Mechanisms of recognition of pathogens and of damaged self by receptors of innate immunity

Prof. Giovanni Bernardini October 23th 2026

Il materiale contenuto in questo documento e distribuito a uso interno e a puro scopo didattico

What are we going to talk about today?

What is the role of the innate immune system and how does it work?

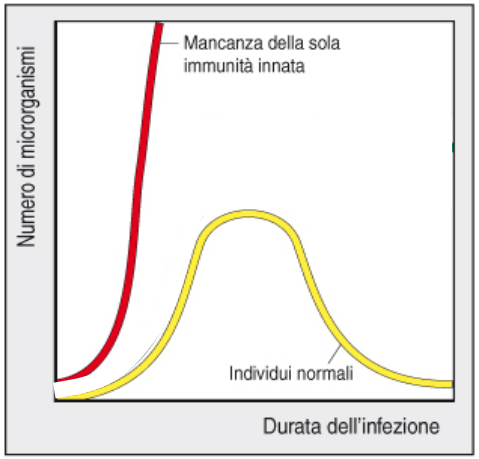
How do innate immune system cells recognize pathogens?

Diversity of recognition molecules and of signaling pathways associated

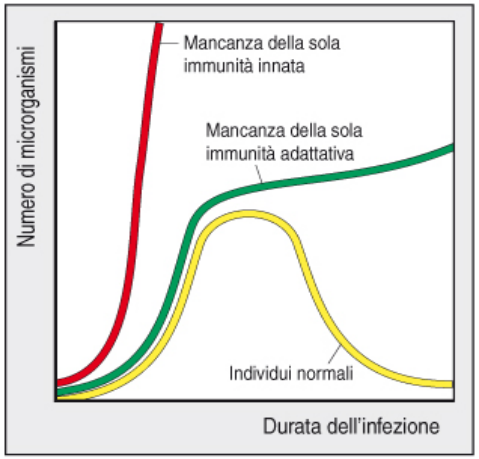
What are the functional outcomes of recognition?

The immune system includes:

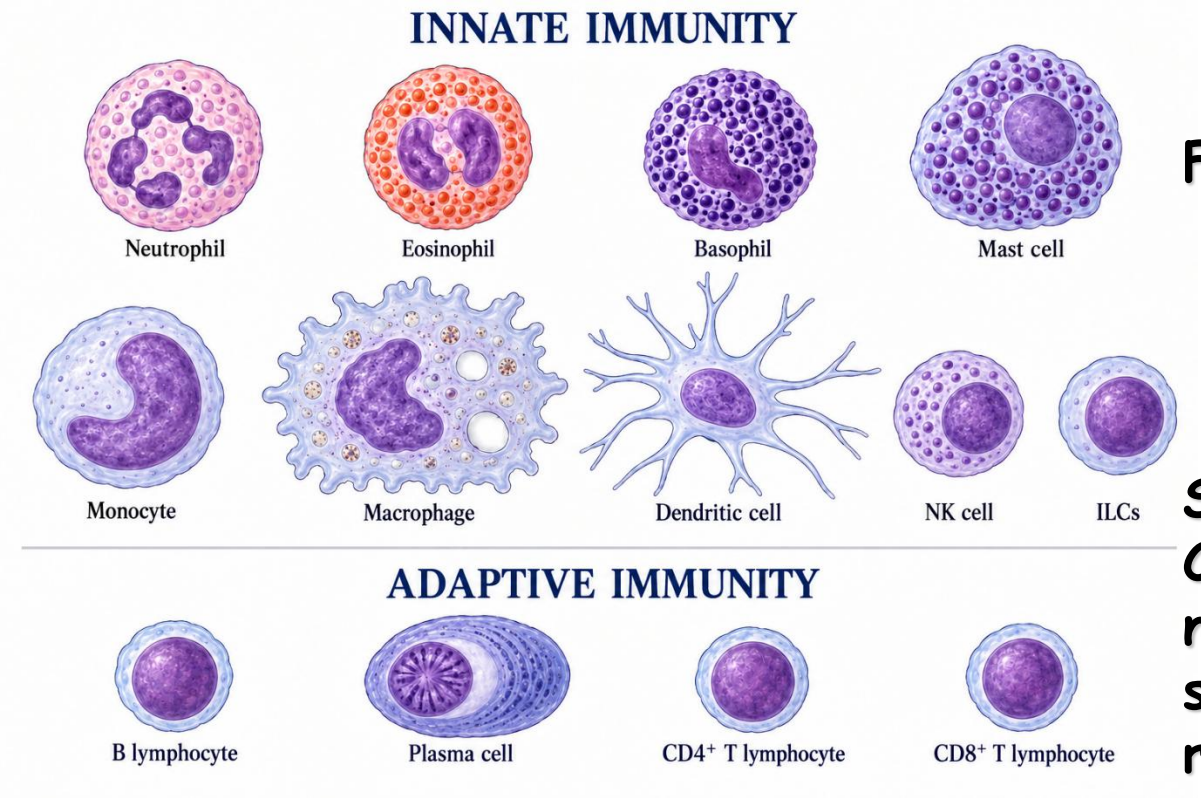
Innate or Natural immunity, Innate immunity provides the **early line** of defence against microbes and exploits **common mechanisms of defence** regardless of the pathogen. Its main function are
Function 1: **to limit infection**
Function 2:to provide signals for activation of adaptive responses



Acquired or adaptive immunity, provides a later response **(its activation requires a long time)** activated by **recognition** of the antigen and by **signals provided by innate responses**. It uses **different mechanisms according to the pathogen type**. Its role is **to eradicate infection and to provide protection**.



The main differences between innate and acquired immunity are in the level of specificity, diversification of recognition and of memory (the ability to remember the pathogen and to react more rapidly and powerfully to a second challenge).



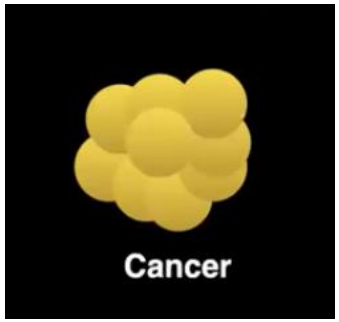
Faster but less specific

Slower but specific. Capacity to recognize a number of molecular structures and to remember them.

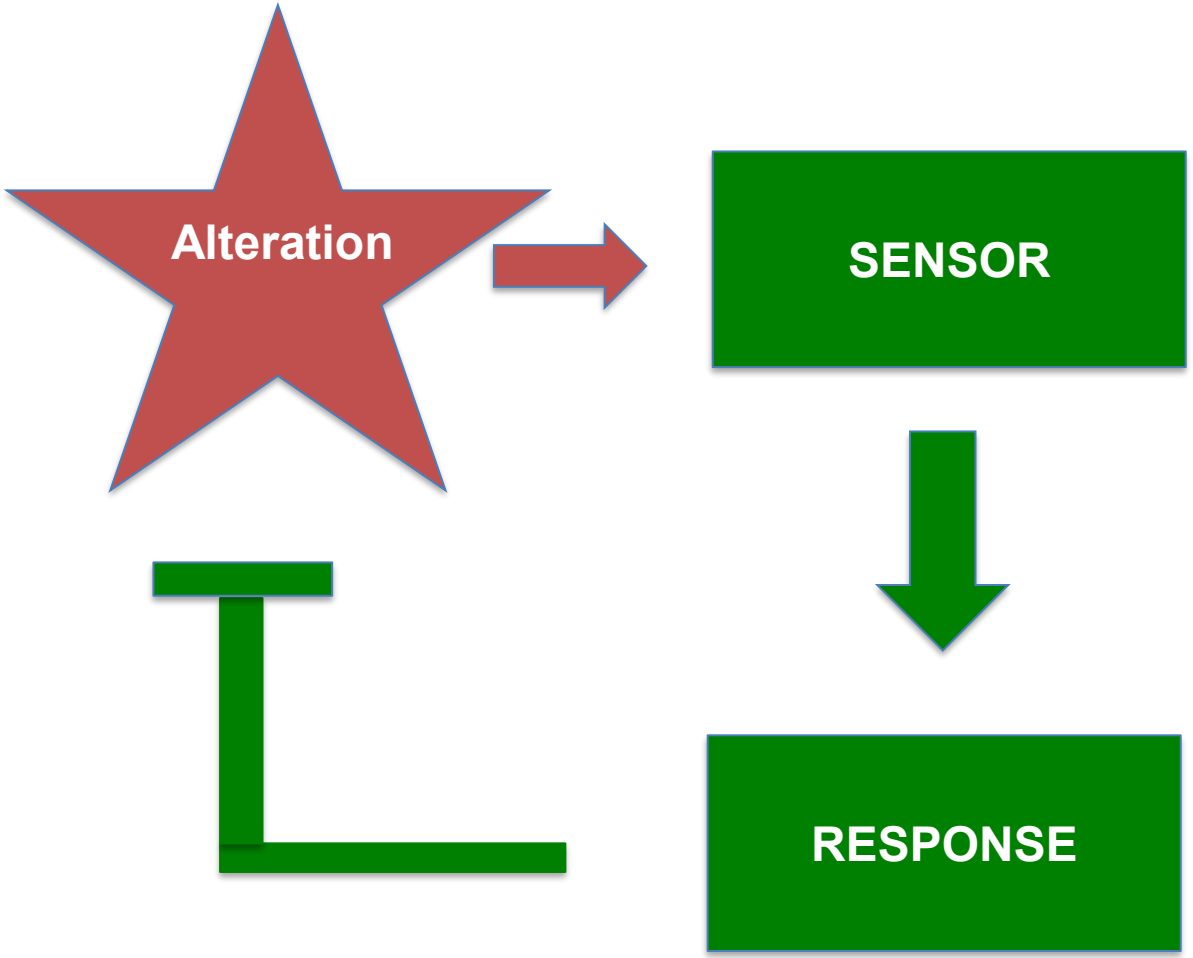
Specificity: ability to discriminate between different molecular structures, even similar.

Immune system responds against TISSUE ALTERATION

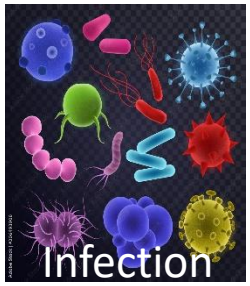


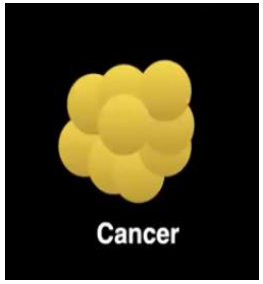


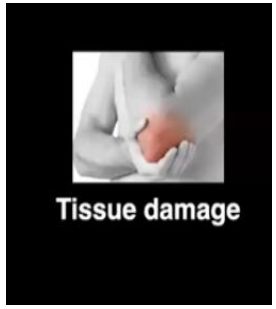




Immune responses against TISSUE ALTERATION



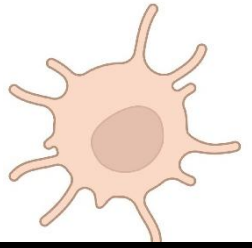




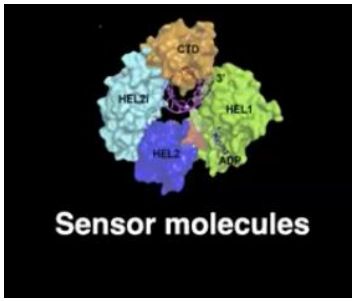
Alteration

SENSOR

RESPONSE



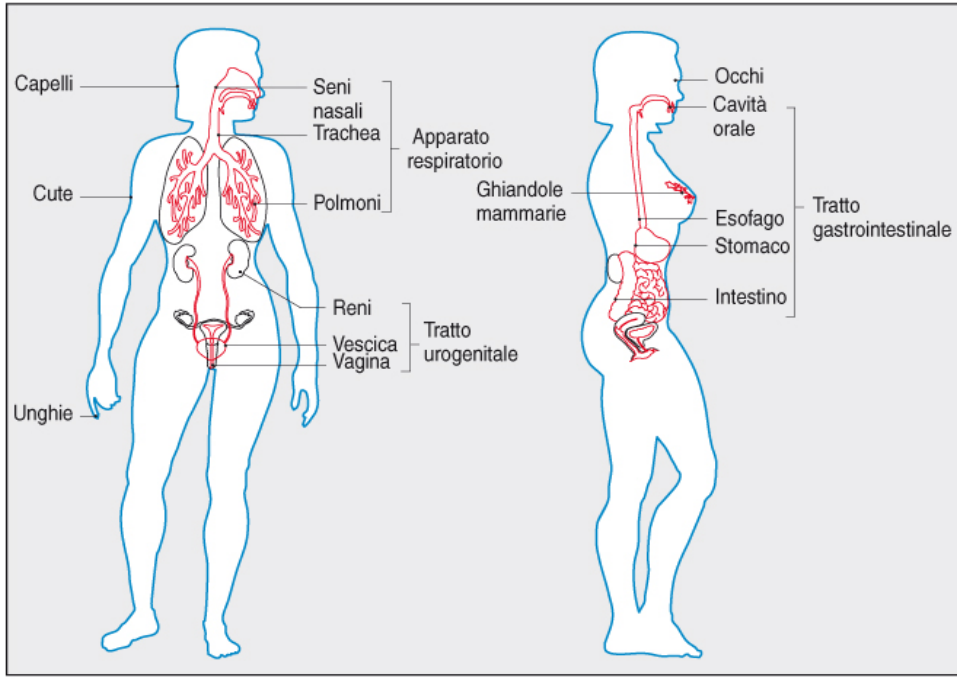
Sensor cells (immune, i.e.DCs, or other non immune cells)



What are the access route for a pathogen?

