S1-2

Chemical Toxicity Assessment of Dietary Fatty Acids

Jooyoung Lee,

Departmentof Life Science, Gwangju Institute of Science and Technology, Gwangju, Korea.

Many epidemiological, clinical, and animal studies have shown the beneficial effect of diets containing n-3 polyunsaturated fatty acids (n-3 PUFAs; DHA, EPA) in reducing the risk of chronic inflammatory diseases and certain cancers while diets rich in saturated fat increase the risk. However, the mechanisms are not fully understood. Toll-like receptors (TLRs) provide critical signals to induce innate immune responses in antigen presenting cells such as macrophages by recognizing invading microbial pathogens. The activation of TLRs triggers the activation of two major downstream signaling pathways, MyD88-dependent and independent pathways, leading to the activation of NF-kappaB and IRF3 and the expression of proinflammatory cytokines and type I IFN genes. Deregulated activation of TLRs can lead to the development of severe systemic inflammation including septic shock with high mortality. Moreover, recent evidence suggests the involvement of TLRs in various chronic inflammatory diseases including atherosclerosis, diabetes, rheumatoid arthritis, and cancer. Results from our studies demonstrated that saturated fatty acids activate TLRs whereas n-3 PUFAs inhibit agonist-induced TLR activation and COX-2 expression in macrophages. N-3 PUFAs, DHA and EPA, are the most potent inhibitors as compared with n-6 PUFAs and n-9 unsaturated fatty acids. Saturated fatty acid-induced TLR activation leads to the activation of both MyD88-dependent and -independent signaling pathway. The target of inhibition by DHA is TLR itself or the proximal events leading to TLR activation, but not the downstream signaling molecules. Our results suggest that inflammatory responses can be differentially modulated by types of dietary fatty acids mediated through the modulation of TLR activation. Therefore, it is important to assess whether certain dietary factors can modulate TLR-mediated signaling pathways and target gene expression in order to manage the deregulation of TLR-mediated inflammatory responses leading to acute and chronic inflammatory diseases. Furthermore, Toll-like receptor inflammasome system can be utilized as a new Toxicity Assessment System to evaluate pro-inflammatory activity of dietary factors.

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Department of Life Science
Gwangju Institute of Science and Technology

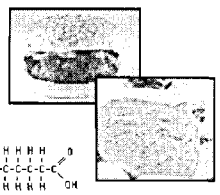
Fatty acids

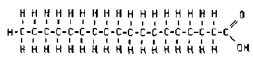
- long-chain hydrocarbon molecules containing a carboxylic acid moiety.

- Major roles
  1) the components of membrane lipids.
  2) the major components of stored fat
  3) signaling components

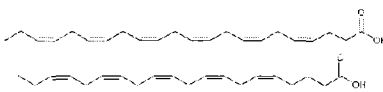
Dietary Fatty acids

- Saturated fatty acids (sFA)
  Lauric acid(C12:0), Myristic acid(C14:0), Palmitic acid(C16:0)
  meat, butter, dairy product, milk, cheese, coconut oil

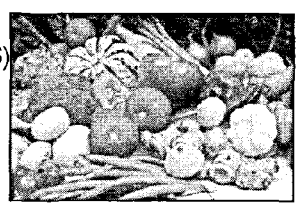




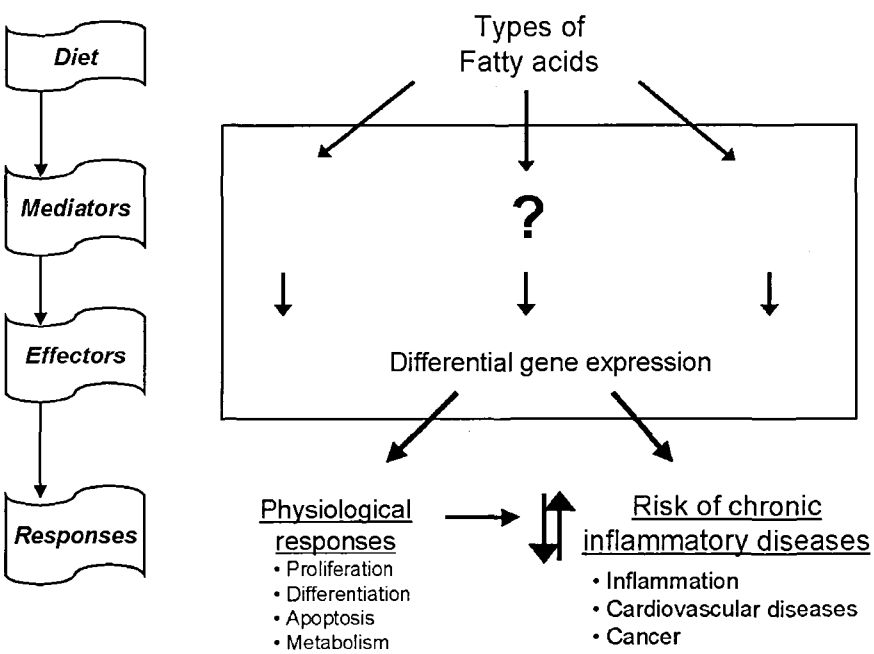
- Unsaturated fatty acids (uFA)
  1) n-3 polyunsaturated fatty acids (n-3 PUFAs)
  DHA(C22:6n-3), EPA(20:5n-3)
  Fish oil

