

REVIEW ARTICLE

Human leukocyte antigens (HLA) associated drug hypersensitivity: consequences of drug binding to HLA

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Abstract

Recent publications have shown that certain human leukocyte antigen (HLA) alleles are strongly associated with hypersensitivity to particular drugs. As HLA molecules are a critical element in T-cell stimulation, it is no surprise that particular HLA alleles have a direct functional role in the pathogenesis of drug hypersensitivity. In this context, a direct interaction of the relevant drug with HLA molecules as described by the p-i concept appears to be more relevant than presentation of hapten-modified peptides. In some HLA-associated drug hypersensitivity reactions, the presence of a risk allele is a necessary but incomplete factor for disease development. In carbamazepine and HLA-B*15:02, certain T-cell receptor (TCR) repertoires are required for immune activation. This additional requirement may be one of the 'missing links' in explaining why most individuals carrying this allele can tolerate the drug. In contrast, abacavir generates polyclonal T-cell response by interacting specifically with HLA-B*57:01 molecules. T cell stimulation may be due to presentation of abacavir or of altered peptides. While the presence of HLA-B*58:01 allele substantially increases the risk of allopurinol hypersensitivity, it is not an absolute requirement, suggesting that other factors also play an important role. In summary, drug hypersensitivity is the end result of a drug interaction with certain HLA molecules and TCRs, the sum of which determines whether the ensuing immune response is going to be harmful or not.

Adverse drug reactions (ADRs) are a major cause of morbidity and mortality worldwide. Up to a third of ADRs are attributable to unpredictable drug hypersensitivity, and a significant proportion of these are immune mediated (1). Drug-specific T cells are frequently involved in the pathogenesis, and a growing number of drug hypersensitivity reactions are found in association with human leukocyte antigen (HLA) alleles. Strongest associations have been described for abacavir hypersensitivity syndrome and flucloxacillin-induced hepatotoxicity with HLA-B*57:01 (2–4), carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) with HLA-B*15:02 in Han Chinese (5, 6), and allopurinol-induced severe cutaneous adverse reactions (SCAR) with HLA-B*58:01 in Han Chinese (7) (Table 1). There are also other implicated HLA alleles with varying degrees of association, but the strongest of these have only been described for MHC I molecules so far. Comprehensive lists of these associations have been published elsewhere and will not be reproduced here (8, 9). This review will focus on using these HLA associations as models to explain how drugs can stimulate T cells.

Immunopathology of T cell-mediated drug hypersensitivity

Hapten/pro-hapten and p-i (pharmacological interactions of drugs with immune receptors) concepts are crucial in understanding how a drug activates the immune system and initiates delayed-type hypersensitivity by activating T cells. These concepts have been extensively reviewed elsewhere and will be discussed briefly here (10).

Because a typical drug is not antigenic on its own because of its small size, it must first bind to a high molecular weight protein to be immunogenic. A stable covalent binding of a chemically reactive drug to a larger protein or peptide is then able to form a neoantigen, which can be recognized directly via immunoglobulin or by T cells after undergoing antigen processing of the hapten-carrier complex. The chemical properties of hapten-like drugs are crucial for the generation of antigenic epitopes and activation of the innate immune system. Some haptens like beta-lactam antibiotics have a tendency to bind to particular amino acids, and this has been

Table 1 Key HLA associations with drug hypersensitivity (OR > 50)¹

Drug	HLA allele	Disease association	Studied population	OR (95% CI)	References
Abacavir	HLA-B*57:01	Abacavir hypersensitivity syndrome	Western Australian	117 (29–481) 960 ²	(3) (2)
Carbamazepine	HLA-B*15:02	SJS/TEN	Han Chinese	1357 (193–8838)	(6)
Allopurinol	HLA-B*58:01	SJS/TEN/DRESS	Han Chinese	580 (34–9781)	(7)
	HLA-B*58:01	SJS/TEN	European	80 (34–187)	(23)
Flucloxacillin	HLA-B*57:01	Drug-induced liver injury	European	80 (23–285)	(4)

¹OR > 50 is arbitrarily chosen as a cutoff, and this is adapted from the review by Phillips and Mallal (8).

²The OR was calculated for definite hypersensitivity syndrome based on positive patch testing.

