

530.304 – General Pathology Lecture Notes

INTRODUCTION TO PATHOLOGY

• Introduction to Pathology

General pathology is the study of the mechanisms of disease (with emphasis on aetiology and pathogenesis), while **systematic pathology** is the study of diseases as they occur within particular organ systems – it involves aetiology, pathogenesis, epidemiology, macro- and microscopic appearance, specific diagnostic features, natural history and sequelae.

Academic pathology includes research and teaching, and the discipline of experimental pathology was derived from this. Clinical pathology is often referred to as laboratory medicine and includes a number of diagnostic disciplines.

Pathology provides the basis for understanding:

- The *mechanisms* of disease
- The *classification* of diseases
- The *diagnosis* of diseases
- The basis of *treatment*
- Monitoring the *progress* of disease
- Determining *prognosis*
- Understanding *complications*

SNOMED – standard classification of disease – considers the following aspects:

- Topography
- Morphology
- Aetiology
- Function
- Disease
- Procedure
- Occupation

• Techniques of Pathology

Gross pathology – macroscopic investigation and observation of disease
Light microscopy – thin section of wax or plastic permeated tissues, snap-frozen tissues
Histochemistry – microscopy of treated tissue sections (to distinguish cell components)
Immunohistochemistry and immunofluorescence – tagged antibodies (monoclonal better)
Electron microscopy
Biochemical techniques – e.g. fluid and electrolyte balance, serum enzymes
Cell cultures – also allowing cytogenetic analysis
Medical microbiology – direct microscopy, culturing and identification
Molecular pathology – in situ hybridisation (specific genes/mRNA), polymerase chain reaction

CELL INJURY

• The Pathogenesis of Cell Injury

Normal cell structure and function requires:

- Nuclear function* for nucleic acid, protein, lipid and carbohydrate synthesis
- Enzyme function* for assembly and degradation of organelles and cell products
- Membrane function* for the transport of metabolites/messengers and for the ionic and fluid homeostasis
- Energy production* and the formation of high-energy compounds by aerobic phosphorylation (and/or anaerobic glycolysis)

Injury to the **nucleus**:

- Genetic defects* – single gene, multiple gene or whole chromosome abnormalities
- Nutritional disturbances* – e.g. pernicious anaemia due to B₁₂ deficiency affecting DNA synthesis in haematopoietic cells

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Toxic injury – may inhibit nuclear functions (synthesis, division)

Standard background radiation is approximately 10^{-3} rads, with minor consequences for dosages lower than 10 rads. A dose of 100 rads will give mild radiation sickness. A dose of 1000 rads will give severe radiation sickness, with pancytopenia. Note that UV is sufficient to create pro-mutagenic damage to DNA and hence has long-term effects.

Ataxia telangiectasia is due to a fundamental failure to repair damaged DNA. Individuals with this condition have hypersensitivity to DNA damage (e.g. radiation). *Fragile X syndrome* is due to an expansion in an unstable codon (6-50 in normal individuals, 250-4000 in affected individuals) which leads to susceptibility to nuclear damage.

Injury to **cell membranes**:

Receptor defects – e.g. familial hypercholesterolemia

Complement related injury – e.g. immunological reactions that activate complement, opening transmembrane channels that alter ionic homeostasis

Free radical injury – atoms/molecules with unpaired e^- (usually O_2 intermediates):

O_2 therapy → Excess O_2

PMNs, macrophages → inflammation

PMNs, xanthine oxidase → reperfusion injury after ischaemia

Mixed function oxidation, cyclic redox reactions → drug-induced/chemical toxicity

Radiotherapy → ionising radiation

Initiators, promoters → chemical carcinogenesis

→ O_2^- , H_2O_2 , $\bullet OH$ – reactive oxygen intermediates

→ membrane damage (lipid peroxidation)

Viruses – direct membrane injury (e.g. polio – viral proteins inserted into membrane forming pores or channels) or indirect membrane injury (e.g. hepatitis B – viral release from the cell exposes viral proteins at the cell surface leading to immune response)

Another example is the alpha toxin produced by *Clostridium perfringens* – this disrupts membrane function.

Lysosomes and cell injury:

Intracellular 'storage' diseases – inherited deficiency of lysosomal enzymes leading to failure to degrade particular substrates that accumulate

Abnormal intracellular release – e.g. gout and silicosis where the ingestion by phagocytic cells of uric acid/silica leads to rupture of phagosomes

Abnormal extracellular release – e.g. rheumatoid arthritis

Cell injury and **energy production**:

Hypoxia or *ischaemia* compromise energy-dependent process like contraction, and transmembrane ionic exchange is affected

Reactions of cells to stress and energy:

Adaptation

Abnormalities of growth – atrophy, hypertrophy, hyperplasia, metaplasia

Abnormal storage – accumulation of products in cytoplasm (e.g. lipofuscin)

Reversible cell damage

Irreversible cell injury – typically cell death by necrosis

Note that there is evidence of **reversible cell injury**:

Cell and organelle swelling – due to failure of energy-dependent ionic exchange and/or membrane injury, also known as intracellular oedema

Fat accumulation – fatty change in the parenchymal cells of the liver, heart and kidney due to failure to utilize or convert the NEFA arriving at the cell (e.g. inadequate synthesis of lipid-acceptor protein in the liver)

• Necrosis and Apoptosis

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The type of **necrosis** is dependent on the nature, intensity and duration of the injurious agent, and the type of cell involved. Note that initial membrane damage allows Ca^{+2} leakage with subsequent activation of Ca-dependent phosphatases and lipases.

Coagulative necrosis – cytoplasm of the necrosed cells becomes eosinophilic and persists for many days (myocardial infarction)

Colliquative necrosis – cells undergo lysis rapidly (brain infarcts)

Caseous necrosis – *Mycobacterium tuberculosis* interacts with macrophages

Gangrenous necrosis – primary (bacterial toxins) or secondary (ischaemia, infection)

Fibrinoid necrosis – smooth muscle necrosis, fibrin release (malignant hypertension)

Fat necrosis – inflammatory response to liberated fat → fibrosis

There are also nuclear changes related to necrosis:

Margination of chromatin – chromatin condensing around the periphery of the nucleus

Pyknosis – small and dense nuclei

Karyolysis – complete lysis of the nuclei

Karyorrhexis – fragmented nuclei (generally seen in apoptosis)

Irreversible cell injury is typically accompanied by:

Release of intracellular enzymes:

Cardiac muscle – creatine kinase (MB isoform), aspartate transaminase, lactate dehydrogenase

Hepatocytes – alanine transaminase

Striated muscle – creatine kinase (MM isoform)

Exocrine pancreas – amylase

Loss of membrane selectivity – may be helpful in diagnosis through uptake of dyes

Inflammatory response – initiated by products (mediators) of the necrotic cells

Cell death can also occur through **apoptosis** – it may be physiological deletion of selected cells (e.g. morphogenesis, cyclic hyperplasia of reproductive processes) or it may occur in response to a pathological stimuli. Note that there are no gross structural changes involved.

The initiation of apoptosis requires two processes:

Priming – a reversible stage in which the specialist machinery for apoptosis (e.g. transglutaminase, calcium/magnesium endonucleases) are activated

Triggering – the irreversible point which leads to a sustained rise in cytosolic calcium, and induction of new mRNA species for c-fos, c-myc and heat-shock proteins

Apoptosis then proceeds:

1. Cytosol and nucleus lost half their *volume*
2. *Fragmentation* of nucleus and cytosol (→ activation of transglutaminase that forms an insoluble layer beneath the intact cell membrane)
3. *Condensation* of chromatin (pyknosis)
4. *Macrophages* bind to cell fragments prior to phagocytosis (non-specific mechanism)

Pathological cell death is more often due to necrosis – this process releases intracellular enzymes (useful diagnostically) and mediators that stimulate inflammation. This is followed by healing by repair, scarring, contracture and distortion of tissue architecture.

	Necrosis	Apoptosis
Histology	Groups of cells, disrupting tissue structure	Single cells within living tissues
Cytology	Cellular swelling, nuclei initially intact	Pyknotic subdivided nuclei, condensed cytoplasm, rounded membrane-bound cell fragments
Dye exclusion	Dyes enter	Dyes initially excluded
Cytoplasm	Dilated organelles – mitochondria show matrix densities, ruptured plasma and internal membranes	Compact and intact organelles, intact plasma membrane
Nucleus	Coarse chromatin patterns with normal distribution	Chromatin condensed, nucleolar disintegration

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Circumstances	Complement-mediated immune reactions, hypoxia, toxins (high dose)	Programmed cell death, atrophy, cell-mediated immune killing, toxins (low dose)
Tissue Effects	Acute inflammation, healing by repair, scarring with distortion of tissue	No inflammation, phagocytosis, rapid involution without affecting tissue structure

TISSUE INJURY

• Introduction to Inflammation

Inflammation is an extravascular process in which the active components of the reaction (cells and fluid) are derived from the blood vessels supplying the tissue area involved. It occurs in the connective tissue components, with a characteristic sequence of events (though the outcome and clinical manifestations vary).

Cause of injury – ischaemic, physical, chemical, infectious, immunological

Time course – rapid and acute, or slow and chronic (depends on the pathogenic mechanism, persistence of the injurious agent and presence of certain cell types)

Initial reactions – localized, non-specific systemic manifestations (e.g. pyrexia)

Redness (rubor), *heat* (calor), *swelling* (tumor), *pain* (dolor), *loss of function* (functio laesa)

1. The initial response involves mediator release from cells and plasma
2. Increased blood flow and vessel permeability, abnormal movement of fluid and plasma proteins into extracellular space
3. Migration and activation of leukocytes in response to attractant substances

If the injury occurs in solid tissue and the causal agent is pyogenic, *suppuration* is likely to occur. On centrifugation, the supernatant contains inflammatory exudates; the deposit consists of polymorphs, bacteria, cell fragments, fat globules and other particulate matter.

Abscess – necrotic, suppurative lesion localised by a fibroblastic boundary

Ulcer – inflammatory lesion involving epithelial surfaces

- i. Sloping edges – healing (granulation tissue formation)
- ii. Punched-out edges – syphilis
- iii. Undermined edges – tuberculosis
- iv. Rolled edges – basal cell carcinoma
- v. Everted edges – squamous cell carcinoma

Cellulitis – inflammatory reaction spreading through connective tissue planes

• Inflammation – Vascular Response

Transient vasoconstriction → vasodilatation of arterioles → hyperaemia (→ rubor, calor)

Arteriolar dilatation occurs after vasoconstriction and results in an opening of the microvascular bed. Increased blood flow to the injured area is called *hyperaemia* and causes redness and heat – note also that there is an increase in the net pressure in capillaries and post capillary venules, leading to an outflow of fluid.

