

EDITORIAL

Inhibition of Immune Tolerance Unmasks Drug-Induced Allergic Hepatitis

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In 1980 Vergani et al. reported that patients with halothane-induced liver injury had antibodies against the cell membrane fraction of hepatocytes taken from rabbits anesthetized with halothane.¹ There followed extensive studies by Lance Pohl's group to characterize the covalent binding of the reactive metabolite of halothane to hepatic proteins and the specificity of the antibodies induced by this covalent binding.² There were also multiple attempts by several groups over the subsequent three decades to produce an animal model of halothane-induced hepatitis; however, none were able to reproduce the delayed onset and other characteristics typical of serious halothane-induced hepatitis in humans. In fact, until recently there were no reproducible animal models of delayed-onset idiosyncratic drug-induced liver injury (IDILI).³

Drugs that cause serious IDILI always cause mild liver injury with a higher incidence than severe injury, and this injury often resolves despite continued treatment with the drug; this is referred to as "adaptation." The general characteristics, in particular the delay in onset of mild injury, are similar to those of severe injury caused by the same drug. This suggests that the mechanisms are similar and, in the case of lumiracoxib, that the human leukocyte antigen (HLA) association for mild injury is the same as for severe injury.⁴ This strongly suggests that the injury is immune-mediated, and furthermore, the mechanism for mild injury is the same as that for severe injury. This implies that adaptation involves immune tolerance. The liver, in particular, responds to most antigens with

immune tolerance, presumably because it is exposed to many antigens from the gut and modified proteins from reactive metabolites formed from food, and tolerance is the appropriate response unless these antigens are associated with significant cell damage. This suggests that overcoming immune tolerance may be a key factor in the development of animal models of IDILI.

In the current issue of HEPATOLOGY, Chakraborty et al.⁵ report the first animal model of halothane-induced IDILI with the delayed onset that is characteristic of halothane-induced IDILI as well as that caused by most other drugs. This was accomplished by depleting myeloid-derived suppressor cells (MDSCs). The basis for this strategy was the finding that treatment of mice with halothane led to a marked increase in MDSCs in the liver. In the model, the initial treatment with halothane after depletion of MDSCs led to an acute increase in alanine aminotransferase, which rapidly resolved. A second treatment with halothane again led to an acute increase in alanine aminotransferase, but this was followed 9 days later by another marked increase in alanine aminotransferase in those animals in which MDSCs were depleted. The delayed liver injury was also accompanied by an infiltration of eosinophils in the liver. This is very similar to the pattern of halothane-induced IDILI in humans, which almost always occurs after two or more exposures to halothane and is often accompanied by eosinophilia.⁶ Not surprising for a model of an idiosyncratic drug reaction, not all of the animals developed this delayed-onset injury.

This is the second recent animal model of IDILI reported in HEPATOLOGY that was achieved by impairment of immune tolerance. In the other report, tolerance was impaired by blocking CTLA-4 and PD-1, and the drug involved was amodiaquine.⁷ In fact, there are many redundant mechanisms of immune tolerance involving multiple cell types and molecules; therefore, there are many ways in which immune tolerance can be modified. It should be noted that, although the liver injury in both of these models was quite significant, overt liver failure was not the result in either model; and this is likely due, at least in part, to this redundancy in mechanisms of immune tolerance. These two successful models are likely to lead to many

Abbreviations: HLA, human leukocyte antigen; IDILI, idiosyncratic drug-induced liver injury; MDSC, myeloid-derived suppressor cell.

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future studies to test other methods to manipulate the immune response. It is revealing to compare these two models. In the halothane model the injury was accompanied by eosinophilia and was abrogated by depleting CD4 T cells but not by depleting CD8 T cells. In the amodiaquine model, the injury was not accompanied by eosinophilia and was abrogated by depleting CD8 T cells (J. Uetrecht, unpublished observation). In humans IDILI can take many different forms, with different drugs and with the same drug in different individuals. The most common severe form is not associated with eosinophilia, and liver histology suggests that it is mediated by CD8 cytotoxic T cells. Also common is an immunoallergic pattern with eosinophilia as observed in this halothane model. Also, IDILI can have a very long delay in onset and features typical of idiopathic autoimmune hepatitis. Autoantibodies are often observed in IDILI associated with halothane⁸ and other drugs⁹ even if the IDILI does not have the typical characteristics of autoimmune hepatitis. The picture that emerges is that some drugs can induce an immune response, which usually resolves with immune tolerance, and only in rare cases does it lead to severe IDILI, as depicted in Fig. 1. The pattern of that immune response and injury depends on both the drug and the individual involved. The same drugs can often cause other types of idiosyncratic drug reactions that presumably reflect where the reactive metabolite is formed; in the case of amodiaquine, covalent binding also occurs in neutrophils, and amodiaquine can also cause agranulocytosis.

