Inflammasomes & inflammation
Kenneth L. Rock, M.D.
The innate immune system uses numerous “sensors” to detect problems

One of the best understood is the Toll-like receptor (TLR) system

TLRs are expressed on the cell surface or in endosomes & monitor the environment for pathogens
Toll-like receptors (TLR) Structure-Function

- **Extra-cellular**
- **Intra-cellular**
- **Leucine-rich repeats**
- **Protein interacting domain**
- **Plasma membrane**

**Recognition**

**Signaling**
TLR recognition - LRR domains

Recognize non-mammalian structures

Gram −

Gram +

Membrane
Peptidylglycan
Lipoprotein
LPS

Lipoteichoic Acid
TLR recognition - LRR domains

Recognize non-mammalian structures

Gram −

Gram +

TLR4

Membrane
Peptidylglycan
Lipoprotein
LPS

Lipoteichoic Acid
TLR recognition - LRR domains

Recognize non-mammalian structures

Gram –

Gram +

TLR4

Membrane
Peptidylglycan
Lipoprotein
LPS

TLR2

Lipoteichoic Acid
TLR recognition - LRR domains

Recognize non-mammalian structures

Gram –

Gram +

Membrane Peptidylglycan Lipoprotein LPS

Lipoteichoic Acid

TLR4

TLR2

A limited number of receptors (10-11 TLR genes) – each “sees” a different set of non-mammalian structures in bacteria, yeast, virus, permitting recognition of a very large number of microbes.
Biochemical & structural evidence of TLR Ligand recognition

Some of the ligand-receptor interactions have been detected biochemically.

Crystal structure shows binding to LRR.

Others are not measurable (affinity too low?)
Cytosolic sensors

One subset = NOD-like receptors (NLR)

23 genes in humans
34 genes in mice

All have Nucleotide Oligomerization Domains (NOD)

All have LRR domains (in this aspect like intracellular TLRs)
Presumed Ligand binding

Oligomerization

Protein interaction
NLRs – 4 families (based in part on C-terminal domains)

- Presumed Ligand binding
- Oligomerization
- Protein interaction

LRR

NOD, NAIP, NLRC, NLRP (NALP)
What do they sense?

NOD1  Peptidyl glycan fragments (e.g. meso-DAP)
NOD2  Peptidyl glycan fragments (e.g. MDP)
NLRC3 (IPAF)  Flagellin
NLRP1 (NALP1)  MDP, Anthrax lethal toxin
NLRP3 (NALP3)  MDP, LTA, pore forming toxins, other

Direct binding has not been measured yet

What most NLRs sense is unknown.
The C-terminal domain determines function

Presumed Ligand binding

Oligomerization

Protein interaction

Homotypic interactions with adaptor and effector molecules

Function
A small subset of NLRs stimulate IL-1 Production

Presumed Ligand binding

Oligomerization

Protein interaction

LRR

CARD

Bir

NOD

NAIP

NOD

NAIP

# human genes

1

14

1

NLRC

NLRP (NALP)

IPAF

NLRP1-3
Summary of key points

2 important sets of microbial sensors are the TLR (extracellular) and NLR (intracellular).

They have LRR regions that are thought to bind ligands (only shown for TLR).

Their C-termini are protein-interacting domains which determine their function.

A subset of NLR participate in the generation of IL-1.
NLRs that trigger IL-1

Have C-terminal protein interaction domains that form a complex called the Inflammasome (more later)
We will focus on the NLRs that trigger IL-1

But first, a primer on IL-1
Interleukin 1 (IL-1)

Discovery
Infection ➔ Fever
Interleukin 1 (IL-1)

Discovery

Infection ➔ Endogenous pyrogen ➔ Fever
Interleukin 1 (IL-1)

Discovery

Infection \( \rightarrow \) Endogenous pyrogen \( \rightarrow \) Fever

IL-1 (also IL-6 & TNF\(\alpha\))
Interleukin 1 (IL-1)

Discovery
Infection → Endogenous pyrogen → Fever

IL-1 (also IL-6 & TNFα)

2 forms: IL-1α & IL-1β
Generation of IL-1

Proinflammatory stimuli

IL-1β gene

Pro-IL-1β (inactive)
Generation of IL-1

Proinflammatory stimuli

procaspase 1 (inactive)

Caspase 1

IL-1β gene

Pro-IL-1β (inactive)

IL-1β (active)
What does IL-1 do?

