



**Modelli sperimentali di  
autoimmunità**

# The immune systems of mice and humans

	<i>Mus musculus</i>	<i>Homo sapiens</i>
Evolutionary divergence		~65 to 80 million years
Genome		~3.1 billion base pairs ~30,000 protein-coding genes On average, 85% of protein-coding sequences are identical Only 50% of non-protein-coding regions are similar
Size	Weight: ~20 to 30 g; length: 7.5 to 10 cm	Weight: ~62 kg; height: 163 cm (females), 176 cm (males)
Some differences in the innate immune system	TLR7 on pDCs, myeloid DCs (150)	TLR7 only on pDCs and B cells (150)
	TLR9 on myeloid cells, pDCs and B cells (150)	TLR9 only on pDCs and B cells (150)
	TLR10: pseudogene (150)	TLR10: widely expressed (150)
Some differences in the adaptive immune system	MyD88 knockout mice: impaired immunity to bacteria, viruses, and parasites (150)	MyD88 mutant humans: susceptible to invasive pathogenic bacterial infections, but normal immunity to many bacteria, viruses, fungi, and parasites (151)
	Ig subclasses: IgA, IgD, IgE, IgG1, IgG2a*, IgG2b, IgG3, IgM (152) *absent in C57BL/6, /10, SJL, and NOD mice, which have IgG2c	Ig subclasses: IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM (152)
	Ig CDR-H3 region: shorter, less diverse (153)	Ig CDR-H3 region: longer, more diverse (153)
	Effect of $\gamma_c$ deficiency: loss of T, NK, and B cells (154)	Effect of $\gamma_c$ deficiency: loss of T, NK, but normal B cells (154)
	Effect of IL-7R deficiency: blocks T and B cell development (154, 155)	Effect of IL-7R deficiency: only blocks T cell development (154, 155)

# Modelli animali per lo studio di patologie autoimmuni

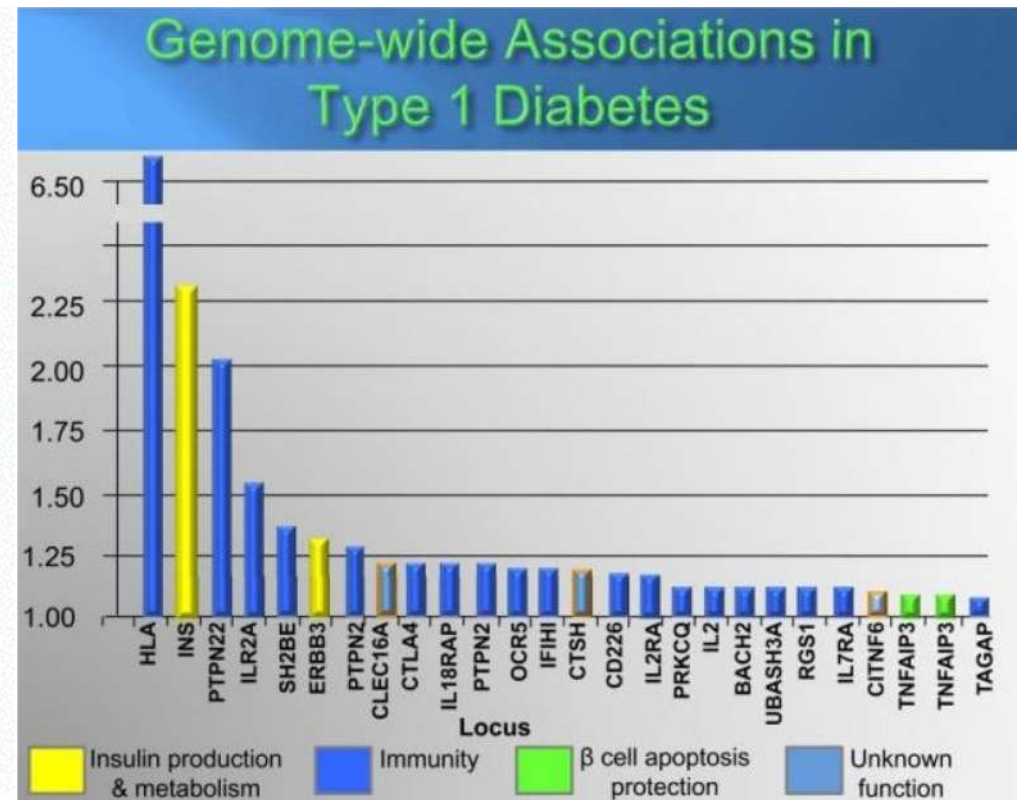
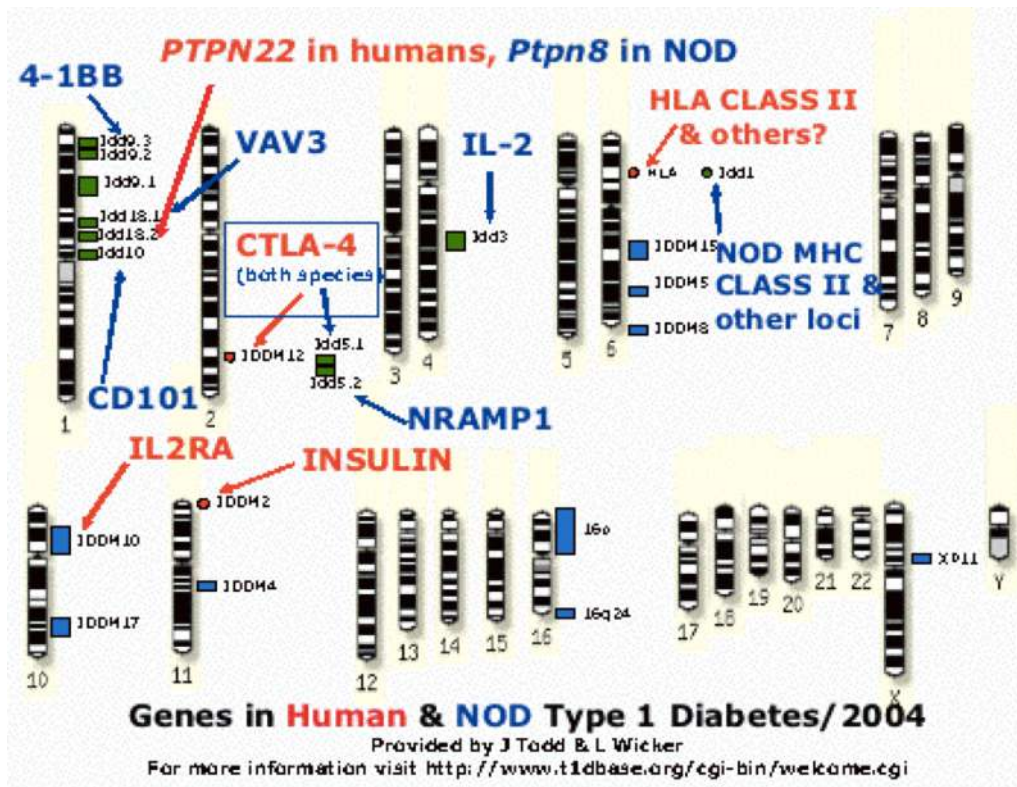


## 1. Modelli poligenici con sviluppo spontaneo di autoimmunità

- La progenie  $F_1$  da incroci di **topi NZB x NZW** sviluppa una **patologia tipo lupus (LES)** con maggiore frequenza nelle femmine. Specifiche infezioni da batteri, virus e parassiti conferiscono protezione.
- Sono modelli per il **LES** anche i ceppi murini “**lpr**” (lymphoproliferation) e “**gld**” (generalized lymphoproliferative disease) omozigoti per mutazioni di Fas e FasL, rispettivamente.
- **Topi NOD**, modello per il **diabete di tipo 1 (T1D)**, hanno un background genetico H-2g7 (omologia tra I-Ag7 ed il DQ8) e sviluppano spontaneamente il diabete in condizioni “germ-free”. In questo ceppo, sono stati identificati più di 20 loci genetici di suscettibilità (*idd* 1-20). La patologia è più frequente nelle femmine. Nei topi NOD, infezione da specifici batteri, virus o parassiti conferiscono protezione dallo sviluppo spontaneo della patologia autoimmune.

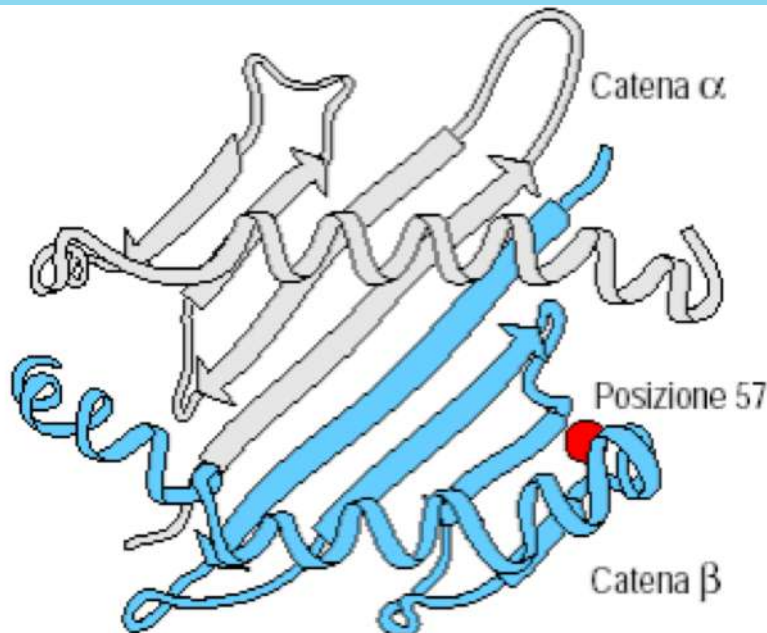


**Topi NOD (nonobese diabetic mice): modello sperimentale per lo studio del TD1 (diabete di tipo I)**



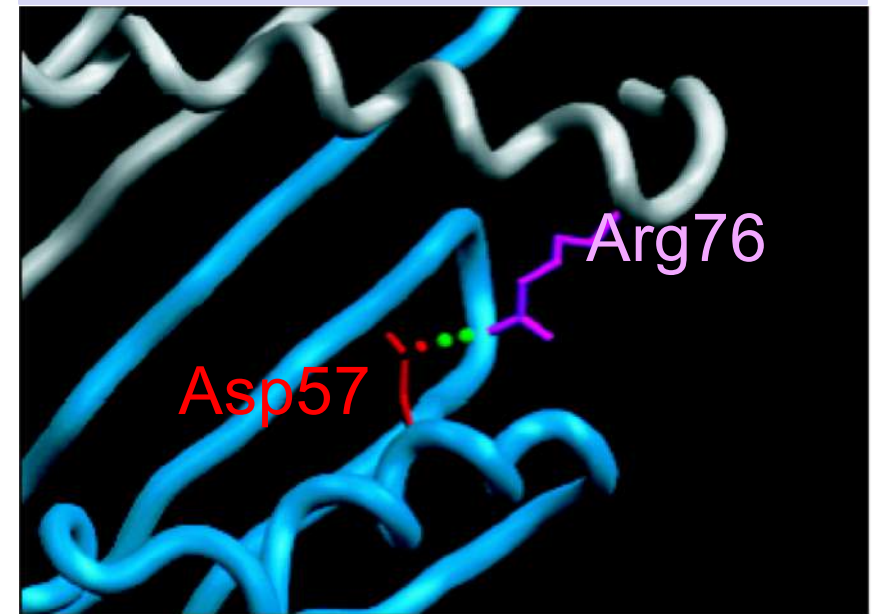
**Nell'uomo, la suscettibilità al diabete di tipo 1 (T1D) associa con la variante Val57 (Ser o Ala) nella catena  $\beta$  del DQ la variante Asp57 è invece protettiva**

La posizione 57 della catena DQ $\beta$  controlla la suscettibilità al T1D

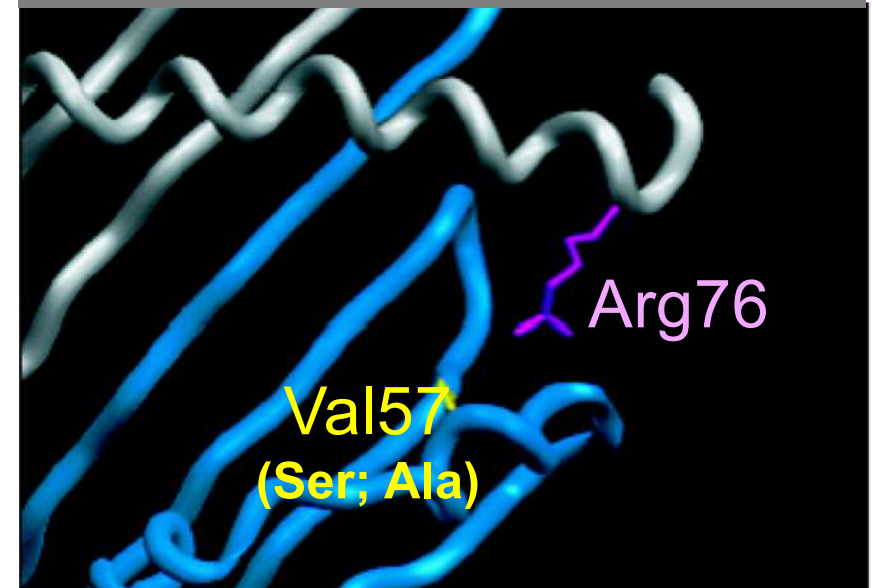


I topi NOD (aplotipo H-2g7) esprimono la molecola I-Ag7 (ortologo del HLA-DQ8 e DQ2) che presenta una Ser in posizione 57 nella catena I-A  $\beta$ ; (questo è uno dei molteplici fattori di suscettibilità al diabete nei topi NOD)

Associato con la resistenza al T1D



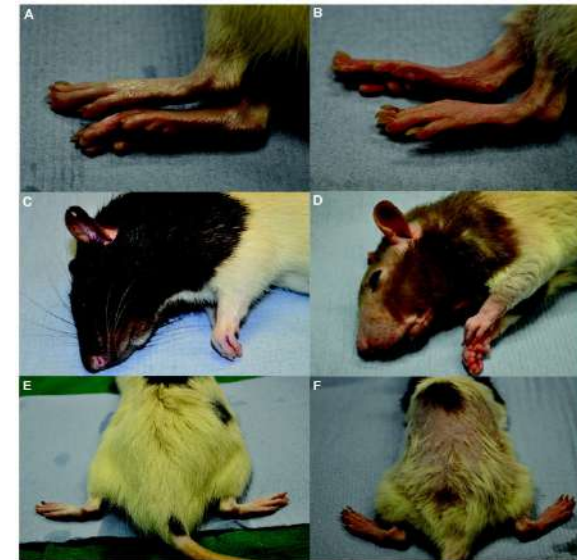
Associato con la suscettibilità al T1D



# Modelli animali per lo studio di patologie autoimmuni

## 2. Modelli transgenici per MHC (umanizzati)

- Ratti transgenici per l'HLA-B27 (esprimenti più di 40 copie di HLA-B27 e 30 di  $\beta$ 2-microglobulina umana) sviluppano, in condizioni "non germ-free", una patologia infiammatoria simile alle **Spondiloartropatie** umane (il cui prototipo è la Spondilite Anchilosante).
- Incrociando topi transgenici per l'**HLA-DQ8** con topi transgenici per B7.1 sotto il controllo di RIP (promotore per l'insulina di ratto) si crea una progenie che sviluppa spontaneamente il **diabete**.



## 3. Modelli transgenici per TCR

I topi transgenici per un TCR (BDC2.5) derivato da un clone T CD4+ diabetogenico reattivo verso un antigene espresso dalle cellule  $\beta$  pancreatiche possono, in particolari condizioni, sviluppare **diabete**.

# Modelli animali per lo studio di patologie autoimmuni

## 4. Modelli knockout

Topi knockout per il gene dell'IL-2 (o per IL-10), per la catena  $\alpha$  del recettore per IL-2 sviluppano spontaneamente delle patologie autoimmuni sistemiche accompagnate da infiammazione cronica dell'intestino (**IBD**) il cui quadro immunopatologico si sovrappone al **morbo di Crohn** dell'uomo.