HLA, human leukocyte antigen; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis; DRESS, drug reaction with eosinophilia and systemic symptoms.

well characterized for penicillin that has a propensity to bind to lysine residues (11). Theoretically, this generates many different new antigenic determinants owing to a vast array of available lysine residues in target proteins *in vivo*. Consequently, the elicited immune responses are expected to be variable, and penicillins are, indeed, known to cause both predominant antibody responses and delayed-type T-cell responses. However, recent reports have shown that piperacil-

lin can bind up to 13 of 59 lysine residues in human serum albumin, indicating that there are some preferential binding sites for haptens (12, 13). While this limits the number of possible antigenic determinants, potential haptenated peptides that can be loaded onto various HLA molecules remain substantial. In other words, drug hypersensitivity to a hapten–peptide complex is less likely to be HLA-restricted as multiple binding sites in a protein imply that after processing, a number of

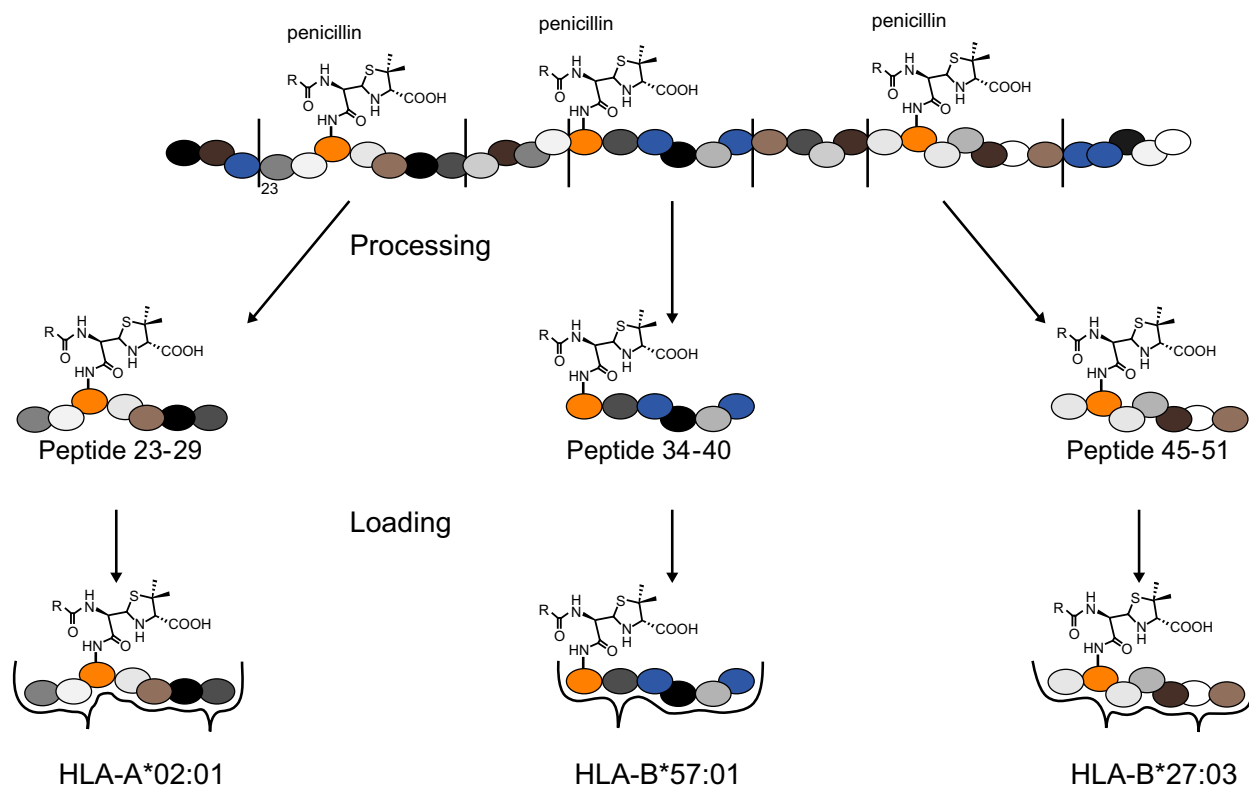


Figure 1 Hapten–protein interaction and human leukocyte antigen (HLA) restriction. A drug may ‘haptensise’ a protein at multiple sites if it has a propensity to form a covalent bond with a particular amino acid. This occurs in penicillin that has a tendency to form covalent bonds with lysine residues. This modified protein is processed into multiple peptide fragments that can be loaded onto different HLA alleles. Furthermore, as a number of peptides can be

shared between closely related HLA alleles, HLA restriction is much likely to be broader, i.e. restricted to serotype rather than to genotype. Therefore, a highly specific HLA restriction is not likely to be seen. An exception may occur if a drug is able to bind covalently only to a specific site in a protein resulting in the presentation of this haptenised peptide by a particular HLA molecule. This allele restricted presentation of hapten has not been shown yet.

potential drug-bound peptides are available for loading onto different types of HLA alleles (Fig. 1). So far, there are no proven examples of a hapten-restricted immune response that is strictly associated with a HLA allele, and although the association of HLA alleles with aminopenicillin hypersensitivity has been reported, the relative risk is quite low (14).

