SHEET NO.

5

IMMUNOLOGY

DOCTOR 2019 | MEDICINE | JU

DONE BY : Doctor 2018

SCIENTIFIC CORRECTION :

GRAMMATICAL CORRECTION :

DOCTOR : Dr.ANAS

⌘We want to study how the cells interact with each other, and how they interact with external environment. We will start with cells of the innate immunity (not specific 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 bounded and .secreted molecules

⌘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 first function in the immune system is to **recognize** →**store**→**remember**, for recognizing foreign bodies, there are certain patterns that the body can detect.

Some of the characteristics of these patterns are:

> **Not found in mammalian cells** but are found in microbes (to distinct them by the immune system as **foreign bodies**).

> **Essential for the survival of microbe**, during the co-evolution the pathogen can't change. (**For better understanding**, cell wall is essential for the survival of bacteria, because it contains structural proteins & glycoproteins & lipids that are important for survival of bacteria, so bacteria can't change them).

Patterns: ****Immune system recognizes them.**

****They are essential for the survival of microbes**

****They are found in microbial cells NOT mammalian cells.**

⌘We call these patterns=>**PAMPs** : The microbial substances that stimulate innate immunity are called pathogen-associated molecular patterns (PAMPs).

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

***Note: Viral nucleic acid are not always coated by a capsid protein.**

***Remember: these patterns targeted by innate immunity.**

\

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

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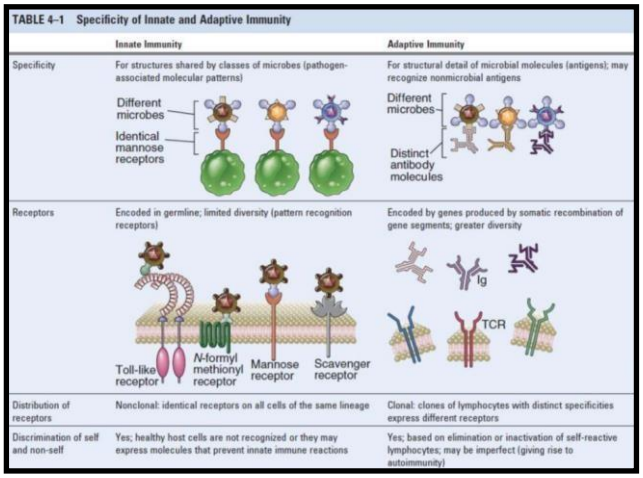


For better understanding>>

We are talking about sensors (receptors) of innate immunity. On the other hand, the adaptive immunity receptors detect fine differences between microbes, so they are very specific. For example, it can detect **L1** protein that isn't found in all bacteria, it is found in some streptococcus and there is some variation. So the adaptive immunity detects these proteins and produce specific antibodies for it.

BUT innate immunity detects [streptococcus (gram +)\ staphylococcus(gram +)\ enterococcus (gram+)],and deals with ALL of them at the same way, because it detects only the known part of them despite of their species. So, it's not specific.

Characteristics of antigens recognized:

→Nucleic acids that are unique to microbes, such as double-stranded RNA, found in replicating viruses, usually in normal mammalian cells they are not found.

→ Certain bases in DNA, **EX>>** CpG on the same strand (not the complementary one)

*When they are found in **mammalian cells**, they are →methylated

*BUT, in **microbes** → unmethylated

So the immune system can detect those unmethylated strands as a foreign bodies.

CpG is shorthand for 5---C---Phosphate---G---3.

Cytosine and guanine are separated by only one phosphate group; phosphate links any two nucleosides together in DNA.

→ Proteins that are found in microbes, such as initiation by N-formylmethionine, which is typical of bacterial protein, and NOT usually made in mammalian cells.

→ Complex lipids and carbohydrates that are synthesized by microbes and 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 innateimmune system has adapted to recognize only a limited number of molecules"

**It is possible to find more than one pattern in one microbe.

>> Receptors for **innate immunity** are **non-clonal** (that means, if we take a group of microphages, we will see that every one of them is displaying the same receptor that can be recognized - not specific)

>> **Adaptive immunity** has **clonality**, there lymphocyte recognizes certain antigen, then makes clones from it. ((Clones of certain antigens have the same receptor.))