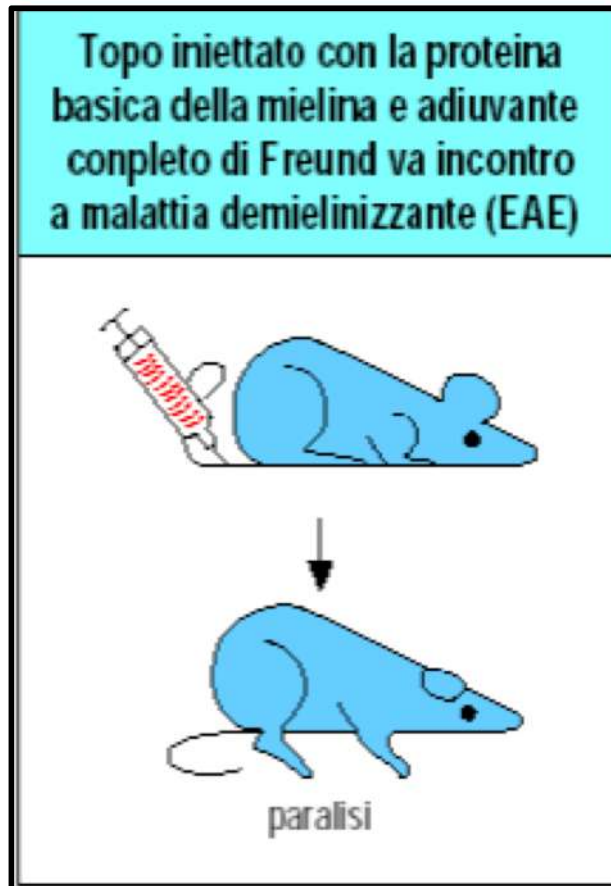
## 5. Modelli di autoimmunità indotta

- ❖ L'immunizzazione con collagene di tipo II (autologo o eterologo) in **adiuvante completo di Freund**, provoca in alcuni ceppi murini una patologia infiammatoria delle articolazioni definita **Artrite indotta da Collagene (CIA)** che presenta un'istopatologia articolare che ricalca quella dell'**artrite reumatoide** nell'uomo.
- ❖ **L'encefalomielite autoimmune sperimentale (EAE)** può essere indotta in topi e ratti mediante immunizzazione con midollo spinale o con specifici antigeni mielinici (MBP; PLP) in **adiuvante completo di Freund**. Modelli per la **sclerosi multipla**.

**L'iniezione di midollo spinale in adiuvante completo di Freund induce l'encefalomielite autoimmune sperimentale (EAE) in ceppi di topo e ratto**



Modello di studio per la sclerosi multipla

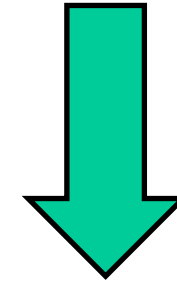


L'EAE è provocata anche da immunizzazione con MBP, PLP, MOG o MAG (antigeni mielinici) **in adiuvante completo di Freund**



## Adiuvanti che stimolano la risposta immunitaria

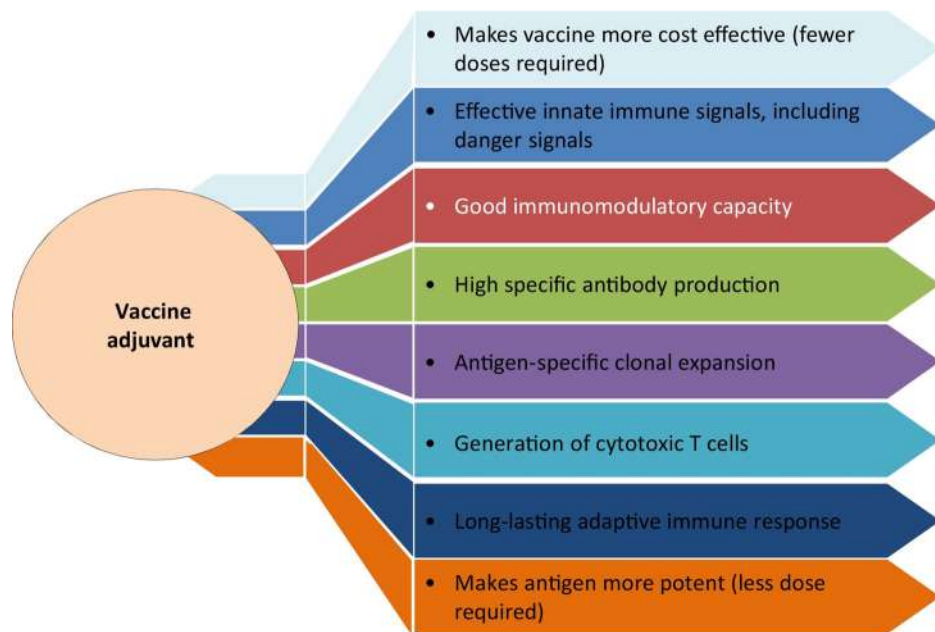
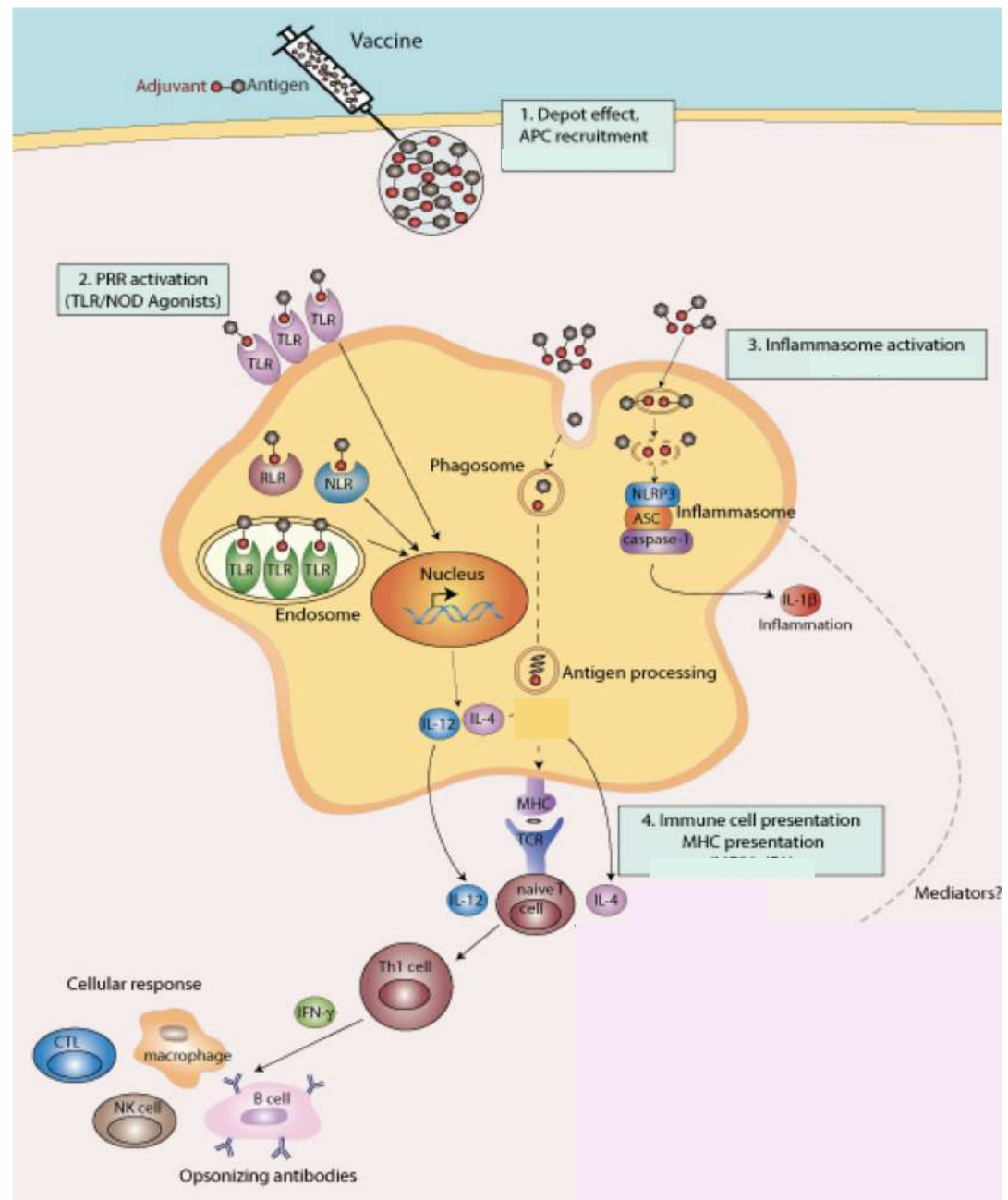
Nome dell'adiuvante	Composizione	Meccanismo d'azione
Adiuvante incompleto di Freund	Emulsione di olio e acqua	Ritarda il rilascio dell'antigene; aiuta la captazione da parte dei macrofagi e DC
Adiuvante completo di Freund	Emulsione di olio e acqua contenente micobatteri morti	Ritarda il rilascio dell'antigene; aiuta la captazione da parte dei macrofagi; induce co-stimolatori dei macrofagi DC
Adiuvante di Freund con MDP	Emulsione di olio e acqua con muramidilpeptide (MDP), un costituente dei micobatteri	Simile a quelle dell'adiuvante completo di Freund
Alum (idrossido di alluminio)	Gel di idrossido di alluminio	Ritarda il rilascio dell'antigene; aiuta la captazione da parte dei macrofagi
Alum plus <i>Bordetella pertussis</i>	Gel di idrossido di alluminio con <i>B.pertussis</i> ucciso	Ritarda il rilascio dell'antigene; aiuta la captazione da parte dei macrofagi; induce co-stimolatori dei macrofagi
Complessi immunitari stimolatori (ISCOMs)	Matrice di Quil A che contiene proteine virali	Rilascia gli antigeni nel citosol; stimola la citotossicità delle cellule T



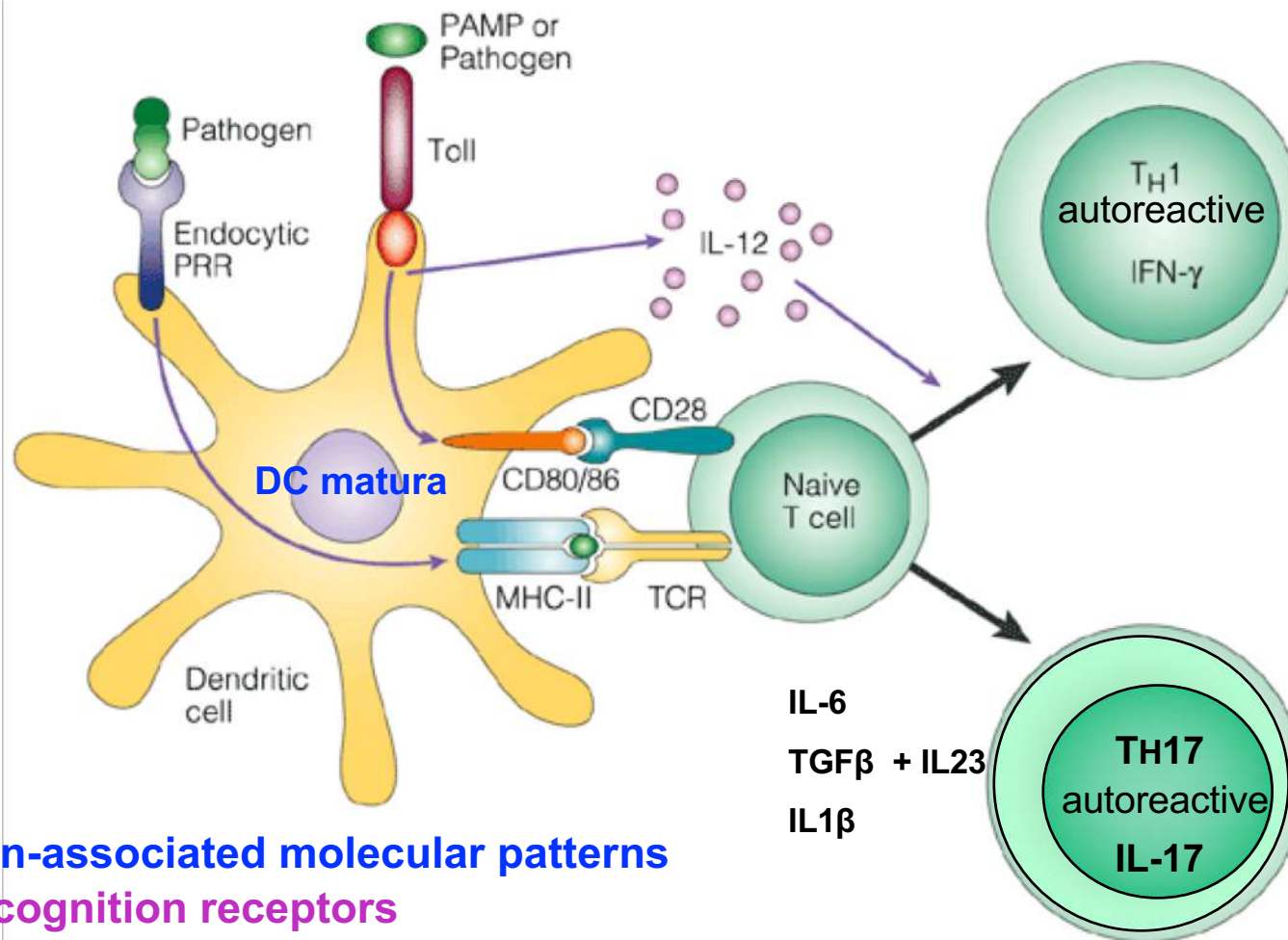
**Stimola la risposta innata nel sito di esposizione all'antigene favorendo la maturazione delle DC**

Stimola l'attivazione dell'inflammasoma

# Adjuvants are essential for enhancing and directing the adaptive immune response setting the inflammatory conditions



## Il ruolo dei patogeni nella maturazione delle cellule dell'immunità naturale e nell'attivazione delle cellule dell'immunità acquisita

