Università degli Studi di Padova

Corso di Laurea in

BIOTECNOLOGIE

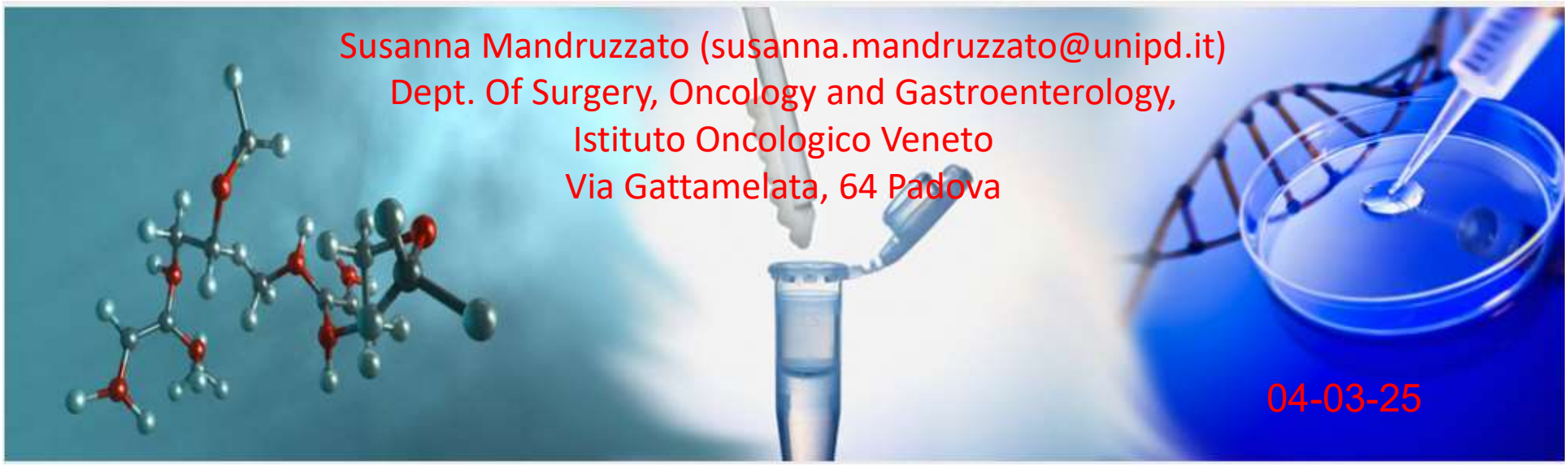
Piano di studi Farmaceutico

Anno Accademico 2045-2025

Insegnamento di

Immunologia Farmaceutica

Immunità innata: i recettori



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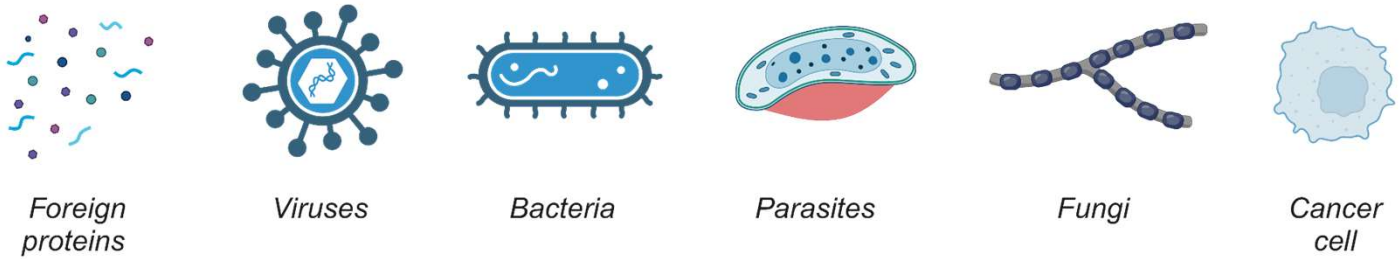
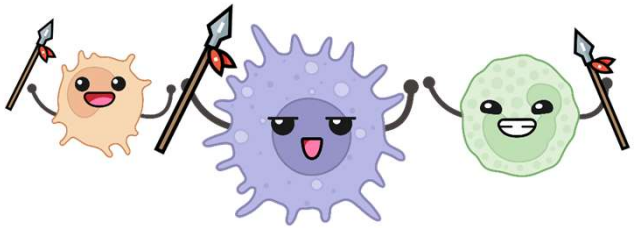
04-03-25

Your active Immune Defenses



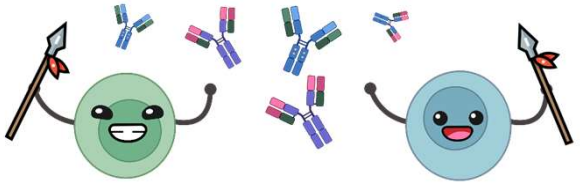
Innate Immunity

- invariant (generalized)
- early, limited specificity
- the first line of defense

