Academic lectures for students of medical schools – 3rd Year updated 2004 - 2015

GENERAL PATHOPHYSIOLOGY

Inflammation - Molecular events

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INNATE IMMUNITY

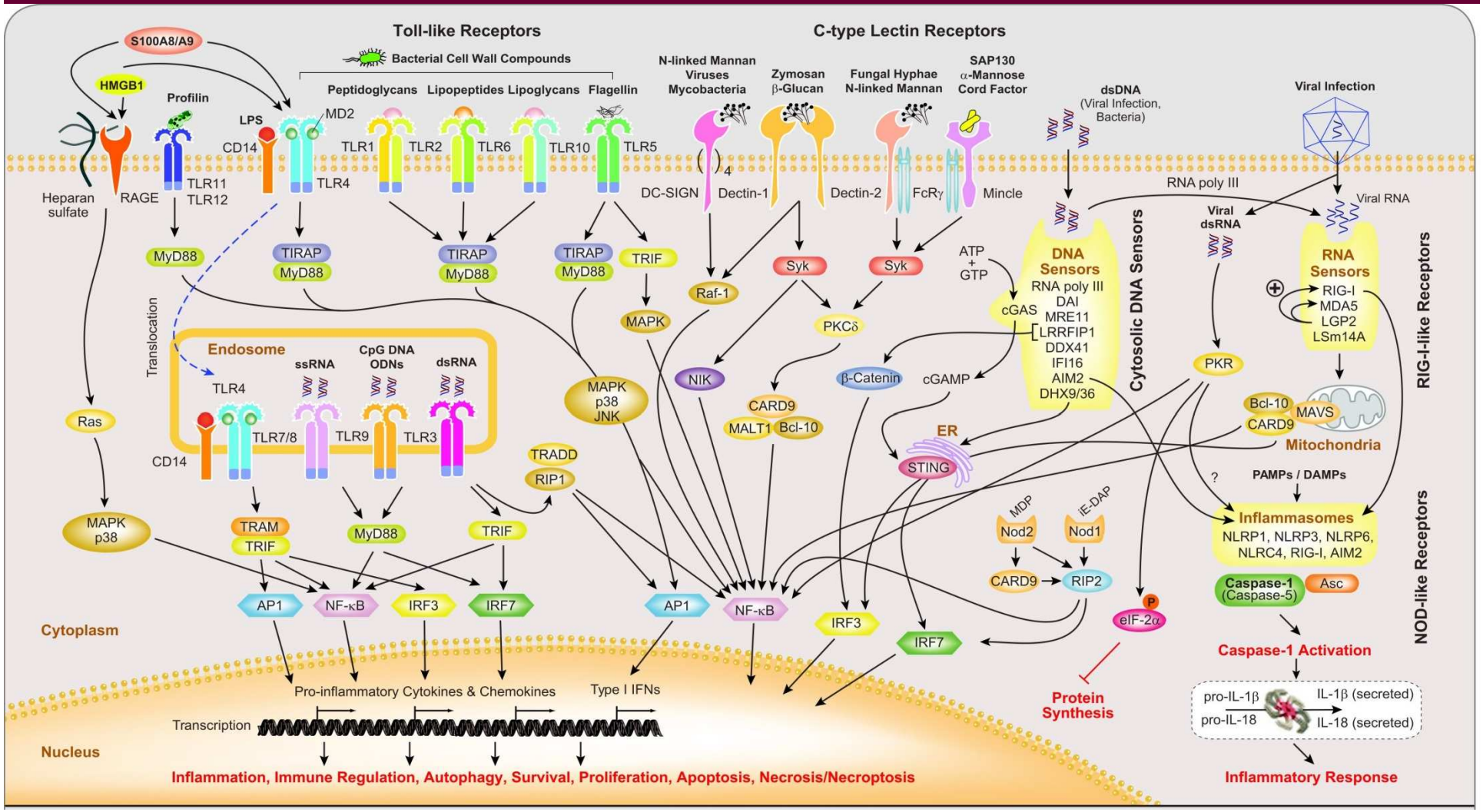
What is recognized by innate immunity cells ?

- **PAMPs (pathogen-associated microbial products)** extracellular or endosomal structurally conserved moleculer that enter the cell via phagocytosis or pores as a part of pathogenic microbes; e.g. bactertial (LPS, ds DNA), fungi (dsDNA), dsRNA, ssRNA, protozoa (hemozoin crystals,

- **MAMPs (microbe-associated molecular patterns)** recently proposed term not only pathogens, express the molecules detected; thy are found in most microbes.

- **DAMPs (damage-associated molecular patterns)** = exogenous or endogenous factors (e.g. released from dying cells**)** of variable nature that are associated with cell stress or damage ; radiation (UVB,UVC, RTG, gamma), uric acid & cholesterol crystals, b-amyloid, asbestos, silica, aluminium, hypoxia, ischemia, synuclein, PrPc prion fibrils, saturatd fatty acids,
- metabolic diseases:obesity, diabetes, cancer, neurodegenerative,

Pattern recognition receptors (PRRs)



- one of the first forms of defense employed by the innate immune response during an infection

Pattern recognition receptors (PRR)

1. Membrane PRRs

- Receptor kinases
- Toll-like receptors (TLR)
- C-type lectin receptors (CLR)
  - Group I: mannose receptors
  - Group II: asialoglycoprotein receptor family

2. Cytoplasmic PRRs

- NOD-like receptors (NLR)
- RIG-I-like receptors (RLR)
- Plant PRRs

3. Secretory PRRs

- Complement receptors,
- Collectins
- Ficolins
- Pentraxins (serum amyloid A, C-reactive protein),
- Peptidoglycan recognition proteins (PGRs)
- Llipid transferases, LRR, XA21DNOD-like receptors (NLR)
- RIG-I-like receptors (RLR)
- Plant PRRs

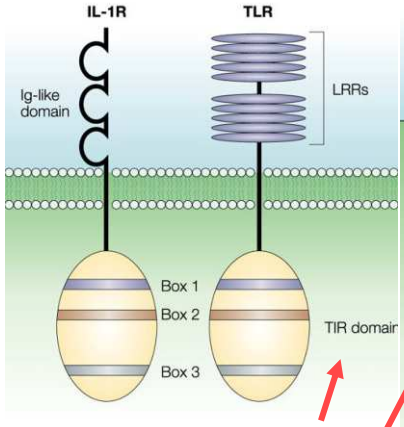
Functional classification

- **Signaling PRRs** = include TLR and cytoplasmic NLR involved in intracellulalr signaling
- **Endocytic PRRs** = include mannose receptors of macrophages, glucan receptors, scavenger receptors that recognize charged ligands, mediate removal of apoptotic cells; promote the attachment, engulfment and destruction of microorganisms by phagocytes

