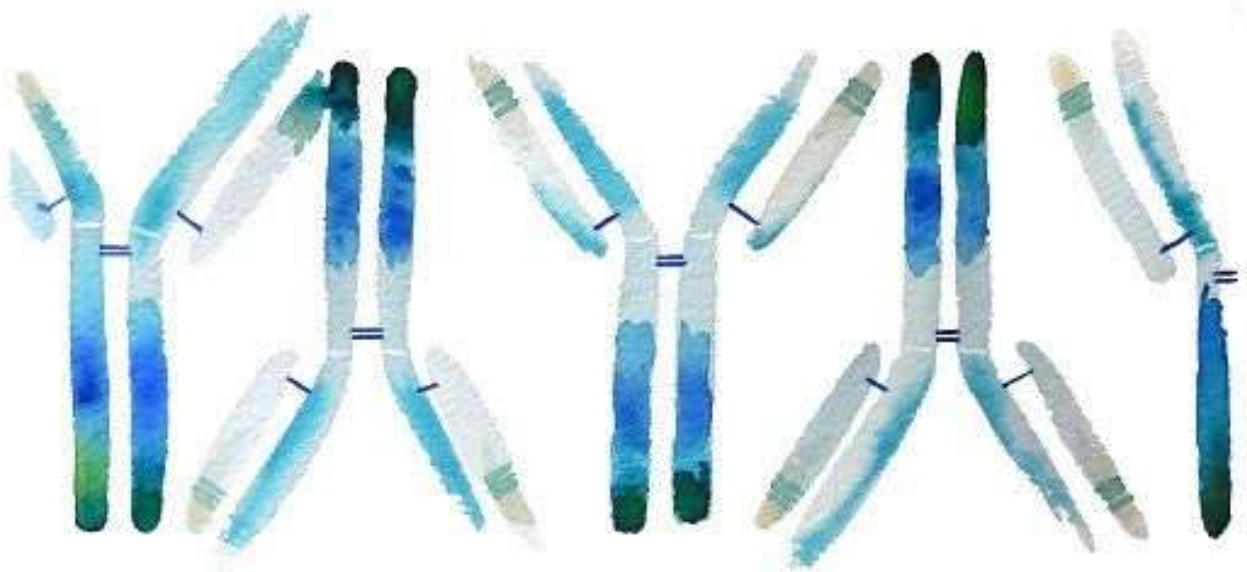
Medical Immunology



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Lecture 5

Molecules of the immune system

- Much of the interactions between cells of the immune system, and between the immune system and foreign introducers depend on the action of cell bound and secreted molecules.

- In this lecture we will discuss some of those molecules.

- Main topics:

DAMPs and PAMPs
TLRs.
NLRs and the inflammasome.
RLRs.
Major inflammatory cytokines (TNF, IL-1, IL-6).

- The cells and soluble molecules of innate immunity either exist in a fully functional state before encounter with microbes or are rapidly activated by microbes

- The innate immune system recognizes molecular structures that are **characteristic of microbial pathogens but not mammalian cells.**

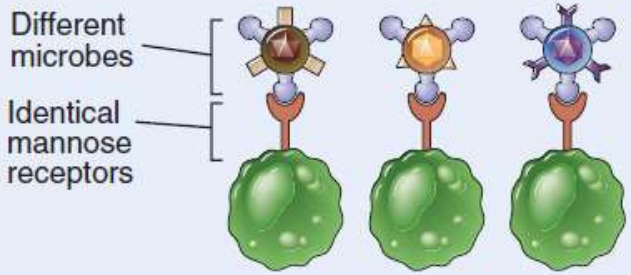
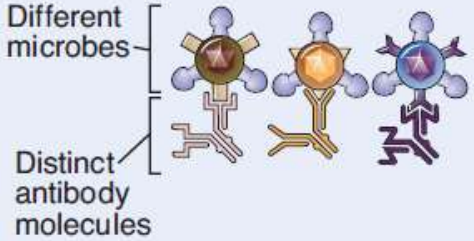
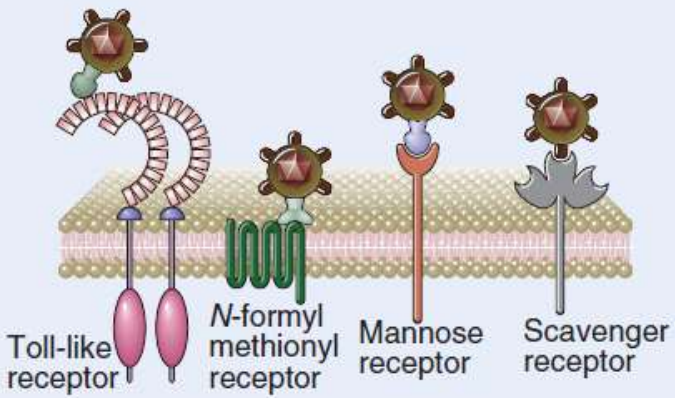
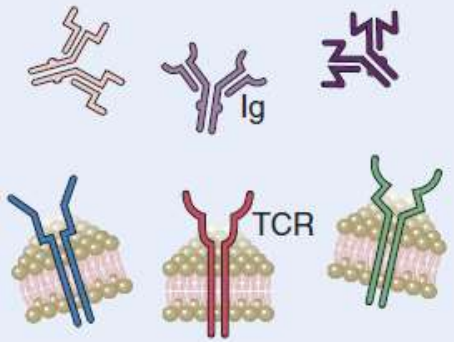
- The innate immune system recognizes microbial products that are often **essential for survival of the microbes.**

- The microbial substances that stimulate innate immunity are called **pathogen-associated molecular patterns (PAMPs).**

- Different classes of microbes (e.g., viruses, gram-negative bacteria, gram positive bacteria, fungi) express different PAMPs.

Molecules of the immune system / Antigen recognition by innate immunity

TABLE 4–1 Specificity of Innate and Adaptive Immunity

	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns) 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination of self and non-self	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

- It is estimated that the innate immune system can recognize about 10^3 molecular patterns. In contrast, the adaptive immune system is capable of recognizing 10^7 or more distinct antigens.

