Molecular Tools for Disease Detection

Molecular Tools for Disease Detection:

Trends and Developments

Edited by

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Ambili Mechoor PhD
Rahana Rathnan PhD

CHAPTER 1

MOLECULAR DIAGNOSTICS: A BRIEF INTRODUCTION

AMBILI MECHOOR & RAHANA RATHNAN

1.1 Introduction

Molecular diagnostic techniques are used to analyse biological markers in the genome and proteome of an individual. Both genomic and proteomic patterns are used by researchers for the expansion of information in diagnostic fields and by clinicians for assisting with diagnosis, prognosis, and therapeutic monitoring. Molecular diagnostics tests the presence of pathological mutations using the direct examination of molecules like genomic DNA, cDNA, RNA, or proteins and helps in detection, diagnosis, prognosis, and response to therapy.

Molecular diagnostics integrates laboratory medicine with the knowledge and technology of molecular genetics and has been enormously modernized over the last decades, benefiting from the discoveries in the field of molecular biology. This technique is used to diagnose and monitor the disease, detect risk, decide which therapies will work best for individual patients, and offer the prospect of personalised medicine, precision medicine, or theranostics. The uses of molecular diagnostics are diverse, including forensics testing, metagenomics, molecular oncology, clinical pathology, epigenetics, immunotherapy and immunosuppression, molecular endocrinology, and toxicology.

Molecular biology has modernised biological and biomedical research and has become an imperative tool in clinical diagnostics (Fig. 1.1). It has developed more than any other science in the last 20 years. Until this time, the clinical laboratory had been illustrative in nature and could measure

events that were currently going on by evaluating haematology, biochemistry, microbiology, or anatomical pathology. The integration of diagnostics and therapeutics signifies a major innovative prospect to emerge as leaders of the new medicine, guiding the selection, dosage, route of administration, and multidrug combinations and producing increased efficacy and reduced toxicity of pharmaceutical products.

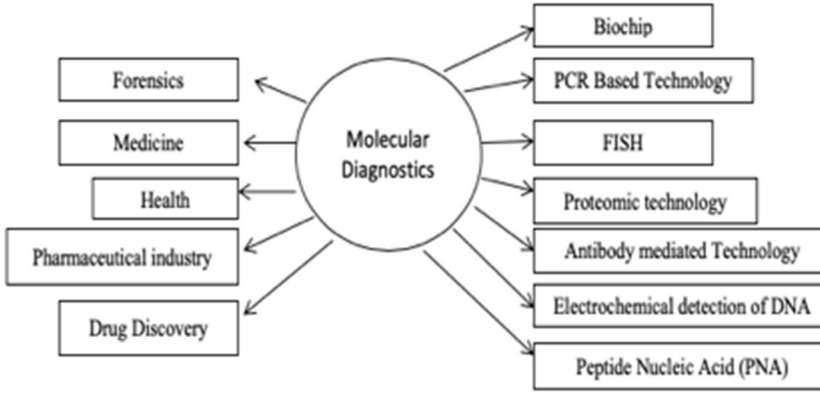


Figure 1.1: Application of molecular diagnostics

The identification and fine characterization of the genetic basis of the disease in question are vital for the accurate provision of a diagnosis. These gene-based markers allow physicians not only to assess disease predisposition but also to design and implement improved diagnostic methods. The revolution in molecular biology is pervading every aspect of medical practice. The rate of disease gene discovery is rising exponentially, which facilitates the understanding of diseases at the molecular level. Gene-based markers allow physicians not only to evaluate disease predisposition but also to design and implement improved diagnostic methods.

Molecular understanding of the disease is translated into diagnostic testing, therapeutics, and eventually, preventive therapies. The main goal of molecular diagnostics is to introduce essential concepts that impact the identification of novel markers of human diseases and to develop and apply useful molecular assays to monitor the disease, determine

appropriate treatment strategies, and predict disease outcomes. Molecular diagnostics is presently a clinical reality with roots deep in the essential study of gene expression pattern and function.

1.1.1 Historical perspective

Linus Pauling and Harvey Itano, in 1949, introduced the term molecular disease in medical vocabulary based on their discovery of a single amino acid change in the beta-globin chain of haemoglobin that leads to sickle cell anaemia. The defect is produced by the substitution of glutamic acid (Glu) by valine (Val) at the sixth position of the beta-globin chain of the haemoglobin molecule. In principle, their findings have laid the foundations for molecular diagnostics.

The first foothold in molecular diagnostics was provided in the early days of recombinant DNA technology. cDNA cloning and sequencing provide basic knowledge on the primary sequence of various genes. DNA sequencing provided a number of DNA probes, allowing the analysis via southern blotting of genomic regions, leading to the concept and application of restriction fragment length polymorphism (RFLP), which tracks a mutant allele from heterozygous parents to a high-risk pregnancy. For the first time, in 1976, Kan and colleagues carried out prenatal diagnosis of alpha-thalassemia using hybridization on DNA isolated from foetal fibroblasts, and Kan and Dozy, in 1978, implemented RFLP analysis to pinpoint sickle cell alleles of African descent. This revolution provided the means of establishing diagnostic methods for the characterization of other genetic diseases, such as phenylketonuria, cystic fibrosis, etc.

