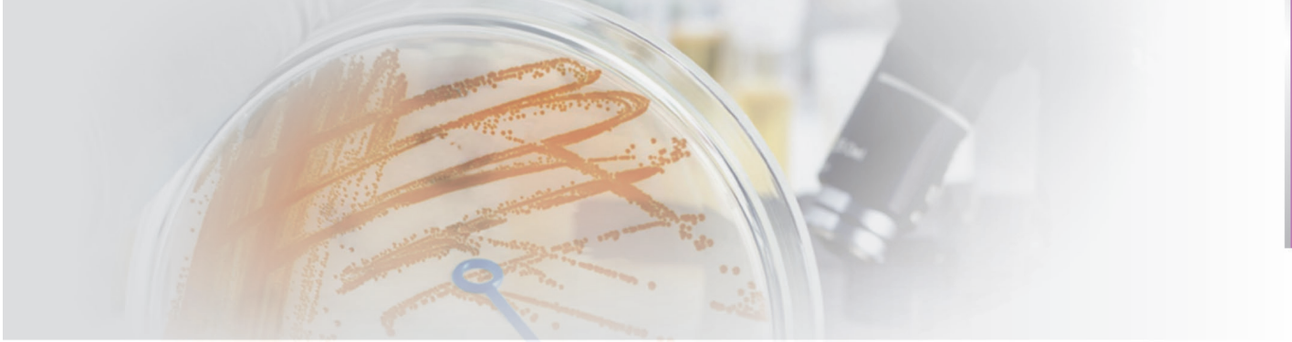

CHAPTER 1

General Microbiology and Immunity

MI 1.1 DESCRIBE THE DIFFERENT CAUSATIVE AGENTS OF INFECTIOUS DISEASES, THE METHODS USED IN THEIR DETECTION, AND DISCUSS THE ROLE OF MICROBES IN HEALTH AND DISEASE

LONG ESSAYS

1. Write in detail about the infectious diseases under the following headings. (1+3+3+3 marks)

A. Definition
B. Sources of infectious diseases
C. Modes of transmission of infectious diseases with examples
D. Prevention and control of common infectious diseases

A. Definition

- Infectious diseases are defined as disorders caused by pathogenic microorganisms like bacteria, viruses, parasites or fungi that can spread directly or indirectly (vector borne) from one individual to another.

B. Sources of Infectious Diseases

1. Humans

- Patient or Carrier.
- *Carrier:* A person who harbors the pathogenic microorganism without suffering any ill effects because of it
- *Healthy carrier:* Who harbors the pathogen without suffering from the disease
- *Convalescent carrier:* Who has recovered from the disease, but continues to harbor the pathogen in his body
- *Temporary carrier:* State lasts less than six months chronic carriage may last for several years
- *Contact carrier:* Acquires the infection from patient, paradoxical carrier acquires the infection from other carrier

2. Animals

- Zoonoses are infections transmitted to human beings from animals.
- *Cattle:* Brucellosis, tuberculosis, anthrax
- *Goat:* Brucellosis
- *Sheep:* Tetanus, anthrax
- *Dog:* Rabies

3. Insects

- *Insects:* Mosquito, ticks, mite, flies, flea and lice: Vectors
- *Mechanical vectors:* Only transmit the agent
- *Biological vectors:* The pathogen undergoes a part of the life cycle in the vectors. Anopheles mosquito in malaria.

4. Soil and Water

- Spores of tetanus bacilli and parasites like roundworm, hookworm.

5. Water

- Cholera, infective hepatitis.

6. Food

- Food poisoning by *Staphylococcus aureus*, salmonellosis.

C. Modes of Transmission of Infection with Examples

Mode of transmission	*Examples*
Contact	• *Direct:* Syphilis, Gonorrhea (Contagious diseases) • *Indirect:* Fomites–inanimate objects like pencils toys
Inhalation	• Droplet nuclei (1–10 μm), e.g. influenza, tuberculosis
Ingestion	• Ingestion of contaminated food, water, e.g. cholera, food poisoning, dysentery

Contd.

Mode of transmission	Examples
Inoculation	• Tetanus spores implanted in deep wounds • Rabies virus by dog bite • HIV and Hepatitis B transmitted through contaminated syringes and needles
Congenital Infection	• From mother to foetus is called vertical transmission

D. Prevention and Control of Common Infectious Diseases

I. Measures: Targeting the Reservoir of Infection

1. **Human reservoirs**
   - Diagnosis and treatment of cases
   - *Isolation:* Especially in easily transmitted diseases, e.g. COVID 19, influenza
2. **Animal reservoirs**
   - Treatment of infected animals
   - Destroying the infected animal (Rabies outbreak: killing of stray dogs)

II. Measures: Targeting the Mode of Transmission

1. **Water**
   - Boiling the water, filtering, or chlorination of water
   - Protection of water sources and through proper use of latrines to prevent faecal contamination of water
2. **Food**
   - Washing and thorough cooking of food items before eating
   - Hand washing and proper use of latrines
3. **Air**
   - Isolation of cases
   - Wearing face mask in crowded places
   - Maintaining proper ventilation in rooms
   - Regular maintenance of ventilation systems
   - To keep the mouth and nose covered when coughing or sneezing
4. **Vectors**
   - Vector breeding can be prevented by proper disposal of faeces and other wastes, eradication of breeding sites, and spraying insecticides to destroy mosquitoes

III. Measures: Targeting the Susceptible Host

- *Vaccination*
- *Chemoprophylaxis:* Refers to the antimicrobials taken by the susceptible hosts to prevent them from contracting an infection, e.g. travellers going to malaria endemic area can take chemoprophylaxis
- *Maintaining a healthy lifestyle:* Proper nutrition and exercise
- *Limiting exposure to reservoirs of infection*
  1. *Safe sex:* Use of condom to prevent transmission of HIV and other sexually transmitted infections
  2. Use of nets, repellants and wearing protective clothing to prevent diseases transmitted by insect vectors
  3. Hand washing with soap and water

2. Discuss the methods of viral cultivation with its uses/advantages, disadvantages, and examples. (10 marks)

- Viruses are obligate intracellular parasites.
- Viruses cannot be cultivated in artificial cell free media.

Methods of Viral Cultivation

1. Animal Inoculation

- Monkeys were used for the isolation of poliovirus, yellow fever; Infant white mice for coxsackie virus; Guinea pig for rabies, herpes.
- Growth is indicated by death, disease, or visible lesions.

Use

1. To study the pathogenesis, immune response, epidemiology and oncogenesis.

Disadvantages

1. Immunity may interfere.
2. Animals harbor latent viruses.
3. Difficulty in handling animals.

2. Embryonated Eggs

- Eggs should be incubated for 5–14 days and then candled to see if germination has occurred.
- *Chorioallantoic membrane*
  - On continued incubation local lesions called as pocks/plagues appear.
  - *Uses*
    1. Titration of viruses by application of serial dilution to CAM and calculating the number of pocks
    2. Titration of antiviral sera
    3. Study of viral morphology
    4. In isolation of the virus from the infected tissue (variola, fowl pox)
    5. In production of vaccinia virus for vaccine
    6. Study of chemotherapy
- *Allantoic cavity*
  - Rich yield of influenza virus, NCD, mumps which is then recognised by chick RBC agglutination.
  - Used when large quantity of virus is required for production of vaccine, study of chemical structure and preparation of Ag.
- *Amniotic sac inoculation*
  - For primary isolation of influenza virus from throat washings.

- *Yolk sac inoculation*
  - For the primary isolation and passage of members of psittacosis—lymphogranuloma, pneumonitis group, *Chlamydia*, *Rickettsia*.

Main Applications of Egg Inoculation

1. For propagation in the laboratory of the stock strains
2. For primary isolation of strains from the pathogenic material
3. For titration of the viruses and antiviral sera
4. For yielding large quantities of the viruses for vaccine production
5. In study of viral morphology

Advantages

1. Ease of handling
2. Many sites available
3. Lack of antibody production
4. Nutritionally rich
5. Self-contained

Disadvantages

1. Narrow range of viruses
2. Age of the egg must be known
3. Contamination

3. Cell/Tissue Culture

- Based on the origin, chromosomal characters and number of generations through which they can be maintained; cell cultures are of 3 types.

1. Primary Cell Culture

- Normal cells are freshly taken from the body and cultured.
- Capable of only limited growth and cannot be maintained in serial culture, e.g. monkey kidney, human embryonic kidney, human amnion, chick embryo.

Advantages

1. Cells in the primary culture possess a diploid component of the chromosome, characteristic of the tissue cells of the donor.
2. Acid accumulation in the medium is slow because of low metabolic activity and hence cells are easily maintained.

Disadvantages

1. Cultures have to be prepared *de novo* from fresh tissue samples which may be difficult to obtain.
2. Endogenous viruses latent in animal host could be hazardous and may give difficulties with cell growth and virus isolation.
3. Primary cultures from organs of different individuals of same animal may vary in their ability to support replication of the same virus.
4. Slow metabolism, slow change in pH.
5. Cell culture from different individuals of the same species may vary in their susceptibility to viral infection.

2. Diploid Cell Lines

- Established from human diploid fibroblasts (embryonic lung/neonatal foreskin)
- Remain virus sensitive for 20–50 passages
- May undergo 50 serial doublings before senescence
- MRC-5, WI-38, HEL, FS-9
- Subculture and feeding once a week
- Maintained by freezing early passage cells in liquid nitrogen.

3. Continuous Cell Lines

- By transformation (spontaneous or engineered) of cell strains or from tumours
- Loss of contact inhibition
- Indefinite subculture
- Chromosome number is not exact multiple of haploid number.
- High plating efficiency.
- Faster growth rate; subculture twice weekly.
- Frequent pH adjustment necessary, e.g.
  1. HEp-2: sq. cell ca. of larynx, HPV16 transfected
  2. McCoy: Mouse cell line of unknown origin
  3. Vero: African green monkey kidney cells

3. Describe the role of various methods of genetic transfer in antibiotic drug resistance. (10 marks)

- The emergence and spread of antibiotic resistance are the major problem among bacteria.
- The bacteria can acquire resistance via mutation or by horizontal gene transfer (HGT) of antibiotic resistance genes among the bacteria.

Mechanism in Antibiotic Resistance in Bacteria

1. Chromosome-mediated Resistance

- Mutation in the gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug.
- The frequency of spontaneous mutations is 1 in 10^7 to $10^{9,}$ cell division.
- To combat this, treatment with two or more drugs is given, e.g. drug resistance in *Mycobacterium tuberculosis.*

2. Plasmid-mediated Resistance

- Occurs in many species
- Responsible for resistance to multiple drugs
- Higher rate of transfer among the bacterial cells by conjugation
- Resistance plasmids (resistance factors, R factors).
  1. Extrachromosomal, circular, double-stranded DNA molecules that carry the genes for a variety of enzymes that can degrade antibiotics and modify membrane transport system

2. May carry one antibiotic resistance gene or may carry two or more of these genes
3. Plasmid mediated resistance
   i. Penicillins and cephalosporins β-lactamase cleavage of β-lactam ring
   ii. Aminoglycosides: Modification by acetylation, adenylylation, or phosphorylation

Structure of R factor (Fig. 1.1.1)

- Resistance transfer factor.
  - Conjugative plasmid
- R determinant
  - Resistance genes
  - Transposons

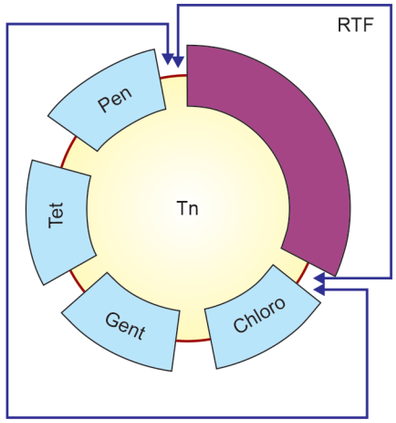

Fig. 1.1.1: Structure of R factor

3. Transposon-mediated Resistance (Fig. 1.1.2)

- Genes that are transferred either within or between larger pieces of DNA such as the bacterial chromosome and plasmids.
- A typical drug-resistance transposon is composed of three genes flanked on both sides by shorter DNA sequences.
- The three genes code for:
  1. Transposase, the enzyme that catalyzes excision and reintegration of the transposon
  2. A repressor that regulates synthesis of the transposase
  3. Drug resistance gene

Methods of Gene Transfer

Horizontal Gene Transfer: Conjugation, Transduction, Transformation

A. Conjugation

- Mating of two bacterial cells, during which DNA is transferred from the donor to the recipient cell.
- F (fertility) plasmid (F factor) carries the genes for the proteins required for conjugation.
- One of the most important proteins is pilin, which forms the sex pilus (conjugation tube).
- The bacteria with F plasmid are F+ and cell with no F plasmid is F^-.

Types of Conjugation

1. *F+ and F^- conjugation:* Recipient is F+ (Fig. 1.1.3).
2. Some F+ cells have their F plasmid integrated into the bacterial DNA and thereby acquire the capability of transferring the chromosome into another cell. These cells are called Hfr (Fig. 1.1.4).
3. *Hfr cells with F-:* F plasmid and a part of donor chromosome is transferred (Figs 1.1.5 and 1.1.6).

B. Transduction

- Transfer of genes (chromosomal or plasmid) among bacteria through bacteriophages.
- Types:
  1. Lytic cycle
  2. Lysogenic cycle

C. Transformation

- It is the transfer of DNA itself from one cell to another.
- Dying bacteria release their DNA which is in turn taken up by recipient cells or by the introduction of foreign DNA into bacteria in the laboratory.

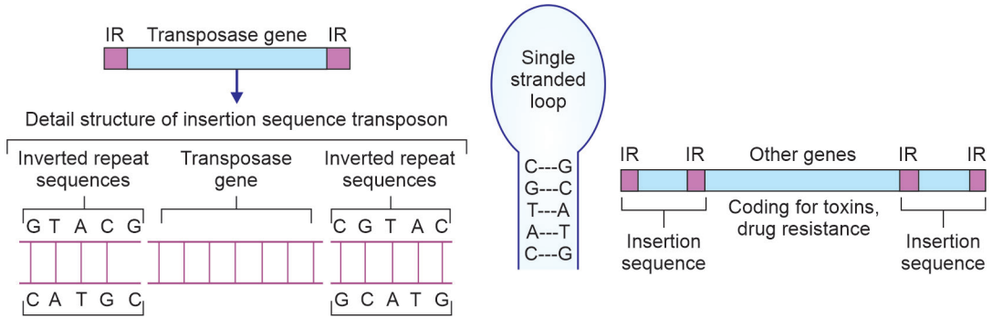

Fig. 1.1.2: Transposase

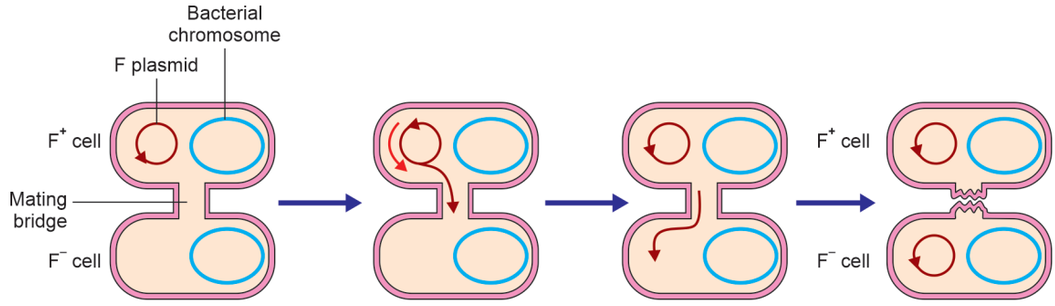

Fig. 1.1.3: Conjugation between F^+ and F^- bacteria

Fig. 1.1.4: Conversion of F^+ male into Hfr male by integration of the F plasmid into the chromosome

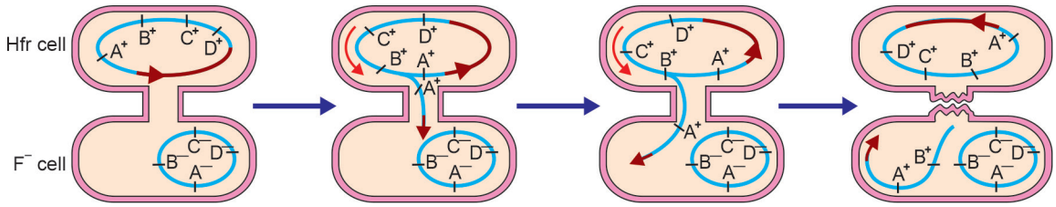

Fig. 1.1.5: *Conjugation between an Hfr and F^- bacteria*

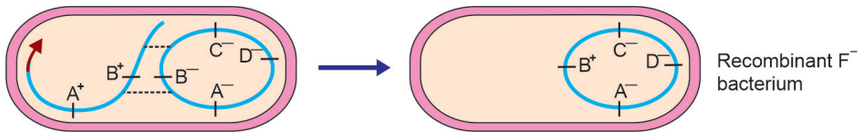

Fig. 1.1.6: *Recombination between the Hfr chromosome fragment and the F^- chromosome*

4. Describe the various methods in diagnosis of viral infections. (10 marks)

- The indications for laboratory diagnosis of viral infections are:.
  1. To confirm or diagnose the viral infection
  2. Screening in blood bank for HIV, hepatitis B and C
  3. Epidemiological surveillance of viral infections

Methods

Specimen Collection

- *Throat swab:* Influenza virus, SARS CoV 2
- *Nasopharyngeal aspirate or swab:* RSV, influenza and parainfluenza virus
- *Nasal swab:* Rhinovirus
- *Bronchial and bronchoalveolar wash:* Adenovirus and influenza virus
- *Rectal swab, stool:* Rotavirus, enterovirus, enteric adenoviruses
- *Urine:* CMV, mumps, rubella, measles, adenoviruses
- *Skin and mucous membrane lesions:* Enterovirus, HSV, VZV, rarely CMV
- *CSF:* Enterovirus, HSV
- *Blood:* CMV, HSV, VZV, enterovirus, adenoviruses

Specimen Transport

- At 2–8°C for a duration of <72 hours.
- For delay of more than 72 hours, viral specimens need to be stored at –70°C.
- Specimens should be transported in viral transport medium.

Microscopic Examination

1. Light Microscopy

- *Inclusion bodies:* The aggregates of many virus particles, seen in nucleus, cytoplasm, e.g. intranuclear inclusion in herpes, Negri body of rabies virus in the corneal smear
- Multinucleated giant cells (Tzanck smear for herpes virus), RSV, measles.

2. UV Microscopy: Direct Immunofluorescence

- Fluorescent antibody staining of virus in infected cell.
- Rabies Ag in corneal smear, respiratory viruses, adenovirus in conjunctival smears.

3. Electron Microscopy

- Virus identified by size, morphology, immune EM.
- Faeces
  - Rotavirus—wheel shaped
  - Astrovirus—star shaped

Rapid Diagnosis Based on the Detection of Viral Antigens

- By ELISA (enzyme-linked immunosorbent assay), ICT (immuno chromatography test), ELFA (Enzyme-linked fluorescent antibody assay)
- Specimens used for various viruses' identification are:
  1. *Serum:* HBsAg, HBeAg, dengue NS1 Ag
  2. *Nasopharyngeal swab:* SARS CoV 2
  3. *Nasopharyngeal aspirate:* RSV, influenza A and B, parainfluenza, Adenovirus
  4. *Faeces:* Rotaviruses, enteric adenoviruses, astrovirus
  5. *Skin:* HSV, VZV

Detection of Viral Antibodies

- Widely used method in the diagnosis of viral infections.
- By ELISA, CLIA, ELFA, ICT, Immunodot.
- IgM or 4-fold rise in IgG titre.
- Antibodies to HIV1/2, hepatitis B, hepatitis C, dengue.

Detection of Viral Nucleic Acid

- Viral genome or mRNA can be detected in patients' blood, tissue with c DNA, c RNA as probe (PCR).
- Methods.
  1. PCR
  2. Reverse transcriptase PCR (for RNA viruses)
  3. Real-time PCR
- Advantage.
  1. More sensitive and specific

Virus Cultivation

- There are three methods of cultivation.
  1. *Cell culture:* Growth is indicated by cytopathic effect (CPE) haemadsorption, immuno-fluorescence
  2. *Embryonated eggs:* Growth is indicated by pocks on CAM, haemagglutination, inclusion bodies
  3. *Animals:* Growth is indicated by disease or death

Cell Cultures

- Cell cultures are most widely used for virus isolation
- There are 3 types of cell cultures:
  1. *Primary cell lines:* Monkey kidney cell line
  2. *Semi-continuous cell lines:* Human embryonic kidney and skin fibroblasts
  3. *Continuous cell lines:* HeLa, Vero, Hep2
- Identification in cell culture
  1. *Cytopathic changes (CPE):* change in shape, size, or fusion of virus infected cell (syncytia). Time taken to produce CPE, type of cell helps in presumptive identification
  2. *Haemadsorption:* Attachment of erythrocytes to the surface of virus infected cells, e.g. mumps, influenza, parainfluenza (haemagglutinin protein)
  3. *Interference:* with formation of CPE by second virus. Rubella and ECHO virus
  4. *Decrease in acid production in infected cells detected by phenol red indicator:* Enteroviruses
  5. *Definitive identification:* CFT, HI, neutralisation, fluorescent antibody, ELISA

5. Briefly describe the various methods in diagnosis of fungal infections. (10 marks)

- Laboratory diagnosis of fungal infections is done to confirm.
  - Clinical suspicion
  - Choose specific antifungal therapeutic regimen
  - Monitor course of disease
  - Confirm mycological cure

Methods

Specimen Collection

- It depends on the type of infection

1. Superficial Mycoses

- Skin scraping (*Malassezia* infections, *Tinea*)
- Hair (*Piedra*, *Tinea capitis*, *Tinea barbae*)
- Nail (*Tinea unguium*)
- Mucous membrane (Candidiasis)
- Scrapings, swabs from the lesions
- *Corneal ulcers:* Corneal scrapings

2. Subcutaneous Mycoses

- Pus, aspirate from nodules
- Swab from the nodules
- Biopsy materials
- Skin scrapings from the superficial part of subcutaneous lesion
- Nasal washings, biopsy of polyp (Rhinosporidiosis).

3. Systemic Mycoses

- *Respiratory infections:* Sputum (early morning), tracheal aspiration, bronchial brushings, bronchoscopy specimen, percutaneous lung biopsy
- *Urinary infection:* Urine, clean–catch specimen in a sterile container

- *CNS infection:* Cerebrospinal fluid (CSF)
- *Blood:* biphasic BHIA broth or automated methods like BacT/alert

Microscopy

1. KOH mount

- *Mycotic elements seen in KOH mount:* Yeast cells, arthrospores, hyphae

Principle

- KOH digests protein debris, clears keratinised tissue, so fungi present in specimen can be seen more readily. The chitinous cell walls of fungi are somewhat resistant to action of KOH.

Procedure

- A drop of KOH (10–20%) is placed in the centre of clean glass slide. The material to be examined is added and mixed. A cover slip is placed over the preparation and slightly warmed over the flame and examined under low power (Fig. 1.1.7).

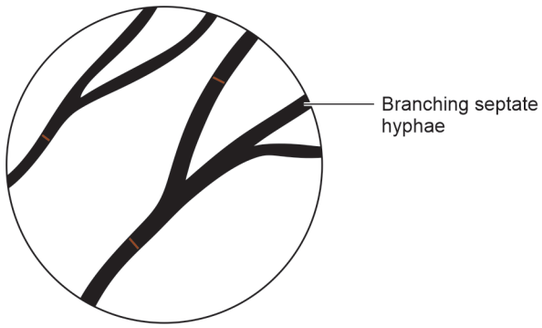

Fig. 1.1.7: KOH mount showing branching septate fungal hyphae

Other Modifications of KOH mount

1. KOH with blue black ink (2:1)
2. KOH with DMSO (dimethyl sulfoxide)
3. Calcofluor white + KOH

2. India Ink

- Detects the encapsulated yeast cells of *Cryptococcus neoformans.*
- Negative stain which creates a dark background against which yeast cells are surrounded by a clear halo (capsule which resists the stain) (Fig. 1.1.8).

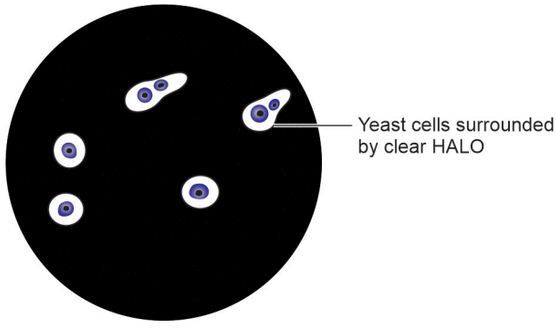

Fig. 1.1.8: India ink positive for *Cryptococcus* spp.

3. Gram's Stain

- Mainly done for the detection of yeast and yeast like budding cells which appear gram-positive.

4. Histopathology of Biopsy with Special Stains

- Haematoxylin and eosin stain
- Periodic acid-Schiff (PAS)
- Grocott's methenamine silver (GMS) stain
- Advantages:
  1. Fungus in tissue sections is almost diagnostic of mycotic infection.
  2. Tissue response of the host can also be studied.

Culture

- *Medium used:* Sabouraud dextrose agar (SDA)
- If the specimens are contaminated (sputum, antibiotics such as chloramphenicol), gentamicin (0.04 mg/ml) is added to SDA.
- Cycloheximide (0.5 mg/ml) can also be incorporated for the isolation of dermatophytes in order to get rid of saprophytic fungi.
- *Other media:* BHI agar, dermatophyte test medium, Caffeic acid agar, potato dextrose agar
- Incubated at 25–30°C for 4 weeks.
- *Identification*
  - Macroscopic features
    1. *Rate of growth*
       - Rapid growers (<5 days): Saprobes, opportunistic fungi
       - Intermediate growers (6–10 days): Dermatophytes, subcutaneous fungi
       - Slow growers (>11 days): Systemic, subcutaneous fungi
    2. *Pigment:* Surface pigmentation and pigmentation on reverse
    3. *Texture:* Glabrous (waxy), velvety, yeast like cottony, granular (powdery)
    4. *Topography:* Flat, folded, rugose, cerebriform, verrucose
       - Microscopic features
       - Lactophenol cotton blue (LPCB) teased preparation

Non-culture Approaches

- Antibody, antigen assays including detection of glucan (*Candida* spp.), mannan (*Aspergillus* spp.), enolase, proteinase by ELISA, ICT.
- *Metabolite detection assays:* mannose, arabinitol by gas-liquid chromatography
- *Molecular identification:* PCR

Examination of Fungal Growth on Primary Media–Yeast

- *Microscopic Examination:* Gram's stain, India ink
- *Biochemical Tests:* Sugar assimilation, fermentation test, urea test
- *Special tests:* Germ tube test, chlamydoconidium formation test

6. Discuss the determinants of bacterial virulence. (10 marks)

- *Pathogenicity:* It refers to the ability of the organism to cause disease.
- *Virulence:* It is the quantitative measure of pathogenicity.
- Virulence of a microbe is determined by its virulence factors.

Determinants of Bacterial Virulence

1. Transmission from an External Source into the Portal of Entry

- *Human to human:* Gonorrhea, syphilis
- *Non-human to human:* Soil, water, animals, fomites

2. Adherence to Cell Surfaces

- Pili, capsule, glycocalyx allow the bacteria to adhere to cell surfaces. These molecules are called as adhesins.

3. Invasion, Inflammation and Intracellular Survival

Invasion

i. Enzymes

- *Collagenase, hyaluronidase:* Allow the bacteria to spread through subcutaneous tissue by degrading collagen and hyaluronic acid, e.g. *Streptococcus pyogenes*
- *Coagulase:* Accelerates the formation of fibrin clot and coats the organism. *Staphylococcus aureus*
- *IgA protease:* Produced by *N. meningitidis, H. influenzae and S. pneumoniae*
- *Leukocidins:* Destroy both neutrophils and macrophages

ii. Other factors

- *Capsule:* antiphagocytic
- *Cell wall proteins of gram-positive cocci:* Antiphagocytic. M protein of *S. pyogenes* and protein A of *Staphylococcus aureus*

Inflammation

- An important host defense induced by the presence of bacteria in the body.
- *Pyogenic inflammation:* Defense against pyogenic bacteria such as *S. pyogenes*, consists of neutrophils, antibody and complement
- *Granulomatous inflammation:* the defense against intracellular granuloma producing bacteria such as *M. tuberculosis*, consists of macrophages and CD4 cells

Intracellular Survival

- *Mycobacteria, Legionella, Brucella* and *Listeria* spp.

Mechanism

- Inhibition of fusion of phagosome with lysosome.
- Inhibition of acidification of the phagosome, reducing the activity of the lysosomal degradative enzymes.
- Escape from phagosome into the cytoplasm.

4. Toxin Production

- Bacteria produces exotoxins and endotoxins.
- Mechanism of action of exotoxins.
  1. *Diphtheria toxin:* Inhibition of protein synthesis by ADP ribosylation of elongation factor 2.
  2. *Tetanus exotoxin:* Neurotoxin, prevents the release of inhibitory neurotransmitter glycine
  3. *Botulinum toxin:* Neurotoxin, blocking the release of Ach at the synapse producing flaccid paralysis
  4. *Toxic shock syndrome toxin (TSST):* Super antigen produced by *Staphylococcus aureus* causes over production of cytokines
  5. Heat labile enterotoxin of *E. coli*, cholera toxin causes watery diarrhoea by stimulating adenylate cyclase and resultant increase in the concentration of cyclic AMP
- Mechanism of action of endotoxin.
  1. Activates macrophages to produce IL 1, TNF and nitric oxide—fever (IL 1), Hypotension, shock and impaired perfusion of the internal organs (bradykinin, nitric oxide)
  2. Activates complement to produce C3a and C5a—inflammation and tissue damage
  3. Activates Hageman factor (coagulation system): DIC resulting in thrombosis, petechial rash and tissue ischaemia

5. The presence of plasmid borne, or bacteriophage borne genes coding for some virulence factor. Enterotoxin of *E. coli* and *Staphylococcus* (plasmid) and toxin of *C. diphtheriae* (bacteriophage).
6. Communicability: Ability of the organism to spread from one host to another.
7. The occurrence of an appropriate route of infection: Streptococci can establish infection whatever be the route of infection whereas *Vibrios* are effective only orally.

SHORT ESSAYS

1. Describe in brief the steps involved in diagnostic microbiology in the diagnosis of skin and soft tissue infections. (5 marks)

- The laboratory diagnostic approach to the diagnosis of skin and soft tissue infections is as follows:.

1. Specimen Collection

- Pus from wound collected by sterile swab.
- Pus from abscess collected by incision and drainage or needle aspiration.

- Subcutaneous infections: From the base of the lesion or biopsy of the deep tissues.
- Skin scrapings, plucked hair or nail clippings in suspected fungal infections.

2. Microscopy

- *Gram staining:* Gram's stain will help study the inflammatory response and morphology of the bacteria causing the infection (Fig. 1.1.9)

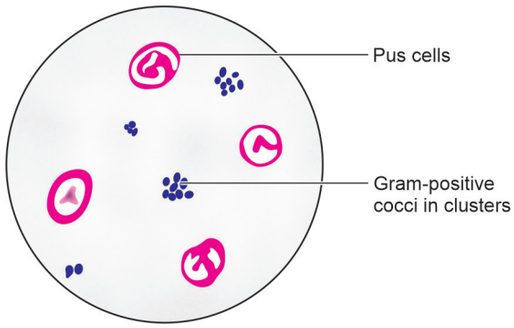

Fig. 1.1.9: Gram's stain of pus showing pus cells with gram-positive cocci arranged in clusters

- *KOH mount for suspected fungal infections:* Yeast cells or hyphae of mold
- *Tzanck smear of the vesicle fluid:* HSV, VZV

3. Culture

- *Bacteria:* Aerobic, anaerobic culture
- Fungal Culture.
- Identification of the growth based on macroscopy, microscopy, biochemical tests.
- Antimicrobial susceptibility testing.

2. Describe in brief the role of blood culture in the diagnosis of blood stream infections. (5 marks)

- Bloodstream infections are characterized by presence of bacteria in blood (bacteremia, septicemia)
- The diagnosis of bloodstream infections relies on isolation of the causative agent in the blood by performing blood culture.

Blood Culture

Specimen Collection

- Blood for culture
- Volume of blood in suspected bacteraemia
  - Ideally 2 sets of blood culture bottles (*aerobic and anaerobic*) has to be collected from two separate venipuncture sites after disinfection of the site.
  - Adults: 8–10 ml of blood per bottle.
  - Pediatrics/neonatal patient: 1–2 ml of blood per bottle.
- *Timing of collection:* Before starting antimicrobial therapy

Specimen Transport

- The inoculated blood culture bottle needs to be transported immediately to the laboratory
- In case of delay, inoculated bottles can be kept at 35^0C or in the incubator

Methods of Culture

1. Conventional Medium

- BHI broth or Castaneda medium.
- *Blood:* broth is 1:5 dilution to dilute the antibacterial substances in blood
- Inoculated bottles are incubated at 35°C for 7 days with periodic subculture onto blood agar and McConkey agar.

2. Automated Systems: BACTEC, BacT/ALERT

- Growth is continuously monitored, and reading is recorded every 15–20 minutes.
- When the growth is detected (CO_2 levels), the system gives a positive signal.
- Then the bottle is removed and processed similarly as done for conventional bottles.
- *Advantages:* Faster isolation and increased sensitivity

3. Antimicrobial Susceptibility Testing

- Done by disk diffusion method or automated system.
- *For endocarditis:* Minimum inhibitory concentration (MIC) determination should be done.

