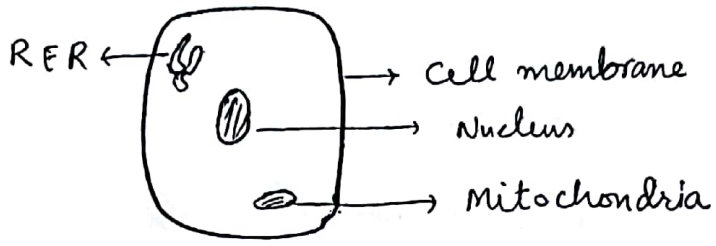
Cell Injury

4 parts of a cell which are vulnerable to injury.



Causes of cell injury

1) Hypoxia (MC)*
   (a) Ischemia* — Atherosclerosis, Thromboembolism, vasospasm
   (b) Anemia
   (c) Cardiopulmonary failure.
   (d) CO poisoning
2) Infections
3) Genetic disorders
4) Hypersensitivity reactions
5) Autoimmune diseases
6) Physical agents - heat, cold, trauma, radiation
7) Chemical agents
8) Nutritional imbalances — Deficiencies (Vit, PEM); Excess (Vit ADEK, Fats)

Outcomes of cell injury

1) Irreversible cell injury / cell death

Necrosis — Necroptosis
Apoptosis — Pyroptosis.

2) Reversible cell injury

Hydrophic degeneration (cloudy swelling)
Fatty denaturation

3) Cellular adaptation

Hypertrophy — Atrophy
Hyperplasia — Metaplasia

* Dysplasia is not a cellular adaptation. It is a premalignant condition.

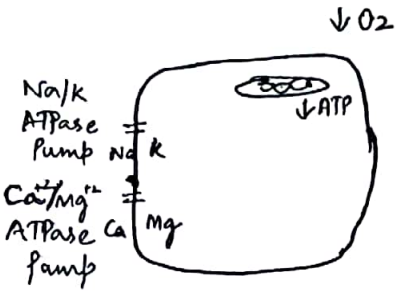
4) Intracellular accumulation & pathological calcification

Proteins
Fats
Glycogen
Pigments - Lipofuscin

Dystrophic cal.
Metastatic cal.

5) Cellular ageing

Mechanism of cell Injury (Reversible)



(a) Pump failure due to lack of ATP.

↳ Efflux of K from cell

↳ Na, Ca influx into the cell

↓

↑ Osmotic load (H_2O enters the cell)

↓

Cellular swelling

Hydrophic changes

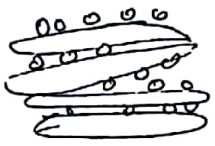
Organelles also swell up.

→ Cell membrane blebs are formed

→ Myelin figures are formed

↳ Due to accumulation of water b/w membrane phospholipids

(b) If ATP is absent - Protein synthesis stops.



↓ ATP

Dispersion of Ribosomes from RER

- Protein synthesis stops
- ↑ lipid synthesis & storage

⇓

Fatty changes

Switch over

(c) to Anaerobic glycolysis

Glycogen in cytoplasm is consumed

Lactic acid is produced

⇓

↓ pH of the cell

Nucleus

↓ pH

Clumping of Nuclear chromatin

Mechanism of Irreversible cell injury

1) Severe mitochondrial damage

Important feature of irreversible cell injury:-

Ⓘ ⇒ NO ATP production by mitochondria

Ⓘ ⇒ Large amorphous flocculant densities in mitochondrial matrix

Composed of
- Calcium
- Phospholipids

2) Severe membrane damage

↑ permeability of plasma membrane

- leakage of important intracellular enzymes & proteins.
- Influx of Ca^{++} into the cell.

3) Release of lysosomal enzymes

↓pH
↑↑ Ca^{++} in cytosol
→ Lysosome receptor irritation
→ Acid hydrolase are released

- Phospholipases → Break membrane phospholipids
- Proteases → Break enzymatic & cytoskeletal proteins
- RNAses → Break RNA
- DNAses → Break the DNA in Nucleus

DNAses

↓

Cause random breaks in the DNA

↓

Extract this DNA and do an agaros gel electrophoresis

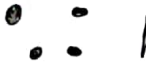
↓

Smear pattern

Nuclear Changes

Pyknosis (condensation of chromatin)

↓



Karyohexis (fragmentation of nucleus)

↓

Karyolysis (dissolution of nucleus)

H&E

↓ Stains Nucleus

↓ Stains cytoplasm & extracellular connective tissue

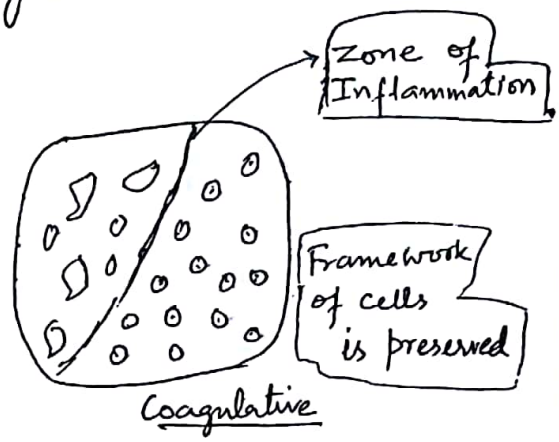
Necrosis

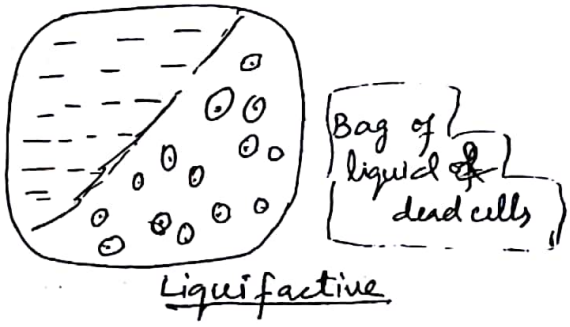
Def. cell death in living tissue

2 Processes that underlie necrosis are (Mech.)

(a) Enzymatic digestion of cells by lysosomal enzymes.

(b) Denaturation of proteins





(1) Coagulative Necrosis

→ Due to denaturation of structural proteins of cells.

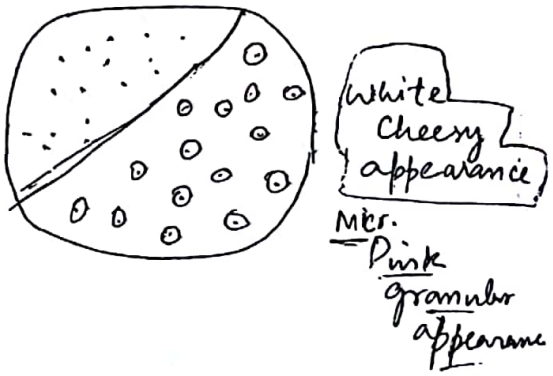
Mc type of Necrosis

a) Mc cause of Coagulative necrosis is Hypoxia except Brain

b) 2nd cause is Severe burns.

c) 3rd Dry gangrene.

d) Zenkers degenerations → Coagulative necrosis of muscle like rectus abdominis seen in pts with severe toxemia e.g typhoid



H/E staining

- Cell outline is preserved but cells do not have a nucleus
- ↑ cytoplasmic eosinophilia → due to denaturation of proteins
  ↘ loss of blue nucleus
- Cytoplasm has a glassy appearance due to loss of glycogen
- Cytoplasm has a "Moth eaten" appearance due to loss of organelles.

M/C organ where coagulative necrosis is seen – HEART (MI)

Others – Liver
Kidney
Spleen.

Infarct

⇒ Area of necrosis, usually coagulative produced due to ischemia.

Infarcts are wedge or triangular shaped.

2 types

White Infarct
(also called as pale/anemic Infarct)
Seen in solid organs with single blood supply
e.g. In Heart, kidney, spleen
Liver (in hypovolumic shock)

Red Infarct
(also called Hemorrhagic Infarct)
Seen in spongy organs
Organs with dual blood supply
Lung
Liver
(Red More common)

organs with collaterals
e.g Intestines
Torsion of Testis/ovaries.

2) Liquifactive Necrosis (colliquative Necrosis)

Due to enzymatic digestion of cells by lysosomal enzymes.

Dead tissue is converted into a bag of liquid.

e.g brain hypoxia (always Liquifactive Necrosis)

Abscess cavity < Bacterial / Fungal

Wet gangrene.

3) Caseous Necrosis

Dead tissue has a white, cheesy appearance and it is friable.

Miscroscopically – Pink granular appearance

Causes
- M. Tuberculosis (Mycolic acid)
- Syphilis
- Fungal infections – Histoplasmosis, Coccidoidomycosis

4) Fat Necrosis

Enzymatic fat Necrosis

- Seen in pts with acute pancreatitis
- Abdominal cavity
  - ↳ Messentry
  - ↳ Omentum
  - ↳ Retroperitoneal fat

Lipase released from pancreatic acinar cells attack the fat cell membrane

↓

FFA released from fat cell membrane

↓

FFA + Ca

↓

Chalky white deposits of fat necrosis

↓

Sponification of fats

Traumatic fat Necrosis

⇒ Breast

⇒ Subcutaneous tissue

↳ Due to trauma

5) Fibrinoid Necrosis

Areas of fibrinoid necrosis has a homogenous pink appearance due to deposition of fibrin

[H & E Staining]

Causes

(a) Vasculitis e.g. PAN.

(b) Malignant HTN

(c) Peptic ulcers

(d) Aschoff nodules of RHD

Fibrinoid Necrosis in the centre surrounded by Caterpillar cells. L. Pc

(e) AID → SLE → vasculitis

→ R A → Rheumatoid nodules contain fibrinoid necrosis

(5)

Free Radicals

Molecules with unpaired electrons in the ordermost orbit.

$O_2 \longrightarrow O_2^{\cdot -}$ (Superoxide)

$H_2 \longrightarrow H_2O_2 \Rightarrow \boxed{OH^-}$ most Powerful free radicle

$Cl \longrightarrow HOCl^-$

$N \longrightarrow ONOO^-$ (Peroxynitrite)

Causes of free radical generation

1. Normal Oxidation & reduction reaction occuring in the cells
2. Radiation → Hydrolysis → Free radicals
3. Oxygen toxicity.
4. Infections.
5. Drugs and chemicals.
6. Reperfusion injury → Heart / Brain

Free radicals damage cells in 3 ways.

1. Lipid Peroxidation of membranes (Most imp. action)
2. DNA Damage (oxidative)
3. Oxidative damage to proteins in cytoplasm

Two reactions that generate ~~Peroxide~~ Free Radicals

Fenton's Rxn

$$H_2O_2 + Fe^{2+} \longrightarrow \boxed{OH^{\cdot}} + OH^- + Fe^{3+}$$

Haber weiss Rxn

$$O_2^{\cdot-} + H_2O_2 + H^- \longrightarrow O_2 + H_2O + \boxed{OH^{\cdot}}$$

Three enzymes that generate Free Radicals.

1. NADPH oxidases (Pagocytic oxidase)
2. Xanthine oxidase
3. Superoxide dismutase [Generates and also inactivates free Radicals]

Free Radical Removal Mechanisms

1. Antioxidants → Vit A, C, E
   Selinium
   Cystein & glutathione containing compounds.
2. Enzymes
   (a) Superoxide dismutase (SOD)-Inactivates Superoxides
   - Cu Zn SOD (SOD I) [Found in Cytosol]
   - Mn SOD (SOD II) [Found in Mitochondria]

$O_2^{\cdot -} \xrightarrow{SOD} H_2O_2 + H_2O$

Generates H_2O_2.

(b) Catalase — Found in peroxisomes

Inactivates H_2O_2

Peroxisomes are organelles where free radicals are generated and inactivated.

(c) Glutathione peroxidase → Cytoplasm
→ Mitochondria

Inactivates — H_2O_2
— $OH^{\cdot}$

(3) Serum Proteins

Albumin → binds Fe & Cu

Lactoferin → Fe binding

Transferrin → Fe binding

Ceruloplasmin → Cu binding.

∴ ⇒ Free radicals can cause cell death by

- Necrosis
- Apoptosis
- Necroptosis

Brain is protected from Free Radical injury by CuZn SOD (SOD I)

Mutation → Amyotrophic Lateral Sclerosis of Brain

Most important Ion involved in cell injury - Ca^{++}

First ion involved - Na

Most susceptible to ischemic injury - Neurons (3-4 min)
followed by Cardiac tissues (20-40 min)

Tissue least susceptible to ischemic Injury - Fibroblasts
followed by Skeletal muscle

First sign in all types of cell injury except apoptosis - Hydrophic Change

Apoptosis

- (Programmed cell death)
- Active process.
- Death of single cell or small group of cells.
- No surrounding inflammation around apoptotic cells.

e.g:

Physiological

- Embryogenesis
- Involution of hormone dependent organs upon hormonal withdrawal
  - ⇒ Breakdown of endometrium during menstruation
  - ⇒ Ovarial follicular atresia following menopause
- Cell death in rapidly proliferating cells like cells in GIT, Skin, Respiratory tract.
- Death of cells that have served their purpose e.g death of neutrophills at the end of acute inflamation.
- Elimination of harmfull self reactive lymphocytes

Pathological

- DNA damage
- Accumulation of misfolded proteins
- Viral infections — Adenovirus/HIV, Viral hepatitis
  ↓
  Councilman bodies
  (Apoptotic hepatocytes)
- Duct obstruction of organs produces atrophy and apoptosis
  e.g: Salivary gland
  Pancreas
  Kidney (ureter obstruction)

Morphology

2 enzymes that bring about apoptosis are

Caspases

Caspases break the framework of the cell → cell will shrink & tight packing of organelles

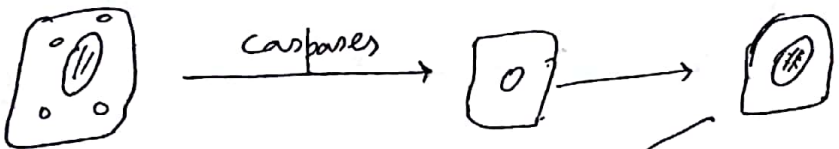
Endonucleases

(Ca, Mg dependent enzymes)

Endonucleases break DNA at specific sites (Internucleosomal Regions)

↓

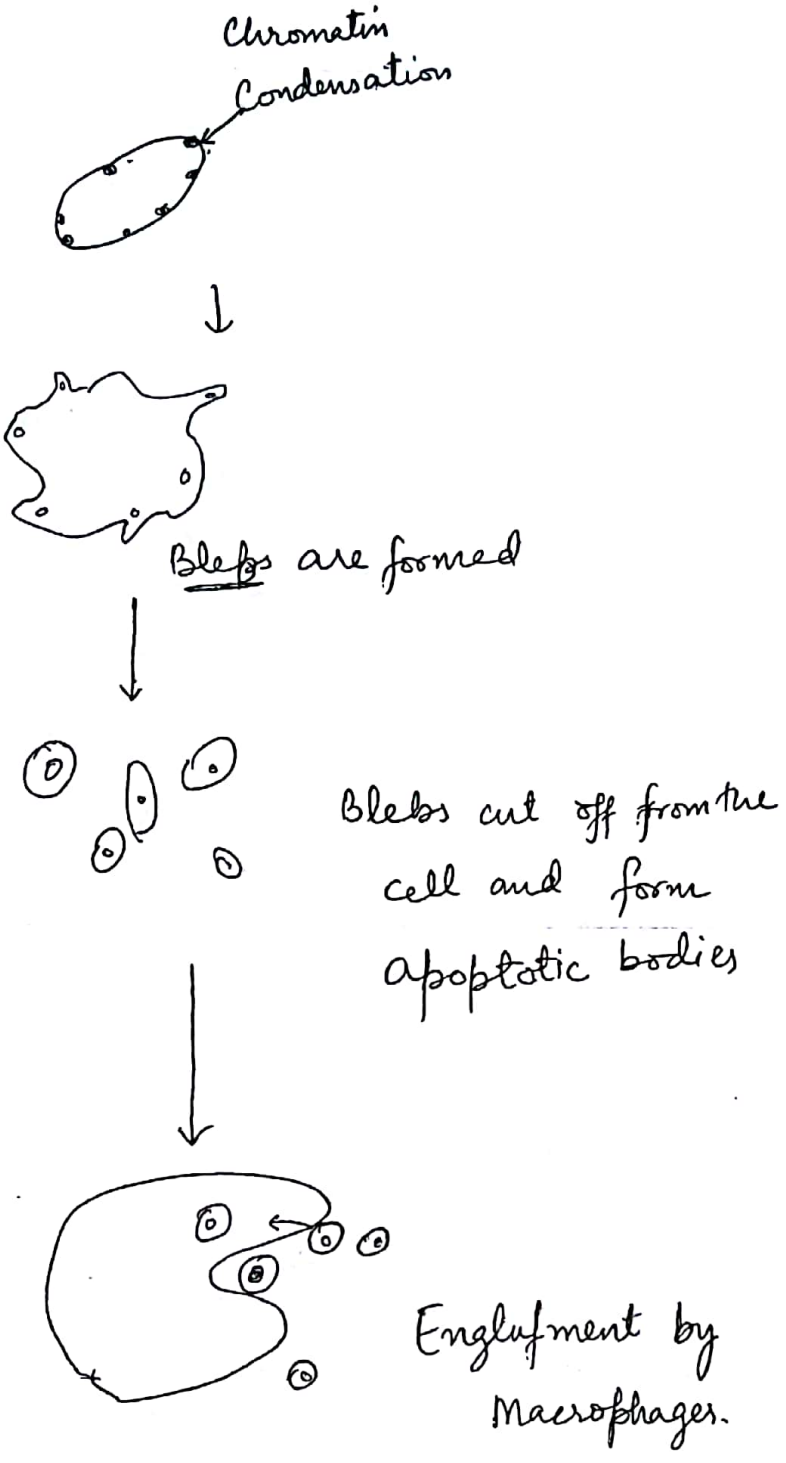
180–200 bp DNA fragments are produced



Chromatin Condensation — most characteristic feature — Pyknosis

Agarose gel ← electrophoroses

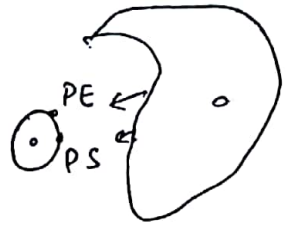
LADDER PATTERN



Apoptotic bodies are removed by Macrophages because.

⇒ Apoptotic bodies express Thrombospondin I on their outer leaflet and macrophages have receptor for Thrombospondin II (absent in normal cells)

- Apoptotic bodies express phosphatidyl serine (PS) and phoshatidyl ethanolamine (PE) on their outer surface & Macrophages have receptors for PS & PE



Annexin V is a marker of cells undergoing apoptosis.

↓

It is an immunostain that stains PS & PE which are expressed on outer leaflet of apoptotic body.

Normal cells have PS & PE on Inner leaflet thus remain unstained by Annexin V

Microscopically

- Shrunken
- Hypereosinophilic
- Chromatin condensation below the nuclear membrane / Pyknosis in nucleus.

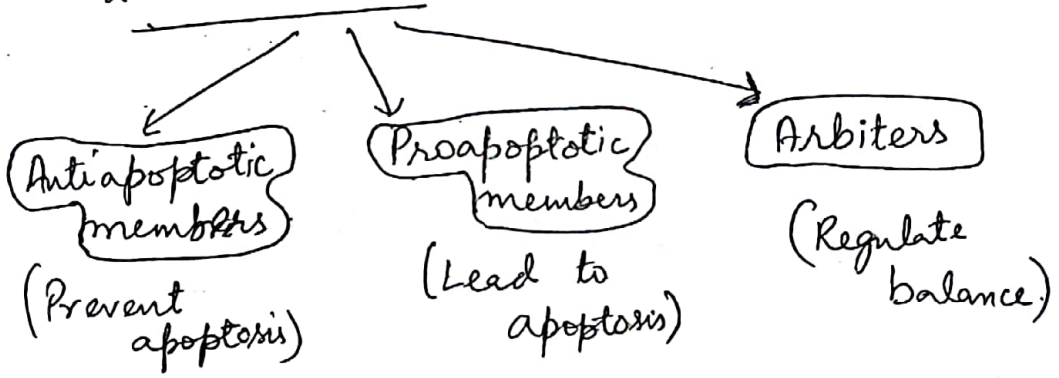
Pathways of Apoptosis

1) Intrinsic / Mitochondrial Pathway
2) Extrinsic / Death receptor initiated Pathway
3) p53 Pathway
4) Perforin granzyme dependent killing.

① Intrinsic / Mitochondrial Pathway

Brought about by BCL2 family of gene.

20 members in the family.



Antiapoptotic members

BCL2 BCL-XL, MCL-1

They reside in the outer mitochondrial membrane, ER membrane & cytosol

↓

Keep the permeability of the membranes Intact

↓

Prevent leakage of Cytochrom C ⇒ No Apoptosis

Proapoptotic members.

BAX BAK

They increase the permeability of outer mitochondrial membrane (drills holes in OMM)

↓

Leakage of cytochrome C from mitochondria into cytosole

↓

which activates Apaf-1 (Apoptosis activating factor-1)

↓

Apoptosome is formed.

↓

Binds and activates Pro Caspase 9.

⇓

Apoptosis

Arbiters of apoptosis

BIM BID BAD PUMA NOXA

Also called as [Sensors of cellular stress] & [BH_3 only proteins]

Function is to regulate balance b/w above two groups

Inhibitors of intrinsic pathway

(IAP → Inhibitor of apoptotic pathway proteins)

↓

Inhibits Procaspase 9

Smac/Diablo – Mitochondrial proteins.

Pro apoptotic

They Inactivate IAP

↓

Thus promoting Apoptosis

Caspases

Initiator Caspases

Caspases 8, 9, 10

Intrinsic → Caspase 9

Extrinsic → Caspase 8, 10

Executioner Caspases

Caspases (3), 6, 7

↘ Most important

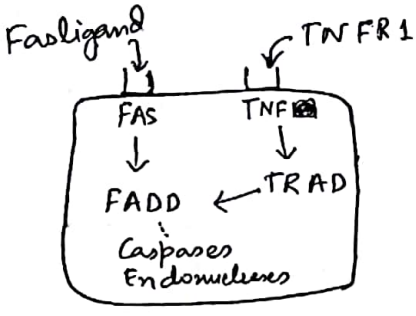
Common for both the pathways

Extrinsic/Death receptor initiated pathway.

Death receptors start this pathway

↳ Belong to TNF receptor family

- FAS (CD95)
- TNFR1



FADD → Fas associated Death domain

TRAD - TNF Receptor 1 Associated Death domain.

Inhibitor of extrinsic pathway

FLIP ⟶ Inhibits procaspase 8

Produced by viruses & normal cells.

p53 Pathway

Tumor suppressor gene

Chromosome 17p 13.1

Called as - (Molecular Policeman)
(Guardian of Genome)
(Critical gate keeper)

It applies Emergency breaks and causes G1 arrest of cells.

Cells with damaged DNA enter the cell cycle

↓

Sensors of DNA damage are activated.
(ATM and RAD family of proteins)

↓

Sensors activate transducers
(CHEK kinase family of proteins are transducers)

↓

p53 activated

↓

p53 recruits p21 (Inhibitor of cyclins & CDKs)

↓

p21 causes G_1 arrest of cells.

⇓

Now p53 assesses DNA Damage

Too much DNA damage

↓

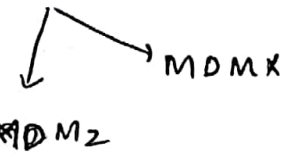
Induces Apoptosis via mitochondrial pathway by BAX.

Little DNA damage

↓

DNA repair gene GADD45 is Recruited & DNA repair done.

$t_{1/2}$ of p53 → 20 minutes



Loss of p53 (both copies) → Li Fraumen's syndrome

↓

⇑ risk of developing carcinomas, Sarcomas, lymphomes, etc.

Gene most commonly mutated in humans carcinoma is p53.

Perforin Granzyme Dependent killing

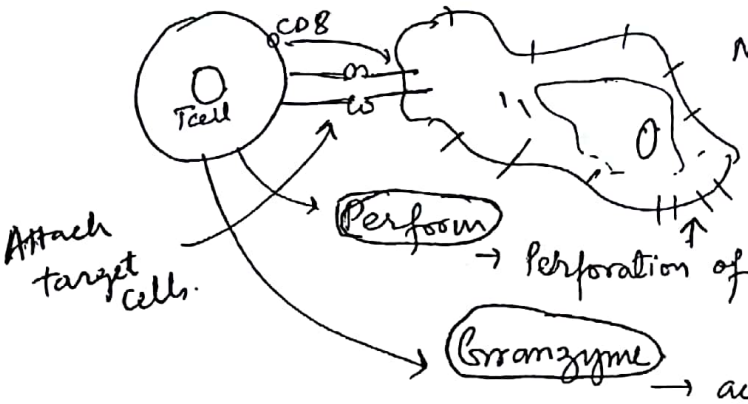
Pathway used by NK cells and Cytotoxic T cells (CD8 cells)

⇓

Induce apoptosis of target cells.

↓

Virally infected cells & Tumor cells.



→ Perforation of cell membrane

→ activates Caspases & endonucleases & leads to apoptosis

Autophagy – cell eats its own contents

Seen in nutrient deprivation.

Membrane of autophagic vacuole is derived from ER

Autophagic vacuole fuses with lysosome

Lysosomal enzymes degrade the contents → used as source of nutrient.

LC3 is a marker of cells undergoing autophagy

(Light chain) Microtuble associated

→ Identifies targets of autophagy

→ Formation of autophagic vacuole.

Differences b/w Necrosis & Apoptosis

Necrosis	Apoptosis
- Passive process	Active process.
- Death of large no. of cells or large parts of the organ.	- Death of single cell or small groups of cells.
- Cells swell up	- Cells shrink.
- Cell membrane permeability is increased	- Cell membrane permeability remains intact.
- Surrounding Inflammation is present	- No surrounding Inflammation is seen.
- DNA is broken by DNAses ↓ Smear pattern	- DNA is broken by endonucleases ↓ Step ladder pattern
↳ Pyknosis ↳ Karyorrhexis ↳ karyolysis	Pyknosis < Chromatin condensation below the clear nuclear membrane.
- Pathological process	- Both physiological & pathological.

Necroptosis

Programmed cell death

Active process.

Also called ~~protosis~~ Programmed Necrosis

Hybrid of Necrosis and apoptosis.

↳ Starts similar to extrinsic pathway

⇓

TNF + TNFR 1

↓

Multiprotein complex called **Necrosome** is formed.

[Necrosome → RIP 1, RIP 3 & Procaspase 8]

RIP

[Receptor Interacting Protein kinase]

~~Procaspase~~

↓

Necrosome (Procaspase 8 is not activated)

↓

Metabolic alteration in cell

↓

↓ ATP

↑ ROS

↓

DNA & protein damage

Membrane damage (by lipid Peroxidase)

↑ membrane Permeability & leakage of contents

⇓

Cell death like Necrosis

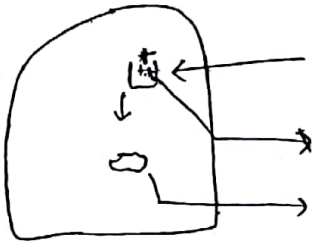
Examples of Necroptosis.

Physiological Occurs during formation of bone growth plate.

Pathological Cell death in Steatohepatitis, acute pancreatitis, reperfusion Injury, & Neurodegenerative diseases e.g. Parkinson's disease.

Pyroptosis

Active process
Programmed cell death.
Also called as pyrogen induced apoptosis.
[accompanied by fever]



Receptors of innate immunity
Microbial products are added
Inflamasome (multiprotein product)
↓
Activates Caspase I (AKA IL 1β converting enzyme)
↓
IL 1
↙ Fever
↘ Inflammation.

⇒ Caspase I & Caspase II induce cell death similar to necrosis

Cellular Adaptations

① Hypertrophy

↑ size of cells → ↑ size of organ
↓
↑ in functional capacity of the organ.

Size of cell increases due to increase in proteins and organelles in the cell.

Cause → ↑ Functional demand
→ ↑ Growth factor / Hormonal stimulation.

Physiological

⇒ Gravid uterus increases in size due to estrogens.
Hypertrophy >> Hyperplasia.

⇒ Skeletal muscles in weight lifters.
⇒ Lactating breast ↑ in size due to Hypertrophy.

Pathological

⇒ Cardiac hypertrophy / LVH due to HTN or aortic valve disease.

Different isoform of myosin in hypertrophic heart

Switch of contractile protein from adult (α myosin) to fetal form (β myosin) which produces slower and energitically economical contractions.

α → β myosin

(2) Hyperplasia

↑ in no. of cells ⟶ ↑ in size of organ
↓
↑ in functional capacity.

Physiological

↙ ⟶

Hormonal Hyperplasia
Seen in breast during puberty & pregnancy

Compensatory Hyperplasia
seen in liver.

Pathological

Due to excessive hormone or GF stimulation.

↙ ⟶

Endometrial Hyperplasia
due to excessive estrogen

BHP
due to androgen.

Pathological hyperplasia can lead to carcinoma.

Endometrial hyperplasia ⟶ Carcinoma
⇒ ± Endometrial adenoca can arise in background of endometrial hyperplasia
⇒ ± Endometrial adenoca can arise in background of endometrial atrophy also.

(3) Atrophy

↓ in size of cells (due to loss of proteins & organelles)

↓

↓ size of organs

↓

↓ in functional capacity of organ.

Causes

↓ work load

↓ Loss of Nerve supply

↓ blood supply

Inadequate Nutrition

Loss of hormonal stimulations

Ageing (Senile atrophy)

Atrophic cells →

- ↓ Protein Synthesis
- ↑ protein degredation by ubiquitin proteosome pathway
- Show autophagy.

Example Brown atrophy of heart.

[Atrophy + Lipofucin accumulation]

(4) **Metaplasia** - One type of adult cell is replaced by another type of adult cell because another one is better suited for the environmental condition.

- Due to stem cell reprogramming.

e.g: (a) Columnar to squamous metaplasia

Seen in smokers & Vitamin A deficient patients (Respiratory Tract)

Protective function of epithelium is lost.

(b) Squamous to columnar metaplasia in Barrett's esophagus in pt of GERD.

(c) Mesenchymal metaplasia → Bone is formed in soft tissue in foci of injury e.g: Myositis ossificans.

Stain used is Mucin

Intracellular accumulations and Pathological Calcification

Pathological Calcification

Dystrophic Calcification

- Seen in dead/damaged tissue.
- Serum Ca. (N).
- Ca metabolism (N)

e.g – dead parasites
- areas of Necrosis
- damaged heart valves (as in RHD)
- Blood vessels — Atherosclerotic plaques
  - Monckeberg's medial Calcific sclerosis: Calcification in media of medium sized arteries in old ages
- Psammoma Bodies – Concentric calcification on necrotic tumor cells.

Metastatic Calcifications

- Seen in normal tissues due to hyper-calcemia
- Serum Ca ↑
- Ca metabolism deranged

Sites
MC – ① Alveolar Septae of lungs
② kidney
③ Gastric mucosa
④ Walls of systemic arteries and pulmonary veins

Causes
① CRF
② Hyperparathyroidism
③ Vit D intoxication
④ Milk alkali syndrome.
⑤ Sarcoidosis
⑥ Paget's diseases Bone disease
⑦ Bone tumor (M.M).

- Calcification starts in mitochondria of all cells except kidney, where it starts in the basement membrane of tubules.

Stains for Calcium → Von Kossa (Black)
→ Alizarine Red S (Red)

- Calcein is another stain for calcium.

Tetracycline labelling index is done to detect bone mineralization.

Intracellular accumulations.

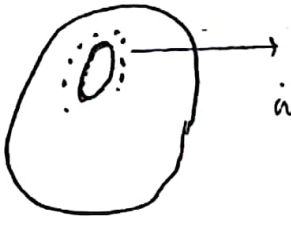
1. Proteins → Russel bodies in Plasma cells in M.M.
   - Intracytoplasmic accumulation of Ig in PC cytoplasm
   → Resorption droplets in PCT cells in nephrotic syndrome

2. Fat → Triglyceride accumulation → Fatty liver.
   → Cholesterol accumulation → Xantomas
   → Atherosclerotic plaques

③ Glycogen → glycogen storage diseases
→ seen in PCT cells of kidney
in Pts ☆ DM → ARMANI EBSTEIN Lesions.

④ Pigments

→ Lipofucin
Lipochrome pigment
Brown in colour
↳ Pigment of ageing
↳ Wear and Tear pigment.
↳ Sign of Free radical injury to cells.



→ accumulates in perinuclear in the lysosomes in the cytoplasm.

→ Hemosiderin
Iron containing pigment
⇒ Stain – Perl's prussian Blue reaction
H & E – golden brown in colour
Accumulation in conditions of Iron overload.
e.g Severe hemolytic anemia (Thal major)
Areas of hematoma
Hemochromatosis

Cellular Ageing

Telomeres

Ends of chromosomes by which chromosomes are attached to nuclear membrane.

Also called as Biological clocks because with each cell division there is telomere shortening And when telomers are shortened beyond a critical limit → Cellular Senescence (terminal non dividing state of cell)

Telomerase

Maintains the length of telomeres.

Not found in somatic cells

Found in
- Stem cells
- Germ cells
- Embryonic cells
- Cancer cells.

Telomere lengthening → Carcinogenesis

90% human cancers are +ve for telomerase enzyme

Werner's Syndrome → Premature ageing
Def. of enzyme DNA helicase
↓
Needed for DNA unwinding & regain.

Calorie restriction promotes longivity.

Calorie restriction promotes longevity

↑ Sirtulins (SIR)

↓ Signaling through IGF pathway
↓
↓ cell growth &
↓ cell metabolism
↓
↓ cell damage.

Sirtulins

NAD dependent protein deacytylases.

Distributed in different components of cell

7 different types known (SIR 7)

⇒ Prevent free radical damage
↓ Apoptosis
Stimulate protein folding
Inhibit cell metabolism
} Promotes Longivity

Associated with ← Ageing
DM
Cancer.

Sirtulins & Lipofucin

Tissue processing

[Paraffin Embedding] (10-12 hrs)

Steps

① **Fixation**

MC fixator used is 10 % Neutral buffer formalin

② **Dehydration**

Putting tissue in ↑ grades of ethyl alcohol.

(30 % Ethanol → 50% → 70% → absolute alcohol)

③ **Clearing**

- Makes the tissue optically clear.
- Clearing agent is miscible with paraffin wax.

MC (Xylene)

④ **Embedding**

Molten paraffin wax enters the tissue and it hardens the tissue

⑤ **Sectioning**

Cut 4-6 microns thick sections and place them on slides → Stain ē H & E.

Fixatives

(1) M/c used fixature for HPE – 10% neutral buffered formaline.

(2) EM → Fixative is 2.5% glutaraldehyde
↓
Followed by post fixation in Osmium Tetraoxide
↓
Ultra thin sections are cut (1-2 micron)

Stain → Uranyl acetate
& observed in EM.

(3) PAP smear fixed in 95% ethanol (Absolute alcohol)

(4) IF examination → fixed in Normal Saline
↓
Frozen section is cut
↓
Section placed on Slide
& stained by IF stains &
↓
observed in UV light.

⑤ Fixatives for Peripheral Smear and FNAC.

↳ Methanol.

Uses of Frozen Section

Also called as (Cryosection) or (Intraoperative biopsy)

Tissue is frozen at -20° to -30°C in a cryostat.

Frozen section ① ⇒ can descriminate benign from malignant

② ⇒ Can analyse resection margins.

③ ⇒ Can detect metastatic deposits in sentinal nodes.

④ ⇒ Used for demonstration of fat in tissue.

⑤ ⇒ Immuno fluoresence examination
- Tissue cut by frozen section.

Special Stains

① MC stain used is H & E

② Fat — Oil Red O (Red)
— Sudan Black
— Orcein

③ Glycogen — PAS

Colour - Pink/Rose Pink

⇒ glycogen is PAS +ve and diastase sensitive -

PAS +ve substances

Amyloid ⇒ PAS +ve diastase resistant.

- Glycogen
- Glycolipids
- Glycoproteins
- Mucins
- Colloid
- Amyloid
- Basement membrane
- All fungi
- Russel bodies.

GGG CAMBRA

Used in

Diagnosis of

- Glycogen storage disorder
- Staining of macrophages in Whipple's disease
- Demonstrating fungi
- Mucins in adenocarcinoma of large intestines.
- Seminoma, rhabdomyosarcoma, Ewig's sarcoma contain glycogen
- Lymphoblasts in AML (Block PAS +ve)

④ **Mucins** (glycosylated proteins – plasma membrane, mucous membrane)

3 Stains → Alcian blue
→ PAS
→ Mucicarmine – Magenta

– Can stain Cryptococcus Capsule also only not the whole fungi, (Red coloured)

& ⑤ **Basement membrane Stains**

– PAS
– Silver Stain (Black colour)

⑥ **Connective tissue Stains**

→ collagen – Massons Trichrome (Blue colour)
→ Reticulin fibre – Silver Stain (Black)
→ Elastic fibers – Verhoff stain

⑦ **Fibrin & Muscle**

PTAH (Phospho Tungstic Acid Hematoxylin)

⑧ **Fat/lipids** (Best) Oil Red O, Sudan black & Orcein

⑨ **Calcium**

Von kossa (Black)
Alzarin Red s (Red)
Calcein

(10) Melanin pigment

Masson Fontana — BLACK (Silver Stain)

Dopa reaction.

(11) Stain for Copper

Rubeanic acid

Rhodamine

Orcein

(12) Hemosiderin pigment

Perl's Prussian Blue reaction

↓

Hemochromatosis

(13) Bile pigment

& Foucret's Technique (green)

(14) Mast cells

Toluidine Blue

Frozen section - Toluidine Blue.

(15) Stains for Microorganism

(a) Mycobacteria Ziehl Neelsen Stain

(b) Lepra Bacilli (Fite Stain).

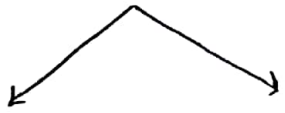
(c) Fungus PAS (Deadly) → Best

GMS (Gomori Methamine Silver) → Live & Dead

(d) Spirochetes Warthin Starry (Silver stain) for H pylori

Inflammation

- It is Response of vascularized connective tissue to injury.
- Protective Injury



Acute | Chronic

Exudate is formed

Exudate	Transudate
- Inflammatory edema	Non Inflammatory edema
- Formed due to increased vascular permeability	- Formed due to increased hydrostatic pressure.
- Rich in proteins & cells	- Poor in proteins & cells.
- Sp. gravity > 1.020	- Sp. gravity < 1.012
- LDH ↑	- LDH ↓

Acute

1. Sudden onset
2. Lasts for a short duration
3. 2 characterstic features
   - extravassation of neutrophils
   - exudate formation
4. Local signs & symptoms are prominent.
5. Usually self limiting
6. Tissue injury is mild.

Chronic

1. Insideous onset.
2. Lasts for long duration (weeks - months - years)
3. 2 characteristic features
   - infiltration of tissue by mononuclear cells
   - Tissue destruction.
4. Local signs & symptoms are not prominent.
5. Progressive
6. Severe & may lead to fibrosis.

Acute Inflammation

- Events of acute inflammation
- Mediators of acute inflammation.

Events of acute inflammation.

- Seen in post capillary venules
  - → Vascular events
  - → Cellular events.

(I) Vascular events

(1) Transient vasoconstriction

↓

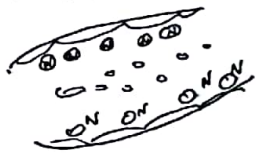
(2) Massive vasodilation

↓

(3) Increased vasular permeability. → exudate formation (Most imp)

↓

(4) Stasis of cells in Blood vessels.



↓

(5) Leucocyte margination to the periphery.

(II) Cellular events

- Phagocytosis
- Chemotaxis
- Adhesion & transmigration

Mechanisms of ↑ vascular permeability

(1) Endothelial contraction (M/c mech)

(occurs in post capillary venules)

Mediators → Histamine
→ Bradykinin
→ Substance P
→ Leukotriene

⇒ Immediate Transient response

② Endothelial retraction / Junctional reorganisation

(venules & capillaries)

Delayed type of contraction

Mediators (cytokines) → IL-1
→ TNF α

⇒ ② Delayed, sustained response

③ Direct Injury

(Seen in venules, arterioles, capillaries)

Severe Injury

e.g Severe burns
Chemicals
Toxins

↓

Cells undergo necrosis & detach.

↓

Fluid leakage which starts immediately

↓

Leakage continues till a new cell regenerates

⇒ ① Immediate sustained response

Mild Injury

e.g mild sunburn.

↓

endothelial cells die after a few hours due to apoptosis.

↓

Fluid leakage starts after few hours

↓

Leakage continues till a new cell regenerates

⇒ ① Delayed sustained response

Transplant Rejection

For a transplant HLA matching is done b/w donor and receipient.

HLA antigens that should be matched are.

- HLA DR
- HLA B
- HLA A

HLA matching is not done for liver, heart, lungs, & cornea because other factors like

- anatomical combatibility
- Severity of underlying disease
- Need to minimize the time of organ storage

Immunosuppressive therapy

1. Steroid - Reduce Inflammation
2. Mycophenolate mofetil - Inhibits lymphocyte proliferation
3. Tacrolimus (FK 506) - Inhibits Phosphatase Calcineurin which is required for activation of NFAT (Nuclear Factor of Activated T cells)

NFAT not activated
↓
No IL 2
↓
T cell inhibition

(4) T & B cell depleting antibodies

(5) Pooled IV immunoglobulins.

Complication of organ transplant.

(1) Infection (MC)

⇒ (CMV) most common infection
Cells show owl eye intranuclear inclusions.

