Innate Immunity

Prof. Roberto Ria

Overview of Innate Immunity

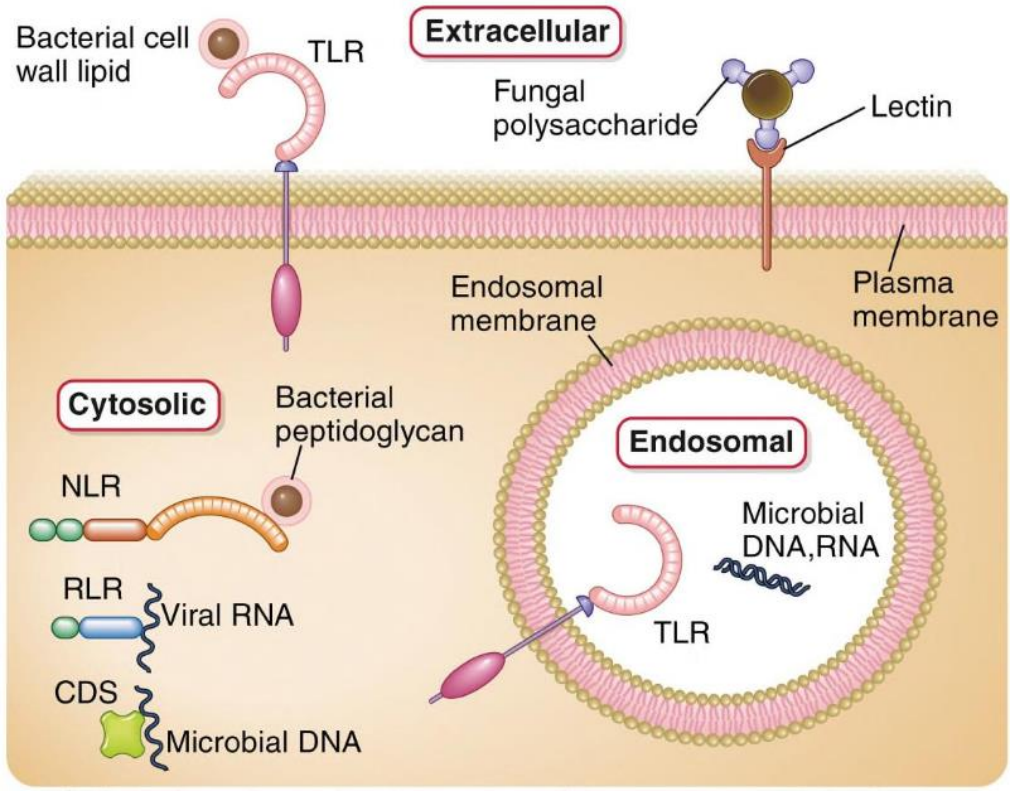
- The term innate immunity refers to defense mechanisms that are always present, ready to combat microbes and other offending agents.
- Innate immunity is the first line of defense against infections and serves several essential functions that protect us against microbes and tissue injury. The major components of the innate immune system are barrier epithelia, which block the entry of microbes; tissue-resident sentinel cells, including macrophages, mast cells, and dendritic cells (DCs), which detect microbes that have breached epithelia and initiate host responses; white blood cells (leukocytes), including neutrophils, monocytes that become macrophages in tissues, natural killer (NK) cells, and other cells, which enter the tissues from the blood and eliminate microbes that have invaded through epithelia and also remove damaged host cells; and several types of plasma proteins that combat microbes within and outside the circulation. We discuss the functions of each of these later in the chapter.

Functions of Innate Immune Responses

- The two major protective reactions of the innate immune system are inflammation and antiviral defense.
- Physical and chemical defenses at epithelial barriers, such as the skin and lining of the gastrointestinal and respiratory tracts, block microbial entry.
- Innate immune responses are the initial reactions to microbes that serve to control or eliminate infection of the host by many pathogens.
- Innate immunity eliminates damaged cells and initiates the process of tissue repair.
- Innate immune responses stimulate adaptive immune responses and can influence the nature of the adaptive responses to make them optimally effective against different types of microbes.

Recognition of Microbes and Damaged Tissue by the Innate Immune System

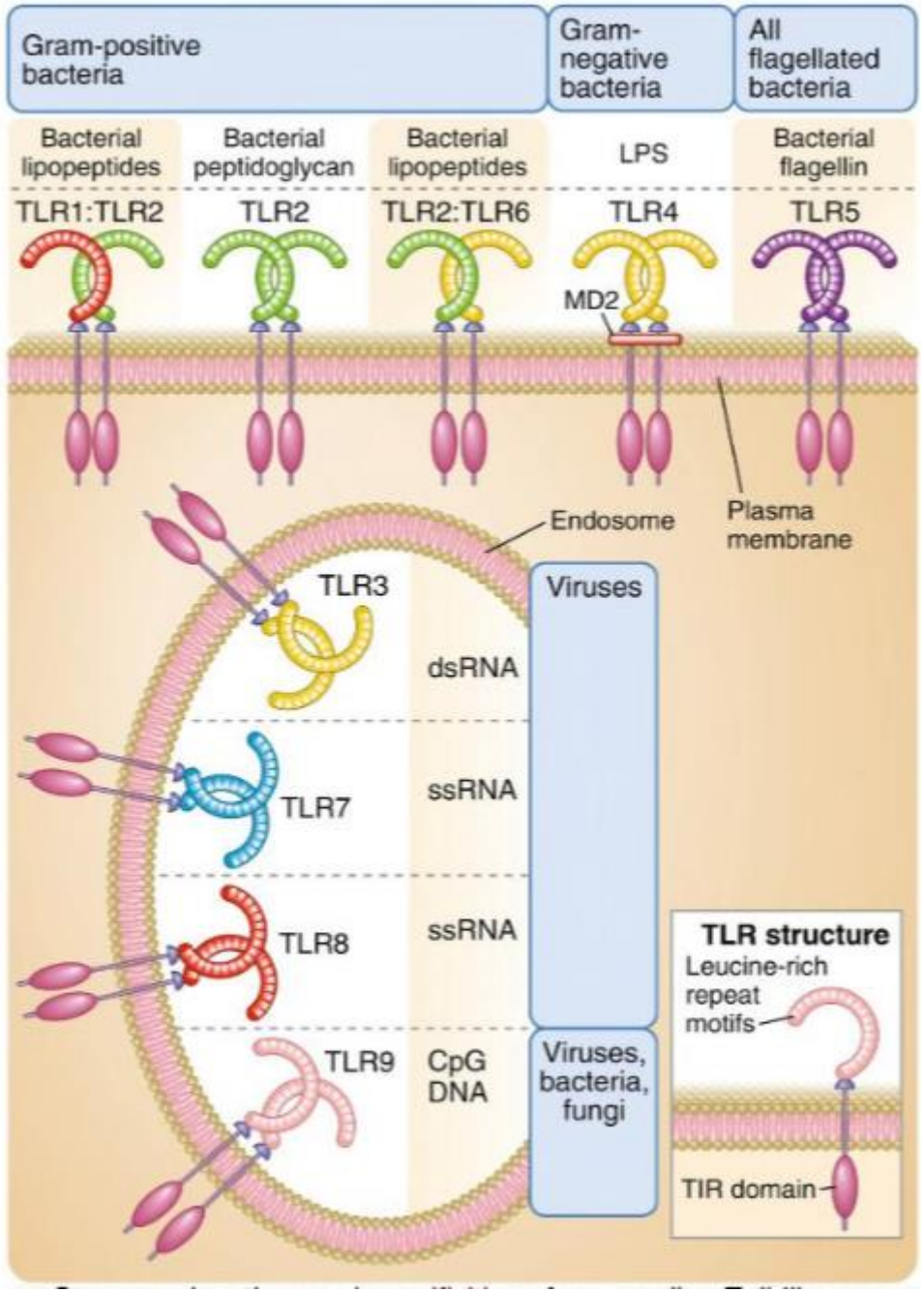
- The innate immune system recognizes molecular structures that are produced by microbial pathogens. The microbial substances that stimulate innate immunity are often shared by classes of microbes and are called pathogen-associated molecular patterns (PAMPs). Different types of microbes (e.g., viruses, gram-negative bacteria, gram-positive bacteria, fungi) express different PAMPs.
- The innate immune system detects the presence of infection but not the specific pathogens.
- The innate immune system recognizes microbial products that are often essential for survival of the microbes.
- The innate immune system also recognizes endogenous molecules that are produced by or released from damaged and dying cells. These substances are called damage-associated molecular patterns (DAMPs).
- The innate immune system uses several types of cellular receptors, present in different locations in cells, and soluble molecules in the blood and mucosal secretions to recognize PAMPs and DAMPs.
- The receptors of the innate immune system are encoded by inherited (germline) genes, whereas the genes encoding receptors of adaptive immunity are generated by somatic recombination of gene segments in the precursors of mature lymphocytes.
- The innate immune system does not react against normal, healthy cells and tissues.



