

**Diagnostic Veterinary
Immunology/VeLT 3113**

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Unit 1

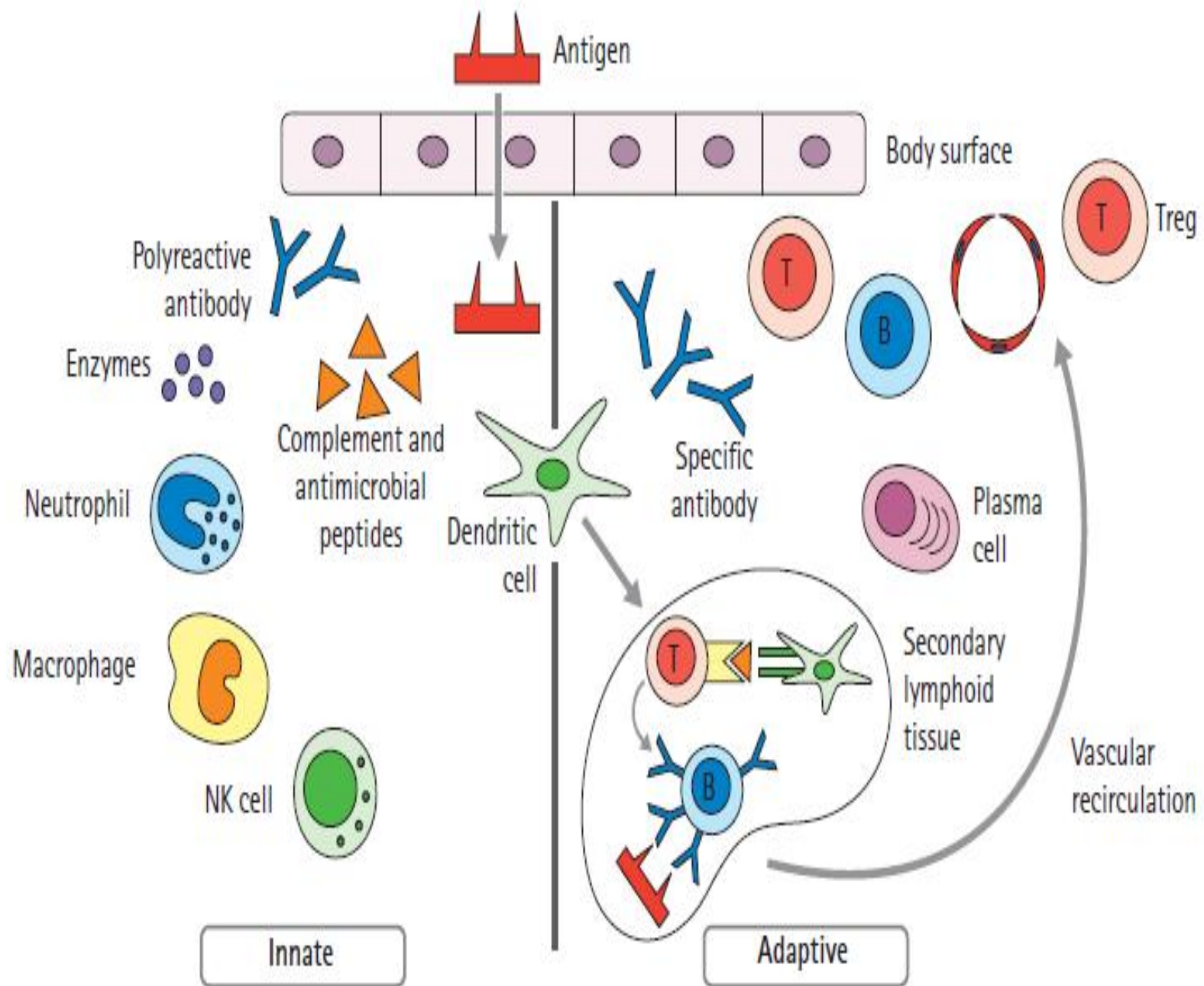
Introduction

The immune system

- A remarkable adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic microorganism and cancer

What is immunology and immunity

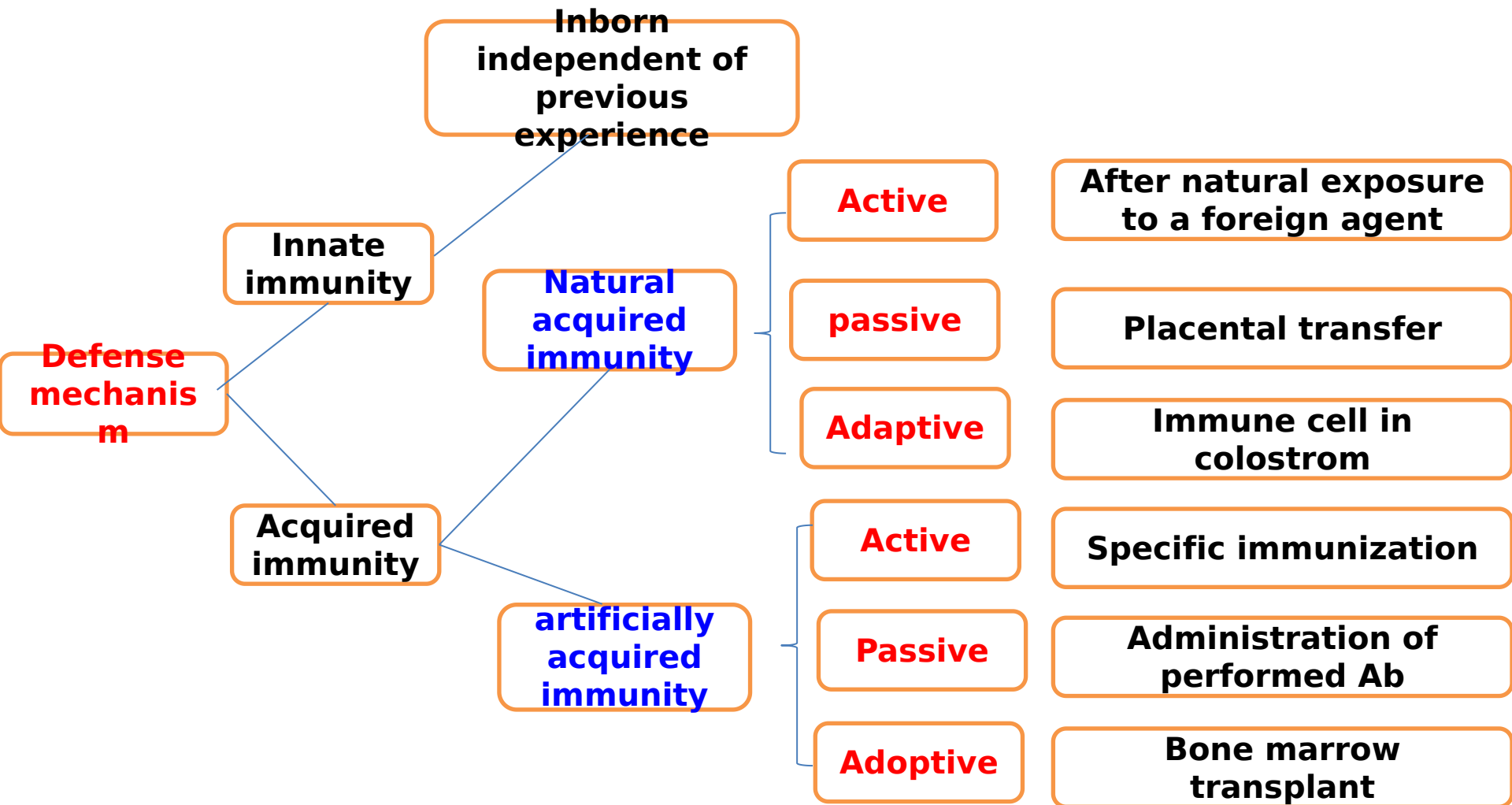
- Immunology is a science deals with immunity
- Immunity the state of protection from infectious diseases, involving specific and non specific elements
- Immunity;- is a biological term that describe a state of having sufficient biological defenses to avoid infection, disease, or other unwanted



standard building blocks of the immune system

- This diagram depicts the various components of the innate and adaptive immune systems responding to challenge by a foreign antigen.
- Innate immune defence is provided by
 - epithelial barriers,
 - resident phagocytic and
 - specialized lymphoid cells,
 - polyreactive antibodies and the alternative pathway of the complement system.
- The dendritic cell is the key link between innate and adaptive immunity and transports sampled antigen to the regional lymphoid tissue.

- T and B lymphocytes of the adaptive immune system are activated within lymphoid tissue and these potent and antigen-specific cells are mobilized to migrate through the vascular system to the site of antigen exposure in order to mount the effector immune response.
- Once antigen is eliminated, the system must be switched off by the action of specific regulatory populations of lymphocytes



1.1. Historical background

- ***Veterinary Immunology***: A branch of biomedical science that covers the study of all aspects of the immune system in all animals.
- Immunis (Latin word) = Exempt (make free)
- Immunity= the state of protection from infectious disease
- The discipline of immunology grew out of the observation that individuals who had recovered from certain infectious diseases were thereafter protected from the disease.

- **In 430 B.C. Thucydides** (The great historian of the Peloponnesian War)

✚ In describing a plague in Athens:

] He wrote that only those who had recovered from the plague could nurse the sick because they would not contract the disease a second time.

- **In the 15th century : China and Turk**

✚ Chinese & Turkish medical practitioners administered dried crusts derived from smallpox pustules (obtained from smallpox patient) in two ways to healthy people:

1) inhaled into the nostrils or

2) inserted into small cuts in the skin (Variolation)

To prevent natural smallpox infection

- **In 1718 : Lady Mary Wortley Montagu** (Wife of the British ambassador in Constantinople).

✚ She observed the positive effects of variolation on native Turkish Population

✚ She performed the technique on her daughter and son.

✚ She was first to introduce variolation in Great Britain

- **In 1796: Edward Jenner** (English physician, Founder of Immunology)

- First to test immunity by challenge with smallpox

- He inoculated James Phipps (8yrs) with fluid from a cowpox pustule taken from the arm of Sara Nelmes (milkmaid) who was suffering from cowpox (Vaccina virus)

- After the infection subsided, he intentionally inoculated the child with smallpox.

The child didn't develop smallpox.

- This technique spread quickly throughout England and elsewhere in the world ultimately leading to eradication of smallpox.

- Invented smallpox vaccine, Laid the first milestone /highlight/ in vaccination

- **In 1880: Louis Pasteur** (French Microbiologist, Father of Immunology)

-  He accidentally observed that chicken Cholera bacillus cultures left on the bench for 2 weeks lost their pathogenicity and retained the ability to protect chickens against subsequent infection by them.

- ↳ He grew Cholera bacillus (fowl cholera) in culture.

- ↳ He then injected chickens with the cultured bacterium.

The chickens developed fatal cholera.

- ↳ He went for summer vacation.