⇒ Polyoma BK viral infection
Decoy cells – PCT cells & intra-nuclear basophilic inclusion.

(2) Transplant rejection

(3) GVHD

(4) ↑ risk of malignancies
- → HPV associated SCC skin
- → EBV ass. lymphomas
- → HHV8 ass. Kaposi's Sarcoma.

Transplant Rejection

Solid organ transplant

Recipient is immunocompetent.

3 types of Rejection.

① Hyperacute rejection
occurs within minutes (< 48 hours)

It is due to preformed antibodies in the recipient.

↳ seen in
- Multiparous women
- Multitransfused individuals
- Past H/o transplant rejection.

* ABO & RH incompatibility can also cause hyperacute rejection.

Gross

Slightly swollen + mottled + cyanosed

kidney — Anuric
Filters few drops of blood urine or no urine at all.

M/E

① Neutrophilic infiltration in glomerular capilaries, arteriales & peritubular capillaries

② Fibrinoid necrosis & thrombosis in the vessels

③ Thrombosis leads to cortical necrosis

Type II Hypersensitivity Reaction.

Acute Rejection

Occurs from weeks – months (< 6 months)
or
later when immunosuppressive therapy is withdrawn.

2 types

Acute cellular Rejection

Brought about by CD4 Tcell / CD8 Tcell

Type IV HR.

Responds to increasing the dose of immunosupressive therapy.

Acute Humoral Rejection

Brought about by antibodies that are produced after transplantation.

Type II > Type III HR.

Doesnot respond to increasing the dose of immuno-supressive therapy but responds to B cell depleting agents.

Biopsy

↓

Microscopical Ex

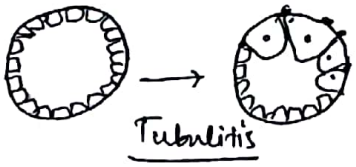
Three types are seen.

Type I / Tubulointerstiteal pattern

- Tubulitis
- Interstitial mononuclear cells Infiltration

(Tcell/macrophage)

M/C vasculitis ↓ Damage to glomeruli & small vessels



Thrombi in small vessels

Fibrinoid Necrosis in vessels

Type II vascular pattern
Endothelitis

Type III vascular pattern
Endothelitis
+
Necrosis in the vessel wall.

& IHC → C4d deposited in vessels
- glomerular Capillaries
- Peritubular Capillaries.

[in immunoperoxidase Stain]

Chronic Rejection

(Months – Years)

Can be due to antibodies or Tcells

More common – Type IV HR.

M/E - ① Obliterative intimal fibrosis of BV.
② Atherosclerosis of graft vessels
③ Glomerulosclerosis
④ Tubular atrophy
⑤ Interstitial fibrosis

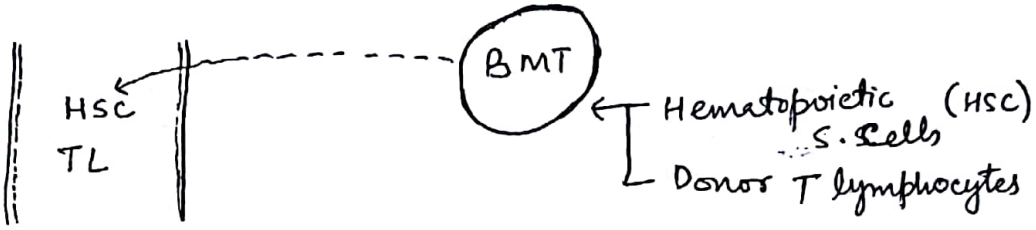
→ Transplant glomerulopathy.

GVHD

Graft versus host disease

It is a complication of Bone Marrow Transplant (BMT)

Type IV HR.



Recipient is immunocompromised

- Due to primary disease for which Transplant is indicated
- Due to Radiotherapy or chemotherapy that is given to form transplant bed.

GVHD

Acute (<100 days)	Chronic (>100 days)
① Skin (Mc) Rashes & ulcers	① Skin Sclerodema like fibrosis
② GIT Oesophageal & Intestinal ulcers Bloody diarrhea Malabsorption	② Strictures Malabsorption.

③ Liver

Cholestatic jaundice — Destruction of the ducts

Inflammation of bile ducts — Cholestatic jaundice

④ Destruction of immune system of the recipient

e.g; LN, Thymus, spleen, etc.

T cell depleted B.M.T.

- No GVHD
- Increased incidence of graft failure
- Relapse of primary disease eg. Acute leukemia.

Also called as graft versus leukemia effect.

- ↑ incidence of EBV. related leukemias & lymphomas.

Neoplasia

- New growth
- Neoplasm - Tumor

Benign

Suffix "oma"

Epithelial origin → Adenoma
Epithelial origin → Papilloma

Mesenchymal origin ⇒
- Lipoma
- Fibroma
- Leiomyoma
- Rhabdomyoma
- Osteoma
- Chondroma

Malignant

Suffix carcinoma/sarcoma

Epithelial origin → Carcinoma
e.g Adenocarcinoma
SCC
Transitional Ca
BCC

Mesenchymal origin → Sarcoma
e.g Liposarcoma
Fibrosarcoma
Leiomyosarcoma
Rhabdomyosarcoma
Osteosarcoma
Chondrosarcoma.

Malignant tumors ~~c~~ Suffix 'oma'.

1. Hepatoma (HCC)
2. Seminoma
3. Melanoma (melanocytes)
4. Chordoma (Malignant tumor of Notocord)

Teratoma

Tumor of Totipotent cells

(a) Mature cystic teratoma [Benign tumor]

- Elements of all three germ layers
- All components are mature.

e.g.; Skin adnexal structures, bone, cartilage, blood vessels, tooth, fat, thyroid tissue

ovary → Dermoid cyst.

(b) Immature Teratoma [Malignant tumor]

Elements are immature or fetal type

e.g. immature cartilage or bone

immature Neural tissue is important

↳ for grading.

(c) Teratoma with malignant transformation

Malignat tumor

One of the component has become malignant.

Pleomorphic adenoma

- Benign tumor
- Salivary glands (M/C)
  (Parotid MC & Breast)

Epithelial component
- glands
- Sq. epithelium

Mesenchymal component
- Cartilage
- Fibrous tissue
- Bone
- Muscle

Cells of origin → Myoepithelial cell.

℞ Wide excision

As Tongues of tumor tissue are seen in the surrounding tissue.

NOT TRUE NEOPLASMS

Choristoma

- Ectopic rest of Normal tissue
- Normal tissue at abnormal location

e.g glial tissue in Nasopharynx
Adrenal rests in the kidney

Hamartoma

Normal tissue in normal location but in a disorganised arrangement.

e.g bronchial hamartoma.
Hemangioma
Lymphangioma.

Benign	Malignant
Well differentiated (Resemble the tissue from which they rise)	Poorly differentiated or well - moderate differentiated or Anaplastic ↓ (Complete lack of differentiation)
Slow growing & may regress on their own	Rapidly growing, have erratic growth.
Usually Encapsulated. Un encapsulated are as - Leiomyoma Hemangioma Lymphangioma	Malignant tumors that show spontaneous regression - R C C - Neuroblastoma - Retinoblastoma - Malignat Melanoma - Chorio Carcinoma. Uncapsulated or may show pseudo capsule (formed by compressed normal tissue)
No local invasion	Local invasion +
No metastasis	Metastasis +

Metastasis is most imp. feature that differentiate ~~that~~ bening from malignant

Malignant tumors that show local invasion but no metastasis ⇒ BCC
Glioma

Features of Malignant cells

Large cells
High N:C ratio
Pleomorphism - variation in shape & size
Hyperchromatic nucleus
Abnormal mitosis

Dysplasia

Premalignant condition e.g. cervical dysplasia

Loss of polarity
Pleomorphism
High N:C Ratio
Abnormal mitosis
Hyperchromatic Nucleus

They are confined in the basement membrane

Cervical dysplasia

CIN I - Abnormal cells confined to 1/3rd of Cx

CIN II - occupy 2/3rd Cx epithelium

CIN III - occupy full thickness of cervical epithelium } BM intact.

Pathways for Spread.

(1) Lymphatic Spread

Preferred mode of spread for a Carcinoma

LM drainage of Ca. → Metastasis
→ Reactive hyperplasia

(2) Hematogenous spread

Preffered mode of spread of a Sarcoma

3 carcinomas first spread by Hemato-genous route:
- RCC
- HCC
- Chorio carcinoma

M/c Spread to - lungs > Liver

Venous invasion is more common than Arterial invasion

(3) Direct Spread

(a) Mesothelioma (MC) arises from mesothelial cells of pleura or peritoneum & spreads on the surface of pleura or peritoneum.

(6) Pseudomyxoma peritonei

Seen in

Mucinous adenoCa Appendix

Mucinous adenoCa ovary

Mucinous adenoCa Colon

Mucin produced by tumor cells

↓

Causes adhesions b/w various organs in the peritoneal cavity.

Tumors that spread via CSF

Medulloblastoma

[Small round cell tumor]

[Childhood tumor]

Site - Posterior fossa (cerebellum)

Extremely radiosensitive

Spreads via CSF & produces DROP METASTASIS in Cauda equina

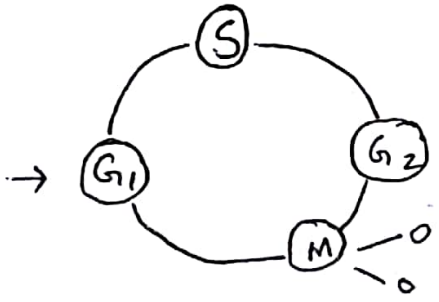
Ependymoma

Arises from ependymal lg lining of ventricles.

Small blue round cell tumors

1. Neuroblastoma
2. Retinoblastoma
3. Medulloblastoma
4. Embryonal rhabdomyosarcoma
5. Lymphoma (Lymphoblastic)

Normal cell cycle regulation.



Orderly progression of cell through cell cycle is brought about by cyclins & cyclin dependent kinases (CDKs) [Proto oncogenes]

Cyclins	CDKs	
Cyclin D	4, 6	G_1 to S transition
Cyclin E	2	G_1 to S transition
Cyclin A	2	G_2 to M transition
Cyclin B	1	G_2 to M transition

G_1 S transition — Cyclin D_CDK4 Most imp.
G_1 S transition — Cyclin E - CDK2
G_2 M t — cyclin A/CDK2
G_2 M t — cyclin B/CDK1

Cyclin A/CDK_2 → Takes the cell through G_2 phase upto prophase of mitosis

Cyclin B/CDK_1 → Regulates all initial events of mitosis beyond prophase.

Inhibitors of cyclins/CDKs

↳ Inactivate cyclins/CDKs and stop the cell in the cell cycle — G_1 arrest, G_2 arrest.

CIP/KIP Family (CDKN1A-D)

- p21 → Induced by p53
- p27 → Induced by TGFβ
- p57

Non specific

INK4 family/(ARF) (CDKN2A-C)

p14, p15, 16, 18, 19

Specifically inhibit cyclin D/CDK

p16 - most imp.

p14 acts by ↑ing p53 by inhibiting MDM_2 activity.

Cell cycle Checkpoint

G_1 S Checkpoint

Sensors → ATM, RAD family

Transducers → CHEK kinase

Effectors → p53 dependent mechanism

G_2 M

Sensors → ATM RAD family

Transducers → CHEK kinase

Effectors → p53 dependent, p53 independent

Rb gene

- Tumor supressor gene - on chromosome 13q14
- Also called as Governer of cell cycle
  or
  Molecular on-off switch of cell cycle.
- Rb causes G_1 arrest of cell cycle.

Rb is located on 13q14

↓

Produces Rb protein

Active form (NP)	Inactive form (P)
- Inhibits cell proliferation	- Allows cell proliferation
- Called under/hypo phosphorylated RB	- Called hyperphosphorylated RB.

⇒ Under phosphorylated RB has pockets in which it hides E2F/DP1 transcription factors - These factors are used in S phase for DNA synthesis.
Thus causes G1 arrest of cell

⇒ Under phosphorylated RB also Recruits enzyme Histone deacetylase causing Compaction of nuclear chromatin thus G1 arrest.

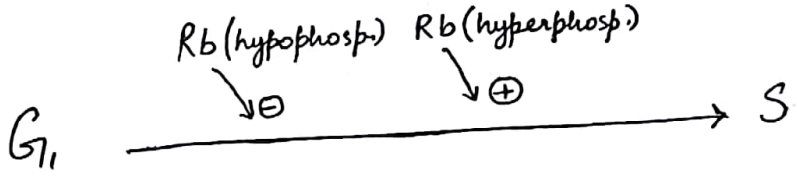
When the cell has to move from G_1 to S phase, a cyclin D/CDK4 phosphorylates the underphosphorylated Rb to hyperphosphorylated Rb

↓

releases E2F/GP1 TF from pockets

↓

Cell will proceed to S phase.



Loss of Rb gene produces
- Retinoblastomas
- Osteogenic sarcomas.

Regulatory genes.

4 classes of regulatory genes.

1. Proto oncogenes
2. Tumor suppressor genes/Anti oncogenes
3. Genes for apoptosis
4. Genes for DNA repair.

1. Protooncogenes → Cause cell proliferation
   Controlled cell proliferation

Protooncogenes can be (GF), (GFR), (STP) (Signal Transduction) (NTP) (Nuclear Transduction Proteins) (cyclins) & (CDKs)

(2) Tumor suppressor gene / Antioncogene

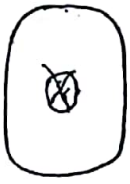
Inhibit cell proliferation by inhibiting

(3) Genes for Apoptosis

- Antiapoptotic: BCL2, BCLXL, MCL1
- Proapoptotic: BAX, BAN
- Arbiters: BIM, BID, BAD, PUMA, NOXA

(4) Gene for DNA repair.

Molecular basis of Cancer



Regulatory gene damage → Undergoes 30 population doublings from a single go transformed cell.

↓

Kinetics of tumor cell growth

1 gm cancer ≈ 10^9 cells is formed

↓ 10 population doublings

1 kg Cancer cells ≈ 10^{12} cells is formed

(Largest Ca mass compatible ≈ life)

Genetic damage lies at the heart of carcinogenesis

Regulatory genes are damaged, tranformed cell enters cell cycle & under goes population doublings leading to cancer.

↓

Tumor cells establish their own blood supply by producing — VEGF, bEGF, PDGF

(Tumor angiogenesis)

↓

Local invasion & distant metastasis

[Phenotypic attributes of Ca cell & are acquired in Step wise fashion]

Mechanism of Metastasis

(1) Metastatic sub clone is formed, which shows decreased expression of E cadherin → loosens up from the main tumor.

(2) Metastatic subclone produces enzymes that degrade BM & extracellular connective tissue

e.g Type IV colagenase
Matrix metalloproteins
Plasminogen activator.

(3) Metastatic subclone shows expression of laminin & fibronectin receptors by which they attach to laminin & fibronectin in the connective tissue.

(4) Some cancers also produce autocrine motility factors which help in ca metastasis.

(5) Cancer cells enter the blood vessels where they attach to

⑥ Tumor emboli gets out of blood vessels at a distant site & produces metastatic deposits.

Epithelial to mesenchymal transition (EMT)

Cancer cells acquire a mesenchymal phenotype (e.g; spindle shape) for metastasis

2 genes for EMT → SNAIL, TWIST } ↓ Ecadherin.

Regulatory genes.

① Proto oncogene (Controlled cell proliferation)

Activated Proto oncogene

→ Translocation
→ Point mutation
→ Amplification
→ Over expression.

POTA

↓

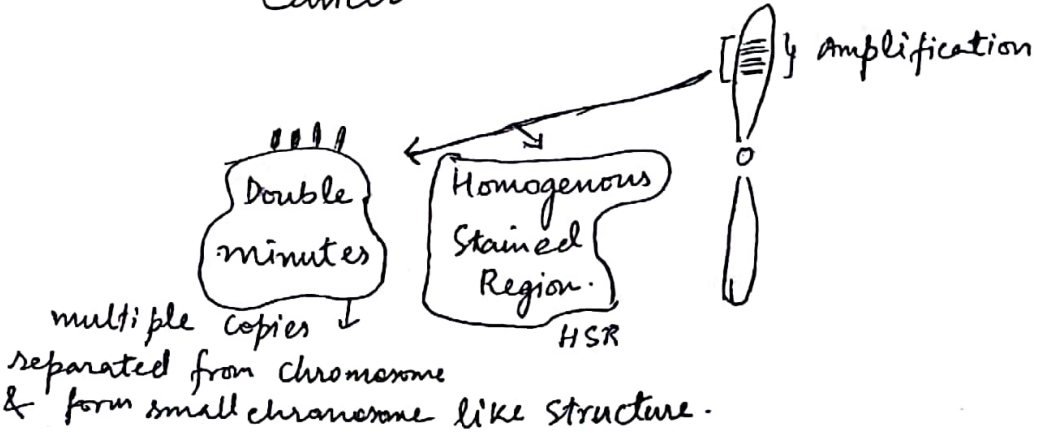
Oncogenes

↓

Produce onco proteins

↓

Cancer.



(I) Growth factors

- SIS (PDGF-B) — Overexpression → Astrocytoma
- HST 1 (FGF) — Overexpression → Osteosarcoma
- INT-2 (FGF3) — Amplification → Bladder Ca, Stomach Ca
- HGF — Overexpression → HCC, Thyroid Ca

(II) GFR (Growth Factor Receptors)

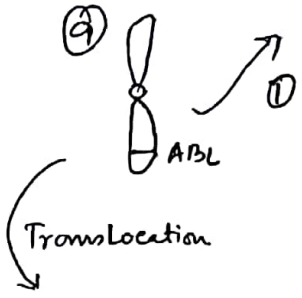
EGFR (Epidermal Growth Factor Receptor)
- ERB-B1 — Overexpression → Adeno Ca Lung
- ERB-B2 (Her-2-neu) — Amplification → Breast Ca, Ovarian Ca

- RET — Point Mutation (Activation) → MEN 2A, MEN 2B, Medullary Ca Thyroid.
- RET — Point Mutation (Inactivation) → ~~Hirschsprung disease~~ HIRSCHSPRUNG Disease [Congenital Megacolon]
- KIT (CD117) — Point Mutation → GIST, Soft tissue tumors
- Alk — Translocation → Adeno ca lung, Lymphoma.

(III) Signal transduction proteins

(a) ABL —t(9:22)→ CML, ALL, AML (Latest)

ABL encodes for a protein with tyrosine kinase activity



Causes signal transduction through Myeloid & lymphoid cells.

→ BCR ABL Fusion gene

↓

Fusion protein ⇒ very high tyrosine kinase activity.

- Myeloid cells → 210 KD size → CML
- Lymphoid cells → 190 KD size → ALL

ⓑ RAS

→ Most common Oncogenic mutations in human Cancers → RAS Mutations.

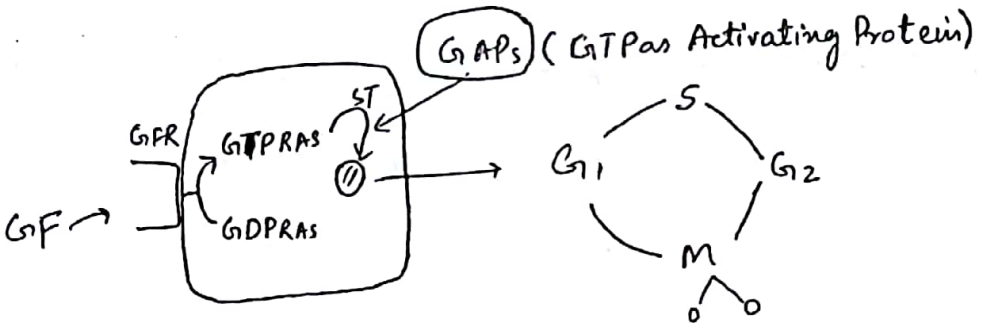
K RAS —PM→ Colon, lung, pancreatic ca

H RAS —PM→ Urinary bladder & kidney tumor.

N RAS —PM→ Melanomas & hematopoietic tumors.

2 forms of RAS protein

Active	Inactive
GTP RAS	GDP RAS
Allows cell proliferation	Prevents cell proliferation



GF + GFR → GDPRAS is converted to GTPRAS

↓

Causes ST

↓

Cell proliferation

After limited cycles

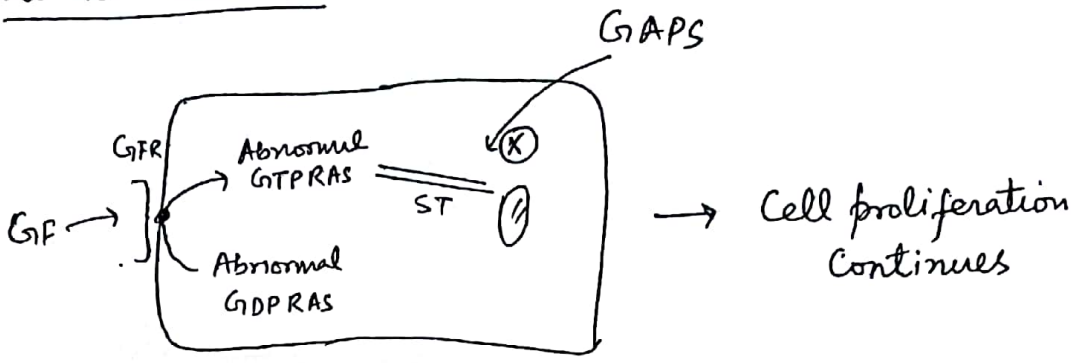
↓

GAPs → Pull the Phosphate group from GTP RAS & Converts in GDP RAS

↓

Stops ST → Stops cell proliferation

Point mutated RAS

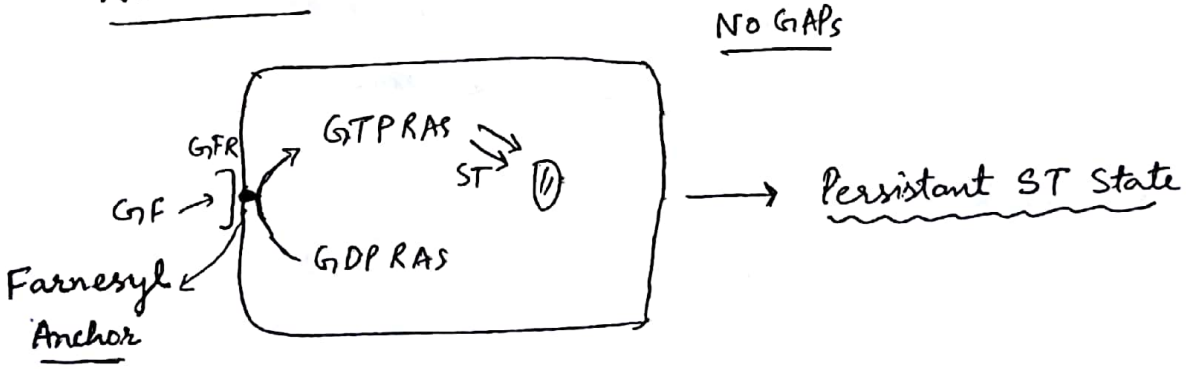


Due to point mutation in RAS, GAPs are unable to pull 'P' group from abnormal GTPRAS.

↓

RAS Remains in persistant Signal Transduction state & produces tumors.

NO GAPS



GAPs are produced by (NF1 gene)

↓

Loss of which leads to Persistant ST state & produces Tumors.

NF1 Syndrome

ⓒ BRAF

BRAF (V600) —PM→
- Hair cell Leukemia
- LCH
- Papillary Ca Thyroid
- Astrocytoma
- Colonic Ca.

HPLC A

Drug – Vemorafinib

ⓓ β Catenin

β Catenin —PM→ HCC
Hepatoblastoma.

ⓔ NOTCH

NOTCH —PM→ Leukemias
Lymphomas.

Ⓘⅴ Nuclear Transcription Proteins

C MYC —t(8;14)→ Burkits Lymphomas

N MYC —Amplification→ Neuroblastoma.

(V) Cyclin / CDKs

Cyclin D_1 —t(11:14)→ Mantle cell Lymphoma.

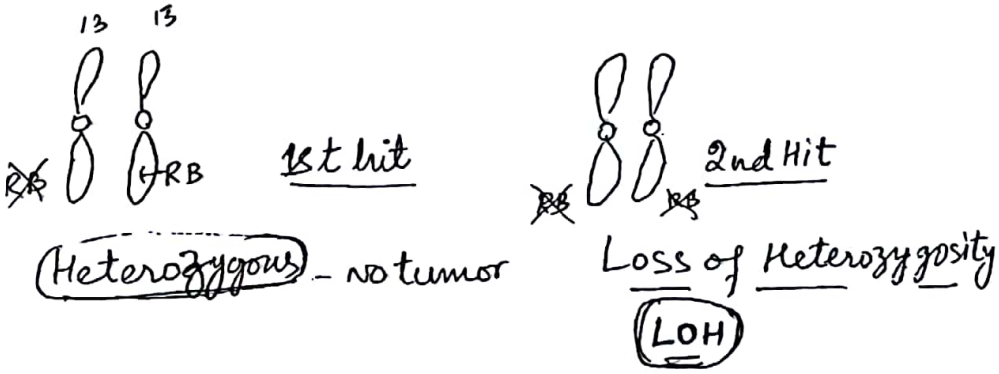
Cyclin E —Overexpression→ Breast Ca

CDK 4 —PM amplification→ Glioblastoma, Melanoma

(2) Tumor suppressor genes

Inhibits cell proliferation

Loss of both copies (Inactivation) of TSG produces Ca.



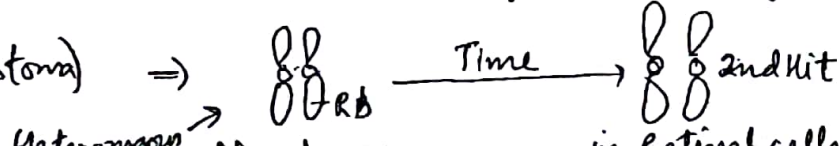
Heterozygous – no tumor

Loss of Heterozygosity (LOH)

1st tumor supressor gene is RB

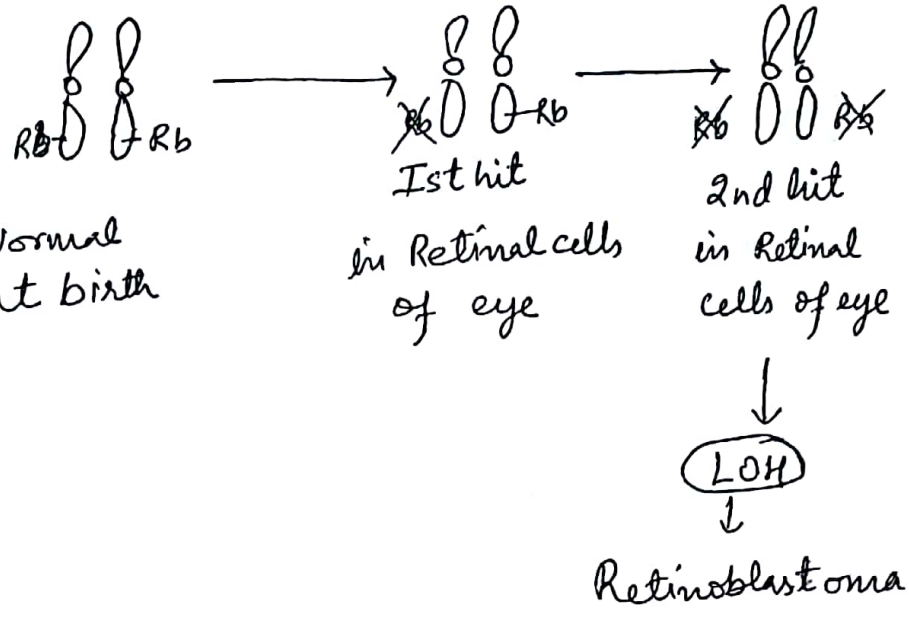
Loss → Retinoblastoma, Osteosarcoma

Knudson's TWO HIT HYPOTHESIS for Heriditary Cases (Retinoblastoma) ⇒



Heterozygous New born → in Retinal cells.

Sporadic Cases



Normal at birth

in Retinal cells of eye

in Retinal cells of eye

Retinoblastoma.

(I) Cell surface

- TGF-β receptor — Function: Growth inhibition — Inactivation → Ca colon
- E cadherin — cell adhesion — Loss → Ca stomach

(II) Under the Plasma Membrane

NF-1 (Neurofibromin) or (GAPs) — Inhibits RAS Signal Transduction → NF 1 Syndrome or (Von Reckling hausen's disease)

GAPs are produced by NF-1

- ↳ Multiple Neurofibromas
- ↳ Cafe au Lait spots
- ↳ Lish nodules in Iris (hamartomas)
- ↳ ↑ risk of brain tumors (optic Nerve glioma)
- ↳ Juvenile myelomonocytic Leukemia (JMML)

(II) Cytoskeleton

NF2 gene (Chr 22) → Cytoskeletal Stability —Loss→ NF2 Syndrome

MERLIN is produced by NF2 gene

B/L Acoustic Schwanoma Syndrome
Meningiomas.

(III) Cytosol

APC gene (Chr-5) → APC protein → Inhibits ST by (WNT pathway) → Familial Adenomatous Polyposis Coli Syndrome (FAP synd.)

- Multiple adenomatous polyps in colon
- Minimum 100 polyps needed for diagnosis.

Polyps appear by teenage
↓
if untreated
↓
Risk of progression to Ca colon is 100%

(IV) PTEN (Chr-10) → Inhibits ST by (PI-3 kinase pathway) → Endometrial & prostate ca.

(V) Nucleus

Rb → Retinoblastoma
Rb → Osteogenic Sarcoma

p53 (Chr. 17) → Li Fraumeni syndrome

M/C gene mutated in human Ca → p53 gene.

WT-1, WT-2 (Chr. 11) → Wilms Tumor

p16 → Malignant Melanoma

ARF/INK4A

BRCA-1 (Chr. 17) → Heriditary breast & ovarian Ca (females)
Prostate Ca

BRCA-2 (Chr 13) → Heriditary breast & ovarian Ca (females)
Prostate Ca.
Heriditary male breast Ca

(3) Genes for Apoptosis

BCL2 $\xrightarrow{t(14:18)}$ Follicular lymphoma.

Chr 14 – IgH gene
Chr 18 – BCL2 gene

(4) Genes for DNA repair

DNA repair defect syndrome

- Lynch / HNPCC syndrome — ADominant
- Xeroderma pigmentosa — ARecessive
- Ataxia Telangectasia — ARecessive
- Bloom's syndrome — ARecessive
- Fanconi's Anemia — ARecessive

Genes for DNA repair are of 3 categories

(I) Mismatch repair genes

Act as spell checkers when a strand of DNA is replicating

Loss of mismatch repair genes → Spelling mistakes accumulate in new strand DNA

the cell which gets this DNA is said to Have (RER phenotype) (Replication ERROR)

Spelling mistakes also produce microsatellite instability.

Microsatellites → Tandem repeats of 1-6 nucleotides scattered throughout our genome.

Fixed for a person & fixed for life.

Also called as Molecular fingerprints.

Loss of mismatch repair gene is associated with Lynch Syndrome (↑ risk of developing Colonic Ca)

(2) Nucleotide Excision Repair Gene (NER)

NER genes remove UV light induced pyrimidine dimers from DNA.

Loss → Xeroderma pigmentosa
- Photosensitivity
- 200 times of ↑ed risk of developing Cutaneous Ca
  SCC
  BCC &
  M. Melanoma

T≡T dimers

(3) Genes for Repair by Homologous Recombination

Repair double Stranded DNA breaks which can be produced by ionizing radiation.

(a) ATM gene – Sensor of DNA Damage

Loss produces Ataxia talangectasia

Cerebellar Ataxia

Oculo cutaneous talengectasia

Def. of Ig A antibody

↓

(Repeated infection) cause of death

↑ed risk of developing Lymphoreticular Tumors.

(b) Gene for enzyme BLM helicase

Loss → Bloom's Syndrome

growth retarelation

mental retardation

↑ed risk of developing lympho reticular tumors.

(c) Fanconi Anemia gene

Loss → Fanconi Anemia

Heriditary aplastic Anemia

Aplasia of radius & thumb bone

Hypoplastic kidney & Spleen.

8 Hallmarks of Cancer & 2 enabling factors.

8 Hallmarks

(1) Self sufficiency of Growth signals.

(2) Insensitivity to growth inhibitory signals.

(3) Altered cellular metabolism

Warburg effect — Also called as Aerobic glycolysis

It provides rapidly dividing Ca cells with metabolic intermediates that are needed for synthesis of cellular components.

Mitochondria manufactures ↓ other building blocks of Ca. cells

(4) Evasion of apoptosis

(5) Limit less replication potential – due to reactivation of telomerase

(6) Sustained angiogenesis

(7) Ability to invade and metastasize

(8) Ability to evade host immune response.

2 enabling factors

- Genomic instability
- Tumor promoting inflammation.

Carcinogens

Chemicals

Arsenic → Lung & Skin

Asbestos → Lung Ca, Mesothelioma
Oesoph., gastric, & colonic Ca

Benzene → AML

Beryllium → Lung Ca

Cadmium & its compounds → Prostate Ca.

Nickel & Chromium → Angiosarcoma, liver

Azo dyes → HCC

β Naphthylamine → Bladder Ca

Nitrosamine & nitrites → Oesophageal & gastric Ca

Aflatoxins → HCC

Carcinogens → Initiators → causes DNA damage
→ Promoters → stimulate genetically damaged cells to proliferate.

e.g Promoters
Hormones like Estrogen, DES (diethyl Stilboestrol)
Saccharin
Phenol
Phorbol esters.

Complete Carcinogens
↳ Capable of both Initiation & promotion.

Carcinogens
↙ ↘
Direct acting — Indirect acting.

e.g alkylating agents
They require no metabolic conversion to become active carcinogen

e.g aromatic amines
benzo pyrines
They require metabolic conversion to become Ultimate Carcinogens.

Radiation

UV light

UVB Associated with cutaneous Ca.

Xeroderma pigmentosa are at ↑ risk of developing cut. Ca
- SCC
- BCC
- & M. Melanoma

Ionizing Radiation

All leukemia except CLL (MC)

Papillary thyroid Ca

Ca breast, Ca lung.

Ca salivary gland (Muco epidermoid Ca).

Biological Carcinogens:

Viruses

DNA	RNA
HPV	HTLV-1
EBV	HCV
HBV	
HHV8	

Bacteria

H. pylori → MALToma, gastric Ca

① HPV

70 serotypes are known

3 groups

Low risk serotypes — 6 & 11 ⇒ Venereal warts (condyloma accuminatum)

Intermediate serotypes — 31, 33

High risk serotypes — 16, 18 → Cancer

- Ca Cervix
- Ca Vulva
- Ca Vagina
- Ca Anal
- Canal
- Ca Penis
- Ca Larynx

Viral DNA encodes for 2 viral proteins

②

E6 —
- Inactivates p53 protein
- ↑ TERT + ↑ telomerase expression

E7 —
- → Inactivates Rb protein
- → Inactivates p21 & p27.

② EBV — DNA virus

Infectious Mononucleosis
Oral Hairy Leukoplakia in HIV(+)] Benign Reactive.

Tumors
Nasopharyngeal Ca
Burkitts lymphoma
Hodgkins lymphoma
Bcell lymphomas in immunocompromised.
Extranodal NK T cell lymphoma.

Produces 2 proteins responsible for excessive cell proliferation
- LMP-1
- EBNA-2

③ HBV → HCC

- Repeated cycles of injury and regeneration lead to accumulation of mutation.
- Some viruses have HBx gene → Interferes with p53 → Ca

④ HHV8 [Kaposi's sarcoma Herpes virus 8]

Causes → Kaposi's Sarcoma
→ Primary effusion lymphoma (variant of DLBCL)

RNA Virus

① HTLV-1

Causes
- Arthritis
- Uveitis
- & Tropical Spastic Para paresis

Tumor – Adult T cell Leukemia/lymphoma.

HTLV-1 codes to TAX protein
↓
Causes uncontrolled cell proliferation.

② HCV
- HCC
- Splenic marginal zone lymphoma

Paraneoplastic syndrome

① Endocrinopathies

(a) Hypercalcemia (MC)

Due to production of PTH related protein by tumor cells.

Tumors – SCC (lung, skin), Ca breast, RCC, Adult T cell leukemia/lymphoma.

(b) Cushing syndrome

Due to ACTH production

Tumor – Small cell Ca lung.

(c) SIADH

Due to ADH production

Tumor – Small cell Ca lung.

(d) Hypoglycemia

Due to production of Insulin or Insulin like substances.

Tumor
- Ovarian Ca
- Fibrosarcoma
- HCC

(e) Carcinoid Syndrome

Due to production of Bradykinin & Serotonin

Tumors
- Bronchial carcinoids
- HCC

(f) Polycythemia
Due to erythropoietin

Tumors — RCC
HCC
Cerebra Hemangioma.

(2) Nerve & muscle

Myasthenia gravis — Ca lung
Thymoma.

Immunologic in origin

(3) Cerebral degeneration

Ca lung
Hodgkins lymphoma

(4) Skin

Acanthosis Nigricans.
Due to production of epidermal growth factor.

Tumor — Gastric Ca
Lung Ca

(5) Hypertrophic osteoarthropathy – Ca lung.

⑤ Trousseau Syndrome
(Migratory Thrombophelibitis)

Tumor cells activate clotting

- Pancreatic Ca
- Lung Ca

⑥ Marantic endocarditis / Non bacterial Thrombotic endocarditis

In advanced Malignancies

Tumor Markers

① Hormones

(a) Calcitonin – Medullary Ca Thyroid.

(b) Catecholamine – Pheochromocytoma

(c) β HCG – Trophoblastic tumors of Chorio Carcinoma.

② Oncofetal antigens

(a) AFP (Alpha Feto Protein) – HCC, Hepatoblastoma
Yolk sac tumor
(no seminomatous germ cell tumor)

(b) CEA (Carcino Embryonic Antigens)
→ Ca colon, Pancreas, lung, Stomach

③ Specific Proteins

(a) Immunoglobulins — Multiple Myeloma.

(b) PSA — Prostate Ca.

④ Iso-enzymes

(a) PAP (Prostate Acid Phosphatase) – Prostate Ca

(b) Neuron specific Enolase — Neuroblastoma
Small cell Ca
Neuroendocrine tumors.

⑤ Mucins

CA 125 → Ovarian Ca

CA 19.9 — Colon & prostate Ca.

CA 15.3 — Breast Ca

⑥ Cell free DNA markers (LIQUID BIOPSY)

TP53, APC, RAS in Stool & serum – Colon Ca

TP53, RAS in stool & serum – Pancreatic Ca

TP53, RAS in Sputum & serum – Lung Ca

TP53, in urine & serum – Bladder Ca

Tumor Markers detected in Tissue by IHC

① Carcinoma
- Cytokinin (M. imp)
- EMA (Epithelial Membrane Antigen)
- CEA (Carcinoembryonic Antigen)

② Lymphoma
- LCA (Leucocyte common Antigen)

③ Sarcoma – Vimentin
- Ewing sarcoma – CD99
- Synovial sarcoma – CD99, BCL2
- Rhabdomyosarcoma – Desmin, MyoD1
- Leiomyosarcoma – Smooth Muscle Actin (SMA) +
- Osteosarcoma
  - Osteopontin
  - Osteonectin
  - Osteocalcin
- Chondrocytoma – S-100
- Liposarcoma – S-100

④ Mesothelioma
- ~~S-100~~
- Cytokeratin 5/6
- Mesothelin
- Calretenin

(5) Malignant Melanoma
- HMB45
- Melan A
- S100

(6) LCH
- CD1a
- S-100
- Langerin (CD 207)

(7) Small cell Ca
Neuroblastoma
Neuro endocrine tumor
- Synaptophysin
- Chromogranein
- Neuro Specific enolase (NSE)
- S-100

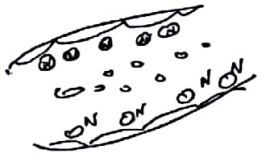
(8) Schwan cells
Neurofibroma
Schwannoma
- S-100

(9) TTF-1 (Thyroid Transcription Factor)
- Lung Ca < Adeno Ca, Small cell Ca
- Thyroid Ca

(10) HCC
- Arginase 3
- Hep Par 1

(I) Vascular events

1. Transient vasoconstriction
↓
2. Massive vasodilation
↓
3. Increased vasular permeability. → exudate formation (Most imp)
↓
4. Stasis of cells in Blood vessels.
↓
5. Leucocyte margination to the periphery.

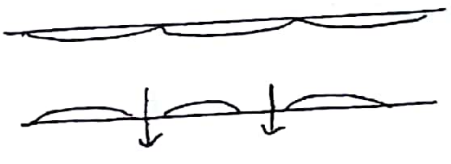


(II) Cellular events

- Phagocytosis
- Adhesion & transmigration
- Chemotaxis

Mechanisms of ↑ vascular permeability

1. Endothelial contraction (M/c mech)
(occurs in post capillary venules)



Mediators → Histamine
→ Bradykinin
→ Substance P
→ Leukotriene

⇒ Immediate Transient response

② Endothelial retraction / Junctional reorganisation

(venules & capillaries)

Delayed type of contraction

Mediators (cytokines) → IL-1
→ TNF α

⇒ ② Delayed, sustained response

③ Direct Injury

(Seen in venules, arterioles, capillaries)

Severe Injury

e.g Severe burns
Chemicals
Toxins

↓

Cells undergo necrosis & detach.

↓

Fluid leakage which starts immediately

↓

Leakage continues till a new cell regenerates

⇒ ① Immediate sustained response

Mild Injury

e.g mild sunburn.