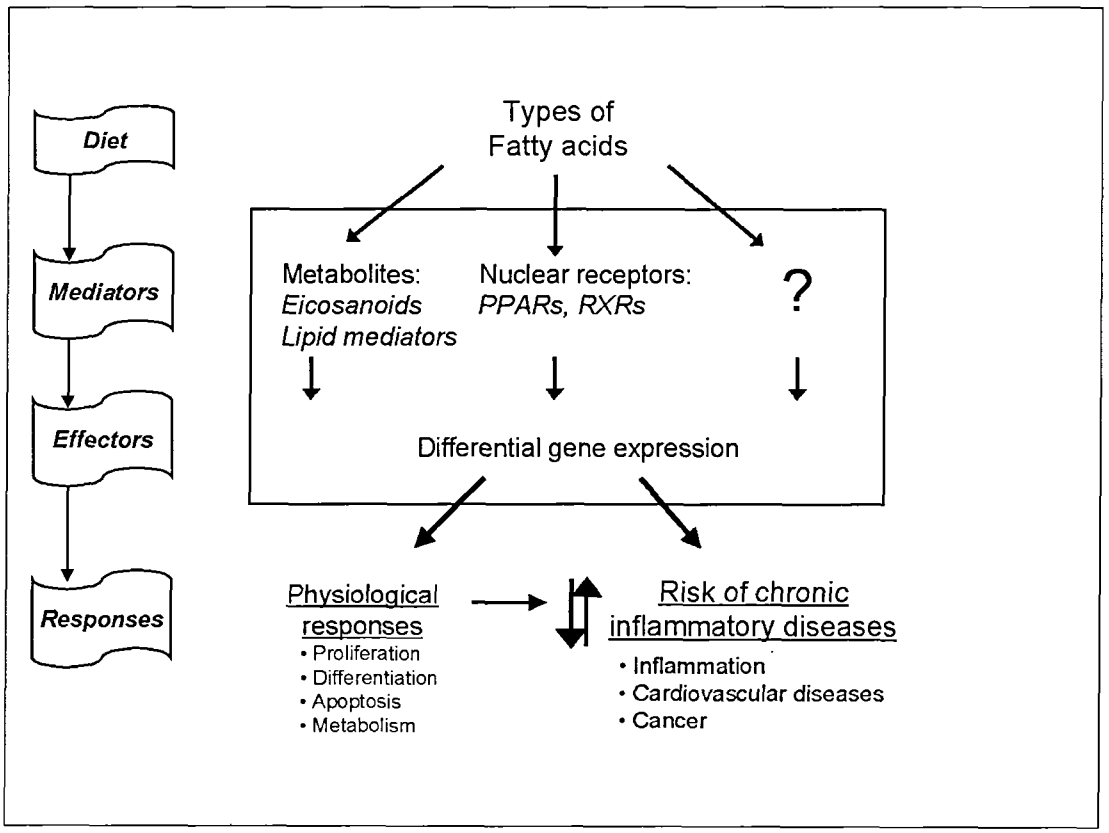


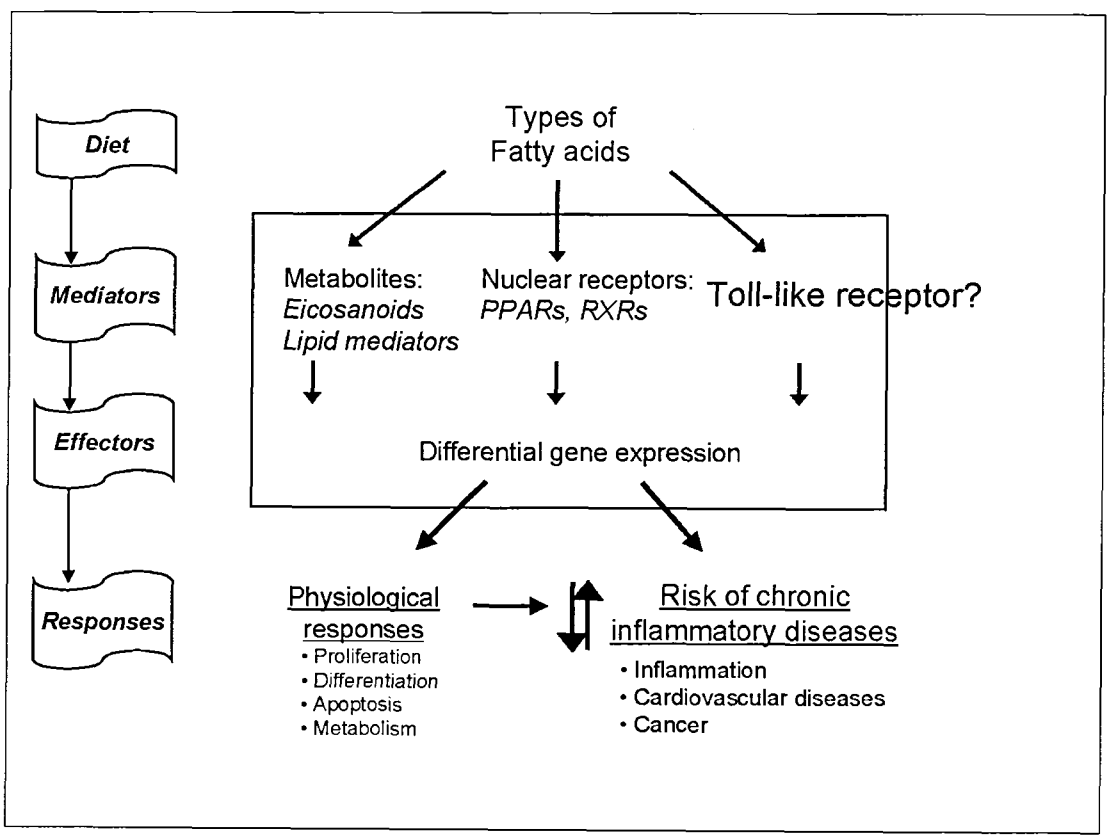
  2) n-6 polyunsaturated fatty acids (n-6 PUFAs)
  Arachidonic acid(20:4n-6), Linoleic acid(C18:2n-6)
  Vegetables, fruits, nuts



  3) n-9 Unsaturated fatty acids
  Oleic acid(C18:1n-9)



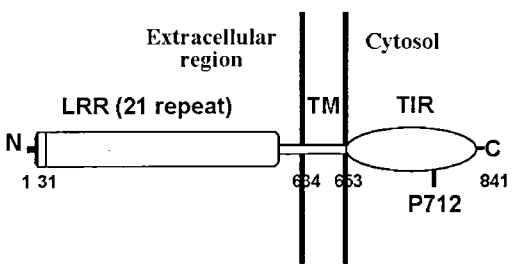

Diet
Mediators
Effectors
Responses
Types of
Fatty acids
Metabolites:
Eicosanoids
Lipid mediators
Nuclear receptors:
PPARs, RXRs
?
Differential gene expression
Physiological responses
• Proliferation
• Differentiation
• Apoptosis
• Metabolism
Risk of chronic inflammatory diseases
• Inflammation
• Cardiovascular diseases
• Cancer


Diet
Mediators
Effectors
Responses
Types of
Fatty acids
Metabolites:
Eicosanoids
Lipid mediators
Nuclear receptors:
PPARs, RXRs
Toll-like receptor?
Differential gene expression
Physiological responses
• Proliferation
• Differentiation
• Apoptosis
• Metabolism
Risk of chronic inflammatory diseases
• Inflammation
• Cardiovascular diseases
• Cancer

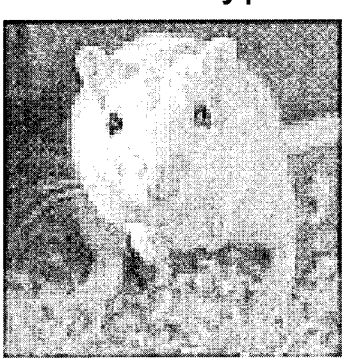
Toll-like receptors (TLRs)

◆ **Toll**
- In *Drosophila*
- Involved in dorsal-ventral patterning in embryos.
- Induce the anti-fungal peptide, dorsomycin.

◆ **Toll-like receptors**
- In mammals (Human homolog of Toll)
- Type I transmembrane receptors with extracellular leucine-rich repeat (LRR) motifs and a cytoplasmic Toll/IL-1R (TIR) homology domain.
- The activation of TLRs leads to the expression of co-stimulatory molecule which is required for the activation of adaptive immunity.



Lipopolysaccharide Hyporesponsive Mice



C57BL/10ScCr

↓

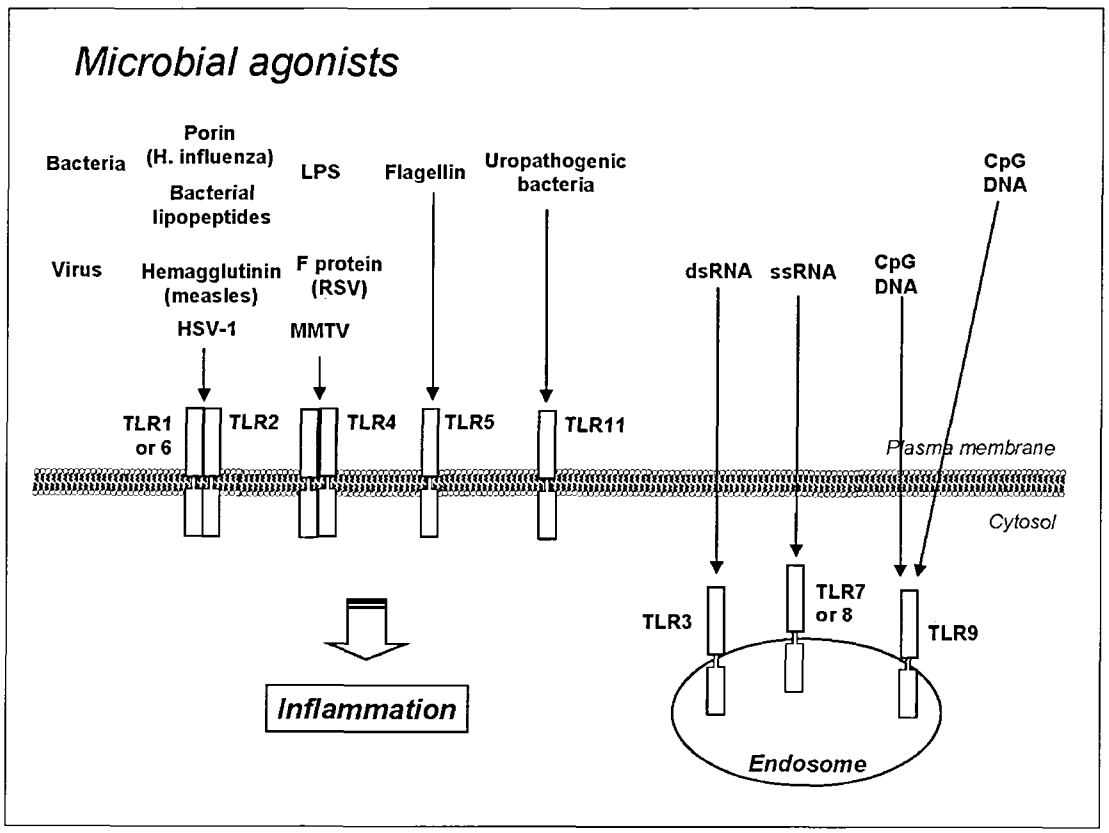
Homozygous for a null mutation of Toll-like receptor 4

