4th SEM B.Sc. BOTANY

CALICUT UNIVERSITY

GENETICS & IMMUNOLOGY

2019 ADMISSION

Prepared by

Jasla Mol K.K & Jamshiya Jaithun C

Assistant professor

Department of Zoology

CPA College of Global of Studies, Puthanathani

FOURTH SEMESTER B.Sc. BOTANY COMPLEMENTARY COURSE

Theory Course- IV

GENETICS AND IMMUNOLOGY

Code: ZOL4C04T

[54 hrs] [3 hours/week] [2 credits]

COURSE OUTCOMES [COs]
COs Course Outcome statements
CO1 Describe human karyotype , chromosomal anomalies and polygenic inheritance
(6 hrs)
CO2 Explain the mechanisms of sex determination (4 hrs)
CO3 Enumerate the concept of genes, gene expression, genetic code, transcription
and translation (8 hrs)
CO4 Illustrate the mechanism of recombinant DNA technology and its practical
applications (13 hrs)
CO5 Explain the types of cancer, causes of transformation and characteristics of
transformed cells (5 hrs)
CO6 Identify the cells and organs of immune system, antigens and antibodies (7 hrs)
CO7 Enumerate antigen-antibody interaction, generation of B-cell and T-cell
response and major immunotechniques (7 hrs)
CO8 Explain primary and secondary immunodeficiency diseases, autoimmune
diseases, vaccination and vaccines (4 hrs)

Question paper pattern for external examination

[Module 1-5 Short answer 8x2=16 marks, Paragraph 5x5=25 marks, Essay 1x10=10 marks
Module 6-8 Short answer 4x42= 8 marks, Paragraph 2x5=10 marks; Essay1x10=10 marks]

Section A: GENETICS (36 hrs)

MODULE 1. Human Genetics (6 hrs)

Normal human karyotype: Classification and grouping of human chromosomes (Patau's & Denver schemes). Chromosomal anomalies and disorders (short note only). Autosomal anomalies: Phenyl ketonuria & Sickle cell anemia. X-linked – Haemophilia and Colour blindness. Y-linked – Y-Chromosome infertility. Polygenic inheritance - Cleft palate or Cleft lip and diabetes mellitus. Prenatal diagnosis. Genetic counselling. Eugenics, Euthenics and Euphenics**.**

[Short answers/Paragraphs/Essays]

MODULE 2. Genetic Control of Sex (4 hrs)
Autosomes and sex chromosomes: Mention Barr body and its significance. Chromosomal mechanism of sex determination: genic balance theory. Control of sex; hormonal influence of sex determination; sex mosaics; gynandromorphism
[Short answers/Paragraphs}

MODULE 3. Genes and gene expression (8 hrs)
Modern concept of genes, split genes, pseudogenes, overlapping genes and transposons. Gene expression. Genetic code, transcription and translation (brief account)
[Short answers/Paragraphs/Essays]

MODULE 4. Genetic Engineering (13 hrs)
Brief account of recombinant DNA technology – role of enzymes (restriction endonucleases, exonucleases, DNA polymerase, DNA ligase, reverse transcriptase, alkaline phosphatase, polynucleotide kinase and terminal transferase). Cloning vectors – plasmid vectors (mention pBR322), phage vectors, cosmids, viruses and YAC vector. Construction of recombinant DNA (preparation of vector DNA and donor DNA, joining of vector and donor DNAs, introduction of recombinant DNA into the host cell and selection of transformants). Methods of gene transfer. Practical applications, advantages and potential hazards.
[Short answers/Paragraphs/Essays]

MODULE 5. Cytogenetics of Cancer (5 hrs)
Types of cancer: brief account of sarcomas, carcinomas, melanomas, leukemia, lymphomas and blastomas. Characteristics of cancer cells: uncontrolled multiplication, loss of contact inhibition, metastasis, reduced cellular adhesion, metaplasia, invasiveness, growth factor secretion, cell surface alterations, alterations in transcriptome and proteome and protease secretion. Origin of Cancer: Carcinogens, oncogenic viruses, polygenic basis, hereditary predisposition to cancer
[Short answers/Paragraphs]

Section B: IMMUNOLOGY (18 hrs)
(Brief account of the following topics)

MODULE 6. Cells and organs of immune system, antigens and antibodies (7 hrs)
Cells and organs of immune system
Innate and adaptive immunity. Cells of immune system- B cell, T cell, NK cell and

Antigen Presenting Cells (dendritic cells, macrophage cells). Organs of the immune system- Primary lymphoid Organs (Thymus, Bone Marrow), Secondary lymphoid Organs (Spleen, lymph node, MALT)

Antigens

Antigenicity, Immunogenicity and Haptens. Factors influencing immunogenicity. Mention human immunoglobulin gene families – λ and κ light chain families and heavy chain family and major histocompatibility complex (MHC) group of genes.

Antibodies

Structure, different classes and Function. Monoclonal antibodies-Hybridoma technology and applications.

[Short answers/Paragraphs/Essays]

MODULE 7. Antigen-Antibody interaction & **Generation of B-cell and T-cell response (7 hrs)**

Antigen - antibody interaction

Strength of Antigen-Antibody interaction. Cross reactivity, Precipitation reactions, and Agglutination reactions. Immunotechniques – Detection of biomolecules using ELISA, RIA, and Western blot. Southern blot, Northern blot and DNA Fingerprinting (Brief)

Generation of B cell and T-cell response:

Humoral and cell-mediated response. Properties of B-cell and T-cell- epitopes. Activation and differentiation of B and T cells. Cytokines- brief

[Short answers/Paragraphs]

MODULE 8. Immunodeficiency diseases, **vaccines & vaccination (4 hrs)**

Immunodeficiency diseases

Primary (Bruton's disease, Di-george syndrome & SCID). Secondary types: AIDSMention Acute, Chronic and Crisis phase, Window period. Autoimmune disease-
Mention Hashimoto's thyroiditis, Grave's disease, Myasthenia gravis and Systemic Lupus Erythematosus.

Vaccines and Vaccination

Principle of vaccination; mention Attenuated vaccines, Inactivated vaccines, Toxoid vaccines and DNA vaccines.

[Short answers/Paragraphs/Essays]

Topics for Assignments/Seminars

(Topics allotted for assignments/ seminars should be considered for internal assessments only, and can be subdivided among students)

1. Human genome
2. DNA tumor viruses

3. Human genome project
4. Structure of immunoglobulins and T-cell receptors

REFERENCES

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MODULE 1 : HUMAN GENETICS

NORMAL HUMAN KARYOTYPE

- *Karyotype* : chromosomal characteristics of a cell
- It signifies the number , size , kind ,shape , structure and other morphological features of chromosomes
- *Karyogram/ideogram* : schematic representation of karyotype with the arrangement of chromosomes in numerical order
- Somatic number / diploid number: chromosome number of somatic cell
- Gametic / haploid number : chromosome number of gamete cell
- Somatic human cell : 46 chromosomes
- Autosomes and allosomes(sex chromosomes)
- *Normal karyotype of human female : 44 A + XX*
- *Normal karyotype of human male : 44 A + XX*

CLASSIFICATION & GROUPING OF HUMAN CHROMOSOMES

- The London system (Patau's system) of classification recognizes seven groups of human chromosomes.
- Considering their morphological features.
- They are groups from A to G.
- Chromosomes are arranged in the order of decreasing length.
- A group has the longest chromosomes.
- G-group has the shortest ones.
- **A** group includes the ***first three pairs of autosomes***.
- **B** group includes the ***4^{th} and 5^{th} pairs***.
- **C** group includes ***6^{th} - 12^{th} pair of autosomes & X chromosomes.***
- **D** group includes the ***13^{th} - 15^{th} pair of autosomes.***
- **E** group includes ***16^{th} - 18^{th} pair.***
- **F** group includes ***19^{th}& 20^{th} pairs***.
- **G** group includes ***21^{st}& 22^{nd} pairs of autosomes, and the Y-chromosome in male.***
- Groups A, E & F are metacentric chromosomes.
- B & C are sub-metacentric
- D & G are acrocentric.
- Autosomes 13, 14, 15, 21 and 22 (D & C groups) have secondary constriction and satellite. So they are ***'sat' chromosomes***.

CHROMOSOMAL ANOMALIES AND DISORDERS

A. DISEASES CAUSED BY CHROMOSOMAL MUTATIONS

- Chromosomal mutations are abnormal changes in the number and structure of chromosomes
- Numerical changes :ploidy changes.
- *Ploidy c*hanges bring about abnormal alterations in the karyotype, which in turn, cause serious genetic disorders.
- The commonest ploidy change is *aneuploidy* :involves the addition or deletion of individual chromosomes or chromosome pairs.
- 2 types of aneuploidy :
  - *Monosomy* : individual chromosome is lost from a *diploid set (2n-1)*
  - *Trisomy* : an individual chromosome is added to a *diploid set (2n+1).*
- Some of the genetic disorders caused by ploidy changes and abnormal karyotypes are the following:
  - Autosomal anomalies: *Down's syndrome, Edward's syndrome, Patau's syndrome, cri-du-chat syndrome.*
  - Sex chromosomal anomalies: *Klinefelter's syndrome, Turner's syndrome.*

B. SINGLE GENE MUTATIONS AND CONGENITAL DISORDERS

Gene mutations can be :

- *Autosomal*
- *Sex-Linked and Dominant or Recessive.*
- Single gene mutations are allelic mutations affecting one or both the alleles of a single gene.
- The disorders caused by them are inherited in the typical Mendelian fashion. So, the probability or chance of their occurence can be predicted in advance.
- Some autosomal recessive gene mutations interfere with the production of enzymes and there by disrupt normal metabolism : *Biochemical mutations.*
- Single gene mutations cause teratogenic development(abnormal and defective development), congenital malformations and disorders, inborn metabolic errors, and hereditary indispositions
- Some Single gene mutations are :
  - Diseases caused by autosomal recessive gene mutations:*Phenylketonuria, Alkaptonuria, Albinism, Galactosemia, Thalassemia, Sickle Cell Anaemia, Tay Sachs Disease, Gaucher's Disease, Microcephaly.*
  - Diseases caused by autosomal dominant gene mutations : *Achonodroplasia, Brachydactyly, Retinoblastoma.*

- Diseases caused by X-linked recessive gene mutations: *Haemophilia, Colour-Blindness, Lesch-Nyhan Syndrome*
- Diseases caused by X-linked dominant gene mutations: *Dermal Hypoplasia*
- Diseases probably caused by Y-linked gene mutations*: Ichthyosis Hystrix ,Gravis, Multiple Sclerosis.*

1. AUTOSOMAL RECESSIVE DISORDERS

PHENYL KETONURIA (PKU)

- Inborn metabolic disorder due to an autosomal recessive mutation.
- Caused by the non-production of the liver enzyme *phenylalanine hydroxylase* that converts *phenyl alanine* to *tyrosine.*
- Accumulation of excessive amounts of phenylalanine and its derivatives (phenyl pyruvic acid and phenyl lactic acid) in blood, cerebro-spinal fluid, and other tissues and their subsequent excretion through urine.
- The accumulation of phenyl pyruvic acid in cerebro-spinal fluid damages brain cells.
- Severe mental retardation and idiocy or imbecility (IQ below 25).
- Some of such unfortunate children will never be able to talk or walk.
- Reduced hair, poor skin pigmentation, irritability, tremors, convulsions,.

SICKLE CELL ANAEMIA

- Sickle-cell anaemia, or sicklemia, is a hereditary disease.
- Severe form of haemolyti canaemia.
- Fatal before puberty : girls
- Common among the Negro populations of Africa and America and also among the people of Mediterranean countries.
- In Kerala, it is found among the tribals of Wayanad District.

Characterized by ;

- Abnormal haemoglobin and sickle-shaped RBCs.
- The abnormal haemoglobin is called *sickle-cell haemoglobin or haemoglobin S (HbS).*
- It partially or almost completely replaces the normal haemoglobin (HbA).
- HbS differs from HbA in having the amino acid *valine* instead of *glutamic acid* in the 6^{th} position of its beta chains.
- In sickle cell anaemia patients (sicklers), the red blood cells become distorted to a crescentic or sickle shape : sickling of RBCs.
- Sickling occurs due to the polymerination of HbS molecules, under conditions of extreme oxygen insufficiency.
- Polymerization of HbS molecules produces pseudocrystalline structures, called *tactoids,* which distort the RBCs.

- HbS has only very low oxygen-binding powers.
- Sickle cells have only very short life span and they undergo heavy destruction in spleen.
- This causes severe haemolytic anaemia (sickle-cell anaemia), which is generally fatal.

Symptoms

- Enlarged spleen,
- Rheumatic complaints,
- Mental disorders,
- Hypoxia
- heart failure
- Renal failure

2. X- LINKED SEX CHROMOSOMAL DISORDERS

HAEMOPHILIA

- "*Bleeder's disease*" or "*royal disease",* is sex linked disease.
- Prolonged bleeding or oozing following an injury, surgery or having a tooth pulled.
- Serious complication can result from bleeding in to the joints, muscles, brain or other internal organs.
- Failure of the blood to clot easily and immediately is due to the deficiency or absence of plasma protein *clotting factor lll or Thromboplastin***,** which is essential for blood clotting.
- Occurs mostly males and very rarely among females.
- A male who is *hemizygous f*or that gene, will be a *haemophilic.*
- A female becomes *haemophilic* only when she is h*omozygous* for the mutant allele.
- Heterozygous female serve only as carriers of haemophilic.
- A girl becomes haemophilic, when she is born to a haemophilic father and a haemophilic or carrier mother.
- Expressed in alternate male generation, female mostly serving as carrier
- *Criss-Cross Inheritance. :* transferred from affected grand father to grand son through a carrier female and never direct like father to son
- 3 types :
    - Haemophilia A(classic Haemophilia)
    - Haemophilia B(Christmas disease)
    - Haemophilia C

- **Diagnostic method :** Blood test
- **Treatment :** Replacing missing blood clotting factor and Gene therapy is possible

HUMAN COLOR BLINDNESS

- *Daltonism*
- Inability to clearly distinguish different colors
- Diagnosis: *Ishihara test charts*
- X-linked recessive trait
- Results from the absence of appropriate color genes in the X-chromosome
- Associated with males ; females : carrier
- Hereditary defect ' exclusively restricted to cone cells
- 4 *types* ;
  - *Monochromatia :* total
  - *Tritanopia :* blue
  - *Protanopia :* red
  - *Deuteranopia* : green
- Common : red-green color blindness
- Follows *criss-cross pattern*: expressed in alternate male generations , females serves as symptom;ess carrier

3. Y-CHROMOSOME INFERTILITY

- Affects the production of sperm and causes male infertility
- Characterized by ;
  - *Spermatogenesis* : disrupts the ability to produce healthy sperm cells
  - *Azoospermia* : blocks the ability to even produce sperm cells
  - *Oligospermia* : defects that results in the very poor or low-quality sperms
- Caused by changes in Y-chromosome (deletion, duplication , insertion etc)
- In particular , genes in areas of Y-chromosome called azoospermia factor regions (AZF)
- Provides instructions for making proteins involved in the sperm cell production and development
- Y-chromosome infertility is caused by the deletions in the genes in the AZF regions

KLINEFELTER SYNDROME

- Trisomy : 47 (44 A + XXY)

Symptoms :

- small testicles
- Enlarged breasts
- Decreased facial and pubic hair
- Small penis

- Low sex drive
- Azoospermia and oligospermia
- Osteoporosis
- Language and speech problems
- Behavioural issues

CYSTIC FIBROSIS

- Single gene mutation
- Absence of part of tube (vas deferens)

4. POLYGENIC TRAITS

CLEFT PALATE/LIP

- Polygenic trait
- Congenital developmental defects (teratogenic defects).
- Cleft-lip may occur without cleft-palate.
- Rarely cleft-palate occurs without cleft-lip.
- Cleft-lip and cleft-palate are considered to be sex-controlled traits.
- Cleft-lip is more common among males than among females
- Cleft palate is more common among females.
- *Cleft-lip* - a wide split in the front part of the upper lip, due to the defective closure of the primary palate during embryonic development.
- *Cleft-palate* - an opening in the middle line of the palate, in between the oral cavity and the nasal cavities, due to the defective closure of the secondary palate during embryonic development.
- Can be corrected by surgery
- Cleft-lip and cleft-palate usually develop during the second or third month of foetal life.

