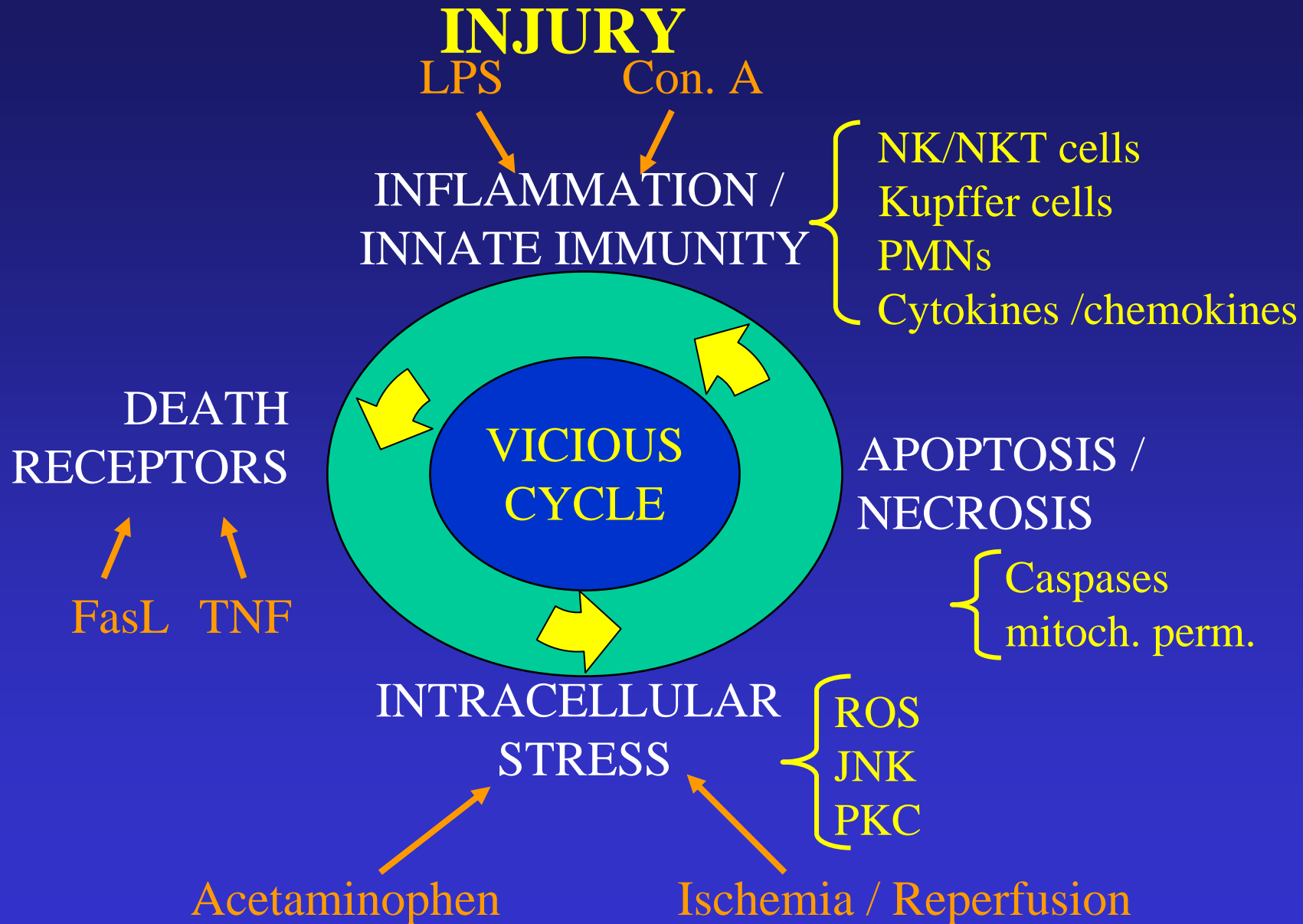


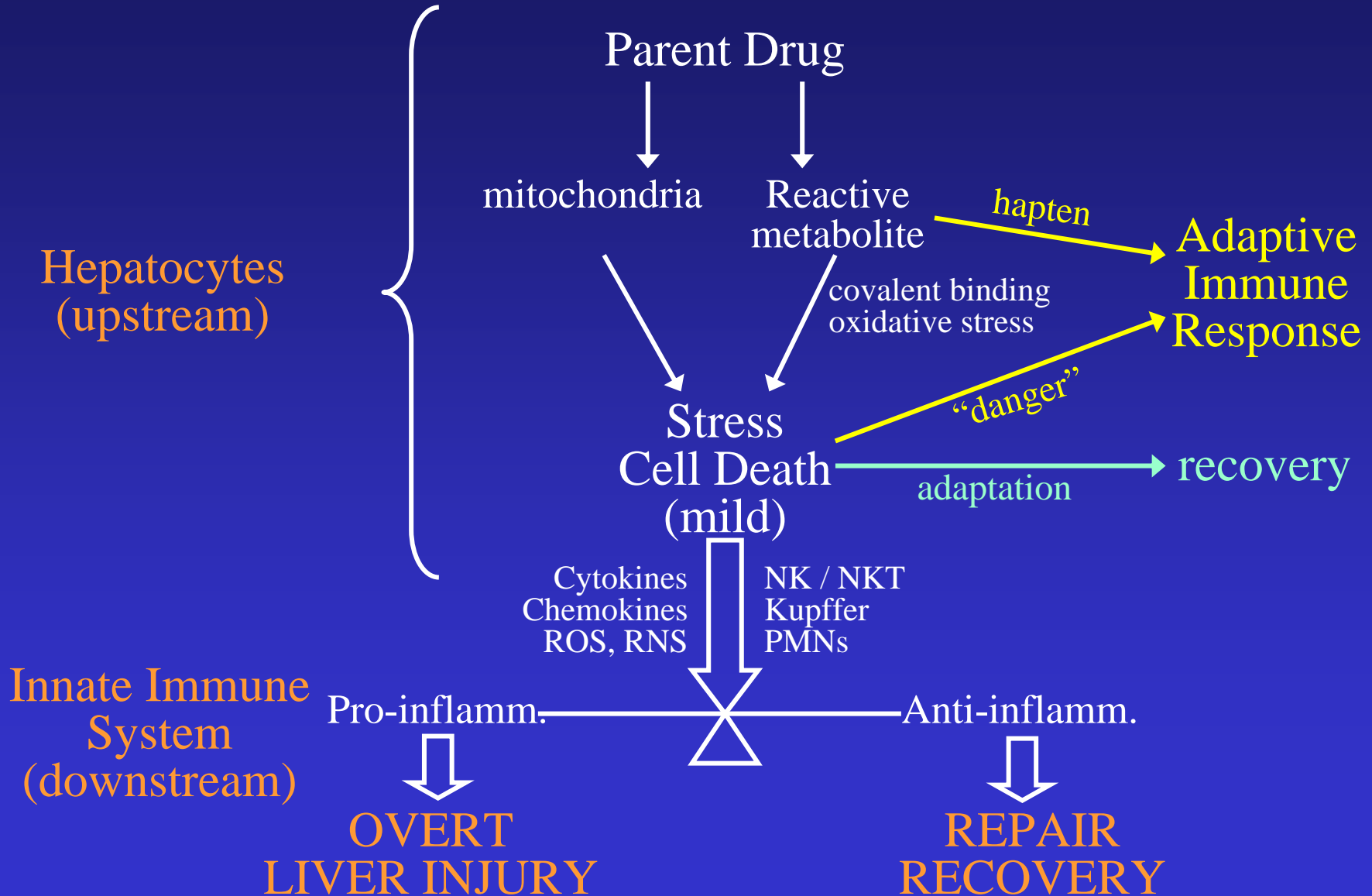
**NON-IMMUNE MECHANISMS OF DILI:
LESSONS FROM THE
ACETAMINOPHEN MOUSE MODEL**

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Keck School of Medicine
University of Southern
California**

