Chapter II : Innate immun response

****I- Introduction****

The innate immune response is the body's first line of defense against aggression (microbial invasion, tissue injury, physical or chemical burns, etc.). It provides a rapid and effective response against a wide range of pathogens. Additionally, it plays a crucial role in initiating adaptive immune responses and in tissue repair and wound healing processes. This innate response is activated immediately at the site of aggression, both in tissues and in the bloodstream, ensuring optimal efficiency.

Unlike adaptive immunity, the innate immune response is not specific to a particular antigen and does not have memory. Most cells involved in innate immunity originate from the myeloid lineage. These include polymorphonuclear cells (granulocytes) and mononuclear phagocytes (monocytes, macrophages, and dendritic cells). There are also lymphoid-derived cells, such as Natural Killer (NK) cells, Innate Lymphoid Cells (ILCs), and plasmacytoid dendritic cells. All these cells are described in detail in Chapter 1. In this chapter, we will examine the individual components of the innate immune system and their roles in defending the host against infections.

****II- Components of Innate Immunity****

The innate immune system consists of epithelial barriers that prevent infections, circulating and tissue-resident immune cells, and several plasma proteins. These components play different but complementary roles in blocking microbial entry and eliminating pathogens that manage to invade host tissues.

****II-1 Epithelial Barriers****

The most common entry points for microbes—the skin, the gastrointestinal tract, and the respiratory tract—are protected by continuous epithelial layers that serve as physical and chemical barriers against infections (Figure 1).

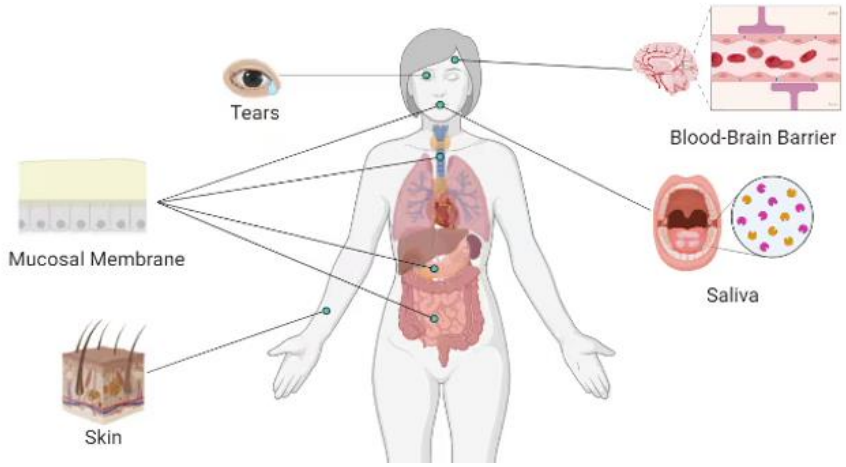

Figure 1: Physical Barriers in innate immunity

Microbes can enter the host from the external environment through these interfaces via physical contact, ingestion, or inhalation. These three entry points are lined with continuous epithelial layers that physically prevent microbial invasion.

Epithelial cells also produce antimicrobial peptides that kill bacteria (Figure 2). Additionally, epithelial tissues contain a specific type of lymphocytes called intraepithelial lymphocytes, which belong to the T cell lineage but express antigen receptors with limited diversity.

