CYTOKINES AND OTHER FACTORS PROTECTING THE LIVER FROM DRUG-INDUCED LIVER DISEASE

Lance R. Pohl, Pharm.D., Ph.D.

Chief, Molecular and Cellular Toxicology

NHLBI, NIH, DHHS

Pohll@nih.gov

THE DILEMMA

- It remains impossible to predict accurately which new drugs will cause DILD and who will be at risk of developing DILD
- This is due to the relatively low incidence of DILD estimated for most drugs to be in the range of 1/10,000-1/100,000
- It is also due to the lack of animal models

STEPS IN DRUG-INDUCED LIVER DISEASE

Initiation → **Progression** → **Secondary Injury** → **Repair**

- **Initiation**
  - Reactive Metabolites
  - Protein Adducts
  - ROS, RNS
  - Injury
  - Release of Protein Adducts
  - Release of Adjuvants
- **Progression**
  - Innate Immune System Activation
  - Kupffer Cells
  - Dendritic Cells
  - NK and NKT Cells, Monocytes, PMNs, and Eosinophils
  - Adaptive Immune System Activation
  - Effector Abs and T Cells
- **Secondary Injury**
  - Death

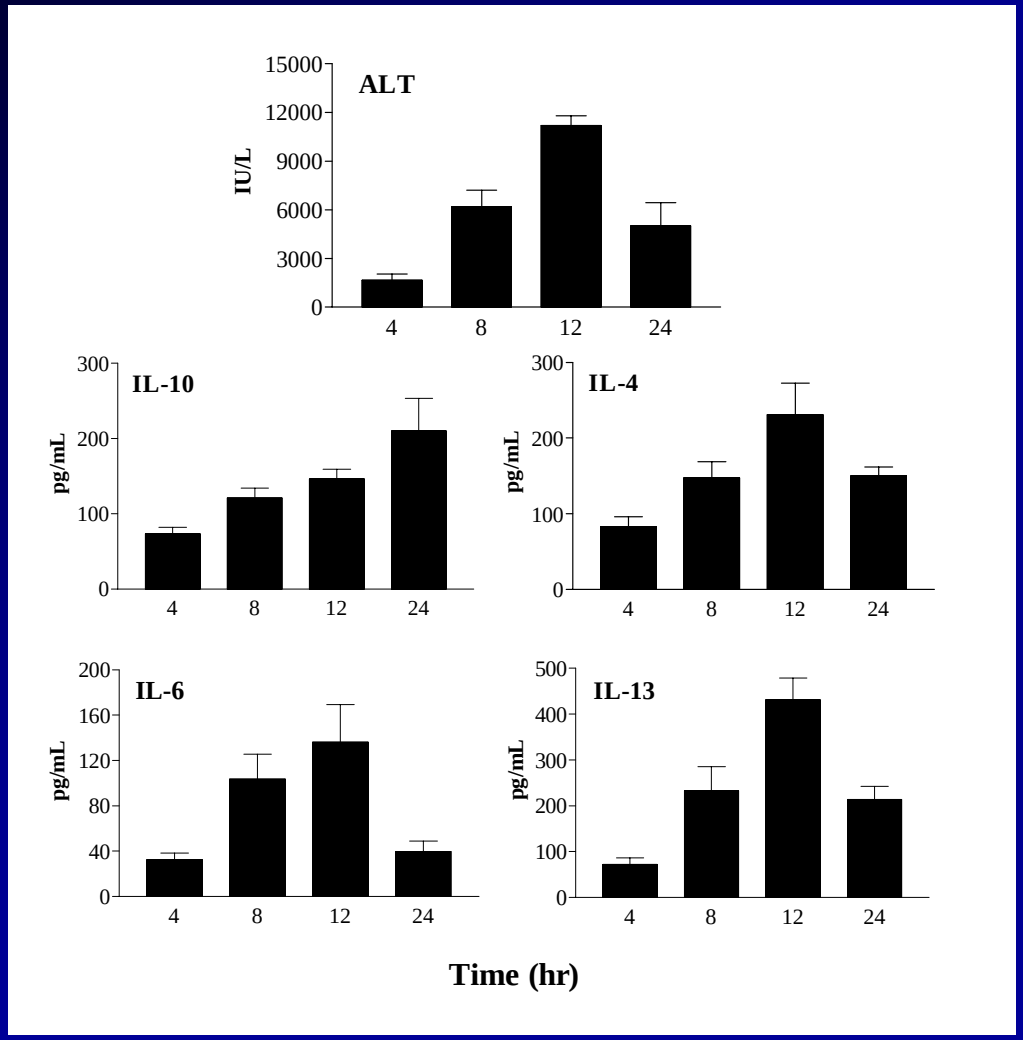
INNATE IMMUNE SYSTEM IN DRUG-INDUCED LIVER DISEASE

- Kupffer cells promote progression of DILD by producing ROS, RNS, TNF-α, IL-18, and other protoxicant factors
- NKT and NK can promote progression of DILD by producing INF-γ and subsequent inflammatory injury by up-regulation of adhesion molecules, chemokines and FasL
- LPS can promote progression of DILD by activating Kupffer cells through binding to their CD14/TLR4 and subsequent activation of NF-kappa B and production of proinflammatory cytokines
- Monocytes, PMNs, Eosinophils?
- Association of DILD with infections

