

## Basic Science Notes

Stimulation of  $\alpha$  receptors leads to:

Vasoconstriction

decrease in gut motility

uterus contraction

decrease in pancreatic exocrine secretion

**Cholera toxin:** activates G protein, which activates *adenylate cyclase*. Elevated cAMP results in unrestricted chloride secretion from villous crypts.

**Iron** is absorbed in upper small intestine. Iron absorption is increased with ascorbic acid. The sulfate form contains more elemental iron per dosage unit than gluconate.

The list of *autosomal dominant* disorders are:

achondroplasia

antithrombin III deficiency

C1 esterase inhibitor deficiency

Ehlers-Danlos syndrome

Familial hypercholesterolaemia

Gilbert's disease

hereditary haemorrhagic telangiectasia

hereditary elliptocytosis,

hereditary spherocytosis

Huntington's disease

idiopathic hypoparathyroidism

intestinal polyposis

marble bone disease

Marfan's syndrome

neurofibromatosis

Peutz Jegher's syndrome

polycystic kidney disease (adult)

protein C deficiency

osteogenesis imperfecta

Treacher Collins syndrome

tuberous sclerosis

Von Willebrand's disease

The list of *autosomal recessive* conditions are:

oculocutaneous albinism

alkaptonuria

Bartter's syndrome

cystic fibrosis

endemic goitrous cretinism

galactosaemia

Gaucher's disease

glycogen storage disease  
homocystinuria  
phenylketonuria  
Sickle cell disease  
Wilson's disease  
xeroderma pigmentosa

The features of **X linked inheritance** are:

Males are all affected  
Females only occasionally show mild signs of disease  
Each son of a carrier a 1:2 chance of being affected  
Each daughter of a carrier has a 1:2 risk of being a carrier  
Daughters of affected males will all be carriers  
Sons of affected males will be affected

**X linked dominant (rare)**

X linked Hypophosphataemic rickets  
von Willebrand's disease

**X linked recessive**

Becker's muscular dystrophy  
Duchenne's muscular dystrophy  
Fabry's disease  
Hunter's syndrome  
Glucose 6 Phosphate dehydrogenase deficiency  
red-green colour blindness  
haemophilia A and B  
Testicular feminisation syndrome  
Wiskott Aldrich syndrome

**Mitochondrial DNA** is inherited from the mother. Mitochondrial DNA codes for proteins in the oxidative phosphorylation / electron transport chain. Leber's optic atrophy is a form of mitochondrial disease.

**DNA**

Bases are paired with hydrogen bonds but the ribose-phosphate complexes are paired with covalent bonds. Histones allow DNA to twirl round it to form stable nucleoprotein complexes.

Coding sequences (exons) are interspersed with introns.

In **RNA**, uracil represents the thymine residues in DNA.

**mRNA** translation into proteins occur in the cytoplasm. *RNA polymerase II* transcribes mRNA

**Transfer RNA** (tRNA) is a small RNA chain (74-93 nucleotides) that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein

synthesis during translation. It has sites for amino-acid attachment and *codon* (a particular sequence of 3 bases) recognition.

The codon recognition is different for each tRNA and is determined by the *anticodon* region, which contains the complementary bases to the ones encountered on the mRNA. Each tRNA molecule binds only one type of amino acid, but because the genetic code is degenerate, more than one codon exists for each amino acid.

Both coding (exons) and non coding regions (introns) exist on RNA. Reverse transcription involves transcription of RNA to DNA (used by retroviruses).

Only about 5% of DNA codes for proteins.

Multiple codons (triplets of nucleotides) code for the same amino acid.

**Antisense oligonucleotides** are sequences of DNA which are complementary to RNA. They bind to RNA and stops it from processing.

## PCR

In PCR, two primers are required for a start and stop sequence to amplify the DNA strand.

*DNA polymerase* needs to be stable at high temperatures and hence thermostable enzyme from *T. aquaticus* is used. The mixture is heated to below 100°C.