BOTH (innate and adaptive) can recognize self from non-self, but sometimes there are some mistakes in **adaptive immunity**, it produces millions of receptors, some of them can stick self and recognize self as non-self.((Autoimmunity))

In **innate immunity,** autoimmunity cannot happen because all patterns are known and found in microbes (PAMPs).

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 many 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.

Why innate immunity can recognize them?

Because these patterns are proteins found in wrong position, (nuclear proteins are normally found inside the nucleus, BUT in this case they are found in EC compartment).So there is certain damage to the cell that it is dying and releasing nuclear proteins that could be recognized in EC.

>>That's why we call them damage associated molecular patterns (DAMPs).

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.

DAMP:

- **HSP**>> under stress, cells start to produce HSP (**Heat shock proteins**).

- **Nuclear protein HMGB**>> a TF, supposed to be in nucleus BUT if it is found in EC, we discover that the cell is damaged enough to release it outside.

PAMPs :(not present in mammalian cells)

- Double stranded RNA\ proteins\ cell wall lipids+ carbohydrates.

The question now is HOW can we sense those PAMPs and DAMPs?

>> We need certain receptors to sense these patterns which is called **PRR (they can know if the pattern either associated with microbes or with damaged).**

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.

Some receptors can be found on the cell membrane **OR** in endosome **OR** in cytoplasm. Some of them is soluble in the tissue fluid, and can be secreted. (So sensors covered every part of the body.)→***SUMMARY***→ **PRR can be cell bounded or soluble\ also, Cell bound PRR can be found on different compartments of the cell (Membrane, cytosol).**

****

The first **cell bound** pattern recognition receptors is >> **(TLR)**

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. (TLRs, are sensory of innate immunity).

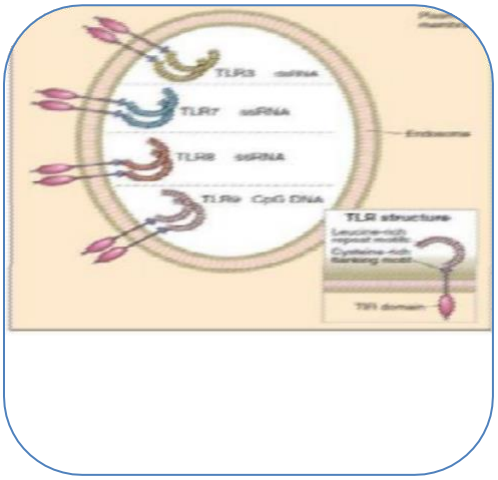
TLR are found in every life form insects up to mammals.

TLRs are also involved in response to endogenous molecules whose expression or location indicates cell damage (DAMP).

They are found either **on the cell surface or in the **endosomes.**

**They called them Toll like receptors because they are similar to Toll proteins that exist in mammalian cells, and they have immune functions.

Most of these receptors have the same structure. There are from 1 to 9 types → they have a part called **leucine-rich domains**, which detects the pattern, then the signal will be transmitted to cytoplasmic part and then associate with adapter proteins that they activate Transcription Factors(TF), like **NF-KB** to start the transcription of certain gene(inflammatory gene) eg. Cytokines & chemokines, and others that help in **inflammatory**



leucine-rich domains → **Reach cytoplasm, and associated with adapter proteins.** → **Activate TF (NF-KB)** → **Transcription of certain gene (inflammatory gene.= cytokines and chemokines → HELP in immune respond**

NOTES>>

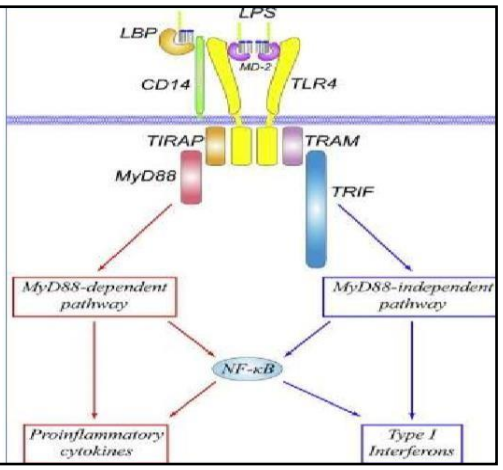
** **TLR** transmit the signal by forming dimers (**Homo**{like TLR4} OR **Hetero**).