DIABETES MELLITUS

- Excessive accumulation of blood sugar and subsequent excretion through urine
- Involves disorders in carbohydrate metabolism and abnormalities in lipid and protein metabolism due to deficiency of insulin
- *Hyperglycemia* : sugar accumulation
- Glucose excreted through urine : *glycosuria*
- Reasons for abnormal increase in blood sugar :
  - Insufficient cellular intake and glucose oxidation
  - Failure of liver to trap glucose

- Inability of liver to store glycogen
- 2 major forms :
  - *juvenile diabetes*
  - *Senile diabetes*

PRE-NATAL DIAGNOSIS OF GENETIC ABNORMALITIES

- Early detection of genetic abnormalities before birth
- Involves analysis of karyotypic , biochemical , physiological and morphological features of the foetus to detect chromosomal abnormalities and anomalies , metabolic disorders and morphological malformations

1. AMNIOCENTESIS

- Widely used , more than 30% of disorders can be detected
- Possible at any stage of pregnancy
- Foetal cells may appear floating in the amniotic fluid
- A sample of 10-20ml of amniotic fluid together with floating foetal cells is taken with the help of syringe
- Cells are cultured and multiplied in a medium and analysed their karyotype
- Biochemical analysis enable : to detect enzyme deficiencies and congenital metabolic disorders
- Structural abnormalities can be further detected by : *banding analysis*

2. CHORIONIC VILLUS SAMPLING (CVS)

- Carried during 8^{th} to 12^{th} weeks of pregnancy
- Involves the biopsy of chorionic villus tissue to detect the chromosomal anomalies , genetic defects ,biochemical disorders and metabolic errors
- Chorionic villus sample is collected (either by inserting a catheter in to the placenta through vagina or by inserting a long and thin cannula of a syringe into the placenta through abdomen
- Sample is subjected to biochemical analysis
- Advantages :
  - Culturing is not needed
  - Defect can be diagnosed in early stage

3. ALPHA FOETOPROTEIN (AFP) ESTIMATION

- Blood test done between 15^{th} and 18^{th} weeks of pregnancy
- AFP is a protein of foetal origin found in amniotic fluid
- High level : abnormalities

4. ULTRASONOGRAPHY

- Applied in the detection of internal organs and tumours and observation of foetus
- Makes use of high frequency sound waves to create an image of the structures
- Ultrasonogram uses intermittent sound waves

- Sonogram is used to record placental and foetal size , location ,age and maturity ,heart rate ,foetal abnormalities
- In computer-enhanced ultrasonography , clear and detailed images of internal organs would be displayed on the oscilloscope

GENETIC COUNSELLING

- Part of medical genetics
- *Provides information to a person about the probabilities and potential dangers of some congenmital disorders and birth defects*
- Primary purpose is to secure voluntarary restriction on child-bearing by high-risk couples , groups or populations with inherited genetic disorders
- Educates parents about the genetic cause and heritable nature of some diseases

EUGENICS

- Set of beliefs and practices that aim to improve the genetic quality of a human population ; historically by excluding inferior people
- Practice or advocacy of improving the human species by selectively mating people with specific hereditary traits
- Reduce human suffering from breeding out disease , disabilities and any undesirable traits
- Deals with race improvement through heredity
- Hygiene for future generations
- Must await careful investigation
- Genetic laws

EUTHENICS

- Study of improvement of human functioning and well-being by improvement of living conditions
- Betterment of living conditions , through conscious endeavor , for the purpose of securing efficient human beings
- Conducted by altering external factors like education and controllable environments ,parasites
- Race improvement through environment
- Hygiene for present generations
- Has immediate opportunity
- Precedes eugenics , developing better men now for the better race in the future
- Better environment

EUPHENICS

- Good appearance / normal appearing
- Symptomatic treatment of genetic disease
- Discipline that aims to improve the outcome of a genetic disease by altering environment
- Example : people with PKU can avoid expression of their diet by staying on a low phenyl alanine diet
- Science of making phenotypic improvements to humans after birth , generally to affect a problematic genetic conditions
- Deals with human race improvement through genetic engineering

MODULE 2 : GENETIC CONTROL OF SEX

SEX DETERMINATION

- Sex determination is the mechanism by which sex is established is termed as sex determination.
- There are various mechanisms operating in determining the sex of different organisms.
- Four major kinds of sex determining mechanisms are,

1. Chromosomal sex determining system
2. Genic balance system
3. Hormonal system
4. Environmental sex determination

- Sex differentiation is the process of the development of the differences between males & females from an undifferentiated zygote.
- Appearance of sertoli cells in males & granulosa cells in females can be thought as the starting point for testicular or ovarian differentiation in many species.

Sex chromosomes

- There are two kinds of chromosomes in organisms, namely ‘
  - ***Autosomes*** : Autosomes carry genes with vegetative characters
  - ***Sex chrormosome*** : carry the genes which control sexual characteristics
- Not the whole sex chromosomes which determine the sex, but only certain genes located in them determine the sex.
- There are two kinds of sex chromosomes, namely X chromsomes & Y chromosomes.
- X chromosome is found in both sexes, where as Y chromososme is found only in one sex.
- In mammals, *Drosophilla*, many plants, Y chromosome is found in males. But in reptiles, birds,.. it is found in females

AUTOSOMES
VERSUS
SEX CHROMOSOMES

AUTOSOMES	SEX CHROMOSOMES
Autosomes determine somatic traits	Sex chromosomes determine the gender
Males and females contain the same copy of autosomes	Different in males and females by their size, form, and behavior
Labeled with numbers, from 1 to 22	Labeled with letters as XY, ZW, XO and ZO
Most chromosomes within a genome are autosomes	Few chromosomes within a genome are sex chromosomes
22 pairs of autosomes are homologous in humans	Female sex chromosomes (XX) are homologous; male sex chromosomes (XY) are non-homologous
Position of the centromere is identical	Position of the centromere is not identical
Show Mendelian inheritance	Show Non-Mendelian inheritance
Contain the number of genes varying from 200 to 2000	X chromosome contains more than 300 genes;Y chromosome contains only a few genes

Chromosome mechanism of sex determination

The mechanisms fall under three main categories,

1. Female homogamesis & male heterogamesis

- XX female & XO male mechanism
- XX female & XY male mechanism
- XX female & XY1Y2 male mechanism.

2. Male homogamesis & female heterogamesis

- XX male & XO female (ZZ male & ZO female) mechanism
- XX male & XY female (ZZ male & ZW female) mechanism.

3. ***Male haploidy & female diploidy***.

1. XX female & XO male mechanism

- This is found in nematodes, spiders, cockroaches, grass hoppers,..
- Also called *Protenor system* because the system has been studied in detail in the bug *Protenor*.
- Female has a pair of X chromosome (XX) & male has a solitary one (XO).
- Chromosomal formula of female is 2A + XX & that of male is 2A + XO.
- During gamete formation each ovum gets an X chromosome where as 50% of sperms will get X chromosome & the remaining 50% will not get it.
- Thus female is homgametic & male is heterogametic.
- If the ovum is fertilized by an X chromosome bearing sperm, an XX will be the result, which give rise to female.
- If it is fertilized by an X chromosome lacking sperm, an XO will be the result, which give rise to male.

2. XX female & XY male mechanism

- It is common among animals.
- It if found in mammals, several insects, some fishes,…
- Here female is homogametic with 2A + XX chromosome complement & male is heterogametic with 2A + XY chromosome complement.
- All the eggs are X chromosome bearing & 50% of sperms are X chromosome bearing & remaining 50% are Y chromosome bearing.

- If the ovum is fertilized by an X chromosome bearing sperm, an XX will be the result, which give rise to female.
- If it is fertilized by a Y chromosome bearing sperm, an XY will be the result, which give rise to male.

3. ZZ male & ZW female mechanism

- Also called XX male & XY female mechanism.
- It is found in moths, butterflies, snakes, birds,…
- Here female is heterogametic with 2A + XY chromosome composition & male is hmogametic with 2A + XX composition.
- All the sperms are X chromosome bearing & 50% of ova are X chromosome bearing & remaining 50% are Y chromosome bearing.
- Fertilization of X bearing ovum results in XX male & that of Y bearing ovum results in XY female.

GENIC BALANCE THEORY

- It was formulated by Calvin Bridges based on his studies on *Drosophila*.
- It is the concept that both autosomes & sex chromosomes have an equal share in sx determination.
- Autosomes carry the genes for maleness , X chromosomes carry the genes for femaleness & Y chromosomes have no role in sex determination.
- A specific ratio between the amounts of male determining genes & female determining genes & a precise balance between their interactions determine the sex of the individual.
- If male determining genes have predominance over female determining genes in their quantity & intensity of action, the individual will be a male.
- If female determining genes have predominance over male determining genes, the individual will be a female.
- 2A + XO & 2A + XY individuals are males because the female tendency of a single X chromosome is not sufficient to override the double dose of the male determiners.
- 2A + XX individuals are females, because the double dose of the female tendency can suppress & mask the male tendency of the diploid set of autosomes.
- Sex index or sex determining ratio – The sex of an individual appears to be determined by the ratio between the number of X chromosomes & the number of haploid autosome sets.

Sex index = Number of X chromosomes (X) i.e., S = X

- Number of haploid set of autosome (A) A

- The strongest support of genic balance theory came from further studies by *Dobzhansky & Schultz* on *Drosophila*.
- They could reveal that in Drsophila the entire Y chromosome & the proximal one-third portion of the X chromosome were inert in sex determination & the distal two- third of the X chromosome carried the genes for femaleness.
- Genic balance theory is not applicable to instances in which Y chromosome has an active role in male- determination.

ENVIRONMENTAL INFLUENCE ON SEX DETERMINATION

Environmentally controlled sex determination has been observed in some annelids, arthropods, molluscs, rotifers & echiuroids.

Hormonal influence on sex determination

In higher animals especially in vertebrates, sex hormones play an important role in sex differentiation.

Sex in Honey Bees

- The mechanism of sex determination found in honey bees is male haploidy & female diploidy.
- Here males are haploid & females are diploid.
- Fertilized egg with diploid number of chromosomes develop to females & unfertilized eggs with haploid number of chromosomes parthenogenetically develops into males.
- Haploid males produce haploid sperms.
- Arrhenotoky – parthenogenetic development of a haploid egg.
- Thelytoky – parthenogenetic development of a diploid egg.
- Arrhenotoky is male producing mechanism & thelytoky is female producing mechanism.

INTERSEXES

- Bridges could discover XXY females & XO males among *Drosophila*.
- He crossed abnormal triploid females (3A + XXX) with normal diploid males (2A + XY), produced a variety of flies.
- They belong to five sexual categories, they are normal males, normal females, super males, super females & intersexes.

Sexual categories	**Sex index**
Normal male	0.5
Normal female	1.0
Super male	<0.5
Super female	>1
Intersexes	0.5 – 1.0

MODULE 3 : GENES AND GENE EXPRESSION

MODERN CONCEPT OF GENES

- Genes are complex hereditary determiners, composed of nucleic acid. Hence, they are not particulate units, but are sequences of nucleoides which contain the coded information necessary for all biological functions, Functionally, 4 basic kinds of genes can be recognized :
  - Structural genes : code for mRNA and proteins
  - Ribosomal RNA genes : code forrRNA
  - tRNA genes : code for tRNA
  - Regulator genes: regulate the functioning of other kinds of genes

- The nucleotide sequence of a gene encodes the amino acid sequence of a polypeptide or protein.
- there is a strict co-linearity" between the nucleotide sequence of a gene and the amino acid sequence of a corresponding protein.
- Genes are mostly sequential and non-overlapping. So, adjacent genes are well demarcated from each other
- Genes are not the units of function, recombination, or mutation, but these are all the functional aspects or properties of genes.
- So, it is now held that genes have the basic attributes of physiogical function, recombination, mutation, complementation, replication and transcription.
  - Cistron
  - Recon
  - Muton
  - Complon
  - Replicon
  - Transcripton

1. Cistron
   - Cistron (Benzer -1957) is the fundamental genic unit, which governs the synthesis of a polypeptide chain or protein molecule
   - A DNA molecule contains many cistrons.
   - The mRNA of eukaryotes is monocistronic while that of prokaryotes is polycistronic.
   - A cistron, in turn, has several functional units, called codons which codes for a specific amino acid.
   - It is formed of a set of three nucleotides and hence called triplet codon.

2. Recon

- Recon (Benzer 1958) isa small genic unit, capable of recombination
- It may be formed of one or more nucleotide pairs.
- It is exchangeable but not divisible by genetic recombination.
- A cistron may contain several recons

3. Muton
   - Muton (Benzer 1957) is the basic unit of gene mutation or, it is a small unit of DNA, that can be altered in the formation of a mutation.
   - Since mutation can occur even by a change in a single base, a single nucleotide pair can serve as a muton.

4. Complon
   - Complon is the unit of allelic complementation.
   - It is almost similar to a cistron.
   - Complementation is the process in which two mutant alleles together perform a function, which cannot be carried out by any one of them alone.

5. Replicon
   - Replicon is the unit of replication, that governs the synthesis of a small stretch of DNA.
   - It can be monocistronic or polycistronic.
   - In prokaryotes, the whole circular DNA represents a single replicon. But in eukaryotes, a single DNA may contain many hundred replicons

6. Transcripton
   - Transcripton is the unit of transcription, andit governs the synthesis of an RNA molecule.
   - It is usually monocistronic in eukaryotes, but polycistronic in prokaryotes.