PATHOGENESIS OF ACUTE LIVER



Pathogenesis of DILI



DYNAMIC DETERMINANTS OF APAP TOXICITY

worsen

protect

Hepatocyte

NAPQI
exposure

CAR

Nrf-2

Stress Response

JNK
PKC

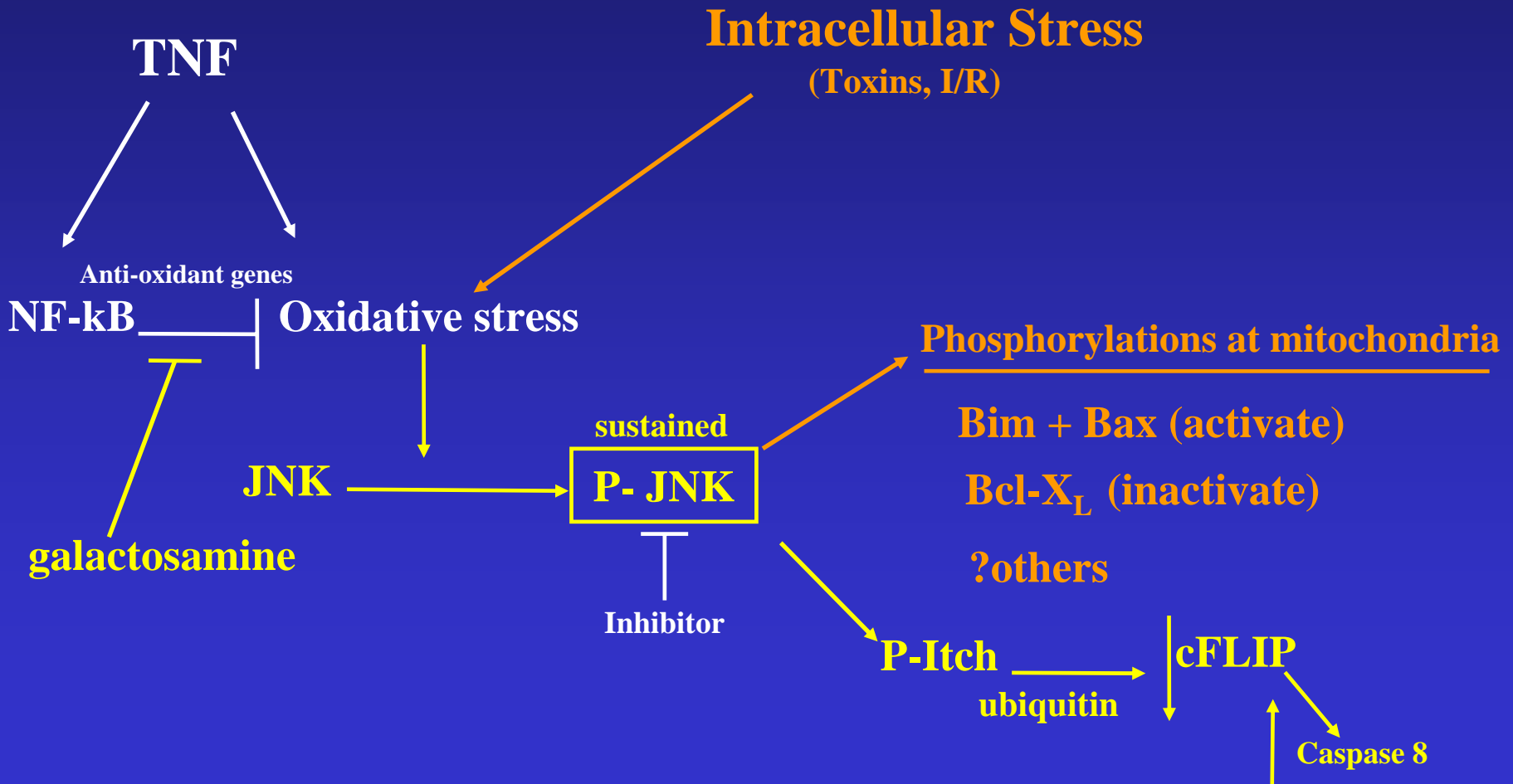
Akt

Innate immune
system

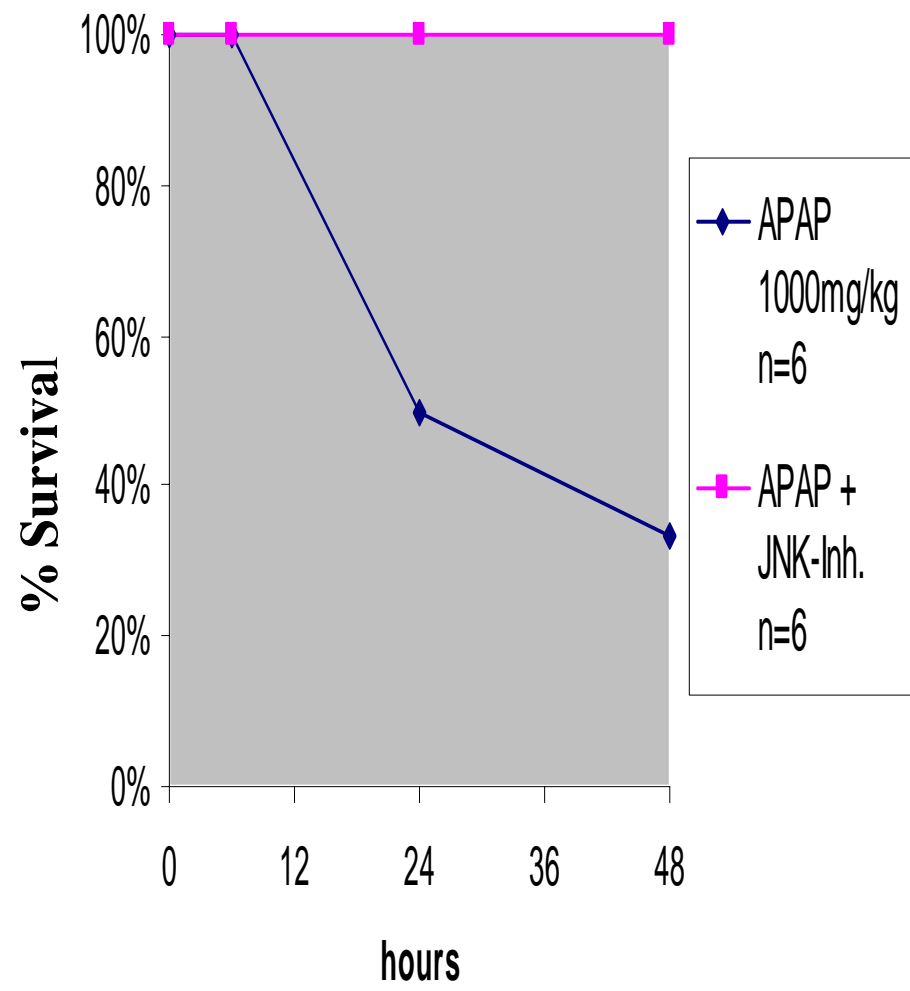