Direct injury to vessels (or venule endothelial cell contraction) causes alteration of **vessel permeability**, leading to leakage of fluid and plasma proteins:

1. *Endothelial cell contraction* and separation of the *endothelial junctions* (in post-capillary venules) in response to mediators
2. Increased *hydrostatic filtration pressure* enhances outward movement of fluid and facilitates the passage of *larger protein molecules*
3. More sustained/serious injury leads to *large gaps* in endothelial junctions and these changes also affect capillaries (increasing the rate of extravascular fluid flux)
4. Intravascular and extravascular osmotic pressure equalise, the hydrostatic pressure in the tissue increases (so fluid loss is dependent on *net hydrostatic pressure*)

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5. A *protein-rich exudates* accumulates extravascularly
6. Due to tissue swelling, collagen fibres anchored in the tissue pull open terminal lymphatic channels – leading to *increased lymph flow*
7. Lymphatic channels assist in draining the fluid and cellular exudate

Swelling – accumulation of excess fluid in the interstitial space → **oedema formation**

1. Changes in the calibre of small vessels
 - a. → changes in blood flow (increase)
 - b. → increased hydrostatic pressure in vessels
2. Changes in vessel wall
 - a. → contraction of endothelial cells → inter-endothelial cell gaps
 - b. OR → damage or destruction of vessel walls
 - c. → changes in permeability of vessel wall

The *fluid exudate* contains a number of proteins including immunoglobulins, fibrinogen and proteins of the complement, kinin and plasmin cascades which act as mediators.

- a. *Serous* – clear watery fluid, low protein content (esp. fibrinogen) – mesothelium
- b. *Fibrinous* – higher protein content (fibrinogen → fibrin) – serosa-lined cavities
- c. *Haemorrhagic* – fibrinous exudate with sufficient damage to small blood vessels
- d. *Purulent* – production of pus, neutrophils dominant and release lysosomal enzymes
- e. *Catarrhal* – mucous membranes

The local structure of connective tissue determines the volume of exudate that can collect, extent of swelling, direction of spread, local tension, and associated pain. Accumulation of excess fluid low in protein also leads to oedema, but the fluid is known as a *transudate*.

Note that lymphatics drain interstitial fluid from the tissues. During inflammation, capillaries open (endothelium, basement membrane, anchoring filaments, intercellular clefts) – while this helps to limit swelling, it raises the possibility of systemic spread of infection agents.

• Inflammation – Mediators

Mediators can be classified functionally:

1. Mediators producing *arteriolar dilatation* and increased *vascular permeability*
2. Mediators that have *effects on leukocytes*

Plasma	Complement cascade Coagulation cascade Fibrinolytic cascade Kininogen-Kinin system Opsonins	C _{3a} C _{5a} C ₅₆₇ Fibrinopeptides Fibrinolytic products Bradykinin Immunoglobulins, Fibronectin, C _{3b}
Tissues	Mast cells, platelets Macrophages, neutrophils Lysosomal products Lymphocytes Many cell types All cell types	Vasoactive amines – histamine, serotonin IL, TNF, MCP, NAP1/IL ₈ Proteases and cationic proteins Lymphokines (cell-mediated immunity) Acidic lipids (prostaglandins, leukotrienes, thromboxane) Breakdown products – lysosomal enzymes
Exogenous	Bacteria	F met peptides Endotoxin Various microbial enzymes and exotoxins

Histamine is a vasoactive amine stored as pre-formed granules in mast cells, basophils and platelets (generally located next to blood vessels). Mast cells degranulate in response to injury and discharge their granule contents locally.

Activation is achieved by:

1. *Phospholipase-A*, an enzyme in the cell membrane
2. *Anaphylotoxins* produced by activation of the complement cascade

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3. An immunological mechanism related to *IgE* which is cytophilic for mast cells

Histamine acts on H1 receptors to mediate vasodilatation, and the increase in permeability during the induction phase of the acute inflammatory response. Effects last for ~60 minutes unless the injury is sustained (i.e. early phases of acute inflammation).

Kinins (mainly *bradykinin*) are released from an inactive plasma precursor (kininogen) by kallikrein, which is in turn activated by Hageman factor (factor XII). Factor XII is activated by a number of mechanisms and can also stimulate complement activation.

Bradykinin is 100,000 times more active than histamine in increasing vascular permeability, and 10 times more potent in respect to vasodilatory activity. It is involved as a mediator of *pain* production by direct nerve stimulation, and activation of arachidonic acid metabolism.

The **eicosanoids** (acidic lipids) have a profound effect on many tissues – arteriolar dilation, venous constriction, increased permeability, and stimulate neutrophil adhesion, fever, and pain. The importance of these arachidonic acid derivatives is demonstrated by the effects from inhibiting their generation.

Activation of *phospholipase A2* stimulates hydrolysis of arachidonic acid from membrane phospholipids. This is metabolised by

1. Cyclo-oxygenase (→ *prostaglandins, thromboxanes*) or
2. Lipoxygenase (→ HETEs and leukotrienes)

The main sources of activity on vascular permeability are:

1. Leukotrienes LTE4 (vasoconstriction) and LTB4 (vasodilatation)
2. Several prostaglandins:
 - a. Thromboxane A2 causing vasoconstriction and platelet aggregation
 - b. PGE2 and PGI2 (*prostacyclin*) causing vasodilatation and pain.

Note that COX1 is physiologically protective (especially prostaglandins on the gastrointestinal tract), while COX2 drives inflammation – hence non-specific NSAIDs/COX inhibitors cause a number of side effects related to COX1 inhibition.

Platelet activating factor is produced by mast cells and leukocytes, inducing platelet aggregation and degranulation (→ histamine). Its production is initiated by phospholipase A2, and also enhances arachidonic acid metabolism in activated neutrophils.

Platelet activating factor also directly causes vasodilatation, promotes increased vascular permeability and is involved in leukocyte aggregation and migration.

Cytokines are polypeptide messenger molecules secreted by cells (lymphocytes → lymphokines, monocytes → monokines). Features:

Some are glycoproteins

Small, not antigen specific

Transient production

Pleiotropic – multiple actions, source cells, target cells, redundancy

Many have names that reflect the actions that were first discovered – not necessarily the most important factors

May be mutually synergistic or antagonistic with other cytokines

Notable cytokines include:

1. Interleukins e.g. IL-1 α – pyrogenic, activates lymphocytes
2. Tumour necrosis factor – pyrogenic, induces adhesion molecules

• Inflammation – Cellular Responses

Polymorphonuclear leukocytes are actively attracted (chemotaxis) to the site of acute inflammation where they ingest foreign and degenerate material:

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1. *Neutrophils* – produced in large numbers in bone marrow, first cells to arrive and can function in poor oxygenated conditions. Involved in the inflammatory response and normal non-specific defences.
2. *Eosinophils and basophils* – limited phagocytic activity, recruited in inflammatory reactions derived from some specific immune responses.
3. *Macrophages* are derived from monocytes (bone marrow) – the majority of macrophages in inflammatory processes migrate directly from blood vessels.

Many lymph node/spleen cells, Kupffer cells, and alveolar/peritoneal macrophages are monocyte-derived. Other similar cells develop specialisation as antigen presenting cells for the immune system.

Leukocyte **migration** (margination, adhesion, emigration, chemotaxis) occurs as follows:

1. Slowing of blood flow and clumping of erythrocytes (*rouleaux formation*) forces leukocytes to the periphery (*margination*). Loss of *central flow* also allows contact between neutrophils, platelets and the endothelium.
2. Expression of adhesion molecules between leukocytes and the endothelium occurs (*pavementing*).
3. *Cell adhesion molecules* facilitate leukocyte adhesion by binding to a single cell surface glycoprotein found on activated monocytes, fibroblasts and vascular endothelial cells.
 - a. Integrins
 - b. Immunoglobulin superfamily
 - c. Selectins
4. *Chemotaxis* – directional movement of phagocytic cells, mediated by a series of chemical messengers
 - a. Diapedesis – passive escape of erythrocytes – may be facilitated by chemotactic leukocyte migration.
5. Note:
 - a. Motile neutrophils and monocytes are actively phagocytic.
 - b. The *PMNs* are the first line of defence, migrating 30-40 minutes after injury occurs and reaching a maximum 6-8 hours later. They have a short life span and limited killing potential.
 - c. *Monocytes* and *macrophages* appear after 4 hours and peak 16-24 hours after injury occurs. They have greater killing potential and have a role in preparing the tissue for healing and repair. They also produce interleukin, stimulating fibroblast proliferation.

Adherence between the phagocyte and unwanted material is the first step in the process of phagocytosis. It is affected by:

1. Lack of *molecular glycosylation* – normal molecules are typically glycosylated
2. *Opsonins*, which facilitate adherence of opsonin coated substances to receptors on phagocytes.

Specific surface receptors are present on phagocytes for immunoglobulin molecules, C3b and fibronectins – note that not all bacteria bind fibronectins and adhere to phagocytes through non-specific mechanisms. *Antibody-mediated opsonization* can be enhanced by activation of complement, and is critical if non-specific opsonization is not effective.

Inflammatory mediators → expression of P-selectin (tethering molecule)
Platelet activating factor co-expressed with P-selecting → activates the PMN
PMN expresses integrins; MAC-1 → MAC-1 binds to ICAM on the endothelium wall
Adhesion of PMN → activation of cytoskeletal elements (pseudopodia)
Pseudopodia → engulfment of particle → phagosome
Phagosome fuses with lysosomes → phagolysosome

Macrophages also carry surface receptors for the Fc fragment of immunoglobulins and C3b – they can be activated by T-lymphocytes or non-immune cell surface interactions. Activated macrophages are larger, have more mitochondria and Lysosomes, and a greater amount of hydrolytic chemicals. These include:

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1. Neutral proteases (e.g. plasminogen activator collagenase, elastase)
2. Oxygen metabolites
3. Arachidonic acid metabolites and cyclo-oxygenase products
4. Interleukin 1 and tumour necrosis factor
5. Neutrophil chemotactic factors
6. Growth promoting factors (for fibroblasts, blood vessels and myeloid cells)
7. Complement compounds
8. Coagulation factors (factor V and thromboplastin)

Note that some bacteria still survive – e.g. *Mycobacterium tuberculosis* → chronic inflammation

• Inflammation – Clinical Manifestations

Defects in leukocyte function → susceptibility to bacterial invasion

1. Number of number of circulation cells – neutropenia
2. Adherence – diabetes, alcoholism, corticosteroids
3. Migration/chemotaxis – Chediak-Higashi syndrome
4. Phagocytosis – opsonising defects, intrinsic cellular defects
5. Microbicidal function - chronic granulomatous disease of childhood

Clinical manifestations of acute inflammation:

1. *Redness* caused by arteriolar dilatation and increased vascularity – congestion may progress to stasis leading to reduction of haemoglobin
2. *Heat* due to increase blood flow – central body temperature may also be elevated
3. *Swelling* due to local accumulation of exudate in loose connective tissue. Processes contained within rigid structures may not swell, but can occlude structures.
4. *Pain* due to physical tension and swelling, as well as the release of bradykinin
5. *Limitation of function* – e.g. noxious influence of the functional parenchyma of an organ. Note that deliberate motion and function may promote the spread of the injurious process through tissue planes and lymphatics.