IL-1α & IL-1β

IL-1R

Cell
What does IL-1 do?

IL-1α & IL-1β → IL-1R

Cell → NFkB (inactive)

→ active genes
What does IL-1 do?

IL-1α & IL-1β → IL-1R → NFkB (inactive) → active genes

Cytokines
- IL-1
- IL-6
- IL-8
- TNFα

Chemokines
- MIP-1
- MCP1
- RANTES
- eotaxin

Cell Adhesion
- ICAM-1
- E-selectin
- VCAM-1
NFκB and Inflammation

- Injury/infection
- Selectins
- Endothelial activation
- Integrin activation
- Integrin ligands
- IL-8, TNFα
- Fever
- IL-1, IL-6
- Chemokines
Contribution of IL-1 to inflammation in different settings
Autoimmunity

IL-1 receptor antagonist (IL-1Ra)
Autoimmunity

IL-1 receptor antagonist (IL-1Ra) → Rheumatoid arthritis patients → Clinical improvement

[but not as effective as anti-TNF; presumably because in autoimmunity IL-1 is one of many factors]
Autoinflammatory diseases

Muckle-Wells, NOMID, Familial cold autoinflammatory syndrome, FMF
Autoinflammatory diseases

Muckle-Wells, NOMID, Familial cold autoinflammatory syndrome, FMF

Genetic syndromes where IL-1 is over produced

Fever, skin, joint, ± neurological symptoms

IL-1Ra

Marked clinical improvement
Other settings where IL-1 plays a major role in inflammation
Sterile cell death

IL-1 is made by the host in response to cell death.

Dead cells

IL-1 is made by the host in response to cell death.

Dead cells

IL-1 is made by the host in response to cell death.

Dead cells

IL-1 is made by the host in response to cell death.

Dead cells
Key role of IL-1-IL-1R in death-induced inflammation

Dead cells

Lack IL-1

Neutrophils

PBS  WT  IL-1α-/-  IL-1β-/-  IL-1αβ-/-

0.0E+00  5.0E+05  1.0E+06  1.5E+06  2.0E+06  2.5E+06  3.0E+06

Lack IL-1

Courtesy of Hajime Kono, submitted
Key role of IL-1-IL-1R in death-induced inflammation

Dead cells

Dead cells i.p.

Lack IL-1

Lack receptor

Neutrophils

PBS  WT  IL-1α/−  IL-1β/−  IL-1αβ/−  IL-1R/−

Bkg  Recipient strain

Courtesy of Hajime Kono, submitted
MSU - gout
MSU-induced inflammation requires the IL-1R

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Other IL-1 dependent inflammation

Irritant particles
Silica
Asbestos
Alum
βAmyloid aggregates
Summary

IL-1β is a cytokine whose maturation requires a cleavage by caspase 1.

Mature IL-1 stimulates NFκB, a transcription factor that controls many key molecules in inflammation.

IL-1 plays an essential role in autoinflammatory conditions, cell death & particle inflammation & also contributes to autoimmune inflammation.
Getting back to the NLRs...
Connecting NLRs to IL-1 production

NLRP3

Stimuli
NFkB activators (TLR, cytokines)

Increase
Synthesis

Pyrin
Connecting NLRs to IL-1 production

Stimuli

The inflammasome

- Pyrin
- CARD
- ASC
- Caspase 1
Connecting NLRs to IL-1 production

The inflammasome
The inflammasome

ASC-CFP

Unstimulated

Stimulated

Courtesy of Eicke Latz
Connecting NLRs to IL-1 production

Stimuli

Pro-IL-1β (inactive)

ACTIVE

caspase 1
Importance of NLRP3 to IL-1 production & inflammation

Inherited inflammatory diseases

Familial cold Auto-inflammatory syndrome
(Fever, cold urticaria, arthralgia)

Muckle-Wells syndrome
(Fever, urticaria, arthralgia, deafness, amyloid)

CINCA (NOMID)
(Fever, urticaria, arthralgia, neurological disease, amyloid)

Mutation NLRP3
How are NLRs normally stimulated?