** Each type from **1-9** can detect certain pattern.

** **TLR4** takes the **LPS** (especially with adapter protein **MD-2.**), then the signal transport to adapter proteins like **TRF** & **MyD88.**Then they get inter complex pathway to activate **NF-KB** or another TF to produce genes help in immune response.

** Pathways are complex and interconnected (so you should know the general idea NOT the details because they are not required).

- 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.



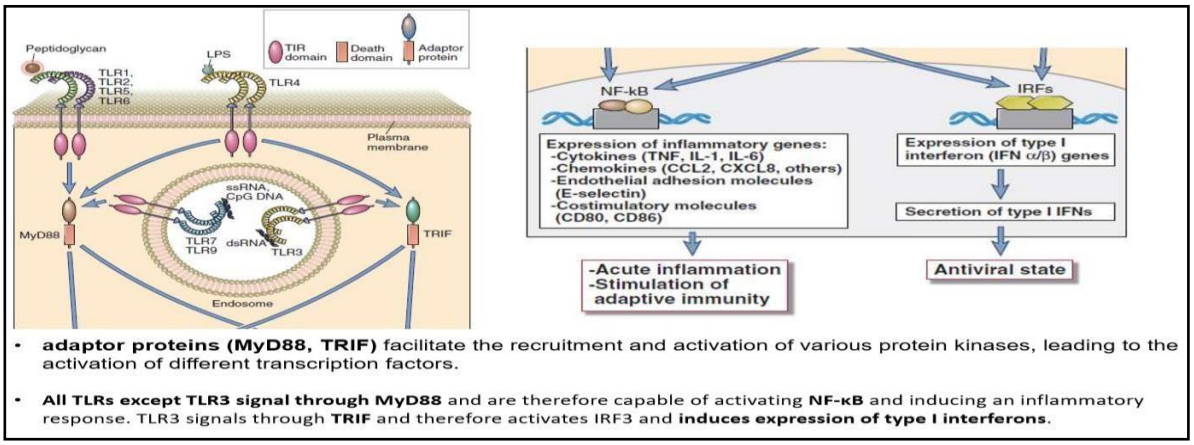
Sometimes for signaling of TLR, there are other proteins that are involved in signaling such as: The signaling of **LPS → CD14**

(TLR detects LPS (homodimer). And it can form heterodimers, for example, by detection of peptidoglycans**).**

TLR that is found inside (endosome) differs from those that are found on the cell surface.

*The sense at outside the cell >> will detect **LPS\ peptidoglycans\ free dendritic of microbe.**

* The sense in endosome>> will detect different types of nucleic acid (single\double strand of DNA\RNA), it could be virus replicated inside the cell OR it could be digested bacteria.



- **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 facilitate the phagocytosis of the microbes and stimulate subsequent adaptive immune response. These receptors belong to the **C-type lectin family**

>> **They bind carbohydrates, so it is called→ lectins**

>> **Ca++-dependent manner → C-type.**

Some of them 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).

➢ **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 chemoattractant that help phagocytic cells trace the bacteria producing it).

For better understanding: These receptors trace certain proteins that are released from bacteria, the question is: HOW neutrophils & macrophages follow microbe? Microbe released certain peptides of protein "the mark that it left behind". So the receptor detect the microbe that released peptides, then phagocytes can trace it".

➢ **Scavenger receptors:** comprise a structurally and functionally diverse collection of cell surface proteins found mainly on macrophages ((for certain type of lipids)).

