IMMUNOLOGY AND AMYLODOSIS

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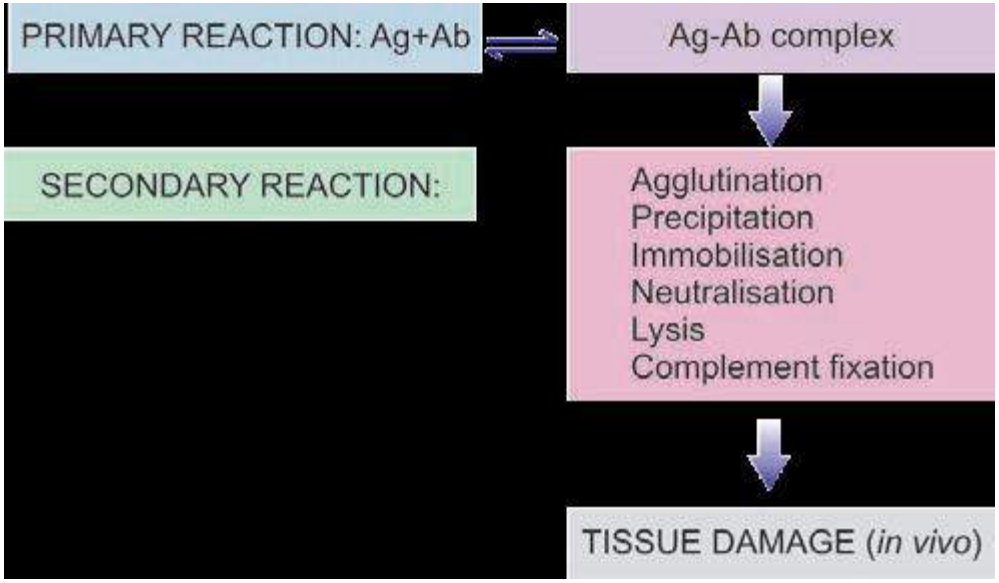
INTRODUCTION

- **Immunity and immunopathology are proverbial two edges of 'double-edged sword'.**
- **An antigen (Ag) is defined as a substance, usually protein in nature, which when introduced into the tissues stimulates antibody production.**
- **Hapten is a non-protein substance which has no antigenic properties, but on combining with a protein can form a new antigen capable of forming antibodies.**
- **An antibody (Ab) is a protein substance produced as a result of antigenic stimulation. Circulating antibodies are immunoglobulins (Igs) of which there are 5 classes: IgG, IgA, IgM, IgE and IgD.**
- **An antigen may induce specifically sensitised cells having the capacity to recognise, react and neutralise the injurious agent or organisms.**
- **The antigen may combine with antibody to form antigenantibody complex. The reaction of Ag with Ab *in vitro may* be *primary or secondary phenomena; the secondary reaction* induces a number of processes. *In vivo, the Ag-Ab reaction* may cause tissue damage**

TYPES OF IMMUNITY

- **TYPES OF IMMUNITY.** Broadly speaking, immunity or body defense mechanism is divided into 2 types, each with
- Humoral and cellular components:



TYPES OF IMMUNITY

- **Natural or innate immunity is *non-specific and is*** considered as the first line of defense without antigenic specificity. It has 2 major components:

a) *Humoral: comprised by complement.*

b) *Cellular: consists of neutrophils, macrophages, and* natural killer (NK) cells.

- **Specific or adaptive immunity is *specific and is***
- characterised by antigenic specificity. It too has 2 main
- components:

a) *Humoral: consisting of antibodies formed by B cells.*

b) *Cellular: mediated by T cells.*

- The various components of both types of immunity are
- interdependent and interlinked for their functions.

STRUCTURE OF IMMUNE SYSTEM

- **ORGANS OF IMMUNE SYSTEM**
- Although functioning as a system, the organs of immune
- system are distributed at different places in the body. These are as under:

a) Primary lymphoid organs:

i) Thymus

ii) Bone marrow

b) Secondary lymphoid organs:

i) Lymph nodes

ii) Spleen

iii) MALT (Mucosa-Associated Lymphoid Tissue located in the respiratory tract and GIT).

CELLS OF IMMUNE SYSTEM

- The cells comprising immune system are as follows:

i) Lymphocytes

ii) Monocytes and macrophages

iii) Mast cells and basophils

iv) Neutrophils

v) Eosinophils

Cells of the Immune System and their Functions.

- **Cells Functions**

1. Lymphocytes (20-50%) Master of immune system

i) B-cells (10-15%) Antibody-based humoral reactions, transform to plasma cells Plasma cells Secrete immunoglobulins

ii) T-cells (75-80%) Cell-mediated immune reactions

a) T-helper cells (CD4+) (60%) Promote and enhance immune reaction by elaboration of cytokines

b) T-suppressor cells (CD8+) (30%) Suppress immune reactions but are directly cytotoxic to antigen

c) NK-cells (10-15%) Part of natural or innate immunity; cause antibody-dependent cell-mediated cytotoxicity (ADCC)

2. Monocytes-macrophages (~5%) Antigen recognition

Phagocytosis

Secretory function

Antigen presentation

3. Mast cells and basophils (0-1%) Allergic reactions **Wound healing**

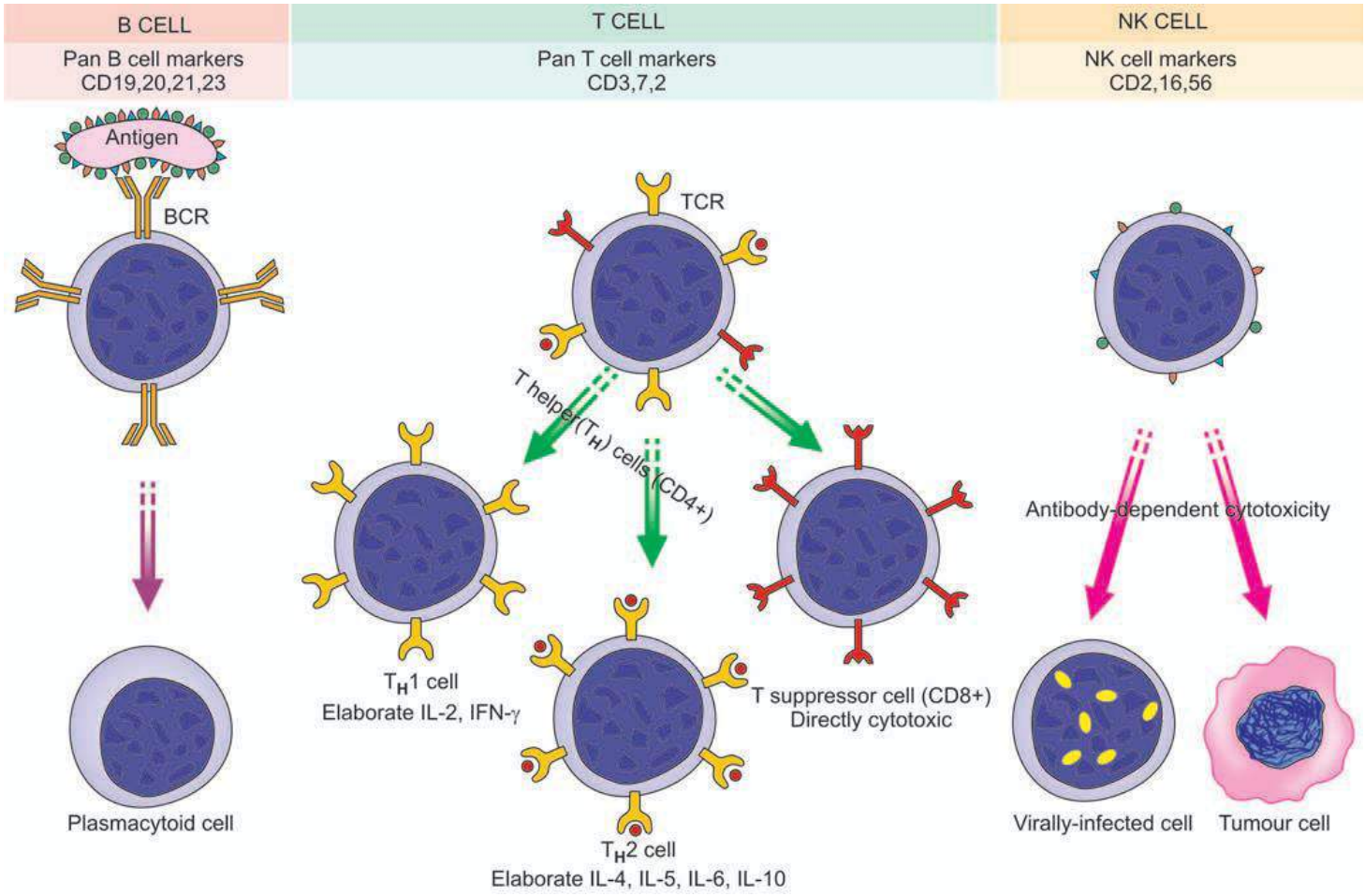
4. Neutrophils (40-75%) First line of dense against microorganisms and other small antigens

5. Eosinophils (1-6%) Allergic reactions **Helminthiasis**

Differences between T and B Lymphocytes.

