and to initiate appropriate preventive measures. The diagnostic cycle begins when the clinician takes a microbiological sample and ends when a clinician receives the laboratory report and uses the information to manage the condition (Figure 12.3).

The steps in diagnostic cycle are

1. Clinical request and provision of clinical information.
2. Collection and transport of appropriate specimens.
3. Laboratory analysis.
4. Interpretation of microbiology report and use of the information.

Specimen Collection and transport: It is important to collect the specimen appropriately and protect it from contamination. Transport media are used that are compatible with the organism believed to be present in the clinical sample. Quality of patient specimens and their transport to the laboratory is important.

Infections and samples used

Respiratory tract infections: Nasal and bronchial washings, throat and nasal swabs, sputum.

Eye infections: Conjunctival swab or scraping.

Wound infections: Pus, skin scraping, wound swap.

Gastrointestinal infections: Stool, rectal swabs.

Genital infections: Vesicle fluid or swab.

Urinary tract infections: Urine.

Blood borne infections: Blood.

Nervous system infections: Cerebrospinalfluid (CSF).

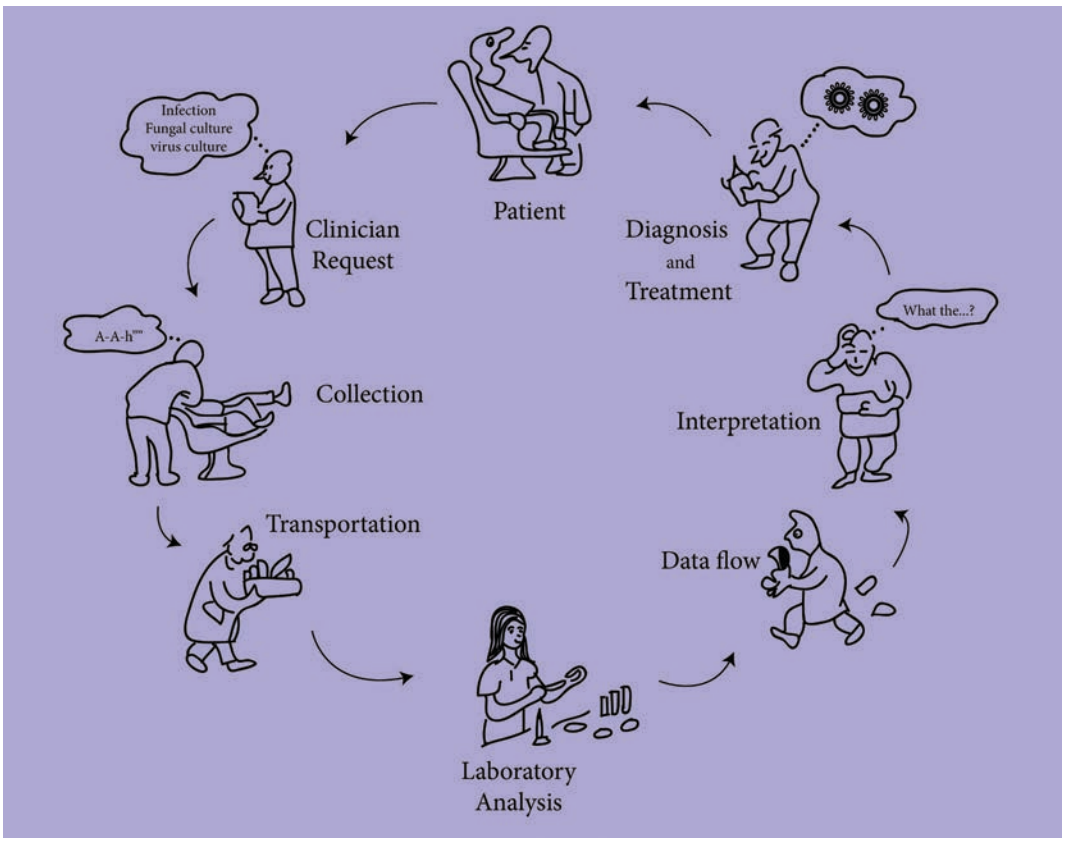


Figure 12.3: The steps in diagnostic cycle

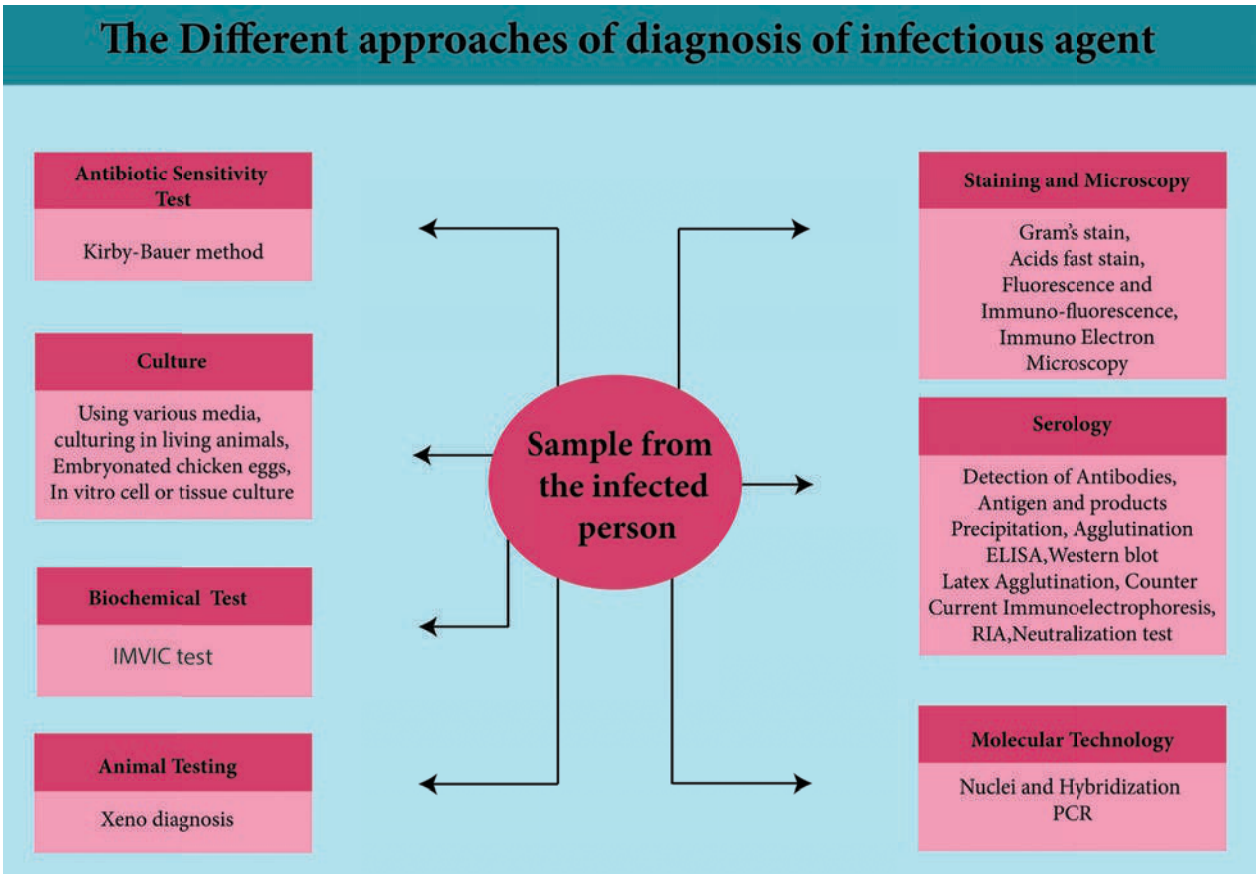


Figure 12.4: Different approaches of diagnosis

Laboratory diagnosis of infectious agents

Direct diagnosis: It is the demonstration of the presence of an infectious agent, antigen or nucleic acids

Indirect diagnosis: It is the demonstration of presence of antibodies to a particular infectious agent, cytopathic effects, haemagglutination, inclusion bodies and neutralization.

The different approaches for diagnosis or identification of infectious agents are shown in Figure 12.4.

12.2 Skin and Wound Infections

The skin, which covers and protects the body, is the body's first line of defense against pathogens. As a physical barrier, it is almost impossible for the pathogens to penetrate it. However, microorganisms can enter through skin breaks that are not readily apparent, and the larval forms of a few parasites can penetrate the intact skin. The skin has up to seven layers (Figure 12.5) of ectodermal tissue and guards the underlying tissues viz; muscles, bones, ligaments and internal organs. Nearly all human skin is covered with hair follicles. Because it interfaces with the environment, skin plays an important role in protecting the body against pathogens and excessive water loss. Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates. Severely damaged skin will try to heal by forming scar tissue. This is often discolored and depigmented.

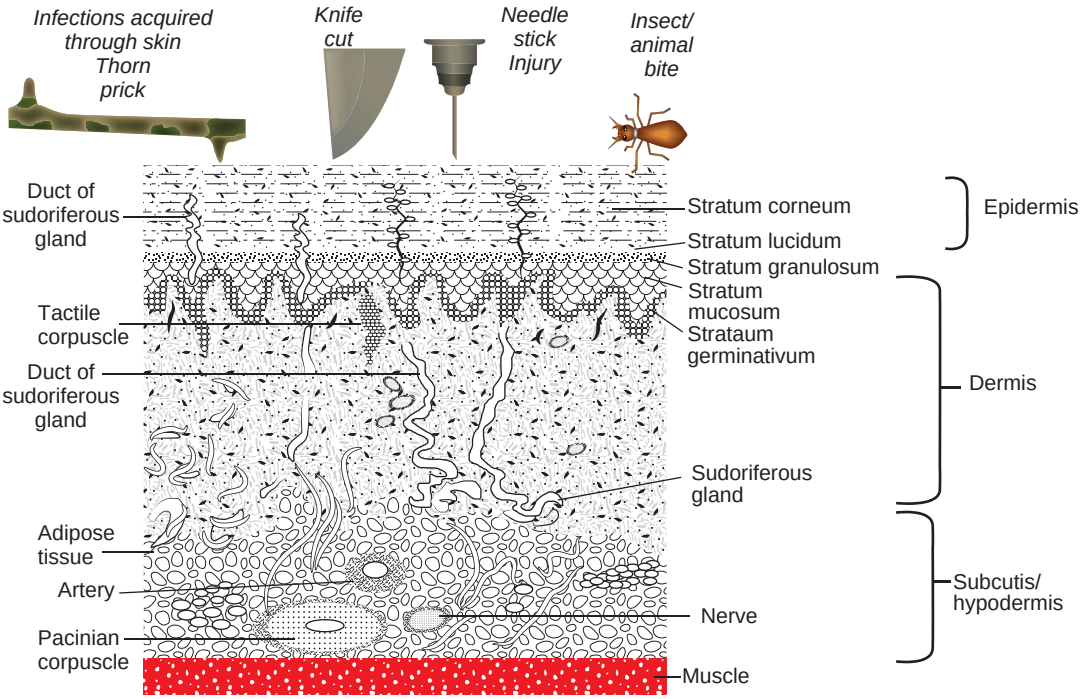


Figure 12.5: Structure of skin

12.2.1 Structure of Skin

Skin is composed of three primary layers: the epidermis, the dermis and the hypodermis.

Epidermis

It forms the water proof, protective wrap over the body's surface. It also serves as a barrier to infection. It is made up of stratified squamous epithelium with an underlying basal lamina. The outermost layer of the epidermis, the stratum corneum, consists of dead cells that contain a waterproofing protein called keratin. The epidermis contains no blood vessels and cells in the deepest layers are nourished exclusively by diffused oxygen from the surrounding air. The main types of cells present in epidermis are Merkel cells, keratinocytes with melanocytes and Langerhan cells.

Dermis

The dermis is the layer of skin beneath the epidermis that consists of epithelial tissue and cushions the body from stress and strain. The dermis is tightly connected to the epidermis by a basement membrane. It also harbors many nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. The blood vessels in the dermis provide nourishment and waste removal from its own cells as well as from the Stratum basale of the epidermis.

Hypodermis

Subcutaneous tissue (also *hypodermis* and *subcutis*) is not part of the skin, and lies below the dermis of the cutis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue, adipose tissue and elastin. The main cells are fibroblasts, macrophages and adipocytes (subcutaneous tissue contains 50% of body

fat). Fat serves as padding and insulation for the body.

The hair follicles, sweat gland ducts, and oil gland ducts in the dermis provide passageways through which the microorganisms can enter the skin and penetrate deeper tissues. Perspiration provides moisture and some nutrients for microbial growth. However, it contains salt, which inhibits many microorganisms; the enzyme lysozyme, which is capable of breaking down the cell walls of certain bacteria and antimicrobial peptides. Sebum, secreted by oil glands, is a mixture of lipids (unsaturated fatty acids), proteins, and salts that prevents skin and hair from drying out. Although the fatty acids inhibit the growth of certain pathogens, sebum, like perspiration, is also nutritive for many microorganisms

12.2.2 Normal Microbiota of the Skin

The skin's normal microbiota contains relatively large numbers of Gram positive bacteria, such as *Staphylococci* and *Micrococci*. Bacteria in the skin tends to be grouped into small clumps. Vigorous washing can reduce their numbers but will not eliminate them. Microorganisms remaining in hair follicles and sweat glands after washing will soon reestablish the normal populations. Areas of the body with high moisture, such as armpits and between the legs, have higher populations of microorganisms. They metabolize secretions from the sweat glands and are the main contributors to body odour.

Also part of the skin's normal microbiota are Gram positive pleomorphic rods called diphtheroids. Some diphtheroids, such as *Propionibacterium acnes*, are typically anaerobic and inhabit hair follicles. These bacteria produce propionic acid, which helps maintain the low pH of skin, generally between 3 and 5

12.2.3 Wound Infection

Wound can be defined as any interruption of continuity of external or internal surfaces caused by violence

Wounds may occur following: surgery, trauma or injections

Wound infections may occur mainly after surgical procedures

Wound sepsis is the result of cross infection from human sources and from other outside sources.

Bacteria associated with wound infections

Many bacteria are associated with wound infection (Figure 12.6). The normal flora may also cause infection. The most common normal flora of the skin are: *Staphylococci*, and various *Streptococci*, *Sarcina* sp, anaerobic Diphtheroids, Gram negative rods and others.

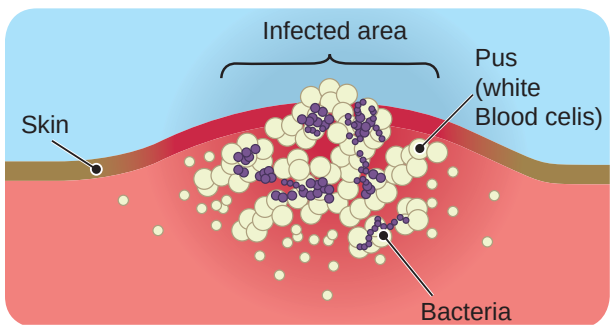


Figure 12.6: Bacterial infection on the skin

Factors determining the ecology of the skin bacteria

The main factors that determine the ecology of skin bacteria:

- Climate: Temperature and humidity
- The effect of free fatty acids
- Maintenance of the flora by products of skin secretions and other bacterial inhibitors.

Defence against infection

- Intact skin: Normal uninterrupted skin provides protection against invasion by bacteria.
- Lysozyme in sweat: The enzyme lysozyme provides protection against Gram positive bacteria by lysing the cell wall.
- IgA antibodies in the sweat and secretions provide first line of defense against infection.
- Inhibitors like unsaturated fatty acids provide protection against bacteria.
- Bacteriocins produced by the normal flora prevent the establishment of other bacteria.

Factors responsible for wound infections

a) Host factors

The following factors help the organisms to survive and produce the infections:

HOTS

1. What are the possible infecting agent you could pick up when you are injured while playing on the ground? List them and name the diseases that they could cause.
2. What are the possible infectious agent that can infect you when you are injured by a rusted nail?

- Extremes of age: Very old and very young people are susceptible to infection.
- Diabetes mellitus: Hormonal imbalance increases susceptibility.
- Steroid therapy: Immune responses are affected.
- Obesity: Increases susceptibility.
- Malnutrition: General health status affected.
- Immunocompromised individual: Immune system will not function properly.
- Presence of remote infection at the time of surgery.

b) Exogenous Factors

- Use of unsterile instruments: They carry pathogens.
- Surgeons hands / from health workers: May carry pathogens.
- Air / Hospital environments: Unclean environment harbur pathogens.

c) Endogenous Factors

- Wound contamination from the patient source: From the normal flora.
- Wound penetrating through structures containing normal flora.
- Surgical procedures involving mucous membranes harbouring normal flora.
- Patients carrying pathogens in their nose, throat, axilla.

Etiological agents

Etiological agents like *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus*, member of enterobacteriaceae anaerobic organisms, anaerobic cocci and bacteroides cause infections.

Post operative infections

Gasgangrene organisms like *Clostridium perfringens, Staphylococcus aureus* and *Clostridium tetani* may cause post operative infections.

Route of entry

Wounds may occur following surgery, trauma or injections. Wound infections may occur mainly after surgical procedures. Wound sepsis is the result of cross infection from human sources and from other outside sources. Infections of skin are listed in Table 12.2.

Mechanisms of damage

1. Organisms enter through the skin, multiply there and produce the disease in the skin.
   For example, impetigo, abscess and cellulitis (Figure 12.7) are caused by *Staphylococcus aureus* and *Streptococcus pyogenes*.

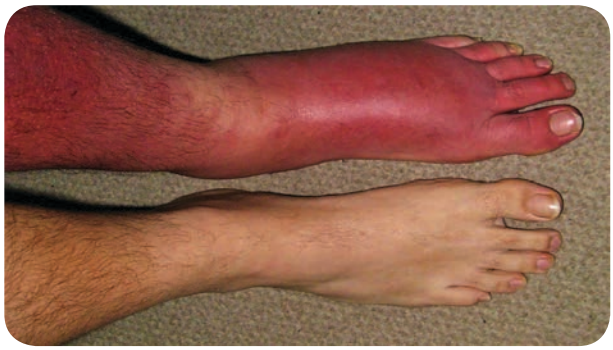


Figure 12.7: Cellulitis

   As soon as the organisms enter the skin they multiply and produce various toxins that kill the cells and produce cellulitis. Further damage leads to necrosis and ulcer formation (Figure 12.8).

2. Organisms multiply in the skin and produce disease in internal organs. For example some Group A *Streptococci* multiply in the skin and produce disease known as Acute Glomerulonephritis

Table 12.2: Bacterial Infections of the skin

Disease	Pathogen	Signs and Symptoms	Transmission
Cellulitis	*Streptococcus pyogenes*	Localised inflammation of dermis and hypodermis; skin red, warm, and painful to the touch	Through cut or abrasion
Erysipelas	*Streptococcus pyogenes*	Inflamed, swollen patch of skin, often on face; may be suppurative	Through cut or abrasion
Impetigo	*Staphylococcus aureus, Streptococcus pyogenes*	Vesicles, pustules, and sometimes bullae around nose and mouth	Highly contagious, especially via contact
Wound infections	*Pseudomonas aeruginosa,* others	Formation of biofilm in or on wound	Exposure of wound to microbes in environment; poor wound hygiene

causing damage to the kidneys. Some times *Corynebacterium diphtheriae* may multiply in the skin and affect the heart due to the toxin

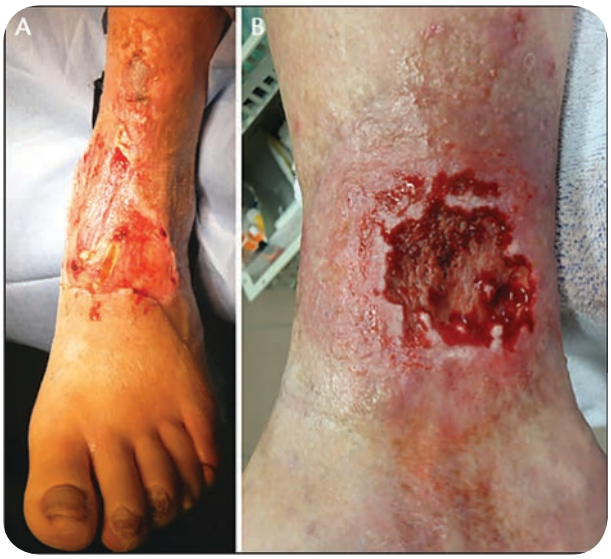


Figure 12.8: Ulcer formation

3. Sometimes organism may multiply in the skin and produce the toxin which affect the Central Nervous System (CNS) and the effects seen. In the case of *Clostridium tetani* infection, convulsions and paralysis occur due to the production of a powerful toxin.

12.3 Respiratory Tract Infections

With every breath, we inhale several microorganisms and therefore the respiratory system is a major portal of entry for pathogens. In fact, respiratory system infections are the most common type of infections and among the most damaging. Some pathogens that enter via respiratory route can infect other parts of the body, such as skin incase of measles, mumps and rubella.

The upper respiratory system has several anatomical defenses against airborne pathogens. Coarse hairs in the nose, filter large dust particles from the air. The nose is lined with a mucous membrane that contains numerous mucous secreting cells and cilia. The upper portion of the throat also contains a ciliated mucous membrane. The mucous moistens inhaled air and traps dust and microorganisms. The cilia help to remove these particles by moving them towards the mouth for elimination.

12.3.1 Structure of Respiratory Tract

The structure of respiratory tract is divided into two main parts viz: upper respiratory tract (URT) and lower respiratory tract (LRT).

Upper respiratory tract includes mouth, nose, nasal cavity, sinuses, throat or pharynx, epiglottis and larynx.

Lower respiratory tract includes trachea, bronchi, bronchioles, lungs and alveoli (Figure 12.9).

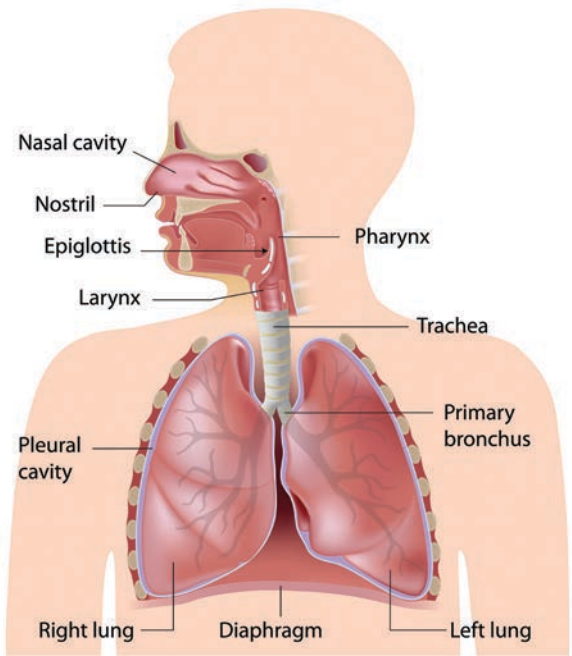


Figure 12.9: Structure of human respiratory tract

12.3.2 Normal Defenses against Infections

1. Arrangement of nose: There is no direct entry of air into (LRT).
2. Broncho constriction helps to trap the microorganisms.
3. Cough reflex expels the microbes out side.
4. Mucociliary blanket traps the organisms.
5. Mucosal factors: Kill the organisms by
   a. Non specific way
      i. Lysozyme: Cell wall of Gram positive organism are lysed
      ii. Influenza virus inhibitors do not allow the virus to multiply
      iii. Resident macrophages kill the organisms
   b. Specific manner

      Secretory IgA antibody: Forms first line of defense

Respiratory tract infection are divided into upper respiratory tract (URT) tract infection and lower respiratory tract (LRT) infection. Infection of the respiratory tract are listed in the Table 12.3.

URT: Infections are Sinusitis, Pharyngitis Laryngitis and Epiglotitis

LRT: Infections are Trachiitis, Tracheo bronchitis, Bronchitis, Alveolitis and Pneumonia (Figure 12.10).

Table 12.3: Microbial diseases of the respiratory system

Upper respiratory system		
Diseases	**Pathogen**	**Symptoms**
Bacterial diseases		
Epigottitis	*Haemophilus influenzae*	Inflammation of the epiglottis
Streptococcal pharyngitis (strep throat)	*Streptococci*, especially *Streptococcus pyogenes*	Inflamed mucous membranes of the throat;
Diphtheria	*Corynebacterium diphtheriae*	Bacterial exotoxin interferes with protein synthesis; damages heart, kidney, and other organs; membrane forms in throat; cutaneous form also occurs;
Otitis media	Several agents, especially *Staphylococcus aureus, Streptococcus pneumonia* and *Haemophilus influenza*	Accumulations of pus in middle ear build up painful pressure on eardrum
Viral diseases		
Common cold	Rhino virus	Familiar symptoms of coughing, sneezing, running nose.

(Continued)

Table 12.3: Microbial diseases of the respiratory system *(Continued)*

Lower respiratory system		
Bacterial diseases		
Pertussis (whooping cough)	*Bordetella pertussis*	Cilia in upper respiratory tract inactivated, mucus accumulates, spasms of intense coughing to clear mucus;
Tuberculosis	*Mycobacterium tuberculosis*	Tubercle bacilli entering lungs survive phagocytosis, reproduce in macrophages; tubercles formed to isolate pathogen; defenses eventually fail, and infection becomes systemic;
Viral diseases		
Respiratory syncytial virus (RSV)	Respiratory syncytial virus	A serious respiratory disease of infants;
Fungal diseases		
Blastomycosis	*Blastomyces dermatitidis*	Abscesses; extensive tissue damage;
Bacterial pneumonia		
Pneumococcal pneumonia	*Streptococcus pneumonia*	Infected alveoli of lung fill with fluids; interferes with oxygen uptake
Haemophilus influenzae pneumonia	*Haemophilus influenzae*	Symptoms resemble pneumococcal pneumonia

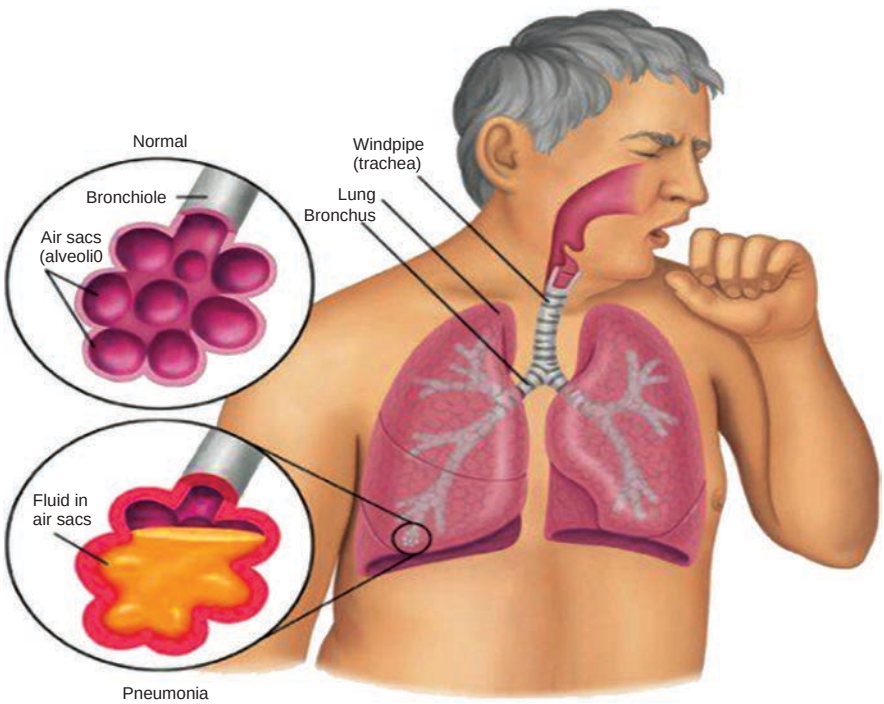


Figure 12.10: Diseased person with Pneumonia

12.4 Gastrointestinal Tract Infections

Human systems function by the energy produced from the digested food molecules. The food is swallowed through mouth and digested in the gastro intestinal tract. The food we consumed should be free of contaminations. The contaminated food causes gastrointestinal infections.

Through contaminated food and water the pathogens are ingested and they enter the GIT. In the small intestine they initiate an infection. Many times the pathogens that cause intestinal infections multiply in the GIT and produce their pathogenic effect in the intestine itself. Example: Shigellosis, Cholera.

The gastrointestinal tract (GIT) or alimentary canal includes the mouth, pharynx, throat, oesophagus (food Tube lead to the stomach), stomach, small and large intestine. It also includes accessory structures salivary glands, liver, gall bladder and pancreas lying outside the GIT. Secretions of these organs enhance the digestion of food molecules (Figure 12.11).

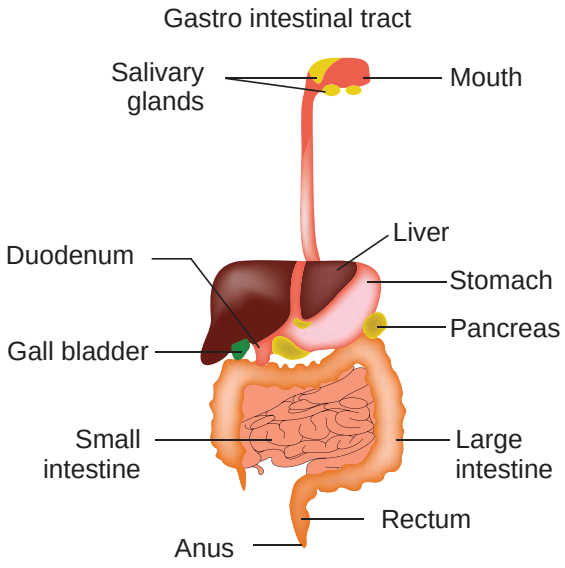


Figure 12.11: The structure of Gastro intestinal tract

Difference between infection and intoxication

Microbial diseases of digestive system are typically transmitted by a fecal oral route. Most such diseases result from the ingestion of food or water contaminated with pathogenic microorganisms or their toxins. These pathogens usually enter the food or water supply after being shed in the feces of people or animals infected with them.

- Each milliliter of saliva can contain millions of bacteria
- Stomach/small intestine has very few microorganisms because of hydrochloric acid present in the stomach.
- Large intestine harbours microbial population exceeding 100 billion of bacteria per gram of feces (40% fecal masses contain microbial cell material)
- Large intestine microbial population mainly contain anaerobes and facultative anaerobes.

After ingestion of pathogenic microorganisms, localization and multiplication of organisms takes place in the GIT and is called infection. Microorganisms may penetrate into intestinal mucosa and grow there or they may penetrate to other organs. Gastroenteritis is usually classified as either infection or intoxication. Food borne diseases can arise from either infection or intoxication. In both cases, bacterial toxins are typically responsible for producing disease signs and symptoms. In a food infection the microbial agent ingested

colonise in the gut and then produces toxins that damage host cells.

In case of food intoxication the toxins produced by bacteria in the food are ingested. Infection and intoxication differ in their onset of symptoms. Infections are characterized by a delay in the appearance of gastrointestinal disturbance until the pathogen increases in number or affects invaded tissue. Infection is correlated with onset of fever, one of the basic body's general responses to an infective organism. In case of intoxication, the symptoms are characterized by sudden appearance of gastrointestinal disturbances like cramping, nausea, vomiting or diarrhoea.

12.4.1 Microbial Flora of Gastrointestinal Tract

The stomach and gastrointestinal tract are not sterile and are colonized by the organisms that perform functions beneficial to the host, including the manufacture of essential vitamins. *Escherichia coli* found in the intestine help the body to produce vitamin K and *Bifodobacteria* can synthesizes vitamins such as vitamin B12, folate, and riboflavin. Humans cannot produce these vitamins. The normal flora changes according to the diet, age, cultural conditions and the use of antibiotics (Table 12.4).

12.4.2 Normal Defences against GI Tract Infections

High acidic nature of stomach acts as natural defensive mechanism. It eliminates potentially pathogenic microorganisms. Many bacteria like *Eschericha coli* and *Salmonella* survive in the acidic environment of stomach for an hour. Nitric oxide produced in the stomach by oxidation of ingested nitrates combines with the stomach acids and kills the bacteria in less than an hour. Lysozyme of saliva has antimicrobial properties. Small intestine contains important antimicrobial defences like specialized granule filled cells called paneth cells. These cells are able to phagocytose bacteria and also produce antibacterial proteins called defensins.

Globet cells secrete a gel forming mucin (major component of mucus), reduce the pathogens reaching the deeper tissues. Peyer's patches of ileum stimulates the host to secrete IgA antibodies (Figure 12.12).

HOTS

What is likely to happen to a child who drinks contaminated water?

Table 12.4: Normal flora of human gastrointestinal tract

At human birth	Stomach and intestine are sterile
Breast fed babies	*Lactobacillus bifidus*
Bottled milk fed babies	Enteric bacteria, *Lactobacillus* bifidus, *Enterococci, Clostridium* sp
Small intestine	*Lactobacilli, Enterococcus faecalis, Escherichia coli*
Large intestine	Anaerobic bacteria, *Streptococci, Bacteroides, Bifidobacteriumbifidum*

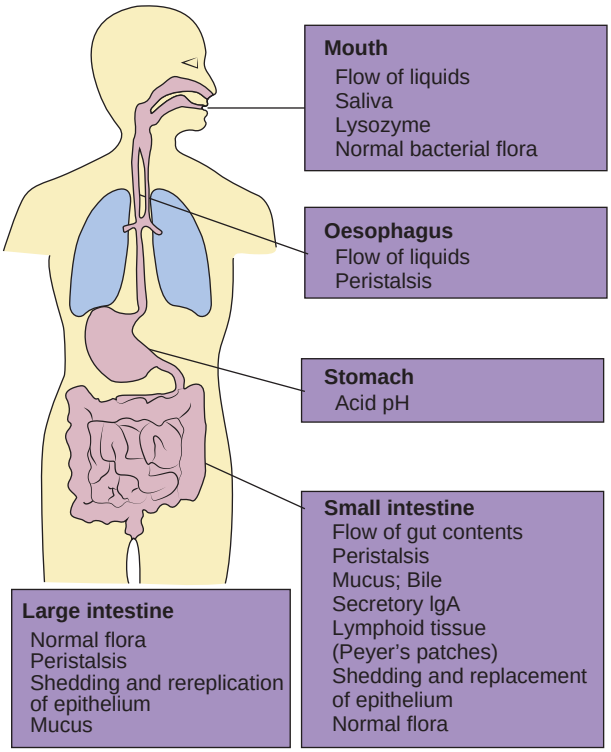
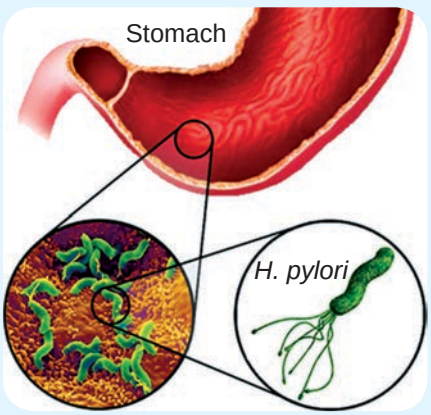


Figure 12.12: Natural defence mechanism in the Gastrointestinal Tract

Stomach is acidic because of the presence of hydrochloric acid. So in this acidic condition organisms generally not survive except one bacterium *Helicobacter pylori*. This bacterium is the leading cause of stomach ulcers. This bacterium has maximum evidence of correlation with the development of stomach and intestinal cancer.



12.4.3 Terms used in GIT Infections

Gastroenteritis: Inflammation of lining of stomach and intestine. It is a syndrome characterized by nausea, vomiting, diarrhea, abdominal discomfort.

Diarrhea: Condition in which feces, are discharged from the bowels frequently and in a liquid form.

Dysentery: Inflammatory disorder of the GIT associated with pus and blood in feces.

Gastritis: Inflammation of the stomach lining that results in swelling.

Enteritis: Inflammation of the intestinal mucosa

Colitis: Inflammation of the colon

Hepatitis: Inflammation of the liver

Enterocolitis: Inflamation involving the mucosa of both large and small intestine.

Peritonitis: Inflammation of peritoneum (it is the serous membrane that forms the lining of the abdominal cavity). Infections of digestive system are listed in Table 12.5.

Botulism is a special case of intoxication because, the ingestion of the preformed toxin affects the nervous system rather than GIT.

Infant Botulism is the infectious form of Botulism which results when spores of *Clostridium botulinum* swallowed colonise in the intestine. Botulism spores can be found in honey.

Table 12.5: Diseases of the digestive system

Infection	Pathogen	Symptoms
Bacterial Diseases		
Staphylococcal food poisoning	*Staphylococcus aureus*	Nausea, vomiting, and diarrhea
Shigellosis (bacillary dysentery)	*Shigella sp-*	Tissue damage and dysentery
Salmonellosis	*salmonella enterica*	Nausea and diarrhea
Typhoid fever	*Salmonella typhi*	High fever, significant mortality
Cholera	*Vibrio Cholerae*	Diarrhea with large water loss
Yersinia gastroenteritis	*Yersinia enterocolitica*	Abdominal pain and diarrhea, usually mild; may be confused with appendicitis
Viral Diseases		
Mumps	*Mumps virus Paramyxoviridae*	Painful swelling of parotid glands
Viral gastroenteritis	*Rotavirus*	Vomiting, diarrhea for 1 week
Fungal Diseases		
Ergot poisoning	*Claviceps purpurea*	Restricted blood flow to limbs; hallucinogenic
Aflatoxin poisoning	*Aspergillus flavus*	Liver cirrhosis; liver cancer

12.5 Ocular Infections

A number of microorganisms cause infection when introduced into the mucosa of the eye. In general, bacterial eye infections can lead to inflammation, irritation, and discharge, but they vary in severity. Some are typically short-lived, and others are chronic and lead to permanent eye damage. Prevention requires limiting the exposure to contagious pathogens. When infections do occur, prompt treatment with antibiotics can often limit or prevent permanent damage.

The external surfaces of the eye viz. the conjunctiva and cornea are susceptible to infection. These are exposed to external world and are easily accessible to infective agents. Particularly the conjunctiva is susceptible because it is covered with eyelid that provides warm, moist and enclosed environment in which contaminating organisms can quickly establish a focus of infection. However, eyelid and tears protect the external surfaces of the eye, both mechanically and biologically (Figure 12.13).

Factors that Protect the External Surfaces of the Eye

1. Eyelid gives mechanical protection to the surfaces 2. Tears (a) make the surfaces moist and prevent drying. (b) contains lysozyme- an enzyme that lyses the cell wall of Gram positive bacteria. (c) contains IgA antibodies that provide first line defense against viruses.

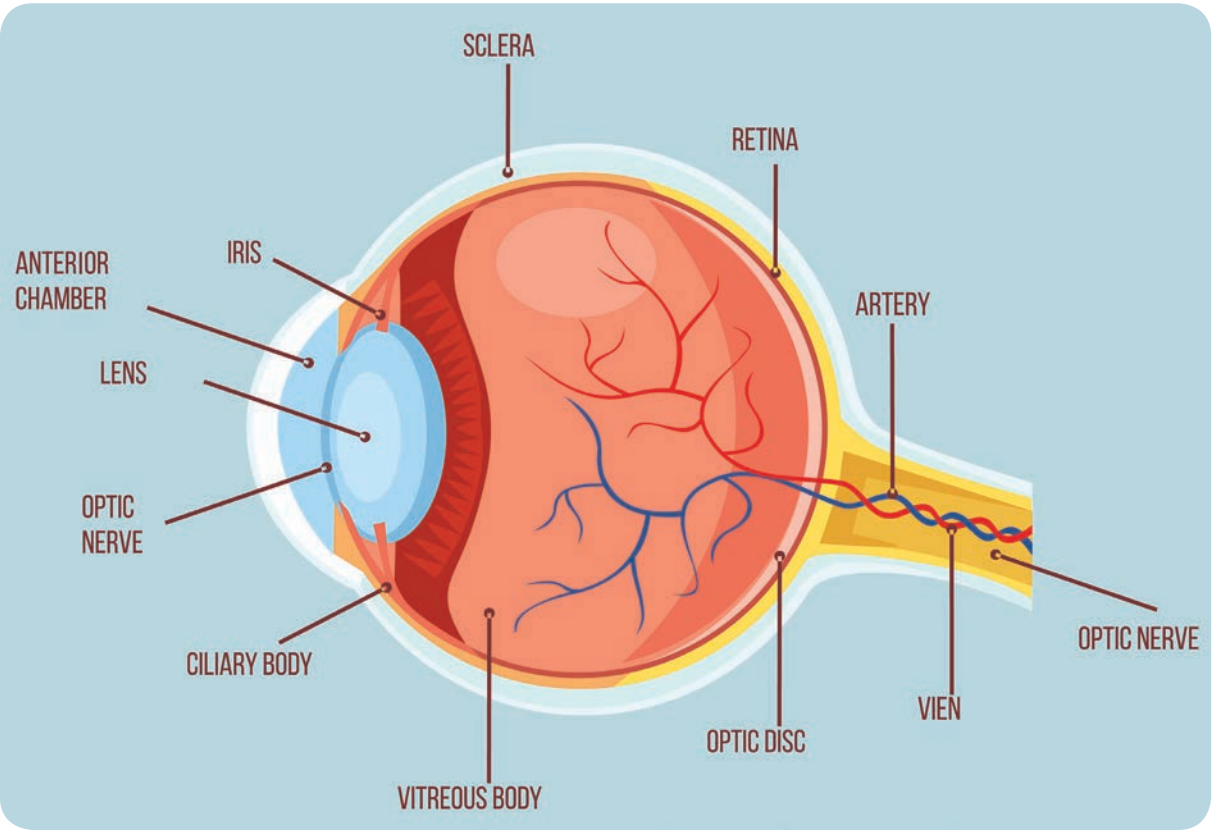


Figure 12.13: Structure and parts of eye

Infection of Eyelid

Most common cause of eyelid infection is *Staphylococcus aureus*.