TLR receptors	**From** 1-9**, found in** plasma membrane and endosome**.**
C-Type receptors	**Recognize carbohydrates** on the surface **of microbes.**
Scavenger receptors	Cell surface **proteins, recognize certain type of lipids.**
N-Formyl receptors	**Recognizing bacterial peptides by neutrophils and macrophages.**

>> In this way, cell membrane and endosome are covered by receptors. BUT, other compartments are not covered yet.

Cytoplasm (cytosol)➲ The two major classes of these cytoplasmic receptors are **NOD-like receptors and RIG like receptors**

WHY we have sensors in these areas? Because there are a lot of viruses and bacteria that replicate within the cytosol. 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.

** These receptors usually detect DNA &RNA nucleic acid.

✧ **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.

BUT the most important types are **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. Also they are important in making **inflammasomes.**

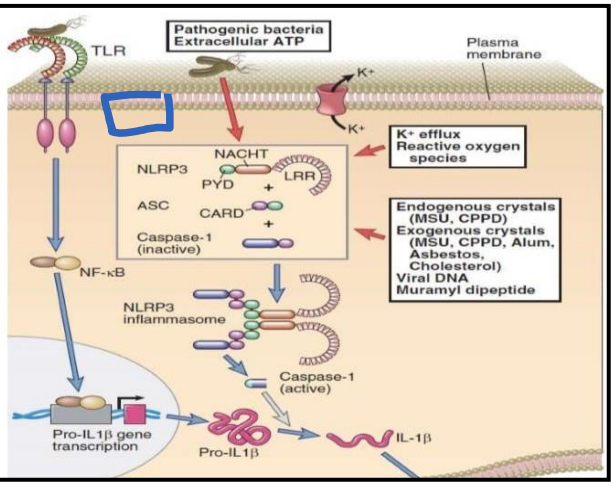
➔ **Inflammasomes:** multi-protein complex, when the danger defected within cytosol, proteins get on each other in a mechanism called inflammasomes. Their function is to

cleave cytokine and re-cytokine into activate form, especially it cleaves pro- interleukin 1b (IL-1b).

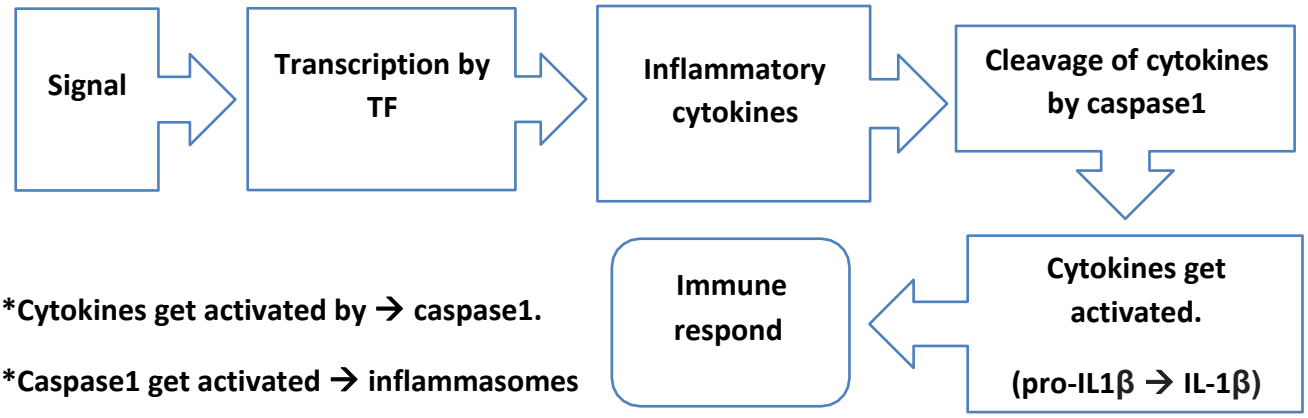
So, the NLRP* subfamily of NLRs respond to cytoplasmic PAMPs and DAMPs by forming signaling complexes called inflammasomes, which generate active forms of the **inflammatory cytokine IL-1.**

*(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.



