

Rezeptoren der angeborenen Immunität

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Rezeptoren der angeborenen Immunität: *The immunologist's dirty little secret* (C. Janeway)

Hypothese (C. Janeway, 1989):

- ➔ Angeborenes Immunsystem entscheidet, ob das adaptive Immunsystem auf Antigene reagiert (Unterscheidung zwischen „Selbst“ und Fremd“)
- ➔ Angeborenes Immunsystem erkennt mikrobielle und virale Bestandteile mit essentiellen Funktionen

Pattern Recognition Receptors (PRR) ↔ Pathogen [!]Associated Molecular Patterns (PAMP)
(Keimbahnkodierte definierte Spezifität !)

Grundlage der Adjuvans-Wirkung ! (lat. adjuvare: helfen)

! Microbial.....(MAMP)
! Danger.....(DAMP)

➔ Erkennung von Lipopolysaccharid (LPS): Schlüssel zur Lösung

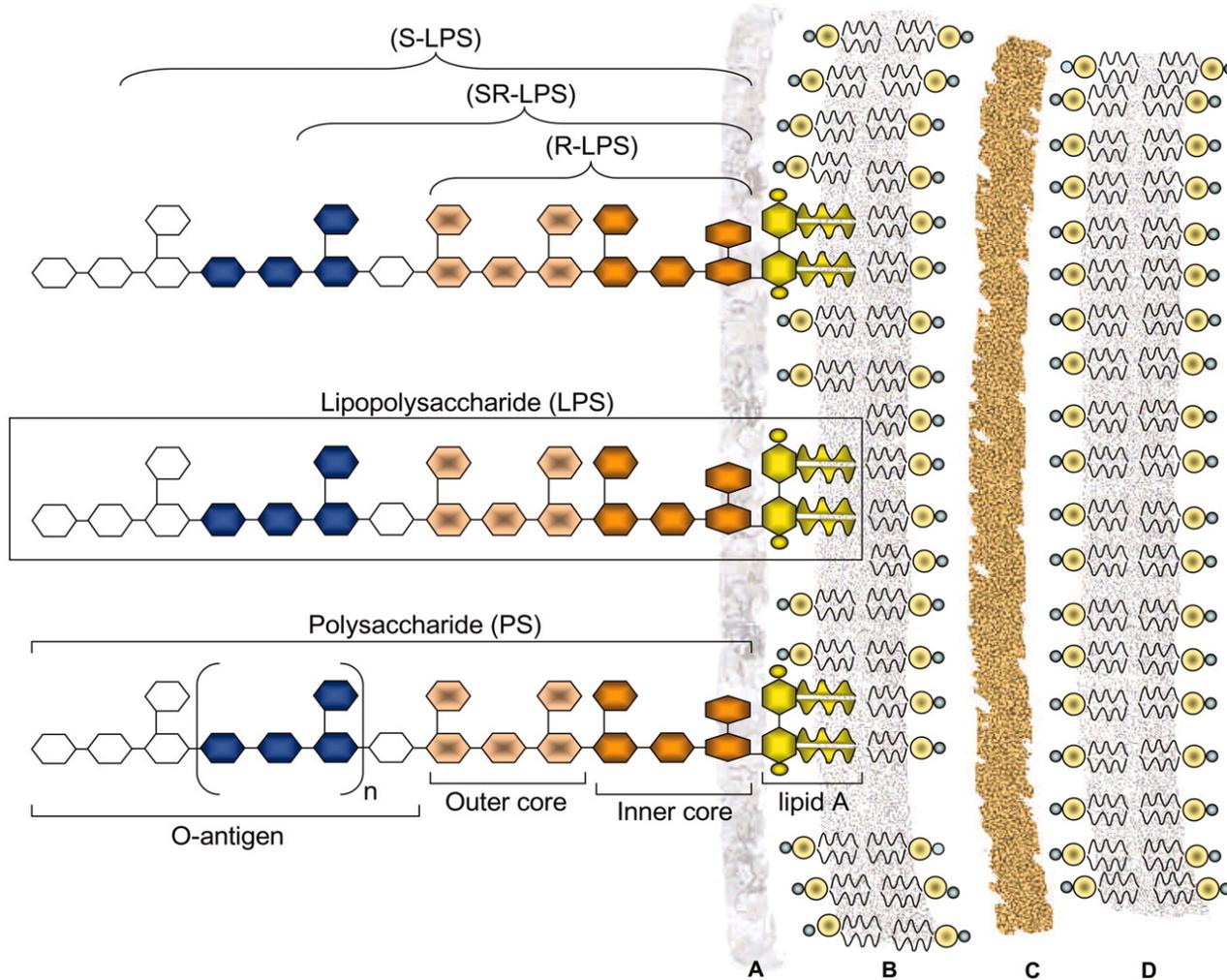


LPS (Endotoxin) löst tödlichen septischen Schock aus („Cytokinsturm“; in C57BL/6 sind $LD_{50}=25\mu\text{g}$ und $LD_{100}=50\mu\text{g}$ LPS)
Positionelles klonieren LPS- insensitiver Mäuse (LPS^d) führte zur Entdeckung des LPS Rezeptors
(Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science, 1998)

Homologien zu IL-1R und zu „toll“ in Drosophila führten zu den Bezeichnungen TIR (Toll IL-1 Rezeptor) und Toll-like Rezeptor

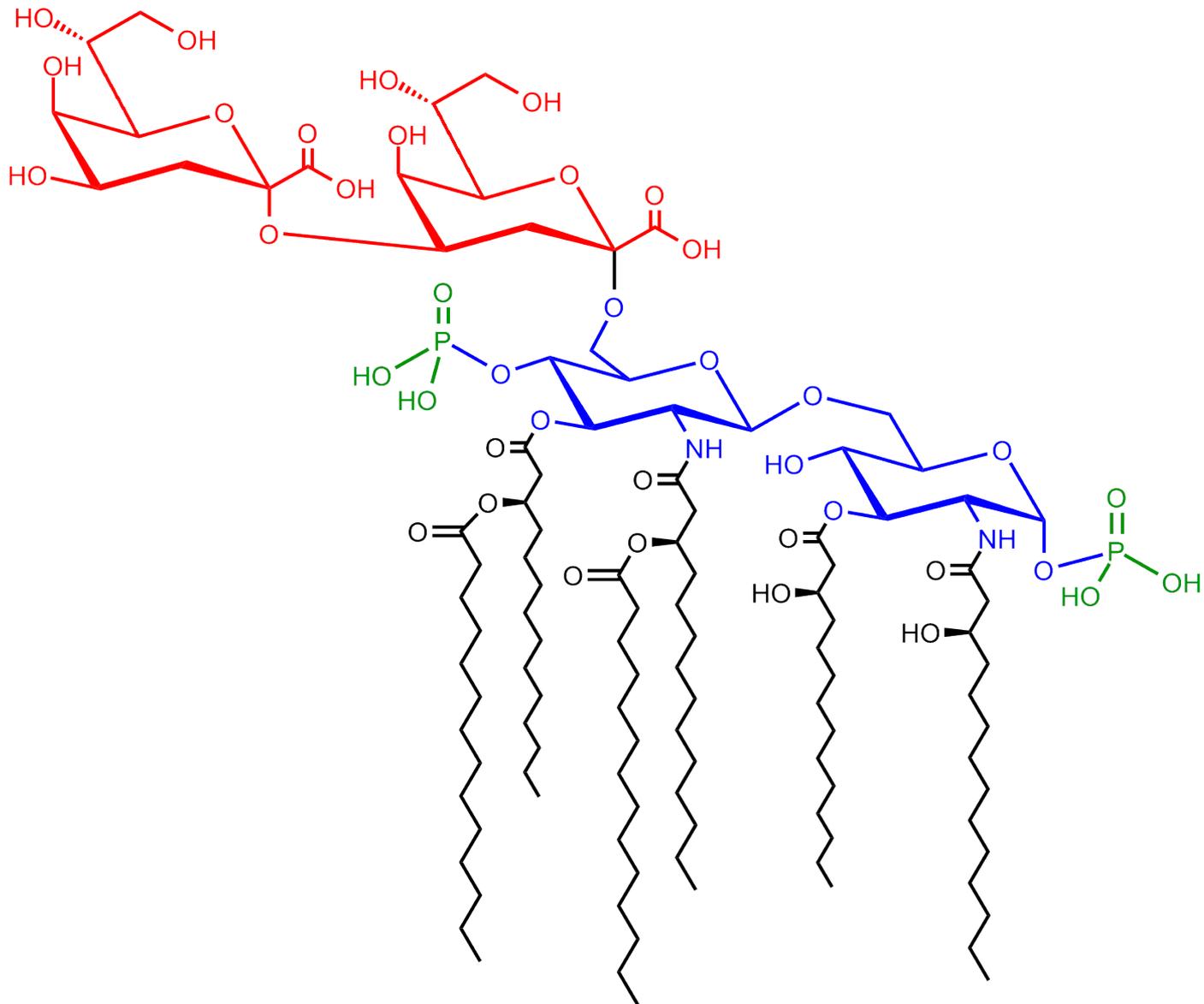
Heutige Bezeichnung des LPS Rezeptors ist TLR4

Lipopolysaccharide (LPS) on the outer membrane of a Gram-negative bacterium.