3. Describe the role of microbiology in the diagnosis of respiratory tract infections. (5 marks)

- The laboratory diagnosis of respiratory infections relies on confirming the clinical diagnosis by identifying the etiological agent and performing antimicrobial susceptibility for bacterial agents.

Specimen Collection

For URTI

- *Throat swab:* 2 swabs (microscopy, culture), e.g. *Corynebacterium diphtheriae.*
- Nasopharyngeal aspirate for viral diagnosis (Influenza, RSV, SARS CoV 2 or for *B. pertussis).*

For LRTI

- Sputum, induced sputum, tracheal aspirate, bronchoalveolar lavage (BAL).

Microscopy

1. Albert Staining

- On throat swab (Fig. 1.1.10).
- *In suspected cases of Bacterial pharyngitis:* Diphtheria caused by *Corynebacterium diphtheriae*

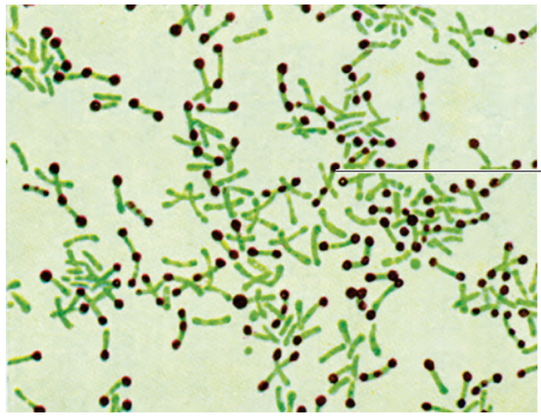

Fig. 1.1.10: Albert's stain showing *Corynebacterium diphtheriae*

2. Gram's Staining

- Of throat swab in bacterial pharyngitis due to Group A *Streptococcus*: pus cells along with gram-positive cocci in chains.

3. Gram's Stain of Sputum/BAL

- Pus cells with the causative bacteria of typical Pneumonia (Fig. 1.1.11).

4. Acid Fast Staining

M. tuberculosis

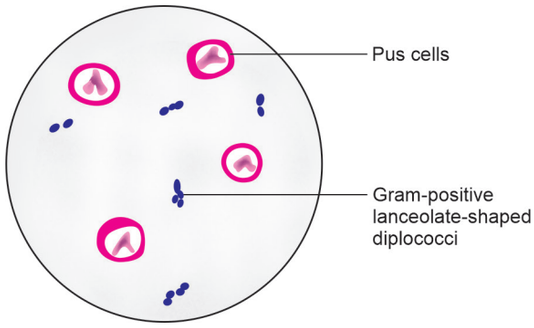

Fig.1.1.11: Gram smear of sputum with pus cells and pneumococcis

5. GMS Stain

Pneumocystis jirovecii

6. Immunofluorescence Microscopy of Nasopharyngeal Aspirate

Respiratory viruses.

Culture

1. For isolation of the bacterial agents of typical pneumonia: Blood agar, chocolate agar and MacConkey agar
2. For isolation of *C. diphtheriae*: Loeffler's serum slope and potassium tellurite agar
3. For *M. tuberculosis*: LJ medium and incubated for up to 6–8 weeks, mycobacteria growth indicator tube.
4. For isolation of fungal pathogens: Sabouraud dextrose agar
5. For isolation of viruses: Appropriate cell lines.

Serology

- Detection of antibodies
- *Mycoplasma:* Cold agglutination test, ELISA formats are available
- Chlamydial antibodies in serum: Micro-IF and CFT.

Molecular Tests

- PCR
- Biofire

4. Add a note on non-cultivable methods in the diagnostic microbiology (5 marks)

Non-cultivable Methods in the Diagnostic Microbiology

Rationale

These methods are useful for:

- Fastidious or slow-growing organisms
- Organisms which cannot be cultured.
- Biosafety concerns with organisms like *Mycobacterium tuberculosis* or *Coxiella burnetii.*
- *Rapid presumptive diagnosis of infections.*

Methods

Microscopy

A. Wet mount

1. *KOH mount:* For fungi.
2. *Hanging drop of stool:* For presumptive identification of *Vibrio cholerae.*
3. *Wet mount stool:* Ova, cysts of parasites.

B. Stained smears

1. *Gram's stain:* Bacteria, yeast.
2. *Auramine O stain: Mycobacterium tuberculosis.*
3. *Fluorescent antibody staining:* On clinical specimen for viruses, ANA.

Antibody Immunoassays

- For retrospective diagnosis of infections after viable microorganisms or nucleic acid have disappeared.
- Demonstration of seroconversion has higher specificity.
- Faster result and safer compared to culture methods for some organisms (e.g. *Coxiella burnetii*).
- Can also rule out acute infection based on serological evidence of previous exposure and immunity.
- Disadvantage:
  1. False-negative IgM
  2. Cross reactions
  3. Sensitivity varies with age
  4. Immunodeficiency

Serology for Antibody Detection

- HIV, hepatitis B, C, syphilis, cytomegalovirus, toxoplasmosis, dengue, chikungunya.

Antigen Detection

- Cryptococcal antigen detection in serum and cerebrospinal fluid, galactomannan antigen in invasive aspergillosis, *S. pneumoniae* antigen.
- Rapid test, results within 15 minutes.
- *Assays for both antigen and antibody:* Dengue virus NS1 antigen with IgM/IgG, or HIV antigen/antibody screening testing, offer reduced diagnostic window periods and enhanced sensitivity and specificity

Molecular Based Methods

High sensitivity and specificity

1. PCR
2. LPA
3. NASBA

5. Add a note on molecular methods available along with the principle for the diagnosis of infectious diseases. (5 marks)

Molecular Methods in Diagnostic Microbiology (Table 1.1)

6. Hanging drop of rice water stool shows actively motile bacilli with darting motility.

A. Name the appendage responsible for the motility in bacteria. (1 mark)

- Flagella is responsible for the motility in bacteria.

B. Discuss the properties, structure, functions, and demonstration of this appendage. (1+2+1+2 marks)

- Flagella are:.
  - Long, whiplike/filamentous unbranched appendages.
  - Organs of locomotion.
  - Present only in motile bacteria.

Properties

1. *Longer than bacteria:* 3–20 μm long; 12–30 nm breadth.
2. Composed of the protein flagellin.
3. Heat labile.
4. *Highly antigenic:* Flagellar antigen is called 'H' antigen.

Table 1.1.1 Molecular methods in diagnostic microbiology

Molecular method	Principle	Applications
Polymerase chain reaction	• Involves extraction of DNA from the bacteria by boiling method or commercial kits • Amplification of DNA ✦ Denaturation ✦ Primer annealing ✦ Extension ✦ Detection of the amplified product by Gel electrophoresis	• More sensitive, specific • Useful for fastidious, non-cultivable organisms • Detection of antibiotic resistance genes • Drawbacks ✦ Qualitative ✦ Detects both live and dead organisms ✦ Liable to contamination
Reverse transcriptase PCR	• RNA—reverse transcriptase forms c DNA and the rest is similar to PCR	• For detection of RNA viruses, 16S RNA of organisms
Nested PCR	• The first set of primers amplify a target sequence, and the second set of primers amplify a region within the first target sequence	• Very specific and alleviates false-positive reactions • Disadvantage: higher contamination rates
Multiplex PCR	• Two or more unique target sequences can be amplified simultaneously	• Diagnosis of respiratory infections, meningitis targeting the aetiological agents

Contd.

Table 1.1.1 Molecular methods in diagnostic microbiology

Molecular method	*Principle*	*Applications*
Real-time PCR	• The amplified target DNA is detected by fluorescently labelled probes as the hybrids are formed. The increase in fluorescence versus cycle number produces amplification plots • Amplification and product detection can be accomplished in one reaction • Quantification can be done	• Prognostic value: viral load—HIV, HBV, HCV • To detect the development of drug resistance, e.g. MRSA, VRE
LAMP (Loop Mediated Isothermal Amplification)	• At constant temperature • Auto-cycling strand displacement DNA synthesis that is performed by a DNA polymerase and a set of two inner and two outer primers	• Genotyping of HBV, HCV • Mutation in HIV, mycobacteria • Detection of HPV subtypes

Structure (Fig. 1.1.12)

- Has 3 parts.

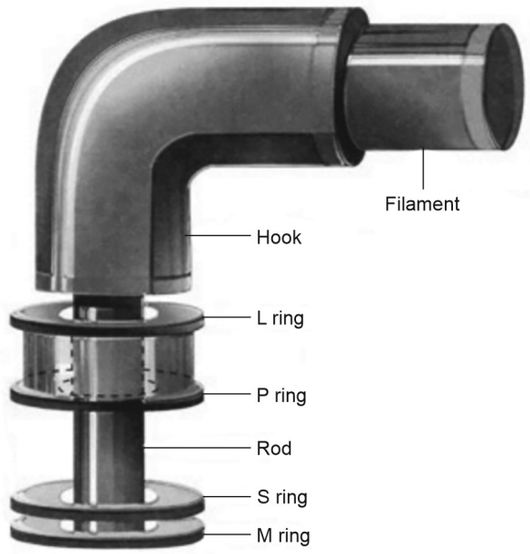

Fig. 1.1.12: Structure of flagella

1. Filament

- It is the part extending to exterior.
- Composed of proteins called flagellin.

2. Hook

- Curved sheath.
- Connects filament to cell.

3. Basal Body

- Anchors flagellum into cell wall and membrane.
- Flagellar arrangements (Fig. 1.1.13).
  - i. *Monotrichous:* Single flagellum at one end: *Vibrio cholerae*
  - ii. *Lophotrichous:* Small bunches arising from one end of cell: *Bartonella bacilliformis*

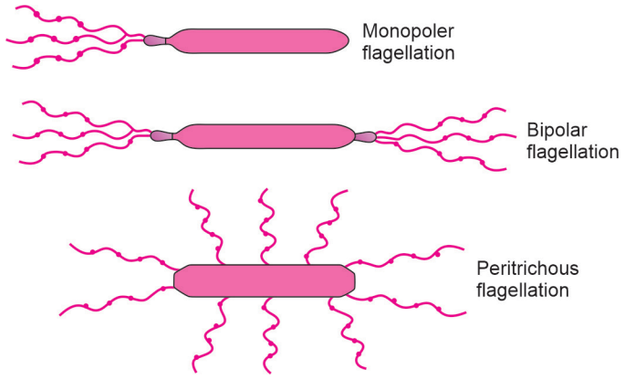

Fig. 1.1.13: Arrangement of flagella

  - iii. *Amphitrichous:* Flagella at both ends of cell: *Spirillum minor*
  - iv. *Peritrichous:* Flagella dispersed over surface: *Escherichia coli*

Functions

1. Organs of locomotion.
2. Locomotion helps the bacterium in the following ways:
   - To move to words the areas of better nutrition.
   - To move away from unfavourable conditions.
3. *Virulence:* Penetration and spread of infection for example: *E. coli* and *Proteus* cause UTI.
4. Antibody to H antigen is detected in Widal test for diagnosis of enteric fever.

Demonstration

Direct Method

1. Electron microscope.
2. *Special flagellar staining:* Leifson's staining (silver impregnation).

Indirect Method

1. Hanging drop Method.
2. Growth in semi-solid agar.

3. *Craigie's tube:* U-shaped tube with semisolid agar.
4. Swarming growth on solid media.
5. Dark ground illumination.
6. By demonstration of H antigen with specific antiserum (agglutination tests).

7. Describe the common vector borne infectious diseases under the following headings.

A. Define a vector and a carrier with examples (3 marks)

B. Vectors for transmission of dengue, JE, malaria (2 marks)

A. Definitions and Examples

Parameter	*Definition*	*Examples*
Vector	Living organisms that can transmit infectious pathogens between humans, or from animals to humans	1. Mosquito i. *Aedes:* Chikungunya, Dengue, Lymphatic filariasis, rift valley fever, yellow fever, Zika ii. *Anopheles:* Lymphatic filariasis, malaria iii. *Culex:* Japanese encephalitis, lymphatic filariasis, West Nile fever 2. Aquatic snails: Schistosomiasis (bilharziasis)
Carrier	Persons/animals that harbour the infectious agent in the absence of any clinical symptoms and shed the organism from the body via contact, air or secretions and risk transmission (inadequate treatment or immune response)	1. *Incubatory carriers:* Measles, mumps, polio, diphtheria, pertussis 2. *Healthy carriers:* Polio, cholera, salmonellosis, diphtheria

B. Vectors for Transmission of Dengue, Japanese B Encephalitis, Malaria

Disease	*Vector*
Dengue	Mosquito—*Aedes*
Japanese encephalitis (JE)	Mosquito—*Culex*
Malaria	Mosquito—*Anopheles*

8. A 20-year-old female is diagnosed with cystitis. Which cell structure of the causative bacteria is responsible for adherence to the uroepithelium? Briefly explain the significance of this cell structure. (1 + 4 marks)

Cell Structure of the Causative Bacteria for Adherence to the Uroepithelium

- Pili or fimbriae.

Pili

- Fine, hair-like appendages that are thinner than flagella and not involved in motility, called as fimbriae or pili (singular fimbria or pilus).
- Pili are made up of protein called pilin.
- Antigenic (but antibodies against fimbrial antigens—not protective).

Significance

- They mediate the attachment of bacteria to specific receptors on the human cell surface, which is a necessary step in the initiation of infection for some organisms, e.g. *Neisseria gonorrhoeae, P. fimbriae* in uropathogenic *E. coli.*
- Specialised kind of pilus, the sex pilus, forms the attachment between the male (donor) and the female (recipient) bacteria during conjugation.
- Transfer of virulence factors or antibiotic resistance genes among bacteria.

9. A 25-year-old man suffers from a major soft tissue injury after RTA. On examination, leg was swollen and bullae exuding. The subcutaneous crepitus extended along the limb and the skin was discoloured from knee to ankle. Extensive gas formation throughout all the muscle compartments of the right leg reaching to the level of knee joint was present. The case was diagnosed as gas gangrene. *Clostridium perfringens* was the organism isolated. What is the source of infection in this case? Explain the role of this cell structure along with the help of a diagram. (1 + 2 + 2 marks)

Source of Infection

- Spores of *Clostridium perfringens.*

Spore (*Fig. 1.1.14*)

Structure

- From inner to outward
  1. **Core**
     - Innermost part
     - Contains the DNA material
     - Surrounded by inner membrane

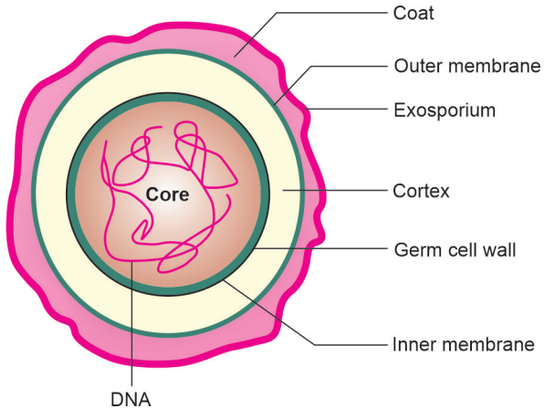

Fig. 1.1.14: Structure of spore

2. **Germ cell wall**
   - Contains normal peptidoglycan; gives rise to future cell wall of the bacterial cell
3. **Cortex**
   - Thickest layer surrounding the core, made up of a special type of peptidoglycan
4. **Spore coat**
   - Multilayered, made of a keratin like protein, impermeable—so spore is resistant to antibacterial agents
5. **Exosporium**
   - Outermost layer
   - Present in some spores

Significance

- Highly resistant to heating; spores are killed at 121°C. Medical supplies must be heated to 121°C for at least 15 minutes to be sterilised.
- Highly resistant to many chemicals, including most disinfectants, due to the thick, keratin-like coat. Only solutions designated as sporicidal will kill spores.
- It survives for many years in the soil. Wounds contaminated with soil can be infected with spores and cause diseases such as tetanus (*C. tetani*) and gas gangrene (*C. perfringens*).
- No metabolic activity, making antibiotics ineffective against spores.
- Spores are not often found at the site of infections because nutrients are not limiting.
- Spores as indicators of sterilisation.
  1. Spores of *Geobacillus stearothermophilus*: Autoclave, plasma steriliser
  2. Spores of *Bacillus atrophaeus*: Hot air oven and ethylene oxide steriliser

10. *Mycoplasma* lacks which of the cell structure component? (1 mark)

- **Give examples of antibiotics acting on this cell structure (2 marks)**
- **Draw a neat, labelled diagram of this structure (2 marks)**

Mycoplasma Lacks

- Cell wall.

Examples of Antibiotics Acting on Cell Wall

- Penicillin, cephalosporins, carbapenem, aztreonam, glycopeptides (vancomycin) inhibit the cell wall synthesis.

Diagram of ***(Fig. 1.1.15)***

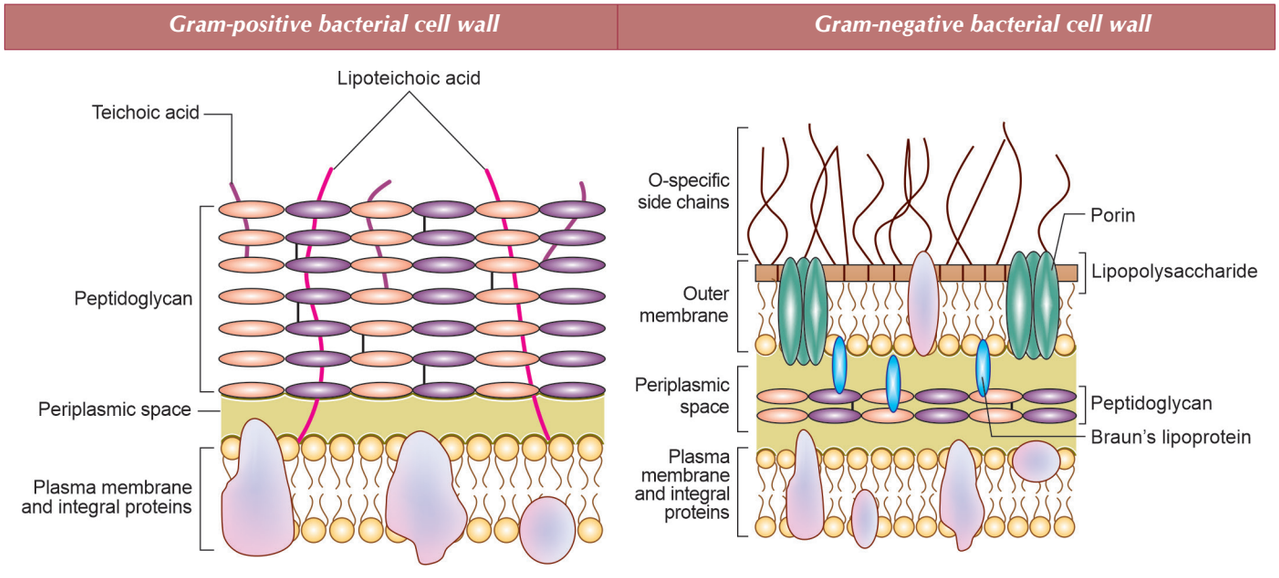

Fig. 1.1.15: Structure of gram-positive and gram-negative bacterial cell walls

11. Antibiotic resistance in *Staphylococcus aureus* is mediated by plasmids. Justify your answer explaining the properties, types, and role of plasmids. (1+1+2+2 marks)

Justification

- Antibiotic resistance in *Staphylococcus aureus* is by plasmid mediated gene blaZ encoding for beta lactamase, and mecA gene coding for altered PBP (MRSA).

Plasmids

Properties

- Extrachromosomal, double-stranded, circular DNA molecules, capable of independent replication.
- Usually extrachromosomal, but can integrate with bacterial chromosome.
- Exist in gram-positive and gram-negative bacteria.

Types

Classification 1

1. Transmissible Plasmids

- Transferred from cell to cell by conjugation.
- Large [molecular weight (MW 40–100 million)], contain genes responsible for synthesis of the sex pilus and enzymes required for transfer.
- Present in few (1–3) copies per cell.

2. Nontransmissible Plasmids

- Small (MW 3–20 million), do not contain the transfer genes; present in many (10–60) copies per cell.

Classification 2

1. Col Plasmids

- Contain genes that code for bacteriocins, proteins that can kill other bacteria.

2. F-plasmid

- Contain genes for expression of sex pili and conjugation.

3. Resistance Plasmids

- Contain genes that provide resistance against antibiotics or poison.

Role

- Plasmids carry the genes for the following functions
- and structures of medical importance.

1. Antibiotic resistance, which is mediated by enzymes, such as the β-lactamase of *S. aureus, Escherichia coli, and Klebsiella pneumoniae.*
2. Exotoxins: enterotoxins of *E. coli*, anthrax toxin of *Bacillus anthracis*, exfoliative toxin of *S. aureus*, and tetanus toxin of *Clostridium tetani.*
3. Pili (fimbriae): Adherence of bacteria to epithelial cells.
4. Resistance to heavy metals: Mercury, the active component of some antiseptics.
5. Resistance to ultraviolet light, which is mediated by DNA repair enzymes.
6. Bacteriocins, which are toxic proteins synthesized by certain bacteria that are lethal for other bacteria.

Plasmids are useful in molecular biology and genetics.

12. A 45-year-old man after RTA is brought to ED. O/E, a surgeon suspect's anaerobic infection of the injured leg. What is the preferred specimen to be collected? Explain the methodology of culture available in this case. (1+4 marks)

Preferred Specimen to be Collected

- Aspirate, biopsy from the lesions.
- Anaerobic bacteria are sensitive to oxygen.

Methods of Anaerobic Culture

1. Production of Vacuum

- The cultures are incubated in a vacuum desiccator.

2. Displacement of Oxygen with Other Gases

- Displacement of oxygen with gases like hydrogen, nitrogen, helium, or CO_2, e.g. Candle jar.

3. Chemical Method

- Alkaline pyrogallol absorbs oxygen.
- *Mcintosh—Fildes' Anaerobic Jar.*
  - Consists of a metal jar or glass jar with a metal lid which can be clamped airtight.
  - The lid has 2 tubes—gas inlet and gas outlet.
  - The lid has two terminals—connected to electrical supply.
  - Under the lid—small, grooved porcelain spool, wrapped with a layer of palladinised asbestos.
  - *Working.*
    - Inoculated plates are placed inside the jar and the lid clamped airtight
    - The outlet tube is connected to a vacuum pump and the air inside is evacuated
    - The outlet tap is then closed, and the inlet tube is connected to a hydrogen supply
    - After the jar is filled with hydrogen, the electric terminals are connected to a current supply, so that the palladinised asbestos is heated
    - Act as a catalyst for the combination of hydrogen with residual oxygen
- *Gaspak.*
  - Plates are kept in the jar with commercially available disposable envelope contains sodium bicarbonate and sodium borohydride which generate H_2 and CO_2 on addition of water.
  - Palladium catalyst is below the jar lid to remove traces of oxygen in the jar.

- Indicator is used—reduced methylene blue.
  - Colourless: anaerobically
  - Blue colour: on exposure to oxygen

4. Biological Method

- Absorption of oxygen by incubation with aerobic bacteria, germinating seeds or chopped vegetables.

5. Reduction of Oxygen

- By using reducing agents—1% glucose, 0.1% thioglycolate.

6. Anaerobic Glove Box and Workstation for Processing and Incubation of Culture

- Chamber for processing of the samples for anaerobic culture.

13. Briefly discuss the role of organisms in health and disease in vaginal tract and gastrointestinal tract. (2.5+2.5 marks)

Microbiome of Vaginal Tract

- Vaginal flora of adult women consists primarily of *Lactobacillus species*.
  1. Lactobacilli are responsible for the acidic pH of the adult woman's vagina
  2. The vaginal pH is high before puberty and after menopause
  3. Lactobacilli as the normal flora in vagina prevent the growth of pathogens. Antibiotics suppress the growth of lactobacilli and can lead to *Candida vaginitis*
- Due to proximity of vagina to the anus, vagina gets colonised by faecal flora. Women are susceptible to recurrent urinary tract infections by *E. coli.*
- Group B streptococci colonize vagina in 15–20% of women of child-bearing age. Associated with neonatal sepsis and meningitis, it is acquired during passage through the birth canal.
- Vaginal colonisation with *Staphylococcus aureus* in 5% of women, predisposes them to toxic shock syndrome.

Microbiome of GIT

- Colon has the complex microbial population in humans.
- *Members are anaerobic bacteria: Bacteroides* spp. and *Prevotella* spp., *Escherichia,* and *Salmonella* spp.
- Change in the microbiome plays role in:
  - Antibiotic associated colitis
  - Weight control (obesity)
  - Crohn disease and ulcerative colitis
  - Patients with IBD have significantly lower numbers of beneficial microorganisms.

14. A 48-year-old man admitted to the hospital with c/o cough and breathlessness. CXR shows left LL consolidation. Gram's stain of sputum showed numerous pus cells along with gram-positive cocci in pairs. Lanceolate shape with clearing was seen around it.

A. What is the cell structure responsible for the invasiveness of the pathogenic bacteria? (1 mark)

B. Briefly describe the role, practical and clinical significance of this cell structure (3 marks)

C. Discuss the methods of demonstration of capsule (2 marks)

A. Cell Structure Responsible

- Invasiveness in *Streptococcus pneumoniae* is attributed to its capsule.

B. Capsule

- A viscid, gelatinous extracellular polymeric substance present just external to cell and in contact with it.

Role of Bacterial Capsule

1. Protect cell wall against attack by antibacterial substances—Lysozyme, complement, bacteriocins.
2. Acts as a virulence factor—anti-phagocytic—adherence factor—attachment to host cell surfaces—loss of capsule makes the bacterium avirulent.
3. Antigenic and confers antigenic specificity on the organism.

Practical and Clinical Significance

1. Detection of capsular material in body fluids (CSF, pleural fluid, blood) helps in rapid diagnosis of infections by capsulated bacteria, e.g. in pneumococcal meningitis, detection of capsular polysaccharide of *Pneumococcus* in CSF sample.
2. Detection of capsular polysaccharide helps in identification of bacterial cultures in the lab.
3. Detection of capsular polysaccharide helps in intraspecies typing of bacteria.
4. Capsular material is antigenic: It can be used for vaccine preparation, e.g. pneumococcal vaccine contains purified polysaccharides of 23 types of pneumococci.

C. Demonstration of Capsule

1. Direct Methods

i. Stained Preparations

- *Negative staining:* Using India Ink/Nigrosin: the black particles in the stain fail to stain the capsule—the presence of a capsule is indicated by a bright halo around the bacteria against a dark background
- *Welch staining method:* Stains the capsule

- *Gram's stain or methylene blue stain:* Appear as unstained halo around the bacteria- not a good or reliable method for capsule demonstration

ii. Unstained

- Can be viewed under dark ground illumination or electron microscopy.

1. Indirect methods

i. Immunofluorescence.
ii. Serological method—Quellung reaction.

---

15. Which cell wall components are present in gram-negative but absent in gram-positive bacteria? Describe their structure and functions. (1+4 marks)

Cell Wall Component Present in Gram-negative but Absent in Gram-positive is

1. Lipopolysaccharide (LPS).
2. Outer membrane proteins (OMP).

Lipopolysaccharide (LPS) (Fig. 1.1.16)

- Integral part of the outer membrane (cell wall) of gram-negative bacteria, which is released only on lysis of the organism.
- It has 3 components:
  1. *Lipid A*
     - A glycolipid which is responsible for the toxicity of the LPS
  2. *Core Polysaccharide*
     - It is the backbone of the LPS molecule and linked to lipid A
     - Similar in all gram-negative bacteria
  3. *Polysaccharide Side Chain*
     - Repeating units of various sugars
     - Unique pattern in each species, therefore, forms the important "O" antigen or somatic Ag of gram-negative bacteria
     - Useful in identification of gram-negative bacteria in the laboratory.

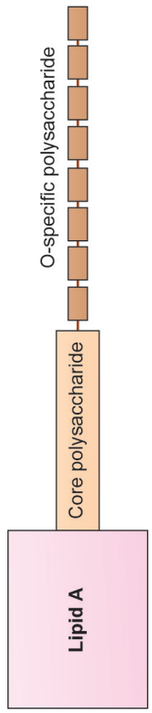

Fig. 1.1.16: Structure of LPS

Functions

1. Fever, hypotension, shock, DIC.
2. Activation of alternate complement pathway.
3. Activation of macrophages—increasing phagocytic action.
4. Activation of B cells, increasing antibody production.
5. The term 'endotoxin' is used to indicate LPS as it is not released into the environment but is an integral part of the cell wall.

Outer Membrane Proteins

Structure

- Protein molecules which traverse both layers of the membrane, and are called 'Porins' which form nonspecific pores or channels, e.g. OmpA, OmpC, OmpD, OmpF, LamB, Tsx.

Functions

1. These channels permit free diffusion/transport of low molecular weight solutes which are required by the cell.
2. These protein molecules may play a role in virulence.
3. Act as receptors for bacteriophages.
4. Anchor outer membrane to peptidoglycan layer.
5. Act as sex-pilus receptor in bacterial conjugation.

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16. Hepatitis B vaccine is an example for recombinant vaccine. Justify your answer. What are the steps involved in recombinant DNA technology and list the applications of recombinant DNA technology? (2+2+1 marks)

Justification

- Recombinant vaccine.
  - *Viruses with large genome (Vaccinia virus):* Gene in vaccinia virus not required for the viral replication is excised and the gene for the surface antigen of hepatitis B virus is introduced into vaccinia virus. The preparation is injected leading to expression of gene of HBsAg in the infected cells

Recombinant DNA Technology (Fig. 1.1.17)

Steps

1. Isolation of genetic material.
2. Cutting the gene at the recognition sites by restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome.
3. Amplifying the gene copies through PCR.
4. *Ligation of DNA molecules:* Cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.
5. Insertion of recombinant DNA into host.

Applications

1. *Production of vaccines, growth hormones, gene therapy:* Therapeutic purposes.
2. Production of antigens used in diagnostic kits.
3. Genetically modified (GM) vegetables, fruits, and transgenic animals.

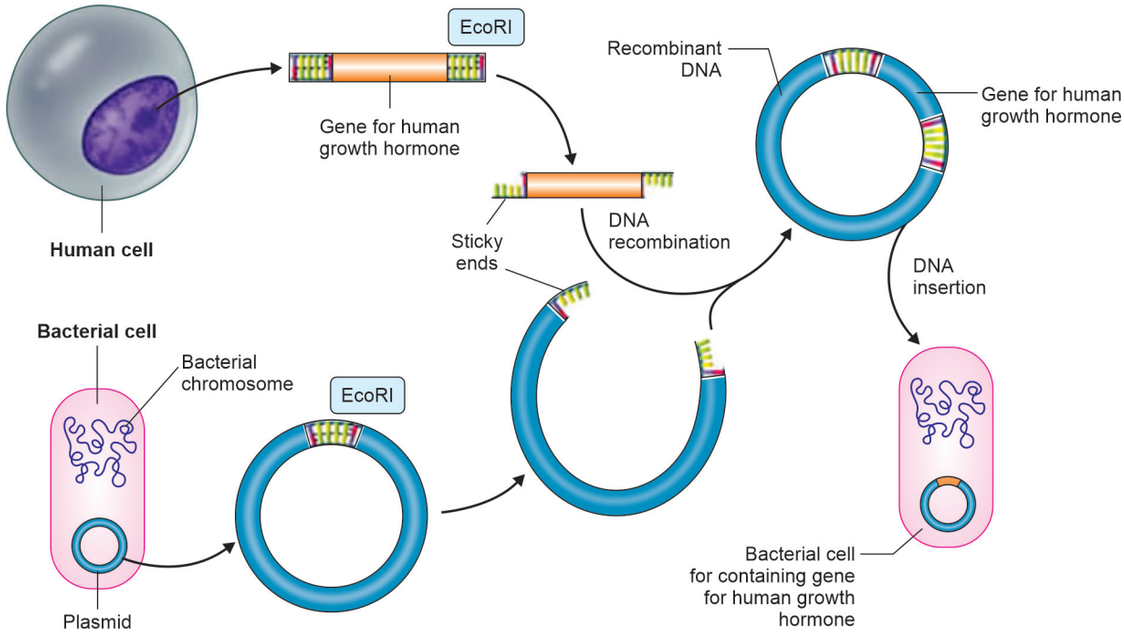

Fig. 1.1.17: Recombinant DNA technology

17. Compare Exotoxins and Endotoxins. (4 marks)

Feature	*Exotoxins*	*Endotoxins*
Definition	• Proteins secreted out of bacteria	• Lipid portion of lipopoly-saccharides (LPSs) are an integral part of outer membrane of bacteria
Source	• Mostly gram-positive bacteria and gram-negative bacteria	• Present only in gram-negative bacteria
Chemical nature	• Polypeptide	• Lipopoly-saccharide
Compo-nents	• 2 subunits 1. A (active) 2. B (binding)	• 3 components 1. O-antigen 2. Core oligo-saccharide 3. Lipid A
Location of genes	• Plasmids, bacteriophage	• Chromosome
Secreted from cell	• Yes	• No
Heat stability	• labile (60–80°C)	• Stable at 100°C
Specificity	• Specific in mode of action and host cells	• Not specific in nature

Contd.