• Feature	T Cells	B Cells
1. Origin	*Bone marrow → Thymus*	*Bone marrow → Bursa (in fowl);* mucosa-associated lymphoid tissue (MALT)
2. Lifespan	*Small T cells: months to years* T cell blasts: several days	*Small B cells: less than 1 month* B cell blasts : several days
3. Location		
(i) *Lymph nodes*	*Perifollicular (paracortical)*	*Germinal centres, medullary cords*
(ii) *Spleen*	*Periarteriolar*	*Germinal centres, red pulp*
(iii) *Peyer's patches*	*Perifollicular*	*Central follicles*
4. Presence in circulation	*75-80%*	*10-15%*
5. Surface markers		
(i) *Ag receptors*	*Present*	*Absent*
(ii) *Surface Ig*	*Absent*	*Present*
(iii) *Fc receptor*	*Absent*	*Present*
(iv) *Complement receptor*	*Absent*	*Present*
(v) *Rosettes*	*E-rosettes (sheep erythrocytes)*	*EAC-rosettes (mouse erythrocytes)*
(vi) *CD markers*	*TH cells CD4, 3, 7, 2* TS cells CD8, 3, 7, 2	*CD19, 20, 21, 23*
6. Functions	*(i) CMI via cytotoxic T cells* positive for CD3 and CD4 (ii) Delayed hypersensitivity via CD4+ T cells (iii) Immunoregulation of other T cells, B cells and stem cells via T helper (CD4+) or T suppressor (CD8+) cells	*(i) Role in humoral immunity by synthesis* of specific antibodies (Igs) (ii) Precursors of plasma cells

Schematic representation of functions of B and T lymphocytes and NK cells. (BCR = B cell receptor, TCR = T cell receptor).



Immunodeficiency Diseases.

- **Disease Defect**

A. PRIMARY IMMUNODEFICIENCY DISEASES

1. Severe combined immunodeficiency diseases (Combined deficiency of T cells, B cells and lgs):

(i) Reticular dysgenesis	**Failure to develop primitive marrow reticular cells**
(ii) Thymic alymphoplasia	**No lymphoid stem cells**
(iii) Agammaglobulinaemia (Swiss type)	**No lymphoid stem cells**
(iv) Wiscott-Aldrich syndrome	**Cell membrane defect of haematopoietic stem cells; associated features are thrombocytopenia and eczema**
(v) Ataxia telangiectasia	**Defective T cell maturation**

2. T cell defect:

DiGeorge's syndrome (thymic hypoplasia)	**Epithelial component of thymus fails to develop**

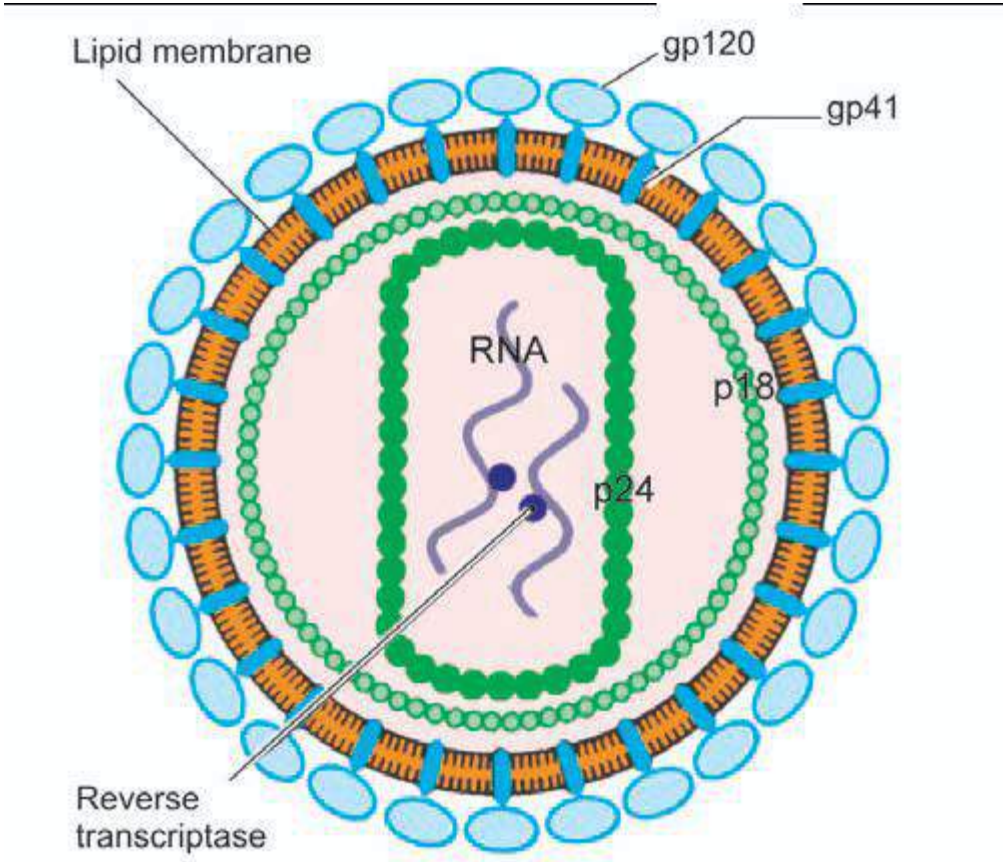
3. B cell defects (antibody deficiency diseases):

(i) Bruton's X-linked agammaglobulinaemia	**Defective differentiation from pre-B to B cells**
(ii) Autosomal recessive agammaglobulinaemia	**Defective differentiation from pre-B to B cells**
(iii) IgA deficiency	**Defective maturation of IgA synthesising B cells**
(iv) Selective deficiency of other lg types	**Defective differentiation from B cells to specific lg-synthesising plasma cells**
(v) Immune deficiency with thymoma	**Defective pre-B cell maturation**

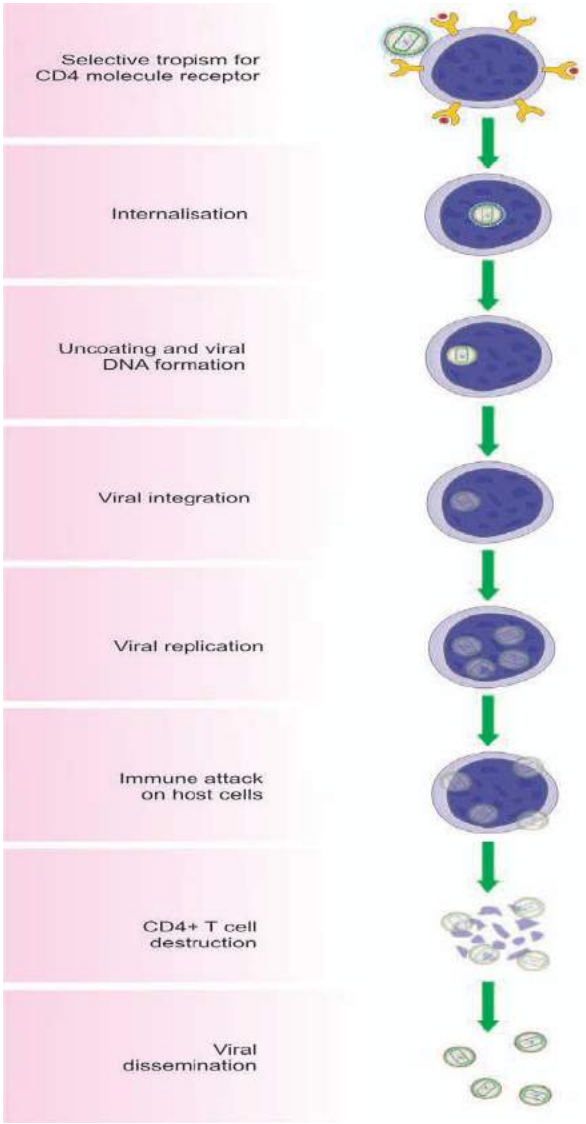
Immunodeficiency Diseases.