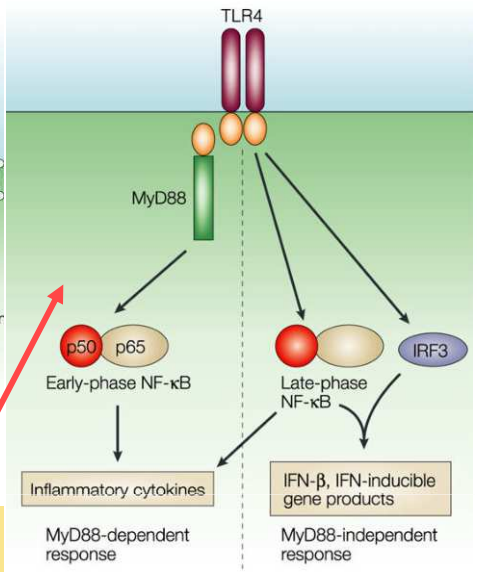
1. Toll-like receptors (TLRs)

- **first discovered in Drosophila**; name according **to similarity** to **Toll gene protein** which is involved in embryonic development in D. melanogaster. (1985 by Christiane Nüsslein-Volhard)
- **Structure:** single protein, membrane-spanning, non-catalytic receptors recognize extracellular or endosomal structurally conserved pathogen-associated microbial products (PAMPs), In addition, TLRs bind to molecules from commensal bacteria and to endogenous damage-associated molecular patterns (DAMPs) from dead and dying cells.
- **Species:** found in many species incl. man; in mammals, these receptors have been assigned numbers 1 to 13 (TLR1-TLR13);.TLR12,TLR13 are not found in humans; TLRs interact with their specific PAMP
- **Cells:** expressed in sentinel cells such as mastocytes, resident macrophages and dendritic cells; NK, monocytes, granulocytes (eosinophiles, basophiles) express the greatest variety of TLR; most tissues express at least one TLR, several expressed all (spleen, peripheral blood leukocytes, intestinal, pulmonary epithelium,
- trigger the synthesis and **secretion of cytokines; activation of defense programs** that are necessary for innate or adaptive immune responses
- **induces NF-κB signaling and the MAP kinase pathway** and therefore the secretion of pro-inflammatory cytokines and co-stimulatory molecules. Molecules released following TLR activation signal to other cells of the immune system making TLRs key elements of innate immunity and adaptive immunity.[4]

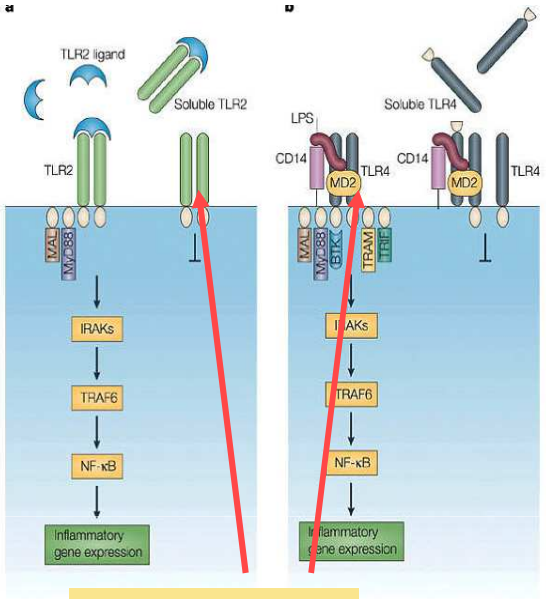
Toll-like receptors (TLRs)