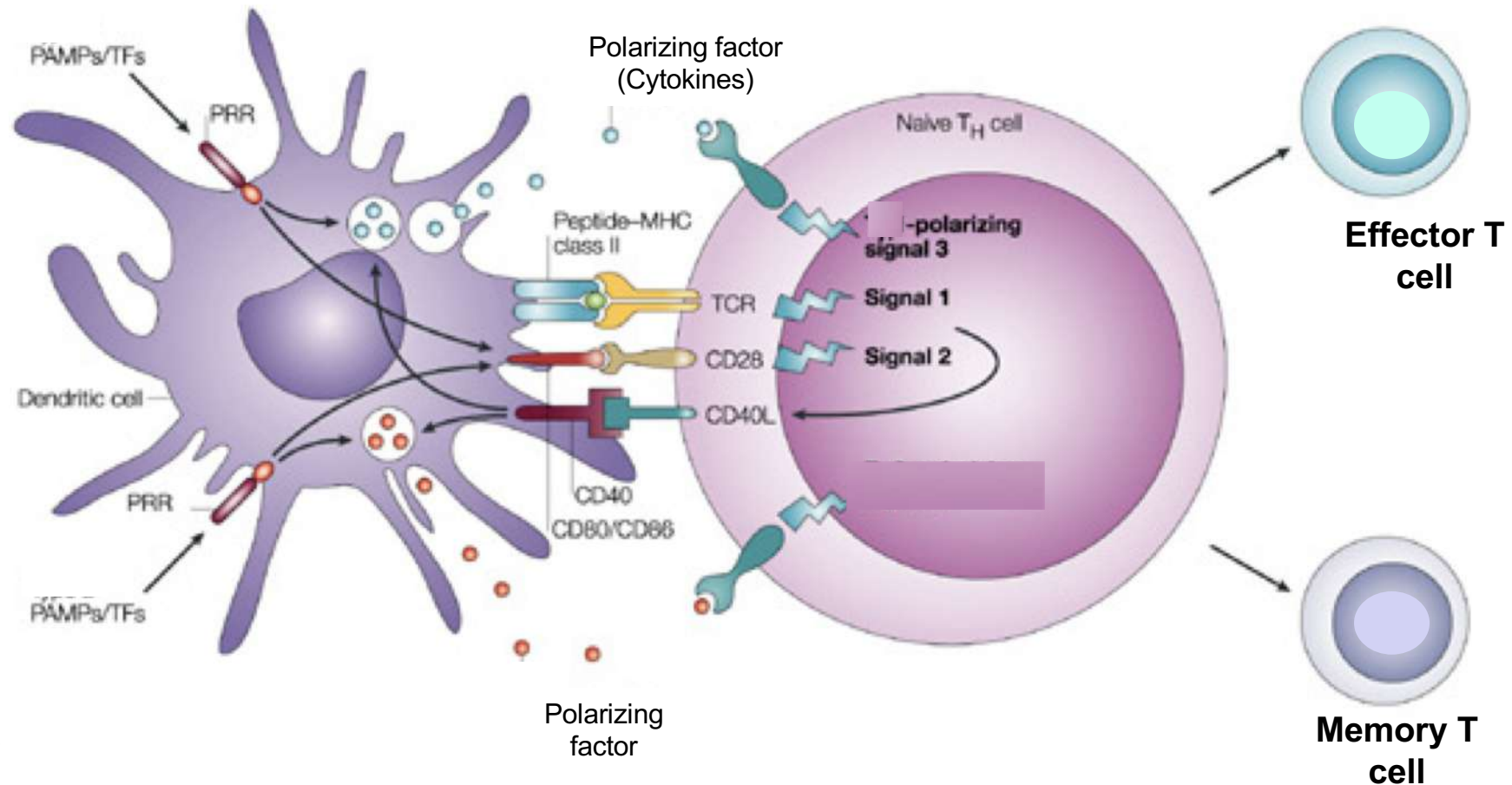


**PAMPs= pathogen-associated molecular patterns**

**PRRs=pattern-recognition receptors**

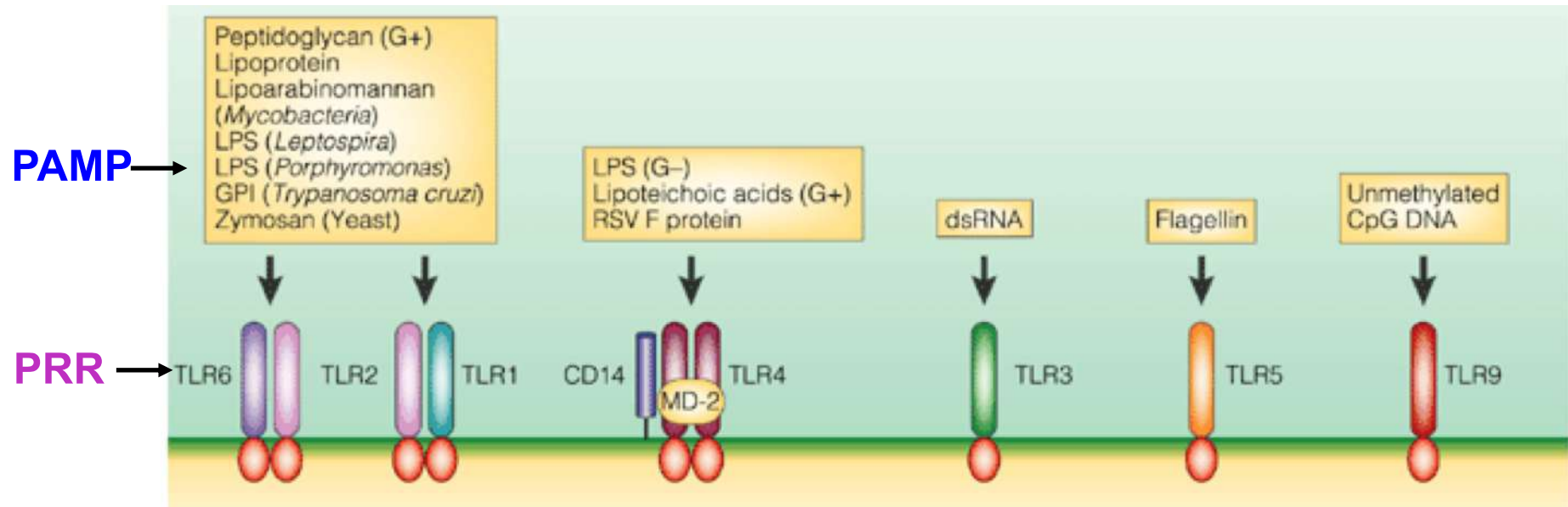
**Role of TLRs in the control of adaptive immunity.** TLRs sense the presence of infection through recognition of PAMPs (pathogen-associated molecular patterns). Recognition of PAMPs by Toll-like receptors (TLRs) expressed on antigen-presenting cells (APC), such as dendritic cells, upregulates cell-surface expression of co-stimulatory (CD80 and CD86) molecules and major histocompatibility complex class II (MHC II) molecules. TLRs also induce expression of cytokines, such as interleukin-12 (IL)-12, and chemokines and their receptors, and trigger many other events associated with **dendritic cell maturation**. Induction of CD80/86 on APCs by TLRs leads to the activation of T cells specific for pathogens that trigger TLR signalling. IL-12 induced by TLRs also contributes to the differentiation of activated T cells into T helper (TH)1 effector cells. It is not yet known whether TLRs have any role in the induction of TH2 responses. IFN-; interferon-; PRR, pattern-recognition receptor.

# T-cell stimulation and T helper polarization require three dendritic cell-derived signal



**Signal 1** is the antigen-specific signal that is mediated through T-cell receptor (TCR) triggering by MHC class-II-associated peptides processed from pathogens after internalization through specialized pattern recognition receptors (PRRs). **Signal 2** is the costimulatory signal, mainly mediated by triggering of CD28 by CD80 and CD86 that are expressed by dendritic cells (DCs) after ligation of PRRs, such as Toll-like receptors (TLRs) that are specialized to sense infection through recognition of pathogen-associated molecular patterns (PAMPs) or inflammatory tissue factors (TFs). **Signal 3** is the polarizing signal that is mediated by various soluble or membrane-bound factors. The nature of signal 3 depends on the activation of particular PRRs by PAMPs or TFs.

## Come le cellule dell'immunità innata “sentono” la presenza dei patogeni



**PAMP= profili molecolari associati ai patogeni (pathogen-associated molecular patterns)**

**PRR= recettori per profili molecolari (pattern-recognition receptors)**

**FAMIGLIE di PRR : Toll-like receptors (TLR)**

**Retinoic acid-inducible gene-I like receptors (RLR)**

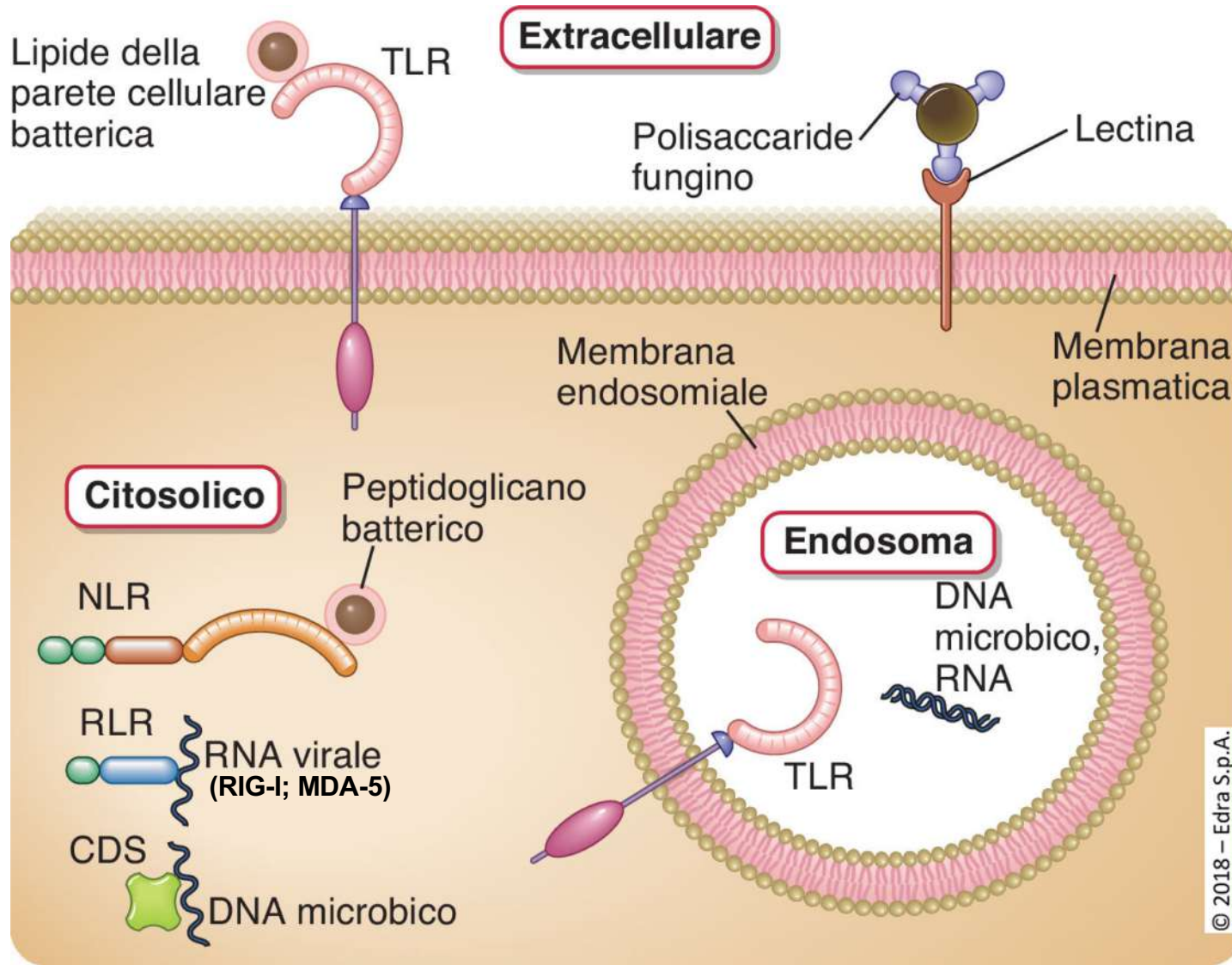
**Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR)**

**Sensori di DNA citosolico (AIM2)**

**C-type lectin receptor (CLRs)**

**Ligand specificities of TLRs.** Toll-like receptors (TLRs) recognize a variety of pathogen-associated molecular patterns (PAMPs). Recognition of lipopolysaccharide (LPS) by TLR4 is aided by two accessory proteins: CD14 and MD-2. TLR2 recognizes a broad range of structurally unrelated ligands and functions in combination with several (but not all) other TLRs, including TLR1 and TLR6. TLR3 is involved in recognition of double-stranded (dsRNA). TLR5 is specific for bacterial flagellin, whereas TLR9 is a receptor for unmethylated CpG motifs, which are abundant in bacterial DNA. G+, Gram-positive; G-, Gram negative; GPI, glycosylphosphatidylinositol; RSV, respiratory syncytial virus.

# Localizzazione cellulare dei PRR



Espressi da tutte le cellule, ma la varietà maggiore di PRR è espressa dai fagociti (macrofagi, neutrofili), DC e cellule epiteliali

- Retinoic acid-inducible gene-I like receptors (RLR)
- Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR)
- Cytosolic DNA sensor (CDS)

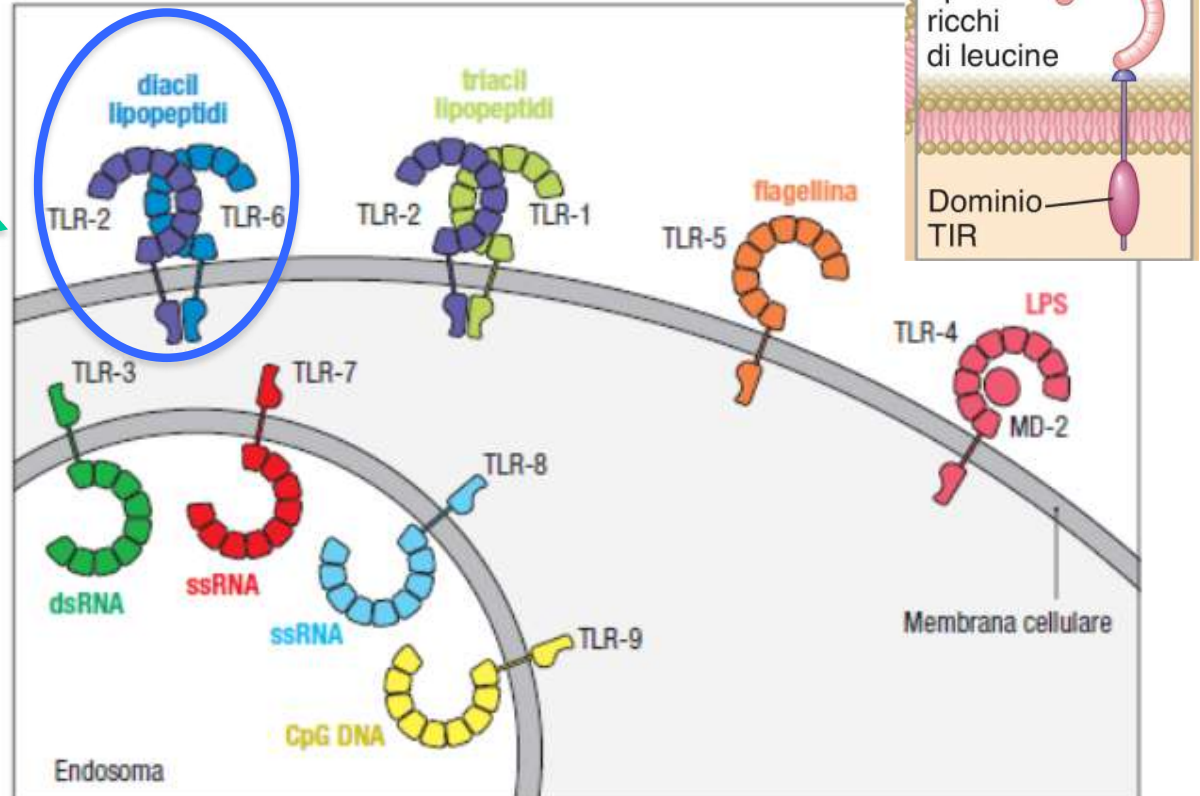
# Localizzazione dei TLR

Espressi da:

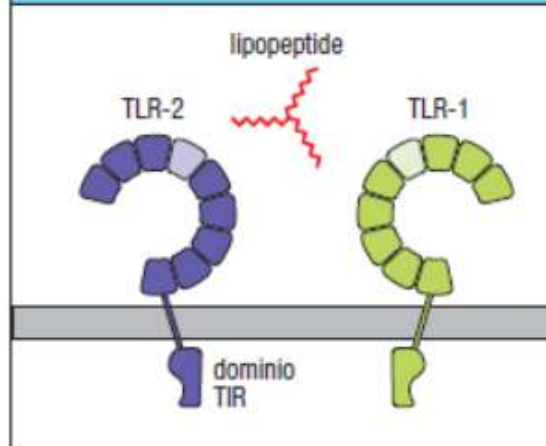
- DC
- macrofagi
- cellule NK
- linfociti B
- Specifici subset di linfociti T
- Cellule non immunitarie
- cellule epiteliali
- fibroblasti

La loro espressione cambia velocemente in presenza di citochine, patogeni e stimoli ambientali