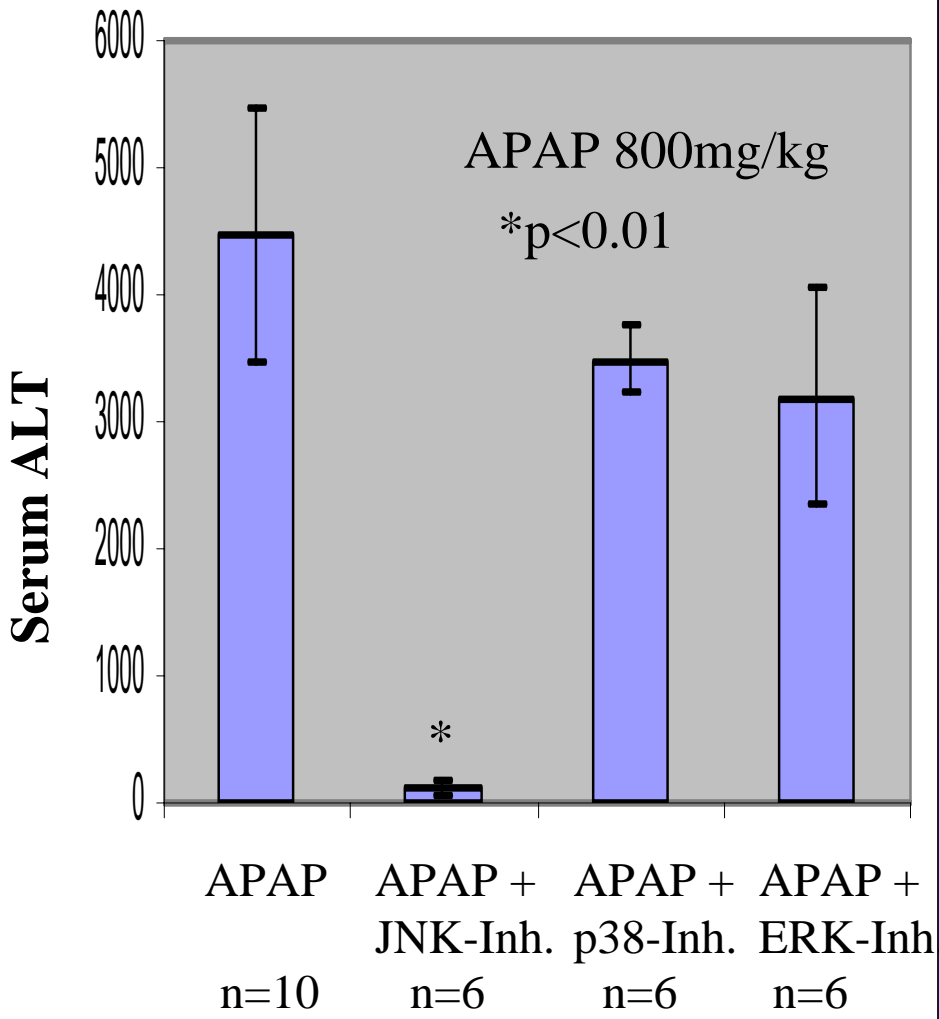
IL-10 KO
CCR5 KO

IFN γ KO
FasL/Fas mutations

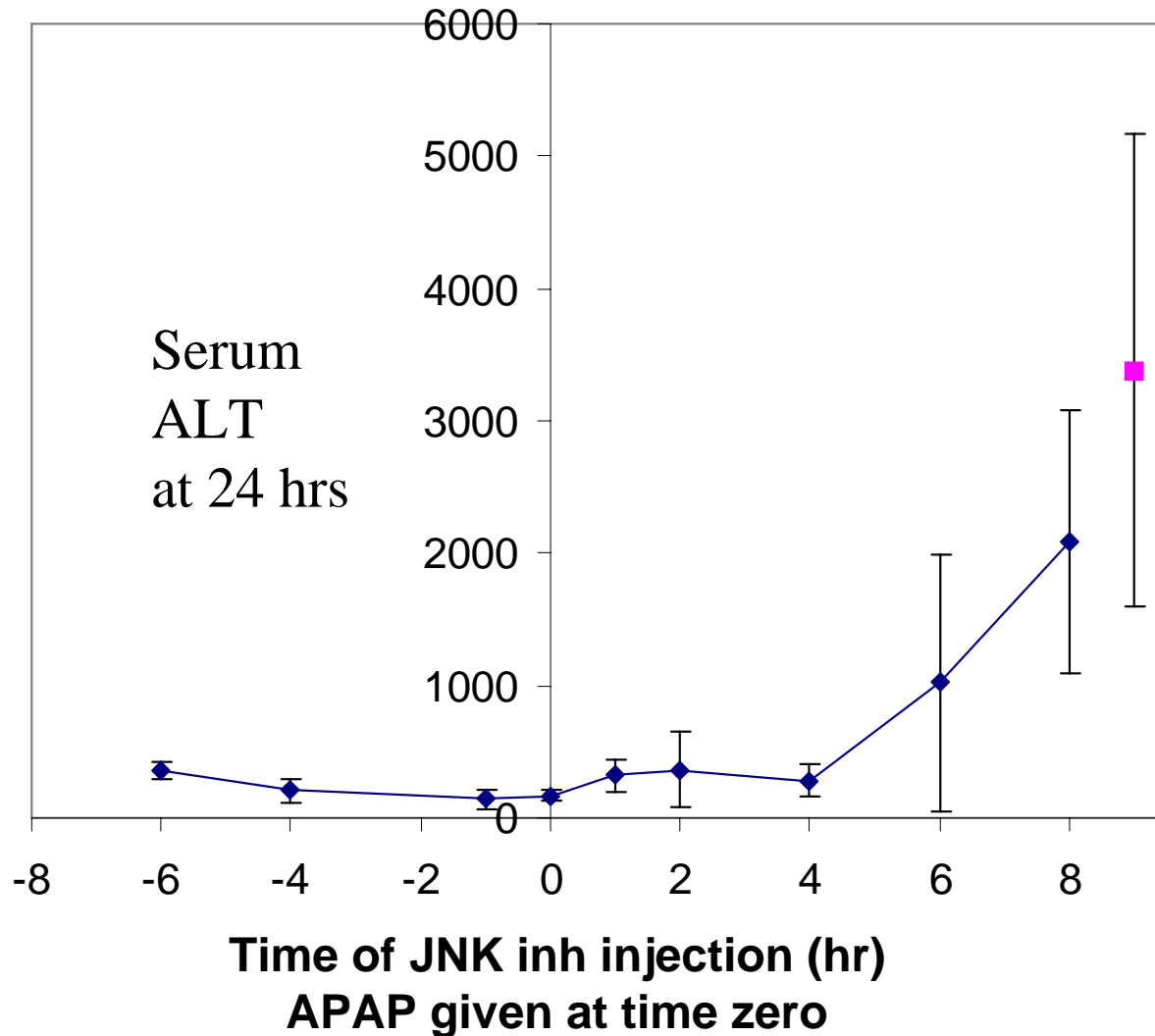
CRITICAL ROLE OF C-JUN-N-TERMINAL KINASE (JNK) IN CELL DEATH



Effect of Stress Kinase Inhibitors on APAP Hepatotoxicity *in vivo* in C57/BL6 Mice