Cellular Pattern Recognition Receptors

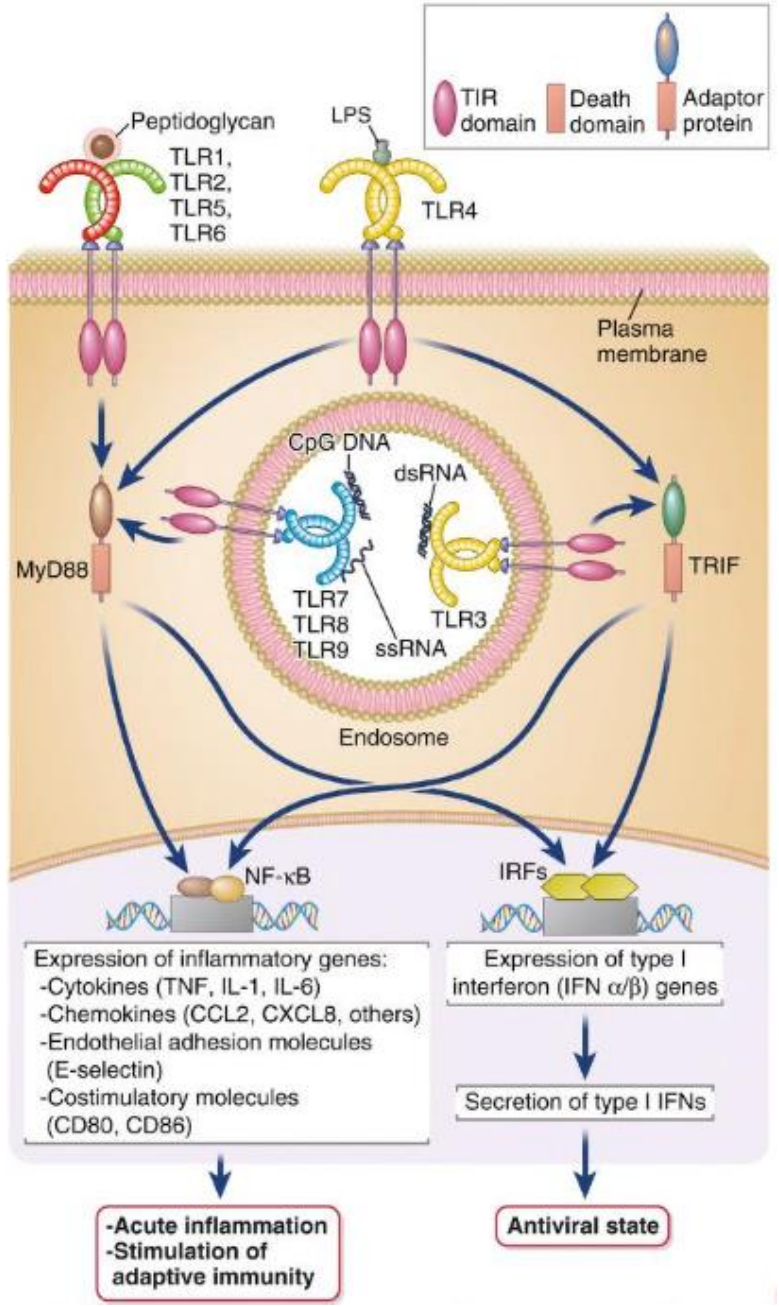
Toll-Like Receptors

- Toll-like receptors (TLRs) are an evolutionarily conserved family of pattern recognition receptors expressed by many cell types that recognize products of a wide variety of microbes, as well as molecules expressed or released by stressed and dying cells.
- TLRs are involved in responses to a wide variety of molecules that are expressed by microbes but not by healthy mammalian cells.
- TLRs are also involved in responses to endogenous molecules whose expression or location indicates cell damage.
- The structural basis of TLR specificities resides in the multiple extracellular or endosomal lumenal leucine-rich modules of these receptors, which bind directly to PAMPs or to adaptor molecules that bind the PAMPs.
- TLRs are found on the cell surface and on intracellular membranes and are thus able to recognize microbes in different cellular locations.
- TLR recognition of microbial ligands results in the activation of several signaling pathways and ultimately transcription factors, which induce the expression of genes whose products are important for inflammatory and antiviral responses



Signaling pathways and functions of Toll-like receptors.

- TLRs 1, 2, Band 6 use the adaptor protein MyD88 and activate the transcription factor NF-kB, which induces inflammatory gene expression. TLR3 uses the adaptor protein TRIF, which activates the IRF3 transcription factor and promotes IFN-B expression and NF-kB. TLR4 uses both MyD88 and TRIF, leading to activation of NF-KB and IRF3 pathways, respectively. TLRs 7, 8, and 9 in the endosome use MyD88, leading to activation of both NF-KB and IRF7, promoting expression of genes whose products mediate inflammation and antiviral defense. dsRNA, Double-stranded RNA; /FN, interferon; /RFs, interferon regulatory factors; NF«B, nuclear factor kappa B; ssRNA, single-stranded DNA; T/A, Toll IL-1 receptor; TLR, Toll-like receptors; TR/F, TIR-domain-containing adapterinducing interferon-B.



Cytosolic Receptors for Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

NOD-Like Receptors: NOD1 and NOD2

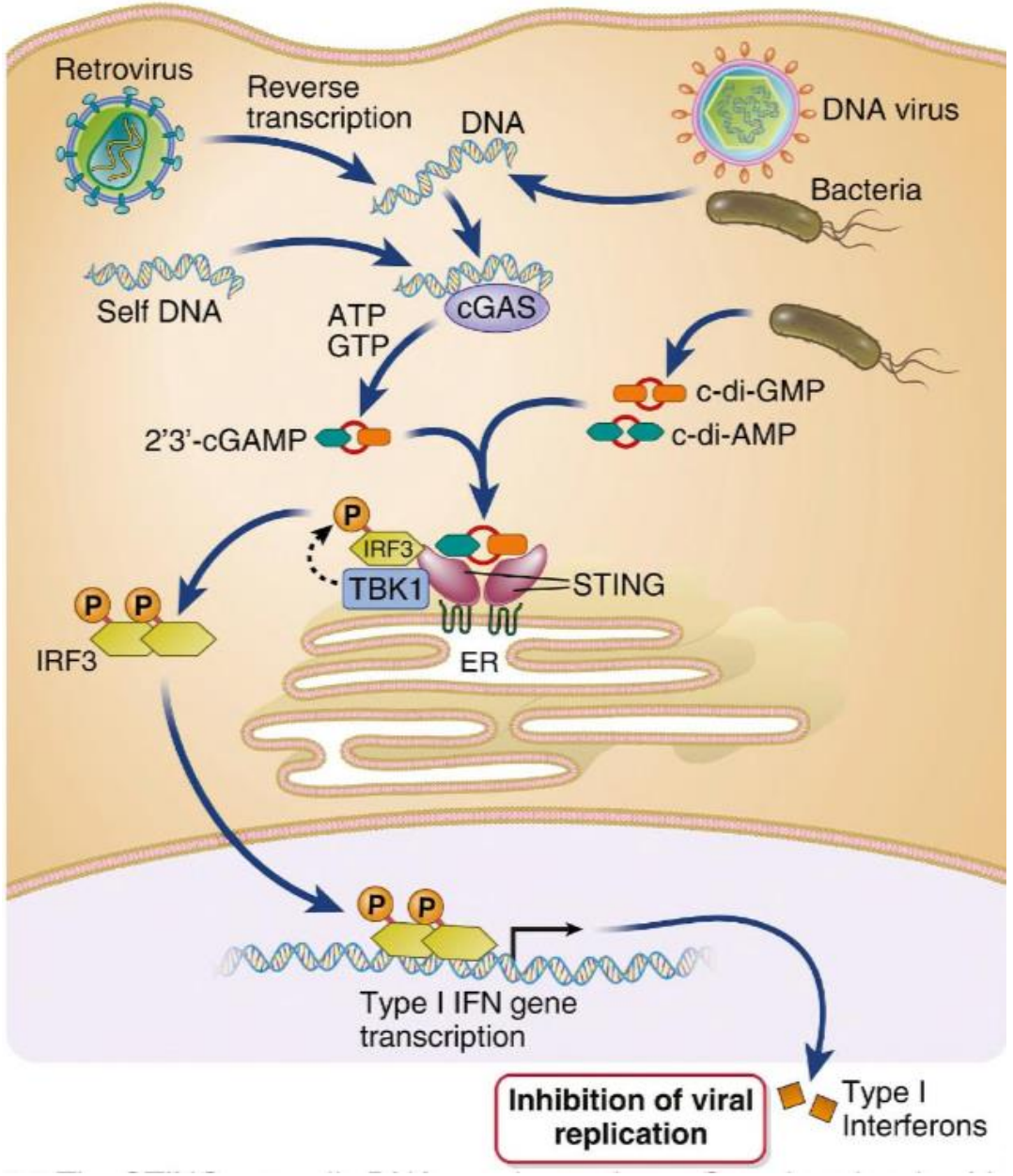
- NOD-like receptors (NLRs) are a family of more than 20 different cytosolic proteins, some of which recognize PAMPs and DAMPs and recruit other proteins to form signaling complexes that promote inflammation.

