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DILIsym User Training – Representation of the Innate Immune System in the DILIsym Software

DILIsym Development Team

Goals for the Training Session on the Innate Immune Representation

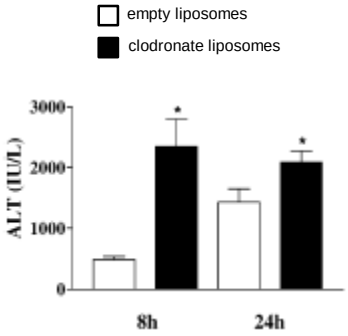
Participants should understand the following general concepts:

- Design concepts behind the DILIsym innate immune representation
  - Available literature to define initial scope of the representation
  - Sterile inflammation in DILI
  - Cell life cycles
  - Mediator production
  - Examples of biological complexity (HMGB1, TNF-α)
- Approach to representing innate immune responses across species
  - Comparison of simulation results with published data
- Relationship of representation to ongoing DILIsym development

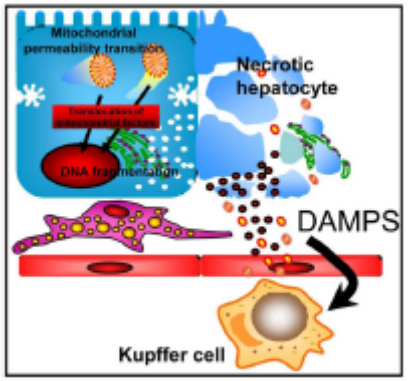
Innate Immune Cells Implicated in DILI and/or Recovery

MICE

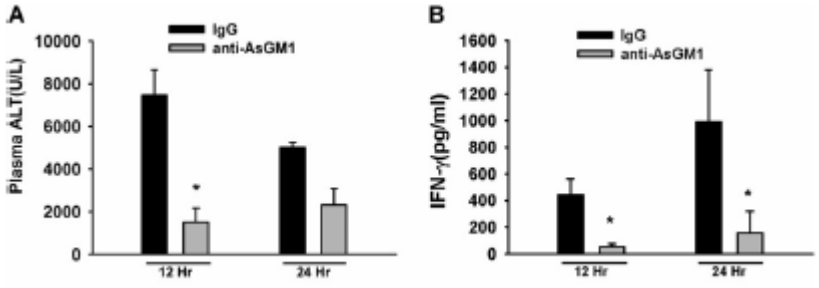
- Immune cell types in DILI
  - APAP (macrophages, DCs, PMNs, NK cells, NKT cells, LSECs)
    - Ju 2002, Campion 2008, Fisher 2013, You 2013, McCuskey 2005, Kato 2011, Connolly 2010, Marques 2015, Huebener 2015, Liu 2004, Liu 2006, Masson 2008, Ishida 2006
  - Halothane (PMNs, NK cells, NKT cells)
    - You 2006, Dugan 2011, Cheng 2010
  - Amodiaquine (NK cells)
    - Metushi 2015
  - Isoniazid (NK cells)
    - Mak 2015
- Interpretation of cell type manipulation studies often challenging
- Initial focus on APAP
  - Macrophages, including Kupffer cells
  - LSECs



Ju et al. 2002



Jaeschke 2015



Dugan et al. 2011

Preclinical Data

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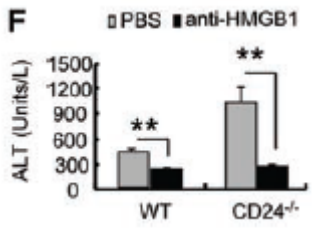
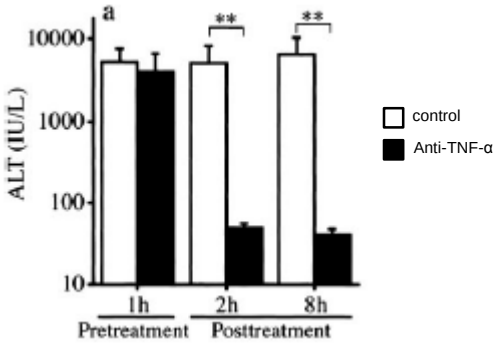
Mediators Derived from Immune Cells Implicated in DILI and/or Recovery

MICE

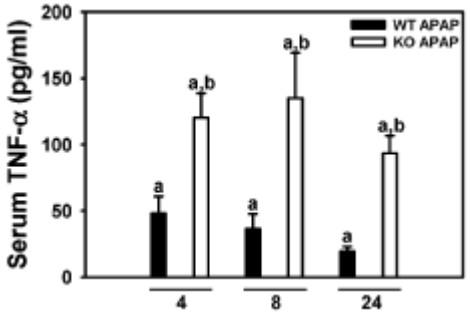
- Functional role in DILI generally defined by addition of exogenous or blockade of endogenous mediator
  - *e.g.,* anti-HMGB1, anti-TNF-α, exogenous HGF
- Mechanistic attributes generally defined by *in vitro* studies
  - May also drive required inclusion
- Exposure profile generally defined by plasma measurements
- Initial focus on APAP
  - HMGB1, TNF-α, IL-10, (VEGF), HGF



