Doctor 021

IMMUNOLOGY

Sheet no.9

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IMMUNE RESPONSES TO EXTRACELLULAR AND INTRACELLULAR PATHOGENS

This lecture is a recap of all the immune mechanisms we've discussed before.

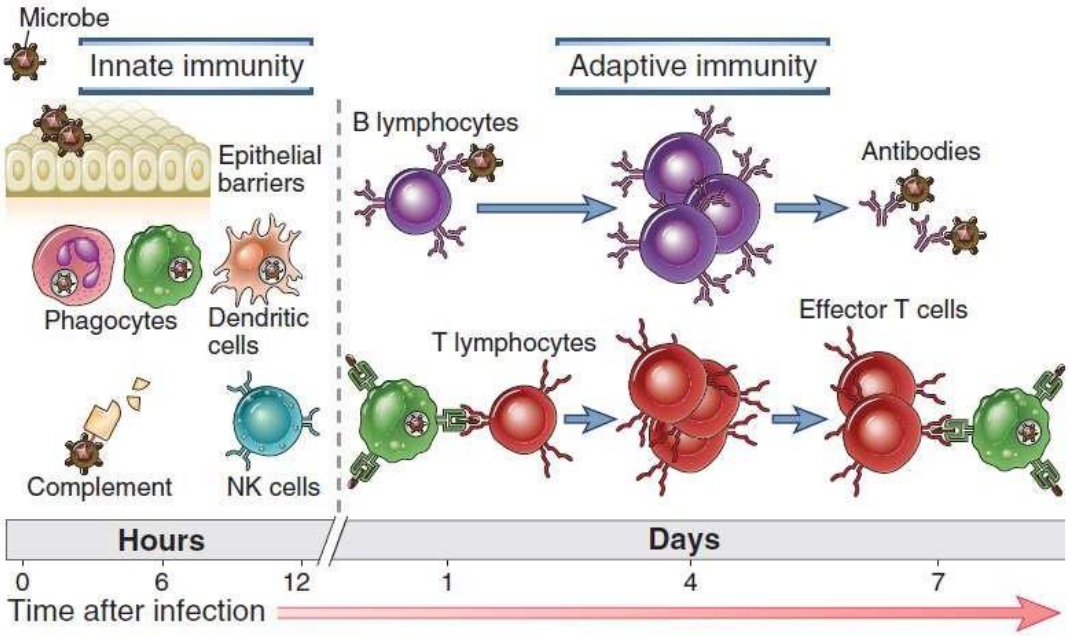


FIGURE 1–1 Innate and adaptive immunity. The mechanisms of innate immunity provide the initial defense against infections. Adaptive immune responses develop later and consist of activation of lymphocytes. The kinetics of the innate and adaptive immune responses are approximations and may vary in different infections.

- NK cells are lymphocytes which are important in case if the pathogen is intracellular.
- Dendritic cells link between innate and adaptive immunity, also they are important producers of cytokines.

GENERAL FEATURES OF IMMUNE RESPONSES TO MICROBES

- **Defense against microbes is mediated by the effector mechanisms of innate and**
- **adaptive immunity**
- **The immune system responds in distinct and specialized ways to different types of microbes to most effectively combat these infectious agents.**
- **The survival and pathogenicity of microbes in a host are critically influenced by the ability of the microbes to evade or resist the effector mechanisms of immunity.**
- **Many microbes establish latent, or persistent, infections in which the immune response controls but does not eliminate the microbe and the microbe survives without propagating the infection.**

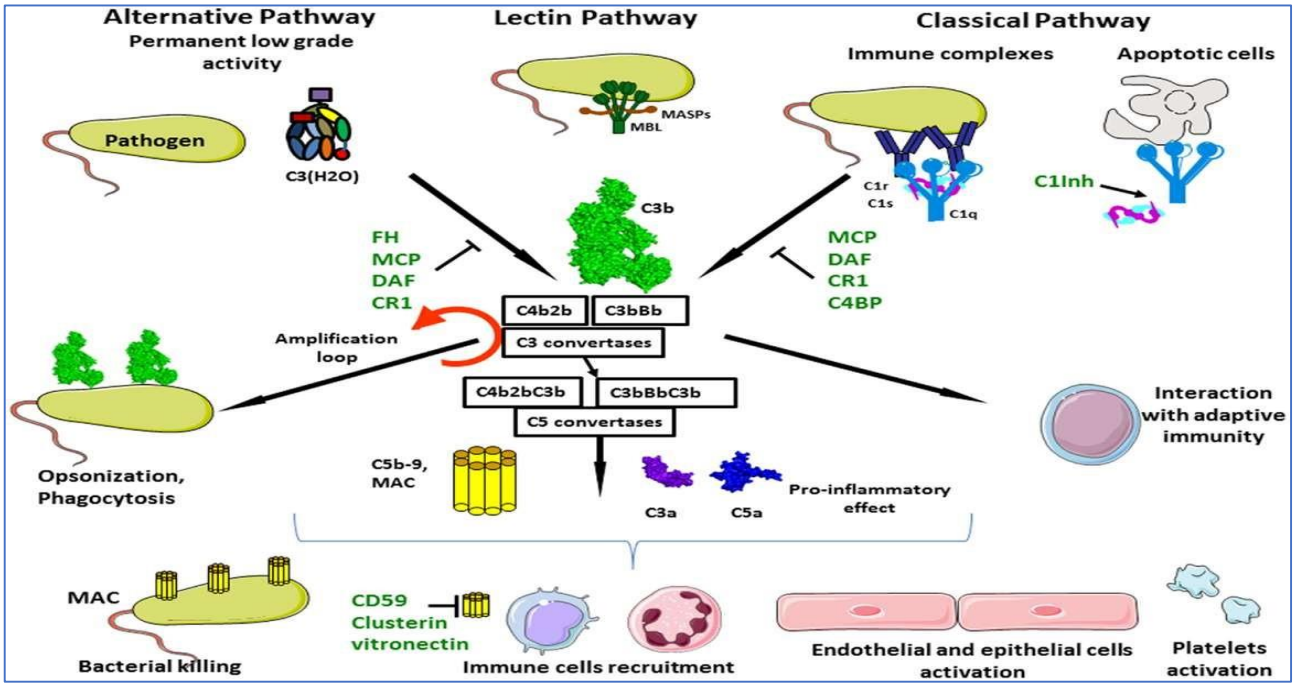
- In many infections, tissue injury and disease may be caused by the host response to the microbe and its products rather than by the microbe itself.