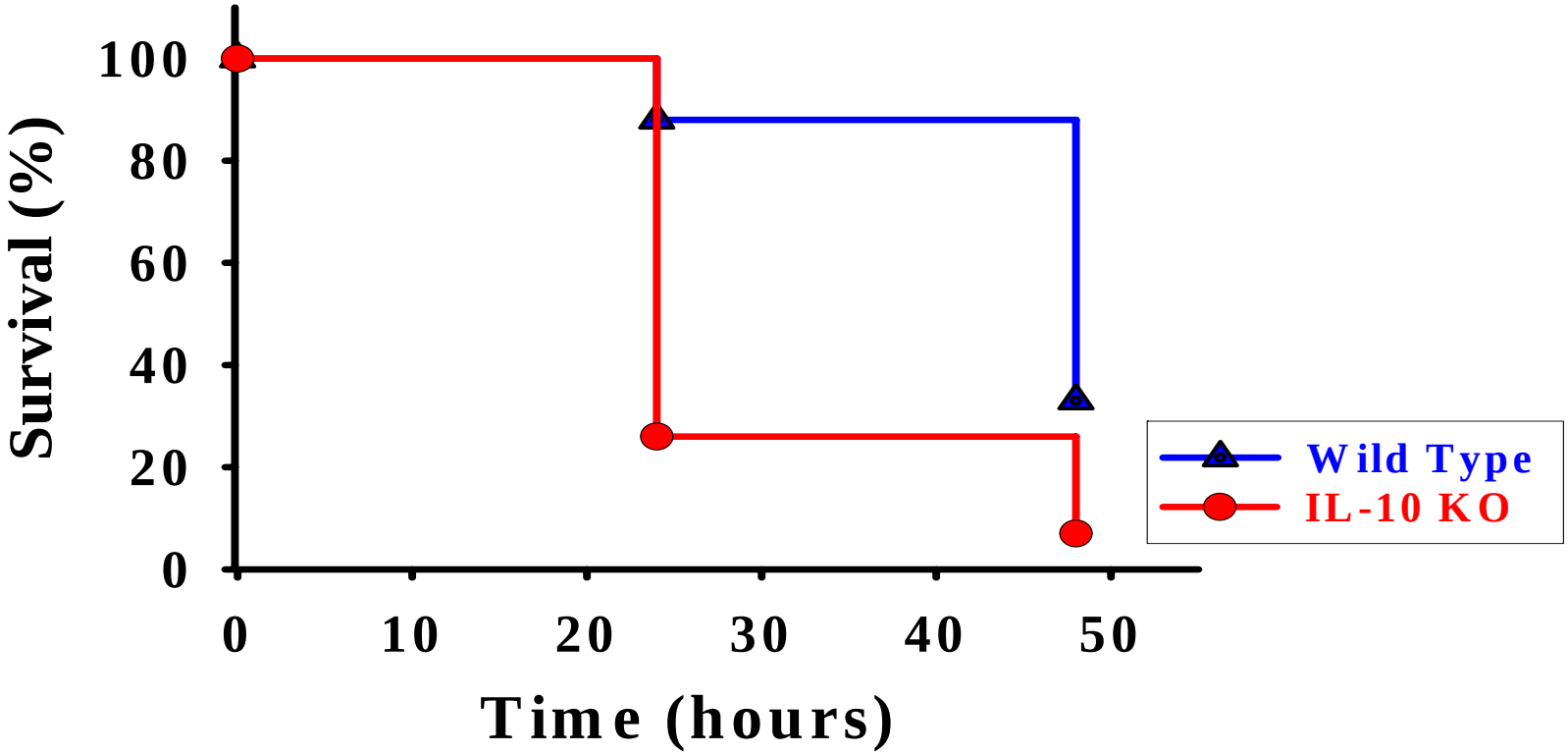
CHARACTERISTICS OF DRUG-INDUCED ALLERGIC HEPATITIS

- Liver injury usually occurs after 7 days of exposure
- Onset of liver injury is often more rapid and severe after the first incidence
- Cross-reactions with structurally similar drugs can occur
- Specific antibodies and T cells that react with drugs, metabolites, and protein adducts have been reported in a number of cases