The discovery of the polymerase chain reaction by Karry Mullis in 1983 and its optimisation have greatly facilitated and revolutionised molecular diagnostics. The most powerful qualities of PCR are the synthesis of a large number of copies of the target sequence by exponential amplification, the generation of DNA fragments for analysis and detection methods, and the rapid identification of a pathogen or mutations within a day.

1.2 Molecular diagnostic methods

Advances in diagnostic medicine, on the other hand, have come through the application of science and technology as a result of a coordinated effort among academia, industry, government, and private institutions. We are now entering the era of molecular diagnostics and pathology, which is bringing forth the newest and most powerful science and technology available for the modern-day practice of diagnostic laboratory medicine. Molecular diagnostic technologies have the potential to move from diagnostics to prognostics.

Molecular diagnostics uncovers sets of changes and captures information as gene expression patterns. The gene expression patterns in diseases are improving clinicians' abilities to diagnose diseases. Molecular diagnostics is constantly translating discoveries and novel technologies into useful clinical tests that provide a molecular fingerprint in the field of pathogenic conditions like cancer and are predictive of the response to specific therapies.

- Diagnostic: Identity of a disease
- Prognostic: Outcome of a disease
- Predictive: Possibility of a disease
- Therapeutic: Response of a disease to treatment

1.2.1 Types of Molecular diagnostic techniques:

- Amplification Techniques
    - PCR: Polymerase Chain Reaction
        - Polymerase chain reaction (PCR) is a technique widely used to make several copies of a specific DNA segment. Copies of DNA sequences are exponentially amplified to generate thousands to millions of more copies of that particular DNA segment using thermostable DNA polymerase enzymes.

- LCR: Ligase Chain Reaction
  - The ligase chain reaction (LCR) is a method of DNA amplification. It involves a thermostable ligase to join two probes or other molecules together, which can then be amplified by standard PCR cycling.
- NASBA: Nucleic-Acid Sequence-Based Amplification
  - Nucleic acid sequence-based amplification (NASBA) is a sensitive, isothermal, transcription-based amplification system specifically designed for the detection of RNA targets. This amplification system uses a mixture of three enzymes (avian myeloblastosis virus reverse transcriptase, RNase H, and T7 RNA polymerase), leading to the formation of amplification products of single-stranded RNA.
- DNA Sequencing
- DNA sequencing is the process of determining the sequence of nucleotides within a DNA molecule. Every organism's DNA consists of a unique sequence of nucleotides. Modern DNA sequencing consists of high-throughput methods that allow entire DNA sequences to be discovered in a matter of hours.

(c) Hybridization Techniques

- Southern hybridization Blot
  - Southern blotting is a method used in molecular biology for the detection of a specific DNA sequence in DNA samples. Southern blotting combines the transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.
- Northern hybridization Blot
  - The northern blot, or RNA blot, is a technique used to study gene expression by detecting RNA (or isolated mRNA) in a sample.

(d) Electrophoretic Methods

- SSCP (single-strand conformation polymorphism)

Single-strand conformation polymorphism (SSCP) is a molecular biology technique used to detect variations in DNA sequences by examining the conformational changes in single-stranded DNA molecules. SSCP is a relatively simple and cost-effective method for identifying mutations, polymorphisms, or sequence differences within a given DNA fragment.

- DGGE (denaturing gradient gel electrophoresis)

Denaturing Gradient Gel Electrophoresis (DGGE) is a molecular biology technique used to separate and analyse DNA fragments based on their sequence variations, specifically by detecting differences in the melting behaviour of double-stranded DNA molecules. DGGE is a valuable tool for identifying sequence variations, mutations, or polymorphisms in DNA fragments, making it useful in fields such as genetics, microbiology, and environmental science.

(e) Recombinant DNA Technology

Recombinant DNA technology, also known as genetic engineering or gene splicing, is a set of molecular techniques that allow scientists to manipulate DNA molecules to create new combinations of genes, alter existing genes, or transfer genes from one organism to another. This technology has had a profound impact on various fields, including medicine, agriculture, and biotechnology.

(f) Biochip Technology

Biochip technology, also known as microarray technology, is a powerful and versatile tool in the fields of molecular biology and biotechnology. It involves the fabrication of small, solid surfaces, usually made of glass or silicon, that are densely packed with miniaturized biological molecules, such as DNA, RNA, proteins, antibodies, or enzymes. These microarrays, or biochips, allow for the simultaneous analysis of thousands to millions of biological interactions or reactions in a high-throughput and parallel manner.

