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Commentary

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Active suppression rather than ignorance: tolerance to abacavir-induced HLA-B*57:01 peptide repertoire alteration

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The discovery of HLA-B*57:01–associated abacavir hypersensitivity is a translational success story that eliminated adverse reactions to abacavir through pretreatment screening and defined a mechanistic model of an altered peptide repertoire. In this issue of the *JCI*, Cardone et al. have developed an HLA-B*57:01–transgenic mouse model and demonstrated that CD4$^+$ T cells play a key role in mediating tolerance to the dramatically altered endogenous peptide repertoire induced by abacavir and postulate a known mechanism by which CD4$^+$ T cells suppress DC maturation. This report potentially explains why 45% of HLA-B*57:01 carriers tolerate abacavir and provides a framework for future studies of HLA-restricted, T cell–mediated drug tolerance and hypersensitivity.

Translational advances in drug hypersensitivity

Drug hypersensitivity reactions have been classified as type B or off-target reactions that are less dependent on dose and unpredictable based on the pharmacological mechanism of the drug. Hypersensitivity is among the most feared, and contributes the highest burden, of adverse drug reactions that threaten both patient safety and new drug development. The perception that type B immune–mediated reactions are unpredictable has fueled the perception that little can be done to avoid the tragic morbidity and mortality caused by these so-called "idiosyncratic" reactions, apart from early recognition, immediate cessation, and permanent avoidance of the causal drug (1). A major paradigm shift in the perception that drug hypersensitivity is unpredictable occurred with the discovery of a strong association between the HLA class I allele HLA-B*57:01 and a severe hypersensitivity syndrome characterized by a potentially fatal CD8$^+$ T cell–dependent cytokine-type storm syndrome induced by the antiretroviral drug abacavir (2, 3). The demonstration that 100% of patients who are hypersensitive to abacavir carry HLA-B*57:01 led to a successful and routine pre-prescription screening strategy that has now eliminated abacavir hypersensitivity as a clinical entity (4). Subsequently, a number of strong, primarily HLA class I but also HLA class II associations were recognized with other CD8$^+$ and CD4$^+$ T cell–mediated drug hypersensitivity syndromes. However, successful screening strategies have only been developed for a minority of drugs, largely because, with the exception of abacavir hypersensitivity, less than 5% of patients carrying an HLA risk allele will develop drug hypersensitivity (1). Ten years after the discovery of an association between HLA-B*57:01 and abacavir hypersensitivity, the mechanistic basis of the exquisite specificity of abacavir for HLA-B*57:01 was explained on the basis of noncovalent, dose-dependent binding of the drug to the floor of the antigen-binding groove observed in crystallography studies (refs. 5, 6 and Figure 1). The binding of abacavir in proximity to the F pocket of HLA-B*57:01 dramatically alters the repertoire of endogenous peptides bound to the HLA molecule, effectively creating a foreign HLA allele in HLA-B*57:01 carriers taking abacavir (5–7). The noncovalent nature of abacavir binding explained the remarkably rapid disappearance of symptoms following abacavir discontinuation (1).

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New models and knowledge gaps

Despite key mechanistic insights into abacavir-induced hypersensitivity in HLA-B*57:01 carriers, several gaps in our understanding remain. For instance, what drives the tissue tropism for specific hypersensitivity reactions that manifest with distinct clinical and laboratory findings? Furthermore, a randomized, double-blind study to examine the utility of HLA-B*57:01 as a screening marker in patients prescribed abacavir confirmed that while HLA-B*57:01 has a 100% negative predictive value for abacavir hypersensitivity, 45% of HLA-B*57:01 carriers will be abacavir tolerant (4). Moreover, for drug-induced liver disease (DILI) that is associated with the antistaphylococcal drug flucloxacillin, the negative predictive value of HLA-B*57:01 is greater than 99%; however, less than 0.1% of those given flucloxacillin develop DILI (1). Thus, how is it possible for HLA-B*57:01 carriers to tolerate abacavir and flucloxacillin, particularly as it has been shown that CD8$^+$ T cells from all HLA-B*57:01 carriers can be driven in vitro by drug to produce abacavir or flucloxacillin polyclonal lines or clones (1, 8, 9)?