Feature	*Exotoxins*	*Endotoxins*
Specific receptors	• Present	• No
Immuno-genicity	• High	• Weak
Fever	• No	• Fever by IL-1 production
Toxicity	• High	• Moderate
Mode of action	• Enzyme-like mechanisms	• TNF and Interlukin-1
Potency	• *High*	• *Low*
Effects	• Cytotoxin, enterotoxin or neurotoxin	• Fever, diarrhea, vomiting
Neutrali-sation by antibodies	• Possible	• Not possible
Detection	• Detected by many tests (neutralisation, precipitation)	• Detected by Limulus lysate assay
Conversion to toxoids	• Possible (On treatment with formalin), e.g. diphtheria, botulism, and tetanus	• Not possible
Availability of vaccines	• Available	• No effective vaccines available.

Contd.

Feature	Exotoxins	Endotoxins
Diseases caused	• Tetanus, diphtheria, botulism	• Meningococcaemia, sepsis by gram-negative rods
Examples	• Toxins produced by *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pyogenes*	• Toxins of *E. coli*, *Salmonella typhi*

18. Enumerate the congenital viral infections. During which stage of gestation, these viruses get transmitted? (3+2 marks)

Congenital Viral Infections

1. *Rubella:* Congenital rubella
2. *CMV:* Congenital CMV
3. *HIV:* Childhood AIDS
4. *VZV:* Skin lesions, musculoskeletal, CNS abnormalities when foetus is infected <20 weeks, later childhood zoster.
5. *HSV:* Neonatal HSV.
6. *HBV:* Persistent infection.
7. *Parvo B 19:* Abortion, hydrops foetalis.

Stage of Gestation, these viruses get transmitted

Virus	Transplacental	During birth	Shortly after birth
Rubella	+ +	–	–
Cytomegalovirus	+	+ +	+ +
Herpes simplex	+	+ +	+
Varicella zoster	+ +	+	+
Parvovirus	+ +	–	–
Enterovirus	+	+ +	+ +
HIV	+	+ +	+
Hepatitis B	+	+ +	+ +
HPV	–	+ +	–

19. Explain in brief the clinical features of any five congenital infections along with the laboratory work-up (5 marks)

See Table 1.1.2

Table 1.1.2 Clinical features of any five congenital infections

Congenital infections	Clinical features	Lab work-up
Congenital rubella syndrome	Triad of: • Patent ductus arteriosus • Microphthalmia • Sensory neuronal deafness	• HAI, ELISA—Rising titres of antibody (mainly IgG) • Presence of rubella-specific IgM—ELISA • WBCs from cord/infant blood– • PCR
Congenital Herpes	• Rare, most devastating • Skin vesicles • Chorioretinitis • Microcephaly • Micro-ophthalmia • IUGR	• Urine culture for CMV (must be in first two weeks of life to confirm congenital infection). • Cord or infant blood for CMV PCR • Serology of blood or Urine. IgM persist for 8 months • Head ultrasound
Congenital CMV infection	• Jaundice • Petechiae • Hepatosplenomegaly • IUGR (33%); Preterm • Microcephaly • Chorioretinitis • Fatal outcome	• Skin vesicles, Swabs from eyes, mouth/nasopharynx, CSF: viral culture and HSV PCR. • WBCs from cord/neonate blood for HSV PCR • CSF-cells, protein, glucose • ELISA-Subtype specific (HSV1 and 2) serology may be useful to child • Ophthalmic consultation
Congenital varicella syndrome	• Scarring of skin • Hypoplasia of limbs • Cortical atrophy, psychomotor retardation • Chorioretinitis, cataracts	• IgM—current acute infection • Immunofluorescence of vesicle fluid • Virus isolation, electron microscopy
Congenital toxoplasmosis	• Abortion, stillbirth • Neonatal disease with encephalitis • Chorioretinitis • Hepatosplenomegaly • Fever, jaundice • Intracranial calcifications • Blindness	• Microscopy: Giemsa stain- crescent-shaped trophozoite • Tissue—Cyst • IgM antibody—Immunofluorescence • IgG—rising in titre • Cell culture • Animal inoculation-mice

SHORT ANSWERS

1. Give 4 examples of organisms which are a part of normal flora and can also cause infections in the host. (4 marks)

Location	*Normal flora*	*Infections caused*
Skin	• *Staphylococcus epidermidis*	• Infections of prosthetic heart valves and prosthetic joints
Skin and nose	• *Staphylococcus aureus*	• Abscesses
Oropharynx	• *Streptococcus sanguinis* • *Streptococcus mutans*	• Subacute endocarditis
Urethra	• *Escherichia coli*	• Urinary tract infection

2. Discuss the contributions of Louis Pasteur to medical microbiology (4 marks)

Louis Pasteur

1. Father of microbiology
2. Disproved theory of spontaneous generation (or Abiogenesis).
3. Put forward theory of microbial fermentation.
4. Introduced liquid media to grow microorganisms.
5. Introduced techniques of sterilisation—Devised Steam steriliser, hot air oven, autoclave. Introduced the process of 'Pasteurisation' for milk.
6. Extensive studies on anthrax, chicken cholera, rabies virus.
7. Discovered the process of attenuation → Development of live attenuated vaccines.
8. Coined the term '*Vaccine*' in honour of Edward Jenner—against smallpox.
9. Vaccines for rabies, anthrax bacilli, chicken cholera.
10. Founder of Pasteur Institute, Paris. In India—at Coonoor, Tamil Nadu.

3. Tuberculosis disease is caused by *M. tuberculosis* and was a discovery by Robert Koch. Enumerate his postulates in the theory of disease and the exceptions. (4 marks)

Koch's Postulates

1. The bacterium should be constantly associated with lesions of the disease.
2. It should be possible to isolate the bacterium in pure culture from the lesions.
3. Inoculation of this pure culture into lab animals should reproduce the lesions of the disease.
4. It should be possible to re-isolate the bacterium in pure culture from the lesions in the lab animal.
5. *Additional criterion was added later:* Specific antibodies should be demonstrable in the serum of the patient suffering from the disease.

Molecular Koch's Postulates

➲ To establish that a **gene** found in a pathogenic microorganism is a virulence gene.

1. The virulence property/phenotype under study should be associated with the pathogenic strains and not with non-pathogenic strains.
2. Inactivation of the gene associated with the suspected virulence trait should lead to loss of pathogenicity or virulence.
3. Replacement of mutated gene with wild type gene should restore virulence.
4. Antibodies/immune system cells directed against the gene products should protect the host.

Exceptions to Koch's Postulates

1. Some microorganisms like Chlamydia, viruses, *Treponema pallidum*, *Mycobacterium leprae*—do not grow in artificial media.
2. *Neisseria gonorrhoeae:* No animal model.
3. *Asymptomatic carriers:* Typhoid.

4. Define true-positive, true-negative, false-positive, false-negative, sensitivity and specificity in the interpretation of test results. (4 marks)

True-positive	• An outcome where the test *correctly* predicts the *positive* result
True-negative	• An outcome where the test *correctly* predicts the *negative* result
False-positive	• An outcome where the test incorrectly predicts the *positive* result
False-negative	• An outcome where the test incorrectly predicts the *positive* result
Sensitivity	• Ability of the test to detect even very minute amounts of Ag or Ab's • Highly sensitive test false-negative results will be absent or minimal
Specificity	• Ability of the test to detect reactions between homologous antigens and antibodies only • In highly specific test, false-positive reactions are minimal or absent

5. "Not all infections are communicable". Define communicable diseases with 2 classical examples. (2+2 marks)

Definition

➲ Communicable diseases are illnesses caused by viruses or bacteria that people spread to one another through contact with contaminated surfaces, bodily fluids, blood products, insect bites, or through the air.

Examples

1. HIV
2. Hepatitis A, B and C
3. Measles
4. Cholera

6. Auramine O dye-stained smear for the detection of *Mycobacterium tuberculosis* is observed under which type of microscope. Discuss the principle and applications of this microscope. (2+2 marks)

Microscope Used

- Fluorescence microscope.

Principle

- Uses fluorescence property to generate an image.
- Fluorescent dye, when exposed to UV light absorb UV light and convert it to visible light.
- Source of light mercury lamp.
- Emitted rays pass through an excitation filter (allows only short wavelength UV light of about 400 nm to pass through).
- Exciting rays get reflected by a dichromatic mirror-fall on the specimen stained by fluorescent dye.
- Barrier filter—removes remaining ultraviolet light (damage the viewer's eyes), or blue and violet light (reduce contrast).

Applications

1. Auramine O stain for *Mycobacterium tuberculosis.*
2. Acridine orange (Quantitative Buffy coat) for *Plasmodium* and filarial nematodes.
3. Direct IF on clinical specimen especially for viruses.
4. *Indirect IF:* Antinuclear antibody, anti-neutrophilic cytoplasmic antibody in patients' serum.

7. The maximum growth rate of bacteria is seen in which phase of the bacterial growth. Explain the different phases in bacterial growth. (1+3 marks)

Maximum growth rate of bacteria is seen in:

- Log phase of the bacterial growth.

Phases in Bacterial Growth (Fig. 1.1.18)

Lag phase	• Period between inoculation and beginning of multiplication of bacteria • Bacteria increase in size due to accumulation of enzymes and metabolites • Bacteria reach their maximum size at the end of lag phase
Log phase	• Bacteria divide exponentially so that the growth curve takes a shape of straight line • Smaller in size • Biochemically active • Uniformly stained—It is the best time to perform the Gram's stain
Stationary phase	• Number of viable cells remains stationary as there is almost a balance between the dying cells and the newly formed cells • Bacterium becomes Gram's variable • More storage granules are formed • Sporulation occurs in this phase • Bacteria produce exotoxins, antibiotics and bacteriocins
Phase of decline	• Bacteria stop dividing completely; while the cell death continues due to exhaustion of nutrients, and accumulation of toxic products • Produce involution forms

Contd.

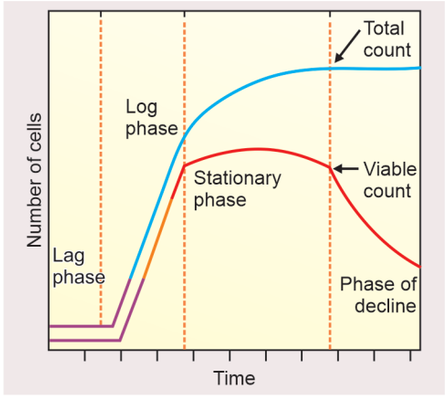

Fig. 1.1.18: Phases in bacterial growth

8. Comment on viral inclusion bodies with examples. (1+3 marks)

Viral Inclusion Bodies

- Inclusion bodies **are nuclear or cytoplasmic** aggregates of stainable substances, usually proteins.
- They typically represent sites of viral multiplication in a eukaryotic cell and usually consist of viral capsid proteins.

Examples

1. Intracytoplasmic Eosinophilic (Acidophilic)

1. Negri bodies in rabies
2. Guarnieri bodies in vaccinia, variola
3. Paschen bodies in variola or smallpox
4. Bollinger bodies in fowl pox
5. Henderson–Patterson bodies in molluscum contagiosum.

2. Intranuclear Eosinophilic (Acidophilic)

1. Cowdry type A in herpes simplex virus and varicella zoster virus.
2. Cowdry type B in polio and adenovirus.

3. Intranuclear Basophilic

1. Cowdry type B in adenovirus.
2. "Owl's eye appearance" in cytomegalovirus.

4. Both Intranuclear and Intracytoplasmic

1. Warthin–Finkeldey bodies in measles.

9. Comment on the differences in the structure of gram-positive and gram-negative bacterial cell wall (2 marks)

Gram-positive cell wall	*Gram-negative cell wall*
• Peptidoglycan layer (thick, 40 sheets)	• Peptidoglycan layer (thinner: 1–2 sheets)
• Teichoic acid	• Lipoprotein • Outer membrane ✦ Phospholipid bilayer ✦ LPS (lipopolysaccharide) ✦ OMP (outer membrane protein) • Periplasmic space

10. What is Lysogenic conversion? Discuss its significance with examples. (3+1 marks)

Lysogenic Conversion (Fig. 1.1.19)

- New properties acquired by bacterium as a result of integrated prophage genes.
- Mediated by transduction of bacterial genes from donor bacterium to the recipient bacterium via bacteriophage.
- Integration of viral DNA into bacterial cell DNA—Prophage.

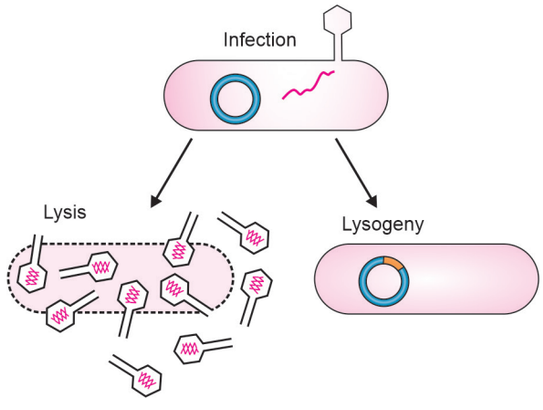

Fig. 1.1.19: Lysogenic conversion

Significance

- Exotoxins of diphtheria, botulinum, cholera and erythrogenic toxins are encoded by prophage.

11. What are L forms in bacteria and note its significance? (2+2 marks)

L-forms

- L forms are the cell wall deficient bacteria, discovered by E. Klieneberger, while studying *Streptobacillus moniliformis.*
- Named it as L form after its place of discovery i.e., Lister Institute, London (1935).
- Damage/removal of cell wall can occur by.
  - Enzymes: Lysozyme, antibiotics acting on cell wall, e.g. penicillin complement system, antibodies.
  - Cell wall damage → cell protoplasm takes up water → swells up → ruptures → cell lysis.
  - In an osmotically protective medium (isotonic solution), cell wall deficient bacteria will survive—These are called L-forms.
- Origin of L-forms.
  - *Spontaneous:* Arise spontaneously without induction, e.g. *Streptobacillus moniliformis*
  - *Induced:* Arise due to induction by certain chemicals, e.g. lysozyme (enzyme), penicillin (antibiotic)
- Classification of L-forms.
  1. *Stable L-forms*
     - Do not revert to parental forms even if cell wall inhibiting agent is removed
     - Multiply, divide and can be maintained for several generations as L- forms
     - Resemble *Mycoplasmas* (a cell wall deficient bacterium) in many properties.
  2. *Unstable L-forms*
     - Revert to parental forms once the cell wall inhibiting agent is removed.
     - Are of two types:
       i. *Protoplasts:* Originate from and revert to Gram +ve bacteria. Protoplasts have only protoplasm and cytoplasmic membrane, whole of cell wall is lost.
       ii. *Spheroplasts:* Originate from and revert to gram –ve bacteria. Spheroplasts retain outer membrane and some amount of peptidoglycan, unlike the protoplasts.

Properties of L-forms

- No regular shape- no cell wall.
- Cannot be demonstrated by ordinary staining methods.
- Resistant to antibiotics which act on cell wall.
- Can be maintained only in osmotically protective media (containing sucrose, NaCl, Mg++).
- On solid media, they grow just below the surface of the agar.
- In liquid media, they do not produce turbidity—they produce clumps.

Significance of L-forms

- Responsible for chronic infections as they are resistant to antibiotics.
- *May result in relapse of infections:* Once the antibiotics are withdrawn, they revert to parental bacterial forms—especially important in endocarditis, UTI

12. Antibiotics like penicillin and cephalosporin act on this structure of the bacterial cell. Discuss the functions and clinical and practical significance of this structure. (1+1+1+1 marks)

Antibiotics like Penicillin and Cephalosporins act on

- Bacterial cell wall.

Functions of Cell Wall

1. Gives characteristic shape, rigidity to bacterial cell.
2. Protects cell from osmotic damage.
3. Helps in cell division—formation of cross wall.
4. *Determines antigenic specificity of bacterium:* Teichoic acid in gram-positive; 'O' antigen in gram-negative bacteria.
5. Site of action for bacteriophages, antibiotics, bacteriocins.
6. *Role in virulence:* Cell wall may contain certain virulence factors (endotoxin).

Clinical Significance of Cell Wall

- Differentiating between gram-positive and gram-negative organisms is important because choice of empirical antibiotic therapy is based on it. Drugs used are different for gram-positive and gram-negative bacteria.
- Endotoxins of gram-negative bacteria are responsible for causing endotoxic shock.
- Teichoic acid of gram-positive bacteria can induce septic shock.

Practical Importance of Cell Wall

- *Due to difference in cell wall composition:* Gram's staining technique can be used to differentiate gram-positive and gram-negative bacteria—useful in identification in laboratory
- 'O" antigen of gram-negative bacteria—useful for identification and classification of bacteria, esp. *Salmonellae*
- 'O" antigen of *Salmonella* used in Widal test for diagnosis of enteric fever.

MI 1.2 PERFORM AND IDENTIFY THE DIFFERENT CAUSATIVE AGENTS OF INFECTIOUS DISEASES BY GRAM'S STAIN, ZIEHL–NEELSEN STAIN AND STOOL ROUTINE MICROSCOPY

MI 8.9 DISCUSS THE APPROPRIATE METHOD OF COLLECTION OF SAMPLES IN THE PERFORMANCE OF LABORATORY TESTS IN THE DETECTION OF MICROBIAL AGENTS CAUSING INFECTIOUS DISEASE

MI 8.10 DEMONSTRATE THE APPROPRIATE METHOD OF COLLECTION OF SAMPLES IN THE PERFORMANCE OF LABORATORY TESTS IN THE DETECTION OF MICROBIAL AGENTS CAUSING INFECTIOUS DISEASE

SHORT ESSAYS

1. An 18-year-old presents with discharge from the laparotomy site for appendicectomy. Pus is sent for culture and sensitivity. Performed Gram stain on the smear provided and reported. (Figs 1.2.1 and 1.2.2)

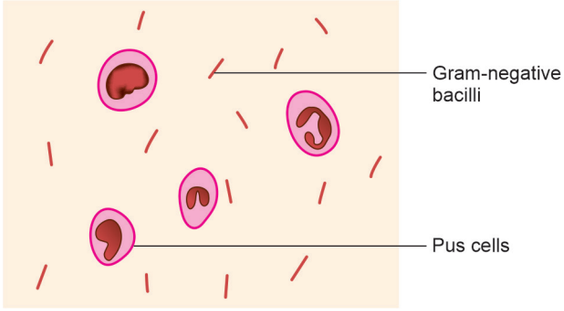

Fig. 1.2.1

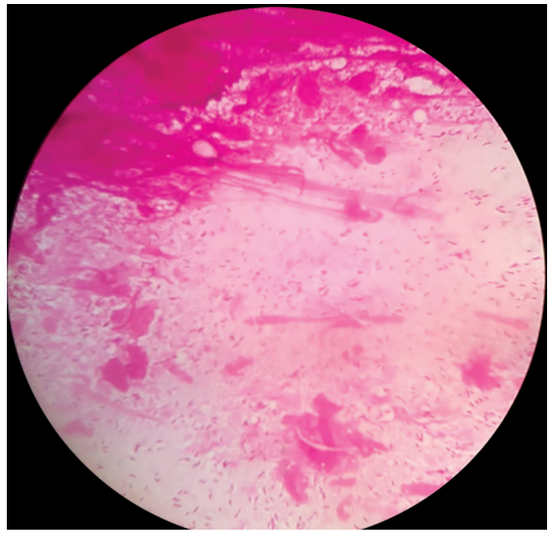

Fig. 1.2.2

Answer the questions below

A. Report on the Gram's stain and draw a neat, labelled diagram. (1 mark)

➲ The given smear shows the presence of polymorphonuclear leucocytes with gram-negative bacilli.

B. What is the probable diagnosis? (1 mark)

➲ Surgical site infection.

C. List out the probable etiological agents in this case based on the Gram's stain report. (1 mark)

1. *Escherichia coli*
2. *Klebsiella* spp.
3. *Citrobacter* spp.
4. *Pseudomonas* spp.

D. What are the further investigations required to confirm the identification of the aetiological agent? (1 mark)

➲ Culture and antibiotic sensitivity testing.

E. What are the clinical applications of Gram's stain? (1 mark)

1. Identification of bacteria on Gram's stain and morphology
2. To start empiric antibiotic in invasive infections: sepsis, meningitis
3. Gram's stain report helps to decide on the culture media to grow and biochemical tests required to confirm the identity of the bacteria
   a. *Clue*
      i. Gram's stain in gonorrhoea is diagnostic in symptomatic male
      ii. Bacterial vaginosis Gram's smear of vaginal swab: clue cells, few pus cells, few or absent lactobacilli, numerous gram-negative coccobacilli (*Gardnerella* spp.)
      iii. Anaerobic infection: Polymicrobial, pale organisms
      iv. Gas gangrene due to *Clostridium perfringens*: Disintegrated pus cells with thick Gram-positive bacilli without spores
      v. *Haemophilus influenzae* (pleomorphic Gram-negative coccobacilli) in sputum, CSF along with pus cells
   b. *Staining of yeasts: Cryptococcus* spp., *Candida* spp.
   c. To judge the quality of sputum sample based on the number of pus cells and epithelial cells/HPF

F. Give reasons for the Gram's staining property of the bacteria. (1 mark)

➲ Affinity to the basic dye (crystal violet) due to more acidic cytoplasm and thick peptidogly can layer which retains the crystal violet iodine complex.

2. A 45-year lady presented with breast abscess to surgery OPD. The surgeon drained the abscess in minor OT and sent to microbiology laboratory for culture and sensitivity. The Gram's stain picture shows as follows (Fig. 1.2.3)

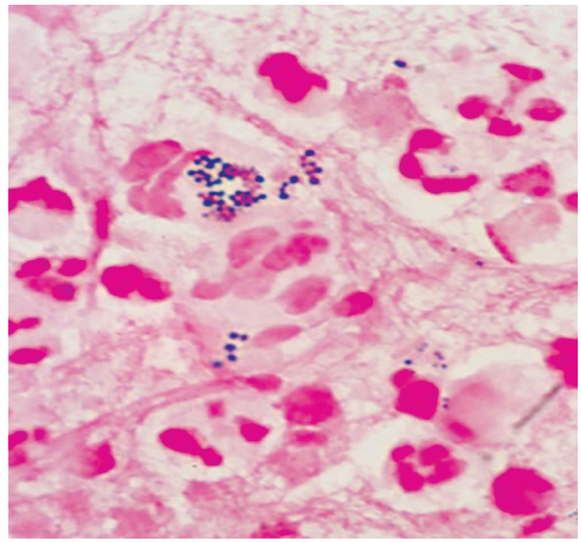

Fig. 1.2.3: Gram-positive cocci in clusters with pus cells

Sensitivity Report of the Organism Isolated

Antibiotic	*S/R*
Amoxiclav	R
Methicillin	R
Cloxacillin	R
Cefazolin	R
Clindamycin	S
Chloramphenicol	S
Erythromycin	R
Vancomycin	S
Teicoplanin	S
Linezolid	S
S: Sensitive, R: Resistant	

A. What is your clinical diagnosis? Identify the aetiological agent and comment on the Gram's stain picture? (3 marks)

Clinical Diagnosis

➲ Breast abscess.

Aetiological Agent

➲ *Staphylococcus aureus.*

Comment on the Gram's Stain Picture

- Gram-positive cocci arranged in clusters morphologically resembling *S. aureus*.

B. List the sample collection methods for skin and soft tissue infection (1 mark)

1. Pus aspirate.
2. Swab from deep wound.
3. Incision and drainage (I and D).

C. What are the recommended drugs in the above case? (1 mark)

- *Antibiotics recommended:* Linezolid, clindamycin, doxycycline, daptomycin, cotrimoxazole, Vancomycin.

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3. In a village, a 25-year-old visited the primary healthcare centre (PHC) with c/o rice watery diarrhea. On eliciting history of food eaten in the past 2 days, he gives a history of having panipoori from a street shop. Stool sample was collected and investigated. Stool sample revealed the following picture. (Fig. 1.2.4)

Fig. 1.2.4

A. Comment on the given stain. What are the preliminary investigations useful for diagnosis of this case? (2+2 marks)

The given smear of dilute carbol fuchsin shows comma-shaped bacilli morphologically resembling *V. cholera*.

- *Hanging drop:* Bacilli with darting Motility
- *Dilute Carbol Fuchsin:* Pink-comma-shaped bacilli
- To be confirmed by culture.

B. Mention the transport media? (2 marks)

1. Alkaline Peptone water.
2. Venkatraman Ramakrishnan media.
3. Cary–Blair medium.
4. Autoclaved sea water.
5. Monsur's taurocholate tellurite peptone water (pH 9.2).

C. What are the culture media required to support your diagnosis? (2 marks)

1. Thiosulphate citrate bile salt sucrose agar (TCBS).
2. Alkaline bile salt agar (BSA, pH 8.2).
3. Monsur's gelatin taurocholate trypticase tellurite agar (GTTA).

MI 1.3 DESCRIBE THE EPIDEMIOLOGICAL BASIS OF COMMON INFECTIOUS DISEASES

SHORT ESSAYS

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1. Xpert® MTB/RIF Ultra is an automated molecular assay for detection of *M. tuberculosis*. The test is highly specific and sensitive when compared to smear microscopy. Positive predictive value is 92% and negative predictive value is 99%

A. Define sensitivity and specificity in the above scenario (2 marks)

B. Define the terms PPV and NPV (2 marks)

C. What type of study is useful for evaluating a new test? (1 mark)

A. Definitions of Sensitivity and Specificity in the Above Scenario

Sensitivity

- The proportion of true-positives correctly identified by the test Xpert MTB/RIF.

Specificity

- The proportion of true negatives identified by the test method.

B. Definitions of the terms PPV and NPV

Positive Predictive Value (PPV)

- The proportion of individuals with a positive test result detected by Xpert who actually have the tuberculosis which is 92% in this case.

Negative Predictive Value (NPV)

- The proportion of individuals with a negative test result detected by Xpert who is free of tuberculosis which is 99% in this case.

C. Type of Study is Useful for Evaluating a New Test

- Cross-sectional study design is useful in evaluating a new test.

2. Classify different types of transmission with examples (5 marks)

Different Types/Modes of Transmission

Type/mode of transmission	Examples	
	Pathogen	Disease
Contact	• MRSA	• Wound infections
Droplet	• *C diphtheria*, • Influenza virus, SARS-CoV2, *N. meningitidis*	• Diphtheria • Influenza • Meningitis
Aerosol	• *M. tuberculosis*	• Tuberculosis
Ingestion	• *Salmonella* • *V. cholerae*	• Typhoid • Cholera
Sexual	• *N. gonorrheae*, *T. pallidum*, Herpes simplex virus 2	• Gonorrhoea, syphilis
Vertical	• *Treponema pallidum*, HSV 2, HIV, hepatitis B virus	• Syphilis, hepatitis B
Vector borne	• *Rickettsia, Borrelia*, Malaria	• Scrub typhus, Endemic typhus
Birth canal	• *Listeria*, group B *Streptococcus*, HSV 2	• Neonatal meningitis
Trans-placental	• TORCH (*Toxoplasma gondii*, Rubella, cytomegalovirus, HSV)	• Toxoplasmosis
Blood	• HIV, hepatitis B virus	• AIDS, hepatitis
Animal bite	• Rabies virus	• Rabies

3. A cook is positive for *S. typhi* in stool culture. He is asymptomatic. Is he a carrier or having the disease? With respect to the history briefly explain carriers and the types of carriers with examples. (5 marks)

Cook is

- A carrier of *S typhi.*

Carriers

Definition

- Are people who harbour infectious agents but are not ill.
- Carriers may present more risk for disease transmission than acute clinical cases.

Types

- Depending on the disease:
  1. Incubatory carriers
  2. In apparent infections (also called subclinical cases)
  3. Convalescent carriers
  4. Chronic carriers

Type of carrier	Feature	Example
Incubatory carriers	• Are going to become ill, but begin transmitting their infection before their symptoms start	Measles
In apparent infections	• People within apparent infections never develop an illness, but can transmit their infection to others	COVID-19
Convalescent carriers	• People who continue to be infectious during and even after their recovery from illness	*Salmonella*
Chronic carriers	• People who continue to harbour infections for a year or longer after their recovery	Hepatitis B

SHORT ANSWERS

1. Give examples for viruses causing diarrhoea and their clinical features. (3 marks)

Virus causing diarrhoea	Clinical Features
Rotavirus	• Fever, diarrhoea, vomiting and dehydration • There is no inflammation or loss of blood
Calicivirus	• Chills, headache, myalgia, or fever as well as nausea, abdominal pain, vomiting and diarrhoea
Astro virus Adenovirus	• Acute diarrhoea

2. Explain the terms infectious period, latent period and incubation period in relation to measles infection. (3 marks)

Infectious Period

- The time when a person is exposed to organism and can transmit or shedding of microorganisms to others.
- Usually for 8–13 days.

Latent Period

- The time frame when a person is exposed to a pathogen up till an infection reappears either in the same form as the primary infection or manifests with different signs and symptoms or able to transmit infection.
- Usually for 6–9 days.

Incubation Period

- The time between the person is exposed to the microbe (or toxin) and the development of symptoms.
- Usually for 6–7 days.

3. What is herd immunity in relation to polio virus? How can it be achieved? (3 marks)

Herd Immunity in Relation to Polio Virus

- Herd immunity occurs with the live polio vaccine primarily because it induces secretory IgA in the gut, which inhibits infection by virulent virus, and prevents its transmission to others.
- An individual is protected from infection by the virtue of the community being not able to transmit the virus to the individual.
- The vaccine containing the live virus replicates in the immunised person and spreads to other members of the population, thereby a greater number of people are protected.

Achieving Herd Immunity

- The important feature as far as herd immunity is concerned is the induction of IgA, which prevents transmission.
- Herd immunity can be achieved either by natural infection or vaccine.
- Here the organism is not capable of being transmitted to others who are not vaccinated.

4. Define epidemic, endemic, sporadic and pandemic diseases with examples for each. (4 marks)

Term	*Definition*	*Example*
Epidemic	• Unusual excess of expected occurrence of a disease	• Cholera
Endemic	• The constant presence of a disease or infectious agent within a given geographic area or population group without importation from outside • When conditions are favorable may burst into an epidemic	• Hepatitis A
Pandemic	• An epidemic usually affecting a large proportion of the population which can spread between two continents	• Influenza pandemics
Sporadic	• Infections occurring at irregular intervals or only in few places, scattered or isolated	• Enteric fever

Contd.

5. A 7-day-old neonate develops vesicular lesions over skin. H/o vaginal herpes in the mother was elicited 15 days ago. What is the most probable mode of transmission of infection in the child? Give examples of other infections transmitted through this route. (1 + 1 marks)

Most Probable Mode of Transmission of Infection in the Child

- Maternal genital tract.

Examples of Other Infections Transmitted through this Route

1. Syphilis.
2. Varicella zoster

MI 1.4 CLASSIFY AND DESCRIBE THE METHODS OF STERILISATION AND DISINFECTION. DISCUSS THE APPLICATION OF DIFFERENT METHODS IN LABORATORY, CLINICAL AND SURGICAL PRACTICE

LONG ESSAY

1. A tertiary care hospital is used to organize its regular board meetings related to infection control on 4th Saturday of every month. Due to an increase in number of post-surgical complications and outbreak of MRSA, the chairperson of infection control committee suggested the lead microbiologist for the proper sterilisation, chemical disinfection methods to be implemented. A training programme is organised by the infection control team for the same. Assuming that one group of the training team are 2nd year UGs, answer the following questions related to sterilisation and disinfection?

A. Definitions of Sterilisation and disinfection. (1 mark)

Sterilisation

- The killing or removal of *all* microorganisms from an item, surface or medium including bacterial spores.

Disinfection

- It is a process that reduces of pathogenic organisms to a level at which they no longer constitute a risk, may or may not destroy spores.

B. Classify the methods of sterilisation, disinfection with proper examples for each. (3 marks)

Sterilisation

Method of sterilisation	*Methods*	*Examples*
Physical method	• Moist heat sterilisation	• Autoclave–Surgical instruments
	• Dry heat sterilisation	• Hot air oven–Glassware
	• Radiation	• Ionisation and non- ionising—Medical devices
Chemical method	• ETO steriliser	• Sutures, catheters, stents
	• Plasma steriliser	• Laparoscopes, arthroscopes

Disinfection

Method of disinfection	*Methods*	*Examples*
High level disinfection	• Aldehydes–Glutaraldehyde • Peracetic acid • Hydrogen peroxide	• Endoscopes • Endoscopes • Dental instruments • Wound cleaning
Intermediate level disinfection	• Alcohols—Ethyl alcohol • Phenolics—Phenol, cresol • Halogens—Iodine and chlorine	• Skin antisepsis • Skin antisepsis • Topical ointment • Skin antisepsis • Blood spill
Low level disinfection	• Quaternary ammonium compounds • Chlorhexidine	• BP cuff • Hand rub

C. Describe the principle, uses and sterilisation controls used in hospital for sterilisation of different materials used for surgery, and labware. (2+2 marks)

Principle

Autoclave

- Water boils at 100°C but when pressure inside the autoclave increases, the temperature at which water boils also increases, and steam is generated.
- The temperature used in autoclave is 121°C for 15 min at pressure of 15 psi.

Hot Air Oven

- This is dry heat method which requires temperatures in the range of 180°C for 2 hours.

Uses

Of Autoclave

1. Surgical instruments, gowns, drapes.
2. Culture media and materials which cannot withstand the higher temperature of hot air oven or media containing water which cannot be sterilised by dry heat.

Of Hot Air Oven

1. This process is used primarily for glassware like glass syringes, Petri dishes, flasks, pipettes, and test tubes.
2. Surgical instruments like scalpels, scissors, forceps, etc.
3. Chemicals such as liquid paraffin, fats, glycerol, and glove dust powder.