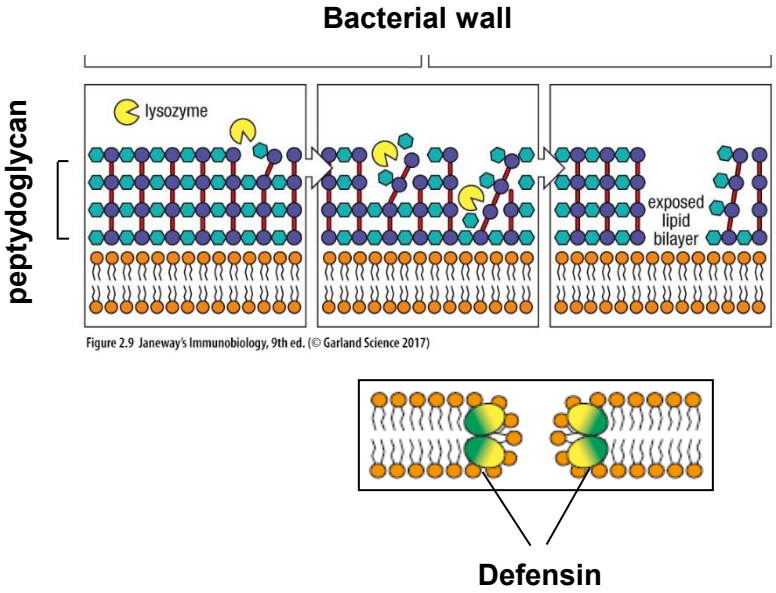
Epithelial surfaces-external and internal:

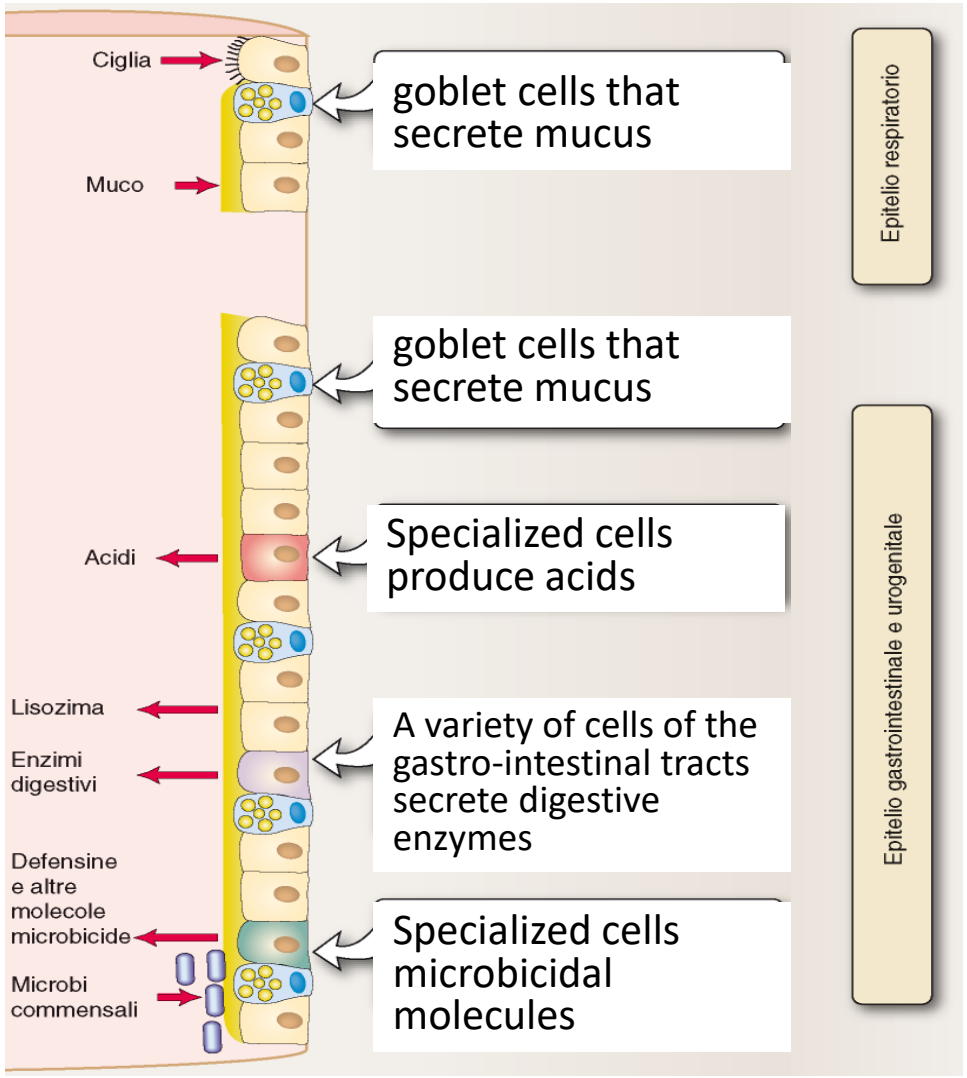
- The skin



- The mucosa: Respiratory
  Urogenital
  Gastrointestinal tract

Remember that epithelial barriers are at the edge of immune defence!!!!





Function 1: to directly limit infection

The two main type of anti-microbial innate immune responses are inflammation and anti-viral defence

During **inflammation**, leukocytes and plasma proteins accumulate in infected tissues where they are activated **to kill and clear pathogens**.
This type of response is active also in the absence of microbes, in **conditions of death/damaged cell accumulation** that may derive from infection or wounds.

During **anti-viral responses**, cells are driven into an **«antiviral state»** that inhibits viral replication while increasing suseptibility of infected cells to **killing by lymphocytes**.

Detection of microbial product or host damaged materials activate inflammation

Inflammation

- A host immune response against noxious agents aimed at:

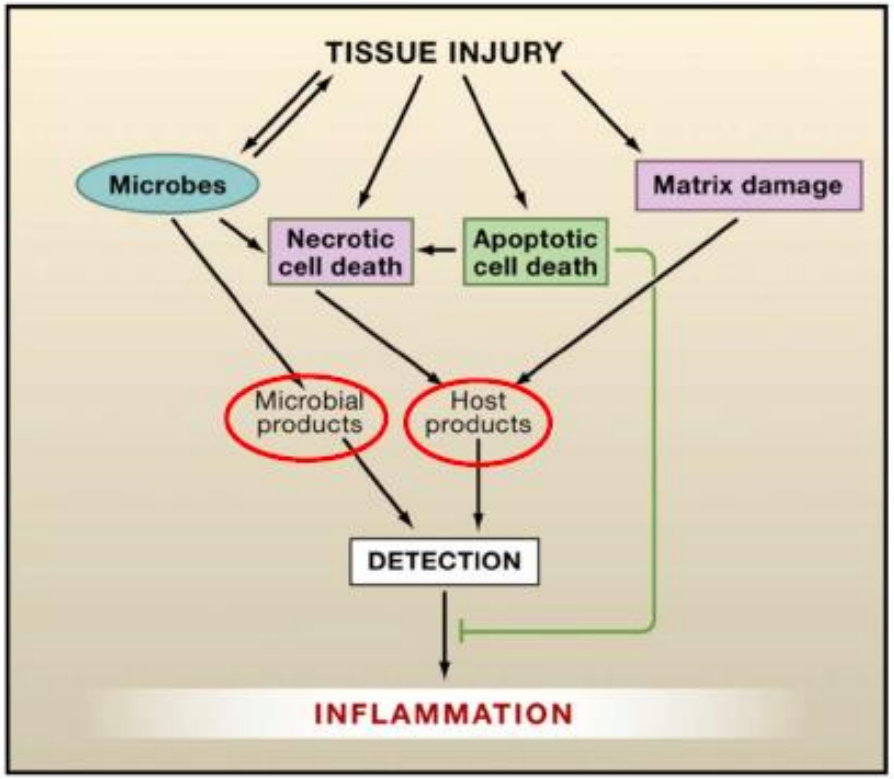
✓**protecting** all living organisms from exogenous pathogens
✓**repairing** tissue damage caused by a variety of noxious stimuli, including infection and injury.

- Inflammatory responses are highly heterogeneous in terms of the cell types and molecular mediators involved.

- Inflammation also comes in different modalities that can be classified as:

✓acute versus chronic

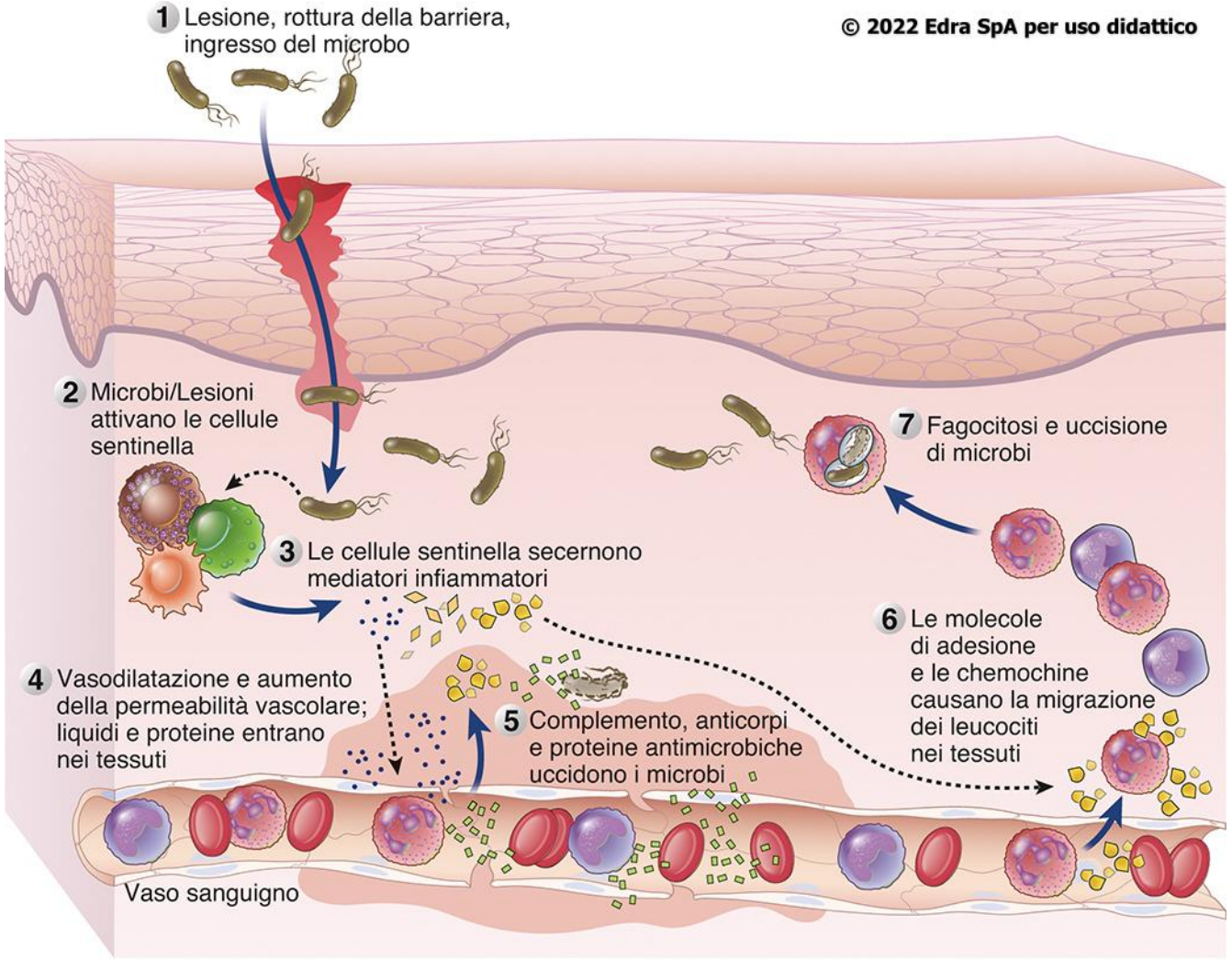
✓local versus systemic



Inflammation is marked by capillary dilatation, leukocyte infiltration, redness, heat, and pain

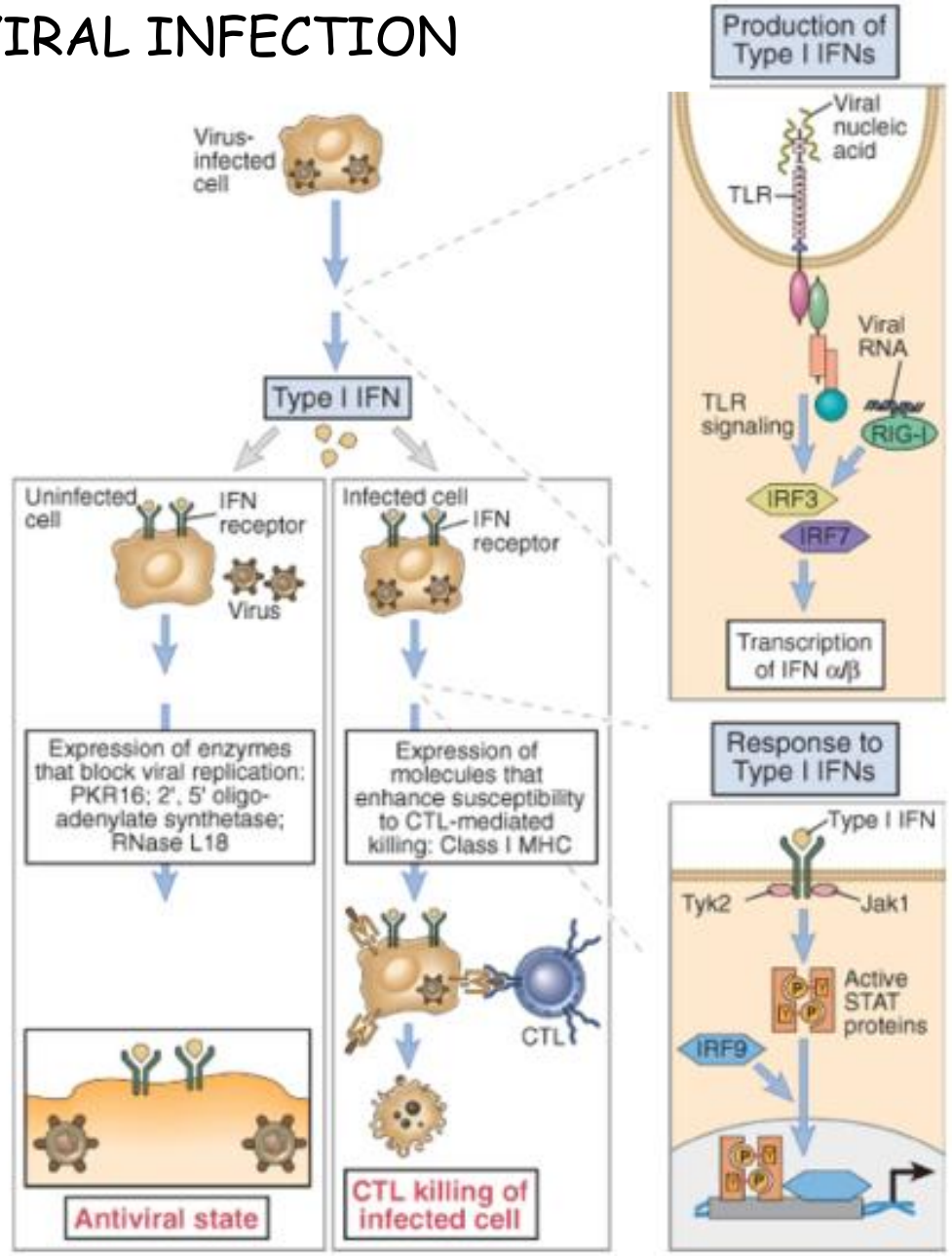
Leukocytes, fluids and proteins come from the blood