Systemic effects of inflammation include:

1. *Pyrexia* mediated by interleukin (IL-1), a cytokine or an inflammatory mediator – prostaglandins may also contribute
2. *Leukocytosis* induced by cytokines (TNF)
3. *Acute phase proteins* (fibrinogen, haptoglobin, α 1-acid glycoprotein, α 2-macroglobulin, some complement proteins, C-reactive protein, serum amyloid protein) produced in the liver in response to IL-1 and TNF.
4. *Endocrine change* – an increase in glucocorticoid steroid hormone production due to stress

• Chronic Inflammation

Chronic inflammation is a prolonged process in which destruction and inflammation are proceeding at the same time as attempts at healing. It occurs:

1. From acute inflammation
 - a. Chronic non-specific inflammation (secondary chemical injury)
 - b. Chronic suppurative inflammation (secondary continuing infection)
2. After repeated bouts of acute inflammation with intervals of healing – gall bladder, kidney and large intestine
3. Insidiously or *de novo*
 - a. Persistent infection
 - b. Prolonged exposure to non-degradable substances
 - c. Immune reactions

Mononuclear cells are the characteristic features of chronic inflammation:

1. Activated *macrophages*, which are motile, capable of phagocytosis, stimulate fibroblasts to divide and are hardy and long lived.

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2. B and T lymphocytes may be involved also, along with plasma cells, eosinophils and fibroblasts

The histological hallmarks of chronic inflammation are:

1. Tissue destruction
2. Infiltration of mononuclear cells
3. Granulation tissue (fibroblasts and small vascular channels)
4. Regeneration
5. Fibrosis

Effects of chronic inflammation:

1. Local
 - a. Tissue destruction (e.g. peptic ulcer perforation, rheumatoid arthritis)
 - b. Fibrosis (e.g. cardiac valves)
2. General
 - a. Hyperplasia of mononuclear/phagocytic cells in lymph nodes, spleen, liver and bone marrow
 - b. Immune response
 - i. Antibody production – increase in γ -globulins
 - ii. Cell-mediated immunity (tissue destruction e.g. tuberculosis)
 - iii. Splenomegaly
 - c. Changes in blood
 - i. Normocytic, normochromic anaemia
 - ii. Persistence of acute phase reaction proteins (e.g. c-reactive protein), serum amyloid A protein, haptoglobins, complement components, fibrinogen, ceruloplasmin
 - iii. Raised erythrocyte synthesis rate
 - d. Non-specific complaints include fatigue, anorexia, low grade fever

A **granulomata** is a focal chronic inflammatory reaction, with macrophages and epithelioid cells in compact masses surrounded by lymphocytes. Modified macrophages are characteristic:

Epithelioid cells are specialised for secretion over phagocytosis – seen in tuberculosis and sarcoidosis

Multinucleate giant cells are formed by fusion of macrophages or epithelioid cells – they may be seen in chronic inflammation without granulomas. Arrangement of nuclei gives naming – foreign body, Langhan, Touton, Warthin-Finkeldy

IL-1 is important in the initiation, while TNF is responsible for its maintenance.

Granulomata can be classified as follows:

1. Morphology
 - a. Non caseating (e.g. foreign body)
 - b. Caseating (e.g. tuberculosis)
 - c. Suppurative (e.g. cat scratch disease)
2. Immunological status
 - a. Immune – stimulate epithelioid cell formation, healing is by fibrosis. T cytotoxic cells may kill cells and produce areas of necrosis
 - b. Non-immune – foreign body may attract macrophages without epithelioid cells or cell-mediated immune response. Fibrosis is less marked.
3. Aetiology
 - a. Biological agents
 1. Bacterial – tuberculosis, syphilis, leprosy
 2. Fungal – cryptococcus, coccidioides
 3. Protozoal – pneumocystis, toxoplasmosis
 4. Parasitic – schistosomiasis
 - b. Non-biological agents – silicosis, berylliosis, talc
 - c. Unknown – sarcoidosis, Crohn's disease

Pathogenesis of **primary tuberculosis**:

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1. Inhalation/ingestion of organisms → acute reaction → organism survives (waxy coat)
2. Macrophages release cytokines → epithelioid cells recruit T lymphocytes
3. → Granuloma with central caseous necrosis
4. → Healing (or development of secondary tuberculosis)
 - a. Macrophages with viable bacilli → Gohn complexes in respiratory lymph nodes
 - b. Immune status change → reactivation of dormant bacteria

Pathogenesis of **syphilis**:

1. Invasion → organisms replicated locally in subepithelial tissue
 - a. Dissemination via lymphatics and blood
2. Within three weeks – primary lesion
 - a. Macule → papule → chancre (circular painless ulcer)
 - b. PMN, lymphocytes, macrophages
3. After six weeks – lesion heals with a tiny scar
4. If untreated, a few months later – secondary lesion
 - a. Systemic spread → skin, mucous membranes, lymph nodes
 - b. Rash – variable shape, colour, distribution (involves palms, soles)
 - c. Ulceration – snail track lesions
 - d. Lymphadenopathy
 - e. Fever, malaise, sore throat, alopecia
5. 35% of cases (years later) – tertiary lesion
 - a. Chronic granulomatous inflammation
 - b. Vasculitis – arteritis → thoracic aorta aneurysm
 - c. Nervous system – dementia, tabes dorsalis
 - d. Bone and skin – gumma necrosis (extensive, few giant cells)

• Healing by Regeneration and Repair

Regeneration is the replacement of lost cells by those of the same type – the capacity of tissues for regeneration depends on the presence of stem cells:

1. *Labile* tissues undergo continual renewal (~1.5% of cells are in mitosis) e.g. bone marrow and lymphoid tissues
2. *Stable* tissues contain stem cells which may become active e.g. parenchyma of glands, mesenchyme
3. '*Permanent*' (highly differentiated) tissues have no stem cells and irreversibly injured cells are replaced with fibrous tissue e.g. nerve, muscle. Exceptions:
 - a. Axons of peripheral nerve cells may regenerate slowly if the nerve sheath is not damaged – a displaced sheath may produce a traumatic neuroma.
 - b. Satellite cells at the basement membrane of skeletal muscle cells can differentiate into myocytes to replace those that have necrosed.

Regeneration is mediated by a number of growth factors from many cell types. The cells are also stimulated to migrate and proliferate until contact is restored with neighbouring cells of the same type ('contact inhibition').

Repair is the regeneration of vascular, fibrous connective tissue – it is characterised by the proliferation and maturation of this tissue to:

1. Replace the loss of more specialised tissue
2. Replace inflammatory exudate and haemorrhage
3. Replace thrombus within blood vessels

There are a number of steps in the repair process:

1. *Phagocytosis*
2. *Fibroendothelial proliferation* stimulated by growth factors – by the 3rd-4th day granulation tissue is evident. This process is also referred to as 'organisation'
3. *Maturation* – capillary blood vessels reduce in number, with some developing into arterial and venous channels. Collagen density continues to increase
4. *Scarring* – fibronectins, glycoprotein and type III collagen are replaced with type I collagen to form a scar. As it matures, the scar contracts (cicatrisation).

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Note that there are differences in the healing of a clean surgical wound with minimal defect (healing by primary intention) and healing where there is a substantial loss of tissue (healing by secondary intention).

• Pathological Calcification

The final event in the death of individual cells is the irreversible failure of membrane function (and hence ionic homeostasis). There is a huge calcium influx when the membrane fails (normal gradient is 10,000:1) – it is debated whether this is the cause or effect of membrane failure, although calcium channel blockers are used to minimise cell injury.

Mitochondria normally maintain a higher Ca^{+2} concentration than the cytosol – hence after membrane failure calcium accumulates in the mitochondria as electron-dense granules or linear deposits.

99% of body calcium is in bone, and 1% in teeth – however, there is a small but significant amount (0.5%) in solution where it is involved as an intracellular messenger (nerve conduction, muscle contraction and blood coagulation).

Hydroxyapatite ($[\text{Ca}(\text{PO}_3)_2]_3 \bullet \text{Ca}(\text{OH})_2$) is the mineral crystal normally deposited in the body as long, flat, narrow crystals with an enormous surface area.

1. Composition varies as OH can be replaced with Cl, CO_3 or F; and Ca can be replaced by Ba or Sr
2. Parathyroid hormone mobilises Ca from bone by osteoclasts
3. Calcitonin increases Ca deposition by osteoblasts
4. Vitamin D is required for Ca absorption in the gut

There are two possible explanations for normal mineralisation:

1. There is a mechanism to locally increase the concentration of Ca^{+2} or PO_4^{-3}
2. There is some local characteristic of the matrix that aids precipitation

Mineral deficiency can be involved in various conditions:

1. Ricketts is caused by vitamin D deficiency in children leading to deficient mineralisation of cartilage and deficient resorption/replacement
2. Osteomalacia is the equivalent in adults with deficient mineralisation of bone
3. Osteoporosis is due to the progressive loss of bone mineral with age as bone formation lags behind bone resorption
4. Dental caries is caused initially by the dissolution of the mineral part of tooth enamel – fluoridation increases the proportion of fluorapatite (less soluble) in the enamel

Pathological calcification:

1. Metastatic calcification can occur in tissues which do not normally mineralise when the circulating levels of Ca^{+2} and/or PO_4^{-3} are increased (e.g. parathyroid tumour)
2. Dystrophic calcification occurs with normal levels of calcium/phosphate where nuclei facilitate precipitation – these include necrotic and injured tissue
3. Lithiasis occurs where degenerating cells or micro-organisms provide a nucleus for mineral deposition. Predisposing factors and consequences are the same:
 - a. Obstruction of flow
 - b. Infection
 - c. Inflammation and ulceration

CARDIOVASCULAR DISEASES

• Arteriosclerosis

There are a number of degenerative disorders of the walls of blood vessels:

Atherosclerosis – intimal thickening with lipid deposition in medium to large arteries
Monkenberg's medial sclerosis – calcification of the media in medium sized arteries

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Arteriosclerosis – hypertrophy and/or fibrosis of the media in arterioles

Narrowing of the lumen → poor tissue perfusion / ischaemia
Vessel wall rigidity → predisposition to rupture / haemorrhage
Alterations to arterial lining → predisposition to thrombosis

Atherosclerosis is the most common form of arteriosclerosis, and its complications produce significant mortality – in NZ, 28% of deaths are due to ischaemic heart disease, and 10% are due to cerebral infarction. It is estimated that complications of atherosclerosis are responsible for half of all mortality in the US.