Protection by Delayed Administration of JNK Inhibitor

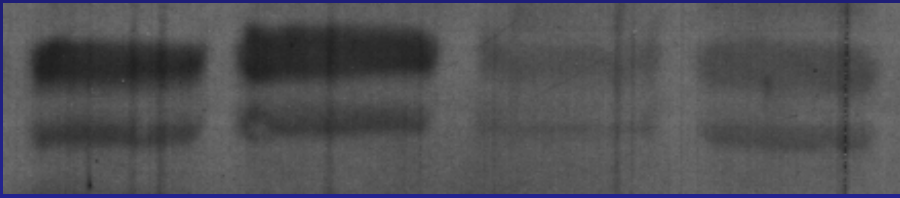


APAP +
vehicle at 8 hr

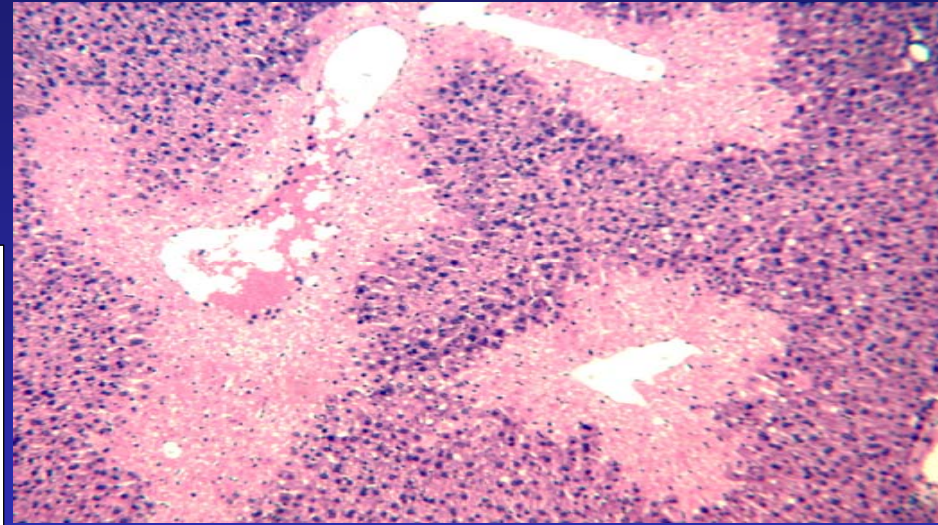
Effect of JNK1+2 Knockdown (antisense) on Acetaminophen Toxicity

Control antisense

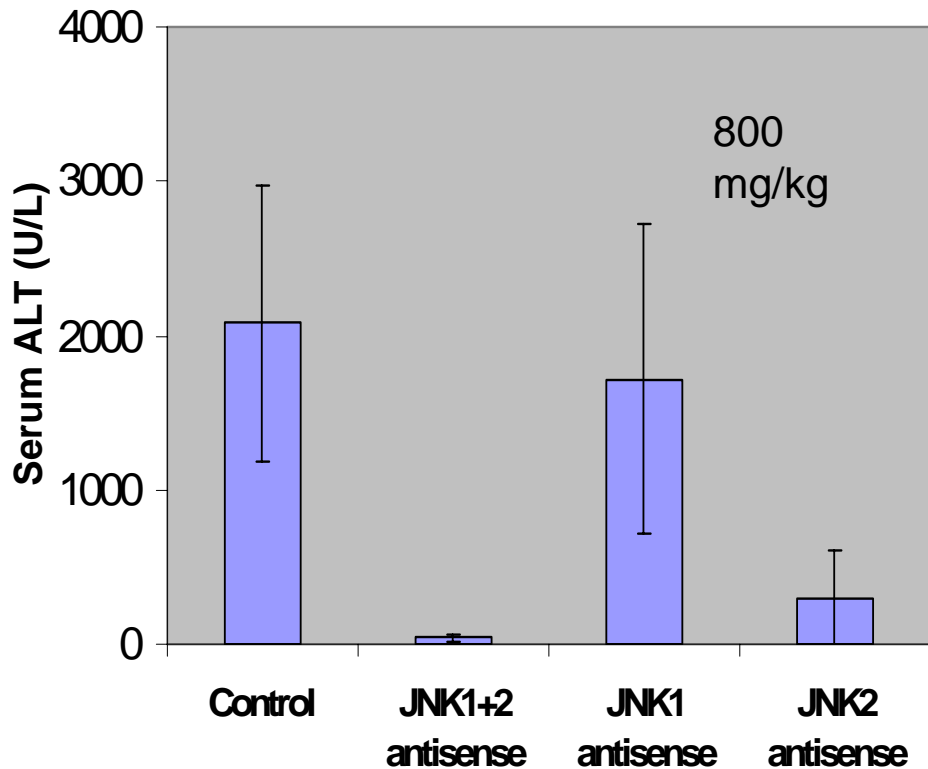
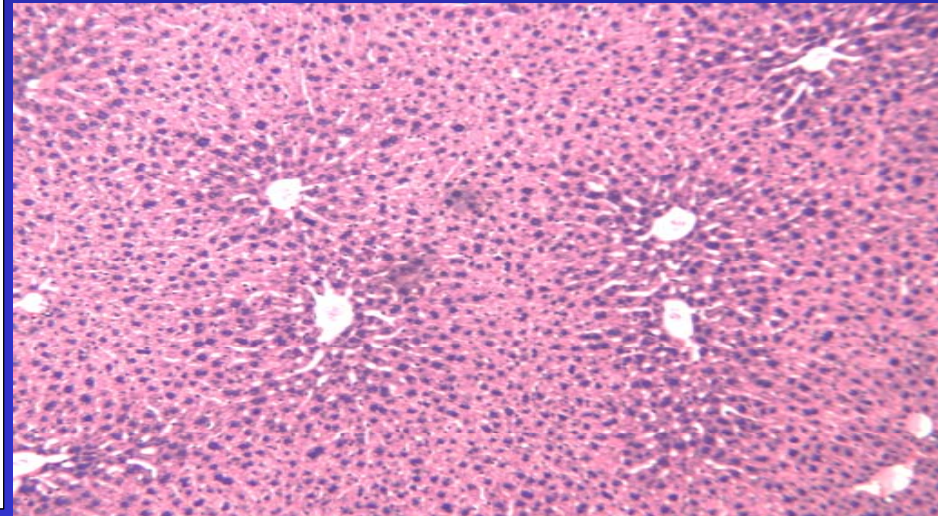
JNK1+2 antisense