Although immune tolerance is a critical factor in the determination of who will develop IDILI, it is unlikely that patients who develop IDILI have the same degree of impaired immune tolerance as the animals in these models. However, as mentioned, there are multiple cells and molecules involved in immune tolerance; therefore, the risk factors for IDILI are likely multifactorial. One molecule involved in immune tolerance is interleukin-10. It has been reported that polymorphisms in interleukin-10 in which there is a decrease in its production were associated with IDILI with lower eosinophil counts, and the mortality appeared to be higher,¹⁰ suggesting that lower levels of interleukin-10 shifted the immune response toward a CD8-mediated liver injury. It is likely that there are many other genetic polymorphisms that affect the immune response to a drug and IDILI risk. The immune response represents a balance, and it is unknown what factors in humans lead to a stronger response that does not resolve with immune tolerance. The IDILI caused by some drugs is associated with

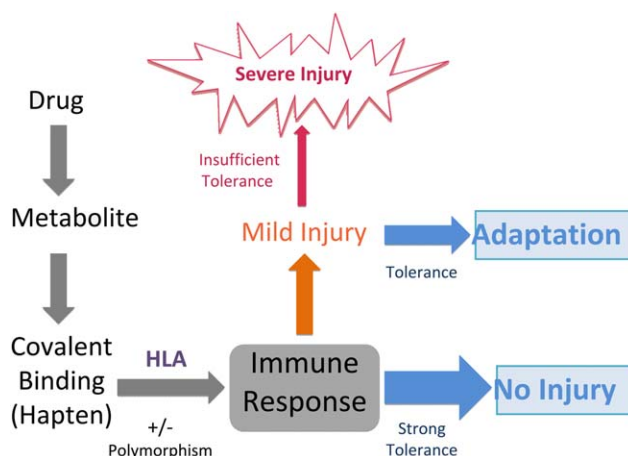


Fig. 1. Conceptual overview of immune-mediated IDILI. Although some IDILI does not appear to involve reactive metabolites, most IDILI appears to involve the formation of a reactive metabolite in the liver that covalently binds to proteins leading to peptides presented by HLA molecules. In some cases a specific HLA is required to induce an immune response, but some reactive metabolites bind to so many proteins that a specific HLA may not be required. These modified proteins, presumably accompanied by some cell damage, can elicit an immune response. In most cases either there is an insignificant immune response or the immune response is so effectively tolerated that no significant liver injury results. In some cases the immune response leads to mild injury, but immune tolerance leads to adaptation so that the injury resolves despite continued treatment. The mechanisms of immune tolerance are highly redundant, involving MDSCs, T-regulatory cells, several checkpoint molecules such as PD-1 and CTLA-4, and many other cells and molecules. It is only rarely that the immune response is so strong that immune tolerance fails to prevent severe liver injury, which can take several forms such as cytotoxic T cell-mediated liver injury, immunoallergic liver injury, or autoimmune liver injury.

specific HLA genotypes; but in most cases no strong HLA association has been found, and even when a strong HLA association is present, most patients with the required HLA phenotype who are treated with the associated drug do not develop IDILI.¹¹ Environmental factors must also be involved. It is surprising that, in most cases, a preexisting liver disease or inflammatory condition such as inflammatory bowel disease in which the liver is exposed to large amounts of lipopolysaccharide does not appear to be a major risk factor. The immune system of an individual is shaped by everything that it has ever been exposed to, and some pathogens may prime the immune system such that it responds to drug-modified proteins and results in a pathogenic response to the drug. Much of this is speculation; however, such animal models will allow us to test such hypotheses.

This new strategy for developing animal models of idiosyncratic drug reactions by manipulation of the immune system provides a very powerful tool for detailed mechanistic studies. In turn, such studies are

likely to lead to methods to predict which drug candidates are likely to cause IDILI and better treatments of IDILI when it does occur. This is likely to be of increasing importance as new drugs are developed that target the immune system, especially anticancer drugs designed to impair immune tolerance. Such drugs are likely to lead to drug interactions and to unmask the potential of other drugs to cause idiosyncratic reactions.

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References

1. Vergani D, Mieli-Vergani G, Alberti A, Neuberger J, Eddleston ALWF, Davis M, et al. Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. *N Engl J Med* 1980;303:66-71.
2. Satoh H, Martin BM, Schulick AH, Christ DD, Kenna JG, Pohl LR. Human anti-endoplasmic reticulum antibodies in sera of patients with halothane-induced hepatitis are directed against a trifluoroacetylated carboxylesterase. *Proc Natl Acad Sci USA* 1989;86:322-326.
3. Ng W, Lobach AR, Zhu X, Chen X, Liu F, Sharma AM, et al. Animal models of idiosyncratic drug reactions. *Adv Pharmacol* 2012;63:81-135.
4. Singer JB, Lewitzky S, Leroy E, Yang F, Zhao X, Klickstein L, et al. A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nat Genet* 2010;42:711-714.
5. Chakraborty M, Fullerton AM, Semple K, Chea LS, Proctor WR, Bourdi M, et al. Drug-induced allergic hepatitis developed in mice when myeloid-derived suppressor cells were depleted prior to halothane treatment. *HEPATOLOGY* 2015; doi: 10.1002/hep.27764.
6. Kaplowitz N, Deleve LD. *Drug-Induced Liver Disease*, 3rd ed. New York: Elsevier; 2013.
7. Metushi IG, Hayes MA, Uetrecht J. Treatment of PD-1 mice with amodiaquine and anti-CTLA4 leads to liver injury similar to idiosyncratic liver injury in patients. *HEPATOLOGY* 2015;61:1332-1342.
8. Bourdi M, Chen W, Peter RM, Martin JL, Buters JT, Nelson SD, et al. Human cytochrome P450 2E1 is a major autoantigen associated with halothane hepatitis. *Chem Res Toxicol* 1996;9:1159-1166.
9. Metushi IG, Sanders C, Lee WM, Uetrecht J; Acute Liver Study Group. Detection of anti-isoniazid and anti-cytochrome P450 antibodies in patients with isoniazid-induced liver failure. *HEPATOLOGY* 2014; 59:1084-1093.
10. Pachkoria K, Lucena MI, Crespo E, Ruiz-Cabello F, Lopez-Ortega S, Fernandez MA, et al. Analysis of IL-10, IL-4 and TNF-alpha polymorphisms in drug-induced liver injury (DILI) and its outcome. *J Hepatol* 2008;49:107-114.
11. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet* 2009;41:816-819.