The arteries most commonly affected are the aorta, coronary, carotid, mesenteric, iliac, femoral and cerebral. There are a number of **lesions** characteristic to this disease:

1. Intimal thickening – sometimes associated with discontinuities in the internal elastic lamina and the presence of smooth muscle cells in the intima
2. Fatty streaks – lipid contained within phagocytic 'foam' cells in the thickened intima
3. Fibrofatty plaques – the lesion becomes raised, and inflammatory responses lead to collagen and capillary generation. The media may become weakened due to pressure, and dystrophic calcification can occur in the centre of the lesion.
4. Complicated lesions
 - a. *Ulceration or rupture* due to loss of intimal surface leading to
 - i. Thrombosis
 - ii. Embolism
 - iii. Haemorrhage
 - b. *Aneurysm* – dilatation of a blood vessel thought to be caused by weakening of the media (abdominal and iliac arteries common).
 - i. Congenital aneurysms (e.g. 'berry')
 - ii. Infective aneurysms (e.g. mycotic)
 - iii. Dissecting aneurysms – blood in the vessel wall forms a false lumen

There are a number of **theories of pathogenesis** for this condition:

1. *Imbibition (Virchow)* – accumulation of lipid due to absorption from blood
2. *Encrustation (Rokitansky)* – thrombus mediating the growth of atheromatous lesions
3. *Design fault (Sims)* – association between discontinuities of the internal elastic lamina and the presence of intimal thickening. Risk factors determine the rate of growth.
4. *Smooth muscle cell proliferation* – muscle cells in some lesions are monoclonal
5. *Infectious theories*
 - a. Marek disease – virus causing atherosclerosis in chickens
 - b. Herpes virus, cytomegalovirus – detected in human lesions
 - c. Chlamydia pneumonia – detected in plaques but not normal vessel wall
6. *Reaction to injury* – local injury to endothelium → entry of lipid → oxidation of lipid → inflammation → growth factors stimulate smooth muscle cell proliferation

Risk factors can be categorised as follows:

1. *Constitutional risks*
 - a. Age
 - b. Sex (M>F up till age 55, after which incidence and severity increases rapidly in women – possibly hormonal protection before menopause)
 - c. Familial traits – familial predisposition independent of hyperlipidaemia
2. *Hard risk factors*
 - a. Hyperlipidaemia – severity of atherosclerosis has a direct correlation with serum levels of cholesterol/LDL (over 3.9mmolL⁻¹). There is a reduced risk with high levels of HDL, while atherosclerosis is more common in patients with type II and III familial hyperlipidaemia.
 - b. Hypertension – especially raised diastolic pressure
 - c. Diabetes mellitus – increased risk related to induced hypercholesterolaemia
 - d. Cigarette smoking – relation between smoking and mortality from coronary artery disease
3. *Soft risk factors*
 - a. Exercise – reduces the incidence of death from ischaemic heart disease

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- b. Overweight individuals have an increased risk of death from IHD
- c. Stress and personality – linked to death from IHD

• Thrombosis

Thrombosis is the process of formation of a solid mass in the lumen of living blood vessels (or in the heart) from the constituents of blood. Haemostasis is the normal equivalent, and this involves the vessel wall, platelets and coagulation.

Excessive thrombosis is normally discouraged by endothelial cell products:

1. *Prostacyclin* (PGI₂) and *nitric oxide* that prevent platelet adhesion and aggregation
2. *Heparin sulphate* which inhibits components of the normal coagulation cascade
3. *Thrombomodulin* that initiates the anti-coagulant effects of proteins C and S
4. *Plasminogen activators* that produce plasmin (lyses fibrin, inhibits parts of the coagulation cascade)

Virchow's Triad

1. Injury to endothelium
 - a. Collagen exposure → platelet adhesion and aggregation
 - b. Release of tissue thromboplastin
 - c. Vasoconstriction
 - d. Factor VIII synthesis
2. Alteration to blood flow
 - a. Stasis (e.g. veins, myocardial injury, ballooned atrium)
 - b. Turbulence (e.g. arterial branching, aneurysms, atherosclerotic plaques)
3. Hypercoagulability due to alterations in the constituents of blood
 - i. Increased platelet numbers and stickiness
 - ii. Elevated fibrinogen levels
 - iii. Increased thrombin production
 - iv. Congenital lack of proteins C or S, or anti-thrombin III
 - v. Increased levels of factors VII, VIII, IX, or XI
 - vi. Abnormal auto-antibodies against platelet phospholipids

Thrombus **morphology**:

Arterial thrombi (small, dry, pale, friable) are formed of alternating layers of platelets and fibrin (mixed with RBCs → lines of Zahn). There may be a red stasis tail.

Venous thrombi (large, gelatinous, red) consist mainly of fibrin with admixed RBCs – occasionally lines of Zahn can be seen near the origin of a venous thrombus.

Arterial thrombosis is a common complication to atherosclerosis. The typical consequence is ischaemic infarction. *Venous thrombosis* of the leg occurs in the femoral, popliteal, iliac and calf veins, and is generally asymptomatic.

DVTs are predisposed by:

1. Post-operative period
2. Pregnancy and postpartum period
3. Disseminated cancer
4. Prolonged bed rest and/or immobility
5. Heart failure
6. Old age

Outcomes of thrombosis include:

1. Removal by fibrinolysis
2. Organisation
3. Recanalisation
4. Propagation
5. Detachment

• Embolism

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An **embolus** is a mass of material in the circulation capable of lodging in a vessel and obscuring its lumen. The term **thromboembolism** reflects the fact that most emboli arise from thrombi.

Pulmonary embolisms are the most common preventable cause of death in hospital patients, but are difficult to diagnose as signs and symptoms are non-specific. The most common sources are DVTs in the lower limb breaking away, passing through the heart and pulmonary artery to impact in the lungs. Consequences vary on situation, but include:

1. Massive emboli → cardiovascular collapse (acute right sided heart failure)
2. Major emboli → pulmonary infarction if the bronchial circulation is also compromised
3. Recurrent minor emboli → progressive pulmonary hypertension → cor pulmonale

Systemic embolisms may also arise and may impact in arteries leading to the brain, lower extremities, spleen, kidneys and gut. Sources include:

1. Myocardial infarction with mural thrombus
2. Valvular heart disease with vegetations
3. Atrial fibrillation with thrombus formation
4. Aneurysms with a mural thrombus

Other emboli:

1. Atheromatous embolisms (plaques of atheroma)
2. Fat (after long bone fracture)
3. Cancer cells (due to metastasis)
4. Foreign bodies (bullets, catheter fragments)
5. Air or gas (decompression sickness)
6. Amniotic fluid

• Ischaemia and Infarction

Ischaemia occurs where there is a reduction in blood flow significant enough to affect function in an organ or tissue. This can occur in a number of conditions, including vascular occlusion (by thrombus), atherosclerosis, embolism, vascular compression, tumour infiltration, trauma and vascular spasm.

It may also occur in conditions affecting venous draining and systemic hypotension. The extent of damage depends on speed of onset, completeness of blockage, anatomy of the local blood supply, and the nature of the underperfused tissue.

With longer periods of ischaemia, cells lose their ability to regulate Na^+ and Ca^{+2} – on reperfusion, free radicals are generated leading to irreversible cell injury. Ischaemia can also lead to plasma membrane damage by accelerating phospholipids metabolism (by activation of phospholipases).

An **infarct** is a localised area of necrosis resulting from ischaemic injury – the majority of these are due to obstruction of the arterial supply to a tissue.

The appearance depends on the amount of blood that escapes through damaged vessels, the solidity of the tissue, and the length of survival of the patient.

1. *Pale infarcts* occur in solid tissue – with complete arterial occlusion with no collateral supply, red cells rapidly lyse. Initially the infarcted tissue swells, but within 48 hrs the tissue become demarcated and the surrounding tissue forms granulation tissue.
2. *Haemorrhagic infarcts* occur in loose expansile tissue due to bleeding or reperfusion. They are deep red, firm and sharply demarcated. Note that any organ can have a haemorrhagic infarct due to venous congestion – typical examples are torsion of the testis, bowel volvulus and strangulated hernia.

Myocardial infarcts are divided into two classes – transmural (e.g. from occlusion of a coronary artery) and subendocardial (e.g. ischaemia secondary to aortic stenosis). They are characterised by coagulation necrosis and inflammatory cell infiltration – repair is by fibrosis

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with collagen deposition and scar formation. Contraction band necrosis may be seen at the margin where blood flow persists or following reperfusion.

12hrs – acute transmural infarct not identifiable macroscopically

24hrs – pallor or reddish-blue colour due to blood vessel congestion

3-5 days – mottled with a central pale necrotic region bordered by a hyperaemic zone

2-3 weeks – infarcted region is depressed, soft and gelatinous

Older infarcts are firm and pale grey

Cerebral infarcts may be pale (slow occlusion) or haemorrhagic (rapid occlusion with vascular spasm), with extensive oedema. Areas of liquefactive or colliquative necrosis are excavated by phagocytes but are not repaired by fibrosis – the lesion becomes a permanent cyst surrounded by glial fibres produced by reactive astrocytes.

Pulmonary infarcts are haemorrhagic and dark red-brown. The blood vessels in the necrotic area are dilated, leaky and engorged due to retrograde filling, and the alveolar spaces become filled with erythrocytes and fibrin. The margins of the infarct show an inflammatory reaction, and the infarct is gradually converted to collagenous scar tissue.

• Oedema

Oedema is the abnormal accumulation of fluid in extracellular spaces or body cavities

Ascites - peritoneal cavity

Hydrothorax (pleural effusion) – pleural cavities

Pericardial effusion – pericardial sac

Under normal circumstances, hydrostatic blood pressure forces water out of capillaries at the arterial end of vessels. At the venous end, however, plasma oncotic pressure (due to albumin) sucks water into capillary beds. A small amount of water drains from the tissues via the lymphatic system.

Oedema occurs when the rate of formation of interstitial fluid exceeds its rate of draining through the lymphatics:

1. Increased permeability of endothelium (e.g. inflammation)
2. Decreased plasma oncotic pressure (e.g. protein malnutrition, proteinuria, reduced albumin synthesis)
3. Increased venous pressure (e.g. heart failure, venous obstruction)
4. Lymphatic blockage (e.g. tumor blockage, resection of lymph nodes, filarial parasites)

Exudate – oedema associated with increased vascular permeability

Transudate – oedema without changed in vascular permeability

	Exudate	Transudate
Total protein	High	Low (1g/100mL)
Protein pattern	As in plasma	Albumin only
Fibrinogen	+ + (and clots)	Nil
Specific gravity	1.020	1.012
Cells	+ +	Few mesothelial cells

Pulmonary oedema occurs rapidly with massive fluid overload of the lungs and frothy fluid exiting the mouth and nose (commonly blood-stained). Note that death from any cause is typically associated with pulmonary oedema – this may confound initial investigation. Acute pulmonary oedema is often referred to as acute left ventricular failure (common cause).

1. LVF from any cause
2. Ventricular and supraventricular tachycardia
3. High pulmonary vein pressure (mitral valve disease)

Cardiac oedema is mainly caused by excess retention of sodium and water in the renal tubules. This is due to decreased perfusion pressure in the kidneys, though increased capillary hydrostatic pressure contributes.

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NEOPLASIA

• Disorders of Growth

Differentiation refers to the process where tissues become different from their precursors and from one another – it is characterised by the inactivation of genes that cause cell proliferation, and activation of genes that produce the specific cell product. Differentiation of tissue occurs mainly in the embryo, though differentiation of cells continues in some tissues – e.g. epidermis (immature cells → squamous cells → loss of nuclei → keratinisation).