The pro-hapten concept proposes that a chemically inert drug may become reactive after undergoing metabolism, after which it is then able to form a hapten and stimulate an immune response (15). A classical example of this is sulfamethoxazole, which is metabolized to a reactive compound and forms a hapten. As many enzymes involved in the metabolism of drugs in target organs have not been clearly defined, the protein targets and the specific sites of modification also remain largely unknown. This limitation has made the role of drug metabolism in T-cell response difficult to study. It is important to note that neither hapten nor pro-hapten necessarily needs to undergo processing to become antigenic. This was illustrated by Horton et al. (16) who showed that amoxicillin, which forms hapten via a covalent bond, is able to stimulate T cells in a processing-independent manner by binding directly to peptide-MHC complex. Thus, hapten formation is not necessarily excluded even when a drug stimulates immune response in a metabolism and processing-independent manner.

It is generally considered that immunogenic hapten or pro-hapten requires the activation of the innate immune system. For example, metabolism of sulfamethoxazole to nitroso sulfamethoxazole in dendritic cells results in their activation (17). However, stimulation of the innate immune system alone is not sufficient to induce a complete immune response, as nickel can stimulate the innate immune system via Toll-like receptor 4 in most people, but only a small proportion of individuals exposed to nickel develop contact dermatitis (18). Similarly, imiquimod, which activates Toll-like receptor 7, rarely causes hypersensitivity. Likewise, drugs that form hapten/pro-hapten in most individuals do not often elicit immune responses.

As an alternative to hapten and pro-hapten concept, p-i concept proposes that a drug is able to stimulate T cells directly without forming a hapten, in a HLA-dependent manner. This may occur if a chemically inert drug that cannot form a covalent bond with a larger carrier interacts directly with T-cell receptors (TCR) or MHC molecules with sufficient affinity. The evidence for p-i concept, derived from both *in vivo* and *in vitro* data, is summarized in the review by Adam et al. (10). In contrast to hapten or pro-hapten, these inert drugs are unlikely to activate the innate immune system. It is hypothesized that T cells that react in p-i manner arise from the memory pool (19). Also, as the drug interacts directly with the MHC molecule or TCR, it is not processing or metabolism dependent.

One should be aware that these distinctions between covalent and noncovalent binding are not straightforward in reality as both may occur together *in vivo*. The drugs known to interact in p-i manner are also known to produce potentially reactive metabolites (10). As there is a paucity of definitive proof on mass spectrometry or crystallography, that HLA-restricted drugs are covalently bound to a carrier, and as the direct binding of a drug to the TCR or MHC molecule often relies on indirect evidence, the controversy

surrounding the immune mechanisms involved will likely persist for some time. The drug-specific immune response, if restricted to T cells, may be clinically similar in both cases as ultimately, drug-specific T cells are expanded and are able to orchestrate the immune response and inflammation.

Epidemiology of HLA association and drug hypersensitivity

The development of drug hypersensitivity depends on both genetic and environmental factors (Table 2). Among genetic factors, HLA alleles play an important role (Table 1). This is particularly well illustrated by abacavir and its association with HLA-B*57:01 allele. Individuals with this allele have approximately a 50% chance of developing abacavir hypersensitivity syndrome, while no one without this allele is predicted to develop an immunologically confirmed hypersensitivity reaction (20). Consequently, the guidelines recommend that the presence of HLA-B*57:01 allele should be excluded before initiating antiretroviral therapy with abacavir, representing one of the first successful examples of personalized medicine (21). Likewise, United States Food and Drug Administration have issued warnings for carbamazepine, stating that HLA-B*15:02 allele should be checked prior to its initiation in patients with ancestry from the areas in which this allele is present. On the other hand, even though the association between flucloxacillin and HLA-B*57:01 allele is very strong, because of its low positive predictive value (PPV), routine testing is not recommended.

These HLA associations with severe manifestations of drug hypersensitivity are intriguing findings because of their exclusive restrictions. HLA-B*57:01 molecule is closely related to HLA-B*58:01 molecule as they only differ by a few amino acid residues and both belong to HLA-B17 serotype. They also share a number of potential peptides that can bind to MHC grooves. However, abacavir hypersensitivity and allopurinol hypersensitivity are exclusively associated with HLA-B*57:01 allele and HLA-B*58:01 allele, respectively. In contrast, HLA-B*15:02 molecule and HLA-A*31:01 molecule are structurally remote but are both associated with carbamazepine hypersensitivity. The reasons for these paradoxical findings are yet to be elucidated.

