Microbial recognition (and evasion) by the host innate immune system

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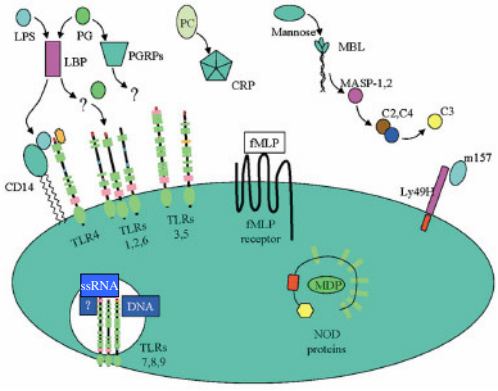
Innate immune system receptors

•Fast response
•Responds to the broad range of pathogenic microbes
•Recognition of **common molecular motifs** - pathogen associated molecular patterns – PAMPs

•PAMPs should be:
- broadly distributed
- different from the host molecules
- essential for the microbe

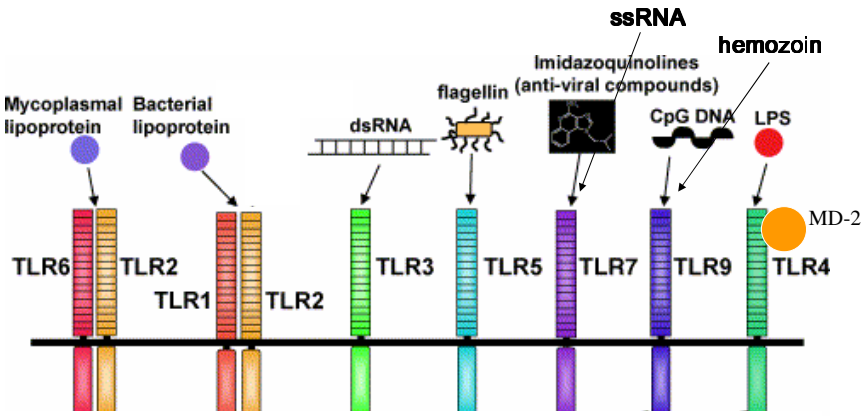


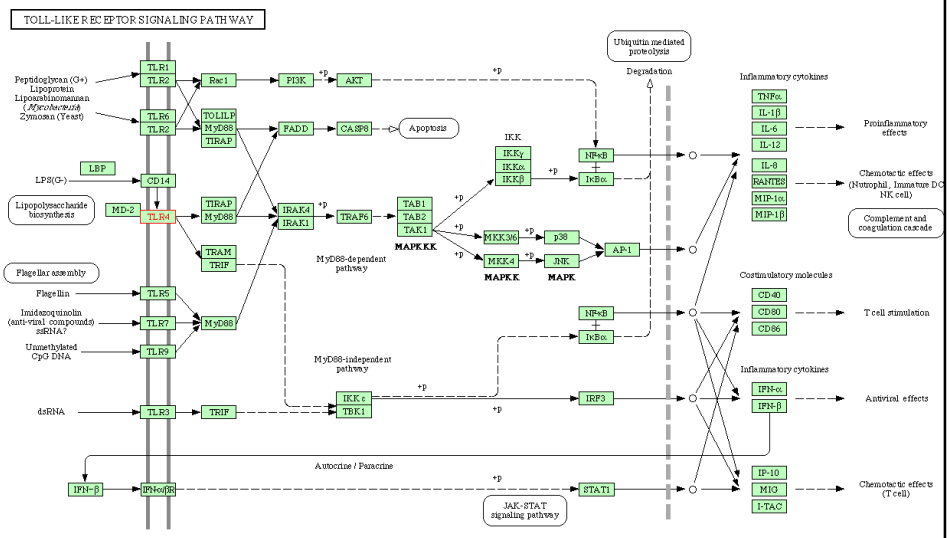
B.Beutler. Immunity 2003

PAMPs – pathogen associated molecular patterns

PAMP	pathogen	biologicaly active fragment	pattern recognition receptors	biological consequence
LPS	most Gram-negative bacteria	lipid A	LBP, CD14, MD-2, TLR4	inflammatory response
lipoprotein, lipopeptide	eubacteria	di(three)Pam. Cys at N-term.	TLR2, TLR6, CD36	inflammatory response