- ↳ After returning from vacation, he injected some chickens with an old bacterial culture.

- ↳ The chickens became ill, but they recovered.

- He then grew a fresh culture of the bacterium.
- He used previously injected chickens since the supply of chickens was limited.

The chickens were completely protected from the disease.

He concluded that aging had weakened the virulence of the pathogen

Lead to the discovery of the process of attenuation and development of live vaccines

- He called the attenuated strain vaccine (from the Latin vacca, meaning cow), in honor of Jenner's work with cowpox inoculation. He coin /invent/ the term 'vaccine'

He invented anthrax vaccine in 1881

→ Pasteur first vaccinated one group of sheep

→ He then challenged the vaccinated sheep and some unvaccinated sheep with a virulent culture of the *B. anthracis*.

◇ *All vaccinated sheep lived and all the unvaccinated sheep dead.*

⌘ ***These experiment marked the beginnings of the discipline of immunology,*** In 1882, 85,000 sheep were immunize

Invented rabies vaccine in 1885

→ Joseph Meister was bitten by a rabid dog

→ Pasteur produced a vaccine for rabies by drying the spinal cord of rabbits

→ The boy was given a series of 14 injections of attenuated rabies virus preparation

→ He lived and later became a custodian at the eponymous Pasteur Institute

- **In 1890, Emil von Behring and Shibasaburo Kitasato**

- ✎ They demonstrated that **serum** from animals previously immunized to diphtheria could transfer the immune state to unimmunized animals.
- ✎ Then, several researchers tried to find
- ✎ out the protective agent from immune serum.

Antitoxin (Neutralize toxins)

Precipitin (Precipitate toxins)

Agglutinin (Agglutinate bacteria)

- **In 1930s, Elvin Abraham Kabat (American immunochemist)**

- ✎ Kabat and Tiselius could separate the protective agent from serum

Microbial invasion

- The world is full of a diverse array of microorganisms
- These include bacteria, viruses, fungi, protozoa and helminths (worms)
- As they struggle to survive, many of these microorganisms see the animal body as a rich source on nutrients and a place to shelter
- They will thus seek to invade animal tissues
- This is normally prevented by our immune defenses
- Sometimes, however these organisms may overcome the immune defenses and cause diseases

- An organism that can cause diseases is said to be a **pathogen**
- These pathogens vary greatly in their ability to cause **disease** or to evade the **body defenses**
- This ability is termed **virulence**
- Thus a **highly virulent** organism has a greater ability to defeat the immune system and cause disease than an organism with lower virulence
- If a bacterium can cause disease almost every time it invades a healthy individual even in low numbers than it is considered a **primary pathogen**
- Examples :
 - **distemper virus**,
 - the **human immunodeficiency virus** which causes **AIDS** and
 - **brucella abortus** the cause of contagious abortion

- Other pathogens may be of such **low virulence** that they will only cause disease if administered in very **high doses** or if the **immune defenses** of the body are impaired first
- These are **opportunistic pathogens**.
- Examples of opportunistic pathogens are those that invade animals whose **immune system** have been destroyed or suppressed
- They include bacteria such as
 - **mannheimia hemolytica** and
 - fungi such as **pneumocystis carinii**

The body defenses

- **Physical barriers**

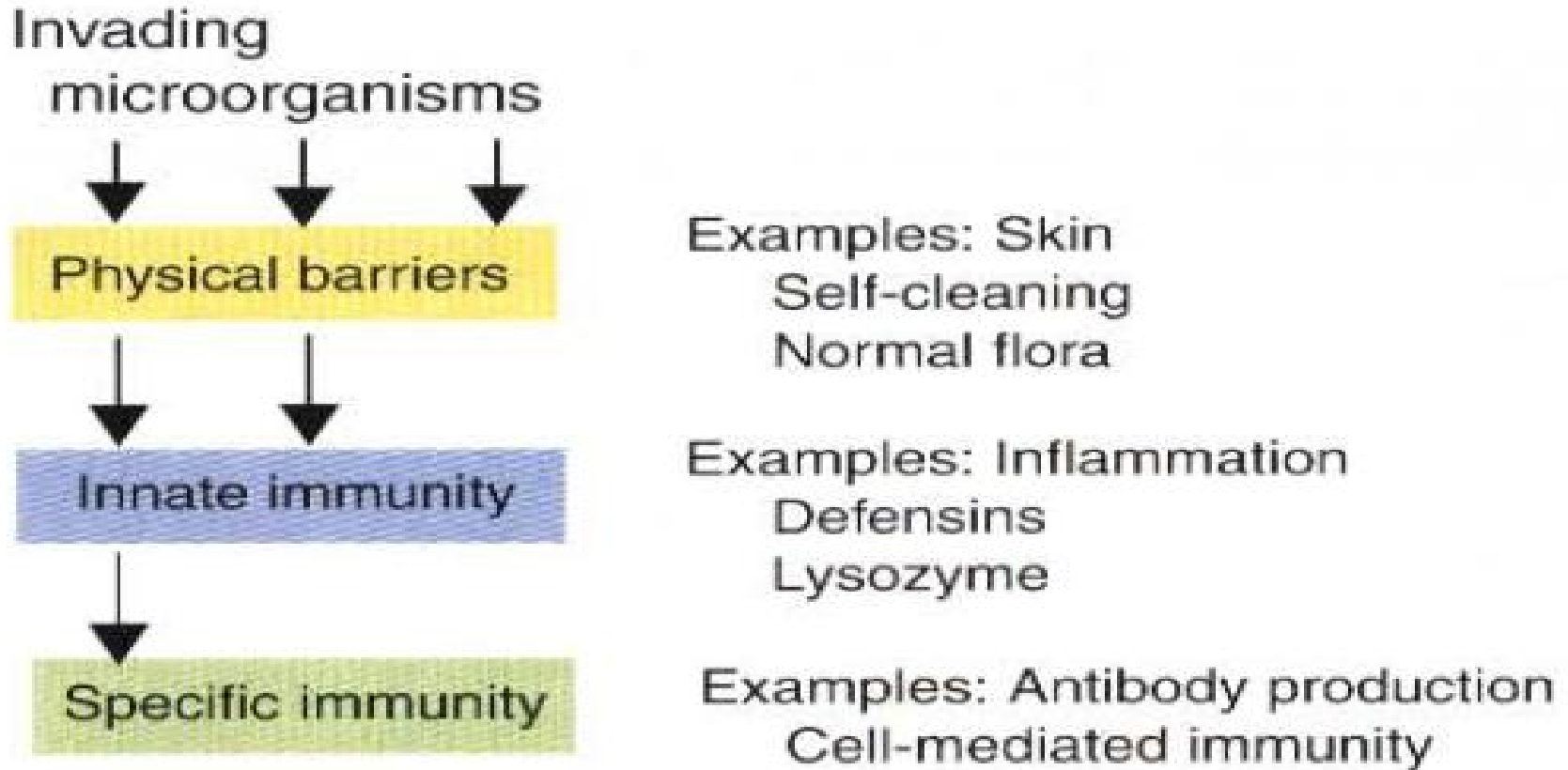


Figure 1-3. The three general ways by which the animal body defends itself against microbial invasion.

- **Anatomic barriers:** physical and anatomical barrier that tend to prevent the entry of pathogens are an organism **first line** of defense against infection
- The skin consists of **two distinct** layers
 - A thinner outer layer – the **epidermis**
 - And a thicker layer – the **dermis**
- The **epidermis** contain several layer of tightly packed **epithelial cells**
- The outer **epidermal layers** consists of dead cells and is filled with a waterproofing protein called **keratin**
- The **dermis** which is composed of **connective tissue**, contains **blood vessels, hair follicles, sebaceous glands** and **sweat glands**
- The **sebaceous gland** are associated with the **hair follicles** and produce an oily secretion called **sebum**
- **Sebum** consists of **lactic** and **fatty acid** which

- This pH inhibits the growth of most microorganism
- A few bacteria that metabolized **sebum** live as commensal on the skin and some times caused a sever form **acne.**
- Not only does intact skin prevent the penetration of most pathogens, but its low pH also inhibits most **bacterial growth**
- Although most pathogens enter the body by binding to and penetrating **mucous membranes** a number of non-specific defense mechanisms tend to prevent the entry
- For examples;- **saliva, tears** and **mucous secretions** act to wash away potential invaders and also contain **antibacterial** and

- The synchronous movement of **cilia** propels mucus entrapped m-o from the tracts
- **Physiological barriers:-** that contribute to **innate immunity** include Temperature, pH and various soluble factors
- Many species are not susceptible to certain disease simply because their normal body **temperature** inhibit the growth of the pathogen
- Chicken for example have **innate immunity** to **anthrax** because their high body temperature inhibit the growth of the pathogen.
- **Simple physical defenses** include the **self clearing** process:
 - coughing, sneezing, and mucous flow in **the respiratory tract**,
 - vomiting and diarrhea in the **gastrointestinal tract** and
 - urine flow in the **urinary tract**

- The **physical barriers** are often bathed by secretions enriched in **antimicrobial molecules** that can cause direct damage to the pathogen (e.g. digestion of bacterial cell walls).
- These molecules include **enzymes** such as,
 - **lysozyme and phospholipase A** and,
 - small **antimicrobial peptides** such as
 - the **α -defensins** and
 - **cathelicidins** produced by **Paneth cells** in the **crypts** of the **intestine** and by **epithelial surfaces** of the

- The **surfactant proteins** that coat the **alveolar** surfaces of the **lung** are also antimicrobial.
- Pathogens coated by these molecules (e.g. surfactant proteins A and D) are more readily removed by **alveolar macrophages**
- Other secreted molecules include the **polyreactive antibodies**, which can potentially bind to a range of foreign invaders, and molecules of the **alternative pathway** of the

Innate immunity(nonspecific) immunity

- Found both in **invertebrates** and **vertebrates**.
- This type of immunity is older **phylogenetically**.
- Stops microorganisms that evade **physical barriers** before inducing infection (disease), Pre-existing / Rapidly responding **chemical** and **cellular defense** mechanisms.
- The characteristics of the innate **immune response** include the following
 - Responses are broad-spectrum (**non-specific**)
 - There is no **memory** or lasting protective immunity
 - There is a limited range of **recognition molecules**
 - The responses are **phylogenetically ancient**
 - Act within **minutes** or **hours**

Recognition of non-self;

- Non-self (Invaders) –Microorganisms and Molecules released by damaged cells.
- Recognition is done by “**Sentinel cells**”

all these cells possesses **surface receptors** that discriminate **self** and **non self**.

