3) Cytogenetic Study

⇒ Used to detect Philadelphia chromosome

- Reciprocal translocation of t(9, 22)/q, 34; q11)

4) PCR for BCR-ABL Fusion Gene

5) Leukocyte Alkaline Phosphatase (LAP) score.

- In CML is is Low, whereas in leukemoid reactions, it is high.
- Helps to differentiate between CML & Leukemoid reaction.

C) Peripheral Smear Findings.

▷ Marked Leukocytosis

- Very high levels of WBC count
→ 2,00,000 /mm³ cells are present. Normal levels are 4000 - 11,000 /μL.

▷ Left shift of Granulocytes

- Presence of immature granulocyte precursors in peripheral blood.

▷ Myelocyte Bulge

- A disproportionately large number of myelocytes compared to other immature cells.

▷ Basophilia / Eosinophilia

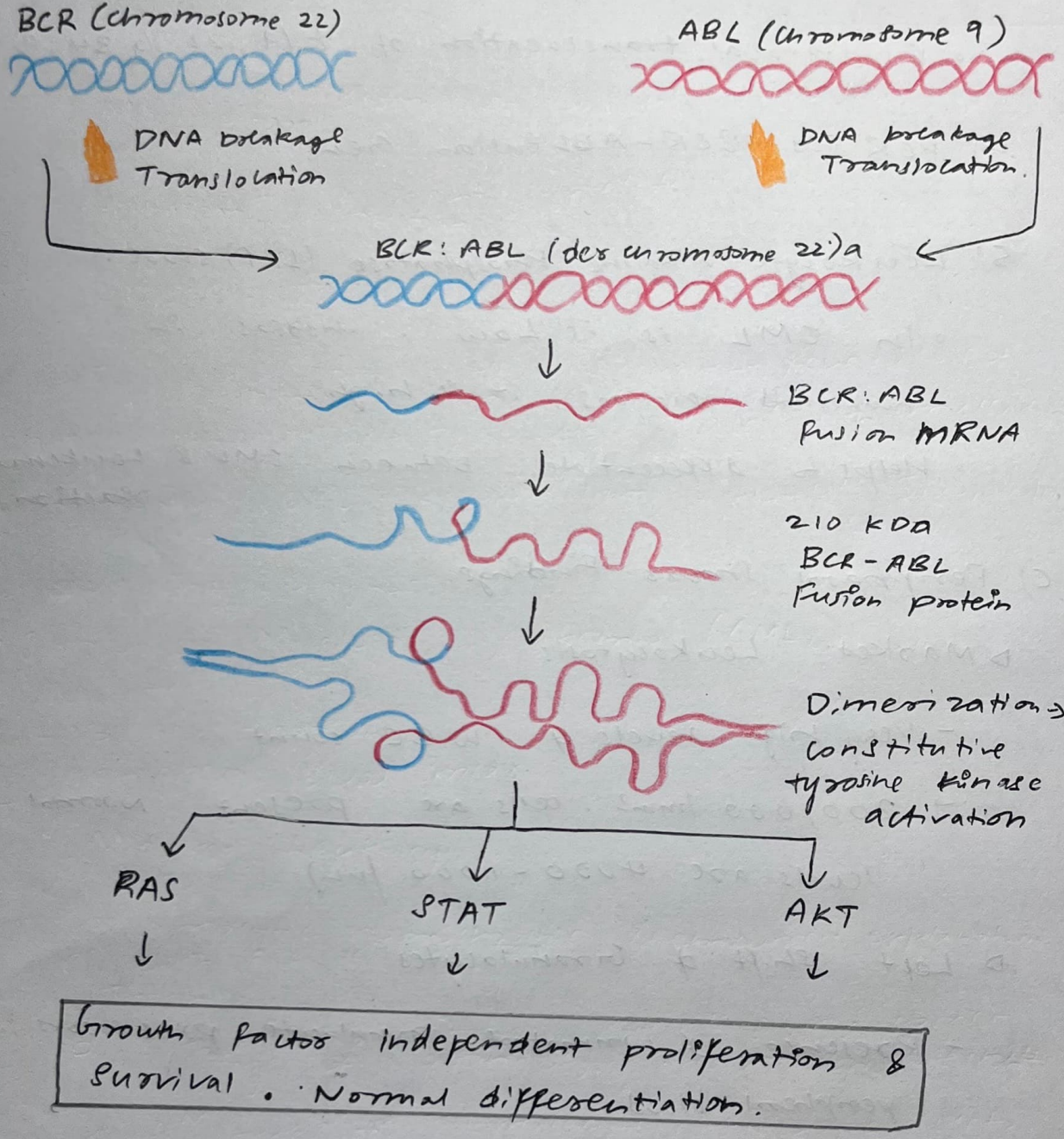
▷ Normocytic Normochromic Anemia

- Number of RBC is reduced.