Subfamily	Examples	Typical domain structure	Activating stimuli	Function
NLRA	NLRA		IFN-γ	Class II MHC expression
NLRB	NAIP		Flagellin	Inflammasome generation of active IL-1 and IL-18; pyroptosis (with NLRC4)
NLRC	NOD1, NOD2, NLRC3-5		DAP (NOD1)	NF-κB activation
			MDP (NOD2)	NF-κB activation, autophagy, type I interferon production
			Flagellin, type III secretion system (NLRC4)	Inflammasome generation of active IL-1 and IL-18; pyroptosis
NLRP	NLRPs 1-10		Extracellular ATP, alum, asbestos, bacterial toxins, silica, ROS, reduced cytosolic K^+ (NLRP3)	Inflammasome generation of active IL-1 and IL-18; pyroptosis
			Lipopeptides (NLRP7)	

Cytosolic Receptors for Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

Cytosolic DNA Sensors and the STING Pathway

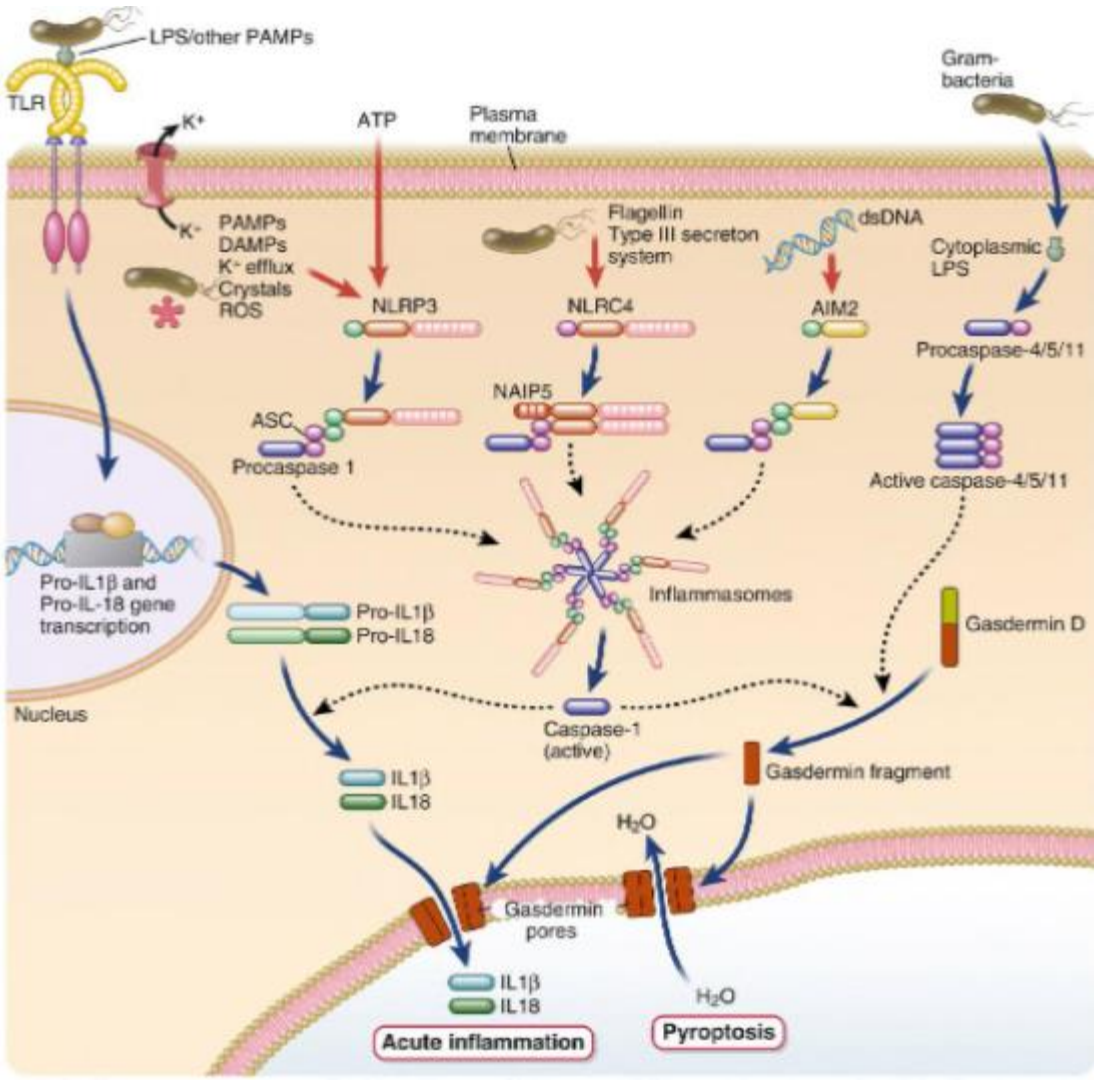
- Cytosolic DNA sensors (CDSs) are molecules that detect double-stranded (ds) DNA in the cytosol and activate signaling pathways that initiate antimicrobial responses, including type I IFN production and autophagy.
- The STING (stimulator of IFN genes) pathway is an important mechanism of DNA-induced activation of type I IFN responses.
- Some cytosolic DNA sensors may work through STING independent pathways.



Cytosolic Receptors for Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

RIG-Like Receptors

- RIG-like receptors (RLRs) are cytosolic sensors of viral RNA that respond by inducing the production of the antiviral type I IFNs. **Inflammasomes**
- Inflammasomes are multiprotein enzymatic complexes that form in the cytosol in response to infections or cell injury, which produce proteolytically active caspase-1 and thereby generate biologically active forms of the inflammatory cytokines IL-1f and IL-18
- Inflammasome activation is induced by a wide variety of cytoplasmic stimuli that are often associated with infections and cell stress, including microbial products, environmentally or endogenously derived crystals, and reduction in cytosolic potassium ion (K +)



Other Cell-Associated Pattern Recognition Receptors

C-Type Lectins: Receptors for Microbial Carbohydrates

- Cellular receptors that recognize carbohydrates on the surface of microbes facilitate the phagocytosis of the microbes and the secretion of cytokines that promote inflammation and subsequent adaptive immune responses: The **mannose receptor** (CD206) is expressed on macrophages, DCs, and lymphatic endothelial cells and is involved in phagocytosis of various types of microbes; **Dectins** (DC-associated C-type lectins) include several different lectins encoded within a gene cluster on human chromosome 12, which also includes genes encoding NK cell receptors; **Langerin** (CD207) and **DC-SIGN** (CD209) are two other lectins expressed on DCs, both of which bind mannose and have roles in immune responses to various microbes.

Scavenger Receptors

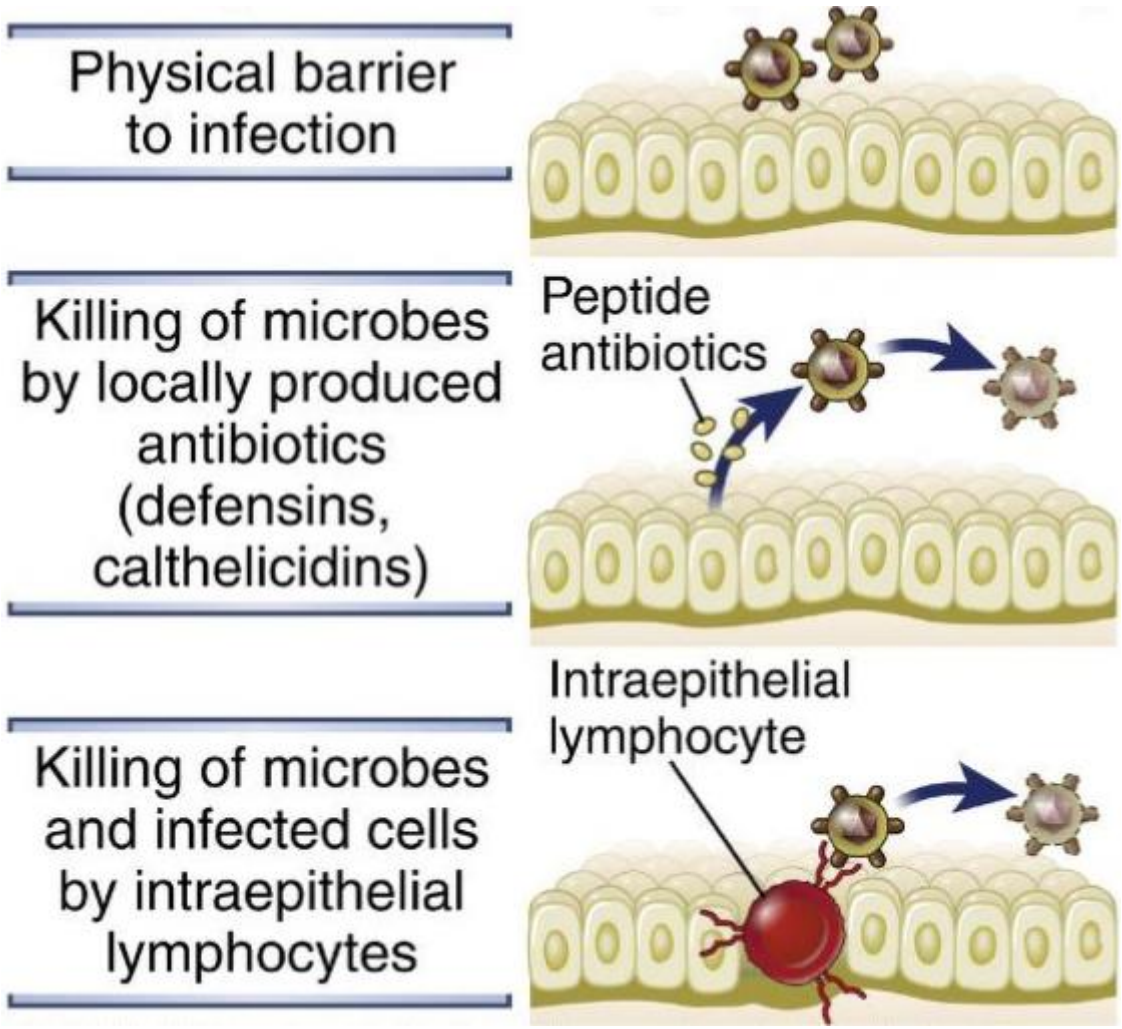
- Comprise a structurally and functionally diverse collection of cell surface proteins that were originally grouped on the basis of the common characteristic of mediating the uptake of oxidized lipoproteins into cells.

Formyl-Peptide Receptors

- (FPR1), expressed on leukocytes, recognizes bacterial peptides containing N-formylmethionyl residues and stimulates directed movement of the cells.