- A Kapsel (fakultativ)
- B äußerer Lipid-Bilayer
- C Peptidoglycan
- D innerer Lipid-Bilayer

Invarianter Teil des LPS wird erkannt: Lipid A



LPS Rezeptorkomplex besteht aus CD14, MD-2 und TLR-4
 LPS wird von LPS Bindeprotein (LBP) gebunden und übertragen

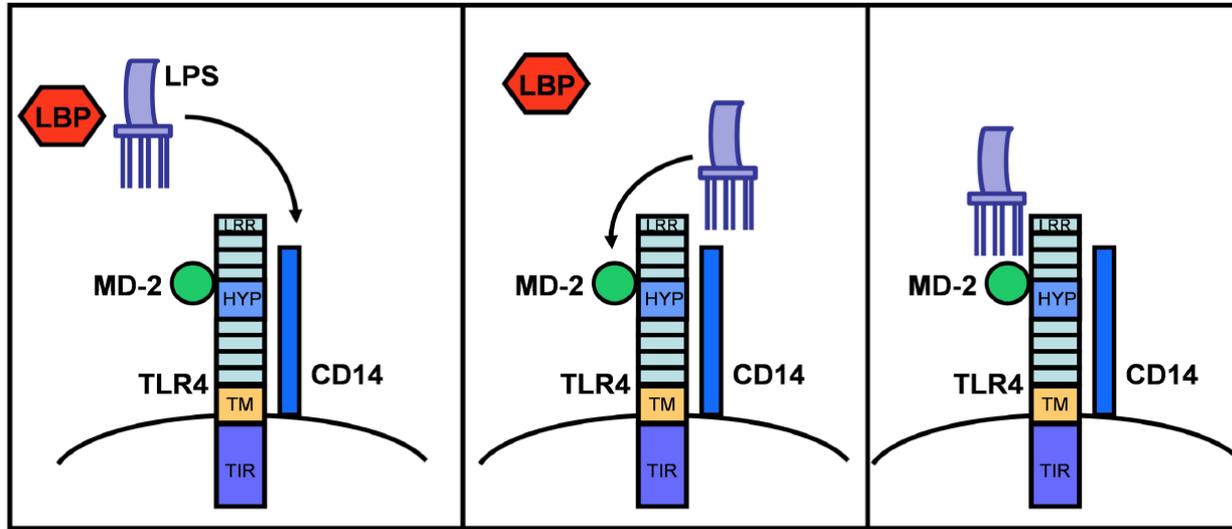
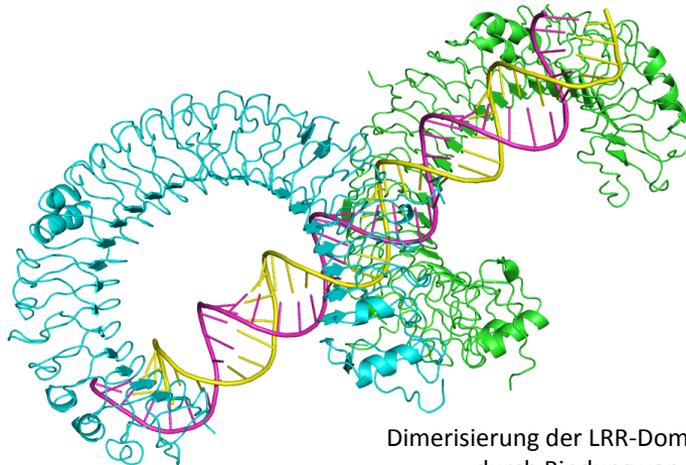
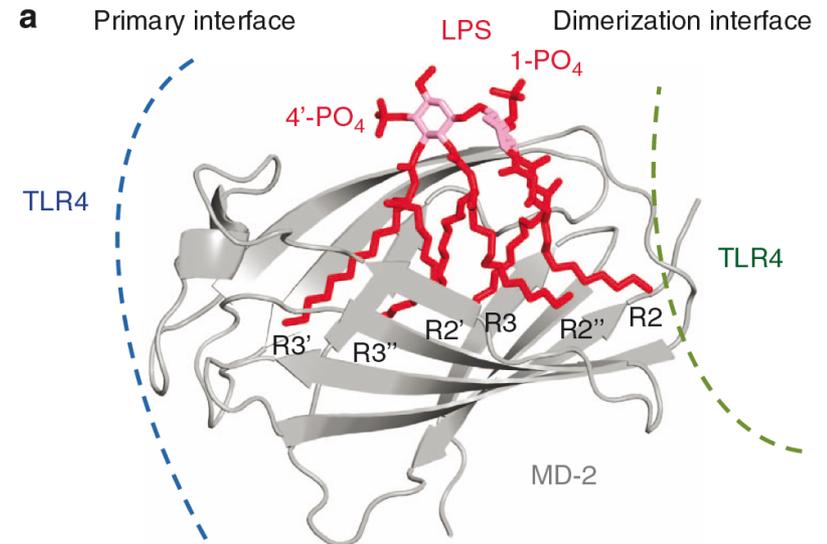
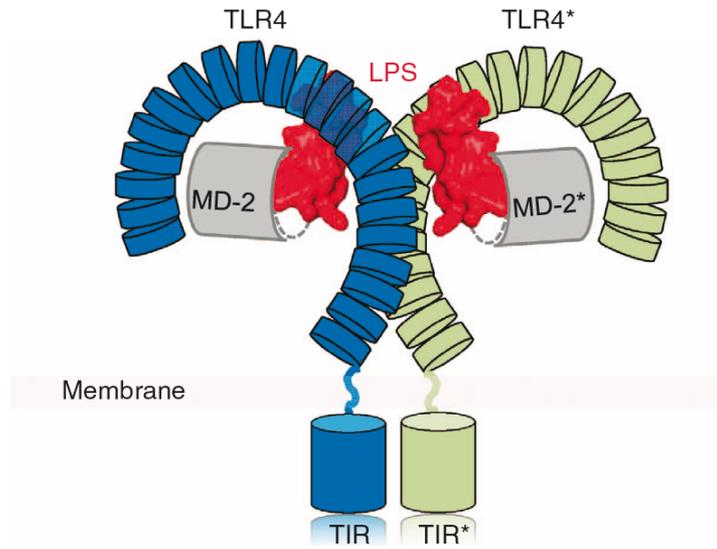


FIGURE 1 | LPS sensing via LBP and the CD14/MD-2/TLR4 receptor complex. TLR4 consists of an extracellular domain with leucine-rich repeats (LRR), a hypervariable domain (HYP), a transmembrane domain (TM), and a

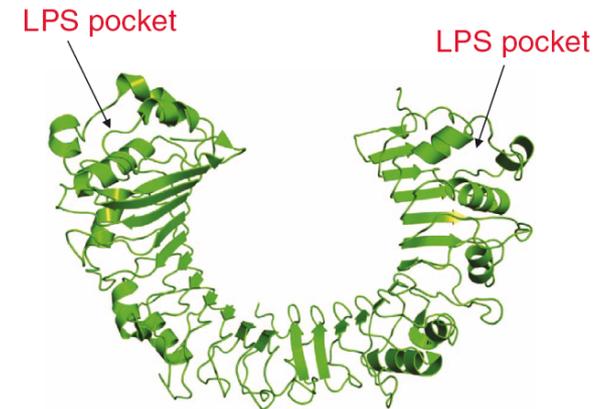
cytoplasmic domain with a highly conserved TIR-domain. After binding to LBP in serum, LPS is transferred to CD14 and then to the MD-2/TLR4 complex. This illustration is based on mouse and human TLR4 knowledge.

☞ CD14 (lösl./membrangebunden) und LBP wirken verstärkend, sind aber entbehrlich

Die ligandenbindenden Domänen der TLR haben Leucin-reiche Sequenzwiederholungen (LRR) und eine hufeisenförmige Struktur

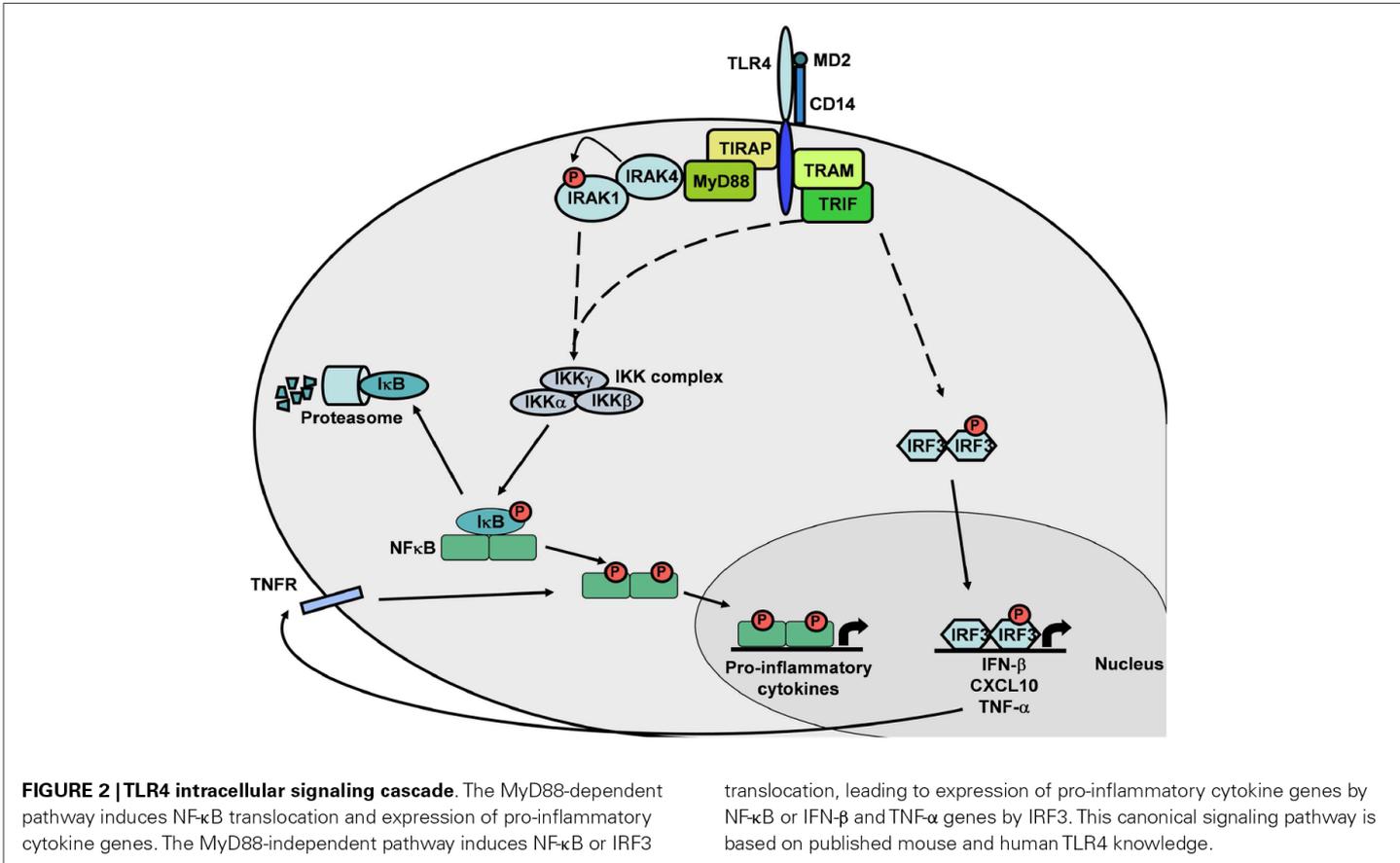


Dimerisierung der LRR-Domänen des TLR3 durch Bindung von dsRNA
(Quelle: Protopedia.org)