TLRs, when they sense a signal (for example, peptidoglycan) it will be transmitted with adapter protein **MID-88,** then start to make **TF**, to produce inflammatory cytokines, such as **IL OR pro-IL1β** BUT it still NOT active, so it has to be cleaved with protein caspase1 (caspase1 become active by **inflammasomes**). To become **IL-1β(** active form).

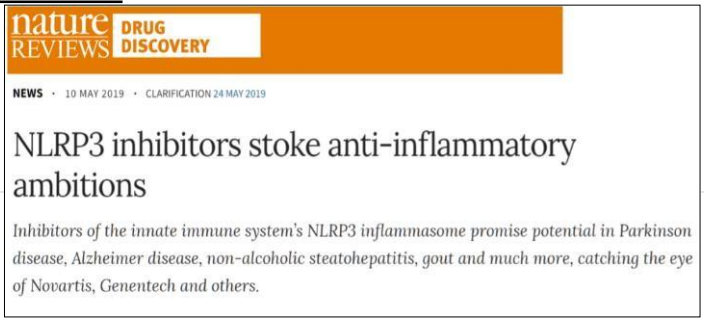


***Cytokines get activated by → caspase1.**

***Caspase1 get activated → inflammasomes**

When Ca2+ level is abnormal OR the presence of crystals ⇨ **Inflammasomes** play important role in inflammation, so they produce drugs that can be used for several diseases that have an inflammation part, like :Parkinson\ Alzheimer\ gout.

[[**Inflammasomes** is a part of detection of crystals, and initiated of immune respond]]


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.

✧ **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 interferon (*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.

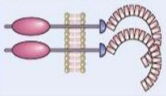
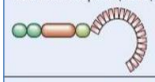
TOI + NLR + RLR →

*Found almost in all cell types, because antigen can be presented anywhere. So, all cell types will express SOME of TOL or NLR or RLR.

*These receptors are not only for immune cells, even epithelial contain some source of TLR.

And inflammatory situation, there will be up-regulation of these TLR, especially epithelial to detect any pathogen.

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

Remember:

Endosome (plasma) →TOL.

Cytosol → NOD\RIG\Other types of receptors.

****

⊙ **Cytokines** ➔ type of communication between cells.

*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 pro-inflammatory 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, also other cell types (non-immune), including endothelial and epithelial cells, can also produce **IL-1 and IL-6.**

*Pro-cytokine initiates inflammatory response >> **TNF\IL-1\IL-6.**

IL-1 & IL-6 are interleukins, the scientists thought that interleukins are found only between leukocytes. BUT, they found that even epithelium can express interleukins. So it takes place in immune and non-immune cells (epi\endo-thelial).

How do cytokines work? By 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.

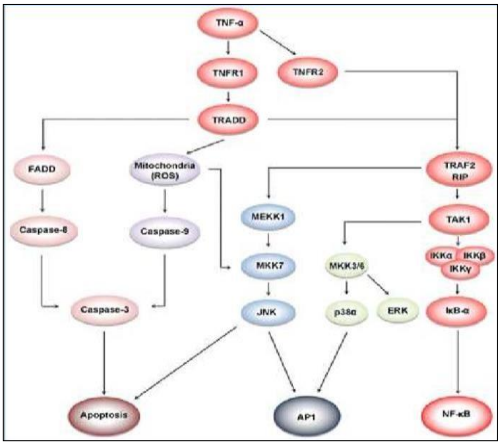
➢TNF can also mediate cell proliferation and in some cases cell death(can kill cancer cell, so it is called Tumor necrosis factor).

➢TNF superfamily plays highly diversified roles in the body.

➢TNF inter the cell and work on TF, like NF-KB, that produce more **IL-1\IL-6\TNF**.

Also, it can lead to apoptosis, and on other place it can induce proliferation of cells.

[Cytokine 1 can have several functions → proliferation + apoptosis]



IL-1 transcription of it induced

by TLR and NOD signaling. Then after **pro-IL1β** formed

it will be **IL1β** by inflammasomes.

Cytokines introduce local inflammation, like producing chemokines. **TF & IL-1** work on endothelial cells to produce **adhesion molecules** (for adhere leukocytes).