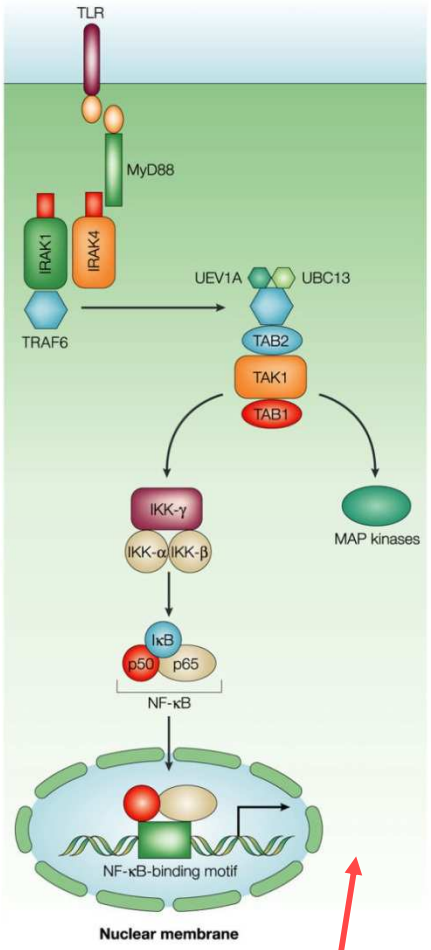
General scheme



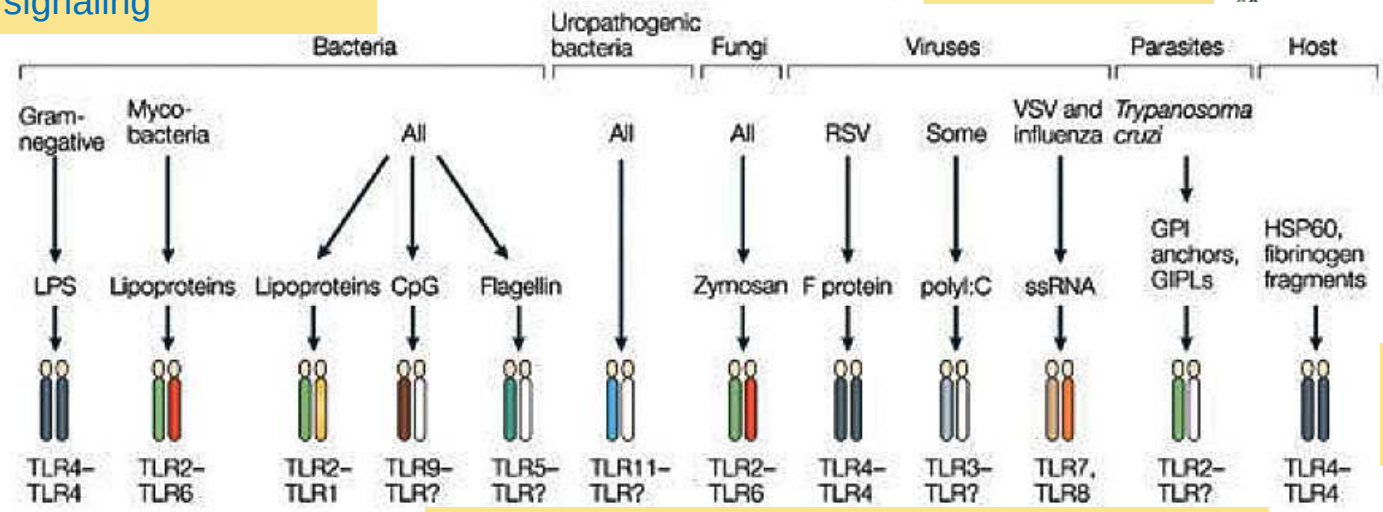
MyD88 independendt signaling



Soluble forms



Classical downstream signalling



Toll receptors according to infection agens

Toll like receptors

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)	Monocytes, dendritic cells, mast cells, eosinophils, basophils
TLR-2:TLR-6 heterodimer		
TLR-3	Double-stranded RNA (viruses)	NK cells
TLR-4 (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)	Macrophages, dendritic cells, mast cells, eosinophils
TLR-5	Flagellin (bacteria)	Intestinal epithelium
TLR-7	Single-stranded RNA (viruses)	Plasmacytoid dendritic cells, NK cells, eosinophils, B cells
TLR-8	Single-stranded RNA (viruses)	NK cells
TLR-9	DNA with unmethylated CpG (bacteria and herpesviruses)	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-10	Unknown	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-11 (mouse only)	Profilin and profilin-like proteins (*Toxoplasma gondii*, uropathogenic bacteria)	Macrophages, dendritic cells, liver, kidney, and bladder epithelial cells

Toll-like receptor mediated diseases

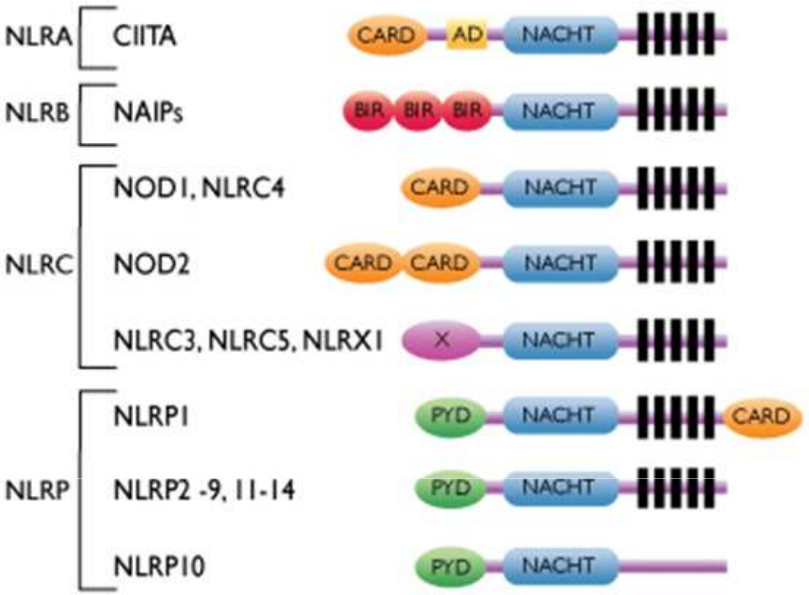
Pathogen	Disease	TLR	Possible mechanism
Infection			
Bacteria	Sepsis	TLR4	LPS induces inflammatory gene expression and organ failure
West Nile virus	Lethal encephalitis	TLR3	Virus double-stranded RNA facilitates infection in the brain
Plasmodium falciparum	Malaria	TLR9	The malaria pigment hemozoin induces inflammatory responses through TLR9
Candida albicans	Candidiasis	TLR2	*Candida albicans* induces immunosuppression through TLR2
Autoimmunity			
Bordetella pertussis	EAE	TLR4	Pertussis toxin recruits autoreactive T cells into the central nervous system
ND	SLE	TLR9	Chromatin–IgG complexes activate B cells and dendritic cells
ND	Diabetes	TLR2,3,4,9	TLR ligands increase innate immunity
ND	Cardiomyopathy	TLR2,3,4,9	TLR ligands promote dendritic cell function by presenting heart antigens
ND	Atherosclerosis	TLR4	TLR signals trigger pro-inflammatory responses
Chronic inflammation			
ND	Asthma	TLR4	LPS induces T_H2-cell responses to inhaled antigens
Bacteria	COPD	TLR4	LPS exacerbates airway inflammation

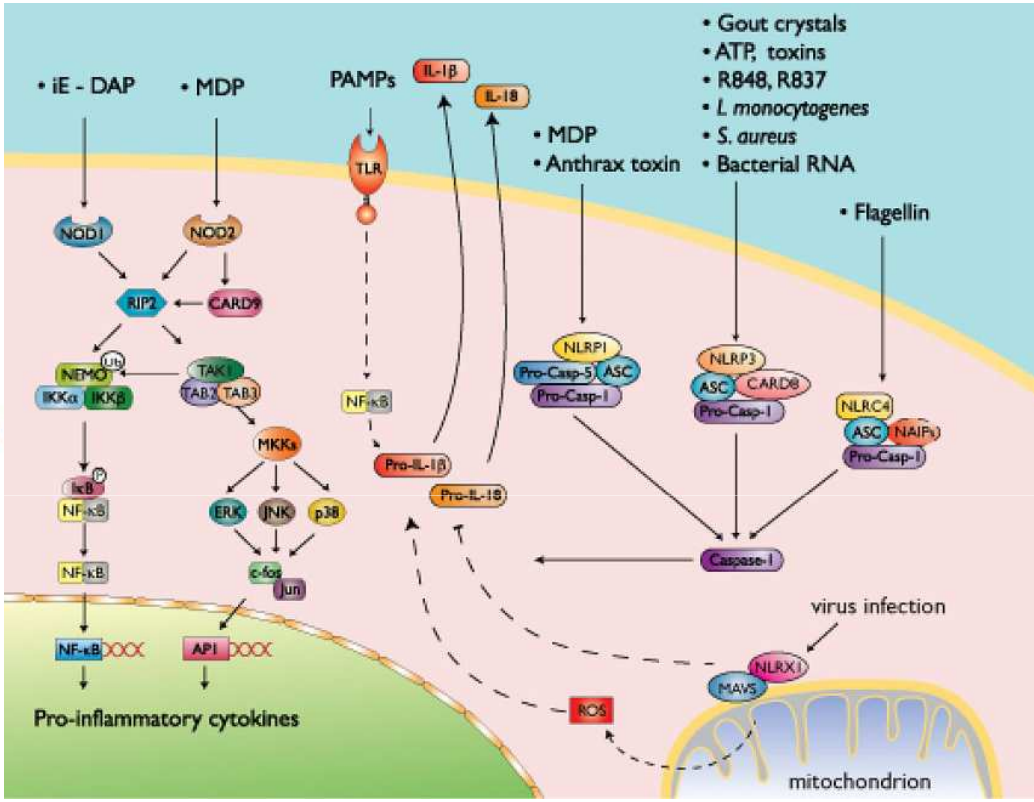
COPD, chronic obstructive pulmonary disease; EAE, experimental autoimmune encephalomyelitis; LPS, lipopolysacc
determined; SLE, systemic lupus erythematosus; T_H2, T helper 2.