Disorders of growth are due to abnormalities in the regulations of cell size, proliferation or differentiation resulting in abnormality of tissue mass, function or morphological appearance:

1. Reduction in tissue mass
 - a. *Agenesis* – congenital absence
 - i. Unilateral renal agenesis – note compensatory hypertrophy
 - b. *Hypoplasia* – congenital reduction in size
 - i. Ageing – brown atrophy of the heart
 - ii. Failure of endocrine stimulation – post-menopausal endometrium
 - iii. Disuse – skeletal muscles in immobile patients
 - c. *Atrophy* – shrinkage due to loss of size or numbers of cells
2. Increases in tissue mass
 - a. *Hyperplasia* – increase in number of cells
 - i. Abnormal immune stimulation in Graves disease → hyperthyroidism (not premalignant)
 - ii. Prostatic, thyroid – no predisposition to malignancy
 - iii. Breast, endometrium – small risk (note higher rate of proliferation)
 - b. *Hypertrophy of cells* – increase in size of cells
 - i. In permanent cells where increase in cell number is not possible
 - c. *Hypertrophy of parts* – increase in size (may be hyperplastic or hypertrophic)
3. Changes in tissue mass – “dedifferentiation”
 - a. *Metaplasia* – change in type of mature tissue (commonly to an adjacent type)
 - i. Typically endothelium → endothelial type of adjacent tissue
 - ii. Some types carry an increased risk of malignancy – this may be due to acquired genetic abnormalities, or due to the irritative stimuli and tissue damage.
 - b. *Dysplasia* – partial transformation to malignancy
 - i. Genetic alteration to the cell, with loss of tumour suppressor genes (and/or activation of oncogenes) but not sufficient for malignancy
 - ii. May occur with increase in tissue mass, or may be associated with a microscopic lesion
 - iii. *Carcinoma in situ* is the extreme end of dysplasia
 - c. *Benign neoplasia* – loss of control leading to stable, non-spreading mass
 - d. *Malignant neoplasia* – more control loss → expansion, infiltration, metastasis
 - e. *Regenerative atypia* – atypical cell features in regenerating cells
 - i. Nuclear enlargement, hyperchromasia, pleomorphism, mitosis
 - ii. Total number of cells is not increased (due to cell loss) – nuclear changes are not associated with DNA damage (but may → Ca)

• What is Cancer?

Virchow (19th century) believed that all disease was a manifestation of cellular dysfunction, including infectious diseases. He was the first to recognise that cancer was a cellular disorder, and showed how it could be diagnosed at the microscopic level on the basis of cellular appearance and arrangement.

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Cancer is **clonal** – all cancerous cells in a tumour are derived from a single cell. The conversion of a single cell to a cancerous cell occurs in steps, with each step governed by a mutation – several subclones may appear before one that has cancerous characteristics.

Clonality is demonstrated by:

1. Activation of the same X chromosome throughout the cancer (in females)
2. Light chain restriction in a lymphoma
3. Specific T cell or B cell gene rearrangement
4. Mutation of a specific cancer gene throughout the tumour

The **incidence** of each type of cancer varies according to age, gender, social class, ethnic origin, geographical location and time. The commonest cancers in adult western populations are those of the lung, breast, bowel, prostate, bladder and stomach.

Studies done on people who migrate between risk areas suggest that most variation is environmental (as opposed to inherited or constitutional), and often highlight environmental factors. For example, bowel cancer is caused by a high animal fat, low plant fibre diet.

Most cancers are **age** related. This has been attributed to the ageing of the immune system, however it is now believed that cancer evolves slowly due to prolonged exposure to environmental carcinogens. Exceptions to this rule are generally due to genetic factors, or alternations of the hormonal environment.

In terms of **gender**, cancer used to be more common in females than males (due to the frequency of cervical and breast cancer, and the rarity of lung cancer). However, the situation has now reversed in most countries – the only cancers that have a higher incidence in the female population are those of the gall bladder, thyroid and malignant melanoma of the skin.

Neoplasia – either a cancer, or a benign disorder of growth and differentiation. Some benign neoplasms are precancerous; others have little or no malignant potential. Flat neoplastic change is typically precancerous and may be termed *dysplasia*.

Tumour – any mass whether inflammatory, cystic or a neoplasm

• Classification of Tumours

	Benign	Malignant
Mode of growth	Expansile, encapsulated	Expansile, infiltrative
Rate of growth	Slow, may cease	Rapid, progressive
Distant spread	Absent	Frequent
End result	Rarely fatal	Always fatal if untreated

Tumour grade is a measure of the **rate** of tumour growth based on tumour histology
Tumour stage is a measure of the **extent** of the tumour, based on clinical, radiological and pathological features.

Clinical stage is based on clinical and radiological grounds (before biopsy)

Pathological stage is the final 'best guess' staging based also on pathological grounds

Rate x Duration = Extent

Cell/Tissue type	Benign	Malignant
Surface epithelium	Papilloma	Carcinoma
Glandular epithelium	Adenoma	Adenocarcinoma
Melanocytes	Melanocytic naevus	Malignant melanoma
Fibrous tissue	Fibroma	Fibrosarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteosarcoma
Fat	Lipoma	Liposarcoma
Blood vessels	Haemangioma	Angiosarcoma

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Smooth muscle	Leiomyoma	Leiomyosarcoma
Lymph nodes		Lymphoma (Hodgkin's, Non-Hodgkin's {high grade, low grade})
Bone marrow		Leukaemia, Multiple myeloma
Central nervous system		Glioma
Salivary gland		Pleomorphic adenoma
Kidney		Wilms tumour
Mesothelium		Malignant mesothelioma
Germ cells	Seminoma, teratoma	

	Cytokeratin	S100	Leukocyte common antigen
Carcinoma	+	-	-
Melanoma	-	+	-
Lymphoma	-	-	+
Sarcoma	-	-	-

Features of the three main tumour types:

1. *Carcinoma* – most common malignant tumours in adults. Preceded by an in-situ carcinoma phase, which may be flat or take the form of a benign tumour.
 - a. Malignancy can be diagnosed by invasion through tissue layers (basement membrane, muscularis mucosae).
 - b. Spread is generally by lymphatics to lymph nodes, then later via the blood stream to the liver, other viscera and bones.
 - c. Treatment is by surgical resection; response to radiation and chemotherapy varies with type.
 - d. Carcinoma cells grow as cohesive groups of polygonal cells that may produce keratin (squamous cell) or mucin (adenocarcinoma). Cells stain for epithelial cell markers – cytokeratin, epithelial membrane antigen.
2. *Melanocytic tumours* – melanocytic naevi → malignant melanoma. Preceded by an in-situ carcinoma phase (note location of melanocytes in dermo-epidermal junction).
 - a. Spread is through lymphatics to regional lymph nodes, and via the blood stream to a number of sites (skin, brain, viscera – small bowel, spleen).
 - b. Treatment is by surgery, with radiotherapy and chemotherapy in disseminated cases.
 - c. Melanoma cells are round or spindle-shaped, with nuclear enlargement, pleomorphism and high mitotic activity. Fine brown melanin may be seen in the cytoplasm. Cells are negative for cytokeratin, positive for S100 protein.
3. *Connective tissue tumours* – benign connective tissue tumours are very common (particularly lipomas) while sarcomas are rare (1% of malignant tumours).
 - a. Sarcomas typically occur in the deep tissues of the limbs or retroperitoneum, less commonly in the head and neck or in viscera.
 - b. Spread is through the blood stream – lymph node involvement is rare.
 - c. Treatment often combines resection with radiation and chemotherapy.
 - d. Sarcomas are more cellular than normal connective tissues – these cells may be spindle-shaped, round or bizarre and pleomorphic. Most are cytokeratin and S100 negative, although specific tissue markers are available.
4. *Lymphomas* – common tumours that may also involve extranodal sites (skin, stomach, small intestine). No in-situ or benign phase is recognisable.
 - a. Treatment is by chemotherapy and radiotherapy, with resection for localised extranodal lymphomas.
 - b. Lymphomas consist of masses of non-cohesive round cells – negative for cytokeratin and S100, but positive for leukocyte common antigen.
5. *Leukaemia* – uncommon neoplasms of haematopoietic cells that infiltrate and replace bone marrow. They may arise from extramedullary sites sometimes; treatment is by chemotherapy.
6. *Glial tumours* – arise from astrocytes, oligodendrocytes and ependymal cells. Most are diffusely infiltrative, but respond to radiation and chemotherapy. Prognosis varies according to grade.

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Tumours which appear undifferentiated to light microscopy typically show features of differentiation using special techniques. For instance, electron microscopy will show melanosomes in melanoma. However, the main technique in use is immunohistochemistry – where antigenic molecules on cell surfaces (or immunoglobulins) are identified.

Monoclonal antibody technology (increasing the number of antibodies available) and techniques for staining antigens in paraffin-embedded tissue (surface antigens for the classifications of lymphomas which do not survive normal processing) have greatly increased the use of immune techniques in tumour diagnosis.

• Cancer Genes

Proliferating cells go through four distinct stages in each life cycle. Transitions between each stage are regulated by cyclin-dependent protein kinases (Cdk).

1. G1 (gap/growth 1) – cells sense growth factors, space & nutrients to decide whether to divide at the restriction point. The length of G1 determines the cell cycle time.
 - a. G1 → S – mediated by Cdk2 (regulated by CycE)
2. S (DNA synthesis) – S-phase cells can be shown by introducing radiolabelled DNA precursors that will be incorporated and/or treatment with anti-precursor antibodies
 - a. S → G2 – mediated by Cdk2 (regulated by CycA)
3. G2 – quality control pathways check for completion of replication (requiring 4n DNA)
 - a. G2 → M – mediated by Cdk1/cdc2 (regulated by CycB)
4. M (mitosis) → cytokinesis

Non-proliferating cells are said to be in G0 – some have reversibly exited the cell cycle (liver cells, fibroblasts, glial cells) while others have irreversibly exited (neurons, striated muscle).

	Proto-oncogenes	Tumour suppressor genes
Normal function	Activate proliferation, promote cell survival	Inhibit proliferation, promote cell death
Carcinogenic change	Mutation → increased or altered activity	Mutation → less activity
Consequence of mutation	Gain of function	Loss of function
Effects on neoplastic change	Anarchistic influence – drives abnormal growth	Loss of restraint – releases cells to an abnormal phenotype

Oncogenes (dominant) were described when it was found that retroviruses underwent recombination with cellular growth genes, acquiring the ability to drive neoplastic change. Examples of viral oncogenes are v-erbB1, v-ras, v-myc, and v-abl.