- In some cases (bacteria) ,some closely associated genes behave as a single functional unit : *operon* which includes :
  - Structural gene:codes an enzyme
  - Operator genes:controls structural gene through activation or inhibition
  - Regulator genes : controls operator gene functioning
  - Promoter genes :transcription initiation

SPLIT GENE

- An interrupted gene (also called a split gene) is a gene that contains expressed regions of DNA called exons, split with unexpressed regions called introns (also called intervening regions).
- Exons provide instructions for coding proteins, which create mRNA necessary for the synthesis of proteins.

- Introns are removed by recognition of the donor site (5' end) and the splice acceptor site (3' end).
- The architecture of the interrupted gene allows for the process of alternative splicing, where various mRNA products can be produced from a single gene.
- Begin and ends with exons
- Allows flexibility in the synthesis of variety of gene products
- Several proteins with same amino acids can be produced
- Enable storage of genetic information within minimum DNA
- Exon-intron- codes for enzyme maturases
- prokaryotes : rare ; eukaryotes : common ;so are translated only after mRNA processing
- E.g. , antibody gene

PSEUDOGENES

- Mutant , imperfect and non-functional genes , which are homologous to functional genes
- They are divergent members of gene families ,become non-functional through mutations in the course of evolution
- They do not have the power of expression
- Regarded as debris
- Mostly repetitive units
- Serve as reservoir of sequences ,having new functions
- Regulates activity of neighbouring genes
- Important in evolution of organisms
- 2 classes :
    - orphans :dispersed and solitary
    - retropseudogenes : defective DNA copies of mRNA
- e.g.,immunoglobulin genes

OVERLAPPING GENES

- Contiguous genes of a single nucleotide sequence that codes for different kinds of functional proteins
- Different proteins are produced by transcription and translation of genes in different reading frames
- Common in bacteria
- Genes within genes
- Gene may be formed partly from one and partly from other gene
- Termination codon of one gene may overlap initiation codon of other
- Termination codon of one may ne initiation codon of other genes
- Single gene : codes for many proteins
- Mutation in this region causes pleiotropy

- Mutate as single unit
- Helps in space saving and gene compacting mechanism
- E.g.,SARS-Co V2 Virus

TRANSPOSONS

- Jumping genes / mobile genes
- A transposable element (TE, transposon, or jumping gene) is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size.
- Transposition often results in duplication of the same genetic material.
- Transposable elements make up a large fraction of the genome and are responsible for much of the mass of DNA in a eukaryotic cell.
- Although TEs are selfish genetic elements, many are important in genome function and evolution
- Transposons are also very useful to researchers as a means to alter DNA inside a living organism.

GENE EXPRESSION

- Process by which the instructions in DNA or gene are converted into a functional product like protein
- Nucleotide language – amino acid language

GENETIC CODE

- Nucleotide sequence of DNA and mRNA that specifies the amino acid sequence of proteins
- Provides biological information for protein synthesis
- Composed of series of codons : set of 3 nucleotide and codes for specific amino acid
- Codons are triplet
- Sum total of codons required for synthesis of a polypeptide chain : cistron or gene
- 64 codons (4^3) which codes for 20 amino acids
- 61 : sense codons
- 3 : non sense codons : stop codons : UAA , UAG and UGA
- AUG : start codon : codes methionine
- UGG : tryptophan
- Codons act collectively and codes for same amino acids : degenerate codons except AUG and UGG
- **Central dogma of molecular biology :**gene expression involves an intrinsic , unidirectional information flow from the information storage system to the final gene product through a message carrier system
- $\mathbf{DNA \xrightarrow{replication} DNA \xrightarrow{transcription} RNA \xrightarrow{translation} Protein}$

- 3 major steps :
    - **Transcription of genetic code : DNA dependent synthesis of mRNA**
    - **Post-transcriptional modification of mRNA**
    - **Translation through mRNA-dependent synthesis of proteins**

Transcription

- Transfer of stored information from DNA to RNA
- Enzyme : RNA polymerase
- DNA-dependent RNA synthesis
- Direction : **5' ⟶ 3'** direction
- Elementary unit : trancripton (operons in prokaryotes)
- Trancripton / template strand / antisense strand/ anticoding strand
- Introns : non-coding
- Exons : coding
- Functionally different regions of transcripton :
    - **Promoter : recognition signal to initiate**
    - **Acceptor / operator : binding site of regulators**
    - **Cistron : structural genes (monocistronic in eukaryotes and polycistronic in prokaryotes)**
    - **Terminator : halt signal**

- 3 stages :
    - **Initiation : RNA polymerase** recognizes recognition site in the promoter and binds to binding site then to promoter region and then to initiation site in cistron : forms transcription bubble
    - **Elongation :** activated ribonucleotide phosphates (ATP,GTP,CTP and UTP) bind to template strand by hydrogen bonding , where as adjascent nucleotides bind by ester bond . **Termination :** occurs when *RNA polymerase* reaches terminator site ; 2 TYPES : rho dependent and rho independent

Post-transcriptional modification

- Pre-mRNA to mature mRNA
- 3 major events :
    - **Splicing :**by nucleases ,non-informative regions are spliced and informative regions are glued by *RNA ligases*
    - **Capping:** 7-methyl guanine nucleotide at 5' end
    - **Tailing :** polyadenylation (200 adenine) at 3' end by *adenylic acid transferase*

Transfer of RNAs from nucleus to cytoplasm : mRNA binds with *informofer* which later binds with cytoplasmic proteins to form *informosome* ;inturn binds with ribosomal subunits to form *polysome*

Translation

- Process by which the genetic code contained within a messenger RNA (mrna) molecule is decoded to produce a specific sequence of amino acids in a polypeptide chain.

- It occurs in the cytoplasm following transcription
- Three stages:
  - **Initiation**
  - **Elongation**
  - **Termination.**
- Components of Translation : mRNA, ribosomes, and transfer RNA (tRNA).

- mRNA nucleotide bases are read as codons of three bases.
- Each 'codon' codes for a particular amino acid.
- Every tRNA molecule possesses an anticodon that is complementary to the mRNA codon, and at the opposite end lies the attached amino acid.
- tRNA molecules are therefore responsible for bringing amino acids to the ribosome in the correct order ready for polypeptide assembly.
- A single amino acid may be coded for by more than one codon. There are also specific codons that signal the start and the end of translation.
- *Aminoacyl-tRNA synthetases* are enzymes that link amino acids to their corresponding trna molecules. The resulting complex is charged and is referred to as an *aminoacyl-tRNA.*

Initiation

- For translation to begin, the start codon 5'AUG must be recognized.This is a codon specific to the amino acid methionine, which is nearly always the first amino acid in a polypeptide chain.
- At the 5' cap of mRNA, the small 40s subunit of the ribosome binds. Subsequently, the larger 60s subunit binds to complete the initiation complex**.**

Elongation

- The ribosome has two tRNA binding sites; the P site which holds the peptide chain and the A site which accepts the tRNA.
- While Methionine-tRNA occupies the P site, the aminoacyl-tRNA that is complementary to the next codon binds to the A site, using energy yielded from the hydrolysis of GTP.
- Methionine moves from the P site to the A site to bond to new amino acid there, and so the growth of the peptide has begun.
- The tRNA molecule in the P site no longer has an attached amino acid, and so leaves the ribosome.
- The ribosome then translocates along the mRNA molecule to the next codon, again using energy yielded from the hydrolysis of GTP.

- Now, the growing peptide lies at the P site and the A site is open for the binding of the next aminoacyl-tRNA, and the cycle continues.
- Whole process is mediated by : *peptide synthetase*
- The polypeptide chain is built up in the direction from the N terminal (methionine) to the C terminal (the final amino acid).

Termination

- One of the three stop codons enters the A site.
- Regulated by certain Release Factors (RF)
- RF first recognize termination codon and bind with A-Site and interact with peptide synthetase to make it hydrolytic
- No tRNA molecules bind to these codons so the peptide and tRNA in the P site become hydrolysed releasing the polypeptide into the cytoplasm.
- The small and large subunits of the ribosome dissociate ready for the next round of translation.

MODULE : 4

GENETIC ENGINEERING

- Genetic engineering, also called genetic modification or genetic manipulation, is the direct manipulation of an organism's genes using biotechnology.
- It is a set of technologies used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms.

RECOMBINANT DNA TECHNOLOGY

- The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources is referred to as Recombinant DNA Technology.
- Recombinant DNA technology is popularly known as genetic engineering.
- The recombinant DNA technology emerged with the discovery of restriction enzymes
- Inserting the desired gene into the genome of the host involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed.
- Thus the recombinant DNA has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring

TOOLS OF RECOMBINANT DNA TECHNOLOGY

The enzymes which include the *restriction enzymes* help to cut, the *polymerases*- help to synthesize and the *ligases*- help to bind.

1. **Enzymes**
2. **Vectors**
3. **Host cells**

1. **Enzymes**

a) Restriction enzymes

- The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome.
- They are two types, namely Endonucleases and Exonucleases.
- The **Endonucleases** cut within the DNA strand whereas the **Exonucleases** remove the nucleotides from the ends of the strands.
- The **restriction endonucleases** are sequence-specific which are usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the **restriction site**.

b) DNA ligase

- DNA ligase is a specific type of enzyme, a ligase, that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- It plays a role in repairing single-strand breaks in duplex DNA in living organisms, but some forms (such as DNA ligase IV) may specifically repair double-strand breaks (i.e. a break in both complementary strands of DNA).
- Single-strand breaks are repaired by DNA ligase using the complementary strand of the double helix as a template,with DNA ligase creating the final phosphodiester bond to fully repair the DNA.
- DNA ligase is used in both DNA repair and DNA replication
- In addition, DNA ligase has extensive use in molecular biology laboratories for recombinant DNA experiments
- Purified DNA ligase is used in gene cloning to join DNA molecules together to form recombinant DNA.
- The ligase joins the two fragments of DNA to form a longer strand of DNA by "pasting" them together.
- The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3' hydroxyl ends of one nucleotide ("acceptor"), with the 5' phosphate end of another ("donor"). Two ATP molecules are consumed for each phosphodiester bond formed

c) Reverse transcriptase

- RNA dependent DNA polymerase
- A reverse transcriptase (RT) is an enzyme used to generate complementary DNA (cDNA) from an RNA template, a process termed reverse transcription.
- Reverse transcriptases are used by certain ***retroviruses*** such as HIV and the hepatitis B virus to replicate their genomes, by retrotransposon mobile genetic elements.

d) Polynucleotide Kinase

- It is a phosphorylating enzyme
- It is concerned with transfer of phosphate group from ATP to dephosphorylated terminus of DNA or RNA
- Polynucleotide kinase (PNK) is an enzyme that catalyzes the reversible phosphorylation of nucleoside monophosphates and nuclic acid
- The enzyme may be used to phosphorylate RNA, DNA and synthetic oligonucleotides prior to subsequent manipulations such as ligation and cloning.

e) Terminal Nucleotidyl Transferase

- It is a polymerising enzyme
- It catalyses the addition of nucleotides onto the the 3'-OH terminus of a DNA primer, without using a template.
- in genetic engineering, it is mainly used for extending the 3' end and also for adding a "tale" to DNA prior to the formation of recombinants

- A small region that overlaps with a nuclear localization signal and binds to the RNA primer contains three aspartates that are essential for catalysis.

1. **Vectors**
   - Cloning Vector : A cloning vector is a small piece of DNA into which a foreign DNA can be inserted for cloning purposes
   - A vector is a DNA molecule that is used to carry a foreign DNA into the host cell.
   - It has the ability to self replicate and integrate into the host cell. These vectors have helped in analysing the molecular structure of DNA.
   - help in carrying and integrating the desired gene.
   - These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism.
   - **Plasmids** and **bacteriophages** are the most common vectors in recombinant DNA technology that are used as they have a very high copy number.
   - The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.

Vectors can be a plasmid from the bacterium, a cell from the higher organism or DNA from a virus. The target DNA is inserted into the specific sites of the vector and ligated by DNA ligase. The vector is then transformed into the host cell for replication.

Features of Cloning Vectors

- The cloning vectors possess the following features:
- A cloning vector should possess an origin of replication so that it can self-replicate inside the host cell.
- It should have a restriction site for the insertion of the target DNA.
- It should have a selectable marker with an antibiotic resistance gene that facilitates screening of the recombinant organism.
- It should be small in size so that it can easily integrate into the host cell.
- It should be capable of inserting a large segment of DNA.
- It should possess multiple cloning sites.
- It should be capable of working under the prokaryotic and eukaryotic systems.

Types of Cloning Vectors

❖ **Plasmids**

- These were the first vectors used in gene cloning.

- These are found in bacteria, eukaryotes and archaea.
- These are natural, extrachromosomal, self-replicating DNA molecules.
- They have a high copy number and possess antibiotic-resistant genes.
- They encode proteins which are necessary for their own replication.
- pBR322, pUC18, F-plasmid are some of the examples of plasmid vectors

❖ **Bacteriophage**

- These are more efficient than plasmids for cloning large DNA inserts.
- Phage λ and M13 phage are commonly used bacteriophages in gene cloning.
- 53 kb DNA can be packaged in the bacteriophage.
- The screening of phage plaques is much easier than the screening of recombinant bacterial colonies.

❖ **Phagemids**

- These are artificial vectors.
- They are used in combination with M13 phage.
- They possess multiple cloning sites and an inducible lac gene promoter.
- They are identified by blue-white screening.

❖ **Bacterial Artificial Chromosomes**

- These are similar to E.coli plasmids vectors.
- It is obtained from naturally occurring F' plasmid.
- These are used to study genetic disorders.
- They can accommodate large DNA sequences without any risk.

2. **Host organism** – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes.

There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

MECHANISM OF RECOMBINANT DNA TECHNOLOGY

The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

Step-1. Isolation of Genetic Material.

The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.

Step-2.Cutting the gene at the recognition sites.

The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called 'restriction enzyme digestions'.

Step-3. Amplifying the gene copies through Polymerase chain reaction (PCR).

It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.

Step-4. Ligation of DNA Molecules.

In this step of Ligation, joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.

Step-5. Insertion of Recombinant DNA Into Host.

In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once after the insertion of the recombinant DNA into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions.

METHODS OF GENE TRANSFER: 6 METHODS

The six methods are:

1. Transformation
2. Conjugation
3. Electroporation
4. Liposome-Mediated Gene Transfer
5. Transduction and
6. Direct Transfer of DNA.

Transformation:

- Transformation is the method of introducing foreign DNA into bacterial cells (e.g. E.coli).
- The uptake of plasmid DNA by E.coli is carried out in ice-cold CaCl2 (0-5°C), and a subsequent heat shock (37-45°C for about 90 sec).
- By this technique, the transformation frequency, which refers to the fraction of cell population that can be transferred, is reasonably good e.g. approximately one cell for 1000 (10-3) cells.
- Calcium phosphate (in place of CaCI2) is preferred for the transfer of DNA into cultured cells.
- Some workers use diethyl amino ethyl dextran (DEAE -dextran) for DNA transfer.