Molecules of the immune system / Antigen recognition by innate immunity

TABLE 4–1 Specificity of Innate and Adaptive Immunity

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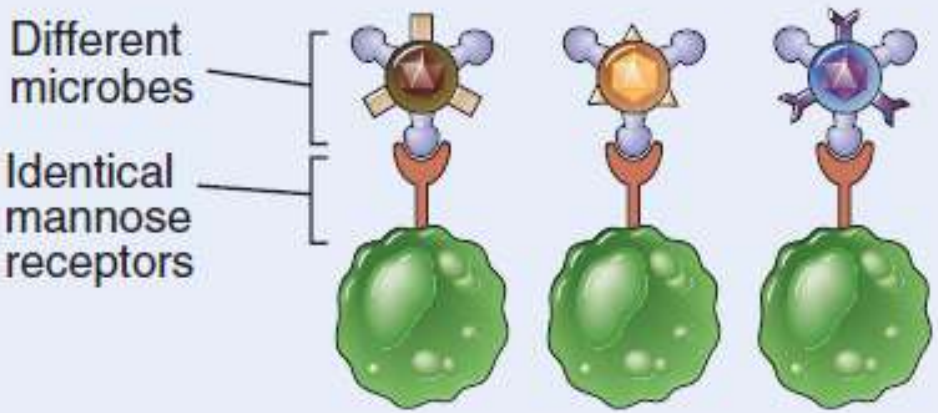
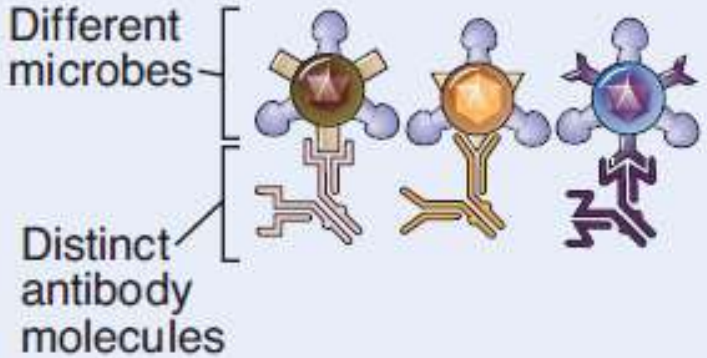
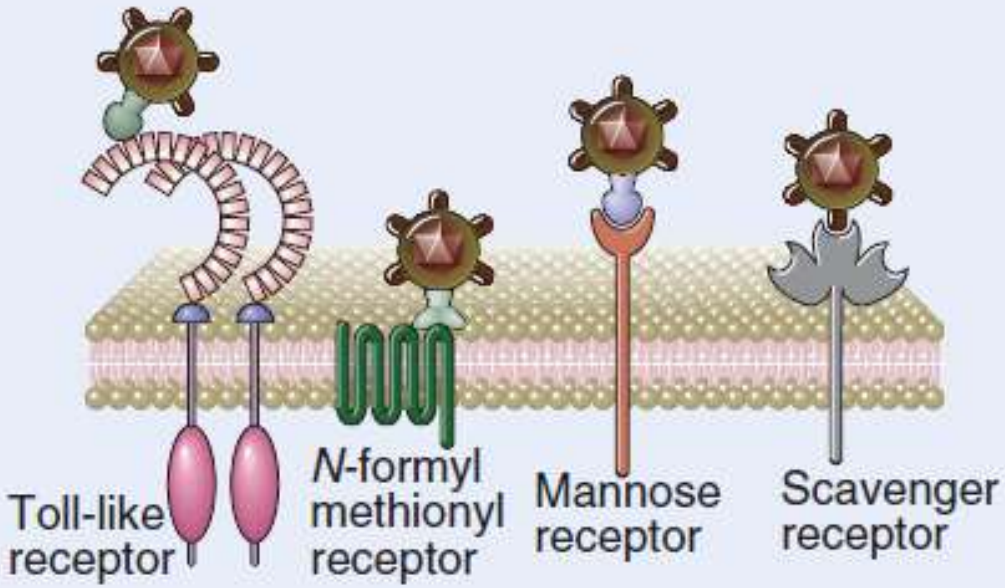
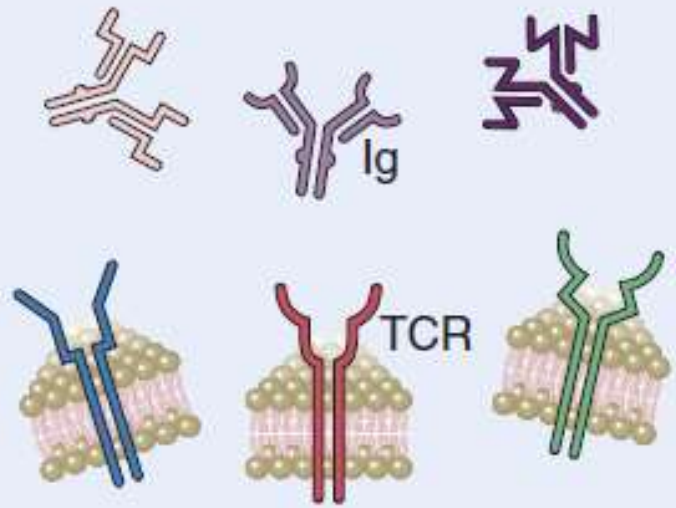
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns)	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens





Molecules of the immune system / Antigen recognition by innate immunity

Receptors	Encoded in germline; limited diversity (pattern recognition receptors) 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination of self and non-self	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

- Characteristics of antigens recognized:

Nucleic acids that are unique to microbes, such as double-stranded RNA found in replicating viruses and unmethylated CpG DNA sequences found in bacteria

Proteins that are found in microbes, such as initiation by N-formylmethionine, which is typical of bacterial proteins.

Complex lipids and carbohydrates that are synthesized by microbes but not by mammalian cells, such as lipopolysaccharide (**LPS**) in gram-negative bacteria, **lipoteichoic acid** or peptidoglycan **(PGN)** in gram positive bacteria, and mannose-rich **oligosaccharides.**

- **limited number of fundamental differences** between microbial molecules and the molecules that higher organisms produce. Thus, the innate immune system has evolved to recognize only a **limited number of molecules**.

- 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).**

- DAMPs may be produced as a result of **cell damage** caused by **infections**, but they may also indicate sterile injury to cells caused by any of myriad reasons, such as **chemical toxins**, **burns**, **trauma**, or **decreased blood supply**.

- DAMPs are generally **not released** from cells dying by apoptosis. In some cases, healthy cells of the immune system are stimulated to produce and release DAMPs, which enhances an innate immune response to infections.

TABLE 4–2 Examples of PAMPs and DAMPs

Pathogen-Associated Molecular Patterns		Microbe Type
Nucleic acids	ssRNA	Virus
	dsRNA	Virus
	CpG	Virus, bacteria
Proteins	Pilin	Bacteria
	Flagellin	Bacteria
Cell wall lipids	LPS	Gram-negative bacteria
	Lipoteichoic acid	Gram-positive bacteria
Carbohydrates	Mannan	Fungi, bacteria
	Dectin glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	
Crystals	Monosodium urate	
Nuclear proteins	HMGB1	

CpG, cytidine-guanine dinucleotide; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; LPS, lipopolysaccharide; ssRNA, single-stranded RNA.

- **Pattern recognition receptors (PRRs)** play a crucial role in the proper function of the innate immune system. PRRs are germline-encoded host sensors, which detect molecules typical for the pathogens.

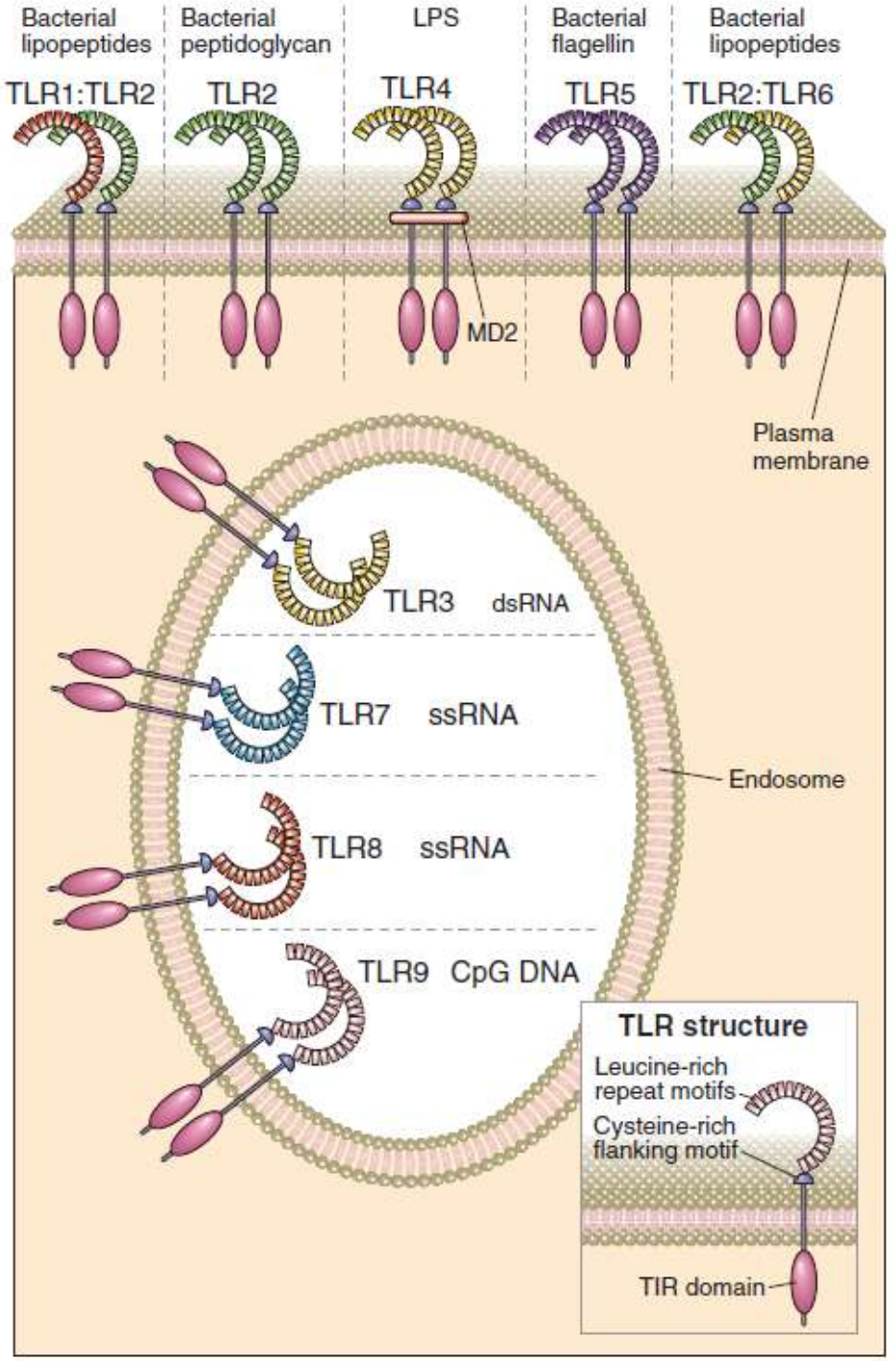
- They are proteins expressed, mainly, by cells of the innate immune system, such as dendritic cells, macrophages, monocytes, neutrophils and epithelial cells, to identify two classes of molecules: **PAMPs** and **DAMPs**.