↓

endothelial cells die after a few hours due to apoptosis.

↓

Fluid leakage starts after few hours

↓

Leakage continues till a new cell regenerates

⇒ ① Delayed sustained response

④ Increased Transcytosis

⇒ Passage of liquid across the channels formed in the endothelial cell cytoplasm

⇒ Channels are formed close to the Junction

Mediator – VEGF

(II) Cellular events

① Adhesion and transmigration

Rolling ⟶ loose adhesions ⟶ firm adhesions

↓

Transmigration (Diapedesis)

↓

Substances that coat the surface of neutrophills and surface of endothelial cells & help them to stick together.

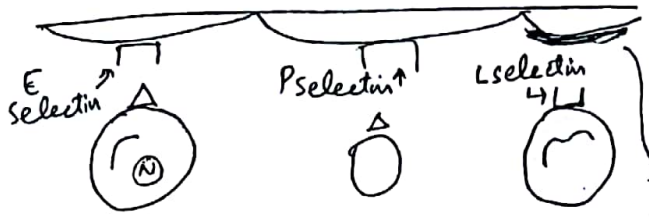
4 Families of adhesion molecules

(I) Selectins Bring about Rolling & loose adhesions

E selectin	CD 62E	Endothelium
P selectin	CD 62P	Platelets, endothelium
L selectin	CD 62L	Leukocytes

Complementary molecules

↳ Sialyl Lewis X
(Sugar formed by fucose metabolism)
[E & P]



Sialyl Lewis X.
(on the surface of Leukocytes)

↳ Sialyl Lewis X in mucin like ~~glycolipids~~ glycoproteins that coat the endothelium.
(for [L])

Leucocyte Adhesion deficiency type II
(LAD type II)

⇓

deficiency of Sialyl Lewis x due to defect in fucose metabolism.

(II) Immunoglobulin Superfamily

ICAM → ① ②
(Intercellular Adhesion Molecule)
VCAM → ① ②
(Vascular cell Adhesion Molecule)

(III) Integrins

β_2 integrins
e.g LFA1 (CD11) / MAC1 (CD18)

β_1 integrins → VLA4

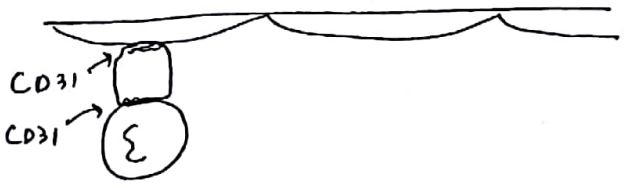
Both are found on endothelium — Found on leucocytes

Responsible for FIRM ADHESIONS.

(IV) CD31 / PECAM-1

(Platelet Endothelial Cell Adhesion Molecule)

- Transmigration
- Homotypic adhesion molecule
- CD31 is found on leukocyte & endothelium.



Neutrophils produce enzyme type IV collagenase
↓
breakes type IV collagen (Basement Membrane) & comes out of the vessel.

LAD type I

Autosomal recessive disorder

Deficiency of β_2 integrins LFA-1/MAC-1

There is mutation in MAC 1 (CD18) gene

C/F - Recurrent bacterial & fungal infection.
Impaired wound healing.
Delayed umbilical cord separation.
Leukocytosis (↑ TLC).

Mechanism of appearance of Adhesion molecules.

① Redistribution → P selectin

↓

Weibel Palade body in endothelial cell cytoplasm
[contains P selectin]

During inflammation

Mediators → Histamine
→ Thrombin
→ PAF

↓

Redistribute P selectin to endothelial cell surface within few minutes

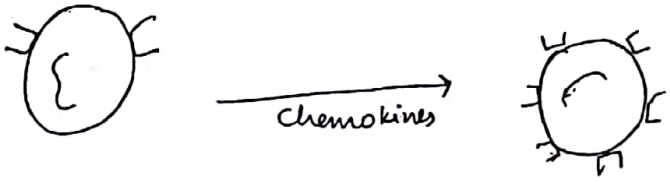
② Induction → Fresh synthesis during inflammation

- E selectin
- ICAM
- VCAM.

Mediators that induce → IL 1, TNFα

Time taken is hours.

③ Increased avidity of binding (Strength) ⇓ Integrins



↑ the no. of integrin molecules on leukocytes

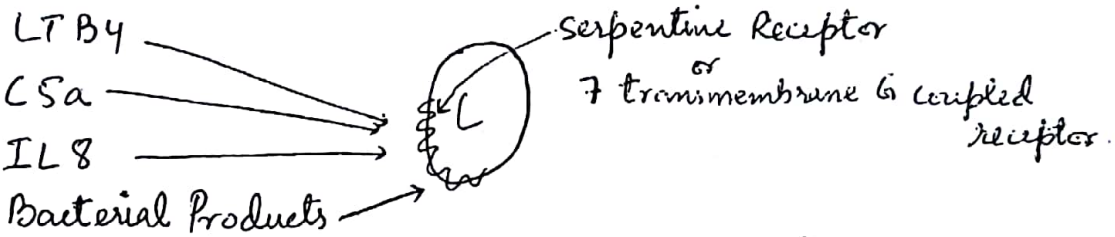
↑ the strength of binding of integrins many times.

⇒ Weibel Palade bodies are ultrastructural markers of endothelial cells (EM)

⑤ Chemotaxis

Def. Locomotion orientend along a chemical gradient.

Chemotactic agents



When these ligands bind to 7 transmembrane G coupled receptors

↓

↑↑ Ca^{2+} in the cytosol

↓

Polymerisation of actin filaments at the leading edge

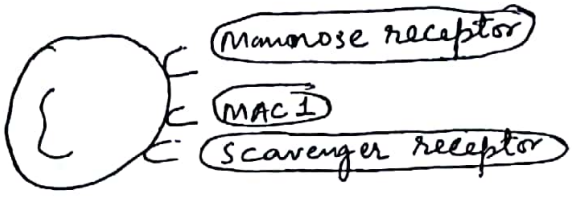
↓

Pseudopod formation

Defects In — DM, Malignancy, Severe burns, CRF

③ Phagocytosis

(a) Recognition & attachment.



3 receptors that help the neutrophil to recognize & attach to the bacteria

- Mannose receptor
- MAC 1 integrin (CD18)
- Scavanger receptor

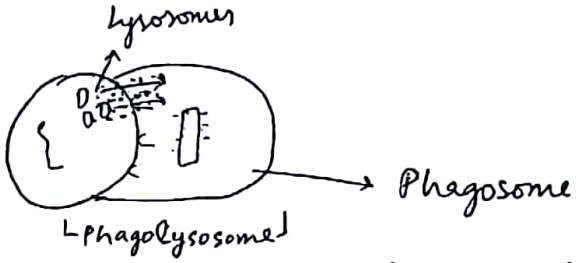
Opsonization

↑es the efficiency of phagocytosis.

Opsonins → Substances that coat the bacteria & make it tasty for neutrophil.

- C3b, C3bi (C4b, C4bi)
- IgG antibody
- Plasma proteins
  - CRP
  - Mannose Binding lectin
  - Fibronectin
  - Fibrinogen.

(b) Engulfment.



Pseudopods flow around the bacteria & bacteria is enclosed in a phagosome

Phagosome fuses with lysosome to form phagolysosome

All enzymes are discharged in phagolysosome.

Chediak Higashi Syndrome – defect in engulfment

↘ Failure of fusion of phagosome & lysosome.

↳ Autosomal recessive disorder

C/F – Repeated infections

- Oculocutaneous albinism & Silvery grey hair.
- Nerve conduction defects.
- Platelet function defects leading to bleeding.

PBS Neutropenia with giant granules in leukocytes

⇒ Lyst gene mutation are seen.

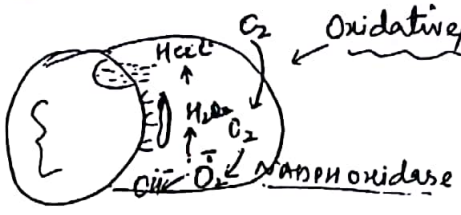
↙

Absence of DOCKING PROTEIN

Needed for fusion of lysosomal membrane with phagosomal membrane.

(C) Killing → O_2 dependent method (Main method)
→ O_2 independent metahod

Oxygen dependent method



$H_2O_2 \xrightarrow[Cl]{MPO} HOCl^-$ kills bacteria

- By lipid peroxidation of bacterial membrane
- Halogenation (bleaching)

H_2O_2 MPO - Halide system kills bacteria, fungi, & parasites

MPO is a lysosomal enzymes.

Defect → Chronic granulomatous diseases
↓
defect of enzymes NADPH oxidase
- XR (MC) (75%)
- AR (25%)

Test for diagnosis Nitroblue tetra zolieum Test. (NBT)

Oxygen independent killing

Lysosomal enzymes kill the bacteria

→ **BPI** Bacterial Permeability Increasing Protein
It is a phospholipase
↓
Breaks phospholipids & bacterial membrane

→ **Lysozyme**
It is a muramidase
↓
Breaks glycopeptide coat of bacteria.

→ **Lactoferrin**
Binds Iron
↓
Iron is unavailable for bacterial growth

→ **Major Basic Protein**
Found in eosinophil granules
↓
Toxic to parasites

→ **Defensins**
→ **Cationic proteins.** } Found in Neutrophils

Mediators of acute inflammation

Cells

Preformed/Primary

- Histamine
- Serotonin
- Lysosomal enzymes

Newly synthesised/Secondary

- Aracadonic Acid mediators
- PAF
- Cytokines
- ROS (Reactive Oxygen Sp.)
- Chemokines
- Nitric oxide
- Substance P

Lipid mediators

Plasma

- Kinin cascade
- Complement cascade
- Coagulation cascade
- Fibrinolytic cascade

Preformed / Primary mediators

① Histamine

First mediator to be produced

Source → Richest source is Mast cell

Others – Basophils, Platelets

Stimuli for release

⇒ IgE Ab binding to receptors on mast cells

⇒ C3a, C5a } Anaphylotoxins (Post Cap. Venules)

⇒ IL-1, IL-8 }

⇒ Histamine Releasing proteins.

⇒ Physical agents like heat, cold & trauma.

Actions

① Vasodilation

② ↑ vascular permeability → causing Immediate Transient response.

③ Vasoconstriction (large vessels due to muscular layer presence)

④ Bronchospasm.

(2) Serotonine (5HT)

Richest source → Platelets

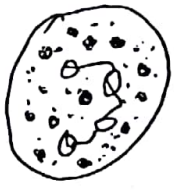
Others → Enterochromaffin cells.

Actions: Platelet aggregation

Other actions are same as histamine.

(3) Lysosomal Enzymes

Found in granules of neutrophils



Primary/Azurophilic granules

- Large coarse granules

- MPO
- BPI
- Acid hydrolases
- Neutral proteases
- Lysozymes
- Phospholipase A_2
- Defensin
- Cationic Proteins

P ANCA → MPO, BPI

C ANCA → Neutral proteases

Secondary/specific granules

- Small fine granules

- Lactoferin
- Alkaline phosphatase
- Type IV collagenase
- Gelatinase
- Lysozyme
- Phospholipase A_2
- Vit B12 binding proteins

ANCA: Antibodies against enzymes found in primary granules of Neutrophils

- PANCA → MPO
- CANCA → Proteinase3 (Neutral proteases)

Newly synthesised mediators.

① Platelet activating Factor (Lipid meddator)

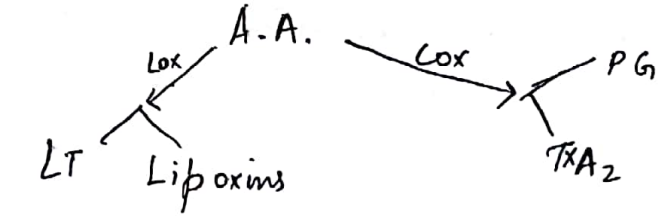
Source - All leukocytes & mast cells.

Actions
- Vaso dilation
- ↑ Vascular permeability
- Vasospasm
- Platelet aggregation
- Brochospasm
- Chemotactic
- Angiogenesis
- Cell to cell signal transduction.

② A. acid mediators

20 Carbon polyunsaturated Fatty Acid
Found esterified in membrane phospholipids.

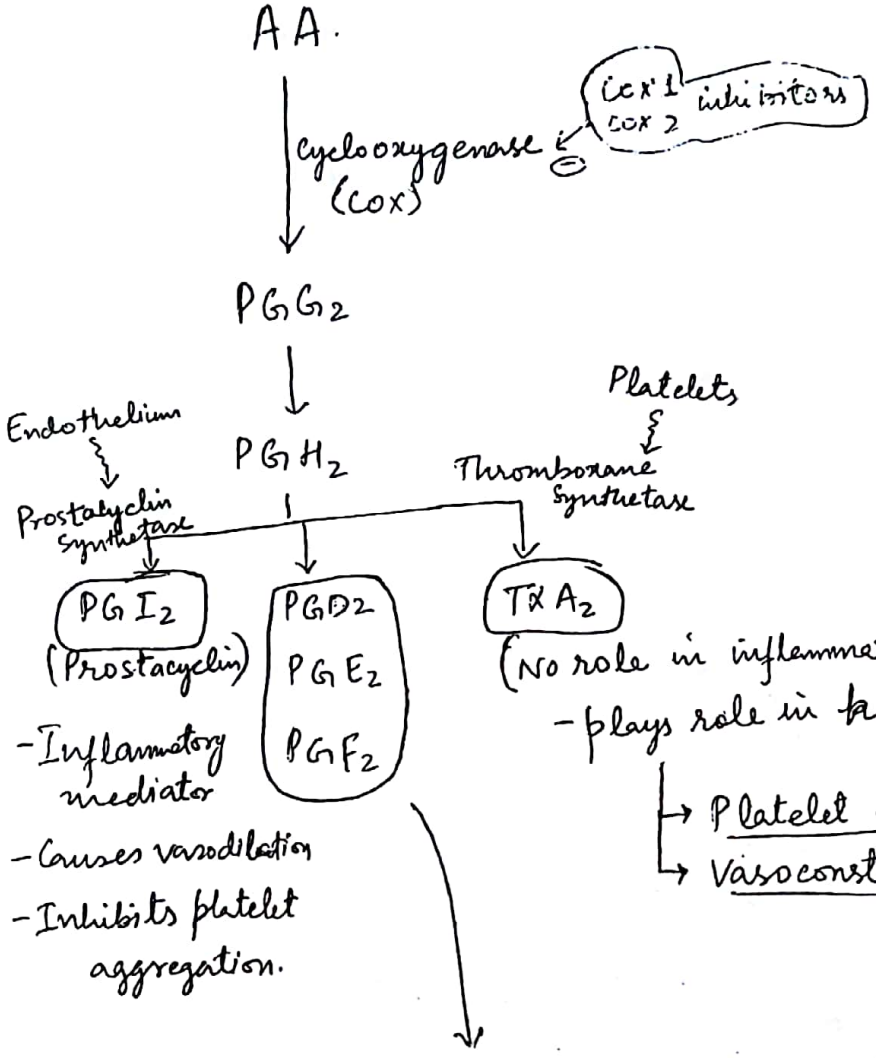
Membrane phospholipids
↓ Phospholipase



Eicosanides

Source - All leucocytes, mass cells, endothelial cells.

(Steroids) inhibit phospholipase



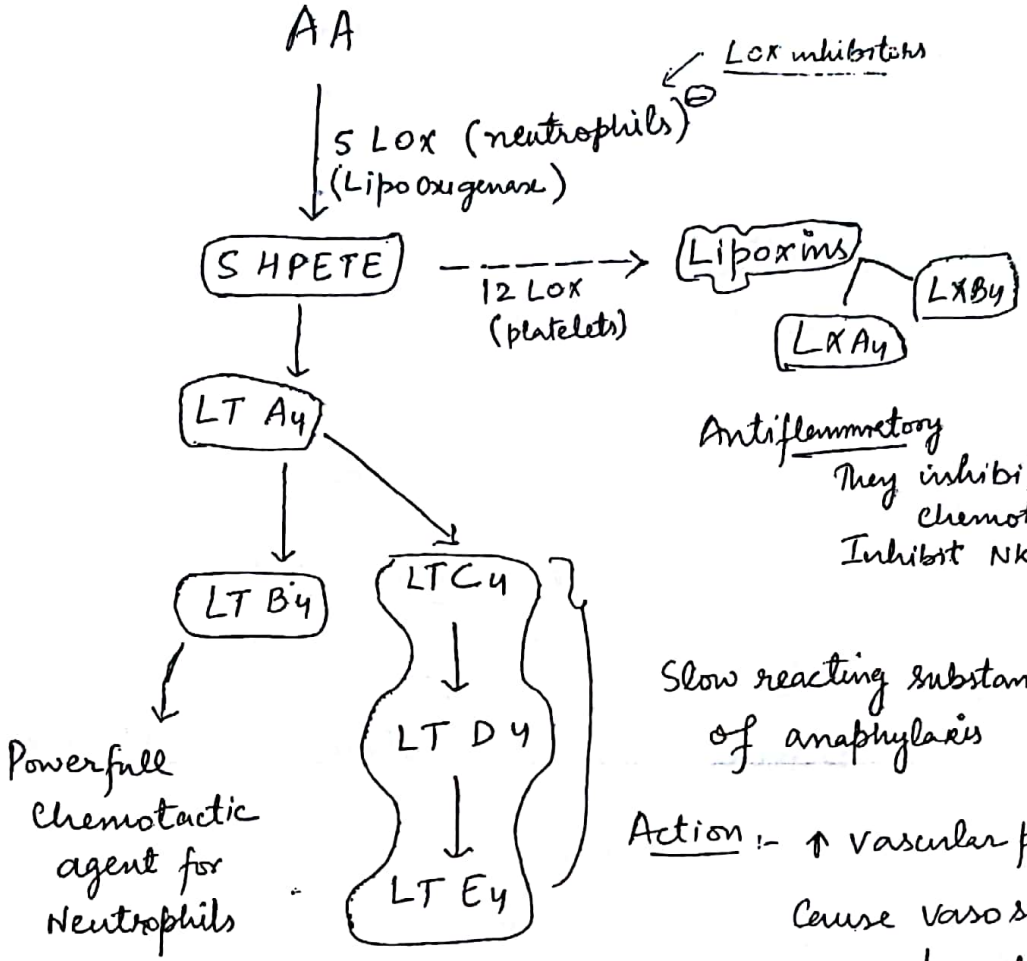
⇒ Increase vascular permeability

⇒ Cause vasospasm & Bronchospasm.

⇒ Pain & Fever (PGE_2)

⇒ PGD_2 & PGF_2 ⇒ Chemotactic.

⇒ $PGF_{2\alpha}$ ⇒ Causes uterine smooth muscle contraction.



Antiflammatory
They inhibit Neutrophil chemotaxis
Inhibit NK cell activity.

Slow reacting substances of anaphylaxis

Action :- ↑ vascular permeability
Cause vasospasm & bronchospasm.

Lipoxins are produced by transcellular biosynthesis
↓
2 Cells are cooperating for production.

③ Chemokines

Belong to family of cytokines

Short chain polypeptides that cause chemotaxis.

4 categories

(I) α chemokines / C x C chemokine

Chemotactic for Neutrophils

e.g.; IL 8

(II) β chemokines / C – C chemokines

Chemotactic for all except Neutrophils.

e.g.; Eotaxin → only for eosinophils

Rantes → eosinophils + Tlymphocytes.

MCP 1 ⟶ Monocytes

(Monocyte chemotactant Protein 1)

MIP-1α ⟶ Monocytes & Macrophages

(Macrophage Inflametory Protein)

(III) gamma chemokines / C – chemokines.

e.g.: lymphotactin → Lymphocytes.

(IV) C X_3C Chemokines

Only one member – Fractalkine – chemotactic for monocytes

Chemokine receptors ⟶ (CXCR4) / (CCR5) } act as co receptors for HIV

④ Cytokines

Source → Macrophage & dendritic cells.

IL-1, TNFα — TNFα in addition is also produced by T cells & mast cells.

Action –

Ⓐ Systemic acid phase reaction

IL-1, TNFα, IL6 → Mc.

Fever, Increased sleep,
↓ appetite
↑ TLC
↑ ESR
↑ CRP

TNFα also regulates energy balance by causing lipid and protein mobilization & suppressing appetite

↓

thus causing ↑ TNF levels

⇓

Cachexia

Cancer cachexia → TNFα.

Ⓑ Endothelial activation

↑ expression of endothelial adhesion molecules

↑ production of mediators – cytokines, chemokines & AA mediators

↑ procoagulant activity

(III) Leukocyte activation

TNF ⟹ Microbicidal activity of leukocytes

(IV) Fibroblast activation

IL 1 - Fibroblast proliferation & Synthesis of collagen.

(5) Nitric Oxide

Arginine $\xrightarrow{\text{NO synthetase}}$ NO

NO synthetase is found in → endothelial cells

Endothelial cells → (eNO) → Vasodilation

Macrophages → (iNO) (inducible) → Produced during inflammation - Microbicidal gas.

Neurons in brain → (nNO) (neuronal) → Neurotransmitter in Brain.

⑥ Substance P (Neuropeptide)

Source - Leukocytes
Sensory Nerves. (CNS & PNS)

Actions
Pain
↑ vascular permeability
Regulation of BP

PAIN mediators.
PGE2
Bradykinin
Substance P → M.Imp

Mediators comming from Plasma

① Kinin Cascades [generate bradykinin]

Prekallikrein —(XIIa (Hegeman factor))→ Kallikrein
↓
High M.W kininogen
↓
Bradykinin

Action
- Pain
- ↑ vascular permeability (↳ M. Imp)
- Vasodilation
- Vasospasm
- Bronchospasm.

② Complement Cascade

[Set of 20 proteins found in plasma]

3 pathways for complement activation

Classical pathway

- Starts ē activation of C1
- C1 is activated by Ag–Ab complex.

Lectin Pathway

- Starts ē activation of C1
- C1 is activated by bacterial lectins and mannose binding lectins

Alternate Pathway

- Starts ē activation of C_3
- No participation of C_1, C_2, C_4
- C_3 is activated by
  - LPS (LipoPS)
  - Cobra venom
  - Aggregates of IgA antibody.

Mediators produced

C3a, C3bi } Opsonins

C3a, C5a } Anaphylotoxins (cause release of histamine from mast cells).

C5a } Chemotactic for Neutrophils, eosinophils & monocytes

C5-9 } Membrane Attack Complex (MAC)

③ Coagulation Cascade

Mediators

ⓐ Fibrinogen → Opsonin

ⓑ Thrombin → Main link b/w inflammation & coagulation.
Causes redistribution of P selectin.
Induces COX enzymes in Endothelial cells.
↑ expression of adhesion molecules on endothelial cells.

ⓒ Fibrinopeptides → Vascular permeability
→ Chemotactic

④ Fibrinolytic Cascade

Plasminogen
↓ Tissue plasminogen Activator (TPA), Urokinase, Streptokinase
Plasmin
↓
clot
↓
Fibrinogen Degradation Products
FDP → ↑ vascular permeability
Chemotactic

NETs

Neutrophil Extracellular Traps.

Extracellur Fibrillar Network formed by Neutrophills to trap bacteria.

Formed from the nuclear chromatin of Neutrophils

Lysosomal enzymes are discharged in the NETs & kill the bacteria

At the end of NET formation, neutrophils die.

Chronic Inflammation

① Infiltration of tissue by mononuclear cells
(monocytes, lymphocytes, & plasma cells)

② Tissue destruction.

Macrophages

⇒ Main cells of chronic inflammation

⇒ Derived from blood monocytes which are produced in bone marrow.

* Resident macrophages eg kupfer cells in liver, microglial cells in brain are derived from stem cells in yolk sac & life span is in years.

⇒ Also called as Histocytes

Liver → Kupfer cells

Brain → Microglial cells

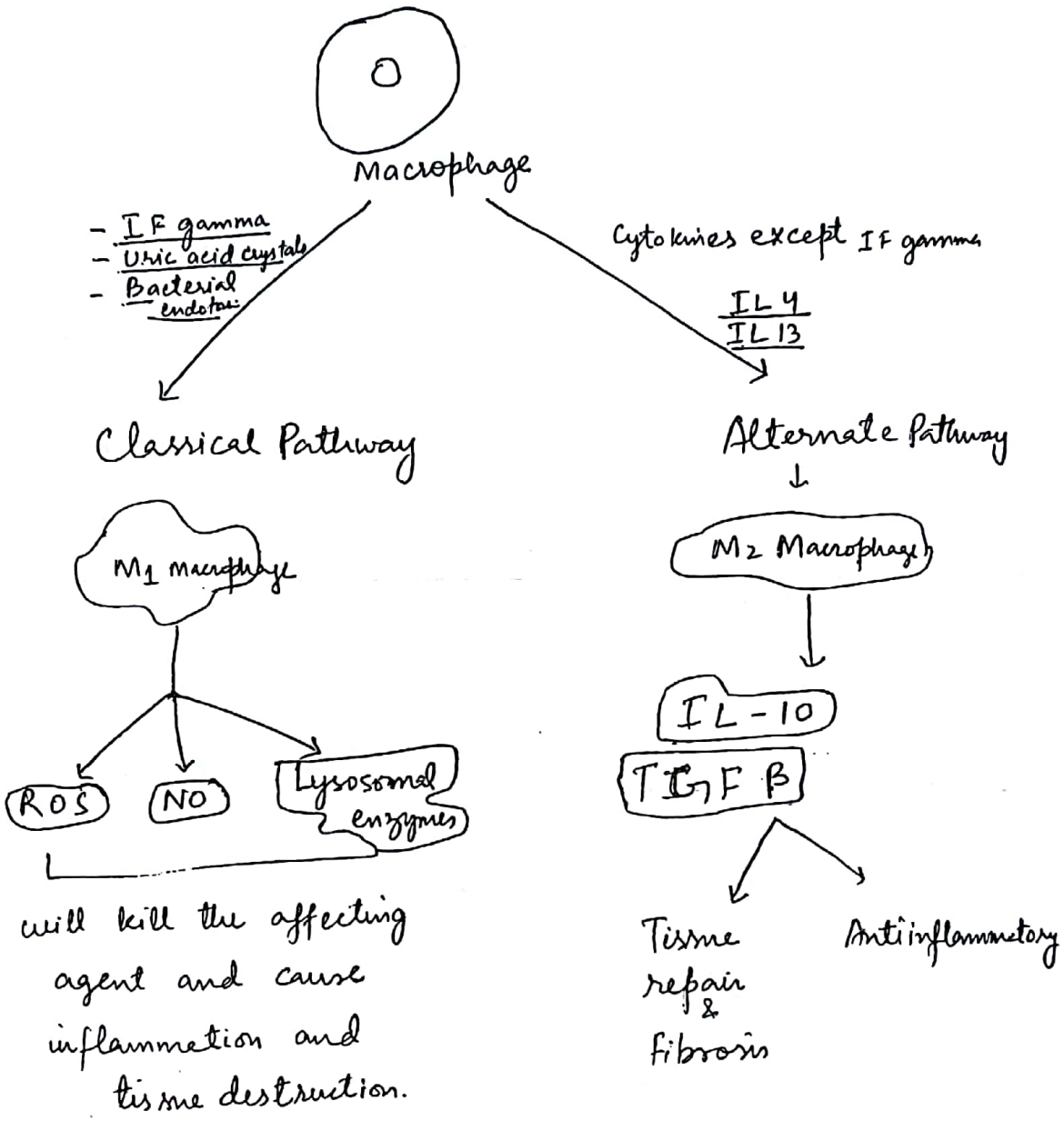
Bone → Osteoclasts

Spleen → Littoral cells
Spleen → Sinus histiocytes

LN → Sinus histiocytes

Lungs → Alveolar macrophages

In chronic inflammation macrophages are activated to kill the bacteria.



Fibrosis - TGF β

Anti inflammatory - IL 10
TGF β
IL 6

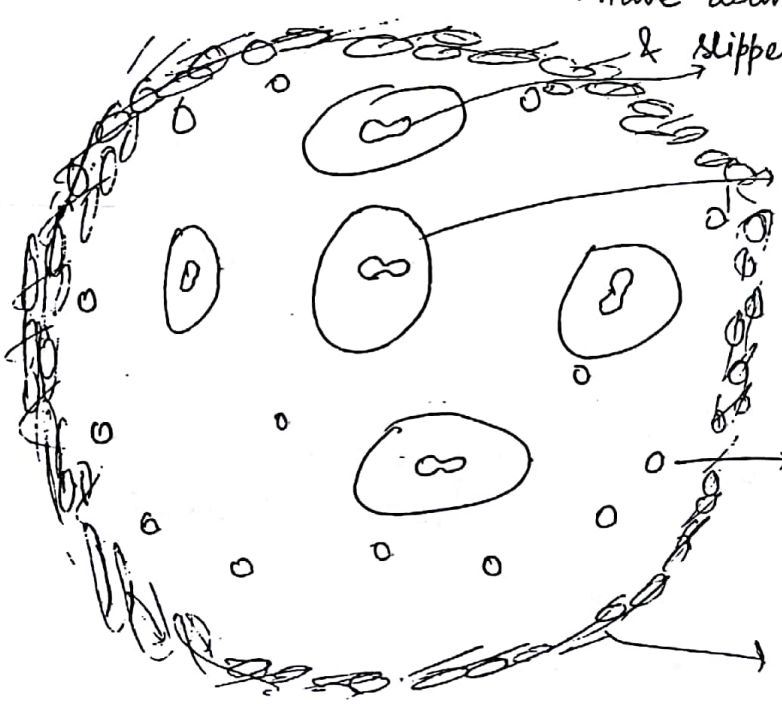
IL 6 is both Pro inflammatory & Anti inflammatory

Chronic granulomatous inflammation

Special type of chronic inflammation characterised by granuloma formation.

→ Collection of specialized macrophages called epithelioid cells

↳ Have abundant cytoplasm & slipper like nucleus.



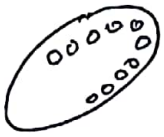
2 types → Immune granuloma
→ Foreign body granuloma.

(1) Immune granuloma — ~~Found~~ Formed in type IV HR.

→ IFN gamma & TNF α plays an important role.

Causes: TB, Sarcoidosis, Leprosy, LGV, Cat scratch disease, Syphilis, Crohn's disease, Hodgkin lymphoma

Immune granulomas may show gaint cells
e.g Langhan's gaint cell.



Multiple nuclei arranged in C or horse shoe shap

⇒ Caseating Granuloma

Caseous neerosis in centre

TB
Syphilis
Fungal infection ← Histoplasmosis
Coccidoidomycosis

⇒ Non Caseating

TB
Sarcoidosis (naked granulomas)
↳ No lymphoid & PC surrounding

Hodgkin's lymphoma
Leprosy (tuberculoid)

⇒ Stellate granulomas → Star shapped granuloma
→ Neutrophilic granuloma

LGV
Cat scratch disease

⇒ Necrotising granuloma

(Small vasculitis) Wegner's granulomatosis

⇛ Eosinophilic granulomas
Churg Strauss
(small vessel vasculitis)

⇛ Durck granulomas
Cerebral malaria.

(2) Foreign body granuloma

Formed around a foreign body eg: talc, suture material, dead parasites, uric acid crystals.

↳ Contains foreign body giant cell (numerous)

Multiple nuclei arranged in haphazard way

May contain foreign body

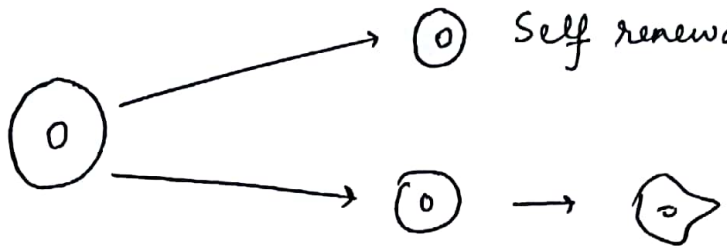
Cytokines for Fever

1. IL1 Most imp.
2. IL6
3. TNF α
4. PG E2
5. Ciliary Neurotrophic factor

Cytokines for Septic Shock
- TNF α

Fibrosis - TGF β

Stem Cells



Capacity to generate differentiated cells by asymmetrical replication

2 types

① Embryonic stem cells — Isolated from blastocyst

Totipotent - can generate all tissues of body.

② Adult or Somatic stem cells

Found in Adult/Normal tissues in special microenvironments called Niches

Can be Pluripotent/Multipotent/Bipotent.

(a) Bone marrow

Hematopoietic stem cells

- Pluripotent - give rise to all Blood cell lineages.
- Can be obtained from
  - (I) Umbilical cord blood
  - (II) Peripheral blood after injecting GM-CSF
  - (III) Bone marrow

Marrow Stromal stem cells

- Mesenchymal SC
- Pluripotent

give rise to adipocytes, endothelial cells, osteoblasts, chondrocytes

Mesenchymal stem cells are also found in abdominal fat.

b) Liver

Called "Oval cells"

Found in Canals of Hering.

Bipotent — Hepatocytes
Bilary cells.

c) Brain

Called Neuronal S.C.

Multipotent.

- Oligodendrocytes.
- Neurons
- Astrocytes

Location → Subventricular zones
→ Dentate gyrus of hippocampus.

d) Skin

Found in adnexa

- Hair follicle bulge
- Sebaceous glands

e) Limbus of cornea

f) Crypts of Intestine

g) Satellite cells - Sc associated with Cardiac & skeletal muscles.

Use of Stem cells

Used to repopulate the damaged tissue

Wound Healing

Regeneration	Repair
Dead cells are replaced by same cells	Dead cells are replaced by fibrous connective tissue.

① Labile cells/Actively dividing cells

They are in G1 phase of cell cycle.

eg epithelia ⇒ Skin, GIT, Resp. tract, urogenital tract.

Stem cells

Hematopoietic cells

Cancer cells

② Stable cells — In G0 phase of cell cycle

They have low replicative potential.

e.g.; i) Parenchymal cells of organ

Hepatocytes
PCT, DCT of kidney
Adrenocortical cells.

(ii) Mesenchymal cells

Adipocytes
Osteoblasts
Chondrocytes
Smooth muscle cell
Endothelial cells.

③ Permanent/Non dividing cells

They have left the cell cycle.

They cannot divide at all.

e.g.: - Neuronal
- Skeletal muscle
- Cardial muscle

Repair occurs by formation of granulation tissue

Pink, moist & has granular appearance.

M/c → Chronic inflammatory cells/Macrophages
lymphocytes, plasma cells.
→ New blood vessels
→ Fibroblasts that synthesize collagen.

Wound healing by Primary intention

Seen in clean, surgical wounds where the edges can be approximated

0 hours → Incision is filled c blood clot.

c in 24 hours →
- Neutrophils from margins infiltrates the clot.
- Mitosis begins in the basal layer of epidermis.

24 - 48 hours → Continuous thin layer of epithelium is formed below the scale/scab.

Day 3 →
- Neutrophils are replaced by macrophages
- Granulation tissue appears.
- Collagen fibers are evident at the margins of the incision.

Day 5 →
- Abundant granular tissue*
- Neovascularization is maximum.
- Collagen fibers bridge the incision (i.e they lay down longitudinally)
- Epidermis regains full thickness c surface keratinization.

2nd week → ↓ edema, ↓ inflammation
↓ vascularization
→ Proliferation of fibroblasts and accumulation of collagen.

↳ Blanching begins due to ↑ collagen deposition & regression of vascularity

3rd week → Scar is formed ē maximum fibrosis.

Wound Strength

1st week → 10% of normal unwounded skin.

3rd month → Maximum wound strength
70 – 80% of unwounded skin.
(100% wound strength is never regained).

⇒ Wound contraction is absent in primary intention healing.

Healing by secondary intention.

Seen in large wounds where wound edges cannot be approximated.

Large amount of granulation tissue is formed

Large scar is formed.

↓

Scar reduces in size – this is called as wound contraction

Brought about by Myofibroblasts.

⇒ Wound contraction is seen in healing by Secondary intention.

Defects in wound healing.

Hypertropic scar :- Raised scar produced due to accumulation of excessive amount of collagen.

⇒ Seen in thermal / traumatic injury.

⇒ Grows rapidly & regresses over several months.

Keloids :- Scar tissue grows beyond the boundaries of the original wound.

⇒ Do not regress

⇒ Genetic predisposition

Exuberent granulation tissue (Proud flesh)

Formation of excessive amount of granulation tissue which protrudes above the level of surrounding skin and blocks the re-epithelialization.

Removed by cautery or surgical excision.

Desmoid / Fibromatosis

Excessive proliferation of fibroblasts and other connective elements on site of injury or surgical scar.

Contrature

Exaggeration of wound contraction produces contracture

Common after severe burns

Site – Palm, sole, anterior thorax

Factors that impair wound healing.

1. Infection
2. DM
3. Poor nutritional status ← Zn def., vit C, PEM
4. Mech. factors ← Local, Torsion
5. Poor perfusion due to ← atherosclerosis, DM, impaired venous drainage
6. Foreign body
7. Type of injury & Site of injury

Positive acute phase proteins

Also called as Acute Phase Reactants.

Production increases during inflammation.

- CRP
- Fibrinogen
- Mannose binding Lectin

} Opsonins

- SAA [Serum Amyloid Associated]
- Ceruloplasmin
- Heptoglobin

} Inhibits the growth of microbes.

- Thrombin
- Factor VIII
- vWF

} Bring about coagulation

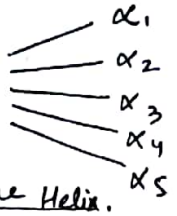
Negative acute phase proteins

Production by liver decreases during Inflammation.

- Albumin
- Transferrin
- Transthyretin
- Transcortin
- Retinol binding proteins.

Collagen

⇒ Triple Helix



→ any three make the Helix.

Vitamin C is essential for crosslinking of collagen fibres.

Type I collagen

Most abundent
Has high tensile strength
Found in skin, bones, tendons, internal organs & blood vessels.

Type II collagen

Cartilage & Vitreous humor

Type III collagen

Granulation tissue
Embryonic tissue
Uterus
Keloid

Type IV Collagen

Basement membrane.

Composition of basement membrane

Laminin
Type IV collagen
Fibronectin
Proteoglycans.

Amyloidosis

Group of diseases that have in common - deposition of abnormal proteinaceous substance extracellularly.

H & E → Pink homogenous appearance

Physical Nature

1) Electron Microscopy

- Long non branching fibrils
- Indefinite length fibrils.
- 7.5 – 10 nm diameter.

2) X ray crystallography & infrared spectroscopy ⇒ β pleated sheet conformation

Chemical Nature

- Fibrillar protein
  - Constitutes 95% of amyloid
  - 15 different types of Fibrillar proteins discovered.
- P-component Glycoprotein
  - Constitutes 5% of amyloid.
  - ⇒ PAS +ve & diastase resistant.

Classification

- Localized amyloidosis
- Systemic amyloidosis — Multisystemic disorder.

Localized Amyloidosis

1) Medullary carcinoma thyroid. — ACal → Amyloid; Calcitonin.
2) Alzheimer's disease — Aβ
3) Isolated atrial amyloidosis — AANF → Atrial Natriuretic Factor
4) Type II DM
   ↳ Amyloidosis in pancreas — AIAPP → Islet Associated Pancreatic Peptide
5) Prion disease — Misfolded prion particles.

Systemic / Generalized Amyloidosis

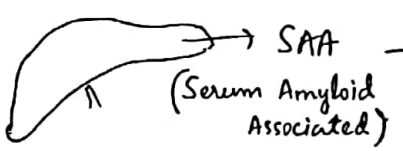
① Primary amyloidosis seen in patients of Multiple myeloma & other plasma cell tumors.

AL (Light chains) type of amyloidosis.

(λ light chains) are more prone to settle down as amyloid

Site - Heart, Kidney, GIT, etc.

② Secondary amyloidosis Also called reactive systemic amyloidosis



Site - kidney, liver, spleen, lungs, etc

M/C organ involved in amyloidosis ⇒ KIDNEY

Causes → Older days - TB, DM, Lung abscess, Bronchi abscess.

→ Now: Ankylosing spondylitis, RA, Ulcerative colitis, RCC

③ Hemodialysis Associated Amyloidosis.

Seen in patients who are on long term hemodialysis for chronic Renal Failure

β_2 microglobulin → ($A\beta_2$)

Sites - Joints, tendons, synovium

↳ Carpel tunnel syndrome.

④ Senile Amyloidosis

Seen in old age

Site - Heart liver, Spleen, etc

Transthyretin (TTR) is deposited as amyloid → (ATTR)

↳ (N) serum protein that transports thyroxine & retinol.

⑤ Familial Amyloidosis

Familial Amyloidotic Polyneuropathy

⇒ ATTR derived from TTR is deposited as amyloid

⇒ Site < Sensory Nerves, Autonomic Nerves

⇒ Mutated Transthyretin is deposited as amyloid

⇒ AD. disorder

Familial mediteranean Fever

⇒ AR disorder. (Recessive)

⇒ AA derived from SAA is deposited as amyloid in many organs.

Fever, effusions seen

* Pyrin gene mutations are seen

Diagnosis

FNAC

Abdominal Fat aspiration

Biopsy

Abdominal Fat aspiration

Rectal biopsy

Gingival biopsy

kidney biopsy is done only when kidney involvement is suspected.

Stains

1. H&E → Pink homogenous appearance
2. PAS → PAS +ve · diastase resistant
3. CONGO RED → Most important stain *

Light microscopy (LM) → Brilliant Pink Colour of amyloid.

Polarized light → Apple green bire fringence

↓

is due to β pleated sheet conformation of amyloid.

Metachromatic stains

Crystal violet & methyl violet.

↓

Megenta coloured amyloid.