1.3 Biomarkers and disease detection

According to the Food and Drug Administration (FDA) and the National Institute of Health (NIH), a biomarker or biological marker is “a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions." A biomarker can be any biological indicator that can be measured. For instance, biomarkers can be cellular or molecular (DNA, RNA, protein, and metabolites). They are measured from a tissue biopsy or a liquid biopsy (blood, urine, saliva, etc.). Other biomarkers (physiological, morphological, etc.) can also be used or measured through clinical or medical imaging. Biomarkers can be either quantitative or qualitative. Qualitative biomarkers could be involved in pathogenic process detection within a yes/no analysis, while quantitative biomarkers are involved in pathogenic process detection with a threshold effect.

Biomarkers are used in research and clinical practice for the following:

- Diagnosing diseases or predicting the risks of disease,
- Monitoring healthy people to detect early signs of disease,
- Determining whether a treatment is efficient or not,
- Targeting specific groups of people for whom a particular drug may be useful,
- Producing safer drugs by predicting the potential for adverse effects earlier,
- Providing researchers with the opportunity to have a global view of the events and changes that are always occurring within a cell.

CHAPTER 2

DISEASE PATHOLOGY

CAROLINE ROSE ALUKKAL
& AMBILI MECHOOR

Pathology comes from the Greek word pathos, which connotes “experience” or "suffering." It relates to an anomalous condition of the body termed a disease, which is materialised when the immune system is weak (in the case of acquired diseases), when the immune system acts against itself (in the case of autoimmune diseases), or when genes result in defective variations (in the case of genetic diseases). In short, any discontented state of the body that is directly visible or invisible is a disease. Pathology is the science of the causes and effects of these diseases or even death, mostly through the laboratory examination of the entire body or samples of body tissue or fluid. It is documented that this scientific field of study commenced at the very beginning when human beings realised that disease is the state of a body caused by physical factors rather than supernatural reasons, with the vaidhya (in the Indian context) being the first pathologists. There are pieces of evidence that date back to the Indus Valley civilization, Chinese civilization, and other Middle East human settlements where detailed assessments of the body with dissection and detailed investigations for particular maladies, along with medications, were given to the upper castes to cure or soothe diseases. The detailed and rigorous causal study regarding the diagnosis and prognosis of the diseases was in progress by the Hellenic period of ancient Greece, with Hippocrates as one of the many distinguished physicians who is admired even in the 21st century for their fundamental research against the odds. The Romans and Byzantines followed the Greek traditions in medical practice until the Classical Era, after which the quest for other scientific areas dominated, which resulted in the torpidity of pathology. The Islamic, Renaissance,

Enlightenment, and Baroque eras were the golden years when empirical studies were promoted and textbooks on convoluted pathological investigations were developed. In the 17th century, the microscope gave a new direction to pathology through the birth of the “germ” theory. The era of scientific pathology starts with the invention of the microscope, which serves even today (in much more improvised and sophisticated modes) as one of the major tools for diagnosis. Modern pathology started to grow as a well-defined science during the 19th century through the philosophers and physicians who studied disease as “morbid anatomy." Pathology was recognized as a specialized area of research with the advent of microbiology. The term “germs” was coined to represent all the disease-causing pathogens, which include bacteria, fungi, protozoa, algae, viruses, and prions, which replaced the spiritual reasons for the incidence of disease that were deep-rooted over 1500 years in European medicine. The pathologists of the 19th century understood that the causative agents have certain characteristic manifestations specific to each disease, and diseases can also be replicated. This approach of widely accepted assumptions and symptoms for the determination of disease is still valid at the present time. The microscopic studies by Rudolf Virchow to analyse the cellular sections marked the pathology, leading to a variety of specialties. By the 20th century, pathology had split into a number of profound fields that were interrelated and essential for complete diagnostic studies.

The scientific studies on causative agents for the diseases first ensued at Heinrich Hermann Robert Koch’s laboratory in 1884. He proposed Koch’s postulates, a series of four generalised principles linking specific microorganisms to specific diseases, especially tuberculosis, cholera, and anthrax. Koch’s studies helped to improve the understanding of diseases and public health. This extensive study gave rise to a serious approach to understanding the pathogen interaction in other diseases as well. Some of the earliest works in this domain are

- first time to observe protozoan parasites in 1880.
- discovery of phagocytes by Élie Metchnikoff in 1882
- discovery of the malaria parasite by Ronald Ross in 1897