- PRR can be **cell bound** or **soluble**.

- Cell bound PRR can be found on **different compartments of the cell**. (membrane, cytosol)

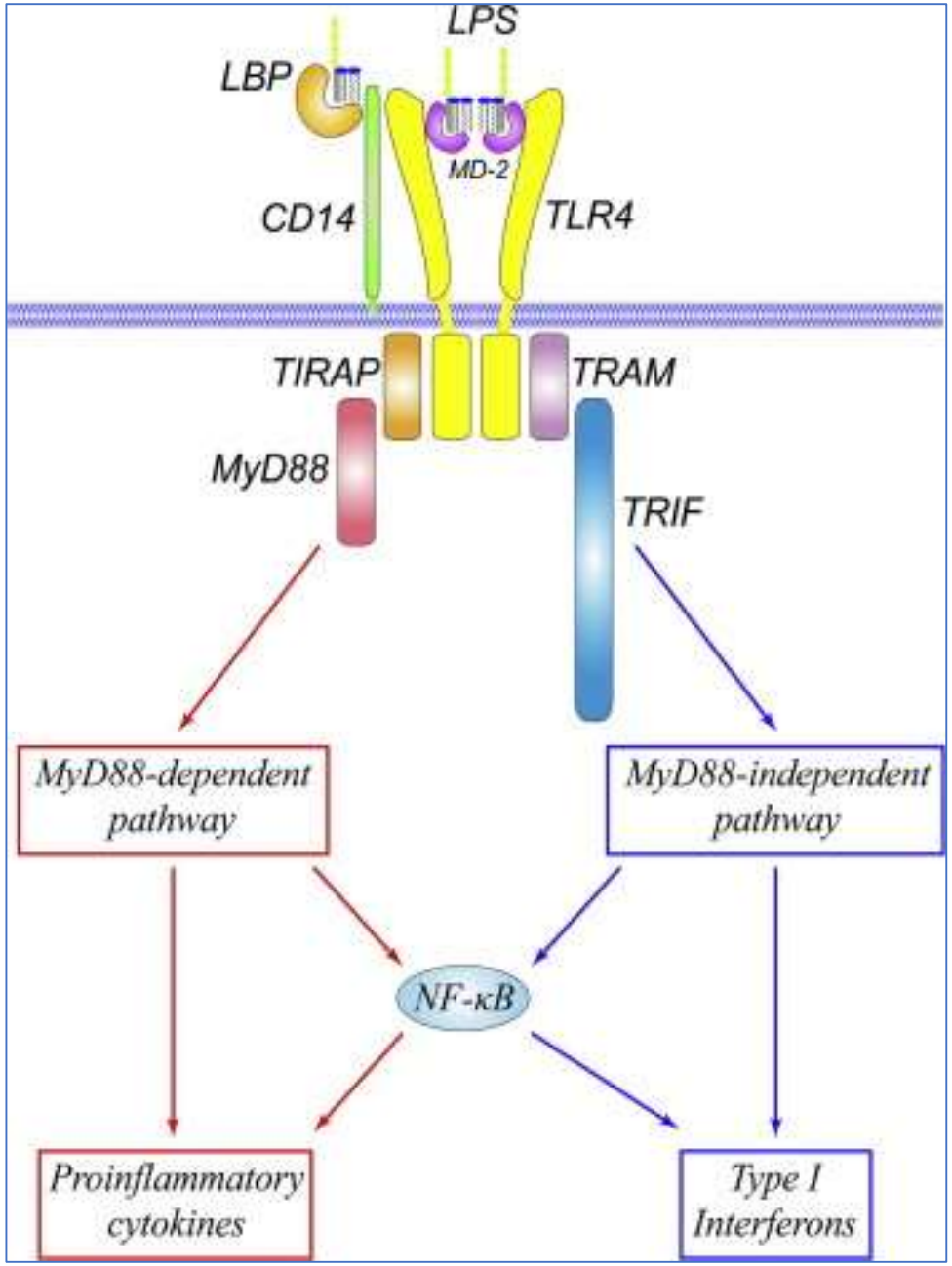
Molecules of the immune system / cell bound PRR/ TLRs

- Toll-like receptors (**TLR**), are proteins that respond to the presence of **pathogenic microbes** by activating antimicrobial defense mechanisms in the cells in which they are expressed.
- TLR are found in every life form in the evolutionary tree from insects up to mammals.
- TLRs are also involved in response to **endogenous molecules** whose expression or location indicates cell damage (DAMP).
- Ligand binding to the leucine-rich domains causes physical interactions between TLR molecules and the formation of **TLR dimers**.