Chen et al. 2009



Ishida et al. 2004



Yee et al. 2007

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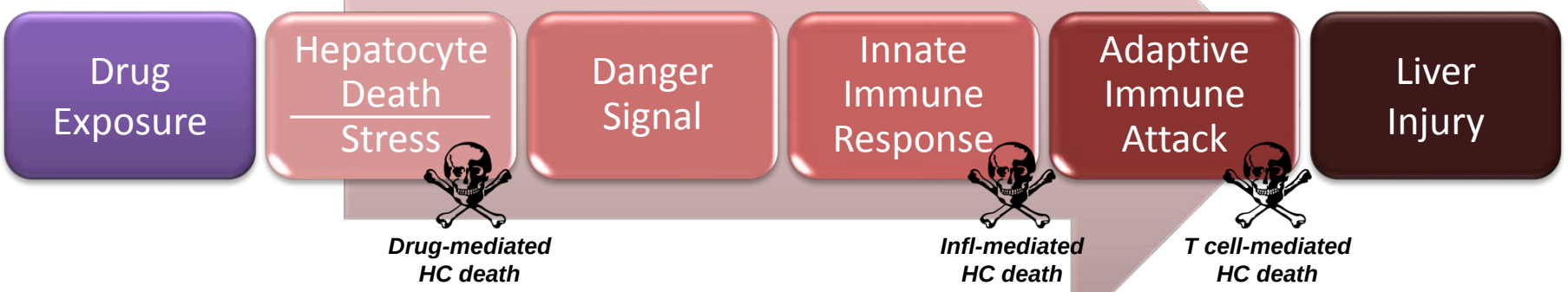
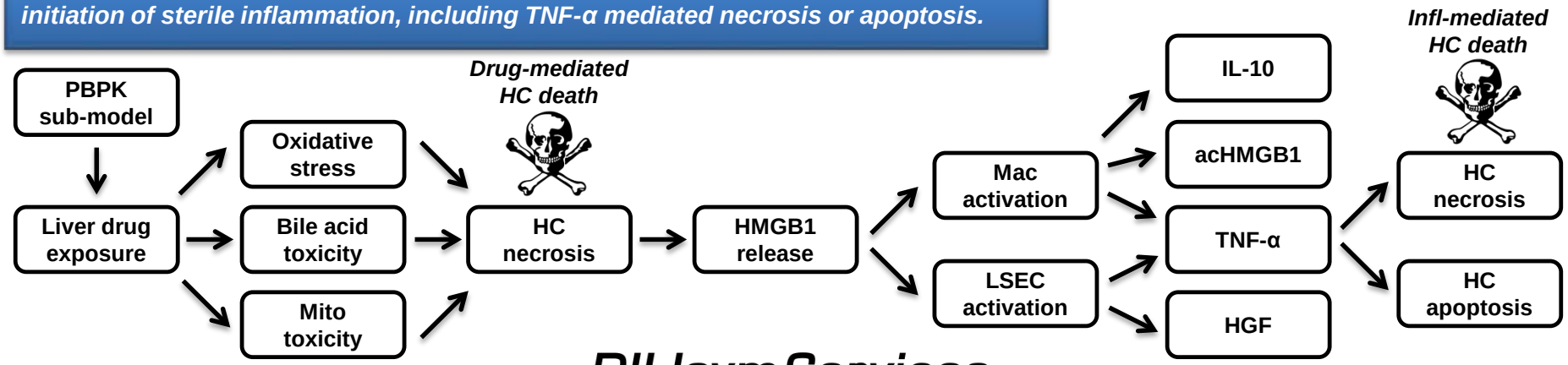
Intrinsic Drug Toxicity and Subsequent DAMP Release Drive Innate Immune Activation

Theory on sequence of events driving potential contributors to liver injury, including intrinsic drug toxicity, sterile inflammation, and adaptive immune attack.



Current DILIsym representation allows for intrinsic drug toxicity, with potential initiation of sterile inflammation, including TNF-α mediated necrosis or apoptosis.



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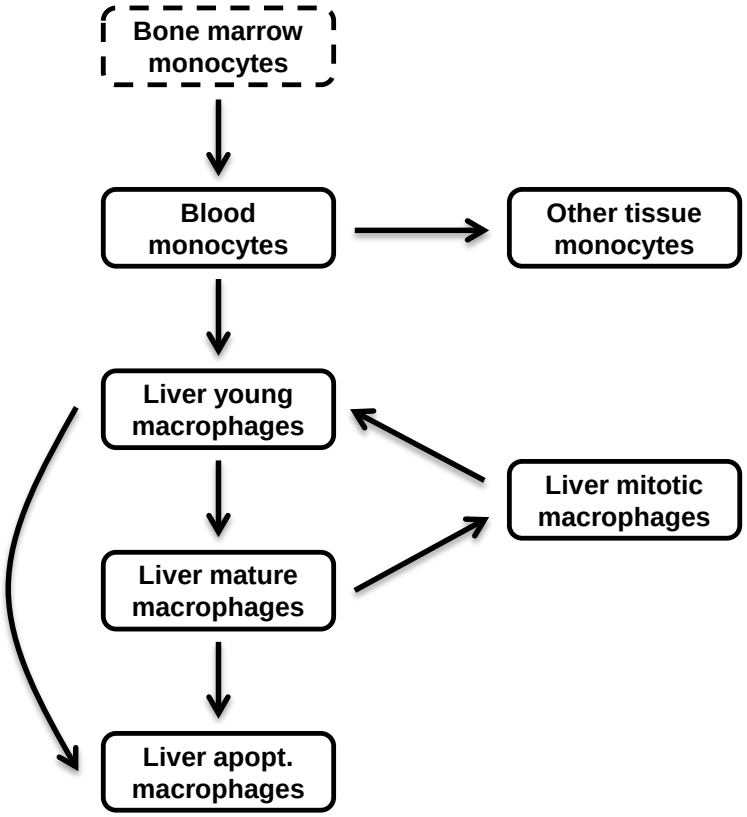
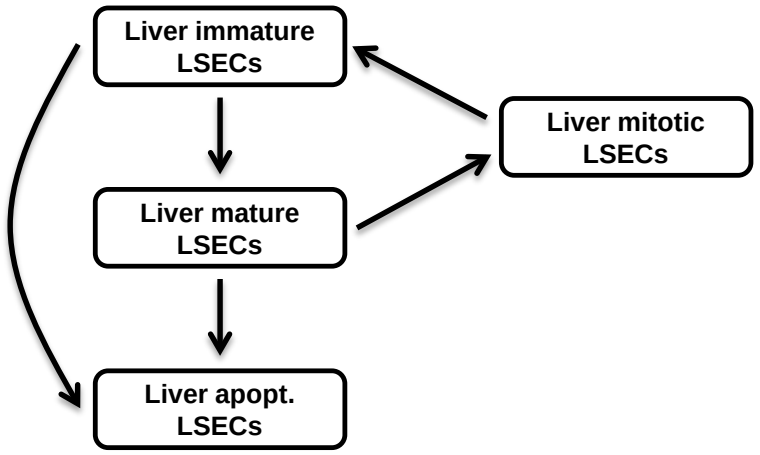
Macrophage and LSEC Cellular Life Cycles

Liver macrophage population maintained by recruitment of blood monocytes from blood and local proliferation