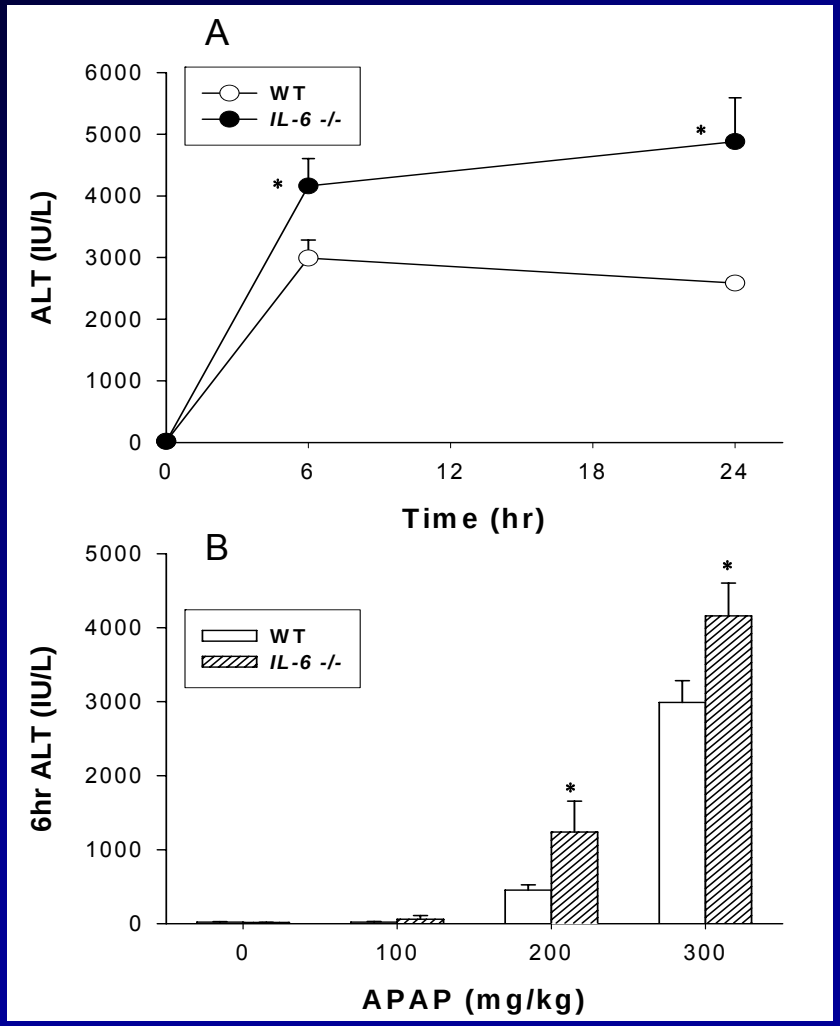
SERUM CYTOKINE LEVELS AFTER APAP TREATMENT OF MICE



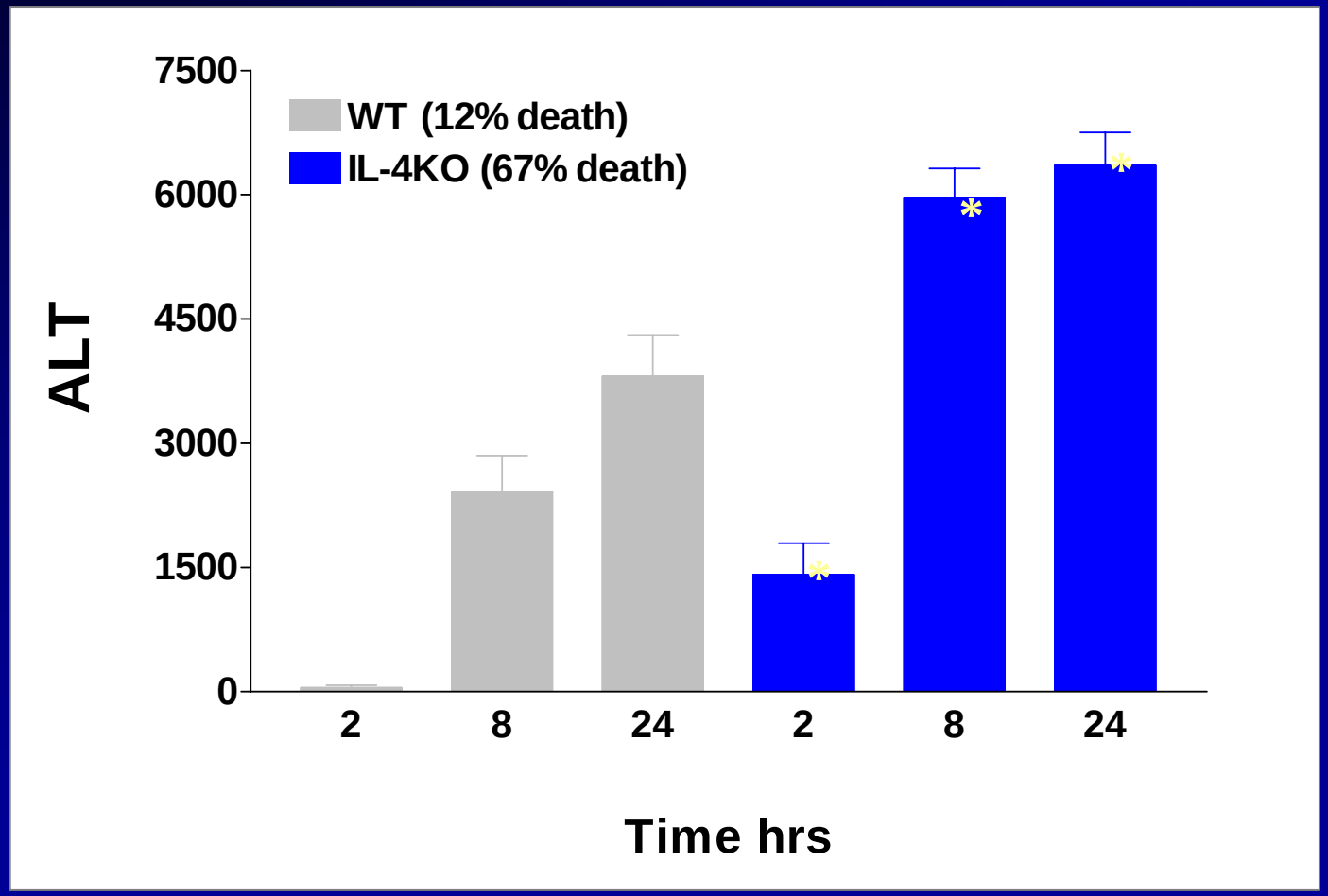
IL-10 DEFICIENCY INCREASES APAP-INDUCED LIVER INJURY AND DEATH