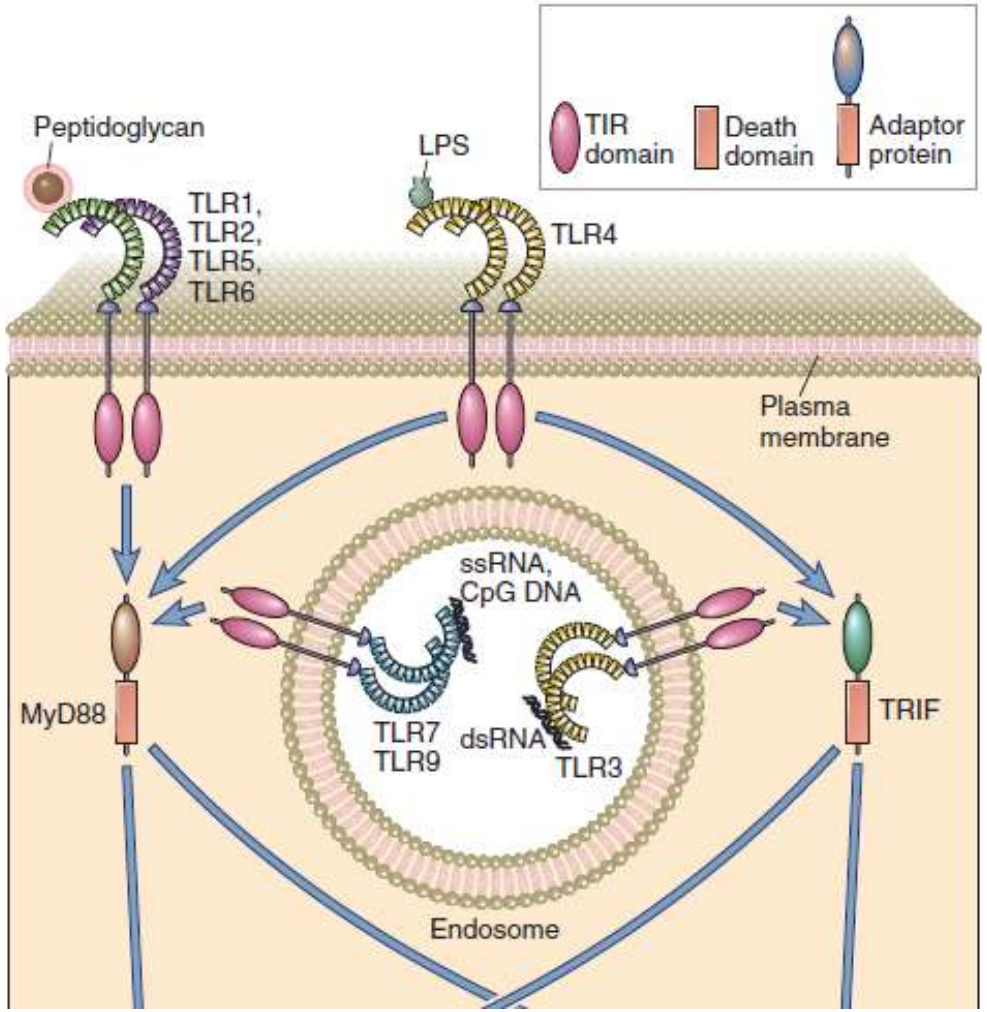


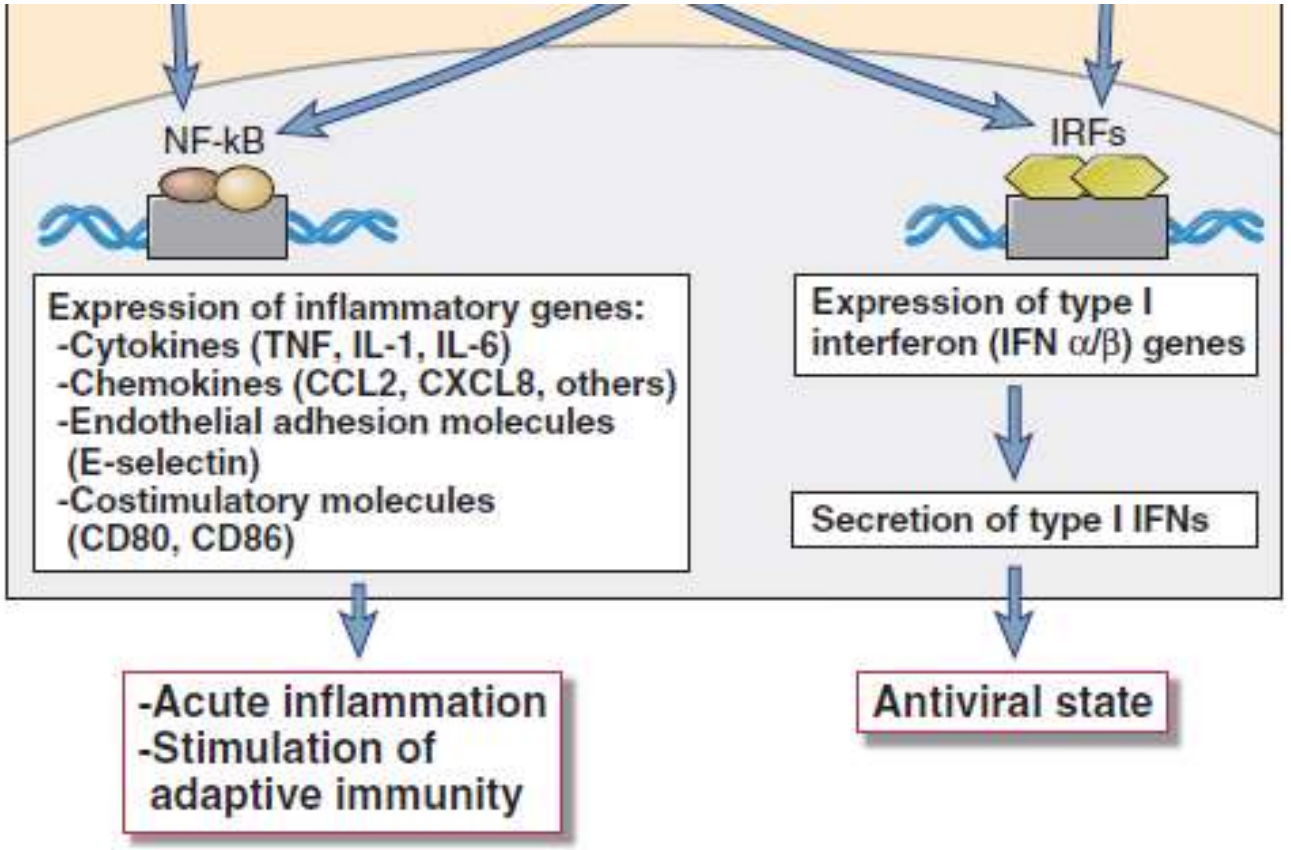
Molecules of the immune system / cell bound PRR/ TLRs

- Adapter and accessory molecules can be needed for proper signalling.
- An extracellular protein called **MD2** (myeloid differentiation protein 2) binds the lipid A component of **LPS**, forming a complex that then interacts with **TLR4** and initiates signaling.
- Another protein called **CD14** is also required for efficient **LPS-induced signaling**.
- Both CD14 and MD2 can also associate with other TLRs.



Molecules of the immune system / cell bound PRR/ TLRs





- **adaptor proteins (MyD88, TRIF)** facilitate the recruitment and activation of various protein kinases, leading to the activation of different transcription factors.

- **All TLRs except TLR3 signal through MyD88** and are therefore capable of activating **NF-κB** and inducing an inflammatory response. TLR3 signals through **TRIF** and therefore activates IRF3 and **induces expression of type I interferons**.

- **Receptors for Carbohydrates** recognize carbohydrates on the surface of microbes, they facilitate the phagocytosis of the microbes and stimulate subsequent adaptive immune responses. These receptors belong to the **C-type lectin family**, so called because they bind carbohydrates (hence, lectins) in a Ca++-dependent manner (hence, C-type). Some of these are **soluble proteins** found in the blood and extracellular fluids; others are **integral membrane proteins** found on the surfaces of macrophages, dendritic cells, and some tissue cells. (examples, **mannose** and **dectin** receptors).

- **Scavenger receptors** comprise a structurally and functionally diverse collection of cell surface proteins found mainly on **macrophages**.

- **N-Formyl met-leu-phe receptors**, expressed by neutrophils and macrophages, recognize bacterial peptides containing **N-formylmethionyl residues** and **stimulate directed movement of the cells. (**i.e those residues are chemoattractans that help phagocytic cells trace the bacteria producing it**)**

Molecules of the immune system / cytoplasmic PRR

- In addition to the membrane-bound TLRs, which sense pathogens outside cells or in endosomes, the innate immune system has evolved to equip cells with pattern recognition receptors that detect infection or cell damage in the **cytoplasm**.

- The two major classes of these cytoplasmic receptors are **NOD-like receptors** and **RIG-like receptors**. These cytoplasmic receptors, like TLRs, are linked to signal transduction pathways that promote inflammation or type I interferon production.

- The normal life cycles of some microbes, such as viral gene translation and viral particle assembly, **take place in the cytoplasm**.

- Some microbes can produce toxins that create pores in host cell plasma membranes, including endosomal membranes, through which microbial molecules can enter the cytoplasm.

Molecules of the immune system / cytoplasmic PRR/ NOD-like receptors (NLRs)