Liver LSEC population maintained by local proliferation



Shoda et al. 2017

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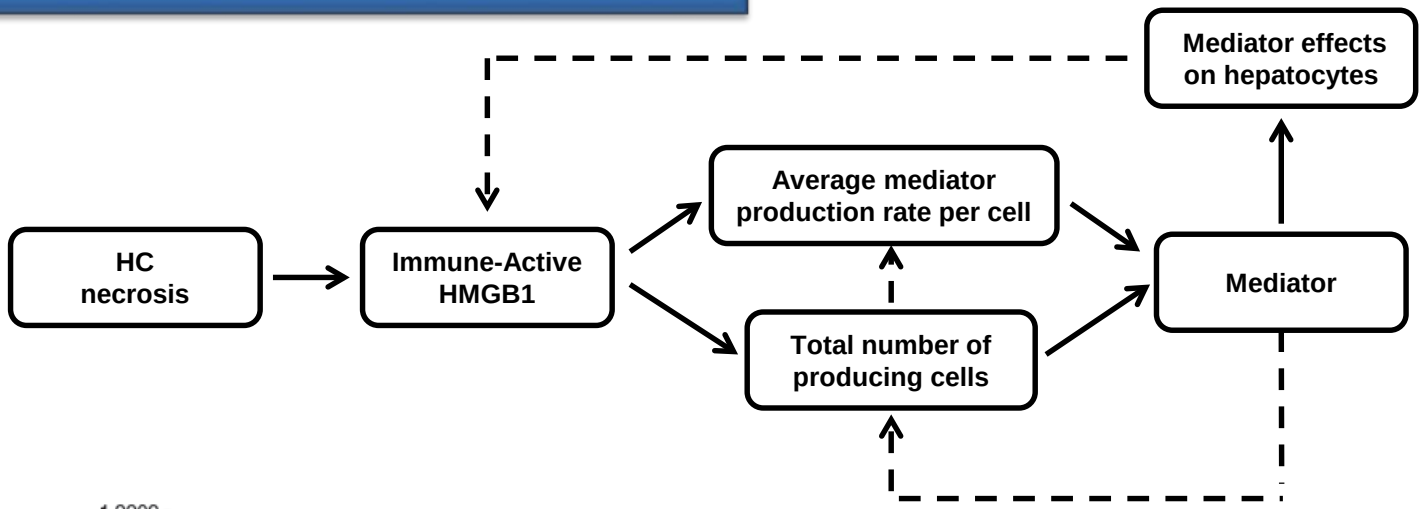
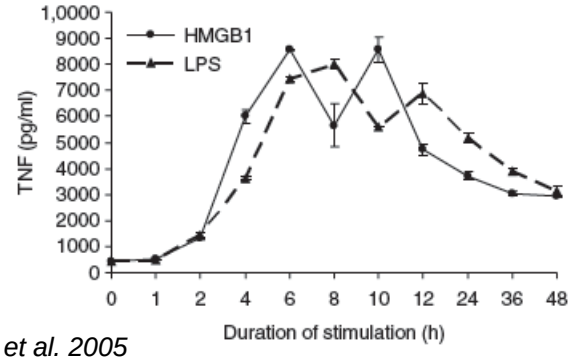
Regulation of Mediator Production

Mediators reflect production rate and cell numbers and include both feed forward and feedback loops



RATS



Kokkola et al. 2005

HMGB1 stimulates macrophage TNF-α production

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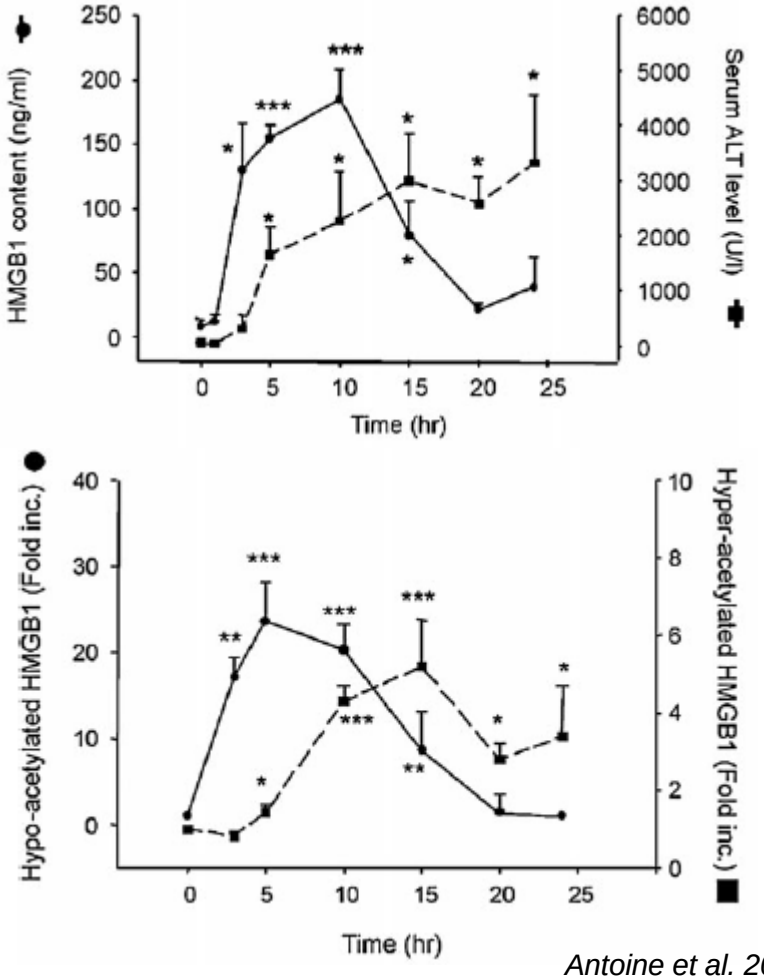
Well-Characterized Dynamics for Total and Acetylated HMGB1 Post-APAP

MICE

- Following single i.p. APAP, quantitative time course data available for HMGB1 elevation
- HMGB1 precedes peak ALT
  - Kinetics allow for HMGB1 contribution to injury
- Measured hypo- *vs*. hyper-acetylated HMGB1
  - Hypo- leakage product
  - Hyper- secreted by activated macrophages
- Used for HMGB1 optimization