Infection involves lid margins and cause blepharitis.

When the eyelid glands or follicles are affected stye (sticky eye) is seen (Figure 12.14).

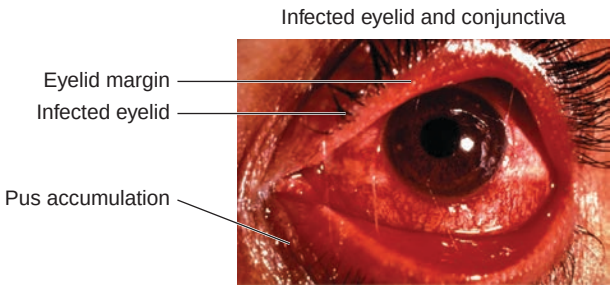


Figure 12.14: Infected eyelid and conjunctiva

Conjunctivitis (inflammation of conjunctiva) Conjunctivitis or pink eye can be caused by many different kinds of viruses and bacteria.

Trachoma

Trachoma, or **granular conjunctivitis**, is a common cause of preventable blindness that is rare in the United States but widespread in developing countries, especially in Africa and Asia. The condition is caused by the same species that causes neonatal inclusion conjunctivitis in infants, *Chlamydia trachomatis*. *Chlamydia trachomatis* can be transmitted easily through fomites such as contaminated towels, bed linens, and clothing and also by direct contact with infected individuals. *Chlamydia trachomatis* can also spread by flies that transfer infected mucous containing *Chlamydia trachomatis* from one human to another. Infections of eye are listed in Table 12.6.

Table 12.6: Bacterial infections of the eye

Disease	Pathogen	Signs and Symptoms	Transmission
Acute bacterial conjunctivitis	*Haemophilus influenza*	Inflammation of conjunctiva with purulent discharge	Exposure to secretions from infected individuals
Bacterial keratitis	*Staphylococcus epidermidis*, *Pseudomonas aeruginosa*	Redness and irritation of eye, blurred vision, sensitivity to light; progressive corneal scarring, which can lead to blindness	Exposure to pathogens on contaminated contact lenses
Neonatal conjunctivitis	*Chlamydia trachomatis*, *Neisseria gonorrhoeae*	Inflammation of conjunctiva, purulent discharge, scarring and perforation of cornea; may lead to blindness	Neonate exposed to pathogens in birth canal of mother with chlamydia or gonorrhea

12.6 Urinary Tract Infections

The urinary system is composed of organs that regulate the chemical composition and the volume of the blood excrete mostly nitrogenous wastes products and water. The urinary system consists of two kidneys, two ureters, a single urinary bladder and a single urethra. Wastes are removed from the blood as it circulates through the kidneys (Figure 12.15).

Infections of the kidney, ureter and bladder constitute Urinary Tract Infections (UTI). When infection occur in the kidney and ureter it is called upper urinary tract infections and bladder downwards is called lower urinary tract infections. Urinary tract infection is common in females than males. The urinary system normally contains few microbes but it is subjected to opportunistic infections that can be quite troublesome. Almost all such infections are caused by bacteria although occasional infections by pathogens such as parasites, protozoa and fungi also occured. Microorganisms invloved in UTI are listed in Table 12.7.

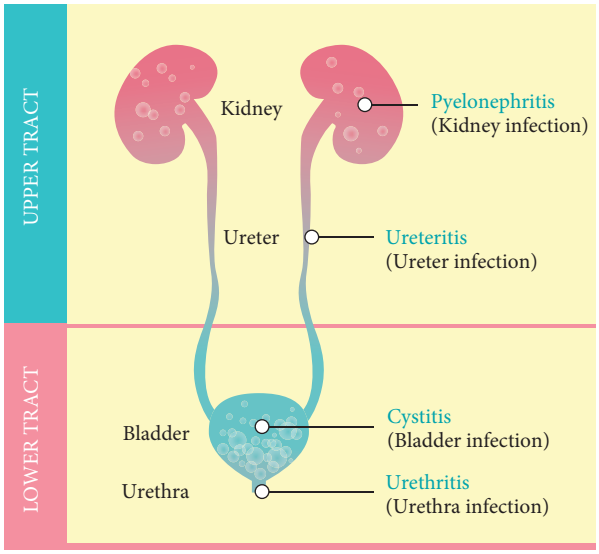


Figure 12.15: Structure of lower and upper urinary tract infection

12.6.1 Predisposing Factors for UTI

Urinary tract infection is common in females than in males. The urethra in females are shorter and wider and is

Table 12.7: Microorganisms involved in UTI

Microorganisms	Examples
Bacteria (most common)	*Escherichi coli, Klebsiella, Enterobacter, Proteus*
Viruses	Adenovirus, Mumps
Fungi	*Trichomonas vaginalis, Schistosoma haematobium*
Parasites	*Candida*

less effective in preventing the bacteria entering the bladder. Sexual intercourse is a predisposing factor in females. High incidence is seen in pregnant women due to hormonal changes and impairment of urine flow due to pressure on urinary tract.

Infobits

Many of the bacteria which cause UTI's have developed resistance to antibiotics. Probiotics are a great defence in such cases. Research has turned to probiotic (*Lactobacillus*) strain that have demonstrated the best result. This particular probiotic bacteria also stimulates immune function, lowers acidity levels in the urinary tract, and discourages the growth of UTI causing organisms.

12.6.2 Urinary Tract Infection caused by *Escherichia coli*

Escherichia coli is the predominant cause of UTI.

It is a normal flora of the gut and can cause extra intestinal infections (UTI, Wound infection.) UTI (it can also be involved in other infections like wound infection peritonitis) UTI is common in (a) married women (b) elderly men with prostate enlargement.

Pathogenesis of cystitis in woman

Bladder infections can result from the downward migration of organisms from an infected kidney. But majority arise by ascent of pathogens from the rectum and vagina to the urethra meatus and bladder, leading to cystitis. If left untreated, the infection can further ascend to involve the kidneys (pyelonephritis) (Figure 12.16).

The rectum and vagina function as the reservoir of bacteria for sporadic infections

In men, the longer urethra is believed to protect against ascending infections.

When *Escherichia.coli* (and other Gram Negative rods) causes UTI, usually the number of organisms in freshly passed urine is more than 100,000 organisms/ml.

This is called "significant bacteriuria". Counts less than this is associated with contaminants from urethra or externalia. Infection of urinary tract are listed in Table 12.8.

Obesity increases the risk of UTI's in men. A 2013 study examineed how obesity affected the chance of developing UTI and it was found that obese men were twice more likely to develop the UTI than obese women.

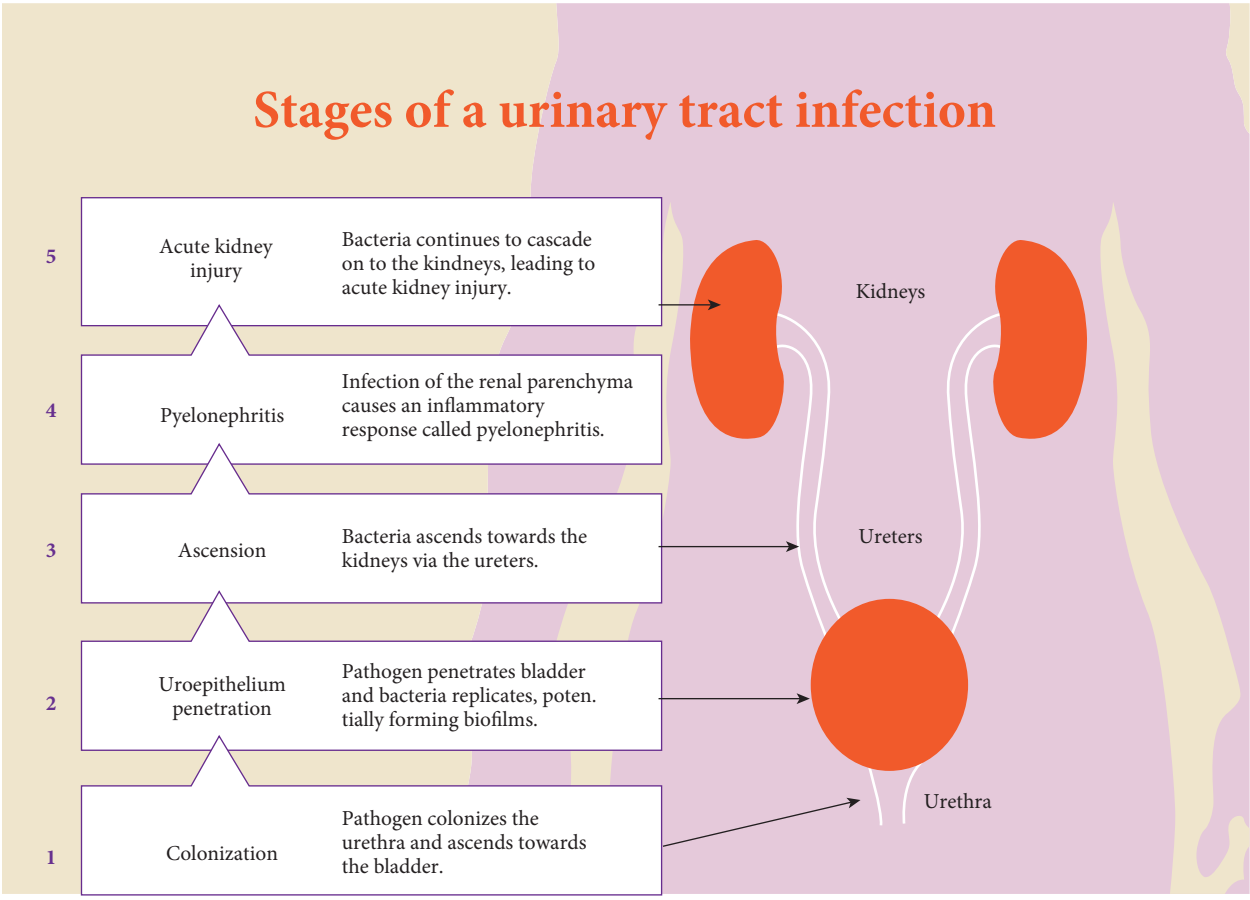


Figure 12.16: Various stages of a urinary tract infection

Table 12.8: Microbial Diseases of the Urinary System

Disease	Pathogen	Symptoms
Bacterial Diseases of the Urinary system Cystitis (Urinary bladder infection)	*Escherichia* coli, *Staphylococcus saprophyticus*	Difficulty or pain in urination
Pyelonephritis (Kidney infection)	Primarily *Escherichia coli*	Fever; back or flank pain
Leptospirosis (Kidney infection)	*Leptospira interrogans*	Headaches, muscular aches, fever; kidney failure a possible complication

12.7 Reproductive Tract Infections

EU9PUE

Reproductive tract infections are caused by organisms normally present in the reproductive or genital tract or introduced from the outside during sexual contact or medical procedures. It occur both in men and women. Based on mode of infection reproductive tract infections are classified into three types:

1. Sexually Transmitted Disease

It is caused through means of sexual contact. Examples: Chlamydia, Gonnorhea, Chancroid, and Acquired Immuno Deficiency Syndrome (AIDS).

2. Endogenous Infections

These are caused by the overgrowth of organisms normally present in the genital tract of healthy women. Example: Bacterial Vaginosis or Vulvo Vaginal Candidiasis.

3. Iatrogenic Infections

These infections are associated with improperly performed medical procedures such as unsafe abortion or poor delivery practices. The endogenous organisms in the vagina or sexually transmitted organisms in the cervix may be transferred during a transcervical procedure into the upper reproductive tract and cause serious infections of the uterus, fallopian tubes, and other pelvic organs.

In men reproductive tract infections transmitted by sexual contact are much more common than by endogenous or iatrogenic reproductive infections. In women reproductive infections spread through non sexual routes are usually more common.

12.7.1 Mode of Transmission

Reproductive tract infections are caused by pathogenic bacteria, parasite, virus. It is mainly caused by pathogens entering into the body through the mucous membranes during unprotected vaginal, oral, anal intercourse with an infected partner. In developing countries bacterial infections like Gonorrhoea, Chlamydia, Syphilis, Bacterial Vaginosis, Lymphogranuloma Venereum, Trichomoniasis, Chancroid, and viral infections caused by Human Papilloma Virus, Hepatitis B Virus, Herpes Simplex Virus, Human Immunodeficiency Virus are very common.

12.7.2 Normal Flora of Reproductive Tract

Mycobacterium smegmatis, a harmless commensal found in the smegma of the genitalia of both men and women. (Figure 12.17 & 12.18). In nomal men aerobic and anaerobic bacteria,

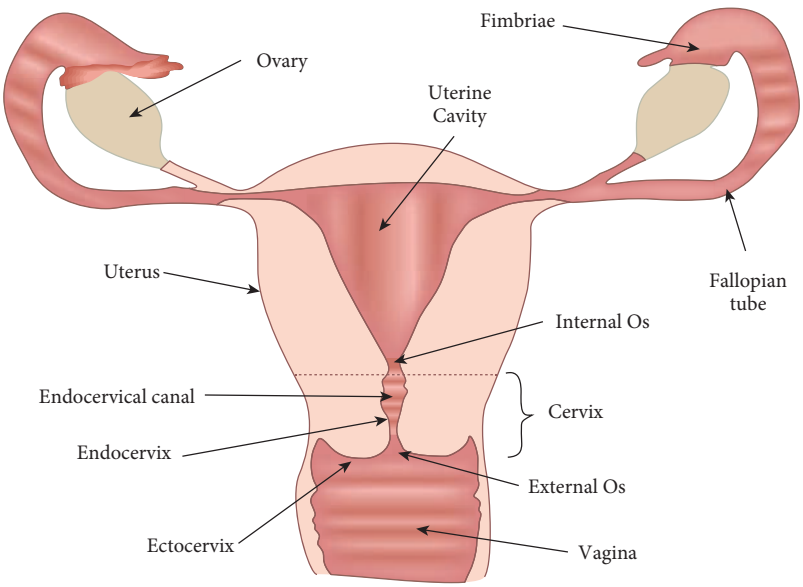


Figure 12.17: Female reproductive system

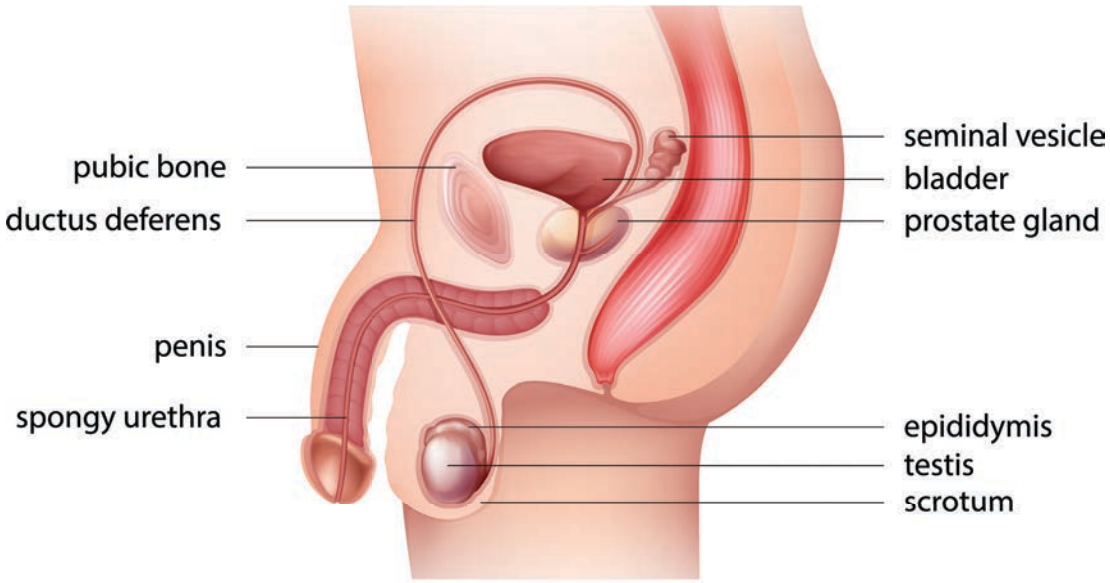


Figure 12.18: Male reproductive system

lactobacilli, alpha haemolytic *Streptococci, Chalmydia trachomatis* and *Ureaplasma urealyticum* may also be present.

The adult female genital tract has a very complex microflora. The character of the population changes with the variation of the menstrual cycle. Mostly the predominant bacteria are acid tolerant *Lactobacilli*. Glycogen is accumulated in the vaginal wall due to ovarian hormonal activity. The breakdown of glycogen by the lactic acid bacteria (Doderlien's bacillus) leads to the formation of acidic pH (4.4-4.5). This acidic nature prevents the vagina from bacterial vaginosis and yeast infections. However before puberty and after menopause there is no glycogen formation. The normal flora during this period contain normal skin microorganisms. The vaginal pH is mild alkaline. The normal vaginal flora often includes *Listeria,* anaerobic *Streptococci, Mycoplasma, Gardnerella vaginalis, Neisseria, Spirochetes, Candida, Staphylococcus epidermidis.*

12.7.3 Pathogenesis

After the entry of pathogenic organisms, with sufficient incubation time, symptoms are clearly manifested in the affected individual. The most common symptoms include unusual vaginal discharge, penile discharge, pelvic pain, itching, abnormal or heavy vaginal bleeding, rashes, warts, lesions, burning or pain during urination. However most of the infections are asymptomatic, which act as a effective control of reproductive tract infections. Diseases of reproductive system are listed in Table 12.9.

Infobits

Tamilnadu has AIDS testing centres at all district head quarters with more than 55 Anti Retroviral Therapy(ART) centres and 750 (ICTC)-Integrated (voluntary) and confidential counselling and testing centres under the national AIDS control programme at district level government hospitals and medical colleges across the state.

Table 12.9: Microbial diseases of the reproductive system

Disease	Pathogen	Symptoms
Bacterial Diseases		
Gonorrhea	*Neisseria gonorrhoeae*	Painful urination, discharge of pus in males, abnormal vaginal discharge in females
Nongonococcal urethritis (NGU)	*Chlamydia trachomatis* or other bacteria, including *Mycoplasma hominis* and *Urea plasma urealyticum*	Painful urination and watery discharge, Chronic abdominal pain in females
Syphilis	*Treponema pallidum*	Initial sore at site of infection, later skin rashes and mild fever; final stages may be severe lesions, damage to cardiovascular and nervous systems.
Lymphogranuloma venereum (LGV)	*Chlamydia trachomatis*	Swelling in lymph nodes in groin
Viral Diseases		
Genital Herpes	*Herpes simplex* virus type 2; HSV type 1	Painful vesicles in genital area
Genital warts	Human papilloma viruses	Warts in genital area
AIDS	Human Immunodeficiency virus (HIV)	loss of appetite, weight loss, persistent cough, attack on T cells (immunocompromise), easily prone to fungal and other bacterial pathogens as secondary opportunistic infections.
Fungal Diseases		
Candidiasis	*Candida albicans*	Severe vaginal itching, yeasty odor, yellow discharge
Protozoan Diseases		
Trichomoniasis	*Trichomonas vaginalis*	Vaginal itching, greenish yellow discharge

12.8 Infections of the Nervous System

Some of the most devastating infectious diseases are those that affect the nervous system, especially the brain and the spinal cord. Damage to these areas can lead to deafness, blindness, learning disabilities, paralysis and death. Microbial infections of CNS are infrequent but often have serious consequences. In pre antibiotic times, they were almost always fatal. An infection of CNS can be life threatening condition, especially for children with weakened immune system. These infections need quick diagnosis and immediate treatment by an infectious disease specialist. Bacteria, Fungi and viruses are the most common causes of CNS infections.

12.8.1 Structure of Nervous System

The human nervous system is organized into two divisions: The Central Nervous System (CNS) and Peripheral Nervous System (PNS). The Central Nervous System (CNS) consists of brain and spinal cord. It controls most functions of the body and mind. The peripheral nervous system (PNS) consists of all the nerves that branch off from the brain and spinal cord. These peripheral nerves are the lines of communication between the CNS, the various parts of the body and the external environment (Figure 12.19).

Brain and spinal cord are covered by three layers of membranes called meninges. These layers are the outermost dura mater, the middle arachnoid mater, and the innermost pia mater. Between the pia mater and arachnoid membranes is a space called the subarachnoid space, in which there is cerebrospinal fluid (CSF) circulating.

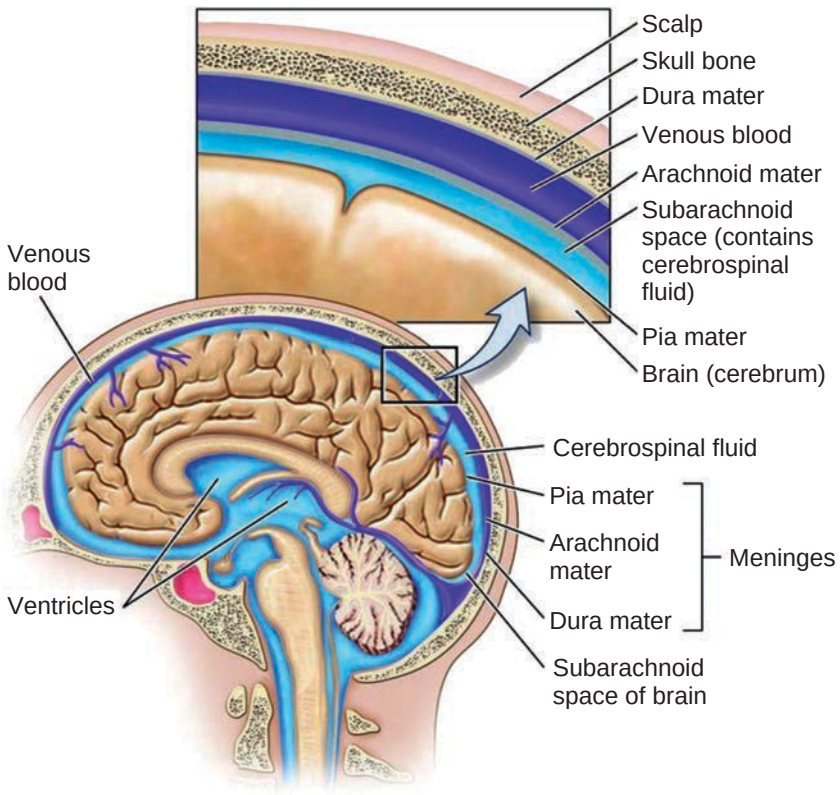


Figure 12.19: Structure of central nervous system

12.8.2 Barriers of CNS

Dyes such as Trypan blue injected into the systemic circulation stain virtually all tissues, with the exception of the brain and spinal cord. This blood brain barrier excludes most macromolecules, microorganisms, immunocompetent cells and antibodies. Even pathogens that are circulating in the bloodstream usually cannot enter the brain and spinal cord because of blood brain barrier. Certain capillaries permit some substances to pass from the blood into the brain but restricts others. These capillaries are less permeable than others within the body and are therefore more selective in passing materials (Figure 12.20). The blood brain barrier (Figure 12.21) is due to the cellular configuration of cerebral capillaries, the choroid plexus and arachnoid cells. It acts as a natural barrier that prevents the invasion of microorganisms into the brain. If this is breached organisms enter the brain. The blood CSF barrier (Figure 12.22) (also calle brain CSF barrier) consists of endothelium with fenestrations, and tightly joined choroid plexus epithelial cells. It acts as a natural barrier that prevents the invasion of microorganisms into the meninges.

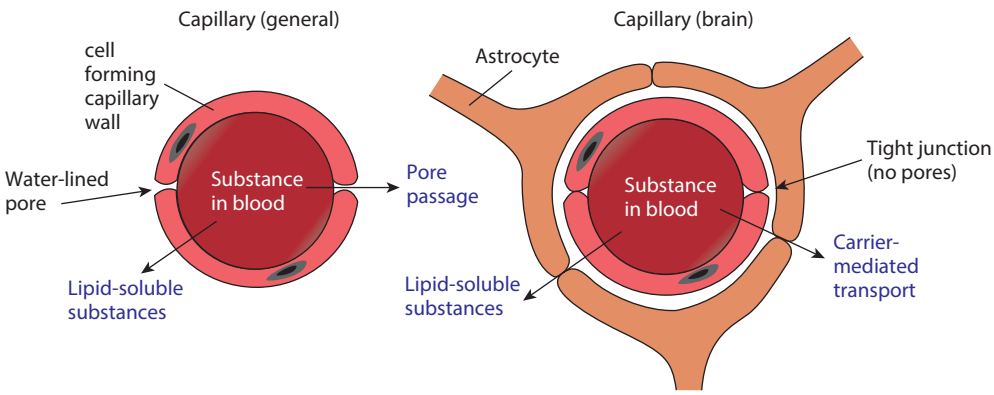


Figure 12.20: Capillaries of brain

Drugs cannot cross the blood brain barrier unless they are lipid soluble. Glucose and many amino acids are not lipid soluble, but they can cross the barrier through special transport systems. The lipid soluble antibiotic Chloramphenicol enters the brain readily. Penicillin is only slightly lipid soluble, but, if it is taken in very large doses, enough may cross the barrier to be effective. Inflammations of the brain tend to alter the blood brain barrier in such a way as to allow antibiotics to cross that would not be able to cross if there were no infection.

Antibodies found in the normal CNS are derived from the serum and are present at low levels compared to serum levels. There are a few phagocytic cells and complement is also largely excluded. CSF is especially vulnerable because it lacks many of the defenses found in the blood, such as phagocytic cells. It is not easy for the microorganisms to enter CNS but it hampers their clearance once it is penetrated.

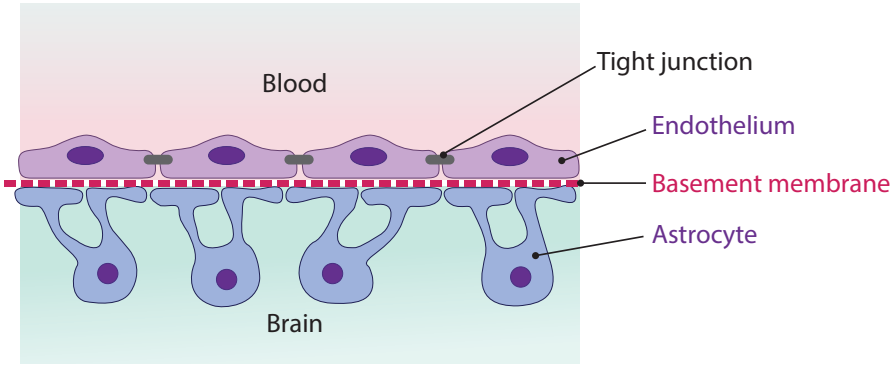


Figure 12.21: Blood brain barrier

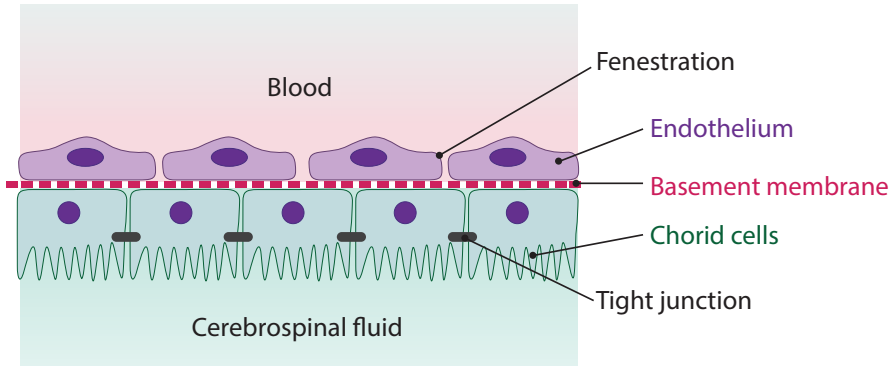


Figure 12.22: Blood CSF barrier

12.8.3 Routes through which microorganisms enter nervous system

- Skull or bone fractures
- Medical procedures
- Peripheral nerves
- Blood or lymph

12.8.4 Clinical Manifestations of Nervous System Infections

Some of the symptoms of nervous System infections are headache, fever, stiff neck, focal signs, seizures, confusion, weakness, hallucinations, stupor, coma, abnormal behavior and sleep disorder

12.8.5 Infections of Nervous System

- Meningitis is an inflammation of the meninges (membrane covering the brain). Meningitis is a diffuse infection caused by a variety of different agents.
- Encephalitis is defined as inflammation of the brain. Unlike an abscess, which is a localised area of bacterial or fungal growth, Encephalitis is usually due to viruses that produce more widespread intracellular infections.
- Brain abcess is a focus of purulent infection and is usually due to bacteria. Brain abscesses develop from either a contiguous focus of infection (such as the ears, the sinuses, or the teeth) or hematogenous spread from a distant focus (such as the lungs or heart, particularly with chronic purulent pulmonary disease, subacute bacterial endocarditis, or cyanotic congenital heart disease). In many cases the source is undetectable.

Etiological agents of Meningitis

This can be caused by a wide range of microorganisms and can be classified as pyogenic and non pyogenic meningitis. In pyogenic meningitis infiltration of pus cells (neutrophils) will be seen. In Non pyogenic or aseptic meningitis infiltration of lymphocytes may be seen. Diseases of nervous system are listed in Table 12.10.

12.9 Systemic Infections

An infection that is in the bloodstream is called a systemic infection. Systemic diseases such as flu and typhoid affect the entire body. Bacteria can enter the circulatory and lymphatic systems through acute infections or breaches of the skin barrier or mucosa. Breaches may occur through fairly common occurrences, such as insect bites or small wounds. Even the act of tooth brushing, which can cause small ruptures in the gums may introduce bacteria in to the circulatory system. In most cases, the Bacteremia result from such common exposure is transient and remains below the threshold of detection. In severe cases, bacteremia can lead to septicemia with dangerous

Table 12.10: Microbial diseases of the Nervous system

Diseases	Pathogen	Portal of Entry	Method of Transmission
Bacterial Diseases			
Haemophilus influenzae meningitis	*Haemophilus. Influenzae*	Respiratory tract	Endogenous infection; aerosols
Meningococcal meningitis	*Neisseria meningitidis*	Respiratory tract	Aerosols
Pneumococcal meningitis	*Streptococcus pneumoniae*	Respiratory tract	Aerosols
Tetanus	*Clostridium tetani*	Skin	Puncture wound
Botulism	*Clostridium botulinum*	Mouth	Food borne intoxication
Viral Diseases			
Poliomyelitis	Poliovirus	Mouth	Ingesting contaminated water (fecal oral route)
Rabies	Lyssavirus, including rabies virus	Skin	Animal bite
Fungal Diseases			
Cryptococcosis	*Cryptococcus neoformans*	Respiratory route	Inhaling soil contaminate with spores
Protozoan Diseases			
African trypanosomiasis	*Trypanosoma brucei Rhodesiense, Trypanosoma brucei gambiense*	Skin	Tsetse fly

complication such as Toxemia sepsis and Septic shock. In these situations, it is often the immune response to the infection that result in the clinical signs and symptoms rather than microbes themselves.

Summary

In the branch of medical microbiology we discussed about prevention, diagnosis and treatment of infectious diseases. Infections are acquired through contact, inhalation, ingestion, inoculation and congenital. Sources of infections are endogenous and exogenous in origin. Normal flora are organisms present in certain areas of the body. Infectious diseases may be generalised or localised. Based on the occurrence of infectious diseases the infection may be epidemic, endemic, or sporadic. There are various virulence factors which are responsibility for the pathogenicity.

Skin is the first line of defence against pathogen. Normal uninterrupted skin provides protection against "invasion by bacteria". Many exogenous and endogenous factors are responsible for wound infections. The mechanism of damage may be in the skin or some cases its spreads to the internal organs and CNS system.

Respiratory system of both lower and upper is the major path for entry of pathogens. The infections of upper respiratory tract are sinusitis, pharyngitis, laryngitis and epiglottitis. The infection of lower respiratory tract are trachitis, tracheobronchitis, bronchitis, and alveolitis.

Gastrointestinal tract infections are infections of the digestive system. The food borne infection and food intoxication are the common cause of gastroenteritis. The gut flora and natural defence mechanism by defensins bacteriocins, globet cells, IgA antibodies protect the individual against pathogenic infection. Diarrhea, dysentery, vomitting are the common symptoms of GIT. Oral rehydration therapy, proper hygiene to be manifested to reduce the risk of gastroenteritis.

The external structure and parts of the eye are easily susceptible to infections. The eyelids, tears, lysozyme, IgA are the natural defence against infections. Conjunctivitis and Trachoma are the common eye diseases. Proper diagnosis and treatment should be suggested.

Uninary tract infections are more common in females than in males. There are many predisposing factors making female prone to the infections. The predominant causative agents in urinary tract infection is *Escherichia.coli*. The number of organisms in freshly passed urine is more than 100,000 organisms/ml. It is called significant bacteriuria.

The infections spread through reproductive tract by direct contact is called sexually transmitted disease. Mostly these infections are asymptomatic in women.

Nervous system infection affect brain and spinal cord. They are of two types meningitis and encephalatis. An infection that is in the blood stream is called systemic infections. Systemic diseases like flu and typhoid affect the entire body.

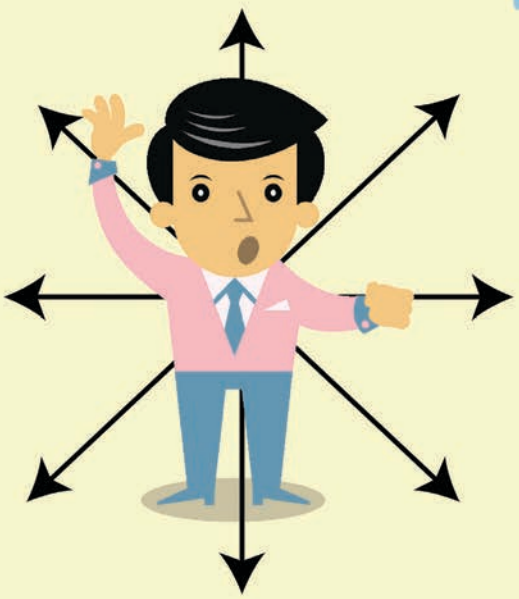
Modes of Infection

Inoculation

Indirect contact

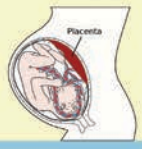
Infected Needle

Direct contact



Dog bite

Food and Drinks



Congenital

Sneezing

ICT CORNER

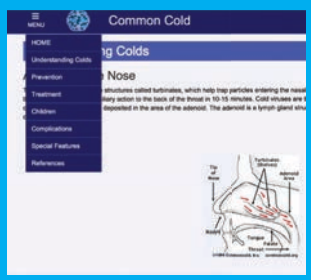
Respiratory Tract Infections

Know the myths of cold

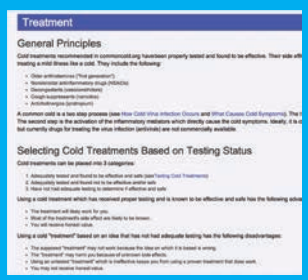
STEPS:

- Use the link or scan the QR code given below. "Cells Alive" home page will open. You can select any topic you wish. For example click "understanding colds"
- "Understanding Colds" page will open. You can go through anatomy of the nose, CAT scan view etc..
- At the top left of the page click on "Menu" and select "Treatments" and analyze.
- Also select "Special features" and go through the topic. Also you can select how penicillin kills bacteria in the "Cells Alive" page, and know the action of penicillin against bacteria.

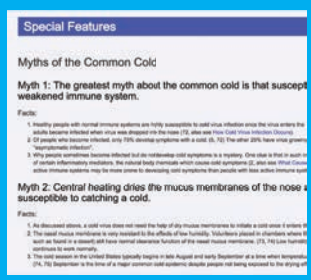
Step1



Step2



Step3



Step4

URL:

https://www.cellsalive.com/toc_micro.htm

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Evaluation

Multiple choice questions

1. Syphilis is _____ disease
   a. Sexually transmitted disease
   b. Respiratory tract disease
   c. Gastro tract disease
   d. Urinary tract disease
2. _____ is the person who harbours the pathogenic microorganisms and suffers from till effect because of it?
   a. Carrier
   b. Healthy carrier
   c. Patient
   d. All the above
3. Circulation of bacteria in the blood is known as _____
   a. Septicimia
   b. Pyemia
   c. Bacterimia
   d. None of the above
4. Stratum corneum is seen in which of the following?
   a. Dermis
   b. Subcutaneous tissue
   c. Muscle
   d. Epidermis*
5. Which of the following is the structure present in epidermis?
   a. Stratum mucosum
   b. Stratumlucidum*
   c. Stratum granulosum
   d. Stratum germinativum
6. Dermis contains which of the following?
   a. Stratum germinativum
   b. Stratum granulosum
   c. Stratum mucosum
   d. All the above*
7. Adipose tissue is present in which layer of the skin
   a. Epidermis
   b. Dermis
   c. Hypodermis *
   d. All the above
8. Which are all the cells present in the epidermis of the skin?
   a. Merkel cells
   b. Keratinocytes
   c. Melanocytes
   d. All the above *
9. Sense of heat and touch is provided by the nerves present in which of the following?
   a. Epidermis
   b. Dermis*
   c. Muscle
   d. Adipose tissue
10. Keratinocytes are present in Dermis (1).
    Epidermis does not contain blood vessels (2). Which of the above statements is/are true?
    a. 1 only
    b. 2 only*
    c. Both 1&2
    d. Neither 1 nor 2
11. From the skull down to the brain, select the arrangement of layers of meninges from the following:
    a. Dura mater/Arachnoid mater/Pia mater *
    b. Arachnoid mater/Dura mater/Pia mater

c. Pia mater/Arachnoid mater/Dura mater
d. Dura mater/Pia mater/Arachnoid mater

12. Cerebrospinal fluid (CSF) is present in which of the following?
    a. Perivascular spaces
    b. Sub arachnoid space *
    c. Between skull and dura mater
    d. Sub dural space
13. Which of the following organisms, notincluded in causing pyogenic meningitis?
    a. Staphylococcus aureus
    b. Streptococcus pyogenes
    c. Neisseria meningitidis
    d. Mycobacterium tuberculosis
    e. Leptospira (ser.var) icterohaemorrhagiae
14. Which of the following organisms, notincluded in causing aseptic meningitis?
    a. Staphylococcus aureus
    b. Streptococcus pyogenes
    c. Neisseria meningitidis
    d. Mycobacterium tuberculosis
15. _____ antibody gives first line defense against respiratory tract infections.
    a. IgM
    b. IgA
    c. IgD
    d. IgE
16. The nose is lined with _____ membrane.
    a. Mucous
    b. Epithelial
    c. Secretion
    d. None of these
17. _____ nature of stomach act as a natural defense mechanism.
    a. Acidic
    b. Neutral
    c. Alkaline
    d. None of the above
18. Traveller"s diarrhea is caused by
    a. *Escherichia coli*
    b. *Staphylococcus aureus*
    c. *Vibrio cholerae*
    d. All the above
19. _____ is the predominant cause of UTI?
    a. *Staphylocous aureus*
    b. *Escherichia coli*
    c. *Salmonella*
    d. *Streptococcus pyogenes*
20. _____ fungi involved in urinary tract infection?
    a. *Klebsiella*
    b. *Candida sp*
    c. *Penicillium*
    d. *Escherichia coli*
21. During the breakdown of glycogen by lactobacilli in the vagina, makes vaginal pH as _____.
    a. Acidic
    b. Neutral
    c. Alkaline
    d. None of the above
22. In typhoid, organisms are acquired through which of the following routes?
    a. Oral*
    b. Respiratory

c. Skin

d. Blood transfusion

23. Which of the following is the causative agent of typhoid?
    a. Salmonella enteritidis
    b. Salmonella typhimurium
    c. Salmonella typhi*
    d. All the above

Answer the following

1. Define congenital infection?
2. What is meant by nosocomial infection?
3. Define the term bacteremia, septicemia pyremia?
4. Explain mode of transfer of infection?
5. Define a wound.
6. What are the causes of wound?
7. Name two types of CNS infections.
8. Give the names of the etiologic agents of wound infection.
9. State the defenses of skin organisms against the bacterial invasion.
10. Describe the factors responsible for wound infections.
11. List out the pathogen that causes Otitis media?
12. List out the normal defense against Respiratory tract infections?
13. Describe microbial disease of upper respiratory tract infection?
14. Define gastroenteritis and enterocolitis?
15. State the difference between dysentery and diarrhoea?
16. What is the difference between food borne infection and intoxication?
17. Give the normal flora of the gastrointestinal tract of humans?
18. Explain the various pathogenic bacteria involved in gastroenteritis?
19. What is called significant bacteriuria?
20. Explain the predisposing factors for urinary tract infection?
21. List out the organisms causing urinary tract infections?
22. Define iatrogenic infection.
23. Explain the role of lactobacilli in the prevention of bacterial vaginosis.
24. Give detailed study of various bacterial, fungal and viral infectious diseases of reproductive tract infection.
25. Give the list of agents causing typhoid and paratyphoid.