Animal model for abacavir tolerance

In this issue, Cardone and colleagues have created an animal model that provides crit-

Conflict of interest: EJP and SAM are co-directors of IIID Pty Ltd, which holds a patent for HLA-B*57:01 screening for abacavir hypersensitivity.

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ical insights to the potential mechanism of abacavir tolerance in the presence of the HLA-B*57:01 risk allele (10) The authors generated HLA-B*57:01–transgenic mice (Tg mice) and were able to show that, as in humans, drug-naive mice had drug-reactive $CD8^+$ T lymphocytes with effector potential that could rapidly respond to abacavir stimulation in vitro. Additionally, drug-reactive $CD8^+$ T cells expanded in vitro were shown to rapidly respond to abacavir stimulation in an HLA-B*57:01–dependent manner. In contrast to the abacavir hypersensitivity syndrome seen in 55% of human HLA-B*57:01 carriers, none of the Tg mice developed a systemic or skin hypersensitivity reaction when challenged with abacavir in vivo for up to 4 weeks. Abacavir-exposed Tg mice accumulated $CD8^+PD1^+$ cells over the first seven days, and these cells had proliferative capacity by day 3. The early-responding cells had a predominant $CD44^{hi}CD62L^{hi}$ memory-like phenotype, suggesting that they may have arisen from preexisting memory $CD8^+$ T lymphocytes, as has been found in human studies (8). The PD1-expressing cells did not express other inhibitory coreceptors, and neutralization of PD1 did not predispose the mice to hypersensitivity, making it unlikely that expression of inhibitory coreceptors on the $CD8^+$ T cells is responsible for abacavir tolerance in Tg mice.

Role of $CD4^+$ T cells

Cardone et al. examined the possibility that $CD4^+$ T cells were contributing to the capacity of the Tg mice to tolerate abacavir by depleting $CD4^+$ T cells with monoclonal anti-mouse CD4. $CD4^+$ T cell–depleted mice exhibited skin hypersensitivity and $CD8^+$ T cell infiltration of the dermis and epidermis at the site of topical abacavir application to the ears by three weeks after the initial treatment (10). By day ten of abacavir treatment, the $CD4^+$ T cell–depleted Tg mice had a higher percentage of $CD8^+PD\text{-}1^+$ T cells than did nondepleted Tg animals, indicating that $CD4^+$ T cells were preventing a systemic reaction in Tg mice. Comparative gene expression analysis of sorted $CD3^+CD4^-CD8^+$ T cells from the lymph nodes (LNs) of abacavir-exposed Tg mice revealed a gene signature associated with anergic T cells that have been stimulated in the absence of costimulation (11). In contrast, the transcriptional profile of $CD8^+$ T cells from $CD4^+$ T cell–depleted Tg mice was similar to that of effector and exhausted cells (12). Flow cytometry of $CD8^+$ T cells from $CD4^+$ T cell–depleted mice on day ten of abacavir exposure confirmed the presence of cells in an active functional state, cells at a more advanced state of activation and exhaustion, and a significant proportion of cells expressing the skin-homing molecules CLA and CXCR3. Skin biopsies revealed that by week three of abacavir exposure, there was effector $CD8^+$ T cell and macrophage infiltration and elevated expression of genes encoding effector markers, cytotoxic factors, inflammatory chemokines, and adhesion molecules. How might $CD4^+$ T cell depletion in Tg mice overcome tolerance to abacavir? Cardone and colleagues considered the possibility that loss of $CD4^+$ Treg activity was inducing abacavir hypersensitivity, in the same way it can favor autoimmunity or graft-versus-host disease, by increasing DC maturation (13, 14). Indeed, DCs from $CD4^+$ T cell–depleted Tg mice had increased expression of CD80 and CD86, but not PD-L1, CD40, or HLA-B*57:01 by day three, with or without abacavir. By day ten, however, the presence of drug enhanced the expression of CD80, CD86, PD-L1, and HLA-B*57:01 compared with anti-CD4 treatment alone. DCs with higher levels of CD80 and CD86 also expressed more PD-L1. CD80 on DCs is known to bind PD-L1 and prevent the inhibitory interaction with PD-1 on $CD8^+$ T cells and to costimulate T cells by CD28. Cardone and colleagues therefore hypothesized that blocking costimulation with CTLA-4–Ig or anti-CD80 in the $CD4^+$ T cell–depleted Tg mice would inhibit the accumulation of reactive $CD8^+$ T cells (10). In Tg mice, CTLA-4–Ig inhibited the accumulation of reactive $CD8^+PD\text{-}1^+$ T lymphocytes in skin at day four, suggesting that basal expression of CD80 and CD86 is required for early transient activation of abacavir-responsive $CD8^+$ T cells. Importantly, costimulatory blockade also inhibited the differentiation of functionally active, skin-homing, abacavir-responsive $CD8^+$ T cells in $CD4^+$ T cell–depleted mice. Taken together, these findings indicated that DC maturation in vivo is increased by the lack of $CD4^+$ T cells and is required to develop pathogenic drug-reactive effector $CD8^+$ T cells.