- Anti-toxin for diphtheria was discovered by Emil Adolf von Behring in 1901.
- usage of phototherapy for the treatment of diseases by Niels Ryberg Finsen in 1903
- The concept of infectious disease was instigated by Robert Koch in 1905.
- The concept of chemotherapy was introduced by Paul Ehrlich in 1909 for treatment against syphilis.
- The discovery of the first antibiotic, penicillin, in 1928
- The study of host-pathogen interaction in mouse typhoid caused by *Salmonella typhimurium* in 1942
- The discovery of streptomycin, the first antibiotic against tuberculosis
- The malaria eradication in Liberia in 1954
- The study on measles by M. S. Bartlet in 1957
- Surface antigen for hepatitis was discovered in 1964.
- The first book on the historical overview of the epidemics, "Plagues and People," by William McNeill in 1976
- The Ebola outbreak of 1976 in West Africa
- The concept of H antigen commenced in 1980.
- Prion was discovered in 1982.
- Multicenter AIDS cohort studies have been initiated since 1983.
- study on stress and immunological response in host-pathogen interactions in 1992
- recognition of virus-infected cells in 1996
- the attempt for the eradication of malaria from Africa in 2007

The understanding of the host-pathogen interaction helps the researchers comprehend the disease pathogenesis and host responses. The study of diseases on a chronological basis has helped to beat or eradicate a large number of diseases.

Pathology, in general, is the study of diseases, which includes a wide range of bioscience research fields and medical practices. In the modern context, the word refers to general pathology, which comprises the specialties needed to diagnose disease through the analysis of tissue and

fluid samples. The state of the disease can be physical, such as in carcinoma, or psychological, in the case of psychopathy. Generally, pathological investigations focus on the cause, pathogenesis (the development of the disease), changes in the cell structure (morphology), and the clinical manifestation of the changes. The primary investigations to diagnose the disease by analysing the clinical abnormalities that are markers or precursors are conducted by two major specialties: anatomical pathology and clinical pathology. The other spheres of pathology are utilised when the diseases remain unrecognized. An inclusive classification of the pathological departments includes:

a) Hematopathology: It deals with the diseases of the blood, the transfusion of the blood, and the products derived from blood.
b) Chemical pathology: It is the biochemical study and investigation of disease processes with particular emphasis on metabolic diseases, which include diabetes, inborn errors, and lipid disorders.
c) Microbiology: This is the scientific study of the microbial causative agents (bacteria, fungi, viruses, etc.) of diseases through the examination of the body fluids.
d) Anatomical pathology: It is the study of organs and tissues to diagnose the disease. This domain is further divided into histopathology and cytology. Histopathology is the microscopic examination of the tissues acquired through a biopsy or resection specimen. The latter is the examination of individual cells for abnormalities.
e) Genetics: It is the study of chromosomal abnormalities (cytogenetics) and mutations in DNA (molecular genetics).
f) Immunopathology: It is the study of the immune response at the molecular level for markers in allergy and autoimmune diseases.
g) Forensic pathology: It is the scientific investigation of the reasons for unexpected deaths to help the police inquest.

General pathology is the study of diseases with the help of more than one of the above-mentioned specialties. By and large, during the hospital visit, the patient confronts the general pathologists for routine or primary examination. Normally, the general pathologist’s service is rendered by

the chemical pathologist, haematologist, and microbiologist as they perform the basic tests for disease diagnosis.

2.1 Host-Pathogen Interaction

The earth is an endless consortium of microbes that are present almost everywhere, even in the extremely toughest situations to survive, which include hot springs, acid mines, etc. Every day, a large number of microbes find their way to our body through inhalation, ingestion, skin lesions, and mucous membranes. Most of the time, the microbes do not cause any harm to us but rather help us with a variety of duties ranging from digestion to nutrient enrichment and many more. At times, they cause disease by bypassing or destroying the various levels of the human defence system, which is called the 'Immune system'. Anything that causes destruction or negative impact in the human body is called a pathogen. Pathogens can be viable (bacteria, fungi, and protists) or unviable (dead microbes, viruses, and prions). The host and the pathogen interact for the manifestation of the disease in the body. The study on the invasion of the pathogen over the biological barriers and survival even inside the immunised individuals is the host-pathogen interaction. This interaction is highly active, meaning that the host defends the pathogen incessantly while the pathogen adapts to changes and develops alternative ways to infect the host. This dynamic characteristic of the host-pathogen interaction makes it very difficult to find a permanent solution for the treatment of many diseases (superbugs, AIDS).

2.1.1 Levels of Host-Pathogen Interaction

The host and the pathogen interact continuously, with the host trying to eliminate the pathogen while the pathogen striving to trounce the defense. The pathogens optimise their traits to suit the balance of the evolutionary forces in the host population. This interaction has been understood at three levels: the population level, the organismal level, and the molecular level. The population-level interaction studies include the pathological investigation of the disease within a population. The organismal level includes parasitic diseases. The host and the pathogen interact with each other through three modes: mutualism, commensalism, and parasitism. Mutualism is the positive