Conjugation:

- Conjugation is a natural microbial recombination process.
- During conjugation, two live bacteria (a donor and a recipient) come together, join by cytoplasmic bridges and transfer single-stranded DNA (from donor to recipient).
- Inside the recipient cell, the new DNA may integrate with the chromosome (rather rare) or may remain free (as is the case with plasmids).
- Conjugation can occur among the cells from different genera of bacteria (e.g Salmonella and Shigella cells).
- Thus by conjugation, transfer of genes from two different and unrelated bacteria is possible.
- The natural phenomenon of conjugation is exploited for gene transfer.

Electroporation:

- Electroporation is based on the principle that high voltage electric pulses can induce cell plasma membranes to fuse.
- Thus, electroporation is a technique involving electric field-mediated membrane permeabilization.
- Electric shocks can also induce cellular uptake of exogenous DNA (believed to be via the pores formed by electric pulses) from the suspending solution.
- Electroporation is a simple and rapid technique for introducing genes into the cells from various organisms (microorganisms, plants and animals).
- The cells are placed in a solution containing DNA and subjected to electrical shocks to cause holes in the membranes. The foreign DNA fragments enter through the holes into the cytoplasm and then to nucleus.
- Electroporation is an effective way to transform E.coli cells containing plasmids with insert DNAs longer than 100 kb.

Liposome-Mediated Gene Transfer:

- Liposomes are circular lipid molecules, which have an aqueous interior that can carry nucleic acids.
- Several techniques have been developed to encapsulate DNA in liposomes.
- The liposome- mediated gene transfer, referred to as lipofection
- On treatment of DNA fragment with liposomes, the DNA pieces get encapsulated inside liposomes.
- These liposomes can adher to cell membranes and fuse with them to transfer DNA fragments. Thus, the DNA enters the cell and then to the nucleus.
- The positively charged liposomes very efficiently complex with DNA, bind to cells and transfer DNA rapidly.

- Lipofection is a very efficient technique and is used for the transfer of genes to bacterial, animal and plant cells. T

Transduction:

- Sometimes, the foreign DNA can be packed inside animal viruses.
- These viruses can naturally infect the cells and introduce the DNA into host cells.
- The transfer of DNA by this approach is referred to as transduction.

Direct Transfer of DNA:

- It is possible to directly transfer the DNA into the cell nucleus. Microinjection and particle bombardment are the two techniques commonly used for this purpose.
- Microinjection:DNA transfer by microinjection is generally used for the cultured cells. This technique is also useful to introduce DNA into large cells such as oocytes, eggs and the cells of early embryos.

The term transfection is used for the transfer DNA into eukaryotic cells, by various physical or chemical means

APPLICATIONS OF GENETIC ENGINEERING

Genetic engineering is used in fields like medicine, agriculture, animal husbandry, pest management etc

1. **Basic genetic study**
    - Genetic engineering technology is used in the study of the minute structure, organization, function and expression of genes, regulation of gene expression, organization of split genes, DNA sequencing protein analysis
2. **Making of proteins**
    - Gene manipulation is used for the synthesis and medical purification of medically important proteins, hormones, enzymes, lymphokines (proteins secreted by T-lymphocytes) and interferon.
    - Therapeutic proteins like insulin, human growth hormone, b endorphin and factor VIII have already been produced by this method.
3. **Protein engineering**
    - Protein engineering is process of developing useful or valuable proteins by modifying the protein structure by making alternation in genetic code through in vitro site-directed mutagenesis.
    - Structural changes in proteins will alter their functional properties making more beneficial.
4. **Vaccine production**
    - Vaccines can be produced by DNA recombinant techniques.

- This avoids the problems of using attenuated strains of intact viruses.
- It is also possible to create recombinant viruses in which many foreign gene can be expressed.
- A vaccine created by using such a virus will be a multiple vaccine in effect.

5. **Diagnosis of genetic diseases**
    - Recombinant DNA technology have revolutionized the diagnosis of genetic diseases.
    - 4000 genetic diseases have already been identified and many of them have been studied extensively using pedigree analysis.

6. **Analysis of the transformation of normal cells to cancer cells**
    - Recombinant DNA technology techniques are extensively used in cancer research to study the process by which normal cells transform to cancer cells.
    - Success outcome of this techniques will definitely pave the way for more effective cancer treatment.
7. **Production of transgenic plants and animals**
    - Recombinant DNA technology is very profitably utilized for making superior varieties of transgenic crop plants, domestic animals, endowed with very high productivity greater resistance to pathogens and pests and greater tolerance and adaptability to unfavorable environmental conditions.
8. **Industrial microbiology**
    - Industrial micro biology is an applied branch concerned with the production of food, drink, pharmaceutical and industrial products etc., and disposal and recycling of sewage organic wastes.
    - It involves the conversion of substrates to end products by enzyme catalyzed biological process.
    - The genes and operons, which govern the fermentation pathways, can be isolated, cloned and manipulated in desirable ways and expressed on industrial scale.

Area which heavily demands the application of genetic engineering :

1. Symbiotic relationship between nitrogen fixing bacteria and some leguminous plant

2. Yeast in alcoholic fermentation

3. Bacterium pseudomonas can produce the enzymes for degrading hydrocarbons

POTENTIAL HAZARDS OF GENETIC ENGINEERING

- Origin of new diseases: gene manipulation may result in the creation of new diseases and organisms carrying fatal genetic elements.

- Drug resistant microorganisms: gene manipulation may produce drug resistant microorganisms and the outbreak of new diseases against which no preventive measures are effective
- Organism with unforeseen properties: the hybrid genome may acquire unforeseen properties and become unfavorable and harmful to organism.
- The spliced gene in the new setting may code for harmful proteins.
- The transplanted gene may induce the target cells to become neoplastic or cancerous
- The products of genetic engineering are likely to develop genetic abnormalities, physical malformation and physiological malfunctions.
- Production of extremely virulent genes:The interspecific gene transfer in genetic engineering using virulent genes as transport vehicles may cause the production extremely virulent genes. A combination of such genes with harmful viral genes may produce new virulent viruses with devastating effects on mankind.
- Local elimination of wild species: Genetic engineering may eventually endanger the original species, threatening biodiversity, and the balance of nature. Moreover, genetically engineered organisms may cause local elimination of wild species by virtue of their greater adaptation, fitness and survival value.
- Biological weapons:Genetic engineering is capable of producing highly destructive “biological weapons” which can spread fatal diseases.
- Allergy: Genetically engineered food products are seriously allergic to sensitive individuals.

MODULE 5.

CYTOGENETICS OF CANCER

TYPES OF CANCER-BASED ON CELL TYPES

INTRODUCTION-CANCER

- Cancer is a broad term. It describes the disease that results when cellular changes cause the uncontrolled growth and division of cells.
- Some types of cancer cause rapid cell growth, while others cause cells to grow and divide at a slower rate.
- Certain forms of cancer result in visible growths called tumors, while others, such as leukemia, do not.
- Most of the body's cells have specific functions and fixed lifespans. While it may sound like a bad thing, cell death is part of a natural and beneficial phenomenon called apoptosis.
- A cell receives instructions to die so that the body can replace it with a newer cell that functions better. Cancerous cells lack the components that instruct them to stop dividing and to die.
- As a result, they build up in the body, using oxygen and nutrients that would usually nourish other cells. Cancerous cells can form tumors, impair the immune system and cause other changes that prevent the body from functioning regularly.
- Cancerous cells may appear in one area, then spread via the lymph nodes. These are clusters of immune cells located throughout the body.
- Cancer cells have two fundamental heritable powers, namely
    - the potentiality for unbridled multiplication
    - the power to invade and colonize other neighboring or far away tissues.

TYPES OF CANCERS

1. Carcinomas

- Carcinoma is a malignancy that develops from epithelial cells.
- Carcinomas occur when the DNA of a cell is damaged or altered and the cell begins to grow uncontrollably and become malignant.
- Nearly 85%of human cancer belong to this group
- E.g. brain cancer, skin cancer, throat cancer etc.

2. Sarcomas

- It originate in mesodermal connective and muscular tissues.
- Nearly 2% of human cancers are sarcomas.
- Sarcoma is the general term for a broad group of cancers that begin in the bones and in the soft tissues (soft tissue sarcoma).

- E.g.: bone cancer (osteosarcoma), cartilage cancer (chondrosarcoma), muscle cancer (myosarcoma).

3. Leukemia

- It is also known as "blood cancer" and "myeloproliferative disorder".
- Leukemia is a cancer of blood cells characterized by the abnormal increase in the number of white blood cells in the tissues.
- There are many types of leukemia and they are classified according to the type of white blood cell involved.
- It originate the haemopoietic tissues (tissues which give rise to blood cells) of bone marrow, lymph nodes, etc.
- Nearly 4% of human cancer are Leukemia.
- Leukemia develops when the DNA of developing blood cells, mainly white cells, incurs damage.
- This causes the blood cells to grow and divide uncontrollably. Healthy blood cells die, and new cells replace them**.**

4. Lymphomas

- Lymphoma is cancer that begins in infection-fighting cells of the immune system, called lymphocytes.
- These cells are in the lymph nodes, spleen, thymus, bone marrow, and other parts of the body.
- When affected with lymphoma, lymphocytes change and grow out of control.
- Nearly 5 % of human cancers are lymphomas
- Symptoms include enlarged lymph nodes, fatigue and weight loss.
- Treatment may involve chemotherapy, medication, radiation therapy and rarely stem-cell transplant.
- E.g.: Hodgkin’s disease.

5. Melanomas

- Melanoma occurs when the pigment-producing cells that give colour to the skin become cancerous.
- Melanoma is a type of skin cancer that occurs when pigment producing cells called melanocytes mutate and begin to divide uncontrollably.
- Most pigment cells develop in the skin.
- Melanomas can develop anywhere on the skin, but certain areas are more at risk than others.
- Symptoms might include a new, unusual growth or a change in an existing mole. Melanomas can occur anywhere on the body.
- Treatment may involve surgery, radiation, medication or in some cases, chemotherapy.

6. Blastomas:

- Blastomas are solid tumors. They form when cells fail to differentiate properly into their intended cell types before birth or in infancy and early childhood.
- As a result, the tissue remains embryonic.
- In a child with blastoma, the condition is usually present at birth.

(a)Retinoblastoma:-

- Retinoblastoma is an eye cancer that begins in the retina — the sensitive lining on the inside of your eye.
- Retinoblastoma most commonly affects young children, but can rarely occur in adults.
- Retinoblastoma may occur in one or both eyes.
- Retinoblastoma has few, if any, symptoms at first. It may be noticed if a pupil appears white when light is shone into the eye, sometimes with flash photography. Eyes may appear to be looking in different directions.
- Treatments include chemotherapy, radiation and laser therapy.

(b)Neuroblastoma

- Neuroblastoma is a cancer that develops from immature nerve cells found in several areas of the body.
- Neuroblastoma most commonly arises in and around the adrenal glands, which have similar origins to nerve cells and sit atop the kidneys.
- It can develop in the stomach, chest, neck, pelvis and bones. Children aged five or younger are most commonly affected.
- Symptoms may include fatigue, loss of appetite and fever. There may be a lump or compression of tissues in the affected area.
- Neuroblastoma usually requires surgery, chemotherapy and sometimes radiotherapy and stem cell transplantation.

(c)Nephroblastoma

- Nephroblastoma is a rare type of kidney cancer that affects the children.
- It is seen in children in the age group of 3 – 4 years.
- Mostly, this type of cancer affects only one kidney. However, in some cases both the kidneys are affected.
- Symptoms include Abdominal swelling, Urine discoloration, Blood in urine, Fever, Pain in abdomen, Nausea, Constipation, Feeling unwell, Vomiting, Unintentional weight loss, Distended veins in abdominal region
- Treatments include surgery, chemotherapy, kidney transplantation, radiation therapy.

CHARACTERISTICS OF CANCER CELLS

1. **Uncontrolled multiplication**
2. **Loss of contact inhibition**
3. **Anchorage -independent**

4. **Metastasis**
5. **Loss of cell recognition and tissue specificity**
6. **Biochemical and physiological despecialization**
7. **Inability to respond to stimuli**
8. **Weak physiological feedback**
9. **Metabolic imbalance**
10. **Cataplasia**
11. **High adaptations to unfavourable conditions**
12. **Enhanced glycolysis and high utilization of sugar**
13. **Reduced cellular adhesion**
14. **Metaplasia**
15. **Invasiveness**
16. **Growth factor secretion**
17. **Cell surface alterations**
18. **Alterations in transcriptome and proteome and protease secretion.**

ORIGIN OF CANCER (CARCINOGENESIS):

- Origin of cancer is called carcinogenesis.
- The switching over or conversion of normal cells to cancer cells by a process called 'cell transformation'.
- ***Carcinogens***:The agents or factors which induce carcinogenesis.
- Many chemical and physical factors,such as ionising radiations,continued physical irritations are shown to be carcinogenic.
- Threshold dose:A single carcinogen may induce carcinogenesis all by itself only when its concentration in the body reaches the optimum level.
- ***Syncarcinogenes*** : A sub-threshold dose of it will not induce cancer by itself.But 2 or more carcinogens in the sub-threshold dose may collectively cause cancer.
- Syncarcinogens: the agents involved in syncarcinogenesis.
- ***Co -carcinogenesis***:A non carcinogenic substance, in combination with the sub threshold dose of a carcinogen may induce cancer.
- Co carcinogen/promoter_the non carcinogen involved in co carcinogenesis.

THEORIES OF CARCINOGENESIS

- **Somatic mutation theory (mutational origin of cancer)**
- **Oncogene theory(genetic origin of cancer)**
- **Viral gene theory(viral origin of cancer)**

1. **Somatic mutation theory:**
   - This theory holds that somatic mutation is the initial cause of neoplastic transformation.
   - Involves either the mutation of the regulator gene,or abnormal alternations in the regulation of the genetic machinery.
   - These mutations are believed to abnormally alter the regulatory mechanism of cell cycle.

- Thereby,a cell loses its control over itself and become cancerous or neoplastic,and undergo uncontrolled multiplication.
- Cancer causing somatic mutations may involve either the activation of normally repressed gene,or the repression of normally active genes.
- Several cancers are caused by autosomal dominant mutation(eg:blastomas) Some are caused by lethal gene mutation(eg:xeroderma pigmentosum)
- There are several physical and chemical mutagens that are potentially carcinogenic.
- They are;arsenic,nickel,chromates,aromatic amines,asbestos fibres,polycyclic hydrocarbons,etc.