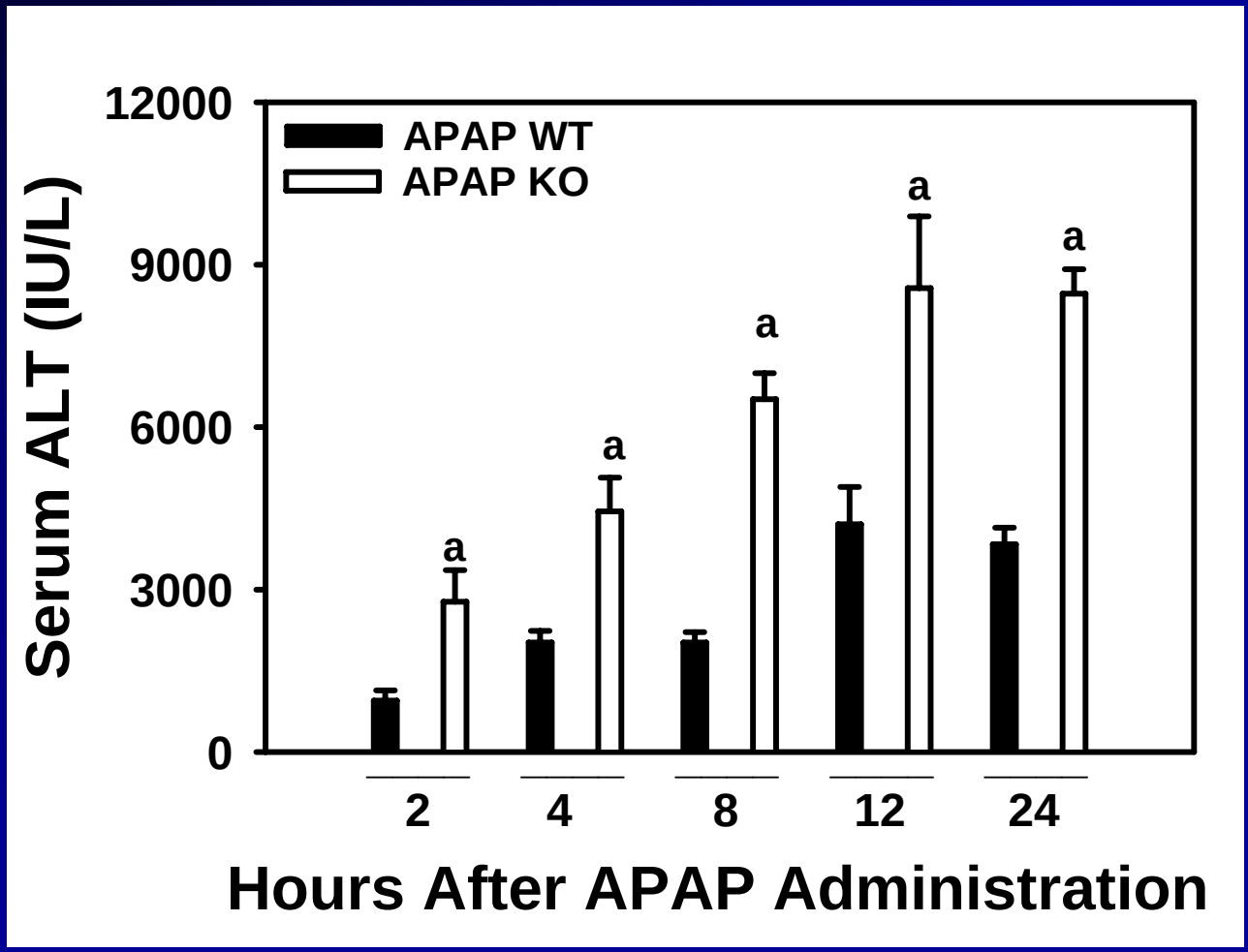
IL-6 DEFICIENCY INCREASES APAP-INDUCED LIVER INJURY



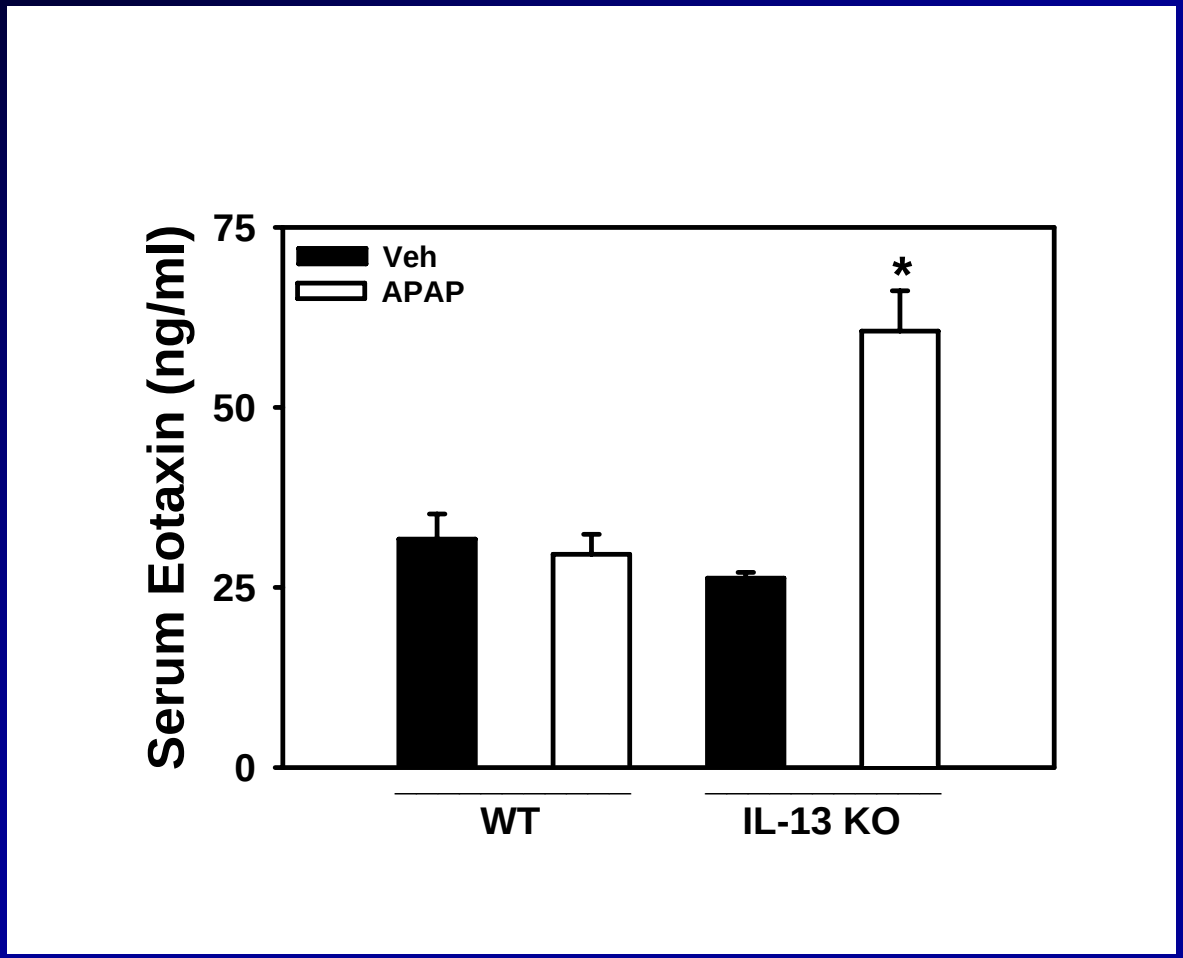
IL-4$^{-/-}$ MICE ARE MORE SUSCEPTIBLE TO APAP-INDUCED LIVER INJURY THAN WT MICE