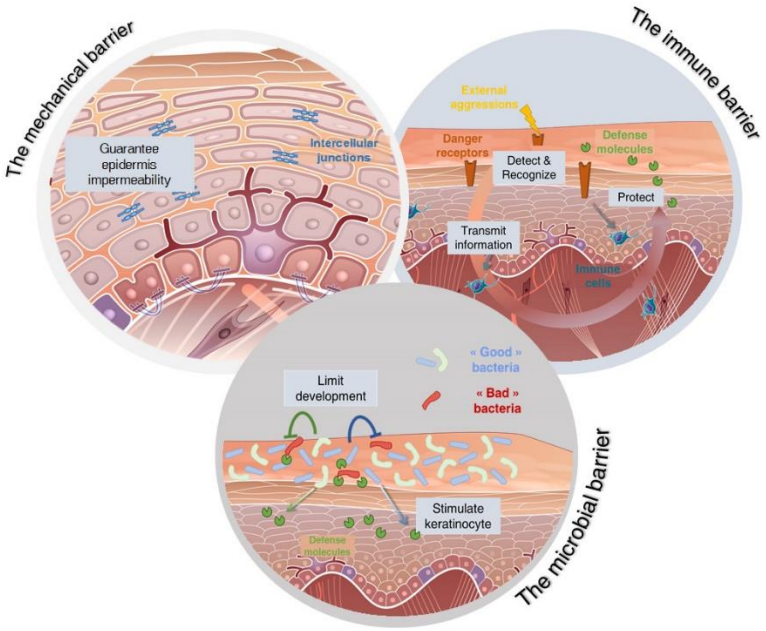

Figure 2: Epithelial barriers function in innate immunity

Some of these T lymphocytes express receptors composed of two chains, known as γ and δ chains, which are similar but not identical to the highly diverse αβ receptors found on most T lymphocytes. Intraepithelial lymphocytes, particularly γδ T cells, frequently recognize microbial lipids or other microbial structures that are shared among similar types of microbes.

These intraepithelial lymphocytes appear to act as sentinels against infectious agents attempting to cross epithelial barriers. However, their specificity and functions remain poorly understood.

II-2 Phagocytes: Neutrophils and Monocytes/Macrophages

The two main types of circulating phagocytes, neutrophils and monocytes, are blood cells recruited to sites of infection, where they recognize, engulf, and destroy microbes within the cell.

- **Neutrophils** (also known as granulocytes, polymorphonuclear cells, or PMNs) are the most abundant leukocytes in the blood, with a count ranging between 4,000 and 10,000 per µL (Figure 3). In response to infections, neutrophil production in the bone marrow increases rapidly, and their count can reach up to 20,000 per µL of blood.
- Neutrophil production is stimulated by cytokines known as **colony-stimulating factors**, which are produced by various cell types in response to infections. These cytokines act on bone marrow stem cells to promote the proliferation and maturation of neutrophil precursors.

- Neutrophils are the first cells to respond to most infections, particularly bacterial and fungal infections. They ingest microbes in the bloodstream and rapidly migrate into extravascular tissues at infection sites, where they continue to engulf microbes before dying within a few hours.
- **Monocytes** are less numerous than neutrophils, with a count ranging between 500 and 1,000 per μL of blood (Figure 4). Like neutrophils, they ingest microbes in both the bloodstream and tissues. However, unlike neutrophils, monocytes that migrate into extravascular tissues can survive for extended periods. Once in the tissues, these monocytes differentiate into cells known as **macrophages** (see Figure 4).
- **Blood monocytes and tissue macrophages** represent two stages of the same cell lineage, often referred to as the **mononuclear phagocyte system**. Resident macrophages are found in connective tissues and throughout all organs of the body, where they perform the same functions as newly recruited mononuclear phagocytes from the bloodstream.

Phagocytosis

Phagocytosis is a process in which the phagocyte extends its plasma membrane around a recognized microbe, enclosing it and forming a vesicle known as a **phagosome**. The phagosome then fuses with lysosomes to create **phagolysosomes (Figure 3)**.

