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Navigating Immunologic Responses to Drugs and Biologics to Predict Clinical Outcomes

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Evidence that IDRs are immune mediated.

- Not much controversy for idiosyncratic drug reactions (IDRs) such as skin rash and autoimmunity, less so for idiosyncratic drug-induced liver injury (IDILI)
  - Characteristic delay in onset, which varies with the type of IDR and even the drug. IDRs can even occur more than a month after discontinuation of the drug. But on reexposure, typically the IDR occurs immediately, although there are many exceptions, especially for mild IDRs.
  - Presence of eosinophilia, fever, and/or rash, but the absence of these characteristics does not argue against an immune mechanism.
  - 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.
Isoniazid (INH)-Induced Liver Injury Was Not Believed to Be Immune Mediated

- 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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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 and 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.

5-10 weeks  10-15 weeks  15-20 weeks

Normal  Normal  Normal

Adaptation

ALT↑
Isoniazid: ALT increase in 15-20% of patients, severe hepatic necrosis only occurs in 1:1000.

Liver failure
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

The graph shows the levels of ALT (U/L) over 6 weeks for control and amodiaquine (AQ) treatments. The y-axis represents ALT levels ranging from 0 to 150, and the x-axis represents weeks from 1 to 6. The data points for AQ are significantly higher than the control at 3, 4, and 5 weeks, indicated by *** and **. This suggests a notable increase in ALT levels due to amodiaquine treatment.
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
Effect of Decreased Immune Tolerance on IDILI Caused by Other Drugs in Animals

• Blocking CTLA-4 and PD-1 also increases the liver injury caused by isoniazid and nevirapine in mice.

• Lance Pohl has a paper recently accepted by Hepatology in which he observed that depletion of myeloid-derived suppressor cells in mice led to an immune response to halothane with liver injury and eosinophilia similar to halothane hepatitis in humans.
IDILI Caused by Biologicals

- It is not surprising that autoimmune hepatitis can sometimes be caused by drugs such as INF-α that “activate” the immune system or inhibit immune tolerance.
- Autoimmune hepatitis can also be caused by “immunosuppressive” biologicals such as infliximab. It appears that this is due to a tip in the immune response balance rather than an immune response to a foreign protein.
- Biological drugs that alter immune response may lead to drug interactions with an increase in IDR risk, e.g. ipilimumab (anti-CTLA-4) increases the risk of IDILI caused by vemurafenib.
We have succeeded in developing an animal model of an IDR that is very similar to the IDR in humans without manipulation of the immune system.

- Nevirapine causes an immune-mediated skin rash in Brown Norway rats similar to the rash that it causes in humans.
### Comparison of Rash in Humans and Rats

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<th>Characteristic</th>
<th>Humans</th>
<th>Rats</th>
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<tr>
<td>Rash</td>
<td>mild morbilliform to TEN</td>
<td>mild to severe but no blisters</td>
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<tr>
<td>Plasma levels</td>
<td>1-10 µg/ml</td>
<td>20-40 µg/ml</td>
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<td>Time to onset</td>
<td>1-3 weeks</td>
<td>2-3 weeks</td>
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<td>Dose response</td>
<td>incidence increases with dose</td>
<td>incidence increases with dose</td>
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<tr>
<td>Sex dependence</td>
<td>incidence greater in women</td>
<td>incidence greater in females but probably metabolic difference</td>
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<tr>
<td>Low dose pretreatment</td>
<td>decreases incidence</td>
<td>prevents rash</td>
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<tr>
<td>Rechallenge</td>
<td>immediate onset and more severe</td>
<td>decreased time to onset and decreased dose required</td>
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<tr>
<td>Histology</td>
<td>little data, lymphocytic infiltrate</td>
<td>T cells and macrophages</td>
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<td>T cell dependence</td>
<td>incidence low if CD4+ count low</td>
<td>depletion of CD4+ T cells is protective.</td>
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<td>In vitro lymphocyte response to NVP</td>
<td>produce IFN-γ</td>
<td>produce IFN-γ</td>
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Nevirapine is oxidized to a free radical intermediate that partitions between oxygen rebound to form the 12-OH-metabolite and loss of another hydrogen to form a quinone methide. The quinone methide binds to hepatic proteins and is presumably responsible for hepatotoxicity. The 12-OH-metabolite is sulfated in the epidermis and the sulfate covalently binds and causes the skin rash.
12-OH-Nevirapine Sulfate Formed in the Skin is Responsible for the Rash

- Topical administration of a sulfotransferase inhibitor prevents covalent binding and the rash where it is applied.
- The required sulfotransferase is not present in mouse skin, and we have not been able to induce the rash in mice.
- This is the first time that a reactive sulfate formed in the skin has been shown to be the cause of skin rashes, and there are several other drugs that probably cause a skin rash by a similar mechanism.
How does the reactive metabolite of nevirapine cause a skin rash?