IMMUNITY TO EXTRACELLULAR BACTERIA

The principal mechanisms of innate immunity to extracellular bacteria:

- complement activation.
- Phagocytosis: Phagocytes use various surface receptors, including mannose receptors and scavenger receptors, to recognize extracellular bacteria, and they use Fc receptors and complement receptors to recognize bacteria opsonized with antibodies and complement proteins, respectively. As well as TLRs and other PRR.
- The inflammatory response: dendritic cells and phagocytes that are activated by the microbes secrete cytokines, which induce leukocyte infiltration, and initiates and propogates adaptive immune responses.

COMPLEMENT ACTIVATION



- The complement system is also important in homeostatic function, so there is an attachment between C1q and apoptotic cells to enhance phagocytosis of these apoptotic cells.

- C1inh: an inhibitor of C1 binding

- Unstable form of C3 (C3+a water molecule) that probes surfaces:

If it recognizes an abnormal surface, a C3b will bind to the surface and the process will start, but in normal human surfaces there are inhibitors that prevent C3 activation. Accordingly, the activation won't do much, it could create a few convertases that will later be degraded by inhibitors such as: **FH, MCP, DAF** and **CR1.**

- The function of MAC:

On host cells: signaling On microbes: degradation

- The main inhibitor of the formation of the pore (MAC) is **CD59**

For better understanding this figure: Click Here

PROFESSIONAL PHAGOCYTES

Phagocytes: Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, are the first line of defense against microbes that breach epithelial barriers.

Macrophages are more important than neutrophils, because they secrete a higher range of cytokines and much longer half-life than the neutrophil

They serve several functions: 1) Internalize and kill microbes. Neutrophils macrophages are particularly good at this function. 2) Phagocytes respond to microbes by producing various cytokines that promote inflammation. Macrophages are particularly good at this.

The essential role that phagocytes play in innate immune defense against microbes is demonstrated by the high rate of lethal bacterial and fungal infections in patients with low blood neutrophil counts caused by bone marrow cancers or cancer therapy, or inherited deficiencies.

Phagocytosis can be done by 2 manners: opsonin-dependent manner and opsonin-independent manner.

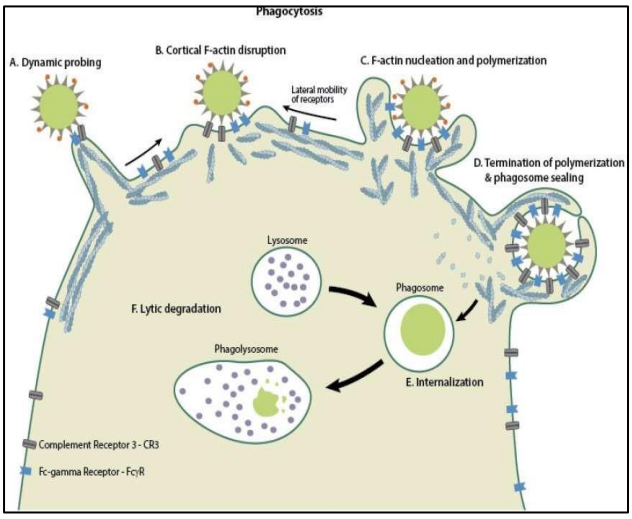
Opsonization enhance phagocytosis up to 10^3 folds.

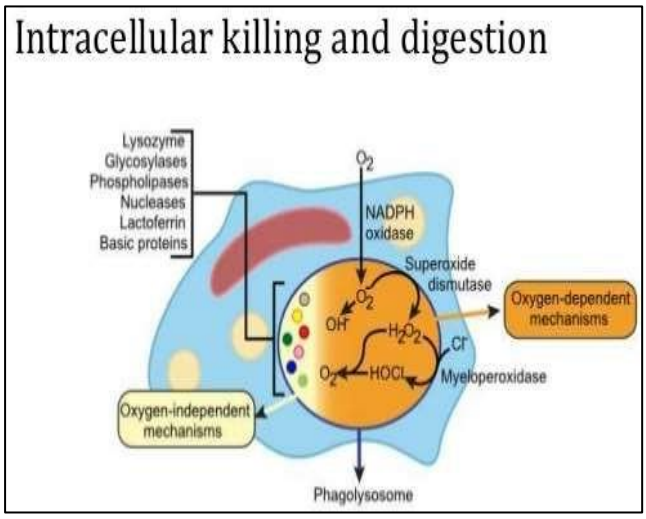
IgG subtypes that bind best to Fc receptors (IgG1 and IgG3) are the most efficient opsonins for promoting phagocytosis. Binding of FcγRI receptors on phagocytes to multivalent antibody-coated particles leads to engulfment of the particles and the activation of phagocytes.

certain receptors of opsonins are: Fcγ receptors and C3b receptors, and if the microbe is not coated by opsonins the detection will be done by certain receptors such as: scavenger receptors and C-lectin receptors.