interaction in which both the partners, i.e., the host and the organism, drive positive results. For example, the gut flora helps in easy digestion, improved uptake, and nutrient fortification for the human hosts, while they themselves use the space and nutrients. Commensalism is a neutral interaction that involves one partner driving a positive result while the other is neither helped nor harmed. This is seen in the case of microbes that endure in the digestive tract but cause diseases when moved to other parts of the body. Parasitism involves negative interaction for the hosts while the parasites survive wholly on the host machine for growth. This is seen in diseases such as dengue and chikungunya, where the pathogens need to be inside the host either to complete its life cycle, gain pathogenicity, or for transmission. The commensalistic and parasitic interactions are of pathological importance due to the inherent disease capacity of the microbes involved in the interaction. The third level of study includes the molecular level of interaction between the host and the pathogen. The ultimate aim of all these studies is to understand this level of interaction for the development of the appropriate medication for the control of the disease. Also, the research on the other two levels of interaction is important to recognise the mode of infection and the control of the diseases. The molecular level of interaction will be studied in the coming chapters with a detailed approach.

2.1.2 Classification of Host-Pathogen Interaction

The term 'host-pathogen interaction' simply implies the ways in which the pathogen infects the host. Every day, a new pathogen will be detected, its infection characteristics will be studied, and prevention or cure will be attained. The research on the interaction between the host and the pathogen is important in this contest. There are different levels of interaction between the host body and the parasitic microbe; each level shows a varied level of complexity and control over the pathogenic progress. The interactions are mainly arranged into four basic levels:

1. Invasion of pathogens through breaches of the host's primary and secondary barriers
2. Evasion of host defences by pathogens
3. Pathogen replication in the host

4. Host's immunological capability to control or eliminate the pathogen

2.1.2.1 Invasion of Pathogen

The pathogen utilises a horde of methods to assault the host's immune system by infringing on the primary resistance barriers such as the skin, mucous membrane, body secretions, etc., damaging tissue, and impeding the responsivity of the immune system. One of the essential components of these strategies to prevail over the host's immune system is the secretion of proteins across phospholipid cell membranes. Secreted proteins promote bacterial virulence, enhance attachment to eukaryotic cells, scavenge resources in an environmental niche, directly intoxicate target cells, and disrupt cell functions. The main types of secreted proteins include effectors, toxins, multivalent adhesion molecules, and urease. These proteins are secreted by the pathogen during contact with the host body, preventing the pathogen from being attacked by the primary line of the immune system of the host.

Bacterial effectors are proteins secreted by the gram negative bacteria into the host for different activities, but usually help the pathogen invade host tissue, suppress its immune system, or otherwise help the pathogen to survive, thus making it vital for virulence. The loss of secretory systems sometimes makes the pathogen avirulent even inside the human bloodstream (the loss of the Type III secretory system makes *Yersinia pestis* nonpathogenic). There are at least six different specialised secretion systems for the secretion of effector proteins, named Type I, Type II, Type III, Type IV, Type V, and Type VI. Of these secretory systems, Types III, IV, and VI are seen to have prominent action in the microbes. The number and diversity of the effectors may vary from a few to even hundreds. The mode of effector protein release to the host varies with the type of microbe being attacked. It has been seen that gram negative bacteria having thin two phospholipid membranes separated by a periplasmic compartment containing a thin layer of peptidoglycan modify the environment to attack or invade the pathogen, utilising bacterial outer membrane vesicles to transfer different virulence factors via the membrane vesicle trafficking secretory pathway, while gram positive bacteria use the *Tat* pathway and *Sec* accessory pathway along with injectsome to secrete the proteins across

the single lipid bilayer surrounded by a thick cell wall. Another class of helper compounds are toxins, which are molecules, peptides, or proteins produced within the living system but are poisonous to human beings once absorbed and reach the human body. Toxins interact with enzymes and cellular receptors, causing an alteration to the protein structure. The change in the protein structure leads to a change or loss of protein function. The effect of the toxins varies from a bite to sudden death. The amount of toxin being secreted into the host ranges from an extremely small quantity during the initial attack so as to minimise the attack on the host's immune system. Urease (absent in mammals) is also an indicator of the invasion of the pathogen in humans because of its toxicity to the epithelium. This enzyme converts urea to carbon dioxide and ammonia, which shortens the life span of cells, and higher concentrations cause tissue necrosis and cytolysis. Another group of supporting proteins that enhances virulence is the Multivalent Adhesion Molecule (MAM). It helps in establishing high affinity binding to host cells during the early stages of infection via protein-lipid (phosphatidic acid) and protein-protein (bronectin) interactions.

2.1.2.2 Evasion of Host Defenses

The pathogens invade the host body, breaching the general primary line of defense. Once inside the human host, the microbe needs mechanisms to dodge the defence offered by the host's immune system. This process is called the evasion of host defenses. Each pathogen develops different dynamic strategies for escaping the host immune system, which makes the treatment of some diseases such as HIV, tuberculosis, and malaria, each killing one to two million people worldwide each year, very difficult. The success of each pathogen in surviving inside the host is directly dependent on its ability to mount an effective anti-immune response within the infected host, which can ultimately result in acute disease or chronic infection; otherwise, it will wipe out of the host. And also, viral and bacterial diseases are often linked, exploiting weaknesses in host defences that are caused by another pathogen. For example, pneumonia attacks occur after influenza infections and HIV occurrences, followed by an incidence of tuberculosis and salmonellosis.