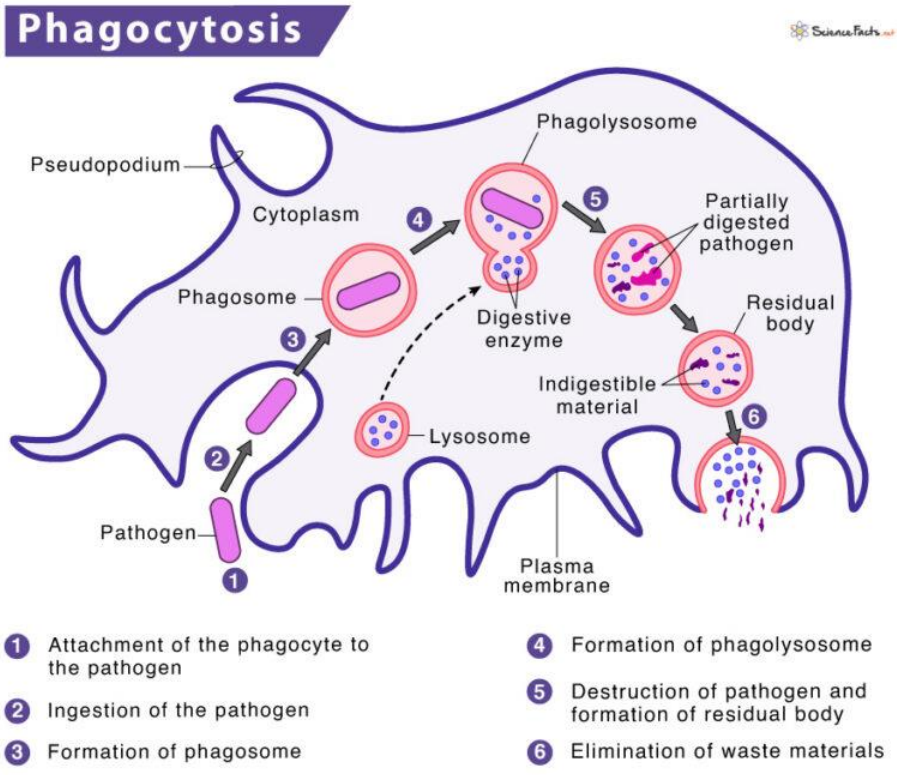

Figure 3: Phagocytosis mechanism

At the same time, microbial binding to phagocyte receptors triggers signals that activate several enzymes within the phagolysosomes:

One of these enzymes, **phagocyte oxidase**, converts molecular oxygen into superoxide anions and free radicals. These molecules, known as **reactive oxygen intermediates (ROIs),** are toxic to ingested microbes. Another enzyme, **inducible nitric oxide synthase (iNOS),** catalyzes the conversion of arginine into nitric oxide (NO), which also has microbicidal properties. A third group of enzymes, **lysosomal proteases**, degrade microbial proteins (Figure 4).

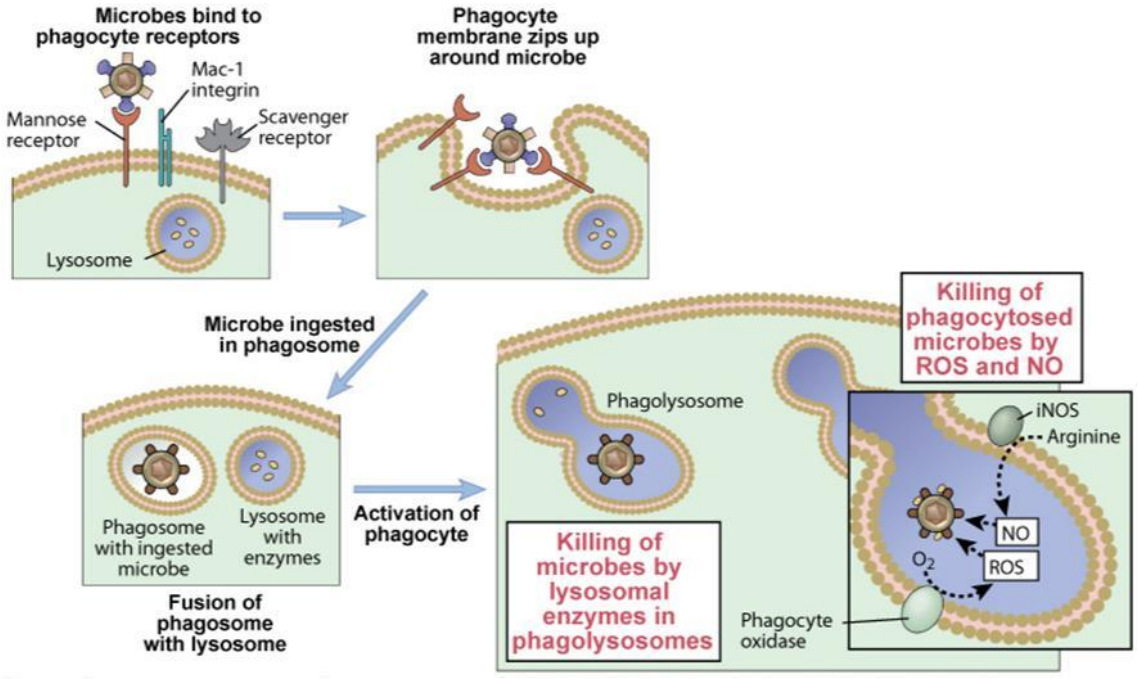

Figure 4: Intracellular Destruction of microbes during phagocytosis

These microbicidal substances are primarily produced in lysosomes and phagolysosomes, where they act on ingested microbes without harming the phagocytes themselves (Figure 5). However, during strong immune responses, these same enzymes can be released into the extracellular environment, potentially causing **tissue damage**. This explains why inflammation, though normally a protective response against infections, can sometimes lead to tissue injury.