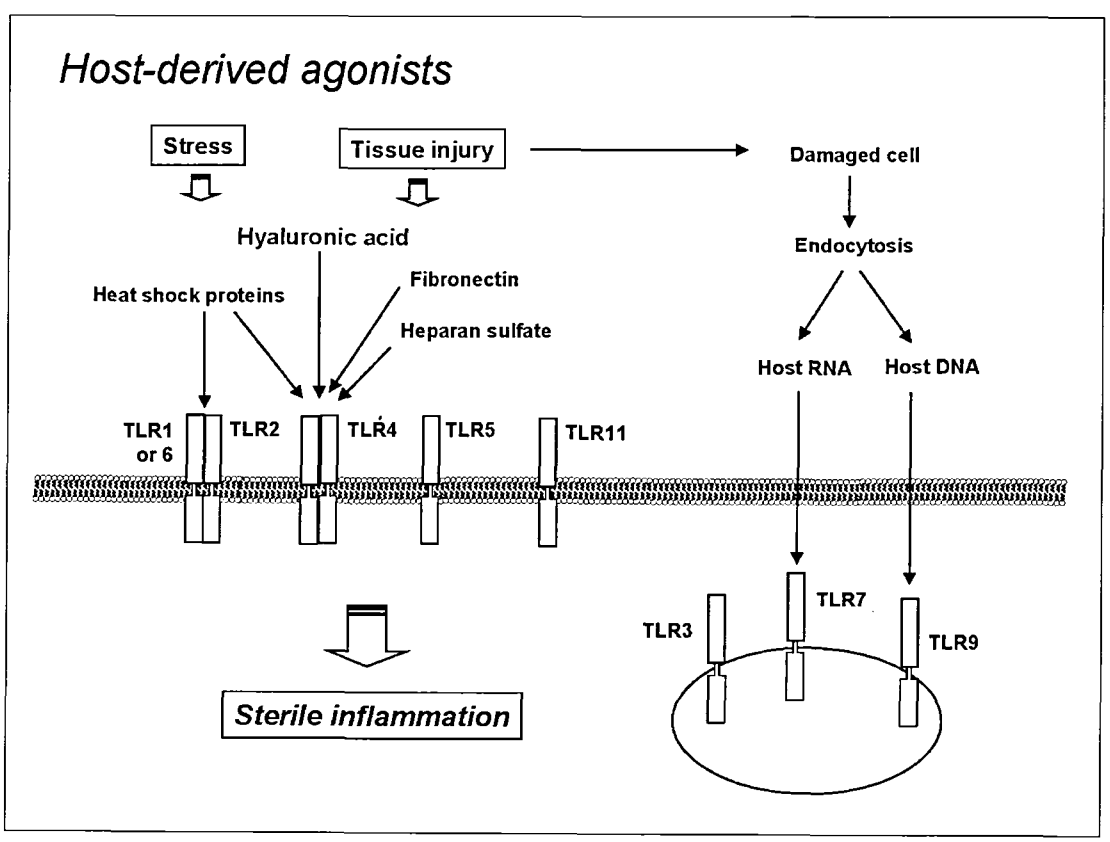
C3H/HeJ

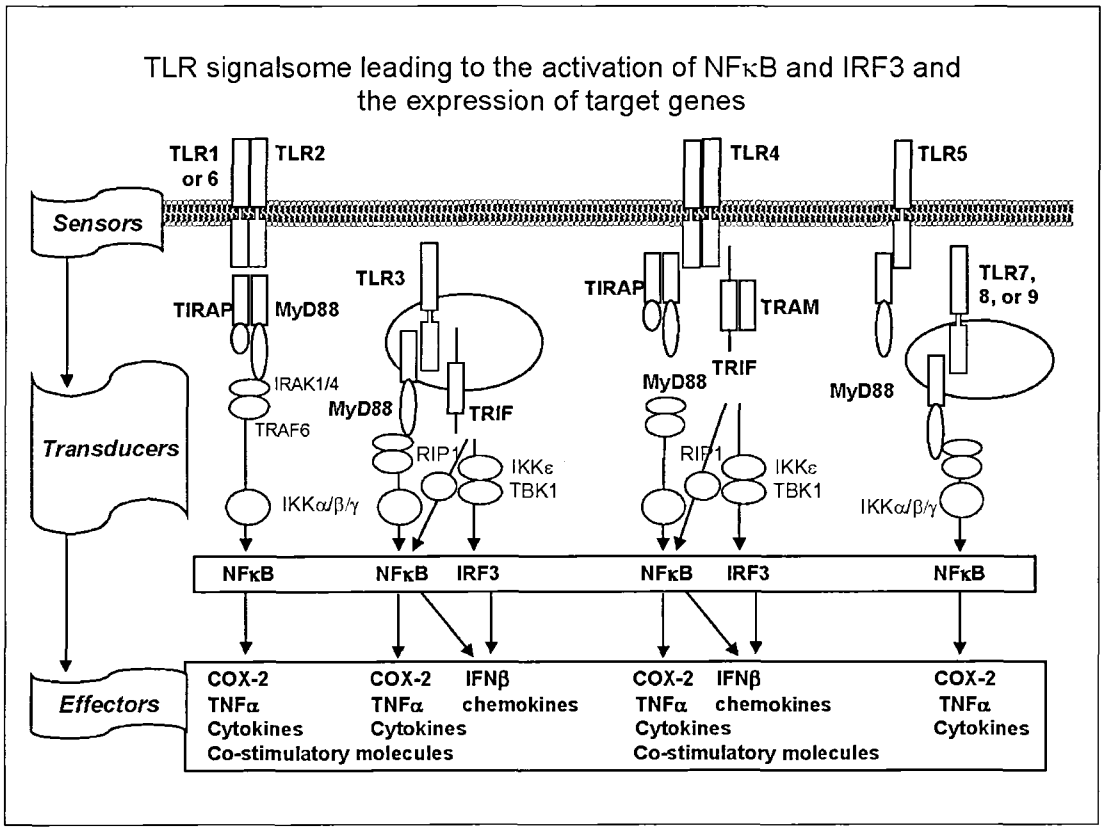
↓

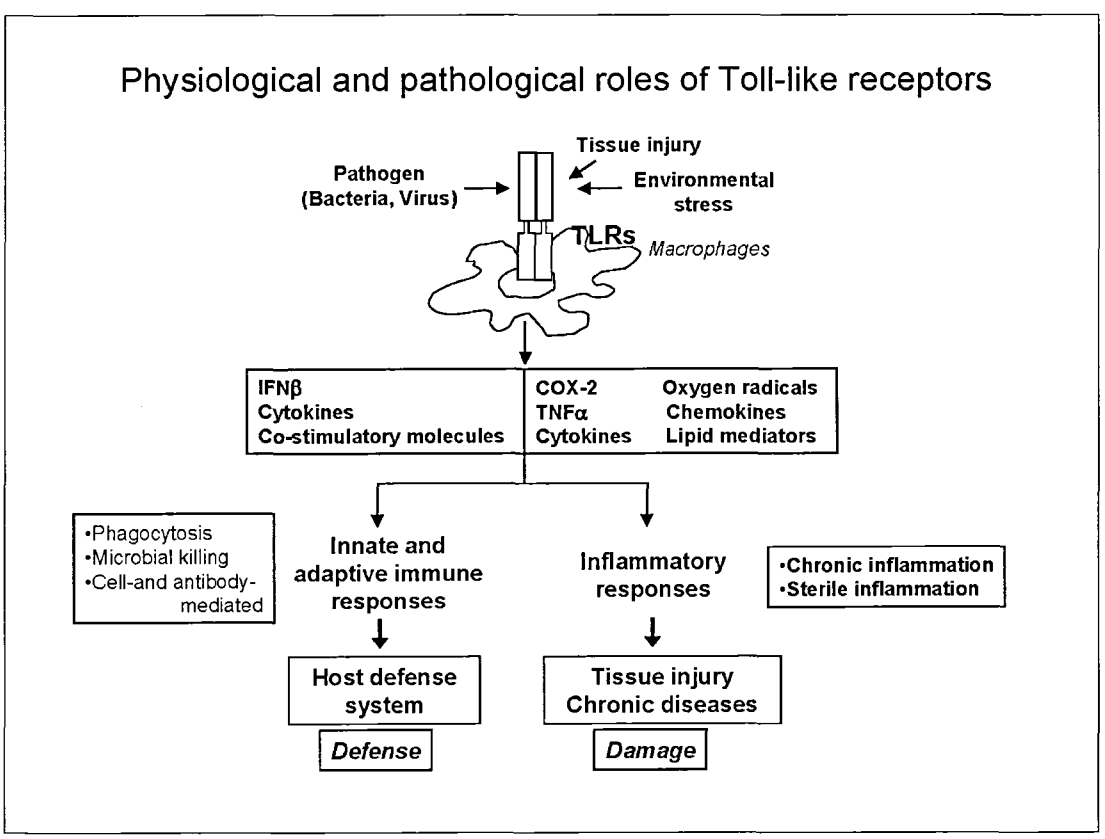
Missense mutation in Toll-like receptor 4 (P712H)

Poltorak *et al. Science* (1998)


Microbial agonists
Bacteria
Porin (H. influenza)
Bacterial lipopeptides
LPS
Flagellin
Uropathogenic bacteria
CpG DNA
Virus
Hemagglutinin (measles)
F protein (RSV)
HSV-1
MMTV
dsRNA
ssRNA
CpG DNA
TLR1 or 6
TLR2
TLR4
TLR5
TLR11
Plasma membrane
Cytosol
TLR3
TLR7 or 8
TLR9
Inflammation
Endosome


Host-derived agonists
Stress
Tissue injury
Damaged cell
Hyaluronic acid
Endocytosis
Heat shock proteins
Fibronectin
Heparan sulfate
Host RNA
Host DNA
TLR1 or 6
TLR2
TLR4
TLR5
TLR11
TLR3
TLR7
TLR9
Sterile inflammation


TLR signalsome leading to the activation of NFκB and IRF3 and the expression of target genes
TLR1 or 6
TLR2
TLR4
TLR5
Sensors
TLR3
TIRAP
MyD88
TIRAP
TRAM
TLR7, 8, or 9
IRAK1/4
MyD88
TRIF
MyD88
TRIF
MyD88
Transducers
TRAF6
RIP1
IKKε
TBK1
RIP1
IKKε
TBK1
IKKα/β/γ
IKKα/β/γ
NFκB
NFκB
IRF3
NFκB
IRF3
NFκB
Effectors
COX-2
TNFα
Cytokines
Co-stimulatory molecules
COX-2
TNFα
Cytokines
IFNβ
chemokines
COX-2
TNFα
Cytokines
Co-stimulatory molecules
IFNβ
chemokines
COX-2
TNFα
Cytokines


Physiological and pathological roles of Toll-like receptors
Tissue injury
Pathogen (Bacteria, Virus)
Environmental stress
TLRs
Macrophages
IFNβ
Cytokines
Co-stimulatory molecules
COX-2
TNFα
Cytokines
Oxygen radicals
Chemokines
Lipid mediators