peptidoglycan	most bacteria	muropeptides	NOD1, NOD2 PGRP	inflammatory response
lipoteichoic acid	most Gram-positive bacteria	glycosidic link, glycolip. anch.	TLR2, TLR6	inflammatory response
CpG	most pathogens	nonmethylated CpG ODN	TLR9	inflammatory response
lipoarabinomannan	mycobacteria	LAM, LM	TLR2, TLR1, CD1	pro and anti-inflammatory response
N-formyl-Met	prokarionts	amino-terminal N-formyl-Met	f-Met receptor 1 and 2	chemotaxis and release of inflammatory mediators
mannanes and mannoproteins	yeasts	unknown	mannose receptor mann. bind. prot.	inflammatory response, phagocytosis, endocytosis
dsRNA	viruses	dsRNA	TLR3	inflammatory response
flagellin	most bacteria	N and C-terminal	TLR5	inflammatory response
zymosan	fungi	β-glucan	TLR2, dectin-1	inflammatory response, fagocytosis

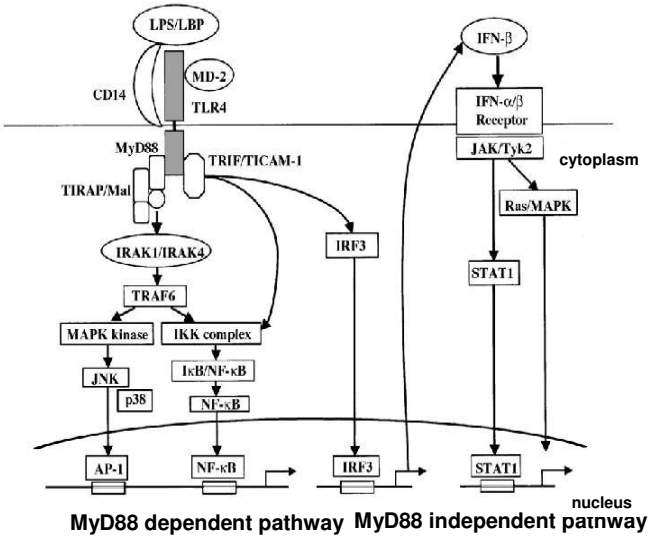
Recognition of a variety of microbial components by Toll-like receptors