Thioflavin T AS

UV light

↘

Secondary fluoresence.

Immunohistochemistry

ORGAN → Site

Liver → 1st site is Space of Disse & causes pressure atrophy of hepatocytes.

ITO ↓ Storehouse of Vit A & synthesis collagen.

Kidney → 1st site is Mesengial matrix

Walls of capillaries of glomerulus, arteries, peripherial capillaries

↓

Sub endothelial deposits.

Heart → Sub endocardium

Between the Myocardial fibres

Will cause Arrythemias (bundle branch block)

Restrictive Cardiomyopathy

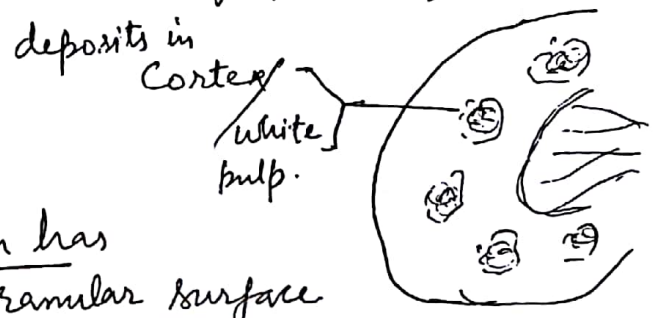
CCF

GIT ⟶ Anywhere from mouth to anus.

Tongue — Macroglossia
\ Amyloid tumor of tongue.

Spleen — Sago Spleen
\ Lardaceous Spleen

SAGO SPLEEN Amyloid deposition in white pulp in lymphoid follicles in cortex.



On cross section ⇓ Gray Translucent bodies like grains of Sago.

⇓
Spleen has granular surface

LARDACEOUS SPLEEN

Amyloid deposition in walls of splenic sinusoids ⟶ produces large map like areas of amyloidosis
(& Red Pulp)

LARD → animal Fat.

IMMUNITY

⇒ Self defence

Innate Immunity

- First line of defence
- Function - Prevention & erradication of infection.
- Innate immunity is non specific
- Innate immune response doesnot become better with each exposure
- No memory cells

Components

1) Epithelia - Skin, GIT, Respiratory Tract.
   - Mech Barrier
   - Produce antimicrobial substances eg: defensins
   - Intraepithelial lymphocytes

Adaptive Immunity

- 2nd line of defence
- Function - eradication of infection
- Adaptive immunity is specific
- Adaptive immunity response becomes better with each response
- Memory cells present.

Components

- T Lymphocytes → Cell mediated immunity
- B lymphocytes → Humoral immunity

2) Cells -

- Neutrophils } Extracellular bacteria & fungi
- Macrophages }

- NK cells – First line of defence against virally infected cells and tumor cells.
- Dendritic cells.
- Mast cells.

3) Plasma proteins

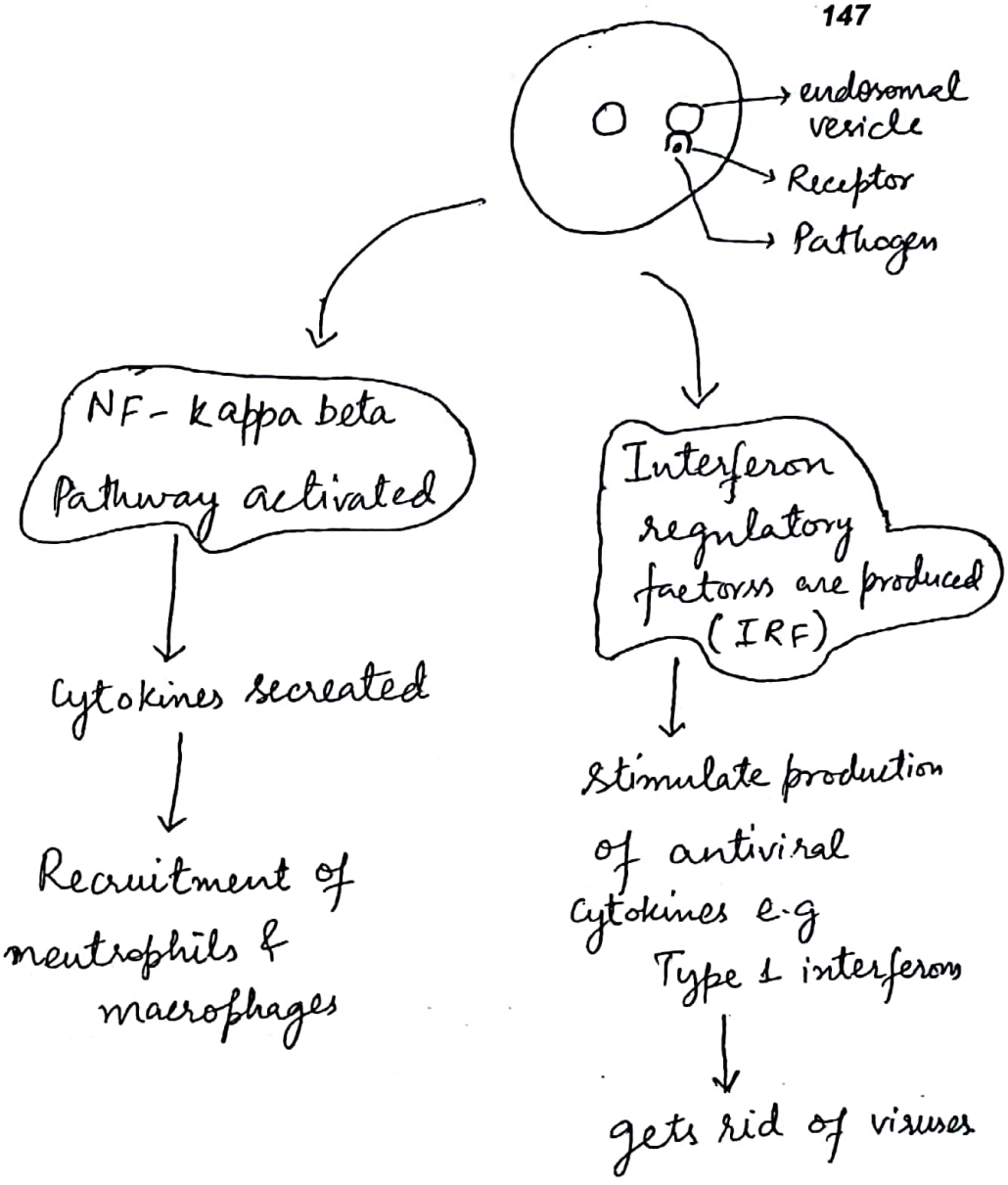
- Mannose binding lectin
- CRP
- Complement < Lectin pathway, Alternate pathway
- Lung surfactant

Pattern recognition receptors.

Cells that participate in innate immunity have receptors that recognize microbial components that are shared by microbes. These are called Pattern Recognition Receptors.

Location

Plasma membrane ✓
Endosomal vesicles ✓
Cytosol ✓



Pattern ~~Rago~~ Recognition receptors.

① Toll like receptors - (TLRs)

Found on — Plasma membrane
Endosomal vesicles

11 TLRs are recognized Till date

e.g TLR-2 — gram +ve bacteria
Fungus
Leptospira

TLR-4 — gram +ve bacteria

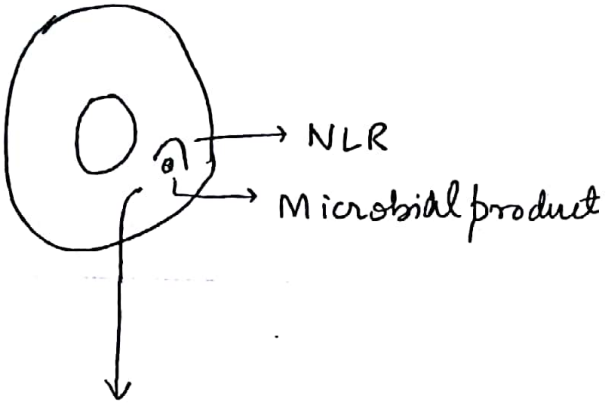
Founder member "TOLL" discovered in Drosophillia

2) NOD like receptors (NLR)

Location - cytosol

Recognize wide variety of substances

- Microbial substances e.g.; Shigella & Salmonella
- Metabolic biproducts e.g.; uric acid
- Recog. ion disturbance
- Necrotic cells.



Inflamasome (multi protein Complex) → activates Caspase I (IL-1β converting enzyme)

↓

IL - 1

↙ Fever

↘ Inflammation

⇒ Gain of function mutation in NLR gene leads fever periodic fever syndrome called auto inflammatory syndrome which responds to treatment with IL 1 antagonist.

③ C-type Lectin receptors

Found on plasma membrane

Detects Fungal glycans and leads to inflammation → gets rid of fungi.

④ Rig like receptors

Found in cytosol

Detects viral nucleic acid

↓

IRF are produced

↓

Type I interferons.

⑤ 7 transmembrane G protein coupled receptors

Recognize bacterial products ≥ N formyl methionyl residues → stimulates chemotaxis.

⑥ Mannose Receptors

Recognize mannose sugar in bacterial wall

↓

Stimulate phagocytosis.

Adaptive Immunity.

Lymphocytes are antigen specific

Mature lympocytes that have not encountered antigen or immunologically inexperienced are called

Naive Lymphocytes

Effector cells

Function- eliminate microbe

Memory cells

Live in a state of heightened awareness

Rapidly combat the microbe if it returns.

Lymphocytes are antigen specific - Lymphocytes of same specificity form a clone.

When an antigen enters, it selectively recruits antigen specific clone, this is called **clonal selection**.

Natural killer cells (NK cells)

Non B & Non T cells

Do not have TCR/BCR.

⇒ Also called large granular lymphocytes.

Function.

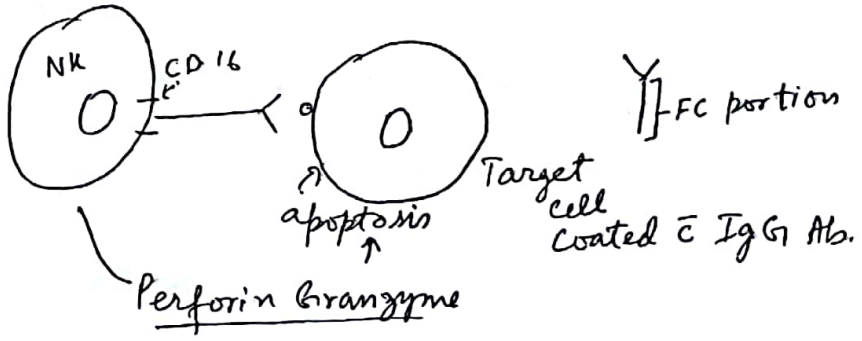
① Innate Immunity — First line of defence against virally infected cells and tumor cells.

② Adaptive immunity — Play a role in ADCC (antibody dependent cellular Cytotoxicity).

Location → Constitute 5-10% of P.B Lympocytes.

Markers → CD 16 → FC receptor for IgG Ab.

CD 56 → Function Not Known.

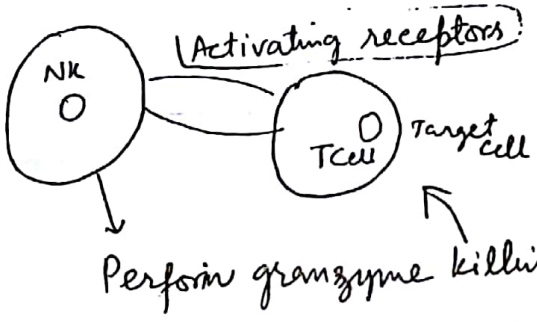


FC Portion of IgG Ab fits into CD16 on NK cells.
NK cells release perforins & granzyme & Kill the Target cell.

Role in Innate Immunity

- Kills virally infected cells & tumor cells.
- NK cells are not MHC restricted.
- NK cells have 2 types of receptors.

① Activating receptors
Activate NK cells to kill the target cells.
- Belong to NKG2D family.



- NK cells attach to target cells by activating receptors & kill the target cells by Perforin granzyme dependent killing.

② Inhibitory receptors.
Prevents NK cells from killing Normal cells.
Inhibitory receptors belong to

CD 94
family of Lectins

KIRS (CD96)
(killer cells Ig like receptors)

Cytokines produced by NK cells – IFN gamma.

↓

activates macrophages by classical pathway

Cytokines that regulate NK cell activity

- IL2
- IL15

Stimulates NK cell proliferation.

IL12

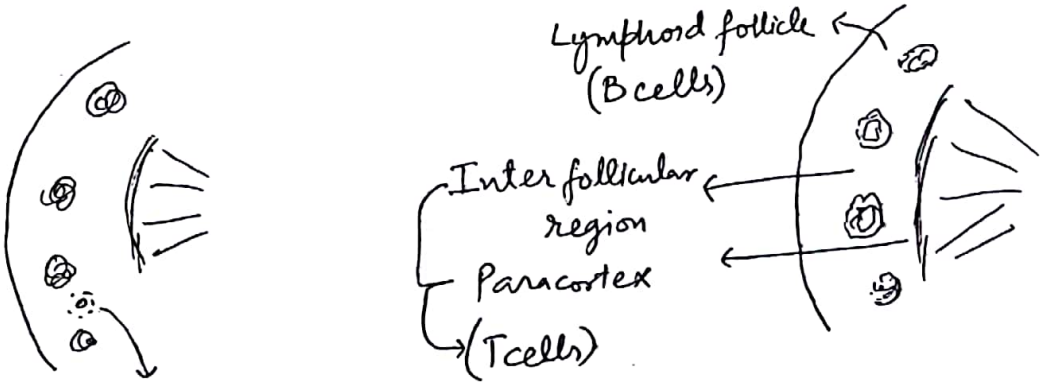
Activates killing & secreation of IFN gamma by NK cells

T cells

Play a role in cell mediated immunity.

Location - · Peripheral blood

60-70% of P.B. lymphocytes.



Periarterioral sheaths of spleen

⇒ Found in Paracortical, Interfollicular region of LN

⇒ Found in Paracortical region & periarterioral sheaths of spleen

T cell Markers

TCR (T cell Receptor)

CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD28.

TCR

- Antigen specific
- 2 types
  - αβ TCR – Found on 95% T cells
    - MHC Restricted.
  - γδ TCR – Found on 5% T cells.
    - Not MHC Restricted.
    - Found in the epithelia like skin, GIT, urogenital etc → provide protection against microbes that try to enter through the epithelia.
    - Do not have CD4 & CD8 on their surface. (γδ)

CD3

⇒ Signal transduction is function
→ Lineage specific T cell marker
⇒ Pan T cell marker

CD7

Pan T cell marker
Not lineage specific.

CD4
CD8 } Found on two mutually exclusive subsets of Tcells.
(CD4 : CD8 :: 2:1)

CD1a — Thymocytes & Langerhan cells.

Tcells

Helper Tcells
CD4 + Tcells
Master regulators

Function
- Help B cells to produce antibodies
- Help macrophages to kill or destroy microbes.

Cytotoxic Tcells
CD8 + Tcells
2nd line of defence against virally infected cells and
→ Tumor cells.
Kill Target cells by Perforin granzyme dependent killing.

Regulatory Tcells.
CD4 + Tcells.
FOX P3 +ve (Transcription Factor)

Function.
Regulate immune response & prevent reaction to self antigens.

Helper T cells

TH1 TH2 TH17

TH1

Cytokine produced – IFN gamma
(Biggest producer, Signature cytokine)

Function

IFN gamma
- Activates macrophages by classical pathway
- Stimulate B cells to produce IgG antibody.

Host defense against – Intracellular microbes

Role in disease – Chronic Auto immune disease
e.g.: IBD, Psoriasis

TH2

Cytokines produced
- IL 4 (signature cytokine)
- IL 5
- IL 13

Function

IL4 stimulates B cells to produce IgE antibody
Activates Macrophages by Alternate pathway

IL5 – Stimulates B cells to produce IgA Ab.
Activates mast cells & eosinophils.

IL 13 → Activates macrophages by alternate pathway
→ Activates epithelial cells to produce mucus

Host defense against – Helmeinthic parasites

Role in disease – Allergies.

TH 17

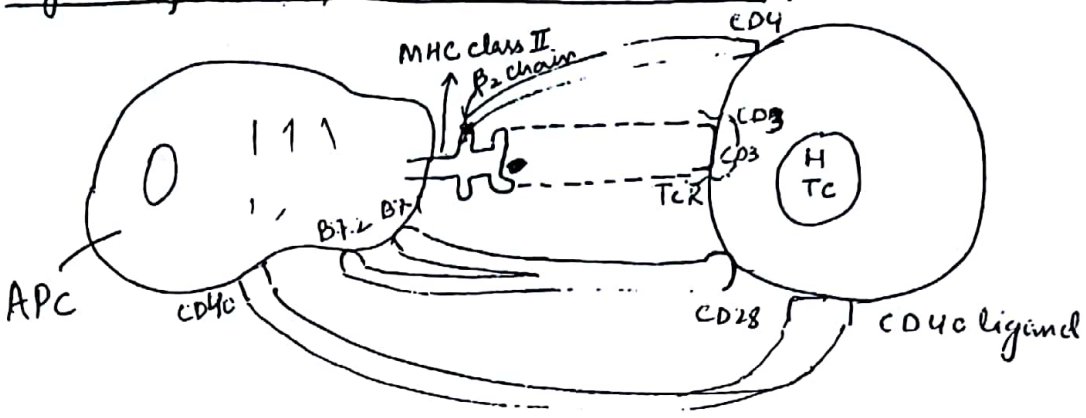
Cytokines produced — IL 17, IL 22, Chemokines

Function — Recruitment of neutrophils and monocytes/macrophages

Host defense against. — Extracellular bacteria & fungi.

Role in diseases — Chronic AID like IBD, Psoriasis & multiple sclerosis.

Signals for Helper T cell activation.



Signal 1

(a) TCR (Tcell) binds to antigen which is presented by APC in context of MHC class II

(b) CD4 of T cells attaches to β_2 chain of MHC class II

Signal 2 (also called- Co- stimulatory signal)

CD28 (Tcells) attaches to [B7.1 (CD 80) / B7.2 (CD86)] of APC

Signal 3

CD40 ligand (Tcells) attaches to CD40 on APC.

To stop T cell (helper) activation

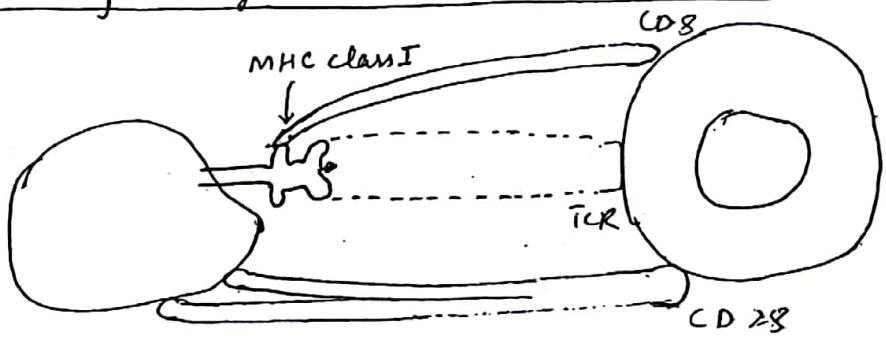
by co inhibitory receptors ← CTLA-4, PD-1

↓

Stops helper T cell activation

Belongs to CD28 family, blocks signals from TCR and CD28 & terminates T cell response.

Signals for Cytotoxic T cell activation.



Signal 1 (a) TCR (T cells) attaches to antigen that is presented by APC in context of Class I MHC

(b) CD8 (T cells) attaches to α_3 chain of MHC class I.

Signal 2 co stimulatory signal

CD28 (T cells) attaches to B7.1 CD80 / B7.2 CD86 of APC

→ CD8 T cell kills Target cells by Perforin granzyme killing.

B cells

Humoral immunity

Sites
- PB → Constitute 15-20% of PB lymphocytes
- Found in lymphoid follicles in LN, Spleen, Peyer's patches, BM, tonsils, etc.

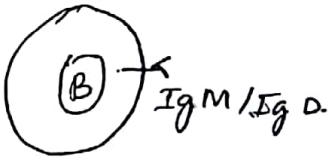
Bcell Markers → BCR (B cell Receptor)

CD19, CD20, CD21, CD22, CD23, CD10 (CALLA)

Igα (CD79a) Igβ (CD79b)

BCR — IgM/IgD antibody (Intramembranous)

- Antigen Specific.



Igα (CD79a)
Igβ (CD79b)
} Signal transduction (like CD3 of Tcells)

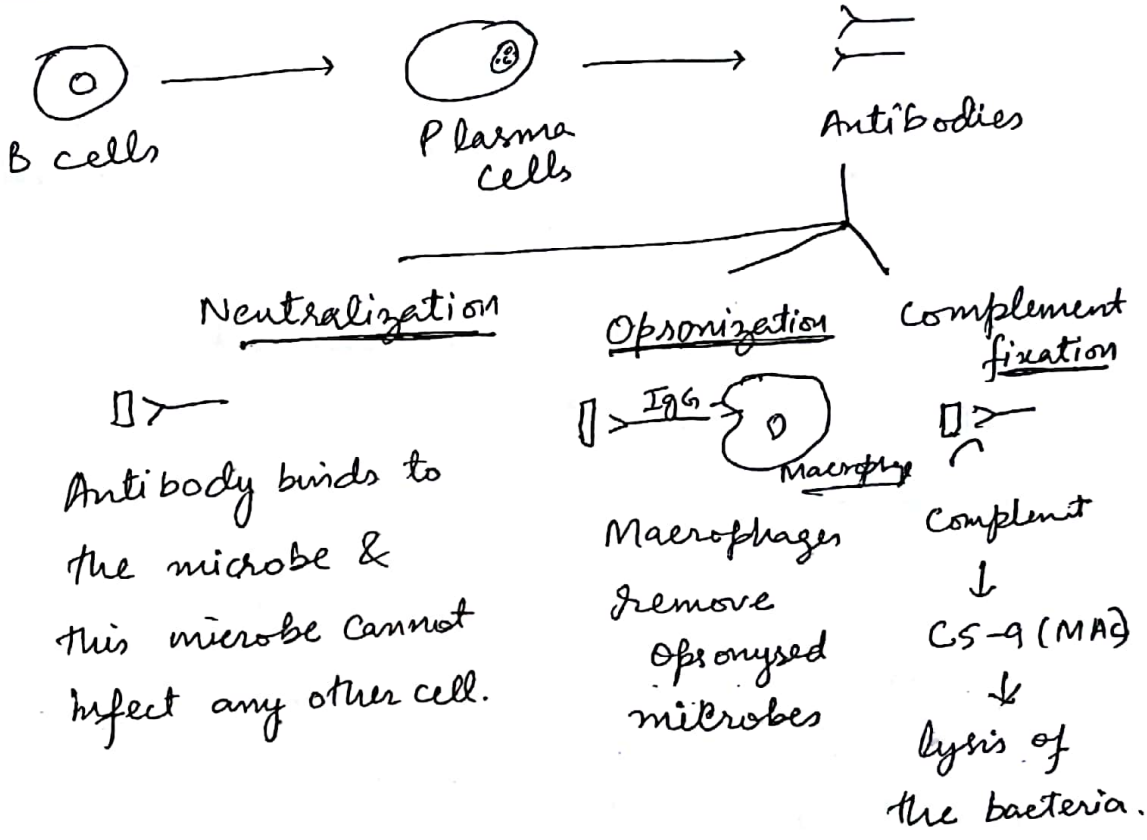
CD19 — Pan B cell marker
Lineage specific

CD20 — Lineage specific IHC

CD21
- Compliment Receptor (CR2)
- EBV receptor

CD40 site where B cells receive signals from Helper T cells.

Function



↳ IgG antibodies cross placenta and provide passive immunity to new born.

↳ IgE antibodies → Parasitic infection

↳ IgA antibodies are produced in the mucosal surface → provide protection on mucosal surface.

2 pathways for Ab production by B cells.

T Independent Pathway

Polysaccharide & lipid antigen occupy a number of antigenic deterinants (i.e BCR) on B cells

↓

B cells → PC → IgM

No help of T cells (HelperT) is taken.

T Dependent Pathway

Protein antigens stimulate B cells which bind to HelperT cells and then B cells produce

↓

IgG
IgA Class of Ab
IgE

Help from HelperTcells is taken.

This is called isotype or class stwitching.

Helper T cells also stimulate B cells to produce antibodies with high affinities for antigen. This is called as affinity maturation.

Dendritic cells

- Antigen presenting cells - Best APC.

Best APC because

① Located at the right place where antigens are encountered.

② They have fine hair like processes that trap antigens.

③ Each Rich in MHC class I & II.
& can present antigen to < CD4 Tcell / CD8 Tcell

④ They are also rich in Co molecule B7.1 & B7.2

Dendritic cells

Interdigitating DC

Present Ags to Tcells

Location -
Skin Langerhan cells.
Interstitia of organs
e.g Lungs, GIT, heart liver. etc.

Langerhan cells
CD1a
S100
Langerin (CD207)

Follicular DC

- Present Ags to Bcells
- Location -
In centre of Lymphoid follicles

E/M Birbeck granules in their cytoplasm

Zipper like

Tennis racquet like.

MHC antigens

RBC do not have MHC Ag.

MHC class I

HLA A

HLA B

HLA C

⇒ Found on all cells except RBCs

CD 8 T cells mount an immune response in context of class I MHC.

MHC class II

HLA DP

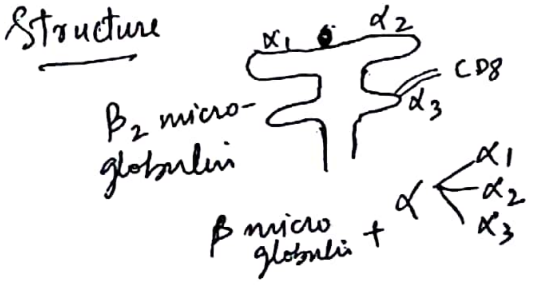
HLA DQ

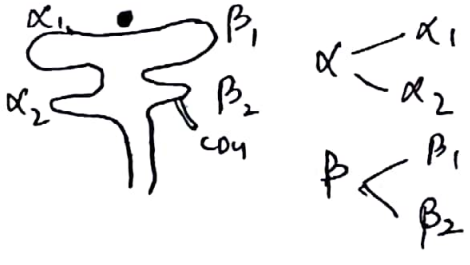
HLA DR

Found on APC i.e; dendritic cells, B lymphocytes, Monocytes/Macrophages

CD4 T cells mount an response in context of class II MHC.

Structure





Antigen binds in the cleft b/w α_1 & α_2

α_3 is the site of CD8 attachment.

Antigen binding cleft is b/w α_1 & β_1

β_2 is the site of CD4 attachment.

Genes for MHC antigen are found on the short arm of chromosome no. 6.

APC

Professional APC

- Dendritic cells
- Monocytes & Macrophages
- B cells

Non Professional APC

Thyroid follicular cells
Thymic epithelial cells
Fibroblasts
Glial cells
Endothelial cells.

Hypersensitivity Reactions

Injurious immune reactions are called Hypersensitivity Reactions.

Type I

Rapidly occuring reaction which occurs within minutes of binding of antigen to IgE antibody on the mast cells in a previously sensitized individual.

eg:

- **Localized** (ATOPY) [Genetic predisposition.]
  - Bronchial Asthma, Hay Fever
  - Allergic conjunctivitis, dermatitis & allergic rhinitis
  - Food allergies
- **Systemic**
  - Anaphylactic Shock
    - Drugs e.g Penicillin
    - Hormones
    - Antisera

Bronchial Asthma

Pollen Antigen binds to APC (dendritic cells)

↓

APC presents Ag to Naive Tcells

↓

TH2 Subset

↓

TH_2 binds to Bcells

↓

Bcells → PC → IgE Ab

IgE antibodies attach to the Fc receptor on mast cells.

↓

Pollen Ag causes cross linking of IgE antibody on mast cells

↓

Mast cells degranulate & release Mediators

Preformed/Primary

Brings about initial phase of Bronchial Asthma

C/F → Vasodilation
→ ↑ Vascular permeability
→ Bronchospasm.

⇒ Histamine, Lysosomal enzymes (Proteases) serotonin.

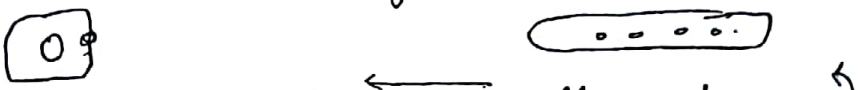
Newly synthesised or Secondary mediators

- Bronchospasm
- Leukocytic infiltration
- Epithelial damage

⇒ PAF, AA mediators (LTB4, LTC4, LD4, PGD2) Cytokine, Chemokine

Type II

- **Antibody Mediated** (IgG/IgM)
- Brought about by antibodies which are directed against fixed antigens



Antigen can be fixed on cell membrane or Connective tissue.

(a)- Antibody attaches to fixed antigen on cellmembrane & causes destruction of Target cell.

(or)

(b)- Antibody attaches to fixed antigen & causes disregulation of function of target cell.

e.g Grave's Disease
Myasthenia gravis

Type V (in Micro) & Type II here

Destruction by — Opsonisation
Compliment fixation

Antibody attaches to Basement membrane
↓
Activate Compliment
C3a C5A
↓
Enzymes released
↓
Breakdown of Basement membrane
↓
Good Pasture Syndrome

e.g. AIH Anemia.
AI granulocytopenia
AI Erythroblastosis fetalis.
Mismatched blood Transfusion reaction.
Pemphigus vulgaris
Pernecious anemia (antibodies against Parietal cells)
Aute Rheumatic Heart disease

↓

No IF
↓
Meg. Anemia.

Type III

[Called Immune complex disease.]

Antigen is not fixed.

3 stages

① Formation of immune complexes (Ic)

Antigen —5days/1week→ IC

Most pathogenic complexes are
- Small/medium size
- Found in antigen excess

② Settling down of IC

Favoured site → Kidneys
- Joints
- Walls of Blood vessels
- Skin, etc

③ Tissue destruction by Abs

Activation of Complement
Activation of Hageman factor.
↓
Tissue destruction

eg.
- Localized → Arthus reaction
  - → APSGN, GN (T. pallidum)
  - → Reactive Arthritis (Yersinia)
- Systemic
  - ① → Serum sickness
  - ② → SLE
  - ③ → PAN (Medium vessel vasculitis & IgG Containing IC are deposited in vessel wall).
  - ④ HSP (Small vessel vasculitis IgA containing IC are deposited in vessel wall)

SLE → All visceral lesions are type III rxn (IC disease)
→ All hematological lesions are Type II — AIHA, AI granulopenia, AI Thrombocytopenia

Type IV

Brought about by T cells (Cytokine mediated)

- CD4 T cells
  - Delayed HR
  - e.g; Tuberculin Reaction
    Immune granulomas
    Contact dermatitis
- CD8 T cells
  - Destruction of Virally infected cells & tumor cells.

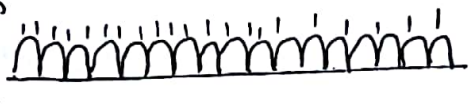
Both CD4 & CD8 ⟹

1. Rheumatoid Arthritis
2. IBD
3. Type I DM
4. Psoriasis
5. Multiple sclerosis.

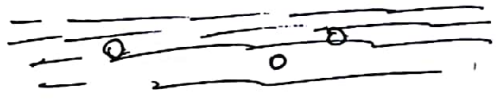
Acute cellular graft Rejection

Graft versus Host disease.

Respiratory System

A  epithelium

B  Mucus gland layer

C  Cartilage

$$\text{Reid's Index} = \frac{B}{A+B+C}$$

N = 0.4
↑ sed = Mucus gland layer

Asthma - Airway Remodelling

1. Sub Basement membrane fibrosis [Type III and Type I collagen]
2. Mucus gland hyperplasia
3. ~~Goblet cell metaplasia~~
3. Goblet cell metaplasia
4. Smooth muscle hypertrophy + hyperplasia

ARDS

[Respiratory failure occuring ⊆in 1wk of a known clinical insult ⊆ b/L opacities on chest imaging, not fully explained by effusion atelectasia cardiac F. or F. overload]

M/C/C — Sepsis > Pneumonia

IL-8, IL-1 and TNF α is involved.

↓

(Chemokine for neutrophils) → So, neutrophils are M/I culprit responsible for ARDS.

→ IL 8 rises ⊆in 30 minutes of injury

→ Cells damaged - endo & epithelial cells

leakage of fluid + cellular debris + leaked plasma proteins

↓

Hyaline

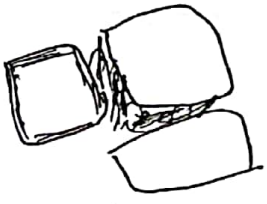
↓

Along the alveoli

↓

making membrane

→ Diffused alveolar damage - Histological Hallmark (DAD)



Coal worker's Pneumoconiosis

→ Pneumoconiosis → To describe non-neoplastic lung reaction to inhalational if mineral dust mainly at workplace ; also includes organic as well as inorganic particulates, chemical fumes & vapours

MC - Silicosis (quartz)

↓

Collagenous nodule in lung ⇒ egg shell Calcification (lymph nodes classically)

Asbestos Related Disease

① Pleural Plaque (MC manifestation)
② Pleural effusion
③ Diffuse Pul. fibrosis → [Asbestosis]
④ Lung Ca – [MC Ca in asbestos exposure]
⑤ Mesothediona → (Most specific Ca) (25-40 yrs)
⑥ Laryngeal Ca
⑦ Ovarian Ca
⑧ Colon Ca
→ Latent period (10-15 yrs)

Giant Cell → Golden brown fusiform/beaded particles

ASBESTOS BODY [Coated c Iron & Proteinaceous material
↓
Perls' Stain +ve

Any other particle coated c Iron & proteinaceous material
↳ FERRUGINOUS BODY

In Anthraconosis → Pigment laiden macrophages are seen

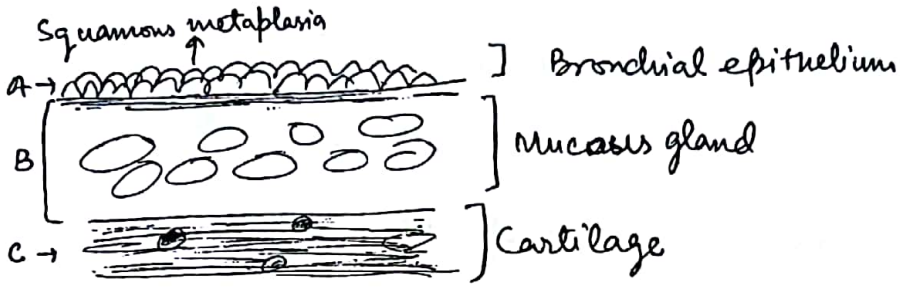
Coal Macule → Aggregated Macrophages (1-2mm)
↓
Coalese to form Nodule
↓
Progressive massive Fibrosis of lung parenchyma

↳ Risk of cancer is more in domestic use of coal i.e; Bituminous coal.

Chronic Bronchitis

MC cause – Smoking

Earliest change – MUCUS HYPERSECREATION



$$\text{REID'S Index} = \frac{B}{A+B+C}$$

done in autopsy (N) = 0.4

↑ed in → ↑ Mucous gland.

Manifestations in Pneumoconiosis

(1) Localised fibrous plaques, or rarely diffuse pleural fibrosis (MC menifestation)
(2) Recurrent Pleural effusion
(3) Parenchymal interstitial Lung fibrosis (Asbestosis)
(4) Lung Ca (MC)
(5) Mesothelioma (most specific)
(6) Laryngeal, ovarian, colon Ca.
(7) ↑ risk of autoimmune disease
(8) CVS disease.

LUNG Ca.

Histological classification (WHO 2015)

① Adeno Ca
- Acinar, papillary, Micropapillary, Solid, lipidic, predominant, Mucinous subtypes.

② Sq cell Ca
- Non keratinising
- Keratinising
- Basaloid

③ Large cell Ca

④ Neuroendocrine Ca
- Small cell Ca
- Large cell neuroendocrine Ca
- Carcinoid Tumor

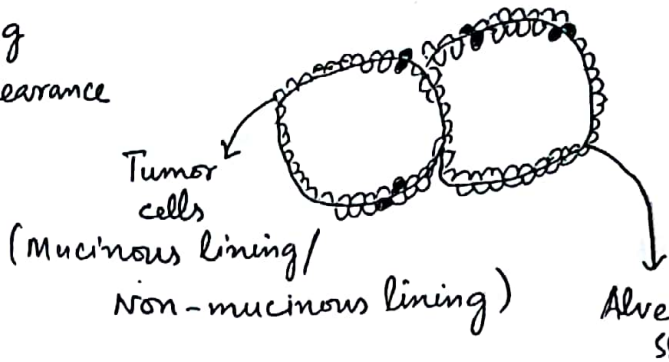
Adeno Ca
- Pre invasive lesions → Atypical Adenomatous Hyperplasia
  → Adeno Ca insitu
- Minimally invasive Adeno Ca
- Invasive Adeno Ca

Adeno Ca Insitu (AIS)

Previously known as Bronchoalveolar Ca.

→ < 3cm
No stromal invasion
No Pleural invasion
No Lymphovascular invasion.

⇒ Butterfly sitting on Fence Appearance



(No desmoplastic Stroma) LEPIDIC PATTERN OF GROWTH

Minimally Invasive

< 5mm stromal invasion & < 3mm in size.

No pleural invasion

No lymphovascular invasion.

→ Basophilic Psamoma bodies

Squamous cell Ca → Keratinising (New) → Eosinophilic

→ Non keratinising

→ Basaloid (New)

Neuroendocrine Origin

Histology — IHC — E.Microscopy.

Salt & Pepper Chromatin/ Stippled Chromatin

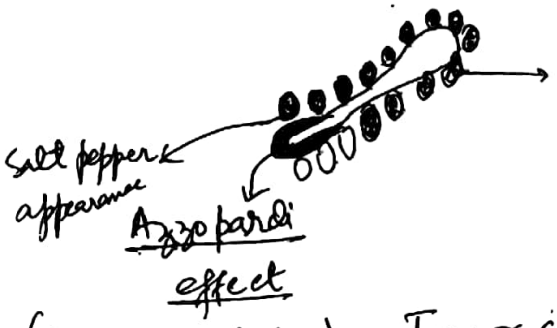
Synotophysin, Chromogranin & CD56, CD57 +ve

Dense Core Neurosecretory granules

In High Gr- Hyperchromatic

Small cell Carcinoma

[Oat cell carcinoma]



Tumor cells have a high turnover & and are fragile ⟶ dissupt easily

↓

Their DNA gets encrusted upon vessel wall [BLUE]

Purplish Blue Powdery discolouration of vessel walls

⇓

Azzopardi effect

Carcinoid [Carcinoma like epitheloid tumor]

	Grade	Mitotic count
Typical	I	< 2/10 HPF
Atypical	II	2-10/10 HPF

HPF = High Power Field
~ 40x

Fibrous septae making nests of ~~cytoplasm~~ Tumor cells.

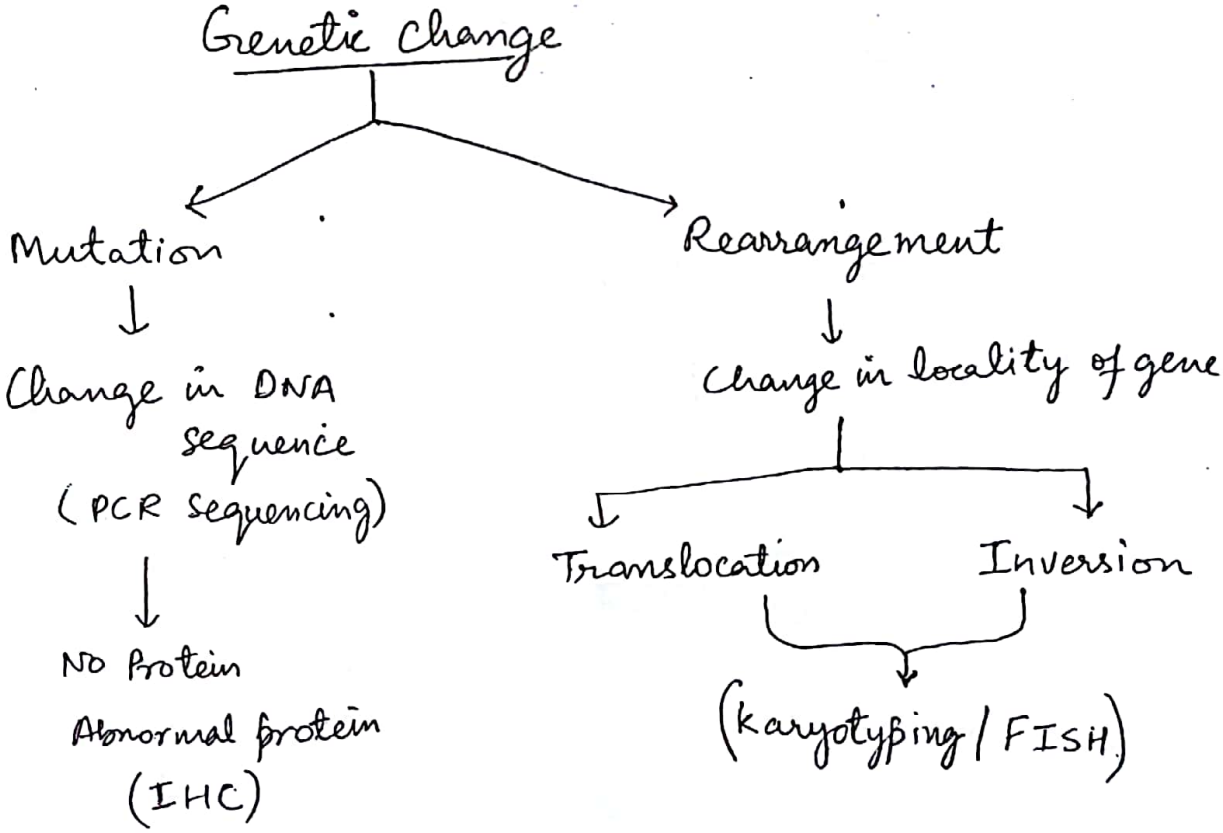
Tumor genetics

Small cell	Squamous cell	Adeno
Rb mutation	P53 mutation	EGFR mutation > amplification
P53 mutation	EGFR ~~mutation~~ amplification (more than mutation)	KRAS mutation
myc amplification	FGFR$_2$ amplification	ALK rearrangement

Amplification is ~~checked~~ by FISH

↓

Overexpression is checked by IHC



Due to Translocation & inversion

① gene 1 + gene 2

↓ Transcription

(Fusion Transcript)

(Fused mRNA) (RT PCR)

↓

(ONCOPROTEIN)

(IHC) [↑ed function than before] → e.g BCR ABL

or

[↓ed function than before]

② gene 1 gene 2 (promoter)

↓

gene 1 ↑↑ed (FISH)

(overexpressed)

↓

Overexpression (IHC)

FISH

A
B
] Normal

Deletion or Monosomy

Trisomy

Amplification

Fusion

FISH INTERPRETATION

ALK [Anaplastic lymphoma kinase]

Receptor Tyrosine kinase

- Rearranged
  - Anaplastic large cell lymphoma (ALCL)
  - ~~Lymphoma~~ Lung Adeno Carcinoma
  - Inflammatory Myofibroblastic tumor
- Mutated
  - Neuroblastoma
  - Hereditary Haemorrhagic Telangiectasia Type II

In lung Adenocarcinoma

EGRF & ALK can be targetted

[EGRF & KRAS are mutually exclusive]

In non smokers & women → EGRF mutation.

