Role of the Adaptive Immune Response in Drug-Induced Liver Injury

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• It is nearly impossible to predict which new drugs will cause hepatotoxicity and who will be susceptible to DILI

• This is due in large part to the idiosyncratic nature of DILI and the lack of animal models for most drugs where mechanisms of liver injury and susceptibility factors can be uncovered

• Our understanding of DILI is improving based on animal model studies of acetaminophen/halothane and clinical findings
• Fever, skin rash, and/or hepatic eosinophilia are often seen
• Hepatic lesions contain mononuclear cells, neutrophils, eosinophils and lymphocytes
• Toxicity occurs often after more than one exposure of the drug
• Susceptible patients have circulating antibodies against protein adducts of drugs or unlabeled carrier proteins
• Susceptible patients have drug metabolite or adducts of drug specific circulating T cells
HLA Associations Suggest Roles For Adaptive Immune Reactions in DILI

Genome-Wide Association Studies in Drug-Induced Liver Injury: Step Change in Understanding the Pathogenesis

Guruprasad P. Aithal and Jane I. Grove, Semin Liver Dis 2015

Genetic association studies in drug-induced liver injury


Human leukocyte antigen (HLA)-B*57:01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury

Naisbitt et al  HEPATOLOGY 2013
Halothane-Induced Liver Injury (HILI)

**Halothane**

\[ \text{Halothane} \rightarrow \text{Trifluoroacetyl Chloride} \]

**Oxidative Metabolism**

\[ \text{CYP450s} \rightarrow \text{Covalent Binding to Hepatic Proteins} \]

**Immune Mediated Hepatotoxicity**

\[ \text{Innate and Adaptive Immune Responses} \]

**Initial Hepatocellular Damage/Necrosis**

**In mice:** NKT, NK, Eosinophils, Neutrophils, IFN-γ, IL-17↑ toxicity

IL-10↓ toxicity

Mouse model of Halothane-Induced Liver Injury (HILI)

**ALT (IU/L)**

<table>
<thead>
<tr>
<th>Hours Post Treatment</th>
<th>Vehicle</th>
<th>Halothane</th>
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<tbody>
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**Leukocytes**

<table>
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<th>Hours Post Treatment</th>
<th>VEH</th>
<th>HAL</th>
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<tbody>
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</tbody>
</table>

24h VEH

24h HAL
Evidence for Liver Tolerance in a Guinea Pig Model of Halothane-Induced Liver Injury

No Secondary Immune Response

M. Chen and J. Gandolfi, Drug Metab. Rev., 29, 103 (1997)
Weak Adaptive Immune Response Occurred After Second Exposure of Halothane

TFA-Protein Serum Antibodies

TFA-Protein Specific T cells

antibody response detected in sera, (1:20)
Hypothesis

The idiosyncratic nature of DILI is due at least in part to immune tolerance in the liver

• Broken immune tolerance in the liver may lead to the development of animal model of DILI mediated by adaptive immune response

• Patients who develop DILI may be deficient in liver tolerance
Steps in Developing a Murine Model of Halothane-Induced Liver Injury Mediated by the Adaptive Immune Response

- Halothane must cause liver injury after first exposure

- Trifluoroacetylated (TFA)-protein adducts of halothane must be released into the circulation

- Tolerogenic mechanisms should be inhibited prior to halothane exposure in order to increase the adaptive immune response and liver injury
Detection of TFA-Protein Adducts in Mouse Liver and Serum after Halothane Treatment

A- Liver Homogenate from Halothane-Treated Mice
B- Extract of Microsomal Fraction from Halothane-Treated Mice
C- Mouse Sera from Halothane-Treated Mice
D- Liver Homogenate from Vehicle-Treated Mice
CD11b$^+$Gr-1$^{\text{high}}$ Cells Infiltrate the Liver after Halothane Treatment
Recent reports indicate that MDSCs are one of the main cell populations responsible for regulating immune responses in cancer, infections, and autoimmune diseases.

MDSCs represent a heterogeneous population of immature myeloid cells that strongly suppress T cells and are identified by expressing both CD11b and Gr-1 and are regulated by STAT3, STAT1,5,6, MyD88 and NF-κB.

Effector molecules include IL-10, PGE2, TGFβ, ROS, NO, and arginase.