Antoine et al. 2009

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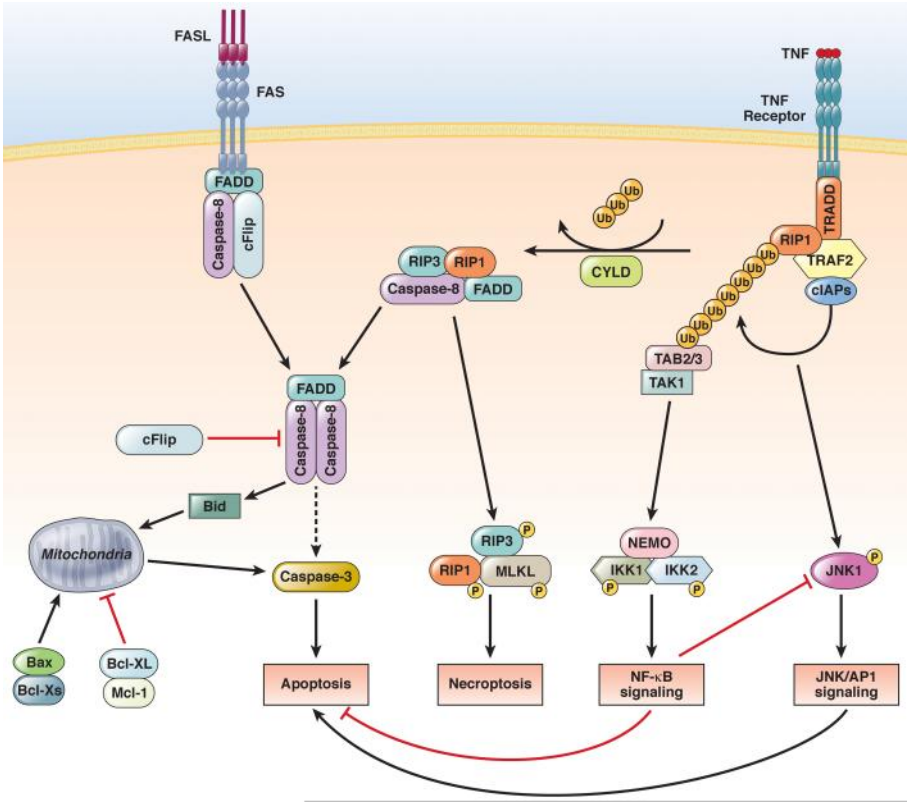
TNF-α Pleiotropic Activity Includes Survival, Proliferation, Apoptosis, and Necrosis

- Alternate signaling pathways characterized
  - Includes newly described pathway of programmed necrosis, “necro-apoptosis” or “necroptosis”
- Liver-specific data, *e.g.*,
  - Proliferation in partial hepatectomy
  - Survival following TNF-α pre-treatment
  - Cell death following LPS or TNF-α and D-galactosamine
- Modeling challenge
  - Hepatocytes must respond dynamically to TNF-α in simulations
  - How will the hepatocyte response be determined?



Luedde et al. 2014

Survival/Proliferation | Apoptosis | Necro-apoptosis

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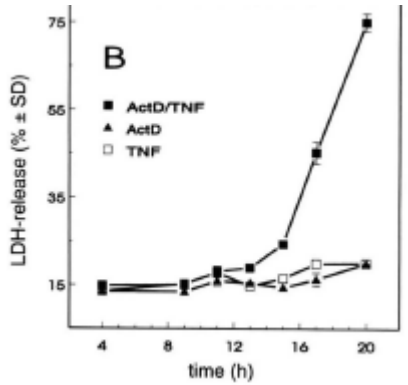
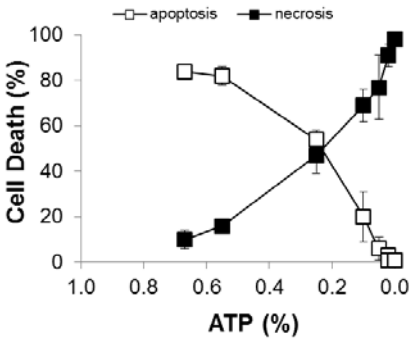
Data Suggest Cell Response to TNF-α Regulated by Cell Health (*i.e.*, ATP)

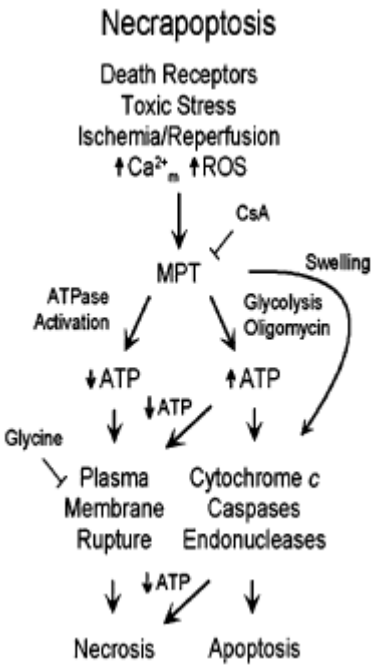
- Healthy cells do not die in response to TNF-α
  - Experimental systems require transcriptional arrest (*e.g.*, actinomycin D, D-galactosamine)
- ATP depletion switches classical apoptotic response to death receptor ligation to necrosis
- Modeled dynamic ATP levels selected as a proxy for cell health



Leist et al. 1994



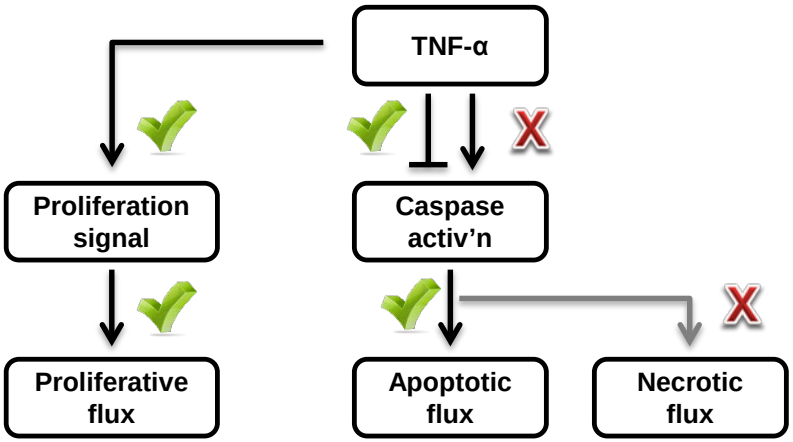
Modified from Leist et al. 1997

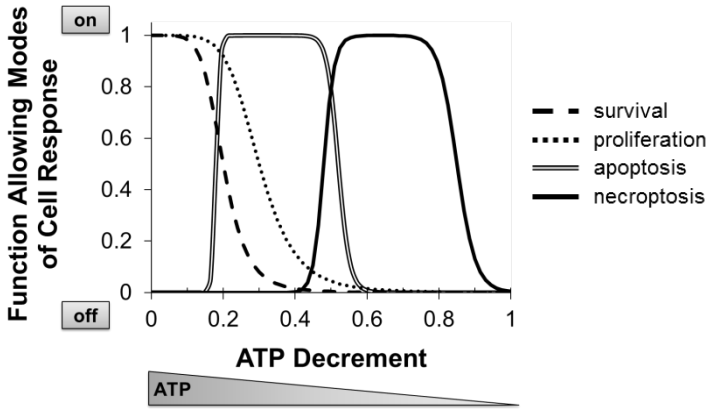


Kim et al. 2003

Dynamic TNF-α Mediated Hepatocyte Response Based on Cell Status Checkpoints

- Modeled TNF-α ATP checkpoints
  - Healthy cells do not die in response to TNF-α
    - ATP sufficient: survival or proliferation
  - Compromised cells (*e.g.*, ActD or D-gal) undergo apoptosis
    - Partial ATP depletion: apoptosis or necroptosis
  - Can cells manage the program energetic requirements?
    - Insufficient ATP diverts cells from apoptosis to necroptosis
- Very low ATP results in necrosis without requirement for TNF-α