*DNA polymerase* causes synthesis of DNA between two primers

*Reverse transcriptase* PCR is used to amplify **RNA**, whilst conventional PCR is used to amplify DNA

**Restriction enzymes** cut DNA at nucleotide sequences specific to each restriction enzyme. HindIII and EcoRI are examples of restriction enzymes

**DNA ligase** and **polymerase** are involved in joining and linking DNA together.

**Plasmids** are circular molecules of bacterial DNA separate from the bacterial chromosome. They are usually small, consisting of a few thousand base pairs. They carry one or a few genes and have a single origin of replication.

A **Barr body** is an inactivated X chromosome.

The human karyotype consists of 22 pairs of autosomes and 1 pair of sex chromosomes totalling 23 pairs altogether.

**Heterochromatin** contains mostly inactivated genes.

**Telomeres** are distal extremities of chromosomal arms but **centromeres** provide a point of attachment for the mitotic spindle.

**Northern blotting** detects RNA

**Southern blotting** detects DNA.

**Western blotting** can be used to detect and quantify proteins

The **karyotype** 46 XX, t (4;8)(q26;p21.3) describes a female with a normal number of chromosomes but a translocation between the long arm of chromosome 4 (q) and the short arm of chromosome 8 (p).

**Genomic imprinting** refers to the difference in phenotypic presentation depending on the origin of the disease chromosome from either maternal or paternal.

Conditions with **genomic imprinting**:

- myotonic dystrophy
- Beckwith-Wiedemann syndrome
- Prader Willi syndrome
- Angelman syndrome

**Anticipation** refers to increase in the number of trinucleotide repeats

The list of **trinucleotide repeat disorders** are:

Fragile X  
Huntington's chorea  
Myotonic dystrophy  
Friedreich's ataxia  
Spinocerebellar ataxia  
Spinobulbar muscular atrophy

Diseases which have **mitochondrial inheritance** (passed on by the mother):

- Kearns Sayre's
- MELAS
- MERRF
- progressive external ophthalmoplegia
- Leber's optic atrophy

There are **cyclins** at various phases of the cell cycle (G1 to S phases) which can bind to enzymes such as threonine and serine kinases (also known as cyclin dependent kinases). These regulate the progression of the cell cycle.

**Eukaryotes** (higher organisms) have multiple chromosomes in a genome which is separated from the rest of the cell by a nuclear membranes.

**Prokaryotes** lack a membrane bound nucleus, their DNA occurs in a circular form.

### **Oncogenes**

Proto-oncogenes and oncogenes encode growth factors. A single aa mutation is enough to change the proto-oncogene into an oncogene.

Mutated proto-oncogenes that cause cancer are called oncogenes.

Examples of oncogenes are:

**Ras** oncogene is involved in sporadic tumours (colon and lung) and rhabdomyosarcomas.

**c-myc** translocation occurs in Burkitt's lymphoma.

**N-myc** proto-oncogene is seen in neuroblastoma.

**SRC** oncogene is associated with sarcoma.

\**Ras* is the commonest oncogene (others include *myc*, *fos* and *jun*)

Fas ligands and caspases trigger apoptosis. Bax, Bad and Bak are members of the oncogenes which promote cell death.

**Bcl-2** *inhibits* apoptosis.

Bcl-2 prevents cell death by blocking apoptosis, preventing p53 mediated cell destruction and prevent cell death.

**Tumour suppressor genes** examples (different from oncogenes):

NF-1 gene in neurofibromatosis

BRCA-1 in breast and ovarian cancer

Rb gene in retinoblastoma

VHL gene in von Hippel Lindau

P53 – Li Fraumeni syndrome

**p53** : The p53 Gene is a tumor suppressor gene. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome. Li Fraumeni syndrome predisposes patients to breast cancer and sarcoma. p53 is also a transcription factor and is not found only in malignant cells. It has a role in downregulating cell division and apoptosis.

**Fragile X syndrome** is typified by delayed mental and motor development with associated seizures. Phenotypic features are prognathism, long face, high arched palate, large ears, cryptorchidism, hypotonia and mitral valve prolapse.

**Marfan's syndrome** is an autosomal dominant condition associated with ocular abnormalities such as upwards lens dislocation and retinal detachment. Aortic regurgitation may occur due to aneurysmal dilatation a feature. Upper to lower body ratio (head- symphysis pubis : Symphysis pubis- toes) is decreased in Marfan Syndrome.