IHC of lung Cancer

	Small cell	Squamous	Adeno
Thyroid Transcription Factor (TTF-1)	+	−	+
NAPSIN-A	−	−	⊕
Other markers	Synaptophysin Chromogranin CD56/CD57	P63	CK7 + CK 20 −

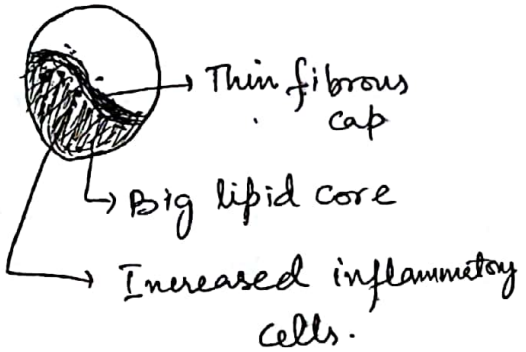
	Mesothelioma	Adenocarcinoma
PAS	−	+
Carcinoembryogenic antigen	−	+
TTF1	−	+
NAPSIN A	−	+
CK7	(+)	(+)
CK5/6	+	−
Calretinin	+	−
WT1	+	−
Electron microscopy	Long slender microvilli	Short Stubby villi

Adenocarcinoma	Squamous cell carcinoma	Small cell carcinoma
- Most common lung Cancer world wide In women In non smokers	- Most common lung Cancer in India in Smokers	- Strongest association with smoking.
- Peripheral location	- Central location	- Central location
- Most common gene mutated is KRAS	- Most common para-neoplastic syndrome is – Hypercalcemia	⇒ Most common Para-neoplastic syndrome. is SIADH.
⇒ Most common Para-neoplastic syndrome is Hematological	⇒ Cavitation (Also in large cell ca)	⇒ SVC obstruction LAMBERT EATON Synd.
⇒ Clubbing is seen	⇒ Pancoast tumor ↓ HORNER's Syndrome	⇒ Worst Prognosis ⇒ Max Risk of Metastasis ⇒ Clubbing is Rare.

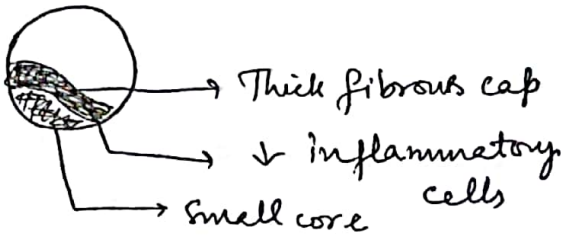
Lung cancer most commonly metastasises to BRAIN & most specifically to Adrenals.

VASCULAR PATHOLOGY

(1) **Atherosclerosis** : Atheroma/Atheromatous plaque.



VULNERABLE PLAQUE



STABLE PLAQUE

(2) **Monckeberg Medial calcific Sclerosis**

- No luminal obstruction
- clinically insignificant

3. Arteriosclerosis :→ Hypertensive changes.

Site → arterioles

Benign

Hyaline Arteriosclerosis

- Wall thickening
- Narrowing of lumen
- Kidney → Benign Nephrosclerosis
- Granular kidney: Leather Grain Appearance

~~Kidney → Malignant~~

Malignant

Hyperplastic Arteriosclerosis

PAS stain → Onion skin appearance

↓

New Basement membrane

Kidney → Malignant Nephrosclerosis

Pinpoint Hemorrhages

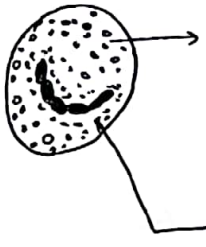
↓

Flea bitten Appearance

Vasculitis → Infectious
→ Non Infectious (Immune)

Mechanisms

1. Anti endothelial or Anti Smooth muscle antibody
2. Immune complex deposition
3. T- cell Mediated Response (granulomatous)
4. ANCA (Anti neutrophilic cytoplasmic Antibody)



→ cytoplasmic : Proteinase-3 (PR3) (diffused)
Antibody against these (c ANCA or PR3 ANCA)

→ Perinuclear - Myeloperoxidase (MPO)
Antibody against these (p ANCA or MPO ANCA)

Leucocyte Activation → Surface exposed to these granules
↓
If ANCA Present (Susceptible individuals)
↓
Binds to the surface granules
↓
Degranulation of Neutrophils
↓
Enzyme Leakage → Tissue damage.

CHAPEL HILL CONSENSUS CLASSIFICATION

Large Vessel
- Giant cell arteritis (Pulse less disease)
- Takayasu arteritis

Medium vessel
- Polyarteritis Nodosa
- Kawasaki disease

Small vessel

Immune Complex vasculitis
- IgA Vasculitis (Henoch schonlein Purpura)
- SLE

ANCA associated vasculitis
- ⇒ Granulomatosis with Polyangiitis (Wegner's Granulomatosis)
- ⇒ Eosinophilic Granulomatosis with Polyangiitis (Churg Straus Syndrome)
- ⇒ Microscopic Polyangiitis

& Variable vessel
- Becket's disease
- Cogan syndrome.

Giant cell Arteritis

> 50yrs

Head & Neck arteries

Temporal – Headache (MC)

Facial → Jaw claudication

Ophthalmic → Blindness

[Most specific]

Diagnosis

Biopsy → Segmental involvement ∴

3–5cm → adequate biopsy Required.

or

at least 1cm

Takayasu Arteritis

< 50yrs

Aorta & Branches

↳ [Aortic arch]

Most commonly: Subclavian

② Common carotid

③ Abdominal aorta

∴ ④ Renal

⑤ Aortic arch or root

∴ ⑥ Coeliac axis

⑦ Coronary

& ↳ (least common)

Diagnosis

Arteriography.

CT Angio

Etiology

Anti endothelial &

Smooth cell antibodies

(?)

Cytokine mediated Response

Granulomatous

Inflammation

Histopathology.

Giant cells (granulomas)

Smooth muscle

Intimal hyperplasia.

Internal Elastic lamina

(Fragmentation)

[Van Geison Stain for Elastin]

Polyarteritis Nodosa (PAN) [Mc - Kidney involved]

(P)AN
(P)ATCHY → (entire circumference of vessel wall is not involved)
(P)ULMONARY ARTERY SPARED
[Bronchial vessels may be involved]

Features

Transmural (Through out wall) : Aneurysms, Fibrosis

Necrotising : Fibrinoid Necrosis

Inflammation : < Acute, Chronic] All stages of activity can be seen at the same time (ongoing Injury)

Associated ⊂ Hep. B. → Immune complex deposition.

⇒ The pathology in the kidney in classic polyarteritis nodosa is that of arteritis without glomerulonephritis

⇒ May involve bronchial vessels but not pulmonary vessels.

⇒ Renal & visceral arteries are very commonly involved. (Musculoskeletal)

⇒ No granulomas. No eosinophilia

Giant cell Aortitis → Aortoarteritis

Kawasaki Disease

- < 5 years
- Anti endothelial cell antibodies ↑
- Acute Necrotising Vasculitis (Transmural)
- Febrile illness
- Strawberry Tongue
- Cervical lymphadenopathy
- Mucocutaneous ulcers

⇒ MUCOCUTANEOUS LYMPH NODE SYNDROME.

Strawberry cervix → Trichomonas infection

Cholesterolosis → Strawberry Gall bladder

- Most common artery involved ⇒ Coronary → Thrombosis, MI, Aneurysms } MC cause of cardiac mortality in Children.

ANCA associated

Wegener's

- c ANCA > p ANCA
- URT + LRT involvement
- Renal involvent
- Necrotising &/or granulomatous vasculitis

Churg Strauss

- p-ANCA
- Asthma
- Allergic Rhinitis
- Nasal polyps
- Peripheral eosinophilia
- Necrotising &/or granulomatous vasculitis.

MPA (Microscopic Poly-Angiitis)

- Hypersensitivity or P ANCA
- LEUCOCYTOCLASTIC VASULITIS ↓ (WBC – Breakdown) ↳ Apoptotic Neutrophils.
- NO granulomas
- Fibrinoid Necrosis

URT – Upper Respiratory Tract involvement

Vascular Tumors

↓

Endothelial cell origin — IHC → PECAM / CD31

Benign

Hemangioma

& Capillary Hemangioma

Cavernous Hemangioma

Boderline

Kaposi sarcoma

HHV-8

1. Classical
2. Endemic (Africa)
3. Transplant associated
4. Immunodeficiency associated

Malignant

Angiosarcoma

1. Hepatic

Exposure to Poly vinyl chloride & Thorium contrast dyes

2. Cardiac

Most common cardiac primary ↑ malignancy in adults.

Microabscesses (Misc)

- Neutrophil Microabscesses in TAO
- Neutrophil crypt abscess in IBD
- Pautrier's Microabscess (Tumor lymphocytes in mycosis Fungoids)
- Munroe Microabscess of Neutrophils in Psoriasis

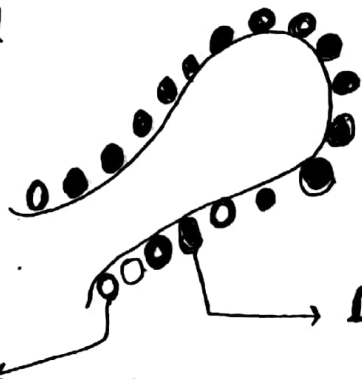
Thyroid Cancers

- **Follicular origin**
  - Papillary : most common
- **Parafollicular origion (C-cells)**
  - ↓
  - CALCITONIN
  - ↓
  - **Medullary**
    - Amyloid (ACal)
    - CEA
    - MEN2 syndrome

Papillary Ca Thyroid

Most common subtype | overall
Children
Thyroglossal cyst
Post Radiation
Hashimoto Thyroiditis

⇒ Lymphatic Spread
⇒ Best prognosis



- Chromatin margination
- Optical clearing of nucleus

ORPHAN ANNIE – EYE NUCLEI

Coffee Bean Nuclei
[Granulosa cell Tumor
also found in
Brenner Tumor
Chondroblastoma
Langerhan cell Histiocytosis]

Follicular Ca

Most common subtype to arise in multinodular goiter

⇒ Capsular or vascular invasion

⇒ Cytology (FNAC) will not help

⇒ Histopathological examination (No biopsy is done) → FNAC
↳ also in Testicular mass.

↓

[Hemithyroidectomy to be done]

⇒ Hematogenous spread (Bones & lungs)

Anaplastic Ca

Bizarre cells

Fibrosis ↑↑

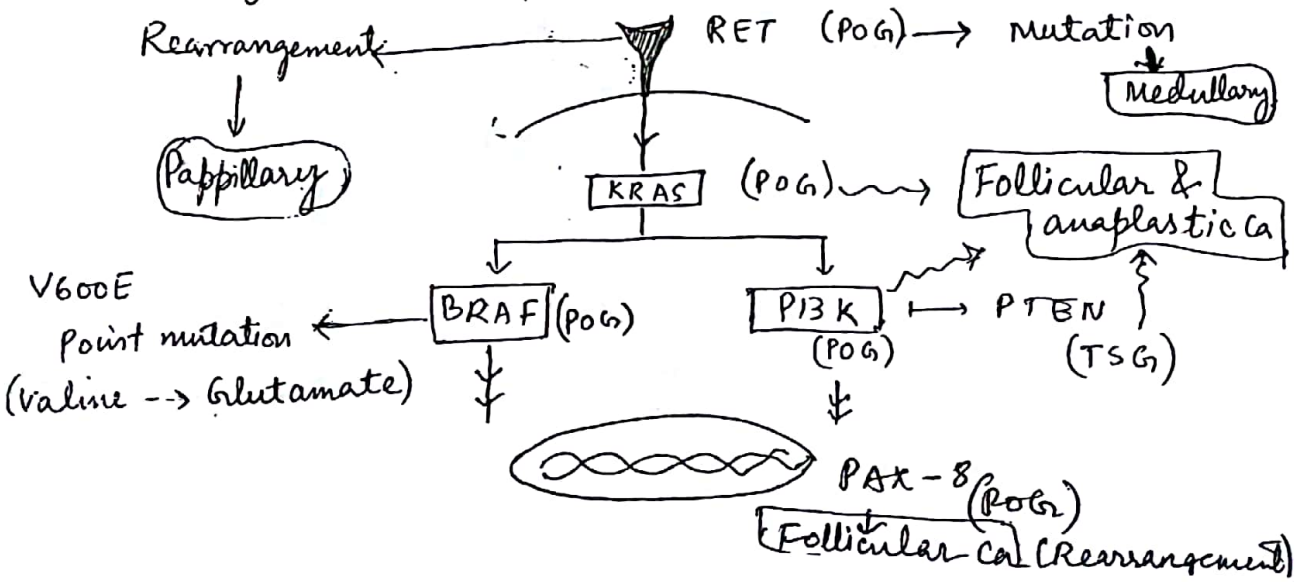
Least common subtype

Most aggressive (rapid onset)

Worst prognosis.

POG → Proto onco gene
TSG → Tumor suppressor gene

Tumor genetics



Most common genes

Papillary : BRAF

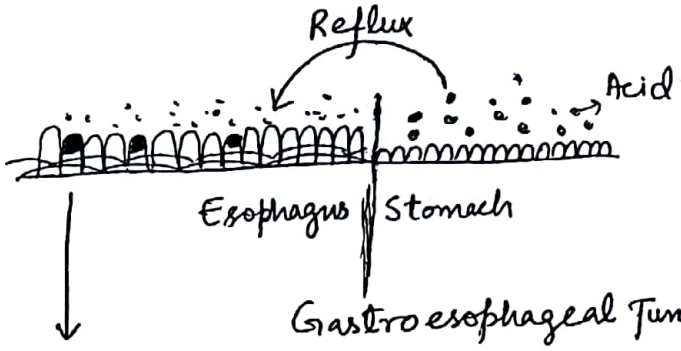
Follicular : KRAS

Anaplastic : p53

Medullary : RET

GIT

Esophagus



Columnar cells
+
Groblet cells ⟶ Acid mucin]→ Alcian blue Stain (Q)

↓

Intestinal metaplasia ⟶ BARRETS Esophagus (Q)

Gross → Red velvety patches in esophagus.

Metaplasia ⟶ Dysplasia ⟶ Anaplasia (Irreversible)

↓

[Some serotypes of H pylori are associated ↓ decreased risk of esophageal adeno.ca. because they cause gastric atrophy → ↓ acid → ↓ Barrett's esophagus] [Adeno-Carcinoma]

Gastro Intestinal Stromal Tumor (GIST)

Origin: Interstitial cells of cajal
(Pacemaker cells in Muscularis propria)

Gross: Nodular or Capsulated tumor
Arising from the wall
Mass effects
Cut section → Fleshy Tumor (Grey Tan) with hemorrhage & necrosis

Stroma ↓ Vimentin – Mesenchymal marker

Microscopy → [Spindle cell (most common)
Epithelioid
Mixed]

Genetics

85% c kit mutation → AML, Seminoma, Mastocytosis

c kit → Receptor Tyrosine kinase (RTK)

Imatinib → Inhibitor →

~ 8% PDGFRA mutations

[c kit & PDGFRA mutations are mutually exclusive]

In a small proportion of non c kit & non-PDGFRA mutated GIST → SDH mutation*

SDH Deficient (Succinate Dehydrogenase)

*Exclusively seen in
↳ Gastric In Location
↳ Indolent Course (slow growing)
↳ Younger Population
↳ Imatinib Resistant
↳ Part of Carney Stratakis Syndrome [Paragangliomas]

Carcinary Triad → GIST, Pulmonary Condroma (Hamartoma), Paraganglioma.

IHC → CD117 (c kit) : +ve (most sensitive)

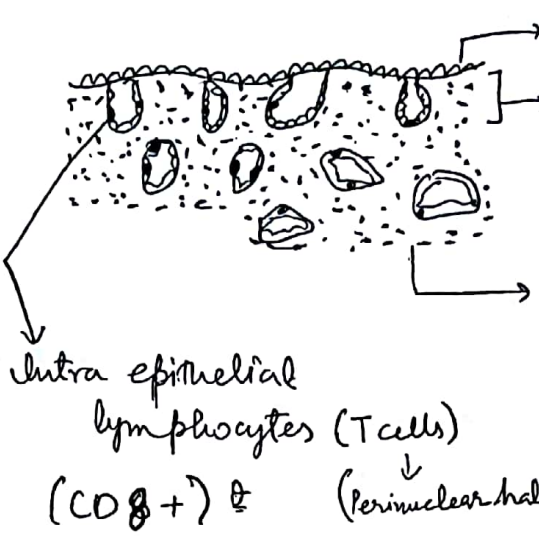
DOG 1 : +ve (most specific)

Myxomas, Skin Lesions, Multiple endocrine involvement

*Prognosis → Location (stomach : better)
Size (< 5 cm : better)
Mitotic count (counted per 50 HPF)

Different cut offs for different sizes at different locations.

Celiac Disease



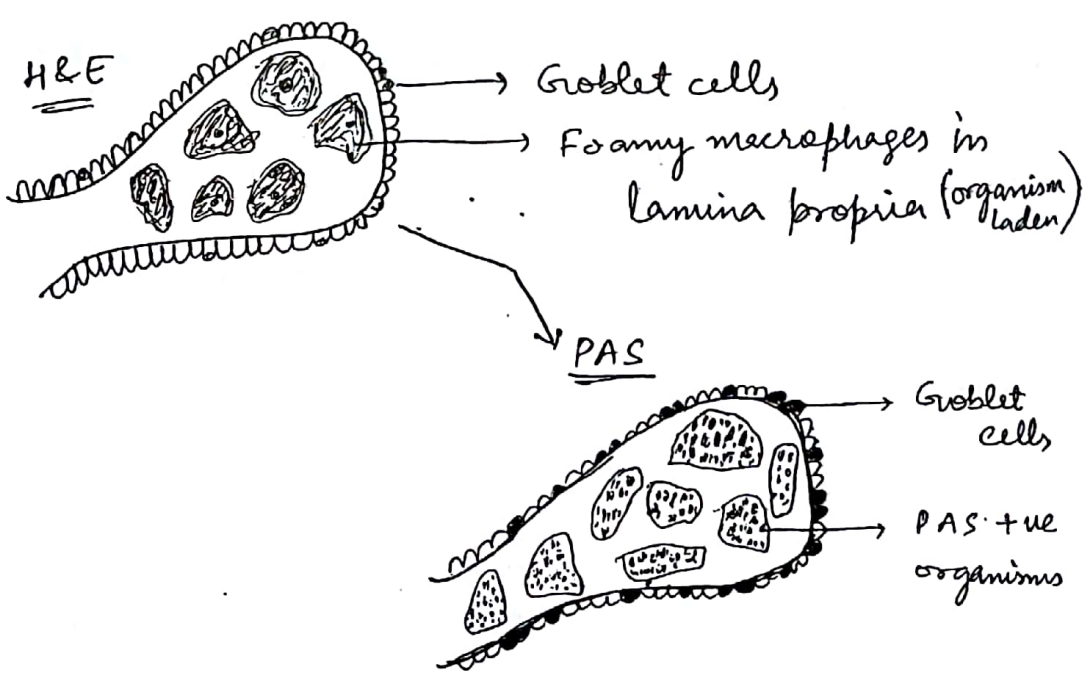
Risk of enteropathy associated T cell lymphoma is high (EATL)

- Adenocarcinoma Risk also increased because of crypt Hyperplasia
- Associated ⊂ HLADQ2 > DQ8

- IgA Nephropathy
- Dermitiis Herpetiformis

Whipple's Disease

(Malabsorptive syndrome)



Intestinal polyps

Non Neoplastic

- Hyperplastic
- Inflammatory (MC)
- Hamartometous ⇒ Indigenous to that organ ± Disorganised mass
  ↳ Now considered Neoplastic
  - Choristoma
    - Normal tissue
    - Abnormal site

Neoplastic polyps

Adenomatous / Adenomas

DYSPLASIA

Peutz Jegher Polyp

Christmas Tree appearance

Multiple PJPs + Mucocutaneous melanosis

⇓

Peutz Jegher Polyp (STK 11 gene)

aka – LKB1

Juvenile Polyp.

Solitary → No risk of cancer

Multiple → Juvenile polyposis
↓ Syndrome
Slight risk of cancer

BMPR 1A – (Primary Pulmonary hypertension)

SMAD 4 – (Pancreatic Cancer)

Lymphoma – MC primary → MALToma (extranodal Marginal zone Ly
↳ H Pylori associated

CD5–, CD10–
CD23–, CD43+

Adenomas / Adenomatous polyps

[Tubular] (mc)

(Villous)

(Tubulovillous)

Familial Adenomatous Polyposis (FAP)

- APC gene mutation
  - Most commonly: Tubular Adenomas
  - Diagnosis > 100 polyps
  - Attenuated FAP < 100 polyps. (variant)

FAP + Skull osteomas
+ Epidermoid cysts
+ Desmoid Tumor (fibromatosis)

→ GARDNER SYNDROME

FAP + Medulloblastoma (& More common) & Glioblastoma] Turcot syndrome &

Colo Rectal Ca

Adenoma - Carcinoma Sequence.

Normal colonic epithelium → (loss of APC & [gate keeper of colonic neoplasia]) → Hyper proliferation → (epigenetic changes) → Early adenoma → (KRAS & NRAS mutations) → Late adenomas → (Loss of P53.) → Adenocarcinoma

Others: EGFR, BRAF &

Right sided colon

Diameter is more → space for tumor to grow → Cauliflower/fungating/Polypoid

↓

Loose consistency stools → easily pass through

↓

Causes erosion of tumor → Bleeding ← (Fecal occult blood test)

↓

Iron deficiency Anemia

Left sided Colon

Diameter is less → No space to grow → Constriction (Napkin Ring constriction)

Bulky stool → Cannot pass easily

↓

features of Intestinal obstruction

Hereditary Non Polyposis colon Cancer (HNPCC)
or LYNCH SYNDROME

Defect in DNA mismatch Repair gene
(MSH2, MLH1)

↓

DNA mistakes accumulate → genomic Instability

[Especially microsattelites (Tandem sequences)] ← **MSI**

[Microsatellite Instability]

Colon Cancers	Extracolonic Cancers
- Mean age ~ 40yrs	Endometrial (MC)
- Proximal to splenic flexure	Gastric
- Mucinous/signet ring cell morphology	Ovarian
- ↑sed lymphocytes in tumor	Transitional
- Better prognosis	Small intestinal

Pancreatic Carcinoma

MC subtype → Ductal Adenocarcinoma

Very aggressive

Procoagulant Tumor mucin + cellular debris

↳ DIC risk is increased

→ Non bacterial Thrombotic endocarditis

→ Migratory thrombophlebitis
↓
(TROSSEAU SYNDROME) → also seen in Breast Ca, Gastric Ca, Lung Ca

Normal Pancreatic epithelium

KRAS ~90% (Oncogene)

CDKN2A/P16 ~95% (TSG) → (Protein)

→ Pancreatic epithelial Neoplasia (PanIN)

SMAD4, BRCA2, P53 } TSG

→ Adenocarcinoma

Most common gene Mutated – CDKN2A

Most common Protooncogene mutated – KRAS.

Most common TSG mutated → CDKN2A/P16

[KRAS changes occur earlier in the Progression]

Chromosome locations (Misc)

BRCA1 – 17q

P53 – 17p

APC – 5q

HFE – 6p

SERPINA1 – 14q

13q – BRCA2, Rb, ATP7B.

MET (Hepatocyte Growth Factor Receptor) – 7 Chr. ↦ Papillary RCC

Mallory Dank Bodies

Mallory Dank Bodies / Mallory Bodies

↓

Remnants of intermediate filaments. Q

following hepatocyte damage.

↓ cytokeratin Q

↓

CK 8/18 Q

→ Eosinophilic Inclusions.

Prognostic Markers for tumors

Stage

Esophagus
Stomach
Gall bladder
Pancreatic
Prostate
Testis

→ T. Stage ↓ Depth of invasion.

Lymph Nodes

Breast (Axillary)
Colon
Penile (Inguinal)
Head & neck

Others

Metastatic Breast
- ER/PR status

Renal
- Pathological Stage

Wilm's
- Histology (Anaplasia)

Soft tissue Sarcoma
- Grade

Melanoma
- Depth

Barrett's Oesophagus → AdenoCa

Early genetic Changes

P53 (TSG)
CDKN2A/p16 (TSG)

Late genetic changes

EGFR (erbb1)
Her 2 Neu (erbb1)
MET
cyclin D1

} Proto oncogenes [POG]

Cardiac Pathology

Myocardial infarction

Coronary Artery Spasm → Hypoxia damage → Myocardial response (Ischemic Injury) ↓ ATP

→ in 30 minutes Reversible (20-40 min)

→ Beyond - Irreversible

Infaretion

TTC (Triphenyl Tetrazolium Chloride)

- → +LDH → BRICK RED : Normal viable myocardium
- → -LDH → PALE YELLOW : Recent infarct

Features	Onset
Onset of ATP Depletion	Seconds
Loss of Contractility	< 2 mins
ATP Reduced to 50% of Normal	10 min
to 10% of Normal	40 min
Irreversible injury	20-40 min
Microvascular injury	> 1 hr.

Valvular H.D.

	Morphologically	Special points
Mitral value Prolapse	Gross Ballooning of value a/w Myxomatous degeneration	Marfan's syndrome Mid systolic click
Rheumatic H.D	Verrucous vegetations along lines of closure [MAC cullum Plaques]	Pathognomic Aschoff nodule
IE (Infective Endocarditis)	Friable vegetations along lines of closure, invading Chordae Tendinae	Risk of Septic embolisation
NBTE (Non Bacterial Thrombotic endocarditis)	Large Fibrin clots along lines of closure ; No Invasion	Seen in Cancer Malnourished (Marantic Endocarditis)
LSE (Libmann Sack Endocarditis)	Vegetations on both sides of Cusp	SLE

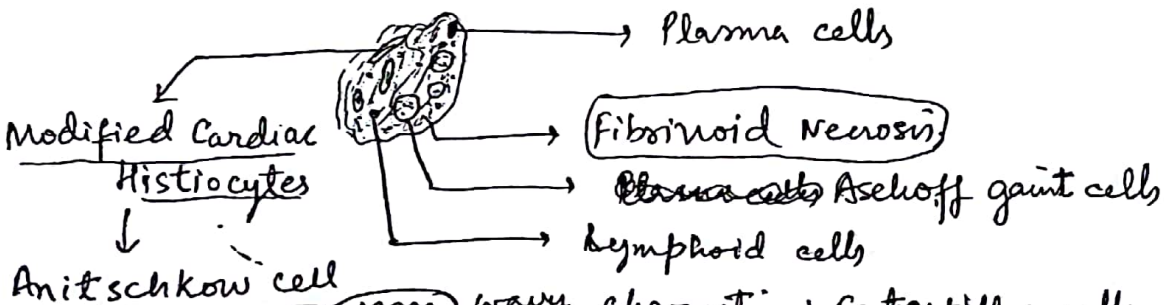
Rheumatic Heart Disease

≡ Molecular Mimicry

Acute = Pancarditis ← Myo, Endo, Peri

↓

Pathognomic feature is Aschoff Nodule which [can be seen in myo, endo, pericardium] Q



Endocarditis

Verrucous vegetations along the lines of closure

↓ warty

Fibrinoid Necrosis

Inflammetory cells, Immune complex

Damage the values ⟶ Regurgitation

Regurgitation Jets into Left Atrium

Overtime can cause (Plaque) formetion especially in posterior wall of (L) atrium (when mitral value involved)

Mac Callum Plaques

Chronic RHD

Due to inflammation

↳ Stenosis of valves

Due to fibrosis of cusps & narrowing of orifice

Fish mouth / Button hole appearance

Most commonly: Mitral > Aortic

Finding in eye →

Superiotemporal dislocation of lens

⇓

MARFAN'S Synd

⇒ Owl's eye → CMV inclusions
→ R.H.D
→ Lymphoma

Fish mouth appearance

Floppy Mitral value

⇓

MVP

CNS & PNS Pathology

Schwanoma — Benign peripheral nerve sheath tumor

↓

Most commonly – Cerebellopontine Angle ✿

NF_2 associated

Variably cellular tumor

↓

- Hypercellular compact areas → **Antoni-A**
- Hypocellular loose areas → **Antoni-B**

→ Tumor nuclei are arranged in parallel to each other ⇒ TRUE PALISADING ✿

✿ VERROCAY BODY

CNS TUMORS

1 Glioma (M.C 1° tumor)
- Astrocytoma (MC) glioma
- Oligodendroglioma
- Ependymoma

2. Meningiomas (2nd m.c 1° tumor)

3. Embryonal Tumors
- Medulloblastoma (M.C 1° CNS malignancy in children)
- Atypical Teratoid/ Rhabdoid Tumor (ATRT)

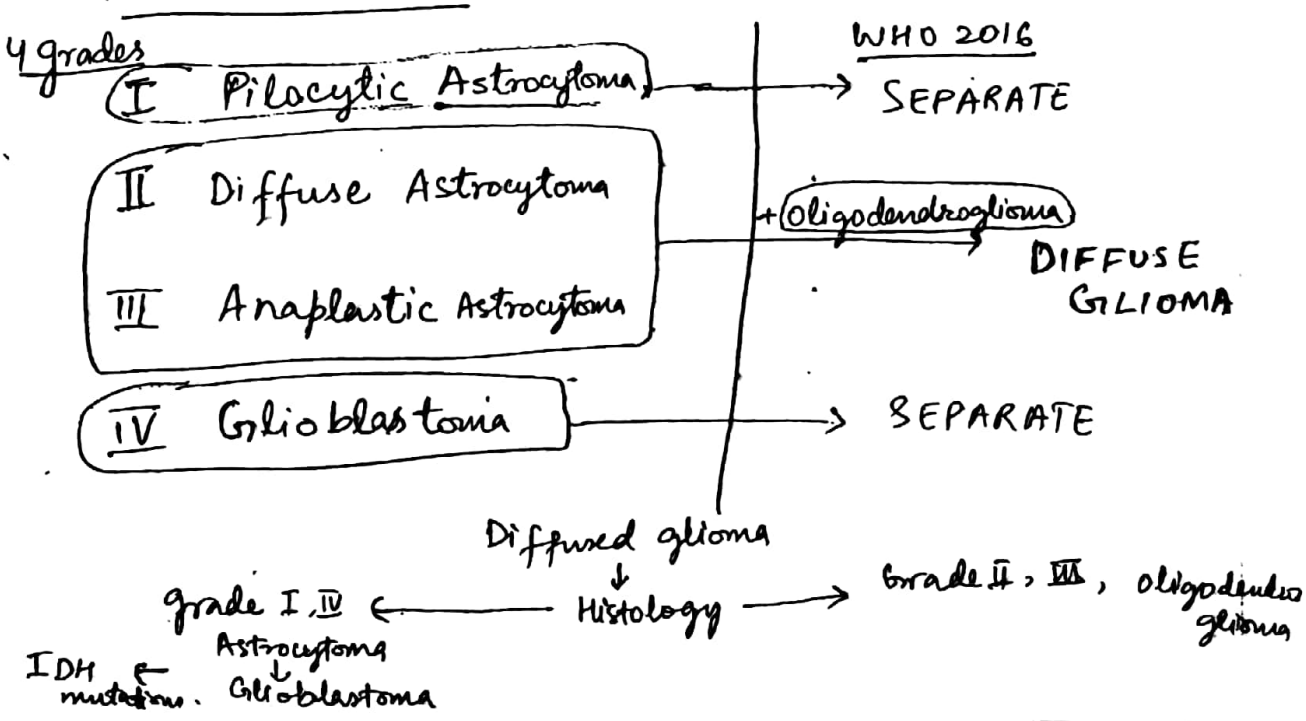
PNET is not included under this anymore WHO 2016

4. Neuronal Tumors (neurofilament +ve)

5. Lymphomas

6. Secondary Tumors (M.C CNS Tumors)
↳ (1° is Lung Ca mc)

ASTROCYTOMAS



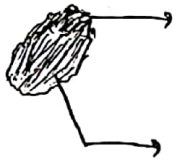
Pilocytic Astrocytoma

Most common 1° CNS Tumor in Children

Seen in Cerebellum

BRAF ← Mutated (V600E)
Rearranged

Gross



↓
(Hemangioblastoma)

Micro

& ROSENTHAL FIBRES
↳ Made of GFAP Proteins & other Proteins
(Non neoplastic reactive change)

Glioblastoma

Most common 1° CNS malignant in Adults

Seen in Frontal & Parietal Lobes

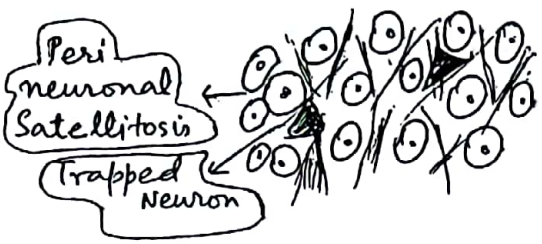
EGFR
P53
CDKN2A / P16
PDGFRA

Gross
- Tumor infiltrating normal brain
↓
crosses midline (Butterfly glioma)
- Areas of Hge & necrosis

Micro

Leaky vessels → Contrast enhancing
↳ Areas of necrosis (Pink)
bordered by viable Tumor cells (blue dots)
↓
PSEUDOPALISADING NECROSIS

OLIGODENDROGLIOMA



Monotonus uniform tumor cells with Perinuclear Halo
[Fried egg appearance
(also in Hairy cell Leukemia
- Mycoplasma)

Delicate anastamosing vasculature
[chicken wire vasculature]

Chicken wire
↳ Fibrosis – Alcoholic liver disease
↳ Calcification – Chondroblastoma

Microcalcification : Imaging
↳(Basophilic on H&E)

Genetics
- IDH1, IDH2 mutation
- 1p/19q codeleted
  - Favourable Prognosis

MEDULLOBLASTOMA [grade IV]

Mc site – cerebellum (Posterior fossa)
CSF dissemination → Drop metastasis

Small round blue cell tumor
→ Neurophil (cytoplasmic feature)

HOMER WRIGHT ROSETTE
↳(PseudoRosette
[True Rossete has Nucleus]
↳ Ependymal Rosette

MENINGIOMA (I - III)

Arises from Araehinoid cap cells / Meningothelial cells

Dura based tumor (DURAL TAIL)

Easily Detachable

⇒ Causes reactive hyperostotic change in overlying bone.

↓

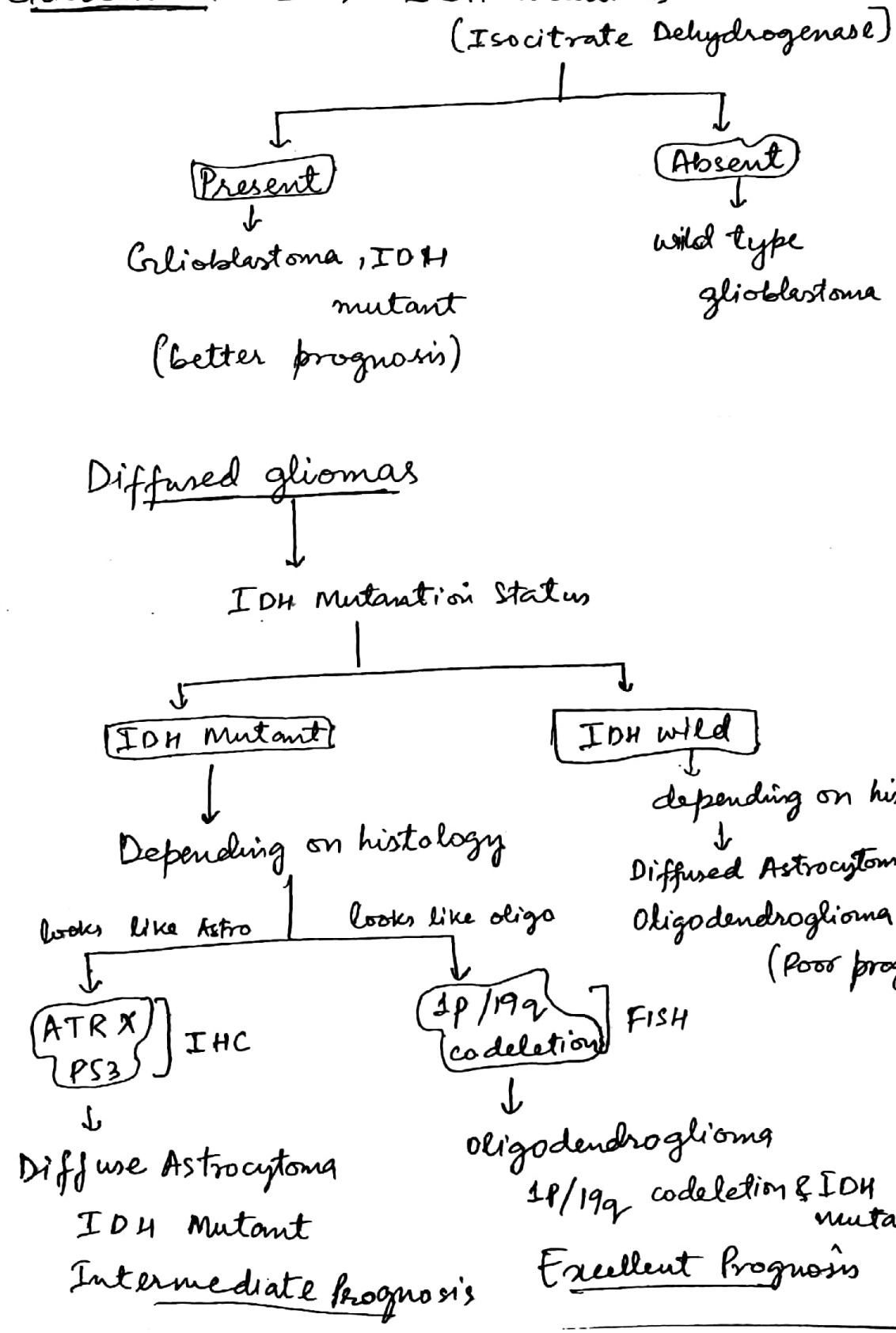
X'Ray

Express PR ⇒ ↑ses in size during pregnancy.

Associated ε NF_2

Most common Histology is - Meningothelial

WHO 2016 → genetic incorporation into histology



Schwanoma

Neoplastic Schwanoma cell

Cerebellopontine angle = Most Commonly

S100 +ve

NF_2 associated.

Variable cellular areas

- Antoni A
- Antoni B

Neurofibroma

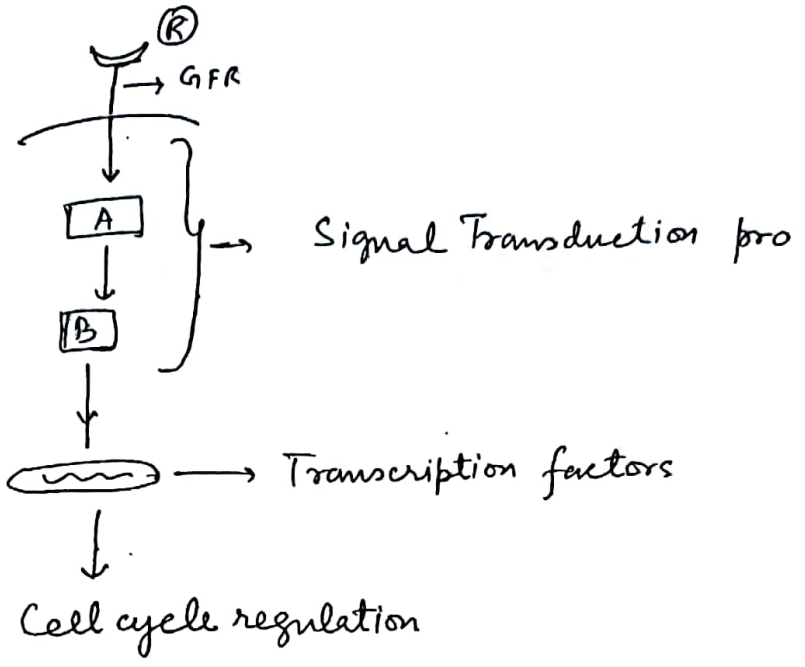
Neoplastic Schwann cells + Fibroblasts + Perineural like cells + Fibroblasts + Spindle cells (CD34 +ve) + Mast cells

S100 +ve

Heterogenous ~ a

NF1 associated ~ a

Wavy collagen & wavy buckled nuclei.