1. Chromosomal amplification – increase in the copies of a gene → over-expression
 - a. Sometimes associated with additional chromatin
 - b. ERBB1 in squamous cell carcinoma
2. Chromosome deletion
 - a. Loss of part of the ERBB1 deregulates the tyrosine kinase activity of its protein by removing negative regulatory domains (carcinoma, glioblastoma)
3. Chromosome translocation
 - a. Deregulation of oncoprotein expression
 - i. Burkitt's lymphoma – c-MYC t(8;14)
 - ii. Mantle lymphoma – BCL-1 t(11;14)
 - iii. Follicular lymphoma – BCL-2 t(14;18)
 - b. Change in protein structure when breakpoint is within the gene
 - i. Chronic myelogenous leukaemia – ABL t(9;22), broken in a 5' intron → BCR-ABL hybrid gene → chimaeric protein
4. Point mutations
 - a. Transfection assay – human cancer DNA → partially transformed mouse cells → full transformation. One of the RAS family – mutant in 25% of cancer

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Tumour suppressor genes (recessive)

1. Genetic change – TSG function inactivated by mutation (typically both alleles)
 - a. Retinoblastoma predisposition gene – hereditary retinoblastomas have single-hit kinetics, sporadic retinoblastomas have two-hit kinetics
 - i. Hit one – mutation of one allele of the RB gene
 - ii. Hit two – loss of the remaining normal allele, often by non-disjunctional loss of c13 at mitosis (also lost in 40% of other Ca)
2. Epigenetic change – methylation of the gene promoter → transcriptional silencing

More clinical examples – cancer cells and the **restriction point**:

1. RB gene products exist in hypo- and hyper-phosphorylated forms
 - a. Hypophosphorylated pRb exists in G1 and G0
 - b. Hyperphosphorylated ppRb exists in the rest of the cell cycle
 - c. Theory is that pRb prevents progression through the R point by binding to transcriptional activators – conversion to ppRb prevents inhibition
2. Mantle cell lymphoma
 - a. BCL-1 gene encodes cycD1, which is over-expressed in many cancers.
 - i. CycD1 activates Cdk4 or Cdk6 (in G1) – substrate for both is pRb
 - ii. Hypophosphorylation of pRb → binding/inhibition of several transcriptional activator proteins
 - iii. Phosphorylation of pRb by CycD-Cdk4 → release of transcriptional activators (e.g. E2F) → CycE
3. Growth factor signalling pathways
 - a. ERBB1 encodes a transmembrane receptor for growth factors (including EGF and TGF α).
 - i. Binding → activation of intracellular tyrosine kinase → signalling molecules (e.g. RAS → CycD1)
4. Melanoma p16 – inactivated TSG gene in hereditary melanoma that is induced by E2F1 and acts as a negative feedback inhibitor of cdk4
5. Oncogenic DNA viruses – produce proteins that cause pRb phosphorylation → entry into the cell cycle and activation of DNA synthetic apparatus
 - a. Hepatitis B, human papilloma virus E7, Epstein-Barr, human herpesvirus-8

• Carcinogenesis

Fas is one of a family of death receptors that induce apoptosis when activated by ligands.

1. Fas → FADD → caspase 8 → caspase 3 → substrate cleavage → death
 - a. Negatively regulated by a decoy receptor (competes with Fas) and a protein called FLIP (prevents activation of caspase 3 by FADD)
2. Decoy receptor over-expression protects cancer cells against cytotoxic T cells, and blocks apoptosis (e.g. that induced by abnormal Myc expression)
3. Mutations in the Fas protein are seen in a proportion of bladder cancers
4. Viral FLIP (inhibitor of caspase activation) is produced by HHV-8 in Kaposi's sarcoma – this correlates with increased resistance to apoptosis in advanced lesions
5. Caspase 8 expression is lost in aggressive neuroblastomas with loss of sensitivity to ligands for death receptors

A family of **apoptosis regulators** are localised to intracellular membrane, particularly the outer mitochondrial membrane – here they regulate transfer of proteins such as pro-caspases and cytochrome C (process involves the anion transporter porin)

1. Anti-apoptotic – Bcl-2, Bcl-X_L close membrane pores
2. Pro-apoptotic – Bax, Bak open membrane pores
3. This type of death regulation is normal in lymphomas and many other cancers:
 - a. Glioblastomas – expression of a mutant EGFR allows aggressive growth by driving Bcl-X_L expression (inhibits apoptosis, resists chemotherapy)
 - b. Infectious mononucleosis (and B cell lymphomas in immunodeficient people) can be caused by EBV. Its latent membrane protein-1 induces Bcl-2 expression, inhibiting the apoptotic response.

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- c. Colon and stomach cancers often have Bax and Bak inactivating mutations

p53 (the 'guardian of the genome') was first found to be up-regulated in cells infected by certain tumour viruses – it has been now shown to be inactivated in 50% of all human cancers, and is the most frequently inactivated TSG known.

1. Inactivation of p53 is mediated by a number of mechanisms
 - a. Typically – a mutation of the first allele is followed by loss of chromatin containing the (normal) second allele, e.g. Li-Fraumeni syndrome
 - b. Connective tissue cancer – the hDM2 protein is overproduced and binds to p53 (→ inactivation and eventually destruction)
 - c. Oncogenic DNA viruses – target/inactivate p53, e.g. HPV, hepatitis B, HHV-8
2. Normal functions of p53:
 - a. DNA damage – p53 is induced and causes the self-elimination of cells that have potentially hazardous genetic damage
 - b. Inappropriate proliferation – if cells cycle uncontrollably (excessive Ras, Myc activity; loss of pRb), p53 → apoptosis
 - i. pRb+, p53+ → proliferation ceases, cells differentiate
 - ii. pRb-, p53+ → proliferation uncontrolled but transient → cell death
 - iii. pRb-, p53- → proliferation uncontrolled and continuous
 - c. Wild-type p53 binds to DNA and activates a genetic programme → DNA repair, cell cycle arrest or apoptosis
 - d. Intermittent hypoxia (like that seen in tumour development) induces p53 (hence → greater aggressiveness if p53 mutants are present)
3. Skin cancer – many oncogenes and TSGs are targeted, but p53 has a vital role:
 - a. p53 is induced following prolonged exposure to UVA/B (which causes potentially mutagenic DNA damage) → apoptosis → peeling sunburn
 - b. p53 may be mutated in very rare cases – normal sun-exposed skin will have clones of p53^{+/-} heterozygote cells
 - i. Further episodes of sunburn – heterozygotes apoptose less effectively than cells with both copies of p53 (hence the frequency of p53^{+/-} clones increases relatively)
 - ii. When the remaining allele is lost from a p53^{+/-} clone → severe loss of control of cell turnover (basal cell carcinoma)
 - c. In mice, DNA damage induces p53-mediated Fas and Fas ligand up-regulation. Loss of p53 → Fas ligand up-regulation in the absence of Fas
 - i. Sunburn keratinocytes will not self-eliminate but may be armed to destroy protective immune cells

Clinical applications of oncogene and TSG studies:

1. Early diagnosis – PCR products characterised for mutant or abnormally methylated genes (colon and bronchial tumours)
 - a. Mitochondrial mutations would increase sensitivity of this approach
2. Definitive diagnosis – CML is characterised by a t(9;22) in which most of the ABL gene is translocated into the breakpoint cluster region (BCR) gene → Philadelphia chromosome
3. Detection of residual disease – CML cells can be detected at a frequency of 10⁻⁶ to 10⁻⁵ using reverse transcription (primers for ABL and BCR sequences)
4. Prognostic variable – amplification of oncogenes is associated with poor outcome – e.g. p53⁺ Vs p53⁻ in breast cancer, epidermal growth factor (EGFR) in head & neck
5. Strategies for therapy – Bcr-Abl protein is a tumour-specific tyrosine kinase which makes cancer cells resistant to apoptosis – studies into molecular weight inhibitors that selectively inhibit this protein

• Tumour-Host Interactions

Benign and malignant solid tumours are comprised of neoplastic cells and a connective tissue framework derived from the host (the tumour stroma). The stroma is formed in response to signals from the neoplastic cells – which stimulate fibroblasts, macrophages and other cells to

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produce collagen fibres, connective tissue mucins and other matrix materials. It also contains new blood vessels and an immune cell infiltrate from the host.

Tumour invasion is a characteristic of nearly all malignant tumours – the lethal effects of these tumours are largely due to invasion and destruction of local tissues, and the ability to metastasise to distant sites where further damage occurs. It requires:

1. Attachment of the cancer cell to the basement membrane – laminin receptors and integrins are important (also in migration/adhesion to endothelial cells)
2. Proteolysis of the basement membrane with hydrolytic enzymes
3. Migration through the gap into surrounding connective tissues

Note that the loss of the basement membrane in malignancy is due to both a decrease in production of membrane components, and an increased degradation by hydrolytic enzymes.

Local spread is by direct invasion of surrounding tissues (note that some are more resistant), leading to firmness and fixation of the part. Further invasion may result in ulceration. Histological evidence of invasion is important, especially in determining the malignant nature of a tumour.

Metastasis is the process whereby a tumour spreads to distant sites via lymphatic vessels, blood vessels, and through body cavities (e.g. pleural and abdominal).

1. Steps:
 - a. Invasion of connective tissue matrix (stroma)
 - b. Invasion of blood and lymphatic vessels
 - c. Circulation of tumour cells
 - d. Invasion from blood vessels into tissues
 - e. Angiogenesis
2. Lymphatic spread occurs mainly in epithelial malignancies – tumour cells that invade a lymphatic vessel may colonise the nearest lymph node and continue to spread from node to node until they enter the circulation.
 - a. Prognosis may depend on lymph node invasion (e.g. breast, bowel, stomach)
3. Venous spread – direct route to distant sites
 - a. This is the main route of dissemination of sarcomas (lymphatic spread rare)
 - b. Involvement of the liver (stomach, large bowel) is due to the portal vein
4. Spread through body cavities
 - a. Usually in serosa-lined cavities, but also in the CSF (high grade gliomas)
 - b. Spread through mucosa-lined cavities has not been demonstrated
5. Spread by implantation – iatrogenic spread/implantation facilitated by surgery
6. Metastatic sites:
 - a. Lymph nodes – carcinomas, melanoma
 - b. Brain – carcinomas, melanoma (perineural spread of prostate cancer)
 - c. Bone – cancer of the breast, prostate, kidney, stomach
 - d. Liver – abdominal carcinomas, breast
 - e. Lung – most tumours

Angiogenesis is the first step in the development of a space-occupying solid tumour. Initially small groups of tumour cells exchange metabolites by diffusion, but growth larger than 1-2mm in diameter needs the ingrowth of new vessels.