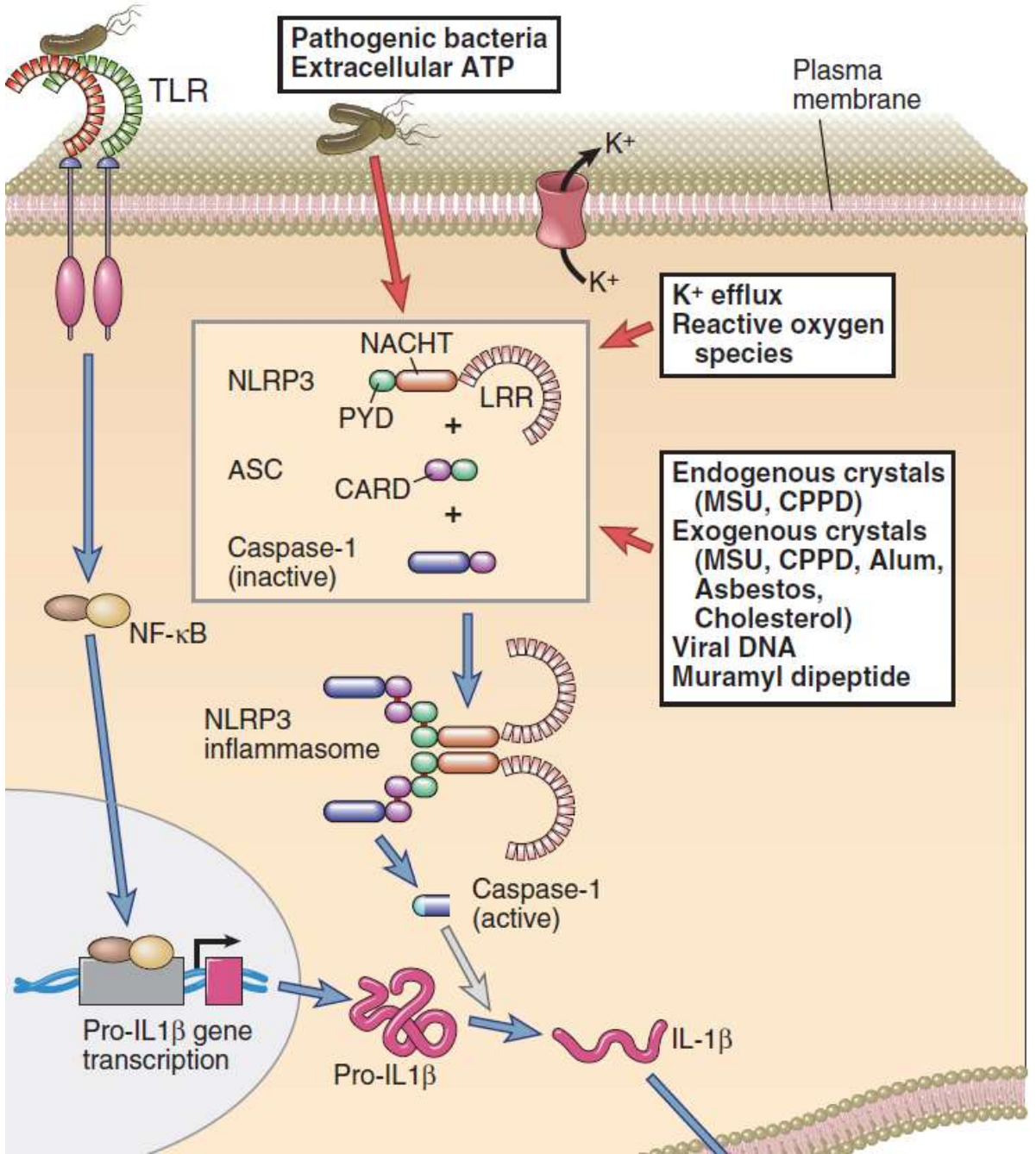
- NOD-like receptors (NLRs) are a family of more than 20 different cytosolic proteins, some of which sense cytoplasmic PAMPs and DAMPs and recruit other proteins to form signaling complexes that promote inflammation.

- **NOD1** and **NOD2**, are expressed in the cytoplasm of several cell types including mucosal epithelial cells and phagocytes, and they respond to bacterial cell wall peptidoglycans.

- The NLRP* subfamily of NLRs respond to cytoplasmic PAMPs and DAMPs by forming signaling complexes called **inflammasomes**, which generate **active forms** of the **inflammatory cytokine IL-1**.

- *(NLR family, pyrin-domain-containing proteins)

- The **inflammasome** is a **multiprotein intracellular complex** that detects pathogenic microorganisms and sterile stressors, and that **activates the highly pro-inflammatory cytokines interleukin-1b (IL-1b)** and **IL-18**. Dysregulation of inflammasomes is associated with a number of autoimmune diseases.



Molecules of the immune system / cytoplasmic PRR/ NOD-like receptors (NLRs)

nature REVIEWS DRUG DISCOVERY

NEWS · 10 MAY 2019 · CLARIFICATION 24 MAY 2019

NLRP3 inhibitors stoke anti-inflammatory ambitions

Inhibitors of the innate immune system's NLRP3 inflammasome promise potential in Parkinson disease, Alzheimer disease, non-alcoholic steatohepatitis, gout and much more, catching the eye of Novartis, Genentech and others.

Molecules of the immune system / cytoplasmic PRR/ RIG-like receptors (RLRs)

- **RIG-like receptors*** RLRs are **cytosolic sensors of viral RNA** that respond to viral nucleic acids by inducing the production of the antiviral type I interferons.

- RLRs can recognize **double-stranded and single-stranded RNA**, which includes the genomes of RNA viruses and RNA transcripts of RNA and DNA viruses

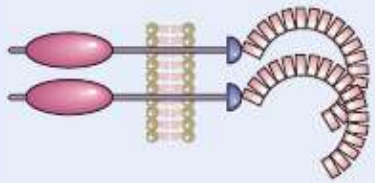
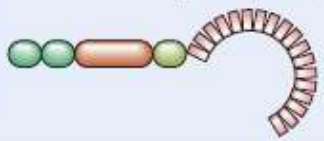
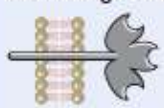
- RLRs also can discriminate viral single-stranded RNA from normal cellular single-stranded RNA transcripts.

- RLRs are expressed in a wide variety of cell types, including bone marrow–derived leukocytes and various tissue cells.

*RIG (retinoic acid–inducible gene)

Molecules of the immune system / cell bound PRR

TABLE 4–3 Pattern Recognition Molecules of the Innate Immune System

Cell-Associated Pattern Recognition Receptors	Location	Specific Examples	PAMP/DAMP Ligands
Toll-like receptors (TLRs) 	Plasma membrane and endosomal membranes of dendritic cells, phagocytes, B cells endothelial cells, and many other cell types	TLRs 1-9	Various microbial molecules including bacterial LPS and peptidoglycans, viral nucleic acids
NOD-like receptors (NLRs) 	Cytoplasm of phagocytes epithelial cells, and other cells	NOD1/2 NALP family (inflammasomes)	Bacterial cell wall peptidoglycans Flagellin, muramyl dipeptide, LPS; urate crystals; products of damaged cells
RIG-like receptors (RLRs) 	Cytoplasm of phagocytes and other cells	RIG-1, MDA-5	Viral RNA
C-type lectin–like receptors 	Plasma membranes of phagocytes	Mannose receptor Dectin	Microbial surface carbohydrates with terminal mannose and fructose Glucans present in fungal cell walls
Scavenger receptors 	Plasma membranes of phagocytes	CD36	Microbial diacylglycerides
N-Formyl met-leu-phe receptors 	Plasma membranes of phagocytes	FPR and FPRL1	Peptides containing *N*-formylmethionyl residues

- One of the earliest responses of the innate immune system to infection and tissue damage is the secretion of cytokines by tissue cells, which is critical for the acute inflammatory response.

- Three of the most important proinflammatory cytokines of the innate immune system are **TNF**, **IL-1**, and **IL-6**.

- Tissue **macrophages** and **mast cells** are the major source of these cytokines, although other cell types, including **endothelial** and **epithelial cells**, can also produce IL-1 and IL-6.

Molecules of the immune system / Important cytokines

Cytokines are a broad and loose category of small proteins that are important in cell signalling.

Cytokines include **chemokines**, **interferons**, **interleukins**, lymphokines, and **tumour necrosis factors**

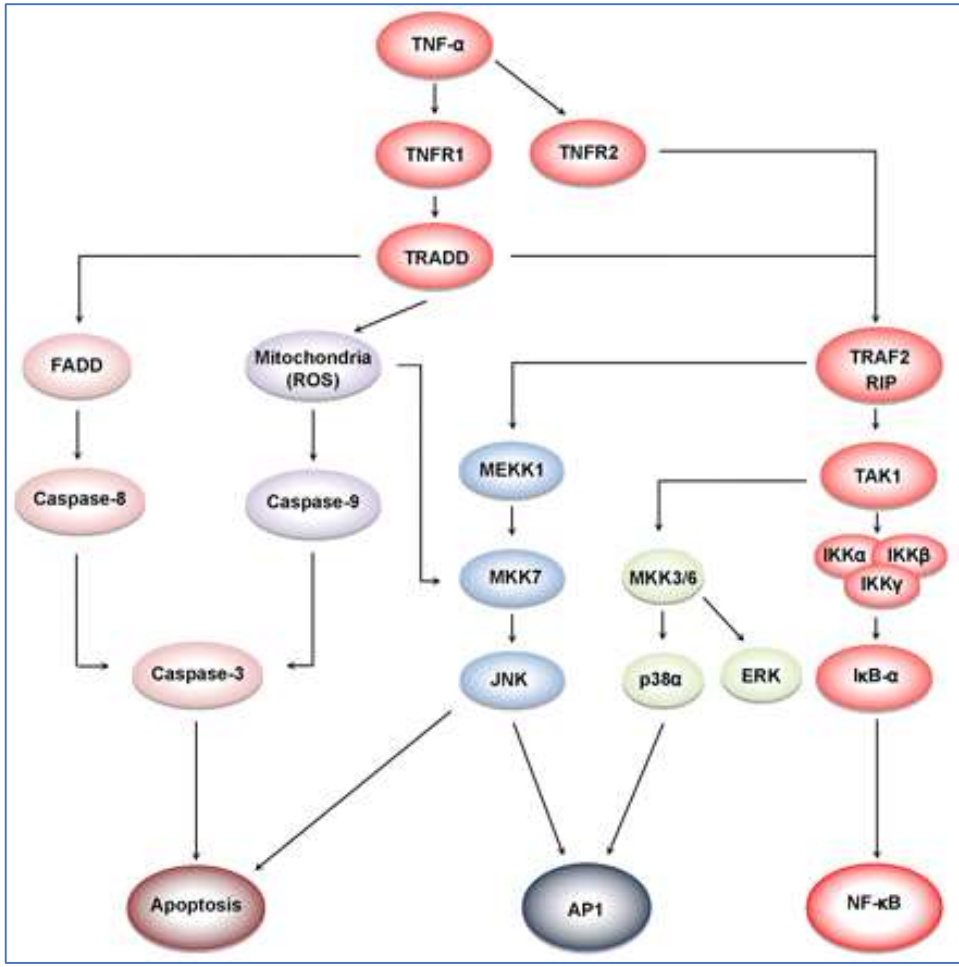
Cytokines are produced by a broad range of cells, including **immune** and **non-immune cells**