•Phagocytosis
•Microbial killing
•Cell-and antibody-mediated
Innate and adaptive immune responses
Inflammatory responses
•Chronic inflammation
•Sterile inflammation
Host defense system
Tissue injury
Chronic diseases
Defense
Damage

Implications of TLRs in chronic inflammatory diseases

Epidemiological studies of gene polymorphism
- **Alzheimer's disease:** TLR4 Asp299Gly
- **Atherosclerosis:** TLR4 Asp299Gly, TLR2 Arg753Gln
- **Coronary heart disease :** TLR4 Asp299Gly in Caucasian
- **Ischemic stroke:** TLR4 intron 1 (A119C) in Chinese
- **Rheumatoid arthritis:** TLR4 Asp299Gly
- **Ulcerative colitis:** TLR4 Asp299Gly, Thr399Ile
- **Inflammatory Bowel Disease:** TLR4 Asp299Gly
- **Systemic lupus erythematosus:** TLR5 stop codon
- **Diabetes:** TLR4 Asp299Gly
- **Prostate cancer:** TLR4 sequence variant in 3'UTR

Mouse knockout studies
- **Atherosclerosis:** TLR4 or MyD88
- **Rheumatoid arthritis:** Knockout of TLR2, 4 or MyD88

Correlation with experimental disease model
- **Diabetes:** TLR3 or TLR7 agonist
- **Ischemia/reperfusion renal injury:** TLR2
- **Systemic lupus erythematosus:** TLR9

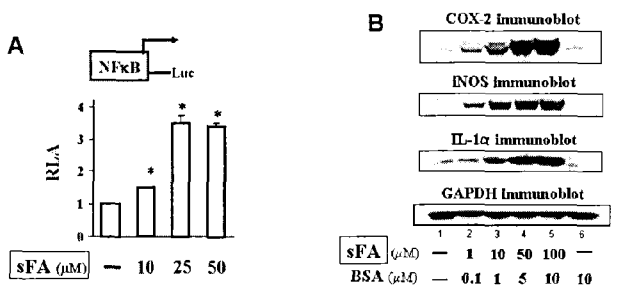
Infection with TLR agonists
- **Atherosclerosis:** *Chlamydia Pneumonia* (TLR2, 4)
- **Gastric cancer:** *Helicobacter Pyroli* (TLR2, 4)

Upregulation of TLR expression
- **Rheumatoid arthritis:** TLR2
- **Gastric cancer:** TLR4 and 5
- **Atherosclerosis:** TLR4
- **Ischemic heart:** TLR4