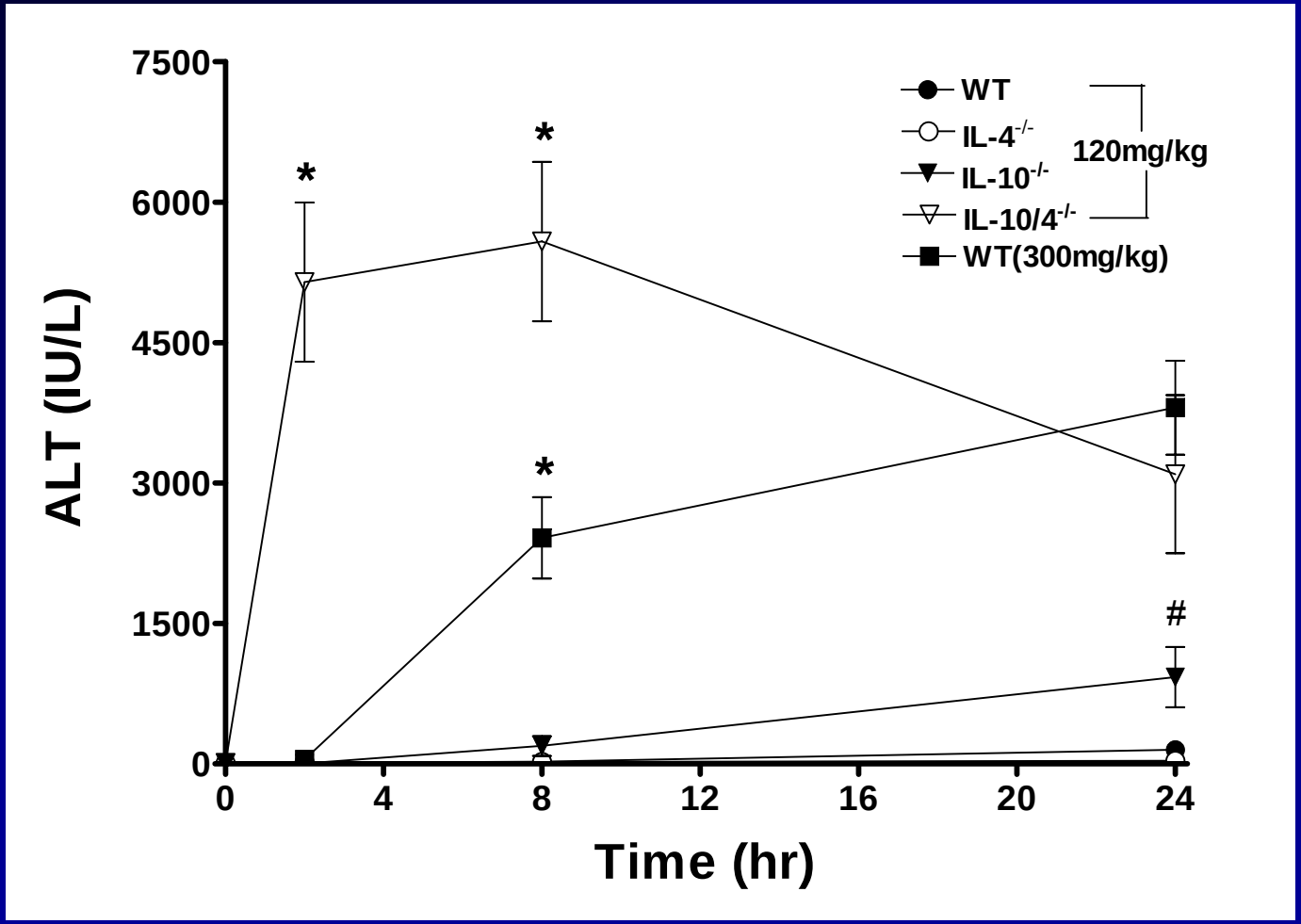
IL-13 KO MICE ARE HIGHLY SUSCEPTIBLE TO APAP



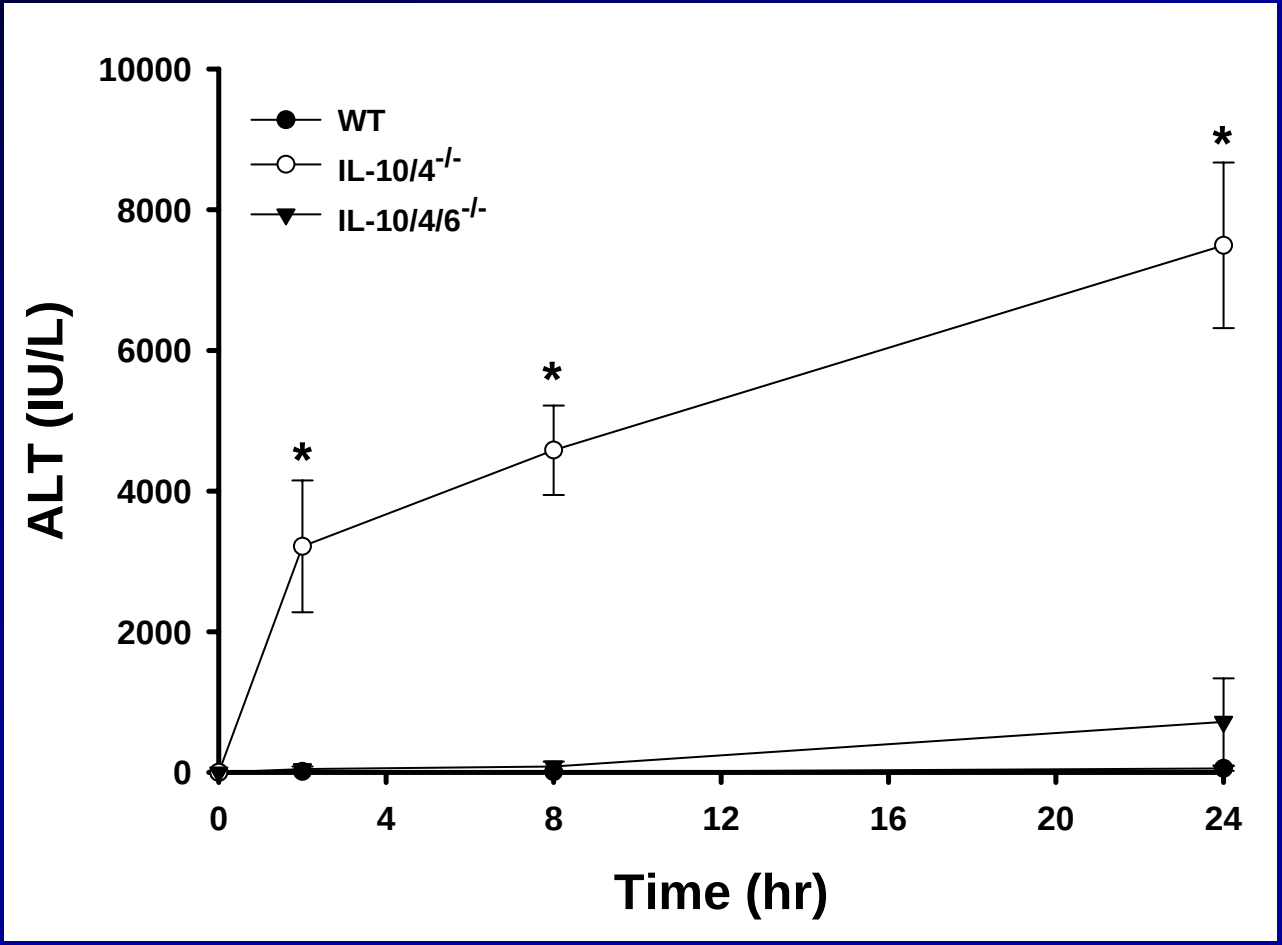
EOTAXIN IS REGULATED BY IL-13 IN MICE TREATED WITH APAP



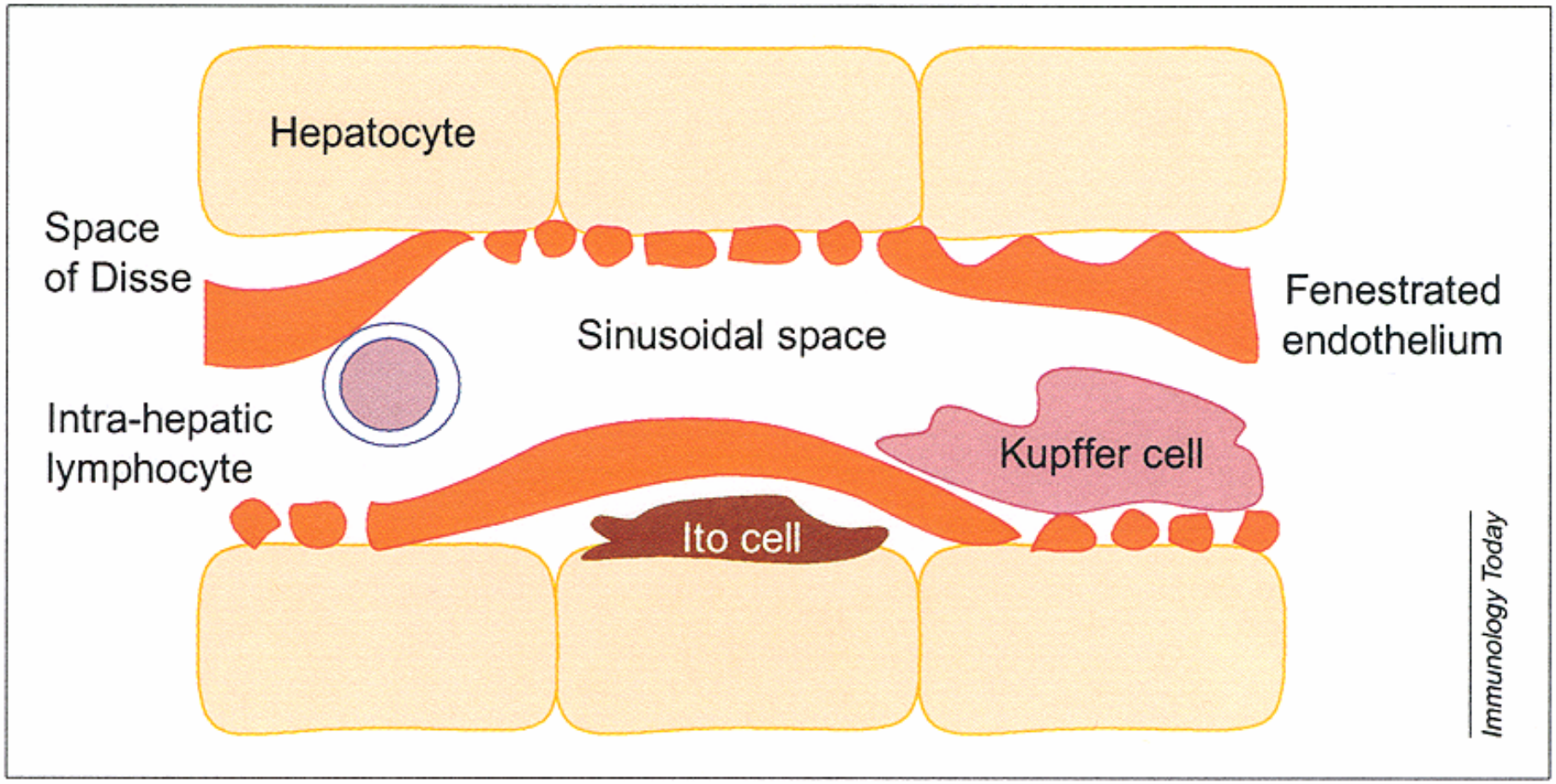
HIGH SENSITIVITY OF IL-10/4$^{-/-}$ MICE TO APAP-INDUCED LIVER INJURY