2. Oncogene theory:

- This theory holds that cancer is genetically programmed or gene controlled cellular phenomenon.
- carcinogenesis and tumourigenesis are controlled by the mutant forms of 4 classes of gene.
- They are;
  - proto oncogene(POG)
  - tumour suppressor gene(TSG)/ anti oncogene(AOG)
  - microRNA genes(miRNA genes)
  - mutator genes
- non mutated type of proto oncogenes are less active.Their more active mutant forms are called ***'oncogenes'.***
- Proto oncogene stimulate cell proliferation.
- Tumour suppressor gene inhibit cell proliferation.
- miRNA genes stimulate post transcriptionally silence the expression of other genes.
- mutator gene govern unerring gene replication,DNA repair and the response of cells to DNA damage.
- mutant forms of all these genes are believed to cause and contribute to the transformation of normal cells to cancer cells
- Oncogenes are the active mutant genes whose expression induces uncontrolled cell proliferation and tumour formation.
- Proto oncogenes governs the controlled growth and multiplication of normal cells.sometimes,under certain unusual condition they get activated and transformed to oncogenes.It stimulate cell growth or prevent cell death.
- Oncogenes are present in viruses(viral oncogenes)and animal cells(cellular oncogenes)
- DNA sequence of cellular oncogene maybe closely similar to viral oncogenes.
- viral oncogene always exist in active state.
- Cellular oncogene exist in active state only in fully transformed cells.
- Proto oncogenes get activated in 4 major ways:
  a) by mutation.

b) by translocation to chromasomal region where there is a strong promoter sequence.
c) by gene amplification
d) by the insertion of viral genome.

- TSGs are the anti oncogenic genes which govern the chech points of cell cycle and thereby restrict uncontrolled cell proliferation and suppress tumour formation.
- In tumour formation,they undergo recessive mutation lose their normal functions,become oncogenic, transform normal cells to cancer cells and induce carcinogenesis and tumourigenesis.
- TSG act in a different way from proto oncogene.
- Proto oncogene transform to active oncogenes through mutation which enhance their activity.
- TSGs become oncogenic through mutation which strip away their normal function.

3. Viral gene theory:

- This hold that some viruses can serve as cancer causing and tumour forming agents.such viruses are called 'Oncoviruses'Or 'Oncogenic viruses'.
- Their cancer inducing genes,responsible for the transformation of normal cell to cancer cells are called 'Viral oncogenes'.
- It is believed to result from the recombination between non oncogenic viral genome and host cell genome.They encode the protiens which are involved in governing the growth of the host cell.
- Viral oncogenes always exist in the active mutated state.
- In retroviruses, they are under the controll of powerful enhancers which enhance the rate of the transcription.
- The viral origin of cancer is often called 'Viral oncogenesis'.
- Some DNA viruses have been implicated in some malignancies.
- Eg:Epstein_Barr virus(a type of herpes virus)which cause Burkitt's lymphoma.
- Oncogenic viruses include DNA and RNA viruses.
- The common oncogenic DNA viruses are papoviruses(eg:polyoma virus) ,Pox viruses,adenoviruses, herpes viruses(Epstein_Barr virus),cytomegaloviruses,etc
- The common Oncogenic RNA viruses include some retroviruses , eg:Rous sarcoma virus(found in chicken) , Murine sarcoma virus(found in mouse) , Murine leukemia virus,avian sarcoma virus,feline leukemua virus(found in cats) , Human T-cell lyphotropic virus(cause T-cell leukemia)
- Oncoviruses donot reproduce within all host cells, nor do they cause the lysis of all host cells.
- The host cell in which viral reproduction is possible is called'permissive cells'.
- The host cell in which viral reproduction is not possible are called 'non permissive cells'.

- The virus usually kills permissive cells,but it leave non permissive cells un effected or sometimes permanently alter their genetic characteristics and transform them to cancer cells.

- In viral oncogenesis,the whole of the viral genome,or a portion of it,gets incorporated with the DNA of the host cell to form the viral oncogene.
- If the oncogene has only the part of the viral DNA,it gets directly inserted into the host DNA by a recombination process, and becomea the integral part of the llatter.
- If it has the part of viral RNA, the RNA serves as the template and guides the synthesis of a complementery single stranded DNA(cDNA) by reverse transcription.
- Soon this single stranded DNA act as a template and guides the synthesis of its complementary campanion strand.Now it transform to a double stranded viral DNA,often called the 'DNA provirus'.
- It gets integrated with the hist cell DNA by recombination.
- Now it can undergo transription to produce copies of viral RNA.
- The integration of the viral oncogene with the host cell genome may result in the transfornation of normal cells to cancer cells.
- The inserted viral oncogene transforms the normal host cell to a cancer cell in 3 major ways:
    - Viral oncogene induces mutational alterations in cellular proto oncogenes and transform them to active cellular oncogene bring about the actual transformation of the cell.
    - The inserted viral oncogene directly induces the transformation of yhe host cell.
    - The inserted viral oncogene inactivates the tumour suppressor gene of the host cell.This in, turn,makes the host cell cancerous.
- Oncovirus are cancer causing and tumour forming viruses
- Their cancer inducing genes, responsible for the transformation of normal cells to cancer cells is called viral oncogenes
- Viral oncogenes always exist in the active mutated state
- The viral origin of cancer is often called viral oncogenesis
- Examples oncogenic DNA viruses
- Papovirus , eg : polyoma virus,simian virus ,Pox viruse
- Oncogenic RNA viruses include some retroviruses , Murine leukaemia,avian sarcoma – cat , HumanT cell lymphotropic virus - T cell leukaemia

"All oncogenic RNA viruses are retroviruses,but all retroviruses are not oncogenic"

4. Polygenic basis

- e.g. breast cancer , liver cancer
- influenced b y environmental factors
- family line also influences

5. **Hereditary predisposition**
    - Cancer-family syndromes
    - Genetic
    - E.g. colon cancer , pancreatic cancer

MODULE 6

CELLS & ORGANS OF IMMUNE SYSTEM, ANTIGENS & ANTIBODIES

Innate & adaptive immunity

Immunity is classified into two major classes

1. Innate immunity
2. Aquired immunity

Innate immunity

- Inborn or naturally inherited.
- Native immunity or natural immunity
- Non specific immunity
- Provides immediate defence against infection

Mechanism of innate immunity

1. Physical & anatomical barrier

- Skin & respiratory mucosa
- Prevents the entry of pathogens into the body
- First line defence
- Skin serves as mechanical & chemical barrier
- Secretions of sweat glands & sebaceous glands make the skin acidic & unfavourable for invading bacteria.
- Lysozyme in tear & sweat, destroys bacteria.
- Acids & other metabolic wastes by resident bacteria inhibits the growth & multiplication of invading pathogens.
- During exfoliation infectious agents landed on the skin are also removed.
- Cilia & mucus in the respiratory tract trap & ingest the bacteria present in the inhaled air.

2. Chemical barrier

- Saliva & gastric juice
- Ingested pathogens are killed by salivary lysozyme & gastric HCL.

3. Physiological barrier

- Temperature, pH, saliva, gastric juice, lysozyme, interferon, complement proteins,..
- Fever responses inhibit the growth of pathogens.
- Acidity kills the ingested microorganisms

4. ***Phagocytic barrier***

- Second line defence
- Major phagocytic cells – neutrophils, monocytes & tissue macrophages
- Ingest & digest invading antigens & microbes

5. ***Inflammatory barrier***

- Inflammation – tissue response to injury or infection
- Clinical symptoms – localized fluid accumulation, swelling, redness, heat & pain
- Physiological changes – histamine & pyrogen production, dilation & increased permeability of blood vessels

6. ***NK- cell mediated response***

- Attack pathogen infected cells & abnormal cells, such as cancerous cells
- Abnormal cells lack the surface antigens of T or B cells – null cells
- NK cells attack null cells

Aquired immunity

- Adapted immunity
- Specific immunity
- Developed by an individual during life time
- Develops in response to artificial or natural stimulation
- Mediated by specific antibodies or sensitized lymphocytes
- Capable of recognizing & selectively eliminating specific microbes, antigens, toxins,..
- Individual specific

Four characteristic attributes

1) ***Diversity*** – ability to recognize a variety of foreign molecules
2) ***Specificity*** – ability to discriminate different kinds of foreign molecules
3) ***Self non-self recognition***
4) ***Immunological memory*** – retain the information about the foreign molecules of previous encounters

Kinds of acquired immunity

- Two types – active immunity & passive immunity

Acquired active immunity

- Elicited against natural or artificial antigenic stimulation

- Two kinds – Antibody mediated – humoral immunity
  Cell mediated – cellular immunity
  Both involves immunological memory
- May be natural or artificial

a) Naturally acquired active immunity

- Developed against by the natural infection by bacteria, viruses,..
- Life-time immunity – small pox, measles, mumps
- Short living immunity – bacterial dysentery

b) Artificially acquired active immunity

- Developed against the antigens introduced artificially by vaccination
- Vaccines – preparations of live attenuated or killed micro organisms or their products (toxoids)

Aquired passive immunity

- Ready made resistance, passively transferred to a recipient
- Transferred from an immune individual to a non immune individual
- Two types – natural & artificial

Advantages

- No antigenic stimulation
- No antibody production
- No latent period

Disadvantages

- Immunity developed is temporary
- No memory cell formation
- Decreases with repeated administration

a) Naturally acquired passive immunity

- Acquired through natural methods
- Transfer of immunity from mother to foetus before the immune system of the foetus becomes functional
- Occurs through transplacental transfer of antibodies
- Enables the foetus to defend against infections until it is able to produce antibodies
- IgG , IgA ,.. obtain from mother body

b) Artificially acquired passive immunity

- Aquire through artificial methods
- Transfer from immunized donor to a non immune recipient by transferring antibodies or immunized lymphocytes
- Administration of specific antibodies or sera which contain specific antibodies
- Used therapeutically for the treatment of tetanus, diphtheria, snake bite,..
- Covalescent serum – serum collected from the patients recovering from a particular infectious disease, which contain high amount of antibodies
- Combined immunization – combination of active & passive methods of immunization.

Adoptive immunity

Passive immunity produced by injecting immunologically competent lymphocytes & not by injecting antibodies

Adopted for the treatment of tuberculosis & leprosy.

Cells of immune system

- Five major groups
- Lymphocytes, antigen presenting cells, granulocytes, agranulocytes & mast cells
- Lymphocytes play the key role in the control & co-ordination
  Recognition of infected or heterologous cells

B – cell

- B lymphocytes
- Uninucleate & non granular
- Produce antibodies – responsible for humoral immunity
- Having a large nucleus & a ring of marginal cytoplasm.
- Found in blood, lymph, lymph nodes & spleen.
- ***Surface markers*** – unique proteins or antibodies found in B cell surface membrane, serve as receptors for antigens.
- Derived from haematopoetic stem cells of red marrow
- Migrate to lymph nodes & spleens for their action.
- An antigenic stimulation occurs, B cells divides to plasma cells & memory cells.
- ***Plasma cells*** – secrete antibodies – bring about primary immune responses – short life.
- ***Memory cells*** – store information – bring about secondary immune responses - long life.

Plasma cells

- Found in lymph nodes & spleen.

- Rarely seen in blood plasma.
- Eccentrically placed nucleus & sparse cytoplasm
- Antibody synthesizing machinery is well developed – densely packed endoplasmic reticulum & ribosomes.
- Devoid of surface receptors & surface immunoglobulins.
- Russel's bodies – immunoglobulins are localised in the spaces of the endoplasmic reticulum.

T – cell

- Uninucleate & non granular.
- Bring about cell mediated immunity.
- Origin in bone marrow & maturation in thymus
- Have membrane receptors for antigens
- Four major sub populations of T cells are
  1) ***Helper T cells***
     - Activated by small quantities of antigens
     - Activated T_H cells secrete lymphokines – increases the response of B & T cells.
     - MMIF – Macrophage Migration Inhibition Factor – lymphokine which causes the accumulation of macrophages & activates phagocytosis.
  2) ***Suppressor T cells***
     - Suppress the activity of B cells & other T cells.
     - Inhibit antibody production.
     - Responsible for immune tolerance – limiting the ability of immune system to attack own boy tissues.
  3) ***Cytotoxic T cells***
     - Killer T cells
     - Kills microbes or body's own tissues.
     - After the binding of receptor proteins with antigens causes the swelling of TC cells – release cytotoxic substances into the target cells – lysosomal enzymes.
     - Distructive to virus infected cells & tissues.
     - Play an important role in destroying cancer cells & allografts.
  4) ***Delayed type hypersensitivity T cells***
     - Bring macrophages to areas where delayed hypersensitivity reactions occur.
     - Secretes macrophage chemotoxin & MMIF.

NK – cell

- ***Natural killer cells – null cells***
- Have no surface markers

- Large granular lymphocytes
- Display cytotoxic activity against tumour cells
- Important role in host defence against tumour cells & the cells infected with viruses.

Antigen presenting cells

- Cells which activate, process & present antigens for lymphocyte action.
- T cells recognize only the antigens processed by APC's.
- Three types

1. Macrophages
2. Dendritic cells
3. Langerhans cells

Dendritic cells

- Very active in stimulating T & B cells.
- Essential for primary immune response.
- Process antigen & present it on the surface of other immune cells.
- Present in skin & the internal lining of nose, lungs, stomach & intestine.
- Found in blood as immature state.
- Once activated, migrate to lymph nodes & spleen

Macrophage cells

- Large mononuclear phagocytic leucocytes.
- Derived from monocytes.
- Crowded in lymphoid organs.
- Immobile at rest.
- Actively mobile when stimulated by lymphokines.
- ***Kupffer cells*** – found in liver
- ***Dust cells*** – lungs
- ***Histiocytes*** – connective tissues
- ***Osteoclasts*** – bones
- ***Microglia*** – nervous system
- ***Mesangial*** cells - kidney

Organs of immune system

- Immune system is formed of different tissues & organs.
- Immune organs can be categorized into two main groups, namely primary lymphoid organs & secondary lymphoid organs

Primary lymphoid organs

- Major centres of lymphopoiesis.
- Lymphoid stem cells undergo proliferation, differentiation & maturation.
- Thymus & bone marrow – organs of mammals.
- Thymus & bursa of Fabricius – organs of birds.
- Primary lymphoid organs are large at birth & they atrophy with age.

Thymus

- Bilobed gland.
- Located above heart & behind the top of the sternum.
- Centre of the chemical modification & functional maturation of T cells.
- Thymic hormones govern T cell maturation .
- It progressively grows in size, reaches maximum size at puberty, then gradually shrinks.
- Two parts – cortex & medulla.
- Cortex – outer part – contains immature T lymphocytes & reticular cells.
- Medulla – inner part – consists of vascular tissue, reticular epithelial cells & scattered lymphocytes.
- T cells are non functional inside thymus.