Sterilisation Controls

1. **Physical indicators**
   - Sterilisers have displays such as temperature, time, pressure, etc.
2. **Chemical indicators**
   - Heat or chemical sensitive materials are used to check the efficacy of sterilisation process, e.g. Bowie Dick tape, autoclave tape.
3. **Biological indicators**
   - The most reliable indicator as it uses spores of bacteria to check the efficacy of the process of sterilisation.
   - The spores are readily destroyed when the process has been effectively completed.
   - The spore vials are incubated, and result is obtained within 24 minutes to 48 hours, e.g.
     1. *Geobacillus stearothermophilus:* For steam steriliser, plasma steriliser
     2. *Bacillus atrophaeus:* Hot air oven

D. Expand CSSD. Mention various compartments of CSSD and functions of CSSD. (2 marks)

CSSD is Expanded as

- Central Sterile Supply Department.

Compartments of CSSD

1. Cleaning area.
2. Packing area.
3. Sterilisation area.
4. Sterile Storage area.

Functions of CSSD

1. Receiving and sorting soiled materials.
2. Determines whether the item should be reused or discarded.
3. The process of decontamination or disinfection is carried out prior to sterilisation.

4. Inspecting and testing instruments, equipments, and linen
5. Assembling and packing all materials for sterilisation
6. Sterilising
7. Labelling and dating materials
8. Storing
9. Issuing and distributing items to various areas in the hospital.

SHORT ESSAYS

1. Describe the chemical methods of Sterilisation under the following headings

A. Uses of commonly used alcohols, aldehydes, Chlorine as disinfectants. (2 marks)

Disinfectants	*Uses/Applications*
Alcohols—Ethyl alcohol	• Hand rub • Thermometers • Stethoscopes
Aldehydes—Formaldehyde	• Fumigation of OT • Preservation of specimens
Chlorine—Sodium hypochlorite	• Spill management

B. Spaulding's classification of medical devices and various levels of disinfectants. (2 marks)

Category	*Description*	*Level of disinfection*	*Examples*
Non-critical	• Items in contact with intact skin	• Low or Intermediate	• BP cuff • Crutches • Non-critical patient items/ surfaces
Semi-critical	• Items in contact with mucous membrane or body fluids	• High	• Respiratory equipment • Scopes
Critical	• Items entering sterile site of the body	• Sterilisation	• Surgical instruments • Implants • Needles

C. Plasma Sterilisation. (1 mark)

- It is a process used to create the plasma state for sterilisation.
- Gas plasma is generated when electrical field is applied which breaks H_2O_2 into free radicals that have the microbicidal action.
- *Duration of cycle:* 24–75 minutes
- *Sterilisation control:* Spores of *Geobacillus Stearothermophiluss*
- *Applications:* In CSSD for sterilisation of heat sensitive materials and devices. e.g. laparoscope, arthroscopes
- *Disadvantages*
  1. High cost of equipment
  2. Bulk items cannot be used (small chamber)
  3. Linen, paper, liquid cannot be processed.

2. The gastroenterology endoscopy room in a tertiary hospital performs 7 procedures approximately on daily basis. Briefly explain the sterilisation process for endoscopes that must be carried out in-between each patient. (5 marks)

Sterilisation Process for Endoscopes

- The endoscopes involve precleaning, manual cleaning, disinfection, and sterilisation.
  1. **Precleaning**
     - It is to remove all the debris by flushing with air and water channels along with detergent solution in-between patients.
     - Debris could include protein, fats, carbohydrates that can inactivate the detergent/ disinfectants action against the microorganisms.
  2. **Manual cleaning**
     - Fill a basin with freshly prepared medical grade detergent solution and brush with help of soft brush and cleaning tools to free the scopes from remnant debris.
  3. **Disinfection and sterilisation**
     - 2–2.4% concentration of glutaraldehyde for 20 minutes for disinfection but 10–12 hours' time to kill spores.
     - The manufacturer's recommendation must be followed for all the steps involved in cleaning and disinfection of endoscopes.

3. A pregnant lady receives blood transfusion after a post-partum haemorrhage due to very low Hb%. Meanwhile the lady is restless and disconnects the IV set and hence there is a blood spill. What are the steps necessary to be taken in management of the above blood spill? Which is the disinfectant used for this purpose. Advantages and disadvantages of the disinfectant. (2+1+1+1 marks)

Steps in Blood Spill Management

1. Wear gloves and other PPE appropriate to the task.

2. When sharps are involved use forceps to pick up sharps, and discard these items in a puncture-resistant container.
3. Cover the spill with a newspaper, blotting paper/tissue paper.
4. Wipe the spill with a tissue paper moistened with freshly prepared hypochlorite solution (1% dilution containing minimum 500 ppm chlorine) for 20–30 minutes. Discard the paper as infected waste. Repeat until all visible soiling is removed.
5. Wipe the area with a cloth mop moistened with 1% hypochlorite solution and allow drying naturally.
6. All contaminated items used in the clean-up should be placed in a bio-hazardous bag for disposal.

Disinfectant for Blood Spill

➲ 1% Sodium hypochlorite.

Advantages of 1% Sodium Hypochlorite

1. Broad spectrum
2. Fast acting, non-inflammable, low cost
3. Widely available

Disadvantages of 1% Sodium Hypochlorite

1. Inactivated by organic matter
2. Unstable and toxic
3. Corrosive, carcinogenic
4. Leaves residues, offensive odour

4. Draw a neat, labelled diagram of an autoclave and enumerate its uses in a CSSD. (3+2 marks)

Diagram of an Autoclave (Fig. 1.4.1)

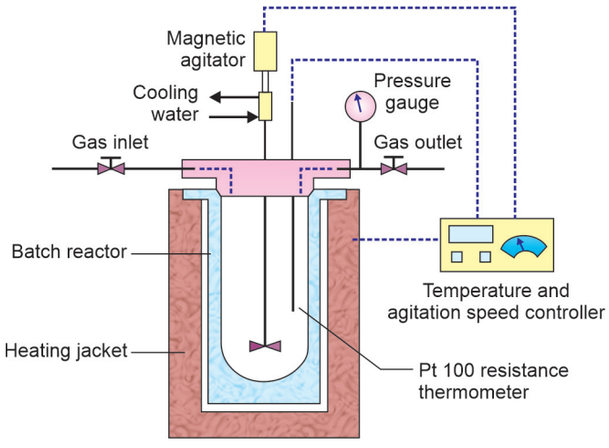


Fig. 1.4.1: *Autoclave*

Use of Autoclave in CSSD

1. Surgical instruments
2. Linen, surgical drapes and linen
3. Anaesthetic equipment
4. Dental equipment
5. Implant medical devices
6. Prosthetic devices

5. ETO steriliser. Mention its applications, advantages, disadvantages, and sterilisation control in a CSSD. (2+1+1+1 marks)

ETO Steriliser

Applications

1. Heart Lung machines
2. Sutures, catheters, and stents
3. Respirators
4. Dental equipment
5. Multi lumen tubing

Advantages

1. Large chamber
2. Suitable for heat sensitive devices
3. Non-corrosive and high penetration

Disadvantages

1. Highly inflammable, irritant, carcinogenic.
2. Long duration of cycle (12–14 hours).
3. High cost of consumables.

Sterilisation Controls

1. *Biological indicator:* Spores of *Bacillus atrophaeus.*
2. *Chemical indicator:* Bowie dick test.
3. *Physical indicator:* Temperature, time and pressure.

SHORT ANSWERS

1. Classify the moist heat methods of sterilisation at and below 100°C and their uses. (3 marks)

Heat methods of sterilisation at and below 100°C	*Uses*
Boiling at 100°C	• Surgical instruments
Steam at 100°C	• Useful for items where heating is unsuitable
Inspissation at 80–85°C for 30 minutes on 3 successive days	• Egg-based media (LJ media)
Pasteurisation (70°C for 30 minutes)	• Milk, respiratory and anaesthesia equipment

2. Applications of alcohol-based products used in a hospital set-up, its mechanisms of action (3 marks)

Alcohol-based Hand Rub

Applications

1. Non-critical item—thermometers (10–15 minutes).
2. Surface disinfection of vaccine bottles, blood culture bottles, hubs of central line.

3. Surface disinfection of stethoscope, ventilators, ultrasound machine.
4. Skin antisepsis.

Mechanisms of Action

- Act on bacteria, fungi, some enveloped virus but do not kill spores.
- Act by denaturing proteins and probably by dissolving membrane lipids.

3. A patient on ventilator support with multi-drug resistant bug gets discharged from the ICU and shifted to the ward. With respect to the case, what is meant by terminal disinfection and its applications? (2+1 marks)

Terminal Disinfection

- Preparing or disinfection of rooms or areas for subsequent patients or residents for them to be treated or cared for without the risk of acquiring an infection.

Applications

1. Subsequent to an outbreak or increased incidence of infection.
2. Following discharge, transfer or death of a patient who has had a known infection.
3. Following isolation/contact precaution nursing of a patient.

4. Add a note on disinfection of operation theatres (OT) (3 marks)

Environmental Cleaning

- Reduces the risk of transmission of infectious agents to healthcare workers and patients.

Surface Disinfection

- Cleaning must be carried out with cleansing agent like detergent followed by using aldehyde-based disinfection.

Procedure

- Disinfection should be carried out as following:

1. First before the cases begin in a day
2. In-between the cases
3. After the last case-terminal disinfection
4. Thorough wash down of the OT complex once a week
5. During any kind of renovation/construction

Note

- Fogging is not routinely recommended and done only in cases of outbreak or newly constructed or after renovation.

5. Enumerate the applications of sterilisation or disinfection process in a microbiology lab. (3 marks)

Applications of Sterilisation/Disinfection in a Microbiology Lab

1. *Sterilisation:* Autoclave-culture media, broth, biomedical waste discard (Vacutainer's culture media plates)
2. *Hot air oven:* Glass ware
3. *Surface disinfection:* 0.5% Sodium hypochlorite
4. *Blood and body fluid Spill:* 1% Sodium hypochlorite
5. *Bio safety cabinet disinfection:* Ethanol

6. Prions are resistant structures to routine methods of sterilisation. Statement is true or false. Justify your answer. (3 marks)

Statement is

- True.

Justification

- Methods used in sterilisation of prions are:
  - Autoclaving at 134°C for 1–1.5 hour.
  - Treatment with 1 N NaOH for 1 hour.
  - 0.5% sodium hypochlorite for 2 hours.

7. Alcohols are sporicidal agents. Statement is True or false. Justify your answer. (3 marks)

Statement is

- False.

Justification

- They act by denaturing proteins and possibly by dissolving membrane lipids but not sporicidal.
- Sporicidal agents are the following:
  - Ethylene oxide, formaldehyde, glutaraldehyde, hydrogen peroxide
  - Peracetic acid, O-phthalic acid, and plasma sterilisation
  - Autoclave and hot air oven.

8. Phenolic compounds can be used as antiseptic and disinfectant agent. Statement is True or False. Justify your answer.

Statement is

- True.

Justification

- Phenols such as cresols have disinfecting properties and retain its activity in the presence of organic matter but cannot be used as an antiseptic as they cause irritation of the skin.
- Phenols such as chlorhexidine, chloroxylenol can be used for skin antisepsis, the active ingredient being Savlon and Dettol, respectively.

9. Active ingredient of lysol is benzalkonium chloride. Statement is True or False. Justify your answer.

Statement is

- True.

Justification

- Quaternary ammonium compounds such as benzalkonium chloride is widely used as floor disinfectant and skin antisepsis.
- These surfactants interact with the lipid in the cell membrane through their hydrophobic chain, water and the polar group—disrupts the membrane.

MI 1.5 CHOOSE THE MOST APPROPRIATE METHOD OF STERILISATION AND DISINFECTION TO BE USED IN SPECIAL SITUATIONS IN THE LABORATORY, CLINICAL AND SURGICAL PRACTICES

LONG ESSAY

1. Describe in detassil about the physical methods of sterilisation under the following headings.

A. Classification of physical methods of sterilisation with appropriate examples. (4 marks)

Method of sterilisation		*Examples*
Physical	• Dry heat	• Flaming • Incineration • Hot air oven
	• Moist heat	
	• Temperature <100°C	• Pasteurisation • Water bath • Inspissation
	• Temperature at 100°C	• Boiling • Steaming • Tyndallisation
	• Temperature >100°C	• Autoclave
Filtration		• Candle filters • Asbestos filters • Membrane filters
Radiation	• Ionising radiation	• X-rays • Y-rays • Cosmic rays
	• Non-ionising radiation	• UV rays • Infrared rays

B. Uses of Cold Sterilisation (2 marks)

Type of method	*Uses*
Ionising radiation X-rays, gamma rays (from cobalt 60 source), and cosmic rays	1. Disposable plastics, e.g. rubber or plastic syringes, infusion sets and catheters 2. Catgut sutures, bone and tissue grafts and adhesive dressings, antibiotics, and hormones
Nonionising radiation Infrared and ultraviolet radiations	1. Sterilisation of clean surfaces in operation theatres, laminar flow hoods as well as for water treatment

C. Mention the specific surgical instruments sterilised in autoclave and hot air oven (2 marks)

Hot Air Oven

- Non sharp surgical instruments, forceps, etc.

Autoclave

- Dressings
- Metal surgical instruments
- Linens
- Glassware
- All suture materials except catgut

D. Importance of CSSD Unit in a tertiary care hospital. (2 marks)

- Bacteriological safe sterilisation
- Assurance of adequate supply of sterile products immediately and constantly available for some time and emergency use
- Conservation of trained staff
- Better quality control
- Prolonged life by proper care of equipment

SHORT ESSAYS

1. Describe the chemicals used as disinfectants in hospital practice under the following headings.

A. Disinfectants used for OT, the purpose and method of OT fogging. (2 marks)

Disinfectant used	*Purpose*	*Method*
Bacillocid • Formaldehyde-free	• Environmental deconta-mination	• These agents are dispersed with the aid of a foggier-like device inside the theatre environment • The contact time—1 hour
Virkon • A non-aldehyde compound • It contains oxone (potassium peroxy-monosulfate), sodium dodecyl-benzenesulfonate, sulphamic acid, and inorganic buffers	• For cleaning up hazardous spills disinfecting surfaces and soaking equipment	

Contd.

Disinfectant used	Purpose	Method
Formaldehyde • Pungent and harmful	• Environmental decontamination • Fumigation is obsolete in many developed nations in view of toxic nature of formalin	• During fumigation, it is tightly closed and sealed before. • The room is opened after fumigation (12–24 hours) • The room can be used once all fumes are out

B. Uses of aldehydes as disinfectants. (1 mark)

Aldehyde	Uses
Formaldehyde	1. Preservation of anatomical specimen 2. Formaldehyde gas is used for fumigation of closed areas such as operation theaters, not used anymore as it is hazardous 3. Preparation of toxoid from toxin. It is toxic, irritant, and corrosive to metals
Glutaraldehyde	1. Less toxic, less irritant, and less corrosive, hence is best used to sterilize endoscopes and cystoscopes
Ortho-phthalaldehyde (0.55%)	1. For sterilisation of endoscopes and cystoscopes 2. It does not require activation 3. Low vapour property 4. Better odour 5. More stable during storage 6. High mycobactericidal activity

C. Grading of disinfectants with examples for each. (2 marks)

Spaulding's Classification

High level disinfection	• Aldehydes—Glutaraldehyde • Peracetic acid • Hydrogen peroxide	• Endoscopes • Endoscopes, dental instruments • Wound cleaning
Intermediate level disinfection	• Alcohols—Ethyl alcohol • Phenolics—Phenol, cresol • Halogens—Iodine, chlorine	• Skin antisepsis • Skin antisepsis • Topical ointment, skin antisepsis, blood spill
Low level disinfection	• Quaternary ammonium compounds • Chlorhexidine	• BP cuff • Hand rub

2. Mention the culture media sterilised by Inspissation. Describe the method of performing inspissation. (2+3 marks)

Inspissation (Fractional sterilisation)

➲ It is a process of heating an article on 3 successive days at 80–85°C for 30 minutes.

Culture Media Sterilised

1. Egg based (LJ and Dorset's egg medium).
2. Serum-based media (Loeffler's serum slope).

Method of Performing Inspissation

Day	Temperature	Time	Purpose
1	85°C	60 minutes ↓ Overnight incubation	• Drying of the medium and killing the organisms in their vegetative form
			• Growth of vegetative forms from spores
2	75 to 80°C	20 minutes ↓ Overnight incubation	• Killing the organisms in their vegetative form
			• Growth of vegetative forms from any remaining spores
3	75 to 85°C	20 minutes	• Killing the organisms in their vegetative form as well as the leftover spores

SHORT ANSWERS

1. Describe the process and mention the culture media sterilised by Tyndallisation. (1+2 marks)

Tyndallisation/Intermittent Sterilisation

Process

➲ Involves steaming at 100°C for 20 minutes for 3 consecutive days.

Culture Media Sterilised

1. Gelatin and egg, serum or sugar containing media.
2. It kills most of the vegetative forms including spores.

2. Enumerate the testing methods of efficacy of disinfection (3 marks)

Testing Methods of Efficacy of Disinfection

1. **Phenol coefficient (Rideal–Walker) test**
   ➲ Determined by the dilution of the disinfectant in question which sterilizes the suspension of *Salmonella typhi* in a given time divided by the

dilution of phenol which sterilizes the suspension at the same time.

2. **Chick Martin test**
   - Modified Rideal and Walker test.
   - Here the disinfectants act in the presence of organic matter (e.g. dried yeast, feces, etc.) to simulate the natural conditions.
3. **Capacity (Kelsey–Sykes) test**
   - It tests the capacity of a disinfectant to retain its activity when repeatedly used microbiologically.
4. **In-use (Kelsey and Maurer) test**
   - It determines the efficacy of chosen disinfectant in hospital practice

MI 1.6 DESCRIBE THE MECHANISMS OF DRUG RESISTANCE AND METHODS OF ANTIMICROBIAL SUSCEPTIBILITY TESTING AND MONITORING ANTIMICROBIAL THERAPY

LONG ESSAYS

1. Describe in detail about the antimicrobial drug resistance under the following headings.

A. Classify antibacterial agents and their mechanism of action. (2 marks)

B. Classify drug resistance and list out the probable reasons for acquired drug resistance. (2 marks)

C. Describe in detail the mechanism of drug resistance with suitable example. (4 marks)

D. Enumerate the various antimicrobial susceptibility testing methods (2 marks)

A. Classification of Drug Resistance (Fig. 1.6.1)

1. **Intrinsic drug resistance**
   - Resistance resulting from the normal genetic, structural, or physiological state of microorganism, e.g. vancomycin resistance among the gram-negative bacilli.
2. **Acquired drug resistance**
   - Resistance that results from altered cellular physiology and structure due to change in the usual genetic makeup of a microorganism.

B. Probable Reasons for Acquired Drug Resistance

- **Acquired drug resistance:** Resistance that result from altered cellular physiology and structure due to change in the usual genetic makeup of a microorganism
- **Mutation and selection**
  - Mutation in genes coding for drug susceptibility leads to resistant bacteria.
  - In presence of antibiotics the susceptible bacteria are eliminated whereas the resistant bacteria survive and multiply, e.g. resistance of *Mycobacterium tuberculosis* to isoniazid and rifampicin.

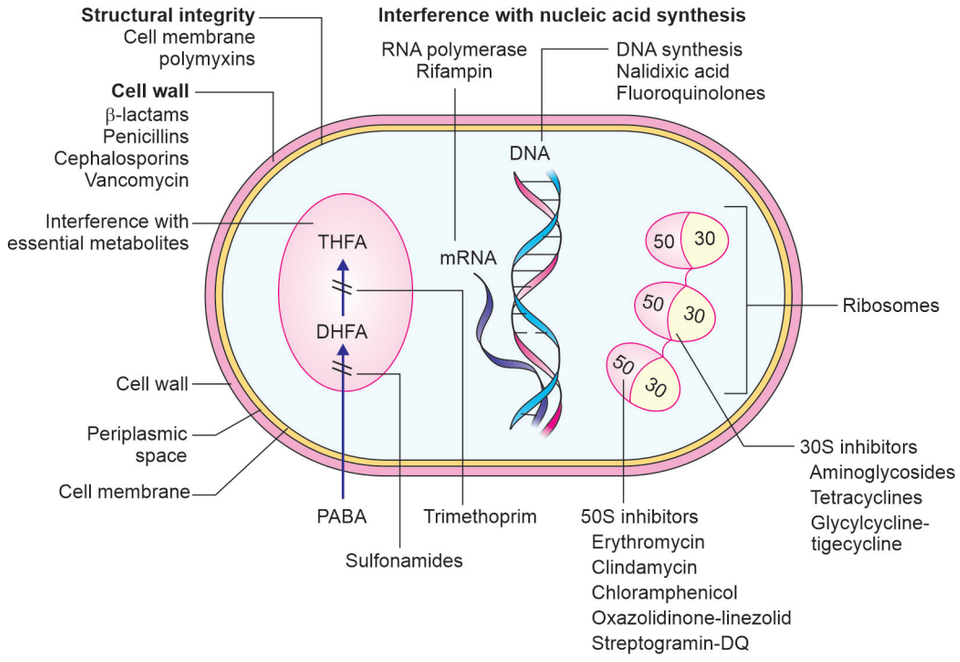


Fig. 1.6.1: Antimicrobial drug resistance

- **Acquiring drug resistant gene/transferable drug resistance**
  - Drug resistant bacteria carry genes for one and often several antimicrobial drugs.
  - The resistant gene can be transferred by transduction, transformation, and conjugation, e.g. *Salmonella typhi* may acquire R plasmid from *Escherichia coli*—conjugation.

C. Mechanisms of Drug Resistance

Mechanism	*Examples*
Enzymatic inactivation of the drug	• Penicillin resistance in *Staphylococcus aureus* • *N. gonorrhoeae* due to production of penicillinases
Altered target site • Resistant bacteria produce altered target for the drug, to which drug does not bind	• Altered penicillin binding protein (PBP2a) in methicillin resistant *Staphylococcus aureus* (MRSA)
Impaired membrane permeability • When the bacteria do not allow entry or influx of antibiotic inside the bacterial cell, they become resistant	• Aminoglycoside resistance in *Pseudomonas aeruginosa*
Active efflux of antibiotics • Efflux pumps remove toxic metabolites and antibiotics from the bacterial cell	• Norfloxacin resistance in *E. coli*
Alteration of metabolic pathway • An altered pathway is developed by the organism that bypasses the reaction inhibited by the drug	• Sulfonamide resistance
Altered enzyme formation • An altered enzyme is produced by the microorganism that can perform similar metabolic function without getting affected by the drug	• Trimethoprim resistance—altered dihydrofolic acid reductase enzyme is inhibited less efficiently by the drug
• Combination of different mechanism	• Carbapenem resistance in *Klebsiella* spp

D. Various Antimicrobial Susceptibility Testing Methods

1. Diffusion Method

- Kirby Bauer disc diffusion method.
- **MRSA detection:** Disk diffusion method—Cefoxitin disc method
- **ESBL detection:** Double disc test
- **E-test or diffusion:** Dilution test

2. Dilution Method

- Agar dilution method.
- Broth dilution method.
  i. Macrodilution method
  ii. Microdilution method

3. Detection of Antibiotic Inactivating Enzymes

i. Automated methods—Vitek 2 Systems.
ii. Molecular Methods—detection of drug resistant genes or genes encoding enzymes.

2. A 35-year-old comes to the emergency department with c/o of fever, breathlessness. On examination patient was febrile with O_2 saturation of 88%. Nasopharyngeal swab sent and positive for influenza H3N2. Oseltamivir was started. Classify the antiviral agents with examples for each type. (10 marks)

Site of action	*Effective drugs*	*Used for*
Early events (entry/uncoating)	• Amantadine • Rimantadine	• Influenza virus
Inhibition of nucleic acid synthesis Nucleoside inhibitors for herpes group	• Acyclovir • Ganciclovir • Cidofovir • Vidarabine • Iododeoxyuridine • Trifluorothymidine	• HSV-1&2, VZV • CMV • CMV, HPV • HSV-Keratitis/encephalitis • HSV-Keratitis • HSV Keratitis
Non-nucleoside inhibitors for herpes virus	• Foscarnet	• HSV, CMV • Esp. resistant to Acyclovir
Inhibition of nucleic acid synthesis Nucleoside inhibitors for HIV	• Azidothymidine • Dideoxyinosine • Dideoxycytidine • Lamivudine • Abacavir • Tenofovir • Stavudine	• HIV
• Nonnucleoside inhibitors for HIV	• Nevirapine • Delavirdine • Efavirenz	• HIV
Cleavage of precursor polypeptides	• Indinavir • Ritonavir • Nelfinavir	• HIV
Inhibition of protein synthesis	• Interferon • Methisazone • Fomivirsen	• HBV, HCV • Smallpox • CMV
Inhibition of viral release	• Zanamivir • Oseltamivir	• Influenza virus

Contd.

Site of action	Effective drugs	Used for
Antiviral	• Interferon α	• Chronic hepatitis B and C • Herpes viral infections • Malignancy: Hairy cell leukemia
Antitumor	• Interferon β	• Autoimmune disorders like multiple sclerosis
Immunoregulatory effects	• Interferon ϒ	• Chronic granulomatous diseases

SHORT ESSAYS

1. Interferons are an important part of the host defense against viral infections. Justify your answer and the role of interferons. (5 marks)

Interferons

- They host coded low molecular weight proteins produced by intact animals or cultured cells in response to viral infections or inducers.
- First line of defense
- Species specific
- Not virus specific

Classification

- Depending on the cell of origin
  1. *Leucocyte interferon:* IFN α
  2. *Fibroblast interferon:* IFN β
  3. *Lymphocyte interferon:* IFN ϒ

Inducers of IFN

- RNA viruses
- Avirulent viruses
- *Nucleic acid:* ds RNA
- Bacterial endotoxins, intracellular bacteria
- Synthetic polymers
- Inducer of IFN ϒ—mitogens like phytohaemagglutinin.

Source

- Pooled human leucocyte or lymphocyte activated by viruses or synthetic polyribonucleotides.
- DNA recombinant technology.

Actions

1. Antiviral effect.
2. Antitumour effect.
3. Immunoregulatory effects.

Uses

1. **IFN α**
   - In treatment of chronic hepatitis B and C
   - In prophylaxis and treatment of herpes viral infections
   - Localised instillation can be done in herpetic keratitis, laryngeal papillomatosis, condyloma acuminata.
   - Used in certain malignancies—lymphoma, hairy cell leukemia, CLL, Kaposi sarcoma, malignant melanoma.
2. **IFN β**
   - Used for treatment of relapsing or remitting autoimmune disorders like multiple sclerosis.
3. **IFN ϒ**
   - Used as immunostimulator in chronic granulomatous diseases and other disorders like Job's syndrome.
   - Used as an adjunct to enhance the response to DNA vaccine.
4. **Pegylated interferon**
   - Improved drug solubility.
   - Enhanced protection from proteolytic degradation.
   - Increase biological half life.
   - Reduced dosage frequency, with same efficacy and potentially decreased toxicity.

2. A 25-year-old man develops cough with blood-tinged sputum. H/o of loss of weight. Chest X ray shows right upper lobe cavity with chest infiltrates. Sputum sent for Gene Xpert® MTB report detected *M. tuberculosis* along with resistance to rifampicin.

A. What is your diagnosis? (1 mark)

- Multidrug resistant tuberculosis.

B. Briefly explain the mechanism of drug resistance in this organism. (4 marks)

- Drug resistance in *Mycobacterium tuberculosis* is chromosomal mediated.
- Drug resistance is acquired through:
  - Alteration of the drug target through mutations.
  - Overproduction of drug targets.
  - MDR occurs through accumulation of individual target genes.

Types

1. **Primary resistance:** Bacteria showing resistance in a patient, who has not received the drug in question before
2. **Acquired/Secondary resistance:** Bacteria susceptible to the drug at the beginning of the treatment but became resistant to the particular drug during the course of treatment

Mutations Conferring Drug Resistance

Drug	Gene	Gene product/ functional role	Cellular target
Rifampicin	• rpoB	• β-subunit of RNA polymerase/ transcription	• Nucleic acids
Isoniazid	• KatG • OxyR-ahpC • KasA	• Catalase-peroxidase/activation of Pro-drug • Alkyl-hydro-reductase/unknown • b-ketoacyl acyl carrier protein	• Cell wall
Ethionamide	• inhA	• Enol-ACP reductase/synthase • Mycolic acid synthesis	• Cell wall
Streptomycin	• rspl • rrs	• Ribosomal protein S12/translation • 16S rRNA/ translation	• Protein synthesis
Fluroquinolones	• gyrA	• DNA gyrase	• Nucleic acid
Pyrazinamide	• pncA	• Amidase/activation of pro-drug	• Unknown
Ethambutol	• embB	• Arabinosyl transferase/ arabinose polymerisation	• Cell wall

3. What is an antimicrobial stewardship programme and enumerate its role in a hospital? (4 marks)

Antimicrobial Stewardship

➲ A well-co-ordinated programme that promotes the appropriate use of antimicrobials (including antibiotics), resulting in improvement in patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms.

Role in a Hospital

Primary Goal

1. To optimize safe and appropriate use of antibiotics.
2. To improve clinical outcomes.
3. Minimise adverse effects of antibiotics.

Secondary Goal

1. To reduce healthcare costs without adversely impacting quality of patient care.
2. To reduce the incidence of antibiotic induced collateral damage.

4. Briefly discuss the factors a clinician should consider while prescribing any antimicrobial therapy? (5 marks)

1. **Obtaining an accurate infectious disease diagnosis**
   - ➲ Specimens are properly obtained and promptly submitted to the microbiology laboratory, preferably before the initiating of antimicrobial therapy.
2. **Timing of initiation of antimicrobial therapy**
   - A. *Empiric therapy*
     - ➲ For septic shock, febrile neutropenic patients, and patients with bacterial meningitis
     - ➲ Clinicians should consider the following
       - i. The site of infection and the organisms most likely to be colonizing that site
       - ii. Prior knowledge of bacteria known to colonise a given patient
       - iii. The local bacterial resistance patterns
   - B. *Definitive therapy*
     - ➲ Once microbiology results have helped to identify the etiologic pathogen and/ or antimicrobial susceptibility data are available, every attempt should be made to narrow the antibiotic spectrum
3. **In stable clinical circumstances**, antimicrobial therapy should be deliberately withheld until appropriate specimens have been collected and submitted to the microbiology laboratory like in subacute bacterial endocarditis and vertebral osteomyelitis/discitis
4. **Different AST interpretations** for different sites of infection (e.g. meningitis and non-meningitis AST results for *S. pneumoniae*)
5. **Bactericidal vs bacteriostatic therapy**
   - ➲ Bactericidal agents are preferred in the case of serious infections such as endocarditis and meningitis
6. **Use of antimicrobial combinations**
   - i. When agents exhibit synergistic activity against a microorganism
   - ii. When critically ill patients require empiric therapy
   - iii. To prevent resistance
7. **Host factors to be considered in selection of antimicrobial agents**
   - i. Renal hepatic function
   - ii. Age
   - iii. Pregnancy/lactation
   - iv. H/o allergy
   - v. H/o recent antibiotic use

5. The reason why methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to methicillin and nafcillin. Justify your answer. (4 marks)

Methicillin-resistant *Staphylococcus aureus* (MRSA)

- Staphylococcus aureus strains emerged that were resistant to the β-lactamase-stable penicillins. These strains were termed "methicillin resistant S. aureus" (MRSA), because methicillin was initially used to detect their resistance to β-lactamase-stable penicillins (oxacillin, methicillin, nafcillin).

Sources

1. Asymptomatically colonised patients and health-care workers.
2. The most common site of MRSA carriage is the anterior nares.
3. A significant risk factor for acquisition of MRSA is the duration of hospital stay.

Mechanism

- Primarily mediated by the *mec*A gene, which codes for the modified penicillin-binding protein 2a (PBP 2a or PBP 2′).
- PBP2a is in the bacterial cell wall and has a low binding affinity for β-lactams.

Detection

A. Phenotypic Detection Systems

1. Dilution Methods

i. *Agar dilution.*
- Tests on MH with 2% NaCl and an inoculum of 10^4 cfu/ml will distinguish most resistant from susceptible strains.
- The method require incubation for 24 hours at 33–35°C.
- In both methods an oxacillin MIC of ≤2 mg/L indicates that the strain is susceptible and >2 mg/L resistant.

ii. *Broth microdilution.*
- The CLSI method, which requires the use of MH broth with 2% NaCl, an inoculum of 5×10^5 cfu/ml and incubation at 33–35°C for 24 hours.

2. E-test Method

- MIC value.

3. Agar Screening Method

- Requires suspending the test organism to the density of a 0.5 McFarland standard and inoculating MH agar containing 4% NaCl and 6 mg/L oxacillin with a spot or a streak of the organism. Plates are incubated at 35°C or less for 24 hours and any growth other than a single colony is indicative of resistance.

4. Cefoxitin Disc Diffusion Test

- Cefoxitin, which is a potent inducer of the *mecA*.
- An inhibition zone diameter of ≤21 mm is reported as methicillin resistant and ≥22 mm is considered as methicillin susceptible.

5. Automated System

- Vitek system.
- Phoenix system.

B. Genotypic Detection System

- PCR for the detection of *mecA* gene.

Treatment

- Vancomycin or daptomycin are the agents of choice for treatment of invasive MRSA infections. Alternative agents that may be used for second-line therapy include ceftaroline, linezolid, cotrimoxazole, clindamycin.