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Goals for the Training Session on the Innate Immune Representation

Participants should understand the following general concepts:

- Design concepts behind the DILIsym innate immune representation
  - Available literature to define initial scope of the representation
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  - Cell life cycles
  - Mediator production
  - Examples of biological complexity (HMGB1, TNF-α)
- Approach to representing innate immune responses across species
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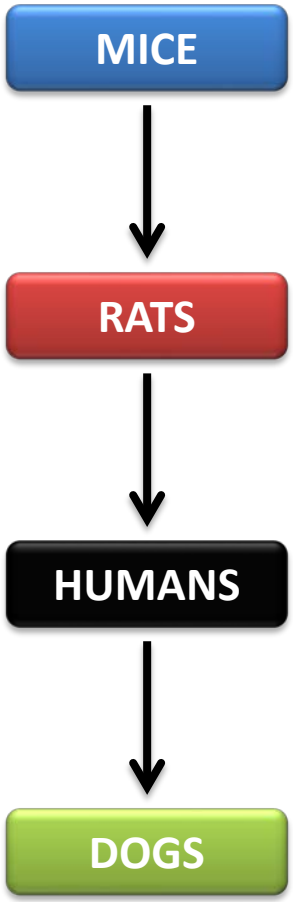
Optimization Order Based on Data Availability



- Optimization primarily w/aggregate time- and dose-dependent data sets from published studies following acute acetaminophen (APAP) dosing
  - HMGB1, cytokines, growth factors, macrophage accumulation
- Exploration leveraged experimental data blocking HMGB1, TNF-α, or adding exogenous HGF

- Optimization primarily w/aggregate data sets from published studies on multiple liver models (i.e., APAP, LPS, TAA, CCl_4, D-Gal, I/R, CLP)
  - HMGB1, cytokines, growth factors, macrophage accumulation

- Optimization w/single or aggregate data sets from published studies on APAP overdose
  - HMGB1, cytokines, growth factors, macrophage accumulation

- Optimization w/aggregate data sets from published studies on LPS liver models
  - TNF-α only

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Species Translation Preserves Parameter Values Unless Dictated by Data

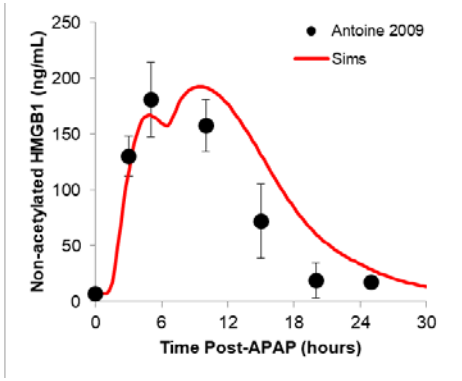
- Parameters values identified by optimization to mouse data preserved unless differences were dictated by the data
  - Intended to leverage largest data set (mice >> rat ≈ human >>> dog)
  - Assumes preservation of mechanistic relationships across species unless indicated by the data
- Measured data applied to make species-specific adjustments to parameter values in two ways
  - Direct measurements reported for different species: extract and use in DILIsym
  - Parameter value will be affected by general species differences (*e.g.*, mass, blood volume): scale by species difference and use

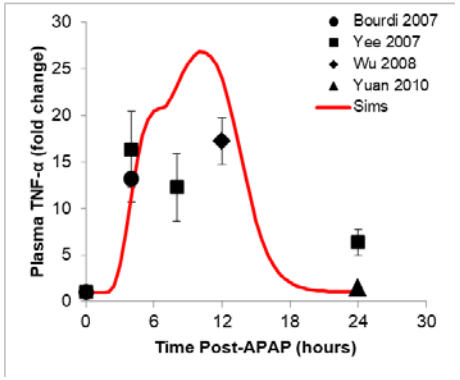
Parameter Value Translation	Parameter	Mice	Rats	Dogs	Humans
Direct measurements	Basal TNF-α level (pg/mL)	4.2	61	13.8	5.0
Regulated by general differences (*e.g.*, mass)	Liver total viable macrophages (1e9 cells)	0.0084	0.13	6.93	20.10

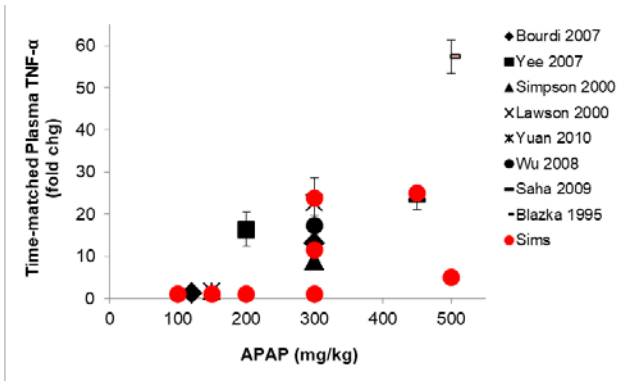
Mouse Simulations Consistent with Preponderance of Data

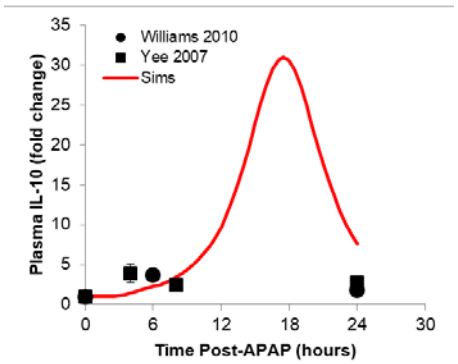
MICE

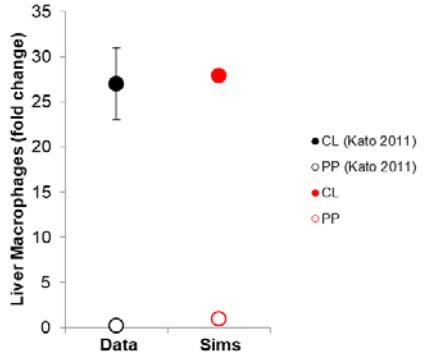
- APAP-centric
- Time- and dose-dependent data comparisons applied wherever possible