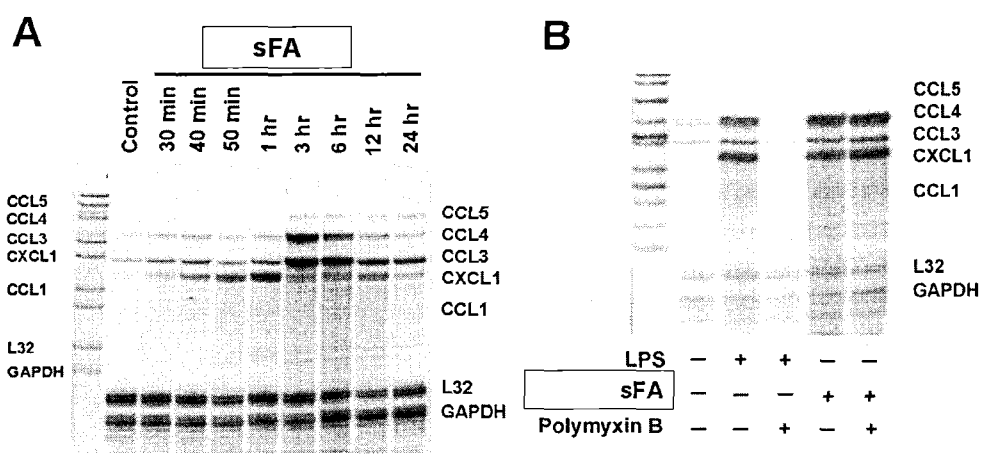
The Aim of the Study

1. To assess the activity of dietary fatty acids to modulate the expression of inflammatory genes in macrophages.
2. To investigate whether the modulation of inflammatory gene expression by dietary fatty acids is mediated through Toll-like receptor.

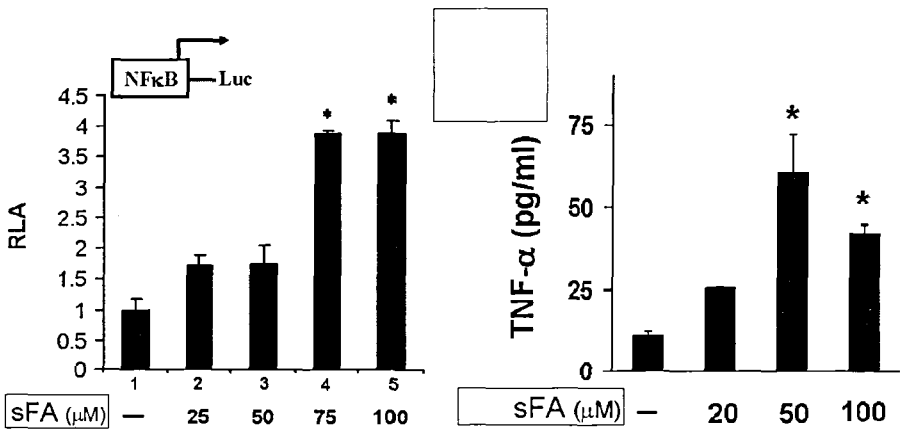
sFA induces NFκB activation and COX-2 expression in macrophages (RAW264.7)



sFA induces the expression of chemokines in macrophages

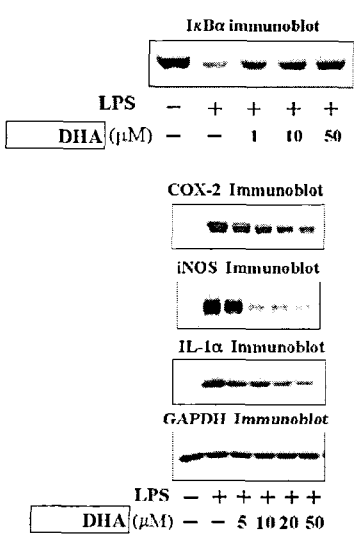


sFA induced NFκB activation in primary rat smooth muscle cells (A) and TNF-α production in human monocytic cell line (THP-1) (B)



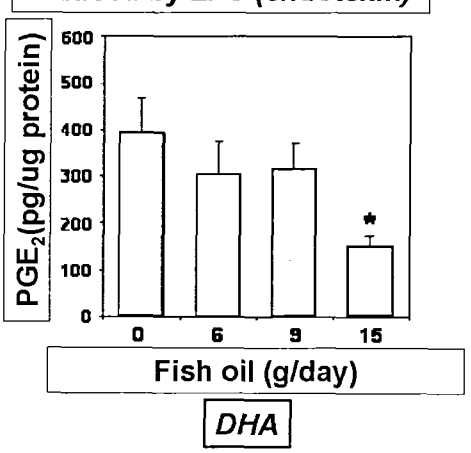
DHA suppresses NFκB activation and COX-2 expression induced by LPS

Murine macrophages



Human Diet Study

COX-2 expression induced by LPS (endotoxin)



The Aim of the Study

1. To assess the activity of dietary fatty acids to modulate the expression of inflammatory genes.
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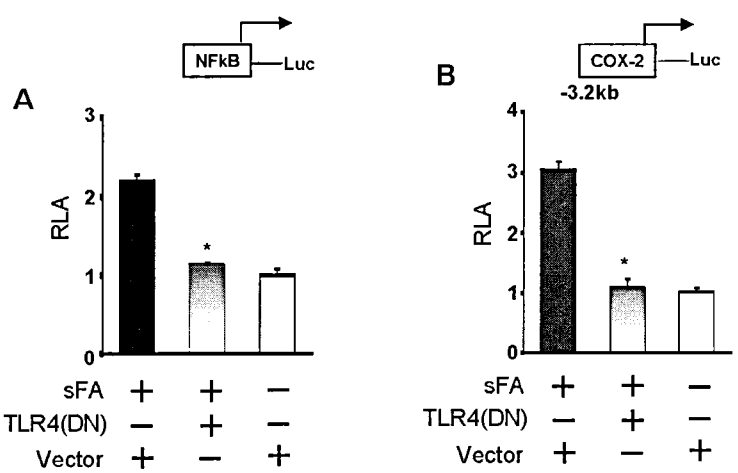
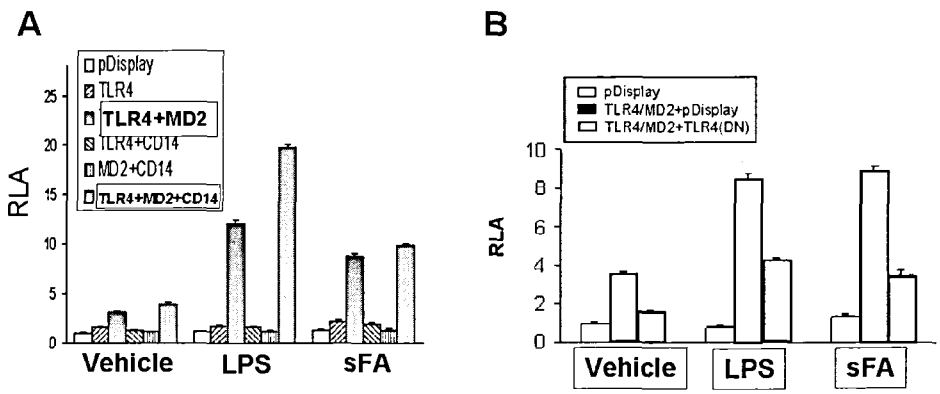
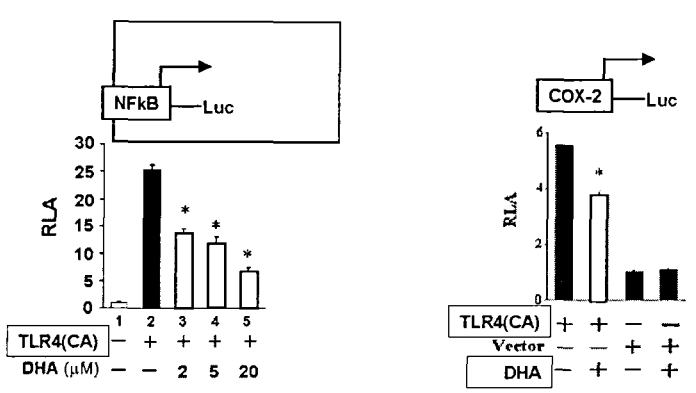
A *dominant-negative mutant of TLR4* inhibits NFκB activation and COX-2 expression induced by sFA in macrophages (RAW264.7)