- ***4. Common variable immunodeficiencies***
- ***(characterised by decreased lgs and serum antibodies and variable CMI):***
- **(i) With predominant B cell defect Defective differentiation of pre-B to mature B cells**
- **(ii) With predominant T cell defect**
- **(a) Deficient T helper cells Defective differentiation of thymocytes to T helper cells**
- **(b) Presence of activated T suppressor cells T cell disorder of unknown origin**
- **(iii) With autoantibodies to B and T cells Unknown differentiation defect**
- **B. SECONDARY IMMUNODEFICIENCY DISEASES**
- ***1. Infections AIDS (HIV virus); other viral, bacterial and protozoal infections***
- ***2. Cancer Chemotherapy by antimetabolites; irradiation***
- ***3. Lymphoid neoplasms (lymphomas, lymphoid leukaemias) Deficient T and B cell functions***
- ***4. Malnutrition Protein deficiency***
- ***5. Sarcoidosis Impaired T cell function***
- ***6. Autoimmune diseases Administration of high dose of steroids toxic to lymphocytes***
- ***7. Transplant cases Immunosuppressive therapy***

Schematic representation of HIV virion or virus particle.
The particle has core containing proteins, p24 and p18, two strands of viral RNA, and enzyme reverse transcriptase. Bilayer lipid membrane is studded with 2 viral glycoproteins, gp120 and gp41, in the positions shown.



Sequence of events in the pathogenesis of HIV infection.



Major Abnormalities in Immune System in AIDS.

1. T CELL ABNORMALITIES
(i) Lymphopenia
(ii) CD4+ T cell depletion
(iii) CD8+ T cell lymphocytosis
(iv) Reversal of CD4: CD8 cell ratio
(v) Decreased production of cytokines by CD4+ T cells
(vi) Decreased antibody-dependent cellular cytotoxicity (ADCC) by CD8+ T cells
2. B CELL ABNORMALITIES
(i) No direct viral damage
(ii) Decreased Ig production
(iii) Polyclonal activation
(iv) Hypergammaglobulinaemia
(v) Circulating immune complexes
3. NK CELL ABNORMALITIES
(i) No direct viral damage
(ii) Depressed number
(iii) Decreased cytotoxicity
4. MONOCYTE-MACROPHAGE CELL ABNORMALITIES
(i) No destruction
(ii) Decreased chemotaxis
(iii) Decreased cytotoxicity

Natural History and Revised CDC HIV/AIDS Classification.

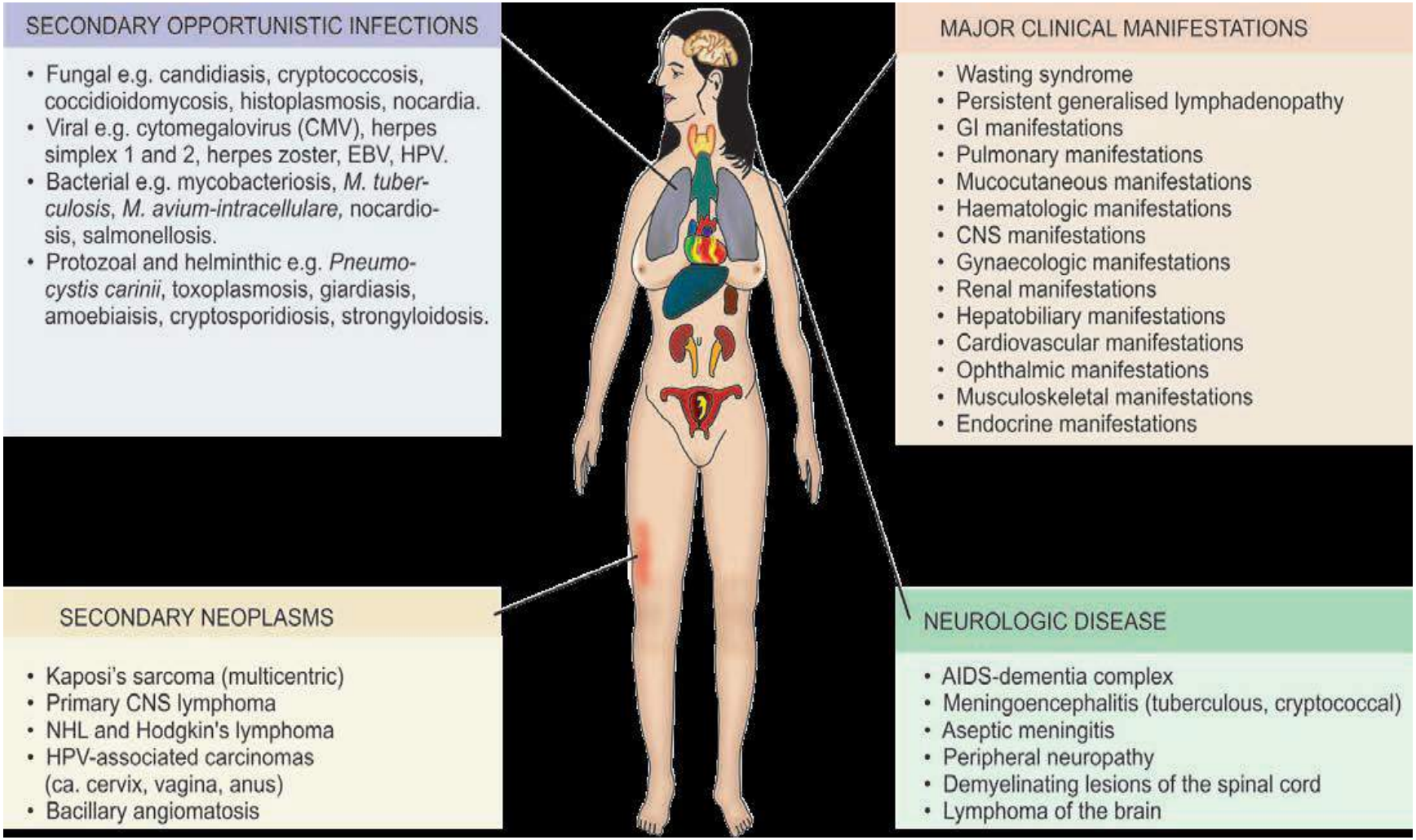
• **Phase Early,**	**Acute Middle,**	**Chronic Final,**	**Crisis**
• ***Period after infection***	***3-6 weeks***	***10 to 12 years***	***Any period up to death***
• ***CDC clinical category***	***Category A:***	***Category B:***	***Category C:***
	Asymptomatic	**Symptomatic**	**AIDS surveillance case**
	Acute HIV syn (neither A nor C)		**definition**
	PGL	**Condition secondary to impaired CMI**	
• ***CDC CD4 + T cell count***	***> 500/µl***	***200-499/µl***	***< 200/µl*** **(AIDS indicator T cell counts)**

(CDC = Centers for Disease Control, Atlanta, USA;

PGL = Persistent generalised lymphadenopathy;

CMI = Cell mediated immunity).

Major pathological lesions and clinical manifestations of HIV/AIDS.



Tests for Diagnosis of HIV/AIDS.

1. TESTS FOR ESTABLISHING HIV INFECTION:

i) Antibody tests:

a) ELISA

b) Western blot

ii) Direct detection of HIV

a) p24 antigen capture assay

b) HIV RNA assay

c) DNA-PCR

d) Culture of HIV

2. TESTS FOR DEFECTS IN IMMUNITY:

i) CD4+ T cell count: Fall

ii) CD8+ cell count: Increased

iii) Ratio of CD4+ T cell/CD8+ T cell count: Reversed

iv) Lymphopenia

v) Hypergammaglobulinaemia

vi) Increased β-2 microglobulin level

vii) Platelet count: Thrombocytopenia

3. TESTS FOR DETECTION OF OPPORTUNISTIC INFECTION AND SECONDARY TUMOURS:

i) FNAC

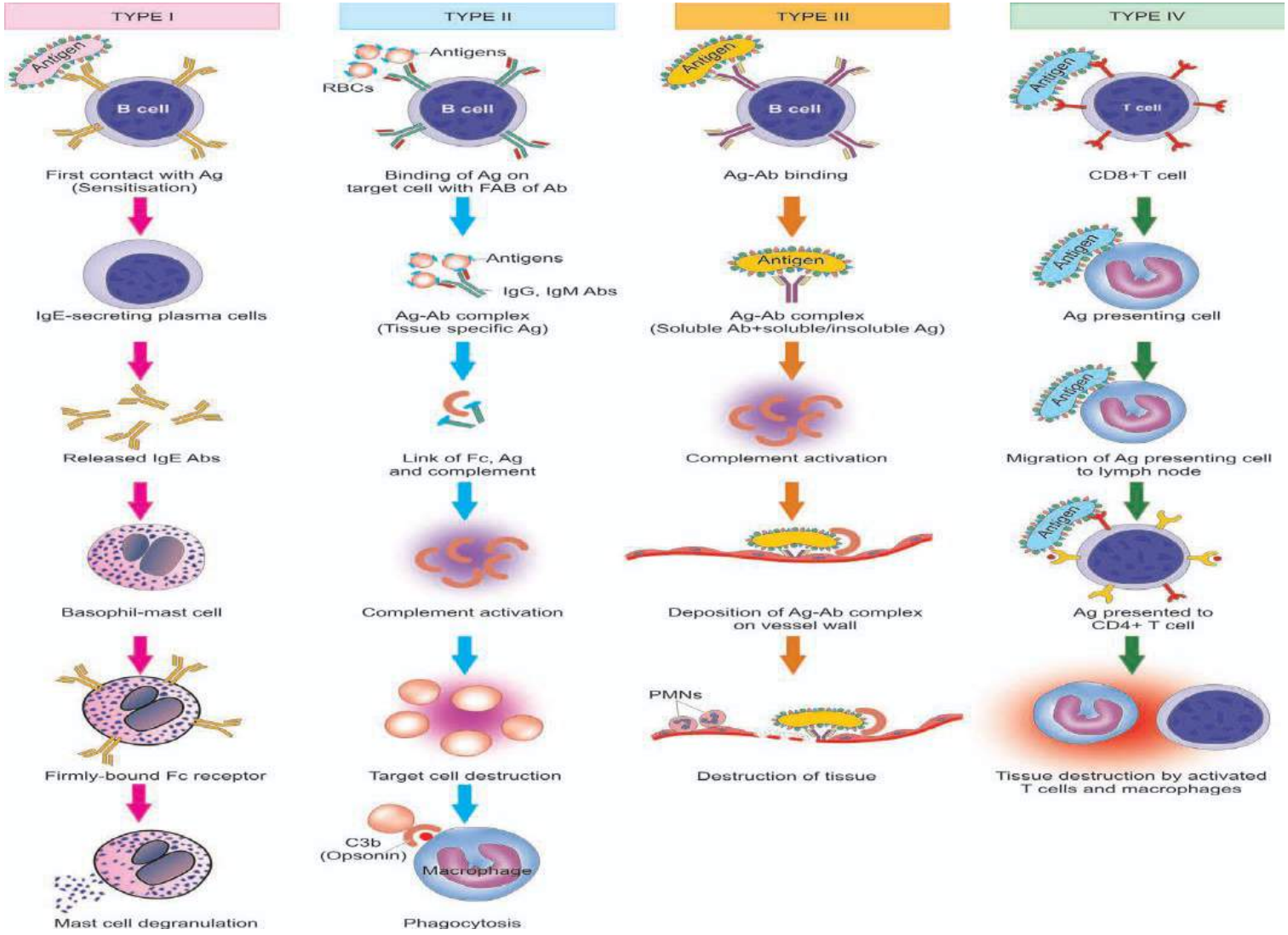
ii) Biopsy

COMPARISION OF FOUR TYPES OF HYPERSENSITIVE REACTIONS

TABLE 4.7: Comparative Features of 4 Types of Hypersensitivity Reactions.

Feature	Type I (Anaphylactic, atopic)	Type II (Cytotoxic)	Type III (Immune-complex, Arthus reaction)	Type IV (Delayed hypersensitivity)
1. *Definition*	Rapidly developing immune response in a previously sensitised person	Reaction of humoral antibodies that attack cell surface antigens and cause cell lysis	Results from deposition of antigen-antibody complexes on tissues	Cell-mediated slow and prolonged response
2. *Peak action time*	15-30 minutes	15-30 minutes	Within 6 hours	After 24 hours
3. *Mediated by*	IgE antibodies	IgG or IgM antibodies	IgG, IgM antibodies	Cell-mediated
4. *Etiology*	Genetic basis, pollutants, viral infections	HLA-linked, exposure to foreign tissues/cells	Persistence of low grade infection, environmental antigens, autoimmune process	CD8+ T cells, cutaneous antigens
5. *Examples*	i. Systemic anaphylaxis (administration of antisera and drugs, stings) ii. Local anaphylaxis (hay fever, bronchial asthma, food allergy, cutaneous, angioedema)	i. Cytotoxic antibodies to blood cells (autoimmune haemolytic anaemia, transfusion reactions, erythroblastosis foetalis, ITP, leucopenia, drug-induced) ii. Cytotoxic antibodies to tissue components (Graves' disease, myasthenia gravis, male sterility, type I DM, hyperacute reaction against organ transplant	i. Immune complex glomerulonephritis ii. Goodpasture's syndrome, iii. Collagen diseases (SLE, rheumatoid arthritis) iv. PAN v. Drug-induced vasculitis	i. Reaction against microbacterial antigen (tuberculin reaction, tuberculosis, tuberculoid leprosy) ii. Reaction against virus-infected cells iii. Reaction against tumour cells

Schematic representation of pathogenesis of 4 types of immunological tissue injury



Autoimmune Diseases

ORGAN NON-SPECIFIC (SYSTEMIC)

1. Systemic lupus erythematosus*

2. Rheumatoid arthritis

3. Scleroderma (Progressive systemic sclerosis)*

4. Polymyositis-dermatomyositis*

5. Polyarteritis nodosa (PAN)

6. Sjögren's syndrome*

7. Reiter's syndrome*

8. Wegener's granulomatosis

ORGAN SPECIFIC (LOCALISED)