**Homocystinuria** is an autosomal recessive disorder. Reduced activity of *cystathionine synthase* results in accumulation of homocysteine methionine. Osteoporosis and osteopetrosis are also seen in homocystinuria.

In pre renal failure / **Acute Tubular Necrosis** (hypovolaemia, dehydration), the kidney attempts to retain sodium, hence urine sodium is <20mmol/l.

The **anion gap** is normal ( $\text{Na} + \text{K} - \text{Cl} - \text{Bicarb}$ ) - normal range is 10 - 16.

**Serum osmolality** is estimated by the formula :  $[2 (\text{Na}) + \text{urea} + \text{glucose}]$ .

**Myosin** is involved in striated muscle contraction.

It forms filaments in a hexameric array of 2 heavy chains and 2 pairs of light chains.

Myosin heavy chain mutation is implicated in familial hypertrophic cardiomyopathy. HOCM and Carney complex are forms of myosin chain disorders.

### **Enzyme deficiencies**

Gaucher's disease – Glucocerebrosidase deficiency  
Tay Sachs disease - Hexosaminidase A deficiency  
Niemann Pick disease - Sphingomyelinase deficiency  
Metachromatic leukodystrophy - Arylsulphatase A deficiency  
Hurler's syndrome - Iduronidase deficiency

**Fabry's disease** is an X-linked lysosomal storage disorder. It is caused by a deficiency of *alpha-galactosidase A*. Ceramide accumulation occurs in various organs including the heart, skin and nerves. The skin lesion is known as angiokeratoma corporis diffusum.

**Gaucher's disease** is associated with the enzyme *glucocerebrosidase*. As a result, glucocerebroside accumulates, principally in the phagocytic cells of the body but also sometimes in the central nervous system neurones.

**Maple syrup urine disease (MSUD)** is an aminoacidopathy secondary to an enzyme defect in the catabolic pathway of the branched-chain amino acids leucine, isoleucine, and valine. Accumulation of these 3 amino acids and their corresponding keto acids leads to encephalopathy and progressive neurodegeneration in the infant who is not treated for MSUD.

**Causes of Retinitis pigmentosa** are:

Kearn Sayre's syndrome  
Usher's disease  
Refsum's disease  
Lawrence Moon Biedl syndrome  
Alports's syndrome  
Friedrich's ataxia

### **SIGNAL TRANSDUCTION**

G protein receptors are 7 transmembrane spanning receptors. Examples are:

- Muscarinic Ach receptor
- Adrenergic receptor (adrenaline)
- Retinal rhodopsin receptor
- FSH
- TSH
- ACTH
- GABA

**G protein second messenger** abnormality is found in:

Cholera  
pituitary adenoma  
McCune Albright syndrome

Albright's hereditary osteodystrophy (pseudohypoparathyroidism)

Examples of tyrosine Kinase receptors:

- Insulin
- PDGF
- VEGF
- Epidermal Growth Factor
- IGF-1
- Macrophage colony stimulating factor

## IMMUNOLOGY

### Complements

C3 is activated by classical and alternative pathways.

#### Classical pathway

C1 (q,r,s), C2, C3, C3 are components

C43b cleaves and activates C3

Activated by Ab-Ag complexes

#### Alternative pathway

C3, factor D, factor B, properdin are components

C3bBb activates C3

Activated by endotoxin, bacterial cell wall

#### Membrane Attack Complex

Helps to penetrate cell membranes, leading to lysis

**Neisseria** infection leading to meningococcal meningitis often occurs in patients with complement deficiencies of C5-9.

Complements C5, C6, C7, C8, C9 form the Membrane Attack Complex.

C3a - mediates inflammation

C3b - cleaves C5, opsonises, activates alternative pathway

C5a – chemotaxin, mediates inflammation

**Cell Adhesion Molecules** are important regulatory molecules in inflammation and cell signalling/migration. ICAM-1 which is widely expressed is also implicated in ulcerative colitis and Crohn's

Rather than T cell receptors, **natural killer** cells express adhesion molecules which allow adhesion to target cells. CD94 receptor is located in Natural Killer (NK) cells and is bound to the cell membrane. It enables NK cells to discriminate between healthy cells and pathogen infected cells

**Phagocytes** have a role in recognition and elimination of microbes by reactive oxygen species or antigens by opsonisation

## T cells

**Cytotoxic (CD8) T cells** recognise MHC class I molecule antigens

**T helper (CD4) cells** recognise MHC class II molecule antigens.

**MHC class I** proteins are present on all nucleated cells. MHC class I molecules (rather than class II) allow self recognition.