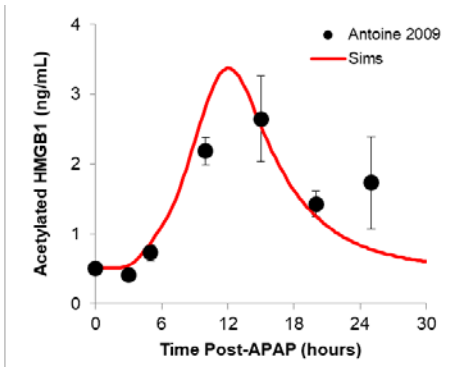












Preclinical Data & Simulation Results

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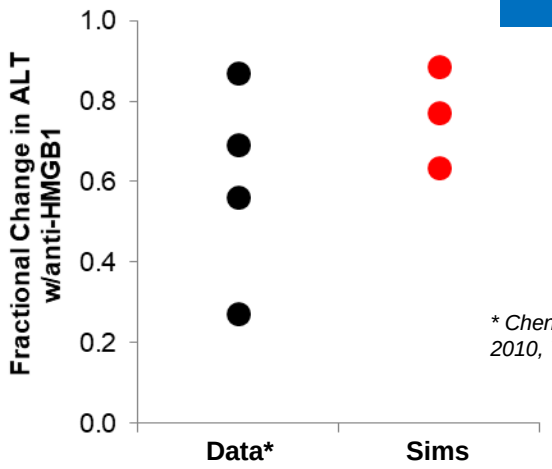
Mechanistic Interventions Test the Innate Immune Dynamics

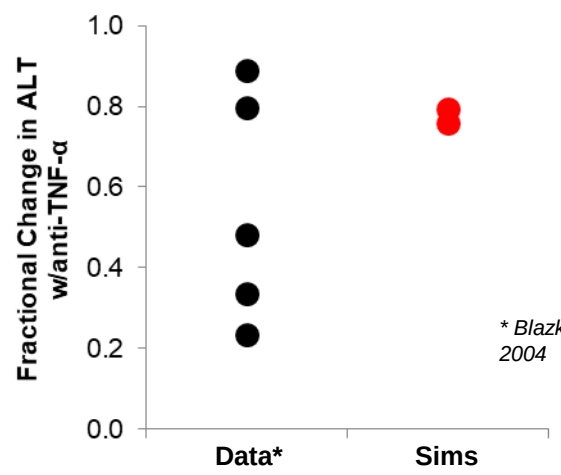
MICE

- Blocking HMGB1
  - Testing the importance of HMGB1 as a DAMP
  - Presumed to block activation/accumulation of innate immune cells
  - Data using antibodies or genetic ablation
  - Consistent reduction in injury observed and reproduced in simulations



** Chen 2009, Antoine 2010, Yang 2012*

- Blocking TNF-α
  - Testing the role of TNF-α
  - Interpretation complicated by pleiotropic nature of TNF-α
  - Variable results reported
  - Net injury reduction due to reduced pro-inflammatory activity observed in simulations



** Blazka 1996, Ishida 2004*

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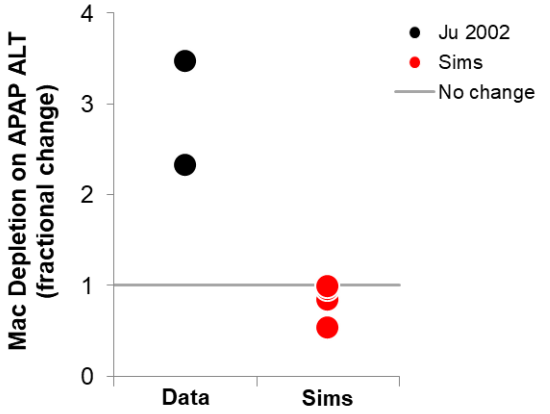
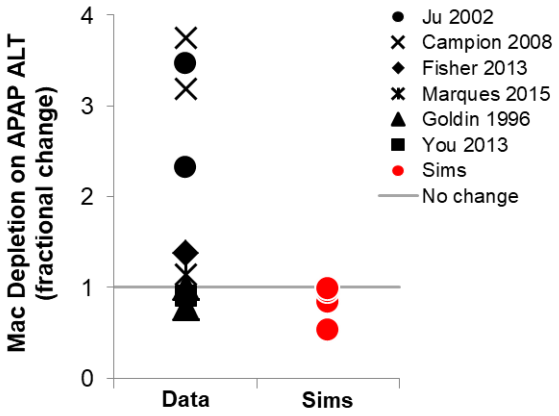
Macrophages: Good Actors? Bad Actors? Both?

MICE

- Initial reports using liposomal clodronate to deplete phagocytic cells suggested macrophages provided early protection from injury
  - Original hypothesis: macrophages protect from APAP toxicity
  - Simulated mac depletion did not reproduce result
  - TNF-α not protective early because of APAP rapid ATP depletion
- Additional data sets reveal more modest and later effects of clodronate
  - Current hypothesis: macrophages support recovery and regeneration
  - Simulated mac depletion more consistent with preponderance of data
  - Mac pro-survival and pro-regenerative mediators consistent with hypothesis
  - Addition of neutrophils may enhance pro-regenerative mac role





Preclinical Data & Simulation Results

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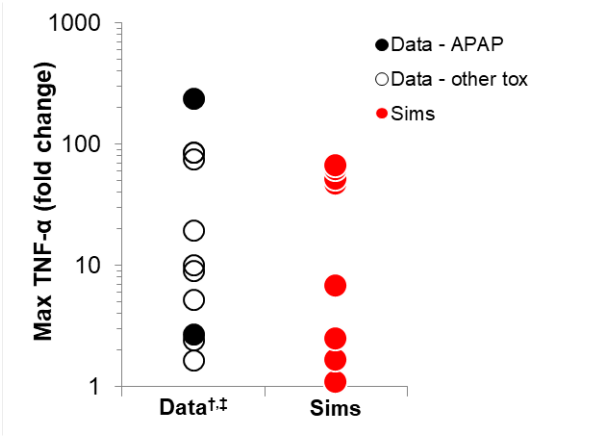
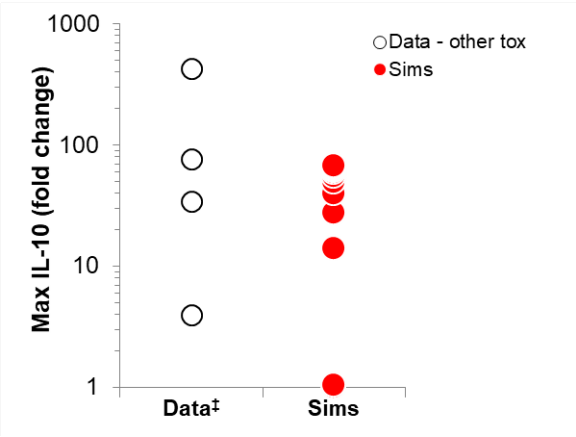
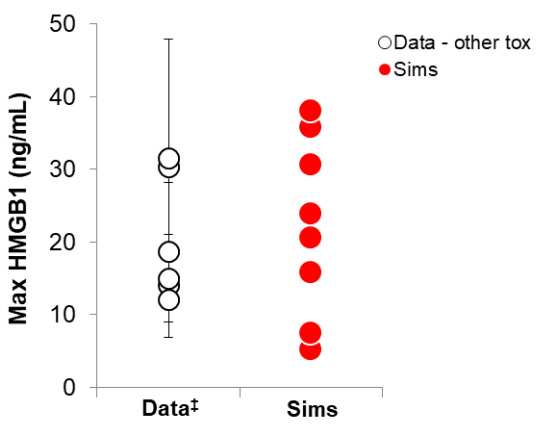
Rat Simulations Consistent with Preponderance of Data