2. Nod-like receptors (NLR)

- **NOD-like receptors (NLRs)(Nucleotide-binding Oligomerization Domain-like**) sense infection and stress through the recognition of cytoplasmic pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)
- play **key roles in regulation of innate immune response,** cooperate with Toll-like receptors and regulate inflammatory and apoptotic response
- highly conserved through evolution; discovered in many different animal species (homologs APAF1) and plant kingdom (disease-resistance R protein)
- **Cells**: lymphocytes, macrophages, dendritic cells and also in nonimmune cells (epithelium).
- **Subfamilies: NLRC** (formely known as NODs**), NLRP** (formerly known as NALPs), **NLRB** (formely known as NAIP or Birc) and **NLRA.**
- High incidence of genetic mutations that are associated with chronic inflammatory or autoimmune disorders.

NOD-like receptors (NLR) - families





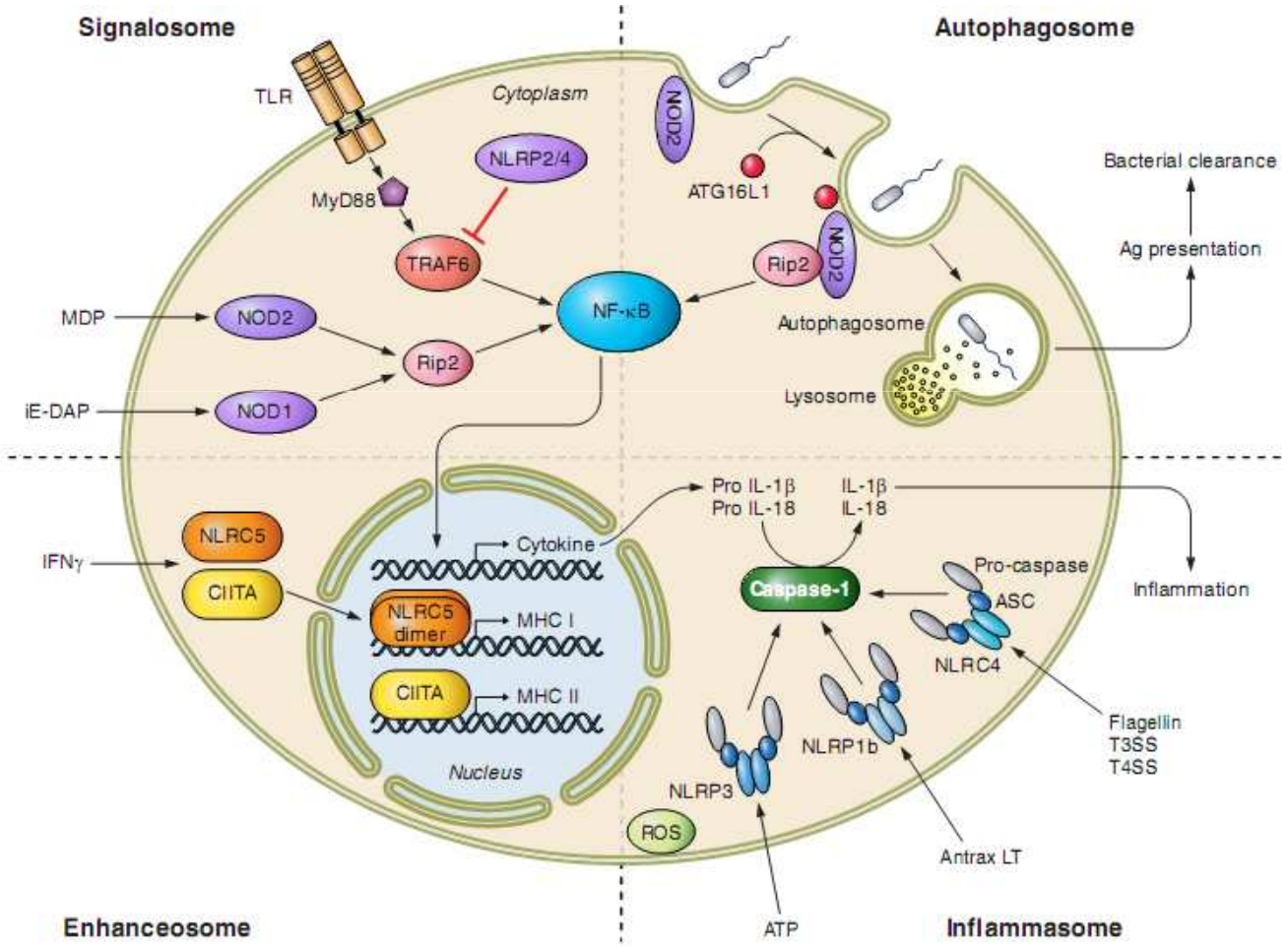
Nod-like receptors (NLRs) - families

- **NOD1 (NLRC1)**and **NOD2 (CARD4** recognize distinct motifs of **peptidoglycan (PGN),** an essential constituent of the bacterial cell wall.
- **NOD1** senses the D-γ-glutamyl-meso-DAP dipeptide (iE-DAP), which is found in PGN of **all Gram-negative** and **certain Gram-positive bacteria**
- **NOD2** recognizes the muramyl dipeptide (MDP) structure found in almost **all bacteria**

.

- **NLRC4** (IPAF, CLAN/CARD12 NLRC subfamily).= key role in flagellin - induced TLR5-independent regulation of caspase-1 by forming a multiprotein “inflammasome”.TLR5 and NLRC4 are distinct sensors that respond to extracellular and cytosolic flagellin
- **NLRX1 (NOD9)** is the first NLR protein shown to be localized at the mitochondria
- NLRX1 negatively impacts antiviral inflammatory response via the RIG-I/IPS-I sensing pathway; controls (NF-κB and JNK) signaling pathway to activate reactive oxygen species (ROS) production in response to TNF-α, and pathogens

