## 1.1.1 Supporting Documents

1 .Academic Calendar

2. Syllabus

3. Logbook

4.Question paper Mapping

# JAYA COLLEGE OF ARTS AND SCIENCE

Thiruninravur - 602 024.

## ACADEMIC CALENDAR JUN' 2023 – NOV' 2023

| DATE | JUNE | JULY | AUGUST | SEPTEMBER | OCTOBER | NOVEMBER |
|---|---|---|---|---|---|---|
| 1 | | LC – 11 | LC – 36 | LC – 57 | HOLIDAY | |
| 2 | | SUNDAY | LC – 37 | LC – 58 | Gandhi Jayanthi | |
| 3 | | LC – 12 | LC – 38 | SUNDAY | LC – 76 | |
| 4 | | LC – 13 | LC – 39 | LC – 59 | LC – 77 | |
| 5 | | LC – 14 | LC – 40 | LC – 60 | LC – 78 | |
| 6 | | LC – 15 | SUNDAY | Janmashtami | LC – 79 | |
| 7 | | LC – 16 | | Ganesh Chaturthi | LC – 80 | |
| 8 | | LC – 17 | REVISION EXAM - I | LC – 61 | SUNDAY | |
| 9 | | SUNDAY | | LC – 62 | | |
| 10 | | LC – 18 | | SUNDAY | | |
| 11 | | LC – 19 | | LC – 63 | MODEL EXAM | |
| 12 | | LC – 20 | LC – 41 | LC – 64 | | |
| 13 | | LC – 21 | SUNDAY | LC – 65 | | |
| 14 | | LC – 22 | LC – 42 | LC – 66 | | |
| 15 | | LC – 23 | Indepen. Day | LC – 67 | SUNDAY | UNIVERSITY OF MADRAS |
| 16 | | SUNDAY | LC – 43 | LC – 68 | LC – 81 / RC - 01 | |
| 17 | | LC – 24 | LC – 44 | SUNDAY | LC – 82 / RC - 02 | THEORY EXAMINATION |
| 18 | | LC – 25 | LC – 45 | REVISION EXAM - II | LC – 83 / RC - 03 | |
| 19 | LC – 01 | LC – 26 | LC – 46 | | Pooja Holidays | |
| 20 | LC – 02 | LC – 27 | SUNDAY | | | |
| 21 | LC – 03 | LC – 28 | LC – 47 | | | |
| 22 | LC – 04 | LC – 29 | LC – 48 | | SUNDAY | |
| 23 | LC – 05 | SUNDAY | LC – 49 | LC – 69 | LC – 84 / RC - 04 | |
| 24 | LC – 06 | LC – 30 | LC – 50 | SUNDAY | LC – 85 / RC - 05 | |
| 25 | SUNDAY | LC – 31 | LC – 51 | LC – 70 | LC – 86 / RC - 06 | |
| 26 | LC – 07 | LC – 32 | LC – 52 | LC – 71 | LC – 87 / RC - 07 | |
| 27 | LC – 08 | LC – 33 | SUNDAY | LC – 72 | LC – 88 / RC - 08 | |
| 28 | LC – 09 | LC – 34 | LC – 53 | LC – 73 | LC – 89 / RC - 09 | |
| 29 | Bakrid | Muharram | LC – 54 | LC – 74 | SUNDAY | |
| 30 | LC – 10 | SUNDAY | LC – 55 | LC – 75 | LC – 90 / RC - 10 | |
| 31 | | LC – 35 | LC – 56 | | Deepavali | |
| WD | 10 | 25 | 26 | 24 | 21 | |

| | | | | |
|---|---|---|---|---|
| LC | LECTURE CLASS | 90 Days | TOTAL WORKING DAYS | 106 DAYS |
| RC | REVISION CLASS | 10 Days | | |
| SLR | SLOW LEARNERS CLASS | 10 Days | | |
| .. | REVISION/MODEL EXAM | 16Days | | |

Note: All science department will conduct Practical Examination in Revision Class days

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# JAYA COLLEGE OF ARTS AND SCIENCE

Thiruninravur - 602 024.

## ACADEMIC CALENDAR DEC' 2023 – APR' 2024

| DATE | NOV | DEC | JAN | FEB | MAR | APR |
|---|---|---|---|---|---|---|
| 1 | | | <= New Year => | LC - 28 | LC - 48 | |
| 2 | | | LC - 12 | LC - 29 | LC - 49 | |
| 3 | | | LC - 13 | LC - 30 | SUNDAY | |
| 4 | | | LC - 14 | SUNDAY | LC - 50 | |
| 5 | | | LC - 15 | LC - 30 | LC - 51 | |
| 6 | | | LC - 16 | LC - 31 | LC - 52 | |
| 7 | | | SUNDAY | LC - 32 | LC - 53 | |
| 8 | | | LC - 16 | LC - 33 | LC - 54 | |
| 9 | | ... | LC - 17 | LC - 34 | LC - 55 | |
| 10 | | | LC - 18 | LC - 35 | SUNDAY | |
| 11 | | | LC - 19 | SUNDAY | MODEL EXAM | |
| 12 | | | LC - 20 | LC - 36 | | |
| 13 | | | LC - 21 | LC - 37 | | |
| 14 | | | SUNDAY | LC - 38 | | UNIVERSITY OF MADRAS<br>THEORY EXAMINATIONS |
| 15 | | | <= Pongal => | LC - 39 | UNIVERSITY OF MADRAS<br>PRACTICAL EXAMINATIONS | |
| 16 | ... | | | LC - 40 | | |
| 17 | | | | LC - 41 | | |
| 18 | | LC - 01 | LC - 22 | SUNDAY | | |
| 19 | | LC - 02 | LC - 23 | REV EXAM - II | | |
| 20 | | LC - 03 | LC - 24 | | | |
| 21 | | LC - 04 | SUNDAY | | | |
| 22 | | LC - 05 | REV EXAM - I | | | |
| 23 | | LC - 06 | | LC - 42 | | |
| 24 | | SUNDAY | | LC - 43 | | |
| 25 | | <= Christmas => | | SUNDAY | | |
| 26 | | LC - 07 | <= Rep. Day => | LC - 44 | | |
| 27 | | LC - 08 | LC - 25 | LC - 45 | | |
| 28 | | LC - 09 | SUNDAY | LC - 46 | | |
| 29 | | LC - 10 | LC - 26 | LC - 47 | | |
| 30 | | LC - 11 | LC - 27 | | | |
| 31 | | SUNDAY | LC - 28 | | | |
| WD | -- | 11 | 22 | 25 | 12 | -- |

| | | | | |
|---|---|---|---|---|
| LC | LECTURE CLASS | 70 Days | TOTAL WORKING DAYS | 070 DAYS |
| RC | REVISION CLASS | ... | | |
| SLR | SLOW LEARNERS CLASS | ... | | |
| .. | REVISION/MODEL EXAM | 12 Days | | |

Note: 1. All Arts Departments will conduct Slow Learners/ Revision Class from 15.03.2024 to 31.03.2024 {Total Working Days: 13}

2. All Science Department will conduct Slow Learners / Revision class on those days where there no practical as per the department practical schedule.

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# IMMUNOLOGY

## Learning outcome:

The students will gain the knowledge about the immune response and reactions, cells involved in immunity, vaccines and tissue rejection.

**Unit 1:**

Introduction – Historical development in Immunology. Cells involved in immune response. Primary and Secondary lymphoid organs – Thymus, Bone marrow, Lymph nodes and Spleen. Hematopoiesis – development of B and T lymphocytes. Types of immunity – Innate and acquired.

**Unit 2 :**

Antigen: Characteristics and types. Antibody – Structure, Types, Properties and their Biological function. polyclonal - monoclonal antibody production and its biomedical applications.

**Unit 3 :**

Antigen – Antibody interactions, Immunodiffusion and Immuno electrophoresis. Principle and application of ELISA and RIA and Flourescent antibody technique. Purification of antibodies.

**Unit 4 :**

The complement system and activation and regulation. Types – Classical, alternative and Lectin pathway. Biological function of C' proteins. Cytokines- Structure and Function. Vaccines – Types , Production and application.

**Unit 5 :**

Hypersensitivity Reactions and Types. Major Histocompatability Complex – MHC genes, MHC in immune responsiveness, Structure and function of Class I and Class II MHC molecules. HLA tissue typing.

**Reference Books:**


- Thomas J. Kindt, Barbara A. Osborne and Richard A Goldsby, 2006. Kuby Immunology. 6th edition, W. H . Freeman and Company.
- Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt, 2011. Roitt.s Essential Immunology, 12 edition, Wiley- Blackwell. USA.

Jaya College of Arts and Science, Thiruninravur-602024,

Affiliated to University of Madras
Accredited by NAAC
Department of Biotechnology

Lesson Plan

- ✍ Syllabus
- ✍ Unit wise Breakup
- ✍ Lecture Notes
- ✍ Question Bank E-materials

Submitted by : Mrs. V. Sudha

Designation : Asst. Professor & Head

Course Name : Immunology

Course Code : SC25C

Class & Section : III B.Sc. Biotechnology

Head of the Department : Mrs. V. Sudha

## Course Syllabus – IMMUNOLOGY

**Learning outcome:**

The students will gain the knowledge about the immune response and reactions, cells involved in immunity, vaccines and tissue rejection.

**Unit 1:** Introduction – Historical development in Immunology. Cells involved in immune response. Primary and Secondary lymphoid organs – Thymus, Bone marrow, Lymph nodes and Spleen. Hematopoiesis – development of B and T lymphocytes. Types of immunity – Innate and acquired.

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Hypersensitivity Reactions and Types. Major Histocompatability Complex – MHC genes, MHC in immune responsiveness, Structure and function of Class I and Class II MHC molecules. HLA tissue typing.

**Course Breakup (Unit wise)- Course Name (Course Code)**

| Unit | Hour | Topic |
|---|---|---|
| 1 | 1 | Introduction About Immune System |
| | 2 | Historical developments in Immunology |
| | 3 | Cells of the Immune System |
| | 4 | Lymphocytes |
| | 5 | Antigen presenting cells |
| | 6 | Macrophage |
| | 7 | Dendritic Cell |
| | 8 | Primary Lymphoid organ |
| | 9 | Secondary Lymphoid organ |
| | 10 | Hematopoies |
| | 11 | Innate Immunity |
| | 12 | Adaptive Immunity |
| | | |
| | | |
| 2 | 1 | Antigen |
| | 2 | Types of Antigen |
| | 3 | Structure of Antibody |
| | 4 | Types of Antibody |
| | 5 | Properties of Antibody |
| | 6 | Biological function of Antibody |
| | 7 | Polyclonal Antibody |
| | 8 | Monoclonal Antibody |
| | 9 | Hybridoma Technology |
| | 10 | Production of Mab |
| | 11 | Biomedical application of Mab |
| | 12 | Application of Mab continuation |
| | | |
| | | |
| 3 | 1 | Ag – Ab Interaction |
| | 2 | Immunodiffusion |
| | 3 | Types of ID |
| | 4 | Immunoelectrophoresis |
| | 5 | Rocket Immunoelectrophoresis |
| | 6 | Counter Immunoelectrophoresis |
| | 7 | ELISA |
| | 8 | Application of ELISA |
| | 9 | RIA |
| | 10 | Application of RIA |

| | | |
|---|---|---|
| | **11** | Fluorescent antibody technology |
| | **12** | Purification of antibody |
| | | |
| | | |
| **4** | **1** | Complement system |
| | **2** | Activation of complement system |
| | **3** | Classical pathway |
| | **4** | Alternative pathway |
| | **5** | Lectin pathway |
| | **6** | Biological function of 'C' protein |
| | **7** | Cytokines |
| | **8** | Structure and function of cytokines |
| | **9** | Vaccines |
| | **10** | Types of vaccines |
| | **11** | Production of vaccines |
| | **12** | Application of vaccines |
| | | |
| | | |
| **5** | **1** | Hypersensitivity reaction |
| | **2** | Types of HS |
| | **3** | Type I HS |
| | **4** | Type II HS |
| | **5** | Type III HS |
| | **6** | Type IV HS |
| | **7** | MHC |
| | **8** | MHC genes |
| | **9** | Class I MHC |
| | **10** | Class II MHC |
| | **11** | HLA typing |
| | **12** | Continuation of HLA typing |
| | | |
| | | |

Question bank

Part A -2 marks

1. Macrophage
2. Precipitation
3. Name two immunosuppressors 4. What are polyclonal antibodies?
5. What are NK cells
6. Expand MMR &MHC
7. Toxoid
8. Define Immunization
9. FACS
10. Complement
11. Innate immunity
12. Hematopoiesis
13. Epitopes
14. Tissue typing
15. Hybridoma
16. Hypersensitivity
17. Cytotoxicity
18. Widal's test
19. Western blot
20. Name two lymphoid organs
21. APC
22. Adaptive immunity
23. Adjuvants
24. Dendritic cell
25. Define antibody
26. Define Hapten
27. Which antibody cross placenta
28. Define Monoclonal antibody
29. Define human leukocyte antigen

Part –B-5-marks

1. Explain the antigen – antibody interaction
2. Write a short note on DNA vaccine
3. Give a brief account on the history of immune system 4. Explain the physiology of immune response
5. What are haptens? Give its importance.
6. Differentiate immunogens from immunoglobulin's
7. Give the vaccination schedule for the newborn.
8. List out the application of monoclonal antibodies.
9. Explain ELISA technique and its importance
10. Explain the RIAanalysis
11. Give the principle of immunofluorescence technique.
12. Discuss about the antigen and its types
13. What do you mean by natural immunity? Explain
14. Give the origin &development of T & B cell
15. What are the types of vaccines? Give one example each
16. Give an account on alternate pathway of complement activation
17. Elucidate the mechanism of delayed type hypersensitivity
18. Comment on Tissue typing
19. Explain the Active and Passive immunity
20. Expalin the lymphoid organs
21. Enumerate the biological function of C- protein
22. Describe the immunoglobulins and its types
23. Explain the humoral & cell mediated immune response
24. Explain the structure and function of class II MHC molecule
25. Explain about the Type II and Type III Hypersensitivity reaction
26. Write a short note on MHC

Part –C-10-marks

1. Discuss the scope and importance of immunology.
2. Describe the structure and function of IgG
3. Illustrate& explain the structure and function of class I MHC molecule
4. Explain in detail the delayed hypersensitivity reaction
5. Write a detailed account on antibody classes and function
6. Explain in detail about monoclonal antibody production
7. Explain in detail about hematopoiesis process
8. Write a detailed account on cells of the immune system
9. Explain in detail about immunodiffusion
10. Write a detailed account on purification of antibodies

**Course Lecture Notes (Unit -I)- IMMUNOLOGY-(Course Code)**

**Unit -1 Hour – 1 Introduction about immune system**

Immune system-The immune system is the body's defense against infections Parts of the Immune System-The main parts of the immune system are:

1. Spleen.
2. Thymus.
3. Antibodies.
4. Bone marrow.
5. White blood cells.
6. Lymphatic system.
7. Complement system

Types of Immune System

Immune System fights against microbes and is divided into different types of reactions. The three types of immunity are: ○ Innate immunity ○ Adaptive immunity ○ Passive and active immunity

**Hour – 2 Historical developments in immunology**

5th Century

The concept of immunity from disease can be traced back at least to Greece in the 5th century BC.10th Century

However, the earliest recognized attempt to intentionally "induce" immunity to an infectious disease was in the 10th century in China, where smallpox was endemic or regularly found.

16th Century

Italian physician Girolamo Fracastoro proposed the theory of contagious diseases as published in his book entitled On Contagion and Contagious Diseases 17th Century The process of "variolation" (or inoculation) which involved the exposing of healthy patients to the material from the lesions caused by the disease was introduced by Circassian traders (Price 2015).

18th Century

Edward Jenner developed a vaccine for smallpox.

19th Century

Robert Koch, German microbiologist published the work on anthrax 20th Century

Karl Landsteiner was awarded the Nobel Prize for identifying human blood groups. In the same year, Rudolf Weigl developed the first vaccine for typhus

**Hour -3- cells of the immune system**

- White blood cells are also called leukocytes. They circulate in the body. White blood cells are on constant patrol and looking for pathogens.
- Our white blood cells are stored in different places in the body, which are referred to as lymphoid organs. These include the following:
- **Thymus** — a gland between the lungs and just below the neck.
- **Spleen** — an organ that filters the blood. It sits in the upper left of the abdomen.
- **Bone marrow** — found in the center of the bones, it also produces red blood cells.
- **Lymph nodes** —small glands positioned throughout the body, linked by lymphatic vessels.There are two main types of leukocyte:

**1. Phagocytes**-These cells surround and absorb pathogens and break them down, effectively eating them. There are several types, including:

- **Neutrophils** — these are the most common type of phagocyte and tend to attack bacteria.
- **Monocytes** — these are the largest type and have several roles.
- **Macrophages** — these patrol for pathogens and also remove dead and dying cells.
- **Mast cells** — they have many jobs, including helping to heal wounds and defend against pathogens.

**2. Lymphocytes-**Lymphocytes help the body to remember previous invaders and recognize them if they come back to attack again. Lymphocytes begin their life in bone marrow. Some stay in the marrow and develop into B lymphocytes (B cells), others head to the thymus and become T lymphocytes (T cells). These two cell types have different roles:

- **B lymphocytes** — they produce antibodies and help alert the T lymphocytes.
- **T lymphocytes** — they destroy compromised cells in the body and help alert other leukocytes.

**Hour -4- Lymphocytes**

Lymphocytes are a type of white blood cell (or leukocyte). They help an organism to fight infections. They occur in the immune system of all vertebrates. Lymphocytes can be found

- In the veins and arteries (in the body's circulation).
- In the lymph nodes and lymph channels of the body's lymphatic system.
- Scattered all over the body eg in the spleen, tonsils, intestines, and in the lining of the airways. Here the lymphocytes represent what is referred to as "lymphoid tissue."

Cell Types

There are three main types of lymphocytes: B cells, T cells, and natural killer cells

- T and B cells originate from stem cells in the bone marrow and are initially similar in appearance. Some lymphocytes migrate to the thymus, where they mature into T cells; others remain in the bone marrow, where—in humans—they develop into B cells.
- Most lymphocytes are short-lived, with an average life span of a week to a few months, but a few live for years, providing a pool of long-lived T and B cells. These cells account for immunologic "memory," a more rapid, vigorous response to a second encounter with the same antigen.
- T cells (thymus cells) and B cells (bone cells) are the main cells of the adaptive immune response **Hour -5- Antigen presenting cells**
- Antigen-presenting cells (APCs) play an important role in adaptive immunity.
- These are required for the functioning of T-lymphocytes.
- The main three antigen-presenting cells are B-lymphocytes, macrophages and dendritic cells.
- These cells present extracellular antigen to T cells for elimination.
- These APCs engulf pathogens and position a piece of antigen on the cell surface with major histocompatibility complex (MHC) molecules, which is recognised by T cells.

**Hour -6-Macrophage**

- Macrophages are mononuclear cells functioning as professional phagocytes in order to remove dying, dead or harmful pathogens.

- Macrophages are a type of white blood cell of the immune system where they engulf and digest particles that are detected as antigens by other blood cells.
- These are larger phagocytic cells that occur in essentially all types of tissues, and their structure and shape depend on the stage of maturation of the cells.
- Macrophages found in different organs have different names like the macrophages of lungs are called alveolar macrophages, while those in the liver are called Kupffer cells.
- Even though phagocytosis is the primary function of macrophages, these also play an essential role in nonspecific defense as well as in adaptive immunity.
- Macrophages are important blood cells that have important roles in almost all aspects of an organism's biology as different subsets of macrophages are involved in different functions in the body.
- Macrophages in the body are produced by the differentiation of monocytes in tissues
- Macrophages keep flowing through the blood where they migrate to and circulate within all tissues, patrolling for pathogens or eliminating dead cells and debris.
- Macrophages consist of a specialized group of receptors called Toll-like receptors that recognize products of bacteria and other microorganisms.

**Hour -7 -Dendritic cell**

- Dendritic cells are important antigen-presenting cells to helper T cells. The dendritic cells have long membranous extensions resembling the dendrites of the nerve.
- Dendritic cells develop from the hematopoietic stem cells in bone marrow.

**The dendritic cells in different sites of body have different names:**

i. Langerhans' cells are present in the skin and mucous membranes

ii. Interstial dendritic cells are present in many organs (such as heart, lungs, kidneys, and gastrointestinal tract) iii. Interdigitating dendritic cells are present in the T cell area of secondary lymphoid follicle and thymic medulla.

iv. Circulating dendritic cells are present in blood and lymph. The dendritic cells in lymph are also called as 'veiled cells'.

**Hour -8 Primary lymphoid organ**

- This is the site where lymphocytes are produced and mature.
- It is also the location where stem cells differentiate and mature into B and T cells
- Humans have two primary lymphatic organs – the thymus gland and the red bone marrow
- B and T cells are formed in the bone marrow
- B cells mature in the bone marrow while T cells mature once they migrate to the thymus.

**(a) Bone Marrow:**

- It is the main lymphoid organ, where all the lymphocytes and all the body cells are produced and Tlymphocytes are developed.

**(b) Thymus:** It is a lobed organ, located near the heart and beneath the breast bone. It is large at the time of birth but with age, the size keep on reducing and becomes very small by attaining puberty. Growth and maturation of T-lymphocytes takes place in thymus only.

**Hour -9 Secondary lymphoid organ**

These organs provide the sites for the interaction of lymphocytes with the antigen, which then proliferate to become effector cells.

**These are of following types:**

(a) Spleen,

(b) Lymph nodes,

(c) Mucosal associated Lymphoid Tissue (MALT)

Peyer's patches of small intestine and appendix are also some of the secondary lymphoid organs.

**(a) Spleen:**

It is a large bean-shaped organ containing lymphocytes and phagocytes. It filters the blood by trapping the pathogens in it.

**(b) Lymph Nodes:**

These are small solid structures located at different points along the lymphatic system. Their function is to trap the microorganisms or other antigens, that enter the lymph and tissue fluid. Therefore, the antigens trapped in the lymph nodes are responsible for the activation of lymphocytes present there and cause the immune response.

**(c) Mucosal Associated Lymphoid Tissue (MALT):**

This is located within the lining of main tracts in the body like respiratory, digestive, urogenital tracts. MALT constitutes about 50% of the lymphoid tissue in human body.

**Hour -10-Hematopoiesis**

Hematopoiesis is the process through which the body manufactures blood cells.

- Hematopoiesis begins during the first weeks of embryonic development.
- All blood cells and plasma develop from a stem cell that can develop into any other cell.

The blood is made up of more than 10 different cell types. Each of these cell types falls into one of three broad categories:

1. **Red blood cells (erythrocytes)**: These transport oxygen and hemoglobin throughout the body.

2. **White blood cells (leukocytes)**: These support the immune system. There are several different types of white blood cells:

- **Lymphocytes**: Including T cells and B cells, which help fight some viruses and tumors.
- **Neutrophils**: These help fight bacterial and fungal infections.
- **Eosinophils**: These play a role in the inflammatory response, and help fight some parasites.
- **Basophils**: These release the histamines necessary for the inflammatory response.
- **Macrophages**: These engulf and digest debris, including bacteria.

**3. Platelets (thrombocytes)**: These help the blood to clot.

## Hour -11 Innate immunity

Immunity is the ability of the body to defend itself against disease-causing organisms.

Types of Immunity

There are two major types of immunity:

1. Innate Immunity or Natural or Non-specific Immunity.
2. Acquired Immunity or Adaptive Immunity.

Innate Immunity

This type of immunity is present in an organism by birth.

This is activated immediately when the pathogen attacks. Innate immunity includes certain barriers and defence mechanisms that keep foreign particles out of the body.

Innate immunity refers to the body's defence system.

This immunity helps us by providing the natural resistance components including salivary enzymes, natural killer cells, intact skin and neutrophils, etc. which produce an initial response against the infections at birth prior to exposure to a pathogen or antigens.

It is a long-term immunity in which our body produces the antibodies on its own. Our body has few natural barriers to prevent the entry of pathogens

## Hour -12 Adaptive immunity

Acquired Immunity

Acquired immunity or adaptive immunity is the immunity that our body acquires or gains over time. Unlike the innate immunity, this is not present by birth.

The ability of the immune system to adapt itself to disease and to generate pathogen-specific immunity is termed as acquired immunity. It is also known as adaptive immunity.

An individual acquires the immunity after the birth, hence is called as the acquired immunity.

It is specific and mediated by antibodies or lymphocytes which make the antigen harmless.

The main function of acquired immunity is to relieve the victim of the infectious disease and also prevent its attack in future.

It mainly consists of an advanced lymphatic defence system which functions by recognizing the own body cells and not reacting to them.

Features of Acquired Immunity

- **Specificity**: Our body has the ability to differentiate between different types of pathogens, whether it is harmful or not, and devise ways to destroy them.
- **Diversity**: Our body can detect vast varieties of pathogens, ranging from protozoa to viruses.
- **Differentiate between self and non-self**: Our body has the unique ability to differentiate between its own cells and foreign cells. It immediately starts rejecting any foreign cell in the body.
- **Memory**: Once our body encounters a pathogen, it activates the immune system to destroy it. It also remembers what antibodies were released in response to that pathogen, so that, the next time it enters, a similar procedure is followed by the body to eliminate it.