RATS

- Minimal APAP data set necessitates use of other liver toxicant/injury models
- Focus on ability to approximate data range using alternate APAP doses







† *APAP: Arafa 2009, Merrick 2006*

‡ *Other tox: Bautista 2010, Chen 2008, Matsuhashi 2005, Nakamoto 2003, DeCicco 1998, Hagiwara 2008, Koga 2012*

‡ *Other tox: Nakamoto 2003, Swain 1999, Hagiwara 2008, Kono 2006*

‡ *Other tox: Takano 2010, Hagiwara 2008, Koga 2012, Oishi 2012, Liu 2010*

Preclinical Data & Simulation Results

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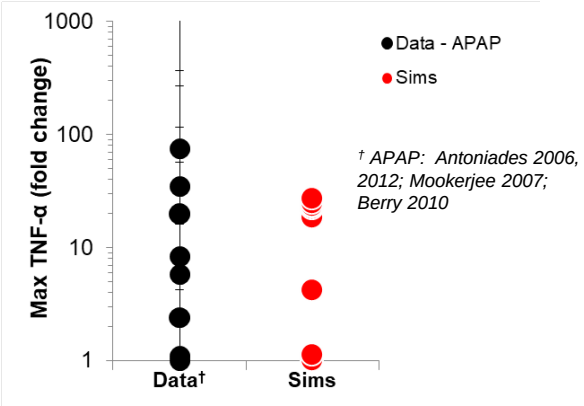
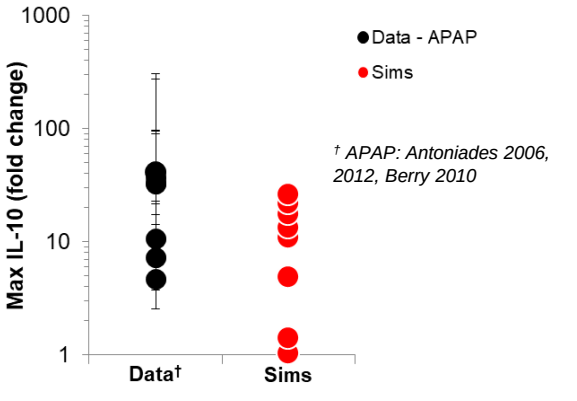
Human Simulations Consistent with Preponderance of Data

HUMANS

- Use of APAP overdose data (minimal data for dose, *i.e.*, model input)
- Focus on ability to approximate data range using alternate APAP doses



† APAP: Antoniades 2006, 2012; Mookerjee 2007; Berry 2010



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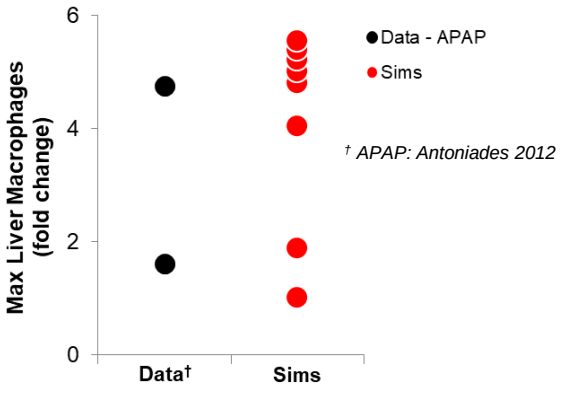

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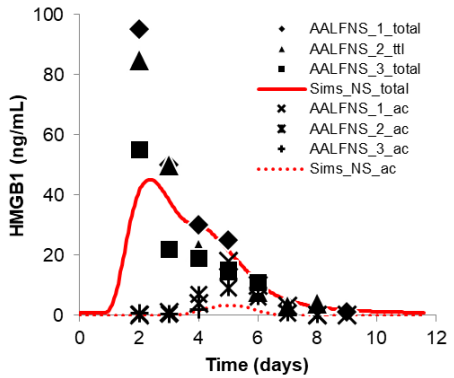

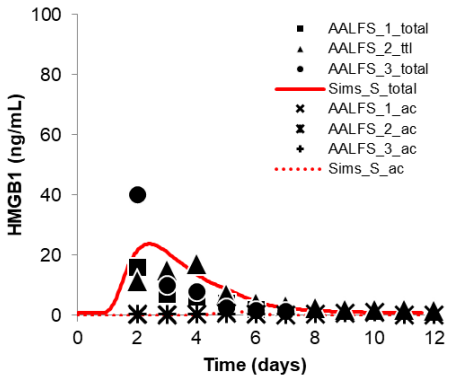

APAP: Antoine 2012

Clinical Data & Simulation Results

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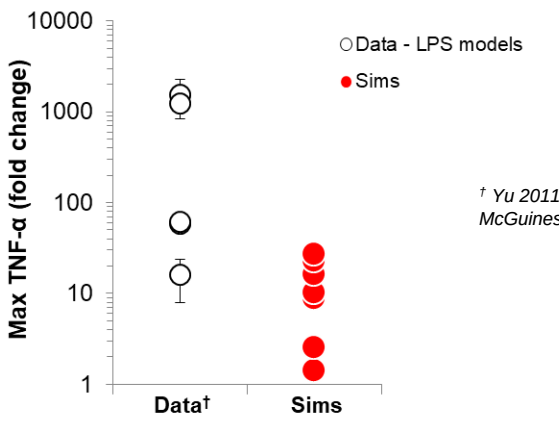
Few Comparisons Available for Dog