Epithelial Barriers

- Intact epithelial surfaces form physical barriers between microbes in the external environment and host tissue.
- Epithelial cells, as well as some leukocytes, produce peptides that have antimicrobial properties.
  - Defensins: Defensins kill microbes by a variety of mechanisms, many of which depend on their ability to insert into and disrupt functions of microbial membranes.
  - Cathelicidin: The active cathelicidins protect against infections by multiple mechanisms, including direct toxicity to a broad range of microorganisms and the activation of various responses in leukocytes and other cell types that promote eradication of microbes.
- Barrier epithelia contain certain types of lymphocytes, including intraepithelial T lymphocytes, which recognize and respond to commonly encountered microbes. These subsets are distinguished mainly by the type of antigen receptors they express conventional αβ or γδ TCR.
- These intraepithelial T lymphocytes are thought to recognize a small number of commonly encountered microbial structures, a typical feature of innate pattern recognition receptors



Cellular Components of the Innate Immune System 1

Phagocytes

- Cells that have specialized phagocytic functions, primarily macrophages and neutrophils, eliminate microbes that breach epithelial barriers.

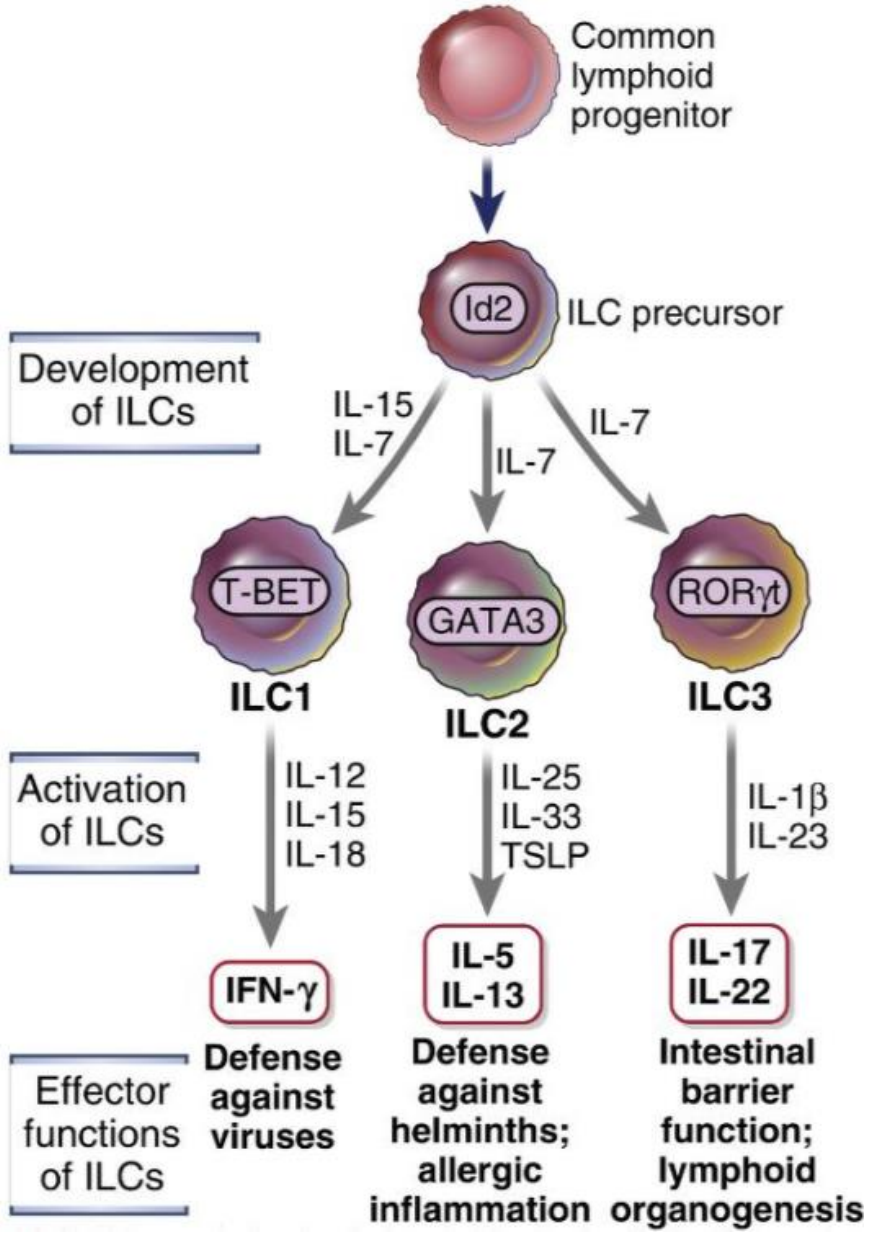
Dendritic Cells

- Dendritic cells rapidly and efficiently detect invading microbes because of their location in tissues and their expression of numerous pattern recognition receptors for PAMPs and DAMPs.
- The ability of DCs to promote T lymphocyte responses after innate immune activation also makes them an important bridge between innate and adaptive immunity.

Cellular Components of the Innate Immune System 2

Cytokine-Producing Innate Lymphoid Cells

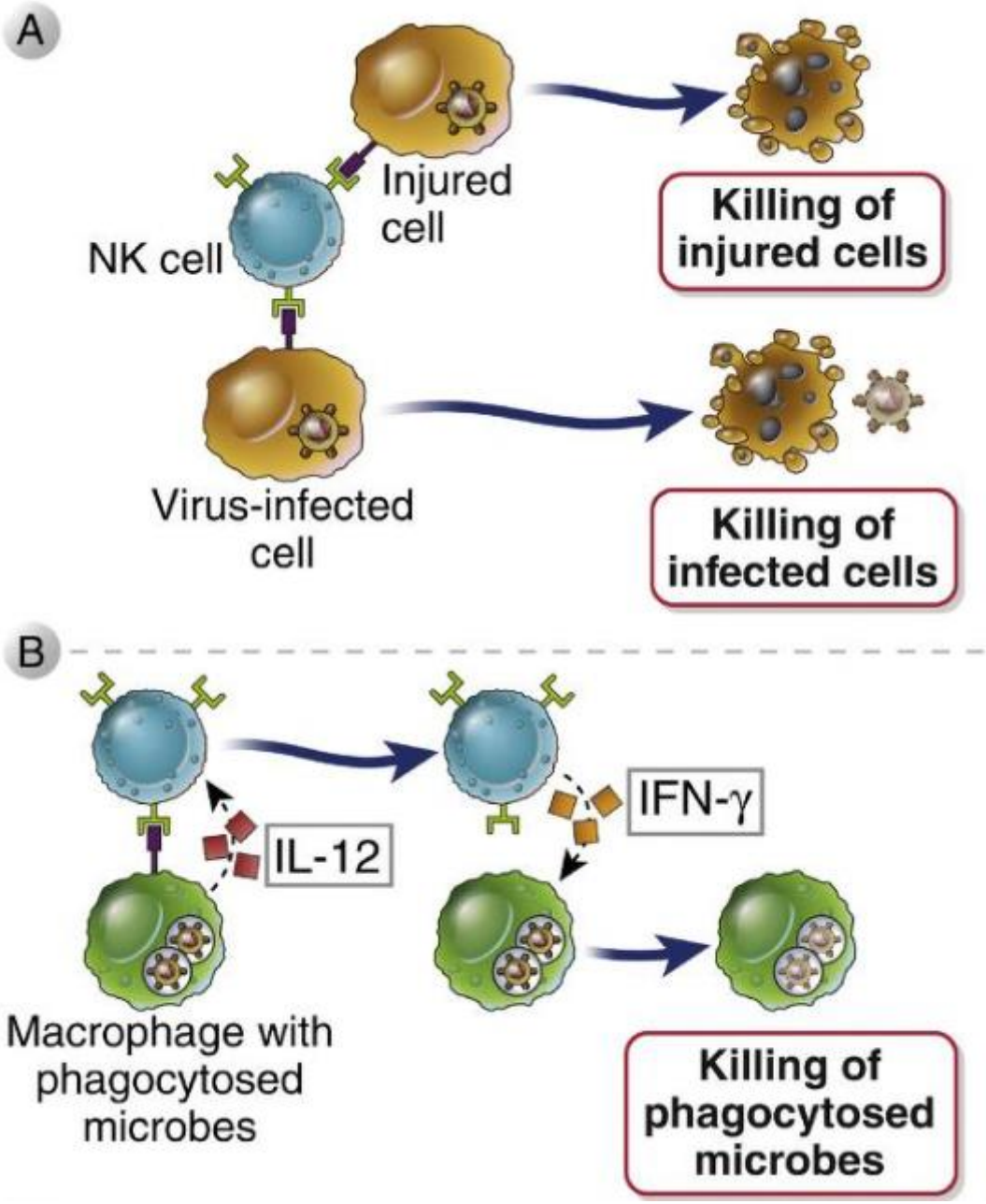
- Three subsets of ILCs, called ILC1, ILC2, and ILC3, produce different cytokines and express different transcription factors, analogous to the Th1, Th2, and Th17 subsets of CD4 + T lymphocytes.
- ILC subsets may participate in host defense against distinct pathogens and also may be involved in inflammatory disorders.