**E-Material**

**Hour – 1 Introduction about immune system** https://my.clevelandclinic.org/health/articles/21196-immunesystem#

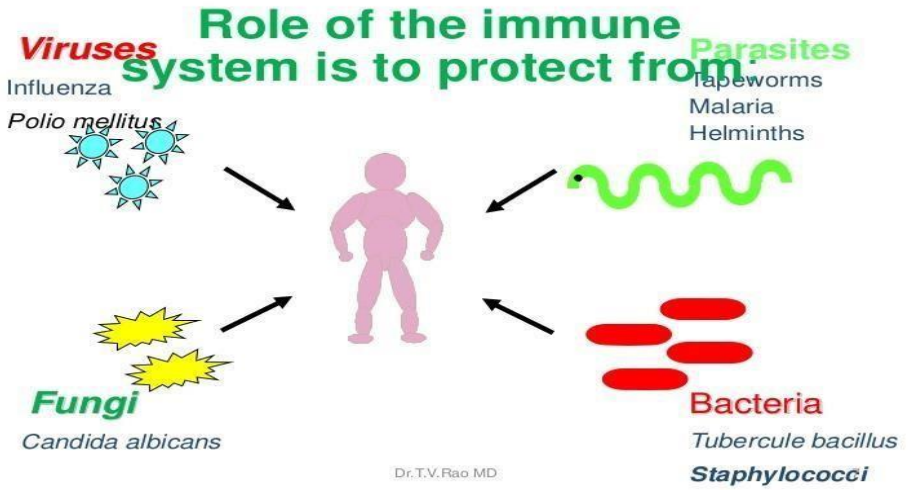
- Branch of biomedical science
- Study of immune system
- Immune system- collection of cells,tissues,and molecules that mediate immune response(resistance to infection)
- The study of how the body protects itself against infectious disease caused by microorganisms such as bacteria, viruses,protozoa and fungi and also parasitic organisms such as helminth
- The study of all aspects of the immune system,including its structure and function, disorders of the immune system, blood banking, immunization and organ transplantation

**PRINCIPAL FUNCTION OF THE IMMUNE SYSTEM**

- To protect human from pathogenic microorganisms
- Pathogenic microorganisms(pathogen)-microorganisms capable of causing disease&or infection
- Infection-ability of pathogen to enter host, multiply and stimulate immune response
- Disease-clinical manifestation associated with infection **What are the parts of the immune system?**

Your immune system is made of up a complex collection of cells and organs. They all work together to protect you from germs and help you get better when you're sick. The main parts of the immune system are:

- **White blood cells:** Serving as an army against harmful bacteria and viruses, white blood cells search for, attack and destroy germs to keep you healthy. White blood cells are a key part of your immune system. There are many white blood cell types in your immune system. Each cell type either circulates in your bloodstream and throughout your body or resides in a particular tissue, waiting to be called into action. Each cell type has a specific mission in your body's defense system. Each has a different way of recognizing a problem, communicating with other cells on the defense team and performing their function.
- **Lymph nodes:** These small glands filter and destroy germs so they can't spread to other parts of your body and make you sick. They also are part of your body's lymphatic system. Lymph nodes contain immune cells that analyze the foreign invaders brought into your body. They then activate, replicate and send the specific lymphocytes (white blood cells) to fight off that particular invader.
  You have hundreds of lymph nodes all over your body, including in your neck, armpits, and groin. Swollen, tender lymph nodes are a clue that your body is fighting an infection.
- **Spleen:** Your spleen stores white blood cells that defend your body from foreign invaders. It also filters your blood, destroying old and damaged red blood cells.
- **Tonsils and adenoids:** Because they are located in your throat and nasal passage, tonsils and adenoids can trap foreign invaders (for example, bacteria or viruses) as soon as they enter your body. They have immune cells that produce antibodies to protect you from foreign invaders that cause throat and lung infections.
- **Thymus:** This small organ in your upper chest beneath your breast bone helps mature a certain type of white blood cell. The specific task of this cell is to learn to recognize and remember an invader so that an attack can be quickly mounted the next time this invader is encountered.
- **Bone marrow:** Stem cells in the spongy center of your bones develop into red blood cells, plasma cells and a variety of white blood cells and other types of immune cells. Your bone marrow makes billions of new blood cells every day and releases them into your bloodstream.
- **Skin, mucous membranes and other first-line defenses:** Your skin is the first line of defense in preventing and destroying germs before they enter your body. Skin produces oils and secretes other protective immune system cells. Mucous membranes line the respiratory, digestive, urinary and reproductive tracts. These membranes secrete mucus, which lubricates and moistens surfaces. Germs stick to mucus in the respiratory tract and then are moved out of the airways by hair-like structures called cilia. Tiny hairs in your nose catch germs. Enzymes found in sweat, tears, saliva and mucus membranes as well as secretions in the vagina all defend and destroy germs.
- **Stomach and bowel:** Stomach acid kills many bacteria soon after they enter your body. You also have beneficial (good) bacteria in your intestines that kill harmful bacteria.




**Hour – 2 Historical developments in immunology**

https://www.encyclopedia.com/science/encyclopedias-almanacs-transcripts-and-maps/history-immunology

**History of immunology**

In Western society, it was not until the late eighteenth century that a rational approach to the origin of disease developed. Prior to the discovery that disease was the result of pathogenic organisms, it was commonly accepted that disease was a punishment from God (or the Gods), or even a witches curse. Eastern cultures perceived disease as an imbalance in the energy channels within the body. Later, the great plagues of Europe were assumed the result of virulent or noxious vapors. Nevertheless, there were intimations as early as 430 B.C. that if one survived a disease, the person thereafter became "immune" to any subsequent exposures. However, this was never recognized as evidence of some type of internal defense system until the later part of the seventeenth century.

**AD**

Although most historical accounts credit **Edward Jenner** for the development of the first **immunization** process, a previous similar procedure had become established in China by 1700. The technique was called variolation. This was derived from the name of the infective agent—the **variola virus** . The basic principal of variolation was to deliberately cause a mild infection with unmodified pathogen. The risk of death from variolation was around two to three percent. Although still a risk, variolation was a considerable improvement on the death rate for uncontrolled infection. **Immunity** to **smallpox** was conferred by inserting the dried exudate of smallpox pustules into the nose. This technique for the transfer of smallpox, as a form of limited infection, traveled to the west from China along the traditional trade routes to Constantinople where it spread throughout Europe. Hearing of this practice, the Royal family of England had their children inoculated against the disease in 1721, but the practice aroused severe opposition as physicians felt it was far too risky.

In 1798, Edward Jenner, noticed that milkmaids were protected from smallpox if they had been first infected with **cowpox** . It was not his intention to make medical history, as his interests were mostly scholarly and

involved the transfer of infections from one species to another, especially from animals to humans. However, Jenner's work led him to the conclusion, that inoculation with cowpox (a bovine analogue of smallpox) could confer immunity to smallpox. Thus, the concept of **vaccination** was initiated. (Incidentally, the Latin word for cow is *vacca* ). Jenner's ideas first made him a medical as well as a social pariah, as they were in opposition to both the church and popular beliefs. Because his method was much safer then variolation, however, the use of vaccinations gradually became widely accepted and most European countries had some form of compulsory program within fifty years of Jenner's discovery.

**AD**

The idea that a pathogenic organism caused disease was not fully realized until certain technological advances had occurred. Initially, **Antoni van Leeuwenhoek** 's development of the **microscope** and the subsequent realization that entities existed that were not visible to the human eye, allowed the concept of germs to be appreciated. That these organisms were the causative agent of disease was not recognized until **Louis Pasteur** developed his **germ theory of disease** . His original interests were in **fermentation** in wine and beer, and he was the first to isolate the organisms that caused the fermentation process. Pasteur's work eventually led him to the development of **pasteurization** (heating) as a means of halting fermentation. While working with silk worms and **anthrax** , he was able to demonstrate that the same method for transferring the fermentation process also worked in transmitting disease from infected animals to unaffected animals. Finally, in 1878, Pasteur accidentally used an attenuated (weakened) chicken cholera **culture** and realized, when he repeated the experiment using a fresh culture, that the weakened form protected the chickens from the virulent form of the disease. Pasteur went on to develop an attenuated **vaccine** against **rabies** and swine erysipelas.

Pasteur was not the only proponent of the germ theory of disease. His chief competitor was **Robert Koch** . Koch was the first to isolate the anthrax microbe and, unaware of Pasteur's work, he was able to show that it caused the disease. Then in 1882, Koch was able to demonstrate that the germ theory of disease applied to human ailments as well as animals, when he isolated the microbe that caused **tuberculosis** . His "Koch's postulates" are still used to identify infective organisms.

**AD**

Much of the basis for modern medicine, as well as the field of **immunology** , can be traced back to these two scientists, but the two major questions still to be answered were how did infection cause the degradation of tissue, and how did vaccines work? The first question was addressed in 1881 by **Emile Roux** and Alexander Yersin when they isolated a soluble toxin from **diphtheria** cultures. Later, **Emil von Behring** and **Shibasaburo Kitasato** were able to demonstrate passive immunity when they took serum from animals infected with diphtheria and injected into healthy animals. These same animals were found to be resistant to the disease. Eventually these serum factors were recognized in 1930 as antibodies. However, thirty years before antibodies were finally isolated and identified, **Paul Ehrlich** and others, recognized that a specific antigen elicited the production of a specific **antibody** . Ehrlich hypothesized that these antibodies were specialized molecular structures with specific receptor sites that fit each pathogen like a lock and key. Thus, the first realization that the body had a specific defense system was introduced. In addition, sometime later, he realized that this powerful effector mechanism, used in host defense would, if turned against the host, cause severe tissue damage. Ehrlich termed this *horror autotoxicus.* Although extremely valuable, his work

still left a large gap in understanding how the **immune system** fights a pathogenic challenge. The idea that specific cells could be directly involved with defending the body was first suggested in 1884 by **Élie Metchnikoff** . His field was zoology and he studied **phagocytosis** in single cell organisms. Metchnikoff postulated that vertebrates could operate in a similar manner to remove pathogens. However, it was not until the 1940s that his theories were accepted and the cell mediated, as opposed to the humoral, immune response was recognized.

The clarification of the immune response and the science of immunology did not progress in a systematic or chronological order. Nonetheless, once scientists had a basic understanding of the cellular and humoral branches of the immune system, what remained was the identification of the various components of this intricate system, and the mechanisms of their interactions. This could not have been accomplished without the concomitant development of **molecular biology** and genetics.

Milestones in the history of immunology include:

- 1798 Edward Jenner initiates smallpox vaccination.
- 1877 Paul Erlich recognizes mast cells.
- 1879 Louis Pasteur develops an attenuated chicken cholera vaccine.
- 1883 Elie Metchnikoff develops cellular theory of vaccination.
- 1885 Louis Pasteur develops rabies vaccine.
- 1891 Robert Koch explored delayed type hypersensitivity.
- 1900 Paul Erlich theorizes specific antibody formation.
- 1906 Clemens von Pirquet coined the word allergy.
- 1938 John Marrack formulates antigen-antibody binding hypothesis.
- 1942 Jules Freund and Katherine McDermott research adjuvants.
- 1949 Macfarlane Burnet & Frank Fenner formulate immunological tolerance hypothesis.
- 1959 Niels Jerne, David Talmage, Macfarlane Burnet develop clonal **selection** theory.
- 1957 Alick Isaacs & Jean Lindemann discover interferon (cytokine).
- 1962 Rodney Porter and team discovery the structure of antibodies.
- 1962 Jaques Miller and team discover thymus involvement in cellular immunity.
- 1962 Noel Warner and team distinguish between cellular and humoral immune responses.
- 1968 Anthony Davis and team discover T cell and B cell cooperation in immune response.
- 1974 Rolf Zinkernagel and Peter Doherty explore **major histocompatibility complex** restriction.
- 1985 Susumu Tonegawa, Leroy Hood, and team identify immunoglobulin genes.
- 1987 Leroy Hood and team identify genes for the T cell receptor.

- 1985 Scientists begin the rapid identification of genes for immune cells that continues to the present.

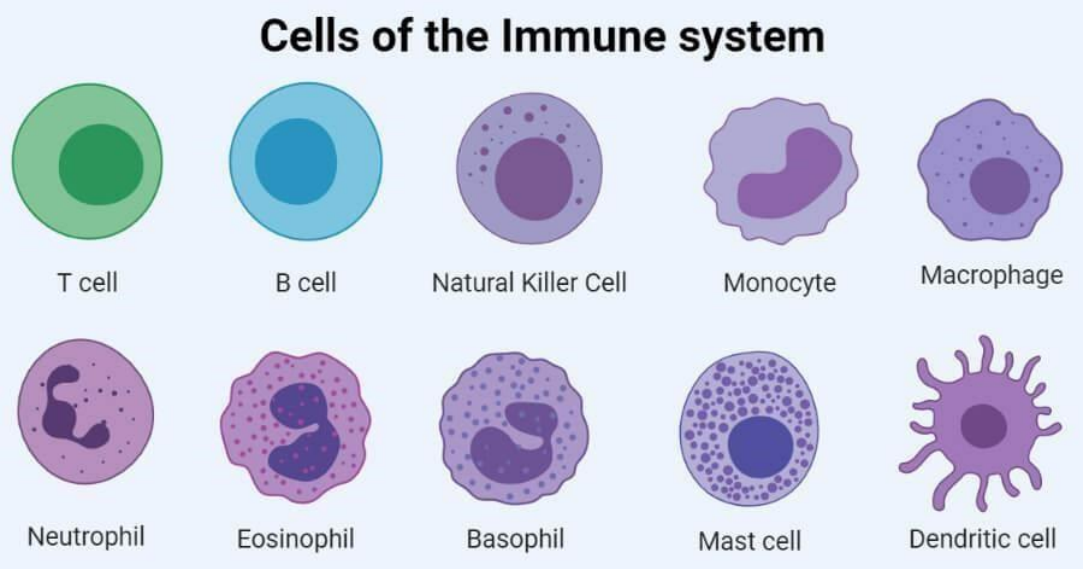
**Hour -3- cells of the immune system** https://microbenotes.com/cells-of-the-immune-system/ https://www.cliffsnotes.com/study-guides/biology/microbiology/the-immune-system/cells-of-the-immune-system

**The cells of immune system are:**

1. Lymphocytes-
   - T-lymphocytes
   - B- lymphocytes
   - NK cell
2. Phagocytic cells
   - Monocytes
   - Macrophages
3. Granulocytic cells
   - Neutrophils
   - Basophils
   - Eosinophils
4. Dendritic cells

Cells of the immune system are associated with the lymphatic system of the body and its specialized cells. Lymphocytes of the lymphatic system are derived from **stem cells** of the bone marrow. These undifferentiated precursor cells proliferate throughout life and replenish the mature cells of the immune system.

**B-lymphocytes and T-lymphocytes.** There are two major pathways for the differentiation of stem cells into immune cells. Certain of the stem cells produce **B-lymphocytes** (B-cells) while other stem cells form **Tlymphocytes** (T-cells). B-lymphocytes are so named because in birds, they are formed in the bursa of Fabricius. The equivalent site in humans has not been identified but is believed to be the bone marrow. Tlymphocytes undergo their conversion in the thymus gland, an organ in the neck tissues near the trachea and thyroid gland




The lymphocytes are the central cells of the immune system which are responsible for adaptive immunity and immunological features of diversity, specificity, memory, and self/non-self recognition. The other immune cells function to engulf and destroy micro-organisms, present antigens and secrete cytokines.

**Hour -4- Lymphocytes Lymphocytes**

- Arise in the bone marrow
- Unlike B- cells (mature within the bone marrow)T-cell migrate to thymus to mature(so it is called TLymphocytes ), T designates thymus
- During its maturation T-cell express TCR on its membrane(cell markers).recognize(Bind) specific group of protein called MHC molecules.
- MHC- Set of cell surface protein help the IS to recognize antigen or foreign substances(In human-complex is HLA).
- B-cell recognize antigen alone. TCR recognize antigen only this bound to cell membrane protein called MHC molecules(Present on APC)
- When T-cell encounters antigen combined with an MHC molecule, T-cell Proliferate, differentiate into memory T-cell and effector T-cell.

**There are two major types of MHC**

**1**.Class I MHC- which are expressed nearly all nucleated cells

**2**.Class II MHC- Expressed only by APC

**The T-Lymphocytes can be divided into three types**

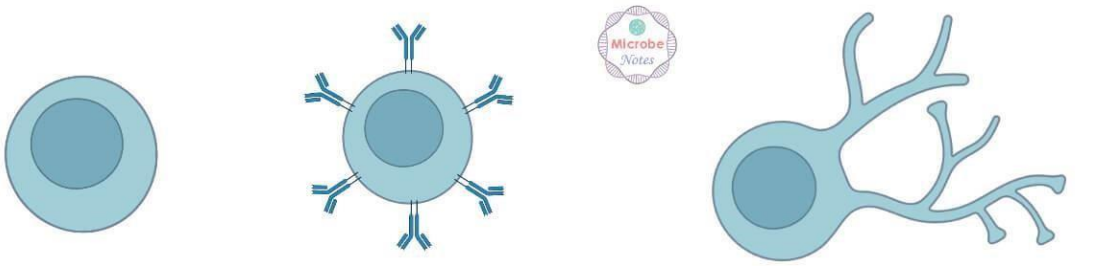
**1**.T-helper cell(T(H)).

**2**.Cytotoxic T-cell(T(C)).

**3**.Suppressor T-cell(T(S)).

**B-lymphocytes**

- B-lymphocytes are also known as B-cells and on lab reports, they are known as CD19 or CD20 cells.
- They are the specialized cells of the immune system whose major function is to produce antibodies also known as immunoglobulins or gamma globulins.
- B-lymphocytes are synthesized and mature in the bone marrow from the hematopoietic stem cells, and after which they mature, migrate, and express themselves by forming unique antigen-binding receptors on their membranes, known as B-cell receptors or antibodies.
- Migration of mature B-cells moves to the bone marrow, lymph nodes, spleen, some parts of the intestines, and the bloodstream.



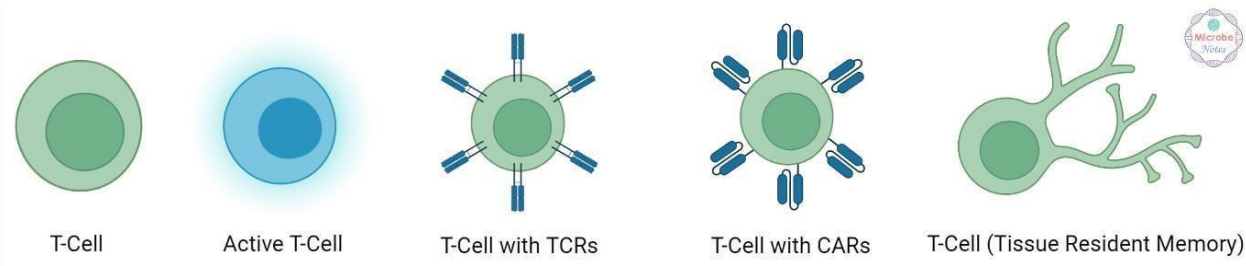

B-Cell B-Cell with Antibodies B-Cell (Tissue Resident Memory)

- When a naive B-cell interacts with an antigen for the first time and it has to match membranebound receptors (antibodies), the antibodies bound to the B-cell bind the antigen causing the B-cell to divide rapidly, and its progenitors to differentiate into **memory B-cells and effector B-cells known as plasma cells.**
- The Memory B-cells have a long life span than the naive cells, expressing the same membranebound antibody as the parent B-cells.
- The plasma cells are responsible for producing the antibodies that can be secreted into the bloodstream, tissues, respiratory secretions, intestinal secretions, and tears.
- Therefore, antibodies are highly specialized serum protein molecules.
- The plasma cells have a short life span of few days but they secrete large amounts of antibodies during this time, with approximately 2000 molecules of antibodies per plasma cell per second.
- The secreted antibodies play the major effector roles in the humoral immune responses.

- **Note that, during maturation, B-cells are trained not to produce antibodies on healthy tissues**. The antibody molecules are specifically designed for every foreign antigen they encounter and interact like a lock and key mechanism.
- Therefore B-cells have the ability to produce vitally a variety of antibodies for all microbes in our environment, however as stated above, each plasma cell produces only one kind of antibody.
- Antibodies' varieties are based on their specialized functions in the body with variations in their chemical structure, which ultimately determine the class of antibody.

**2. T-Lymphocytes**

- T-lymphocytes are also known as T-cells, often named in lab reports as CD3 cells. They also arise in the bone marrow but migrate to the thymus gland for maturation, where they express a unique antigen-binding molecule on its membrane known as the T-cell receptor.
- The name **T** originated from its site of maturation, the **Thymus.**
- Mature T-cells leave the thymus and populate other organs of the immune system, such as the spleen, lymph nodes, bone marrow, and blood.
- Unlike the B-cell receptors that can recognize antigens alone, T-cell receptors only recognize antigens that are bound to cell membrane proteins known as Major Histocompatibility Complex (MHC) molecules.
- The MHC molecule recognizes antigens that are presented to them by antigen-processing cells (APCs) on their cell membrane.
- The two major classes of MHC molecules are **Class I MHC molecules**, which are expressed by nearly all nucleated cells of vertebrate species, consist of a heavy chain linked to a small invariant protein called 2-microglobulin. **Class II MHC molecules**, which consist of an alpha and a beta glycoprotein chain, are expressed only by antigen-presenting cells.
- When a naive T cell encounters an antigen combined with an MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells.




- The T-cells are classified into three categories: **T helper (Th), T cytotoxic (Tc), and T suppressor** T Cells **(Ts) cells.**
- The Th and Tc cells are differentiated from each other with the **presence of their CD4 and CD8 membrane glycoproteins** on their surfaces.

- T cells naturally **displaying CD4 function as T helper (Th) cells** while those **displaying CD8 naturally function as T cytotoxic (Tc)** cells.
- The Th cells recognize and interact with antigens that are presented on the MHC class II molecule complex, then they become activated becoming effector cells that are able to secrete various growth factors that are collectively known as **cytokines.**
- The cytokines that are secreted are actively involved in the activation of B-cells, T-cytotoxic cells, macrophages, and other immune cells.
- The cytokine patterns produced by the activated Th cells result in different immune responses. The Thderived cytokines enable the recognition of an antigen-MHC class I molecule complex by the Tc cells which then proliferate and differentiate into effector cells known as **Cytotoxic T-lymphocytes (CTL).**
- The T-cytotoxic cells have the ability to induce cytokine secretion, unlike the Cytotoxic T-Cells which do not induce secretion of cytokines, rather **they exhibit cell-killing or cytotoxic activity.**
- Cytotoxic T-lymphocytes (CTL) play a key role in monitoring the body cells and eliminating any of these cells that display antigens such as tumor cells, cells infected with viruses, and cells of a foreign tissue graft.
- CTLs target foreign antigen (altered self-cells) complexes displayed by the class I MHC molecule.

**3. Natural killer cells (NK cells)**

- These are large granular lymphocytes, that do not express surface markers like the B and T-cell lineages
- They were first described in 1976 by indications of the presence of a small population of large granular lymphocytes that had a cytotoxic effect against a wide range of tumor cells in the absence of any previous immunization with the tumor.
- These cells also indicated that they play key roles in host defense against tumor cells and cells infected with some, not all viruses.
- They constitute 5-10% of lymphocytes in the human peripheral blood.

**Granulocytic Cells**

- Granulocytes are white blood cells (leukocytes).
- They are classified based on their cellular morphologies and cytoplasmic staining characteristics and they include **neutrophils, eosinophils, basophils, or mast cells.**
- All granulocytes have multilobed nuclei that make them visually distinctive and easily distinguishable from lymphocytes, whose nuclei are round. The cytoplasm of all granulocytes is replete with granules that are released in response to contact with pathogens.
- These granules contain a variety of proteins with distinct functions: Some damage pathogens directly; some regulate trafficking and activity of other white blood cells, including lymphocytes; and some contribute to the remodeling of tissues at the site of infection.
- Neutrophils have a multilobed nucleus and a granulated cytoplasm that stains with both acid and basic dyes; it is often called a **polymorphonuclear leukocyte (PMN)** for its multilobed nucleus.
- The eosinophils have a bilobed nucleus and a granulated cytoplasm that stains with the acid dye eosin red (hence its name).

- The basophil has a lobed nucleus and heavily granulated cytoplasm that stains with the basic dye methylene blue.
- Both neutrophils and eosinophils are phagocytic, whereas basophils are not.
- Neutrophils constitute the majority (50% to 70%) of circulating leukocytes and are much more numerous than eosinophils (1%–3%), basophils (≤ 1%), or mast cells (≤ 1%). **Neutrophils**

Neutrophils are produced by hematopoiesis n the bone marrow. They are released into the peripheral blood and circulate for 7–10 h before migrating into the tissues, where they have a life span of only a few days. In the bone marrow, a surmountable level of neutrophils is produced in response to the types of infections and they are normally the first cells that arrive at the site of inflammation.

The resulting transitory increases in the number of circulating neutrophils known as leukocytosis, which is an indicator of an infection, medically.

The movement of circulating neutrophils into tissues is also known as **extravasation.**

Extravasation takes place in several steps:

Adherence to the vascular endothelium

Penetration into the gap between adjacent endothelial cells lining the vessel wall

Penetration into the vascular basement membrane and moving out into the tissue spaces.

Several substances can be generated during an inflammatory reaction which serves as chemotactic factors. They promote the accumulation of neutrophils at the site of inflammation. Some of these chemotactic factors include complement components, blood-clotting system components, and several cytokines secreted by activated Th Cells and macrophages.

The functions include:

Neutrophils are also active phagocytes just like macrophages and the mechanism of phagocytosis is similar to that of macrophages except for the lytic enzymes and bactericidal substances in neutrophils which are contained within primary and secondary granules.

The neutrophils have larger denser primary granules which are a type of lysosome containing peroxidase, lysozyme, and various hydrolytic enzymes, and smaller secondary granules that contain collagenase, lactoferrin, and lysozyme.

Both the primary and the secondary granules fuse with the phagosomes and digest and eliminate the contents similar to macrophages.

Neutrophils also employ both oxygen-dependent and oxygen-independent pathways to generate antimicrobial substances.

Neutrophils exhibit a larger respiratory burst than macrophages and they are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates.

Neutrophils also express higher levels of defensins than macrophages.

**Eosinophils**

They are motile phagocytic cells that can migrate from the blood into the tissue spaces.

They have a phagocytic mechanism of eliminating antigens but their role as phagocytic cells is much less significant than that of neutrophils.

They play a role in defense against multicellular parasitic organisms including worms.

The secreted contents of eosinophilic granules may damage the parasite membrane. They can be found clustering around invading worms, whose membranes are damaged by the activity of proteins released from eosinophilic granules. Like neutrophils and basophils, eosinophils may also secrete cytokines that regulate B and T lymphocytes, thereby influencing the adaptive immune response.

In areas where parasites are less of a health problem, eosinophils are better appreciated as contributors to asthma and allergy symptoms.

**Basophils**

Basophils are nonphagocytic granulocytes containing large granules that are filled with basophilic proteins that stain blue in standard H & E staining methodologies.

Naturally, basophils are in the body's normal circulation but they can be very potent.

They function by binding to circulating antibodies and react by the content of their granules which are pharmacologically active substances found in their cytoplasm.

These substances play a major role in certain allergic responses. For example, histamines are the most common and well-known protein in that basophilic granules. They play a role in increasing blood vessel permeability and smooth muscle activity.

Additionally, just like the eosinophils, basophils are also crucial in response to parasites, and particularly the helminths (worms).

Basophils also secrete cytokines that assist in the modulation of the adaptive immune response.

**Mast Cells**

Mast cells are formed in the bone marrow.

They are released from the bone marrow into the blood as undifferentiated cells, and when they enter the tissues they then mature.

Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs, and mucosal epithelial tissue of the respiratory, genitourinary, and digestive tracts. **Hour -5- Antigen presenting cells**

https://courses.lumenlearning.com/wm-biology2/chapter/antigen-presenting-cells/

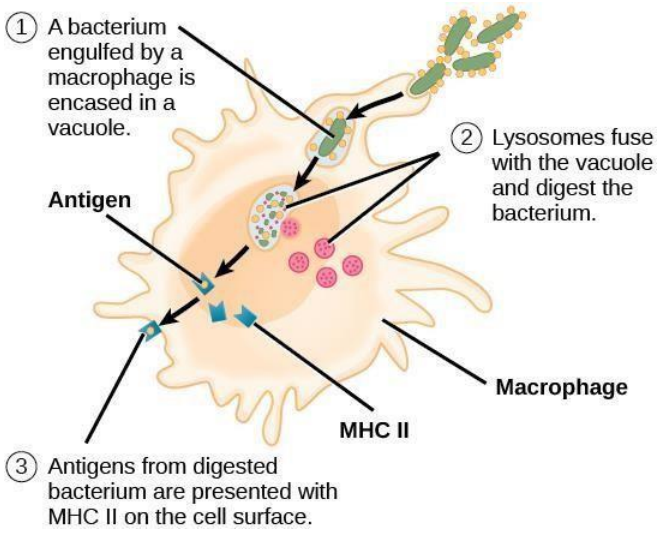
Unlike NK cells of the innate immune system, B cells (B lymphocytes) are a type of white blood cell that gives rise to antibodies, whereas T cells (T lymphocytes) are a type of white blood cell that plays an important role in the immune response. T cells are a key component in the cell-mediated response—the specific immune response that utilizes T cells to neutralize cells that have been infected with viruses and certain bacteria. There are three types of T cells: cytotoxic, helper, and suppressor T cells. Cytotoxic T cells destroy virusinfected cells in the cell-mediated immune response, and helper T cells play a part in activating both the antibody and

the cell-mediated immune responses. Suppressor T cells deactivate T cells and B cells when needed, and thus prevent the immune response from becoming too intense.