▷ Thrombocytosis.

d) Molecular Pathogenesis of CML

The hall mark event is chromosomal translocation.



▷ Breakage & joining of BCR & ABL creates a chimeric BCR:ABL fusion gene that encodes a constitutively active BCR-ABL tyrosine kinase.

▷ BCR-ABL activates multiple downstream pathways, which drive growth factor independent proliferation & survival of BM progenitors.

▷ Because BCR-ABL does not interfere with differentiation, the net result is an increase in mature elements in peripheral blood, granulocytes & platelets.

3. Neoplasia is the process of new, uncontrolled growth of cells that results in the formation of a neoplasm.

"Neoplasia is an abnormal mass of tissue, the growth of which exceeds & is uncoordinated with that of normal tissues & persists even after the cessation of the stimulus that evoked the change".

Characteristics	Benign Tumor	Malignant Tumor.
Differentiation/ Anaplasia	Well Differentiated, structure often typical of tissue of origin	Some lack of Differentiation (Anaplasia) Structure often deranged
Rate of Growth	Usually progressive & slow	Erratic, may be slow to rapid.
Local Invasion	Usually cohesive, expansile, well-demarcated masses that do not invade the surrounding normal tissues	Locally invasive, Infiltrating surrounding tissue, Misleadingly Cohesive & expansile.
Metastasis	Absent	Frequent
Mitotic Activity	Rare mitoses	Numerous Abnormal mitoses
Recurrence after removal	Rare	Common.

Routes of Metastasis

Metastasis means the spread of malignant tumor cells from primary site to distant sites forming secondary tumors.

Main routes of metastasis.
1. Direct seeding of body cavities or surfaces.
2. Lymphatic spread
3. Hematogenous spread.

1. Seeding of Body cavities & Surfaces

- occurs when malignant neoplasms spread to a natural "open ~~cavity, but any body cavity~~ field" lacking physical barriers.
- Most often involved is peritoneal cavity
  → Ovarian carcinomas.

2. Lymphatic spread

Transport.
▷ ↑ Through the lymphatic vessels.

▷ Most common pathway for initial dissemination of carcinomas.

- The pattern of spread follows the natural routes of lymphatic drainage.
  → Carcinoma of breast

3. Hematogenous spread.

▷ It is a typical of sarcomas but is also seen with carcinomas

▷ Involved vessels are usually small veins, as arteries, with their thicker walls, are more resistant to penetration.

4. Explain the aetiopathogenesis of Septic Shock.

Ans) ▷ Etiology

Septic shock usually occurs due to severe microbial infections. Common causes include:

A. Bacterial Infections

- Gram negative bacteria
  - eg: E.coli, Klebsiella, Pseudomonas
  - Endotoxin lipopolysaccharide (LPS) plays a major role.
- Gram positive bacteria
  - eg: Staphylococcus aureus, Streptococcus pyogenes.
  - Produce exotoxins & super antigens.

B. Other microorganisms.

- Fungi ~~causes~~ → candidiasis.
- Viruses
- Parasites.

▷ Pathogenesis

→ It is a complex process & involves immune, inflammatory & coagulation pathways.

1. Microbial products trigger immune response.
   → Endotoxin (LPS), Peptidoglycans, Lipoteichoic acid.
   - Activates the pattern recognition receptors (PRRs) such as Toll-like receptors on macrophages, neutrophils & endothelial cells.
   - This leads to release of pro-inflammatory cytokines TNF-α, IL-1, IL-8, IL-6

2. Systemic Inflammatory response

These cytokines cause Fever, Leukocytosis, widespread inflammation, increased acute phase proteins.

