Doctor 021

IMMUNOLOGY

Sheet no. 5

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MOLECULES OF THE IMMUNE SYSTEM

In this sheet we will talk about the important molecules of the immune system, which are responsible for the identification for non-self-structures, whether protiens, carbs or lipids. These non-self-structures sometimes carry a signal that the innate immune system can recognize, and we will also talk about the receptors of our innate immunity that can recognize foreignness.

- We discussed before how adaptive immunity recognize foreign structures, but we didn't talk about how innate immunity does that.
- Receptors in innate immunity that recognize foreignness can't do genetic recombination.

➢ **The diversity of patterns they can recognize is much less than in adaptive immunity.**

❖ **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).**

ANTIGEN RECOGNITION BY INNATE IMMUNITY

❖ **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.**

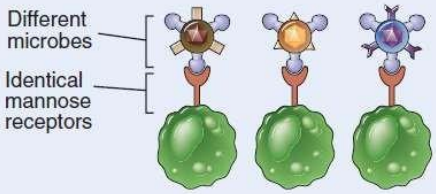
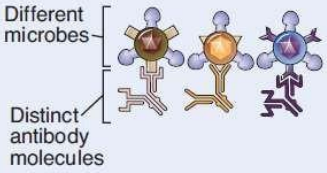
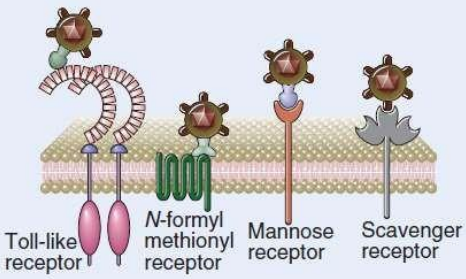
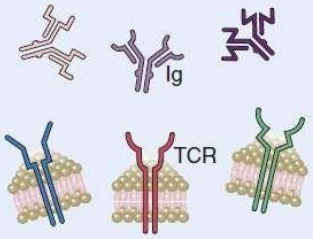
❖ **The innate immune system recognizes microbial products that are often essential for survival of the microbes.** (PGN,LPS,LTA…etc.)

❖ **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.**

- The recognized patterns must be essential, for example plasmids and capsules aren't essential so they will not be recognized by the innate system.
- you only have a limited number of receptors in innate immunity so you should choose only the most conserved and essential patterns.
- If these molecular patterns are associated with pathogens, they are called PAMPs.
- There are certain molecules that should be present inside the cell, so if they are found outside the cell, this indicates a damage that happened to the cell, like the transcription factors and ATP, such patterns are called damps.
- Innate immunity has a limited set of receptors that can detect conserved PAMPs and DAMPs, and receptors are called pattern recognition receptors or PRR.

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 can recognize 10^7 or more distinct antigens.**

- Now the innate immune system responses to the LPS as a pathogen, but also there is antibodies specific for the LPS, and other antibodies that recognize pathogens which are not recognized by the innate immune system.

- Usually there is an important type of antibodies that target a specific part of the microbe, for example in SARCS covid 2, the antibodies that target the protein that help in the attachment of the virus to the epithelial cell are the most important antibodies, even though we have other types of antibodies that target this virus.
- antigens differ in their abilities to stimulate the production of antibodies.
- The receptors are encoded in the germline, so macrophages all share the same gene to produce certain PRR (nonclonal), while lymphocytes are all different in the receptors except in clones, with the same receptor's specificity.

❖ To sum, innate immunity has its receptors coded in the germline a limited set of receptors equals a limited set of specificity.

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.**

***CpG:** Cytidine and guanine base pairs.

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

- Microbe Proteins are unique in their structure compared to mammalian proteins, if we find **N-formyl methionine** for example, we will know its microbial.

❖ **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.** these sort of lipids and carbs are not found in humans.

❖ **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).**

- ATP can function as 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.**

➢ Cells are constantly dying in our bodies why don't we have activation of innate immune responses?

-Because in our bodies we have a programmed cell death (apoptosis) where intracellular DAMPs are not released to the extracellular environment, so the cell and its content will be engulfed by macrophages.

➢ Innate immunity doesn't always respond to microbes, such as in burns, trauma and decreased blood supply. Another example is necrosis in the tissues that releases DAMPs which lead to inflammation without an infection.

❖ **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.**

doctor's notes about the pic:

➢ -proteins: -

- Pilin is used in bacterial structure in pili (for adhesion and motion and transmission of DNA through conjugation).
- Flagellin used in flagella used for movement.

➢ So Innate immunity can cover most of the microbes through the presence a variety of receptors and these are just a few examples.