• Chemically reactive compounds applied to the skin cause a skin rash (contact hypersensitivity).
• This is known to involve inflammasome activation because ASC- or NALP3-deficient mice (ASC and NALP3 are components of the inflammasome) have impaired responses to contact sensitizers.
Inflammasome Activation

- To test whether the IL-1β is produced by inflammasome activation a caspase inhibitor (Z-VAD) is used to block IL-1β production.
Can Inflammasome Activation Differentiate Reactive Species That Are Likely to Cause IDRs?

telaprevir

boceprevir

ethacrynic acid
Telaprevir is associated with TEN while boceprevir is not; dimethyl fumarate causes hypersensitivity reactions and ethacrynic acid does not.

Fig 1. Telaprevir activates inflammasomes in THP-1 cells with production of IL-1β, which is inhibited by ZVAD, while boceprevir does not. Likewise dimethyl fumarate activates inflammasomes while ethacrynic acid does not.
Contrasting Clozapine and Olanzapine

- Clozapine causes agranulocytosis with an incidence of about 0.2%
- It causes an increase in IL-6, other cytokines, C-reactive protein, fever, and paradoxical neutrophilia in >50% of patients.
- Clozapine also causes neutrophilia and an increase in alpha-1-glycoprotein (equivalent to C-reactive protein in humans) in rats.
- Olanzapine does not cause a significant incidence of agranulocytosis in humans, and it does not cause neutrophilia in rats even though both are oxidized to a reactive nitrenium ion by myeloperoxidase.
Clozapine

- Clozapine also covalently binds to THP-1 cells and activates inflammasomes.
- Clozapine causes agranulocytosis and other idiosyncratic drug reactions, but olanzapine, which has a very similar structure and forms a similar reactive metabolite does not.
Inflammasome Activation by Amodiaquine

Amodiaquine Treated THP-1 Macrophages

IL-1B Sec (pg/ml)

Sample (ug/ml)

Med  DMSO  AQ-2  AQ-5  AQ-10  AQ-10z

*  **  *
Inflammasome Activation as a Biomarker of IDR Risk

• These results suggest that the ability of a reactive drug or reactive metabolite to activate inflammasomes may be a biomarker of IDR risk.

• The assay is simple for drugs with intrinsic chemical reactivity or are easily oxidized by peroxidases, but it is more of a challenge for drugs that require bioactivation by P450.

• Possible approaches include co-cultures and in vivo studies of inflammasome activation.

• The release of microvesicles containing molecules such as HMGB-1 by hepatocytes is an attractive mechanism by which inflammasomes may be activated in the liver.
Immune Responses to Drugs That Cause IDR s

• Drugs such as clozapine clearly cause an immune response in most humans and rodents treated with the drug.

• A careful study of the immune response to drugs, especially by phenotyping mononuclear leukocytes, may provide important mechanistic clues and ultimately provide biomarkers of IDR risk.
What are the IDR 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 have an IDR to the drug – there must be additional factors. Many IDRs do 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 IDR 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.
Treatment of Serious IDR

- If there were an effective treatment for serious IDR it would markedly decrease their significance.
- Many serious IDR 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 idiopathic autoimmune hepatitis, efficacy is unclear.
- An effective treatment for aplastic anemia, whether idiopathic or drug-induced, is anti-thymocyte globulin.
- 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

• Valid animal models are important for mechanistic studies, but they are difficult to produce; most animal models of IDRs do not represent the same mechanism as human IDRs.

• There is compelling evidence that most IDRs are immune mediated, and reactive metabolites are responsible for initiating the immune response; however, not all reactive metabolites carry the same risk.

• Genetic factors play an important role in who is at increased risk, but other factors are also important.

• Mild IDRs are always more common than serious IDRs, and many resolve despite continued treatment. If they are immune mediated, this adaptation must involve immune tolerance.

• One mechanism by which drugs appear to induce an immune response is through activation of inflammasomes. This may represent a good biomarker to predict which drugs that form reactive metabolites are likely to pose a significant risk of serious IDRs.

• Serious IDRs persist after the drug is stopped. If they are immune mediated, there could be a window in which serious IDRs could be effectively treated.