An **antigen** is a foreign or "non-self" macromolecule that reacts with cells of the immune system. Not all antigens will provoke a response. For instance, individuals produce innumerable "self" antigens and are constantly exposed to harmless foreign antigens, such as food proteins, pollen, or dust components. The suppression of immune responses to harmless macromolecules is highly regulated and typically prevents processes that could be damaging to the host, known as tolerance.

The innate immune system contains cells that detect potentially harmful antigens, and then inform the adaptive immune response about the presence of these antigens. An **antigen-presenting cell (APC)** is an immune cell that detects, engulfs, and informs the adaptive immune response about an infection. When a pathogen is detected, these APCs will phagocytose the pathogen and digest it to form many different fragments of the antigen. Antigen fragments will then be transported to the surface of the APC, where they will serve as an indicator to other immune cells. **Dendritic cells** are immune cells that process antigen material; they are present in the skin (Langerhans cells) and the lining of the nose, lungs, stomach, and intestines. Sometimes a dendritic cell presents on the surface of other cells to induce an immune response, thus functioning as an antigen-presenting cell. Macrophages also function as APCs. Before activation and differentiation, B cells can also function as APCs.

After phagocytosis by APCs, the phagocytic vesicle fuses with an intracellular lysosome forming phagolysosome. Within the phagolysosome, the components are broken down into fragments; the fragments are then loaded onto MHC class I or MHC class II molecules and are transported to the cell surface for antigen presentation, as illustrated in Figure 1. Note that T lymphocytes cannot properly respond to the antigen unless it is processed and embedded in an MHC II molecule. APCs express MHC on their surfaces, and when combined with a foreign antigen, these complexes signal a "non-self" invader. Once the fragment of antigen is embedded in the MHC II molecule, the immune cell can respond. Helper T- cells are one of the main lymphocytes that respond to antigen-presenting cells. Recall that all other nucleated cells of the body expressed MHC I molecules, which signal "healthy" or "normal."



**Hour -6-Macrophage** https://www.physio-pedia.com/Macrophages https://www.immunology.org/public-information/bitesized-immunology/cells/macrophages https://www.britannica.com/science/macrophage

Macrophages are specialised cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms. In addition, they can also present antigens to T cells and initiate inflammation by releasing molecules (known as **cytokines**) that activate other cells.

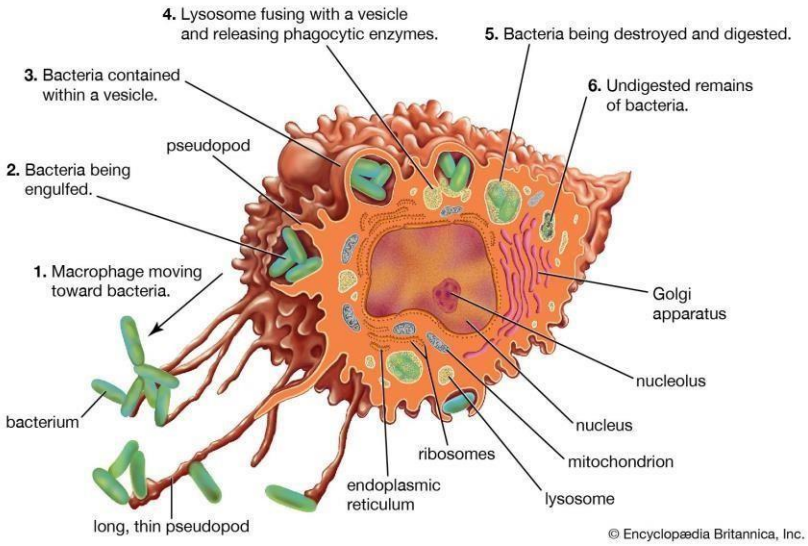
- Macrophages are mononuclear cells functioning as professional phagocytes in order to remove dying, dead or harmful pathogens.
- Macrophages are a type of white blood cell of the immune system where they engulf and digest particles that are detected as antigens by other blood cells.
- These are larger phagocytic cells that occur in essentially all types of tissues, and their structure and shape depend on the stage of maturation of the cells.
- Macrophages found in different organs have different names like the macrophages of lungs are called alveolar macrophages, while those in the liver are called Kupffer cells.
- Even though phagocytosis is the primary function of macrophages, these also play an essential role in nonspecific defense as well as in adaptive immunity.
- Macrophages are important blood cells that have important roles in almost all aspects of an organism's biology as different subsets of macrophages are involved in different functions in the body.
- Macrophages in the body are produced by the differentiation of monocytes in tissues
- Macrophages keep flowing through the blood where they migrate to and circulate within all tissues, patrolling for pathogens or eliminating dead cells and debris.
- Macrophages consist of a specialized group of receptors called Toll-like receptors that recognize products of bacteria and other microorganisms.

Macrophages originate from blood **monocytes** that leave the circulation to differentiate in different tissues. There is a substantial heterogeneity among each macrophage population, which most probably reflects the required level of specialisation within the environment of any given tissue. This heterogeneity is reflected in their morphology, the type of pathogens they can recognise, as well as the levels of inflammatory cytokines they produce (i.e. **IL-1**, **IL-6**, **tumour necrosis factor alpha**). In addition, macrophages produce reactive oxygen species, such as **nitric oxide**, that can kill phagocytosed bacteria.The heterogeneous nature of these cells may not solely be the result of their differentiation process, but it is likely to be inherited from their monocyte precursors.

Macrophages migrate to and circulate within almost every tissue, patrolling for pathogens or eliminating dead cells. The table below describes the location and function of a few different macrophage populations. Location and Function of Macrophages

Below describes the location and function of a few different macrophage populations.

1. Alveolar macrophage: Lung alveoli - Phagocytosis of small particles, dead cells or bacteria. Initiation and control of immunity to respiratory pathogens. The first line of defense against invading respiratory pathogens. They reside in pulmonary alveoli and the inter-alveolar septum in close proximity with pneumocytes.
2. Histiocytes: Connective Tissue - Motile, phagocytic cells found in the loose connective tissue throughout the body, help the body heal after an injury or infection by removing dead cells, blood, micro-organisms (such as bacteria and fungus), and foreign material from the body.[2]
3. Osteoclasts: Bone - Mediators of the continuous destruction of bone
4. Interstitial macrophage (IM): gut - represent the largest pool of tissue macrophages in the body. [3]Recent evidence supports the notion that IMs perform important immune functions in the lung, notably in terms of innate immunoregulation[4].
5. Kupffer cells: Liver- Initiate immune responses and hepatic tissue remodelling. Kupffer cells are the most abundant tissue macrophages as they constitute 80-90% of them.
6. Microglia: Central nervous system - Elimination of old or dead neurons and control of immunity in the brain.
7. Splenic macrophages (marginal zone, metallophilic and red pulp macrophages): Spleen marginal zone, red and white pulp - Elimination of dysfunctional or old red blood cell




**Hour -7 -Dendritic cell** https://microbenotes.com/dendritic-cells/

Dendritic cells are key antigen-presenting cells of the immune system that inform other effector immune cells to fight against invasive pathogens.

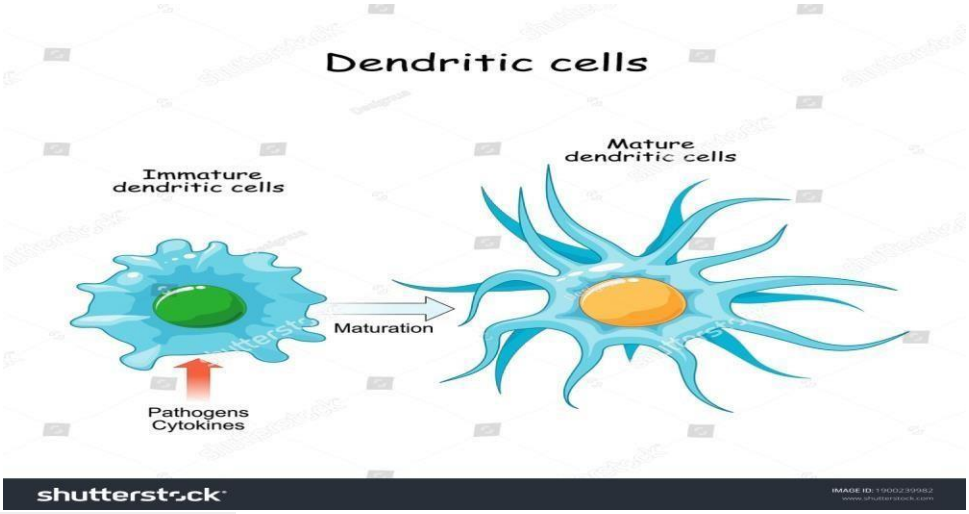
- These are also called professional antigen-presenting cells as these present antigens or parts of antigen to the receptors on different immune cells in order to elicit an immune response against them.

- Dendritic cells represent a distinct type of white blood cells capable of alerting the immune system about the presence of infections and activating the innate and adaptive immune response.
- The term ‘dendritic cell’ was given due to the tree-like or dendritic appearance of the cell. These are also considered the sentinels of the immune system as these play an essential role in the initiation of both innate and adaptive immune responses.
- As a part of antigen presentation, dendritic cells also process antigens to ensure the association between them and the major histocompatibility complex molecules.
- Dendritic cells can only perform their function after a complete maturation process initiated by direct exposure to Toll-like receptor ligands.
- Dendritic cells are easy to modify, and the modification of the cells is determined by signals which in turn depend on the local microenvironment.
- Besides antigen presentation, dendritic cells also work to maintain immune tolerance by ensuring that effector T cells are not produced against the normal or self-antigens under normal conditions.
- Dendritic cells exist in two distinct forms; immature and mature dendritic cells.
- Immature dendritic cells are present in peripheral tissues where they patrol for pathogens and **antigens**. Immature dendritic cells are defined by their inefficiency to present antigens to MHC receptors, but they do secrete few **cytokines** and express some ligands.
- The trigger for the activation of dendritic cells is brought by the encounter of pathogen-derived TLR ligands, intracellular sensors, or proinflammatory molecules.
- The maturation of dendritic cells involves the activation of lysosomes and antigen-processing machinery, which enhances the efficiency of peptide-MHC production.
- Dendritic cells are larger antigen-presenting cells with large cytoplasmic projections that are similar in structure to dendrites of nerve cells.
- The cells are irregular in shape with phase-dense granules, an irregular nucleus, and a small nucleolus.
- The projections from the cell extend in many directions from the cell body, which are involved in patrolling for invading pathogens.
- The distinctive dendrite formation by dendritic cells is an important feature for the morphological identification of DC in a blood sample.
- The cytoplasm of dendritic cells does not have any filaments, but cell organelles like mitochondria and Golgi complex can be observed.
- Similarly, dendritic cells, as different stages of maturation, have different types of granules. The size and occurrence of granules in dendritic cells are diverse, but the most common granules occurring in dendritic cells are melanin granules

**Functions of Dendritic cells**

The following are some of the functions of dendritic cells;

- Dendritic cells are professional antigen-presenting cells that perform the most important function of presenting antigens to different receptors on different immune cells for their activation.
- Dendritic cells are responsible for eliciting an immune response in T-cells and are also involved in the differentiation of T-helper cells.
- In the innate immune system, dendritic cells are involved in the activation of natural killer cells by the secretion of different cytokines.
- Dendritic cells have also been observed to be involved in the functional control of regulatory T cells.




**Hour -8 Primary lymphoid organ**

https://www.biologydiscussion.com/immunology/lymphoid-organs-primary-andsecondary-with-diagram/56268

| |
|---|
| Primary Lymphoid Organs are the centers of the Immune System where Lymphocyte development and |
| Maturation occur. That is to say, they are responsible for the Lymphocyte Proliferation, Differentiation and |
| Maturation. The initial Cells in Primary Lymphoid Organs are therefore the undifferentiated Stem Cells in |
| the Lymphoids. Such sites provide the Environment in which the Stem Cells grow into either T Cells or B |
| Cells . |

In addition, Bone Marrow is a type of Primary Lymphoid Organ, where Lymphoid stem Cells proliferate
and differentiate. Therefore, these stem Cells are differentiated into both T Lymphocytes and B Lymphocytes in the bone marrow. Immature T Cells then travel through the blood to the thymus, which is
another Primary Lymphoid Organ for maturation. But B Lymphocyte maturation occurs in the bone
marrow itself.

**These are of two types**

(a) Bone marrow

(b) Thymus

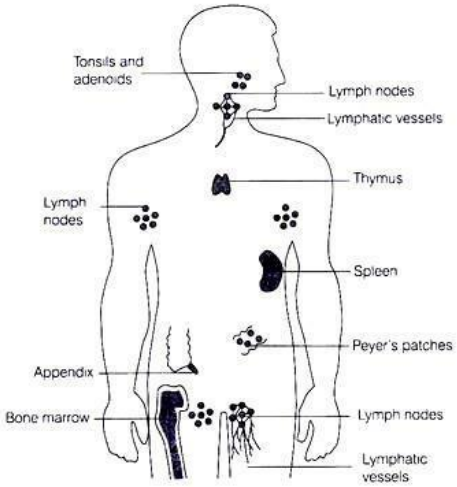
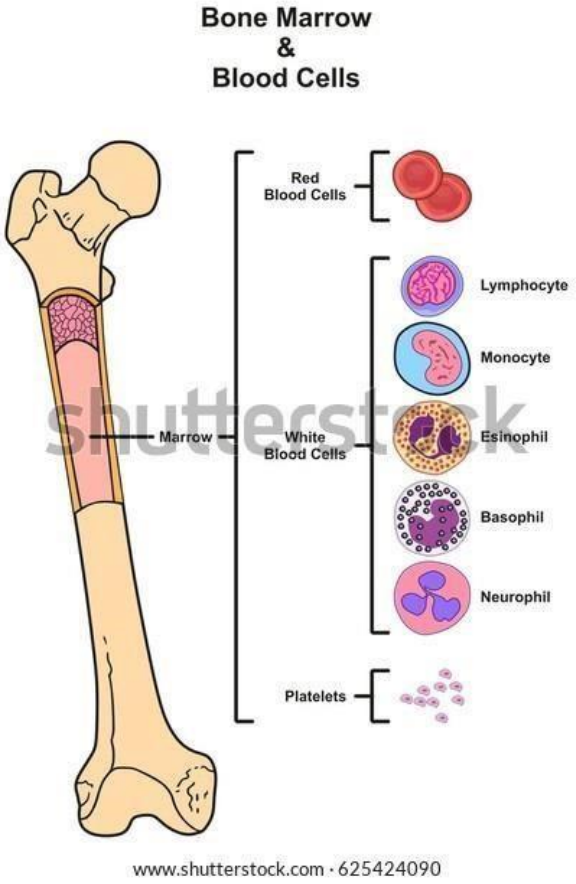



**Fig. 8.3** Human immune system

**(a) Bone Marrow:**

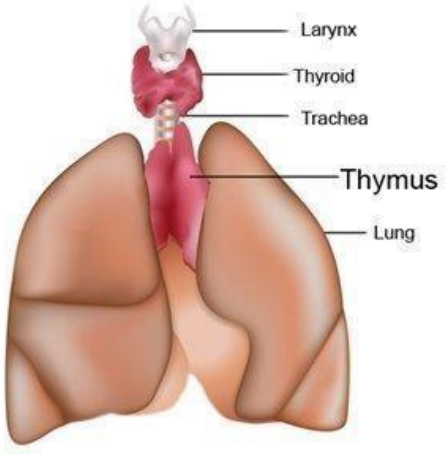
It is the main lymphoid organ, where all the lymphocytes and all the body cells are produced and Tlymphocytes are developed.

Bone marrow is **found in the center of most bones and has many blood vessels**. There are two types of bone marrow: red and yellow. Red marrow contains blood stem cells that can become red blood cells, white blood cells, or platelets. Yellow marrow is made mostly of fat.




**(b) Thymus:**

It is a lobed organ, located near the heart and beneath the breast bone. It is large at the time of birth but with age, the size keep on reducing and becomes very small by attaining puberty. Growth and maturation of Tlymphocytes takes place in thymus only.



**Hour -9 Secondary lymphoid organ** https://www.biologydiscussion.com/immunology/lymphoid-organs-primary-and-secondarywith- diagram/56268
https://www.nursingtimes.net/clinical-archive/immunology/the-lymphatic-system-2-structureand-function-of-the-lymphoid- organs

These organs provide the sites for the interaction of lymphocytes with the antigen, which then proliferate to become effector cells. **These are of following types:**

(a) Spleen,

(b) Lymph nodes,

(c) Mucosal associated Lymphoid Tissue (MALT)

Peyer's patches of small intestine and appendix are also some of the secondary lymphoid organs. **(a) Spleen:**

It is a large bean-shaped organ containing lymphocytes and phagocytes. It filters the blood by trapping the pathogens in it.

- Filtering out and destroying unwanted pathogens;
- Maintaining the population of mature lymphocytes (which are white blood cells) to enable the adaptive immune response to begin.

When foreign antigens reach these organs, they initiate lymphocyte activation and subsequent clonal expansion and maturation of these important white blood cells. Mature lymphocytes can then leave the secondary organs to enter the circulation, or travel to other areas, and target foreign antigens.

The spleen is the largest lymphoid organ. Situated in the upper left hypochondriac region of the abdominal cavity, between the diaphragm and the fundus of the stomach, it primarily functions as a filter for the blood, bringing it into close contact with scavenging phagocytes (white blood cells in the spleen that will seek out and 'eat' any pathogens in the blood) and lymphocytes.

Due to its extensive vascularisation, the spleen is a dark-purplish oval-shaped organ; in adults it is approximately 12cm long, 7cm wide and weighs around 150g. However, the size of the spleen can vary with circumstance: it diminishes in starvation, after heavy exercise and following severe haemorrhage (Gujar et al, 2017), and recent investigations indicate an increase in size in well-fed individuals and during the ingestion of food (Garnitschnig et al, 2020).The spleen is enclosed in a dense, fibro-elastic capsule that protrudes into the organ as trabeculae; these trabeculae constitute the organ's framework. Blood enters the spleen from the splenic artery and leaves via the splenic vein, both of which are at the hilum; the splenic vein eventually becomes a tributary of the hepatic portal vein.

The spleen is made up of two regions:

- Stroma – comprising the dense outer capsule with its trabeculae, some fibres and fibroblasts (cells that secrete connective tissue collagen);
- Parenchyma – composed of two types of intermingling tissue called white pulp and red pulp.

White pulp is a mass of germinal centres of dividing B-lymphocytes (B-cells), surrounded by T-cells and accessory cells, including macrophages and dendritic cells; these cells are arranged as lymphatic nodules around branches of the splenic artery. As blood flows into the spleen via the splenic artery, it enters smaller, central arteries of the white pulp, eventually reaching the red pulp. The red pulp is a spongy tissue, accounting for 75% of the splenic volume (Pivkin et al, 2016); it consists of blood-filled venous sinuses and splenic cords.

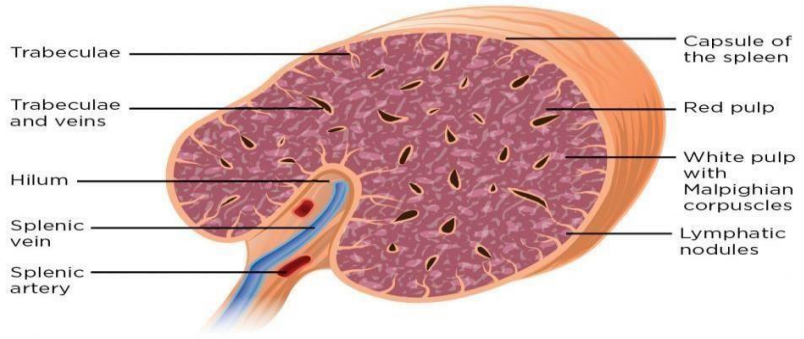
Splenic cords are made up of red and white blood cells and plasma cells (antibody-producing B-cells); therefore, the red pulp primarily functions as a filtration system for the blood, whereas the white pulp is where adaptive T- and B-cell responses are mounted. The colour of the white pulp is derived from the closely packed lymphocytes and the red pulp's colour is due to high numbers of erythrocytes (Stewart and McKenzie, 2002).

Functions

The spleen has three major functions:

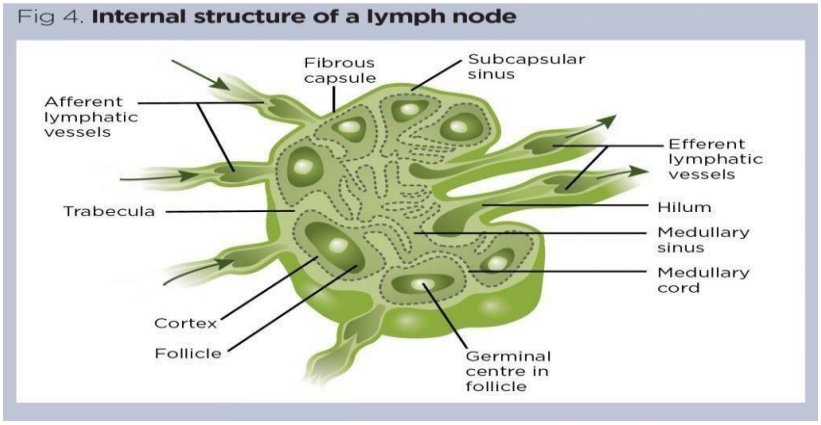
- To mount an immune response and remove micro-organisms from circulation;
- To destroy damaged and worn-out red blood cells;  To store platelets (and blood).

Fig 3. **Cross-section of the spleen**




**(b) Lymph Nodes:**

These are small solid structures located at different points along the lymphatic system. Their function is to trap the microorganisms or other antigens, that enter the lymph and tissue fluid. Therefore, the antigens trapped in the lymph nodes are responsible for the activation of lymphocytes present there and cause the immune response.




The central portions of the lymph node are essential to its function; here, there are large numbers of fixed macrophages, which phagocytose foreign material such as bacteria on contact, and populations of B- and Tcells. Lymph nodes are crucial to most antibody-mediated immune responses: when the phagocytic macrophages trap pathogenic material, that material is presented to the lymphocytes so antibodies can be generated.

Each lymph node is supplied by one or more afferent lymphatic vessels, which deliver crude, unmodified lymph directly from neighbouring tissues. A healthy, fully functioning node removes the majority of pathogens from the lymph before the fluid leaves via one or more efferent lymphatic vessels. In addition to its lymphatic supply, each lymph node is supplied with blood via a small artery; the artery delivers a variety of leucocytes, which populate the inner regions of the node.

When infection is present, the lymph nodes become increasingly metabolically active and their oxygen requirements increase. A small vein carries deoxygenated blood away from each node and returns it to the major veins. In times of infection, this venous blood may carry a variety of chemical messengers (cytokines) that are produced by the resident leucocytes in the nodes. These cytokines act as general warning signals, alerting the body to the potential threat and activating a variety of specific immune reactions. Structure

The structure of a lymph node is not unlike that of the spleen. Each lymph node is divided into several regions:

- Fibrous capsule – this forms a protective outer sheath and has trabeculae that extend periodically into the node, subdividing it into small compartments;
- Outer cortex (nodular cortex) – just inside the capsular margin, this consists of numerous follicles that are rich in B-cells. When pathogens are present, these follicles expand to reveal prominent germinal centres containing actively dividing, antibody-secreting B-cells;
- Inner cortex (paracortex) – this is just below the outer cortex and is particularly rich in T-cells, which also continually circulate throughout most other regions of the node;
- Medulla – the central inner portion of the node that contains large numbers of fixed phagocytic macrophages. These continually monitor the lymph for potentially pathogenic foreign material (a process known as immuno-surveillance), which they phagocytose on contact.

**(c) Mucosal Associated Lymphoid Tissue (MALT):**

This is located within the lining of main tracts in the body like respiratory, digestive, urogenital tracts. MALT constitutes about 50% of the lymphoid tissue in human body **Hour -10-Hematopoiesis** https://www.nursingtimes.net/ https://app.pulsenotes.com/medicine/haematology/notes/haematopoiesis

Haematopoiesis describes the process by which the cellular components of the blood are formed.

In adults, the predominant site of haematopoiesis is the bone marrow. Here we find the multipotent hematopoietic stem cells (HSCs).

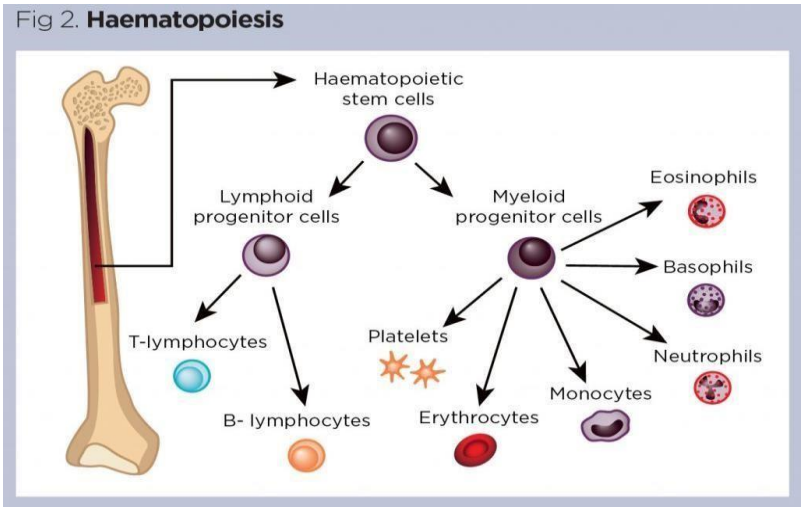
The HSCs are able to differentiate into both myeloid or lymphoid cell lines. Furthermore, the ability to selfrenew facilitates continued production of blood cells.

Haematopoiesis is essential to the continued production of all blood cell lineages. Three major cell types exist; red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes).

Leucocytes are further divided into a number of specialised cell types, including monocytes, basophils, neutrophils, eosinophils, B & T lymphocytes, dendritic cells and natural killer (NK) cells.

Haematopoiesis is a highly regulated process and its dysregulation may lead to deficiencies (e.g. anaemia, leucopaenia, thrombocytopaenia) or over-production (e.g. haematological malignancies)

Fig 2. **Haematopoiesis**




Myeloid and lymphoid lineages

Pluripotent haematopoietic stem cells are able to give rise to two further stems cells, named multipotent myeloid stem cells and multipotent lymphoid stem cells.

These lymphoid stem cells are able to differentiate into a number of progenitor cell lines that eventually give rise to B lymphocytes, T lymphocytes and natural killer cells.

The myeloid stem cells differentiate into a number of different progenitor cells that give rise to the rest of the blood cell lineages including erythrocytes, thrombocytes, neutrophils, monocytes, basophils, eosinophils and mast cells.

