Inhibition of Immune Tolerance Unmasks IDILI Potential

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Evidence that most IDILI is immune mediated

- Not much controversy for idiosyncratic drug reactions such as skin rash and autoimmunity, less so for IDILI
  - Characteristic delay in onset, which varies with the type of IDILI and even the drug. IDILI can even occur more than a month after discontinuation of the drug. But on reexposure, typically the IDILI occurs immediately, although there are many exceptions, especially for mild IDILI.
  - Presence of eosinophilia, fever, and/or rash, but the absence of these characteristics does not argue against an immune mechanism.
  - Histology similar to other immune mediated DILI
  - Presence of anti-drug antibodies.
  - HLA associations
  - Positive lymphocyte transformation test.
Mechanistic Studies of IDRs

- Given their unpredictable nature, it is very difficult to study IDRs in humans.
- As in other areas of biomedical research, animal models are essential for controlled studies; however, IDRs are also idiosyncratic in animals.
- It is essential that an animal model have essentially the same mechanism as the IDR in humans; therefore, the characteristics must be similar.
- If IDRs are immune mediated, it should be possible to develop animal models by stimulation of the immune system; however, multiple attempts using this strategy have failed. This fits with the observation that, with notable exceptions, preexisting liver disease or inflammation are not major risk factors for IDRs.
A classic example of IDILI that was not thought to be immune mediated is Isoniazid (INH)-IDILI

- It was shown that acute INH toxicity in rats is caused by bioactivation of the metabolite N-acetylhydrazine.
- This is the wrong type of toxicity in the wrong species.
- Most of the covalent binding in mice and humans involves direct oxidation of INH to a reactive metabolite.
Most patients with INH-induced liver failure have antibodies against INH-modified proteins and/or P450s

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Other evidence that INH IDILI is Immune Mediated

• Positive lymphocyte transformation tests – with mild injury the lymphocytes only responded to INH-modified proteins, but with severe injury the lymphocytes also responded to INH itself.

• With severe injury there is immune memory, i.e. rapid onset on rechallenge.
Why is it So Difficult to Develop Valid Animal Models of IDRs?

- The animals do not have the right MHC or T cell receptor
  - However, reactive metabolites of drugs such as isoniazid and nevirapine bind to thousands of proteins, each of which is processed to several peptides. One of the several MHCs should be able to present one of the modified peptides to the large repertoire of T cell receptors.

- There is insufficient activation of antigen presenting cells
  - We have used multiple agents to activate antigen presenting cells - it does not work.

- We have also used methods to increase reactive metabolite formation, deplete glutathione, cotreated with cytotoxic drugs, stimulated AHR, inhibited IDO, etc.

- The biggest factor appears to be overcoming immune tolerance, especially in the liver.
Clinical Picture of Idiosyncratic Liver Injury

If it is immune mediated and the mechanism of mild liver injury and liver failure are the same, adaptation must represent immune tolerance.

Isoniazid: ALT increase in 15-20% of patients, severe hepatic necrosis only occurs in 1:1000.
Patients with mild INH IDILI have an increase in Th17 cells and IL-10+ T cells.
Amodiaquine causes idiosyncratic liver injury and agranulocytosis that appear to be immune mediated

Bioactivation of Amodiaquine
Amodiaquine-Induced ALT Release in Mice
BSO depletes glutathione but paradoxically prevents amodiaquine-induced liver injury.
If it is difficult to develop animal models because the default response is immune tolerance, it might be possible to overcome tolerance by immunization

- Amodiaquine alone causes mild immune-mediated liver injury that resolves despite continued treatment.
- Immunization with amodiaquine-modified hepatic proteins followed by treatment with oral amodiaquine should lead to severe hepatitis.
Immunization with amodiaquine-modified proteins + soluble adjuvant paradoxically prevented the mild liver injury caused by amodiaquine.
Immunization with amodiaquine-modified hepatic proteins followed by oral amodiaquine led to a marked increase in myeloid-derived suppressor cells and Tregs in the liver.
If the dominant response to drugs that cause idiosyncratic liver injury is immune tolerance, it might be possible to develop an animal model by inhibiting molecules involved in immune tolerance.