A number of studies have been conducted across various ethnic groups, and some of these have concluded that drug hypersensitivity and HLA association are dependent on ethnicity (22, 23). Linkage disequilibrium has been considered as a potential explanation for these discrepancies, but

Table 2 Risk factors other than the associated human leukocyte antigen alleles for drug hypersensitivity

Immunogenicity of the drug (53)
Factors that affect drug levels <i>in vivo</i> , such as dosage, other drugs that interfere with metabolism or chronic renal insufficiency (7, 53)
Viral infections: HIV and herpes viruses (46, 54)
T-cell receptor repertoires (40)
Other genetic polymorphisms (55)
Female sex (56, 57)

differences in allele frequencies (AF) must also be taken into account. For example, in the Han Chinese population, carbamazepine-induced SJS/TEN is strongly associated with HLA-B*15:02 (OR = 1357) (6). However, this association was not found in European or Japanese studies where the prevalence of this allele is <1% and 0.1%, respectively (Table 3) (22, 24, 25). Even though the risk of developing carbamazepine-induced SJS/TEN is substantially higher for those who carry this allele, these studies likely failed to pick up this association as the AF is very low in these cohorts. On the other hand, carbamazepine hypersensitivity is found to be associated with HLA-A*31:01 (OR = 25.93–33.9) in these groups where the AF of this gene is higher (Table 3) (24, 26). Interestingly, HLA-A*31:01 allele was also identified by Hung et al. (6) to be associated with carbamazepine-induced maculopapular exanthem (MPE) in Han Chinese. This is expected because Han Chinese also have the AF of 1.8% for HLA-A*31:01.

The usefulness of predictive testing is also influenced by AF. The association of HLA-B*57:01 allele with abacavir hypersensitivity syndrome was mainly confirmed in Caucasian populations where the AF is high. However, the studies performed in Taiwanese and Korean population where HLA-B*57:01 is rare (<1%) showed that there were HLA-B*57:01-negative patients with clinically suspected abacavir hypersensitivity (27, 28). In these populations, it is not clear whether HLA-B*57:01 testing is clinically useful or not. Furthermore, it must be noted that 100% sensitivity of HLA-B*57:01 testing for abacavir hypersensitivity was found after eliminating the patch test–negative group, but when clinically diagnosed hypersensitivity reaction was used as the criterion, the sensitivity became much lower at 45.5% (20). This discrepancy between patch test–positive and patch test–negative patients was interpreted as a sign for true or ‘false’ immune-mediated abacavir hypersensitivity. However, many variables may play a role in patch test reactions, and a negative patch test does not necessarily exclude an immune-mediated mechanism.

Immunology of abacavir hypersensitivity in HLA-B*57:01 – unique interaction of drug with HLA

Abacavir hypersensitivity syndrome is manifested clinically by a combination of fever, rash, malaise, nausea, vomiting,

and diarrhea, and it can sometimes be fatal upon rechallenge (29). The first discovery of its association with HLA-B*57:01 allele approximately 10 years ago was an exciting development for drug hypersensitivity research (3, 30). Because of its narrow HLA restriction and high PPV of 47.9%, it provides a unique model to study the pathogenesis of drug hypersensitivity.

An initial study by Martin et al. (31) suggested an involvement of the innate immune system in abacavir hypersensitivity reactions whereby abacavir activates antigen-presenting cells (APC) via HSP70-mediated Toll-like receptor pathway. However, there have been no further reports that confirm this finding (9). The generation of abacavir-specific T-cell lines from abacavir-naïve HLA-B*57:01-positive donors demonstrated that abacavir-specific T cells require HLA-B*57:01 molecule for activation (29). The restriction to HLA-B*57:01 was exclusive as CD8 T cell–driven, drug-specific response was abolished if HLA-B*57:01 was replaced by closely related HLA-B*57:03 or even by a single, crucial amino acid mutation. This implies that a single amino acid in HLA-B*57:01 may be the determining factor in the development of abacavir hypersensitivity.

Because abacavir reactivity was considered to be TAP and tapasin dependent, it was suggested that processing is required for abacavir presentation (29). In this model, abacavir or its metabolite would be haptenized to a protein that then undergoes processing before it is loaded onto the HLA-B*57:01 peptide-binding groove (9, 32). Yet, this hypothesis does not explain the extreme selectivity for HLA-B*57:01, because this pool of potentially generated abacavir-modified peptides would likely be loaded onto various other MHC I molecules. An alternative explanation that reconciled the exclusive association of HLA-B*57:01 molecule with abacavir reactivity and the requirement for TAP and tapasin was recently illustrated by Adam et al. (33). Their study demonstrated that T-cell reactivity to abacavir was determined by the TCR avidity for the drug. Because of the diminished expression of MHC I on TAP and tapasin-deficient cells, T-cell reactivity to abacavir was therefore very likely reduced as well. Furthermore, abacavir was shown to be neither metabolized nor processed inside APC. This supports a noncovalent, yet rather strong interaction of abacavir molecules with HLA-B*57:01, which is consistent with p-i concept (10, 33).