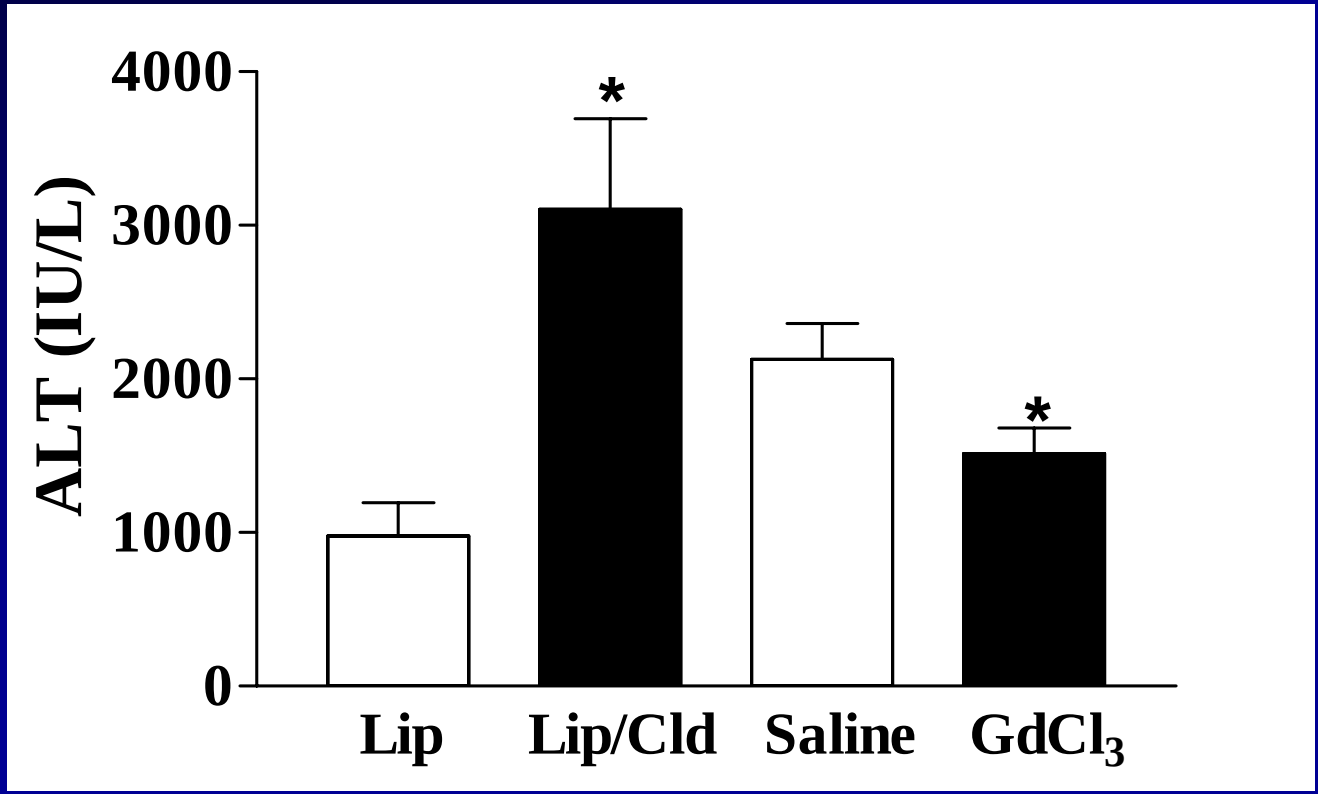
IL-10/4/6$^{-/-}$ ARE LESS SUSCEPTIBLE THAN IL-10/4$^{-/-}$ MICE TO APAP



HEPATIC SINUSOID

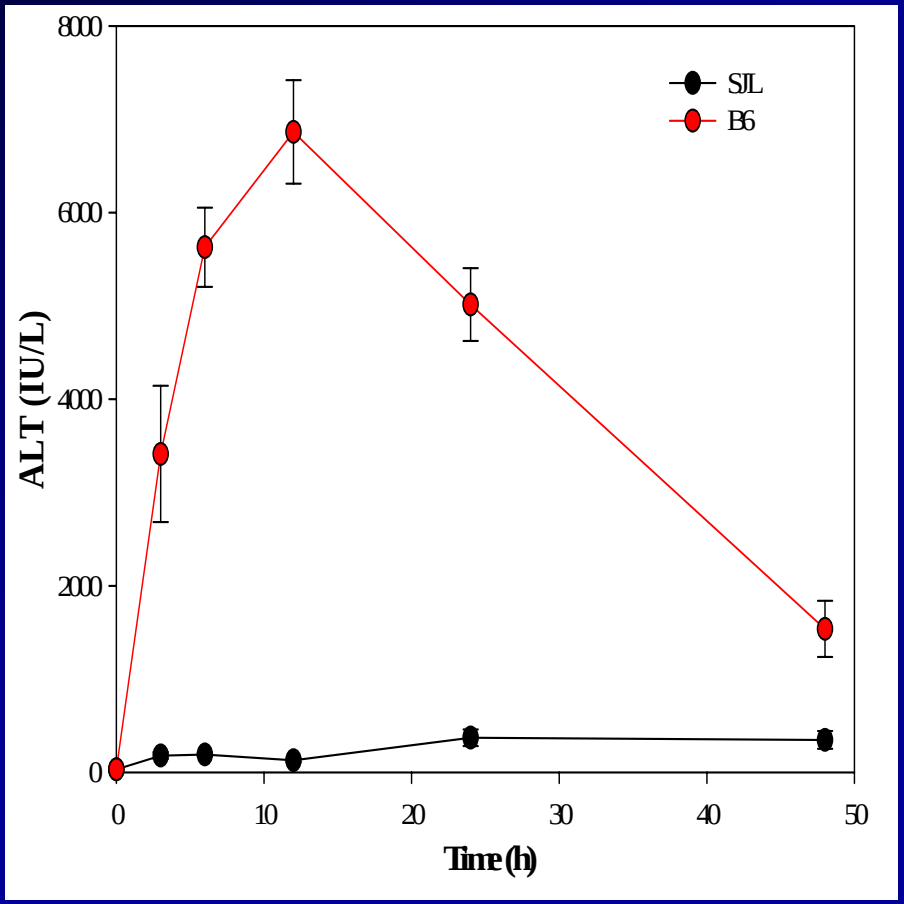


KC DEPLETION INCREASES APAP-INDUCED LIVER INJURY



STRAIN DIFFERENCES IN SUSCEPTIBILITY TO APAP

Serum ALT



PROTEINS EXPRESSED HIGHER IN SJL THAN B6 CONTROL MICE

Protein	Function
Lactoferrin Precursor	Scavages Iron, Antiinflammatory
Galectin-1	Antiinflammatory, Liver Regeneration
Proteasome Subunit Beta Type 1	Protein Turnover
Tripeptidyl Peptidase II	Protein Turnover
DNAJ Homolog Subfamily A Member 1 (HSP40 Member)	HSP 70 Cochaperone