Pathogens employ different approaches to obstruct and evade the host immune attack. In the case of *Mycobacterium tuberculosis*, it actively transcribes a number of genes involved in defence and evasion from the host system. They also trigger signals for anti-inflammation by glycolipids to prevent an extreme host response. *Staphylococcus aureus* expresses immune-evasive proteins, which are present in most of the strains. On the other hand, the recognised targets of these proteins vary in different hosts, which implies that these proteins are not crucial for virulence. Pathogens generally affect the essential pathways in the host, thus making the host weak enough to launch a strong defence mechanism. Many bacteria and viruses attack the hub proteins, which are pivotal in several essential pathways, to deteriorate the defence system. One of the crucial elements of the human immune system is the macrophage (a type of white blood cell), which is another important tool used for pathogenic evasion. Macrophage performs dual functions: scavenger activity of this cell ensures the uptake and destruction of bacteria in phagolysosomes and activation of the adaptive component of the immune system through the presentation of bacterial antigens. The pathogens have evolved dynamic methods to circumvent this fatal environment, utilising normal host cell function. The viral infections antagonise the sensing of infection, production of interferons, and synthesis and activity of interferon-stimulated genes to overcome the host's immune attack. In general, pathogens defend the host immune system using the 12 different strategies described in Section 2.1.4.

2.1.2.3 Replication of Pathogen

The pathogen needs suitable means to replicate inside the host for survival and to transmit to most hosts, even when the pathogens are in continuous battle with the host's immune system. The means and modes of support drawn from the host for the growth of the pathogen differ depending on the type of pathogen. On one hand, viable parasites such as bacteria, fungi, and protists are complete cells utilising their own machinery for replication, transcription, and translation, whereas dead pathogens such as viruses and prions utilise the host's package of enzymes and proteins for the different stages of growth. Inside the host, they require very few genes and proteins when compared to survival outside the host. For example, *Salmonella typhimurium* has 1083 genes that catalyse 1087 metabolic and

transport reactions, implying a minimal set of pathways are required for the survival of the host. In the erythrocytic stage of the malaria parasite, *Plasmodium* species needs proteases for a number of its cellular processes in order to survive in the host. The hijack of the host cell system after the pathogens infect the host. Some pathogens, such as *Mycobacterium,* respond to the microenvironment by changing the metabolic state for survival and growth. Some pathogens have functionally distinct proteinases (enzymes performing proteolysis) in the genome so as to have enough flexibility to multiply and survive in the host. Sometimes the host factors (the host's facilities that promote or inhibit the pathogen) promote the pathogen's entry, replication, transcription, integration, growth, propagation, and interactions. A set of 116 Dengue Virus Host Factors (DVHF) is needed for the propagation of dengue virus type 2. For HIV-1, a set of 311 host factors is essential. Also, a set of 213 host factors and 11 HIV-encoded proteins have been found responsible for HIV-1 replication. Among them, a few proteins help in the regulation of RNA splicing, ubiquitin conjugation, nuclear import or integration of viral DNA, initiation and/or kinetics of DNA synthesis, DNA damage response, and proteolysis. Some pathogens cannot survive independently, and hence they convert the glial cells of the host into progenitor cells to survive and spread disease in the host. E.g., *Mycobacterium leprae* alters the genetic structure of adult Schwann cells to progenitor cells and de-differentiates back to adult Schwann cells. Pathogens also cause the apoptosis of host defence cells to survive in an extreme environment. Biofilm is yet another established and successful tactic used by the microbes to accustom to their host surroundings. Biofilm helps the pathogen survive in the extreme conditions of nutrient starvation, immune system attack, and reproduction to release numerous cells for further infection.

2.1.2.4 Immunological Capability to Control Disease

Once the pathogen has surpassed the primary line of defence and entered the host, the host's immune system will always try to eradicate it from the body until it is exonerated from the host body. The purpose of most of the medications administered during a diseased state is to support or help the immune system recover from its earlier stage without any illness. The host body also prevents the occurrence of infection or disease by launching an