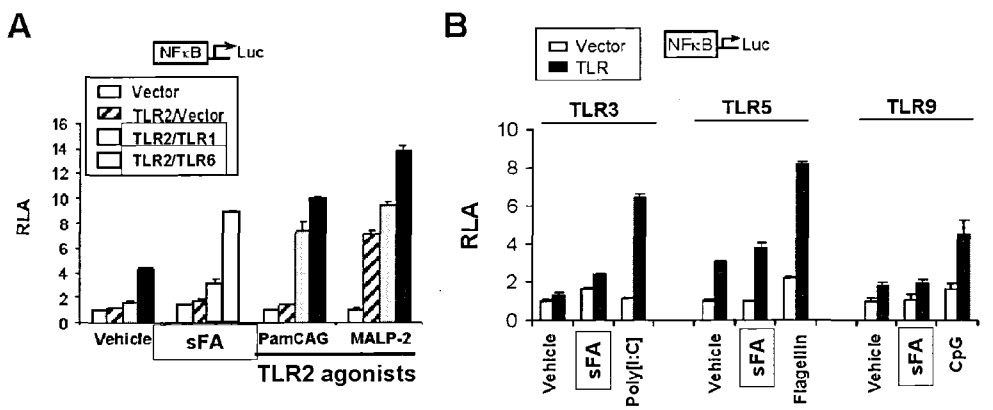
sFA activates TLR4 ectopically expressed with MD2/CD14



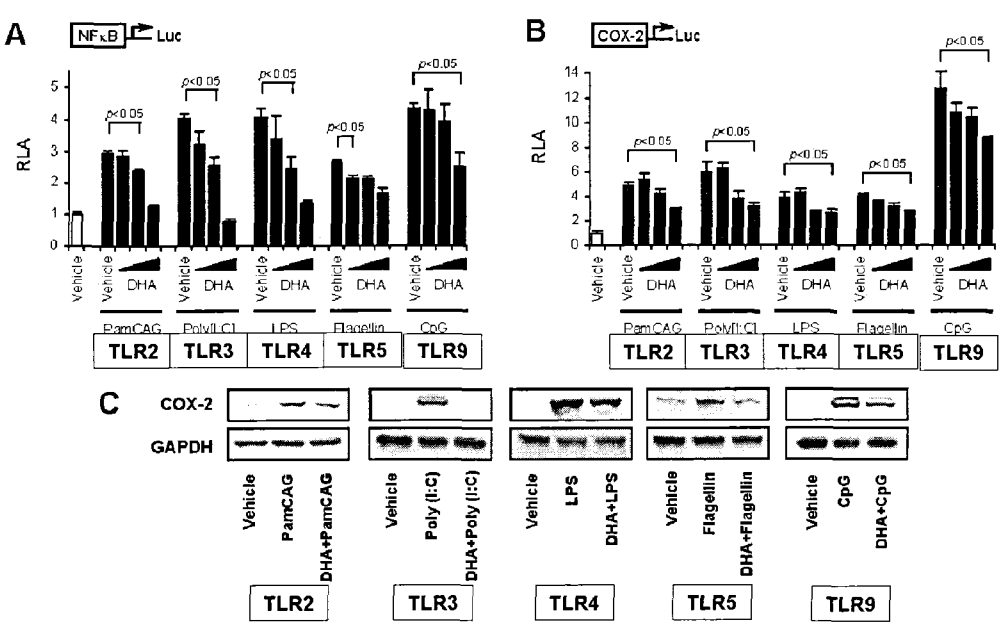
DHA inhibits constitutively active TLR4-induced NFκB activation and COX-2 expression



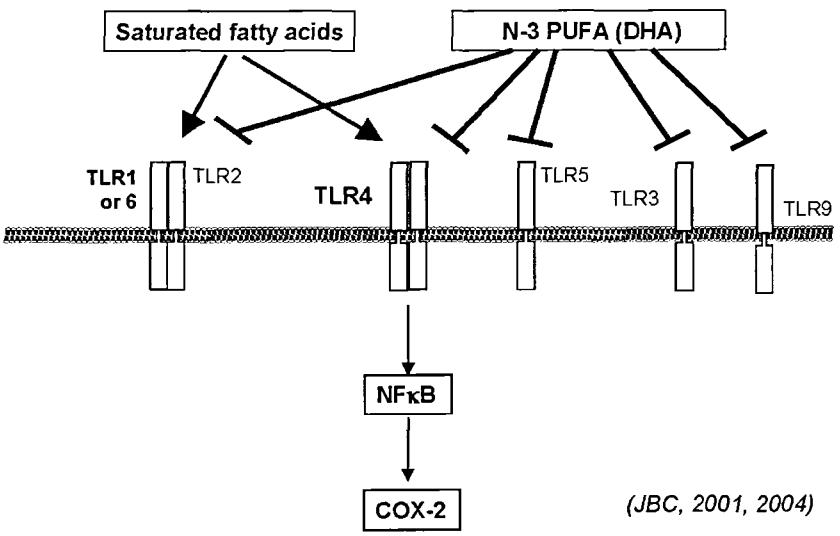
sFA activates TLR2/1 or TLR2/6 dimer, however, does not activate TLRs for which cognate ligands do not require fatty acid acylation



DHA suppresses the activation of various TLRs

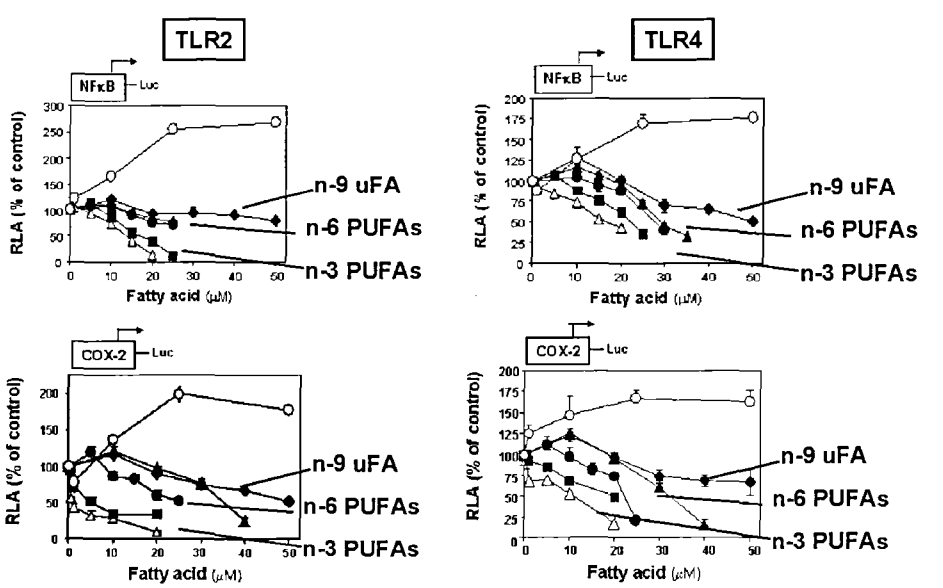


Differential modulation of inflammatory genes by different dietary fatty acids mediated through Toll-like receptors