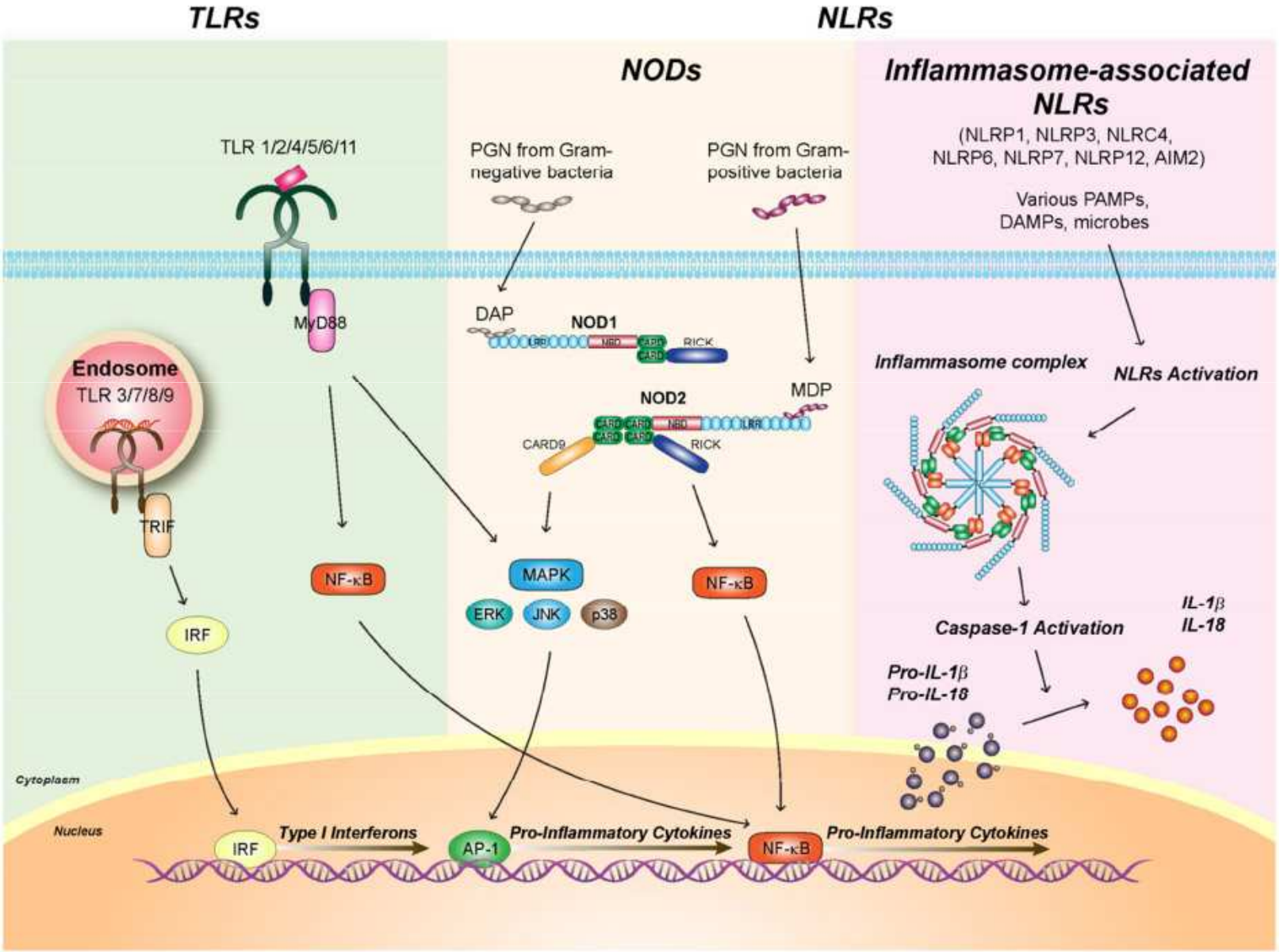
Overview of NOD-like receptor cellular pathways



NLR family can be divided into 4 broad functional tags: **transduction signaling, autophagy, transcriptional activation, and inflammasome assembly.**

NOD1 and NOD2 are known to activate the NF-B pathway, NLR receptors (e.g., NLRP2 and NLRP4) are negative regulators of this pathway. Inflammasomes can be assembled by various NLR receptors. NOD2 is also known to recognize bacteria at the cell entry site and initiate the autophagosome formation around the intracellular bacteria. NLRC5 have been described as transactivators of major histocompatibility complexes

Comparison of various PRR



Other PRRs

RIG-I-Like Receptors (RLRs)

- family of **cytoplasmic RNA helicases** critical for host **antiviral responses**.
- **RIG-I** and **MDA-5** sense double-stranded RNA (**dsRNA),** a replication intermediate for RNA viruses, leading to production of type I interferons (IFNs) in infected cells. LGP2 contains a RNA binding domain but lacks the CARD domains and thus acts as a negative feedback regulator of RIG-I and MDA-5.

C-type lectin receptors (CLRs)

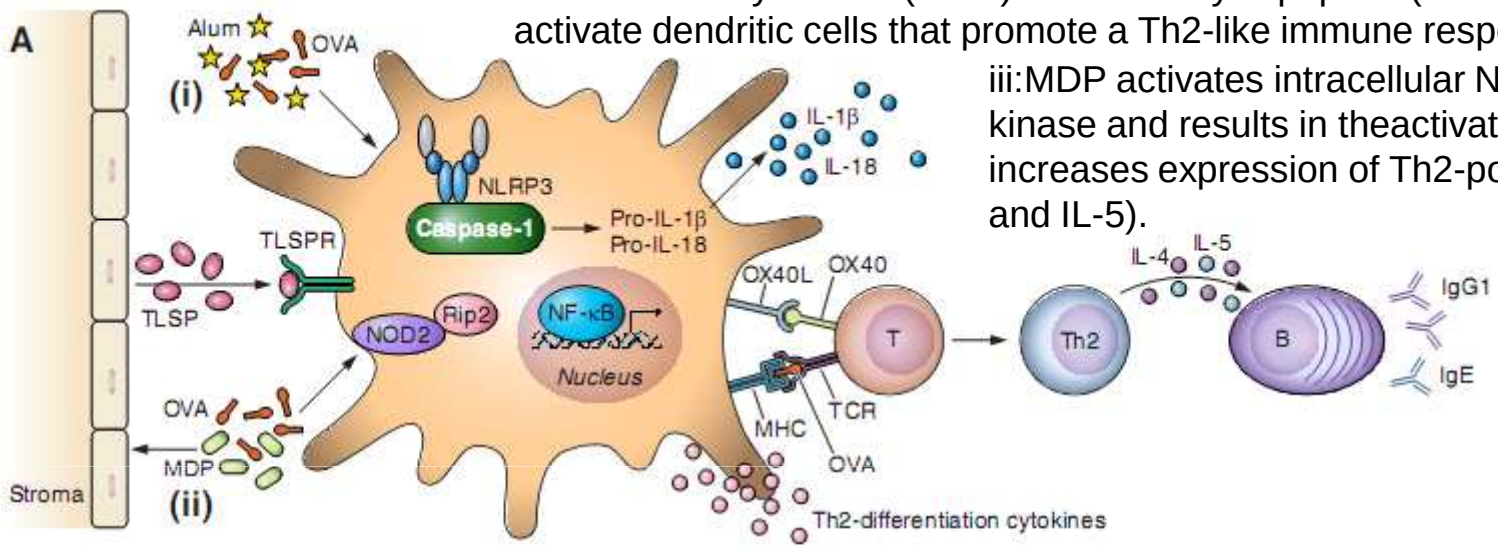
- large family of receptors that **bind to carbohydrates in a calcium-dependent manner; family** share one or more CRD (carbohydrate-recognition domains).
- involved in **fungal recognition** and the moulation of the innate immune response
- Group I: mannose receptors
- Group II: asialoglycoprotein receptor
- CLRs include **Dectin-1, Mincle, DC-SIGN, DC-SIGNR** and **MBL**.