Growth factors

The regulation of haematopoiesis is dependent on glycoprotein growth factors, which drive the proliferation and differentiation of progenitor cells.

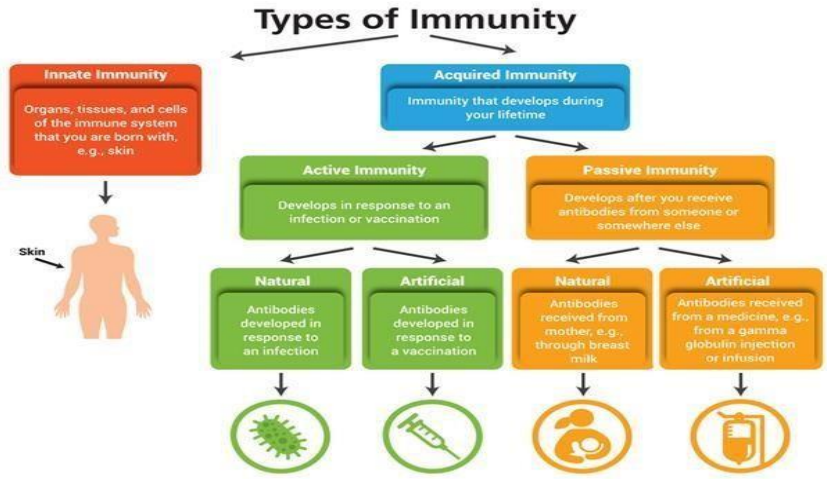
Examples of growth factors include:

- Erythropoietin (EPO)
- Thrombopoietin (TPO)
- Interleukins (e.g. IL-3, IL-6, IL-7, IL-11)
- Colony-stimulating factors (e.g. M-CSF, G-GSF)
- Negative regulators (e.g. TNF-alpha, TGF-beta)

EPO is essential for the proliferation and maturation of red blood cells. Its release is triggered by low haemoglobin concentration.The gene for EPO is located on chromosome 7 and the product is a 165 amino acid polypeptide hormone.

TPO is produced by the liver and essential for the control of platelet production.

**Hour -11 Innate immunity** https://medlineplus.gov/




INNATE IMMUNITY

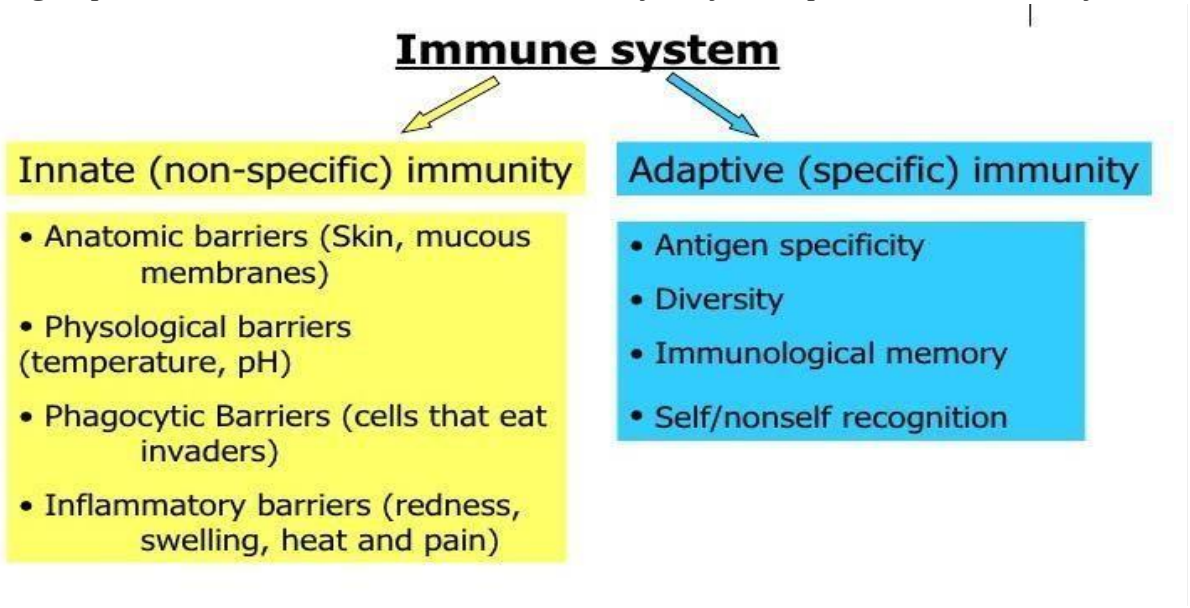
Innate, or nonspecific, immunity is the defense system with which you were born. It protects you against all antigens. Innate immunity involves barriers that keep harmful materials from entering your body. These barriers form the first line of defense in the immune response. Examples of innate immunity include:

- Cough reflex
- Enzymes in tears and skin oils
- Mucus, which traps bacteria and small particles
- Skin

- Stomach acid

Innate immunity also comes in a protein chemical form, called innate humoral immunity. Examples include the body's complement system and substances called interferon and interleukin-1 (which causes fever).

If an antigen gets past these barriers, it is attacked and destroyed by other parts of the immune system.




1. ANATOMICAL BARRIER

Epithelial surface-skin and mucus membrane covering body protect it against from invading pathogen

- SKIN-Mechanical barrier
- Possesses bactericidal activity due to high salt concentration in drying sweat
- Normal flora on skin prevents colonization by pathogen

2.PHYSIOLOGICAL BARRIER-pH and temperature

- pH- In digestive tract -swallowed pathogens destroyed by acidic pH of gastric juice
- Temp-normal body temp of 37C Inhibit the growth of some microbes
- Fever –Rise in temp after infection limits or prevents the growth of many microbes especially viruses(fever stimulates the production of interferons and helps in recovery from viral infection

3.PHAGOCYTIc BARRIER

- Pathogens invading blood and tissue destroyed by phagocytic cells Phagocytes- macrophage, dendritic cell and neutrophil

Phagocytic action of phagocytes is divided in 4 stages:

**Chemotaxis:**

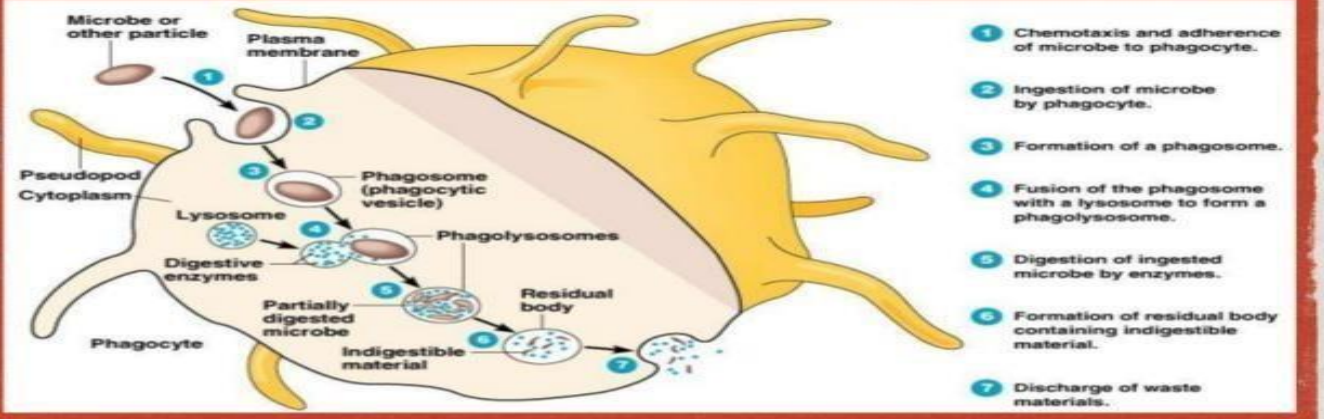
- Phagocytic cells attracted by chemotactic substances → reach site of inflammation

**Attachment:**

- Infective agent gets attached to phagocytic membrane

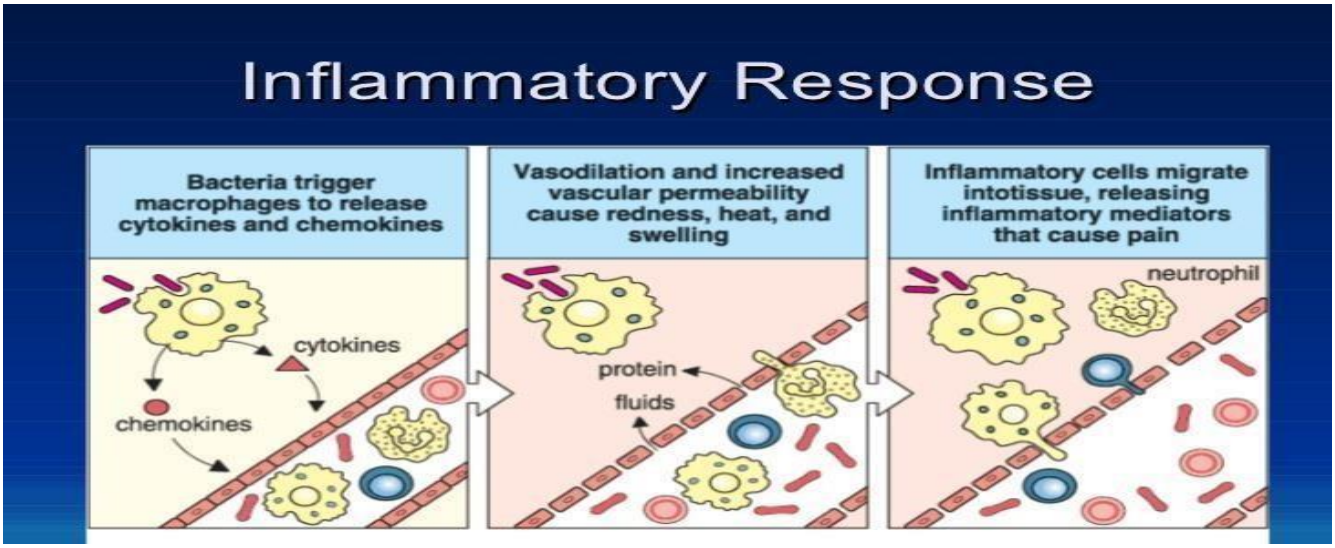
**Ingestion:**

- Bacteria are engulfed by phagocytes
- Membrane of phagosome fuses with lysosome to form phagolysosome




**Intracellular killing:**

- Lytic enzymes in phagolysosome destroy bacteria
- Some bacteria (mycobacteria) resist such killing & can multiply within phagolysosome
- A class of lymphocyts called Natural killer (NK) cells play important role in non-specific defence against viral infections & tumor



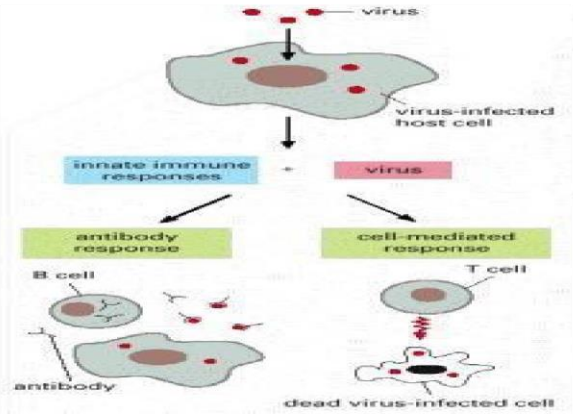

**Hour -12 Adaptive immunity** https://www.technologynetworks.com/immunology/articles/innate-vs-adaptive-immunity-335116 Organisms are not born with adaptive immunity and it is not "hard wired" in their genes like innate immunity. It is acquired during their lifetime as a result of exposure to specific antigens, be that through natural means such as infection or by vaccination. Consequently, it is also known as acquired immunity. An adaptive immune response is much slower than an innate response, taking days or even weeks to develop on first encounter (the primary immune response), but is specific to the antigen(s) present and can retain a long term "memory" to enable a faster response if it is encountered again in the future. Adaptive immunity does it necessarily last throughout an organism's entire lifespan, especially if it is not regularly re-exposed, although it can.

If the innate immune system alone is insufficient to control a foreign threat, the adaptive immune system is activated via signaling molecules and/or the presentation of antigens by antigen presenting cells. Professional antigen presenting cells, such as dendritic cells, have **major histocompatibility complex class II (MHC class II)** molecules on their surface that are involved in the presentation of foreign peptides, helping to ensure appropriate immune activation. The adaptive response consists of the **cell-mediated immune response**, which is executed by T cells, and the **humoral immune response**, which is controlled by activated B cells and antibodies. Clonal expansion of T and B cells that specifically recognize epitopes of the antigens present occurs.

T cells have diverse functions and may cause direct killing of infected cells or help to stimulate B cells towards antibody production. Once stimulated, naïve B cells differentiate into memory cells and plasma cells. Plasma cells produce and secrete large quantities of antigen-specific antibodies for the remainder of their lifecycle to help neutralize and destroy their target. Memory cells can survive for decades, reactivating in response to the presence of their target antigen to produce antibodies. Consequently, the response to repeated exposures may be faster and more robust.

| Innate Immune Response | Adaptive Immune Response |
|---|---|

| | | |
|---|---|---|
| **Takes Effect** | Immediately | Over time (days to weeks) |
| **Response Ty** | Non-specific | Specific |
| **Types** | • Physical barriers<br>• Chemical barriers<br>• Chemical defenses<br>• Cellular defenses | • Cell-mediated response<br>• Humoral response<br>• Active immunity<br>• Passive immunity |
| **Also Known As** | Natural immunity; genetic immunity | Acquired immunity |
| **Length of Efficacy** | Lifelong | Short-term, long-term, lifelong |

**Course Lecture Notes (Unit -II)- IMMUNOLOGY-(Course Code) Unit -2 Hour – 1 Antigen**

An antigen is a molecule that initiates the production of an antibody and causes an immune response.

1. The antigen should be a foreign substance to induce an immune response.
2. The antigens have a molecular mass of 14,000 to 6,00,000 Da.
3. They are mainly proteins and polysaccharides.
4. The more chemically complex they are, the more immunogenic they will be.
5. Antigens are species-specific.
6. The age influences the immunogenicity. Very young and very old people exhibit very low immunogenicity.

**Hour – 2 Types of Antigen**

On the basis of Origin

There are different types of antigens on the basis of origin:

Exogenous Antigens

Exogenous antigens are the external antigens that enter the body from outside, e.g. inhalation, injection, etc. These include food allergen, pollen, aerosols, etc. and are the most common type of antigens.

Endogenous Antigens

Endogenous antigens are generated inside the body due to viral or bacterial infections or cellular metabolism.

Autoantigens

Autoantigens are the 'self' proteins or nucleic acids that due to some genetic or environmental alterations get attacked by their own immune system causing autoimmune diseases.

Tumour Antigens

It is an antigenic substance present on the surface of tumour cells that induces an immune response in the host, e.g. MHC-I and MHC-II. Many tumours develop a mechanism to evade the immune system of the body.

Native Antigens

An antigen that is not yet processed by an antigen-presenting cell is known as native antigens.

**Hour – 3 Structure of Antibody**

Antibody Structure

An antibody has a Y-shaped structure, made up of four polypeptide subunits. Each subunit has two identical light and heavy chains.

The N-terminus of each heavy chain forms an antigen-binding domain with a light chain. There are two antigen-binding domains forming the arms of the "Y" shape. They are known as 'fragment antigenbinding' (Fab) domains.

The C-terminus of the heavy chains forms 'fragment crystallization' (Fc) domain, which helps in the interaction with the effector cells.

All four polypeptide subunits are held together by disulfide and non-covalent bonds.

The heavy chains of the antibodies contain a variable region and three constant regions. Each antibody has two identical antigen-binding sites and they differ in the antibodies.

**Hour – 4 Types of Antibody**

Antibodies or immunoglobulins(Ig) are of five different isotypes. This classification is on the basis of their H chains

**The Five Immunoglobulin (Ig) Classes**

| | IgM pentamer | IgG monomer | Secretory IgA dimer | IgE monomer | IgD monomer |
|---|---|---|---|---|---|
| |  |  |   |  |  |
| Heavy chains | μ | γ | α | ε | δ |
| Number of antigen binding sites | 10 | 2 | 4 | 2 | 2 |
| Molecular weight (Daltons) | 900,000 | 150,000 | 385,000 | 200,000 | 180,000 |
| Percentage of total antibody in serum | 6% | 80% | 13% | 0.002% | 1% |
| Crosses placenta | no | yes | no | no | no |
| Fixes complement | yes | yes | no | no | no |
| Fc binds to | | phagocytes | | mast cells and basophils | |
| Function | Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor | Main blood antibody of secondary responses, neutralizes toxins, opsonization | Secreted into mucus, tears, saliva, colostrum | Antibody of allergy and antiparasitic activity | B cell receptor |

**Hour – 5 properties of Antibody**

- IgG is the most predominant antibody found in the body and constitutes for 80% of the total antibody content in the **serum**.
- It is the only antibody with the ability to cross the placental membrane and provide immunity to the fetus. There are four sub-classes of the IgG molecule: IgG1, IgG2, IgG3, and IgG4.
- Among these, IgG 3 and IgG 4 possess the ability to cross the placenta. IgG1 is the most common antibody subclass among the four.

- IgA is found in the mucous membranes of the gastrointestinal and respiratory tracts. It is located in mucus secretions, saliva, tears, and the colostrum.
- It constitutes 13% of total antibody content found in the serum. There are two subclasses of the IgA antibody – IgA1 and IgA2.
- The IgA1 antibody is the most prevalent and is also called secretory immunoglobulin or sIgA, and is most commonly found in secretions in high quantities.
- IgM is the largest antibody found in the body and is the first to be produced after an antigen enters the body.
    - It is found in the blood and the lymph fluid. It constitutes 6% of the total antibody content of the serum.
- IgE antibody is also known as the reaginic antibody and is involved in hypersensitivity reactions or allergic responses.
- It is found in the linings of the respiratory and intestinal tracts.
- It is the least abundant antibody which constitutes about 0. 002% of the antibody content in serum.The antigenic site binds to mast cells or basophils that are known to be involved in hypersensitivity reactions.
- IgD antibody makes up less than 1% of the total antibody content of serum.
- It is usually co-expressed on the surface of B cells with IgM.
- Its specific function is still unknown. However, it is thought to be involved in the process of B cell activation.

**Hour – 6 Biological function of Antibody**

Following are some of the key functions of antibody:

- Binds to pathogens
- Activates the immune system in case of bacterial pathogens
- Directly attacks viral pathogens
- Assists in phagocytosis
- Antibody provides long-term protection against pathogens because it persists for years after the presence of the antigen.
- It neutralizes the bacterial toxins and binds the antigen to enhance its efficiency.
- They also act as the first line of defence for mucosal surfaces.
- They ingest cells by phagocytosis.
- Few antibodies bind the antigen present on the pathogens. These aggregates the pathogen and they remain in secretions. When the secretion is expelled out, the antigen is also expelled.

**Hour – 7 poly clonal antibody**

- They are produced by immunizing animals with antigens, typically with adjuvant.
- As there are different antibodies usually existing which can associate with a specific antigen or epitope, the B cells generating these antibodies are activated.
- The immune response as a result will have many different antibodies against the antigen.
- These are derived from different clones of B cells hence the serum having them is polyclonal serum. The Abs have different binding strengths (affinities).

**Hour – 8 Monoclonal antibody**

Monoclonal antibodies are artificially engineered in laboratories by scientists as a form of medication. This is because they are characterised by their ability to help a human body combat viral infections better. These can target only one specific type of antigen.

- The short-form for them is Moabs or Mabs.
- The body responds by producing antibodies to counteract the virus or antigens, whenever a person falls ill. These antibodies are specific to a particular antigen. Therefore, scientists can replicate these antibodies and help in the treatment of a disease.

**Hour – 9 Hybridoma technology**

- Hybridoma technology is composed of several technical procedures, including antigen preparation, animal immunization, cell fusion, hybridoma screening and subcloning, as well as characterization and production of specific antibodies.
- mAb generation by the hybridoma approach requires knowledge of multiple disciplines and practice of versatile technical skills, ranging from animal handling, immunology to cellular and molecular biology.
- Generation and identification of high-quality hybridoma clones is a comprehensive and laborintensive process, and requires months of work during the time frame from immunization to specific hybridoma identification

**Hour – 10 Production of Mab**

1. **Immunization of mice & isolation of splenocytes -** Mice are immunized with an antigen and later their blood is screened for antibody production. The antibody-producing splenocytes are then isolated for *in vitro* hybridoma production.
2. **Preparation of myeloma cells -** Myeloma cells are immortalized cells that, once fused with spleen cells, can result in hybridoma capable of unlimited growth. Myeloma cells are prepared for fusion.
3. **Fusion -** Myeloma cells and isolated splenocytes are fused together to form hybridomas in the presence of polyehthylene glycol(PEG), which causes cell membranes to fuse.
4. **Clone screening and picking -** clones are screened and selected on the basis of antigen specificity and immunoglobulin class.
5. **Functional characterization -** Confirm, validate and characterize (e.g. ELISA) each potentially high-producing colony.
6. **Scale up and wean -** Scale up clones producing desired antibodies and wean off selection agent(s).
7. **Expansion -** Expand clones producing desired antibodies (e.g. bioreactors or large flasks).

**Hour – 11 &12 Biomedical application of Mab Diagnostic Testing**

 Once mAbs are produced for a specific substance, they can be then used to test for the presence of that substance in a vessel. This can include toxins, drugs or hormones.

 **Pregnancy Testing**

MAbs that have been developed to detect human chorionic gonadotropin (HCG) are now present in pregnancy test kits.

 **Radioimmunodetection (RID) of Cancer**

 An imaging technique used to detect the presence of cancerous or cancer-specific cells has been developed deploying radio-labelled antibodies, which can be produced as mAbs.

**Radioimmunotheraphy (RIT) of Cancer**

Similar to RID, RIT uses mAbs to specifically target antigen cells that are associated with tumours, and then blast these with a lethal dose of radiation, whilst minimising the level of radiation absorbed by normal cells.

**Treatment of Cancer through Drugs**

Many different drugs are being developed in clinical trials with the ultimate hope of being able to treat various strains of cancer. In fact, some of these are already on the market. In 1997, a drug named Ritoxin was approved by the FDA for commercial use which is based on mAb technology.

**Viral Disease Treatment**

- Doctors hope that with further research into mAbs and an increased knowledge of their properties, treatments will become available for diseases previously thought to be incurable, such as AIDS.
- **Identifying Pathogens**
- MAbs can now be used to identify strains of a single pathogen, for example *neisseria gonorrhoeae.*

**Tracing Specific Cells and their Functions**

Scientists can use mAbs to first identify and then track certain cells or molecules in a living thing, and determine its function. For example, scientists at the University of Oregon are using such practices to determine which proteins are responsible for differentiation amongst cells in the respiratory system.

**Organ Rejection**

A certain mAb named OKT3 (developed as an antibody to the T3 antigen) is able to be used to alleviate the effects and likelihood of organ rejection when transplanting new organs into a subject.

**Rhesus disease Immunisation**

Anti-rhesus antiserum is becoming increasingly hard to find, and the UK Blood Products laboratory has been researching the possibility of substituting mAb rhesus immunisation, with a view to ultimately replacing the serum.

**E material Hour 1 Antigen**

https://en.wikipedia.org/wiki/Antigen

In immunology, an antigen (Ag) is a molecule or molecular structure or any foreign particulate matter or a pollen grain that can bind to a specific antibody or T-cell receptor. The presence of antigens in

the body may trigger an immune response. The term antigen originally referred to a substance that is an antibody generator. Antigens can be proteins, peptides (amino acid chains), polysaccharides (chains of monosaccharides/simple sugars), lipids, or nucleic acids.

Antigens are recognized by antigen receptors, including antibodies and T-cell receptors. Diverse antigen receptors are made by cells of the immune system so that each cell has a specificity for a single antigen. Upon exposure to an antigen, only the lymphocytes that recognize that antigen are activated and expanded, a process known as clonal selection.In most cases, an antibody can only react to and bind one specific antigen; in some instances, however, antibodies may cross-react and bind more than one antigen.

The antigen may originate from within the body ("self-protein") or from the external environment ("non-self").The immune system identifies and attacks "non-self" external antigens and usually does not react to self-protein due to negative selection of T cells in the thymus and B cells in the bone marrow.

Vaccines are examples of antigens in an immunogenic form, which are intentionally administered to a recipient to induce the memory function of the adaptive immune system towards antigens of the pathogen invading that recipient. The vaccine for seasonal influenza is a common example.

Etymology

Paul Ehrlich coined the term antibody (German: Antikörper) in his side-chain theory at the end of the 19th century. In 1899, Ladislas Deutsch (László Detre) named the hypothetical substances halfway between bacterial constituents and antibodies "antigenic or immunogenic substances" (French: substances immunogènes ou antigènes). He originally believed those substances to be precursors of antibodies, just as zymogen is a precursor of an enzyme. But, by 1903, he understood that an antigen induces the production of immune bodies (antibodies) and wrote that the word antigen is a contraction of antisomatogen (Immunkörperbildner). The Oxford English Dictionary indicates that the logical construction should be "anti(body)-gen".

Terminology

Epitope – the distinct surface features of an antigen, its antigenic determinant.

Antigenic molecules, normally "large" biological polymers, usually present surface features that can act as points of interaction for specific antibodies. Any such feature constitutes an epitope. Most antigens have the potential to be bound by multiple antibodies, each of which is specific to one of the antigen's epitopes. Using the "lock and key" metaphor, the antigen can be seen as a string of keys (epitopes) each of which matches a different lock (antibody). Different antibody idiotypes, each have distinctly formed complementaritydetermining regions.

Allergen – A substance capable of causing an allergic reaction. The (detrimental) reaction may result after exposure via ingestion, inhalation, injection, or contact with skin.

Superantigen – A class of antigens that cause non-specific activation of T-cells, resulting in polyclonal Tcell activation and massive cytokine release.

Tolerogen – A substance that invokes a specific immune non-responsiveness due to its molecular form. If its molecular form is changed, a tolerogen can become an immunogen.

Immunoglobulin-binding protein – Proteins such as protein A, protein G, and protein L that are capable of binding to antibodies at positions outside of the antigen-binding site. While antigens are the "target" of antibodies, immunoglobulin-binding proteins "attack" antibodies.

T-dependent antigen – Antigens that require the assistance of T cells to induce the formation of specific antibodies.

T-independent antigen – Antigens that stimulate B cells directly.

Immunodominant antigens – Antigens that dominate (over all others from a pathogen) in their ability to produce an immune response. T cell responses typically are directed against a relatively few immunodominant epitopes, although in some cases (e.g., infection with the malaria pathogen Plasmodium spp.) it is dispersed over a relatively large number of parasite antigens.

Antigen-presenting cells present antigens in the form of peptides on histocompatibility molecules. The T cells selectively recognize the antigens; depending on the antigen and the type of the histocompatibility molecule, different types of T cells will be activated. For T-cell receptor (TCR) recognition, the peptide must be processed into small fragments inside the cell and presented by a major histocompatibility complex (MHC).The antigen cannot elicit the immune response without the help of an immunologic adjuvant.Similarly, the adjuvant component of vaccines plays an essential role in the activation of the innate immune system

An immunogen is an antigen substance (or adduct) that is able to trigger a humoral (innate) or cellmediated immune response. It first initiates an innate immune response, which then causes the activation of the adaptive immune response. An antigen binds the highly variable immunoreceptor products (B-cell receptor or T-cell receptor) once these have been generated. Immunogens are those antigens, termed immunogenic, capable of inducing an immune response.