GFR

EGFR (erbb1) — Her2neu

PDGFRA

RET

MET

STDPs

KRAS

BRAF

PI3K

Tyrosine kinase — Receptor TK

ALK

Ckit

Transcription f

PAX 8

myc

Cell cycle inhibitors CDK N2A/P16

Repair genes : BRCA 1 & 2

p53

Rb

Lung

⇓

Mutually exclusive

Colon

KRAS/NRAS mutation analysis is mandatory now before starting anti EGFR Therapy.

Q Mutation analysis of which of the following genes will not help in progrostication of colon cancer?

(A) EGFR

(B) KRAS

(C) P53

(D) MSH2

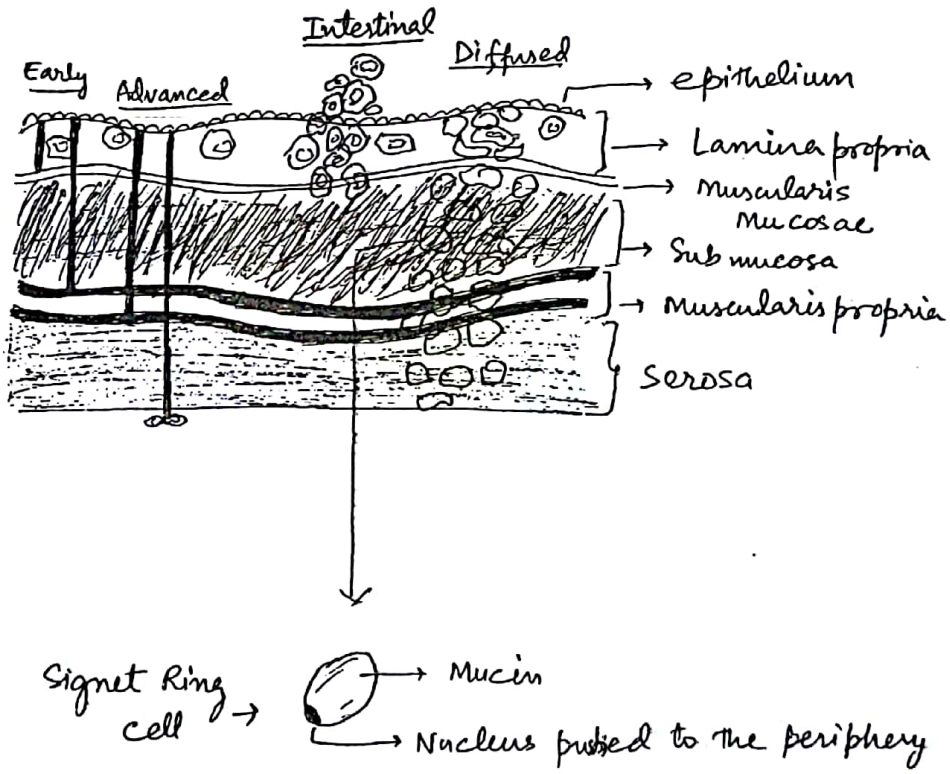
↓ DNA mismatch

↓

Ly Syndrome

Gastric Adenocarcinoma

- Depth of Invasion
  - Early
  - Advanced
- Histology
  - Diffuse
  - Intestinal



- No mucosal mass
- Loss of Rugal folds
- Transmural thickening – Infiltration → wall thickening ↓ Leather bottel appearance ← LINITIS PLASTICA

Disease	Efect on			
	Intraluminal digestion	Terminal digestion	Transepithelial Transport	Lymphatic Transport
Celiac disease		⊕	⊕	
Lactase		⊕		⊖
whipples				⊕
Abeta			⊕	
Chronic Pancreatitis	⊕			

Hematology 23/04/18

Hematooncology

Hematopoietic Stem cells

- Common Myeloid progenitor
  - DIFFERENTIATION
    - Erythroblasts → RBC
    - Myeloblast → Granulocytes (IMATURATION)
      - Neutrophils
      - Eosinophils
      - Basophils.
    - Monoblast → Monocytes
    - Megakaryoblast → Platelets
- Common Lymphoid progenitor
  - Lymphoblasts (B/T/NK)
  - Lymphocytes (B/T/NK)

BEN

Myeloblast → Promyelocyte → Myelocyte → Metamyelocyte → Band form → Neutrophil

- Myeloblast → Prominent nucleoli
- Promyelocyte → Azurophilic primary granules
- Myelocyte
- Metamyelocyte
- Band form
- Neutrophil
  - Segmented Nucleus
  - Increased no. of granules

While Maturation
- Increased Segmentation
- Increased functional granules
- Decreased size

Right Shift ⇒ (Immature cells) (Infection)

Left Shift (Hypoplastic marrow) ⇒ ARNETH Index or ARNETH COUNT

(↑ Immature cells)
(Few lobes of Neutrophils Nucleus)

Leukemia ⟷ Lymphoma

White in blood.

Liquid tumor

Origin - Bone Marrow

Malignant

Inv - Flowcytometry of peripheral blood or B. M. aspirate

Lymphocytes → Tumor

Solid tumor

Origin - Lymphoid organs → L.N, thymus, spleen, tonsils. any where in body.

Benign/Malignant.

Inv. Immunohisto chemistry on Histopath sample. (IHC)

Gene Rearrangement

Change in location

Karyotyping / FISH → Translocation

F In Situ Hyb. → Inversion

Translocation / Inversion → gene1 + gene2

mRNA (Now fused) [FUSION TRANSCRIPT] — Inv. RT. PCR.

New protein (oncoprotein) — IHC / Flow cytometry

Cancer.

(↑ Leukemias)

gene1 → gene2

↑ed copies (Amplification → FISH)

↓

Increased protein → (IHC) [Overexpression]

↓

Increased unregulated proliferation

↓

Cancer.

(↑ Lymphomas)

Mutation

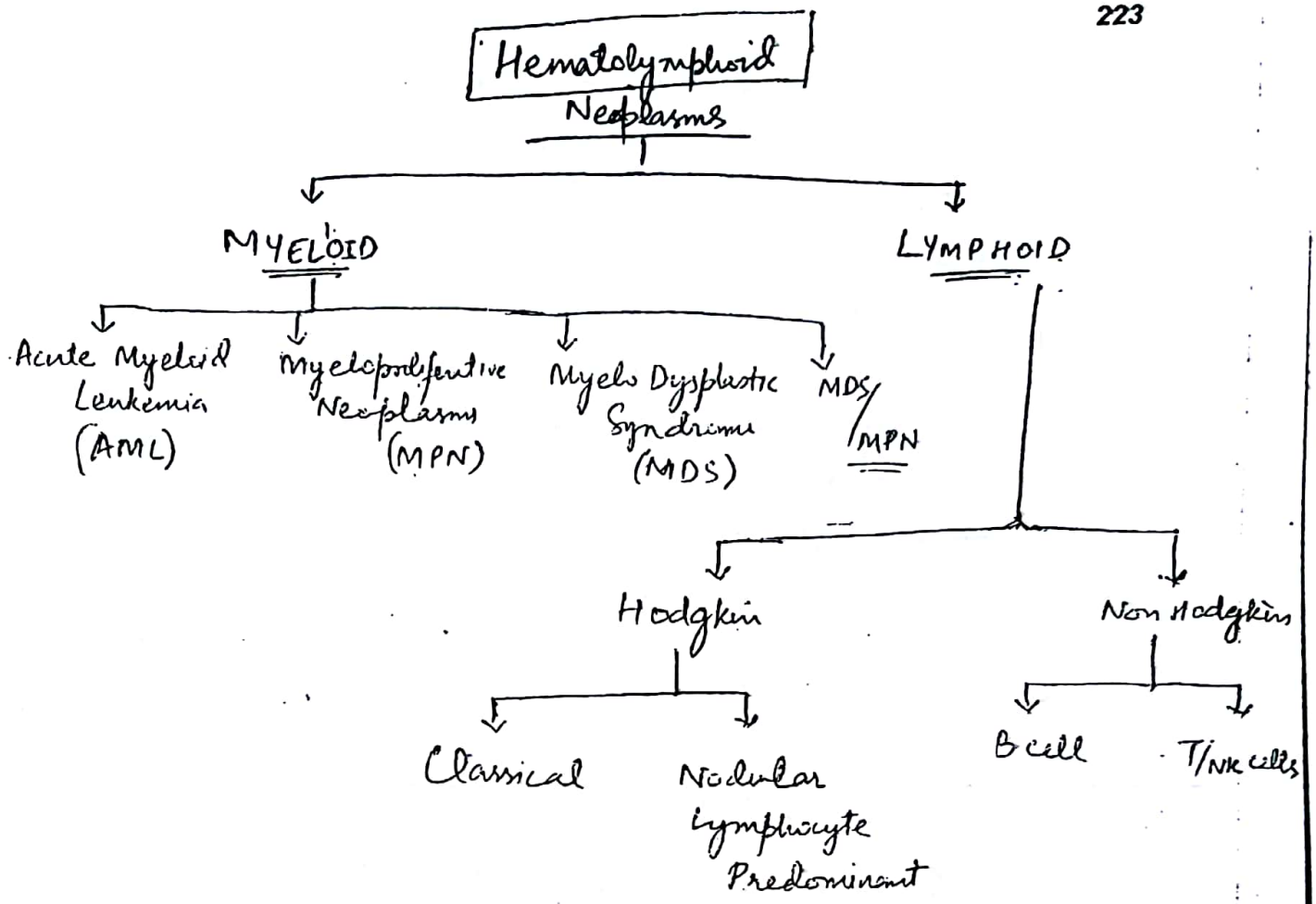
Change in sequence (PCR + Screening)

→ Commonly seen in Solid tumors.

→ Increased expression or Decreased expression

↓

New protein (IHC)



Acute Myeloid Leukemia (AML)

⇒ Rise in Immature cells in marrow.

Origin :- Haematopoietic stem cells / progenitor

Marrow is taken over by bad cells → Thus no place for good cells

- Anemia
- Thrombocytopenia
- Recurrent Infection

HSCs go to the extramedullary sites

↓

Extramedullary Hematopoiesis

↓ Causing

Spleenomegaly or Hepatomegaly

⇒ Combination of Maturation arrest + Clonal proliferation

↓↓

Increased Immature cells

FAB classification

AML - with minimal differentiation (M0)
AML - without maturation (M1)
AML - with maturation. (M2)
AML - with promyelocytic maturation (M3)
AML - with myelomonocytic differentiation. (M4)
AML - with monocytic differentiation (M5)
AML - with erythrocytic differentiation (M6)
AML - with megakaryotic differentiation. (M7)
↳ (↑ risk of myelofibrosis)

Indented nucleus ⇒ Monocyte

2017 WHO AML Classification.

≥20% Blastcells in Marrow. (<20% - MDS)

1. AML with Recurrent genetic abnormalities.

Do not require 20% Blast count for diagnosis (exceptions)

- AML with t(8;21) — Favourable Prognosis
- AML with t(15;17) M3 — Favourable Prognosis
- AML with t(16;16) or inv(16) — Favourable Prognosis
- AML with t(6;9) — Unfavourable Prognosis
- AML with 11q23 rearrangements (MLL gene) — Unfavourable Prognosis
- AML with t(9;22) (WHO 2017) — Phil. Chromosome, 22nd chr. formed after Translocation
- AML with minimal karyotype:
  - (i) Biallelic CEBPA mutation (2017) — Favourable prognosis
  - (ii) NPM mutation (2017) — Favourable prognosis

8;21
15;17
16;16
6;9
11q23
9;22

2. AML, therapy related.

Alkylating Agents
Epipodophyllotoxins

3. AML, with dysplasia related changes.
   With prior MDS
   Without prior MDS

4. AML, Not otherwise Specified (NOS)
   Previous FAB M0 - M2, M4 - M7. (30-40%) More common in adults.

AML with t(8;21)

RUNX1T1 | RUNX1 → Important for myeloid & lymphoid differentiations & maturation.

→ Fine chromatin
→ Auer rods
→ Prominent Nucleoli

Fused azurophilic granules (MPO +ve granules)

- with M2 Morphology.

AML with t(15;17)

PML (Promyelocytic Leukemia) | RARA (Retinoic acid receptor α)

PML-RARA Fusion

Myeloblast → Promyelocyte ✗→ Myelocyte

PML-RARA fusion blocks the maturation

Thus ↑ Promyelocyte

→ Granules MPO+ve
→ Stacks of Auer rods

Atypical promyelocyte + Stacks of Auer rods ⇒ FAGGOT cells

↑ Granules → Procoagulate in nature

↓

[Widespread thrombosis

↓

[Consumption of all platelets & coagulation factors.

↓

Bleeding

↙

Consumption Coagulopathy.

[Disseminated Intra Vascular Coagulation] ← DIC

(#) ↙ All Trans Retinoic Acid (ATRA) Remove the block!

Arsenic

[AML ⊂ DIC = M3

[AML ⊂ best prognosis = M3

AML with t(16;16) or Inv(16)

Gene - Core binding factor β (CBFβ)

Usually shows [M4] & [M5] morphology.

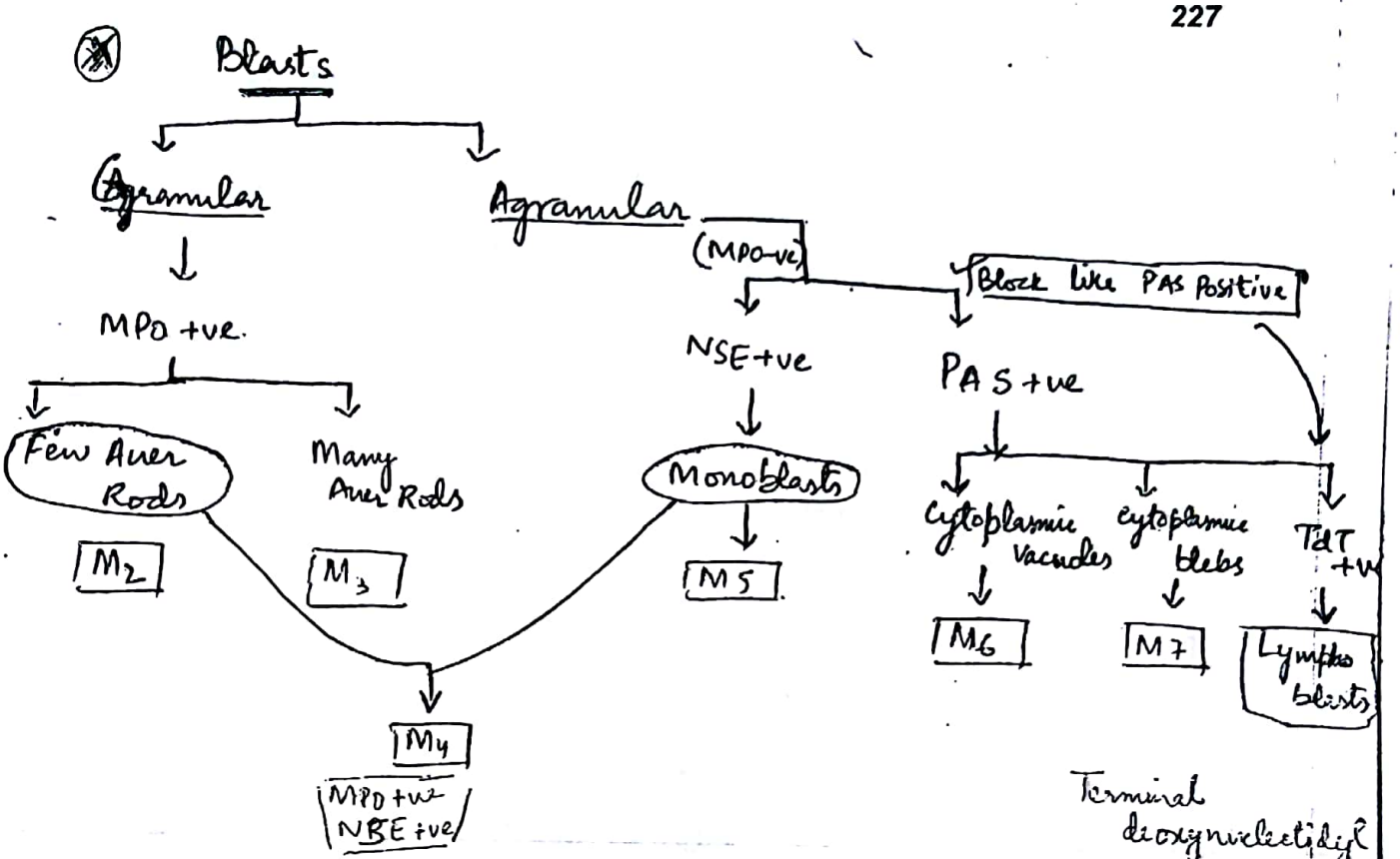
(#) → Nuclear creases / folds / Indentation.

Monoblast (NSE +ve)

✓ M4 = MPO + NSE +ve.

✓ M5 = NSE +ve

Myeloblast → Auer Rods



MYELOPROLIFERATIVE NEOPLASMS (MPNs)

Origin - Hematopoietic cells (stem cells)/Progenitor cells

PANMYELOSIS → ALL, Myeloid cells

with ↑ ⇒ GRANULOCYTE DOMINANCE

⇓

Chronic Myeloid Leukemia (CML)

t (9; 22) = fusion of BCR - ABL 1 on Chr 22 ⇓ Philadelphia Chr.

(Tyrosine kinase)

⇒ Variable excess of BCR may fuse with a fixed sequence of ABL1

(i) Major BCR (M-BCR) + ABL 1 = 210 KD = P210 ↓ CML

(ii) Minor BCR (m-BCR) + ABL1 = 190 KD = P190 ↓ ALL

⇓

Unregulated Tyrosine Kinase ⇓ Using TYROSINE KINASE

(#) AML can have both (P210 & P190) WHO 2017

In CML Chronic phase

TLC ↑ → Differential C. ⇒ Marrow preferable over blood

- Myelocyte peak
- Neutrophil peak
- Blasts < 2%
- Basophilia
- Eosinophilia +/-

→ Garden Party Appearance

→ College Girl Appearance

Additional cytogenetic changes

⇓

Progression to Accelerated Phase

↑ TLC → Differential C.

Blasts 10-19% (marrow > blood)

Basophilia > 20% (blood)

Thrombocytosis / Thrombocytopenia

↑ uig Splenomegaly.

Unresponsiveness to Tyrosine Kinase Inhibitors (WHO 2017)

⇓

Additional Genetic changes.

⇓

Blast Crisis

Blasts ≥ 20% (marrow > Blood) OR Extra medullary Blast Proliferation (Skin, CNS)

75% (Myeloid) Blasts

25% (Lymphoid) Blasts

RT PCR → To monitor response to treatment & to followup

⇒ Neutrophil Alkaline Phosphatase (NAP)
(Leukocyte ALP)

NAP → Number – ↑ses (Leukemoid reaction)
→ Function – ↓ses (CML)

2. Erythrocyte Dominance :- Polycythemia vera

[redacted]

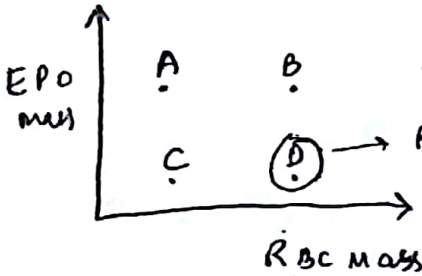
Epo (erythropoietin)
↓
EPO receptor
↓
JAK2 (JANUS KINASE) (Non receptor kinase Tyrosin kinase)
↓
Nucleus
↓
Erythrocyte formation & proliferation.

Unrequired & Unregulated Erythropoiesis
↓
- RBC mass ↑
- Hb > 16.5 g/dl (M)
  > 16.0 g/dl (F)
- Hematocrit
  > 49 % (M)
  > 48 % (F)

Polycythemia vera → JAK 2 → Activating Point Mutation
Essential thr. →
Myelofibrosis →
↓↓
Proto oncogene
↓
Oncogene
↓↓
Unrequired & Unregulated Erythropoiesis
↓
POLYCYTHEMIA VERA

Biopsy - Erythroid hyperplasia
-EPO Level = Normal/Subnormal

[redacted]
↓↓
At 617th position
Valine → Phenylalanine



↑ Risk of thrombosis ⇒ Budd Chiari Syndrome
Platelet function defects may be present → Bleeding manifestation

③ Essential Thrombocytosis / Thrombocythemia
(Megakaryocyte dominance)

Platelet count > 450 x 10^9/L

Biopsy ⇒ Mature enlarged Megakaryocytes

Genes – JAK2 (40%)
MPL } (NHO 2017)
CALR }

↑ Thrombosis (+)

Thrombopoietin
Thrombopoietin Receptor
JAK2
Activating point mutation (JAK2 V617F) 40%
Thrombopoiesis

Bone Marrow Aspirate & Biopsy indicated ⇒ Essential Thrombocytosis.

④ Primary Myelofibrosis

De novo Marrow Collagen deposition

Panmyelosis (JAK2, MPL, CALR) ⟶ Atypical Megakaryocytes

↓

TGF β, PDGF ⇒ Fibrosis

– Tear Drop cells / Dacrocytes (distorted RBCs)

↓ cellularity in marrow

⇒ Extra Medullary Hematopoiesis (EMH)

– Precursors of RBCs & Granulocytes.

Erythroblasts, Leucoblasts } Leuco Erythro Blastic Reaction (LEBR) ⇒ Myelofibrosis

Biopsy is diagnostic ✓
↳ Aspirate is Dry tap

Demonstrate fibrosis ✓ (Masson)
↳ Reticulin Stain (Silver → Black)

AML ⇄ Myelofibrosis

Biopsy indicated

Visualize Reticulate fibers
(Fibrosis of liver)

Myelofibrosis secondary to some inciting events

↓

Myelophthisic Anemia.

→ Metastatic Carcinoma
→ Storage disorders
→ Granulomatous Inflammation
→ Radiotherapy/Chemotherapy.

MYELODYSPLASTIC SYNDROME

Disordered growth in marrow ⟶ Cytopenia in blood.

At least 10% of the cells of a series should have dysplasia to call it significant.

MDS can occur in a single lineage ⇒ Single Lineage Dysplasia (MDS-SL)

or Multi lineage ⇒ (MDS-ML)

[MDS with ring sideroblasts
MDS with excess blasts.]

	Blood cytopenia	Bone marrow dysplasia	Ringed Sideroblasts	Blasts in marrow.
MDS-SL	Uni/Bi cytopenia	One cell line	<15%	<5% (No Auer Rod cells)
MDS-ML	Uni to Pancytopenia	More than one cell line	<15%	<5% (No A.R. cells)
MDS-(RS) Ring Sideroblasts SL	Uni cytopenia	One or two cell lines	>15%	<5% (No A.R. cells)
MDS-(RS) MLD	Uni to Pancytopenia	More than one cell line	>15%	<5% (No A.R. cells)
MDS-EB1 Excess blasts	Uni to Pancytopenia	One to three cells lines	None or any	5-9% (No A.R. cells)
MDS-EB2	Uni to Pancytopenia	One to three cell lines	None or any	10-19% Auer Rods

Erythroid Dysplasia

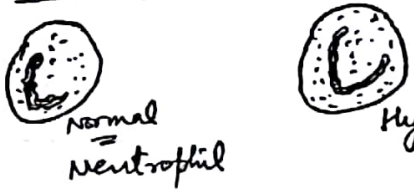
Perlic Stain

→ Prussian blue deposits (Iron laddon Mitochondria around the nucleus)

↓

Ring Sideroblasts

Granulocyte Dysplasia



Pelger Huet Anomaly :-

- Inherited condition
- Neutrophils are morphologically abnormal but functionally normal.

↓

Psedo Pelger Huet anomaly

PINCE NEZ Appearance

Megakaryocyte Dysplasia

Large cell → Dysplastic Small cell
Single nucleus → Multinucleated
Multilobed → Hyper/Hypolobulated

⇒ Most significant Dysplasia for diagnosis of MDS.

→ Pawn ball megakaryocytes - small megakaryocytes & multinucleation & Hypolobation

✓ Most common cytogenetic abnormality in MDS

In Adults
In Children
Overall

⇒ **Monosomy 7** (who 2017)

MDS/MPN

$>10^9/L$ ↑ monocytosis ≥10% of all Leucocytes

Chronic Myelomonocytic Leukemia (CMML)
(Dysplasia ⇒ Proliferation)

Juvenile Myelomonocytic Leukemia (JMML)

Atypical Chronic Myeloid Leukemia (ACML)
(Philadelphia Chr. −ve)

New confirmed addition by WHO.
↳ MDS/MPN with ring sideroblasts and thrombocytosis

Lymphoid Neoplasms

HODGKIN LYMPHOMA

- Involves contiguous group of lymph nodes.
  (Contiguous → sharing common border)
- Almost always Nodal (Axial L.N.)
- Bimodal age distribution (young adults & old)
- Neoplastic cells <<< Non neoplastic cells (1:10 – 1:100)

HL ⇒ Classical R-S cells
Lacunar cells
Mononuclear cells
L&H cell.

NON HODGKIN LYMPHOMA

- Non contiguous group of lymph Nodes
- Can be Nodal / Extranodal (Waldeyer's Ring, Mesenteric LN) commonly affected
- No specific age group.
- Almost all cells are neoplastic.

NHL

- B cell (more common)
  - Precursor → B-acute Lymphoblastic Leukemia/Lymphoma
  - Peripheral/mature
- T/NK Cell
  - Precursor → T/NK-Acute Lymphoblastic Leukemia/Lymphoma
  - Peripheral/Mature

Acute lymphoblastic Leukemia/lymphoma

Most common Cancer in children

B-ALL

- Children
- Leukemia (↑ Tendency)

T-ALL

- Adults
- Lymphoma (↑ tendency)
- Thymic mass → SVC obstruction

Also seen with
- small cell CA.
- Hodgkin Lymphoma

Lymphoblast

Large cell
↑ N:C ratio
Less/No prominent Nucleoli
Scanty cytoplasm
Agranular
Block like PAS +vity

Prognosis of ALL

Unfavourable

<1yr , >10yrs
CNS involvement
Testicular involvement
Male
T. phenotype
t (9;22) (P190)
11q23 MLL gene
Hypoploidy (<45 chr)

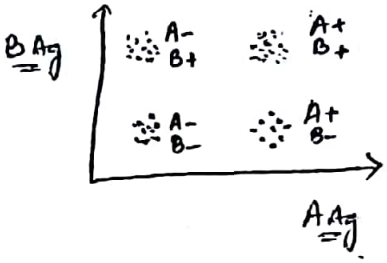
Favourable

2-9 years
Absent
Female
B phenotype
t 12;21
RUNX1
Hyperploidy (>47 chr.)

Priority for ALL prognosis

① – Response to treatment (steroids) ✓
② – Cytogenetics – Polidy
                  – Translocations
③ – Clinical features.

Flowcytometry :→



Myeloid Markers :-

Myeloperoxidase (MPO) :- Lineage Specific marker for granulocyt

CD 13 CD 33	] :→	Seen in granulocytes + Monocytes (Myeloid Lineage marker)
CD 11c CD 14 CD 64	] :→	Monocytic markers.
CD 71 CD 235	] :→	Erythrocytic markers
CD 41 CD 61	] :→	Megakaryocytic markers.

Lymphoid Markers :-

T cell

CD 1a → Thymocytes, Langerhan cells ✓
✓CD 2 → Almost all T cells
✓CD 3 → Part of T receptor (more diagnostic)
✓CD 4 → Helper T cell.
... T cell + Subset of B cells

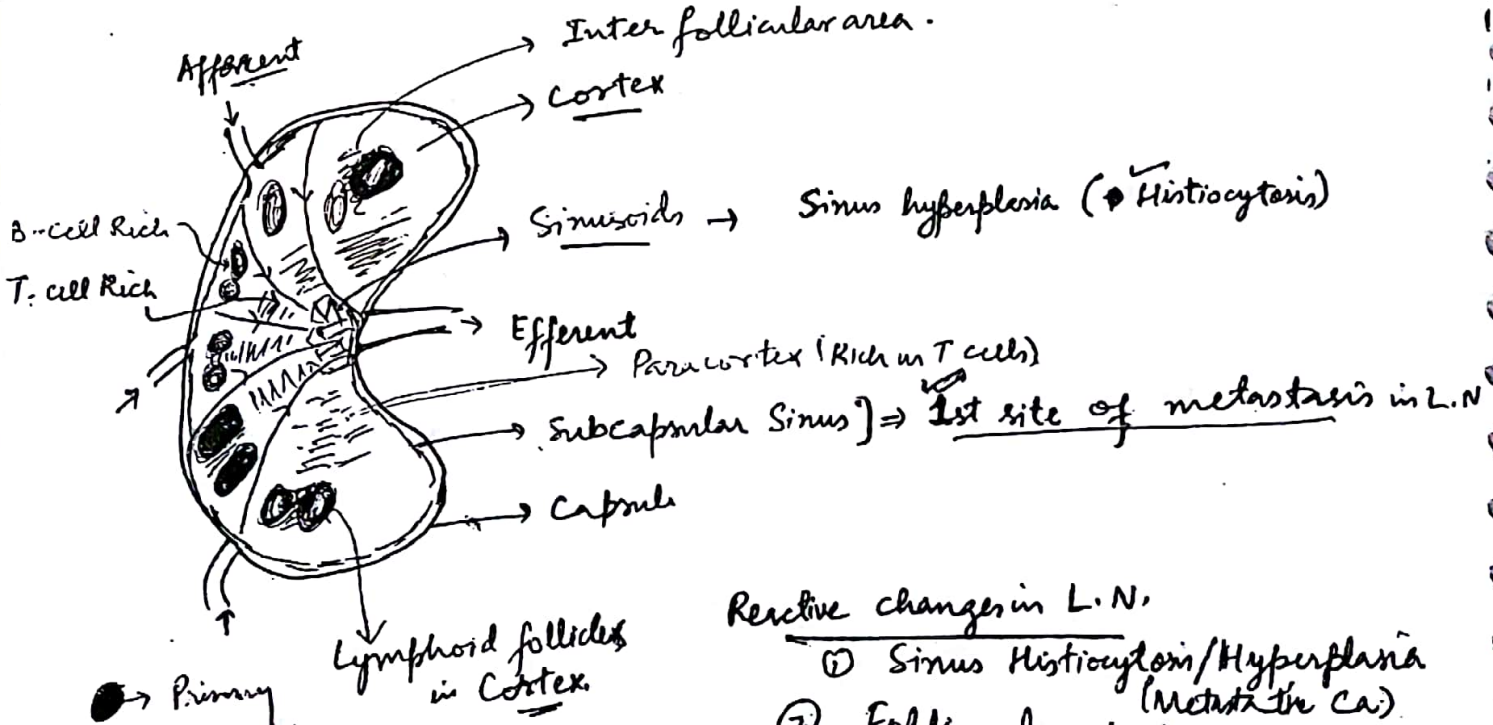
Pan T cells
2, 3, 7

B-cell markers

- CD19 → Lineage specific and consistently expressed.
- CD20 → Marker of choice for B cell lymphomas (Mature)
- CD21 → EBV receptor
- CD23 → Activated B cells
- CD79a → Almost all B cells
- PAX 5 (New marker) → B cell trancription factor (PAIR)
  [♡ → First love of B cell: PAX 5]

Miscellaneous markers

- CD34 :- Hematopoietic stem cells + Progenitors
- HLA DR : Blasts (immaturity)
- CD10 : Precursor B cell + T cells
  ⟸ Germinal centre - B cells
- CD15 - Mature granulocytes

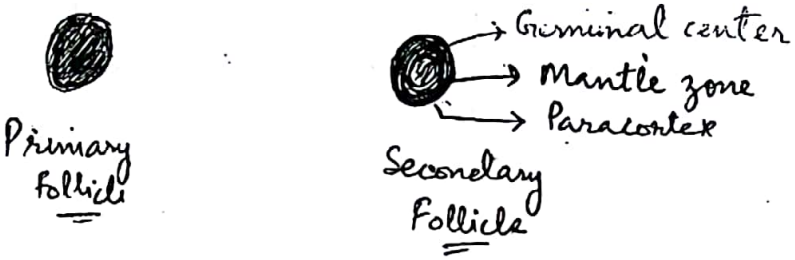


Reactive changes in L.N.

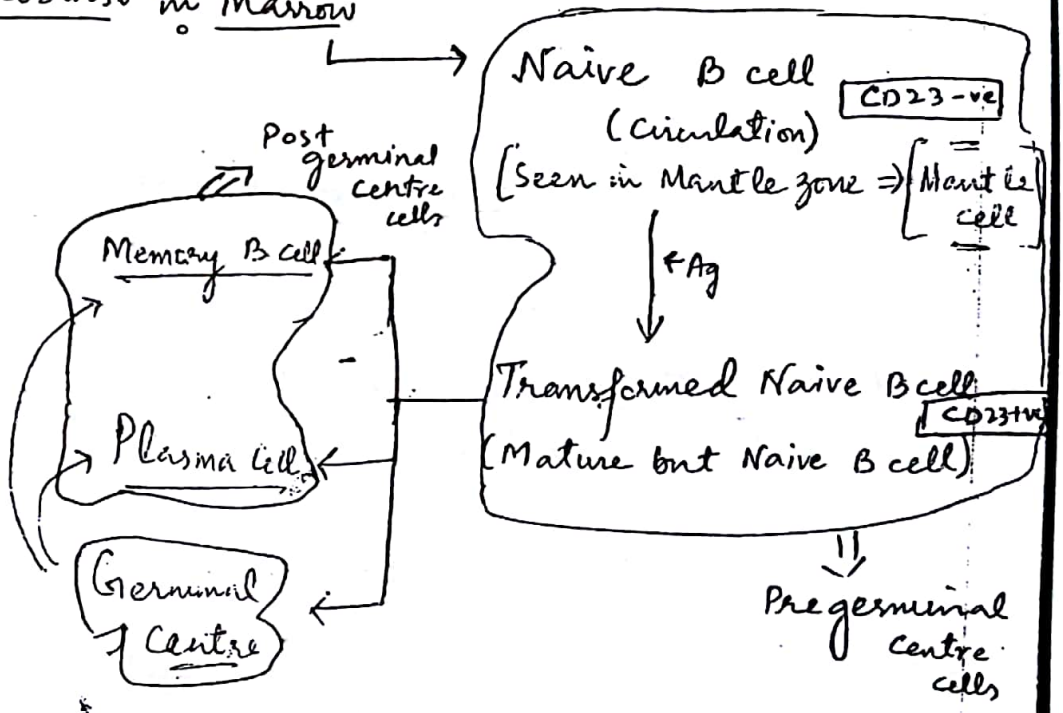
1. Sinus Histiocytosis/Hyperplasia (Metastatic Ca.)
2. Follicular Hyperplasia

- Follicular hyperplasia – Humoral immunity requirement ↑↑
- Paracortical hyperplasia – ↑ T cell mediated immunity
  ↳ infected B cells but increases T cells.

⇒ Plasmal cells and Histiocytes are formed in Medulla.



B Lymphoblast in Marrow



Pre Germinal centre	Germinal centre	Post Germinal centre
→ Mantle cell lymphoma (CD23-ve)	Burkitt lymphoma	Marginal zone lymphoma
→ Chronic lymphocytic lymphoma/Leukemia. (CD23+ve)	Follicular lymphoma	Plasma cell neoplasm
	Diffused Large B cell lymphoma (DLBCL)	Lymphoplasmacytic Lymphomas (LPL)
		CLL
		DLBCL
CD5 +ve, CD10 -ve	CD10 +ve, CD5 -ve	CD5 -ve, CD10 -ve

① Mantle cell lymphoma

Elderly

Nodal & Extranodal

↓

GIT - Polyploidal appearance.

May be high grade. (small cells & cleaved Nucleus)

✓ t(11;14)

→ IgH (Heavy chain Ig) (Promoter)

→ Cyclin D1 (cell cycle regulator which pushes the cell cycle forward)

↓

✓ Overexpressed (IHC)

New marker for cyclin D1 Negative MCL

→ [redacted] May AHMS17

SOX 11

IHC

CD20 +ve

CD79a +ve

CD5 +ve

CD23 -ve

CD10 -ve

✓ Cyclin D1 +ve

(2) Chronic Lymphocytic Leukemia
Small Lymphocytic Lymphoma (CLL/SLL)

↓ Resembling small lymphocyte

Small cell
Non cleaved
Low grade


→ Coarse clumped Chromatin
→ Scanty cytoplasm.

"Soccer ball appearance"

- CLL is most common Leukemia in adults
- Almost exclusively in the elderly

Peripheral Blood :- Uniform monotonous population
[Convent School Girl appearance]

Tumor cells have very less Vimentin
↓
Intermediate filament of cytoplasm

↓
Become fragile
↓
Making a smear disrupt easily.
↓

→ Smudge cells / Basket cells

Lymph Node Histology


Diffused effacement of LN architecture (No demarcation between cortex & medulla seen) Most common Pattern

Pro lymphocyte (another type of tumor cell)


(Rapidly multiplying cell)
↳ enough cytoplasm.
Thus lighter staining.


→ Proliferation centres
& Pseudo follicular pattern

Most Pathognomonic pattern of CLL

⇒ More the proliferation centres Poor the prognosis (Richter's)

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Most common cytogenetic Change

50% – Deletion 13q

20% – Trisomy 12 (47 XX +12)

New marker for CLL – LEF1

	MCL	CLL
Nuclear	+	+
CD5	+	+
CD23	–	+
CD20	+	+
Cyclin	+	–

Best marker to differentiate b/w MCL & CLL → CD23

(3) Follicular Lymphoma

Nodal >> Extranodal

Most common NHL in western hemisphere

Dual population.

Multiple peripheral Nucleoli

Centroblast

Cleaved Nuclei

Centrocyte

Who grading of FL = No. of centroblasts per high power field.

gr I < 5

gr II 6-15

gr III >15

t (14 ; 18)
↓ ↳ BCL2 (anti apoptotic)
IgH ⇓
(Promotor) over expression (1yc)

⇒ Back to back closely packed follicles with very little interfollicular space.

To differentiate from follicular hyperplasia
↳ BCL2 is the marker of choice
⇓
+ve in germinal centres of follicular lymphoma & -ve in germinal centre of follicular hyperplasia

BCL2 is normally expressed in mantle zone.

IHC
CD 20+
CD 79a +
CD5 -
CD10 +
BCL6 (germinal center)+
BCL2 +

(4) Burkitt Lymphoma
↳ Extranodal >>> Nodal
↳ EBV +ve

Types
- Endemic (African continent) — most common site is Jaw
- Sporadic — most common site Ileocecum
- Immunodeficiency associated (HIV) — most common site - CNS, GIT

↳ 8 = B
↓
Chr. No 8.
"C myc" Transcription factor
⇒ t(8;14)
↳ IgH ⇒ Kappa (κ) Chr 2 t(2;8)
Lambda (λ) Chr 22 t(8;22)

To assess proliferation of tumor cells – marker used is

Ki-67 (Mib-1)
(% of cells expressed)

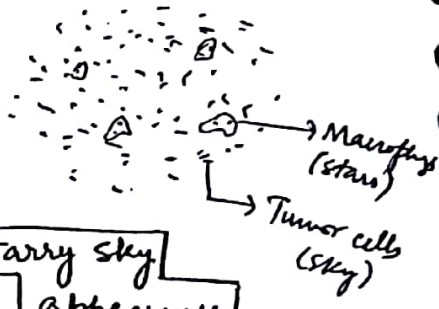
Lymphomas
- <40% – low grade
- >40% – Highgrade

[different cut offs for different tumors]

Ki 67 in Burkitt lymphoma ≃ 100%

i.e; rapidly proliferating & rapidly dying cells

High Mitotic Index ⟹



Starry sky appearance

IHC
- CD 20 – +ve
- CD 79a +ve
- CD 5 – -ve
- CD 10 +ve
- BCL6 +ve
- BCL2 – -ve
- ~~Translocation~~ Cmyc +ve

Also seen in
- ALL
- BLBCL
- Variants of MCL
- Reactive lymph nodes.

New marker is [redacted] who 2017

(6) DLBCL (Diffused Large B Cell Lymphoma)

- Diffused effacement of architecture
- Nuclei of tumor cell is 3x – 5x that of normal lymphocyte nucleus

Most common NHL worldwide

↳ Molecularly heterogenous.

* Most common Ca arising in HIV patients

	Frequency
BCL6 rearrangent	
BCL2 rearrangent	
Cmyc rearrangent	

DLBCL is high grade lymphoma.

Large cell

Histological variants — Centroblastic
Immunoblastic
Plasmoblastic → Most common in Immunodeficiency.

IHC

CD20 +ve
CD79a +ve
CD5 −ve
CD10 +ve
CMyC +/−
BCL2 +/−
BCL6 +ve.