Detection of microbial products or host damaged materials activate inflammation



Le risposte infiammatorie acute iniziano quando i microbi invadono i tessuti o in seguito ad un danno

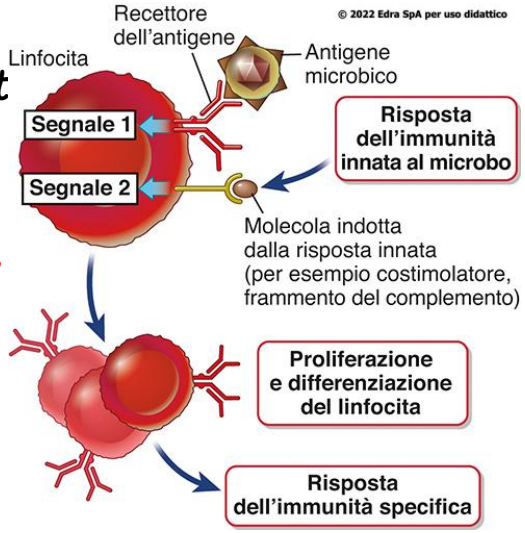
RESISTANCE TO VIRAL INFECTION



Function 2: Innate immune recognition at the cross-roads between adaptive and innate immune responses

-Activation of the adaptive immune response is controlled by the more ancient innate immune system.

*"The requirement for **two signals** to initiate **the adaptive immune response** may reflect the evolutionary history of host defences.*



Early phases of host defence involve receptors and ligands that may have controlled immune responses prior to the development of clonally-distributed receptors encoded in rearranging genes.

Double role of innate recognition:

"The former receptors persist in contemporary vertebrates both to trigger innate or nonclonal responses and to signal to lymphocytes that a particular antigen is associated with a microorganism"

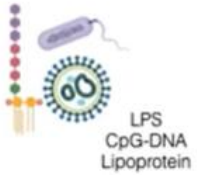


Charles Janeway Immuology Today 1992

Dinamics of the immune response

Microbes

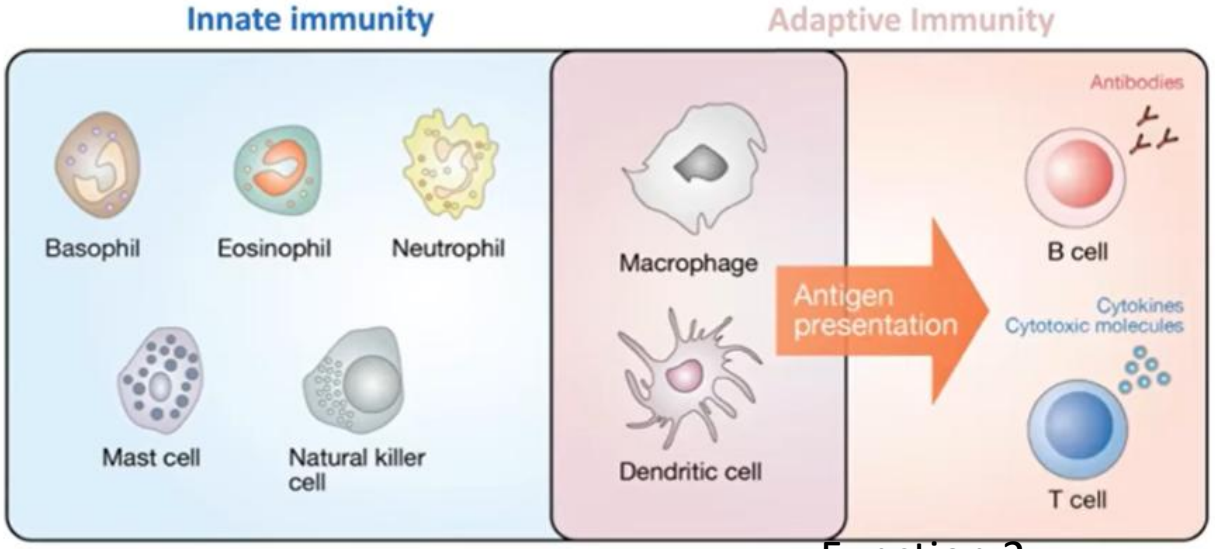
Pathogen
Associated
Molecular
Patterns

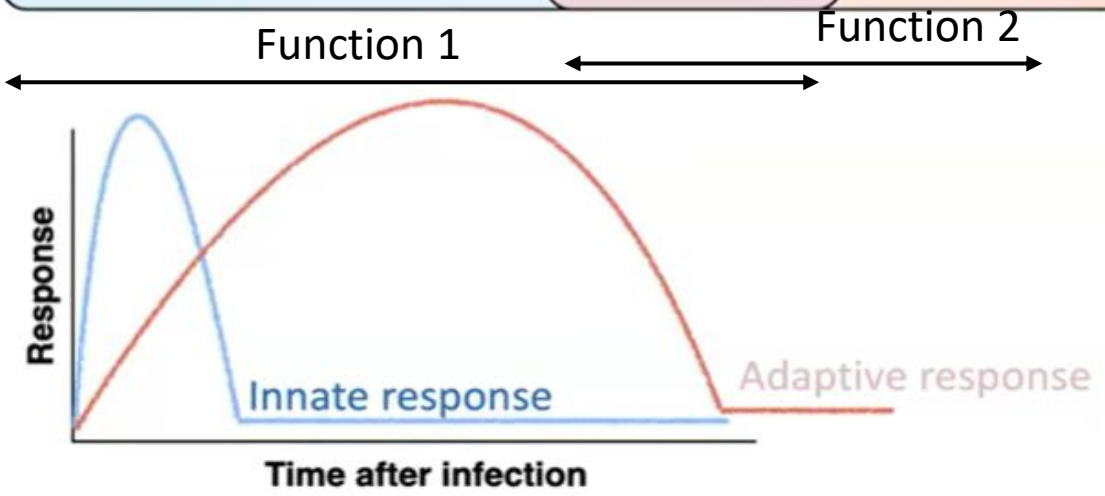


Self-molecules

Danger
Associated
Molecular
Patterns







First of all, recognition!!

The ability to sense microbes is used by the immune system to maintain host-microbial homeostasis and/or to induce antimicrobial defence mechanisms

Pathogen-associated molecular patterns PAMPS
The pattern recognition theory

In 1989 C. Janeway proposed that innate immune recognition is based on nonclonal, germline-encoded receptors, which he termed pattern recognition receptors (PRRs) (the pattern recognition theory)

Our organism perceives the presence of pathogens-associated molecules uniquely expressed by microorganisms and not associated with host cells.
These molecules are called ***pathogen-associated molecular patterns (PAMPs) or MAMPS*** (microbial associated molecular patterns)

pathogen-associated molecular patterns: ***profili molecolari associati a patogeni***

Immune response to infection develops in three phases

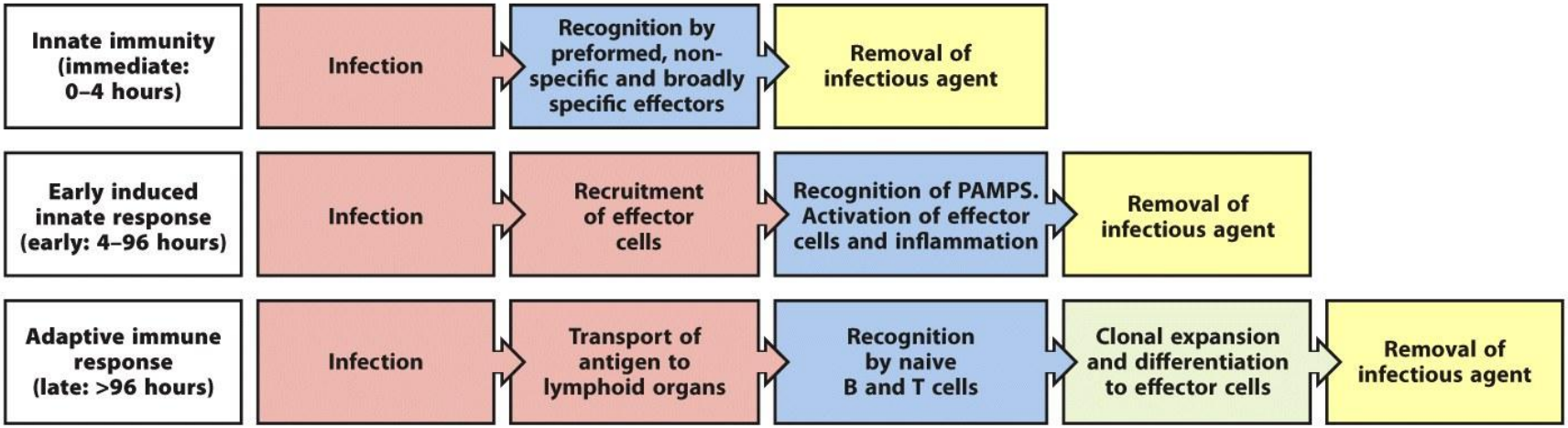
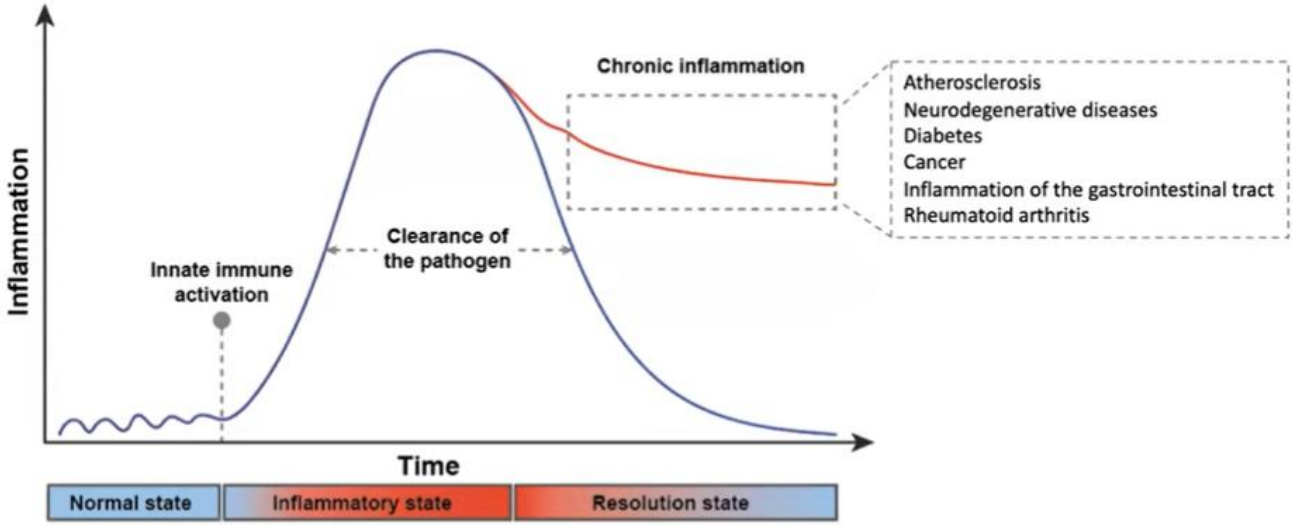


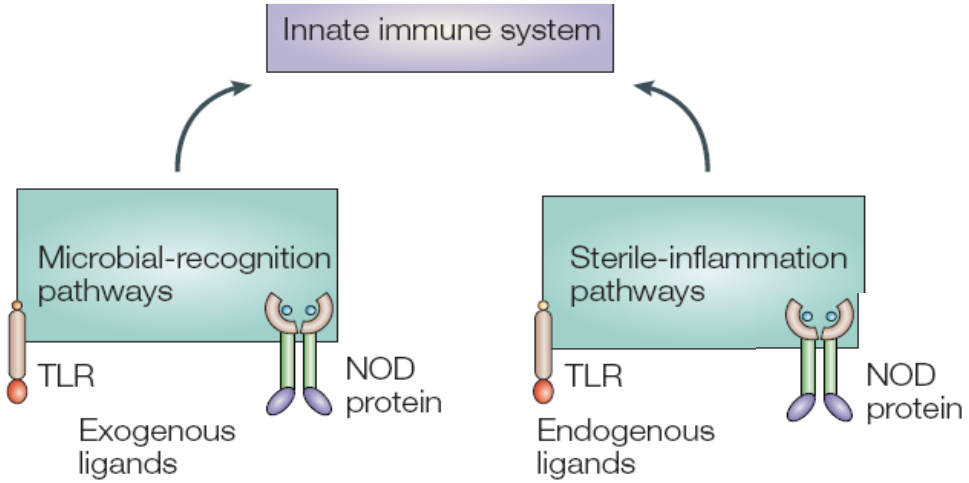
Figure 2.1 Janeway's Immunobiology, 8ed. (© Garland Science 2012)



PAMPS: Patogen-associated molecular Pattern

Innate immunity at the crossroads between health and disease

Both **patogen-associated** molecules and endogenous molecules produced by **damaged or death cells** are recognized



NOD, nucleotide-binding oligomerization domain
TLR, Toll-like receptor

Microbial invasion:

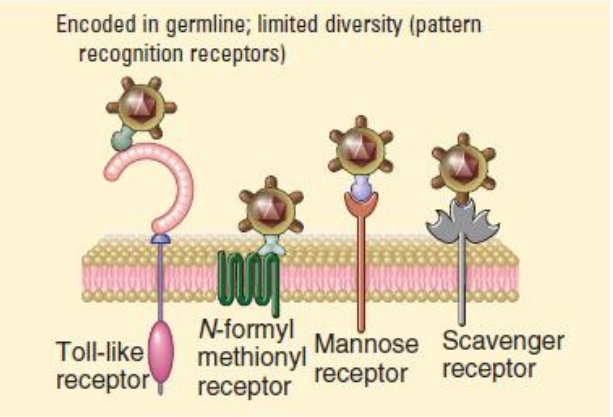
How do innate immune system cells recognize pathogens?