- They recognize **molecules** that are normally expressed by **microbes** and never found in higher animals.
- These target molecules or molecular patterns are called **pathogen associated molecular patterns** (PAMPs)

Pathogen associated molecular patterns (PAMPs)

- **are LPS** (Gram negative bacteria),
- **LTA** (Gram positive bacteria) (lypo-ticoic acid),
- **Mannans** (Yeasts),
- **Glycolipids** (Mycobacteria),
- **bacterial DNA** Highly conserved and are widespread among the entire class of pathogens.
- Restricted to microorganisms and not found in animal tissues, essential for **microbial survival**,
- receptors that recognize **PAMPs** are referred to as **PRRs** (pattern recognition receptors)

Pattern recognition receptors are,

- cell surface **PRRs**.
- **Toll like** receptors.
- **Complement receptors** designated CRs- (binds to complement components such as C3b and C4b which **opsonize microorganisms** as a consequence of the activation of the **complement cascade**).
- **Macrophage Mannose receptor** (Binds to Mannose residues commonly present on surface of **microorganisms**).
- **Scavenger receptors**- at least 6 different scavenger receptors with different specificities have been described (recognize certain anionic polymers and acetylated low-density lipoproteins).
- e.g. CD36 – bind bacterial **lipoproteins** and, CD14 binds **microbial glycolipids**. CD14 (receptor on the surface of phagocytes which allows for the **recognition** of **LPS**

Secreted PRRs

- LPS binding protein (LBP)

The innate immune system generally comprises seven types of defensive barriers:

No	Types	Mechanism
1	Anatomic barrier Skin Mucous membrane	Mechanical barrier retards entry of microbes Normal flora compete with microbes for attachment site and nutrients, mucous entraps foreign microorganism, cilia propels microorganism out of the body
2	Phagocytic cell	Specialized cell (blood monocytes, neutrophils, tissue macrophage) internalized (phagocytized) kill and digest whole microorganism
3	Physiologic barrier Temperature Low pH Chemical mediator	Normal body temperature inhibits growth of some pathogens Fever response inhibits growth of some pathogens Acidity of GIT content kills most ingested microorganism Lysozyme cleaves bacterial cell wall Interferon induces antiviral state in an infected

4	Inflammatory barriers	Tissue damaged and infection induce leakage of vascular fluid, containing serum proteins with antibacterial activity and influx of phagocytes cells into the affected area
5	Normal Microbiological Flora (Microbiota)	Non-pathogenic organisms associated with epithelial surfaces compete with invading organisms for attachment sites, Compete for nutrients. Flora can produce anti-microbial substances (e.g. colicins made by <i>Escherichia coli</i> , oleic acid made by <i>Propionibacterium acnes</i>). Often displaced by antibiotics enabling colonization by opportunistic bacteria
6	Alternative pathway of complement activation	Does not require antibody, Acts immediately. Activates the terminal complement components which destroy bacteria by creating holes (pores) in the bacterial membrane Membrane Attack Complex (MAC). Opsonization also enhanced (C3b binds to CR1)
7	Lymphoid lineages	Natural killer (NK) cells (aka large granular lymphocytes - LGL)

Adaptive Immunity/Acquired immunity/

- Acquired /adaptive or specific/ immunity is capable of recognizing and selectively eliminating specific foreign microorganism and molecules i.e. foreign antigenes unlike innate immune response,
- adaptive immune response are not the same in all members of a species but are reaction to specific antigen challenges
- Specific elements include : cell lymphocytes
- B-cells secretes specific molecules called antibodies
- Acquired immunity came into play relatively late, in evolutionary terms and is present only in vertebrates
- Although an animal is borne with the capacity to mount an immune response to foreign invaders

- Acquired immunity display four characteristics
 - Antigen specificity
 - Diversity
 - Immunological memory
 - Self/non-self recognition
- Antigenic specificity of the immune system permits it to distinguish subtle differences among antigens
- Antibodies can distinguished between two proteins molecules that differ in only a single amino acid
- Diversity ; the immune system is capable of generating tremendous diversity in its recognition molecules allowing it to recognize billion of unique different structure on foreign

- Memory;- once the immune system has recognized and respond to an antigen, it exhibits immunologic memory, that is a second encounter with the same antigen induces a heightened state of immune reactivity
- Self and non-self recognition ;- able to distinguished between self and non-self and respond only to non-self molecules.
- The outcome of an inappropriate response to self molecules can be fatal

Type of adaptive immunity

- There are two arm (branches) of acquired immunity that have different sets of participants and different sites of purposes but with one common aim, that is to eliminate the antigen
 - These two arms interact with each other and collaborate to achieve the final goal of eliminating the antigen.
1. **Humoral immunity:** the one mediated by B-cells and circulated antibodies referred as humoral immunity
 - The word humors was formerly used to define body fluid.
 2. **Cell mediated immunity:** the antigen specific arm of cell mediated immunity consists of the T-lymphocytes
 - Unlike B-cells which produce soluble antibody

A comparison of Innate and acquired immunity

	Innate immunity	Acquired immunity
Evolutionary history	Ancient	Recent
onset	Rapid (min-hrs)	Slow (days-weeks)
Specificity	Common microbial structure	Unique antigen
Cell engaged	Macrophages, dendritic cell, neutrophils, NK-cells	T & B-cells
Memory	Lacks memory	Exhibits immunological memory

Antibody mediated Immune responses

- Soon after Louis Pasteur discovered that it was possible to produce immunity to infectious agents by vaccination, it was recognized that the substances that provided this immunity could be found in the blood serum
- For example;- if serum is taken from a horse that has been vaccinated against tetanus (or has recovered from tetanus) and injected into a normal horses, the recipient animal will become resistant to tetanus for several weeks
- The protective molecules found in the serum of an immunized animal are protein called antibodies
- Antibodies against tetanus toxin are not found in the serum of normal horses but are produced following exposure to tetanus toxin as a result of infection or vaccination

- The general term for such a substance is called an antigen
- If an antigen is injected into an animal, then antibodies will be produced which can bind to that antigen and ensure its destruction
- Antibodies are specific and only bind to the antigen that stimulate their production
- For example ;- the antibodies produced in response to tetanus toxin bind only tetanus toxin
- If serum containing this antibodies is mixed with a solution of tetanus toxin the antibodies bind and “neutralize” the toxin so that it is no longer toxic for animals
- In this way antibodies protect animals against

Primary and secondary immune

response

- The primary response is divided into several phases
- The lag or latent phase: is the time between the initial exposure to an immunogen and the detection of antibodies in the circulation (1 week in humans)
- The exponential phases: is marked by a rapid increase in the quantity of circulating antibodies and reflects the increasing numbers of secretors plasma cell
- Steady state or plateau phase: the interval during which the antibody level remains constant because secretion and degradation occur at approximately equal intervals
- Decline phase: the antibody level gradually decline as synthesis of new antibody wanes

Primary and secondary immune response

Primary immune response	Secondary immune response
Naïve B cells involved	Memory B-cells
Longer lag period (4-7 days)	Shorter lag period (1-3days)
Lower immune response	High immune response (100-1000 times higher)
IgM pre-dominates	IgG predominates
Lower Ab affinity	Higher Ab affinity

Cell mediated immune response

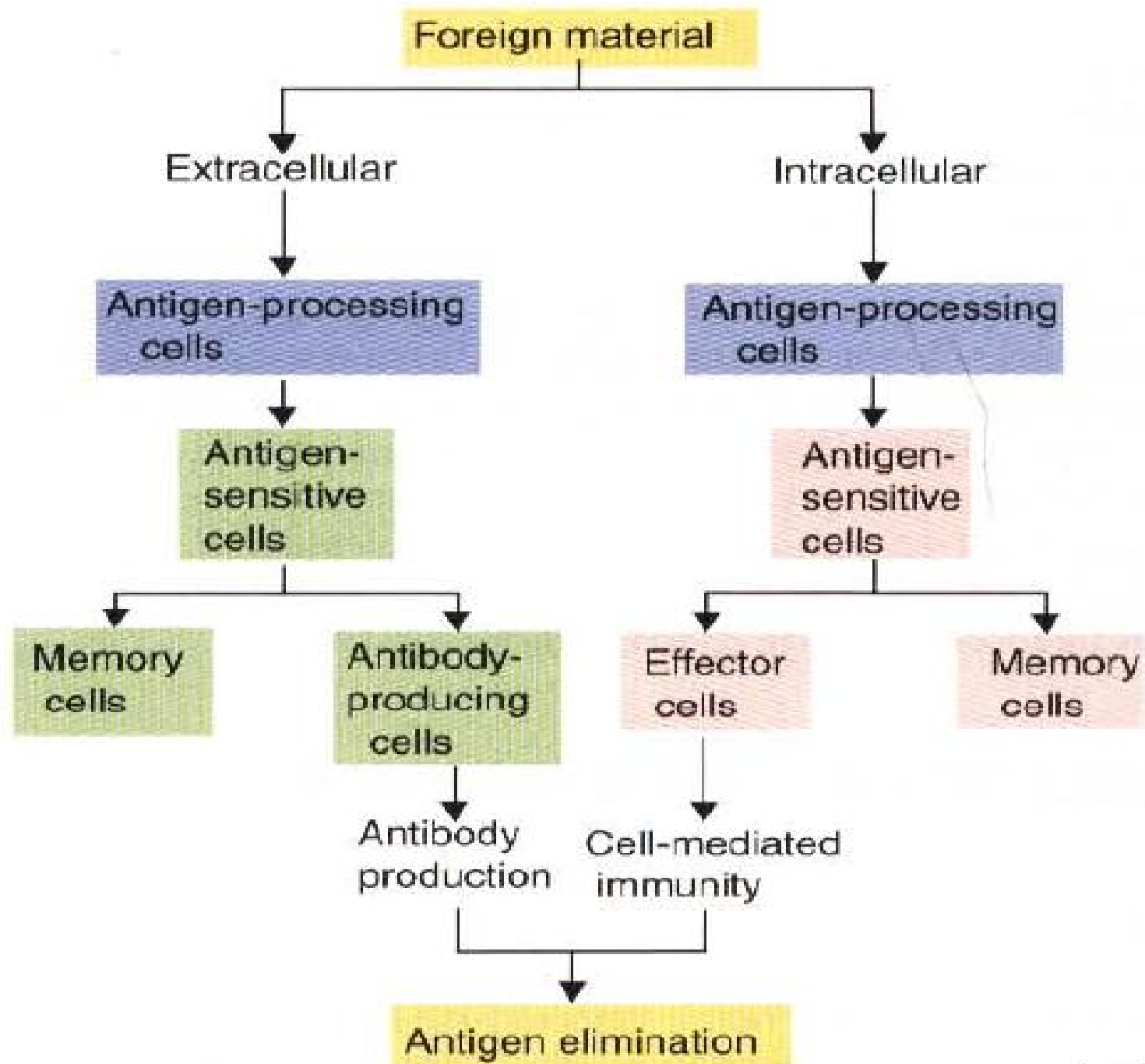
- Humoral immunity is responsible for defending organisms in the blood and extracellular fluid
- As antibodies are unable to access the inside of the cell, humoral immunity involvement in defending intracellular infection is limited
- Cellular immunity is therefore responsible in defending intracellular infection
- Cellular immunity is also involved in cancerous (abnormal cells) foreign grafts and in clearing aged cells