Student Activity (1)

1. Get information from your parents/neighbor about types of diseases one gets due to contamination. Example: If you drink contaminated water, you get diarrhoea.

No	After certain activity	Getting a disease	Preventive method to advocate
1	Contaminate drinking water	Diarrhoea	Don't drink or Boil, cool and drink
2			
3			
4			
5			

2. Give a list of organisms present as normal flora of the skin (include other than that is given in the text book).

3. Prepare model of respiratory tract with innovations.

   Prepare a list of URT infections with the etiologic agents and prevention.
   Observe a chronic smoker. He coughs very often. List out the reasons for his cough.
   collect information from nearby neighbors kids (10). How many of them are immunized DTP vaccinated?

No	Kid's name	DOB	Immunized on	Where corporation or pvt
1				
2				
3				

4. Student is asked to prepare a model of GIT with their innovations.
   See for example:
   What all the organisms that can be transmitted through the fly contaminated food. Give a list.
5. (1) Write an assignment on Madras eye (conjunctivitis due to viruses)
   (2) Write Dos and Don's when a dust particle comes into your eye.
6. 1) Draw the structure of urinary tract in a chart board using your innovation. Label the parts (make a poster presentation material with flow of urine from kidney to urethra).
7. Prepare a chart showing all sexually transmitted diseases.

   Collect the disease photographs from the net.
8. Write a chart showing differences between pyogenic and aseptic meningitis.

Chapter 13

Immunology

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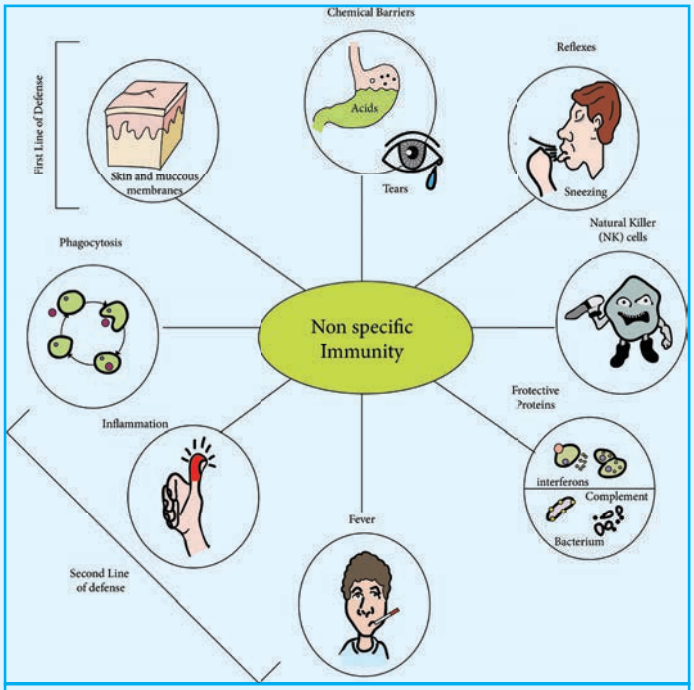
Chapter Outline



Non specific immunity or Innate immunity has four types of defense barriers namely anatomical barriers, chemical barriers, phagocytic barriers and inflammatory barriers.

Learning Objectives

After studying this chapter the student will be able,

- *To gain knowledge on the history of immunology and Know the Nobel prize winners in immunology.*
- *To know the structure and functions of primary and secondary lymphoid organs.*
- *To know the cells of immune system and understand the role of granulocytes, mast cells, macrophages, dendritic cells and lymphocytes.*
- *To define immunity and Differentiate between innate immunity and acquired immunity.*
- *To understand the properties of antigen.*
- *To describe the basic structure and function of immunoglobulin (antibodies).*
- *To explain the mechanism of antigen-antibody interactions and their applications in clinical laboratory.*

13.1 Historical Background

Immunology is the study of immunity to diseases. Immunology began as a branch of Microbiology. Its origin is usually attributed to **Edward Jenner** who introduced variolation in 1796.

The success of vaccination enabled the World Health Organization to announce in 1979 that small pox had been eradicated. Late in 19th century **Robert Koch** proved that infectious diseases are caused by microorganisms. The discoveries of Koch stimulated the extension of Jenner's strategy of vaccination to other diseases.

Pasteur used attenuated culture and called it **vaccine** (Latin *vacca*, cow) in honour of Edward Jenner. Table 13.1 and Figure 13.1 list and shows the scientist who contributed to the field of immunology.

Table 13.1: Scientists and their contributions to immunology

Year	Name of the Scientists	Contributions to immunology
1796	Edward Jenner	Discovered that cowpox or vaccinia, induced protection against human small pox
Discovery of humoral and cellular immunity		
1890	Von Behring and Kitasto (von Behring earned the Nobel Prize in medicine in 1901)	Gave the first insights into the mechanism of immunity
1930's	Kabat	Showed that gamma - globulin (now immunoglobulin) a fraction of serum exhibited the active component of immunity
1883	Elie Metchnikoff	He observed that certain white blood cells, which he termed **phagocytes**, were able to ingest microorganisms and other foreign material
1903	Sir Almoth Wright	Reported that antibodies could aid in the process of phagocytosis. Wright called these antibodies '**opsonins**'
1996	Claman, Chaperon and Triplett	Discovered the presence and cooperation of B cells and T cells

(Continued)

Year	Name of the Scientists	Contributions to immunology
Specificity of immune response		
Around 1900	Jules Bordet	Demonstrated that nonpathogenic substances, such as red blood cells from other species, could also serve as antigens
	Karl Landsteiner	Showed that injecting an animal with almost any non-self, organic chemical could induce production of antibodies that would bind specifically to the chemical
Molecular immunology		
1959	Porter	Separated fragments of immunoglobulin
	Edelman	Heavy and light chains of antibodies were separated by him
1965	Putnam, Hirschmann and Craig	Discovered constant and variable regions of immunoglobulin
1979	Kung et al.	Described the first monoclonal antibody identifying a T cell subset
1982-83	Allison et al and Haskins et al.	Isolated T cell receptor
Immunogenetics and Genetic Engineering		
1936	Gorer	Discovered the major histocompatibility antigens
1968	McDevitt and Tyan	Showed that immune response genes were linked to the genes of the major histocompatibility complex (MHC)
1974	Doherty and Zinkernagel	Reported that recognition of antigen by T cells was restricted by MHC molecules
1978	Tonegawa et al.	Demonstration of immunoglobulin gene rearrangement

Infobits

Nobel Prizes for immunologic research

Year	Recipient	Country	Research
1908	Elie Metchnikoff Paul Ehrlich	Russia Germany	Role of phagocytosis (Metchnikoff) and antitoxins (Ehrlich) in immunity
1913	Charles Richet	France	Anaphylaxis
1919	Jules Bordet	Belgium	Complement-mediated bacteriolysis
1930	Karl Landsteiner	United States	Discovery of human blood groups
1972	Rodney R. Porter Gerald M. Edelman	Great Britain United States	Chemical structure of antibodies
1977	Rosalyn R. Yalow	United States	Development of radioimmunoassay
1980	George Snell Jean Dausset Baruj Benacerraf	United States France United States	Major histocompatibility complex
1984	Cesar Milstein George E. Kohler	Britain Germany	Technological advances in the development of monoclonal antibodies
1991	E. Donnall Thomas Joseph Murray	United States United States	Transplantation immunology
2002	Sydney Brenner H. Robert Horvitz J. E. Sulston	South Africa United States Great Britain	Genetic regulation of organ development and cell death (apoptosis)
2008	Harald zurHausen Françoise Barré-Sinoussi Luc Montagnier	Germany France France	Role of HPV in causing cervical cancer (Hausen) and the discovery of HIV (Barré-Sinoussi and Montagnier)
2011	Jules Hoff man Bruce Beutler Ralph Steinman	France United States United States	Discovery of activating principles of innate immunity (Hoff man and Beutler) and role of dendritic cells in adaptive immunity (Steinman)

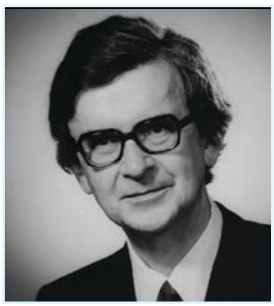
Elie Metchnikoff

karl Landsteiner

Emil von Behring

Paul Ehrlich

Robert Koch



Niels K.Jerne

Jules J.B.V.Bordet

Max Theiler

Rodney R.Porter

Figure 13.1: Notable Scientists who contributed to the development of Immunology

13.2 Organs of the Immune System

The immune system consists of structurally varied organs that are distributed throughout the body. Based on function, the organs can be divided into primary and secondary lymphoid organs (Figure 13.2). The primary lymphoid organs are responsible for providing the appropriate microenvironments for the development and maturation of antigen sensitive B and T cells. The thymus is the primary lymphoid organ for development of T cells and the bone marrow is the primary lymphoid organ for development of B cells. The secondary lymphoid organs serve as sites where lymphocytes interact with antigen and undergo proliferation and differentiation into antigen specific effector cells. The spleen, lymph nodes and mucosal associated lymphoid tissues (MALT) are secondary lymphoid organs. These are discussed in more detail below.

13.2.1 Primary Lymphoid Organs

a. Thymus

The **thymus** is a highly organized lymphoid organ located above the heart. The thymus consists of **two lobes**. Each

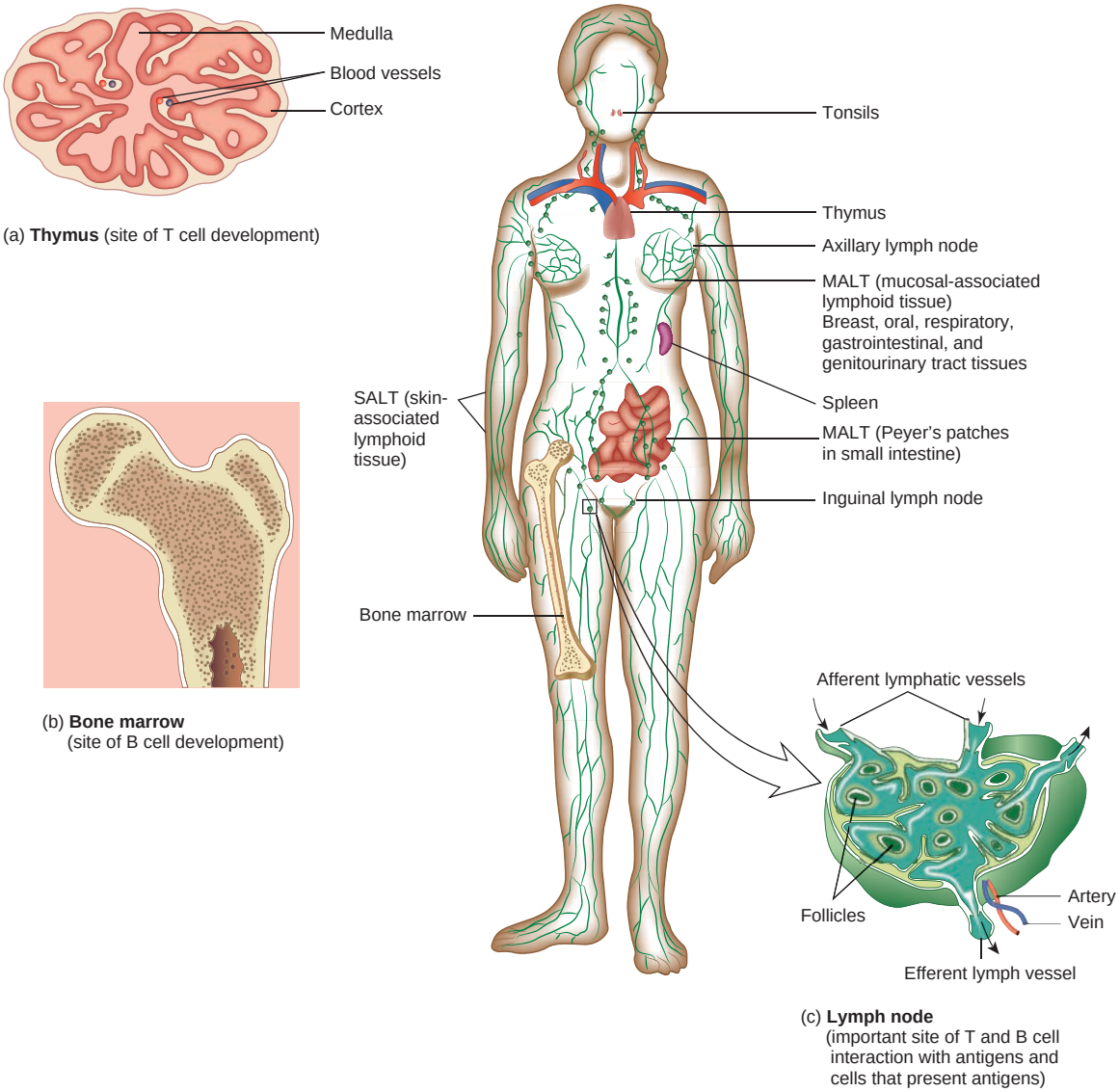


Figure 13.2: The distribution of Lymphoid tissues in the body

lobe is surrounded by a **capsule** and is divided into several **lobules** by strands of connective tissue called **trabeculae**. Each lobule contains an outer **cortex** and an inner **medulla**. The cortex contains many dividing immature lymphocytes. The medulla consists of reticular and epithelial cells with fewer lymphocytes and isolated Hassall's corpuscles (Figure 13.3). The primary function of the thymus is the production of mature T cells. Precursor cells from the bone marrow migrate into the outer cortex where they proliferate. As they mature, about 98% die. This is due to a process known as **thymic selection** in which T cells that recognize host (self) antigens are destroyed. The remaining 2% move into the medulla of the thymus, become mature T cells and subsequently enter the blood stream. These T cells recognize non host (non self) antigens.

b. Bone marrow

In mammals, **the bone marrow** (Figure 13.4) is the site of B cell maturation. **Stromal cells** within the bone marrow interact directly with the

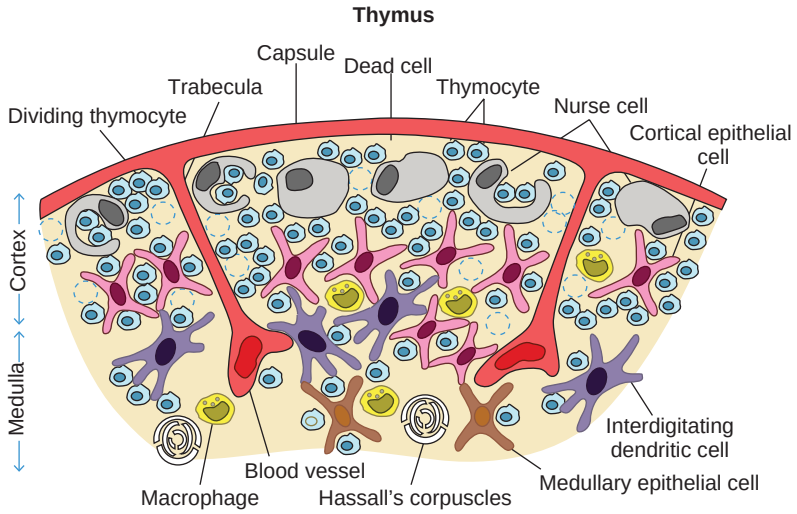


Figure 13.3: Diagrammatic Cross-section of a portion of the thymus

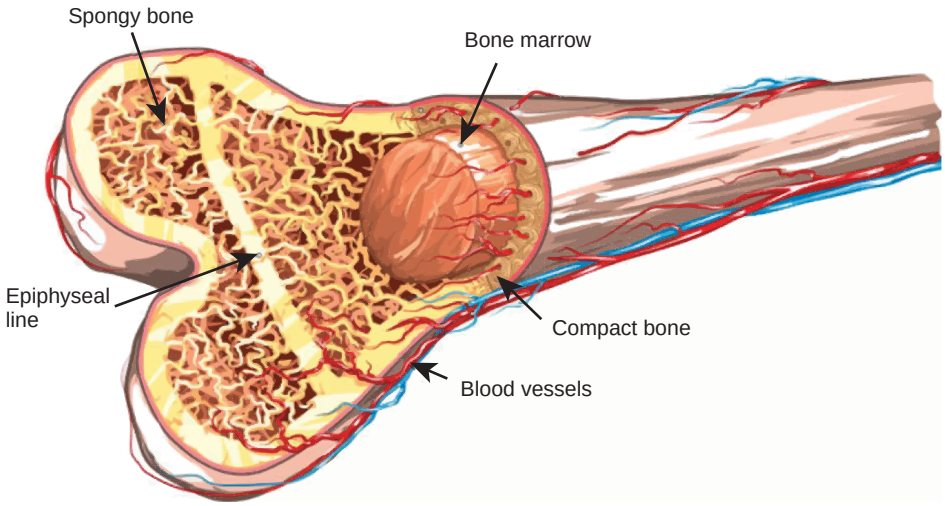


Figure 13.4: Structure of Bone marrow

B cells and secrete various cytokines that are required for B cell development. Like thymic selection during T cell maturation, a selection process within the bone marrow eliminates non functioning B cells and those bearing self reactive antigen receptors. In birds, undifferentiated lymphocytes move from the bone marrow to the **Bursa of Fabricius**, where B cell mature; this is where B cells were first identified and how they came to be known as " B" (for bursa) cells.

13.2.2 Secondary Lymphoid Organs

a. Spleen

The **spleen** is the most highly organized secondary lymphoid organ. The spleen is a fist sized organ just behind the stomach. It collects and disposes of aged red blood cells. Its organization is shown schematically in Figure 13.5. The bulk of the spleen is composed of **red pulp** which is the site of red blood cell disposal. The spleen is not supplied by lymphatic vessels. The lymphocytes surround the

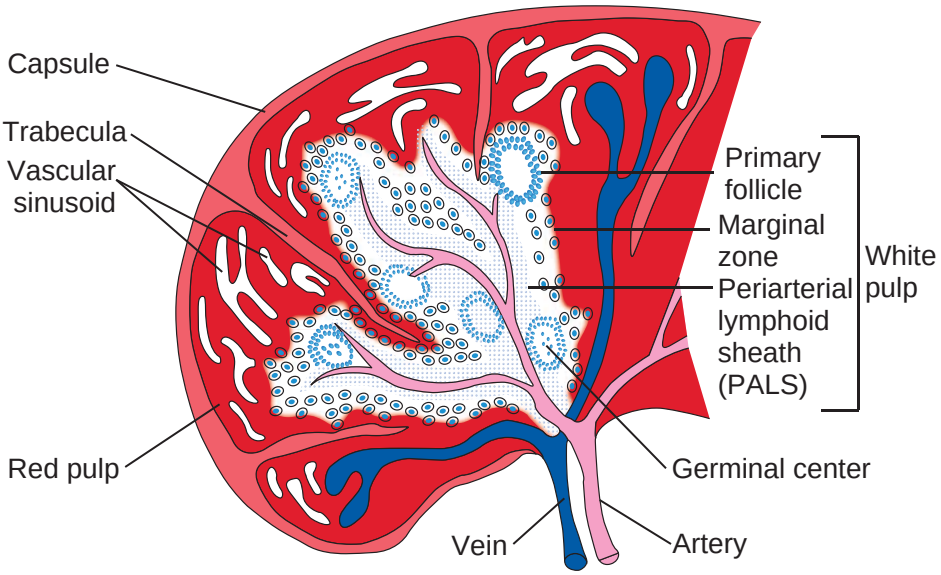


Figure 13.5: Structure of Spleen

arterioles entering the spleen, forming areas of **white pulp.** The inner region of white pulp is divided into a **Periarteriolar Lymphoid Sheath** (PALS) containing mainly T cells. The spleen filters the blood and traps blood borne microorganisms and antigens. Once trapped by splenic macrophages or dendritic cells, the pathogen is phagocytosed, killed and digested.

b. Lymph nodes

The **lymph nodes** are encapsulated round structures located at the junction of major **lymphatic vessels**. Lymph node is morphologically divided into three regions: the cortex, the paracortex and the medulla (Figure 13.6). The outer most layer, the **cortex** contains lymphocytes (mostly B cells), macrophages and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal centre. Beneath the cortex is the **paracortex** which is populated largely by T lymphocytes and also interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dentritic cells express high levels of class II MHC molecules, which are necessary for presenting antigen to T helper (T_H) cells. Lymph nodes taken from neonatally thymectomized (removal of thymus from new born animal) mice have unusually few cells in the paracortical region; the paracortex is therefore sometimes referred to as a **thymus dependent area** in contrast to the cortex, which is a **thymus independent area**. The inner most layer of a lymph node, the **medulla**, is more sparsely populated with lymphoid lineage cells; of those present many are plasma cells actively secreting antibody molecules. Lymph nodes are specialized to trap antigen from regional tissue spaces. As antigen is carried into a lymph node by the lymph, it is trapped, processed and presented together with class II MHC molecules by interdigitating dendritic cells in the paracortex, resulting

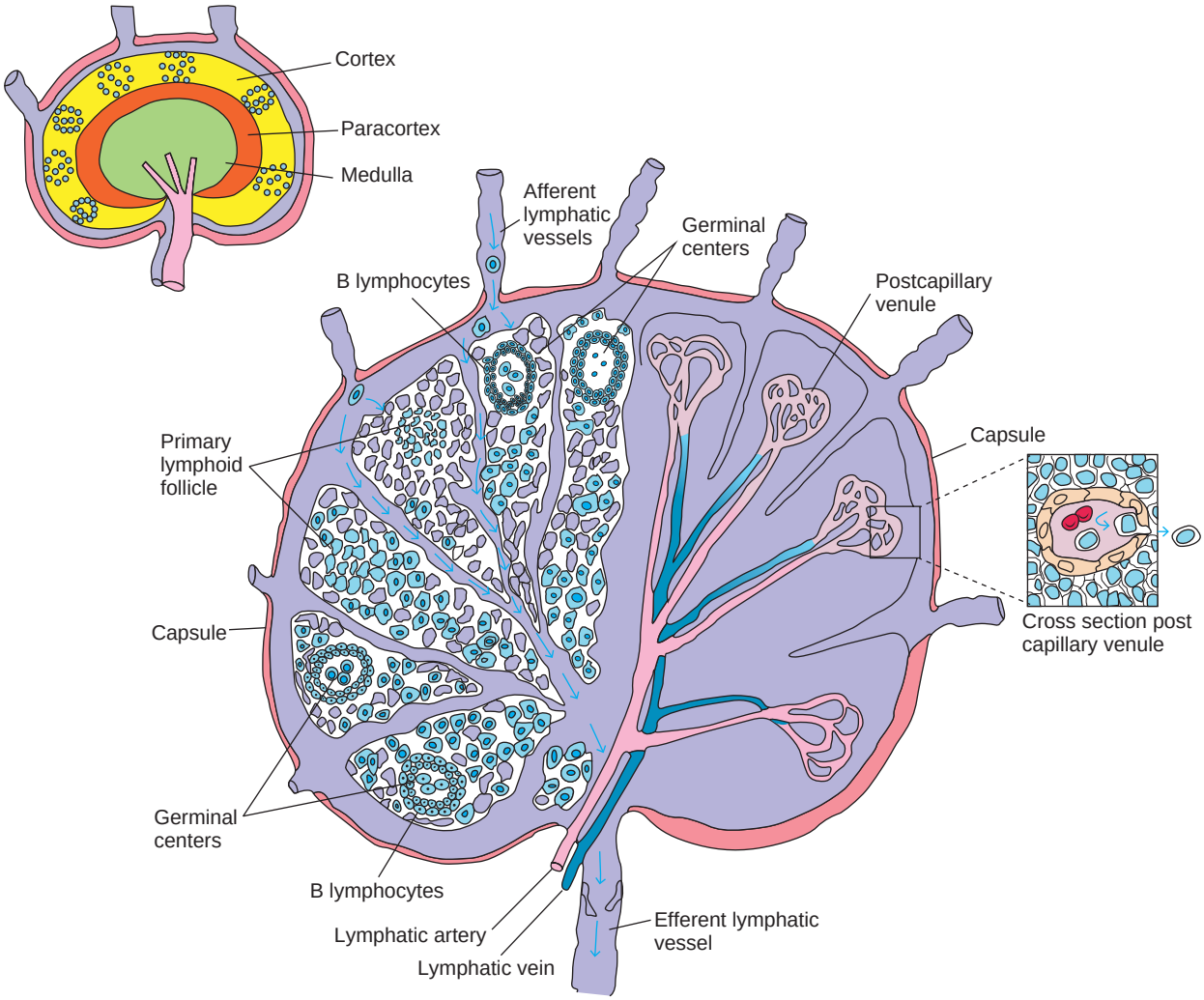


Figure 13.6: Structure of Lymph node

in the activation of T_H cells. Activated T_H cells release cytokines needed for B cell activation. Thus lymph nodes represent one environment where B cells differentiate into memory cells and antibody – secreting plasma cells.

Lymph draining the extra cellular spaces of the body carries antigen from the tissues to the lymph node through the **afferent lymphatics**. Lymph leaves by the **efferent lymphatic** in the medulla. Naive lymphocytes (mature lymphocytes not yet exposed to an antigen) enter the node from the blood stream through specialized **post capillary venules** and leave with the lymph through the efferent lymphatic.

c. MALT and SALT

The specialized lymphoid tissue in mucus membranes is called **mucosal associated lymphoid tissue** (MALT). There are several types of MALT. The system most studied is the **gut associated lymphoid tissue** (GALT). GALT include the **tonsils, adenoids, and appendix** and specialized structures called **peyer's patches** (Figure 13.7) in the small intestine, which collect antigen from the epithelial surfaces of the gastrointestinal tract. In peyer's patches, the antigen is collected by specialized epithelial cells called **M cells** (Figure 13.8). The lymphocytes form a follicle consisting of a large central dome of B lymphocytes surrounded by

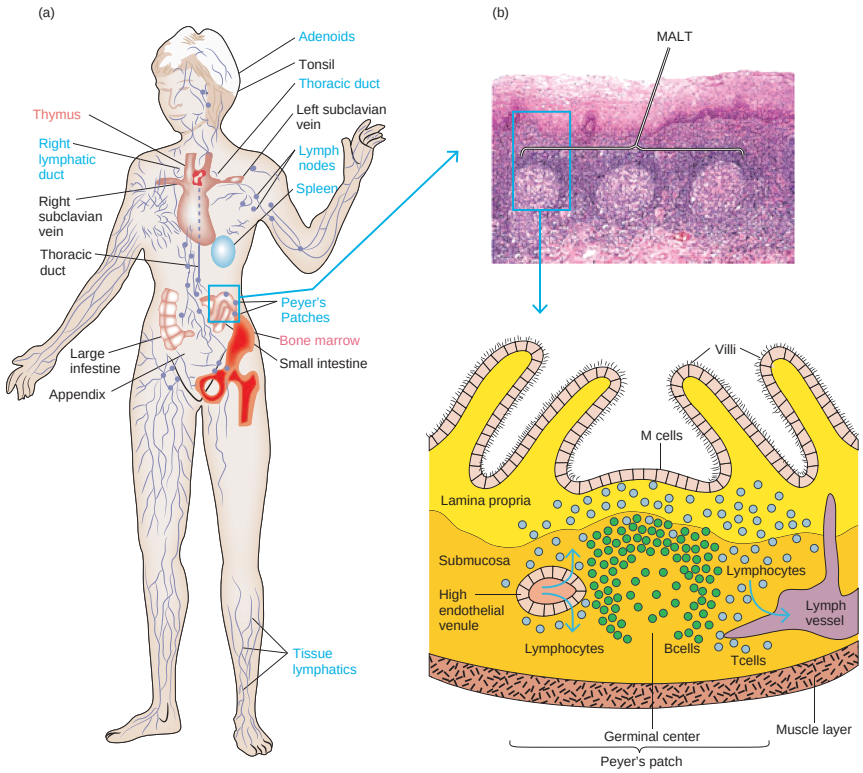


Figure 13.7: Malt Mucosa Associated Lymphoid Tissue (MALT)

(a) The Peyer's patch is a representative of the extensive MALT system that is found in the intestine. (b) A stained tissue cross-section of Peyer's patch lymphoid nodules in the intestinal submucosa is schematically diagrammed in (c). The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

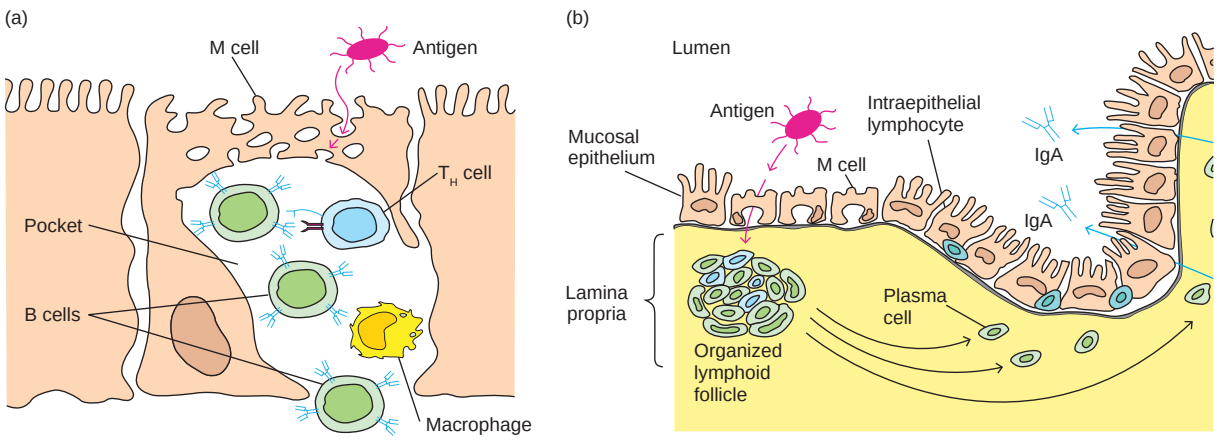


Figure 13.8: Structure of M cells and production of IgA:

(a) M cells, situated in mucous membranes, endocytose antigen from the lumen of the digestive, respiratory, and urogenital tracts. The antigen is transported across the cell and released into the large basolateral pocket. (b) Antigen transported across the epithelial layer by M cells at an inductive site activates B cells in the underlying lymphoid follicles. The activated B cells differentiate into IgA-producing plasma cells, which migrate along the lamina propria, the layer under the mucosa. The outer mucosal epithelial layer contains intraepithelial lymphocytes, of which many are T cells.

small numbers of T lymphocytes. Similar but more diffusely organized aggregates of lymphocytes protect the respiratory epithelium, where they are known as **bronchial- associated lymphoid tissue** (BALT).

Despite the skin's defenses, at times pathogenic microorganisms gain access to the tissue under the skin surface. Here, they encounter a specialized set of cells called the **skin associated lymphoid tissue (SALT)** (Figure 13.9). The major function of SALT is to confine microbial invaders to the area immediately underlying the epidermis and to prevent them from gaining access to the blood stream. One type of SALT is the **langerhans cell**, a specialized myeloid cell that can phagocytose antigens.

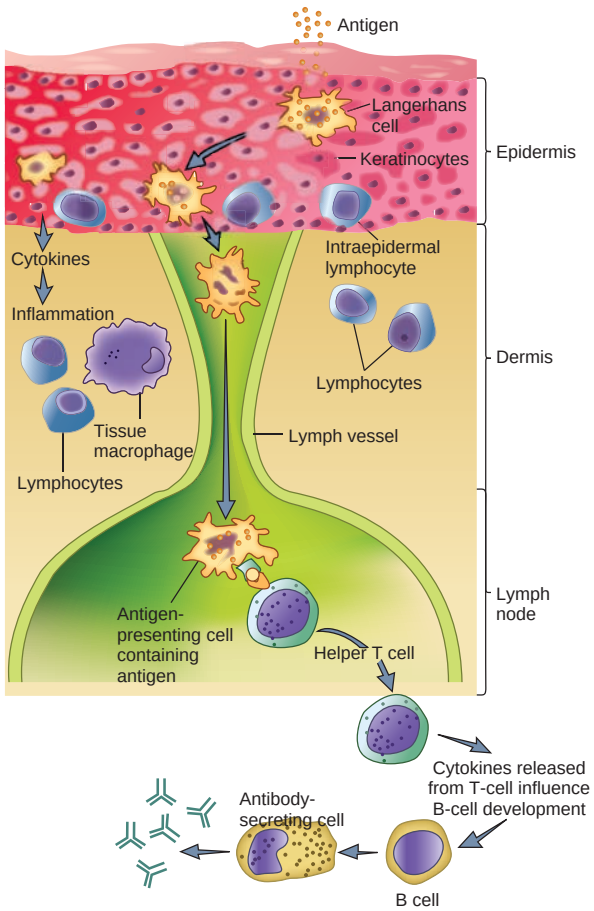


Figure 13.9: Skin-Associated Lymphoid Tissue (SALT)

HOTS

What happens when thymus is removed from the human body?

Infobits

A Second Thymus: No one expected a new anatomical discovery in immunology in the 21st century. However, in 2006 Hans Reimer Rodewald and his colleagues reported the existence of a second thymus in mice. The conventional thymus is a bilobed organ that sits in the thorax right above the heart. Rodewald and his colleagues discovered thymic tissue that sits in the neck, near the cervical vertebrae of mice. Rodewald's findings raise the possibility that some of our older observations and assumptions about thymic function need to be reexamined. The evolutionary implications of this thymus are also interesting. Thymus are found in the neck in several species, including the **Koala** and **Kangaroo**.

Nasal Associated Lymphoid Tissue (NALT): Secondary lymphoid microenvironments in the nose that support the development of the T and B lymphocyte respond to antigens that enter nasal passages. Part of the mucosa associated lymphoid tissue system (MALT).

13.3 Cells of the Immune System

All blood cells arise from a type of cell called the **hematopoietic stem cell(HSC)**. Stem cells are cells that can differentiate into other cell types. They are self renewing and they maintain their population level by cell division. This chapter describes the formation of blood cells and the properties of the various cells of the immune system.

13.3.1 Hematopoiesis

Hematopoiesis is the formation and development of blood cells of all types. In humans, hematopoiesis begins in the yolk sac in the first weeks of embryonic development. In the third month of gestation, the stem cells migrate from the yolk sac to the fetal liver and then to the spleen. Hematopoiesis continues in these two organs from the third to the seventh month of gestation. As gestation continues, the site of hematopoiesis gradually shifts to the bone marrow such that it becomes the principle site at the time of birth.

As hematopoietic stem cells can give rise to all of the different types of blood cells, they are often known as **pluripotent stem cells**. The different types of blood cells and their lineage relationships are summarized in Figure 13.10. We shall be concerned here only with the cells derived from the **myeloid progenitor** and the **common lymphoid progenitor**.

The myeloid progenitor gives rise to erythrocytes, neutrophils, eosinophils, basophils, monocytes, mast cells and platelets. The common lymphoid progenitor gives rise to B lymphocytes, T lymphocytes and natural killer (NK) cells.

13.3.2 Types of Leukocytes

The cells responsible for both innate immunity and acquired immunity are the leukocytes (Greek *leukos*, white and *kytos* cell). The average adult has approximately 7400 leukocytes (white blood cells) per cubic millimeter of blood (Table 13.2). The average value shifts substantially during an immune response. In defending the host against pathogenic microorganisms, leukocytes cooperate with each other first to recognize the pathogen as an invader and then to destroy it. The different types of leukocytes are now briefly described.

a. Granulocytes

Granulocytes have irregularly shaped nuclei with two or five lobes. Their cytoplasm has granules that contain reactive substances that kill microorganisms and enhance inflammation. Three types of granulocytes exist: basophils, eosinophils, and neutrophils. Because of the many lobed (3-5) nuclei, neutrophils are also called **polymorphonuclear neutrophils** or **PMNS** (Figure 13.11).

i) Basophils

Basophils are found in blood. Basophils have irregularly shaped nuclei with two lobes and granules that stain bluish black with basic dyes. Basophils are non phagocytic cells that release specific compounds from their cytoplasmic granules which include histamine, prostaglandins, serotonin, and leukotriens. Because these compounds

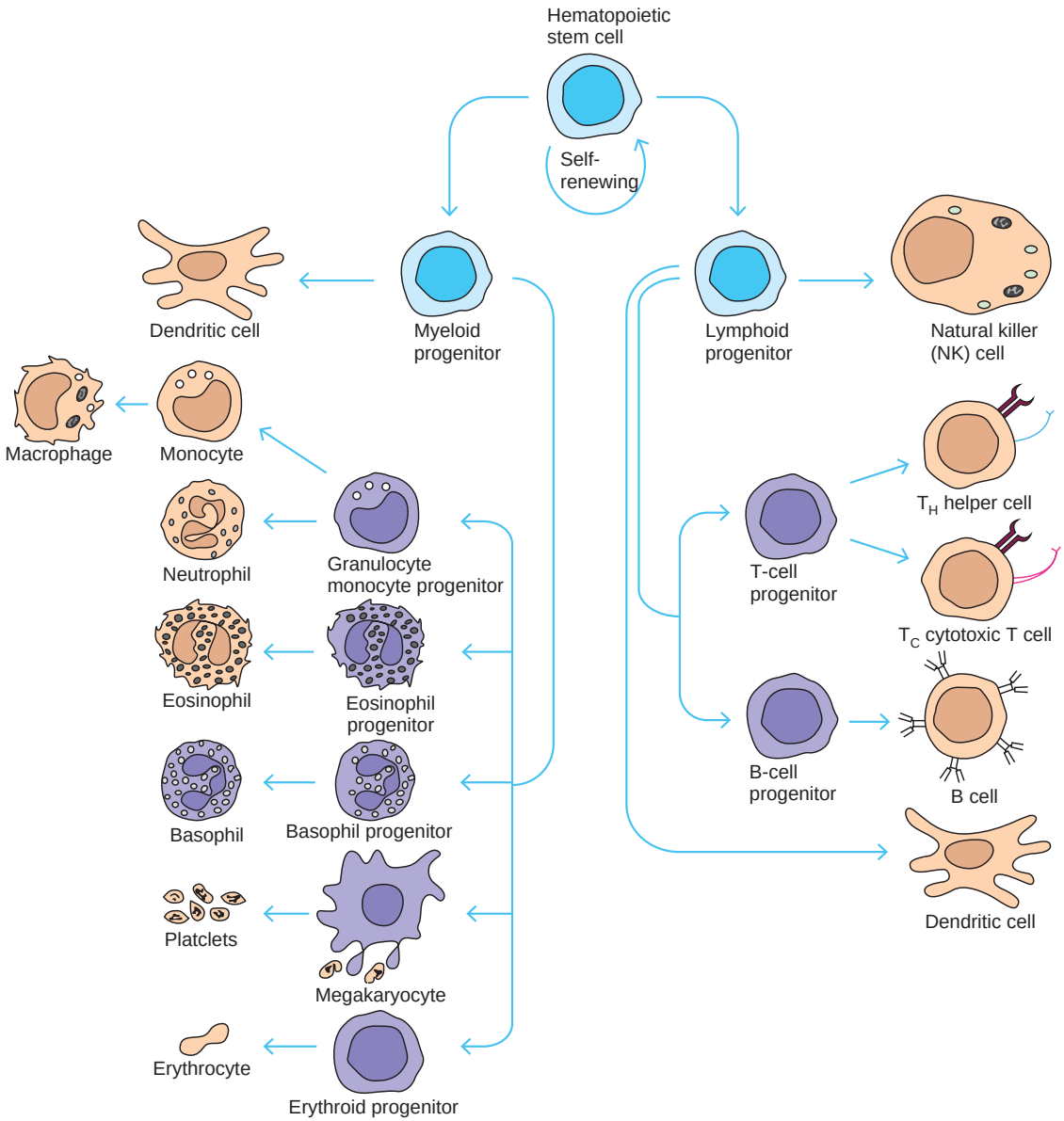


Figure 13.10: Hematopoiesis

affect vascular permeability they are termed **vasoactive mediators**. Vasoactive mediators play a major role in certain allergic responses such as eczema, hay fever and asthma.