- **Granulocytic-MDSC**: $\text{CD11b}^+ \text{Gr-1}^{\text{high}} - \text{CD11b}^+ \text{Ly-6G}^+ \text{Ly-6C}^{\text{low}}$
- **Monocytic-MDSC**: $\text{CD11b}^+ \text{Gr-1}^{\text{low}} - \text{CD11b}^+ \text{Ly-6G}^- \text{Ly-6C}^{\text{high}}$

*Trends in Immunology, 32, 19 (2013)*
CD11b^+Gr-1^{high} Cells Produce High Levels of ROS after Halothane Treatment
MDSC Suppression Assay

- Coat plate with Anti-CD3ε

- Sorted CD4⁺CD25⁻ and CD8⁺CD25⁻ cells from naïve mice spleen used as target cells

- Irradiated CD3⁻ HLA-DR⁺ splenocytes were used as APCs

- Sorted CD11b⁺ Gr-1<sup>high</sup> cells (MDSC) from the livers of halothane or vehicle treated mice were used as effector cells

- Plates were incubated for 72hrs
Hepatic MDSC From Halothane Treated Mice Suppressed T Cell Proliferation by Producing Nitric Oxide

**CD4⁺ T cells**

- **Proliferation (CPM)**
  - Anti-CD3ε: −, +, +, +, +
  - MDSC: −, −, +, ++, +
  - L-NIL: −, −, −, −, +

**CD8⁺ T cells**

- **Proliferation (CPM)**
  - Anti-CD3ε: −, +, +, +, +
  - MDSC: −, −, +, ++, +
  - L-NIL: −, −, −, −, +

**Nitrite (μM)**

- **CD4⁺ T cells**
  - Anti-CD3ε: +, +, +, +, +
  - MDSC: −, +, ++, +
  - L-NIL: −, −, −, +

- **CD8⁺ T cells**
  - Anti-CD3ε: +, +, +, +, +
  - MDSC: −, +, ++, +
  - L-NIL: −, −, −, +
CD11b^+Gr-1^{high} Cells Infiltrate the Liver after Halothane Treatment
Liver Injury after depletion of MDSCs Prior To Halothane Treatment

9 Days Post 2nd Halothane Treatment

Isotype Treated

Anti-Gr-1 Treated
Liver Injury after depletion of MDSCs Prior To Halothane Treatment

Anti-Gr-1 Treated

Anti-Gr-1 Treated
Histology of Severely Injured Liver Treated With Anti-Gr-1

ALT - 1513
Similar Levels of Liver TFA-Protein adducts 9 Day Post 2\textsuperscript{nd} Exposure of Halothane

\begin{itemize}
\item 1 - Anti Gr -1
\item 2 - Isotype
\end{itemize}

$\beta$-Tubulin
Depletion of MDSCs Prior To Halothane Treatment Increased The Titer of Anti-TFA Antibodies

- **Graphs:**
  - TFA-Protein Serum IgG (OD 405):
    - 7 Days Post 1st Exposure of Halothane
    - 9 Days Post 2nd Exposure of Halothane
  - TFA-Protein Serum Ab (OD 405):
    - lgG1
    - lgG2a
    - lgG2b
    - lgG3
    - lgM
    - lgA
    - lgE
  - IL-4:
    - Serum conc. (pg/mL)
    - 9 Days Post Halothane Rechallenge
Depletion of MDSCs Prior to Halothane Exposure
Increased Eosinophil Infiltration

9 Days Post Halothane Rechallenge

Isotype

Anti Gr-1

CCL11 (pg/ml)

CCL24 (pg/ml)
Depletion of Hepatic MDSC Prior To Halothane Treatment Resulted In Increased TFA-Protein Specific CD4⁺ T Cells In The Liver
Decreased Toxicity After The Depletion of CD4⁺ T Cells

![Graph showing ALT levels in Anti-Gr-1 treated mice with Isotype (CD4), Anti-CD4, Isotype (CD8), and Anti-CD8 treatments.]

**9 days Post 2nd Exposure of Halothane**

**Isotype**

**Anti-CD4**
Cytokines after Halothane Rechallenge

**IL-10**

- Serum conc. (pg/mL)
- 9 Days Post Halothane Rechallenge

**TGF-β**

- Serum conc. (pg/mL)
- 9 Days Post Halothane Rechallenge
Summary

- Protein adducts of halothane produced in the liver and released in the blood were able to induce both specific humoral and T cell responses against protein adducts when tolerogenic MDSC were depleted from the liver prior to halothane treatment.

- This approach also led to a significant inflammatory liver injury that appeared to be mediated at least in part by adaptive immune system.

- We provide the evidence for the development of an animal model of drug induced liver injury mediated by adaptive immune system.
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