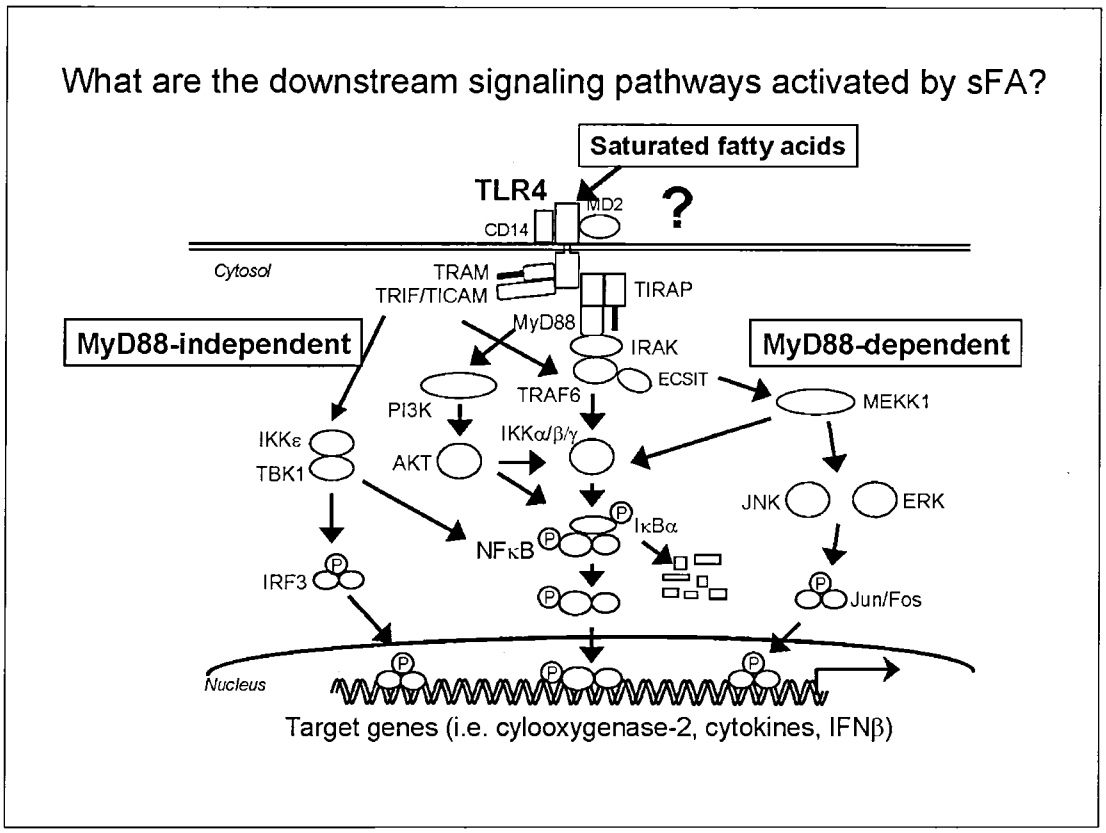


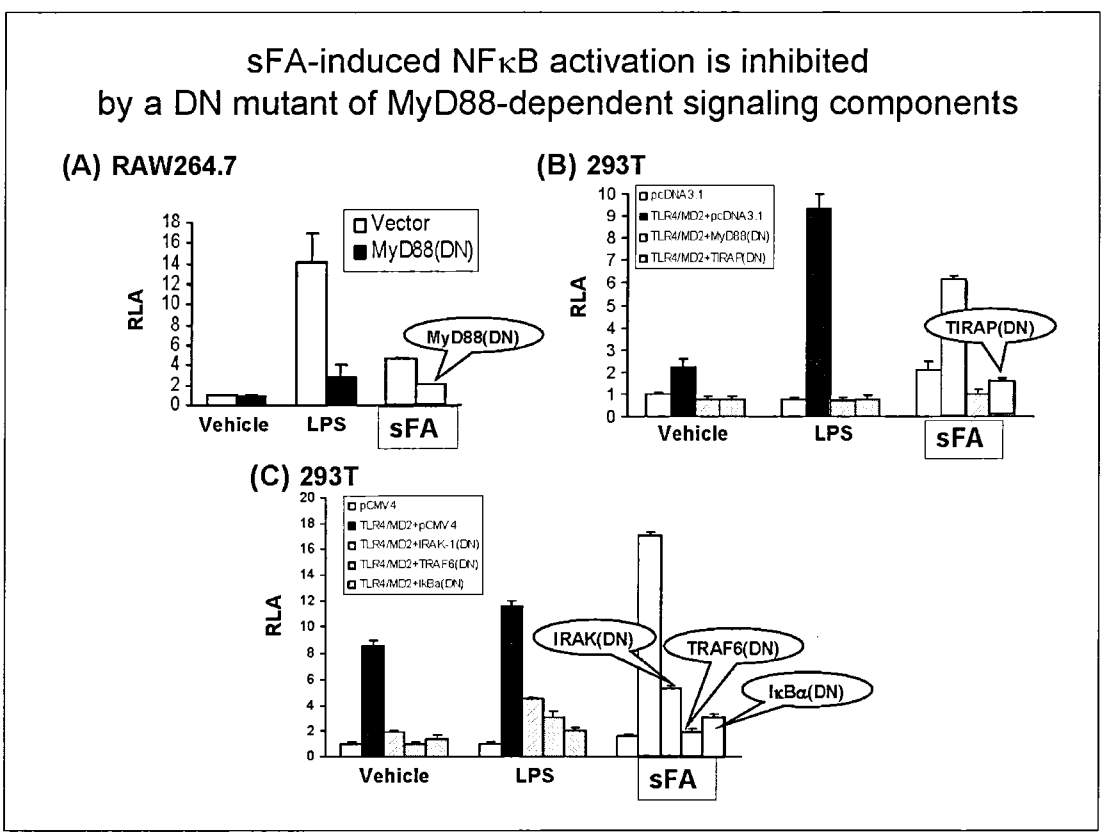
(JBC, 2001, 2004)

Relative inhibitory potency of different unsaturated fatty acids on TLR activation in reporter macrophages

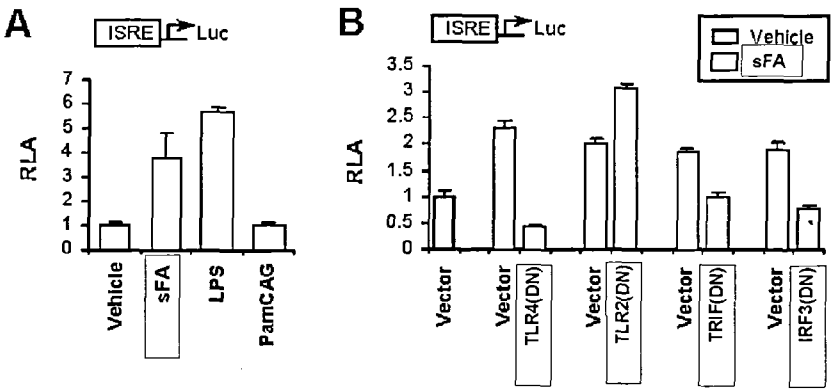


(J. Lipid Res., 2003)