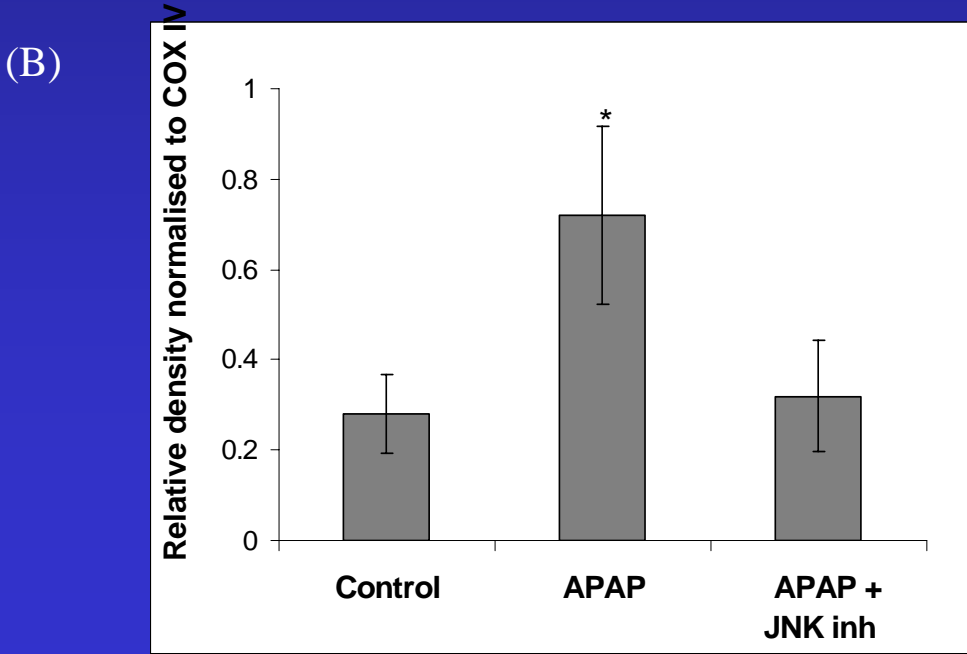
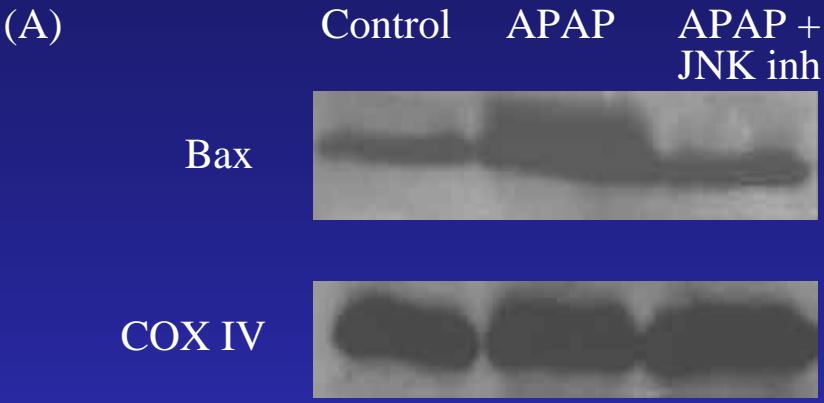
APAP + control antisense



APAP + JNK1+2 antisense



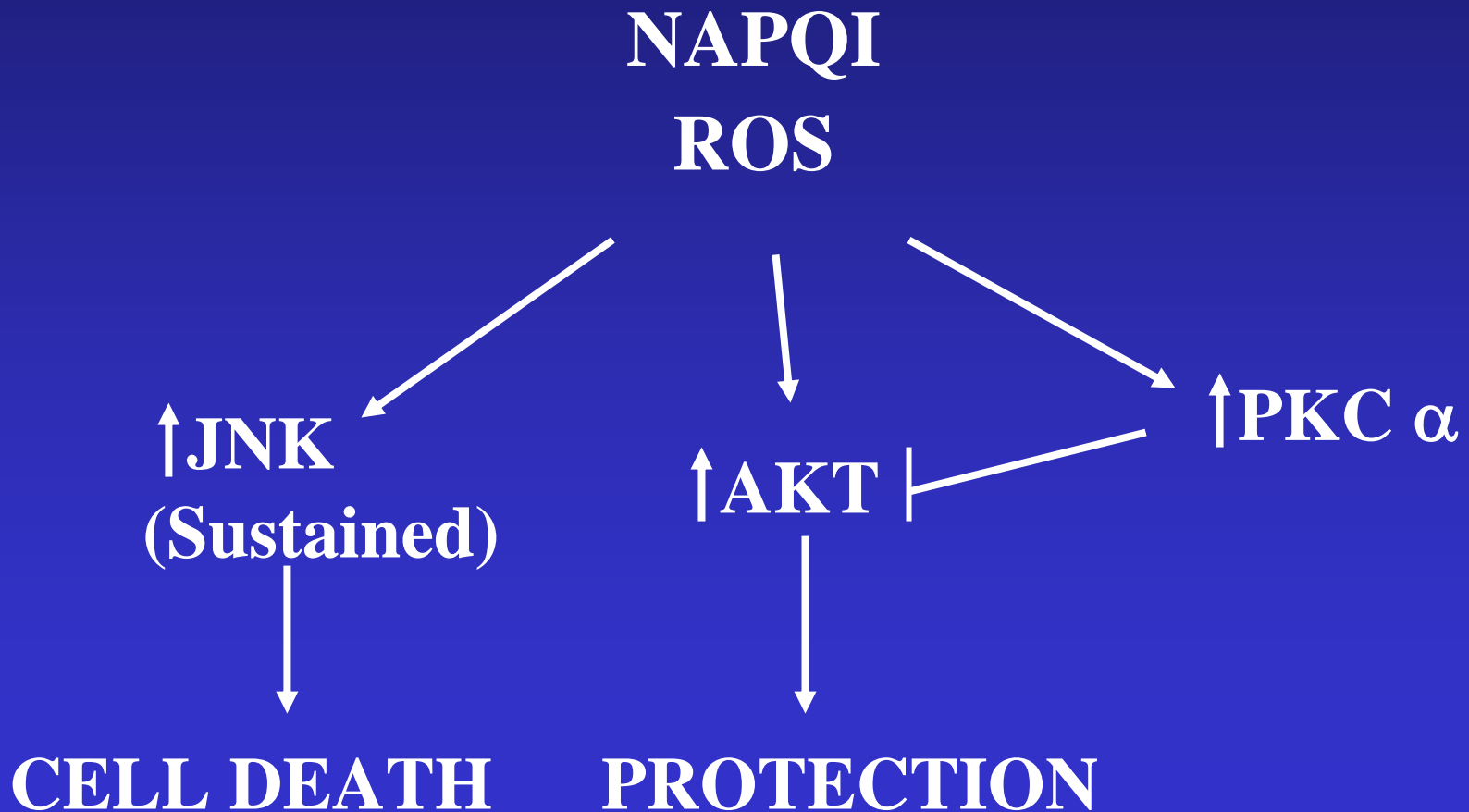
APAP Induces JNK-Dependent BAX Translocation