Activation leads to:

- **Production of the enzyme phagocyte oxidase, which catalyzes the intracellular generation of reactive oxygen species that are cytotoxic for phagocytosed microbes. This process is called the respiratory burst.**
- **Activation of an enzyme called inducible nitric oxide synthase (iNOS), which triggers the production of nitric oxide that also contributes to the killing of pathogens.**
- **Secretion of hydrolytic enzymes and reactive oxygen intermediates into the external milieu that are capable of killing extracellular microbes too large to be phagocytosed. The same toxic products may damage tissues.**





The first thing that will happen after the phagocyte recognizes the pathogen is repolymerization (rearrangement) of Actin filaments to surround the pathogen preparing to be engulfed.

Now, after the engulfment of the pathogen, it should be degraded by lysozymes in many mechanisms, one of them is ROS mechanism.

The phagocyte starts to uptake O_2 from the outside in a process called oxidative/respiratory burst, to convert it to ROS by an enzyme called NADPH oxidase.

$$O_2 \rightarrow O^-_2 \text{ (highly reactive)} \xrightarrow{\text{Superoxide dismutase}} H_2O_2$$

$$H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} HOCl$$

Another mechanism is an Oxygen-independent mechanism such as:

Lysozymes: work on cell wall

Nucleases: work on nucleic acids

Basic proteins: anti-bacterial effects

Lactoferrins: due to the reliance of the bacterial metabolism on Fe, these lactoferrins bind Fe and sequester it, which will lead to abnormal metabolism and the bacteria can't replicate properly.

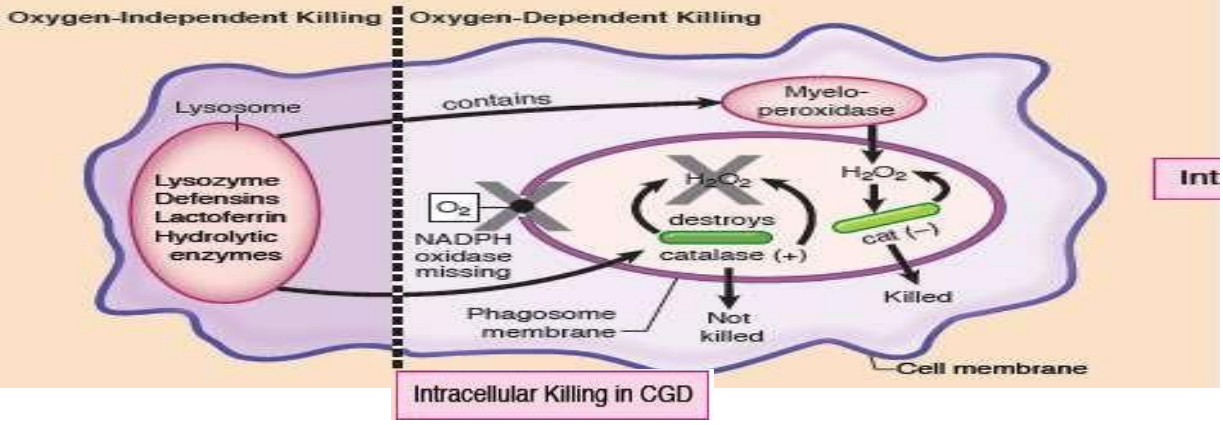
CHRONIC GRANULOMATOUS DISEASE

Clinical Correlate

When defects prevent phagocytes from performing their critical functions as first responders and intracellular destroyers of invading antigens, clinically important pathologic processes ensue. Such defects tend to make the patient susceptible to severe infections with extracellular bacteria and fungi.

Chronic granulomatous disease (CGD) is an inherited deficiency in the production of one of several subunits of NADPH oxidase. This defect eliminates the phagocyte's ability to produce many critical oxygen-dependent intracellular metabolites ($\cdot O_2^-$, $\cdot OH$, $^1O_2$, and H_2O_2). The 2 other intracellular killing mechanisms remain intact (myeloperoxidase + $H_2O_2 \rightarrow$ HOCl and lysosomal contents).

- If the patient is infected with a catalase-negative organism, the H_2O_2 waste product produced by the bacterium can be used as a substrate for myeloperoxidase, and the bacterium is killed.
- If the patient is infected with a catalase-positive organism (e.g., *Staphylococcus*, *Klebsiella*, *Serratia*, *Aspergillus*), the myeloperoxidase system lacks its substrate (because these organisms destroy H_2O_2), and the patient is left with the oxygen-independent lysosomal mechanisms that prove inadequate to control rampant infections. Thus, CGD patients suffer from chronic, recurrent infections with catalase-positive organisms.



Intracellular Killing in CGD

The doctor didn't talk about this table:

TABLE 12–3 Fc Receptors

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation (inefficient)
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes	Feedback inhibition of B cells
FcγRIIC (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-6}$ M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, glycophosphatidylinositol; NK, natural killer.