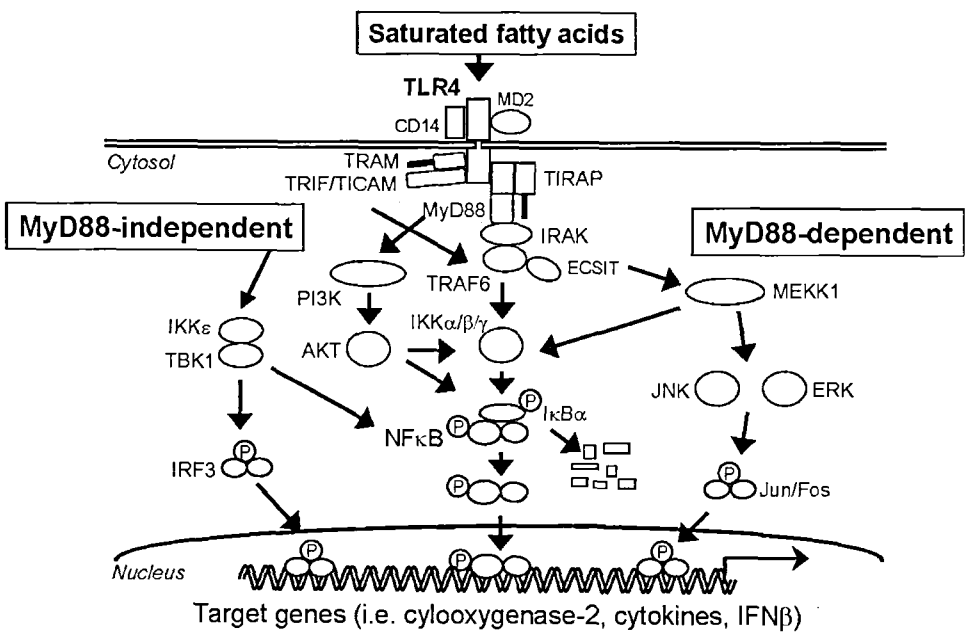

What are the downstream signaling pathways activated by sFA?
Saturated fatty acids
TLR4
MD2
CD14
?
Cytosol
TRAM
TRIF/TICAM
TIRAP
MyD88
IRAK
MyD88-independent
MyD88-dependent
ECSIT
PI3K
TRAF6
MEKK1
IKKε
TBK1
AKT
IKKα/β/γ
JNK
ERK
IκBα
NFκB
IRF3
Jun/Fos
Nucleus
Target genes (i.e. cylooxygenase-2, cytokines, IFNβ)


sFA-induced NFκB activation is inhibited
by a DN mutant of MyD88-dependent signaling components
(A) RAW264.7
Vector
MyD88(DN)
RLA
MyD88(DN)
Vehicle
LPS
sFA
(B) 293T
pcDNA3.1
TLR4/MD2+pcDNA3.1
TLR4/MD2+MyD88(DN)
TLR4/MD2+TIRAP(DN)
RLA
TIRAP(DN)
Vehicle
LPS
sFA
(C) 293T
pCMV4
TLR4/MD2+pCMV4
TLR4/MD2+IRAK-1(DN)
TLR4/MD2+TRAF6(DN)
TLR4/MD2+IκBa(DN)
RLA
IRAK(DN)
TRAF6(DN)
IκBα(DN)
Vehicle
LPS
sFA

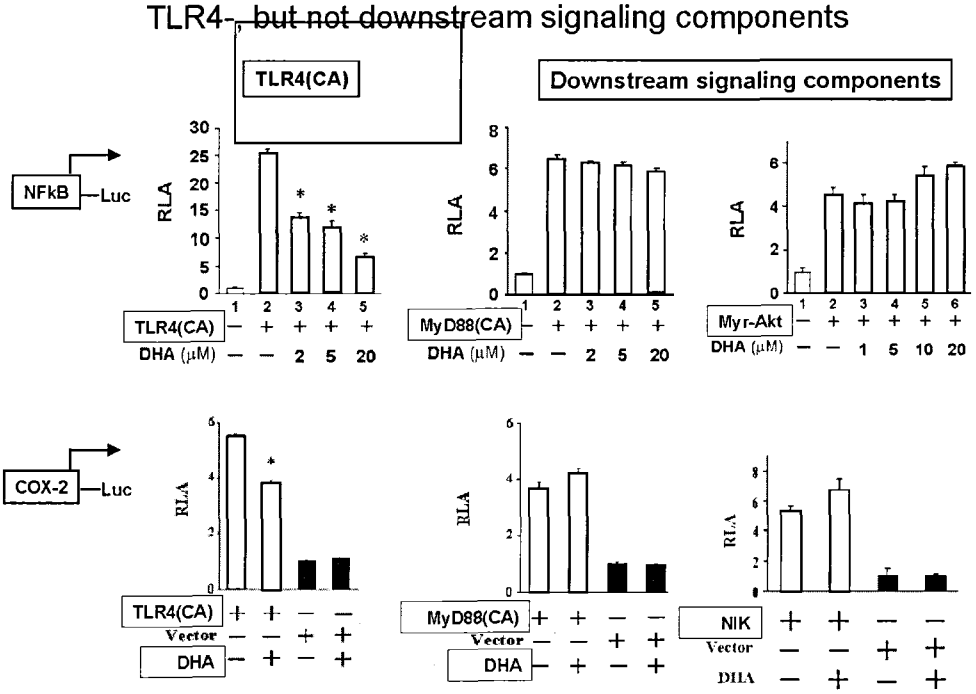
sFA activates MyD88-independent signaling pathways



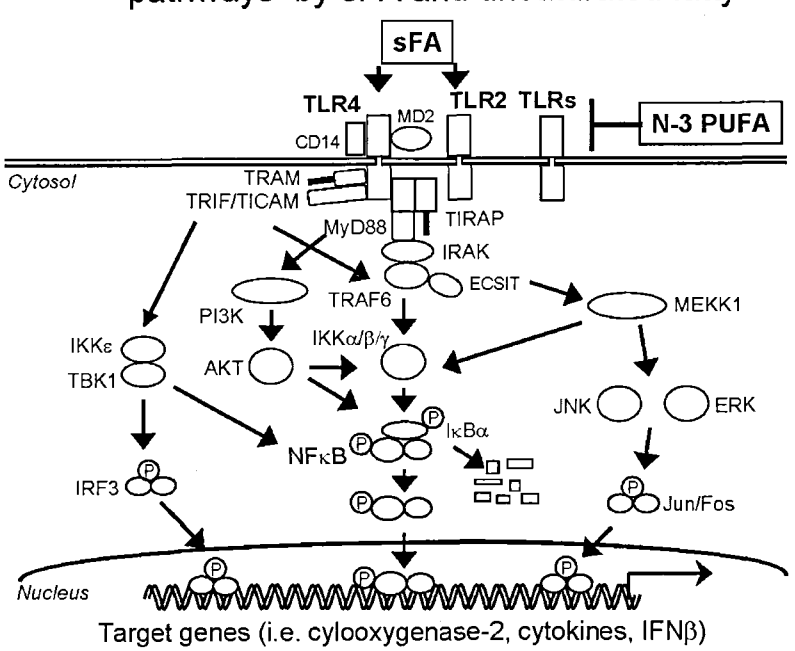
sFA activates both MyD88-dependent and –independent signaling pathways of TLR4



DHA inhibits NFκB activation and COX-2 expression induced by TLR4-, but not downstream signaling components



The modulation of TLR-mediated downstream signaling pathways by sFA and unsaturated fatty acids



(JBC, 2003)

Summary

- Saturated fatty acids induce, but unsaturated fatty acids inhibit NFkappaB activation and COX-2 expression mediated through the reciprocal modulation of Toll-like receptor.
- Saturated fatty acid activates TLR4 and TLR2 dimers while docosahexaenoic acid (DHA) is a pan inhibitor for all TLRs tested.
- N-3 PUFAs, DHA and EPA, are the most potent inhibitors as compared with n-6 PUFAs and n-9 unsaturated fatty acids.
- Saturated fatty acid-induced TLR4 and TLR2 activation leads to the activation of both MyD88-dependent and –independent signaling pathway.
- The target of DHA may be signaling components upstream of MyD88 or the proximal events leading to TLR activation.

Conclusion

- Inflammatory responses can be differentially modulated by types of dietary fatty acids mediated through the modulation of TLR activation.
- It is important to assess whether certain dietary factors can modulate TLR-mediated signaling pathways and target gene expression in order to manage the deregulation of TLR-mediated inflammatory responses leading to acute and chronic inflammatory diseases.

Future Perspectives

Toll-like receptor inflammasome system:
A new Toxicity Assessment System
to evaluate pro-inflammatory activity of dietary factors.