to microfilariae in lung.

Secondary chronic pulmonary eosinophilia

Idiopathic chronic eosinophilic pneumonia Diagnosis of exclusion after excluding parasites, Fungus, Bacteria, Hypersensitivity pneumonitis, Drug allergy, Asthma, Allergic bronchopulmonary aspergillosis, Churgh strauss syndrome etc.

Respond to steroids.

Boop (Bronchiolitis Obliterans Organizing Pneumonia):

Response to infection or inflammatory injury to lung. May follow toxic damage, drugs, collagen vascular disease, bronchial obstruction.

Rheumatoid Lung:

Feature include Chronic pleuritis, DIP, Fibrosis, Intrapulmonary rheumatoid nodules, Caplan's syndrome, Pulmonary hypertension.

Pulmonary Alveolar Proteinosis:

Homogeneous granular precipitate in alveoli. PAS positive with lipid. Material is surfactant like. On EM necrotic macrophages. Type 2 cells and osmiophillic bodies are seen.

Secondary PAP is associated with Acute Silica exposure, immunocompromised. Haematolymphoid malignancies, and Opportunistic infections.

Acquired PAP accounts for 90% cases. Autoimmune anti GM-CSF antibodies may be pathogenic.

Radiation Damage:

Acute radiation Pneumonitis – Shows a picture of DAD.

Chronic radiation Pneumonitis – Interstitial fibrosis.

Characteristic feature are epithelial cell atypia with foam cells in vessel wall.

Surfactant Dysfunction Disorders:

Surfactant dysfunction disorders are diseases caused by mutations in genes encoding proteins involved in surfactant trafficking or secretion. The mutated genes include the following:

- **ATP-binding cassette protein member 3** (**ABCA3**) is the most frequently mutated gene in sufactant dysfunction disorders. It is an autosomal recessive disorder and usually presents in the first few months of life with rapidly progressive respiratory failure followed by death. Less commonly it comes to attention in older children and in adults with chronic interstitial lung disease.
- **Surfactant protein C** is the second most commonly mutated gene in sufactant dysfunction disorders. It is autosomal dominant with variable penetrance and severity in 45% and sporadic in 55%. It has a highly variable course.
- **Surfactant protein B** is the least commonly mutated gene and is associated with an autosomal recessive form of sufactant dysfunction disorder. Typically, the infant is full term and rapidly develops progressive respiratory distress shortly after birth. Death ensues between 3 and 6 months of age.

Tumours:

Bronchogenic carcinoma is the most common Ca.

Predisposing factors are:

- Tobacco smoking.
- Radiation.
- Uranium.
- Asbestosis, Berylliosis.
- Nickel, Chromates, mustard gas, Arsenic, Iron.
- Gold miners.
- Newspaper workers.

Histologic Classification of Malignant Epithelial Lung Tumors:

Tumor Classification
Squamous cell carcinoma
Papillary, clear cell, small cell, basaloid
Small-cell carcinoma
Combined small-cell carcinoma
Adenocarcinoma
Minimally Invasive adenocarcinoma (nonmucinous, mucinous)
Lepidic, acinar papillary, solid (according to predominant pattern}
Mucinous adenocarcinoma
Large-cell carcinoma
Large-cell neuroendocrine carcinoma
Adenosquamous carcinoma
Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements
Carcinoid tumor
Typical, atypical
Carcinomas of salivary gland type

Previous terminology of Bronchioalveolar carcinoma is not used anymore. That pattern is called Adenocarcinoma in situ according to the latest WHO classification.

More than 90% of lung cancers develop as a direct result of exposure to tobacco smoke. Approximately 10% of smokers eventually develop lung cancer.

The gene known to be mutated most frequently in lung cancers is P53. P53 mutations are found in about 50% of NSCLC and in over 90% of SCLC.

Many smoking-associated mutations are G→T transversions that occur in known hotspots of the P53 open reading frame.

These characteristic mutations can be directly attributed to bulky adducts caused by exposure to BPDE, a carcinogen in cigarette smoke.

RB is inactivated in 30–40% of NSCLC and in nearly all SCLC tumors.

Among NSCLC, RB mutations are associated with more advanced tumors, implying that RB loss occurs during later stages of tumorigenesis.

Scar carcinoma – Mostly adenocarcinoma. Follows old infarcts, Tuberculosis, Metallic foreign bodies and wounds.

Commonest type in smokers- Squamous cell carcinoma.

Commonest type in women and non smokers- Adenocarcinoma.

Ca associated with smoking – SCC, Small Cell Ca.

SCC- In and about hilus, Central tumour. Fast growing. Associated with hypercalcemia.

Adeno Ca- Peripheral and slow growing.

Small cell Ca- Hilar or central, Oat cells growing in clusters, EM shows dense core neurosecretory granules. More often associated with Ectopic hormone production, Most aggressive, Respond to chemotherapy and radiation.

Large cell Ca – Anaplastic, undifferentiated

Other changes with Lung CA.

Emphysema, Atelectasis, Bronchitis, Bronchiectasis, Lung Abscess, SVC syndrome.

Pericarditis, Pleuritis.

Clinically – Present with cough, weight loss, Chest pain, Dyspnoea, Increased sputum,

Paraneoplastic syndrome associated with lung CA.

- SIADH.
- Cushings.
- Hypercalcemia.
- Hypoglycemia.
- Gynaecomastia.
- Carcinoid syndrome.
- Eaton Lambert Myaesthenic syndrome.
- Peripheral neuropathy.
- Acanthosis nigricans.
- Leukemoid reactions.
- Hypertrophic pulmonary osteoarthropahty and clubbing.

Bronchioalveolar Carcinoma

(now called Adenocarcinomain situ - with Mucinous features):

Peripheral lesion, May be multiple nodules or pneumonia like consolidation.

30 yrs onwards.

Tall columnar mucin secreting cells in papillary formation May be bronchiolar cells, Clara cell or rarely type II pneumocytes.

Tumor does not involve major bronchi, therefore atelectasis, emphysema are infrequent Metastasis not widely disseminated.

Neuroendocrine tumours:

Tumorlets / Carcinoids / Small cell CA

Carcinoids form 1-5% of lung tumors. Of tumours diagnosed as Bronchial adenoma, 90% are carcinoids. Others are adenoid cystic and mucoepidermoid carcinoma.

Grossly – 3-4 cm polypoidal lesion projecting into the bronchial lumen or producing a **collar button lesion** by fanning into peribronchiolar tissue.

Mostly do not have secretory activity and do not metastasize.

Lung hamartoma - Coin lesion on X ray. 3-4 cm. Composed of mature haline cartilage mixed with other tissues.

Metastasis:

Most common Brain.

Most Specific adrenal.

Mesothelioma:

Affects visceral or parietal pleura. Very rare without asbestos exposure. Asbestos bodies may be seen. Shows epithelial, sarcomatoid or mixed pattern.

Positive for acid mucopolysaccharides which is inhibied by hyaluronidase. Lack of CEA or LeuM1, Positive for Keratin.

EM- Shows long microvilli and abundant tonofilaments. No microvillous rootlets and lamellar bodies seen.

Lung transplantation:

The most common indications are end-stage emphysema, idiopathic pulmonary fibrosis, cystic fibrosis, and idiopathic / familial pulmonary arterial hypertension.

HEMATOLOGY

13 Hematology in Newborn and Pregnancy

Red Blood Cells:

- Hematopoiesis is first established soon after implantation of the blastocyst, with the appearance of primitive erythroid cells in blood islands of the yolk sac beginning at day 18 of gestation.
- **After 7 weeks' gestation, hematopoietic progenitors are no longer detected in the yolk sac** .
- The liver serves as the primary source of red cells from the 9th to the 24th week of gestation.
- In contrast to the yolk sac, where hematopoiesis is restricted to maturing primitive erythroid, macrophage, and megakaryocytic cells, hematopoiesis in the fetal liver consists of definitive erythroid, megakaryocyte, and multiple myeloid, as well as lymphoid lineages.
- Hematopoietic cells are first seen in the marrow of the 10- to 11-week embryo, and they remain confined to the diaphyseal regions of long bones until 15 weeks' gestation.
- Lymphopoiesis is present in the lymph plexuses and the thymus beginning at 9 weeks' gestation.
- The aorta-gonad-mesonephros (AGM) region generates hematopoietic stem cells that seed the liver and the marrow to provide lifelong hematopoiesis (*).
- Hgb Gower-1 ($\zeta_2\ \varepsilon_2$) is the major hemoglobin in embryos younger than 5 weeks. (*).
- Hgb F ($\alpha_2\ \gamma_2$) is the major hemoglobin of fetal life (*).
- The mean hemoglobin level in cord blood at term is 16.8 g/Dl (*)
- The red cells of the newborn are macrocytic, with a mean corpuscular volume (MCV) in excess of 110 fl/cell. (*).

- The red cell, hemoglobin, and hematocrit values decrease only slightly during the first week, but decline more rapidly in the following 5 to 8 weeks, producing the physiologic anemia of the newborn.
- **Segmented neutrophils are the predominant leukocytes in the first few days after birth.**
- **As their number decreases, the lymphocyte becomes the most numerous cell and remains so during the first 4 postnatal years.**
- The absolute number of CD3+ and CD4+ (helper/inducer phenotype) T-cell subsets in blood of newborns is significantly higher than in adults.
- In the newborn, approximately 15 percent of lymphocytes have immunoglobulin on their surface, with all immunoglobulin (Ig) isotypes represented.
- The term newborn has reduced mean plasma levels (<60% of adult levels) of factors II, IX, X, XI, and XII, prekallikrein, and high-molecular-weight kininogen.
- In contrast, the plasma concentration of factor VIII is similar and von Willebrand factor is increased compared to older children and adults.

Embryonic Hemoglobins (AIIMS Question):

Hemoglobin	Chain Composition	Primary Site	Appearance
Gower-1	ζ2 ε2	Yolk sac	<5-6 weeks
Gower-2	α2 ε2	Yolk sac	4-13 weeks
Portland	ζ2 γ2	Yolk sac	4-13 weeks
Fetal (F)	α2 γ2	Liver	Early, 53-95% at term
Adult (A]	α2 β2	Marrow	9 weeks, 5-45% at term

- The ζ -to-α-globin switch precedes the ε-to-γ-globin switch as the liver replaces the yolk sac as the main site of erythropoiesis.
- Hgb A_2 has not been detected in fetuses.
- Normal adult levels of Hgb A_2 are achieved by 4 months of age (*).
- Decreased levels of Hgb F at birth are found in trisomy 21 (*).

- By 1 year of age the i antigen is undetectable, and the ABH antigens increase to adult levels by age 3 years.
- The life span of the red cells in the newborn infant is shorter than that of red cells in the adult.
- The average of several studies of mean half-life of newborn red cells is 60 to 80 days (*).

White Blood Cells:

- The absolute number of neutrophils in the blood of term and premature infants usually is greater than that found in older children.
- In term infants, opsonic activity is normal for **Staphylococcus aureus** , but it is low for yeast and **Escherichia coli** .
- Diminished opsonic antibody is associated with group B streptococcal infection and represents one risk factor for neonatal infection.
- In premature infants, opsonic activity is low for **S. aureus** and **Serratia marcescens** , but is normal for **Pseudomonas aeruginosa.**
- **Complement components appear in fetal blood before 20 weeks' gestation and increase markedly during the third trimester.**
- The absolute number of CD3+ and CD4+ (helper/inducer phenotype) T-cell subsets in blood of newborns is higher than in adults. (*).
- This is a result of an increased total lymphocyte count in neonates (and older children) as compared with adults.
- There is a trend toward increased CD4 and decreased CD8 lymphocytes in newborns and children, resulting in an increased CD4:CD8 ratio.
- In spite of this, T-cell suppressor activity may be increased in newborns.
- Humoral (B-cell) immunity also develops early in gestation, but it is not fully active until after birth. In the newborn, approximately 15 percent of lymphocytes have immunoglobulin on their surface, with all Ig isotypes represented.

Coagulation:

- The term newborn has reduced mean plasma levels (<60% of adult levels) of factors II, IX, X, XI, XII, prekallikrein, and high-molecular-weight kininogen.
- In contrast, the plasma concentration of factor VIII is similar and von Willebrand factor is increased compared to that of older children and adults.
- In spite of the lower levels of factors, the functional tests (prothrombin and partial Thromboplastin times) are only slightly prolonged compared to adult normal values.
- Near-adult values are achieved for most components by 6 months of age.
- Factors II (prothrombin), VII, IX, and X require vitamin K for the final gamma-glutamyl carboxylation step in their synthesis.
- These factors decrease during the first 3 to 4 days after birth. This fall may be lessened by administration of vitamin K, effectively preventing classic, early occurring (first few days after birth) hemorrhagic disease of the newborn.

A hemorrhagic diathesis also may occur later, 2 to 12 weeks after birth, as a result of lack of vitamin K, and is called late hemorrhagic disease of the newborn or acquired prothrombin complex deficiency.

The etiology of the vitamin K lack is unclear but may result from poor dietary intake, particularly related to breast feeding, alterations in liver function with cholestasis and decreased vitamin K absorption, or a toxic or infectious impairment of hepatic utilization. Unfortunately, intracrania l hemorrhage frequently is the presenting event in this condition.

The current recommendation of the American Academy of Pediatrics suggests that vitamin K1, 0.5 to 1 mg, be administered intramuscularly at birth.

- Significant bleeding occurs more often in low-birth-weight infants than in term newborn infants. Increased capillary fragility is frequently found in premature infants in the first 2 days after birth and is not associated with thrombocytopenia.
- The levels of proteins C and S, which are vitamin K-dependent, as well as antithrombin and heparin cofactor II, are low in the newborn.

Haematological Effects of Maternal Drugs on Fetus and Newborn:

Drug	Effect	Certainty*	Mechanism
Antiretroviral agents in combination	Decreased hemoglobin	Established	Unknown—only seen with combination of zidovudine, lamivudine + nelfinavir.
Aspirin	Bleeding; kernicterus	Established; potential	Interference with platelet function.
			Displacement of bilirubin from albumin.
Diazoxide	Bleeding	Questionable	Thrombocytopenia.
Nalidixic acid	Hyperbilirubinemia	Potential	Oxidant damage to hemoglobin.
Nitrofurantoin	Hyperbilirubinemia	Potential	Oxidant damage to hemoglobin
Phenytoin (Dilantin/phenobarbital)	Bleeding	Suspected	Depletion of vitamin K-dependent coagulation factors by hepatic enzyme induction and factor degradation.
Rifampin/isoniazid	Bleeding	Suspected	Depletion of vitamin K-dependent coagulation factors.
Sulfonamides	Kernicterus	Established	Displacement of bilirubin from albumin.
Thiazides	Bleeding	Suspected	Thrombocytopenia.
Warfarin (Coumadin]	Bleeding	Established	Known depletion of vitamin K-dependent coagulation factors by blocking carboxylation.

Hematology In Pregnancy:

- Maternal blood volume increases by an average of 40 to 50 percent above the nonpregnant level.
- Plasma volume begins to rise early in pregnancy, with most of the escalation taking place in the second trimester and prior to week 32 of gestation.
- Red cell mass increases significantly beginning in the second trimester and continues to expand throughout pregnancy, but to a lesser extent than plasma volume.
- Erythropoietin levels increase throughout pregnancy, reaching approximately ١٥٠ percent of their prepregnancy levels at term.
- The overall effect of these changes in most women is a slight drop in hemoglobin concentration, which is most pronounced at the end of the second trimester and slowly improves approaching term.
- During labor and the early puerperium, there is a rise in the leukocyte count. Leukocytosis appears to be linearly related to the duration of labor.
- The levels of some plasma proteins also increase during pregnancy.
- In particular, C-reactive protein concentration is higher in pregnant women and rises even further during labor.
- Erythrocyte sedimentation rate (ESR) rises during pregnancy, and is affected by both hemoglobin concentration and gestational age.
- The rise in ESR during pregnancy, in large part a result of an increase in levels of plasma globulins and fibrinogen, makes its use as a marker of inflammation difficult.
- The levels of many of the procoagulant factors increase during pregnancy whereas activity of the fibrinolytic system diminishes in preparation for the hemostatic challenge of delivery.
- Plasma levels of von Willebrand factor (VWF), fibrinogen, and factors VII, VIII, and X all increase markedly, whereas factors II, V, IX, and XII are essentially unchanged and factor XIII declines.[11]
- Levels of protein C and antithrombin remain stable throughout pregnancy whereas total and free protein S fall with increasing gestational age.

- Fibrinolysis is also impaired by increases in plasminogen activator inhibitors I and II, the latter a product of the placenta.

14

Red Blood Cell & Disorders of Iron Metabolism

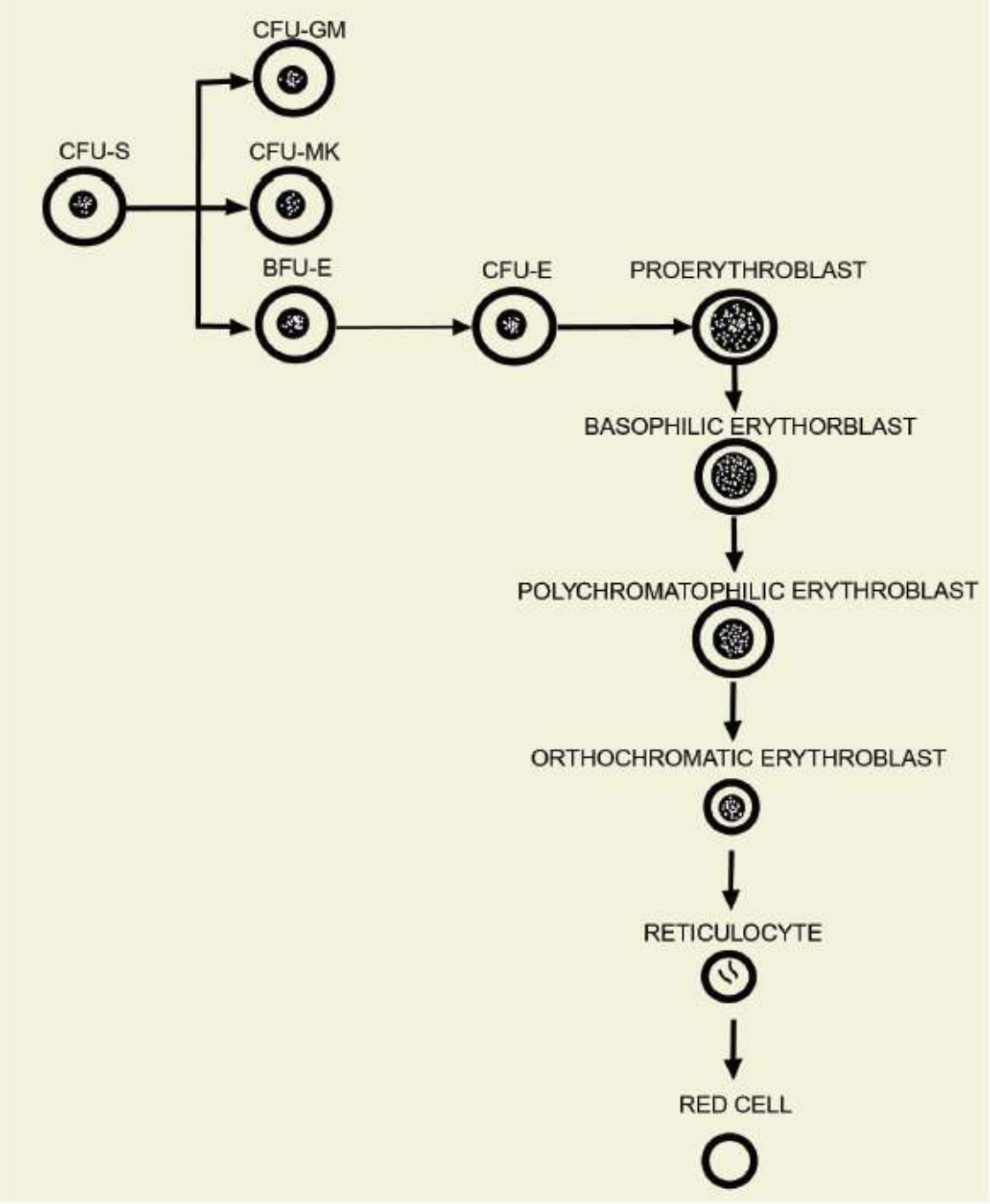


Fig. 14.1

Hemoglobin s first formed at the stage of proerythroblast but at this stage it cannot be stained by Giemsa and can only be seen on electron microscpopy. (*)

The first faint blush of Hb can be seen at the stage of Intermediate normoblast/ Polychromatophilic normoblast. (*)

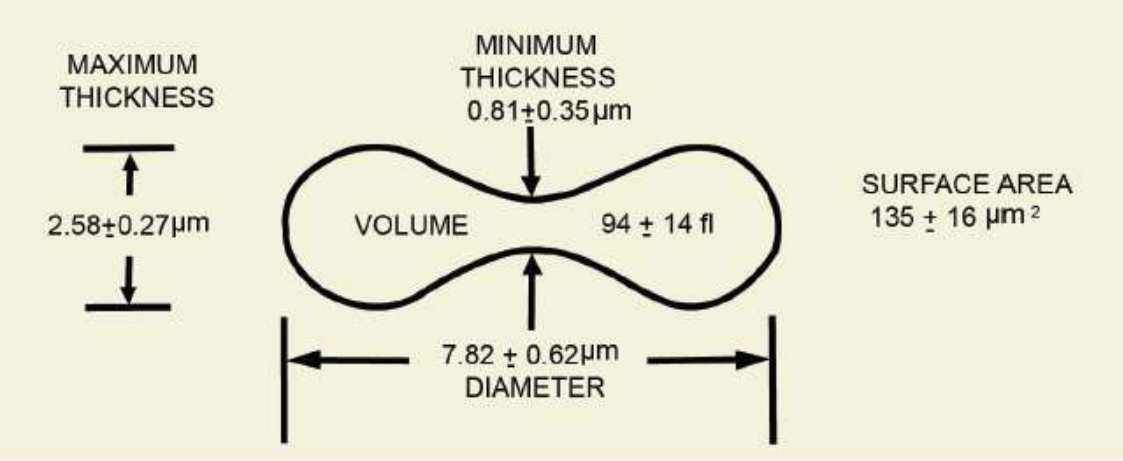


Fig. 14.2

- Historically, membrane proteins were thus first characterized by whether they were stainable by protein-binding or carbohydrate-specific dyes.
- Now, however, they are classified on the basis of their relationship to the membrane or their functions.
- One common classification of membrane proteins comprises the categories of integral membrane proteins and peripheral membrane proteins.
- Integral membrane proteins are most often globular and amphipathic; in their folded, three-dimensional form, they have distinct hydrophobic and hydrophilic domains. Of the major Coomassie-stainable proteins, only bands 3, 4.5, and 7 are integral membrane proteins.
- The two predominant erythrocyte transmembrane proteins are glycophorin A (GPA) (most common integral protein) (*) and the anion channel (AE1, formerly known as Band 3).
- The most abundant of the peripheral/cytoskeletal proteins are those that make up the so-called spectrin-actin cytoskeletal complex (*).
- Erythrocytes have an abundant and highly active water channel protein, aquaporin-1, which contributes as much as 85% of the osmotic water permeability pathway.

In disease, abnormality in the red cell picture stems from four main causes:

1.	Abnormal erythropoiesis that may be effective or ineffective
2.	Inadequate haemoglobin formation
3.	Damage to, or changes affecting, the red cells after leaving the bone marrow, including the effects of reduced or absent splenic function
4.	Attempts by the bone marrow to compensate for anaemia by increased erythropoiesis.

These processes result, respectively, in the following abnormalities of the red cells:

a.	Increased variation in size (anisocytosis) and shape (poikilocytosis) and punctate basophilia.
b.	Reduced or unequal haemoglobin content (hypochromasia, anisochromasia, or dimorphism).
c.	Spherocytosis, irregular contraction, elliptocytosis, or fragmentation (schistocytosis); the presence of Pappenheimer bodies, Howell–Jolly bodies, and a variable number of certain specific poikilocytes (target cells, acanthocytes, and spherocytes).
d.	Signs of immaturity (polychromasia and erythroblastaemia).

A clear explanation for the mechanism of red cell senescence remains elusive. It may be that human erythrocyte aging and

destruction results from a combination of the abnormalities described in the preceding paragraphs, or from some as yet unrecognized phenomenon. Contrary to long-held concepts, it probably is not due to enzyme or energy depletion. The role of membrane protein alterations also has not been documented. The one intriguing possibility relates to changes in RBC membrane phospholipid asymmetry associated with phosphatidyl serine exposure on the external bilayer. This mechanism would fit into a general concept of how cells are removed from the circulation.

Under normal conditions approximately 80 to 90% of normal erythrocyte destruction occurs without release of hemoglobin into plasma.

Because of this fact, the major part of the destructive process is considered to be extravascular, within macrophages of the spleen and, to a lesser extent, the liver and bone marrow. Only 10 to 20% of normal destruction occurs intravascularly.

Haptoglobins:

Haptoglobins are a family of α_2 -glycoproteins that bind hemoglobin.

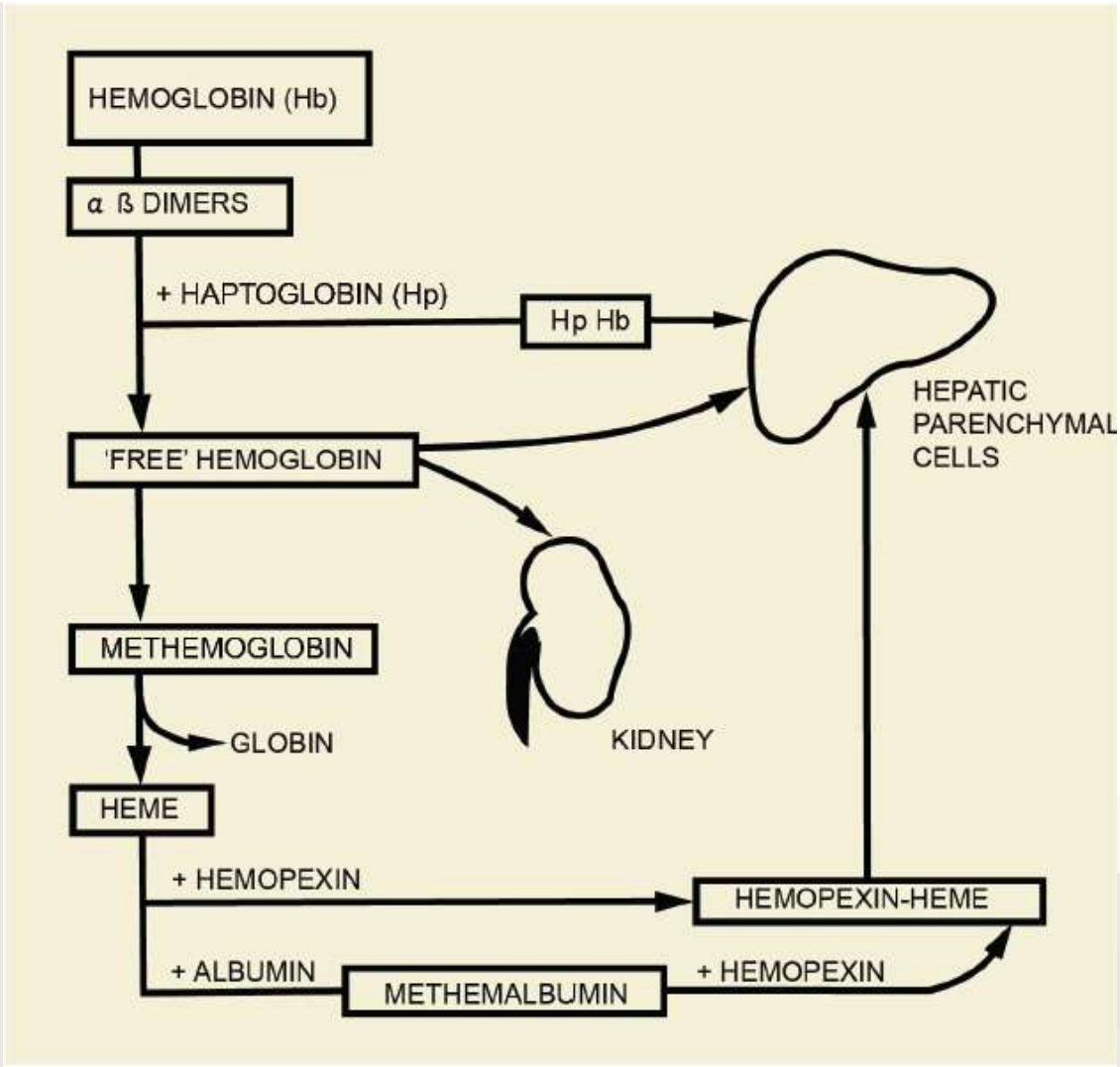


Fig. 14.3

The tetrameric molecule resembles certain immunoglobulins in that it has two light (α) chains and two heavy (β) chains linked in humans by disulfide bonds.

Haptoglobin is synthesized as a single polypeptide chain that is cleaved posttranslationally within the endoplasmic reticulum to generate its α and β subunits Transcriptional activity of the haptoglobin gene is promoted by interleukin-1, interleukin-6, and glucocorticoids as a part of the acute-phase response to systemic inflammation and related physiologic disturbances thereby explaining why haptoglobin levels are increased with inflammation.

An increased haptoglobin level is recognized as a nonspecific sign of disease with much the same significance as an accelerated sedimentation rate.

Pathways for the Disposal of Hemoglobin in Plasma:

Hemoglobin freely dissociates into αβ dimers. These are bound by haptoglobin with subsequent removal of the hemoglobin–haptoglobin complex by hepatic parenchymal cells. Hemoglobin in excess of the haptoglobin-binding capacity circulates as the unbound (free) protein. In this form it is partially removed by hepatic cells, but it may also follow two other pathways; it may be excreted by the kidney or oxidized to methemoglobin, from which heme is easily dissociated. Heme is initially bound to hemopexin, which transports it to the hepatic parenchymal cell. Heme may also be bound nonspecifically by albumin, forming methemalbumin. This complex probably transfers its heme to hemopexin as the latter becomes available.

Iron Deficiency Anemia:

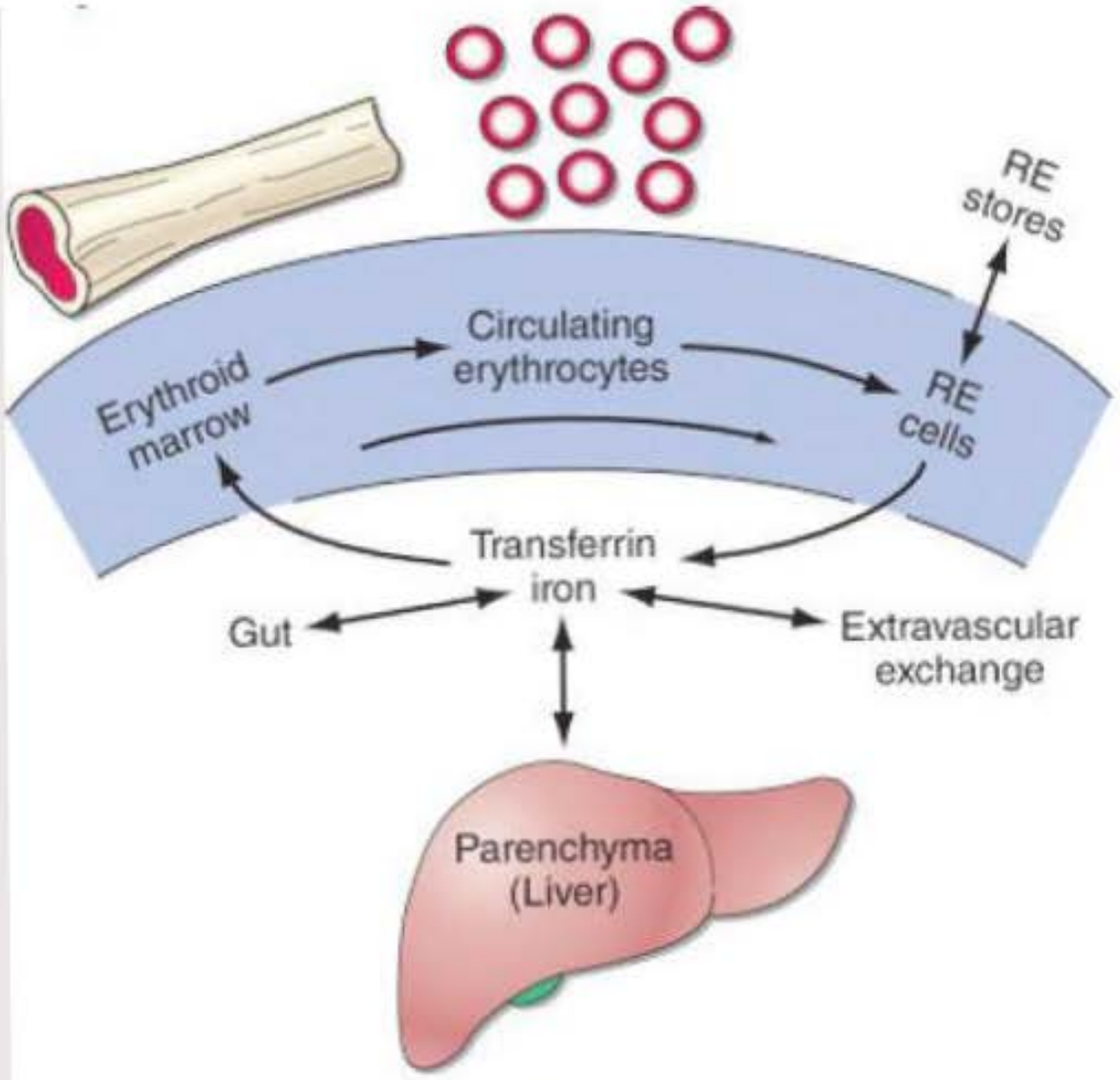

RE
stores
Circulating
erythrocytes
Erythroid
marrow
RE
cells
Transferrin
iron
Gut
Extravascular
exchange
Parenchyma
(Liver)

Fig. 14.4

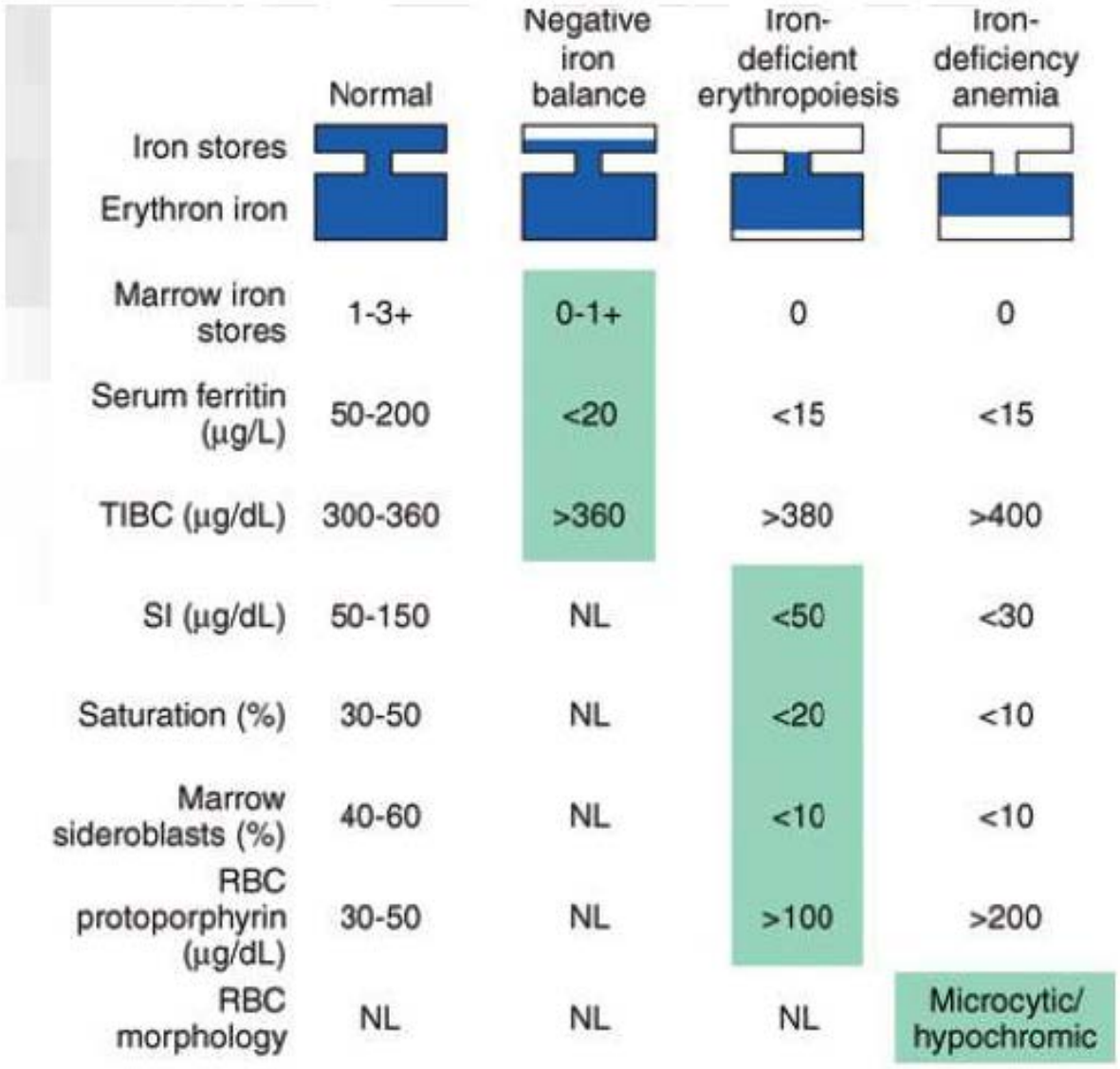

Normal
Negative iron balance
Iron-deficient erythropoiesis
Iron-deficiency anemia
Iron stores
Erythron iron
Marrow iron stores
1-3+
0-1+
0
0
Serum ferritin (µg/L)
50-200
<20
<15
<15
TIBC (µg/dL)
300-360
>360
>380
>400
SI (µg/dL)
50-150
NL
<50
<30
Saturation (%)
30-50
NL
<20
<10
Marrow sideroblasts (%)
40-60
NL
<10
<10
RBC protoporphyrin (µg/dL)
30-50
NL
>100
>200
RBC morphology
NL
NL
NL
Microcytic/ hypochromic

Fig. 14.5

Cause of Iron Deficiency
Increased Demand for Iron
Rapid growth in infancy or adolescence Pregnancy Erythropoietin therapy
Increased Iron Loss
Chronic blood loss Menses Acute blood loss Blood donation Phlebotomy as treatment for polycythemia vera
Decreased Iron Intake or Absorption
Inadequate diet Malabsorption from disease (sprue, Crohn's disease) Malabsorption from surgery (postgastrectomy) Acute or chronic inflammation

Clinical Features:

- When anemia develops rapidly, shortness of breath, tachycardia, dizziness or faintness (particularly upon arising from a sitting or recumbent posture), and extreme fatigue are prominent.
- In chronic anemia, only moderate dyspnea or palpitation may occur, but in some patients, congestive heart failure, angina pectoris, or intermittent claudication can be the presenting manifestation.
- Heart murmurs are a common cardiac sign associated with anemia. They usually are systolic in time and best heard in the pulmonic area.