IMMUNITY TO EXTRACELLULAR BACTERIA / ANTIGEN PRESENTATION

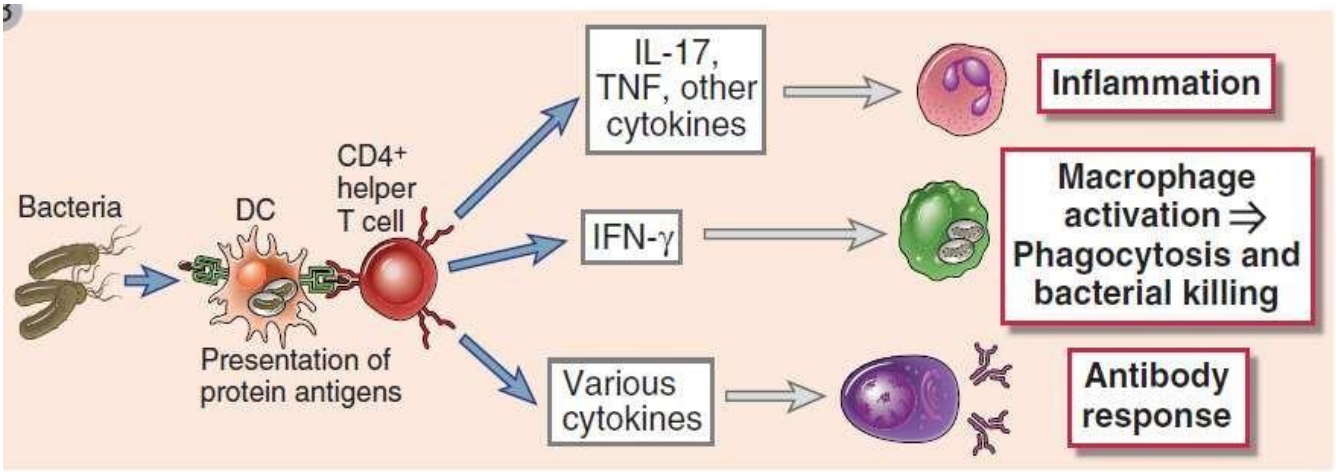
Macrophages and dendritic cells function as antigen-presenting cells (APCs). They present peptide antigens derived from digested bacteria on the major histocompatibility complex class II and activate acquired immunity by activating helper T cells.

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.

Dendritic cells are a heterogeneous family of bone marrow-derived cells with long dendrite-like cytoplasmic processes that are constitutively present in epithelia and most tissues of the body.

Most versatile sensors of PAMPs and DAMPs among all cell types in the body.

TLR signaling induces dendritic cell expression of molecules, including costimulatory 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.



The dendritic cell will ingest the bacteria and degrades it, then it presents a part of it on its surface to be recognized by T-cells.

The attachment is formed by: T-cell receptor and MHC II receptor

For people who suffer from CGD (the disease in the previous slide) it's beneficial to give them INF-γ to reduce infections and enhance bacterial killing processes because they have deficiencies in oxygen-dependent mechanisms.

INJURIOUS EFFECTS FOLLOWING IMMUNITY TO EXTRACELLULAR

Inflammatory reactions are usually self-limited and controlled. Cytokines secreted by leukocytes in response to bacterial products also stimulate the production of acute-phase proteins and cause the systemic manifestations of the infection. Sepsis is a severe pathologic consequence of disseminated infection by some gram-negative and gram-positive bacteria.

Sepsis when there is an overactivation of the immune system. it is referred to cytokine storm (large number of cytokines being produced) so equal large number of immune cells activated. leads to abnormal metabolism and increased # of reactive O species that leads to organ dysfunction and maybe death.

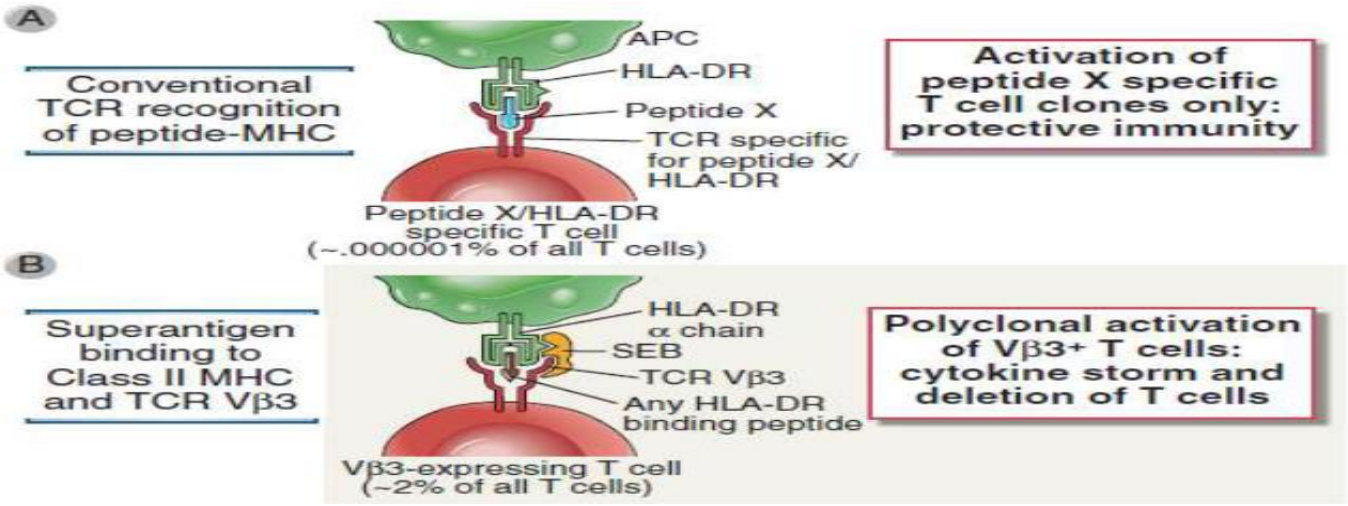
A late complication of the humoral immune response to bacterial infection may be the generation of disease-producing antibodies. The best-defined examples are two rare sequelae of streptococcal infections of the throat or skin. Infection leads to the production of antibodies against a bacterial cell wall protein (M protein). Some of these antibodies cross-react with self antigens.

In some cases, antigen on the pathogen is structurally similar to antigens in the body. Antibodies produced against the pathogen (even after it is cleared) bind antigen in our own tissue (molecular mimicly).

Certain bacterial toxins stimulate all the T cells in an individual that express a particular family of Vβ T cell receptor (TCR) genes. Such toxins are called superantigens because they resemble antigens in that they bind to TCRs and to class II MHC molecules (although not to the peptide-binding clefts) but activate many more T cells than do conventional peptide antigens

Bind abnormal way to T cell receptor causing activation of large number of T cell clones (polyclonal activation).