KEGG database

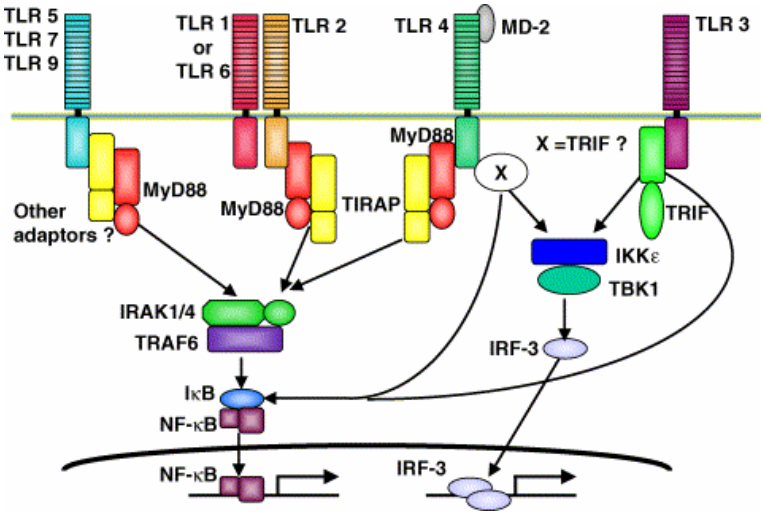
LPS signal transduction pathway



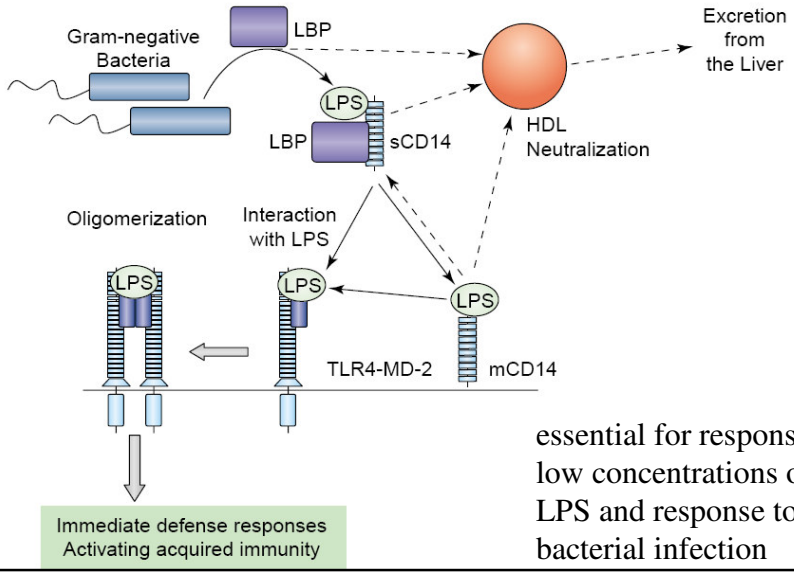
LPS or live *E.coli* affects transcription of more than 1000 genes in human macrophages

74% are MyD88 independent

Signalling of TLRs through TRIF and TIRAP/Mal adaptors

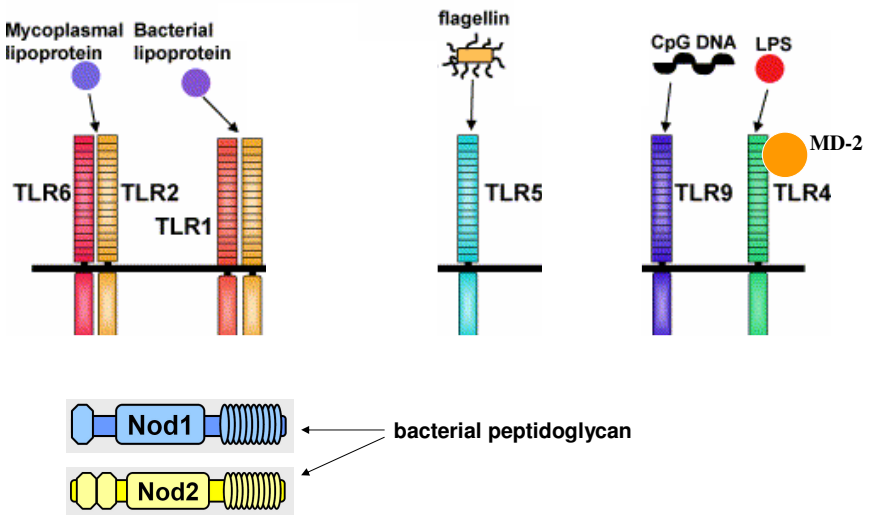


Additional members of the extracellular LPS recognition cascade



essential for response to low concentrations of LPS and response to bacterial infection

PRRs recognizing bacteria



Microbial motivation for modifying PAMPs:

- to avoid detection by the receptors
- to decrease the effect of host defense such as AMPs

Possible bacterial strategies:

- modification of PAMPs
- downregulation of PAMPs during the selected phase of the microbial life cycle

Possible modifications of PAMPs

CpG – not much possibility for variation

LPS: lipid A phosphate modification, number and length of acyl chains

Flagellin sequence, downregulation

Lipoproteins - recognition by TLRs is provided by the lipid chain as well as peptide sequence

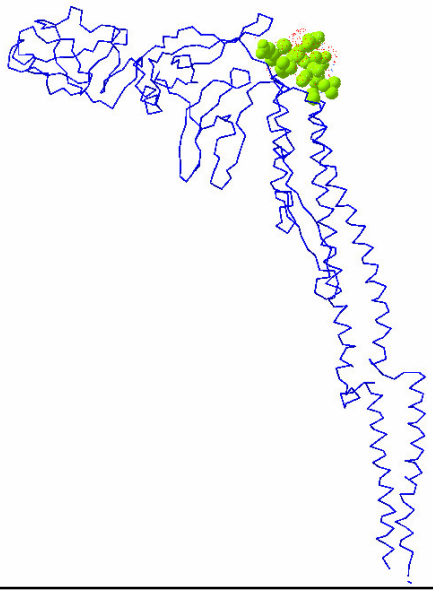
Peptidoglycans – conserved motif MDP, iE-DAP – variation in PGN composition (e.g. Spirochetes) effects of sequence variation on the integrity of cell wall