6. A patient with UTI whose urine culture and sensitivity report revealed *E. coli* resistant to all the antibiotics tested under the panel. Briefly discuss the possible types of mechanism of antibiotic resistance prevailing in this case scenario. (4 marks)

Antibiotic Resistance in Uropathogenic *Escherichia coli*

1. **Resistance to β-lactams**
   - Production of different types of β-lactamase enzymes (penicillin, cephalosporin, monobactams, and carbapenems).
   - ESBL are enzymes that confer resistance to β-lactam antibiotics (all penicillins, cephalosporins, and monobactams), except for carbapenems, cephamycins, and β-lactamase inhibitors. Result of mutations in the genes coding for ancestral enzymes responsible are blaTEM-1, blaTEM-2, and blaSHV-1.
2. **Quinolone resistance**
   - Mutation in the genes *gyrA* and *gyrB* that catalyze DNA supercoiling.
   - Presence of efflux pumps.
   - Decreased uptake of the antibiotics due to changes in the outer membrane porin proteins .
3. **Fosfomycin resistance**
   - Fosfomycin uptake is reduced by the bacterial cells due to mutations in the genes related to the inactivation of fosfomycin by enzymatic cleavage.
4. **Nitrofurantoin resistance**
   - Mutations in genes *nsfA* and *nfsB* encoding the oxygen-insensitive nitroreductases.
5. **Resistance to sulphonamides, aminoglycosides**
   - Associated with the presence of specific plasmids. Resistance to sulphonamides is determined

by three genes (*sul1*, *sul2* and *sul3*) and a*adB*, *aac(3)-II* and *aac(3)-IV* genes are related to the gentamicin, tobramycin, neomycin resistance.

SHORT ANSWERS

1. Amphotericin B is noted for both its antifungal efficacy and side effects when administered to humans. Statement is true or false. Classify antifungal agents and their mechanism of action with appropriate examples. (3 marks)

Statement is

- True.

Classification of Antifungal Agents

Mechanism of action	*Examples*	
• Inhibit lanosterol 14α-demethylase—the enzyme required to convert lanosterol into ergosterol	**Imidazoles**	
	1. Clotrimazole 2. Oxiconazole 3. Miconazole	4. Econazole 5. Tioconazole 6. Ketoconazole
	Triazoles	
	1. Fluconazole 2. Itraconazole 3. Terconazole	4. Voriconazole 5. Isavuconazole 6. Posaconazole
• Inhibits mitosis in dermatophytes	• Griseofulvin	
• Pyrimidine analogue—converted into S-fluorouracil by the fungal enzyme cytosine deaminase	• Flucytosine	
• Inhibit cell wall synthesis by targeting glucans—inhibit 1,3-β-glucan synthase	• Micafungin • Anidulafungin • Caspofungin	
• Inhibits squalene epoxidase	• Amorolfine • Naftifine • Terbinafine	

2. Define intrinsic resistance in bacteria. Give 4 examples. (1 + 2 marks)

Intrinsic Resistance

Definition

- Drug resistance resulting from normal genetic, structural, or physiologic state of the bacteria.

Examples

1. Penicillin resistance in cell wall deficient bacteria like *Mycoplasma.*
2. *Enterococci* are resistant to cotrimoxazole and cephalosporins.
3. Vancomycin resistance in gram-negative bacilli.
4. Tigecycline in *Pseudomonas aeruginosa.*

3. Some bacteria become resistant to penicillin. Which are the different mechanisms for development its resistance? (3 marks)

1. Plasmid-encoded penicillinase .
2. Inactivation of the antibiotic by production of penicillinase.
3. Antibiotic efflux.
4. Modification of target Penicillin binding protein (PBP).
5. Impaired penetration of the drug to the target PBP.

4. Lower the MIC of an antimicrobial agent better is the therapeutic efficacy. Justify your answer. (3 marks)

- MIC is a reasonable approximate order of magnitude of concentration of free drug needed at the site of infection.
- Minimal concentration of an antimicrobial needed to inhibit bacterial growth.
- A lower MIC is an indication of a better antimicrobial agent.
- An MIC number for one antibiotic CANNOT be compared to the MIC number for another antibiotic.
- Minimum inhibitory concentrations (MICs)—useful in selecting the best antimicrobial agent for a given patient with known culture and susceptibility results.
- Antibiotics with high MICs may still be effective if it concentrates at the site of infection (e.g. treatment of UTI with gentamicin, which concentrates in the urine).

MI 1.7 DESCRIBE THE IMMUNOLOGICAL MECHANISMS IN HEALTH

LONG ESSAY

1. Describe in detail about immunity and immunological mechanisms under the following headings.

A. Define and classify immunity. (1 + 1 marks)

Definition

- Resistance of the individual to the damage done by microbe or the microbial product.

Classification of immunity (Fig. 1.7.1)

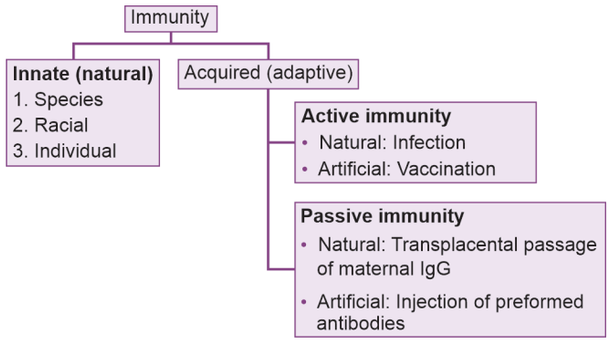


Fig. 1.7.1: Classification of immunity

B. Cilia present in the respiratory tract helps in clearance of microorganisms and provides immunity. Discuss the components and mechanism of this type of immunity. (1 + 7 marks)

- Innate immunity is the inborn resistance against infections that an individual possesses right from the birth, due to his genetic or constitutional makeup.

Components

1. **Mechanisms defined at body surface.**
   - Physiological factors, mechanical barriers and surface secretions.
2. **Systemic natural immunity.**
   - Cellular factors.
   - Other body defense mechanisms of systemic natural immunity.

Mechanisms of Innate Immunity (Fig. 1.7.2)

Barriers

Skin

- Protective integument prevents entry of microorganisms.
- Skin secretions-antibacterial activity of sweat and sebaceous secretions—acidic pH, high salt content, long chain saturated fatty acids.
- Epithelial shedding.
- Normal flora—prevents adherence, colonisation.

GIT

- Hydrolytic enzymes in saliva, gastric acidity kill the bacteria.
- Mucous membrane, mucus entraps the organism.
- Proteolytic enzymes, bile: Antibacterial activity.
- Peristaltic movement expelling the organism.
- Normal intestinal flora (microbial antagonism): Block the receptors, produce bacteriocins, compete for nutrition.
- Gut-associated lymphoid tissue, mucosa associated lymphoid tissue.

Respiratory System

- Architecture of nose prevents entry of organisms.
- Mucus entraps the organism.
- Antibacterial and antiviral substances—lysozyme, secretary IgA.
- Cilia, sneezing, cough reflex, help to remove the organisms out.
- Alveolar macrophages—phagocytosis.

Genitourinary Tract

- Flushing action of urine
- Uromucoid—entraps the bacteria.
- High acidic pH of vagina.
- Antibacterial secretions of glands (prostatic).

Conjunctivae

- Lacrimal secretions
- Lysozyme
- Shedding of microorganisms: Salivation, urination, defaecation, desquamation of skin, lacrimal secretions.

Lysozyme

- Present in tears, saliva, respiratory and cervical secretion. Hydrolytic enzyme breaks the cell wall.

Systemic Mechanisms

1. **Cellular systemic immunity**
   - Natural defense against tissue and blood invasion. Immune cells as a part of Innate immunity come into play when the integrity of the barrier (first line of defence) has been breached.
   - Phagocytosis—intracellular killing by neutrophils, macrophages.
   - Reticuloendothelial system present in liver, spleen and bone marrow.
   - Fixed macrophages clear organisms from circulation.

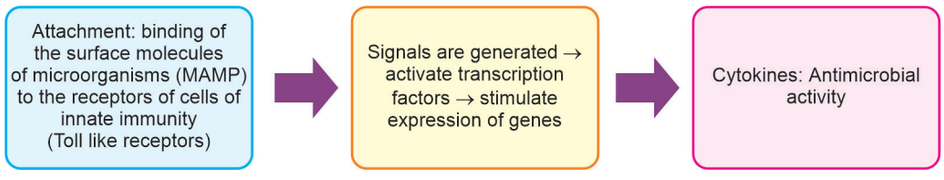


Fig. 1.7.2: Mechanism of innate immunity

- *Eosinophils:* Line of defense against the migratory larvae of parasites: *Trichinella* and *Strongyloides*
- *Natural killer cells:* Virus infected and cancer cells

2. **Inflammation**
   - Response of the body to the damage done by pathogen.
   - Vasoconstriction → vasodilatation → migration of PMNLs → phagocytosis.
   - There is accumulation of phagocytes at the site of infection with outpouring of natural antibacterial substances, fibrin deposition and localisation of infection.
3. **Fever**
   - Endotoxin, antigen antibody complex activate macrophages, which produces interleukin-1.
   - IL-1 acts on thermoregulatory centre of hypothalamus → Fever.
   - Elevated temperature retards bacterial growth.
4. **Acute phase proteins**
   - C-reactive protein (CRP), mannose binding protein, alpha-1-acid glycoprotein, serum amyloid P component.
   - *Action:* activation of complement system, prevent tissue injury, promote repair of inflammatory lesions
5. **Interferons:** Antiviral activity.
6. **Complement system**
   - Alternate pathway (bacterial endotoxin), mannose binding pathway (mannose residue on bacterial surface).
   - Destroys pathogenic organisms by various biological activities (lysis, opsonisation).
7. **Antibacterial substances in blood and tissues**
   - β-lysin-active against many bacteria like *Bacillus anthracis.*
   - Basic polypeptides—leukins.
   - Acidic substances like lactic acid seen in inflammatory tissue.
   - Lactoperoxidase in milk.
8. **Toll-like receptors (pathogen-associated molecular pattern PAMP)**
   - 13 TLRs on the macrophages, dendritic cells, mast cells recognise pathogens, enhance phagocytosis led to inflammation at the site.
9. **Defensins**
   - Positively charged (cationic) peptides, produced in the gastrointestinal and lower respiratory tracts.
   - Create pores in lipid membranes of bacteria, fungi, and viruses.
   - *Neutrophils and Paneth cells in the intestinal crypts:* α-defensins (antiviral activity)
   - *Respiratory tract:* β-defensins (antibacterial)
10. **Apolipoprotein B RNA editing enzyme**
    - Antiretroviral activity.
    - Causes hypermutation in retroviral DNA and mRNA.

2. Discuss the type of immunity acquired by an individual during life under the following headings.

A. Definition. (1 mark)

- Immunity acquired by an individual during life is Acquired Immunity or Adaptive Immunity.
- It is the resistance acquired by an individual during life.

B. Characteristics. (3 marks)

- It has two components.
  1. Cell-mediated immunity (T cells)
  2. Humoral or antibody mediated.
- Features—diversity, memory, specificity.
- Mediators.
  - T cells and B cells
  - Classical complement pathway
  - Antigen presenting cells
  - Cytokines (IL-2, IL-4, IL-5)
- Immunity acquired through effective prior contact with antigen.
- Effective contact of immunogen with immune system → Leads to immune response against the immunogens → Resulting in specific immunity against antigen.
- Immunity—both humoral and cellular immunity.
- Associated with immunological memory (improves upon repeated exposure).
- Duration of immunity.
  1. Lifelong as in mumps, measles.
  2. Short as in influenza.

C. Types with examples. (6 marks)

1. Active Immunity

- Protection based on exposure to the organism in the form of overt disease, subclinical infection, or a vaccine.

Characteristics

- Active involvement of the host in mounting an immune response consisting of antibodies and activated T lymphocytes.
- Develops slowly and persists for long time
- Specific
- Negative phase
- Lag phase
- Primary and secondary immune response
- Booster effect observed during subsequent injection.

A. Natural Active Immunity

- Resistance developed by the host in response to infection
- Immunity is long lasting—measles, chickenpox or short-live (influenza).
- Premunition or concomitant immunity—Immunity may last as long as the microbe is present. Once the disease is cured, the patient becomes susceptible to the microbe again (*Spirochaetes* and *Plasmodium).*

B. Artificial Active Immunity

- Produced by vaccination.

2. Passive Immunity

Characteristics

- Specific immunity
- No active immune response in the host
- Gives immediate protection
- Short lived or temporary protection.

A. Natural Passive Immunity

- Placental transfer of antibodies (IgG) from mother to faetus gives protection up to 3–6 months
- Through milk and colostrum
  - Secretary IgA antibodies
  - Protection against enteric infection

B. Artificial Passive Immunity

- Preformed antibodies acquired artificially through immune serum (antisera) and immunoglobulins.
- Given for immediate protection of non-immune host.
- Antisera-obtained by hyperimmunisation of horses ARS for rabies, ADS for diphtheria, ATS for tetanus.
- Advantage: Immediate protection.
- Disadvantages.
  1. Risk of hypersensitivity (foreign protein)
  2. Large doses required
  3. Immunity short lived.

SHORT ESSAYS

1. What are macrophages and classify macrophages based on their location? Discuss the functions of different types of macrophages. (3 + 2 marks)

Macrophages

They are:

- A type of white blood cell of the immune system that engulfs and digests cancer cells, microbes, cellular debris, foreign substances by phagocytosis.
- *Function:* To defend the host against infection and injury
- Derived from bone marrow histiocytes.

Classification of Macrophages

1. Free macrophages (Monocytes).
2. Fixed macrophages.
   i. Kupffer cells in liver.
   ii. Alveolar macrophages in lungs.
   iii. Microglial cells in brain.

Functions

1. **Phagocytosis**
   - Ingestion of bacteria, viruses, and other foreign particles
   - Fusion of phagosome containing microbe with lysosome
   - Killing of the microbe within the phago-lysosome
     - Reactive metabolites such as H_2O_2, superoxide anions
     - Reactive N_2 metabolite—nitric oxide
     - Lysosomal enzymes—proteases, nucleases, and lysozyme
2. **Antigen presentation**
   - Capture and process antigen.
   - Present antigen in association with class II MHC protein.
   - Display B7 protein which acts as co-stimulatory signal for T cells activation.
3. **Production of cytokines**
   - IL-1 (Endogenous pyrogen).
   - TNF (Inflammatory mediator).
   - IL-8 (Chemoattractant).

2. Describe the barriers of innate immunity with appropriate examples. (5 marks)

Anatomic site	*Mechanical barriers*	*Chemical barriers*	*Biological barriers*
Skin	• Keratinised squamous epidermis cells	• Fatty acids • Defensins	• Normal Skin flora
Gastro-intestinal tract	• Mucins: A sticky mixture of glycoproteins produced by secretory epithelial cells • Peristalsis • Normal shedding of epithelial cells	• Gastric acid • Digestive enzymes • Defensins lysozyme • Iron-binding protein	• Gut flora • IgA
Genito-urinary tract	• Urine flow	• Low pH	• Vaginal flora • IgA

Contd.

Anatomic site	Mechanical barriers	Chemical barriers	Biological barriers
Respiratory tract	• Airflow • Ciliated airway cells • Coughing	• Surfactant proteins: lipoproteins produced in the lung alveoli that bind to the surface of microbes, which can facilitate their phagocytosis (i.e. opsonin function) or can be directly bactericidal	• Nose, mouth, and pharyngeal flora • IgA

3. Define herd immunity. Discuss the role of herd immunity in a community/city with respect to COVID-19 infection (2+3 marks)

Herd Immunity

Definition

- Overall immunity of persons in a community against infectious disease or
- Indirect protection from an infectious disease that happens when a population is immune either through vaccination or immunity developed through previous infection, e.g. in OPV, MMR.

Role of herd immunity in a community/city with respect to COVID-19 infection

- Herd immunity against COVID-19 can be achieved by vaccinating all the individuals in the community and ideally not by exposing the individuals to infection.
- To achieve herd immunity against COVID-19, a substantial proportion of a population would need to be vaccinated.
- This reduces the amount of virus spread in the community.

4. An elderly gets a pneumococcal vaccine. Which type of specific immunity plays a significant role in this case scenario? Define vaccine. List the different types of vaccines. (1+1+3 marks)

Type of specific immunity plays a significant role in this case

- Artificial active immunity attained by vaccination.

Vaccine Definition

- Preparation of live, attenuated or killed microorganisms or their antigens or active materials derived from them (toxoids) used for immunisation.

Types of Vaccines

They are:

1. Live attenuated vaccines
2. Killed vaccines
3. Toxoids
4. Subunit vaccines
5. Recombinant vaccines
6. Synthetic peptides
7. DNA vaccines
8. Edible vaccines
9. Anti-idiotype vaccines.

5. Compare and contrast active and passive immunity. (1+4 marks)

Active Immunity

- Protection based on exposure to the organism in the form of overt disease, subclinical infection, or a vaccine.

Passive Immunity

- Protection based on the transfer of preformed antibody from one person (or animal) to another person.

Differences between Active Immunity and Passive Immunity

Feature	Active immunity	Passive immunity
Method of acquisition	• Effective contact with antigen	• Preformed antibodies
Immune response	• Present	• Absent
Duration of immunity	• Long lived months to years	• Short lived
Immunological memory	• Present	• Absent
Lag phase	• Present	• Absent
Utility in immunocompromised	• No	• Yes

6. Enumerate the systemic mechanisms of innate immunity and their mode of action. (5 marks)

Systemic mechanisms of innate immunity	*Mode of action*
Cellular systemic immunity • Natural defense against tissue and blood invasion	• *Phagocytosis:* Intracellular killing by neutrophils, macrophages • *Reticuloendothelial system:* Present in liver, spleen and bone marrow • *Fixed macrophages:* Clear organisms from circulation • *Eosinophils* (defense against parasites) and *natural killer cells* (virus infected and cancer cells)
Inflammation	• Vasoconstriction → vasodilatation → migration of PMNLs → phagocytosis • Accumulation of phagocytes at site of infection with outpouring of natural antibacterial substances, fibrin deposition and localisation of infection
Fever	• Endotoxin, antigen antibody complex → Activate macrophages → which produces interleukin-1 • IL-1 acts on thermoregulatory centre of hypothalamus • Fever increases rate of phagocytosis and antibody production
Acute phase proteins	• C-reactive protein (CRP), mannose binding protein, alpha-1-acid glycoprotein, serum amyloid P component • Activate complement, prevent tissue injury, promote repair of inflammatory lesions
Interferons	• Antiviral activity
Complement system	• Alternate pathway (activated by bacterial endotoxin), mannose-binding pathway (activated by mannose residue on bacterial surface) • Destroys pathogenic organisms by various biological activities (lysis, opsonisation
Antibacterial substances in blood and tissues	• β-lysin-active against many bacteria like *Bacillus anthracis* • Basic polypeptides-leukins • Acidic substances like lactic acid seen in inflammatory tissue • Lactoperoxidase in milk
Toll like receptors (pathogen associated molecular pattern PAMP)	• TLRs on the macrophages, dendritic cells, mast cells recognise pathogens • TLR stimulate expression of genes encoding cytokines and enzymes → antimicrobial activity
Defensins	• Highly positively charged (cationic) peptides that create pores in the membranes of bacteria, which kills them. • Neutrophils and Paneth cells in the intestinal crypts contain α-defensins • Respiratory tract produces β-defensins
Apolipoprotein B RNA editing enzyme	• Antiretroviral activity

7. Enumerate the proteins that either increase or decrease exponentially during acute inflammatory conditions. Justify your answer. Elaborate on two such important proteins. (1+1+2 marks)

Proteins that either increase or decrease exponentially during acute inflammatory conditions are:

- Acute phase reactants (APR)
- The positive acute phase proteins include procalcitonin, C-reactive protein, ferritin, fibrinogen, hepcidin, and serum amyloid A.
- Synthesized by endothelial cells, fibroblasts, monocytes, and adipocytes.

Justification

- These markers show significant changes in serum concentration during inflammation.
- Produced in the liver during acute and chronic inflammatory states.

Examples of APR

1. CRP

- *Normal range for CRP:* 2–10 mg/L.
- CRP levels can increase 100–1000-fold during acute inflammation.
- Levels of CRP increase in:
  - Malignancies, pancreatitis, myocardial infarction.
  - *Marked increase* (>10 mg/dl)—acute bacterial infections, major trauma, and systemic vasculitis.
  - hs (high sensitivity) CRP is used to determine the risk for cardiovascular diseases.
- *Function:* Promote phagocytosis and facilitate the innate immune response against infectious pathogens
- Measured by latex agglutination or on automated platforms by nephelometry and turbidometry.

2. Procalcitonin

- LPS, microbial toxins, or inflammatory mediators can activate the procalcitonin gene in the liver,

kidney, adipocytes, pancreas, colon, and brain during inflammation.
- Sensitive marker for following the progression of infections, especially for pneumonia and sepsis. Levels of procalcitonin are used to guide antibiotic therapy.
- Measured by ELFA, ECLIA.

SHORT ANSWERS

1. Mention two examples for live attenuated vaccines and killed vaccines (2 marks)

Examples of Live Attenuated Vaccines

1. BCG vaccine for tuberculosis.
2. Measles, mumps, rubella (MMR) vaccine.

Examples of Killed Vaccines

1. TA vaccines for enteric fever.
2. Polio vaccine (Salk vaccine).

2. An intern has an accidental needle stick injury while drawing blood from hepatitis B patient. She is not vaccinated against hepatitis B. Which approach must be taken to prevent hepatitis B for the intern? (2 marks)

- In passive–active immunity (combined immunisation) the intern gets both preformed antibodies to provide immediate protection and a vaccine to provide long-term protection. These preparations are given at different sites in the body to prevent the antibodies from neutralising the vaccine. This approach is used to prevent hepatitis B.

3. Mr XYZ must travel 3 days from now to a country where hepatitis A is endemic. She knows that both vaccine and serum globulin preparation are available for prophylaxis. She asks which you would recommend? Justify your answer and which type of immunity does it confer? (2 marks)

- In this scenario, the serum globulin preparation containing antibodies against the virus is best because it provides immunity in the shortest time. The type of immunity provided by preformed antibodies is artificial passive immunity.

4. Describe in detail about Pattern Recognition Receptors with appropriate examples. (3 marks)

- Components of the innate immune arm have receptors, called pattern recognition receptors (PRRs) that recognise a molecular pattern, called a pathogen-associated molecular pattern (PAMP), that is present on the surface of many microbes.
- Two classes of receptors (Toll-like receptors and C-type lectin receptors)—recognise microbes that are outside the cells or within the cells' vesicles.
- Two classes of receptors in the cytoplasm of cells (NOD-like receptors and RIG-I helicase receptors) recognise microbes in the cells cytoplasm.
- Any mutations in the genes encoding these pattern receptors result in a failure to recognise pathogens and predispose to severe bacterial, viral, and fungal infections.
- Immune response is shaped by the combination of PRRs activated during the initial encounter with innate immunity.

5. Live viral polio vaccine induces what type of immunity. Define this type of immunity. Mention the significance of this type of Immunity. (1 + 2 marks)

Type of Immunity Induced by Live Viral Polio Vaccine

- Local or mucosal immunity.

Local Immunity Definition

- Immunity at the site of entry of infectious agents provided by secretory IgA which is produced by plasma cells present in submucosa.

Significance of Mucosal Immunity

1. To protect the mucous membranes from the colonisation and invasion by pathogens.
2. *To prevent antigen uptake:* Foreign proteins derived from ingested food, air-borne matter, and commensal microorganism.
3. To prevent the development of harmful immune responses to these antigens if they do reach the body interior.

6. Enumerate the bridges between Innate and Acquired Immunity and discuss their role clinically. (4 marks)

Feature	*In Innate Immunity*	*In Acquired Immunity*
Macrophages	• Engulfs and kills many classes of microbes, removal of debris, tissue repair	• Have surface IgG receptors that facilitate phagocytosis (opsonisation) • activated by IFN-γ, TNF-α from T cells • Professional APC expressing class II MHC

Contd.

Feature	In Innate Immunity	In Acquired Immunity
Dendritic cells	• Antigen uptake and presentation	• Professional APC expressing class II MHC
Neutrophils	• Engulfs and kills bacteria and fungi, digests cellular debris	• Attracted into tissues by chemokines, which are increased by T cell-derived IL-17
Eosinophils	• Granule proteins are toxic to cells • Involved in asthma and allergic diseases • Protective against helminth infections	• Surface IgE receptors; maturation and survival supported by IL-5 from T cells
Mast cells and basophils	• Release histamine, proteases, chemokines, and cytokines; contribute to allergic disease and anaphylaxis	• IgE receptors hold IgE molecules that survey for antigen
Complement system	• Alternate and mannose binding pathways activated by Endotoxin, mannose residue on the bacteria	• Classic pathway activated by Ag Ab complex
Cytokines	• Released by cells of innate immunity	• Activate cells of acquired immunity, e.g. IL1 by macrophages activate T cells
Antibody dependent cell-mediated cytotoxicity	• Cells of innate immunity such as NK cell, eosinophils, and neutrophils destroy (by cytotoxic effect) the target cells coated with specific antibodies	

7. Compare and contrast Innate and Acquired Immunity. (4 marks)

Feature	Innate Immunity	Acquired Immunity
Presence	• Since birth	• Acquired later in life
Specificity	• Nonspecific • No exposure to antigen required	• Specific response developing after exposure to foreign antigen
Response	• Rapid	• Slower (1-2 weeks)
Specificity of response	• Response not specific to some microbe, rather shared by many microbes (called as microbes-associated molecular patterns)	• Response is targeted to specific Antigens
Immunological memory	• Absent	• Present
Components	• Barrier • Phagocytes • NK cells, Mast cells, Dendritic cells • Alternate and mannose binding pathways • Fever and inflammatory responses • Normal resident flora • Cytokines: TNF-α, certain interleukin (IL-1, IL-6, IL-8, IL-12, IL-16, IL-18), IFN-α, β and TGF- β • Acute phase reactant proteins (APRs)	• T cell • B cell • Classical complement pathway • Antigen presenting cells • Cytokines (IL-2, IL-4, IL-5, IFN-γ) • Types • Active and passive immunity • Artificial and natural immunity

MI 1.8 DESCRIBE THE MECHANISMS OF IMMUNITY AND RESPONSE OF THE HOST IMMUNE SYSTEM TO INFECTIONS

LONG ESSAYS

1. Discuss the principle, types, and applications of agglutination reactions. (2+6+2 marks)

Agglutination Reactions

Principle

➲ When a particulate Ag reacts with Ab in the presence of electrolytes at suitable temperature and pH, the particulate Ag is clumped or agglutinated.

- *Marrack's hypothesis:* Zone phenomenon in agglutination occurs when Ag and Ab are present in optimal proportions
- Agglutination is more sensitive than precipitation for the detection of antibodies, better with IgM.
- For agglutination the Abs should be at least bivalent.
- Incomplete or monovalent Abs do not cause agglutination, though they combine with Ag. They act as blocking antibodies and are responsible for false-negative results.

Types Based on Principle

1. Active/direct agglutination tests.
2. Passive agglutination tests.
3. Reverse passive agglutination.

Active/Direct Agglutination Tests

- Particulate Ag is directly agglutinated by the Ab.

1. **Slide agglutination test**
   - Smooth suspension of the particulate Ag on the slide + a drop of antiserum
   - Clumping of the Ag within seconds
   - *Uses*
     1. To identify unknown bacteria using known antisera.
     2. ABO grouping of blood.
2. **Tube agglutination test**
   - Convenient method to detect Ab in the patient serum
   - Can be done to quantitate antibodies
   - Patient's serum is diluted in physiological saline 2 folds and fixed amount of Ag is added to each of the dilution
   - Highest dilution of the patient's serum which shows agglutination is the endpoint. The reciprocal of endpoint is the titre
   - Examples
     1. *Widal test:* Tube agglutination test to demonstrate O and H agglutinins against typhoid and paratyphoid bacilli in patient serum.
     2. *Standard agglutination test:* for brucellosis
     3. *Weil-Felix test:* Heterophile agglutination test for the serodiagnosis of typhus fever.
     4. *Streptococcal MG agglutination test:* Heterophile agglutination test used in the diagnosis of primary atypical pneumonia.
     5. *Paul Bunnell test:* Based on the presence of sheep cell agglutinins in the sera of infectious mononucleosis patients, which are adsorbed by ox RBCs, not by Guinea pig kidney extract.
     6. *Cold agglutination test:* Positive in mycoplasmal primary atypical pneumonia. Patient's sera agglutinate human O group erythrocytes at 4°C, being reversible at 37°C.
3. **Coombs' test (Antiglobulin test)**
   - Used to detect anti Rh antibody.
   - Performed to diagnose Rh incompatibility by detecting Rh antibody from mother's and baby's serum.
   - This Ab is incomplete.
   - It binds on the surface of Rh + erythrocytes but does not agglutinate.
   - Such sensitized erythrocytes agglutinate when antiglobulin (Rabbit antibody to human globulin–antiglobulin/Coombs' serum) is added.

Passive Agglutination Tests

- Ag coated inert carrier particles are agglutinated by Abs.
- It is possible to label carrier particle with soluble Ag.
- It is possible to use soluble Ag in agglutination by binding the Ag to an inert carrier particle.
- More convenient and sensitive for Ab detection.
- Characters of the inert particle.
  - Should be coloured.
  - Should be antigenically inert.
  - Must be stable and preservable.
  - Must be easy to label the carrier particle with the Ag.
- Carrier particles.
  - RBCs (not stable, susceptible to hemolysis)
  - Latex particles
  - Bentonite

1. Passive Haemagglutination Test

- *Uses.*
  1. *Streptozyme test:* to detect Abs against enzymes and toxins of Streptococcus pyogenes
  2. *TPHA (Treponema Pallidum Haemagglutination Test):* Used to detect specific antitreponemal Abs in syphilis

2. Passive Latex Agglutination Test

- Polystyrene latex are uniform spherical particles.
- 0.8–1 μm in diameter.
- Latex particles labelled with the Ag are used to detect specific Ab.
- *Use:* Detection of ASO, CRP, RA, hCG
- *Advantages*
  1. Easy to perform
  2. Rapid
  3. Highly sensitive
  4. Relative stability of the reagents
- *Disadvantages*
  1. False-positive results due to cross reacting antibodies.
  2. False-negative results due to presence of Ab's in low titre.

Reverse Passive Agglutination

- Carrier particles are labelled with Ab and the Ab labelled carrier particles are used to detect the Ag.
- *Uses.*
  1. In the diagnosis of cryptococcal meningitis, acute bacterial meningitis.
  2. Detection of *Streptococcus pyogenes* Ag in the throat swab.
  3. Detection of Vi Ag of *Salmonella typhi* in serum, urine.
  4. *In virology:* detection of Rotavirus Ag in the stool, HbsAg in serum

2. Which is the serological test method used to detect dengue IgM antibodies in the patient serum employing enzyme substrate complex? Describe in detail the principle, types, applications, advantages and disadvantages of the above test method. (1+3+4+2 marks)

- The test used to detect the presence of dengue IgM is ELISA.

ELISA

Principle

- Enzyme-linked immunosorbent assay is a plate-based assay technique designed for the detection and quantification of peptides, proteins, antibodies, and hormones.
- Antigen must be immobilised to a solid surface and then complexed with an antibody that is linked to an enzyme.
- Detection is done by assessing the conjugated enzyme activity via incubation with a substrate to produce a measurable product.
- Colour is detected by spectrophotometer.
- Substrates are specific for each enzyme (o-phenyl-diamine-dihydrochloride for Horse radish peroxidase).

Types (Fig. 1.8.1)

1. Noncompetitive Binding Assay

i. **Direct**
- Antigen measuring system (well + serum (Ag) + primary antibody labeled with enzyme + substrate → Colour)

ii. **Indirect**
- Antibody measuring system (Titre wells coated with antigens + serum (Ab) + Enzyme labelled antiantibodies + substrate → Colour)

2. Sandwich ELISA

- Well is coated with capture antibody + Serum (for Ag detection) + Antibody specific for Ag + substrate → Colour.
- Indirect Sandwich ELISA.
  - Primary antibody and the capture antibody belong to different species.
  - Another enzyme-labelled secondary antibody targeted against the primary antibody is added.
  - More specific than direct sandwich ELISA.

3. Competitive ELISA

- Unlabelled antibody is incubated with the serum to be tested for antigen.
- The antibody/antigen complexes are then added to the antigen coated well.
- Free antibody will bind to the well, more the test Ag in serum, less amount of free Ab will bind to the well.
- The plate is washed to remove unbound antibody.
- Antiglobulin specific to the primary antibody, coupled to enzyme is added.
- A substrate is added, and remaining enzymes elicit a chromogenic signal.
- For competitive ELISA, the higher the original antigen concentration, the weaker the eventual signal.

4. IgM capture ELISA

- Microtitre wells coated with anti IgM + Patient's serum (IgM) + Ag + Enzyme labelled secondary antibody + Substrate → Colour.

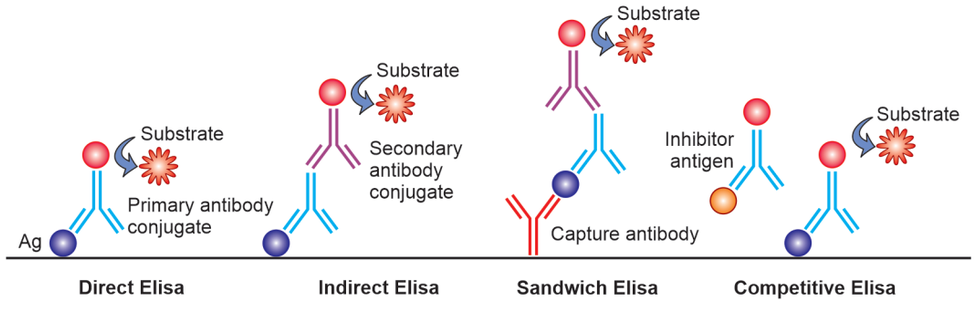


Fig. 1.8.1: Types of ELISA

5. Modifications of ELISA—Cassette ELISA. (Fig. 1.8.1)

- Ag is fixed on solid phase which is porous.
- Underneath the solid phase absorbing material is kept.
- The test sample where the Ab has to be detected is put on the solid phase.
- If the sample has Ab it will bind to the solid surface and then enzyme labelled antiglobulin is added, followed by substrate.
- Colour change occurs if the sample has Ab.