Table 3 HLA alleles associated with carbamazepine hypersensitivity

	Han Chinese (6)	Japanese (24)	European (26)
HLA-B*15:02			
OR (95% CI)	1357 (193.4–8838.3) for SJS/TEN	Association not found ¹	Association not found
AF (25)	8.6%	0.1%	<1%
HLA-A*31:01			
OR (95% CI)	Association not found for SJS/TEN 17.5 (4.6–66.5) for MPE	33.9 (3.9–295.6) for SJS/TEN 8.0 (3.9–16.6) for other cADR ²	25.93 (4.93–116.18) for SJS/TEN 8.33 (3.59–19.36) for MPE
AF	1.8%	9.1%	2–5%

¹In the study by Ozeki et al., (24) there were no individuals with HLA-B*15:02 alleles in either the control population or those with cADR.

²Other cADR excludes DRESS and SJS/TEN.

AF, allele frequency; cADR, cutaneous adverse drug reactions; DRESS, drug reaction with eosinophilia and systemic symptoms; HLA, human leukocyte antigen; OR, odds ratio; MPE, maculopapular exanthem; SJS/TEN, Stevens–Johnson syndrome/toxic epidermal necrolysis.

This noncovalent binding was lately verified by modeling and crystallography data, demonstrating the incorporation of abacavir into the F anchor pocket of HLA-B*57:01 during its formation (34, 35). This drug binding alters the landscape of the peptide-binding cleft, affecting the potential peptide repertoire that is loaded onto this MHC I molecule (Fig. 2A, B). Peptide elution experiments revealed that in addition to the usual B*57:01 binding peptides, abacavir-treated HLA-B*57:01-positive APC presented also altered peptides compared to the untreated control APC. In the absence of abacavir, HLA-B*57:01 molecules particularly displayed peptides with a tryptophan or phenylalanine at position 9 fitting into the F pocket of the HLA-B*57:01 molecule. In the presence of the drug, however, some identified HLA-B*57:01-embedded peptides bore smaller amino acid residues at position 9, predominantly valine, leucine, or isoleucine (34–36). Thus, the noncovalent MHC interaction of abacavir alters self-peptide loading onto HLA-B*57:01 molecules. Consequently, new endogenous peptides are selected to be displayed on the cell surface of APC, and these neoantigens then initiate polyclonal T-cell response to self-epitopes. This abacavir-induced alteration in the presented peptide repertoire provides a novel explanation for drug-dependent autoimmunity. Analogous to altered peptide repertoire by abacavir, drug-affected peptide loading has been already described for MHC class II system by using compounds known as ‘MHC loading enhancer’ (MLE) (37). These MLE can improve the transfer of peptides onto MHC II molecule, resulting in an increased immune response in a manner similar to abacavir that increases the loading of peptides with valine, leucine, or isoleucine at C-terminus. However, it remains to be determined to what extent this phenomenon contributed to the disease manifestation in abacavir hypersensitivity and whether it can be generalized to other HLA-associated drug hypersensitivity reactions.

Although the altered peptide hypothesis outlined above provides a novel possible explanation for abacavir-induced hypersensitivity reactions, the suggested model does not consider the full spectrum of this specific allergic response. The study from Adam et al. (33) revealed that approximately 40% of all analyzed abacavir-specific T-cell clones (TCC) reacted immediately or very fast (<2–5 min) to the addition

of abacavir in solution (Adam J, Yerly D and Pichler WJ, unpublished data). This is too fast to allow peptide loading and presentation, strongly suggesting that at least part of abacavir-specific TCC react with abacavir itself and not an altered peptide. Interestingly, the very same immediately reacting clones were also stimulated by APC that were abacavir-treated for more than 12 h after which abacavir in solution was removed. How these clones can recognize immediately presented abacavir on the APC surface and after a long incubation remains to be determined. The possibilities of how abacavir could be directly recognized are illustrated in Fig. 2C. The drug could, for example, bind to ‘incompletely’ filled HLA-B*57:01 molecule or even empty molecule. Alternatively, abacavir may be recognized by TCR in the presence of self-peptides. However, these possibilities need to be proven.

Immunology of carbamazepine hypersensitivity in HLA-B*15:02 – complex interactions of a drug with TCR and HLA allele

As mentioned above, HLA-B*15:02 allele has been strongly associated with carbamazepine-induced SJS/TEN in Han Chinese. Why HLA-B*15:02 is a risk factor for only SJS/TEN and not DRESS or MPE is unknown. It is tempting to hypothesize that because SJS/TEN, DRESS, and MPE are distinct clinical manifestations, HLA-B*15:02 molecule is only able to recruit T cells with specific phenotypes. However, this explanation is inadequate as HLA-B*31:01 allele is associated with carbamazepine-induced SJS/TEN, DRESS, and MPE. In fact, this association with different clinical manifestations points to the possibility that these clinical entities must somehow share common features in drug, TCR, and HLA interactions.