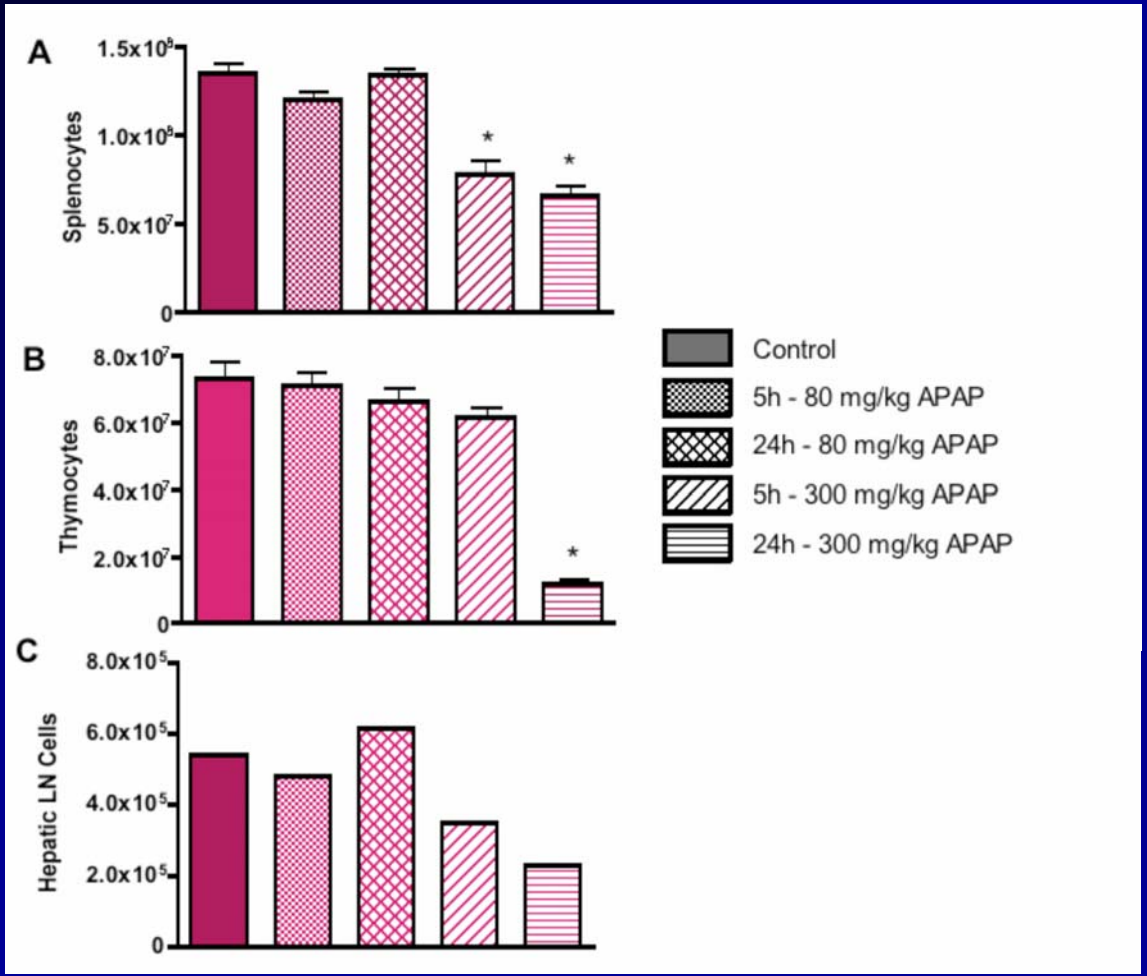
PROTEINS EXPRESSED HIGHER IN SJL THAN B6 MICE 6 HOURS AFTER APAP

Protein	Function
170 kDa GRP	Endoplasmic Reticulum Chaperone
HSP 70 Binding Protein	Cochaperone of HSP70
Small Ubiquitin-Related Modifier (SUMO)-1 Acti-vating Enzyme Subunit 2	Protein Sumoylation
Proteasome Subunit Alpha Type 1	Protein Turnover
Thioredoxin mitochondrial precursor	Antioxidant
Peroxiredoxin 1	Antioxidant
Peroxiredoxin 6	Antioxidant
Complement C5 Precursor	Liver Regeneration

PROTEINS EXPRESSED HIGHER IN SJL THAN B6 MICE 6 HOURS AFTER APAP

Protein	Function
Microsomal Glutathione S-Transferase-1	Reduces Fatty Acid Hydroperoxides
Biliverdin Reductase	Antioxidant
Senescence Marker Protein-30 (Regucalcin)	Antiapoptotic and Cell Survival

LYMPHOCYTOLYSIS FOLLOWING HEPATOTOXIC DOSE OF APAP



OTHER HEPATOPRTECTIVE FACTORS IN APAP-INDUCED LIVER INJURY

- COX-2 deficient mice are more susceptible to APAP
- Transgenic mice over-expressing superoxide dismutase or plasma glutathione peroxidase are resistant to APAP
- CCR2 deficient mice are more susceptible to APAP due to elevated liver levels of IFN-γ and TNF-α
- IFN-γ-inducible protein-10 (IP-10) protects against APAP-induced liver injury by up-regulating CXCR2-dependent proliferation response of hepatocytes
- Nrf2 deficient mice are more susceptible to APAP-induced liver injury possibly due to low levels of γGC and GSH synthetases, GSH, catalase, superoxide dismutase, HSP32, UGT1a6, and GSTpi transferases

PROTOXICANT FACTORS IN APAP-INDUCED LIVER INJURY

- Interferon- γ deficient mice are less susceptible to APAP-induced liver injury
- LPS-binding protein deficient mice are less susceptible to APAP-induced liver injury
- MIF deficient mice are less susceptible to APAP-induced liver injury
- Osteopontin deficient mice are less susceptible to APAP-induced liver injury

CONCLUSION

- Susceptibility to DILD is likely dependent upon multiple factors
- Genomic and proteomic approaches should lead to the discovery of additional protective and protoxicant factors
- Studies with KO mice, inhibitory antibodies, and siRNAs are needed however to confirm the toxicologic relevance and mechanisms of action of the discovered factors
- Polymorphisms and environmental factors that alter the levels and activities of protective and protoxicant factors may contribute to patient susceptibility to DILD

ACKNOWLEDGEMENTS

- Mohammed Bourdi
- Hamid Amouzadeh
- Timothy P. Reilly
- Yasuhiro Masubuchi
- Kevin Welch
- Steven Yee
- Michael Adams
- Cynthia Ju
- Mary Jane Mason
- Pauline Ryan
- Daniel Dieras
- Michael Holt
- Marie Webster
- Christine Chung
- Tami Graf
- John George
- Sidney Nelson, Bo Wen, David Goodlett, Eugene Yi, and Hookum Lee, University of Washington