Finally, Cardone et al. considered why, in contrast to the in vivo findings in their mouse model, drug-reactive $CD8^+$ T cells can be activated in vitro with or without $CD4^+$ T cells (10). The authors compared DC maturation levels in LN cells from drug-naive mice ex vivo and in culture. Strikingly, the costimulatory molecules HLA-B*57:01, CD86, and CD80 were upregulated as a consequence of cell culture, even in the absence of abacavir, and addition of CTLA-4–Ig or anti-CD80 mAb to cultures impaired the production of IFN-γ by LN cells treated with abacavir. This critical observation explains the apparent paradox that drug-reactive $CD8^+$ T cells can be activated in vitro from all abacavir-naive individuals carrying HLA-B*57:01, while only 45% of these HLA-B*57:01 carriers would tolerate the drug in vivo, without developing symptoms or becoming skin-patch test positive. Importantly, these findings also caution that the drug-reactive T cells obtained from in vitro studies may not be relevant to the pathogenesis of hypersensitivity in vivo.

Conclusions and future directions

Cardone et al. have now provided a model system to study the potential mechanism of abacavir tolerance in the presence of HLA-B*57:01 that could be applied to other drug hypersensitivity phenotypes and drug-HLA combinations (Figure 1). There are still many unanswered questions surrounding the immunopathogenesis of severe T cell–mediated adverse drug reactions. For instance, what is the mechanism that explains the occurrence of abacavir hypersensitivity within two days of first exposure, what drives long-lasting memory, and what is the basis for differing tissue distribution and clinical and laboratory phenotypes for each drug-HLA combination (1)? A possible explanation comes from studies of another "experiment of man" — rejection of HLA-mismatched renal transplants in humans. In this case, preexisting memory $CD8^+$ T cell responses to chronic human herpes virus infection can cross-recognize allo–MHC-I–peptides to rapidly mediate organ rejection, even in the absence of $CD4^+$ T cell help (15). According to a similar heterologous model of drug hypersensitivity (1), DCs and Tregs are unable to tolerize an established high-avidity T cell memory response to an immunodominant epitope from a chronic, persistent virus that cross-reacts