Analogous to abacavir and HLA-B*57:01, carbamazepine interacts specifically with HLA-B*15:02 molecules. However, it was found that other structurally related HLA-B*75 family members can also present carbamazepine (38). This interaction is processing and metabolism independent, in a manner that is most consistent with the p-i concept (39). The study by Ko et al. (40) shed further light on this subject by showing that in addition to the HLA allele, the availability of TCR is crucial

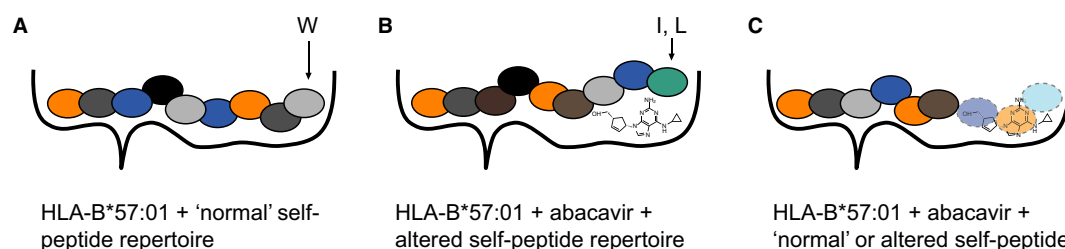


Figure 2 Schema for possible antigenic conformations of HLA-B*57:01 plus abacavir. Without abacavir: (A) HLA-B*57:01 molecules present ‘normal’ self-peptide and this does not trigger T cell activation. With abacavir: (B) HLA-B*57:01 molecules bind abacavir and present altered self-peptides, where peptides bearing tryptophan (W) at C-terminus are preferentially replaced by those that

carry Isoleucine (I) or leucine (L) at this site. (C) HLA-B*57:01 molecules bind abacavir, which is clearly recognizable by the TCR. The peptide might be a self peptide, be altered or shorter (i.e. without dotted circles), or even be absent. TCR, T-cell receptors. HLA, human leukocyte antigen.

for the development of carbamazepine-induced SJS/TEN. They found that two most immunodominant CDR3 clonotypes of TCR, VB-11-ISGSY and VB-11-GLAGVDN, were shared among five and two of the eight patients with carbamazepine-induced SJS/TEN, respectively. Further analysis revealed that these clonotypes could be found in PBMC or blister cells of patients with carbamazepine-induced SJS/TEN, but not in carbamazepine-tolerant control subjects, including those with HLA-B*15:02 allele. Interestingly, these clonotypes were present at a low frequency (7–14%) in healthy subjects with HLA-B*15:02 alleles. When PBMC from these healthy individuals were cultured with carbamazepine *in vitro*, carbamazepine-specific T-cell response could be demonstrated, whereas this could not be obtained in HLA-B*15:02-positive donors who do not have these clonotypes. Thus, this study provides strong evidence that in addition to the associated HLA allele, particular TCR also play a role in the development of the immune response against carbamazepine.

This finding may explain, at least in part, why most individuals with HLA-B*15:02 allele do not develop carbamazepine hypersensitivity. This is in sharp contrast to abacavir hypersensitivity and HLA-B*57:01 allele where drug-specific T cells were reported to contain multiple TCR clonotypes without shared TCR variable beta gene usages between individuals, implying that particular sequences of TCR do not appear to play a major role (9, 34). In summary, the available repertoire of TCR in combination with HLA-B*15:02 is a risk factor for carbamazepine-induced SJS/TEN, but this requirement for TCR does not seem to be the case for abacavir hypersensitivity and HLA-B*57:01. Because this additional prerequisite is not required for the generation of abacavir-specific T cells, it should be no surprise that the PPV for HLA-B*57:01 allele is higher than that for HLA-B*15:02.

Allopurinol hypersensitivity and HLA-B*58:01 – one association for diverse manifestations

HLA-B*58:01 allele is strongly associated with allopurinol-induced SJS/TEN. While the initial study by Hung et al. (7) did not analyze allopurinol-induced SJS/TEN and DRESS separately, more than half of the patients in that study had DRESS and all of them were found to have the HLA-B*58:01 allele, making it highly likely that allopurinol-induced DRESS is also associated with HLA-B*58:01. This strong association with allopurinol-induced DRESS was also confirmed in a Korean study (41). As is the case for HLA-A*31:01 and carbamazepine-induced SJS/TEN, DRESS, and MPE, how one HLA allele restriction can manifest itself in diverse clinical manifestations is yet to be clarified. However, in contrast to HLA-A*31:01, a recent Australian study found that none of 12 patients with allopurinol-induced MPE had HLA-B*58:01, possibly hinting that this association may be limited to more severe phenotypes (42).