3. Endothelial activation & injury

→ Cells became activated leading to Increased vascular permeability, vasodilation, Leukocyte adhesion.

→ As a result plasma leakage, edema & reduced effective circulating volume is seen.

4. Vasodilation & hypotension.

→ Large amounts of NO cause peripheral vasodilation, Decreased systemic vascular resistance. severe hypotension.

- This results in circulatory changes.

5. Coagulation abnormalities

- Inflammation activates the coagulation cascade leading to DIC. & formation of microthrombi in small vessels.
- Consequences are tissue ischaemia, organ damage & consumption of clotting factors.

6. Metabolic abnormalities

- Due to impaired tissue perfusion there is increased lactic acidosis, cellular hypoxia, mitochondrial dysfunction.

7. Multiorgan Dysfunction

- Reduced blood flow & inflammation lead to failure of kidneys, Liver, heart, lungs, brain.

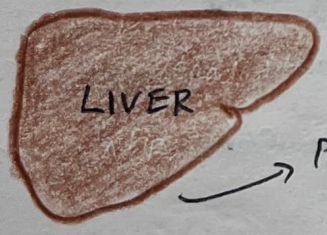
5. Chemical mediators of ~~amyloid~~ Inflammation

These are the biologically active substances released from cells or plasma that regulate the inflammatory process, causing vasodilation, increased vascular permeability leukocyte migration & tissue damage.

Cell Derived

		Source	Action
Preformed mediators in secretory granules	Histamine	Mast cell, Basophil	Increased permeability,
	Serotonin	Platelets	Vasodilation
Newly synthesized	PGL	All leukocytes	Vasodilation
	LT → B4	Chemotactic for phagocyte cells	
	↳ C4, D4, E4	Smooth muscle contraction, vasoconstriction, Bronchoconstriction	
	PAF	Increases permeability	
	ROS	Endothelial cell damage, Tissue damage	
	NO	vasodilation, microbicidal action.	
	Neuropeptides	Transmission of painful stimuli	
	Cytokines	Fever	

Plasma-protein Derived



Plasma

- Complement Activation
  - C3a, C5a → Anaphylatoxins
  - C3b → Increased Permeability.
  - C5b-9 → Membrane attack complex
- Factor XII [Hagemann factor] Activation
  - Kinin System - Bradykinin
  - Coagulation / Fibrinolysis system - Fibrin split products.

7. Stains for Amyloid

1. Stain on Gross:
→ The oldest method, by Virchow for demonstrating amyloid on cut surface of a gross specimen or on frozen/paraffin section is iodine stain. Lugol iodine.

2. H&E
- Appears as extracellular, homogenous, structureless & eosinophillic hyaline-like material, especially deposited in relation to blood vessels & basement membranes.
- Pink, hyaline, homogenous.

3. Metachromatic stains

▷ Rosaniline Dyes.

- Property of ~~metaplasia~~ metachromasia
- Metachromatic stains employed are rosaniline dyes (eg. methyl violet & crystal violet) which imparts rose-pink colouration to amyloid deposits.

4. Congo red & Polarised Light

- Amyloid of all types stains pink-red colour.
- If Congo red stained section is viewed in polarised light, the amyloid characteristically shows apple-green birefringence due to cross-β-pleated sheet configuration of amyloid fibrils.

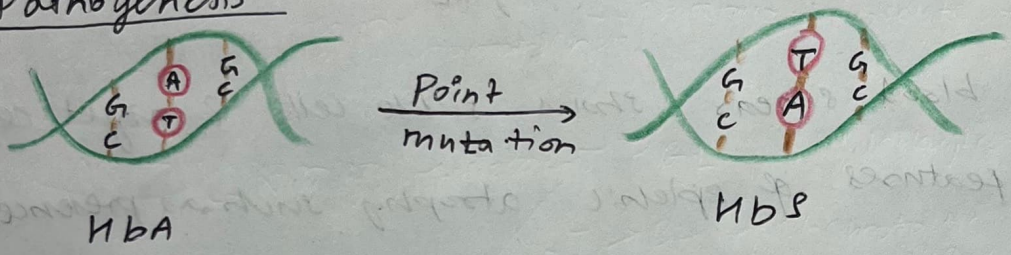
5. Fluorescent stains
- Thioflavin-T binds to amyloid & fluoresces yellow under ultraviolet light, ie. amyloid emits secondary fluorescence. Thioflavin-S is less specific.