1. Steps:
 - a. Secretion of tumour angiogenic factors
 - b. Local degradation of post-capillary basement membrane
 - c. Migration of EC to form a solid sprout
 - d. Lumen formation in the sprout by curvature of endothelial cells
 - e. Proliferation to lengthen the sprout
 - f. Fusion of pairs of sprouts to form loops
2. Tumour angiogenic factors – acidic/basic FGF, TNF- α , angiogenin
3. Anti-angiogenic factors – interferon- α , thrombospondin 1
4. Characteristics of the tumour vasculature:
 - a. Highly disorganised with variable flow (note shunts)

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- b. Hypoxia and necrosis develops in areas with poor supply or those furthest from the blood supply (more necrosis in high grade tumours)
- c. Cells may also become transiently hypoxic as blood flow stops and starts (due to high interstitial pressure)
- d. Hypoxic cells are more resistant to radiotherapy and chemotherapy
- e. Normal pre-existing vessels may be incorporated into the tumour mass
 - i. Loss of some innervation → altered physiological responses
 - ii. Enlargement to cope with increased flow

The **immune system** may be involved in killing tumour cells as they emerge (and become antigenetically detectible), or in suppressing tumour growth after treatment has reduced tumour load.

1. There is some debate of the exact role of the immune system:
 - a. Tumours are self-derived and unlikely to have great antigenic differences
 - b. Tumours more common in immunosuppressed individuals are mainly those of the lymphatic system (which is already normal) or those caused by viruses
 - c. Many tumours have immune cells infiltrating their substance or stroma
 - d. Degree of infiltration may correlate with improved prognosis (eg large bowel)
2. Tumour antigens have been identified on a variety of tumours
 - a. Many are not specific for tumour cells, but are still useful for stains/assays or as targets for antibodies or cytotoxic cells used in immunotherapy
 - b. However, specific tumour antigens may be involved in immunosurveillance, but this has only been demonstrated in experimental tumours.
 - i. Mechanism of tumour specificity:
 1. Mutation of a normal protein causing a change in antigenicity
 2. Mutation → binding to the class I MHC molecule
 3. Transcription of a normally silent gene
 - ii. MAGE-1 in human melanomas is found on 40% of melanomas, 20% of breast carcinomas and a number of other malignant tumours (lung carcinomas, gliomas, leukaemias)
 1. Expressed as a short peptide associated with the class 1 MHC molecule, eliciting a cytotoxic T cell response
 2. Also found in spermatogonia and healing skin wounds
3. Anti-tumour effector mechanisms:
 - a. Cytotoxic T lymphocytes – may be related to cells with antigens bound to class 1 MHC molecules, especially those that are virus-induced
 - b. Natural killer cells – stimulated by IL-2 to lyse tumour cells not detected by T cells (mechanism for specificity is unknown). Can lyse antibody-coated cells
 - c. Macrophages – activated by a range of stimuli (endotoxin, interferon gamma)
 - i. Activation → anti-tumour substances (oxygen metabolites, TNF-α)
 - ii. May kill by a direct contact mechanism similar to NK cells
 - d. Antibodies – potentially kill tumour cells by fixing complement and facilitating killing by macrophages and NK cells (type II hypersensitivity reaction)
4. Immunotherapy:
 - a. Adoptive cellular therapy – lymphocytes from the patient's blood or from the resected tumour are cultured with IL-2 in vitro and reinjected with more IL-2
 - b. Cytokine therapy – injected to stimulate immune cells, and to increase the expression of MHC molecules (e.g. interferon-α for hairy cell leukaemia)
 - c. Antibody therapy – investigated as agents for delivering cytotoxins or enzymes which induce local cytotoxin synthesis

Note – para-neoplastic syndrome in neuropathies, myopathies, acanthosis nigricans

• Spread of Malignant Tumours

Metastasis is an inefficient process – primary tumours may shed large numbers of cells into the circulation, but relatively few metastases form.

1. Angiogenesis – a primary mediator in the mouse model is vascular endothelial growth factor (VEGF). It is expressed widely in human cancers (e.g. liver cancer)
 - a. Induced in skin by treatment with promoter

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- b. Expressed constitutively at the premalignant papilloma stage
 - c. Further upregulated at the transformation to carcinoma
- 2. Detachment (loss of structural cohesiveness) occurs by reduced cell-cell adhesiveness and alterations in integrins that mediate cell adhesion to the BM
 - a. E-cadherin loss at adherens junctions is a marker of poorly differentiated carcinomas. These junctions may also be disassembled by phosphorylation of proteins by activated tyrosine kinase oncoproteins (e.g. EGFR)
 - b. The best in vitro correlation of malignancy is the proliferation in suspension
 - i. Integrins clustered at focal adhesions generate anti-apoptotic signals
 - ii. Activation of tyrosine kinase oncoproteins or over-expression of integrin-linked kinases suppresses detached cell apoptosis (anoikis)
- 3. Tissue penetration – intravasation and extravasation
 - a. Matrix metalloproteinases are a family of secreted or transmembrane proteins that degrade the ECM and BM. One domain maintains latency, and another ligates Zn^{+2} (required for activity)
 - i. Three classes of MMPs exist:
 1. Collagenases – fibrillar collagen
 2. Stromelysins – proteoglycans, glycoproteins
 3. Gelatinases – nonfibrillar denatured collagens
 - ii. Evidence for a role in metastases:
 1. MMPs are found where the BM has been breached
 2. The number of types of MMPs increases with progression
 3. Concentration increases with tumour progression
 4. Tissue inhibitors of MMPs suppress experimental metastasis
 5. Transfection of MMPs increases metastases
 - iii. MMPs may modify the environment of the metastatic site by:
 1. Releasing growth factors sequestered in the ECM
 2. Promoting angiogenesis
 - b. Plasminogen activators are associated with tumour spread
 - i. Urokinase-type plasminogen activator cleaves plasminogen to plasmin, which
 1. Degrades components of the ECM (fibronectin, laminin)
 2. Activates proenzyme forms of MMPs
 3. Activates/liberates growth factors (HGF, bFGF, TGF- β)
 - ii. UPA binds cyclically/transiently to vitronectin → molecular motor
- 4. Motility
 - a. Motility consists of a cycle of:
 - i. ECM proteolysis (mediated by proteinases at the edge of the cell)
 - ii. Pseudopod extension/attachment to macromolecular components
 - iii. Translocation of the whole cell body
 - iv. Detachment of the trailing processes of the cell
 - b. Stimulated by three classes of chemical signals:
 - i. Autocrine motility factors – e.g. hepatocyte growth factor → HGF receptor autophosphorylation → activation of Ras protein and protein kinase cascade. Two types of induced migration:
 1. Scattering of individual cells
 2. Cohort migration – spreading of intact sheets of cells
 - ii. ECM proteins can stimulate both
 1. Chemotaxis to part-degraded fragments of matrix proteins
 2. Haptotaxis to intact macromolecules (bound substrate)
 - iii. Host-secreted growth factors – ‘homing factors’
- 5. Platelet adhesion – thrombospondin acts as a bridge between tumour/platelet integrins. Possible effects of platelet binding includes:
 - a. Protection of tumour cells from NK cell mediated lysis
 - b. Enhancement of tumour cell attachment to ECM
 - c. Enhanced release of 12(S)-HETE → endothelial cell retraction, extravasation
- 6. Homing to organs is determined by the blood flow from the primary tumour, and the extent to which the new location can provide for colonisation. Bone is a source of:
 - a. Chemotactic factors – collagen (I) fragments, growth factors (TGF- β , PDGF)
 - b. Cues for attachment – vitronectin receptor ($\alpha_v\beta_3$ integrin)

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Metastasis is mediated by a number of factors, but driven by classical oncogenes. Ras → activation of the activator protein-1 complex → transcription of many genes:

1. Angiogenic proteins – VEGF
2. Proteinases – MMP-3, MMP-7, uPA, cathepsin L
3. Proteins involved in cell-cell interactions – osteopontin

OTHER ASPECTS OF GENERAL PATHOLOGY

• Effects of Ionising Radiation (wr.wilson@auckland.ac.nz)

Carcinogenesis – chemicals > viruses > radiation (UV 3%, ionising 1-5% total cancers)

Types of biologically active radiation:

1. Non-ionising electromagnetic radiation
 - a. ELF electromagnetic fields (<300Hz)
 - b. RF (0.3-30MHz) and microwave (30MHz-300GHz)
 - c. UV light (200-300nm, 5-10eV, 10^{15} Hz) – excitation of pyrimidine bases (DNA)
2. Ionising radiation (>10eV) – ejection of an orbital electron
 - a. Types:
 - i. Particulate – electrons, neutrons, α particles, pi mesons
 - ii. Electromagnetic – X and gamma rays
 - b. Linear energy transfer – energy transferred to the medium per unit track length of the ionising particle.
 - i. High LET radiation (most particulate) has straight tracks with a dense column of ionising events – e.g. α particles
 - ii. Low LET radiation – non-uniform tracks (sparse ionisation events) with clusters due to ejection of an electron with sufficient energy to cause subsequent ionisation events

SI Unit of absorbed dose – Gray (Gy) = 1 J/Kg = 100 rads

Unit of dose equivalent – Sievert (Sv) = Gray x RBE (relative biological effectiveness)

Human LD₅₀ for whole-body irradiation is around 4Sv (8Sv with medical intervention)

Ionising radiation delivers small amounts of energy in large quanta – the individual random ionisations (hits) can inactivate critical biological structures (structures).

Medical significance of ionising radiation exposure:

1. Background (low dose, low dose rate) exposure – 2.1mSv/year NZ, 3mSv/year world
 - a. Main source is radon – $^{222}\text{Rn} \rightarrow ^{218}\text{Po}$ (binds to dust → lungs)
 - b. Risk estimate is 3-5 excess fatal cancers per 100 person-Sv
 - i. Hence ~0.5% will develop a fatal cancer, which is ~2% of all fatal cancers (consistent with epidemiological data)
 - ii. Based on high dose, high dose rate exposure using a zero threshold linear extrapolation model, with correction for dose rate effects
 - c. Note that there is a small risk of inducing fatal cancer whenever using ionising radiation in medicine – there is no threshold
2. High dose, whole body exposure (accidents, nuclear war)
 - a. Stochastic effects due to DNA damage and chromosome breakage
 - b. Non-stochastic effects due primarily to cell death
 - i. Threshold is only because a certain number of cells must die before clinical symptoms present – the underlying process is stochastic
3. High dose, partial body exposure (radiotherapy)
 - a. Killing of tumour cells
 - b. Damage to normal tissues in the radiation field, particularly haemopoetic and gastrointestinal. Late effects in most tissues due to vascular endothelium damage and chronic inflammation/fibrosis

Effects of ionising radiation on individual cells:

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1. Activation of radiation-response genes – triggered by DNA/membrane damage
2. Cell division delay – at the G2 and G1 phase checkpoints
3. Mutation – germ line and somatic cells
4. Cell killing:
 - a. Apoptosis – programmed cell death (particularly lymphocytes, Sertoli cells)
 - b. Reproductive (post-mitotic) cell death – only significant for proliferating tissues. 'Dead' cell may maintain structure/function, but cannot divide.