- The pallor associated with anemia is best detected in the mucous membranes of the mouth and pharynx, the conjunctivae, the lips, and the nail beds.
- In the hands, the skin of the palms first becomes pale, but the creases may retain their usual pink color until the Hb concentration is <7 g/dl (*).
- A distinctly sallow color implies chronic anemia. (*).
- A lemon-yellow pallor suggests pernicious anemia, but it is observed only when the condition is well advanced. (*).
- Definite pallor associated with mild scleral and cutaneous icterus suggests hemolytic anemia. (*).
- Marked pallor associated with petechiae or ecchymoses suggests more generalized bone marrow failure due to acute leukemia, aplasia, or myelodysplastic syndromes.
- The nails may lose their luster, become brittle, and break easily.
- This finding is especially noticeable in chronic iron deficiency anemia, in which the nails may actually become concave instead of convex (koilonychia).
- Approximately 20% of such patients have flame-shaped hemorrhages, hard exudates, cottonwood spots, or venous tortuousness affecting the retina.
- The craving to eat unusual substances, for example, dirt, clay, ice, laundry starch, salt, cardboard, and hair, is a classic manifestation of iron deficiency and is usually cured promptly by iron therapy (PICA).

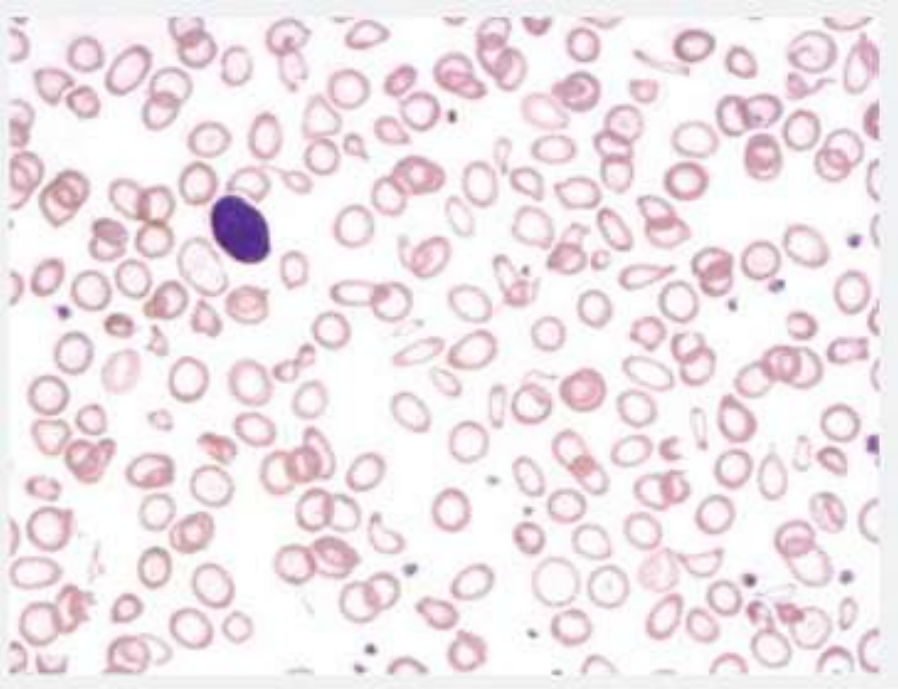


Fig. 14.6

Differential Diagnosis of Microcytic Anemia:

Diagnosis of Microcytic Anemia				
Tests	Iron Deficiency	Inflammation	Thalassemia	Sideroblastic Anemia
Smear	Micro/hypo	Normal micro/ hypo	Micro/hypo with targeting	Variable
SI	<30	<50	Normal to high	Normal to high
TIBC	>360	<300	Normal	Normal
Percent saturation	<10	10–20	30–80	30–80
Ferritin (g/L)	<15	30–200	50–300	50–300
Hemoglobin pattern on electrophoresis	Normal	Normal	Abnormal with beta thalassemia; can be normal with alpha thalassemia	Normal
Serum Ferritin. Most sensitive and specific test for diagnosis of iron deficiency anemia. It decreases even before the appearance of anemia. Correlates with body iron stores (1 µg/L = 10 mg storage iron). Levels less than 12 µg/L are highly specific for diagnosis of iron deficiency anemia. Not suitable for diagnosing iron deficiency anemia in patients with concomitant inflammation, neoplastic or liver disorders.				

Most Commonly Asked Questions:

1. Most important finding of iron deficiency anemia- anisocytosis (earliest also).
2. First sign of improvement after iron therapy- improvement in symptoms.
3. Diagnosis of iron deficiency in pregnancy- serum ferritin.

Once the Diagnosis of Iron-Deficiency Anemia and its Cause is Made, There are Three Major Therapeutic Approaches:

1. Transfusion therapy is reserved for individuals who have symptoms of anemia, cardiovascular instability, continued and excessive blood loss from whatever source, and require immediate intervention. The management of these patients is less related to the iron deficiency than it is to the consequences of the severe anemia. Not only do transfusions correct the anemia acutely, but the transfused red cells provide a source of iron for reutilization, assuming they are not lost through continued bleeding.
2. In the asymptomatic patient with established iron-deficiency anemia, treatment with oral iron is usually adequate.

Oral Iron Preparations		
Generic Name	Tablet (Iron Content), mg	Elixir (Iron Content), mg in 5 ml
Ferrous sulfate	325 (65) 195 (39)	300 (60) 90 (18)
Extended release	525 (105)	
Ferrous fumarate	325 (107) 195 (64)	100 (33)
Ferrous gluconate	325 (39)	300 (35)
Polysaccharide iron	150 (150) 50 (50)	100 (100)

Of the complications of oral iron therapy, gastrointestinal distress is the most prominent and is seen in 15–20% of patients. Typically, the reticulocyte count should begin to increase within 4–7 days after initiation of therapy and peak at 1–11/2 weeks.

1. Intravenous iron can be given to patients who are unable to tolerate oral iron; whose needs are relatively acute; or who need iron on an ongoing basis, usually due to persistent gastrointestinal blood loss.
2. Parenteral iron therapy may be indicated when the patient.

a. has severe iron deficiency anemia;

b. is unable to tolerate iron compounds given orally;

c. repeatedly does not heed instructions or is incapable of accepting or following them;

d. loses iron (blood) at a rate too rapid for the oral intake to compensate for the loss, such as in hereditary hemorrhagic telangiectasia;

e. has a disorder of the gastrointestinal tract, such as ulcerative colitis, in which symptoms may be aggravated by iron therapy;

f. is unable to absorb iron from the gastrointestinal tract;

g. is unable to maintain iron balance on treatment with hemodialysis; or

h. has functional iron deficiency because of concurrent treatment with erythropoietin (e.g., in the anemia of renal failure, in the anemia of inflammation, or for autologous blood donation).

Hemochromatosis:

Classification of Hemochromatosis
I. Hereditary Hemochromatosis
A. Classical hemochromatosis (hereditary hemochromatosis; HFE hemochromatosis) (type 1)
B. Juvenile hemochromatosis (type 2)
1. Abnormality in hemojuvelin
2. Abnormality of hepcidin
C. Transferrin receptor-2 deficiency (type 3)
D. Ferroportin deficiency (includes some cases of African iron overload)(type 4)
E. Ferritin H-chain IRE mutation
F. African iron overload
G. Neonatal hemochromatosis (?)
II. Secondary Hemochromatosis

Pathophysiology:

Normal humans absorb and lose approximately 1 mg of iron each day.

Duodenal absorption of iron increases when iron deficiency occurs, then drops to 1 mg/day after iron deficiency is corrected.

In iron-loaded subjects with hemochromatosis, iron absorption usually is >2 mg/day at a time when iron absorption should have decreased to nearly zero.

The progressive accumulation of iron increases plasma iron, saturation of transferrin, and results in a progressive increase of plasma ferritin

HFE gene (High FE- High iron gene):

The HFE gene is located on the short arm of chromosome 6, approximately 4 megabases telomeric to the HLA region.

The HFE gene is structurally somewhat similar to other HLA class I–like genes.

The HFE gene is composed of seven exons, of which the first six exons encode for the six domains of the HFE protein.

The seven exons of HFE result in formation of a messenger RNA transcript that is approximately 4.2 kilobases (kb) in size. This in turn results in the synthesis of the HFE product, which consists of 343 amino acids.

The most common iron-loading mutation of HFE (C282Y) is caused by a mutation of one nucleotide base (845G → A) in exon 4 of the HFE gene

Of the known mutations of the HFE gene, 16 are missense mutations that result in substitution of the normal amino acid by another.

The HEIRS Study (HEmochromatosis and IRon Overload Screening Study) isthe largest study of the prevalence of mutations of HFE and of serum iron tests.

Clinical Features:

- Initial symptoms are often nonspecific and include lethargy, arthralgia, change in skin color, loss of libido, and features of diabetes mellitus.
- Hepatomegaly, increased pigmentation, spider angiomas, splenomegaly, arthropathy, ascites, cardiac arrhythmias, congestive heart failure, loss of body hair, testicular atrophy, and jaundice are prominent in advanced disease.
- Gray or bronze pigmentation is the most common physical examination abnormality (*).
- The **liver** is usually the first organ to be affected, and hepatomegaly is present in more than 95% of symptomatic patients (*).
- Hepatocellular carcinoma develops in about 30% of patients with cirrhosis, and it is the most common cause of death in treated patients—hence the importance of early diagnosis and therapy.
- The characteristic metallic or slate-gray hue is sometimes referred to as **bronzing** and results from increased melanin and iron in the dermis.
- Pigmentation usually is diffuse and generalized, but it may be more pronounced on the face, neck, extensor aspects of the lower forearms, dorsa of the hands, lower legs, and genital regions, as well as in scars.
- The joints of the hands, especially the second and third metacarpophalangeal joints, are usually the first joints involved, a feature that helps to distinguish the chondrocalcinosis associated with hemochromatosis from the idiopathic form.
- The most common cardiac manifestation is congestive heart failure, which occurs in about 10% of young adults with the disease, especially those with juvenile hemochromatosis.
- **Hypogonadism** occurs in both sexes and may antedate other clinical features.
- Manifestations include loss of libido, impotence, amenorrhea, testicular atrophy, gynecomastia, and sparse body hair. These changes are primarily the result of decreased production of gonadotropins due to impairment of hypothalamic-pituitary function by iron deposition.

- Adrenal insufficiency, hypothyroidism, and hypoparathyroidism are rare manifestations.

Representative Iron Values in Normal Subjects, Patients with Hemochromatosis, and Patients with Alcoholic Liver Disease					
Determination	Normal	Symptomatic Hemochroma-tosis	Homozygotes with Early, Asymptomatic Hemochroma-tosis	Heterozygotes	Alcoholic Liver Disease
Plasma iron, mmol/L (mg/dL)	9–27 (50–150)	32–54 (180–300)	Usually elevated	Elevated or normal	Often elevated
Total iron-binding capacity, mmol/L (mg/dL)	45–66 (250–370)	36–54 (200–300)	36–54 (200–300)	Elevated or normal	45–66 (250–370)
Transferrin saturation, percent	22–46	50–100	50–100	Normal or elevated	27–60
Serum ferritin, mg/L		900–6000	200–500	Usually <500	10–500
Men	20–250				
Women	15–150				
Liver iron, mg/g dry wt	300–1400	6000–18,000	2000–4000	300–3000	300–2000
Hepatic iron index	<1.0	>2	1.5–2	<2	<2

The most common laboratory abnormalities in subjects with hemochromatosis are elevations of serum iron concentration, the percent saturation of transferrin, and the serum ferritin concentration.

The next most common laboratory abnormality in hemochromatosis patients is elevation of serum alanine aminotransferase and aspartate aminotransferase.

Treatment:

- The therapy of hemochromatosis involves removal of the excess body iron and supportive treatment of damaged organs.
- Iron removal is best accomplished by weekly or twice-weekly phlebotomy of 500 mL.

- Each milliliter of packed red cells contains approximately 1 mg of iron.
- Thus, the removal of 500 mL of blood with a hematocrit of 40 percent removes approximately 200 mg of iron.
- As the red cell mass is restored to its prephlebotomy size, iron is mobilized from the stores.
- When the stores have been exhausted the signs of iron deficiency develop, and this is the endpoint of the initial part of the phlebotomy program.
- The patient is then followed and a schedule of maintenance phlebotomies is established with the frequency of phlebotomies tailored to maintain the serum ferritin level, the best indicator of body stores, below 100 ng/mL.
- Chelating agents such as deferoxamine, when given parenterally, remove 10–20 mg iron per day, which is much less than that mobilized by once-weekly phlebotomy.
- Subcutaneous infusion of deferoxamine using a portable pump is the most effective means of its administration

15 Megaloblastic Anemia

Megaloblastic Anemia

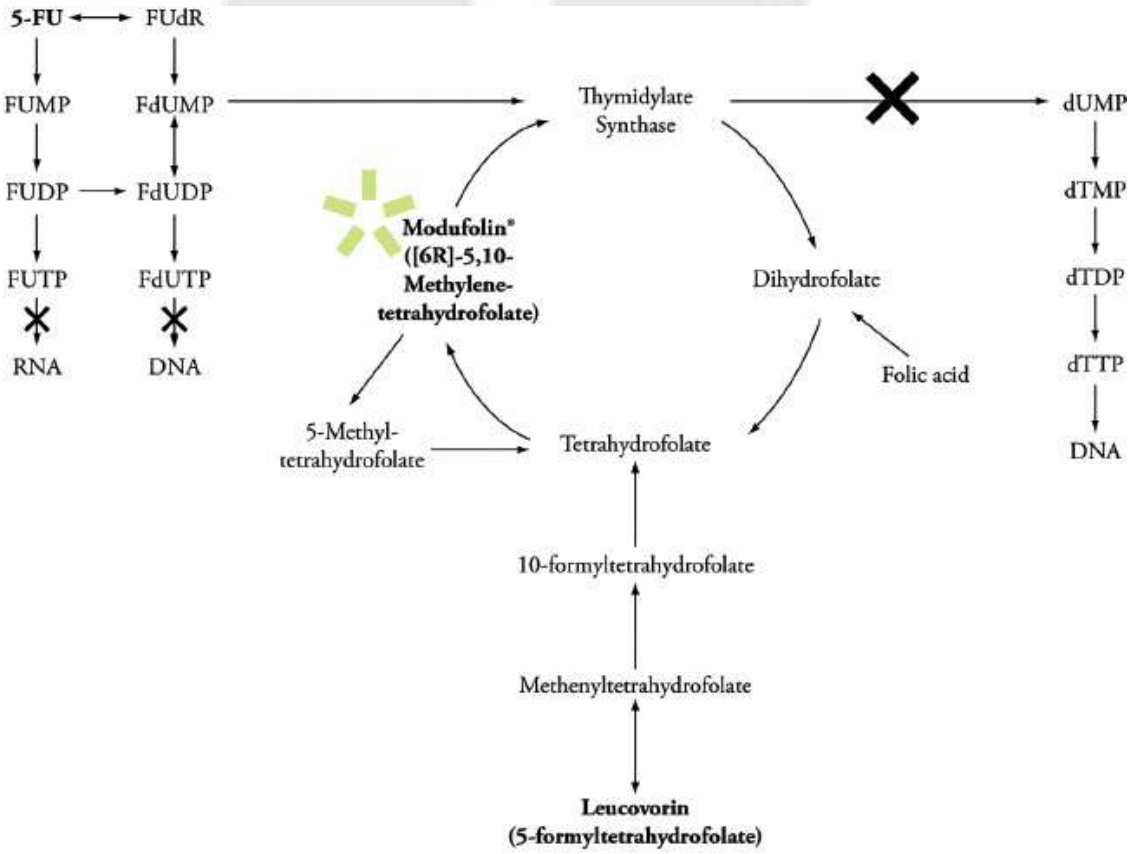


Fig. 15.1

Causes of Megaloblastic Anemia
Cobalamin deficiency or abnormalities of cobalamin metabolism (see Tables 105–3 and 105–4)
Folate deficiency or abnormalities of folate metabolism (see Table 105–5)
Therapy with antifolate drugs (e.g., methotrexate)
Independent of either cobalamin or folate deficiency and refractory to cobalamin and folate therapy:
Some cases of acute myeloid leukemia, myelodysplasia
Therapy with drugs interfering with synthesis of DNA [e.g., cytosine arabinoside, hydroxyurea, 6-mercaptopurine, azidothymidine (AZT)]
Orotic aciduria (responds to uridine)
Thiamine-responsive

Cobalamin-Binding Proteins		
Protein	Source	Function
Intrinsic factor	Gastric parietal cells	Promotes absorption uptake of cobalamin by ileum
Transcobalamin	Probably all cells	Promotes uptake of cobalamin by cells
Haptocorrin	Exocrine glands, phagocytes	Helps dispose of cobalamin analogues (?)

Folate deficiency typically evolves rapidly, and it is often associated with other deficiencies and with alcohol abuse.

In contrast, cobalamin deficiency has a slow onset usually measured in years, and it tends to be a purer deficiency state because of the frequent restriction of malabsorption to cobalamin alone.

Megaloblastic anemia is a panmyelosis:

- The common feature of all megaloblastic anemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow.
- All conditions that give rise to megaloblastic changes have in common a disparity in the rate of synthesis or availability of the four immediate precursors of DNA: the deoxyribonucleoside triphosphates (dNTPs)—dA(adenine)TP and dG(guanine)TP (purines), dT(thymine)TP and dC(cytosine)TP (pyrimidines).
- In deficiencies of either folate or cobalamin, there is failure to convert deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), the precursor of dTTP (Fig. 105-1).

- This is the case because folate is needed as the coenzyme 5,10-methylene-THF polyglutamate for conversion of dUMP to dTMP; the availability of 5,10-methylene-THF is reduced in either cobalamin or folate deficiency.
- An alternative theory for megaloblastic anemia in cobal-amin or folate deficiency is misincorporation of uracil into DNA because of a buildup of deoxyuridine triphosphate (dUTP) at the DNA replication fork as a consequence of the block in conversion of dUMP to dTMP.

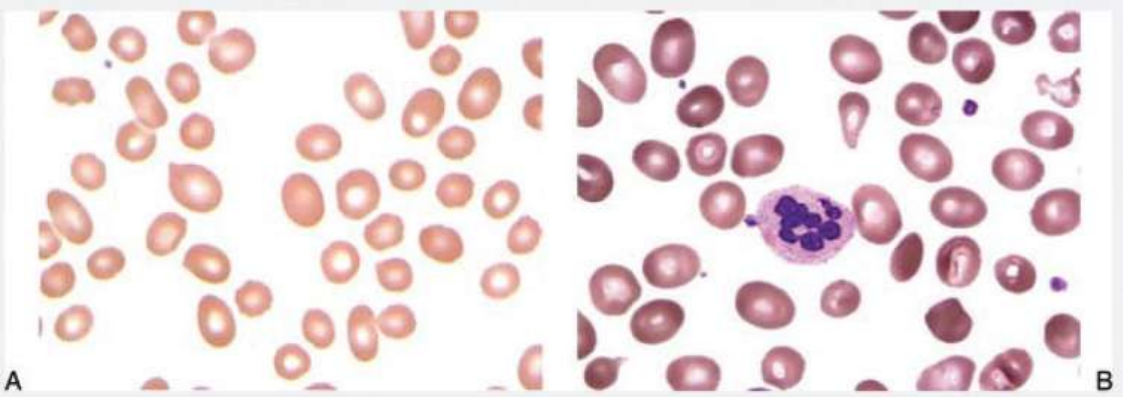


Fig. 15.2

Clinical Features:

- Epithelial Surfaces.
- After the marrow, the next most frequently affected tissues are the epithelial cell surfaces of the mouth, stomach, and small intestine and the respiratory, urinary, and female genital tracts. The cells show macrocytosis, with increased numbers of multinucleate and dying cells. The deficiencies may cause cervical smear abnormalities.
- Complications of Pregnancy.
- The gonads are also affected, and infertility is common in both men and women with either deficiency. Maternal folate deficiency has been implicated as a cause of prematurity, and both folate deficiency and cobalamin deficiency have been implicated in recurrent fetal loss and neural tube defects, as discussed below.

- Neural Tube Defects.
- Folic acid supplements at the time of conception and in the first 12 weeks of pregnancy reduce by around 70% the incidence of neural tube defects (NTDs) (anencephaly, meningomyelocele, encephalocele, and spina bifida) in the fetus. Most of this protective effect can be achieved by taking folic acid, 0.4 mg daily at the time of conception.
- Cardiovascular Disease.
- Children with severe homocystinuria (blood levels > 100 µmol/L) due to deficiency of one of three enzymes, methionine synthase, MTHFR, or cystathionine synthase (Fig. 105-1), have vascular disease, e.g., ischemic heart disease, cerebrovascular disease, or pulmonary embolus as teenagers or in young adulthood.
- Neurologic Manifestations.
- Cobalamin deficiency may cause a bilateral peripheral neuropathy or degeneration (demyelination) of the posterior and pyramidal tracts of the spinal cord and, less frequently, optic atrophy or cerebral symptoms.
- Neuromyelopathic symptoms are the most common neurologic features of cobalamin deficiency.
- Earliest neurological sign due to cobalamin deficiency is said to precede other neurological findings by months- loss of position sense in second toe and loss of vibration sense for 256 Hz tuning fork; not 128 Hz fork.
- Earliest indicator of folate deficiency is a low serum folate.
- LDH1 and LDH2 are both increased- megaloblastic LDH1> LDH2.

Laboratory Features:

- Megaloblastic anaemia resulting from impaired DNA synthesis is characterised by the presence of megaloblastic red cell precursors in the bone marrow and occasionally also in the blood.
- Megaloblasts have a characteristic chromatin pattern and increased cytoplasm as a result of asynchrony of nuclear and

cytoplasmic maturation with a relatively immature nucleus for the degree of cytoplasmic haemoglobinisation. (sieve like chromatin) (*).

- The morphologic hallmark is nuclear-cytoplasmic dissociation, which is best appreciated in precursor cells in the bone marrow aspirate.
- The delay in nuclear maturation caused by delay in DNA synthesis resulting from lack of vitamin B_{12} or folate is also seen in all lineages, particularly granulocytic marrow precursors with giant metamyelocytes and polylobed neutrophils with increased lobe size as well as number of nuclear segments.
- In severe pernicious anaemia a progressive increase in mean red cell volume (MCV) up to 130 fl occurs, with oval macrocytes, poikilocytes, and hypersegmentation of neutrophils (greater than 5% with more than 5 nuclear lobes).
- The mean platelet volume is decreased, and there is increased platelet anisocytosis, as detected by the platelet distribution width (PDW).
- The MCV falls to 110–120 fl as megaloblastic change advances. Howell–Jolly bodies and basophilic stippling are seen in the red cells.
- The functional pathophysiology of megaloblastic anemia is ineffective hematopoiesis in all three hematopoietic cell lines; bone marrow hyperplasia is intense but reticulocytosis does not occur.
- Ineffective hematopoiesis causes a minor component of hemolytic anemias causing jaundice.
- Low serum glutathione has been reported as the most significant metabolic predictor of anemia in cobalamin deficiency.

Causes of Cobalamin Deficiency Sufficiently Severe to Cause Megaloblastic Anemia	
Nutritional	Vegans
Malabsorption	Pernicious anemia
Gastric causes	Congenital absence of intrinsic factor or functional abnormality Total or partial gastrectomy
Intestinal causes	Intestinal stagnant loop syndrome: jejunal diverticulosis, ileocolic fistula, anatomic blind loop, intestinal stricture, etc. Ileal resection and Crohn's disease Selective malabsorption with proteinuria Tropical sprue Transcobalamin II deficiency Fish tapeworm

Pernicious Anemia:

- Pernicious anemia (PA) may be defined as a severe lack of IF due to gastric atrophy.
- This usually shows atrophy of all layers of the body and fundus, with loss of glandular elements, an absence of parietal and chief cells and replacement by mucous cells, a mixed inflammatory cell infiltrate, and perhaps intestinal metaplasia.
- The infiltrate of plasma cells and lymphocytes contains an excess of CD4 cells. The antral mucosa is usually well

preserved.

- Two types of IF immunoglobulin G antibody may be found in the sera of patients with PA.
- One, the “blocking,” or type I, antibody, prevents the combination of IF and cobalamin, whereas the “binding,” or type II, antibody prevents attachment of IF to ileal mucosa. Type I occurs in the sera of >55% of patients, and type II in 35%.
- IF antibodies cross the placenta and may cause temporary IF deficiency in a newborn infant.
- Patients with PA also show cell-mediated immunity to IF.
- Parietal cell antibody is present in the sera of almost 90% of adult patients with PA but is frequently present in other subjects.
- Thus, it occurs in as many as 16% of randomly selected female subjects age >60 years.
- The parietal cell antibody is directed against the α and β subunits of the gastric proton pump (H+, K+-ATPase).

FIGLU Excretion Test:

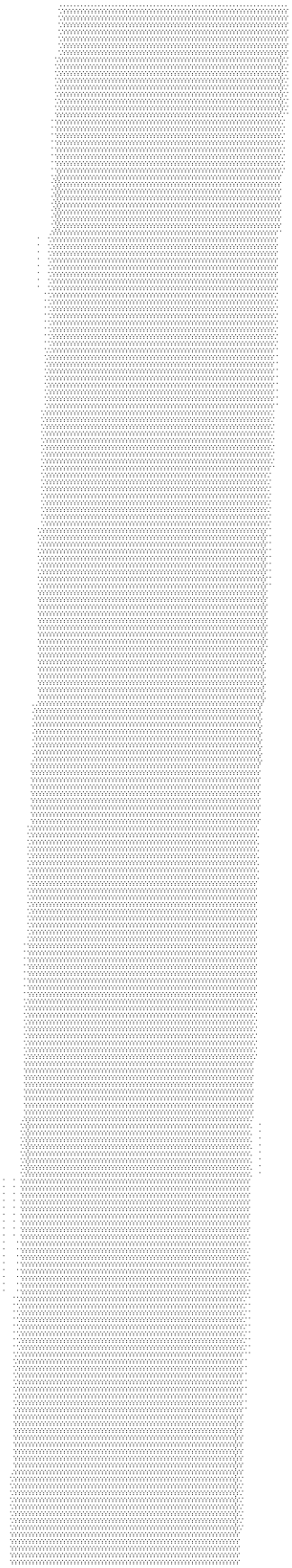
FIGlu (formiminoglutamate) is excreted in excessive amounts in folate deficiency. In this test 15 gm oral dose of histidine is given to the patient and the urinary excretion of FIGlu is measured spectro photo metrically.

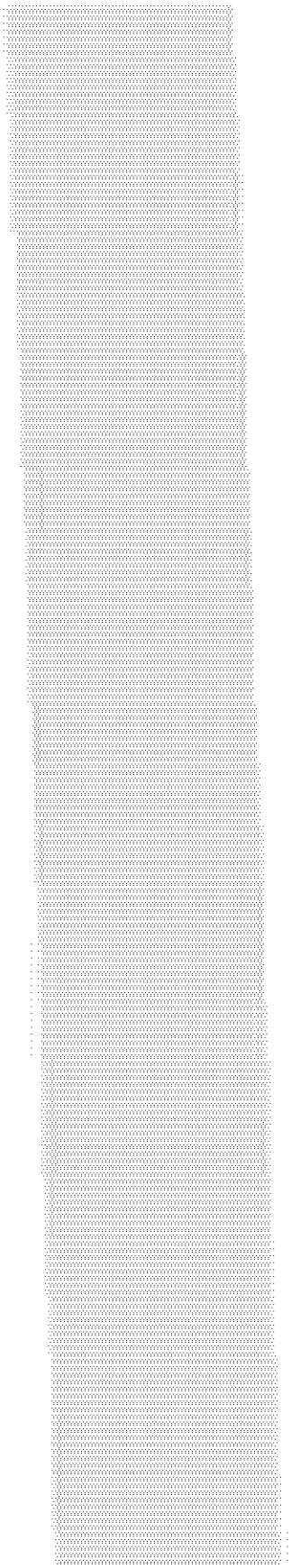
Treatment:

- Megaloblastic anemia should never be empirically with folic acid alone unless vitamin B12 levels are normal.
- Folate deficiency is treated by 1 to 2 mg folic acid per day orally.
- The aims of vitamin B12 replacement therapy are correction of hematocrit to improve neurological abnormalities and to refill storage pools. Initial therapy consists of 1000 µg of hydroxycobalamin every day for one week. Then maintenance every 3 months.
- Patients of pernicious anemia require maintenance therapy for indefinite period.

16 Aplastic Anemia

Differential Diagnosis of Pancytopenia	
Pancytopenia with Hypocellular Bone Marrow	
Acquired aplastic anemia Constitutional aplastic anemia (Fanconi's anemia, dyskeratosis congenita) Some myelodysplasia Rare aleukemic leukemia Some acute lymphoid leukemia Some lymphomas of bone marrow	
Pancytopenia with Cellular Bone Marrow	
Primary bone marrow diseases Myelodysplasia Paroxysmal nocturnal hemoglobinuria Myelofibrosis Some aleukemic leukemia Myelophthisis Bone marrow lymphoma Hairy cell leukemia	Secondary to systemic diseases Systemic lupus erythematosus Hypersplenism B12, folate deficiency Overwhelming infection Alcohol Brucellosis Sarcoidosis Tuberculosis Leishmaniasis
Hypocellular Bone Marrow ± Cytopenia	
Q fever Legionnaires' disease Anorexia nervosa, starvation Mycobacterium	





Etiologic Classification of Aplastic Anemia
Acquired
Autoimmune
Drugs
See next table
Toxins
Benzene
Chlorinated hydrocarbons
Organophosphates
Viruses
Epstein-Barr virus
Non-A, -B, -C, -D, -E, or -G hepatitis virus
Human immunodeficiency virus (HIV)
Paroxysmal nocturnal hemoglobinuria
Autoimmune/connective tissue disorders
Eosinophilic fasciitis
Immune thyroid disease (Graves disease, Hashimoto thyroiditis)
Rheumatoid arthritis
Systemic lupus erythematosus
Thymoma
Pregnancy
Iatrogenic
Radiation
Cytotoxic drug therapy
Hereditary
Fanconi anemia
Dyskeratosis congenita
Shwachman-Diamond syndrome
Other rare syndromes

Other Rare Syndromes Associated with Aplastic Anemia				
Disorder	Findings	Inheritance	Mutated Gene	References
Ataxia-pancytopenia (myelocerebellar disorder).	Cerebellar atrophy and ataxia; aplastic pancytopenia; ± monosomy 7; increased risk of AML.	AD	Unknown	256–258
Congenital amegakaryocytic thrombocytopenia.	Thrombocytopenia; absent or markedly decreased marrow megakaryocytes; hemorrhagic propensity; elevated thrombopoietin; propensity to progress to aplastic pancytopenia; propensity to evolve to clonal myeloid disease.	AR (compound heterozygotes).	MPL	259, 260
DNA ligase IV deficiency.	Pre- and postnatal growth delay; dysmorphic facies; aplastic pancytopenia.	AR (compound heterozygotes).	LIG4	261–263
Dubowitz syndrome.	Intrauterine and post-partum growth failure; short stature; microcephaly; mental retardation; distinct dysmorphic facies; aplastic pancytopenia; increased risk of AML and ALL.	AR	Unknown	264, 265
Nijmegen breakage syndrome.	Microcephaly; dystrophic facies; short stature; immunodeficiency; radiation sensitivity; aplastic pancytopenia; predisposition to lymphoid malignancy.	AR	NBS1	266, 267
Reticular dysgenesis (type of severe immunodeficiency syndrome).	Lymphopenia; anemia and neutropenia; corrected by hematopoietic stem cell transplantation.	XLR	Unknown	268, 269
Seckel syndrome.	Intrauterine and post- partum growth failure; microcephaly; characteristic dysmorphic facies (bird-headed profile); aplastic pancytopenia; ? increased risk of AML.	AR	ATR (and RAD3-related gene); PCNT.	270–273
WT syndrome.	Radial/ulnar abnormalities; aplastic pancytopenia; increased risk of AML.	AD	Unknown	

Fanconi's Anemia:

- Fanconi anemia (FA) is an inherited chromosomal instability syndrome with a variable clinical presentation that includes congenital anomalies, progressive pancytopenia, and cancer susceptibility.
- The diagnostic hallmark of FA is increased chromosomal breakage in response to DNA-damaging agents such as mitomycin C (MMC) or diepoxybutane (DEB).
- There are currently 13 known FA subtypes (A, B, C, D1, D2, E, F, G, I, J, L, M and N).
- With the exception of subtype B, which is X-linked recessive, all the other FA subtypes follow an autosomal recessive pattern of inheritance.

Physical Findings Associated with Fanconi Anemia
Skeletal. Short stature. Radial ray anomalies (thumbs, hands, radii). Hip and spine anomalies.
Skin. Hyperpigmentation (café au lait spots). Hypopigmentation.
Genitourinary. Renal structural anomalies. Hypogonadism.
Craniofacial. Microcephaly. Ophthalmic anomalies (microphthalmia, epicanthal folds). Otic anomalies (external and middle ear anomalies, deafness).
Gastrointestinal malformations Esophageal atresia or tracheoesophageal fistula Imperforate anus Cardiac malformations

- Growth hormone deficiency has been observed in some FA patients, and treatment with growth hormone improved growth in a subset of these patients.
- Additional endocrine disorders associated with Fanconi anemia include hypothyroidism with or without thyroid hormone–

binding globulin (TBG) deficiency, abnormal glucose tolerance, and diabetes mellitus.

Laboratory Features:

- Blood counts and marrow cellularity are often normal until 5 to 10 years of age, when pancytopenia develops over an extended interval.
- The hematologic complications of FA typically present within the first decade of life. Early manifestations include moderate single or bilineage cytopenia with red cell macrocytosis.
- Thrombocytopenia may precede the development of granulocytopenia and anemia.
- The marrow becomes hypocellular, and **in vitro** colony assays reveal a decrease in CFU-GM and BFU–E.
- Random chromatid breaks are present in myeloid cells, lymphocytes, and chorionic villus biopsy samples.
- The diagnosis of Fanconi anemia is based on the demonstration of increased chromosomal breakage in the presence of DNA cross-linking agents, such as mitomycin C (MMC) or diepoxybutane (DEB).
- The hypersensitivity of the chromosomes of marrow cells or lymphocytes to the latter agent is used as a diagnostic test for this condition.
- Patients with FA are at increased risk of developing myelodysplasia (MDS) or acute myeloid leukemia (AML).

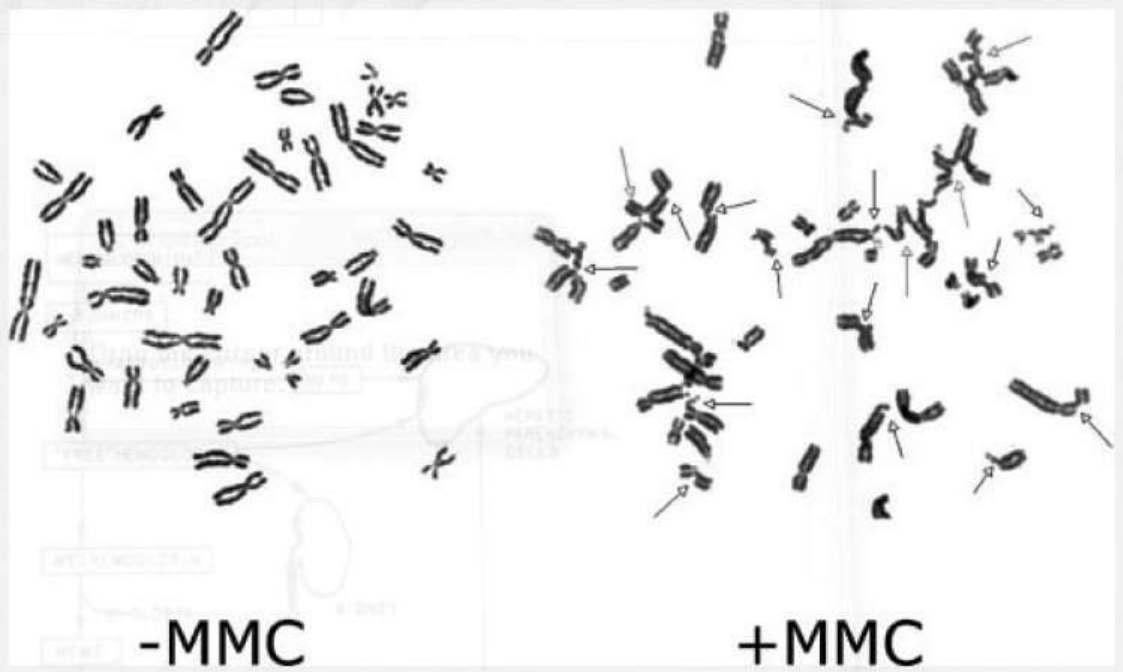


Fig. 16.1

Chromosomal Breakage in Fanconi Anemia:

Peripheral blood lymphocytes from a Fanconi anemia patient were cultured without (left) or with (right) mitomycin C.

Treatment:

- Most patients with Fanconi anemia do not respond to ATG or cyclosporine but do improve with androgen preparations, often for as long as several years.
- Cancer surveillance and education plays an important role in the management of Fanconi anemia patients. Physicians should counsel patients regarding established behavioral and environmental risk factors associated with increased cancer risk.
- Because of the increased risk of MDS and leukemia in patients with bone marrow failure syndromes, frequent complete blood counts and annual bone marrow aspirates and biopsies with cytogenetic analysis are recommended.
- Hematopoietic stem cell transplantation is the only curative therapy for the hematologic manifestations of FA.