At the molecular level, an antigen can be characterized by its ability to bind to an antibody's paratopes. Different antibodies have the potential to discriminate among specific epitopes present on the antigen surface. A hapten is a small molecule that can only induce an immune response when attached to a larger carrier molecule, such as a protein. Antigens can be proteins, polysaccharides, lipids, nucleic acids or other biomolecules. This includes parts (coats, capsules, cell walls, flagella, fimbriae, and toxins) of bacteria, viruses, and other microorganisms. Non-microbial non-self antigens can include pollen, egg white, and proteins from transplanted tissues and organs or on the surface of transfused blood cells. **Hour-2 Types of Antigens**

https://en.wikipedia.org/wiki/Antigen

Exogenous antigens

Exogenous antigens are antigens that have entered the body from the outside, for example, by inhalation, ingestion or injection. The immune system's response to exogenous antigens is often subclinical. By endocytosis or phagocytosis, exogenous antigens are taken into the antigen-presenting cells (APCs) and processed into fragments. APCs then present the fragments to T helper cells (CD4+) by the use of class II histocompatibility molecules on their surface. Some T cells are specific for the peptide:MHC complex. They

become activated and start to secrete cytokines, substances that activate cytotoxic T lymphocytes (CTL), antibody-secreting B cells, macrophages and other particles.

Some antigens start out as exogenous and later become endogenous (for example, intracellular viruses). Intracellular antigens can be returned to circulation upon the destruction of the infected cell.

Endogenous antigens

Endogenous antigens are generated within normal cells as a result of normal cell metabolism, or because of viral or intracellular bacterial infection. The fragments are then presented on the cell surface in the complex with MHC class I molecules. If activated cytotoxic CD8+ T cells recognize them, the T cells secrete various toxins that cause the lysis or apoptosis of the infected cell. In order to keep the cytotoxic cells from killing cells just for presenting self-proteins, the cytotoxic cells (self-reactive T cells) are deleted as a result of tolerance (negative selection). Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens. Sometimes antigens are part of the host itself in an autoimmune disease.

Autoantigens

An autoantigen is usually a self-protein or protein complex (and sometimes DNA or RNA) that is recognized by the immune system of patients with a specific autoimmune disease. Under normal conditions, these selfproteins should not be the target of the immune system, but in autoimmune diseases, their associated T cells are not deleted and instead attack.

Neoantigens

Neoantigens are those that are entirely absent from the normal human genome. As compared with nonmutated self-proteins, neoantigens are of relevance to tumor control, as the quality of the T cell pool that is available for these antigens is not affected by central T cell tolerance. Technology to systematically analyze T cell reactivity against neoantigens became available only recently. Neoantigens can be directly detected and quantified through a method called MANA-SRM developed by a molecular diagnostics company, Complete Omics Inc., through collaborating with a team in Johns Hopkins University School of Medicine.

Viral antigens

For virus-associated tumors, such as cervical cancer and a subset of head and neck cancers, epitopes derived from viral open reading frames contribute to the pool of neoantigens.

Tumor antigens

Tumor antigens are those antigens that are presented by MHC class I or MHC class II molecules on the surface of tumor cells. Antigens found only on such cells are called tumor-specific antigens (TSAs) and generally result from a tumor-specific mutation. More common are antigens that are presented by tumor cells and normal cells, called tumor-associated antigens (TAAs). Cytotoxic T lymphocytes that recognize these antigens may be able to destroy tumor cells

Tumor antigens can appear on the surface of the tumor in the form of, for example, a mutated receptor, in which case they are recognized by B cells.

For human tumors without a viral etiology, novel peptides (neo-epitopes) are created by tumor-specific DNA alterations.

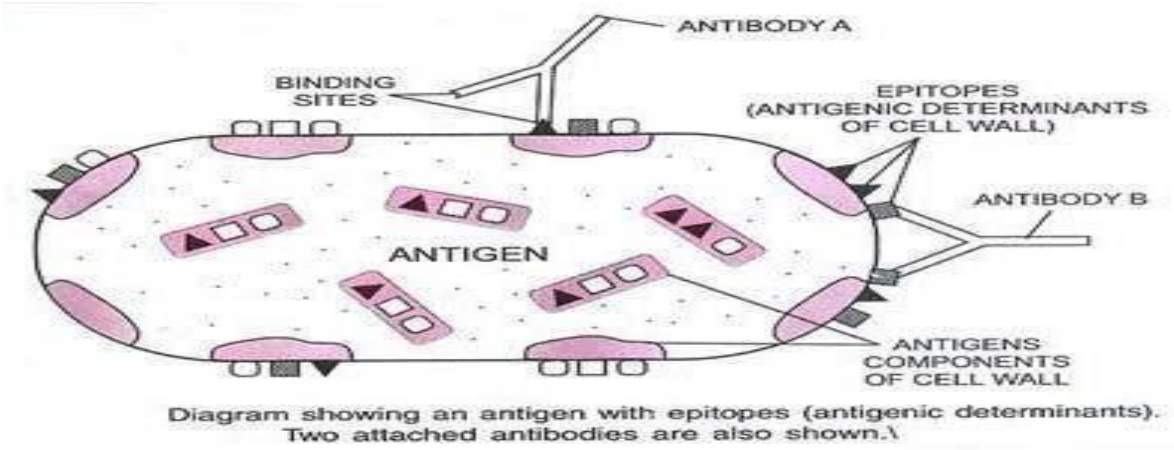
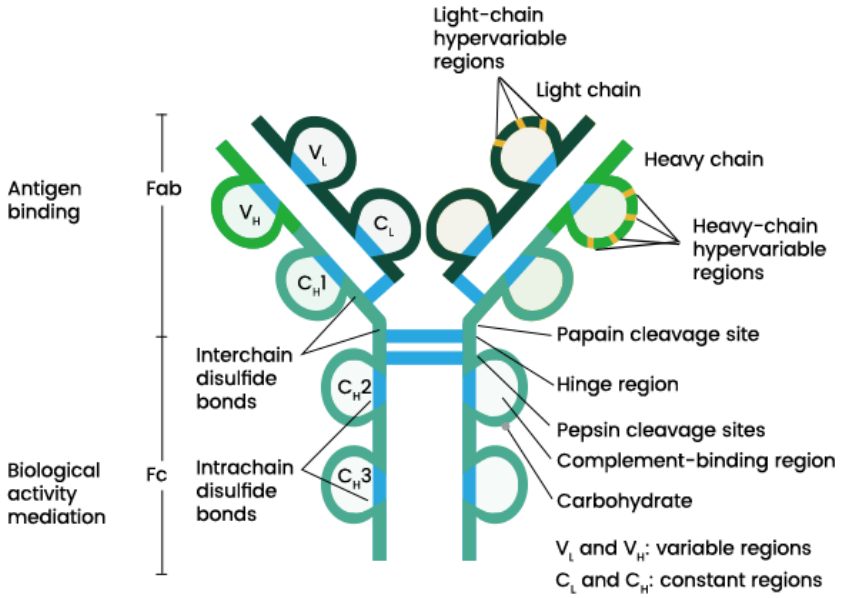



Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

**Hour-3 Structure of Antibody** https://www.sinobiological.com/resource/antibody-technical/antibodystructure-function




An antibody, also known as an immunoglobulin, is a Y-shaped structure which consists of four polypeptides — two heavy chains and two light chains. This structure allows antibody molecules to carry out their dual functions: antigen binding and biological activity mediation.

Each function is carried out by different parts of the antibody: fragment antigen-binding (Fab fragment) and fragment crystallizable region (Fc region).

Fab fragment is a region on an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy and the light chain. These domains shape the paratope — the antigenbinding site — at the amino terminal end of the monomer.

Fc region is the tail region of an antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system. This property allows antibodies to activate the immune system. The Fc regions of immunoglobulin Gs bear a highly conserved N-glycosylation site.

**Hour – 4 Types of Antibody** https://microbiologyinfo.com/antibody-structure-classes-and-functions/

https://www.sinobiological.com/resource/antibody-technical/antibody-structure-function

Types Of Antibodies

Antibodies or immunoglobulins(Ig) are of five different isotypes. This classification is on the basis of their H chains. Let's look at the different types of immunoglobulins and their functions.

**IgM**

IgM is the first antibody produced in response to a microbial attack by B cells.

It is the largest antibody and is found in a pentameric form.

It circulates in the blood and lymph and constitutes 6% of the total antibody content in the serum.

It is involved in agglutination and opsonization.

It has a large number of antigenic sites on its surface and therefore facilitates efficient activation of the immune system.

**IgG**

Most abundant isotype in the plasma, and comprises 80% of the total antibody content in the serum. It detoxifies substances that are harmful and recognizes the antibody-antigen complex.

It is transferred to the placenta through the foetus and protects the infant until its birth.

IgG is divided into four subclasses- IgG1, IgG2, IgG3, and IgG4. Among these, only IgG3 and IgG4 possess the ability to cross the placenta.

The heavy chains of IgG have two antigen-binding sites and are of the sub-class gamma.

It facilitates the process of phagocytosis and provides immunity to the developing foetus. It neutralizes the toxins and pathogens and offers protection to the body.

**IgA**

Usually found in liquids such as breast milk, serum, saliva, fluids of the intestine. IgA in breast milk protects an infant's gastrointestinal tract from microbial activity.

It constitutes 13% of the total antibody content in the serum and is divided into 2 sub-classes- IgA1 and IgA2. Among these, IgA1 is highly found in the secretions and is also called the secretory immunoglobulin.

It exists in both monomeric as well as dimeric forms.

It provides the first line of defence against the pathogens and limits inflammation. It also activates the complement pathway and participates in the immune response.

**IgD**

It is involved in the production of the antibody by B cells.

It is present as a monomer and weighs around 1,80,000 dalton.

It comprises less than 1% of the total antibody content in serum.

It acts as a receptor on B cell surface and participates in B cell activation and differentiation.

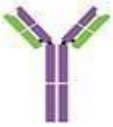
**IgE**

IgE is present in the least amounts, around 0.02% of the antibody content in the serum.

These are present in the linings of the respiratory and intestinal tracts and respond to allergic reactions.

This is found as a monomer in the body and weighs about 200,000 Dalton.

**The Five Immunoglobulin (Ig) Classes**

| | IgM pentamer | IgG monomer | Secretory IgA dimer | IgE monomer | IgD monomer |
|---|---|---|---|---|---|
| |  |  |   |  |  |
| Heavy chains | μ | γ | α | ε | δ |
| Number of antigen binding sites | 10 | 2 | 4 | 2 | 2 |
| Molecular weight (Daltons) | 900,000 | 150,000 | 385,000 | 200,000 | 180,000 |
| Percentage of total antibody in serum | 6% | 80% | 13% | 0.002% | 1% |
| Crosses placenta | no | yes | no | no | no |
| Fixes complement | yes | yes | no | no | no |
| Fc binds to | | phagocytes | | mast cells and basophils | |
| Function | Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor | Main blood antibody of secondary responses, neutralizes toxins, opsonization | Secreted into mucus, tears, saliva, colostrum | Antibody of allergy and antiparasitic activity | B cell receptor |

**Hour-5 Properties of Antibody**

https://microbenotes.com/properties-and-function-of-different-classes-of-antibodies/

**Properties of IgG**

The IgG antibodies exist in the serum in the monomeric form, and these can cross the placenta from the mother to the fetus.

Each IgG antibody has two paratopes that bind to two different epitopes on different antigens.

IgG has four subclasses classified on the basis of the subclasses of the γ heavy chains.

IgG antibodies participate predominantly in secondary immune response as these are generated as a result of class switching and maturation of the response.

**Properties of IgM**

IgM is the largest and the only pentameric antibody in humans. It is also the first antibody to be produced in response to the initial exposure to an antigen.

IgM is the first immunoglobulin to be synthesized by the fetus, beginning at about 20 weeks of age.

IgM is a pentameric molecule with 10 antigen-binding sites and 5 Fc portions held together by disulfide linkages.

The monomeric form of IgM occurs as the major antibody receptor on the surface of B lymphocytes.

IgM is relatively short-lived and usually disappears earlier than IgG.

The large size of the molecules do not allow effective diffusion of the antibody, and thus, it is found in very low concentration in the intracellular fluids.

**Properties of IgA**

IgA is the second most abundant immunoglobulin in humans, with a concentration of 2-4 mg/ml. It accounts for about 10-15% of the total serum concentration but is the most abundant antibody in external secretions.

IgA is the first line of defense as it works by inhibiting bacterial and viral adhesion to epithelial cells and by neutralizing viral and bacterial toxins intracellularly.

The secretory IgA mostly occurs in dimeric form with two monomeric units linked together by a joining peptide.

**Properties of IgD**

IgD is found in low concentration in serum, and its exact function in the immune system is not yet clearly understood.

It represents about 0.25% of the total serum immunoglobulins with a relative molecular mass of 185 kDa and a half-life of 2.8 days.

It also accounts for about 1% of the proteins present in the plasma membranes of B lymphocytes. Here, it usually coexpressed with another cell surface antibody, IgM. Hour-6 Biological function of Antibody

**Hour-6 Mechanisms**

https://www.creative-diagnostics.com/blog/index.php/the-biological-function-of-antibodies/

Antibody is an immunoglobulin produced by the body's immune system and stimulated by antigen to proliferate and differentiate from B lymphocytes or memory cells and specifically bind to the corresponding antigen.

**1.Specific binding of the corresponding antigen**

Antibody hypervariable region and antigenic determinants of the three-dimensional structure must be consistent in order to bind the antibody and the antigen binding is highly specific. Antibody molecules that specifically bind antigen can mediate a variety of physiological and pathological effects *in vivo*.

Antibody and antigen binding by non-covalent bond is reversible, and electrolyte concentration, PH, temperature and the integrity of the antibody structure can affect the ability of antibodies and antigen binding. The binding valence of IgG is bivalent; the binding valence of IgM is theoretically deca-valent but is practically pentavalent due to steric hindrance; and the dimeric IgA is tetravalent.

**2. Activation of complement**

When the IgG1, IgG2, IgG3 and IgM antibody molecules specifically bind to the corresponding antigen, their conformation changes. The complement of the complement binding site, CH2 of IgM or CH2 of IgG is bound to Clq and the complement system is activated by the traditional pathway. For IgG, at least two closely adjacent IgG molecules are needed to activate complement when they are bound to the corresponding antigen. Aggregates of other Ig molecules, such as IgG4 and IgA, activate complement by alternative pathways. Human natural anti-A and anti-B blood group antibody is IgM, and when blood group does not meet the blood transfusion, the antigen-antibody reaction activates complement hemolysis, causing rapid and serious transfusion reactions. **3. Binding Fc receptors**

After binding the corresponding antigen through the V region, Ig can bind trough Fc segment to a variety of cell surface Fc receptors, and stimulate different effector functions.

**3.1 Opsonization promotes phagocytosis**

IgG molecules binds to bacteria and other particulate antigen, then pass through the Fc segment and mononuclear phagocytes and neutrophils corresponding receptors (FcγR), and thus promotes its phagocytosis called opsonization. Complement and antibody play the role of conditioning phagocytosis, known as the joint conditioning effect. Neutrophils, monocytes and macrophages have high affinity or low affinity for FcγRI (CD64) and FcrRII (CD32), and IgG, particularly human IgGl and IgG3 subclasses, plays major roles in opsonophagocytosis. Eosinophils have affinity FcyRII, and IgE and the corresponding antigen can promote phagocytosis of eosinophils.

**3.2 Mediated allergic reactions**

Fc fragments of IgE, upon binding to the corresponding receptors on the surface of mast cells and basophils (FcεR), sensitize these cells and under the action of allergens, degranulate these cells to release bioactive

substances such as Histamine, bradykinin, causing local telangiectasia, increased permeability, stimulate type I hypersensitivity.

### 3.3 Antibody-dependent cellular cytotoxicity, ADCC effect

IgG binds to corresponding target cells, such as virus-infected cells and tumor cells, and exerts an ADCC effect by binding its Fc fragment to the corresponding receptor (FcγR) on NK cells. Mononuclear phagocytes and neutrophils, which have IgG Fc receptors on the surface, also produce ADCC effects on target cells that bind to IgG as described above.

### 4. Through the placenta

Among the five types of Ig, IgG is the only Ig that can be transferred from the mother to the fetus through the placenta, and the immunity obtained by the fetus in this manner is called natural passive immunity. Studies have shown that maternal IgG may be transported to the fetus by binding to the corresponding receptor on the surface of the placental trophoblast—FcγR.

### 5. Immune regulation

Antibodies have a positive and a negative regulatory effect on immune response, and through the unique and anti-unique type of network involve in the body's immune regulation. The above briefly described the five biological functions of antibodies, which are a specific function with the antigen, activation of complement, binding of Fc receptors and transplacental and immunoregulation. Resulting from a single B cell clone, monoclonal antibody is highly uniform and only binds to specific antigenic epitopes; and polyclonal antibodies are hybrid antibodies that stimulate various types of monoclonal antibodies produced by various epitopes. All of these antibodies have the basic biological function of antibodies and are widely used in many types of research and diagnosis.

## Hour-7 Polyclonal Antibody

https://www.news-medical.net/life-sciences/Polyclonal-Antibodies.aspx

**Polyclonal antibodies (pAbs) are a complex mixture of several antibodies that are usually produced by different B-cell clones of an animal. These antibodies recognize and bind to many different epitopes of a single antigen and hence can form lattices with the antigens.**

### How are polyclonal antibodies generated?

### Antigen preparation

The quality and quantity of antigen used directly affects the immune response. Even small amounts of impurities will lead to antibodies reacting more to the impurity than to the desired antigen. Too little or too much antigen may cause sensitization, suppression, or other unwarranted immunomodulatory effects. Thus, the purification of the antigen is a crucial process to achieve increased antibody specificity.

The antigen should be prepared under sterile conditions to make sure it is endotoxin free. The quantity of the antigen is dependent on several factors such as properties of the particular antigen, the animal species chosen, injection route, frequency of injection, and the purity level of the antigen.

**Animal species selection**

The factors that influence the choice of animal species are amount of pAb required, the phylogenetic relationship between the animal and antigen, the age of the animal, the ease of obtaining blood samples, and the application in which the pAb is used.

Commonly used animal species in the laboratory are rabbits, rats, mice, guinea pigs, hamsters, goats, chicken, and sheep. Rabbits are preferred due to their size and relatively long life span. However, in order to produce larger quantities of pAbs, farm animals such as goats, sheep, and horses are used.

**Immunization protocol**

The immunization protocol differs for different animal species. Adjuvants are compounds used as a form of stimulus in cases where the induced immune response would otherwise be inadequate. The most commonly used adjuvant for production of pAbs is the Freund's complete adjuvant (FCA).

FCA induces high antibody titers to most types of antigens. However, care should be taken that FCA is not over administered. Use of FCA should be limited to a single time as FCA can cause severe tissue injury.

The smallest volume of antigen capable of inducing an effective immune response is injected into the animal. However, the injection route depends on the nature of the antigen as well as the animal being used. The antigen can be injected as a single volume or as several low volumes at different sites.

Booster injections are provided if the antibody titer concentration has reached a plateau or is declining. Such injections do not always require an adjuvant and very small quantities of the antigen are sufficient to improve the antibody concentration. A maximum of three booster injections is recommended.

**Post-immunization observation**

Animals are monitored daily to evaluate the side effects of immunization and blood is withdrawn at regular intervals. Serum from the animals is analyzed to monitor the antibody responses and to extract the antibodies when sufficient quantity is produced.

**Advantages of polyclonal antibodies**

This is a relatively inexpensive process and it can be used to isolate large quantities of an antibody in a single extraction. PAbs are a heterogeneous mix of antibodies that can bind to wide range of antigenic epitopes. Hence, small change in the epitopes of an antigen is less likely to affect pAbs. These antibodies are very stable across a wide range of salt concentrations and pH values.

**Disadvantages of polyclonal antibodies**

The affinity of pAbs to antigens may change over time thereby leading to a lot of variability between batches. Moreover, the amount of pAbs produced is limited by the size and lifespan of the animal. The purity and concentration levels of a specific antibody are lower in pAbs than that in monoclonal antibodies.

**Hour-8 Monoclonal antibody**

https://www.cancerresearchuk.org/about-cancer/cancergeneral/treatment/immunotherapy/types/monoclonal-antibodies

What are monoclonal antibodies?

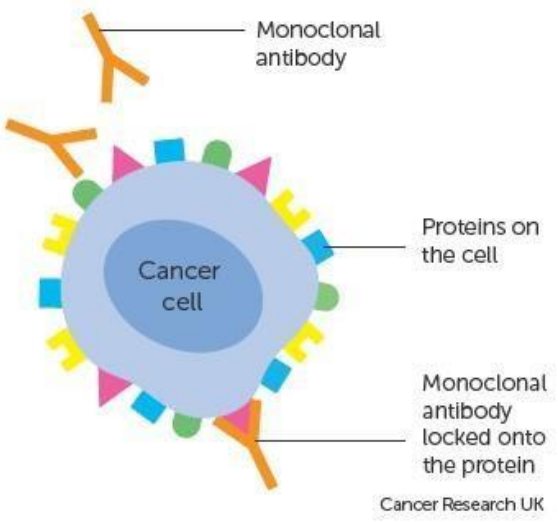
Antibodies are found naturally in our blood and help us to fight infection. MAB therapies mimic natural antibodies but are made in a laboratory. Monoclonal just means all one type. So each MAB is a lot of copies of one type of antibody.

Many different MABs are available to treat cancer. They work in different ways and some work in more than one way.

How do monoclonal antibodies (MABs) work?

A MAB works by recognising and finding specific proteins on cells. Some work on cancer cells, others target proteins on cells of the immune system.

Each MAB recognises one particular protein. They work in different ways depending on the protein they are targeting.




MABs work as an immunotherapy in different ways. Some MABs work in more than one way.

They can:

- trigger the immune system to attack and kill cancer cells
- act on cells to help the immune system attack cancer cells

Trigger the immune system

Some MABs trigger the immune system to attack and kill cancer cells.

Although cancer cells are abnormal, they develop from normal cells so they can be difficult for the immune system to spot.

Some MABs attach themselves to cancer cells, making it easier for the cells of the immune system to find them. This process is called antibody-dependent cell-mediated cytotoxicity or ADCC.

**Hour-9 Hybridoma Technology**

https://en.wikipedia.org/wiki/Hybridoma_technology

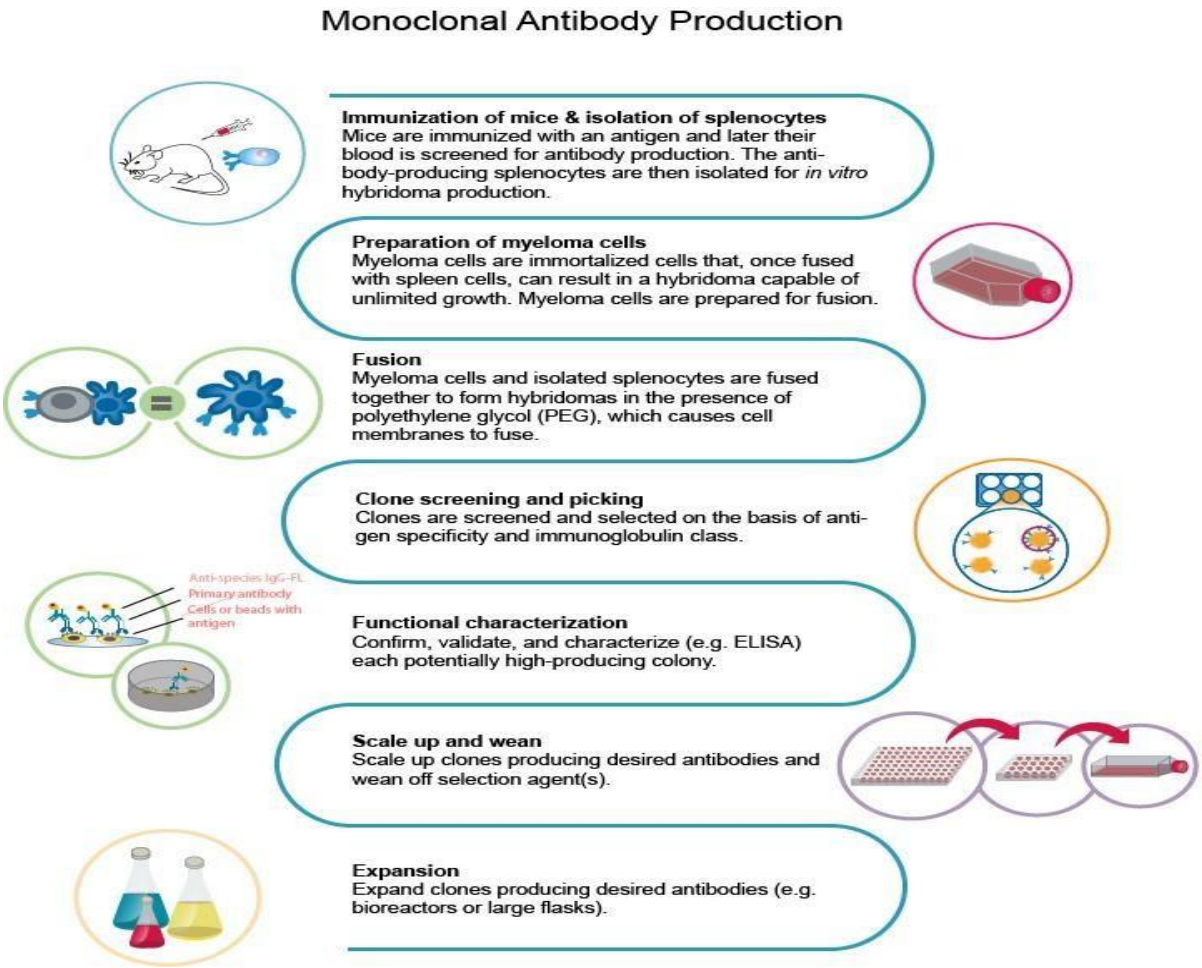
**Hybridoma technology** is a method for producing large numbers of identical antibodies (also called monoclonal antibodies). This process starts by injecting a mouse (or other mammal) with an antigen that provokes an immune response. A type of white blood cell, the B cell, produces antibodies that bind to the injected antigen. These antibody producing B-cells are then harvested from the mouse and, in turn, fused with immortal B cell cancer cells, a myeloma,[*clarification needed*] to produce a hybrid cell line called a **hybridoma**, which has both the antibody-producing ability of the B-cell and the longevity and reproductivity of the myeloma. The hybridomas can be grown in culture, each culture starting with one viable hybridoma cell, producing cultures each of which consists of genetically identical hybridomas which produce one antibody per culture (monoclonal) rather than mixtures of different antibodies (polyclonal). The myeloma cell line that is used in this process is selected for its ability to grow in tissue culture and for an absence of antibody synthesis. In contrast to polyclonal antibodies, which are mixtures of many different antibody molecules, the monoclonal antibodies produced by each hybridoma line are all chemically identical.

