PKR is required for macrophage apoptosis after activation of TLR-4

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Introduction
Macrophage activation leads to clearance of Bacteria (LPS, Gram -ve) and LTA (Gram +ve).
Figure 1

Signaling through TLR4 while inhibiting p38 leads to apoptosis.
HKBA = heat killed B. anthracis

HeJ = TLR4 wt
HeN = TLR4 mut
SB = p38 inhibitor

Apoptosis

IFN-inducible genes
Antagonistic inflammatory and death cytokines

HKBA or LPS
When p38 is inhibited (by SB) only signalling via TLR4 leads to increased apoptosis.
iCAD

ProCaspase 9 → Caspase 9

Cytochrome C

Caspase 8 ← ProCaspase 8

Caspase 3 ← ProCaspase 3

insult

nucleus

FAS-L

FAS-L

Fas

Fas

Fas

Fas

Fas

Fas

Fas
Again this LPS/p38-inhibitor dependent apoptotic response is specific to signaling through TLR4.

(see last slide for notes on DAPI and TUNEL)
Figure 2

The role of MyD88 and TRAF6
MyD88 -/-  $\rightarrow$ more apoptosis
TRAF6 -/- → more apoptosis
TRAF6 -/-  → less JNK phosphorylation
     → same p38 phosphorylation
     → IKK less degradation
Role of PKR in apoptosis induced by TLR signaling
Induction of IFN in response to TLR signaling

IFNbeta IFNalpha
PKR is necessary for LPS-induced INF signaling in macrophages

PKR activation by LPS
PKR acts downstream of TRIF

Normal MAPK and IKK activation in PKR−/−
PKR is required for INFbeta induction but is dispensable for induction of anti-apoptotic genes.

PKR is required for iNOS induction and STAT1 phosphorylation.
PKR is required for LPS-induced macrophage apoptosis

PKR deficient BMDMs are resistant to LPS-induced apoptosis
Bacillus anthracis Exotoxin

Edema Factor (EF)  
adenylylate cyclase, inhibits phagocytosis

Lethal Factor (LF)  
Zn dependent protease, cleaves MAPKs and lyses macrophages

Protective Ag (PA)  
binds to cellular receptors

PKR is required only for LPS induced apoptosis

INF beta sensitizes BMDM to LPS-induced apoptosis
Type 1 IFN signaling is not required for LPS-induced macrophage apoptosis

PKR activation inhibits A1/Bfl1 expression
eIF2alpha phosphorylation is required for induction of macrophage apoptosis

IRF3 is required for induction of macrophage apoptosis

LPS → PKR

IFN induction → macrophage apoptosis

BUT macrophage apoptosis does not require IFN induction
Figure 5
Effect of PKR on bacteria-induced apoptosis

- Both G-ve and G+ve bacteria induce apoptosis
- PKR−/− strain much less susceptible
- In addition to TLR4 engagement, bacterium needs to make its own contribution
Effect of PKR on bacteria-induced apoptosis

- Non-infected population shows comparable number of live cells and little death
- Infected PKR^{+/+} population displays much more apoptosis than PKR^{-/-} population
Dependence of Apoptosis on eIF-2α Phosphorylation and IRF3

- S51A: cannot phosphorylate eIF-2α, cannot shut down translation
- IRF3 activated by dsRNA much like PKR; logical downstream signaling partner
Dependence of Apoptosis on a Functional TLR4

- TLR4 receptor in HeJ mice is inactive
- Inactive TLR4 receptor associated with greatly diminished apoptotic response to bacteria
Summary

TLR4 signaling is an inducer of apoptosis in macrophages.

MyD88, TRAF6, and IKK all contribute to the anti-apoptotic pathway.

PKR, already known to induce TI IFN, is a crucial component of TLR4-induced apoptosis in macrophages.

Acts in part through suppression of synthesis of anti-apoptotic products as well as through IRF3.

IFNβ sensitizes the cell to apoptosis but is not required.
Apoptosis Through TLR4: It Takes Two to Tango

“2nd Hit” Needed

Homo: SB202190

B. anthracis: LT

Yersinia: YopJ

Salmonella: SPI2
Downstream PKR Effectors: Is Anyone Out There?

Double KO → Immunity?
Co-precipitation?
Microarray Analysis?
Epilogue: PKR Targeting as Anti-Bacterial Therapy

PKR inhibition:
- reduction of apoptosis
- better control of pathogens
- suppression of iNOS

PKR needed for virus suppression

Short term inhibition → siRNA?

Blocking of “2nd Hit” instead?
For Your Reference

**TUNEL** – Tdt is used to add labeled nucleotides on to the ends of DNA. The more the DNA is broken (apoptosis) the more nucleotides can be added and the stronger the signal *(Janeway 647-8)*.

**DAPI** – Binds to the minor groove of DNA and stains chromosomes.
Hold Me, Thrill Me, Kiss Me, Kill me