SHEET NO. 8

IMMUNOLOGY

DOCTOR 2019 | MEDICINE | JU

DONE BY : **Doctor 2018**

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

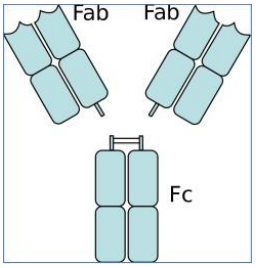
DOCTOR : **Dr. Anas**

Outline:

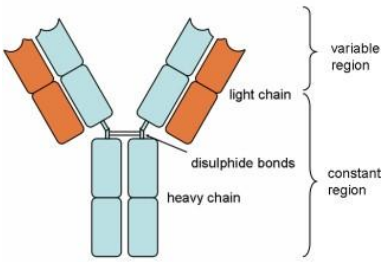
- ***Antibodies.***
- ***Innate immunity response to extracellular pathogens:***
  - *Complement activation.*
  - *Phagocytosis; macrophages and dendritic cells.*
  - *Adaptive immunity activation.*
- ***Innate immunity response to intracellular pathogens:***
  - *Natural killer cells.*

Antibodies

- Antibodies (immunoglobulins) are made up of:

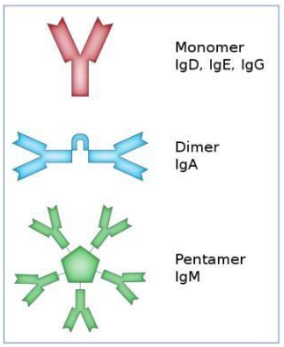


  a- **Antigen-binding region (Fab):** 2 heavy chains and 2 light chains, those chains combined give us Fab which binds to antigens. Random recombination of the genes encodes different antigen-binding sites; thus, it is the **variable** part of the antibody.



  b- **Fragment crystallizable region (Fc):** 2 heavy chains make up the tail region of an antibody that interacts with **cell surface receptors** *(Fc receptors)* and some proteins of the **complement system**. The Fc region is **constant** in contrast to Fab.

- Immunoglobulins can come in different varieties known as **isotypes** or classes. Those of different classes **differ** in their **location** around the body and appear at **different stages** of an adaptive immune response.



- There are **5** known isotypes: IgM, IgG, IgA, IgD, IgE.
- **IgM** is the first immunoglobulin expressed during **B cell** development as a monomer on the surface of B naive cells.

Antibodies Functions:

1. Neutralization of infectivity.
2. Phagocytosis.
3. Antibody-dependent cellular cytotoxicity (ADCC).
4. Complement-mediated lysis of pathogens or of infected cells.
5. Transcytosis, mucosal immunity, and neonatal immunity.

Innate Immunity Response to Extracellular Pathogens

In the last lecture, we discussed the role of the **epithelial barrier** acting as the **first line** of defense against invading pathogens. If extracellular pathogens manage to penetrate this barrier, the **innate immunity** acts against it through multiple mechanisms:

1- Activation of the **complement cascade**: this cascade enhances the ability of **antibodies** and **phagocytic** cells to **clear** microbes, **promote inflammation**, and **attack** the pathogen's cell membrane.

2- Identification and removal of the pathogens by **phagocytosis**:

Recall: phagocytes use various surface receptors, including **mannose receptors, scavenger receptors, TLRs, and PRRs,** to recognize extracellular bacteria.

They also use Fc receptors and complement receptors to recognize bacteria opsonized with antibodies and complement proteins, respectively.

3- Recruiting immune cells to sites of infection **'inflammation'** through the production of **cytokines**: **dendritic cells** and **phagocytes** that are activated by the microbes secrete those cytokines.

4- **Activation** of **the adaptive immune system** through antigen presentation by APCs.

Complement cascade: *'discussed before'*

The cascade can be **activated** via **three** different pathways:

a- Classical Pathway

This pathway is triggered by **antibody-antigen complexes** binding to **C1**, which itself has three subcomponents C1q (which binds to Fc), C1r and C1s, forming a C3 convertase (**C4b2a**).