TABLE 2–2 Some Cytokines Acting in Infection

	CELL SOURCE	FUNCTIONS
Interleukins (IL)		
IL-1	Macrophages, endothelium, fibroblasts, epithelial	Differentiation and function of immune effectors, PMN response (T_H17)
IL-2	T cells (T_H1)	T-cell proliferation, cytolytic activity of natural killer (NK) cells
IL-4	T cells (T_H2), macrophages, B cells	Differentiation of naïve T cells to helper T cells, proliferation of B cells
IL-5	T cells (T_H2)	Eosinophil activation
IL-8	Macrophages, endothelial, T cells, keratinocytes, PMNs	Chemoattractant for PMNs and T cells, PMN degranulation, migration of PMNs
IL-17	T cells (T_H17)	Inflammation, PMN response
IL-22	T cells (T_H17)	Antimicrobial peptides
Interferons (IFN)		
IFN-α/β	T cells, B cells, macrophages, fibroblasts	Antiviral activity, stimulates macrophages, MHC class I expression
IFN-γ	T cells (T_H1, CTLs), NK cells	T-cell activation, macrophage activation, PMNs, NK cells, antiviral, MHC class I and II expression
Tumor Necrosis Factor (TNF)		
TNF-α	T cells, macrophages, NK cells	Expression of multiple cytokines, (growth and transcription factors), stimulates inflammatory response, cytotoxic for tumor cells
TNF-β	T cells, B cells	Same as TNF-α

MHC, Major histocompatibility complex; PMN, Polymorphonuclear neutrophil

- Tumor necrosis factor (TNF) is a **mediator of the acute inflammatory response** to bacteria and other infectious microbes.
- TNF production by **macrophages** is stimulated by PAMPs and DAMPs. TLRs, NLRs, and RLRs can all induce TNF gene expression, in part by activation of the NF-κB transcription factor.
- TNF can also **mediate cell proliferation** and in some cases **cell death**.
- TNF superfamily plays highly diversified roles in the body.



- Interleukin-1 (IL-1) is also a mediator of the acute inflammatory response and has many similar actions as TNF.

- Unlike TNF, IL-1 is also produced by many cell types other than macrophages, such as neutrophils, epithelial cells (e.g., keratinocytes), and endothelial cells.

- There are two forms of IL-1, called IL-1α and IL-1β, The main biologically active secreted form is IL-1β.

- IL-1β gene **transcription is induced by TLR and NOD signaling pathways** that **activate NF-κB**, whereas **pro–IL- 1β cleavage is mediated by the NLRP3 inflammasome**.

- IL-1 mediates its biologic effects through a membrane receptor called the **type I IL-1 receptor**

- IL-6 is another important cytokine in acute inflammatory responses that has both local and systemic effects, including the induction of liver synthesis of a variety of other inflammatory mediators, the stimulation of neutrophil production in the bone marrow, and the differentiation of IL-17–producing helper T cells

Molecules of the immune system / The Major Proinflammatory Cytokines

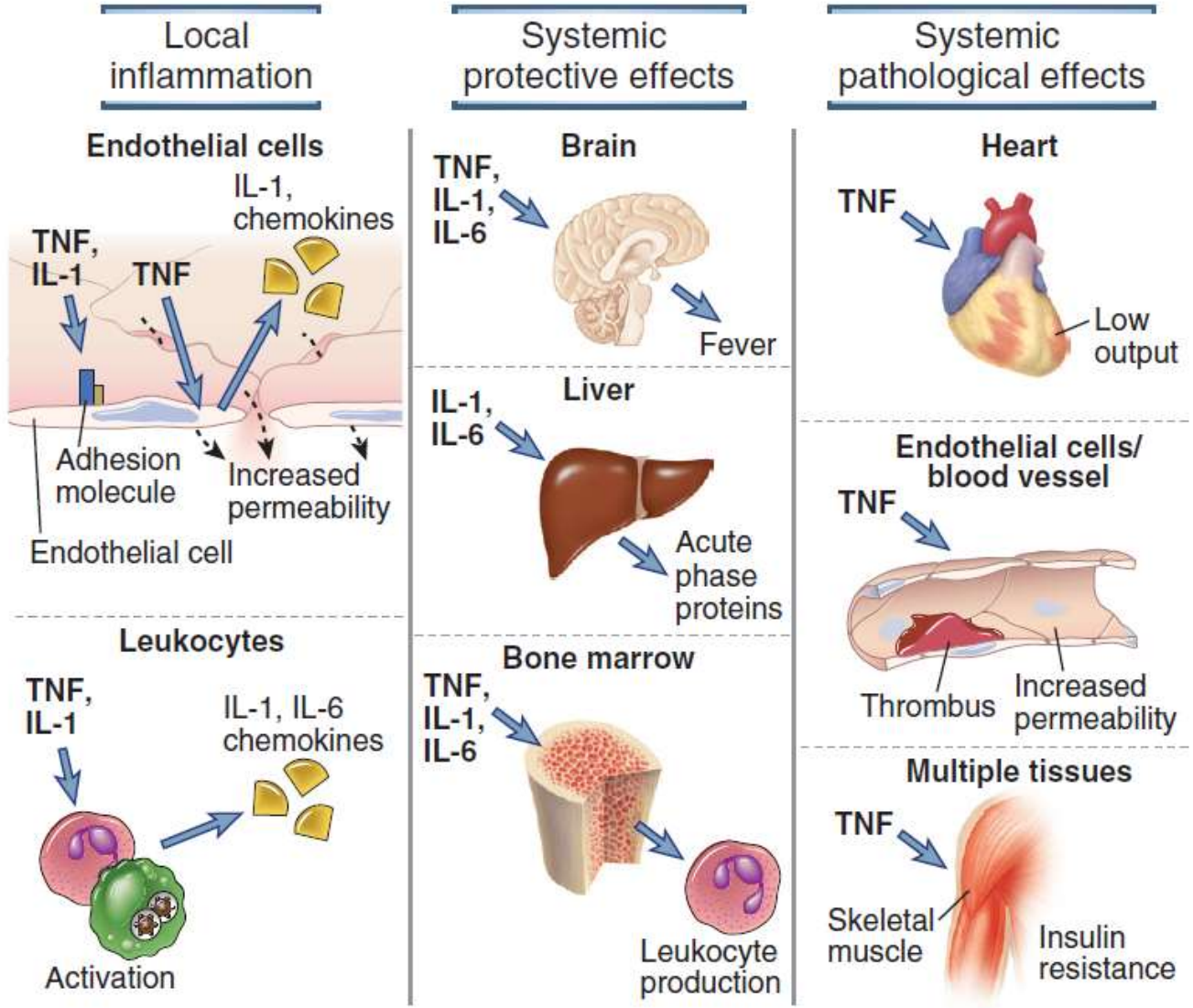


FIGURE 4–14 Local and systemic actions of cytokines in inflammation. TNF, IL-1, and IL-6 have multiple local and systemic inflammatory effects. TNF and IL-1 act on leukocytes and endothelium to induce acute inflammation, and both cytokines induce the expression of IL-6 from leukocytes and other cell types. TNF, IL-1, and IL-6 mediate protective systemic effects of inflammation, including induction of fever, acute-phase protein synthesis by the liver, and increased production of leukocytes by the bone marrow. Systemic TNF can cause the pathologic abnormalities that lead to septic shock, including decreased cardiac function, thrombosis, capillary leak, and metabolic abnormalities due to insulin resistance.