Unlike normal way where 1 or 2 clones identify this antigen.



IMMUNITY TO INTRACELLULAR BACTERIA

Innate immunity has a brief role since intracellular pathogens are mainly dealt with adaptive immunity.

The innate immune response to intracellular bacteria is mediated mainly by phagocytes and natural killer (NK) cells.

Phagocytes, initially neutrophils and later macrophages, ingest and attempt to destroy these microbes, but pathogenic intracellular bacteria are resistant to degradation within phagocytes.

Products of these bacteria are recognized by TLRs and cytoplasmic proteins of the NOD-like receptor (NLR) family, resulting in activation of the phagocytes

Intracellular bacteria activate NK cells by inducing the expression of NK cell–activating ligands on infected cells and by stimulating dendritic cell and macrophage production of IL-12 and IL-15, both of which are NK cell–activating cytokines.

The major protective immune response against intracellular bacteria is T cell–mediated immunity.

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 eliminating the pathogen. Tyrosine kinase can be inhibited by inhibitory-receptor-associated phosphatases by removing the phosphate group causing NK cell inactivation.

- Antibody-dependent cytotoxicity:

• Antibodies that bind to antigens can be recognised by FcϒRIII (CD16) receptors expressed on NK cells, resulting in NK activation, release of cytolytic granules and consequent cell apoptosis. This allows NK cells to target cells against which a humoral response has been gone through and to lyse cells through antibody-dependant cytotoxicity (ADCC).

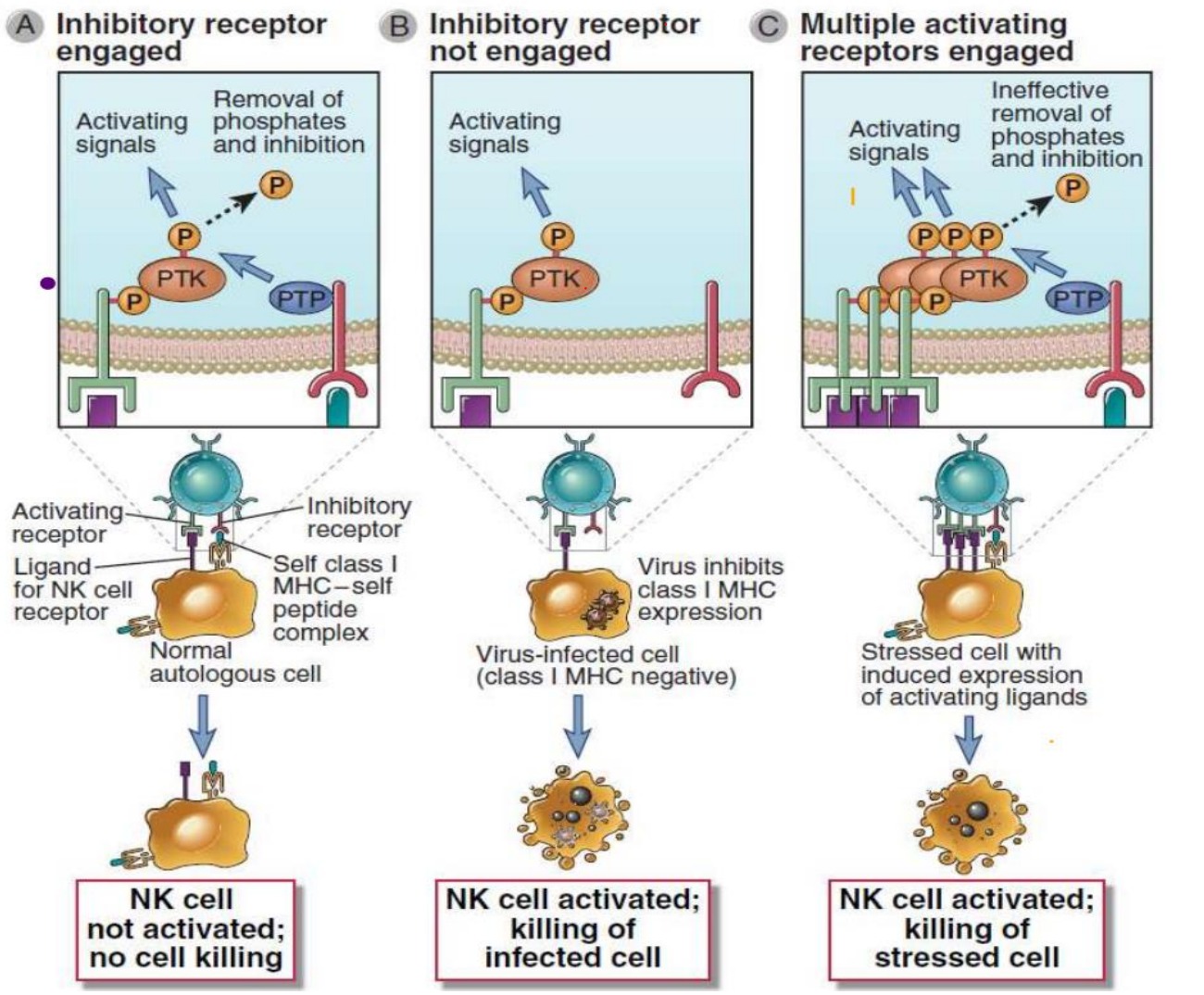
• NK cells work to control viral infections by secreting IFNγ and TNFα. IFNγ activates macrophages for phagocytosis and lysis, and TNFα acts to promote direct NK tumor cell killing.

❖ Possible scenarios showing the balance of inhibitory and activating receptor stimulation:

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.