HOTS

Heard about stem cell treatment!

Why do we need stem cells bank?

Table 13.2: Normal Adult Blood Count

Cell type	Cells/ mm3	% WBC
Red blood cells	50,00,000	–
Platelets	2,50,000	–
Leukocytes	7,400	100
Neutrophil	4320	60
Lymphocytes	2160	30
Monocytes	430	6
Eosinophils	215	3
Basophils	70	1

ii) Eosinophils

Eosinophils have a two lobed nucleus connected by a slender thread of chromatin and granules that stain with acidic dyes. Unlike basophils, eosinophils, migrate from the blood stream into tissue spaces, especially mucous membranes. They are important in the defense against protozoan and helminth parasites, mainly by releasing cationic peptides and reactive oxygen intermediates, into the extracellular fluid. These molecules damage the parasite plasma membrane, killing it. Eosinophils also play a role in allergic reactions.

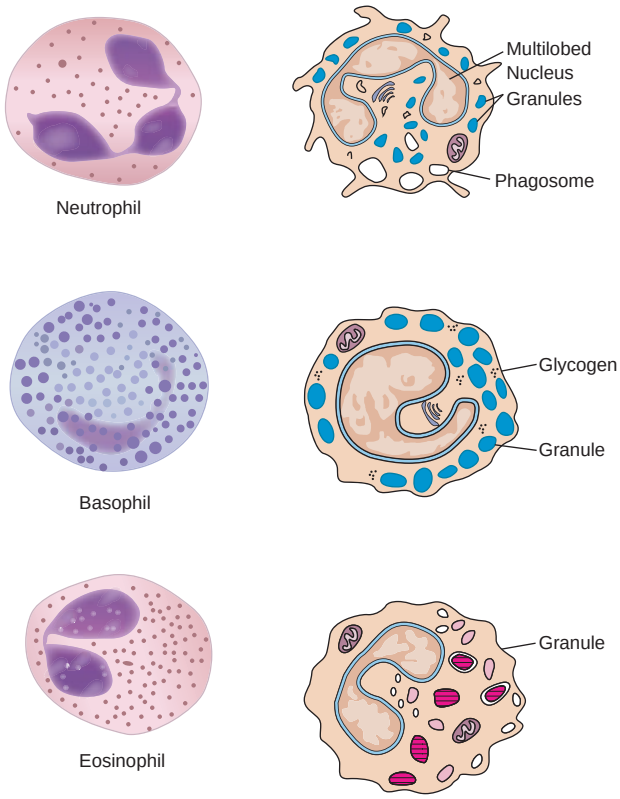


Figure 13.11: Structure of granulocytes

iii) Neutrophils

Neutrophils have three to five lobed nucleus. Like macrophages, neutrophils have receptors for antibodies and complement proteins and are highly phagocytic. However, unlike macrophages, neutrophils do not reside in healthy tissue but circulate in blood so they can rapidly migrate to the site of tissue damage and infection, where they become the principle phagocytic and microbicidal cells.

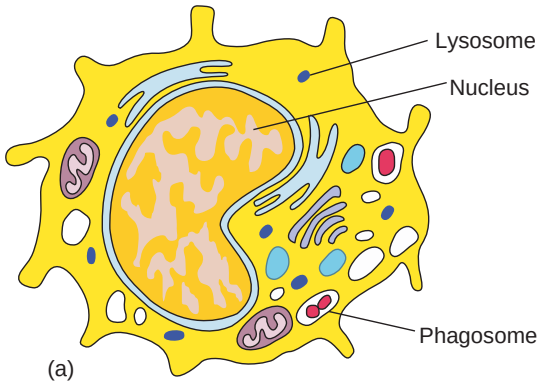
b. Mast cells

Mast cells are bone marrow derived cells that differentiate in the blood and connective tissue. Although they contain granules with histamine and other pharmacologically active substances similar to those in basophils, they arise from a different cellular lineage. Mast cells, along with basophils, are important in the development of allergies and hypersensitivities.

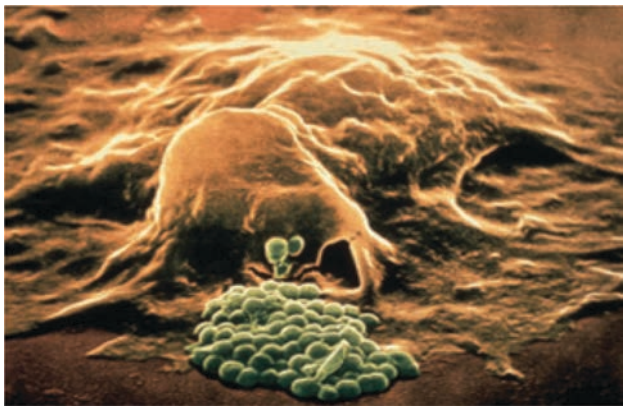
c. Monocytes and Macrophages

Monocytes are mononuclear leukocytes. They are produced in the bone marrow and enter the blood, circulate for about eight hours, enlarge, migrate to the tissues and mature into macrophages or dendritic cells (Figure 13.12 a).

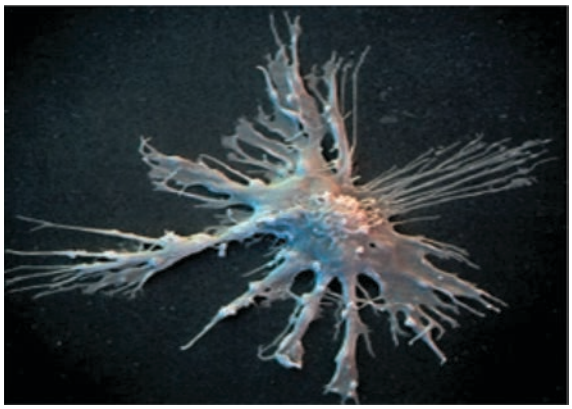
Macrophages are derived from monocytes and are classified as mononuclear phagocytic leukocytes. However, they are larger than monocytes, contain more organelles that are critical for phagocytosis and have a plasma membrane with microvilli. Macrophages have receptors to recognize common components of pathogens. These receptors include mannose and fructose receptors and a special class of molecules called **toll like receptors**. Toll like receptors bind lipopolysaccharide (LPS), peptidoglycan, fungal cell wall component called zymosan, viral nucleic acids and foreign DNA. These microbial molecules are examples of **pathogen associated molecular patterns** (PAMPs) (Figure 13.12 c).



(a)



(b)



(c)

Figure 13.12: (a) Structure of Monocytes (b) Phagocytosis by a Macrophage (c) Dendritic Cell

PAMPs enable macrophages to distinguish between potentially harmful microbes and other host molecules. After the pathogen is recognized, the macrophages', **pattern recognition receptors** (Example: Toll like receptors) bind the pathogen and phagocytose it. Macrophages also have receptors for antibodies and complement proteins. Both antibody and complement proteins can coat microorganisms and enhance their phagocytosis. This enhancement is termed **opsonization**. Macrophages spread throughout the body and take up residence in specific tissues. Macrophages serve different functions in different tissues and are named according to their tissue location.

- **Alveolar macrophages** in the lung
- **Histiocytes** in connective tissue
- **Kupffer cells** in the liver
- **Mesangial cells** in the kidney
- **Microglial cells** in the brain
- **Osteoclasts** in bone

d. Dendritic cells

Dendritic cells are not a single cell type. They are a heterogeneous group of cells so named because of their Dendron (neuron) like appendages (Figure 13.12d). They arise from various hematopoietic cell lineages. Most dendritic cells are tissue bound, where they play an important role in bridging innate immunity and acquired immunity.

Dendritic cells can be classified by their location:

- **Langerhans cells** found in the skin and mucus membranes
- **Interstitial dendritic cells** which populate most organs (heart, lungs, liver, kidney, gastrointestinal tract)
- **Interdigitating cells** present in T cell areas of secondary lymphoid tissue and the thymic medulla.
- **Circulating dendritic cells** in the blood and lymph.

All the above dendritic cells express high levels of both class II MHC molecules. They are more potent antigen presenting cells than macrophages and B cells. Another type of dendritic cell, called the **Follicular dendritic cell** has a different origin and function from antigen presenting dendritic cells described above. Follicular dendritic cells do not express class II MHC molecules and therefore do not function as antigen presenting cells. Follicular dendritic cells express high level of membrane receptors for antibody and complement. Binding of circulating antibody-antigen complexes by these receptors facilitates B cell activation in lymph nodes.

Dendritic cells are similar to macrophages in their ability to recognize specific PAMPs on microorganisms. They also posses **pattern recognition receptors** (PRRs) to bind and phagocytose the pathogen.

e. Lymphocytes

Lymphocytes are the major cells of the specific immunity. Lymphocytes can be divided into three populations: T cells, B cells, and NK (natural killer) cells. Clusters of differentiation are group of monoclonal antibodies that identify the same cell surface molecule. The cell surface molecule is designated CD (cluster of differentiation followed by a number (CD1, CD2).

i) B Lymphocytes

B lymphocytes mature within the bone marrow. When they leave bone marrow, each expresses a unique antigen binding receptor on its membrane. The B cell receptor is a membrane bound antibody molecule (Figure 13.13a). When a naive B cell, first encounters the antigen that matches its membrane bound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly. Its progeny differentiate into **memory B cells** and **effector B cells** called **plasma cells**

Memory B cells have a longer life span than native cells. They express the same membrane bound antibody as their parent naive B cell. Plasma cells do not express membrane bound antibody. Plasma cells secrete large quantities of antibodies. Secreted antibodies are the major effector molecules of humoral immunity.

ii) T Lymphocytes

T lymphocytes also arise in the bone marrow. T cells then migrate to the thymus to mature. During its maturation within thymus, the T cells express a unique antigen binding molecule called the **T cell receptor** (Figure 13.13b) on its membrane. Unlike membrane bound antibodies on B cells, which can recognize antigen alone, T cell receptor can recognize only antigen that is bound to MHC molecules. There are two major

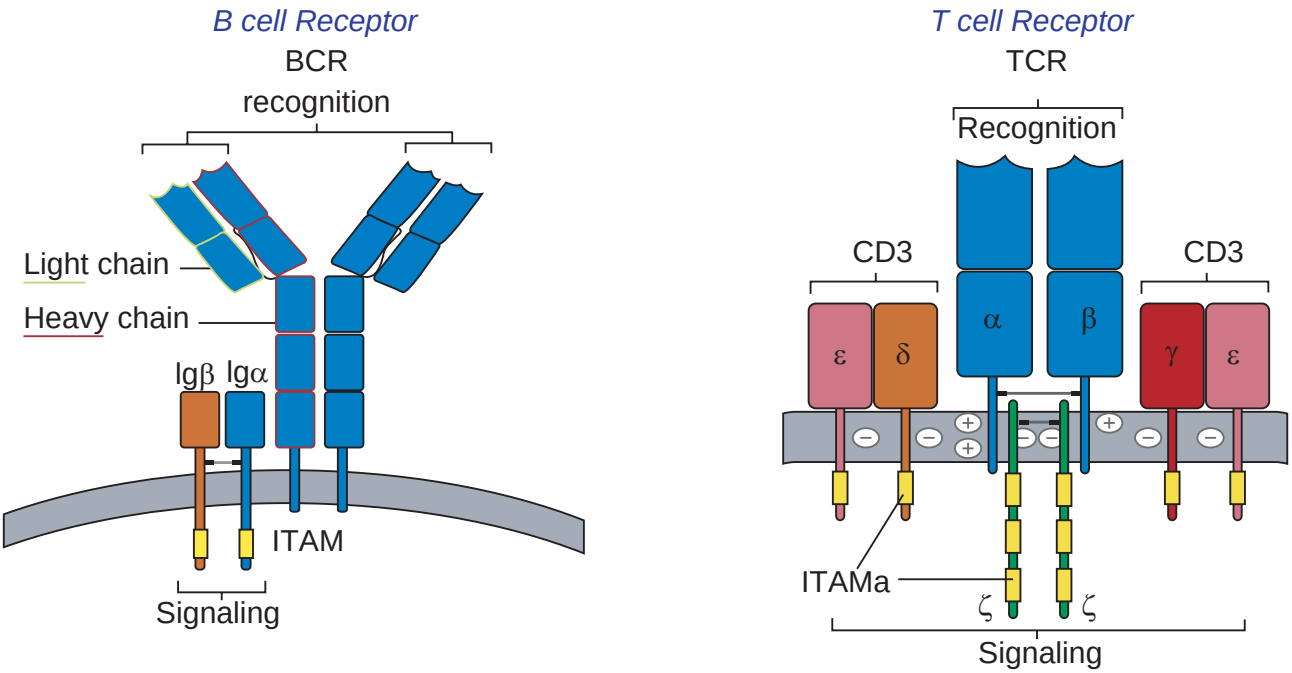


Figure 13.13: (a) B cell receptor. (b) T cell receptor

types of MHC molecules. Class I MHC molecules are expressed by all nucleated cells. Class II MHC molecules are expressed only by antigen presenting cells. When a naive T cell encounters antigen combined with an MHC molecule on a cell the T cell proliferates and differentiates into memory T cell and various effector T cells.

There are two subpopulations of T cells: **T helper** (T_H) and **T cytotoxic** (T_C) cells. Although a third type of T cells called a T suppressor (T_S) cell, has been postulated, recent evidence suggests that it may not be distinct from the T_H and T_C subpopulations. T cells displaying CD4 function as T_H cells whereas; those displaying CD8 function as T_C cells (Figure 13.14).

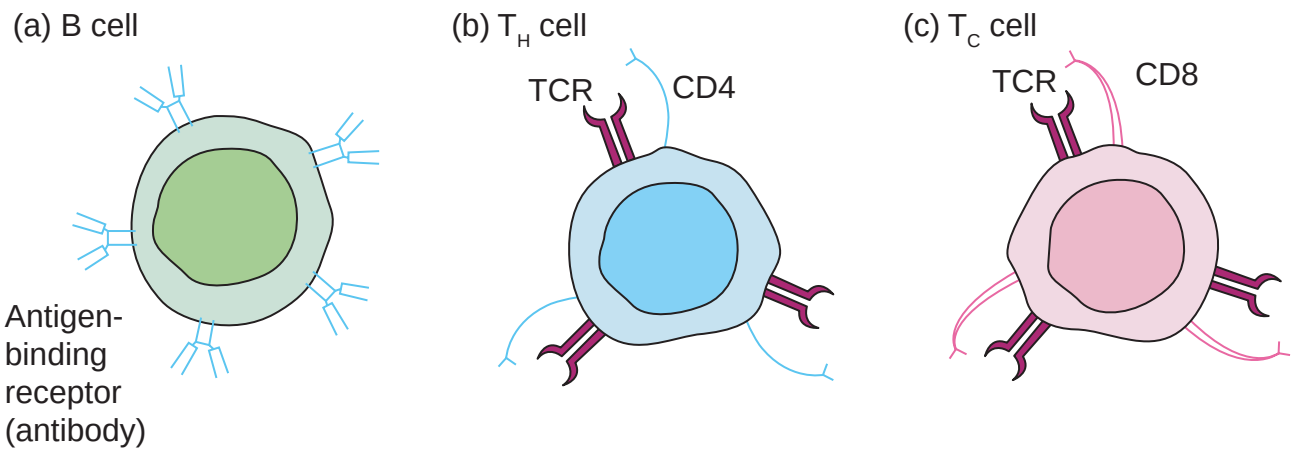


Figure 13.14: Distinctive membrane molecules on lymphocytes

After a T_H cell recognizes and interacts with an antigen-MHC class II molecule complex, the cell is activated. It becomes an effector cell that secretes cytokines. The secreted **cytokines** activate B cells, T_C cells, macrophages and various other cells that participate in the immune response.

Under the influence of T_H derived cytokines, a T_C cell that recognizes an antigen-MHC class I molecule complex proliferates and differentiates into a **cytotoxic T lymphocyte** (CTL). Cells that display foreign antigen complexed with a class I MHC molecule are called **altered self cells**. CTL destroy virus infected cells and tumor cells.

iii) Natural killer (NK) Cells (Null cells)

NK cells are a small population of large, non phagocytic granular lymphocytes that play an important role in innate immunity. The major NK cell function is to destroy cancer cells and cells infected with microorganisms. They recognize their targets in one of two ways. They can bind to antibodies that coat infected or cancer cells. Thus the antibody bridges the two cell types. This process is called **antibody dependent cell mediated cytotoxicity (ADCC)** (Figure 13.15) The second way that NK cells recognize infected cells and cancer cells relies on the presence of specialized proteins on the surface of all nucleated host cells known as class II MHC molecules. If a hosts cell loses this MHC protein, as when some viruses or cancers overtake the cell, the NK cells kill it by releasing pore forming proteins and cytotoxic enzymes called **granzymes** (Figure 13.16).

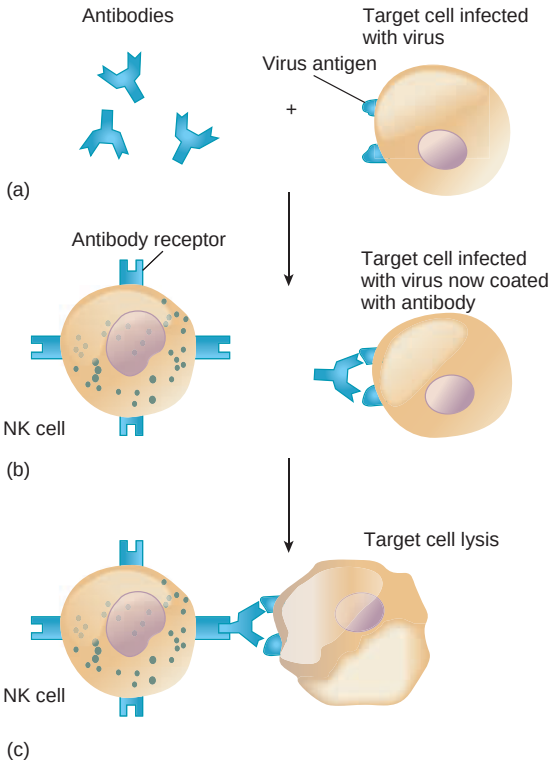


Figure 13.15: Antibody-Dependent Cell-Mediated Cytotoxicity

Stem Cell: A cell from which differentiated cells are derived. Stem cells are classified as totipotent, pluripotent, multipotent, or unipotent depending on the range of cell types that they can generate.

Necrosis: Morphologic changes that accompany death of individual cells or groups of cells and that release large amounts of intracellular components to the environment, leading to disruption and atrophy of tissue.

13.4 Immunity

To establish an infection, an invading microorganism must first overcome many surface barriers, such as skin, degradative

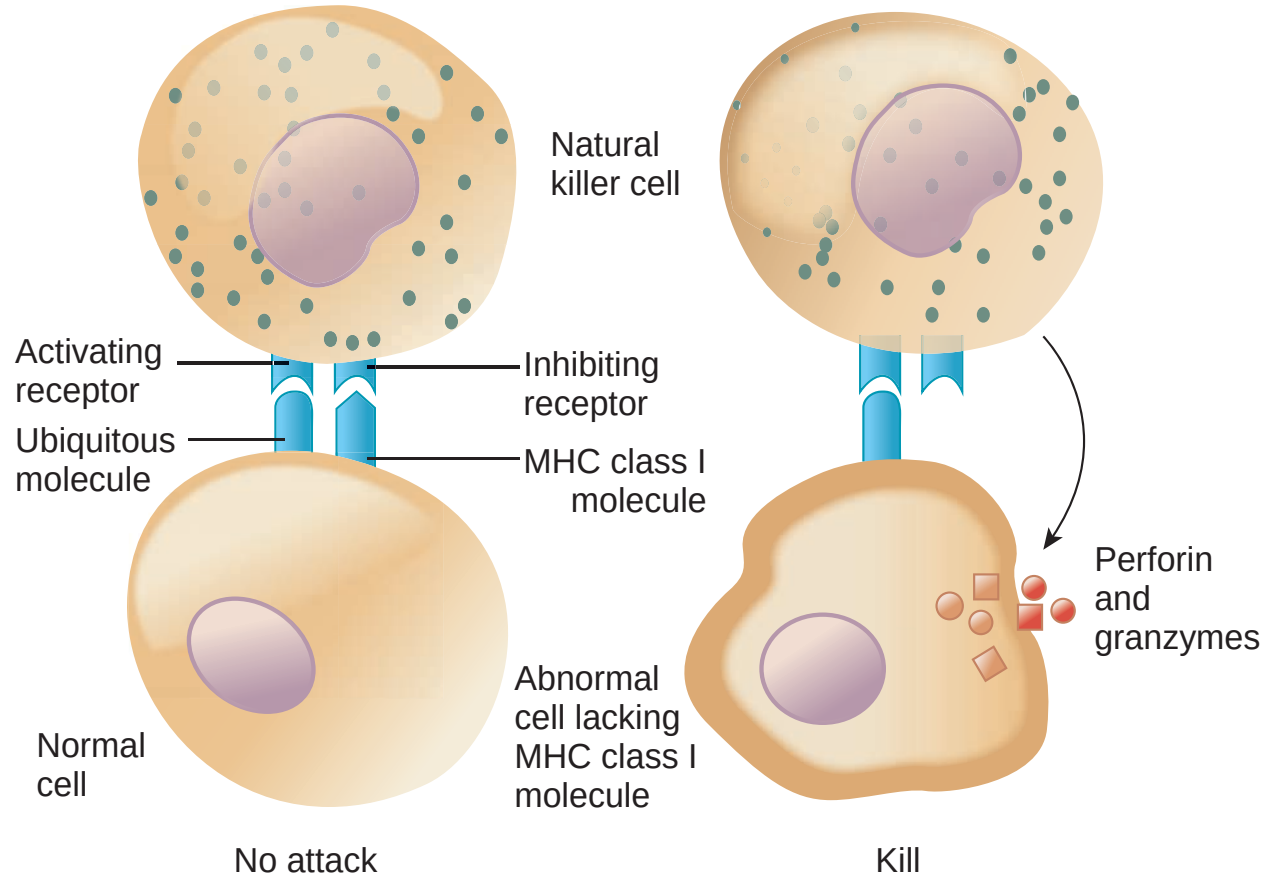


Figure 13.16: The system used by natural killer cells to recognize normal cells and abnormal cells that lack the Major Histocompatibility Complex Class I surface molecule

enzymes and mucus. These surface barriers have either direct antimicrobial activity or inhibit attachment of the microorganism to the host. Any microorganism that penetrates these barriers encounters two levels of resistance: nonspecific resistance mechanisms and the specific immune response.

13.4.1 Types of Immunity

The term immunity (Latin ***immunis***, free of burden) refers to the general ability of a host to resist infection or disease. There are two interdependent components of the immune response to invading microorganisms and foreign material. They are **non-specific immune response** or **innate immunity** or **natural immunity** and **specific immune response** or **acquired immunity** or **adaptive immunity**.

I. Innate immunity

Innate immunity refers to those general defence mechanisms that are inherited as part of the innate structure and function of each animal (such as skin, mucus and lysozyme). Innate immunity is the first line of defence against any microorganism or foreign material encountered by the vertebrate host. Innate immunity defends against foreign invaders equally and lacks immunological memory.

II. Acquired immunity

Acquired immunity refers to the type of specific immunity that develops after exposure to a suitable antigen. The effectiveness of acquired immunity increases on repeated exposure to foreign agents such as viruses, bacteria or toxins. So acquired immunity has memory. The innate immunity and acquired immunity work together to eliminate pathogenic microorganisms and other foreign agents. Although innate systems predominate immediately upon initial exposure to foreign substances, multiple bridges occur between innate and acquired immune system components (Figure 13.17).

13.4.2 Mechanisms of Innate Immunity

A potential microbial pathogen invading a human host immediately confronts a vast array of nonspecific defence mechanisms. Many direct factors (nutrition, physiology, fever, age, genetics) and equally as many indirect factors (personal hygiene, socioeconomic status, living conditions) influence all host microbe relationships. In addition to these direct and indirect factors, a vertebrate host has the following four non specific defence mechanisms.

A. Physical barriers

B. Chemical mediators

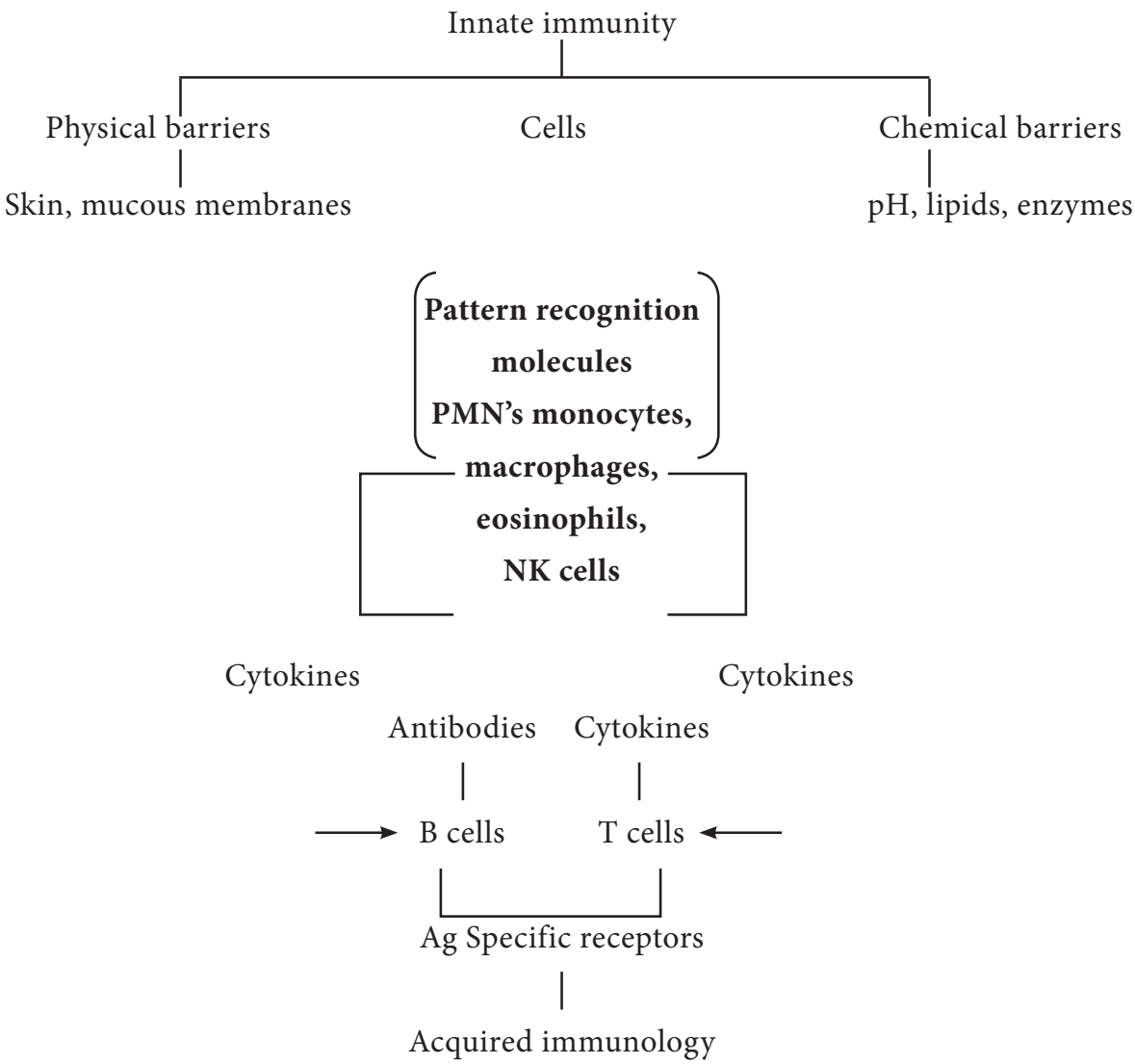


Figure 13.17: The interrelationship between innate and acquired immunity

C. Phagocytosis

D. Inflammation

A. Physical barriers

i) Skin

Intact skin contributes greatly to host resistance. It forms a very effective mechanical barrier to microbial invasion. Its outer layer consists of thick, closely packed cells called **keratinocytes**, The skin is slightly acidic (around pH 5-6) due to skin oil, secretion from sweat glands and organic acids produced by commensal *Staphylococci*. It also contains a high concentration of sodium chloride and is subject to periodic drying.

ii) Mucous membranes

The mucous membranes of the eye (conjunctiva), the respiratory, digestive and urogenital systems withstand microbial invasion. The intact stratified squamous epithelium and mucus secretions form a protective covering that resists penetration and traps many microorganisms. Many mucosal surfaces are bathed in specific antimicrobial secretions. One antibacterial substance in these secretions is **lysozyme**, an enzyme that lyses bacteria. Mucous secretions possess the iron binding protein, **lactoferrin**. Lactoferrin sequesters iron from the plasma reducing the amount of iron available to invading microbial pathogens and prevents their ability to multiply. Mucous membranes produce **lactoperoxidase**, an enzyme that catalyzes the production of superoxide radicals, reactive oxygen intermediate that is toxic to many microorganisms.

iii) Respiratory system

The mammalian respiratory system has strong defense mechanisms. The average person inhales at least eight microorganisms a minute or 10,000 each. Microbes larger than 10μm are trapped by hairs and cilia lining the nasal cavity. The cilia in the nasal cavity beat toward the pharynx, so that mucus with its trapped microorganisms is moved toward the mouth and expelled. Microbes smaller than 10μm pass through the nasal cavity and are trapped by the **mucociliary blanket** and the trapped microbes are transported by ciliary action that moves them away from lungs. Coughing and sneezing reflexes clear the respiratory system of microorganisms by expelling air forcefully from the lungs through the mouth and nose, respectively. Salivation also washes microorganisms from the mouth and nasopharyngeal areas into the stomach.

iv) Gastrointestinal tract

Most microorganisms that reach the stomach are killed by gastric juice. (pH 2-3). However, organisms embedded in food particles are protected from gastric juice and reach the small intestine. There microorganisms are damaged by various pancreatic enzymes, bile, enzymes in intestinal secretions and GALT system. Normal microbiota of the large intestine is important in preventing the establishment of pathogens. The mucous membranes of the intestinal tract contain **paneth cells**. These cells produce lysozyme and cryptins (toxic for bacteria).

v) Genitourinary tract

Under normal circumstances, the kidneys, ureters and urinary bladder of mammals are sterile. Urine within the urinary bladder is also sterile. In addition to removing microbes by flushing action, urine kills some bacteria due to its low pH and the presence of urea and other metabolic end

products (uric acid, hippuric acid, indican, fatty acids, mucin, and enzymes). The acidic environment (pH 3-5) of the vagina is unfavorable to most microbes.

vi) Eye

The conjunctiva is specialized mucus secreting epithelial membrane that lines the interior surface of each eyelid and the exposed surface of the eye ball. It is kept moist by the continuous flushing action of tears. Tears contain large amounts of lysozyme, lactoferrin, and antibody and thus provide chemical as well as physical protection (Figure 13.18).

B. Chemical mediators

- **Antimicrobial peptides**

They are low molecular weight proteins that exhibit broad spectrum antimicrobial activity toward bacteria.

i) Cationic peptides

Cationic peptides are found in humans. There are three generic classes of cationic peptides that have the ability to damage bacterial plasma membrane.

Classes of Cationic Peptides	Cells that produce
Cathelicidins	neutrophils, respiratory cells and alveolar macrophages
Defensins	primary granules of neutrophils, intestinal paneth cells and in-testinal and respiratory epithelial cells
Histatin	Found in human saliva. It has antifungal activity.

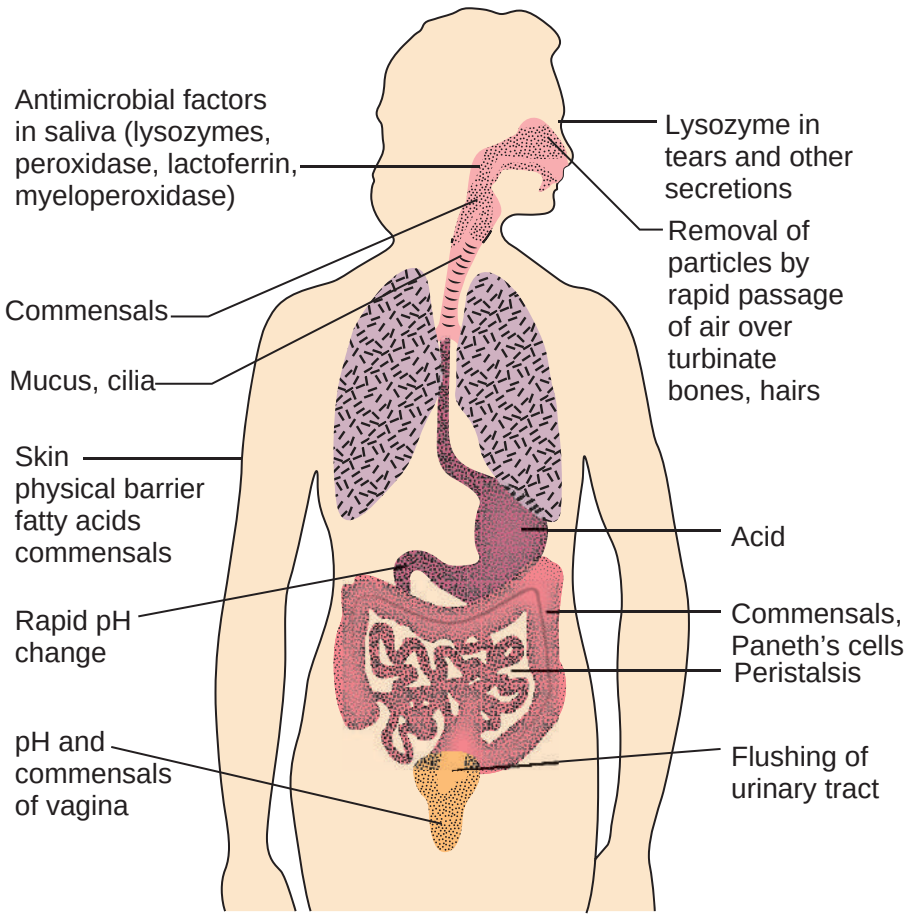


Figure 13.18: Physical Barriers

ii) Bacteriocins

Bacteriocins are produced by gram negative and gram positive bacteria. For example, *Escherichia coli* synthesize bacteriocins called **colicins**. Colicins causes cell lysis.

- **Cytokines**

Cytokines are proteins made by cells that affect the behavior of other cells. When released from mononuclear phagocytes, they are called **monokines**. When released from T lymphocytes they are called **lymphokines.** When released from leukocytes they are called **interleukins**. Cytokines are required for regulation of both the nonspecific and specific immune responses. **Interferons** (IFNS) are a group of cytokines produced by virus infected cells. Several classes of interferons are recognized. IFN γ is synthesized by virus infected leukocytes, antigen stimulated T cells and natural killer cells. IFN α / β is derived from virus infected fibroblasts. Interferons prevent viral replication and assembly, thereby limiting viral infection.

Another group of noteworthy cytokines are endogenous pyrogens which elicit fever in the host. Examples of **endogenous pyrogens** include interleukin – 1, Interleukin – 6 and tissue necrosis factor. All are produced by host macrophages in response to pathogens.

- **Complement system**

The complement system is a part of the immune system, consists of a series of proteins that interact with one another in a highly regulated manner, in order to eliminate pathogens. Complements are soluble proteins and glycoproteins mostly produced by hepatocytes. More than 20 types of complements are present in serum found circulating normally in human body in inactive forms (called as zymogens or proenzymes). Complement activation is triggered by an antibody when it is bound to the antigen. It can also be triggered by some components of innate immunity. Thus the complement system works in both innate and acquired immunity.

Complement activation and cell lysis

The complement activation occurs via three pathways which are:

1. Classical pathway
2. Alternative pathway
3. Lectin pathway (or mannose binding lectin pathway)

Classical pathway, activated by antigen-antibody reaction, Alternative pathway, activated on microbial cell surfaces, and Mannose binding Lectin pathway, activated by a plasma lectin that binds to mannose residues on microbes (Figure 13.19).

Functions of complements

Some major functions of complements are:

- Opsonization and phagocytosis
- Cell lysis
- Chemotaxis
- Activation of mast cells and basophils and enhancement of inflammation
- Production of antibodies

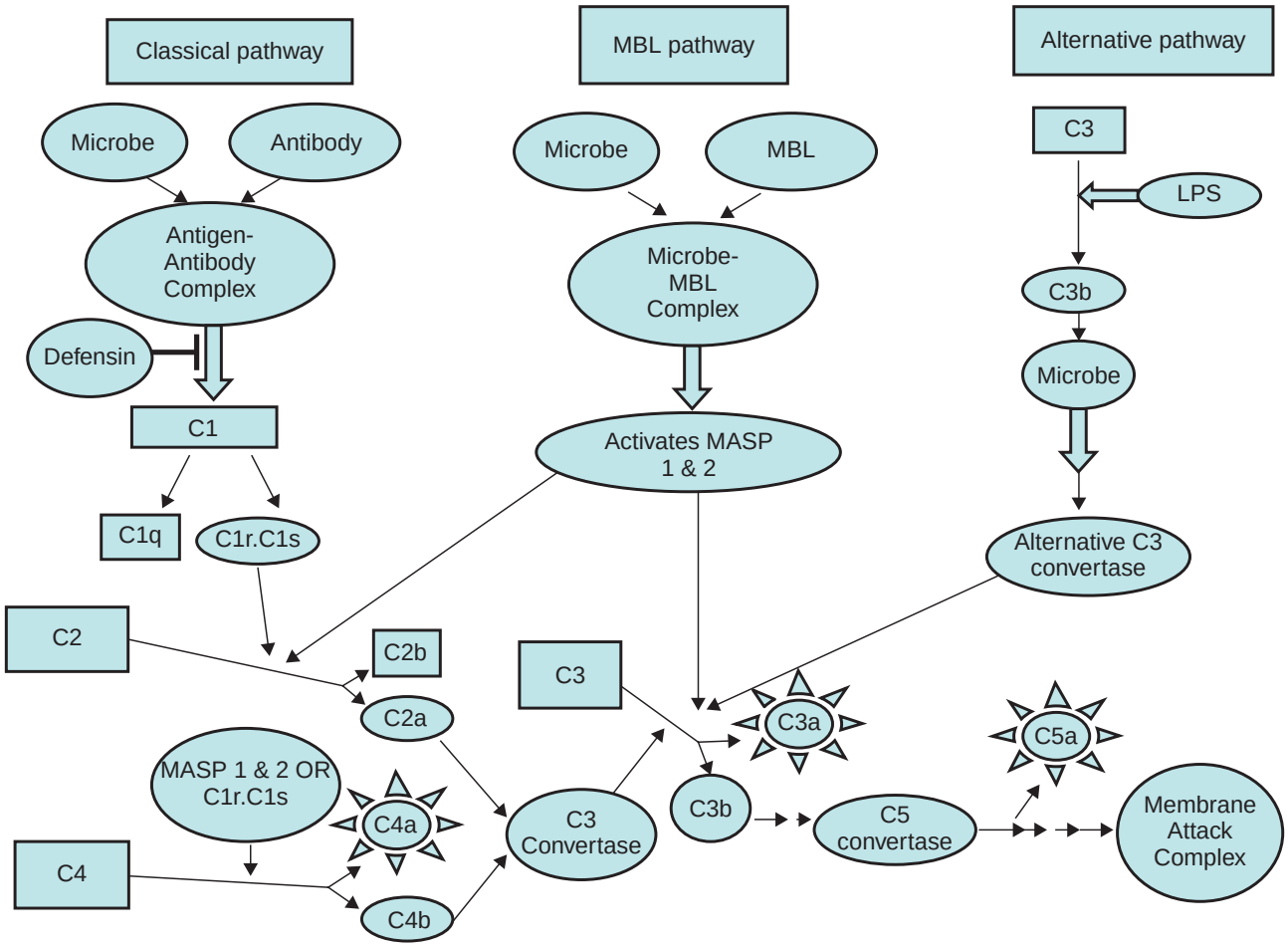


Figure 13.19: Complement pathways

- Immune clearance and inflammation by attracting macrophages and neutrophils.