- 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**.

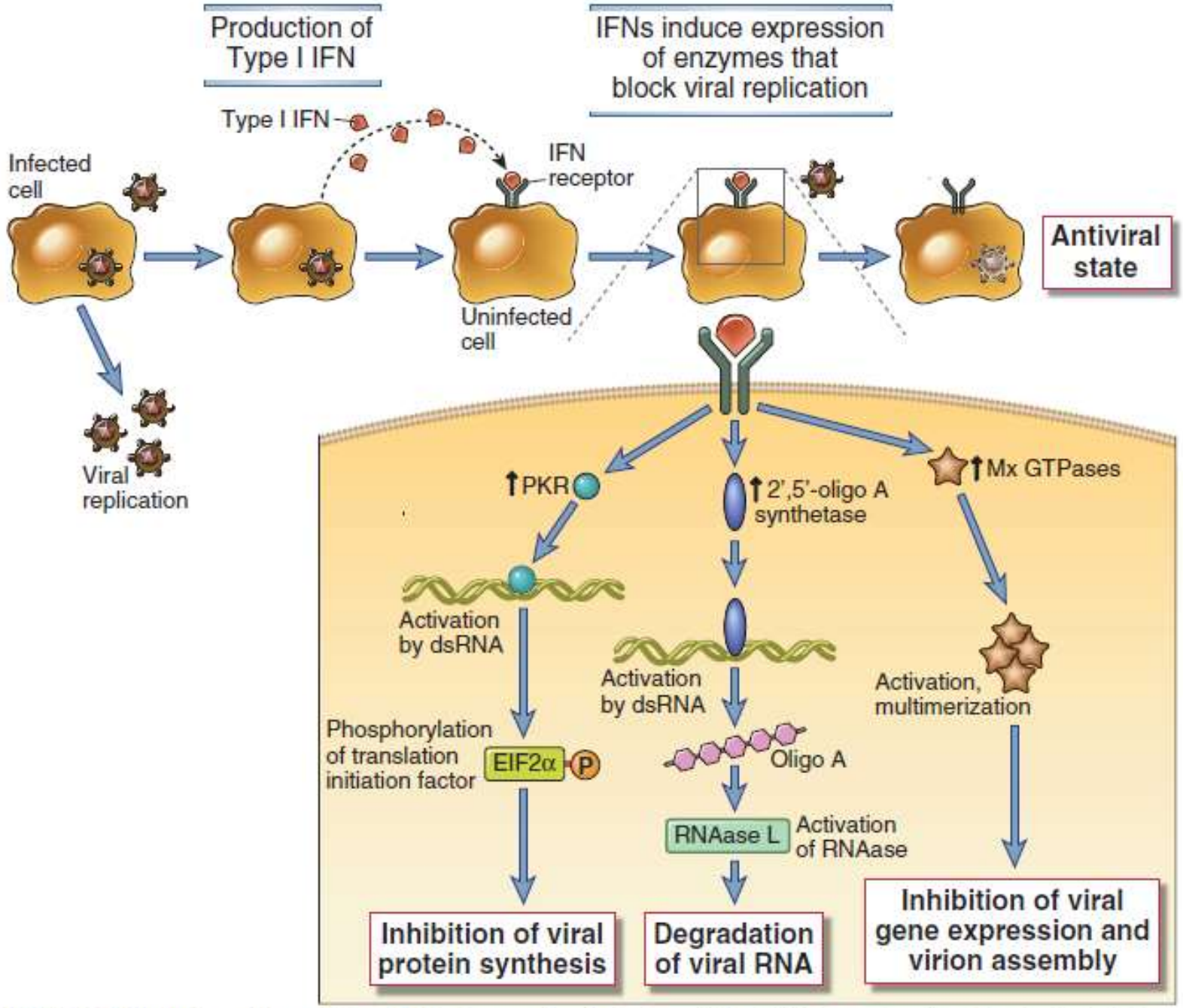
- Type I interferons, signaling through the type I interferon receptor, activate transcription of several genes that confer on the cells a resistance to viral infection, called an **antiviral state**.

- Type I interferons cause **sequestration of lymphocytes in lymph nodes**, thus maximizing the opportunity for encounter with microbial antigens.

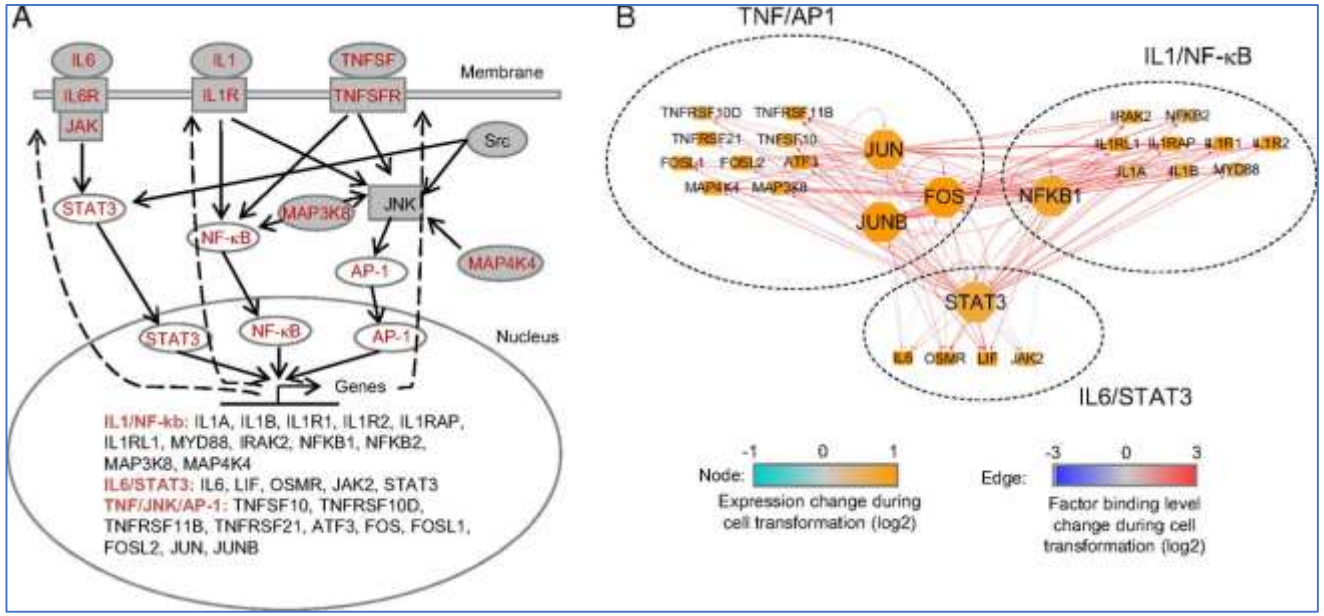
- Type I interferons **increase the cytotoxicity of NK cells** and **CD8+ CTLs**

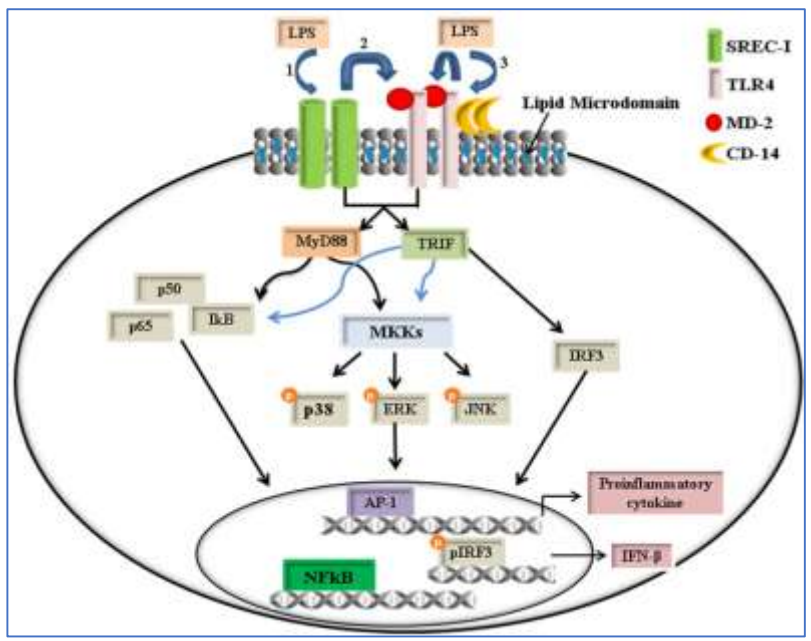
- **Upregulate expression of class I MHC** molecules and thereby increase the probability that virally infected cells will be recognized and killed by CD8+ CTLs.