The production of monoclonal antibodies was invented by César Milstein and Georges J. F. Köhler in 1975. They shared the Nobel Prize of 1984 for Medicine and Physiology with Niels Kaj Jerne, who made other contributions to immunology. The term **hybridoma** was coined by Leonard Herzenberg during his sabbatical in César Milstein's laboratory in 1976–1977.

**Hour-10 Production of Mab**

https://en.wikipedia.org/wiki/Monoclonal_antibody

The traditional monoclonal antibody (mAb) production process usually starts with generation of mAb-producing cells (i.e. hybridomas) by fusing myeloma cells with desired antibody-producing splenocytes (e.g. B cells). These B cells are typically sourced from animals, usually mice. After cell fusion, large numbers of clones are screened and selected on the basis of antigen specificity and immunoglobulin class. Once candidate hybridoma cell lines are identified, each "hit" is confirmed, validated, and characterized using a variety of downstream functional assays. Upon completion, the clones are scaled up where additional downstream bioprocesses occur.




**Hour-11 and 12 Biomedical application of Mab**

https://www.h-h-c.com/an-overview-of-monoclonal-antibodies-and-its-applications/

THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF MABS

Monoclonal antibodies are valued reagents for an abundance of applications. Due to their selectivity, specificity, high binding affinity, and immunogenicity/low toxicity, monoclonal antibodies are suitable for clinical, environmental, elemental research and more. They are used in applications of therapeutics and diagnostics, including medical devices, vitro tests, and medical imaging.

Through therapeutic applications, monoclonal antibodies can be used in their naked form or as carriers by being conjugated to a tiny molecule or drug. While performing diagnostic and research applications, monoclonal antibodies are often conjugated with fluorescent tags for visual detection of targets or enzymes.

1.**MAbs in Biochemical Analysis**

Symptomatic tests based on MAbs are reagents that are routinely used in radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) in labs. Those assays estimate the circulating concentrations of hormones like insulin, human chorionic gonadotropin, growth hormone, progesterone, thyroxine,

triiodothyronine, thyroid-stimulating hormone, and several other tissue and cell products. In current years, various diagnostic kits using MAbs have grown to be commercially available. Now it is helpful for diagnosis of different diseases:

- **In Pregnancy**: Pregnancy by indirect detection of the urinary levels of human chorionic gonadotropin.
- **In Cancers**: Cancers estimation of plasma carcinoembryonic antigen in colorectal cancer and prostate-specific antigen for prostate cancerous cell. Besides diagnosis, evaluation of tumour markers is also essential for the diagnosis of cancers. A constant fall in specific tumour antigens is observed with a contraction in tumour size following treatment.
- **In Hormonal disorders:** Hormonal dysfunctions analysis of triiodothyronine, thyroxine and thyroidstimulating hormone for thyroid disorders.

**2. Use of MAbs in Therapy against Complications of Viral Infections**

Cytomegalovirus (CMV) induces severe immunocompromised illnesses, such as patients with AIDS and those going through organ transplants. Infection frequencies may increase up to 75% in those negative for CMV who receive kidneys from seropositive patients. CMV infection can result in retinitis and gastroenteritis in HIV-infected patients and may also cause chronic pain intrauterine disease.

About 40,000 cases of congenital CMV infection are recorded each year; mental retardation and hearing loss might occur in around 25% of those cases. Presently, there is no vaccine against CMV. Ganciclovir, foscarnet, and (S)-1-[3-hydroxy-(2 phosphonylmethoxy) procytosine are a few of the possible treatments for Cytomegalovirus infection.

Another method of medication is via regulation of anti-CMV hyper immunoglobulin obtained from combined sera of CMV-seropositive persons. Passive immunisation has been attested to reduce the severity of CMV and block mother-to-infant transference.

Furthermore, humanized antibodies may evacuate the virus from infected tissues, and a purpose earlier thought to be exclusive to cytotoxic T lymphocytes. Many doctors use a blend of antiviral agents and immunoglobulins in patients in danger of CMV infection. MAbs may also reduce the number of antiviral agents needed for treatment. MAbs against murine CMV polypeptides are shielding in animal models.

**3. Use of MAb in Cancer: Radioimmunotherapy**

Advances in radiolabeling have allowed immuno-conjugates to be delivered to cells and showed promise in clinical trials. Radioimmunotherapy uses a radiolabeled MAb to provide radioactive isotopes in targeted cells. Radioisotopes such as iodine-131 and yttrium-90, which are β emitters, can cause damage not only to the bound cell but also to cells adjacent to tumour cells that humanized antibodies may not be able to reach within the tumours.

The absence of knowledge about the suitable dose, shedding of target antigen hinders radioisotopes and biodistribution. Radiolabeled MAbs may also attack healthy cells, depending on the degree to which reticuloendothelial cells exposing Fc receptors bind to the connected regions of unimpaired antibody molecules. Using antibody particles or constructs might modify this nonspecific uptake.

**4. Use of MAbs in Therapy of Asthma**

Enormous levels of IgE might cause bronchial hyperresponsiveness, an uncertainty factor for asthma. Immune responses propitiated by IgE are crucial in the pathogenesis of allergic asthma.

Two two-week injections of recombinant humanised anti-IgE antibodies were performed in a recent study with patients reporting mild to acute allergic asthma.

It forms complexes with unfettered IgE and obstructs its communication with mast cells and basophils, directed to a fall in serum IgE levels and slightly reduced asthma symptom scores compared to the placebo group. Subjects receiving anti-IgE were capable of decreasing dependence on corticosteroids.

**5.Use of MAbs in the treatment of AIDS**

Immunosuppression is the hallmark of AIDS, and it is induced by a reduction in cluster determinant antigen 4 (CD4) cells of T-lymphocytes. The HIV or human immunodeficiency virus binds to specific receptors on CD4 cells by using exterior membrane glycoprotein (gp).

Genetic engineers have been prospering in connecting the Fc portion of rat monoclonal antibodies to human CD4 molecules. This complex has a great affinity to attach to membrane glycoprotein gp120 of virus-infected cells. The Fc remnant induces cell-mediated eradication of HIV infected cells.

6 **Use of MAbs in COVID-19 treatment**

In May 2021 the FDA issued an emergency use authorization (EUA) for the investigational monoclonal antibody therapy Sotrovimab for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (12 years and older weighing at least 40 kilograms) with positive results of direct SARS-CoV-2 viral testing and who are at high risk for progression to severe COVID-19, including hospitalization or death. This includes patients of 65 years of age and older or individuals who have certain medical conditions.

Sotrovimab is a monoclonal antibody that is specifically directed against the spike protein of SARS-CoV-2 and is designed to block the virus' attachment and entry into human cells. Sotrovimab is not authorized for patients hospitalized due to COVID-19 or those requiring oxygen therapy due to COVID-19.

**CONCLUSION**

Immunology has developed at a speedy pace and has produced many critical developments. Although vaccination has thus far been determined to be the most cost-effective method of limiting and preventing diseases universally, the development of MAbs that utilise the specificity of immunological responses is one of the most successful applications of immunology to date.

Chimeric and humanised antibodies have reduced the risk of allergenicity from exposure to nonself antibodies and heightened the clinical effectiveness of MAb treatments. Advancements in radiology and pharmacology have permitted radiolabeled and immunoconjugate antibodies to be produced. Fab fragments, heteropolymers, and bispecific antibodies are now available in addition to whole MAbs. These assuring advancements may soon allow humanized monoclonal antibodies to be used to treat diseases as varied as lung cancer, asthma, viral infection, septicemia, and poisoning.

UNIT: III

**Hour:1 Ag – Ab interaction**

There are three stages to the interactions between Ag and Ab.

- The first stage of the reaction entails the formation of the Ag-Ab complex.
- The second stage results in visible phenomena like agglutination, precipitation, etc.
- The third stage involves the destruction of Ag or neutralisation of Ag.
- Significantly specific reaction
- Occurs in a noticeable manner
- Non-covalent reactions (Ionic bonds, Van der Waals forces, Hydrophobic interactions, Hydrogen bonds)
- Antibodies and antigens are not denatured
- Reversible
- Affinity: This refers to how strongly an antigen binds to a certain antigen-binding site on an antibody. • Avidity: It is a more general concept than affinity. It represents the Ag-Ab complex's total strength. It depends on:

1. The antibody's affinity
2. Antibody and antigen valencies (the number of binding sites)
3. How epitopes and paratopes are structurally arranged.

- Cross-Reactivity: This term describes an antibody's capacity to bind to similar epitopes on other antigens.

**Hour: 2 Immunodiffusion**

- Immunodiffusion is the term for a precipitation test on agar gel.
- This test involves the addition of reactant to the gel, and the diffusion of the antigen-antibody mixture.
- The size of the particles, temperature, gel viscosity and amount of hydration all affect the diffusion rate.
- Agar concentrations between 0.3 and 1.5% allow diffusion of most reactants.

**Hour: 3 Types of ID**

1 Single diffusion in one dimension (Oudin procedure)

2 Double diffusion in one dimension (Oakley Fulthorpe procedure)

3 Single diffusion in two dimension (radial immunodiffusion or Mancini method)

4 Double diffusion in two dimensions (Ouchterlony double immunodiffusion)

**Hour:4 Immunoelectrophoresis**

Immunoelectrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies, reacting with the proteins to be separated or characterized.

**Hour: 5 Rocket Immunoelectrophoresis**

Is one-dimensional quantitative immunoelectrophoresis. The method has been used for quantitation of human serum proteins before automated methods became available.

**Hour: 6 Counter Immunoelectrophoresis**

In comparison to other conventional methods of diagnosis e.g. for viral infection testing, counterimmunoelectrophoresis is a highly specific, simple, and speedy method that does not require sophisticated, expensive tools, input materials, or long-term capacity building.

**Hour: 7 ELISA**

The enzyme-linked immunosorbent assay (ELISA) is a commonly used analytical biochemistry assay, first described by Eva Engvall and Peter Perlmann in 1971.

The assay uses a solid-phase type of enzyme immunoassay (EIA) to detect the presence of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured. ELISA has been used as a diagnostic tool in medicine, plant pathology, and biotechnology, as well as a quality control check in various industries.

**Hour:8 Application of ELISA**

ELISA can be performed to evaluate either the presence of antigen or the presence of antibody in a sample, it is a useful tool for determining serum antibody concentrations (such as with the HIV test or West Nile virus)

**Hour:9 RIA**

A radioimmunoassay (RIA) is an immunoassay that uses radiolabeled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive in vitro assay technique used to measure concentrations of substances, usually measuring antigen concentrations (for example, hormone levels in blood) by use of antibodies.

**Hour: 10 Application of RIA**

Radioimmunoassay allows for the measurement of wide range of materials of clinical and biological importance.

**Hour:11 Fluorescent antibody technique**

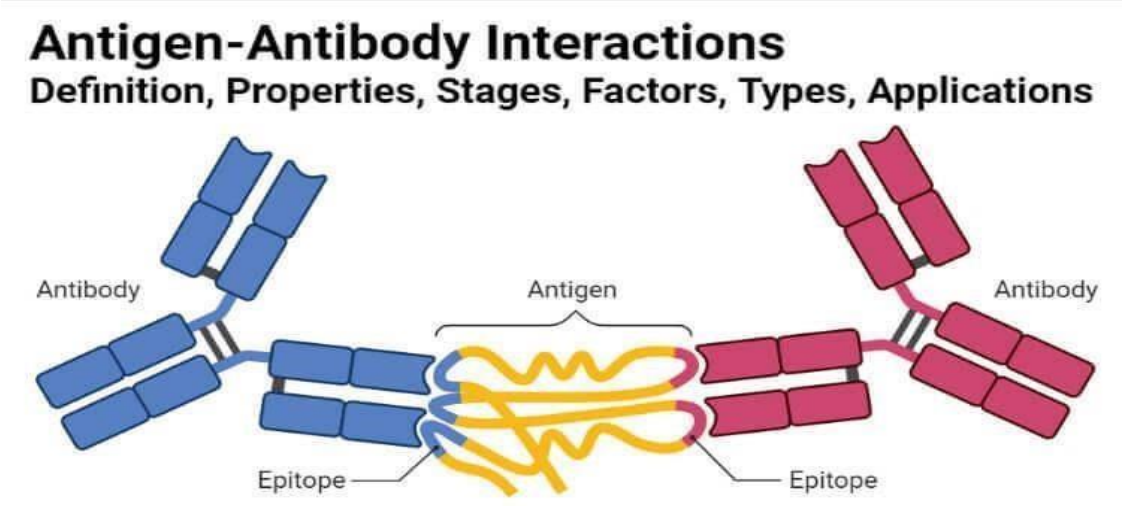
Immunofluorescence is a technique used for light microscopy with a fluorescence microscope and is used primarily on microbiological samples. This technique uses the specificity of antibodies to their antigen to target fluorescent dyes to specific biomolecule targets within a cell, and therefore allows visualization of the distribution of the target molecule through the sample. The specific region an antibody recognizes on an antigen is called an epitope.

**Hour: 12 Purification of antibody**

Antiserum from an immunized animal can be used directly for certain applications, but more often some form of antibody purification is required to obtain an antibody probe that is effective for multiple types of detection methods. This article summarizes the main approaches and tools available for accomplishing antibody purification.

**E- Material Hour: 1 Ag- Ab interaction**

The interactions between antigens and antibodies are known as ***antigen-antibody reactions***. The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens. Antibodies recognize molecular shapes (epitopes) on antigens. Generally, the better the fit of the epitope (in terms of geometry and chemical character) to the antibody combining site, the more favorable the interactions that will be formed between the antibody and antigen and the higher the affinity of the antibody for antigen. The affinity of the antibody for the antigen is one of the most important factors in determining antibody efficacy *in vivo*.




**Antigen-Antibody (Ag-Ab) Interaction**

The antigen-antibody interaction is a bimolecular irreversible association between antigen and antibody. The association between antigen and antibody includes various non-covalent interactions between epitope (antigenic determinant) and variable region (VH/VL) domain of an antibody.

**Antigen-Antibody Interaction Properties**

– Highly specific reaction

– Occurs in an observable manner

– Non-covalent interaction ( Van der Waals forces, Ionic bonds, Hydrogen bonds, Hydrophobic interactions )

– No denaturation of antibodies and antigens – Reversible

– **Affinity**: It is the strength with which one antigen binds on a single antigen-binding site on an antibody.

– **Avidity**: It is a broader term than affinity. It is a measure of the overall strength of the Ag-Ab complex. It depends on:

- The affinity of the antibody
- Valency(no. of binding sites) of antibody and antigen
- And the structural arrangement of epitopes and paratopes.

– **Cross-Reactivity:** It refers to the ability of an antibody to bind to similar epitopes of different antigens.

**Stages of Antigen-Antibody Interaction**

1. **Primary Stage:** It is the initial interaction between antigens and antibodies.

– Rapid

– Reversible

– Without any visible effects

2. **Secondary Stage:** It is the irreversible interaction between antigens and antibodies.

– Slow

– With visible effects

**Types of Antigen-Antibody Interaction**

**Ag-Ab reactions are basically of two types:**

**1. In Vivo (Occurring in natural condition):** It includes the general antibody-mediated immune response occurring in our body against any antigen.

**2. In Vitro (Done in artificial conditions):** It includes a series of serological tests performed in laboratories to detect antigens or antibodies in case of many diseases. **In Vivo Reactions** • Agglutination

- Precipitation
- Complement fixation
- Neutralization
- Ab Dependent Cell-Mediated Toxicity
- Immobilization
- Opsonization

**In vitro Reactions**

- Agglutination
- Precipitation
- Complement fixation
- Neutralization
- ELISA
- Radioimmunoassay (RIA)
- Western Blotting

**Hour:2 Immunodiffusion:**

https://www.biosciencenotes.com/immunodiffusionreaction/

**Immunodiffusion** is a diagnostic test which involves diffusion through a substance such as agar which is generally soft gel agar (2%) or agarose (2%), used for the detection of antibodies or antigen.

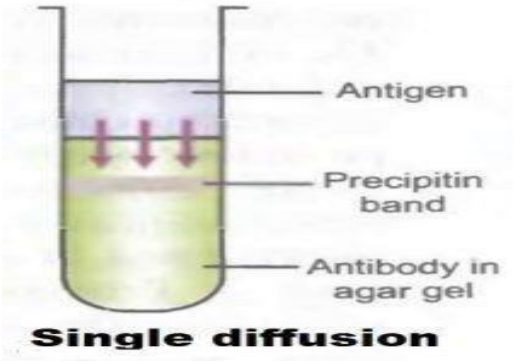
- The precipitation test in a agar gel is termed as immunodiffusion test.
- In this test, reactant are added to the gel and antigen-antibody combination occurs by the means of diffusion.
- The rate of diffusion is affected by the size of the particle, temperature, gel viscosity, amount of hydration and interaction between the matrix and reactants.
- An agar concentration of 0.3-1.5% allows for diffusion of the most of the reactants.
- One of the major advantages of immunodiffusion reaction is that the line of precipitation is visible as a band which can also be stained for preservation.
- Another advantage is that it can be used to detect, identify the cross reaction and non-identify between different antigens in a reacting mixture.

**Hour:3 Types of ID**

https://microbenotes.com/immunological-techniques/

Immunodiffusion reaction are classified based on the ;

- Number of reactant diffusing and Direction of diffusion, as follows;




**Single diffusion in one dimension:**

- As the name suggests, it is the single diffusion of antigen in a agar in one dimension.
- In this method, antibody is incorporated into agar gel in a test tube and the antigen solution is poured over it.
- During the course of time, the antibody in agar gel and a line of precipitation is formed.
- The number of precipitate band shows the number of different antigen present in antigen solution.

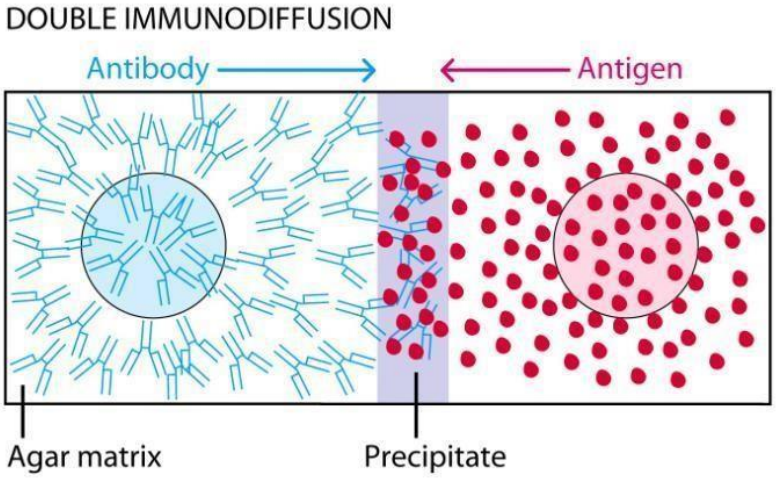
**Single diffusion in two dimension:**

- It is also called radial immunodiffusion.
- In this method, antiserum solution containing antibody is incorporated in agar gel on a slide or petridish.
- The wells are cut on the surface of the gel.
- The antigen is then applied to the well cut into the gel.
- When antibody already present in gel reacts with antigen which diffuses out of the well, a ring of precipitation is formed around the wells.
- The diameter of the ring is directly proportional to the concentration of the antigen.
- The greater the amount of antigen in the well, the farther the ring will be from the well. ▪ Radial immunodiffusion has been used for the quantitative estimation of antibody and antigen in serum.
- It is used to
- measure IgG, IgM, IgA and complement components in the serum ▪ measure antibodies to influenza virus in the sera.

**Double diffusion in one dimension:**

- In this method, the antibody is incorporated in agar gel in a test tube above which a layer of plain agar is placed.
- The antigen is then layered on the top of this plain.

- During the course of time, the antigen and antibody move toward each other through the intervening layer of plain agar.
- In this zone of plain agar, both antigen and antibody react with each other to form a band of precipitation at their optimum concentration.




**Double diffusion in two dimension:**

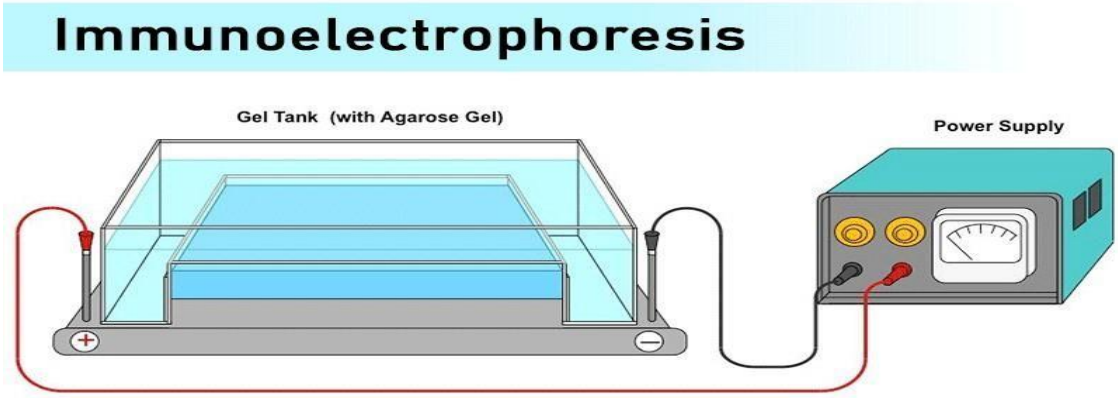
- In this method, both the antigen and antibody diffuse independently through agar gel in two dimensions, horizontally and vertically.
- The test is performed by cutting wells in the agar gel poured on a glass slide or in a petri dish.
- The antiserum consisting of antibody is placed in the central well and different antigens are added to the wells surrounding the center well.
- After an incubation period of 12-48 hours in a moist chamber, the lines of precipitin are formed at the sites of combination of antigen and antibody.

**Hour-4 Immunoelectrophoresis**

https://en.wikipedia.org/wiki/Immunoelectrophoresis
https://microbenotes.com/immunoelectrophoresisprinciple-procedure-results-and-applications-advantagesand-limitations/

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- It is a process of a combination of immuno-diffusion and electrophoresis.
- An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion.
- Antigens are placed into wells cut in a gel (without antibody) and electrophoresed. A trough is then cut in the gel into which antibodies are placed.
- The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.

- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953.




**Procedure of Immunoelectrophoresis**

1. Agarose gel is prepared on a glass slide put in a horizontal position.
2. Using the sample template, wells are borne on the application zone carefully.
3. The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
4. Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
5. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.
6. After electrophoresis completes, 20 µl of the corresponding antiserum is added to troughs in a moist chamber and incubated for 18- 20 hours at room temperature in a horizontal position.
7. The agarose gel is placed on a horizontal position and dried with blotter sheets.
8. The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
9. The gel is dried at a temperature less than 70°C and may be stained with protein staining solution for about 3 minutes followed by decolorizing the gel for 5 minutes in distaining solution baths.
10. The gel is dried and results evaluated.

**Results of Immunoelectrophoresis**

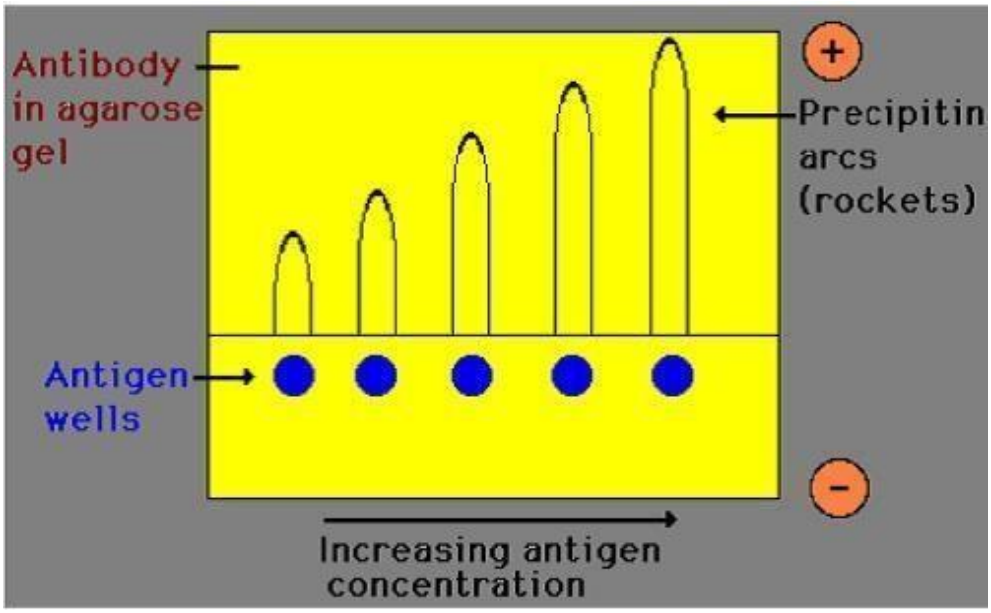
1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.
2. The absence of the formation of precipitate suggests no reaction.

3. Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

**Hour-5 Rocket immunoelectrophoresis:**

**https://www.biosciencenotes.com/rocket-immunoelectrophoresis/**

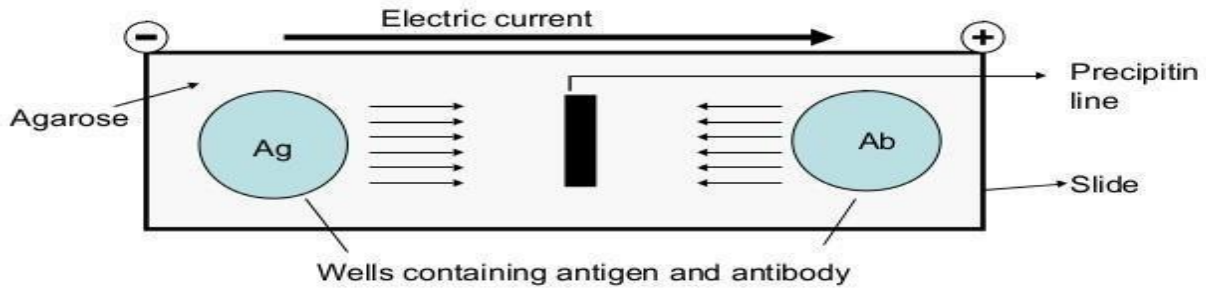
- Rocket immunoelectrophoresis (also referred to as electroimmunoassay) is a simple, quick, and reproducible method for determining the concentration of a specific protein in a protein mixture. ▪ The method, originally introduced by Laurell (1) involves a comparison of the sample of unknown concentration with a series of dilutions of a known concentration of the protein, and requires a monospecific antiserum against the protein under investigation.
- This quantitative one dimensional immunoelectrophoresis method involves a comparison of antigen sample ofunknown concentration with a series of dilutions of a known concentration of the antigen and requires a monospecific antibody against the antigen under investigation. ▪ In this method, antigen migrates from the well through agarose gel containing antiserum, forming rocket shaped precipitin peaks. The height of this peak is proportional to the concentration of the antigen loaded in the corresponding well.




**Hour-6 Counter Immuno Electrophoresis**

https://microbeonline.com/serologic-methods-counterimmunoelectrophoresis-cie/

Counterimmunoelectrophoresis (CIE) is a modification of the Ouchterlony method that speeds up the migration of an antigen and antibody by applying an electrical current.