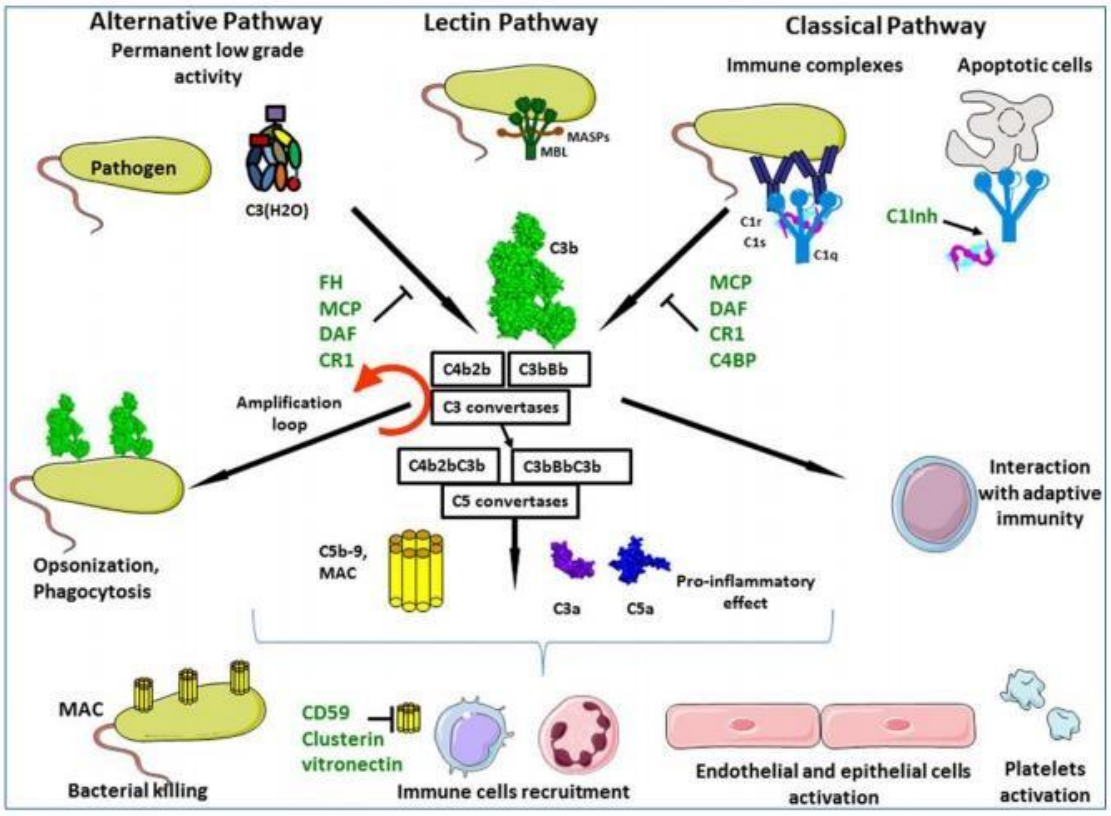
b- Alternative Pathway

This pathway is triggered when the **C3b protein directly binds a microbe**, forming a C3 convertase (**C3bBb**) which can activate more C3, hence the pathway is sometimes called 'the amplification loop'.

Activation of the loop is promoted in the presence of bacterial and fungal cell walls but inhibited by molecules on the surface of normal mammalian cells.

c- Mannose-binding Lectin Pathway

This pathway is activated by the **binding** of mannose-binding lectin (**MBL**) to **mannose residues** on the pathogen surface. This, in turn, activates the MBL-associated **serine proteases**, MASP-1 and MASP-2, which activate C4 and C2, to form the C3 convertase (**C4b2a**).



⇨ Each pathway ends up forming a **C3 convertase** which activates **C3** by splitting it into 2 fragments**:**

1- **C3b** (the large fragment), attaches to pathogens and opsonize them.
2- **C3a** (the small fragment), activates mast cells promoting inflammation.