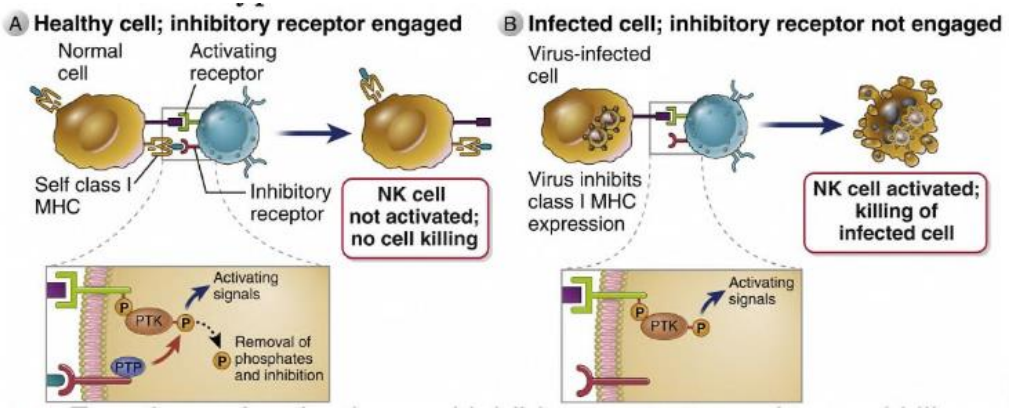
Cellular Components of the Innate Immune System 3

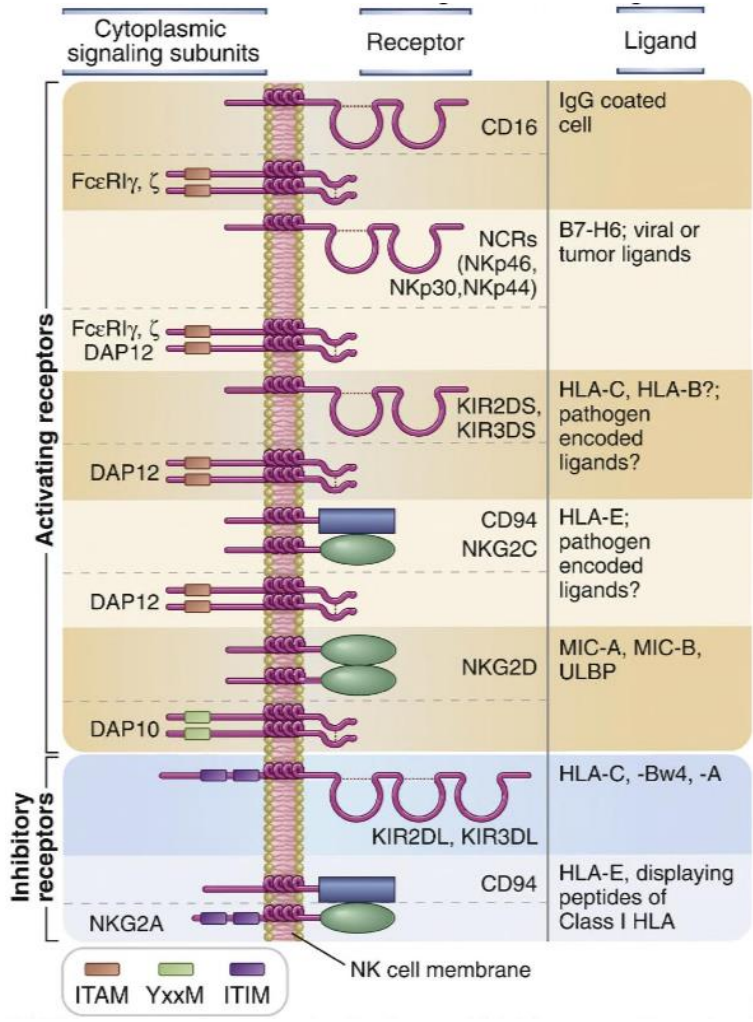
Natural Killer Cells

- NK cells are cytotoxic cells that play important roles in innate immune responses, mainly against viruses and intracellular bacteria.
- The effector functions of NK cells are to kill infected cells and to produce IFN-γ, which activates macrophages to destroy phagocytosed microbes.



Cellular Components of the Innate Immune System 4





Activating and Inhibitory Receptors of Natural Killer Cells

- NK cells distinguish infected and stressed cells from healthy cells, and NK cell function is regulated by a balance between signals generated by activating and inhibitory receptors.
- Activating receptors on NK cells recognize a heterogeneous group of ligands, some of which may be expressed on normal cells and others mainly on cells that have undergone stress, are infected with microbes, or are neoplastic.
- Inhibitory receptors of NK cells recognize class I MHC molecules, which are cell surface proteins normally expressed on all healthy nucleated cells in the body.
- Activating and inhibitory NK receptors contain structural motifs in their cytoplasmic tails that engage the signaling pathways that respectively promote or inhibit target cell killing and cytokine secretion.
- Cytokines can enhance the functional responses of NK cells.

Cellular Components of the Innate Immune System 5

T and B Lymphocytes With Limited Antigen Receptor Diversity

- Small populations of lymphocytes that express antigen receptors that are structurally the same as those of T and B cells, but these receptors have very little diversity.
- These T and B cell subsets may recognize structures expressed by many different or commonly encountered microbial species.
- T cells with limited antigen receptor diversity include invariant natural killer T (iNKT) cells, γδ T cells, mucosa-associated invariant T (MAIT) cells, and intraepithelial T cells with αβ TCRs.
- B cell subsets that produce antibodies with a limited set of specificities include B-1 cells and marginal-zone B cells.

Mast Cells

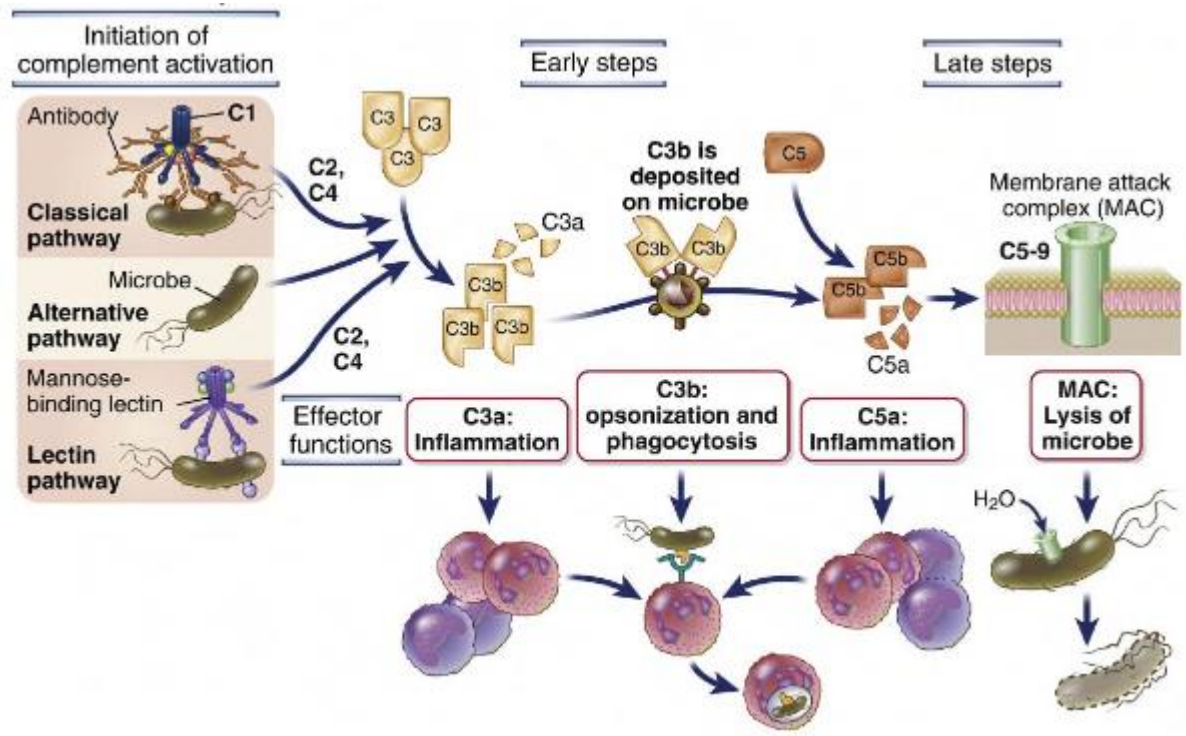
- Mast cells are sentinel cells present in the skin, mucosal epithelium, and connective tissues that rapidly secrete proinflammatory cytokines and lipid mediators in response to infections and other stimuli.

Soluble Effector Molecules of Innate Immunity

- Several different kinds of molecules that recognize microbes and promote innate responses exist in soluble form in the blood and extracellular fluids.
- These molecules provide early defense against pathogens that enter the circulation or are present outside host cells at some stage of their life cycle.
- The soluble effector molecules function in two major ways:
  - By binding to microbes, they act as opsonins and enhance the ability of macrophages and neutrophils to phagocytose the microbes. This is because the phagocytic cells express membrane receptors specific for the opsonins, and these receptors can efficiently mediate the internalization of the complex of opsonin and bound microbes and subsequent destruction of the ingested microbes.
  - After binding to microbes, soluble mediators of innate immunity promote inflammatory responses that bring more phagocytes to sites of infections, and they may also directly kill microbes.

The Complement System

- The complement system consists of several plasma proteins that work together to opsonize microbes, to promote the recruitment of phagocytes to the site of infection, and in some cases to directly kill the microbes.
- The first step in activation of the complement system is recognition of molecules on microbial surfaces, and this occurs in three ways:
  - The classical pathway
  - The alternative pathway
  - The lectin pathway
- Recognition of microbes by any of the three complement pathways results in sequential recruitment and assembly of additional complement proteins into protease complexes.