In addition to eliminating phagocytosed microbes, **macrophages** perform several crucial functions in the body's defense against infections (see Figure 6):

- They produce **cytokines** that recruit and activate leukocytes.
- They secrete **growth factors and enzymes** that help repair damaged tissue and replace it with connective tissue.
- They stimulate **T lymphocytes**, thereby amplifying the adaptive immune response.
- They also respond to T lymphocyte signals and function as **effector cells** in cell-mediated immunity.

II-3 Dendritic Cells

Dendritic cells respond to microbes by producing **cytokines** that recruit leukocytes and trigger adaptive immune responses. These cells play a crucial role in bridging innate and adaptive immunity. In fact, they are the **primary antigen-presenting cells (APCs)** for T lymphocytes, which is one of their key functions.

II-4 Natural Killer (NK) Cells

- **Natural killer (NK) cells** are a class of lymphocytes that recognize infected or stressed cells and respond by **killing** them and secreting **IFN-γ**, a cytokine that activates macrophages (Figure 5).

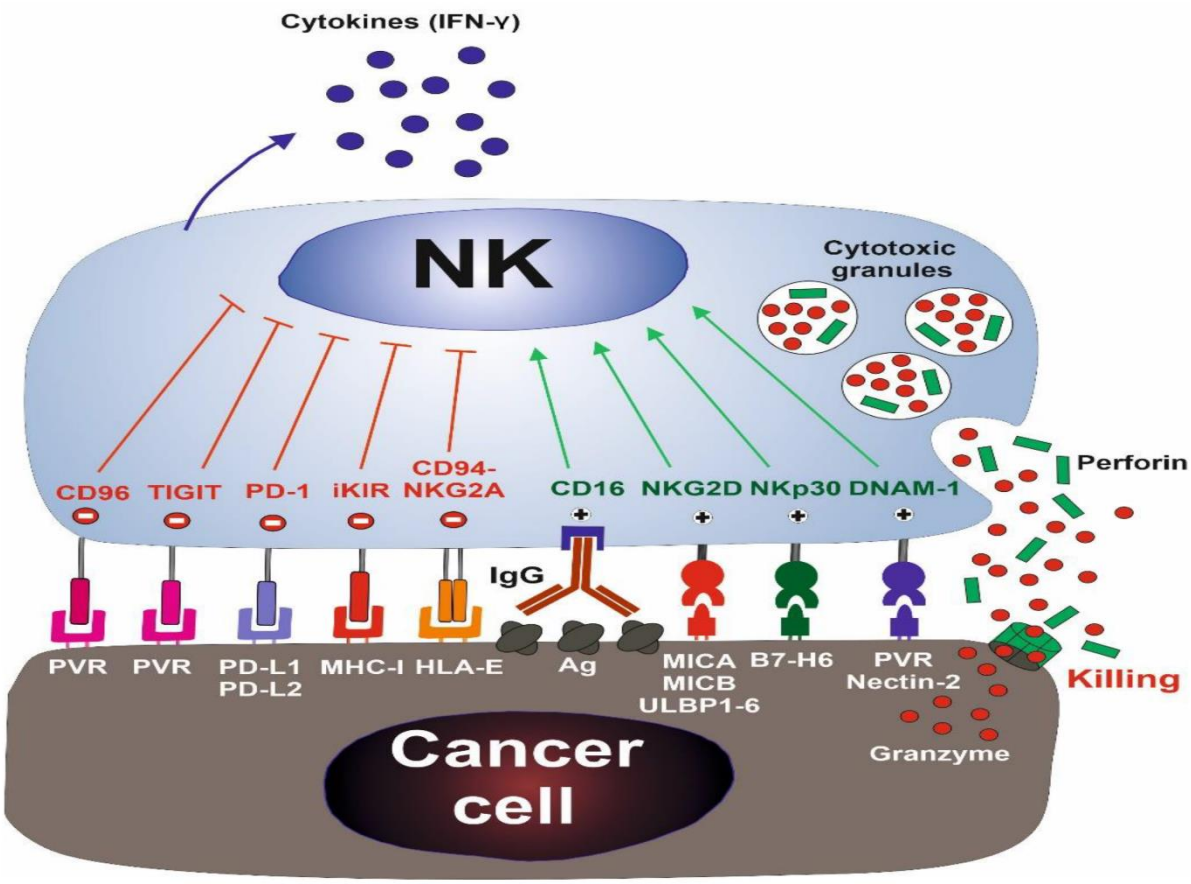

Figure 5: Natural Killer cells

- NK cells make up about **10% of lymphocytes** in the blood and peripheral lymphoid organs. They contain **abundant cytoplasmic granules** and express **specific surface markers**, but they **do not express immunoglobulins or T cell receptors**, which are the antigen receptors of B and T lymphocytes, respectively.