⇨ Activated C3 can trigger the '**Lytic pathway'**, this pathway is initiated by the splitting of **C5** into:

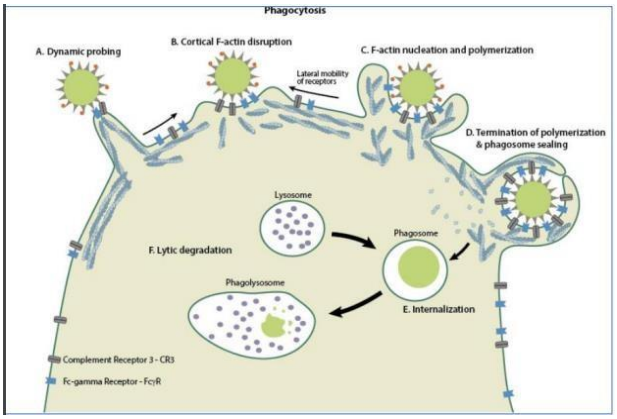
1- **C5b**: which unites with C6, C7, C8, and C9 on the target's surface. This membrane-attack complex (**MAC**) contributes in **lysing** the pathogens' membrane promoting its death.
2- **C5a**: attracts **macrophages** and **neutrophils** and also activates **mast cells**.

Professional phagocytes

- Cells that have specialized phagocytic functions, primarily **macrophages** and **neutrophils**, are part of the first line of defense against microbes that breach epithelial barriers.
- They serve several functions:
  1- **Internalize and kill microbes**. Neutrophils and macrophages are particularly good at this function.
  2- **Producing various cytokines** that promote inflammation. **Macrophages** are particularly good at this.
- Bacterial and fungal infections in patients with **low** blood neutrophil count caused by bone marrow cancers or cancer therapy, or inherited deficiencies, are **lethal**. This reflects the essential role that phagocytes play in innate immunity defense against microbes.

1- Macrophages:

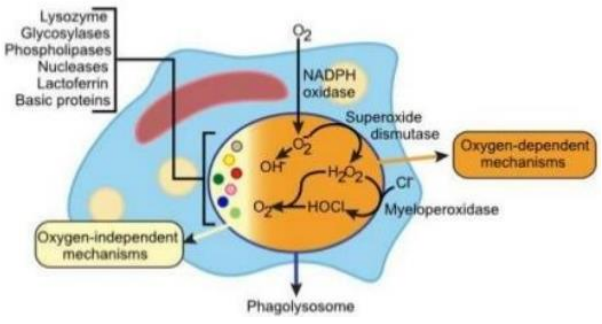
- Macrophages surface contains **Complement receptors** *(for opsonized antigens)* and **Fc-gamma receptors** *(for antibody-coated antigens)*. The binding of a pathogen to these receptors induces nucleation and polymerization of **F-actin** which forms a phagosome, seals it, and internalize it. A lysosome then binds to the phagosome forming a phagolysosome in which lytic degradation of the pathogen takes place using either:



  a- Oxygen-independent mechanisms: lysozymes, phospholipases, nucleases, etc.

  b- Oxygen-dependent mechanisms:

  Binding of **Fc receptors** causes an **increase** in **oxygen** uptake by the phagocyte. This influx of oxygen, called the **respiratory burst**, is used in a variety of mechanisms to cause damage to microbes inside the phagolysosome, but the common theme is the creation of **highly reactive** small molecules that damage the biomolecules of the pathogen.



- Macrophages are **plastic cells** *(able to switch between different phenotypes)*, different stimuli will affect macrophage phenotypes differently.