What additional risk factors could contribute to allopurinol hypersensitivity? Renal failure has long been associated with this syndrome, and this association appears to be maintained even when HLA-B*58:01 alleles were considered (7, 43). In

addition, a Korean study of patients with chronic renal insufficiency taking allopurinol revealed that 18% (9/50) of HLA-B58-positive patients developed allopurinol-induced SCAR (44). This is much higher than the previously estimated PPV of 2.7% with HLA-B*58:01 (8). Furthermore, a recent study has found that a daily dose of 200 mg or more was associated with an increased risk for SJS/TEN (45). Putting these together, it can be speculated that either renal impairment or higher dosage results in higher serum levels of allopurinol and/or its metabolite, oxypurinol, which in turn increases the risk of generating drug-specific T cells.

In addition to the availability of drug levels and HLA associations, viral infections have long been known to play a role in drug hypersensitivity. This is particularly relevant for DRESS in which the reactivation of herpes virus seems to play a key role in its pathogenesis (46, 47). According to the study by Daubner et al. (48), patients with multiple drug hypersensitivity often had DRESS and the drug-specific T cells from these patients were found to have preactivated T-cell phenotypes. Therefore, it was suggested that this *in vivo* T-cell activation is attributable to chronic stimulation by latent herpes viruses, which result in upregulation of costimulatory molecules, subsequently providing additional signals for the generation of drug-specific T cells upon exposure. Alternative to this hypothesis, the Japanese group has suggested that DRESS is mainly driven by antiviral T cells that can cross-react with drugs (46). Finally, Picard et al. (47) postulated that the culprit drug may somehow induce the reactivation of herpes viruses, which then triggers an antiviral immune response. In the context of allopurinol-induced DRESS, how viral reactivation, HLA-B*58:01 molecules, and allopurinol interact in the context of T cell-driven antiviral/antidrug immune responses is yet to be elucidated.

As outlined above, the available TCR repertoire is another important factor for drug hypersensitivity. The TCR repertoire is highly variable and this is determined by TCR gene rearrangements and thymic selection, which in turn, is also determined by MHC molecules and self-peptide repertoires, as well as by the history of antigen load such as infections. Therefore, it is possible that a repertoire of TCR required for drug-specific T-cell response is deleted in the process of negative selection as other HLA alleles may present self-peptides of sufficient affinity, resulting in cell death. If such 'protective' HLA alleles were absent, then the risk of developing drug hypersensitivity would be higher. On the other hand, the converse may also be true and the potentially dangerous TCR repertoire may be selected in those with risk alleles. In support of this hypothesis, the association between proportion of shared TCR sequences and shared HLA class I alleles has been recently demonstrated (49). Furthermore, Kosmrlj et al. (50) showed that HLA alleles play an important role in TCR selection and illustrated that those with HLA-B*57:01 allele are likely to have more T cells that can cross-react with mutants of target epitopes. Finally, as a history of infections in an individual can affect the pool of circulating TCR repertoires, this may also result in an expansion of T cells that react with a drug.

There are other known risk factors but whether they are also relevant for HLA-associated drug hypersensitivity is

unknown (Table 2). Combining these known risk factors together, the following scenarios can be considered. The presence of HLA-B*58:01 allele alone is inadequate as although allopurinol itself may interact specifically with the MHC molecule, this in itself is insufficient to generate drug-specific T cells (Fig. 3A). This can explain why the PPV is only 2.7% (8). However, when there is drug accumulation because of an alteration in dosage or renal failure, more allopurinol or oxypurinol may be presented by HLA-B*58:01 molecules, culminating in sufficient signals for T-cell activation (Fig. 3B). This can be postulated from the abacavir-specific TCC data discussed above, which shows that TCR avidity determines T-cell reactivity; T cells with lower avidity will be recruited with increasing concentration of the drug (33). In the absence of HLA-B*58:01 molecules, the chance of developing allopurinol hypersensitivity is very low (Fig. 3C). However, if a concurrent herpes viral infection results in strong enough costimulation, expansion of cross-reactive T cells, or favorable allopurinol interaction with viral peptide loaded on MHC I molecules, then this may be enough to generate drug-specific T cells (Fig. 3D). Therefore, the presence of HLA-B*58:01 allele is preferential but not essential for the development of allopurinol hypersensitivity, and the barrier for the development of drug hypersensitivity may be overcome if other risk factors are present.