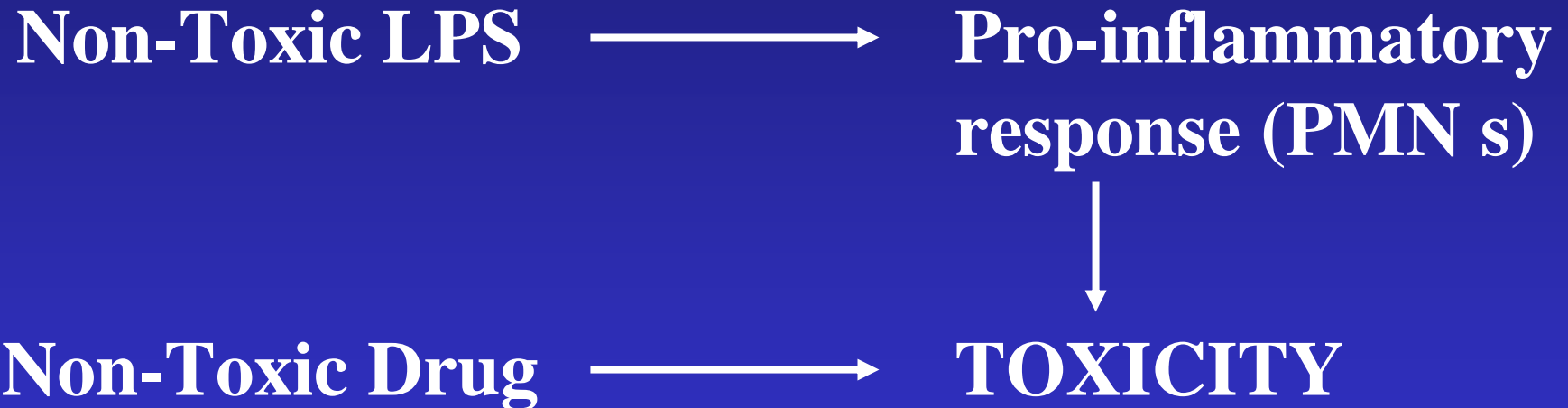
Additional Findings Related to Inhibition of JNK in the APAP Model

1. JNK1 $-/-$ mice were not protected whereas JNK2 $-/-$ were protected (comparable to ASO-JNK2)
2. No evidence of Bid cleavage or Bim activation
3. JNK inhibitor protects against APAP in TNF-R1 $-/-$ and Kupffer cell depleted mice
4. JNK inhibitor or ASO did not protect against CCl_4 or concanavalin A

Interplay of Signal Transduction in APAP Toxicity



Roth Model: Non-Toxic Doses of LPS enhance DILI



Diclofenac

Trovafloxacin

Ranitidine

Role of the Innate Immune System in Susceptibility to Acetaminophen

Protection

IFN γ knockout

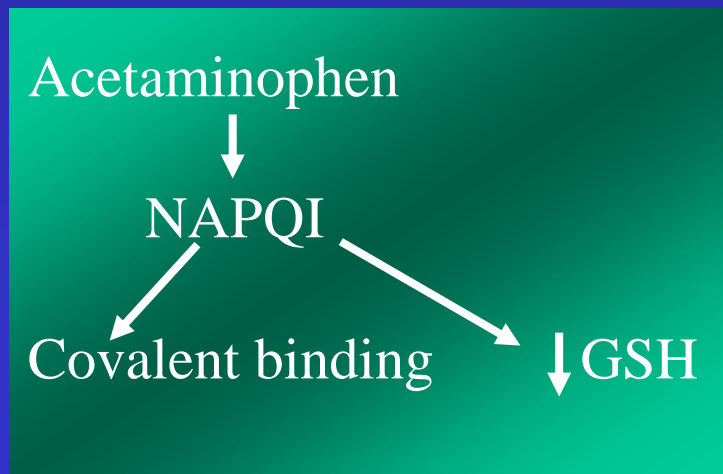
Fas antisense

NK / NKT depletion

Worsening

IL-10 knockout

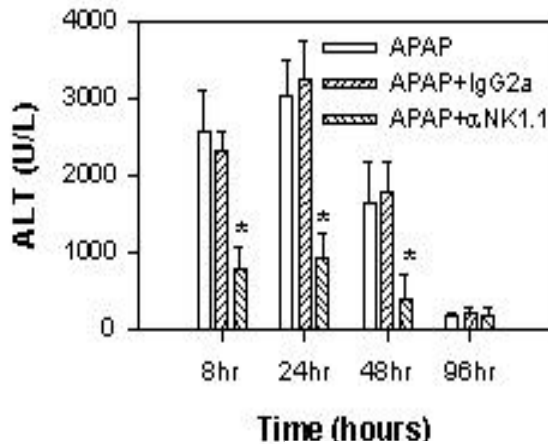
IL-6 knockout



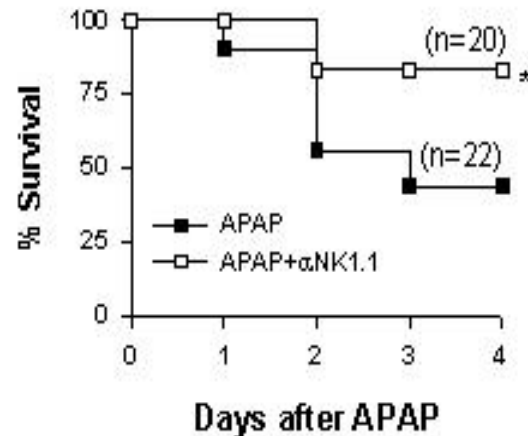
$\xleftarrow{\text{injury}}$ $\xrightarrow{\text{repair}}$ Cytokine/chemokine balance