Counterimmunoelectrophoresis

Principle of CIE Test

Most bacterial antigens are negatively charged in a slightly alkaline environment, whereas antibodies are neutral. When an electric field is applied in an electrophoresis apparatus filled with buffer (pH 8.4) and containing known antibodies and unknown antigens in the agarose well, the antibodies will migrate towards the negative end. In contrast, antigens will migrate towards the positive end.

As the antibody and antigen move toward each other in an electric field, they will soon meet in optimal proportion (a zone of equivalence is formed) at some points between the well, and visible precipitation occurs.

Counterimmunoelectrophoresis is used to detect the presence of bacterial (H. influenzae, N. meningitidis, S. pneumoniae) or fungal (Cryptococcus neoformans) capsular polysaccharide antigens in cerebrospinal fluid (CSF).

**Hour: 7 ELISA**

https://en.wikipedia.org/wiki/ELISA

ELISA is the basic assay technique, known as enzyme-linked immunosorbent assay (also referred to as EIA: Enzyme Immunoassay) that is carried out to detect and measure antibodies, hormones, peptides and proteins in the blood.

Antibodies are blood proteins produced in response to a specific antigen. It helps to examine the presence of antibodies in the body, in case of certain infectious diseases.

ELISA is a distinguished analysis compared to other antibody-assays as it yields quantitative results and separation of non-specific and specific interactions that take place through serial binding to solid surfaces, which is normally a polystyrene multiwell plate.

Types Of ELISA

ELISA tests can be classified into three types depending upon the different methods used for binding between antigen and antibodies, namely:

- **Indirect ELISA** – Antigen is coated to the microtiter well
- **Sandwich ELISA** – Antibody is coated on the microtiter well

- **Competitive ELISA** – Microtiter well which is antigen-coated is filled with the antigen-antibody mixture.

Indirect ELISA

- Indirect ELISA detects the presence of an antibody in a sample.
- The antigen is attached to the wells of the microtitre plate.
- A sample containing the antibodies is added to the antigen-coated wells for binding with the antigen.
- The free primary antibodies are washed away and the antigen-antibody complex is detected by adding a secondary antibody conjugated with an enzyme that can bind with the primary antibody.
- All the free secondary antibodies are washed away. A specific substrate is added which gives a coloured product.
- The absorbance of the coloured product is measured by spectrophotometry.

Sandwich ELISA

- Sandwich ELISA helps to detect the presence of antigen in a sample.
- The microtitre well is coated by the antibody.
- The sample containing the antigen is added to the well and washed to remove free antigens.
- Then an enzyme-linked secondary antibody, which binds to another epitope on the antigen is added. The well is washed to remove any free secondary antibodies.
- The enzyme-specific substrate is added to the plate to form a coloured product, which can be measured.

Competitive ELISA

- Competitive ELISA helps to detect antigen concentration in a sample.
- The microtitre wells are coated with the antigen.
- Antibodies are incubated in a solution having the antigen.
- The solution of the antigen-antibody complex is added to the microtitre wells. The well is then washed to remove any unbound antibodies.
- More the concentration of antigen in the sample, lesser the free antibodies available to interact with the antigen, which is coated in the well.
- The enzyme-linked secondary antibody is added to detect the number of primary antibodies present in the well.
- The concentration is then determined by spectrophotometry.

**Also Read**: **Antigens and Immunology**

Principle of ELISA

ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding. In order to increase the sensitivity and precision of the assay, the plate must be coated with antibodies with high affinity. ELISA can provide a useful measurement of antigen-antibody concentration.

ELISA Procedure

ELISA is one of the easiest blood tests that can be carried out. It is rapid, quick and requires a blood sample of the patient. The entire procedure of ELISA is mentioned below.

- An antibody is attached to a polystyrene plate which is a solid surface and is attracted or has an affinity towards bacteria, other antibodies and hormones.
- A microtiter coated with antigen is filled with this antigen-antibody mixture after which free antibodies are removed by washing.
- A second antibody specific to primary antibody is added which is usually conjugated with an enzyme.
- Free enzyme-linked secondary antibodies are removed by washing the plate.
- Finally, the substrate is added. The substrate is converted by the enzyme to form a coloured product, which can be measured by spectrophotometry.
- **Hour-8 Application of ELISA**
- https://microbiologynotes.com/elisa-principle-types-and-applications/

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody concentrations in a virus test.
3. Used in food industry when detecting potential food allergens.
4. Applied in disease outbreaks- tracking the spread of disease e.g. HIV, bird flu, common, colds, cholera, STD etc.

**Hour- 9 RIA**

https://microbenotes.com/radioimmunoassay-principle-uses-and-limitations/

**Radioimmunoassay is one of the sensitive immunoassay techniques which helps in the determination of antigens or antibodies in a sample with the use of radioisotopes**.

It is an in vitro type of antigen-antibody interaction.

When radioisotopes instead of enzymes are used as labels to be conjugated with antigens or antibodies, the technique of detection of the antigen-antibody complex is called radioimmunoassay (RIA). Radioimmunoassay (RIA) is an *in vitro* assay that measures the presence of an antigen with very high sensitivity. RIA was first described in 1960 for the measurement of endogenous plasma insulin by **Solomon Berson and Rosalyn Yalow** of the Veterans Administration Hospital in New York.

The classical RIA methods are based on the principle of competitive binding. In this method, an unlabeled antigen competes with a radiolabeled antigen for binding to an antibody with the appropriate specificity. Thus, when mixtures of radiolabeled and unlabeled antigen are incubated with the corresponding antibody, the amount of free (not bound to antibody) radiolabeled antigen is directly proportional to the quantity of unlabeled antigen in the mixture.

**Hour-10 Application of RIA**

https://en.wikipedia.org/wiki/Radioimmunoassay

- It was first used for the detection of peptide hormones.
- Detection of different viral antigens
- Detection of many hormones and drugs
- Detection of Hepatitis B surface antigens
- Detection of mycotoxins
- Detection of the early stage of cancer

**Hour-11 Fluorescent antibody technique**

https://microbenotes.com/immunofluorescence/

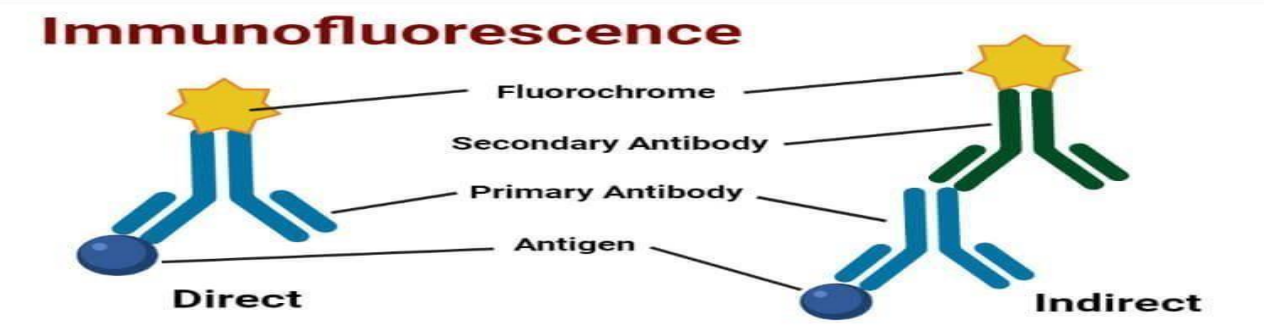
**Immunofluorescence is a type of assay performed on biological samples to detect specific antigens in any biological specimen or sample and vice-versa.** The specificity of antibodies to their antigen is the base for immunofluorescence.

It was described in 1942 and refined by Coons in 1950, which used a fluorescence microscope able to read the specific immunological reaction and cellular slide preparations.

It is an effective method for visualizing intracellular processes, structures, and conditions as well.

- In Vitro type of Ag-Ab Interaction.
- Detects surface antigens or antibodies.
- Fluorescent dyes are used for the visualization of Ag-Ab reactions.

The property of certain dyes absorbing light rays at one particular wavelength (ultraviolet light) and emitting them at a different wavelength (visible light) is known as **fluorescence.** In the immunofluorescence test, a fluorescent dye that illuminates in UV light is used to detect/show the specific combination of an antigen and antibody. The dye usually used is fluorescein isothiocyanate, which gives yellow-green fluorescence. Immunofluorescence tests are also termed fluorescent antibody tests **(FAT).**




**Hour-12 Purification of antiboby**

https://www.thermofisher.com/in/en/home/lifescience/antibodies/antibodies-learning-center/antibodiesresource-library/antibody-methods/introductionantibody-production-purification.html

Introduction to antibody purification

Antibody purification involves selective enrichment or specific isolation of antibodies from serum (polyclonal antibodies), ascites fluid, or cell culture supernatant of a hybridoma cell line (monoclonal antibodies). Purification methods range from very crude to highly specific and can be classified as follows:

Physicochemical fractionation—differential precipitation, size-exclusion or solid-phase binding of immunoglobulins based on size, charge, or other shared chemical characteristics of antibodies in typical samples. This isolates a subset of sample proteins that includes the immunoglobulins.

Class-specific affinity—solid-phase binding of particular antibody classes (e.g., IgG) by immobilized biological ligands (proteins, lectins, etc.) that have specific affinity to immunoglobulins. This purifies all antibodies of the target class without regard to antigen specificity.

Antigen-specific affinity—affinity purification of only those antibodies in a sample that bind to a particular antigen molecule through their specific antigen-binding domains. This purifies all antibodies that bind the antigen without regard to antibody class or isotype.

Antibodies that were developed as monoclonal antibody hybridoma cell lines and produced as ascites fluid or cell culture supernatant can be fully purified without using an antigen-specific affinity method (third type) because the target antibody is (for most practical purposes) the only immunoglobulin in the production sample. By contrast, for polyclonal antibodies (serum samples), antigen-specific affinity purification is required to prevent co-purification of nonspecific immunoglobulins. For example, generally only 2–5% of total IgG in mouse serum is specific for the antigen used to immunize the animal. The type(s) and degree of purification that are necessary to obtain usable antibody depend upon the intended application(s) for the antibody.

UNIT-IV

**Hour-1 Complement System**

The complement system, also known as complement cascade, is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism, promote inflammation, and attack the pathogen's cell membrane. It is part of the innate immune system, which is not adaptable and does not change during an individual's lifetime. The complement system can, however, be recruited and brought into action by antibodies generated by the adaptive immune system.

**Hour-2 Activation of complement system:**

The complement system activates through a triggered-enzyme cascade. In such a cascade, an active complement enzyme generated by cleavage of its zymogen precursor then cleaves its substrate, another complement zymogen, to its active enzymatic form.

**Hour-3 Classical Pathway**

The classical complement pathway is one of three pathways which activate the complement system, which is part of the immune system. The classical complement pathway is initiated by antigenantibody complexes with the antibody isotypes IgG and IgM.

**Hour-4 Alternative Pathway**

The alternative pathway is a type of cascade reaction of the complement system and is a component of the innate immune system, a natural defense against infections.

**Hour-5 Lectin Pathway**

Lectins are carbohydrate-binding proteins that are highly specific for sugar groups that are part of other molecules, so cause agglutination of particular cells or precipitation of glycoconjugates and polysaccharides. Lectins have a role in recognition at the cellular and molecular level and play

**Hour-6 Biological function of ‘c’ protein**

it activates complement, binds to Fc receptors and acts as an opsonin for various pathogens.

**Hour-7 Cytokinesis**

Cytokinesis (/ˌsaɪtoʊkɪˈniːsɪs/) is the part of the cell division process during which the cytoplasm of a single eukaryotic cell divides into two daughter cells. Cytoplasmic division begins during or after the late stages of nuclear division in mitosis and meiosis.

**Hour-8 Structure and Function of Cytokinesis**

Cytokines are a broad group of signalling proteins that are produced transiently, after cellular activation, and act as humoral regulators which modulate the functions of individual cells, and regulate processes taking place under normal, developmental and pathological conditions.

**Hour-9 Vaccines**

A vaccine is a biological preparation that provides active acquired immunity to a particular infectious or malignant disease.[1] The effectiveness of vaccines has been widely studied and verified.[2] A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as a threat, destroy it, and to further recognize and destroy any of the microorganisms associated with that agent that it may encounter in the future.

**Hour-10 Types of Vaccines:**

1) Attenuated
2) Inactivated
3) Subunit
4) Conjugate
5) DNA
6) Recombinant vaccine

**Hour-11 Production of Vaccines**

Vaccines are made from dead (inactivated) or modified (attenuated live) whole microbes, or from inactivated or recombinant parts of microbes that are responsible for disease (such as toxins or surface proteins). Although it can take a long time to produce vaccines, they are not difficult to produce.

**Hour -12 Application of Vaccine:**

Vaccines help protect against many diseases that used to be much more common. Examples include tetanus, diphtheria, mumps, measles, pertussis (whooping cough), meningitis, and polio. Many of these infections can cause serious or life-threatening illnesses and may lead to life-long health problems.

**E- Material Hour-1 Complement System**

https://www.physio-pedia.com/Complement_System#

The complement system helps or “complements” the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the innate immune system.

The complement system consists of a number of small proteins found in the blood, made by the liver. Normally they circulate as inactive precursors. When stimulated by a trigger, proteases split these small proteins to release active cytokines. This starts a series (a cascade) of further cleavages which release more cytokines. This amplifies the response. So, if the original stimulus was an invading bacterium, the cytokines disrupt the phospholipid bilayer cell membrane of the target, which kills it [1].[2][3]

Complements are soluble proteins and glycoproteins, more than 20 types of complements are present in serum, found circulating normally in human body in inactive forms.

Complements are activated only during inflammatory reactions. During the inflammation, more amount of complements reaches to the interstitial area of the infected tissue through dilated blood vessels, which are then activated by proteolytic cleavage

Image: **Membrane Attack Complex (MAC)** is the terminal complex of the complement system of the innate immune system. It forms pore-like holes in cell membranes, causing swelling that ultimately leads to cell death

**Hour-2 Activation of Complement System**

https://teachmephysiology.com/immune-system/innateimmune-system/complement-system/

**Activation of the Complement System**

There are three ways to activate the complement system, involving different molecules initially but converging to produce the same effector molecules. Each involves activation of enzymes that cleave their substrates to form a cascade, so that the complement response is amplified.

1. The **Classical** Pathway
2. The **Mannose-Binding Lectin** Pathway
3. The **Alternative** Pathway

All three pathways produce **C3 convertase**, an enzyme which triggers further effects downstream. The effects of C3 convertase

**Hour-3 Classical Pathway:**

https://en.wikipedia.org/wiki/Classical_complement_pathway

The **classical complement pathway** is one of three pathways which activate the complement system, which is part of the immune system. The classical complement pathway is initiated by antigen-antibody complexes with the antibody isotypes IgG and IgM.

Following activation, a series of proteins are recruited to generate C3 convertase (C4b2b, historically referred C4b2a), which cleaves the C3 protein. The C3b component of the cleaved C3 binds to C3 convertase (C4b2b) to generate C5 convertase (C4b2b3b), which cleaves the C5 protein. The cleaved products attract phagocytes to the site of infection and tags target cells for elimination by phagocytosis. In addition, the C5 convertase initiates the terminal phase of the complement system, leading to the assembly of the membrane attack complex (MAC). The membrane attack complex creates a pore on the target cell's membrane, inducing cell lysis and death.

The classical complement pathway can also be activated by apoptotic cells, necrotic cells, and acute phase proteins

**Hour-4 Alternative Pathway**

https://en.wikipedia.org/wiki/Alternative_complement_pathway

https://microbenotes.com/alternative-pathway-of-the-complement-system/

The components of this pathway include:

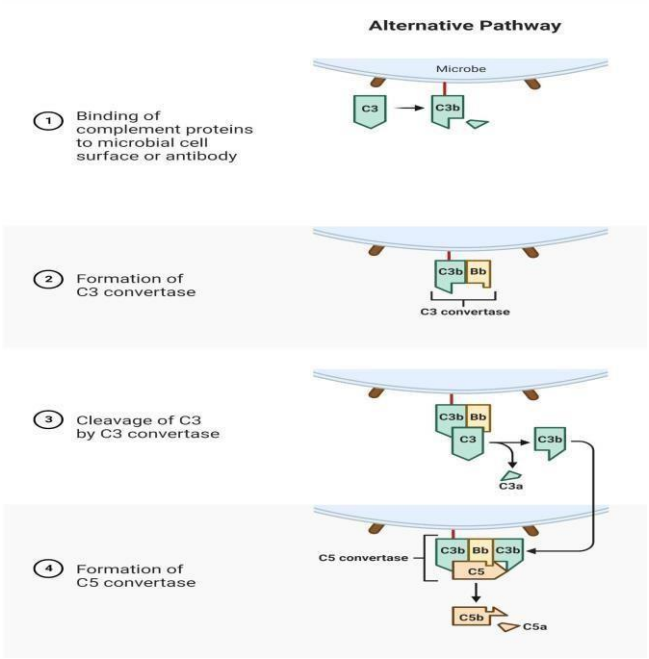
C3, C5-C9

Factor B

Factor D

Factor P (properdin)

1. Upon encountering foreign surfaces, the 3b protein binds directly to these surfaces and, unlike the classical pathway, does not require the formation of antigen-antibody complexes.
2. The hydrolyzed thioester domain of C3 undergoes structural change exposing the binding site for Factor B. $Mg^{2+}$ is the only ion required for functional activation of the alternative pathway that stabilizes the interaction between C3b and factor B.
3. This C3($H_2O$) bound Factor B is cleaved by serine protease Factor D, forming $C_3(H_2O)Bb$, C3 convertase. C3 convertase in the alternative pathway is unique as it is made up of subunits C3b and Bb. This complex can now activate more C3 via a powerful amplification cycle; therefore, this pathway is also called "the amplification loop".
4. The convertase complex, in turn, cleaves C3 to C3a and C3b. The 3a fragment is released into the fluid phase.
5. When a second C3b adds to it, the C5 convertase is synthesized.
6. The membrane attack complex is formed following the same pathway as the classical pathway of the complement system. C5 convertase cleaves C5 into subunits C5a and C5b. C5b recruits C6, C7, C8, and C9 forming MAC that induces cell lysis.




Alternative pathway of the complement system steps

**Hour-5 Lectin Pathway**

https://microbenotes.com/complement-activation-classical-alternative-and-lectin-pathways/

**The lectin pathway**

It is triggered by a plasma protein called mannose-binding lectin (MBL), which recognizes terminal mannose residues on microbial glycoproteins and glycolipids, similar to the mannose receptor on phagocyte membranes described earlier. MBL is a member of the collectin family (discussed later) with a hexameric structure similar to the C1q component of the complement system. After MBL binds to microbes, two zymogens called MASP1 (mannose-associated serine protease 1, or mannan-binding lectinassociated serine protease) and MASP2, with similar functions to C1r and C1s, associate with MBL and initiate downstream proteolytic steps identical to the classical pathway.

The central event in complement activation is proteolysis of the complement protein C3 to generate biologically active products and the subsequent covalent attachment of a product of C3, called C3b, to microbial cell surfaces or to antibody bound to antigen.

Complement activation depends on the generation of two proteolytic complexes: the **C3 convertase,** which cleaves C3 into two proteolytic fragments called C3a and C3b; and the **C5 convertase**, which cleaves C5 into C5a and C5b.

**Hour-6 Biological Function of 'C' protein**

**https://teachmephysiology.com/immune-system/innate-immune-system/complement-system/** •

**Opsonisation**

- **Lysis** of pathogens
- **Chemotaxis**
- **Inflammation**

**Opsonisation**

C3 convertase is a product of all the pathways triggering the complement cascade and is responsible for converting factor C3 into C3a and C3b. **C3b** binds to antigens on the pathogen, which stimulates neutrophils and macrophages to phagocytose pathogens – this is called opsonisation.

**Lysis of Pathogens**

Lysis of pathogens is facilitated by the formation of the **membrane attack complex** (MAC). C3 convertase is vital to the production of the MAC because it generates C3a and C3b. C3b combines with other factors to produce C5 convertase, an enzyme which converts factor C5 to C5a and C5b.

C5b combines with several factors to produce the MAC. The MAC ruptures the bacterial cell membrane, allowing fluid to enter the bacteria and causing cell **lysis.** However, because they possess a cell wall, gram positive bacteria and fungi do not swell and hence cannot be lysed by the complement system.

**Chemotaxis**

The production of C5a by **C5 convertase** attracts neutrophils and macrophages to the site of infection and causes extravasation of leucocytes from capillaries to tissues. C3a is another complement component that acts as a chemotaxin.

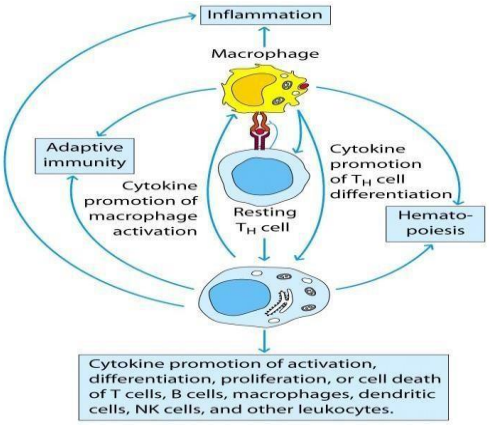
**Inflammation**

C3a, C4a and C5a are the complement components responsible for causing inflammation. They bind to mast cells and basophils to cause degranulation. The histamine and serotonin released increase vascular permeability. C3a, C4a and C5a also promote synthesis of pro-inflammatory **cytokines**

**Hour-7Cytokine**

https://www.biosciencenotes.com/cytokines/#:~:text=Cytokines%20are%20those%20molecules%20that,soluble%20molecules%20or%20membrane%2Dbound.

- Cytokines are those molecules that communicate among the cells of the immune system.
- They are low molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli.
- They are either soluble molecules or membrane-bound.
- Mostly different immune cells are responsible for secreting cytokines.
- The activity of cytokines was first recognized in the mid-1960's when the supernatant derived from the in-vitro cultures of lymphocytes were found to contain soluble factors; usually protein or glycoprotein, that could regulate proliferation, differentiation, and maturation of immune system cells.
- Cytokines transmit signal from cell to cell in an organism.
- The interaction of cytokines with its receptor on target cells can cause changes in the expression of adhesion molecules and chemokines receptor on the target membrane, thus allowing it to move from one location to another.
- Cytokines can also signal an immune system to increase or decreases the activity of particular, enzymes or to change its transcriptional program thereby altering and enhancing its effector functions.

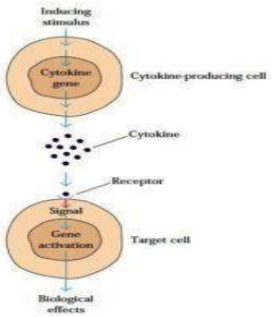



- Cytokines are divided into 6 families:

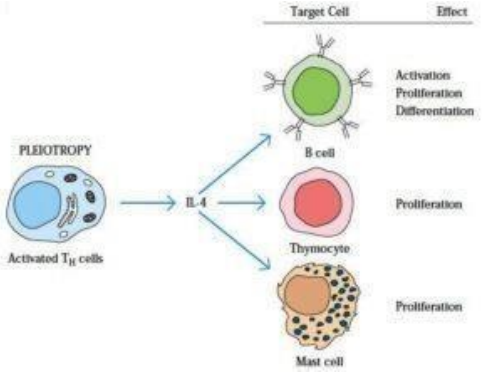
1. Interleukin 1 family (IL-1)
2. Hematopoietin family (Class 1 cytokine)
3. Interferon (Class 2 cytokines)
4. The tumor necrosis factor (TNF) family
5. The interleukin 17 family (IL-17)
6. Chemokine family

**Hour-8 Structure and Function of Cytokine**

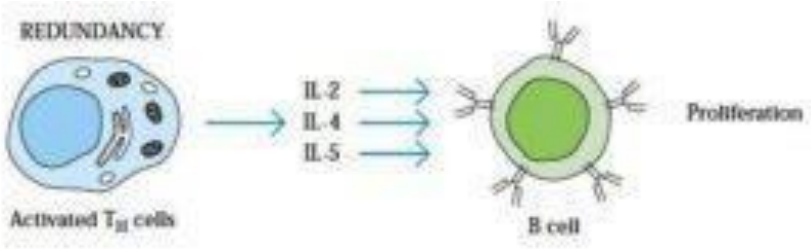
https://microbiologynotes.org/cytokines-introductionproperties-and-functions/



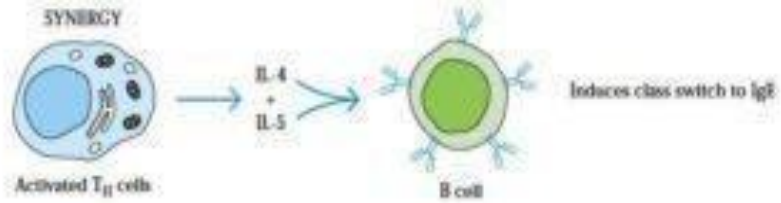

**Pleiotrophy**: An individual cytokine has different effects on distinct target cells, exhibits **pleiotropic action.**



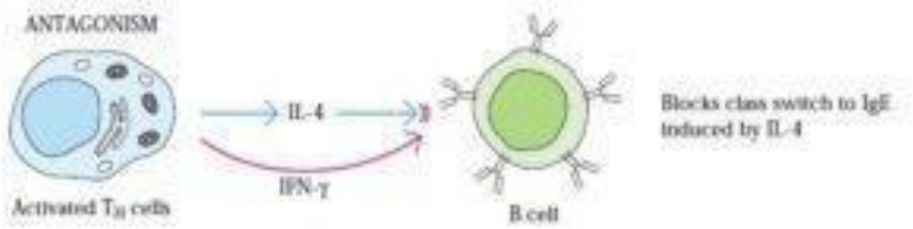

**Redundancy**: Similar biological activity can be mediated by one or more cytokines



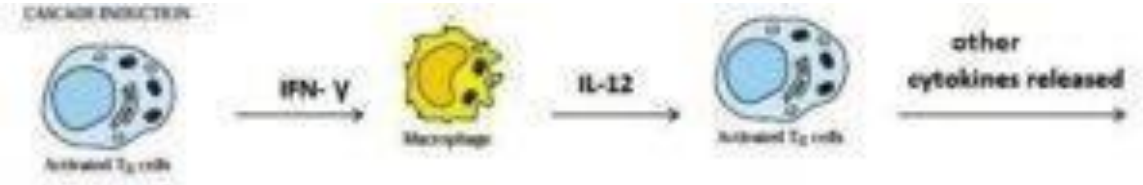

**Synergy**: Combined effect on cellular function by two cytokines




**Antagonism:** One cytokine blocks the effect of the other cytokine




**Cascade Induction**: When individual cytokine action on target cell promotes the production of other cytokines, which may induce different target cells to produce the other cytokine.



**Hour-9 vaccines:** https://www.vedantu.com/biology/vaccine

A vaccine is a substance that is used for the production of antidotes in the body and provides immunity against one or a few diseases. In biological terms, a vaccine is defined as a biological and formulated preparation to provide acquired immunity for a particular disease.

Generally, a vaccine is an agent which contains a weakened or killed form of the disease-causing agent, its surface, or its toxins. When this solution is introduced to the human body, the immune system is able to identify the threat and destroy it. More than this, the human body will recognize the threat and can initiate an appropriate response in the future also.