Mechanisms of acquired immunity

- When a foreign antigen enters the body it first must be trapped and processed so that it can be recognized as being foreign
- If so recognized, then this information must be conveyed either to the antibody forming system or to the cell mediated immune system
- These system must then respond by the production of specific antibodies and /or cells that are capable of eliminating the antigen
- The acquired immune system must also remember this events so that the next time an animal is exposed to the same antigen, its response will be faster and more efficient
- The immune system also learn how to make antibodies or cells that can bind more strongly to

- Therefore consider that the acquired immune system includes four major components
 1. cells that can trap and process antigen and then present it for recognition to the cells of the immune system
 2. cells that have receptors for the processed antigen
 - These cells can thus bind and respond to the antigen (antigen-sensitive cells)
 3. cells that once activated by antigen will produce specific antibodies or will participate in the cell-mediated immune response against the antigen (effector cells)
 4. cells that will retain the memory of the event and react rapidly to that specific antigen if it is encountered at a later time
- A fifth type of cell is also required if the acquired

- Antigen is trapped, processed and presented by several cell types including
- Dendritic cells and macrophages
- Lymphocytes called B and T cells have specific receptor for foreign antigen and are thus able to bind the processed antigen and respond appropriately
- Lymphocytes also function as memory cells and therefore initiates a secondary immune response
- The lymphocytes that mediate the cell mediated response are “T” cells
- The lymphocytes that mediated the antibody mediated response are “B” cells
- The immune response is mainly regulated by population of T cells
- Those that promote immune response are called helper T-cell
- Those that inhibit immune response are called



Unit 2

Cells and Organs of the Immune System

- **Definitions**
 - **Stem cells:** Cells that can differentiate into other cell types and have self-renewal capability
 - **Progenitor cells:** Cells that have lost the capacity for self-renewal and are committed to a particular cell lineage
 - **Hematopoiesis:** The formation and development of RBCs and WBCs
- All blood cells arise from a type of cell called the hematopoietic stem cell (HSC)
- Hematopoiesis begins in the embryonic Yolk sac

Cells of the immune system

- lymphocytes are the central cells of the immune system, responsible for adaptive and the immunologic attributes of **diversity**, **specificity**, **memory** and **self and non self** recognition
- The other types of **white blood cell** play important role **engulfing** and **destroying** microorganism, presenting **antigen**, and secreting **cytokines**
- All blood cells originated from a type of cells called **hematopoietic stem cells** (HSC)
 - Stem cells are cells that can differentiate into other cell types
 - They are self renewing,
 - they maintain their population level by cell division
- **Unipotent cells** are cells which differentiate into a single cell type
- A **haematopoietic stem cell** is a **multipotent** or

- Early in **haematopoiesis** a **multipotent stem cell** differentiate along one of two pathways giving rise to either
 - a common **lymphoid progenitor** cell or
 - a common **myeloid progenitor cell**.
- Common **lymphoid progenitor** cells give rise to B, T and NK cells and some dendrite cells
- **Myeloid stem cells** generate progenitor of
 - red blood cells (erythrocytes)
 - many of the white blood cells
 - neutrophils, eosinophils,
 - basophils, monocytes,
 - mast cells, dendritic cells and platelets
- Red and white blood cells pass into **bone**

Lymphoid cells;

- **Lymphocytes** are small, round cells, 7-15 μ m in diameter
- Each contains a large, **round nucleus** that stains with **hematoxylin**
- **Lymphocytes** constitute 20-40% of the body's WBCs and 99% of the cells in the **lymph**
- These **lymphocytes** continually circulate in the **blood** and **lymph** and are capable of migrating into the **tissue space** and **lymphoid organ** there by integrating the **immune system** to a high degree
- Based on function and cell membran components (immunophenotypes), lymphocytes are broadly subdivided into 3 major populations: **B cells**, **T cells** and **Natural killer cells**
- **Natural killer cell** are large, granular,

- Resting **B** and **T lymphocytes** are small motile, nonphagocytic cells, which can not be distinguished morphologically
- **B** and **T-lymphocytes** that have not interacted with antigen referred to as **naïve** or **unprimed**
- The interaction of **lymphocytes** with **antigen** induces these cell to enter the **cell cycle**.
- As they progress the cell cycle they changed to **lymphoblasts**
- **Lymphoblasts** proliferate and eventually differentiate into **effector cells** or into **memory cells**
- **Effectors cells** function in vareous ways to eliminate antigen
- These cells have short life span

Plasma cells- the antibody secreting **effector cells** of **B-cell lineage**

- The effectors cells of the **T-cells lineage** include the cytokine secreating **T-helper cell** and the **T-**

A) B lymphocytes (B cells)

- The designation of *B-lymphocytes* is derived from its site of maturation in the ***bursa of fabricius*** in birds and
- ***bone marrow*** in a number of mammalian species including **humans** and **mice** is the major sit of maturation of **B-lymphocytes**.
- Account for 10 % to 40 % of **blood lymphocytes**
- Mature **B cells** are definitively distinguished from other lymphocytes and all other cells by their synthesis and display of membrane bound **immunoglobulins** (B cell receptors, BCRs) (antibody) molecules which serve as receptors for *antigen*.
- BCRs are shed in large amounts into **tissue fluid** and the **blood** where they are called

B) T lymphocytes (T cells)

- Derive their letter designation from their site of maturation in the **thymus**
- Account for 60-80% of the **lymphocytes** in blood
- Each expresses a unique **antigen binding** site on its membrane called **T cell-receptor**
- Two major populations of T cells differentiated by their TCR:- TCR- α/β 85-95% (Mouse), 5-30% (Bovine) TCR- γ/δ 5-15% (Mouse), 45-50% (Bovine)
- CD3 is found in all T cells
- CD4 is found in T helper cell, Is the receptor for MHC class II molecule
- CD8 is found in T cytotoxic cells, Is the receptor for MHC class I molecule
- In human, about 65% of T cells are CD4⁺CD8⁻ and 30% of T cells are CD4⁻CD8⁺
- The ratio of CD4⁺ to CD8⁺ cells in normal human peripheral blood is approx 2:1

But, it may be altered by immunodeficiency diseases and autoimmune diseases

- T regulatory cells are identified by the presence of both CD4 and CD25 on their membrane

C) Natural killer cells (NK cells)

- Identified as a result of the presence of detectable cytotoxic activity in **unsensitized animals**
- Are large granular, **nonphagocytic** lymphocytes
- Account for 5-10% of **blood lymphocytes**
- Part of the **innate immune system**
- Involved in immune defenses against **viruses**, other **intracellular pathogens** and **tumors**
- Are first line of defense **against virus infection**
- Do not have **antigen receptors** like T cells and B cells
- Employ two different categories of receptors to recognize potential target cells:

θ When NK cells encounter **healthy normal cells, inhibitory signal** predominates

MHC class I molecules expressed on the surface of **healthy normal cells** provide inhibitory signals sufficient to block NK cell killing

θ When they encounter infected, damaged, or malignant cells, the activation signals prevail

Reduction of MHC class I molecules expression provide activating signals and the target cells will be killed

Viruses may suppress MHC class I expression in an attempt to hide within infected cells

Metastatic  tumor cells often fail to express MHC class I molecules
cell attack

θ Because NK cells express receptor (CD16) for Fc region of antibodies, they can attach to the antibodies and subsequently destroy the targeted cells (The process is called antibody-dependent cell-mediated cytotoxicity [ADCC])

→ Produce IFN- γ Activate macrophage
Produce IL-12 Activate NK cells + T_H1 cells
ω NKT cell

- Has some of the characteristics of both T cells and NK cells
- Like T cells, they have TCRs
- Unlike T cells, their TCRs interact with MHC like molecule called CD1
- Like NK cells, they have CD16, other receptors typical of NK cells and can kill target cells

Cluster of differentiation;

- **Definition:** cluster of differentiation (CD): the set of monoclonal antibodies that recognize a *single protein* on a cell surface.
- A *CD antigen* therefore by extension a defined protein on the surface of a cell.
- All of the *monoclonal antibodies* that react with a particular membrane molecule are grouped together as *cluster of differentiation* (CD).
- Although the CD nomenclature was originally developed for membrane molecule of human leukocytes, the homologous membrane molecules of other species such as *mice* are commonly referred by the same CD designation.
- In general expression of *CD4* and *CD8* also

- *CD4+ T-cells* generally function as *T-helper (TH)* cell and are restricted to *class-II MHC - molecules*;
- *CD8+ T-cells* generally function as *T-cytotoxic (TC)* cell and are *MHC-class-I restricted*.
- Thus the ratio of *TH* to *TC* T-cells in a sample can be approximated assaying the number of *CD4+* and *CD8+* T-cells.
- This ration approximately 2:1 in normal human peripheral blood, but it may be significantly altered by *immunodeficiency disease* , *outoimmune disease*, and other disorder

Myeloid cell

A. Mononuclear phagocytes: Have single, rounded nuclei and are thus described as “mononuclear” cells

→ The mononuclear phagocytic system consists of monocytes circulating in the blood, and macrophage in the tissues

→ Monocytes circulate in the blood stream for about 8 hours during which they enlarge, they then migrate into the tissue and differentiate into specific macrophages or dendritic cells.

→ Two types of Macrophages

- i. M1 cells that defend against microbial invaders and produce pro-inflammatory cytokines

- ii. M2 cells reduce inflammation and produce

- ♣ **Microphages** are dispersed throughout the body
- ♣ Some take up residence in particular tissue becoming **fixed macrophages**
- ♣ Whereas other remain motile and are called **free** or **wandering macrophage**.
- ♣ Macrophage like cell serve different function in different tissue and named according to their tissue location
 - Intestinal macrophages (gut), Alveolarmacrophages (Lung),
 - Histocytes (connective tissues), Kupffer cells (liver),
 - Mesangial cells (kidney)
 - Microglial cells (brain), and Osteoclasts (bone)
- Macrophages are capable of ingesting and digesting exogenous antigens such as whole microorganism

B. Granulocytes cells

- o Have characteristic lobulated, irregular nuclei and are thus described as “**Polymorphonuclear**” cells
- o Classified into **3 populations** based on the **staining properties** of their **granules**

i) Neutrophils

- Produced in the bone marrow and released into the peripheral blood and migrates into tissues at time of inflammation or infection.
- Like macrophages neutrophils are active phagocyte cells
- circulate for 7-10h migrate into the tissues
die after a few days
- Constitute 60-75% (carnivores), 50% (horses), 20-30% (cattle)
- There are 2 pools of neutrophils in the blood, the **circulating pool** and the **Sequestered pool**
 - Are active phagocytic cells
 - Employ both oxygen-dependent and oxygen-

- Exhibit a larger respiratory burst than macrophages
- Express higher levels of defensin than macrophages do.

ii. Eosinophils

- These cells like neutrophils are motile phagocytic cells that can migrate from the blood into tissue spaces.
- Their phagocytic role is significantly less important than that of neutrophils and is thought that they play a role in the defense against parasitic organism.
- The secreted contents of the eosinophilic membrane are damage the parasitic membrane
 - Their granules take up **acidic dyes**, Produced in the bone marrow released into the peripheral blood circulate for 30 mins migrate into the tissues die after a few days
 - Normal values range from 2% (dogs) to about 10% (cattle)
 - Play a role in the defense against **parasitic**

iii) **Basophils**

- Their granules take up **basic dyes**, Constitute about 0.5% of blood leukocytes, and are **nonphagocytic** granulocytes
- Function by releasing **pharmacologically** active substances from their cytoplasmic granules (histamin)
- Basophils have blue-staining granules, containing histamine which is secreted during allergy.
- These substances play a major role in certain allergic responses

C. Mast cells

- Are large, round cells, Found in the skin, connective tissues of various organs, and
- mucosal epithelial tissue of the respiratory, genitourinary and digestive tracts
- Produced in the bone marrow circulate in the blood

- Have large numbers of **cytoplasmic granules** that contain **pharmacologically** active substances (histamine)
- Mast cells together with other blood basophils play an important role in the development of allergies

D. Dendritic cells (DCs)

- Acquired its name because it is covered with long membrane extensions that resemble the dendrites of nerve cells
- Four types of dendritic cells are known:
 - Langerhans cells, - interstitial dendritic cells,
 - myeloid cells, and lymphoid dendritic cells
- All constitutively express high levels of both **class II MHC molecules** and **members of the co-stimulatory B7 family**
- The major function of mature dendritic cells is the presentation of antigen to T_H cells.