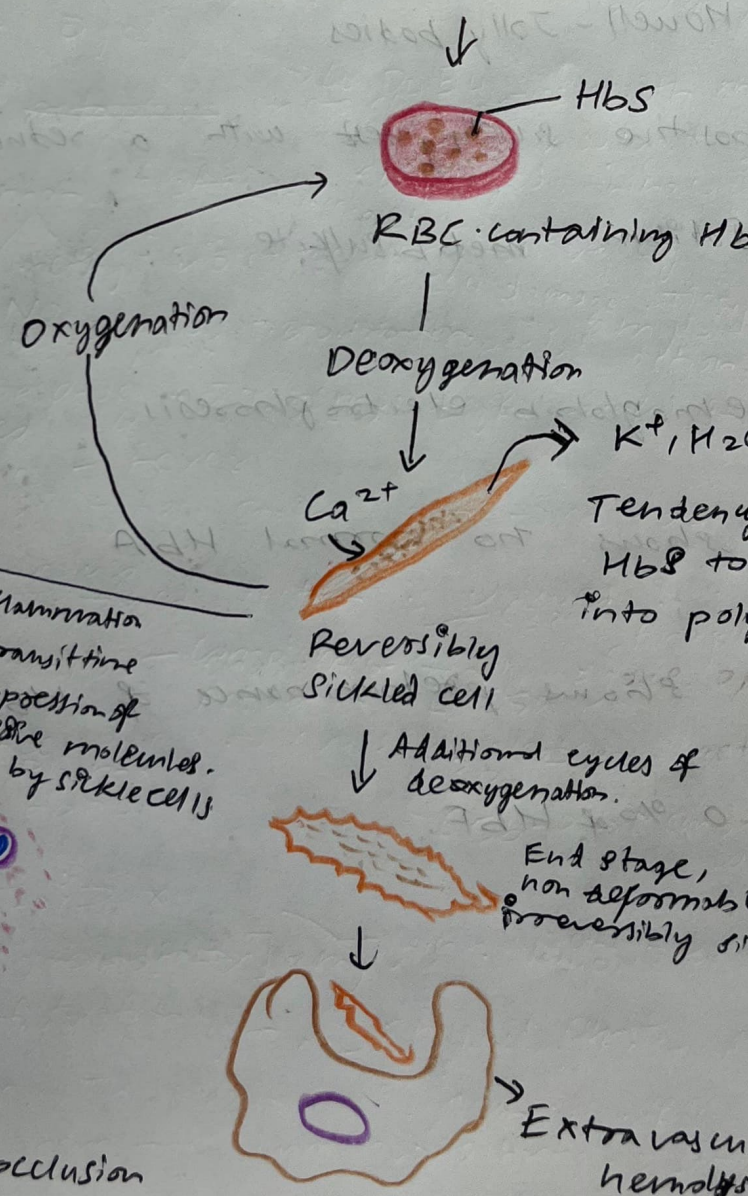
6. Immunohistochemistry: Immunoreactivity - positive

8. Sickle Cell Disease - pathogenesis & lab diagnosis.

Sickle cell disease is a common hereditary hemoglobinopathy caused by a point mutation in β-globin. that promotes the polymerization of deoxygenated hemoglobin, leading to sed cell distortion, hemolytic Anemia, microvascular Obstruction, & ischemic tissue damage.

▷ Pathogenesis





Lab Diagnosis

▷ The diagnosis of SS is considered high in blacks with haemolytic anaemia

- The Laboratory findings are as under:

i) Moderate to severe anaemia

→ Hb concentration . 6-9 g/dL

ii) The blood smear shows sickle cells & target cells & features of splenic atrophy such as presence of Howell-Jolly bodies.

iii) A positive sickling test with a reducing substance as Sodium metabisulfite.

iv) Haemoglobin electrophoresis

→ It shows no normal HbA

→ But shows predominance of HbS &

▷ 2-20 % of HbF.

9. ITP - Pathogenesis & Lab Diagnosis

- Caused by auto antibodies against platelet antigens.
- May be triggered by drugs, infections or lymphomas or may be idiopathic.