Bridging innate-adaptive immune responses

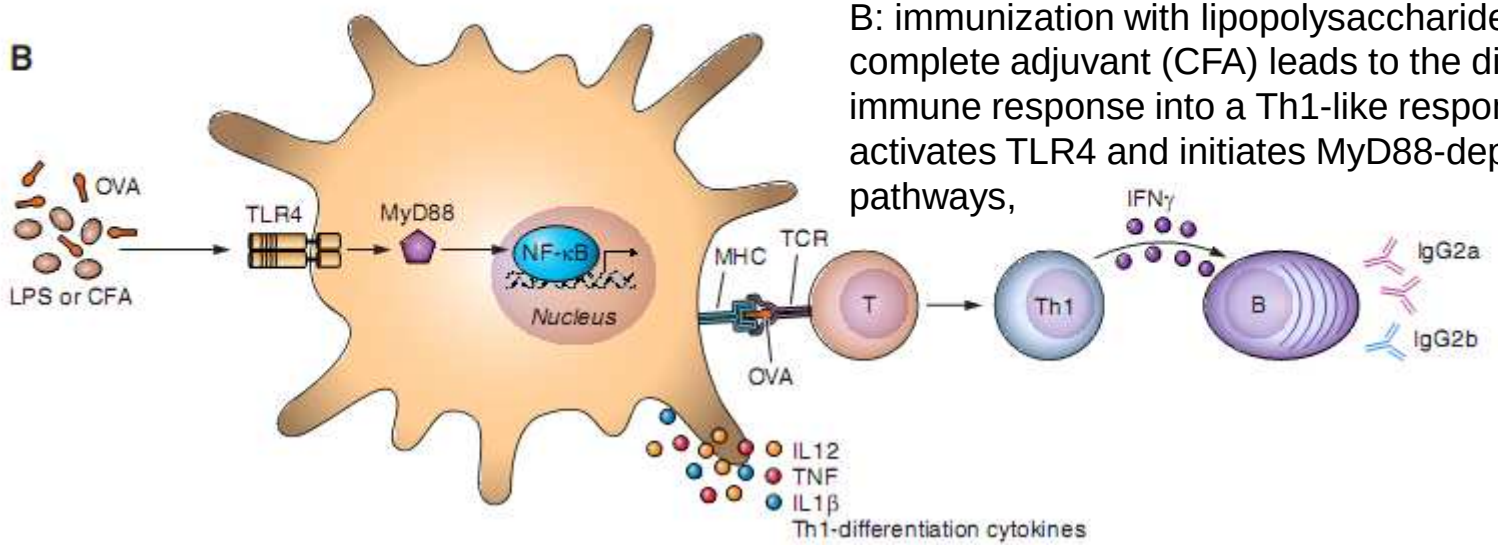
A: aluminum hydroxide (Alum) and muramyl dipeptide (MDP) differentially activate dendritic cells that promote a Th2-like immune response.

iii:MDP activates intracellular NOD2 that recruits RIP2 kinase and results in theactivation of NF-B that increases expression of Th2-polarizing cytokines (IL-4 and IL-5).

Alum activates the NLRP3 inflammasome leading to the processing and release of proinflammatory cytokines, IL-1 and IL-18.



B: immunization with lipopolysaccharide (LPS) or Freund's complete adjuvant (CFA) leads to the differentiation of the immune response into a Th1-like response. LPS or CFA activates TLR4 and initiates MyD88-dependent signaling pathways,



CHEMOTAXIA

Chemotaxia

INFLAMMASOME

Inflammasome

- **Definition**: = multimeric protein complex that assemble in the cytosol of activated myeloid cells as part of intracelullar inflammatory signalling cascade in response to PAMPs or DAMPs; it is a part of innate immune system machinery
- **History:** discovered by Tschopp (2002) ; Martinon et al.[2002] =subset of NLRs named NLRP1 assemble and oligomerize into a multi-molecular complex (dubbed 'inflammasome) which collectively activated the caspase-1
- **Cells:** myeloid cells (neutrophiles, basophiles, eosinophiles) analogous to the apoptosome (activates apoptotic cascades); present in distinct intracellular compartments (cytoplasm, secretory vesicles)
- **Composition:** common canonical inflammasomes serve as a scaffold to recruit the inactive zymogen **pro-caspase-1**. Steps:
  - Inflammasome binds to and apposits many **pro-caspase-1 molecules** (caspase-1 precursor, protein p45, 45 kDa) via its own **CARD (Caspase Activation and recruitment domain)** or through CARD of the adaptor **protein ASC** which it binds to during inflammasome formation.
  - Pro-caspases-1 **autocatalyticaly cleave** into p20 and p10 subunits, which then assemble into active cysteine-dependent protease **caspase-1** (heterodimer consisting of p20 and p10)
  - Caspase -1 cause proteolytic cleavage and of **inactivation of IL-33,** cleavage and activatiopn of pro-IL-1B into **IL1β**, of pro-IL-18 into **IL-18** to induce **IFN-γ secretion** and **natural killer cell activation,** cleavage and
  - **pyroptosis =** .inflammatory form of cell death - DNA fragmentation, cell pore formation,
  - activation of lipid biosynthesis, inhibition of glycolytic enzymes,
  - secretion of tissue-repair mediators such as pro-IL-1α.

Families of inflammasomes & related disorders

- **NLR family** — NLRP1, NLRP3, NLRC4 ; two common features: the first is a nucleotide-binding domain (NBD) which is bound by ribonucleotide-phosphates (rNTP) and is important for self-oligomerization.C-terminus leucine-rich repeat (LRR), which serves as a ligand-recognition domain for other receptors (e.g. TLR) or microbial ligands.
- **PYHIN family** (pyrin and HIN domain-containing protein) - AIM2 (Absent In Melanoma 2) (dsDNA).