- For this reason they are more potent antigen presenting cells than macrophages and B-cells both of which need to be activated before they can function as antigen presenting cells (APCs).
- Following microbial or during inflammation mature or immature forms of langerhans cells and intestinal dendritic cell migrates into draining lymphnodes where they make the critical presentation of antigen to TH-cells that is required for the initiation of responses by those cells
- **Follicular dendritic cell**, Does not arise in bone marrow
 - Does not express class II MHC molecules
 - Does not function as APC

Organs of the Immune System

- Lymphocytes are small round cells that predominate in organs such as the spleen, lymph nodes and thymus, these are called lymphoid organs
- These organs can be classified by function as primary and secondary lymphoid organs

Comparison of primary and secondary lymphoid organ

No		primary	secondary
1	Time of development	Early in embryonic life	Late in fatal life
2	Persistence	Involves after puberty	Persists in adults
3	Effect of removal	Loss of lymphocytes	No or minor effect
		Loss of immune response	
4	Response to antigen	unresponsive	Full reactive

Primary lymphoid organs

- o Are also called **central lymphoid** organs, Developed early in **fetal life**
- o The organs that regulate the maturation of lymphocytes are called primary lymphoid organs
- o Indeed lymphocytes fall into two major populations called T-cell and B-cells based on the organs in which they mature.
- o Thus all T-cells mature in the thymus; B-cells mature within different organs depending of species.
- o This includes the bursa fibricious in birds,
- o the bone marrow in primates and rodent and
- o intestinal lymphoid tissue in rabbits ruminants

- o As animals developed newly produced, immature lymphocytes migrate from the bone marrow to the primary lymphoid organs where they mature
- o Include: **Thymus**, **Bone marrow**, **bursa** of **Fabricius**, and **intestinal lymphoid tissues**

Thymus: The site of T-cell development and maturation

- θ it is located in the thoracic cavity in front of and below the heart
- θ In horse, cattle, sheep, pigs and chickens it also extended up the neck as far as the thyroid gland.
- θ The thymus consists of lobules which has two parts, the outer parts is called cortex and the inner part is medulla
 - The cortex is densely populated with large number of rapidly dividing T-lymphocytes called thymocytes
 - of these about 10% are CD4- and CD8-, these are primitive T-cell precursor.
 - About 15% are CD4+CD8- and CD4-CD8+ and most abundant in the thymic medulla.

- Also in the outer cortex are thymic nurse cells which are specialized epithelial cells with long membrane extension that surrounds as many as 50 thymocytes.
- The inner part medulla has fewer thymocytes,
 - epithelial cells,
 - fewer macrophage and
 - dendritic cells are found.
- The functions of the thymus are best demonstrated by studying the effect of its surgical removal in rodents (thymectomy).
- Neonatal thymectomy: mice thymectomized within a day of birth become susceptible to infection and may fail to grow.
- These animals have very few circulating lymphocytes and cannot reject foreign organ grafts because they lose the ability to mount cell mediated immune response

- On the other hand the antibody response to protein antigens, such as bovine serum albumin, or sheep red blood cells is significantly reduced.
- But the antibody response to polysaccharide antigen may be unaffected

⌘ **Positive selection**

- **Thymocytes** that survive the negative selection process but can still recognize specific **MHC class II-antigen** complexes with moderate affinity are stimulated to grow
- As an animal ages, the **thymus** shrinks and is gradually replaced by fat
- However, the aged **thymus** still contains small amounts of **lymphoid tissue** and is functionally active
- **Thymic epithelia** cells secrete **thymulin** which is **zinc** containing **peptide**

θ **Bursa of Fabricius**

- Found only in **birds**, Round sac located just above the **cloaca**
- Reaches its greatest size in chick about 1-2 weeks after hatching, Shrinks as the bird ages
- The site of development and maturation of B lymphocytes in birds
- Lymphoid follicles divided into **cortex** and **medulla**
- **Cortex** contains **lymphocytes, plasma cells, macrophages** and **dendritic cells**
- **Medulla** contains **lymphocytes, epithelial cells, macrophages** and **dendritic cells**
- The bursa may be removed either surgically or by infecting new borne chickens with a virus that destroys the bursa (infectious bursal disease or Gumborow disease)
- Bursaectomized birds have very low level of antibodies in their blood and antibodies producing cells disappear from lymphoid organs.
- However posses circulating lymphocytes and can

- About 90%-95% immature B cells that migrate from bone marrow to bursa will die by **apoptosis**
- Like **thymus**, two-stage selection process takes place in **bursa**

Negative selection: Self-reactive B cells are killed

Positive selection: B cells that do not react will survive

- Produce **hormone (bursin)** that activates B cells

θ **Peyer's Patches (PPs)**

- Located in the walls of the **small intestine**

ileal PPs (80%-90% found in the ileum, **group I animals** [ruminants, pigs, horses, dogs and human])

jejunal PPs (found in the jejunum, group I animals)-persist lifelong
-consists of **B cells** and **T cells**
(30%)

Group II animals (rabbit and rodents, located at random intervals in the ileum and jejunum
-do not develop until 2-4 weeks after birth
-persist into old age

θ **Bone marrow**

- The site of B cell origin and development in mice and human
- Negative selection eliminates B cells that possess self-reactive BCR
- There is no exclusive B cell development site in the bone marrow although it is suggested that precursor B cells develop at

Secondary lymphoid organs

- **In contrast to the primary lymphoid organs the secondary lymphoid organs arise late in fetal life and persist in adults**
- Unlike the primary lymphoid organ they enlarge in response to antigenic stimulation
- Surgical removal of the secondary lymphoid organ does not significantly reduce immune capability
- **Example of secondary lymphoid organ include the spleen, bone marrow, the lymph node, the tonsil, and other lymph tissue in the intestinal, respiratory and urogenital tracts**
 - o This organ contain DCs that trap and process antigens and lymphocytes that mediate the immune responses
 - o Provide conducive environment for mature

- o Less organized lymphoid tissues, collectively called mucosa-associated lymphoid tissue (MALT) is found in various body sites

BALT (Bronchi-associated lymphoid tissue)

NALT (Nasopharynx-associated lymphoid tissue)

GALT (Gut-associated lymphoid tissue)

θ Lymph nodes (LNs)

- Are round or bean shaped filters strategically placed on lymphatic vessels in such a way as to trap antigens carried in lymph
- Encapsulated bean shaped structures containing a reticular network packed with lymphocytes, macrophages, and dendritic cells
- Lymph nodes have three layers which support distinct microenvironment, -a peripheral cortex a central medulla and the paracortex (located between cortex and medulla)
- Macrophages and Dc which trap antigen are present in

- The medulla is largely populated by antibody producing plasma cells
- LNs taken from neonatally thymectomized mice have unusually few cells in the paracortical region
- The paracortex is therefor called thymus-dependent region in contrast to the cortex which is a thymus-independent region
- The lymphoid follicles in the cortex are of two types the primary and secondary follicles
- Primary follicles contain predominantly mature, resting lymph nodes prior to exposure to antigen in lymph nodes that have been stimulating by antigen the cell within the primary follicles expand to form germinal center

- Lymphocytes circulation-T-cell constantly circulate around the body in the blood and tissue fluids are the predominant lymphocytes in blood
- As they travel they survey the body for foreign antigen and preferentially home to sites and microbial invasion and inflammation
- They also spent time in the secondary lymphoid organ

θ **Spleen**

- Just as lymph nodes filter antigen from lymph, so the spleen filters from blood
- The filtering process removes both antigenic particles and aged blood cells
- The spleen also stores red-cells and platelets and undertakes red cell production in the fetus. It is therefore divided into two compartments
- One for antigen trapping and for red cell storage called Red pulp-
- And one rich in lymphocytes and is the site where immune responses occur called white pulp
- Intravenously administered antigen trapped in the spleen and taken up by DC in the marginal zone and in the sinusoids of the red pulp
- The DC carry the Ag to the primary follicles of white pulp from which after a few days Ab producing cells migrate

- The white pulp surrounds the branches of the splenic artery, forming a periarteriolar lymphoid sheath (PALS) populated mainly by T lymphocytes
- Primary lymphoid follicles are attached to the PALS and are rich in B cells

Site where clonal expansion, isotype switching and somatic hypermutation take place

- The marginal zone is located peripheral to the PALS and is populated by lymphocytes and macrophages
- Blood-borne antigens and lymphocytes enter the spleen through the splenic artery, which empties into the marginal zone
- In the marginal zone, antigen is trapped by interdigitating DCs, which carry it to the PALS

θ **Mucosal-associated lymphoid tissue**

- The tonsils defend against antigens entering through the nasal and oral epithelial routes
- In ruminants and pigs, jejunal peyer's patches are secondary lymphoid organ
- Jejunal PPs plays a major role in the intestinal defense
- PPs consists of lymphocytes arranged in follicles and covered with an epithelium that contains microfold cells (M cells)
- M cells take up antigens from the intestinal lumen and present them directly to nearby lymphocytes
- T lymphocytes present in the GIT are called intraepithelial lymphocytes(IELs).