C. Phagocytosis

i. Phagocytosis is the ingestion by phagocytic cells of invading foreign particles such as bacteria. After ingestion, the foreign particle is entrapped in a phagocytic vacuole **(phagosome)**, which fuses with **lysosomes** forming the **phagolysosome.** The lysosomes release their powerful lytic enzymes which digest the particle. (Figure 13.20). Phagocytosis is conducted by blood monocytes, neutrophils and tissue macrophages. Phagocytosis may be enhanced by a variety of factors collectively referred to as opsonins which consist of antibodies and various serum components of complement.

ii. Phagocytic cells use two basic mechanisms for the recognition of microorganisms. Opsonin dependent and opsonin independent

iii. Phagocytes use pathogen recognition receptors to detect pathogen associated molecular patterns on microorganisms. Toll like receptors are a distinct

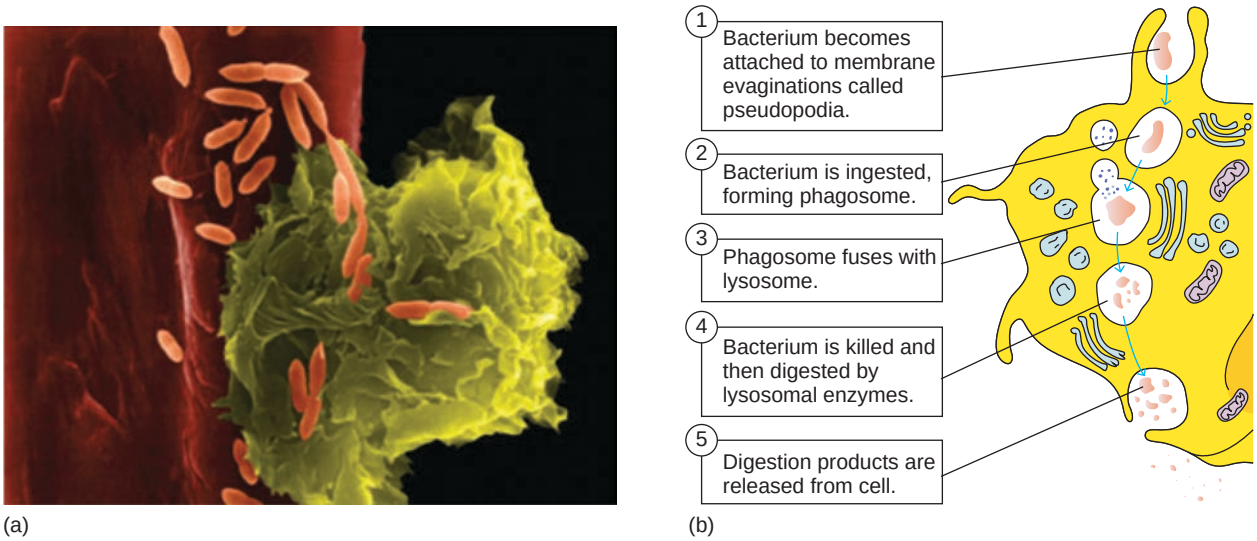


Figure 13.20: (a) Scanning electron micrograph of alveolar macrophage phagocytosis of E. coli bacteria on the outer surface of a blood vessel in the lung pleural cavity. (b) Steps in the phagocytosis of a bacterium.

class of pathogen recognition receptors.

D. Inflammation

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as **inflammatory response**. Inflammation can either be acute or chronic. The gross features were described over 2000 years ago and are still known as the cardinal signs of inflammation: redness (*rubor*), warmth (*calor*), pain (*dolor*), swelling (*tumor*), and loss of function (*functiolaesa*)

The cardinal signs of inflammation reflect the three major events of an inflammatory response.

1. **Vasodilation** (an increase in the diameter of blood vessels) of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict. This results in engorgement of the capillary network. The engorged capillaries are responsible for tissue redness (erythema) and an increase in temperature.
2. An increase in **capillary permeability** facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates (**exudate**) has much higher protein content. Accumulation of exudate contributes to tissue swelling (**edema**)
3. Influx of phagocytes from the capillaries into the tissues is facilitated by increased capillary permeability. As phagocytic cells accumulate at the site and begin to phagocytoses bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material and fluid forms substances called **pus**.

Infobits

Reactive Nitrogen Species: Highly cytotoxic antimicrobial compounds formed by the combination of nitric oxide and superoxide anion within phagocytes such as neutrophils and macrophages.

Reactive Oxygen Species (ROS): Highly reactive compounds such as superoxide anion O_2, hydroxyl radicals (OH)(OH^-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HClO) that are formed from oxygen under many conditions in cells and tissues, including microbe-activated innate responses of phagocytic cells; have anti-microbial activity.

13.4.3 Acquired Immunity

Lower animal forms possess so called innate or non-specific immune mechanisms such as phagocytosis of bacteria by specialized cells. Higher animals have evolved an adaptive or acquired immune response. This acquired immune response provides a flexible, specific and more effective reaction to different infections.

- **Definition of Acquired (Adaptive) Immunity**

Acquired (adaptive)immunity refers to the type of specific immunity that a host develops after exposure to a suitable antigen.

- **Important features of acquired immunity**

This is the immunity one develops throughout life time. Adaptive or acquired immunity has four important features namely (1) Memory (2) Specificity (3) diversity and (4) discrimination between self and non self.

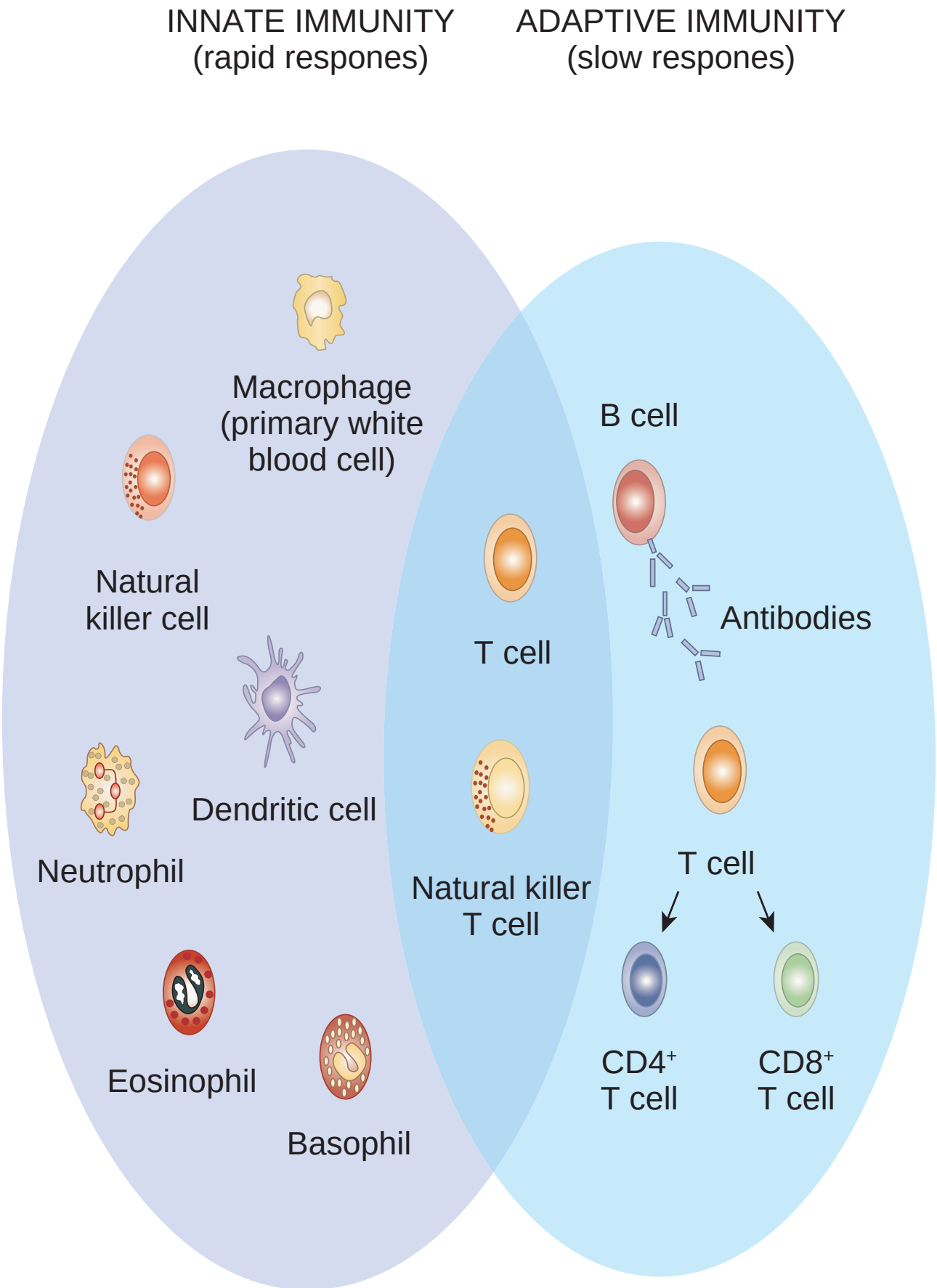
1) Memory

We rarely suffer twice from diseases such as measles, mumps, chicken pox, whooping cough and so on. The first contact with an infectious organism clearly imprints some memory so that the body is effectively prepared to repel any later invasion by that organism.

By following the production of antibody on the first and second contact with antigen, we can know the basis for the development of immunity. For example, when we inject a bacterial product such as staphylococcal toxoid into a rabbit, several days elapse before antibodies can be detected in the blood. These reach a peak and then fall. If we now allow the animal to rest and then give a second injection of staphylococcal toxoid, the cause of events is dramatically altered. Within two to three days the antibody level in the blood raises steeply to reach much higher values than were observed in the primary response. This secondary response is characterized by a more rapid and more abundant production of antibody. This explosive production of antibodies is due to the tuning up of the antibody forming system to provide a population of memory cells after first exposure to antigen. The principle of memory is involved in vaccination.

2) Specificity

The establishment of immunity by one organism does not provide protection against another unrelated organism. After an attack of measles we are immune to


INNATE IMMUNITY
(rapid respones)
ADAPTIVE IMMUNITY
(slow respones)
Macrophage
(primary white
blood cell)
Natural
killer cell
Dendritic cell
Neutrophil
Eosinophil
Basophil
B cell
T cell
Antibodies
Natural killer
T cell
T cell
CD4+
T cell
CD8+
T cell

further infection but are susceptible to polio or mumps viruses. Thus the body can differentiate specifically between the two organisms.

3) Diversity

The immune system is able to generate an enormous diversity of molecules such as cellular receptors and soluble proteins, including antibodies that recognize trillions of different foreign substances.

4) Discrimination between self and nonself

The specific immune system almost responds selectively to non self and produces specific responses against the stimulus. This is possible because host cells express a unique protein on their surface, making them as residents of that host or as self. Thus the introduction of materials lacking that unique self marker results in their attack by the host.

13.4.4 Humoral and Cellular Immunity

Two branches or arms of specific immunity are recognized: humoral (antibody mediated) immunity and cellular (cell mediated) immunity (Figure 13.21).

Humoral (antibody mediated) immunity

The antigen specific arm of the humoral immunity consists of the B cells. Each B cell expresses a unique antigen binding receptor on its membrane. The B cell receptor (BCR) is membrane bound antibody molecule. When a naive B cell first encounters the antigen that matches its membrane bound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly. Its progeny differentiate into memory B cells and antibody secreting plasma cells. A single plasma cell can secrete more than 2000 molecules of antibody per second. Circulating antibodies bind to microorganisms, toxins and extracellular viruses, neutralizing them or tagging them for destruction by phagocytes and other mechanisms.

The cellular (cell mediated) immunity consists of the T cells. Each T cell expresses antigen receptors called T cell receptors (TCRS). Unlike membrane bound antibody on B cells, which can recognize antigen alone, T cell receptors can recognize only antigen that is bound to MHC molecules. There are two major types of MHC molecules. Class I MHC molecules are expressed by all nucleated cells. Class II MHC molecules are expressed only by antigen presenting cells such as dendritic cells, macrophages and B cells. When a naive T cell encounters antigen combined with an MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells (helper T cells, cytotoxic T cells and regulatory T cells). Specific kinds of T cells directly attack target cells infected with viruses or parasites, transplanted cells or organs and cancer cells. T cells can induce target cell suicide (apoptosis), lyse targets cells, or release chemicals (cytokines) that enhance specific immunity and non specific defences such as phagocytosis and inflammation.

13.4.5 Types of Specific Immunity

Specific immunity can be acquired by natural means actively through infection or passively through receipt of preformed

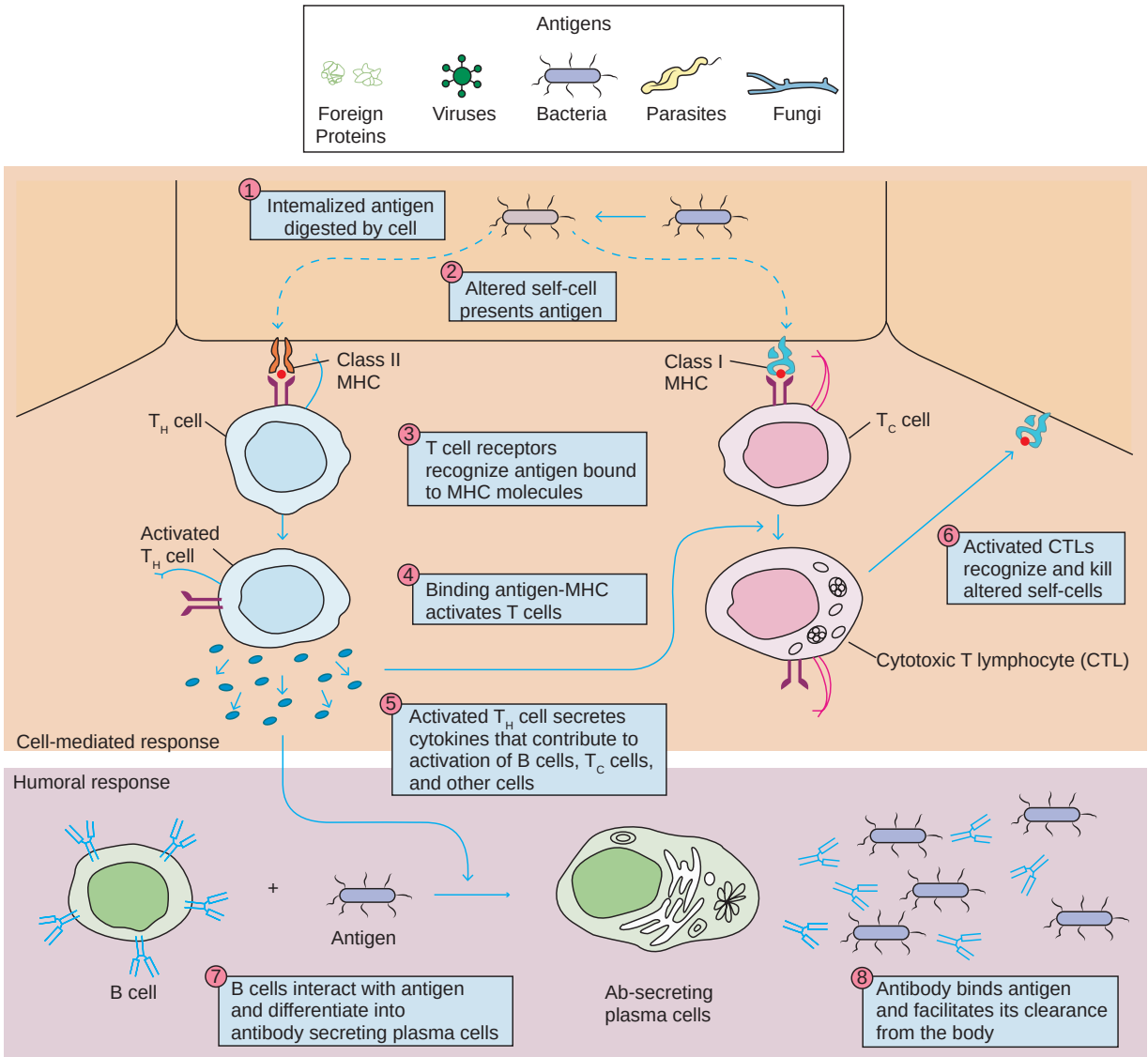
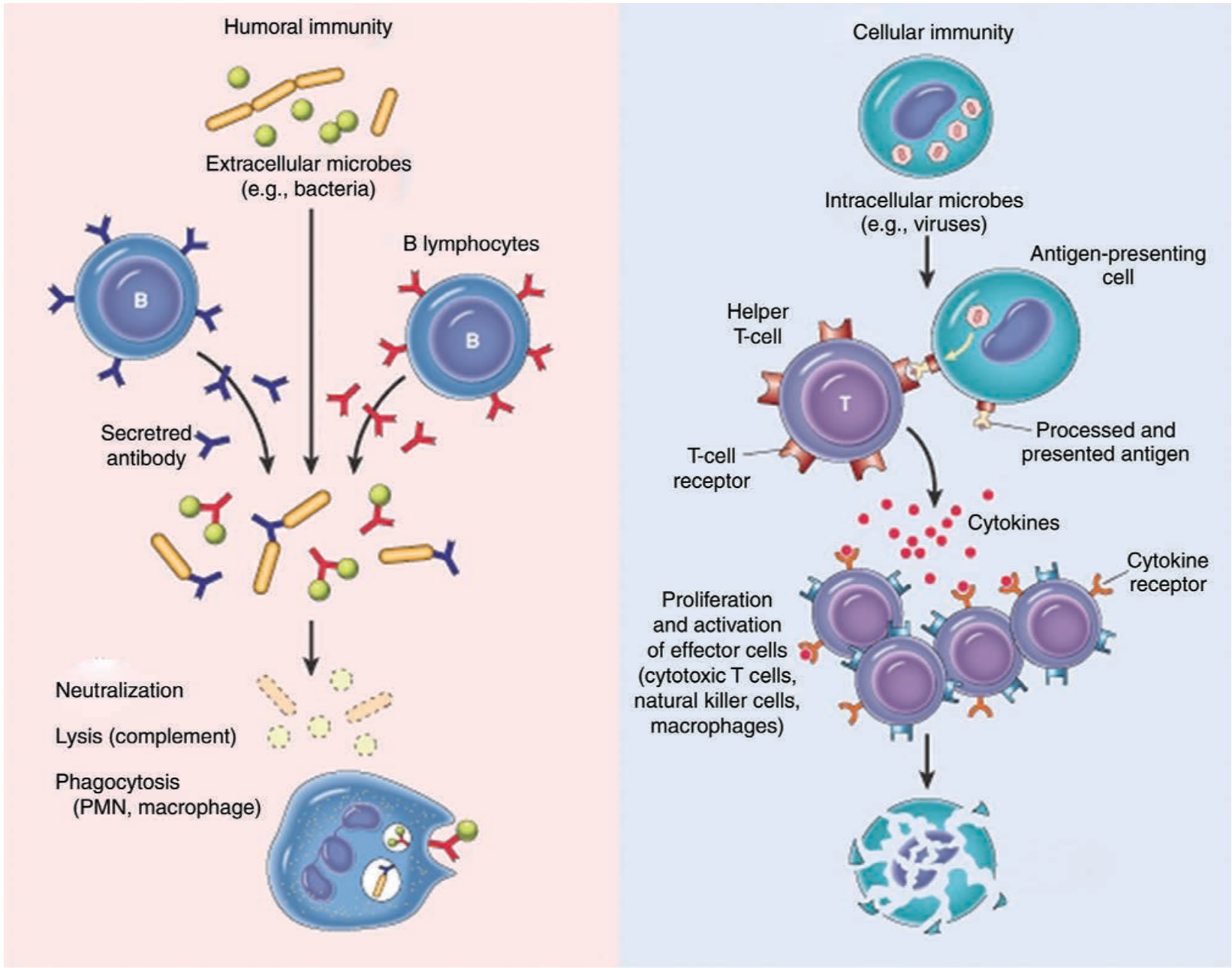


Figure 13.21: Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (Example: virus-infected cells).

Passive Immuno-therapy: Treatment of an infectious disease by administration of previously generated antibodies specific for the infectious pathogen.

antibodies as through colostrum. Specific immunity can be acquired by artificial means actively through immunization or passively through receipt of preformed antibodies as with antisera.


Humoral immunity
Extracellular microbes
(e.g., bacteria)
B lymphocytes
B
B
Secretred
antibody
Neutralization
Lysis (complement)
Phagocytosis
(PMN, macrophage)
Cellular immunity
Intracellular microbes
(e.g., viruses)
Antigen-presenting
cell
Helper
T-cell
T
Processed and
presented antigen
T-cell
receptor
Cytokines
Cytokine
receptor
Proliferation
and activation
of effector cells
(cytotoxic T cells,
natural killer cells,
macrophages)

13.5 Antigens

Substances capable of inducing a specific immune response are called **antigens**. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system.

13.5.1 Immunogenicity Versus Antigenicity

Two properties are exhibited by **antigens**; they are **immunogenicity** and **antigenicity**. Immunogenicity is the ability of an antigen to induce a humoral and / or cell mediated immune response.

B cells + antigen ⟶ effector B cells (Plasma cells) + memory B cells

T cells + antigen ⟶ effector T cells (T_C, T_H cells) + memory T cells

Although a substance that induces a specific immune response is usually called an **antigen**, it is more appropriately called an **immunogen**. **Antigenicity** is the ability of an antigen to combine specifically with the final products of the above responses. (antibodies and/or cell surface receptors). All immunogens are antigens but all antigens are not immunogens. Some small molecules called **haptens** are antigenic but incapable, by themselves, of inducing a specific immune response. In other words haptens lack immunogenicity. Examples of haptens are dinitrophenol, penicillin and m-amino benzene sulphonate.

13.5.2 Factors that Influence Immunogenicity

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. The factors that influence immunogenicity can be divided under two categories.

1. Contribution of the immunogen to immunogenicity
2. Contribution of the biological system to immunogenicity

1. Contribution of the immunogen to immunogenicity

Immunogenicity is determined in part, by the following four properties of the immunogen.

A. Foreignness

The immune system normally discriminates between self and non self, so that only molecules that are foreign to the host are immunogenic. For example, albumin isolated from the serum of a rabbit and injected back into the same or another rabbit will not induce an immune response but the same protein when injected into other vertebrate species (rat) will induce an immune response.

B. Molecular size

There is a correlation between the size of a macromolecule and its immunogenicity. The best immunogens tend to have molecular mass approaching 100,000 daltons (Da). Generally, substances with a molecular mass less than 5000-10000 Da are poor immunogens; however a few substances with a molecular mass less than 1000 Da have proven to be immunogenic.

C. Chemical composition and complexity

Proteins are the most potent immunogens with polysaccharides ranking second. In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides (examples-

lipoprotein or nucleo - protein). For example, attachment of tyrosine chains to the weakly immunogenic protein **gelatin** markedly enhances its immunogenicity.

D. Susceptibility to antigen processing and presentation

The development of both humoral and cell mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC (Major Histocompatibility Complex) molecules. To T_H cells, the antigen must be presented with class II MHC molecules on an antigen presenting cell; to T_C Cells the antigen must be presented with class I MHC molecule on an altered self cell. Macromolecules that cannot be degraded and presented with MHC molecules are poor immunogens. This can be illustrated with polymers of D-amino acids, which are stereoisomers of the naturally occurring L-amino acids. Because the degradative enzymes within antigen presenting cells can degrade only proteins containing L-amino acids, polymers of D-amino acids cannot be processed and thus are poor immunogens (Figure 13.22).

2. Contribution of the biological system to immunogenicity

Even if a macromolecule has the properties that contribute to immunogenicity, its ability to induce an immune response will depend on the following properties of the biological system that the antigen encounters.

A. Genetic constitution of the host animal

The genetic constitution (genotype) of an immunized animal plays an important role in determining whether a given substance will stimulate an immune response. Genetic control of immune responsiveness is largely made by genes mapping within the MHC

B. Immunogen dosage and route of administration

Whether an immunogen will induce an immune response also depends on the dose and mode of administration. A quantity of an immunogen that has no effect when injected intravenously may evoke a good antibody response when injected subcutaneously, particularly if it is accompanied by an adjuvant.

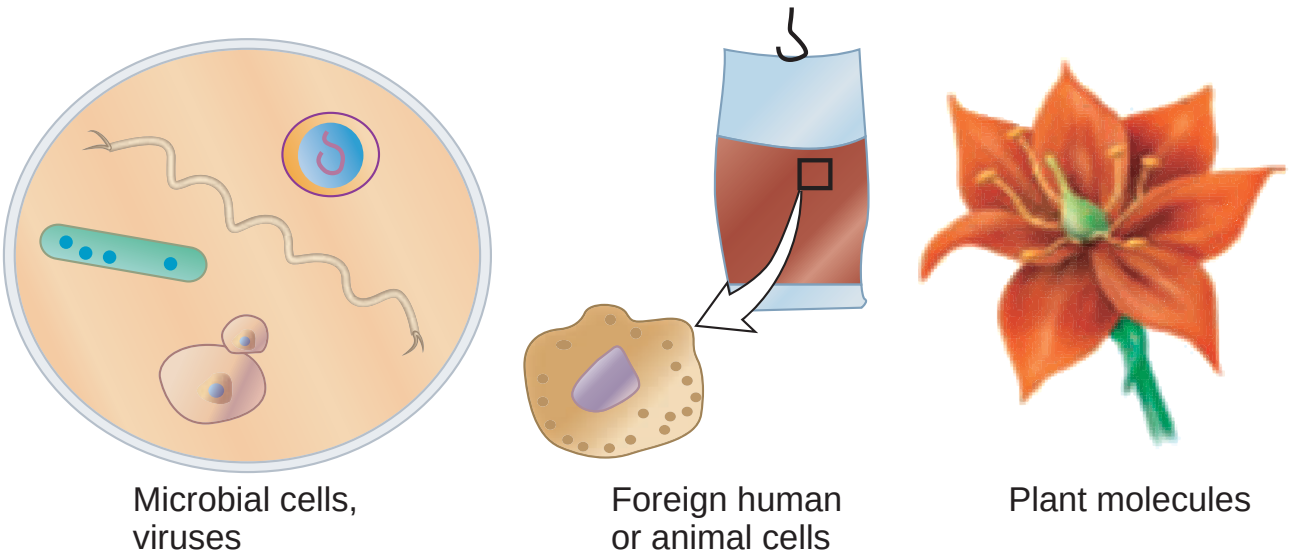


Figure 13.22: Diverse Antigens

C. Adjuvants

The response an immunogen is often enhanced if it is administered as a mixture with **adjuvants.** Adjuvants are substances that enhance the immunogenicity of an antigen. Adjuvants function in one or more of the following ways. (1) by prolonging retention of the immunogen (2) by increasing the effective size of the immunogen or (3) by stimulating the local influx of macrophages and/ or other immune cell types to the injection site and promoting their subsequent activities. Example: Freund's incomplete antigen, Freund's complete antigen, *Mycobacterium tuberculosis,* Aluminum potassium sulphate (alum) and Bacterial lipopolysaccharide (LPS).

13.5.3 Epitopes

F3S8RU

Immune cells do not interact with or recognize an entire immunogen molecule instead; lymphocytes recognize discrete sites on the macromolecule called **epitopes or antigenic determinants**. Epitopes are the immunologically active regions of an immunogen that bind to antigen specific membrane receptors on lymphocytes or to secreted antibodies. Antigenic epitopes may consist of a single epitope or have varying number of the same epitope on the same molecule (Example: polysaccharides).

The size of a single epitope may be 4 or 5 aminoacid or monosaccharide residues. The recognition of antigens by T cells and B cells is fundamentally different B cells recognize soluble antigen when it binds to their membrane-bound antibody. (Figure 13.23 & 13.24). Most T cells recognize only peptides combined with MHC molecules on the surface of antigen presenting cells and altered self cells.

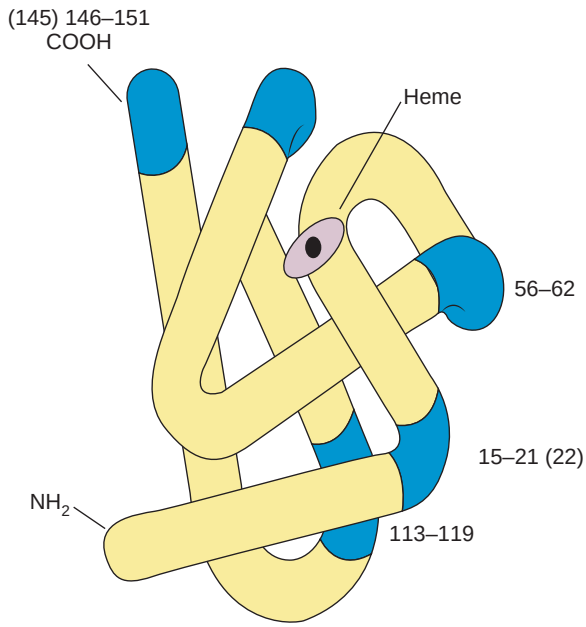


Figure 13.23: Diagram of sperm whale myoglobin showing locations of five sequential B-cell epitopes

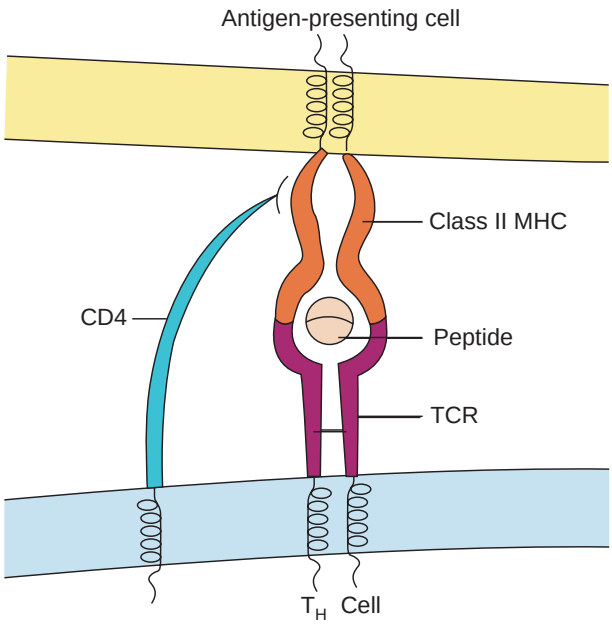


Figure 13.24: Schematic diagram of the ternary complex formed between a T-cell receptor (TCR) on a T_H cell, an antigen and a class II MHC molecule

13.5.4 Haptens and the Study of Antigenicity

The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Landsteiner employed various **haptens**(small organic molecules that are antigenic but not immunogenic). Chemical coupling of a hapten to a large protein called a **carrier,** yields an immunogenic **hapten-carrier conjugate.**

13.5.5 Cross-Reactivity

When two antigens possess structurally similar antigenic determinants, the antibodies obtained to one of these antigens tend to react with the other antigen. These reactions are called **cross reactions.**

Infobits

Penicillin Allergy: New antigens are produced by altering epitopes. This can be done by conjugating haptens to the molecule. A classic example in human medicine is the **allergic response** of some persons to penicillin. A derivative of penicillin, **penicilloic acid** acting as a hapten, can couple with body protein and elicit an immune response that can be harmful, even life threatening, thus excluding this antibiotic from use in certain individuals.

13.6 Antibodies

The first real chemical information regarding the structure of antibodies was provided by **Tiselius** and **Kabat** in the early 1940s. They demonstrated that the gamma globulin fraction of serum proteins that migrated most slowly in electrophoresis contained most of the serum antibodies. This section deals with the structural and biological properties of antibodies (immunoglobulins).

Definition of antibodies

Antibodies are glycoproteins present in serum gamma globulins produced by B-lymphocytes **(B cells)** or **Plasma cells** in response to exposure to antigen. Antibodies are also known as immunoglobulins. They react specially with that antigen *in vivo* or *in vitro* and are hence a part of the **adaptive immune response** specifically, **humoral immunity**.

13.6.1 Structure of an Immunoglobulin Molecule

1. Basic unit

The basic structural unit (monomer) of an immunoglobulin molecule consists of four polypeptide chains linked covalently by disulfide bonds (Figure 13.25). The four-chain structure is composed of two identical light (L) and two identical heavy (H) polypeptide chains. Every immunoglobulin can be represented by the general formula $(H_2L_2)_n$.

a) Light chains

Light Chains have a molecular weight of approximately 25000 Da and are composed of about 220 amino acids. Light chains are common to all immunoglobulin classes and are of two types – **kappa** (κ) or **lambda** (λ) - based on their structural differences. A given immunoglobulin molecule may contain either identical κ or λ chains but never both.

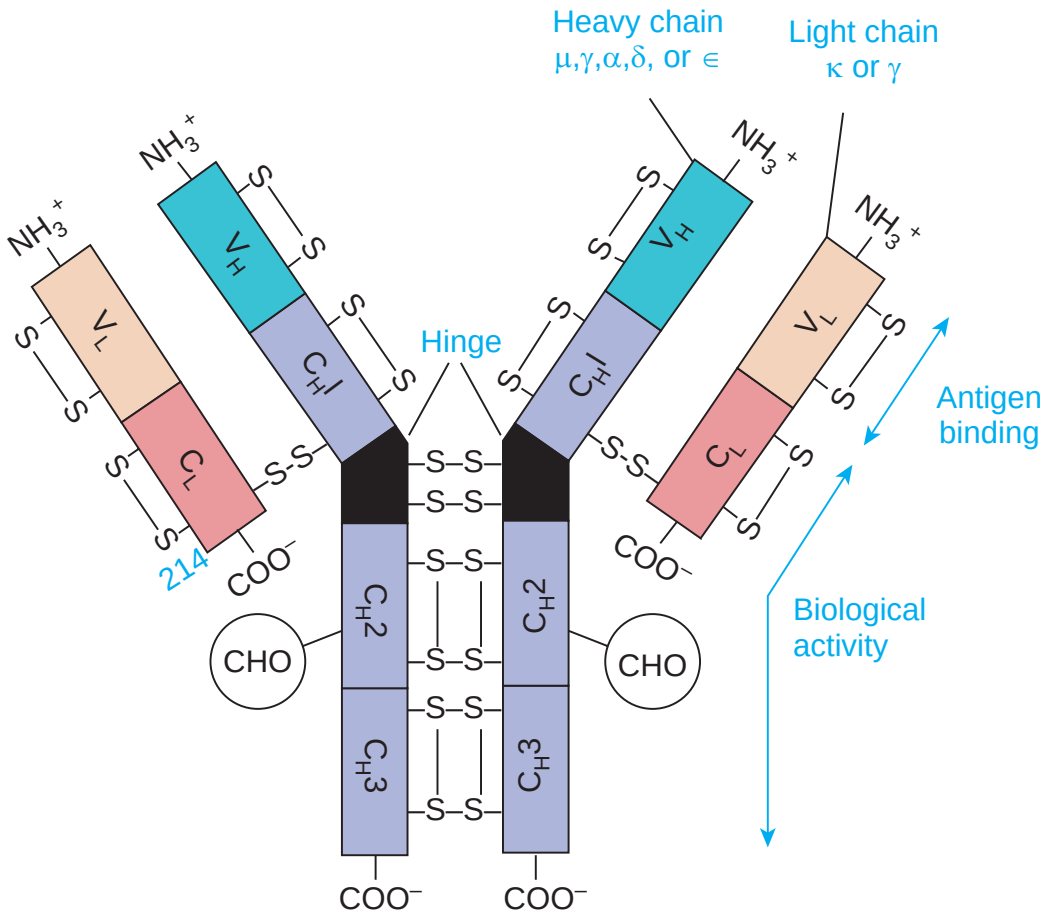


Figure 13.25: Structure of Immunoglobulin

b) Heavy chains

Heavy chains have a molecular weight of approximately twice that of light chains (57000-70000 Da) and twice the number of amino acids (about 440). Five antigenically distinct isotypes of heavy chains are recognized-gamma (γ), alpha (α), mu (μ), delta (δ) and epsilon (ε) – based on structural differences in the carboxy terminal portion of heavy chains. The heavy chains isotypes form the basis of five classes of immunoglobulin molecules – IgG (contains γ chain), IgA (contains α chain), IgM (contains μ chain), IgD (contains δ chain) and IgE (contains ε chain). Five heavy chain classes of immunoglobulin can be easily remembered as GAMDE. Heavy chain classes are again subdivided into subclasses of molecules.

i. Four known subclasses of the γ chain exist – γ1, γ2, γ3 and γ4 - which yield IgG1, IgG2, IgG3 and IgG4.

ii. Two subclasses of the α chain are known – α1 and α2 - which yield IgA1 and IgA2.

iii. Two subclasses of the μ chain are known – μ1 and μ2 - which yield IgM1 and IgM2.

iv. No subclasses of the δ and ε (IgD and IgE) are known.

2. Disulfide bonds

Disulfide bonds hold together the four polypeptide chains in normal immunoglobulin molecules and are of two types namely **interchain bonds** and **intrachain bonds**.

a. **Inter chain bonds** occur between heavy chains (H-H), heavy and light chains (H-L) and light chains (L-L). H-H bonds occur primarily in the hinge region and can vary in number from 1-15 depending on the class and subclass of the immunoglobulin molecules.

b. **Intra chain bonds** are stronger than interchain bonds and occur within the individual chain type, with the number of bonds varying depending on the type (light chains have two, human γ, α and δ heavy chains have four and human μ and ε heavy chains have five). The distribution of intrachain disulfide bonds forms the basis for division of each immunoglobulin into **domains.**

3. Regions

Each heavy and light chain consists of two segments, **the variable region** and **the constant region**. The variable (V) region shows a wide variation in amino acid sequence in the amino terminal portion of the molecule. The areas of high variability in the variable region of heavy (VH) and light (VL) chains are called **hypervariable regions** or **complementarity determining regions (CDRs).** Hypervariable regions are most intimately involved in formation of the antigen binding site.

4. Domains

Each immunoglobulin chain consists of a series of globular regions enclosed by disulphide bonds. Each heavy chain consists of four or five domains - one in the variable region (VH) and three or four in the constant region (CH1, CH2, CH3, and CH4). Each light chain consists of two domains – one in the variable region (VL) and one in the constant region (CL).

5. Fragments.

Proteolytic (peptide bond -splitting) enzymes such as **papain** and **pepsin** are used to degrade immunoglobulin molecules into definable fragments to facilitate study of their structure.

i. Treatment of the monomeric basic unit with the enzyme papain splits it into two Fab fragments (**Fragment-antigen binding**) and one Fc fragment. These Fab fragments can bind but cannot precipitate the antigen; therefore, they are monovalent, possessing only one combining site each.

ii. Treatment of the immunoglobulin molecule with pepsin results in digestion of most of the Fc fragment, leaving one large fragment that consists of two Fab fragments joined by covalent bonds, termed the **F(ab')2 fragment**. The F(ab')2 fragments has two antigen combining sites. Therefore it is bivalent, possessing the ability to bind and precipitate an antigen (Figure 13.26).

6. Hinge region

Hinge region is the portion of heavy chain between the CH1 and CH2 domains. It is highly flexible and allows for movement of the Fab arms in relation to each other. The S values (**sedimentation coefficient** that is expressed in **Svedberg units(s)**) of immunoglobulins range from 7S- 19S.