1. *ENDOCRINE GLANDS*

(i) Hashimoto's (autoimmune) thyroiditis

(ii) Graves' disease

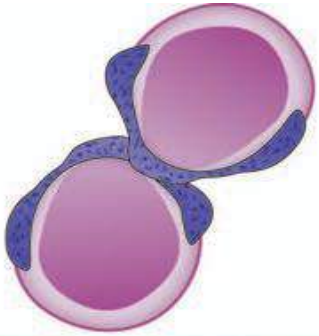
(iii) Type 1 diabetes mellitus

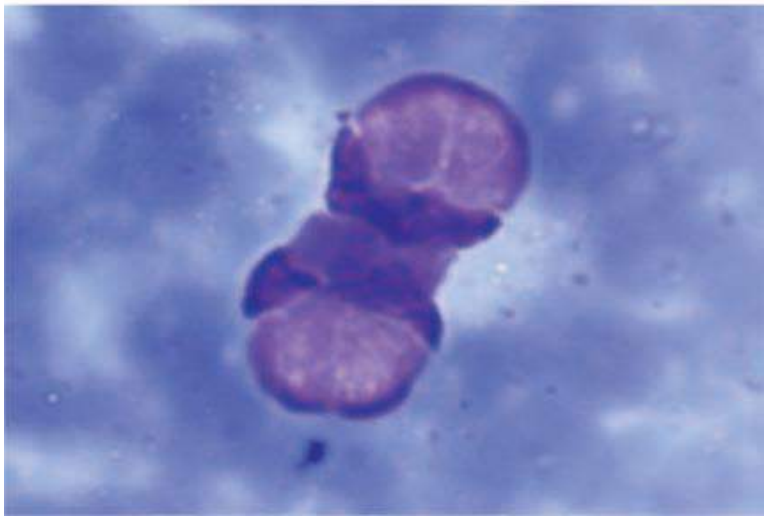
(iv) Idiopathic Addison's disease

Autoimmune Diseases

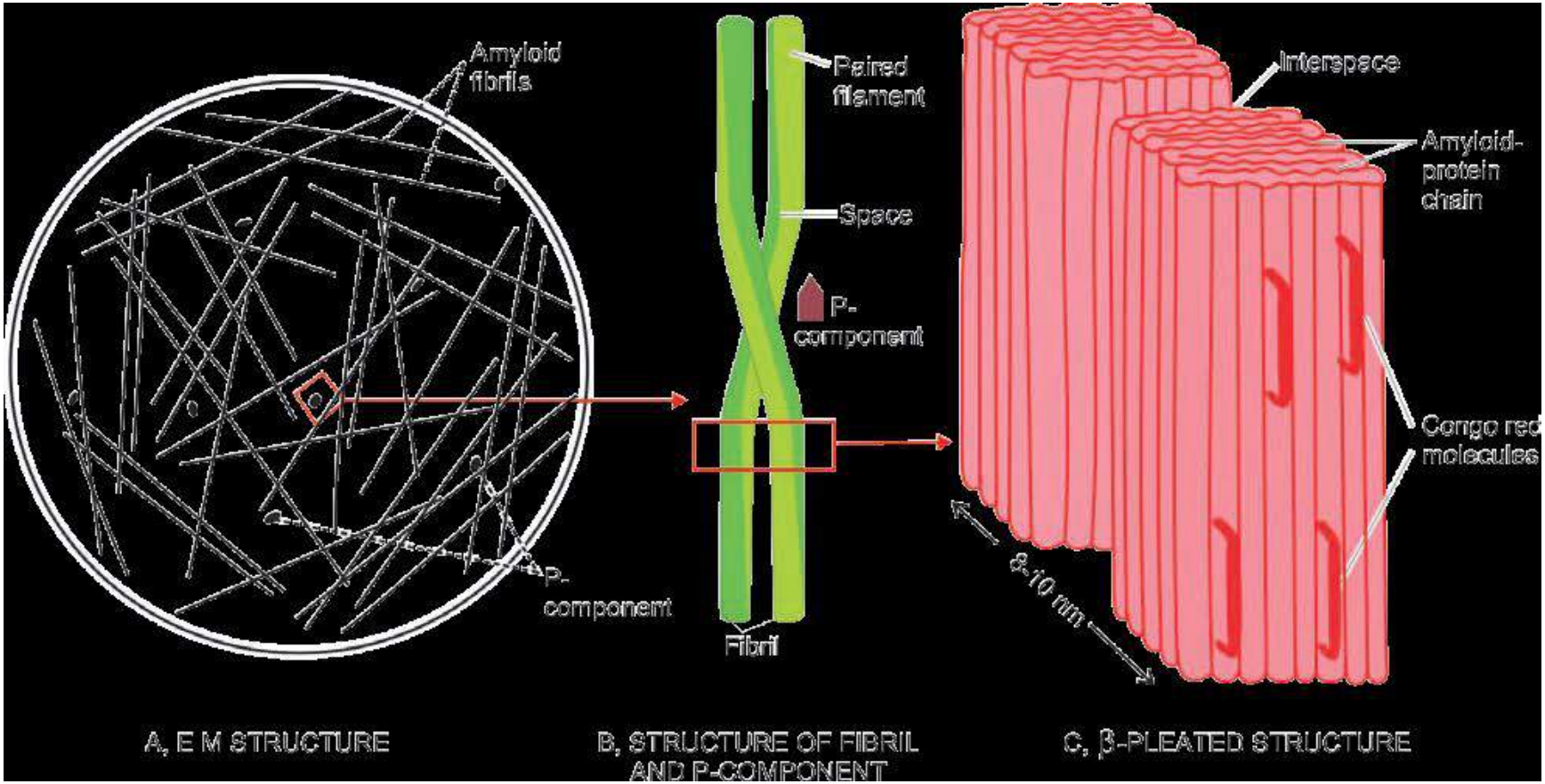
2. *ALIMENTARY TRACT*
(i) Autoimmune atrophic gastritis in pernicious anaemia
(ii) Ulcerative colitis
(iii) Crohn's disease
3. *BLOOD CELLS*
(i) Autoimmune haemolytic anaemia
(ii) Autoimmune thrombocytopenia
(iii) Pernicious anaemia
4. *OTHERS*
(i) Myasthenia gravis
(ii) Autoimmune orchitis
(iii) Autoimmune encephalomyelitis
(iv) Goodpasture's syndrome
(v) Primary biliary cirrhosis
(vi) Lupoid hepatitis
(vii) Membranous glomerulonephritis
(viii) Autoimmune skin diseases

Typical LE cell. There are two LE cells having rounded masses of amorphous nuclear material (LE body) which has displaced the lobes of neutrophil to the rim of the cell.

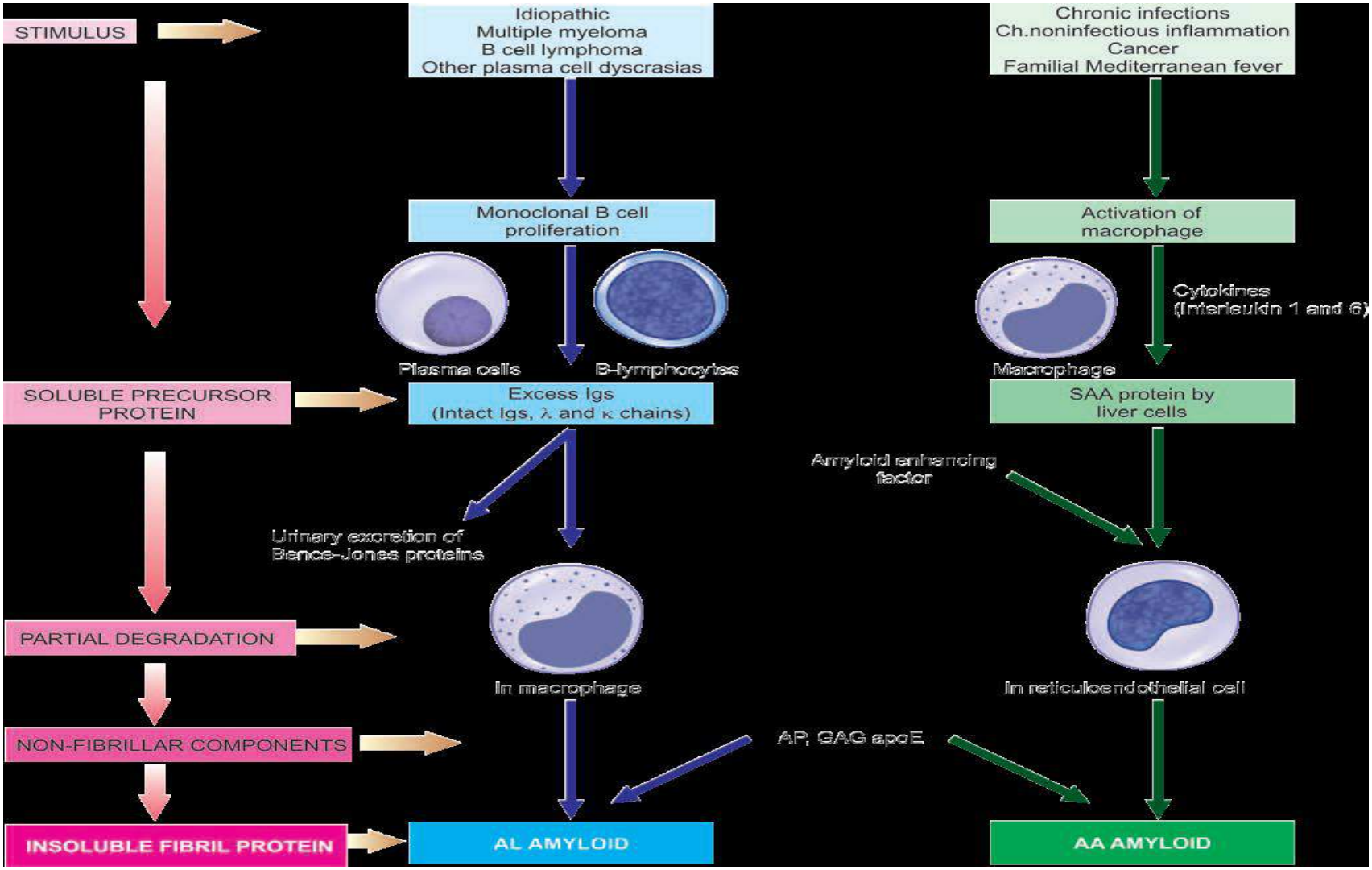




AMYLOIDOSIS



Pathogenesis of two main forms of amyloid deposition (AL = Amyloid light chain; AA = Amyloid-associated protein; GAG = glycosaminoglycan; AP = Amyloid P component). The sequence on left shows general schematic representation common to both major forms of amyloidogenesis.



CLASSIFICATION OF AMYLODOSIS

TABLE 4.10: Classification of Amyloidosis.