Bone marrow

- Soft tissues within the cavities of bones.
- Origin of B & T cells & maturation of B cells.
- Divisible into two regions – vascular adipose region & the haemopoietic region.
- Vascular adipose region – contains blood vessels – supplies nutrients & oxygen – removes wastes.
- Haemopoietic region – haemopoiesis – red marrow – contains multipotent stem cells.
- Major site of antibody synthesis.

Secondary lymphoid organs

- Include lymph nodes, spleen, MALT, CALT,…
- Contain distinct regions of T cell & B cell activity.
- Rich in macrophages & dendritic cells

Spleen

- Large, ovoid, located in left abdominal cavity below pancrease.

- Specialized for filtering blood & trapping blood borne antigens.
- Concerned with destruction of old, dead & worn out RBC's.
- Reserve site for haematopoiesis.

Lymph node

- Small, capsulated, solid, rounded or bean shaped bodies.
- Located on lymphatic vessels.
- Numerous in arm pits, groins, mesenteries,..
- Filter out foreign material from lymph.

MALT

- Mucosa Associated Lymphoid Tissue.
- Very active in elicitating immune responses for defence against invading pathogens.
- Major groups of MALT are,

a) Gut associated lymphoid tissue (GALT)
b) Nose associated lymphoid tissue (NALT)
c) Larynx associated lymphoid tissue (LALT)
d) Trachea associated lymphoid tissue (TALT)
e) Bronchus associated lymphoid tissue (BALT)
f) Eye associated lymphoid tissue (EALT)
g) Vulvo vagina associated lymphoid tissue (VALT)

Peyer's patches

- Elongated thickenings of the intestinal epithelium.
- ***Microfold cells*** – specialized cells on *Payer's patches*.
  Collect & deliver antigen to antigen presenting cells.

Tonsils

- Located around the pharynx.
- Similar to thymus.
- Contains lymphoid tissues & T lymphocytes.
- Three group of tonsils
  a) ***Palatine*** – found on palate
  b) ***Lingual*** – base of the tongue
  c) ***Pharyngeal*** – adenoids – roof of nasopharynx

  These three forms ***tonsillar ring*** or ***Waldeyer's tonsillar ring***.

- Defend against antigens entering through the nasal & oral epithelial routes.

Antigens

- An exogenous substance which can sensitize a specific lymphocyte & evoke or induce a specific immune response.

Antigenicity

- Ability of a substance to behave as an antigen & to be recognized by an antibody.

Immunogenicity

- Potentiality of an antigenic substance to stimulate the immune system & to evoke an immune response.

Haptens

- Low molecular weight compounds.
- Antigenic not immunogenic.
- ***Hapten – carrier conjugate*** – chemical coupling of hapten with a carrier protein results in hapten – carrier complex.
  Serve as immunogen.
- A hapten can combine with an antibody, but cannot induce antibody formation & immune response.
- Some substances act as haptens in some animals, where as they act complete antigens.
- Eg:- Pneumococcal polysaccharide
  Antigen in man
  Hapten in rabbits

Factors influencing immunogenicity

1. ***Nature of immunogen***
   - **Foreigness**
     Molecule must be recognized as non self by the biological system
   - **Molecular size**
     Active immunogens – 100000 Da.
     Poor immunogens – 5000 – 10000 Da.
   - **Chemical composition & heterogeneity**
     Chemical complexity contributes to immunogenicity
   - **Lipids as antigens**
     Appropriately presented lipoidal antigens can induce B cell & T cell responses.
   - **Susceptibility to antigen processing & presentation**

Larger molecules are more readily phagocytosed & processed.

2. ***Biological systems***
    - Genotype of recipient animal
    - Immunogen dosage & route of administration
    - adjuvants

Human immunoglobulin gene families

- Heavy chain & light chain of immunoglobulin are encoded by separate gene families, located on different chromosomes.
- Gene segments – multigene families containing several coding sequences.
- They are
    1. Heavy chain multigene family – chromosome 14
    2. Kappa light chain multigene family – chromosome 22
    3. Lamda light chain multigene family – chromosome 2.

λ light chain

- Four kinds – λ_1, λ_2, λ_3, λ_4.
- Not all of them may present in all species.
- In human beings all are present.

κ light chain

- Differ from λ chain in the amino acid sequence.

Heavy chain families

- 5 different types
- Gamma, alpha, mu, delta, epsilon.

Major histocompatibility complex (MHC)

- Also called Human Leucocyte Antigen Complex (HLAC).
- Large genomic region with many highly polymorphic genes.
- Located on chromosome 6.
- Govern the production of antigen presenting molecules & complement proteins.
- Minor histocompatibility locus – genomic region outside MHC – encodes the antigens which contribute to graft rejection.
- MHC plays a central role in immune response against antigens.

- MHC is divided into three regions :
  1. Class I
  2. Class II
  3. Class III.
- Class I MHC genes – Code for the MHC found on the surface of most nucleated cells.
  Associated with the stimulation of T cells.
- Class II MHC genes - Code for the MHC genes code for antigen presentation molecules that are not found on the cell surface.
  Associated with the stimulation of T cells.
- Class III MHC genes – codes for the proteins of the complement system, soluble serum proteins & the tumour necrosis factor.

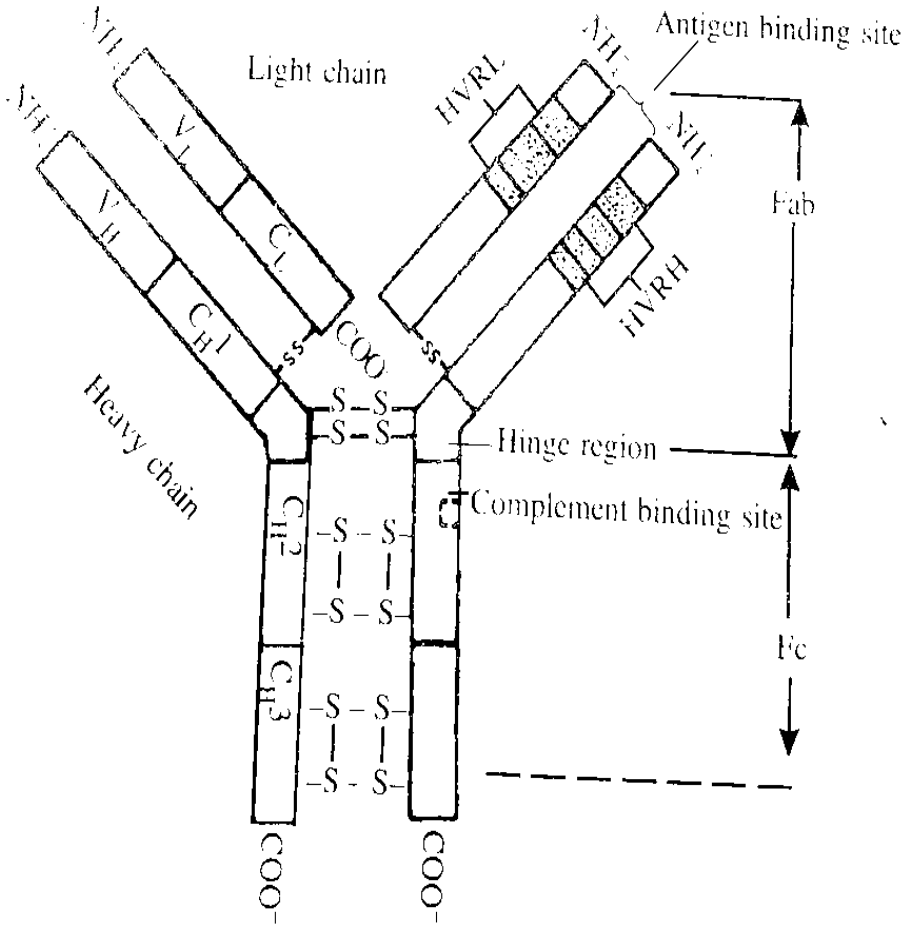
Antibodies

- Immunoglobulins
- Synthesized by plasma cells
- Present on B lymphocyte membrane
- Responsible for humoral immunity
- Recognize & inactivate specific antigens
- Immunoglobulin – represents the resting or non reacting state.
- Antibody – represents the active or reacting state.

Structure

- Y – shaped
- Symmetrical & tetrameric glycoprotein.
- It has a tail & two arms
- Arm has specific antigen binding properties – Antigen Binding Fragment (Fab).
- Tail – crystallizable – crystallizable fragment (Fc).
- Antibody consist of four polypeptide chains – two heavy chains & two light chains.
- Heavy chains are longer than light chains
- Each chain has two terminals :
  1. Amino terminal – N
  2. Carboxyl terminal –C
- These chains are held together by disulphide bond, salt linkages, hydrogen bonds & hydrophobic interactions.
- Each chain has two distinct regions – variable & constant region.
- Variable region of H chain – V_H
- Variable region of L chain – V_L
- Constant region of H chain – C_H

- Constant region of L chain - C_L



- Amino acid sequence of V region is highly variable, different in different antibodies.
- Amino acid sequence of C region is almost same.
- C region of H chain has three domains – C_H1, C_H2 & C_H3.
- Hinge region – between C_H1 & C_H2, contains 12 amino acids – proline rich – gives flexibility for the movement of Fab.
- Two types of light chains – λ & κ.
- Five types of heavy chains - Gamma, alpha, mu, delta, epsilon.
- Based on function five kinds of immunoglobulins –
  Neutralins – anti toxins
  Agglutinins – clumping antigens
  Precipitins – precipitate antigens
  Opsonins – inactivate antigens by opsonization
  Lysins – disintegrate antigens.

Different classes & functions

Ig class	Heavy chain	No. of paratopes	Major functions	Location
IgG	Gamma (γ)	2	Activation of complement system & macrophages, agglutination serves as anti-toxin	Blood, tissue fluid Can cross placenta
IgA	Alpha (α)	2 or 1	Prevents bacterial growth & multiplication on mucosa, inhibits bacterial adhesion to host cells	Saliva, intestinal secretions, urinogenital secretions, bronchial mucus, tear, milk,..
IgM	Mu (μ)	10	Activation of complement system, agglutination of RBC's, neutralization of viral infections	Blood, tissue fluid
IgD	Delta (δ)	2	Not definitely known	Lining of thoracic & abdominal tissues
IgE	Epsilon (ε)	2	Activation of mast cells to release histamine, defence against allergens & parasitic worms	Tissues

Monoclonal antibodies

- Same type of ultra-pure & identical antibodies
- Produced by a single clone of hybridoma cell line against a single type of epitope.
- Derived from one & the same progenitor cell
- Structurally, chemically & genetically identical
- Production of monoclonal antibodies involves three major events

1. Production of hybridomas
2. Separation of hybridomas by cloning
3. Continuous propagation of hybridomas

Hybridoma technology

- Hybridomas are genetically engineered cells.
- Used to produce a desired antibody in large quantities
- Hybridoma technology is the invitro production & culturing of hybridomas for the production of monoclonal antibodies.
- Hybridomas are produced by fusing B lymphocytes with myeloma cells.
- B cells – short living, antibody producing cells
- Myeloma cells – malignant bone marrow cells, proliferate rapidly

- Hybridomas have the properties of both their parent cells.
- They are of three major categories

1. Mediating specific immune responses
2. Producing specific T cell factors
3. Secreting non specific lymphokines.

Applications

- Identification of pathogenic & infectious viruses, bacteria & protozoans.
- Diagnosis of allergies, hepatitis,…
- Detection of cancer at an early stage
- Preparation of vaccines to counteract tissue rejection
- Pregnancy test
- Destruction of auto antibodies produced in auto immune diseases
- Analysis of hormonal disorders

MODULE 7

ANTIGEN - ANTIBODY INTERACTION & GENERATION OF B-CELL AND T- CELL RESPONSE

Antigen - antibody interaction

- Bimolecular association similar to enzyme – substrate interaction.
- Interaction between the chemical groups on the surface of the epitope & paratope.

Strength of Antigen-Antibody interaction

- Formation of immune complex
- Lock & key mechanism
- Non covalent bonding
- Specificity
- Reversibility
- Cross reactivity
- Affinity – binding capacity of an antibody with a univalent antigen in the complexed state.
- Avidity – binding strength of antigen antibody complexing.

Cross reactivity

- Cross reactivity is the ability of a particular antibody or T-cell receptor to react with two or more antigens which have the same type of epitope.
- Most antigen-antibody reactions are highly specific.
- Some antigens show cross reactivity with non-specific or unrelated antibodies.
- This occurs only when different types of antigens have the same or similar type of epitopes.
- Cross reactivity common among ABO blood group antigens.

Precipitation reactions

- These are the preciptate-forming antigen-antibody interactions.
- The reaction between soluble antigens and antibodies results in precipitate formation.
- Antibodies that are capable of precipitate formation on reacting with antigens are called ***precipitins***.
- The antibody must be bivalent, i.e, with more than one Fab site for reacting with antigen,

- The antigen should also be bivalent or polyvalent, with two or more similar or dissimilar epitopes for reacting with antibodies.
- Precipitate formation occurs when antigen and antibody are present in equal concentration.

Agglutination reactions

- These are the antigen-antibody reactions.
- Clumping or agglutination of antigen molecules.
- The antibodies which can bring about the agglutination of antigen molecules are called ***agglutinins***.
- Occurs when antigens and antibodies exist in equal concentrations.
- The inhibition of the agglutination of antigens by high concentrations of antibody is called ***prozone effect***.
- The antibodies which cannot agglutinate antigen molecules are sometimes called ***incomplete antibodies.***
- When bacterial antigens are agglutinated, the reaction is called ***bacterial agglutination*** - diagnose bacterial infections.
- The agglutination of red blood cells is termed ***haemagglutination***.

Immunotechniques – Detection of biomolecules

ELISA

- Enzyme - linked immunosorbent assay.
- Sensitive technique used to detect small quantitues of specitic proteins by antigenic means.
- Immunological test for the detection antigens and antibodies in the human blood.
- It was first carried out by Pearlmann and Engwall.
- It is done as a clinical test for the diagnosis of AlDS, Hepatitis B, syphilis, gonorrhoea, etc.
- ELISA test makes use of the principle of high antigen-antibody specificity.
- There are two types of ELISA test, namely double antibody sandwich ELISA (DAS ELISA) and direct antigen coating ELISA (DAC ELISA).

DAS ELISA test

- This test is done to detect the presence of HIV antigen in the human blood.
- The antibody of the suspected antigen is coated on the surface of a plastic well.
- The blood sample of the person (test solution) who is suspected to have HIV infection is added to the well and incubated for 30 minutes at 37 C.
- If the blood sample contains HIV antigen, the antibody will immobilize it and interact with, forming an antigen- antibody complex.
- Uncomplexed antibodies are now washed out.
- A second lot of antibodies is taken.
- They are linked to an enzyme, which is specific to the HIV antigen.
- This forms an antibody - enzyme complex.
- The antibody-enzyme complex is now introduced to the well.
- It may link with the antigen-antibody complex, forming an antibody-antigen-antibody enzyme complex.
- In this complex the antigen remains sandwiched between two antibodies.
- Free, uncomplexed antibodies are again washed out.
- Then, an appropriate
  chromogen or colour reagent (Indicator) is added to the well.
- If the secondarily-added enzyme-linked antibody has already bound with the antigen, the enzyme will react with the indicator generating a colour.
- This colour generation reveals that HIV antigen is present in the sample blood.