**Definition**

The process of implementing the vaccine is called vaccination. It is responsible for the clearance of many diseases, especially infectious diseases like smallpox and chickenpox. The word "vaccine" is derived from the Latin word "vaccines" which means "from the cows".

**Invention of Vaccine**

The practice of immunization of the body dates back hundreds of years, but the first official vaccination was developed by Edward Jenner who is considered the founder of vaccinology. In 1796, he injected a 13 yearold-boy with cowpox(vaccinia virus) and established immunity to smallpox. In 1798, the very first smallpox was developed. During the 18th and 19th centuries, systematic implementation of mass smallpox immunization culminated in its global establishment in 1979.

**Hour-10 Types of vaccines**

https://www.vedantu.com/biology/vaccine

**Types of Vaccines**

There are many initiations to vaccine development, but vaccines can be mainly classified by how the antigen, active component, that produces a specific immune response against the disease-causing organism, are prepared.

**Classification of Vaccines**

**A. Live Attenuated Vaccines:**

Attenuated vaccines are developed in many several ways. The common methods include passing the disease-causing virus through a series of cell cultures or animal embryos. When the vaccine virus is implemented in a human, it will be unable to replicate enough to cause illness, but still promotes an immune response that can protect against future infection.

**B. Inactivated Vaccine:**

Vaccines of this category are developed by inactivating a pathogen, typically using chemicals or even heat such as formaldehyde or formalin. This destroys the pathogen's ability to replicate but keeps it intact so that the immune still remembers it.

**C. Toxoid Vaccine:**

There are some bacterial diseases that are not directly caused by a bacteria itself, but by producing toxins by the bacterium. For this type, immunization of pathogens can be developed by inactivating the toxin that causes disease symptoms. As the viruses or organisms used to kill or inactivate vaccines, this can be done through treatment with a chemical such as formalin or by heat.

**D. Subunit Vaccine:**

Subunit vaccines are only used as part of a target pathogen to promote a response from the immune system. This can be done by isolating a specific protein from a pathogen and presenting it as an antigen on its own.

**E. Conjugate Vaccine:**

Conjugate vaccines are somehow similar to recombinant vaccines, they are made up of a combination of two different components. Conjugate vaccines, however, are made up of using the pieces from the coat of bacteria. These coats are chemically linked to a carrier immune protein, and this is how a combinational vaccine is used.

**F. Valence Vaccine:**

Vaccines may be monovalent. The monovalent vaccine is designed to be immune against a single microorganism or single antigen. A multivalent or polyvalent vaccine is made to immunize against two or more viruses of the same microorganism.

**G. Heterotypic Vaccine:**

Heterologous vaccines are also called "jennerian vaccines". These vaccines are pathogens of different animals that either do not cause disease or cause disease or cause mild disease in the organism being treated.

**H. mRNA Vaccine:**

An mRNA Vaccine (or RNA Vaccine) is a different type of vaccine which is a combination of nucleic acid RNA, packaged within a vector such as lipid nanoparticles.

**Hour-11 Production of vaccines**

https://en.wikipedia.org/wiki/Vaccine https://www.vedantu.com/question-answer/give-application-of-a-vaccine-class-12-biologycbse5fafea738824db450e94f561

Vaccination is based on the memory of the immune system. Vaccines contain attenuated disease-causing organisms or toxins that initiate an immune response in the body. The first step in vaccine production is to prepare the antigen. Viruses are grown in the primary or cultured cells, bacteria are grown in the bioreactors. With the advent of biotechnology, recombinant antigenic protein is produced in bacteria or yeast. Then antigen is isolated and inactivated, the recombinant protein is processed and ultrafiltrated. Then vaccine is prepared by adding adjuvant, preservatives and stabilisers. Adjuvants enhance the immune response and stabilisers increase the shelf life. The final step in vaccine production is packaging. Every vaccine has to be

licensed by FDA before being brought into use. A vaccine needs to go through extensive tests and clinical trials to confirm its safety before approval by the FDA.

**Hour-12 Application of Vaccines**

https://www.vedantu.com/question-answer/give-application-of-a-vaccine-class-12-biologycbse5fafea738824db450e94f561

- It is used to induce long term humoral as well as cell-mediated immune response against diseasecausing pathogens.
- Vaccines help in developing immunity against specific diseases.
- It initiates a primary immune response, generating memory cells without making a person ill. Later, if the same or very similar pathogens attack, a specific memory cell already exists. They recognize the antigen and evoke secondary immune response producing large numbers of antibodies that quickly overpower the invaders.
- The immune system is strongest in adulthood that means infants; children and elderly are particularly susceptible to a dangerous infection. Vaccines strengthen their immune system and bypass this risk. - The use of vaccines has been effective in developing resistance of infection of microorganisms that cause cholera, diphtheria, measles, mumps, whooping cough, rabies, smallpox, tetanus, typhoid, yellow fever and poliomyelitis.
- Vaccines can be a key tool in managing threat or pandemic situations such as Covid-19 caused by a coronavirus.

| |
|---|
| Hypersensitivity reaction |
| Types of HS |
| Type I HS |
| Type II HS |
| Type III HS |
| Type IV HS |
| MHC |
| MHC genes |
| Class I MHC |
| Class II MHC |
| HLA typing |
| Continuation of HLA typing |

**UNIT V**

**Hour 1** Hypersensitivity reaction

**Hypersensitivity** (also called **hypersensitivity reaction** or **intolerance**) refers to undesirable reactions produced by the normal immune system, including allergies and autoimmunity. They are usually referred to as an over-reaction of the immune system and these reactions may be damaging and uncomfortable

**Hour 2** Types of Hypersensitivity reaction

Type I: IgE mediated immediate reaction

Type II: Antibody-mediated reaction (IgG or IgM antibodies)

Type III: Immune complex-mediated reaction

Type IV: Cytotoxic, cell-mediated, delayed hypersensitivity reaction

**Hour 3** Type I Hypersensitivity reaction

Type I hypersensitivity occurs as a result of exposure to an antigen. The response to the antigen occurs in two stages: the sensitization and the effect stage. In the "sensitization" stage, the host experiences an asymptomatic contact with the antigen. Subsequently, in the "effect" period, the pre-sensitized host is reintroduced to the antigen, which then leads to a type I anaphylactic or atopic immune response

**Hour 4** Type II Hypersensitivity reaction

Type II hypersensitivity reaction refers to an antibody-mediated immune reaction in which antibodies (IgG or IgM) are directed against cellular or extracellular matrix antigens with the resultant cellular destruction, functional loss, or damage to tissues.

**Hour 5** Type III Hypersensitivity reaction

In type III hypersensitivity reaction, an abnormal immune response is mediated by the formation of antigenantibody aggregates called "immune complexes". They can precipitate in various tissues such as skin, joints, vessels, or glomeruli, and trigger the classical complement pathway. Complement activation leads to the recruitment of inflammatory cells (monocytes and neutrophils) that release lysosomal enzymes and free radicals at the site of immune complexes, causing tissue damage

**Hour 6** Type IV Hypersensitivity reaction

Type IV hypersensitivity reactions are, to some extent, normal physiological events that help fight infections, and dysfunction in this system can predispose to multiple opportunistic infections. Adverse events can also occur due to these reactions when an undesirable interaction between the immune system and an allergen happens

**Hour 7** MHC **major histocompatibility complex (MHC)**, group of genes that code for proteins found on the surfaces of cells that help the immune system recognize foreign substances. MHC proteins are found in all higher vertebrates. In human beings the complex is also called the human leukocyte antigen (HLA) system.

**Hour 8** MHC genes

In humans these molecules are encoded by several genes all clustered in the same region on chromosome 6. Each gene has an unusually large number of alleles (alternate forms of a gene that produce alternate forms of the protein). As a result, it is very rare for two individuals to have the same set of MHC molecules, which are collectively called a tissue type. The MHC also contains a variety of genes that code for other proteins—such as complement proteins, cytokines (chemical messengers), and enzymes—that are called class III MHC molecules.

**Hour 9 Class I** MHC

The structure of Class I MHC molecule consists of two polypeptide chains α and β. These two chains are connected together by non-covalent bonds. The α chain is characterized as an internal membrane glycoprotein with a molecular weight of 45000 Da (in humans). B chain, on the other hand, is an extracellular microglobulin with a molecular mass of 12kDa

**Hour 10 Class II** MHC

Class II MHC molecules are heterodimers and characterized by two non-covalently connected polypeptide chains. The chains are termed a heavy chain (α, 30kDa) and light chain (β, 26kDa).

**Hour 11 &12** HLA typing

Human leukocyte antigen (HLA) typing is used to match patients and donors for bone marrow or cord blood transplants. HLA are proteins -- or markers -- found on most cells in your body. Your immune system uses these markers to recognize which cells belong in your body and which do not.

**UNIT V E-materials**

**Hour-1 Hypersensitivity reaction**

https://www.biologyonline.com/dictionary/hypersensitivity

**Hypersensitivity** is the *exaggerated* immune response to protect the human from foreign bodies known as antigens. When the antigen is detected by the immune system, a *hyperimmune* response starts and the hypersensitivity reaction starts. This reaction is not always desirable as it may harm humans. Hypersensitivity reactions may lead to various consequences ranging from mild symptoms to severe shock causing death. Antigens or causative agents may be either small particles such as pollen grains or large particles such as drugs including antibiotics. Antigens are detected by T cells or antibodies by recognizing epitopes on the surface of the antigen. Some antigens may have the same epitope, so antibodies are able to detect more than one antigen with the same epitope and interact with them. This phenomenon is known as **cross-reactivity**.

**Hour 2 Types of Hypersensitivity reaction**

https://www.biologyonline.com/dictionary/hypersensitivity

Gell and coombs are British scientists who published a book in 1963 describing the classification of hypersensitivity disorder. Gell and coombs classified the reaction into 4 types of hypersensitivity according to the difference in the latency; the following are types of hypersensitivity reactions (or types of immune responses):

- **Type 1 hypersensitivity**: it is an immediate type as physiological symptoms appear within seconds to minutes.
- **Type 2 hypersensitivity**: it is called cytotoxic hypersensitivity. Its symptoms appear within minutes to hours.
- **Type 3 hypersensitivity**: this type is an immune complex-mediated type where symptoms appear within several hours.
- **Type 4 hypersensitivity**: it is a delayed allergic reaction. As it occurs after hours to days for symptoms to appear which is a long time for response.

**Hour 3** Type I Hypersensitivity reaction

https://www.biologyonline.com/dictionary/hypersensitivity

**Type 1 hypersensitivity** occurs within seconds to minutes. Therefore, it is called *immediate hypersensitivity*. Generally, this type is not harmful, however, the antigen in this reaction may be either harmless as pollen grain, drugs, and food or harmful antigen as venoms. Symptoms of the reaction may affect any part of the body such as the following hypersensitivity examples:

- *Rhinitis*: allergy in the nose.
- *Conjunctivitis:* it is an ocular allergy.
- *Dermatological allergy*: hypersensitive skin suffers from dermal hypersensitivity reactions like eczema.

Etiology

The main antibody exaggerated by the immune response during this reaction is called immunoglobulin E (IgE) which attacks soluble antigens. The interaction between IgE and the antigen releases histamine and inflammatory mediators.

There are two stages of this immune response after exposure:

- ***First stage***, called the *sensitization stage*, is where the host contacts the antigen for the first time. This contact is asymptomatic as the host recognizes the antigen for the first time.
- ***Second stage*** is the *late phase reaction* at which the sensitized host is exposed to the antigen again leading to the development of type I hypersensitivity reaction.

Types of antigens involved

What causes hypersensitivity? There are various types of antigens that can exaggerate the immune response such as:

- **Food**: some foods may cause allergies like Nuts, Soy, and wheat.
- **Animal source**: Bee bites, cats, and rat dender
- **Environmental source**: Dust, pollen, and molds.
- **Drug allergy**: antibiotics are the main drugs that induce allergic reactions. However, other such as propofol and isoflurane anesthesia drugs may induce hypersensitivity as well.

**Hour 4** Type II Hypersensitivity reaction

https://www.biologyonline.com/dictionary/hypersensitivity

- This reaction occurs within about 24 hours. It is known as *cytotoxic type* as the main antibodies directed to the cellular antigens are IgE and IgM. This interaction damages the cell, affecting its function, and damaging tissues. This interaction takes place by three mechanisms:
- Antibodies can alter the activity of the cell by binding to its receptors
- Antibodies can also activate the complement pathway
- Induce their cytotoxic effect on the cell leading to its death

*What is immune tolerance?* **Immune tolerance** is a phenomenon in which the immune system can detect antigens of the body and recognize them as self-antigens. self-antigens are not attacked by the immune system, So the immune system does not produce antibodies against them.

In *type 2 hypersensitivity*, self-antigens on the cell surface may be changed and modified by their contact with the external antigen. Thereafter, the self-antigen turns into an antigenic epitope that can be detected and attacked by the immune system. Some medications are the most common cause of type 2 hypersensitivity such as *penicillin*, *methyldopa*, and *cephalosporins*. These medications break down the immune tolerance of the cell as the drug binds to the cells changing self-antigens into epitopes.

**Hour 5** Type III Hypersensitivity reaction

https://www.biologyonline.com/dictionary/hypersensitivity

Type 3 hypersensitivity is an immune complex-mediated reaction. In this reaction, an antibody-antigen complex is formed. This aggregation can be precipitated in different tissues leading to the activation of the complement pathway. When the complement pathway is activated, inflammatory cells such as monocytes and neutrophils are recruited to the complex site. Lysosomal enzymes that damage cells are released by monocytes and neutrophils. This damage takes place in three stages which are:

- **Immune complex formation**: at this stage, the antigen is detected by the antibody and interacts with it forming an immune complex.
- **Immune complex deposition**: the antigen-antibody complex precipitates in joints and glomeruli of the kidney. This occurs when the ratio of antigens is higher than that of antibodies.
- **Inflammatory reaction**: the classical pathway begins when the complex is precipitated which leads to the release of C3a, C5a, macrophages, and neutrophils. The release of these mediators damages tissue. hypersensitivity symptoms differ according to the site of inflammation; it is presented as arthritis when the inflammation is at joints and glomerulonephritis when glomeruli are inflamed.

*Serum sickness bee sting* is an allergic reaction but less dangerous and slower than anaphylaxis. when a bee stings a human, it releases its toxins in the blood and causes serum sickness which can be observed within a few days to weeks

*Serum sickness*, *rheumatoid arthritis*, and *systemic lupus erythematosus* are the most common diseases as a result of type 3 hypersensitivity

**Hour 6** Type IV Hypersensitivity reaction

https://www.biologyonline.com/dictionary/hypersensitivity

Type 4 hypersensitivity is considered as delayed anaphylaxis in which the T-cells play the main role.

The reaction happens as follows:

1. The body is exposed to the antigen.
2. Leukocytes are attracted to the antigen.
3. Macrophages engulf the antigen.
4. Monocytes are presented to T-cells.
5. T-cells begin to be activated and sensitized.
6. T-cells begin to secrete cytokines and chemokines.
7. Tissue damage occurs.

There are four subtypes of type 4 hypersensitivity reaction; *IVa*, *IVb*, *IVc*, and *IVd*. These subtypes are dependent on the type of T-cells included in the reaction. Delayed reaction is important for the protection of the body against pathogens, such as mycobacteria and certain fungi, and tumors.

**Hour 7** MHC

https://microbenotes.com/mhc-molecules/#class-i-mhc-molecule

- The Major Histocompatibility complex is a genetic locus that encodes the glycoprotein molecules (transplantation antigens) which are responsible for tissue rejection of grafts between genetically unidentical individuals.
- It is also the molecule that binds the peptide antigens processed by Antigen-presenting Cells and presents them to T-cells, hence they are responsible for antigen recognition by the T-cell receptors.
- Unlike the B-cell receptors that directly interact with the antigens, the T-cell receptors have an intertwined relationship with the MHC molecule, in that T-cell receptors can only receive and bind processed antigens in form of peptides that are bound to the MHC molecule, and therefore, T-cell receptors are specific for MHC molecules. In humans, the Major Histocompatibility complex is known as Human Leukocyte Antigen (HLA). There are three common MHC molecules i.e class I, class II, and class III MHC proteins.
- The genes of the MHC exhibit genetic variability; and the MHC has several genes for each class hence it is polygenic.
- The MHC is also polymorphic, meaning a large number of alleles exist in the population for each of the genes.

- Therefore, a large number of alleles exist in the population for each of the genes. Each individual inherits a restricted set of alleles from his or her parent. Sets of MHC genes tend to be inherited as a block or haplotype. There are relatively infrequent cross-over events at this locus.
- The structure of the MHC class I have two domains that are distant from each other, made up of two parallel α helices on top of a platform that is created by a β-pleated sheet. The general structure looks like a cleft whose sides are formed by the α helices and the floor is β-sheet.
- Generally, the MHC molecules have a broad specificity for peptide antigens and many different peptides can be presented by any given MHC allele binding a single peptide at a time.
- The α helices forming the binding clefts are the site of the amino acid residues that are polymorphic (varying allelic forms) in MHC proteins, meaning that different alleles can bind and present different peptide antigens. For all these reasons, MHC polymorphism has a major effect on antigen recognition.
- The function of T-cells on interaction with the MHC molecules reveals that the peptide antigens associated with class I MHC molecules are recognized by CD8+ cytotoxic T-lymphocytes (Tc cells) and MHC class-II associated with peptide antigens that are recognized by CD4+ Helper Tcells (Th cells).
- The MHC in humans is known as **human leukocyte antigens (HLA) complex**.

**Hour 8** MHC genes

https://microbenotes.com/mhc-molecules/#class-i-mhc-molecule

**Gene Products of HLA complex**

1. **Class I MHC genes** encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of endogenous peptide antigens to $CD8^+$ T cells.
2. **Class II MHC genes** encode glycoproteins expressed predominantly on APCs (macrophages, dendritic cells, and B cells), where they primarily present exogenous antigenic peptides to $CD4^+$ T cells.
3. **Class III MHC genes** encode several different proteins, some with immune functions, including components of the complement system and molecules involved in inflammation.

**Hour 9 Class I** MHC

https://microbenotes.com/mhc-molecules/#class-i-mhc-molecule

**Class I MHC Molecule**

- The structure of Class I MHC molecule consists of two polypeptide chains α and β. These two chains are connected together by non-covalent bonds. The α chain is characterized as an internal membrane glycoprotein with a molecular weight of 45000 Da (in humans). B chain, on the other hand, is an extracellular microglobulin with a molecular mass of 12kDa.
- The α chain is made up of approximately 350 amino acids and also divided into three globular domains $\alpha_1$, $\alpha_2$ and $\alpha_3$. Each of these domains contains roughly 90 amino acids. The N terminal of α chain is the place of $\alpha_1$ domain, while $\alpha_2$ and $\alpha_3$ are present after $\alpha_1$ The $\alpha_2$ domain is characterized by the formation of a loop of 63 amino acids; the loop is formed due to intrachain disulfide bond. $\alpha_3$ also contains a disulfide bond enclosing 86 amino acids. The $\alpha_1$ and $\alpha_2$ domains interact to form peptide-binding units of class I MHC molecule.
- Moreover, α chain also consists of a stretch of 26 hydrophobic amino acids which holds the α chain on the plasma membrane. This transmembrane segment is present as a form of α helix at the hydrophobic region of the plasma membrane. An intracellular domain or the carboxyl-terminal of α chain is located inside the cell and it contains around 30-40 amino acids.
- In humans the β chain is non-polymorphic and it is dimorphic in mice. $\alpha_3$ and β chain are structurally similar to the immunoglobulin C domain and also characterized as a disulfide loop. A peptide binding platform is formed by β plated sheets of $\alpha_1$ and $\alpha_2$
- $T_{cyt}$ Cell (cytotoxic T cell) has specificity towards cells containing peptides associated with Class I MHC due to the presence of CD8 antigen on the surface of Tcyt Cell. CD8 antigen has an affinity towards the $\alpha_3$ domain of Class I MHC molecules.

**Hour 10 Class II** MHC

https://microbenotes.com/mhc-molecules/#class-i-mhc-molecule

**Class II MHC Molecule**

- Class II MHC molecules are heterodimers and characterized by two non-covalently connected polypeptide chains. The chains are termed a heavy chain (α, 30kDa) and light chain (β, 26kDa).

Similar to class I MHC molecules, class II MHC molecules are also characterized by an extracellular amino-terminal domain, a transmembrane domain, and an intracellular carboxyterminal tail.

- The class II MHC molecules are expressed on the surface of the antigen-presenting cells such as B cells, dendritic cells, and macrophages.
- The α chain is divided into two domains $\alpha_1$ and $\alpha_2$, while the β chain is also divided into two groups $\beta_1$ and $\beta_2$. The $\beta_2$ domain is responsible for the binding of T cell co-receptor CD4. The $\alpha_1$ and $\beta_1$ domains, on the other hand, are involved in the formation of the antigen-binding sites. Peptides containing 13-20 amino acids can bind at the antigen-binding site of class II MHC.
- The presence of disulfide bonds in $\alpha_2$, $\beta_1$, and $\beta_2$ domains is also an important structural feature of the class II MHC molecules.
- **Hour 11&12 HLA TYPING**

https://www.fujirebio.com/en/insights/tissue-typing-and-transplantation/hla-human-leukocyteantigensand-tissue-typing

HLA stands for human leukocyte antigens.

These are normal glycoproteins expressed to varying degrees on the surface of almost every cell in the body.

The molecules form part of the system of immune recognition, essentially the ability to distinguish "self" from "non-self".

Their role is to present peptides to cytotoxic T cells.

Specific destruction of non-self cells is induced when peptides derived from non-self or foreign antigens are presented.

The highest level of expression of these molecules is on white blood cells (leukocytes).

**Importance of HLA**

**Tissue typing: Target indication and market potential** There are approximately 45 000 organ transplants performed in the United States and Europe each year and more than 160 000 individuals with end-stage organ failure are waiting for organ transplants.

The demand for transplant organs far surpasses the number of organs donated each year.

National and international organizations act as mediators between donors and recipients and play a key role in the acquisition and distribution of donor organs/bone marrow for transplant operations.

Data of all potential organ recipients, including blood group and tissue characteristics (HLA groups) are stored in a centralized computer database. Subsequently, the patient is put on a waiting list.

As soon as a donor organ becomes available, the regional tissue-typing laboratory establishes the donor's blood and tissue characteristics.

All relevant information about the donor is then passed on to the registration center where the patient is selected.

The HLA match is an important factor in this selection.

For bone marrow transplantation, the blood group and HLA type of all donors are also stored in a central database.

Currently, more than 8.8 million volunteer donors are registered worldwide

**NOVEMBER 2023** **502351/ SC25C**

Time : Three hours Maxumum : 75 marks

PART A — (10 x 2 = 20marks)

Answer any TEN questions, each in 30 words.

1. What is Immunology?
2. Write the contribution of Edward Jenner.
3. Define Haematopoiesis.
4. What is epitope?
5. Comment on polyclonal antibody
6. What is agglutination?
7. Write short notes on immune electrophoresis
8. What is complement?
9. Define vaccine.
10. What is allergen?
11. Write short notes on MHC.
12. What is humoral Immunity?

**PART B — (5 × 5 = 25 marks)**

Answer any FIVE questions each in 200 words.

13.Briefly explain the structure and functicm of lymph nodes .

14.Describe the development T lymphocyte.

15.Write the general characteristics of antigen.

16.Explain the principle and application of hybridoma techniques.

17.Write the principle and applications of fluorescent antibody techniques.

18.What is cytokines? Write the function different types of cytokine.

19. Briefly explain different types of vaccine.

PART C — (3 x 10=30 marks)

Answer any THREE questions each in words.

20. What is immunity? Explain about the innate immunity.

21. Describe the structure and function of lgG.

22. Explain about the principle and application of ELISA .

**23.** Explain about the classical complement activation pathway

24. Describe the mechanism of antibody mediated cytotoxicity hypersensitivity reaction.

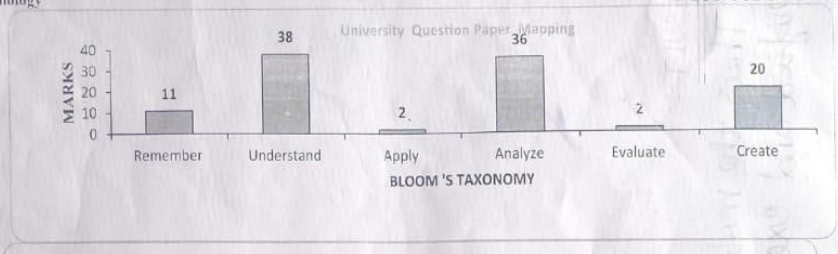
50285/SC25C

JAYA COLLEGE OF ARTS AND SCIENCE , THIRUNINRAVUR 602024
DEPARTMENT OF ~~COMMERCE~~ BIOTECHNOLOGY
UNIVERSITY QUESTION PAPER MAPPING

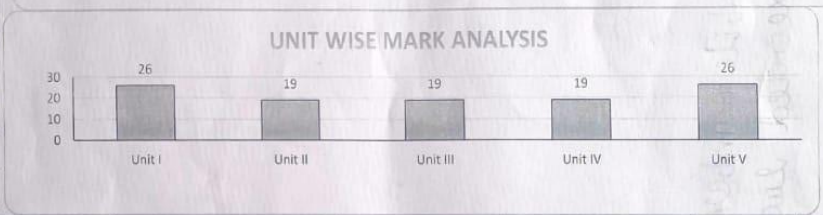
DATE: 23.11.2022
SUBJECT NAME : Immunology

CLASS: III B.Sc
SUB CODE SC25C

| Particulars | Marks |
|---|---|
| Remember | 11 |
| Understand | 38 |
| Apply | 2 |
| Analyze | 36 |
| Evaluate | 2 |
| Create | 20 |




| Particulars | Marks |
|---|---|
| Unit I | 26 |
| Unit II | 19 |
| Unit III | 19 |
| Unit IV | 19 |
| Unit V | 26 |




| Unit | REMEMBER | | | UNDERSTAND | | | APPLY | | | ANALYZE | | | EVALUATE | | | CREATE | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2M | 5M | 10M | 2M | 5M | 10M | 2M | 5M | 10M | 2M | 5M | 10M | 2M | 5M | 10M | 2M | 5M | 10M | |
| I | 2(1) | | | 2(2) 2(3) | 5(13) | | | | | | 5(19) | 10(20) | | | | | | | 26(6) |
| II | 2(4) | 5(14) | | 2(5) | | | | | | | | | | | | | | 10(21) | 19(4) |
| III | | | | | | | | | | 2(6) 2(7) | 5(15) | 10(22) | | | | | | | 19(4) |
| IV | 2(9) | | | 2(8) | 5(16) | | | | | | | | | | | | | 10(23) | 19(4) |
| V | | | | | 5(17) 5(18) | 10(24) | 2(11) | | | 2(11) | | | 2(12) | | | | | | 26(6) |
| Total | 11(4) | | | 38(9) | | | 2(1) | | | 36(7) | | | 2(1) | | | | | | 109 |

Note : Past five Years university paper were revised , Slow learners class conducted , Pre model exam conducted University question paper blue print given to students to score marks in exam

( ) Inside the bracket Question number ( ) Outside the bracket Mark

Signature of the HOD

Signature of the Principal
PRINCIPAL
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