Ki 67 > 40% (not as high as Burkitt)

Low grade Small cell Lymphoma → (can transform) → High grade Large cell Lymphoma

⇓

Richter transformation

(6) Marginal Zone Lymphoma

⇒ Diagnosis of exclusion
(CD5−, CD10−) (CD43 +ve)

⇓

NODAL & Extranodal ⇒ MALToma

⇓

Chronic inflammatory states
- Sjogren's Syndrome
- Hashimoto thyroiditis
- H. Pylori Gastritis
(regression of MALToma on treating H.Pylori)

New marker for Marginal zone lymphoma

⇓

IRTA 1
MNDA.

MALT (Mucosal-associated lymphatic tissue)

(7) Lymphoplasmacytic Lymphomas

Commonly associated with Hepc.

- Lymphocytes
- Plasmacells ↓

LPL in CNS ⊆ WM
⇓
Bing Neel Syndrome

IgM ⟶ forming pentamers ↓

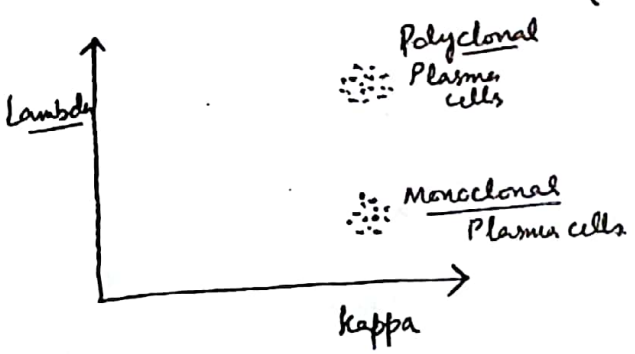
↑ Viscosity in blood
⇓
Waldenstorm's Macroglobulinemia (WM)
⇓
Hyperviscosity

JIPMER 24

(8) Plasma Cell Neoplasms

Clonal plasma cells ⟶ Abnormal Immunoglobulins + Excess of Light chains ↓

Monoclonal Proteins (M-Proteins)



Deletion 13q

Monoclonal Gammopathy of Uncertain Significance (MGUS)

Heavy chain disease
↓
Rare monoclonal gammopathy
Occurs in malnourished population
A/E/A ↳ Mediterranean Lymphoma.

Smoldering Myeloma

Plasma cell Myeloma

	Monoclonal Gammopathy of Uncertain Significance (MGUS)	Smoldering Myeloma	Plasma cell Myeloma
M-protein (g/dl)	< 3	≥ 3	≥ 3
Bone marrow clonal plasmal cell (%)	< 10%	≥ 10%	≥ 10%
Symptoms	Absent	Absent	Present

MGUS → Premalignant condition.

MGUS
- Ig M-MGUS (MYD88 mutation) → Lymphoplasmacytic lymphoma progression
- IgG/IgA-MGUS → Plasma cell myeloma progression

(MYD88 L265P Mutation.)

Plasma cell Myeloma

Tumor plasma cells

- Abnormal Igs + Excess light chains
  - Recurrent Infection → M/C/C Death in M.M.
  - ⇒ Excreated in Urine as Bence Jones protein
  - ⇒ Light chain Nephropathy → 2nd M/C/C of R.F.
  - → Renal Insufficiency → 2nd M/C/C death in MM
  - ↓ erythropoietin ⇒ Anemia (Normocytic normochromic) (Multifactorial)
- Osteoclast Activating Factors
  - Stimulates Osteoclasts → [redacted]
  - Inhibits Osteoblasts → ALP levels (N/↓)

Bone resorption

Hypercalcemia → Most common Cause of Renal failure in Multiple Myeloma

Blanched out Lytic bone lesion ✓

[redacted]

CRAB Symptoms of M.M.
- Hypercalcemia
- Renal insufficiency
- Anemia
- Bone Resorption/lesion

Tumor plasma cells → IL-6 → Survival, proliferation & level correlate with disease activity.

Symptoms of P.C. Myeloma

> 20% of Plasma cells in peripheral blood

⇓

Plasma cell leukemia ✓

How to Diagnose Multiple Myeloma As Per 2014 — IMWG Criteria

1st → Look for >10% clonal plasma cells in bone marrow or biopsy proven plasmocytoma (either bony or extramedullary)

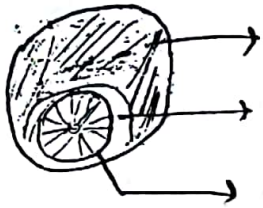
2nd → Any of the myeloma defining events.

End Organ Damage (CRAB)
- Hypercalcemia
- Renal Insufficiency
- Anemia
- Bone lesions

Any one or more of malignancy biomarkers
1) >60% of plasma cells in Bone marrow.
2) Involved/uninvolved Serum free light chain ratio ≥100
3) >1 focal lesion on MRI (≥5 mm)

In Multiple Myeloma order of frequency of Ig secreated

⇒ Ig G (50%).
Ig A (20%)
Ig D
Ig E
Ig M



Globular inclusions
⇓
Mott cell/Grape cell

Intra Nuclear inclusion
⇓
Dutcher Body

Intra cytoplasmic
⇓
Russell Body

common

Malignant plasma cells are ⇒ Binucleate/ Multinucleate
⇒ Have Inclusions

Most common cytogentic abnormality associated is
Rearrangements of 14q (IgH)

Most common gene involved is cyclin D1

Drugs used ⇒ Ibrutinib
Rituximab.

(9) Mature T cell lymphomas

1. Mycoses Fungoides (Most common cutaneous T cell Lymphoma)

↓

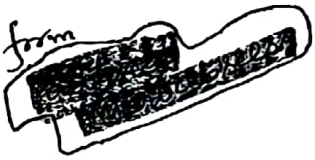
Leukemia when developed is called [redacted]

CD4+

 → Cerebriform Nuclei

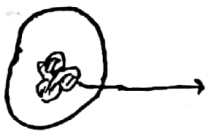
(Tumefaction of skin Lesions)

SEZARY LUTZNER Cell → Invade epidermis & form



(10) Adult T cell Lymphoma/Leukemia

Caused by HTLV-1

 → Flower shaped/cloverleaf shaped Nuclei

(11) Anaplastic Large cell Lymphoma

ALK positive

→ Anaplastic Lymphoma Kinase (Receptor tyrosine kinase)

T cell lymphoma

↓ Rearranged in

- ALCL
- Lung Adenocarcinoma
- Inflammatory Myofibroblastic tumor ~~or~~ AIIMS

↓ Mutated in

- Neuroblastoma
- Hereditary hemorrhagic telengiectasia II (JIPMER)

"Hallmark cells"

 Embryoid Nuclei

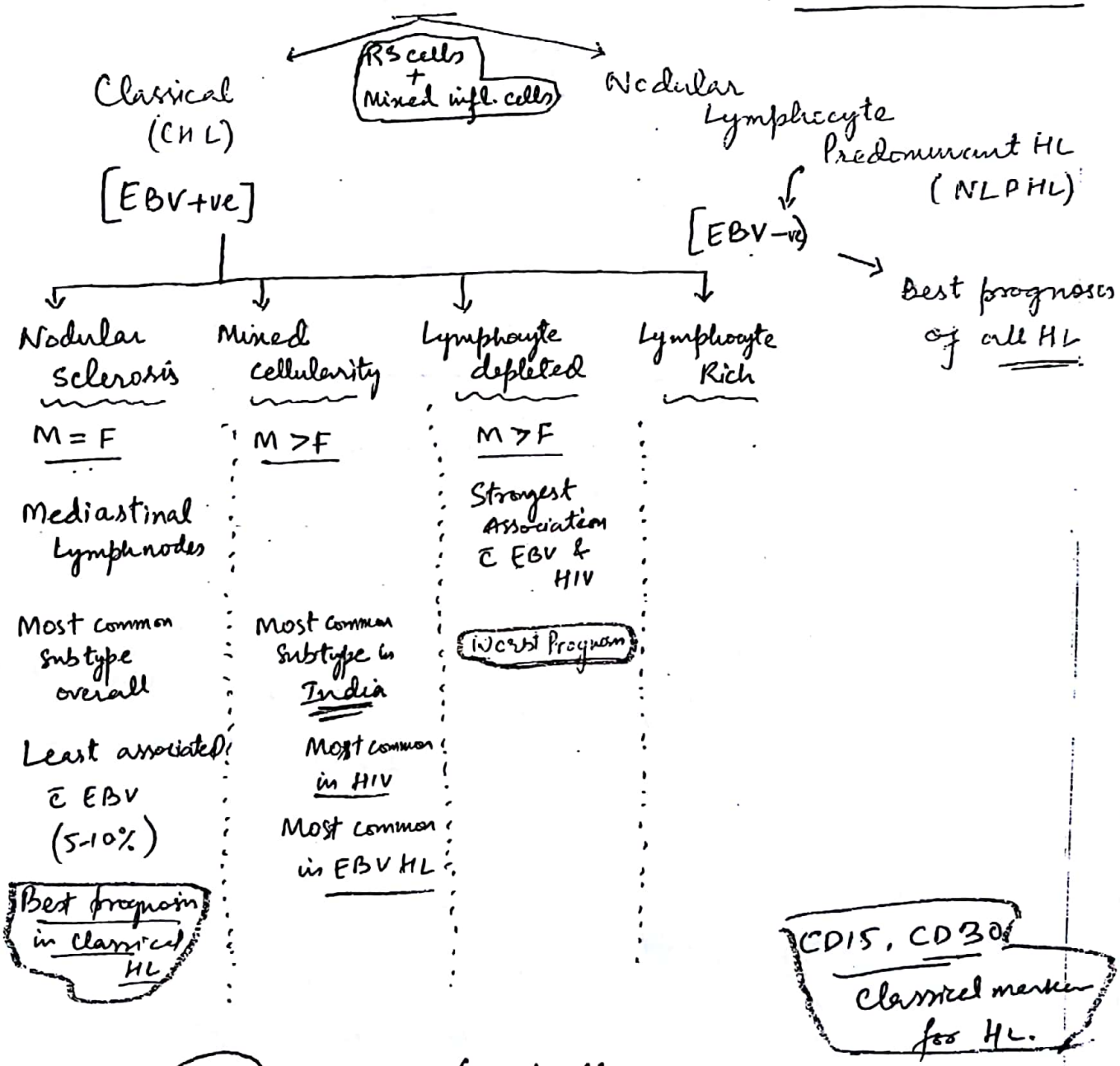
 Horseshoe Shaped Nuclei

 R.S like cells

HODGKIN Lymphoma

- Involves Contiguous group of LN.
- Almost always Nodal —— Most common is cervical group. (~~Axial~~/Axial)
- Bimodal age
- Neoplastic cells LL Non neoplastic cells

ASSOCIATED WITH EBV

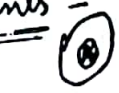


→ Large/giant cell
Binucleate
Prominent macronucleoli

Owl's eye appearance

origin - germinal centre B cells
Size - 45 μm

Variants – Mononuclear cell/ Hodgkin cell.



- Lacunar cell → Nodular Sclerosis

- Popcorn cell / LP cell / L & H cell

NLPHL

	RS cell	LP cell
CD45 (Leucocyte common Ag)	–	+
CD20	–	+
CD10	–	+
BCL6	–	+
CD30	+ 100%	–
CD15	+ 75-85%	–
PAX 5	Weakly +ve 95%	Strongly +ve

Hairy cell Leukemia

- Tumor cells resemble the features of memory B cells.
- Mutation has been identified in the hematopoietic stem cells.

→ Signal transduction protein

→ both position Val → Glutamate

Also seen in- Langerhan cell histiocytosis
- Pilocytic Astrocytoma
- Papillary Carcinoma Thyroid
- Malignant Melanoma

BRAF inhibitor
↳ VEMURAFINIB

Hairy cells (frayed borders)

Bm marrow → Individual cell fibrosis

→ Fried egg appearance → also seen in Oligodendroma Mycoplasma c.

Aspirate - Dry Tap

Biopsy - Diagnostic

- Also infiltrates Spleen → Splenomegaly (involves Red pulp)
- Pancytopenia
- Tumor cells stain positive for acid phosphatase
  ↓
  wash them with tartarate
  ↓
  Stain is retained.

Tartarate Resistant Acid Phosphatase (TRAP) +ve ⇐

Associations
- Monocytopenia ⇒ Used Risk of Infection with atypical Mycobacterium
- Erythema nodosum
- Polyarthritis Nodosa

IHC ✓ Annexin A1 most specific marker

CD 11c
CD 103
CD 25

Langerhan Cell Histiocytosis

- Tumor of Immature Dendritic cells (Langerhan's cells)
- [redacted] mutation
- Clinically

Unisystem

Unifocal
- Eosinophilic granuloma

Multifocal
- Calvarial bone defects
- Exophthalmos
- Diabetes Insipidus

→ Hand Schuller Christian Trad

Multisystem
- Cutaneous
- Hepatic
- Splenic
- Pulmonary

→ Letterer Siwe Disease

Histology

Coffee Bean Nuclei (Longitudinal groove)

- Langerhan cells
- Eosinophil.

Also found in
- Granulosa cell tumor
- Brenner tumor
- Papillary Ca. Thyroid
- Chondroblastoma

Electron Microscopy

Pentalaminar Crystalline Structures with fusiform dilatation (Tennis Racquet appearance)

⇒ Most specific marker ⇒ LANGERIN (CD 207)

⇒ Birbeck Granules

Pulmonary LCH

- Neoplastic → BRAF V600E
- Reactive → Smoking → Cessation of smoking causes regression of disease

IH

CD 1a

S-100 → Neuroectodermal marker

HLA-DR

- Malignant Melanoma
- Schwannoma
- Lipoma / Liposarcoma
- Sustentacular cells of pheochromocytoma.

RBC Disorders

Anemia

Means Absence → Blood

↓ sed O_2 Carrying Capacity

Reflected on red Hb

- Too little production
  - Aplastic anemia
  - Nutritional deficiency
- Too much Destruction → Hemolytic anemia
- Blood loss.
  - Acute
  - Chronic

Hemolytic Anemia

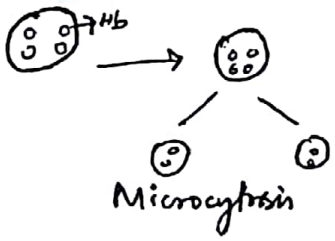
Microcytic anemia

⇓

Defective in formation of Hb.

Protoporphyrin → Heme → Globin → Hemoglobin

- Protoporphyrin: Sideroblastic anemia
- Fe: ↓ Iron deficiency Anemia of Chronic disease
- Globin: Thalassemia



Reticulocyte :- Immature RBC with retained RNA due to improper enucleation & maturation

→ Polychromatophilic cell seen as routine peripheral smear

→ Purplish due to bluish staining of RNA.

On Supravital Stain → Reticulocytes meshwork/ reticulum of blue deposits of RNA

Routine - Polychromatophilic

Supravital : Reticulocyte
- → New methylene blue
- → Brilliant cresyl blue
- → Crystal violet

Reticulocytosis → Marrow is normally functioning
→ Marrow is in hurry.

Normal Retic count = 0.5 - 1.5%

Hemolytic Anemia

↑sed RBC destruction

~~Extrinsic~~

Intrinsically wrong ~~c~~ RBC
↓
or There is Intracorpuscular defect.
⇓
Extravascular Hemolysis
↳ Spleenomegaly.

Extrinsically wrong ~~c~~ RBC
↓
or There is Extracorpuscular defect.
⇓
Intravascular Hemolysis

Lab findings

✓ Signs of ↑sed defect/destruction
- Hemoglobinemia / uria
- ↑sed Indirect Bilirubin
- ↑sed LDH
- → ↓sed Haptoglobulin

Signs of compensatory production
- Reticulocytosis
- Bone marrow hyperplasia

Hereditary Spherocytosis

Defect in membrane proteins

- **Integral**
  - Glycophorin (CD235) — almost never involved in H.S
  - Band 3 (most abundant Integral glycoprotein)
- **Peripheral**
  - ⇒ Ankyrin – Most common def.
  - ⇒ Spectrin – most severe def. — Responsible for biconcave shape of RBC
  - ⇒ Protein 4.1
  - ⇒ Protein 4.2 (Palladin)

Most common inheritence is Autosomal Dominant.

Loss of Surface area → Spherical Shape → Splenic macrophagic destruction of RBC formed

↓ Mean corpuscular Hb Conc. (MCHC)

Less deformable

⇒ Osmotically ~~[illegible]~~ more fragile.

⇒ RBCs without any central pallor

↳ Characteristic feature of Spherocytes

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Episodic
- Intravascular hemolysis

Decreased pH in body during night activates complement.

⇓

Acquired genetic defect

PIGA gene

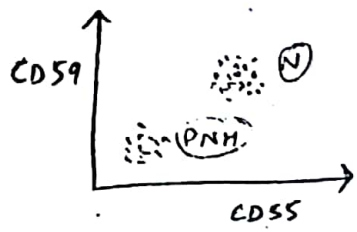
GPI anchored proteins

⇓

(Protect cells from complement mediated damage)

GPI anchored Proteins

- Membrane Inhibitor of Reactive lysis (MIRL) (CD59) — Most common & important
- Reactive Decay Accelerating Factor (DAF) (CD55)
- C8 binding protein



Triad of:

- Hemolytic anemia
- Pancytopenia
- Thrombosis (50%) – common cause of Death. (Multifactorial)

5-10% ⇒ Progress to AML/MDS

Stem cell Transplant ⇓ Treated

Diagnosis

- Ham's test
- Sucrose lysis test
- Flowcytometry ⇒ Best technique

G-6 PD Deficiency

HMP Shunt ⟶ Detoxify H_2O_2

In G6PD deficiency → when there is oxidative damage
↓
H_2O_2 is not detoxified
↓
Free radical damage to Sulfhydryl groups of Hb.
↓
Denaturation of Hb
↓
HEINZ Body
↓
Can be visualized on **Supravital Stain**

→ Denatured Hb doesn't take other stains.
← When stained Routinely

Heinz body → capable of damaging the membrane
↓
EV Hemolysis
without membrane damage - IV Hemolysis

G6PD deficiency → X linked recessive
Males full expression
Females - Carriers.

Oxident damage

Infections
Drugs
Fava beans

- Antimalarials
- Dapsone
- Co-trimoxazole
- Nitrofurantoin

cDNA

Adult Hb

HbA	$\alpha_2\beta_2$	96%
HbA_2	$\alpha_2\delta_2$	3%
Hb F	$\alpha_2\gamma_2$	1%

⇒ α is coded by a pair of genes on chr. 16 ✓
⇒ β is coded by a single gene on chr. 11. ✓

Sickle cell Anemia

Glutamate changes to valine at 6th position of β globin chain
↓
Hb becomes sticky.

In reduced state → the stickiness of Hb causes polymerization
↓
HbS polymers distort the shape of RBC

O → Sickle cell Drepanocyte

- Fragile → Hemolysis
- Can cause microvascular occlusion → Hypoxia → Ischemia → Tissue damage.

If there is repeated occlusion in splenic tissue,
↓
Repeated cycles of damage & fibrosis
↓
Autosplenectomy → ↑ risk of infection by encapsulated organism

Autosomal Recessive

ββ – (N)

$\beta^s\beta \rightarrow \alpha_2\beta_2$ = HbA

$\alpha_2\beta^s$ ← Heterozygous → HbA → Interferes ⊂ polymerization of HbS.
↓
Sickle cell trait

$\beta^S \beta^S \Rightarrow \alpha_2 \beta^S_2$ – HbS Homozygous condition

Diagnosis

Hb Electrophoresis]– Qualitative

High performance liquid chromatography [HPLC]

↳ Quantitative & Qualitative

Sickling test

Na Meta bisulphite is used.

or Na dithionate

THALASSEMIA

α or β thalasemia (α↓ or β↓)

↓ globin production → Microcytic cells

If α ↓ ⇒ β↑, δ↑, γ↑

β ↓ ⇒ α↑

Thus getting Excess unpaired chains

↓

form tetramers

↓

Precipitate ⇒ Hemolysis

$\beta\uparrow \Rightarrow \beta_4$ – HbH (Golf ball Inclusions)

$\gamma\uparrow \Rightarrow \gamma_4$ – Hb Bart's

α thalasemia

Most common Genetic Change ⇒ Deletions

αα | αα → (N)

αα | α– → ¼ deletion | Silent carriers

αα | –– → | 2/4 deletion α thal-trait

or

α– | α– → 3/4 HbH disease

α– | –– → ¼ deletion ⇒ Hydrops fetalis

–– | –– →

β thalasemia

Clinically → Major, Intermedia, Minor

β - Genetic Changes

β - (N)

β^+ - Reduced production of β] ⇒ Splicing mutations

β^0 - Absent production of β] ⇒ Chain Termination mutation

When both alleles are mutated → Homozygous (Major)

When only one allele is mutated → Heterozygous (Minor)

Major	Minor
- Severe anemia	- Mild to moderate anemia
- Dependence on blood transfusion.	- No history of blood transfusion
Target cell +ve Microcytic cell	- Target cell +ve Microcytic cell
⇒ Considerable amount of anisocytosis & reticulocytosis	⇒ Uniformly microcytic so no anisocytosis

Target cell OR CODOCYTE

Microcytic cell

Anisocytosis ⇒ Variation of Size

↓ Absence

Poikilocytosis ⇒ Variation of Shape

RDW - Red cell Distribution Width

Range over which RBCs are distributed.

→ Anisocytosis confirmation by RDW.

β thal. Minor

At least on allele is functional (β)

Mild reduction in β

↓

Mild excess of (α) → binds ε ζ

β thal major

Both alleles are affected

Severe reduction in β

Severe excess of (α) → binds more ε ζ → HbF ↑

& ζ also → HbA2 ↑

HPLC - Quick Interaction

	HbA	HbA2	HbF	Additional.
Normal	~96%	< 3.5%	< 1%	—
β thal. Trait	< 96%	3.5-9%	Normal or ↑sed	—
β thal Major	~ 3%	Variable	> 85%	—
Sickle Cell Trait	40-60%	~ 3.5%	Normal or Slightly ↑sed	HbS - 30-40%
Sickle cell Homozygous	Markedly Reduced	Slightly ↑ or Normal	5-25%	HbS - 70-90%

Autoimmune Hemolytic Anemia (AIHA)

↓

Auto antibodies targetted against RBC Ags

Warm Antibody type (IgG Antibodies Active at 37°C) (No clumping in PB Smear)

- Primary
- Secondary
  - Autoimmune disorder ⇒ SLE
  - Drugs
  - Lymphoid Neoplasms ⇒ CLL

Cobid Agglutinin Type (IgM antibodies active below 37°C)

- Acute (mycoplasmal Infection)(Infectious mononucleosis)
- Chronic (Clumping is found in PBS)
  - Idiopathic
  - Lymphoid neoplasms.

Cold Hemolysin Type (IgG antibody active below 37°C)

→ Donath Landsteiner Ab.

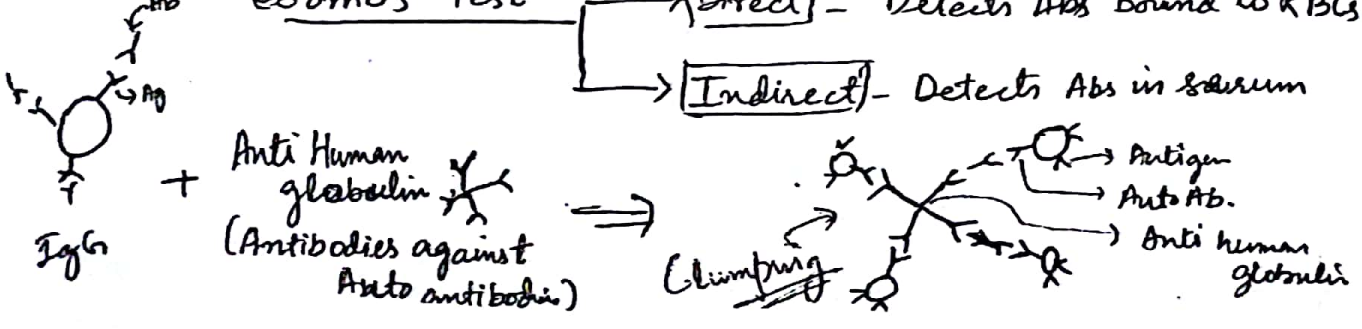
Rare; Occurs mainly in children following viral inf.

As RBC is covered with antibodies

- Compliment mediated membrane damage
  - ↓ IVH
  - ↓ Spherocytes
    - ↓ Most common cause is AIHA
- Splenic macrophages destroy them
  - ↓ EVH

Coomb's Test

- → Direct – Detects Abs bound to RBCs.
- → Indirect – Detects Abs in serum

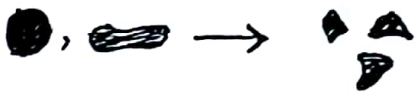


Microangiopathic Hemolytic Anemia (MAHA)

Small Vessel pathology

- In Vasculitis (SLE)
- DIC
- TTP/HUS

| Thrombi formation



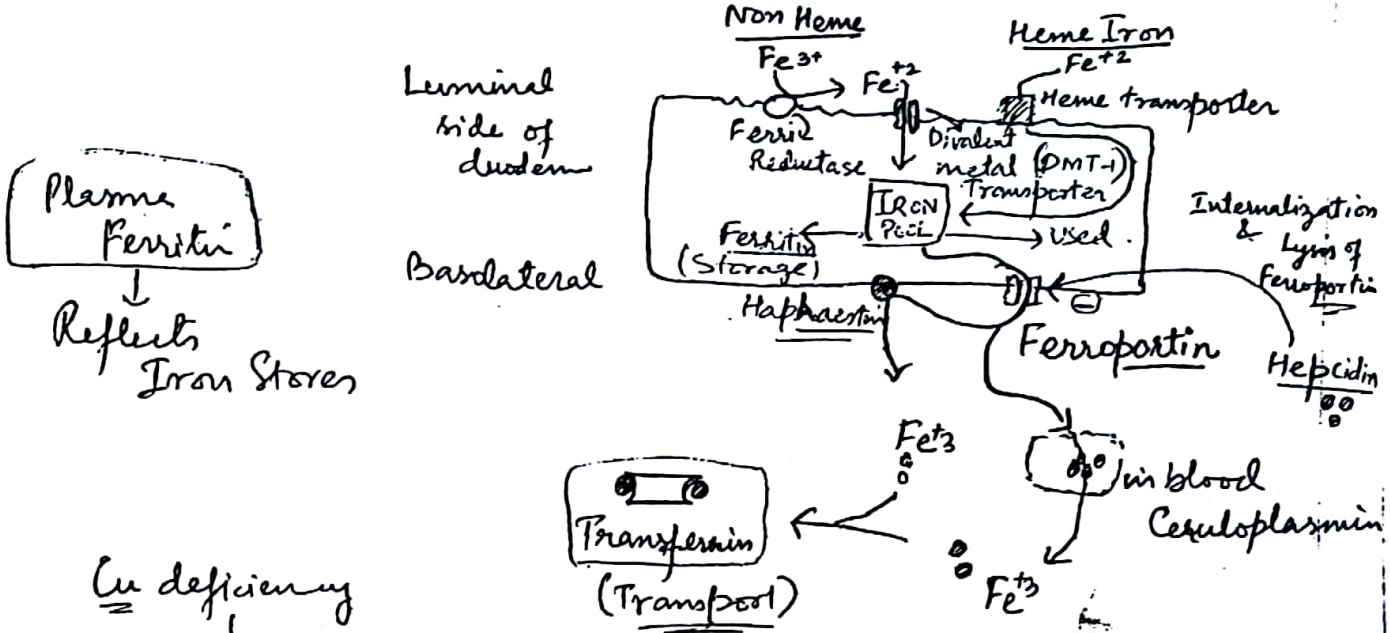
- Schistocytes
or
Schizocytes
or
Fragmented RBCs
or
Helmet Shaped

Macroangiopathic Hemolytic Anemia

⇓

Prosthetic valves
Coarctation of Aorta

Iron Absorption



Cu deficiency
↓
can also lead to
Iron deficiency
As Haphaestin is ⇒

Hepcidin
↳ ↓ing the exit of Iron from cell to blood.

TIBC ⇒ Reflection of Transferrin Production in body.

Transferrin % Saturation

Iron deficiency Anemia

- Earliest smear finding is: **Anisocytosis (RDWT)**
- After treatment first response: **Improvement of Mood**
  Sign is Reticulocytosis
  (↑se in Reticulocyte Hb)
- Pencil Shape cells +ve ↳ characteristic of Iron def. anemia

Megaloblastic Anemia :-

- It is a macrocytic anemia, but every macrocytic anemia is not a megaloblastic anemia

- Macrocytic anemia ⇒ MCV > 100

Folic acid / Vit B_{12} ↓ ⟶ Retarded DNA Synthesis

↓

Immature Nuclei + Mature cytoplasm + Big cells

Megaloblasts in marrow

↓

Macrocytes / Ovalocytes in blood

↓

Earliest finding

Other cells are also affected

↓

Giant metamyelocytes + Hypersegmented Neutrophils

↓

Most characteristic

Ineffective erythropoiesis ⟶ Improperly formed cells

↓

Destroyed in marrow itself

↓

Hemolytic phase

↓

Jaundice

Platelets

Injury to vessel
↓
Vasoconstriction
↓
Primary Platelet Plug (unstable)

Fibrin clot
↑
Coagulation Cascade.

Primary Hemostasis

- Disorders will present ~ Superficial haemorrhages
- Not associated with family history

Secondary Hemostasis

Deep haemorrhages

Family history +ve

Thrombocytopenia
Immune Thrombocytopenic Purpura (ITP)
Thrombotic Thrombocytopenic Purpura (TTP)

Hemophilia (A/B/C)
Clotting factor inhibitors

Von Willebrand Disease

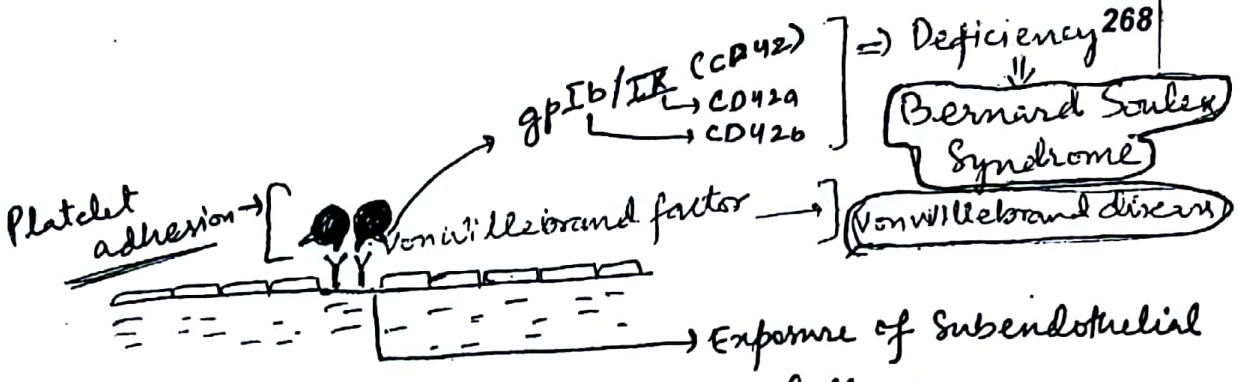
Disseminated Intra Vascular Coagulation.

Platelets release ① granules.

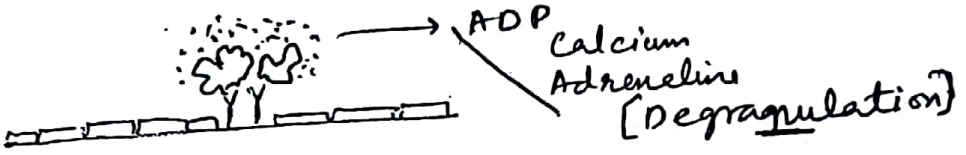
α — δ — Serotonin, ADP, Ca+

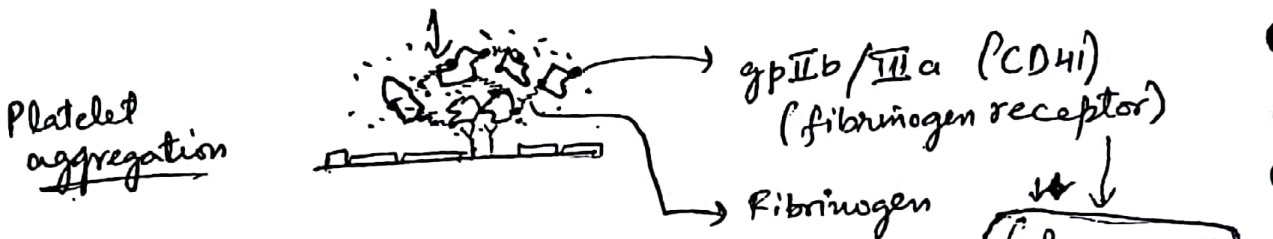
② Thromboxin A_2

Fibrinogen
↓
Coagulation
↓
Fibrin
(on the surface of primary platelet plug)



↓ Platelet activation





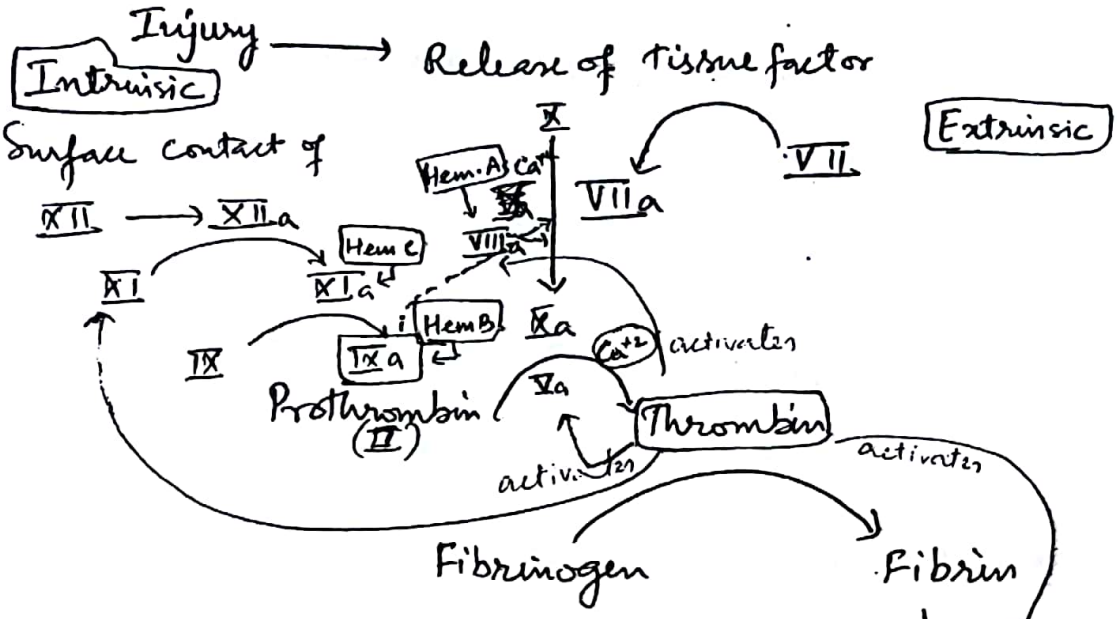
Platelet function Aggregometry (PFA)

Umbilical Stump bleeding

Agonists are given to stimulate Primary Plug formation

- Ristocetin → Plt. adhesion
  - → If no curve/flat curve
    - ↓
    - VWF or gpIb/IX ↓↓
    - ↓
    - VWD or gpIb/IX def.
    - ↓
    - BSS

Injury to vessel
↓
Release of Tissue factor



XII def → Asymptomatic

Polymerises & Stablises the CLOT.

Intrinsic Pathway

Measured by

APTT (Activated Partial Thromboplastin Time)

[25 - 40 sec (N)]

Extrinsic Pathway

Measured by PT.

[11 - 14 sec (N)]

Factor VII - K dependent factor

Prothrombin time is measured as

Platelet Poor Plasm + Tissue factor [Thromboplastin]

↓

Phospholipids (platelets are missing)

↓

Calcium (Monitor stopwatch)

APTT

Platelet poor plasma + Surface Activator (glass, silica, kaolin)

↓

Phospholipids (Platelets are missing)

↓

Calcium → (Start stopwatch)

Immune Thrombocytopenic Purpura

Auto antibodies against Plt. Ags.

Most common – gp IIb/IIIa

Destruction of plts. by splenic macrophages [TOC – Splectomy]

Children (Acute) – Following Infection

Adults (chronic) – No H/o Infection
Auto immune disorders.

Marrow → Megakaryocytic Hyperplasia (not required of Dx) as it is non specific

VWF (von Willebrand Factor)

Produced as large multimers —ADAM TS 13 enzyme→ broken in small molecules

↓

Act as carrier for factor VIIIa

Deficiency of ADAM TS 13 enzyme

↓

Large multimers of VWF will cause widespread platelet ~~aggre~~ adhesion followed by aggregation.

↓↓

Thrombotic Thrombocytopenic Purpura

If VWF is deficient ⟶ No primary plug formation
↓
Bleeding

If Severe
↳ APTT ↑

Functional deficiency of VIII
will lead to ↑ APTT in VWD.

DIC

- Procoagulant molecules (cancers, Autoimmune)
- Extensive endothelial injury

↓↓

Wide spread thrombosis → PT ↑, APTT ↑ } due to Coagulation factor consumption.
Bleeding

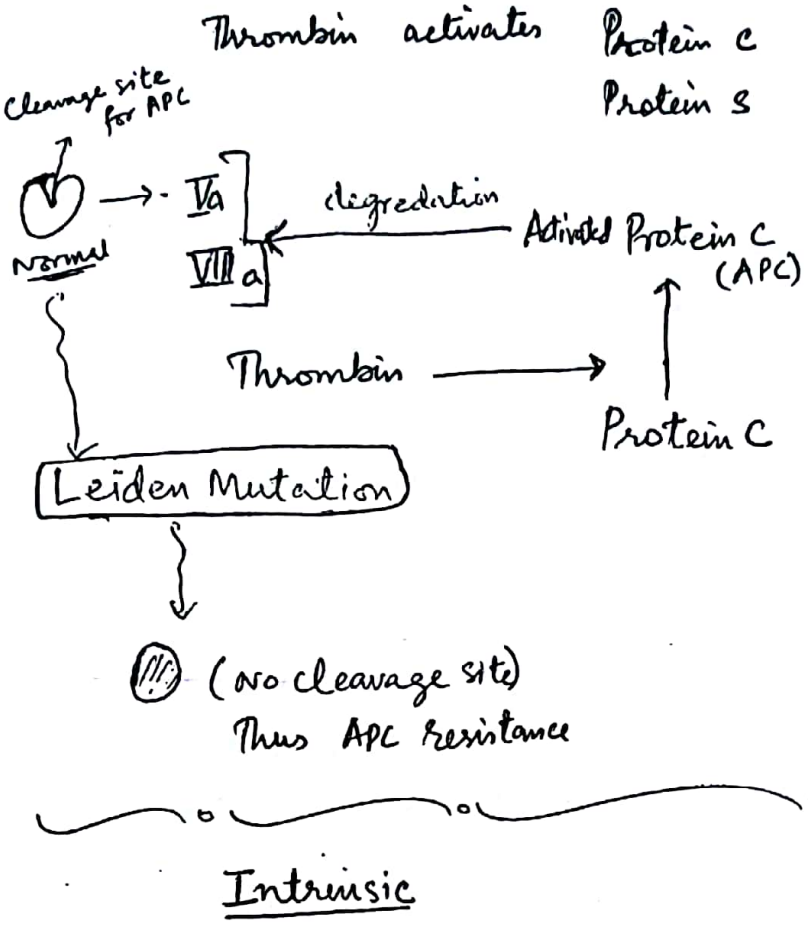
Also called as Thrombohaemmorhagic Disorder.

Bleeding Pt. with APTT ↑
↓
Mixing Studies ⇒ Pt plasma + Normal pooled plasma
50:50
↓
Repeat APTT

APTT Normalize
Confirms Coagulation factor deficiency
↓
Factor Assay.