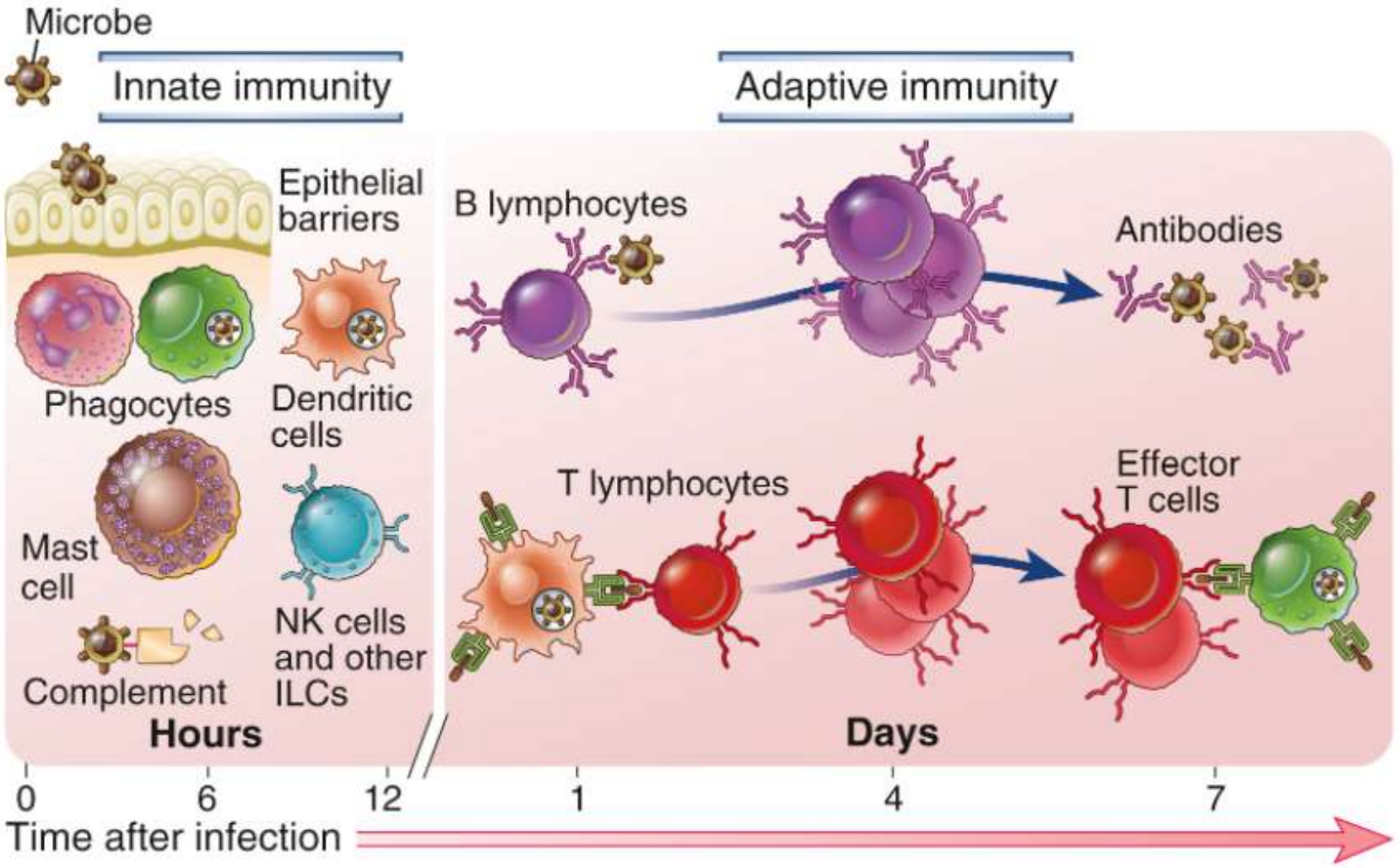


Adaptive Immunity

- variable (custom)
- later, highly specific
- ‘‘remembers’’ infection



Caratteristiche principali dell'immunità innata e di quella adattativa



Phases of the immune response			
Response		**Typical time after infection to start of response**	**Duration of response**
Innate immune response	Inflammation, complement activation, phagocytosis, and destruction of pathogen	Minutes	Days
Adaptive immune response	Interaction between antigen-presenting dendritic cells and antigen-specific T cells: recognition of antigen, adhesion, co-stimulation, T-cell proliferation and differentiation	Hours	Days
	Activation of antigen-specific B cells	Hours	Days
	Formation of effector and memory T cells	Days	Weeks
	Interaction of T cells with B cells, formation of germinal centers. Formation of effector B cells (plasma cells) and memory B cells. Production of antibody	Days	Weeks
	Emigration of effector lymphocytes from peripheral lymphoid organs	A few days	Weeks
	Elimination of pathogen by effector cells and antibody	A few days	Weeks
Immunological memory	Maintenance of memory B cells and T cells and high serum or mucosal antibody levels. Protection against reinfection	Days to weeks	Can be lifelong

Figure 1.7 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

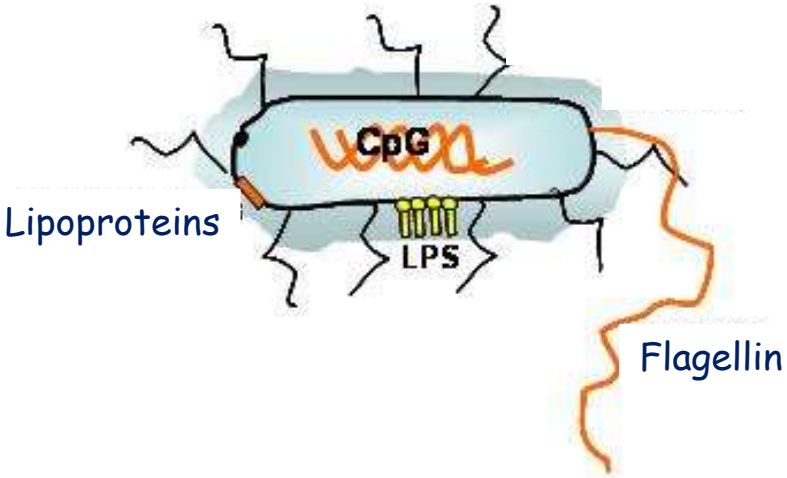
Come l’immunità innata riconosce i microrganismi ed il self danneggiato:

Riconoscimento di strutture molecolari che sono prodotte dai patogeni: PAMP, profili molecolari associati ai patogeni

Molecular patterns

Structures common for certain groups/classes of pathogens

- essential for their life, replication and/or infectivity
- not present on human cells



Examples:

structures of bacterial cell wall (LPS, peptidoglycan, flagellin...)

nucleic acids of pathogens (dsRNA, unmethylated CpG dinucleotides...)

L’immunità innata è anche in grado di riconoscere molecole endogene prodotte o rilasciate da cellule danneggiate o morenti.
Queste strutture sono denominate DAMPS (*Damage-Associated Molecular Pattern*)

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.

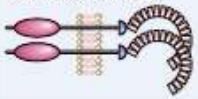
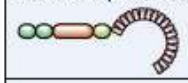
L'immunità innata riconosce i DAMP ed i PAMP utilizzando:
sia recettori cellulari, distribuiti su diversi compartimenti,
sia molecole solubili presenti nel sangue e nelle secrezioni mucose.