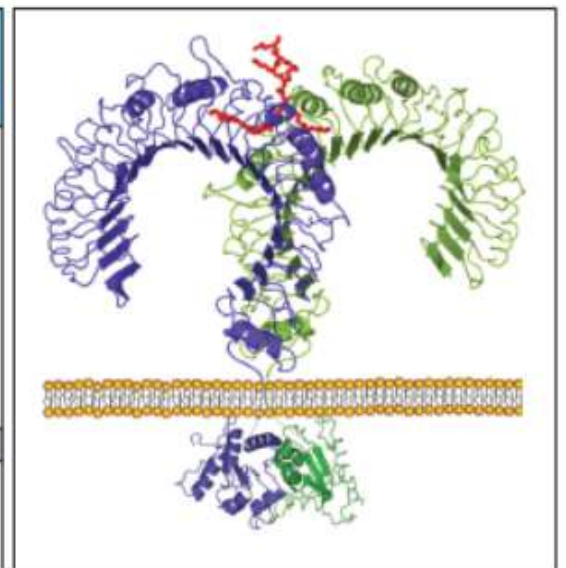
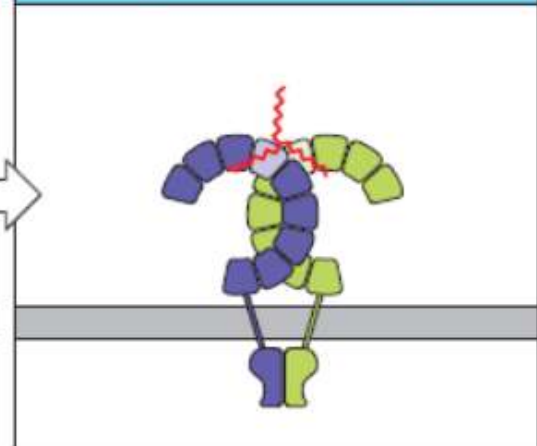
**dimerizzazione**



Le superfici convesse di TLR-1 e TLR-2 hanno siti di legame per le catene laterali lipidiche dei lipopeptidi triacilici

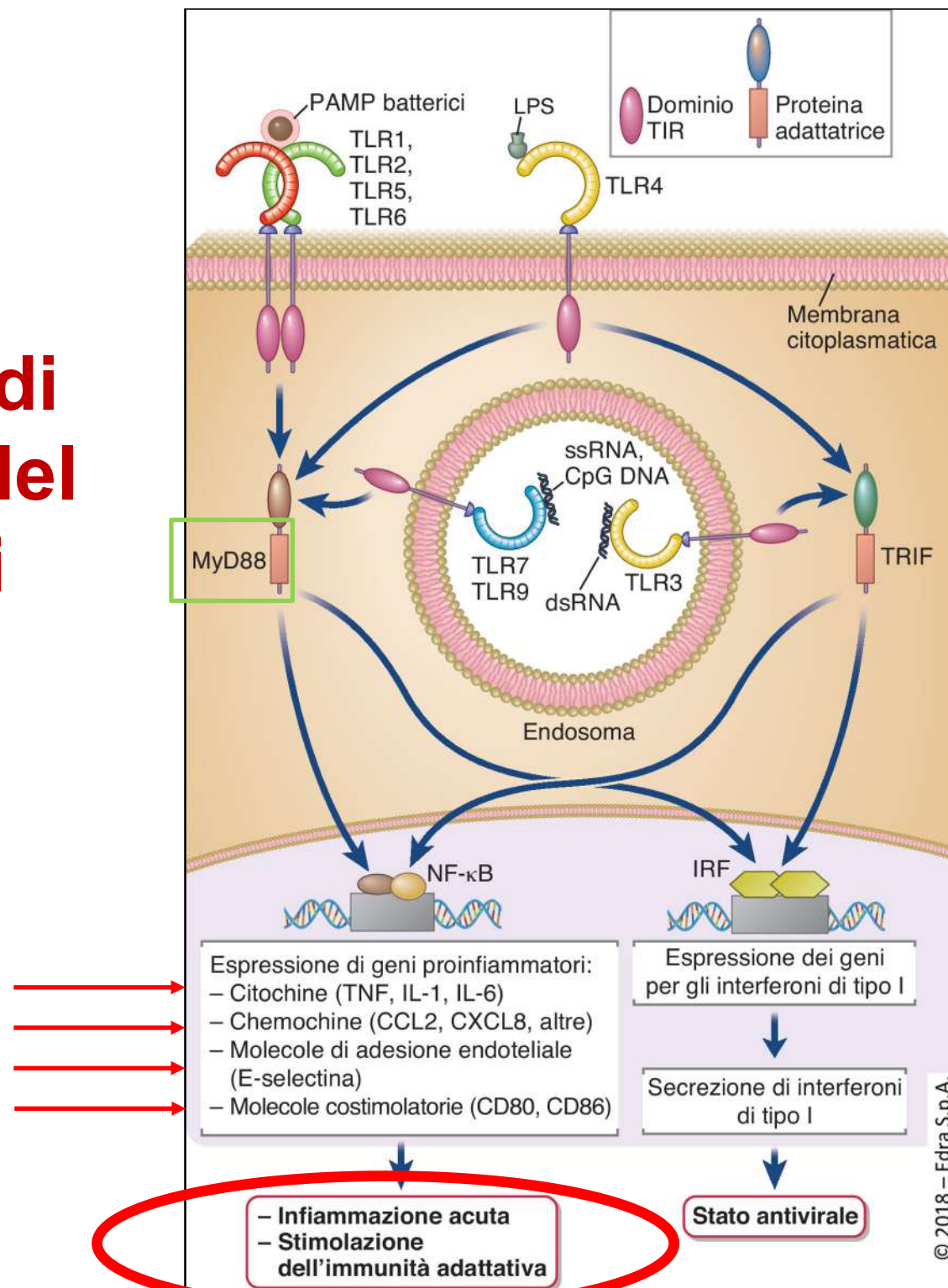


Il legame di ogni TLR allo stesso lipopeptide induce la dimerizzazione, portando i loro domini citoplasmatici TIR in stretta prossimità



Riconoscimento diretto dei PAMPs da parte di TLR1 e TLR2

# Meccanismi di trasduzione del segnale dei TLR





**Health**



**Pathogens  
and  
commensals**

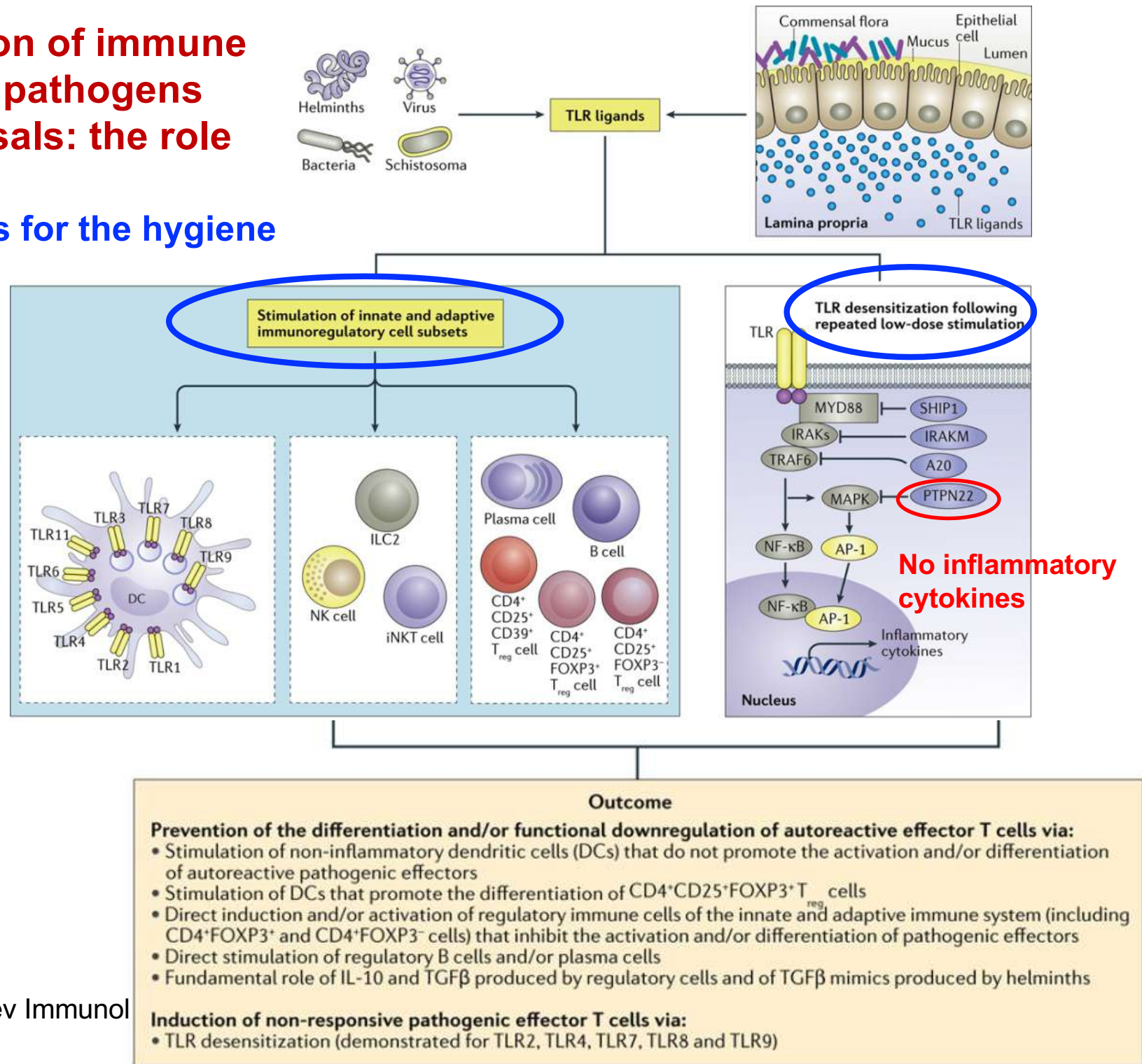


**Autoimmunity  
Autoinflammation**



# The stimulation of immune regulation by pathogens and commensals: the role of TLRs

## Molecular basis for the hygiene hypothesis



The protective effect of pathogens and commensals on autoimmune diseases is triggered by the presence of pathogen-associated molecular patterns (PAMPs), including Toll-like receptor (TLR) ligands. PAMPs can have indirect or direct effects on antigen-presenting cells (APCs) and on various cells at the interface of innate and adaptive immunity, such as natural killer (NK) cells, invariant NKT (iNKT) cells and innate lymphoid cells (ILCs), in particular group 2 ILCs (ILC2s). These cell populations, through their capacity (via cell–cell contact and/or cytokine production) to interact with the adaptive immune system efficiently control pathogenic autoimmune responses. In addition, CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> or FOXP3<sup>-</sup> regulatory T (T<sub>reg</sub>) cells are induced by pathogens and commensals and potentially regulatory B cells and plasma cells.

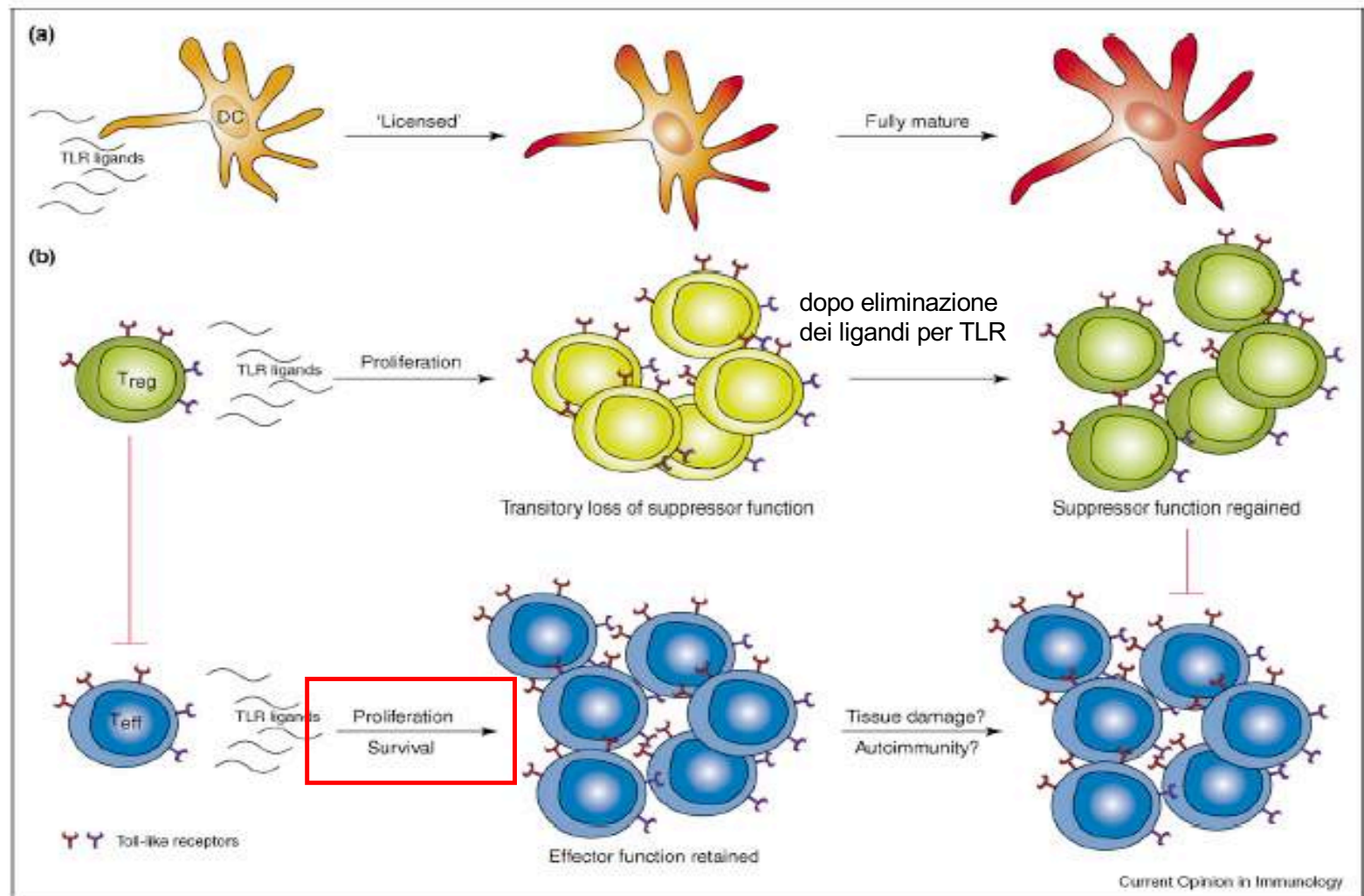
Concerning cytokines, animal models outline the role of IL-10 and/or transforming growth factor- $\beta$  (TGF $\beta$ ). APCs and regulatory lymphocytes are the likely source of these cytokines. In addition, some parasites may produce mimics of TGF $\beta$ .

The figure also addresses putative mechanisms that link TLR signalling and triggering of a 'non-response'. **The interaction of TLRs with their ligands delivered at a low dose and repeatedly results in desensitization.** As the response of TLRs to conventional stimulation is an inflammatory response, desensitization leads to a lack of inflammation, which may protect against autoimmunity. The desensitization effect has been shown for TLR2, TLR4, TLR7, TLR8 and TLR9. There is concordant data showing that, from a signalling point of view, desensitization involves phosphatases including SHIP1, IRAKM or **PTPN22**, but also molecules of unclear function, such as A20. Known for many years as an inhibitor of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and apoptosis, A20 also has ubiquitin-regulatory properties and negatively regulates various inflammatory signalling cascades.