CD14

TLR-4 aktiviert 2 Signalwege

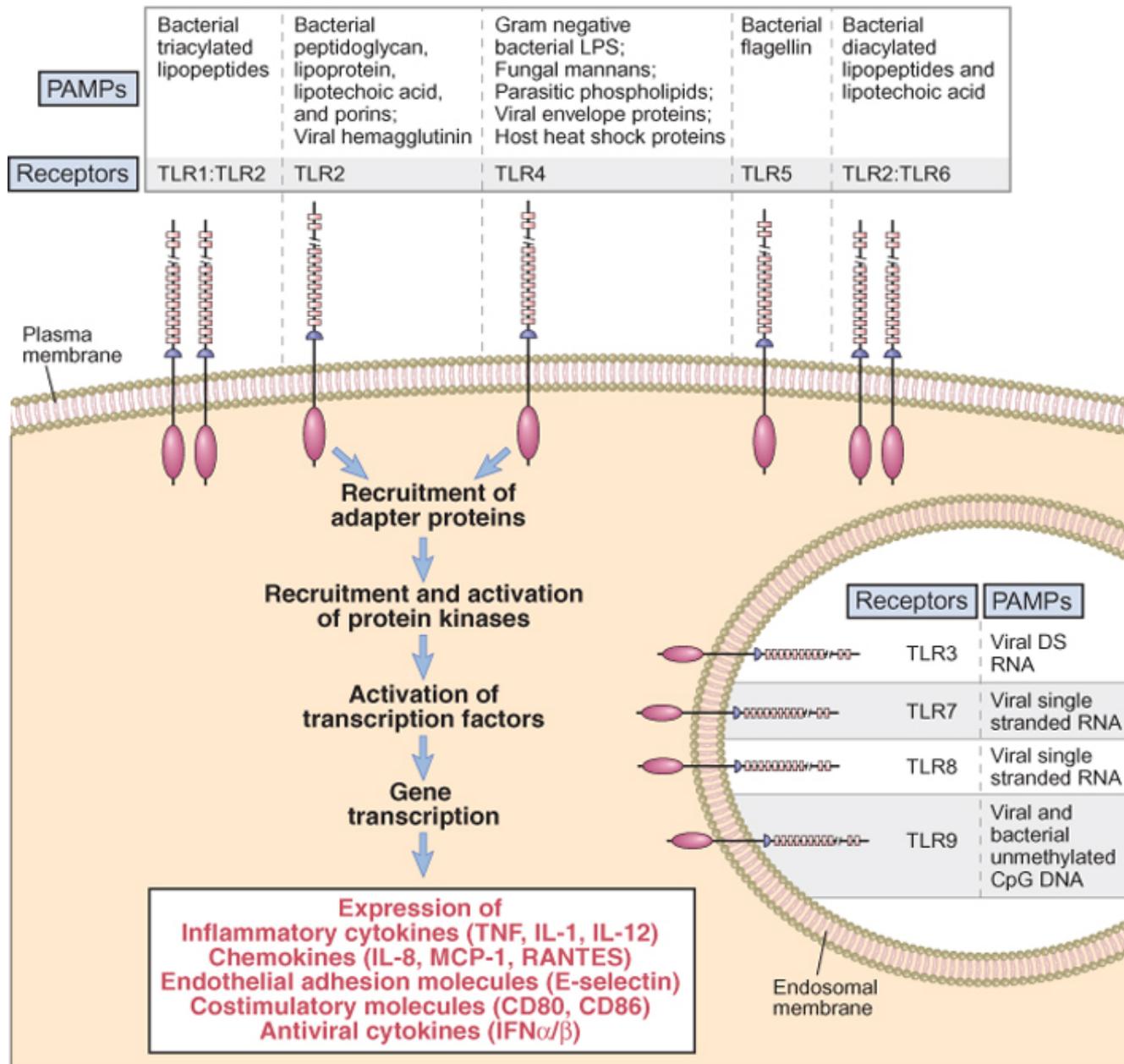


MyD88 Signalweg bewirkt Produktion pro-inflammatorischer Cytokine

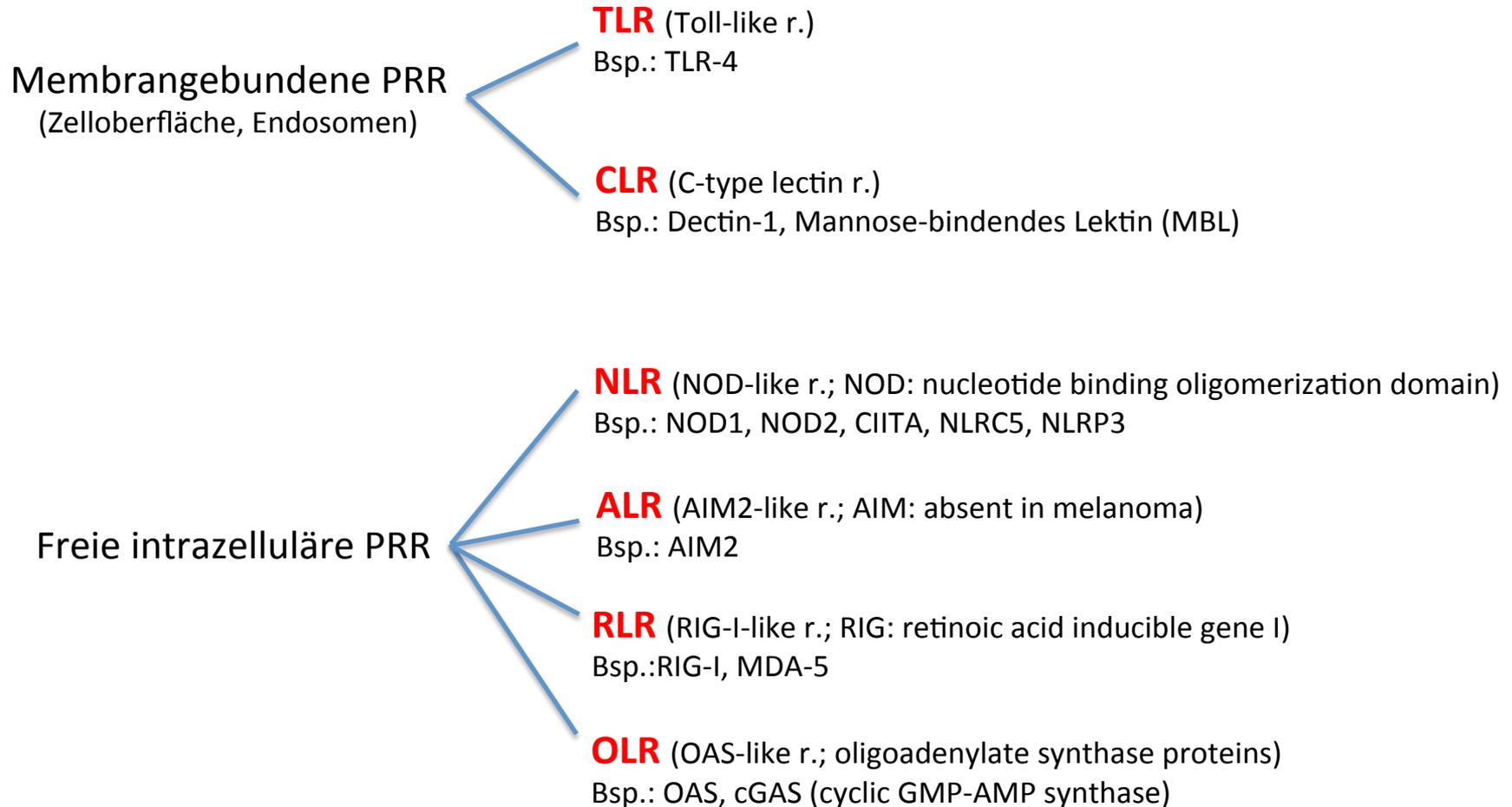
TRIF Signale (von endosomalem TLR-4) wichtig für DC-Reifung (Expression von CD40, CD80, CD86), Produktion von Typ I –Interferonen und späte Phase der NF-κB Aktivierung



TRIF-Signalweg erst nach CD14-induziertem Transport des LPS-Rezeptorkomplexes in Endosomen; Erst dort Kontakt mit TRIF/TRAM



PRR können auf Grund von Proteinhomologien in 6 Familien oder nach Lokalisation in 2 topologische Klassen gegliedert werden



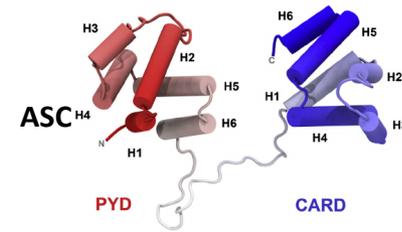
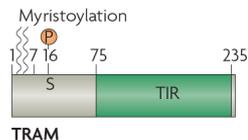
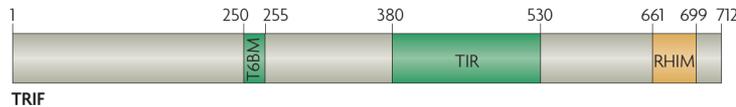
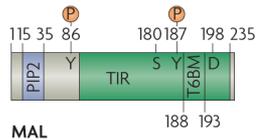
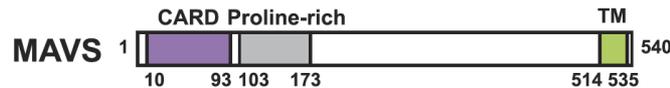
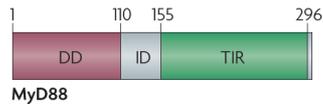
**Transkription proinflammatorischer Cytokine und Interferone
Induktion von Phagozytose, Autophagie, Zelltod („Pyroptosis“)**

Adapter-Proteine verbinden PRR mit Signalkaskaden



Adapter-Proteine integrieren Signale mehrerer PRR: Herausragende Bedeutung
 Adapter-Proteine definieren zugehörige Signalwege