θ **Bone marrow**

- The largest mass of 2^o lymphoid tissue in adult
- Antigen given intravenously can be trapped in the BM
- During 1^o immune response, antibodies are largely produced in the LNs and spleen
- During 2^o immune response, large quantities of antibodies (up to 70%) are produced in the BM

Unit - Three

Antigen

- Any foreign substances that can bind to a specific lymphocytes receptor and induce immune response is called an antigen

Factor that influence immunogenecity

- **Foreignness;** in order to elicit an immune response, a molecule must be recognized as non-self by the biological system
- The capacity to recognize non self is accompanied by tolerance of self, a specific un responsiveness to self antigen
- Much of the ability to tolerate self antigen arises during lymphocyte development, during which immature lymphocytes are exposed to self components
- Antigen that have not been exposed to immature lymphocytes during this critical period may later recognized as non-self or goreign by the immune

- Generally the greater the phylogenetic distance between two species, the greater the structural (antigenic) disparity between them

Molecular size:- the most active immunogens tend to have a molecular size of 100,000 daltons(Da) or more

- Generally substance with a molecular mass of less than 5000-10000 Da are poor immunogens
- Although a few substances with a molecular mass less than 1000 Da have proven to be immunogenic

Chemical composition and heterogeneity:- size and foreignness are not by themselves sufficient to make a molecule immunogenic

- Other properties are needed as well chemical complexity contributes to immunogenicity
- **Host genetics:-** the genetic constitution of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response

- Examples:- two different inbred strains of mice respond very differently to a synthetic polypeptide immunogen.
- **chemical stability**: - nucleic acids are poor antigens as they are not stable
- Simple polysaccharides of starch or glycogen also not stable
- Lipids:- poor antigens, because rapidly degraded
- Protein are more effective antigens

Immunogen dosage and route of administration:- An insufficient dose will not stimulate an immune response either because it fails to activate enough lymphocytes or because in some cases certain ranges of low doses can induces a state of immunogenic unresponsiveness or tolerances

- A single doses of most experimental immunogens will not induce a strong responses, rather repeated administration or booster increase the clonal proliferation of antigen-specific T cells or B

- Route of administration are generally parental: IV, SC, ID, IM, IP –commonest ones
- Antigen administered IV is carried first to the spleen, whereas antigen administered subcutaneously moves first to the local lymph nodes
- Differences in lymphoid cells that populate these organs may be reflected in the subsequent immune response

Adjuvants:- from Latin word to help, are substances that when mixed with an antigen and injected together with it, enhance the immunogenicity of that antigen.

- Adjuvant is used to boost the immune response when an antigen has low immunogenicity or only small amounts are available
- Adjuvant may help by protecting antigen from rapid degradation, assisting effective delivery of antigens to antigen presenting cells (APCs) or through

Example of some common adjuvants

Type	Adjuvant	Mode of action
Depot adjuvant	Aluminum phosphate	Slow release antigen
Immunostimulatory adjuvant	BCG	Macrophage stimulator
Particulate adjuvant	Liposomes	Stimulate antigen processing

recognize an entire immunogen molecule

- Instead lymphocytes recognize discrete sites on the macromolecule called epitopes or antigen determinants
- Epitopes are the immunologically active regions of an immunogen that bind to antigen specific membrane receptor on lymphocytes or to secreted antibodies.
- In a large complex protein molecule, many different epitopes may be recognized by the immune system, but some are much more immunogenic than others.

- Thus animal may response to a few fevered epitopes
- Such epitopes are said to be immunodominant.

Haptens:- small molecule ($<1000\text{Da}$) chemical groups that can function as epitopes when bound to other molecules are called haptens.

- The antigenic molecules to which the haptens are attached is called the carrier
- Many drug allergies occur because the drug molecules although small can bind covalently to normal body proteins and so act as haptens.

Crossreaction :- identical or similar epitopes may sometimes be found on apparently unrelated molecules

- As a result antibodies directed against one antigen may react unexpectedly with unrelated antigen
- This situation is called cross reaction
- Example:- cross reactivity occurs between

Genetic basis of immune diversity:

The immune system is capable of generating tremendous diversity in its recognition molecules, allowing it to recognizing billions of unique structures of foreign antigens

- The main distinction between the innate and acquired immune system lies in their use of receptor to recognize foreign invaders.
- The innate system uses pre-existing receptors that can bind to molecules and molecular patterns commonly found on many different microbial pathogen
- In contrast the cells of the acquired immune system randomly generate enormous numbers of structurally unique receptors
- These receptors can bind an enormous array of foreign molecules
- Because the binding repertoire of these receptors is

Unit four

Lymphocyte activation and Response to Antigen

- **Response of B-cells to antigen**
- The developmental process that results in production of plasma cell and memory B-cell can be divided in three broad stage
 1. generation of mature and immunocompetent cells (maturation)
 2. activation of mature B-cells when they interact with antigen
 3. differentiation of activated B-cells into plasma and memory cells
- After export of B-cell from bone marrow activation, proliferation and differentiation occur in the periphery and require antigen
- Antigen driven activation and clonal selection of naïve B-cells leads to generation of plasma cell and memory cells.

B/T/APCs cells collaboration in antibody production

- Thymus dependent (TD) and thymus independent (TI) B-cell activation
- Depending on the nature of the antigen B-cell activation proceeds by two different ways
- One dependent up on T_H - cells and the other not.
- The B-cell response to thymus dependent (TD) antigen requires direct contact with T_H cell not simply exposure to T_H derived cytokines
- Antigen that can activate B-cell in the absence of this kind of direct participation by Th cells are known as thymus independent (TI) antigens.
- The humoral response to thymus independent

- The response to TI antigen is generally weaker, no memory cells are formed and IgM is the predominant antibody secreted and reflecting low level of class switching
- These differences highlight the important role played by Th cells in generation of memory cells and class switching
- This is because of Th cytokines.

Deference between TD and TI antigen

Proper ty	TD- antigen	TI- antigen	
		Type -1	Type - 2
Chemic al nature	Soluble protein	Bacteri al cell wall	Polymeric protein antigens, capsular polysaccharides
Humora l response	strong	weaker	weaker
Isotype	Yes	No	No

- T independent antigens are usually is simple repeating polymers such as E. coli LPS, polymerized salmonella flagellin and pneumococcal polysaccharide
- T independent antigen bind directly to B cell
- The ability of B-cells to respond directly to these antigen provides a rapid response to many important pathogens irrespective of the limitation
- Antibodies induced by microbial antigens alone are therefore less variable and less functionally versatile than those induced with T helper cells
- Antigen receptors
- TCR – T-cell receptor
- BCR – B- receptor
- MHC – class I – expressed by all nucleated

Antigen processing and presentation

- Recognition of foreign protein antigen by T- cells requires that the peptides derived from the antigen be displayed within the cleft of an MHC molecule on the membrane of a cell.
- The formation of these peptide -MHC complexes require that a protein antigen be degraded into peptides by a sequences of events called antigen processing
- The degraded peptide then associate MHC molecule within the cell interior and the peptide MHC complex are transported to the membrane where they are displayed called antigen presentation
- Endogenous antigen;- Ag that have been produced within the infected cells (e.g. normal cell proteins, tumor proteins, viral and bacterial

Primary and secondary immune responses

- The primary response is divided into several phases
 1. the lag or latent phases;- is the time between the initial exposure to an immunogen and the detection of antibodies in the circulation (1 week in human)
 2. the exponential phases:- is marked by a rapid increase in the quantity of circulating antibody and reflects the increasing numbers of secretory plasma cells.
 3. steady state or plateau phases:- the interval during which the antibody level remains constant because secretion and degradation occur at approximately equal intervals.
 4. decline phase;- the antibody level gradually declines as synthesis of new antibody wanes

Primary and secondary immune response

Primary	Secondary
Naïve B-cells involved	memory B-cell
Longer lag period (4-7 days)	Shorter lag period (1-3 days)
Lower immune response	High immune response (100-1000 times higher)
IgM predominates	IIgG production predominates
Lower Ab affinity	Higher Ab affinity

Unit-five

Nature of Antibody/ immunoglobulin

- ♣ **Antibody** are the **antigen -binding proteins** present on **B-cell** membrane and secreted by **plasma cell**
- ♣ **Membrane bound** Ab confers **Ag specificity** on B-cell
- ♣ Antigen-specific proliferation of B-cell clones is elicited by the interaction of **membrane antibody** with antigen
- ♣ **Secreted Ab** circulate in the blood , where they serve as the **effectors** of **humoral immunity** by searching out and **neutralizing Ag** or marking them for **elimination**
- The Ab produced in response to a particular Ag are **heterogeneous** /mixed/
- Most Ag are **complex** and contain many different **antigenic determinants**, and the **immune system** usually respond by

- Their out put are **monoclonal Ab**, each of which specifically binds to a **single antigen determinants**
- Together these **monoclonal antibodies** make up the **polyclonal** and **heterogeneous serum antibody** response to an **immunizing antigen**
- Blood can be separated in a **centrifuge** into a **fluid** and **cellular fraction**
- The **fluid fraction** is the **plasma** and the **cellular fraction** contain blood cell (RBC, WBC, plateletes)
- **Plasma contains** all of the **soluble small molecules** and **macromolecules** of blood, including **fibrin** and other **protein** required for the formation of **blood clotes**

2.1. Basic structure of Immunoglobulins

- Antibody molecules are **glycoproteins** called **immunoglobulins (Ig)**,
- Thus an Ig molecules consists of **three globular region**
- 2-Fab region and 1 Fc region linked by a flexible hinge
- In a key experiment a brief digestion of IgG with the **enzyme papain** produced **three fragments** and a **third** that was quite different.
- The two identical fragment (each of a MW 45,000dal) had binding activity and were called **Fab fragment** (fragment Ag binding)
- The other fragment (MW of 50,000dal) have **no Ag activity** at all, because it was found to **crystallize** during **cold storage**. It was called

The term immunoglobulin is used to describe all soluble BCRs

- **Antibodies are made up of 4 polypeptide chains:**

- 2 identical light (L) chains ~22 KDa
- 2 identical heavy (H) chains ~55 Kda

- **Each Light chain bound to Heavy chain by:**

- θ Disulfide bond
- θ Noncovalent interactions (ionic bonds, hydrogen bonds, hydrophobic interactions)

- Similar noncovalent interactions and disulfide bonds link two identical L and H chain combinations to each other