Dyskeratosis Congenita:

- Dyskeratosis congenita (DC) is an inherited disorder characterized by lacey reticular skin pigmentation, nail dystrophy, and leukoplakia (the diagnostic triad).
- X-linked recessive (most common), autosomal dominant, and autosomal recessive inheritance patterns have been reported.
- Patients exhibit a predisposition to bone marrow failure, malignancy, and pulmonary dysfunction.

Clinical Features Associated with Dyskeratosis Congenita
Skin pigmentary abnormalities.
Nail dystrophy.
Leukoplakia.
Epiphora.
Cognitive/developmental delay.
Pulmonary disease.
Short stature.
Dental caries/tooth loss.
Esophageal stricture.
Hair loss/gray hair/sparse eyelashes.
Hyperhidrosis.
Intrauterine growth retardation.
Gastrointestinal disorders.
Ataxia.
Hypogonadism/undescended testes.
Microcephaly.
Urethral stricture/phimosis.
Osteoporosis/aseptic necrosis/scoliosis.
Deafness.
Bone marrow failure.
Malignancy.

The cutaneous findings usually appear after 5 years of age and include.

- reticulated, tan to gray, hyperpigmented and hypopigmented cutaneous macules;

- alopecia of scalp, eyelashes, and eyebrows;
- adermatoglyphia (loss of dermal ridges on fingers and toes);
- hyperkeratosis of palms and soles;
- mucosal leukoplakia in 75 percent of patients;
- and dystrophic nails in more than 85 percent of patients.

Diagnosis:

- The diagnosis results from the combination of phenotypic findings and blood cell deficiencies.
- Genetic analysis for telomerase complex gene mutations should be used to confirm the clinical conclusion.
- Shortened telomere length in leukocytes also can be assessed by flow cytometric fluorescence in situ hybridization studies.

Treatment:

The only curative treatment for bone marrow failure in dyskeratosis congenita remains allogeneic stem cell transplantation.

Shwachman-Diamond Syndrome:

- Shwachman-Diamond syndrome (SDS, also called the Shwachman-Diamond-Oski syndrome) is characterized by the combination of exocrine pancreatic insufficiency and bone marrow failure.
- Exocrine pancreatic insufficiency typically presents in infancy with failure to thrive and loose, foul-smelling stools consistent with steatorrhea.
- Exocrine pancreatic insufficiency may improve with age to become clinically asymptomatic in a subset of patients.
- The pancreas in patients with SDS shows fatty replacement of the pancreatic acini, with sparing of the ducts and islets.
- Fat-soluble vitamin deficiencies (vitamins A, D, E, K) may be seen.
- Neutropenia is the most common feature of marrow failure in SDS. Neutropenia may be either intermittent or persistent.

- Patients are predisposed to infections (e.g., pneumonia, abscess, and recurrent otitis media) caused by Staphylococcus aureus, Haemophilus influenzae, and Gram-negative rods including Pseudomonas species.
- SDS is the second most common cause of inherited pancreatic insufficiency, after cystic fibrosis.
- Regular monitoring of peripheral blood counts and periodic bone marrow examination are recommended.
- Therapy in SDS is initiated based on clinical manifestations. Fat malabsorption is treated with the administration of oral pancreatic enzymes. Exocrine pancreatic functions wax and wane over time, and thus regular assessment by a gastroenterologist is recommended.
- Bone marrow transplantation can cure the hematologic aspects of SDS and has been evaluated as a potential therapy in SDS patients with marrow failure, MDS, or AML.

Bone Marrow Picture:

A hypocellular bone marrow is required for the diagnosis of aplastic anemia.

Spicules from an aspirate may be surprisingly cellular in some patients despite overall marrow hypocellularity as most patients will have residual pockets of ongoing hematopoiesis.

Thus, a 1- to 2-cm core biopsy is essential for assessing cellularity.

Bone Marrow Findings that Help to Discriminate Aplastic Anemia from Myelodysplasia		
Characteristic	**Myelodysplastic Syndromes**	**Aplastic Anemia**
Cellularity	Usually increased or normala	Decreased
CD34 count	Normal to increased	Decreased
Erythropoiesis		
Megaloblastosis	Common	Common
Dyserythropoiesis	Common	Sometimes
Ringed sideroblasts	Common	Never
Myelopoiesis		
Increased blasts	Common	Never
Megakaryocytes		
Dysplastic	Common	Never
[a]A hypocellular bone marrow is found in up to 15% of cases of myelodysplastic syndromes.		

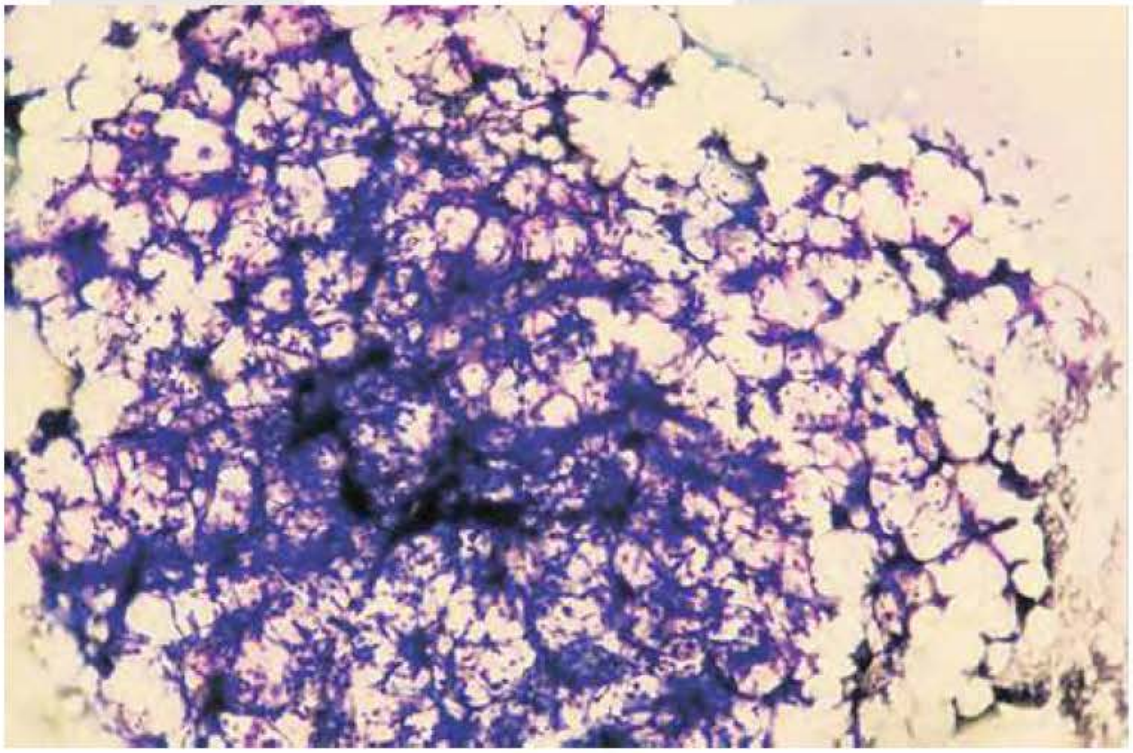


Fig. 16.2

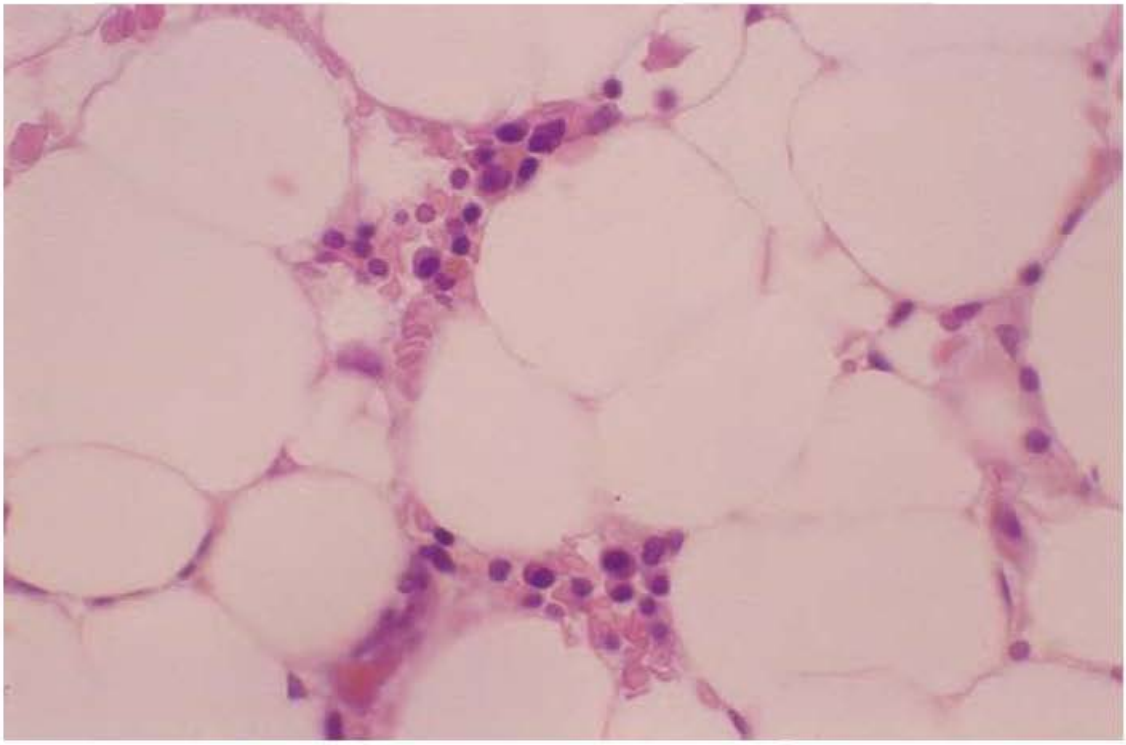


Fig. 16.3 (a): Bone Marrow Aspirate In Aplastic Anemia (High power)

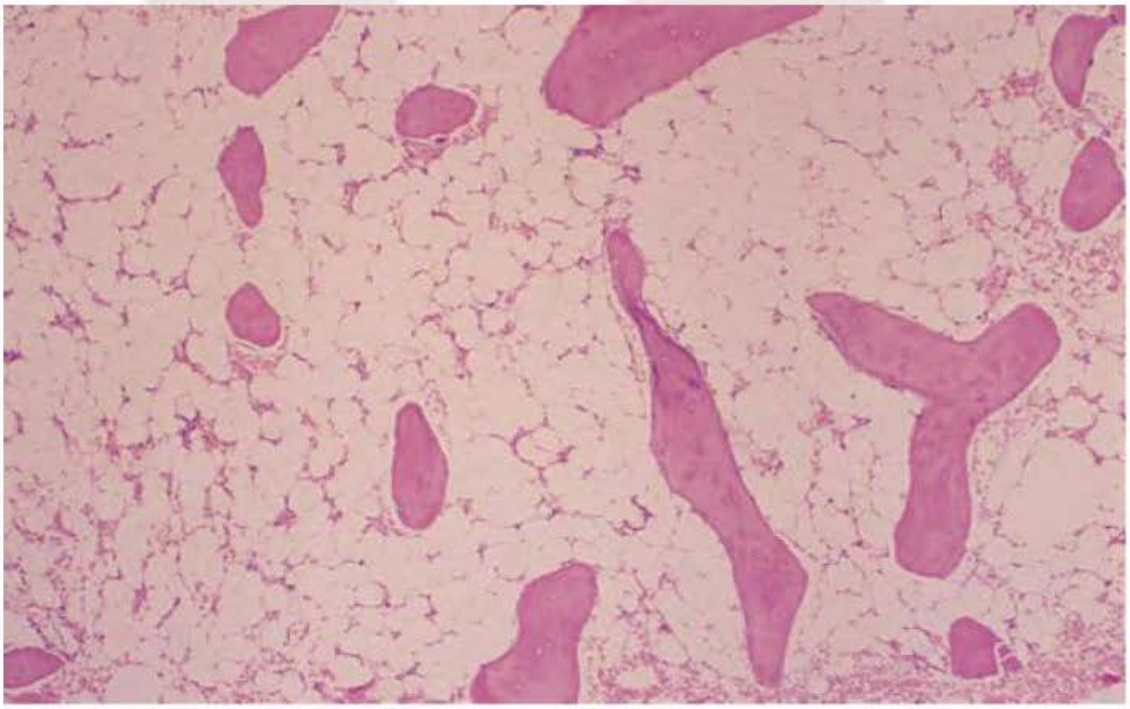


Fig. 16.3 (b): Bone Marrow Aspirate In Aplastic Anemia (Low power)

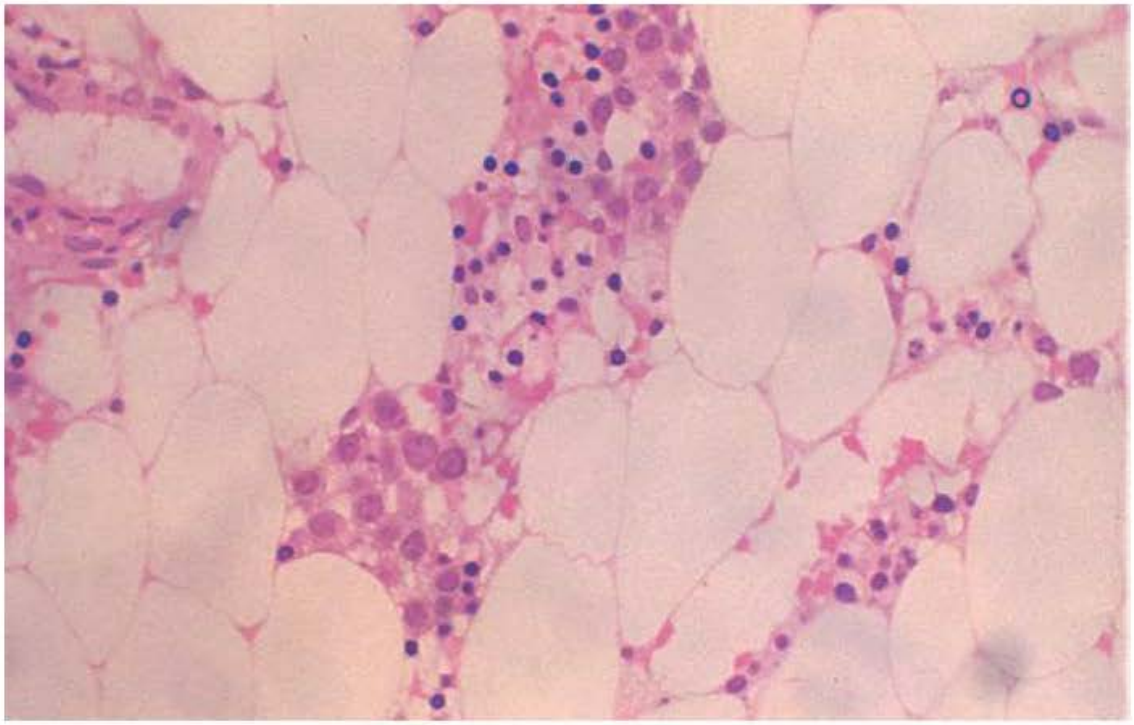


Fig. 16.4: Bone Marrow Trephine Biopsys

17 An Overview of Lymphoid Neoplasms

Nonrandom chromosomal abnormalities, most commonly translocations, are present in the majority of white cell neoplasms

Leukemia is used for neoplasms that present with widespread involvement of the bone marrow and (usually, but not always) the peripheral blood. Lymphoma is used for proliferations that arise as discrete tissue masses.

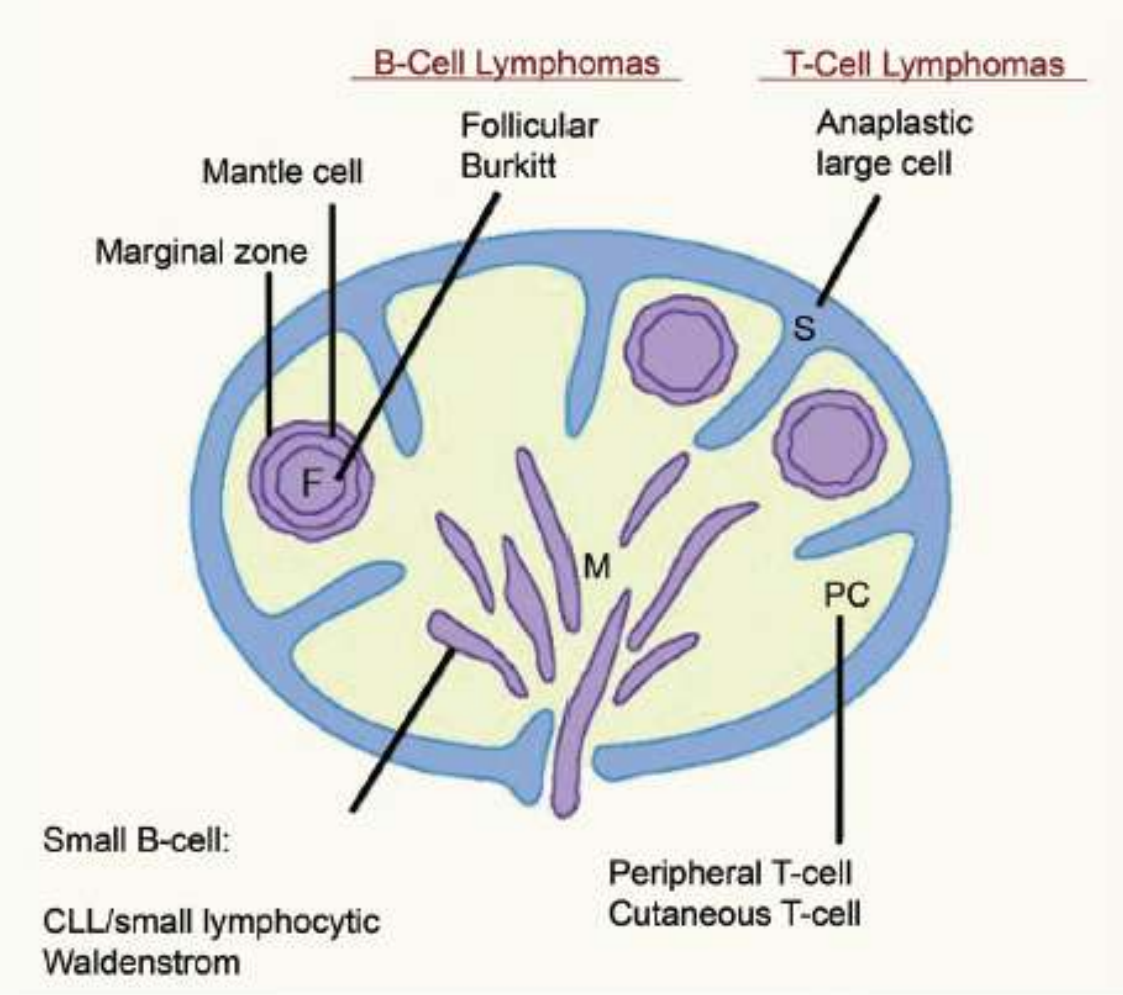


Fig. 17.1

Sites of origin of malignant lymphomas in a lymph node according to anatomic and functional compartments of the immune system. CLL, chronic lymphocytic leukemia; F, follicles with germinal centers; M, medullary cords; PC, paracortex, or interfollicular areas; S, sinuses.

The Who Classification of the Lymphoid Neoplasms:

- The vast majority (85% to 90%) of lymphoid neoplasms are of B-cell origin, with most of the remainder being T-cell tumors; only rarely are tumors of NK cell origin encountered.
- Most lymphoid neoplasms resemble some recognizable stage of B- or T-cell differentiation.

Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia

- Splenic marginal zone lymphoma
- Hairy cell leukemia
- Splenic B-cell lymphoma/leukemia, unclassifiable
  - Splenic diffuse red pulp small B-cell lymphoma
  - Hairy cell leukemia-variant
- Lymphoplasmacytic lymphoma
  - Waldenström macroglobulinemia
- Monoclonal gammopathy of undetermined significance (MGUS), IgM*
- μ heavy-chain disease
- γ heavy-chain disease
- α heavy-chain disease
- Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Monoclonal immunoglobulin deposition diseases*
- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
  - Pediatric nodal marginal zone lymphoma
- Follicular lymphoma
  - In situ follicular neoplasia*
  - Duodenal-type follicular lymphoma*
- Pediatric-type follicular lymphoma*
- Large B-cell lymphoma with IRF4 rearrangement*
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
  - In situ mantle cell neoplasia*
- Diffuse large B-cell lymphoma (DLBCL), NOS
  - Germinal center B-cell type*
  - Activated B-cell type*
- T-cell/histiocyte-rich large B-cell lymphoma
- Primary DLBCL of the central nervous system (CNS)
- Primary cutaneous DLBCL, leg type
- EBV+ DLBCL, NOS*
- EBV+ mucocutaneous ulcer*
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK+ large B-cell lymphoma
- Plasmablastic lymphoma
- Primary effusion lymphoma
- HHV8+ DLBCL, NOS*
- Burkitt lymphoma
- Burkitt-like lymphoma with 11q aberration*
- High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements*
- High-grade B-cell lymphoma, NOS*
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Mature T and NK neoplasms

- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorder of NK cells
- Aggressive NK-cell leukemia
- Systemic EBV+ T-cell lymphoma of childhood*
- Hydroa vacciniforme–like lymphoproliferative disorder*
- Adult T-cell leukemia/lymphoma
- Extranodal NK-/T-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Monomorphic epitheliotropic intestinal T-cell lymphoma*

- Indolent T-cell lymphoproliferative disorder of the GI tract*
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30+ T-cell lymphoproliferative disorders
  - Lymphomatoid papulosis
  - Primary cutaneous anaplastic large cell lymphoma
- Primary cutaneous γδ T-cell lymphoma
- Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
- Primary cutaneous acral CD8+ T-cell lymphoma*
- Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder*
- Peripheral T-cell lymphoma, NOS
- Angioimmunoblastic T-cell lymphoma
- Follicular T-cell lymphoma*
- Nodal peripheral T-cell lymphoma with TFH phenotype*
- Anaplastic large-cell lymphoma, ALK+
- Anaplastic large-cell lymphoma, ALK−*
- Breast implant–associated anaplastic large-cell lymphoma*

Hodgkin lymphoma

- Nodular lymphocyte predominant Hodgkin lymphoma
- Classical Hodgkin lymphoma
  - Nodular sclerosis classical Hodgkin lymphoma
  - Lymphocyte-rich classical Hodgkin lymphoma
  - Mixed cellularity classical Hodgkin lymphoma
  - Lymphocyte-depleted classical Hodgkin lymphoma

Posttransplant lymphoproliferative disorders (PTLD)

- Plasmacytic hyperplasia PTLD
- Infectious mononucleosis PTLD
- Florid follicular hyperplasia PTLD*
- Polymorphic PTLD
- Monomorphic PTLD (B- and T-/NK-cell types)
- Classical Hodgkin lymphoma PTLD

Histiocytic and dendritic cell neoplasms

- Histiocytic sarcoma
- Langerhans cell histiocytosis
- Langerhans cell sarcoma
- Indeterminate dendritic cell tumor
- Interdigitating dendritic cell sarcoma
- Follicular dendritic cell sarcoma
- Fibroblastic reticular cell tumor
- Disseminated juvenile xanthogranuloma
- Erdheim-Chester disease*

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
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.	

Pathologic Features in the Differential Diagnosis of Small B-Cell Lymphomas

Lymphoma Type	Growth Pattern	Cytology	Immunophenotype			Surface Ig	Genetics
			CD5	CD10	CD23		
Follicular lymphoma	Nodular (follicular)	Lymphocytes with irregular cleaved nuclei (centrocytes) and admixed large cells (centroblasts)	-	+	-	Bright	t(14;18)(q32; q21) in >85%
B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma	Diffuse with proliferation centers	Small lymphocytes with round nuclei and scant cytoplasm	+	-	+	Weak IgM and IgD > IgG > IgA	Trisomy 12 deletions of 13q, 6q, 11q and 17p; rearranged 14q 32

Lymphop-lasmacytic lymphoma	Diffuse or interfollic-ular	Small lymphocytes, plasma cells, and plasmacytoid lymphocytes	-	-	-	Moderate IgM	Deletion 6q
Mantle cell lymphoma	Diffuse or vaguely nodular	Small lymphocytes with irregular nuclei, scant cytoplasm, and few admixed large cells	+	-	-	Moderate IgM and IgD; λ>κ	t(11;14) (q13; q32)
Nodal marginal zone B-cell lymphoma	Interfol-licular and perisinu-soidal	Small lymphocytes with round, folded nuclei and abundant cytoplasm plasma cells	-	-	-	Moderate IgM	None
Splenic marginal zone B-cell lymphoma	Nodular	Biphasic: inner core of small lymphocytes with irregular nuclei and scant cytoplasm; outer core of medium-size lymphocytes with round nuclei and abundant clear cytoplasm ± plasma cells	-	-	-	IgM ± IgD	Deletion 7q
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue	Diffuse	Small lymphocytes with round, folded nuclei and abundant cytoplasm ± plasma cells	-	-	-	IgM	Trisomy 3 or t(11;18) (q21;q21)
Ig, immunoglobulin; +, positive; -, negative.							

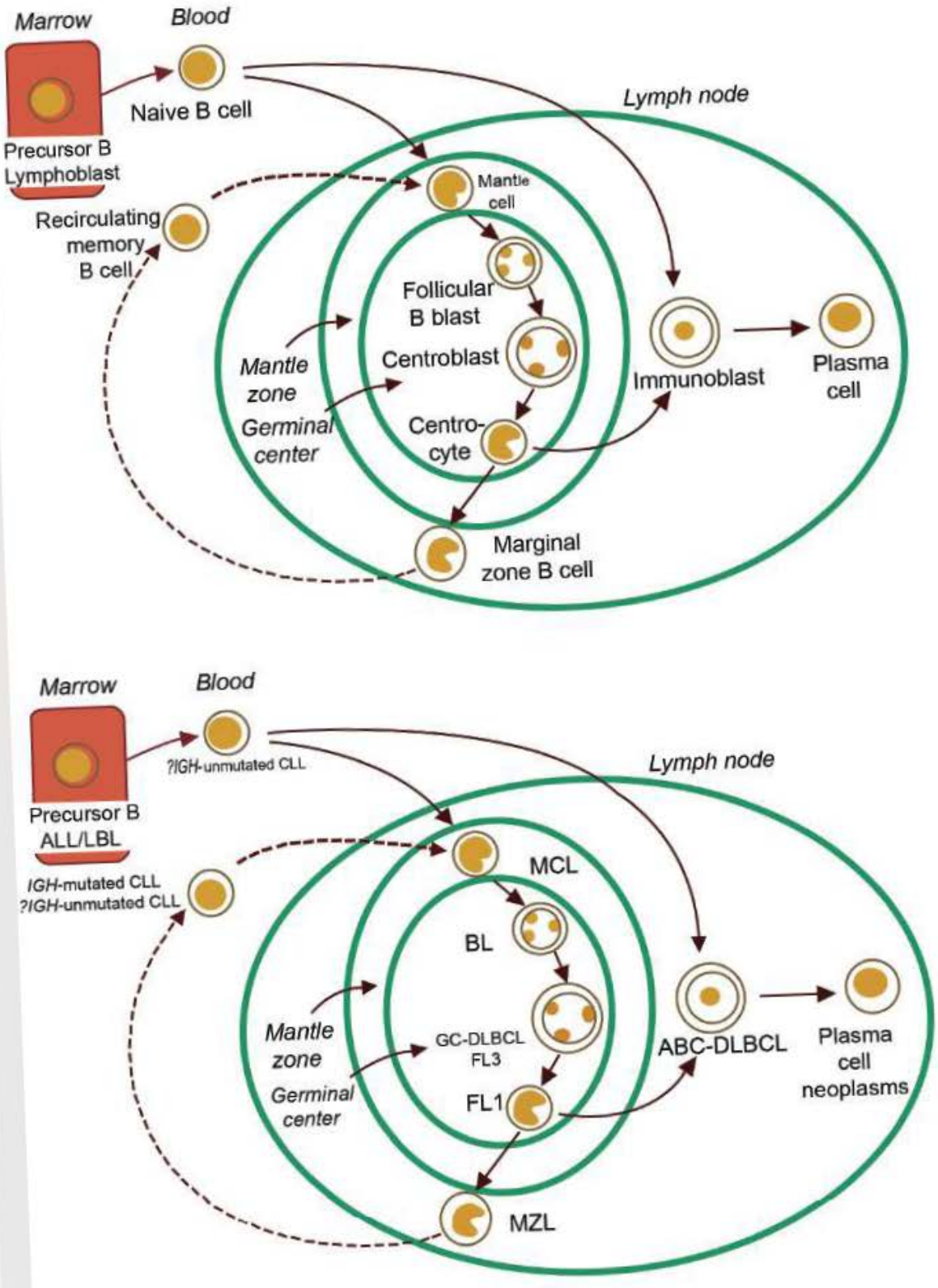

Marrow
Blood
Naive B cell
Precursor B
Lymphoblast
Lymph node
Mantle
cell
Recirculating
memory
B cell
Follicular
B blast
Centroblast
Centro-
cyte
Mantle
zone
Germinal
center
Immunoblast
Plasma
cell
Marginal
zone B cell
Marrow
Blood
?IGH-unmutated CLL
Lymph node
Precursor B
ALL/LBL
IGH-mutated CLL
?IGH-unmutated CLL
MCL
BL
GC-DLBCL
FL3
FL1
MZL
Mantle
zone
Germinal
center
ABC-DLBCL
Plasma
cell
neoplasms

Fig. 17.2

Inherited Syndromes Predisposing to Lymphoma				
Syndrome	Altered Genes		Mechanism	Leukemia Type
	Inheritance	Description		
Ataxia telangiectasia	R	ATM homozygotes	Genomic instability	T-cell lymphoma, T-cell ALL, T-cell PLL, B-cell lymphoma
		Dominant-negative missense mutations	Increased translocations in T cells formed at the time of V(D)J recombination	
Bloom	R	BLM	Genomic instability	ALL, lymphoma
Nijmegen breakage	R	NBS1	Genomic instability Altered telomere maintenance	Lymphoid tumors, especially B-cell lymphoma
Li-Fraumeni*	D	p53	Defect in tumor suppressor	CLL, ALL, Hodgkin and Burkitt lymphoma
Common variable immunodeficiency	R and D	Defect in CD40 signaling	Failure of B-cell maturation	Burkitt, MALT, other B-cell lymphomas, Hodgkin lymphoma
Severe combined immunodeficiency disease (SCID)	R	ADA	Defective T- + B-cell function	B-cell lymphoma
Wiskott-Aldrich	X	WASP	Signaling and apoptosis	Hodgkin and non-Hodgkin lymphoma
X-linked immunodeficiency with normal or increased IgM	X	CD40L	CD40 ligand defect on T cell	Hodgkin and non-Hodgkin lymphoma
X-linked lymphoproliferative syndrome (XLP)	X	SAP	Defect in immune signaling	EBV-related B-cell lymphoma
Autoimmune lymphoproliferative syndrome (ALPS)	D	APT (FAS)	Germ-line heterozygous FAS mutations; defective apoptosis	Lymphoma

Infectious Agents Associated with the Development of Lymphoid Malignancies	
Infectious Agent	Lymphoid Malignancy
Epstein-Barr virus	Burkitt's lymphoma Post–organ transplant lymphoma Primary CNS diffuse large B cell lymphoma Hodgkin's disease Extranodal NK/T cell lymphoma, nasal type
HTLV-I	Adult T cell leukemia/lymphoma
HIV	Diffuse large B cell lymphoma Burkitt's lymphoma
Hepatitis C virus	Lymphoplasmacytic lymphoma
Helicobacter pylori	Gastric MALT lymphoma
Human herpesvirus 8	Primary effusion lymphoma
	Multicentric Castleman's disease

Infections And Associations With Lymphoma	
Agent	Lymphoma Type(s)
Hepatitis C virus	Splenic marginal zone lymphoma; other B-cell lymphomas
Campylobacter jejuni	Immunoproliferative small intestinal disease
Borrelia burgdorferi	Primary cutaneous B-cell lymphoma
Chlamydia psittaci	Ocular adnexal lymphoma
aExtranodal marginal zone lymphoma, MALT-type.	

The Ann Arbor Staging System for Hodgkin's Disease	
Stage	Definition
I	Involvement of a single lymph node region or lymphoid structure (e.g., spleen, thymus, Waldeyer's ring)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (the mediastinum is a single site; hilar lymph nodes should be considered "lateralized" and, when involved on both sides, constitute stage II disease)
III	Involvement of lymph node regions or lymphoid structures on both sides of the diaphragm
III1	Subdiaphragmatic involvement limited to spleen, splenic hilar nodes, celiac nodes, or portal nodes
III2	Subdiaphragmatic involvement includes paraaortic, iliac, or mesenteric nodes plus structures in III1

IV	Involvement of extranodal site(s) beyond that designated as "E"
	More than one extranodal deposit at any location
	Any involvement of liver or bone marrow
A	No symptoms
B	Unexplained weight loss of >10% of the body weight during the 6 months before staging investigation
	Unexplained, persistent, or recurrent fever with temperatures >38°C during the previous month
	Recurrent drenching night sweats during the previous month
E	Localized, solitary involvement of extralymphatic tissue, excluding liver and bone marrow

International Prognostic Index for NHL	
Five clinical risk factors:	
Age 60 years Serum lactate dehydrogenase levels elevated Performance status 2 (ECOG) or 70 (Karnofsky) Ann Arbor stage III or IV >1 site of extranodal involvement Patients are assigned a number for each risk factor they have Patients are grouped differently based upon the type of lymphoma	
For diffuse large B cell lymphoma:	
0, 1 factor = low risk:	35% of cases; 5-year survival, 73%
2 factors = low-intermediate risk:	27% of cases; 5-year survival, 51%
3 factors = high-intermediate risk:	22% of cases; 5-year survival, 43%
4, 5 factors = high risk:	16% of cases; 5-year survival, 26%
For diffuse large B cell lymphoma treated with R-CHOP:	
0 factor = very good:	10% of cases; 5-year survival, 94%
1, 2 factors = good:	45% of cases; 5-year survival, 79%
3, 4, 5 factors = poor:	45% of cases; 5-year survival, 55%

Clinicopathologic Differences Between Childhood and Adult Non-Hodgkin Lymphomas		
	Children	Adults
Incidence	Rare	Common
Median age	10–15 y	55–70 y
Presentation	Extranodal > nodal	Nodal > extranodal

Most common histologic diagnoses	B cell: Burkitt; diffuse large cell T cell: Lymphoblastic; ALK+ anaplastic large cell	B cell: Diffuse large cell (DLBCL); small cleaved (follicular center) cell T cell: Peripheral T-cell, unspecified; anaplastic large cell; angioimmunoblastic
Immunophenotype	50–70% B cell	85–90% B cell (United States, Europe); 50–70% T cell (Asia)
Paraprotein	None	Rare (<5%)
Clinical course	Aggressive	Variable–often indolent
Curability	70–90%	<30%, except 40–70% in aggressive subtypes, particularly DLBCL

18 Lymphoid Neoplasms

- Within the large group of lymphomas, **Hodgkin lymphoma** is segregated from all other forms, which constitute the **non-Hodgkin lymphomas (NHLs)** .
- The other important group of lymphoid tumors is the **plasma cell neoplasms** . These most often arise in the bone marrow and only infrequently involve lymph nodes or the peripheral blood.
- **The clinical presentation of the various lymphoid neoplasms is most often determined by the anatomic distribution of disease** .
- Two thirds of NHLs and virtually all Hodgkin lymphomas present as enlarged nontender lymph nodes (often >2 cm).
- The remaining one third of NHLs present with symptoms related to the involvement of extranodal sites (e.g., skin, stomach, or brain).
- The lymphocytic leukemias most often come to attention because of signs and symptoms related to the suppression of normal hematopoiesis by tumor cells in the bone marrow.
- Finally, the most common plasma cell neoplasm, multiple myeloma, causes bony destruction of the skeleton and often presents with pain due to pathologic fractures.
- However, it should also be kept in mind that **certain lymphoid tumors cause symptoms through the secretion of circulating factors**

International Prognostic Factor Index for Non-Hodgkin Lymphoma
Risk Factors
Age >60 years
Serum lactic dehydrogenase greater than twice normal
Performance status ≥2
Stage III or IV
Extranodal involvement at 11 site

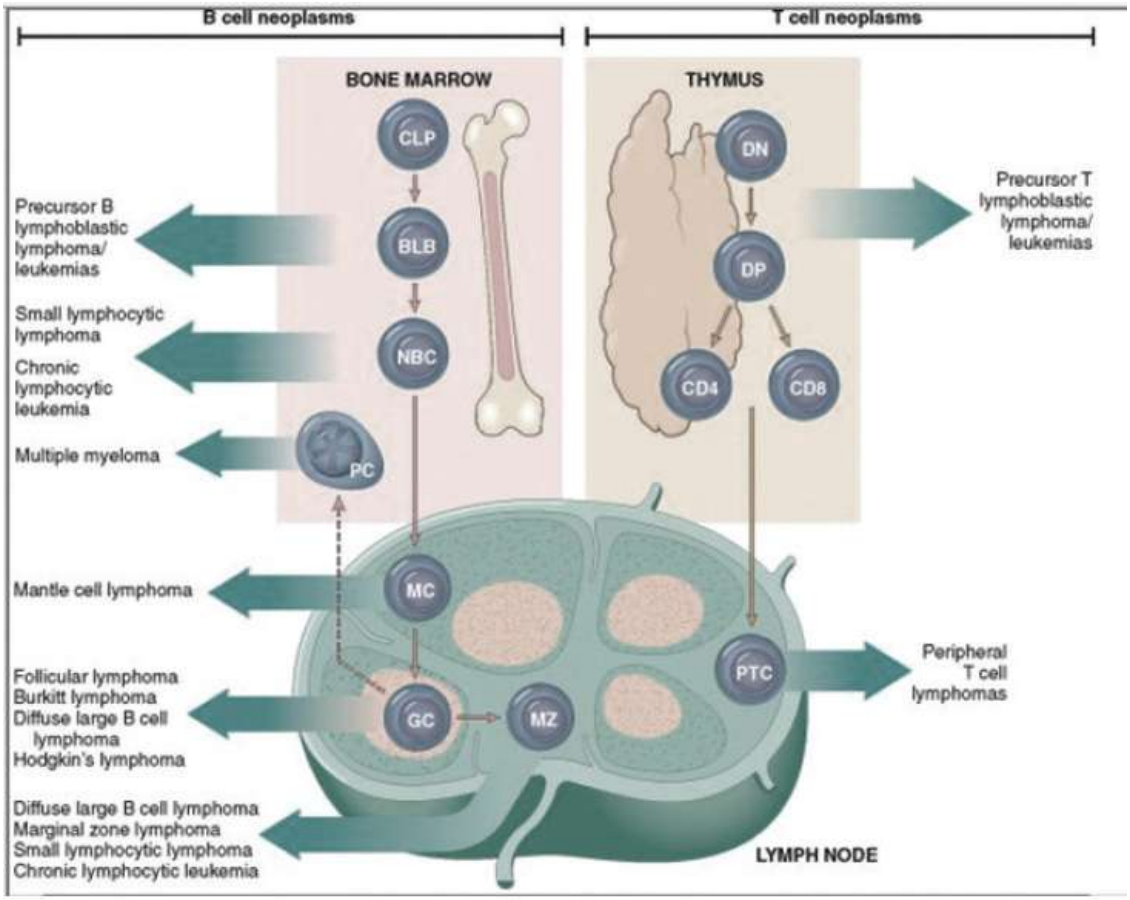


Fig. 18.1

Summary of Major Types of Lymphoid Leukemias and Non-Hodgkin Lymphomas:

Diagnosis	Cell of Origin	Genotype	Salient Clinical Features
Neoplasms of Immature B And T Cells			
B-cell acute lymphoblastic leukemia/lymphoma	Bone marrow precursor B cell	Diverse chromosomal translocations; t(12;21) involving CBFα and ETV6 present in 25%	Predominantly children; symptoms relating to marrow replacement and pancytopenia; aggressive
T-cell acute lymphoblastic leukemia/lymphoma	Precursor T cell (often of thymic origin)	Diverse chromosomal translocations, NOTCH1 mutations (50% to 70%)	Predominantly adolescent males; thymic masses and variable bone marrow involvement; aggressive
Neoplasms of Mature B Cells			
Burkitt lymphoma	Germinal-center B cell	Translocations involving c-MYC and Ig loci, usually t(8;14); subset EBV-associated	Adolescents or young adults with extranodal masses; uncommonly presents as "leukemia"; aggressive
Diffuse large B-cell lymphoma	Germinal-center or post–germinal-center B cell	Diverse chromosomal rearrangements, most often of BCL6 (30%), BCL2 (10%), or c-MYC (5%)	All ages, but most common in adults; often appears as a rapidly growing mass; 30% extranodal; aggressive
Extranodal marginal zone lymphoma	Memory B cell	t(11;18), t(1;14), and t(14;18) creating MALT1-IAP2, BCL10-IgH, and MALT1-IgH fusion genes, respectively	Arises at extranodal sites in adults with chronic inflammatory diseases; may remain localized; indolent
Follicular lymphoma	Germinal-center B cell	t(14;18) creating BCL2-IgH fusion gene	Older adults with generalized lymphadenopathy and marrow involvement; indolent
Hairy cell leukemia	Memory B cell	No specific chromosomal abnormality	Older males with pancytopenia and splenomegaly; indolent
Mantle cell lymphoma	Naive B cell	t(11;14) creating CyclinD1-IgH fusion gene	Older males with disseminated disease; moderately aggressive
Multiple myeloma/ solitary plasmacytoma	Post–germinal-center bone marrow homing plasma cell	Diverse rearrangements involving IgH; 13q deletions	Myeloma: older adults with lytic bone lesions, pathologic fractures, hypercalcemia, and renal failure; moderately aggressive
			Plasmacytoma: isolated plasma cell masses in bone or soft tissue; indolent

Small lymphocytic lymphoma/chronic lymphocytic leukemia	Naive B cell or memory B cell	Trisomy 12, deletions of 11q, 13q, and 17p	Older adults with bone marrow, lymph node, spleen, and liver disease; autoimmune hemolysis and thrombocytopenia in a minority; indolent
Neoplasms of Mature T Cells or Nk Cells			
Adult T-cell leukemia/ lymphoma	Helper T cell	HTLV-1 provirus present in tumor cells	Adults with cutaneous lesions, marrow involvement, and hypercalcemia; occurs mainly in Japan, West Africa, and the Caribbean; aggressive
Peripheral T-cell lymphoma, unspecified	Helper or cytotoxic T cell	No specific chromosomal abnormality	Mainly older adults; usually presents with lymphadenopathy; aggressive
Anaplastic large-cell lymphoma	Cytotoxic T cell	Rearrangements of ALK	Children and young adults, usually with lymph node and soft-tissue disease; aggressive
Extranodal NK/T-cell lymphoma	NK-cell (common) or cytotoxic T cell (rare)	EBV-associated; no specific chromosomal abnormality	Adults with destructive extranodal masses, most commonly sinonasal; aggressive
Mycosis fungoides/ Sézary syndrome	Helper T cell	No specific chromosomal abnormality	Adult patients with cutaneous patches, plaques, nodules, or generalized erythema; indolent
Large granular lymphocytic leukemia	Two types: cytotoxic T cell and NK cell	No specific chromosomal abnormality	Adult patients with splenomegaly, neutropenia, and anemia, sometimes, accompanied by autoimmune disease

Acute Lymphoblastic Leukemia:

Classification of Acute Lymphoid Leukemia (ALL)			
Immunologic Subtype	**% of Cases**	**FAB Subtype**	**Cytogenetic Abnormalities**
Pre-B ALL	75	L1, L2	t(9;22), t(4;11), t(1;19)
T cell ALL	20	L1, L2	14q11 or 7q34
B cell ALL	5	L3	t(8;14), t(8;22), t(2;8)

- **ALL is the most common cancer of children**
- Approximately half of patients present with fever, which often is induced by pyrogenic cytokines (e.g., interleukin-1, interleukin-6, and tumor necrosis factor) released from leukemic cells
- In these patients, fever resolves within 72 hours after the start of antileukemic therapy.

- Among the frequently evident findings are pallor, petechiae, and ecchymosis in the skin and mucous membranes, and bone tenderness as a result of leukemic infiltration or hemorrhage that stretches the periosteum.
- Liver, spleen, and lymph nodes are the most common sites of extramedullary involvement, and the degree of organomegaly is more pronounced in children than in adults.
- In leukemic presentations, the marrow is hypercellular and packed with lymphoblasts, which replace the normal marrow elements.
- Mediastinal thymic masses occur in 50% to 70% of T-ALLs, which are also more likely to be associated with lymphadenopathy and splenomegaly.
- In both B- and T-ALL, the tumor cells have scant basophilic cytoplasm and nuclei somewhat larger than those of small lymphocytes.
- B-ALLs are arrested at various stages of pre-B cell development. The lymphoblasts usually express the pan B-cell marker CD19 and the transcription factor PAX5, as well as CD10. In very immature B-ALLs, CD10 is negative. Alternatively, more mature “late pre-B” ALLs express CD10, CD19, CD20, and cytoplasmic IgM heavy chain (μ chain).
- Similarly, T-ALLs are arrested at various stages of pre-T cell development. In most cases the cells are positive for CD1, CD2, CD5, and CD7. The more immature tumors are usually negative for surface CD3, CD4, and CD8, whereas “late” pre-T cell tumors are positive for these markers.
- **Approximately 90% of ALLs have numerical or structural chromosomal changes** . Most common is hyperploidy (>50 chromosomes).
- The total WBC count at the time of diagnosis is the single most powerful clinical determinant of remission induction, remission duration, and long-term survival for all age groups.
- Examination of the cerebrospinal fluid (CSF) is an essential diagnostic procedure.
- Traditionally, CNS leukemia is defined by the presence of at least 5 leukocytes per microliter of CSF (with leukemic blast

cells apparent in a cytocentrifuged sample) or by the presence of cranial nerve palsies.

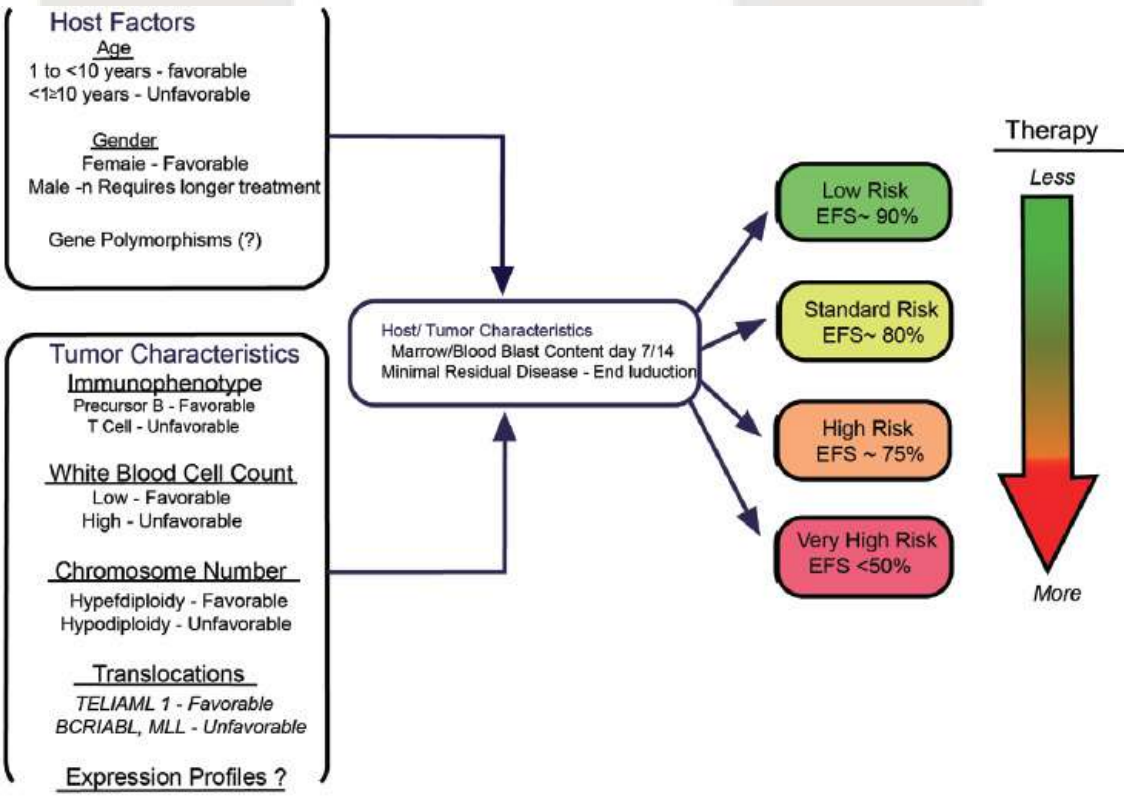


Fig. 18.2

EFS: event free survival:

Prognostic Factors For Remission Duration in Adults with Acute Lymphoblastic Leukemia (All)	
Patient Features	Prognostic Factor
Age (y)	
<30	Favorable
30	Unfavorable
White blood cell count ×(106/ml)	
<30,000	Favorable
30,000 (>100,000 for T cell)	Unfavorable
Immunophenotype	
T-cell ALL	Favorable
Mature B-cell ALL; early T-cell ALL	Unfavorable
Cytogenetics	
12p abnormality; t(10;14)(q24;q11)	Favorable
Normal; hyperdiploid	Intermediate
t(9;22), t(4;11), t(1;19), hypodiploid, −7, +8	Unfavorable
Response to therapy	
Complete remission within 4 wk	Favorable
Persistent minimal residual disease	Unfavorable

Prognostic Factors In Acute Lymphoblastic Leukemia		
Determinants	Favorable	Unfavorable
White blood cell counts	<10 × 109/L	>200 × 109/L
Age	3–7 y	<1 y, >10 y
Gender	Female	Male
Ethnicity	White	Black
Node, liver, spleen enlargement	Absent	Massive
Testicular enlargement	Absent	Present
Central nervous system leukemia	Absent	Overt (blasts + pleocytosis)
FAB morphologic features	L1	L2
Ploidy	Hyperdiploidy	Hypodiploidy <45
Cytogenetic markers	Trisomies 4, 10, and/or 17	t(9;22) (BCR-ABL)
	t(12;21) (TEL-AML1)	t(4;11) (MLL-AF4)
Time to remission	<14 d	>28 d
Minimal residual disease	<10-4	>10-3

Treatment and Supportive Care:

- Hyperuricemia and hyperphosphatemia with secondary hypocalcemia are frequently encountered at diagnosis, even before chemotherapy is initiated, especially in patients with B-cell or T-cell ALL or precursor B-cell leukemia with high leukemic cell burden.
- Patients should be given intravenous fluids; allopurinol or rasburicase (recombinant urate oxidase) to treat hyperuricemia; and a phosphate binder, such as aluminum hydroxide, calcium carbonate (if the serum calcium concentration is low), lanthanum carbonate, or sevelamer to treat hyperphosphatemia.
- The most effective contemporary treatment regimens for B-cell ALL are drug combinations that include cyclophosphamide given over a relatively short time (3–6 months).
- Systemic treatment including high-dose methotrexate, intensive asparaginase, and dexamethasone, as well as optimal intrathecal therapy, is important to control CNS leukemia. Triple intrathecal therapy with methotrexate, cytarabine, and hydrocortisone is more effective than intrathecal methotrexate in preventing CNS relapse.
- The induction regimen typically includes a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and L-asparaginase for children or an anthracycline for adults.
- Consolidation phase: More commonly used regimens for childhood ALL include high-dose methotrexate with or without mercaptopurine, high-dose L-asparaginase given for an extended period, or a combination of dexamethasone, vincristine, L-asparaginase, and doxorubicin, followed by thioguanine, cytarabine, and cyclophosphamide.

Side Effects Associated with Antileukemic Therapy		
Treatment	Acute Complications	Delayed Complications
Prednisone (or prednisolone)	Hyperglycemia, hypertension, changes in mood or behavior, acne, increased appetite, weight gain, peptic ulcer, hepatomegaly, myopathy	Avascular necrosis of bone, osteopenia, growth retardation
Dexamethasone	Same as prednisone, except for increased changes in mood or behavior and myopathy but less salt retention	Same as prednisone
Vincristine	Peripheral neuropathy, constipation, chemical cellulitis, seizures, hair loss	None
Daunorubicin, idarubicin, doxorubicin, or epirubicin	Nausea and vomiting, hair loss, mucositis, marrow suppression, chemical cellulitis, increased skin pigmentation	Cardiomyopathy (with high cumulative dose)
L-Asparaginase	Nausea and vomiting, allergic reactions (manifested as rashes, bronchospasm, severe pain at intramuscular injection site), hyperglycemia, pancreatitis, liver dysfunction, thrombosis, encephalopathy	None

Mercaptopurine	Nausea and vomiting, mucositis, marrow suppression, solar dermatitis, liver dysfunction: increased hematologic toxicity in persons lacking thiopurine methyltransferase	Osteoporosis (long-term use), acute myeloid leukemia in persons with thiopurine methyltransferase deficiency
Methotrexate	Nausea and vomiting, liver dysfunction, marrow suppression, mucositis (resulting from high-dose treatment), solar dermatitis	Leukoencephalopathy, osteopenia (resulting from long-term use)
Etoposide, teniposide	Nausea and vomiting, hair loss, mucositis, marrow suppression, allergic reactions (bronchospasm, urticaria, angioedema, hypotension)	Acute myeloid leukemia
Cytarabine	Nausea and vomiting, fever, skin rashes, mucositis, marrow suppression, liver dysfunction, conjunctivitis (resulting from high-dose treatment)	Decreased fertility (with high cumulative dose)
Cyclophosphamide	Nausea and vomiting, hemorrhagic cystitis, marrow suppression, syndrome of inappropriate secretion of antidiuretic hormone, hair loss	Bladder cancer or acute myeloid leukemia (rare), decreased fertility (with high cumulative dose)
Rituximab	Infusion reactions, mucocutaneous reactions, cardiac arrhythmias, lymphopenia	Reaction of virus infections, progressive multifocal leukoencephalopathy from JC virus infection
Intrathecal methotrexate	Headache, fever, seizure, marrow suppression, mucositis (in patients with renal dysfunction)	Encephalopathy or myelopathy (with high cumulative dose)
Brain irradiation	Hair loss, postirradiation somnolence syndrome (6–10 weeks after treatment)	Seizure, mineralizing microangiopathy, growth hormone deficiency, thyroid dysfunction, obesity, osteopenia, brain tumors, basal cell carcinoma, parotid gland carcinoma, hair loss, cataract (rare), dental abnormalities

Burkitt's Lymphoma:

It was the first tumor to be etiologically associated with

1. a virus, specifically Epstein-Barr virus,
2. a specific chromosomal translocation involving chromosome 8, and
3. one of the first cancers shown to be curable by chemotherapy alone.

- It presents in three clinically distinct forms: endemic, sporadic, and immunodeficiency associated.

- The unifying feature of all three types of BL is activation of the **MYC** gene via immunoglobulin (Ig) translocation leading to high levels of MYC protein, which activates transcription of a plethora of genes involved in cell growth.
- The endemic (African) form often presents as a jaw or facial bone tumor. It may spread to extranodal sites, especially to the marrow and meninges. Almost all cases are EBV positive.
- The nonendemic or American form presents as an abdominal mass in approximately 65 percent of cases, often with ascites. Extranodal sites, such as the kidneys, gonads, breast, marrow, and central nervous system (CNS) may be involved. Involvement of the marrow and CNS is much more common in the nonendemic form. Patients with more than 25 percent marrow involvement with malignant cells often are referred to as having **acute Burkitt cell leukemia** . In addition, in contrast to the endemic form, only 15 percent of the nonendemic cases are EBV positive.
- Immunodeficiency-related cases often involve the lymph nodes and are associated with EBV in 30 percent of the cases.
- **The tumor exhibits a high mitotic index and contains numerous apoptotic cells** , the nuclear remnants of which are phagocytosed by interspersed benign macrophages.
- These phagocytes have abundant clear cytoplasm, creating a characteristic **“starry sky” pattern** .
- When the bone marrow is involved, aspirates reveal tumor cells with slightly clumped nuclear chromatin, two to five distinct nucleoli, and royal blue cytoplasm containing clear cytoplasmic vacuoles.

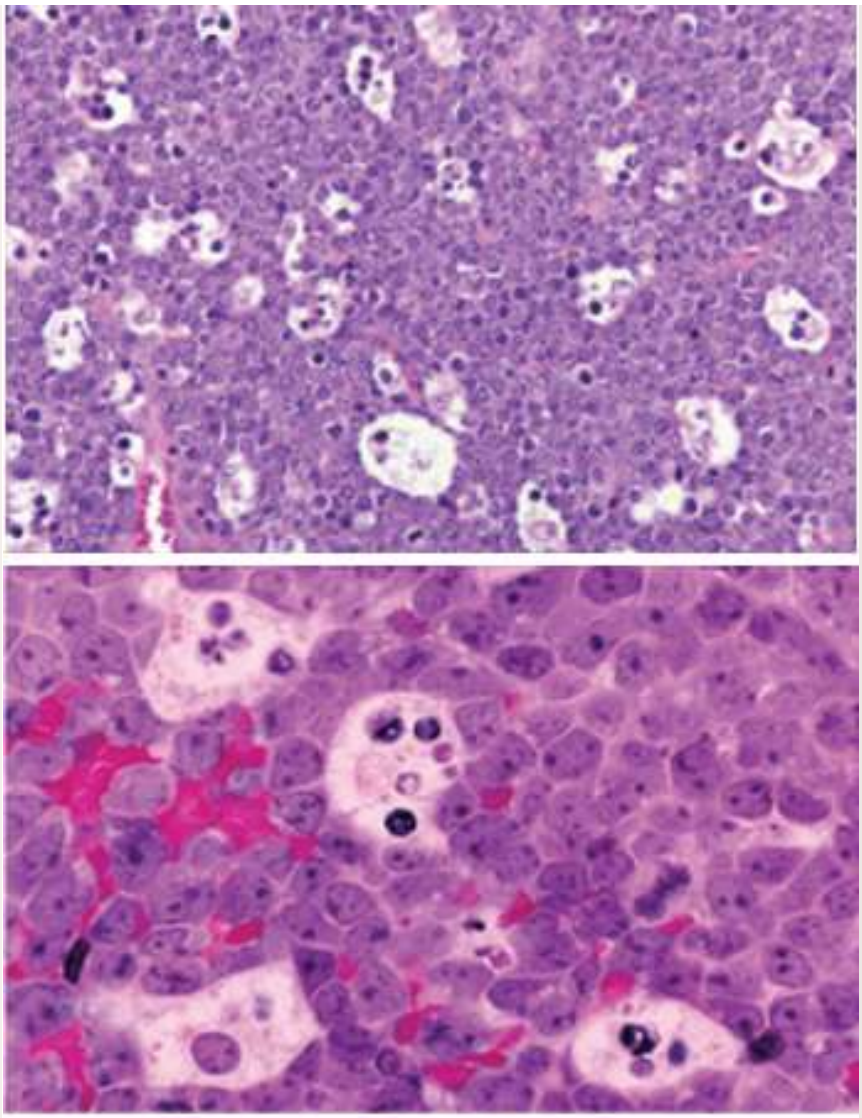


Fig. 18.3

- All cases of BL have a translocation between the long arm of chromosome 8, the site of the **MYC** protooncogene (8q24), and one of three translocation partners: the Ig heavy-chain region on chromosome 14; the κ light-chain locus on chromosome 2; or the λ light-chain locus on chromosome 22.
- A key feature of BL is the relative simplicity of their karyotype: In a good proportion of cases, the **MYC** translocation is the sole abnormality. This distinguishes it from diffuse large B-cell lymphoma.
- These are tumors of mature B cells that express surface IgM, CD19, CD20, CD10, and BCL6, a phenotype consistent with a

germinal center B-cell origin. Unlike other tumors of germinal center origin, Burkitt lymphoma almost always fails to express the anti-apoptotic protein BCL2.

- The regimens employ multiple non–cross-resistant drugs used over a short period. These drugs include high-dose cyclophosphamide, methotrexate, vincristine, prednisone, high-dose methotrexate, high-dose cytarabine, etoposide, and sometimes ifosfamide.
- CNS prophylaxis therapy, either intrathecal or systemic, is given in almost all patients with BL. Radiation therapy does not play a role in the treatment of BL.

Diffuse Large B Cell Lymphoma:

- **Diffuse large B-cell lymphoma (DLBCL) is the most common form of NHL (*)**
- Incidence increases with age; the median age at presentation is in the seventh decade.
- The disease typically presents as a nodal or extranodal mass with rapid tumor growth associated with systemic symptoms.
- Patients with DLBCL typically present with a rapidly enlarging, symptomatic, lymphatic masses. The typical presentation is of a rapidly enlarging lymph node in the neck or an abdominal mass.
- Extranodal disease occurs in approximately 40 percent of patients, most commonly involving the gastrointestinal tract (*). Other sites that may be affected include the testis, bone, thyroid, salivary glands, skin, liver, breast, nasal cavity, paranasal sinuses, and central nervous system (CNS).
- DLBCL can be highly invasive, with local compression of vessels (e.g., superior vena cava syndrome) or airways (e.g., tracheobronchial compression) requiring urgent treatment.
- These mature B-cell tumors express CD19 and CD20 and show variable expression of germinal center B-cell markers such as CD10 and BCL6. Most have surface Ig.
- One frequent pathogenic event is dysregulation of BCL6, a DNA-binding zinc-finger transcriptional repressor that is required for the formation of normal germinal centers.

- About 30% of DLBCLs contain various translocations that have in common a breakpoint in **BCL6** at chromosome 3q27.

Several other subtypes of DLBCL are sufficiently distinctive to merit brief discussion:

- Immunodeficiency-associated large B-cell lymphoma occurs in the setting of severe T-cell immunodeficiency (e.g., advanced HIV infection and allogeneic bone marrow transplantation). The neoplastic B cells are usually infected with EBV, which plays a critical pathogenic role. Restoration of T-cell immunity may lead to regression of these proliferations.
- Primary effusion lymphoma presents as a malignant pleural or ascitic effusion, mostly in patients with advanced HIV infection or the elderly. The tumor cells are often anaplastic in appearance and typically fail to express surface B- or T-cell markers, but have clonal IgH gene rearrangements. In all cases the tumor cells are infected with KSHV/HHV-8, which appears to have a causal role.
- The common features are a relatively large cell size (usually four to five times the diameter of a small lymphocyte) and a diffuse pattern of growth.
- Treatment: CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone and R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone.

Marginal Zone Lymphomas:

The category of marginal zone lymphoma encompasses a heterogeneous group of B-cell tumors that arise within lymph nodes, spleen, or extranodal tissues.

Marginal zone B-cell lymphomas comprise three distinct clinicopathologic entities with variable clinical presentations, namely, the extranodal marginal zone lymphomas also known as mucosa-associated lymphatic tissue (MALT) lymphoma, the nodal marginal zone lymphoma, and the splenic marginal zone lymphoma. The

extranodal type is the most common, accounting for approximately 7.5 percent of all cases of non-Hodgkin lymphoma.

The extranodal tumors were initially recognized at mucosal sites and are often referred to as mucosa-associated lymphoid tumors (or “maltomas”).

In most cases, the tumor cells show evidence of somatic hypermutation and are considered to be of memory B-cell origin.

Although all marginal zone lymphomas share certain features, those occurring at extranodal sites deserve special attention because of their unusual pathogenesis and three exceptional characteristics.

- They often arise within tissues involved by chronic inflammatory disorders of autoimmune or infectious etiology; examples include the salivary gland in Sjögren disease, the thyroid gland in Hashimoto thyroiditis, and the stomach in Helicobacter gastritis.
- They remain localized for prolonged periods, spreading systemically only late in their course.
- They may regress if the inciting agent (e.g., Helicobacter pylori) is eradicated.
- **These characteristics suggest that extranodal marginal zone lymphomas arising in chronically inflamed tissues lie on a continuum between reactive lymphoid hyperplasia and full-blown lymphoma.**
- The disease begins as a polyclonal immune reaction.
- With the acquisition of still-unknown initiating mutations, a B-cell clone emerges that still depends on antigen-stimulated T-helper cells for signals that drive growth and survival. At this stage, withdrawal of the responsible antigen causes tumor involution.
- A clinically relevant example is found in gastric “maltoma,” in which antibiotic therapy directed against **H. pylori** often leads to tumor regression.
- With time, however, tumors may acquire additional mutations that render their growth and survival antigen-independent, such as the (11;18), (14;18), or (1;14) chromosomal translocations,

which are relatively specific for extranodal marginal zone lymphomas.
- All of these translocations up-regulate the expression and function of BCL10 or MALT1, protein components of a signaling complex that activates NF-κB and promotes the growth and survival of B cells.

Follicular Lymphoma:

The tumor likely arises from germinal center B cells and is strongly associated with chromosomal translocations involving BCL2 .

- Patients with FL usually present with painless diffuse lymphadenopathy.
- The classic cytogenetic finding detected in FL is the t (14;18) (q32; q21) translocation that juxtaposes the **BCL** -2 gene on band q21 of chromosome 18 with the immunoglobulin (Ig) heavy-chain gene on band 32 of chromosome 14.
- **Histologic transformation occurs in 30% to 50% of follicular lymphomas** , most commonly to diffuse large B-cell lymphoma.
- **Two principal cell types are present in varying proportions:**
- **small cells with irregular or cleaved nuclear contours and scant cytoplasm, referred to as centrocytes (small cleaved cells); and larger cells with open nuclear chromatin, several nucleoli, and modest amounts of cytoplasm, referred to as centroblasts.**

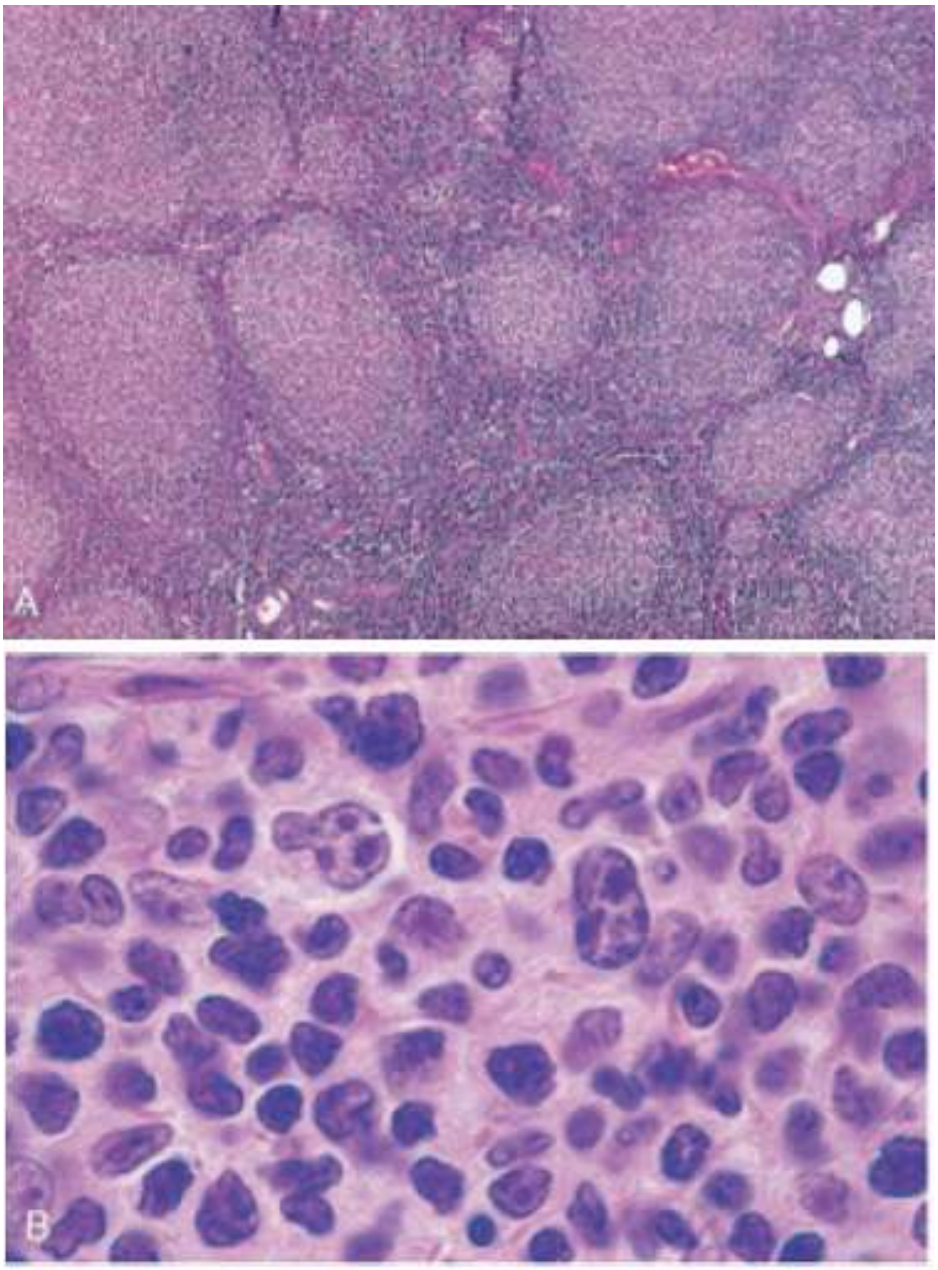


Fig. 18.4

Follicular lymphoma (lymph node). A, Nodular aggregates of lymphoma cells are present throughout lymph node. B, At high magnification, small lymphoid cells with condensed chromatin and irregular or cleaved nuclear outlines (centrocytes) are mixed with a population of larger cells with nucleoli (centroblasts).

Hairy Cell Leukemia/Leukemic Reticuloendotheliosis:

- It is predominantly a disease of middle-aged white males, with a median age of 55 and a male-to-female ratio of 5:1.
- The characteristic morphologic appearance of hairy cells on a Wright-stained peripheral blood smear is the single most important diagnostic finding.
- The cells are mononuclear with relatively abundant cytoplasm and a cell diameter in the range of 10 to 25 μm.
- The cytoplasm is pale blue-gray and agranular with a variable number of elongated (hairy) projections.
- The nuclei are round, oval, reniform, or dumbbell-shaped with a nuclear chromatin pattern that is homogeneous and less clumped and lighter staining than that of normal mature lymphocytes and those seen in classic CLL and prolymphocytic leukemia.
- A prominent nucleolus is rarely seen
- The marrow is involved by a diffuse interstitial infiltrate of cells with oblong or reniform nuclei, condensed chromatin, and pale cytoplasm.
- Because these cells are enmeshed in an extracellular matrix composed of reticulin fibrils, they usually cannot be aspirated (a clinical difficulty referred to as a **“dry tap”**) and are only seen in marrow biopsies.
- The spleen is almost always involved in HCL, and the pattern of hairy cell involvement, as with that in the bone marrow, is nearly pathognomonic for HCL.
- The infiltrates are confined to the red pulp, and, unlike other lymphoproliferative disorders, the white pulp is not expanded and is actually atrophic.
- Hairy cell leukemias typically express the pan-B-cell markers CD19 and CD20, surface Ig (usually IgG), and certain relatively distinctive markers, such as CD11c, CD25, and CD103. Analysis of Ig gene sequences has revealed a high incidence of somatic hypermutation, suggesting a post-germinal center memory B-cell origin.
- Chromosome 5 is involved in clonal aberrations in approximately 40 percent of patients with HCL, most commonly as trisomy 5 or as pericentric inversions and interstitial deletions involving band 5q13.

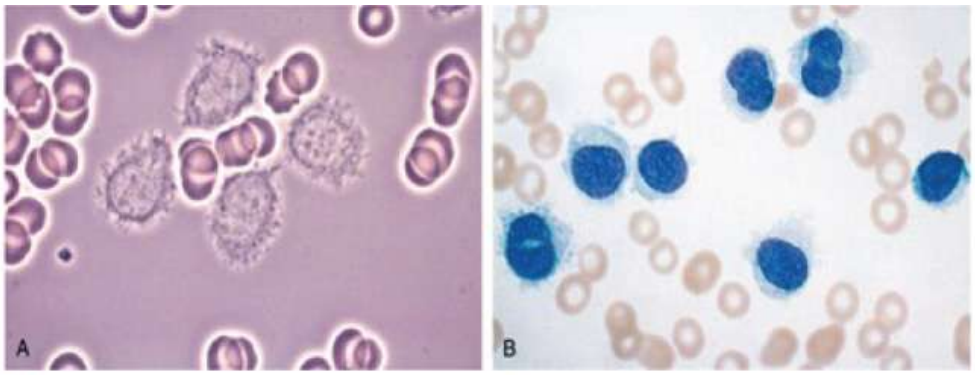


Fig. 18.5: Hairy cell leukemia (peripheral blood smear.) A, Phase-contrast microscopy shows tumor cells with fine hair like cytoplasmic projections. B, In stained smears, these cells have round of folded nuclei and modest amounts of pale, agranular cytoplasm.

Mantle Cell Lymphoma:

- It usually presents in the fifth to sixth decades of life and shows a male predominance.
- Mantle cell lymphoma (MCL) is a lymphoma subtype that usually is characterized by cells carrying an immunophenotype similar to lymphocytes in the mantle zone of normal germinal follicles, secretory immunoglobulin (sIg) M+, sIgD+, CD5+, CD20+, CD10–, CD43+, and the cytogenetic abnormality t(11;14) (q13;q32) in the tumor cells, resulting in the overexpression of cyclin D1.
- MCL is still considered incurable. Because of the presence of advanced disease at presentation, most patients require systemic therapy. Current strategies involve intensification of therapy with or without consolidation with stem cell transplantation (SCT).

NOTE: Differential diagnosis- SLL/CLL AND FOLLICULAR LYMPHOMA.

- The immunophenotype of MCL has some similarities to that of chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) in that the lymphoma cells express surface IgM and IgD and the B-cell–associated antigens CD19 and CD20, and have aberrant expression of the T-cell antigen CD5.

- In contrast to CLL or SLL, MCL cells are positive for FMC7 and typically do not express CD23.
- Like follicular lymphoma, MCL is positive for CD20 and BCL-2, but in contrast to follicular lymphoma, MCL is negative for CD10 and BCL-6.
- This finding occurs because most cases do not originate in the germinal center but rather arise from naïve cells in the mantle zone of the follicle.
- More importantly, almost all cases of MCL overexpress cyclin D1, and no other lymphoma shows overexpression of cyclin D1.

Plasma Cell Neoplasms:

Plasma cell neoplasms (PCNs) are monoclonal expansions of a single B lymphocyte characterized by plasma cell morphology and monoclonal immunoglobulin gene rearrangement. The vast majority of PCNs produce monoclonal immunoglobulin or immunoglobulin fragments.

Some Biologic Features of Monoclonal Gammopathy			
	Myeloma	Monoclonal Gammopathy	Immunodeficiency
Clonal size	Large	Medium	Small
Immunoglobulin production	>30 g/L	<30 g/L	<3 g/L
Time course	Progressive	Persistent	Transient
Abnormal immunoglobulin structure	Frequent	Rare	Never
Bone destruction	Frequent	Never	Never
Mouse models			
Transformed clone		+	–
Transplantable generations	<4	–	
Autonomous growth	+	+?	–
Immortality	+	–	–

Terms used to describe the abnormal Igs include monoclonal gammopathy, dysproteinemia, and paraproteinemia. The following

clinicopathologic entities are associated with monoclonal gammopathies.

- Multiple myeloma (plasma cell myeloma), the most important monoclonal gammopathy, usually presents as tumorous masses scattered throughout the skeletal system. Solitary myeloma (plasmacytoma) is an infrequent variant that presents as a single mass in bone or soft tissue. Smoldering myeloma refers to another uncommon variant defined by a lack of symptoms and a high plasma M component.
- Waldenström macroglobulinemia is a syndrome in which high levels of IgM lead to symptoms related to hyperviscosity of the blood. It occurs in older adults, most commonly in association with lymphoplasmacytic lymphoma.
- Heavy-chain disease is a rare monoclonal gammopathy that is seen in association with a diverse group of disorders, including lymphoplasmacytic lymphoma and an unusual small bowel marginal zone lymphoma that occurs in malnourished populations (so-called Mediterranean lymphoma). The common feature is the synthesis and secretion of free heavy-chain fragments.
- Primary or immunocyte-associated amyloidosis results from a monoclonal proliferation of plasma cells secreting light chains (usually of γ isotype) that are deposited as amyloid. Some patients have overt multiple myeloma, but others have only a minor clonal population of plasma cells in the marrow.
- Monoclonal gammopathy of undetermined significance (MGUS) is applied to patients without signs or symptoms who have small to moderately large M components in their blood. MGUS is very common in the elderly and has a low but constant rate of transformation to symptomatic monoclonal gammopathies, most often multiple myeloma.