I recettori cellulari sono noti come: RECETTORI CHE RICONOSCONO I PROFILI MOLECOLARI (*Pattern recognition receptors, PRR*) .

La localizzazione dei PRR può essere:

- Membrana citoplasmatica
- Membrana endosomiale
- Citoplasma
- Plasmatica

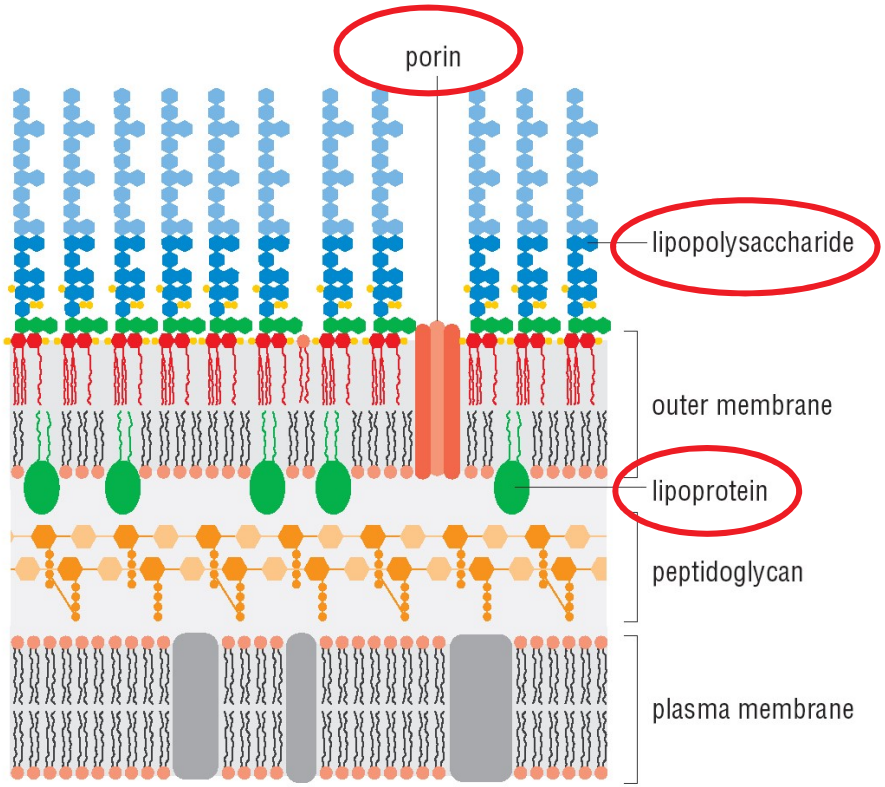
TABLE 4–3 Pattern Recognition Molecules of the Innate Immune System

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
Soluble Recognition Molecules	**Location**	**Specific Examples**	**PAMP Ligands**
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma Alveoli	Mannose-binding lectin Surfactant proteins SP-A and SP-D	Carbohydrates with terminal mannose and fructose Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-Acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	C3	Microbial surfaces
Natural antibodies 	Plasma	IgM	Phosphorylcholine on bacterial membranes and apoptotic cell membranes

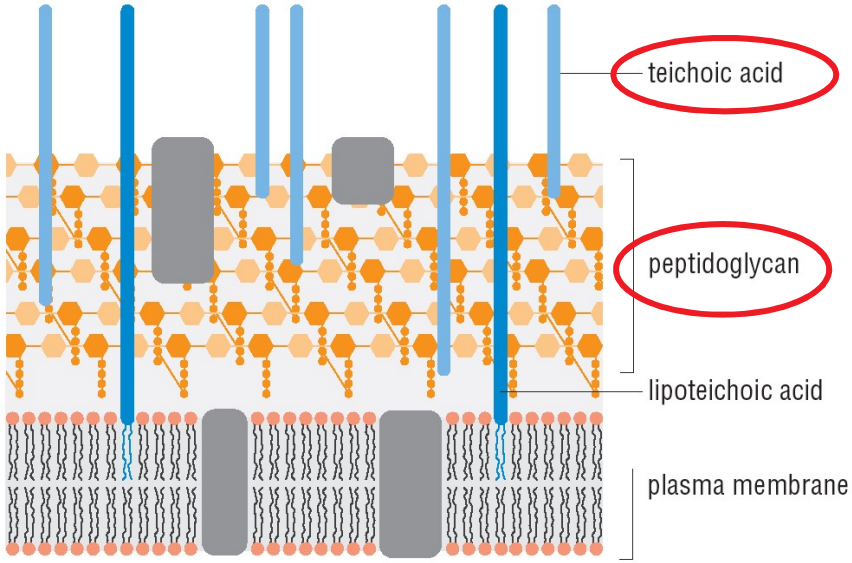
Identification of DAMP and PAMP by the innate immune system

- Recognition
  - Receptors – Pattern Recognition Receptors (PRRs)
    - Encoded in the germline, fixed in the genome, i.e. gene rearrangement is not needed. Can recognize about 10^3 molecular patterns.
  - Distribution
    - Non-clonal, i.e. all cells of a class are identical

Examples of innate immune recognition of bacterial cell wall components



Gram-negative bacteria



Gram-positive bacteria

PRR cellulari

Quasi tutte le cellule esprimono PRR e possono quindi prendere parte alla risposta immunitaria innata.

Le cellule che presentano la varietà e le quantità maggiori di PRR sono i fagociti (*neutrofili, macrofagi e cellule dendritiche*)

I PRR attivano vie di trasduzione del segnale che determinano risposte cellulari come la produzione di molecole proinfiammatorie e antimicrobiche.