# Toll like receptors e autoimmunità

Anche i linfociti T e B esprimono i TLRs che possono mediare una efficiente costimolazione

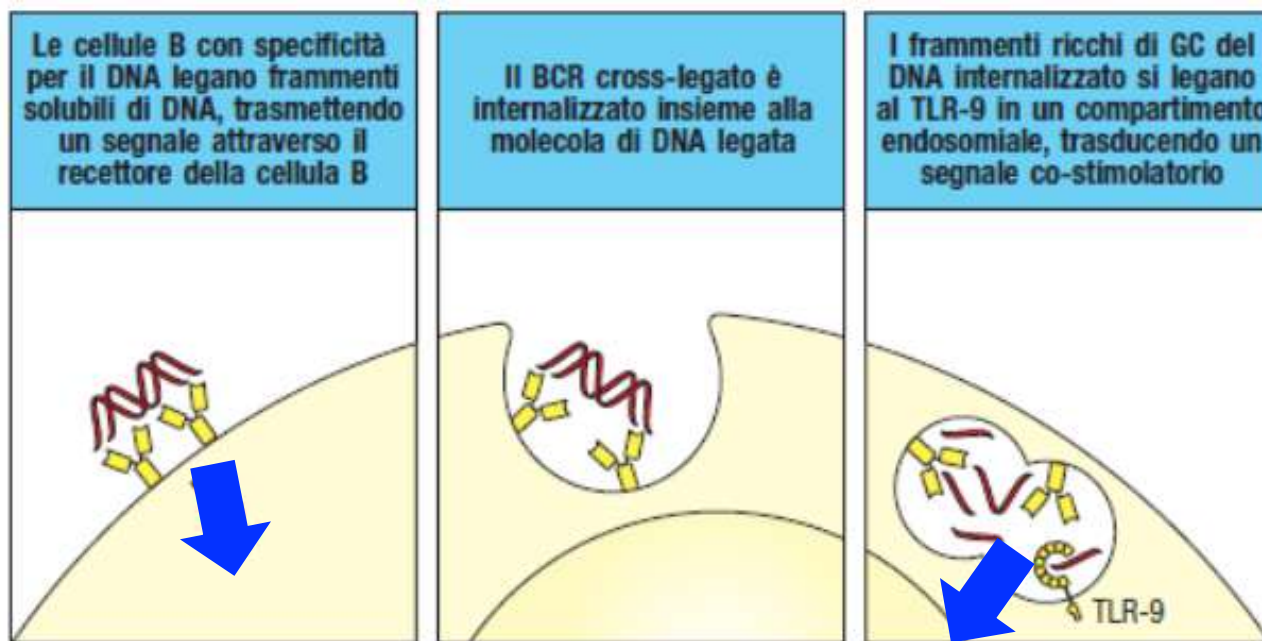
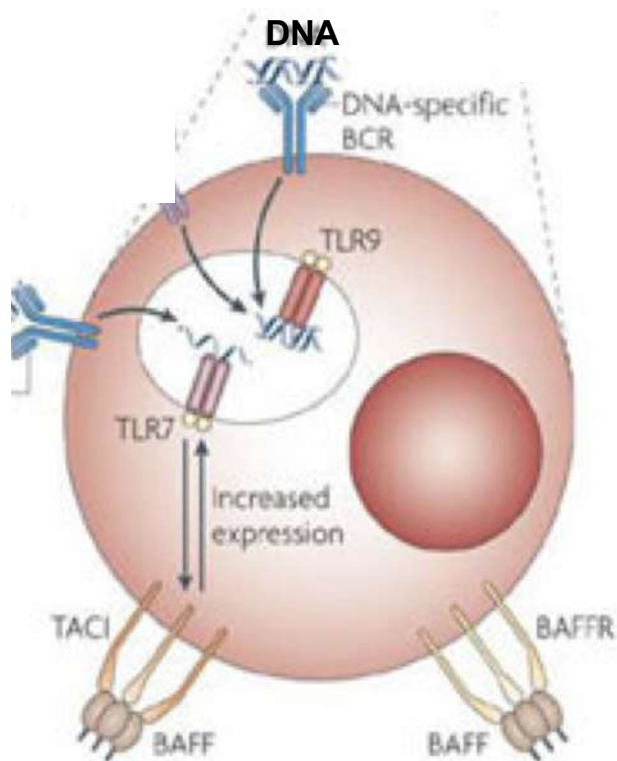
Il coinvolgimento dei TLRs nelle patologie autoimmune rende i loro pathways potenziali target di nuovi trattamenti terapeutici



Possible role of TLR signaling on T cell driven autoimmunity. (a) TLR stimulation of dendritic cells (DC) licenses them for full maturation which occurs en route to and within lymphoid tissue. (b) TLR stimulation of regulatory T cells (Treg) results in their proliferation and a transient loss of suppressor function and Foxp3 expression, which is regained following removal of the TLR stimulus. This expanded population of Treg cells may display enhanced suppressive activity due to increased cell numbers. TLR stimulation of effector T cells (Teff) results in rapid polyclonal proliferation and upregulation of anti-apoptotic factors. In the presence of TCR stimulation and polarizing cytokines, Teff cells maintain their effector function. Expansion of self-specific T cells, and the lowering of the required strength of signal by TLR-'costimulation', may lead to increased tissue damage and autoimmunity.

# I TLRs riconoscendo antigeni self forniscono costimolazione ai linfociti B autoreattivi attivandoli in assenza di “help” da parte dei linfociti T

## L'esempio del TLR9 e dei motivi CpG non metilati del DNA



**Primo segnale via BCR**

**Secondo segnale via TLR9**

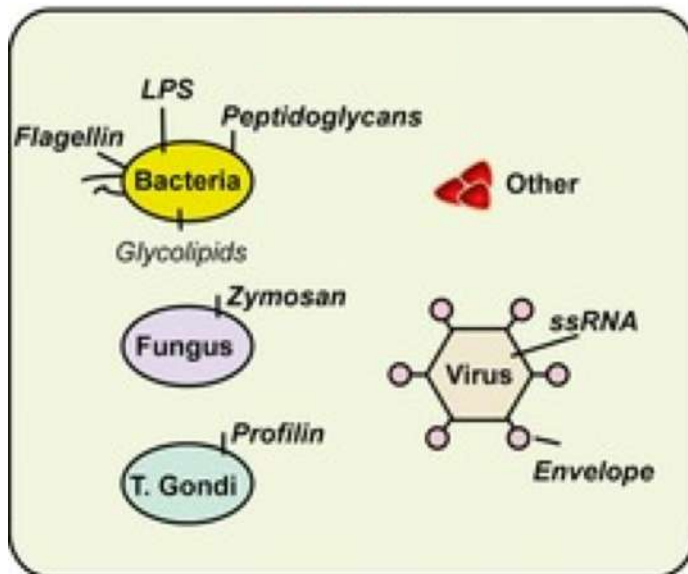
### Situazione documentata nel LES

Over-espressione del TLR9 nei linfociti B di pazienti con LES

Manifestazioni lupus-simili indotte dalla procainamide con produzione di autoanticorpi anti-DNA

# PAMPs

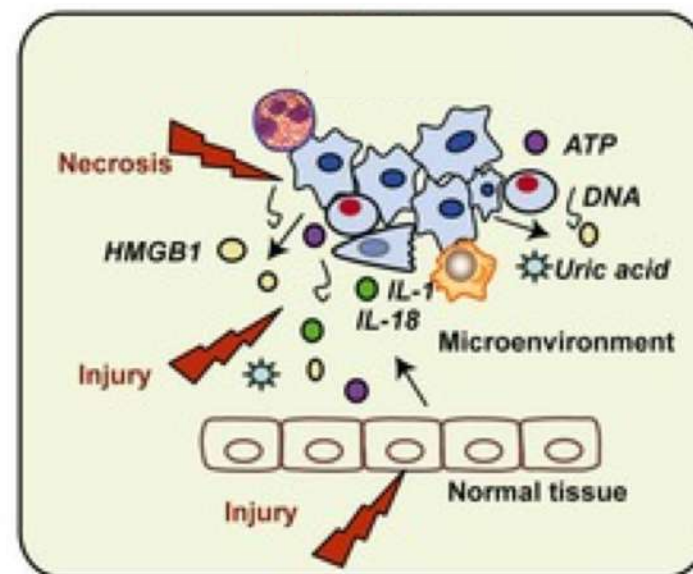
Pathogen Associated molecular patterns



<b>Acidi nucleici</b>	ssRNA dsRNA CpG	Virus Virus Virus, batteri
<b>Proteine</b>	Pilina Flagellina	Batteri Batteri
<b>Lipidi della parete cellulare</b>	LPS acido lipoteicoico	Batteri Gram- Batteri Gram+
<b>Carboidrati</b>	Mannano Glucani	Funghi, batteri Funghi

# DAMPs

Damage(danger) Associated Molecular patterns



<b>Proteine nucleari</b>	HMGB1
<b>Proteine indotte da stress cellulare</b>	HSP
<b>Cristalli</b>	Urato monosodico
<b>Mitocondri e loro componenti</b>	ATP peptidi formilati
<b>Prodotti derivati da matrice extracellulare</b>	peptidi di proteoglicani

# Damage or danger-associated molecular patterns (DAMPs)

List of alarmins, molecules, which contain endogenous damage- or danger-associated molecular patterns often recognized by TLRs and/or other PRR

**ANNEXINS** Bind Ca<sup>2+</sup>-dependent negatively charged phospholipids

**CATHELICIDINS** Antimicrobial cathelin-containing cationic peptides

**DEFENSINS** Antimicrobial cysteine-rich cationic peptides

**ENDOTHELINS** Endothelial peptides, constrict blood vessels, raise blood pressure

**GALECTINS** Beta-galactoside binding lectins

**HDGF** Hepatoma-derived growth factor

**HIGH MOBILITY GROUP BOX-1** A protein, binds to and distorts the minor groove of DNA

**HEAT SHOCK PROTEINS** Chaperones, assist proteins to fold correctly

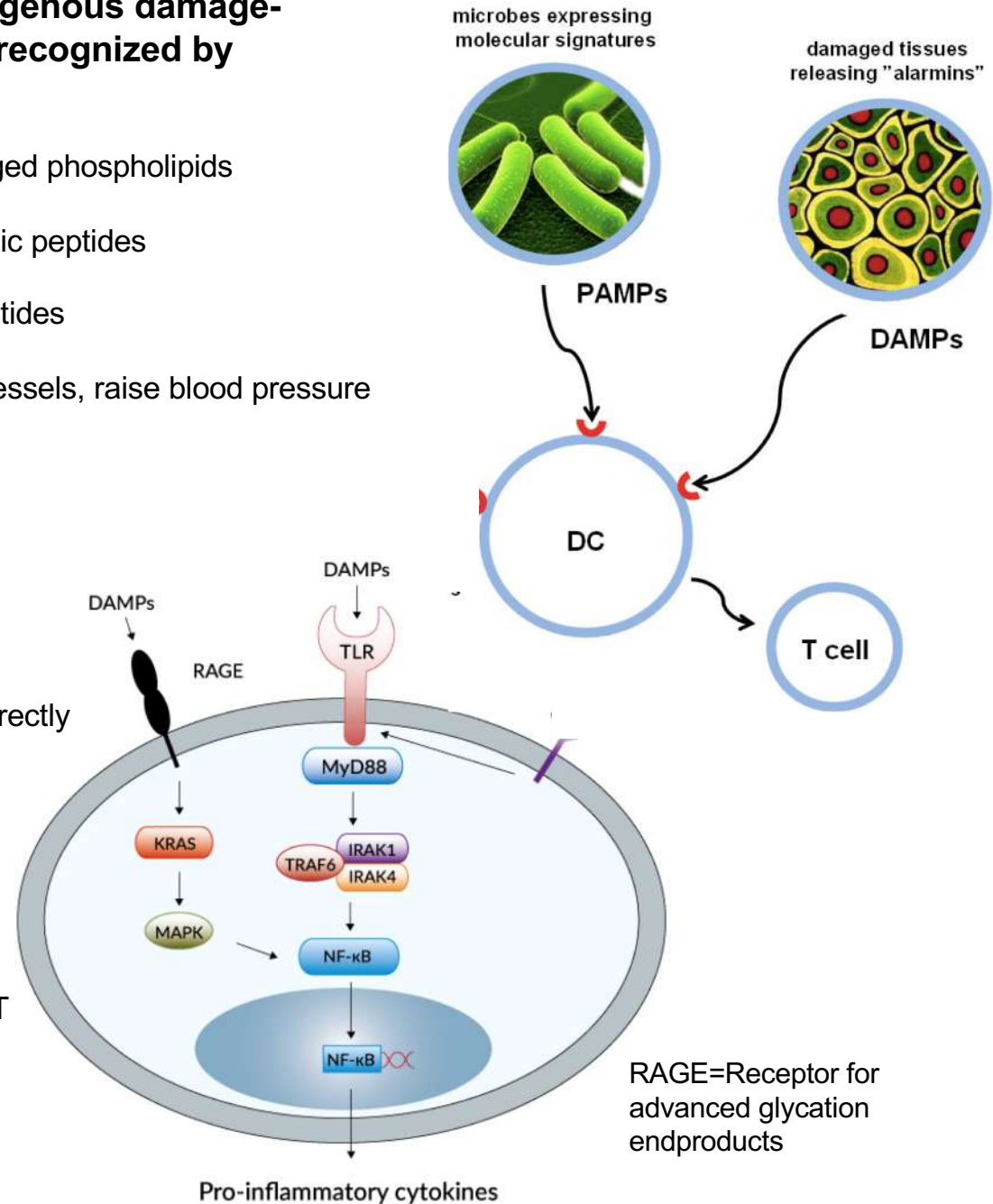
**INTERLEUKIN-1a** Pro-inflammatory cytokine

**NUCLEOLIN** Eukaryotic nucleolar phosphoprotein, role in ribosome biogenesis





**THYMOSINS** Thymic polypeptide hormones, role in T lymphocyte maturation

**URIC ACID** Urate, an oxidation product of purines, anti-oxidant

DAMPs=Alarmins



# NLR (NOD like receptor) dell'immunità innata

Famiglia	Esempi	Struttura tipica	Stimoli attivatori	Funzioni
NLRA	CIITA		IFN- $\gamma$	Espressione di molecole MHC di classe II
NLRB	NAIP		Flagellina	Controllo dell'infezione da <i>Legionella pneumophila</i>
NLRC	NOD1, NOD2, NLRC3-5		DAP (NOD1)	Attivazione di NF- $\kappa$ B
			MDP (NOD2)	Attivazione di NF- $\kappa$ B, autofagia, produzione di IFN di tipo I
			Flagellina (NLRC4)	Attivazione della caspasi-1, morte cellulare
NLRP	NLRP, 1-10	 <b>NOD</b> o <b>NBD</b>	ATP extracellulare, alluminio, asbesto, tossine batteriche, silice, urato di sodio, ROS, ridotta concentrazione di ioni K nel citosol (NLRP3)	Attivazione della caspasi-1
			Lipopeptidi (NLRP7)	Attivazione della caspasi-1

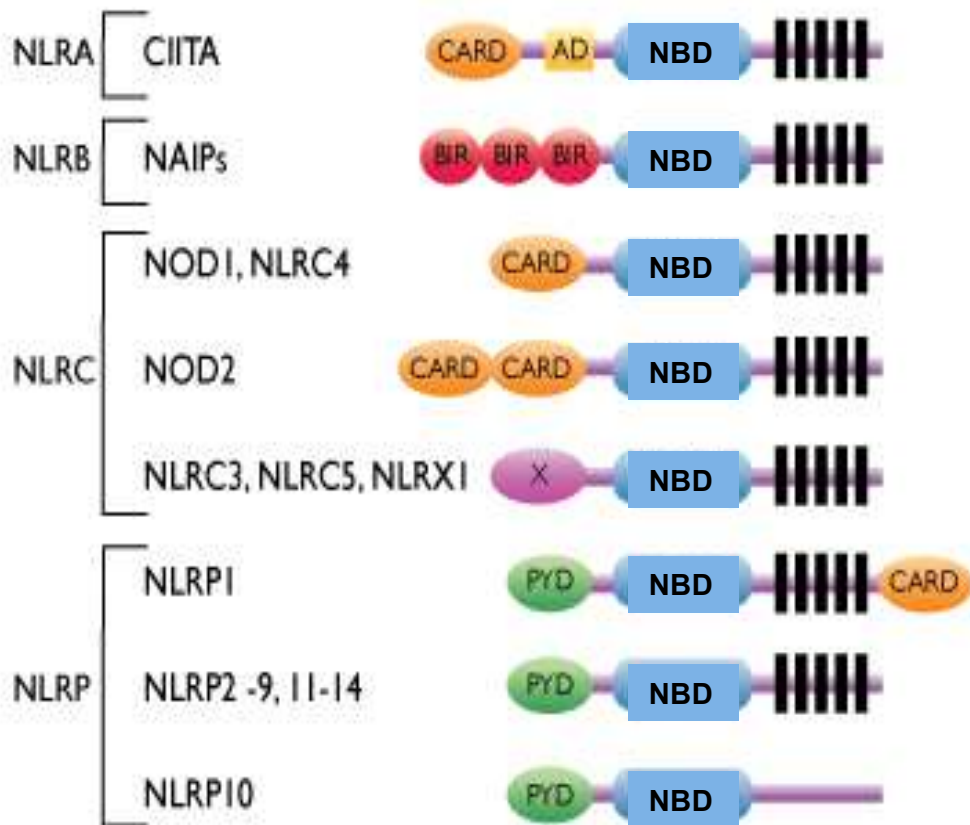
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NLR dell'immunità innata riconoscono **PAMP** e **DAMP**. I membri della famiglia NLR che svolgono funzioni tipiche dell'immunità sono 4: **NLRA**, **NLRB**, **NLRC**, **NLRP** ciascuno con un differente dominio effettore amino-terminale.