PCM:

Clonal bone marrow plasma cell percentage ≥ 10% or biopsy-proven plasmacytoma and ≥ 1 of the following myeloma-defining events:

End-organ damage attributable to the plasma cell proliferative disorder:

- Hypercalcaemia: serum calcium > 0.25 mmol.L (>1mg / Dl) higher than the upper limit of normal or > 2.75 mmol/L (>11mg/Dl).
- Renal insufficiency:
- Creatinine clearance < 40 Ml/minute or serum creatinine > 177 umol/L (>2 mg/Dl).
- Anaemia: a haemoglobin value of >20 g/L below the lower limit of normal or a haemoglobin value < 100g/L.
- Bone lesions: ≥ 1 osteolytic lesion on skeletal radiography, CT, or PET/CT.
- ≥ of the following biomarkers of malignancy:
- Clonal bone marrow plasma cell percentage ≥ 60%.
- An involved-to-univolved serum free light chain ratio ≥ 100.
- > 1 focal lesion on MRI.

Smouldering (asymptomatic) PCM.

Both criteria must be met:

- Serum M protein (lgG or lgA) ≥ 30 g/L or urinary M protein ≥ 500 mg/24 hours and/or clonal bone marrow plasma cell percentage of 10-60%.
- Absence of myeloma-defining events or amloidosis.

Essential monoclonal gammopathy is defined by two key features: (1) the presence of a monoclonal immunoglobulin in the serum or of monoclonal light chains in the urine and (2) the absence of evidence for an overt malignancy of B lymphocytes or plasma cells (e.g., lymphoma, myeloma or amyloidosis).

Diagnostic Criteria for Multiple Myeloma, Myeloma Variants, and Monoclonal Gammopathy of Undetermined Significance
Monoclonal Gammopathy of Undetermined Significance (MGUS).
M protein in serum <30 g/L Bone marrow clonal plasma cells <10% No evidence of other B cell proliferative disorders No myeloma-related organ or tissue impairment (no end organ damage, including bone lesions)a
Asymptomatic Myeloma (Smoldering Myeloma)
M protein in serum >30 g/L and/or Bone marrow clonal plasma cells >10% No myeloma-related organ or tissue impairment (no end organ damage, including bone lesions)a or symptoms
Symptomatic Multiple Myeloma
M protein in serum and/or urine Bone marrow (clonal) plasma cellsb or plasmacytoma Myeloma-related organ or tissue impairment (end organ damage, including bone lesions).
Nonsecretory Myeloma
No M protein in serum and/or urine with immunofixation Bone marrow clonal plasmacytosis >10% or plasmacytoma Myeloma-related organ or tissue impairment (end organ damage, including bone lesions)a
Solitary Plasmacytoma of Bone
No M protein in serum and/or urinec Single area of bone destruction due to clonal plasma cells Bone marrow not consistent with multiple myeloma Normal skeletal survey (and MRI of spine and pelvis if done) No related organ or tissue impairment (no end organ damage other than solitary bone lesion)a

a Myeloma-related organ or tissue impairment (end organ damage) (ROTI): Calcium levels increased: serum calcium >0.25 mmol/L above the upper limit of normal or >2.75 mmol/L; renal insufficiency: creatinine >173 mmol/L; anemia: hemoglobin 2 g/dL below the lower limit of normal or hemoglobin <10 g/dL; bone lesions: lytic lesions or osteoporosis with compression fractures (MRI or CT may clarify); other: symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (>2 episodes in 12 months).

b If flow cytometry is performed, most plasma cells (>90%) will show a “neoplastic” phenotype.

c A small M component may sometimes be present.

The most common clinical feature of multiple myeloma is anemia.

Myeloma bone disease is a major source of morbidity and may present as an area of persistent pain or as a vague migratory bone pain, often in the lower back and pelvis. The type, location, and duration of the pain has no characteristic features. At times, pain and tenderness may be sudden in onset, especially when associated with a pathologic fracture, and is most commonly precipitated by movement. Persistent localized pain also may be associated with a pathologic fracture.

1. Myeloma cells adhere to stroma.
2. Stromal cells secrete OAFs.
3. OAFs induce stroma and osteoblasts to secrete RANKL.

4a. RANKL is blocked by OPG, syndecan from MM cells traps and internalizes OPG.

4b. Excess RANKL is available to stimulate osteoclasts.

5. Increased cytokines stimulate myeloma cell growth.
6. These cytokines stimulate myeloma cell growth.
7. These cytokines also cause release of PTHrP from MM cells, which activates stromal cells to secrete additional RANKL.

Clinical Features of Multiple Myeloma	
Clinical Finding	Underlying Cause and Pathogenetic Mechanism.
Hypercalcemia, osteoporosis, pathologic fractures, lytic bone lesions, bone pain.	Tumor expansion, production of osteoclast activating factor by tumor cells, osteoblast inhibitory factors.
Renal failure	Hypercalcemia, light chain deposition, amyloidosis, urate nephropathy, drug toxicity (nonsteroidal anti-inflammatory agents, bisphosphonates), contrast dye.
Easy fatigue/anemia.	Bone marrow infiltration, production of inhibitory factors, hemolysis, decreased red cell production, decreased erythropoietin levels.
Recurrent infections.	Hypogammaglobulinemia, low CD4 count, decreased neutrophil migration.
Neurologic symptoms.	Hyperviscosity, cryoglobulinemia, amyloid deposits, hypercalcemia, nerve compression, antineuronal antibody, POEMS syndrome, therapy-related toxicity.
Nausea and vomiting	Renal failure, hypercalcemia
Bleeding/clotting disorder	Interference with clotting factors, antibody to clotting factors, amyloid damage of endothelium, platelet dysfunction, antibody coating of platelet, therapy-related hypercoagulable defects.
Abbreviation: POEMS, polyneuropathy, organomegaly, endocrinopathy, multiple myeloma, and skin changes.	

Diagnostic Criteria for Myeloma Requiring Therapy
Presence of an monoclonal immunoglobulin in serum and/or urine plus clonal plasma cells in the marrow and /or a documented clonal plasmacytoma.
PLUS one or more of the following:
Calcium elevation (>11.5 mg/dL) [>2.65 mmol/L]
Renal insufficiency (creatinine >2 mg/dL) [177 µmol/L or more]
Anemia (hemoglobin <10 g/dL or 2 g/dL <12.5 mmol/L‡ or 1.25mmol/L
Bone disease (lytic lesions or osteopenia)

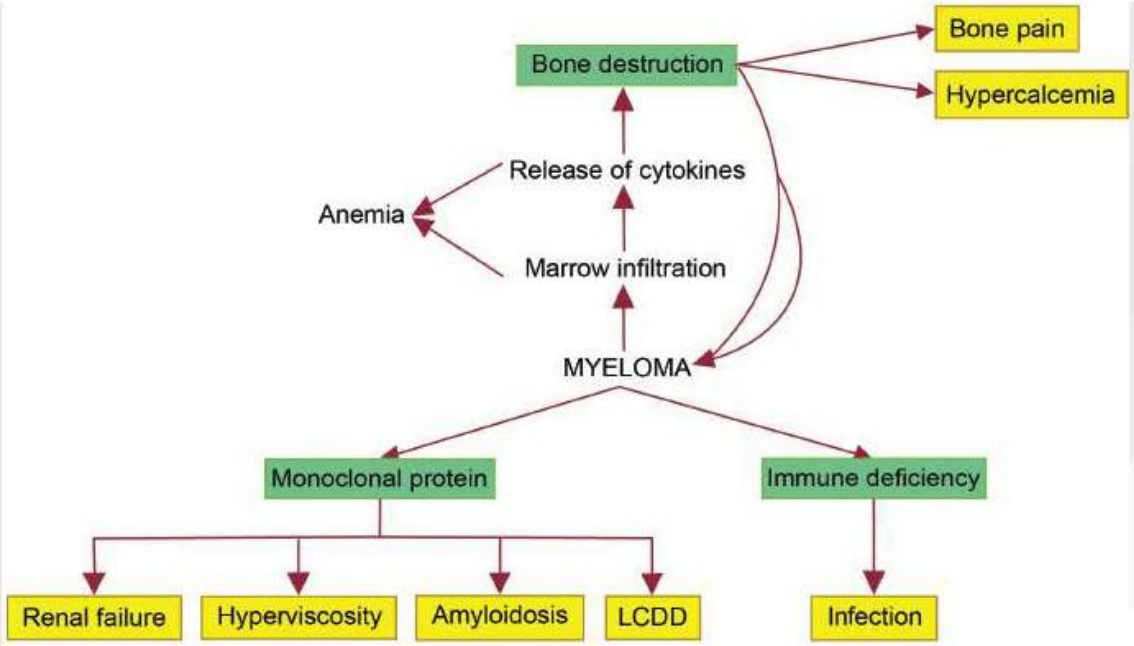

Bone pain
Bone destruction
Hypercalcemia
Release of cytokines
Anemia
Marrow infiltration
MYELOMA
Monoclonal protein
Immune deficiency
Renal failure
Hyperviscosity
Amyloidosis
LCDD
Infection

Fig. 18.6

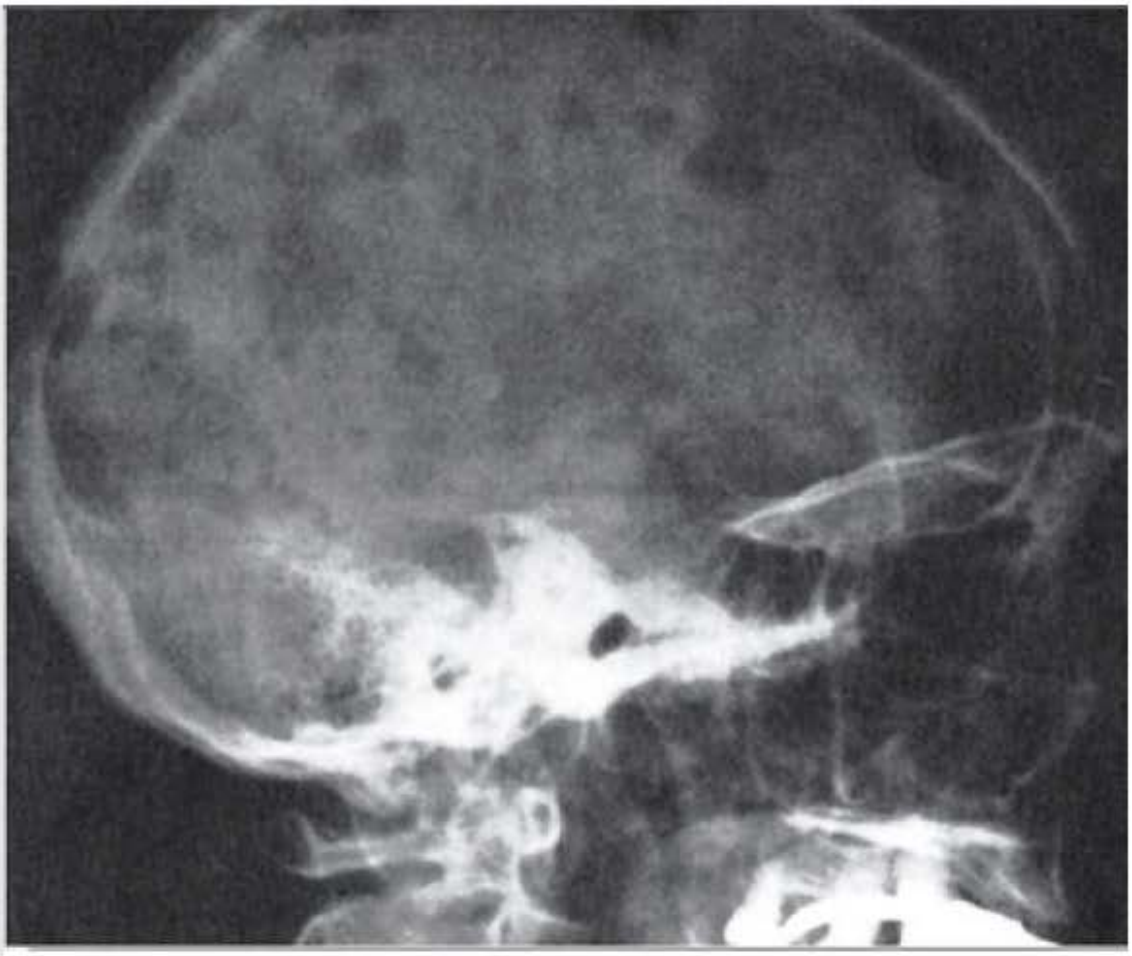


Fig. 18.7 Punched out lesions in the skull

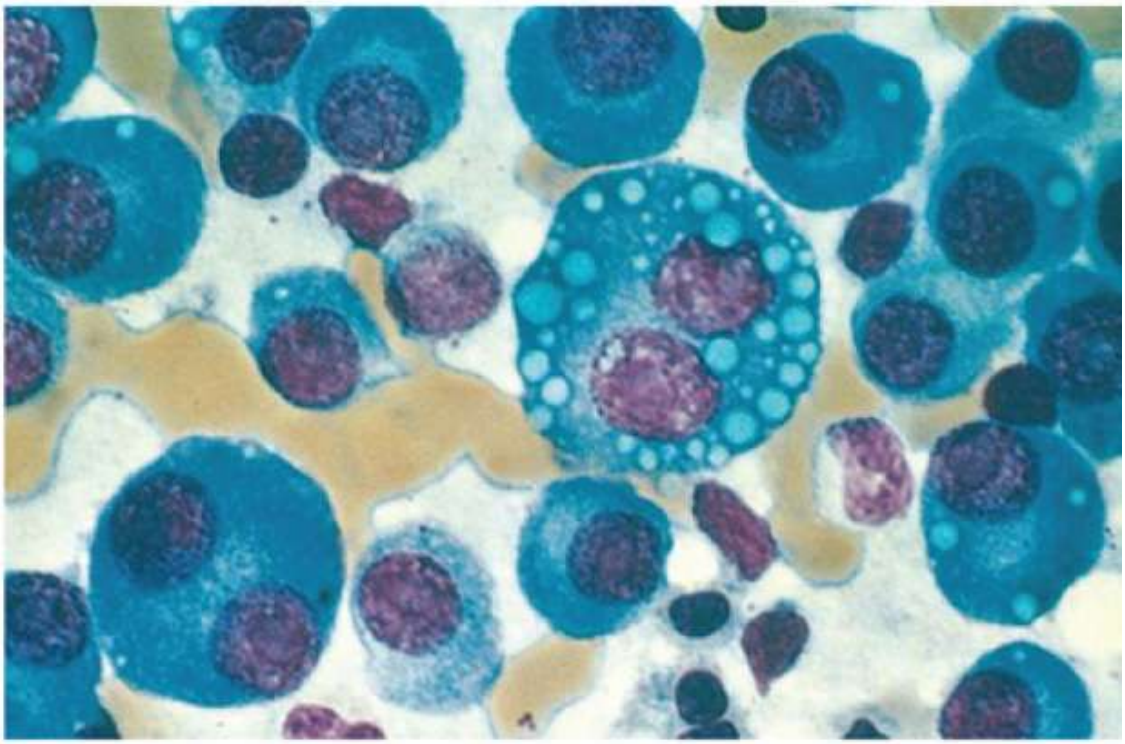


Fig. 18.8

- Relatively normal-appearing plasma cells, **plasmablasts** with vesicular nuclear chromatin and a prominent single nucleolus, or **bizarre, multinucleated cells** may predominate.
- Other cytologic variants stem from the dysregulated synthesis and secretion of Ig, which often leads to intracellular accumulation of intact or partially degraded protein.
- Such variants include **flame cells** with fiery red cytoplasm, **Mott cells** with multiple grapelike cytoplasmic droplets, and cells containing a variety of other inclusions, including **fibrils, crystalline rods, and globules** .
- The globular inclusions are referred to as **Russell bodies** (if cytoplasmic) or **Dutcher bodies** (if nuclear).
- In advanced disease, plasma cell infiltrates may be present in the spleen, liver, kidneys, lungs, lymph nodes, and other soft tissues.
- Commonly, the high level of M proteins causes red cells in peripheral blood smears to stick to one another in linear arrays, a finding referred to as **rouleaux formation** .
- Rouleaux formation is characteristic but not specific, in that it may be seen in other conditions in which Ig levels are elevated,

such as lupus erythematosus and early HIV infection.

- Rarely, tumor cells flood the peripheral blood, giving rise to **plasma cell leukemia** .
- The traditional age limit for autotransplantation is 65 years, although older patients should be considered for transplantation provided good organ function is present.
- Physiologic rather than chronologic age is more suitable for determining transplantation eligibility.
- The standard of care for elderly patients for many years has been melphalan and prednisone (MP).
- However, the introduction of the novel drugs thalidomide, lenalidomide, and bortezomib has changed the treatment paradigm for the elderly patient population.

Chronic Lymphocytic Leukemia/Small Lymphocytic Leukemia:

- The median age at diagnosis is 60 years, and there is a 2 : 1 male predominance.
- Deletions in the long arm of chromosome 13 are the most common genetic abnormality in CLL, occurring in approximately half of all CLL cases.
- More than 25 percent of patients are asymptomatic at diagnosis. Such patients generally are detected because of the discovery of nontender lymphadenopathy or an unexplained absolute lymphocytosis. Otherwise, patients may have only mild symptoms of reduced exercise tolerance, fatigue, or malaise.
- Nearly 80 percent of all CLL patients have nontender lymphadenopathy at diagnosis, most commonly involving the cervical, supraclavicular, or axillary lymph nodes.The diagnosis of CLL requires a sustained monoclonal lymphocytosis greater than 5000/µL (5×10^9 /L).
- Lymph nodes are diffusely effaced by an infiltrate of predominantly small lymphocytes 6 to 12 µm in diameter with round to slightly irregular nuclei, condensed chromatin, and scant cytoplasm.

- Admixed are variable numbers of larger activated lymphocytes that often gather in loose aggregates referred to as **proliferation centers** , which contain mitotically active cells. When present, **proliferation centers are pathognomonic for CLL/SLL.**
- Some of these cells are usually disrupted in the process of making smears, producing so-called **smudge cells** .

Immunophenotype of Chronic B-Cell Leukemias/Lymphomas										
Disease Entity	**sIg**	**CD5**	**CD10**	**CD11c**	**CD19**	**CD20**	**CD22**	**CD23**	**CD25**	**CD103**
Chronic lymphocytic leukemia	+/–	++	–	–/+	+	+/–	–/+	++	–/+	–
Prolymphocytic leukemia	++	+/–	–	–/+	+	+/–	+	+/–	–	–
Hairy cell leukemia	+	–	–	++	+	+	++	–/+	+	++
Mantle cell lymphoma	+	++	–	–	+	+	+	–	–	–
Splenic marginal zone lymphoma	+	–/+	–	+/–	+	+	+/–	–	–	–
Lymphoplasmacytoid lymphoma	–/+	–/+	–	–	+	+/–	+/–	–/+	+/–	–
Follicular center lymphoma	+	–	+	–	+	++	+	–/+	–	–

RAI Clinical Staging System			
Revised Staging System	**Original Staging System**	**Clinical Features at Diagnosis**	**Median Survival, Years***
Low risk	0	Blood and marrow lymphocytosis	12
	I	Lymphocytosis and enlarged lymph nodes	11
Intermediate risk	II	Lymphocytosis and enlarged spleen and/ or liver.	8
High risk	III	Lymphocytosis and anemia (hemoglobin below 11 g/dL).	5
	IV	Lymphocytosis and thrombocytopenia (platelets below 100,000/L).	

Binet Clinical Staging System		
Stage	**Clinical Features at Diagnosis**	**Median Survival, Years**
A	Blood and marrow lymphocytosis and less than 3 areas§ of palpable lymphoid-tissue enlargement.	12
B	Blood and marrow lymphocytosis and 3 or more areas of palpable lymphoid-tissue enlargement.	9
C	Same as B with anemia (hemoglobin below 11 g/dL in men or 10 g/dL in women) or thrombocytopenia (platelets less than 100,000/μL.	

Indications for Therapy in CLL
Anemia
Thrombocytopenia
Disease-related symptoms
Markedly enlarged or painful spleen
Symptomatic lymphadenopathy
Blood lymphocyte count doubling time <6 months
Prolymphocytic transformation
Richter transformation

- Patients whose presentation is typical B cell CLL with no manifestations of the disease other than bone marrow involvement and lymphocytosis (i.e., Rai stage 0 and Binet stage A) can be followed without specific therapy for their malignancy. These patients have a median survival >10 years, and some will never require therapy for this disorder.
- Patients who present with bone marrow failure (i.e., Rai stage III or IV or Binet stage C) will require initial therapy in almost all cases.
- The most common treatments for patients with typical B cell CLL/small lymphocytic lymphoma have been chlorambucil or fludarabine, alone or in combination. Chlorambucil can be administered orally with few immediate side effects, while

fludarabine is administered IV and is associated with significant immune suppression. However, fludarabine is by far the more active agent and is the only drug associated with a significant incidence of complete remission.

Mycoses Fungoides/Sezary Syndrome:

- Mycosis fungoides and Sézary syndrome are different manifestations of a tumor of CD4+ helper T cells that home to the skin.
- Two different clinical types of malignant T-cell disorders were originally recognized: **mycosis fungoides** , a chronic proliferative process; and a more aggressive nodular eruptive variant, **mycosis fungoides d'emblée**
- Clinically, the cutaneous lesions of mycosis fungoides typically progress through three somewhat distinct stages, an inflammatory premycotic phase, a plaque phase, and a tumor phase.
- Histologically, the epidermis and upper dermis are infiltrated by neoplastic T cells, which often have a cerebriform appearance due to marked infolding of the nuclear membrane.

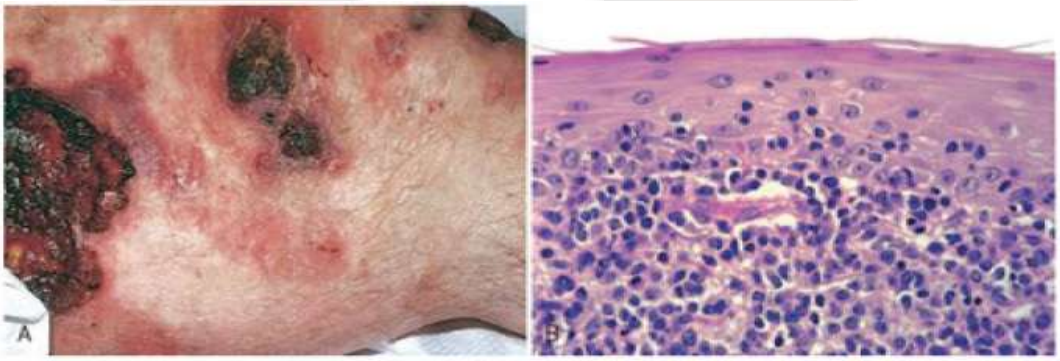


Fig. 18.9: Cutaneous T-cell lymphoma. A, Several ill-defined, erythematous, often scaling, and occasionally ulcerated plaques. B, Microscopically, there is an infiltrate of atypical lymphocytes that show a tendency to accumulate beneath the epidermal layer and to invade the epidermis.

- Late disease progression is characterized by extracutaneous spread, most commonly to lymph nodes and bone marrow.
- Sézary syndrome is a variant in which skin involvement is manifested as a generalized exfoliative erythroderma. In contrast

to mycosis fungoides, the skin lesions rarely proceed to tumefaction, and there is an associated leukemia of “Sézary” cells with characteristic cerebriform nuclei.

- The histologic hallmark of CTCL of the mycosis fungoides type is the presence of the **Sézary-Lutzner cells** . These are T-helper cells (CD4+) that characteristically form band-like aggregates within the superficial dermis and invade the epidermis as single cells and small clusters **(Pautrier microabscesses)** .
- These cells have markedly infolded nuclear membranes, imparting a hyperconvoluted or cerebriform contour.
- Although patches and plaques show pronounced epidermal infiltration by Sézary-Lutzner cells (epidermotropism), in more advanced nodular lesions the malignant T cells often lose this epidermotropic tendency, grow deeply into the dermis, and eventually spread systemically.
- The tumor cells characteristically express the adhesion molecule CLA and the chemokine receptors CCR4 and CCR10, all of which contribute to the homing of normal CD4+ T cells to the skin.
- Although cutaneous disease dominates the clinical picture, sensitive molecular analyses have shown that the tumor cells circulate through the blood, marrow, and lymph nodes even early in the course. Nevertheless, these are indolent tumors, with a median survival of 8 to 9 years. Transformation to aggressive T-cell lymphoma occurs occasionally as a terminal event.
- Topical therapy with steroids or UV light is often used for early lesions of CTCL, whereas more aggressive systemic chemotherapy is indicated for advanced disease.

Non Hodgkins Lymphomas(Nhl) Revision Points:

Most common form of NHL - diffuse large B cell lymphoma.

Most common extra nodal site of NHL – stomach > CNS.

Most lymphoid neoplasms are of B cell origin (85-90%).

Most common site of endemic Burkitt s lymphoma - jaw or mandible. Most common site of sporadic burkitt lymphoma is ileocaecum or

peritoneum.

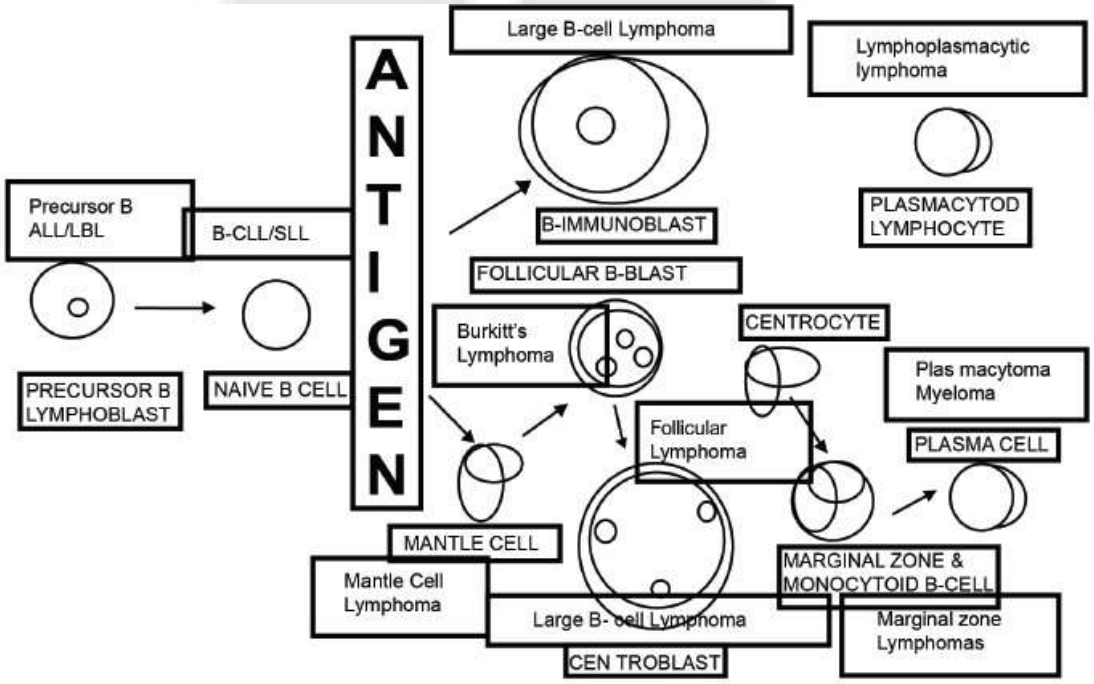


Fig. 18.10: Schema of B-cell differentiation, showing postulated normal counterpart of B-cell neoplasms.

Lymphomas in HIV AIDS:

Predominantly aggressive B cell lymphomas.

Most common include.

- burkitts lymphoma.
- DLBCL.
- primary effusion lymphoma (especially associated with HHV 8).
- plasmablastic lymphoma.
- Hodgkin's lymphoma (unexpected increase after HAART).

Role of EBV (40%) and HHV 8 Disruption of cytokine network leading to high levels of IL 6 and IL 10- a feature of HIV related lymphomas associated with EBV and HHV 8.

19 Hodgkin's Lymphoma

- From a historical point of view, HL was the first cancer in which the curative potential of combination chemotherapy was demonstrated.
- Second, because affected patients are often young, there is a great potential for adding years of productive life by giving curative therapy.
- Third, because patients with HL are often cured, HL serves as a clinical laboratory for investigating the late effects of cancer therapy.
- HL usually presents as solitary or generalized lymphadenopathy and most commonly occurs in young adults, although any age group may be affected.
- The disease appears to spread in a contiguous fashion, and most patients present with disease limited to the lymph nodes or to the lymph nodes and spleen.
- Even when the disease is advanced, cure is possible.
- Overall, cure can be achieved in approximately 80% of patients with HL.
- Treatment of limited disease often incorporates radiation therapy and combination chemotherapy, whereas treatment of advanced disease is generally limited to combination chemotherapy alone.

Staging of Hodgkin and Non-Hodgkin Lymphomas (Ann Arbor Classification):

Stage	Distribution of Disease
I	Involvement of a single lymph node region (I) or a single extra-lymphatic organ or site (IE).
II	Involvement of two or more lymph node regions on the same side of the diaphragm alone (II) or localized involvement of an extra-lymphatic organ or site (IIE).
III	Involvement of lymph node regions on both sides of the diaphragm without (III) or with (IIIE) localized involvement of an extra-lymphatic organ or site.
IV	Diffuse involvement of one or more extra-lymphatic organs or sites with or without lymphatic involvement.

Pathologically, HL is distinguished from other lymphomas by the presence of large binucleated or multinucleated cells (i.e., Reed-Sternberg cells) generally surrounded by a benign reactive host response consisting of lymphocytes, histiocytes, granulocytes, eosinophils, and plasma cells.

Reed-Sternberg cells are large cells with abundant cytoplasm and generally contain two or more nuclei and two or more inclusion like nucleoli.

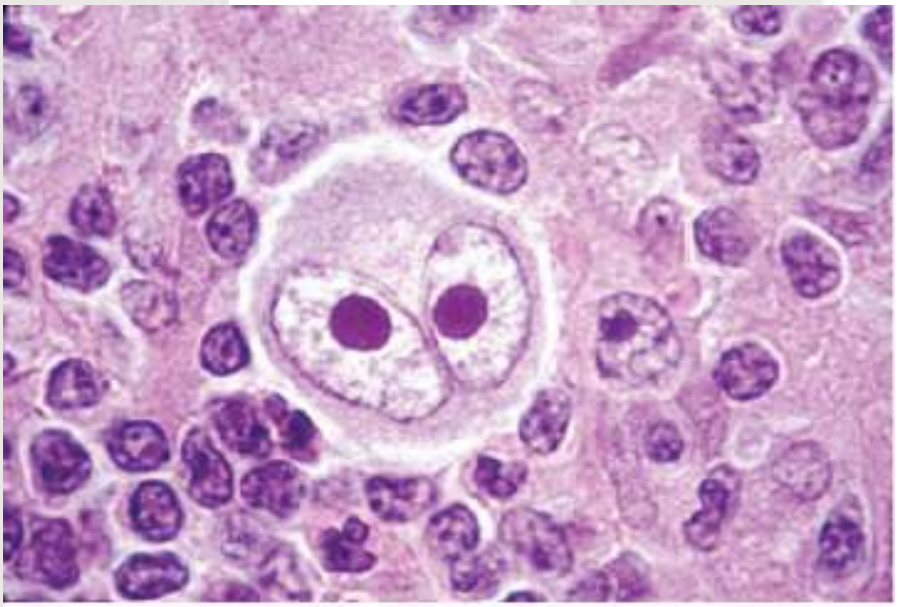


Fig. 19.1

- Reed-Sternberg cells are not absolutely specific for HL and have been noted in cases of infectious mononucleosis and other malignancies including lymphoma, carcinomas, and sarcomas

The WHO classification recognizes five subtypes of HL:

1.	Nodular sclerosis
2.	Mixed cellularity
3.	Lymphocyte-rich
4.	Lymphocyte depletion
5.	Lymphocyte predominance

In the first four subtypes–nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte depletion–the Reed-Sternberg cells have a similar immunophenotype. These subtypes are often lumped together as **classical** forms of HL. In the remaining subtype, lymphocyte predominance, the Reed-Sternberg cells have a distinctive B-cell immunophenotype that differs from that of the "classical" types.

Classification of Hodgkin Lymphoma	
Histological Subtype	Immunopheno type
Nodular lymphocyte-predominant	CD20+ CD30– CD15– Ig+
Classical	CD20–* CD30+ CD15+ Ig–
Nodular sclerosis	
Mixed cellularity	
Lymphocyte-rich	
Lymphocyte-depleted	

- Lymphocyte-predominant HL is associated with the least tendency to have advanced disease and with the most favorable

prognosis, whereas lymphocyte-depleted HL is associated with the greatest tendency to have advanced disease and the worst prognosis.

- Nodular sclerosing HL and mixed cellularity HL are intermediate in this regard, with nodular sclerosing HL being more favorable than mixed cellularity HL.

Subtypes of Hodgkin Lymphoma:

Subtype	Morphology and Immunopheno type	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:

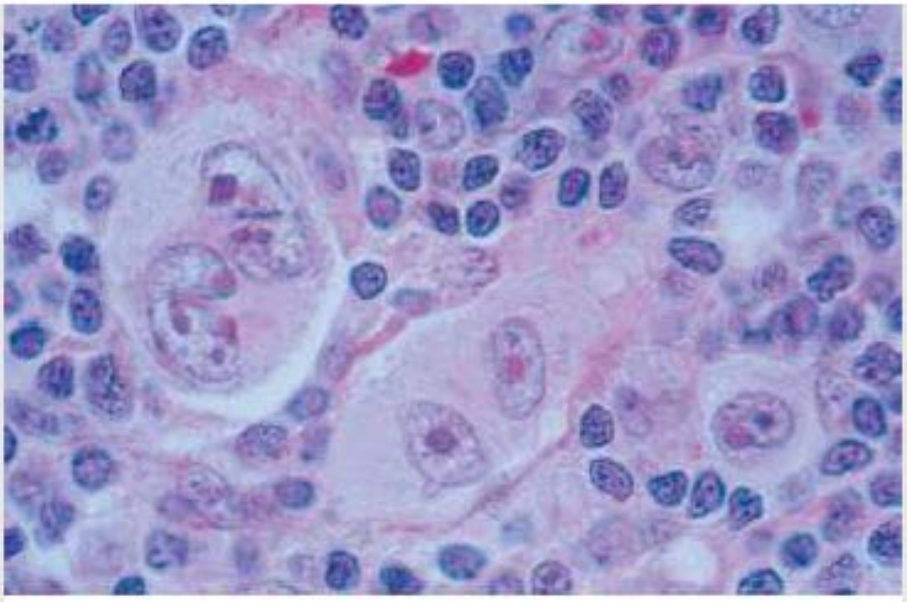


Fig. 19.2: Nodular sclerosing hodgkin lymphoma. High magnification shows Reed-Sternberg cells and lacunar variants in B5 fixed material.

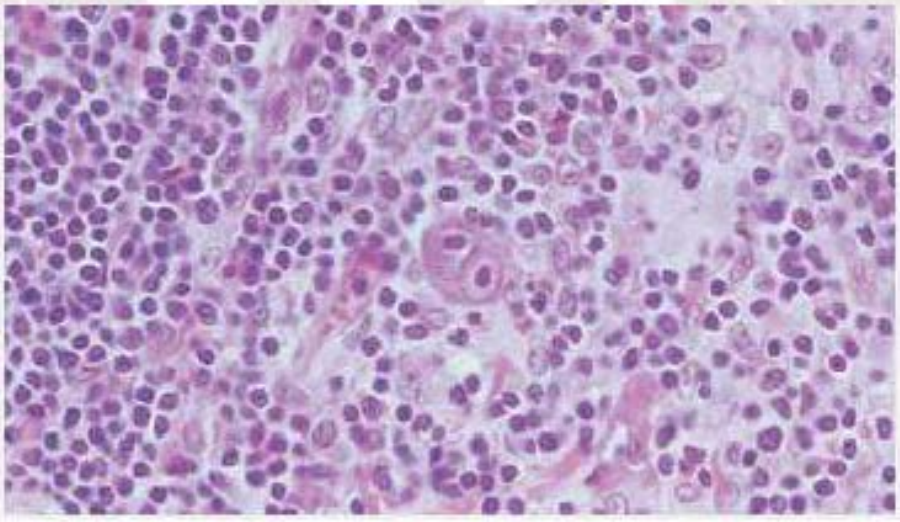


Fig. 19.3: Mixed cellularity-type Hodgkin lymphoma. High magnification shows a classic Reed-Sternberg cell in a mixed background of small lymphocytes, plasma cells, and eosinophils.

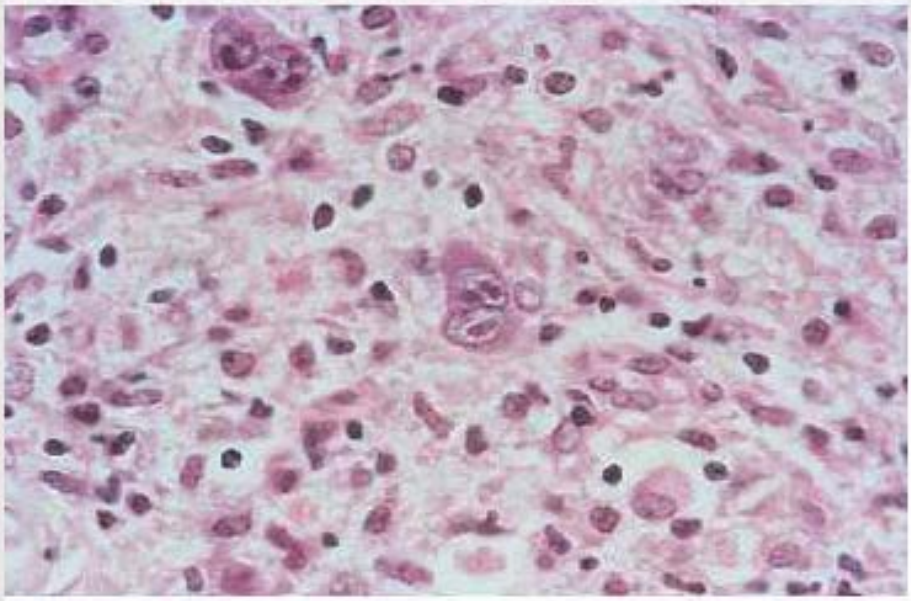


Fig. 19.4: Lymphocyte-depleted type hodgkin lymphoma, diffuse fibrosis subtype. Reed-Sternberg cells are esaily foun, and the background is depleted of cellularity and composed of amorphous eosinophilic connective tissue.