Toll-like receptors: un antico sistema di riconoscimento del patogeno

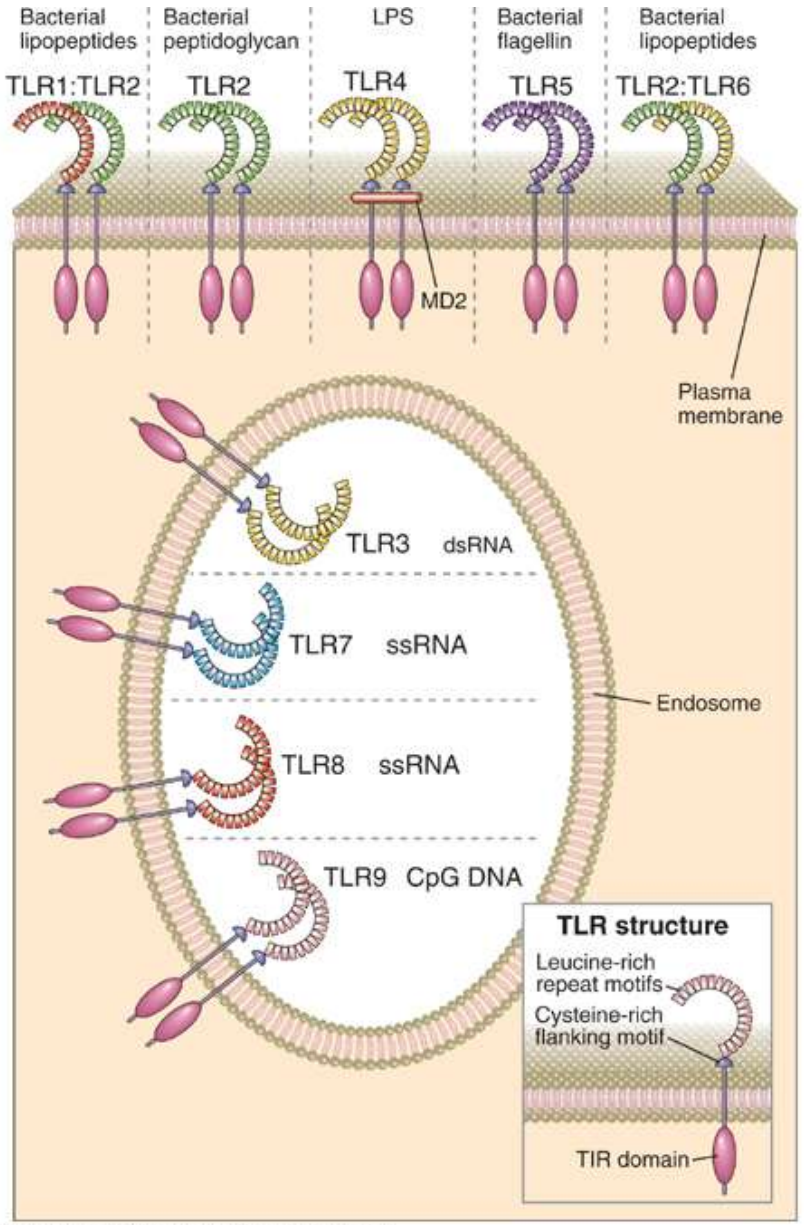
- Importante famiglia di PRR espressi da molti tipi cellulari
- Nell'uomo sono espressi 10 geni *TLR* (13 nel topo), ognuno dedicato al riconoscimento di un insieme distinto di forme molecolari che NON si trovano nelle cellule sane del vertebrato.
- I TLR del mammifero riconoscono forme molecolari caratteristiche di batteri G+ e G-, funghi e virus.
- Sono anche coinvolti nella risposta a molecole endogene la cui espressione o localizzazione indica la presenza di un danno cellulare
  - Es HSP e HMGB-1 normalmente intracellulari, vengono rilasciate da cellule danneggiate o morte. Una volta rilasciate, attivano TLR2 e TLR4 espressi da DC, macrofagi e altri tipi cellulari.
- La base strutturale della specificità dei TLR risiede nei moduli extracellulari che sono ripetuti, ricchi in leucina, fiancheggiati da caratteristici motivi ricchi in cisteina. Essi legano i PAMP direttamente o attraverso molecole adattatrici.
- Le loro code citoplasmatiche contengono un dominio denominato TIR (*Toll-IL-1 Receptor*), essenziale per l'attivazione cellulare.
- Il dominio TIR si trova anche nelle code citoplasmatiche di IL-1 e IL-18, citochine che attivano una cascata di trasduzione del segnale simile a quella dei TLR.

Riconoscimento immunitario mediato dai recettori Toll-like		
Recettori Toll-like	**Ligando**	**Distribuzione cellulare**
TLR-1:TLR-2 eterodimero	Peptidoglicani Lipoproteine Lipoarabinomannano (micobatteri) GPI (*T. cruzi*) Zymosan (lievito)	Monociti, cellule dendritiche, mastociti, eosinofili, basofili
TLR-2:TLR-6 eterodimero		
TLR-3	dsRNA	Cellule NK
TLR-4 dimero (più MD-2 e CD14)	LPS (batteri gram-negativi) Acidi lipoteicoici (batteri gram-positivi)	Macrofagi, cellule dendritiche, mastociti, eosinofili
TLR-5	Flagellina	Epitelio intestinale
TLR-7	ssRNA	Cellule dendritiche plasmacitoidi, cellule NK, eosinofili, cellule B
TLR-8	Oligonucleotidi G-ricchi	Cellule NK
TLR-9	Cpg DNA non metilato	Cellule dendritiche plasmacitoidi, eosinofili, cellule B, basofili
TLR-10	Sconosciuto	Cellule dendritiche plasmacitoidi, eosinofili, cellule B, basofili
TLR-11 (solo il topo)	Proteine profilina e profilina-simile (*Toxoplasma gondii*, batteri uropatogeni)	Macrofagi, cellule dendritiche, cellule epiteliali di fegato, rene e intestino