NLRC4

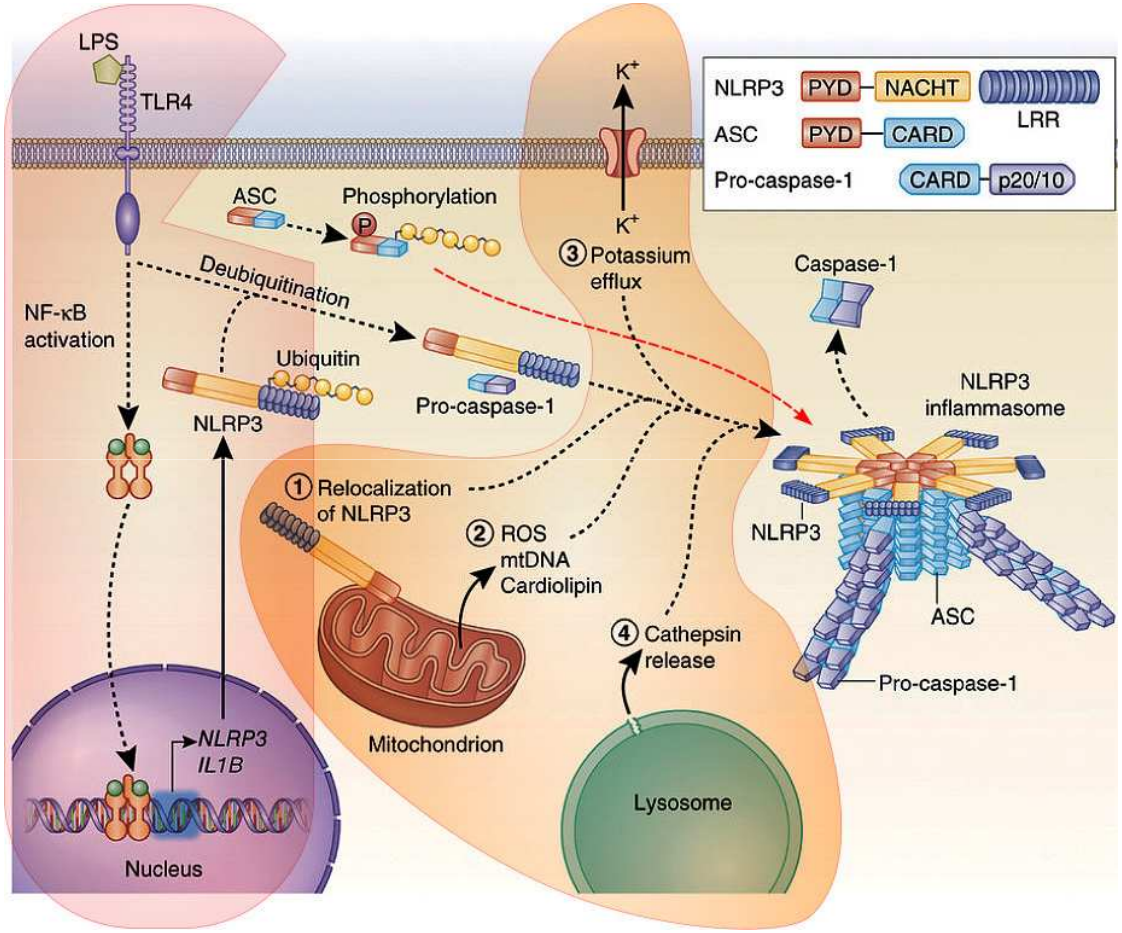
- activating mutations in humans cause **autoinflammatory syndrome** (acute fever, hepatitis, very high serum ferritin, and other features suggestive of **Macrophage Activation Syndrome (MAS).**
- potentially life-threatening **enterocolitis during early childhood**; in these patients, chronic and extraordinary elevation of serum IL-18
- **cold-induced urticaria** that was caused by a dominantly inherited NLRC4 mutation