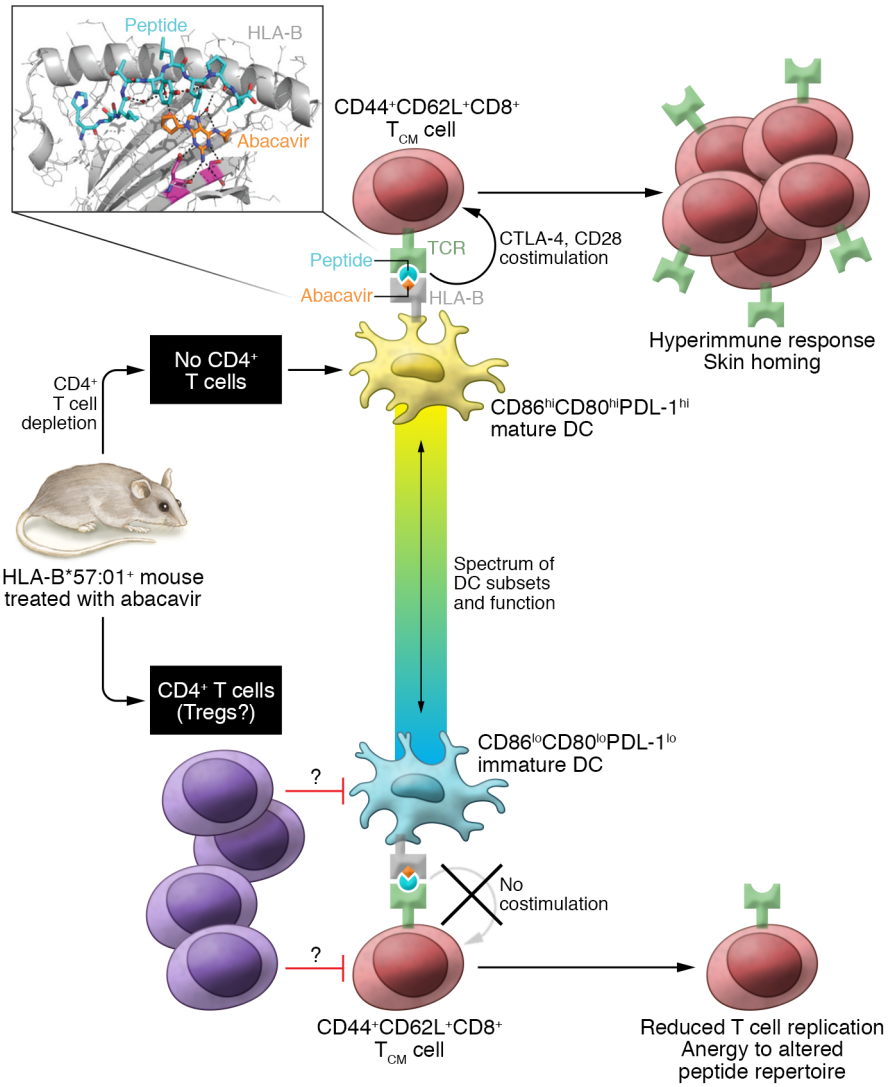

Figure 1. Model of the mechanism of tolerance or hypersensitivity to abacavir in HLA-B*57:01–Tg mice. Model of the mechanism of tolerance or hypersensitivity to abacavir in HLA-B*57:01–Tg mice. Abacavir binds noncovalently to residues on the floor of the peptide-binding groove of the HLA-B*57:01 molecule and the overlying endogenous peptide, dramatically altering the repertoire of endogenous peptides presented to early-responding $CD44^{hi}CD62L^{hi}CD8^{+}$ Tcm cells (see inset). $CD4^{+}$ T cell depletion results in the maturation of DCs to a $CD86^{hi}CD80^{hi}PD\text{-}L1^{hi}$ phenotype that is capable of costimulation and induction of effector $CD8^{+}$ T cells that can home to the skin and induce hypersensitivity. Without $CD4^{+}$ T cell depletion, DCs remain in an immature state ($CD86^{lo}$ $CD80^{lo}PD\text{-}L1^{lo}$), and the mouse remains tolerant to the altered peptide repertoire. Tcm, T central memory cell; TCR, T cell receptor.

with a drug-induced neoepitope. Together, this model would explain why $CD4^{+}$ T cell help is not required for induction of the $CD8^{+}$ T cell response, why hypersensitivity may be seen within two days of drug initiation, why different drugs induce very different characteristic clinical syndromes, and why lifelong memory responses to the drug persist after drug withdrawal (1).