- Upon activation, NK cells release **granule proteins** into infected cells. These proteins include **pore-forming molecules** that disrupt the plasma membrane and **enzymes** that enter the cell to induce **apoptotic death**.
- The **cytolytic mechanisms** used by NK cells are the same as those employed by **cytotoxic T lymphocytes (CTLs)** to kill infected cells.

Additionally, activated NK cells **synthesize and secrete IFN-γ**, which enhances macrophage activity, increasing their ability to destroy phagocytosed microbes. Thus, **NK cells and macrophages work together** to eliminate intracellular microbes:

- **Macrophages** ingest microbes and produce **IL-12**.
- **IL-12 stimulates NK cells** to secrete **IFN-γ**.
- **IFN-γ then activates macrophages**, enabling them to efficiently destroy ingested microbes.

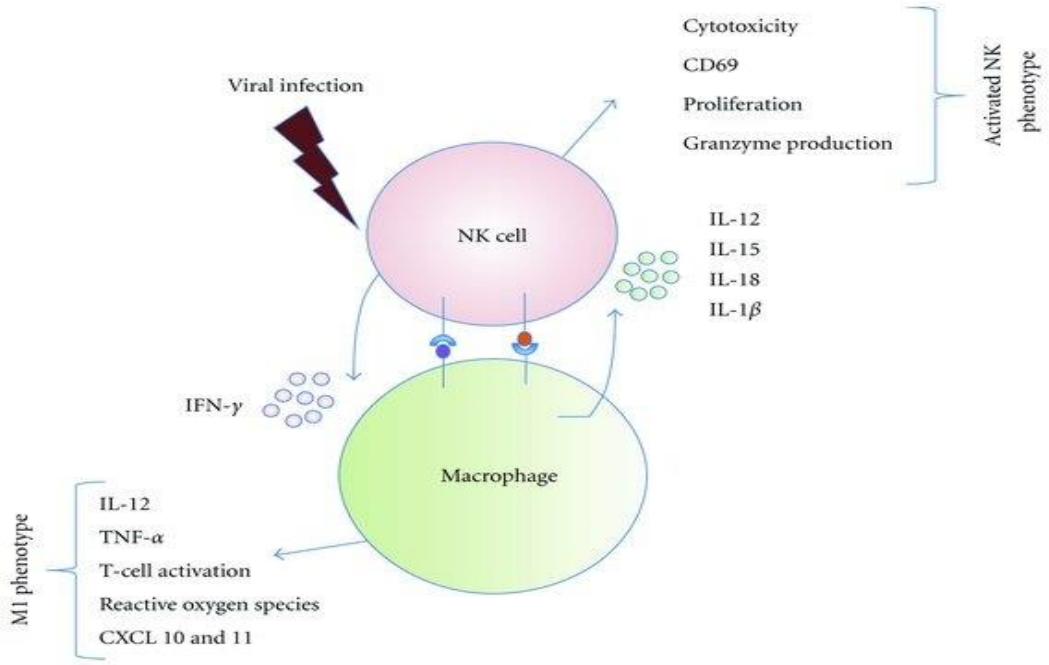

Figure 6: Reverse activation and interaction between Natural killers and macrophages

Complement System

The **complement system** consists of **proteins mainly synthesized by the liver**, circulating in **plasma** or present on **cell surfaces**. It is part of **innate immunity**, and its activation relies on **physicochemical interactions**.