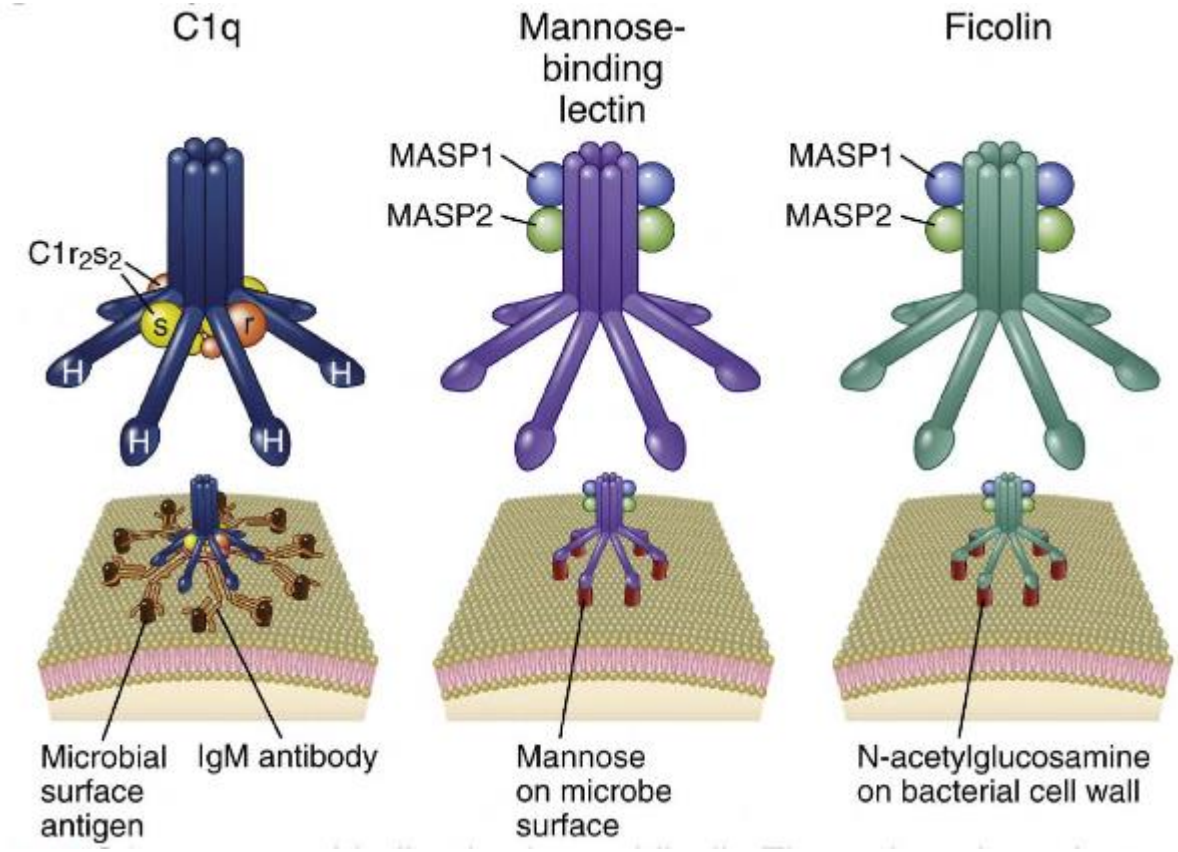


Pentraxins

- Several plasma proteins that recognize microbial structures and participate in innate immunity belong to the pentraxin family, which is a phylogenetically old group of structurally homologous pentameric proteins. Prominent members of this family include the short pentraxins, C-reactive protein (CRP) and serum amyloid P (SAP), and the long pentraxin PTX3.
- Both CRP and SAP bind to several different species of bacteria and fungi.
- The molecular ligands recognized by CRP and SAP include phosphorylcholine and phosphatidylethanolamine, respectively, which are found on bacterial membranes and become exposed on apoptotic cells.
- PTX3 is produced by several cell types, including DCs, macrophages, and endothelial cells, in response to TLR ligands and inflammatory cytokines, such as TNF.
- PTX3 is also stored in neutrophil granules and released as neutrophils die.
- PTX3 recognizes various molecules on fungi, certain bacteria, viruses, and apoptotic cells, and activates the classical complement pathway.

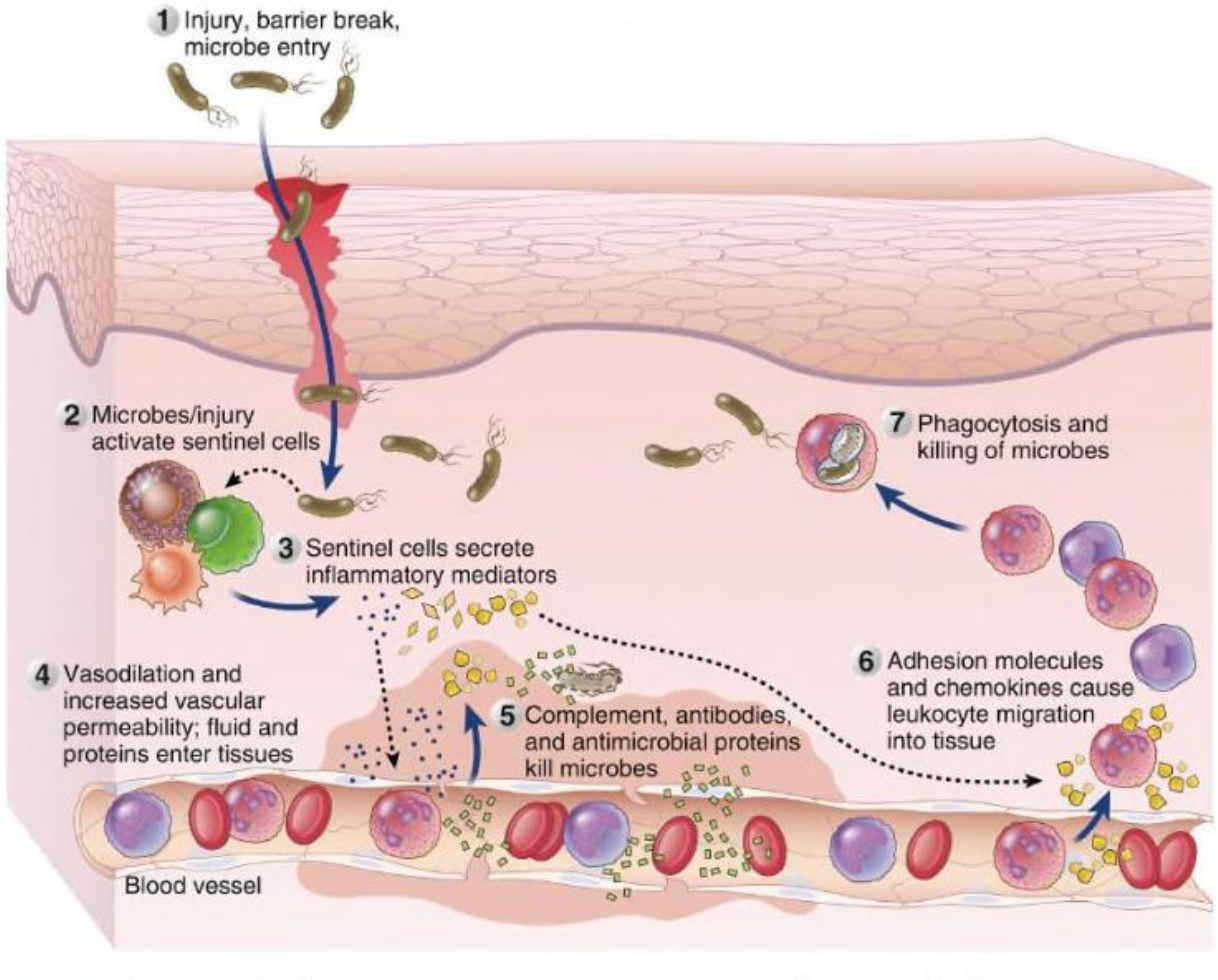
Collectins and Ficolins

- Collectins are a family of trimeric or hexameric proteins, each subunit of which contains a collagen-like tail connected by a neck region to a calcium-dependent (C-type) lectin head.
- MBL, which is a soluble pattern recognition receptor that binds carbohydrates with terminal mannose and fucose, was discussed earlier in relation to the lectin pathway of complement activation. MBL also functions as an opsonin by binding to and enhancing phagocytosis of microbes.
- Surfactant protein A (SP-A) and surfactant protein D (SP-D) are collectins with lipophilic properties shared by other surfactants. They bind to various microorganisms and act as opsonins, facilitating ingestion by alveolar macrophages.
- Ficolins are plasma proteins that are structurally similar to collectins. Bind several species of bacteria, opsonizing them and activating complement in a manner similar to that of MBL.



The Inflammatory Response

- Acute inflammatory responses begin when microbes transgress epithelial barriers or when tissue is injured (1), and then pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) activate sentinel cells, such as macrophages, dendritic cells, mast cells (2), to secrete cytokines and other mediators (3). Some of these mediators (e.g., histamine, prostaglandins) increase the permeability of capillaries (4), leading to the entry of plasma proteins (e.g., complement proteins) into the tissues (5), and others (interleukin-1, tumor necrosis factor) increase expression of endothelial adhesion molecules and chemokines that promote the movement of leukocytes from the post-capillary venules into the tissues (6), where the leukocytes destroy microbes, clear damaged cells (7), and promote more inflammation and repair.