Applications of ELISA

1. *For antigen detection:* HBsAg, HBeAg, Dengue NS1 Ag.
2. *For antibody detection:* Hepatitis B, Hepatitis C, Toxoplasmosis, Dengue Leptospirosis.
3. Measuring hormone levels HCG (as a test for pregnancy), LH (determining the time of ovulation) TSH, T3 and T4 (for thyroid function).
4. For the detection of allergens in food and house dust.
5. Measuring rheumatoid factor and other auto-antibodies in autoimmune diseases: Anti CCP, anti-dsDNA
6. Measuring toxins in contaminated food.
7. Therapeutic drug monitoring—barbiturates, morphine, digoxin.

Advantages of ELISA

1. Economical.
2. 2–3 hours.
3. *High sensitivity:* Screening for HIV, Hepatitis B and C in blood bank.

Disadvantages of ELISA

1. More time compared to rapid tests.
2. Not preferred when sample load is low.

3. Discuss immune response under the following headings.

A. Define and classify the immune response with main functions (3 marks)

Immune Response

Definition

- Specific reactivity induced in the host by an antigenic stimulus.

Types

1. Humoral (antibody mediated).
2. Cellular (cell mediated).

Functions

Humoral immunity	• Directed primarily against toxin-induced diseases • Infections with capsulated bacteria (Pneumococci, Meningococci, *Haemophilus influenzae*) • Viral infections • Participates in the pathogenesis of immediate (1,2,3) hypersensitivity and certain autoimmune disorders
Cellular immunity	• Responsible for • Resistance to intracellular pathogens bacterial infections—tuberculosis, leprosy, listeriosis, brucellosis, viruses—measles, mumps • Resistance to fungal and protozoal infections • Resistance to tumours

B. What type of immunity is activated in toxin-mediated diseases? Explain the basis of this type of immune response. (1 + 6 marks)

- Humoral Immunity is active against toxin-mediated diseases.

Basis of Humoral Immune Response

- Humoral immunity is mediated by antibodies.
- Antibodies provide resistance through the following mechanisms.
  1. Antitoxin neutralises bacterial toxins (diphtheria, tetanus).
     - Antitoxins are formed by previous infection or through artificial immunisation
     - Neutralisation of toxin with antitoxin nullifies the effect of toxin
  2. Antibodies attach to the surface of bacteria and.
     - Act as opsonins and enhance phagocytosis
     - Prevent the adherence of microorganisms to their target cells, e.g. IgA in the gut
     - Activate the complement and that leads to bacterial lysis
     - Clump bacteria (agglutination) leading to phagocytosis

Three Steps in Antibody Production

1. Entry of antigen, its distribution in the tissue and contact with the appropriate immunocompetent cell (afferent limb).
2. Antigen processing by the cell and control of antibody production (Central limb).
3. Secretion of antibody, its distribution in the tissue and its effects (Efferent limb).

T-cell Independent Response

- *T cell independent Ag:* These are large multivalent structures, mostly polysaccharides that cross-link many IgM receptors—Signal 1

- Signal 2 has complement C3b derivatives bound to the bacterial cell or pathogen-associated molecular patterns.
- Short-lived responses dominated by IgM plasma cells, although some IgG is also generated.

T-cell Dependent Response

- T-cell–dependent antigen has some protein component.
- Ag is recognised followed by binding to the B-cell receptor (BCR) and the Ag is then endocytosed by the B cell.
- peptides are processed and complexed with class II MHC proteins.
- B cells present antigen to peptide-specific T follicular helper (Tfh) cells that had been previously activated by dendritic cells presenting the same peptide fragment of the antigen.
- Activated T cells now produce IL2,4,5 that stimulate the growth and differentiation of B cell.
- In the germinal center, the B-cell clones that receive more CD40 ligand (CD40L) and cytokines are able to proliferate, class switch, and become long-lived memory B cells and plasma cells.

Primary vs Secondary Immune Response (Fig. 1.8.2)

Feature	*Primary immune response*	*Secondary immune response*
Occurrence	• The immune response developing to the first exposure to any antigen	• Occurs following re-exposure to the same Ag
Responding cells	• B and T cells	• Memory cells
Lag phase duration	• 4–7 days	• 1–4 days
Time taken for immune establishment	• Longer time	• Quick
First antibody produced during response	• IgM	• IgG
Level of antibodies	• Declines quickly	• Remains for a long period

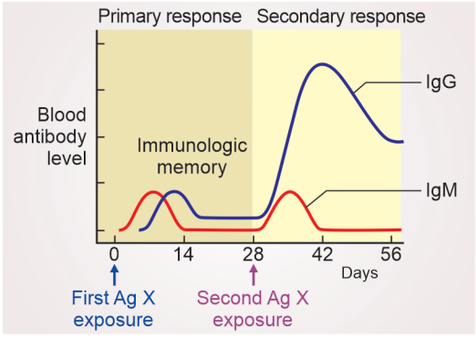


Fig. 1.8.2: Primary response vs secondary response

Tests for Evaluation of Humoral Immunity

1. Measuring the immunoglobulins (i.e., IgG, IgM, and IgA) in the patient's serum by nephelometry or by enumeration of B-cell numbers by flow cytometry.
2. *B-cell function in vivo:* absence of a normal rise in the concentration of IgM and IgG following immunisation points to either an intrinsic defect in the B cells or an extrinsic defect that inhibits T cells' capacity to provide help in activating B cells.

4. Which type of immunity plays a role in resistance against tuberculosis infection? Explain the different types of cells involved with the mechanism of this type of immune response. Add a note on evaluation of this type of immunity. (1+6+3 marks)

- Cell-mediated immune response plays a role in resistance against *Mycobacterium tuberculosis*.

Cell-mediated Immunity

- Immune response that does not involve antibodies, but rather is mediated by cytotoxic T-lymphocytes, NK cells, macrophages and granulocytes and cytokines in response to an antigen.
- Exist in two forms.
  1. Delayed type hypersensitivity mediated by CD4+ Th1 cells.
  2. Cell-mediated lysis mediated by CD8+ cytotoxic T lymphocytes.

Mechanism of Cell-mediated Immunity (Figs 1.8.3 and 1.8.4)

Activation of Cytotoxic T Cells

1. **Ag processing and presentation**
   - Cytosolic pathway.
   - Endogenous (intracellular) antigens (viral antigens and tumour antigens) are processed.
   - Presented along with MHC class I molecules to CD8 T cells.
2. **Activation of T-cells**
   - Activation of T cells requires 3 signals.
     - Ag specific signal is by binding MHC I + Ag to TCR
     - Co-stimulatory signal CD28(CTL)/B7 (APC)
     - IL-2 signaling inducing proliferation (CTL-P do not express IL-2 R)
       - IL-2 is provided by T_{H1} or CTL-P itself
       - IL-2R is expressed only after activation
   - Activated CD8 TC-cells proliferate and differentiate into a clone of effectors cells CTLs.
3. **Killing of the target cells by cytotoxic T cells**
   - Effectors CTLs kill target cells, i.e., nucleated cells (expressing MHC-I) infected with viruses, tumour cells or graft cells.

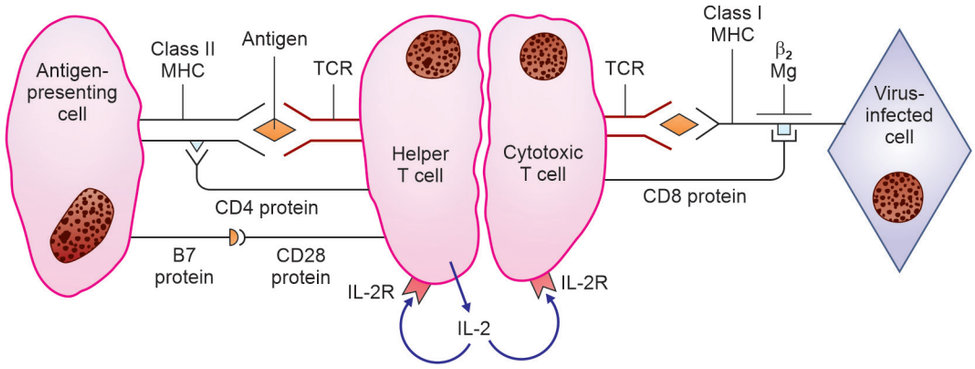


Fig. 1.8.3: Activation of T cells

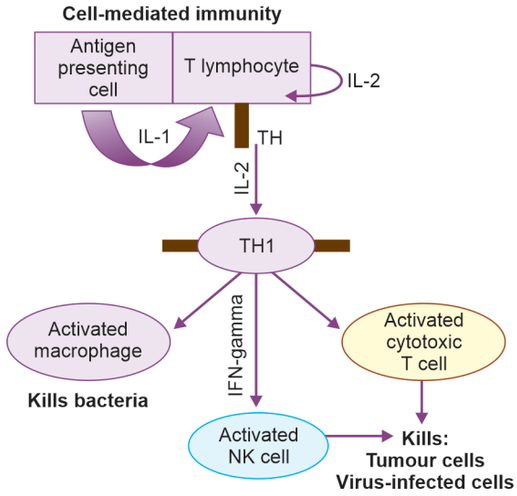


Fig. 1.8.4: Th1 immune response

- Perforins produce pores in the target cell membrane; through which granzymes are released inside.
- Granzymes are serine proteases; they induce cell death by apoptosis through caspase pathway.

Activation of Macrophages and delayed Type Hypersensitivity (DTH)

- Activated macrophages can also kill abnormal host cells (tumour cells).
- Cytotoxicity is nonspecific and stimulated by TNF, nitric oxide, enzymes, and oxygen metabolites.
- If infection is not fully resolved, activated macrophages cause tissue injury and fibrosis, i.e. DTH reaction.

Natural Killer Cells

- Play two important roles in immunity.
  1. Kill virus-infected cells and tumour cells.
  2. Produce gamma interferon that activate macrophages to kill the ingested bacteria.

Antibody-mediated Cell Cytotoxicity

- Antibodies bound to the infected cells are recognised by IgG receptors on the surface of macrophages, NK cells and the infected cell is killed.
- Antibody provides the specificity, e.g. macrophages, NK cells, neutrophils, eosinophils.
- Killing of target cell is accomplished.
  - Through perforin, granzyme (NK, Eosinophils).
  - Through TNF (Macrophages, NK).
  - Through lytic enzymes (macrophages, neutrophils, eosinophils, NK).

Delayed Type Hypersensitivity

- *Induction phase:* Memory T cells recognise their MHC plus peptide complex presented by APC and are activated
- TH1 cells secrete cytokines that activate local macrophages and recruit more macrophages and TH1 cells to area.
- If chronic antigen is present, a large mass of activated macrophages and TH1 cells may form a granuloma.
- *Granuloma:* Walled off portions of tissue within which microbes are trapped causing tissue damage

Evaluation of Cell-mediated Immunity

In Vivo Tests for Lymphoid Cell Competence

1. *Skin tests for the presence of delayed type of hypersensitivity:* Normal persons respond with delayed type of reactions to skin test antigen – *Candida*, streptokinase- streptodornase or mumps.
2. *Skin tests for the ability to develop delayed type hypersensitivity* to simple chemicals—DNCB.

In Vitro Tests for Lymphoid Cell Competence

1. *Lymphocyte blast transformation test:* When sensitized T lymphocytes are exposed to specific antigen, they transform into large blast cells with greatly enhanced DNA synthesis as measured by incorporation of tritiated thymidine.
2. Macrophage migration inhibitory factor is elaborated by cultured T cells when exposed to the Ag to which they are sensitized. Its effect can be measured by observing the reduced migration of macrophages in the presence of the factor compared with the levels in the controls.
3. Enumeration of T and B cell, subpopulations by flow cytometry.

5. Explain the immunological process of activation of T cells. Mention its effector and regulatory functions (8+2 marks)

Immunological Process of Activation of T cells

T cells

- Constitutes 65–80% of lymphocytes.
- Present in inner and subcortical region of lymphocytes.

Types of T cells

- T helper (CD4) cells.
  - Th1 cells
  - Th 2 cells
  - Th17 cells: produce IL17---Maintains mucosal barrier, recruit neutrophils
- Cytotoxic T cells (CD8 cells)
- Regulatory T cells (Suppressor T cells) CD4, CD 25

Activation of T Cells

Ag Presentation

- Cells present antigenic peptides (endogenous) with MHC Class I to cytotoxic T cells (CD8).
- Antigen presenting cells (dendritic cells, macrophages) present Ag with MHC Class II to helper T cells (CD4).

Antigen Processing

- *Cytosolic pathway:* For endogenous antigens (viruses, tumor)
- *Endocytic pathway:* For exogenous antigens (extracellular bacteria, toxins)

Activation of Helper T Cells (Figs 1.8.2 and 1.8.3)

- Two signals are required to activate T cells.
  - Interaction of antigen and the MHC protein with the T cell receptor specific for that antigen.
  - Second co-stimulatory signal is the B7 protein on APC must interact with CD28 protein on the helper T cell.
  - Interleukin-1 (IL-1) produced by the macrophage.
- T cell now produces IL-2 which is also known as T-cell growth factor.
- This stimulates the T cell to multiply into a clone of antigen specific helper cells capable of performing regulatory, effector and memory functions.

Effector T Cells

- Are of two subsets.
  1. Th1 cells.
     i. *IL2:* Activation of T cells, NK cells.
     ii. *IFN:* Activates macrophages, inflammation in delayed type of hypersensitivity, inhibits Th2 proliferation.
     iii. *TNF:* Enhances phagocytic activity of macrophages.
  2. Th2 cells.
     i. *IL4:* Inhibits Th1 differentiation, stimulates B cells to produce IgE.
     ii. *IL5:* Protection against helminths.
     iii. *IL 6:* B cell proliferation.

Memory T Cells

- Derived from activated helper T cells.
- Get activated on subsequent antigenic stimulus into effector cells.

Functions of T Cells

Effector Functions

1. *Th1 cells and macrophages:* Delayed type of hypersensitivity that protects against intracellular organisms.
2. *Th2 cells:* protection against helminths by IL 4, IL5.
3. *CD 8 cells:* killing of the virus infected cells, tumour cells, graft cells.

Regulatory Functions

1. **Antibody production**
   - T cell dependent: all classes of Ig, memory cells
   - T cell Independent: Polysaccharide Ag, only IgM, no memory
2. **Cell mediated immunity**

6. Discuss the activation and biological effects of complement system. (6+4 marks)

Complement System

- Components.
  - C1-C9, C1 has 3 subunits C1q, C1 r, C1 s.
  - Properdin system: factor B.
- Synthesised in the liver.
- Heat labile (inactivated at 56°C—1 hour).
- Complement plays a role in inflammatory responses of both the innate and adaptive immune responses.
- Present as proenzymes, which must be cleaved to active form.
- Activation of complement system is by either by.
  - Ag-Ab complex: Classic pathway.
  - Endotoxin: Lectin and alternative pathway.
- All the 3 pathways result in formation of C3b and Membrane attack complex (C5b,6,7,8,9).
- In the pathway, b fragment continues in the main pathway and a fragment is split off and has other functions.

Pathways of Complement Activation (Fig. 1.8.5)

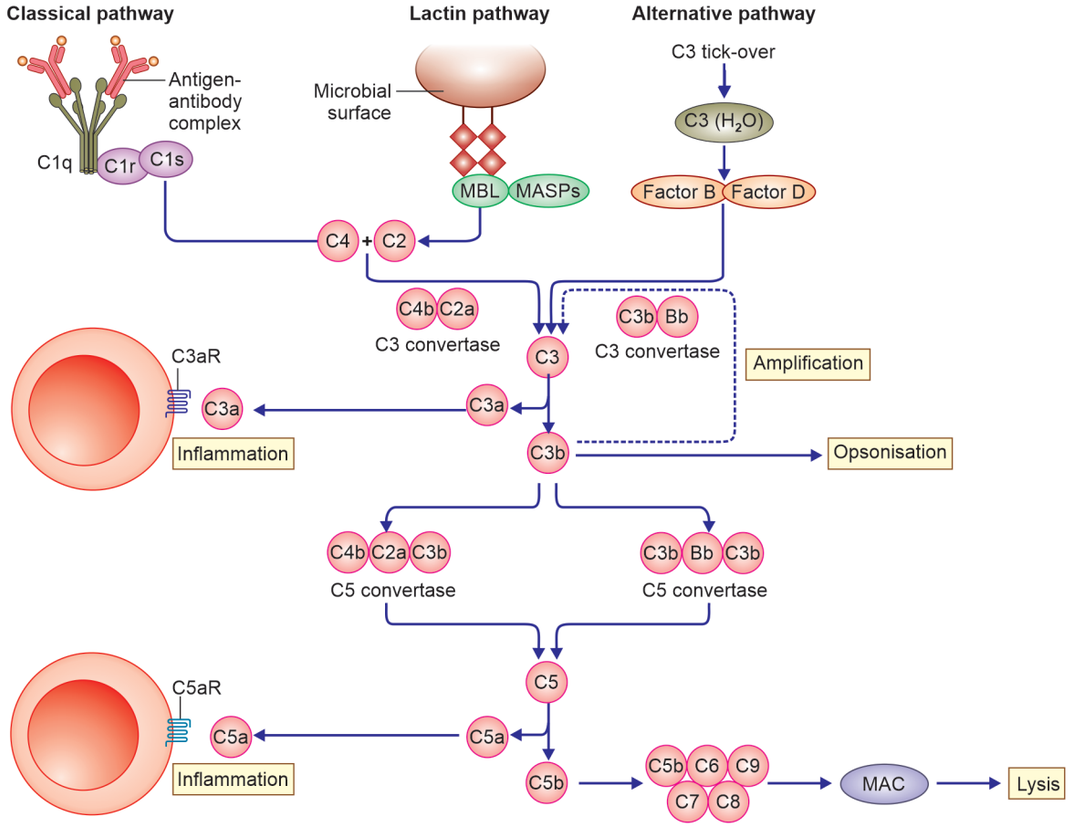


Fig. 1.8.5: Pathways of complement activation

Biological Functions of Complement

1. **Opsonisation:** Bacteria and viruses are phagocytized better in the presence of C3b due to the presence of C3b receptors on the surface of phagocytes.
2. **Chemotaxis**
   - C5a and the C5,6,7 complex attract neutrophils to the area.
   - C5a also enhances the adhesiveness of neutrophils to the endothelium.
3. **Anaphylatoxin:** C3a, C4a, and C5a—mast cell degranulation with release of mediators (e.g., histamine), leading to effects like increased vascular permeability and smooth muscle contraction, especially contraction of the bronchioles, leading to bronchospasm.
4. **Cytolysis**
   - Insertion of the C5b,6,7,8,9 membrane attack complex into the cell membrane forms a "pore" in the membrane.
   - *Results in lysis of cell:* Erythrocytes, bacteria, and tumour cells
5. **Enhancement of antibody production:** The binding of C3b derivatives to its receptors on the surface of activated B cells (complement receptor 2 [CR2]) provides the signal 2, which greatly enhances antibody production.

SHORT ESSAYS

1. Cytokine storm syndrome is one of the host responses post viral infections. Classify and enumerate the role of important cytokines with examples (2 + 3 marks)

Cytokines

- Low molecular weight biologically active proteins/glycoproteins produced by cells (lymphocytes, macrophages, platelets, and fibroblasts) that are activated by some stimulus.
- Peptide mediators, intracellular messengers, who regulate immunological, inflammatory, and reparative host cell responses.

Classification of Cytokines

1. *Lymphokines:* Biologically active substance released by activated T Lymphocytes.
2. *Monokines:* Substances secreted by monocytes and macrophages.

3. *Interleukins:* Produced by lymphocytes which exert a regulatory effect on other cells.
4. Type-1 cytokines are cytokines produced by Th1 T-helper cells. Include IL-2 (IL2), IFN-gamma (IFN-G), IL-12 (IL12) and TNF beta (TNF-b).
5. Type-2 cytokines are those produced by Th2 T-helper cells. Include IL-4 (IL4), IL-5 (IL5), IL-6 (IL6), IL-10(IL10), and IL-13 (IL13).
6. *Mediators of natural immunity:* TNF-α, IL-1, IL-10, IL-12, type I interferons (IFN-α and IFN-β), IFN-γ, and chemokines.
7. *Mediators of adaptive immunity:* IL-2, IL-4, IL-5, TGF-β, IL-10 and IFN-γ.

Role of Important Cytokines with Examples

Cytokines	*Example*	*Functions*
T_H1	• IL-2	1. Promotes activation of T_H and T_C cells 2. Activates NK cells to become LAK* cells
	• IFN-γ	1. Activates the resting macrophages into activated macrophage 2. Activates B cells to produce IgG 3. Promotes inflammation of delayed type of hypersensitivity (along with TNF-β) 4. Inhibits T_H2 cell proliferation
	• TNF-β	1. Enhances phagocytic activity of macrophage
T_H2	• IL-4	1. Inhibits T_H1 cell differentiation 2. Stimulates B cells to produce IgE and also IgG4 and IgG1
	• IL-5	1. Enhances proliferation of eosinophils 2. Both IL-4 and IL-5 together provide protection against helminthic infections and also mediate allergic reaction
	• IL-6	1. Promotes B cell proliferation and antibody production
	• IL-10	1. Inhibits T_H1 cell differentiation

2. Explain the immunological process of activation of B cells. Mention the functions of B cells (4+2 marks)

➲ B cells perform two important functions.
1. differentiate into plasma cells and produce antibodies.
2. Antigen presentation to helper T cells.

B Cell Activation (Fig. 1.8.6)

➲ **Antigens that activate B cells fall into two categories:**
- T cell dependent (TD).
  - Activate B cells indirectly via activation of T cells

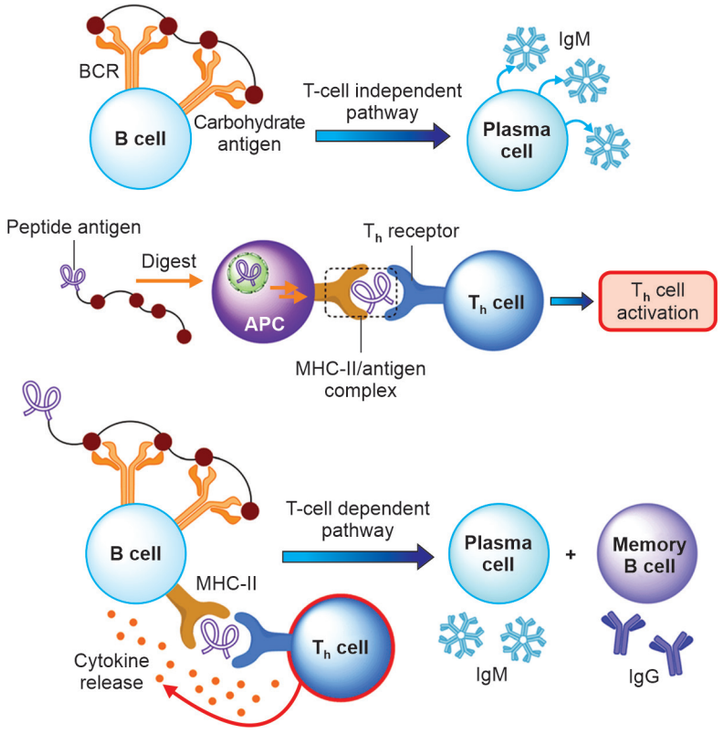


Fig. 1.8.6: Activation of B cells

- TD antigens are processed by APCs → presented to T_H cells following which the activated T_H cells → cytokines that in turn activate the B cells
- T cell independent (TI) antigens (e.g. bacterial capsule) are not processed by APC. They can directly activate B cells without the help of T cell induced cytokines.

- IL-2, IL-4 and IL-5 stimulate the growth and differentiation of the B cell.
- The activated B cells get converted to plasma cells and produce large amounts of immunoglobulins.
- Some activated B cells form memory cells which can remain quiescent for long periods but are capable of being activated rapidly on re-exposure to antigen.
- Effector function.
  - Secreted antibodies by plasma cells which in turn counter act with the microbes in many ways such as neutralisation, opsonisation, complement activation, etc.
  - Mediates mucosal immunity.

3. Discuss the biological effects of complement. (5 marks)

1. **Opsonisation**
   - Bacteria and viruses are phagocytized better in the presence of C3b due to the presence of C3b receptors on the surface of many phagocytes.
2. **Chemotaxis**
   - C5a and the C5,6,7 complex attract neutrophils.
   - C5a also enhances the adhesiveness of neutrophils to the endothelium.
3. **Anaphylatoxin**
   - C3a, C4a, and C5a cause mast cell degranulation with release of mediators (histamine), leading to the effects of increased vascular permeability and smooth muscle contraction, especially contraction of the bronchioles, leading to bronchospasm.
4. **Cytolysis**
   - Insertion of the C5b,6,7,8,9 membrane attack complex into the cell membrane forms a "pore" in the membrane.
   - results in the killing (lysis) of many types of cells, including erythrocytes, bacteria, and tumour cells.
5. **Enhancement of antibody production**
   - The binding of C3b derivatives to its receptors on the surface of activated B cells (complement receptor 2 [CR2]) provides the signal 2, which greatly enhances antibody production compared with that by B cells that are activated by antigen alone.

4. Define monoclonal antibodies. Write their applications. (1+4 marks)

Monoclonal Antibodies

- These are the antibodies produced by a single clone of cells or cell line and consisting of identical antibody molecules.

Applications

1. **Diagnostic uses**
   i. Identification of leucocytes.
   ii. HLA typing.
   iii. Identification of microorganisms.
   iv. Preparation of serological kits used for serodiagnosis of infection.
2. **Therapeutic uses (Table 1.8.1)**

5. In DPT vaccine, which component acts as an adjuvant. Write the mechanism of action of an adjuvant with 2 examples. (1+3+1 marks)

- In DPT vaccine, killed *Bordetella pertussis* component acts as Adjuvant.

Adjuvant

- A substance which enhances immunogenicity of an Ag

Mechanisms of Action

1. Depot action (slow release of Ag).
2. Modulation of immune system
3. Increase uptake by macrophage.
4. Enhance T cell activation.
5. Increase T cell proliferation.

Table 1.8.1 Therapeutic uses

Function	*Name*	*Target*	*Uses*
Transplant related immunosuppression	• Basiliximab • Daclizumab	• IL 2 receptor • CD 3 on T cell	• Prevent or treat allograft rejection and GVH
Treatment of autoimmune diseases	• Infliximab	• TNF—alpha	• Treatment of rheumatoid arthritis, Crohn's disease
Prevention of infectious diseases	• Palivizumab	• Fusion protein for RSV	• Prevents pneumonia in susceptible neonates
Treatment of cancer	• Rituximab	• CD 20 on B cell • Epidermal growth factor receptor	• Treatment of non-Hodgkin lymphoma, breast cancer.

Examples

1. Alum—used in vaccines (depot action).
2. Mineral oil.
3. Freund's incomplete adjuvant (water in oil emulsion + protein).
4. Freund's complete Adjuvant (water + protein with killed Tubercle bacilli + oil).

6. Detection of antinuclear antibody (ANA) by IF is suggested for the diagnosis of systemic lupus erythematosus (SLE). Discuss the principle, types, and applications of Immunofluorescence. (1+3+1 marks)

Immunofluorescence

Principle

- Ag–Ab reaction which involves antibody labelled with fluorochromes.
- Fluorescence is defined as the property of absorbing light rays of one wavelength and emitting light rays with a different wavelength.
- Fluorochromes are substances which get excited after absorption of UV light. After excitation the fluorochrome returns to normal level and while doing so emits visible light.
- Fluorochromes used.
  - *Fluorescein isothiocyanate*
    - Absorbs light of wavelength 490–495 nm and emits light of wavelength of 470 nm
    - It produces apple green colour
  - *Lissamine rhodamine*
    - Absorbs light of wavelength 460 nm and emits light of 480 nm
    - It produces orange red colour

Classification/Types of Immunofluorescence or Fluorescent Antibody Technique (Fig. 1.8.7)

1. Direct immunofluorescence

- Clinical sample for the detection of Ag is smeared on the slide.

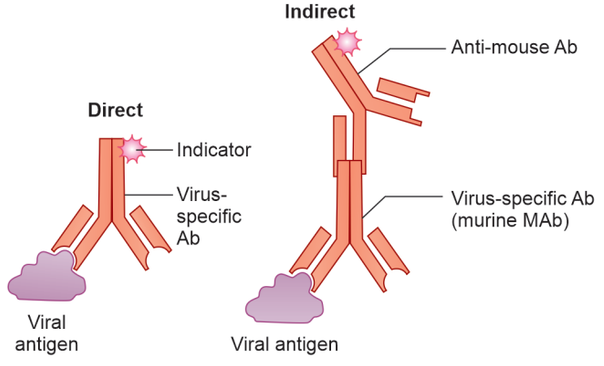


Fig. 1.8.7: Types of immunofluorescence or fluorescent antibody technique

- It is stained using specific Ab which is labelled with fluorochrome.
- If Ag is present, fluorescence is seen.

Use/Application

1. Detection of microbial Ag in the clinical samples.
   - Rabies Ag in the corneal smears
   - Herpes virus Ag in skin scraping

Advantages

1. Rapid diagnosis.
2. More specific than indirect IF.

Disadvantages

1. Requires labelling of each specific Ab.
2. Less sensitive than indirect IF.

2. Indirect Immunofluorescence

- Two-step procedure.
- Detection of either Ag or Ab.
- Ag is fixed onto the solid phase and unlabeled Ab is made to react with it.
- Free Abs are removed by washing.
- Fluorescent labelled antiglobulin is added and after the reaction free antiglobulin is washed.
- If Ag –Ab reaction has taken place, fluorescent labelled antiglobulin binds to the complex.

Uses/Application

1. Detection of specific Abs in *Chlamydia* infection, *Legionella* infection viral infections, extra-intestinal amoebiasis toxoplasmosis, kala azar.
2. Detection of antinuclear antibodies (ANA) in patients' serum.
3. Detection of antineutrophilic cytoplasmic antibody (ANCA) in patients' serum.

Advantages

1. More sensitive.
2. Requires only 1 Ab labelled with the fluorescent compound.
3. Possible to differentiate between IgM and IgG Ab in the clinical sample.

Disadvantages

1. Nonspecific fluorescence.
2. Longer procedure.

SHORT ANSWERS

1. Describe in brief about antigenic determinants of immunoglobulins with a neat labelled diagram. (2+2 marks)

Immunoglobulin Structure

- Glycoprotein made up of 2 light (L) and 2 heavy (H) polypeptide chains.
- Y shape.
- 4 chains are linked by disulfide bonds.

- L and H chains are subdivided into variable and constant regions. Regions are composed of 3 dimensionally, folded repeating segments called domains.
- L chain has 1 VL and 1 CL domain.
- H chain has 1 VH and 3 CH domains.
- *Variable region:* Antigen binding sites
- Within the variable region, there are some zones (hot spots) that show relatively higher variability in the amino acid sequences. Called as hypervariable regions or complementarity determining regions (CDRs). Form the antigen-binding site.
- *Constant region of H chain:* Biological functions (complement activation, binding to cell surface receptors)
- L chain 2 types—Kappa and lambda. Both occur in all the types, but 1 Ig has only 1 type of L chain.
- H chains are specific for each class.

Labelled Diagram of Immunoglobulin (Fig. 1.8.8)

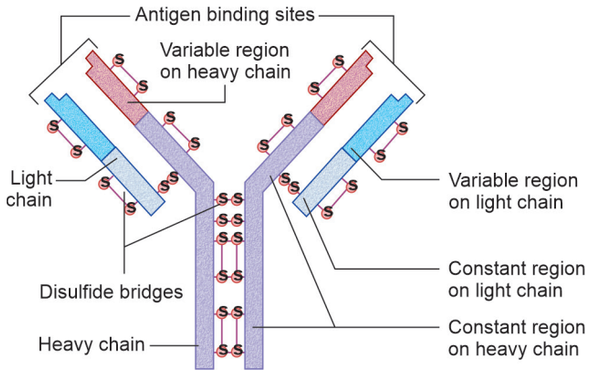


Fig. 1.8.8: Structure of immunoglobulin

2. Draw a neat, labelled diagram of Immunoglobulin M molecule. Enumerate its few properties and clinical significance. (2+2 marks)

Labelled Diagram of Immunoglobulin M (Fig. 1.8.9)

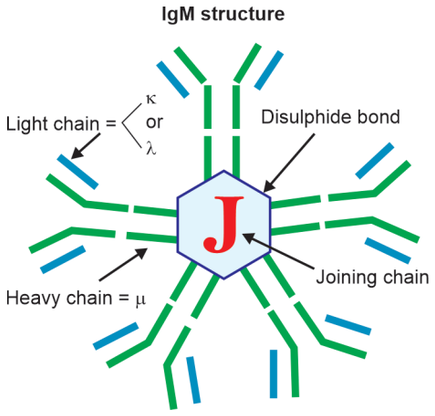


Fig. 1.8.9: Structure of immunoglobulin M

Properties of IgM

- Pentamer with 5 subunits and J chain.
- Highest molecular weight among all immunoglobulins.
- 5–10% of serum proteins.
- Distributed intravascularly.
- 2 forms.
  - *Monomeric:* Bound on the surface of B cells
  - *Pentameric:* Secreted form

Clinical Significance of IgM

1. Demonstration of IgM used in diagnosis of acute infections and congenital infections.
2. Protection against intravascular organisms.
3. Very efficient in agglutination opsonisation, complement fixation.