When they want to reach the site of infection, they need adhesion molecules to catch it to enter inside the tissue.

- 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

Inflammatory cytokine increases the expression of adhesion molecules, and gives enough space for leukocytes to pass. In the same time; they produce more **IL-1** and chemokine to bring more cells to the damaged area.

And they work, not only on epi\endo-thelia, they also work on leukocytes, they activate them in order to lose more cytokine OR to be able to kill more pathogens.

↑activated more ↑able to kill pathogen.

**Cytokines have systemic effects:

✓ **They are the cause of fever in case of inflammation or infection (they go to the brain, to hypothalamus).**

✓ **They can reach bone marrow to induce the protection of more leukocytes, for that leukocytes increase in case of infection.**

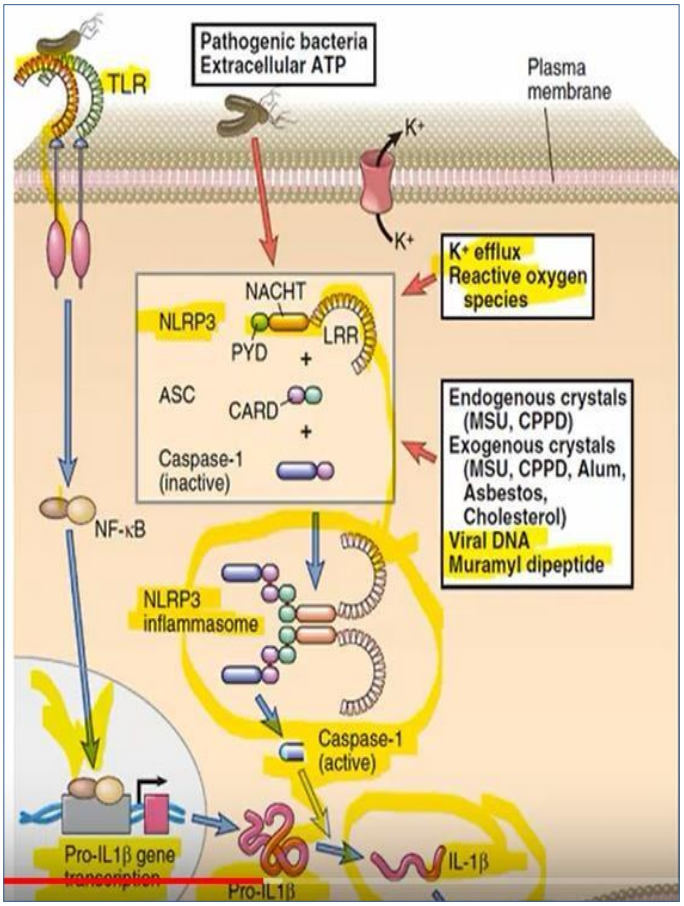
✓ **They can reach hepatocytes.**

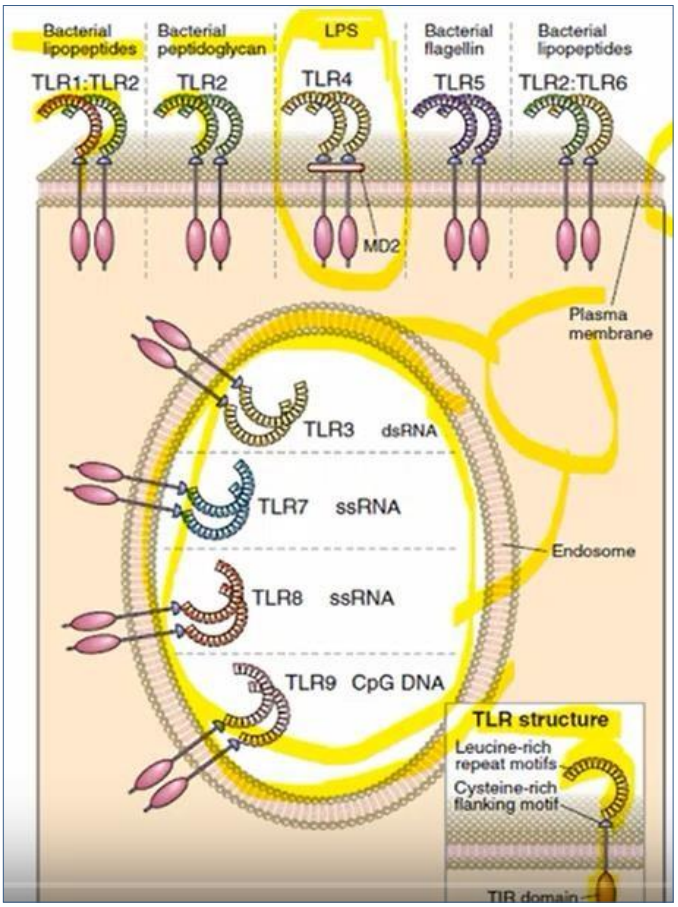
✓ **Heart, and cause systemic effects.**