Flagellin

Identified region within flagellin that activates TLR5

Modification: flagellin from *H.pylori* is 1000x less potent than from other G-bacteria

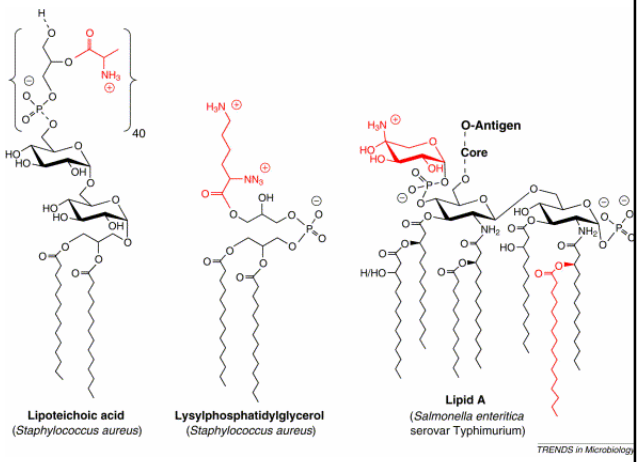
Downregulation: *Listeria monocytogenes* shuts-off flagellin expression at 37℃ in 80% of clinical isolates (bacteria are less motile, no effect on virulence in murine infection)



Modification of lipid-derived PAMPs by positive charges

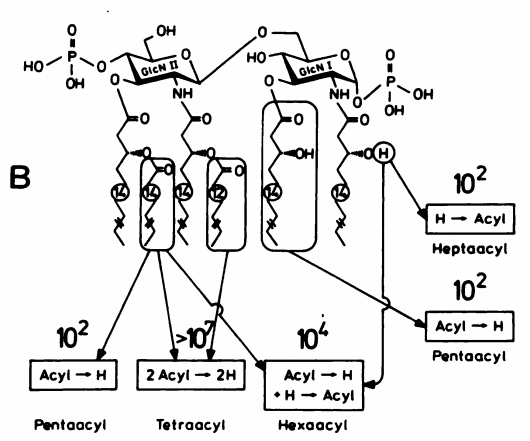
Introduction of positive charges (L-Lys to phospholipids, D-Ala to lipoteichoic acids), aminoAra to lipid A

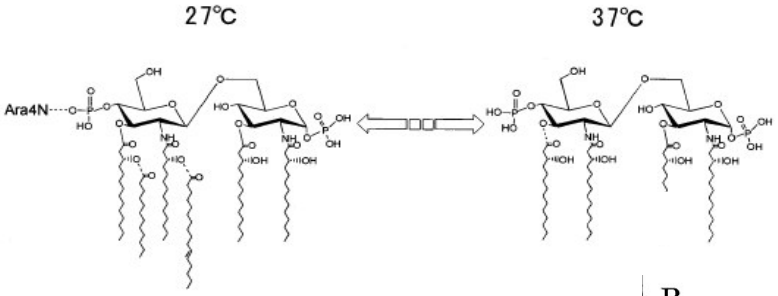
Decreased susceptibility to AMPs and recognition by TLRs



Heterogeneity of the LPS affects the immune response

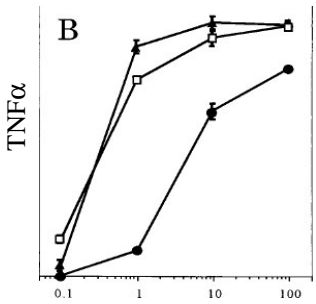
Number of acyl chains of *lipid A*





Different LPS acylation pattern:
Porphyromnas, Leptispira…

Regulation of LPS acylation pattern :
Temperature dependent shift in lipid A acylation pattern of *Yersinia pestis* and its effect on immune response.