Remember = cytosolic molecules
NLRC4 (IPAF)

Type III & IV secretion systems

salmonella, pseudomonas

Legionella

Pannexin (channel)

e.g. Flagellin

NLRC4
NLRP3 (NALP3)
Maybe the same model

Pore–forming toxins
Listeria lysin O
Aerolysin
maitolysin

ATP
K+
P2X7
K+

Maybe the same model
But in addition…
Gout-associated uric acid crystals activate the NALP3 inflammasome

Fabio Martinon, Virginie Petrilli, Annick Mayor, Aubry Tardivel & Jurg Tschopp

Other particulates activate the NLRP3 inflammasome

Silica

Wild-type | NLRP3−/−

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<th>250</th>
<th>500</th>
<th>1000</th>
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<td>0</td>
<td>0.2</td>
<td>0.6</td>
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Silica crystals (µg/ml)

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<th>500</th>
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Alum

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<th>Wild-type</th>
<th>Nalp3−/−</th>
<th>ASC−/−</th>
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<tr>
<td>++</td>
<td>−/−</td>
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IL-1β (ng/ml)

Yet other particles/crystals stimulate IL-1 production from Mθ via NLRP3 (NALP3) inflammasomes

Asbestos (Flavell)
Summary

Pyrin-domain containing NLRs assemble into inflammasomes

Simulated inflammasomes activate caspase 1 that in turn generates active IL-1β

Gain of function mutations in these NLRs causes IL-1 dependent autoinflammatory syndromes.

These NLRs can “sense” microbial ligands & also somehow particles and crystals.
How can intracellular NLRP3 sense extracellular particles?

Crystals

CFP-ASC (inflammasome)
Phagocytosis of MSU

Control

Cytochalasin D

Courtesy of Eicke Latz
Is phagocytosis important for triggering IL-1 production?
Phagocytosis is required for crystal activation of the inflammasome

How do internalized crystals activate inflammasomes?
Postulated mechanism 1

NADPH Oxidase

O₂

ROS

Thyroidoxin binding-protein

Thyroidoxin
Data for another, maybe unifying mechanism
Visualize the fate of crystals

Mθ

Cholera toxin B
Membrane stain
Rupture of MSU-phagosomes

Red = membrane
Green = MSU

Courtesy of Eicke Latz
Conclusions

Triggering of NLRP3 inflammasomes by particles requires phagocytosis

Some phagosomes containing crystals (MSU+others) rupture & deliver their contents to the cytosol
What is NLRP3 sensing?

- MSU
- Silica
- Alum

Rupture (Common)
Structurally Distinct
Particle-free sterile rupture of lysosomes

Leu-Leu-OMe

Hypertonic sucrose

Feed to M\(\theta\)

Osmotic rupture of vesicles
Rupture of lysosomes is sufficient to activate inflammasomes

Wild type M\(\theta\)

![Graph showing IL-1\(\beta\) levels in response to different treatments.](image-url)

Rupture of lysosomes is sufficient to activate inflammasomes

Wild type Mϕ

Conclusions

Crystal containing phagosomes rupture

Rupture of lysosomes is sufficient to cause NLRP3 activation

NLRP3 senses internal cell damage (lysosomal rupture)
NLRP3 sensing of endosome-phagosome rupture

This might also explain why pore-forming toxins trigger NLRP3
How does NLRP3 sense lysosomal rupture?
Acidification is required
Acidification is required

WHY?