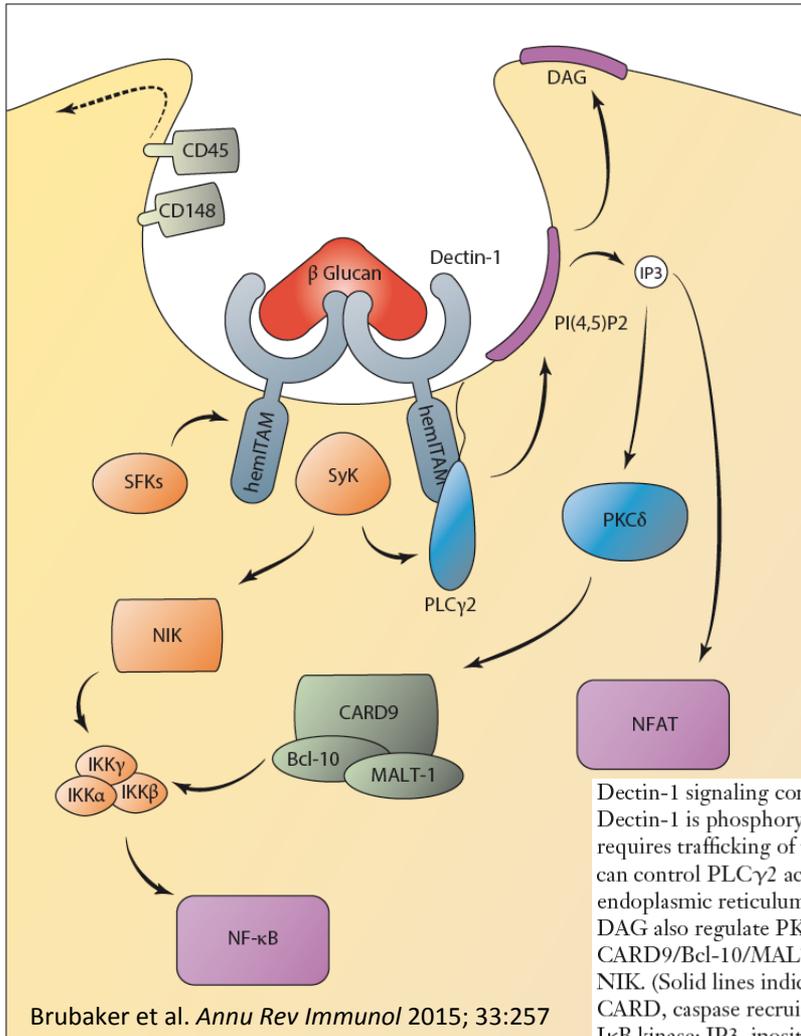
Adapter/Adapter Set	Interaktion mit PRR	Interaktion mit Signalmolekülen	Lokalisation
TIRAP (MAL)/MyD88	TIR-Domäne	Death-Domäne	Zelloberfläche, Endosomen
TRAM/TRIF	TIR-Domäne	TRAF-binde-Domäne, RHIM-Domäne	Zelloberfläche, Endosomen
MAVS	CARD-Domäne	Prolinreiche Region	Mitochondrien- u. Peroxisomenmembran
ASC	PYRIN-Domäne	CARD-Domäne	Cytosol, Mitochondrien



ASC: apoptosis-associated speck-like protein containing a CARD
 CARD: caspase recruitment domain
 MAVS: mitochondrial antiviral signaling protein
 RHIM: RIP homotypic interaction motif
 TIR: Toll/IL-1 receptor
 TIRAP: TIR-containing adapter protein
 TRAF: TNF receptor-associated factor
 TRAM: TRIF-related adapter molecule
 TRIF: TIR-domain-containing adapter inducing IFN- β

Dectin-1: Ein C-type lectin receptor (CLR) induziert Phagozytose und Genexpression

Hunderte CLR bilden heterogene Gruppe, die Kohlenhydrate oft Calcium-abhängig (Name!) binden. CLR können löslich oder membrangebunden sein, viele wirken als Opsonine



Brubaker et al. *Annu Rev Immunol* 2015; 33:257

Dectin-1 ist exprimiert auf DC, Makrophagen, Monocyten, PMN, Mastzellen (myeloide Zellen)

Hauptligand β -1,3-Glucane aus Pilzen

Benötigt kein Calcium zur Ligandenbindung

Dectin-1 signaling controls NF- κ B and NFAT activation. Upon ligand binding, the hemITAM domain of Dectin-1 is phosphorylated by Src family kinases (SFKs), which recruit the signaling kinase Syk. This requires trafficking of the phosphatases CD45 and CD148 away from the forming phagosome. Syk activation can control PLC γ 2 activity to induce the formation of IP3 and DAG. IP3-mediated Ca^{2+} release from the endoplasmic reticulum induces a CRAC-dependent Ca^{2+} influx and consequent NFAT activation. Ca^{2+} and DAG also regulate PKC δ activation, which controls canonical NF- κ B activation following induction of a CARD9/Bcl-10/MALT-1 complex. In parallel, Syk also controls noncanonical NF- κ B activation through NIK. (Solid lines indicate signal transduction; dotted lines indicate trafficking events.) (Abbreviations: CARD, caspase recruitment domain; CRAC, calcium-release-activated calcium; DAG, diacylglycerol; IKK, I κ B kinase; IP3, inositol 1,4,5-trisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; NFAT, nuclear factor of activated T cells; NIK, NF- κ B-inducing kinase; PKC, protein kinase C.)

NOD-like Rezeptoren: Intrazelluläre Sensoren für bakterielle Infektionen und Zellschädigung

Subfamily	Gene	Structure
NLRA	CIITA	
NLRB	NAIP	
NLRC	NOD1, NLRC4	
	NOD2	
	NLRC3, NLRC5, NLRX1	
NLRP	NLRP1	
	NLRP2-9, 11-14	
	NLRP10	

Fig. 1. Classification and protein structure of human NOD-like receptor family (based on Ref. 6). AD, acidic transactivation domain; NACHT, for NAIP, CIITA, HET-T, and TP-1; BIR, baculovirus inhibitor of apoptosis repeat; CARD, caspase activation and recruitment domain; X, unidentified; PYD, pyrin domain, FI-IND, function to find domain; , leucine-rich repeat; NOD, nucleotide-binding and oligomerization domain.



Die NOD-Domäne der NLR wird auch als NACHT-Domäne bezeichnet
Wichtig zur Ligandenbindung ist der C-terminale „leucine-rich repeat“

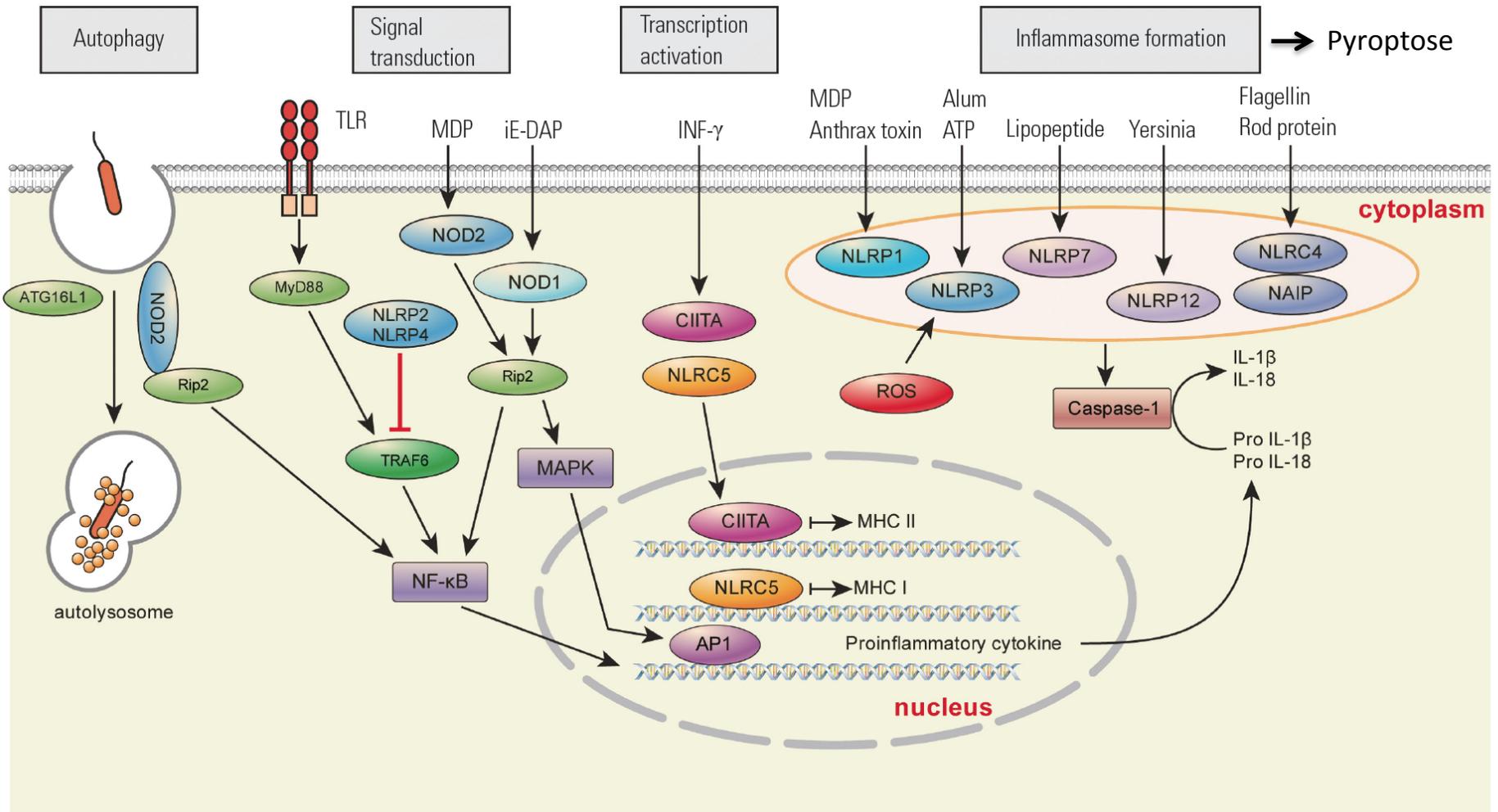


Fig. 2. Functions of NOD-like receptors. The NLRs activities can be divided into four broad categories; autophagy, signal transduction, transcription activation, and inflammasome formation. NOD2 induces autophagy to remove pathogens by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. NOD1 and NOD2 recognize γ -D-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP) respectively; thereafter they activate the NF- κ B and MAPK signaling pathways. NLRP2 and NLRP4 act as negative regulators of NF- κ B pathway by modifying TRAF6. CIITA and NLRC5 are transactivators of major histocompatibility complexes (MHC). Inflammasome-forming NLRs (orange circle) convert procytokines to active IL-1 β and IL-18 by activating caspase-1. NOD, nucleotide-binding and oligomerization domain; NLRs, NOD-like receptors; NF- κ B, nuclear factor kappa B; MAPK, mitogen-activated protein kinase; TRAF, tumor necrosis factor (TNF) receptor-associated factor; IL, interleukin; INF- γ , interferon- γ .