Kawahara et al., Immunity 2002

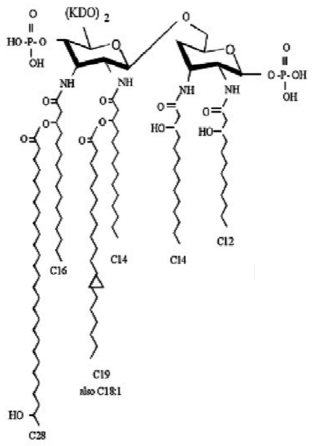
Long chain fatty acids in LPS of *Brucella*

LPS characteristics:
•long chain fatty acid chains
•absence of negatively charged groups besides KDO

Decreased susceptibility to cationic antimicrobial peptides – increases permeability to hydrophobic compounds

LPS from *Brucella* induces weak immune response – depends primarily upon lipoproteins

Involvement of the BacA in biosynthesis of LCFA



Variability in the host receptors

Allelic variants of TLRs and their accessory proteins

TLR4: D299G, cosegregates with T399I, frequency ~8%, correlation with increased susceptibility to bacterial infection (decreased risk of atherosclerosis), novel study indicated resistance to Legionellosis

LPS detection cascade: **LBP, CD14, MD-2**

Variability of response in **different vertebrates**:

Mouse – different response to LPS chemotypes than in human, connected to MD-2 and TLR4

Evolution of immune system, evolutionary recent genes (MD-2 only occurs in higher vertebrates)

Veterinary importance – poultry, cattle ...

Variability in the host receptors

TLR5: N592S, R392X with frequency of 10%, associated with susceptibility to Legionellosis

NOD-2: several mutations connected with susceptibility to Crohn's disease, Blau syndrome, diminished response to LPS

TLR2, TLR6, TLR1, TLR9 ?

CD14 and LBP are also involved in presentation of lipopeptides, peptidoglycan

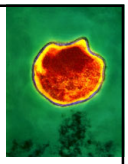
Involvement of bacterial infection in inflammatory chronic diseases

•Atherosclerosis, Crohn's disease, arthritis ...

•Initiation of inflammation by bacterial infection or similarity between PAMPs and endogenous ligands (sterile inflammation) ?

•Involvement of *Chlamydial* infection in atherosclerosis

Chlamydia

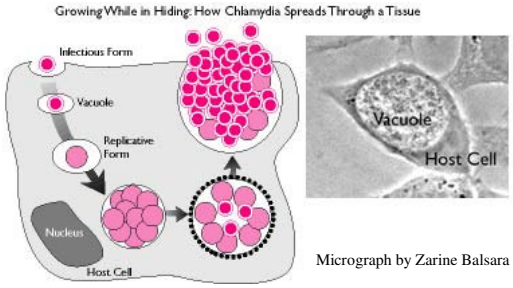


Unique cell wall of *Chlamydia trachomatis* is thought to be one of its **virulence factors**, as it inhibits phagolysosome fusion in phagocytes.

Cell wall of Chlamydia contains an outer lipopolysaccharide membrane and **lacks peptidoglycan** although it contains genes which encode peptidoglycan biosynthetic activity. It contains cysteine-rich proteins that are likely the functional equivalent of peptidoglycan.

Determined genomes of *Chlamydia trachomatis* and *Chlamydia pneumoniae*

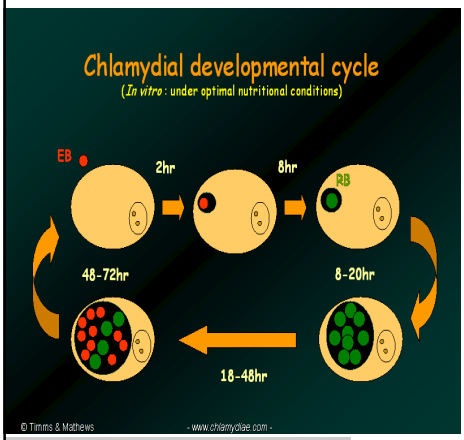
Stealth replication of *Chlamydia* within the host cells



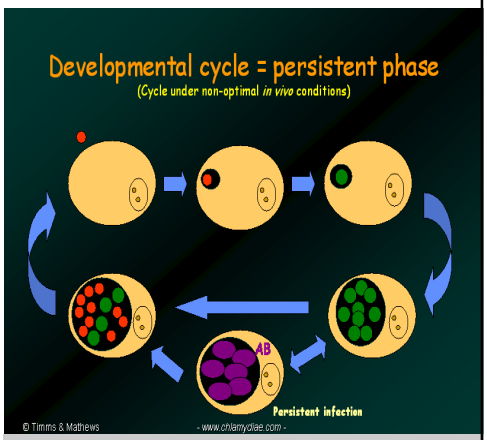
Micrograph by Zarine Balsara

Chlamydia has evolved a stealth operation to grow while evading the host's immune system.