DAC ELISA TEST

- Detect the presence of HIV antibody in the human blood.
- HIV antigen is coated on the surface of the plastic well.
- The blood sample of the person who is suspected to have HIV infection is added to the well.
- If HIV antibody is present in it, it will interact with the antigen, forming an antigen-antibody complex.
- Uncomplexed antigens are now washed out.
- Then, a suitable colour reagent which is anti-human immunoglobulin in action, is linked to an enzyme and added to the well.
- If HIV antibody is present in the sample blood, it will react with indicator producing a colour.
- This colour generation therefore indicates the presence of HIV antibody in the sample blood.

RIA

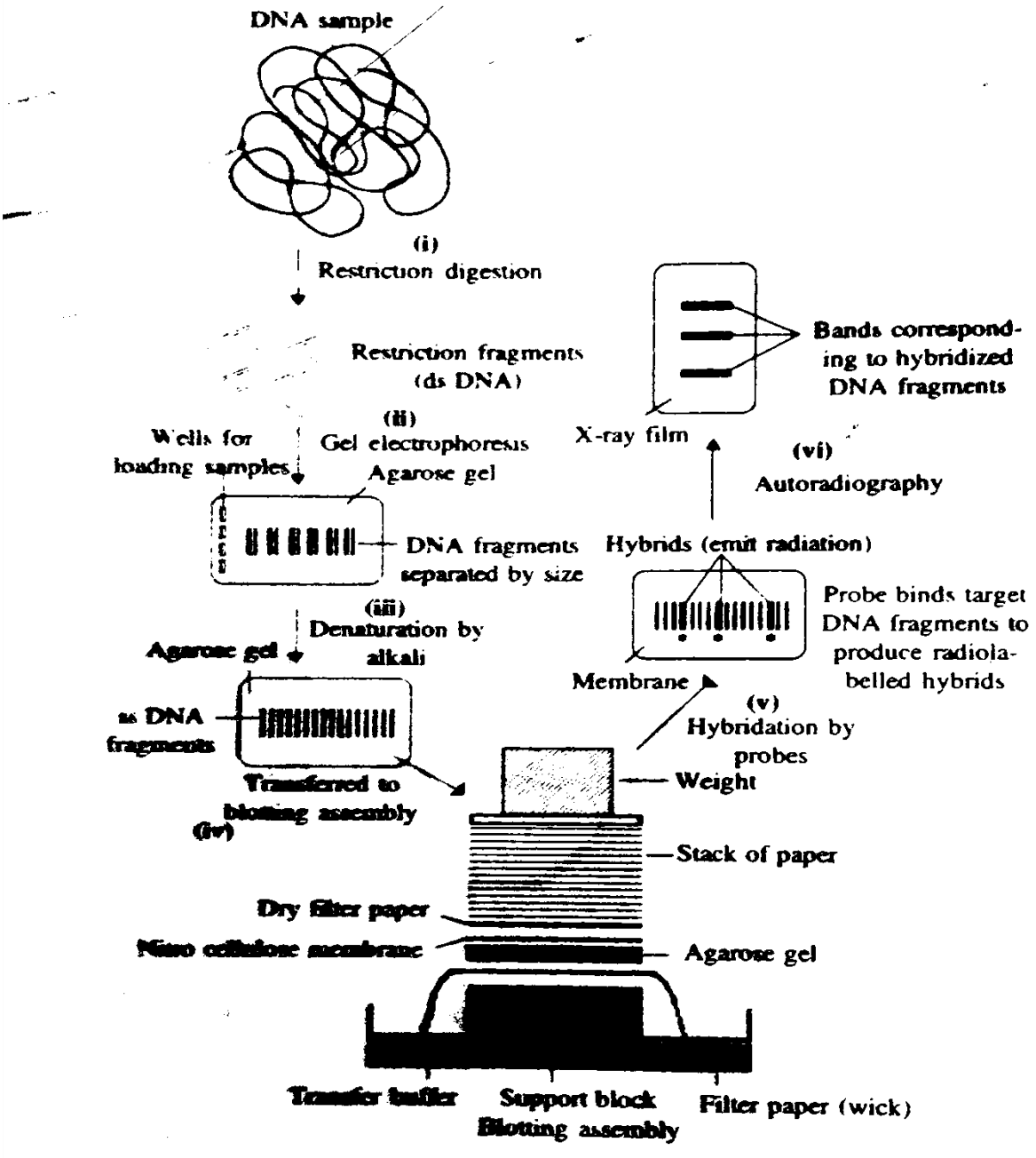
- Radioimmunoassay (RIA)
- Sensitive techniques for detecting antigen or antibody.
- The principle of RIA involves competitive binding of radio labelled antigen and unlabelled antigen to a high-affinity antibody.
- To perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine.
- This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another.
- Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added.
- This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites.
- As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free
  radiolabeled antigen.
- The bound antigens are then separated and the radioactivity of the free (unbound) antigen remaining in the supernatant is measured using a gamma counter.

Western blot

- Introduced by Burnette.
- Variant of Southern blotting.
- Commonest method of protein detection.
- The proteins of cell extracts are first separated by a special type of electrophoresis, called ***sodiumdodecyl sulphate-polyacrylamide gel electro- phoresis (SDS-PAGE).***
- SDS is negatively charged.
- It denatures proteins and makes them negatively charged.
- The negatively charged proteins then migrate to the $+^{ve}$ pole.
- The rate of this movement depends on their size.
- The electrophoretically separated proteins are then transferred from polyacrylamide gel and blotted on a suitable immobilizing matrix, such as nitrocellulose sheet or nylon sheet.
- Proteins strongly bind to nitro cellulose or nylon.
- The bound proteins are then probed for their detection.
- Different kinds of probes are used for hybridizing different kinds of proteins.
- Radioactive antibodies are used for antigens.
- Double - stranded radioactive DNA probes are used for DNA-binding proteins.
- Lectins are used for glycoproteins.

Southern blot

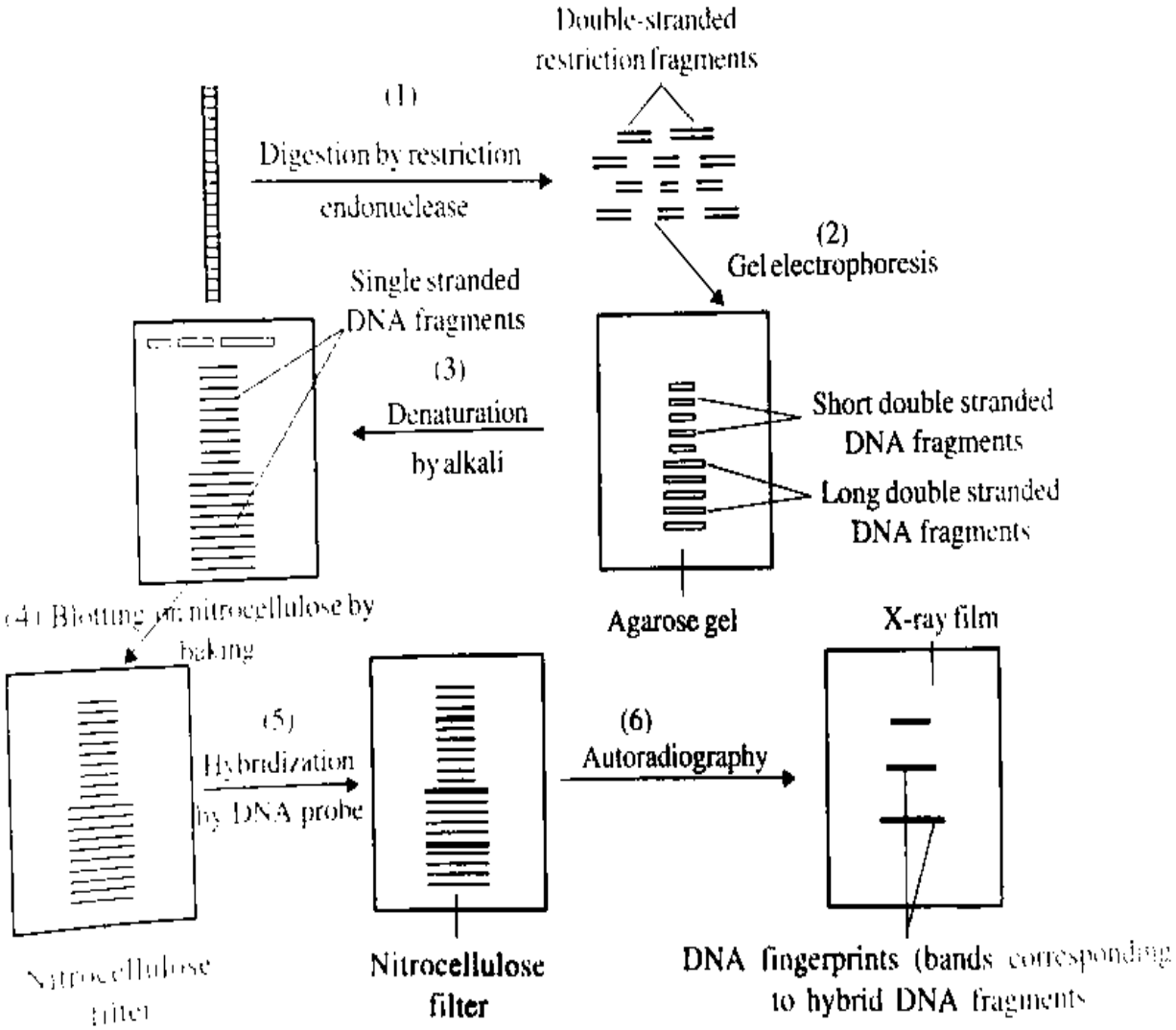
- Blotting technique of DNA hybridization.
- Developed by EM Southern
- DNA is seperated by electrophoresis
- DNA is denatured before blotting
- Nitrocellulose membrane is used
- Produces DNA – DNA hybrid



Northern blot

- Blotting technique of RNA hybridization.
- Developed by Alwine et al
- RNA is seperated by electrophoresis
- Denaturation step is altogether absent
- Nitrocellulose membrane is not generally used. Instead diazobenzyloxy – methyl paper
- Produces RNA – DNA hybrid

DNA Fingerprinting



DNA fingerprinting technique

Generation of B cell and T-cell response:

- Immune response – complex defence mechanism – initiated by the immune system – protect the body against pathogens

Cell - mediated response

- Antibody – independent ímmune mechanism.
- Mediated by T cells.
- Effective against fungi, parasites, intracellular viral infection,…
- There are many thousand kinds of competent T-cells.
- Each of them is specific to a particular antigen.
- When an antigen invades the body - antigen-presenting cells (APC), rush to it, ingest and degrade it into antigenic fragments and then bring it to T-cells – Presentation of antigen.
- A specific type of T-cell, especially sensitive to it, gets activated - ***sensitized T-cell***.
- Sensitized T-cell undergoes rapid and repeated proliferation and forms 1arge number of clones or colonies - This is known as ***clonal amplification***.
- Each clone differentiate into several sub-populations, such as ***effector cells and memory cells***.
- Effector cells include helper T-cells, cytotoxic T-cells, suppressor T-cells, initiator T-cells, amplifier T-cells and regulator cells.
- Some effector T-cells soon undergo rapid division and form ***killer cells***.
- Memory T-cells stay in the lymphatic station.
- All other cells leave the lymphatic organ and move to the site of infection or inflammation.
- Killer cells bind with the antigen or virus-infected target cells and secrete ***lymphokines and***
  some activating factors.
- Lymphokines are powerful toxins – directly destroy the antigen or kill the pathogen.
- Activating factors are of three main groups, ***transfer factors, chemostaxis factors and macrophage-activating factors (MAF).***
- Transfer factors - transforms non-sensitised T-cells to active killer cells.
- Chemostaxis factors - promotes inflammatory changes and attracts macrophages.
- MAF – Stimulates macrophages and promotes their phagocytic activity.
- On receiving appropriate cytokines, helper T-cells differentiate into two groups – T_H1 cells and T_H2 Cells.
- T_H1 cells activate macrophages and stimulate the differentiation and maturation of cytotoxic T cells.
- T_H2 cells are mainly involved in the activation and differentiation of B-cells into antibody producing plasma cells.

- Mature T_C cells will directly destroy virus-infected cells.
- Initiator T-cells are concerned with the recruitment of killer T-cells for quick action.
- Amplifier T-cells stimulate the activity of all kinds of T-cells, B-cells and lymphoid stem cells.
- Suppressor T-cells suppress or inhibit all kinds of immune responses by other lymphocytes.
- Regulator T-cells regulate the functioning of helpers and suppressors.
- Those regulators which inactivate suppressor T-cells are called ***counter suppressors***.
- Memory T-cells are concerned with storing the information about antigens.

Humoral response

- This is the antibody-dependent immune response.
- Mediated by B-cells.
- For the recognition of antigens, B-cells have specific receptors on their surface.
- When an antigen invades the body, antigen-presenting macrophages will ingest them and carry
  them to the location of B-cells.
- The naive B-cell, having receptors for the invading antigen, would recognize, ingest and process the antigen - activate the B-cell.
- The activated B-cell would undergo rapid multiplication and produce several clones -This is called clonal expansion.
- The cells of these clones differentiate into effectors, helpers, suppressors and memory cells.
- Effector B-cells soon undergo rapid multiplication and produce plasmablasts which, in turn
  mature and transform to active anibody-producing plasma cells.
- Helper B-cells promote the multiplication of all kinds of T cells.
- Suppressor B cells inhibit the rapid proliferation of effector B cells.
- Memory B-cells are functionally similar to memory T-cells - can store informations about the antigens they have encountered.
- Plasma cells do not normally leave the lymphatic system, but secrete specific immunoglobulins which serve as the arsenals of the chemical weaponry of immunity.
- They are carried to infected areas by blood or lymph.
- Antigen-antibody complexing and antigen-antibody interaction follow - inactivates or destroys the antigen by agglutination or precipitation, promotes their phagocytosis by macrophages.

Properties of B-cell and T-cell- epitopes

- Epitopes – immunologically active regions of an immunogen that bind to antigen specific membrane receptors on secreted antibodies.
- B cells recognize soluble antigens when it binds to membrane bound antibodies.
- They bind antigen that is free in solution.
- B cells rrecognize folded amino acid sequences.
- T cells recognize only peptides combined with MHC molecules on the surface of APC's & altered self cells.
- T cell recognizes linear amino acid sequences.