Structure, location and specificities of mammalian TLRs

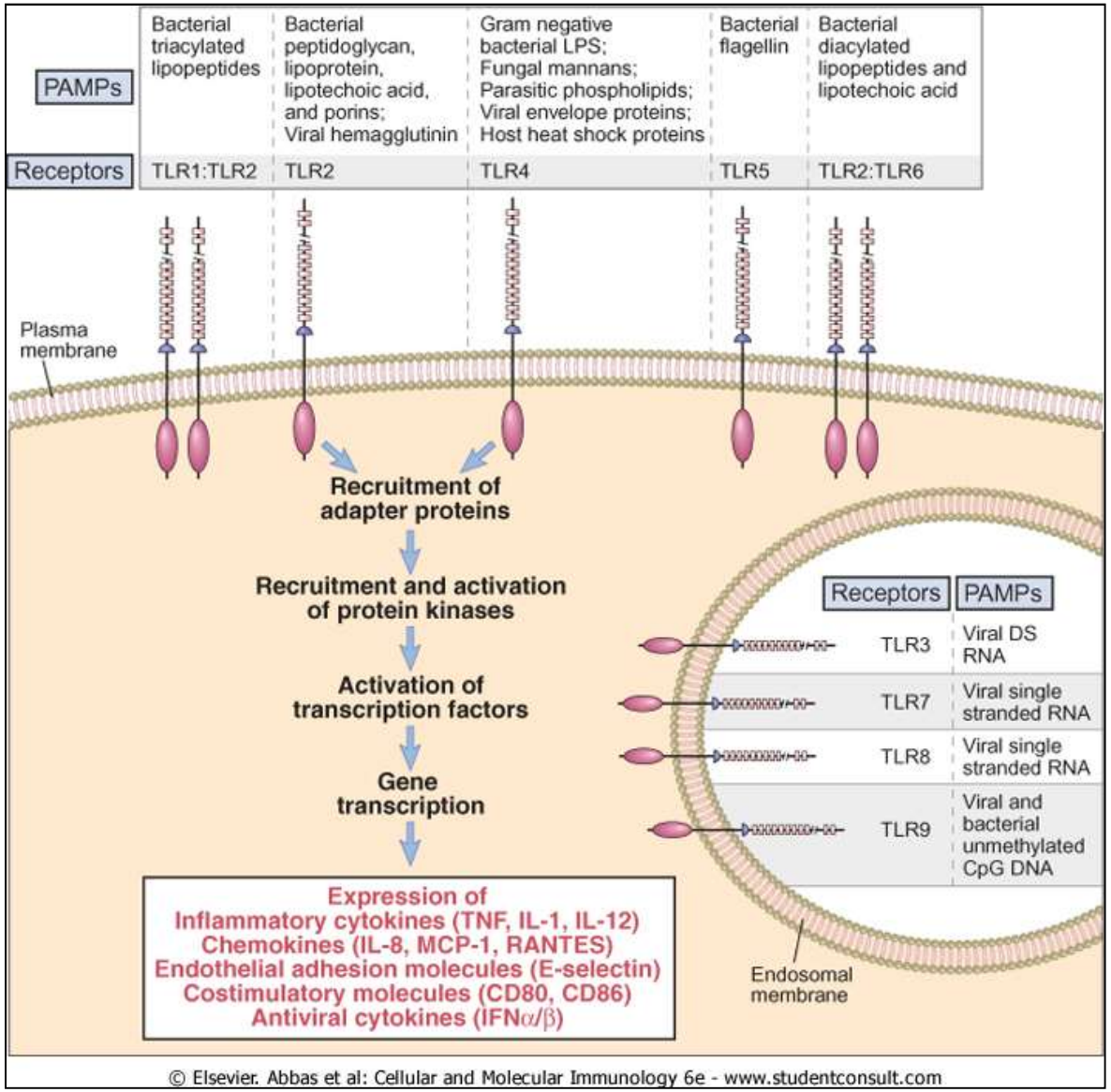
TLR 1,2,4,5 e 6 sono espressi sulla membrana citoplasmatica, dove riconoscono i PAMP presenti nell'ambienti extracellulare.

TLR 3, 7, 8 e 9 sono intracellulari, espressi prevalentemente nel RE e sulle membrane endosomiali, dove riconoscono ligandi composti da acidi nucleici.
(RNA e DNA dell'ospite non sono normalmente presenti negli endosomi)



Meccanismi di trasduzione del segnale dei TLR

Quando i TLR riconoscono i propri ligandi, essi innescano diverse cascate di trasduzione del segnale che portano all'attivazione di fattori trascrizionali i quali inducono l'espressione di geni fondamentali per le risposte infiammatoria e antivirale



Meccanismi di trasduzione del segnale dei TLRs

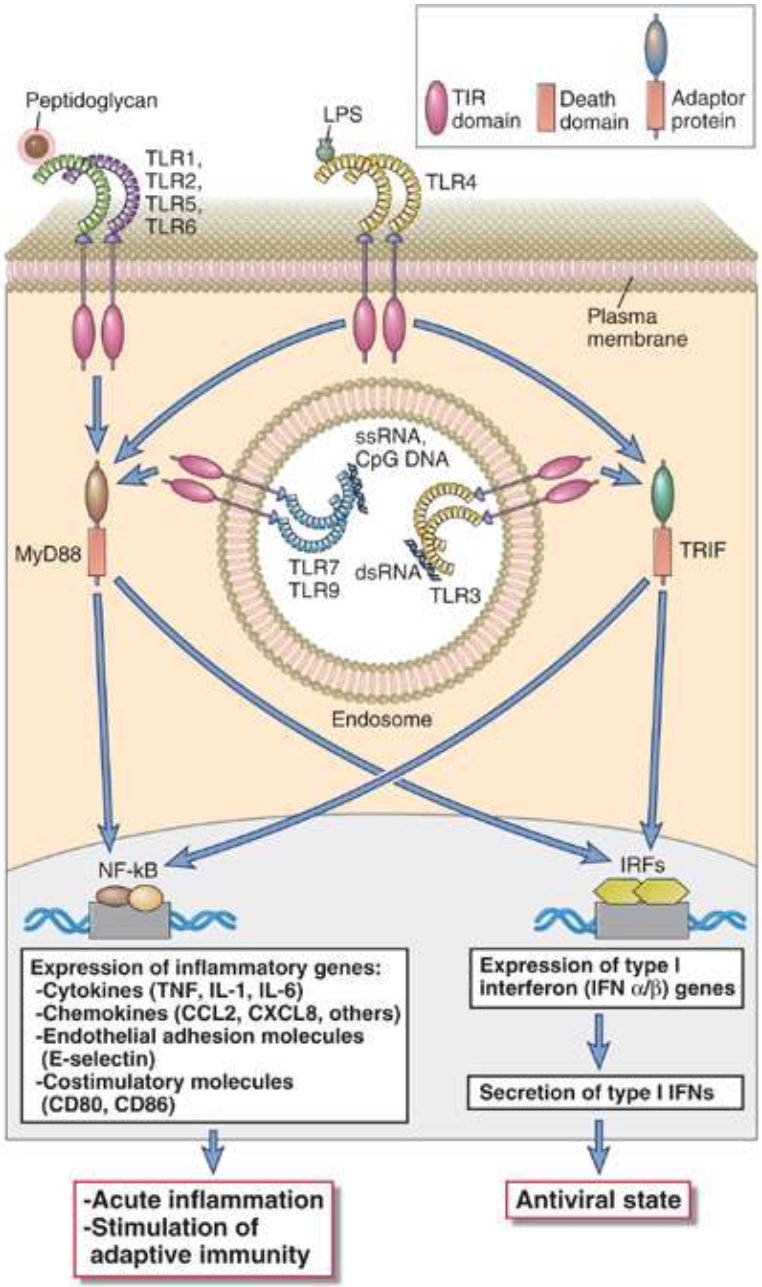
I principali fattori trascrizionali attivati dai TLR sono:

- NF-κB
- AP-1

Responsabili della maggior parte delle molecole necessarie per la risposta infiammatoria

- IRF3
- IRF7

Responsabili della risposta antivirale

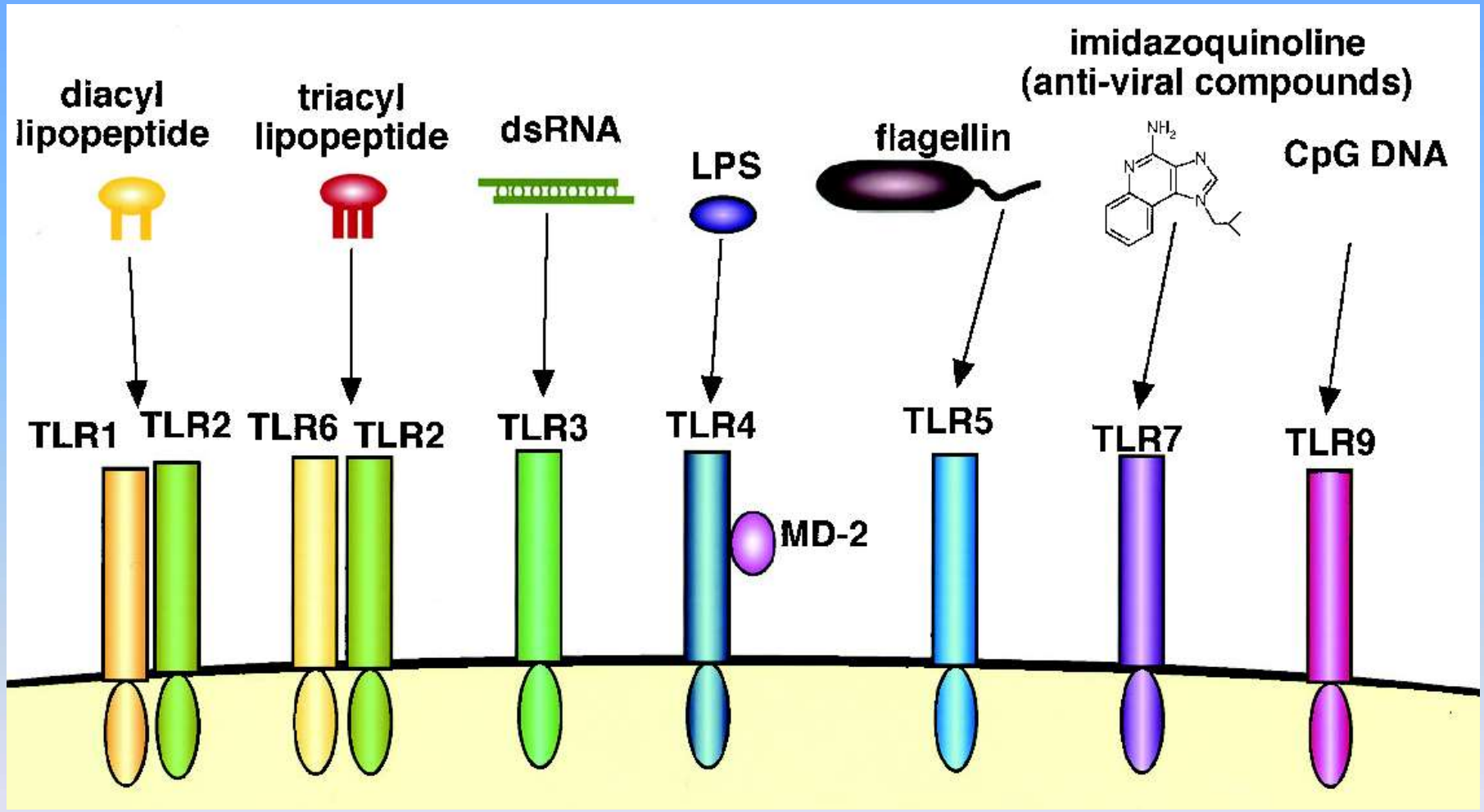


Genes regulated by NF-κB

Genes under the control of NF-κB
Inflammatory cytokines
TNF
IL-1
IL-6
IL-12
Lymphotoxin α/β
GM-CSF
IFN-β
Chemokines
IL-8
MIP-1a
MCP
RANTES
Eotaxin

Adhesion molecules
ICAM-1
VCAM-1
E-selectin
Immune effector molecules
FasL
iNOS
COX-2
β-defensins
Pro-survival molecules
Bcl-XL
A1
c-IAP1, 2