**MHC class II** proteins are only expressed on dendritic cells, macrophages, B-cells as antigen presenting cells .

**B cells** usually require T cell help for full activation. B cells are activated in primary immune response which initially produce IgM. Deficiency of **CD40** impairs class switching. Most T cell antigens have repetitive epitopes (e.g.LPS/endotoxin), which are able to cross link to B cell surface immunoglobulin to activate these cells.

**IL-1** causes pyrexia and local inflammation. Recombinant forms of interleukin receptor antagonists (IL-1Ra) are useful as disease modifying drugs in rheumatoid arthritis.

**IL-2, IL-4, IL-5 and IL-10** are commonly produced by **T helper** cells. Antibody production is promoted. Immature dendritic cells are stimulated to mature by T helper cells.

**Tumour necrosis factor** can bind to either p55 or p75 receptors and activates nuclear factor kappa B (NFkB) transcription factor. It promotes interleukin activity and can induce apoptosis. TNF  $\alpha$  is procoagulant and can lead to thrombosis. TNF is produced by natural killer, T cells and macrophages. It enhances dendritic cell migration. It also increases adhesion molecule expression on vascular endothelium, allowing for increased permeability.

**IFN  $\alpha$**  and **IFN  $\beta$**  are antiviral cytokines. I

FN  $\gamma$  is produced by natural killer and T cells.

IFN  $\alpha$  and IFN  $\beta$  (not  $\gamma$ ) bind to the same receptor known as type I receptors. IFN  $\beta$  is used therapeutically in multiple sclerosis.

**IgG** is the commonest antibody in the serum. There are 2 light and 2 heavy chains, making a total molecular weight of 150,000 kDa. It comprises 2 Fab fragments, and is divalent rather than monovalent. The reference range is 8-20 g/l.

**Systemic Mastocytosis** is due to excessive mast cell stimulation. It leads to anaphylactic like states - urticaria, flushing and also GI symptoms such as diarrhoea and nausea. Mast cells are Ig E mediated but can be triggered by injury, drugs and complements.

It is caused by mast cell release of histamine in the skin or connective tissue.

Diagnosis can be confirmed by raised levels of *urinary N-methyl imidazole*, *blood eosinophilia* and *thrombocytopenia*. It classically presents with episodes of flushing, vomiting, diarrhoea and abdominal pain.

### **Hypersensitivity reactions**

Anaphylaxis is a type I reaction (atopic diseases).

Good pasture's syndrome and Coomb's positive haemolytic anaemia are type II reaction (antibody to antigenic components).

Serum sickness is a type III reaction (immune complex disease).

An example of type IV reaction is contact dermatitis or allograft rejection (T cell mediated response).

**Common Variable Immunodeficiency (CVID)** is a disorder characterized by low levels of serum immunoglobulins and an increased susceptibility to infections. A clear mode of inheritance is not defined (there are multiple modes) and there is a <10 % chance of passing on the disease.

**X linked** immunodeficiency include SCID and Bruton's hypogammaglobulinaemia.

**Prions** are glycoproteins which codes for a membrane protein. Prion proteins are usually resistant to protease digestion and are not broken down by heat. Prions do not contain genetic material. CJD, Kuru and scrapie are prion diseases. Prions are thought to have a role in cell signalling rather than DNA replication. Normal prion proteins are encoded by chromosome 20. PrP<sup>sc</sup> is characterised by increased  $\beta$  pleated sheets. PrP<sup>sc</sup> proteins are anchored by glycoproteins (GPI).

O<sub>2</sub>-Hb dissociation curve is **shifted to the right** when there is:

an increase in CO<sub>2</sub>

increase in hydrogen ions (fall in pH)

increase in temperature

increase in lactate

The curve is **shifted to the left** by

increased carboxyhaemoglobin

methaemoglobin

fetal haemoglobin.