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.

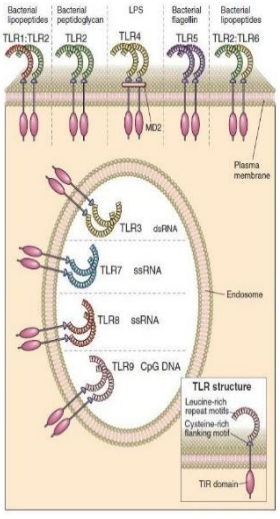
The receptors that recognize PAMPs and DAMPs are called:

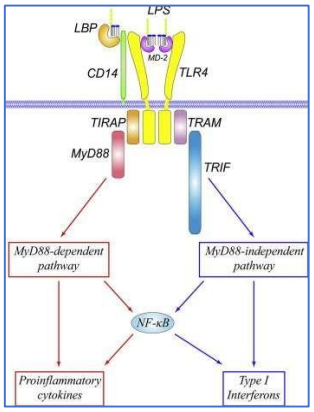
- **Pattern recognition receptors (PRRs) play a crucial role in the proper function of the innate immune system. PRRs are germline-encoded host sensors, which detect molecules typical for the pathogens.**
- **They are proteins expressed, mainly, by cells of the innate immune system, such as dendritic 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)**
- PRRs should be distributed on all compartment in the tissue that may have PAMPs or DAMPs that's why it is found in cytosol, endosome and extracellular compartment.
- All nucleated cells in the body at least have one PRR (even epithelial and non-immune cells and they will initiate the inflammation) but they are more diverse and abundant in immune cells.

CELL BOUND PRR

TLRs

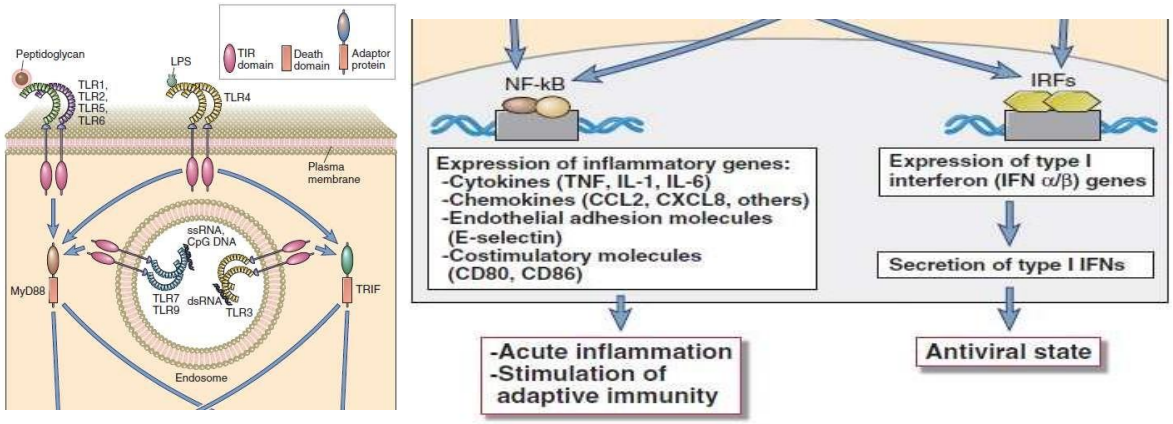
- **Toll-like receptors (TLR) are proteins that respond to the presence of pathogenic microbes by activating antimicrobial defense mechanisms in the cells in which they are expressed.**
- The name comes from Toll proteins that were discovered in fruit fly then similar ones were found in humans, so it held the name.
- **TLR are found in every life form in the evolutionary**
- They are found on cell surface and endosomes.
- **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.** (They work as dimers mainly to enhance and amplify the signal).





- **Adapter and accessory molecules can be needed for proper signaling.**
- **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.**
- **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.**

➢ PAMPs bind to the TLR's extracellular compartment, the intracellular part transmits a signal and activates adaptive protiens (MyD88 and TRIF) until it activates a transcription factor and enters the nucleus to bind to a certain part of DNA and starts transcription of genes related to immunity.



❖ In the pic we see that in the endosome there's TLRs because some pathogens enter through this endosome (phagosome in phagocytes) they usually detect nucleic acids of bacteria origin.