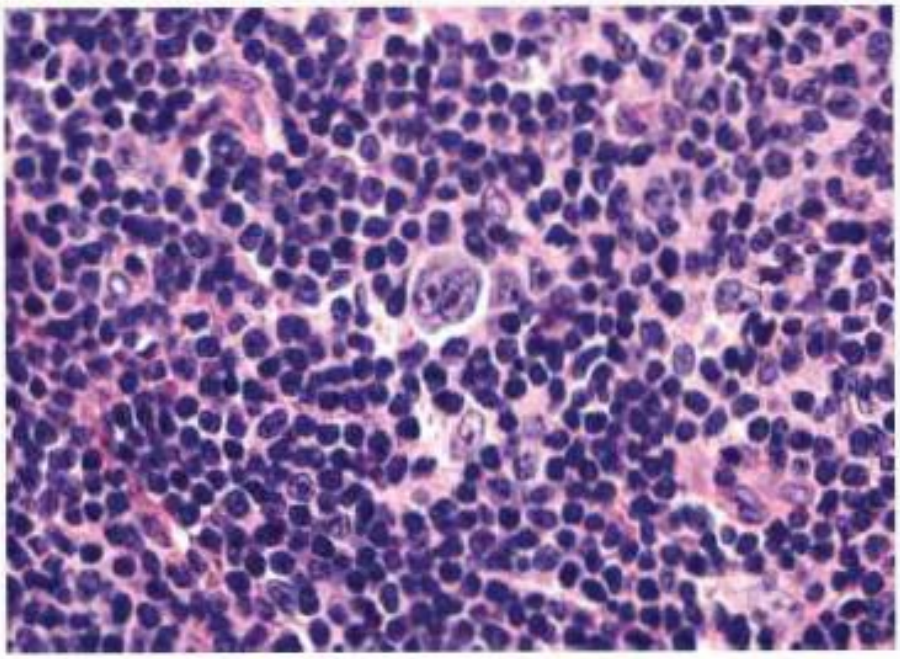


Fig. 19.5: Lymphocyte-rich "classic" Hodgkin lymphoma. The background is primarily lymphocytes, and the Reed-Sternberg cells are usually CD1S+ and CD30+ and negative for the B-cell marker CD20.

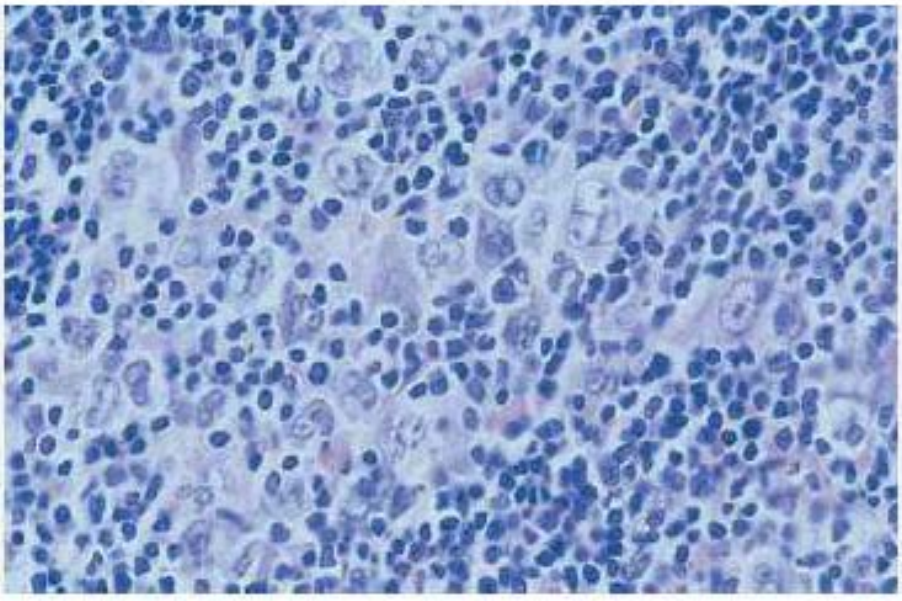


Fig. 19.6: Lymphocyte-predominant Hodgkin lymphoma. High magnification shows variant lymphocytic and histiocytic cells (L and H cells), which have "popcorn" nuclei. A background of small lymphocytes and his tiocytes is present.

Recommended Staging Procedures for Patients with Hodgkin Lymphoma
History and physical examination. Special attention to history of B symptoms (i.e., fever, night sweats, weight loss of >10% in past 6 mo).
Examination of all peripheral lymph node regions, liver, and spleen.
Radiologic studies. Chest radiograph. CT scan of thorax. CT scan of abdomen and pelvis. PET scan.
Laboratory studies. Hematocrit, white blood cell count, differential, platelet count. Erythrocyte sedimentation rate (optional). Blood urea nitrogen, creatinine. Bilirubin, alkaline phosphatase, lactic dehydrogenase, "hepatocellular" enzymes.
Bone marrow aspiration and biopsy (optional)
CT, computed tomography; PET, positron emission tomography.

Patients with localized Hodgkin's disease are cured >90% of the time. In patients with good prognostic factors, extended-field radiotherapy has a high cure rate.

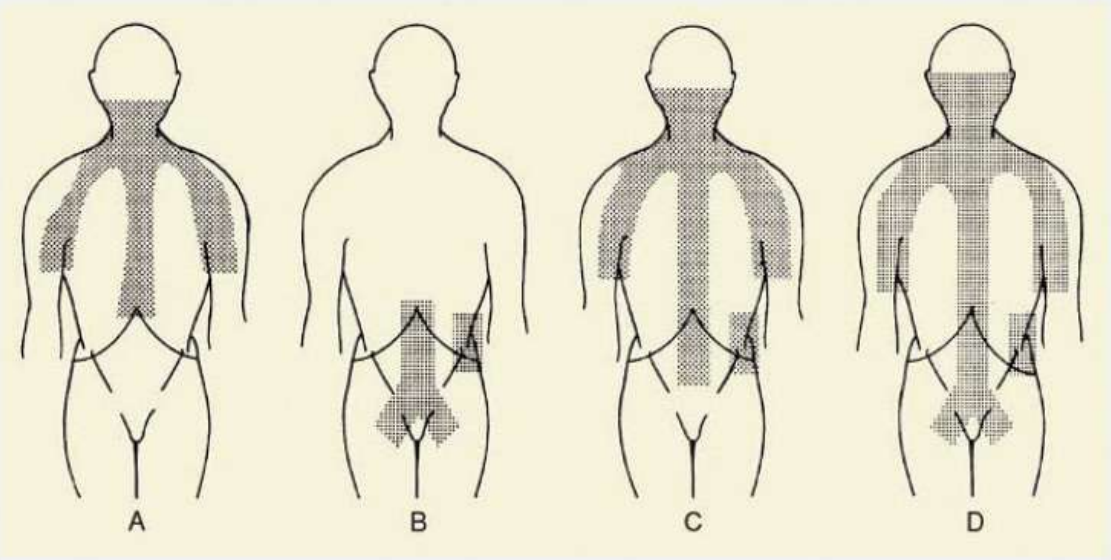


Fig. 19.7: Treatment fields used as extended field irradiation of Hodgkin disease. A. Mantel field. **B.** Inverted Y field. **C.** Mantle and para-aortic field (extended mantle field). **D.** Total nodal field. The spleen is irradiated in conjunction with the fields in **B**, **C**, and **D**, unless it has been surgically removed.

Increasingly, patients with all stages of Hodgkin's disease are treated initially with chemotherapy. Patients with localized or good-prognosis disease receive a brief course of chemotherapy followed by radiotherapy to sites of node involvement. Patients with more extensive disease or those with B symptoms receive a complete course of chemotherapy. The most popular chemotherapy regimens used in Hodgkin's disease include doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) and mechlorethamine, vincristine, procarbazine, and prednisone (MOPP), or combinations of the drugs in these two regimens. Today, most patients in States receive ABVD, but a weekly chemotherapy regimen administered for 12 weeks the United called **Stanford V** is becoming increasingly popular, but includes radiation therapy, which has been associated with life-threatening late toxicities such as premature coronary artery disease and second solid tumors.

Therapeutic Options for Hodgkin Lymphoma	
Stage	Therapeutic Options.
IA and IIA	Combination chemotherapy.
	Abbreviated combination therapy (e.g., four cycles ABVD) plus involved field radiation therapy.
	Extended field radiation therapy .
	Involved field radiation therapy .
IB and IIB	Combination chemotherapy plus involved field radiation.
	Combination chemotherapy.
	Extended field radiation therapy plus combination chemotherapy.
	Extended field radiation therapy .
IX and IIX	Combination chemotherapy with involved field radiation therapy.
	Combination chemotherapy.
IIIA, IIIB, IVA, and IVB	Combination chemotherapy
	Combination chemotherapy with radiation boost to large tumor masses

Final Revision Points for Hodgkin's Lymphoma:

Most common Hodgkin's lymphoma - nodular sclerosis.

Most common HL in India - mixed cellularity

Most common HL in western countries - nodular sclerosis.

Least common HL- lymphocyte depleted.

Maximum RS cells - mixed cellularity.

Minimum RS cells - lymphocyte rich.
Best or most specific marker for classical RS cells - CD 30.

Most sensitive marker- CD 15.

Best prognosis - lymphocyte predominant followed by nodular sclerosis. Worst prognosis - lymphocyte depleted. Maximum HIV association - lymphocyte depleted. EBV frequency is maximum in - mixed cellularity.

EBV frequency minimum in - nodular sclerosis.

General Points about Hodgkin's Lymphoma:

1. Mostly cervical lymph nodes are involved.
2. Seen in young adults.
3. Large monocuclear/multinucleated tumor cells (RS cells) in abundant heterogenous mixture of non-neoplastic inflammatory and accessory cells.
4. Often ringed by T cells in a rosette like manner.
5. Constitutes ~30% of all lymphomas.
6. Classified into Nodular Lymphocytic Predominant HL and Classical HL.

Classical HL:

- **95% of HL.**
- **bimodal presentation : 15-35 years and in late life.**
- **patients with Infectious Mononucleosis have a higher incidence.**
- **75% cases present with cervical lymph nodes followed by mediastinal, axillary and paraortic.**

- **60% patients have lo** calized disease.
- 40% patients have B symptoms.
- CD 30+ in all cases.
- CD 15+ in 75-85% cases.
- CD 45 - in all cases.
- maximum EBV in mixed cellularity (75%).
- minimum EBV in nodular sclerosis (10-40%),.

Nodular Lymphocyte Predominant Hodgkin Lymphoma:

- large neoplastic cells called Popcorn or LP or LH cells.
- 5% of HL.
- mostly in Males.
- most frequently in 30-50 years age group.
- latent EBV infection is consistently absent from LP cells.
- CD 20/79a/75/45 positive, BCL 6 positive.

20 Acute Myelogenous Leukemia

Phenotype	Usually Positive
Myeloblastic	CD11b, CD13, CD15, CD33, CD117, HLA-DR
Myelomonocytic	CD11b, CD13, CD14, CD15, CD32, CD33, HLA-DR
Erythroid	Glycophorin, spectrin, ABH antigens, carbonic anhydrase I, HLA-DR
Promyelocytic	CD13, CD33
Monocytic	CD11b, 11c, CD13, CD14, CD33, CD65, HLA-DR
Megakaryoblastic	CD34, CD41, CD42, CD61, anti-von Willebrand factor
Basophilic	CD11b, CD13, CD33, CD123, CD203c
Mast cell	CD13, CD33, CD117

WHO 2017 update of AML classification.

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
AML with t (8;21) (q22;q22.1); RUNX1-RUNX1TI
AML with inv (16) (p”l3.1 q22) Or t(16;-l6) (p13.1;q22); CBFB-MYH11
APL with PML-RARA
AML with t(9:11)(p2l.3m23J3):MLLT3-KMT2A
AML with (6.9) (p23:q34.1); DEK-NUP214
AML with inv (3) (q21_3q26_2) or t (3;3) (q21 ,3; q26_2); GATA2. MECOM
AML (megakaryoblastic) with t(1:22)(pi3.3gq 13L3); RBM15-MKL1
Provisional entity: AML with BCR-ABL1

AML with mutated NPM1
AML with biallelic mutations of CEE3PA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML NOS
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic / monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

Signs and symptoms that signal the onset of AML include pallor, fatigue, weakness, palpitations, and dyspnea on exertion. The signs and symptoms reflect the development of anemia; however, weakness, loss of sense of well-being, and fatigue on exertion can be disproportionate to the severity of anemia.

Easy bruising, petechiae, epistaxis, gingival bleeding, conjunctival hemorrhages, and prolonged bleeding from skin injuries reflect thrombocytopenia and are frequent early manifestations of the disease.

Skin involvement may be of three types: nonspecific lesions, leukemia cutis, or granulocytic (myeloid) sarcoma of skin and subcutis.

AML with t (8;21), t (15;17), t (16;16) or inv (16) - are diagnosed as AML irrespective of blast count (AIIMS question).

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); CBFα/ETO fusion gene.	Favorable	M2	Full range of myelocytic maturation; Auer rods easily found; abnormal cytoplasmic granules.
AML with inv(16) (p13;q22); CBFβ/ MYH11 fusion gene.	Favorable	M4eo	Myelocytic and monocytic differentiation; abnormal eosinophilic precursors with abnormal basophilic granules.
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.
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.

The diagnosis of AML is based on the presence of at least 20% myeloid blasts in the bone marrow.

- Several types of myeloid blasts are recognized, and individual tumors may have more than one type of blast or blasts with hybrid features.
- **Myeloblasts** have delicate nuclear chromatin, two to four nucleoli, and more voluminous cytoplasm than lymphoblasts.
- The cytoplasm often contains fine, peroxidase-positive azurophilic granules. **Auer rods** , distinctive needle-like azurophilic granules, are present in many cases; they are particularly numerous in AML with the t (15;17) (acute promyelocytic leukemia).
- **Monoblasts** have folded or lobulated nuclei, lack Auer rods, and are nonspecific esterase-positive. In some AMLs, blasts show megakaryocytic differentiation, which is often accompanied by marrow fibrosis caused by the release of fibrogenic cytokines.
- Rarely, the blasts of AML show erythroid differentiation.
- The number of leukemic cells in the blood is highly variable. Blasts may be more than 100,000 per mm^3 , but are under

10,000 per mm in about 50% of patients.

- **Occasionally, blasts are entirely absent from the blood** (aleukemic leukemia). For this reason, a bone marrow examination is essential to exclude acute leukemia in pancytopenic patients.

Better prognosis than average of all patients
Early blast clearance during remission induction therapy.
Leukemic cells contain t(8;21), t(15;17), inv(16) t(16;16), trisomy 21.
CEBPA mutations in cytogenetically normal AML.
Absence of exaggerated dysmyelopoiesis.
Residual normal metaphases admixed with clonal cytogenetic abnormalities.
High telomerase activity levels.
Low levels of TdT expression by flow cytometry (<5%).
High BAX expression and high BAX/BCL-2 ratios.
High expression of integrin CD11b.
Absence of VLA-4 expression on AML blast cells.
High levels of soluble VCAM-1 binding to AML blast cells.
High levels of caspase-3.
Mutant CEBPA expression.
NPM1 gene expression in adults or children (usually present in cytogenetically normal cases).
Higher neutrophil and higher platelet counts at time of complete remission.
<5% blasts on day 14 marrow predicts for complete remission but not for overall survival

Poorer prognosis than average of all patients
Older age: Age at the time of diagnosis has the greatest impact on the probability of remission and on duration of survival. Children in the first 15 years of life, exclusive of the neonatal period, have the highest rate of remission and longest relapse-free remission; patients older than age 60 years have only half the chance of a young adult to enter remission and less likelihood of a long relapse-free remission. There is a gradient of poor response to treatment through adulthood, with the largest decrease after the sixth decade of life.
Unfavorable karyotypes: The cytogenetic pattern of leukemic blast cells influences outcome, but the relationship is complex. The presence of 5–, 7–, 5q–, 7q–, or of exaggerated hyperdiploidy (>47 chromosomes), trisomy 8, t(6;9), trisomy 11, and multiple chromosomal abnormalities in leukemic cells are poor prognostic signs.
Multidrug resistance phenotype: Leukemic cells expressing P-glycoprotein, a unidirectional drug efflux pump, encoded by the MDR1. Expression of this gene product can result in decreased accumulation of anthracyclines, amsacrine, mitoxantrone, and etoposide. Expression of P-glycoprotein does not influence outcome of treatment, but if rhodamine-123 efflux also is increased, relapse is more common. Frequently observed in AML cells after relapse. Associated with CD34 expression and chromosome 7 abnormalities. Alternative non-MDR1–mediated drug efflux mechanisms are important also. MDR1 expression is low in favorable prognosis subtypes of AML.
Presence of mutated KIT with t(8;21): Associated with higher relapse risk and poorer overall survival.
Prior clonal hemopathy: Chemotherapy or radiotherapy remission rates are one-third to one-half that of de novo AML in the same age group. Remission duration is shorter with remissions >3 years very uncommon. AML developing from the clonal hemopathy may relapse as a smoldering leukemia. It then reverts to AML but can be treated with remissions lasting several years.

Higher white cell count: Count >30,000/μL (30 x 109/L) or a blast cell count >15,000/μL (>15 x 109/L).
Very low platelet count (<30,000/μL [<30 x 109/L]).
High serum lactic dehydrogenase.
High stem cell mobilizing capacity during complete remission predicts for relapse risk.
Another medical disorder: extreme obesity, diabetes mellitus, chronic renal disease.
Low serum albumin or prealbumin.
Need for intubation or ventilator support during induction therapy.
Autonomous clonal growth of leukemic blast cells.
High BCL-2 expression.
High MCL-1 expression: Elevated at the time of leukemic relapse. Suggests prognostic importance or that chemotherapeutic regimen selects for leukemia cells with elevated levels of apoptosis inhibitors.
Low expression of retinoblastoma gene.
High levels of WAF/Cip1 protein: This is a regulator at the G1 checkpoint of cell cycle.
High CD34 expression: High CD34 antigen expression often in AML subtypes M0, M1, and M4. Remission rate of 61% vs. to 88% in AML not expressing CD34. Correlation is stronger between high-intensity expression of CD34 and lower remission rate. CD34 expression in APL.
GATA-1 expression.
Neural cell adhesion molecule (CD56) expression.
Elevated soluble L-selectin: Seen especially in extramedullary disease.
Higher expression of interleukin-1β gene.
Low FMS expression.
Expression of the thrombopoietin receptor (c-MPL) mRNA.
FLT3 mutations.
Increased angiogenesis/vascular endothelial growth factor levels.
High β2-microglobulin levels in adults younger than 60 years old.
MN1 (meningioma 1) gene overexpression in AML patients with normal cytogenetics.
Young adults with the genotype WT1(mutation)/FLT3-ITD(positive) have a lower complete remission rate and an inferior relapse-free and overall survival compared to those with the genotype WT1(mutation)/ FLT3-ITD(negative).
WT1 gene mutations in patients with AML and a normal karyotype.
Patients with AML with a large number of AML stem cells.
Elevated expression of IL-3Rα.
MLL tandem duplications and 11p23/MLL abnormalities.
CD56 expression in APL.High incidence of CNS involvement, especially with CD7 expression. Also contributes to poorer outcomes in t(8;21) cases.
P15 methylation.
Microsatellite instability (may not be independent of age and t-AML).

AC133 expression (shorter remissions and disease-free survival).
Constitutive activity of signal transducer and activator of transcription 3 protein (shorter disease-free survival).
BAALC gene expression.
High S-phase activity in cells surviving after 7 days of induction.
High EVI1 expression.
Overexpression of CXCR4.
Increased marrow angiogenesis as measured by magnetic resonance imaging.
The presence of the CTLA4 CT60 A/G genotype adult patients with AML.

Chromosome findings at diagnosis are currently the most important independent prognostic factor. Patients with t (15;17) have a very good prognosis (approximately 85% cured), and those with t (8;21) and inv (16) a good prognosis (approximately 55% cured), while those with no cytogenetic abnormality have a moderately favorable outcome (approximately 40% cured). Patients with a complex karyotype, t (6;9), inv (3), or -7 have a very poor prognosis.

Treatment:

- The most commonly used CR induction regimens (for patients other than those with APL) consist of combination chemotherapy with cytarabine and an anthracycline.
- Cytarabine is a cell cycle S-phase–specific antimetabolite that becomes phosphorylated intracellularly to an active triphosphate form that interferes with DNA synthesis.
- Anthracyclines are DNA intercalators.
- Their primary mode of action is thought to be inhibition of topoisomerase II, leading to DNA breaks.
- Cytarabine is usually administered as a continuous intravenous infusion for 7 days.
- Anthracycline therapy generally consists of daunorubicin intravenously on days 1, 2, and 3 (the 7 and 3 regimen).
- Treatment with idarubicin for 3 days in conjunction with cytarabine by 7-day continuous infusion is at least as effective as daunorubicin in younger patients.
- The addition of etoposide may improve the CR duration.

- When combined with cytarabine in a 7 and 3 regimen, a higher dose of anthracycline (i.e., daunorubicin 90 mg/m^2) improves outcome compared with a lower dose (i.e., daunorubicin 45 mg/m^2).
- After induction chemotherapy, if persistence of leukemia is documented, the patient is usually re-treated with cytarabine and an anthracycline in doses similar to those given initially, but for 5 and 2 days, respectively

AML with best prognosis, highest incidence of DIC, maximum Auer rods: APML (M3) (*).

21 Myeloproliferative Neoplasms

2017 World Health Organization Classification of Myeloproliferative Neoplasms (Mpn)
Chronic myeloid leukemia.
Polycythemia vera.
Essential thrombocythemia.
Primary myelofibrosis.
Chronic neutrophilic leukemia.
Chronic eosinophilic leukemia/not otherwise categorized.
MPNs, unclassifiable.

Chronic Myeloid Leukemia:

- Chronic myeloid leukemia (CML) is the classic chronic myeloproliferative disorder.
- It is a clonal stem cell disorder characterized by the acquisition of an oncogenic BCR/ABL fusion protein [usually the result of a reciprocal translocation (9;22) q34;q11)] and by proliferation of granulocytic elements at all stages of differentiation.
- The t(9;22) is also referred to as the Philadelphia chromosome (Ph), in honor of the city in which it was identified by Nowell and Hungerford in 1960.

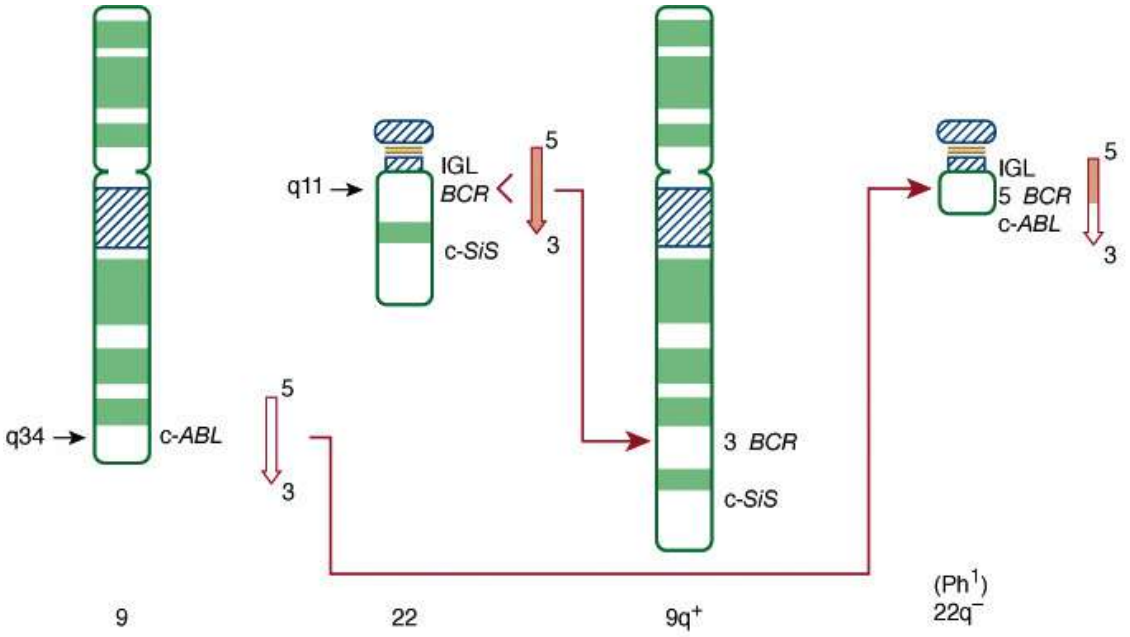


Fig. 21.1

Schematic of normal chromosome 9 showing the ABL gene between band q34 and qter of chromosome 22, which has the BCR and SIS genes between band q11 and qter. The t(9;22) is shown on the right. The ABL from chromosome 9 is transposed to the chromosome 22 M-bcr sequences, and the terminal portion of chromosome 22 is transposed to the long arm of chromosome 9. The 22q– is the Ph chromosome. bcr, breakpoint cluster region; c-SiS, cellular homologue of the viral simian sarcoma virus-transforming gene; IGL, gene for immunoglobulin light chains.

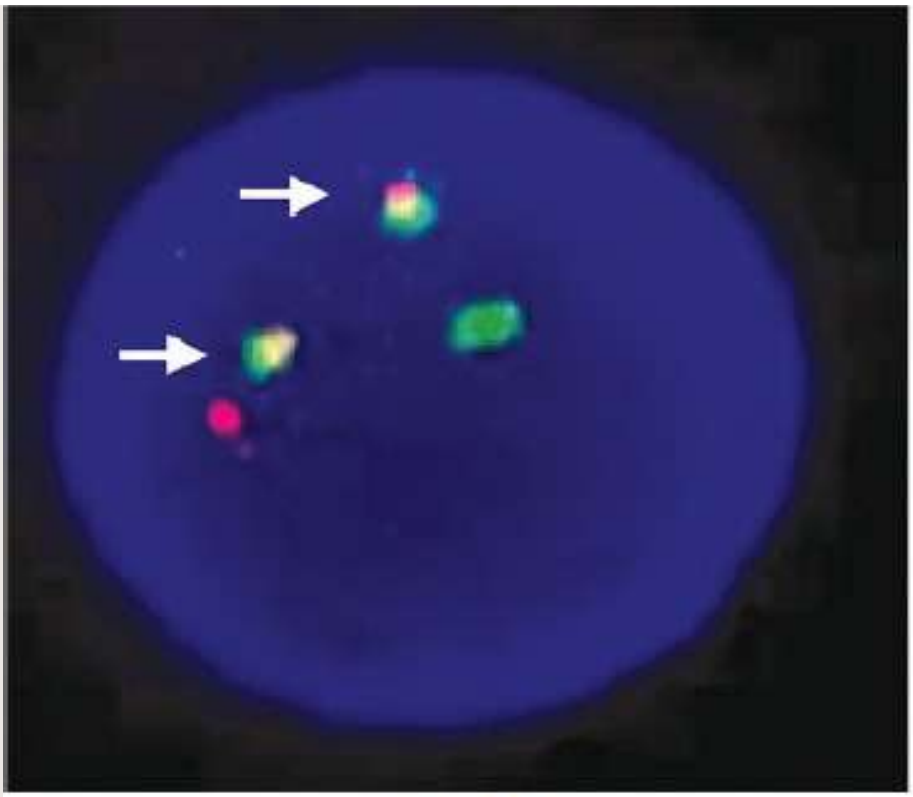


Fig. 21.2

Fluorescence in situ hybridization for BCR/ABL using a dual-color, dual-fusion probe set - Positive for BCR/ABL fusion. Typical abnormal pattern with one red, one green, and two fused (yellow) signals.

CML is often referred to as the disease of "firsts". It was the first disease.

a. in which the term leukemia was utilized,

b. to be associated with a consistently recurring chromosomal abnormality,

c. to be recognized as the result of material reciprocally translocated from one chromosome to another,

d. to be the direct result of a specific gene fusion (as a result of the translocation), and

e. to have a therapy particularly targeted against the fusion protein.

- The most frequent complaints include easy fatigability, loss of sense of well-being, decreased tolerance to exertion, anorexia, abdominal discomfort, early satiety (related to splenic enlargement), weight loss, and excessive sweating.
- A physical examination may detect pallor and splenomegaly. The latter was present in approximately 90 percent of patients at diagnosis.
- Elevated white blood (cell) counts (WBCs), with increases in both immature and mature granulocytes, are present at diagnosis.
- Usually <5% circulating blasts and <10% blasts and promyelocytes are noted, with the majority of cells being myelocytes, metamyelocytes, and band forms.
- Cycling of the counts may be observed in patients followed without treatment.
- Platelet counts are almost always elevated at diagnosis, and a mild degree of normocytic normochromic anemia is present. Leukocyte alkaline phosphatase is low in CML cells.
- Phagocytic functions are usually normal at diagnosis and remain normal during the chronic phase.
- Histamine production secondary to basophilia is increased in later stages, causing pruritus, diarrhea, and flushing.

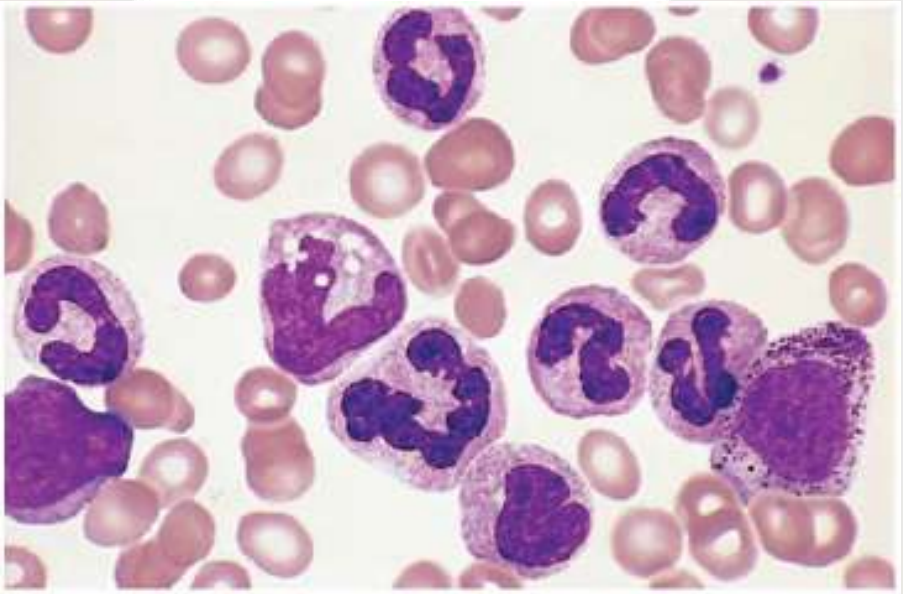


Fig. 21.3

Disease acceleration is defined by the development of increasing degrees of anemia unaccounted for by bleeding or therapy; cytogenetic clonal evolution; or blood or marrow blasts between 10 and 20%, blood or marrow basophils >20%, or platelet count <100,000/µL. **Blast crisis** is defined as acute leukemia, with blood or marrow blasts >20%. Hyposegmented neutrophils may appear (Pelger-Huët anomaly). Blast cells can be classified as myeloid, lymphoid, erythroid, or undifferentiated, based on morphologic, cytochemical, and immunologic features.

Diagnosis of Accelerated and Blast Phase in CMLa
Accelerated phase Blasts 10–19% in the peripheral blood and/or bone marrow Basophils ≥20% in the peripheral blood Persistent thrombocytopenia Increasing spleen size and white blood cell count despite therapy Cytogenetic evidence of clonal evolution
Blast phase Blasts ≥20% Extramedullary blast proliferation Large aggregates or clusters of blasts in the bone marrow.

Treatment:

- Imatinib mesylate (imatinib) is now used as initial therapy in almost all patients with CML presenting in the chronic phase.
- In cases where the white cell count is markedly elevated, hydroxyurea can be used prior to or in conjunction with imatinib.

- If rapid cytoreduction is required because of signs of the hyperleukocytic syndrome, leukapheresis and hydroxyurea often are combined.
- Patients with newly diagnosed chronic phase CML should be started on imatinib, 400 mg/day by mouth. Imatinib is easier to use, induces a higher frequency of hematologic remission, a higher frequency of complete cytogenetic remission, and greater suppression of the CML clone (molecular remission) than therapy with interferon (INF)-α.
- The goal of imatinib therapy is to decrease the cells bearing the t (9;22) translocation (leukemic cells) to the lowest levels possible, under which conditions normal (polyclonal) hematopoiesis is restored.
- Allografting continues to play a prominent role in the treatment of patients with suboptimal imatinib responses, who are refractory or intolerant to tyrosine kinase inhibitors, and remains the optimal therapy in those who progress to accelerated phase or blast crisis.
- Patients in the chronic phase of CML who are younger than 65 years and who have an identical twin, or a histocompatible sibling, or who are younger than 55 years with access to a histocompatible unrelated donor, can be transplanted after intensive therapy, usually with cyclophosphamide and fractionated total-body irradiation (TBI) or a combination of busulfan and cyclophosphamide. Busulfan can be administered as an intravenous preparation and as a single daily dose.

Polycythemia Vera:

- Polycythemia vera (PV), also called polycythemia rubra vera, is a chronic clonal myeloproliferative disorder characterized by a striking absolute increase in the number of red blood corpuscles and in the total blood volume, and usually by leukocytosis, thrombocytosis, and splenomegaly.
- The bone marrow is typically hypercellular and exhibits hyperplasia of myeloid, erythroid, and megakaryocyte lineages.

- PV usually has an insidious onset, most commonly during the sixth decade of life.
- Thrombotic episodes are the most common and the most important complications of PV, occurring in about one-third of the patients.
- **Most symptoms are related to the increased red cell mass and hematocrit** . Usually, there is also an increased total blood volume.
- Together, these factors cause abnormal blood flow, particularly on the low-pressure venous side of the circulation, which becomes greatly distended.
- Patients are plethoric and cyanotic due to stagnation and deoxygenation of blood in peripheral vessels. Headache, dizziness, hypertension, and gastrointestinal symptoms are common.
- Intense pruritus and peptic ulceration may occur, both possibly resulting from the release of histamine from basophils.
- High cell turnover gives rise to hyperuricemia; symptomatic gout is seen in 5% to 10% of cases.

The diagnosis of polycythaemia vera requires either all 3 major criteria or the first 2 major criteria plus the minor criterion3.

Major criteria:

Elevated haemoglobin concentration (>16.5 g/dL in men; >16.0 g/dL in women) or Elevated haematocrit (>49% in men; >48% in women) or Increased red blood cell mass (>25% above mean normal predicted value).

Bone marrow biopsy showing age-adjusted hypercellularity with trilineage growth (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size).

Presence of JAK2 V617F or JAK2 exon 12 mutation.

Minor criterion:

Subnormal serum erythropoietin level a Major criterion 2 (bone marrow biopsy) may not be required in patients with sustained

absolute erythrocytosis haemoglobin concentrations of >

18.5 g/dL in men or > 16.5 g/dL in women and haematocrit values of> 55.5% in men or > 49.5% in women), if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in as many as 20% of patients) can only be detected by bone marrow biopsy, and this finding may predict a more rapid progression to overt myelofibrosis (post-PV myelofibrosis) {253}.

- PV is generally an indolent disorder, the clinical course of which is measured in decades, and its management should reflect its tempo.
- Thrombosis due to erythrocytosis is the most significant complication, and maintenance of the hemoglobin level at <140 g/L (14 g/dL; hematocrit <45%) in men and <120 g/L (12 g/dL; hematocrit <42%) in women is mandatory to avoid thrombotic complications.
- Phlebotomy serves initially to reduce hyperviscosity by bringing the red cell mass into the normal range.
- Periodic phlebotomies thereafter serve to maintain the red cell mass within the normal range and to induce a state of iron deficiency that prevents an accelerated reexpansion of the red cell mass.
- In most PV patients, once an iron-deficient state is achieved, phlebotomy is usually only required at 3-month intervals.
- Extended survival with treatment has revealed that **PCV tends to evolve to a "spent phase," during which clinical and anatomic features of primary myelofibrosis develop** .
- The disease undergoes this transition in about 15% to 20% of patients after an average period of 10 years.
- It is marked by the appearance of obliterative fibrosis in the bone marrow (myelofibrosis) and extensive extramedullary hematopoiesis, principally in the spleen, which enlarges greatly

Primary Myelofibrosis:

- **The hallmark of primary myelofibrosis is the development of obliterative marrow fibrosis** .
- The replacement of the marrow by fibrosis suppresses bone marrow hematopoiesis, leading to cytopenias and extensive neoplastic extramedullary hematopoiesis.
- **Activating JAK2 mutations are present in 50% to 60% of cases and activating MPL mutations in an additional 1% to 5% of cases** .
- Pathogenetic mechanisms in PMF include (a) megakaryocyte-weighted clonal myeloproliferation, (b) reactive bone marrow stromal changes, and (c) extramedullary hematopoiesis (EMH).
- The chief pathologic feature is the extensive deposition of collagen in the marrow by non-neoplastic fibroblasts.
- The fibrosis inexorably displaces hematopoietic elements, including stem cells, from the marrow and eventually leads to marrow failure.

Disorders Causing Myelofibrosis	
Malignant	Nonmalignant
Acute leukemia (lymphocytic, myelogenous, megakaryocytic)	HIV infection
Chronic myelogenous leukemia	Hyperparathyroidism
Hairy cell leukemia	Renal osteodystrophy
Hodgkin's disease	Systemic lupus erythematosus
Idiopathic myelofibrosis	Tuberculosis
Lymphoma	Vitamin D deficiency
Multiple myeloma	Thorium dioxide exposure
Myelodysplasia	Gray platelet syndrome
Metastatic carcinoma	
Polycythemia vera	
Systemic mastocytosis	

Hepatomegaly is detectable in two-thirds of patients, and splenomegaly is present on palpation or imaging studies in almost all patients at the time of diagnosis.