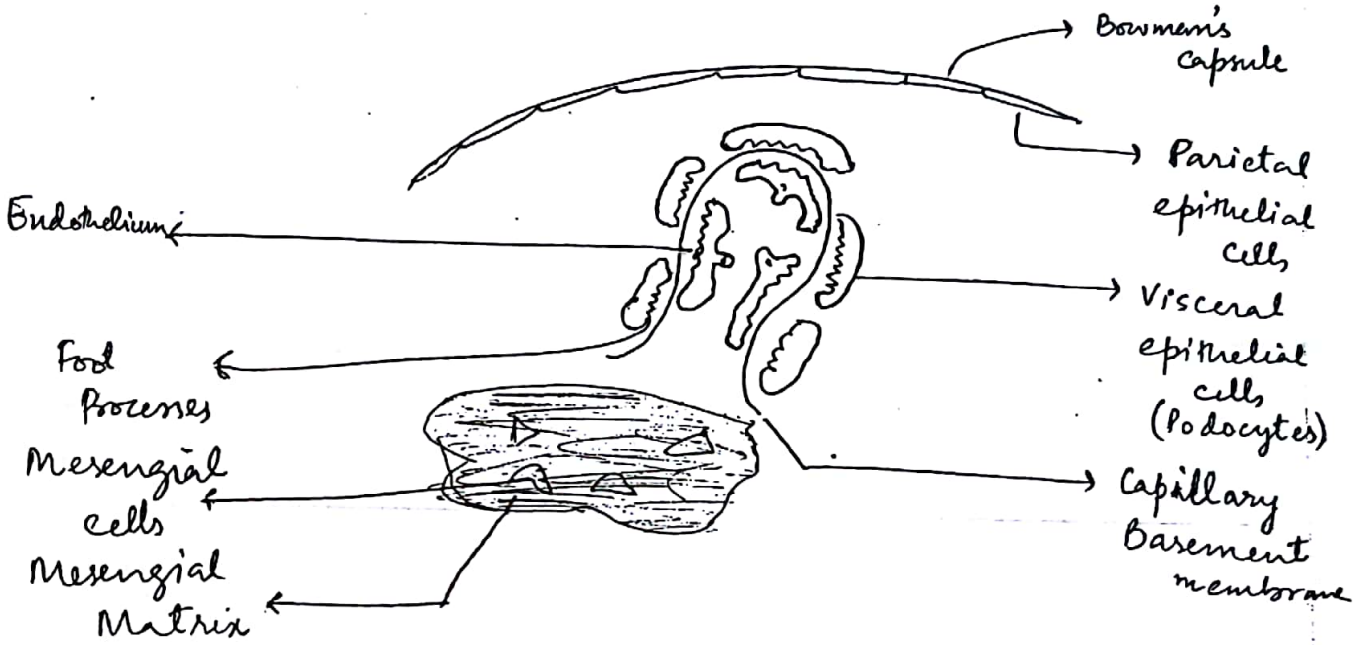
APTT is Still ↑ed
↓
Confirms presence of Clotting factor Inhibitors
↓
Lupus Anticoagulant ← Dilute Russel Viper venom Test

Inherited Thrombophilia



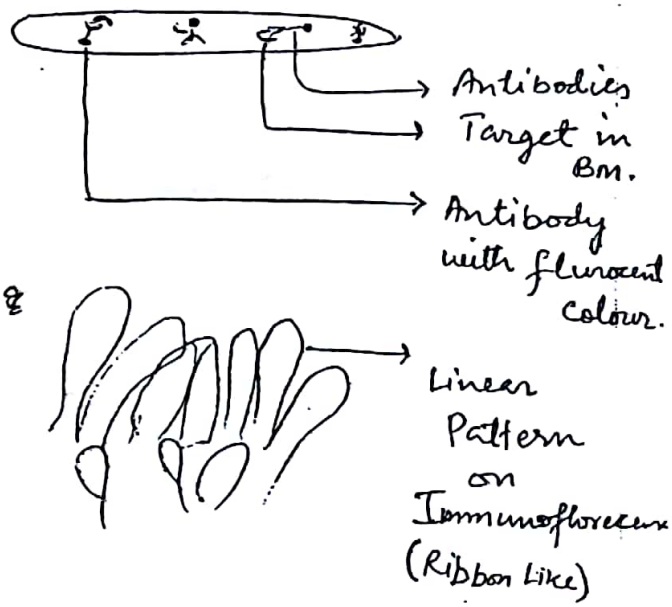
Renal Pathology

Ultrastructure of Glomerulus



Mechanisms of Glomerular Injury

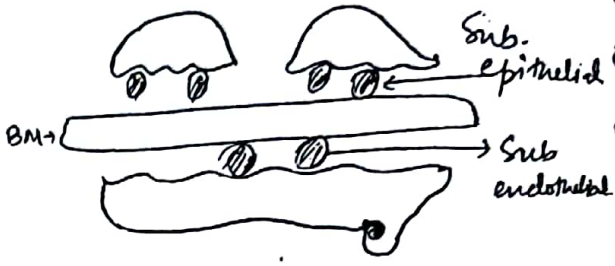
① Antibodies to components of Basement membrane.



2) Immune Complex deposition

↓ Circulating

↓ Insitu



⇓

→ Granular Pattern (Coarse, Irregular texture).

3) ANCA mediated.

(Anti Neutrophil cytoplasmic Antibody)

⇓

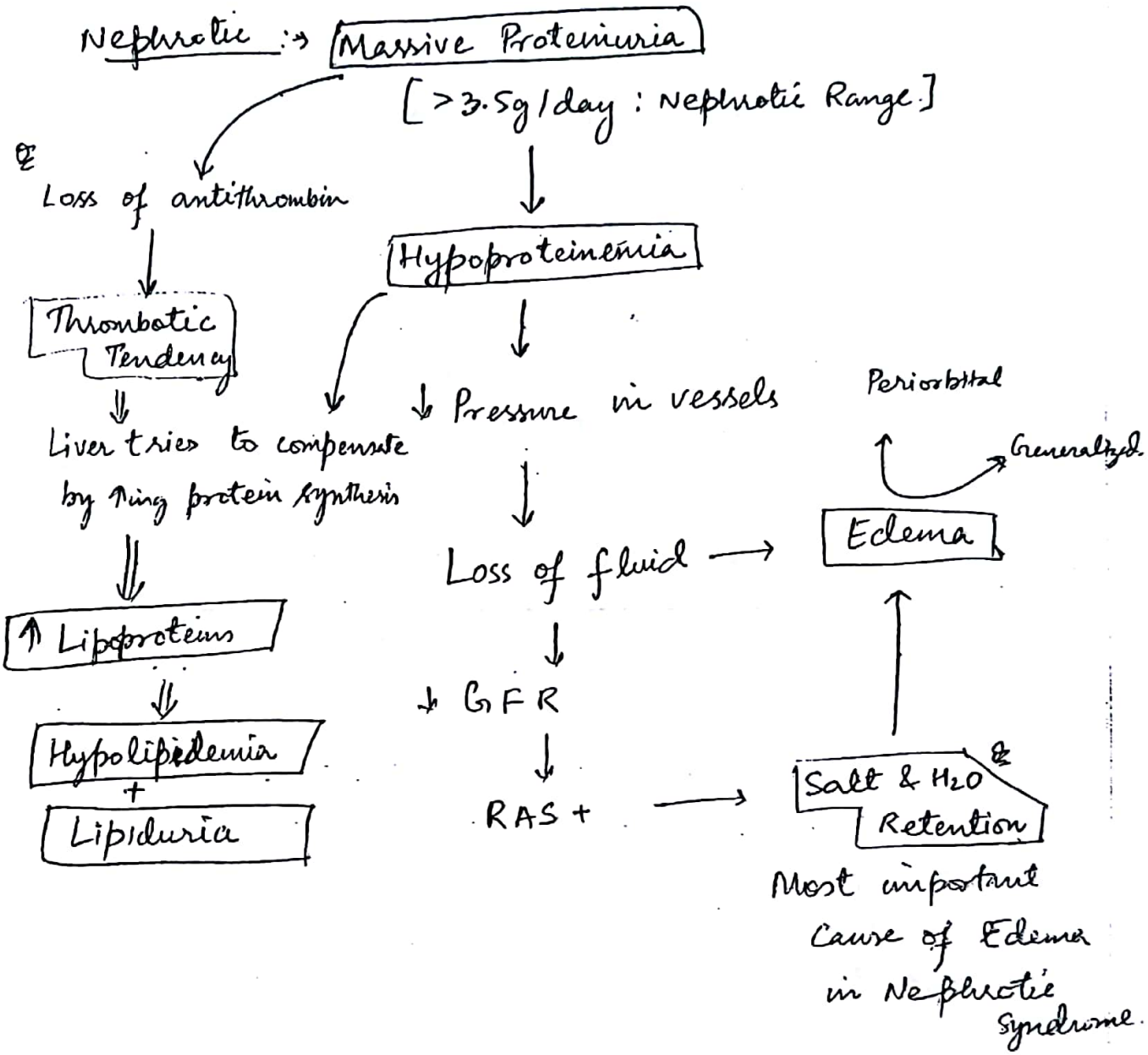
(No pattern on immunofloresense.)

Clinically

Hematuria - Damage to glomerular basement membrane (most often due to inflammation)

↓ Nephritic

Proteinuria - Derangement of capillary wall + Podocytes.



Proteinuria
Lipiduria
Hypolipidemia
Hypoproteinemia

Clinicopathological features

1. Minimal change disease (Nephrotic)
2. Focal segmental glomerulosclerosis (Nephrotic +/- Nephritic)
3. Membranous Nephropathy (Nephrotic)
4. Membroproleferative glomerulonephritis (Nephrotic + Nephritic)
5. IgA Nephropathy (Nephritic)
   ↳ (BERGER's Disease)
   (BUERGER ✗ → Thrombangitis Obliterans)
6. Post infectious glomerulonephritis (Nephritic)
7. Rapidly progressive glomerulonephritis (RPGN) (Nephritic)

Pathological Assessments

① Light Microscopy
- H & E
- Special Stains → PAS (Magenta)
  → Jone's Methanamine Silver (Black)

② Immunofluoresence → No pattern.
→ Linear
→ Granular
→ Any other

③ Electron microscopy → Site of deposition
→ Associated changes

Minimal Change Disease

(NIL LESION or LIPID NEPHROSIS)

? Cytokine mediated podocyte Injury.

↳ Dramatic Response to steroids.

↳ This condition is associated with Hodgkin lymphoma, & Tcell lymphomas

LM ⇒ None

IF ⇒ None

EM ⇒

→ Effacement/Loss of foot processes

Focal Segmental glomerulosclerosis

- Only some of the glomeruli are involved
- Only a part of glomerulus is involved
- Thickening/Hardening → Hyaline deposition

Eosinophilic Amorphous Acellular Homogenous glassy.

[Intracellular/Extracellular]

Tiny Tiny damage to BM → Tiny Tiny leakage of plasma proteins

↓

Podocyte damage → Dying Podocyte → Debris → Deposited outside the capillary wall (Hyalinosis)

↓

Loss of foot processes.

+

Lipid Deposition.

Obliteration of Capillaries and sclerosis of glomerulus

⇒ Idiopathic (Primary)

⇒ Secondary focal segmental glomerulosclerosis

→ Virus : HIV / HepB / Parvovirus

→ Hypersensitivity Nephropathy

⇒ Reflux Nephropathy.

Cholesterol emboli

⇒ Drugs: Heroin / analgesic / Pamidronate.

⇒ Oligomeganephronia

⇒ Renal dysgenesis

⇒ Alport's syndrome

⇒ Sickle cell disease

⇒ Lymphoma

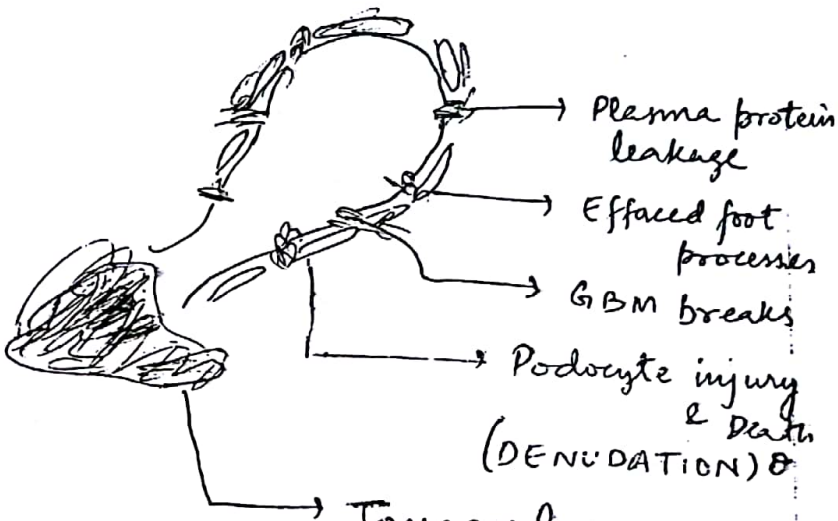
⇒ Radiation nephritis

⇒ Familial podocytopathies

Gene	Protein
NPHS 1	mutation/nephrin
NPHS 2	mutation/podocin

LM: Mesangial widening by sclerosis
↓
Obliterate capillaries
↳ Hyaline nodules.

IF: Usually no pattern > Granular.

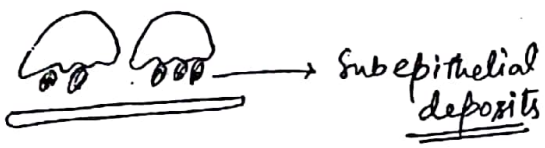


Membranous Glomerulopathy

Primary

Antibodies against

Phospholipase A_2 Receptors on podocytes



Secondary

① Drugs – Gold
NSAIDs
Penicillamine

② Infections – Hep B
– Hep C
– HIV
– Leprosy; Syphilis

③ Cancers – Lung &
– Colon &
– Melanoma

④ Autoimmune
Hashimoto & SLE &

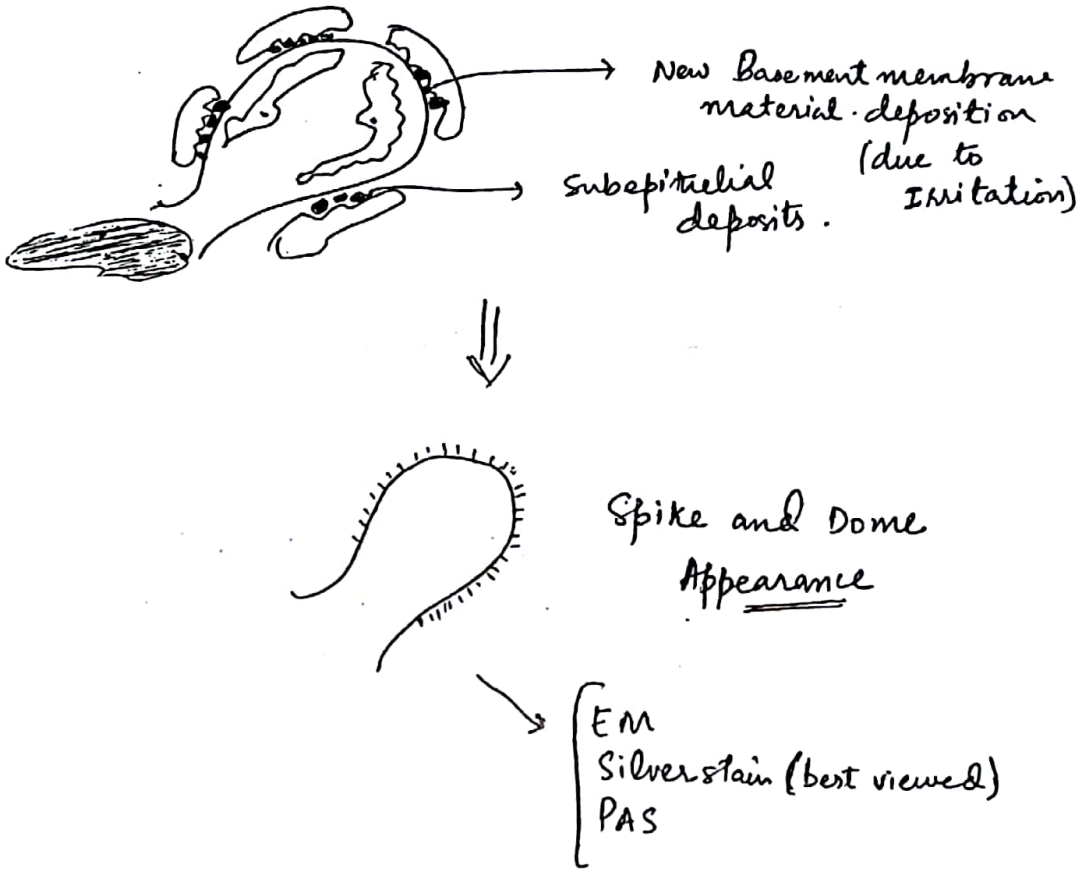
LM :-

- Thickened basement membrane H & E

IF

Granular IF.

EM



Membrano proliferative Glomerulonephritis

- Thickened BM → ⊕ Subendothelial Deposits
- ↑sed cellularity (↑ cells) → ⊕ Mesangial & Endothelial (Reactive)
- Inflammation → ⊕ GBM damage & Podocyte Damage.

Type I (Most common)

Type II (Dense deposit disease)

Type III (Now removed)

Type I

Subacute bacterial endocarditis
Systemic Lupus erythematous
Hepatitis C ± cryoglobeulinemia
Mixed cryoglobulinemia
Hepatitis B
Cancer: Lung, breast & Ovary (germinal)

Type II

C_3 Nephritic factor - associated

Type III

Complement Receptor deficiency

LM

Hypercellular glomerulus.

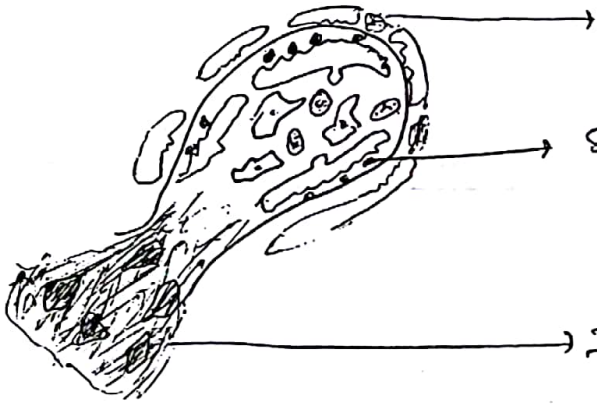
- Mesangial cells ↑ed (more) } AKA Mesangio Capillary Glomerulonephritis
- Endothelial cells ↑ed

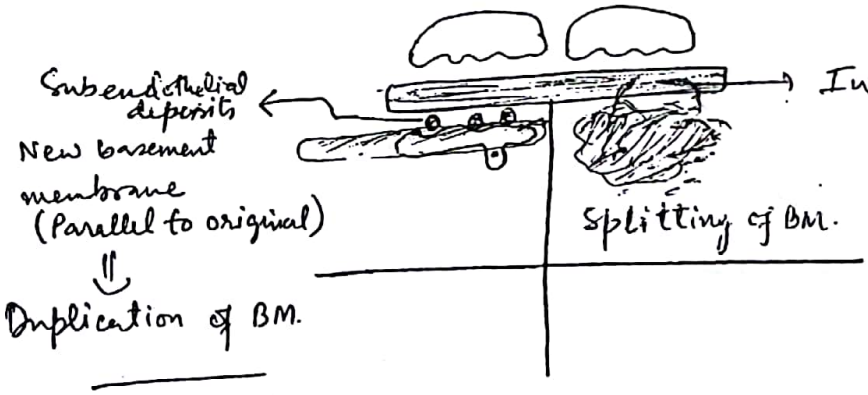
Inflammatory cells ⊕

Silver

IF - Granular.

EM



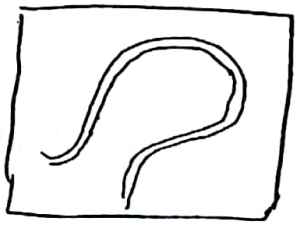


⇓

Duplication of BM.

⇓

TRAM TRACK APPEARANCE
or
DOUBLE CONTOURING



IgA Nephropathy (BERGER'S Disease)

↑sed IgA: Following Respiratory/GIT infections.
< 1 week ♁.

↓sed IgA clearance: Liver diseases
(↓ed hepatobillary clearance)

Abnormal IgA: Celiac disease ♁

Also attacks anchoring filaments in hemidesmosomes of Dermal papillae thus leading to
⇓
Dermatitis herpetiformis.

LM
Mesangial widening.
(even on PAS)

IF: Mesangial pattern.

EM:

→ Mesangial deposits

↳ ↑ mesangial matrix & cells.
⇓
Mesangio proliferative
(Mesangial widening)

PSGN

POST INFECTIOUS

After 1-3 wks of throat infection or 3-5 weeks of skin Infection

(POST) INFECTIVE → Streptococcal, Staphylococcal, Viruses, Parasitis

or

(Acute) Proliferative → Glomerulonephritis

Onset is quick: Transient. Rarely leads to chronic renal failure.

Increase in number of cells. ↓ Hypercellular glomeruli.

Inflamation → Damages GBM

LM :- Hypercellular glomerulus

increased (Endothelial cells) — More ↑ (Max seen)
Mesangial cells
Inflammatory cells

IF : Granular

EM

→ Subepithelial deposits (MC & characteristic)

→ Endothelial ↑

→ Subendothelial (early)

→ Mesangial cells ↑

Most common cause of Nephrotic Syndrome.

In adults – Focal segmental Glomerulosclerosis

In Children – Minimal change Disease

In Elderly – Membranous nephropathy.

Most common cause of Glomerulonephritis.

Primary GN in world : IgA Nephropathy

Primary GN in India : Post Streptococcal glomerulonephritis

Secondary GN : Diabetes Mellitus.

Most common type of Glomerular Disorder in.

⇒ Leprosy:- MPGN

⇒ Syphilis : Membranous Nephropathy.

⇒ Malaria : Mesangioproliferative.

⇒ Hepatitis C : Cryoglobulinemic glomerulonephritis > membranous glomerulopathy > type 1 MPGN.

⇒ Hepatitis B : Membranous Nephropathy (Component of Hep B virus Responsible - HBsAg).

⇒ SLE – Diffuse glomerulonephritis (class IV) Lupus Nephritis

⇒ Colon cancer / lung cancer – Membranous glomerulopathy.

Rapidly Progressive GlomeruloNephritis

Onset — Has progressed from something.

Type I

Anti GBM antibody

- Renal Limited
- Good Pasture's Syndrome

Type II

Immune Complex mediated

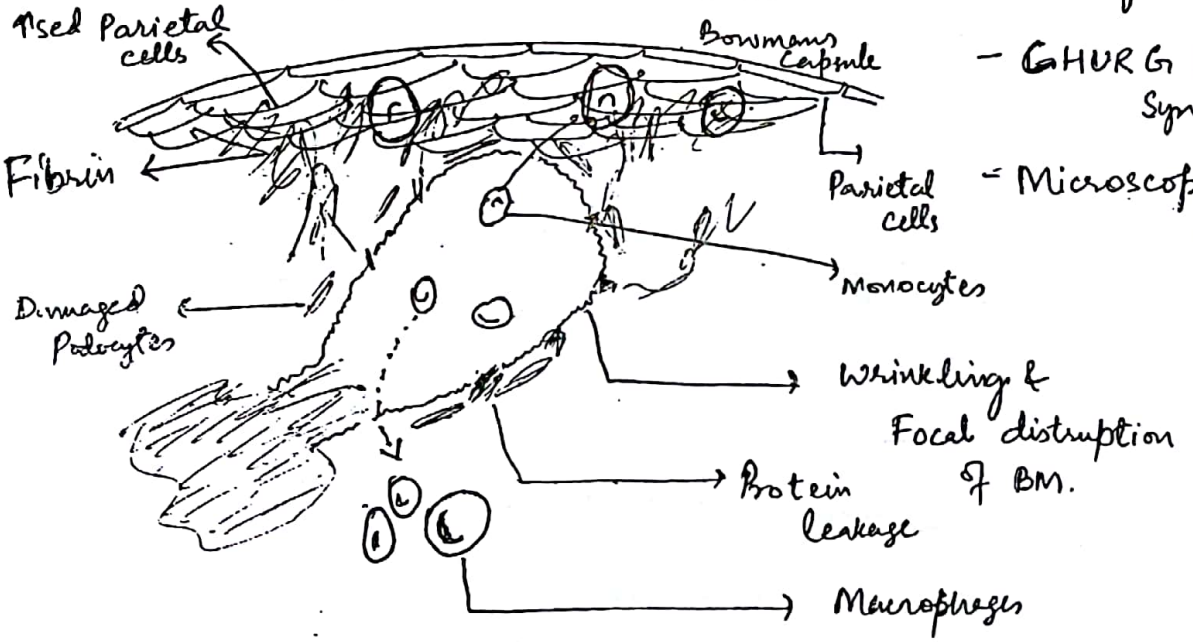
- IgA Nephropathy
- PSGN
- SLE

Type III

ANCA mediated.

(Pauci Immune)

- Wegener's granulomatosis
- CHURG STRAUSS Syndrome
- Microscopic Polyangitis



⇒ CRECENT ⇒
- ↑ Parietal cells
- Fibrin
- Macrophages

Subepithelial
Deposits on EM
+
GRANULAR IF

- Nephrotic → Membranous Nephropathy
- Nephritic → PSGN

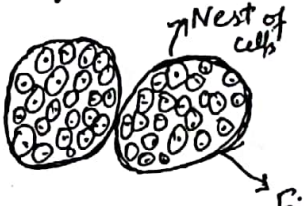
Hypercellular
Glomerulus
+
GRANULAR IF

- Subepithelial → Nephritic → PSGN
- Subendothelial. → Nephritic + Nephrotic → MPGN

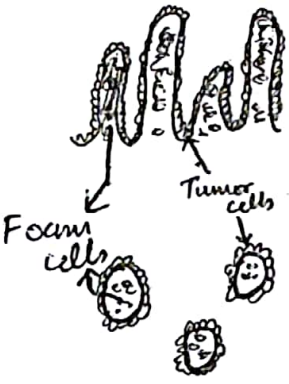
Renal cell Carcinoma

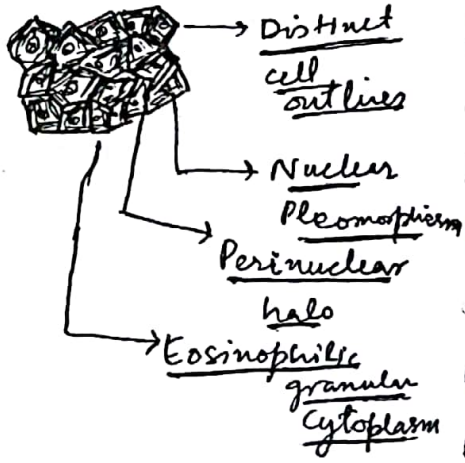
	Clear cell	Papillary	Chromophobe
ORIGIN	← Proximal tubule →		Intercalated cells of CD.
Genetics	3p deletions → VHL gene	Trisomy (7) 17, Loss of Y, & Met-proto-oncogene	Extreme Hypoploidy.

Histology



Pleomorphism with Clear cytoplasm (because of glycogen/lipid)





+
- CK7 +ve
- Hem's colloidal iron +ve

Psammoma Bodies (Dystrophic Calcification)

[ARISE FROM THE INFARCT and CALCIFICATION OF PAPILLAE TIPS.]

P - Papillary Ca thyroid
P - Prolactinoma
S - Serous CAO
Sa - Somatostatinoma
M - Meningioma
Mo - Mesothelioma

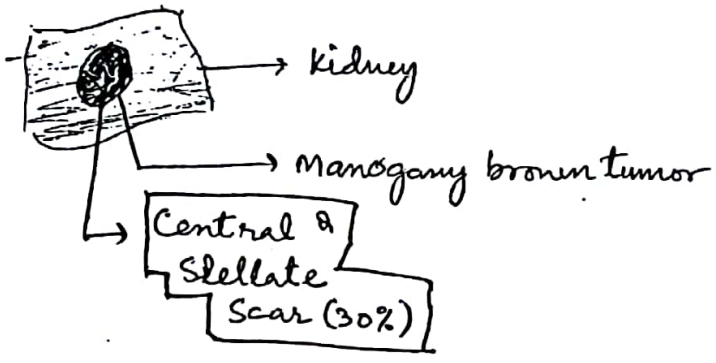
KFI
- Papillary Ca thyroid
- Serous cystadenocarcinoma ovary
- Meningioma
- Mesothelioma

Intercalated cells of collecting duct

↓ gives Rise to

Benign Tumor

Oncocytoma



Histology

Same as chromophobe except

- No perinuclear halo
- No nuclear pleomorphism

(cells are eosinophilic & granular because of increased number of mitochondria)

Angiomyolipoma
Picoma

Pancreas
↓
Central scar

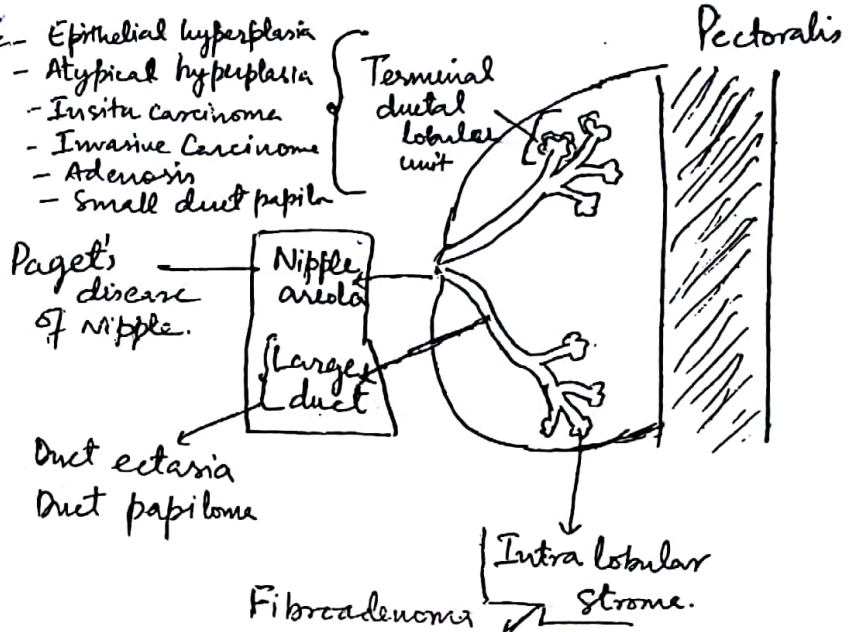
Renal cell carcinoma

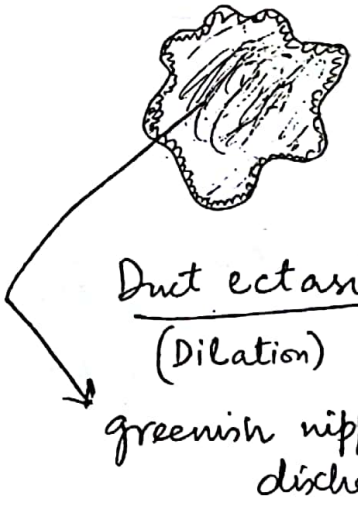
- Most common subtype: Clear cell carcinoma
- Most common subtype in sickle cell anemia: Medullary Ca
- Most favourable prognosis: Chromophobe
- Least favourable prognosis: Sarcomatoid > Medullary > Collecting duct > Clear cell

BREAST PATHOLOGY

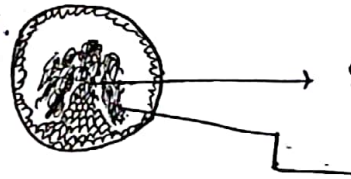
- Epithelial hyperplasia
- Atypical hyperplasia
- In situ carcinoma
- Invasive Carcinoma
- Adenosis
- Small duct papilla

} Terminal ductal lobular unit

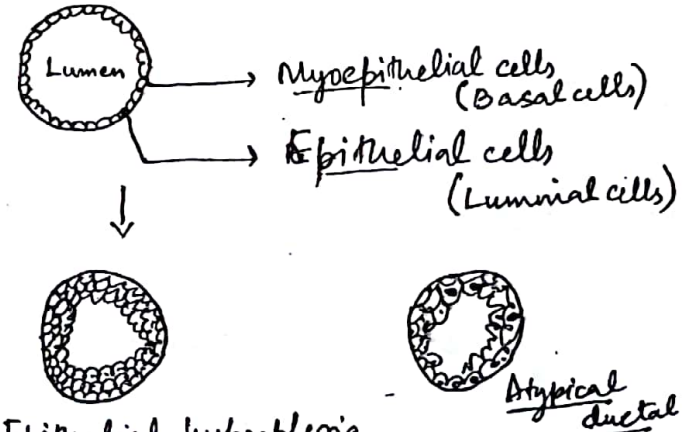




Duct ectasia
(Dilation)
greenish nipple discharge

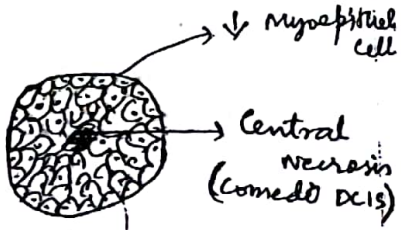


Duct papilloma
(Benign papillary tumor of lining Epithelium)

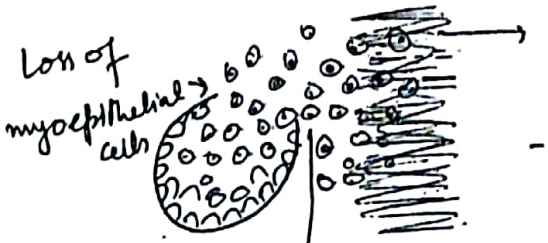


Epithelial hyperplasia
(NO pleomorphism of Nuclei)

Atypical ductal Hyperplasia



Ductal carcinoma in situ (DCIS)



→ Desmoplastic Stroma (invaded)
- Stroma reacts with fibrosis

Invasive Carcinoma

→ Invasion of basement membrane.

Benign epithelial Lesions

Non Proliferative
(No relative Risk)

→ Duct ectasia
Fibrocystic change
Apocrine Metaplasia

Proliferative

Without Atypia (Slight Risk)
- Duct Papilloma
- Epithelial Hyperplasia
- Sclerosing Adenosis

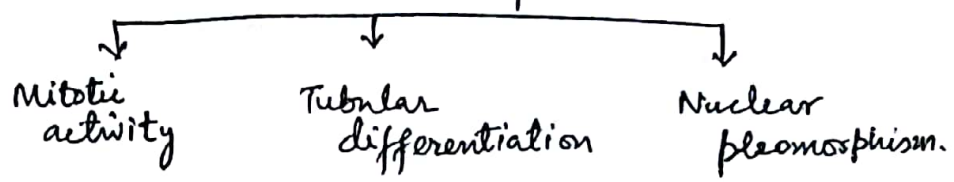
With Atypia (Moderate Risk)
- Atypical ductal hyperplasia
- Atypical lobular hyperplasia

Higher Risk
- DCIS
- LCIS

Histological Subtypes of Invasive Carcinoma

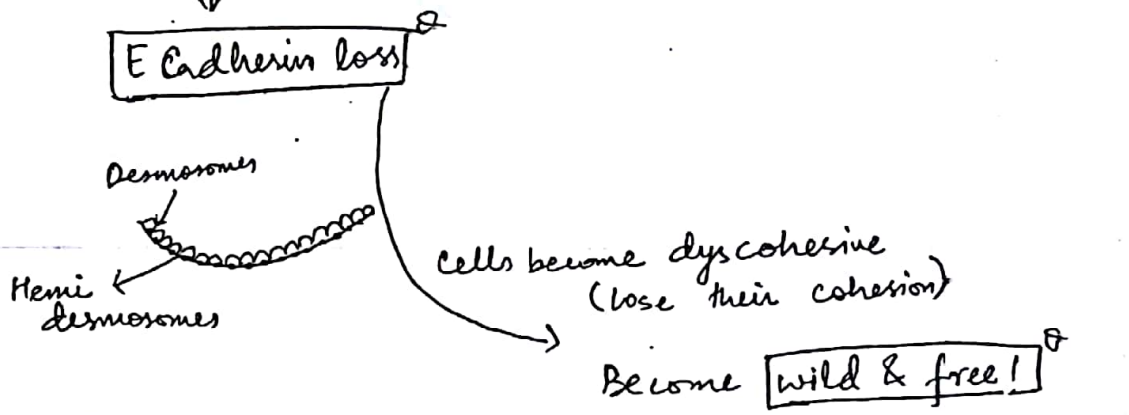
1. **Invasive Ductal Carcinoma, Not otherwise specified** (IDC, NOS)
   - Most common histological subtype
   - Firm, grey white, Irregular

- Grading based on **NOTTINGHAM CRITERIA**



② Invasive Lobular Carcinoma. (ILC)

CDH 1 gene mutation (Loss of function for making protein E-Cadherin)



Single File / Indian File Pattern

⇒ Fail to elicit a desmoplastic response

[difficult to detect as a well defined mass on imaging]

⇒ Involve contralateral side commonly.

⇓

Metastasize to
- Leptomeningis (arach. + Pia)
- Peritoneum
- Ovaries

③ Medullary Carcinoma

Sheets of tumor cells interspersed with lymphocytes.

↓ Poorly differentiated tumor

↓ Improves treatment response

↓ Thus better prognosis as compared to other poorly differentiated tumors.

Usually associated with BRCA 1 gene mutation.

- Triple Negative (ER−, PR−, Her2 Neu−)

④ Tubular Carcinoma — Best prognosis

⑤ Inflammatory Carcinoma

Dermal lymphatics are involved by tumor cells

↓ Peau de orange appearance.

Tumor genetics

Sporadic (more common)[Q]

Most common: p53

Familial

- BRCA1 (most common)[Q] → Ovarian Cancer
- BRCA2 → Male breast Cancer, Pancreatic Cancer, Prostate Cancer
- p53 → Li Fraumeni Syndrome

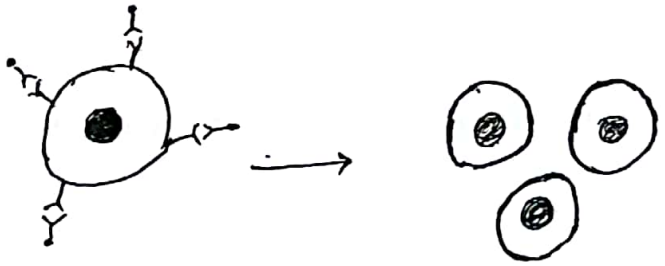
- Triple Negative (ER-, PR-, Her2Neu-)
- ER+ (Her 2 Neu -ve)
  - ~~Her 2 neu~~
  - ~~Her 2 Hard~~
- Her 2 Neu +ve
  - ER+
  - ER-

IHC

Chromogen: Di Amino Benzidine (DAB)

BROWN

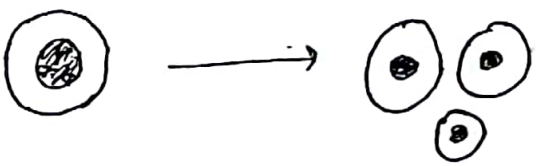
diamino Benzidine



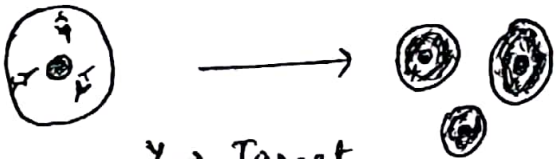
Membranous Positivity [Her 2 Neu]

IHC ⇓ Brown on blue

Test / Contrast/control



Nuclear Positivity [ER, PR]



Cytoplasmic Positivity [cytokeratin]

Y → Target
Y → Antibody against
● → Chromogen

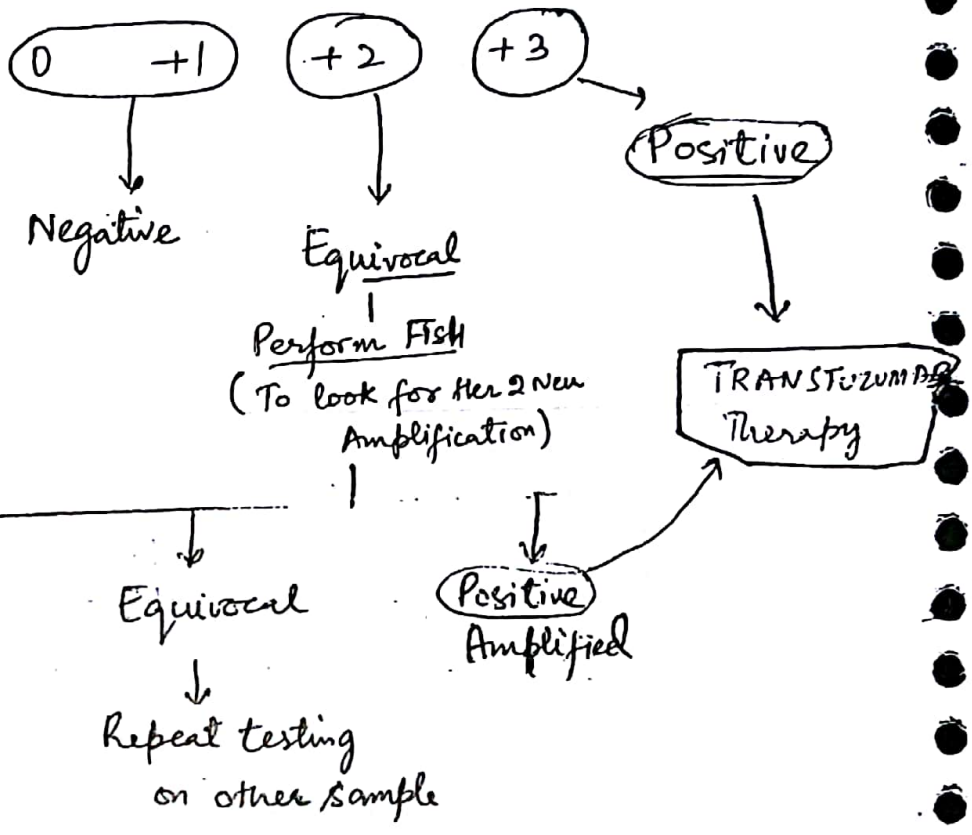
IHC in Breast Cancer

① ER/PR: Allred Score - [0-8]

Intensity + % of positive cells.

② Her-2-Neu

% of cells positive & intensity.



Breast Carcinoma

Most common histological subtype - IDC, NOS

Most common molecular subtype - Luminal A

Histological subtype with Best prognosis - Tubular > Mucinous

Molecular subtype with Best prognosis - Luminal A

Most common genetic mutation - p53.

Most common familial genetic mutation - BRCA 1.

Highest susceptibility seen with which mutation - BRCA2

Association of male breast cancer, prostate cancer, pancreatic Ca, melanoma and gastric cancer - BRCA 2

Association of ovarian cancer (serous) - BRCA1 > BRCA2

Most common molecular group associated with BRCA1 - Basal

Most common molecular group associated with BRCA2 - Luminal B

Most important prognostic marker - Lymph node status.

Most important marker (prognostic) for metastatic Cancer - ER/PR status

Least desmoplastic tumor - Lobular Carcinoma.