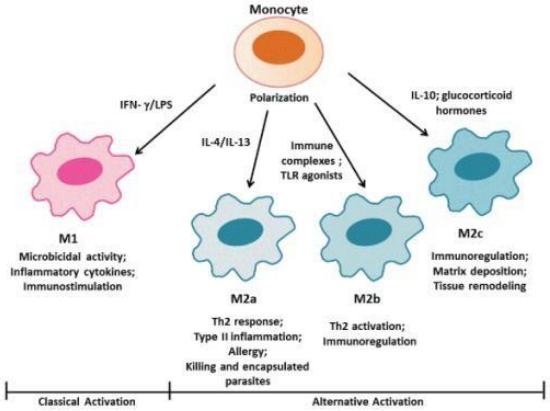
- Macrophages are found in **all tissues** exhibiting great **functional diversity**. They have roles in development, homeostasis, tissue repair, and immunity.
- Generally, it is considered that **embryonic-derived** macrophages play a strong role in the **maintenance** of tissue homeostasis and that **macrophages** derived from **bone marrow** monocytes are related to host defense reactions and **inflammatory** diseases.
- Unlike neutrophils, macrophages are **not** terminally differentiated and do not undergo cell division at an inflammatory site. Therefore, macrophages are the **dominant** effector cells of the **later stages** of the innate immune response, several days after infection.
- Macrophages are categorized as:

1- **M1**: activated by the invasion of pathogens to destroy them.

Induced by: PAMPs, DAMPs, and inflammatory cytokines such as TNF-α and IFN-γ.

2- **M2**: cause chronic inflammation because of allergic reactions, fat metabolism, wound healing, and cancer invasion and metastasis.

Induced by: anti-inflammatory cytokines such as IL-10, and IL-13.

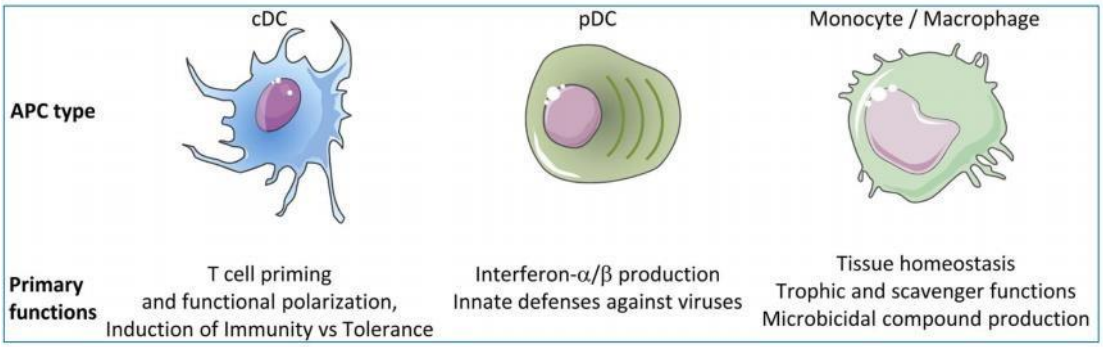


2- Dendritic Cells (DCs):

- Heterogeneous family of **bone marrow-derived** cells with long dendrite-like cytoplasmic processes are constitutively present in epithelia and most tissues of the body.
- They are the **most versatile sensors** of **PAMPs** and **DAMPs** among all cell types in the body.
- **TLR** signaling induces **dendritic cell** expression of molecules, including co-stimulatory molecules and cytokines that are needed, in addition to antigen, for the **activation** of the **naive T cells**. Activation into effector **T cell subtypes** depends on the **nature** of the **pathogen**.
- DCs include two main cell types:

1- **Plasmacytoid DC** (pDC): expert in **type I interferon** synthesis upon **viral stimulation**.

2- **Conventional DC** (cDC): specialized in **antigen** capture, processing, and presentation **for T-cell priming**.



Adaptive Immunity Role

- If neither the complement system nor the phagocytes eliminated the pathogen, the **adaptive immunity** *(acquired immunity)* is activated.
- **Macrophages** and **dendritic cells** function as antigen-presenting cells (**APCs**). They present peptide antigens derived from digested bacteria on the **MHC-II** and activate acquired immunity by activating **helper T cells**.
- While **macrophages** present antigens within **tissues**, **dendritic** cells present antigens in the **lymph node**. Only **dendritic** cells can **activate naïve T cells** to become effector T cells and are the **most powerful APCs**.

This figure summarizes the 'adaptive' immune response to extracellular microbes.