Depletion of NK / NKT Cells Protects Against APAP

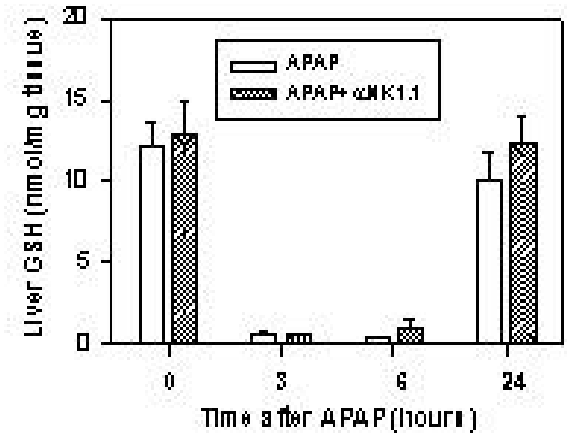
A) Serum ALT



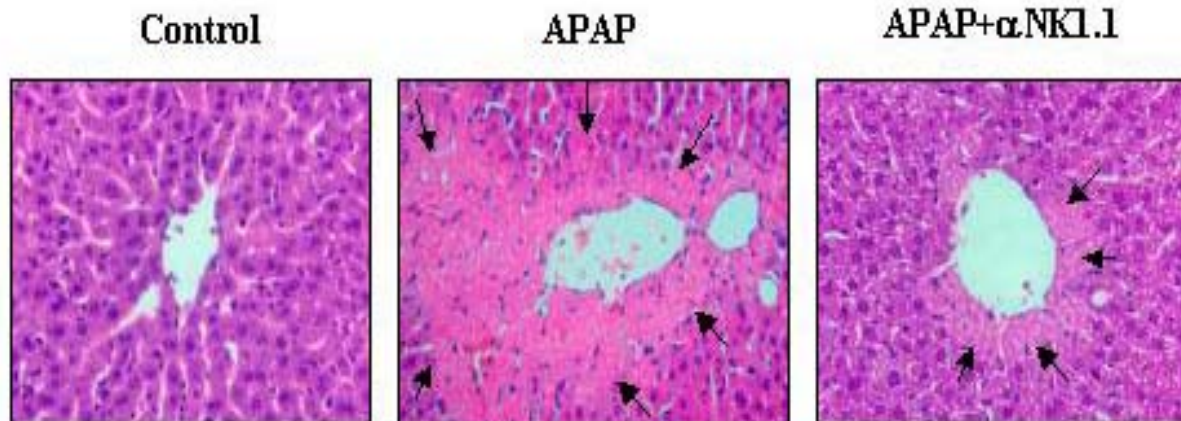
B) Survival



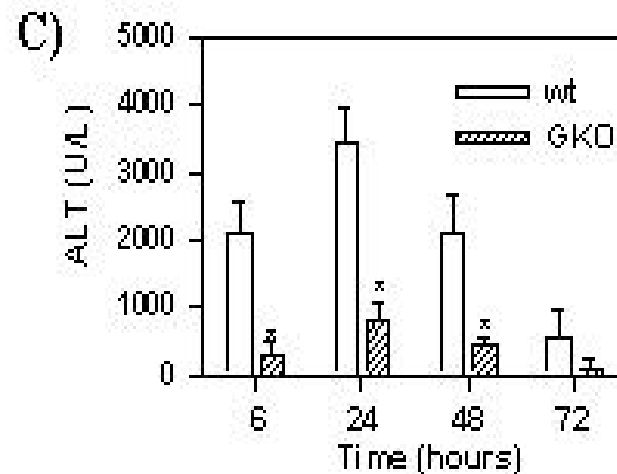
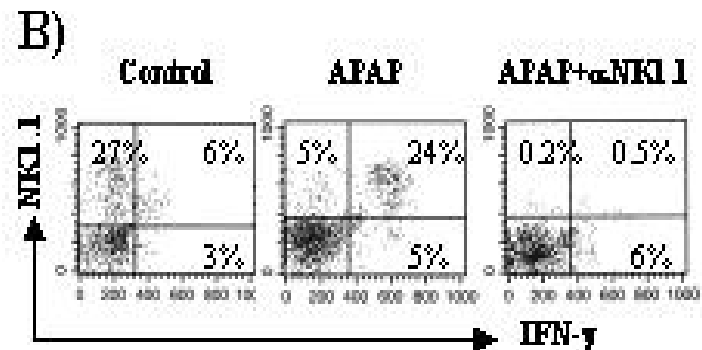
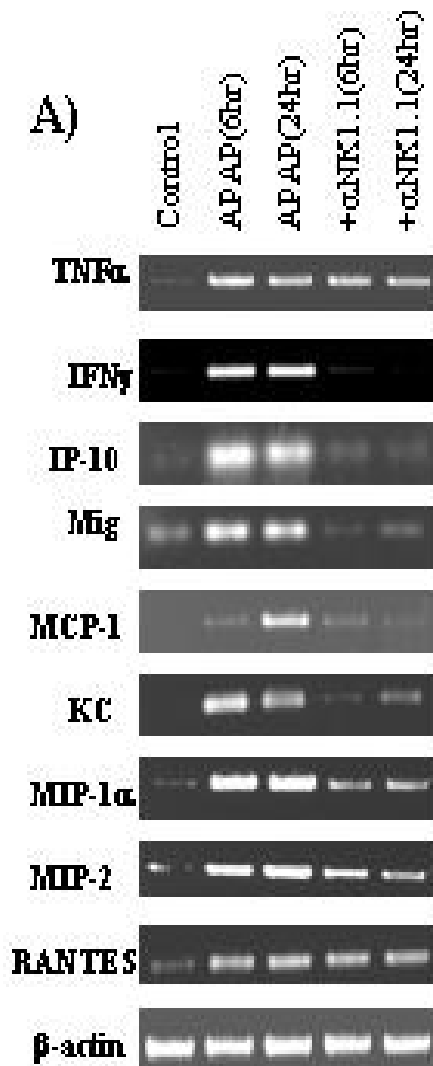
C) Hepatic GSH