NOD1 und NOD2 werden durch Abbauprodukte bakterieller Zellwände aktiviert

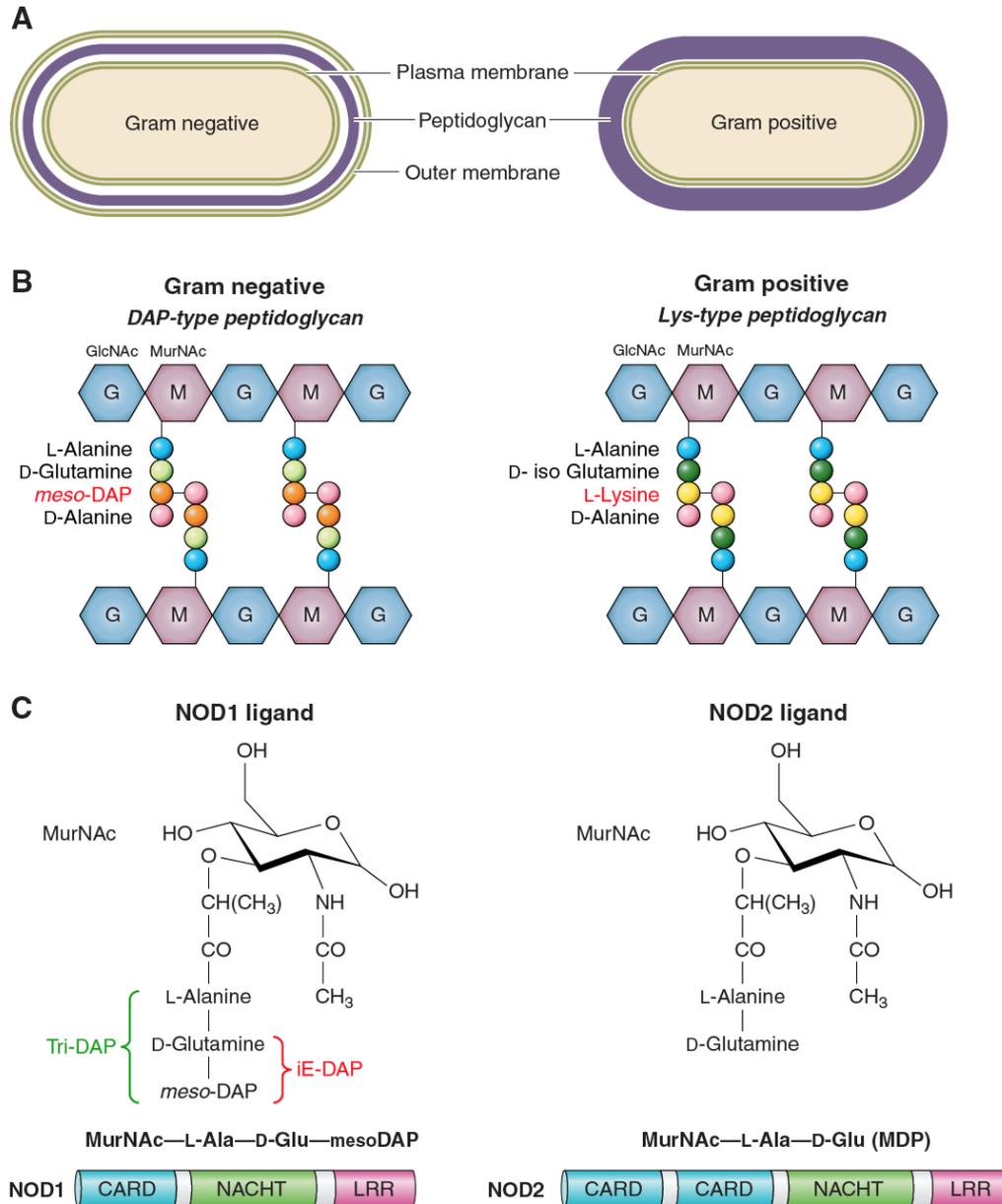


FIGURE 3. Peptidoglycan drives NOD1 and NOD2 activation. **A:** distribution of peptidoglycan (PG) in Gram-negative and Gram-positive bacteria. **B:** schematic illustrating the basic structure of PG found in Gram-negative (DAP-type PG) and Gram-positive (Lys-type PG) bacteria. **C:** NOD1 and NOD2 ligand specificity displaying the minimal structures detected by NOD1 (iE-DAP) and NOD2 (MDP). Tri-DAP, L-Ala-D-Glu-*meso*-diaminopimelic acid; iE-DAP, D-Glu-*meso*-diaminopimelic acid; MDP, muramyl dipeptide; GlcNAc, *N*-acetyl-D-glucosamine; MurNAc, *N*-acetylmuramic acid.

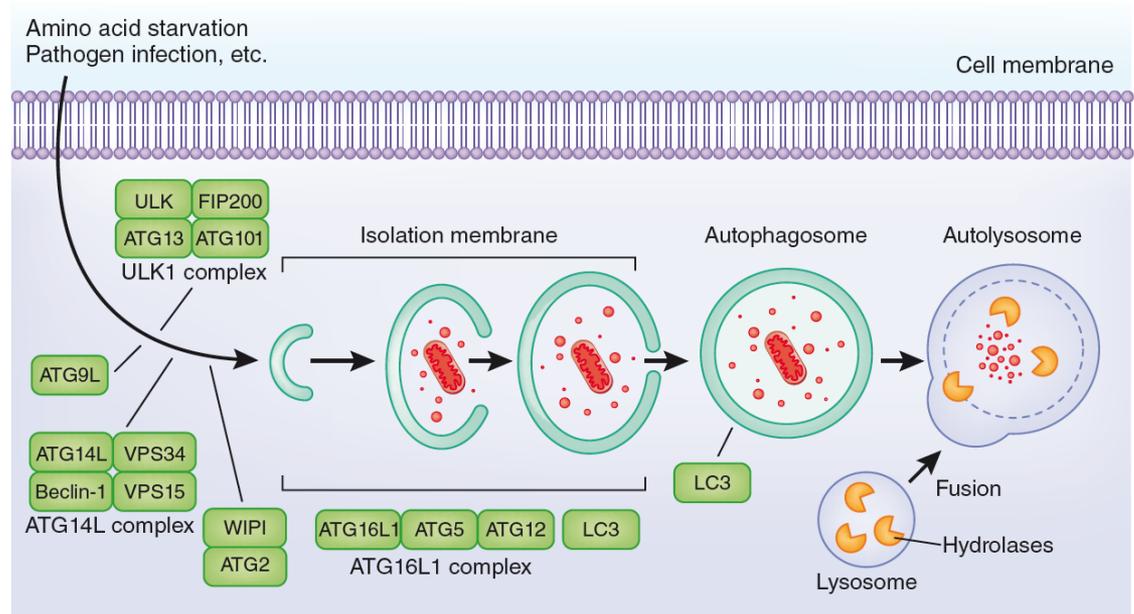
Autophagie als Mechanismus des angeborenen Immunsystems



Abbau von cytoplasmatischem Material und Organellen in Autophagosomen mit doppelter Membran
Dient grundsätzlich der zellulären Homöostase („Grundreinigung“, Wiederverwertung von Rohstoffen)
Steigerbar durch Stress (z. B. Aminosäuremangel)

Kann auch in das Cytoplasma eingedrungene Pathogene eliminieren („Xenophagie“)

Figure 1 The autophagy pathway. Autophagy-inducing signals (for example, amino acid starvation and pathogen infection) initiate formation of the isolation membrane (or ‘phagophore’). Closure of the isolation membrane results in formation of the autophagosome. The subsequent lysosome-autophagosome fusion leads to degradation of the contents of the autophagosome by lysosomal hydrolases in the autolysosome. Labels in green boxes indicate core ATG proteins required for autophagosome formation.



ATG: Autophagy-related proteins
LC3: Erleichtert Fusion mit Lysosomen



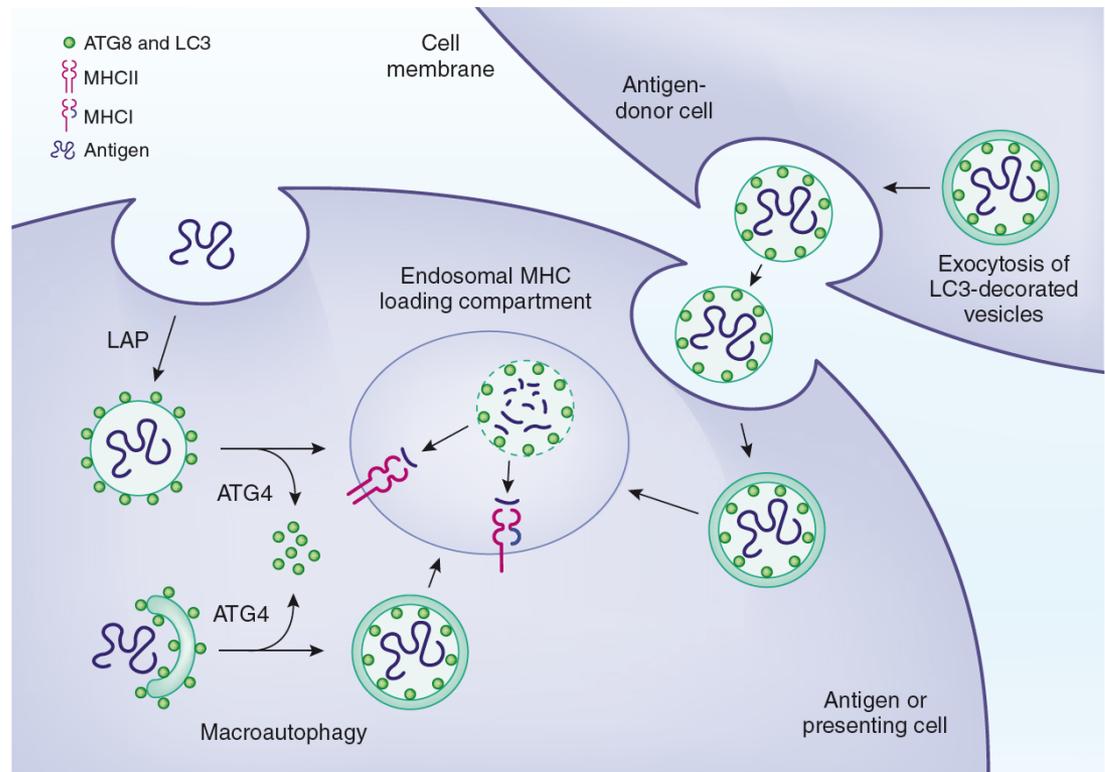
NOD1 und NOD2 können nach Bindung ihrer Liganden (Peptidoglycane) mit ATG Proteinen interagieren und Bildung von Autophagosomen induzieren