OTHER RECEPTORS

- **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 chemoattractants that help phagocytic cells trace the bacteria producing it)**

- These types of receptors are important for recognition and binding (they serve an important role in phagocytosis that's why they are mainly on macrophages)

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.**

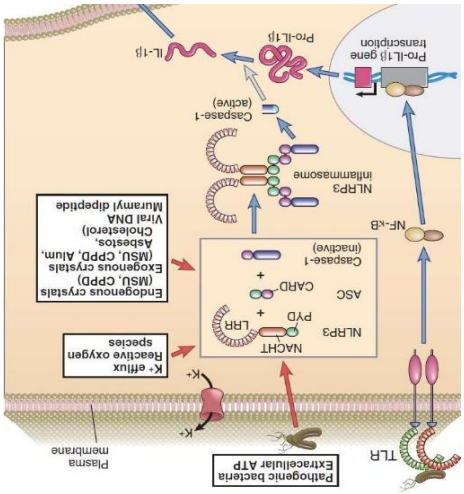
  They are in the cytoplasm especially for viruses

- **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.**

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 several autoimmune diseases.**



- The inflammasome is formed intracellularly after the activation of NLRs, then it activates a certain enzyme which called Caspase-1 which cleaves Pro-IL1β to form IL-1β to enhance inflammation.

You may ask where is Pro-IL1β come from? It's a complementary process in which the same microbe that activated the NLR might be found earlier in the extracellular environment, and it had activated another PRR which signals to translate the Pro-IL1β then the Caspase-1 will do its work.

Recently, these PRRs have become a target for drug discovery to modulate the inflammation, as we said before, the signal maybe enhances the inflammation too much in a way that is going to harm the body, like in sepsis.

Sepsis: is an activation of the immune response following infection leading to destruction of organs.

- Pro means inactive

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.

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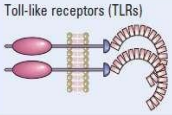
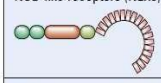
RIG-LIKE RECEPTORS (RLRS)

- **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.**

  *RIG (retinoic acid–inducible gene)

- **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.**

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

THE MAJOR PROINFLAMMATORY CYTOKINES

- 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.

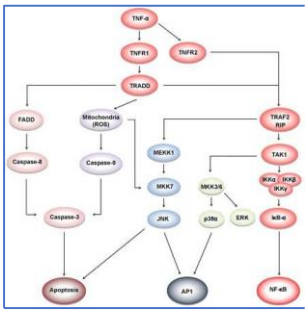
TABLE 2–2 Some Cytokines Acting in Infection

	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

- Cytokines are a broad and loose category of small proteins that are important in cell signaling.
- Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors.
- Cytokines are produced by a broad range of cells, including immune and non-immune cells.

TNF



- 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.

IL-1

- 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

- 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.

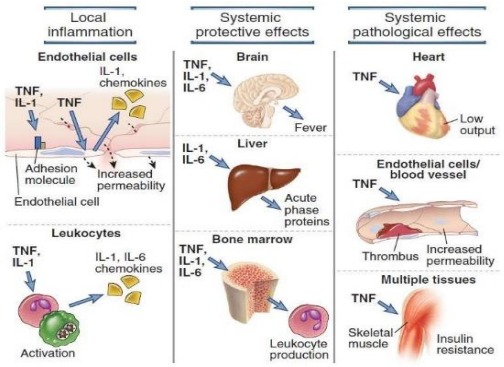
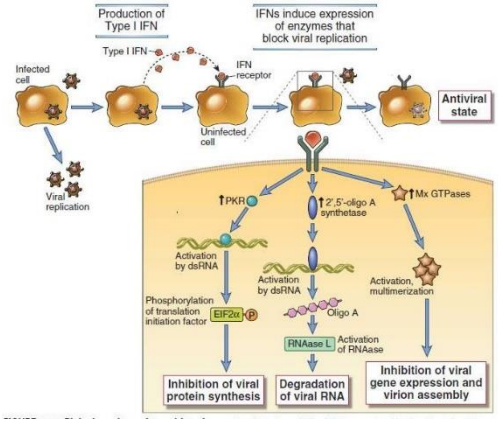


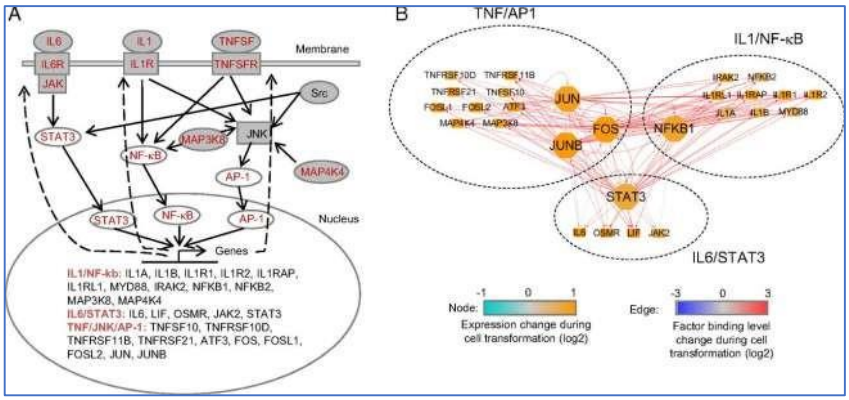
FIGURE 4–14 Local and systemic actions of cytokines in inflammation. TNF, IL-1, and IL-6 have multiple local and systemic inflammatory effects. TNF and IL-1 act on leukocytes and endothelium to induce acute inflammation, and both cytokines induce the expression of IL-6 from leukocytes and other cell 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.