- **Each L and H chain has got two regions**

- θ Amino (NH_2 , N) -terminal region
- θ Carboxy (COOH , C) -terminal region

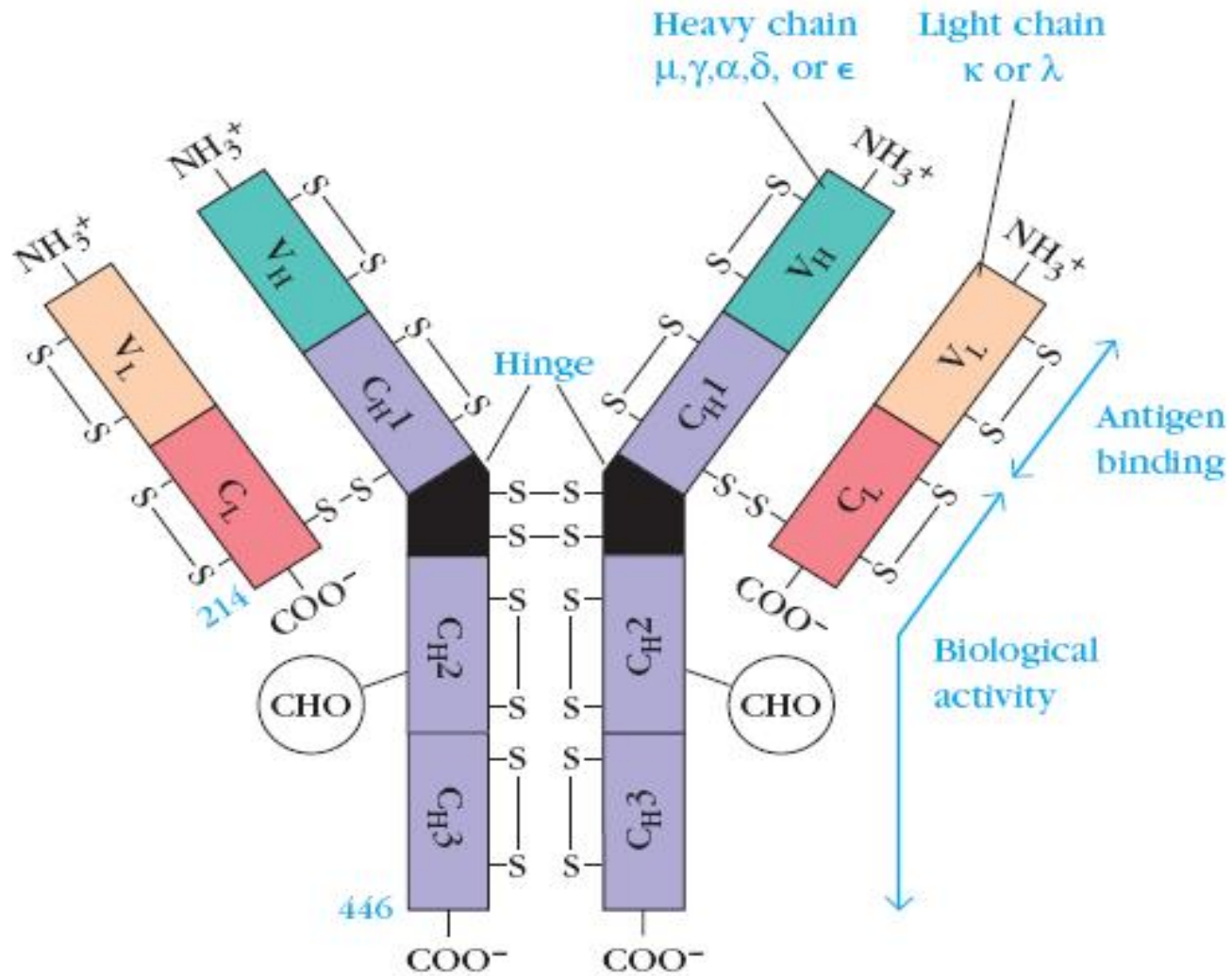
An antibody has got the following structure

Variable region (antigen binding site/V-region)

- The first 110 or so **amino acids** (aas) of the **amine terminus (N-terminal)** region of a L or H chain varies among antibodies of different **antigen specificity**
- All of the difference in specificity displayed by different Ab can be traced to differences in the **amino acids** of the **V-region**
- This region is called **Variable (V) region** (V_H and V_L)
- Most of the difference among Ab fall within areas of **V-region** called **complementary determining region (CDR)**
- And it is this CDRs. **both light** and **heavy**

Constant region ; The region of relatively constant sequence of aas is called constant (C) region (C_H and C_L)

- This region of antibody molecule is involved in **biological function of antibody**
- That is it binds with molecules of **the body** other than the Ag.
- This region also determine the class of Ab.



Structure of antibody molecule

θ **Hinge region**

- └ Consists of about 12 aas (two prominent /important/ aas are proline and cysteine)
- └ Gives antibody molecules segmental flexibility

2.2. Antibody classes and Biological activities

- Each class is distinguished by unique aa sequences in Heavy chain C-region
- **The 5 major classes of immunoglobulins include:**

1) Immunoglobulin G (IgG)

- Made and secreted by plasma cells in spleen, LNs and BM
- Found in highest concentration in the blood
- Major secretory immunoglobulin in colostrum and milk of cattle
- Plays the major role in antibody-mediated

Function

- Mediate opsonization
- Agglutinate bacteria
- Activate classical complement pathway

2. Immunoglobulin M (IgM)

- Do not possess a **hinge region**
- Made and secreted by **plasma cells** in **spleen**, **LN**s and **BM**
- Occurs in the **second-highest concentration** after IgG in most mammals
- IgM is a **180 kDa Ig monomer** (on B cell surface)
- The **secreted form IgM** consists of **5 subunits** linked by **disulfide bonds** in a circular fashion,
- Its total molecular weight is **900kDa**
- More efficient than IgG at **activating complement**, **opsonization**, **neutralization** of viruses and **agglutination**
- Rarely enter **tissue fluids** even at sites of **acute inflammation**

3) Immunoglobulin A (IgA)

- Made and secreted by **plasma cells** in **GIT, RT, UT, skin** and **mammary glands**
- Predominant Ig class in **external secretions** e.g. saliva, tear, milk (human)
- Occurs in two forms: **Monomer** 150 kDa and **polymer** (dimer, trimers and some tetramers)
- All **polymeric forms** contain a **J-chain polypeptide**
- Involved in the protection of GIT, RT, UGT, mammary glands and eyes
- Can agglutinate **particulate antigens** and **neutralize viruses**
- Prevents the **adherence** of invading microbes to **body surfaces**
- Does not activate the **classical complement pathway**

4) Immunoglobulin E (IgE)

- Do not possess a **hinge region**
- Made and secreted by **plasma cells** located beneath body surface like IgA
- A typical **Y-shaped Ig** and has a molecular weight of 190 kDa
- Present in extremely **low concentration** in serum
- Mediate **Type I hypersensitivity reaction**
- Responsible for immunity to **parasitic worms**

5) Immunoglobulin D(IgD)

- Like IgM, it is the major **membrane bound Ig** expressed by **mature B cells**
- Has a molecular weight of 170 kDa
- Its role in the physiology of **B cells** is under investigation
- No **biological effector function** has been identified for IgD

The complement system

- This was discovered by Jules Bordet many years ago as a heat-labile component of normal plasma that augment the opsonization and killing of bacteria by antibodies
- This activity was said to be 'complement' the antibacterial activity of antibody.
- The complement system is made of many distinct *plasma proteins* that react one another to opsonize pathogens and induce a series of inflammatory response that help to fight infection.
- Complement is a system of plasma protein that can be activated directly by pathogens or indirectly by pathogen bound antibody, leading to cascade of reactions
- There are three ways in which the complement system protect against infection.
 - It generates large numbers of activated complement proteins that bind covalently to pathogens, opsonizing them for engulfment by phagocytes bearing receptors for complement.

- The terminal complement damages certain bacteria by creating pores in the bacterial membrane.
- The complement system is inactive in healthy uninfected animals
- The complement system recognizes microbes by 3 ways:
 - A. The classical pathway,
 - B. The alternative pathway
 - c) The Lectin pathway

Function: -Lysis of cells, bacteria and virus,

- Opsonization (C3b and C4b),
- Chemotaxis (C3a, C5a and C5b67), Inflammation (C3a, C4a and C5a), Immune clearance

Activation

A) The classical pathway

- o So called because it was discovered 1st
- o Triggered by Ag-Ab complex

→ **Part of the acquired immune system**

- o IgM and IgG (IgG1, IgG2, IgG3, and but not IgG4) can activate this pathway
- o Involves 4 serum proteins: C1 (C1q, C1r and C1s), C2, C3 and C4

B) The alternative pathway

- Evolutionary ancient pathway that is even found in invertebrates
- Does not require Ag-Ab complex for initiation, but only through the presence of certain antigen

Component of the innate immune system

- Involves 4 serum proteins: C3, factor B, factor D and **Properdin**
- Triggered by the binding of spontaneously generated C3b to foreign surface Ags (bacterial cell walls, yeast cell walls and certain viral envelop)

c) **The lectin pathway**

- Synonymous: Mannan-binding lectin pathway
- Does not require Ag-Ab complex for initiation

- Involves MBL, MASP-1, MASP-2 (mannose amyloid serum protein), C2 and C4
- Triggered by the binding of MBL to mannose residues on glycoproteins or carbohydrates on the surface of Bacteria, fungi and some viruses (Including HIV-1)

Membrane attack complex (MAC)

- The complex of complement components (C5b, C6, C7, C8 and C9 (10-17 molecules)), which is formed in the terminal step of activation of the complement pathways
- Tubular structure of **70-100 Å** diameter
- Form a large channel through the membrane of the target cell

Ions and small molecules can diffuse freely through the central channel of MAC

Unit 6

CYTOKINES

- Low molecular weight (<30kDa) **regulatory protein** or **glycoproteins** secreted by **WBCs** and various **other cells** in the body in response to a number of **stimuli**
- The two principal producers are the **T_H cell** and the **macrophage**
- **Interleukins (ILs)**: cytokines secreted by some leukocytes and act on other leukocytes.
- They are numbered sequentially in the order of their discovery
- **Interferons (IFNs)**: cytokines produced in response to virus infection or immune stimulation
- **Tumor necrosis factors (TNFs)**: cytokines secreted by macrophages and T cells.

- The cell of the immune system secrete a bewildering variety of protein whose function is to regulate the immune response by communicating among cells
- These protein are called cytokines
- Cytokines differ from conventional hormones in several important respect

For example

1. unlike classical hormones which tend to effect a single target organ cytokines affect a wide variety of cell and organs
2. cells rarely secrete only one cytokines at a time

- For instance, macrophages secrete at least 15 different cytokines: IL-1, IL-6, IL-12, IL-18, IL-23, IL-27, TNF- α , TNF- β , GM-CSF, IL-10, IL-13, IL-15, IL-16, IL-17, and IL-18.