Activation Pathways of the Complement System

Three major pathways can activate the complement system:

1. **The classical pathway**: Initiated when **C1q** binds to **antibody-antigen complexes** (mainly **IgG1, IgG2, IgG3, and IgM**). This leads to the activation of **C1r and C1s**, which cleave **C4 and C2**, forming **C3 convertase (C4b2a)**.
2. **The lectin pathway**: Triggered by **carbohydrates on microbes or apoptotic cells**. It functions similarly to the classical pathway but uses **Mannose-Binding Lectin (MBL)** and associated **MASP-1 and MASP-2 enzymes** to cleave **C4 and C2**, forming the same **C3 convertase (C4b2a)**.
3. **The alternative pathway**: Activated by **bacterial LPS, Gram-positive bacteria, viruses, or infected/transformed cells**. Here, **C3b** binds to **factor B**, which is cleaved by **factor D**, forming the **alternative C3 convertase (C3bBb)**.

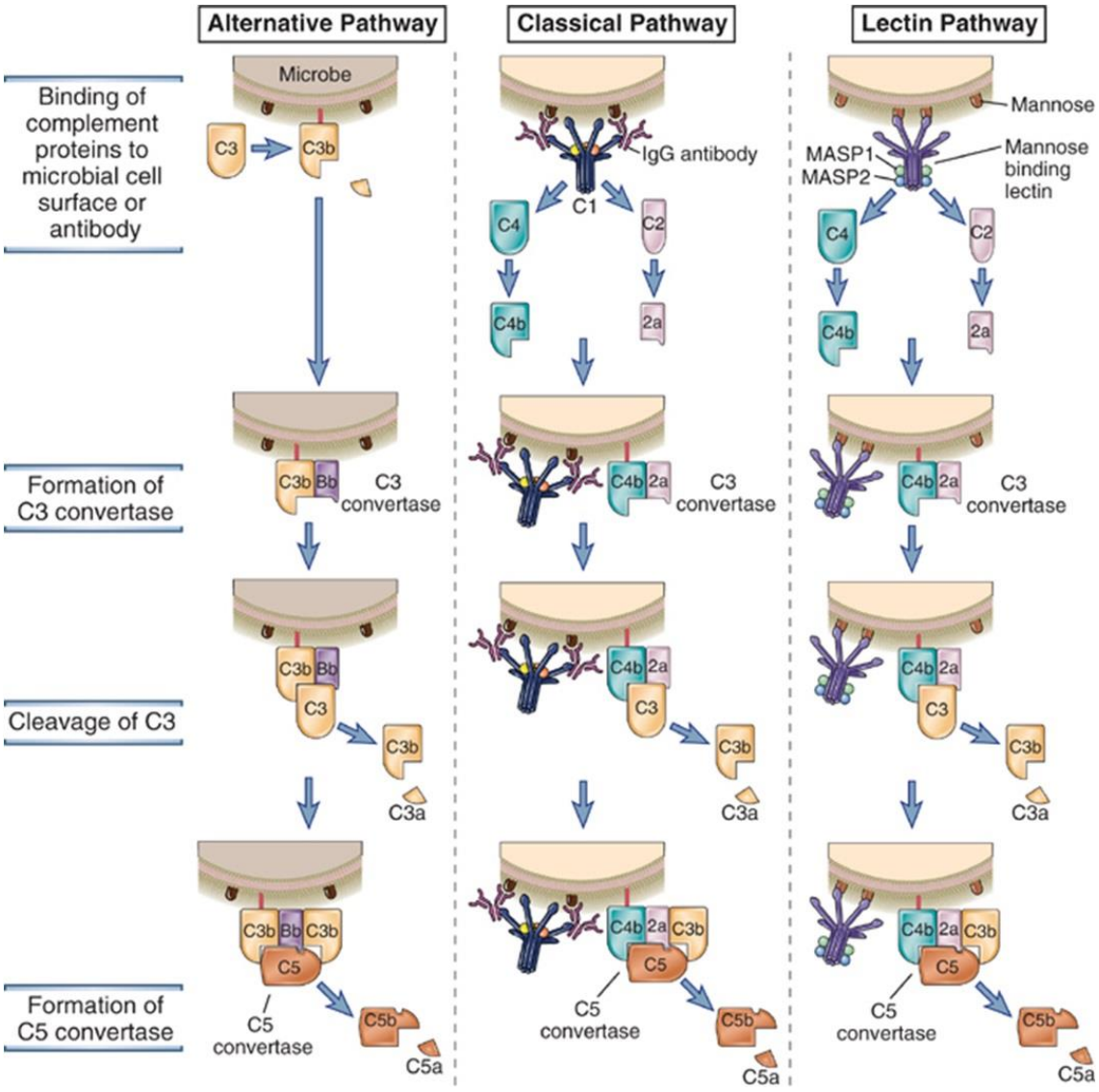

Figure 7: The three pathways of Complement activation

Effector Functions of the Complement System

Once activated, the complement system **defends against infections** using three key mechanisms:

1. **Opsonization**: **C3b** coats pathogens, enhancing **phagocytosis** by neutrophils and macrophages.
2. **Inflammatory recruitment**: **C3a and C5a** act as **anaphylatoxins**, recruiting and activating **immune cells** to intensify inflammation.
3. **Membrane Attack Complex (MAC) Formation**:
    - **C5b binds to C6, C7, and C8**, anchoring to the pathogen's membrane.
    - Multiple **C9 molecules** polymerize, forming **pores** in the membrane.
    - This **osmotic lysis** leads to the **death of the pathogen or infected cell**.

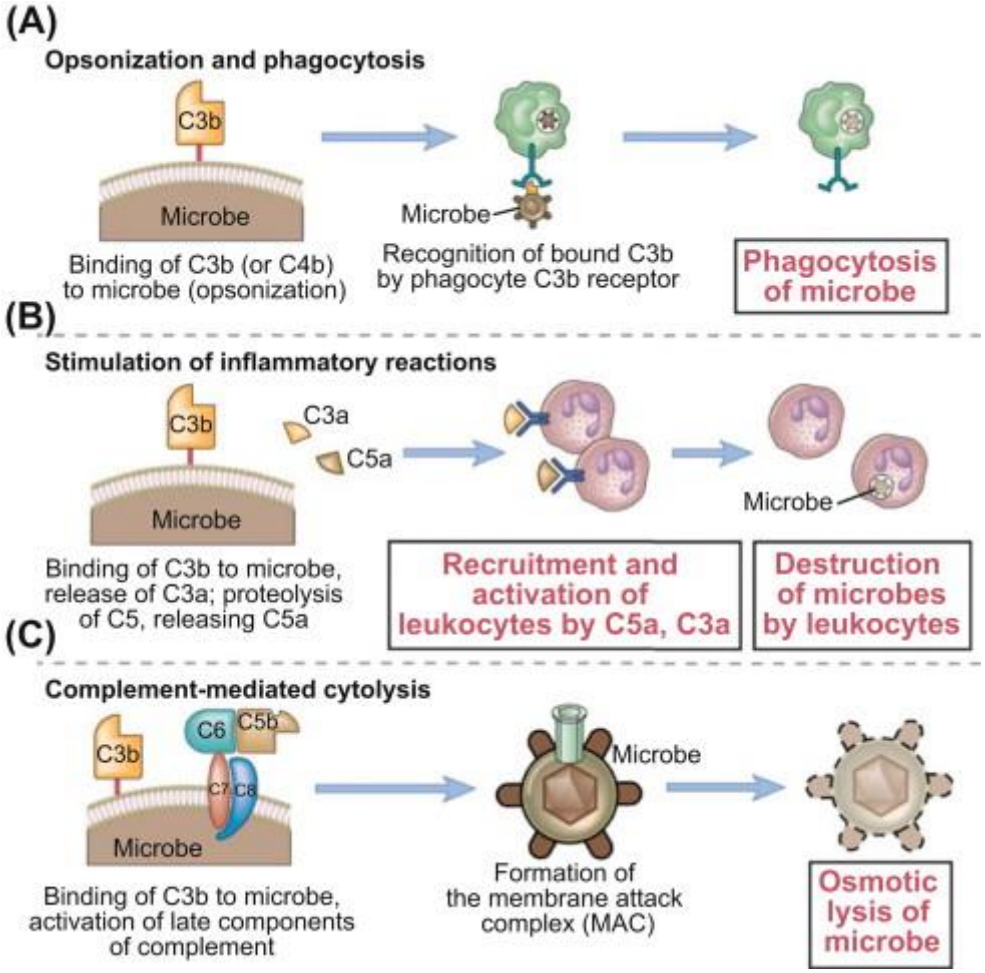

Figure 8: Different Functions of complement system

Final Common Pathway

- Both **C3 convertases (C4b2a and C3bBb)** cleave **C3 into C3a and C3b**.
- C3b binds to form a **C5 convertase**, which cleaves **C5 into C5a (a potent anaphylatoxin) and C5b**.
- C5b initiates the formation of the **Membrane Attack Complex (C5b-C6-C7-C8-C9)**, leading to **pathogen lysis**.