Exkurs: Autophagie und Antigenpräsentation

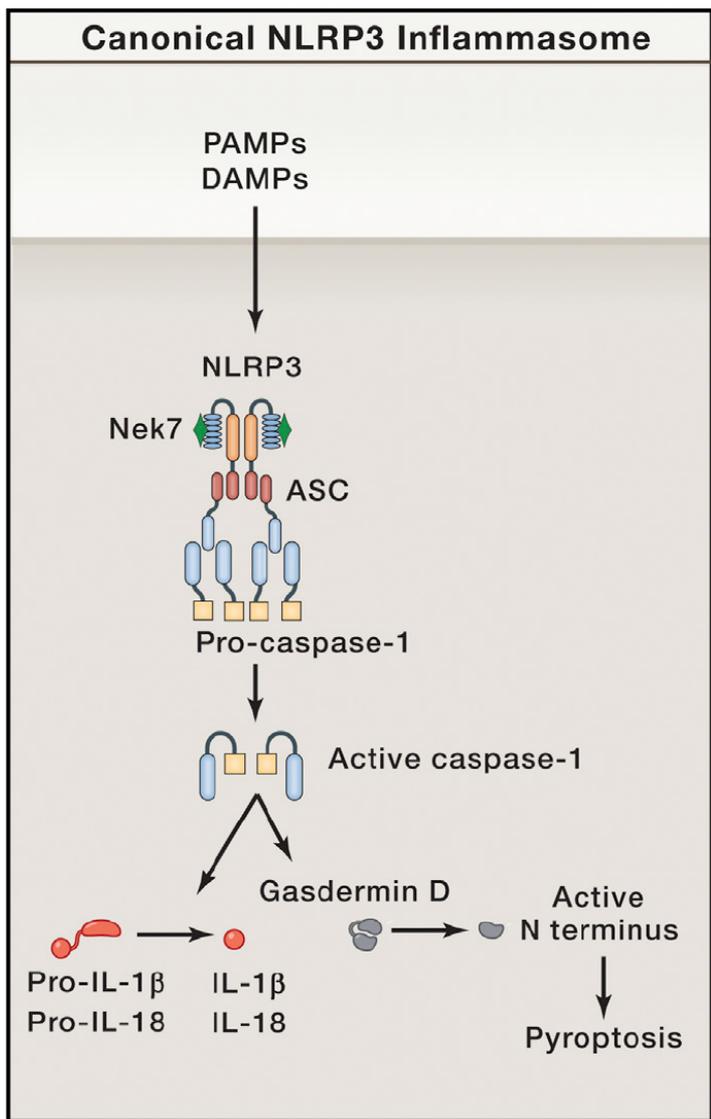


Autophagosomen liefern cytosolische Antigene für die MHC II-Beladung
mTEC betreiben intensive Autophagie für Negativselektion von CD4⁺ T-Zellen
Antigentransfer von Donorzellen auf APC für Kreuzpräsentation durch MHC I

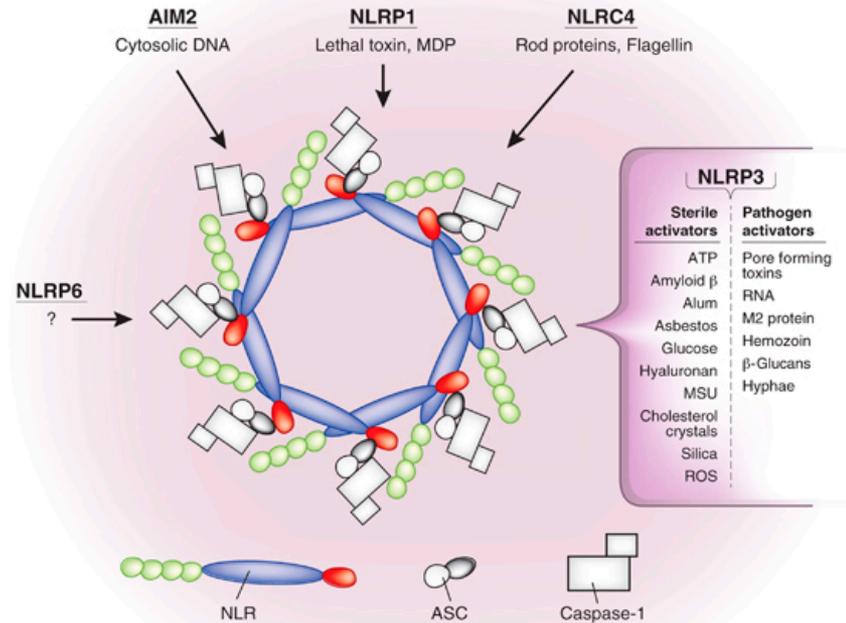
Figure 4 ATG proteins in MHC-restricted antigen presentation. Autophagy-related proteins contribute, by at least three pathways, to the loading of vesicular antigens onto MHC molecules. Autophagosomes can fuse with endosomal MHC loading compartments and deliver cytoplasmic constituents for loading onto MHC class II (MHCII). Loading of MHC class I (MHCI) might also occur in these vesicular compartments, especially under conditions in which the classical processing of classical MHC class I antigens, including import into the ER by TAP, is inhibited. LAP can deliver extracellular antigens to this loading compartment. LC3 might need to be deconjugated from the outer autophagosomal and the LAP phagosome membrane by ATG4 for efficient fusion with the endosomal MHC loading compartment. Finally, signal peptide-independent but autophagy-dependent exocytosis seems to efficiently transfer antigens from donor cells to antigen-presenting cells, mainly for cross-presentation by MHC class I molecules.



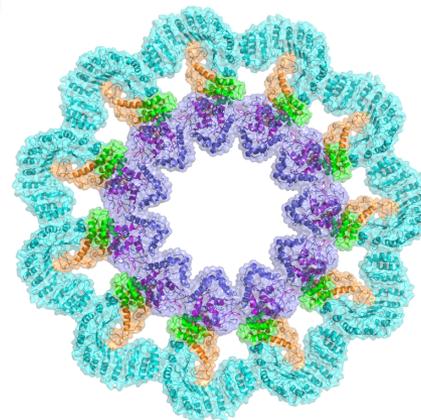
NLR bilden Inflammasomen und aktivieren Caspase-1*



Rathinam & Fitzgerald *Cell* 2016; 165:792

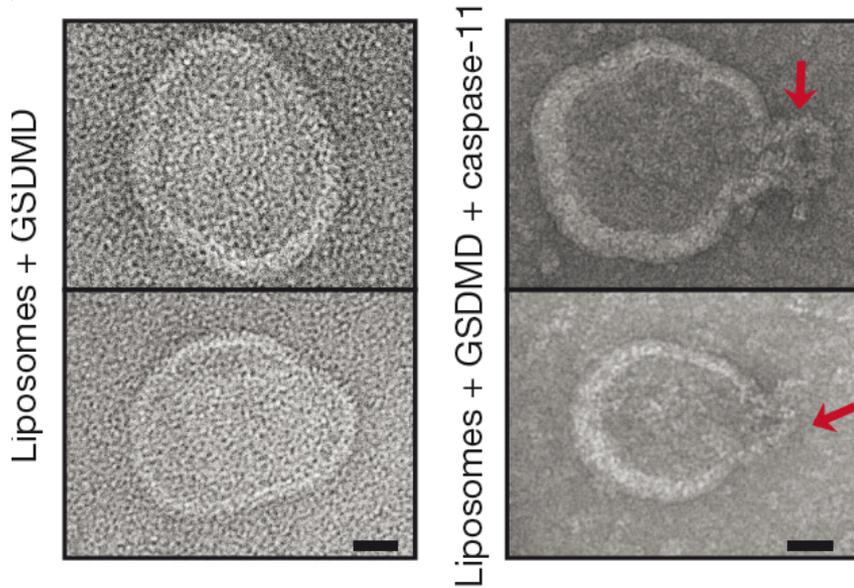


www.researchgate.net/figure/233849027_fig1_Figure-1-Activators-of-the-inflammasomes-The-NLRP3-NLRC4-NLRP1-AIM2-and-NLRP6