**NLRA** meglio conosciuta come CIITA è un fattore di trascrizione che presenta un dominio transattivante aminotermiale (TA) che permette l'espressione dei geni MHC di classe II. **NLRB** presenta il dominio effettore BIR (Baculovirus inhibition of apoptosis protein repeat) con funzione sconosciuta. **NLRC** presenta domini effettori CARD (caspase activation and recruitment domains) coinvolti nell'attivazione della caspasi 1 e **NLRP** che utilizzano i domini della pirina (PYrin Domain = PYD) anch'essi coinvolti nell'attivazione della caspasi 1. Tutti gli NLR presentano un dominio centrale NACHT o NOD (nucleotide oligomerization domain) coinvolto nel legame con i nucleotidi e domini ripetuti ricchi di leucina all'estremo carbossi-terminale che sono coinvolti nel riconoscimento del ligando. La figura mostra alcune delle principali funzioni dei recettori NLR e dei loro ligandi. DAP Diaminopimelic acid; LRR leucine rich repeat; MDP muramyl dipeptide;



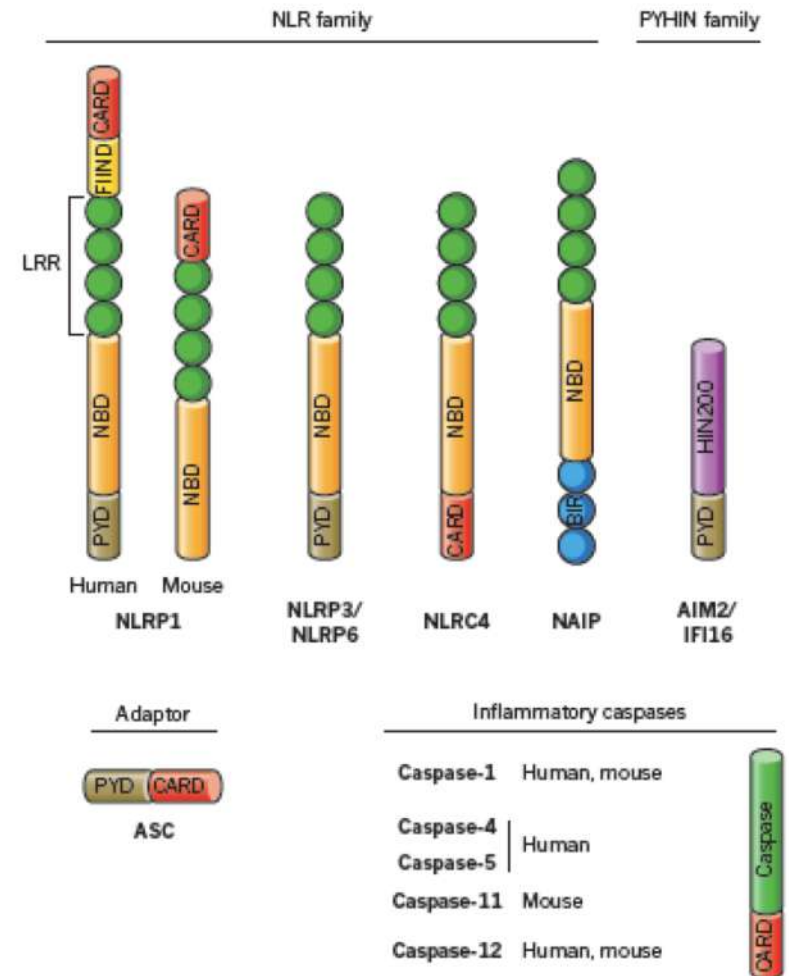
# Sottogruppi della famiglia dei recettori di tipo NOD (NLR)



I membri della famiglia NLR sono suddivisi in 4 sottogruppi in base al dominio N-terminale

**Nell'uomo ci sono 22 membri della famiglia NLR**

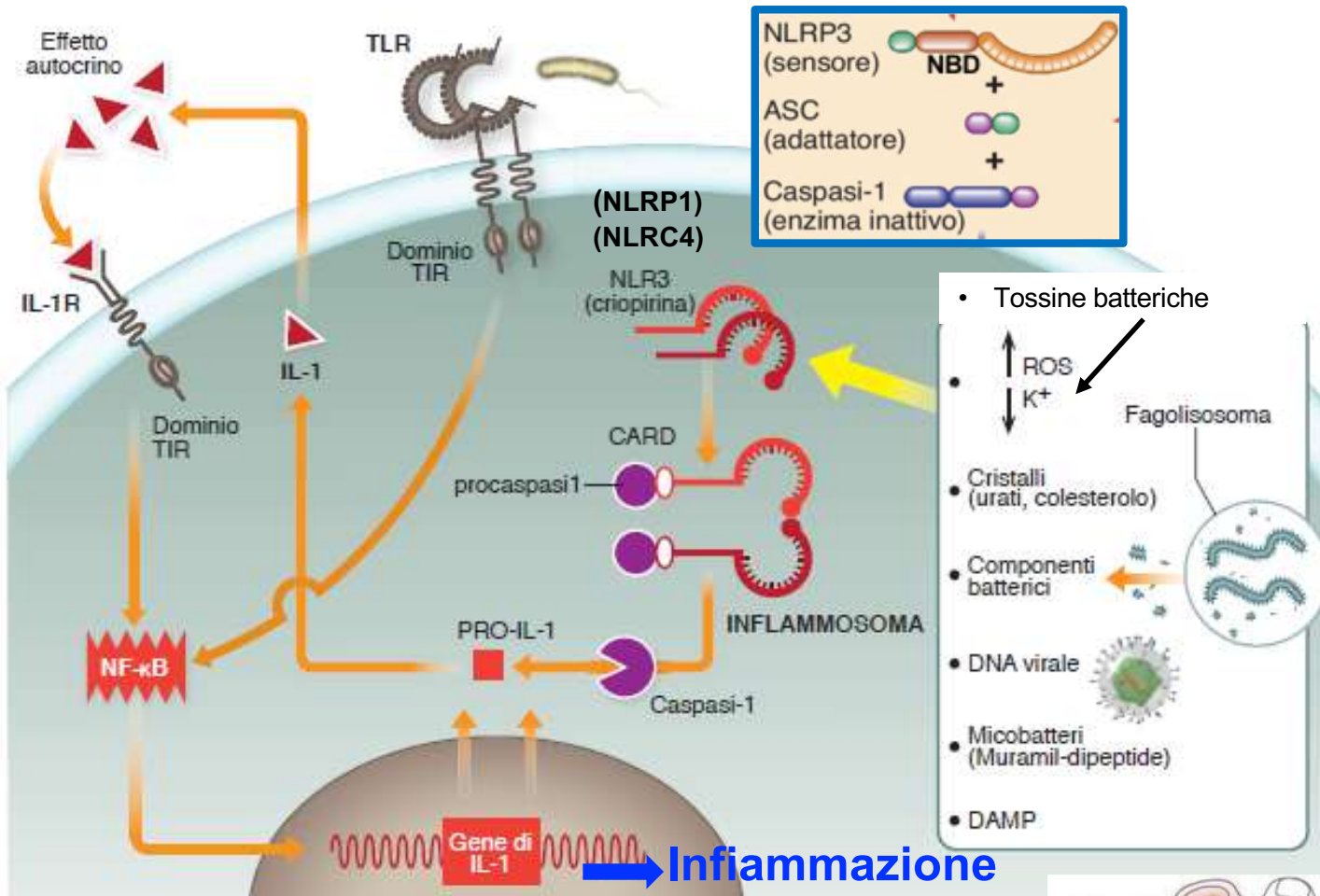
## Proteine del complesso dell'inflammasoma



**Queste proteine citosoliche riconoscono PAMP e DAMP**

Figure 1 | Domain organization of inflammasome proteins. The identified core components belong to two families, the NOD-like receptor (NLR) family and the PYHIN (pyrin and HIN200 (haematopoietic interferon-inducible nuclear antigens with 200 amino-acid repeats) domain-containing protein) family. The NLR family members include NLRP1, NLRP2 (not shown), NLRP3, NLRP6, NLR4 and, potentially, NLRP12. They all contain a nucleotide-binding domain (NBD), carboxy-terminal leucine-rich repeat (LRR), and can contain either a PYD or a caspase activation and recruitment domain (CARD) or both. The PYHIN family members AIM2 and IFI16 are characterized by having, in addition to a PYD, a HIN200 domain, which is involved in ligand binding. BIR, baculoviral inhibition of apoptosis repeat domain; FIIND, domain with function to find.

# L'attivazione dell'inflammasoma induce la produzione della forma attiva della IL-1 $\beta$ (e IL18)



Nell'uomo malattie definite autoinfiammatorie possono essere causate da mutazioni dei geni NLRPs

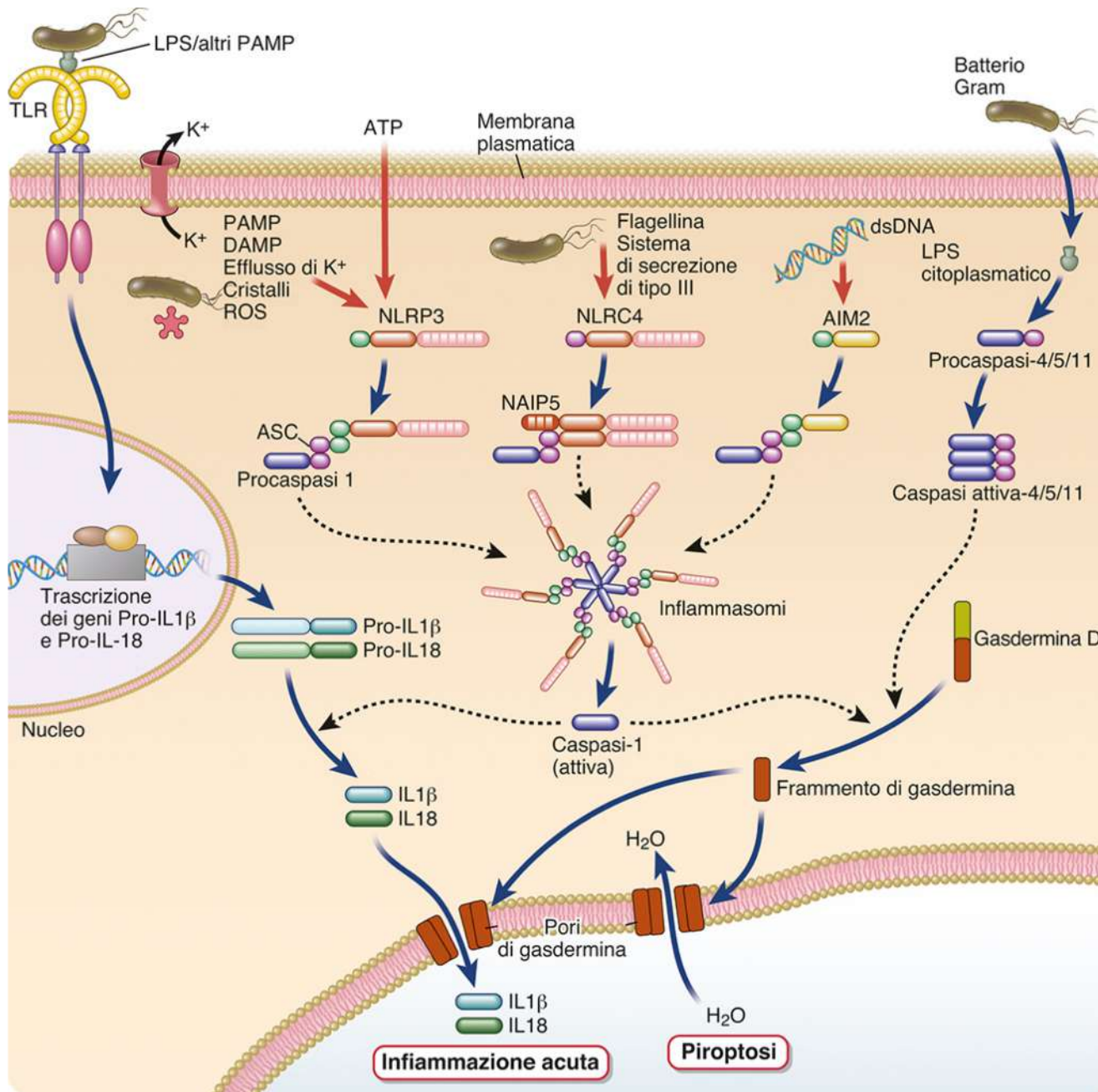
Es. mutazioni di NLRP3 sono causa delle criopirinopatie ma NLRP3 è coinvolto anche in numerose patologie multigeniche complesse e disordini metabolici

**Nella gotta** (patologia autoinfiammatoria) i cristalli dell'urato monosodico attivano l'inflammasoma NLRP3



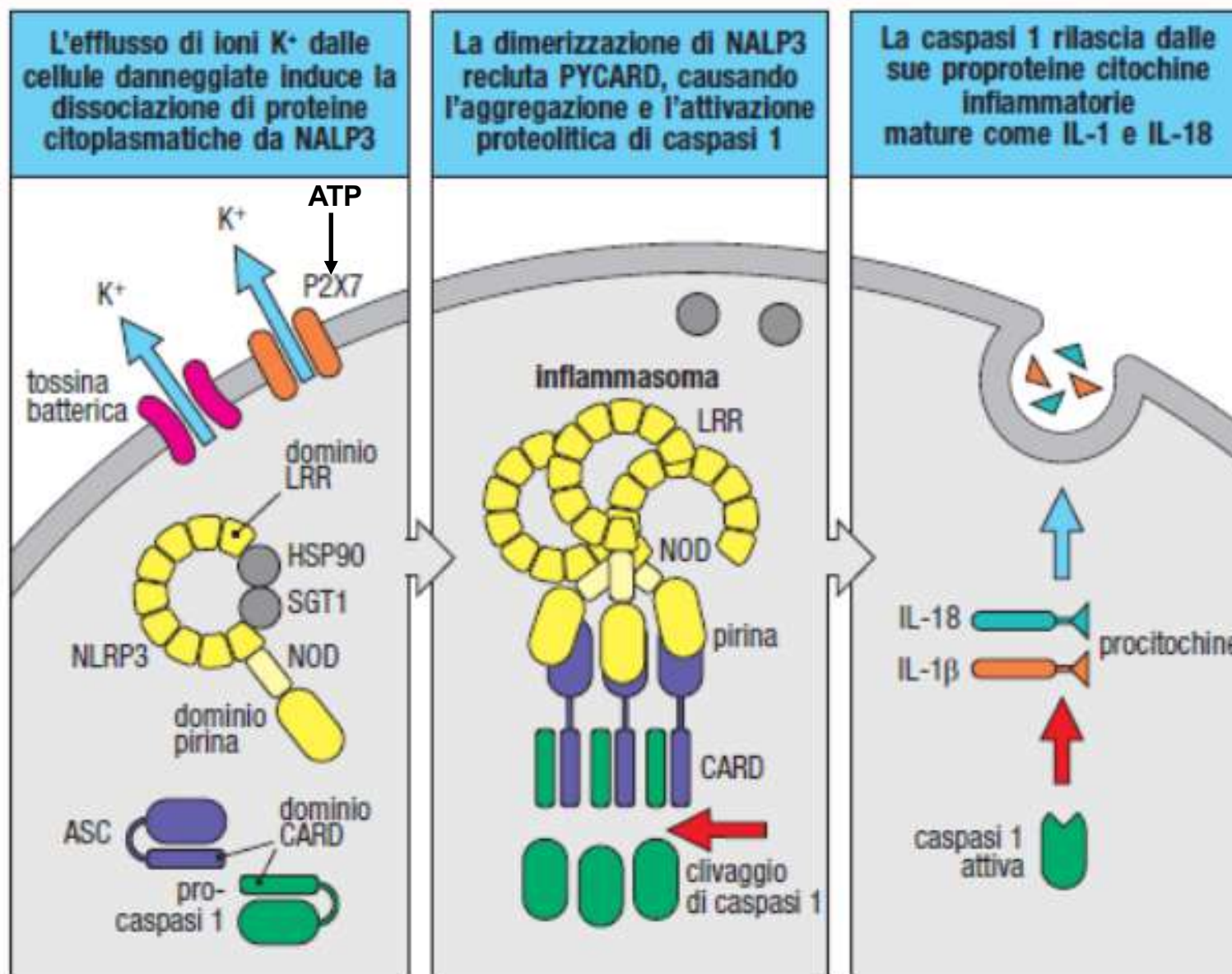
**Farmaco biologico (anticorpo monoclonale) per la gotta è un antagonista dell'IL-1**

# Inflammasomi



Attivazione di tre diversi inflammasomi canonici, che utilizzano NLRP3, NLRC4, o AIM2 come sensori, e degli inflammasomi non canonici composti da caspasi 4, 5, o 11. Sono indicati alcuni dei ligandi o condizioni cellulari che inducono l'assemblaggio di ciascuno di questi inflammasomi. Gli inflammasomi canonici si assemblano come complessi multimerici che includono i sensori NLR, proteine adattatrici come ASC e la procaspasi 1, portando alla generazione proteolitica della caspasi 1 attiva, che processa pro-IL-1 $\beta$  e pro-IL-18 in IL-1 $\beta$  e IL-18 attive. La caspasi-1 attivata dall'inflammasoma pu $\acute{o}$  anche scindere proteoliticamente la proteina citosolica gasdermina D, generando un frammento N-terminale che polimerizza nella membrana plasmatica, formando un poro che lascia uscire IL-1 $\beta$  e fa entrare acqua e ioni nella cellula, portando alla morte cellulare per lisi osmotica. Questo percorso di morte cellulare  $\acute{e}$  chiamato piroptosi, perch $\acute{e}$   $\acute{e}$  accompagnato da un'inflammatione dovuta a IL-1 rilasciata dalle cellule morenti. L'LPS citoplasmatico induce l'assemblaggio di molecole di procaspasi-4, -5, -11 per formare inflammasomi non canonici che sono multimeri di caspasi attive 4, 5, o 11, che possono anche scindere la gasdermina D, portando alla formazione di pori di gasdermina-N e alla piroptosi.