Cytokines of Innate Immunity

- Cytokines are **soluble or membrane-bound mediators** that enable communication between cells. During the **innate immune response**, all immune cells, as well as **epithelial and endothelial cells**, can produce cytokines. The main types of cytokines include:
    - **Pro-inflammatory cytokines**, such as **TNF, IL-1, IL-6, IL-12, IFN-α, IFN-β, IFN-γ, and IL-15**.
    - **Chemoattractant cytokines (chemokines)**, such as **CXCL8 (IL-8)**.
    - **Regulatory cytokines** that control inflammation, such as **IL-10 and TGF-β**.

Examples are shown in the following table:

Table 1: Main cytokines with their actions and sources

Fig. 8.4 Important cytokines and their actions

Cytokine	Main sources	Main actions
IL-1	Macrophages	Fever T-cell and macrophage activation
IL-2	T helper 1 cells	Growth of T cells Stimulates growth of B cells and NK cells
IL-4	T helper 2 cells	Activation and growth of B cells IgG1, IgE and MHC class II induction of B cells Induces CD4 T cells to differentiate into T helper 2 cells
IL-6	Macrophages	Lymphocyte activation Increased antibody production Fever, induces acute phase proteins
IL-8	Macrophages	Chemotactic factor for neutrophils Activates neutrophils
IL-10	T helper 2 cells Macrophages	Inhibits immune function
IL-12	Macrophages	Activates NK cells Induces CD4 T cells to differentiate into T helper 1 cells
IL-17	T helper 17 cells	Proinflammatory Recruits neutrophils
Interferon-γ	T helper 1 cells NK cells	Activation of macrophages and NK cells Produces antiviral state in neighbouring cells Increases expression of MHC class I and II molecules Inhibits T helper 2 cells
TNF	T helper cells Macrophages	Activates macrophages and induces nitric oxide production Proinflammatory Fever and shock

IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; TNF, tumour necrosis factor.

- It is important to note that although **neutrophils produce fewer cytokines** than monocytes or macrophages, they are the **first immune cells to infiltrate an**

inflammatory site in large numbers. Their **local production of cytokines** plays a crucial role in the **early stages of the innate immune response**.

- The **targets** of innate immunity cytokines include:
    - **Innate immune cells themselves**, promoting **self-sustaining inflammation and regulation**.
    - **Organs**, such as:
        - The **liver**, which synthesizes **acute-phase proteins** (e.g., **C-reactive protein (CRP)**).
        - The **hypothalamus**, which induces **fever**.
        - **Endothelial cells**, which activate the **coagulation process**.

Other Plasma Proteins and Soluble Mediators of Innate Immunity

Antimicrobial Enzymes and Peptides

- **Neutrophils and mast cells** rapidly release **antimicrobial and inflammatory proteins** via **granular exocytosis**.
- These proteins have different mechanisms of action:
    - **Direct antimicrobial effects**, such as **proteases** and **myeloperoxidase**.
    - **Indirect effects**, such as **lactoferrin**, which **sequesters essential nutrients** needed by microbes.
    - **Degrading the extracellular matrix**, via **elastase and metalloproteases**, which contributes to the inflammatory response.

Other Soluble Mediators

- **Lipid Mediators of Inflammation**:
    - Produced **de novo** from **membrane phospholipids** by **innate immune cells** upon activation.
    - Includes **leukotrienes, prostaglandins, and Platelet-Activating Factor (PAF)**.
    - These molecules have **diverse effects**, such as:
        - **Endothelial activation**.
        - **Recruitment of immune cells (chemotaxis)**.
        - **Pain signaling (nociception)**.
- **Histamine**:
    - Released by **mast cells and basophils** during **degranulation**.

    - Causes **vasodilation** and **increased capillary permeability**, facilitating the **recruitment of immune cells** to the site of infection or injury.

- **Substance P**:
    - A **neuropeptide** produced by **mast cells** and other cells.
    - Contributes to **pain signaling**, acting as a mediator of **inflammatory pain responses**.