Other unresolved issues and the future direction in HLA and drug hypersensitivity

Daly et al. (4) have recently identified the association between HLA-B*57:01 and flucloxacillin-induced hepatotoxicity. However, as this study dealt exclusively with hepatotoxicity, it is not known whether other manifestations such as rash or nephritis are also associated with B*57:01 or not. Nonetheless, as rash is not a prominent feature of flucloxacillin-induced hepatotoxicity, why other target organs are spared remains a mystery (51). Furthermore, how HLA-B*57:01 allele can be associated with hypersensitivity to two structurally unrelated drugs with completely different clinical manifestations is also unknown. In contrast to abacavir hypersensitivity, the PPV of flucloxacillin-induced hepatotoxicity is extremely low at 0.12%, which implies that different mechanisms may be involved in these disease manifestations (8).

With increasing technology and sophisticated tools available to analyze genes, more HLA associations with drug hypersensitivity are likely to emerge. In addition, advances in computer modeling and screening tools will likely improve the prediction of drug hypersensitivity. It is interesting to note that Yang et al. (52) were able to predict the interaction of HLA-B*57:01 molecule with abacavir using a bioinformatics approach rather than *in vivo* or *in vitro* models. Such methods, when coupled with

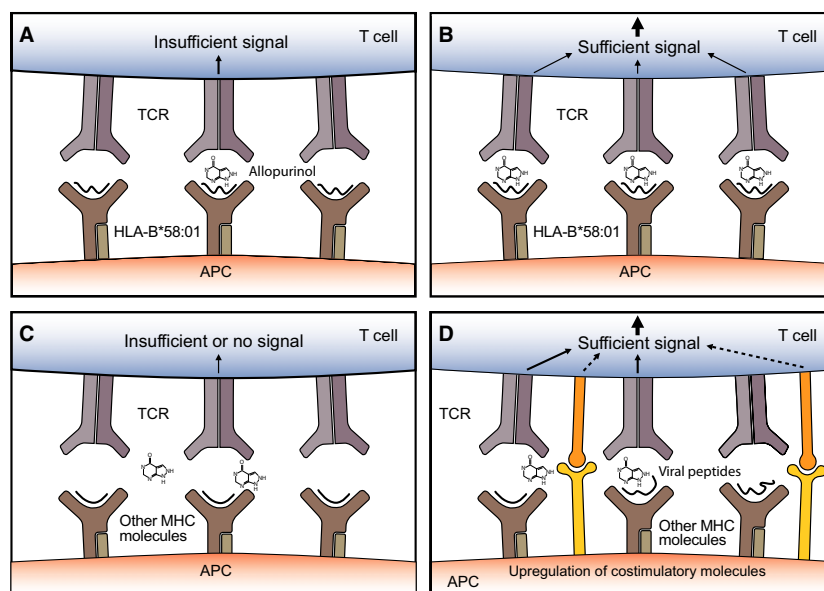


Figure 3 Possible mechanisms involved in allopurinol recognition by T cells. (A) HLA-B*58:01 molecules are able to present allopurinol specifically but when the drug concentration is low and the danger signal is absent, there is insufficient signal to activate T cells. (B) When the drug concentrations are increased, e.g. due to renal failure, then more allopurinol molecules are presented on HLA-B*58:01 molecules, resulting in sufficient signal for T cell activation. (C) In non-HLA-B*58:01 individuals, MHC I molecules do

not interact specifically with allopurinol and there is an insufficient or absent signal for T cell activation. (D) In the presence of a viral infection, costimulatory molecules are upregulated, lowering the threshold that is required for T cell activation. Alternatively, viral peptide specific T cells may cross-react with allopurinol, or allopurinol may interact more favourable with viral peptides loaded MHC molecules, stimulating T cell responses. HLA, human leukocyte antigen.

experimental data, will prove to be invaluable tools for drug hypersensitivity research and for the pharmaceutical industry.

Conclusion

The recent discovery of strong HLA associations with drug hypersensitivity has opened an exciting chapter in drug hypersensitivity research as it provides a unique model to study drug hypersensitivity. While *in vitro* data so far have been limited, complex interactions of drugs with HLA molecules, peptides, and TCR are beginning to be understood. The role of processing and direct interaction of abacavir with HLA-B*57:01 molecule have been both illuminating and puzzling, particularly in view of the recently described altered self-peptide repertoire and T-cell reactivity kinetics (33–36). The study of HLA-B*15:02 and its pathogenic role in carbamazepine-induced SJS/TEN has highlighted that the presence of particular CDR3 in TCR is also crucial in the immunopathogenesis (40). In addition, the complexity of allopurinol-induced SCAR with its diverse clinical manifestations in the context of other

relevant risk factors such as viral infection will need further investigations. Over the coming years, it is likely that further evidence for more complex interactions between drug and immune system will emerge, demanding more holistic explanations that will require inputs from multiple disciplines. Further advances in this perplexing field will make these presently unpredictable reactions more predictable and preventable.

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Conflict of interest

None.

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