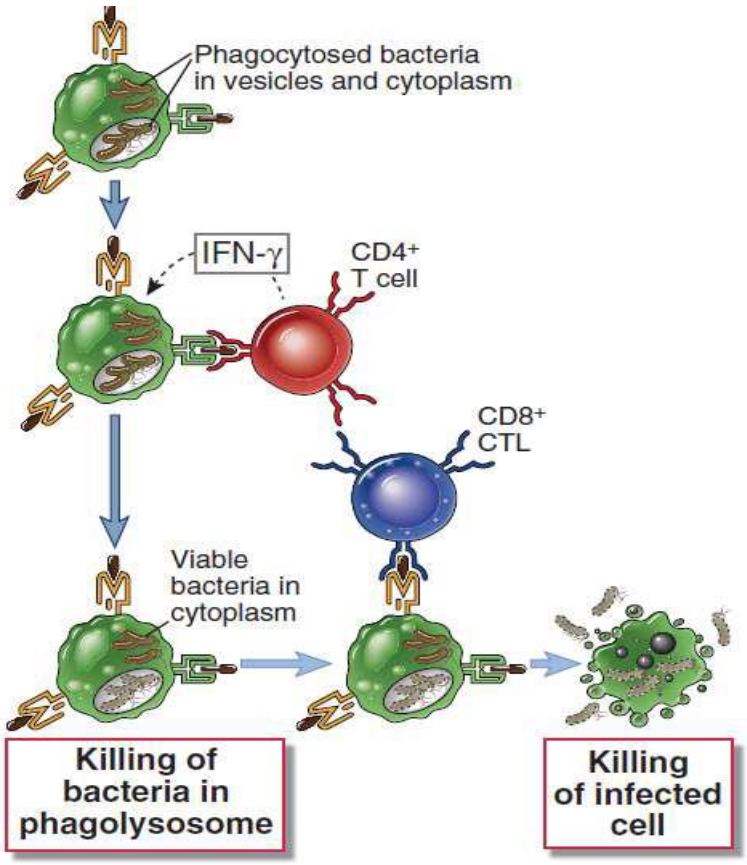
B- Virus infections or other stresses inhibit MHC-I expression on infected cells while inducing the expression of activating ligands. Therefore, the NK cell inhibitory receptor is not engaged and the activating receptor dominates and kills the targeted cells.

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.



Immunity to Intracellular bacteria / Adaptive immunity:

Cooperation of CD4+ and CD8+ T cells in defense against intracellular microbes. Intracellular bacteria such as L. monocytogenes are phagocytosed by macrophages and may survive in phagosomes and escape into the cytoplasm.



CD4+ T cells respond to class II MHC– associated peptide antigens derived from the intravesicular bacteria. These T cells produce IFN-γ, which activates macrophages to destroy the microbes in phagosomes. CD8+ T cells respond to class I–associated peptides derived from cytosolic antigens and kill the infected cells.

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

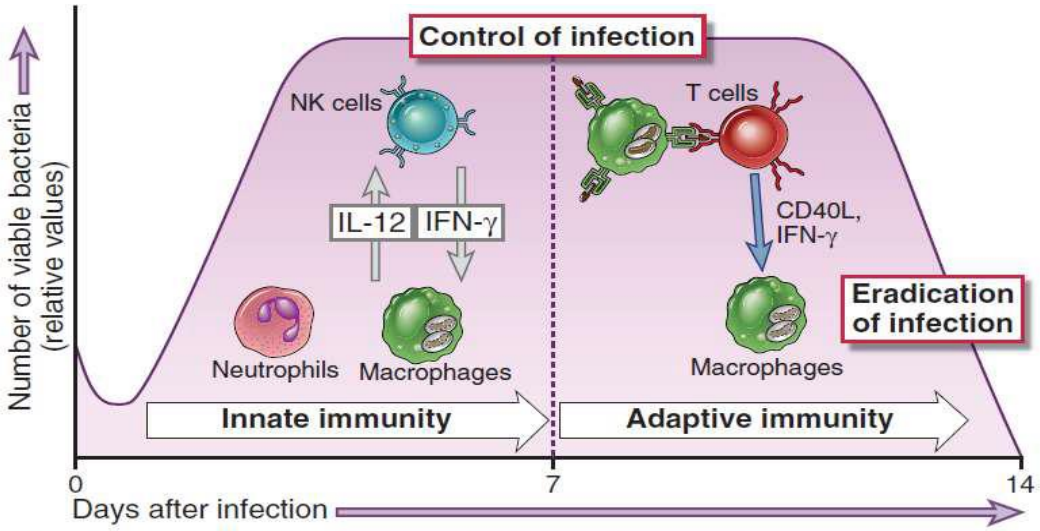


FIGURE 15–3 Innate and adaptive immunity to intracellular bacteria. The innate immune response to intracellular bacteria consists of phagocytes and NK cells, interactions among which are mediated by cytokines (IL-12 and IFN-γ). The typical adaptive immune response to these microbes is cell-mediated immunity, 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 from Unanue ER. Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. Immunological Reviews 158: 11-25, 1997.)*

INTERFERONS AND THEIR EFFECT TO INTRACELLULAR PATHOGENS:

The major way by which the innate immune system deals with viral infections is to induce the expression of type I interferons. Type I interferons are a large family of structurally related cytokines that mediate the early innate immune response to viral infections.