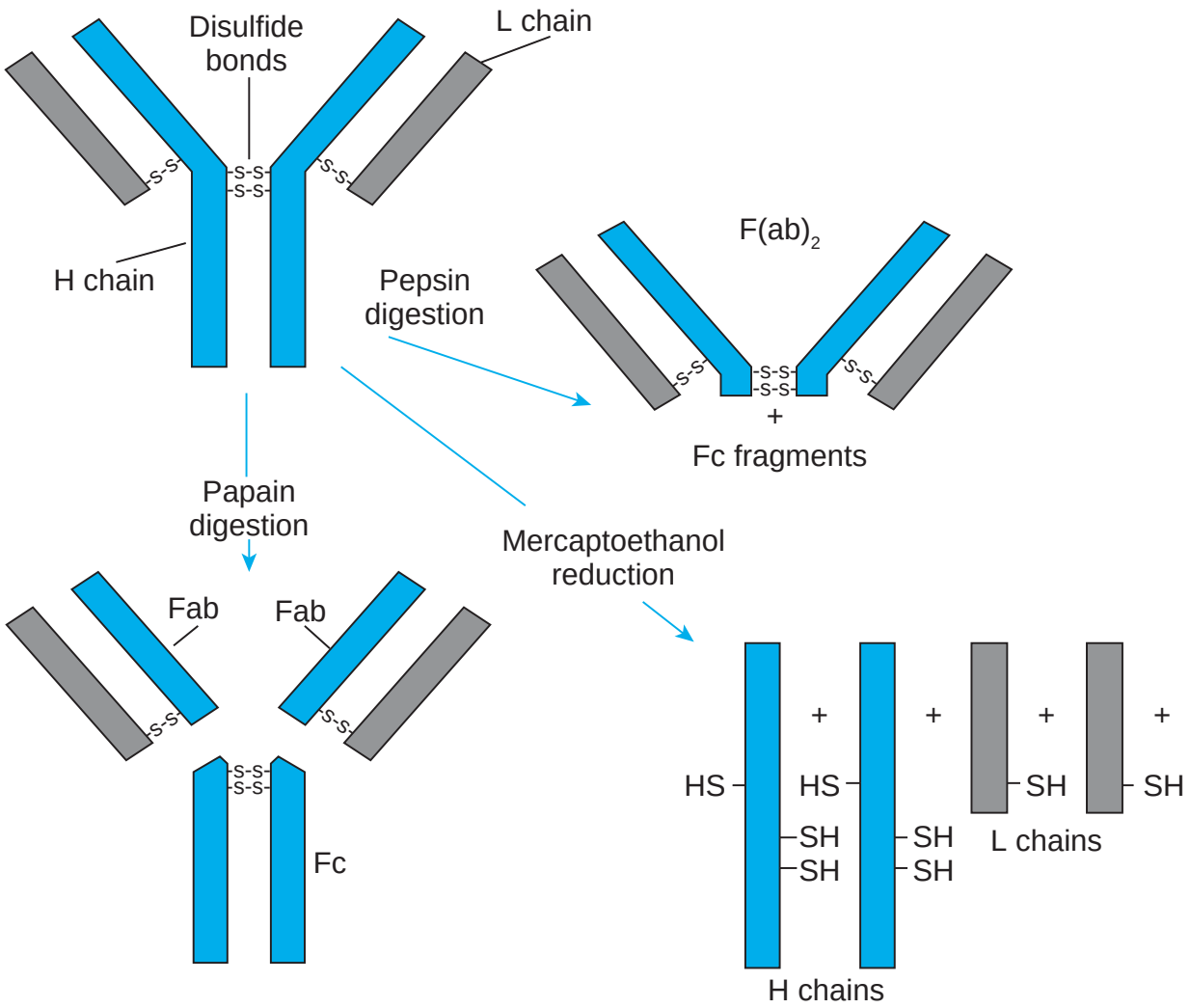


Figure 13.26: Prototype structure of IgG, showing chain structure and interchain disulfide bonds

13.6.2 Immunoglobulin Function

There are three major effector functions that enable antibodies to remove antigens and kill pathogens. **Opsonization** promotes antigen phagocytosis by macrophages and neutrophils. **Complement activation** by IgM and IgG can activate a pathway that leads to the generation of a collection of proteins that can perforate cell membranes. **Antibody-dependent cell-mediated cytotoxicity** (ADCC) can cause NK cell mediated death of target cells when antibody bound to the target cells associates with Fc receptors of natural killer (NK) cells.

HOTS

Which antibody protects the new born for few months against infections?

13.6.3 Properties and Activities of Immunoglobulin Classes

Each immunoglobulin class differs in its general properties, distribution in the body and interaction with other components of the host defensive systems.

i) IgG

1. IgG is the major immunoglobulin in human serum, accounting for 80% of the immunoglobulin pool.
2. It is present in blood plasma and tissue fluids. It has a monomeric structure.
3. IgG class acts against bacteria and viruses by opsonizing the invaders and neutralizing toxins and viruses.
4. IgG molecules are capable of fixing complement, except for IgG4.
5. It is the major antibody in the secondary immune response and it has half life of 23 days.
6. IgG is the only immunoglobulin molecule able to cross the placenta and provides natural immunity *in utero* and to the neonate at birth.

ii) IgA

It is present in the serum and in various bodily secretions and thus takes two forms – serum IgA and secretory IgA (sIgA)

A) Serum IgA

1. It accounts for about 12% of serum immunoglobulin.
2. In humans, over 80% of serum IgA exists in a monomeric form and the remaining existing as polymers in the form of dimers, trimers or tetramers. In polymeric IgA, the monomeric units are linked by disulphide bonds and joining (J) chain.
3. Serum IgA fixes complement via the alternative pathway. It has a half life of 5 days.

B) Secretory IgA

1. Secretory IgA is the primary immunoglobulin of mucosal associated lymphoid tissue (MALT). It is also found in saliva, tears, and breast milk.
2. It consists of two monomeric units plus J chain and secretory component (Figure 13.29).
3. The dominant subclass of sIgA is sIgA2 which is unique for its absence of a covalent bond between the light and heavy chains. In this subclass, light chains are linked by disulphide bonds.
4. It has a half life of 5-6 days. It is responsible for local immunity.
5. The sIgA molecules protect mucosal surfaces by reacting with the surface of potential pathogens and interfering with their adherence and colonization. It also plays a role in the alternative complement pathway.

iii) IgM

1. IgM accounts for about 5-10% of the serum immunoglobulin pool.
2. It has a pentameric structure consisting of five monomeric units linked by J chain and disulphide bonds at the Fc fragment (Figure 13.27).
3. It is the predominant antibody in the primary immune response to most antigens and the predominant antibody produced by the fetus.
4. It is the first immunoglobulin made during B cell maturation and individual IgM monomers are expressed on B cells, serving as the antibody component of the B cell receptor (BCR).

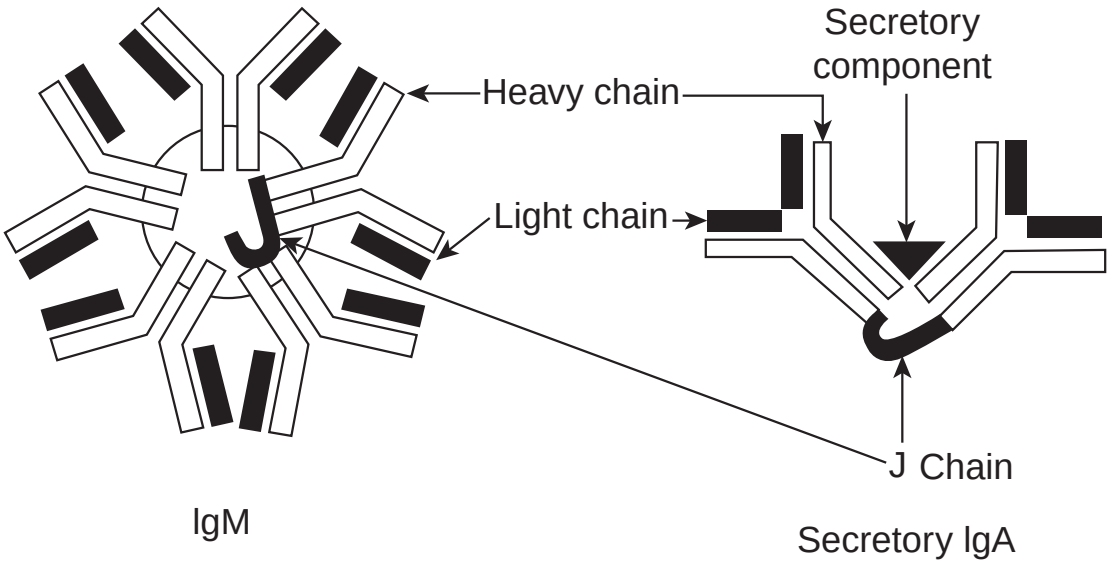


Figure 13.27: Structural models of IgM and secretory IgA. IgM has a pentameric structure linked by J chain at the Fc fragment. Secretory IgA has a dimeric structure plus J chain plus secretory component and is shown in the dominant IgA2 subclass, which is unique for its absence of a convalent bond between the light and heavy chains. Light chains are linked by disulfide bonds.

5. IgM tends to remain in the bloodstream, where it agglutinates (clumps) bacteria, activates complement by the classical pathway and enhances the ingestion of pathogens by phagocytic cells.
6. It has a half life of approximately 5 days.

iv) IgD

1. IgD accounts for about less than 1% of the total immunoglobulin pool.
2. One unique structural feature is the presence of only a single H-H inter chain bond along with two H-L interchain bonds.
3. It has a monomeric structure similar to that of IgG.
4. IgD antibodies are abundant in combination with IgM on the surface of B cells and thus are part of the B cell receptor complex. Therefore their function is to signal the B cell to start antibody production upon initial antigen binding.
5. It has a half life of 2-3 days.

v) IgE

1. IgE accounts for only 0.004% of serum immunoglobulin. It has a monomeric structure. It is also called reagin or reaginic antibody.
2. The skin sensitizing and anaphylactic antibodies belong to this class.
3. The Fc portion of IgE can bind to Fc receptors specific for IgE that are found on mast cells, eosinophils and basophils. Thus these cells can become coated with IgE molecules. When two cell-bound IgE molecules are cross linked by binding to the

Infobits

i) Isotype

Isotypic determinants are constant region determinants that collectively define each heavy chain class and subclass and each light chain type and subtype within a species. Each isotype is encoded by a separate constant region gene and all members of a species carry the same constant region genes. Within a species, each normal individual will express all isotypes in the serum. Different species inherit different constant region genes and therefore express different isotypes. Therefore, when an antibody from one species is injected into another species, the isotypic determinant will be recognized as foreign, inducing an antibody response to the isotypic determinants on the foreign antibody.

ii) Allotype

Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle aminoacid differences, called **allotypicdeterminnats**that occur in some.

The unique amino acid sequence of the VH and VL domains of a given antibody can function not only as an antigenic binding site but also as a set of antigenic determinants. Therefore, the idiotypic determinants are generated by the conformation of the heavy and light chain variable regions. Each individual determinant is called an idiotope and the sum of the individual idiotopes is the idiotype. Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized.

Polyclonal Antibody: A mixture of antibodies produced by a variety of B-cell clones that have recognized the same antigen. Although all of the antibodies react with the immunizing antigen, they differ from each other in amino acid sequence.

Breast Milk: Breast milk is uniquely suited to the human infant's nutritional needs and is a live substance with unparalleled immunological and anti-inflammatory properties that protect against a host of illnesses and diseases for both mothers and children. All five classes of immunoglobulins have been found in human milk, but by far the most abundant type is IgA, specifically the form known as secretory IgA.

Antitetanus Serum: Antitetanus serum, also known as tetanus immune globulin (TIG) is made up of antibodies against the tetanus toxin. It is used to prevent tetanus in those who have a wound that is at high risk and have not been fully vaccinated with tetanus toxoid. It is also used to treat tetanus along with antibiotics and muscle relaxants. It is given by injection into a muscle.

same antigen, the cells degranulate. This degranulation releases histamine and other mediators of inflammation.

4. IgE also stimulates production of an excessive number of eosinophils in the blood (eosinophilia) and increased rate of movement of the intestinal contents (gut hypermotility) which aid in the elimination of helminthic parasites. IgE has a half life of 2-3 days.

13.6.4 Antigenic Determinants on Immunoglobulins

Since antibodies are glycoproteins, they can themselves function as potent immunogens to induce an antibody response. Such anti-Ig antibodies are powerful tools for the study of B cell development and humoral immune response. The antigenic determinants or epitopes, on immunoglobulin molecules fall into three major categories: **isotypic, allotypic and idiotypic**deteminants, which are located in characteristic portions of the molecule.

13.7 Antigen – Antibody Reactions

Antigen and antibody combine with each other specifically and in an observable manner. The exquisite specificity of antigen-antibody interactions has led to the development of a variety of immunological assays. These assays can be used to detect the presence of either antibody or antigen. These assays are also helpful in diagnosing diseases, monitoring epidemiological surveys and identifying molecules of biological or medical interest. Antigen-antibody reactions in vitro are known as serological reactions.

13.7.1 Three stages of Antigen – Antibody Reactions

a) Primary stage

The reactions between antigen and antibody occur in three stages. The **primary stage** is the initial interaction between the two without any visible effects. This reaction is rapid and obeys the general laws of physical chemistry and thermodynamics. The reaction is reversible. The combination between antigen and antibody is effected by the weaker intermolecular forces such as electrostatic forces, hydrogen bonds, Van der Waals forces and hydrophobic forces. The primary reaction can be detected by estimating free and bound antigen or antibody separately in the reaction mixture by a number of physical and chemical methods including the use of markers such as radioactive isotopes, fluorescent dyes or enzymes.

b) Secondary stage

The primary stage is followed by the secondary stage leading to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralization of motile organisms, complement fixation and enhancement of phagocytosis.

c) Tertiary stage

Some antigen-antibody reactions occurring in vivo initiate chain reactions that lead to neutralization or destruction of injurious antigens or to tissue damage. These are the tertiary reactions and include humoral immunity against infectious diseases as well as clinical allergy and other immunological diseases.

13.7.2 General Features of Antigen – Antibody Reactions

Antigen-antibody reactions have the following general characteristics:

1. The antigen-antibody reaction is specific. An antigen combines only with its homologous antibody and vice versa. However, the specificity is not absolute and cross reactions may occur due to antigenic similarity or relatedness.
2. An entire molecule reacts and not fragments.
3. There is no denaturation of the antigen or the antibody during the reaction.
4. The combination occurs at the surface.
5. The combination is firm but reversible. The firmness of the union is influenced by the affinity and avidity of the reaction. **Affinity** is the strength of binding of one molecule to another at a **single site**, such as the binding of a monovalent Fab fragment of antibody to a monovalent antigen. **Avidity** is the sum total of the strength of binding of two molecules to one another at **multiple sites**.
6. Both antigen and antibody participate in the formation of agglutinates or precipitates.
7. Antigens and antibodies can combine in varying proportions, unlike chemicals with fixed valence. Both antigens and antibodies are multivalent. Antibodies are bivalent. Antigens may have valencies up to hundreds.

13.7.3 Measurement of Antigen and Antibody

Many methods are available for the measurement of antigens and antibodies participating in the primary, secondary and tertiary reactions. Measurement may be in terms of mass (Example: mg Nitrogen) or more commonly as units or titre. The antibody titre of a serum is the highest dilution of the serum which gives an observable reaction with the antigen in the particular test. The titre of a serum is influenced by the nature and quantity of the antigen and the type and conditions of the test. Antigens may also be titrated against sera.

Two important parameters of serological tests are sensitivity and specificity**. Sensitivity** refers to the ability of the test to detect even very minute quantities of antigen and antibody. When a test is highly sensitive, false negative results will be absent or minimal. **Specificity** refers to the ability of the test to detect reactions between homologus antigens and antibodies only. When a test is highly specific, false positive results will be absent or minimal. Some tests are qualitative and others are quantitative. The various tests used for detection of antigen and antibodies are given below:

1. Precipitation tests
2. Agglutintion tests
3. Complement Fixation test
4. Immunofluorescence
5. Radio immuno assay
6. Enzyme linked immuno sorbent assay
7. Western Blotting technique
8. Neutralization test

In this section Agglutination and Precipitation reactions will be described in detail.

1. Precipitation reactions

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen-antibody complex, forms an insoluble (visible) precipitate and this reaction is called **precipitation**. When instead of sedimenting, the precipitate remains suspended as floccules, the reaction is known as **flocculation.**

- **Applications of precipitation reactions**

The following types of precipitation tests are in common use:

a) Ring test

This test consists of layering the antigen solution over a column of antiserum in a narrow tube. A visible precipitate forms at the junction of the two liquids. Examples of ring precipitation test are the C- reactive protein test, Ascoli's thermoprecipitin and the grouping of streptococci by the Lancefield technique.

b) Slide test

When a drop of antigen and a drop of antiserum are placed on a slide and mixed by shaking, floccules appear. The VDRL test for syphilis is an example of slide flocculation.

c) Tube test

A quantitative tube flocculation test is used for the standardization of toxins and toxoids. Serial dilution of the toxin / toxoid is added to the tube containing a fixed quantity of the antitoxin. The toxin or toxoid that flocculates optimally with one unit of the antitoxin is defined as the Lf (Lethal Flocculation) dose.

Precipitation reaction in gels

There are several advantages in allowing precipitation to occur in a gel rather than in a liquid medium. The reaction is visible as a distinct band of precipitation, which is stable and can be stained for preservation, if necessary. Imunodiffusion is usually performed in 1% agarose gel. Different modifications of the test are available.

- Single Diffusion in One Dimension (Oudin Procedure)
- Double Diffusion in One Dimensions (Oakley-Fulthorpe Procedure)
- Single Diffusion in Two Dimensions (Mancini Procedure)
- Double Diffusion in Two Dimensions (Ouchterlony Procedure)

Immunoelectrophoresis

Immunoelectrophoresis was devised by Grabar and Williams (1953). This method consists of two steps. The first step is agarose electrophoresis of the antigen. Rectangular trough is then cut into the agarose gel parallel to the direction of the electric field and is filled with the antiserum. By diffusion, lines of precipitation develop with each of the separated compounds (Figure 13.28). This method is used to detect normal and abnormal serum proteins.

1. Semisolid agar layered on the glass slide. A well for antigen and a trough for antiserum cut out of agar.
2. Antigen well filled with human serum.
3. Serum separated by electrophoresis.
4. Antiserum trough filled with antiserum to whole human serum.
5. Serum and antiserum allowed to diffuse into agar.

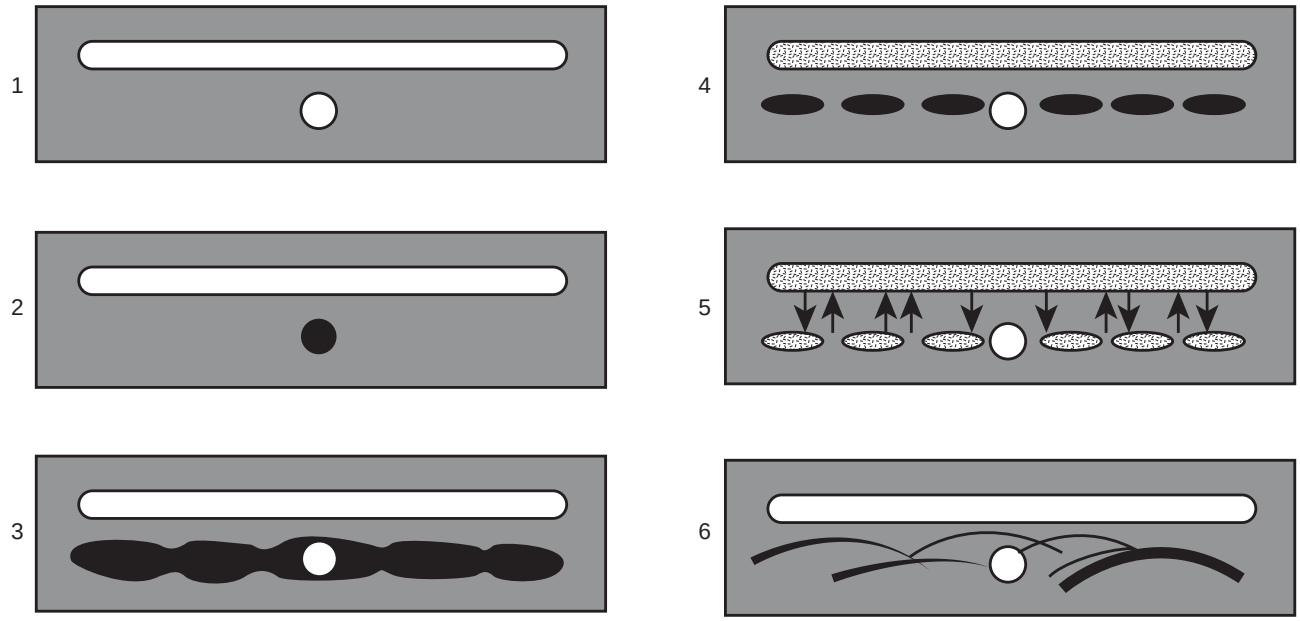


Figure 13.28: Immunoelectrophoresis

6. Precipitin lines form for individual serum proteins
   - Counterimmunoelectrophoresis
   - Rocket Electrophoresis

2. Agglutination reactions

When a particulate antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated, and the reaction is called **agglutination.**

Agglutination is more sensitive than precipitation for detection of antibodies. Agglutination occurs optimally when antigens and antibodies react in equivalent proportions. Incomplete or monovalent antibodies (having only one antigen combining site) do not cause agglutination, though they combine with the antigen. They may act as blocking antibodies inhibiting agglutination by the complete antibody added subsequently.

Direct agglutination test

In **the direct technique**, a cell or insoluble particulate antigen is agglutinated directly by antibody. An example is the agglutination of group A erythrocytes by anti-A sera.

Indirect (Passive) agglutination test

Passive agglutination refers to agglutination of antigen coated cells or inert particles (bentonite or latex particles) which are passive carriers of soluble antigens. An example is the latex agglutination for detection of rheumatoid factor. When instead of the antigen, the antibody is adsorbed to carrier particles in test for estimation of antigen, this technique is known as **reverse passive agglutination**.

Hemagglutination inhibition method

The inhibition of agglutination of antigen-coated red blood cells by homologous antigen is a highly sensitive and specific method for detecting small quantities of soluble antigen in blood or other tissue fluids. The principle of this method is that antibody preincubated with soluble homologous antigen will be inactivated when incubated with antigen coated red blood cells.

This method is used in the detection of HBs Ag in hepatitis and in the detection of factor VIII antigen in hemophilia.

Hemagglutination inhibition is also used to detect antibodies against certain viruses (Arbovirus, Influenza, Measles and Rubella). These viruses are able to agglutinate red blood cells because they possess hemagglutinins on their outer surfaces.

- **Applications of agglutination reactions**

a) Slide agglutination

When a drop of the appropriate antiserum is added to a smooth uniform suspension of a particulate antigen in a drop of saline on a slide, agglutination takes place. A positive result is indicated by the clumping together of the particles and the cleaning of the drop. Mixing the antigen and the antiserum by gently rocking the slide facilitates the reaction.

It is essential to have on the same slide a control consisting of the antigen suspension in saline, without the antiserum, to ensure that the antigen is not autoagglutinable. Agglutination is visible to the naked eye but may sometimes require confirmation under the microscope. Slide agglutination is a routine test for the identification of many bacterial isolates from clinical specimens. It is also the method used for blood grouping and cross matching.

b) Tube agglutination

This is a standard quantitative method for measurement of antibodies. When a fixed volume of a particulate antigen suspension is added to an equal volume of serial dilution of an antiserum in test tubes, the agglutination titre of the serum can be estimated. **Widal test** done for **typhoid** and Weil Felix test done for **rickettsial infections** are examples of Tube agglutination.

Latex agglutination test

Here latex particles are used as passive carriers for adsorbed soluble antigens. The most widespread application of latex agglutination has been in the detection of rheumatoid factor. In rheumatoid arthritis, the patient's produces rheumatoid factor. Rheumatoid factor is a pentameric IgM antibody directed against IgG. The test consists of coating latex particles with IgG and reacting them with the patient serum. Agglutination indicates a positive test. Latex agglutination tests are also employed in the clinical laboratory for detection of HBs Ag, ASO (Antistreptolysin O) and CRP (Carbohydrate Reactive Protein)

Coombs test (antiglobulin test)

This test was devised by Coombs, Mourant and Race (1945) for the detection of anti-Rh antibodies that do not agglutinate Rh-positive red blood cells in saline. When sera containing incomplete anti-Rh antibodies are mixed with Rh- positive red blood cells, the antibody globulin coats the surface of the red blood cells, though they are not agglutinated. When such red blood cells coated with antibody globulin are washed free of all unattached protein and treated with a rabbit antiserum against human gammaglobulin (antiglobulin or Coombs serum), the cells are agglutinated. This is the principle of the Coombs test (Figure 13.29).

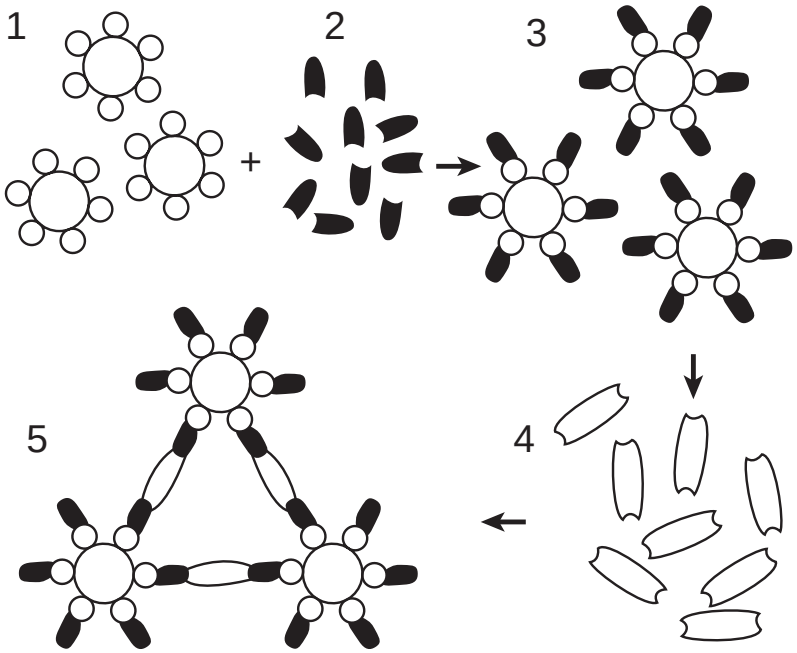
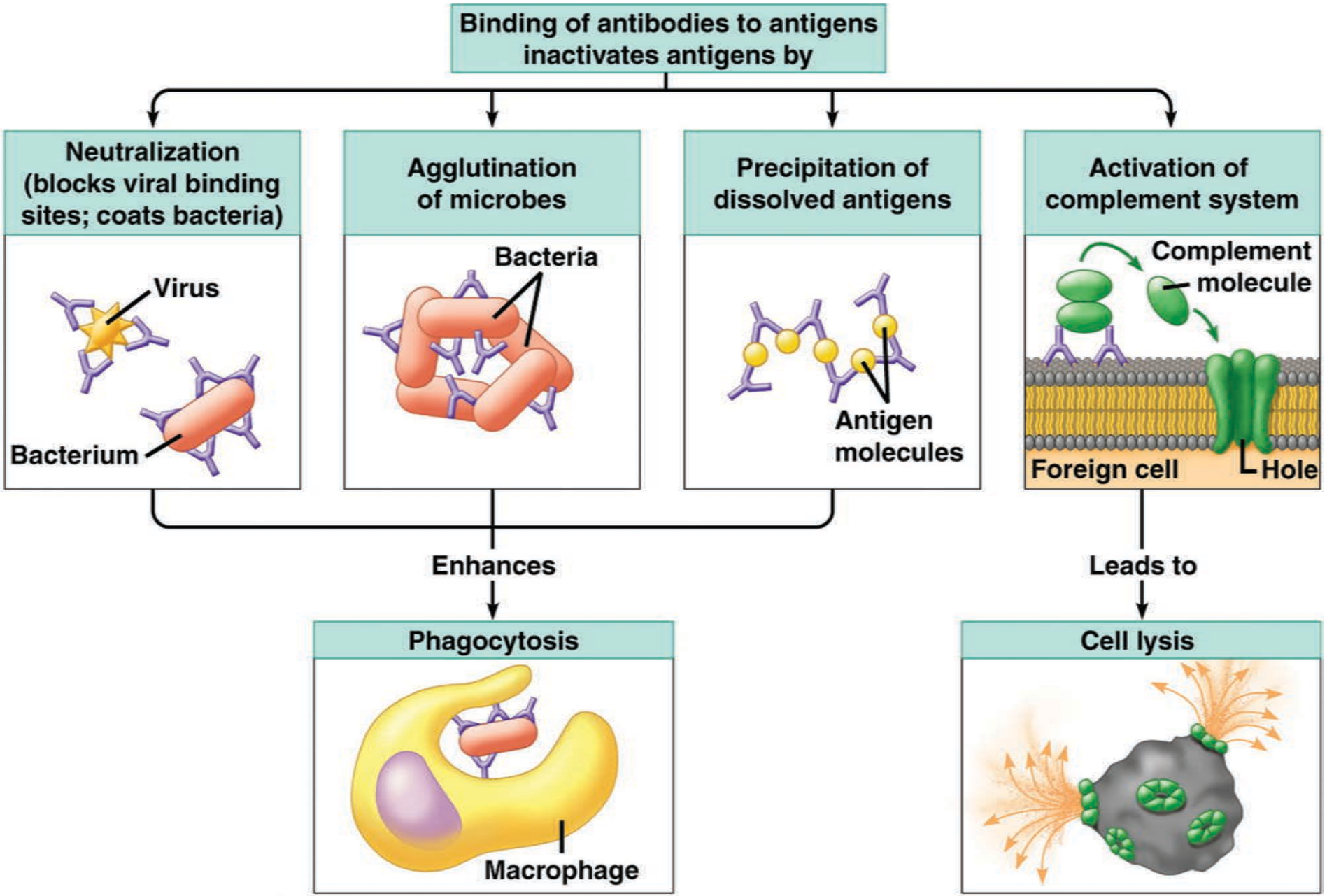


Figure 13.29: Antiglobulin (Coombs) test Rh positive erythrocytes (1) are mixed with incomplete antibody (2). The antibody coats the cells (3) but, being incomplete, cannot produce agglutination. On addition of antiglobulin serum (4) which is complete antibody to immunoglobulin, agglutination takes place.

The Coombs test may be of the direct or the indirect type.

Applications of coombs test

1. Erythrocyte typing in blood banks.
2. The evaluation of hemolytic disease of the newborn.
3. The diagnosis of autoimmune hemolytic anemia.


Binding of antibodies to antigens inactivates antigens by
Neutralization (blocks viral binding sites; coats bacteria)
Virus
Bacterium
Agglutination of microbes
Bacteria
Precipitation of dissolved antigens
Antigen molecules
Activation of complement system
Complement molecule
Foreign cell
Hole
Enhances
Leads to
Phagocytosis
Macrophage
Cell lysis

Summary

Immunology began as a study of the response of the whole animal to infection. Over the years, it has become progressively more basic, passing through phases of emphasis on serology, cellular immunology, molecular immunology and immunogenetics.

The thymus and bone marrow are the primary lymphoid organs. The primary lymphoid organs provide sites for the development and maturation of B and T lymphocytes.

The secondary lymphoid organs function to capture antigen and provide sites where lymphocytes interact with that antigen and undergo clonal proliferation and differentiation into effector cells. The lymphatic system drains the tissue spaces and interconnects many organized lymphoid tissues. The spleen, lymphnodes and mucosal associated tissues (GALT and SALT) are secondary lymphoid organs. Lymph nodes are specialized to trap antigen from regional tissue spaces, whereas the spleen traps blood- borne antigens.

The cells that participate in the immune response are white blood cells or leukocytes. All of the white blood cells develop from a common pluripotent stem cell in hematopoiesis. Lymphocytes are the central cells of the immune system and are responsible for acquired immunity. The other types of white blood cells play ancillary roles such as engulfing and destroying microorganisms, presenting antigens and secreting cytokines. Basophills and mast cells are non phagocytic granulocytes that play a role in allergic responses. Eosinophils are motile phag ocytic cells. Their phagocytic role is less important than that of neutrophils. They play a role in the defense against parasitic organisms. Macrophages and neutrophils are the accessory cells of the immune system that phagocytose and degrade antigens. Dendritic cells are antigen presenting cells. They play an important role in T_H cell activation by processing and presenting antigen bound to class II MHC molecules. Lymphocytes can be subdivided into B lymphocytes, T lymphocytes and null cells (NK cells). The two major subpopulations of T lymphocytes are T helper (T_H) cells and T cytotoxic (T_C) cells.

Immunity is the state of protection against foreign organisms or substances (antigens). Innate immunity offers resistance to any microorganism or foreign material. It has no immunological memory. Acquired immunity resists particular foreign agent. Moreover, acquired immunity improves on repeated exposure to the agent. Mechanisms of innate immunity include physical barriers, chemical mediators, phagocytosis and inflammation. Acquired (adaptive) immunity refers to the type of specific immunity that a host develops after exposure to a suitable antigen. Two branches or arms of acquired immunity are humoral (antibody mediated) immunity, and cellular (cell mediated) immunity. The important features of acquired immunity are memory, specificity, diversity and discrimination between self and nonself. The humoral immunity is best suited for elimination of extracellular antigens. The cellular immunity is best suited for elimination of intracellular antigens. Acquired immunity can be obtained actively or passively by natural or artificial means.

Immunogenicity is the ability of an antigen to induce an immune response by either the humoral or cell mediated branch of the immune system. Antigenicity is the ability of an antigen to interact specifically with free antibody and/or antigen binding receptors on lymphocytes. The foreignness, molecular size, chemical composition and susceptibility to antigen processing and presentation influence the immunogenicity of a substance. In addition, several properties of biological system that an antigen encounters affect its immunogenicity; these include the genetic constitution of the host animal, the immunogen dosage and the route of administration and the presence or absence of adjuvants. Epitopes are the regions or sites of the antigen that bind to a specific antibody or T cell receptor. Haptens are small molecules that can bind to antibodies but cannot by themselves induce an immune response. The conjugate formed by coupling a hapten to a large carrier protein is immunogenic and elicits production of antihapten antibodies when injected into an animal. The cross reactivity is the ability of a particular antibody or T cell receptor to react with two or more antigens that possess a common epitope. Antibodies are immunoglobulins which are produced by B cells or plasma cells in response to antigenic stimulation.

Antibodies are a group of glycoproteins present in the blood tissue fluids and mucous membranes of vertebrates. All immunoglobulins have a basic structure composed of four polypeptide chains (two lights and two heavy) connected to each other by disulphide bonds. In any given antibody molecule, the constant region contains one of five basic heavy chain sequences (γ, α, μ, δ and ε) and one of two basic light chain sequences (k or γ). There are three major effector functions of immunoglobulins are Opsonization, Complement activation and Antibody-dependent cell-mediated cytotoxicity (ADCC).

In vitro antigen-antibody reactions (serological reactions) provide methods for the diagnosis and for the identification and quantitation of antigens and antibodies.

The reactions between antigen and antibody occur in three stages viz, primary stage, secondary stage and tertiary stage.

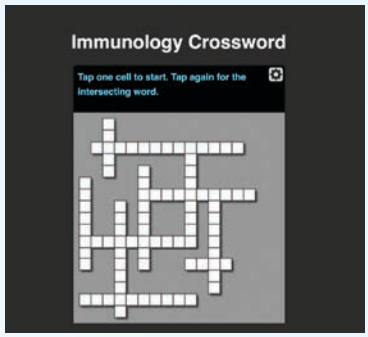
The interaction between a soluble antigen and precipitating antibody (precipitin) produces visible precipitate or precipitation. Precipitation reactions can be performed in liquids or gels. Most are useful primarily for quantitative comparison of antibodies or antigens. Electrophoresis can be combined with precipitation in gels a technique called immunoelectrophoresis.

The interaction between a particulate antigen and agglutinating antibody (agglutinin) produces visible clumping or agglutination. In some cases, the antigen is membrane protein on a bacterial cell or red blood cell. In other cases, the antigen may be attached to a latex particle or adsorbed on the surface of a red blood cell. Agglutination reactions are more sensitive and much faster than precipitation reactions and can detect 100 or 1000 fold lower levels of antigen or antibody than can be detected with a precipitation reaction.

ICT CORNER

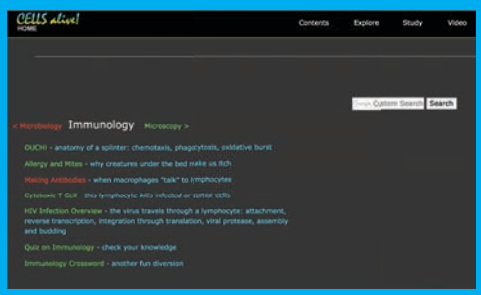
Immunology

How are we protected from microbes?

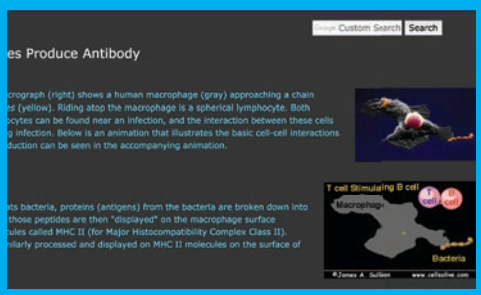


STEPS:

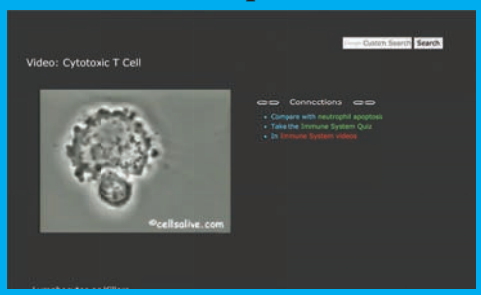
- Use the link or Scan the QR code given below. "Cells Alive-Immunology" will open. You can select any topic you wish. For example click "Making Antibodies"
- 'Making Antibodies' page will open. You can go through How 'Lymphocytes Produce Antibody', 'Antigen Processing', etc….
- From the top of the page click on 'Video' and select 'watch' view video topics. Select 'Cytotoxic T Cells'.
- From the top select 'Study' and then 'Quiz' to answer the questions for the topic you choose.



Step1



Step2



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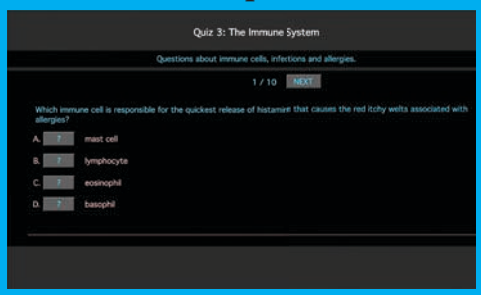

Step4

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https://www.cellsalive.com/toc_micro.htm

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Evaluation

Multiple choice questions

1. Who coined the term vaccine?
   a. Jenner b. Pasteur
   c. Koch d. Roux
2. Who advanced the idea that immunity was primarily due to white blood cells?
   a. Metchinikoff b. Ehrlich
   c. Wright d. Kitasato
3. Which of the following does apply uniquely to secondary lymphoid organs?
   a. Presence of precursor B and T cells.
   b. Circulation of lymphocytes.
   c. Terminal differentiation.
   d. Cellular proliferation.
4. Which of the following is the major function of the lymphoid system?
   a. Acquired immunity.
   b. Innate immunity.
   c. Inflammation.
   d. Phagocytosis.
5. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the
   a. Paracortex b. Cortex
   c. Medulla d. Thymus
6. The myeloid progenitor gives rise to
   a. Erythrocytes, neutrophils, eosinophils, basophils, monocytes, mast cells and platelets.
   b. Erythrocytes, eosinophils, basophils, monocytes, mast cells, platelets and B lymphocytes.
   c. Erythrocytes, eosinophils, basophils, monocytes, mast cells, platelets and T lymphocytes.
   d. Erythrocytes, eosinophils, neutrophils, basophils, monocytes, mast cells and NK cells.
7. Which of the following is a correct statement about NK cells?
   a. They kill target cells by phagocytosis and intracellular digestion.
   b. They proliferate in response to antigen.
   c. They kill target cells in an extracellular fashion.
   d. They are a subset of polymorphonuclear cells.
8. Which of the following cells play an important role in the development of allergies?
   a. Neutrophils b. Mast cells
   c. Monocytes d. Dendritic cells
   c. Absence of specificity
   d. Activation by a stimulus
9. All of the following will act as opsonins except
   a. Complement b. Antibody
   c. Acute – phase proteins
   d. Lactoferrin
10. Which of the following is not the important feature of acquired immunity?
    a. Phgocytosis b. Memory.
    c. Specificity
    d. Discrimination between self and non-self

11. Cell mediated immunity is brought about by
    a. B cells b. T cells
    c. NK cells d. Null cells
12. Vaccines induce immunity that is
    a. Naturally acquired active immunity.
    b. Naturally acquired passive immunity.
    c. Artificially acquired passive immunity.
    d. Artificially acquired active immunity.
13. Haptens
    a. Require carrier molecules to be immunogenic.
    b. Interact with specific antibody, even if the haptens are monovalent.
    c. Cannot stimulate immune responses without carriers.
    d. All of the above.
14. The protection against small pox virus infection afforded by prior infection with cowpox represents
    a. Antigenic specificity
    b. Antigenic cross reactivity.
    c. Innate immunity.
    d. Passive protection.
15. An adjuvant is a substance that
    a. Enhances the immunogenicity of haptens.
    b. Increases the chemical complexity of the immunogen.
    c. Enhances the immune response to the immunogen.
    d. Enhances the immunologic cross – reactivity.
16. Antigenic sites with which antibodies react are called
    a. Immunogens b. Carriers
    c. Epitopes d. haptens
17. Basic structural unit of an immunoglobulin molecule includes
    a. Identical λ light chains only.
    b. One constant and three variable regions.
    c. Two identical heavy and two identical light chains.
    d. A total of five domains.
18. J chain is a glycopeptides chain associated with which of the following immunoglobulins?
    a. IgA b. IgG c. IgD d. IgE
19. Primary interactions between antigens and antibodies involve all of the following except which?
    a. Van der Waals forces
    b. Hydrophobic forces
    c. Electrostatic forces
    d. Covalent bonds
20. When instead of the antigen, the antibody is adsorbed to carrier particles in test for estimation of antigen, this technique is known as
    a. Indirect agglutination
    b. Direct agglutination
    c. Reverse passive agglutination
    d. Hemagglutination inhibition

Answer the following

1. What is immunology?
2. Define the term vaccination.
3. What are M cells?

4. What is the role of primary lymphoid organs and secondary lymphoid organs?
5. Define hematopoiesis.
6. What are pluripotent stem cells?
7. What is acquired immunity?
8. What is immunological memory?
9. Define the term active/passive immunity.
10. What is immunogenicity?
11. Define the term immunogen.
12. What are haptens?
13. What is antigenicity?
14. Define epitopes.
15. Define the term antibodies.
16. What is opsonization?
17. What is immunity/complement?
18. What is precipitation/agglutination?
19. Write short notes on eosinophils/ neutrophils.
20. Give a short account of natural killer cells.
21. How do intact mucous membranes resist microbial invasion of the host?
22. Briefly explain the various stages involved in phagocytosis.
23. Write short notes on interferons.
24. Write short notes on primary immune response/secondary immune response?
25. How do adjuvants function?
26. Explain immunoglobulin structure and function?
27. Describe briefly the structure and function of thymus.
28. Describe the structure and function of spleen/lymph node.
29. Describe the characteristics of macrophages
30. Write the characteristics of B/T cells.
31. Briely explain the three major events in the inflammatory response.
32. Briefly explain humoral immunity.
33. Write an account of cell mediated immunity.
34. Mention the properties of IgM.
35. List out the general features of antigen-antibody reactions.