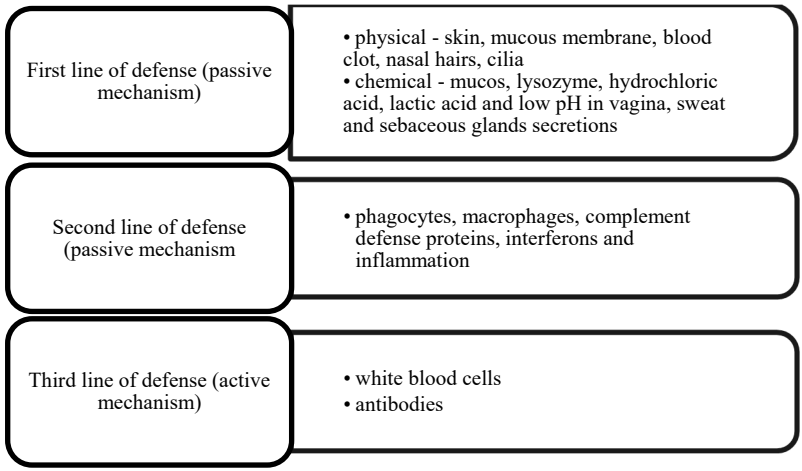
immune response with respect to the pathogenic invasion, which includes high expression of certain genes, autophagy, dendritic cell attack, glycoconjugates, and iron in the activation or alteration of the host immune system. The research has revealed that a large number of genes are expressed when a pathogen is attacking the host body, i.e., gene expression plays an important role in the immune response. E.g., a set of 67 genes was expressed in immune-competent mice within 21 days compared to immune-deficient mice when *Mycobacterium tuberculosis* was infected in mice. Autophagy is another strategy used by the host body to extirpate the pathogen. Autophagy is a regulated natural self-degradative mechanism of the cell that disassembles unnecessary or dysfunctional components and recycles the cellular components to balance the sources of energy at critical times in development and in response to nutrient stress. Autophagy has multiple roles, such as housekeeping with the removal of misfolded or aggregated proteins and damaged organelles such as mitochondria, endoplasmic reticulum, and peroxisomes, as well as eliminating intracellular pathogens, which is generally a survival mechanism. Autophagy also promotes cellular senescence and cell surface antigen presentation, protects against genome instability, and prevents necrosis, playing a key role in preventing diseases such as cancer, neuro-degeneration, cardiomyopathy, diabetes, liver disease, autoimmune diseases, and infections. Dendritic cells are another important member of the immunological activation pathway in humans upon encountering a pathogen. Sometimes the pathogen itself activates dendritic cells, or these cells are recruited to the lamina propria of the mucous lining with the release of chemokines and cytokines (strong mediators of dendritic cell activation). Most of the time, these cells are helpful, while at times they act as carriers of pathogens as they can migrate very quickly to connective fluids such as lymph for the activation of T cells. Glycoconjugates on the cell surface also help in the presentation of the pathogen to the immune cells, which helps to control the disease. Iron helps the host in cytokine secretion and activation of transcription factors related to the immune system, which in turn showcases a method to control the pathogen through judicious homeostasis.

Recent developments in the field of host-pathogen interaction have evolved software to predict this parasitic communication. Different *in-silico* prediction methods are used nowadays for the primary screening of the possible highly dynamic and diverse interactions for experimental validation.

2.1.3 Host Defense Mechanism

Our body encounters a large number of pathogens every day. In spite of all these rigorous attacks, we remain healthy most of the time. The reason behind a healthy body is that the pathogen encounters different stages of resistance from the host before causing the disease. The disease becomes noticeable once the host's defence system is surmounted and the pathogen can survive inside the host. The detailed flow chart for the human defence mechanism is described below:

The primary physical or chemical resistance is offered by the skin, mucosal membrane, blood clotting, sebaceous and sweat glands, lysozyme, nasal hairs, cilia, hydrochloric acid, and lactic acid, along with a low pH in the vagina. Skin, which is composed of epithelial cells, is the preliminary mechanical barrier for entry into the human body. The mucosal membrane secretion involves a large number of defence effector molecules, including antimicrobial peptides and proteinase inhibitors. This membrane is continuous with the skin at various body openings such as the eyes, ears, inside the nose, inside the mouth, lip, vagina, the urethral opening, and the anus, thus safeguarding the body externally from pathogens. It also covers the intestinal tract, where it lubricates and protects the epithelial cells. The blood clots form a mesh-like structure and prevent the entry of microbes into the blood stream. The nasal hair prevents the entry of pathogens via the respiratory route. Cilia punches to force mucus to the pharynx for swallowing in the stomach, and coughing helps in this process. The above-mentioned five render physical resistance.



The chemical barriers are the lysozyme, hydrochloric acid in the gut, the intestinal natural flora, lactic acid, the low pH in the vagina, and the secretions at the hair follicle. Lysozyme is the major innate antimicrobial enzyme produced by the host body. The high pH due to the hydrochloric acid in the stomach kills the pathogen, which finds entry to the stomach through the food. The natural flora in the human intestinal tract defends against pathogen invasion under normal healthy conditions. The normal flora, which persists for a longer time in the host, along with the transient flora, which is built up under special conditions, preclude the pathogens from infecting the human body either by covering the adhesion surfaces, producing toxic compounds, or modifying the essential nutrients so as to prevent the pathogen from consuming them. The disease-causing pathogen invades only when there is a perturbing condition, such as a pH change or toxic chemicals that obliterate the natural flora. The lactic acid produced by the bacteria, along with the low pH, protects the vagina from antigens. Sebaceous and sweat glands secrete antimicrobials in the hair follicles, which support the skin in its defence against the pathogen.