- **Type I interferons, signaling through the type I interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection, called an antiviral state.**
- **Type I interferons cause sequestration of lymphocytes in lymph nodes, thus maximizing the opportunity for encounter with microbial antigens.**
- **Type I interferons increase the cytotoxicity of NK cells and CD8+ CTLs**
- **Upregulate expression of class I MHC molecules and thereby increase the probability that virally infected cells will be recognized and killed by CD8+ CTLs**

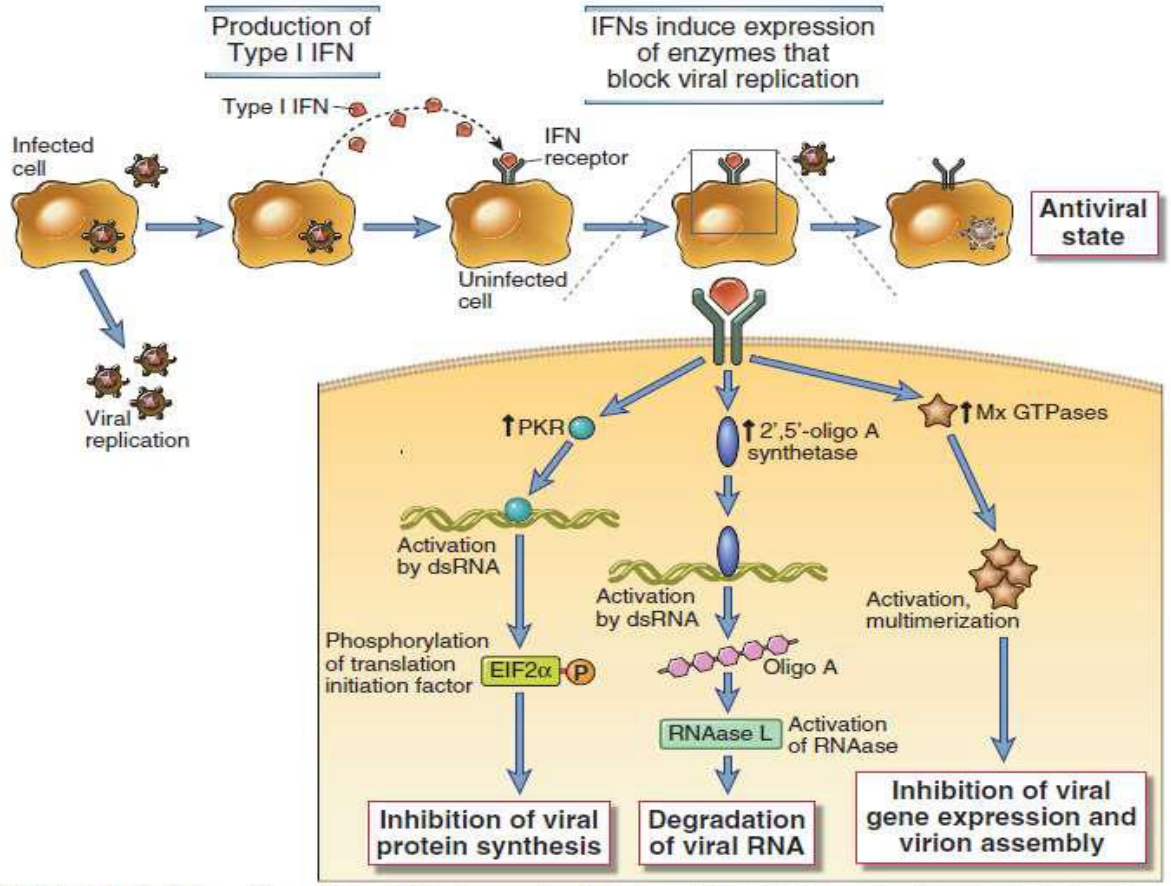


FIGURE 4–15 Biologic actions of type I interferons. Type I interferons (IFN-α, IFN-β) are produced by virus-infected cells in response to intracellular TLR signaling and other sensors of viral RNA. Type I interferons bind to receptors on neighboring uninfected cells and activate JAK-STAT signaling pathways, which induce expression of genes whose products interfere with viral replication. Type I interferons also bind to receptors on infected cells and induce expression of genes whose products enhance the cell's susceptibility to CTL-mediated killing.

Clinical Correlate

Therapeutic Use of Interferons

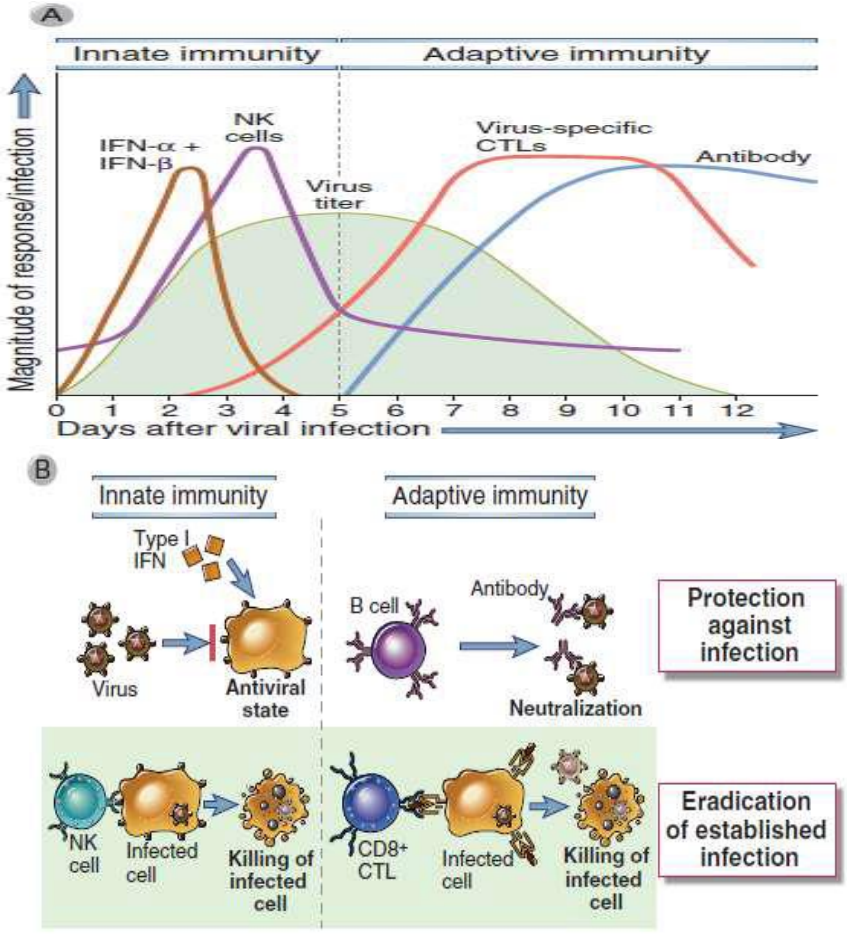
Since the first description of interferons (IFN) almost 50 years ago, a multitude of dramatic immunomodulatory roles have been discovered for this group of proteins. As a group, IFNs induce increases in the expression of class I and II MHC molecules and augment NK cell activity. They increase the efficiency of presentation of antigens to both cytotoxic and helper cell populations. Cloning of the genes that encode IFNs α, β, and γ has made it possible to produce sufficient amounts to make their use clinically practical.