Diagnostic Findings in Idiopathic Myelofibrosis
Prefibrotic stage.
Anemia may be absent or mild.
Leukocytosis may be absent or slight.
Thrombocythemia very frequent.
BCR-ABL fusion gene absent .
Presence of JAK2 mutation indicative of diagnosis of myeloproliferative disease .
Cellular marrow with mild increase in granulopoiesis; increased megakaryocytes, clusters of very dysmorphic megakaryocytes and megakaryocytic nuclei; no to very slight increase in reticular fibers on silver stain.
Palpable splenomegaly infrequent.
Absent or slight anisopoikilocytosis including teardrop red cells.
Fully developed stage.
Marrow reticulin fibrosis plus or minus collagen fibrosis.
BCR-ABL fusion gene absent .

JAK2 mutation in approximately 50% of patients.
Splenomegaly.
Anisopoikilocytosis with teardrop red cells in every oil immersion field.
Immature myeloid cells in blood.
Increased CD34-positive cells in blood.
Erythroblasts in blood.
Marrow usually hypercellular but invariably has increased megakaryocytes, clusters of highly dysmorphic megakaryocytes, and megakaryocyte bare nuclei regardless of overall marrow cellularity.

Diagnostic criteria for pre fibrotic phase of PMF:

The diagnosis of prefibrotic/early primary myelofibrosis requires that all 3 major criteria and at least 1 minor criterion are met.

Major criteria:

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis grade > 1a, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and (often) decreased erythropoiesis.
2. WHO criteria for BCR-ABL1-pos\i\ve chronic myeloid leukaemia, polycythaemia vera, essential thrombocythaemia, myelodysplastic syndromes, or other myeloid neoplasms are not met.
3. JAK2, CALR, or MPL mutation

or

Presence of another clonal marker [b]

or

Absence of minor reactive bone marrow reticulin fibrosis [C]

Minor criteria:

Presence of at least one of the following, confirmed in 2 consecutive determinations:

- Anaemia not attributed to a comorbid condition.
- Leukocytosis ≥11 × 109/L.
- Palpable splenomegaly.
- Lactate dehydrogenase level above the upper limit of the institutional reference range.

Diagnostic criteria for overt/fibrotic phase of PMF:

The diagnosis of overt primary myelofibrosis requires that all 3 major criteria and at least 1 minor criterion are met.

Major criteria:

1. Megakaryocyte proliferation and atypia, accompanied by reticulin and/or collagen fibrosis grades 2 or 3 [a]
2. WHO criteria for essential thrombocythaemia, polycythaemia vera, BCR-ABL1-pos\i\ve chronic myeloid leukaemia, myelodysplastic syndrome, or other myeloid neoplasms5 are not met.
3. JAK2, CALR, or MPL mutation

or

Presence of another clonal marker 0

or

Absence of reactive myelofibrosis’1

Minor criteria:

Presence of at least one of the following, confirmed in 2 consecutive determinations:

- Anaemia not attributed to a comorbid condition.
- Leukocytosis ≥ 11 × 109/L.

- Palpable splenomegaly.
- Lactate dehydrogenase level above the upper limit of the institutional reference range.
- Leukoerythroblastosis.

Essential Thrombocythemia:

- Essential thrombocythemia is a clonal stem cell disorder characterized by an overproduction of platelets and associated with mutations in **JAK2** or **MPL** .
- Complications include thrombosis (predominantly arterial), hemorrhage, and progression to myelofibrosis or acute myeloid leukemia.
- Diagnosis requires exclusion of reactive thrombocytosis and other myeloid malignancies associated with a raised platelet count.
- Therapy is aimed at reducing thrombotic complications and includes modification of known cardiovascular risk factors and antiplatelet therapy for the majority of patients.
- Those at high risk of thrombosis are also considered for cytoreductive therapy with agents such as hydroxyurea, anagrelide, or interferon-α. Although survival in the first decade following diagnosis appears similar to controls, mortality rates increase thereafter as a consequence of disease complicaions.
- The most frequent symptom complex (∼30% prevalence rate) is recognized as “microvascular symptoms” consisting of headaches, visual symptoms, lightheadedness, atypical chest pain, acral dysesthesia, and erythromelalgia. Among these symptoms, erythromelalgia is the most impressive but least prevalent, occuring in <5% of patients.
- Erythromelalgia is characterized by painful erythematous discoloration of the feet and/or hands that results from abnormal platelet–endothelium interaction.

Causes of Thrombocytosis	
Clonal Thrombocytosis	Reactive Thrombocytosis
Essential thrombocythemia Polycythemia vera Myelofibrosis with myeloid metaplasia (overtly fibrotic) Myelofibrosis with myeloid metaplasia (cellular phase) Chronic myeloid leukemia Myelodysplastic syndrome Atypical myeloproliferative disorder Acute leukemia	Infection Tissue damage Chronic inflammation Malignancy Rebound thrombocytosis Renal disorders Hemolytic anemia Postsplenectomy Blood loss

Choice of Cytoreductive Agent in Essential Thrombocythemia		
Age Group	First Line	Second Line
<40 years old	Interferon-	Hydroxyurea
		Anagrelide
40–75 years	Hydroxyurea	Interferon-
		Anagrelide
>75 years	Hydroxyurea	Anagrelide
		Pipobroman
		Busulphan
		Radioactive phosphorus

Diagnostic criteria for ET:

The diagnosis of essential thrombocythaemia requires that either all major criteria or the first 3 major criteria plus the minor criterion are met.

Major criteria:

1. Platelet counts ≥450 × 109/L.
2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; very rarely a minor (grade 1a) increase in reticulin fibres.
3. WHO criteria for BCR-ABL1-positive chronic myeloid leukaemia, polycythaemia vera, primary myelofibrosis, or other myeloid neoplasms are not met.
4. JAK2, CALR, or MPL mutation.

Minor criterion:

Presence of a clonal marker or

Absence of evidence of reactive thrombocytosis.

22 Platelet and Coagulation Disorders

Classification of Disorders of Hemostasis		
Major Types	Disorders	Examples
Acquired	Thrombocytopenias	Autoimmune and alloimmune, drug-induced, hypersplenism, hypoplastic (primary, myelosuppressive therapy, myelophthisic marrow infiltration), disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome.
	Liver diseases	Cirrhosis, acute hepatic failure, liver transplantation thrombopoietin deficiency.
	Renal failure	
	Vitamin K deficiency	Malabsorption syndrome, hemorrhagic disease of the newborn, prolonged antibiotic therapy, malnutrition, prolonged biliary obstruction.
	Hematologic disorders	Acute leukemias (particularly promyelocytic), myelodysplasias, monoclonal gammopathies, essential thrombocythemia.
	Acquired antibodies against coagulation factors.	Neutralizing antibodies against factors V, VIII, and XIII, accelerated clearance of antibody-factor complexes, e.g., acquired von Willebrand disease, hypoprothrombinemia associated with antiphospholipid antibodies.
	DIC	Acute (sepsis, malignancies, trauma, obstetric complications) and chronic (malignancies, giant hemangiomas, retained products of conception).
	Drugs	Antiplatelet agents, anticoagulants, antithrombins, and thrombolytic, hepatotoxic, and nephrotoxic agents.
	Vascular	Nonpalpable purpura ("senile," solar, and factitious purpura), use of corticosteroids, vitamin C deficiency, child abuse, thromboembolic, purpura fulminans; palpable-purpura (Henoch-Schönlein, vasculitis, dysproteinemias; amyloidosis.

Inherited	Deficiencies of coagulation factors	Hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency), deficiencies of fibrinogen factors II, V, VII, X, XI, and XIII and von Willebrand disease.
	Platelet disorders	Glanzmann thrombasthenia, Bernard-Soulier syndrome, platelet granule disorders.
	Fibrinolytic disorders	α2-Antiplasmin deficiency, plasminogen activator inhibitor-1 deficiency.
	Vascular	Hemorrhagic telangiectasias.
	Connective tissue disorders	Ehlers-Danlos syndrome.

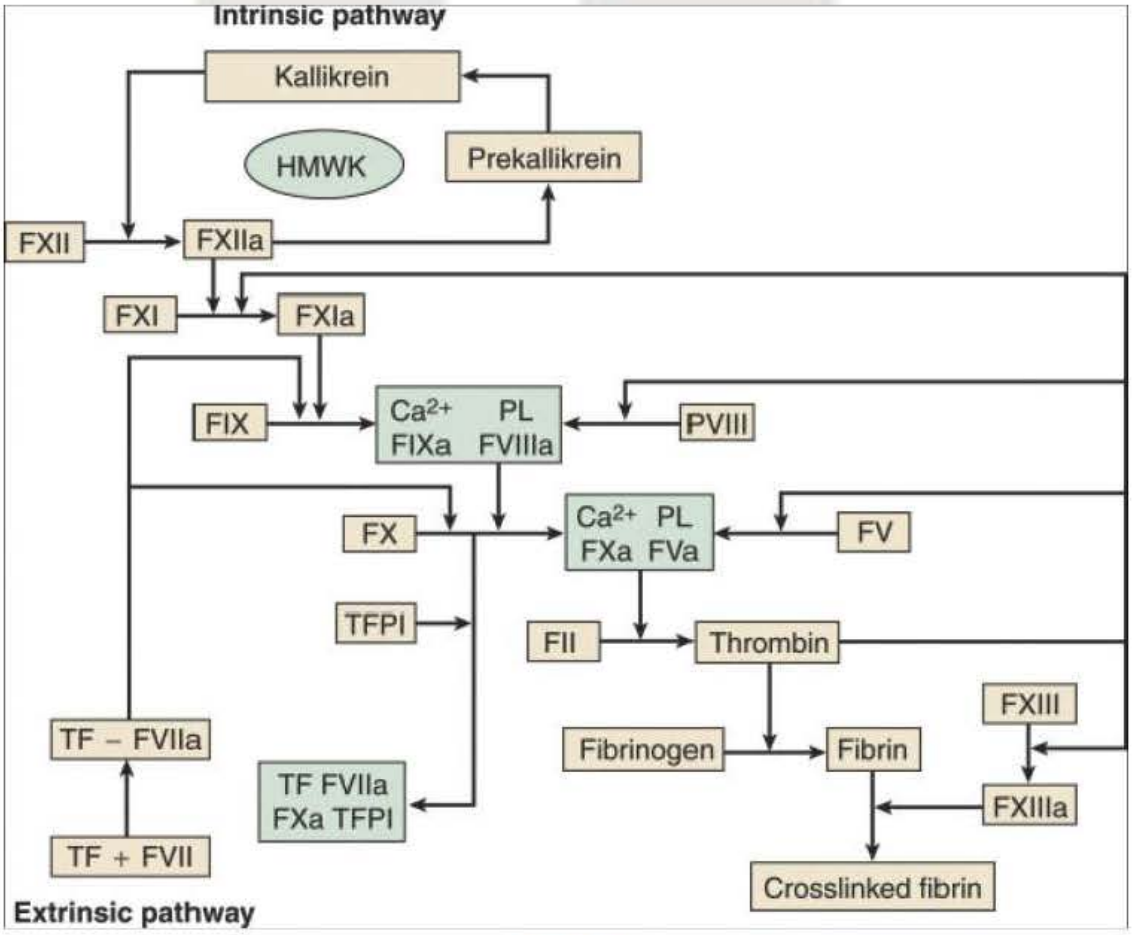


Fig. 22.1

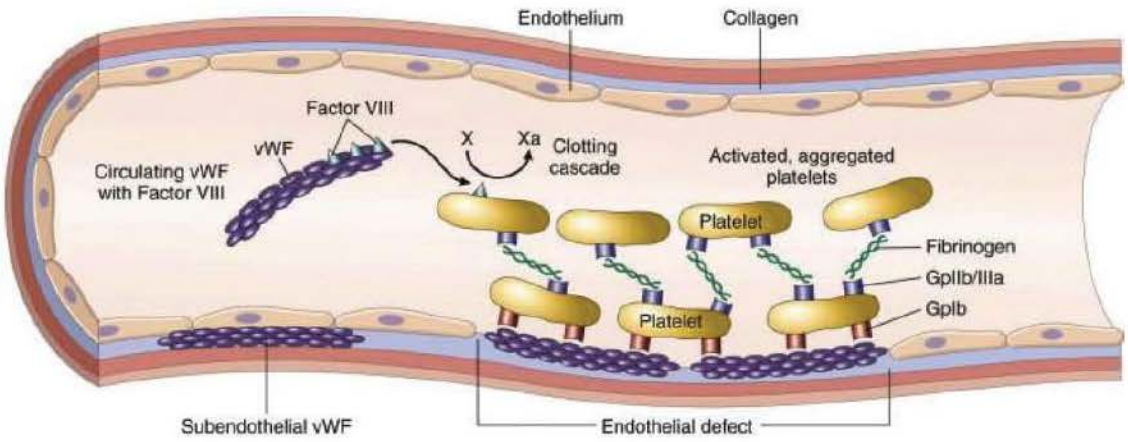


Fig. 22.2

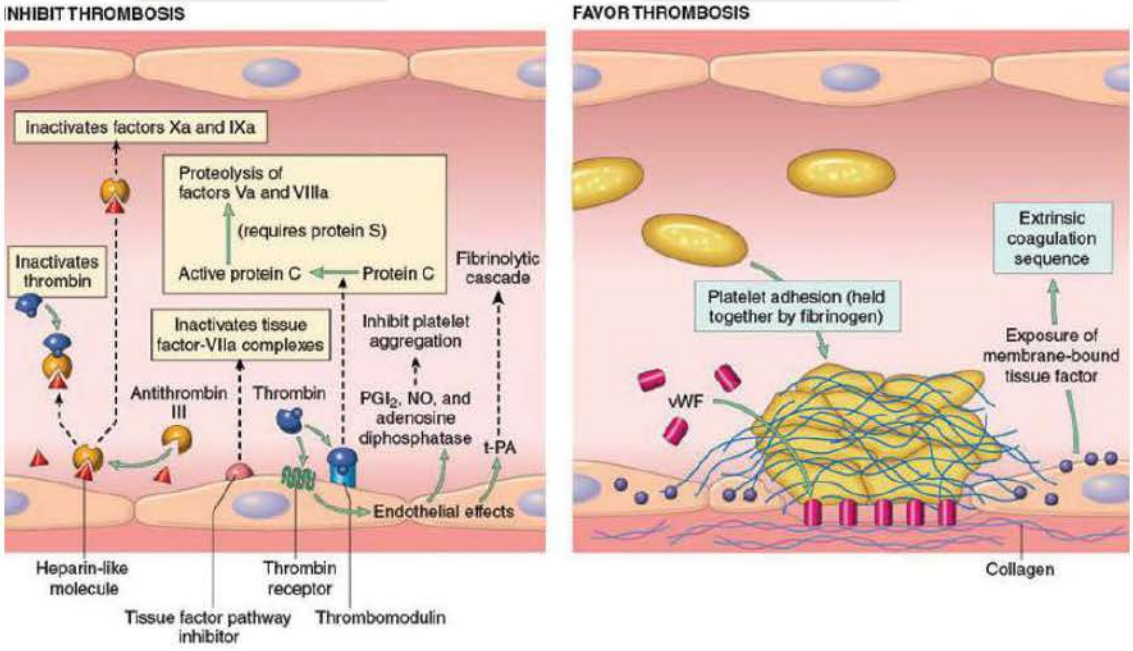


Fig. 22.3

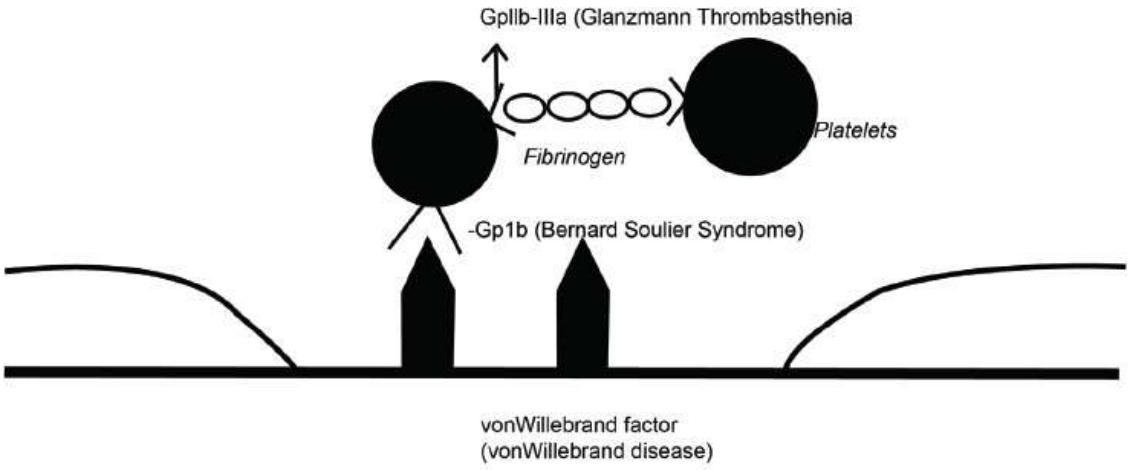


Fig. 22.4

The vitamin K–dependent factors group includes coagulation factors II, VII, IX, and X. However, it is important to remember that the anticoagulant proteins S, C, and Z are also vitamin K-dependent. Each of these proteins contains a number of glutamic acid residues at its amino terminus that are γ-carboxylated by a vitamin K–dependent mechanism. This results in a novel amino acid, γ-carboxyglutamic acid, which is important in promoting a conformational change in the protein that promotes binding of the factor to phospholipid. Because this binding is crucial for coordinating the interaction of the various factors, the proteins produced in the absence of vitamin K (PIVKAs) that are not γ-carboxylated are essentially functionless. The vitamin K–dependent factors are proenzymes or zymogens, which require cleavage sometimes with release of a small peptide (activation peptide) to become functional. Measurement of these activation peptides has been used as a means of assessing coagulation activation

Factors VIII and V are the two most labile of the coagulation factors, and they are rapidly lost from stored blood or heated plasma. They share considerable structural homology and are cofactors for the serine proteases FIX and FX, respectively; they both require proteolytic activation by factor IIa or Xa to function. Factor VIII circulates in combination with VWF, which is present in the form of large multimers of a basic 200 kD monomer. One function of VWF is to stabilize factor VIII and protect it from degradation. In the absence of VWF the survival of factor VIII in the circulation is extremely

short (i.e., <2 hours instead of the normal 8–12 hours). VWF may also serve to deliver factor VIII to platelets adherent to a site of vascular injury. Once factor VIII has been cleaved and activated by thrombin it no longer binds to VWF.

Prothrombin Time:

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, and X and on the fibrinogen concentration of the plasma.

The common causes of prolonged one-stage PTs are as follows:

1.	Administration of oral anticoagulant drugs (vitamin K antagonists).
2.	Liver disease, particularly obstructive.
3.	Vitamin K deficiency.
4.	Disseminated intravascular coagulation.
5.	Rarely, a previously undiagnosed factor VII, X, V, or prothrombin deficiency or defect. Note: With prothrombin, factor X, or factor V deficiency the APTT will also be prolonged.

Activated Partial Thromboplastin Time:

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicates the overall efficiency of the intrinsic pathway.

The common causes of a prolonged APTT are as follows:

1.	Disseminated intravascular coagulation.
2.	Liver disease.
3.	Massive transfusion with plasma-depleted red blood cells.
4.	Administration of or contamination with heparin or other anticoagulants.
5.	A circulating anticoagulant (inhibitor).
6.	Deficiency of a coagulation factor other than factor VII.

The APTT is also moderately prolonged in patients taking oral anticoagulant drugs and in the presence of vitamin K deficiency. Occasionally, a patient with previously undiagnosed haemophilia or another congenital coagulation disorder presents with an isolated prolonged APTT.

The common causes of prolonged TT are as follows:

1.	Hypofibrinogenaemia as found in DIC and, more rarely, in a congenital defect or deficiency.
2.	Raised concentrations of FDP, as encountered in DIC or liver disease.
3.	Extreme prolongation of the TT is nearly always a result of the presence of heparin, which interferes with the thrombin–fibrinogen reaction. If the presence of heparin is suspected, a Reptilase time test should be carried out (see p. 407). Low molecular weight heparin (LMWH) produces only a slight prolongation at therapeutic levels.
4.	Dysfibrinogenaemia, either inherited or acquired, in liver disease or in neonates.
5.	Hypoalbuminaemia.

Shortening of the TT occurs in conditions of coagulation activation.

Clinical Distinction between Disorders of Vessels and Platelets and Disorders of Blood Coagulation		
Finding	Disorders of Coagulation	Disorders of Platelets or Vessels
Petechiae	Rare	Characteristic
Deep dissecting hematomas	Characteristic	Rare
Superficial ecchymoses	Common; usually large and solitary	Characteristic; usually small and multiple
Hemarthrosis	Characteristic	Rare
Delayed bleeding	Common	Rare
Bleeding from superficial cuts and scratches	Minimal	Persistent; often profuse

Sex of patient	80–90% of inherited forms occur only in male patients.	Relatively more common in females.
Positive family history	Common	Rare (except von Willebrand disease and hereditary hemorrhagic telangiectasia).

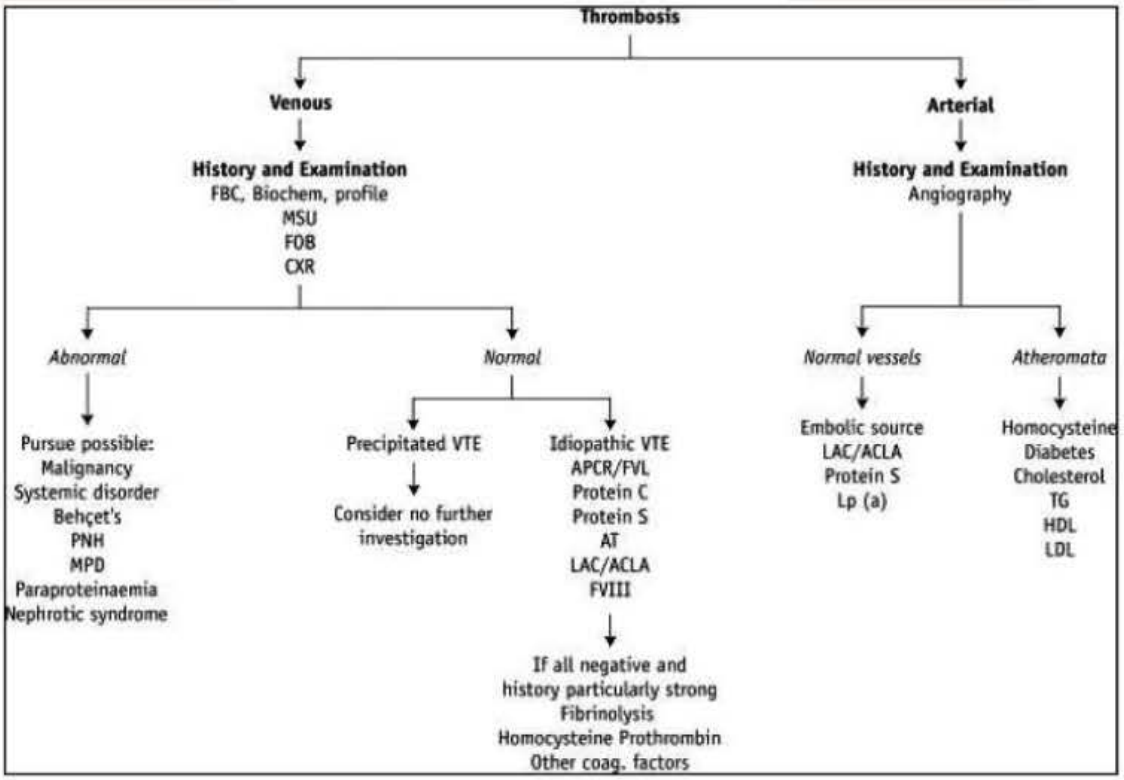


Fig. 22.5

Clinical Manifestations Typically Associated with Specific Hemostatic Disorders	
Clinical Manifestations	**Hemostatic Disorders**
Mucocutaneous bleeding.	Thrombocytopenias, platelet dysfunction, von Willebrand disease.
Cephalhematomas in newborns, hemarthroses, hematuria, and intramuscular, intracerebral, and retroperitoneal hemorrhages.	Severe hemophilias A and B, severe deficiencies of factor VII, X, or XIII, severe type 3 von Willebrand disease, afibrinogenemia.
Injury-related bleeding and mild spontaneous bleeding.	Mild and moderate hemophilias A and B, severe factor XI deficiency, moderate deficiencies of fibrinogen and factors II, V, VII, or X, combined factors V and VIII deficiency, 2-antiplasmin deficiency

Bleeding from stump of umbilical cord and habitual abortions.	Afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, factor XIII deficiency.
Impaired wound healing.	Factor XIII deficiency.
Facial purpura in newborns.	Glanzmann thrombasthenia, severe thrombocytopenia.
Recurrent severe epistaxis and chronic iron deficiency anemia.	Hereditary hemorrhagic telangiectasias.

Thrombokinetic Patterns in Various Forms of Thrombocytopenia

Decreased Production:				
Measurement	Hypoproliferation or Hypoplasia[a]	Ineffective Thrombopoiesis[b]	Accelerated Destruction[c]	Pooling Abnormal
Total megakaryocyte mass[d]	Decreased	Increased	M increased	V increased
Megakaryocyte number	Decreased	M increased	Increased	V increased
Megakaryocyte volume	Increased	Normal or V decreased	Increased	V increased
Platelet turnover rate or production rate[e]	Decreased	Decreased	Increased	V increased
Total platelet mass	Decreased	Decreased	Decreased	? Normal
Splenic platelet pool	Decreased	Decreased	Decreased[f]	Increased
Platelet survival	Normal	V shortened	Shortened	V shortened

M, markedly; V, variably.
[a] Includes myelophthisic processes.
[b] Mainly in megaloblastic hematopoiesis; component of accelerated destruction present in some cases.
[c] Minor component of ineffective thrombopoiesis present in some cases.
[d] Equated to total thrombopoiesis.
[e] Equated to effective thrombopoiesis.
[f] Not representative of sequestered antibody-sensitized platelets.

Pathophysiologic Classification of Thrombocytopenia

Artifactual thrombocytopenia:
Platelet clumping caused by anticoagulant-dependent immunoglobulin (pseudothrombocytopenia)
Platelet satellitism
Giant platelets

Decreased platelet production:
Hypoplasia of megakaryocytes
Ineffective thrombopoiesis
Disorders of thrombopoietic control
Hereditary thrombocytopenias

Increased platelet destruction:
Caused by immunologic processes
Autoimmune
Idiopathic
Secondary: Infections, pregnancy, collagen vascular disorders, lymphoproliferative disorders, drugs, miscellaneous.

Alloimmune
- Neonatal thrombocytopenia
- Posttransfusion purpura

Caused by nonimmunologic processes
- Thrombotic microangiopathies
  - Disseminated intravascular coagulation
  - Thrombotic thrombocytopenic purpura
  - Hemolytic-uremic syndrome
- Platelet damage by abnormal vascular surfaces
- Miscellaneous
  - Infection
  - Massive blood transfusions

Abnormal platelet distribution or pooling
Disorders of the spleen (neoplastic, congestive, infiltrative, infectious, of unknown cause)
Hypothermia.
Dilution of platelets with massive transfusions.

A count below 100,000 platelets/μL is generally considered to constitute thrombocytopenia.

However, spontaneous bleeding does not become evident until platelet counts fall below 20,000 platelets/μL.

Platelet counts in the range of 20,000 to 50,000 platelets/μL can aggravate post-traumatic bleeding.

Bleeding resulting from thrombocytopenia is associated with a normal PT and PTT.

Causes of Immune-Mediated Thrombocytopenia
1. Primary.
A. Idiopathic autoimmune thrombocytopenic purpura.
2. Secondary.
A. Autoimmune diseases: systemic lupus erythematosus, antiphospholipid syndrome, autoimmune hepatitis, autoimmune thyroiditis.
B. Lymphoproliferative disorders: chronic lymphocytic leukemia, Hodgkin lymphoma, large granular lymphocytic leukemia.
C. Infections: HIV, hepatitis C, Helicobacter pylori .
D. Myelodysplastic syndrome.
E. Agammaglobulinemia, hypogamma-globulinemia, immunoglobulin A deficiency.
F. Drugs: quinidine, gold, heparin, penicillin, procainamide, α-methyldopa, sulfamethoxazole.

Features of Acute and Chronic Idiopathic Thrombocytopenic Purpura (ITP)		
Feature	Acute ITP	Chronic ITP
Peak age of incidence	Children, 2–6 yr	Adults, 20–40 yr
Sex predilection	None	3:1 female to male
Antecedent infection	Common 1–3 wk before	Unusual
Onset of bleeding	Abrupt	Insidious
Hemorrhagic bullae in mouth	Present in severe cases	Usually absent
Platelet count	<20,000/μl	30,000–80,000/μl
Eosinophilia and lymphocytosis	Common	Rare
Duration	2–6 wk; rarely longer	Months or years
Spontaneous remissions	Occur in 80% of cases	Uncommon

The autoantibodies, most often directed against platelet membrane glycoproteins IIb-IIIa or Ib-IX, can be demonstrated in the plasma and bound to the platelet surface in about 80% of patients. In the

overwhelming majority of cases, the antiplatelet antibodies are of the IgG class.

Thrombocytopenia is defined as a blood platelet count less than 150 x 109/L. The blood film usually demonstrates isolated thrombocytopenia without erythrocyte or leukocyte abnormalities. Platelet anisocytosis is a common finding in ITP. Mean platelet volume and platelet distribution width are increased. Platelets may be abnormally large or abnormally small. The former reflect accelerated platelet production and the latter platelet microparticles reflect platelet destruction.

Almost all patients respond to glucocorticoids (which inhibit phagocyte function), but many eventually relapse. In such individuals, splenectomy normalizes the platelet count in about two thirds of patients, but with the attendant in-creased risk of bacterial sepsis. Immunomodulatory agents such as intravenous immunoglobulin or anti-CD20 antibody (rituximab) are often effective in patients who relapse after splenectomy or for whom splenectomy is contraindicated.

Heparin Induced Thrombocytopenia:

Heparin-induced thrombocytopenia (HIT) has a distinctive pathogenesis and is of particular importance because of its potential for severe clinical consequences.

- Thrombocytopenia occurs in about 5% of persons receiving heparin.
- Most develop so-called type I thrombocytopenia, which occurs rapidly after the onset of therapy and is of little clinical importance, sometimes resolving despite the continuation of therapy.
- It most likely results from a direct platelet-aggregating effect of heparin.
- Type II thrombocytopenia is less common but of much greater clinical significance.
- It occurs 5 to 14 days after therapy begins (or sooner if the person has been sensitized to heparin) and, paradoxically, often

leads to life-threatening venous and arterial thrombosis.

- This severe form of HIT is caused by antibodies that recognize complexes of heparin and platelet factor 4, which is a normal component of platelet granules.
- Binding of antibody to these complexes activates platelets and promotes thrombosis, even in the setting of thrombocytopenia.
- Unless therapy is immediately discontinued and an alternative nonheparin anticoagulant instituted, clots within large arteries may lead to vascular insufficiency and limb loss, and emboli from deep venous thrombosis can cause fatal pulmonary thromboembolism.
- The risk of severe HIT is lowered, but not completely eliminated, by the use of low-molecular-weight heparin preparations.
- Unfortunately, once severe HIT develops even low-molecular-weight heparins exacerbate the thrombotic tendency and must be avoided.

Thrombotic Thrombocytopenic Purpura:

Thrombotic Microangiopathies: Causes and Associations.

Thrombotic Thrombocytopenic Purpura
Deficiency of ADAMTS13
Inherited
Acquired (autoantibodies)
Hemolytic Uremic Syndrome
Epidemic: Escherichia coli strain O157 : H7 infection
Endothelial damage by Shiga-like toxin

Nonepidemic: alternative complement pathway inhibitor deficiencies (complement factor H, membrane cofactor protein (CD46), or factor I)
Inherited
Acquired (autoantibodies).
Miscellaneous associations .
Drugs (cyclosporine, chemotherapeutic agents).
Radiation, bone marrow transplantation.
Other infections (HIV, pneumococcal sepsis).
Conditions associated with autoimmunity (systemic lupus erythematosus, HIV infection, lymphoid neoplasms.

- TTP is an important diagnosis to consider in any patient presenting with thrombocytopenia and microangiopathic hemolytic anemia, since delays in diagnosis can be fatal. With plasma exchange, which removes autoantibodies and provides functional ADAMTS13, TTP (which once was uniformly fatal) can be treated successfully in more than 80% of patients.
- In contrast, HUS is associated with normal levels of ADAMTS13 and is initiated by several other distinct defects.
- **Epidemic, "typical" HUS is strongly associated with infectious gastroenteritis caused by Escherichia coli strain O157:H7, which elaborates a Shiga-like toxin .**
- This toxin is absorbed from the inflamed gastrointestinal mucosa into the circulation, where it alters endothelial cell function in some manner that results in platelet activation and aggregation.
- Children and the elderly are at highest risk.
- Those affected present with bloody diarrhea, and a few days later HUS makes its appearance. With appropriate supportive care complete recovery is possible, but irreversible renal damage and death can occur in more severe cases.
- **Nonepidemic, "atypical" HUS is often associated with defects in complement factor H, membrane cofactor protein (CD46),**

or factor I, three proteins that normally act to prevent excessive activation of the alternative complement pathway .

- Deficiencies of these proteins can be caused by inherited defects or acquired inhibitory autoantibodies and are associated with a remitting, relapsing course.
- Unlike TTP, the basis for the platelet activation in HUS is unclear; presumably, both Shiga-like toxin produced by pathogenic **E. coli** and defects in complement-regulatory proteins alter endothelial cell function in some way that promotes platelet activation.

Inherited disorders of platelet function can be classified into three pathogenically distinct groups: (1) defects of adhesion, (2) defects of aggregation, and (3) disorders of platelet secretion (release reaction).

- Bleeding resulting from defective adhesion of platelets to subendothelial matrix is best illustrated by the autosomal recessive disorder Bernard-Soulier syndrome, which is caused by an inherited deficiency of the platelet membrane glycoprotein complex Ib-IX. This glycoprotein is a receptor for vWF and is essential for normal platelet adhesion to the subendothelial extracellular matrix.
- Bleeding due to defective platelet aggregation is exemplified by Glanzmann thrombasthenia, which is also transmitted as an autosomal recessive trait. Thrombasthenic platelets fail to aggregate in response to adenosine diphosphate (ADP), collagen, epinephrine, or thrombin because of deficiency or dysfunction of glycoprotein IIb-IIIa, an integrin that participates in “bridge formation” between platelets by binding fibrinogen.
- Disorders of platelet secretion are characterized by the defective release of certain mediators of platelet activation, such as thromboxanes and granule-bound ADP.

Among the **acquired defects** of platelet function, two are clinically significant.

- The first is caused by ingestion of aspirin and other nonsteroidal anti-inflammatory drugs. Aspirin is a potent, irreversible

inhibitor of the enzyme cyclooxygenase, which is required for the synthesis of thromboxane A2 and prostaglandins These mediators play important roles in platelet aggregation and subsequent release reactions The antiplatelet effects of aspirin form the basis for its use in the prophylaxis of coronary thrombosis.

- Uremia is the second condition exemplifying an acquired defect in platelet function. The pathogenesis of platelet dysfunction in uremia is complex and involves defects in adhesion, granule secretion, and aggregation.

Inherited Disorders of Platelet Function
I. Abnormalities of Glycoprotein Adhesion Receptors.
A. (Glycoprotein IIb/IIIa; CD41/CD61): Glanzmann thrombasthenia.
B. Glycoproteins Ib (CD42b,c)/IX(CD42a)/V: Bernard-Soulier syndrome.
C. Glycoprotein GPIb (CD42b): platelet-type (pseudo-) von Willebrand disease.
D. Glycoprotein Ia/IIa; very-late antigen [VLA]-2; CD49b/CD29) .
E. CD36 (Glycoprotein IV).
F. Glycoprotein VI.
II. Abnormalities of Platelet Granules.
A. Alpha-Storage pool deficiency.
B. Gray platelet syndrome (alpha-storage pool deficiency).
C. Storage pool deficiency.

D. Quebec platelet disorder.
III. Abnormalities of Platelet Coagulant Activity (Scott syndrome).
IV. Abnormalities of Platelet Signaling and Secretion.
V. Abnormalities of a Cytoskeletal Structural Protein: beta1 Tubulin .
VI. Abnormalities in Cytoskeletal Linking Proteins.
A. Wiskott-Aldrich syndrome protein (WASP)
B. Kindlin-3: Leukocyte adhesion defect-III (LAD-III); LAD-1 variant, integrin activation deficiency disease defect (IADD).
VII. Abnormalities of Ttranscription Factors Leading to Functional Defects.
A. RUNX1 (familial platelet dysfunction with predisposition to acute myelogenous leukemia).
B. GATA-1.
C. FLI1 (dimorphic dysmorphic platelets with giant alpha granules and thrombocytopenia; Paris-Trousseau/Jacobsen syndrome).

Wiskott Aldrich Syndrome:

- **Wiskott-Aldrich syndrome is an X-linked recessive disease characterized by thrombocytopenia, eczema, and a marked vulnerability to recurrent infection, ending in early death .**

- The thymus is morphologically normal, at least early in the course of the disease, but there is progressive secondary depletion of T lymphocytes in the peripheral blood and in the T-cell zones (paracortical areas) of the lymph nodes, with variable loss of cellular immunity.
- Patients do not make antibodies to polysaccharide antigens, and the response to protein antigens is poor. IgM levels in the serum are low, but levels of IgG are usually normal.
- Paradoxically the levels of IgA and IgE are often elevated. Patients are also prone to developing non-Hodgkin B-cell lymphomas.
- The Wiskott-Aldrich syndrome is caused by mutations in the gene encoding **Wiskott-Aldrich syndrome protein (WASP)** , which is located at Xp11.23.
- This protein belongs to a family of proteins that are believed to link membrane receptors, such as antigen receptors, to cytoskeletal elements.
- The WASP protein may be involved in cytoskeleton-dependent responses, including cell migration and signal transduction, but the essential functions of this protein in lymphocytes and platelets are unclear. The only treatment is bone marrow transplantation.