- Idiopathic / Immune thrombolytopenic purpura (ITP) is characterised by immunologic destruction of platelets & normal or increased megakaryocytes in the Bone marrow.

▷ Pathogenesis:

- On the basis of duration of illness, ITP is classified into acute & chronic forms.

i) Acute ITP.

▷ self limited disorder, seen in children following recovery from viral illness or upper respiratory illness.
▷ Onset is sudden & severe thrombocytopenia but recovery occurs within a few weeks to 6 months.
▷ Formation of immune complexes containing viral antigens, & formation of antibodies against it which cross-react with platelets & lead to their immunologic destruction.

ii) Chronic ITP

▷ More commonly in adult, especially in women of child bearing age (20-40 years)
▷ Develops insidously & persists for several years.
▷ Similar immunologic thrombocytopenia may be seen associated with SLE, AIDS & autoimmune thyroiditis.

- Formation of antiplatelet antibodies, usually by platelet associated IgG humoral antibodies synthesised mainly in spleen. These are directed against target antigens on platelet glycoproteins, GpIIb-IIIa & GpIb-IX complex.
- Sensitised platelets are destroyed mainly in spleen & rendered susceptible to phagocytosis by cells of reticuloendothelial system.

- Laboratory Features:

1. Platelet count is markedly reduced, usually in range of 10,000-50,000/μL.
2. Blood smear shows only occasional platelets which are often large in size
3. Bone marrow shows increased number of megakaryocytes which have large non-lobulated single nuclei & may have reduced cytoplasmic granularity.
4. With sensitive techniques, anti-platelet IgG antibody can be demonstrated on platelet surface or in serum of patients.
5. Platelet survival studies reveal markedly reduced platelet lifespan, sometimes less than one hour, compared to normal lifespan of 7 to 10 days.

- Typical Laboratory findings are reflective of isolated thrombocytopenia. A low platelet count, normal or increased megakaryocytes in the bone marrow, & large platelets in peripheral blood are taken as presumptive evidence in accelerated platelet destruction.
- The PT & PTT are normal

11. Breaking Bad News: SPIKES Protocol

S - Setting - Choose a private, comfortable, non-threaten Setting for the discussion.

P - Perception - Uncover what patient & family think is happening.

I - Invitation - Ask the patient what they would like to know.

K - Knowledge - Explain the Disease & care options in a plain/simple language

E - Emotion - Respect feelings, respond with empathy

S - Summarize - Recap & decide what to do next.

- Breaking Bad News (BBN) refers to the skill of communicating difficult medical information to a patient or family in a sensitive, ethical & structured manner.

eg: cancer diagnosis, poor prognosis, death

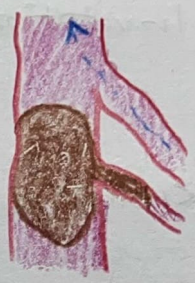
- The most commonly taught approach is the SPIKES protocol.

12. Fate of Thrombus.

- If a patient survives the initial thrombosis, in the ensuing days to weeks thrombi undergo some combination of 4 events.

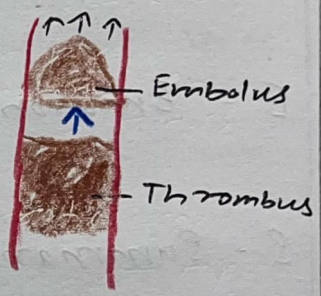
i) Propagation

- Thrombi accumulate additional platelets & fibrin enlarge



ii) Embolization.

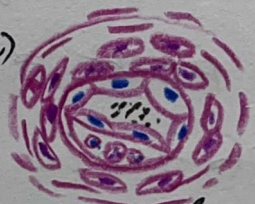
- Thrombi dislodge & travel to other sites in vasculature.



iii) Dissociation

- It is the result of fibrinolysis, which can lead to rapid shrinkage & total disappearance of recent thrombi.
- In contrast, extensive fibrin deposition & cross-linkage in older thrombi render them more resistant to lysis.

iv) Organization & Recanalization →



- Older thrombi become organized by ingrowth of endothelial cells, smooth muscle cells, & fibroblasts.
- Continued recanalization may convert a thrombus into smaller mass of connective tissue that becomes incorporated into the vessel wall.
- Eventually, with remodelling & contraction of the mesenchymal elements, only a fibrous lump may remain to mark the original thrombus.