Category	Associated Disease	Biochemical Type	Organs Commonly Involved
A. SYSTEMIC (GENERALISED) AMYLOIDOSIS			
1. *Primary*	Plasma cell dyscrasias	AL type	Heart, bowel, skin, nerves, kidney
2. *Secondary (Reactive)*	Chronic inflammation, cancers	AA type	Liver, spleen, kidneys, adrenals
3. *Haemodialysis-associated*	Chronic renal failure	$A\beta_2M$	Synovium, joints, tendon sheaths
4. *Heredofamilial*			
i. *Hereditary polyneuropathies*	—	ATTR	Peripheral and autonomic nerves, heart
ii. *Familial Mediterranean fever*	—	AA type	Liver, spleen, kidneys, adrenals
iii. *Rare hereditary forms*	—	AApoAI, AGel ALys, AFib, ACys	Systemic amyloidosis
B. LOCALISED AMYLOIDOSIS			
1. *Senile cardiac*	Senility	ATTR	Heart
2. *Senile cerebral*	Alzheimer's, transmissible encephalopathy	Aβ, APrP	Cerebral vessels, plaques, neurofibrillary tangles
3. *Endocrine*	Medullary carcinoma type 2 diabetes mellitus	Procalcitonin Proinsulin	Thyroid Islets of Langerhans
4. *Tumour-forming*	Lungs, larynx, skin, urinary bladder, tongue, eye	AL	Respective anatomic location

(AL= Amyloid light chain; AA= Amyloid-associated protein; $A\beta_2M$= Amyloid β_2-microglobulin; ATTR= Amyloid transthyretin; APrP=Amyloid of prion proteins, Aβ= β-amyloid protein).

DIFFERENCE BETWEEN PRIMARY AND SECONDARY AMYLODOSIS

TABLE 4.11: Contrasting Features of Primary and Secondary Amyloidosis.

Feature	Primary Amyloid	Secondary Amyloid
1. *Biochemical composition*	AL (Light chain proteins); lambda chains more common than kappa; sequence homology of chains	AA (Amyloid associated proteins); derived from larger precursor protein SAA; No sequence homology of polypeptide chain
2. *Associated diseases*	Plasma cell dyscrasias e.g. multiple myeloma, B cell lymphomas, others	Chronic inflammation e.g. infections (TB, leprosy, osteomyelitis, bronchiectasis), autoimmune diseases (rheumatoid arthritis, IBD), cancers (RCC, Hodgkin's disease), FMF
3. *Pathogenesis*	Stimulus → Monoclonal B cell proliferation → Excess of Igs and light chains → Partial degradation → Insoluble AL fibril	Stimulus → Chronic inflammation → Activation of macrophages → Cytokines (IL1,6) → Partial degradation → AEF → Insoluble AA fibril
4. *Incidence*	Most common in US and other developed countries	Most common worldwide, particularly in developing countries
5. *Organ distribution*	Kidney, heart, bowel, nerves	Kidney, liver, spleen, adrenals
6. *Stains to distinguish*	Congophilia persists after permanganate treatment of section; specific immunostains anti-λ, anti-κ	Congophilia disappears after permanganate treatment of section; specific immunostain anti-AA

STANING CHARACTERS OF AMYLOID

TABLE 4.12: Staining Characteristics of Amyloid.

	Stain	Appearance
1.	*H & E*	Pink, hyaline, homogeneous
2.	*Methyl violet/Crystal violet*	Metachromasia: rose-pink
3.	*Congo red*	Light microscopy: pink-red Polarising light: red-green birefringence
4.	*Thioflavin-T/Thioflavin-S*	Ultraviolet light: fluorescence
5.	*Immunohistochemistry (antibody against fibril protein)*	Immunoreactivity: Positive
6.	*Non-specific stains:*	
	i) Standard toluidine blue	Orthochromatic blue, polarising ME dark red
	ii) Alcian blue	Blue-green
	iii) PAS	Pink

AMYLODOSIS OF KIDNEY

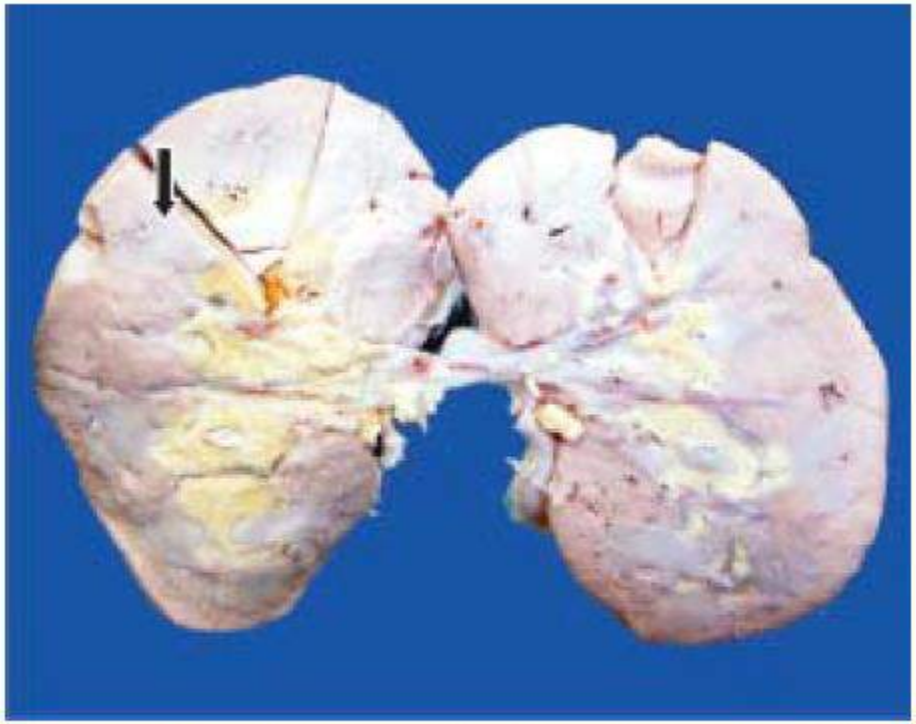


Figure 4.11 Amyloidosis of kidney. The kidney is small and pale in colour. Sectioned surface shows loss of cortico-medullary distinction (arrow) and pale, waxy translucency.

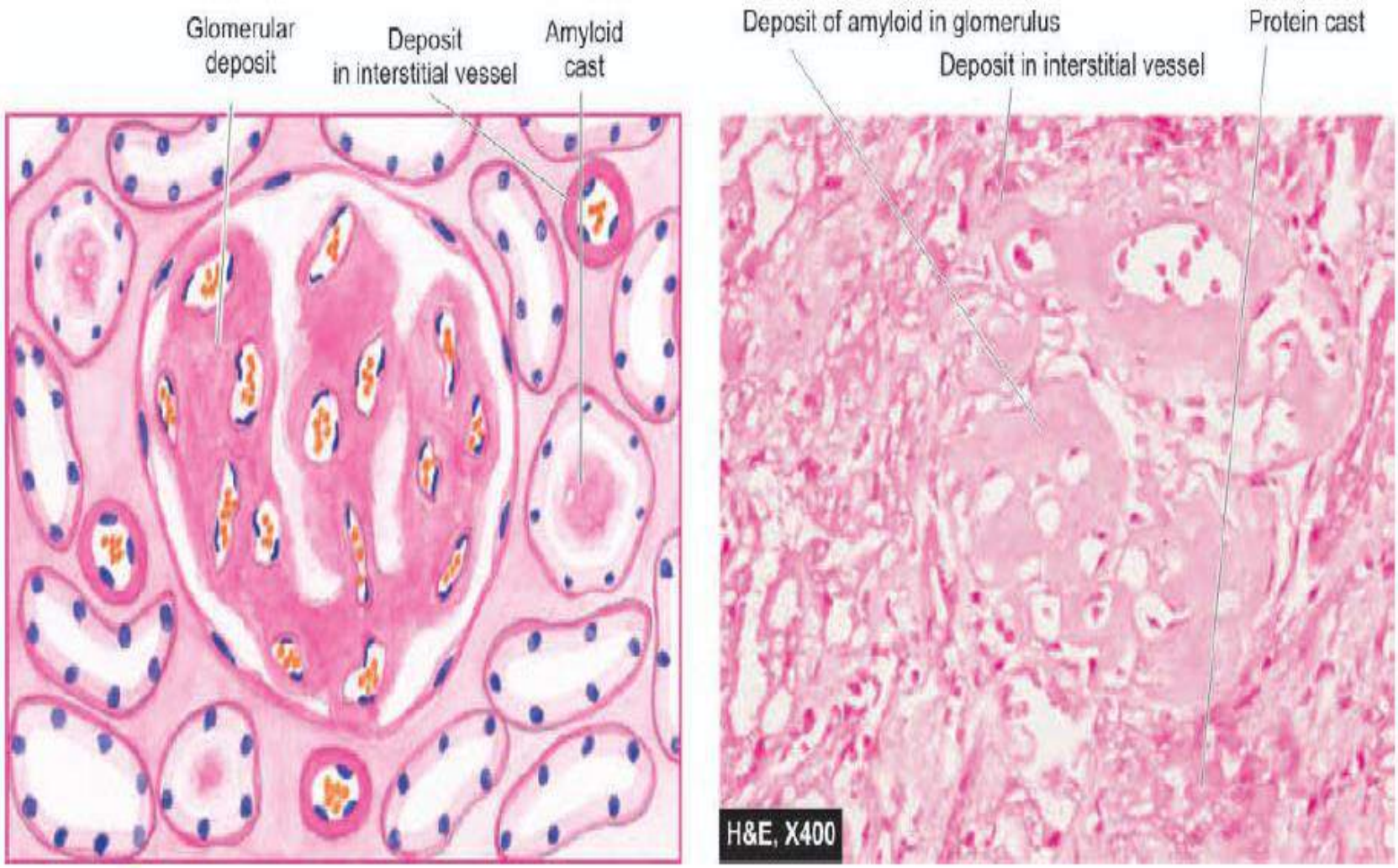


Figure 4.12 Amyloidosis of kidney. The amyloid deposits are seen mainly in the glomerular capillary tuft. The deposits are also present in peritubular connective tissue producing atrophic tubules and amyloid casts in the tubular lumina, and in the arterial wall producing luminal narrowing.