Inherited Disorders of Coagulation
X-linked recessive traits
Hemophilia A
Hemophilia B (i. e., CRM+ and CRM- variants; hemophilia Bm, B Leyden, etc.)
Autosomal recessive traits
Factor XI deficiency
Prothrombin deficiency
Factor V deficiency
Factor VII deficiency
Factor X deficiency (i.e., Prower variant, Stuart variant, Friuli variant, others)
Afibrinogenemia
Hypofibrinogenemia

Factor XII deficiency Factor XIII deficiency **Autosomal dominant traits:** von Willebrand disease Dysfibrinogenemias **Combined abnormalities:** Associated with factor VIII deficiency (i.e., factor V deficiency, hemophilia B, factor XI deficiency, factor VII deficiency, von Willebrand disease, dysfibrinogenemias, platelet dysfunction) Involving vitamin K–dependent factors (i.e., factors II, VII, IX, and X; factors IX and XII; others). **Miscellaneous:** Prekallikrein deficiency High-molecular-weight kininogen deficiency Deficiency of physiologic inhibitors (i.e., α2-antiplasmin, abnormal α1-antitrypsin [antithrombin Pittsburgh])
CRM, cross-reacting material.

Haemophilia:

- Hemophilia is an X-linked recessive hemorrhagic disease due to mutations in the **F8** gene (hemophilia A or classic hemophilia) or **F9** gene (hemophilia B).
- The disease affects 1 in 10,000 males worldwide, in all ethnic groups; hemophilia A represents 80% of all cases.
- Male subjects are clinically affected; women, who carry a single mutated gene, are generally asymptomatic.

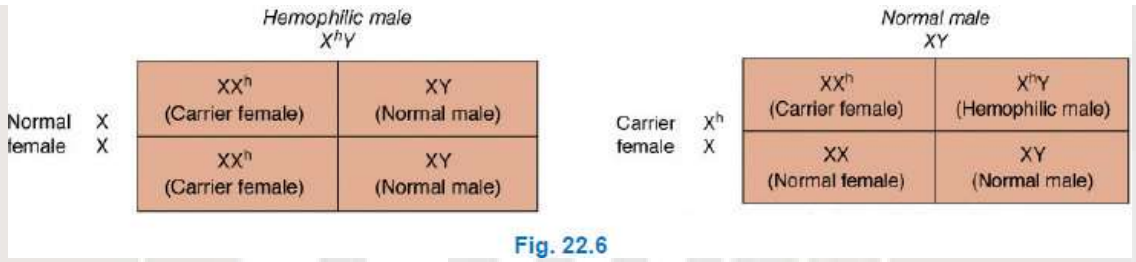


Fig. 22.6

- More than 500 different mutations have been identified in the **F8** or **F9** genes of patients with hemophilia A or B, respectively.
- One of the most common hemophilia A mutations results from an inversion of the intron 22 sequence, and it is present in 40% of cases of severe hemophilia A.
- **Hemophilia A is the most common hereditary disease associated with life-threatening bleeding.**
- Clinically, hemophilia A and hemophilia B are indistinguishable.
- The disease phenotype correlates with the residual activity of FVIII or FIX and can be classified as severe (<1%), moderate (1–5%), or mild (6–30%). In the severe and moderate forms, the disease is characterized by bleeding into the joints (hemarthrosis), soft tissues, and muscles after minor trauma or even spontaneously.

Clinical Classification of Hemophilia		
Classification	**Factor VIII Level**	**Clinical Features**
Severe	≤1% of normal (≤0.01 U/mL)	1. Spontaneous hemorrhage from early infancy
		2. Frequent spontaneous hemarthroses and other hemorrhages, requiring clotting factor replacement
Moderate	1–5% of normal (0.01–0.05 U/mL)	1. Hemorrhage secondary to trauma or surgery
		2. Occasional spontaneous hemarthroses
Mild	6–30% of normal (0.06–0.30 U/mL)	1. Hemorrhage secondary to trauma or surgery
		2. Rare spontaneous hemorrhage

- Typically, the global tests of coagulation show only an isolated prolongation of the aPTT assay. Patients with hemophilia have normal bleeding times and platelet counts.
- The diagnosis is made after specific determination of FVIII or FIX clotting activity.

- Factor replacement therapy for hemophilia can be provided either in response to a bleeding episode or as a prophylactic treatment.
- Primary prophylaxis is defined as a strategy for maintaining the missing clotting factor at levels 1% or higher on a regular basis in order to prevent bleeds, especially the onset of hemarthroses.
- Hemophilic boys receiving regular infusions of FVIII (3 days/week) or FIX (2 days/week) can reach puberty without detectable joint abnormalities.

Von Willebrand Disease:

- **Von Willebrand disease is the most common inherited bleeding disorder of human.**
- The most common symptoms are spontaneous bleeding from mucous membranes (e.g., epistaxis); excessive bleeding from wounds; menorrhagia; and a prolonged bleeding time in the presence of a normal platelet count. It is usually transmitted as an autosomal dominant disorder, but rare autosomal recessive variants have been described.
- VWD is associated with either quantitative deficiency (type 1 and type 3) or qualitative abnormalities of VWF (type 2).
- The uncommon type 3 variant is the most severe form of VWD and is characterized by very low or undetectable levels of VWF, a severe bleeding diathesis, and a generally autosomal recessive pattern of inheritance.
- Type 1 VWD, the most common variant, is characterized by VWF that is normal in structure and function but decreased in quantity (in the range of 20–50% of normal).
- In type 2 VWD, the VWF is abnormal in structure and/or function.
- Type 2A VWD is associated with selective loss of the largest and most functionally active VWF multimers.
- Type 2A is further subdivided into group 1, as a result of mutations that interfere with biosynthesis and secretion, and group 2, in which the mutant VWF exhibits an increased sensitivity to proteolysis in plasma.

- Type 2B VWD is caused by mutations clustered within the VWF A1 domain, in a segment critical for binding to the platelet glycoprotein Ib (GPIb) receptor.
- These mutations produce a “gain of function” resulting in spontaneous VWF binding to platelets and clearance of the resulting platelet complexes, leading to thrombocytopenia and loss of the most active (large) VWF multimers.
- Type 2N VWD is characterized by mutations within the factor VIII binding domain of VWF, leading to disproportionately decreased factor VIII and a disorder resembling mild hemophilia A, but with autosomal recessive inheritance.
- Type 1 VWD can often be effectively managed by treatment with desmopressin (DDAVP), which transiently produces a two- to threefold increase in plasma VWF level.
- Response to DDAVP is generally poor in type 3 and some type 2 VWD variants.
- These disorders often require treatment with factor replacement in the form of factor VIII concentrates containing large quantities of intact VWF multimers.
- Patients with von Willebrand disease have **defects in platelet function** despite a **normal platelet count** .
- The plasma level of active vWF, measured as the ristocetin cofactor activity, is reduced.
- Because vWF stabilizes factor VIII, a deficiency of vWF gives rise to a secondary decrease in factor VIII levels. This may be reflected by a prolongation of the PTT in von Willebrand disease types 1 and 3.
- However, except in rare type 3 patients, adverse complications typical of severe factor VIII deficiency, such as bleeding into the joints, are not seen.

Disseminated intravascular coagulation:

Disseminated intravascular coagulation (DIC) is a clinicopathologic syndrome characterized by widespread intravascular fibrin formation in response to excessive blood protease activity that overcomes the natural anticoagulant mechanisms.

Common Clinical Causes of Disseminated Intravascular Coagulation	
Sepsis	**Immunologic disorders**
Bacterial: Staphylococci, streptococci, pneumococci, meningococci, gram-negative bacilli Viral Mycotic Parasitic Rickettsial	Acute hemolytic transfusion reaction Organ or tissue transplant rejection Graft-versus-host disease
Trauma and tissue injury	**Drugs**
Brain injury (gunshot) Extensive burns Fat embolism Rhabdomyolysis	Fibrinolytic agents Aprotinin Warfarin (especially in neonates with protein C deficiency) Prothrombin complex concentrates Recreational drugs (amphetamines)
Vascular disorders	**Envenomation**
Giant hemangiomas (Kasabach-Merritt syndrome) Large vessel aneurysms (e.g., aorta)	Snake Insects
Obstetrical complications	**Liver disease**
Abruptio placentae Amniotic-fluid embolism Dead fetus syndrome Septic abortion	Fulminant hepatic failure Cirrhosis Fatty liver of pregnancy
Cancer	**Miscellaneous**
Adenocarcinoma (prostate, pancreas, etc.) Hematologicmalignancies (acute promyelocytic leukemia)	Shock Respiratory distress syndrome Massive transfusion

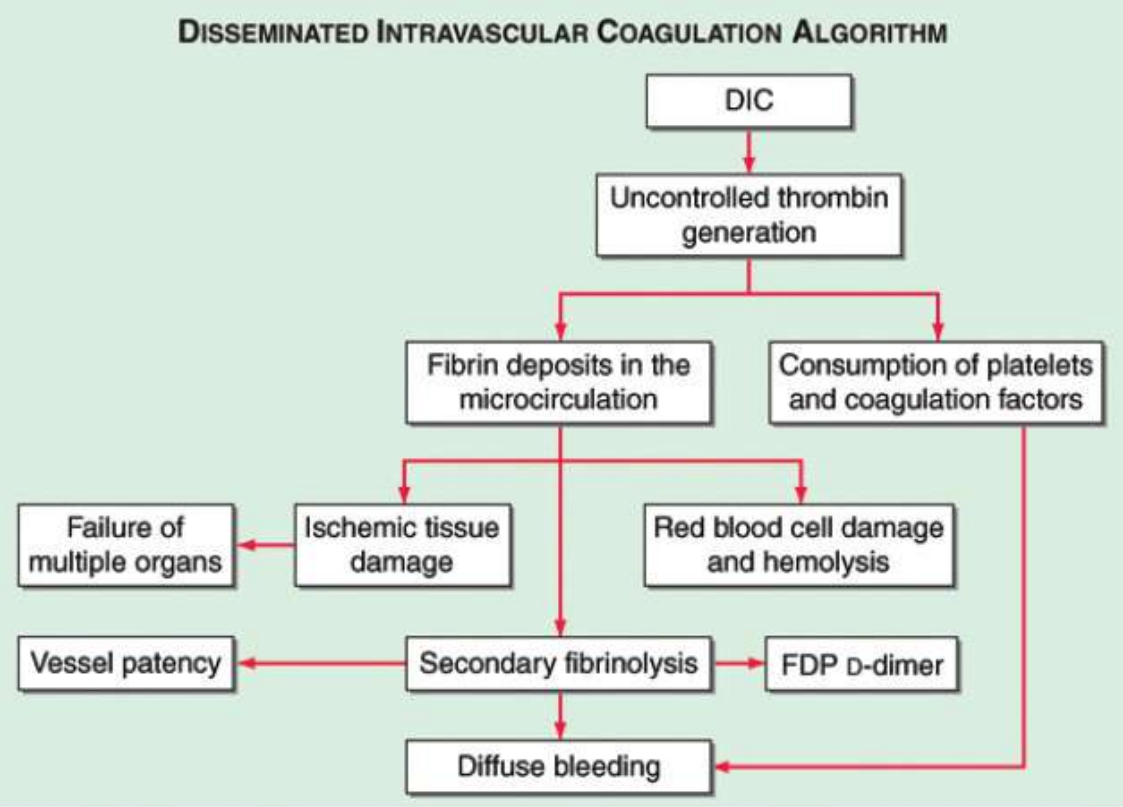


Fig. 22.7

- Two major mechanisms trigger DIC: (1) release of tissue factor or thromboplastic substances into the circulation, and (2) widespread injury to the endothelial cells.
- Common findings include the prolongation of PT and/or aPTT; platelet counts <100,000/µL3, or a rapid decline in platelet numbers; the presence of schistocytes (fragmented red cells) in the blood smear; and elevated levels of FDP.
- The most sensitive test for DIC is the FDP level. DIC is an unlikely diagnosis in the presence of normal levels of FDP.
- The D-dimer test is more specific for detection of fibrin—but not fibrinogen—degradation products and indicates that the cross-linked fibrin has been digested by plasmin.
- Because fibrinogen has a prolonged half-life, plasma levels diminish acutely only in severe cases of DIC. High-grade DIC is also associated with levels of antithrombin III or plasminogen activity <60% of normal.

- It is almost impossible to detail all the potential clinical presentations, but a few common patterns are worthy of description.
- These include microangiopathic hemolytic anemia; dyspnea, cyanosis, and respiratory failure; convulsions and coma; oliguria and acute renal failure; and sudden or progressive circulatory failure and shock.
- In general, acute DIC, associated with obstetric complications or major trauma, for example, is dominated by a bleeding diathesis, whereas chronic DIC, such as occurs in cancer patients, tends to present with thrombotic complications.

Coagulation Disorders and Hemostasis in Liver Disease
Bleeding
Portal hypertension
Esophageal varices
Thrombocytopenia
Splenomegaly
Chronic or acute DIC
Decreased synthesis of clotting factors
Hepatocyte failure
Vitamin K deficiency
Systemic fibrinolysis
DIC
Dysfibrinogenemia
Thrombosis
Decreased synthesis of coagulation inhibitors: protein C, protein S, antithrombin
Hepatocyte failure
Vitamin K deficiency (protein C, protein S)
Failure to clear activated coagulation proteins (DIC)
Dysfibrinogenemia
Iatrogenic: Transfusion of prothrombin complex concentrates
Antifibrinolytic agents: EACA, tranexamic acid

23 Blood Bank– Important Points

Donors should be between the age of 18 and 65 years.

The haemoglobin should be not less than 12.0 gm/dl or the packed cell volume (haematocrit) should be not less than 36%. The screening should be carried out by using any appropriate and validated methodology. A more sensitive method of hemoglobin testing should be available as a reference or control.

Blood collection from donors weighing 45-55 Kg should be 350 ml blood and from those weighing 55 Kg and above should be 450 ml.

The systolic blood pressure should be between 100 and 160 mm of mercury and the diastolic pressure should be between 60-90 mm of mercury.

Temperature should not exceed 37.50C/ 99.5oF

Pulse should be between 60 to 100 beats per minute and regular.

Vaccinations:

Individuals who have taken vaccination against TAB/TT/CHOLERA/ HEPATITIS-A - should be accepted if free of symptoms.

Those who have received Hepatitis B vaccination should be accepted after 7 days of vaccination.

Yellow fever/measles/polio - should be deferred for 2 weeks.

Rabies vaccination - should be deferred for 1 year.

Those bitten by any animal should be deferred for one year.

Hepatitis B Immunoglobulin-should be deferred for 1 year.

Donation Interval:

The interval between two blood donations should be at least 12 weeks.

At least 48 hours must elapse after plasma pheresis or Cytapheresis before whole blood is collected from a donor.

Apheresis should be done only after 90 days of whole blood collection or in an event when red cells are not returned at the end of pheresis.

One of the following solutions should be used in the indicated volumes

Citrate-Phosphate-Dextrose (CPD) Solution.14 ml solution is required for 100 ml of blood.

Citrate-Phosphate-Dextrose-Adenine (CPDA-1) solution.14 ml solution is required for 100 ml of blood.

100 ml SAG-M/ADSOL or any approved additive solution containing saline adenine and glucose (or with mannitol) is added to packed cells after separation of plasma for storage.

Temperature:

Immediately after collection, the blood should be placed at 40C to 60C + 20C except if it is used for component preparation it will be stored at 220C + 20C until the platelets are separated.

Tests:

Test for Syphilis Each donation of whole blood should be subjected to a serological test for syphilis by VDRL / RPR Method / TPHA.
Test for Viral HepatitisA test for hepatitis B (HBsAg) and hepatitis C (anti-HCV) by ELISA/Rapid test which is a validated method should be done on each unit of blood. Any technology with similar or higher sensitivity may be used additionally to improve blood safety.
Screening for HIV AntibodiesAll blood units collected should be tested for HIV 1&2 antibodies using ELISA/Rapid which is a validated method. Any alternative technology with similar or higher sensitivity may be used.

Test for Malaria All blood units should be tested for malarial parasites using a validated and sensitive antigen test.

The sterility of the blood should be checked on 1% of the blood units collected or 4 per month whichever is higher.

The following colour code is used to differentiate the ABO group label

- Blood group O - Blue
- Blood group A - Yellow
- Blood group B - Pink
- Blood group AB – White

Red blood cells:

RBCs are prepared from whole blood by centrifugation and removal of plasma
The most commonly used anticoagulant preservative solution for RBCs is CPDA-1 (*)
This is supplemented with dextrose and adenine to preserve red cell ATP levels. RBCs in CPDA-1 may be stored for up to 35 days at 1-6°C.

Plasma:

Plasma may be stored in the liquid state at 1-6°C or it may be frozen for extended preservation.

In the liquid state at refrigerator temperature, there is loss of labile clotting factors particularly factor VIII and factor V (*).

FFP is separated from the RBCs and is placed at -18°C within 8 hours of collection

Plasma frozen within 24 hrs after phlebotomy (FP24) is manufactured similarly to FFP.

The coagulation factor content of FP24 is equivalent to FFP.

Frozen plasma may be stored for upto a year at -18°C or lower.

Before transfusion both FFP and FP24 are thawed at 37°C and must be transfused within 24 hrs.

Cryoprecipitated Antihemophilic Factor:

Cryoprecipitated antihemophilic factor (cryoprecipitate or cryo) is the cold insoluble portion of plasma remaining after FFP has been thawed at refrigerator temperatures.

It contains approximately 50% of Factor VIII and 20-40% of the fibrinogen present in the original plasma unit (*)

Cryo also contains von Willebrand factor (vWF) and factor XIII (*)

FDA regulations require that a unit of cryoprecipitate contain at least 80 IU of factor VIII.

A unit of cryoprecipitate contains approximately 250 mg of fibrinogen (but testing for it is not required)

Currently cryo is used mainly as a source of fibrinogen (*).

Platelet concentrates:

Platelet concentrates are prepared from whole blood by centrifugation of platelet rich plasma and expression of platelet poor plasma
Platelet concentrates must contain at least 5.5 x 10(10) platelets per unit
They are stored at room temperature (20-24°C) because platelets stored at refrigerator temperature (1-6°C) have greatly diminished post transfusion survival.
Current FDA regulations allow PCs to be stored for upto 5 days with continuous gentle agitation (*)
At the end of storage the pH of PCs must be 6.0 or higher.

It is typically necessary to pool five or more PCs to obtain a therapeutic dose for a typical adult patient.

Red cell transfusion guidelines:

1. Symptomatic anemia in a euvolemic patient.
2. Acute blood loss of >15% of estimated blood volume.
3. Preoperative Hb < 9 g/DL with expected blood loss of over 500 ml.

4. Hb < 7 g/dL in a critically ill patient.
5. Hb < 8 g/dL in a patient with acute coronary syndrome.
6. Hb < 10 g/dL with uremic or thrombocytopenic bleeding.
7. Some cases of sickle cell disease.

Platelet transfusion guidelines:

Thrombocytopenia due to decreased production.

1. Stable patient : platelet count < 10,000/ microL.
2. Fever : platelet count < 20, 000/ microL.
3. Bleeding, invasive procedure or surgery: platelet count < 40,000-50,000/ microL.
4. Retinal or central nervous system bleeding: platelet count < 100,000/ microL

Micro vascular bleeding due to platelet dysfunction.

Plasma transfusion guidelines:

1. Coagulation factor deficiency, factor concentrate unavailable.
2. Dilutional coagulopathy.
3. Hemorrhage in liver disease.
4. DIC.
5. Coumadin reversal.
6. TTP.
7. Acute trauma resuscitation.

Cryoprecipitate transfusion guidelines:

1. Factor VIII deficiency, factor concentrate unavailable.
2. Von Willebrand disease, factor concentrate unavailable.
3. Hypofibrinogenemia.
4. Factor XIII deficiency.
5. Uremic bleeding (DDAVP preferred).

First factor to reduce in strored blood is factor 8 followed by factor 5.

24 Miscellaneous

Classification	Criteria
Severe	BM cellularity < 25% (or < 50% if < 30% of BM is hematopoietic cells). AND ≥ 2 of the following: • Peripheral blood neutrophil count < 0.5 X 109/L. • Peripheral blood platelet count < 20 X 109/L. • Peripheral blood reticulocyte count < 20 X 109/L.
Very severe	As above, but peripheral blood neutrophil count must be < 0.2 X 10^9/L
Nonsevere	Hypocellullar BM with peripheral blood values not meeting criteria for severe aplastic anemia.

Names and Definitions of Vasculitides from the Chapel Hill Consensus	
Definition	
Large-Vessel Vasculitis Giant cell (temporal) arteritis	Granulomatous arteritis of the aorta and its major branches, with a predilection for the extracranial branches of the carotid artery. Often involves the temporal artery. Usually occurs in patients older than 50 and is often associated with polymyalgia rheumatica.
Takayasu's arteritis Medium-Sized Vessel Vasculitis Polyarteritis nodosa (classic)	Granulomatous inflammation of the aorta and its major branches. Usually occurs in patients younger than 50.
Kawasaki disease	Necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis of the arterioles, capillaries, or venules.
Small-Vessel Vasculitis Wegener's granulomatosis	Arteritis involving the large, medium-sized, and small arteries, and associated with mucocutaneous lymph node syndrome. Coronary arteries are often involved. Aorta and veins may be involved. Usually occurs in children.
Churg-Strauss	Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels (capillaries, venules, arterioles, and arteries). Necrotizing glomerulonephritis is common.
Microscopic polyangiitis	Eosinophil-rich and granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels and associated with asthma and eosinophilia.
Henoch-Schonlein purpura	Necrotizing vasculitis, with few or no immune deposits, affecting small vessels (capillaries, venules, or arterioles).

Essential cryoglobulinemic vasculitis	Vasculitis with immunoglobulin (Ig)A-dominant immune deposits, affecting small vessels (capillaries, venules, or arterioles).
Cutaneous leukocytoclastic vasculitis	Vasculitis with cryoglobulin immune deposits, affecting small vessels (capillaries, venules, or arterioles). Typically involves skin, gut, and glomeruli, and is associated with arthralgias and arthritis. Isolated cutaneous leukocytoclastic angiitis without systemic vasculitis or glomerulonephritis.

Antibodies in Common Paraneoplastic Neurologic Syndromes and Associated Tumors

Antibody	Clinical syndrome	Associated tumors and conditions
Anti-Yo	Cerebellar degeneration	Ovary, breast
Anti-Hu (ANNA-1)	Brain and cerebellar dysfunction	SCLC, neuroblastoma
Anti-Ri (ANNA-2) Antiamphiphysin	Opsoclonus-myoclonus Stiff-man syndrome Encephalitis-neuropathy	Breast, ovary, SCLC Breast, ovary, SCLC Diabetes mellitus
Anti-VGCC	Lambert-Eaton myasthenia syndrome	SCLC

SCLC, Small cell lung cancer; VGCC, voltage-gated calcium channels (anti-body).

Typical Lumbar CSF Findings in Meningitis

Test	Bacterial	Viral	Fungal	Tuberculous
Opening pressure Leukocyte count Cell differential Protein Glucose CSF/serum glucose ratio Lactic acid	Elevated ≥1000/uL Mainly neutrophils* Mild-marked increase Usually ≤40 mg/dL Normal-marked decrease Mild-marked increase	Usually normal <100/uL Mainly lymphocytes† Normal-mild increase Normal Usually normal Normal-mild increase	Variable Variable Mainly lymphocytes Increased Decreased Low Mild-moderate increase	Variable Variable Mainly lymphocytes Increased Decreased: may be <45 mg/dL Low Mild-moderate increase

CSF Proteins and Central Nervous System Diseases

Protein	Major diseases/disorders.
α2-Macroglobulin β–Amyloid and τ proteins β–Microglobulin C-reactive protein Fibronectin Methemoglobin Myelin basic protein Protein 14-3-3 Transferrin	Subdural hemorrhage, bacterial meningitis Alzheimer's disease Leukemia/lymphoma, Behçet's syndrome Bacterial and viral meningitis Lymphoblastic leukemia, AIDS, meningitis Mild subarachnoid/subdural hemorrhage Multiple sclerosis, tumors, others Creutzfeldt-Jakob disease CSF leakage (otorrhea, rhinorrhea).

Condition	Hla Type
Sarcoidosis	Al, B8
Good pasture's syndrome	DRB*1501, *1502
Celiac disease	DQ2, DQ8
Ankylosing spondylitis	B27*2705, *2702
Reactive arthritis	B27
Primary sclerosing cholangitis	B8
Primary membranous nephropathy	DQA1
Grave's disease	DR3
Diabetes mellitus Type 1	DR3, DR4, DQ8
Psoriasis	Cw*0602
Rheumatoid arthritis	DRB1
Psoriatic arthritis	B27 and Cw6
21 Hydroxylase deficiency	BW47
Hereditary hemochromatosis	A
Systemic lupus erythematosis	DRB1*0301, *1501
SLE with anti dsDNA, anti Sm and antiphospholipid antibodies	DQ
Subacute cutaneous SLE	DR3
Hydralazine induced SLE	DR4
Procainamide induced SLE	DR6
Sjogren's syndrome	B8, DR3, DRW52, DQA1/B1
Behcet's disease	B51

DNA Repair Pathways	
Direct repair	Repairs certain types of DNA damage in a single-step reaction.
Mismatch repair	Checks for errors made when DNA is replicated. Any mispaired bases in the daughter strand are removed and replaced with the correct match.
Base excision repair	Repairs small, nonhelix-deforming adducts such as those produced by methylation, oxidation, reduction, or base fragmentation by ionizing radiation.
Nucleotide excision repair	Removes bulky DNA adducts such as thymine dimers and certain photoproducts as well as chemical adducts and cross-links.
Double-strand break repair	Repairs double-strand breaks that result from physiologic processes or from ionizing radiation and oxidative insults.

Key Features of Hodgkin Lymphomas				
Lymphoma	presentation	Morphology	Cell surface markers	Prognosis
Nodular, lymphocyte predominant (NLPHL).	M > F, 30-50 years, with peripheral lymphadenopathy.	Mononuclear cells with convoluted nuclei (popcorn or LHS cell) loosely aggregated in nodules of small & cells.	CD45, CD20, bcl-6,s J-chain, Oct-2, BOB.1, EBV absent in LP cells.	Excellent for stages 1, II
Nodular sclerosis	M = F, <30 years with mediastinal mass, occasional spleen or lung involvement; 40% have B symptoms; most patients present with stage II disease.	Broad bands of collagen, nodules of lymphoid tissue with aggregates of HRS cells and lacunar cells, multinucleated variants.	CD15, CD30, CD45-EBV in 1%-40%.	Good with systemic therapy.
Mixed cellularity	M > F; median age, 38 years; peripheral lymphadenopathy common, spleen, BM; B symptoms common; patients often stage III or IV.	Classic HRS cells in mixture of lymphocytes, plasma cells, eosinophils, histiocytes.	CD15, CD30, CD45-EBV in 75%.	Good with systemic therapy.
Lymphocyte depletion.	M > F; median age, 30-37 years; B symptoms, advanced stage common; associated with HIV.	Classic HRS cells common with paucity of background lymphocytes; pleomorphic HRS cells mimic sarcoma.	CD15, CD30, CD45- EBV pos in HIV- affected patients.	Associated with advanced stage.
Lymphocyte-rich classical.	M > F, older age; peripheral lymphadenopathy; B symptoms rare; most patients with stage 1 or II disease.	Scattered classic HRS cells among numerous small lymphocytes; nodular growth pattern.	CD15, CD30; Oct2 and BOB.1 vary; J-chain absent; EBV in 40%-75%.	Good, similar to NLPHL.

Disease	Macroscopic urinalysis	Microscopic urinalysis
Acute glomerulonephritis	Gross hematuria "smoky" turbidity Proteinuria.	Erythrocyte and blood casts Epithelial casts Hyaline and granular casts Neutrophils Erythrocytes.
Chronic glomerulonephritis	Hematuria Proteinuria	Granular and waxy casts Occasional blood casts Lipid dorplets.
Acute pyelonephritis	Turbid Occasional odor Occasional proteinuria.	Numerous neutrophils (many in clumps) Few lymphocytes and histiocytes Leukocyte casts Renal epithelial cells Epithelial casts Granular and waxy casts Bacteria.
Chronic pyelonephritis	Occasional proteinuria	Leukocytes Broad waxy casts Granular and epithelial cells Occasional leukocyte cysts Bacteria Erythrocytes.
Nephrotic syndrome	Proteinuria	Fatty and waxy casts. Cellular and granular casts. Oval fat bodies and/or vacuolated renal epithelial cells occurring single or as cellular cluters.
Acute tubular necrosis	Hematuria Occasional proteinuria.	Necrotic or degenerate renal epithelial cells. Neutrophils and erythrocytes. Granular and epithelial casts. Waxy casts. Board casts. Epithelial tissue fragment.
Cystitis	Hematuria	Numerous leukocytes. Erythrocytes. Transitional epithelial cells occurring singly or as fragments. Histiocytes and giant cells. Bacteria. Absence of casts.
Dysuria-pyuria syndrome.	Slightly turbid	Numerous leukocytes, bacteria. Erythrocytes. No casts.
Acute renal allograft rejection (lower nephrosis).	Hematuria Occasional proteinuria.	Renal epithelial cells. Lymphocytes and plasma cells. Neutrophils. Renal epithelial casts. Renal epithelial fragments. Granular, bloddy, and waxy casts.

Urinary tract neoplasia	Hematuria	Atypical mononuclear cells with enlarged irregular hyperchromatic nuclei and sometimes containing.
Diseases	Macroscopic urinalysis	Microscopic urinalysis. Neutrophils. Erythrocytes. Transitional epithelial cells.
Viral infection	Hematuria Occasional proteinuria.	Enlarged mononuclear cells and/or multinucleated cells with prominent intranuclear and/or cytoplasmic inclusions. Neutrophils. Lymphocytes and plasma cells. Erythrocytes.

Urine and Fecal Findings in Jaundice				
Finding	Normal	Obstruction to bile flow.	Hemolysis, hemolytic anemia.	Liver damage, hepatitis, cholestasis.
Urinary bilirubin	Absent	Increased, dark urine.	Absent	Increased early.
Urinary urobilinogen	Present	Neoplasm–low or absent; gallstones–variable.	Increased	Decreased early; increased late.
Fecal color	Dark	Pale; intermittent with gallstones in common bile duct; persistent with neoplasm in duct or pancreas.	Dark	Pale early and dark late in hepatitis; pale with cholestasis.

EBV Associations

Mononucleosis-type illness.

Hodgkin lymphoma: MC > LR > NS; LD seen in HIV patients.

Immunodeficiency-associated lymphoproliferative disorders.

Posttransplant lymphoproliferative disorders.

Some DLBCL cases (associated w/inflammations, in elderly).

Lymphomatoid granulosis.

Primary effusion lymphoma.

EBV+ lymphoproliferative disorders of childhood.

Burkitt lymphoma (endemic and HIV associated).

Nasal NK-T cell lymphoma.

Angioimmunoblastic lymphoma.

Plasmablastic lymphoma.

Guidelines for Neonatal Transfusion

RBC Transfusion:

Hct <20% with symptomatic anemia.

Hct <30% with supplemental O2 <35% or mechanical ventilation with MAP <6 cm H2O.

Hct <35% with supplemental O2 >35% or mechanical ventilation with MAP >6 cm H2O.

Hct <45% with cyanotic congenital heart disease or extracorporeal oxygenation.

Plasma Transfusion:

Coagulation factor deficiency, factor concentrate unavailable.
Disseminated intravascular coagulation (DIC).

Platelet Transfusion:

Platelet count <30,000/μL in term infant with platelet production failure Platelet count <50,000/μL in stable premature infant Platelet count <100,000/μL in unstable premature infant.

Red Cell Transfusion Guidelines
Symptomatic anemia in a euvolemic patient Acute blood loss of >15% of estimated blood volume. Preoperative Hb <9.0 g/dL with expected blood loss >500 mL Hb <7.0 g/dL in a critically ill patient. Hb <8.0 g/dL in a patient with an acute coronary syndrome. Hb <10.0 g/dL with uremic or thrombocytopenic bleeding. **Sickle cell disease:** Acute sequestration: Hb <5.0 g/dL or decrease of 20% from baseline. Acute chest syndrome: Target Hb = 10 g/dL, HbS.

fraction <30% Stroke prophylaxis: Target HbS fraction <30%
General anesthesia: Target Hb = 10.0 g/dL, HbS fraction <60%

Cryoprecipitate Transfusion Guidelines
• Factor VIII deficiency, factor concentrate unavailable von Willebrand disease, factor concentrate unavailable. • Hypofibrinogenemia. • Factor XIII deficiency. • Uremic bleeding (DDAVP preferred). • Topical fibrin sealant (commercial product preferred).

Salient features of Germ cell and sex cord stromal tumors of ovary:

Neoplasm	Peak Incidence	Usual Location	Morphologic Features	Behavior
Germ Cell Origin				
Dysgerminoma	Second to third decade of life Occur with gonadal dysgenesis.	Unilateral in 80%-90%	Counterpart of testicular seminoma sheets or cords of large clear cells Stroma may contain lymphocytes and occasional granulomas.	All malignant but only one-third metastasize; all radiosensitive; 80% cure rate.
Choriocarcinoma	First 3 decades of life	Unilateral	Identical to placental tumor Two types of epithelial cells: cytotrophoblast and syncytiotrophoblast.	Metastasizes early and widely Primary focus may degenerate, leaving only metastases Resistant to chemotherapy.
Sex Cord Tumors				
Granulosa-theca cell	Most postmenopausal, but may occur at any age.	Unilateral	Composed of mixture of cuboidal granulosa cells and spindled or plump lipid-laden theca cells Granulosa elements may recapitulate ovarian follicle as Cell-Exner bodies.	May elaborate large amounts of estrogen Granulosa element may be malignant (5%-25%).

Thecoma-fibroma.	Any age	Unilateral	Yellow (lipid-laden) plump thecal cell.	Most hormonally inactive About 40% produce ascites and hydrothorax (Meigs syndrome) Rarely malignate.
Sertoli-Leydig cell	All ages	Unilateral	Recapitulates development of testis with tubules or cords and plump pink Sertoli cells.	Many masculinizing of defeminizing Rarely malignant.
Metastases to Ovary				
	Older ages	Mostly bilateral	Anaplastic tumor cells, cords, glands, dispersed through fibrous background Cells may be "signet ring" mucin-secreting.	Primaries are gastrointestinal tract (Krukenberg tumors), breast, and lung.

Factors associated with risk of invasive carcinoma in breast:

Factor	Relative Riska	Absolute Lifetime Riska
Women with no risk factors	1.0	3%
First-degree relatives(s) with breast cancerb	1.2-9.0	4%-30%
Germline tumor suppressor gene mutation (e.g. BRCA / mutation)	2.0-45.0	6% to >90%
Menstrual History		
Age at menarche <12 years	1.3	4%
Age at menopause >55 years	1.5-2.0	5%-6%
Pregnancy		
First live birth <20 years (protective)	0.5	1.6%
First live birth 20-35 years	1.5-2.0	5%-6%
First live birth >35 years	2.0-3.0	6%-10%
Never pregnant (nulliparous)	3.0	10%
Breast-feeding (slightly protective)	0.8	2.6%
Benign Breast ssDisease		
Proliferative disease without atypia	1.5-2.0	5%-6%
Proliferative disease with atypia (ALH and ADH)	4.0-5.0	13%-17%

Carcinoma in situ (ductal or lobular)	8.0-10.0	25%-30%
Lonizing radiation	1.1-1.4	3.6%-4.6%
Mammographic density	3.0-7.0	10%-23%
Postmenopausal obesity and weight gain	1.1-3.0	3.6%-10%
Postmenopausal hormone replacement	1.1-3.0	3.6%-10%
Alcohol consumption	1.1-1.4	3.6%-4.6%

Summary of the major biological types of breast cancer:

Feature	ER Positive / HER2 Negative	HER2 Positive (ER Positive or Nagative)	Triple Negative (ER, PR, and HER2 Negative)
Overall frequency	50%-65%	20%	15%
Typical patient groups.	Older women; men; cancers detected by screening; germline BRCA2 mutation carries.	Young women; germline TP53 mutation carries.	Young women; germline BRCA / mutation carries.
Ethnicity			
European/American	70%	18%	12%
African/American	52%	22%	26%
Hispanic	60%	24%	16%
Asian/ Pacific Islander	63%	26%	11%
Grade	Mainly grade 1 and 2	Mainly grade 2 and 3	Mainly grade 3
Complete response to chemotherapy	Lower grade (<10%), higher grade (10%)	ER positive (15%), ER negative (>30%)	30%
Timing of relapse	May be late (>10 years after diagnosis)	Usually short (<10 years after diagnosis)	Usually short (<8 years after diagnosis
Metastatic sites	Bone (70%), viscera (25%), brain (<10%)	Bone (70%), viscera (45%), brain (30%)	Bone (40%), viscera (35%), brain (25%)
Similar group defined by Mrna profiling	Luminal A (low grade), luminal B (high grade).	Luminal B (ER positive), HER2-enriched (ER negative).	Basal-like.
Common special histologic types.	Lobular, tubular, mucinous, papillary.	Apocrine, micropapilary.	Carcinoma with medullary features.
Common somatic mutations	PIK3CA (40%), TP53 (26%)	TP53 (75%), PIK3CA (40%)	TP53 (85%)

Targetted treatment of breast cancer :

Target	Treatment	Assay	Comments
ER	Estrogen deprivation (oophorectomy. Aromatase inhibitors) Blockage of ER (tamoxifen).	IHC for nuclear ER.	Effective cytostatic (but not cytotoxic) therapy for ER-positive cancer.
HER2	Antibodies to HER2 Cytotoxic therapy linked to HER2 antibody Tyrosine kinase inhibitors.	IHC for membrane HER2 ISH for HER2 gene amplification.	Effective for HER2-positive cancers.
Susceptibility to DNA damage resulting from BRAC I and BRAC2 mutations that cause defect in HRR.	Chemotherapy with agents causing DNA damage that requires HRR (e.g. platinum agents) Inhibition of alternative DNA repair pathway (poly-ADS ribose polymerase or PARA inhibitors).	Sequencing of BRCAI and BRCA2.	May be effective for carcinomas arising in patients with germline BRCA / or 2 mustations or cancers with somatic loss of BRCA function.
P13K/AKT pathway	Inhibitions or proteins in the pathway .	Activating mutations or pathway activation-not yet validated .	>80% of breast cancers have alternations in this pathway Effectivencess or treatment not yet demonstrated.
Immune checkpoint proteins.	Blocking antibodies to PD-LI, PD-I, and other immune checkpoint proteins.	IHC for immune checkpoint proteins-not yet validated.	Under investigation in patients with triple-negative breast cancer.

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