What is different from adaptive cells?

Microbial invasion:

The **innate immune system** is genetically programmed to detect invariant molecular features of invading microbes that **differ** from those of host cells

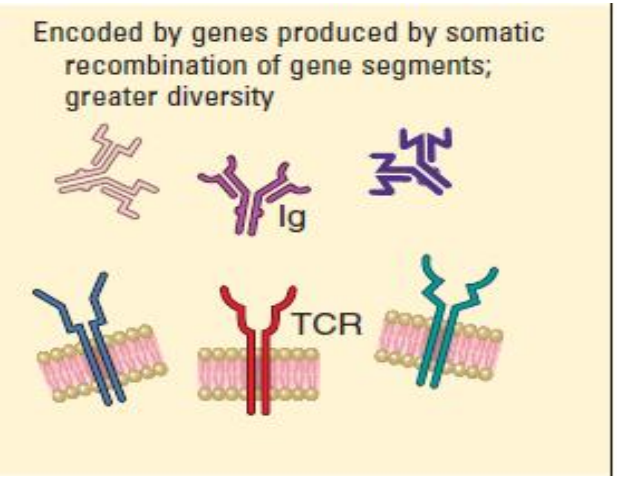
All cells of the same lineage express identical receptors called pathogen **recognition** receptors



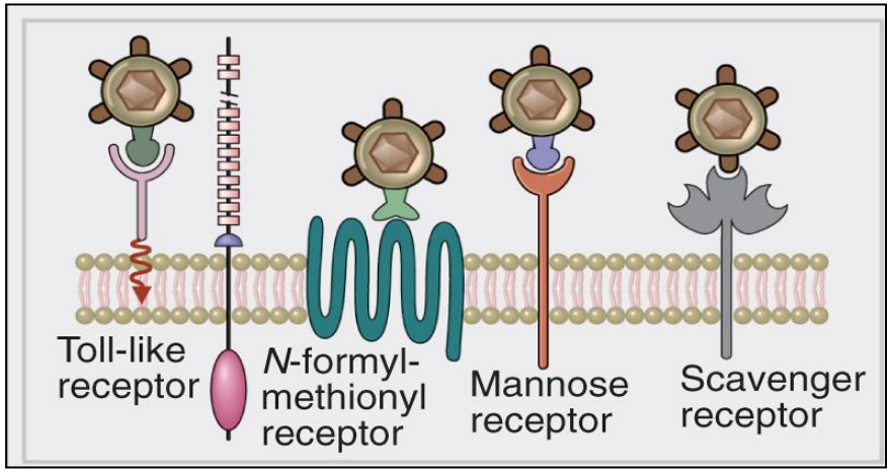
In contrast, **adaptive immune cells** employ antigen receptors that

- Discriminate a number of structural components of microbial molecules (**antigenes**)
- are not encoded in the germline
- are generated de novo in each organism in a random gene recombination process occurring during cell differentiation.

Thus, each **B or T cell (clone) express** a different antigen receptor.



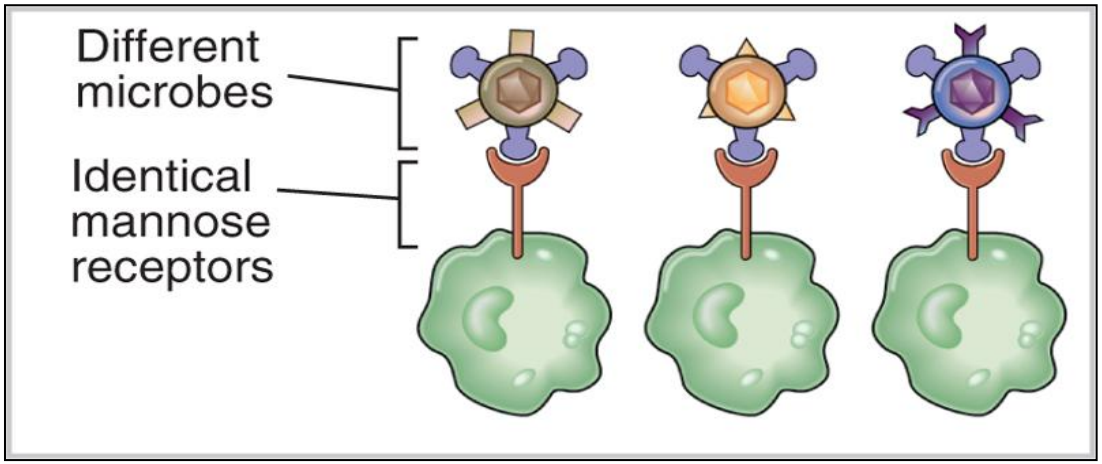
Pathogen recognition receptors (PRR)



Recognition is mediated by specific invariant receptors for pathogen molecular structures which are not expressed by mammal cells ("molecular patterns").

Each cell type expresses several receptor types and is thus able to recognize different molecular patterns.

In addition different microorganisms share same molecular patterns and can thus be recognized by the same receptor type.



Membrane glycoproteins on fungal cell wall

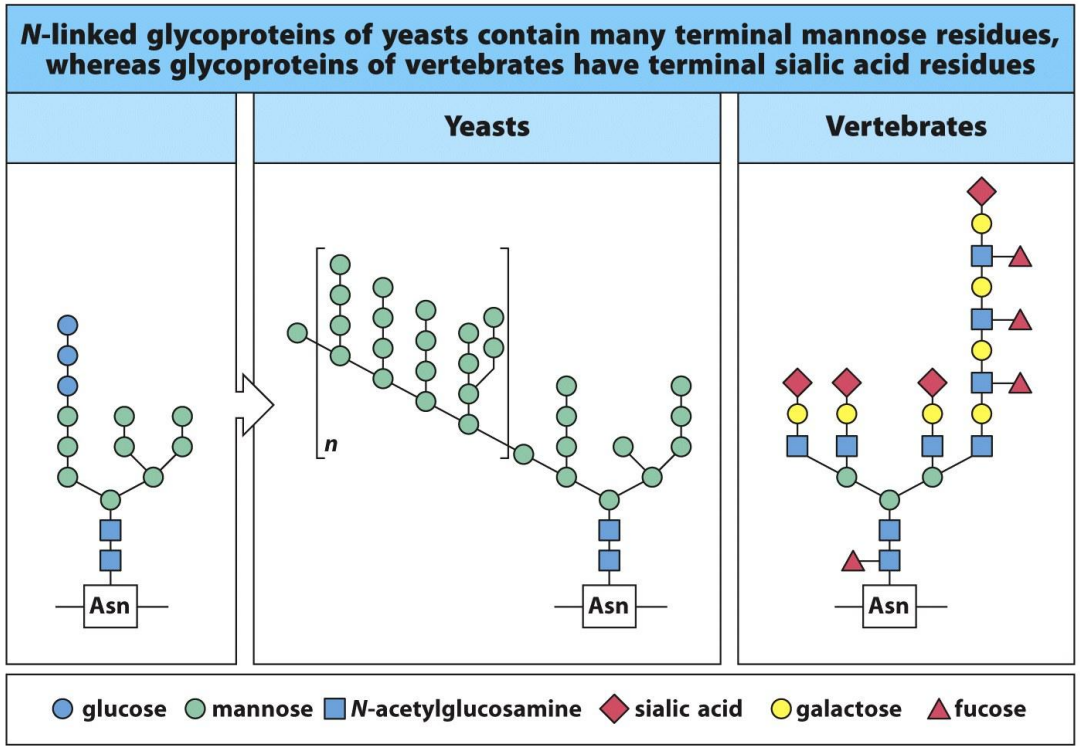
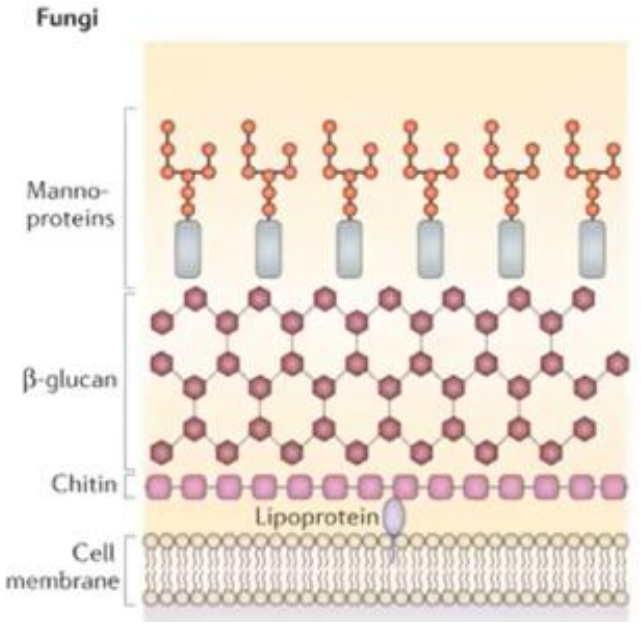


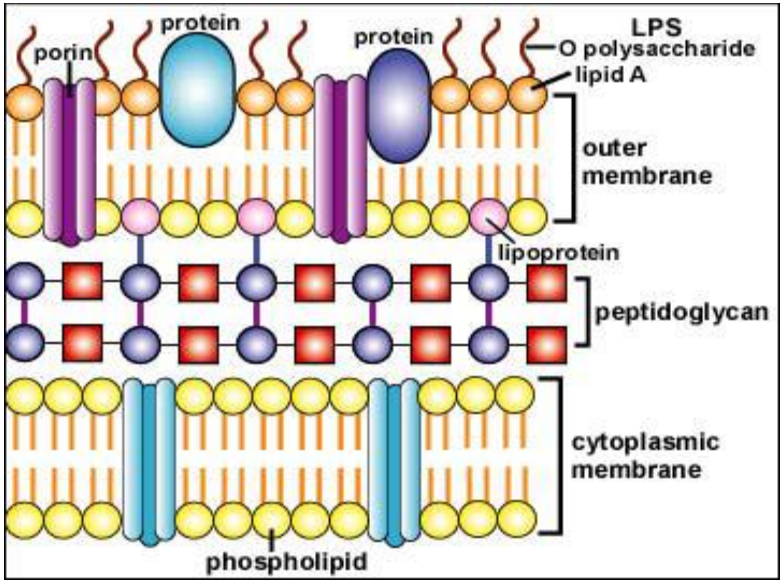
Figure 2.14 Janeway's Immunobiology, 8ed. (© Garland Science 2012)



Mannose can be recognized by membrane or soluble receptors

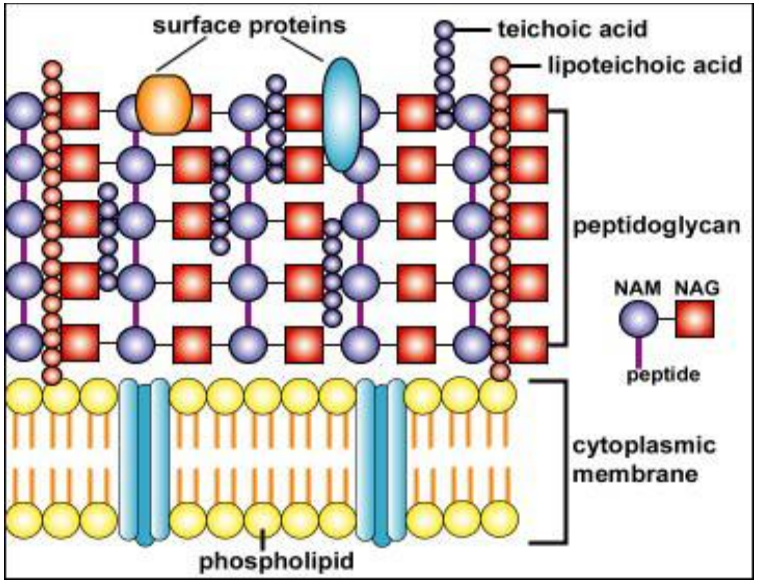
Structural components of the bacteria cell wall

Gram-Negative



The cell wall of the Gram-negative bacterium consists of a thin inner layer of peptidoglycans and an outer membrane containing phospholipids, lipoproteins and lipopolysaccharide (LPS)

Gram-Positive



The cell wall of the Gram-positive bacterium is thick and composed of various layers of peptidoglycans and other components