Other key issues to address in future studies include defining the drug neoepitopes that mediate the immunopathogenesis of drug hypersensitivity and tolerance. Most T cell responses to the myriad of drug-induced neoantigens are of low avidity and, therefore, are likely to alter the phenotype and function of DCs to favor tolerance (16). To understand this process, it will be necessary to distinguish at a single-cell level the likely pathogenic, high-avidity responses with effector function and tissue homing that also favor DC maturation and function from the low-avidity responses driving tolerance. Such studies of HLA-restricted, T cell–mediated drug hypersensitivity may provide key mechanistic insights and approaches to inform the study of natural forms of hypersensitivity or autoimmunity.

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1. White KD, Chung WH, Hung SI, Mallal S, Phillips EJ. Evolving models of the immunopathogenesis of T cell-mediated drug allergy: The role of host, pathogens, and drug response. *J Allergy Clin Immunol*. 2015;136(2):219–234; quiz 235.
2. Hetherington S, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet*. 2002;359(9312):1121–1122.
3. Mallal S, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet*. 2002;359(9308):727–732.
4. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. *N Engl J Med*. 2008;358(6):568–579.
5. Ostrov DA, et al. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc Natl Acad Sci U S A*. 2012;109(25):9959–9964.
6. Illing PT, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature*. 2012;486(7404):554–558.
7. Norcross MA, et al. Abacavir induces loading of novel self-peptides into HLA-B*57: 01: an autoimmune model for HLA-associated drug hypersensitivity. *AIDS*. 2012;26(11):F21–F29.
8. Lucas A, et al. Abacavir-reactive memory T cells are present in drug naïve individuals. *PLoS One*. 2015;10(2):e0117160.
9. Chessman D, et al. Human leukocyte antigen class I-restricted activation of CD8+ T cells provides the immunogenetic basis of a systemic drug hypersensitivity. *Immunity*. 2008;28(6):822–832.
10. Cardone M, et al. A transgenic mouse model for HLA-B*57:01–linked abacavir drug tolerance and reactivity. *J Clin Invest*. 2018;128(7):2819–2832.
11. Safford M, et al. Egr-2 and Egr-3 are negative regulators of T cell activation. *Nat Immunol*. 2005;6(5):472–480.
12. Kaech SM, Hemby S, Kersh E, Ahmed R. Molecular and functional profiling of memory CD8 T

cell differentiation. *Cell*. 2002;111(6):837–851.
13. Muth S, Schütze K, Schild H, Probst HC. Release of dendritic cells from cognate CD4$^+$ T-cell recognition results in impaired peripheral tolerance and fatal cytotoxic T-cell mediated autoimmunity. *Proc Natl Acad Sci U S A*. 2012;109(23):9059–9064.
14. Schildknecht A, et al. FoxP3$^+$ regulatory T cells essentially contribute to peripheral CD8$^+$ T-cell tolerance induced by steady-state dendritic cells. *Proc Natl Acad Sci U S A*. 2010;107(1):199–203.
15. Amir AL, et al. Allo-HLA reactivity of virus-specific memory T cells is common. *Blood*. 2010;115(15):3146–3157.
16. Mailliard RB, et al. Selective induction of CTL helper rather than killer activity by natural epitope variants promotes dendritic cell-mediated HIV-1 dissemination. *J Immunol*. 2013;191(5):2570–2580.