The Major Proinflammatory Cytokines of Innate Immunity

TNF

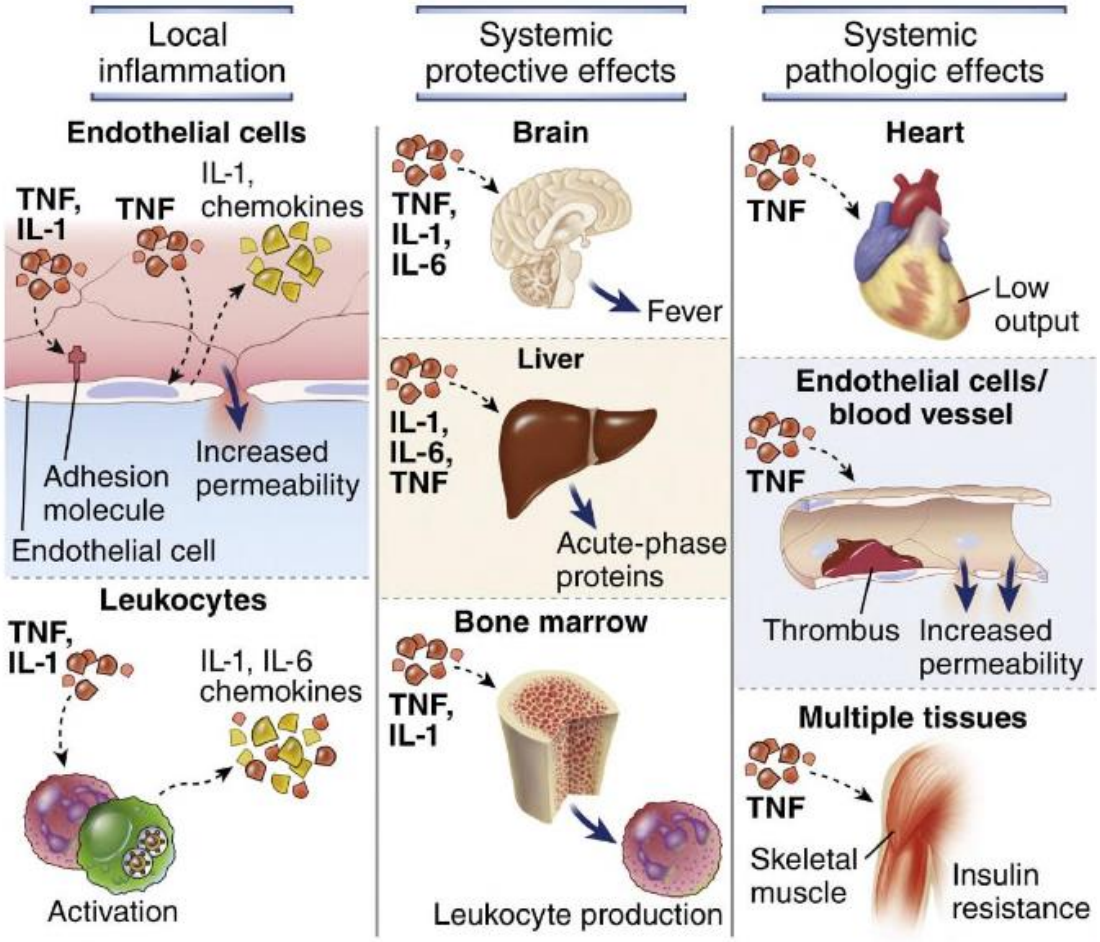
- Is a mediator of the acute inflammatory response to bacteria and other infectious microbes.

Interleukin-1

- Is also a mediator of the acute inflammatory response and has many actions like TNF.

Interleukin-6

- is another important cytokine in acute inflammatory responses with local and systemic effects.



Other Cytokines Produced During Innate Immune Responses

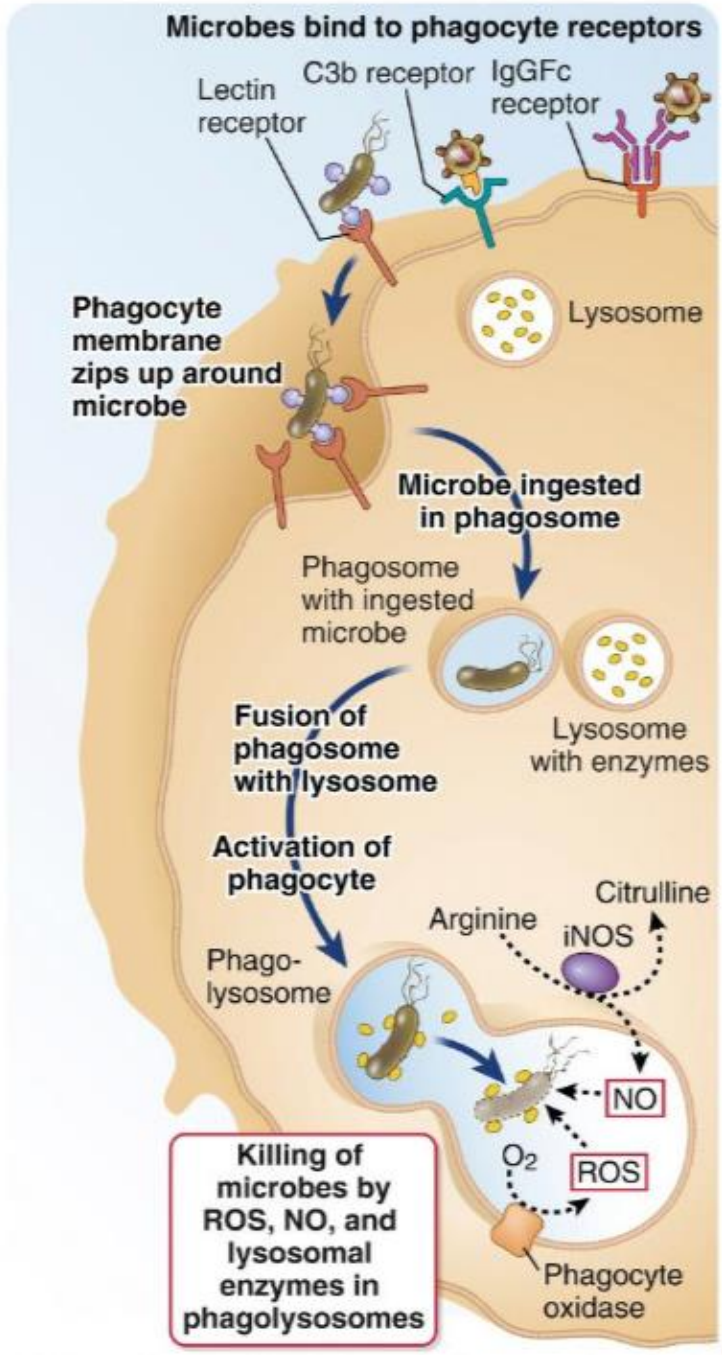
- IL-12 is secreted by DCs and macrophages and stimulates IFN-y production by ILC1s, NK cells, and T cells; enhances NK cell- and CTL mediated cytotoxicity; and promotes differentiation of Th1 cells.
- IL-18 enhances the functions of NK cells, similar to IL-12.
- IL-15 stimulates the growth and functions of ILCls, NK cells, and some T cells.
- IL-25, thymic stromal lymphopoietin (TSLP), and IL-33 are structurally unrelated cytokines produced by epithelial barrier cells, as well as other cell types, which stimulate ILC2s, Th2 cells, and mast cells to produce IL-4, IL-5, and IL-13.
- Other cytokines play important roles in both innate and adaptive immune responses, including IL-5, IL-17, and IFN-y produced by helper T cell subsets.

Sequence of Events in Inflammation: Vascular Changes and Leukocyte Migration Into Tissues

- Acute inflammatory responses are initiated when sentinel cells, including mast cells, tissue-resident macrophages, and DCs, which are present in normal tissues before infection, use TLRs and cytosolic innate pattern recognition receptors to sense microbes and injured cells.
- Recruitment of large numbers of neutrophils, followed by monocytes, from blood into tissues typically occurs as part of the acute inflammatory response to infections and tissue injury.
- Postcapillary venule endothelial cells increase surface expression of adhesion molecules for leukocytes.
- TNF and IL-1 also stimulate various cells to secrete chemokines, such as CXCL8 and CCL2, which bind to receptors on neutrophils and monocytes, respectively.
- The leukocytes accumulate in the tissues, forming an inflammatory infiltrate.
- Neutrophils and monocyte-derived macrophages phagocytose and kill microbes.

Ingestion and Killing of Microbes by Activated Phagocytes

- Neutrophils and macrophages that are recruited into sites of infections ingest microbes into vesicles by the process of phagocytosis and destroy these microbes.
- Neutrophils and macrophages express receptors that specifically recognize microbes, and binding of microbes to these receptors is the first step in phagocytosis.
- Activated neutrophils and macrophages kill phagocytosed microbes by the action of microbicidal molecules in phagolysosomes.
- Activated neutrophils and, to a lesser extent, macrophages convert molecular oxygen into ROS, which are highly reactive oxidizing agents with free radicals that destroy microbes (and other cells).
- Macrophages produce reactive nitrogen species, mainly NO, by the action of an enzyme called inducible nitric oxide synthase (iNOS).
- Activated neutrophils and macrophages produce several proteolytic enzymes in the phagolysosomes that function to destroy microbes.
- Neutrophils also kill microbes by extruding their DNA and granule contents, which form extracellular threads on which bacteria and fungi are trapped and killed.