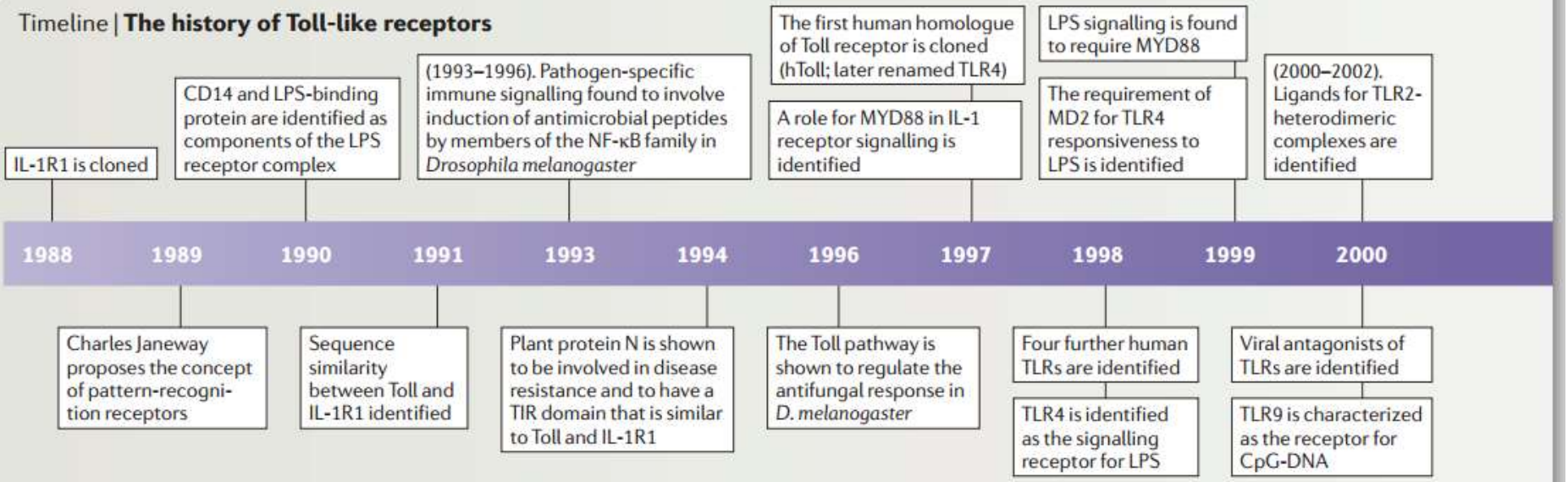
Toll-like receptors and recognition of pathogens



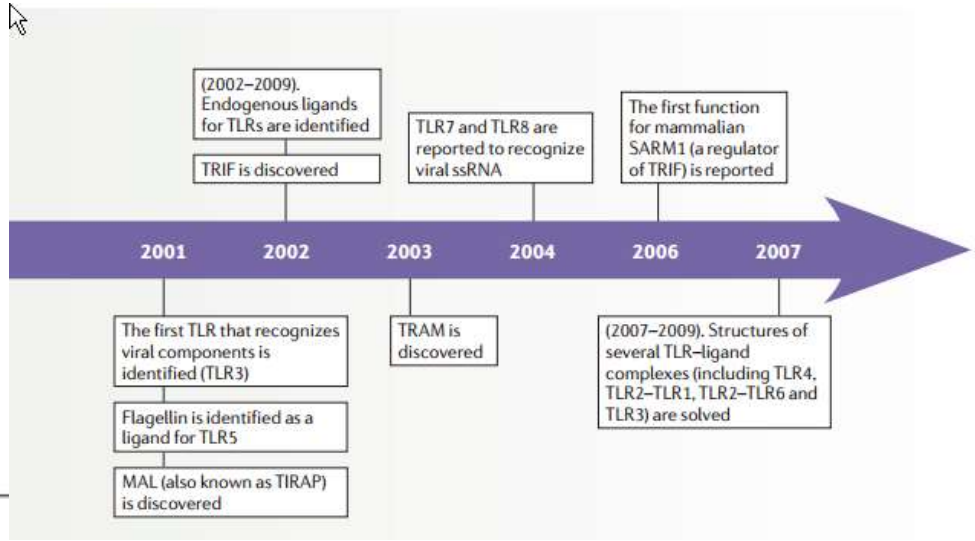
LRR extracellular domain
TIR domain inside

K. Takeda & S. Akira, Cell. Microbiol. 5: 143-53, 2003

Timeline | **The history of Toll-like receptors**



IL-1R1, interleukin-1 receptor type 1; LPS, lipopolysaccharide; MAL, MYD88-adaptor-like protein; MD2, myeloid differentiation factor 2; MYD88, myeloid differentiation primary-response protein 88; SARM1, sterile-α- and armadillo-motif-containing protein 1; ssRNA, single-stranded RNA; TIR, Toll–IL-1-resistence; TLR, Toll-like receptor; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor protein inducing IFNβ.



RECETTORI CITOSOLICI PER I PAMP E I DAMP

Oltre ai TLR di membrana, le cellule dell'immunità innata esprimono PRR che possono riconoscere i prodotti delle infezioni o del danno cellulare a ***livello citoplasmatico***.

Le 2 classi principali sono:

- Recettori di tipo **NOD**
- Recettori di tipo **RIG**

Come I TLR, essi attivano vie di trasduzione che promuovono l'infiammazione o la produzione di IFN di tipo I

Intracellular PRRs: Present in the Cytosol of Host Cells

NOD proteins or Nucleotide-binding Oligomerization Domain

- Recognize intracellular peptidoglycan-derived structures and transduce signals
- three distinct functional domains
  - carboxy-terminal ligand-recognition domain (LRD)
  - centrally located NOD
  - amino-terminal effector-binding domain (EBD)
    - CARD domains in mammals
    - Interacts and activates RIP2 inducing NF-kB and MAP-kinase pathways

NOD Proteins

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 inflamation.

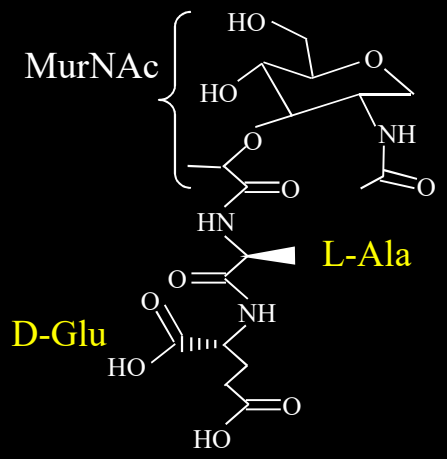
NOD 1 and NOD2 are expressed in the cytoplasm of several cell types including Mucosal epithelia cells and phagocytes, and they respond to bacterial cell wall peptoglycans.