Families of inflammasomes

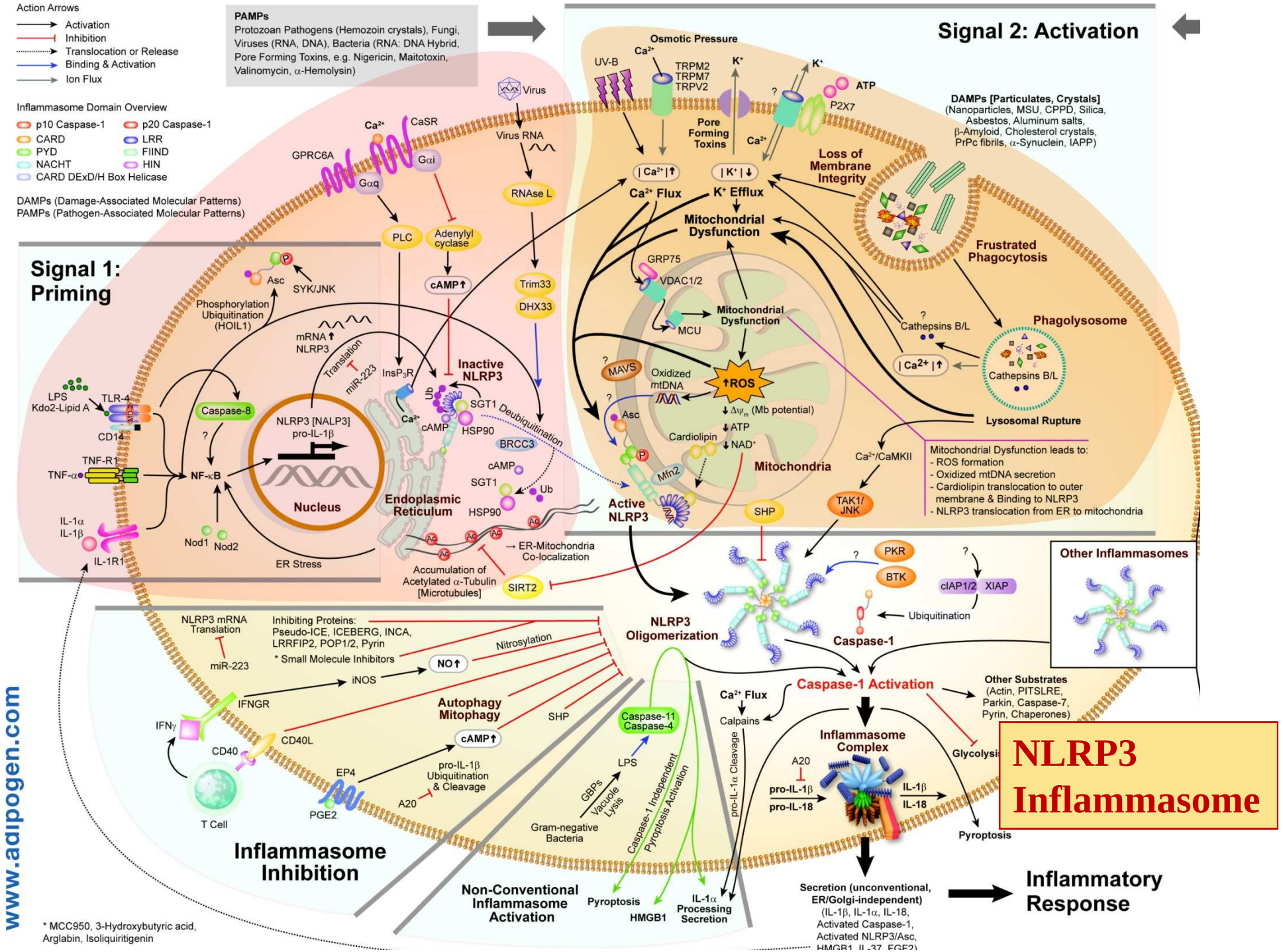
NLRP3

- expressed predominantly in **macrophages**
- mutations in the NLRP3 gene associated with a number of **organ specific dominantly inherited autoimmune diseases**, called **cryopyrin-associated periodic syndrome (CAPS).** This includes familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), chronic infantile neurological cutaneous and articular (CINCA) syndrome, and neonatal-onset multisystem inflammatory disease (NOMID)
- defects in this gene have also been linked to **familial Mediterranean fever**
- role in the pathogenesis **of gout, in atherosclerosis, neuroinflammation- protein-misfolding diseases Alzheimer's, Parkinson's, and Prion diseases**.
- **carcinogenesis;** downregulated or completely lost in human hepatocellular carcinoma.
- oligomerization is activated by a large number of stimuli,
- viruses e.g. influenza A, Neisseria gonorrhoeae, bacterial toxins e.g. nigericin and maitotoxin, liposomes, urban particulate matter, inorganic particles like Titaniumdioxide, Siliciumdioxide, asbestos, crystallized endogenous molecules (cholesterol crystals, monosodium urate crystals)

Mechanisms of NLRP3 inflammasome activation



- **NLRP3** must be **primed before activation**. Priming involves two distinct steps.
- **NF-κB–activating stimulus**, such as LPS binding to TLR4, induces elevated **expression of NLRP3** (as well as **IL1B**) followed bt its deubiqutination.
- The **adaptor protein ASC**is ubiquitinated and phosphorylated for inflammasome assembly
- After priming, canonical NLRP3 inflammasome activation requires a second, **distinct signal to activate NLRP3** and lead to the formation of the NLRP3 inflammasome complex. The most commonly accepted activating stimuli for NLRP3 include **relocal-ization of NLRP3 to the mitochondria,** mitochondrial factors release into the cytosol (mitochondrial ROS, mitochondrial DNA, or cardiolipin), **potassium efflux** through ion channels, and **cathepsin release f**ollowing destabilization of lysosomal mem-branes.


Action Arrows
Activation
Inhibition
Translocation or Release
Binding & Activation
Ion Flux
Inflammasome Domain Overview
p10 Caspase-1
p20 Caspase-1
CARD
LRR
PYD
FIIND
NACHT
HIN
CARD DExD/H Box Helicase
DAMPs (Damage-Associated Molecular Patterns)
PAMPs (Pathogen-Associated Molecular Patterns)
PAMPs
Protozoan Pathogens (Hemozoin crystals), Fungi, Viruses (RNA, DNA), Bacteria (RNA: DNA Hybrid, Pore Forming Toxins, e.g. Nigericin, Maitotoxin, Valinomycin, α-Hemolysin)
Signal 1: Priming
Signal 2: Activation
Virus
Virus RNA
RNAse L
Ca2+
CaSR
GPRC6A
Gαi
Gαq
PLC
Adenylyl cyclase
cAMP↑
Trim33
DHX33
Asc
SYK/JNK
Phosphorylation Ubiquitination (HOIL1)
mRNA↑ NLRP3
Translation
miR-223
InsP3R
Inactive NLRP3
Ub
SGT1
HSP90
Deubiquitination
BRCC3
LPS
Kdo2-Lipid A
TLR-4
MD-2
CD14
TNF-R1
TNF-α
Caspase-8
NF-κB
NLRP3 [NALP3]
pro-IL-1β
Nucleus
Endoplasmic Reticulum
IL-1α
IL-1β
IL-1R1
Nod1
Nod2
ER Stress
→ ER-Mitochondria Co-localization
Accumulation of Acetylated α-Tubulin [Microtubules]
SIRT2
Osmotic Pressure
UV-B
TRPM2
TRPM7
TRPV2
Pore Forming Toxins
ATP
P2X7
DAMPs [Particulates, Crystals]
(Nanoparticles, MSU, CPPD, Silica, Asbestos, Aluminum salts, β-Amyloid, Cholesterol crystals, PrPc fibrils, α-Synuclein, IAPP)
Loss of Membrane Integrity
Ca2+ Flux
K+ Efflux
Mitochondrial Dysfunction
Frustrated Phagocytosis
GRP75
VDAC1/2
MCU
Cathepsins B/L
Phagolysosome
Lysosomal Rupture
MAVS
Oxidized mtDNA
↑ROS
↓Δψm (Mb potential)
↓ATP
↓NAD+
Cardiolipin
Mitochondria
Mfn2
Active NLRP3
SHP
Ca2+/CaMKII
TAK1/ JNK
Mitochondrial Dysfunction leads to:
- ROS formation
- Oxidized mtDNA secretion
- Cardiolipin translocation to outer membrane & Binding to NLRP3
- NLRP3 translocation from ER to mitochondria
PKR
BTK
cIAP1/2
XIAP
Ubiquitination
Caspase-1
NLRP3 Oligomerization
Other Inflammasomes
NLRP3 mRNA Translation
miR-223
Inhibiting Proteins: Pseudo-ICE, ICEBERG, INCA, LRRFIP2, POP1/2, Pyrin
* Small Molecule Inhibitors
Nitrosylation
NO↑
iNOS
IFNGR
IFNγ
CD40L
CD40
T Cell
EP4
PGE2
cAMP↑
pro-IL-1β Ubiquitination & Cleavage
A20
Autophagy Mitophagy
SHP
Caspase-11 Caspase-4
LPS
GBPs
Vacuole Lysis
Gram-negative Bacteria
Caspase-1 Independent Pyroptosis Activation
Caspase-1 Activation
Ca2+ Flux
Calpains
pro-IL-1α Cleavage
Other Substrates (Actin, PITSLRE, Parkin, Caspase-7, Pyrin, Chaperones)
Glycolysis
Inflammasome Complex
A20
pro-IL-1β
pro-IL-18
IL-1β
IL-18
Pyroptosis
Inflammasome Inhibition
Non-Conventional Inflammasome Activation
Pyroptosis
HMGB1
IL-1α Processing Secretion
Secretion (unconventional, ER/Golgi-independent)
(IL-1β, IL-1α, IL-18, Activated Caspase-1, Activated NLRP3/Asc, HMGB1, IL-37, EGF2)
Inflammatory Response
NLRP3 Inflammasome
www.adipogen.com
* MCC950, 3-Hydroxybutyric acid, Arglabin, Isoliquiritigenin

Other inflammasomes