The second line of defence against the pathogen is executed by the non-specific passive general defence system, which includes phagocytes, macrophages, complement defence proteins, interferons, and inflammation. These are specific cells or cell interactions that mediate the pathogen arrest

or modification for the active immune system attack or extermination. Phagocytes are white blood cells that engulf the pathogens and digest them in a way similar to amoebal digestion. Macrophages, on the other hand, are also white blood cells that move around the body as scavengers or are present at the site of infection. They are also present in the lymphatic system as immune sentinels and trophic effectors, mediating the detection, engulfment, and destruction of pathogens and apoptotic cells. The third component of the general defence system is the complement defence proteins. They are produced in response to the presence of antigen, which triggers and participates in a complement reaction, resulting in a series of enzymatic reactions that finally lead to the engulfment and razing of the pathogen. The interferons are also defence proteins produced by the host cells, but under the virus infection, they prevent the neighbouring cells from further viral attack. The most generalised line of attack against the pathogen by the host body is inflammation, which occurs when the tissues or the epithelial surface layer are injured by bacteria, trauma, toxins, heat, or any other cause that induces the damaged cells to release chemicals including histamine, bradykinin, and prostaglandins. These chemicals trigger blood vessels to discharge fluid into the tissues, resulting in swelling, so as to isolate the foreign substance from further contact with body tissues. The inflammation-inducing chemicals also attract white blood cells, causing phagocytosis, which is the engulfment of the germ and the dead or damaged cells, resulting in the formation of pus.

The final stage of defence by the host is the specific immune responses, which are specific to the invader, and hence it is called the active immune response. The human body tries out all the defence mechanisms of the passive immune system, and if it fails, the active system takes over the charge to exterminate the pathogen. This is mediated by a certain type of white blood cells (lymphocytes B and T) and proteins (antibodies). They either attack the pathogen directly or help the other members of the system attack the foreign body more effectively. The B type produces antibodies that attach to a specific antigen (pathogen), which makes the pathogen more susceptible to immune cell attack. On the other hand, the T lymphocytes directly attack the antigens and release certain chemicals called cytokines, which are signalling molecules that mediate and regulate

immunity, inflammation, and hematopoiesis, thus controlling the entire immune response. The lymphocytes differentiate between their own cells and those of the foreign body. It also provides the additional advantage of memory for the immune system to protect against illness when a subsequent pathogen attack occurs in the host. When all the levels of the immune system fail to complete the eradication of the pathogen, the disease occurs. At the same time, the body will be continuously seeking to remove the pathogen, which results in the disease calming down after a short while when the body regains immunity.

2.1.4 Pathogen Defense Mechanism

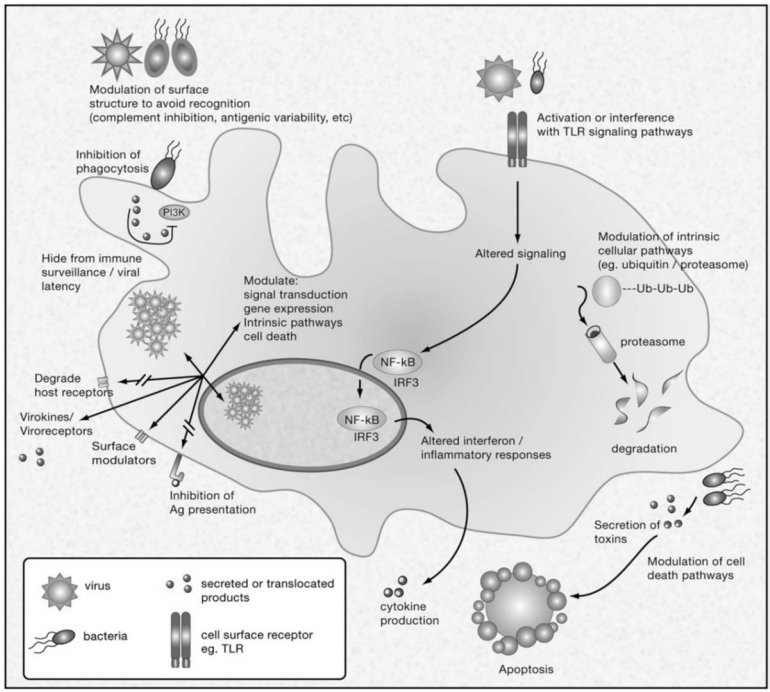


Figure 1: Pathogen defense mechanism

The pathogen evolves different infectious strategies to overcome the host's defensive mechanisms and to adapt and establish a successful infection. Many bacteria can destabilise and hijack the host signalling cascades and