NOD2 is particularly highly expressed in intestinal Paneth cells , where it stimulates expression of antimicrobial *defensins* in response to pathogens.

NOD1 and NOD2 are important in innate immune responses to bacterial pathogens in the gastrointestinal tract (such as *H. pylori*)

NOD1 and NOD2 motif recognition

NOD2

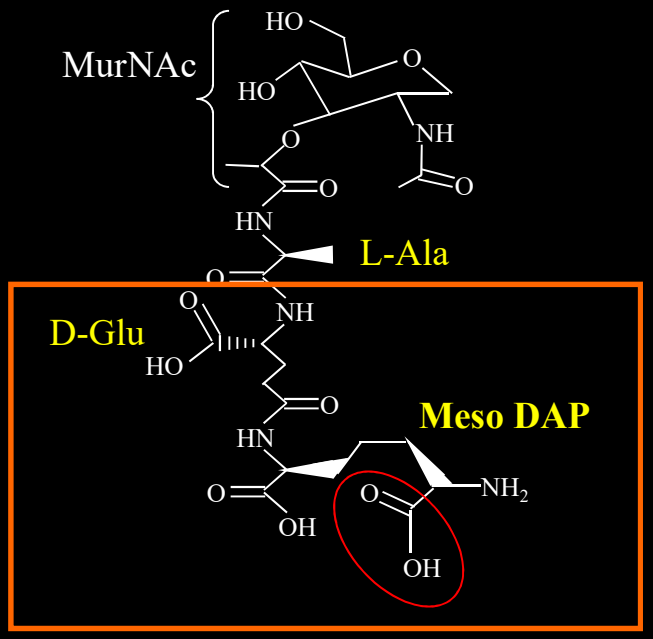


MurNAc-L-Ala-D-Glu

Minimal structure detected by NOD2

Muramyl dipeptide
(from both G- and G+ bacteria)

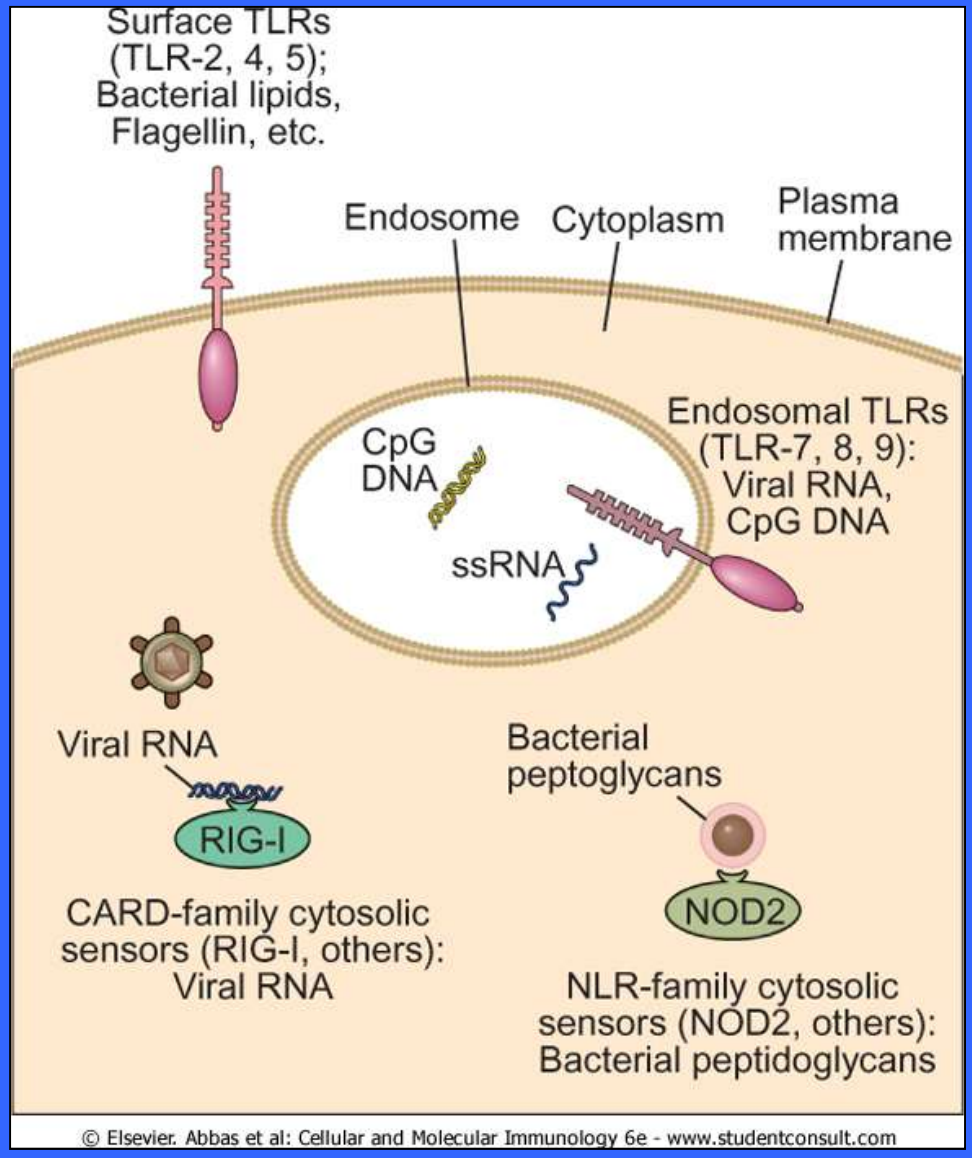
NOD1



MurNAc-L-Ala-D-Glu-mesoDAP

Minimal structure detected by NOD1

(mainly from G- bacteria)


Surface TLRs
(TLR-2, 4, 5);
Bacterial lipids,
Flagellin, etc.
Endosome
Cytoplasm
Plasma
membrane
CpG
DNA
ssRNA
Endosomal TLRs
(TLR-7, 8, 9):
Viral RNA,
CpG DNA
Viral RNA
RIG-I
CARD-family cytosolic
sensors (RIG-I, others):
Viral RNA
Bacterial
peptoglycans
NOD2
NLR-family cytosolic
sensors (NOD2, others):
Bacterial peptidoglycans
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