Role of Macrophages in Tissue Repair

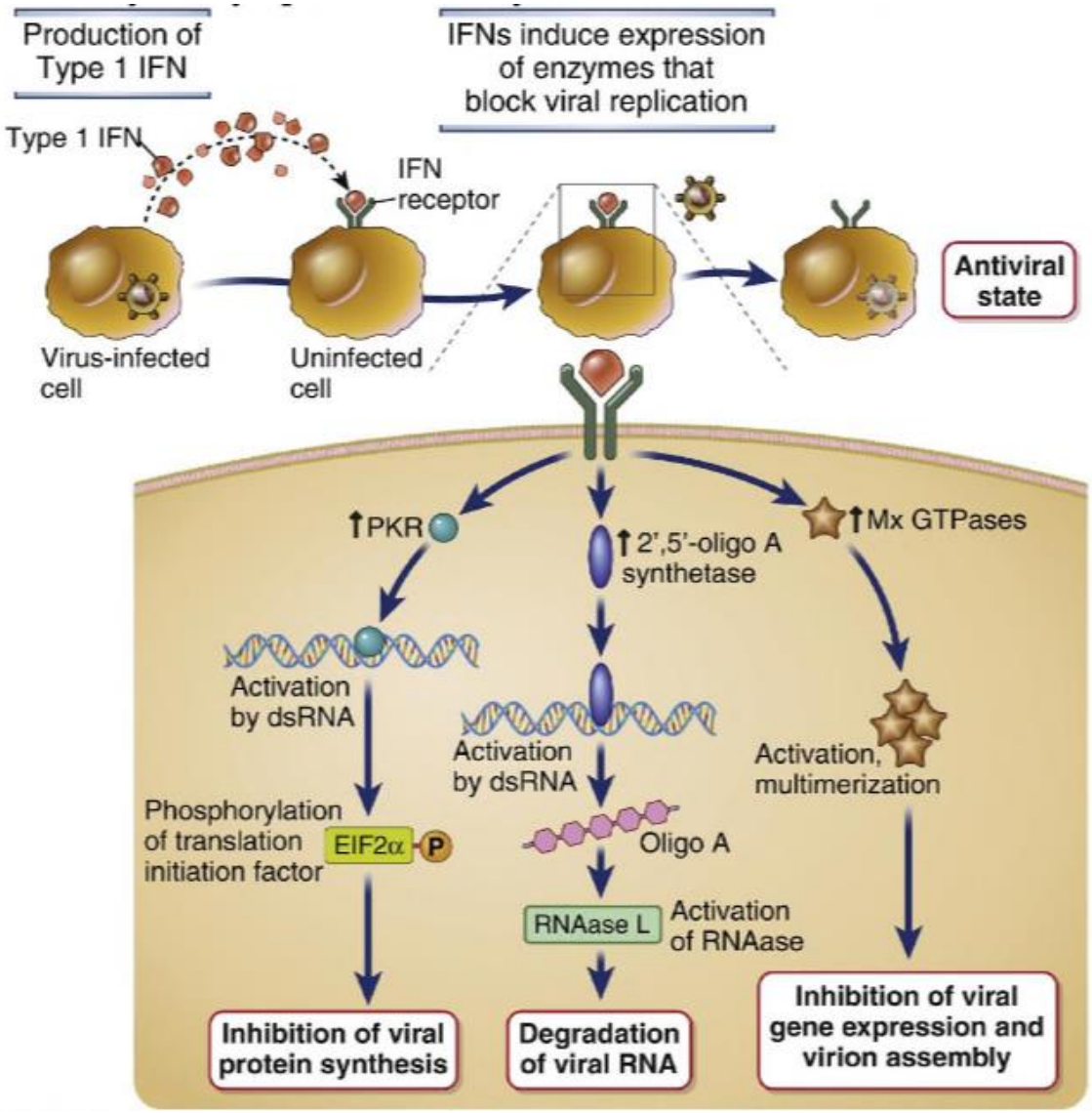
- Acute inflammation is often associated with significant death of host cells, including tissue cells killed by microbes or from collateral damage by microbicidal activities of recruited leukocytes.
- Once the offending agents are eliminated, the damaged tissue has to be repaired. Macrophages play a critical role in the repair process because of several activities.
- Macrophages clear dead cells and secrete growth factors that promote regeneration and angiogenesis.
- Macrophages also secrete TGF-B and other cytokines that stimulate collagen synthesis by fibroblasts, thus promoting the formation of scar tissue to replace the damaged parts.
- Macrophages activated in different ways are responsible for different functions: classically activated macrophages are microbicidal and promote inflammation early in the reaction, and alternatively activated macrophages promote tissue repair later.

Systemic and Pathologic Consequences of Inflammation

- TNF, IL-1, and IL-6 produced during the innate immune response to infection or tissue damage have systemic effects that contribute to host defense and are responsible for many of the clinical manifestations of infection and inflammatory disease.
  - Fever. TNF and IL-1 act on the hypothalamus to induce an increase in body temperature (fever).
  - Leukocytosis. TNF, IL-1, and IL-6 produced at inflammatory sites circulate to the bone marrow and promote the release of neutrophils and monocytes, and other cytokines called colony-stimulating factors stimulate the production of these cells, leading to elevated numbers of white cells in the blood (leukocytosis).
  - Acute-phase response. TNF, IL-1, and IL-6 induce hepatocytes to produce acute-phase proteins, including CRP, SAP, and fibrinogen, which are secreted into the blood.
- In severe infections, TNF may be produced in large amounts and causes systemic clinical and pathologic abnormalities (myocardial contractility and vascular smooth muscle tone, intravascular thrombosis, wasting of muscle and fat cells, called cachexia).
- Acute inflammation may cause tissue injury because the effector mechanisms that leukocytes use to kill microbes are also toxic to host tissues.

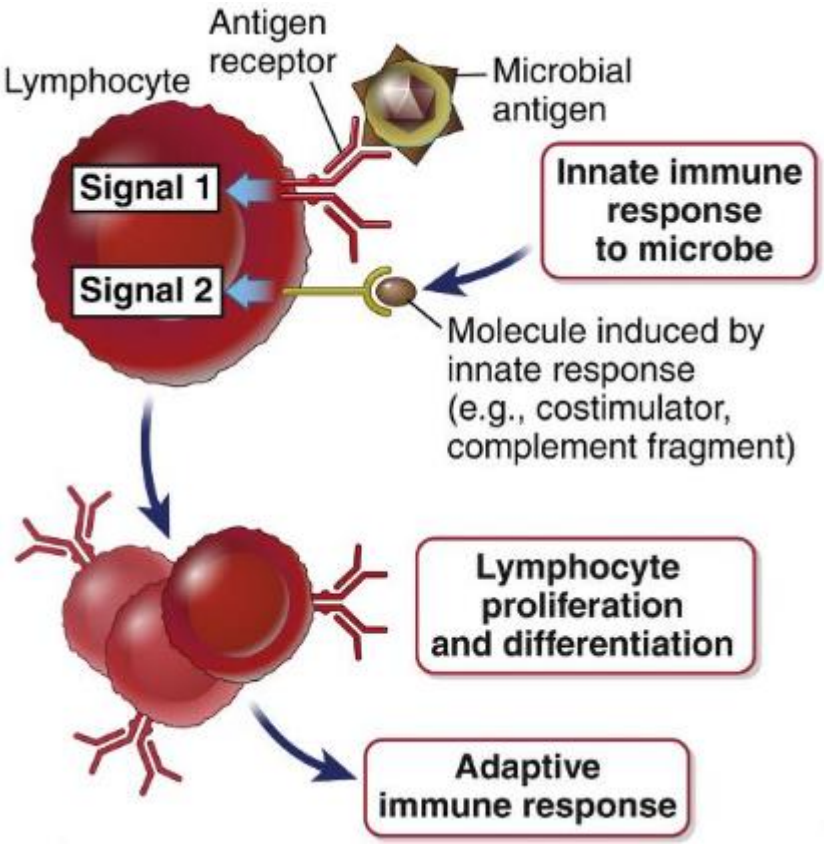
The Antiviral Response

- The major way by which the innate immune system blocks viral infections is to induce the expression of type I IFNs, whose most important action is to inhibit viral replication.
- Type I IFNs are a large family of structurally related cytokines that mediate the early innate immune response to viral infections.
  - Type I IFNs activate transcription of several genes that confer on cells a resistance to viral infection.
  - Type I IFNs cause sequestration of lymphocytes in lymph nodes, thus maximizing the opportunity for encounter with microbial antigens.
  - Type I IFNs increase the cytotoxicity of NK cells and CD8 + CTLsand promote the differentiation of naive T cells to the Th1 subset of helper T cells.
  - Type I IFNs upregulate expression of class I MHC molecules and thereby increase the probability that virally infected cells will be recognized and killed by CD8 + CTLs.
- Protection against viruses is due in part to the activation of intrinsic apoptotic death pathways in infected cells and enhanced sensitivity to extrinsic inducers of apoptosis.



Stimulation of Adaptive Immunity

- The innate immune response provides signals that function in concert with antigen to stimulate the proliferation and differentiation of antigen-specific T and B lymphocytes.
- The second signals generated during innate immune responses to different microbes not only enhance the magnitude of the subsequent adaptive immune response but also influences the nature of the adaptive response.
- Cytokines produced by cells during innate immune responses to microbes or cell injury stimulate the proliferation and differentiation of lymphocytes in adaptive immune responses.
  - IL-12 stimulates the differentiation of naive CD4+ T cells to the Th1 subset of effector cells (see Chapter 10) and naive CD8+ T cells to CTLs.
  - IL-1, IL-6, and IL-23 stimulate the differentiation of naive CD4+ T cells to the Th17 subset of effector cells.
  - IL-25, IL-33, and TSLP stimulate the differentiation of naive CD4+ T cells to the Th2 subset of effector cells.
  - IL-15 promotes the survival of memory CD8+ T cells.
  - IL-6 promotes the survival of antibody-producing plasma cells.



Mechanisms that Limit Innate Immune Responses

- The magnitude and duration of innate immune responses are regulated by a variety of inhibitory mechanisms that limit potential damage to tissues.
- IL-10 is a cytokine that is produced by and inhibits activation of macrophages and DCs.
- There are numerous negative regulatory signaling pathways that block the activating signals generated by pattern recognition receptors and inflammatory cytokines.