3. A nurse caring for a patient with varicella-zoster infection in an isolation ward. Which immunoglobulin titre measurement is required for nurse and patient to be tested? Briefly discuss the clinical significance of both these antibodies. (1+2 marks)

- IgM has to be detected in the patient for acute infection and IgG in the nurse for the antibody titre.

Clinical Significance of IgM

- Demonstration of IgM used in diagnosis of acute infections and congenital infections.
- Very efficient in agglutination opsonisation, complement fixation.
- *Protection against intravascular organisms:* Deficiency of IgM predisposes to septicaemia

Clinical Significance of IgG

- Predominant antibody in secondary response and defense against bacteria and viruses.
- Only antibody that can cross placenta—passive immunity to the newborn.
- Activates complement.
- Opsonisation and enhances phagocytosis.
- Participates in precipitation.
- Major role in neutralisation of toxins.

4. A 45-year-old man, alcoholic and h/o IV drug abuse for 15 years comes with severe jaundice. He has raised AST and ALT levels and has hepatitis B, due to which complement components are reduced. Briefly describe the clinical significance of complement deficiency. (3 marks)

Clinical Significance of Complement Deficiency

• Inherited (or acquired) deficiency of some complement components, especially C5–C8	• Neisseria bacteraemia

Contd.

• C3 deficiency	• Recurrent pyogenic sinus and respiratory tract infections.
• C1 esterase inhibitor deficiency	• Angioedema (acquired or genetic disease called hereditary angioedema
• Acquired or inherited deficiency of decay-accelerating factor (DAF)	• Paroxysmal nocturnal haemoglobinuria (increased complement mediated hemolysis)
• Complement levels: low	• Immune complex diseases (acute glomerulonephritis and systemic lupus erythematosus)
	• Severe liver disease (alcoholic cirrhosis or chronic hepatitis B) → predisposed to pyogenic infections

5. A 1-year-old baby admitted for recurrent *Staphylococcus* furuncles and fungal diaper rash. On examination, the paediatrician noticed mild hepatosplenomegaly with chronic lymphadenopathy and suspected as chronic granulomatous disease (CGD). Which cell type is affected in this case? Role of this type of cell in innate immunity. (1+2 marks)

- CGD is due to a defect in the intracellular microbicidal activity of phagocytes as a result of a lack of NADPH oxidase activity (or similar enzymes).
- Neutrophils are affected in this condition.

Role of Neutrophils in Innate Immunity

- Abundant, 50–60% of the circulating WBC.
- White blood cells, subgroup called granulocytes, named for their cytoplasmic granules.
- Phagocyte of innate immunity and is the first cell to respond in inflamed or necrotic tissue.
- Engulfs and kills bacteria and fungi, digests cellular debris.
- severe bacterial and fungal infections occur if they are too few in number (neutropenia) or are deficient in function.
- Neutrophils have surface receptors for IgG, making it easier for them to phagocytize opsonised microbes.
- "Two-edged" sword. The positive edge of the sword is their powerful microbicidal activity, but the negative edge is the tissue damage caused by the release of degradative enzymes.

6. Compare and contrast T lymphocytes and B lymphocytes (3 marks)

Feature	*T lymphocytes*	*B lymphocytes*
Percentage in peripheral blood	• 80% of circulating lymphocytes	• 20% of circulating lymphocytes
Life span	• Longer	• Shorter
Origin and maturity	• Originate in thymus and mature in bone marrow	• Bone marrow
Location	• Outside the lymph node	• Inside the lymph node
Distribution	• Parafollicular areas of cortex in lymph nodes, periarteriolar in the spleen	• Germinal centers of lymph nodes, spleen, gut, respiratory tract; also, subcapsular and medullary cords of lymph nodes
Surface receptor	• Immunoglobulin surface receptors	• T cell receptors
Cell surface marker	• CD3 in T cells	• CD19 in B cells
Types	• T cells: Cytotoxic T cells (CD8+ T cells), helper T cells (CD4+ T cells) and suppressors cells along with memory cells	• B cells: Plasma cells and memory cells
Secretory product	• Cytokines	• Antibodies
Functions	• Help lyse virus-infected cells tumour cells and intracellular bacterial pathogens	• Provide immunity against most foreign antigens and bacteria

7. Compare MHC 1 and MHC class II proteins. What is meant by MHC restriction? (3+1)

Feature	*MHC Class I*	*MHC Class II*
Nomenclature	• HLA-A, HLA-B, HLA-C	• HLA-DP, HLA-DQ, HLA-DR
Found on	• All nucleated somatic cells	• Macrophages, B-cells, Dendritic cells, Langerhans cells of skin and activated T cells
Recognised by	• CD8 TC cells	• CD4 TH cells
Functions	• Presentation of Ag to cytotoxic T cells leading to elimination of tumour or infected host cell	• Presentation of Ag to helper T cells which secrete cytokines

Contd.

MHC Restriction

- Means that different T cells are restricted to either Class I or Class II MHC antigens.
- Cytotoxic T cells are activated only by antigens presented in association with Class I MHC protein present on nucleated body cells, thus play a role in protecting against virus-infected cells or tumour cells.
- Helper CD4 cells are activated only by antigens presented along with Class II MHC proteins on the Antigen presenting cells, thus play a role in increasing the humoural immune response.

8. Comment on the principle of conjugate vaccine with one example. (3 marks)

- The immunogenicity of polysaccharide capsule vaccine is enhanced by coupling it with a carrier protein resulting in a stronger immunological response.
- T cell dependent response occurs, characterised by antibody class switching from IgM to IgG, memory cells and longer response, e.g. pneumococcal 'conjugate' vaccine.
- Conjugation of capsular polysaccharides on common serotypes of *Streptococcus pneumoniae* with a highly immunogenic protein, i.e. diphtheria toxoid, induces a B- and T-cell response resulting in mucosal immunity and thus protects against vaccine serotypes and also reduces vaccine serotype carrier rates.

9. Write about.

A. Class Switch over (2 marks)
B. Paratope (1 mark)

A. Class Switching

- Also called as Isotype switching.
- Mechanism that changes B cell production of antibodies from one type to another (IgM to IgG).
- In immune response, IgM is the first class of Ig to be formed, followed by other types—IgG, IgA.
- The new antibodies formed uses the same VH but different CH chains. Since the variable region does not change, class switching does not affect antigen specificity.
- *Significance of this phenomenon:* 4 isotypes have specialised functions. IgG is the major antibody in interstitial fluids, whereas IgA is the protective antibody of mucosal surfaces

B. Paratope

- The site on the hypervariable regions that make actual contact with the epitope of an antigen is called as paratope.

10. Describe in brief the mechanisms of "antibody diversity". (2 marks)

- 10^{11} possible heavy chain–light chain combinations.
- Antibody diversity depends on:
  - Multiple gene segments.
  - rearrangement into different sequences.
  - The combining of different l and h chains in the assembly of immunoglobulin molecules.
  - Mutations.
  - Junctional diversity applies primarily to the antibody heavy chain. Junctional diversity occurs by the addition of new nucleotides at the splice junctions between the V–D and D–J gene segments.
- The resulting antibodies have the potential to recognise the three-dimensional structure of a wide range of proteins, carbohydrates, nucleic acids, and lipids.

11. A clinician suspects parasitic infection in his patient. Blood tests reveal raised eosinophil counts. Which is the immunoglobulin raised in this condition? Enumerate the importance of this immunoglobulin. (1 + 3 marks)

- IgE levels in the serum is increased in parasitic infections.

Importance of IgE

- Produced in the linings of the respiratory tract and GIT.
- Least abundant serum Ig.
- Shortest half life.
- heat labile antibody (inactivated at 56°C in one hour).
- Has affinity for the surface of tissue cells (mainly mast cells) of the same species (homocytotropism).
- Extravascular in distribution.
- Mediates immediate hypersensitivity and participates in host defenses against certain parasites.

12. Compare T cell dependent and T cell independent antigens. (4 marks)

Feature	*T cell dependent antigen*	*T cell independent antigen*
Nature	Proteins	Lipopolysaccharide Capsular polysaccharide
Antigen processing and presentation	By APC to helper T cells is required	Ag directly stimulate B cells for production of antibodies without the assistance of T cells
Immunogenicity	Immunogenic over wide range of dose	Dose dependent immunogenicity

Contd.

Feature	*T cell dependent antigen*	*T cell independent antigen*
Polyclonal activation	No	Polyclonal activation of B cells occurs in high doses
Immunologic memory	Present	No immunologic memory
Affinity maturation	Yes	No
Isotype switching	Occurs (*i.e., antibodies of all classes are produced*)	No isotype switching

13. Write about the determinants of antigenicity. (3 marks)

Determinants of Antigenicity

1. Size
   - >100,000 Daltons. Molecules of lesser size are weakly immunogenic.
2. Foreignness.
   - Self-antigens are non-immunogenic because of self-tolerance.
   - To be immunogenic, molecules must be recognised as non self or foreign.
3. Chemical nature.
   - Proteins are highly immunogenic, then carbohydrates, lipid, nucleic acid.
4. Chemical complexity.
   - Amino acid homopolymers are less immunogenic than heteropolymers containing 2 or 3 different amino acids.
5. Susceptible to tissue enzyme digestion.
   - Latex particles are less immunogenic.
6. Dosage, route and timing of antigen administration.
7. Genetic constitution of the host (HLA genes) determines whether a molecule is an immunogen.

14. Describe Superantigens with examples. (2+1 marks)

Superantigens

- Superantigens are a subclass of antigens that cause overstimulation of the immune system.
- non-specific activation of T-cells which ends with polyclonal activation of T cells and massive cytokine release (IL-2 from the T cells and IL-1 and TNF from macrophages).
- Superantigens bind to the outside of MHC Class II protein and TCR of T cell without any specificity, resulting binding and activation of large numbers of T cells.
- **Cytokines:** IL2, TNF alpha, IL8 are responsible for nausea, vomiting, fever, endothelial damage, acute respiratory distress syndrome, DIC, shock, and multiple organ failure, e.g. staphylococcal enterotoxins, and toxic shock syndrome toxin
- Responsible for the effects seen in toxic shock syndrome, scalded skin syndrome, food poisoning.

15. Define antigen/immunogen, hapten, epitope/paratope. (1+1+1 marks)

Antigen

- Any substance which when introduced parenterally into the body stimulates production of antibody and reacts with it in a specific and observable manner.

Immunogen

- It is a substance which can induce immune response.
- Two attributes of complete antigen.
  - Ability to induce antibody production (immunogenicity).
  - Ability to react specifically with antibody (immunologic reaction).

Hapten

- A low molecular weight substance that cannot induce immune response by itself but can react with the specific antibody.
- Are not immunogenic.

Epitope/Paratope

- Small chemical groups on the antigen that can elicit and react with antibody.

16. Enumerate various abnormal immunoglobulins in different diseases conditions. (3 marks)

Abnormal Immunoglobulins

1. **Bence Jones proteins**
   - Monoclonal light chain excreted in urine of people suffering from multiple myeloma.
   - L chain made up of kappa or lambda.
   - Precipitates when heated to 60°C but dissolves at 70°C.
2. **Macroglobulinaemia (Waldenstorm's)**
   - Myeloma of IgM producing cells.
3. **Heavy chain disease**
   - Fc portion of heavy chain is excessively produced and excreted in urine in association with myeloma.

17. Discuss immunochromatography: Principle and applications. (2+2 marks)

Immunochromatography Assay (ICA)/Lateral Flow Test

- *Principle:* Combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions

- Device to detect the presence of Ag or Ab.
- Cassette or strip format.
  - *Test band:* At the test line, the Ag-labelled Ab complex is immobilised by binding to the monoclonal Ab in the test line to form a Coloured band.
  - *Control band:* The free colloidal gold labelled Ab can move further and binds to the anti-human Ig to form a colour control band. Control band should be positive for the test to be valid.

Types

1. Sandwich assay
2. Competitive assay
3. Multiplex assay

Applications

1. Detection of HIV, hepatitis B antibodies in patient's serum.
2. Detection of dengue NS 1 Ag, IgG, IgM in patient serum.
3. Urine pregnancy test by detection of hCG.

18. Write about the principle and applications of Immunoblot. (3 marks)

Immunoblotting (Western Blotting)

- Is sensitive assay for the detection and characterisation of proteins based on the specificity of antigen-antibody recognition.

Principle

1. Involves the solubilisation and electrophoretic separation of proteins, glycoproteins, or lipopolysaccharides by gel electrophoresis (separates the proteins by size, charge).
2. Quantitative transfer of proteins and irreversible binding to nitrocellulose.
3. Immunoprobing, and visualisation using chromogenic or chemiluminescent substrates.

Applications

1. Identification of a specific protein in a complex mixture of proteins.
2. Estimation of the size and amount of protein in the mixture.
3. Supplemental test for HIV diagnosis.
4. To detect antibody in Lyme's disease, herpes simplex virus infection, cysticercosis, hydatid disease and toxoplasmosis.

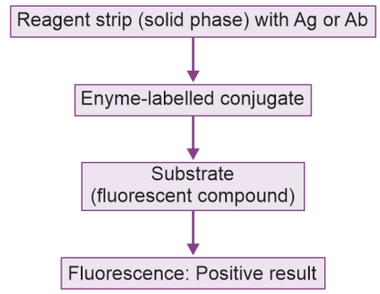
19. Enzyme-linked fluorescent assay: Describe the principle and applications. (2+2 marks)

Enzyme-linked Fluorescent Assay (ELFA)

- Automated system.
- Single test format.
- 10 different analytes can be tested simultaneously.

Principle

- Based on the principle of detection of Ag–Ab enzyme complex by fluorescence.



Applications

1. Detection of IgM and IgG of TORCH panel, hepatitis markers, measles, IgG, varicella IgG, Toxin of *Clostridium difficile*.
2. *Biomarker:* Procalcitonin.

20. Chemiluminescent-linked immunassay (CLIA). Describe the principle and applications. (2+2 marks)

Chemiluminescent-linked Immunassay (CLIA)

Principle

- Chemiluminescence (CL) is defined as the emission of electromagnetic radiation caused by a chemical reaction to produce light.
- An assay that combines chemiluminescence technique with immunochemical reactions.
- **CLIA utilize chemical probes:** Luminol, acridinium ester which could generate light emission through chemical reaction to label the antibody
- Detected by Luminometer.

Applications

1. Detection of antigens or antibodies to HIV, Hepatitis B, Hepatitis C, SARS CoV2.
2. *Biomarker:* PCT Q.

21. What are the major functions of T cells and B cells? (2+2 marks)

T cells

Regulatory Functions

1. Help B cells to develop into antibody producing plasma cells.
2. Help CD8 T cells to become activated cytotoxic T cells.

Effector Functions

1. Help macrophages effect delayed hypersensitivity.
2. CD8 cells perform cytotoxic functions; that is, they kill virus-infected, tumour and allograft cells.

B cells Functions

1. Differentiate into plasma cells and produce antibodies.
2. Antigen presentation to helper T cells.

22. Principle and application of flow through assay. (1 + 1 marks)

Flow Through Assay

Principle

- Sample flows *vertically* through the nitrocellulose membrane (NCM) as compared to lateral flow in ICT.

Application

- Flow-through tests can be used for both antigen and antibody detection, e.g. HIV TRIDOT test.

MI 1.9: DESCRIBE THE IMMUNOLOGICAL BASIS OF VACCINES AND DESCRIBE THE UNIVERSAL IMMUNISATION SCHEDULE

LONG ESSAY

1. Discuss in detail about methods of preparation of Vaccine under the following headings.

A. Define Vaccine and classify the types of Vaccines with examples (3 marks)

Definition of Vaccine

- A vaccine is a preparation that consist of either live, live attenuated or killed microorganisms, or microbial products or its genetic elements, used to induce active immunity in an individual, against that specific microorganism or infection caused by the same.

Type of Vaccines

Type of vaccine	*Examples*
Live vaccines (Historic)	• Smallpox vaccine
Attenuated live vaccines	• BCG vaccine
Inactivated (killed vaccines)	• DPT vaccine
Subunit vaccines and toxoids	• Hepatitis B virus vaccine • DPT vaccine
Conjugate vaccines	• *H. influenzae* b conjugate vaccine
Recombinant (Surface antigen) vaccines	• Hepatitis B Virus (HBV) vaccine
DNA vaccines	• H5N1 DNA vaccine
Anti-idiotypic vaccines	• HBV vaccine in animals
Edible vaccines	• Norwalk virus vaccine

B. Describe in brief different types of vaccines and its active component and their role. (4 marks)

Type of vaccine	*Active component and their role*
Live attenuated vaccines	• Virulent pathogenic organisms are treated to become attenuated and avirulent but retain antigenicity and immunogenicity
Inactivated vaccines	• Vaccine strain stimulates immunity, but microorganism cannot multiply
Subunit vaccines and Toxoids	• A particular antigenic determinant induces a good protective immune response
	• Modified bacterial toxin made nontoxic but retains immunogenicity
Conjugate vaccine	• Covalent coupling of polysaccharide antigen to a carrier protein can improve the T cell mediated immune response
Recombinant (Surface antigen) vaccines	• Produced using recombinant DNA technology or genetic engineering where genes for desired protective antigens are inserted into a plasmid vector and introduced into an expression system and protein is expressed in large quantities and then purified which induces humoural immunity
DNA vaccines	• DNA that codes protein antigen of the pathogen and inserted into a plasmid vector which induced both humoural and cellular immunity
Anti-idiotypic vaccines	• Antibodies (anti-idiotypes) can be raised against the idiotype in the antibody by injecting the antibody into another animal that has the potential to neutralise the virus
Edible vaccine	• Antigenic proteins that are genetically engineered into a consumable crop. When the crop is digested, some of the protein makes its way into the blood stream causing an immune response

C. Distinguish between a Vaccine and a toxoid. (2 marks)

Vaccine	*Toxoid*
• Live, live attenuated or killed microorganisms or their products • Made nontoxic by heating, disinfectant • Retains the capacity to stimulate active immunity, e.g. BCG, HBV vaccine	• Modified bacterial toxin • Made nontoxic but retains immunogenicity • Retain the capacity to stimulate the formation of antitoxin, e.g. tetanus toxoid

D. Give 2 examples for bacterial live attenuated vaccines (1 mark)

1. Bacillus Calmette–Guérin (BCG).
2. Typhoid vaccine.

SHORT ESSAYS

1. With respect to N-COVID 19, describe the available vaccines, the type, dose, schedule, efficacy and adverse effects. (5 marks)

➲ Refer Table 1.9.1

2. Describe the vaccines given for children included in Universal Immunisation schedule (from neonate to 9 years of age under the following headings.

A. BCG (Type of Vaccine, age of administration, route of administration, dose). (2 marks)

➲ *Type of vaccine:* Bacilli, Calmette Guerin—live attenuated vaccine—Danish strain
➲ *Age of administration:* Given at birth
➲ *Route of administration:* Intradermal
➲ *Dose:* 0.05 ml diluted with saline

B. MR (Type of Vaccine, age of administration, route of administration, dose). (2 marks)

➲ *Type of vaccine:* Measles, rubella—live attenuated vaccine
➲ *Age of administration:* 1st dose—9 months; 2nd dose—16–24 months
➲ *Route of administration:* Subcutaneous
➲ *Dose:* 0.05 ml

C. HBV Vaccine (age of administration, Site of administration). (1 mark)

➲ *Type of vaccine:* Hepatitis B vaccine—recombinant, subunit vaccine
➲ *Age of administration:* 0, 1, 6 months or at birth or 6, 10, 14 weeks
➲ *Route of administration:* Intramuscular
➲ *Dose:* 0.05 ml

3. Define "cold chain". Discuss various compartments of cold chain with examples. (1 + 4 marks)

Cold Chain Definition

➲ Refers to maintaining an optimum temperature required for manufacturing storage, transport, and handling of vaccines.

Various Compartments of Cold Chain

➲ The optimum temperature for refrigerated vaccines between 2°C and 8°C.
➲ *For frozen vaccines:* –15°C or lower
➲ Improper temperature maintenance leads to vaccine failure especially vaccines such as oral polio vaccine.
➲ *Vaccine storage in freezer compartment:* Polio and measles vaccines
➲ *Vaccine storage in cold part but not allowed to freeze:* DPT, *H influenzae* type B, hepatitis B vaccine

Vaccine Vial Monitor (VVM)

➲ A tool to monitor the potency of vaccine
➲ To check the efficiency of vaccines

Table 1.9.1 Available vaccines, the type, dose, schedule, efficacy and adverse effects

Vaccine Brand	*Type*	*Dose*	*Schedule*	*Efficacy*	*Adverse Effects*
Covishield (AstraZeneca)	Viral vector vaccine	2	12 weeks apart	90%	• Fever • Pain at the site of injection • Myalgia • Thrombosis • Thrombocytopenia
Covaxin	Inactivated virus vaccine	2	4 weeks apart	82%	• Fever • Pain at the site of injection • Myalgia
Moderna	mRNA vaccine	2	4 weeks apart	94.5%	• Anaphylaxis (<0.01)
Pfizer BioNTech	mRNA vaccine	2	3 weeks apart	95%	• Anaphylaxis (<0.01)
Johnson and Johnson's Janssen	Viral vector vaccine	1	—	66%	• Anaphylaxis (<0.01) • Thrombosis • Thrombocytopenia
Sinopharm-BBIBP	Inactivated virus vaccine	2	3 weeks apart	79%	• Fever • Pain at the site of injection • Myalgia
	Viral vector vaccine	2	3 weeks apart	90%	• None
	Inactivated virus vaccine	2	2 weeks apart	50%	• None

Features

- Heat sensitive label lining the vaccine vial.
- Outer blue circle, inner white square.
- Temperature, time affects inner square.

Staging of VVM

Stage 1

- **Inner square:** White → can be used

Stage 2

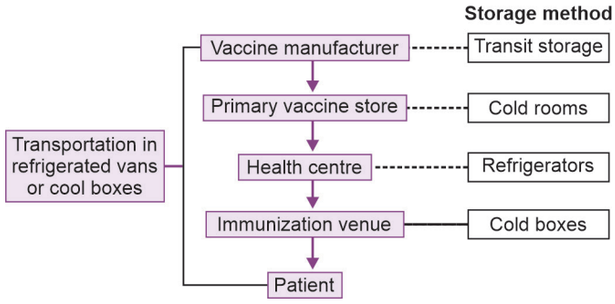
- **Inner square:** Pale blue → can be used

Stage 3

- **Inner square:** Blue → discard the vial

Stage 4

- **Inner square:** Dark blue → discard



4. Enumerate live attenuated and killed vaccines. (4 marks)

Bacterial	
Live attenuated	***Killed***
BCG	• Typhoid
Typhoral	• Cholera
	• Pertussis
	• Plague
	• Diphtheria, pertussis, tetanus (DPT)

Viral	
Live attenuated	***Killed***
OPV (Sabin)	• IPV(Salk)
Live attenuated Influenza	• Killed influenza vaccine
Japanese B encephalitis (14–14-2 strain)	• Japanese B encephalitis
MMR	• (Nakayama strain)
Yellow fever 17 D vaccine	• Rabies
Hepatitis A	• Hepatitis A
Rotavirus	

SHORT ANSWERS

1. Compare and contrast the features in OPV and IPV (3 marks)

Feature	*Inactivated polio vaccine*	*Oral polio vaccine*
Type	Killed formalised vaccine	Live attenuated vaccine
Route of administration	Intramuscular	Orally
Type of immunity induces	Humoural immunity only	Humoural and local immunity quickly
Paralysis	Prevents paralysis	Prevents paralysis and reinfection
Herd immunity	No	Herd immunity is produced
Epidemic control	Not useful	Useful in epidemics control
Storage requirement	Does not require stringent storage and transport conditions	Requires stringent storage and transport conditions
Shelf life	Longer	Shorter
Price	Costlier	Cheaper

2. Mention the complete schedule of DPT vaccine. What is the role of adjuvant in it? (1+2 marks)

Diphtheria, Pertussis, Tetanus Toxoid Vaccine (DPT Vaccine)

Schedule

- At 6, 10, 14 weeks.
- Booster dose DPT → 1st: 16–24 months DT-I 2nd: 5–6 years, TT-3rd: 10 years and 16 years.
- *Dose:* 0.5 ml
- *Route of administration:* Intramuscular

Role of Adjuvant

- Adjuvants have a component which increase the immunogenicity of the vaccine antigen.
- Alum and pertussis component acts as adjuvant in DPT vaccine.

3. Enumerate the diseases treated with hyper-immune human immunoglobulins and its indications. (3 marks)

Immunoglobulin preparation	*Indications*
Diphtheria	• Respiratory Diphtheria
Tetanus Ig	• Post exposure prophylaxis (PEP) of tetanus for inadequately immunised individuals

Contd.

Immunoglobulin preparation	*Indications*
Botulinum antitoxin	• Botulism
Varicella zoster Ig	• PEP for immunosuppressed contacts of acute cases or newborn contacts
Rabies Ig	• Treatment of rabies and PEP in previously unimmunised individuals
Hepatitis B Ig	• PEP as percutaneous/mucosal/sexual exposure, newborn of HBsAg positive mothers
Rubella	• Women exposed in early pregnancy

4. Mention the properties of live attenuated vaccines and give 2 examples. (3 marks)

Properties

1. Induces long-lasting immunity.
2. Induces herd-immunity.
3. Induces good cell-mediated immunity as well as local immunity.
4. Multiple booster doses may not be required.
5. Mimics natural infection.
6. Immunoglobulins IgA and IgG produced.

Examples

1. Bacillus-Calmette Guérin (BCG vaccine).
2. Sabin polio vaccine.

5. Conjugate vaccines. Give 2 examples. Write advantages and disadvantages. (1 + 1 + 1 marks)

Conjugate Vaccines

- Covalent coupling of polysaccharide antigen to a carrier protein can improve the immune response elicited.

Examples

1. *H. influenzae* b conjugate vaccine.
2. Typhoid Vi conjugate vaccine.

Advantages

- Conjugation of a polysaccharide antigen to a carrier protein brings about a T-cell dependent immune response, and thereby memory.

Disadvantages

- Infants/young children have immature immune systems, may not recognising certain antigens.

6. Subunit vaccine. Give 2 examples, the merits and demerits. (1 + 1 + 1 marks)

Subunit Vaccine

- Contains only a particular antigenic determinant which induces a good protective immune response.

Examples

1. Influenza vaccines.
2. Herpes simplex vaccines.
3. Hepatitis B vaccine.
4. Typhoid Vi polysaccharide vaccine.

Advantages

1. Safe.
2. Induces specific immune response.

Disadvantage

1. Expensive.

7. A 3-year-old c/o of severe diarrhoea with dehydration. Stool sample was positive for capsid antigens of the virus. Briefly explain the prophylaxis available for this condition for this child (3 marks)

Prophylaxis Required

- Rotavirus vaccine.
- Rotavac—live attenuated G9P(10) strain and Rotarix-G1P(8) stain.
  - *Dose:* 5 drops
  - *Schedule:* At 6,10, 14 weeks
  - *Route of administration:* Oral
  - *Efficacy:* 55%
  - *Side effects:* Crying, irritability, fever, diarrhoea

8. A 28-year-old woman who had tested positive for hepatitis B surface antigen delivered a child. Explain in detail the recommended therapy to minimise the transmission to the neonate. (3 marks)

- **Hepatitis B vaccination.**
  - *Route of administration:* Intramuscular
  - *Dose:* 0.5 ml dose
  - *Schedule:* Within 12 hours of birth, 2nd dose: 1 month, 3rd dose: 6 months
- **Hepatitis B Immunoglobulin.**
  - *Route of administration:* Intramuscular
  - *Dose:* 300–500 IU
  - *Schedule:* Immediately within 12 hours of birth.

MI 1.10 DESCRIBE THE IMMUNOLOGICAL MECHANISMS IN IMMUNOLOGICAL DISORDERS (HYPERSENSITIVITY, AUTOIMMUNITY, IMMUNODEFICIENCY DISORDERS) AND THE LABORATORY METHODS USED IN THE DETECTION

LONG ESSAY

1. A 32-year-old business executive at the department of emergency medicine and critical care developed breathlessness, repeated hitting sensation in the head and chest tightness followed by an intramuscular injection of penicillin for treatment of cellulitis. IV antihistamines and IM adrenaline was given. O_2 therapy also administered. Patient recovered on the same day.

A. Define and classify hypersensitivity reactions (2 marks)

Hypersensitivity

Definition

➲ Hypersensitivity is an augmented immune response in a sensitised host, following subsequent contact with specific antigen which is harmful to the host.

Classification (Combs and Gell)

A. **Immediate hypersensitivity reaction**
   1. Type I hypersensitivity
   2. Type II hypersensitivity
   3. Type III hypersensitivity

B. **Delayed hypersensitivity reaction**
   1. Type IV hypersensitivity.

B. Mechanism, mediators and manifestations of hypersensitivity in this case. (7 marks)

➲ Type I Hypersensitivity is mediated in this case.

Mechanism

1. **Sensitisation phase**
   - The allergen induces IgE antibody which binds to mast cells and basophils when exposed to the allergen the first time (priming dose).
2. **Effector phase**
   - The allergen crosslinks the bound IgE when exposed to allergen again and induces degranulation and release of mediators (shocking dose).

Mediators

1. **Primary mediators (preformed)**

Histamine	• The most important vasoactive amine in human anaphylaxis • Formed by decarboxylation of histidine found in granules of mast cells, basophils, and platelets • It causes vasodilatation, increased capillary permeability and smooth muscle contraction
Serotonin (s-hydroxy tryptamine)	• Base derived from decarboxylation of tryptophan • Found in intestinal mucosa, brain tissue and platelets • It causes smooth muscle contraction, increased capillary permeability and vasoconstriction
Chemotactic factor	• Accumulate at the site of injury • Eosinophilic chemotactic factor—chemotaxis • Neutrophilic chemotactic factor—chemotaxis
Enzymatic mediators	• Proteases and hydrolases—bronchial mucous secretion, degradation of blood vessel basement membrane

2. **Secondary mediators**
   - ➲ These are either synthesized after target cell activation or released by the breakdown of membrane phospholipids during degranulation.

Slow reacting substance of anaphylaxis (SRS-A)	• Contraction of smooth muscles in lungs and cause increased vascular permeability
Prostaglandins and thromboxane A2	• Prostaglandins cause dilatation and increase vascular permeability of capillaries and bronchoconstriction • Thromboxane aggregates platelets and cause vasodilatation and increased mucous secretion
Platelet activating factor (PAF)	• Released from basophils which cause aggregation of platelets and release of their vasoactive amines

Manifestations (Table 1.10.1)

C. Common allergens associated with this type of hypersensitivity. (2 marks)

1. *Drugs:* Aspirin, antibiotics (penicillin, streptomycin), vitamins (thiamine, folic acid).
2. *Insect venom:* Honeybees, wasps, hornets.
3. Fish.
4. *Legumes:* Peanut beans.
5. *Seeds:* Sesame, mustard.
6. Nuts, berries.
7. *Hormones:* Insulin.
8. *Crustaceans:* Lobster, crab.

Table 1.10.1 Manifestations

Main Organ Affected	*Disease*	*Allergens*	*Clinical System*	*Route of Exposure*
Lung	• Asthma	• Egg • Feather • Grass pollen • House dust mite	• Wheezing • Dyspnoea • Tachypnoea	• Inhalation
Eye	• Rhinitis • Conjunctivitis • Hay fever	• Pollen	• Running nose, • Redness and itching of eye	—
Skin	• Eczema (atopic dermatitis) • itching	• food	• Pruritic vascular lesions • Pruritic bullous lesions	• Ingestion
Intestinal tract	• Allergic • Gastroenteropathy	• Foods	• Vomiting and diarrhoea	• Ingestion
Systemic	• Anaphylaxis	• Insect venom • Drugs • Nuts	• Shock • Hypotension • Wheezing	• Sting • Various • Ingestion

D. How do you detect and treat this type of hypersensitivity? (2+2 marks)

Detection

History

- Type, duration, season.

Allergy skin testing

- Cutaneous test, patch test, scratch test.
- **Advantages**
  1. Allows screening of a large number of allergens at one time.
  2. Results are available with no delay.
- **Disadvantages**
  1. May sensitize to new allergens.
  2. May rarely induce systemic anaphylaxis.
  3. Principle disadvantage is the need to discontinue certain inhibitory drugs.

Tests for IgE levels

- Radioallergosorbant test (RAST).

Treatment

- Drugs to counteract the action of mediators.
- Ensuring a protected airway.
- Support of respiratory and cardiac function.
- Single or in combination epinephrine, antihistamines, corticosteroids, or cromolyn sodium, should be given. Cromolyn sodium prevents release of mediators (e.g., histamine) from mast cell granules.
- Prevention by identifing the allergen by a skin test and avoidance of that allergen.