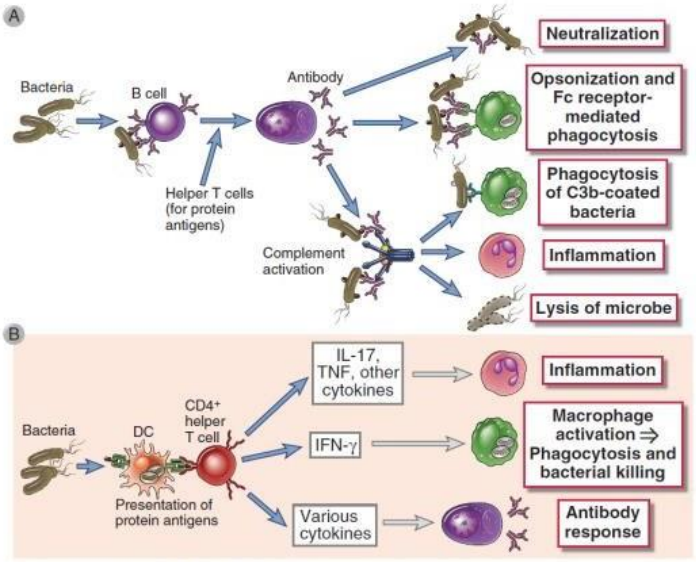


FIGURE 15–1 Adaptive immune responses to extracellular microbes. Adaptive immune responses to extracellular microbes such as bacteria and their toxins consist of antibody production **(A)** and the activation of $CD4^+$ helper T cells **(B)**. Antibodies neutralize and eliminate microbes and toxins by several mechanisms. Helper T cells produce cytokines that stimulate inflammation, macrophage activation, and B cell responses. DC, dendritic cell.

This marks the end of 'Innate Immunity Response to Extracellular Pathogens', which is either by:

1- *The complement system and phagocytes.*
2- *Activation of adaptive immunity.*

Innate Immunity Response to Intracellular Pathogens

- Innate immunity has a brief role since intracellular pathogens are mainly dealt with adaptive immunity. The major protective immune response against **intracellular** bacteria is **T cell-mediated immunity**.
- The innate immune response to intracellular bacteria is mediated mainly by **phagocytes** and **natural killer (NK) cells**.
- Products of bacteria are **recognized** by **TLRs** and **cytoplasmic proteins** of the NOD-like receptor (**NLR**) family, resulting in **activation** of the phagocytes.
- Phagocytes, **initially** neutrophils and **later** macrophages, ingest and attempt to destroy these microbes, but pathogenic intracellular bacteria are **resistant** to **degradation** within **phagocytes**.

Natural killer cells (NK):

- NK are **lymphocytes** important in innate immunity. The term **natural killer** derives from the fact that these cells are capable of performing their killing function **without** a need for **clonal expansion** or **differentiation**.
- NK cells are unique as they distinguish **infected** and **stressed** cells from **healthy** cells in the **absence** of **antibodies**, allowing for a much faster immune reaction.
- Natural killer cell **activation** is determined by the **balance** of inhibitory and activating receptor stimulation. For example, if the **inhibitory** receptor signaling is **more** prominent, then NK cell activity will be **inhibited**; similarly, if the **activating** signal is **dominant**, then NK cell **activation** will result.

a- Activating receptors:

In general, the activating receptors recognize ligands on **infected** and **injured cells**.

Intracellular bacteria stimulate dendritic cells' and macrophages' production of IL-12 and IL-15, both of which are NK cell-activating cytokines.

b- Inhibitory receptors:

Inhibitory receptors recognize **healthy** normal cells.

Regular cells express **MHC-I**. NK cells express inhibitory receptors that recognize MHC-I, thus it won't act on normal cells.