Selection between productive and persistent infection



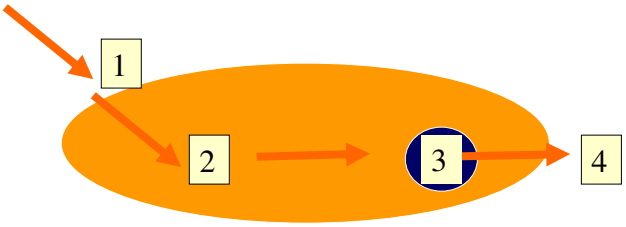
Optimal nutrition leads to **productive infection**.



Non-optimal conditions may lead to interrupted development, the presence of aberrant bodies (AB) and persisting infection.

Chlamydia

Micro-environmental signals – direct and indirect signals that may be used by C.pneumonie – e.g. IFN-γ, other proinflammatory cytokines, LDL, other biochemical markers derived from the host



1. cell attachment – TLR2, heparan-sulfate, type II mechanism
2. cytosol – LDL uptake, oxidation, response to microenvironment
3. nucleus – transcription factors

Chlamydia in atherosclerosis

There is considerable evidence that atherogenesis is partly driven by inflammatory stimuli, notably the pro-inflammatory cytokines.

C. pneumoniae is able to infect most of the key cells involved in atherogenesis, including macrophages, endothelium and smooth muscle cells, bacteria or its components have been detected in atheromatous lesions of patients. In murine model *C. pneumoniae* infections stimulated atherogenesis. Antibiotics cleared the infection, but did not influence the atherogenic properties.

Chlamydiae are capable of interacting with macrophages or epithelia to generate a variety of pro-inflammatory cytokines: IL-6,IL-8, IFN-γ, TNF-α, IL-1β and IL-18. Other effects: induction of macrophage foam cell formation, oxidation of LDL, blockade of apoptosis of inflammatory cells in blood vessel walls. IFN-γ is stimulated through unknown receptor utilizing MyD88 pathway (not TLR2 ot TLR4).

Chlamydia

A RESEARCH APPROACH in optimal (*in-vitro* and non-optimal (*in-vivo*) conditions:

1. **Transcription factors,**
2. **mRNA expression and stability,**
3. **Post-translational modificiations,**
4. **Protein-stability**

DNA- comparative genomics,

RNA-expression arrays,

Proteins and

protein-DNA; protein-protein, protein-lipid interactions

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Some open questions in host-patogen interactions

•Distribution of enzymes involved in biosynthesis and variability of PAMPs (TLR-ligands) in different microbial genomes

•Regulation of PAMP expression upon host infection

•Synegistic effects of presentation of a range of PAMPs by a microbe

•(Co)evolution of microbial genomes and host immune response receptors in different vertebrates

•Micronvironmental signals that affect microbial (e.g. *Chlamydial*) interactions with the host in the development of atherosclerosis (particul. in case of chronic kidney failure or rheumathoid arthritis)

Expected results and their potential application

• molecular **mechanism of recognition** of pathogenic microbes based on the bioinformatic, genomic, transcriptomic, proteomic (lipidomic?) analysis

• microbial **strategies to evade detection** and immune response by the host

• identification of **potential targets** for therapeutic intervention in pathogenic microorganisms as well as in host, particularly for potential bioterroristic microorganisms

•potentials for **diagnostics** of strains with increased virulence

•microbial infection in chronic diseases - identification of etiological agents, development of **novel targets or therapies** to prevent excessive immune stimulation (sepsis, Crohn's disease...)

Is any of the omics still available ?

Collection of all microbe-specific molecules that interact with host innate immune system:

Microbial PAMPome ®

or innatome or Tollome …

Hvala lepa za pozornost !