- **PD-1** is expressed on activated T cells, B cells, and macrophages. It negatively regulates TCR signals.
- **CTLA-4** is expressed on T cells and competes with CD8 for binding to CD80 and CD86 on antigen presenting cells to inhibit immune responses.
- These molecules are being targeted to treat cancer.
Treatment of PD-1⁻/⁻ mice with amodiaquine + anti-CTLA-4 leads to liver injury that looks like IDILI in patients with piecemeal necrosis.
AQ treatment increases the number of PD-1⁺ and CTLA-4⁺ T cells and in PD-1⁻/⁻ mice cotreated with anti-CTLA-4 increases cytotoxic T cells.
Depletion of CD8 T cells prevents amodiaquine-induced liver injury
Checkpoint Block Also Unmasks INH-Induced Liver Injury

![Graph showing GLDH levels over weeks for different groups: Control, INH, INH/Anti-CTLA-4.](image)
And Nevirapine-Induced Liver Injury
This model differentiates troglitazone, which causes IDILI, from pioglitazone, which does not.
Other Methods of Decreasing Immune Tolerance

- Lance Pohl has shown that depletion of myeloid-derived suppressor cells with anti-GR-1 antibodies led to an immune response to halothane with liver injury and eosinophilia in mice similar to halothane hepatitis in humans.
- Anti-GR-1 antibodies also increased amodiaquine-induced DILI; however, it antagonized the effects of anti-PD-1 and anti-CTLA-4.
- Other immunomodulators used to treat cancer did not increase amodiaquine liver injury: 1-methyltryptophan had no effect, and anti-CD137 caused liver injury, but adding amodiaquine produced no additional injury.
Use of this Model to Test Hypotheses

• Two other hypotheses for IDILI are mitochondrial injury and BSEP inhibition; these are attractive hypotheses for activation of the immune system.

• Troglitazone IDILI has been hypothesized to be due to BSEP inhibition; if that is a major factor then cotreatment with other BSEP inhibitors should increase injury.

• Isoniazid IDILI has been hypothesized to be due to mitochondrial injury, and in vitro, rotenone increases INH cytotoxicity. Now we can test this in our in vivo model.

• Testing of the role of inflammasome activation and involvement of exosomes.

• Testing potential drug interactions, e.g. vemurafinib, CD137
What are the IDILI risk factors in Humans?

- Genetic – all known strong associations are with HLA genes, but with the exception of abacavir, even if a person has the required HLA gene they are still unlikely to develop IDILI—there must be additional factors. IDILI caused by many drugs does not appear to have a strong HLA association.
- T cell receptors – formed by random gene recombination
- Activation of the immune system/inflammatory condition – with some exceptions - this does not appear to be important.
- Deficiency in immune tolerance – although most patients do not have marked deficiency in immune tolerance, immune system-related genes are associated with IDILI risk.
- The immune system is a product of everything it has ever been exposed to - some patients have antibodies against drugs that they have never been exposed to, which is presumably due to cross-reactivity with some pathogen.
Heterologous T cell Immunity

• Although the number of T cell receptors that can be produced is almost limitless, the number of T cells in the body is limited, and the number is too small to have a specific T cell receptor for every pathogen-derived antigen.

• However, the same T cell receptor can recognize more than one antigen even if the antigens are not similar because there are many binding sites and configurations by which an antigen can interact with a T cell receptor. This greatly increases the number of antigens that can be recognized.

• It has been shown in mice that infection with one virus can markedly effect the response to other unrelated viruses.

• It is likely that the response to a virus or other pathogen can shape the response to an unrelated antigen produced by a drug.

• It is plausible that this “shaping” of the immune response by viruses and other pathogens is a major determinant of who will develop a serious IDR.
Treatment of Serious IDRs

• If there were an effective treatment for serious IDRs it would markedly decrease their significance.

• Many serious IDRs appear to be mediated by CD8\(^+\) T cells.

• The usual care for toxic epidermal necrolysis is IVIG; steroids do not appear to decrease mortality.

• Liver injury is often treated with steroids, but with the exception of autoimmune hepatitis, efficacy is unclear.

• An effective treatment for aplastic anemia, whether idiopathic or drug-induced, is anti-thymocyte globulin + cyclosporin.

• The most serious cases of IDILI appear to be mediated by CD8 T cells and depleting CD8\(^+\) T cells prevented liver injury in our model.

• Targeting CD8\(^+\) T cells may be a more effective treatment; various treatments should be studied.
Conclusions

• There is compelling evidence that most IDILI is immune mediated.
• Mild IDILI is always more common than serious IDILI, and most resolves despite continued treatment. If it is immune mediated, this adaptation must involve immune tolerance.
• Most animal models of IDILI do not represent the same mechanism as human IDILI.
• Impairment of immune tolerance leads to models of IDILI similar to IDILI in humans. This model can be used to test hypotheses such as involvement of mitochondrial injury and BSEP inhibition.
• Genetic factors can play an important role in who is at increased risk, but other factors are also important; heterologous immunity may play a dominant role in who is at risk.
• Serious IDILI persists after the drug is stopped. If they are immune mediated, there could be a window in which serious IDILI could be effectively treated.