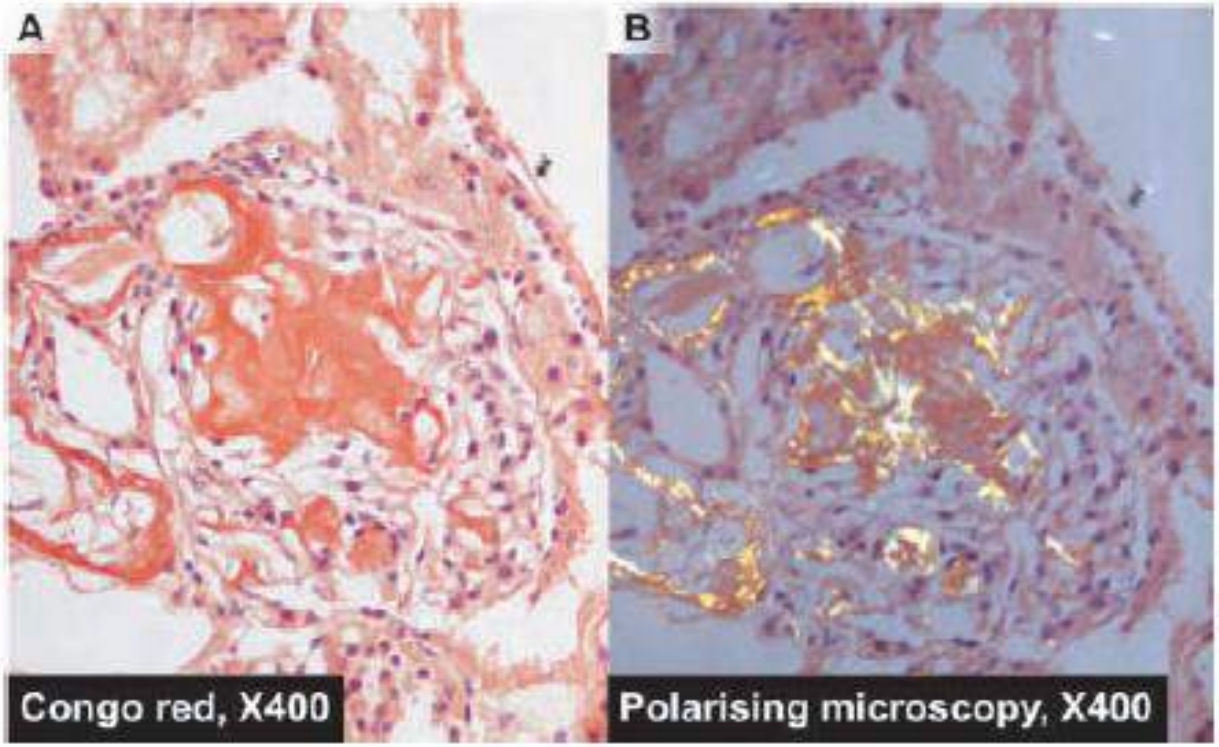


Figure 4.13 Amyloidosis kidney, Congo red stain. A, The amyloid deposits are seen mainly in the glomerular capillary tuft stained red-pink (Congophilia). B, Viewing the same under polarising microscopy, the congophilic areas show apple-green birefringence.

GROSS PATTERN OF AMYLODOSIS OF SPLEEN

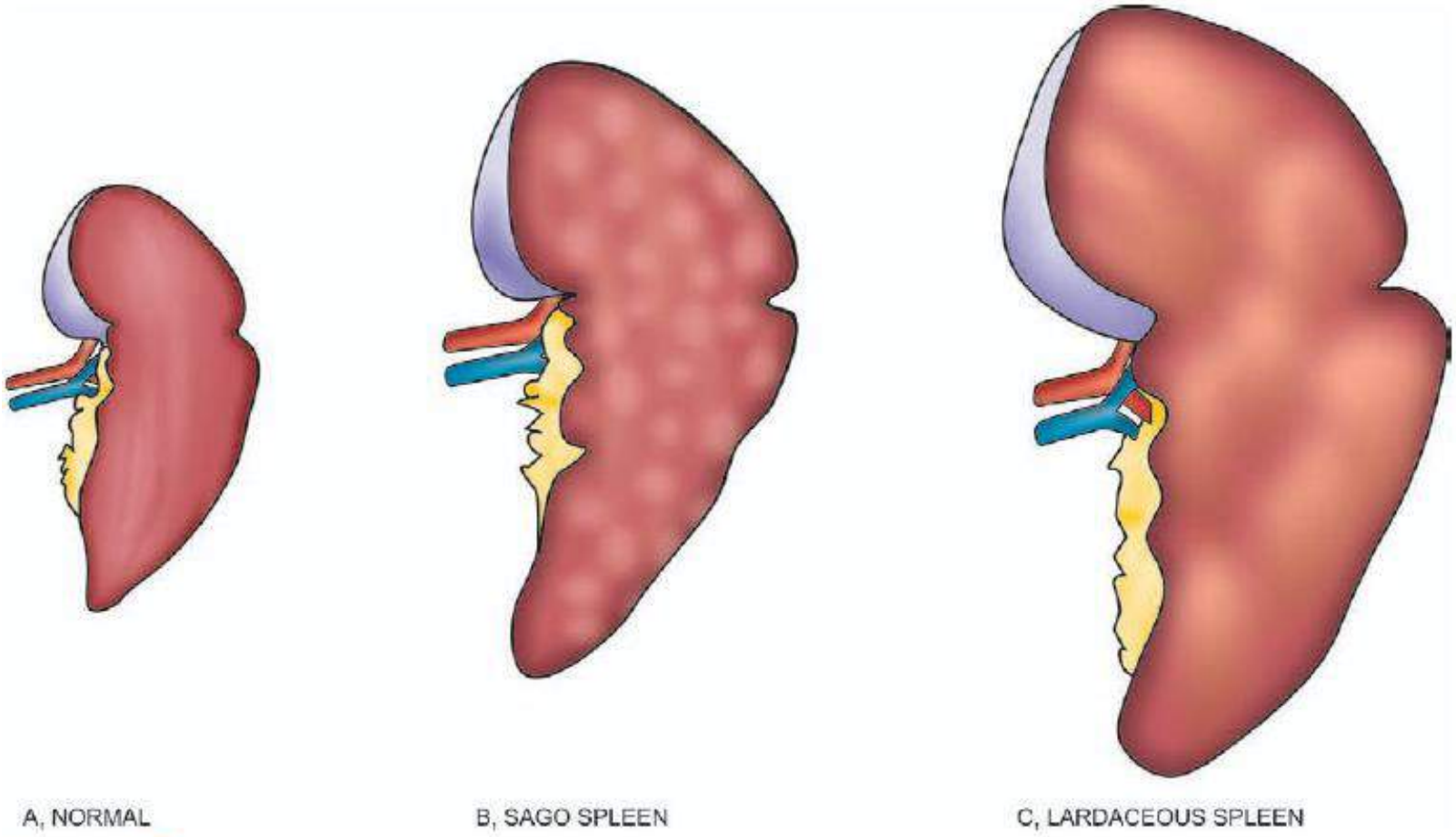


Figure 4.14 ◆ Gross patterns of amyloidosis of the spleen.

Figure 4.15 ◆ Lardaceous amyloidosis of the spleen. The sectioned surface shows presence of plae waxy translucency in a map-like pattern.