13. Exfoliative Cytology

- It is a branch of diagnostic cytology that involves the study of cells spontaneously shed off from the epithelial surfaces into the body cavity or fluids.

▷ The cells shed (exfoliated) from epithelial surfaces are collected, stained, & examined microscopically to detect premalignant & malignant changes.

▷ It is widely used for early detection of cancers arising from epithelial linings.

▷ Types of samples for exfoliative cytology obtainable from different organ systems / body sites.

i) Gynaecologic exfoliative cytology

- Female Genital tract - a) Lateral vaginal smears (LVS)
  b) Vaginal 'pool' smears
  c) Cervical smears
  d) Endocervical/endometrial aspiration

ii Non-Gynaecologic exfoliative cytology

- Respiratory tract - Sputum, Bronchial washings, Bronchio-alveolar lavage (BAL)
- Gastrointestinal tract - Endoscopic lavage / brushing
- Urinary tract - Urinary sediment, Bladder washings.
- Body Fluids - Effusions, CSF, Hydrocoele, Nipple discharge
- other samples - Buccal smears (sex chromatin)

▷ Pap smears are the smears obtained from the female genital tract.

▷ Combined smears contain:

→ Normal epithelial cells - superficial, immediate, basal.

→ Variants - navicular cells, endocervical & endometrial cells.

→ Other cells - leucocytes & Doderlein bacilli.

□ Pap smears are applied for categorising their abnormalities into non-neoplastic and benign & malignant neoplastic types.

▷ Cellular material from respiratory tract can be obtained from sputum, washing or brushing, & bronchoalveolar lavage (BAL) during bronchoscopic procedures (BB, BW, BAL)

▷ Alimentary tract material is obtained by direct vision by brushing or lavage through fibreoptic endoscope.

□ Techniques in Exfoliative Cytology.

~~Preparation of Pap smears.~~

A. Collection of Samples

B. Fixation & Fixatives

C. Processing in the Laboratories

D. Staining -

1. Papanicolaou stain
2. H & E stain
3. Romanowsky stain

Necrosis	Apoptosis
◦ Cell death along with the release of hydrolytic enzyme	◦ Programmed & coordinated cell death
- Always pathological	- May be pathological or physiological.
◦ ~~Also~~ Always Inflammatory reaction	◦ No inflammatory reaction
◦ Cell swelling initially	◦ Cell shrinkage
◦ Membrane disruption, damaged organelles & nuclear disruption	◦ Cytoplasmic blebs on membrane, Apoptotic bodies, chromatin condensation.
- Phagocytosis of cell debris by macrophage	- Phagocytosis of apoptotic bodies by macrophages.
- Lysosomal breakdown with liberation of hydrolytic enzyme	- Lysosomes & other organelles intact.

Aetiopathogenesis of SLE

◦ It is a chronic autoimmune disease characterized by production of autoantibodies against nuclear antigens, leading to immune complex deposition & multisystem inflammation.

- The pathogenesis involves genetic susceptibility, environmental triggers, & immune dysregulation

Etiology

1. Genetic factors
   - ▷ Genetic predisposition
   - ▷ Association with certain HLA genes.

▷ Defects in complement proteins

- C1q deficiency, C2 & C4 deficiency

2. Immunologic factors

- Failure of self tolerance in B cells
- TLR engagement by nuclear DNA & RNA contained in immune complex may activate B lymphocytes.