# Attivazione dell'inflammasoma



Il danno cellulare attiva l'inflammasoma NLRP3 (NALP3) e induce la produzione di citochine pro-infiammatorie

# Struttura dell'inflammasoma

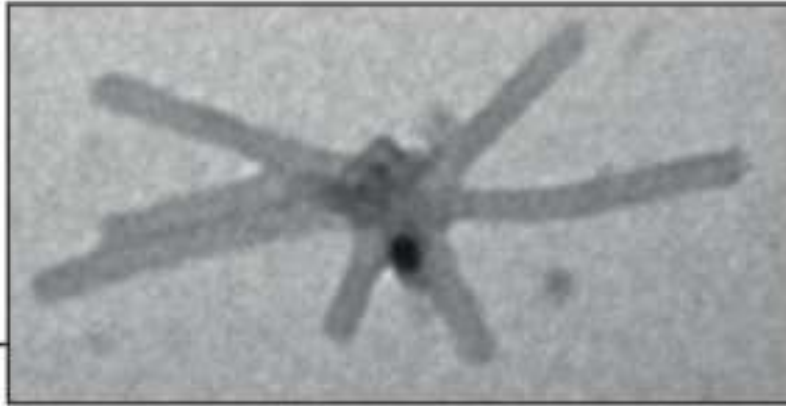
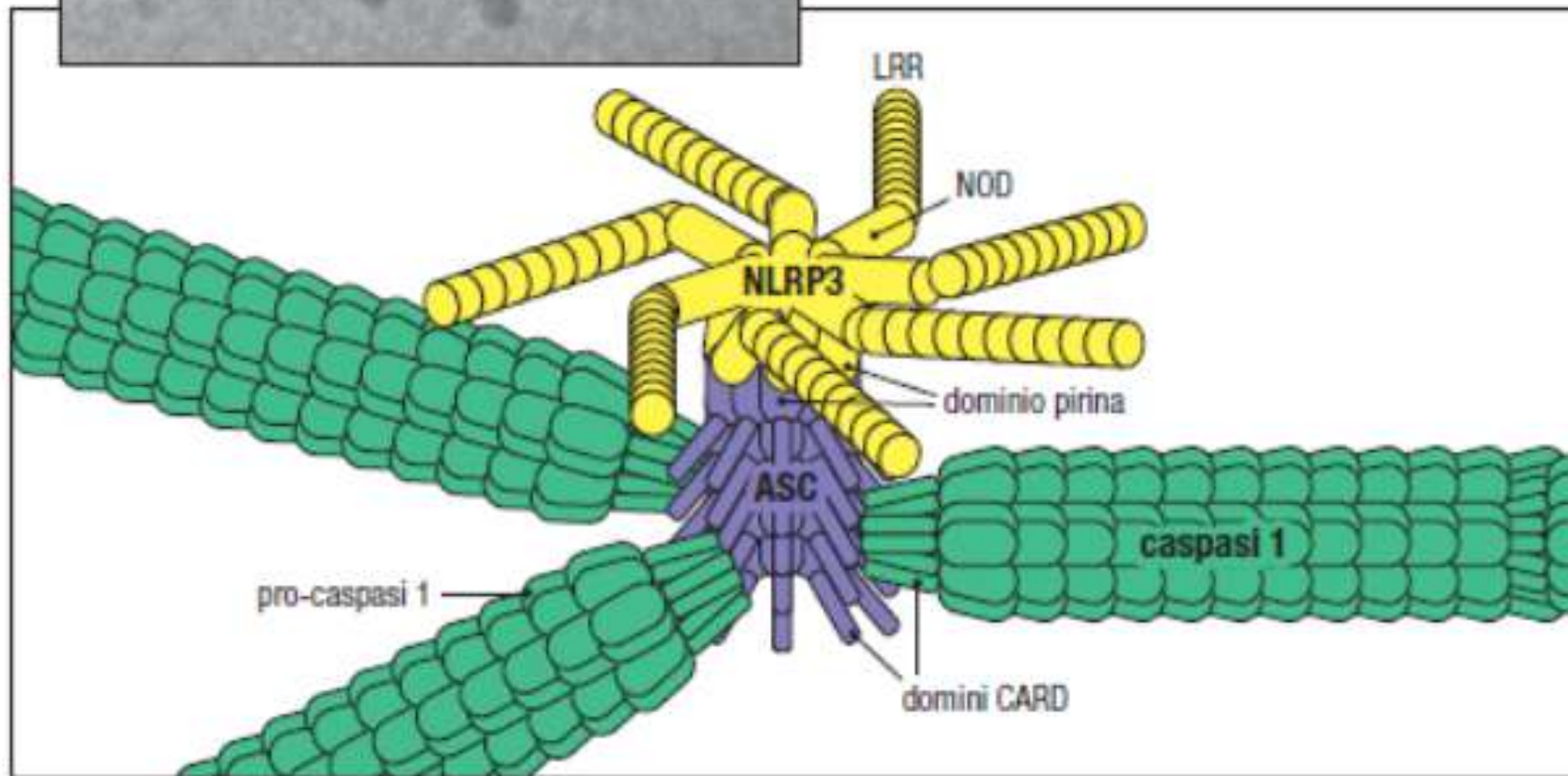
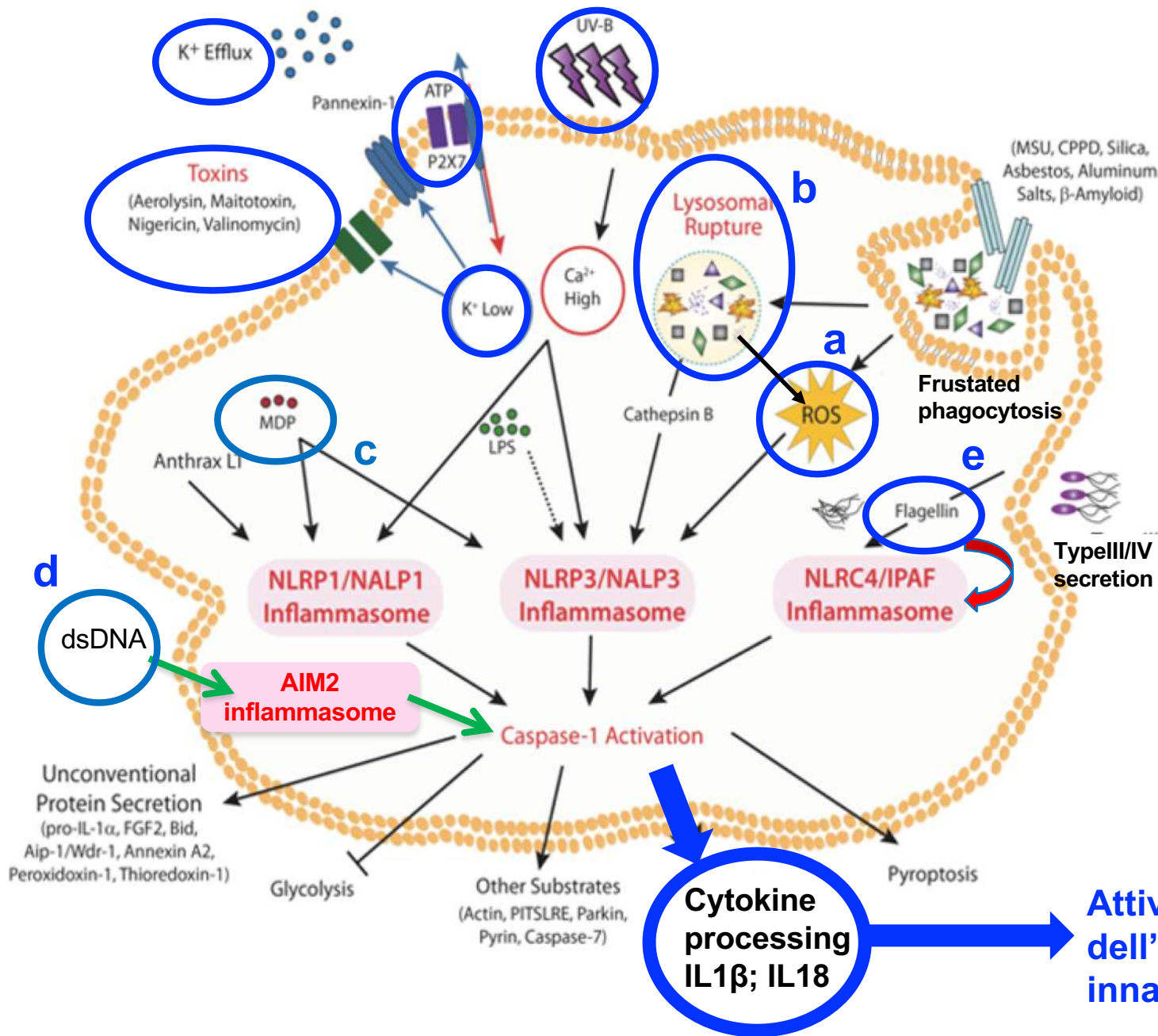


Immagine di microscopia elettronica di ASC ed il dominio pirina di AIM2 ed il dominio CARD della caspasi 1



**L'inflammasoma è composto da numerosi filamenti polimerici di proteine creati da aggregazione di domini pirinici e CARD**

# Segnali che inducono attivazione del complesso dell'inflammasoma



**a)** NLRP3 senses the reactive oxygen species (ROS);

**b)** NLRP3 is activated after lysosome destabilization;

**c, d)** NLRP1 and AIM2 sense the ligand directly: muramyl dipeptide (MDP) and double-stranded DNA (dsDNA);

**e)** NAIP proteins sense bacterial proteins resulting in the recruitment of NLRC4

**Attivazione dell'immunità innata ed adattativa**

Inflammasomes are assembled after sensing a structurally diverse repertoire of PAMPs and DAMPs. Several models have been proposed to explain how these signals are sensed, including models based on recognition of general cellular stress (Fig. **a** and **b**) or on direct and indirect recognition of activation signals (Fig. **c–e**).

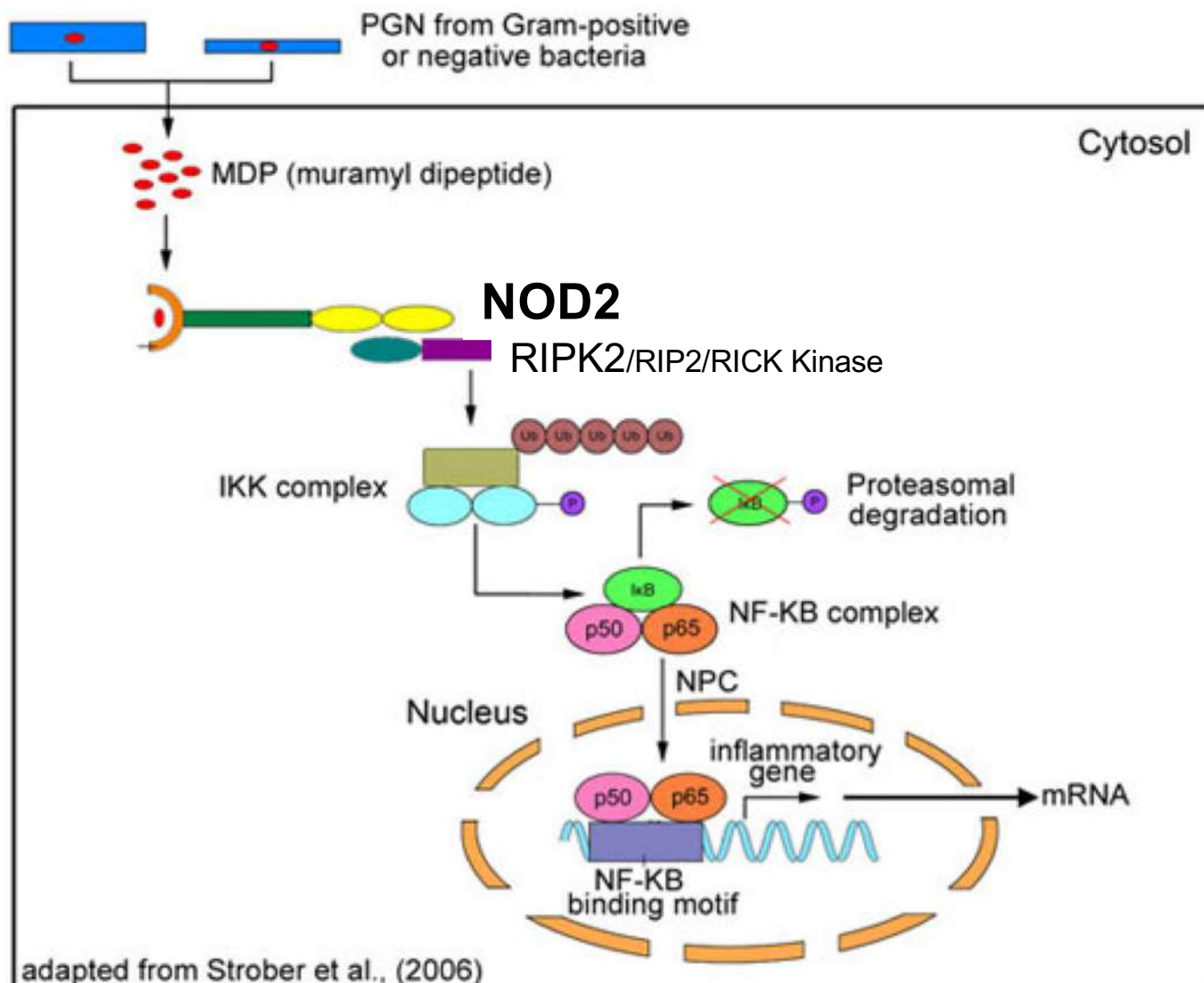
**a) NLRP3 senses the reactive oxygen species (ROS)**, which is produced in the cell (potentially by mitochondria) directly or indirectly by activators of the NLRP3 inflammasome. Increased amounts of ROS are sensed by a complex of thioredoxin and thioredoxin-interacting protein (TXNIP), leading to the dissociation of this complex. Subsequent binding of TXNIP to NLRP3 leads to the activation of NLRP3, the recruitment of ASC and pro-caspase-1 and formation of the active inflammasome complex.