There are some pictures, which the doctor mentioned during lecture.

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)





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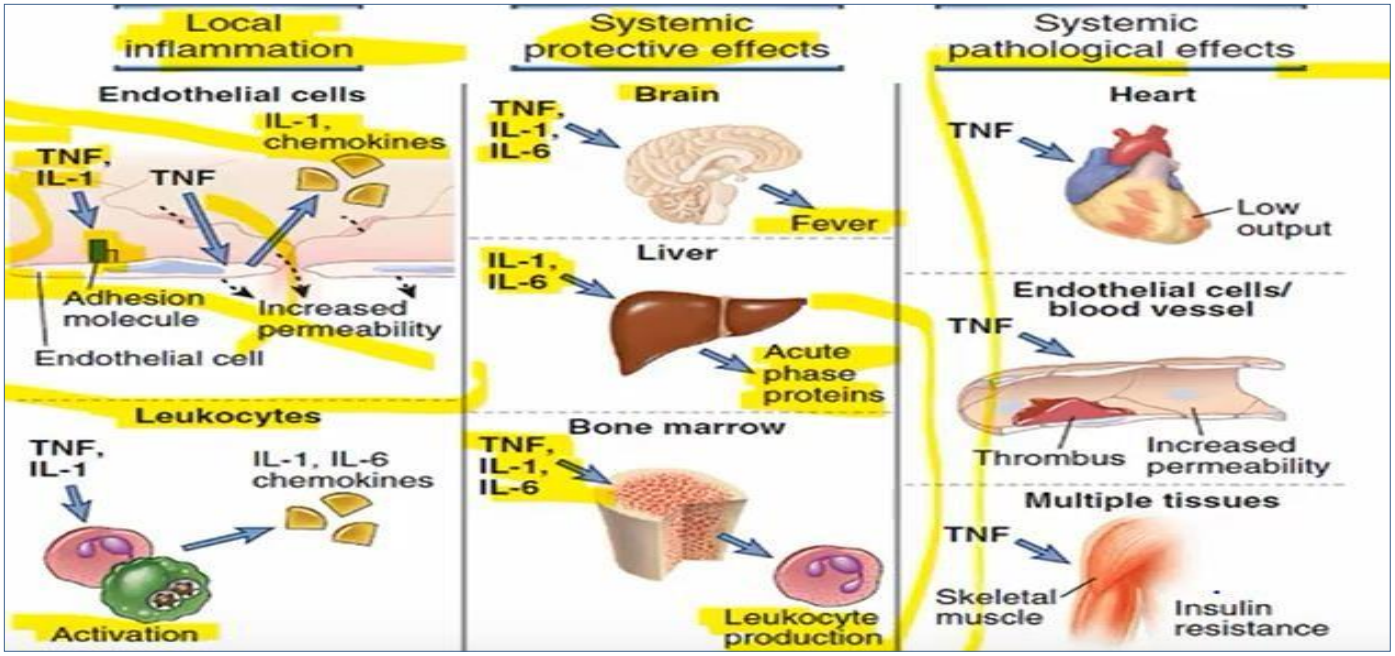

GOOD LUCK^^