3. Environmental factors.

→ UV radiation

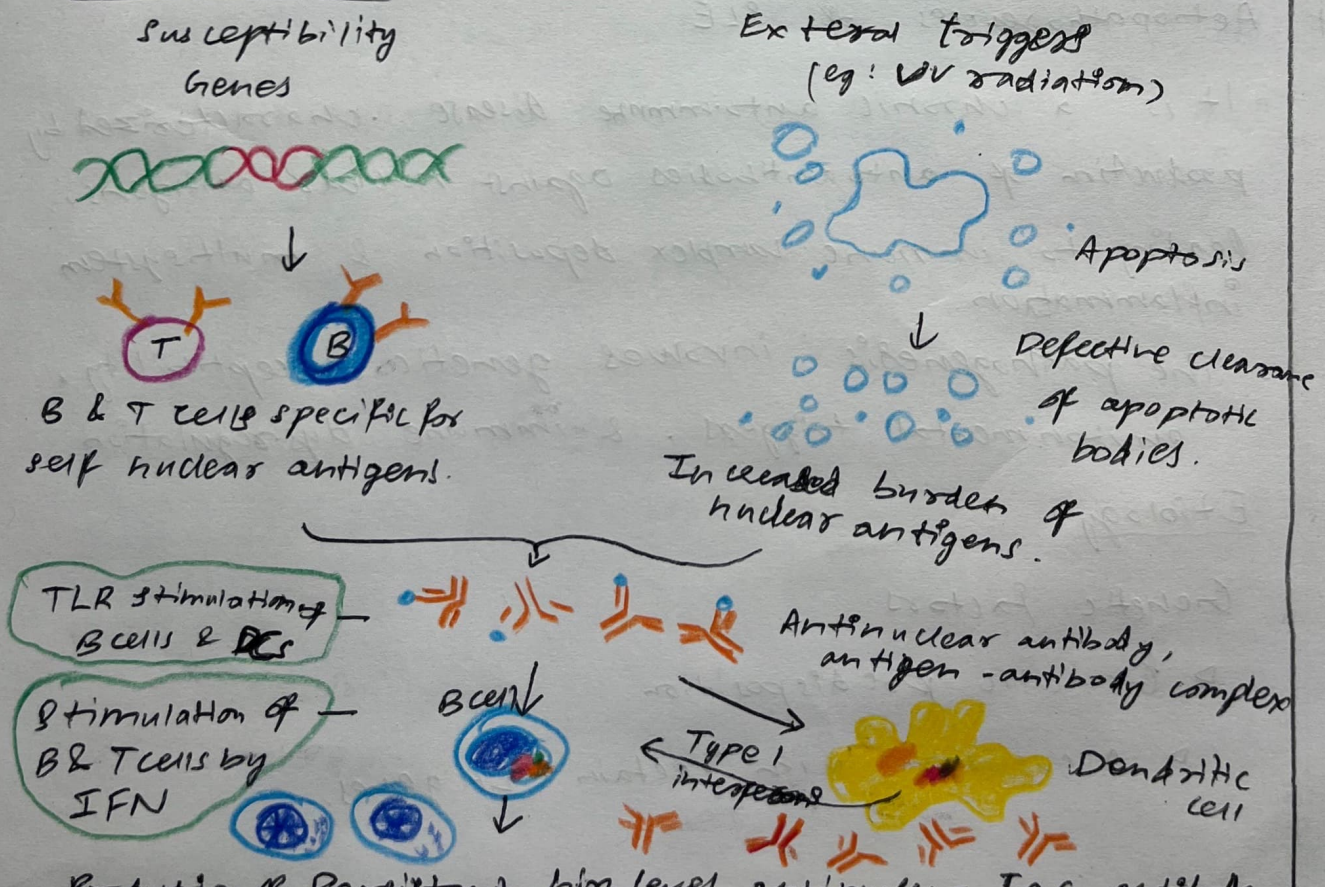
→ Infections.

4. Hormonal factors.

SLE is common in women of reproductive age.

- Estrogen enhances B cell activation
- Female : male ratio = 9:1

□ Pathogenesis



15. Opportunistic infections in AIDS.

- OIs are infections caused by organisms that normally do not cause disease in immunocompetent individuals but became pathogenic due to severe immunosuppression.
- In AIDS, these infections cause because of progressive depletion of CD4+ T-lymphocytes caused by HIV.

Pathogenesis

1. HIV infection of CD4+ T cells.
2. Progressive decline in CD4 count
3. Defective macrophage & dendritic cell function
4. Impaired B-cell regulation.
5. Reactivation of latent infections & development of opportunistic infections.

Infections

A. Bacterial infections

- Mycobacterium tuberculosis → TB
- Salmonella → recurrent septicemia
- Dissemminated infection is common.

B. Fungal infections.

- Pneumocystis pneumonia
- Candidiasis
- Cryptococcosis