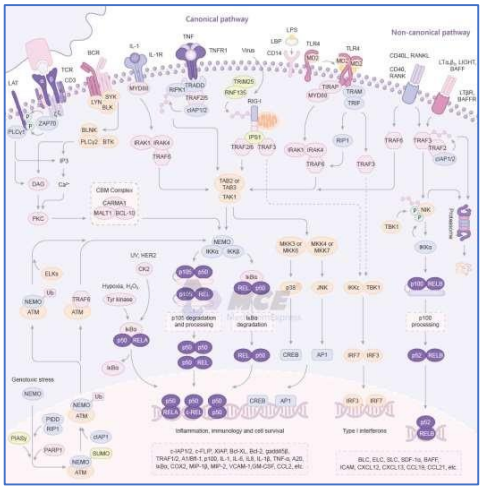
INTERFERONS

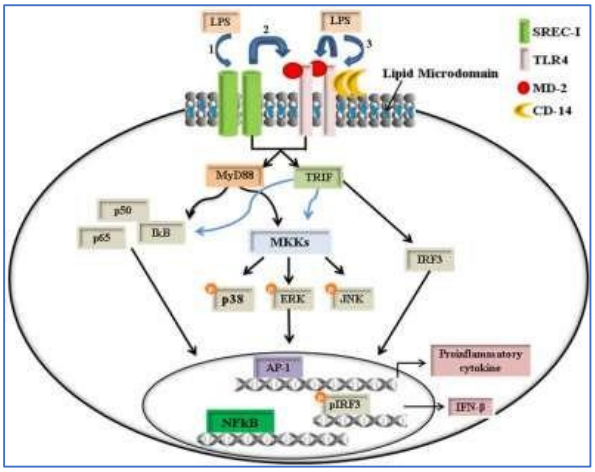
- The major way by which the innate immune system deals with viral infections is to induce the expression of type I interferons. Type I interferons are a large family of structurally related cytokines that mediate the early innate immune response to viral infections.
- Type I interferons, signaling through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







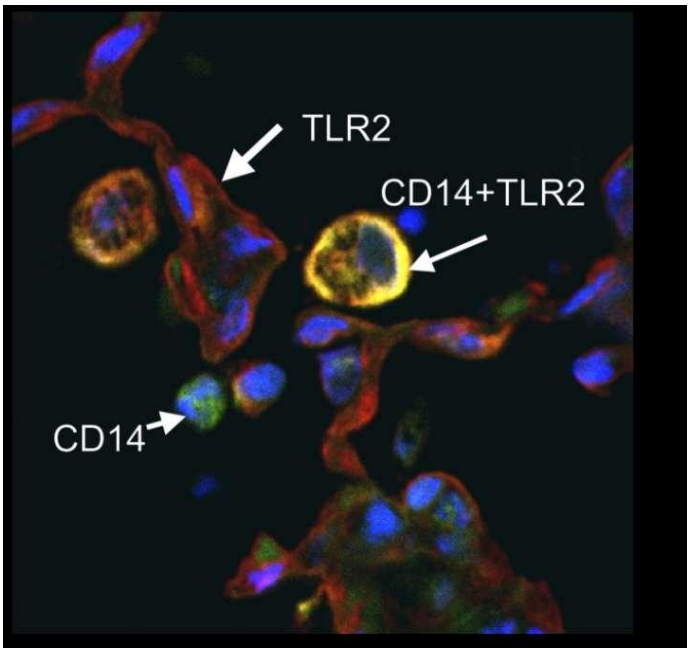


Figure 3. TLR2 and CD14 in the lungs of a rabbit. TLR2 is labeled *red* and CD14 is labeled *green*. Colocalization of TLR2 and CD14 is shown in *yellow*. TLR2 is visible on the alveolar epithelium and on alveolar macrophages in the airspace. CD14 is visible on alveolar macrophages, and neutrophils in 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.