D) Histology

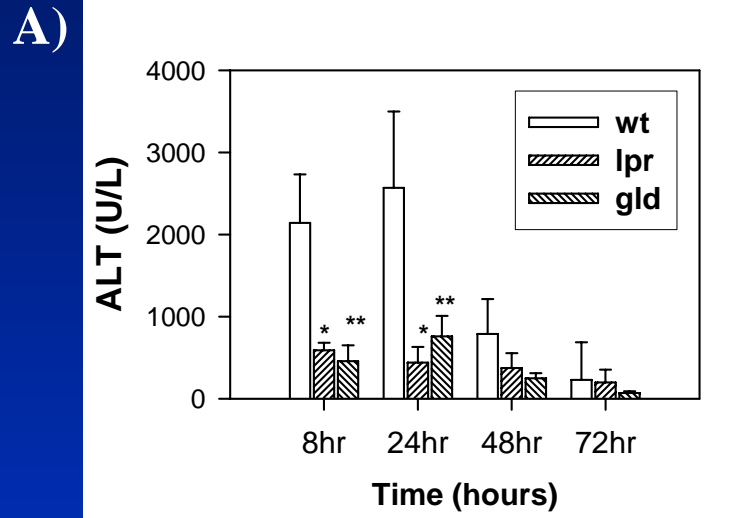


CYTOKINE/CHEMOKINE EXPRESSION IN APAP HEPATOTOXICITY

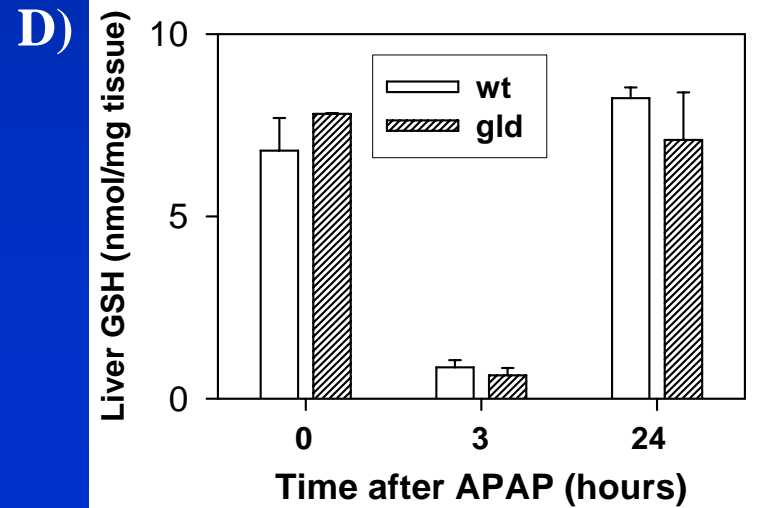
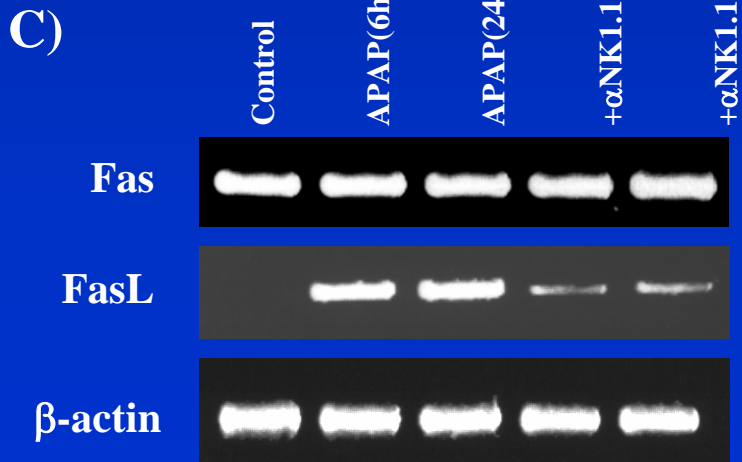
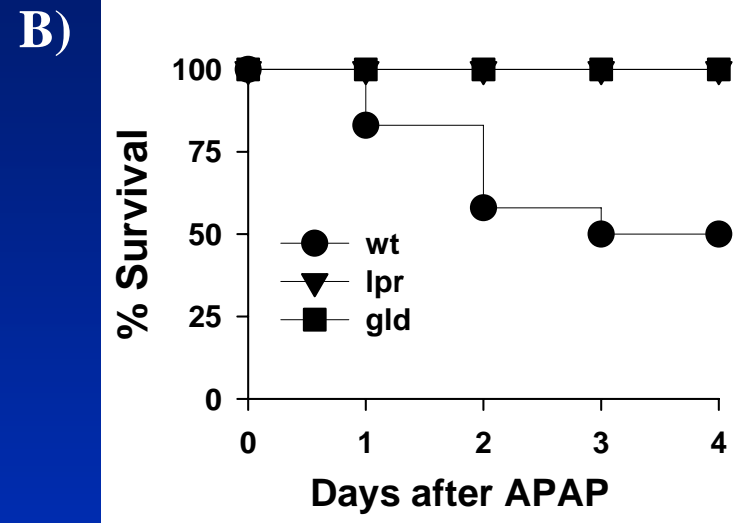


Effect of Acetaminophen on Expression of Fas, FasL and Toxicity in Fas (lpr) and FasL (gld) Deficient Mice

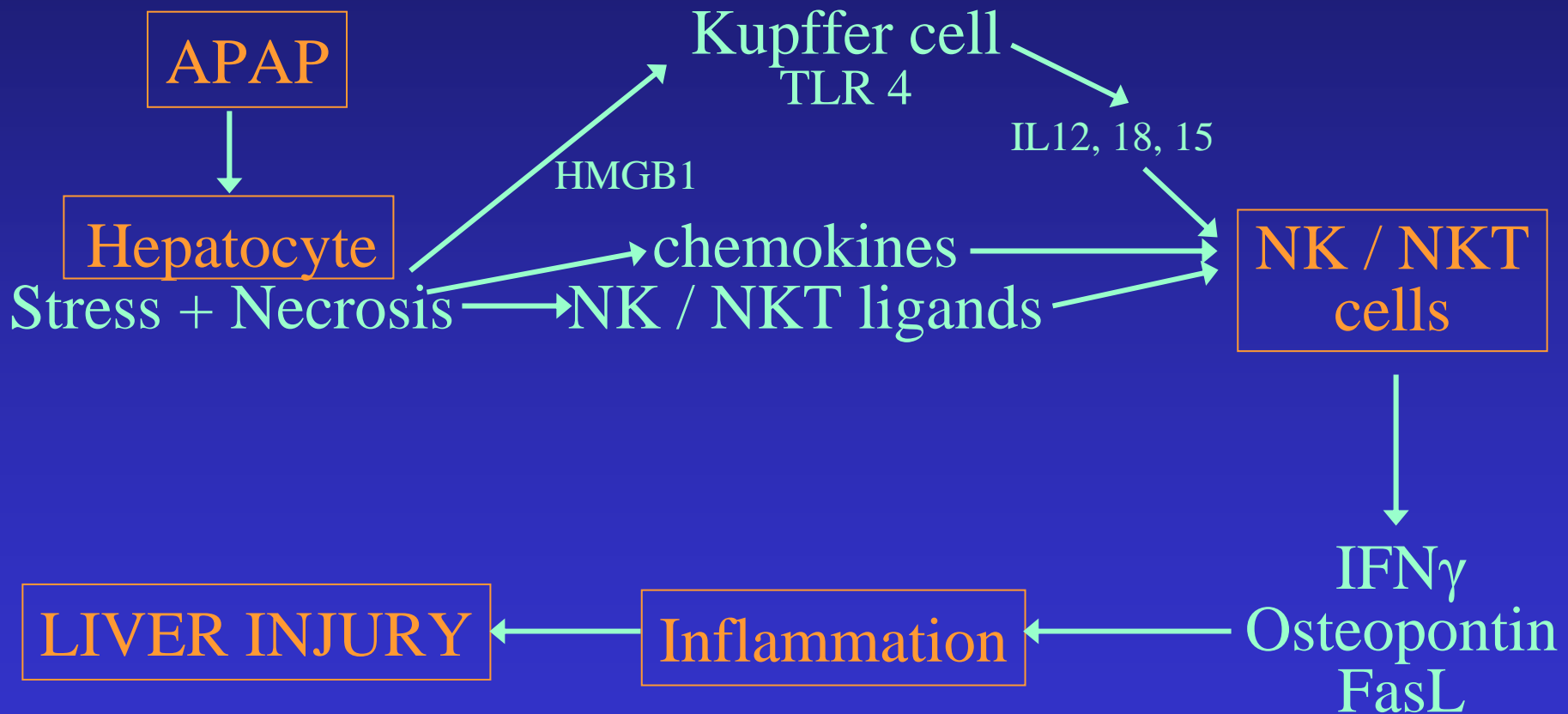
Serum ALT



Survival



Working Model of Innate Immune System in APAP Toxicity



CONCLUSIONS: LESSONS FROM APAP

- 1. Drug hepatotoxicity is usually triggered by toxic metabolites.**
- 2. The outcome (spectrum from no, mild, severe liver injury) is determined by counteracting injurious and protective phenomena which occur in response to toxic metabolites and their chemical effects.**
 - a. Within the hepatocytes, stress simultaneously activates signal transduction and transcription factor pathways which are protective or toxic (directly or through sensitization)**
 - b. Pro- and anti-inflammatory cascades of the innate immune system are simultaneously activated, the balance of which play a major role in the outcome.**
- 3. The environmental and genetic control of these intracellular and intercellular responses to toxic metabolites may be of critical importance in determining susceptibility to idiosyncratic drug hepatotoxicity.**

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