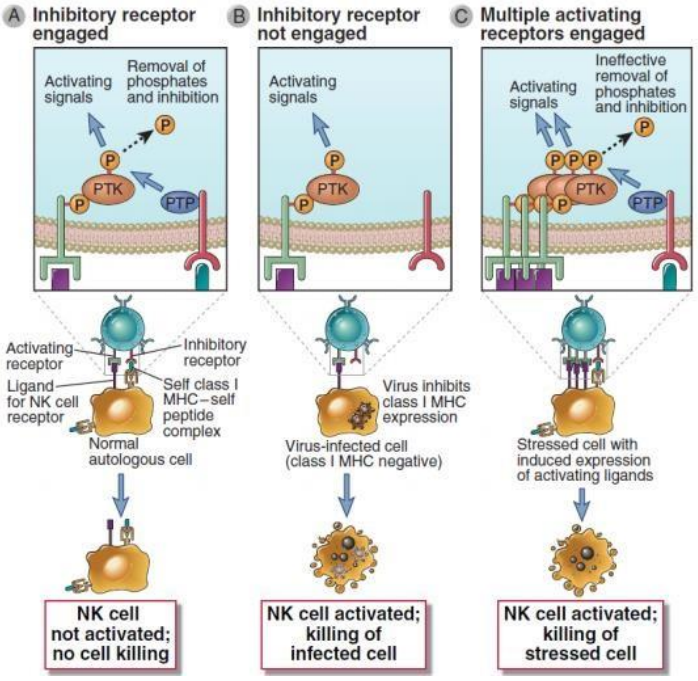
When NK cells become activated by host cells that **lack** MHC-I, it is called **'recognition of missing self'**.

⇨ When stimulating the **activating** receptors, **protein tyrosine kinase** is activated inducing more tyrosine **phosphorylation** resulting in **<u>eliminating</u>** the pathogen. Tyrosine kinase can be **inhibited** by inhibitory-receptor-associated **phosphatases** by removing the phosphate group causing **<u>NK cell inactivation</u>**.

<u>Possible scenarios showing the balance of inhibitory and activating receptor stimulation:</u>

A- Activating receptors of NK cells recognize ligands on target cells and activate protein tyrosine kinase (**PTK**). However, the presence of **MHC-I** stimulates **inhibitory** receptors that activate protein tyrosine **phosphatases** (PTP) inhibiting the action of NK cells.

B- Virus infections or other stresses **inhibit** MHC-I expression on infected cells while **inducing** the expression of activating ligands. Therefore, the NK cell inhibitory receptor is **not** engaged and the **activating** receptor **dominates** and **kills** the targeted cells.



C- Cells stressed by **infection** or **neoplastic** transformation may express **increased** amounts of **activating** ligands, which bind NK cell-activating receptors and induce more tyrosine phosphorylation resulting in **killing** of the stressed cells.

- **<u>Antibody-dependent cytotoxicity:</u>**

  Antibodies that bind to antigens can be **recognized** by **FcϒRIII** (CD16) receptors expressed on **NK cells**, resulting in NK **activation**, the release of **cytolytic granules** and, consequently, **cell apoptosis**.

- NK cells can work by secreting **IFNγ** and **TNFα**:

  IFNγ ❼ to control **viral** infections activating **macrophages** for **phagocytosis** and lysis.
  TNFα ❼ acts to promote direct NK **tumor cell killing**.

Innate and adaptive immunity to intracellular pathogens: *'read the captions'*

This figure just shows how innate immunity may control bacterial growth, but complete eradication requires adaptive immunity.