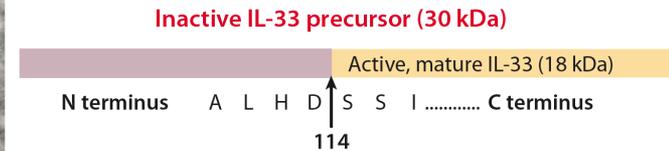
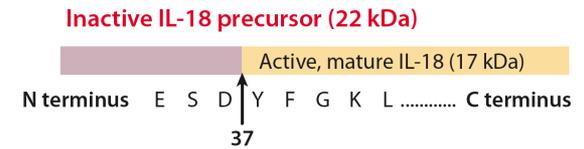
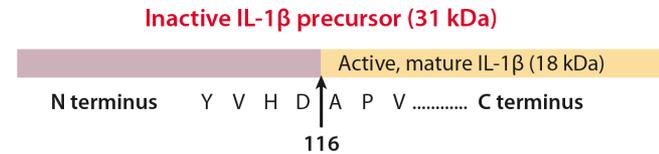
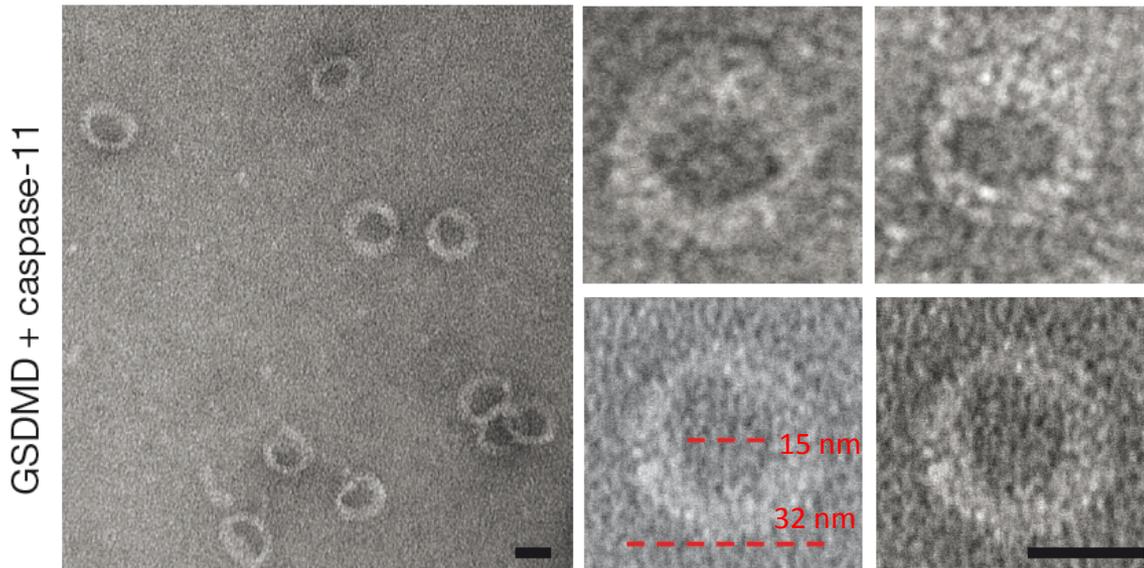
* "C" für Cystein-Protease, „-aspase“ für Spaltung nach Asp-Resten
Alte Bezeichnung „ IL-1 converting enzyme“ (ICE)

Das N-terminale Spaltprodukt von Gasdermin D bindet an Membranlipide und bildet Poren



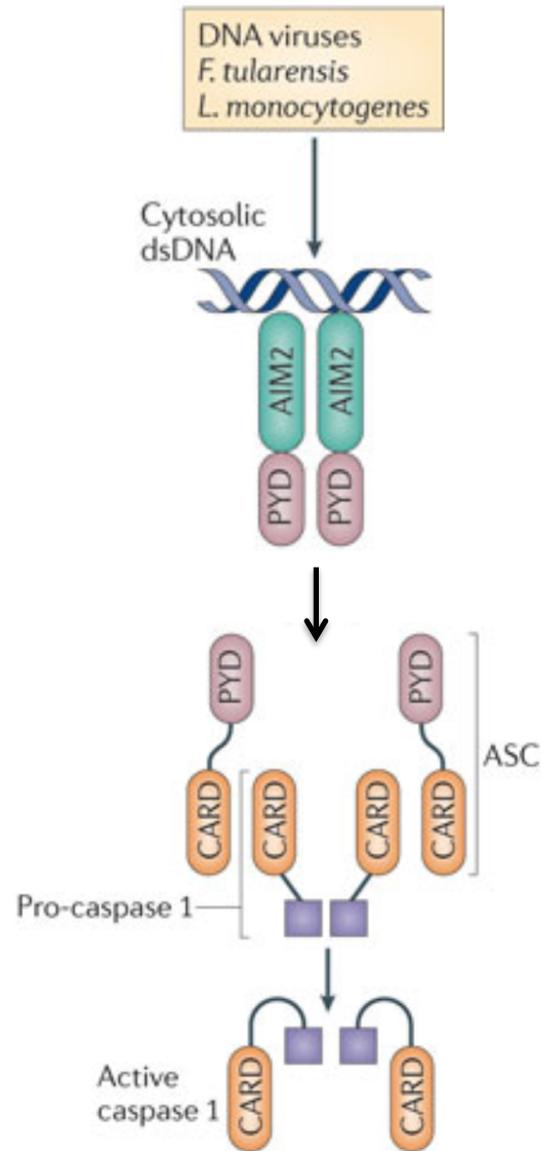
Gasdermin D lysiert eu- und prokaryotische Zellen und tötet cytolasm. Erreger ab

Der Prozeß wird als „**Pyroptose**“ bezeichnet u. dient auch der Freisetzung von IL-1 β und IL-18 sowie weiterer „DAMP“

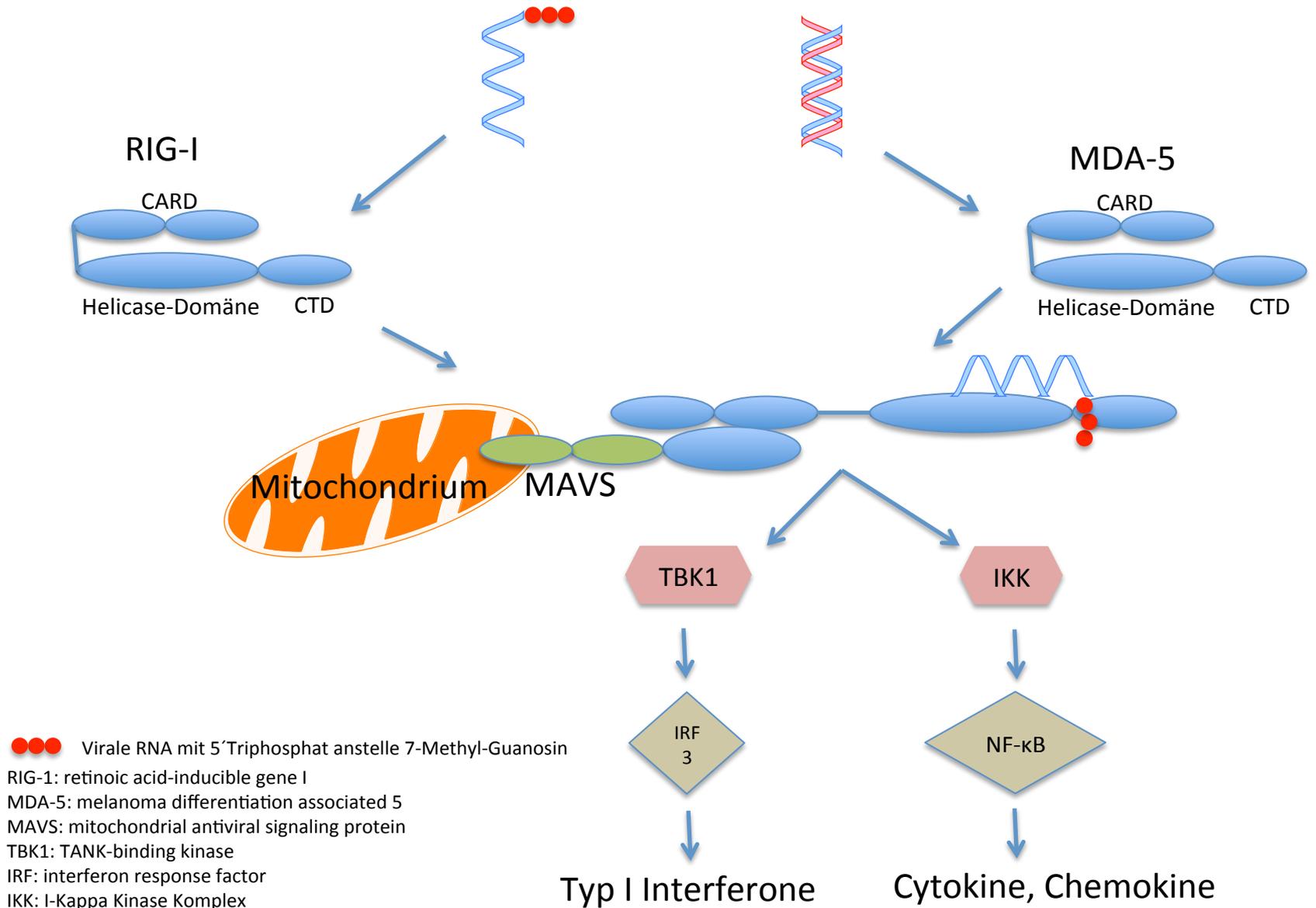


Dinarello *Annu Rev Immunol* 2009.27:519

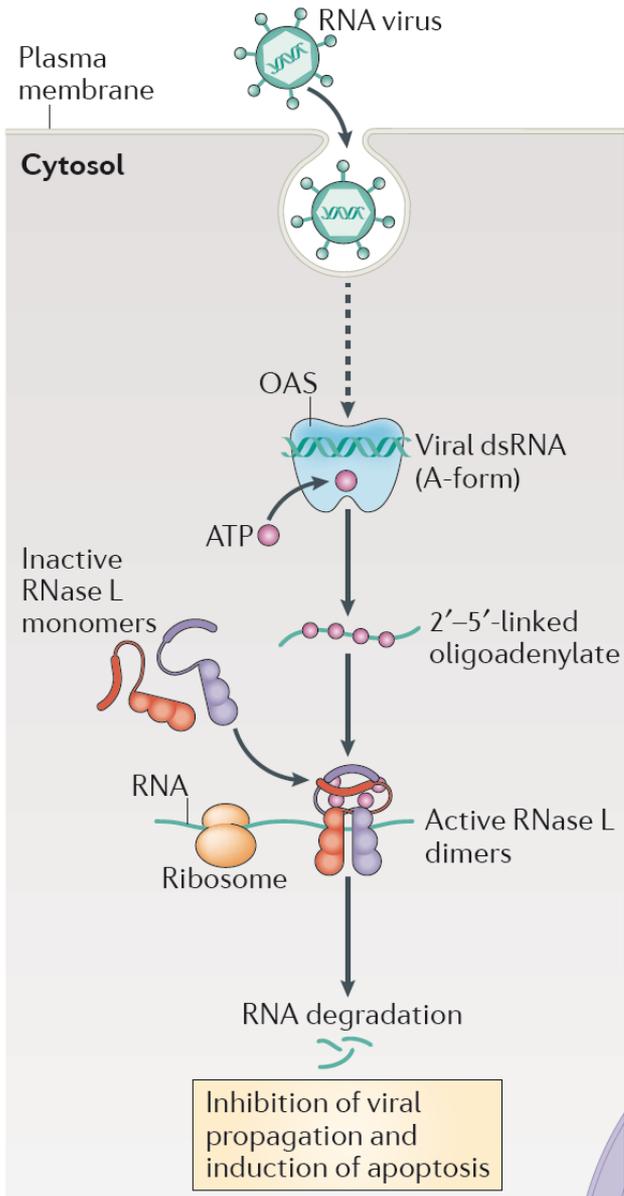
Absent in melanoma 2 (AIM2) erkennt dsDNA im Cytosol und bildet das AIM2 Inflammasom