3. cytokines appear to be “redundant” in their biological activities in that many different cytokines may have similar effects

- For examples;- IL-1, TNF α , TNF- β , IL-6, HMGP-1 and the chemokine CCL3 all cause fever
- Interferons are antiviral cytokines that are produced in response to viral infection or immune stimulation
- Their name is derived from the fact that they interfere with viral RNA and protein synthesis
- The two most important type I interferone are interferon alpha (IFN- α) and interferon

- Many interferons also play a role in the maintenance of pregnancy
- Tumor necrosis factor (TNFs) are cytokines derived from macrophages and T-cells
- As their name suggests they can kill tumor cells although this is not their primary function
- Thus TNF- α is the key mediator of acute inflammation
- Many cytokines serve as growth factor (or colony – stimulating factors) and control leukocyte production by regulating stem cell growth
- They thereby ensure that the body is

- Chemokines are a family of cytokines that play an important role in leukocyte circulation and migration especially in inflammation
- They act primarily as chemotactic factors and leukocyte activator
- A typical example of a chemokines is CXCL8 (also known as interleukin 8)
- A family of small (8-10kDa) proteins that control cellular migration
- Since they regulate the movement of specific cell populations, they can dictate the course of many inflammation and immune response
- Produced by divers cell types including Macrophage and mast cells
- At least 50 different chemokines have been identified (90-130 amino acid residues)

A comparison of cytokines and hormones

Property	Hormones	Cytokines
Sources	Specific endocrine glands	Many different cell types
Targets	Specific cell targets	Many different cell types
Functional redundancy	Very low	Very high
Effect	Endocrine	Autocrine Paracrine Endocrine
Function	Homeostasis	Tissue repair Resistance to infection

The Different Forms of Interferons

Class	Interferon	Source	Function
Type I	α	Dendritic cells	Antiviral
	β	Fibroblasts	Antiviral
	δ	Trophoblast	Pregnancy signaling
	ω	Trophoblast	Pregnancy signaling
	τ	Trophoblast	Pregnancy signaling
Type II	γ	Th1 cells	Immune activation

TABLE 12-4

The Tumor Necrosis Factors

Name	Produced Mainly by	Major Targets
TNF- β	T cells, other	Tumor cells
TNF- α	Macrophages, other	Endothelium

Some Selected Growth Factors

Name	Produced Mainly by	Major Targets
G-CSF	Fibroblasts, macrophages	Hematopoietic cells
GM-CSF	Fibroblasts, macrophages	Monocytes, stem cells
M-CSF	T cells, others	Hematopoietic cells
TGF- β	Platelets, nucleated cells	Many targets

θ **Properties of cytokines**

- A particular cytokines may bind to receptors on the membrane of the **same cell** that secrete it, exerting **autocrine action**
- It may bind to receptors on a **target cell** in close proximity to the **producer cell**, exerting **paracrine action**
- It may bind to **target cells** in **distant parts** of the body, exerting **endocrine action**
- **Cytokines** exhibit the attributes of **pleiotropic**, **redundancy**, **synergy**, **antagonism** and **cascade** induction
 - └ A given **cytokine** that has different biological effects on **different target cells** has a **pleiotropic action**
 - └ Two or more cytokines that mediate similar

- Cytokine **synergism** occurs when the combined effect of **two cytokines** on **cellular activity** is greater than the **additive effects** of the **individual cytokine**
- Cytokines, in some cases, exhibit **antagonism**; that is, the effects of **one cytokine** inhibit or offset the effects of **another cytokine**
- **Cascade induction** occurs when the action of **one cytokine** on a target cell induces that cell to produce one or more cytokines, which in turn may induce other target cell to produce **other cytokines**

θ **Biological Functions of cytokines**

- Involved in the development of cellular and humoral immune responses
- Induce inflammatory response
- Play a role in the regulation of hematopoiesis
- Control cellular proliferation and differentiation

Unit 7

Inflammatory barriers:

- Inflammation: The responses of tissues to invading microorganism or tissue injury /damage/
- it is also a vital protective process, because it is the by which defensive cells and molecules are concentrated rapidly at site of microbial invasion

Cardinal signs of inflammation ;

- *rubor* (redness)
 - *Calor* (heat)
 - *functio laesa* (loss of function)
- Tumor* (swelling)
Dolor (pain) and *functio laesa*

- *The cardinal signs of inflammation reflects the three major events of an inflammatory response*

1. vasodilation- an increase the diameter of blood vessel
2. An increase in capillary permeability
3. Influx of phagocytes from the capillaries into the tissues is facilitated by the increase capillary permeability

Inflammation and innate immunity

- Tissue damaged caused by a wound or by an invading pathogen microorganism induces a complex sequence of events collectively known as the inflammatory response.
- The cardinal signs of inflammation reflect the three major events of an inflammatory response
 1. **Vasodilatation**:- an increase the diameter of blood vessels of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict resulting in engorgement of the capillary networks.
 - The engorged capillaries are responsible for tissue redness (erythema) and an increase in tissue temperature.
 2. **An increase capillary permeability** facilitates an influx of fluid and cells from the engorged capillaries into the tissue.
 - The fluids that accumulate (exudates) have a much higher protein contents than fluid normally

- 3. Influx of phagocytes from the capillaries** into the tissue is facilitated by the increase capillary permeability.
- The emigration of phagocytes in a complex series of events, including adherence of the cell to the endothelial wall of the blood vessels (margination)
 - followed by their emigration between the capillary endothelial cell into the tissue (diapedesis or extravasations) and
 - finally their migration through the tissue to the site of the inflammatory response (Chemotaxis).
 - As phagocytic cell accumulate at the site and begin to phagocytose bacteria they release lytic enzyme, which can damage nearby healthy cells.
 - The accumulation of dead cells digested materials, and fluid forms a substance called pus.
- Inflammatory response is initiated by chemical mediators.
 - Some of these mediators are derived from
 - Invading microorganism, Some are released from damaged cells, in response to tissue injury
 - Some are generated by several plasma enzyme system, and some are product of various white blood cells participating in the inflammatory response

- Among the chemical mediator released in response to tissue damage are various serum protein called **acute phase proteins**.
- The concentration of these proteins increase dramatically in **tissue damaging** infection
- **C-reactive protein** is a major **acute phase protein** produced by the liver in response to tissue damage.
- Its name derives from the fact that it binds to the **C-polysaccharide** cell wall components found on a variety of bacteria and fungi.
- These bindings activate the **complement system** resulting in increased clearance of the pathogen either by **complement mediated lysis** or by complement mediated increase in **phagocytosis**.
- One of the principal mediators of the inflammatory response is **histamine**, a chemical released by a variety of cells in response to tissue injury

- Another important group of **inflammatory mediators**' small peptide called **kinins** is normally present in blood plasma in an inactive form.
- Tissue injury activate the peptide, which then cause **vasodilation** and increased permeability of capillaries
- A particular **kinin** called **bradykinin** also stimulates pain receptor in the skin (pain normally cause an individual to protect the injured area).
- Once the inflammatory response has subsided and most of the debris has been cleared away by **phagocytic cell**, tissue repair and regeneration of new tissue begin.
- Capillaries grow into the fibrin of a **blood clot**.
- New connective tissue cells called **fibroblast**

Extra cellular killing;-

- Alter feature of the membranes of abnormal cells such as those found on virus infected cells (cancer cell) are recognized by cytotoxic or killer cells that destroy target cells not by phagocytosis but by releasing biological potent molecules that within a very short time kill the target cells.
- Such killer cell include the antigen specific cytotoxic T-lymphocytes which constitute an arm of acquired immune response
- And the natural killer (NK) cells, a component of the innate immune system
- NK cells are large granular lymphocytes that are able to lyse certain virus infected cell and tumor cells without prior stimulation
- Killing is achieved by the release of various cytotoxic molecules
- Some of these molecules cause the formation of pores

- because they were found to interfere with virus replication in previously uninfected tissue culture cells.
- They are believed to have a similar role *invivo*, blocking the spread of viruses to uninfected cells.
- These antiviral effector molecules called interferon $INF-\alpha$ and β , and are quite distinct from interferon ($IFN-\delta$).
- $IFN-\delta$ this cytokine is not directly induced by viral infection but is produced later (T-cell and NK-cells) and does have an important role in adaptive immune response to *intracellular pathogen* (cellular immunity)
- Interferon: antiviral proteins produced by cell

- *By increasing the cellular immune response to these (viruses) pathogen, i.e. increase MHC-class I expression and antigen presentation in all cells*
- *Activate NK – cells to kill the infected cells.*

Killing of the invaders

- Once the invaders are recognized, they will be destroyed by:
 - i) Phagocytosis
 - ii) Antimicrobial molecules
- i) Phagocytosis
 - Cellular uptake of invaders by engulfment
 - Performed by phagocytes (Neutrophils, monocytes and Macrophages)
 - Neutrophils are martyrs of the immune system.
 - Reasons-Reach and attack invaders 1st
 - In dying they attract macrophage to the site of invasion

- Macrophage can undertake sustained, repeated phagocytic activity.
- Destruction mechanisms:
 - 1) Oxidative mechanism
 - 2) Nonoxidative mechanism

Oxygen-dependent killing

Reactive oxygen intermediates

$O_2^{\bullet -}$ (superoxide anion)

$OH^{\bullet}$ (hydroxyl radicals)

H_2O_2 (hydrogen peroxide)

ClO^- (hypochlorite anion)

Reactive nitrogen intermediates $ONOO^-$ (Peroxynitrite)

NO (nitric oxide)

NO_2 (nitrogen dioxide)

Oxygen-independent killing

Defensins

Tumor necrosis factor α
(macrophage only)

Lysozyme

Hydrolytic enzymes

Antimicrobial molecules

- Antimicrobial peptides
 - More than 800 have been identified to date
 - Have a net cationic charge and the ability to form amphipathic structures (i.e., have both hydrophobic and hydrophilic regions)
 - Hydrophobic regions can insert themselves into the lipid-rich membranes of bacteria
 - Hydrophilic regions can form channel-like pores or simply cover the membrane
 - Resulting in membrane disruption and microbial death
 - Can kill most Spps of bacteria, some fungi, protozoa, enveloped viruses and tumor cells

→ Kills microbes, within minutes (e.g. Defensins) up to 90 minutes (slow acting antimicrobial peptides).

→ Mechanisms of actions

- └ Disruption of microbial membranes
- └ Inhibit synthesis of DNA, RNA and Proteins
- └ Activate antimicrobial enzymes that lyse components of the microbe

→ Potential for therapeutic use

- θ The breadth of the above activities
- θ Their proven antimicrobial effectiveness
- θ The increasing emergence of resistance to the existing antibiotics

has stimulated investigation of their suitability

- However, questions remain about their in vivo stability, toxicity and efficacy when administered in a clinical setting
- They are not yet in clinical use
 - Reason- Fear of development of resistance to them
- Example:
 - Defensins (α , β and Θ), Cathelicidins
 - Serprocidins
 - Granulysins

Lysozyme

- Cleaves the bond between N-acetyl muraminic acid and N-acetyl glucosamine.
 - Destroys the peptidoglycans of G+ bacteria
- Found in all body fluids except CSF and urine
- Found in high concentration in tear and egg white

A potent innate opsonin (bind to bacteria and

Pentraxins; The family includes C-reactive protein (CRP), and

Serum amyloid P (SAP) (Acute-phase proteins)

- It is a serum protein and the concentration increase dramatically in tissue damage infection
- C-reactive protein is the major acute phase protein produced by liver in response to tissue damage
- Function: - Opsonization
 - Activate the classical complement pathway

Collectins

- The family includes
 - Mannose-binding lectin (MBL)
 - Pulmonary surfactant proteins SP-A and SP-D
- Function: -Opsonization
 - Activate the complement pathway (MBL)