Pathogen-associated molecular patterns PAMPS

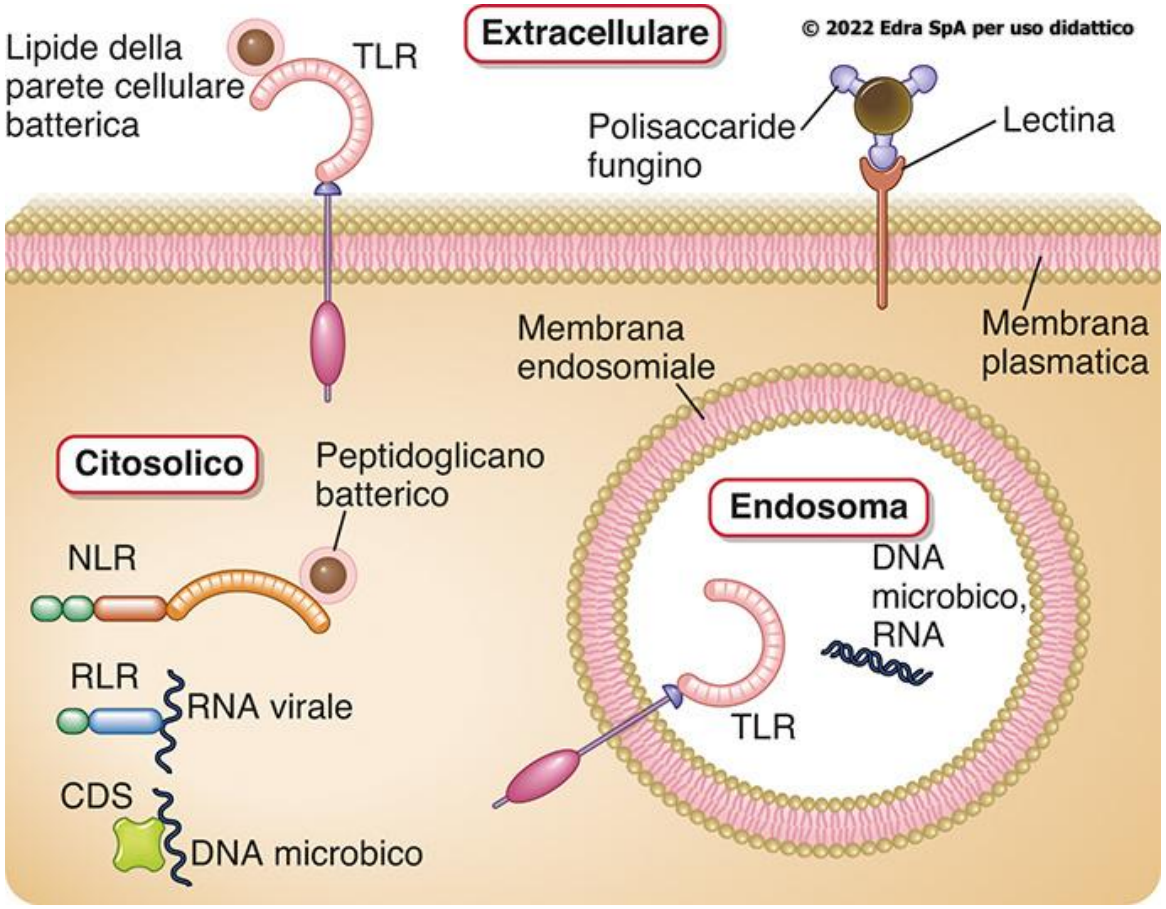
Our organism perceives the presence of pathogens by recognizing molecules unique to microorganisms that are not associated with host cells.

Some examples are:

1. **lipopolysaccharide (LPS)** from the wall of gram-negative bacteria;
2. **peptidoglycans** mainly in gram positive bacteria; to a lesser extent in the gram negative
3. Gram positive **lipoteicoic acids**;
4. **Mannose** (a carbohydrate common in micro-organisms poorly detectble in humans);
5. **Flagellin**: bacterial flagella;
6. **Pilin**: bacterial pili ;
7. **Nucleic acids. (CpG)** (presence of non-methylated cytosine-guanine in bacteria and viruses. In mammalians the frequency of *CG* is much lower and they are methylated);
8. **Single-stranded or double-stranded RNA** unique to many viruses;
9. **Lipoteicoic acids, glycoproteins (i.e. beta-gulcan) and zymosan** from the yeast wall.

Where are pathogen recognition receptors located in the cell?

Cell localization of patogen recognition receptors (PRR)



NLR, NOD-LIKE receptors
TLR, Toll-like receptor
CDS cytosolic DNA sensors
RLR: RIG-LIKE receptors

Microbial Pathogens are recognized through multiple PRRs that have different cellular location:

-TRANSMEMBRANE: include receptors either expressed on the **plasma membrane** or **in endosomal/lysosomal** compartment with specificities for cell-surface associated and intracellular (mainly nucleic acids) PAMPs, respectively.

- CYTOSOLIC: Include **N**ucleotide-binding oligomerization domain-**L**ike **R**eceptors (NLRs) that can detect **pathogen or stress signals** and **R**etinoic acid-**I**nducible **G**ene-**1** (RIG-1) that **recognize viral RNA**.

-SECRETED: include several families of soluble molecules (collectins, ficolins, pentraxins, etc.) that bind to microbial cell surface **and activate the classical or lectin pathway of the complement sytems** and/or **opsonize** pathogens for phagocytosis by neutrophils and macrophages

Innate immunity can also recognize endogenous molecules produced or released from damaged cells (altered self)

TABLE 4.2 Examples of Pathogen-Associated Molecular Patterns and Damage-Associated Molecular Patterns

		Microbe Type
Pathogen-Associated Molecular Patterns		
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
	Glucans	Fungi
Damage-Associated Molecular Patterns		
Stress-induced proteins	HSPs	—
Crystals	Monosodium urate	—
Proteolytically cleaved extracellular matrix	Proteoglycan peptides	—
Mitochondria and mitochondrial components	Formylated peptides and ATP	—
Nuclear proteins	HMGB1, histones	—

Microbial-derived:
PAMPS or MAMPS (microbial associated molecular patterns)

Host-derived:
Damage-Associated Molecular Pattern; **DAMP**

Two main functions of PRRs

Although some PRRs may be involved primarily in **phagocytic clearance of invading organisms**, others engage a plethora of signaling pathways that lead to the expression of genes that encode **chemokines, cytokines, and other mediators** of innate immune responses to infection. (**function 1:** promotion of inflammation and anti-viral state)

Among other mediators, PRR signaling in APC such as DCs renders them competent to prime T cells, thereby initiating adaptive immunity **(function 2)**

Functional classes of microbial recognition receptors capable of binding conserved portions of these molecules

1. **Endocytic pattern-recognition receptors**
   Engulfment of pathogens; can induce cytokine expression

2. **Chemotactic pattern-recognition receptors**
   Migration to infection site

3. **Signaling pattern-recognition receptors**
   Production of effector molecules that participate to the immune response and affect the nature of adaptive response

1-Endocytic pattern-recognition receptors

Expressed on the membrane of cells that can do phagocytosis (phagocytes), they promote the incorporation of the microorganism and its subsequent destruction

a. C-type lectin receptors
This includes the mannose receptor and the beta-glucan receptor (dectin-1)

b. Scavenger receptors
In addition to the modified LDL, the scavenger receptors of macrophages bind innumerable microbes by LPS, peptidoglycans etc, as well as apoptotic cells of the host

C-type Lectin receptor (CLR) family

Molecules containing domains that bind carbohydrates in a calcium-dependent fashion

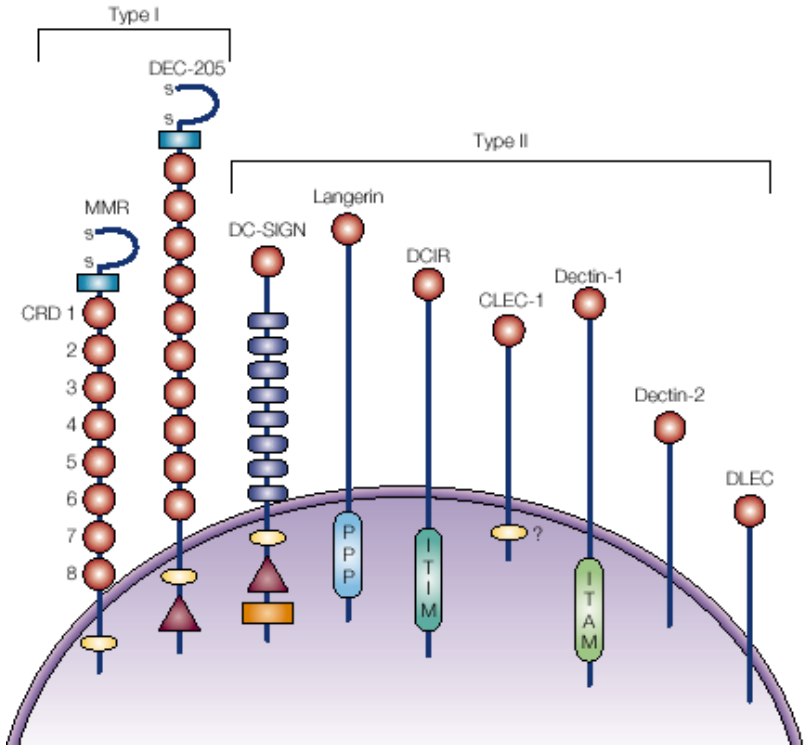
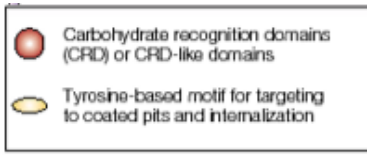


Table 2 Ligands for myeloid CLRs

CLR	Carbohydrate specificity	Pathogen binding	Recognition of self and altered self
MR (CD206)	Mannose, fucose, sLe^X	HIV, *P. carinii*, *M. tuberculosis*, *C. albicans*	Lysosomal hydrolases, thyroglobulin, L-selectin, MUC-1
DC-SIGN (h) (CD209)	Mannan, high-mannose, ManLAM, fucose, Le^X, Le^A, Le^Y, Le^B, $6SLe^A$	HIV, HCV, CMV, filoviruses, dengue, *H. pylori*, *M. tuberculosis*, *S. mansoni*, *C. albicans*, *A. fumigatus*, *Leishmania* spp.	ICAM-2, ICAM-3, CEACAM-1–Mac-1 (PMN), CEA
Dectin-1 (CLEC7A, β-glucan receptor)	β1,3-glucans	*P. carinii*, *C. albicans*, *A. fumigatus*	Ligand on T cells



Type I C-type lectins (MMR, macrophage mannose receptor and DEC-205) they contain 8-10 **carbohydrate recognition domains (CRDs**) in the extracellular portion (N-terminal), which bind sugars in a calcium dependent manner.

Type II C-type lectins contain only one CRD in their extracellular portion (carboxy-terminal)

CRD:
Carbohydrate recognition domain

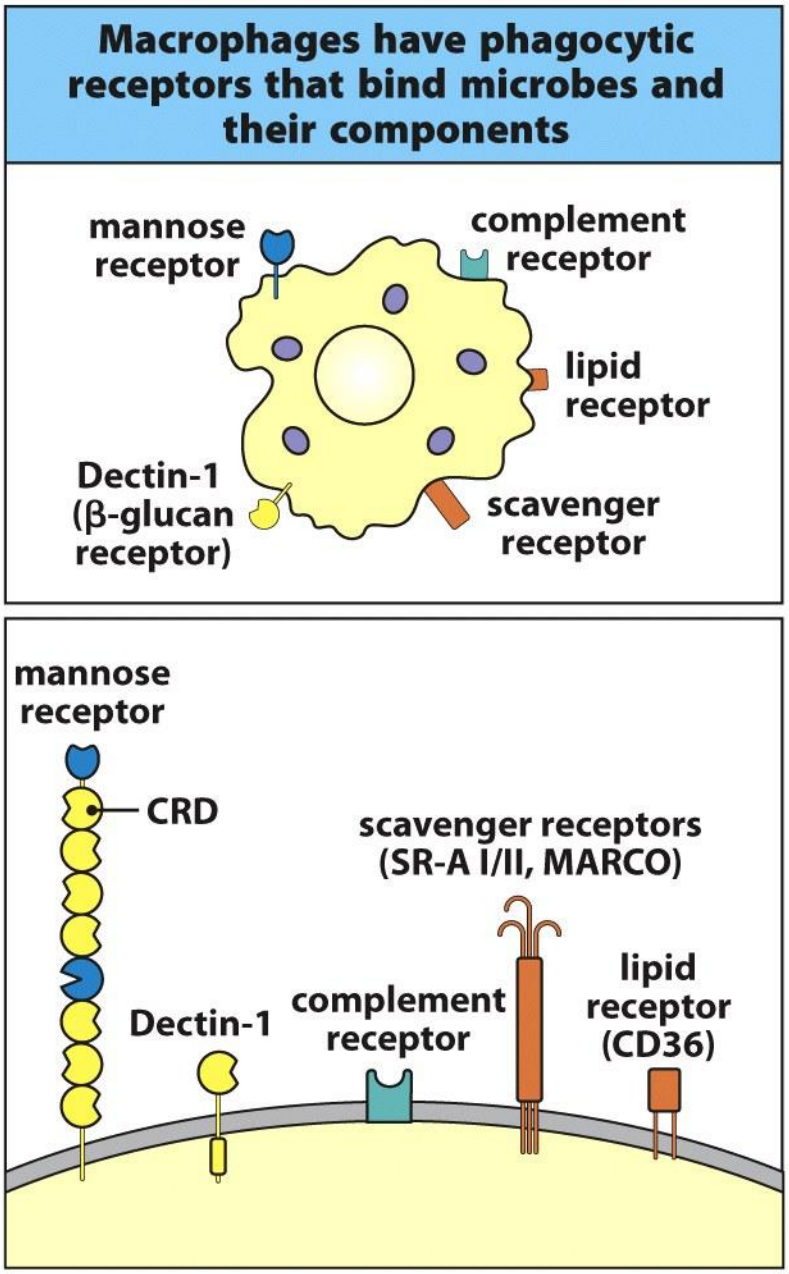


Figure 3.2 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Thanks to endocytic PRR, phagocytes are cells able to engulf microbes through a process called phagocytosis