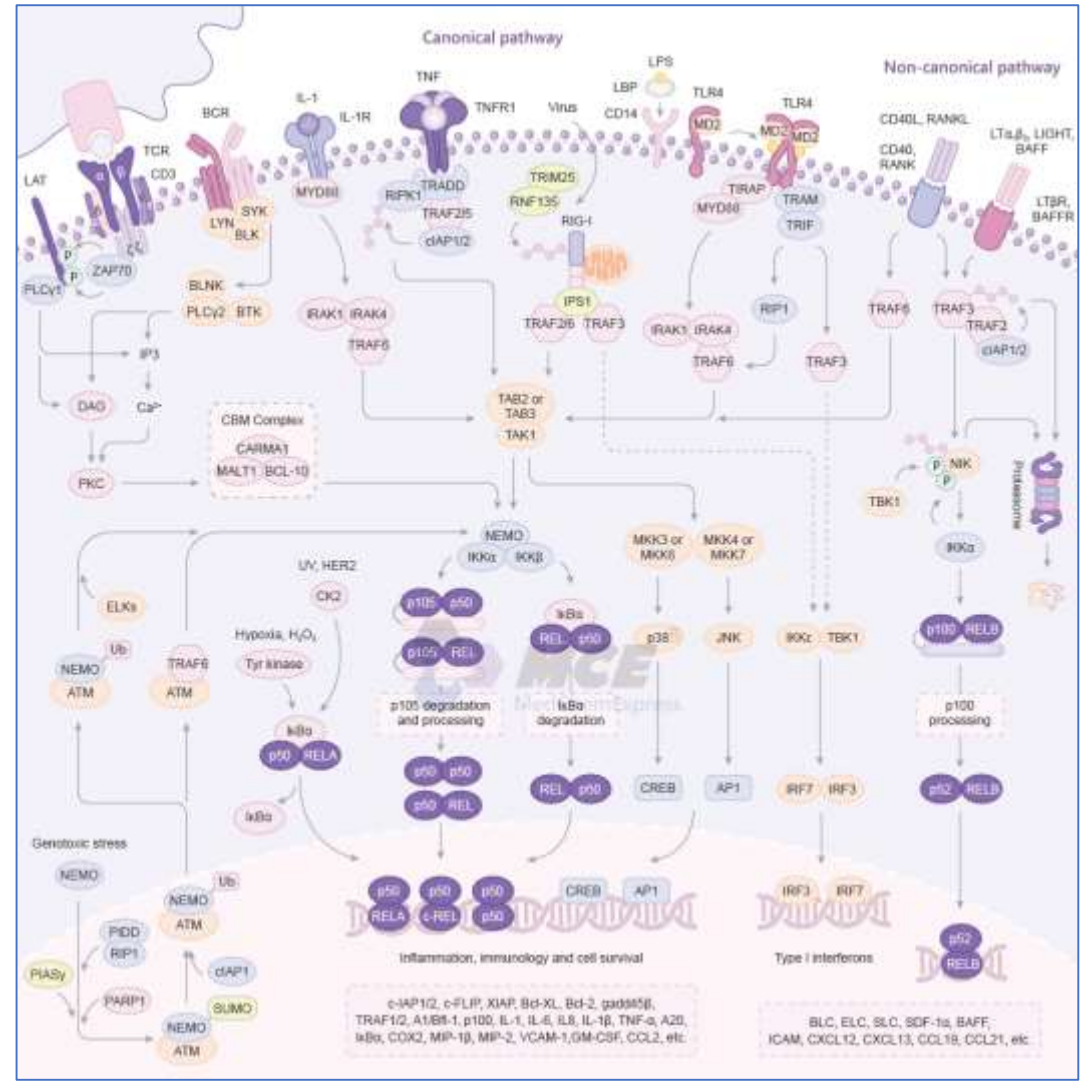
Molecules of the immune system / Important cytokines / Interferons



Molecules of the immune system / The Major Proinflammatory Cytokines







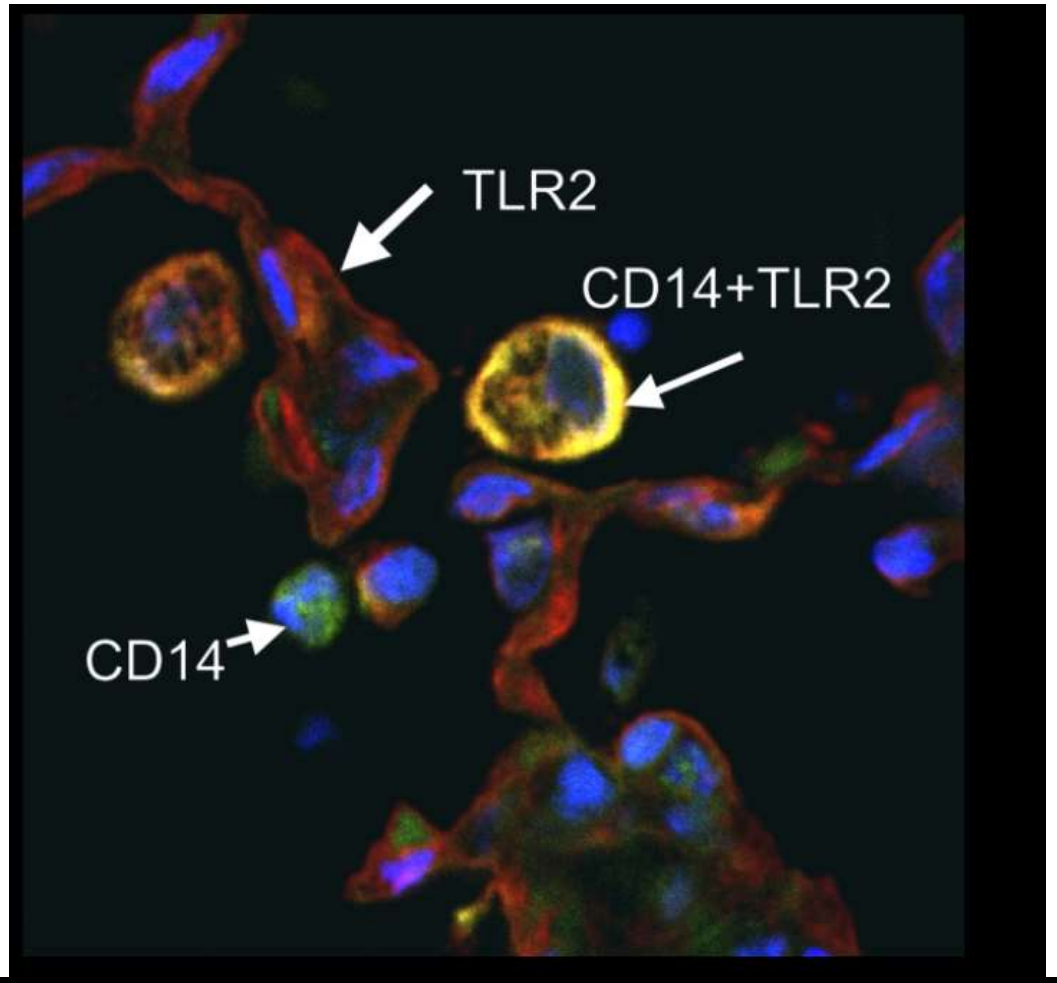


Figure 3. TLR2 and CD14 in the lungs of a rabbit. TLR2 is labeled *red* and CD14 is labeled *green*. Colocalization of TLR2 and CD14 is shown in *yellow*. TLR2 is visible on the alveolar epithelium and on alveolar macrophages in the airspace. CD14 is visible on alveolar macrophages, and neutrophils in the intravascular and alveolar space. The *bright yellow* alveolar macrophage shows high levels of expression of both TLR2 and CD14. Similar results are found when the sections are labeled for TLR4 and CD14.

Further reading:

- Cellular and Molecular Immunology. 7th Edition..

Chapter 4. Innate immunity

CELL-ASSOCIATED PATTERN RECOGNITION RECEPTORS OF INNATE IMMUNITY

SOLUBLE RECOGNITION AND EFFECTOR MOLECULES OF INNATE IMMUNITY