**b) NLRP3 is activated after lysosome destabilization.** The phagocytosis of specific crystalline and particulate structures can lead to lysosome destabilization and the release of lysosomal content, including proteases. These proteases could lead to proteolytic inactivation of a negative regulator or to proteolytic activation of a positive regulator of NLRP3, resulting in inflammasome assembly.

**c, d) NLRP1 and AIM2 sense the ligand directly.** The direct binding of specific ligands (muramyl dipeptide (MDP) and double-stranded DNA (dsDNA)) can lead to conformational changes in NLRP1 and AIM2, resulting in inflammasome activation. Inflammasome formation in NLRP1 is independent of ASC.

**e) NAIP proteins sense bacterial proteins** resulting in the recruitment of NLRC4 and assembly of the NLRC4 inflammasome.

## NOD2: A Critical Regulator of Ileal Microbiota and Crohn's Disease

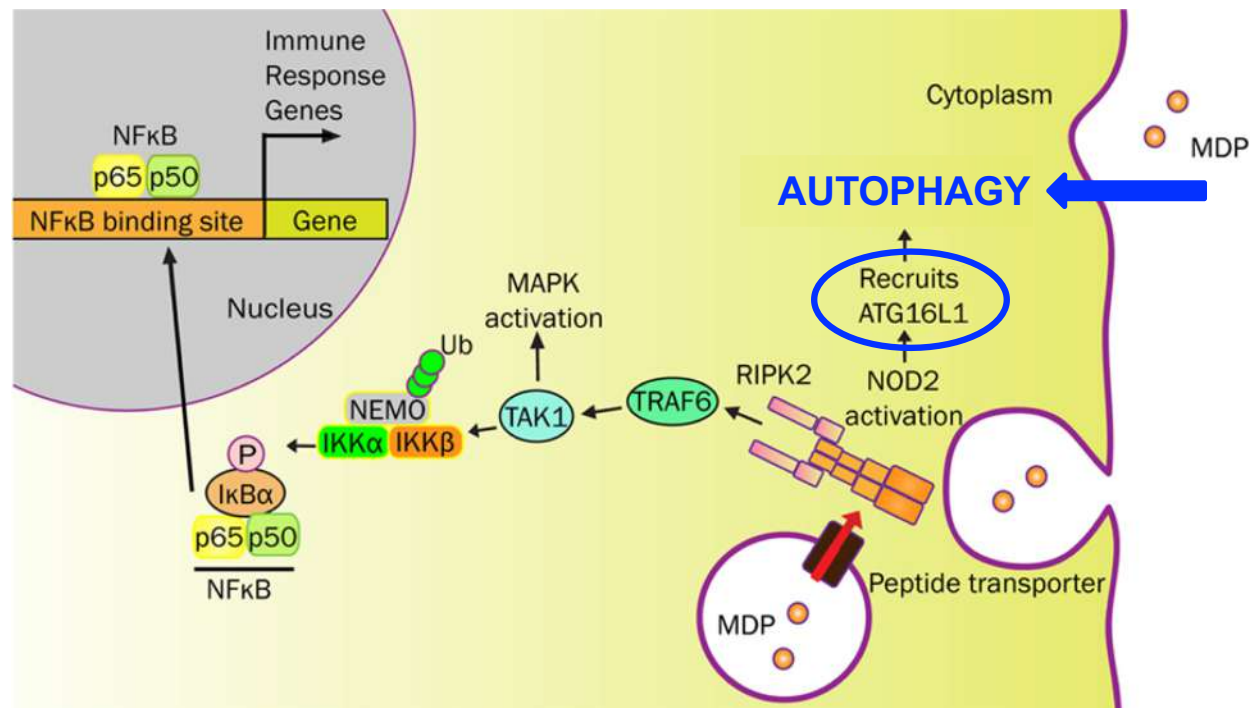


The **human NOD2** protein from the Card15 gene is of particular interest because polymorphisms in this protein have been implicated in **Crohn's disease**, a chronic severe inflammation of the small intestine. Mutations in **NOD2** may also give rise to early onset granulomatous arthritis (Blau's syndrome). During NFκB activation, a CARD in the NOD2 complex binds to the adaptor molecule **RIPK2** (RIP2 or RICK). The CARD and kinase domain of RIPK2 are separated by a central region that binds to the regulatory subunit (IKKγ or NEMO) of the IKK complex. Thus, NOD2 assembly may recruit RICK through CARD-CARD interactions, and this kinase may then transiently recruit the IKK complex to this platform. Phosphorylation of IKK by RIPK2 then activates this downstream kinase leading to phosphorylation of the IκB regulatory subunit of NFκB.

This promotes ubiquitination of the IκB subunit and its subsequent degradation. Released NFκB then translocates into the nucleus where it up-regulates genes involved in innate and adaptive immunity. We are studying the structure and function of recombinant NOD2 complexes to provide an understanding of assembly and signaling by this platform.

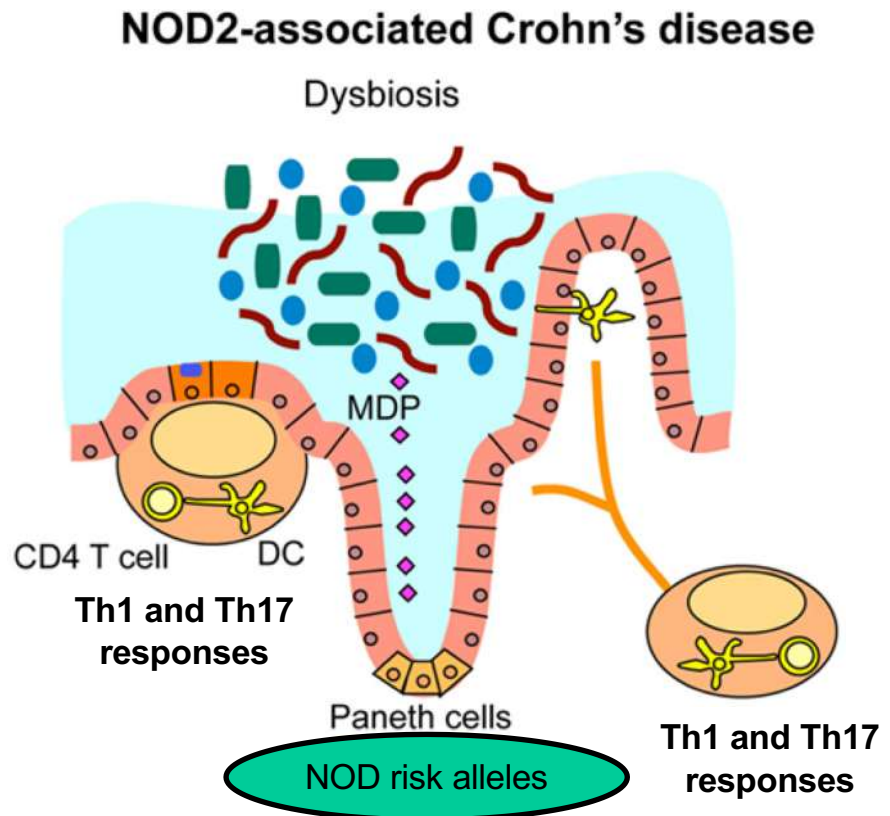
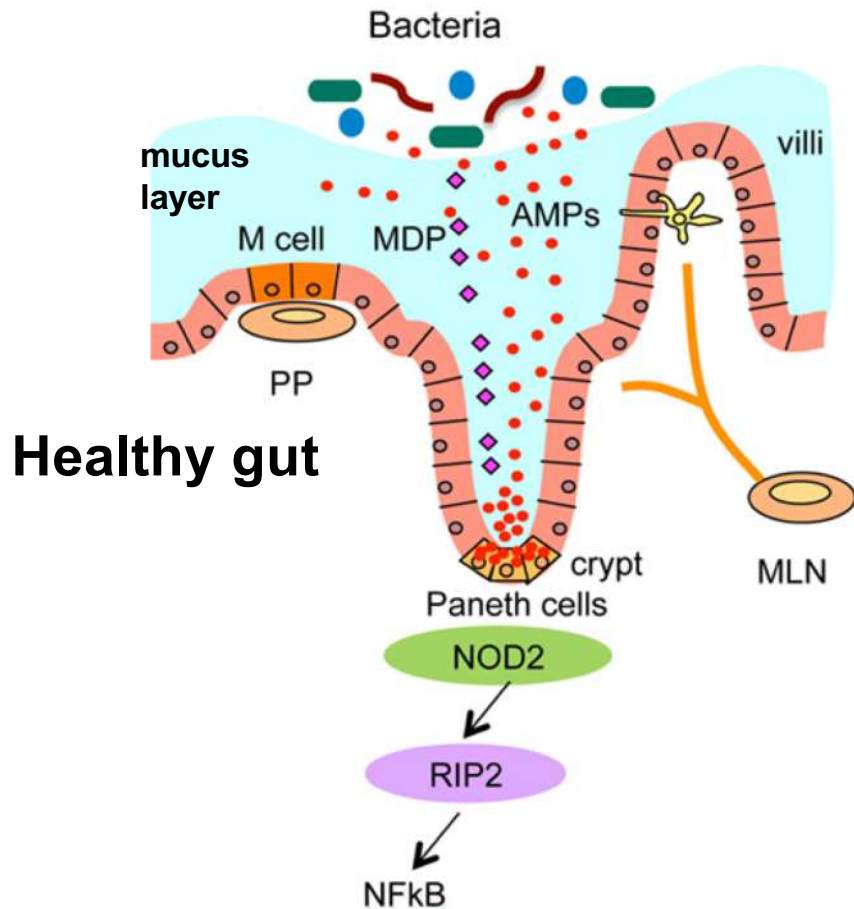


# NOD2: A Critical Regulator of Ileal Microbiota and Crohn's Disease



**NOD2 senses MDP and elicits immune responses.** MDP-derived from bacterial cell wall can be transported into the cytosol by endo/phagosome through peptide transporters (e.g., SLC15A4). **MDP** activates **NOD2** to initiate **RIPK2** recruitment resulting in platform formation. This cytosolic NOD2-initiated structure localizes E3 ligases (e.g., TRAF6) to ubiquitinate NEMO, for which NEMO and TAK1 forms a complex near IKK. In addition to activating the mitogen-activated protein kinase (MAPK) pathway, TAK1 phosphorylates IKK to initiate the NF- $\kappa$ B signaling pathway. I $\kappa$ B- $\alpha$  is phosphorylated by IKK, which targets I $\kappa$ B- $\alpha$  to proteasome degradation. No longer restricted, free NF- $\kappa$ B translocates into the nucleus and activates various immune response genes. NOD2 also recruits **ATG16L1** near the plasma membrane to promote autophagosome formation, known to degrade antigenic structures. The release of cytokines and chemokines, initiated by NOD2, recruits immune cells to influence Th1 and Th2 responses.

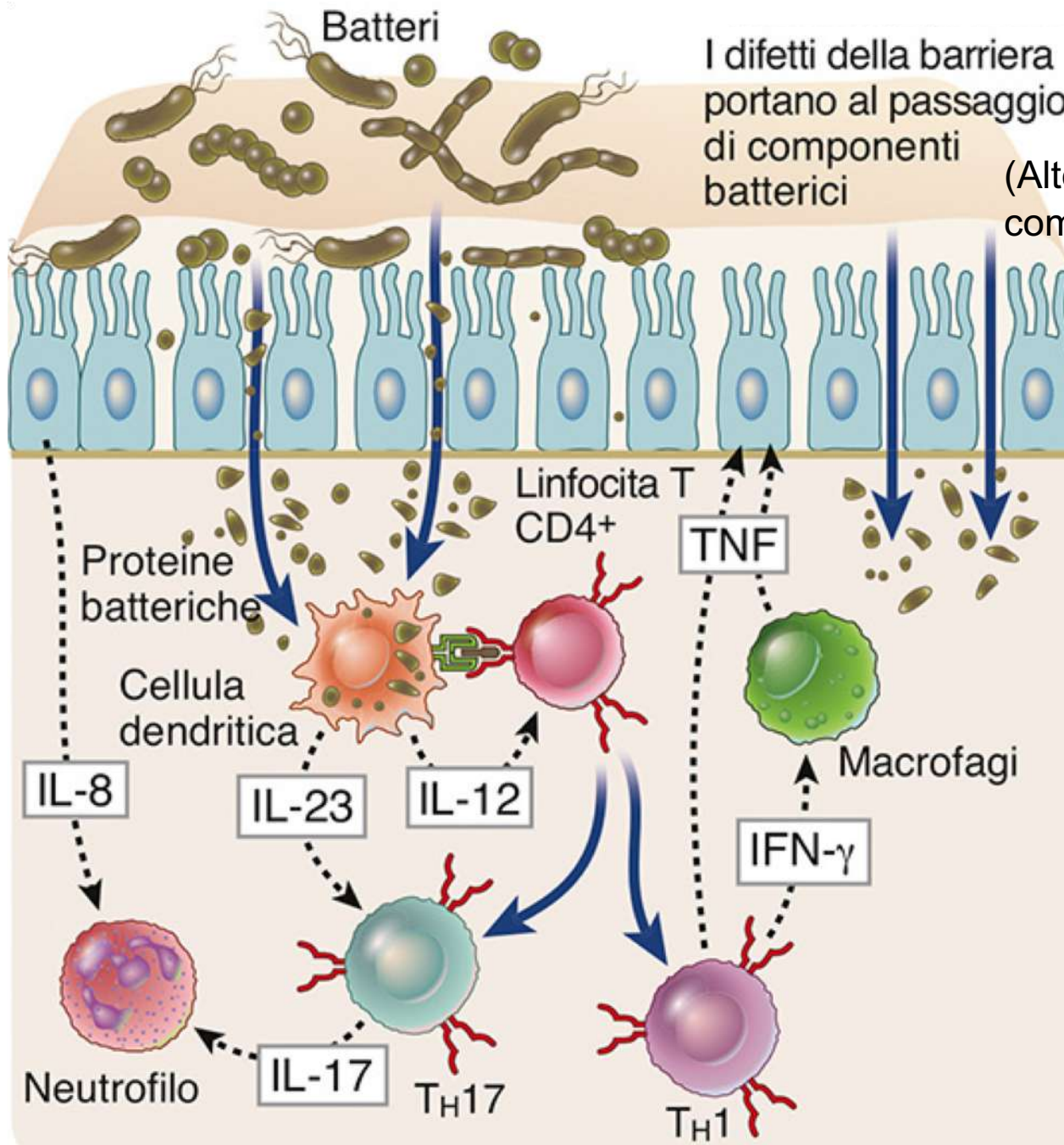
# NOD2-associated dysregulated microbiota leads to the susceptibility of CD



**Ileum in healthy individuals (left).** NOD2 senses microbiota-derived MDP and activates NF-κB through the downstream kinase receptor-interacting protein 2 (RIP2), which is critical for the bacterial killing activity of Paneth cells via the secretion of anti-bacterial compounds.

**Ileum with loss of NOD2 function (right).** In CD, that is associated with *NOD2* polymorphisms, CD-associated *NOD2* polymorphisms disturb many characteristics of **gut immune homeostasis**, including reduced MDP sensing, and impaired antimicrobial responses in Paneth cells, leading to defective bacterial clearance. This dysbiosis caused by impaired Paneth cell function is characterized by increased load of bacteria and abnormalities of Peyer's patches (PP) and mesenteric lymph nodes (MLN) that stimulate the mucosal immune system to induce Th1 and Th17 immune response, leading to chronic inflammation.

# Modello patogenetico della malattia di Crohn



I difetti della barriera portano al passaggio di componenti batterici (Alterata secrezione di composti antimicrobici)

I batteri del lume intestinale penetrano nella lamina propria, dove inducono lo sviluppo di linfociti Th1 e Th17. Le citochine prodotte da queste cellule causano infiammazione e danni ai tessuti. IFN- $\gamma$  interferone- $\gamma$ ; TNF fattore di necrosi tumorale.