SHORT ESSAYS

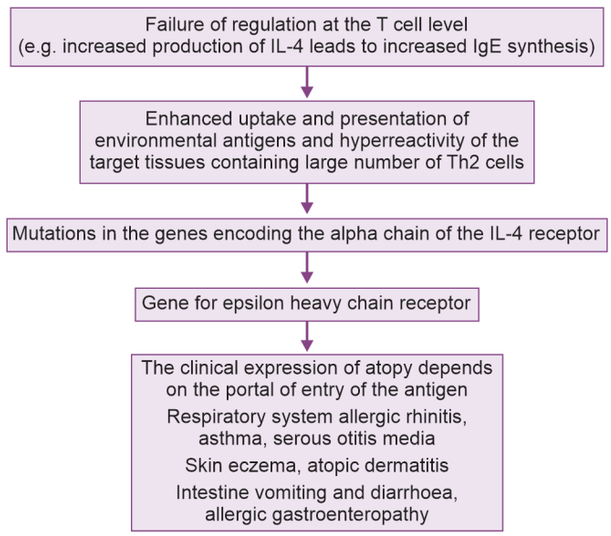
1. Define atopy and its mechanism with examples. (1+4 marks)

Atopy

Definition

- Refers to an inheritance propensity to respond immunologically to common naturally occurring inhaled or ingested allergens with the continual production of IgE antibodies.

Mechanism



Examples

1. Pollen allergens.
2. House dust mite.
3. *Mould allergens:* Fungal spores.
4. *Arthropod allergens: Dermatophagoides pteronyssinus.*
5. *Animal allergens:* Household pets like cats and dogs.
6. *Food allergens:* Legume, cow's milk, egg white.

2. A 9-month-old child with h/o diarrhoea, several episodes of respiratory tract infections. O/E paediatrician noticed gingivostomatitis due to HSV along with oral thrush. X-ray revealed absence of thymic shadow and blood picture showed absence of T lymphocytes. Mother gives a h/o rash after birth.

A. What is the most likely diagnosis of this case? (1 mark)

- DiGeorge syndrome.

B. Briefly describe about this condition and its treatment. (2 marks)

DiGeorge Syndrome

- Absence of T cells and suppressed antibody responses.
- Defective development of pharyngeal pouches, associated with chromosome 22 deletions, congenital defect in development of thymus.
- Viral, fungal, and protozoal infections; tetany caused by hypoparathyroidism.
- *Treatment:* Thymus transplant.

C. Define and classify immunodeficiency disorders. (2 marks)

Definition

- Immunodeficiency is the condition where
  - Impaired defense mechanism of the body.
  - Repeated microbial infection of varying severity.
  - Enhanced susceptibility to malignancies and autoimmune diseases.

Classification

I. Primary/Congenital Immunodeficiency

1. **B cell immunodeficiency disorders.**
   i. X linked gammaglobulinaemia selective IgA deficiency.
   ii. Immunodeficiencies with hyper IgM.
   iii. Transient hypogammaglobulinaemia of infancy.
2. **T cell immunodeficiency disorders.**
   i. Thymic aplasia (DiGeorge's).
   ii. Chronic mucocutaneous candidiasis.
3. **Combined B cell and T cell deficiencies.**
   i. Severe combined immunodeficiency (SCID).
   ii. Wiskott-Aldrich syndrome.
   iii. Ataxia–Telangiectasia.
4. **Disorders of phagocytosis.**
   i. Chronic granulomatous disease.
   ii. Chediak–Higashi syndrome.
   iii. Job's syndrome.
5. **Disorders of complements.**
   i. Hereditary angioedema.
   ii. Paroxysmal nocturnal Haemoglobinuria.
   iii. Recurrent pyogenic infections.
   iv. Autoimmune disorders.

II. Secondary/Acquired Immunodeficiency

i. *Viral infections:* HIV, measles.
ii. *Bacterial infections:* Lepromatous leprosy.

3. Describe the immunodeficiency disorders and their molecular defect with clinical manifestations. (5 marks)

See Table 1.10.2

4. A 36-year-old auditor comes to the hospital, he shows signs of excessive nervousness, irritability, and complaints it's too hot in the room. O/E he has goitre and exophthalmia. Laboratory analysis of his blood reveals high antibody titres against the thyroid-stimulating hormone (TSH) receptor.

A. What is the most likely diagnosis? (1 mark)

- Graves' disease.

B. Define Autoimmunity. Classify auto-Immune diseases with examples. (1 + 3 marks)

Definition

- Autoimmunity is a condition in which the body's own immunologically competent cells or antibodies act against its self-antigens resulting in structural or functional damage.

Classification

I. *Local/Single Organ Autoimmune Diseases*

1. **Addison's disease**
   - Characterized by lymphocytic infiltration of adrenal glands and the presence of circulating antibodies directed against the cells of zona glomerulosa.
2. **Graves' disease**
   - Auto antibodies that the receptor for TSH, activating adenylate cyclase and resulting in over production of hormones.
   - Low TSH levels.

Table 1.10.2 Manifestations

Deficient component and name of disease	*Specific deficiency*	*Molecular defect*	*Clinical features*
B cell			
• X-linked (Bruton's)	• Absence of B cells; very low Ig levels	• Mutant tyrosine kinase	• Pyogenic infections (*S. aureus, H, influenza, S. peumoniae*)
• Selective IgA	• Very low IgA levels	• Failure of heavy-chain gene switching	• Sinus and lung infections
T cell			
• Thymic aplasia (DiGeorge's)	• Absence of T cells	• Defective development of pharyngeal pouches; not a genetic disease	• Viral, fungal, and protozoal infections • Tetany
• Chronic mucocutaneous candidiasis	• Deficient T-cell response to *Candida*	• Unknown	• Skin and mucous membrane infections with *Candida*
Combined			
• Severe combined immunodeficiency (SCID)	• Both B-cell and T-cell function deficiency	• Either defective IL-2 receptor, defective recombinases, defective kinases, absence of class II MHC proteins, or ADA or PNP deficiency	• Bacterial, viral, fungal, and protozoal infections
Complement deficiencies			
• Hereditary angioedema	• Deficiency of C1 protease inhibitor	• Too much C3a, C4a, and C5a generated	• Edema, especially laryngeal edema
• C3b	• Insufficient C3	• Unknown	• Pyogenic infections, especially with *S. aureus*
• C6,7,8	• Insufficient C6,7,8	• Unknown	• *Neisseria* infections
Phagocyte deficiencies			
• Chronic granulomatous disease	• Defective bactericidal activity because no oxidative burst	• Deficient NADPH oxidase activity	• Pyogenic infections, especially with *S. aureus*

3. **Myasthenia gravis**
   - Autoantibodies that bind the acetylcholine receptors on the motor end plates of muscles.

II. Systemic Type

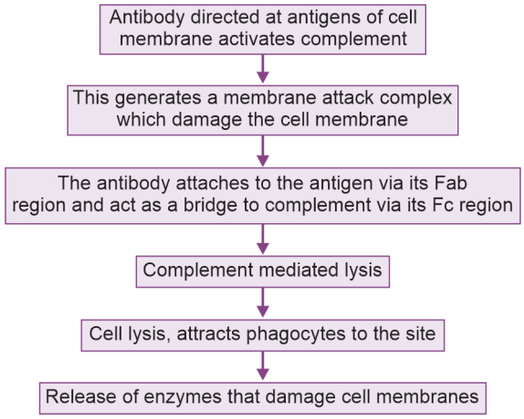
1. **Rheumatoid arthritis**
   - Chronic inflammatory response ultimately destroys cartilage and bone, rendering the affected joints immobile.
   - The rheumatoid factors are an IgM antibody that binds to circulatory IgG forming. IgM-IgG complexes that are deposited in the joints.
2. **Sjogren's syndrome**
   - Triad of keratoconjunctivitis sicca (dry eyes), xerostomia (dryness of mouth) with or without salivary gland enlargement and rheumatoid arthritis.
3. **SLE**
   - Affected individual produces autoantibodies to vast array of tissue antigen such as DNA, histones, RBCs, platelets, leucocytes, and clotting factors.

5. Briefly describe type 2 hypersensitivity with examples. (5 marks)

Type 2 Hypersensitivity

- These reactions involve a combination of IgG (rarely IgM) antibodies with the antigenic determinants on the surface of cells leading to cytotoxic or cytolytic effects.

Mechanism ***(Fig. 1.10.1)***



Examples

I. **Hypersensitivity reactions against erythrocytes**
   1. Non compatible blood transfusion -mismatch of ABO blood group, severely destroy RBCs.
   2. Rh incompatibility—Hemolytic disease of newborn.
   3. Autoimmune haemolytic anaemia—Conversion of a hapten to a full antigen by drug induce self-antibody.
   4. Drug induced haemolytic anemia—Penicillin, quinidine.

II. **Hypersensitivity reactions due to infections**
   1. Rheumatic fever after group A *Streptococcus* infection.
   2. Haemolytic anaemia after *Mycoplasma pneumoniae* infection.

III. **Superacute rejection** in allogenic organ transplantation—due to prior sensitisation

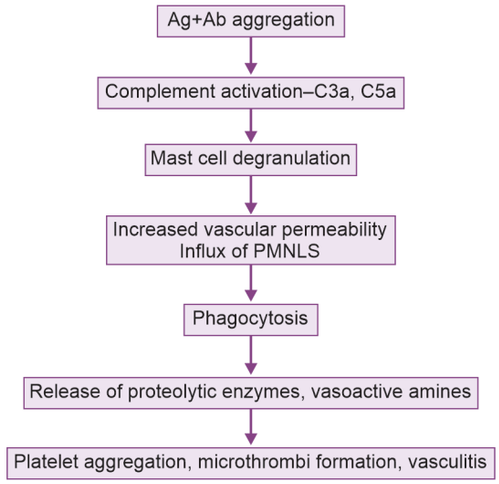
IV. **Hypersensitivity against solid organs**
   1. *Good pasture syndrome:* Antibody to basement membrane of kidneys and lungs.
   2. *Graves' disease:* Antibody to TSH receptors.
   3. *Myasthenia gravis:* Antibody to acetylcholine receptors.

6. Briefly describe type 3 hypersensitivity with examples. (5 marks)

Type 3 Hypersensitivity

- An inflammatory response in tissues caused by the deposition of antigen-antibody complexes.
- There is inflammation of blood vessels, kidney glomerular membranes, joints and skin.

Mechanism ***(Fig. 1.10.2)***



Reactions

1. Cutaneous Arthus Reaction

- It is the inflammation caused by the deposition of immune complexes at a localised site.
- Arthus response are slow and more persistent.
- **Clinical manifestations**
  i. Farmer's lung.
     - Hypersensitivity pneumonitis or allergic alveolitis
     - Associated with the repeated inhalation of thermophilic actinomycetes growing in plant material (hay)

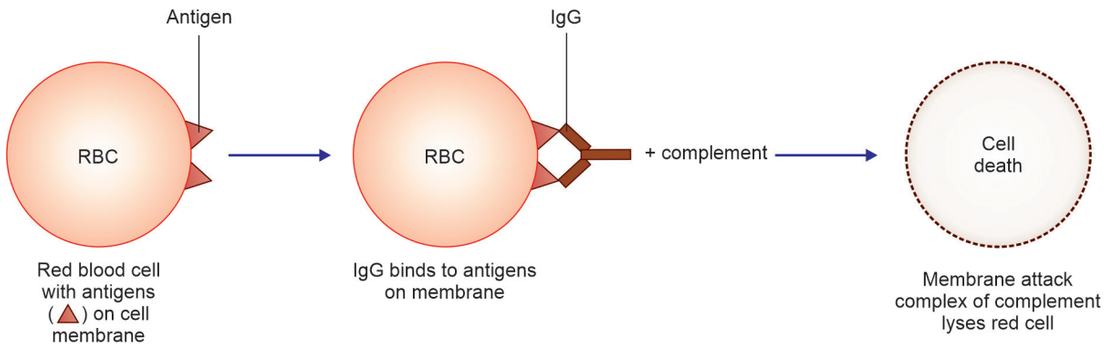


Fig. 1.10.1: Mechanism of type 3 hypersensitivity

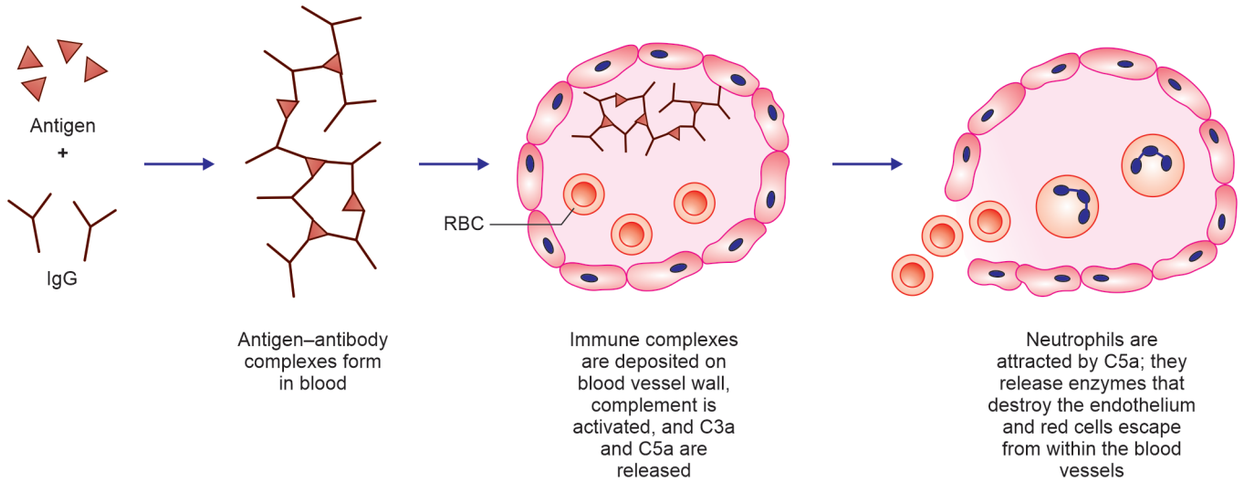


Fig. 1.10.2: Mechanism of type 3 hypersensitivity

ii. Cheese worker's lung.
   - Extrinsic allergic alveolitis caused by the inhalation of spores of *Penicillium casei* from moldy cheese

iii. Wood worker's lung.
   - Inhalation of wood particles in an occupational setting can cause various lung symptoms

iv. Mushroom worker's lung.
   - Inhalation of thermophile actinomycetes in the compost

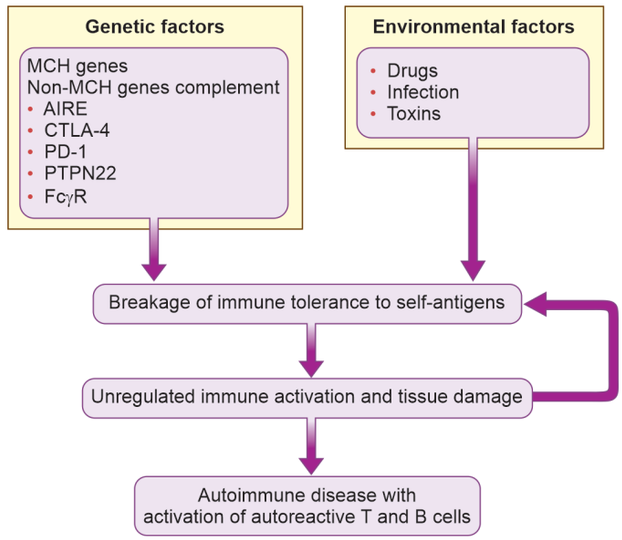
2. Systemic Serum Sickness

- Systemic inflammatory response due to the presence of immune complexes deposited in many areas of the body.
- This condition occurs after administration of a large dose of foreign antigen (antiserum or certain drugs).
- **Clinical manifestations**
  - First sign is often a pruritic rash, which may be urticarial, maculopapular, or erythematous.
  - Fever, arthralgia, lymphadenopathy, splenomegaly and eosinophilia complete the clinical features.
  - Occasionally there are headache, nausea and vomiting.
  - Recovery takes 7–10 days.

Examples

1. Glomerulonephritis.
2. Rheumatoid arthritis.
3. Systemic lupus erythematosus.

7. "Autoimmunity results due to breakdown of immunological tolerance". Describe the mechanism of autoimmunity in support of the statement given. (5 marks)



Release of Sequestered Antigens

- Sperm, central nervous system, lens, and uveal tract of eye are sequestered so that the antigens are not exposed to immune system.
- Immunologically privileged sites.
- Bacterial and viral infections → damage cells cause release of sequestered antigens → elicit immune response, e.g. lens antigen of eye.
- Lens protein is enclosed in its capsule and does not circulate in the blood.
- Hence immunological tolerance against this antigen is not established during fetal life.
- When antigen leaks out, following injury, it may induce an immune response causing damage to lens of other eye.

8. An 18-year-old student gets a new watch with metal strap. Next day he notices rash over his wrist in the area where he has worn his watch. What is your likely diagnosis? Briefly describe this type of reaction. (1+4 marks)

Likely Diagnosis

- Contact hypersensitivity.

Contact Hypersensitivity

- It is an eczematous skin disease caused by cell mediated hypersensitivity to an environment allergen.
- It occurs in people sensitized to chemicals, plant materials, topically applied drugs, some cosmetics, soaps and other substances.
- The small molecules acting as haptens enter the skin, attach to body proteins, and modify those proteins enough to 'break tolerance.' For example, normal skin proteins, to which the T cells tolerate as 'self' due to negative thymic selection, binding to metal ions, they are recognised as foreign.
- The skin proteins are taken up, processed, and presented to CD4-positive T cells by dendritic cells. The T cells differentiate into Th-1 and Th-17 cells, and later skin contact with metal, the Th cells cause inflammation when they recognise the metal bound peptides presented by antigen-presenting cells in the metal-exposed skin.
- The sensitized person develops contact dermatitis characterized by erythema, itching, vesicles, eczema, or necrosis of skin within 12–48 hours.
- *Patch testing* can be done for diagnosing contact hypersensitivity.
- *Prevention:* Avoidance of known allergen.

SHORT ANSWERS

1. Briefly describe the Laboratory diagnosis of SLE. (3 marks)

Laboratory Diagnosis of SLE

1. **Immunofluorescence**
   - Direct immunofluorescence.
   - LE factor antibody detection.
2. **Antinuclear antibodies tests**
   - Antibodies to DNA, histones, non-histones bound to RNA, nucleolar antigens.
3. **ELISA**
   - Antibodies to dsDNA.
4. **Immunoblot assay**
   - Antibodies to dsDNA.
5. **Other tests**
   - Platelets, RBC`s, WBC count.

2. Enumerate the serological/laboratory tests applicable for the diagnosis of Rheumatoid Arthritis. (3 marks)

1. RA factor for rheumatoid arthritis.
2. Antibodies to cyclic citrullinated peptide.
3. Low complement levels.

3. Define 'molecular mimicry' with an example. (3 marks)

Definition

- Infectious agents possess antigen or similar amino acid sequence that elicit an immune response that cross reacts with components of human cells because of the similarity in the structure with the host.

Examples

- Antibodies against M proteins cross react with cardiac myosin, leading to rheumatic fever *Streptococcus pyogenes*—Rheumatic fever.

4. Give examples for intracellular microbes inducing delayed type of hypersensitivity and its mechanism. (1+2 marks)

Examples

1. *M. tuberculosis.*
2. *Coccidioides immitis.*

Mechanism (Fig. 1.10.3)

- CD4 T cells and macrophages are the immune cells involved in granuloma formation when exposed to components of intracellular microbes.

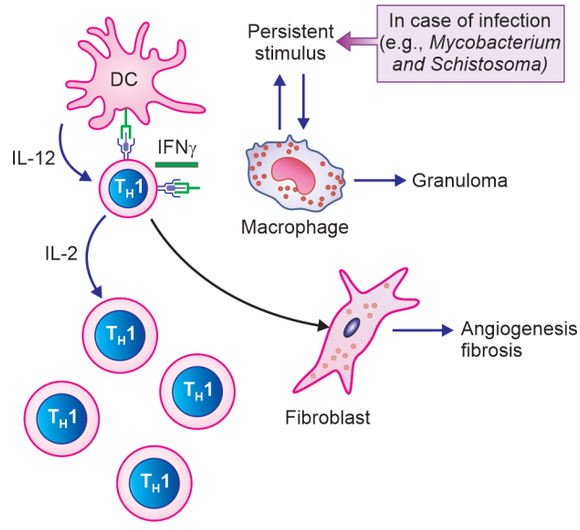


Fig. 1.10.3: Mechanism of delayed hypersensitivity

5. List out the drugs used in the treatment of Type-I Hypersensitivity (3 marks)

Anaphylaxis

- Single or in combination—Epinephrine, antihistamines, corticosteroids, or cromolyn sodium, should be given.
- Cromolyn sodium prevents release of mediators (e.g. histamine) from mast cell granules.

Asthma

- Inhaled β-adrenergic bronchodilators.
- Corticosteroids, such as prednisone, are also effective but carry significant toxicity if used chronically.
- *A monoclonal anti-IgE antibody:* Omalizumab
- *Leukotriene antagonist:* Montelukast

6. Briefly describe X-linked disorders leading to immunodeficiency. (3 marks)

1. **Bruton Disease (X-linked Agammaglobulinaemia).**
   - Failure of pre-B-cells to differentiate into immature B-cells in the bone marrow.
   - Absence of an enzyme tyrosine kinase leading to total absence of B-cells, plasma cells and all classes of Ig.
   - The B-cell maturation stops at pre B cell stage; after the synthesis of heavy-chain without forming the light chains, leading to incomplete immunoglobulins synthesis.
   - Seen primarily in males; rarely in females.
   - *Onset:* after 6 months of life
   - *Secondary infection:* Recurrent bacterial infections, viruses (enteroviruses) and parasites (*Giardia lamblia*)
   - Autoimmune diseases (such as SLE and dermatomyositis) also occur in up to 20% of cases.
3. **X-linked Severe Combined Immunodeficiency (SCID)**
   - Defect in IL2 receptor on T cells with lack of gamma chain of IL2 receptor which is essential for the development of T cells.
4. **Wiskott–Aldrich syndrome**
   - Decreased IgM and elevated IgA and IgE level.
   - Decreased T-cell function with increased susceptibility to autoimmunity and malignancy.
   - Syndrome of eczema, recurrent pyogenic infections, and thrombocytopenia.

MI 1.11 DESCRIBE THE IMMUNOLOGICAL MECHANISMS OF TRANSPLANTATION AND TUMOUR IMMUNITY

LONG ESSAY

1. A 65-year-old male patient with chronic kidney disease underwent renal transplantation donated by an unrelated donor. Patient developed rejection reaction within 1 month. With respect to this case/transplantation answer the following questions.

A. Define a graft. Classify the types of graft. (2 marks)

Graft

- A tissue or an organ transplanted surgically in the same or genetically different individual is termed as graft.
- The organ/tissue is called 'transplant'.

Classification of Grafts

Based on Genetic Relation

1. *Autograft:* is an organ or tissue taken from an individual and grafted in the same individual.
2. *Isograft:* is a graft/ tissue taken from an individual and placed on another individual of the same genetic constitution.
3. *Allograft:* is tissue transferred between genetically non identical members of the same species.
4. *Xenograft:* is tissue transferred between members of different species.

Based on Anatomical Site

1. *Orthotopic graft:* when tissues/organs are transplanted in their same anatomical position Examples: Skin graft.
2. *Heterotopic graft:* when tissues/organs are placed at abnormal sites Examples: thyroid tissue is placed in subcutaneous pocket.

Based on Organ Transplanted

1. *Vital graft:* Live grafts.
2. *Static grafts:* Nonliving graft.

B. Define Transplantation Antigens. (1 mark)

- Types of tumour antigens.
  1. **Tumour specific transplantation antigen.**
     - An antigen that induces the immune response against the transplant cells that are transformed by virus
  2. **Tumour associated transplantation antigen.**
     - Antigens which develop on the cells surface during the process of neoplastic transformation and are considered as "nonself"

C. Enumerate the types of graft rejection and distinguish between each other. (5 marks)

Types of Graft Rejection (Table 1.11.1)

1. Acute graft rejection
2. Chronic rejection
3. Hyperacute rejection (White graft rejection).

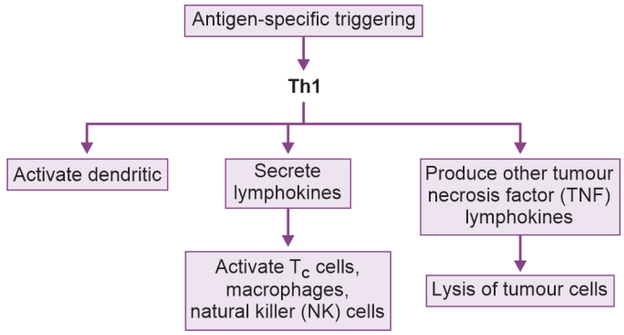
D. Add a note on immunosuppressive therapy. (2 marks)

- Immunosuppression of the recipient—by administration of.
  1. **Immunosuppressive drugs**
     - *Cyclosporine:* Prevents activation of T cells
     - *Azathioprine:* Disrupt the synthesis of DNA and RNA and cell division
     - *Tacrolimus:* Calcineurin inhibitor
  2. **Corticosteroids**
     - Inhibit cytokine production and hence inflammation
  3. **Monoclonal antibodies**
     - Block IL-2 receptors
     - To suppress the activity of subpopulation of T-cells
     - To block co-stimulatory signals
     - Examples: Daclizumab
  4. **Antilymphocyte serum**
     - Selectively destroys mature T cells (in GVH)

SHORT ESSAYS

1. Discuss in brief about Immunosurveillance theory. (5 marks)

- Refers to the policing or monitoring function of immune system cells to recognise and destroy clones of transformed cells prior to their development into neoplasms and to destroy tumours after they develop.
- It is mediated by the cellular limb of immune response.
- T cells surveillance system detects and eliminate newly arising clones of neoplastic cells.
  - Less expression of major histocompatibility complex (MHC) class I–complexed TAAs.
  - Release of soluble factors that promote the activity of immunosuppressive leukocytes, including T regulatory (Treg) cells.
  - Expression of cell surface molecules that inhibit the function of cytotoxic T cells and NK cells.



- Immune system kills and induces changes in the tumour resulting in tumour escape and recurrence-Cancer Immuno-editing by.
  - Elimination: Innate and adaptive immunity.
  - Equilibrium: Cancer persistence-genetic instability and immune selection.
  - Escape-cancer progression—chronic inflammation.

Table 1.11.1 Types of graft rejection

Feature	*Acute graft rejection*	*Chronic rejection*	*Hyperacute rejection (White graft rejection)*
Duration	• Occurs after one week	• Occurs after months to years	• Occurs in min to hours
Cells involved	• Cytotoxic T-cell, macrophage and antibody mediated, • myocyte and endothelial damage,	• T cells react with the graft alloantigens secrete cytokines resulting in proliferation of fibroblasts in the vascular intima	• Due to preformed anti-ABO antibodies the graft will be immediately rejected
Aetiology	• Inflammation: damage the graft	• Due to atherosclerosis of endothelium of blood vessels	• Vasospasm of blood vessels
Factors	• Current immunosuppressive drugs prevent the acute rejection by blocking the activation of alloreactive T cells	• Appears due to side effects of immunosuppressive therapy/alterations in minor MHC antigens • Occurs in most solid organ transplants, e.g. heart, kidney • Mostly refractory to therapy	• Exposure to foreign HLA antigens due to previous blood transfusion, transplants, or pregnancy • This type is uncommon as it avoided by prior matching of donor and recipient

2. A 15-year-old with acute lymphocytic leukaemia fails all standard therapy. A transplant was carried out before which he received antibiotics and immunosuppressive agents. After 21 days duration, he developed sever rash, diarrhoea and jaundice.

A. What is the most likely diagnosis? (1 mark)

- Acute allograft rejection.

B. Explain the immunological process of this condition. (2 marks)

- Rapidity of rejection depends on:
  1. Degree of dissimilarity between donor and recipient HLA Ags.
  2. Immunocompetency of the recipient.
  3. Prior sensitisation of the recipient to donor tissues/blood.
     - Recognition of transplanted cells that are self or foreign is determined by polymorphic genes (MHC) that are inherited from both parents and are expressed co-dominantly
     - Alloantigens elicit both cell-mediated and humoral immune responses

Direct Pathway

- Direct presentation.
- Recognition of an intact MHC molecule displayed by donor APC in the graft.
- Basically, self MHC molecule recognises the structure of an intact allogeneic MHC molecule.
- Involves both $CD8^+$ and $CD4^+$ T cells.
- CD4 T cells—damages the graft by delayed type of hypersensitivity and CD8 T cell kill nucleated cells of graft.

Indirect Pathway

- Indirect presentation
- Donor MHC is processed and presented by recipient APC
- Basically, donor MHC molecule is handled like any other foreign antigen
- Involve only CD4+ T cells
- Antigen presentation by class II MHC molecules
- Donor APCs migrate to regional lymph nodes and are recognised by the recipient's T_H cells.
- Alloreactive T_H cells in the recipient induce generation of CTLs, delayed type of hypersensitivity and B cell activation (by complement mediated lysis and Antibody dependent cell cytotoxicity) then migrate into the graft and cause graft rejection.

C. Mention the pre-transplantation workup and prevention for such case. (2 marks)

1. Blood group matching.
2. HLA matching of donor and recipient: Micro-cytotoxicity, mixed lymphocyte reaction, PCR.
3. Immunosuppression of the recipient—by administration of.
   - *Cyclosporine:* Prevents activation of T cells
   - *Corticosteroids:* Inhibit cytokine production
   - *Monoclonal antibodies:* Block IL-2 receptors
   - Antilymphocyte serum selectively destroys mature T cells (in GVH).
   - Chronic rejection is considered irreversible.

3. Explain the structure of MHC with the help of diagram and its clinical significance. (2+2+1 marks)

Structure of MHC (Fig. 1.11.1)

- MHC molecules or human leukocyte antigens (HLA) serve as a unique identification marker for every individual as the genetic sequence of MHC genes differs for every individual.
- In humans, HLA complex coding for MHC proteins is located in short arm of chromosome *6*.
- The genes are clustered in three regions named as MHC region-I, II and III.
- MHC I and II help in antigen presentation to T-cells.
  - MHC I present intracellular antigen on viral/tumor cells to cytotoxic T-cells.

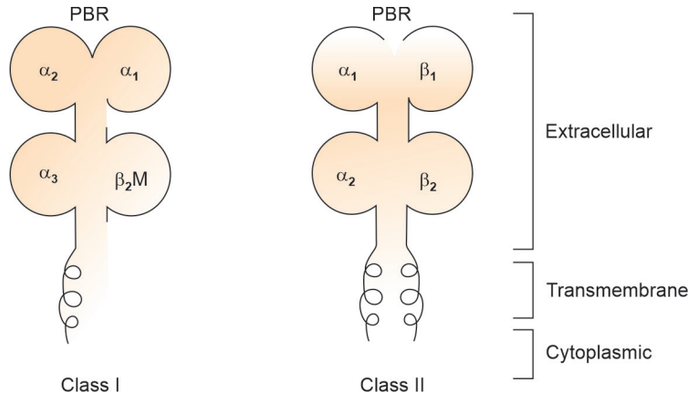


Fig. 1.11.1: Structure of MHC

- MHC II presents extracellular antigen on APCs to helper T-cells.
- MHC III does not help in Ag presentation, but code for various proteins such as complement factors (C2, C4, C3 convertase, factor B and properdin), heat shock protein.

- TNF-α and β and steroid 21-hydroxylases.

Clinical Significance

- It determines the histocompatibility between the donor graft and the recipient.

SHORT ANSWERS

1. A 50-year-old businessman c/o of vague abdominal pain and notices blood in stool. The gastroenterologist performs a sigmoidoscopy and notices a mass in the colon. Which is the tumour marker the clinician should order for? Give examples of tumour-associated transplantation antigens. (1 + 2 marks)

Tumor Marker the Clinician should Order for is

- Carcinoembryonic antigen—Tumour marker for carcinoma colon.

Examples of Tumour Associated Transplantation Antigens

1. *Alfa fetoprotein:* Hepatoma, testicular cancer.
2. *Carcinoembryonic antigen:* Ovarian, lung, GIT cancers.
3. *CA125:* Ovarian cancer.
4. *Prostatic specific antigens:* Prostate cancer.

2. Write in brief about GVH Reaction. (3 marks)

- The disease produced by the reaction of immunocompetent T lymphocytes of the donor graft that are histoincompatible with the tissues of the recipient.
- The recipient must be either immunologically immature, immunosuppressed by irradiation or drug therapy, or tolerant to the administered cells, and the grafted cells must also be immunocompetent.
- The recipient must express antigens foreign to the donor cells.
- The donor`s cytotoxic T cells play a key role in destroying the recipient's cells.
- GVH is usually seen in.
  - Bone Marrow transplant cases.
  - After random blood transfusion in neonates.
  - Patients with congenital immunodeficiencies.
  - Cancer (leukaemia) patients.

3. Define cancer immunotherapy. Explain the role of monoclonal antibodies and cytokine therapy in the treatment of cancers. (1 + 2 marks)

Cancer Immunotherapy

- It is the artificial stimulation of the immune system to treat cancer, improving on the immune system's natural ability to fight the disease.

Role of Monoclonal Antibodies

- Most successful with targeted therapy.
- *Rituximab (anti CD20 Ab):* treatment of relapsed low-grade NHL and follicular NHL
- *Daclizumab:* treatment of human T lymphotropic virus (HTLV)
- *Trastuzumab—ErbB2:* treatment of breast cancer

Role of Cytokine Therapy

- Increased class I MHC expression on tumour cells, thereby increasing CTLs activity against tumours.
- Inhibits angiogenesis—TNF.
  - IFN α: in Hairy cell leukaemia, Kaposi sarcoma.
  - IL-2: Malignant melanoma, renal cell carcinoma