DOGS

- Relatively few published dog data sets
- Focus on reasonable comparison to measured TNF-α from LPS model



† Yu 2011, Moeniralam 1997, 1998; McGuiness 1996, DeClue 2008

Preclinical Data & Simulation Results

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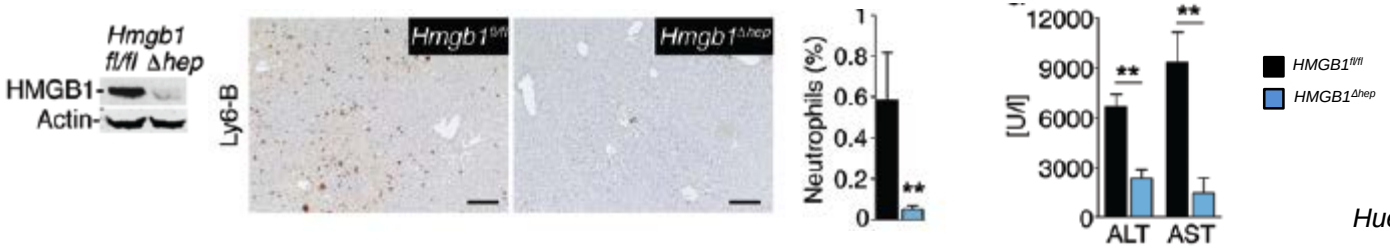
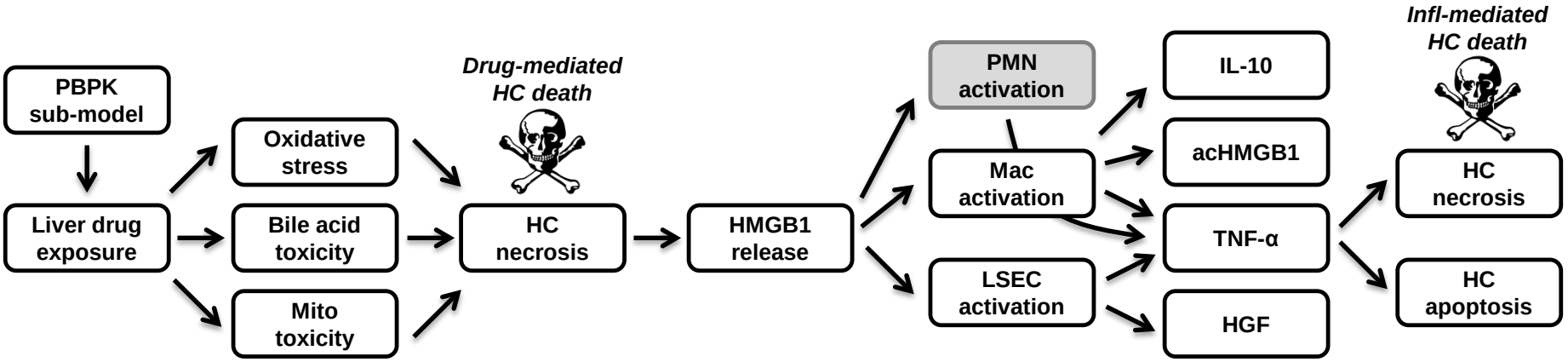
Model Development Underway to Enhance the Innate Immune Representation

MICE



Huebener et al. 2015

Conditional HMGB1 knockout (HC only) has reduced PMN infiltration and reduced injury at 24h



PMNs as a potential contributing cell type to injury amplification

Preclinical Data

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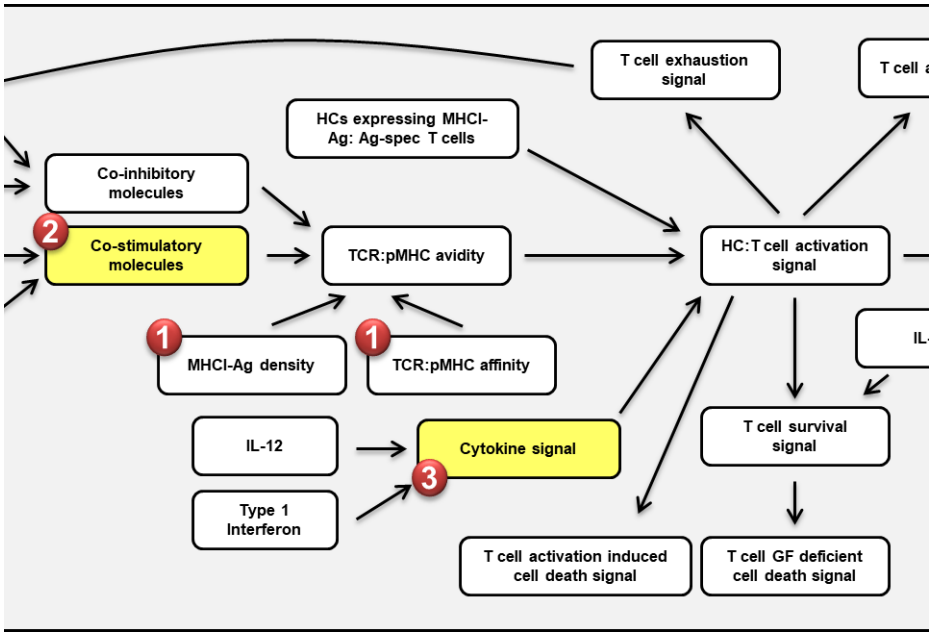
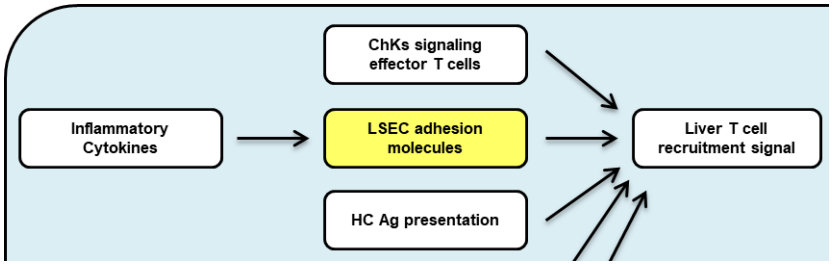
Innate Immune Response Contributes to CD8+ T cell Response

- Drug-mediated release of DAMPs sets the stage for breaking immune tolerance
  - Macrophage, PMN, endothelial cell activation
  - Upregulation of costimulatory molecules
  - Release of pro-inflammatory mediators (amplification)
- Provision of signal 2 (costimulation)
- Provision of signal 3 (cytokines)
- Dampening of regulatory signals (cellular or cell-associated)





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