Phagocytosis (from Ancient Greek «*phagein*» , **meaning** 'to eat', and «*kytos*» , **meaning** 'cell') Greek *–ōsis* meaning a process

In summary phagocytosis is the cell uptake of particulate material



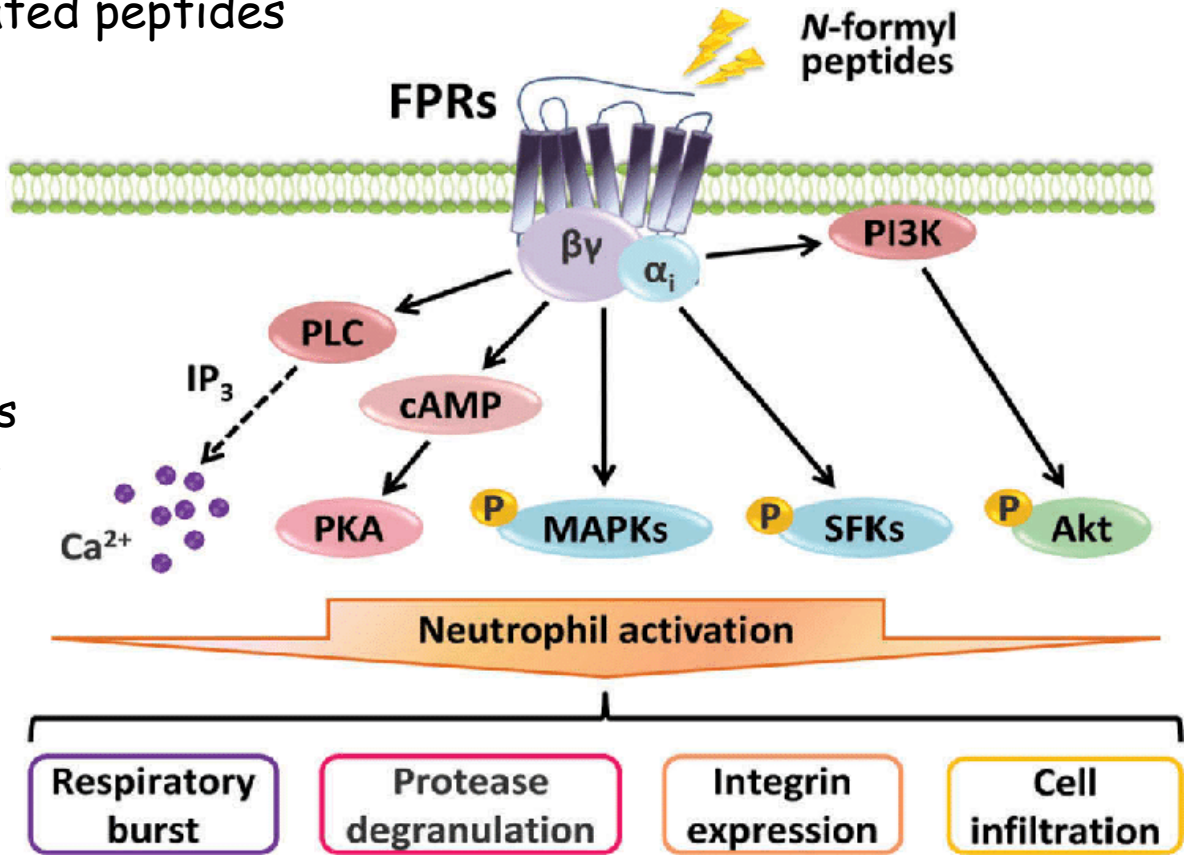
2-Formyl-peptide receptors (FPRs)

FPRs are G protein-coupled receptors that bind bacterial and mitochondrial formylated peptides

Main function:

Promotion of chemotaxis

During infection, pathogens target and destroy host tissue with the simultaneous release of **both bacteria-derived** (when the pathogen is of bacterial origin) **and host-derived formylated peptides** (from host mitochondria), thereby linking FPRs in both infective and sterile inflammatory processes



The main responders are neutrophils:

50% to 70% of circulating human leukocytes, neutrophils patrol the vasculature and rapidly migrate into tissues in response to chemotactic signals

3. Signaling pattern-recognition receptors

a. Toll-like receptors (TLRs)

b. CD14

c. NOD-like receptors; RIG-like, CDS…. (cytoplasmic)

Christiane Nüsslein – Volhard, Nobel Prize 1995





TOLL RECEPTOR in DROSPHILA DEVELOPMENT

The Nobel Prize in Physiology or Medicine 2011

Bruce A. Beutler, Jules A. Hoffmann, Ralph M. Steinman



Photo: Mosimann for Balzan

Bruce A. Beutler



Photo: Mosimann for Balzan

Jules A. Hoffmann

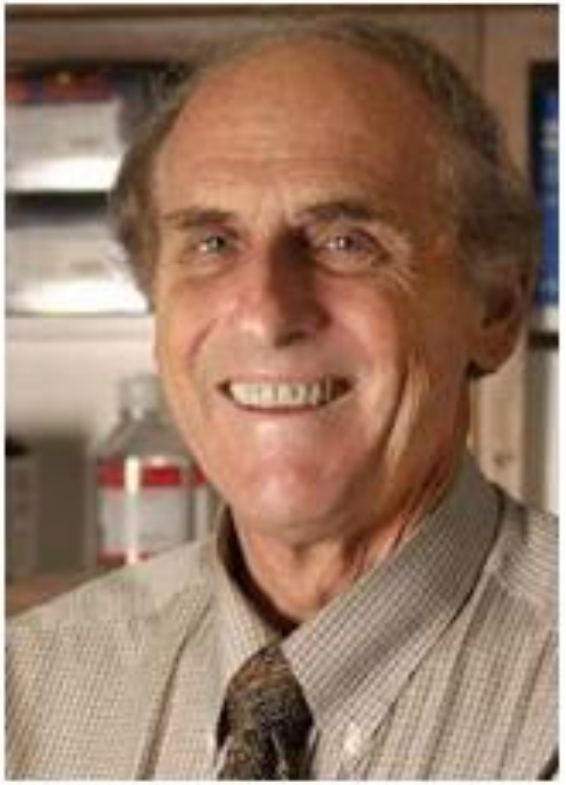


Photo: Rockefeller University Press

Ralph M. Steinman

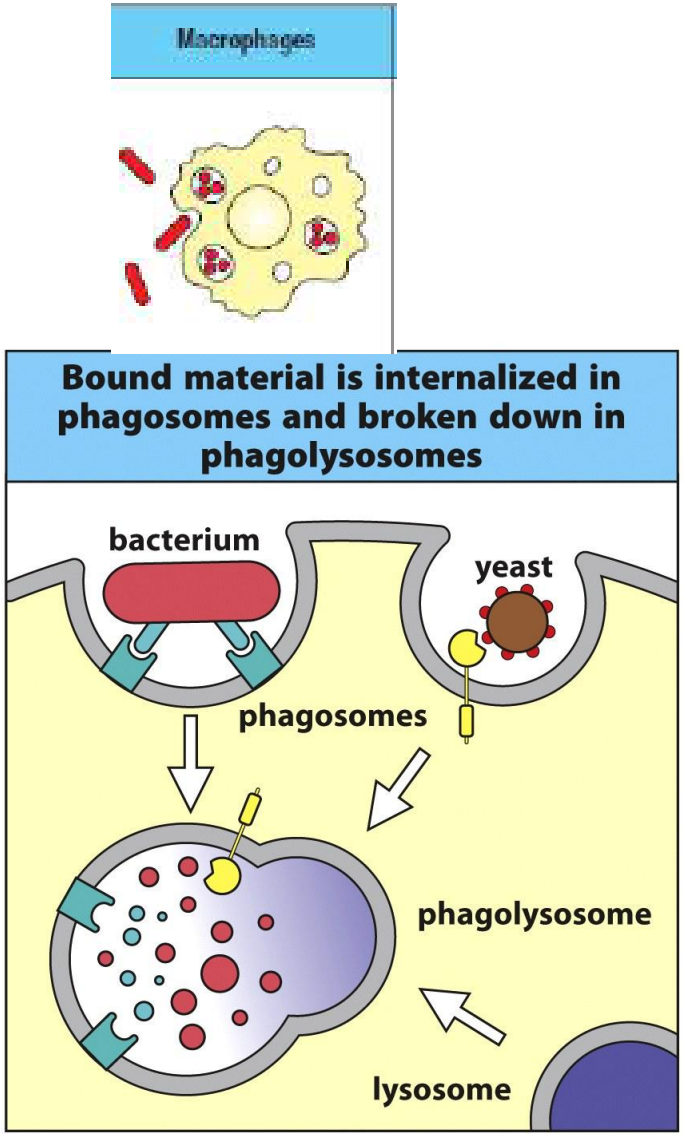
The Nobel Prize in Physiology or Medicine 2011 was divided, one half jointly to Bruce A. Beutler and Jules A. Hoffmann "for their discoveries concerning the activation of innate immunity" and the other half to Ralph M. Steinman "for his discovery of the dendritic cell and its role in adaptive immunity".

Negli eucarioti i recettori Toll-like sono responsabili della risposta ad un gran numero di molecole espresse da diversi microbi ma non da cellule dell'organismo

Riconoscono anche «induced self» o «damaged self» molecules

HOW DOES THE INNATE IMMUNE SYSTEM RECOGNIZE PAMPS PRESENT ON THE MICROBIAL SURFACE OR INSIDE THE MICROBE?

Phagocytosis and endocytosis generate material that can be detected by PRRs in vescicles



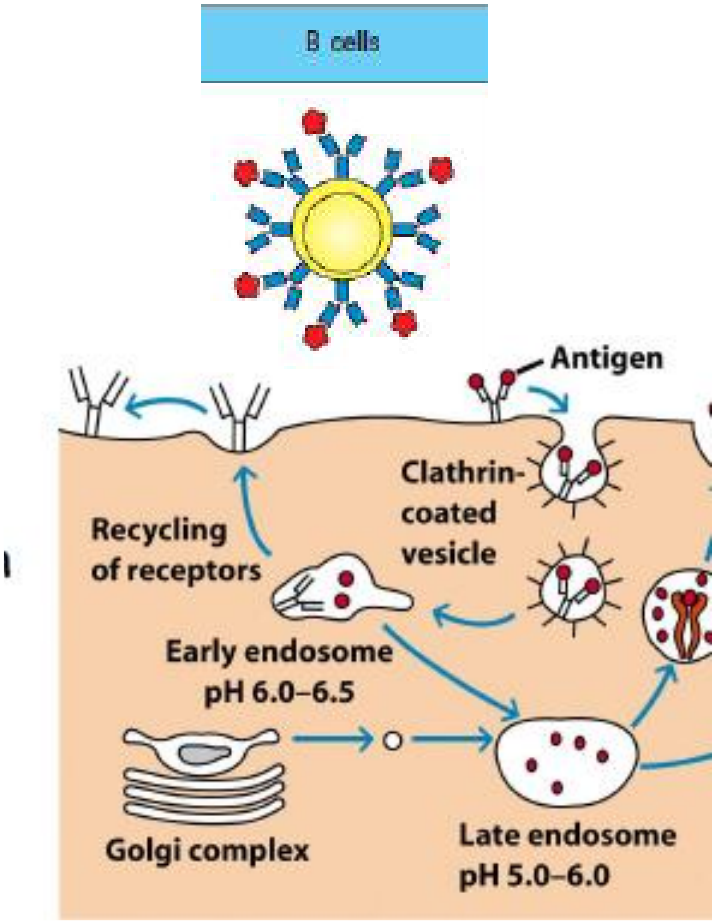
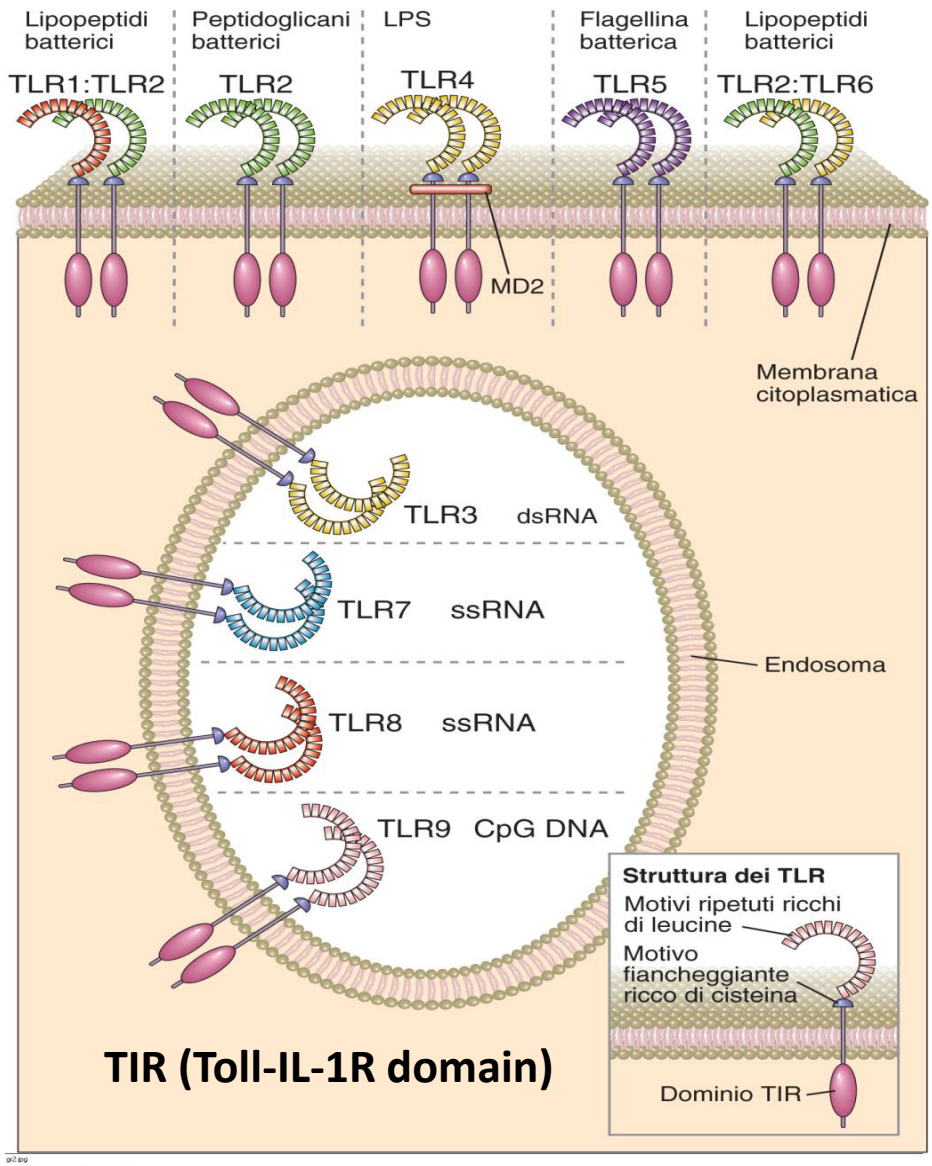