Chapter 14

Microbial Genetics

Chapter Outline

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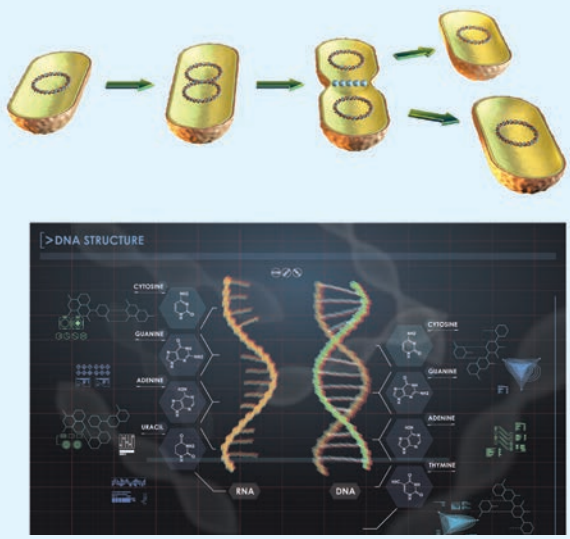


Microbial genetics provides powerful tools for deciphering the regulation, as well as the functional and pathway organization of cellular processes. The genetic study of microbes has played a highly significant role in the developments of Molecular Biology, Recombinant DNA Technology and in the preparation of useful products. Microbial Genetics makes microbes beautiful, beneficial and bountiful.

Learning Objectives

After studying this chapter the student will be able,

- To *review the historical discoveries that led to establishing DNA as the genetic material.*
- *To identify the role of genetic material.*
- *To recognize the contributions of Griffith, Avery, MacLeod, and McCarty, and Hershey and Chase.*
- *To explain the structure of DNA.*
- *To recognize the contributions of Chargaff, Rosalind Franklin, Maurice Wilkins, Watson and Crick.*
- *To describe the Watson and Crick model of DNA.*
- *To compare structure of DNA and RNA.*
- *To know Meselson and Stahl's experiment.*
- *To explain the steps of replication.*
- *To know the enzymes and their roles involved in DNA replication.*

14.1 Genetic Information is Stored in DNA

Microorganisms are diverse in nature. A particular bacterium can be identified based on certain characteristics. When a bacterial cell grows and divides, it gives rise to cells with similar characteristics. Have you ever pondered as to why some of the characteristics of progeny cells are similar and a few dissimilar?

In the middle of the 19th century it was assumed that there was some particle present somewhere in the cell which

was the controlling factor to carry the characteristics from one generation to another.

Genetics, a branch of science aims to understand the working of the controlling factor. The factor governing the transfer of information is now very well known as Gene. Gene can be defined as a unit of heredity which is transferred from parent to progeny.

Although there were experiments to prove the inheritance pattern due to gene, there was no real understanding of the molecular nature of the gene. Work of Frederick Griffith introduced the transforming principle which was further confirmed as Deoxyribonucleic acid (DNA) by experiments of Avery, Mac Leod and Mac Carty in 1944 followed by Hershey and Chase in 1952.

14.1.1 Frederick Griffith's Experiment

In 1928, British bacteriologist Frederick Griffith (Figure 14.1) was trying to develop a vaccine against pneumonia. In his experiments Griffith used two related strains of *Streptococcus pneumoniae* (Figure 14.2).

Figure 14.1: Frederick Griffith

1. Rough strain (R) – avirulent, non-capsulated strain, forming rough colonies on culture media.
2. Smooth strain (S) – virulent, capsulated strain (resists phagocytosis), forming smooth colonies on culture media.

Griffith injected live smooth strain into mice which caused disease and killed it. When he injected live rough strain into mice, it did not cause disease and mice remained alive. He heat killed the smooth strain and injected into mice, the mice remained alive. But the experiment gave surprising results when a mixture of harmless live rough strain and heat-killed smooth strain was injected into mice. Griffith observed that the mice developed pneumonia and died. Further, when he analysed the blood sample from dead mouse, he found that it contained live smooth strain. This accidental discovery made Griffith to conclude that the rough strain changed (transformed) into smooth strain by taking up a substance which he called a "transforming principle" from heat killed smooth bacteria. This phenomenon is called "Bacterial Transformation" Griffith's experiment is summarized in Figure 14.3.

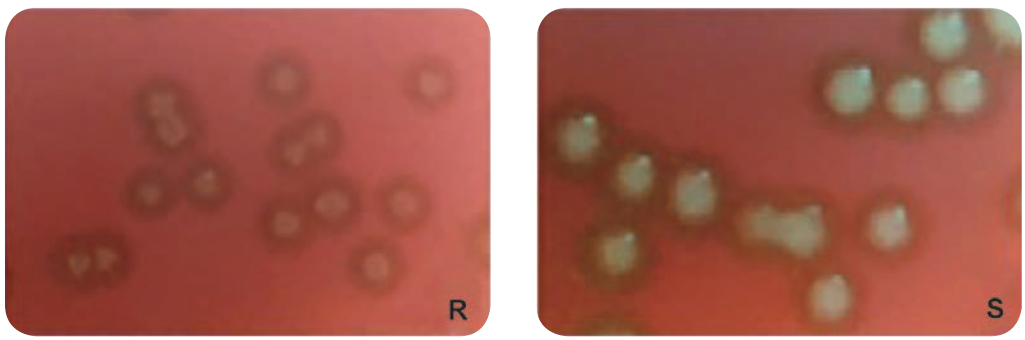


Figure 14.2: Rough and Smooth colonies of *Streptococcus pneumoniae*

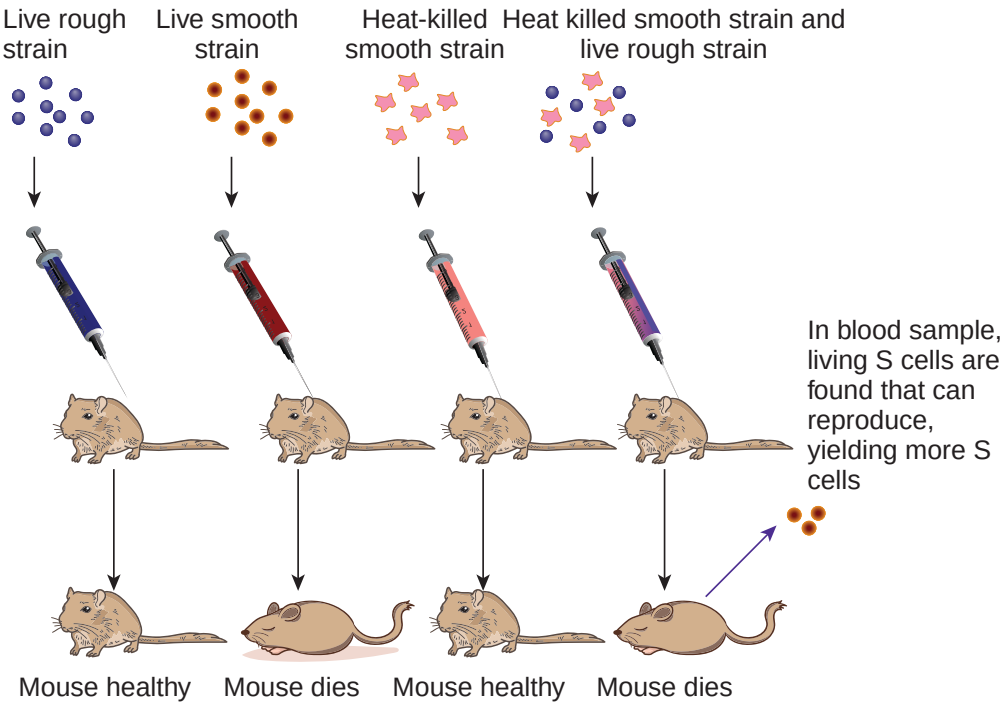


Figure 14.3: Summary of Griffith's experiment

HOTS

What did Griffith expect to happen to mouse when he injected it with live rough strain and heat killed smooth strain

14.1.2 Oswald T. Avery, Colin Mac Leod and Maclyn Mc Carty's Experiment

Figure 14.4: Avery *et al.*,

Griffith's experimental results led to the curiosity to explore the transforming principle. Avery and his colleagues (Figure 14.4) used the extracts of heat-killed smooth bacteria and treated it with enzyme protease, RNase, DNase to eliminate proteins, RNA and DNA respectively. Each of the treated extracts were mixed with live rough bacteria and injected into mice. The mice injected with a mixture of DNase treated extract and live rough strain did not die. This partially proved that DNA was responsible for changing the rough strain of *Streptococcus pneumoniae* bacteria into smooth bacteria. Avery *et al.*, experiment is summarized in Figure 14.5. Later Hershey and Chase's experiment on T2 bacteriophage confirmed that genetic information is present in DNA.

These important early experiments and many other lines of evidence have shown that DNA bears the genetic information of living cells and it is responsible for transfer of characteristics from one generation to another. This is true in all organisms, the notable exceptions being RNA viruses

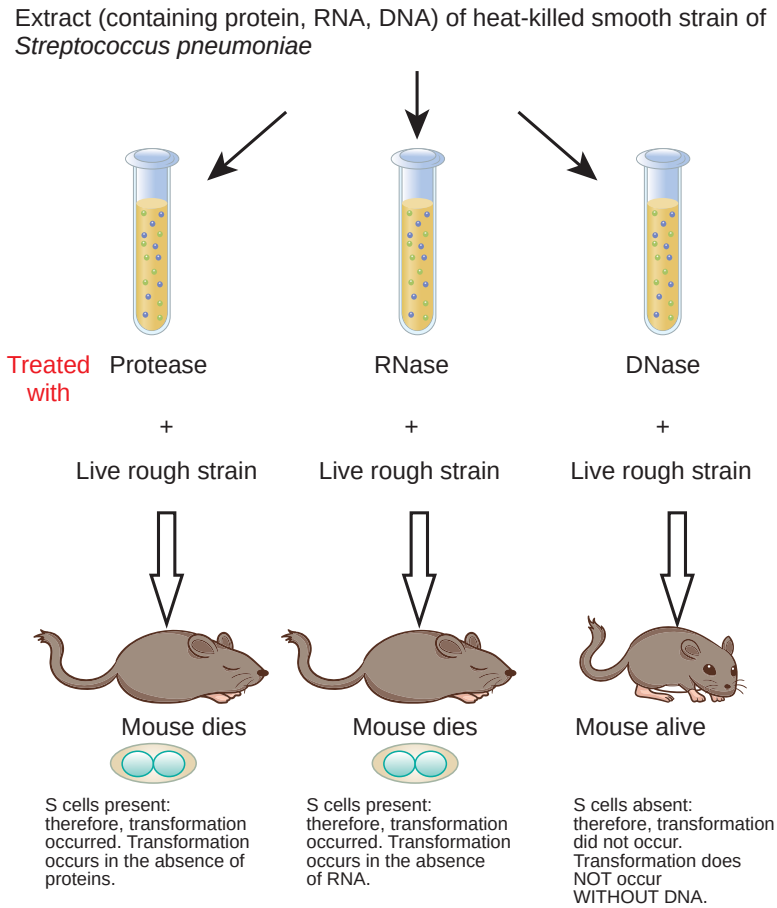


Figure 14.5: Avery, Mc Cleod and Mac Carty's experiment

Infobits

Bacterial transformation

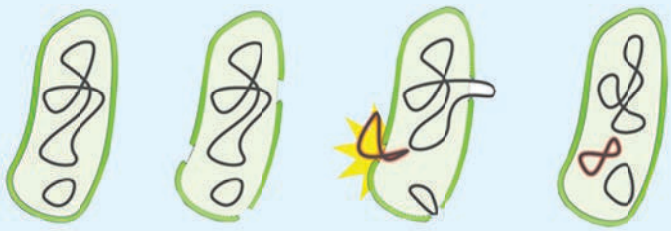
Getting a plasmid into a bacterium

Here is an E.coli. bacterium in natural state. (Notice how bacterial DNA is circular)

Extreme cold causes pores (small holes) to appear in the bacterial membrane.

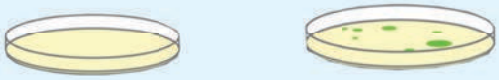
Small DNA molecules like plasmid can move through these holes.

When the bacteria are heated again, some of them end up with plasmid inside them. These are the transformed bacteria.



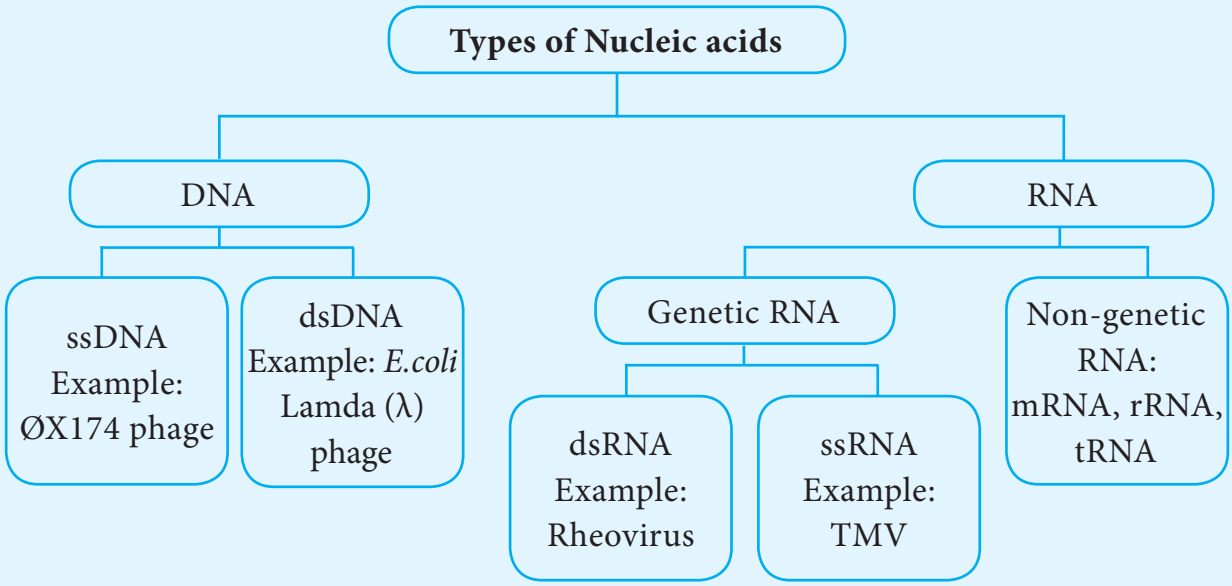
We can filter out the untransformed bacteria (the ones that got no plasmid) by growing all the bacteria in an antibiotic containing medium.

Untransformed bacteria are killed by the antibiotic in the medium. (They don't have the plasmid with the antibiotic resistance gene.)



The transformed bacteria grow. Now we can pick them off the plate and grow more if we want.

The entire genetic content of a cell is known as its genome and the study of genomes is genomics. In eukaryotic cells, but not in prokaryotes, DNA forms a complex with histone proteins to form chromatin, the substance of eukaryotic chromosomes. A chromosome may contain tens of thousands of genes. Many genes contain the information to make protein product. DNA controls all of the cellular activities by turning the genes "on" or "off."



which store genetic information in RNA. The understanding of DNA's role in heredity has led to variety of practical applications including forensic analysis, paternity testing and genetic screening.

14.2 DNA Structure

- DNA is a polymer of simple monomeric units, the nucleotides (Figure 14.6).
- Each nucleotide is made up of three components:
  1. Nitrogenous base
  2. Sugar
  3. Phosphate group
- Nucleotide without phosphate group is known as nucleoside.
- The sugar present in DNA is deoxyribose sugar.
- The nitrogenous bases present in DNA are
  - * Purines – Adenine (A), Guanine (G)
  - * Pyrimidines – Thymine(T), Cytosine (C)
- The nucleotide as a unit is formed by
  - * Glycosidic bond between nitrogenous base and sugar,
  - * Ester bond between phosphate group and sugar
- Each of the nucleotides is bonded by a phosphodiester bond to form a polynucleotide chain (strand) (Figure 14.7a).

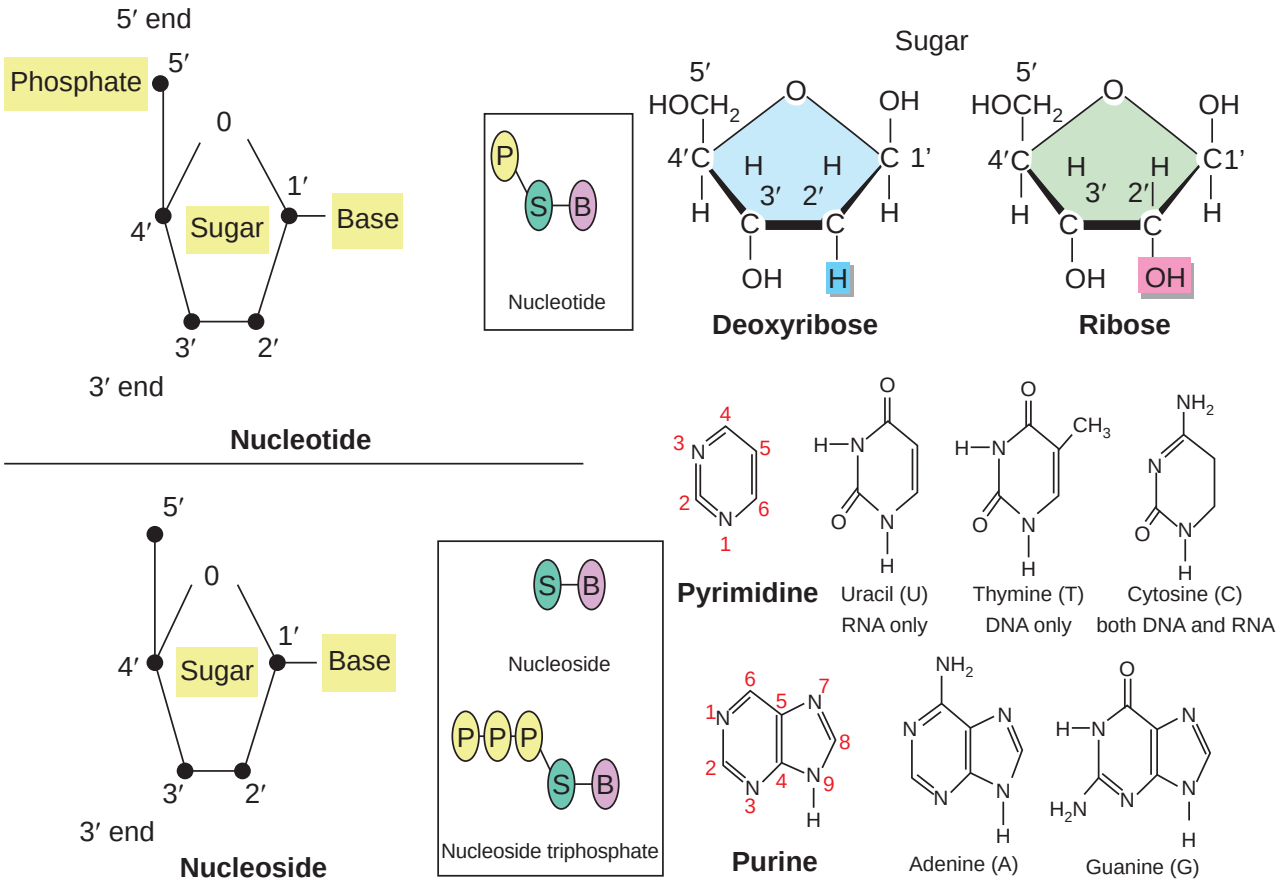


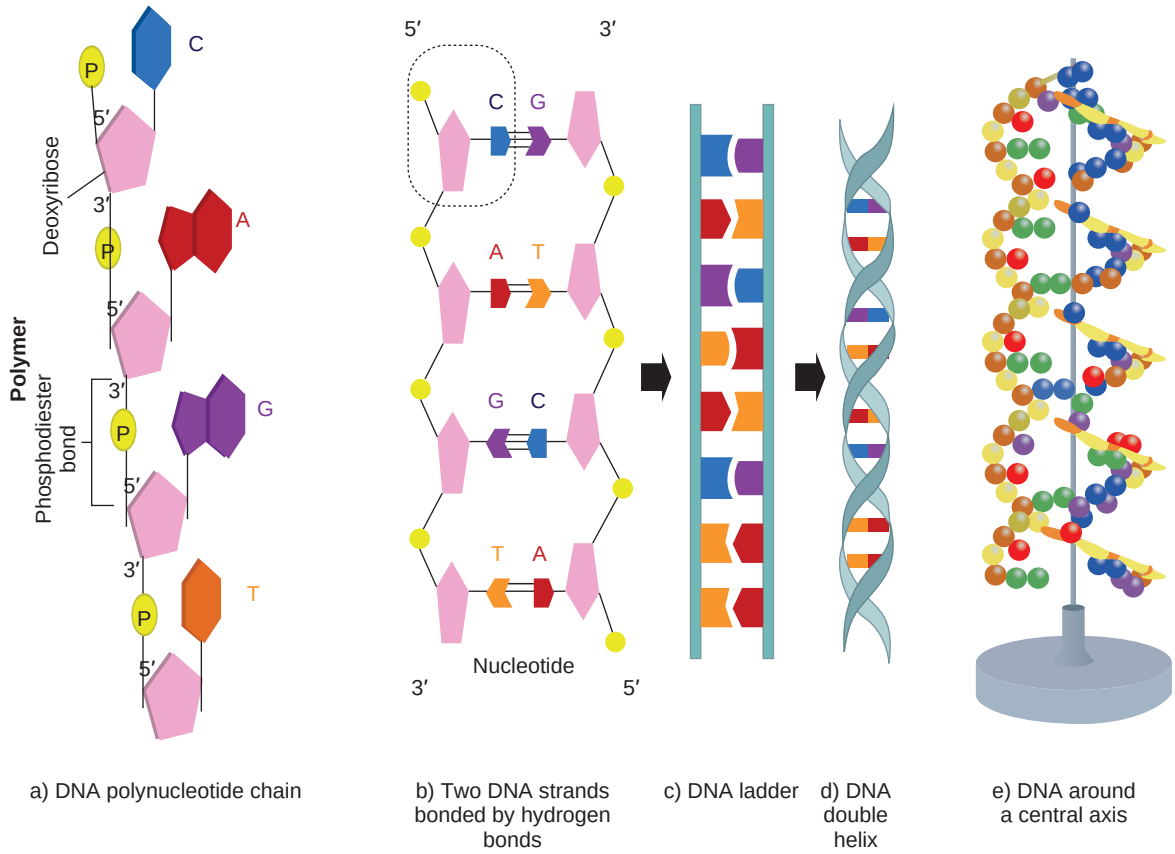
Figure 14.6: Structure of nucleotide, nucleoside, deoxyribose, ribose and nitrogenous bases

- Two polynucleotide chains join together through hydrogen bonds between nitrogenous bases, to form double stranded DNA (Figure 14.7b).
- Two hydrogen bonds exists between adenine and thymine and three hydrogen bonds between guanine and cytosine.
- DNA is coiled in the form of a double helix, in which both the strands of DNA coil around an axis (Figure 14.7d & e).
- The further coiling of this axis upon itself produces DNA supercoiling an important property of DNA structure.

All DNA, whether large or small, possess the same sugar phosphate backbone. What distinguishes one DNA from another is the length of the polymer and distribution of four bases along the backbone. The variety of sequences that can be made from the four nitrogenous bases is limitless, as is the number of melodies possible with a few musical notes. RNA differs from DNA by having a ribose sugar instead of deoxyribose and nitrogenous base Uracil instead of Thymine.

14.2.1 Watson And Crick Model of DNA Double Helix

In the early 1950's, Rosalind Franklin and Maurice Wilkins used the powerful method of X-ray diffraction to shed more light on the structure of DNA. From the X-ray Diffraction pattern it was deduced that DNA molecules are helical. In 1953 Watson and Crick (Figure 14.8) postulated a three dimensional model of DNA structure based on Franklin's X-ray crystallographic studies. In recognition of their work leading to



a) DNA polynucleotide chain | b) Two DNA strands bonded by hydrogen bonds | c) DNA ladder | d) DNA double helix | e) DNA around a central axis

Figure 14.7: Structure of a single polynucleotide chain of DNA, hydrogen bonding between two DNA strands, Double helix around axis

the double helix model, Nobel prize was awarded in 1962 to Watson, Crick and Wilkins. According to Watson and Crick model (Figure 14.9),

- The DNA consists of two helical polynucleotide chains wound around the same axis to form a right handed helix.
- The Purine and Pyrimidine bases of both strands are stacked inside the double helix.
- Each nitrogenous base of one strand is paired in the same plane with a base of the other strand.
- According to Watson and Crick rule Adenine base pairs with Thymine and Guanine base pairs with Cytosine.
- Two hydrogen bonds are present between A and T (symbolised as A=T)

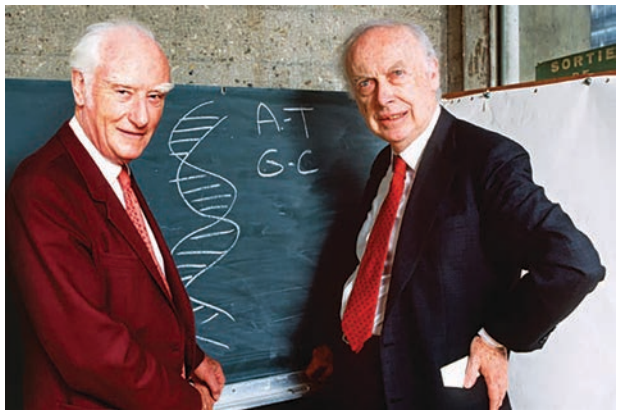


Figure 14.8: Watson and Crick

and three hydrogen bonds are present between G and C (G ≡ C).

- The hydrogen bonds provide chemical stability essential to hold the two chains together.
- The specific A equal to T and G equal to C base pairing is the basis for the complementarity concept. This complementarity concept is very

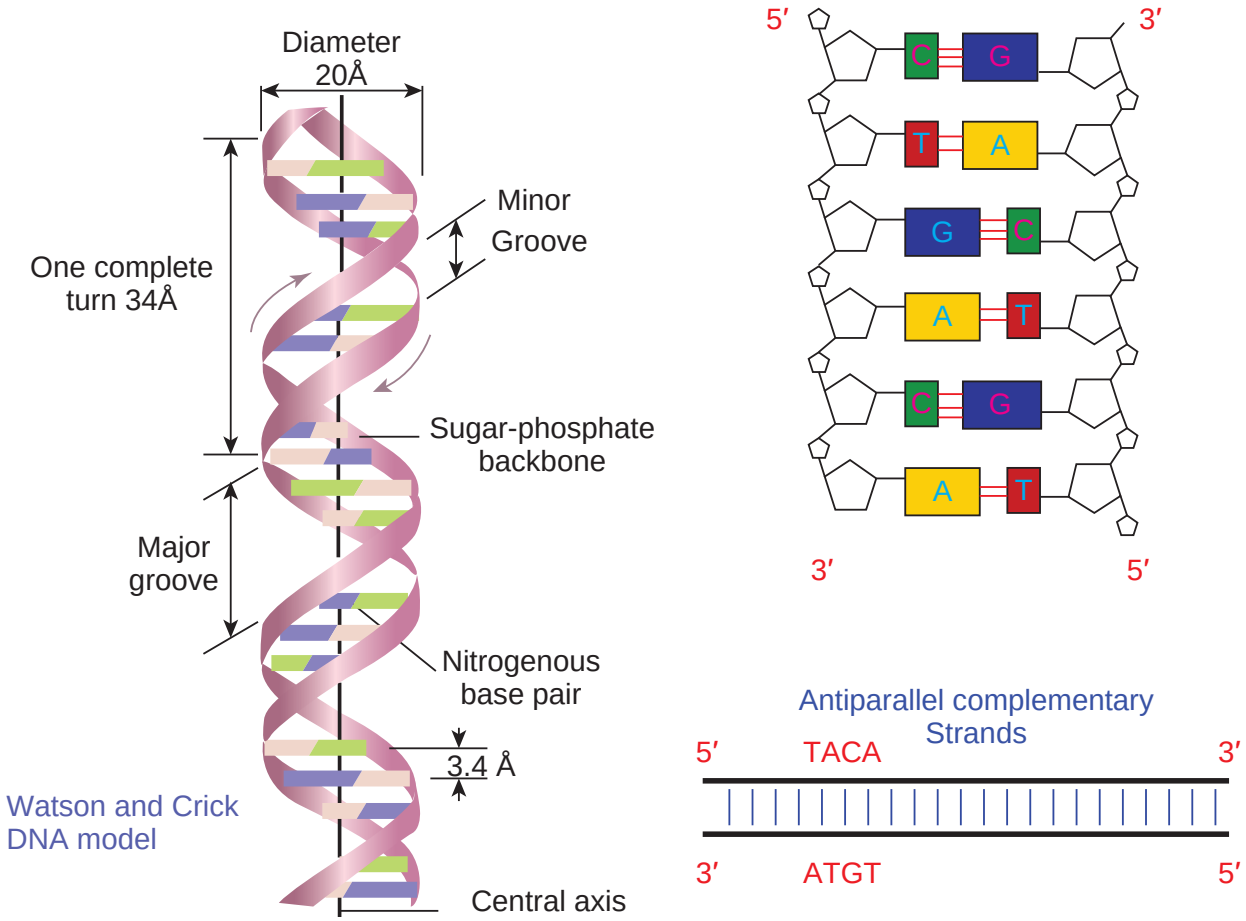


Figure 14.9: Watson and Crick DNA model, Antiparallel dsDNA

important in the process of DNA replication and gene expression.

- The pairing of two strands creates a major groove and minor groove on the surface of the duplex.
- The two strands are antiparallel, that is their 5′, 3′ phosphodiester bonds run in opposite directions.
- The vertically stacked bases are 3.4 Aº apart.
- Each complete turn of double helix contain base pairs, which is 34 Aº units long.

14.2.2 Erwin Chargaff's Rule

In the late 1940s Erwin Chargaff and his colleagues found that the four nucleotide bases of DNA occur in different ratios in the DNA of different organisms. Erwin Chargaff measured the quantity of the bases in DNA and noticed that the number of Adenine is equal to the number of Thymine and the number of Guanine is equal to the number of Cytosine residues. Hence the sum of Purine residues equal to the sum of the Pyrimidine residues.

Quantitatively A=T or A/T = 1
C=G or C/G = 1
A+G = T+C

HOTS

If percentage of adenine in one of the DNA strand is 20. Can you determine the percentage of other bases. If yes how?

14.2.3 Alternative Forms of DNA

DNA is a remarkably flexible molecule. Considerable rotation is possible around a number of bonds in sugar-phosphate backbone and thermal fluctuations can produce bending, stretching and unpairing of the strands. Watson and Crick model of DNA is called as B-DNA or B-form. However DNA can exist in A or Z form. In 1979 Alexander Rich discovered Z form (Figure 14.10). Recently, several alternative forms of DNA have been discovered C-form, D-form and E-form. The B-form of DNA is the most stable structure and is therefore the standard point of reference in any study of the properties of DNA (Table 14.1).

Table 14.1: Properties of different forms of DNA

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~ 26A°	~ 20 A°	~ 18 A°
Base pairs per helical turn	11	10	12
Distance between adjacent bases	2.6 A°	3.4 A°	3.7 A°

Bacterial genome size

- Bacterial genomes are typically expressed in Mb
- The length of Bacterial genomes are typically in the mm range and therefore 1000X bigger than the typical bacterial size.
- The mass of Bacterial genomes are typically in 10^{-3} pg(picogram) range.

Conversions

1 Kb = 10^3 bp (base pairs)

1 Mb = 10^6 bp

1 Gb = 10^9 bp

1 bp ≈ 0.33nm

1 kb ≈ 0.33μm

1 Mb ≈ 0.33mm

1 Gb ≈ 0.33m

1 pg = 10^{-12} g

1pg = 978 Mb

Number of base pairs = mass in pg × (0.978×10^9)

1 kb ≈ 10^{-6} pg

1 Mb ≈ 10^{-3} pg

1 Gb ≈ 1 pg

	Bacteria	Virus, organelles	Eukaryotes
Genomes	Small(Mb)	Tiny (Kb)	Large (Gb)
Gene Density	High	High	Low
Example	*E.coli* 5000 genes	Bacteriophages 10-100 genes	*Homo sapiens* 25000 genes

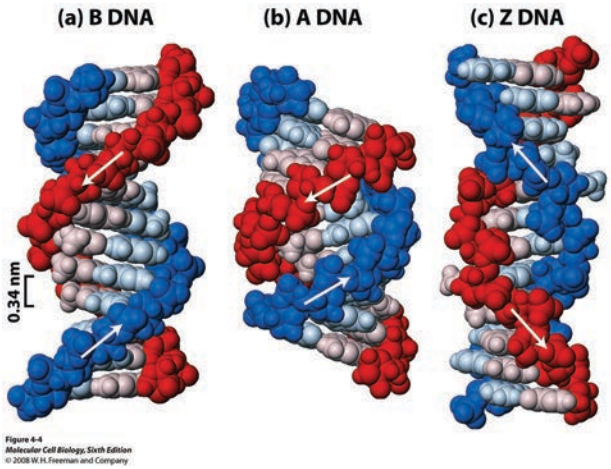


Figure 14.10: Forms of DNA

HOTS

Write the base sequence of complementary DNA and RNA strand for the following.

5′GCGCAATATTTCT3′

14.3 DNA Replication

DNA is a marvelous device for the stable storage of genetic information. DNA replication is a process in which copies of DNA molecules are faithfully made. Here double stranded DNA molecule is copied to produce two identical dsDNA molecules. Replication is an essential process because whenever a cell divides, the two new daughter cells must contain the same genetic information in DNA as parent cell. DNA replication occurs during the S (DNA synthesis) phase and precedes cell division.

Watson and Crick proposed the hypothesis of semiconservative replication. According to them each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules each with one old strand and one new strand. (Figure 14.11).

Infobits

Max Delbruck suggested that there could be three possible ways in which DNA could replicate.

Semiconservative – DNA replication that produce two copies of double stranded DNA (dsDNA) each containing one old strand and one new strand.

Conservative – DNA replication that produces two daughter ds DNAs, one of which consists of two original strands whereas the other daughter DNA consists of two newly synthesised strands.

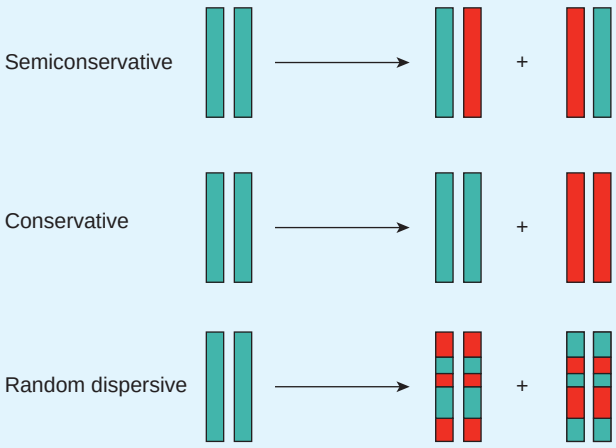


Figure 14.11: Semiconservative, conservative and dispersive replication

Dispersive – DNA replication in which the original dsDNA undergoes fragmentation, the fragments synthesize complementary structure both of which assemble to form two replicas.

14.3.1 Meselson and Stahl's Experiment

Mathew Meselson and Franklin Stahl in 1957 gave experimental evidence for semiconservative replication process.

Steps

1. *E.coli* cells were grown for many generations in a medium containing radioactive isotope (heavy isotope) of nitrogen source ^{15}N.
2. After many generations all nitrogen containing molecules in *E.coli* cells, including nitrogen bases of DNA contained ^{15}N.
3. The cells labeled with ^{15}N were then transferred to a medium containing only ^{14}N (light isotope). Hence all subsequent DNA synthesised during replication contained ^{14}N.
4. Cell samples were removed at periodic time intervals from the growth medium.
5. From each of the above samples, DNA was isolated and subjected to density gradient centrifugation (Caesium chloride (CsCl) centrifugation).

Expected results

- The heavy isotope ^{15}N containing DNA, will reach equilibrium in a gradient point closer to the bottom of the tube.
- ^{14}N containing DNA will reach equilibrium at a gradient point closer to the surface of the tube.

Observed Results

- After first generation the isolated DNA occupied an intermediate density band.
- After second generation two bands were observed, one at intermediate density and the other at lighter density corresponding to the ^{14}N position in the gradient.

These experimental results and other experiments repeated by Meselson and Stahl with prokaryotes suggested that semiconservative mechanism of replication is universal (Figure 14.12).