Mechanism of stochastic effects (mutation and post-mitotic cell death)

1. Radiation giving rise to DNA free radicals → single strand breaks in DNA
 - a. Direct – direct ionisation of DNA
 - b. Indirect – formation of reactive radicals by radiolysis of water → ionises DNA
 - c. Damage fixation – irreversible oxidation of DNA free radicals to a form that cannot be repaired via rapid reduction by thiols (especially glutathione)
 - d. Enzymatic DNA repair – acts slowly on DNA breaks rather than the radicals
2. Clustering of ionisation events → single strand breaks on opposite strands
 - a. Within a few base pairs → double strand break → clastogenesis
3. Misrepair – especially if there are multiple double strand breaks
 - a. Non-reciprocal translocations (→ post-mitotic cell death)
 - i. Acentric → micronucleus, dicentric → anaphase bridge
 - b. Reciprocal translocations (e.g. Philadelphia chromosome)
 - i. May activate a proto-oncogene or inactivate a tumour suppressor gene (→ carcinogenesis)
4. Observations/evidence:
 - a. Hypoxic cells resistant to ionising radiation – O₂ sensitises, thiols protective
 - b. Proliferating cells are more radiosensitive (greater reproductive cell death)
 - i. Note indirect damage via endothelial cell damage
 - c. G2 checkpoint is important
 - d. Shape of dose-response (log) curves for cell death, mutagenesis, aberrations
 - i. High LET is stochastic (linear) – single particle track creates multiple sublesions (DNA double strand breaks) → aberrant chromosome
 - ii. Low LET is non-linear – at high doses the probability that sparse sublesions will interact to form an aberrant chromosome increases

• Disorders of Immunity

Hypersensitivity is an excessive and injurious response to an exogenous antigen (which may be harmless). It can be used to describe various immune mechanisms that harm the individual, including transplant rejection and autoimmune diseases.

1. Type I – systemic/local anaphylaxis
 - a. Mast cell release of histamine (→ oedema), proteases, chemotoxins, cytokines, arachidonic metabolites
 - b. Degranulation induced by antigen binding, C3a, C5a, opiates, temperature
2. Type II – transfusion reaction, immune thrombocytopenic purpura, drug reactions, erythroblastosis fetalis
 - a. IgG fixes complement (direct lysis), attack by macrophages and NK cells
3. Type III – arthritis, glomerulonephritis, serum sickness
 - a. Immune complexes in tissues activate and fix complement
4. Type IV – contact dermatitis, granulomatous inflammation
 - a. T-cell mediated cytotoxicity
 - b. Granulomatous inflammation – epithelioid and giant cells

Autoimmune diseases are classified into two major groups, those affecting a single organ and those that are systemic (connective tissue or collagen diseases). Note that there is some overlap among systemic diseases (SLE and rheumatoid arthritis), and some single organ diseases may occur together (Schmidt's syndrome – Addison's + Hashimoto's + IDDM).

1. Criteria for autoimmune disease:
 - a. An autoimmune tissue reaction is present
 - b. This reaction is primary

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- c. No other cause of tissue injury is present
- 2. Mechanisms involved in autoimmune diseases:
 - a. Loss of self tolerance (failure of clonal deletion, failure of T-cell suppression)
 - b. Antigen modification
 - c. Cross-reaction with infectious agent
 - d. Emergence of sequestered antigen
 - e. Genetic factors (mainly class II MHC)
- 3. Examples:
 - a. Systemic lupus erythematosus is a multisystem autoimmune disease.
 - i. Aetiology:
 - 1. Failure of self-tolerance – antinuclear antibodies (unknown mechanism), toxic blood antibodies (type II hypersensitivity)
 - 2. Genetic factors – some early complement deficiencies
 - 3. Non-genetic factors – UV light, drugs, sex hormones
 - ii. Pathogenesis:
 - 1. Type III hypersensitivity – glomerulonephritis, vasculitis
 - 2. Type II hypersensitivity – antibodies against blood cells
 - iii. Pathology:
 - 1. Kidney – glomerulonephritis (diffuse proliferative)
 - 2. Skin – macular papular erythematous lesion
 - 3. Joints – arthralgia, destructive arthritis (uncommon)
 - 4. CNS – damage via vascular occlusion, vessel damage from anti-phospholipid antibodies (lupus anticoagulant)
 - iv. Clinical features – rash, fever, joint and pleuritic chest pain with waves of remission. Corticosteroids and immunosuppressants usually decrease activity. Deaths (30% over 10 yrs) are mainly due to renal failure, infections or diffuse CNS disease)
 - b. Sjogren's syndrome – keratoconjunctivitis sicca and xerostomia, may be primary (sicca syndrome) or secondary (to rheumatoid arthritis or SLE)
 - i. Aetiology and pathogenesis:
 - 1. Glandular destruction with infiltration of T helper and cytotoxic T cells – localisation to salivary glands unexplained
 - 2. Autoantibodies, rheumatoid factor, antinuclear antibodies
 - 3. Antibodies to ribonucleic proteins are useful diagnostically
 - ii. Pathology:
 - 1. Salivary glands – lymphocyte infiltration → fibrosis, ulceration/inflammation of cornea, conjunctiva, oral mucosa
 - 2. Extra-glandular – kidney may show interstitial nephritis
 - iii. Clinical features – chronic and progressive, but does not decrease life expectancy to the extent of SLE. Lymphocytic infiltration predisposes to lymphoma (40x risk).
 - c. Scleroderma – characterised by fibrosis of many organs (skin, GI tract, heart and lungs). Intrarenal artery fibrosis → malignant hypertension, renal failure
 - i. Aetiology and pathogenesis:
 - 1. Immunologic theory – T cells stimulate fibroblasts → collagen
 - 2. Vascular theory – intimal fibrosis in arteries
 - ii. Pathology and clinical features:
 - 1. Skin – increase in collagen (extends into SC fat, replaces muscular tissue of various organs), SC calcification
 - 2. Vessels – lymphocytic infiltrates, intimal thickening
 - 3. Hands and face most affected – claw fingers, mask face
 - 4. Raynaud's syndrome → fingertip ulceration and gangrene
 - d. Inflammatory myopathies – dermatomyositis, polymyositis
 - i. Proximal muscle weakness – lymphocyte/macrophage infiltration
 - ii. Lilac discoloration of the upper eyelids, periorbital oedema

Vasculitis refers to (primary) inflammation of the vessels – this may occur without existing tissue damage and mediates tissue damage.

- 1. Pathogenesis seems to involve hypersensitivity reactions – initiating factors unknown
 - a. Type III hypersensitivity – immune complex deposition within vessel walls

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- b. Type II hypersensitivity – anti-BM antibodies (Goodpasture's syndrome), anti-endothelial antibodies (Kawasaki disease)
 - c. Anti-neutrophil cytoplasmic antibodies – perinuclear (p-ANCA) and cytoplasmic (c-ANCA) → myeloperoxidase in neutrophil granules
2. Examples:
- a. Large vessel vasculitis
 - i. Giant cell (temporal) arteritis, over 50 – inflammatory damage with giant cells on the IEL. Retinal artery involvement → blindness
 - 1. Responds well to corticosteroids
 - 2. Headaches, tenderness over temporal arteries, facial pain
 - 3. Associated with polymyalgia rheumatica
 - ii. Takayasu's arteritis (pulseless disease), females 15-40 – granulomatous inflammation of vessels including the aorta
 - b. Medium vessel vasculitis
 - i. Kawasaki syndrome (mucocutaneous lymph node syndrome), children and infants
 - 1. Acute presentation with fever and erythema of conjunctiva, mouth, hands and feet → skin desquamation with cervical node enlargement
 - 2. 20% develop vasculitis of the coronary artery, 2% fatally
 - 3. Beings in the intima → fibrinoid necrosis with inflammation
 - ii. Polyarteritis nodosa (PAN) – fibrinoid necrosis of arteries of kidney/viscera, particularly young adults
 - 1. Systemic – pyrexia, weight loss, progressive hypertension
 - 2. Alimentary tract – abdominal pain, nausea, vomiting, melaena, occasional perforation
 - 3. Nerves – peripheral neuropathy, mononeuritis
 - 4. Other – hepatitis B antigenaemia, positive p-ANCA, segmental arteritis (kidney and skin)
 - 5. Responsive to corticosteroids, cyclophosphamide
 - c. Small cell vasculitis
 - i. Wegener's granulomatosis, middle-aged adults – necrotising respiratory tract granulomas, necrotising vasculitis, necrotising focal glomerulonephritis. Responds to corticosteroids/cyclophosphamide.
 - ii. Churg-Strauss syndrome (allergic granulomatosis and angiitis) – vascular lesions, bronchial asthma, eosinophilia
 - 1. Skin, peripheral nerves, lungs. Renal involvement rare.
 - 2. p-ANCA positive in 75% of patients
 - iii. Microscopic polyangiitis
 - iv. Leukocytoclastic vasculitis – Henoch-Schonlein purpura, essential cryoglobulinaemia, allergic vasculitis, serum sickness vasculitis, lupus vasculitis, hepatitis B microscopic polyarteritis

Amyloidosis is the deposition of an abnormal extracellular material between cells in various tissues, leading to pressure atrophy or loss of function. The two main types are sequelae of extensive prolonged inflammatory activity (secondary amyloidosis) or due to excessive production of plasma cell derived immunoglobulin light chain (primary amyloidosis).

1. Amyloid appears microscopically as non-branching fibrils (95%). These are derived from proteins with a β -pleated sheet configuration (stable, digestion-resistant). The remaining 5% is composed of P component (glycoprotein) → starch-like features.
2. Classification is based on whether deposition is systemic or localised, then on the clinical associations and type of protein the amyloid is derived
3. Primary (immune cell) amyloidosis
 - a. May complicate plasma cell neoplasms (e.g. multiple myeloma) – monoclonal gammopathy (M band) seen in serum
 - b. Large amounts of immunoglobulin → isolated light chains (Bence-Jones protein) in serum and urine. May precipitate in renal tubules or as amyloid in glomeruli and arteries
4. Secondary amyloidosis

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- a. Incidence has decreased via control of tuberculosis, bronchiectasis and osteomyelitis. Usually due to non-infective inflammation and inflammatory bowel disease.
- b. Occurs in 3% of patients with rheumatoid arthritis
5. Pathology – enlarged, firm, rubbery, waxy organs with affinity for Congo red
 - a. Kidneys – normal/enlarged, sometimes smaller due to vascular occlusion and atrophy, deposition in glomeruli, arteries, arterioles and interstitium. BM deposition → nephrotic syndrome → renal failure
 - b. Spleen – granular deposition in follicles (sago spleen) or red pulp deposition (lardaceous spleen)
 - c. Liver – deposition in the space of Disse, few clinical effects
 - d. Heart – deposition between fibres → restrictive cardiomyopathy, subendocardial deposition common with age
6. Clinical features:
 - a. Nephrotic syndrome common, cardiac less common
 - i. Proteinuria (>4g/day)
 - ii. Hypoalbuminaemia
 - iii. Oedema
 - b. Gastrointestinal – enlargement of the tongue, or malabsorption and diarrhoea if the intestines are involved
 - c. Diagnosis – biopsy, cytological examination of abdominal fat aspirate
 - d. Prognosis poor – mean survival 2 years (slightly longer for secondary)
 - e. Alkylating agents may be useful in secondary amyloidosis to stop deposition