Figure 3.2 part 2 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Toll-like receptors (TLRs) are expressed both on cell surface and intracellularly



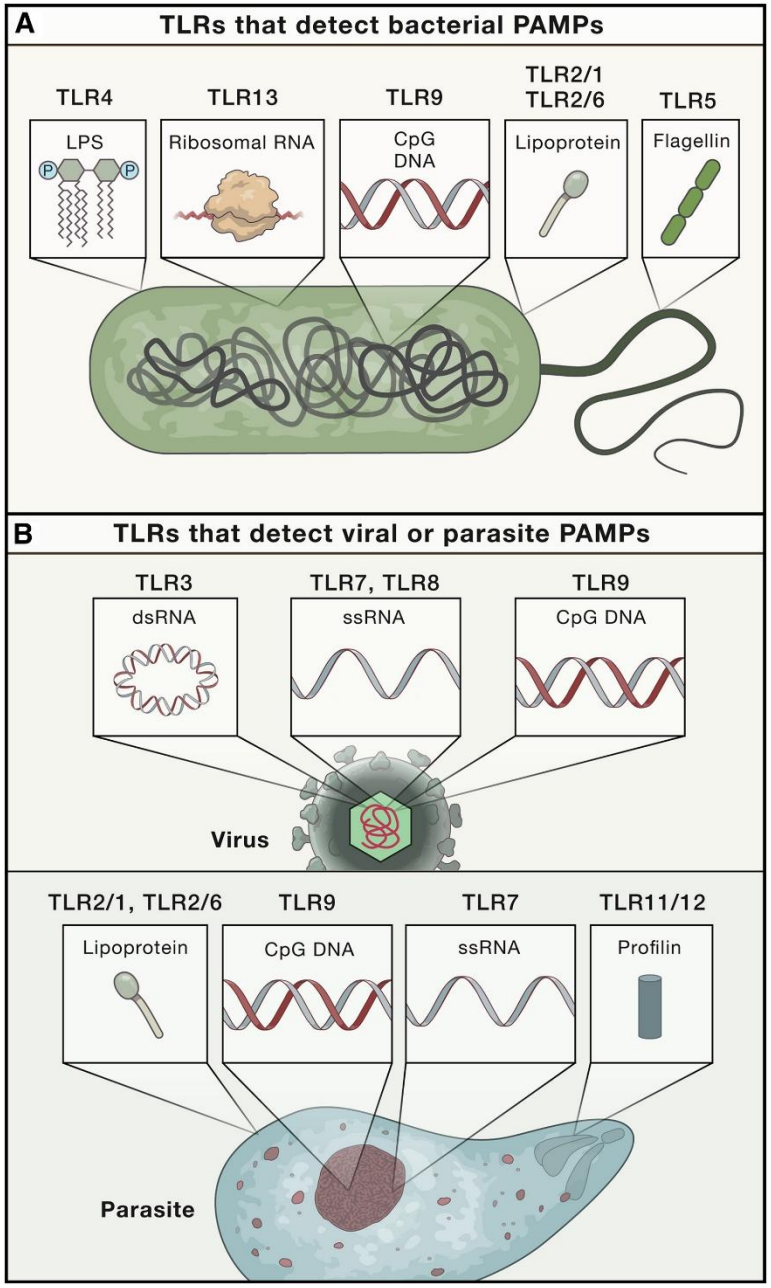
Glycoproteins known as TLRs (Toll-like Receptors) are expressed both on the surface and in the phagosomes of the phagocytes.

The receptors expressed on the membrane recognize molecules expressed on the surface of microbes such as components of the bacterial wall.

Receptors expressed in the vesicles recognize microbial molecules released following phagocytosis / endocytosis of the microbe.

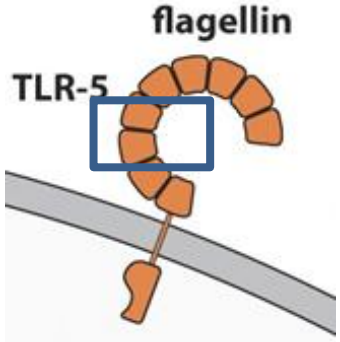
However, they can also recognize proteins involved in the response to cellular stress such as:

- High-Mobility group Box-1 (HMGB-1)
- Heat shock proteins



Toll-like receptors recognize different molecules derived from pathogens by forming homo- or hetero-dimers

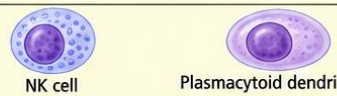
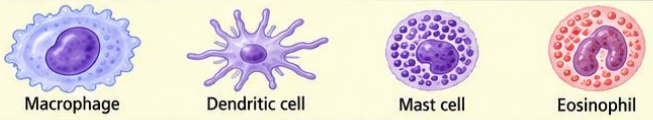
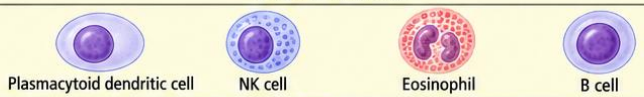
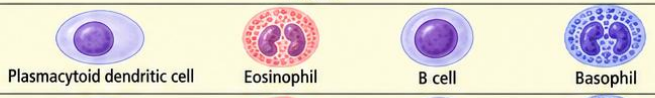
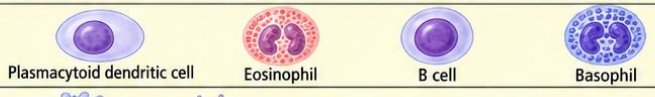
16-20 leucine rich Motifs **LXXLXLXXN**



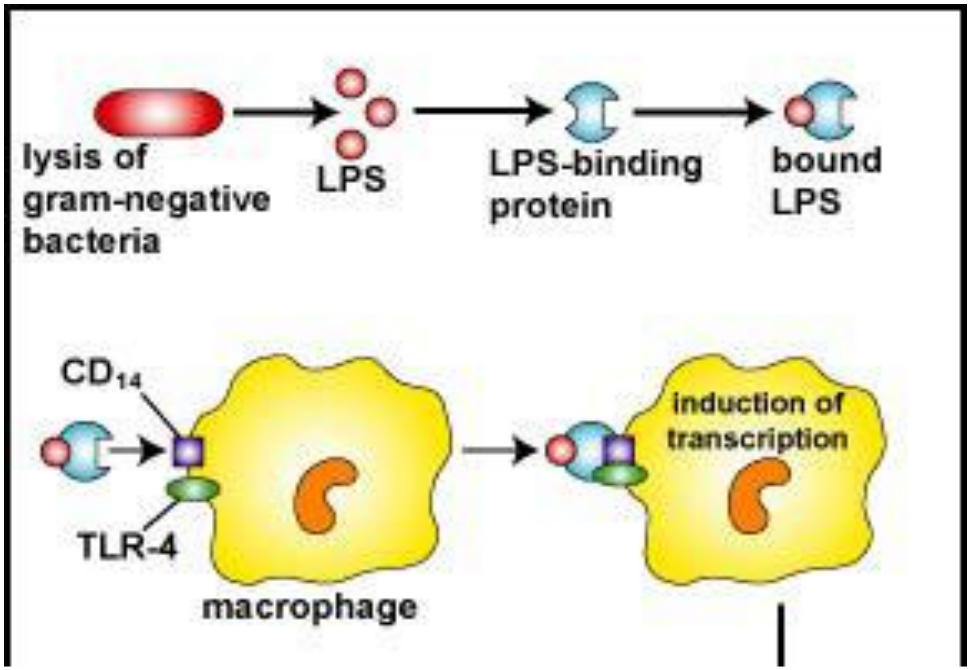
Microbe binding to leucine rich domains in the convex portion promote homo or heterodimerization of TLR

L'espressione cellulare dei recettori Toll-like è direttamente collegata alla funzione delle cellule…….

Innate immune recognition by mammalian Toll-like receptors

Toll-like receptor	Ligand	Cellular distribution
TLR-1:TLR-2 heterodimer	Lipomannans (mycobacteria) Lipoproteins (diacyl lipopeptides; triacyl lipopeptides) Lipoteichoic acids (Gram-positive bacteria) Cell-wall β-glucans (bacteria and fungi) Zymosan (fungi)	
TLR-2:TLR-6 heterodimer		
TLR-3	Double-stranded RNA (viruses)	
TLR-4 (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)	
TLR-5	Flagellin (bacteria)	
TLR-7	Single-stranded RNA (viruses)	
TLR-8	Single-stranded RNA (viruses)	
TLR-9	DNA with unmethylated CpG (bacteria and herpesviruses)	
TLR-10	Unknown	
TLR-11 (mouse only)	Profilin and profilin-like proteins (*Toxoplasma gondii*, uropathogenic bacteria)	

Lypopolysaccharid (LPS) recognition and cell activation

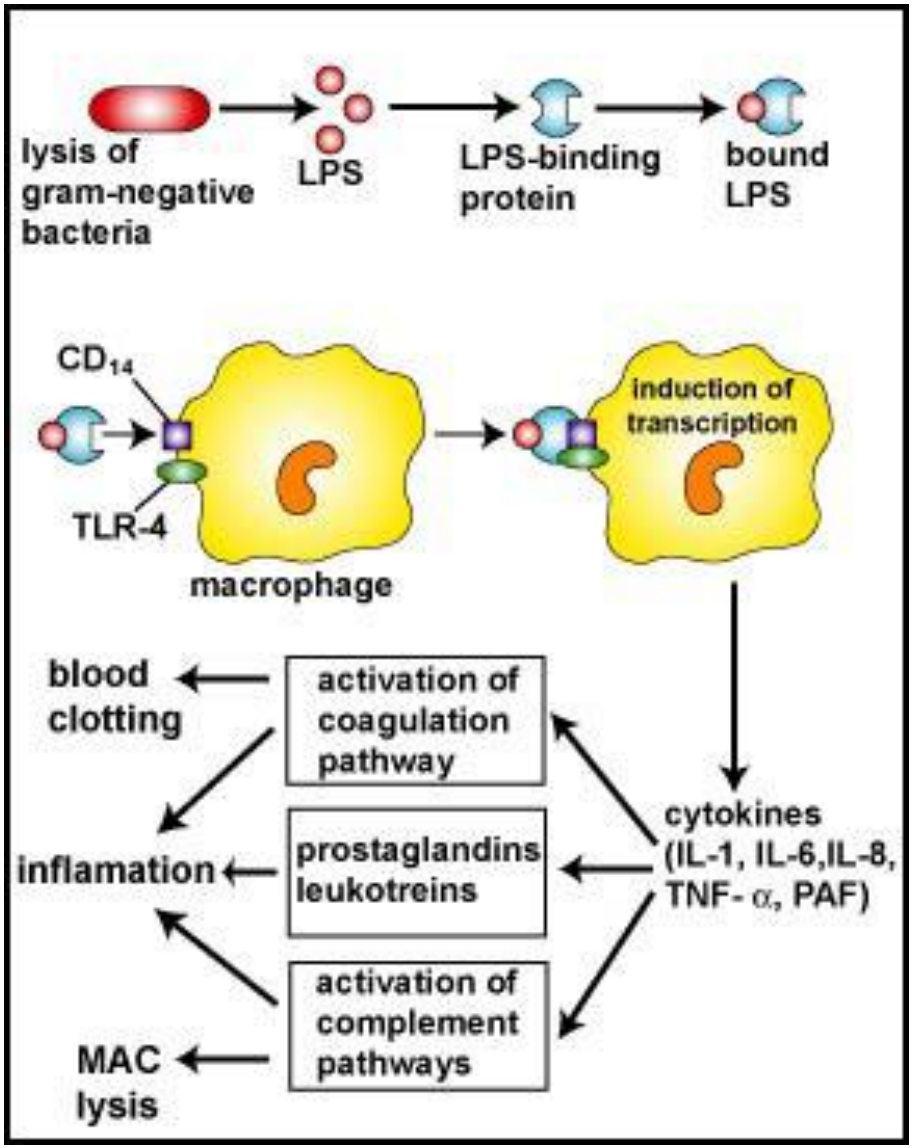


The lysis of negative GRAM bacteria allows to release LPS from their external membrane.

LPS binds to an LPS binding protein in the bloodstream and this complex is able to bind CD14 (a receptor located on the macrophage membrane).