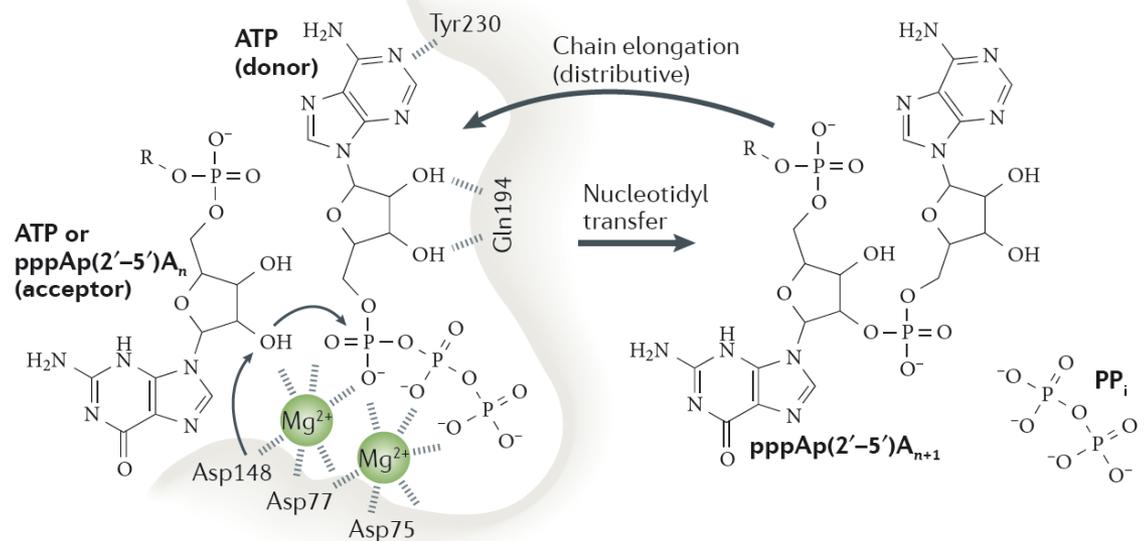
RIG-I und MDA-5 erkennen ssRNA mit 5'Triphosphat bzw. lange (!) dsRNA im Cytoplasma



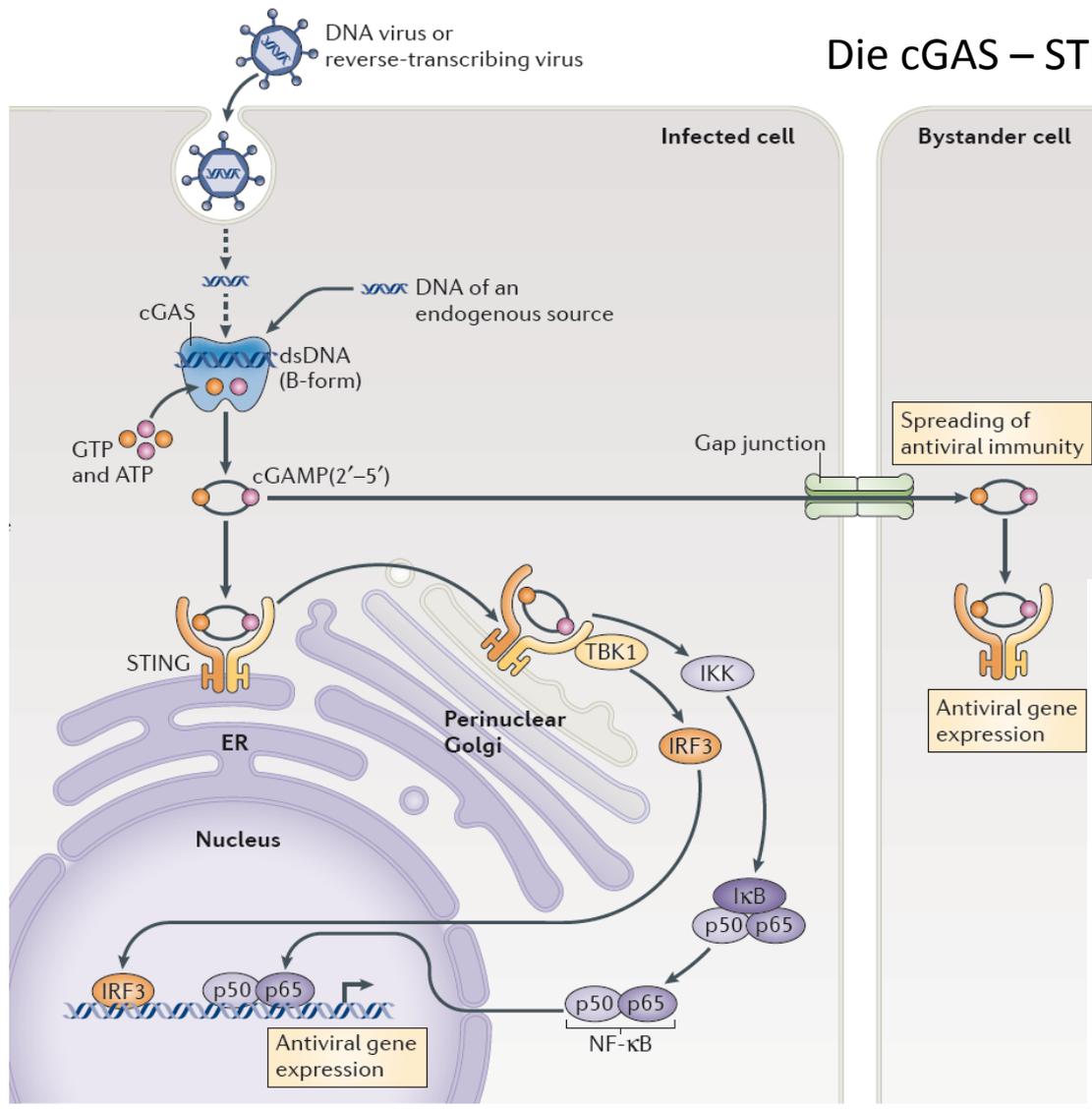
OAS-like Rezeptoren sind Matrizen-unabhängige Nukleotidyl-Transferasen und produzieren 2'-5'-verknüpfte second messenger



b Formation of 2'-5'-linked oligoadenylates by OAS1

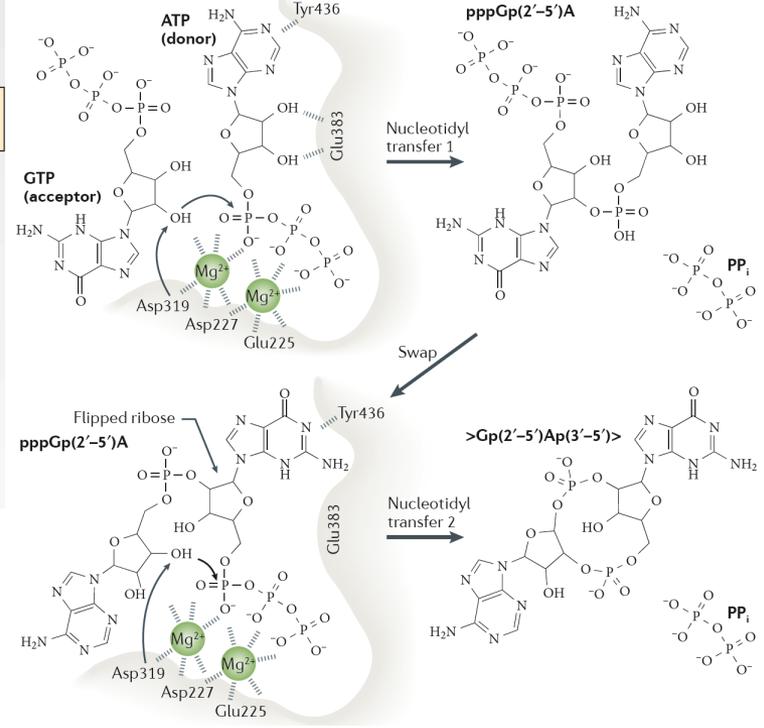


Die cGAS – STING - Achse (stimulator of IFN genes)



cGAMP: cyclic GMP-AMP

a Formation of cGAMP by cGAS



Hornung et al. *Nat Rev Immunol* 2014. 14:521

Figure 1 | Simplified schematic comparison of the OAS1–RNase L and cGAS–STING axes in innate immune signalling and antiviral defence. Upon double-stranded RNA (dsRNA) binding, oligoadenylate synthase (OAS) enzymes undergo a conformational switch, which results in their catalytic activity — that is, the synthesis of 2′–5′-linked oligoadenylates using ATP as a substrate. 2′–5′-linked oligoadenylates subsequently act as second messenger molecules by activating the latent endoribonuclease RNase L in the cytoplasm. RNase L then forms a crossed dimer and degrades RNA that is of both cellular and viral origin, leading to the inhibition of viral propagation. On the other hand, cyclic GMP–AMP (cGAMP) synthase (cGAS) is activated by cytosolic B-form dsDNA to synthesize the non-canonical cyclic dinucleotide (CDN) cGAMP(2′–5′)

as its second messenger molecule (using the substrates ATP and GTP). cGAMP(2′–5′) binds to and activates the endoplasmic reticulum (ER)-resident receptor stimulator of interferon genes (STING), which subsequently translocates to a perinuclear Golgi compartment where it obtains its signalling-competent state. This results in the activation of transcription factors that initiate antiviral and pro-inflammatory gene expression. At the same time, cGAMP(2′–5′) can also diffuse through gap junctions to initiate antiviral activity in bystander cells. In addition to its role in sensing the endogenous second messenger molecule cGAMP(2′–5′), STING responds to exogenous CDNs that are derived from prokaryotes (not shown). IκB, inhibitor of NF-κB; IKK, IκB kinase complex; IRF3, interferon-regulatory factor 3; NF-κB, nuclear factor-κB; TBK1, TANK-binding kinase 1.