H^+

Bafilomycin

NLRP3
Role for Cat B in crystal induced inflammasome activation

Model

- H^+ + Cat B → Rupture
- NLRP3
- Substrate
Medical Significance
Inflammation is a double-edged sword

Rapid defense
Repair

Tissue damage
Cost-benefit in sterile inflammation
Sterile inflammation leads to or exacerbates a number of diseases
Collateral damage after sterile cell death

Toxic injury

C57BL/6

IL-1R−/−

Also in ischemic injury (MI, Stroke)

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Crystal-based diseases
Gout
Silicosis

Kumar et al Robbins and Cotran Pathologic Basis of Disease 8/E (Fig 15-18)
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Other diseases?

Maybe.....
Atherosclerosis

Kumar et al Robbins and Cotran Pathologic Basis of Disease 8/E (Fig 15-21)
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Consequences of atherosclerosis

16.7 million deaths from cardiovascular disease each year. > 29% of all deaths globally and 38% of all deaths in North America


Also:

Peripheral vascular disease

Other ischemic organ damage
Atherosclerosis - pathogenesis

Kumar et al Robbins and Cotran Pathologic Basis of Disease 8/E (Fig 11-10)
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Cholesterol crystal in Atherosclerosis

Fibrous plaque (on opposite wall)

Optical "empty", needle-like cholesterol crystals

Endothelium

"Fibrous cap"

Vascular lumen

Atheroma core

Cholesterol crystal in Atherosclerosis

atherosclerosis
(inflammation)

cholesterol crystal
Cholesterol crystal in Atherosclerosis

monosodium urate crystal

gout (inflammation)
Cholesterol crystal in Atherosclerosis

silica crystal

silicosis (inflammation)
Atherosclerosis

A crystal-based disease???

Eicke Latz

Hajime Kono

Peter Duewell
Franz Bauerfeind
Cherlyn Sirois
George Abela
Luigi Franchi

Gabriel Nunez
Max Schnurr
Terje Espevik
Kathryn Moore
Kate Fitzgerald

Sam Wright
Veit Hornung
ApoE−/− mice

Regular Diet

High Fat Diet
X 12wk


nuclei (Hoechst)

macrophage (MoMa-2)
crystals (reflection)
Uptake of modified LDL leads to the formation of cholesterol crystals

Cholesterol crystal stimulates M0s to produce IL-1 in vitro

![Graph showing IL-1B levels in response to varying concentrations of cholesterol crystals]

Cholesterol crystal stimulates Mφs to produce IL-1 in vitro
Cholesterol crystal stimulation of IL-1 requires:

- Phagocytosis
- Acidification
Rupture of cholesterol-phagosomes

Plasma membrane
Free crystals
Crystals in phagosomes

Cholesterol crystal stimulation of M\(\theta\)s to produce IL-1 in vitro requires cathepsins
Cholesterol stimulates IL-1 production by the same pathway as other crystals

Cholesterol
Uric acid
Silica
Etc.

Inflammation
What about in vivo?

LDL receptor-/-  High Fat Diet
What about in vivo?

LDL receptor-/-

High Fat Diet

What about in vivo?

LDL receptor-/-

→ NLRP3 → IL-1

High Fat Diet

What about in vivo?

LDL receptor-/-

→ NLRP3 → IL1

High Fat Diet

Inflammasome role in atherosclerosis \textit{In Vivo}

LDLR-/-
Inflammasome role in atherosclerosis *In Vivo*

Bone marrow

WT B6
ASC-/-
NLRP3-/-
IL-1AB-/-

LDLR-/-
And the results ....

**plaque percentage**

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<th>WT</th>
<th>IL-1ABKO</th>
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And the results …..

Summary

• Cholesterol crystal appears in the early atherosclerosis

• Cholesterol crystal stimulates macrophage / DCs to secrete IL-1 beta, which is dependent on phagocytosis / lysozomal acidification and damage / CatB / CatL / NLRP3 / ASC / Casp1.

• LDLR-/- mice with inflammasome deficient bone marrow showed reduced atherosclerosis lesion.

• Atherosclerosis might be a crystal-based disease
Overall summary

Microbes & particles

NLR

Inflammasomes

IL-1

Inflammation

Disease
The End