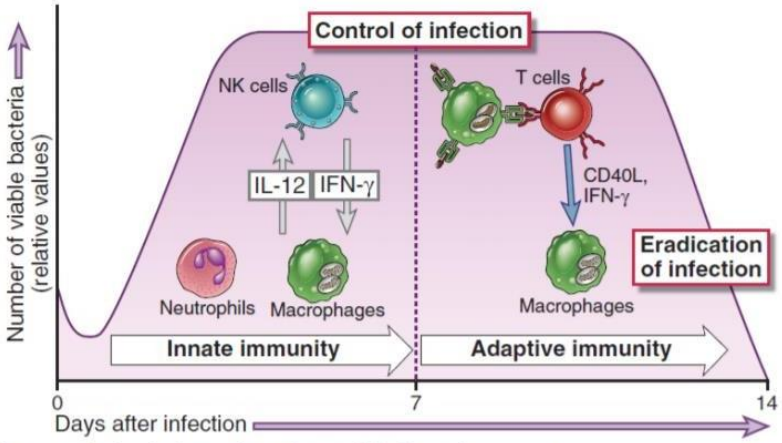


FIGURE 15–3 Innate and adaptive immunity to intracellular bacteria. The innate immune response to intracellular bacteria consists of phagocytes and NK cells, interactions among which are mediated by cytokines (IL-12 and IFN-γ). The typical adaptive immune response to these microbes is cell-mediated immunity, in which T cells activate phagocytes to eliminate the microbes. Innate immunity may control bacterial growth, but elimination of the bacteria requires adaptive immunity. These principles are based largely on analysis of *Listeria monocytogenes* infection in mice; the numbers of viable bacteria shown on the y-axis are relative values of bacterial colonies that can be grown from the tissues of infected mice. (Data

A: *shows the viral infection response to innate and adaptive immunity. Adaptive immunity is what mainly eradicates the virus.*

B: *shows the mechanism by which innate and adaptive immunity protect against / eradicate infected cells.*

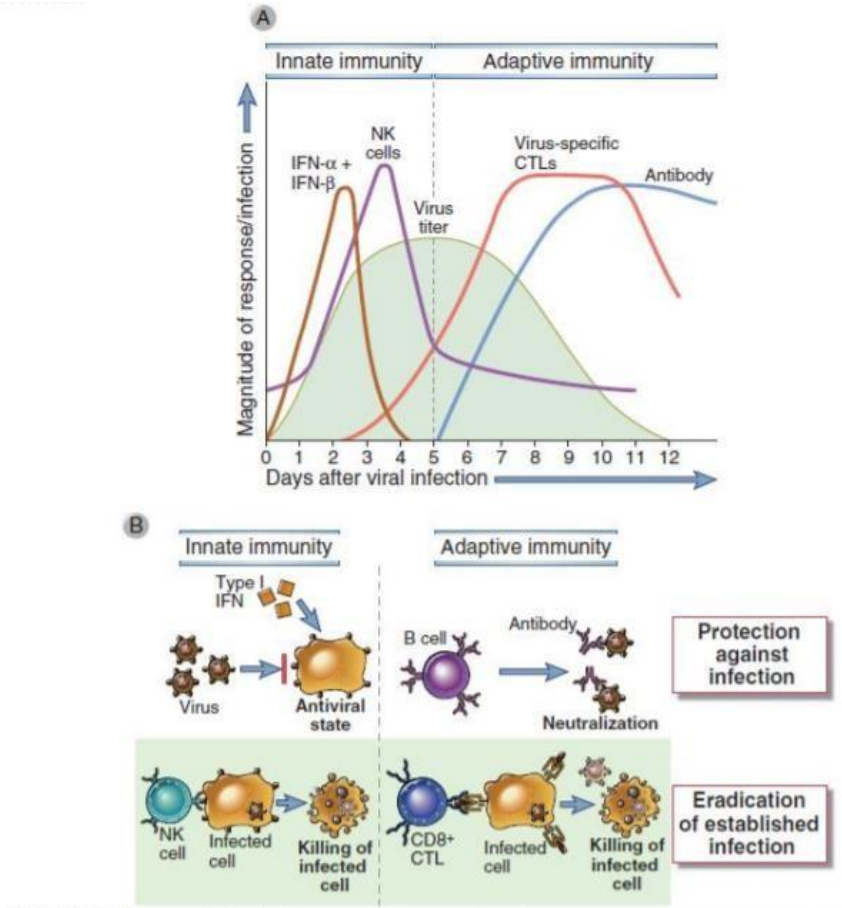


FIGURE 15–6 Innate and adaptive immune responses against viruses. A, Kinetics of innate and adaptive immune responses to a virus infection. **B,** Mechanisms by which innate and adaptive immunity prevent and eradicate virus infections. Innate immunity is mediated by type I interferons, which prevent infection, and NK cells, which eliminate infected cells. Adaptive immunity is mediated by antibodies and CTLs, which also block infection and kill infected cells, respectively.

Good Luck