Cytokines

- Low - molecular weight chemical messengers, or Immunomedators.
- secreted by white blood cells.
- Functionally they are regulatory proteins or glycoproteins which mediate immune responses.
- They bind to specific receptors on the target cell membrane and induce signal transduction which ultimately triggers gene expression in target cells.
- The cytokine secreted by a particular cell binds to the receptor of the same cell and stimulates that particular cell, it is called ***autocrine***.
- The cytokine secreted by a particular cell acts on a target cell in the immediate vicinity, it is called ***paracrine***.

Functions of cytokines

- Accomplish the activation, proliferation and differentiation of B-cells and T cells.
- Promote the proliferation and differentiation of haematopoietic stem cells.
- Mediate the chemotaxis and tissue access of macrophage, neutrophils, eosinophils, etc, at the site of infection.
- Stimulate the growth, differentiation and activation of granulocytes.
- Mediate inflammation and innate immunity.
- Cytotoxic action against foreign antigens, foreign tissue grafts and cancer cells.

Examples of cytokines

1. **Interleukins**

- Produced by T cells

- Participate in the production of immune cells, interaction between leucocytes & inflammatory responses.
- Different classes of interleukins are IL-1, IL-2, IL-3,IL-4,…

2. MCSF

- Monocyte colony stimulating factor
- Secreted by macrophages, endothelial cells,..
- Acts on progenitors that are already commited to develop to monocytes.

3. **GCSF**

- Granulocyte colony stimulating factor
- polypeptide
- Produced by monocytes, endothelial cells,..
- Promotes the maturation of neutrophils.

4. GM – CSF

- Granulocyte monocyte colony stimulating factor
- Glycoprotein
- Secreted by activated T cells
- Promotes macrophage activity.

MODULE 8

IMMUNODEFICIENCY DISEASES, VACCINES & VACCINATION

Immunodeficiency diseases

- Immunodeficiency - Condition in which the immune system of a person fails to protect him from antigens, pathogens, or malignant cells.
- Occurs when the immune system becomes abnormal, or some of its constituents become defective or absent.
- Congenital immunodeficiencies – due to abnormalities in certain genes which control the formation of leucocytes or the production of immunglobulins.
- Acquired immunodeficiencies – due to prolonged pathogenic infection, malnutrition, effects of drugs,…

Primary Immunodeficiency diseases

- Disorders in which a part of the body's immune system is missing or malfunctioning.
- Also called congenital immunodeficiency disorders.

Bruton's disease

- X – linked agammaglobulinemia (XLA).
- Seen in male infants.
- Characteised by extreme deficieny or absence of B – lymphocytes & antibodies.
- B – cells fails to mature & become functional
- Caused by the mutation in the X – linked gene located in the long arm of X chromosome.
- Humoral immunity does not operate.
- Victims are susceptible to frequent infections.
- The level of serum immunoglobulins will be extremely low.

Di – George syndrome

- Congenital thymic aplasia (CTA).
- T – lymphocyte deficiency disorder.
- Starts during foetal development due to an abnormality in the 22^{nd} chromosome.
- Thymus will be absent or underdeveloped.
- Susceptible to viral & fungal infections.
- Can occur in both male & female.

- Physical abnormalities include
    1. Low set ears
    2. Small receding jaw bone
    3. Wide spaced eyes
- They may have endocrine & cardiovascular problems – seizures, congenital heart diseases, oesophageal atresia,…

SCID

- Inherited primary immunodeficiency disease.
- Typically present at infancy.
- Resulting in a weak immune system that is unable to fight off even mild infections, so it is considered to be a serious disease.
- It is caused by gene mutation in different genes that result in the defective development or malfunctioning of both B-cells and T-cells which, in turn, causes impaired humoral and cellular immune responses.
- SCID is a congenital disease, due to biochemical gene mutation that result in the non-production of enzyme *adenosine deaminase* - enzyme involved in development and maintenance of the immune system.
- SCID is inherited as an autosomal recessive pattern.
- Mostly expressed by male.
- SCID is usually recognized during the first year of life
- By using newborn screening test it is possible to detect SCID before symptoms appear.
- If the child don't get proper treatment, they deemed to die from infections before the age of two years.

Symptoms

- Occur in infancy and include serious life threatening infections, such as pneumonia, chronic diarrhea, oral thrush(infection caused by *candida* yeast)and many viral infections.

Treatments

- Gene therapy
- Hematopoietic stem cell transplantation
- Enzyme replacement therapy (PEG-ADA)

Secondary Immunodeficiency diseases

AIDS

- Perhaps the most fatal of all known secondary or acquired immunodeficiency disease is the acquired immunodeficiency syndrome (AIDS).
- AIDS is a much dreaded viral disease of human immune system.
- Interferes with the immune system, making the victims easiliy susceptible to other infections and tumours.
- The disease is most common among homosexual males, intra venous drug abusers, recipients of blood transfusion, etc.
- The causative agent of the disease in an RNA virus, called human immunodeficiency virus (HIV).
- AIDS is characterized by
  (1) extremely low count of T-cells
  (2) abnormally high ratio of suppressor T-cells (the cells which suppress the activity of other lymphocytes)
  (3) abnormally low ratio of helper T-cells (the cells which activate other lymphocytes, including B-cells).
- The major symptoms include
  Low -grade fever, coughing, shortness of breath, muscle-ache, fatigue, loss of weight, enlarged lymph nodes, brain damage heavy destruction to WBCs, etc.
  AlDS has a slow onset, but its damages are rapid an drastic.
- The commonest secondary diseases that kill AIDS Victims are *Kaposi's sarcoma,* B-cell lymphoma and a rare type of pneumonia.

  Channels of infection

  (i) Intimate sexual contact and sexual intercourse (homosexual and heterosexual, including vaginal oral and anal sex).
  (ii) Transfusion of contaminated blood.
  (ii) Parental transmission from infected mother to baby through transplacental circulation and breast-feeding.
  (iv) Sharing of hypodermic injection needles.
  (v) Organ transplantation and artificial insemination.
  (vi) Contact of the mucous membrane or bloodstream of a normal person with an HIV-containing bodily fluid, such as blood, semen, vaginal fluid, seminal fluid, or breast milk, from an infected person.
- Diagnosis of AlDS is possible through ELISA (enzyme-linked immunosorbent assay) test and Western blotting.

- These tests give positive results only 2 to 24 weeks after HIV infection.

Stages of HIV infection

Acute infection

- Lasts for several weeks.
- Its symptoms include fever, lymphadenopathy (swollen lymph nodes), pharyngitis (sore throat), rashes, myalgia (muscle pain), oral and oesophageal sores, etc.
- During this stage, large numbers of HIV may be present in the peripheral blood, and the immune system begins to respond to the virus by producing anti-HIV antibodies and cytotoxic lymphocytes. This process is called *seroconversion*.

Latency stage

- Involves little or no symptoms and it may last from two weeks to twenty years or more, depending on the individual.
- During this stage, the level of HIV in the peripheral blood falls very low. Still antibody production may continue.

Symptomatic HIV infection stage

- The final stage of HIV infection
- Low T-cell count.
- Various opportunistic infections, cancers and other conditions.

Autoimmune disease

Hashimoto's thyroiditis

- The disease is due to the production of autoantibodies and sensitized T cells, that are specific against thyroid antigens.
- Frequently affects middle aged women.
- The sensitized T-cells involved in it are concerned with delayed type hypersensitivity (DTH) reactions (TDTH cells).
- DTH response results in sever inflammatory reactions.
- The thyroid gland gets infiltrated by lymphocytes, macrophages and plasma cells.
- This leads to the formation of lymphocytic follicles and germinal centers, that are characteristic of lymph nodes, as a result thyroid develops goitre.
- Autoantibodies are also produced against thyroid globulin and thyroid peroxidase.

- Binding of autoantibodies to these thyroid protein products severely impairs iodine uptake, it results in hypothyroidism.

Grave's disease

- Autoimmune disease.
- Due to the production of agonistic autoantibodies.
- Characterized by Goitre : due to the autoantibodies which stimulate the growth of thyroid gland.
- ***Hyperthyroidism*** : due to the autoantibodies which stimulate the excessive production of thyroid hormone.
- ***Exophthalmus*** : due to the antibodies which stimulate the retro -orbital tissue around the eyeball.
- TSH from pituitary gland - stimulate the production of thyroid hormone.
- In grave's disease - autoantibodies are produced against TSH. Binding of these receptors by this autoantibodies mimics the action of TSH which stimulate the production of excess thyroid hormone.
- ***Long- acting thyroid stimulating antibodies*** (LATS) : autoantibodies continuously stimulate thyroid gland for hormone secretion.

Myasthenia gravis

- Myasthenia gravis is a serious autoimmune disease.
- Characterized by muscular weakness and a strong tendency for fatigue which comes on suddenly.
- It is caused by antagonistic autoantibodies, which bring about negative reaction by blocking the receptor of the neurotransmitter, acetyl choline.
- Acetyl choline receptors are abundant in skeletal muscles.
- Binding of autoantibodies with these receptors inhibits acetyl choline binding and induces complement-activated muscle lysis, resulting in progressive weakening of skeletal muscle response.
- In myasthenia, the body develops autoantibodies which interfere with the functioning of neuromuscular junctions and thereby block the transmission of impulses from nerves to muscles.
- In myasthenia, the autoantibodies inhibit binding of acetyl choline with the receptors on muscles.
- This blocks impulse transmission, with the result that depolarisation of muscles fails to occur and muscular contraction does not take place.

Systemic Lupus Erythematosus

- Autoimmune disease.
- The immune system of the body mistakenly attacks healthy tissue.
- Prevalent in female between 20 and 40 years of age
- It is characterized by butterfly shaped rashes on face, whole body rash, arthritis and kidney dysfunction.
- Individuals affected with this disease may produce autoantibodies against wide variety of self antigens, such as DNA, thrombocytes, RBC, leukocytes, clotting factor etc.
- Action of autoantibodies against these antigens can initiate complement reaction and immune complex mediated type III hypersensitivity reaction.
- The amount of circulating neutrophils decreases and occlusions of small blood vessels occur. These occlusions can cause vasculitis and widespread tissue damage.
- It can affect the skin, joints, kidneys, brain, and other organs.

Causes

1. Genetic
2. Environmental
3. Hormonal
4. Certain medicines

Signs and symptoms

1. Fatigue
2. Skin rashes
3. Fevers
4. Pain or swelling in the joints

Treatment

- Immunosuppressive drugs that inhibits the activity of the immune system.
- Hydroxychloroquine and corticosteroids (e.g., prednisone) are often used to treat SLE.

Vaccines & vaccination

- Vaccine is an immunogenic biological agent or preparation, which can provide immunity against a specific pathogen, infection or disease.
- Vaccines are usually preparations of attenuated (weakened), inactivated, or killed microorganisms or their products, which can increase resistance against infectious diseases.

- Vaccines can be prophylactic or preventive (to prevent or reduce the effects of a future infection by a natural or wild pathogen), or therapeutic or curative (Eg:-cancer vaccines).
- Stimulate the production of antibodies and cytokines and generate a primary immune response. During this, memory T-cells and memory B- cells will be produced.

Principle of vaccination

Attenuated vaccines

- These are the preparations of organisms, weakened by various methods, including genetic alterations.
- They produce only a mild, sub-clinical infection which can provide strong resistance and protection.
- These vaccines are not as safe as inactivated vaccines, because their reversion to virulent state is possible and so they can cause diseases in immunologically weak individuals.
- Oral polio vaccine, mumps vaccine, measles vaccine, whooping cough vaccine, etc. are live attenuated vaccines.
- Attenuation is the process of reducing the virulence of a pathogen.
- Attenuated vaccines induce both humoral and cell-mediated immunity.
- Many of these vaccines require a booster dose for effective disease prevention.
- Booster dose is a dose of antigen given after the primary dose to stimulate accelerated production of large amounts of antibody (e.g., polio vaccine).

Inactivated vaccines

- These are the vaccines in which the microorganisms are killed by various methods.
- They are safer than live attenuated vaccines.
- Examples are cholera vaccine, injectable polio vaccine, pertussis vaccine, etc.
- Inactivated vaccines are produced by the inactivation of virulent pathogens by chemical treatment or irradiation with gamma rays.
- They require multiple boosters.
- They are more stable than attenuated vaccines, and so they will not revert to virulent forms.
- These vaccines produce mainly humoral immunity.
- They may produce a general toxic effect in the organism, and sometimes induce allergies.

Toxoid vaccines

- Some bacteria release toxins (poisonous proteins) when they attack the body.
- The immune system recognizes these toxins in the same way that it recognizes other antigens on the surface of bacteria, and is able to mount an immune response to them.
- Some vaccines are made with inactivated version of these toxins - Toxoids.
- They look like toxins but are not poisonous.
- They triggers a strong immune response.
- ***Toxoid*** is an inactivated toxin whose toxicity has been suppressed either by chemical or heat treatment, while other properties typically immunogenecity are maintained.
- Toxins are secreted by bacteria, whereas toxoids are altered form of toxins which are not secreted by bacteria.
- ***Toxoid vaccines*** - Vaccines made from a toxin (poison) that has been made harmless but that elicits immune response against toxin.
- These vaccines are used when a bacterial toxin is the main cause of illness.
- Eg :- Crotalus atrox toxoid is used to vaccinate dogs against rattle snake bite.
- Toxoid vaccines are used to protect against
  1.Diphtheria
  2.Tetanus
- A Toxin is both toxic and immunogenic.
- A Toxoid is no longer toxic but immunogenic as the toxin from which it was derived.
- Some bacterial pathogens produces Exotoxins.
- Vaccines can be produced by purifying these exotoxins with the help of chemicals like formaldehyde to form a Toxoid.
- Toxoid vaccines induce anti-toxoid antibodies, which are also capable of binding to the toxin and neutralizing its effects.
- Eg:- diphtheria vaccines and tetanus vaccines.

Advantages

- Toxoid vaccines are safe because they cannot cause the disease.
- They prevent and there is no possibility of reversion to virulence.
- They are stable as they are less susceptible to change in humidity and light.

Disadvantages

- Toxoid vaccines tend not to be highly immunogenic unless large amounts or multiple doses are used.
- One problem with using larger doses is that tolerance can be induced to the antigen.

DNA vaccines

- DNA vaccine is a foreign DNA sequence, introduced to an organism to direct the synthesis of a protein.
- The protein, thus produced, forms an antigen.
- It activates the immune system of the host to elicit an immune response.
- DNA vaccination, also called genetic immunization.
- In this case,
  DNA does not directly serve as an antigen and so it does not directly induce immediate immune response. On the other hand, its genetic expression in the host results in the production of an antigenic foreign protein in the host and this protein induces an immune response.
- DNA vaccine is a third generation vaccine.
- DNA vaccination is a simple and straight forward technique, with easy method of vaccine production and a wide range of applicability.
- The technique is relatively more cost-effective and it does not require refrigeration and other costly and complex methods of storage, transportation & delivery.