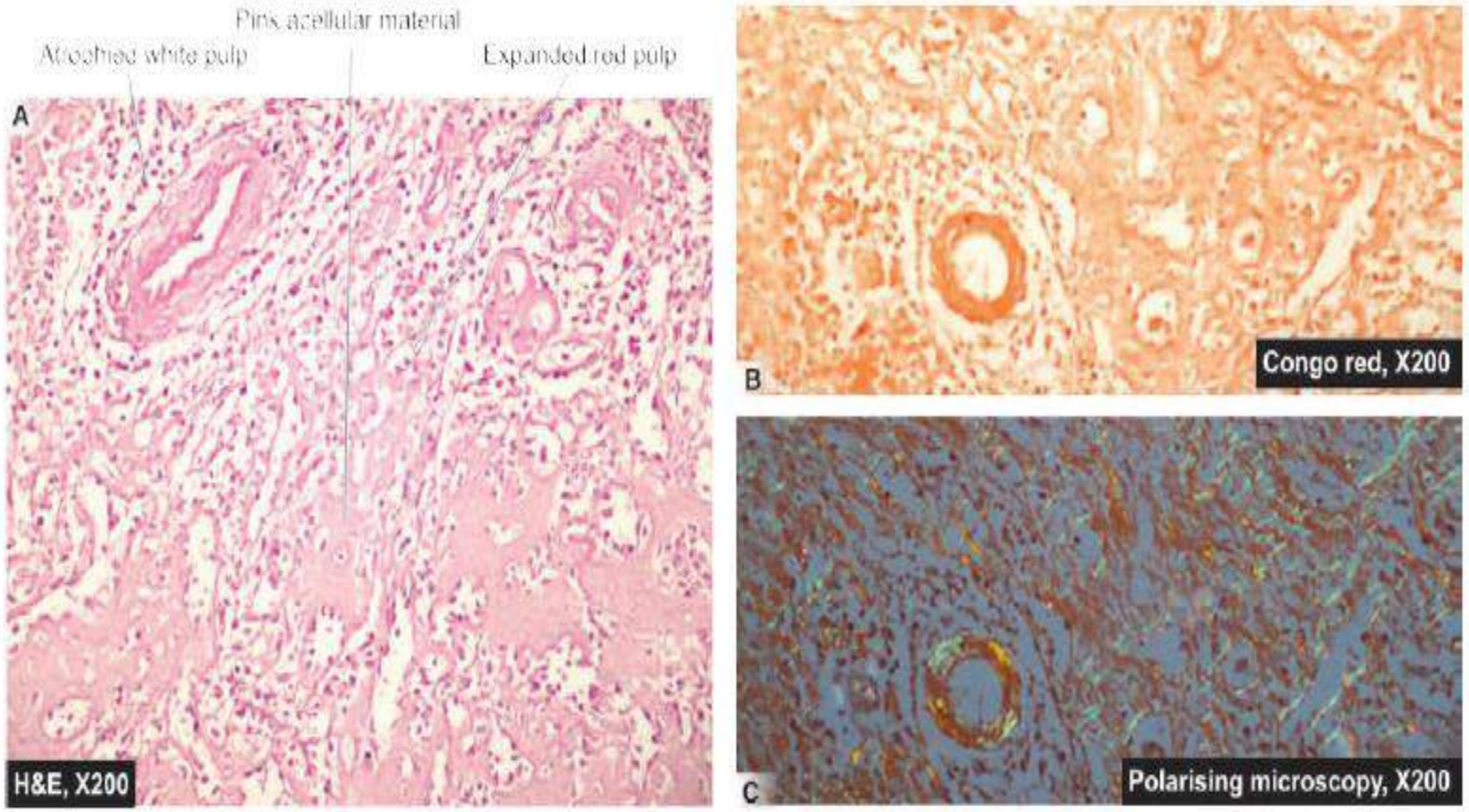
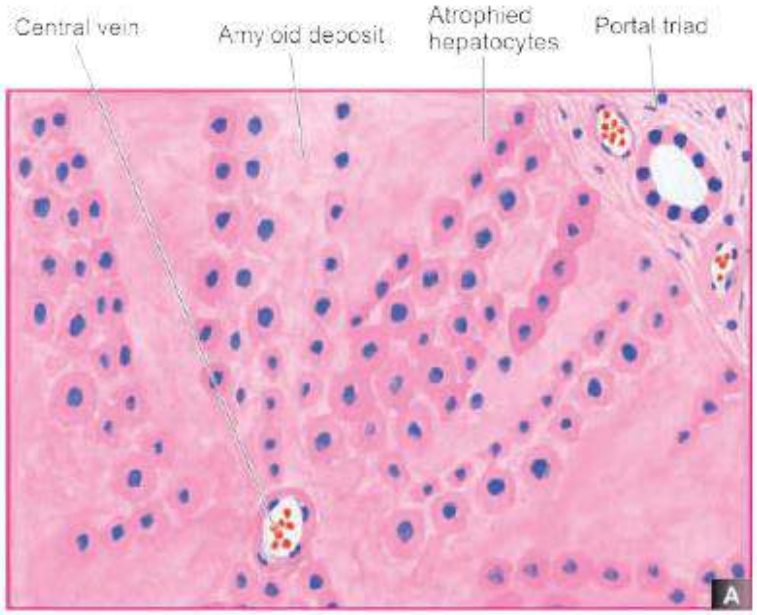
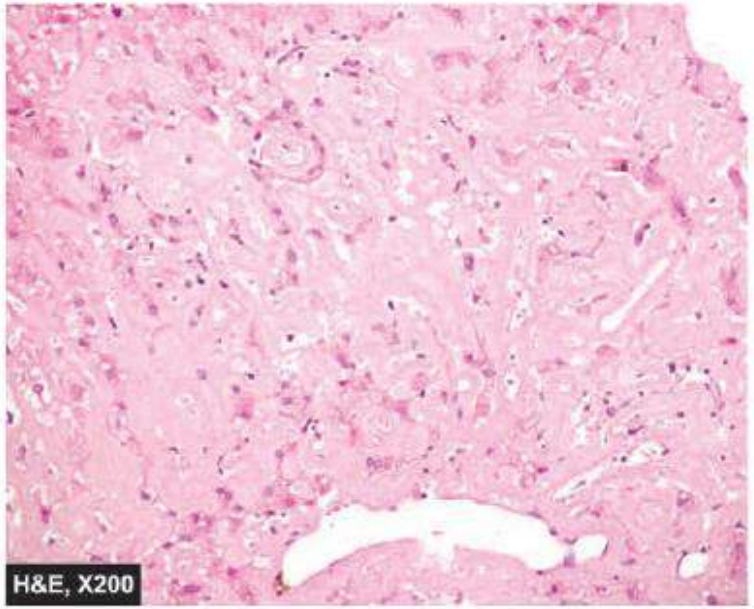


Figure 4.16 ◆ Amyloidosis spleen. A, The pink acellular amyloid material is seen in the red pulp causing atrophy of while pulp. B, Congo red staining shows Congophilia as seen by red-pink colour. C, When viewed under polarising microscopy the corresponding area shows apple-green birefringence.





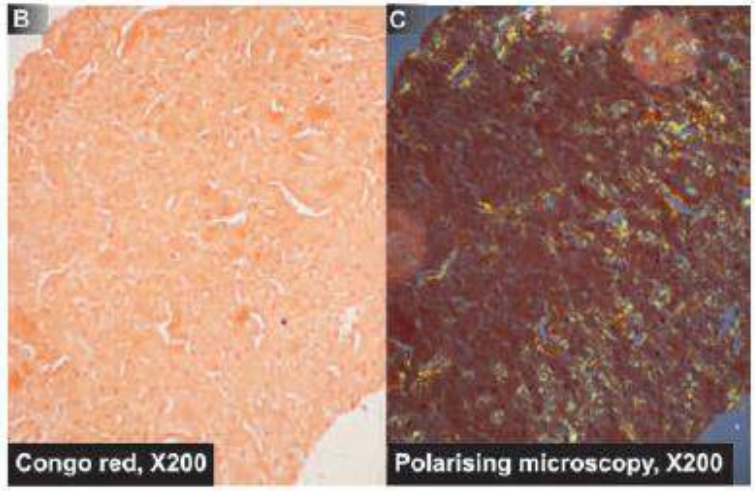


Figure 4.17 Amyloidosis of the liver. A, The deposition is extensive in the space of Disse causing compression and pressure atrophy of hepatocytes. B, Congo red staining shows congophilia which under polarising microscopy. C, shows apple-green birefringence.