The forming complex promotes the toll-like receptor TLR-4 binding to the LPS

Lypopolysaccharid (LPS) recognition and cell activation



The activation of the resulting macrophage leads to the release of chemicals involved in the defensive response called cytokines, including IL-1, IL-6, IL-8, TNF-alpha and PAF.

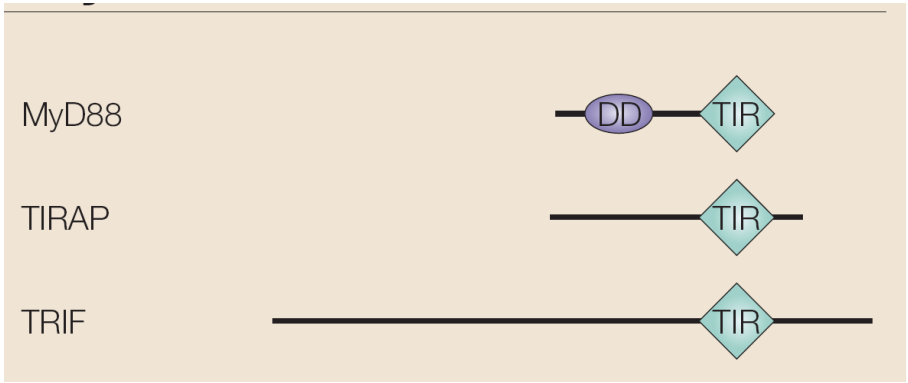
The cytokines then bind to cytokine receptors on target cells and begin the inflammatory process

How do TLRs activate gene transcription?

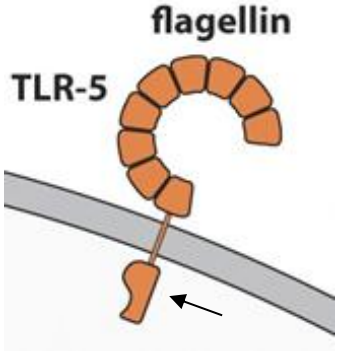
TLR triggering starts a signaling cascade that lead to production of:

- Inflammatory cytokines
- Chemotactic factors
- Anti-microbial peptides
- Co.stimulatory molecules
- Antiviral cytokines
- …….

Adaptors of MyD88 family



TIR domain:
Toll/interleukin-1 receptor-domain



TIR domain

MyD88:
myeloid differentiation primary-response protein 88

TIRAP (TIR-domain containing adaptor protein)

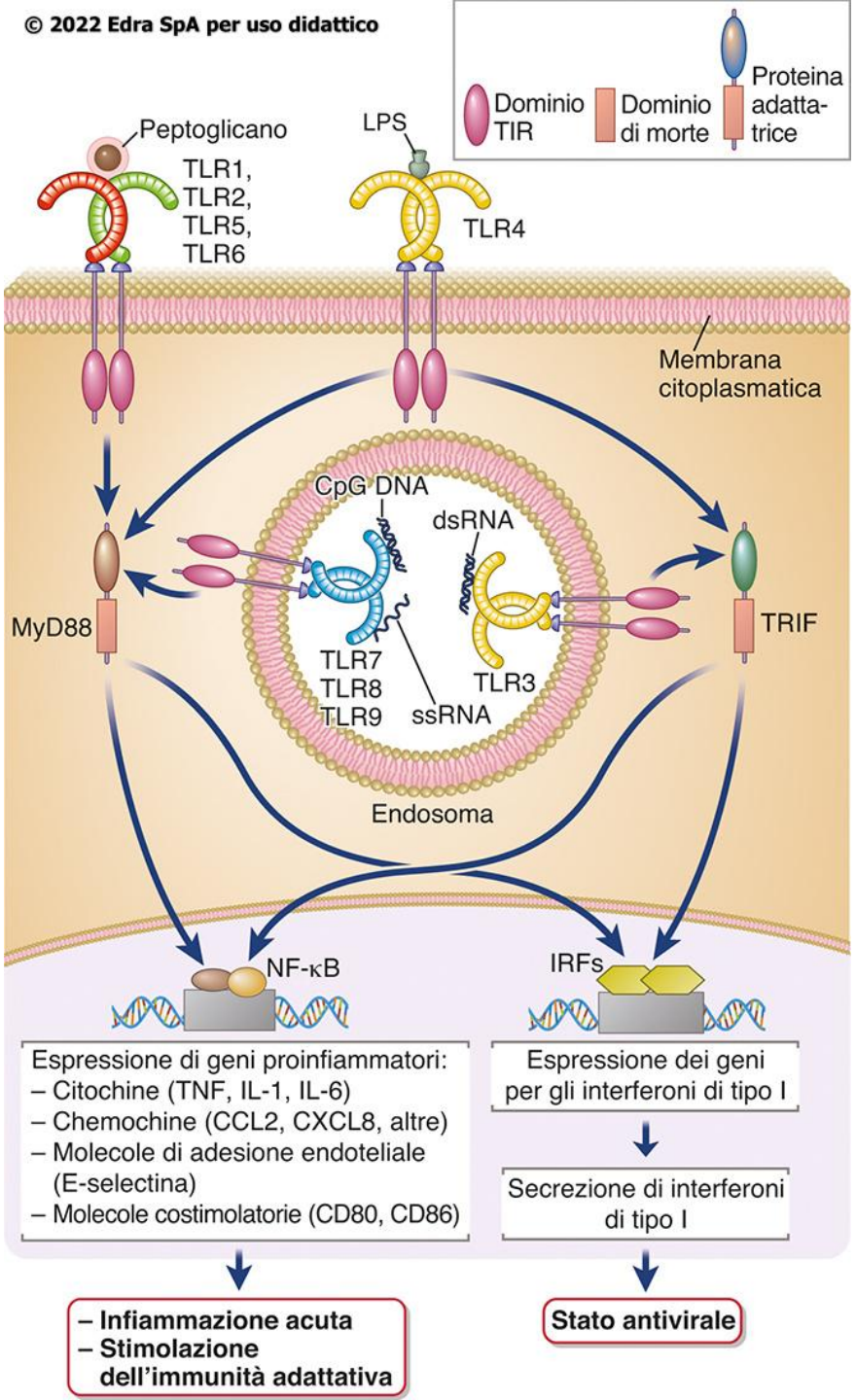
TRIF adapter (TIR-domain containing adaptor protein inducing interferon-β

Signal transduction of TLR

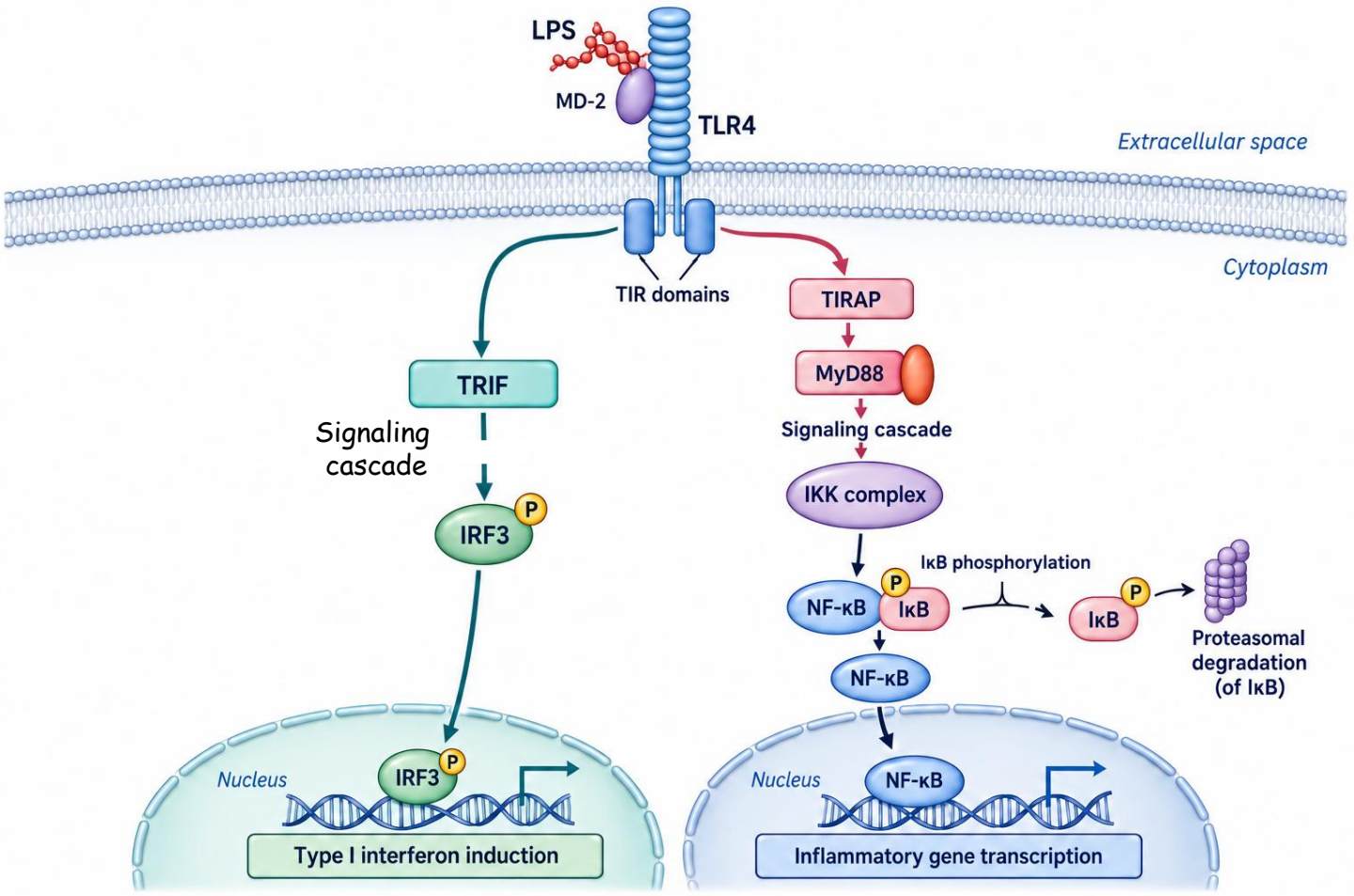
La dimerizzazione dei domini TIR intracellulari dei rec TOLL-like porta ad interazione con adattatori molecolari e la conseguente attivazione di **Fattori di Trascrizione**

MyD88:
myeloid differentiation primary-response protein 88

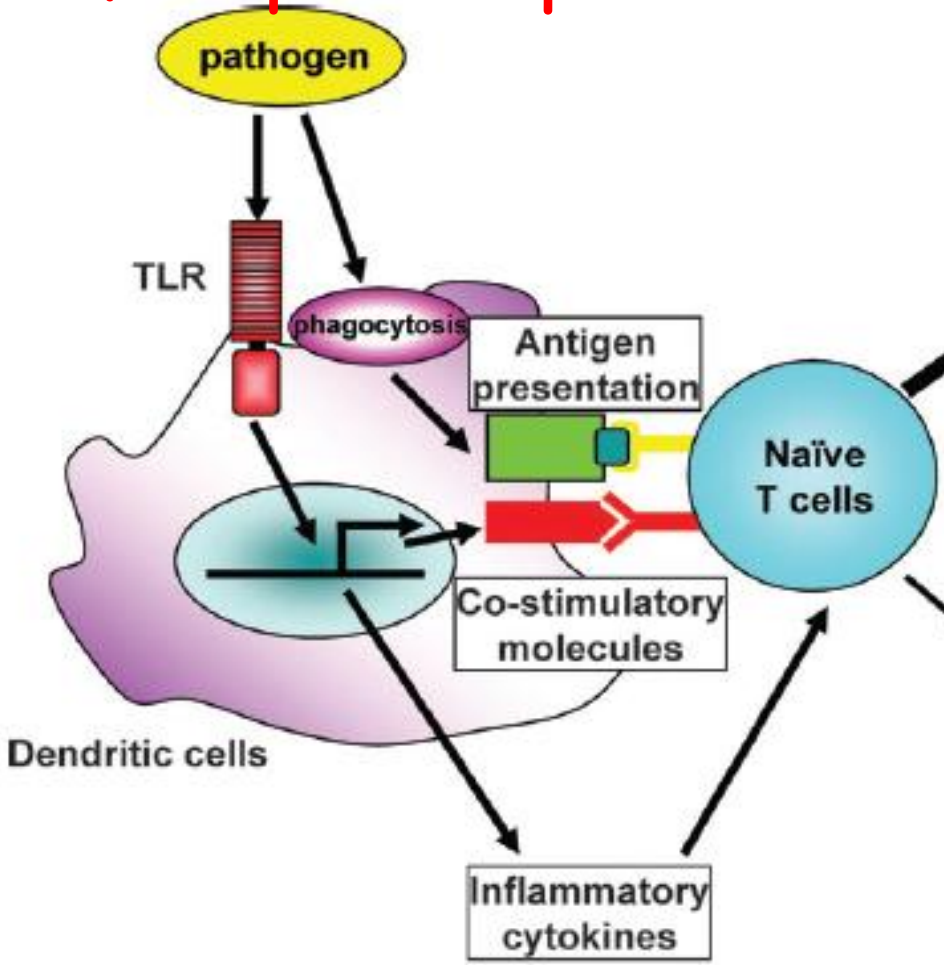
TRIF:
TIR-domain-containing adaptor inducing interferon-beta



TLR-4 signal transduction



Fuction 2: innate immune cells such as dendritic cells and macrophages provide signals for activation of adaptive responses



Phagocytosis-mediated **antigen presentation**, together with TLR-mediated expression of **co-stimulatory molecules**, provide first and second signal for development of antigen-specific adaptive immunity

Innate immunity → Acquired immunity

In conclusion, TLRs work as sensors for extracellular pathogens that activate innate immune cells for several functions

What happen if a pathogen reaches the cytoplasm or uses the cytoplasm during its life cycle?