- **Interferon-α** has well-known antiviral activity and has been used in the treatment of hepatitis B and C infections. Within cancer therapy, IFN-α has shown promise in treatment of hairy B-cell leukemia, chronic myelogenous leukemia, and Kaposi sarcoma.
- **Interferon-β** was the first drug shown to have a positive effect on young adults with multiple sclerosis. Patients treated with IFN-β enjoy longer periods of remission and reduced severity of relapses.
- **Interferon-γ** is being used in the treatment of chronic granulomatous disease (CGD). This molecule is a potent inducer of macrophage activation and a promoter of inflammatory responses. Its application appears to significantly reverse the CGD patient's inability to generate toxic oxygen metabolites inside phagocytic cells.

The side effects of IFN therapy are fortunately mild and can be managed with acetaminophen. They include headache, fever, chills, and fatigue, and they diminish with continued treatment.

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.



IMMUNITY TO FUNGI

Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans

The principal mediators of innate immunity against fungi are neutrophils and macrophages. Patients with neutropenia are extremely susceptible to opportunistic fungal infections

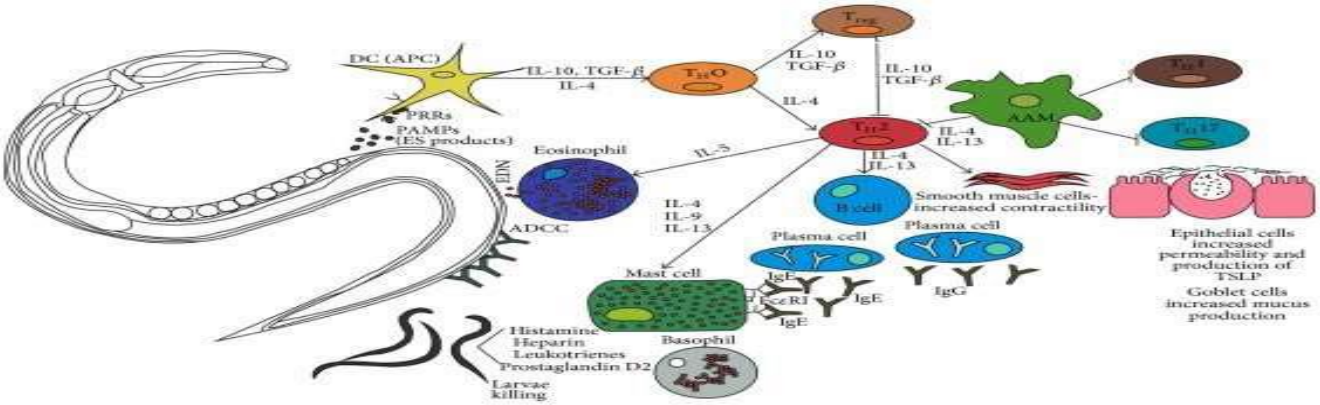
less is known about antifungal immunity than about immunity against bacteria and viruses. This lack of knowledge is partly due to the paucity of animal models for mycoses and partly due to the fact that these infections typically occur in individuals who are incapable of mounting effective immune responses.

IMMUNITY TO HELMINTHS

• Antibodies, mast cells, and eosinophils function with antibodies to mediate the expulsion and killing of some helminthic parasites. Helminths (worms) are too large to be engulfed by phagocytes, and their integuments are relatively resistant to the microbicidal products of neutrophils and macrophages.

• IgE, IgG, and IgA antibodies that coat helminths can bind to Fc receptors on eosinophils and cause the degranulation of these cells, releasing the major basic protein, a toxic cationic protein, present in the granules of eosinophils. Other eosinophil granule contents also aid in killing the parasites.

• IgE antibodies that recognize antigens on the surface of the helminths may initiate local mast cell degranulation through the high-affinity IgE receptor. Mast cell mediators may induce bronchoconstriction and increased local motility, contributing to the expulsion of worms.



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Q1: if a bacterial cell binds C046 to in surface during the course of infection, then we expect to find:

A- increased formation of C5b-9 on the bacterial surface

B- Decreased C3b deposition on the bacterial surface

C- Increased formation of C3 convertase

D- Decrease in C1q binding to the bacteria

E- increased phagocytoses of the bacteria

Q2: which is an example of antimicrobial peptides:

A- C3

B- Human Cathelidins LL-37 .

C- Mucin

D- Pentraxin

E- Natural antibodies.

Q3: Inhibition of one of the following receptors can significantly inhibit immunity to Helminths:

A- Fc gamma receptor 1

B- Fc gamma receptor 2A

C- Fc gamma receptor 2B

D- Fc epsilon receptor 1

E- NOD-like receptors 1

Answers: E , B , D