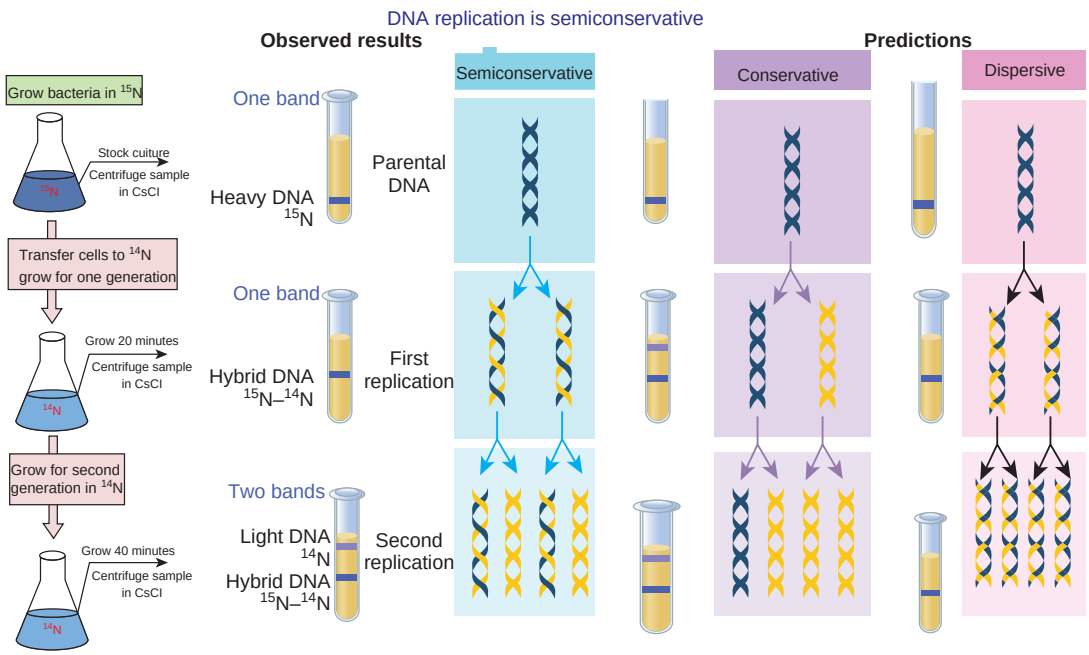


Figure 14.12: Meselson's and Stahl's experiment

14.3.2 Enzymes Involved in DNA Replication

DNA replication in *E.coli* requires many enzymes and proteins, each performing a specific task. The entire complex is called the DNA replicase system or replisome. The major enzymes and proteins involved with their functions are tabulated in Table 14.2.

Table 14.2: Enzymes involved in DNA replication

Enzyme	Function
Helicase	Unwinds DNA
DNA gyrase	Relieves stress created by unwinding
SSB protein	Binds to single stranded DNA and stabilises it
Primase	Synthesis of RNA primer
DNA pol I	Excision of primers and filling of gaps with nucleotides
DNA pol III	New strand elongation
DNA ligase	Joins the nick

Infobits

- First in vitro synthesis of DNA with a template was carried out by Kornberg in 1959.
- First in vitro synthesis of DNA without template was carried out by H.G. Khorana in 1965.

14.3.3 Events in DNA Replication in *E.coli*

1. Initiation
2. Elongation
3. Termination

DNA replication is depicted in Figure 14.14.

Initiation

- DNA replication is initiated at replication origin known as oriC (245 base pairs in *E.coli*).
- Dna A protein molecules bind to the origin of replication.
- Helicase (DnaB) denatures the DNA helix by breaking the hydrogen bonds between base pairs.
- Many molecules of SSB (single stranded binding) proteins bind cooperatively to single stranded DNA, stabilizing the separated strands and preventing renaturation.
- Gyrase (topoisomerase) releases the topological stress produced by helicase.
- RNA primers are synthesised by primase.

The separated polynucleotide strands are used as templates for synthesis of complementary strands. The area of the DNA opened by helicase for DNA synthesis is referred to as the replication fork. At the replication fork there are four strands of DNA, two are conserved and two are newly synthesised. Replication may occur in either a unidirectional or bidirectional manner (Figure 14.13) from each origin. Bidirectional replication can be explained as two replication forks moving in opposite directions around the circular chromosome. Both forks move along the double helix away from the origin of replication in opposite directions and around the circular chromosome.

Elongation

- DNA synthesis proceeds in a 5′→3′ direction(read as 5 prime to 3 prime).
- One strand is synthesised continuously and is known as leading strand.

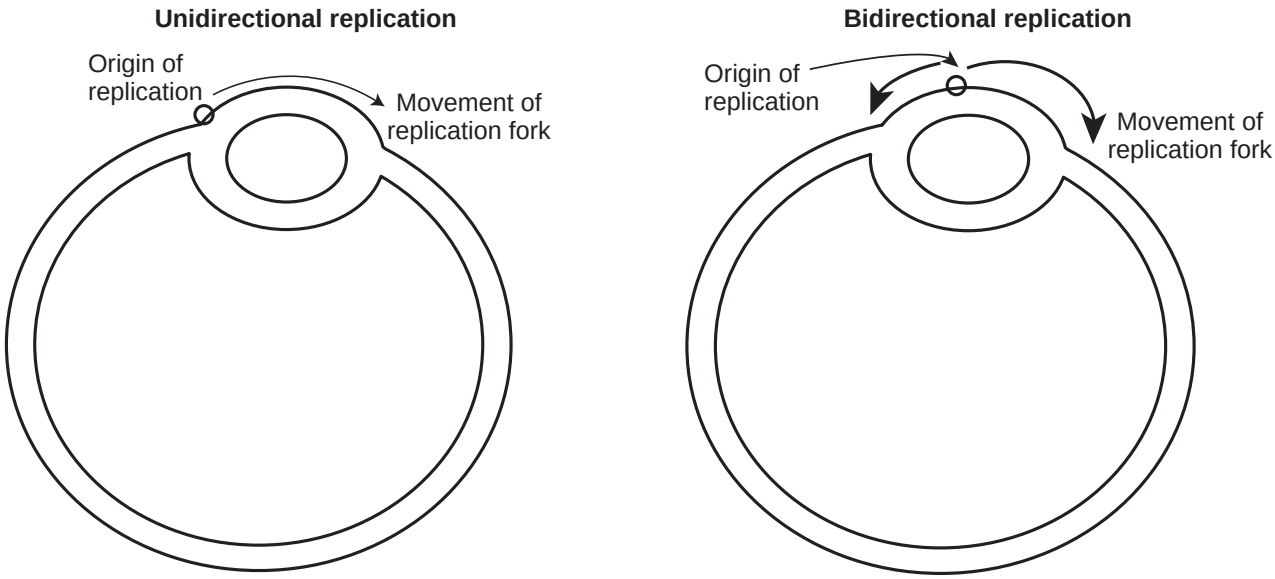


Figure 14.13: Unidirectional and bidirectional replication

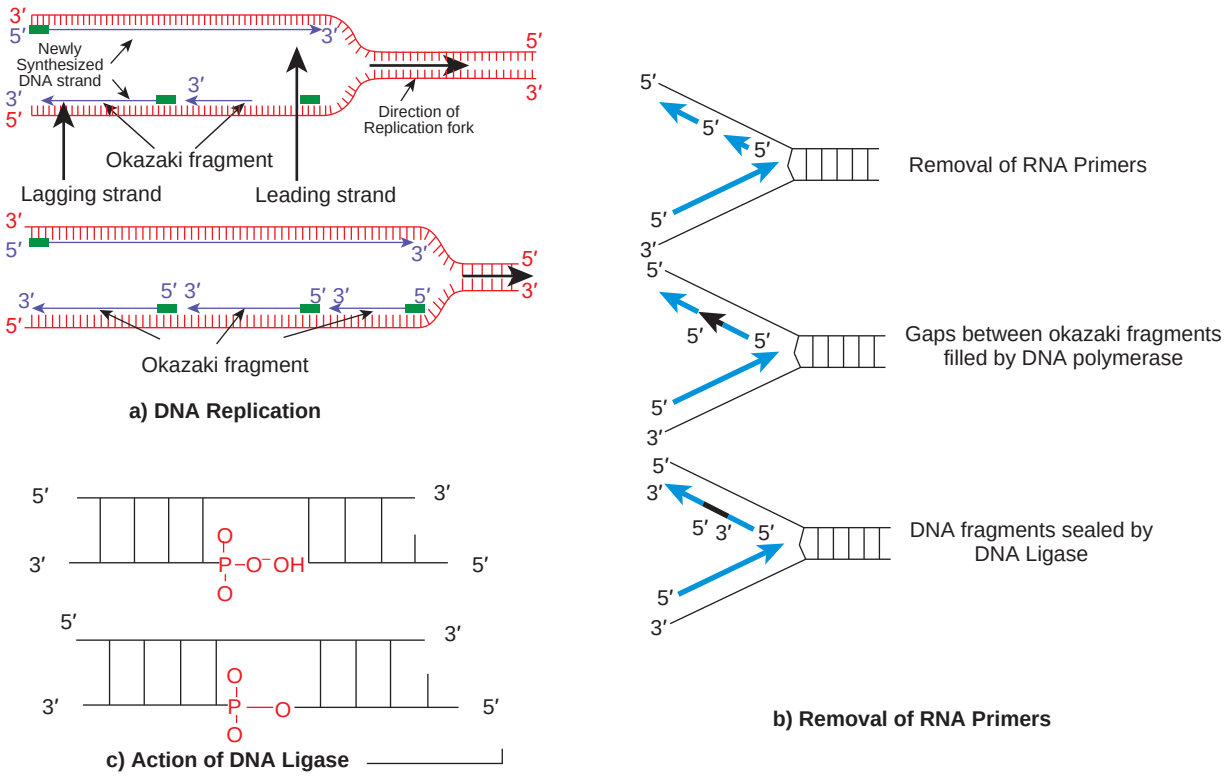


Figure 14.14: a) DNA replication b) Removal of RNA primers c) Action of Ligase and

- Other strand is synthesised discontinuously and is known as lagging strand.
- The enzymes that are able to synthesise new DNA strands on a template strand are called DNA polymerases. Kornberg was awarded Nobel Prize for discovering DNA polymerase in 1956.
- There are three known enzymes in *Escherichia.coli* viz., DNA polymerase I, II and III.
- All of the known DNA polymerases can extend a deoxyribonucleotide

chain from a free 3′OH end, but none can initiate synthesis.

- Synthesis of DNA requires nucleoside triphosphates or nucleotides - deoxyadenosinetriphosphate (dATP), deoxythymidinetriphosphate (dTTP), deoxycytidinetriphosphate (dCTP), deoxyguanosinetriphosphate (dGTP). When a nucleoside triphosphate bonds to sugar in a growing DNA strand, it loses two phosphates.
- RNA primer synthesised during initiation is removed and replaced with DNA by DNA polymerase I
- Sealing of the nick by DNA ligase which catalyses the formation of phosphodiester bond between a 3′ hydroxyl end of one DNA fragment and 5′ phosphate at the end of another strand.

The elongation phase of replication includes two distinct but related operations

- Leading Strand Synthesis
- Lagging Strand Synthesis

Leading strand synthesis begins with the synthesis of short (10 to 60 nucleotide long) RNA primer at the replication origin. Deoxyribonucleotides are added to this primer by DNA polymerase III. Leading strand synthesis then proceeds continuosly, keeping pace with the unwinding of DNA at the replication fork. The continuous strand or leading strand is one in which 5′→3′ synthesis proceeds in the same direction as replication fork movement.

Lagging strand or discontinuous strand is one in which 5′→3′ DNA synthesis proceeds in the direction opposite to the direction of fork movement. This strand is synthesised as short fragments, known as Okazaki fragments named after R. Okazaki. Okazaki fragments range in length from a few hundred to a few thousand nucleotides depending on the cell types. Each okazaki fragment must be initiated by the action of primase. Once an Okazaki fragment has been completed its RNA primer is removed and replaced with DNA by DNA polymerase I and the nick is sealed by DNA ligase.

The fidelity of DNA replication is maintained by (1) base selection by the polymerase, (2) a 3′→5′ proofreading exonuclease activity that is part of most DNA polymerases, and (3) specific repair systems for mismatches left behind after replication.

Infobits

RNA dependent DNA polymerases, also called reverse transcriptases, were first discovered in retroviruses, which convert their RNA genomes into double-stranded DNA as part of their life cycle. These enzymes transcribe the viral RNA into DNA, a process that can be used experimentally to form complementary DNA.

Termination

Eventually, the two replication forks of the circular *E.coli* chromosome meet at a terminus region called Ter (for terminus). The Ter sequence function as binding sites for protein Tus (Termination utilization substance). The Tus-Ter complex can arrest a replication fork from only one direction. When either replication fork encounters a functional Tus-Ter complex, it halts. The other fork halts when it meets the first (arrested) fork (Figure 14.15).

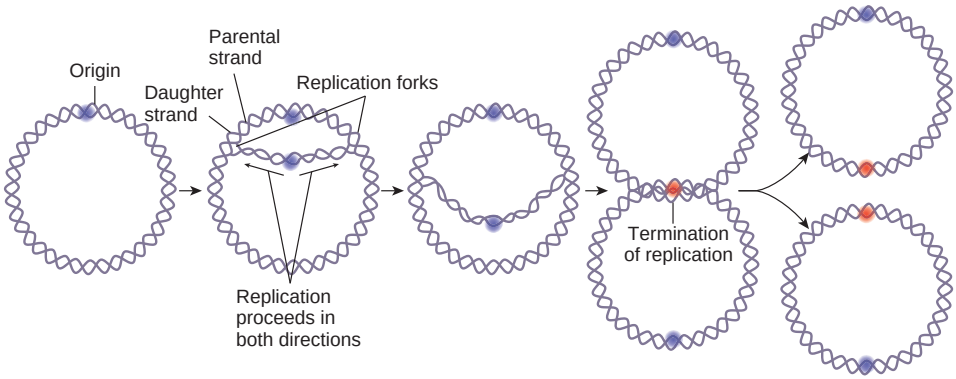


Figure 14.15: Termination of replication in a circular DNA

14.3.4 Eukaryotic DNA Replication

Replication in Eukaryotic cells is more complex. The DNA molecules in Eukaryotes are considerably larger than those in bacteria and are organized into complex nucleoprotein structures (chromatin). The essential features of DNA replication are same in eukaryotes and prokaryotes. However, some interesting variations do occur. Initiation of replication in all eukaryotes requires a multisubunit protein. Multiple origins of replication are probably a universal feature in eukaryotic cells. Like bacteria,

Infobits

DNA molecules exists in circular form in prokaryotic microorganisms, viruses and in organelles of eukaryotic organisms. However not all DNA molecules are circular. The chromosomes of eukaryotic organisms and of many viruses consists of linear DNA molecules. There are three general methods of replication of DNA molecule

1. Theta (θ) mode
2. Sigma (σ) mode
3. Linear mode

The two essential functions of genetic material are replication and expression. Genetic material must replicate accurately so that progeny inherit all of the specific genetic determinants (the genotype) of the parental organism. A gene is a DNA sequence that encodes a protein, rRNA, or tRNA molecule (gene product).

Gene expression usually involves transcription of DNA into messenger RNA and translation of mRNA into protein. Genetic information encoded in DNA is expressed by synthesis of specific RNAs and proteins, and information flows from DNA to RNA to protein.

Expression of specific genetic material under a particular set of growth conditions determines the observable characteristics (phenotype) of the organism. Bacteria have few structural or developmental features that can be observed easily, but they have a vast array of biochemical capabilities and patterns of susceptibility to antimicrobial agents or bacteriophages. These latter characteristics are often selected as the inherited traits to be analyzed in studies of bacterial genetics.

eukaryotes have several types of DNA polymerases (Example: DNA polymerase α [alpha] and DNA polymerase δ [delta]). Some have been linked to particular functions, such as the replication of mitochondrial DNA. The termination of replication on linear eukaryotic chromosomes involves the synthesis of special structures called telomeres at the ends of chromosome.

HOTS

What will happen if single stranded binding proteins are not present during replication of DNA?

Summary

Many lines of evidence show that DNA bears genetic information. Frederick Griffith showed transformation of bacteria. Avery, Mac Leod, Mc Carty experiment and further experiment by Hershey Chase confirmed that DNA is the transforming principle.

There are two types of nucleic acid: RNA and DNA. Nucleic acids are polymers of nucleotides, joined together by phosphodiester linkages between the 5′hydroxyl group of one pentose and the 3′hydroxyl group of the next. The nucleotides in RNA contain ribose, and the common pyrimidine bases are uracil and cytosine. In DNA, the nucleotides contain deoxyribose sugar, and the common pyrimidine bases are thymine and cytosine. The primary purines are adenine and guanine in both RNA and DNA.

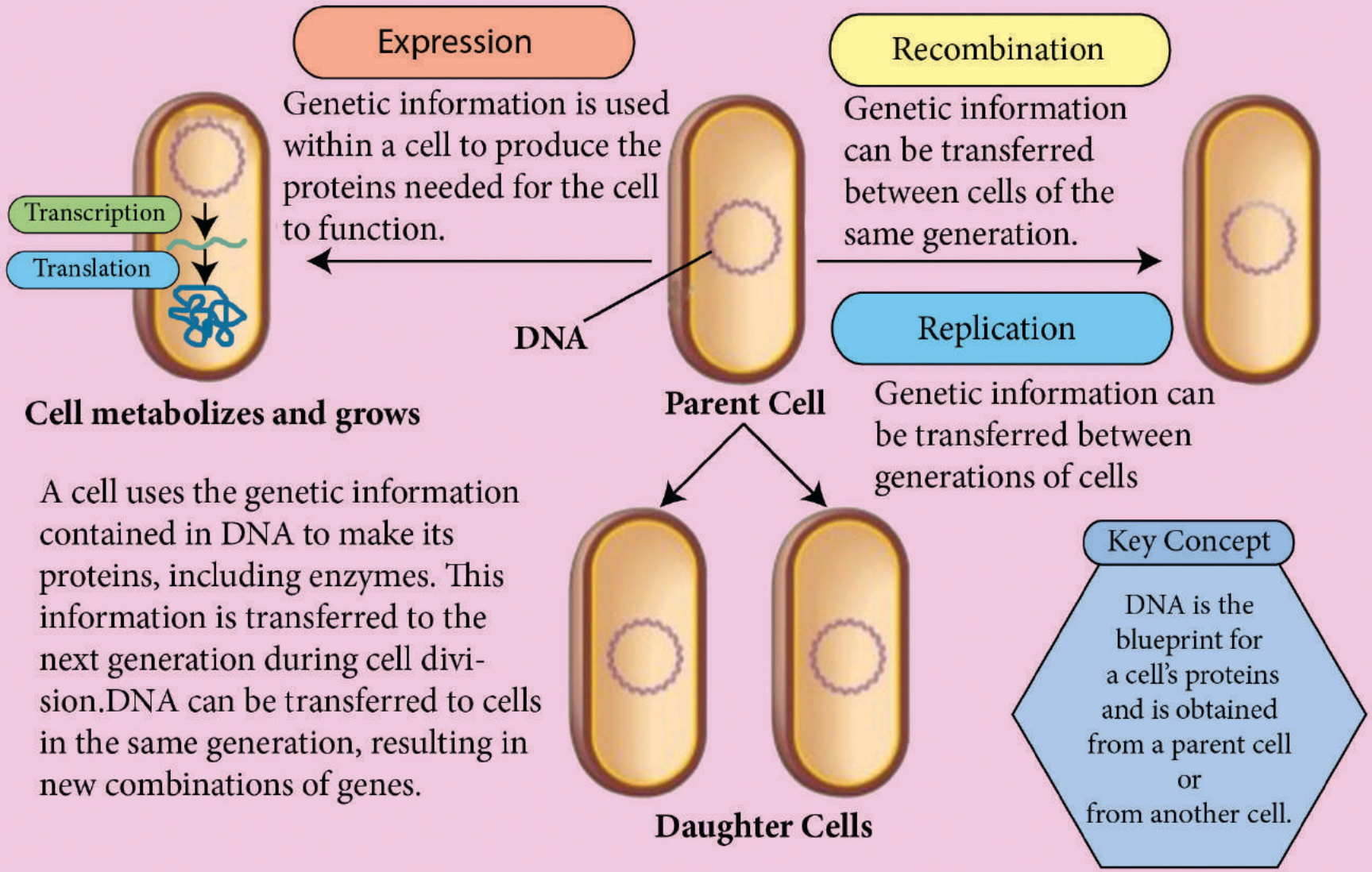
Erwin Chargaff rules states that A = T and G = C. Watson and Crick postulated that DNA consists of two antiparallel chains in a right-handed double-helical arrangement. Complementary base pairs, A = T and G ≡ C, are formed by hydrogen bonding within the helix. The basepairs are stacked perpendicular to the long axis of the double helix. DNA can exist in several structural forms. Two variations of the Watson-Crick form, or B-DNA, are A-DNA and Z-DNA.

Replication of DNA occurs with very high fidelity and at a designated time in the cell cycle. Replication is semiconservative, each strand acting as template for a new daughter strand. It is carried out in three phases: initiation, elongation, and termination.

The reaction starts at the origin and usually proceeds bidirectionally. DNA is synthesized in the 5′→3′ direction by DNA polymerases. At the replication fork, the leading strand is synthesized continuously in the same direction as replication fork movement; the lagging strand is synthesised discontinuously as Okazaki fragments, which are subsequently ligated by DNA ligases.

Most cells have several DNA polymerases. In *E. coli,* DNA polymerase III is the primary replication enzyme. Replication of the *E. coli* chromosome involves many enzymes and protein factors. Replication is similar in eukaryotic cells, but eukaryotic chromosomes have many replication origins.

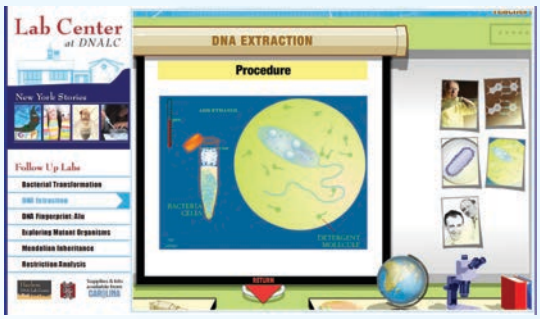
The Flow of Genetic Information



ICT CORNER

Bacterial DNA extraction

Lets separate DNA from bacteria



STEPS:

- Scan the QR code
- Click DNA extraction on the left tab
- Select student lab notebook and click open Bacterial Extraction Protocol
- Press return and read students protocol on the left table
- Click producer and follow the steps

OBSERVATIONS :

- Select base pair interactions at the right side and join nitrogenous base pairs as in DNA.

Step1

Step2

Step3

URL:

http://labcenter.dnalc.org/labs/dnaextraction/dnaextractiond.html

B177_11_MBIO_EM

Evaluation

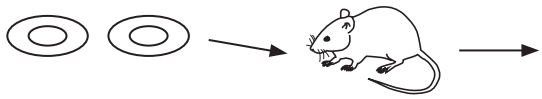
Multiple choice questions

1. The genetic material of virus is
   a. DNA
   b. RNA
   c. a or b
   d. None
2. _____ is used to denature RNA
   a. DNase
   b. Protease
   c. Nuclease
   d. RNase
3. In DNA molecule, the sugars
   a. Bond to nitrogenous bases by hydrogen bonds
   b. Bond to nitrogenous bases by glycosidic bonds
   c. Bond to phosphate by hydrogen bonds
   d. Bond to phosphate by glycosidic bonds
4. Which of the following is not present in DNA
   a. Adenine
   b. Guanine
   c. Uracil
   d. None
5. A nucleoside contains
   a. Sugar
   b. Nitrogenous base
   c. Both a and b
   d. Only b
6. Glycosidic bond is present between
   a. Phosphate and sugar
   b. Sugar and nitrogenous base
   c. Nitrogenous bases
   d. All the above
7. According to Chargaff the base composition of DNA in a given species does not change with
   a. Age
   b. Nutritional State
   c. Changing environment
   d. None
8. The bond between adenine and thymine in a DNA double helix is
   a. Hydrogen
   b. Double hydrogen
   c. Vander Waal's
   d. Triple hydrogen
9. Watson and Crick DNA model is
   a. A form
   b. B form
   c. Z form
   d. D form
10. In the first generation of Meselson and Stahl's experiment, the results showed a hybrid band of DNA containing both ^{14}N and ^{15}N. Which of the following is the best interpretation of these results?
    a. The results are consistent with semi-conservative replication
    b. The results support conservative replication
    c. The results support both semi conservative and conservative replication
    d. Neither dispersive nor conservative replication can take place.
11. A form of DNA is
    a. Left Handed helix with 20 nucleotide pairs per turn
    b. Right handed helix with II nucleotide pairs per turn
    c. Right handed helix with 12 nucleotide pairs per turn
    d. Left handed helix with 11 nucleotide pairs per turn

12. DNA polymerase is required for the synthesis of
    a. RNA from DNA
    b. DNA from RNA
    c. DNA from DNA
    d. RNA from RNA
13. Okazaki segments are
    a. Segment of a chain of nucleotides removed during replication of DNA
    b. Segment of a chain of nucleotides formed during replication of DNA
    c. Segments of gene which undergo recombination
    d. Segments of DNA capable of replication
14. DNA replication is aided by
    a. DNA Polymerase only
    b. Both DNA polymerase and primase only
    c. DNA ligase only
    d. RNA Polymerase.
15. The semi-conservative mode of DNA replication was proved by
    a. Beadle and Tatum
    b. Meselson and Stahl
    c. Watson and Crick
    d. H.G Khorana
16. Enzymes involved in unwinding of DNA at replication are
    a. Ligases
    b. Helicases
    c. Endonucleases
    d. DNA Polymerases
17. DNA replication is semiconservative because the _____ strand will become half of the _____molecule
    a. RNA, DNA
    b. Template, finished
    c. Sense, mRNA
    d. Condon, anticodon
18. In DNA adenine is complementary base for _____ and cytosine is the complementary for _____
    a. Guanine, thymine
    b. Uracil, guanine
    c. Thymine, guanine
    d. Thymine, uracil

Answer the following

1. Define gene
2. DNA is not always the genetic material, what are the exceptions?
3. Define Nucleotide.
4. List any two difference between DNA and RNA
5. In what sense are the two strands of DNA antiparallel.
6. What is a nucleoside?
7. Depict Erwin Chargaff rule by an equation.
8. Give examples of nitrogenous bases.
9. Draw the structure of Deoxyribose.
10. State Watson and Crick rule.
11. List 2 characteristics of Z DNA
12. Define replication.
13. What is a template DNA?
14. Explain **semiconservative** replication.
15. List major events in replication
16. Write two main events during initiation of replication.
17. What is the role of topoisomerase?
18. What do you understand by leading strand/lagging strand?
19. What is RNA primer?
20. Name two enzymes that make primers for DNA synthesis
21. What is the origin of replication?
22. Label the following diagram of Griffith.



23. Point out the mistake in the following scheme:

    Extracts of smooth strain + R**Nase**

    ⟶ Mouse ⟶ Alive
24. Differentiate between right handed and left handed DNA forms with any three salient features.
25. What are the types of DNA polymerases present in *E.coli*? Write their functions.
26. Explain replication fork.
27. Explain bidirectional replication.
28. Explain continuous replication
29. Define okazaki fragments.
30. Why are RNA primers required
31. Describe what is meant by the antiparallel arrangement of DNA
32. Why is one strand of DNA synthesised discontinuously?
33. How is the faithfulness of DNA replication maintained? Write the name of the enzymes with its function.
34. Outline the experiment of Griffith.
35. Relate the experiments of Griffith and Avery.
36. Discuss Avery, Mc Cleod's experiment.
37. What was the motive of Avery and colleagues for conducting the experiment.
38. Differentiate between R and S strains of *Streptococcus pneumoniae*.
39. Describe the various characteristics of the Watson and Crick double helix model for DNA
40. Discuss the various bonds present in DNA double helix
41. Discuss various forms of DNA double helix structure.
42. Diagramatically explain the DNA double helix structure
43. Draw a four base pair segment of a DNA molecule, including each nucleotide and associated bonds involved in the maintenance of the double helix.
44. Diagrammatically represent the results of Meselson and Stahls experiment.
45. Explain how Meselson's and Stahl ruled out dispersive model of replication.
46. Tabulate the enzymes involved in DNA replication with their functions
47. Explain Elongation of DNA during replication

Student Activity

1. Fun with beads – students will understand the concept of polymer – polynucleotide and different sequences of DNA by preparing a chain of 20 beads of four different colours.
2. Prepare a model of DNA
3. Supercoiling of DNA – students will hold the ends of the rubber band and twist it. The two ends will be joined to feel the stress of coiling relieved due to supercoiling.
4. On paper replicate the following segment of DNA

   5′ATCGGCTACGTTCAC3′

   3′TAGCCGATGCAAGTG5′

   Show the direction of replication of the new strands and explain what the lagging and leading strands are? Explain how this is semiconservative replication. Are the new strands identical to the original segment DNA?

Glossary

1. Acute disease: A disease in which symptoms develop rapidly but lasts for only a short time.
2. Antibiotic: An organic substance produced by one organism that in low concentrations kills or inhibits the growth of other organisms.
3. Antibiotic Susceptibility Test: It is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in *vivo*.
4. Antiserum: A blood derived fluid containing antibodies.
5. Aseptic techniques: Laboratory techniques to minimize contamination.
6. Assimilation: The absorption and digestion of nutrients by any biological system.
7. Axenic: Pure cultures of micro organisms, which are not contaminated by any foreign organisms.
8. Bacterial transformation: The production of a new phenotype as a result of introduction of novel genetic material.
9. Bactericide: A substance capable of killing bacteria
10. Base Stacking: Stacking implies vertical interactions between bases as they sit on top of one another.
11. Bio-augmentation: The use of pollutant acclimated microbes or genetically engineered microbes for bioremediation.
12. Bioreactor: A fermentation vessel with controls for environmental conditions; temperature and pH.
13. Bioremediation: The use of microorganisms to remove an environmental pollutant.
14. Biotechnology: The industrial application of microorganisms, cells, or cell components to make a useful product.
15. Caramel: Sugar or syrup heated until it turns brown, used as a flavouring or colouring agent in food or drink.
16. Clone: Cells which have descended from a single parent cell organisms having identical copies of DNA structure which is obtained by replication.
17. Coagulation: The action or process of a liquid, especially blood, changing to a solid or semi-solid state.
18. Coal-tar dyes: Liquid produced by distilling coal containing benzene naphthalene, phenols, aniline and many other organic chemical.
19. Coliforms: Aerobic or facultatively anaerobic, Gram negative, non endospore forming, rod shaped bacteria that ferment lactose with acid and gas formation within 48 hours at 350 °C.
20. Colony: A Colony is defined as a visible mass of microorganism all originating from a single mother cell.
21. Competition: A rivalry between two or more species for limiting factor in the environment.
22. Cover slip: A small, thin piece of glass used to cover and protect a specimen on a microscope slide.
23. CsCl Density gradient centrifugation: DNA is mixed with CsCl and centrifuged at very high speeds in an ultracentrifuge for many hours. A linear gradient of CsCl is produced with the highest density at the top and the heaviest at the bottom. As CsCl gradient forms, the DNA comes to equilibrium

in the gradient where its density equals the density of the surrounding CsCl.

24. Denaturation of DNA: Separation or unwinding of dsDNA strands into single strands.
25. Denature: To deprive something of its natural character and properties.
26. Depyrogenation: Removal of pyrogens from solutions mostly from injectable pharmaceuticals.
27. Dermatomycosis: A fungal infection of skin.
28. Diatomaceous earth: A soft, crumbly, porous sedimentary deposit formed from the fossil remains of diatoms.
29. DNA amplification: The production of multiple copies of a sequence of DNA.
30. Electromagnetic spectrum: The range of wavelengths or frequencies over which electromagnetic radiation extends.
31. Enzymes: A molecule that catalyzes biochemical reactions in a living organism, usually a protein.
32. Exudate: Low molecular weight metabolites that enter the soil from plant roots.
33. Fermentation: The enzymatic degradation of carbohydrates in which the final electron acceptor is an organic molecule, ATP is synthesized by substrate level phosphorylation, and oxygen is not required.
34. First line of defense: Includes physical and chemical barriers that are always ready and prepared to defend the body from infection.
35. Flake: A small flat thin piece of which has broken away or been peeled from a larger piece.
36. Fluorescent antibody (FA) technique: A diagnostic tool using antibodies with fluorochromes and viewed through a fluorescence microscope; also called immunofluorescence.
37. Fluoresence: The property of absorbing light of short wave length and emitting light of longer wave length.
38. Folliculitis: An infection of hair follicles, often occurring as pimples.
39. Fulminating: A condition that develops quickly and rapidly increases in severity.
40. Furuncle: A pus filled, painful infection of a hair follicle.
41. Gene: A unit of heredity which is transferred from parent to progeny.
42. Genetic code: The mRNA codons and the amino acids they encode.
43. Genetics: The study of heredity and variation of inherited characteristics.
44. Genome: One complete copy of the genetic information in cell.
45. Genomics: Study of genes and their functions.
46. Genotype: The genetic makeup of an organisms.
47. Gestation: The development of something over a period of time.
48. Heavy isotope: A stable atom in which there are more neutrons than in the normal isotope of the element, giving it a greater mass. For example, 15N is the heavy isotope, 14N the common form.
49. Histology: The study of microscopic structure of tissues.
50. Horizontal gene transfer: The transfer of genes between two organisms in the same generation.

51. Hypersensitivity: An altered, enhanced immune reaction leading to pathological changes it is also called allergy.
52. Hypotonic environment: Environment with higher water concentration and less solutes.
53. Immunodiffusion test: A test consisting of precipitation reactions carried out in an agar gel medium.
54. Immunoelectrophoresis: The identification of proteins by electrophoretic separation followed by serological testing.
55. Immunotherapy: Making use of immune system to attack tumour cells, either by enhancing the normal immune response or by using toxin – bearing specific antibodies.
56. Incubation: The microbial growth are obtained by placing the medium in the bacteriological incubator.
57. Indicator: Device or an organisms providing specific information on the state or condition of something, in particular.
58. Inflorescence: The arrangement of the flowers on a plant.
59. Inoculation loop: They are made of platinum or nichrome wire. They are used to make smears.
60. Inoculum: The material used to introduce an organism into a certain medium for growth or culture medium in which microorganisms are implanted.
61. In vivo: Process taking place in a living organisms.
62. Ionizing radiation: Radiation consisting of particles, X-rays, or gamma rays with sufficient energy to cause ionization in the medium through which it passes.
63. Latent infection: A condition in which a pathogen remains in the host for long periods without producing disease.
64. Lymph: A colourless fluid containing white blood cells, which bathes the tissues and drains through the lymphatic system into the bloodstream.
65. Lysis: Destruction of a cell by the rupture of the plasma membrane, resulting in a loss of cytoplasm.
66. Lysozyme: An enzyme capable of hydrolyzing bacterial cell walls.
67. MHC: Major histocompatibility complex – The genes that code for histocompatibility antigens; also known as human leucocyte antigens.
68. Microaerophile: An organism that grows best in an environment with less molecular oxygen (O2) than is normally found in air.
69. Molasses: It is a viscous product resulting from refining sugar cane or sugar beets into sugar.
70. Monomer–A small molecule that collectively combines to form polymers.
71. Mucigel: Mucilage or complex polysaccharide forming a layer around plant roots.
72. Mycorrhiza ("fungus root"): The association, usually symbiotic, of specific fungi with the roots of higher plants.
73. Neutralism: A lack of interaction between two organisms in the same ecosystem.
74. Nick: It is discontinuity in a dsDNA molecule where there is no phosphodiester bond between

adjacent nucleotides of one strand.

75. Non-ionizing radiation: Any type of electromagnetic radiation that does not carry enough energy per quantum (photon energy) to ionize atoms or molecules—that is, to completely remove an electron from an atom or molecule.
76. Normal microbiota: The microrganisms that colonize a host without causing disease; also called normal flora.
77. Occupational health: The branch of medicine dealing with the prevention and treatment of job related injuries and illnesses.
78. Ophthalmic: Relating to the eye and its diseases.
79. Osmotic lysis: Rupture of the plasma membrane resulting from movement of water into the cell.
80. Osmotic pressure: The force with which a solvent moves from a solution of higher solute concentration.
81. Oxidation: The removal of electrons from a molecule.
82. Oxidation reduction: A coupled reaction in which one substance is oxidized and one is reduced also called redox reaction.
83. Pathogenecity: The ability of a microorganism to cause disease by overcoming the defence of host.
84. Pathogenesis: The manner in which a disease develops.
85. Pathologist: A scientist who studies the causes and effects of disease. He examines laboratory samples of body tissue for diagnostic or forensic purposes.
86. PCR: Polymerase chain reaction, a technique using DNA polymerase to make multiple copies of a DNA template in vitro.
87. Pellicle: scum on the surface of the liquid medium.
88. Phagocytes: Cells capable of ingesting bacteria or other substances.
89. Phagocytosis: Ingestion of bacteria or other substances by phagocytes.
90. Phylogenetic: Relating to the evolutionary development and diversification of a species or group of organisms, or of a particular feature of an organism.
91. Plasmolysis: Loss of water from a cell in a hypertonic environment.
92. Pluripotent: A cell that can differentiate into a many different types of tissue cells.
93. Polynucleotide: Chain of nucleotides.
94. Pore: A minute opening in a surface, especially the skin or integument of an organism, through which gases, liquids, or microscopic particles may pass.
95. Predation: A relationship between two organisms whereby one organism (predator) engulfs and digests the second organism (prey).
96. Prevalence: The fraction of a population having a specific disease at a given time.
97. Progeny: Offspring, decendant of a cell.
98. Protein sequencing: The practical process of determining the amino acid sequence of all or part of a protein or peptide.

99. Protocooperation: An association of mutual benefit to two or more species but without the cooperation or without being obligatory for their existence or the performance of some function.
100. Pustule: A small pus filled elevation of skin.
101. Renaturation/Annealing: Process in which ssDNA or ssRNA pair to form double stranded DNA.
102. Salmon-GAL (6 chloro 3- indolyl –β – D galactopyranoside): It is a chromogenic substrate capable of detecting LacZ gene encoded β galactosidase.
103. Semi-transparent: Partially admitting the passage of light through its substance.
104. Serial dilution: Series of step wise dilutions normally done in sterile water which is done to reduce microorganism population to manageable numbers.
105. Serological methods: Methods for identifying microorganisms based on its reactions with antibodies.
106. Smear: A thin spread of bacterial suspension from a clinical spemicen or from a culture on a glass slide.
107. Spectrophotometer: An apparatus for measuring the intensity of light in a part of the spectrum, especially as transmitted or emitted by particular substances.
108. Stab culture: A long straight wire dipped in culture is punctured into a solid medium usually to see the motility.
109. Strain: A population of cells all of which arise from a single pure isolate. Strain is a subtype of a microbe.
110. Sutures: A stitch or row of stitches holding together the edges of wound or surgical incision.
111. Template: A structure that would allow molecules to be lined up in a specific order and joined, to create a macromolecule with a unique sequence and function.
112. Three-dimensional: having or appearing to have length, breadth, and depth.
113. Topological stress: stress created due to over winding or repeated interwinding of DNA during replication.
114. Topography: the arrangement of the natural and artificial physical feature of an area.
115. Toxigenic: (especially of a bacterium) producing a toxin or toxic effect.
116. Turbo blower: It is a fan that blows the air.
117. Vaccine: A preparation of killed, inactivated, or attenuated microorganisms or toxoids to induce artificial immunity.
118. Vacuoles: A space or vesicle within the cytoplasm of a cell enclosed by a membrane and typically containing fluid.
119. Vegetative cells: A bacterial cell growing actively under favorable conditions.
120. Virulence: The degree of a pathogenicity of a pathogenic microorganism.
121. X-ray diffraction method: Technique used for determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of incident X rays to diffract into many specific directions.

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Microbiology Weblinks

Chapter - 1

Web link: http://www.britannica.com / biography/Alexender-Fleming

Chapter - 2

Working of compound microscope

https://youtu.be/cmzWDkOYTjM

Chapter – 3

Gram Staining

https://youtu.be/L9bats-vGDY

Endospore Staining

https://youtu.be/o1uYmUW4qe8

Chapter – 4

Quick review of sterilization

https://youtu.be/ZDmP14twN8g

Chapter – 5

Streak Plate

http://youtu.be/NDMNGnxCZ1Q

Bacterial colony description

https://youtu.be/gH--8YWdyyk

Chapter – 6

Photosynthesis

https://youtu.be/1Dn_zdAZN0I

Chapter – 7

Bacterial flagellum

https://youtu.be/PIOfMifowP4

Chapter – 8

Classification of microbes

https://youtu.be/W2nNIRUs6Wo

Taxonomy and Classification

https://youtu.be/yCMDHd44ekQ

Chapter – 9

Can microbes clean up oil

https://youtu.be/a_HWLFzgQiM

Composting

https://youtu.be/VNgFXvL9ZH8

Chapter – 10

soil horizons types

https://youtu.be/OEvLuucpYw8

Chapter – 11

Nitrogen fixation

https://www.youtube.com/watch?v=qzh7ZzJQJ84

Late blight of potato

https://youtu.be/2Y77KEYuw_g

Chapter – 12

Bacterial meninigitis

https://youtu.be/HhWjA1xq3Ig

Chapter – 13

Agglutination Reaction

https://www.youtube.com/watch?v=3W67OH3v2lU

Coomb's Test

https://www.youtube.com/watch?v=sUHsX3xrlFM

Chapter – 14

Structure of DNA

https://youtu.be/F5JazhVvlm4

Difference between prokaryotic and eukaryotic DNA

https://youtu.be/0CoZT6hYemk

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